Reference Manual 2.90



Multi-channel data acquisition software





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Title Page: Cultivated Nerve Cell, soma contacted by a patch pipette; Courtesy of Prof. Sakmann, Max-Planck-Institute for Medical Research, Heidelberg, Germany

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COMDCM/3

Contents

1	Intr	roduction	1
	1.1	Disclaimer	1
	1.2	Scope of the Program	1
	1.3	The Chartmaster Concept	1
	1.4	Supported Data Acquisition Interfaces	4
	1.5	Supported System Software	4
	1.6	Naming Conventions	4
		1.6.1 Interfaces	4
		1.6.2 Windows Versions	4
		1.6.3 Syntax	4
	1.7	Windows and Mac Key Conventions	5
	1.8	Installing Chartmaster	5
	1.9	Starting Chartmaster	5
	1.10	Get Online Help on Keys	6
	1.11	Get Online Help Controls	7
	1.12	Closing Chartmaster	7
	1.13	Support Hotline	7
2	Use	er Interface	9
2	Use 2.1	er Interface Dialog Controls	9 9
2	Use 2.1	pr Interface Dialog Controls 2.1.1 Numerical Input	9 9 10
2	Use 2.1 2.2	er Interface Dialog Controls 2.1.1 Numerical Input Modifying Dialogs and Controls	9 9 10 10
2	Use 2.1 2.2	Printerface Dialog Controls 2.1.1 Numerical Input Modifying Dialogs and Controls 2.2.1 Dialog Control Window	 9 10 10 11
2	Use 2.1 2.2	Pri Interface Dialog Controls 2.1.1 Numerical Input Modifying Dialogs and Controls 2.2.1 Dialog Control Window 2.2.2 Hiding Controls	 9 10 10 11 13
2	Use 2.1 2.2	pr Interface Dialog Controls 2.1.1 Numerical Input Modifying Dialogs and Controls 2.2.1 Dialog Control Window 2.2.2 Hiding Controls 2.2.3 Deleting Controls	 9 10 10 11 13 13
2	Use 2.1 2.2 2.3	Prior Interface Dialog Controls 2.1.1 Numerical Input Numerical Input Modifying Dialogs and Controls 2.2.1 Dialog Control Window 2.2.2 Hiding Controls 2.2.3 Deleting Controls Saving Dialogs and Controls	 9 10 10 11 13 13 14
2	Use 2.1 2.2 2.3 2.4	Pare Interface Dialog Controls 2.1.1 Numerical Input Modifying Dialogs and Controls 2.2.1 Dialog Control Window 2.2.2 Hiding Controls 2.2.3 Deleting Controls Saving Dialogs and Controls Toggling between Windows	 9 10 10 11 13 13 14 14
2	Use 2.1 2.2 2.3 2.4 2.5	Dialog Controls	 9 10 10 11 13 13 14 14 15
2	Use 2.1 2.2 2.3 2.4 2.5 Key	Part Interface Dialog Controls 2.1.1 Numerical Input Modifying Dialogs and Controls 2.2.1 Dialog Control Window 2.2.2 Hiding Controls 2.2.3 Deleting Controls Saving Dialogs and Controls Copy and Paste Functions	 9 10 10 11 13 13 14 14 15 17
2	Use 2.1 2.2 2.3 2.4 2.5 Key 3.1	Printerface Dialog Controls 2.1.1 Numerical Input Modifying Dialogs and Controls 2.2.1 Dialog Control Window 2.2.2 Hiding Controls 2.2.3 Deleting Controls Saving Dialogs and Controls Toggling between Windows Copy and Paste Functions VS	 9 9 10 10 11 13 13 14 14 15 17 17
2	Use 2.1 2.2 2.3 2.4 2.5 Key 3.1 3.2	bialog Controls	 9 10 10 11 13 13 14 14 15 17 18
2 3 4	Use 2.1 2.2 2.3 2.4 2.5 Key 3.1 3.2 Men	bialog Controls 2.1.1 Numerical Input Modifying Dialogs and Controls 2.2.1 Dialog Control Window 2.2.2 Hiding Controls 2.2.3 Deleting Controls Saving Dialogs and Controls Toggling between Windows Copy and Paste Functions Ys The Key Listing The CHARTMASTER.key File	 9 9 10 11 13 13 14 14 15 17 17 18 21
2 3 4	Use 2.1 2.2 2.3 2.4 2.5 Key 3.1 3.2 Men 4.1	Printerface Dialog Controls 2.1.1 Numerical Input Modifying Dialogs and Controls 2.2.1 Dialog Control Window 2.2.2 Hiding Controls 2.2.3 Deleting Controls Saving Dialogs and Controls Toggling between Windows Copy and Paste Functions rs The Key Listing The CHARTMASTER.key File File Menu	 9 9 10 11 13 13 14 14 15 17 17 18 21 21

	4.3	Windows Menu	25
	4.4	Replay Menu	26
	4.5	Display Menu	31
		4.5.1 3D-Mode	32
		4.5.2 Trace Properties Dialog	33
	4.6	Buffer Menu	35
		4.6.1 Handling of Parameters by the Buffer	36
	4.7	Notebook Menu	36
	4.8	Protocols Menu	37
	4.9	AD-board	38
	4.10	Help Menu	39
5	Con	nfiguration Window	41
	5.1	Individualize Chartmaster	41
	5.2	Save	42
	5.3	General	42
		5.3.1 Max Shown Traces and Values	42
		5.3.2 Window Scaling	42
		5.3.3 Memory Allocation	43
		5.3.4 Minimum Wait Time	43
		5.3.5 Batch Communication	43
		5.3.6 User Name	43
	5.4	Hardware	44
		5.4.1 Amplifier and Digitizer Selection	44
		5.4.2 LockIn	44
		5.4.3 Spectroscopy	45
		5.4.4 Imaging	45
		5.4.5 Photometry	45
		5.4.6 Serial Out	45
		5.4.7 Further Options	46
	5.5	Files	47
		5.5.1 General Advice on Naming Folders and Files	48
		5.5.2 Miscellaneous Settings	48
	5.6	Display	50
		5.6.1 Fonts and Colors	50
		5.6.2 Notebook, Display, and Analysis	50
		5.6.3 Show Options	51
	5.7	I/O Control	51
		5.7.1 AD/DA Input/Output Scaling	52
		5.7.2 List of I/O Parameters	55

		5.7.3 Show Digital In / Out	55
	5.8	Trace Assign	56
	5.9	Miscellaneous	59
6	Osc	illoscope Window	61
	6.1	Display	61
	6.2	Navigation	62
	6.3	Measurements on Traces	62
	6.4	Display Refreshing	62
	6.5	Overlay Options	62
	6.6	Display Scaling	63
	6.7	Multi-Channels	64
7	Con	ntrol Window	65
	7.1	Information about the Experiment	65
	7.2	Controlling Data Acquisition	66
	7.3	Starting Pulse Generator Sequence	66
	7.4	Starting a Protocol	67
	7.5	Fixed Control Protocols	67
8	Rep	blay Window	69
	8.1	Main window functions	69
		8.1.1 Tree Handling	70
0	ות		70
9	Puls	Different Viewe of the Deley Consector	73
	9.1	Different views of the Pulse Generator	75
	9.2	Sequence Deel Handling	75
	9.5		76
	9.4 0.5	Timing	76
	9.0	Charle and Evocuto	70
	9.0 0.7	Sweep and Channel Longth	78
	9.1	Wave Parameters	78
	5.0	9.8.1 Sine Wave Parameters	78
		0.8.2 Squara Wave Parameters	80
		0.8.3 Chirp Wave Parameters	83
		0.8.4 Photometry Wave Parameters	85
		0.8.5 Imaging Wave Parameters	86
	90	Channel Settings for DA Output and AD Input	87
	0.0	Chamics Seconds for Dir Carpar and the input	01
		9.9.1 DA output channel settings	87
		9.9.1 DA output channel settings 9.9.2 AD input channel settings	87 89

9.10	Segments	2
	9.10.1 Segment Classes	2
	9.10.2 Scan Rates	4
	9.10.3 Increment Modes	4
9.11	Miscellaneous	6
9.12	Stimulus Template Preview	7
9.13	V-membrane	7
9.14	PGF Parameters	8
9.15	Error Handling	8
10 Pro	ocol Editor Window	9
10 1 10	Protocol Handling	g
10.1	Event Handling	0
10.2	Becurring Functions	1
10.0	Events - Overview 10	1
10.4	10.4.1 Protocol Sequence	1 2
	10.4.2 Acquisition 10	2 7
	10.4.3 Hardware	8
	10.4.4 Data/Display	0
	10.4.5 Value/Parameter	5
	10.4.6 Mossage 11	7
	10.4.0 Messages	1
	10.4.7 Extensions 11	8
	10.4.7 Extensions	8
11 Ana	10.4.7 Extensions	8 9
11 Ana 11.1	10.4.7 Extensions	8 9 0
11 Ana 11.1 11.2	10.4.7 Extensions	8 9 0 0
11 Ana 11.1 11.2 11.3	10.4.7 Extensions 11 lysis Window 11 Stimulus Control 12 Analysis Methods 12 Analysis Functions 12	8 9 0 1
11 Ana 11.1 11.2 11.3	10.4.7 Extensions 11 lysis Window 11 Stimulus Control 12 Analysis Methods 12 Analysis Functions 12 11.3.1 Analysis Function Settings 12	8 9 0 1 1
11 Ana 11.1 11.2 11.3	10.4.7 Extensions 11 lysis Window 11 Stimulus Control 12 Analysis Methods 12 Analysis Functions 12 11.3.1 Analysis Function Settings 12 11.3.2 Analysis Function Types 12	8 9 0 1 1 4
11 Ana 11.1 11.2 11.3 11.4	10.4.7 Extensions 11 lysis Window 11 Stimulus Control 12 Analysis Methods 12 Analysis Functions 12 11.3.1 Analysis Function Settings 12 11.3.2 Analysis Function Types 12 Analysis Graphs 12	8 9 0 1 1 4 8
11 Ana 11.1 11.2 11.3 11.4	10.4.7 Extensions 11 lysis Window 11 Stimulus Control 12 Analysis Methods 12 Analysis Functions 12 11.3.1 Analysis Function Settings 12 11.3.2 Analysis Function Types 12 11.3.1 Scale Axis 12	8 9 0 1 1 4 8 9
11 Ana 11.1 11.2 11.3	10.4.7 Extensions 11 lysis Window 11 Stimulus Control 12 Analysis Methods 12 Analysis Functions 12 11.3.1 Analysis Function Settings 12 11.3.2 Analysis Function Types 12 11.4.1 Scale Axis 12 11.4.2 Graph Entries 13	8 9 0 1 1 4 8 9 1
11 Ana 11.1 11.2 11.3 11.4	10.4.7 Extensions 11 lysis Window 11 Stimulus Control 12 Analysis Methods 12 Analysis Functions 12 11.3.1 Analysis Function Settings 12 11.3.2 Analysis Function Types 12 11.4.1 Scale Axis 12 11.4.2 Graph Entries 13 Graph Positions 13	8 9 0 1 1 4 8 9 1 2
 11 Ana 11.1 11.2 11.3 11.4 11.5 11.6 	10.4.7 Extensions11lysis Window11Stimulus Control12Analysis Methods12Analysis Functions1211.3.1 Analysis Function Settings1211.3.2 Analysis Function Types12Analysis Graphs1211.4.1 Scale Axis1211.4.2 Graph Entries13Graph Positions13Multi-Channels Analysis13	8 9 0 0 1 1 4 8 9 1 2 3
 11 Ana 11.1 11.2 11.3 11.4 11.5 11.6 12 Par 	10.4.7 Extensions 11 lysis Window 11 Stimulus Control 12 Analysis Methods 12 Analysis Functions 12 11.3.1 Analysis Function Settings 12 11.3.2 Analysis Function Types 12 11.3.2 Analysis Function Types 12 11.4.1 Scale Axis 12 11.4.2 Graph Entries 13 Graph Positions 13 Multi-Channels Analysis 13 ameters Window 13	8 9 0 1 1 4 8 9 1 2 3 5
 11 Ana 11.1 11.2 11.3 11.4 11.5 11.6 12 Par 12.1 	10.4.7 Extensions 11 lysis Window 11 Stimulus Control 12 Analysis Methods 12 Analysis Methods 12 Analysis Functions 12 11.3.1 Analysis Function Settings 12 11.3.2 Analysis Function Types 12 11.3.2 Analysis Function Types 12 11.4.1 Scale Axis 12 11.4.2 Graph Entries 13 Graph Positions 13 Multi-Channels Analysis 13 Parameter Tabs 13	8 9 0 0 1 1 4 8 9 1 2 3 5 6
 11 Ana 11.1 11.2 11.3 11.4 11.5 11.6 12 Par 12.1 	10.4.7 Extensions 11 lysis Window 11 Stimulus Control 12 Analysis Methods 12 Analysis Methods 12 Analysis Functions 12 11.3.1 Analysis Function Settings 12 11.3.2 Analysis Function Settings 12 11.3.2 Analysis Function Types 12 11.4.1 Scale Axis 12 11.4.2 Graph Entries 13 Graph Positions 13 Multi-Channels Analysis 13 Parameter Tabs 13 12.1.1 Root 13	8 9 0 0 1 1 4 8 9 1 2 3 5 6 6 6
 11 Ana 11.1 11.2 11.3 11.4 11.5 11.6 12 Par 12.1 	10.4.7 Extensions 11 lysis Window 11 Stimulus Control 12 Analysis Methods 12 Analysis Functions 12 11.3.1 Analysis Function Settings 12 11.3.2 Analysis Function Settings 12 11.3.1 Analysis Function Types 12 11.3.2 Analysis Function Types 12 11.4.1 Scale Axis 12 11.4.2 Graph Entries 13 Graph Positions 13 Multi-Channels Analysis 13 Parameter Tabs 13 12.1.1 Root 13 12.1.2 Group 13	8 9 0 0 1 1 1 4 8 9 1 2 3 5 6 6 6 7
 11 Ana 11.1 11.2 11.3 11.4 11.5 11.6 12 Par 12.1 	10.4.7 Extensions 11 lysis Window 11 Stimulus Control 12 Analysis Methods 12 Analysis Functions 12 11.3.1 Analysis Function Settings 12 11.3.2 Analysis Function Types 12 11.3.2 Analysis Function Types 12 11.3.4 Analysis Function Types 12 11.3.1 Canalysis Function Types 12 11.3.2 Analysis Function Types 12 11.4.1 Scale Axis 12 11.4.2 Graph Entries 13 Graph Positions 13 Multi-Channels Analysis 13 Parameter Tabs 13 12.1.1 Root 13 12.1.2 Group 13 12.1.3 Series 13	8 9 0 0 1 1 4 8 9 1 2 3 5 6 6 6 7 8
 11 Ana 11.1 11.2 11.3 11.4 11.5 11.6 12 Par 12.1 	10.4.7 Extensions 11 lysis Window 11 Stimulus Control 12 Analysis Methods 12 Analysis Functions 12 11.3.1 Analysis Function Settings 12 11.3.2 Analysis Function Settings 12 11.3.2 Analysis Function Types 12 11.3.1 Analysis Function Types 12 11.3.2 Analysis Function Types 12 11.4.3 Craphs 12 11.4.1 Scale Axis 12 11.4.2 Graph Entries 13 Graph Positions 13 Multi-Channels Analysis 13 Parameter Tabs 13 12.1.1 Root 13 12.1.2 Group 13 12.1.3 Series 13 12.1.4 Sweep 13	8 9 0 0 1 1 1 4 8 9 1 1 2 3 5 6 6 6 7 8 9

12.1.5 Trace $\dots \dots \dots$	10
12.1.6 Marked Items	11
12.2 Flagging	12
3 Notebook Window 14	3
4 I/O Control Window 14	15
14.1 Dig-In / Out	15
14.2 DA / AD-Channels	15
14.3 Values	16
14.4 Serial Output	16
14.5 Solutions $\ldots \ldots \ldots$	16
14.6 Parameters	46
5 Solution Base 14	17
15.1 Activating the Solution Base	17
15.2 Using Solution Indices	18
15.3 Solution Data Base	18
6 Solution Changer 15	51
7 Markers 15	63
17.1 New Marker	53
17.1 New Marker 15 17.2 Edit Marker 15	53 55
17.1 New Marker 15 17.2 Edit Marker 15 8 Calculator Window and Equations 15	53 55 5 7
17.1 New Marker 15 17.2 Edit Marker 15 8 Calculator Window and Equations 15 18.1 Equation Syntax 15	53 55 5 7 57
17.1 New Marker 15 17.2 Edit Marker 15 8 Calculator Window and Equations 15 18.1 Equation Syntax 15 18.2 Equations - Where to Use 15	53 55 57 57
17.1 New Marker 15 17.2 Edit Marker 15 8 Calculator Window and Equations 15 18.1 Equation Syntax 15 18.2 Equations - Where to Use 15 18.2.1 Analysis 15	53 55 57 57 58 58
17.1 New Marker 15 17.2 Edit Marker 15 17.2 Edit Marker 15 8 Calculator Window and Equations 15 18.1 Equation Syntax 15 18.2 Equations - Where to Use 15 18.2.1 Analysis 15 18.2.2 Trace Buffer 16	 53 55 57 57 58 58 58 58 50
17.1 New Marker 15 17.2 Edit Marker 15 8 Calculator Window and Equations 15 18.1 Equation Syntax 15 18.2 Equations - Where to Use 15 18.2.1 Analysis 15 18.2.2 Trace Buffer 16 18.2.3 DA Scaling 16	 53 55 57 57 58 58 58 50 50
17.1 New Marker 15 17.2 Edit Marker 15 8 Calculator Window and Equations 15 18.1 Equation Syntax 15 18.2 Equations - Where to Use 15 18.2.1 Analysis 15 18.2.2 Trace Buffer 16 18.2.3 DA Scaling 16 18.2.4 Values 16	53 55 57 57 58 58 58 58 50 50 50 51
17.1 New Marker 15 17.2 Edit Marker 15 17.2 Edit Marker 15 18 Calculator Window and Equations 15 18.1 Equation Syntax 15 18.2 Equations - Where to Use 15 18.2.1 Analysis 15 18.2.2 Trace Buffer 16 18.2.3 DA Scaling 16 18.2.4 Values 16 19 Photometry Extension 16	53 55 57 57 57 58 58 58 50 50 50 51 51 53
17.1 New Marker 15 17.2 Edit Marker 15 17.2 Edit Marker 15 8 Calculator Window and Equations 15 18.1 Equation Syntax 15 18.2 Equations - Where to Use 15 18.2.1 Analysis 15 18.2.2 Trace Buffer 16 18.2.3 DA Scaling 16 18.2.4 Values 16 19.1 Photometry Configuration T.I.L.L. 16	53 55 57 57 58 58 58 58 50 50 51 51 51 51 53 54
17.1 New Marker 18 17.2 Edit Marker 15 17.2 Edit Marker 15 17.2 Edit Marker 15 18.1 Equation Window and Equations 15 18.1 Equation Syntax 15 18.2 Equations - Where to Use 15 18.2.1 Analysis 15 18.2.2 Trace Buffer 16 18.2.3 DA Scaling 16 18.2.4 Values 16 19.1 Photometry Extension 16 19.1.1 Wavelength Calibration 16	53 55 57 57 58 58 58 50 50 50 51 51 53 54 54
17.1 New Marker 15 17.2 Edit Marker 15 17.2 Edit Marker 15 18 Calculator Window and Equations 15 18.1 Equation Syntax 15 18.2 Equations - Where to Use 15 18.2 Equations - Where to Use 15 18.2.1 Analysis 15 18.2.2 Trace Buffer 16 18.2.3 DA Scaling 16 18.2.4 Values 16 19.1 Photometry Extension 16 19.1 Photometry Configuration T.I.L.L. 16 19.2 Photometry Configuration DG4/DG5 and Lambda-10 16	 53 55 57 57 58 58 50 50 50 51 53 54 54 56
17.1 New Marker 15 17.2 Edit Marker 15 17.2 Edit Marker 15 18 Calculator Window and Equations 15 18.1 Equation Syntax 15 18.1 Equation Syntax 15 18.2 Equations - Where to Use 15 18.2.1 Analysis 15 18.2.2 Trace Buffer 16 18.2.3 DA Scaling 16 18.2.4 Values 16 19.1 Photometry Extension 16 19.1.1 Wavelength Calibration 16 19.2 Photometry Configuration DG4/DG5 and Lambda-10 16 19.2.1 PGF Sequence 16	53 55 57 57 57 58 58 50 50 50 50 50 50 50 50 51 54 54 54 54 57 57 57 57 57 57 57 57 57 57 57 57 57
17.1 New Marker 15 17.2 Edit Marker 15 17.2 Edit Marker 15 18.1 Equation Syntax 15 18.1 Equation Syntax 15 18.2 Equations - Where to Use 15 18.2.1 Analysis 15 18.2.2 Trace Buffer 16 18.2.3 DA Scaling 16 18.2.4 Values 16 19.1 Photometry Extension 16 19.2 Photometry Configuration T.I.L.L. 16 19.2 Photometry Configuration DG4/DG5 and Lambda-10 16 19.3 Photometry Configuration PTI DeltaRAM 16	53 55 57 57 58 58 58 58 58 50 50 50 50 51 53 54 54 54 56 57 58 50 50 50 50 50 50 50 50 50 50 50 50 50
17.1 New Marker 15 17.2 Edit Marker 15 17.2 Edit Marker 15 18.1 Equation Syntax 15 18.1 Equation Syntax 15 18.2 Equations - Where to Use 15 18.2 Equations - Where to Use 15 18.2.1 Analysis 15 18.2.2 Trace Buffer 16 18.2.3 DA Scaling 16 18.2.4 Values 16 19.1 Photometry Extension 16 19.1 Photometry Configuration T.I.L.L 16 19.2 Photometry Configuration DG4/DG5 and Lambda-10 16 19.2.1 PGF Sequence 16 19.3 Photometry Configuration PTI DeltaRAM 16 0 Imaging Extension 16	53 55 57 57 58 58 50 50 50 50 50 50 50 50 50 50 50 50 50
17.1 New Marker 15 17.2 Edit Marker 15 17.2 Edit Marker 15 18.1 Equation Syntax 15 18.1 Equation Syntax 15 18.2 Equations - Where to Use 15 18.2 Equations - Where to Use 15 18.2.1 Analysis 15 18.2.2 Trace Buffer 16 18.2.3 DA Scaling 16 18.2.4 Values 16 19.1 Photometry Extension 16 19.1 Photometry Configuration T.I.L.L 16 19.2 Photometry Configuration DG4/DG5 and Lambda-10 16 19.3 Photometry Configuration PTI DeltaRAM 16 19.3 Starting the Imaging Extension 16 20.1 Starting the Imaging Extension 17	53 55 57 57 57 58 58 58 50 50 50 50 51 53 54 54 54 54 54 57 57 57 57 57 57 57 57 57 57 57 57 57

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	2	.2.1 Imaging Configuration T.I.L.L	71
	2	.2.2 Imaging Configuration DG4/DG5 and Lambda-10 $\ldots \ldots $	72
	2	.2.3 Imaging Configuration PTI DeltaRAM	73
	20.3 II	nage Window	74
	20.4 II	nage Control Window	74
	2	.4.1 Camera Setup	74
	2	.4.2 PGF Primer	75
	2	.4.3 ROI Manager	77
	2	.4.4 Image File Selector	78
	2	.4.5 Imaging Configuration	79
	2	.4.6 Imaging Log	80
21	Softw	re LockIn Extension 1	81
	21.1 L	ockIn Configuration Window	81
	21.2 T	ne LockIn Traces	82
:	21.3 C	apacitance Measurements - Step by Step	83
	2	.3.1 Activating the LockIn Extension	84
	2	.3.2 LockIn Configuration	84
	2	.3.3 Creating a PGF sequence	85
	2	.3.4 Amplifier Settings	87
	2	.3.5 Running the PGF sequence	.87
	2 2	.3.5 Running the PGF sequence .1 .3.6 Analysis .1	.87 .88
22	2 2 Spect	.3.5 Running the PGF sequence .1 .3.6 Analysis .1 oscopy Extension 1	.87 .88 91
22	2 2 Spect 22.1 In	.3.5 Running the PGF sequence .1 .3.6 Analysis .1 oscopy Extension 1 troduction .1	.87 .88 91 .91
22	2 2 Spect 22.1 In 2	.3.5 Running the PGF sequence .1 .3.6 Analysis .1 oscopy Extension 1 troduction .1 .1.1 Chirp Wave Forms .1	.87 .88 91 .91
22	2 2 Spect 22.1 In 2 2	.3.5 Running the PGF sequence .1 .3.6 Analysis .1 oscopy Extension 1 troduction .1 1.1 Chirp Wave Forms .1 1.2 Transfer Function Ratio .1	87 88 91 91 91 91
22	2 2 Spect 22.1 In 2 2 22.2 A	.3.5 Running the PGF sequence .1 .3.6 Analysis .1 oscopy Extension 1 troduction .1 1.1 Chirp Wave Forms .1 1.2 Transfer Function Ratio .1 ctivating the Spectroscopy Extension .1	87 88 91 91 91 91 91 92
22	2 2 Spect 22.1 In 2 2 22.2 A 2	.3.5 Running the PGF sequence .1 .3.6 Analysis .1 oscopy Extension 1 troduction .1 1.1 Chirp Wave Forms .1 1.2 Transfer Function Ratio .1 ctivating the Spectroscopy Extension .1 2.1 The Spectroscopy Traces .1	 87 88 91 91 91 91 92 94
22	2 2 Spect 22.1 In 2 22.2 A 2 22.3 S	.3.5 Running the PGF sequence .1 .3.6 Analysis .1 oscopy Extension 1 troduction .1 1.1 Chirp Wave Forms .1 1.2 Transfer Function Ratio .1 ctivating the Spectroscopy Extension .1 2.1 The Spectroscopy Traces .1 tting up a Spectroscopy Acquisition .1	 87 88 91 91 91 91 92 94 95
22	2 2 Spect 22.1 h 2 22.2 A 2 22.3 S 2 2	.3.5 Running the PGF sequence 1 .3.6 Analysis 1 oscopy Extension 1 troduction 1 1.1 Chirp Wave Forms 1 1.2 Transfer Function Ratio 1 ctivating the Spectroscopy Extension 1 2.1 The Spectroscopy Traces 1 1.2.1 The Spectroscopy Acquisition 1 1.3.1 The Chirp Wave Dialog 1	 87 88 91 91 91 92 94 95 95
22	2 2 Spect 22.1 In 2 22.2 A 2 22.3 S 2 2 2 2 2 2 2	.3.5 Running the PGF sequence 1 .3.6 Analysis 1 oscopy Extension 1 troduction 1 1.1 Chirp Wave Forms 1 1.2 Transfer Function Ratio 1 ctivating the Spectroscopy Extension 1 2.1 The Spectroscopy Traces 1 1.3.1 The Chirp Wave Dialog 1 3.2 Parametrization of the Chirp Stimuli 1	 87 88 91 91 91 92 94 95 95
22	2 2 Spect 22.1 In 2 22.2 A 2 22.3 S 2 2 2 2 2 2 2 2 2	.3.5 Running the PGF sequence 1 .3.6 Analysis 1 .3.6 Analysis 1 oscopy Extension 1 troduction 1 .1.1 Chirp Wave Forms 1 .1.2 Transfer Function Ratio 1 .1.3.1 The Spectroscopy Extension 1 .3.1 The Chirp Wave Dialog 1 .3.2 Parametrization of the Chirp Stimuli 1 .3.3 Adding Chirp Analysis Traces 1	 87 88 91 91 91 92 94 95 95 96
22	2 2 Spect 22.1 In 2 22.2 A 2 22.3 S 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	.3.5 Running the PGF sequence 1 .3.6 Analysis 1 oscopy Extension 1 troduction 1 .1.1 Chirp Wave Forms 1 .1.2 Transfer Function Ratio 1 .1.3 The Spectroscopy Extension 1 .3.1 The Chirp Wave Dialog 1 .3.2 Parametrization of the Chirp Stimuli 1 .3.3 Adding Chirp Analysis Traces 1 .3.4 Minimizing Swing-In Effects 1	 87 88 91 91 91 92 94 95 95 95 96 96
22	2 2 2 22.1 In 2 22.2 A 2 22.3 S 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	.3.5 Running the PGF sequence 1 .3.6 Analysis 1 .3.6 Analysis 1 oscopy Extension 1 troduction 1 .1.1 Chirp Wave Forms 1 .1.2 Transfer Function Ratio 1 .1.2 The Spectroscopy Extension 1 .2.1 The Spectroscopy Traces 1 .3.1 The Chirp Wave Dialog 1 .3.2 Parametrization of the Chirp Stimuli 1 .3.3 Adding Chirp Analysis Traces 1 .3.4 Minimizing Swing-In Effects 1 splay of Spectra in the Oscilloscope Window 1	 87 88 91 91 91 92 94 95 95 95 96 98
22 23	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	.3.5 Running the PGF sequence 1 .3.6 Analysis 1 .3.6 Analysis 1 oscopy Extension 1 troduction 1 .1.1 Chirp Wave Forms 1 .1.2 Transfer Function Ratio 1 .1.2 Transfer Function Ratio 1 .1.2 Transfer Function Ratio 1 .2.1 The Spectroscopy Extension 1 .2.1 The Spectroscopy Traces 1 .3.2 Parametrization of the Chirp Stimuli 1 .3.3 Adding Chirp Analysis Traces 1 .3.4 Minimizing Swing-In Effects 1 .3.5 Adding I Spectra in the Oscilloscope Window 1 .3.6 Adir I: File Overview 2	87 88 91 91 91 92 94 95 95 95 95 96 96 98 01
22	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	.3.5 Running the PGF sequence 1 .3.6 Analysis 1 .3.6 Analysis 1 oscopy Extension 1 troduction 1 .1.1 Chirp Wave Forms 1 .1.2 Transfer Function Ratio 1 .1.2 Transfer Function Ratio 1 .1.2 Transfer Function Ratio 1 .1.2 Transfer Spectroscopy Extension 1 .2.1 The Spectroscopy Traces 1 .2.1 The Spectroscopy Acquisition 1 .3.1 The Chirp Wave Dialog 1 .3.2 Parametrization of the Chirp Stimuli 1 .3.3 Adding Chirp Analysis Traces 1 .3.4 Minimizing Swing-In Effects 1 splay of Spectra in the Oscilloscope Window 1 dix I: File Overview 2 le Types 2	87 88 91 91 91 92 94 95 95 95 95 96 96 98 01
22 : 23 : 23 : 24 :	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	3.5 Running the PGF sequence 1 .3.6 Analysis 1 .3.6 Analysis 1 oscopy Extension 1 troduction 1 .1.1 Chirp Wave Forms 1 .1.2 Transfer Function Ratio 1 .1.3.1 The Spectroscopy Traces 1 .3.2 Parametrization of the Chirp Stimuli 1 .3.3 Adding Chirp Analysis Traces 1 .3.4 Minimizing Swing-In Effects 1 .3.5 play of Spectra in the Oscilloscope Window 1 dix I: File Overview 2 le Types 2 <t< td=""><td>87 88 91 91 91 92 94 95 95 95 95 95 96 96 98 01 03</td></t<>	87 88 91 91 91 92 94 95 95 95 95 95 96 96 98 01 03

	1.1.1 Raw Data File	13
	.1.2 Markers File	13
	.1.3 Protocol Methods File	13
	.1.4 Analysis Methods File	14
	1.5 Stimulation Template File	14
	.1.6 Acquistion Parameters File	14
	.1.7 Solutions File	14
	.1.8 Notebook File	15
24.2	he Tree Format	15
24.3	le Template	16
	.3.1 Filename	16
	A.3.2 Data Format of the File Template)7

1. Introduction

1.1 Disclaimer

This *Product* relies on the tools of Microsoft Windows (Windows Vista, Windows 7, Windows 8) or Mac 10.6 (or more recent). HEKA is not responsible for: i) the contents of these third party products, ii) any links contained in these third party products, iii) changes or upgrades to these third party products and iv) for any consequential damages resulting from the use of these products.

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In no event shall HEKA be responsible for any incidental, punitive, indirect, or consequential damages whatsoever, (including but not limited to loss of data, privacy of data or other pecuniary loss), arising from or relating to the use, or the inability to use, this *Product* or the provision, or lack of provision, of support services.

In all cases where HEKA is liable, the extend of HEKA's liability shall be limited to the actual cost of the *Product* or to the provision of a replacement version of the *Product*.

1.2 Scope of the Program

The CHARTMASTER program provides versatile tools for electrophysiological and electrochemical experiments. Pulse generation, data acquisition, storage, and analysis are among them.

Extended analysis functions for CHARTMASTER data are provided by the FITMASTER software. FITMASTER features analysis and fitting routines designed to fully support the extended CHARTMASTER data structure. Analysis can be performed on the levels of *Traces*, *Sweeps* and *Series*. There are standard fit functions such as "Polynomial, Exponential, Gaussian, and Boltzmann" tailored functions to fit e.g. whole-cell current traces according to the Hodgkin & Huxley gating formalism, current-voltage relationships and dose-response curves.

Users familiar with PULSE might first read the chapter **PATCHMASTER** for **PULSE** Users in the PATCHMASTER Tutorial and then go on with specific chapters of this manual.

1.3 The Chartmaster Concept

The following section presents a rather simplified view on the overall structure of the CHARTMASTER program. It should help to understand the general way how to operate the program and should give the reader an idea about the capabilities of CHARTMASTER.

The main objects of CHARTMASTER are:

- Protocol scripts
- Pulse Generator sequences (stimulation and data acquisition) and experiment specific Extensions, such as:
 - Software Lock-In
 - Photometry
 - Imaging
 - (Impedance/Admittance) Spectroscopy
- Oscilloscope (display)
- Data Tree (storage)

• Analysis methods (analysis)

The new concepts in comparison to the well known PULSE software are the *Protocols*, *Global Variables*, and a powerful *Analysis*.

CHARTMASTER – New Concepts Protocol Editor Global Variables Powerful Analysis

- **Protocols:** Are the top structuring layer of the program organizing the interplay between the different parts of the program. The protocol script is created and managed in the **Protocol Editor**.
- **Global Variables:** Are a kind of *glue* between the program parts (see chapter **Global Variables** in the CHART-MASTER Tutorial.)
- Analysis: For convenient representation of results during and after the experiment.

With these new concepts CHARTMASTER accomplishes two important points: **Standardization** and **Automation** of experiments.

CHARTMASTER accomplishing Standardization and Automation Reproducible Experimental Conditions Data collected at different setups by different personnel

The overall aim in good laboratory practices is to work under **Reproducible Experimental Conditions**. Data collected from different personnel and at different setups should have the same conditions that they can be used for **Data Pooling**. User errors should be prevented as much as possible.

In order to accomplish the above mentioned points you can create an experimental protocol script in the Protocol Editor. By the use of such a protocol script the experimental approach is very much standardized. The Protocol Editor gives you access to all parts of the CHARTMASTER program. You can get input from these parts, make decisions, and control other parts of the program.

Protocol Editor – Access Network



The Protocol Editor is the central part of CHARTMASTER, keeping the experimental cycle running. E.g. the Protocol Editor starts a data acquisition. Data are recorded, stored and analyzed by the Analysis. Results from the Analysis can be feed back into the Protocol Editor, e.g. making a decision in a conditional "If"-statement. Furthermore the Protocol Editor can react on input from external devices or the user. The following picture tries to visualize the work-flow of the program.



1.4 Supported Data Acquisition Interfaces

CHARTMASTER supports the following data acquisition interfaces:

- ITC-16
- ITC-18
- LIH 1600 / ITC-1600
- LIH 8+8

1.5 Supported System Software

CHARTMASTER is supported on all recent MS Windows Versions: Windows Vista, Windows 7, Windows 8.

CHARTMASTER is supported on MacOS X version 10.6 (or more recent). Older MacOS X versions and MacOS 9 are not supported.

1.6 Naming Conventions

1.6.1 Interfaces

Throughout this manual we will address all data acquisition interface types as LIH 8+8. We will explicitly mention the particular interface if required.

1.6.2 Windows Versions

Throughout this manual we will address all the supported Microsoft Windows versions as "MS Windows". We will explicitly mention the particular MS Windows versions if required.

1.6.3 Syntax

- CHARTMASTER: Small Capitals denote a HEKA program / hardware or keys on the keyboard.
- Replay: Typewriter font denotes menu entries and chapters or filenames
- Protocol Editor: Sans serif font denotes window names or dialogs.
- Sinewave: Roman font denotes options or buttons
- *Italic*: Denotes general emphasis.
- Bullet list: Lists points.
- Numbered list: Lists actions to be performed sequentially.

 \blacksquare & \blacksquare or (MS Windows) & (Macintosh) denotes system-specific keys and actions.

1.7 Windows and Mac Key Conventions

In CHARTMASTER, all key commands are saved in the file Chartmaster.key and will be read at the program start (see chapter 3 on page 17).

Note that all key commands specified in this manual refer to the default setting in Chartmaster.key. Since all commands can be customized by the user, the settings in your working version of CHARTMASTER might differ from these default settings.

Please also be aware that in case the file Chartmaster.key is not available at the program start, no key commands are available!

The basic conventions for the system keys on MS Windows and Mac are as follows:

- CTRL: Stands for the CONTROL key.
- ALT: Stands for the ALT key.

é

- CTRL: Stands for the CONTROL key.
- CMD: Stands for the COMMAND key (apple or cloverleaf symbol).
- ALT: Stands for the OPTION key.

1.8 Installing Chartmaster

Please install the hardware and the software according to the Installation Guide which is supplied with your order. It can also be obtained from the HEKA homepage.

1.9 Starting Chartmaster

Upon clicking on CHARTMASTER the software starts and various controls and windows become available:

- The drop-down menus File, Edit, Windows, Replay, Display, Buffer, Notebook, Protocols, EPC10, Help.
- Frequently used CHARTMASTER windows such as Amplifier, Control Window, Analysis Window 1, Oscilloscope, Replay.
- A scrolling text window called Notebook.

Most of these windows can be minimized; they can be re-opened by clicking on the window bar or by selecting them in the drop-down menu Windows.

1.10 Get Online Help on Keys

The option Show Keys in the drop-down menu Help (compare chapter 4.10 on page 39) displays the shortcut keys that are assigned to various controls of the active windows.

Oscilloscope	
	(Measure) (Scan) (Freeze) (backspace Repaint)
	Overl.Swp
	(Overl.Ser)
	[nu 5]nu 4]
	Dig. Filter
	Off
	Y-sca <u>nu</u> *
	sh-nu -
	Auto Swp
	(Auto Ser
	Start Time
	0.0 %
	100.0 % Page (P)

By selecting List Keys in the drop-down menu Help you can list all keys in the Notebook window. This is useful e.g., if you want to print the complete list.

For further information on the assigned keys, see chapter 3 on page 17.

1.11 Get Online Help Controls

The option Show Tooltips in the drop-down menu Help (chapter 4.10 on page 39) displays a short description of the control under the mouse tip.



1.12 Closing Chartmaster

To exit from CHARTMASTER, choose Quit from the drop-down menu File or



press CTRL + Q.



press CMD + Q.

You have three possibilities:

- Save + Exit: Saves configuration and data files and quits the program.
- Exit: Saves data files and quits the program.
- Cancel: Exit process will be aborted, you return to the program.

Note: If you changed the protocols, the Pulse Generator pool, or the Analysis, then you will be asked independently if you want to save your changes.

Note: Data files are always saved.

1.13 Support Hotline

If you have any questions, suggestions, or improvements, please contact HEKA's support team. The best way is to sent an e-mail to "support@heka.com" specifying as much information as possible:

- Your contact information
- The program name: e.g. PATCHMASTER, POTMASTER or CHARTMASTER software
- The program version number: e.g. v2.65, v2.73.1
- Your operating system and its version: e.g. Mac OS 10.8, Windows 7 64-bit

- Your type of computer: e.g. Intel Core i3 3.2 GHz with 2 GB RAM
- Your acquisition hardware, if applicable: e.g. EPC 10 USB, EPC 800 + LIH 8+8 or PG 340 USB
- The serial number and version of your amplifier, if applicable: e.g. EPC 9 single, version "920552 D"
- The questions, problems, or suggestions you have
- Under which conditions and how often the problem occurs

We will address the problem as soon as possible.

Contact Address: HEKA Elektronik Dr. Schulze GmbH A Division of Harvard Bioscience Wiesenstrasse 71 D-67466 Lambrecht/Pfalz Germany phone: +49 (0) 6325 9553 0 fax: +49 (0) 6325 9553 50 e-mail: support@heka.com web: http://www.heka.com

2. User Interface

The following chapter describes the user interface of CHARTMASTER. It consists of four sections:

Dialog controls explains all types of control controls available in the CHARTMASTER user interface.

Modifying the Dialogs and Controls describes how size, color, and arrangement can be changed.

Saving modified Dialogs and Controls describes how to save the dialog settings.

Toggling between Windows describes how to switch very fast in between different CHARTMASTER dialog windows.

2.1 Dialog Controls

Controls are defined as parts of the dialogs that allow user input (data, options or execution of actions).

The general rules for dialog controls in this program are:

- Whole Cell : Box items with a drop shadow enclose changeable values, either as
 - list item or
 - pop-up menu list or
 - $-\,$ drag item.
- **SETUP** : Rounded rectangles are items that perform some action.
- **496. MO** : Simple rectangles (without drop shadow) display a measured value.
- Recording Mode : Plain text is for titles only.

2.9 kHz Drag: A number in a box with a drop shadow. The parameter value in a drag item can be changed by clicking on it and dragging the mouse up and down. Alternatively, you can double-click on it, or SHIFT-click, or right-click (MS Windows), and then type in a new value. Terminate input with RETURN or ENTER. Using TAB will cycle through all *Drag* items of the active dialog window.

Whole Cell List: Similar in appearance to a *Drag* item. Clicking on it will pop up a menu list from which one can choose a setting.

Success Edit Text: A text string in a box with a drop shadow. Clicking on it will allow editing the displayed string.

SETUP Button: Rounded corner rectangle. Clicking on it will cause the respective action to occur.

Store Switch: Rounded corner rectangle. Clicking on it toggles the parameter value. The switch is "On" or activated if the item is highlighted. A switch can optionally also execute some action.

Prediction Radio Button / Checkbox: Identical to the standard dialog items. Clicking on them will toggle the respective parameters.

-70 mV Framed Text / Number / Boolean: Simple box with optionally some text. The Boolean value is indicated by its color, inactive controls are gray **100 0.00**.

Enter: Pressing ENTER on the extended keyboard always brings you back to edit the control that was edited last. The feature is very useful when one often edits the same control (e.g., a duration of a specific segment in the Pulse Generator or the *Display Gain* in the Oscilloscope window).

Background Color: The color that appears while the user is dragging or entering a value is set by the *Highlight Color* in the Mac OS control panel.

Important note: Be careful if changing – the user will not be able to read the edited number if the highlight color is set to a very dark color. The MS Windows version displays highlighted controls with white text on a black background.

2.1.1 Numerical Input

The numerical values can be entered in scientific notation (e.g., "2.3e-3", "2.3E-3") or in engineering format (e.g., "2.3m"). Numbers outside the range for engineering numbers (see table) are always displayed in scientific notation. The old value is erased as soon as the user starts to type. To preserve the old string, move the LEFT or RIGHT cursor first. To leave the previous value unchanged although a new one has been entered already, just clear the input by pressing ESC, then RETURN or ENTER.

Name	eng.	sci.
Tera	Т	E12
Giga	G	E9
Mega	М	E6
kilo	k	E3
milli	m	E3
micro	μ / u	E-6
nano	n	E-9
pico	р	E-12
femto	f	E-15

SI Units: CHARTMASTER expects most units to be SI units, i.e., meters, seconds, amperes, or derived units like Hertz, etc. However, for convenience there are exceptions to that rule. In such cases the item title contains an identifier for what unit is to be used, e.g., "mV" if a voltage is to be entered in millivolts rather than in volts.

String Buffer: Whenever an edit process is finished with RETURN the edited string is entered into a cyclic buffer of edit strings consisting of 10 entries. These strings can be accessed during editing using CURSOR UP and CURSOR DOWN. This feature is useful when identical or similar strings have to be typed into various string items.

2.2 Modifying Dialogs and Controls

Dialog items can be modified in many different ways, e.g. background or item color, text font, position of one item, position of all items in the window.

To modify dialogs and control items in the CHARTMASTER user interface, you have to select Enable Icon Configuration from the Windows menu.

Select Enable Icon Configuration. Now you can customize the windows:

- To drag and resize an item, right-click on the item and drag.
- To drag a group of items, press CTRL and right-click on the group and drag. A group of items is indicated by a grey background field.
- To bring up the Dialog Control window, press CTRL and left-click on the item. Here you can modify the item settings e.g. such as color, text font, dragging speed (see below).

Important note: Be careful when e.g. changing positions, sizes or colors of buttons. If you deselect the option visible in the Dialog Control window the button will disappear. Be reminded that it is not possible to get the button back after that action.

Select Enable Icon Configuration. Now you can customize the windows:

- To drag and resize an item, press ALT and left-click on the item and drag. The new item position will be ignored if ALT is up when the mouse button is released.
- To drag a group of items, press CMD and ALT and left-click on the group and drag.
- To bring up the Dialog Control window, press CMD and click on the item. Here you can modify the item settings e.g. such as color, text font, dragging speed (see below).

Important note: Be careful when e.g. changing positions, sizes or colors of buttons. If you deselect the option visible in the Dialog Control window the button will disappear. Be reminded that it is not possible to get the button back after that action.

The following table summarizes all actions (Enable Icon Configuration has to be selected!):

Action	Mac OS	MS Windows
Open a configuration	CMD + click	CTRL + left-click
dialog		
Move one item	ALT + left-click +	ALT + right-click +
	drag	drag
Move group of items	CMD + ALT + left-	CTRL + right-click +
	click+drag	drag

All windows except the **Configuration** window can be iconized i.e. reduced to a minimal size window. Such a window can easily be expanded to the original size (and shrunk again) by clicking in its zoom box.

2.2.1 Dialog Control Window

In the Dialog Control window, you can control the properties of an item.

🔜 Configure: "E RSeries" (drag,macro)	— ×-
Rect I: 70 b: 435 w: 84 h: 17 Position I: 1 b: 13 center Text MOhm inverted Length 11 Digits 1 any modifier Font: Arial Size 12 Back Col normal Key	 ☑ executable ☑ visible ☑ enabled ○ integer ④ fixed ○ scientific ○ engineering
WMF Goto Item 189 Update Canc	el Done

Here you can see the Dialog Control window for a *Drag* button, in this case the amplifier *R*-series button. For other buttons, e.g., with numbers or execution commands, there may be more or less controls available.

Here is the complete list of the controls:

- **Rectangle:** Gives the position of the rectangle in the dialog window (left **l**, bottom **b**) and the size of the item (width, height) in pixel.
- **Position:** Gives the position of the text in the item (left **l**, bottom **b**) in pixel. You can select a position from the list: left/center/right/last. "Last" is useful if you have text that may be longer than the specified button and where the end of the text is more important than the beginning, e.g. a complete file path and name. In this case, only the end of the text will be displayed, cutting off the beginning.

Text: Text label of the item.

Note: The label of a button can only be changed when there is already a name in the text field inserted. Buttons with the default entry "——" will not be effected by a change of the label.

- Unit: Unit of numeric values.
- inverted: Changes the text color from black to white or vice versa.
- Length: Maximum number of characters for this field. If the number is too low no number/value is displayed.
- **Digits:** Number of decimal places.
- Font: Select a font type from the list of available fonts on the system.
- Size: Change the font size of the text.
- Style: Define the style of the text: normal, bold, italic, underline, outline, shadow, condense, extend.
- **Key:** Define the character which is assigned to that item. This enables the item to be executed from the keyboard. When a key is applied to a **list** item, there are two keys assignments. E.g. one for increment and one for decrement.
- front only: If marked, the key will only work if this window is in front, i.e. the active one. This prevents key command collision in case you want to use the same key settings in several windows.
- any modifier: If marked, any of the modifier keys can be used.

Note: In newer MS Windows versions this function is limited.

- **executable:** The button starts action by clicking. If not executable, the button will stay in its original color and is inactive.
- **visible:** The button is visible.

Note: If not activated, the button will be invisible and cannot be edited anymore! To restore the original setting you have to delete the file Chartmaster.set.

enabled: The button can be activated or edited. If not enabled, the button will be gray and inactive.

Back Col: Color for a switch button that is not active (default: pink) or for any other button.

High Col: Color for a switch button that is active (default: red). For other buttons this feature is disabled.

Format of values:

- integer: Value has to be entered as integer, e.g., "5".
- fixed: Value has to be entered as floating point with maximum as many decimal places as given in **Digits**, e.g., "0.001".
- scientific: Value has to be entered in scientific format, e.g., "e-9" for "nano".
- engineering: Value has to be entered in engineering format, e.g., "n" for "nano".

WMF: Saves the window as Windows Meta File (WMF, MS Windows) or PICT (Mac).

Goto Item: Index number of the button. Please do not change this number, it is for internal handling only.

E Configure: "E Mode" (list,macro) In the caption of the dialog, you can see, if the item can be called from a *Macro Command* in a protocol ('macro').

To complete the input, you have the following possibilities:

- Click on Update to see the change in the item of the respective window.
- Click on Cancel to leave the Dialog Control window without changes.
- Click on Done to leave the Dialog Control window and save all changes.

2.2.2 Hiding Controls

If you want to hide controls that are usually not necessary for your tasks, the easiest way to hide them is to pull them to the side of the window.

Proceed as follows:

- 1. Select Enable Icon Configuration from the Windows menu.
- 2. Increase the size of a window. For that pull with the mouse on the lower end of the window.
- 3. Press CTRL and click with the right mouse button (MS Windows) or while holding the CMD key down click (Mac OS) on a control and drag it to the edge of the window. While moving, you will see a gray rectangle underneath it.
- 4. Re-size the window again to the original size. The controls are now hidden in the not visible area.
- 5. Now you can save it using the Save Front Dialog function in the Windows menu. The window setting will be saved as *.dia file.
- 6. To restore the old default settings, delete the ***.dia** files from the home path as set in the **Configuration** window.

2.2.3 Deleting Controls

If you want to customize CHARTMASTER for special purposes, e.g., for not allowing the user access to controls, the secure version is to delete the controls. For this, you have to edit the button properties in the Dialog Control window.

Proceed as follows:

- 1. Select Enable Icon Configuration from the Windows menu.
- 2. Press CTRL and click with the left mouse button (MS Windows) or while holding the CMD key down click (Mac OS) on a control. The Dialog Control window opens.
- 3. Deselect enable or executable to simply deactivate the control. It will still be visible then.
- 4. Alternatively deselect visible the control disappears completely.
- 5. Click on Update to see the effect, or on Done to save the control setting.
- 6. Now you can save it using the Save Front Dialog function in the Windows menu. The window setting will be saved as *.dia file.

To prevent users from changing your settings, place the .dia files into access restricted folders and change the home path in the Configuration window accordingly.

To restore the old default settings, delete the *.dia files from the home path as set in the Configuration window.

2.3 Saving Dialogs and Controls

Position and size of all CHARTMASTER windows are saved in the configuration file (e.g., Chartmaster.set).

However, position, size, and state (iconized/not iconized) of each window can also be stored using the menu option Save Front Dialog in the Windows menu (e.g., IO_Control.dia). If a dialog file for a window exists, then the settings from that dialog file overrule the settings from the configuration file.

Note: When installing a new version of CHARTMASTER these customized dialogs are likely to become incompatible, because additional items may have been introduced in the new version. It is, therefore, best to discard these custom dialogs when upgrading.

Note: When you have saved a wrongly configured window by chance, delete the corresponding file from the CHARTMASTER directory, the naming convention is [windowname].dia, e.g., Oscilloscope.dia.

2.4 Toggling between Windows

Hitting the ESC key will close whatever window is in front. Systematically pressing the ESC key will close all windows one at a time (except the Notebook window).



The SPACE key can be used to quickly toggle between windows. The sequence of switching can be set in the *Switch to* control. This control is extremely useful in several respects, firstly, it enables you to switch from one window to another, depending upon what *Switch to* window option is selected.

Secondly, switching between windows can be performed very quickly and easily if you assign a keystroke to perform the function. Simply select the desired *Switch to* window option and then assign a key to this window in the previously described **Icon Configuration** window. To achieve this press the CTRL and select the *Switch to* button with your mouse. The SPACE key is the default key but this can be changed. Thirdly, having the *Switch to* control, enables window switching to be included within macro recordings in the **Protocol Editor**.

Note: Since this feature is not needed normally, it is available only if either Enable Icon Configuration is selected or you are in the Macro Recording mode. You can then find the control in the invisible window area.

Furthermore, this control is also available in the Protocol Editor, event *Switch Window* (see chapter 10.4.1.12 on page 106) where it allows the inclusion of window switching in a protocol sequence.

2.5 Copy and Paste Functions

In this section we give an overview of the Cut and Paste functions within the different CHARTMASTER windows and dialogs.

While editing an item text in a dialog the Cut and Paste functions will operate on the edited text of the active, highlighted item.

The menu function $\texttt{Edit} \rightarrow \texttt{Copy}$ is enabled for the following windows:

- Notebook Window: Standard copy functionality
- Equation Editor Dialog: Complete equation is copied.
- Calculator Window: Result of the equation if the SHIFT is not pressed and equation plus result if the SHIFT is pressed.
- Amplifier Window: Amplifier state as defined in the Parameter window (Amplifier tab).
- Analysis Window 1 & 2: Respective Analysis Graphs are copied
- Analysis Window: The function list of the active Analysis Method, equivalent to the List function.
- Oscilloscope Window: Oscilloscope graph is copied.
- PGF Editor Window: Text description of selected stimulus is copied. The stimulus cartoon is copied instead of the text listing, if the Cartoon View tab is active.
- Protocol Editor Window: Parameters as defined in the active tab are copied.
- Replay Window: Text listing of the data tree is copied.
- Protocol Methods: The protocol method is copied.
- Markers Window: Copies Series and Markers to the clipboard, equivalent to the function "Write to Notebook".
- In most other functions or when the OPTION is down during the Copy function in the above functions the window content is copied to the clipboard as PICT and WMF for MacOS and MS Windows, respectively. The graph is stored to disk, if the SHIFT is hold down while selecting the Copy function. Be aware that *Trace* representations will be a bit map (not vectors) when exported as part of the dialog window.

The menu function $\texttt{Edit} \rightarrow \texttt{Paste}$ is enabled for the following windows:

- Notebook Window: Standard paste functionality
- Equation Editor Dialog: Insert a complete equation
- Calculator Window: Insert an equation
- Protocol Editor Window: Insert a protocol. This includes a method copied in the Protocol Methods window. Events are inserted at the active position, if the pasted text contains events without a leading protocol definition. New protocols are appended to the present protocol pool.

Thus, Copy and Paste operations are target specific. E.g., pasting an equation while a sub-equation is being edited in FITMASTER, will paste the new text into the selected sub-equation string, while pasting when no item is selected, will replace the complete equation. 16

3. Keys

Controls within windows can be accessed from the keyboard. The key assignments are saved in the Chartmaster.key file and will be read upon starting the program. All commands can be customized by the user.

Please be aware that in case the Chartmaster.key file is not available when the program is started, no key commands are assigned!

The option Show Keys in the Help menu displays the key assignments in the various windows. For further information see chapter 1.10 on page 6.

To list the keys, choose List Keys in the Help menu. The complete key list is displayed in the Notebook window.

To save the keys, choose Save Keys in the Help menu. The keys are saved in the Chartmaster.key file. Old keyboard assignments will be automatically saved with an incrementing extension, e.g., *.k00, *.k01, *.k02....

You can freely customize the key commands by:

- changing and saving key assignments via the dialog control (chapter 2.2 on page 10) or by
- directly modifying the Chartmaster.key file, e.g., in a text editor.

3.1 The Key Listing

All keys can be listed in the Notebook by choosing List Keys in the Help menu. Alternatively open the Chartmaster.key file with a text editor.

In the following the notation of the key listing is described:

; denotes remark – will not be executed

@ denotes menu command.

B, **P**, **N**... denotes the window (B = Protocol Editor, P = Pulse Generator, N = Notebook...)

; B -> Protocol Editor B WindowSwitch Key SPACE any

char denotes a letter – simply press it.

R UnmarkIt Key Char U

any denotes that any modifier key may be pressed and the key will function.

R WindowSwitch Key SPACE any

back denotes that this key will also work when the window is in the background (reverse to the "front only" function in the Dialog Configuration window).

E HoldDec2 Key CursorLeft option back

shift, option, caps, command, control requires that the respective modifier key must be pressed.

HOME, END denotes the corresponding keys on your keyboard.

R ScrollHome Key HOME any

NUM denotes that you have to use the numeric keypad for these numbers.

E Macro3 Key Numeric3

3.2 The Chartmaster.key File

900

MACRO-FILE

;	Q	-> Menu Keys						
	0	File	"New"	Key	"N"	[Press	"Key"	+ CTRL]
	0	File	"Open Read Only"	Key	"0"	[Press	"Key"	+ CTRL]
	0	File	"Open Modify"	Key	"M"	[Press	"Key"	+ CTRL]
	0	File	"Update File"	Key	"U"	[Press	"Key"	+ CTRL]
	Q	File	- "File Status"	Kev	"I"	[Press	"Kev"	+ CTRL]
	0	File	"Quit"	Key	"Q"	[Press	"Key"	+ CTRL]
;	0	the standard ke	eys should not be changed "Undo"	l:	"7"	[Progg	"Kow"	
;	@	Edit	"Cu+ "	Kow	ے ۱۱۷۱	[Progg	"Kow"	
,	0			кеу	л "	[FIess	Key	
;	0 A		"Copy"	кеу		LPress	"key"	+ CIRL]
;	0 Q	Edit	"Paste"	кеу		LPress	"key"	+ CIRL]
	Q	Edit	"Select All"	Key	"A"	LPress	"Key"	+ CIRL]
	Q	Edit	"Find"	Key	"F"	[Press	"Key"	+ CTRL]
	Q	Edit	"Find Same"	Key	"G"	LPress	"Key"	+ CTRL]
	0	Edit	"Find Selection"	Key	"H"	[Press	"Key"	+ CTRL]
	0	Edit	"Replace"	Key	"R"	[Press	"Key"	+ CTRL]
	0	Edit	"Replace Same"	Кеу	"T"	[Press	"Key"	+ CTRL]
	6	114 - 4	"O	V	E40			
	Q	windows	"USCILIOSCOPE"	Кеу	F12			
	Q	Windows	"Replay"	Key	F10			
	Q	Windows	"Protocol Editor"	Key	F9			
	0	Windows	"Pulse Generator"	Key	F8			
	0	Windows	"Analysis"	Key	F7			
	0	Windows	"Notebook"	Key	F5			
	0	Windows	"Close Front Window"	Кеу	"W"	[Press	"Key"	+ CTRL]
			"	.,		5		
	Q	Notebook	"Save"	Key	"S"	[Press	"Key"	+ CIRLJ
	0	Notebook	"Print"	Key	"P"	[Press	"Key"	+ CTRL]
	Q	Notebook	"Clear"	Key	"B"	LPress	"Key"	+ CTRL]
	Q	Notebook	"Zoom In"	Key	"K"	LPress	"Key"	+ CTRL]
	Q	Notebook	"Zoom Out"	Кеу	"L"	[Press	"Key"	+ CTRL]
;	A	-> Analysis						
	A	WindowSwitch	Key SPACE any					
,	R	-> Protocol Edito	n r					
	B B	-> Protocol Edito	or Key SDACE any					
	B B P	-> Protocol Edito WindowSwitch	Key SPACE any					
	B B B B	-> Protocol Edito WindowSwitch End	Key SPACE any Key END any Kay HOME any					
	B B B B	-> Protocol Edito WindowSwitch End Home	or Key SPACE any Key END any Key HOME any					
	B B B B B	-> Protocol Edito WindowSwitch End Home DownPage	or Key SPACE any Key END any Key HOME any Key PageUp any Key PageUp any					
	B B B B B B B	-> Protocol Edito WindowSwitch End Home DownPage UpPage	or Key SPACE any Key END any Key HOME any Key PageUp any Key PageDown any					
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	B B B B B B B B	-> Protocol Edito WindowSwitch End Home DownPage UpPage Down Up	or Key SPACE any Key END any Key HOME any Key PageUp any Key PageDown any Key CursorUp any Key CursorDown any					
;	B B B B B B B C	-> Protocol Edito WindowSwitch End Home DownPage UpPage Down Up -> Solution Base	or Key SPACE any Key END any Key HOME any Key PageUp any Key PageDown any Key CursorUp any Key CursorDown any					
;	B B B B B B C C	-> Protocol Edito WindowSwitch End Home DownPage UpPage Down Up -> Solution Base Done	or Key SPACE any Key END any Key HOME any Key PageUp any Key PageDown any Key CursorUp any Key CursorDown any Key RETURN any					
;	B B B B B B B C C D	-> Protocol Edito WindowSwitch End Home DownPage UpPage Down Up -> Solution Base Done -> Parameters	or Key SPACE any Key END any Key HOME any Key PageUp any Key PageDown any Key CursorUp any Key CursorDown any Key RETURN any					
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• • • •	B B B B B B B B B B B C C D D H H I I	-> Protocol Edito WindowSwitch End Home DownPage UpPage Down Up -> Solution Base Done -> Parameters WindowSwitch -> Photometry WindowSwitch -> I/O Control WindowSwitch	or Key SPACE any Key END any Key HOME any Key PageUp any Key PageDown any Key CursorUp any Key CursorDown any Key RETURN any Key SPACE any Key SPACE any Key SPACE any					
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•••••••••••••••••	BBBBBBCC DD HH II JK	-> Protocol Edito WindowSwitch End Home DownPage UpPage Down Up -> Solution Base Done -> Parameters WindowSwitch -> Photometry WindowSwitch -> I/O Control WindowSwitch -> Calculator -> Markers	or Key SPACE any Key END any Key HOME any Key PageUp any Key PageDown any Key CursorUp any Key CursorDown any Key RETURN any Key SPACE any Key SPACE any Key SPACE any					
•••••••••••••••••••••••••••••••••••••••	BBBBBBBCCDDHHHIIJKK	-> Protocol Edito WindowSwitch End Home DownPage UpPage Down Up -> Solution Base Done -> Parameters WindowSwitch -> Photometry WindowSwitch -> I/O Control WindowSwitch -> Calculator -> Markers WindowSwitch	or Key SPACE any Key END any Key HOME any Key PageUp any Key PageDown any Key CursorUp any Key CursorDown any Key RETURN any Key SPACE any Key SPACE any Key SPACE any					
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• • • • • •	BBBBBB CC DD HH II J KK L	-> Protocol Edito WindowSwitch End Home DownPage UpPage Down Up -> Solution Base Done -> Parameters WindowSwitch -> Photometry WindowSwitch -> I/O Control WindowSwitch -> Calculator -> Markers WindowSwitch -> LockIn	<pre>br Key SPACE any Key END any Key HOME any Key PageUp any Key PageDown any Key CursorUp any Key CursorDown any Key RETURN any Key SPACE any Key SPACE any Key SPACE any Key SPACE any</pre>					

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L	LockInDone	Key RETURN any
. M	-> Control Window	
; N	-> Control Window	Kow SDACE one
N	TimerSet	Key Char T any back
N	Resume	Key Char B control back
N	Wait	Key Char W control back
N	Novt	Key Char N control back
N	Break	Key Char B control back
N	Stop	Key Char S control back
N	PC6	Key Char 6
N	PG5	Key Char 5
N	PG4	Key Char 4
N	PG3	Key Char 3
N	PG2	Kev Char 2
N	PG1	Key Char 1
; 0	-> Oscilloscope	
0	WindowSwitch	Key SPACE any
0	YOffsetDec	Key Numeric- shift back
0	YOffsetInc	Key Numeric+ shift back
0	YScaleDec	Key Numeric- back
0	YScaleInc	Key Numeric+ back
0	YCenter	Key Numeric. back
0	ResetY	Key Numeric* back
0	DispTrace	Key Numeric4 back
0	DispTrace	Key2 Numberic5 back
0	Wipe	Key DeleteLeft any back
0	MoveRRight	Key Char > any [for "Scan" function, jumps 10 points]
0	MoveRLeft	Key Char < any [for "Scan" function, jumps 10 points]
0	MoveLRight	Key Char . [for "Scan" function, point by point]
0	MoveLLeft	Key Char, [for "Scan" function, point by point]
; P	-> PGF-Editor	
Р	WindowSwitch	Key SPACE any
; R	-> Replay	
R	WindowSwitch	Key SPACE any
R	ScrollEnd	Key END any
R	ScrollPageDown	Key PageDown any
R	ScrollDown	Key CursorDown any
ĸ	ScrollUp	Key CursorUp any
ĸ	ScrollPageUp	Key PageUp any
ĸ	ScrollHome	Key HUME any
ĸ	Scrollkight	Key Cursorkight any
ĸ	ScrollLeft	Key CursorLeft any
ĸ	Unmarkit	Key Char U
ĸ	Markit	Key Char M
ĸ	Snowit	Key REIORN any
. c	-> Configuration	
, ວ ຕ	-> configuration	Kou SDACE one
5	WINDOWSWICCH	Key SPACE any
. т	-> Trace Properties	
, т	Done	Key RETURN any
T	P0116	INCY INDIVIENT CHIY
: U	-> Online 1	
, U	WindowSwitch	Key SPACE any
0		
; V	-> Online 2	
V	WindowSwitch	Key SPACE any
		· · ·

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4. Menus

The following section describes the various drop-down menus in CHARTMASTER.

4.1 File Menu

The File menu has all options to handle CHARTMASTER experiment files. A single CHARTMASTER "Experiment", that can hold a variable number of single electrophysiological

experiments, can consist of several types of files:

- The *.dat file contains only the actual raw data without any timing or scaling information (=<u>dat</u>a).
- The *.mac file contains macros (=<u>Mac</u>ro). [Disposed since version 2x52]
- The *.mrk file contains the used marker information (=<u>Mark</u>er).
- The *.onl file contains Analysis information (=<u>Onl</u>ine Analysis).
- The *.pgf file contains the used stimulus templates (= <u>Pulse Generator File</u>).
- The *.pro file contains Protocol Editor information (=<u>Pro</u>tocol Editor).
- The *.pul file contains the complete data tree (=<u>Pul</u>sed Tree).
- If the solution database is used, then a file with the extension *****.**sol** is stored with the data files (=<u>Sol</u>ution). It contains information on the solutions used in the stored experiments.
- In the *.txt file (=<u>Text</u>), the contents of the Notebook are stored (If Auto Store is activated in the Notebook menu).

If the option Make Bundle File is checked in the Configuration window (see chapter 5.5.2 on page 48), then the files *.pul, *.pgf, *.onl, *.mrk and *.dat are merged into one single *.dat file.

Raw data acquired by CHARTMASTER is only written to disk in the so called *Store* mode (i.e. if the *Store* button in the **Control** window is switched on).

The data are written to disk either:

- upon completion of a Sweep or Series or
- during the acquisition, in case of a continuous Sweep.

The structural information is kept in RAM; they are stored to disk only when:

- a new file (File \rightarrow New), a new group (File \rightarrow New Group) or a new experiment (File \rightarrow New Experiment) is created or
- the Update File function is executed (File \rightarrow Update File) or
- the program is terminated (File \rightarrow Quit)

New...: Creates a new, empty data file that is ready for data acquisition. The file has read and write permission.

Open Read Only...: Loads an existing file in *Read Only* mode. Modification of the file is not allowed. Use this option when you want to analyze data and to make sure not to change or delete anything.

Open Modify...: Loads an existing file with read and write permission. Modification of the file such as appending or deleting data is allowed.

Important note: Deleting entries in the data file is not reversible. Make sure to always have a backup of the original files when modifying an experiment. The exception is, of course, when you really want to delete a part of the stored data.

Merge...: Appends the data tree from an existing data file to the data tree of the actually opened file.

Update File: Updates the whole experiment to disk. This includes all files involved (see above). If you encounter computer crashes leading to data loss, use this option frequently or enable one of the settings to be found via File \rightarrow Write Tree Files.

Close: Closes the actual file.

File Status: Prints information about the status of the currently opened file such as the path, length, etc. to the **Notebook** window. A typical output could look as follows:

Storing to disk ENABLED, read-write file: "C:\HEKA\Data\Exp001.dat" length: 284 kb; free disk space: 44.33 GB.

New Group: Generates a new *Group* in the output data tree of the **Replay** window if the file is opened without write protection. After the addition of a new *Group* a file update is automatically performed.

New Experiment: Generates a new experiment and a new *Group* (see above) and increments the experiment number.

Write Tree File: Defines when the data are flushed to disk. Choose one of these options:

- After Update File: Files are updated after an *Update File* command, and after creating a new experiment or *Group*.
- After Series: Writes raw data to disk after each acquired Series.
- After Sweeps: Writes raw data to disk after each Sweep.

The second option will make sure that the system file cache gets written to disk (i.e., "flushed") after acquiring a *Sweep*, and the third option performs a cache flush after acquiring a complete *Series*. Deselecting both options will suppress file cache flushing. In that case, the operating system will flush the file cache when it overflows.

The flushing of the file cache may take some seconds, depending on its size. Thus, if one lets the system decide when to flush, it may occur at an inappropriate moment, such as in the middle of a series. On the other hand, if CHARTMASTER would always force file cache flushing (as it does when the option *Write After Sweep* is active), one could not take advantage of the fact that writing to the file cache in RAM is faster than physically writing to disk.

It is safest to select the Write After Sweep option. This ensures that the data are immediately written to disk. The timing between Sweeps is also not interrupted by the system when a possibly large file cache is written to disk. If one must get the fastest disk performance possible, one can deselect the options. In that case, data are written to RAM, not directly to disk. However, this can only work as long as fewer data are acquired than there is space in the file cache.

Note: CHARTMASTER writes only that part of the tree files which were added or moved, thereby drastically reducing the time required for updating the files to disk.

 $\mathbf{22}$

New	Ctrl+N
Open Read Only	Ctrl+O
Open Modify	Ctrl+M
Merge	
Update File	Ctrl+U
Close	
File Status	Ctrl+I
New Group	
New Experiment	
Write Tree Files	+
Convert To PPC Format	
Convert To Intel Format	
Page Setup	
Page Properties Notebook	
Page Properties Traces	
Quit	Ctrl+Q

Convert to PPC: Converts the raw data to PowerPC format ("big endian"). This is required if a third party program is used that can not distinguish between Intel ("little endian") and PPC data format but requires data to be read in PPC format.

Convert to Intel Format: Converts the raw data to Intel ("little endian") format. This is required if a third party program is used that can not distinguish between Intel and PPC data format but requires data to be read in Intel format.

Page Setup...: Calls the Printer/Page Setup dialog of the operating system.

Page Properties Notebook...: Calls a dialog to set the page margins (left, right, top and bottom) and the font for printing. These settings apply for printing the Notebook.

Page Properties Traces...: Calls a dialog to set the number of columns and rows, the page margins (left, right, top and bottom) and the font for printing. These settings apply for printing data graphs.

Quit: Exits CHARTMASTER.

The default quit options are:

- Save + Exit: Saves configuration and data files and then quits the program.
- Exit: Saves data files and quits program.
- Cancel: Exit process will be aborted, you return to the program.

These default behavior can be influenced by the options Save Settings and Verify Quit in the configuration dialog of CHARTMASTER.

Note: If you changed the Protocol Editor, the Pulse Generator pool or the Analysis you will be asked independently if you want to save your changes.

Important note: Data files are always saved.

Text Font	System				
Left Margin	0.125				
Right Margin	0.05				
Top Margin	0.05				
Bottom Margin	0.05				
🔽 Text Wrapp	ing				
ОК	Cancel				
Set Margins					
Number Of Ro	ws 3				
Number Of Col	umns 1				
Text Font	Arial				
Left Margin	0.1				
Right Margin	0.04				
Top Margin	0.02				
Bottom Margin	0.04				
🔽 Print Frame	ä				
Print Comm	ent				
ОК	Cancel				

Set Margins

4.2 Edit Menu

The Edit menu applies to text manipulation in the Notebook window. The *Copy* function can also be used to copy graphs from the Oscilloscope or from an Online window into the clipboard.

Please note that most entries of this menu are disabled unless the Notebook window is in front!

The menu entries conform to the typical functions of the actual operating system (MacOS or MS Windows).

 $\mathbf{Undo:}\ \mathbf{Cancels}\ \mathbf{the}\ \mathbf{last}\ \mathbf{action}\ \mathbf{performed}\ \mathbf{in}\ \mathbf{the}\ \mathbf{Notebook}\ \mathbf{window}.$

 $\mathbf{Cut:}$ Cuts the text selection from the Notebook window.

Copy: Copies a text selection from the Notebook. The *Copy* function can also be used to copy graphs from the Oscilloscope or from an Online Window into the clipboard. The active window (Notebook, Oscilloscope or Analysis) determines what is copied.

Paste: Pastes text from or to the clipboard.

Clear: Removes any content of the Notebook window.

Select All: Selects the whole Notebook window content.

Find...: Finds the entered search string.

Find Same: Finds the next appearance of the entered search string.

Find Selection...: Finds the search string that was marked (highlighted) in the Notebook window.

Replace...: Replaces the entered search string by some new string.

Replace Same: Finds and replaces the next appearance of the entered search string.

Undo	Ctrl+Z
Cut	Ctrl+X
Сору	Ctrl+C
Paste	Ctrl+V
Clear	
Select All	Ctrl+A
Find	Ctrl+F
Find Same	Ctrl+G
Find Selection	Ctrl+H
Replace	Ctrl+R
Replace Same	Ctrl+T

4.3 Windows Menu

F12

F10 F9

F8

F7

F5

Ctrl+W

Analysis

I/O Control

Spectroscopy

Solution Base

Solution Changer

Protocol Methods Calculator

Close Front Window

Reset Front Window Position

Enable Icon Configuration

Save Front Dialog

LockIn

Markers

Notebook

Analysis Window 1

Analysis Window 2 Parameters

Photometry/Imaging

The Windows menu applies to the windows in CHARTMASTER. Clicking	Control Window
on a menu entry either opens the respective window or brings the already	Configuration
open window to the front. For most windows there are key commands	Oscilloscope
assigned which will be displayed in the menu (see image beneath and also	Replay
chapter 3 on page 17).	Protocol Editor
Control Window: Opens the Control Window or brings it to the front (see chapter 7 on page 65).	Pulse Generator

Configuration: Opens the Configuration window or brings it to the front (see chapter 5 on page 41).

Oscilloscope: Opens the Oscilloscope window or brings it to the front (see chapter 6 on page 61).

Replay: Opens the Replay window or brings it to the front (see chapter 8) on page 69).

Protocol Editor: Opens the Protocol Editor window or brings it to the front (see chapter 10 on page 99).

Pulse Generator: Opens the **Pulse Generator** window or brings it to the front (see chapter 9 on page 73).

Analysis: Opens the Analysis window or brings it to the front (see chapter 11 on page 119).

Analysis Window 1, 2: Opens the specified Analysis Window window or brings it to the front (see chapter 11.4 on page 128).

Parameters: Opens the Parameters window or brings it to the front (see chapter 12 on page 135).

I/O Control: Opens the I/O Control window or brings it to the front (see chapter 14 on page 145).

Photometry/Imaging: Opens the Photometry Configuration or Imaging Configuration window or brings it to the front if the Photometry or Imaging Extension is activated (see chapter 5.4.5 on page 45 or chapter 5.4.4 on page 45).

LockIn: Opens the LockIn Control window or brings it to the front if the LockIn Extension is activated (see chapter 5.4.2 on page 44).

Spectroscopy: Opens the Spectroscopy Control window or brings it to the front if the Spectroscopy Extension is activated (see chapter 5.4.3 on page 45).

Solution Base: Opens the Solution Base window (see chapter 15 on page 147). If no solution base file is loaded you will be prompted to create a ***.sol** file.

Solution Changer: Opens the Solution Changer dialog (see chapter 16 on page 151).

Markers: The Markers window allows to set sweep markers and/or trace markers during a continuous recording (see chapter 17 on page 153).

Protocol Methods: Opens a dialog which provides information about the used *Protocol Method*.

Calculator: The Calculator window allows to perform numeric calculations in CHARTMASTER (see chapter 18 on page 157).

Notebook: Opens the Notebook window or brings it to the front (see chapter 13 on page 143).

Close Front Window: Closes the window that is in front display.

Save Front Dialog: Dialogs modified by the user can be saved via this menu entry (see chapter 2 on page 9).

Reset Front Window Position: The position of the active (front) window is reset to a predefined "home" position. This function is essential when accidentally the window position has a huge offset and cannot be brought back into the field of view.

Enable Icon Configuration: If selected, the dialogs and controls in the CHARTMASTER user interface can be modified (see chapter 2.2 on page 10).

4.4 Replay Menu

The Replay menu functions are active when the Replay window is selected.

Show: Displays the content of the selected target. *Traces* are displayed according to the settings specified in the Display menu and the Oscilloscope window. If the *Root* is selected for *Show*, all *Traces* are displayed one after another. This can be aborted by mouse click on the *Break* or *Stop* buttons in the Oscilloscope window.

Show PGF Template: This option opens the stimulation file (PGF-Template) of the corresponding selected *Series* in the Replay tree (or the first *Series*, if a *Group* is the target). Leave the window open and scroll through your acquired data in the Replay tree to get an overview of the stimulation settings of your recordings (read-only).

Copy PGF to PGF-Pool: Copies the stimulus protocol of a selected *Trace, Sweep* or *Series* into the current **Pulse Generator** file.

Show Method: Displays the name and the used settings of the *Protocol Method* of the selected target (read-only). There are two additional options:

- Copy To Analysis: Copies the used Analysis Method of the Protocol Method to the Analysis Methods pool of the Analysis.
- Copy To Protocol: Copies the protocol event lines of the used *Protocol Method* into the active protocol at the selected position of the **Protocol Editor**.

Note: By using the Copy function from the **Edit** menu one can copy the the method text to the clipboard. Then one can paste the method at any position in the **Protocol Editor** by the Paste option.

Reference Series: Selects a target as reference with the *Set* function. The *Reference Series* is subtracted from all replayed data, if *Subtract Ref. Series* in the **Display** menu is active. The subtraction of the *Reference Series* not only affects the display of the data in the **Oscilloscope** window, but also data analysis (see chapter 4.5.2 on page 33) and export. A previously selected *Reference Series* can be turned off with the *Off* function.

Delete: Deletes the selected target.

Print: Prints the selected target and the corresponding analysis results.

Export: Exports the selected target. Display gain, leak subtraction or zero line subtraction will be applied (special case for *Igor Binary* export see below).

Note: The Export option will try to keep a "what-you-see-is-what-you-get" behavior. This means that the display options define the export options; e.g., only the displayed data are exported; or when Show Leak Traces is on, the leak Traces are also exported. For full Sweep export please use Export Full Sweep or make sure that the display is set to full time range, i.e. Start Time = 0%, End Time = 100%.

Export Full Sweep: Exports the full *Sweep*. This function works independent from the time range settings in the Oscilloscope window. Display gain, leak subtraction or zero line subtraction will be applied.

Show	
Show PGF-Template	
Copy PGF to PGF-Pool	
Reference Series	+
Delete	
Print	
Export	
Export Full Sweep	
Export Format: ASCII	×
Export Mode	•
ASCII Text Format	•
Import Trace	•
Export Trace	×
With Marked Target and Children	
Show Target	
Print Target	
Export Target	
Export Full Target	
Recompute Zero Offset	
Average Sweep	
Average Series	
Duplicate Target	
Compress Target	
Collapse Target	
Delete Target	
Export Format: This determines the output device and the type of output to be created. Output is generated in the way the data are displayed in the Oscilloscope window, e.g. when the digital filter is on, filtered data are output.

The following export formats are implemented:

• ASCII: Sweeps are output as columns of ASCII numbers representing the data Traces (all in scientific format). Each Sweep and Series starts with an identifier. The separator can be modified (space, comma, or tab separators) by using the ASCII-Text format option in the Replay menu.

Note: The output style, either scientific notation or engineering format, is defined in the Notebook menu, 4.7 on page 36.

Please note, that this may create huge ASCII files when the output target is a Group, for example.

- Igor Pro: Exports Igor Pro format. For further format options, see the lower part of the menu.
- MatLab: Sweeps are exported as MatLab file.
- PULSE v8.6: Exports data in PULSE format. This feature is useful when CHARTMASTER data are to be analyzed with PULSEFIT, PULSETOOLS or PULSESIM.
- PICT: Sweeps are exported as Mac OS PICT file. Each file contains a single Sweep. When a Series is output, the Sweep files are generated automatically with the same name convention as waves for Igor Pro files: indices of "Group_Series_Sweep" are appended to the name.
- WMF: Sweeps are exported as Windows Meta Files. Each file only contains a single Sweep. When a Series is output, the Sweep files are generated automatically with the same name convention as waves for Igor Pro files: indices of "Group_Series_Sweep" are appended to the name.

Note: Instead of exporting graphs in PICT or WMF format, you might want to copy (CTRL+C) the traces and to paste (CTRL+V) them into another application.

- Info File: Sweep information are exported as Igor Info file (*.inf), e.g. labels, sample intervals, total points
- Printer: Direct output to a connected printer. The page setup magnification determines the line width; usually, 50 % gives good results. The numbers of columns and rows per page have to be entered. This determines how many items are placed on a page. In any case, a form feed is output after the selected target is output. Thus, if one prints a *Group* with 3 *Series* to a page with 2 columns and 2 rows, three quarters of the page will be filled, then the page is released from the printer. If one wants to have individual *Sweeps* rather than a complete *Series* plotted in the page sections, one has to turn off *Overl. Ser.* in the Oscilloscope window.

Further export parameters can be set here:

- Trace Time relative to Sweep: Individual Sweeps will be exported in an overlaid fashion. Time of the first data point of each Sweep starts with 0.
- Trace Time relative to Series: Individual *Sweeps* will be exported in an overlaid fashion. Time of the first data point of each *Sweep* starts with the difference between the start time of the *Series* and the start time of the 1. *Sweep* of that *Series*.
- Trace Time relative to Timer: Individual *Sweeps* will be exported in a concatenated fashion. The *Trace* time is offset by the *TimerTime* at *Sweep* start.
- Relative Trace Time: Individual Sweeps will be exported in a concatenated fashion. Same as above, but the time is subtracted by the *TimerTime* at the start time of the 1. Sweep. Resulting that the time of the first Sweep starts with 0.

The following format options refer to Igor Pro format only:

- Igor Allow Raw Data Access: Whenever possible an Igor Info file is generated allowing access to raw CHARTMASTER data which should be stored in the same folder. Using this output option assures fastest output and the smallest output files.
- Igor Create Binary Wave: Export of Sweeps to Igor Pro as binary data. This function generates an Igor macro which contains the instructions for Igor on how the data are to be loaded, scaled, and displayed. It has the extension *.itx. A double-click on it will make Igor Pro load that macro file and execute the instructions in it, importing, scaling, and displaying the data. The actual data are not really exported when using the Allow Raw Data Access option. That option will make use of the "GBLoadWave" Igor extension to read the data directly from the CHARTMASTER raw data file, i.e., the *.dat file. The data are converted to Igor binary waves when the simple Export option is used. However, even in that case a macro file is generated and you should load the data via that file. When you want to import data from within Igor Pro, use the option Load...Igor Text to load the macro file. Use the option Load...Igor Binary only when you want to explicitly load one of the generated Igor binary waves (file extensions *.ibw or *.wav).

Note: It is much faster to work with "Create Binary Wave" than with "Create Text Wave", and the created files are considerably smaller.

- Igor Create Text Wave: Export of Sweeps as ASCII waves in "Igor Text" format for the analysis and display program Igor. In Igor Pro, each wave is identified by indices "Group_Series_Sweep_Trace" (e.g., "Name2_4_3_1"). If the file name starts with a number, a "W" is placed in front of it, because in Igor Pro, waves are not allowed to start with a number. The created file has the extension ***.itx** and is recognized by Igor Pro, i.e., double-click on this file will start Igor Pro, load and display the file content (not for Sweeps). The waves will immediately be displayed in Igor Pro only when the Igor: Make Graphs option was selected. Otherwise, the Sweeps will be loaded, but must be displayed by Igor Pro's "Display Wave" or "Make Graph" command. When loading Igor Text output files, do not use the "General Text" import option in Igor; always use the option "Load...Igor Text".
- Igor Make Graphs: The Sweeps are exported as Igor graphs as they appear in CHARTMASTER in the Oscilloscope window. The created files have the extension *.itx and are recognized by Igor Pro, i.e., double-click on this file will start Igor Pro, load and display the file content.
- Igor Make Layouts: This option is useful if several *Series* shall be exported to Igor Pro. The exported *Traces* are arranged on an Igor Pro layout page. The created files have the extension ***.itx** and are recognized by Igor Pro, i.e., double-click on this file will start Igor Pro, load and display the file content.
- Igor One Graph per Full Sweep: With this option, a *Trace* that consists of several pages (e.g. continuous data) is exported as one single IGORPRO wave. Without this option, one wave per page is exported.
- Igor for Windows: If selected, the data will be exported for the MS Windows version of Igor Pro.
- Igor for Mac OS: If selected, the data will be exported for the Mac OS X version of Igor Pro.
- Set PULSE v8.6 Traces (1,2): If Export Format is set to Pulse v8.6 (see chapter 4.4 on the preceding page) this option gets active. Specify two traces of your selected Series which will be converted as first and second trace of the PULSE file format.

Export Mode: Shows a sub-menu that allows determining what will be exported:

- Traces: Exports the selected Traces.
- Stimulus: Exports information on the used stimulation pattern (segment duration and amplitude).

Note: This option can only be used when either Traces or Analysis - Graphs is selected.

- Analysis Graphs: The graphs of the Analysis get exported.
- Analysis Notebook: The Analysis function results which are displayed in the Notebook get exported. Please note that the option *Notebook* in the Analysis window has to be selected.

Note: This option is not available when using the Igor Pro Export Format.

ASCII-Text Format: Shows a sub-menu that allows to specify the type of separator used when generating ASCII tables, and the format of the exported text:

- Space/Comma/Tab Separator: Specifies how values are separated.
- Include Headers: If checked, a header that specifies various parameters of the exported data will precede the actual values.
- Mac OS 9 format: Lines are terminated by carriage returns. This generates standard Mac OS 9 text files.
- Mac OS X format: Lines are terminated by line feeds, generating Mac OS X text files.
- Windows format: Lines are terminated by line feeds plus carriage returns. This generates text files that can be read by programs running under DOS or MS Windows.

Import Trace: Imports data into a selected Trace.

When importing data you first have to generate a corresponding data structure (Tree) in CHARTMASTER. Make sure that the *Gain* and *Sampling Interval* of the data structure match the data you intend to import:

- Gain mismatch might result in loss of resolution or saturation of the data.
- The length of the imported *Trace* must be equal or greater than the *Trace* selected in the **Replay** window. Points exceeding the length of the *Trace* in the **Replay** window are discarded.

You can import the following three formats:

- from ASCII file: Imports data which were saved as CSV (= \underline{C} omma- \underline{S} eparated \underline{V} alues).
- from Binary file: Imports data which were saved in the Igor binary format.
- from Igor Wave: Imports data which were saved in the Igor Wave format.

Note: Since the Traces will be imported into the open file, the import function does not work when your file is read-only.

Export Trace: Exports data. You have four possibilities:

- as ASCII file: Exports data in ASCII file format.
- as Binary file: Exports data in Binary file format.
- as Igor Wave: Exports data in Igor wave format.
- as Stimulus Template file: Exports data as a stimulus template file.

With marked Target and Children...

Actions executed from this section of the Replay menu are performed on all marked <u>children</u> of the selected *Target* in the data tree:



In example "A" the With Marked Target and Children \rightarrow Export command would export only Sweep 3 to 8 of the first IV, while in example "B" all marked Sweeps would be exported.

- Show: Displays marked Targets in the selected tree branch.
- Print: Prints the marked Targets and children of the selected tree branch.
- Export Target: Exports marked targets and children of the selected tree branch. The display options in the Oscilloscope window define the export options.
- Export Full Target: Exports the full marked targets and children independent from the time range settings in the Oscilloscope window.
- Recompute Zero Offset: The zero offset value of all *Traces* in the selected target is recomputed. The zero offset value is used by the *Subtract Zero Offset* function (see chapter 4.5 on the next page).

	×
Recompute zero offset of vis	ible traces:
target trace (132)	1
left bound [%] (099)	10
right bound [%] (1100)	90
segment (0 = use zero seg.)	0
ОК	Cancel

- target trace (1...32): Selects the target Trace for the zero offset recomputing.
- left bound [%](0...99) & right bound [%](0...100): Defines the range in between CHARTMASTER should recompute the zero offset.
- segment (0=use zero seg.): Defines the Segment (of the PGF) for the zero offset calculation.

Note: Only visible Traces are recomputed.

Note: Only Traces originating from an AD-channel are recomputed; virtual Traces are not affected.

Note: The zero offset of leak Traces are only recomputed if the "Show Leak Traces" is selected from the **Display** menu.

• Average Sweep: Averages all Sweeps of a Series and stores them as Series with one Sweep. The Sweeps have to have the same length.

- Average Series: Generates one new Series in which each Sweep is the average of all marked Sweeps with the same Sweep index and each Trace with the same Trace index.
- Duplicate: Creates a new *Group* containing all marked targets. This is e.g. convenient when one needs to compress a *Trace* for export.
- Compress: Compresses all marked *Traces* by a given compression factor. Optionally one can supply a maximal compression factor. This can be used e.g., if one has *Traces* which are already compressed and having much fewer points per second, e.g., a FURA trace. The *Traces* cannot reside in a read-only file. To compress a *Trace* in a read-only file, duplicate the required targets.
- Collapse: The Sweeps of all marked Series are copied into a new Series. This is typically used when one acquired many Series with one single Sweep and one wants to combine them into one Series for easier Analysis.
- Delete: Deletes the marked targets and children of the selected tree branch.

Note: Delete and Delete Marked Target Children are disabled when a data file is opened with File \rightarrow Open Read Only.

4.5 Display Menu

This menu sets some parameters for the display of data in the Oscilloscope window.

Auto Show: Automatically displays the data selected in the Replay window. When this option is disabled, a double-click on the targets in the tree is required for displaying the data.

Trace Properties: In the **Trace Properties** dialog the user can set Show, Vector (connect individual data points) and Center flags, select the Marker type and the Reference Series properties. All settings are explained in detail in chapter 4.5.2 on page 33.

Also, the *Trace Properties* can be set via macro commands to allow automatic display adjustment depending on type of acquisition. Have a look for the macro commands starting with T (Help menu \rightarrow List All Macro Items).

The color of *Traces* can be set in the Line Colors dialog of the Configuration window (see chapter 5.6.1 on page 50).

Show Zero Line: Draws a reference zero line in the Oscilloscope window.

Subtract Zero Offset: Subtracts the zero offset from the measured signals. The zero offset is the mean value, calculated from the Zero segment. The Zero segment is defined in the PGF (see chapter 9.9.2 on page 92). Usually, the zero offset value is determined online, but it can also be computed offline by use of the *Recompute Zero Offset* function from the **Replay** menu (see chapter 4.4 on the preceding page).

Subtract Ref. Series: If this item is checked, then the *Reference Series* is subtracted from all replayed data (see chapter 4.5.2 on page 33).

Subtract Trace Buffers: If this item is checked, then the Trace Buffer is subtracted from all replayed Traces (see chapter 4.5.2 on page 33).

Dimmed Overlay: Turns the *Dimmed Overlay* mode on or off. In *Dimmed Overlay* mode, the last displayed *Trace* is drawn in another color than the previously plotted *Traces*.

Overlay Pages: During continuous acquisition the data are displayed in pages. The pages can be overlayed.

Overlay Traces: Overlay of all *Traces* of a selected *Sweep*.

Overlay Sweeps: Overlay of all Sweeps of a Series.

Overlay Series: Overlay of all Series, i.e. overlay of all.

Auto Y-scale: The Y-scaling is automatically adapted to the replayed data to fit minimal and maximal data of a Series onto the screen. The range of the Y-scaling depends on the Gain settings.

√	Auto Show	
	Trace Properties	
✓	Show Zero Line	
	Subtract Zero Offset	
	Subtract Ref. Series	
	Subtract Trace Buffers	
✓	Dimmed Overlay	
	Overlay Pages	
✓	Overlay Traces	
✓	Overlay Sweeps	
	Overlay Series	
	Auto Y-scale	
	Fixed Y-scale	
	Fixed X-width	
	Freeze Zero Line	
	Labeling	•
	3D Mode	•
	Show Tree Info	

Fixed Y-scale: The Y-scale is fixed. In the Oscilloscope window the controls *Y*-scale and *Y*-offs. turn into *Y*-max and *Y*-min.

Note: Auto Y-scale has priority over Fixed Y-scale.

Fixed X-width: The X-scale is fixed. In the Oscilloscope window the controls *Start Time* and *End* turn into *X-min* and *X-max*.

Freeze Zero Line: This checkbox determines the behavior of the display scaling in the Oscilloscope window. When *Freeze Zero Line* is selected, the Oscilloscope behaves like an oscilloscope, i.e. when the display gain is changed the zero line stays at its position. When deselected, display scaling is performed with reference to the center of the display. I.e., when *Y*-offs is not zero, the zero line may alter its position on the screen.

Labeling: Determines the labels in the main display of the Oscilloscope window and the *PGF-Editor Grid* independently:

- Labels Only: Draws calibration bars.
- Grids + Labels: Draws a grid and units/division.
- Grids + Values: Draws a labeled grid.
- No Labels: No labels, no grids.

Show Tree Info: When this option is selected, information about the replayed target is shown in the Notebook window.

4.5.1 3D-Mode

Shows a sub-menu for selection of the pseudo three-dimensional graphing.



The following settings can be made:

• 3D-Graph - On: The results are displayed in black and white in pseudo three-dimensional mode by displaying subsequent *Sweeps* with a horizontal and vertical offset. The dX and dY values for the 3D feature can be entered in the Configuration window. The default value for both is 0.010.

- 3D-Graph Color: The results are displayed in pseudo colors leading to a two-dimensional height profile. You can set the color selection and the contrast by changing the values in *Y*-scale and *Y*-offs.
- 3D-Graph On and 3D-Graph Color: The results are displayed in pseudo colors and 3D-mode to give the impression of contour lines, thus leading to a 3D height profile.



4.5.2 Trace Properties Dialog

Trace Properties						
Copy 1. to al	Extend	Extend	Extend	Extend	Extend	Extend
Trace 1	Show	⊠ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 2	Show	☑ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 3	Show	☑ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 4	Show	☑ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 5	Show	☑ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 6	Show	☑ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 7	Show	Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 8	Show	☑ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 9	Show	☑ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 10	Show	☑ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 11	Show	☑ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 12	Show	☑ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 13	Show	☑ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 14	Show	☑ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 15	Show	☑ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Trace 16	Show	☑ Vectors	No Marker	Ref. Series Corr.	Center	No TBuffer Corr.
Buffer 1	Show	Vectors	No Marker	Use Separate Scal	ing	
Buffer 2	Show	☑ Vectors	No Marker	Use Separate Scal	ing	Panaint
Buffer 3	Show	⊠ Vectors	No Marker	Use Separate Scal	ing	Repaint
Buffer 4	Show	☑ Vectors	No Marker	Use Separate Scal	ing	Done

The Trace Properties dialog provides access to certain display settings of individual *Traces* and *Buffers*. **Extend:** Extends the setting of the first *Trace* to all other *Traces*.

Trace 1...16, Buffer 1...4: Up to 16 Traces and 4 buffers can be individually modified.

Note: The amount of traces (default: 16) is defined in the Configuration (General pane).

Show: Here, one can decide if a Trace shall be displayed (Show) in the Oscilloscope window or not.

Vectors: When option *Vectors* is checked then the markers are connected by lines.

Marker: Markers, such as

- Point
- Plus
- Star
- Diamong
- Cross
- Square

can be used for plotting data points. This is particularly useful, if LockIn or FURA data are acquired.

Ref. Series Corr.: If *Ref. Series Corr.* is checked for a *Trace*, then the *Reference Series* is subtracted from that *Trace.* A *Reference Series* can be defined by use of the *Reference Series - Set* item from the *Replay* menu. The *Ref. Series Corr.* can be turned 'On' or 'Off' globally in the *Display* menu.

Center: If this option is set the traces get centered during acquisition. Therefore the left 10% of the first page are brought to the center of the display.

Trace Buffer Corr.: If *Trace Buffer Corr.* is set to one of the four available *Trace Buffers* then the contents of the chosen *Buffer* are subtracted from that *Trace.* This can be used for subtracting a control *Trace* from other *Traces.* The *Trace Buffer* subtraction can be turned 'On' or 'Off' globally in the Display menu.

Use Trace Scaling: With this option, the display scaling of a *Trace Buffer* can be coupled to the display scaling of a certain *Trace*.

Repaint: Repaints the last displayed Sweep in the Oscilloscope with the new Trace Properties.

Done: Closes the Trace Properties dialog.

Note: Like other menu settings, the Trace Properties are stored in the CHARTMASTER configuration file.

4.6 Buffer Menu

CHARTMASTER offers four independent Trace Buffers for storing and processing of intermediate Trace data.

Buffer 1...Buffer 4: Select (make active) the buffer to work on.

Use Full Trace: If this option is selected the functions *Add Trace*, *Subtract Trace*, and *Accumulate Trace* work on the complete *Trace*. If this option is not selected the above mentioned functions work on the part of the *Trace* which is displayed in the Oscilloscope window only.

Clear All: Clears all Buffers (Buffer 1...4).

Clear: Clears the selected Buffer.

Show: Shows the selected *Buffer* in the Oscilloscope.

Scale: Scales the selected Buffer with Scale and Offset.

Equation: Allows to perform a *Buffer* calculation based on an equation. For details see chapter 18 on page 157 and chapter 18.2.2 on page 160.

Import: Imports data into a selected Buffer.

Please make sure that the key parameters *Gain* and *Sampling Interval* of the imported data match the parameters of the *Buffer*. In case a *Buffer* is filled with data for the first time, the parameters are retrieved from the *Trace* currently selected in the **Replay** window.

You can import the following three formats:

- from ASCII file: Imports data which were saved as CSV (=<u>C</u>omma-<u>S</u>eparated <u>V</u>alues).
- from Binary file: Imports data which were saved in the IGOR binary format.
- from Igor Wave: Imports data which were saved in the IGOR Wave format.

Export: Exports data from a selected *Buffer*. You have four possibilities:

- as ASCII file: Exports data in ASCII file format.
- as Binary file: Exports data in Binary file format.
- as Igor Wave: Exports data in Igor wave format.
- as Stimulus Template file: Exports data as a stimulus template file (see Using a Recorded Waveform as Stimulus in the CHARTMASTER Tutorial).

Add Trace: Adds the Trace selected in the Replay window to the active Buffer.

Subtract Trace: Subtracts the Trace selected in the Replay window from the active Buffer (Trace).

Accumulate Trace: Accumulates the Trace selected in the Replay window to the active Buffer:

Buffer(n+1) = (Buffer(n) * n + Trace)/n+1

with n = number of Traces in the Buffer.

Deaccumulate Trace: Deaccumulates the Trace selected in the Replay window from the active Buffer:

$$Buffer(n-1) = (Buffer(n) * n - Trace)/n-1$$

with n = number of Traces in the Buffer.

Buffer 1	
Buffer 2	
Buffer 3	
Buffer 4	
Use Full Trace	
Clear All	
Clear	
Show	
Scale	
Equation	
Import	•
Export	•
Add Trace	
Subtract Trace	
Accumulate Trace (0)	
Deaccumulate Trace	
With Marked Target and Children	
Use	×
Add	
Subtract	
Accumulate	
Deaccumulate	
Replace Target Trace	

With Marked Target and Children: Multiple Traces can be marked in the Replay window and processed simultaneously.

- Use: Specify which Trace of the marked Sweeps should be processed.
- Add, Subtract, Accumulate, Deaccumulate: Work on all marked targets. For further explanations of the functions refer to individual functions explained above (see chapter 4.4 on page 29).

Replace Target Trace: The *Trace* selected in the Replay window (target *Trace*) will be replaced with the active *Buffer*.

4.6.1 Handling of Parameters by the Buffer

When a *Buffer* is filled with data for the first time (after a *Clear* command) all parameters of the target *Trace* are transferred to the *Buffer* data structure. When adding further *Traces* to the *Buffer* these parameters are not changed. It is assumed that all *Traces* that are added to the *Buffer* are of same type. When replacing a target *Trace* with the data of a *Buffer*, data only are transferred. The parameters of the target *Trace* stay unchanged.

Important note: It is the responsibility of the user that the key parameters such as Gain and Sampling Interval are chosen adequately when adding importing data to a Buffer or processing different Traces in a Buffer. Once a Trace is replaced by a Buffer, most parameters i.e. Zero Offset or C-slow become meaningless.

4.7 Notebook Menu

The Notebook is used to display experiment information. The ASCII-table separator setting of the Replay menu is used for the Notebook as well. This enables e.g. to transfer data directly to spread sheets requiring a TAB separator, such as MS Excel, via "Cut and Paste". The options in the Notebook menu are:

Save: Saves the Notebook under its default name: "Notebook_Date".

Save As...: Asks for a file name before saving.

Merge...: Merges a text file to the content of the Notebook.

Print...: Output the content of the Notebook to a printer.

Clear when Saved: Automatically clears the Notebook after the present content is saved to disk.

Clear: Clears the Notebook.

Set Length...: Specifies the maximal number of text lines in the Notebook. The maximal number of lines is given in parentheses.

Line Numbers: Shows line and column numbers when moving with the cursors inside the **Notebook**.

Font Size...: Allows to select the font size of the Notebook text.

Zoom In: Shrinks the Notebook window to default (i.e. small) size.

Zoom Out: Expands the Notebook window to full program screen size.

Scientific Notation: If set, the results of the Analysis are written to the Notebook in scientific notation (e.g., 1.23e-12). The default is engineering format (e.g., 1.23p). The scientific notation is mostly used when the user wants to copy results from the Notebook to a spread sheet program by copying to the clipboard.

Note: This setting also applies to the ASCII-export format entry in the Replay menu.

Auto Store: This option will automatically store the Notebook together with the data file ([data file name]*.txt). Upon opening a data file, its Notebook file will automatically be loaded as well.

Ctrl+S
Ctrl+P
Ctrl+B
Ctrl+K
Ctrl+L

4.8 Protocols Menu

The Macros menu of older CHARTMASTER versions has been replaced by the new Protocols menu (since version 2x52). This drop-down menu can be used for the conversion of macro files from older CHARTMASTER versions to protocols.

Append Macro File: Converts an existing macro file (*.mac) to a protocol and appends this new protocol to the actually opened protocol file (*.pro).

Execute while Recording: If selected, every button you press during macro/protocol recording will be executed. If not selected, this option allows recording a protocol without executing the buttons. Thus, during protocol recording, commands and values are recorded, listed in the Notebook, and then the parameters are immediately set back to their previous value.

Note: Macro/protocol recording can be started from the Protocol Editor dialog (see chapter 10.1 on page 99).

Execute: Executes the selected, user defined protocol. Alternatively, protocols can be executed from:

• the Control window, if the option *Hide Protocol-Bar* in the Control window is not activated on the Display tab (see chapter 5.6.3 on page 51).

Append Macros File ~ **Execute while Recording** Execute Exampl1 Execute Example2 Execute Link Execute Buffer Execute SETUP Execute SEAL Execute WHOLE-CELL Execute 8 Execute 9 Execute 10 Execute 11 Execute 12 Execute 13 Execute 14 Execute 15 Execute 16 Execute 17 Execute 18 Execute 19 Execute 20 Execute 21 Execute 22 Execute 23 Execute 24 Execute 25 Execute 26 Execute 27 Execute 28

4.9 AD-board

 Image: A start of the start of	Enable Batch Control Enable Background
	Initialize LIH 8+8
	LIH 8+8

Enable Batch Control: Allows CHARTMASTER to be remotely controlled by another program (see chapter Controlling PATCHMASTER in the PATCHMASTER Tutorial).

Enable Background: Allows background process to keep running even when CHARTMASTER is in the background.

Initialize LIH 8+8: This will re-initialize (restart) the connected AD/DA interface.

LIH 8+8: Displays the type of AD/DA interface being used along with serial number and board version, if available.



4.10 Help Menu

With the additional tools listed in the Help menu you can get more familiar with the available functions of CHARTMASTER.

Show Tooltips Show Keys Hide Keys List Keys Save Keys List All Items List All Macro Items About ChartMaster

Show Tooltips: When activated information about the button that is located under the mouse tip is displayed.



Show Keys: Displays the key commands with the corresponding buttons.

🔜 Control Window		
idle	13:56:57	03:58:38 Store Ctr-Bik Ctr-Sip Ctr-Nit Ctr-Wit Ctr-Rime
Comment		Average 1
PGF 00 1	1 IV 2(2R	Ramp 3 3 tinuous 4 4 Hinf 5 5 Tails 6 6 tSeries 1 00
Protocol 00 1	SETUP 2 S	SEAL 3(WHOLE-CE) 4 IV 5 IV_2 6 Link 6 00

Hide Keys: Hides the key commands.

List Keys: Lists all keys to the Notebook window (see chapter 3 on page 17).

Save Keys: Saves the actual key command settings to the default key file. The old Chartmaster.key file will be saved with the extension *.kXX (X = consecutive index number).

List all Items: Lists all items and their values in the Notebook window.

List all Macro Items: Lists all macro items and their values in the Notebook window.

About Chartmaster: Displays the CHARTMASTER software version and contact information.

5. Configuration Window

Configuration: C:\Program Files (x86)\HEKA\2x90\Char\ChartMaster					
SAVE	SAVE AS	Default Windows	Default Settings		
General Hard	dware Fil	es Display	I/O Control	Trace Assign	Misc.
Max. Shown Traces Max. Shown Values Window Scaling Max. Sample Points Max.Stim.Traces x Points Min. Wait Time Batch Communication User Name	16 16 500000 5 2000000 100. ms Disable Username	⊠ Logging	1 Connection		

Settings like sources for external parameters, default values, display settings, colors, fonts, default files, ... can be edited in the Configuration window. To access the Configuration window select the drop-down menu Windows \rightarrow Configuration. These and other settings can be stored in *.set files; by default this file is Chartmaster.set. By means of different *.set files every user can define their individual program layout to meet specific requirements.

5.1 Individualize Chartmaster

The name of a CHARTMASTER setting file can be used as a command line parameter upon starting the program. That way, one can start CHARTMASTER with various configurations which is very useful when several users work on one setup and have a need for different CHARTMASTER settings.

The procedure for an individualized CHARTMASTER starting procedure is as follows:

- Rename the *.set file in your HEKA folder (e.g. "Chartmaster_User.set").
- Create a shortcut of Chartmaster.exe on the desktop.

Note: The term "User" is just a place holder for individual names.

- Rename the shortcut (e.g. "Chartmaster.exe_User").
- Right-click on the shortcut: Properties Link Target.
- The following command line should be written in the "Target" field, including the inverted comas and empty spaces:

"C:\Program Files (x86)\HEKA\Chartmaster\Chartmaster.exe" "C:\Program Files (x86)\HEKA\Chartmaster_User.set".

Note: Please be aware that your installation path of your CHARTMASTER may vary on your PC.

5.2 Save

SAVE SAVE AS ... Default Windows Default Settings

SAVE: Saves the configuration file with its current name.

SAVE AS...: Saves the configuration file under a new name.

Default Windows: Resets the position of most windows. This helps to restore the windows position once one gets dragged off screen (for example after reducing the screen resolution).

Default Settings: Sets all settings back to the original settings. Note that this needs a restart of the program!

Note: There is no Load option because CHARTMASTER has to be restarted upon substantial changes of the settings. Thus, in order to use another setting, quit CHARTMASTER and restart. Before you do so, make certain that there is no Chartmaster.set file available. In this case, upon start-up, CHARTMASTER will prompt you to select a new setup file (this can have any name).

5.3 General

5.3.1 Max Shown Traces and Values

Max. Shown Traces: This number defines how many *Traces* are used by CHARTMASTER. The user interface is adapted to show only the defined number of *Traces*. A small number makes the dialogs and lists within CHARTMASTER

Max. Shown Traces	16
Max. Shown Values	16

shorter. The new setting only takes effect after saving the configuration file and upon restarting the program. When using the *Probe Selector* it may be necessary to increase *Max. Shown Traces.*

Max. Shown Values: This number defines how many values are used by CHARTMASTER. The user interface is adapted to show only the defined number of values. A small number makes the dialogs and lists within CHARTMASTER shorter. The new setting only takes effect after saving the configuration file and upon restarting the program.

5.3.2 Window Scaling

JO):
000
Cancel

Window Scaling: This feature is used to scale (change size of all controls) all windows. This may be useful when there is too little space for all necessary windows on a single monitor. The new scaling only takes effect after saving the configuration file and upon restarting the program. The default *Window Scaling Factor* value is '1.2', if you enter a '0' the option is inactive.

5.3.3 Memory Allocation

Max. Sample Points: Sets the maximum number of points that can be acquired in one *Trace* (see chapter 9.7 on page 78). This parameter is limited by the amount of available memory.

Max. Stim. Traces x Points: Maximum number of stimulation *Traces* and their respective number of points. This parameter is limited by the amount of available memory.

Note: In case you want to drive the number of Max. Sample Points to its limit you might reduce the number of Max. Stim. Traces to make more memory available for allocating sample points.

5.3.4 Minimum Wait Time

Min. Wait Time: Sets the time CHARTMASTER reserves to wait for the correct time to start when executing individual repeat loops such as acquisition of a Series of Sweeps or an Repeat loop in the Protocol Editor.

For example, to ensure an Acquire Each Sweep repeat loop keeping the same repeat time as set by the Duration in the Repeat event, the program will wait Min. Wait Time before executing the next acquisition to hit the scheduled starting time of the next Sweep as exactly as possible.

Note: In case you are observing incorrect and unstable repeat times, please try to increase the Min. Wait Time.

This parameter can also be set via the Acquire Properties event in the Protocol Editor.

5.3.5 Batch Communication

Batch Communication: The *Batch Communication* with other applications can be disabled or enabled (see Controlling CHARTMASTER in the CHARTMASTER Tutorial).

- Disable: If this option is checked, the Batch Communication is turned off.
- Enable as Receiver: CHARTMASTER is configured to serve as *Receiver* and will wait for command inputs of other applications.
- Enable as Sender: CHARTMASTER is configured to serve as *Sender*, thereby controlling another program, e.g. FITMASTER.
- Synchronize Files: With this option the Sender application will send the "FileOpened" message, when a ***.dat** file, ***.pgf** file, ***.pro** file or ***.on1** file is opened, and the "FileUpdated" message, when the data file is updated. The "FileClosed" message is send, when the data file is closed. The Receiver will then open, update, or close the same data file as the Sender application.
- Polling: In the *Polling* mode, the master application polls the slave application for commands. The slave then answers to the query and appends all pending commands, i.e., it no longer sends commands independently. In doing so, commands do no longer get lost, if heavy bi-directional communication is ongoing.

Logging: If the checkbox is activated the communication via the *Batch Communication* interface is written to the Notebook.

5.3.6 User Name

User Name: A user name that is entered here will be stored with the raw data.

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5

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Min. Wait Time	100. ms
	-

Max. Sample Points

Max.Stim.Traces x Point

5.4 Hardware

E Configuration: C:\Program Files (x86)\HEKA\2x90\Char\ChartMaster						
SAVE SAVE AS Default Windows Default Settings						
General Hardware	Files	Display	I/O Control	Trace Assign	Misc.	
⊠ DA/AD: Demo Mode LIH 8+8						
Lockin Off Spectroscopy Off Imaging Off Photometry Off Serial I/O Off Multi-Channels Off						

5.4.1 Amplifier and Digitizer Selection

 ${\bf Digitizer \ Selection: \ Select \ the \ appropriate \ AD/DA-converter, \ if \ it \ is \ not \ automatically \ assigned \ via \ the \ amplifier \ selection. }$

CHARTMASTER supports the following AD/DA-converters:

- ITC-16
- ITC-18
- LIH 1600 / ITC-1600
- LIH 8+8
- 1...3 PCI: Additional set-up information, if there is more than one possible card slot to connect to the digitizer.
- USB: InstruTech's USB-16 or USB-18 adapters allow for connecting an ITC-16, EPC 9, ITC-18 to the computer via an USB 2.0 port. In case, such an adapter is used, it should be enabled here.

5.4.2 LockIn

Here, the LockIn amplifier of CHARTMASTER can be activated. For a detailed description see chapter Software LockIn Extension, 21 on page 181, and the CHARTMASTER Tutorial "Capacitance Measurements using the LockIn Extension".

	ПС-16 ПС-18 ЦН 1600 ЦН 8+8	
	PCI	
	PCI	
	PCI	
✓	USB	
	USB	
	USB	

5.4.3 Spectroscopy

Here, the Spectroscopy Extension of CHARTMASTER can be activated. For a detailed description see chapter Spectroscopy, 22 on page 191, and the CHARTMASTER Tutorial "Using the Spectroscopy Extension".

5.4.4 Imaging

Here, the Imaging Extension of CHARTMASTER can be activated. Depending on the fluorescence excitation light source or wavelength switcher you have installed in your setup, you can choose between different kinds of Imaging Extensions:

- T.I.L.L.: "T.I.L.L. Photonics' Polychrome", can be controlled via an analog output of the amplifier or any AD/DA converter supported by CHARTMASTER. The exposure of the camera should then be triggered via a digital output channel.
- DG4/DG5: The "DG-4/DG-5" can be controlled via the digital output of the EPC 10, ITC-16 or ITC-18. The exposure of the camera should then be triggered via an analog output channel.
- Lamba-10: The "Sutter filter wheel Lambda-10" can be controlled via the digital output of the EPC 10, ITC-16 or ITC-18. The exposure of the camera should then be triggered via an analog output channel.
- pti DeltaRAM: The "High-Speed Random-Wavelength Illuminator" can be controlled via the digital output of the EPC 10, ITC-16 or ITC-18. The exposure of the camera should then be triggered via a digital output channel.
- no device: No light source control.

For details on the Imaging Extension please refer to the chapter Imaging, 20 on page 169.

5.4.5 Photometry

This will activate stimulation of a monochromator and acquisition via a photo-multiplier or photo diode.

You can choose four kinds of Photometry Extensions:

- T.I.L.L.: "T.I.L.L. Photonics' Polychrome", can be controlled via an analog output of the amplifier or any AD/DA converter supported by CHARTMASTER.
- DG4/DG5: The "DG-4/DG-5" can be controlled via the digital output of the EPC 10, ITC-16 or ITC-18.
- Lamba-10: The "Sutter filter wheel Lambda-10" can be controlled via the digital output of the EPC 10, ITC-16 or ITC-18.
- pti DeltaRAM: The "High-Speed Random-Wavelength Illuminator" can be controlled via the digital output of the EPC 10, ITC-16 or ITC-18.

For details on the Photometry Extension please refer to the chapter Photometry, 19 on page 163.

5.4.6 Serial Out

This control is used to open and set up the serial port communication mode. If a so-called *Serial Communication* has been established between the computer and another device, CHARTMASTER can send strings over a serial port, but it will not receive instructions.

The options for the serial communication are:





- Off: No connected device.
- Comm 1...4: Any device that can receive strings through the serial port. Up to 4 ports can be defined and triggered.

When opening a serial communication, CHARTMASTER will allow you to configure the serial device. Make sure that the settings match on both communicating machines.

- Serial Port: No Port, Comm 1...Comm 8.
- Baud Rate: 300, 600, 1200, 1800, 2400, 3600, 4800, 7200, 9600, 19200, 57600, 115200, 128000 or 256000 bps.
- Stop Bit: 1.0, 1.5 or 2.0.
- Parity: No Parity, Even Parity or Odd Parity.
- Data Bit: 5, 6, 7 or 8 data bits.
- XOn/XOff: On or Off.
- Rts/Cts: On or Off.

To change all settings or leave the dialog, do one of the following:

- Edit Start String: Allows to enter a specific start string. The *Start String* (Initialization String) is send once after opening the COM port.
- Open: Opens the configured port (icon text changes to "Close")
- To Notebook: Commands sent and received are written to the Notebook.
- Get Defaults: Sets the default settings given in CHARTMASTER.
- Undo: Cancels all changes without closing the dialog.
- Cancel: Cancels all changes and closes the dialog.
- Done: Saves all changes and closes the dialog.

If a serial out is active then command strings can be entered and sent via the I/O Control window (see chapter 14 on page 145). There is also a *Serial Out* event available in the Protocol Editor, 10.4.3.1 on page 108.

5.4.7 Further Options

Multi-Channels: Enabling *Multi-Channels* has major implications on the Oscilloscope window (see chapter 6.7 on page 64) and the Analysis (see chapter 11.6 on page 133).



Multi-Channels

Off

5.5 Files

In this section, the user has to specify the paths for several files. CHARTMASTER will use these paths when saving or retrieving files.

Configuration: C	\Program Files (x86)\H	HEKA\2x90\Char\Ch	nartMaster			
SAVE SAVE AS Default Windows Default Settings						
General	Hardware	Files	Display	I/O Control	Trace Assign	Misc.
Home Path C Data File C Protocol File C PGF Pool File C Analysis File C Solution Base E Equations File C Batch Path C "*.dia" Path C Temp. Path C Save Settings Ask for Data F Make Bundle Startup Protocol Experiment Num Max. File Size Al	Constant Service Ser	86)\HEKA\2x90\Ch 5)\HEKA\2x90\Char 5)\HEKA\2x90\Char 5)\HEKA\2x90\Char 5)\HEKA\2x90\Char 5)\HEKA\2x90\Char Break ase ATE2]_[001] 1B	artMaster\ \Username_2015-1 tMaster\DefProtCha tMaster\DefPgfCha tMaster\DefAnalCh	2-09_001.dat art.pro rt.pgf art.onl		

To rename a path, click on the button and set a new path.

Home Path: Sets the path to the folder LookupTables and to the key assignment file (Chartmaster.key). It is also the path where the default files DefPgf_v9.pgf, DefAnal.onl and DefProt.pro are searched automatically as long as no configuration file was saved.

The path to the Configuration file is shown in the title of the Configuration window. This path can be changed by saving the Configuration under another path using the SAVE...AS function.

Data File: Sets the path to the data file (file extension: *.dat).

Protocol File: Sets the path to the protocol files (file extension: ***.pro**).

PGF Pool File: Sets the path to the Pulse Generator file (file extension: *.pgf).

Analysis File: Sets the path to the Analysis file (file extension: *.onl).

Solution Base: Sets the path to the solution data base files (file extension: *.sol).

Equations File: Sets the path to the equation file, which can contain equation formulas. This path can be cleared by use of the \bigcirc button.

Batch Path: Path for batch file control protocols, e.g., E9BatchIn and E9BatchOut. This path can be cleared by use of the ^O button. For further information we refer to the CHARTMASTER Tutorial: "Controlling CHARTMASTER".

Dialog Files: Sets the path to the dialog files, in which button placements of the different windows can be stored in case they should deviate from the default. This path can be cleared by use of the \bigcirc button.

Temp. Path: Optionally a path different from the home path can be specified for storing temporary data. This path can be cleared by use of the ^O button.

I-gain Lookup: Path to the *Current Gain Lookup Table* if selected in the configuration (see chapter 5.7.2 on page 55). Only necessary for telegraphing amplifiers.

5.5.1 General Advice on Naming Folders and Files

Here is some advice to keep in mind when naming folders and files:

- The use of invisible characters and spaces is not recommended (a blank in a file name is very often overlooked).
- Names should not begin with a number, because some other applications, e.g., Igor, do not allow names with a number as the first character (exported Igor waves inherit characters of the data file).
- CHARTMASTER only shows the first 14 letters of the file name in the title of the Oscilloscope window.
- The first few letters of a name are the more important ones, because they ease file selection with the file selector. The file selector continuously selects the files while the user types.
- Experiment data should not be stored within the HEKA software folder. This will ease upgrading the software without moving the data.

5.5.2 Miscellaneous Settings

Save Settings File: The Configuration set-	Save Settings File	⊠ Verify Quit
tings will be saved automatically on exit, thus	Ask for Data File	Save after Break
overwriting the previous settings.	Make Bundle Files	Solution Base
Ask for Data File: When this option is en-	Auto Filename	
abled CHARTMASTER will ask for a data file	Startup Protocol	
upon start up	Experiment Number	1
upon soar o-up.	Max. File Size Alert	500. MB

Make Bundle Files: When this option is se-

lected, all files generated for a data set (e.g. the raw data *.dat, *.pgf, *.pul,...) are combined into one *.dat file.

Verify Quit: When this option is enabled, CHARTMASTER will ask to save modified setting files (*.pgf, *.pro, *.onl) when you quit the program. When it is not enabled, the Configuration settings will not be saved on quit!

Save after Break: When this option is enabled, CHARTMASTER will save the data in a *Sweep* that have been acquired when the acquisition was terminated via the *Break* button. With this option disabled the entire *Sweep* will be discarded if during its acquisition the *Break* button was pressed.

Solution Base: The Solution Base is activated by default, therefore the default solution base (DefSolutionBase.sol) is loaded (see chapter 15 on page 147).

Auto Filename: Allows an automatic file name generation. The file names can be generated out of a set of special components.

- [DATE=<descriptor>] = descriptor compounded by "YYYY" or "YY", "MMM" or "MM", and "DD". Characters separating the blocks are copied to the name, e.g. [DATE=DD-MMM-YYYY] = 31-Dec-2015
- [DATE] or [DATE1] = DD-MMM-YYYY (e.g. "31-DEC-2015")
- [DATE2] = YYYY-MM-DD, international custom data format, directly sorts according to dates e.g. "2015-12-31"
- [DATE3] = YYYYMMDD, analog to [DATE2], but without hyphens e.g. "20151231"
- [DATE4] = YYMMDD, e.g. "151231"
- [TIME] or [TIME1] = HH-MM-SS (e.g. "24-59-59")
- [TIME2] = HHMMSS, analog to [TIME1], but without hyphens (e.g. "245959")
- [USERxx] = username, "xx" is a number giving the maximal number of characters. No "xx" means maximal allowed characters, presently 32
- [000] = an incrementing counter, each digit being a placeholder. The given number is the starting value of the counter. The counter will increment until a unique filename is created

• [NAME] = a placeholder for the data file name

The components are composed to a file name by the insertion of an underscore "_".

A typical entry could look like: HERG_[DATE]_[001] = "HERG_31-Dec-2015_001.dat"

Do not use the following characters:

- path separators: "/", "\", ":" (unacceptable)
- extension separator: "." (unacceptable)
- blank: " " (compatibility problems, danger of oversight)

Startup Protocol: Here, you can enter the name of a protocol that is executed after the end of program initialization (i.e. after the configuration file is loaded, the amplifier initialized and the other settings activated). E.g., this allows for additional settings like the immediate activation of the second amplifier of an EPC 10 Double at start-up.

Experiment Number: This number can be used to identify experiments. It is automatically incremented when a new Experiment is generated with File \rightarrow New Experiment.

Max. File Size Alert: This feature will not limit the size of any file written to disk, but can be used as a reminder so that no files are created that e.g., do not fit onto a CD. A warning is written to the Notebook if the size of the raw data file exceeds this value. Entering a very large number will - in practice - suppress any warnings.

Important note: The maximum file size is limited to 2 GB. If you exceed this number during data acquisition you have to consider data loss.

5.6 Display

Configuration: C:\Program Files (x86)\HEKA\2x90\Char\ChartMaster						
SAVE SAVE AS Default Windows Default Settings						
General Hardware Files Dis	play I/O Control Trace Assign Misc.					
Fonts Button Colors Line Colors Ø Wipe at Start Digits: Notebook Time 5 8 Grid Digits: X Y 6 6 Graph-3D: dX dY 0.010 0.010 Hide PGF-Bar in Control Window Hide Protocol-Bar in Control Window Show no Fixed Control Protocols						

5.6.1 Fonts and Colors

Fonts / Button Colors / Line Colors:

Colors and text fonts for the program layout can be selected here. These are global settings for all window

Fonts) Button Colors	Line Colors

dialogs and they are installed upon restart of CHARTMASTER. The colors and fonts are stored in the configuration file (*.set), i.e., they are independent of the dialog files (see chapter 2 on page 9).

Note: If dialog files are present, they will overwrite the Configuration settings.

Note: You can make the background colors of the windows dark (useful when doing light-sensitive experiments) by selecting the option Button Colors. In this case, you may also have to change the color of lines, like the Trace Colors, for example, using the option Line Colors.

5.6.2 Notebook, Display, and Analysis

Wipe at Start: If this option is activated the Analysis and Oscilloscope windows will be wiped before data acquisition.

Digits:

- ☑ Wipe at Start

 Digits: Notebook | Time
 6
 8

 Grid Digits: X | Y
 6
 6

 Graph-3D: dX | dY
 0.010
 0.010
- Notebook: Numbers of digits for data display in the Notebook window (range 3 24).
- Time: Numbers of digits for the *Time* and *Timer* function in the Oscilloscope window (range 8 11). The maximal number is 11, this results in "hh:mm:ss:ms".

Grid Digits X|**Y**: Number of digits to be shown in grid annotation of the Oscilloscope and the stimulation template preview of the Pulse Generator (range 6 - 10).

Graph 3D dX|**dY:** Allows to specify the horizontal and vertical offset of subsequent *Sweeps* to be displayed in the 3D graphing mode (range 0.001 - 1.000). You can also enter 0 for one or both. Note that the *3D*-*Graph* feature has to be on for this option to take effect (see chapter 4.5 on page 31).

5.6.3 Show Options

The appearance of some windows can be configured using the following options:

Hide PGF-Bar in Control Window
 Hide Protocol-Bar in Control Window
 Show no Fixed Control Protocols

Hide PGF-Bar in Control Window: The complete PGF pool in the **Control** window can be hidden.

Hide Protocol-Bar in Control Window: The complete protocol pool in the Control window can be hidden.

Show Fixed Control Protocols: In case the user does not want to scroll through the protocol pool, a specified number of protocols (0, 7, 15, 23, 31 or 39) can be shown as buttons in the Control window.

5.7 I/O Control

Selected options in this section of the Configuration window will appear in the I/O Control window (see chapter 14 on page 145) and can be edited there.

Parameters such as e.g. Temperature, Gain, R-series, are used to customize the recording environment of a given experimental setup.

The checkbox on the left determines whether the parameter is displayed (and updated) in the I/O Control window. For each I/O parameter you have to enter a default value and a source.

Configuration: C:\P	rogram Files (x86)\	HEKA\2x90\Char\C	hartMaster			X
SAVE	SAVE	AS Defa	ult Windows	efault Settings		
General	Hardware	Files	Display	I/O Control	Trace Assign	Misc.
I/O Parameter scale AD-0 DA-0 User 1 V User 2 V Temperature Show Solutions	Default 1.000 1.000 0.000 V 0.000 V 20.00 C	Source Default Default Default Default	list ⊠ S cale ⊠ S ⊠ S 0 12 Nan	J Show Digital In Show Digital Out Show AD Controls Show AD Values ©⊠ØØØ 23 4 5 6 7 Show 4 Values ne of Value-1 Value	1	

 ${\bf Default:}$ The default values can be edited in this column.

Source: In this row one can determine how the value is obtained. For most of the parameters there are three alternatives:

- Default: The specified default value is taken.
- AD-Channels: Parameters are sampled via a specified AD-channel.

List : Pressing this button writes all scaling and offset values for the I/O parameter in the Notebook window. Use this feature for getting a quick overview on the scaling of all your AD- and DA-channels.

5.7.1 AD/DA Input/Output Scaling

In the I/O Control pane of the Configuration window you can define the scaling methods for the DA- and ADchannels. Please note that the I/O Control window always displays the raw (unscaled) voltage values of the channels. The scaling of DA- and AD-channels can be used for stimulus output and for data acquisition via the Pulse Generator.

In case of an AD-channel you can choose between a Scaling Factor and a Lookup table:

	X
Use Scaling factors or Lookup table?	
Scaling	Lookup table

For direct input of scaling factors, enter a Scale Factor and a Scale Offset:

1.000000
0.000000
Cancal

Example: A temperature controller has a measure range from $10 \,^{\circ}\text{C}$ to $50 \,^{\circ}\text{C}$ and a corresponding voltage output range of +/-10 V. The correlation between measured temperature and voltage output is linear (see figure below).



In this example, *Scaling Factor* should be set to "2" and *Scale Offset* should be set to "30". If the correlation between measured signal and voltage output is not linear then a *Lookup table* should be used. For input via a *Lookup table*, you have to choose the file from which the program will read the data.

Select existing Adc-0 Lookup Table:						
Coo V 📕 « Lokaler Datenträger (C:)	▶ Programme (x86) ▶ HEKA ▶ Ch	artMaster 🕨 LookupTables	•	 LookupTables durchsucher 	n 🔎	
Organisieren 🔻 Neuer Ordner				•	0	
☆ Favoriten	Name	Änderungsdatum	Тур	Größe		
🧮 Desktop	AdcScale	24.05.2005 11:54	Datei	1 KB		
🐌 Downloads	DacLookup	11.03.2009 10:32	Datei	1 KB		
🖫 RecentPlaces 😑						
	1					
詞 Bibliotheken						
Documents						
🚽 Music 🗕	1					
Pictures						
Subversion						
📑 Videos						
Lokaler Datenträger (C:)						
🖵 transfer (\\server2\public) (0:)						
🖙 Programme (\\server2) (P:)						
Dateiname:			•	All Files (*.*)	•	
				Abbre	cnen	

In case of a DA-channel you have in addition the the possibility to apply a scaling based on an equation. The equation has to be entered here in the Control window, whereas the scaling has to be activated in the Pulse Generator (see chapter 9.9.1 on page 88).

		×			
Scale Dac output using:					
Equation	Lookup	Scaling			

The options *Lookup* and *Scaling* work similar to the methods described for the AD-channel scaling. In case you choose *Equation* the following dialog appears:

Scaling Equation of Dac-0:	
	Results:
ampl*2+0.5	2.5000
Equation User Defined Select Equation Save Equation	Cancel

In the command field an equation can be entered, the calculated result is displayed in the Results field beneath.

Equation User Defined : A label for the equation can be entered in this field.

Select Equation : Already saved equations can be selected in this pop-up menu.

Save Equation : After defining a name for the entered equation it can be saved. After doing this the formula is available in the pop-up menu of *Select Equation*.

Note: The new equations is saved in a default Equations.txt. file. If you want to change the file or the file name for your equations you have to this via the Save button.

? : Prints a help text on the equation syntax to the Notebook.

LOAD : Brings up a file selector to open an equation file.

SAVE: Defines the place and the name of the file in which your equations are stored.

For a detailed description on the equation syntax please refer to the chapter Calculator and Equations, 18 on page 157.

As was mentioned earlier, *Lookup tables* for commonly used amplifiers are supplied. If your amplifier is not one of those provided, a *Lookup Table* can be created as an ASCII file.

Note: Have a look for our video tutorial "Using Telegraphing Inputs" to learn how to create your own Lookup table.

Note: It takes CPU time to read AD-channels and to convert the data to the desired values. In order to minimize handling overhead, only activate those parameter fields that are actually used.

5.7.2 List of I/O Parameters

					list
□ scale	AD-	0	1.000	Default	scale
	DA-	0	1.000		scale
User	1	۷	0.000 V	Default	
User 2	2	V	0.000 V	Default	
Tempe	rature		20.00 C	Default	
□ Show	Solutio	ns			

scale:

- AD-Channel: If data Traces from AD-channels are acquired in a PGF sequence, the voltages can be scaled to convert them to appropriate units. For scaling you can use a fixed value or Lookup tables (see chapter 5.7.1 on page 52). In case you have an EPC 8 or EVA 8 amplifier connected as second amplifier then use EPC 8 Gain to scale the AD-channel that samples the Current Monitor signal of the EPC 8. The corresponding EPC 8 has to be connected via the parallel digital I/O interface and the amplifier set to local mode. If you want to use external noise filters you should pick up the Filter 1 or Filter 2 signal of the EPC 10. After filtering your signal you have to select the corresponding AD-input channel (AD-0...). Furthermore you have to select the Epc 10 Gain option in the Source pop-up menu. Changes in the Gain adjustment of the active amplifier are now displayed in the Default box.
- DA-Channel: Free DA output channels that can be used for stimulation during a PGF sequence can be scaled with a factor entered in the *Value* field.

User 1, 2: There are two parameter fields reserved for user-specific assignments. These fields act like other parameter fields with the addition that parameter name and unit can be specified (e.g., "pH" and "U"). Name and unit are also stored in the output *.pul file on the level of each *Sweep*, so they can be changed during an experiment, if required.

Temperature: Temperature (from a recording device).

Show Solutions: If the checkbox is active you can enter/change the number of the external and internal solutions in the I/O Control window.

5.7.3 Show Digital In / Out

Show Digital In: If the checkbox is deselected the Digital Inputs in the I/O Control window will be hidden.

Show Digital Out: If the checkbox is deselected the Digital Outputs in the I/O Control window will be hidden.

Show DA Controls: If the checkbox is deselected the drag-able DA-channel fields in the I/O Control window will be hidden.

Show AD Values: If the checkbox is deselected the AD-channel display fields in the I/O Control window will be hidden.

Show AD Channels: Via these checkboxes the user can deactivate any AD Value field in the I/O Control window.

Show Values: You can specify how many values (4, 8, 12, 16, 20, 24, 28 or 32) should be listed in the I/O Control window.

Name of Value: A user defined name can be specified for each value (right text field) in order to make the parameter input and output more meaningful. A list of all value names pops up if you select the left box.



5.8 Trace Assign

Configuration: C:\Program Files (x86)\HEKA\2x90\Char\ChartMaster											
	SAVE S	AVE AS	Default Windows Default Settings								
General Hardward		e Files	Display	I/O Control	Trace Assign	Misc.					
Trace-1	AD-Channel	Adc-0]								
Trace-2	AD-Channel	Adc-1									
Trace-3	Digital	Dig-in 1									
Trace-4	Photometry	Photo_R	1								
Trace-5	Photometry	Photo_Ca									
Trace-6	Virtual	Virtual-5	ī								
Trace-7	Trace Count	Trace 7]								
Trace-8	Trace Count	Trace 8									
Trace-9	Trace Count	Trace 9]								
Trace-10	Trace Count	Trace 10	j								
Trace-11	Trace Count	Trace 11]								
Trace-12	Trace Count	Trace 12]								
Trace-13	Trace Count	Trace 13									
Trace-14	Trace Count	Trace 14									
Trace-15	Trace Count	Trace 15]								
Trace-16	Trace Count	Trace 16]								
	Undo	Reset									

The Trace Assignment function is very useful if your number of input and output channels of a PGF is increasing. This function allows you a defined labeling of each acquired Trace. E.g. if you define acquisition channels for current, voltage, ADC input, LockIn and Leak, they get labeled according to their origin, i.e. Imon-1, Vmon-1, Adc-0, Adc-1, LockIn_CM, LockIn_GM, LockIn_GS and Leak-8, instead of simple Trace counts (e.g. Trace 1...8). It is also possible to assign several Traces with the same input signal, so you can define e.g. three times Imon-1 but the compression rate for each channel can be set differently. The benefit comes with the consistent labeling of these Traces within CHARTMASTER (e.g. Analysis, Trace Properties, Oscilloscope etc.) which helps the user to keep the overview of the data.

In the Trace Assign dialog it is possible by default to assign labels for up to 16 Traces (Trace 1...16). If you increase the number of Max. Shown Traces in the General tab of the Configuration window this number will also increase.

For each *Trace Assignment* you have to select the type of channel:

Trace Count: Define a Trace number for the selected Trace:

• Trace 1...32

Amplifier: Define a current or voltage monitor for the selected *Trace*:

- V-mon
- I-mon

AD-Channel: Define an AD-channel for the selected *Trace*:

• Adc-0...15

	Trace Count				
	AD-Channel				
	Leak				
	Digital				
	Virtual				
	LockIn				
	Spectroscopy				
	Photometry				
	Imaging				

Leak: Define a leak channel for the selected Trace:

• Leak-1...32

Digital: Define a digital channel for the selected Trace:

- Dig-in(word)
- Dig-in 0...15

Virtual: Define a virtual channel for the selected Trace:

• Virtual-1...32

LockIn: Define a LockIn channel for the selected Trace. The following channels are available:

- LockIn_CM
- LockIn_GM
- $\bullet \ LockIn_GS$
- $LockIn_Real(Y)$
- $LockIn_Imag(Y)$
- $LockIn_DC$
- LockIn_Avg
- LockIn_CV
- LockIn_GP
- $LockIn_Admit(Y)$
- $\bullet \ LockIn_Phase$
- $LockIn_Real(Z)$
- $LockIn_Imag(Z)$
- $LockIn_IMP(Z)$

Spectroscopy: Define a spectroscopy channel for the selected Trace. The following channels are available:

- Chirp_Avg
- Chirp_Phase
- $Chirp_Admit(Y)$
- $Chirp_Real(Y)$
- $Chirp_Imag(Y)$
- $Chirp_Real(Z)$
- $Chirp_Imag(Z)$
- $Chirp_Imp(Z)$

Photometry: Define a photometry channel for the selected Trace. The following channels are available:

- Photo_W1
- $Photo_W2$
- Photo_W3
- $Photo_R$
- Photo_Ca

Imaging: Define an imaging channel for the selected Trace. The following set of channels is available for six times:

- Image_W1
- Image_W2
- Image_W3
- Image_R
- Image_Ca

S.W.Voltammetry: Define a square wave voltammetry channel for the selected Trace. The following channels are available:

- I-forward
- I-reverse
- I-diff
- V-mean



Undo: The last change will be reset.

Reset : Resets all *Trace* assignments to the default value.

5.9 Miscellaneous

Configuration: C:\Program Files (x86)\HEKA\2x90\Char\ChartMaster											
SAVE	SAVE A	S Defau	It Windows	efault Settings							
General	Hardware	Files	Display	I/O Control	Trace Assign	Misc.					
Analysis: Sweeps Analysis: Mode Analysis: Max.Sw Replay Scroll Rate ⊠ Analysis: Com □ Clear Commen	Results 1024 Fix p.Redrawn 10 100. press Vectors t	32 eed ms									

Analysis: Sweeps | Results: The Analysis needs to allocate memory for storage of its results. The Analysis Result buffer is a 2-dimensional buffer (table).

- Analysis: Sweeps: Maximum Analysis results per Analysis function (default = 1024)
- Analysis: Results: Maximum Analysis functions (default = 36)

For example if you use the Analysis for chart like recording during your experiment. The experiment lasts for about 10 minutes and you analyze one set of *Analysis: Results* per second then the online buffer has space for 600 *Analysis: Sweeps.*

Note: The Analysis buffer is cleared after each Wipe.

Important note: In case you select more Analysis functions in your Analysis Method than specified here, then all results exceeding this number will not be computed, hence not displayed!

Analysis Mode: Handling of Analysis: Sweeps | Results:

- Fixed: The number of *Analysis: Sweeps* | *Results* given above is fixed, i.e. limited to that number. For long chart like recordings and many *Analysis* results the number of *Sweeps* and results might have to be set to such high values that available memory become a problem.
- Extend: The number of *Analysis: Sweeps* | *Results* given above will be extended, if more data are acquired. With this mode selected the system might run into trouble when no further free memory is available.

Analysis | **Max.Swp.Redraw:** Defines the maximum number of *Sweeps* which can be displayed in an Analysis window.

Replay Scroll Rate: Defines the time interval when scrolling through the Replay tree.

Analysis: Compress Vectors: If this checkbox is selected, the data of the Analysis results get compressed to increase the drawing speed of the graphs in the Analysis Windows.

Clear Comment: Clears the Comment field in the Control window after the Comment is stored with the data.

6. Oscilloscope Window



The Oscilloscope window is mainly used for monitoring the data. The *Traces* of a *Sweep* are displayed versus the time. Controls for display scaling and data handling are provided. The title of the window contains the currently active data file name.

6.1 Display

Zooming – **Lassoing:** It is possible to set the display scaling in the Oscilloscope window by "lassoing" a screen region while pressing the left mouse button. When you release the mouse button, the marked area will be set to fill the Oscilloscope screen. The scaling has to be done for each *Trace* separately.

If you want to display data that is outside the active screen area, you can either enter some values into Y-scale or Start/End Time or drag the mouse *outside* the active screen area. The display gains gets reduced by 20% as long as the mouse is outside, and stops when it moves back on the active display area. When the mouse is below or above the screen, the Y-scale is changed, and when the mouse is to the left or right of the screen, the X-scale is changed.

Cursor shapes: To accommodate different user preferences there are multiple cursor shapes available. Press the CTRL key while the "cross" cursor is displayed and you can cycle through all available cursor shapes.

6.2 Navigation



Group_Series_Sweep of Sweep: Currently active *Group*, *Series* and *Sweep* number within the data tree. The total number of *Sweeps* per *Series* is given after "of". The numbers are updated with every start of a new *Sweep* acquisition. During data replay these information are replaced by the information of the replayed data. Note that this can interfere with the above mentioned updating process if data are replayed in the wait time between data acquisition cycles.

6.3 Measurements on Traces

[Measure : Allows to measure amplitudes and duration. With this option the user can measure the displayed data by pointing and dragging the mouse or by clicking on $\leq \geq$ and \vee \wedge . Two mouse-driven horizontal lines are provided to measure signal differences. By clicking on the button **To Notebook**, the measurement data is written to the **Notebook** window.

Scan: Allows to measure signal amplitudes by scanning the individual data points. When selected, a marker is displayed on the *Trace*. This marker can be moved forward and backward by dragging with the mouse or by clicking on and and . By clicking on the button notebook, the measurement data are written to the Notebook window.

Note: The functions "Measure" and "Scan" work on the selected Trace in the **Replay** window. If anything other than a Trace is selected in the **Replay** window, then the functions work on the Trace that is selected in the **Oscilloscope** window (right panel).

6.4 Display Refreshing

Freeze: Freezes the present content of the Oscilloscope. Any change in display parameters, such as scaling etc. will unfreeze the display again, as does a *Wipe* or switching to the Amplifier window.

(Wipe) : Clears the Oscilloscope completely.

Repaint: Clears the Oscilloscope first, then redraws the last displayed Trace. This is useful e.g., if you had the Overlay Sweep option activated, but then decided that you only want to see the last Sweep of the Series.

6.5 Overlay Options

[Overl.Swp] : Displays all Sweeps of a Series without erasing the screen in between Sweeps. The next Series, however, will erase the screen.

Overl.Ser: Displays all incoming Sweeps without erasing the screen. This allows Sweeps of different Series to be computed. The screen can be wiped by pressing BACKSPACE.
6.6 Display Scaling

Trace Color: Displays the color of the selected Trace.

Trace 1...16 (default): Selects the *Trace* to which the scaling setting applies. The color of the selected *Trace* is displayed above the **Trace** control.

Note: When using the Trace Assignment function (see chapter 5.8 on page 56) the names of the Traces may be different.

Dig. Filter: Shows the currently selected bandwidth of a digital non-lagging Gaussian low-pass filter (i.e. software filter). The -3dB cutoff frequency is specified in Hertz. This allows to reduce the original bandwidth by the factor 0.0025 (1/400). It is for display purposes only; no changes to the data are performed.

Y-scale: For each *Trace* you can control the display scaling. The value of 1 corresponds to full scale of \pm 10.24 V. This scaling does not affect the display of *Test Pulses*. Pressing + from the numeric keypad (take care not to have NUMLOCK activated!) increases the display scaling by a factor of 2 and - decreases it by a factor of 2. It is also possible to auto-adjust (*Auto Y-scale*) or fix the Y-scale (*Fixed Y-scale*) of the Oscilloscope. The reset button (B) sets the *Y-scale* to "1". For further information we refer to chapter 4.5 on page 31.

Note: This display scaling does not affect the display of the Test Pulse Current Trace. If you want to be able to scale the Test Pulse as well, activate the option Scale Test Pulse in the **Configuration** window and use I-scale in the **Amplifier** dialog. We advise against activating that option because one can easily overlook that the amplifier Gain is not correctly set, when the current Trace is scaled by the display scaling.

 ${\bf R}{:}$ Resets the Y-scale to the default values.

Y-offs.: For each Trace you can control the offset of the zero line. Offsets of Traces may be between -1 and 1 relative to full scale of display (default = 0). SHIFT + and SHIFT - (from the numeric keypad) increase and decrease the display offset by 0.1.

Solution: Clicking on the Center button automatically centers the respective Trace on the screen.

Auto Swp: Pressing the Auto Swp button calculates the maximum Y-scaling factors (Y-scale, Y-offs.) to display the selected Trace of a Sweep as large as possible in the Oscilloscope. The basis for the calculation are the Y-min and Y-max values of the selected Trace of the Sweep (see chapter 12.1.5 on page 140).

Auto Ser: Pressing the Auto Ser button calculates the maximum Y-scaling factors (Y-scale, Y-offs.) to display the selected Traces of a Series as large as possible in the Oscilloscope. The basis for the calculation are the Y-min and Y-max values of the Sweep Traces of the Series (see chapter 12.1.5 on page 140).

Start Time / End: Section of the Sweep to be shown on the screen in % (Start - End).

R: The Reset button sets the full Sweep length (0 to 100 %).

Note: The full time scale provided for Sweep display is based on the longest Sweep within a Series. Alternatively, one can use the Fixed Scale option (see below).

Page: Page of display during replay of continuous data *Sweeps* or when time axis is chosen to be less than 100%. Clicking on the right/left arrow control will display the next/previous page of the current *Sweep*. Dragging the page number scrolls the data forward or backward; entering a page number will display that particular page. Page is highlighted whenever there is more than one page available.

R: The *Reset* button sets the page number to "1.0".



6.7 Multi-Channels

If the *Multi-Channel* option is activated in the Configuration (see chapter 5.4.7 on page 46) the *Traces* are displayed in separate, clipped viewports, one beneath the other. All viewports share the same scaling in the Oscilloscope. The figure below shows an example for data recorded with two joined EPC 10 Quadro and *Multi-Channels* set to "8".

Amplifier 1 Amplifier 2 Amplifier 3 Amplifier 4 Amplifier 4 Amplifier 4 Amplifier 4 Amplifier 1 Overl.	Ser) ilter
Amplifier 2 Amplifier 3 Amplifier 4 Amplifier 4	Ser ilter R Ser
Amplifier 2 Amplifier 3 Amplifier 4 Amplifier 4 Dig. F OT Y-sca 10. Y-offs 0.0 Auto Start 0.0	
Amplifier 2	
Amplifier 3 Amplifier 4 Amplifier 4	<mark>ଖିଞ୍ଚ</mark> ା _ (ମ ଁ ଅ
Amplifier 3 10. Y-offs 0.0 Amplifier 4 X	
Amplifier 3 Amplifier 4 Amplif	Swp
Amplifier 3	Ser
Auto Auto Amplifier 4	Ser.
Amplifier 4	Ser
Amplifier 4	-
	Ime
	//
	R
	70
Amplifier 5	
	<u> </u>
Amplifier 6	
Amplifier 8	
X: 5.00 ms Y: 1.00 nA	

Note: When using Multi-Channels only 1 AD channel (Trace) per amplifier is allowed.

7. Control Window

Control Window		
idle	17:15:28	00:13:48 Set Store Break Stop Next Wait (Resume)
Comment		Average 1
PGF 🚺 👌 1	IV 2⊂ F	Ramp 3 (Continuous) 4 Hinf 5 Tails 6 (TestSeries) 1 🗘 🗘
Protocol 🚺 🗘 1	Exampl1 2(Ex	ample2) 3 Link 4 Buffer 5 SETUP 6 SEAL 1 🗘 🗘
Exampl1 Exa	mple2 Link	Buffer SETUP SEAL (WHOLE-CELL) Assign

From this window, protocols or Pulse Generator sequences are started or stopped. It allows comments to be entered and it provides timing information. The number of buttons for executing protocols can be set in the Display section of the Configuration dialog (see chapter 5.6.3 on page 51).

7.1 Information about the Experiment

State: Current state of the experiment. All possible states are listed below:

- "aq: waiting": CHARTMASTER is waiting for the user to click on the *Resume* button during an acquisition (because the *Wait* button was activated by the user).
- "acquiring": Acquiring data in the Interactive mode.
- "ex: waiting": CHARTMASTER is waiting for the user to click on the *Resume* button during a protocol (because of a *Wait* event with the wait type "Resume Icon" or "wait for key").
- "gap-free": Acquiring data in the Gap-free mode.
- "idle": All else.
- "locked": User is locked out (PATCHMASTERPRO only).
- "noise": Measures noise.
- "recording": Recording a macro.
- "run macro": Runs a macro (may be part of a protocol).
- "run protocol": Runs a protocol.
- "test": Test Pulse is running.
- "test (demo)": Test Pulse in Demo mode.

Time: Actual system time measured since midnight.

Timer: This item functions as a stopwatch or timer called *TimerTime*. It can be reset at any time by clicking on the *Set* item or by pressing T. It may be useful to keep track of the experiment (e.g., to monitor the time spent in a specific experiment mode). The *TimerTime* is updated also during the *Series* execution. The timer value is stored at the beginning of the *Sweep* acquisition. The internal *Timer* tick corresponds to 1 ms. When you click on *Set*, the *Timer* will be reset to zero and started again.

Comment: Comment to the currently active Series. This field can be edited. It will result in a modification of the text of the present Series if a file was opened with write permission. This means that one has to enter a text to a Series after it has been acquired. The new comment will be copied into all incoming new Series until a new text is entered.

7.2 Controlling Data Acquisition



Store: Store is used to save the data that will be acquired from now on. A dialog window opens and you have to enter the name of the new data file.

In case Store is not activated but the file is opened with write permission, the last Sweep will be stored temporarily. This Sweep is marked with a "#" in its label. It will be deleted when:

- the next Sweep is acquired
- or a new *Group* is created or
- the file is closed.

Break: Break is used to stop *Series* acquisition or replay. The *Break* flag is reset, when the next target is displayed or executed. If *Break* is pressed during acquisition of a *Sweep*, this particular *Sweep* will not be completed and the data acquired for this *Sweep* thus far will be discarded. All previous *Sweeps* of the *Series* will be saved; thus, there can be a *Series* with fewer *Sweeps* than specified in the Pulse Generator. The *Break* button can also be used in *Replay* mode when performing a lengthy operation.

Stop: This button is used similarly to *Break*. However, during acquisition of a *Sweep*, this particular *Sweep* will be completed. The *Stop* button can also be used in *Replay* mode when performing a lengthy operation.

Next: At the end of the actually acquired Sweep the Series is stopped and the next protocol event will be executed.

Wait: This button is used to pause *Series* execution. Its command key is W. Series execution is resumed by a click on *Resume* or by R. Wait will become effective after completion of the currently acquired *Sweep*. The Wait button can also be used in **Replay** mode when performing a lengthy operation; e.g., it allows inspecting a particular *Sweep*, when replaying a collection of *Sweeps*.

Resume: Opposite of Wait. Press Resume to continue data acquisition or replay.

Average: By use of this function, a PGF sequence can be repeated several times. Only the average is stored to disk. Please enter the number of averages acquired for one Sweep.

Note: The Average function uses the Trace buffers for temporary storage. Thus, the average is limited to 4 Traces per Sweep. Also, it will overwrite whatever is in the Trace buffers at time of acquisition.

7.3 Starting Pulse Generator Sequence



If you have not activated the option *Hide PGF-Bar in Control Window* on the Display tab of the Configuration (see chapter 5.6.3 on page 51) then you will see a row with buttons listing the various sequences of the loaded Pulse Generator file (*.pgf). Six buttons are shown at a time. But the row of buttons can be scrolled using the arrow buttons on the left and right side.

- A single click on one of those buttons will start the respective Pulse Generator sequence.
- By entering a number into the field to the right of the button row, the sequence with this index is started.

7.4 Starting a Protocol

If you have not activated the option *Hide Protocol-Bar in Control Window* on the Display tab of the Configuration (see chapter 5.6.3 on page 51) then you will see a scroll-able row with buttons listing the various protocols of the loaded protocol file (*.pro). Six buttons are shown at a time. But the row of buttons can be scrolled using the arrow buttons on the left and right side. If you have activated the option *Show fixed Protocols in Control Window* on the Display tab of the Configuration (see chapter 5.6.3 on page 51) then you will see a buttons listing the various protocols of the loaded protocol file (*.pro). A single click on one of those buttons will start the respective *Protocol*.

- A single click on one button in the scroll bar will start the respective protocol.
- By entering a number into the field to the right of the button row, the protocol with this index is started.
- A single click on one button in the row of the fixed protocols will start the respective protocol .

7.5 Fixed Control Protocols

If you have activated the option Show X Fixed Control Protocols in the Configuration window (see chapter 5.6.3 on page 51) there will be a certain number of buttons available below the protocol bar.

Exampl1 Example2	Link Buffer) SETUP SEAL	WHOLE-CELL)
			Assign

The position of the protocols is fixed. The order can only be changed when using the Assign button. In the upcoming dialog the user is asked to enter the name of an existing protocol and the position to which button it should be moved within the Fixed Control Protocols.

8. Replay Window



If data has been acquired in the *Store* mode or if an old data file has been opened, they can be reviewed and edited in the **Replay** window. To open it select $Windows \rightarrow Replay$. Up to five levels of the data tree are displayed.

8.1 Main window functions

Label show Root to Trace tracing Series visible Trace apply Text

Text: Displays the comment to the target (either *Root*, *Group* or *Series*). Use this function to edit the comment. The entered text can be seen in the field *Text* of the tab for *Root* and *Group* or in the field *Comment* of the tab for *Series* in the Parameter window.

Note: A comment for a Series can be written directly in the field Comment of the Oscilloscope window.

Label: Displays the target label (Groups/Series/Sweep/Trace). All objects have default labels assigned:

- Group: E-1
- Series: Name of Pulse Generator sequence
- Sweep: No label
- Trace: Name of acquired AD channel or assigned Trace name of the Pulse Generator

You may use the Label function to edit the label.

When creating a new *Experiment* or a new *Group* with the *File Operation* event or acquiring a *Series* with the *Acquire* event in the Protocol Editor, then you can specify a custom label.

show / to: Defines which part of the data tree will be displayed. The highlighted part of the data tree is referred to as target throughout this manual. Available parts of the data tree are *Root*, *Group*, *Series*, *Sweep*, and *Trace*.

tracing: Specify the part of the data tree that will be traced during replay. This feature might be of importance if the **Replay** window is very narrow (allowing to display three levels of the tree only) and many tree entries exit. Setting the *Tracing* item to *Series* then the active items during replay are the series objects and still *Root*, *Group* and *Series* are shown in the **Replay** window. If you would set the *Tracing* item to *Trace* keeping the same narrow **Replay** window, then the whole tree would be scrolled to the right and only *Series*, *Sweep* and *Trace* are shown in the **Replay** window.

visible/Apply: This feature can be used to expand or minimize the whole tree. If we specify *Series* and click *Apply* then the tree is reduced to the level of the *Series*, all *Sweep* and *Trace* objects are hidden.

Show Mark Unmark 🔽 🕨 (Mark All) repeat rate [0.100 s]

Show: Replay the selected target. Alternatively, you can perform a double-click on the target.

Mark: Mark the selected target. You can mark more than one entry. Several functions in the Replay menu apply for the marked entries (see chapter 4.4 on page 26). Note that set marks will be written into the *.pul file of the data when you save the data. That means that marks will also be reloaded when you open such a marked data file.

Unmark: Unmark the selected marked target.

Note: You can also quickly mark or unmark your targets by selecting them and pressing 'M' or 'U', respectively.

Show/Hide Arrows: These buttons can be used to expand and close the child tree.

Mark All: Mark all targets in the selected data range with the named label.

repeat rate: Enter a time interval for controlling the scroll speed when keeping a scroll button (arrows) pressed. Every time value between 0.01 and 1 s is possible.

 $(\Rightarrow |\frac{\overleftarrow{a}}{\diamond}, \frac{\overleftarrow{b}}{\diamond})$ Window Scroll Arrows: The arrow buttons on the window frame can be used to scroll/jump through the data tree.

8.1.1 Tree Handling

Interpreting the Tree objects: Besides a running index, text is displayed in the icons of the tree entries. The displayed text is the *Label* of the object. Usually the *Root* icon contains the file name, the *Group* icon contains the experiment number, and the *Series* icon shows the name of the *Stimulation Sequence*. The *Sweep* icon holds the *Sweep* index and the *Trace* icon the *Trace* name.

Example: In the screenshot of the Replay window below you can see the following entries in the first line:

- File name: Demo
- Group name: HEK; The group "HEK" is the first group of the file Demo.
- Series name: Lock 1; The Series Lock is the first Series of the Group.
- Sweep name: 1; the Sweep carries no name or label. It is the first Sweep of the Series.
- Trace names: Imon-1, CM 2



Labels starting with a "#" (Series "6") denote a currently acquired object that will not be stored after acquisition.

Maneuvering through the Tree: The cursor keys (LEFT, RIGHT, UP and DOWN) of the keyboard allow you to scroll through the data structure of the tree. PAGE UP and PAGE DOWN can be used to scroll one window up or down. HOME and END will move to the start or end of the tree.

Selecting/Marking multiple objects: An example: For analysis purposes you would Mark All Lock

like to export all data obtained with Lock protocols for a given cell. Thus, enter Lock in the name field, select the Group for the cell under consideration, and click on Mark All. Then all Series named Lock of this group can be exported with Replay \rightarrow Export Marked Target Children.

Mark a Target and its Children:

SHIFT+CLICK = Marks Target and Children between two selections.

STRG+CLICK = Unmark a specific Target Children

9. Pulse Generator Window

🔤 Pulse Generator File: DefPgfChart 📃 📼
Full View Condensed View Cartoon View
Pool LOAD (MERGE) SAVE Name 2-Pulsed LIST COPY MOVE UNDO DELETE
Interactive Mode O Gap Free Mode
Timing No wait before 1. Sweep Not Triggered Checking EXECUTE
No of Sweeps 1000 Use Durations Sweep Length Total 10.00 ms 200 pts
Sample Interval 50.0 µs (20.0kHz StartTime 0.000 Channel Length Stimulus 10.00 ms 200 pts
1 DA Unit Stimulus -> DA AD Unit Link Compr. Points Store Zero
Ch-1 DA-0 V absolute voltage AD-0 V 1 1 C 200 Ø 0
Ch-Z off V absolute voltage AD-1 V 1 1 C 200 ⊠ 0
✓ ✓ </td
Segments 0 0 1 Stored 2 Not Store 3 Constant 4 Constant 5 Constant 6 C 0 0 0 Common Timing
Segment Class Constant Constan
Voltage [V] val 0.000 val val val val val val Analysis: (Edit)
V-incr. Mode Increase Increase Increase Increase Increase Rel X-seg 1
V-fact./incr. [V] 1.00 0.000 Rel Y-seg 1
t-fact /increase Increase Increase Increase Increase Increase
Draw: Active Channel, all Sweeps) Delay: DA 0.00 s AD 0.00 s Voltage IVI (display)
0.000 Set Last Seg. Amplitude
100.004
p1 p2 p3 p4 p5 p6 p7 p8 p9 p10 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000
Traces 2

The Pulse Generator window defines the layout of stimulation pulse patterns. On the level of stimulation, they are called "sequence" throughout this manual. The result of a sequence stimulation, i.e. the families of sampled data *Traces*, are called a *Series*. Entries that are not default (e.g. adjustable parameters) are highlighted in bright background colors (*active* parameters), the rest is shown in dim background colors (*inactive* parameters). Because of this automatic feature, the color of individual editable controls cannot be changed in this dialog. All other controls can be modified.

9.1 Different Views of the Pulse Generator

There a three different views available for the Pulse Generator window.

Full View: Shows the complete Pulse Generator window (see figure above).

Note: You can use the "Copy to Clipboard" function to copy the text listing of the PGF setting somewhere else.

Condensed View: The condensed view provides access to the most important functions of a simple experiment. In case segment parameters have to be changed frequently, then the Pulse Generator window may stay opened during the entire experiment with minimal space requirement.

📃 Pulse Generator Fil	e: DefPgfChar	t					
Full View	Conder	nsed View	Cartoon	View)		
4 1 2-Pulse	ed 2 (2-Co	ontinuous) 3		4	5	6	
Segments 👌 👌	1 Stored	2 Not Store	3	4	5 000000		
Segment Class	Constant	Constant	Constant	Consta	ant Constant	Constant	
Voltage [V]	val 0.000	val	val	val -	val	val	Analysis: (Edit)
Duration [s]	val 10.00m	val	val	val -	val	val	
V-incr. Mode	Increase	Increase	Increase	Increa	se Increase	Increase	
V-fact./incr. [V]	1.00 0.000						
t-Incr. Mode	Increase	Increase	Increase	Increa	se Increase	Increase	
t-fact./incr.	1.00 0.00						
					No of Swoons	1000	
					No of Sweeps	1000	
					Sample Interval	50.0 µs (20.0)kHz
						EVECUTE	
100						EXECUTE	
100.μν1							
1.00ms							

Cartoon View: Shows only the graphical representation of the selected Pulse Generator sequence.

Note: If you use the "Copy to Clipboard" function when the Cartoon View pane is active, the stimulus cartoon is copied instead of the text listing of the PGF settings.

Pulse Generator File:	DefPgfChart	
Full View	Condensed View	Cartoon View
100.μV		
1.00ms		

9.2 Sequence Selector

001	IV 2	Ramp) 3(Continuous) 4 (Hinf) 5 (Tails) 6 (TestSeries		l
-----	------	------	------	------------	-------	------	-------	-------	-------	------------	--	---

Sequence Buttons: The first row displays a section of the pool of available sequences. It is a paging bar in units of six sequences. Two arrows at each side allow scrolling through the available pulse protocols (the innermost arrows move in increments of one page, i.e., six sequences; the outermost arrows move to the start/end of the sequence list). A sequence is selected by clicking on it.

The pool of sequences is read from a file, usually the default file DefPgf_v9.pgf.

Note: If no Pulse Generator file is available, CHARTMASTER creates the default file. This file only contains one stimulation sequence, called "Test". This sequence can also be edited.

More sequences are added to the pool by copying an already existing sequence (*Copy* button, see below) or by selecting a free position in the pool.

9.3 Sequence Pool Handling

LOAD: Loads the pool of available stimulation sequences (*.pgf file). The present file name is indicated in the title bar of the Pulse Generator window, e.g., "Pulse Generator File: DefPgf_v9".

MERGE: Loads individual stimulation sequences from another (.pgf file) into the currently opened file. In case a sequence name is not unique, you are prompted to either skip this sequence, mark it invalid, or give it a new name.

SAVE: Saves the pool of available stimulation sequences. After modification of the existing pool of sequences, the entire Pulse Generator File (PGF) should be saved to disk (*Save* button). It can be saved under any name. CHARTMASTER automatically appends the extension ***.pgf** to the file name. If this new PGF file should be loaded into the Pulse Generator as a default, the new name of the PGF file has to be specified in the Configuration window and the configuration file has to be saved.

Name: Editable name of the present sequence.

LIST: Writes the settings of the actual *Series* into the **Notebook** window. Use this option, if you want to create a listing of your sequence to be able to recreate it on another machine. A PGF listing could look as follows:

```
PGF-stimulus: pulse, Sequence: 4 - polarogr
TIMING:
NumberSweeps: 1, Averages: 0, SweepInterval: 0.000 s,
SampleInterval: 10.00 us
Wait before 1. sweep: FALSE
CHANNEL: 1, channel 1 time.
AD-Channel [A]: AD-6, Comp.Factor: 1, Comp.Mode: 00000000,
CompSkip: 0
Rel.DacChannel: 1, Rel.Segments - X: 2, Y: 2, Write: TRUE,
Holding: 0.000 V
LEAK SUBTRACTION:
Rel.DacChannel: 1, Rel.Segments - X: 2, Y: 2, Write: TRUE,
Holding: 0.000 V no leak pulses
DA-Channel [V]: DA-3, use StimScalerelative to Vmemb
Amplifier mode: VoltageClamp, Set last Seg. Ampl.: FALSE
SEGMENTS: Voltage Duration VFact VIncr TFact Tincr
```

COPY: Duplicates the actual sequence into the first free position. A new name has to be entered.

Note: Copy is one way of creating a new sequence. Another way is to activate an unassigned sequence button and name the new sequence in the prompted window. The pulse pattern can be edited in the Segments section of the **Pulse Generator** window.

MOVE: Moves the sequence to a new position. A number for the new position has to be entered. Use this option to move the most commonly used sequences to one of the handy first six positions, or to rearrange your pool.

UNDO: Discards all changes that had been carried out since the last opening of the Pulse Generator window within one PGF sequence.

Note: When closing the window, all changes will be saved temporarily for this session. When closing CHARTMASTER, you will be asked once more if you want to save the PGF pool, if it was changed but not saved.

DELETE: Removes the present sequence from the pool.

9.4 Acquisition Modes

Interactive Mode: In the *Interactive Mode* there is a minimum waiting period between the recording of single Sweeps, the so-called "Sweep Gap". The size of

Interactive Mode O Gap Free Mode

the gap depends on the speed of your computer system and the complexity and duration of the stimulus to be calculated and output. If you need to do any modification (e.g. *C-slow*, recording modes...) during the acquisition which effects the *Sweep* recording you have to select the *Interactive Mode*.

Gap Free Mode: The *Gap Free Mode* allows a timely precise acquisition of *Sweeps* without any gap in between. If you do not want to modify the recording settings during acquisition we highly recommend to select the *Gap Free Mode*.

9.5 Timing

Timing:

- Wait before 1. Sweep: Forces CHARTMASTER to wait the time indicated by Sweep Interval before executing the sequence after activating it.
- No Wait before 1. Sweep: If you want the sequence to start immediately without a delay.

Timing No w	ait before 1. Sweep	Not Trig	gered
No of Sweeps	1	Use Dura	ations
Sweep Interval	0.00 s	StartSeg	0
Sample Interval	50.0 µs (20.0kHz	StartTime	0.00

• Allow Continuous Redraw: Check this option to allow continuous display of the data in the Oscilloscope during acquisition. Minimum required *Sweep* length is 1 second to activate this function.

No. of Sweeps: Determines the number of Sweeps within one sequence, e.g., how many times the specified pulse pattern is run.

Sweep Interval: The first *Sweep* of a sequence has a waiting period during which the stimulation template is computed and loaded to the AD/DA interface. Then the first *Sweep* is acquired. The next *Sweep* will start *Sweep Interval* seconds after the beginning of the first *Sweep*.

Sample Interval: The timing of data acquisition is given as Sample Interval (in seconds) and as sampling frequency (in Hz). The shortest Sample Interval (in case only one channel is used) is 5 μ s (200 kHz); the longest interval is 1 s (1 Hz). Only the Sample Interval has to be entered, the sampling frequency is just displayed as a reference.

Note: If the Sample Interval is so long as to yield only one point per segment, CHARTMASTER asks whether the segment duration should be fixed, e.g., set to a higher value.

Trigger Mode: Determines if and how data acquisition is triggered by an external TTL pulse. The default is *Not Triggered*, which means that stimulation is immediately elicited by the user within CHARTMASTER. Otherwise you have to activate the sequence and then one or more external triggers have to be applied.

- Not Triggered: No external triggering.
- Trigger Series: One trigger at the start of a sequence.
- Trigger Sweeps: One trigger at the start of a Sweep.

Use Durations / Use Scan Rates:

Segments 👌	⊠St	ore 1	⊠S	tore 2	⊠S	tore 3
Segment Class	Co	nstant		Ramp	C	onstant
Voltage [mV]	hold	V-memb	val	50	holo	V-memb
Duration [ms]	val	50.00	val	800.00	val	50.00
V-incr. Mode	Inc	crease	In	crease	In	crease
V-fact./incr. [mV]	1.00	0 0	1.0	0 0	1.0	0 0
t-incr. Mode	Inc	crease	In	crease	In	crease
Scan-fact./incr.	1.00	0.00	1.0	0 0.000	1.0	0 0.00
Scan Rate [V/s]	1		6	2.50m		

Ramp segments can be specified either by their duration or by their Scan Rate. If the Scan Rate is given by the user, the duration will be calculated, and vice versa. The desired mode can be selected from the list:

- Use Durations: The time between the ending and beginning of a *Ramp* is set in seconds in the *Duration* field of the sequence.
- Use Scan Rates: The Scan Rate is set in 'V/s' in the Scan Rate field of the sequence and is calculated as Voltage/Duration.

Start Seg: Sets the start segment.

Start Time: Sets the start time within the start segment.

Important note: Start Seg and Start Time determine the start from where acquired data are stored. The full stimulation template is always output.

9.6 Check and Execute

Not Checking / Checking: This option determines whether a check is performed for any inconsistencies that might occur when entering values.

NOT Checking: When Not Checking is enabled, the validity checking of the sequence editing is suspended. This is convenient when one wants to perform multiple changes, especially when some intermediate steps would result in a (temporarily) faulty sequence.

Checking: When *Checking* is enabled, the active sequence is checked after any modification of segments and when storing, switching, or leaving the PGF editor. If the input is faulty, the user is notified and the last operation is canceled, until the sequence is valid. The checking should be done at least at the end of the sequence input, before executing or storing a sequence.

EXECUTE: Allows to output the presently active stimulation sequence. Upon termination of data acquisition, the program returns to the Pulse Generator window. In this way, pulse patterns can be adjusted interactively without changing windows until they yield the desired responses.

9.7 Sweep and Channel Length

Sweep Length: Is the duration of the *Sweep*. It is given by the longest *Trace* (stored or not stored) in a *Sweep*.

Shows the length of the executed *Sweep* and stored data as determined by the timing, compression and segments settings.

• Total: Denotes the total duration of the longest Trace of the Sweep in 'ms' and 'points'.

Note: If the total Sweep length exceeds Max. Sample Points (see chapter Memory Allocation, 5.3.3 on page 43) a warning appears. If you would like to acquire longer Sweeps or a Sweep at higher time resolution, you might have to increase the Max. Sample Points parameter in the Configuration window.

• Stored: Denotes the total duration stored of one Sweep of the given sequence in 'ms'. The Sweep size is given in 'bytes'.

Note: All Traces contribute to the Sweep size but not to the Sweep Length. Total and Stored duration may be different when a Start Seg and Start Time are set.

Channel Length: Length of the actual DA stimulation. This can be shorter than the *Sweep* length, e.g., a short trigger impulse.

• Stimulus: Denotes the duration of the selected stimulus signal in 'ms' and 'points'.

9.8 Wave Parameters

Depending on the Segment type (see chapter Segment Classes, 9.10.1 on page 92) and the $Stim \rightarrow DA$ setting (see chapter 9.9.1 on page 88) different wave parameter buttons will appear.

9.8.1 Sine Wave Parameters

Sine Wave For segments of the Sine type. This button will appear between the buttons Checking and Execute and allows the specification of the wave characteristics.

9.8.1.1 Use as Simple Sinewave: Common Frequency

Use this setting if all wave segments within a Sweep should have the same frequency.

Peak Ampl. [mV]	10.0	value
Requested Freq.	100.0 Hz	
Actual Frequency	100.0 Hz	
Points / Cycle	500	

Peak Ampl. [mV]: Defines the size of the Peak Amplitude. The amplitude is half of the peak-to-peak amplitude.

Sweep Length	Total	30.00 ms	1500 pts
	Stored	25.00 ms	5000 bytes
Channel Length	Stimulus	30.00 ms	1500 pts

Value or parameter: If *Value* is selected the amplitude of the sine wave can be entered in the field *Peak Ampl.*. Alternatively, the *Peak Ampl.* can be determined by a *PGF parameter*. This allows to automatically change the *Peak Ampl.* of the sine wave between different series acquisitions.

Requested Freq.: Desired frequency of the sine wave in Hertz (Hz). Once the Wave Parameters window is closed, the *Requested Freq.* is set to the *Actual Frequency*.

Actual Frequency: Only certain frequencies are possible because they are generated by dividing a fundamental clock frequency by an integer. Nevertheless, the *Actual Frequency* is usually within a couple of percent of the *Requested Freq.*. The *Actual Frequency* is a function of the *Requested Freq.*.

Points / **Cycle:** Number of sample points per full wave length. This number is calculated and can not be edited. It is a display value only and gives the number of points per sinus wave which is the cycle length divided by the sample interval.

Below a sine wave created with a Peak Ampl. of 10 mV and a Actual Frequency of 100 Hz is shown.



9.8.1.2 Use as Simple Sinewave: Separate Frequency

Use this setting if the frequency of the wave is determined by the segment duration. Each segment will contain one wave cycle only. This option allows to create a *Sweep* containing wave cycles of different frequencies.

Use as Simple Si	newave: sepa	rate frequency
eak Ampl. [mV]	10.0	value
		Cancel

All available settings have been explained before (see chapter 9.8.1.1 on the preceding page).

9.8.1.3 Use as LockIn Sinewave

Use this setting if the sine wave should be analyzed with the software LockIn.

Use as	s Lockln Sinew	ave
Use Th	eoretical Stim	ulus
Peak Ampl. [mV]	10.0	value
Requested Freq.	100.0 Hz	
Actual Frequency	100.0 Hz	
Points / Cycle	200	
Cycles to Skip	0	Checking
Cycles to Average	1	
Total Cycles	1	Cancel
V-reversal [mV]	0.0	Done

Note: For the LockIn Sinewave to be an active option, the LockIn Extension must first be turned on in the Configuration window (see chapter 5.4.2 on page 44).

Use Theoretical Stimulus: Specify whether to use the theoretical stimulus or a measured stimulus Trace for computing real and imaginary traces.

Note: This option is needed when using the Protocol Method "Impedance Spectroscopy", also know as "EIS".

Peak Ampl. [mV]: See chapter 9.8.1.1 on page 78.

Requested Freq.: See chapter 9.8.1.1 on page 78.

Actual Frequency: See chapter 9.8.1.1 on page 78.

Points / Cycle: See chapter 9.8.1.1 on page 78.

Cycles to Skip: When a Sinewave segment begins, there is a "capacitive" transient current response just as there is a transient response when a voltage step is given. In order to prevent this from causing an artifact in the C_m Trace, sine wave cycles are skipped at the beginning of each Sinewave segment. If the frequency of the sinusoidal wave is chosen appropriately, then the transient should decay within a single cycle.

Note: A value of 1 Cycle to Skip is recommended.

Cycles to Average: If a value larger than '1' is entered, the specified number of cycles will be averaged. This reduces the noise and the time resolution (number of data points) in the results.

Total Cycles: Number of all cycles.

Checking: Allows to deactivate the internal checking procedures during the editing process.

For more information concerning the LockIn extension, we refer to the chapter Software LockIn Extension, 21 on page 181, and the CHARTMASTER Tutorial Capacitance Measurements using the LockIn Extension.

9.8.2 Square Wave Parameters

Square Wave For segments of the Square type. This button will appear above the Execute button and allows the specification of the wave characteristics.

9.8.2.1 Use as Simple Squarewave: Common Frequency

Use this setting if all wave segments within a Sweep should have the same frequency.

Use as Simple So	uarewave: com	nmon frequency
Peak Ampl. [mV]	10.0	value
Requested Freq.	100.0 Hz	
Actual Frequency	100.0 Hz	
Points / Cycle	200	
Neg. Ampl. [mV]	-10.0	value
Ampl. Incr. [mV]	0.0	
Pos. Dur. Factor	0.000	Cancel

Peak Ampl. [mV]: Defines the size of the Peak Amplitude. The amplitude is half of the peak-to-peak amplitude.

Value or parameter: If Value is selected the amplitude of the square wave can be entered in the field *Peak Ampl.*. Alternatively, the *Peak Ampl.* can be determined by a *PGF parameter*. This allows to automatically change the *Peak Ampl.* of the square wave between different *Series* acquisitions.

Requested Freq.: Desired frequency of the square wave in Hertz (Hz). Once the Wave Parameters window is closed, the Requested Freq. is set to the Actual Frequency.

Actual Frequency: Only certain frequencies are possible because they are generated by dividing a fundamental clock frequency by an integer. Nevertheless, the Actual Frequency is usually within a couple of percent of the Requested Freq.. The Actual Frequency is a function of the Requested Freq..

In contrast to the sine wave the square wave allows to use different positive and negative amplitudes as well as different half cycle times. By the use of the following two parameters, e.g. stimulation trains can be easily parameterized.

Neg. Ampl. [**mV**]: If a *Pos. Dur. Factor* unequal zero is used, the negative amplitude of the square wave can be entered.

Ampl. Incr. [**mV**]: Defines an increment amplitude which causes a baseline shift of the square waves ("Staircase" effect).

Below a square wave created with a Peak Amplitude of 10 mV and an Ampl. Incr. of 5 mV) is shown.



Pos. Dur. Factor: Factors between 0 and 1 are allowed. A factor of '0.5' means that both half cycles have the same duration. A factor smaller than '0.5' shortens the positive half cycle of the square wave.

Below a square wave created with a Pos. Dur. Factor of '0.2' and a Peak Ampl. of 10 mV and a Neg. Ampl. of -5 mV is shown.



9.8.2.2 Use as Simple Squarewave: Separate Frequency

Use this setting if the frequency of the wave is determined by the segment duration. Each segment will contain one wave cycle only. This option allows to create a Sweep containing wave cycles of different frequencies.

Use as Simple So	dualewave. set	barate frequency
Peak Ampi. [mv]	10.0	value
Neg. Ampl. [mV]	-10.0	value
Neg. Ampl. [mV] Ampl. Incr. [mV]	-10.0	value

All available settings have been explained before (see chapter 9.8.2.1 on page 80).

9.8.2.3 Use as LockIn Squarewave

Use this setting if the square wave should be analyzed with the LockIn Extension.

Use as	LOCKIN Square	wave			
Use Th	neoretical Stimu	ulus			
Peak Ampl. [mV]	10.0	value			
Requested Freq.	100.0 Hz				
Actual Frequency	100.0 Hz				
Points / Cycle	200				
Cycles to Skip	0	Checking			
Cycles to Average	0.0				
Fotal Cycles 1 Cancel					
V-reversal [mV]	0.0	Done			

Note: For the LockIn Squarewave to be an active option, the LockIn Extension must first be turned on in the Configuration window (see chapter 5.4.2 on page 44).

Use Theoretical Stimulus: Specify whether to use the theoretical stimulus or a measured stimulus *Trace* for computing real and imaginary traces.

Note: This option is needed when using the Protocol Method "Impedance Spectroscopy", also know as "EIS".

Peak Ampl. [mV]: See chapter 9.8.2.1 on page 80.

Requested Freq.: See chapter 9.8.2.1 on page 80.

Actual Frequency: See chapter 9.8.2.1 on page 80.

Points / **Cycle:** Number of sample points per full wave length. This number is calculated and can not be edited. It is a display value only and gives the number of points per square wave which is the cycle length divided by the sample interval.

Cycles to Skip: When a Squarewave segment begins, there is a "capacitive" transient current response just as there is a transient response when a voltage step is given. In order to prevent this from causing an artifact in the

 C_m Trace, square wave cycles are skipped at the beginning of each Squarewave segment. If the frequency of the square wave is chosen appropriately, then the transient should decay within a single cycle.

Note: A value of '1' Cycle to Skip is recommended.

Cycles to Average: If a value larger than '1' is entered, the specified number of cycles will be averaged. This reduces the noise and the time resolution (number of data points) in the results.

Total Cycles: Number of all cycles.

V-reversal [mV]: Applicable in the Square + DC mode. Since the DC current is used in processing estimates of the equivalent circuit parameters, the reversal potential must be known (see Gillis, 1995). For our purposes, V-reversal is the zero current potential extrapolated from the slope conductance about V-membr., which is not necessarily the same as the actual zero current potential. If you expect a membrane conductance to be activated during the course of the experiment, set V-reversal to the zero current potential of the activated conductance. This setting is actually not very critical if G_m is low.

Note: A value of zero is often used in the common situation where G_m is low and the actual reversal potential is unknown.

Checking: Allows to deactivate the internal checking procedures during the editing process.

For more information concerning the LockIn extension, we refer to the chapter Software LockIn Extension, 21 on page 181, and the CHARTMASTER Tutorial Capacitance Measurements using the LockIn Extension.

9.8.3 Chirp Wave Parameters

Chirp Wave For segments of the *Chirp* type. This button will appear between the buttons *Checking* and *Execute*, allowing specification of the wave parameters. The *Chirp* wave's characteristic is either an increasing or decreasing frequency over time. This frequency modulation can be in a linear, exponential or spectroscopic manner. An example for a *Linear Chirp* and an *Exponential Chirp* is given below:



9.8.3.1 Linear Chirp

Use this setting if the chirp frequency should change in a linear manner.

	Linear Chirp	
Amplitude [mV]	10.0	value
Start Frequency	0.000 Hz	
End Frequency	10.00 kHz	
Min. Points / Cycle	2.0	Checking Cancel
Segment Points	500	Done

Amplitude [mV]: Defines the size of the *Peak Amplitude*. The amplitude is half of the peak-to-peak amplitude.

Value or parameter: If *Value* is selected the amplitude of the chirp wave can be entered in the field *Peak Ampl.*. Alternatively, the *Peak Ampl.* can be determined by a *PGF parameter*. This allows to automatically change the *Peak Ampl.* of the chirp wave between different *Series* acquisitions.

Start Frequency: Defines the frequency where the chirp wave starts.

End Frequency: Defines the frequency where the chirp wave ends.

Min. Points / Cycle: Indicates the minimum number of sample points per full wave length for the End Frequency.

Segment Points: Total amount of points for the Chirp segment. It is a display value only.

9.8.3.2 Exponential Chirp

Use this setting if the chirp frequency should change in an exponential manner.

Ex	ponential Chirp	
Amplitude [mV]	10.0	value
Start Frequency	25.00 kHz	
End Frequency	250.0 Hz	
Min. Points / Cycle	2.0	Checking
Min. Points / Cycle	2.0	Can
Segment Points	5000	Done

All available settings have been explained before (see chapter 9.8.3.1 on the preceding page).

9.8.3.3 Spectroscopy Chirp

Use this setting if the Chirp wave should be analyzed with the Spectroscopy Extension.

Spe	ctroscopy Chir	p
Amplitude [mV]	10.0	value
Start Frequency	6.104 Hz	Pre-Chirp
End Frequency	12.50 kHz	
Min. Points / Cycle	4.0	Checking
Chirps to Skip	0	Cancel
Segment Points	5000	Done

Note: For the Spectroscopy Chirp to be an active option, the Spectroscopy Extension must first be turned on in the Configuration window of Chartmaster (see chapter 5.4.3 on page 45).

Amplitude [mV]: See chapter 9.8.3.1 on page 83.
Value or parameter: See chapter 9.8.3.1 on page 83.
Start Frequency: See chapter 9.8.3.1 on page 83.
End Frequency: See chapter 9.8.3.1 on page 83.
Min. Points / Cycle: See chapter 9.8.3.1 on page 83.
Chirps to Skip: Defines the number of skipped chirp segments.
Segment Points: See chapter 9.8.3.1 on page 83.

Note: End Freq. = Min. Points / Cycle * Sampling Freq.

Pre-Chirp: Attaches a small chirp fragment to the beginning of the chirp wave to avoid

Checking: Allows to deactivate the internal checking procedures during the editing process.

For details on the parametrization of *Chirp* wave form we refer to the chapter Spectroscopy Extension, 22 on page 191 and the CHARTMASTER tutorial Using the Spectroscopy Extension.

9.8.4 Photometry Wave Parameters

Photometry Wave If the option Use for Wavelength is active for a DA output channel, then the Photometry Wave button will appear above the Checking button. It assists you in creating repetitive stimulation pattern for controlling the excitation wavelengths.

Photometry Parameters	
Number of Segments	3
Adapt to Maximal Sv	veep Length
Number of Cycles	1
Trunc Expand	Done

Number of Segments: The number of creating a single cycle for a measurement. Typically there are three segments used for a ratio-metric measurement. Segment '1' and '2' for the wavelength setting and a third segment for a waiting time.

Adapt to Maximal Sweep Length: If checked, then the number of cycles is calculated by the software in respect to the duration of the longest output channel.

Number of Cycles: As input determines how many times the first *Number of Segments* are duplicated with the *Expand* command. In case *Adapt to Maximal Sweep Length* is checked, then it shows how many cycles have been fit into the stimulus.

Expand: Creates the complete photometry stimulus. The first Number of Segments are duplicated until the Maximal Sweep Length or the Number of Cycles is reached.

Trunc: Removes all segment except the first *Number of Segments*. This option should be used to clean the stimulus before changing any parameters in the photometry stimulus. After parameter adjustment an *Expand* creates the new stimulus.

Checking: Allows to turn off the internal consistency checks. Turning this transiently off might be necessary when multiple changes of parameter would lead to intermediate invalid configurations.

Segments 👌 👌	St	ore 1	Sto	ore 2	⊠Sto	ore 3
Segment Class	Co	nstant	Cor	nstant	Cor	nstant
Wavelength [nm]	valu	340	valu	380	hold I	Resting
Duration [ms]	val	25.00	val	25.00	val	10.00
V-incr. Mode	Inc	rease	Inc	rease	Inc	rease
W-fact./incr. [nm]	1.00) 0	1.00	0	1.00	0
t-incr. Mode	Inc	rease	Inc	rease	Inc	rease
t-fact./incr. [ms]	1.00	0.00	1.00	0.00	1.00	0.00
Draw: Active Chapr	nol a	II Sween	പറ	alaw: DA	0.00	s AD
10.0nm 50.0ms	_					

In this example a single cycle consists of 3 segments. You have just to edit the first three segments. A click on *Expand* adds 15 additional segments to the PGF sequences. The resulting PGF sequence then consists of 6 * 3 = 18 segments. *Trunc.* removes all segments except the initial 3 segments.

9.8.5 Imaging Wave Parameters

In case you want to perform image acquisition during the execution of a Pulse Generator sequence, please we recommend reading first the chapter Imaging Extension, 20 on page 169.

The Imaging Wave parameters are usually set in the PGF Primer of the Imaging Extension and automatically integrated into the Pulse Generator sequence by the Prime function of the Imaging Extension. Hence, usually it is not necessary to modify the parameters in this dialog.

Imaging Parameters	
Adapt to Maximal S	weep Length
Number of Cycles	1
Cycle Time	500. ms
Readout Time	100. ms
Exposure Time 1	50.0 ms
Wavelength 1	0.00
Exposure Time 2	0.00 s
Wavelength 2	280.
Exposure Time 3	0.00 s
Wavelength 3	280.
Resting Wavelength	280.
Dead Time	2.00 ms
ROI Traces	1
Trunc	Prime
Checking	Done

For a detailed description of the imaging parameters we refer to the chapter PGF Primer, 20.4.2 on page 175.

9.9 Channel Settings for DA Output and AD Input

	1	DA	Unit	Stimulus -> DA	AD	Unit	Link	Compr.	Points	Store	Zero
	Ch-1	DA-0	V	absolute voltage	AD-0	V	1	1 (200		0
(Ch-2	off	V	absolute voltage	AD-1	V	1	1 (200		0
		off		absolute voltage	off			(2		
]	off		absolute voltage	off			(X		

Ch-1...16: Settings for each channel. The default is "1", the other channels (Channels = 2...) may be used to simultaneously record the potential or an amperometric signal, for example.

The DA section allows you to set the properties of the DA output, e.g., the stimulus signal.

The AD section allows you to set the properties of the AD input, e.g., the acquired data. DA and AD settings are basically independent from each other. Their reference is only given by the variable *Link* in the AD settings! This allows you to associate several AD inputs to the same DA stimulation.

9.9.1 DA output channel settings

DA: The DA channel for stimulation has to be specified. The figure below shows the options for the EPC 10 Single amplifier.

• DA-0...3: Analog output. DA-0 DA-1 • off: No output of stimulus, disables this channel. DA-2 • Dig-out (word): Output as digital word. DA-3 DA-4 • Dig-0...15: Digital output channel. DA-5 DA-6 DA-7 ~ off Dig-out (word) Dig-0 Dig-1 Dig-2 Dig-3 Dig-4 Dig-5 Dig-6 Dig-7 Dig-8 Dig-9 Dig-10 Dig-11 Dig-12 Dig-13 Dig-14 Dig-15

Stim to DA

relative to hold

use for LockIn

use DA-Scaling

use for Imaging

use for Wavelength

use for Spectroscopy

load from file template

Unit: Units for output channels. Defaults are "V" for voltage, and "A" for current.

Stimulus \rightarrow DA: Conversion between the stimulus and the actual analog output.

- relative to hold: Calculates the signal relative to V-memb.
- load from file template: Load a stimulus template from file. A detailed description how to use a recorded waveform as a stimulus template can be found in the chapter Using a Recorded Waveform as Stimulus in the CHARTMASTER Tutorial.
- use for LockIn: Has to be activated if the stimulus should be used for the software LockIn. For details please refer to the chapter Software LockIn Extension, 21 on page 181.
- use for Wavelength: Has to be activated if the stimulus should be used to control a wavelength. Amplitudes of segments can then be entered in "nm". This option is available if the Photometry or Imaging Extension is activated.
- use DA-Scaling: Has to be activated if the stimulus should be scaled depending on the settings made in the I/O Control tab of the Configuration. For further information we refer to the chapter AD/DA Input/Output Scaling, 5.7.1 on page 52.
- use for Spectroscopy: Has to be activated if a chirp wave form should be used for impedance or admittance analysis with the Spectroscopy Extension. For details please refer to the chapter Spectroscopy Extension, 22 on page 191.
- use for Imaging: Has to be activated if the stimulus should be used to control the exposure of the camera. This option is available if the Imaging Extension is activated. For further information we refer to the chapter Imaging Extension, 20 on page 169.

If nothing is selected, "absolute voltage" will be applied.

Use of digital Outputs: Digital outputs are mainly used for controlling (triggering) other devices, such as perfusion systems, filter wheels, flash lamps, or others. The EPC 10 amplifier (and the LIH 8+8) are equipped with 16 digital output lines. These outputs can be accessed from the rear panel of the amplifier. The first three of the 16 available digital outputs can be accessed also from the front panel of the amplifier (interface). The BNC outputs ("Trigger-Outputs") "Out-0", "Out-1", "Out-2" correspond with the out channels "Dig-0", "Dig-1", and "Dig-2" in the Pulse Generator dialog of CHARTMASTER.

There are two ways to control the digital lines:

If only one digital line is intended to be used, then one of the "Dig-Out" lines ("Out-0" to "Out-15") can be selected as output channel. In the Segments section of the Pulse Generator the state of the digital line can be set either to "1" (active = TTL level), "-1" (inactive = 0 V), or to "0" (don't change, use the state that is set in the I/O control dialog).

The Dig-Out word output allows to switch several digital lines simultaneously. What channels are switched is defined in the Segments section by the corresponding bit value. If Voltage in the Segments section is set to "7", then the first three digital lines are turned on: $1 * 10^2 + 1 * 10^1 + 1 * 10^0 = 4 + 2 + 1 = 7$.

Note: For such trigger channels it is suggested to switch from Common Timing to Separate Timing. With this setting, the duration and number of segments is completely independent from the first (main) channel.

9.9.2 AD input channel settings

AD: The AD-channel for input.

- AD-0...7: AD channel.
- off: No input.
- Virtual: Virtual input. A virtual *Trace* is used to generate a *Trace* where the data come from a source different from an ADC, e.g., external data loaded via the option *Import Trace*, or data which generate virtual *Traces*, such as the LockIn.
- Dig-in (word): Digital input.
- Dig-0...15: Digital input channels.
- Chirp Traces: Storage of particular Spectroscopy values, such as e.g. Admittance, Phase

✓ AD-0 AD-1 AD-2 AD-3 AD-4 AD-5 AD-6 AD-7 AD-8 AD-9 AD-10 AD-11 AD-12 AD-13 AD-14 AD-15 off Virtual Dig-in (word) Dig-0 Dig-1 Dig-2 Dig-3 Dig-4 Dig-5 Dig-6 Dig-7 Dig-8 Dig-9 Dig-10 Dig-11 Dig-12 Dig-13 Dig-14 Dig-15 Chirp_Avg Chirp_Phase Chirp_Admit(Y) Chirp_Real(Y) Chirp_Imag(Y) Chirp_Real(Z) Chirp_Imag(Z) Chirp_Imp|Z|

- LockIn Traces: Storage of particular LockIn values, such as e.g. $C_m, G_m, G_s \dots$
- Imaging/Photometry Traces: Storage of particular Imaging or Photometry values (e.g. mean fluorescence, calcium concentration, fluorescence ratio.)

Unit: Unit for input channels. Default is "A" for current (in voltage clamp mode). Use "V" if a plain AD channel is sampled or any other applicable unit if virtual *Traces* are used or the channel is scaled.

Link: Number of the stimulus channel that is associated with this input. This setting is important for later analysis of the *Trace*. It links the stimulus segments to the recorded *Trace*.

Compr.: Enter the number of points for the compression (compression factor) and choose the compression method from the list. The compression factor should be chosen such that the compression interval (or range) covers the complete period of interest. The number of data points divided by the number of compressed data points yields the compression factor.

Note: Normal Traces are acquired and can be compressed. Virtual Traces are generated.

- Compression Mode:
 - single sample: Takes one point per compression interval.
 - mean: Averages all points of a compression interval.
- 2-byte integer / 4-byte real: Data type for storage.
- Set Skip: With the Set Skip function, a specified number of points of the compression interval can be skipped before the compression starts. In a compression interval, you can only skip data points at the beginning of the interval.
- Set Offset: With this function, the start of the compression interval can be influenced. A negative offset moves the start of the compression interval to the left and a positive offset to the right. A negative offset essentially adds dummy data points on the negative time axis. Since compression starts at the first recorded point, the compression range is moved to the left (towards smaller times) with respect to the data points.

Note: For an example describing the use of Skip and Offset compression functions please refer to the chapter High Speed Fluorescence Measurements in the CHARTMASTER Tutorial.

- Digital Filter: If selected, the raw data are filtered digitally before compression. The filter factor is given by the compression factor. Example: If a *Trace* is acquired with at sampling rate of 5 kHz and the compression factor is 10, then the digital filter is set to 500 Hz. With a compression factor of 5 the filter bandwidth is increased to 1 kHz.
- Set Defaults: Sets all settings back to default.

✓	Compression Mode: single sample mean
v	2-byte integer 4-byte real
	Set Skip (Skip = 0) Set Offset (Offset = 0)
	Digital Filter
	Set Defaults
	Build Instructions: empty

Lock	dn_Avg
Lock	dn_Phase
Lock	dn_Real(Y)
Lock	dn_Imag(Y)
Lock	dn_Admit(Y)
Lock	dn_Real(Z)
Lock	dn_Imag(Z)
Lock	dn_Imp Z
Imag	ge_W1
Imag	ge_W2
Imag	ge_W3
Imag	ge_R
Ima	ne Ca

Build Instructions: Allows computing a virtual *Trace* from other *Traces* and inputs. The instruction string starts with the math operation. The formula is terminated by a semicolon (;). Then a list with additional instructions all separated by a comma (,) can follow.

- Mathematical Operations:
 - $\circ\,$ An: Anodic integral of Trace with index n
 - $\circ\,$ Bn: Trace buffer with index n
 - $\circ\,$ Cn: Cathodic integral of Trace with index n
 - $\circ\,$ Dn: Differential of the Trace with index n
 - $\circ\,$ In: Integral of the Trace with index n
 - $\circ\,$ M: Mean of a Trace. It must be followed by a bracket enclosing 3 numbers, separated by comma:
 - 1. The Trace count of the Trace from which to compute the mean
 - 2. The time offset (in [s]) from the beginning of the stored Trace data.
 - 3. The time span (in [s]) over which to compute the mean. Example: "M(2,0.0,0.005)"
 - $\circ\,$ Tn: Trace with index n
 - $\circ~$ Vn: Value with index n
 - $\circ\,$ Zn: Zero data of Trace with index n
 - \circ Operators: +, -, *, /
 - Brackets: ()
- Other Assignments:
 - Name="String" (separated from the math operation by a semicolon ';'). "String" is just a placeholder for any name.
 - $\circ\,$ No Lock In: Suppressing LockIn computation
 - $\circ\,$ T count: Trace count with a value between 1 and 16
 - S: Stands for "Shift". It must be followed by a bracket enclosing 2 numbers, separated by comma:
 - 1. The source Trace index
 - 2. The number of samples by which to shift the source Trace Example: "S(2,50)" = Trace with index "2" shifted by 50 samples to the beginning of the Trace. Negative numbers shift the data towards the end of the trace. The undefined data will be set to invalid (NaN). Invalid data points are not displayed.
 - Slave=[number]: Copying the Trace data from the "slave" CHARTMASTER instance via the batch communication protocol. The equal sign must immediately follow "slave", e.g. "Slave=2". The given number is the Trace count of the Trace to get. In the given example data from the source Trace with Trace count = '2' is read.
 - StimSwp(): Only Sweeps defined in the list will output the respective segment amplitudes. The other Sweeps will use the amplitude of segment '1' for all segments. Values are to be separated by commas, and Sweep ranges can be defined by a hyphen, e.g.: "StimSwp(1,3-5,7-9)".
 - Yrange=real (separated by a colon ','). The "Yrange" sets the scaling of the Oscilloscope display. The value of "Yrange" corresponds to the total length of the Y-axis. 1/10 of "Yrange" corresponds to the scale bar displayed in the Oscilloscope window.
 - Yrange([N],[factor]). Sets the "Y-range" of a Trace proportional to the value of another Trace. "N" is the Trace index of the source Trace and "factor" is the factor to multiply.

Note: The total length of the Build Instruction is limited to 128 characters.

Example 1: V1*T1;Name="m1", Yrange=1E-9

Trace 1 is multiplied with Value 1. The name of the new virtual Trace is "m1" and the total range of the Y-axis is 1e-9.

Example 2: V1*T1+(T6-T7);Name="Baseline",Yrange=1E-6

Trace 1 is multiplied with Value 1 and the difference between Trace 6 and Trace 7 is added. The name of the new virtual Trace is "Baseline" and the total range of the Y-axis is 1e-6.

Example 3: I1; Name="Charge", TCount=5, Yrange=1E-5

Trace 1 is integrated. If Trace 1 is a current Trace we get the charge. The new virtual Trace gets the name "Charge", the total range of the Y-axis is 1e-5 (i.e. 10 C). The Trace # is set to "5".

Important note: No other operator priority is considered besides the brackets.

The *Build Instruction* can also be used to give a particular *Trace* a name (label). This is convenient for having the name of the *Trace* displayed in the **Replay** window.

It is also possible to specify an equation as a build instruction similar to the equation handling in the Analysis (see chapter 11.3.2.7 on page 126), the Buffer menu (see chapter 4.6 on page 35) or the Calculator dialog (see chapter 4.3 on page 25). It is important to put the equation in double-quotes and start at the first character.

Example 4: "t[1]-t[2]" or "sin(y)".

Points: Number of resulting points for this channel.

Store: If checked, data acquired for this channel will be stored.

Note: In case Store is not activated but the file is opened with write permission, the last Sweep will be stored temporarily. This Sweep is marked with a "#" in its label. It will be deleted when the next Sweep is acquired or a new Group is created or the file is closed.

Zero: Number of the segment on which base the Zero Offset will be calculated. Usually the Zero Offset is calculated from the second half of that segment to avoid contamination by tails of capacitive transients. In order to specify a custom range for the Zero Offset calculation please specify a StartSeg and a StartTime >0. Then the Zero Offset will be calculated between StartTime and end of the segment.

Note: When the segment number is set to "0", no Zero Offset subtraction will be calculated.

9.10 Segments

9.10.1 Segment Classes

Segments 👌 👌	1	Stored	2	Stored	3	Stored	4	Not Store	5	COLUMN TO A	6	00
Segment Class	Co	onstant	C	onstant	C	onstant	C	onstant		distant		nstant d
Voltage [mV]	hold	V-memb	val	-60	holo	V-memb	val		val	1.12.008	val	
Duration [ms]	val	10.00	val	10.00	val	10.00	val		val		val	
V-incr. Mode	In	crease	In	crease	In	crease	In	crease	Inc	crease	Inc	crease
V-fact./incr. [mV]	1.0	0 0	1.0	0 10	1.0	0 0	1200	•				
t-incr. Mode	In	crease	In	crease	In	crease	In	crease	Inc	crease	Inc	crease
Scan-fact./incr.	1.0	0 0.00	1.0	0 0.00	1.0	0 0.00		- (
Scan Rate [V/s]									1			

A pulse pattern consists of an arbitrary number of segments. Segments are shown as a horizontally scrolling matrix; clicking on the arrows does scrolling.

Store: On the top of the segment column there is a *Store* field with a selection list determining whether or not the data of that segment is to be stored to disk.

The following options are available:

• Not-Stored: The data for that segment are sampled but removed from the *Trace* before storage. A non-stored segment must *not* be a *StartSegment* or a *Relevant Y*-segment. For a description on the usage of non-stored segments please read Non-Stored segments in the Pulse Generator in the CHARTMASTER Tutorial.

- Stored: The data are sampled and stored.
- First Sweep: These segments are output only with the first Sweep of the Series but are not stored.
- Last Sweep: These segments are output only with the last Sweep of the Series but are not stored.

Segment Class:

Segments can be the following:

Segment olass Constant Ramp Sine Square Chirp Continuo	Segment Class	Constant	Ramp	Sine	Square	Chirp	Continuous
--	---------------	----------	------	------	--------	-------	------------

- Constant: Segment of constant amplitude.
- Ramp: Segment with ramp from the amplitude of the previous segment to the amplitude of this segment.
- Continuous: Identifier for continuous data acquisition.

Note: Only the last segment can be of that class.

- Sine: Segment with sine characteristics. Amplitude and cycle duration are defined in Sine Wave parameters (see chapter 9.8.1 on page 78).
- Square: Wave segment with rectangular characteristics. Amplitude and cycle duration are defined in *Square Wave* parameters (see chapter 9.8.2 on page 80).
- Chirp: Wave segment with increasing sine wave frequency. Wave characteristics are defined in *Chirp Wave* parameters (see chapter 9.8.3 on page 83).

The list entries Insert, Duplicate, and Delete are used to create or remove segments.

- Insert: Inserts a constant segment (default duration = 0) at the actual location (moves the selected segment to the right).
- Duplicate: Creates a copy of the selected segment and inserts it at the actual location.
- Delete: Deletes the selected segment at once.
- Duplicate...: Creates multiple copies of a number of segments. You have to enter how many segments you want to copy. Segments are counted starting with the selected segment. How often specifies the number of copies from these segments. They will be inserted at the actual location.
- Delete...: Deletes a specified number of segments at once.

Voltage: The Voltage of a segment is either a numeric value (val, in mV), a holding value at time of sequence execution (hold), or a value from the PGF parameters list (p1-p10). The Voltage field may turn into "Current (pA)" in Current Clamp mode, into "Wavelength (nm)" when the segments are used for photometry stimulation or into "Amplitude" when the Use for DA scaling is used. The valid amplitude range is a function of the given DA output voltage range of the interface and the appropriate stimulus scaling factors (e.g. VC: Stim Scale in Voltage Clamp mode, CC:Stim Scale (or CC-Gain) in current clamp or the wavelength to voltage conversion formalism of the Photometry Extension.)

Duration: Duration of a segment, entered in milliseconds (ms). The value may be adjusted by dragging the mouse or typing the number. For segment duration, *PGF parameters* can also be used. In this case, all data are taken in seconds. Make sure that the duration of segments are even multiples of the *Sample Interval*. A warning is given if they are not, in case *Checking* is activated.

val / hold / p1...10: Allows setting the value.

- val: Standard value.
- hold: Keeps the potential at *V*-membrane during voltage clamp measurements. If the channel is used for photometry, the resting wavelength as specified in the Photometry window is set.
- p1...10: Sets the value to the value given in the corresponding PGF parameter. For a description of usage of the PGF parameters please read PGF Parameters, chapter 9.14 on page 98.



9.10.2 Scan Rates

The Scan Rate options are only available if Use Scan Rates is activated (see chapter 9.5 on page 77).

- Scan-fact./incr.: If a value for the increment is entered the field t-incr. Mode gets active.
- Scan Rates [V/s]: The user can enter a defined value into the field *Scan Rates* whereby the duration of the segment will be calculated or vice versa. The *Scan Rate* is calculated as Voltage/Duration.

9.10.3 Increment Modes

The various increment modes are flexible tools to create sequences with varying durations or amplitudes. Please note that first the amplitudes or durations in logical order are calculated as specified by the parameters *Voltage/Duration*, *V-fact./t-fact* and *V-incr./t-incr.*. Then the logical order is converted to the physical output by applying the selected *Increment* Mode.

Increment Mode: This determines the order of incrementing the segment voltage and/or duration. The options are as follows (the numbers give an example for a *Series* with 6 *Sweeps*):

- Increase: first Sweep comes first (e.g. 1, 2, 3, 4, 5, 6)
- Decrease: last Sweep comes first (e.g. 6, 5, 4, 3, 2, 1)
- Interleave +: ascending interleaved (e.g. 1, 3, 2, 5, 4, 6)
- Interleave -: descending interleaved (e.g. 6, 4, 5, 2, 3, 1)

Example: If you apply a Series of 6 Sweeps at an increment of 10 mV starting at -40 mV the logical sequence will be: -40, -30, -20, -10, 0 and +10 mV. With the mode Interleave - activated the pulses will be output in the following (physical) order: +10, -10, 0, -30, -20 and -40 mV.

- Alternate: first, last, second, penultimate...(e.g. 1, 6, 2, 5, 3, 4)
- Toggle: Starts with the first Sweep, second the voltage increment is added and third the voltage segment is subtracted. Then twice the voltage segment is added.... E.g. using 6 Sweeps with a V-incr.of 1 mV and the first Sweep steps to 10 mV. The output voltages are as follows: 10, 11, 9, 12, 8, 13 mV.



9.10.3.1 Logarithmic increment modes

V * Factor: In mode V * Factor the logarithmic increment is based on the voltage of the first Sweep. Therefore the voltage cannot be zero. The segment's voltage of the *i*th Sweep is then calculated as:

 $V_i = Voltage + Voltage * VFactor^{i-1} + (i-1) * dVincr$

In mode V * Factor the increment may be zero. Let Voltage be 10 mV, $\Delta VIncr. = 0$ mV, and VFactor = 2 then the Series 10, 20, 40, 80 mV, ... is obtained.



dV * Factor: In mode dV * Factor the logarithmic increment is based on the linear increment step. Therefore the voltage increment cannot be zero. The segment's voltage of the *i*th Sweep is then calculated as: for V-factor = 1:

$$V_i = Voltage + (i - 1) * dV-incr$$

for V-factor $\neq 1$:

$$V_1 = Voltage V_{i,i>1} = Voltage + dV-incr * V-Factor^{i-2}$$

In mode dV * Factor the first segment may be zero and is then logarithmical incremented. Let Voltage = 0 mV, dV-Incr = 1 mV, and V-Factor = 2 then the Series 0, 1, 2, 4 mV, ... is obtained.

In some kind of experiments it might be useful to toggle between two fixed potentials. To achieve this, you have to:

- Define your starting "Voltage".
- Select "Increase" and "V*Factor".
- Select "V-fact." to "-1".

There are three cases for setting the voltage increment:

- 1. "0": E.g. +100 mV, -100 mV, +100 mV, -100 mV,...
- 2. "positive" (e.g. +20 mV): E.g. +100 mV, +80 mV, +100 mV, +80 mV,...
- 3. "negative" (e.g. -20 mV): E.g. +100 mV, +120 mV, +100 mV, +120 mV,...

		Segments 👌 🔇	⊠S	tore 1	Sto	re 2	⊠Sto	ore 3	□St	ore 4
		Segment Class	Co	onstant	Cor	nstant	Cor	nstant	Co	onsta
		Voltage [mV]	hold	V-memb	valu	60	hold \	/-memb	valu	-
		Duration [ms]	val	10.00	val	10.00	val	10.00	val	
		V-incr. Mode	In	crease	Inc	rease	Inc	rease	In	creas
		V-fact./incr. [mV]	1.0	0 0	-1.00	0	1.00	0		
		t-incr. Mode	In	crease	Inc	rease	Inc	rease	In	creas
1		t-fact./incr. [ms]	1.0	0 0.00	1.00	0.00	1.00	0.00		8
•	Increase									
	Decrease	Draw Active Chan	nal c	II Cureer			0.00			
	Interleave +	Draw. Active Chan	nei, a	ili Sweep	DS DE	ay. DA	0.00	SAU	0.00	JS
	Interleave -									
	incencerce									
	Alternate									
	Toggle									
-	V * Factor	10.0mV								
	dV * Factor	2.00ms								

In analogy to the logarithmic amplitude increments the duration increments are calculated as follows:

t * Factor: In mode t * Factor the logarithmic increment is based on the duration of the first Sweep. The segment's duration of the *i*th Sweep is then calculated as:

 $t_i = Duration + Duration * tFactor^{i-1} + (i-1) * dtIncr$

In mode t * Factor the increment may be zero. Let Duration be 10 ms, dt-Incr. = 0 ms, and t-Factor = 2 then the Series 10, 20, 40, 80 ms, ... is obtained.

dt * Factor: In mode dt * Factor the logarithmic increment is based on the linear increment step. Therefore the duration increment cannot be zero. The segment's duration of the *i*th Sweep is then calculated as:

for t-Factor = 1:

 $t_i = Duration + (i - 1) * dt-incr$

for t-Factor $\neq 1$: $t_1 = Duration$

 $t_{i,i>1} = Duration + dt-incr * t-Factor^{i-2}$

In mode dt * Factor the duration of the first segment may be zero and is then logarithmical incremented. Let Duration = 0 ms, dt-Incr = 1 ms, and t-Factor = 2 then the Series 0, 1, 2, 4 ms, ... is obtained.

9.11 Miscellaneous



Common Timing / Separate Timing: If *Common Timing* is selected, the segments of all channels are timed like the first one. If *Separate Timing* is selected, all channels are timed separately, i.e. segment boundaries may be different in different channels.

Break Condition: For each acquired *Trace* of the AD input that is longer than 1 second a *Break* condition can be specified. The first condition that is *TRUE* terminated the acquisition of the *Sweep*.

- No Break: No break condition for that Trace.
- Break if >: Terminates the acquisition if the acquired values become larger than the value entered in the field to the right.
- Break if <: Terminates the acquisition if the acquired values become smaller than the value entered in the field to the right.
- Break if abs >: Terminates the acquisition when an absolute sample value of that trace exceeds the given threshold.
- Break on "next": Terminates the acquisition of the Sweep if the user presses the Next button in the Control window. The acquisition then continues with the next Sweep.

Note: The delay between the break condition = TRUE and leaving the acquisition routine can be up to 50 ms.

Filter Factor: The *Filter Factor* is implemented for the EPC 9 and EPC 10 amplifiers. It is used to define the automatic filter setting relative to the sample rate (activated by *Auto Filter* in the Configuration window).

Example: for Sample Interval = 250 μ s (4 kHz) and Filter Factor = 4, a filter cutoff frequency closest to 1 kHz (= 4 kHz / 4) will be selected. The suggested filter frequency is shown in parentheses.

Analysis: Enter an *Analysis Method* that should be executed automatically after the data acquisition. Note that *Automatic Stimulus Control* has to be activated in the Analysis window for this feature to become effective.

Relevant Segments: The *Rel X-seg* (relevant X-segment) specifies a segment of interest that is mainly used as X-axis reference for later analysis. The *Rel Y-seg* (relevant Y-segment) specifies the segment where the analysis is performed (e.g., determination of peak current). For the measurement of an h-infinity curve, for example, the *Relevant X-Segment* would be the conditioning segment of variable voltage, while the *Relevant Y-Segment* would be the test segment, where the peak current is determined.

9.12 Stimulus Template Preview



After each editing operation, the stimulus template is refreshed to reflect the changes made. Sweep other than the first one are shown as dashed lines. If part of the pulse pattern exceeds the DA limits, the forbidden voltage region is indicated in the picture by shading. The corresponding warning box "Segment with too large voltage encountered" only appears once. Segments drawn in red color refer to the *relevant* Y-segment. The type of graph scaling is chosen in Display \rightarrow Labeling \rightarrow PGF-Editor Grid.

Preview Mode: Select from the list what shall be displayed in the preview:

- Draw: Active Channel, all Sweeps: Displays the settings of the active channel for all Sweeps. Recommended if you use increment modes in your settings.
- Draw: Active Channel, 1. Sweep: Displays the settings of the active channel for the first Sweep.
- Draw: Active Channel, Last Sweep: Displays the settings of the active channel for the last Sweep.
- Draw: All Channels, all Sweeps: Displays the settings of all channels for all Sweeps. Recommended if you use *Increment* modes in your settings.
- Draw: All Channels, 1. Sweep: Displays the settings of all channels for the first Sweep.
- Draw: All Channels, Last Sweep: Displays the settings of all channels for the last Sweep.
- Draw no cartoon: Disables the display of the stimulus template.

Delay: Displays the hardware delay time for the DA output and the AD input with The transformation of the first DA output channel.

9.13 V-membrane



V-membrane (V): This control displays the presently selected membrane potential. It is **only** used for the sequence cartoon as reference and can be changed without actually affecting the membrane potential in the Amplifier window (*V-membrane*). When the PGF template of a stored file is reviewed, V-membrane is the holding voltage of that particular experiment.

Set Last Seg. Amplitude: At the end of the *Sweep*, *V*-membrane is set to the amplitude of the last segment, e.g., -90 mV, if this was the potential of the last segment. Normally, the potential is reset to holding.

9.14 PGF Parameters

p1	p2	p3	p4	p5	p6	р7	p8	p9	p10
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Use the PGF parameters as variables in the segment settings for Voltage or Duration. This allows you to change multiple settings in one or more sequences with changing only one parameter. CHARTMASTER provides 10 PGFparameters which will be stored with the Pulse Generator file. Instead of p1 to p10 you can assign use specific names to make the parameter input more intuitive.

Example: You want to increase the duration of three segments during a loop in a protocol. Enter the start duration in field "p1", set the duration of all segments to "p1" and then increase "p1" during loop execution.

For a description of usage of the PGF parameters please we refer to the chapter Global Variables in PATCHMASTER in the PATCHMASTER Tutorial.

9.15 Error Handling

If CHARTMASTER encounters an unreasonable value in the Pulse Generator window, the user is requested to change the corresponding parameter before proceeding. In case of multiple errors, one cannot exit the dialog until all parameters are set correctly. If it seems impossible to solve the error situation and you consistently get error messages, you can load a valid PGF file from disk.

Values in the Pulse Generator controls are rounded to the exact values as displayed. This prevents unexpected results caused by rounding problems, such as the *Sample Interval* being an odd number.
10. Protocol Editor Window

In the Protocol Editor window you can assemble complex experimental arrangements by combining PGF-templates with other operations (e.g. breaks, IF-THEN loops, setting changes). This window is the heart of the CHARTMAS-TER software concerning the automation of experiments.

Protocol Editor: DefProt	
Image: Constraint of the second se) 4 Buffer 5 SETUP 6 SEAL 5 00
1: Command: " E Reset" 2: Command: " E Mode 3; Whole Cell" 3: Command: " E PulseAmp 5.0mV" 4: Command: " E PulseDur 5.0ms" 5: Command: " E CSlow 50.00pF" 6: Command: " E RSeries 20.0MOhm" 7: Command: " E Gain 10; 5.0 mV/pA -> r 8: Command: " E AutoZero" 9: Command: " E PulseOn 9: Command: " E SaveRpip" TRUE" 10: Command: " E SaveRpip" TRUE"	Contract Contract Set of the set of th

With protocols, very complex sequences can be edited. During protocol execution no opening/closing of windows is necessary, thus the execution is pretty fast. Furthermore, in protocols it is possible to use conditional events, depending on e.g., analog or digital input, analysis results or key commands.

10.1 Protocol Handling

00	1 SETLID	2 SEAL	3 WHOLE-CEL) 4 Exampl1	5	Example?	6	Link)	1	N	N
1 1 1	SLIDE	ZU SLAL	J JWHOLL-CLLJ 4(LXamph	\mathbf{j}		υυ	LIIIK		4	4

Protocol Pool: The first row displays a section of the pool of available protocols. It is a paging bar in units of six protocols. Two arrows at each side allow scrolling through the available protocols (the innermost arrows move in increments of one page, i.e., six protocols; the outermost arrows move to the start/end of the sequence list). A protocol is selected by clicking on it or by entering the protocol number in the blue field on the right side next to the arrows.

After modification of the existing pool of protocols, the entire Protocol Editor file (*.pro) should be saved to disk (see below). It can be saved under any name. The default name is DefProt.pro. If a custom *.pro file should be loaded into the Protocol Editor as a default, the new name of the *.pro file has to be specified in the Configuration window as Protocol File (see chapter 5.5 on page 47) and the Configuration File has to be saved.

LOAD : Loads the pool of available protocols (*.pro file).

SAVE : Saves the pool of available protocols (*.pro file).

Exampl1: The name of the present protocol is displayed in the blue field. To edit this name, click into the field. End editing either by pressing RETURN or by clicking with the mouse into another area of the window.

NEW: Creates a new protocol in the pool.

(DELETE) : Removes the present protocol from the pool.

STEP : Steps to the next line in the event list and executes the event.

TOEND : Runs the protocol through to its end.

Write : Writes the events to the Notebook window during execution of a protocol. The Write button should also be activated if you want to log the execution of single PGFs in the Notebook.

LIST: Lists the present protocol in the Notebook window.

MOVE : Moves the present protocol to another position in the protocol pool.

Record Macros: Activates the macro recording. In the following the command lines for all activated events are recorded and inserted into the active protocol. Press again on *Record Macros* to finish the macro recording.

Relative Value: Interprets the subsequent *Vhold* potential as a relative value ("True"). It is a macro command and has to be activated via the *Record Macro* button. It has to be repeated prior to each *Vhold* macro command.

10.2 Event Handling

In the event area, you can select events for the actual event list that should be performed during the experiment.



Events: Here the position of the present event in relation to the total number of events is displayed.

Insert Before / Insert After: Here you can choose a new event from the drop-down list. It will be positioned either above or below the present entry.

Duplicate: Duplicates the present event. The duplicate will automatically be inserted above the duplicated event.

Delete: Deletes the present event from the list without further notice.

Move: Allows you to move the present event in the event list to another position by entering a new position number.

Note: Multi-line events such as Repeat loops or conditional statements can not be moved.

Note: Since protocol files are stored in text format, editing of protocols with standard text editors is also possible.

10.3 Recurring Functions

These functions and information fields exist in every event area.

 \square Skip : Omits this step in the protocol execution. This option is included to allow the user a quick disabling of events without losing all settings.

Delay 0.00 s : Sets a delay time in seconds. Note that the delay is executed before the events.

Repeat Status	Repeat at line 2: 2 of 5		
IF etc. Result	Auto C-slow = 1.0000 < 1.0000 => FALSE		

Repeat Status: Displays the status of loops. The following example shows that the repeat step happens at line 2 of the protocol and that there have been 2 repeats in a 5 cycle loop.

IF etc. Result: Displays the last IF condition that has been triggered.

10.4 Events - Overview

The available events are categorized into eight groups. Not all of the events are explained in detail here. Please follow the given links to other chapters.

✓ Insert After	
Protocol Sequence	
Break	
Chain Protocol	
Clear Key	
Goto	
Goto_Mark	
IFThen	
ElselfThen	
Else	
Launch	
Macro Command	
Repeat	
Switch Window	
Wait	
Acquisition	
Acquire Each Sweep	
Acquire Properties	
Acquire Series	
Set Sweep Label	
Test Pulse	
Hardware	
Amplifier	23.0290 00210
Serial Output	Value/Parameter
Set DAC	PGF Parameters
Set Digital Bit	Set Solutions
Set Digital Word	Set Value
Data/Display	Messages
Analysis	Annotation
Digital Filters	Веер
Display Properties	Write Icon Value
Export	Extensions
File Operation	LockIn
Replay	ElProScan
Trace Buffer	Photometry/Imaging

10.4.1 Protocol Sequence

10.4.1.1 BREAK

Use to terminate a repeat loop or the protocol.

BREAK		Skip
Туре	End Protocol	

Type:

- End Protocol: This kind of *BREAK* stops the protocol execution completely at this point.
- End Repeat: This kind of *BREAK* ends the execution of a repeat loop (e.g. *Acquire Each Sweep...*) and jumps out of it to the following events. If this *BREAK* is used outside of a loop, the execution jumps to the end of the protocol.
- End If: This kind of *BREAK* ends the execution of an IF-THEN statement and jumps out of it to the following events. If this *BREAK* is used outside of a loop, the execution jumps to the end of the protocol.

10.4.1.2 Chain Protocol

Use this event to link one protocol to another protocol.

hain Protoco	ol 🗆 Sk	ip	Delay [0.00 s]
Protocol Na	ame				
Return	Edit Protocol	Se	elect Proto	col:	

Protocol Name: The name of the protocol is displayed in this field after one protocol is select in the *Select Protocol* selection list.

Return: If this option is not checked the protocol sequence will stop after executing the selected protocol. If the *Return* option is enabled the protocol sequence will come back to the main protocol and will be continued.

Edit Protocol: Opens the protocol named in the field Protocol Name.

Select Protocol: Select a protocol to define the link. After a selection is made the name of the protocol is displayed in the field *Protocol Name*.

10.4.1.3 Clear Key

Normally a keystroke is stored during the execution of a protocol until a new key is pressed. In case the key buffer has to be cleared, the *Clear Key* event can be used. This is typically used when a protocol should react once on a single key stroke (e.g. execute an acquisition). In case the key would not be cleared the protocol would react repeatedly until another key is entered.

Clear Key

10.4.1.4 GOTO

Use to jump to a *GOTO_Mark*.

GOTO	🗆 Skip	Delay	0.00 s
Mark			

Mark: Enter the name of the mark that denotes the destination of the GOTO jump (see below).

10.4.1.5 GOTO_MARK

Creates a GOTO_Mark.

GOTO_MARK	🗆 Skip	
Mark		

Mark: Enter the name of the mark for a possible destination of a GOTO jump.

10.4.1.6 IF...THEN

Allows the definition of conditional events. Note that the list of available channel variables depends upon the chosen source.

	🗆 Skip	Delay 0.00 s
Left Source		Right Source
ADC	>	Number
AD-0		0.0000

Left Source:

- ADC: AD channel to be read
- Digital In: Digital Input
- Parameters: Choose from a list of parameters, e.g., Imon, C-slow....
- Analysis: Choose from the list of available functions (1...16)
- Value: Use one of the Values 1...16 that can be defined in the event Set Value. In addition, results from the Analysis can be stored in these "Values" (see below). Therefore, the "Values" constitute an important interface between the Protocol Editor and the Analysis.
- Key: Input a key command
- Icon Value: Reads the value of an icon.
- Repeat Count: Enter a number that will be compared with the actual Repeat Count. If the Repeat Count is...then....
- Sweep Count: Enter a number that will be compared with the actual Sweep Count. If the Sweep Count is...then....
- Last Sweep: If this condition is true
- Sweep Aborted: In case a *Sweep* has been aborted with a *Break* command or condition in the Pulse Generator sequence.



- Selection Failed: If the defined condition for a selection fails the loop can be interrupted.
- Break Alert: The "IF Break Alert" event pops up a dialog with a title (can be specified by the user) and the two buttons *Break* and *Continue*. A click on *Continue* proceeds with the first event after the *IF...THEN* loop. *Break* executes the events within the *IF...THEN* loop.
- Batch Comm.: Checks if the last received batch control string contains a user-defined phrase (see Controlling CHARTMASTER in the CHARTMASTER Tutorial).
- Serial Comm.: Checks if the last received string of the serial port communication contains a user-defined phrase (see chapter 5.4.6 on page 45).
- Series Name: Checks if the last name of a Series contains the user-defined phrase.
- Overflow: Checks if an FIFO overflow occurred ("True") on the AD/DA board.
- True / False: Choose if the IF loop shall be used (True) or not (False).

Right Source:

- ADC: AD channel to be read
- Digital In: Dig-0...15. The operators for the Digital Input set the voltage.
- Parameters: Choose from a list of parameters, e.g., *Imon*, *C-slow*.... Additionally, the *Timer* parameter allows to time processes, e.g., reset a timer with the *Display Properties* event and measure time lapse since this event.
- Analysis: Function 1...16
- Value: One of the global variables (Value-1 to Value-n) can be specified.
- Number: User-defined values are entered manually.
- True / False: Choose if the IF loop shall be used (True) or not (False).

If Operators: Select the appropriate logical operator for the IF... THEN operation.

- >: Greater than
- <: Smaller than
- ABS >: Absolute value greater than
- ABS <: Absolute value smaller than
- =: Equal to
- <>: Between the ranges of
- MOD: A Modulo operation; it is an integer operation. The modulo operator will return TRUE, if the operation parameter value of *MOD* equals zero.
- invalid: Condition of a channel, value etc. has to be *invalid*.

10.4.1.7 ELSEIF...THEN

Allows a secondary conditional event, e.g. a check after an IF-THEN step. Works identically to IF-THEN, see above.

ELSIF THEN	🗆 Skip		
Left Source		Right Source	
ADC	>	Number	
AD-0		0.0000	



10.4.1.8 ELSE

Final step of the IF condition.

ELSE 🗆 Skip

10.4.1.9 Launch

The Launch event allows to start another application. The path to the application is defined relative to CHART-MASTER's home path.

aunch	🗆 Skip	Delay 0.00 s
Application		
Arguments		
Arguments		

- Application: Enter path where the application is located.
- Arguments: Enter command line arguments if necessary.

10.4.1.10 Macro Command

Use to execute an individual *Macro Command*. Many controls that can not be accessed via special events can be controlled using *Macro Command*.

A listing of all *Macro Command* can be plotted to the Notebook using the function *List All Macro Items* from the Help menu (see chapter 4.10 on page 39.)

Alternatively, you can view the *Macro Command* for individual icons via opening the Dialog Control window (see chapter 2.2 on page 10.)

Macro Command	🗆 Skip	Delay	0.00 s
Command			

Command: Enter the name of the Macro Command you wish to include, e.g., "E Zap".

10.4.1.11 REPEAT

Inserts a repeat loop. There are two types of repeat loops.

RE	PEAT	🗆 Skip	Delay	0.00 s
	Repeat Counts	Duration	0.00 s	
	No. of Repeats	Increment	0.00 s	

- Repeat Counts: Repeats for a given number of repeats.
- Repeat Indefinite: Repeats in an indefinite loop.

Duration: Time between repeats.

Increment: Increments duration.

Note: REPEAT events cannot be moved in the event list!

10.4.1.12 Switch Window

Brings the selected window in front.

Switch Window	🗆 Skip	Delay	0.00 s
Switch to Control Window			

In the drop-down menu you can select the a CHARTMASTER window you want to switch to:

√	Switch to Control Window	
	Switch to Configuration	
	Switch to Oscilloscope	
	Switch to Amplifier	
	Switch to Replay	
	Switch to Protocol Editor	
	Switch to Pulse Generator	
	Switch to Analysis	
	Switch to Analysis Window 1	
	Switch to Analysis Window 2	
	Switch to Parameters	
	Switch to I/O Control	
	Switch to Photometry/Imaging	
	Switch to LockIn	
	Switch to Spectroscopy	
	Switch to Solution Base	
	Switch to Solution Changer	
	Switch to Markers	
	Switch to Protocol Methods	
	Switch to Calculator	
	Switch to Notebook	

10.4.1.13 WAIT

Wait	🗆 Skip	
Wait Type	Absolute	
Duration	0.00 s	

Wait type:

- Absolute: Waits exactly for the given delay time after the previous event.
- Relative: Waits for the given delay time after the actual end of the previous event (e.g., plus additional delay times).
- Resume Icon: Waits until the Resume button is clicked (i.e. sets the WAIT button).
- Key: Waits until the specified key is entered. You can either enter a key or mark the Any Key option.
- Comment Alert: Will display a standard alert window where the user has to enter a new comment text. This comment will be used as long as no other is given.

10.4.2 Acquisition

10.4.2.1 Acquire Series

Used to execute an entire PGF sequence.

Acquire Series	🗆 Skip	Delay	0.00 s
Edit PGF Template	Select F	PGF-Temp	late
Sequence Comment	La	bel	
□ Averages 1 Break Keys			

Edit PGF Template: Opens the PGF template of the selected sequence in the Pulse Generator window.

Sequence: Indicates the name of the selected PGF.

Label: Enter a Series label, e.g. "Drug A".

Comment: Enter a comment to the Series. The entry is stored with the Series and therefore available in the Comment field for the Series in the Parameter window.

Averages: A PGF sequence can be repeated several times. Only the average is stored to disk. Please enter the number of averages acquired for one *Sweep*. If the box in front of the "Averages" label is not checked, then this setting is ignored.

Break Keys: Define a key to break the acquisition of the running Series.

10.4.2.2 Acquire Each Sweep

This event allows executing other protocol events between the acquisition of *Sweeps*. E.g. use the Amplifier event to execute an *AutoCSlow* update before acquisition of each *Sweep* in a *Series*. A repeat loop is inserted with an *Acquire Each Sweep* event inside.

REPEAT Each Sweep	□ Skip	Delay [0.00 s
	Duration	0.00 s	
	Increment	0.00 s	

Duration: Defines the total cycle time for one Sweep.

Increment: Sets a defined time increment for the Sweeps.

🗆 Skip	Delay 0.00 s
Select PC	GF-Template
Labe	el
	_
	Select PC

For a description of the Acquire Each Sweep event please refer to the Acquire Series event above.

10.4.2.3 Acquire Properties

Acquire Pro	perties	🗆 Skip	Delay	0.00 s
⊠ Set It	⊠Update R-me	mbrane an	d I-pipette	
□ Set It	⊠Wipe Display	at Start		
□ Set It	□Write Events	to Noteboo	ok	
🗆 Set Mir	n. Wait Time	100.00 r	ns	

Update R-membrane and I-pipette: Compute and update the calculated *I-pipette* and *R-membrane* after every acquired Sweep. Enable the checkbox Set It in front of this option if you really want to execute it.

Wipe Display at Start: Determines whether the display is cleared at the start of a protocol or not. Enable the checkbox *Set It* in front of this option if you really want to execute it.

Write Events to Notebook: Writes the events to the Notebook window. This function is identical to the Write button. Enable the checkbox Set It in front of this option if you really want to execute it.

Set Min. Wait Time: Sets the time CHARTMASTER reserves to wait for the correct time to start when executing individual repeat loops such as acquisition of a *Series* of *Sweeps* or an *Repeat* loop in the Protocol Editor. Enable the checkbox *Set It* in front of this option if you really want to execute it. The *Min. Wait Time* can also be set in the Configuration window (see chapter 5.3.4 on page 43).

Note: A long Min. Wait Time will make the program to react very slowly. A very short Min. Wait Time might reduce the accurate timing of the starting of events. The timing within an acquisition; e.g sampling rate, is not affected by this parameter. Sampling within a Sweep is always accurate.

10.4.2.4 Set Sweep Label

Allows to put a label on the Sweep to be acquired next.

Sweep Label	🗆 Skip	Delay 0.00 s
Sweep Label		
Set in Next Sv	weep	

Sweep Label: Enter the name of the label into this entry field.

Sweep Label Target:

- Set in Next Sweep: The Sweep Label is acquired in the next Sweep.
- Set in Selected Sweep: The Sweep Label is acquired already in the selected Sweep.

The Sweep Label can be edited after acquisition in the Replay window by pressing the Label button.

10.4.3 Hardware

10.4.3.1 Serial Output

Use to send a string to the opened serial interface. For configuration of the Serial Output see Configuration Window, chapter 5.4.6 on page 45.

Serial Output	🗆 Skip	Delay 0.00 s
String		

String: Enter a string that will be sent to a device via the Serial Output.

Examples:

GO	The command "GO" is send.
$GO \backslash r$	The command "GO" $+ <$ Carriage Return> is send.
$GO \ l$	The command "GO" $+ <$ Line Feed> is send.
$GO \ 032$	The command "GO" + ASCII Code 32 (<space>) is send.</space>

10.4.3.2 Set DAC

Set DAC		□ Skip D		lay	0.00 s
Channel	DA-0	Volt	age	0.	000V

Channel: Select a channel for analog output.

Voltage: Enter a voltage that will be output via the given channel.

Note: The value gets scaled as defined in the Configuration window.

10.4.3.3 Set Digital Bit

Set Digital Bi	t	🗆 Skip	Delay	0.00 s
Channel	Dig-0	□low		

Channel: Select a channel for digital output.

low: If selected, 0 Volt will be sent via the channel. If not selected, 5 Volt (standard TTL setting) will be sent.

10.4.3.4 Set Digital Word

Set Digital Wo	rd	Skip	Delay	0.00 s
Digital Word	0			
Digital Bits	0 1 2 3 4	56789		5

Digital Word: The entered number will be automatically translated into the digital bits. **Digital Bits:** Allows selecting the digital bits individually.

10.4.3.5 Set Solution Changer

Solution Changer	🗆 Skip	Delay 0.00 s
Off		
Set Marker		

Solution: Select a Solution (1-16) at the connected Perfusion System.

Note: The output channels and their aligned solutions have to be defined first in the Solution Changer dialog (chapter 16 on page 151).

Set Marker: If selected, a *Marker* will be set when the *Solution* is changed by the *Solution Changer* event. The label of the *Marker* can be entered in the entry field behind.

10.4.4 Data/Display

10.4.4.1 Analysis



Analysis Mode:

- Analysis: Keep: Preserves the current settings of the Analysis.
- Analysis: Use Given Method: Sets the Analysis mode to Use Selected Method and selects the Analysis Method specified in the field Name.
- Analysis: Use Selected Method: Sets the Analysis mode to Use Selected Method and uses the active Analysis Method.
- Analysis: Auto Stim Control Pool: Sets the Analysis mode to Automatic Stimulus Control: Pool and uses the Analysis Method specified in the PGF sequence.
- Analysis: Auto Stim Control Assigned: Sets the Analysis mode to Automatic Stimulus Control: Assigned and uses the Analysis Method which was assigned to the PGF sequence.
- Analysis: No Analysis: The Analysis is deactivated.

Name: Enter the Analysis Method you wish to start.

Edit: Opens the Analysis window with the given method for direct editing.

Wipe: Can be used to wipe individual graphs or all graphs at once.

- No Wipe
- Wipe All Graphs
- Wipe Graph 1...16

Set Graph Pos.: Activate the checkbox in front to apply the Graph Positions to the selected Analysis Method.

10.4.4.2 Digital Filters

Defines the cut-off frequencies of digital filters in Hz for Trace 1...16 (1 - 16) and Buffer 1...4 (B1 - B4). The checkbox is used to mark which of the filters are to be set.

Digita	al Filters		C] Skip	Del	ay 🗌	0.00 s
□1	0.000	□2	0.000	□3	0.000	□4	0.000
□5	0.000		0.000	07	0.000		0.000
□9	0.000	□ 10	0.000	□11	0.000	□ <u>12</u>	0.000
□13	0.000	□ 14	0.000	□15	0.000	□ 16	0.000
□B1	0.000	□B2	0.000	□B3	0.000	□B4	0.000

10.4.4.3 Display Properties

Use to control settings of the Display menu and the Oscilloscope window.

Display Properties	□Skip Delay 0.00 s
🗆 Wipe Osci 🛛 Wip	e Analysis 🔲 Reset Timer
Overlay Series: keep	Overlay Sweeps: keep
Subtract Leaks: keep	Subtract Zero: keep
Show Leaks: keep	Show Tree Info: keep
1234 □ Show Traces ⊠⊠⊠	4 5 6 7 8 9 0 1 2 3 4 5 6 XXXXXXXXXXXXXXX

 $\label{eq:Wipe Osci: Deletes all displayed data in the Oscilloscope window.$

Wipe Analysis: Deletes all displayed data in the Online windows.

Reset Timer: Resets the timer in the Oscilloscope window. These options are also described in section 4.5 on page 31.

Overlay Series and Overlay Sweeps:

- keep: Keep the state that was set before this event.
- ON: Overlay on.
- OFF: Overlay off.

Subtract Leaks:

- keep: Keep the state that was set before this event.
- ON: Subtract leak signals on.
- OFF: Subtract leak signals off.

Subtract Zero:

- keep: Keep the state that was set before this event.
- ON: Subtract zero offset on.
- OFF: Subtract zero offset off.

Show Leaks:

- keep: Keep the state that was set before this event.
- ON: Show leak signals on.

• OFF: Show leak signals off.

Show Tree Info:

- keep: Keep the state that was set before this event.
- ON: Show Tree Info in the Notebook on.
- OFF: Show Tree Info in the Notebook off.

Show Traces 1...9, 0...6: Changes the show flag of all 16 Traces. This function can be used to show only a subset of acquired Traces in the Oscilloscope window (see chapter 4.5 on page 31). Note that you have to mark Show Traces for the changes to take effect.

10.4.4.4 Export

Exports the acquired data according to the export settings made in the **Replay** menu. Unless you activate the checkbox *Full Sweep* only the displayed data is exported (see chapter 4.4 on page 26).

xport	🗆 Skip	Delay	0.00 s
Filename			
Target			
Overwrite	Full Sweep		

Filename: Define a name for exported file according to the auto filename components described in the **Configuration** (see chapter 5.5.2 on page 48).

Target: Declares the *Target Specifier* in the tree. 4 comma separated integers are allowed, specifying the index of *Group*, *Series*, *Sweep* and *Trace* (Gr_Se_Sw_Tr). "+" and "-" define relative index from the present selection. An index of "0" defines the present selection.

Examples:

- "0": Exports the present *Group*.
- "0,0": Exports the present Series.
- "0,0,0,0": Exports the present Trace.
- "0,-1": Exports the previous Series of the present Group.
- "1,4": Exports the 4^{th} Series of the 1^{st} Group.
- "1,1,4,1": Exports the 1^{st} Trace of the 4^{th} Sweep of the 1^{st} Series of the 1^{st} Group.

Overwrite: Enables to overwrite already existing output files.

Full Sweep: The full Sweep is exported independent of what is shown in the Oscilloscope.

10.4.4.5 File Operation

File Operation	🗆 Skip	Delay 0.00 s
Store Data: ke	ep Save a	fter Break: keep
Close File		
□ New Group	Label	
New Experiment	t Laber	
□ New File	□Update File	□File Status

Store Data:

- keep: Keeps the status that was set before this event (either ON or OFF of the Store button).
- ON: Acquired data will be saved to disk. This corresponds to the active Store button.
- OFF: Data will not be saved to disk. This corresponds to the deactivated Store button.

Save after Break:

- keep: Keeps the status that was set before this event (either ON or OFF of the Save after Break parameter in the Configuration window).
- ON: Acquired Sweep data will be stored if the Sweep was terminated with the Break command.
- OFF: Sweep data will not be saved to disk if the Sweep was terminated with the Break command.

Close File: The data file will be closed.

New Group, New Experiment, New File: A new group, experiment or file can be created. The ability to be able to create a new data file is very convenient when the auto file name generation is used.

Label: It is active when a new group or a new experiment is created and it specifies the group label.

Update File: Updates and saves the file.

File Status: Prints the file status to the Notebook (see chapter 4.1 on page 21).

10.4.4.6 Replay

Data of the opened file can be replayed.

Replay	🗆 Skip	Delay 0.00 s
Series	Select Last	
Marked Only		
Wipe Osci		
Wipe Online		

Select the target level of data which should be replayed:

- Nothing: Nothing is selected.
- Root: Select the *Root*.
- Group: Select the *Group*.
- Series: Select the Series.
- Sweep: Select the Sweep.
- Trace: Select the *Trace*.

The options refer to the target level selection (see above).

- Select Next: Selects the next target (no replay).
- Select Present: Selects the present target (no replay).
- Select Last: Selects the last target (no replay).

- Replay Next: Selects the next target and replays the data.
- Replay Present: Selects the present target and replays the data.
- Replay Last: Selects the last target and replays the data.

More options can be selected via the checkboxes:

Marked Only: Only marked data in the Replay tree will be affected.Wipe Osci: Deletes all displayed data in the Oscilloscope window.Wipe Online: Deletes all displayed data in the Online window.

10.4.4.7 Trace Buffer

Trace Buffer	🗆 Skip	Delay 0.00 s
Source Trace-1	no operation	Buffer-1
Clear Buffer		no replace
no operation		
Update Display	Update Analy	vsis

Trace Buffer Source: Define the data source you want to operate with.

Operation: Define the operation which should be applied to data before storing it in a *Buffer*.

- no operation
- $\bullet~$ add to
- subtract from
- accumulate in
- deaccumulate from

Target: Select the target Buffer (Buffer $1 \dots 4$).

Replace: Replaces the selected *Trace* with the *Buffer*

Clear Buffer: Clears the target Buffer before storing the data.

Scaling:

- no operation
- Scale: Scales the Trace Buffer Source (Trace is selected) or Buffer (Buffer is selected) with a specified Factor and Offset.

```
Scale Trace Factor 1.0000 Offset 0.0000
```

• Equation: Calculates the scaling of the *Buffer* via an equation. For details on the equation syntax please refer to Equation Syntax, chapter 18.1 on page 157.

	Equation	Edit	
1		5 (199) - C	

Update Display: Updates the display of the Buffer in the Oscilloscope window.

Update Analysis: Updates the display of the *Buffer* in the *Analysis* graphs.

10.4.5 Value/Parameter

10.4.5.1 PGF Parameters

PGF parameters can be set or modified during the protocol to automatically adapt the acquisition sequences.

PGF Parameters	🗆 Skip	Delay 0.	00 s
PgfParameter-4	=	0.0000	

PGF Parameters 1...10: Specify a PGF parameter to be set.

Operation: To modify a PGF parameter the following functions can be used.

- Functions working on manually entered Values:
 - =
 - increase by
 - decrease by
 - multiply by
 - divide by
- Functions working on global Values:
 - =value
 - inc by value
 - dec by value
 - mul by value
 - div by value

Value: Manually entered value or selection of a global Value (see PGF Parameters, chapter 9.14 on page 98).

Note: Please keep in mind that the stimulus amplitude is given relative to 1 V or 1 nA in voltage clamp and current clamp mode, respectively. I.e., to obtain a stimulus amplitude of 100 pA in current clamp mode you have to enter a value of "100m" (= 0.1 * 1 nA).

10.4.5.2 Set Solutions

Solutions	🗆 Skip	Delay 0.00 s
Set Internal	0]
□ Set External	0	Ī
□ Set Marker		_

Set Internal: User-defined index number for the internal solution. Activate the checkbox to apply the setting.

Set External: User-defined index number for the external solution. Activate the checkbox to apply the setting.

Set Marker: Set a solution marker when the solution is changed. In the entry field a label for the marker can be defined. Activate the checkbox to apply the setting. See also Solutions, chapter 15 on page 147.

10.4.5.3 Set Value

Allows you to freely define and store values that can be used in an IF... THEN event such that the If event can make decisions based on these stored values. The values can also be set via "batch control", thus allow triggering an IF decision by an external input, see Controlling Chartmaster in the CHARTMASTER Tutorial. In addition, results from the Analysis can be stored in these values (see Analysis Functions, chapter 11.3 on page 121). The values, therefore, constitute an important interface between the Protocol Editor and the Analysis.

Set	Value	C	Skip	Delay	0.00 s
	Value-1	=	0.0	0000	
	d	on't copy			

Value 1...16, User 1-2: Refers to the global Values and the User Parameters. User Parameters can be defined in the Configuration window, see I/O Parameters, chapter 5.7.2 on page 55.

peration: Set value to	=
• manually ontored values:	increase by
• manually entered values.	multiply by
o =	modulo
\circ increase by	keep
• multiply by	= item
\circ divide by	inc by item
∘ modulo	dec by item
\circ keep: no change to the value	mul by item
• on Itom:	div by item
• an item:	= param
$\circ = item$	inc by param
\circ inc by item	dec by param
\circ dec by item	mul by param
\circ mul by item	div by param
\circ div by item	
• Parameters	= value
• 1 arameters.	inc by value
$\circ = param$	dec by value
\circ inc by param	mul by value
\circ dec by param	div by value
\circ mul by param	= analysis
\circ div by param	inc by analysis
Clobal Values:	well by analysis
• Giobai values.	div by analysis
$\circ = $ value	- equation
\circ inc by value	= input
\circ dec by value	= batch comm. value
• mul by value	
\circ div by value	
• Analysis results:	
$\circ = $ online	

- \circ inc by online
- \circ dec by online
- \circ mul by online

0

- \circ div by online
- equation results: = equation. An equation can be entered to compute a value. For details on the equation syntax please refer to Equation Syntax, chapter 18.1 on page 157.
- = input: A dialog opens for user input of a new numeric value.
- = batch comm. value: A value received via the batch communication interface is assigned to the respective value.

Value:

- in case of constant value: Enter manually the constant value.
- in case of *Item* operation: Enter the item ID, e.g. "E CFastTot" to read the value of a program control (here compensated *C-fast* value). For a description how to get info on the control IDs please refer to Modifying Dialogs and Controls, chapter 2.2.3 on page 13.
- in case of *Parameter* operation: Choose a parameter from the selection.
- in case of Value operation: Choose one of the 16 global Values.
- in case of Analysis operation: Choose one of 16 Analysis results.

Copy:

- don't copy: No further operation.
- copy to item: Copies the value to a specified item.
- add to item: Adds the value to a specified item.
- multiply to item: Multiplies specified item with the value.

10.4.6 Messages

10.4.6.1 Annotation

Use this function to add comments to your protocol and improve the readability.

An	notation		
	Annotation		

Text can be entered that will be shown in the event list.

10.4.6.2 Beep

Use to execute acoustic alerts.

Beep				
	🗆 Skip	Delay	0.00 s	
			-	

An acoustic signal will be output. This only works if your computer is properly equipped for sound (e.g. sound card, loudspeakers...).

10.4.6.3 Write Icon Value

Outputs the value of the specified icon into the Notebook like this: [Title] [ItemID]: [value]

Write Icon V	alue	🗆 Skip	Delay 0.00 s
Title			
Item ID			
	☑ Notebook☐ Alertbox	🛛 LF	Icon Text

Title: Title for the Item ID, leads to an improved readability of the output but is not mandatory.

Item ID: Unique identifier of the icon. Can be inspected by opening the **lcon configuration** dialog (see Modifying Dialogs and Controls, chapter 2.2 on page 10) – it is given in the title of that window.

Notebook: Writes to Notebook.

Alertbox: Opens an Alertbox containing the text.

LF: When LF is on, a linefeed signal is appended.

Icon Text / Icon Value:

- Icon Text: Writes the text from a field. For example from the icon E Gain would return "1.0 mV/pA".
- Icon Value: Writes the value of a field. For example from the icon E Gain would return "8.0", meaning the 8th item of the Gain list corresponding to "1.0 mV/pA".

10.4.7 Extensions

10.4.7.1 Photometry/Imaging

Note that the Photometry Extension has to be active to allow the editing of the following events (see Configuration, chapter 5.4.5 on page 45 and Photometry Extension, chapter 19 on page 163).

Photometry	🗆 Skip	Delay 0.00 s
□ New Resting Waveler	igth 0	
□ Set Resting Wavelen	gth	
□ Set New Wavelength	0	
□ Set New Speed	0	
□ Open Shutter		
Close Shutter		

New Resting Wavelength: Sets the variable Resting Wavelength to the given value.

Set Resting Wavelength: Sets outputs *Resting Wavelength*. Note that this function is called "Close shutter" for those systems that have a shutter. In this case, the option New Resting Wavelength disappears.

Set New Wavelength: Sets a specified wavelength.

Set New Speed: Sets a new speed setting of the filter wheel.

Open Shutter / Close Shutter: Note that this function is called *New Resting Wavelength* for those systems without shutter (see above).

Note: The amount of available options for the Photometry event depend on the type of photometry device selected in the Configuration window.

11. Analysis Window

Analysis: DefAnalChart	
Auto Stim C	ontrol: Pool
Analysis Methods: Analysis Pool	
	3 4
Load Merge Save Cha	rt New Delete Move
Analysis Functions	List
1 Timer 2 Mean_1	3 Extr_2 4 00
Function Type Timer Time Change	New Delete Move
Name Timer	
Input	Output
	🛛 Notebook
	Not Stored in Values
Cursors	Operation Options
Cursor Bounds (%) 0.0 100.0	
Analysis Graphs	
Save to Tree Conv Print	
Scale Axis Overlay Wrap + wipe (Granh Entries
Min Max Scale	
X 0.00 30.0 Fixed X	Timer Timer Mean_1 Mean_1
Y -10.0 10.0 Fixed Y	Mean_1 Extr_2 Mean_1 Mean_1
(<u>Modify Axis</u>) ⊡Share X-axis (
Graph Positions 1234567890	1 2 3 4 5 6
Graph in Window 2	

The Analysis allows you to immediately calculate and display data based on the acquired *Traces*, thus giving you a fast overview of your results.

CHARTMASTER will automatically plot the analysis to Analysis Windows 1 or 2 after or during execution of a Series (based on the settings made in the various controls inside this window).

Nomenclature: The Analysis is structured as follows. An Analysis file with the extension *****.onl holds a set of Analysis Methods. Each method is an assembly of Analysis Functions and Analysis Graphs. Based on the type of incoming data, the user can specify a number of Analysis Functions. These functions produce analysis results which may be displayed in the Notebook and/or shown in an Online Graph inside either Analysis Window 1 or Analysis Window 2. Elements of a graph are Graph Entries, i.e. couples of analysis results to be used as X- and Y-references. Up to 4 Graph Entries can fit into one graph and multiple graphs can fit into one Analysis Window.

Thus, based on the hierarchy of the components involved in constructing an Analysis Method, Analysis Functions must first be defined because only then the respective analysis results can be placed as Graph Entries in graphs and windows.

11.1 Stimulus Control

Stimulus Control: There are four possible options for controlling the particular *Analysis Method* of choice:

- Use Selected Method: Uses the active (selected) Analysis Method.
- Auto Stim Control: Pool: Uses the Analysis Method stored in and loaded from the Analysis file, e.g. 'DefAnal.onl'. With this kind of Analysis one defines one Analysis Method for all Series until the Analysis Method is changed.
- Auto Stim Control: Assigned: Uses the Analysis Method stored in and loaded from the data file bundle. With this kind of Analysis one creates an permanent link of an Analysis Method to a type of Series.
- No Analysis: No Analysis will be performed.

11.2 Analysis Methods

Analysis Methods: Analysis Pool								
001	IV	2	Ramp	3 (Recover	_4	(Hinf	
Load) (Merge) (Save		IV	New		Delete)(Move)

An Analysis Method is defined by all the information stored in the areas of Analysis Graphs and Analysis Functions. CHARTMASTER provides a pool of such Analysis Methods and such pools of methods are stored on disk as Analysis files (*.onl).

1...4 DO: Scrolling bar of available Analysis Methods within this pool. The active (selected) Analysis Method is highlighted.

Load: Loads a pool of Analysis Methods.

Merge: Adds Analysis Methods from an Analysis file (*.onl) to the already loaded pool of methods.

Save: Saves the actual pool of Analysis Methods.

Analysis Method Name: Text field in which the name of the current *Analysis Methods* can be edited – "IV" in this example.

New: Creates a new Analysis Method.

Delete: Deletes the active Analysis Method.

Move: Changes the positioning of a particular Analysis Method within the scroll bar.

If Auto Stim Control: Assigned is selected the three buttons Load, Merge and Save are replaced by:

Analysis Methods: Assigned Pool			
<mark>₫₫</mark> 1 IV 2	3	4 (<mark>0</mark> 0
(Duplicate) Assign To Pool	IV	New	Move

Duplicate: Makes a copy of the currently selected *Analysis Method*.

Assign: Assigns the active Analysis Method to the selected Series in the data tree. The corresponding PGF template is automatically linked to this Analysis Method.

To Pool: Copies the active Analysis Method to the standard analysis pool (Auto Stim Control: Pool).

11.3 Analysis Functions

In this section of the Analysis dialog, Analysis Functions can be added to the Analysis Method and parameters of already existing Analysis Functions can be modified.

New Analysis Functions can be added by clicking on an empty button in the list at the top of the section. For changing an already existing Analysis Function just click on the Change button (see below).

List: Writes a list with the name of all Analysis Functions into the Notebook window.

Function Type: Here the *Function Type* of the presently activated *Analysis Function* is displayed, e.g. "Amplitude".

Name: Here you can rename the selected Analysis Function Type.

Change: Click on this button to open the Function Type dialog for changing the Analysis Function.

New: Creates a new Analysis Function. You can also create a function by clicking on an empty Function button.

Delete: Deletes the selected Analysis Function.

Move: Changes the position of a particular Analysis Function within the scroll bar.

11.3.1 Analysis Function Settings

The Analysis provides four sections for customizing the selected Analysis Function. Here, we describe which settings are in general available. The availability depends of course on the selected Analysis Function and may vary from function to function.

Input

Input						
Use Given Value 0.00						
Y-Trace	T	race 1				
X-Trace Compute Theoretical Amplitud						

Value: Either use "Use Given Value" entered into the field beneath the function or "Get Value-1...16" which takes the value stored in *Value-1...16*.

Y-Trace: Select which Trace (1...16) or Buffer (1...4) shall be analyzed using the Analysis Functions. The Trace index usually corresponds with the channel number in the PGF sequence (if the Trace number is not set to another value in the build instruction of the Trace (see 9.9.2 on page 91)).

X-Trace: The voltage can either be calculated from a theoretical stimulus (*Compute theoretical Amplitude*), e.g. when there is no voltage *Trace* available, or it can be taken from a recorded *Trace* (*Amplitude from Trace* 1...16, *Amplitude from Buffer* 1...4).

Threshold: Sets the Threshold value for one of the AP Analysis functions.

Threshold	20.0 %
Tra	ace 1
	Threshold Tra

Operation: Refer to the result of an already existing *Analysis Method*. In this example the *Math* operation 'a + b' refers to the *Analysis Method* index '3' and '4'.



Cursors

Cursors X-, Y-seg. Offset	0	0		
Cursor Bounds (%)	0.0	100.0		
Cursors Relative to Segment				
Adjust to Position of	Off			

X-, Y-seg. Offset: The X- and Y-Segment offsets are set relative to the *Relevant X- and Y-Segments* as defined in the Pulse Generator window.

Cursor Bounds: Within the relevant segment, a left bound and a right bound (in %) determine the actual time period for analysis. Pressing the button lets pop-up the cursors in the Oscilloscope window. When moving the cursors in the Oscilloscope window, the cursors position is automatically transferred to the fields to the right of the button. The cursor limits can be outside the limits of the selected segment, i.e., they can be smaller than 0 % and greater than 100 %.

Cursor Type: Sets the definition of the cursor type. The following options are available:

- Cursors relative to Segment
- Cursors relative to Trace
- $\circ\,$ Cursors: Copy from Function-1...16: The active function gets the cursor bounds and cursor type from the selected function.

Adjust to Position of Function: Specify an existing Analysis Function to set the cursor bounds relatively to the found position of a peak or threshold. E.g. if one function analyzes the *Time to Maximum* and the second function analyzes the *Tau*, one can use Adjust to Position of Function in the second function to set the cursor settings in respect to the found *Time to Maximum* results. This means every time the *Time to Maximum* value changes the cursor settings (here: 1-15%) for the calculation of *Tau* will be adapted.



Note: Be aware that in this example the cursor range for the Tau function gets shifted to the right by Time to Maximum because the new 0% value is now the value of Time to Maximum.

Output

Output			
	Notebook		
	Not Stored in Values		

Notebook: If selected, the Analysis Result of this function will be written into the Notebook window.

Not Stored in Values / Store in Value-1...16: Analysis Results can be stored in one of the Values-1...16. They can then be retrieved and used as a Constant math function. In addition, they can be used in the Protocol Editor (see Set Value, 10.4.5.3 on page 116). The Values-1...16 are used to exchange information between different Analysis Methods and between the Analysis and the Protocol Editor.

Operation Options

Operation Options	
🗆 Fit	
Baseline: Zero	

Fit: If selected, the peak value is determined by a polynomial fit around the first numeric estimate of the peak. With this function, the influence of noise can be reduced in the determination of *Peak Amplitudes*.

Important note: The fit function must not be used when non uniformly continuous changes are expected around the peak value. E.g. if you want to analyze the peak value of a saw-tooth signal, the Fit function easily lead to erroneous results, because of the quick jump from the peak level to base line at the end of the saw-tooth.

Baseline: Define the Baseline for the selected Analysis Function. There are three options available:

- Baseline: Zero: Calculates the integral between the two intersection points of the cursors and the *Trace*, starting at the zero line.
- Baseline: Cursor Intersections: Calculates the integral, starting at a virtual baseline between the two intersections points of the cursors and the *Trace*.
- Auto Bounds: Makes a derivation of the *Trace* and sets the cursors to the minimum and maximum value of the derivation. This option can be helpful to find the proper peak if some other global maximum (or minimum) exists.

Operation Options		
Positive and	Negativ	e Crossing
Average Points	1	

Crossings: Set the direction of the Crossing. There are three options available:

- Positive and Negative Crossing: Every threshold crossing is detected.
- Positive Crossing: A crossing is only detected if the signal runs through the threshold from negative to positive values.
- $\circ\,$ Negative Crossing: A crossing is only detected if the signal runs through the threshold from positive to negative values.

Average Points: Enter the number of points to be used for a running average to apply before extracting the voltage. The function applies to the source *Trace* as well as to the result *Trace*.

Ramps only: The Analysis Function does only work on Ramp segments if the checkbox is selected.

Operation Options		
□ Ramps only		
Equation Edit		

Equation: Enter an equation into the text field or modify an already existing one by clicking on the *Edit* button.

Histogram Bins: Enter the number of bins for the histogram.

Operation Options		
Histogram Bins (128	

11.3.2 Analysis Function Types

When selecting or changing an Analysis Function the Function Type dialog pops up. Here, you can select an Analysis Function. The functions are organized in several groups.

Timina	Moasuromonts	Lockin	Math	Traco	Dowor Coostra
Count	∩ Extromum		∩ Equation	1 Trace	() log(Eroquonov)
O Assessia ladau	O Maviation	O Lockin_Phase	C V(v) v et ess = v	O Faustian	O Density
O Analysis index	O Maximum	C LOCKIN_Freq	O f(x). y at pos – x	CEquation	O Density
O Time			() Constant	Q Q = Integral	
Timer Time	C Extr. Amplitude	Trace Param.	()a+b	() 1 / (trace)	Histogram
O Series Time	O Min. Amplitude	C Trace Count	🗘 a - b	01/(Q)	O Histogram Ampl
O Real Time	O Max. Amplitude	O Int. Solution	⊖a*b	O In (trace)	O Histogram Bins
	O Time to Extremum	O Int. Sol. Value	⊖ a / b	O In (Q)	
Stim. Properties	O Time to Maximum	O Ext. Solution	🔾 a in b	O log (trace)	
O Amplitude	O Time to Minimum	O Ext. Sol. Value	O abs	O log (Q)	
O Duration	C Time to Threshold		O log	🔿 dt = Differential	
O Rel. Seg. Time	O Threshold Ampl.	Sweep Param.	O sqrt	O Trace x-axis (time)	
O Abs. Seg. Time	O Thres. Crossings	O Temperature	O arctan	O Stimulus	
O Scan Rate	O Mean	O Digital-In	O 1/a		
	O Variance	O User_1	O 1/log		
	O Integral	O User_2	O 1/sqrt		
	O Anodic Q		O 1/arctan		
	Cathodic Q				
	C Zero Crossing				
	Slope				
	O Intercept				
	() Tau			Cancel	Done

11.3.2.1 Timing

Sweep Count: Index of the Sweep within a Series.

Analysis Index: Index of the Analysis during the experiment. The Analysis Index is initialized with a Wipe command. The maximum number of Analysis Sweeps that can be handled, can be set in the Configuration window (see chapter 5.6.2 on page 50).

Time: Time starting with the relative "first sweep" analyzed (or created) since the last Wipe Analysis command.

Timer Time: Time of the *Timer* in the Oscilloscope at the start of *Sweep* acquisition.

Series Time: Time in respect to the Sweep acquisition of the corresponding Series.

Real Time: Time elapsed since midnight at the start of Sweep acquisition.

11.3.2.2 Stim. Properties

These are functions that are usually used as an X-reference in a graph. They are based on the *Relevant X-segment*, i.e. they are also sensitive to the X-segment offset.

Amplitude: Amplitude of the Relevant X-segment (usually "Voltage").

Duration: Duration of the *Relevant X-segment*. This may be useful if the length of the *Relevant X-segment* is changing during sweep acquisition.

Rel. Seg. Time: The *Relative Segment Time* function returns the start time of the *Relevant X-segment* with respect to the first stored point of the stimulus. The duration of non-stored segments is not taken into account.

Abs. Seg. Time: The Absolute Segment Time function returns the start time of the Relevant X-segment with respect to the first point of the stimulus. In opposite to Relative Segment Time the duration of non-stored segments is taken into account.

Scan Rate: Scan rate of the Relevant X-segment. Can be used e.g. to analyze the slope of a ramp.

Note: Do not mix up the Scan Rate with the Slope analysis function! While the Slope is calculated from the recorded Trace, the Scan Rate is taken from the stimulus.

11.3.2.3 Measurements

These are functions that are usually used as an Y-reference in a graph. They are based on the relevant Y-segment, i.e. they are also sensitive to the Y-segment offset.

Extremum: Extreme value of data in the cursor region; either a maximum or a minimum.

Maximum: Maximum value of data in the cursor region.

Minimum: Minimum value of data in the cursor region.

Extr. Amplitude: Calculates the voltage applied at the position of the peak current (either minimum or maximum).

Min. Amplitude: Calculates the voltage applied at the position of the minimum peak current.

Max. Amplitude: Calculates the voltage applied at the position of the maximum peak current.

Time to Extremum: Time from the beginning of the *Trace* or the relevant segment to the extreme value in the cursor range.

Time to Maximum: Time from the beginning of the *Trace* or the relevant segment to the maximum value in the cursor range.

Time to Minimum: Time from the beginning of the *Trace* or the relevant segment to the minimum value in the cursor range.

Time to Threshold: Time from the beginning of the *Trace* or the relevant segment to the specified threshold value in the cursor range.

Threshold Ampl.: This function returns the corresponding Y-axis value (often called amplitude) of the threshold crossing point. Use this function e.g. if you want to know the current value for a specific (recorded) voltage value.

Thres. Crossings: Counts the number of threshold crossings between the two cursors.

Mean: Mean value of the data in the cursor region.

Variance: Variance value of data in the cursor region (square of standard deviation).

Integral: Integral value of data in the cursor region.

Anodic Q: Integral of positive current in the cursor range.

Cathodic Q: Integral of negative current in the cursor range.

Reversal: This function searches for the zero crossing of the target *Trace* and computes the corresponding value of a second *Trace* (i.e. voltage).

Intercept: Calculates the intercept (a) in the defined region, using a linear regression (y = a + bx).

tau: Calculates the time constant (τ) from an exponential curve. Computes τ in two steps:

- 1. semi-logarithmic regression of x vs ln(y)
- 2. abs(1/slope)

11.3.2.4 Lock-In

The following parameters can be retrieved from the LockIn Extension. Please note that the LockIn returns a mean value of all data of the Trace. In case a mean value in the cursor range is desired, then please acquire/store the parameter as individual Trace and use the Mean function of the Measurements.

LockIn_Phase: Returns the phase setting of the software LockIn.

LockIn_Freq: Returns the frequency of the sinusoidal wave used for LockIn measurements.

11.3.2.5 Trace Parameters

The following parameters can be retrieved for analysis from the parameter set that is stored with the data (see Parameter window, 12 on page 135).

Trace Count: Gives back the index number of the *Trace* which is set in *Trace* #. If no *Trace* with the index number exists a "NAN" result will be reported.

Int. Solution: Index number of the internal solution.

Int. Sol. Value: Relevant concentration value of the internal solution.

Ext. Solution: Index number of the external solution.

Ext. Sol. Value: Relevant concentration value of the external solution.

11.3.2.6 Sweep Parameters

The following parameters can be retrieved for analysis from the parameter set that is stored with the data (see Parameter window, 12 on page 135).

User_1: Parameter as set by the user in the I/O Control section of the Configuration window, 5 on page 41.

User_2: Parameter as set by the user in the I/O Control section Configuration window, 5 on page 41.

Temperature: Temperature (from a recording device).

Digital-In: State of the Digital-In port of EPC 10 amplifiers.

11.3.2.7 Math

Math supplies various calculations on one or two function results. By combining several *Math* functions, complex expressions can be generated.

The following control shows: "Result 1" "- Operand" "Result 2".



Equation: An equation can be entered to compute a value. For details on the equation syntax please refer to the section **Equation Syntax**, 18.1 on page 157.

Y(x) : y at pos = x: Calculates the Y-value at a defined X-position (e.g. time, frequency...). The X-position value can be entered into the field beneath the function.

Constant: Sets a constant value if Use Constant Value is selected. The constant value may be determined either by entering a value in the input field beneath the function name or by using a value (see also function Y(x): y at pos = x above.)

 $\mathbf{a} + \mathbf{b}$: Calculates the sum of two Analysis results.

Note: Depending on the Math function one or two results of the Analysis are available for the calculation.

 ${\bf a}$ - ${\bf b}{\bf :}$ Calculates the difference of two Analysis results.

 \mathbf{a} * b: Calculates the product of two Analysis results.

a / b: Calculates the quotient of two Analysis results.

a in b: Calculates the amount of one Analysis result that is present within another Analysis result.

abs: Calculates the absolute value of an Analysis result.

log: Calculates the logarithmic value of an Analysis result.

sqrt: Calculates the square root of an Analysis result.

arctan: Calculates the arc tangent of an Analysis result.

1 / a: Calculates the reciprocal value of an Analysis result.

1 / log: Calculates the reciprocal logarithmic value of an Analysis result.

1 / sqrt: Calculates the reciprocal square root of an Analysis result.

1 / arctan: Calculates the reciprocal arc tangent of an Analysis result.

11.3.2.8 Trace

This analysis is applied to a selected Trace. The result is again a Trace.

Trace: Sets a Trace as an Analysis result.

Equation: An equation can be entered to compute a *Trace*. For details on the equation syntax please refer to the section Equation Syntax, 18.1 on page 157.

Q=integral: Calculates the integral over time of the *Trace*.

1 / (trace): Calculates the reciprocal of a Trace.

1 / (Q): Calculates the reciprocal of the integrated Trace.

ln (trace): Calculates the natural logarithm of the Trace.

In (Q): Calculates the natural logarithm of the integrated Trace.

log (trace): Calculates the logarithm of the Trace.

log (Q): Calculates the logarithm of the integrated Trace.

dt = Differential: Calculates the differential over time of the recorded Trace.

Trace x-axis (time): Returns the Trace time (first data point of Trace has time = 0). You may use this as X-reference when plotting a Trace versus time in the Online Window.

Stimulus: The stimulus that is linked to the Trace is returned.

11.3.2.9 Power Spectra

For calculation of the power spectrum. Useful for measurement of the noise performance. Please note, that at least 1024 data points are required for compiling such a power spectrum.

log(Frequency): Returns the log(Frequency) for a *Power Spectrum*. It should be used as X-axis in a *Power Spectrum* display. E.g. a value of '2' means 100 Hz, '3' means 1000 Hz.

Density: Returns the log(Distribution) of a Power Spectrum. The distribution is also called spectral density. It should be used as Y-axis in a Power Spectrum display. The displayed values are the linear exponents, e.g. '-25' means '1 $\times 10^{-25}$ '. The unit is in A²/Hz.

11.3.2.10 Histogram

The histogram functions can be used e.g. for a fast online detection of different amplitude levels.

Histogram Ampl: Returns the *Histogram Amplitude* distribution. You can select the *Trace* from which the amplitude should be calculated.

Histogram Bins: Defines the number of *Histogram Bins* for the analysis of *Histogram Amp*. The number of bins is entered manually in the *Function Type* window:

The width of a bin is computed as:

bin width = Y-range / number of bins

Note: A fixed Y-range is used to analyze all Traces of one Series. In Fixed mode it is the Y-range of the first analyzed Trace, in Auto mode it is the largest Y-range of all analyzed Traces of the parent Series.

11.4 Analysis Graphs

Ana	Analysis Graphs							
0	(小) Graph 1 On Graph 2 On Graph 3 On On On							
S	ave to Tre	e Co	py Prin	t (Redraw			
Sca	Scale Axis Overlay No Wrap Graph Entries							
	Min	Max	Scale		⊠ 1	□ 2	□ 3	□ 4
X	-100.m	100.m	Auto Ser.	X	Ampl_1	Ampl_1	Ampl_1	Ampl_1
Y	-200.p	2.00n	Auto Ser.	Y	Mean_1	Mean_init	Ampl_1	Ampl_1
	Modif	y Axis	Share X-a	xis	()	()	()	()

Here, the properties of the graph display for the Analysis results are defined.

Graph 1, Graph 2, Graph 3 - DO : Scrolling bar of Analysis Graphs (currently to a maximum of 12 graphs). All information shown in the lower part of this panel is only valid for the highlighted graph.

Graph 1 On (Graph Display Mode) : A graph can either be turned On or Off.

- On: Displayed in Analysis Window 1 or Analysis Window 2.
- Off: Not displayed.

Save to Tree: Saves the active Graph Entries of the selected Analysis Graphs to the end of the data tree as a new Series.

Copy: Allows to automatically copy the settings (functions, scaling etc.) of a graph into the selected graph, rather than having to enter each entry manually. Enter the index number of the graph from which you want to copy the settings.

Print: Prints the Analysis results as displayed.

Redraw: Redraws the Analysis results displayed in the Online windows, e.g. after changing the axis scaling.

11.4.1 Scale Axis

Overlay: The default setting in the Analysis is to wipe the data before a new Series is started. If you mark the *Overlay* option, the graph plot in the Online window will not be cleared when performing a new analysis (new Series acquisition).

Scale Axis		Overlay	No Wrap
Min		Max	Scale
Х	-100.m	100.m	Auto Ser.
Y	-200.p	2.00n	Auto Ser.
Modify Axis			Share X-axis

Note: This setting is overruled by the setting Wipe Display at Start and Wipe at Start (see 10.4.2.3 on page 108 and 5.6.2 on page 50).

Wrap: Selecting *Wrap* leads to a wrapping of the data at the end of the display, e.g. the display will start again at the left edge.

- No Wrap: No adaption of the X-axis scaling when the amount of data points exceeds the X-axis scaling.
- Wrap + wipe: Wiping of the online graph after the wrapping around. This behavior is typically used during Chart Recording (see CHARTMASTER Tutorial).
- Wrap, no wipe: No wiping of the online graph after wrapping around. Old data points are overlayed by newer data points when the *Series* exceeds the length of the graphical display.

Note: The Wrap option is limited to Fixed Scaling of the X-axis and Y-axis and the X-axis mode has to be linear.

If both Overlay and Wrap are selected, the data will wrap around at the end of the graph without clearing the graph.

More complex and custom display behavior can be controlled from a protocol, e.g. wiping individual graphs only, by using the Analysis protocol event (see 10.4.4.1 on page 110).

In case *Trace* data are displayed in the graphs, the options *Overlay* and *OvrlSwp* are available.

Scale Axis		⊠ Overlay	⊠OvrlSwp	
2	Min	Max	Scale	
Х	-3.40m	120.	Auto Swp.	
Y -877.n		40.5µ	Auto Swp.	
		fy Axis	Share X-ax	d

- No selection: If neither *Overlay* nor *OvrlSwp* is selected the online graph is wiped after every *Sweep* acquisition.
- Overlay: General overlay, see also description above. Overlays Sweeps of different Series. Compare to Overl. Ser in the Oscilloscope window.
- OvrlSwp: Overlay Sweep function overlays all Sweeps within a Series. Compare to Overl. Swp in the Oscilloscope window.

Note: The autoscale functions Auto Ser. and Auto Swp. are incompatible with the OvrlSwp function. Only the last Sweep will be drawn after re-scaling the graph.

 $\mathbf{X},\,\mathbf{Y}\text{:}$ Here you can enter the analysis display settings.

- Min: Minimum of axis range.
- Max: Maximum of axis range.
- Scale: There are several possible scaling options available once you click on the Scale field.
 - Fixed: Analysis results can be shown in the graph immediately after acquisition or replay of a Sweep, if the scaling is known from the beginning. Therefore, a Fixed Scaling option is provided and the X-, Y-scaling of the analysis graph can be specified.
 - Auto after a Series: After all Sweeps of a Series have been acquired or replayed, the maximal and minimal values of the currently selected abscissa and ordinate values are determined and used to scale the graph.

- Auto after each Sweep: After every Sweep, the maximal and minimal values of the currently selected abscissa and ordinate values are determined and used to scale the graph.
- Fixed with last Min/Max: Uses the maximum/minimum from the last graph drawing to copy into the Min/Max fields. They can now be used as scaling factors when the mode is switched to Fixed.

Modify Axis: Clicking on this button opens the Scale Properties window.

- Unit: The units of the first graph entry are shown on the graph display.
- Zero-line: The zero-line is depicted in the online graph.
- Position: Y-(X)-min, Y-(X)-zero, Y-(X)-max). Determines the position of the axis either at the minimum, at zero or at maximum of the other axis, respectively.
- Mode: Defines how the respective axis is drawn: linear, log, inverse, sqrt, square.
- Tics / Direction: Sets the number of tics plotted on the axis. A value of zero suppresses the display of axis tics. Direction *up*, *down*, *both* determines in which direction the tics point.
- Grid, Factor: A grid will be displayed in the given Analysis Graph. Enter a Factor for the grid density. If Factor is set to "1" then one line per Tic is drawn. A factor <1 increases the grid density.





- Include Zero: The Y- and X-axis always contains "0" at their origin independent of wrapping events.
- Nice Values: If selected, the axis limits will be rounded to the next possible "nice" value (e.g. 0, 1, 2, 5...).
- Centered: If selected, the first data point will be set in the center of the graph. This allows the monitoring of ongoing experiments. The displayed scaling defined by the values entered in the *Fixed Scaling* is not considered as an absolute value range but defines a relative one.

Note: This option is only available if Fixed Scaling is on and if Nice Values is off.

• Labels: If selected, the name of the Analysis Function is used as label for X- and/or Y-axis

- Header: Text for the graph header can be entered.
- Normalize: Select the type of normalization of the data:

None
y' = y / max
y' = (y - min) / (max - min)

• Sort: If selected, the data will be sort the data according to ascending X-values.

Note: The Sort flag should be set for summary data and non-ordered, non-continuous Traces before performing the Save to Tree operation.

Share X-axis: If *Share X-axis* is checked, then the same X-axis parameters (e.g. "Min", "Max", "Scale") are used for all graphs

11.4.2 Graph Entries

1...4: Activate up to four graph entries from this list by checking the box.

	Graph Entries				
	⊠ 1	□ 2	□ 3	□ 4	
Х	Timer	Timer	Timer	C-slow_1	
Y	C-slow_1	TraceX_1	Trace_1	C-slow_1	
	<u> </u>	<u> </u>	<u> </u>	<u> </u>	

Axis: Now choose from the list of available analysis result variables for the X- and the Y-axis. An example could look like that shown on the right in the diagram above.

The following variables are shown in the figure beneath:

Max_1: Maximum value between two cursor of Trace 1.
Timer: Time of the Timer.
C-slow_1: C-slow value of Trace 1.
Int_1: Integral value between two cursors of Trace 1.
5...16: Empty item.
Select by index...: Select the variables by their index number. This is necessary when more than 16 analysis results are defined.



Clicking on _____ opens the Marker Properties window:

Marker Propert	ies	
Туре	Square	
Size	3	
Connect		
Set	Color)	
Same C	olor as Trace	
Ca	ancel	Done

Type: Choose between the available symbols (point, plus, star, diamond, cross, square).

Size: Size of the symbols.

Connect: If selected, the symbols will be connected with a line.

Set Color: Choose a color for the symbols and the lines.

Same Color as Trace: Use the Trace color for the symbols and the lines. The Set Color setting is ignored.

11.5 Graph Positions

Graph Positions	123456789012
Graphs in Window 1:	880000000000000000000000000000000000000
Graphs in Window 2:	

Graph Positions: In this section of the Analysis dialog, one can specify if and to what Analysis window (1 or 2) a graph is plotted. The currently selected graph (in the *Graph Entries* section) is indicated by red color.

Note: If a graph is not checked in the Graph Positions section, then it is not displayed, even if it is turned on in the Graph Entries section.

11.6 Multi-Channels Analysis

The *Multi-Channels* option facilitates the Analysis when using multiple amplifiers, e.g. EPC 10 Double, Triple, Quadro or 2x Quadro. Therefore, the user should keep in mind that the workflow is different.

Note: When using Multi-Channels only 1 AD channel (Trace) per amplifier is allowed.

🔛 Online Analysis: DefAnal	- • •
Analysis Methods: Analysis Pool	Auto Stim Control: Pool
(ecover 4 Hinf 0
Load Merge Save IV	New Delete Move
Analysis Graphs	
Graph 1 On Graph 2 On	
Save to Tree Copy Print Redraw	
Scale Axis Overlay No Wrap Graph E	ntries
Min Max Scale 🛛 🛛 🖊	
X -100.m 100.m Auto Ser. X Ampl	Ampl Ampl Ampl
Y -200.p 2.00n Auto Ser. Y Mean	Mean_init Ampi Ampi
Analysis Functions	
Amp 2 Mean 3 Mean init 4 Mean end 0	
Function Type Amplitude Change Delete Move	
Name Ampl	
Input Output	t in the second s
🖂 Not	ebook
	Not Stored in Values
X-, Y-seg. Offset 0 0 Operation	tion Options
Cursor Bounds (%) 0.0 100.0	

In the Analysis only 2 graphs per amplifier/Trace are available. Graph 1 and 2 of a Trace are arranged in a row, thus graphs 1 and 2 from the amplifiers are arranged in a column (see figure below).

Instead of defining every graph for every amplifier *Trace*, the user has to define the *Analysis Functions* for graph 1 and graph 2 only once. Subsequently, all graphs for all amplifiers are created automatically.


12. Parameters Window

Parameters								-	- • •
Marked Items	Root Items	Group	Items	Series Items	Sweep Items	Trac	ce Items		
⊠ TRACE	□ Titles	L	F 🖪	ag Group 1 Fla	g Group 2) (Flag (Group 3) (Flag Group 4)	
Header	TRACE 1 1	11 0	Cop	y Flags from Gr.1) Gr.2] Gr.3] Gr.4) Info t	o Notebook Gr.1		
Label	Current			efault Flags) (T	arget to Notebook) (Tar	get to File		
Count Count	1	[n Ca	ear all Flags	arked to Notebook	Mar	ked to File		
Data Points	680	0	- <u></u>			<u> </u>			
File Offset	256		2						
Average Count	1	0		Y-range	102.4µA		Marker-1	0.000	S 🗆
Data Kind	Adc0-Imon	1 [Y-min	-68.75nA		□ Marker-2	0.000	S 🗆
Data Format	2-byte integ	er C		Y-max	31.25nA		□ Marker-3	0.000	s 🗆
Data Factor	3.125n	0		Zero Offset	-33.53nA		Marker-4	0.000	s 🗆
Time Offset	0.000 s	0		DA Channel	1		□ Marker-5	0.000	S 🗆
Y-unit	A	[AD Channel	1		□ Marker-6	0.000	S 🗆
□ X-unit	S			Holding	0.000 V		□ Marker-7	0.000	S 🗆
X-interval	250.0µs			Internal Solution	0		□ Marker-8	0.000	s 🗆
□ X-start	50.00ms			Int. Sol. Value	0.000 M		□ Marker-9	0.000	S 🗆
Y-Offset	0.000 A			External Solution	10010		□ Marker-10	0.000	S 🛛
				Ext. Sol. Value	0.000 M				

Parameters are additional values describing the experimental and measurement conditions. The Parameters are required to interpret the data correctly. In CHARTMASTER we distinguish different types of Parameters depending to which level of the data structure they relate.

Note: If possible CHARTMASTER will use internal values to set the Parameters. E.g. C-slow values will be automatically read from the amplifier and stored with the Parameters. For some Parameters it might be necessary to assign a source (e.g. AD channel or manual input) in the I/O Control section of the Configuration window. For other amplifiers than EPC 10 or EPC 9 most of the external Parameters have to be entered manually via the I/O Control window.

In the Parameter window either the Parameters that are saved with already acquired data, or the Parameters of the last acquisition (stored or not stored) are displayed.

12.1 Parameter Tabs

12.1.1 Root

Parameters						
Marked Items	Root Items	Group Items	Series Items	Sweep Items	Trace Items	
□ ROOT □ Date □ Entries □ Program Version □ Text	L Titles 09-Jan-199 10 v8.41	LF Flag 7 Copy 0 Det 0 Clea	Group 1 Flag Flags from Gr.1 <u>ault Flags</u> <u>Ta</u> ar all Flags Ma	Group 2 Flag (Gr.2) Gr.3 Gr.4 rget to Notebook	Group 3) (Flag Grou Info to Notebook (Target to File) Marked to File	<u>p4</u> <u>jr.1</u>

Level 0 of the data tree. The Parameters of the Root level are:

- Date: Date at the time of acquisition.
- Entries: Number of *Groups* in the file.
- Program Version: Version number and date of CHARTMASTER.
- Text: A descriptive text can be entered and stored if the data file is opened in the Open Modify... mode.

12.1.2 Group

Parameters			
Marked Items	Root Items Group I	Items Series Items Sweep Items Trace Items	
GROUP GROUP Header Label Count	GROUP 1 GROUP	Flag Group 1 (Flag Group 2) (Flag Group 3) (Flag Group 4) Copy Flags from (Gr.1) (Gr.2) (Gr.3) (Gr.4) Info to Notebook (Gr.1) Default Flags Target to Notebook Target to File (Grear all Flags) (Marked to Notebook) (Marked to File)	
□ Entries ⊠ Exp. Number □ Text			

Level 1 of the data tree. The Parameters of the Group level are:

- Header: Index number of the Group (e.g. GROUP_1).
- Label: Text identifier; default label is the experiment number.
- Count: Number of *Group* entries.
- Entries: Number of *Series* in the *Group*.
- Exp. Number: Number of the experiment. The Exp. Number can be incremented by creating a new Experiment. An Experiment might contain a collection of different Groups with the same Exp. Number.
- Text: A descriptive text can be entered and stored if the data file is opened in the Open Modify... mode.
- MatrixWidth: Width of the acquired image in the *Matrix Scan* mode (only for data acquired with the ELPROSCAN).
- MatrixHeight: Height of the acquired image in the Matrix Scan mode (only for data acquired with the ELPROSCAN).

12.1.3 Series

Parameters						
Marked Items	Root Items Gro	oup Items	Series Items	Sweep Items	Trace Items	
SERIES	□ Titles	LF Fla	g Group 1 Flag	g Group 2) (Flag G	Group 3) (Flag G	roup 4
Header	SERIES 1 1	Cop	y Flags from Gr.1) Gr.2) Gr.3] Gr.4) Info to Notebook	Gr.1
Label	IV		fault Flags) (T	arget to Notebook	Target to File	
Count Count	1		ar all Flags	arked to Notebook	Marked to File	<u>í</u>
Entries	12				<u></u>	
Number Sweeps	12					
User Name						
⊠ Date	01-Nov-1996					
☑ Time	11:04:16.486					
Timer	00:59:06.585					
User Param 1	0.000 V					
User Param 2	0.000 V					
□ Aux3	0.000					
□ Aux4	0.000					
□ Aux5	0.000					
□ Aux6	0.000					
Comment	hKv1.5, alternating IV					

Level 2 of the data tree. The Parameters of the Series level are:

- Header: Index number of the Group and the Series (e.g. SERIES_1_4).
- Label: Text identifier; default label is the name of the Series.
- Count: Index number of the Series.
- Entries: Number of Series in the Group.
- Number of Sweeps: Original number of Sweeps in the Series. The number of Entries gets lower after deleting individual Sweeps.
- User Name: Name of the user which was entered in the Configuration (5.3.6 on page 43).
- Date: Date at the time of acquisition.
- Time: Time of the acquisition.
- Timer: Timer time of the acquisition.
- User Param 1/2: User-defined external values (see Configuration, 5.7.2 on page 55).
- Aux3...6: Auxiliary Parameter fields only used when certain extension are switched on.
- Comment: Text identifier (see Control window, 7.1 on page 65).

12.1.4 Sweep

Parameters			
Marked Items	Root Items Gro	up Items Series Items Sweep Items Trace Items	
SWEEP	□ Titles	LF Flag Group 1 (Flag Group 2) (Flag Group 3) (Flag Group 4)	
Header	SWEEP 1 1 1	Copy Flags from Gr.1 Gr.2 Gr.3 Gr.4 Info to Notebook Gr.1	
Label		Default Flags Target to Notebook Target to File	
Count	1	Clear all Flags Marked to Notebook Marked to File	
Entries	2] • • • • • • • • • • • • • • • • • • •	
Stim. Count	1		
⊠ Time	11:04:18.056		
Timer	00:59:08.156		
Marker-1	0.000 s		
Marker-2	0.000 s		
□ Marker-3	0.000 s		
Marker-4	0.000 s		
Temperature	20.00 C		
□ User1	0.000		
□ User2	0.000		
Digital In	000000000000000000000000000000000000000		
Digital Out	000000000000000000000000000000000000000] 0	

Level 3 of the data tree. The Parameters of the Sweep level are:

- Header: Index number of the Group, the Series and the Sweep (e.g. SWEEP_1_4_1).
- Label: Text identifier; label of the Sweep (not the index number).
- Count: Index number of the Sweep.
- Entries: Number of Traces in the Sweep.
- Stim. Count: Total number of Series in the data tree.
- Time: Time of the acquisition.
- Timer: Timer time of the acquisition.
- Marker-1...4: Position of the Sweep Marker (see Markers, 17 on page 153).
- Temperature: Temperature value from an external device (see Configuration, 5.7.2 on page 55).
- User 1/2: User-defined external values (see Configuration, 5.7.2 on page 55).
- Digital In: Status of the input bits of the digital channels (see I/O Control, 14 on page 145).
- Digital Out: Status of the output bits of the digital channels (see I/O Control, 14 on page 145).

12.1.5 Trace

Parameters								
Marked Items	Root Items Gr	roup Items	s Series Items	Sweep Items	Trac	e Items		
⊠ TRACE	□ Titles	LF 🔵	Flag Group 1 Fla	g Group 2) (Flag G	iroup 3) (Flag Group 4)		
Header	TRACE 1 1 1 1		opy Flags from (Gr.	1] Gr.2] Gr.3] Gr.4) Info to	o Notebook Gr.1		
Label	Current		Default Flags	arget to Notebook	Tar	get to File		
Count Count	1		Clear all Flags	larked to Notebook	Mar	ked to File		
Data Points	680							
File Offset	256							
Average Count	1		Y-range	102.4µA		Marker-1	0.000 s	
Data Kind	Adc0-Imon		□ Y-min	-68.75nA		□ Marker-2	0.000 s	
Data Format	2-byte integer		Y-max	31.25nA		□ Marker-3	0.000 s	
Data Factor	3.125n		Zero Offset	-33.53nA		□ Marker-4	0.000 s	
Time Offset	0.000 s		DA Channel	1		□ Marker-5	0.000 s	
Y-unit	A		AD Channel	1		□ Marker-6	0.000 s	
□ X-unit	S		Holding	0.000 V		□ Marker-7	0.000 s	
X-interval	250.0µs		Internal Solution	0		□ Marker-8	0.000 s	
X-start	50.00ms		Int. Sol. Value	0.000 M		□ Marker-9	0.000 s	
□ Y-Offset	0.000 A		External Solution	10010		Marker-10	0.000 s	
			Ext. Sol. Value	0.000 M				

Level 4 of the data tree. The Parameters of the Trace level are:

- Header: Index number of the Group, the Series, the Sweep and the Trace (e.g. TRACE_1_1_2).
- Label: Text identifier; label of the Trace.
- Count: Index number of the *Trace*.
- Data Points: Number of stored data points.
- File Offset: Offset of the data in the according memory block (minimum number is 256 which is the size of the header).
- Average Count: Number of acquired Traces for the calculation of the stored average Trace.
- Amplifier Mode: Recording mode of the amplifier during recording (e.g. "Whole-Cell").
- Amplifier: Notification field of any Amplifier event (e.g. "Clipping" CLIP-1 = clipping, NoClip-1 = clipping).
- Data Kind: AD channel index number and kind of acquired data (e.g. Adc6-Imon).
- Data Format: Format of the data (either 2-byte integer, 4-byte integer, 4-byte real or 8-byte real).
- Data Factor: Scaling factor for the conversion of interface values into e.g. current or voltage values.
- Time Offset: Time delay of DA or AD channels (see Delay, 9.12 on page 97).
- Y-unit: Unit of the Y-axis.
- X-unit: Unit of the X-axis.
- X-interval: Sample Interval (see Timing, 9.5 on page 76).
- X-start: Indicates any delay of the X-axis, mostly a time delay when using a *Start-Segment* (see Timing, 9.5 on page 76). But it may also indicate other delays when X-axis is not time but e.g. frequency.
- Y-Offset: Y-axis offset in the Oscilloscope.
- Y-range: Maximal range of the Y-axis (one direction only). This depends on the Gain settings.
- Y-min: Minimum Y-value of the Trace (e.g. current or voltage).
- Y-max: Maximum Y-value of the Trace (e.g. current or voltage).
- Zero Offset: Calculated Zero Offset (see AD input channel settings, 9.9.2 on page 92).

- Bandwidth: Filter frequency of the acquired Trace.
- DA Channel: DA channel number (see Channel Settings for DA Output and AD Input, 9.9 on page 87).
- AD Channel: AD channel number (see Channel Settings for DA Output and AD Input, 9.9 on page 87).
- Holding: Holding potential/current value.
- Int. Sol.: Name of the used internal solution.
- Int. Sol. Value: Value entered in the selected solution (see Solution Data Base, 15.3 on page 148).
- Ext. Sol.: Name of the used external solution.
- Ext. Sol. Value: Value entered in the selected solution (see Solution Data Base, 15.3 on page 148).
- X-position: X-position value in relation to the origin.
- Y-position: Y-position value in relation to the origin.
- Z-position: Z-position value in relation to the origin.

12.1.6 Marked Items

All marked (ticked checkboxes) Parameters will be displayed in this tab sorted according to their origin (Root, Group, Series, Sweep, Trace or Amplifier).

Parameters		×
Marked Items	Root Items	Gro
GROUP		
Header	GROUP 1	
Exp. Number	2	
SERIES		
Header	SERIES 1	1
Label	IV	
User Name		
Date	01-Nov-1996	
Time	11:04:16.486	5
SWEEP		
Header	SWEEP 1 1	1
Label		
Time	11:04:18.056	6
Timer	00:59:08.156	6
TRACE		
Header	TRACE 1 1 1	1
Label	Current	
Data Kind	Adc0-Imon	

Parameter Options:

SERIES This checkbox is available in every Parameter tab, if there is no tick set all Parameters of this tab will not be displayed in the Marked Items tab independent how many Parameter checkboxes are ticked.

SERIES A highlighted red background color indicates that the display level of the tree is identical to the display tab of the *Parameters*. Here, a *Series* in the *Replay* tree and the *Series Parameters* have been displayed.

Titles If selected, the *Parameter* names will be exported together with their corresponding values. Otherwise, only the values will be exported.

I If selected, values and/or *Parameter* names will be separated with a linefeed when exported.

12.2 Flagging

Flag Group 1	Flag Group 2) (Flag Group 3) (Flag Group 4)							
Copy Flags from I	Gr.1 Gr.2 Gr.3 Gr.4 Info to Notebook	Gr.1						
Default Flags	Target to Notebook Target to File							
Clear all Flags	(Marked to Notebook) (Marked to File)							

Via the checkboxes (flag options) you can select information that you want to export to the Notebook window or to a file (see section before).

Flag Groups 1...4: Four different flag groups can be defined, each containing the information for all checkboxes in this window.

By default, Flag Group 1 contains the following flags:

- Group: Header, Exp. Number.
- Series: Header, Label, User Name, Date, Time.
- Sweep: Header, Label, Time, Timer.
- Trace: Header, Label, Data Kind.
- Amplifier State: Amplifier, Recording Mode, V-Pipette, Gain, Filter 2, C-fast, C-slow.

Copy Flags from: Copy flag definitions from one group to another. First click on the *Flag Group* that you want information copied into, then on the copy group button of the group whose flags you want to copy to the active group.

Info to Notebook: The contents of the Parameters of the selected Flag Group are written to the Notebook window when the data is replayed and when $Replay \rightarrow Show$ Tree Info is on.

Default Flags: Set the Flag Group 1 back to the default setting.

Clear all Flags: Reset all flags, i.e. no selected Parameters.

Target to Notebook: Copy all flagged information of the actual target in the Replay window to the Notebook.

Marked to Notebook: Copy all flagged information of the marked target in the Replay window to the Notebook.

Target to File: Copy all flagged information of the actual target in the **Replay** window to a file. You will be asked for a file name.

Marked to File: Copy all flagged information of marked targets in the Replay window to a file. You will be asked for a file name.

13. Notebook Window

The Notebook window is used to display messages and warnings of the program, such as error messages, analysis results, and information about displayed data. In many of the CHARTMASTER windows (e.g. Parameters, Configuration, Online Analysis), there are checkboxes or options that can be selected to target settings and results to be written into the Notebook window. The content of the Notebook can be stored on disk; its maximal size has to be specified in the drop-down menu Notebook \rightarrow Set Length. To keep a better log of an experiment, the names of opened files and of executed series are written to the Notebook window.

<u>_</u>

When the window is activated (by clicking with the mouse pointer into it), the text editing functions are activated and applicable in the Notebook window. Thus, the Notebook window is an editor window of the memory-resident text file *.txt. Therefore, one can modify text in the Notebook window just as in any other text file. This option can be used to add further information to the text file, or to get rid of messages that should not be stored to the disk file.

The applicable menu commands are described in the Edit and Notebook menus (see Menus, 4.2 on page 24 and 4.7 on page 36).

Note: The Cut, Copy and Paste commands copy to and from the clipboard.

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14. I/O Control Window

The I/O Control window can have the following sections:

Digital In/Out, DA-Channels, AD-Channels, Values, Serial Out, and Parameters. Which sections and items are shown in the I/O Control window can be selected in the I/O Parameters section of the Configuration window (see 5.7 on page 51).

The idea of the I/O Control window is that the settings in the Configuration window ideally should be edited only during major software setups, whereas the I/O Control window should be used for *Parameter* editing during data acquisition. Hence, only those *Parameter* should be displayed in the I/O Control window that are not set internally and are subject to change during the experiment. These *Parameters* do not have an internal source. That means, are not defined by EPC 10 or read from a specified AD-channel. E.g. some amplifier settings such as recording bandwidth can be entered when a non-telegraphing amplifier is used.

I/O Contro	bl							
Dig-In 000000000000000000000000000000000000								
Dig-Out								
Clear	0	⊠Single B	it					
DA-Channels								
0.000 V	0.000 V 0.000 V 0.000 V 0.000 V							
AD-Cha	AD-Channels							
V 000.0	0.000 V	0.000 V	0.000 V					
0.000 V	0.000 V	0.000 V	0.000 V					
Value-1	Value-2	Value-3	Value-4					
0.000	0.000	0.000	0.000					
Comm-1 Configure								
Internal Sol	ution	0						
External So	lution	0						
Temperatur	e	20.00 C						

14.1 Dig-In / Out

Dig-In/Out: Control field of the digital channels. The digital input bits are only displayed. The digital output bits can be set by selecting or deselecting the checkboxes.

Clear: Clear all settings for the digital output, e.g., deselect all bits. The number in the blue field represents the output bits. It can be used to set the bits. Clear actions are written to the Notebook.

Single Bit: If selected, only one bit can be set for Dig-Out. The outputted digital value is set to the highest bit.

14.2 DA / AD-Channels

DA/AD-Channels: This section allows you to set the output of the DA-channels. The currently sampled voltages at the AD-channels are only displayed.

Note: When using these DA-channels to control external devices from the I/O Control window, make sure that the same DA-channels are not used by other parts of CHARTMASTER, *i.e.* as a stimulation channel in the Pulse Generator.

Dig-In								
– Dig-Out	23	4 5		89		12	34	15
Clear	0		ØS	ingle	Bi	t		

DA-Cha	nnels		
0.000 V	0.000 V	0.000 V	0.001 V
AD-Cha	nnels		
-0.000 V	-0.001 V	-0.001 V	0.001 V
0.000 V	0.000 V	-0.003 V	0.012 V

0.000

Value-4

0.000

14.3 Values

146

Values (1-32) are global variables which can be used for calculations in the Protocol Editor and in the Analysis. In addition, they are used to exchange information between these two program modules. In the I/O

Control dialog the Values are displayed and they can be manually changed. The number of displayed Values (4, 8, 12, 16, 20, 24, 28 or 32) can be specified in the Configuration dialog (see 5.7.3 on page 55). In addition, the names of the Values are displayed above the numeric Values. The name can be set in the Configuration dialog.

14.4 Serial Output

Serial Port: A string can be sent (*Send* button) to a device via the serial port (RS-232) of the computer. The *Configure* button opens the Serial Port Configuration window (see Serial Out, 5.4.6 on page 45). An example is shown in the Protocol Editor, 10.4.3.1 on page 108.

Note: This section is only visible, if the serial communication is turned on in the Configuration dialog, 5.4.6 on page 45.

14.5 Solutions

Here, it is possible either to monitor the presently active solution or to manually change the solution index. The solution information provided here will be stored in the data file of CHARTMASTER.

Note: When changing the solution in the I/O Control window there will be no active setting of external solutions. If you want to do that please use the Solution Changer event in the Protocol Editor (chapter 10.4.3.5 on page 109) and the Solution Changer dialog (chapter 16 on page 151).

14.6 Parameters

Only the *Temperature* option or *User Parameters* can be selected in the Configuration window to be listed in the I/O Control window for display or editing.

The individual Parameters are described in the context of the Configuration window, 5.7 on page 51.

0.000

0.000

Comm-1	Configure
Send	

Internal Solution	0
External Solution	0

Temperature 20.00 C

15. Solution Base

In most electrophysiological experiments bathing solutions are changed during the experiment. Thus, it is of great importance to keep track, which solutions were on both sides of the membrane.

CHARTMASTER provides two ways to handle solution changes during your experiments:

1. A simple one is to use the entries *Internal Solution* and *External Solution* in the I/O Control window. To activate this option please go to the Configuration window and active the *Show Solution* checkbox in the I/O Control tab.

E 1 10 1 1 0
External Solution 0

You can set the entry manually or via the event *Set Solutions* in the Protocol Editor (10.4.5.2 on page 115). The numbers can be the entry of an external list of solutions, or the index of a specific solution in a CHARTMASTER Solution Data Base (see 15.1). The solution indices are stored together with the recorded data and can be reviewed in the Parameters window (12.1.5 on page 140).

2. A more advanced method is to create a ***.sol** file. Such a data base is a *Tree* of solution entries ordered by the given identifying index.

This way the indices stored with the data are converted to solutions from the Solution Data Base. Here, it is also possible to use the event Set Solutions in the Protocol Editor (10.4.5.2 on page 115).

15.1 Activating the Solution Base

First, turn on the Solution Base in the Configuration window (5.5.2 on page 48). If no Solution Data Base has been loaded before and stored with the current configuration, a dialog box shows up asking you if you want to open an existing data base or creating a new data base.

No Solution Base de	efined yet:	
Cancel	Create New	Find File

During the experiment, solutions can be set either via the I/O Control window (14.5 on the facing page) or via the Set Solutions event in the Protocol Editor (10.4.5.2 on page 115).

15.2 Using Solution Indices

The indices can be given to solutions in any arbitrary way but it certainly is of advantage if one sticks to some consistent concept in order to be able to identify solutions by their index easily. Since the solution indices are numbers between 0 and 2'147'483'648 (many more than you will find bottles in the laboratory), there is plenty of freedom to organize them.

Here is an example: Usually one has several standard solutions, which are frequently used and modified slightly for various experiments. One could assign numbers divisible by 1000 or 10000 to them. Then one has 999 or 9999 possibilities for modifications of this solution, respectively. An example would be that certain concentrations of toxin are added to the standard solution 1000 yielding numbers 1001 through 1099. Another toxin could occupy the numbers 1100 through 1199, etc..

Note: The indices given for internal and external solutions are pointing into the same Solution Data Base. It is therefore a good idea to index internal and external solutions such that they are consistent and easily identifiable. For example, you could use odd thousands (or ten thousands) for external solutions and even thousands (or ten thousands) for internal solutions.

	1	Normal Frog Rin	ger		1 entry
Nume	ric Nam	Free Ca		1.80m	Create Ent
pН	7.20	NaOH	Osmol	285.m	(Duplic, Ent
Index	Ingred	ient	Co	nc.	Delete Ent
1	NaCl		<u> </u>	140. mM	Next Entr
2	KCI			2.50 mM	Last Entr
3	CaCl2			1.80 mM	Export Lab
4	Hepes	1		10.0 mM	Export List
					SAVE UNDO DONE

15.3 Solution Data Base

To open the Solution Data Base you have to select Solution Base in the Windows menu of CHARTMASTER.

Solution Index: Index number for the solution. By clicking into the field you are able to enter a defined index number directly which might be helpful if you have a large *Solution Data Base*.

Name: Name of the solution.

Numeric Name: An editable field that may hold a feature of the solution that is not easily determined from its ingredients (e.g., free calcium concentration, etc.).

Numeric Name Value: An editable field containing a number or value (Osmolarity, pH) which is in relation to the name entered in the field *Numeric Name*.

pH: Holds the value of the pH of the solution and the substance used to adjust it.

Osmol.: Holds the value of osmolarity of the solution.

Ingredient / Conc.: Each ingredient is defined by its index number and its concentration in the solution.

Insert, Append, Delete: By clicking into an index number of the solution ingredients options for modifying the solution content get available:

- Insert: Creates a new entry line for an ingredient before the selected index number.
- Append: Creates a new entry line for an ingredient at the end of the ingredient list.
- Delete: Deletes the selected ingredient.

Entries: Number of entries in the solution data base.

Create / Duplic. / Delete Entry: Used to generate, copy, and remove solutions in the data base.

Next / Last Entry: This option moves through the data base by selecting the next or last available solution. **Export Label:** This is used to output labels of the shown solution:

- ASCII File: Exports the solution information as an ASCII text file (*.label).
- Printer: Two labels with two different size containing the solution information are created.
- Igor Text: Exports the solution information as IGOR text (*.label).
- Notebook: Exports the solution information to the Notebook.

Export Listing: This is used to output a list of the entire solution file:

- ASCII File: Exports the index number and the name of the solutions as an ASCII text file (*.list).
- Printer: Prints the index number and the name of the solutions.
- Notebook: Lists the index number and the name of the solutions in the Notebook.

SAVE: Saves the file to disk.

UNDO: Reverts the edited solution to its original form.

DONE: Exits the dialog.

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16. Solution Changer

The Solution Changer dialog can be accessed via the Windows menu of CHARTMASTER. The Solution Changer dialog has two main purposes:

- 1. Manage analog or digital outputs of your perfusion system
- 2. Align external or internal solutions to the perfusion system

Thus, using the Solution Changer helps to keep track with the solutions used during an perfusion experiment. The solution information is stored in the data file of CHARTMASTER. To look up this information use the Parameters dialog.

Any external perfusion system with analog or digital inputs can be used together with the Solution Changer and e.g. an EPC 10 USB amplifier.

🔛 Solution Changer 📃 📼 🔤				
Control Configure				
		Index	Solution	
Off	Set			
Solution 1	O Set	0	undefined	
Solution 2	O Set	0	undefined	
Solution 3	O Set	0	undefined	
Solution 4	O Set	0	undefined	
Solution 5	O Set	0	undefined	
Solution 6	O Set	0	undefined	
Solution 7	O Set	0	undefined	
Solution 8	O Set	0	undefined	
Solution 9	O Set	0	undefined	
Solution 10	O Set	0	undefined	
Solution 11	O Set	0	undefined	
Solution 12	O Set	0	undefined	
Solution 13	O Set	0	undefined	
Solution 14	O Set	0	undefined	
Solution 15	O Set	0	undefined	
Solution 16	O Set	0	undefined	

The Solution Changer dialog has two main tabs. In the *Control* tab one sets the active perfusion channel manually ("Set"). Further the solution index and the solution name from the Solution Base is displayed here. By clicking into the selection field it is possible to change the solution index/solution name.

Note: The solution selection does only work if a Solution Base is defined and loaded.

Solution Char	nger		- • •
Control	Configure		
16 Solutions	S		
Off		off	
Solution 1	External Solution	off	
Solution 2	External Solution	off	
Solution 3	External Solution	off	
Solution 4	External Solution	off	
Solution 5	External Solution	off	
Solution 6	External Solution	off	
Solution 7	External Solution	off	
Solution 8	External Solution	off	
Solution 9	External Solution	off	
Solution 10	External Solution	off	
Solution 11	External Solution	off	
Solution 12	External Solution	off	
Solution 13	External Solution	off	
Solution 14	External Solution	off	
Solution 15	External Solution	off	
Solution 16	External Solution	off	

In the *Configure* tab you can define up to 16 solutions used in the perfusion experiment. Further you can define if the used solutions is an internal or an external solution. In the last column one defines the output channel e.g. analog or digital. If an analog output is selected you will have to define the voltage amplitude necessary to activate the perfusion channel.

Note: Please do not forget to define an output channel for the "Off" position.

	DA-0
	DA-1
	DA-2
	Stim-DA
	DA-4
	DA-5
	DA-6
	DA-7
1	off
	Dig-out (word)
	Dig-0
	Dig-1
	Dig-2
	Dig-3
	Dig-4
	Dig-5
	Dig-6
	Dig-7
	Dig-8
	Dig-9
	Dig-10
	Dig-11
	Dig-12
	Dig-13
	Dig-14
	Dig-15
	Clear all

17. Markers

🔛 Markers			
New Marker			
Add Marker	Text	Marker 1	
	Trace	Sweep	
	Time		From Sweep
Edit Marker		1 of 1	
Show Sweep	Text	Marker 1	
Show Marker	Trace	Sweep	
Delete	Time	14-Dec-2015 14:48:13.290	
		Write to Notel	book

The Markers dialog can be opened by selecting Markers from the Windows menu.

With this dialog, *Markers* can be set at any time during a running experiment even between *Sweep* acquisitions. A *Marker* is drawn as a blue vertical line in the Oscilloscope.

17.1 New Marker

In this section one can add Markers and define the name of the label.

Add Marker: Adds a new Marker to the data file. The Marker Text and the target Sweep/Trace information is stored as well.

Text: Enter a label for the Marker you want to add.

Trace: A Marker can be linked either to a Sweep or a Trace which can be specified by this selection menu.

If the *Marker* is defined for a *Sweep* it is labeled "[i] Text". The i in square brackets indicates the number of the *Marker* in the list (index number) and "Text" shows the label of the *Marker*.

Username_2015-0	2-09_001	
1_1_1 of 1	Measure Scan (Freeze)	Wipe Repaint
	[1] Marker 1	Overl.Swp Overl.Ser
		Trace 1 Dig. Filter
		Y-scale 1.00
		Y-offs. S
		Auto Ser Start Time
		0.0 % End R
X: 100. ms	Y: 2 00 nA Y: 200 mV	Page (R)

If the Marker is defined for a Trace it is labeled "[i] i: Text". The i in square brackets indicates the number of the Marker in the list (index number) whereas the index number thereafter indicates the Trace index number. "Text", again, shows the entered label for the Marker.

Username_2015-02	2-09_001 / Continuous	
1_5_1 of 1	(Measure) Scan) (Freeze	Wipe Repaint
	[1] 2: Marker	1 Overl.Swp Overl.Ser
		Trace 1 Dig. Filter Off Y-scale 1.00 Y-offs. S 0.00
X: 100. ms	Y: 2.00 nA Y: 200. mV	Auto Ser Start Time 0.0 % End R 100.0 % Page R 3.0 0

Time: The time of a new *Marker* is the actual time if the field is empty. It can be the given time in the time format "hh:mm:ss.mmm" e.g. if you add a *Marker* offline. Optionally the time format may be preceded by a date "yyyy/mm/dd".

From Sweep: Gets the time information of the Sweep selected in the Replay tree.

17.2 Edit Marker

Marker Index: The Marker index e.g. "1 of 7" indicates the index number of the selected Marker in the Marker list.

Show Sweep: Selects the according Sweep in the Replay tree and replays it in the Oscilloscope.

Show Marker: Shows the appropriate *Marker* information in the *Marker* list if the selected *Series* in the data tree owns a *Marker*.

Delete: Deletes the selected *Marker*.

Text: Modifies the label of the set *Marker*.

Trace: Modifies the link (Trace or Sweep) of the set Marker.

Time: Displays the time information of the selected Marker.

O: The two arrow buttons enable to scroll through the list of added *Markers*.

Write to Notebook: Writes the time information of acquired Series and set Markers into the Notebook.

Notebook_09	9-Feb-2015		
2015/02/09	08:42:24.731	series[1_1]	Continuous
2015/02/09	08:42:59.707	series[1_2]	Continuous
2015/02/09	08:43:15.572	series[1_3]	Continuous
2015/02/09	08:43:43.091	series[1_4]	Continuous
2015/02/09	08:44:34.212	series[1 5]	Continuous
2015/02/09	11:06:49.682	series[1_6]	IV
2015/02/09	11:11:15.726	series[1_7]	Continuous
2015/02/09	11:17:49.161	series[1 8]	Continuous
2015/02/09	11:18:00.471	series[1_9]	Continuous
2015/02/09	11:20:27.845	series[1_10] IV
2015/02/09	11:20:32.962	marker[0] H	ello
2015/02/09	11:20:37.236	marker[0] M	arker 2 -

Note: In the Protocol Editor event "Set Solutions" it is possible to add Solution Markers. The Markers set there are also included into the Markers list.

18. Calculator Window and Equations

Equations are a very flexible way to perform complex calculations within CHARTMASTER. Equations can be used at various places, such as Analysis, *Trace Buffer*, or for *DA-Scaling*. In addition, a separate Calculator window allows to use the equations syntax as replacement of the calculator provided by the operating system.

Calculator	
pi+2*3^2	21.142
Equation Select Equation Save Equation	

In case an equation has to be edited in the Analysis, Protocol Editor or Trace Buffer, then the following dialog can be opened by pressing on the Edit button.

Equation:	
	Results:
	0.0000
Equation Select Equation Save Equation	
? LOAD SAVE Cancel	Done //

Command Line: Enter the equation string into this field.

Results: The result of the equation will be shown here.

Equation: You can enter an equation name which will be loaded into the *Command Line* after pressing RETURN. Alternatively if an equation was selected by the option *Select Equation* the equation name will be displayed in this field.

Select Equation: After loading an equation file you can select already stored equations.

Save Equation: For saving an equation enter a name in the *Equation* field and press the *Save Equation* button. When the entered name does not already exist in the equation file you will be asked if you want to save it as a new entry.

? : Prints a help text on the equation syntax to the Notebook.

LOAD: Brings up a file selector to open an equation file (e.g. Equation.txt).

SAVE: Saves the modifications made in the equation file.

Cancel: Closes the dialog without accepting the equation.

Done: Accepts the new equation string and closes the dialog.

18.1 Equation Syntax

Normal math syntax is to be used. All 3 types of brackets ('('and')', '['and']', '{'and'}') are equivalent and can be freely used and nested. The opening and closing brackets must match.

Math functions:

- +, -, *, /, $^{\wedge}$ (power), % (remainder)
- & (and), nor, or, xor: bit operation on integer values
- >, <, =: boolean operations: true=1, false=0
- round: rounds towards nearest value using the banking rules $(-2.5 \rightarrow -3.0)$
- trunc: rounds towards the smaller amount $(-2.5 \rightarrow -2.0)$
- ceil: rounds towards the positive $(-2.5 \rightarrow -2.0)$
- floor: rounds towards the negative $(-2.5 \rightarrow -3.0)$
- exp, sqrt, ln, log
- \sin, \cos, \tan
- arcsin/asin, arccos/acos, arctan/atan
- abs, rad, deg
- random: returns random number between 0.0 and 1.0

Constants:

• e, pi

Special case:

'@' as the first character will replace the given label by the formula in the equation file with that label. E.g. '@first 1+2' results in '1+2'.

Comments:

Anything after ';' is considered a comment.

18.2 Equations - Where to Use

18.2.1 Analysis

Trace Param.	Math	Trace	Spectra
C Trace Count	Equation	O Trace	O Frequency
C-slow	○ Y(x): y at pos = x	C Equation	O Distribution
O R-series	O Constant	🗘 Q = Integral	
⊖ Rs-value	⊖a+b	0 1 / (trace)	Histogram
C Leak Comp.	⊖a-b	O1/(Q)	O Histogram Ampl
O M-conductance	⊖a*b	O In (trace)	O Histogram Bins
Cell Potential	⊖ a / b	O In (Q)	
O Seal Resistance	🔿 a in b	O log (trace)	
O Pip. Pressure	() abs	🗘 log (Q)	
O Int. Solution	O log	🔘 dt = Differential	
O Int. Sol. Value	O sqrt	O Trace x-axis (time)	
C Ext. Solution	O arctan	O Stimulus	
O Ext. Sol. Value	() 1/a		
	O 1/log		
Sweep Param.	O 1/sqrt		
O User_1	O 1/arctan		
O User_2			
O Temperature			
O Digital-In	Equation 4*pi-10		1
		Edit	-
		<u></u>	

The *Equation* functions in the *Math* and *Trace* section of the Analysis Function dialog provide the possibility to assign the result of an equation to a function. The advantage of the equation is to perform multiple steps of a calculation with just one function. In an equation of the Analysis function the results of other Analysis functions can be easily combined.

Math - Equation The following additional identifiers can be used in the Math - Equation function:

- f[i], i = online function index = 1...n
- p[i], i = amplifier parameter index = 1...n
- v[i], i = value index = 1...n
- icon[icon descriptor], e.g. 'icon[E VHold]'

An example: A parameter of the Parameters window can be easily plotted in the Analysis graph.

Trace Param.	Math	Trace	Spectra
C Trace Count	Equation	O Trace	O Frequency
C-slow	O Y(x): y at pos = x	O Equation	O Distribution
O R-series	Constant	Q = Integral	
⊖ Rs-value	🔾 a + b	○ 1 / (trace)	Histogram
🗘 Leak Comp.	🗘 a - b	O1/(Q)	O Histogram Amp
O M-conductance	⊖a*b	O In (trace)	O Histogram Bins
Cell Potential	⊖ a / b	O In (Q)	
O Seal Resistance	🗘 a in b	O log (trace)	
O Pip. Pressure	🔿 abs	🗘 log (Q)	
O Int. Solution	O log	🗘 dt = Differential	
O Int. Sol. Value	⊖ sqrt	O Trace x-axis (time)	
C Ext. Solution	O arctan	O Stimulus	
C Ext. Sol. Value	◯ 1/a		
	O 1/log		
Sweep Param.	O 1/sqrt		
O User_1	O 1/arctan		
O User_2			
O Temperature			
O Digital-In	Equation Licon ID A	mplCslowl	1
	Edución	Edit	1

As equation string you choose the icon descriptor of the *C*-slow parameter from the Parameters window. Please note that the Parameters window has to stay open to access the value of this icon. However, you may minimize the window. The advantage of using the *C*-slow parameter from the Parameters window instead of the Amplifier window is that the Parameter is available also during offline replay and analysis of the acquired data.

Analysis Functions				Switch to	Control Windov List
4 Mean_end	5	Time	🔵 6 🗲 🖉	C-slow"	1 7 0 0
Op-Equa	ation			NEW	DELETE MOVE
X-, Y-seg. Offset	0	0	Trace #	Trace 1	🛛 Notebook
Cursor Bounds (%)	0.0	100.0	not store	ed in Value	

Trace - Equation

The Analysis Function Trace - Equation provides the possibility to scale a Trace before plotting it in the online graph. The advantage of the equation is to perform multiple steps of a calculation with just one function. In an equation of the Analysis function the results of other Analysis functions as well as other trace data of the selected trace can be easily combined.

In addition to the identifiers of Math - Equation the following identifiers can be used:

- y: The trace y-value, i.e. "amplitude"
- x: The trace x-value, i.e. "x-axis value = x-start + index * x-increment"
- Q: The integral up to the respective index, i.e. "(y[i] + y[i-1]) / 2 * dx"
- dt: The differential between present and preceding y-value, i.e. (y[i] y[i-1] / dx)
- i: The index of the trace data point
- tb[i]: The y-value of equivalent sample in buffer i, i = buffer index, 1...4
- tr[i]: The y-value of equivalent sample in trace i, 1...n. One can use the trace-ID (e.g. "I-mon") to specify a trace instead of the numeric trace-count "i". The text must be identical to the text in the trace-count menu.

18.2.2 Trace Buffer

In case you would like to permanently modify (rescale) acquired data or at least let the rescaled data then run through the Analysis then you can use the *equation* functionality of the *Trace Buffer* menu or protocol event.

An example: As *Trace* 3 we acquire the frequency dependence of the *Admittance* (Gain). This is an output *Trace* of the Spectroscopy Extension. The X-axis of the *Admittance Trace* is scaled in frequency units. The *Admittance* itself is scaled in linear units. For some applications it is useful to rescale the *Admittance* in dB units.

Irace Buffer	Skip	Delay 0.00 s
Source Trace-3	add to	Buffer-3
⊠ Clear Buffer		Replace Trace-3
Equation		Edit
Update Display	Update Analysi	is

The protocol event above shows the following: We take Trace 3 as source, clear the destination buffer, add the source to the buffer-3, apply the equation (here we calculate $20*\log(y)$, y = values in the destination buffer after performing the operation), and then put the result back into Trace 3. Now we can run Trace 3 through the Analysis again and apply the analysis function on an Admittance (Gain) Trace, which is now scaled in decibel. E.g. we can apply the Time to Threshold function with a threshold of -3 (dB) to search for the cutoff frequency.

The operands are identical to the ones used in Trace - Equations (see 18.2.1 on the preceding page).

18.2.3 DA Scaling

Special Operands:

• ampl = The value for output or amplitude as defined by the PGF-stimulus. Used for equations in *DA-Scaling* only.

In the following we will describe how to output two consecutive pulses with exponentially increasing voltages.

We first enter in the I/O Control pane of the Configuration window for the DA Scaling an equation of a single exponential (5.7.1 on page 52). As Parameters we use the values 1 to 3. As time or x-value we use the Ampl Parameter of the stimulus.

Scaling Equation of Dac-0:	
	Results:
v[1]+v[2]*exp(ampl*v[3])	0.0000
Equation Select Equation Save Equation	
? LOAD SAVE	Cancel Done

In a second step, we define the appropriate segment pattern for the DA 0 channel and select Use DA Scaling. In segments of amplitude 0 a constant voltage is put out. In case we want to generate the exponentially increase, we set the segment type to Ramp and choose for amplitude and duration the same value (1 Volt corresponds to 1 Second). In case the amplitude has to be reset to zero, we insert a constant segment with duration 0 and amplitude 0.

Segments 📢 👌	⊠ Store 1	Store 2	Store 3	⊠Store 4	⊠Store 5	Stor 00
Segment Class	Constant	Ramp	Constant	Ramp	Constant	Constant
Amplitude	valu 0.000	valu 100.0m	valu 0.000	valu 100.0m	valu 0.000	valu
Duration [ms]	val 100.00	val 100.00	val 0.00	val 100.00	val 100.00	val
	Increase	Increase	Increase	Increase	Increase	Increase
fact./incr.	1.00 0.000	1.00 0.000	1.00 0.000	1.00 0.000	1.00 0.000	
t-incr. Mode	Increase	Increase	Increase	Increase	Increase	Increase
t-fact./incr. [ms]	1.00 0.00	1.00 0.00	1.00 0.00	1.00 0.00	1.00 0.00	
Draw: Active Chann	nel, all Sweep	s Delay: DA	0.00 s AD	0.00 s An	nplitude (dis	play) □ ⊡ Set La
10.0mV l				Le No Le Le	ak Pulses of Leaks ak Delay ak Size ak Hold	0 10.0 ms 0.250

We now set the Values 1 to 3 as desired via the I/O Control window and execute the sequence. The result is shown in the Oscilloscope window. By changing the values 1 to 3 the stimulus can be easily adjusted.



18.2.4 Values

Set Value			Skip	Delay	0.00 s
Valu	e-1	= equation			
	do	n't copy		Edit	<u> </u>

The protocol event Set Value provides the possibility to assign the result of an equation to a value. The advantage of the equation is to perform multiple steps of a calculation with just one protocol event. In an equation of the Set Value event results of the Analysis can be easily combined. Therefore the definitions are identical to the ones in Math - Equation (see 18.2.1 on page 159).

19. Photometry Extension

This chapter provides a brief overview of the settings for each of the Photometry Extensions mentioned above. A complete tutorial/exercise for photometry measurements using CHARTMASTER is provided in the CHARTMASTER Tutorial.

After starting CHARTMASTER, open the Configuration window and select the appropriate Photometry Extension in the *Hardware* section of the dialog. The options are:

- T.I.L.L
- DG4 / DG5
- Lambda-10
- PTI DeltaRAM

Some controls are independent from the photometry hardware. These controls are described in this section. Hardware specific options will be described below.

R-max	1.0000E+00	R-min	0.0000E+00	Kd * Sf	1.0000E+00
Background1	0.0000E+00	Background2	0.0000E+00	Background3	0.0000E+00
Dead Time	2.00 ms		Compute	Traces of Marke	d Targets

R-max, R-min: Minimum and maximum ratio (F1/F2).

Kd*Sf: Effective Kd (dissociation constant) of the dye.

Note: These values are required for calculating the free calcium concentration (according to the Grynkiewicz formalism). The calcium concentration can be stored as a Trace by use of the "Photo_Ca" input channel in the Pulse Generator dialog.

Background 1...3: Background fluorescence for the three wavelengths F1, F2, F3.

Note: The parameters R-max, R-min, Kd*Sf and Background 1...3 are stored as "Series Parameters" with the raw data file and are listed in the Parameters dialog (see figure below).

D Photo1 Backgroui	0.000	
D Photo2 Backgroui	0.000	
D Photo3 Backgroui	0.000	
PhotoCa Kd	1.000	
PhotoCa Rmin	0.000	
PhotoCa Rmax	1.000	

Dead Time: Time to allow for settling of the commanded wavelength before starting the image exposure.

Compute Traces of Marked Targets: This function allows to recompute stored "Photo" *Traces* (Photo_W1, Photo_W2, Photo_W3, Photo_R, and Photo_Ca) with modified fluorescent background values.

19.1 Photometry Configuration T.I.L.L.

Photometry: T.I.L.L.
R-max 1.0000E+00 R-min 1.0000E+00 Kd * Sf 1.0000E+00 Background1 0.0000E+00 Background2 0.0000E+00 Background3 0.0000E+00
Dead Time 2.00 ms Compute Traces of Marked Targets
Resting Wavelength 280 nm Set Excit. DA-1 -9.95V 280 nm
Undo Defaults Emit-1 AD-1 0.00V Emit-2 off 0.00V
No Shutter
Hide Show Calibration
Wavelength Calibration from -10.00V to 10.00V Compute Factors
wavelength - lower - volt wavelength - upper - volt
380 nm Scan -4.50V 505 nm Scan 2.50V
Wavelength to Volts
Slope Offset Angle ß
7.2500E-02 1.8200E+00 30°

Resting Wavelength: Wavelength to be set during resting periods.

Set: Only after pressing the Set button the wavelength entered in the Resting Wavelength field is output.

Excit.: Select the DA channels for the voltage output. This function is used to control the monochromator. Once it is calibrated, the output can be defined either in Volts (left) or in nm (right).

Emit-1, Emit-2: Select the AD channels for sampling the photometry responses. *Emit-1* is always active whereas *Emit-2* can be turned off. It may be used for dual emission fluorescence experiments.

Shutter Selection:

- No Shutter: No shutter available.
- Shutter Control: Shutter can be closed/opened via a button.
- Digital-out 7: Shutter control via digital output.

19.1.1 Wavelength Calibration

Hide	Show Cali	bration							
Wav	elength Cal	libration	from -	-10.00V	to 10.00	DV]	Comp	oute F	actors
wa	avelength	- lower	- volt	1	waveleng	th -	upper	- vo	olt
38	0 nm	Scan	-4.50V		505 nm		can	2.	50V

In this area the monochromator is calibrated, i.e. the relationship between output voltage and the wavelength is defined. To do so, voltage ramps ranging between the values specified by *from...to* are output when the *Scan* buttons are pressed. This is done for two bandpass filters with known transmission maxima. The corresponding wavelengths are entered in the *wavelength* fields. After both scan operations, i.e. when the voltages corresponding to the peak transmission have been determined successfully, the calibration constants for the control of the monochromator are calculated upon *Compute Factors* is pressed.

from - to: Scan range for voltage ramps. For a single bandpass filter usually the full range of ± 10 V can be scanned. However, when a single multi-band filter is used, the scan range has to be limited in order to detect only the peak of interest.

Compute Factors: Compute calibration constants based on the determined relationships of wavelength and voltage.

wavelength: Enter here the maximum of the bandpass filter (in nm).

lower - Scan: Do the scan for the low-wavelength calibration filter.

Volt: This field shows the voltage corresponding to the peak transmission.

upper - Scan: Do the scan for the high-wavelength calibration filter.

Volt: This field shows the voltage corresponding to the peak transmission.

Wavelength to Volts: Slope, Offset and Angle are internal calibration constants used to control the monochromator. These values are the result of the calibration procedure.

Wavelength to Volts							
Slope Offset Angle ß							
7.2511E-02	1.8235E+00	30°					

The conversion between voltages and wavelength is done as follows:

$$F(\lambda) = \arcsin(\lambda/1666.67 * \cos(\pi/360 * \beta))$$
(19.1)

$$V = (2\pi * F(\lambda) - Offset)/Slope$$
(19.2)

$$\lambda = (1666.67 * \cos(\pi/360 * \beta) * \sin((Slope * V + Offset)/2\pi)$$
(19.3)

$$Slope = 2\pi * (F(\lambda_1) - F(\lambda_2)) / (V_1 - V_2)$$
(19.4)

$$Offset = 2\pi * F(\lambda_1) - Slope * V_1 \tag{19.5}$$

with

- β = typically 30 degree; depends on your hardware. Please refer to the manual of the monochrometer.
- λ = requested wavelength in [nm]; λ_1 = calibration wavelength 1.
- V = DA voltage in [V]; $V_1 = DA$ voltage of calibration wavelength 1.
- arguments of trigonometry functions in [RAD].

Here an example: We calibrate our monochrometer at the wavelength 380 nm and 505 nm. With the 380 nm and 505 nm filters we measure an intensity maximum at -4.5 V and 2.5 V, respectively. Now, we calculate $F(\lambda)$ for both wavelength: $F(\lambda_1) = 0.2383$, $F(\lambda_2) = 0.3191$. Then the Slope is 0.0725 and the Offset is 1.8235.

19.1.1.1 Calibrating the different T.I.L.L. monochrometers

Polychrome I and Polychrome II: Two bandpass filters are provided with the Polychrome I and II. Enter the peak wavelength in the Wavelength fields of the Wavelength Calibration section, then put the first filter in place and press the left Scan button. The voltage of the peak intensity will be automatically detected and shown in the Volt field. Now, put the second filter in place and press the right Scan button. Finally, press Compute Factors to calculate all calibration parameters.

Polychrome IV: The Polychrome IV comes with a triple-band filter. When using this filter the scan range to detect the first or the last peak of the three has to be limited. Therefore, you should limit the scan range for the first peak from -10 V to -2 V and for the third peak from +2 V to +10 V. We omit the center peak in this calibration. Please enter the peak wavelengths in the corresponding *Wavelength* fields and the scan range before performing each scan in the *from* – *to* fields. Then, perform the two scans and finally, press *Compute Factors* to calculate all calibration parameters.

Polychrome V: The Polychrome V features an auto-calibration. Please ask T.I.L.L. Photonics for a pair of calibration values. E.g. which analog control voltages refer to the wavelength 380 nm and 505 nm. Then, please enter the wavelength and voltages in the *Wavelength* and *Volts* fields and press *Compute Factors* to calculate all calibration parameters.

19.2 Photometry Configuration DG4/DG5 and Lambda-10

🔛 Photometry: DG4 / DG5								
R-max	1.0000E+00	R-min	0.0000E+00	Kd * Sf	1.0000E+00			
Background1	0.0000E+00	Background2	0.0000E+00	Background3	0.0000E+00			
Dead Time 10.0 ms Compute Traces of Marked Targets								
Emit-1 AD-0 -0.00V Emit-2 off 0.00V								
Filter Position 0 Shutter: Open Digital-out 7 Resting Wavelength 0 Set								

Emit-1, **Emit-2**: Select the AD channels for sampling the photometry responses. *Emit-1* is always active whereas *Emit-2* can be turned off. It may be used for dual emission fluorescence experiments.

Filter Position: Enter a number (Filter Position) between 0 and 15.

Shutter Open/Close: Toggles between an open and a closed shutter.

Shutter Selection:

- No Shutter: No shutter available.
- Shutter Control: Shutter can be closed/opened via a button.
- Digital-out 7: Shutter control via digital output.

Resting Wavelength: Wavelength to be set during resting periods.

Set: Only after pressing the *Set* button the wavelength entered in the *Resting Wavelength* field is output. Speed: Enter a number between 0 and 7.

19.2.1 PGF Sequence

When using the DG4/DG5 or Lambda-10 you need to define the *Filter Position* in your PGF sequence. Therefore you have to set the DA output channel to *Dig Out (word)* and use for wavelength. Then you can enter the *Filter Position* directly in the segments (Wavelength) of the PGF sequence.

🔣 Pulse Generator F	ile: DefPgf							
Full View	Conden	sed View	Cartoon V	/iew				
0 Time_Ex	pand) 10 🔄	LTP 11(LockIn) 12(TestS	eries) 1	3 DG4/DG5	14	<mark>0</mark> 0
Pool LOAD	MERGE) (SAV	/E Name	DG4/DG	5 CLIS		PY MOV		O DELETE
Interactive Mode	O Gap Free	Mode	(Photometry	<mark>/ Wave</mark>)			
Timing No w	ait before 1. Sw	veep Not Trig	gered	Checki	ng			EXECUTE
No of Sweeps	1	Use Dur	ations	Sweep Le	ngth	Total	1.000 s	20000 pts
Sample Interval	50.0 µs (20.0	kHz StartTime	0.00	Channel L	enath	Stored	1.000 s	20000 pts
2 DA	Unit	Stimulus -> DA	Leak	AD U	nit Link	Compr. Po	ints Store	Zero Leak
△(Ch-1) Stim-E	A V	StimScale		Imon2 /	A 1	1 C 20	000 🖾	0 No Leak
Ch-2 Dig-out	(woi nm	Wavelength		Photo W1	2	4000 C	5 🖾	0 No Leak
✓ Ch-3 01	V a	absolute voltage		Photo R	2	4000 C	5 🖾	0 No Leak
Segments 00	1 Stored	2 Stored 3	3 Stored	4 Stored	15 [St	ored 6 Sta	া বব	Separate Timing
Segment Class	Constant	Constant	Constant	Constant	Const	ant Const	ant N	lo Break 0.00
Wavelength	val 2	val 4 v	al 2	val 4	val	2 val	4	Voltage Clamp
Duration [ms]	val 100.00	val 100.00 v	al 100.00	val 100.00	val 10	0.00 val 10	0.00	Filter Factor
	Increase	Increase	Increase	Increase	Increa	ase Increa	ase	0.01
VV-fact./incr.	1.00 0	1.00 0 1	1.00 0	1.00 0	1.00	0 1.00	0 /	Analysis: (Edit)
t-Incr. Iviode	Increase	Increase	Increase	Increase	Increa	ase Increa	ase	
t-fact./incr. [ms]	1.00 0.00	1.00 0.00	1.00 0.00	1.00 0.00	1.00	0.00 1.00	0.00 R	el X-seg 1
							R	el Y-seg 1
Draw: Active Char	inel, all Sweeps	Delay: DA	0.00 s AD	55.0 ms				
							Set Last	Seg. Amplitude
				Le	eak Puls	es		
				N	o of Leaks	s 0		
				Le	eak Delay	10.0	ms (Leak Alternate
00.mnm				Le	eak Size	1.0	00 (Alt.Leak Average)
100 ms				Le	eak Hold		-	wait = abs. hold
p1 p	2 p3	p4	p5	p6	p7	p8	P9	p10
100.00m 0.00	100 100.00r	n 90.000m	10.000m	45.000m	0.0000	0.0000	0.000	0 0.0000
Traces 4								

Note: To control the DG4/DG5 or Lambda-10 via the CHARTMASTER software you need a special cable connecting the Digital Outputs of the interface and the DG4/DG5 or Lambda-10. Please contact HEKA (sales@heka.com) if you need such a cable.

19.3 Photometry Configuration PTI DeltaRAM

🔛 Photometry: p	oti DeltaRAM					
R-max	1.0000E+00	R-min	0.0000E+00	Kd * Sf	1.0000E+00	
Background1	0.0000E+00	Background2	0.0000E+00	Background3	0.0000E+00	
Dead Time	20.0 ms		Compute Traces of Marked Targets			
Resting Wav	elength 300 nm	n <mark>Set</mark>	Excit. DA-1	300 nm		
Undo	Defaults E	mit-1 AD-1	3.21V	Emit-2 off	0.00V	
W	avelength to V	olts	No Shutter			
Volts/n	m Wav	elength at 0V				
-2.1414E	-02 4.	5000E+02				

Resting Wavelength: Wavelength to be set during resting periods.

Set: Only after pressing the Set button the wavelength entered in the Resting Wavelength field is output.

Excit.: Select the DA channels for the voltage output. This function is used to control the monochromator. Once it is calibrated, the output can be defined either in [V] (left) or in [nm] (right).

Undo: Resets all changes made since the last opening of the Photometry window.

Defaults: Resets all changes to the default entries.

Emit-1, **Emit-2**: Select the AD channels for sampling the photometry responses. *Emit-1* is always active whereas *Emit-2* can be turned off. It may be used for dual emission fluorescence experiments.

Wavelength to Volt Volts/nm and Wavelength at 0 V are internal calibration parameters, which must be given as described in the specifications of your DeltaRAM system. The PTI system has a linear relationship between the applied voltage and the wavelength of the excitation light.

Shutter Selection:

- No Shutter: No shutter available.
- Shutter Control: Shutter can be closed/opened via buttons.
- Digital-out 7: Shutter control via digital output.

20. Imaging Extension

The Imaging Extension allows to perform simultaneous image acquisition and electrophysiological recordings. The timing of a fluorescence excitation light source, the camera and the electrophysiological command signals are synchronized by the Pulse Generator of CHARTMASTER. Fluorescence mean values from regions of interest (ROI), which can be marked by the user in the image, are plotted together with e.g. the current *Trace* in the Oscilloscope window and stored together with the other data in CHARTMASTER's data tree. When stepping through the data tree, the Imaging Extension shows the corresponding image. In case more images have been acquired during a single *Sweep*, the individual images are shown when stepping through the data points of the fluorescence *Trace* using the scan function of the Oscilloscope.



The Imaging Extension consists of an Imaging window in CHARTMASTER, an additional Control window and an Image window.

The Image Extension supports the following hardware:

- Fluorescence Excitation light sources which can be controlled in the same way as:
 - Polychrome and Oligochrome from T.I.L.L. Photonics
 - DG-4/DG-5 wavelength switcher from Sutter Instrument Company
 - Lambda-10 filter wheels from Sutter Instrument Company
 - DeltaRAM monochromator from PTI
- Cameras supporting the so-called "Bulb Trigger Mode" from Andor, Jenoptic, QImaging and Hamamatsu.

Image files are stored as 16-bit tiff stacks and can be opened by other programs such as ImageJ, IGOR Pro etc....

20.1 Starting the Imaging Extension

After starting CHARTMASTER, open the Configuration window and select the appropriate Imaging Extension in the hardware section of the dialog. The Imaging Extension has to be selected to support the fluorescence excitation light source of your setup (see above).

After activating the IMAGING EXTENSION, the Imaging window is opened in CHARTMASTER, the Image Control window and the Image window are opened.

20.2 Imaging Configurations

This section provides a brief overview of the settings for each of the fluorescence excitation light source used with the Imaging Extension.

Once the Imaging Extension has been activated the Imaging window can be opened from the menu Windows.

Some controls are common for all configurations describing the different fluorescence excitation light sources. These controls are described in this section.

R-max, R-min: Minimum and maximum ratio (F1/F2).

 $\mathbf{Kd}\mathbf{*Sf:}$ Effective Kd (dissociation constant) of the dye.

Note: These values are required for calculating the free Calcium concentration (according to the Grynkiewicz formalism). The Calcium concentration can be stored as a Trace by use of the "Photo_Ca" input channel in the Pulse Generator dialog.

Background 1...3: Background fluorescence for the three wavelengths F1, F2, F3. The background values are stored as *Series Parameters* with the raw data file and are listed in the **Parameters** dialog.

Dead Time: Time to allow for settling of the commanded wavelength before starting the image exposure.

Traces to create: Check the *Traces* which should be automatically generated by the **Pulse Generator** or created by the *Recompute* function.

Compute Traces of Marked Targets: ALL: Recomputes the complete fluorescence data set starting with the calculation of the mean fluorescence values from the regions of interest (ROIs). Use this function when you have modified the set of ROIs.

Compute Traces of Marked Targets: Ratio + Ca: Recomputes the based on the fluorescence values already stored in CHARTMASTER. Use this function when you modify the *Background* values or the calibration *Parameters* for the calculation of the calcium concentration *R-min*, *R-max*, *Kd*Sf*.

Compute Histogram of Marked Targets: A histogram *Trace* is created for every image connected with your recordings and added at the end of the **Replay** tree. If you have drawn a ROI in your image the histogram is created only for the ROI and not the total image. When several ROIs exist CHARTMASTER will create histogram *Traces* for every ROI.

Camera Trigger: Select the output channel which is used for triggering the camera.
20.2.1 Imaging Configuration T.I.L.L.

🔜 Imaging: T.I.L.L.				
R-max 1.0000E+00 Background1 0.0000E+00 Dead Time 2.00 ms	R-min Background2	0.0000E+00 0.0000E+00	Kd * Sf Background	1.0000E+00 0.0000E+00
		Compute Traces	ate: V2 ⊠ W3 I of Marked Targei	□ Ratio □ Ca ts: All Ratio + Ca
Resting Wavelength 280 nm	Set	Excit. DA-1	9.95V	280 nm
Undo Defaults				
Camera Trigger Dig-0			No Shutt	er
Wavelength Calibration	from -10	.00V to 10.	00V Com	pute Factors
wavelength - lower - 380 nm Scan	volt -4.50V	wavelen 505 nm	ngth - upper	- volt 2.50V
Wavelength to Volts	i Angla (
7.2500E-02 1.8200E+00	Angle Is			

Resting Wavelength: Wavelength to be set during resting periods.

Set: Only after pressing the Set button the wavelength entered in the Resting Wavelength field is output.

Excit.: Select the DA channels for the voltage output. This function is used to control the monochromator. Once it is calibrated, the output can be defined either in Volts (left) or in nm (right).

Shutter Selection:

- No Shutter: No shutter available.
- Shutter Control: Shutter can be closed/opened via a button.
- Digital-out 7: Shutter control via digital output.

20.2.1.1 Wavelength Calibration:

In this area the monochromator is calibrated, i.e. the relationship between output voltage and the wavelength is defined. To do so, voltage ramps ranging between the values specified by from...to are output when the Scan buttons are pressed. This is done for two bandpass filters with known transmission maxima. The corresponding wavelengths are entered in the wavelength fields. After both scan operations, i.e. when the voltages corresponding to the peak transmission have been determined successfully, the calibration constants for the control of the monochromator are calculated upon Compute Factors is pressed.

- from to: Scan range for voltage ramps. For a single bandpass filter usually the full range of ± 10 V can be scanned. However, when a single multi-band filter is used, the scan range has to be limited in order to detect only the peak of interest.
- Compute Factors: Compute calibration constants based on the determined relationships of wavelength and voltage.

- wavelength: Enter here the maximum of the bandpass filter (in nm).
- lower Scan: Do the scan for the low-wavelength calibration filter.
- Volt: This field shows the voltage corresponding to the peak transmission.
- wavelength: Enter here the maximum of the bandpass filter (in nm).
- upper Scan: Do the scan for the high-wavelength calibration filter.
- Volt: This field shows the voltage corresponding to the peak transmission.

20.2.1.2 Calibrating the different T.I.L.L. monochrometers

Polychrome I and Polychrome II: Two bandpass filters are provided with the Polychrome I and II. Enter the peak wavelength in the Wavelength fields of the Wavelength Calibration section, then put the first filter in place and press the left Scan button. The voltage of the peak intensity will be automatically detected and shown in the Volt field. Now, put the second filter in place and press the right Scan button. Finally, press Compute Factors to calculate all calibration parameters.

Polychrome IV: The Polychrome IV comes with a triple-band filter. When using this filter the scan range to detect the first or the last peak of the three has to be limited. Therefore, you should limit the scan range for the first peak from -10 V to -2 V and for the third peak from +2 V to +10 V. We omit the center peak in this calibration. Please enter the peak wavelengths in the corresponding *Wavelength* fields and the scan range before performing each scan in the *from* - *to* fields. Then, perform the two scans and finally, press *Compute Factors* to calculate all calibration parameters.

Polychrome V: The Polychrome V features an auto-calibration. Please ask T.I.L.L. Photonics for a pair of calibration values. E.g. which analog control voltages refer to the wavelength 380 nm and 505 nm. Then, please enter the wavelength and voltages in the *Wavelength* and *Volts* fields and press *Compute Factors* to calculate all calibration parameters.

20.2.2 Imaging Configuration DG4/DG5 and Lambda-10

🔛 Imaging: DG4	/ DG5				- • ×	
R-max	1.0000E+00	R-min	0.0000E+00	Kd * Sf	1.0000E+00	
Background1	0.0000E+00	Background2	0.0000E+00	Background3	0.0000E+00	
Dead Time	10.0 ms		Traces to crea	ite:		
			⊠ W1 ⊠ W	/2 ⊠ W3 C] Ratio 🛛 Ca	
Compute Traces of Marked Targets: All Ratio + Ca						
Resting Wav	elength 0	Set)	Compute H	listograms of Mar	ked Targets	
Filter Positio	n 0 (Shutter: Open) Digital-	out 7		
Camera Trig	ger DA-1				//	

Filter Position: Enter a number (*Filter Position*) between 0 and 15. Shutter Open/Close: Toggles between an open and a closed shutter. Shutter Selection:

- No Shutter: No shutter available.
- Shutter Control: Shutter can be closed/opened via a button.
- Digital-out 7: Shutter control via digital output.

20.2.3 Imaging Configuration PTI DeltaRAM

🔛 Imaging: pti D)eltaRAM						
R-max	1.0000E+00	R-min	0.0000E+	00	Kd * Sf	1	1.0000E+00
Background1	0.0000E+00	Background2	0.0000E+	00	Backgroun	d3 (0.0000E+00
Dead Time	20.0 ms		Traces to ⊠ W1	creat	e: 2 ⊠ W3		Ratio 🗆 Ca
			Compute Tra	aces of	f Marked Tarç	gets: .	All Ratio + Ca
			Comp	oute His	stograms of N	Marke	ed Targets
Resting Wav	elength 300 nm	<mark>Set</mark>	Excit.	A-1	3.21\	/	300 nm
Undo	Defaults						
N	avelength to Vo	olts				No S	Shutter
Volts/n -2.1414E	m Wav E-02 4.1	elength at 0V 5000E+02					
Camera Trig	ger Dig-0						

Resting Wavelength: Wavelength to be set during resting periods.

Set: Only after pressing the Set button the wavelength entered in the Resting Wavelength field is output.

Excit.: Select the DA channels for the voltage output. This function is used to control the monochromator. Once it is calibrated, the output can be defined either in Volts (left) or in nm (right).

Shutter Selection:

- No Shutter: No shutter available.
- Shutter Control: Shutter can be closed/opened via a button.
- Digital-out 7: Shutter control via digital output.

Wavelength to Volt Volts/nm and Wavelength at 0 V are internal calibration parameters, which must be given as described in the specifications of your DeltaRAM system. The PTI system has a linear relationship between the applied voltage and the wavelength of the excitation light.

20.3 Image Window



The Image window displays the image during image acquisition or when loading an image file.

The pixel position of the mouse tip is shown as X- and Y-value on the left side in the foot line of the image. In the middle of the foot line the *pixel* intensity (brightness or fluorescence value) is shown. On the right side the computer's time is shown.

The icons on the right side of the window are short cuts to the corresponding tabs of the Image Control window.

20.4 Image Control Window

20.4.1 Camera Setup

Kinning	Live View Darameters
birining	Live view Parameters
Ix1	
© 2x2	
3x3 1360 x 1024	Exposure Time [ms] 10
© 4X4	
O JAJ	
Cooling	Scaling
- attice	auto 🔽
	min max.
	0 16383
	Histogram

Camera Reference: Lists the identification of the camera used by the Imaging Extension.

Binning: Allows to set the camera binning factor. The resulting pixel dimensions of the image are shown to the right.

Note: The binning factor is typically set once for a complete experiment.

Live View Parameters: Camera parameters used during Live View mode only.

• Exposure Time [ms]: Time for image exposure during live image acquisition.

Scaling: Parameters determining the intensity scaling of the image.

- auto: When activated each image is scaled with respect to the darkest and brightest pixel.
- min / max: When *auto* is activated, *min/max* display the intensity values of the darkest and the brightest pixel. When *auto* is not active, the user can set intensity values for the darkest (black) and brightest (white) pixel manually.
- histo: Calculates an intensity histogram of all pixels and shows it in a separate graph (see below).



Live View: Starts and stops the live acquisition of the camera. In "Live Mode" the camera is running with its maximal speed.

Snapshot: A click to this button acquires a single image (YYYY-MM-DD-000.tiff). The path is defined in the *Imaging Configuration* (20.4.5 on page 179).

20.4.2 PGF Primer

Imaging related information about wavelength, exposure times and repetition rates can be automatically integrated in already existing Pulse Generator sequences. All imaging related manipulation of the sequences should be done through the *PGF Primer*. Once the desired imaging *Parameters* are found, the user can integrate them into multiple Pulse Generator sequences.

Update	from PGF		
Acquisitio	n parameters		
Readout 1	Time [ms]	160	
Cycle Time	e [ms]	0	
Cycles:		1	Adapt to length
	no imaging Wave	elength	Exposure [ms]
W1		1	20
W2		2	20
W3		0	0

PGF Listing: A list which contains all **Pulse Generator** sequences currently loaded in CHARTMASTER. You can select a specific sequence to work on. In case this sequence contains already imaging related *Parameters* the values are updated in the fields below.

Prime: Transfers the imaging related Parameters to the selected Pulse Generator sequences.

Update List: Reads the complete Pulse Generator pool from CHARTMASTER and updates the PGF Listing.

Update from PGF: If this option is selected the imaging related *Parameters* of already existing **Pulse Generator** sequences is read out and displayed in the fields of this dialog.

Note: In case you want to integrate the same imaging related Parameters to multiple **Pulse Generator** sequences then this option has to be deselected.

Acquisition Parameters:

- Readout Time [ms]: Displays the approximate camera read out time for one image. This setting usually depends on the binning factor.
- Cycle Time [ms]: Total duration of one cycle of image acquisition. One cycle can contain 1 to 3 individual image acquisitions.
- Cycles: Enter the number of cycles (repetitions). In case the option Adapt to length is selected, this field displays the maximal number of repetitions after the priming process.
- Adapt to length: This option allows to repeat the parent imaging cycle as many times until the duration of the longest sweep in the Pulse Generator sequences is reached.
- No. of wavelengths list: You have to specify how many different wavelengths you want to use in a single imaging cycle.
- Wavelength: Enter the wavelength (in nm when using a T.I.L.L. monochromator) or the filter position number (when using a filter wheel or DG-4/5).
- Exposure [ms]: Enter the exposure times for the different wavelength.

20.4.3 ROI Manager

When the *ROI Manager* tab is selected the user can draw ROIs into the image. The ROIs are listed in the main field of the tab.

💦 ROI Manager	8 ×
ROI_1 ROI_2 ROI_3	Delete ROI
	Save ROIs Load ROIs
Show ROIs	
<	

List of ROIs: Shows the names of all ROIs from the current set. The selected ROI from the list is shown in bright yellow color.

Delete ROI: Deletes the selected ROI.

Save ROI: Saves the current set of ROIs to a separate file with extension ***.roi**. In case an image acquisition is started from CHARTMASTER the ROIs, which belong to this image set, are automatically stored to a file with extension ***.roi** and the name of the image. When reloading this image file the corresponding set of ROIs is also loaded.

Load ROI: Loads a set of ROIs from file.

Show ROIs: When activated the ROIs are shown as overlay to the image.

20.4.4 Image File Selector



Filename: Shows the name and path of the active image.

Page Selector: In case the image file contains several image frames you can use the left and right arrow buttons to step through the individual frames of the image stack.

Info Text: Shows the additional text information which is stored with the image file.

Open Image File: Allows to open an image file.

Close File: Closes the current image file.

20.4.5 Imaging Configuration

Paths Snapshots: C:/F	Program Files ((x86)/HEKA/Pat	chMaster	
Other settings				
Show Tab Bar				~
Keep Aspect R	Ratio			
Default Pixel Dept	th	14-bit		•

Paths:

• Snapshots: Shows the path to the folder in which all snapshots will be stored. Press the button on the right in order to change the folder.

Note: Images acquired with the **Pulse Generator** are stored in the same folder as the CHARTMASTER data file.

Other Settings:

- Show Tab Bar: If activated the Tab Bar is shown in the foot line.
- Keep Aspect Ratio: If activated the aspect ratio of the camera sensor is kept proportional when enlarging the window.
- Default Pixel depth: Select the dynamic range of the digitalization (8, 12, 14 or 16 bit).

20.4.6 Imaging Log



Log File Text: Protocols the communication between the Imaging Extension and CHARTMASTER. This information is used to facilitate the troubleshooting.

21. Software LockIn Extension

21.1 LockIn Configuration Window

For the activation and the configuration of the *LockIn Extension* you have to enable the software lock-in function in the Configuration window (see 5.4.2 on page 44). After switching on the *LockIn*, the LockIn Configuration window opens, which is later on available in the Window menu.

Important note: In the CHARTMASTER manual only the main user functions are explained. If you are interested in a detailed description how the LockIn Extension works or what might be the best approach to determine membrane capacitances in different recording modes we refer to the PATCHMASTER Tutorial: "Capacitances Measurements using the LockIn Extension".

📃 LockIn C	onfiguration	E	- 0 X				
Lockin M	ode [C	Dn				
Phase S	hift [0.0°					
Attenuati	on [1.000					
Parent Tr	ace [Trace 1					
U Write	to Notebook						
Points to	Average [C	Dff				
Offline C ⊠ Real(\ ⊠ Imag(□ Admit □ Phase	Offline Computation - Traces to create: \Box Real(Y) \Box Real(Z) \Box Imag(Y) \Box Imag(Z) \Box Admit(Y) \Box Imp Z \Box Phase \Box Sine Average						
(<u>Co</u>	mpute LockIn	of Marked T	argets				
Default	-ranges:		Set Defaults				
Real(Y)	200.0n	Real(Z)	1.000				
Imag(Y)	200.0n	Imag(Z)	1.000				
Admit(Y)	200.0 nS	Imp Z	1.000 Ohm				
Phase	180.0°	Skip	0				

LockIn Mode: Set a LockIn Mode. You have these possibilities:

- Off: No LockIn mode is set.
- On: The *LockIn* mode is turned on.
- Phase Range: 0..180: Limits the phase to a range of 0° to 180°.
- Phase Range: 0..360: Allows to use the full phase range.

Phase Shift: The user can specify a *Phase Shift* to be applied to the residual admittance before the equivalent circuit parameters are calculated. This *Phase Shift* is only applied to the actual current that is measured and not the component of admittance which is "nulled out" by *C*-slow compensation.

This feature can be used, for example, to "tweak" the phase determined by Measured or Calculated Calibration. Another example where this would be useful is if data are recorded with a set of "critical parameters" which differs from those that were used when a Measured Calibration was performed (this is prevented by the software if an EPC 9 or EPC 10 is used, but can occur for other amplifiers). The phase difference can be determined by performing a new Measured Calibration and can be specified as the Phase Shift for replay of the recorded data.

When Manual Calibration is used, the Phase Shift sets the absolute phase of the software lock-in.

Note: Generally the Phase Shift should be left at 0.0°.

Attenuation: It is an amplitude scaling factor for the current signal that can be used to correct the transfer function of the complete system. This attenuation factor is applied to the residual admittance before the equivalent circuit parameters are calculated.

Note: Generally the Attenuation should be left at 1.0°.

When Manual Calibration is used the Attenuation should be set to the value estimated by the Measured Calibration.

Generally, the *Attenuation* might be set to a value unequal to 1 when the transfer function of the system is not ideal, i.e. the sine wave frequency is higher than the current filter frequency or the sine wave frequency approaches the overall bandwidth of the amplifier.

Parent Trace: This option is only available if Manual Calibration is selected. Select the corresponding Trace (e.g. current or voltage Trace) for the calibration, either the Linked Trace assigned in the PGF (Link) or any other Trace (Trace 1...16).

Write to Notebook: Enabling this option will print out the LockIn results to the Notebook window.

Note: The Write to Notebook option does only work if you acquire at least one LockIn Trace in your PGF.

Points to Average: A global number of points to average can be entered.

Offline Computation - Trace to create: These sections of the LockIn window are used for calculating LockIn data from pre-recorded current Traces (for further details see The LockIn Traces, 21.2).

Compute LockIn of Marked Targets: Computes the LockIn *Traces* selected in *Offline Computation* - *Trace to create* of the marked targets in the in the Replay tree.

Default Y-ranges: The user has to define *Default Y-ranges* for the resulting *LockIn Traces*. CHARTMASTER does not know the range of the forthcoming data because this dependents on cell size. Thus, the user has to provide a default range (e.g. 0 to 40 pF for the C_m Trace would be a reasonable estimate).

SetDefaults: Resets the Default Y-ranges to the default values.

Skip: A global number of cycles to skip can be entered.

Important note: The global setting Skip, when activated, overrules the setting made in the Wave Parameters of the PGF.

21.2 The LockIn Traces

The LockIn Extension provides several output Traces which can be selected in the AD-channel section of the Pulse Generator (9.9.2 on page 89).

The following Traces which are independent from the LockIn mode are available:

LockIn_Phase: The phase angle between stimulus and measured signal.

$$Phase = \frac{180}{\pi} * \arctan(B/A)$$

LockIn_Real(Y): The real part of the admittance.

Voltage Clamp: Real(Y) = A (Siemens). Current Clamp: $Real(Y) = \frac{A}{A^2 + B^2}$ (Ohm).

LockIn_Imag(Y): The imaginary part of the admittance.

Voltage Clamp: Imag(Y) = B (Siemens). Current Clamp: $Imag(Y) = \frac{B}{A^2+B^2}$ (Ohm).

LockIn_Admit(Y): The admittance (Siemens).

Voltage Clamp: $Admit(Y) = \sqrt{A^2 + B^2}$. Current Clamp: $Admit(Y) = \frac{1}{\sqrt{A^2 + B^2}}$.

LockIn_Real(Z): The real part of the impedance.

Voltage Clamp: $Real(Z) = \frac{A}{A^2 + B^2}$. (Siemens) Current Clamp: Real(Z) = A (Ohm).

LockIn_Imag(Z): The imaginary part of the impedance.

Voltage Clamp: $Imag(Z) = \frac{B}{A^2+B^2}$. (Siemens) Current Clamp: Imag(Z) = B (Ohm).

 $LockIn_Imp|Z|$: The impedance (Ohm).

Voltage Clamp: $Imp(Z) = \frac{1}{\sqrt{A^2 + B^2}}$. Current Clamp: $Imp(Z) = \sqrt{A^2 + B^2}$.

Abbreviations:

- A = Real part of the admittance.
- B = Imaginary part of the admittance.
- $I_{dc} = DC$ current.
- E_{rev} = Reversal potential.
- V_h = Stimulus voltage.
- $f_{sine} =$ Stimulation frequency.

A detailed description of all terms can be found in the literature (see reference list of the PATCHMASTER Tutorial: "Capacitances Measurements using the LockIn Extension").

21.3 Capacitance Measurements - Step by Step

Important note: The following section was made for PATCHMASTER and HEKA EPC 9 or 10 amplifier. If you are using CHARTMASTER or POTMASTER several modifications may be necessary!

Here, we make our first capacitance measurement using a model circuit and the LockIn Extension. We used an EPC 10 Single for this tutorial, but most settings are identical for EPC 10 Double, Triple or Quadro amplifiers and for EPC 9 Single, Double and Triple amplifiers. We will explicitly mention the particular amplifiers, where it is required.

General	Hardware	Files	Display	I/O Control	Trace Assign	Misc
EPC10_USB A	mplifier	VC Stim. Scale	0.100	Auto Filter		
LIH 8+	8	CC Stim. Scale	100. fA/mV	Zap OnCel	l only	
		Voltage In Scale	0.100			
nd	Off	V-membrane Out	Stim-DA	□ 3-Electrod	e Headstage	
dn 🗸	On	Test Trigger Out	off	Multi-Channel	s Off	
ctroscopy		Current In	Imon2	Probe Selecto	or Off	
jing	Off	Voltage In	Vmon			
tometry	Off					
oScan	Off					
	Off					

21.3.1 Activating the LockIn Extension

By default, the LockIn Extension of CHARTMASTER is inactive. To activate the extension, we have to open the Hardware tab of the Configuration window and turn on the LockIn. This step only has to be done the first time one uses LockIn. The setting is stored in the CHARTMASTER configuration file Chartmaster.set.

21.3.2 LockIn Configuration

Immediately after switching on the LockIn Extension, the LockIn Configuration window comes up. Please set the LockIn mode to Sine + DC. This is the recommended mode, if you are using an EPC 10 or EPC 9 amplifier. At the moment, no other settings need to be made. We will come back to the LockIn Configuration window later.

Important note: If you want LockIn calculations to be written to the Notebook, Write to Notebook should be checked and at least one LockIn trace has to be selected as an AD input channel in the PGF.

Lockin Mode	Sine + DC	Offline Computation	Traces to	create:
Calibration Mode	Calculated	Real(Y) Real(A	Z) 🛛 CM	
Phase Shift	0.0°	□ Imag(Y) □ Imag(Z) 🗆 GN	
Attenuation	1.000	Phase Sine	Average	
Parent Trace	Linked Trace	Compute Lockin	of Marked T	argets
PL-Phase	0.0° Compute	Compute CV + GP fro	om Real + Ir	mag. Trace
Calib. Sequence	IV	Default Y-ranges:	(Set Defaults
Perform Mea	sured Calibration	Real(Y) 200.0n	Real(Z)	1.000
Write to Notebook		Imag(Y) 200.0n	Imag(Z)	1.000
Points to Average	Off	Admit(Y) 200.0 nS	Imp Z	1.000 Ohm
		Phase 180.0°		
				1 000 -0
		CM 40.00 pF	DC	4.000 nS
		CM 40.00 pF GM 4.000 nS	DC CV	40.00 nS
		CM 40.00 pF GM 4.000 nS GS 400.0 nS	DC CV GP	40.00 pF 400.0 nS

Note: The Default Y-ranges section can be used to set reasonable display ranges according to the expected values.

21.3.3 Creating a PGF sequence

The next step is to prepare a PGF sequence, that can be used for C_m measurements. Please open the Pulse Generator window. We could either modify an existing sequence or create a new one from the scratch. Let us create a new sequence this time. A click on an empty button in the sequence pool will create a new sequence and you are prompted to enter a name for the new sequence. We name the sequence we want to create "LockIn".

CHARTMASTER automatically creates a new and very simple protocol: it just consists of one *Constant* segment with a duration of 10 ms. 10 ms is quite short, therefore we will increase the duration to 100 ms. Furthermore, we have to change the *Segment Class* to *Sine*, since at lease one sine wave segment is required for the *LockIn*. Of course, one can also use other segment classes (Constant, Ramp, Square) in the same sequence, for example to define a depolarizing voltage step at the beginning of the *Sweep*. But *LockIn* data can only be calculated from *Sine* segments.

🔛 Pulse Gene	erator File: D	efPgf_v9							
Full V	iew	Condense	d View	Cartoon \	/iew				
00 1	Lockin	2	30) 4 🦲		5 (6	<mark>0</mark> 0
Pool LOA	D MERC	E) SAVE	Name	Lockin		ST CC	OPY MC	OVE UND	O DELETE
Interactive	Mode O	Gap Free M	ode				Sine V	Nave	
Timing	No wait be	fore 1. Swee	p Not Trig	gered	Chec	king			EXECUTE
No of Swe	eps	1	Use Dur	ations	Sweep L	ength	Total	100.0 ms	2000 pts
Sweep Int Sample In	erval 50.0	0.00 s	z StartSeg	0.00	Channel	Longth	Stored	100.0 ms	4000 bytes
		Luit Chi-		Lask	AD	Lengui	Carran	Deinte Ctere	7 Lask
Ch.1	Stim-DA		nuius -> DA StimScale	Leak	Imon2		Compr. 1	2000 0	0 No Leak
<u> </u>	off	abs	olute voltage		off		C	0	No Leak
	off	abs	olute voltage		off		Ci	0	No Leak
⊻()	off	abs	olute voltage		off		CI	0	No Leak
Segments	📢 🖓 🛇 St	ore 1	Store 2	Store 3	□Store 4	Store	5 🗆 Sto	or 🗘 🗘 re	Common Timing
Segment Cla	ass	Sine C	onstant	Constant	Constan	Const	aht Co		lo Break 👘 🛶
Voltage [mV] hold	V-memb value	J Va	alu	valu	valu	valu		Voltage Clamp
V-incr. Mode	sj Val	crease	V		Val	var Incre	Val	10200	Filter Factor
V-fact./incr.	[mV] 1.0	0 0 -							
t-incr. Mode	In	crease I	ncrease	Increase	Increase	e Increa	ase Inc	rease	anarysis. (<u>con</u>)
t-fact./incr. [ms] 1.0	0 0.00 -						R	el X-seg 1
								R	el Y-seg 1
Draw: Active	e Channel, a	II Sweeps	Delay: DA	0.00 s AD	0.00 s	V-membra	ne [mV] (display)	
	\langle					0		□ Set Last	Seg. Amplitude
					1	Leak Puls	es		
						No of Leak		0	
						Leak Delay	10).0 ms	Leak Alternate)
2.00mV			1			Leak Size	(0.250	Alt.Leak Average)
1	0.0ms					Leak Hold	[mV] [wait = abs. hold
p1	p2	р3	p4	p5	p6	p7	p8	p9	p10
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	0 0.000	0 0.0000
	Tra	ces 1							

The Sample Interval is set to 50 μ s. This corresponds to a sampling rate of 20 kHz. Please keep that sampling rate in mind. We will need that information later on. For the calculation of the LockIn parameters, we need to know the whole-cell conductance. The conductance can only be measured, if the holding potential is not "0". Therefore, you should not forget to enter a holding potential for the Sine segment. One can either enter a fixed value, for example "-70 mV" or set Voltage to "hold". If Voltage is set to "hold", the holding potential from the Amplifier window is used.

If a Sine segment exists in the PGF sequence an additional button (Sine Wave) appears in the dialog. This button can be used to modify the parameters (frequency, amplitude,...) of the sine wave segments in a protocol (see Sine Wave Parameters, 9.8.1 on page 78).

In this dialog, you can decide, if the Sine segments in the sequence should be used for LockIn measurements (Use as LockIn Sinewave) or just as a simple sine wave stimulus (Use as Simple Sinewave: common/separate frequency). Of course, we want to use it as LockIn sine wave.

Use a	s Lockln Sinew	/ave
Peak Ampl. [mV]	10.0	value
Requested Freq.	800.0 Hz	
Actual Frequency	800.0 Hz	
Points / Cycle	25	
Cycles to Skip	0	Checking
Cycles to Average	1	
Total Cycles	80	Cancel
V-reversal [mV]	0.0	Done

Now, we increase the *Requested Frequency*. A frequency of 800 Hz is quite good for most approaches. All segment durations must be integral multiples of the sine wave period. Therefore the *Actual Frequency* might be slightly different from the *Requested Frequency*. An appropriate value is calculated by the software and cannot be changed.

Please note, that the Sample Interval in the PGF sequence is adopted, upon changing the setting for Requested Frequency or Points/Cycle. It is intuitive that for higher frequencies higher sampling rates are required. If you want to reduce the sampling rate, you can decrease the number of Points/Cycle. We will do that and set Points/Cycle to "25".

Important note: The number of Points/Cycle should never be below 10, otherwise the accuracy of the C_m measurement is dramatically reduced.

The setting of 10 mV for the sine wave amplitude is fine and a V-reversal of 0 mV is also correct for our model circuit. The V-reversal setting is actually not very critical if G_m is low. A value of zero is often used in the common situation where G_m is low and the actual reversal potential is unknown. Now, we can go back to the PGF sequence.

Since we decided to use the Sine segment in the sequence for LockIn measurements, the entry Stimulus \rightarrow DA in the PGF sequence has automatically been changed from "StimScale" to "StimScale, LockIn" upon closing the Sinewave Parameters dialog. This LockIn flag is important. Without that flag, no C_m data can be calculated.

	1 DA U		Unit	Stimulus -> DA	Leak
	Ch-1	Stim-DA	V	StimScale, LockIn	
	()	off	í an ai	absolute voltage	
▽	()	off		absolute voltage	
	()	off		absolute voltage	

The AD input is set to "Imon-2", the current input of your amplifier. This is undoubtedly a good idea, however, we need an additional Trace to store the C_m value. A click in an empty AD field opens a long list of available inputs. What we need is a LockIn_CM Trace. You will see, that the compression factor is automatically set to "25" (the value, we have set for Points/Cycle in the Sinewave Parameters dialog). That means, 25 data points are in one cycle and one C_m value is calculated from each cycle, therefore you will get 80 C_m data points in that 100 ms Sweep.

AD	Unit	Link	Comp	r,	Points	Store	Zero	Leak
Imon2	Α	1	1	C	2000		0	No Leak
Lockin CM	F	1	25	C	80		0	No Leak
off				C				No Leak
off				C				No Leak

Please note, that *Link* is set to "1". This tells CHARTMASTER to use the first *Trace* (the current *Trace*) for calculating the *LockIn* information. This point is not crucial as long as you have only one current *Trace* and one LockIn *Trace*, however, it is important if you should have more then one current *Trace* (e.g. if you are using a Double or Triple amplifier).

Store is active for both Traces. It would also be possible to store only the LockIn information and to discard the current data. However, in most cases, it is reasonable to store both Traces.

21.3.4 Amplifier Settings

Now it is time for the experiment. Switch the model circuit into the "10 M" position to simulate a 10 M Ω pipette that is open to the bath solution.

In the Amplifier window you can correct pipette offset potentials by adjusting the V_0 value or you can alternatively click on the Auto V_0 button to let CHARTMASTER do this correction automatically for you. The same is done by calling the protocol "SETUP".

Now simulate a pipette sealed to the membrane by switching the model circuit into the middle position. Make an automatic fast capacitance cancellation by clicking on the *Auto C-fast* or the "SEAL" protocol button.

To break into the cell, set the switch of the model circuit to the "0.5 G" position. Make an automatic slow capacitance cancellation by clicking on the *Auto C-slow* or "WHOLE-CELL" protocol button. With the *V-membrane* control change the pipette holding potential to "-70 mV". Now we are ready to run the PGF sequence we defined before.

21.3.5 Running the PGF sequence

In CHARTMASTER, PGF sequences can be run from the Pulse Generator directly (*Execute* button) or from the Control window. We click on the *LockIn* button to start the sequence.

Control V	Vindow							• ×
id	le	12:45:34	00:54:18	Set Sto	re) (Break) (Stop Nex	t Wait	(Resume)
Comment							Average	1
PGF	<mark>\$ \$</mark> 1	Lockin 2(3	4(5(6		1 00
Protocol	00 1	Exampl1 2(Example2) 3	Link 4	Buffer 5	SETUP 6	SEAL)	1 00

The results are displayed in the Oscilloscope. As defined in the PGF sequence, you can see the current Trace (black) and a second Trace (red) with the LockIn data.

🚹 ChirpTutorial					
1_2_1 of 1		(Measure)(Scan) (Fre	eze) (Wipe)	Repaint
1.00 nA				39.1 pF	OverI.Sw
	1 1			24.2 5	(Overl.Se
800. pA				31.3 p⊢	Trace 1
_600_pA				23.4 pF	Dig. Filte
					Off
400. pA				15.6 pF	Y-scale
200 04				7.91 pE	1.00
200. pA				7.01 pr	Y-offs.
0.0 A				0.0 F	0.00
mannam	mannin	mmmm	mmmm	Antoninanta	(Auto Sv
-200. pA				-7.81 pF	Auto Se
.400 pA				-15.6 pE	Start Tir
400. pr				-15.0 pi	0.0 %
-600. pA				-23.4 pF	100.0.9
				24.2.5	Page (
-800. pA				-31.3 p⊢	1.0
10.0 ms	30.0 ms	50.0 ms	/0.0 ms	90.0 ms	<u> </u>

Note: Change the display labeling to Grid + Values in order to display the axis scaling.

By default, all data points are connected by lines. If you want to see the individual data points, you can modify the *Trace Properties* in the Display menu. The MC-10 model cell circuit in the "0.5 G" position has a capacitance of 20-22 pF. The calculated C_m values are well within this range which can be seen in the Notebook window (only if Write to Notebook is activated in the LockIn Configuration).

21.3.6 Analysis

In the Oscilloscope, the *LockIn* information is displayed with a high time resolution. Even fast membrane capacitance changes can be observed in that way.

However, in some experiments, the expected C_m changes are in the range of several seconds or minutes. In that case, we should use the Analysis to monitor the changes in membrane capacitance. For that purpose, it is necessary to modify the *LockIn* PGF sequence. We will increase the *No of Sweeps* from 1 to 100. That is all we have to do for the moment.

📰 Analysis: DefAnal	
Use Select	ed Method
Analysis Methods: Analysis Pool	
001 Lockin 2	3 4 000
Load Merge Save Lock	dn New Delete Move
Analysis Functions	List
(1) SweepC 2 LI_CM_2	3 4 00
Function Type Sweep Count Change	New Delete Move
Name SweepC	
Input	Output
	🖾 Notebook
	Not Stored in Values
Cursors	Operation Options
X-, Y-seg. Offset 0 0	
Cursor Bounds (%) 0.0 100.0	
Analysis Graphs	
Graph 1 Un Graph 2	
Save to Tree Copy Print (1	
Scale Axis Overlay No Wrap	Graph Entries
X 0.00 1.00 Auto Swp. x	SweepC SweepC SweepC SweepC
Y 10.0p 30.0p Fixed Y	LI CM 2 SweepC SweepC SweepC
(Modify Axis □Share X-axis	
Graph Positions 1234567890	123456
Graph in Window 1	

Click on an empty button to define a new *Analysis Method*. We name the new analysis "LockIn". We decline the question if we want to copy the settings from an previous method. Again we will start from the scratch. Further, you have to do the following modifications:

- Graph Positions: Activate checkbox "1" in the row "Graphs in Window 1". This enables the display of the Graph 1 (which will be defined below) in the Analysis Window 1. Enable "Use Selected Method" to make sure that the now specified Analysis Method is used.
- Analysis Functions: Click on an empty field and the Function Type dialog will open. First, select Sweep Count for the X-axis analysis then click again on an empty button field and select LockIn_CM for Y-axis analysis. Please take care that Trace # for the proper C_m values has to be Trace 2.
- Analysis Graphs: Switch on the Graph 1.
- Graph Entries: Activate the checkbox "1" and select SweepC (Sweep Count) for X-axis and LI_CM_2 (CM for trace 2) for Y-axis.
- Scale Axis: Select Auto Swp. for X-axis and Fixed for Y-axis. 10 to 30 pF (10 p / 30 p) are sufficient for our model cell.

• Analysis Window: If not visible, go to the Windows menu and activate Analysis Window 1.

Function Type						
Timing	Measurements	Lockin	Trace Param.	Math	Trace	Power Spectra
O Sweep Count	O Extremum	Lockin_CM	C Trace Count	O Equation	O Trace	O log(Frequency)
O Analysis Index	O Maximum	O Lockin_GM	C-slow	O Y(x): y at pos = x	O Equation	O Density
O Time	O Minimum	O Lockin_GS	O R-series	O Constant	🗘 Q = Integral	
O Timer Time	O Extr. Amplitude	O Lockin_Phase	O Rs-value	() a + b	O 1 / (trace)	Histogram
O Series Time	O Min. Amplitude	O Lockin_Freq	C Leak Comp.	⊖a-b	01/(Q)	O Histogram Ampl
O Real Time	O Max. Amplitude		O M-conductance	⊖a*b	O In (trace)	O Histogram Bins
	O Time to Extremum	AP Analysis	Cell Potential	⊖ a / b	O In (Q)	
Stim. Properties	O Time to Maximum	O Baseline	O Seal Resistance	🔿 a in b	O log (trace)	
O Amplitude	O Time to Minimum	O AP Amplitude	O Int. Solution	O abs	(Q) log (Q)	
O Duration	O Time to Threshold	O Time to AP Ampl	O Int. Sol. Value	O log	🔿 dt = Differential	
O Rel. Seg. Time	C Threshold Ampl.	O Repol Ampl	O Ext. Solution	O sqrt	O Trace x-axis (time)	
O Abs. Seg. Time	O Thres. Crossings	O Time to Repol Ampl	O Ext. Sol. Value	O arctan	O Stimulus	
O Scan Rate	O Mean	O Rise Time		🗘 1/a		
	O Variance	O Up Slope	Sweep Param.	O 1/log		
	O Integral	O Rise Time Delay	O Temperature	O 1/sqrt		
	O Anodic Q	O Decay Time	O Pip. Pressure	O 1/arctan		
	Cathodic Q	O Down Slope	O Digital-In			
	O Reversal	O Decay Time Delay	O User_1			
	O Slope	O Decay Tau	O User_2			
	O Intercept					
	() Tau				Cancel	Done

If we now run the LockIn PGF sequence, one LockIn data point per Sweep is plotted in the Analysis window. This single point is the mean value from all C_m values within a Sweep.



190

22. Spectroscopy Extension

22.1 Introduction

The Spectroscopy Extension allows to measure the transfer functions of a system. The complex transfer function can be presented as two real traces: *Magnitude* (or *Gain*) and *Phase*. E.g. if we send a sine wave stimulus of amplitude 1 into a system and we measure as response a sine wave of the same frequency but amplitude of 0.5, then we would get a *Magnitude* or *Gain* of 0.5.

In case the system converts a voltage stimulus to a current signal, we can interpret the Magnitude (or Gain) as Admittance (Y) or Impedance (Z).

The CHARTMASTER LockIn Extension performs this type of analysis at a given single frequency. In order to obtain a complete spectrum, repetition of the measurement at different frequencies is required. This ends up in a very elaborate procedure. The Spectroscopy Extension, however, uses a stimulus containing a set of multiple frequencies, allowing to calculate a complete spectrum from a single measurement.

22.1.1 Chirp Wave Forms

The Pulse Generator of CHARTMASTER provides three different chirp wave forms:

Linear Chirp:

In a Linear Chirp, the frequency f(t) varies linearly with time:

$$f(t) = f_0 + kt (22.1)$$

where f_0 is the starting frequency (at time t = 0), and k is the rate of frequency increase or chirp rate. The corresponding time-domain function for a sinusoidal *Linear Chirp* is:

$$x(t) = \sin[2\pi(f_0 + \frac{k}{2}t)t]$$
(22.2)

Exponential Chirp:

In an *Exponential Chirp*, the frequency of the signal varies exponentially as a function of time.

$$f(t) = f_0 k^t \tag{22.3}$$

where f_0 is the starting frequency (at t = 0), and k is the rate of exponential increase in frequency. Unlike the Linear Chirp, which has a constant chirp rate, an Exponential Chirp has an exponentially increasing chirp rate. The corresponding time-domain function for a sinusoidal Exponential Chirp is:

$$x(t) = \sin\left[\frac{2\pi f_0}{\ln(k)}(k^t - 1)\right]$$
(22.4)

Spectroscopy Chirp:

A chirp wave form created by inverse *Fourier Transform* from a spectrum with frequencies increasing with t^2 and constant amplitude. The Spectroscopy Extension uses this stimulus wave form as input only.

22.1.2 Transfer Function Ratio

The transfer function (H) of two signal processing units in series is given by:

(22.5)

Reference

Response

In case one is interested the transfer function of an individual signal processing unit, the "ratio" of the transfer function can be calculated:

 $H(A+B) = H(A) \otimes H(B)$

$$H(B) = \frac{H(A+B)}{H(A)}$$
 (22.6)

Overall

Response

The Spectroscopy Extension provides a so-called *Correction Mode* to calculate the *Transfer Function Ratio* of the recorded response (nominator) and the reference response (denominator).

In some application a reference element (e.g. calibrated resistor) is used for a calibration measurement of the system. In this case the value of the reference element has to be taken into account when calculating the transfer function of the measuring system without reference element. We offer the option to use the measured resistance or a given resistance for this correction.

22.2 Activating the Spectroscopy Extension

The Spectroscopy Extension is activated in the Configuration window on the tab Hardware.

General	Hardware	Ì						
EPC10_USB Amplifier								
LIH 8+8								
Sound	LIH 8+8 Sound							
Lockin	Off							
Spectroscopy	On							
Imaging	Off							
Photometry	Off							
ElProScan	Off							
Serial Out	Off							

Once the extension is turned On, the Spectroscopy window can be opened via the Windows menu list in CHART-MASTER.

🛃 Spectroscopy Configur	ation 🗖 🗖 💌						
Correction Mode	Buffer 1						
Input Source	Raw Data Trace						
 Reference Element Correction Measured Resistance Given Resistance 10.00 MO Log Compression Comp.Factor 1.40 							
Offline Computation - Traces to create: ☑ Admit(Y) ☑ Phase ☑ Average ☑ Real(Y) ☑ Imag(Y) □ Real(Z) □ Imag(Z) □ Imp Z Parent AD-Trace Trace 1							
Compute Spectrosc	opy of Marked Targets						
Default Y-ranges:							
Admit(Y) 1.000 S	Phase 180.0 °						
Real(Y) 1.000 S	Imag(Y) 1.000 S						
Real(Z) 1.000 O Imp Z 1.000 O	-Imag(Z) 1.000 O						

Correction Mode: It is possible to correct the spectra during online and offline calculation with respect to another chirp response (see Transfer Function Ratio, 22.1.2 on page 191).

- In case a simultaneous recording of the reference chirp is possible: Under certain circumstances it might be possible to record the reference *Trace* simultaneously with the chirp response. In this case you can select the respective *Trace* which contains the reference chirp response in the *Correction Mode*.
- In case the reference chirp is recorded in advance: The reference chirp has to be copied in one of the four *Trace Buffers* and in the *Correction Mode* the respective *Buffer* has to be selected.

If a correction *Trace* is selected, the admittance of the recorded response is divided by the normalized magnitude of the reference. In addition, the phase of the reference is subtracted from the phase of the recorded response.

Input Source: Defines the data type from which the spectra are calculated. In case a *Correction Mode* is used, the type of input data for the acquired data and the reference have to be the same.

Reference Element Correction: Must be used in case the measurement system has been calibration with respect to a reference element (e.g. a 10 M Ω resistor), which is removed and replaced by the cell under study. Two options are available:

- 1. Measured Resistance: The resistance of the reference element is measured at the lowest frequency.
- 2. Given Resistance: A given resistance can be used to normalize the calibration measurement.

In case Reference Element Correction is not activated the unscaled raw data of the reference transfer function are used. This method usually applies only if it is possible to measure from two different locations in the signal processing path.

Log Compression: Often the results of the chirp analysis are plotted on a logarithmic frequency scale. In this case the higher frequencies contain much more measuring points than the lower frequencies. For better display and post analysis (e.g. fitting of the spectra) a equidistant scaling on the logarithmic frequency axis is of advantage. Therefore we offer the option of a logarithmic compression. The *Compression Factor* (usually a value between 1 and 2) defines the strength of the compression. When logarithmic compression is used the frequency axis is scaled in units of logarithm to basis 10.

Offline Computation - Traces to create: You can mark by using the checkboxes which Traces (Admit(Y), Real(Y), Real(Z), Phase, Imag(Y), -Imag(Z), Average, Imp(Z)) should be created during re-calculation of the spectra.

Parent AD-Trace: Select the corresponding *Trace* for computing spectroscopy, either *All* or any other *Trace* (Trace 1...16).

Compute Spectroscopy of Marked Targets: Recalculates and generates the selected *Traces* (select and mark the target (*Sweeps* or *Series*) first).

Default Ranges: For the various spectroscopy *Traces* the default Y-ranges for display in the Oscilloscope window are defined. E.g. a Y-range of 1 S (Siemens) defines that at scaling of 1 and offset of 0, the Oscilloscope shows a range from -1 S to +1 S.

22.2.1 The Spectroscopy Traces

Chirp_Avg: Average of the chirp response. In case multiple *Chirp* segments are used in one *Sweep*, the *Chirp_Avg* contains the average of all *Chirp* segments.

Chirp_Phase: The phase angle between stimulus and response versus frequency. The default unit is degree(°). This is a frequency based *Trace* (X-unit is Hertz).

Chirp_Admit(Y): Contains the magnitude of the spectrum normalized to the stimulus amplitude. In case of a recorded current response this parameter can be interpreted as admittance. The default unit is Siemens (S). This is a frequency based *Trace* (X-unit is Hertz).

Chirp_Real(Y): The real part of the admittance. This is a derived frequency based *Trace* (X-unit is Hertz), calculated as follows:

$$Real(Y) = \frac{Admittance}{\sqrt{1 + \tan\left(Phase\right)^2}}$$
(22.7)

The default unit is Siemens (S).

Chirp_Imag(Y): The imaginary part of the admittance. This is a derived frequency based *Trace* (X-unit is Hertz), calculated as follows:

$$Imag(Y) = \tan(Phase) * Real(Y)$$
(22.8)

The default unit is Siemens (S).

Chirp_Real(Z): The real part of the impedance. This is a derived frequency based *Trace* (X-unit is Hertz), calculated as follows:

$$Real(Z) = \frac{Real(Y)}{Real(Y)^2 + Imag(Y)^2}$$
(22.9)

The default unit is Ohm (Ω) .

Chirp_Imag(Z): The imaginary part of the impedance. This is a derived frequency based *Trace* (X-unit is Hertz), calculated as follows:

$$-Imag(Z) = \frac{Imag(Y)}{Real(Y)^2 + Imag(Y)^2}$$
(22.10)

The default unit is Ohm (Ω) .

Chirp_Imp|Z|: The impedance. This is a derived frequency based Trace (X-unit is Hertz), calculated as follows:

$$Imp|Z| = \frac{1}{\sqrt{Real(Y)^2 + Imag(Y)^2}}$$
(22.11)

The default unit is Ohm (Ω) .

22.3 Setting up a Spectroscopy Acquisition

Important note: The following section was made for PATCHMASTER and HEKA EPC 9 or 10 amplifier. If you are using CHARTMASTER or POTMASTER several modifications may be necessary!

Create a new PGF sequence with one *Chirp* segment as stimulus. When at least one segment contains a chirp wave form then a button Chirp Wave appears in the top right section of the Pulse Generator window.

22.3.1 The Chirp Wave Dialog

A detailed description of the available chirp wave parameters can be found in Chirp Wave Parameters, 9.8.3 on page 83.

22.3.2 Parametrization of the Chirp Stimuli

Possible Frequency Range: The maximal possible frequency in a chirp wave is half the sampling frequency (*Min. Points / Cycle* = 2).

• Linear and Exponential Chirp: The Start and End Frequency can be directly entered in the Chirpwave Parameters dialog. According to Start and End Frequency and given segment duration the rate of frequency increase (k) and the Min. Points / Cycle are calculated.

	Linear Chirp	
Amplitude [mV]	10.0	value
Start Frequency	78.13 Hz	
End Frequency	10.00 kHz	
Min. Points / Cycle	2.0	Checking
		Cancel
Segment Points	256	Done

• Spectroscopy Chirp: Start Frequency and End Frequency can not be entered directly. Both parameters are defined by the Sampling Frequency, Segment Duration, and the Min. Points / Cycle. The End Frequency is given by Sampling Frequency divided by Min. Points / Cycle. The Start Frequency then depends on the duration of the segment. The longer the segment the smaller the starting frequency of the chirp.

Spectroscopy Chirp							
Amplitude [mV]	10.0	value					
Start Frequency	78.13 Hz	Pre-Chirp					
End Frequency	10.00 kHz						
Min. Points / Cycle	2.0	Checking					
Chirps to Skip	0	Cancel					
Segment Points	256	Done					

Useful Frequency Range: In the preceding section we have discussed the possible frequency range in a chirp stimulus. Correct analysis of the chirp response, however, has to obey some additional boundary conditions. The most important rule is to obey the *Sampling Theorem* or *Nyquist Theorem*! That means that the response has to be low pass filtered to prevent aliasing artifacts. The filtering can be either done by the system under study itself or by additional filters. When using filters with steep cut-off (e.g. 8-Pole filter) unbiased analysis can be extended to about 80 % of the possible frequency range.

22.3.3 Adding Chirp Analysis Traces

Once you have configured the chirp stimulus you can add multiple chirp analysis *Trace* to the AD selection in the Pulse Generator.

22.3.4 Minimizing Swing-In Effects

In order to minimize swing-in effects, two methods can be used:

1. Use at least two Chirp Segments: We duplicate the *Chirp* segment and set the *StartSeg* to "2". In case of very long chirp stimuli this method is very time consuming.

Note: When you have more than one Chirp segment, please deactivate the checking routines during editing the parameters of the segments. Once you have finished the edition, turn the Checking on again.

In the following screenshot you see a sample Pulse Generator sequence for a chirp acquisition.

E Dulce Generator Fi	le: chim							
Full View	Condense	d View	Cartoon \	liew				
dd 1 Chirp	2	3	Guiteoni) 4 (5 (6	
Pool LOAD	MERGE SAVE	Name	Chirp					DELETE
Interactive Mode	O Gap Free M	ode						
Timing No wa No of Sweeps Sweep Interval Sample Interval	it before 1. Swee 1 0.00 s 5.00 μs (200.kh	p Not Trig Use Dur StartSeg z StartTime	gered ations 2 0.00	Che Sweep Channe	cking Length el Length	Chirp V Total Stored Stimulus	Vave 327.7 ms 163.8 ms 327.7 ms	EXECUTE 65536 pts 320 kb 65536 pts
1 DA	Unit Sti V StimSc V abs V abs V abs	mulus -> DA ale, Spectro solute voltage solute voltage solute voltage	Leak	AD Vmon-2 Chirp Avg Chirp Pha Chirp Adm	Unit Link V 1 A 1 s ° 1 ii S 1	Compr. F 1 C 2 C 2 C	Points Store 32768 Ø 32768 Ø 16384 Ø 16384 Ø	Zero Leak 0 No Leak 0 No Leak 0 No Leak 0 No Leak 0 No Leak
Segments () Segment Class Voltage [mV] Duration [ms] V-incr. Mode V-fact./incr. [mV] t-incr. Mode t-fact./incr. [ms]	Store 1 Store 1 Chirp valu valu valu 163.84 valu 1.00 0 11.00 1.00 0 11.00 1.00 0 11.00	Store 2 C Chirp u u u 0 va l 163.84 v ncrease 00 0 ncrease 00 0 ncrease 00 0	Store 3 Constant alu al Increase Increase Increase	Store 4	Store	5 Sto valu val ase Incr ase Incr 	ease ease R R	Common Timing Voltage Clamp Filter Factor Analysis: Composition el X-seg 1 el Y-seg 1
Draw: Active Channel 2.00mV I 20.0ms	nel, all Sweeps	Delay: DA	0.00 s AD	0.00 s	V-membra Leak Puls No of Leak Leak Delay Leak Size Leak Hold	ane [mV] (c es s / 10 [mV]	lisplay) Set Last 0 .0 ms .250 	Seg. Amplitude Leak Alternate Alt Leak Average wait = abs. hold
p1 p2	p3	p4	p5	p6	p7	p8	p9	p10
0.000	Traces 4	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	0.0000

2. Use of a Pre-Chirp Segment: In order to reduce the time for swing in to a minimum, a *Constant* segment of fixed length can be defined before the *Chirp* segment. When the option *Pre-Chirp* is activated in the **Chirpwave Parameters** dialog, then this segment is automatically filled with appropriate swing-in stimulus data. Please note that the *Pre-Chirp* segment should not be recorded (use *Start Segment* "2") to ensure accurate chirp analysis.



Pulse Gene	rator File: ch	nirp						C	- 0 X
Full V	iew	Condensed	d View	Cartoon	View				
00 1	Chirp	2	30		4		5 🤇	6	<mark>q q</mark> (
Pool LOA	D MERC	SE) SAVE	Name	Chirp		ST CC	OPY MOVE		DELETE
Interactive	Mode O	Gap Free Mo	ode						
Timing	No wait be	fore 1. Swee	p Not Trig	gered	Chec	king	Chirp Wa	ve 🤇	EXECUTE
No of Swe	eps	0.00 e	Use Dur	ations	Sweep L	ength	Total	2.671 s	267144 pts
Sample In	terval 10.0) µs (100.kH	z StartTime	0.00	Channel	Length	Stored Stimulus	2.621 s 2.671 s	2560 kb 267144 pts
1	DA	Jnit Stir	nulus -> DA	Leak	AD	Unit Link	Compr. Poir	nts Store Z	ero Leak
Ch-1	Stim-2	V StimSc	ale, Spectro	scop 🗆	Imon-2	A 1	1 C 262	144 🛛	0 No Leak
\bigcirc Ch-2	off	V abs	olute voltage		Vmon-2 Chirp Admi	V 1 S 1	2 C 131	144 🔤	0 No Leak
♥ Ch-4)	off	V abs	olute voltage		Chirp Phas	° 1	2 C 131	072 🛛 🗌	0 No Leak
Segments		tore 1 🖂 S	Store 2	Chirpwave P	arameters			D	mmon Timing
Segment Cla	ass Co	onstant	Chirp		Spe	ctroscopy	Chirp		Break 0.00
Duration Ims	j inolo sl val	50 00 val	2621 44	Amplitude	[mV]	10.0	value		ilter Eactor
V-incr. Mode	In	crease li	ncrease	Start Free	uencv	381.5 m	Hz Pre-0	Chirp	inter i deter
V-fact./incr.	[mV] <u>1.0</u>	0 0 1.0	0 0	End Frequ	Jency	50.00 kl	Hz	n	alysis: (Edit)
t-fact./incr. [ms] 1.0	0 0.00 1.0	00 0.00	Min. Point	ts / Cycle	2.0	Chec	king	X-seg 1
				Chirps to	Skip	0	Can	cel	Y-seg 1
Draw: Active	Channel, a	II Sweeps	Delay: DA	Segment	Points	262144	f Dor	1e)	
	\wedge							Ser Last Se	eg. Amplitude
				$/ \setminus$	1	Leak Puls	es		
			/			No of Leak	s 0		
2.00mV					$\setminus / $	Leak Delay	0.25	ms Le	eak Alternate
20	0 ms		\bigvee		\mathbf{V}	Leak Hold	[mV]	wa	iit = abs. hold
20	n2	n3		n5	p6	p7	n8	n9	10
0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
	Tra	ices 4							

In the following screenshot the swing in signal of a *Pre-Chirp* segment is shown. The first 5 ms of the stimulus are the *Pre-Chirp* segment which provides an optimal swing-in of the chirp segment.



22.4 Display of Spectra in the Oscilloscope Window

The chirp analysis Traces are stored in the Replay window.

1 - Imon-1	1
- Vmon-1	2
- Phase	10
- Admit(Y)	11
- Real(Y)	12
- Imag(Y)	13
- Real(Z)	14
-Imag(Z)	15
Imp Z	16

Their X-unit is Hertz and the number of points differs from the raw data *Trace*. Since raw data *Trace* and spectra have different X-axis scaling it is difficult to show them at the same time on appropriate scaling in the Oscilloscope, which supports only one X-scaling. By default you will see the raw data *Trace* in the Oscilloscope, since the Oscilloscope takes its X-scaling from the first *Trace* in the *Sweep*.



In order to show also the chirp analysis *Traces* in the Oscilloscope, please select *Fixed X-width* from the Display menu and enter as *X-max* (panel to the right in the Oscilloscope window) the maximum frequency in the spectrum (e.g. 12k, what stands for 12 kHz).



Trace 10 (orange) and Trace 11 (green) show the phase and the admittance, respectively.

http://www.heka.com

23. Appendix I: File Overview

23.1 File Types

file type	extension	default file	see also
Data file (includes the	*.dat	-	-
measured data)			
Dialog setting for the	*.dia	-	Chapter User
dialog of windows (if		(*.dia files are	Interface, 2.3
no dialog file exists,		always named	on page 14.
the software will start		after the window	
with its own default		that is saved, e.g.,	
values.)		Uscilloscope.dia.)	<u></u>
Key file for key com-	*.key	Chartmaster.key	Chapter Keys, 3
mands (includes all			on page 17.
key commands)			NT / 1
Macro file for macros;	*.mac	-	Now protocols
not supported any-			are used in-
more starting with			Stead. Chapter
version 2.40.			Vinden 10 om
			window, 10 on
Markors file for all	* mrlz		page 99. Chapter
markers information	*.III K	-	Markorg 17
marker miormation.			on page 153
Online file for Analysis	* 0.01	DofAnal onl	Chapter
settings (can include	*.011	DerAnar.onr	Analysis
more than one Analy-			Window 11
sis Method)			on page 110
Stimulus file for the	* ngf	DefPaf naf	Chapter Pulse
Pulse Generator set-	· • P6-	Dell Br. PBr	Generator
tings			Window 9 on
ungo.			nage 73
Protocol file for a pro-	*.pro	DefProt.pro	Chapter
tocol pool (can include	. 11.0	Dollios.bio	Protocol Editor
more than one proto-			Window, 10 on
col).			page 99.
Equation file for stor-	*.txt	Equation.txt	Chapter
ing equation strings.		1	Calculator
			Window and
			Equations, 18 on
			page 157.
Acquisition parame-	*.pul	-	-
Settings file	* 90+	Chartmastor sot	
Solution file for storing	* sol		- Chapter
the entries of the Solu-	*.BUT	Trampic. 201	Solution
tion Database			Base 15 on
tion Database.			nage 147
Filename template file	* tnl	_	Chapter "Using
i nename template me.	br		a Recorded
			Waveform as
			Stimulus" in the
			CHARTMASTER
			Tutorial.
Notebook file.	*.txt	-	Chapter
			Notebook
			menu, 4.7 on
			page 36.

Table 23.1: The different file types

24. Appendix II: Data Format

In this chapter we describe the general structure of the files generated by CHARTMASTER.

24.1 Data Files

CHARTMASTER generates up to 8 files, when a data file is created:

- 1. The Raw Data File (*.dat).
- 2. The Marker File (*.mrk).
- 3. The Analysis File (*.onl).
- 4. The Stimulation Sequence File (*.pgf).
- 5. The Acquisition Parameters File (*.pul).
- 6. The Solution File (*.sol).
- 7. The Notebook File (*.txt).

If *Make Bundle Files* is checked in the Configuration window (5.5.2 on page 48), then all files will be merged into one single bundle file with the file extension *.dat.

Except for the Raw Data File and the Notebook File, all other files have a "Tree" structure. The entire trees are kept in memory, whereas the raw data Traces are always loaded from disk, when needed.

24.1.1 Raw Data File

This Raw Data File has an optional header, followed by a continuous data stream. Each data point is a 16-bit signed integer or 32-bit IEEE real as defined in the respective PGF template. When a Sweep is stored, CHARTMASTER stores the various Traces (if available) sequentially as defined in the respective PGF template. Traces with leak pulses are stored leak subtracted. The leak Traces are normally stored after the Traces themselves.

Very long traces with "continuous" segments may be stored as interleaved blocks. The size of such a block is given in the "InterleaveSize" field (in bytes) of the *Trace* record, the distance to the next block in the "InterleaveSkip" field (in bytes).

The structure of the Raw Data File (*.dat) is defined by the file DataFile_v9.txt.

24.1.2 Markers File

It contains the Marker records. The structure of the Markers File (*.mrk) is defined by the file MarkerFile_v9.txt. It has a tree structure:

Record	Description
Root	Version information
Marker	Description of one marker event

24.1.3 Protocol Methods File

It contains possibly used protocol method records. The structure of the *Protocol Methods File* (*.mth) is defined by the file MethodFile_v9.txt.

24.1.4 Analysis Methods File

It contains the assigned analysis method for every Series. The structure of the Analysis Methods File (*.onl) is defined by the file AnalysisFile_v9.txt. It has a tree structure:

Record	Description
Root	Version information
Method	Description of one Analysis Method
Function	Description of one Analysis Function

24.1.5 Stimulation Template File

Stores the stimulation protocol. The structure of the *Stimulation File* (*.pgf) is defined by the file StimFile_v9.txt. It has a tree structure:

Record	Description
Root	Version information
Stimulation	Description of an ensemble of pulse patterns;
	e.g., I-V curve
Channel	Combines the definition for one output (DAC)
	and one input (ADC) Trace
Segment	Individual segment of a pulse pattern

Stimulation Files can be loaded into the Pulse Generator. In fact, the Pulse Generator Files for the stimulation protocols used during the experiments have the same data structure as the PGF files, which belong to the recorded data. In this way it is possible to exactly repeat an experiment by using a copy of a PGF file as Pulse Generator File.

24.1.6 Acquistion Parameters File

Stores parameters, such as e.g. *Gain*, *Capacitance...*. The pointer to the data stored in the *Raw Data File* is also contained in this file. The structure of the *Acquisition Parameters File* (*.pul) is defined by the file PulsedFile_v9.txt. It has a tree structure:

Record	Description
Root	Version information
Group	Larger section of an experiment; e.g., cell or
	patch
Series	Description of an ensemble of Sweeps
Sweep	Description of a Sweep, i.e. one collection of
	Traces
Trace	Description of an individual data Trace

A graphical template of the Pulsed File (Tree) is shown in the Replay window. It contains information necessary to reconstruct the experimental conditions as the data were recorded.

24.1.7 Solutions File

The file is only generated, when the option Solution Base is activated. It contains the solution record for every Series. The structure of the Solutions File (*.sol) is defined by the file SolutionsFile_v9.txt. It has a tree structure:

Record	Description
Root	Version information
Solution	Description of one solution
Chemical	Description of one chemical compound

24.1.8 Notebook File

The Notebook File is a standard ASCII text file with line breaks.

24.2 The Tree Format

The idea of CHARTMASTER is to order the data of an experiment in "Trees". The trunk of the tree (*Root*) is the main descriptor of a data file (it could, for example, correspond to one cell or the entire experiments of one day). The next level is the *Group*. This level can be defined by the user to identify data that belong together. An example would be to open a new *Group* for each patch. The *Group* may contain several families of records. Such a family (e.g., records of a current–voltage relationship) is called *Series*. The individual records of a family are called *Sweeps*. Finally, each *Sweep* may be composed of *Traces*. A copy of this data tree is accessible to the user throughout the experiment (so one has an overview of what was recorded, and one can immediately edit the entries (e.g., discard bad records).

The following is a description of the Tree format. An example tree with 5 levels can be diagrammed as follows:



The above tree has five levels:

- 0 : Root
- 1 : Group
- $2\,$: Series
- 3 : Sweep
- 4 : Trace

The format of a tree stored to a file is as follows:

1. Magic number: 054726565 (hex) = "Tree"

- 2. Number of levels
- 3. Level sizes, one per level
- 4. Tree records, top down, left-to-right. Each record has the format:
 - Record contents
 - Number of children

All of the values (except the record contents) are INT32 values, i.e., 32-bit (4 bytes) values.

Note: Check the record sizes in the file headers. The record sizes may differ from what you are expecting, e.g., because the file has been created by an older program version which used fewer fields than it is currently using, or a newer version with additional fields. **You must use the record sizes stored in the files themselves.**

Note: The "Magic2 number will be 065657254 (hex) = "eerT", if the file was written on an operating system with opposite byte ordering (e.g. written under Mac OS, read under MS Windows). In that case, appropriate byte swapping has to be performed (highest \rightarrow lowest, etc.), when the data is read in.

Important detailedA description oftheCHARTMASTER file note: dataserver: format forprogrammers isavailable fordownload from our FTPftp://server.hekahome.de/pub/FileFormat/Patchmasterv9/.

24.3 File Template

24.3.1 Filename

In CHARTMASTER you can use so-called template files for stimulation.

You have the following options how to use the file templates:

Option 1: One template per DA channel common to all Sweeps of a Series

In this case, the name of the template file would be:

[stimulus name]_[channel number].tpl

E.g., if the stimulus name is "IV", then CHARTMASTER looks for the Template File IV_1.tpl to be used as Template File for the first DA channel of all Sweeps.

These *Template Files* must be in a sub-folder of the folder with the pgf files. The sub-folder must named identically to the stimulus, e.g., "IV".

Option 2: A different template per DA channel and Sweep

In this case, the name of the *Template File* would be:

[stimulus name]_[sweep index]_[channel number].tpl

E.g., if the stimulus name is "IV", then CHARTMASTER looks for the Template File $IV_1_1.tpl$ to be used as Template File for the first DA channel of the first Sweep, $IV_1_2.tpl$ for the first DA channel of the second Sweep and so on.

These *Template Files* must be in a sub-folder of the folder with the pgf files. The sub-folder must be named identically to the stimulus, e.g., "IV".
24.3.2 Data Format of the File Template

24.3.2.1 Voltage Clamp Stimulation or plain DA output

The file should contain one voltage value per stimulus point. CHARTMASTER fills missing samples with the holding value, if *Apply StimScale* is active, or alternatively with zero. Excessive samples are ignored.

The voltage must be a *short* (4 byte), binary IEEE-floating point format number. All values must be in Volt, i.e., if a voltage of -80 mV has to be output, the required value is -0.080. The total number of samples should be equal to the total number of input samples.

24.3.2.2 Current Clamp Stimulation

Template Files for stimulation can be used in Voltage Clamp and in Current Clamp mode. In Current Clamp mode, the stimulation data is defined in volts, where 1 mV corresponds with 1 pA current injection.

Example: A value of 0.1 would result in a current injection of 100 pA.

Index

AD-board Enable Background, 38 Enable Batch Control, 38 interface, 38 Re-Initialize, 38 Analysis Analysis Graphs Scrolling Bar, 128 Analysis Methods Scrolling Bar, 120 Input X-Trace, 121 Y-Trace, 121 Analysis Window, 119 Analysis Functions, 121 1/(Q), 1271/(trace), 1271/a, 127 1/atan, 127 $1/\log, 127$ 1/sqrt, 127 a in b, 127 a*b, 127 a+b, 127 a-b, 127 a/b, 127 abs, 127 Absolute Segment Time, 125 Amplitude, 125 Analysis Function Types, 124 Analysis Index, 124 Anodic Charge, 125 atan, 127 Cathodic Charge, 125 Change, 121 Constant, 127 Cursors, 122 Delete, 121 Differential, 127 Digital-In, 126 Distribution, 128 Duration, 125 Equation, 126, 127 External Solution, 126 External Solution Value, 126 Extremum, 125 Extremum Amplitude, 125 Frequency, 127 Function Type, 121 Histogram, 128 Histogram Amplitude, 128 Histogram Bins, 128 Input, 121 Integral, 125 Intercept, 126

Internal Solution, 126 Internal Solution Value, 126 List, 121 $\ln/(Q), 127$ $\ln/(\text{trace}), 127$ LockIn, 126 LockIn_Frequency, 126 LockIn_Phase, 126 log, 127 $\log/(Q), 127$ $\log/(\text{trace}), 127$ Math, 126 Maximum, 125 Maximum Amplitude, 125 Mean, 125 Measurements, 125 Minimum, 125 Minimum Amplitude, 125 Move, 121 Name, 121 New, 121 **Operation Options**, 123 Output, 122 Power Spectra, 127 Q=integral, 127 Real Time, 124 Relative Segment Time, 125 Reversal, 125 Scan Rate, 125 Scrolling Bar, 121 Series Time, 124 Slope, 126 sqrt, 127 Stimulation Properties, 125 Stimulus, 127 Sweep Parameters, 126 Tau, 126 Temperature, 126 Threshold Amplitude, 125 Threshold Crossings, 125 Time, 124 Time to Extremum, 125 Time to Maximum, 125 Time to Minimum, 125 Time to Threshold, 125 Timer Time, 124 Timing, 124 **Trace**, 127 Trace Count, 126 Trace Parameters, 126 Trace Time, 127 User_1,2, 126 Variance, 125 y at pos = x, 126 Analysis Functionss

Sweep Count, 124 Analysis Graphs, 128 Auto after a Series, 129 Auto after each Sweep, 130 Centered, 130 Copy, 128 Fixed, 129 Fixed with last Min/Max, 130 Grid, Factor, 130 Header, 131 Include Zero, 130 Labels, 130 Max, 129 Min, 129 Modify Axis, 130 Nice Values, 130 Normalize, 131 On/Off, 128 Overlay, 129 Position, 130 Print, 128 Redraw, 128 Save to Tree, 128 Scale, 129 Scale Axis, 129 Share X-axis, 131 Sort, 131 Tics/Direction, 130 Units. 130 Wrap, 129 X, Y, 129 Zero Line, 130 Analysis Methods, 120 Assign, 120 Automatic Stimulus Control: Assigned, 120 Automatic Stimulus Control: Pool, 120 Delete, 120 Duplicate, 120 Load, 120 Merge, 120 Move, 120 Name, 120 New, 120 No Analysis, 120 Save, 120 To Pool, 120 Use Selected Method, 120 Cursors Adjust to Position of Function, 122 Cursor Bounds, 122 Cursor Type, 122 X-, Y-seg. Offset, 122 Graph Entries, 131 Axis, 131 Connect, 132 Same Color as Trace, 132 Set Color, 132 Size, 132

Type, 132 Graph Positions, 132 Input Operation, 121 Threshold, 121 Value, 121 Multi-Channels Analysis, 133 Nomenclature, 119 **Operation Options** Average Points, 123 Baseline, 123 Crossings, 123 Equation, 124 Fit, 123 Histogram Bins, 124 Ramps Only, 124 Output Notebook, 123 Store in Value, 123 Stimulus Control, 120 Appendix I: File Overview, 201 File Types, 201 *.amp, 201 *.dat, 201 *.dia, 201 *.epc, 201 *.key, 201 *.mac, 201 *.mrk. 201 *.mth, 201 *.onl, 201 *.pgf, 201 *.pro, 201 *.pul, 201 *.set, 201 *.sol, 201 *.tpl, 201 *.txt, 201 Appendix II: Data Format, 203 Data Files, 203 *.dat, 203 *.mrk, 203 *.onl, 203 *.pgf, 203 *.pul, 203 *.sol, 203 *.txt, 203 Acquisition Parameters File, 204 Analysis Methods File, 204 Markers File, 203 Notebook File, 205 Protocol Methods File, 203 Raw Data File, 203 Solutions File, 204 Stimulation Template File, 204 File Template, 206 Tree Format, 205 Current Clamp Stimulation), 207

Data Format of the File Template, 207 Filename, 206 Voltage Clamp Stimulation), 207 Calculator Window, 157 ?, 157 Command Line, 157 Equation, 157 Equation Syntax, 157 Comments, 158 Constants, 158 Math Equation, 159 Math functions, 157 Special Case, 158 Equations Analysis, 158 DA Scaling, 160 Trace Buffer, 160 Values, 161 Equations - Where to Use, 158 Load. 157 Results. 157 Save, 157 Save Equation, 157 Select Equation, 157 Configuration Hardware Photometry, 45 Configuration Window, 41 **Batch** Communication Disable, 43 Enable as Receiver, 43 Enable as Sender, 43 Polling, 43 Synchronize Files, 43 Display, 50 3D Graph, 51 Fonts and Colors, 50 Grid Digits, 51 Hide PGF-Bar in Control Window, 51 Hide Protocol-Bar in Control Window, 51 Notebook. 50 Notebook Digits, 50 Show Fixed Control Protocols, 51 Show Options, 51 Time Digits, 50 Wipe at Start, 50 Files, 47 Ask for Data File, 48 Auto Filename, 48 Batch Path, 47 Data File, 47 Dialog Files, 47 Equations File, 47 Experiment Number, 49 Home path, 47 I-gain Lookup, 47 Make Bundle Files, 48 Maximum File Size Alert, 49

Miscellaneous Settings, 48 Naming Folders and Files, 48 Online File, 47 PGF Pool File, 47 Protocol File, 47 Save after Break, 48 Save Settings File, 48 Solution Base, 47, 48 Startup Protocol, 49 Temporary Path, 47 Verify Quit, 48 General, 42 Batch Communication, 43 Max Shown Traces and Values, 42 Max. Shown Traces, 42 Max. Shown Values, 42 Maximum Number of Stimulation Traces x Points, 43 Maximum Sample Points, 43 Memory Allocation, 43 Minimum Wait Time, 43 User Name, 43 Window Scaling, 42 Hardware, 44 ITC-1600, 44 ITC-16, 44 ITC-18, 44 Lih 1600, 44 LIH 8+8.44 Amplifier and Digitizer Selection, 44 Digitizer Selection, 44 Further Options, 46 Imaging, 45 LockIn, 44 Multi-Channels, 46 Serial Out, 45 Spectroscopy, 45 I/O Control, 51 AD scale, 55 AD/DA Input Scaling, 52 AD/DA Output Scaling, 52 DA scale, 55 Default, 51 List, 52, 55 Name of Value, 55 Show AD Values, 55 Show AD-channels, 55 Show DA Controls, 55 Show Digital In, 55 Show Digital In/Out, 55 Show Digital Out, 55 Show Solutions, 55 Show Values, 55 Source, 52 Temperature, 55 User Parameters, 55 Individualize CHARTMASTER, 41 Miscellaneous, 59

Analysis Maximum Sweeps Redraw, 59 Analysis Mode, 59 Analysis: Compress Vectors, 60 Analysis: Sweeps | Results, 59 Clear Comment, 60 Replay Scroll Rate, 59 Save. 42 Default Settings, 42 Default Windows, 42 **SAVE.** 42 SAVE AS..., 42Trace Assignment, 56 AD-Channel, 56 Amplifier, 56 Digital, 57 Imaging, 58 Leak, 57 LockIn, 57 Photometry, 57 S.W.Voltammetry, 58 Spectroscopy, 57 Trace Count, 56 Virtual, 57 Control Window, 65 Controlling Data Acquisition, 66 Average, 66 Break, 66 Next. 66 Resume. 66 Stop, 66 Store, 66 Wait, 66 Fixed Control Protocols, 67 Information, 65 Comment, 65 State, 65 Time, 65 Timer, 65 Start PGF Sequence, 66 Starting a Protocol, 67 I/O Control Window, 145 AD-Channels, 145 DA-Channels, 145 Dig-In, 145 Dig-In/Out Clear, 145 Dig-In/Out, 145 Single Bit, 145 Dig-Out, 145 Parameters, 146 Serial Output, 146 Serial Port, 146 Solutions, 146 Values, 146 Image Extension Camera Setup Binning, 175 Camera Reference, 175

Live View, 175 Live View Parameters, 175 Scaling, 175 Snapshot, 175 Image Control Window Camera Setup, 174 Imaging Extension, 169 Calibration Polychrome II, 172 Calibration - T.I.L.L., 172 Polychrome I, 172 Polychrome V, 172 Calibration-T.I.L.L. Polychrome IV, 172 DG4/DG5Filter Position, 172 Shutter Open/Close, 172 Shutter Selection, 172 Image Control Window, 174 Image File Selector, 178 Imaging Configuration, 179 Imaging Log, 180 PGF Primer, 175 ROI Manager, 177 Image File Selector Close File, 178 Filename, 178 Info Text, 178 Open Image File, 178 Page Selector, 178 **Imaging Configuration** DG4/DG5, 172 Lambda-10, 172 Other Settings, 179 Paths, 179 PTI DeltaRAM, 173 Imaging Configurations, 170 Background 1...3, 170 Camera Trigger, 170 Compute Histogram of Marked Target, 170 Compute Traces of Marked Targets: All, 170 Compute Traces of Marked Targets: Ratio + Ca, 170 Dead Time, 170 Kd*Sf, 170 R-max, 170 R-min, 170 T.I.L.L., 171 Traces to create, 170 Imaging Log Log File Text, 180 Lambda-10 Filter Position, 172 Shutter Open/Close, 172 Shutter Selection, 172 PGF Primer Acquisition Parameters, 176 PGF Listing, 176

Prime, 176 Update from PGF, 176 Update List, 176 PTI DeltaRAM Excitation, 173 Resting Wavelength, 173 Set. 173 Shutter Selection, 173 Wavelength to Volt, 173 **ROI** Manager Delete ROIs, 177 List of ROIs, 177 Load ROIs, 177 Save ROIs, 177 Show ROIs, 177 Starting, 170 T.I.L.L. Excitation, 171 Resting Wavelength, 171 Set. 171 Shutter Selection, 171 Wavelength Calibration T.I.L.L., 171 Imaging Extensions Image Window, 174 Introduction, 1 Closing Chartmaster, 7 Disclaimer, 1 Installing Chartmaster, 5 Key Conventions, 5 Naming Conventions, 4 Syntax, 4 Windows versions, 4 Online Help Controls, 7 Online Help on Keys, 6 PATCHMASTER Concept, 1 Scope, 1 Starting Chartmaster, 5 Support Hotline, 7 Supported System Software, 4 Keys, 17 Key file, 18 Listing, 17 Markers, 153 Edit Marker, 155 Delete, 155 Marker Index, 155 Scrolling Marker List, 155 Show Marker, 155 Show Sweep, 155 Text, 155 Time. 155 Trace, 155 Write to Notebook, 155 New Marker, 153 Add Marker, 153

Text, 153 Time, 154 Trace, 153 Menu AD-board, 38 Buffer Menu, 35 Accumulate Trace, 35 Add Trace, 35 Buffer 1...Buffer 4, 35 Clear, 35 Clear All, 35 Deaccumulate Trace, 35 Equation, 35 Export, 35 Import, 35 Parameter Handling, 36 Replace Target Trace, 36 Scale, 35 Show, 35 Subtract Trace, 35 Use Full Trace, 35 With Marked Target and Children, 36 Display Menu, 31 3D-Mode, 32 Auto Show, 31 Auto Y-scale, 31 Buffer, 33 Center, 34 Dimmed Overlay, 31 Done, 34 Extend, 33 Fixed X-width, 32 Fixed Y-scale, 32 Freeze Zero Line, 32 Labeling, 32 Marker, 34 Overlay Pages, 31 Overlay Series, 31 Overlay Sweeps, 31 Overlay Traces, 31 Reference Series Correction, 34 Repaint, 34 Show, 34 Show Tree Info, 32 Show Zero Line, 31 Subtract Reference Series, 31 Subtract Trace Buffers Series, 31 Subtract Zero Offset, 31 Trace, 33 Trace Buffer Correction, 34 Trace Properties, 31, 33 Use Trace Scaling, 34 Vectors, 34 Edit Menu, 24 Clear, 24 Copy, 24 Cut, 24 Find Same, 24

From Sweep, 154

Find Selection, 24 Find..., 24 Paste, 24 Replace Same, 24 Replace..., 24Select All, 24 Undo. 24 File Menu, 21 Close, 22 File Status, 22 Merge..., 22New Experiment, 22 New Group, 22 New..., 22 Open Modify..., 22Open Read Only..., 22 Page Properties Notebook..., 23 Page Properties Traces..., 23 Page Setup..., 23 Quit, 23Update File, 22 Write Tree File, 22 Help Menu, 39 About Chartmaster, 40 Hide Keys, 39 List all Items, 40 List all Macro Items, 40 List Keys, 39 Save Kevs. 40 Show Keys, 39 Show Tooltips, 39 Notebook Auto Store, 36 Notebook Menu, 36 Clear, 36 Clear when Saved, 36 Font Size..., 36 Line Numbers, 36 Merge..., 36 Print..., 36 Save, 36 Save as..., 36Scientific Notation, 36 Set Length..., 36 Zoom. 36 Protocols Menu, 37 Append Macro File, 37 Execute, 37 Execute while Recording, 37 Replay Menu, 26 ASCII, 27 ASCII-Text Format, 29 Copy PGF to PGF-Pool, 26 Delete, 26 Export, 26 Export Format, 27 Export Full Sweep, 26 Export Mode, 28

Export Trace, 29 Igor Pro, 27, 28 Import Trace, 29 Info File, 27 MatLab, 27 PICT, 27 Print. 26 Printer, 27 PULSE v8.6, 27 Reference Series, 26 Show, 26 Show Method, 26 Show PGF Template, 26 Trace Time, 27 With marked Target and Children..., 29 WMF, 27 Windows, 25 Windows Menu Analysis Window, 25 Calculator, 25 Close Front Window, 25 Configuration Window, 25 Control Window, 25 Enable Icon Configuration, 26 I/O Control Window, 25 Imaging Window, 25 LockIn Window, 25 Markers, 25 Notebook, 25 Oscilloscope Window, 25 Parameters Window, 25 Photometry Window, 25 Protocol Editor Window, 25 Protocol Methods, 25 Pulse Generator Window, 25 Replay Window, 25 Reset Front Window Position, 25 Save Front Dialog, 25 Solution Base, 25 Spectroscopy, 25 Menus, 21 Notebook Window, 143 Oscilloscope Display Cursor shapes, 61 Measurements on Traces Measure, 62 **Overlay Options** Overlay Series, 62 Overlay Sweep, 62 Oscilloscope Window, 61 Display, 61 Auto Series, 63 Auto Sweep, 63 Digital Filter, 63 Display Scaling, 63 Lassoing, 61

Overlay Options, 62 Page, 63 Page Reset, 63 Reset, 63 Start / End Time, 63 Sweep Length Reset, 63 Trace Color, 63 Trace Selection, 63 Y-offset, 63 Y-Scale, 63 Y-Scale Centering, 63 Zooming, 61 Display Refreshing, 62 Freeze, 62 Repaint, 62 Wipe, 62 Measurements on Traces, 62 Scan, 62 Multi-Channels, 64 Navigation, 62 Statistics Group/Series/Sweep, 62 Parameters Window, 135 Flagging, 142 Clear all Flags, 142 Copy Flags from, 142 Default Flags, 142 Flag Groups 1...4, 142 Info to Notebook, 142 Marked to File, 142 Marked to Notebook, 142 Target to File, 142 Target to Notebook, 142 Parameter Tabs, 136 Group, 137 LF, 141 Marked Items, 141 Parameter Options, 141 Root, 136 Series, 138 Sweep, 139 Titles, 141 Trace, 140 Photometry Extension, 163 Background 1...3, 163 Compute Traces of Marked Targets, 163 Dead Time, 163 DG4/DG5, 166 Emit-1, 166 Emit-2, 166 Filter Position, 166 Open/Close, 166 PGF Sequence, 167 Resting Wavelength, 166 Set, 166 Shutter Selection, 166 Speed, 166 Kd*Sf, 163

Lambda-10, 166 PTI DeltaRAM, 168 Defaults, 168 Emit-1, 168 Emit-2, 168 Excitation, 168 Resting Wavelength, 168 Set, 168 Shutter Selection, 168 Undo, 168 Wavelength to Volt, 168 R-max, 163 R-min, 163 T.I.L.L., 164 Calibrating T.I.L.L. monochrometers, 165 Compute Factors, 165 Emit-1, 164 Emit-2, 164 Excitation, 164 from - to, 165 lower - Scan, 165 Polychrome I, 165 Polychrome II, 165 Polychrome IV, 165 Polychrome V, 166 Resting Wavelength, 164 Set, 164 Shutter Selection, 164 upper - Scan, 165 Volt, 165 wavelength, 165 Wavelength Calibration, 164 Wavelength to Volts, 165 Protocol Editor Window, 99 Acquire Each Sweep Duration, 107 Increment, 107 Acquire Properties Set Min. Wait Time, 108 Update R-membrane and I-pipette, 108 Wipe Display at Start, 108 Write Events to Notebook, 108 Acquire Series Averages, 107 Break Keys, 107 Comment, 107 Edit PGF Template, 107 Label, 107 Sequence, 107 Analysis Analysis Mode, 110 Edit, 110 Name, 110 Set Graph Position, 110 Wipe, 110 BREAK Type, 102 Chain Protocol

 $\mathbf{214}$

Edit Protocol, 102 Protocol Name, 102 Return, 102 Select Protocol, 102 **Display Properties** Overlay Series, 111 Overlay Sweep, 111 Reset Timer, 111 Show Leaks, 111 Show Traces, 112 Show Tree Info, 112 Subtract Leaks, 111 Subtract Zero, 111 Wipe Online, 111 Wipe Osci, 111 Event Handling, 100 Delete, 100 Duplicate, 100 Events, 100 Insert After, 100 Insert Before, 100 Move, 100 Events, 101 Acquire Each Sweep, 107 Acquire Properties, 108 Acquire Series, 107 Acquisition, 107 Analysis, 110 Annotation, 117 Beep, 117 **BREAK**, 102 Chain Protocol, 102 Clear Key, 102 Data/Display, 110 Digital Filters, 110 **Display Properties**, 111 ELSE, 105 ELSEIF... THEN, 104 Export, 112 Extensions, 118 File Operation, 112 GOTO, 102 GOTO_MARK, 103 Hardware, 108 IF... THEN, 103 Imaging, 118 Launch, 105 Macro Command, 105 Messages, 117 PGF Parameters, 115 Photometry, 118 Protocol Sequence, 102 REPEAT, 105 Replay, 113 Serial Output, 108 Set DAC, 109 Set Digital Bit, 109 Set Digital Word, 109

Set Solution Changer, 109 Set Solutions, 115 Set Sweep Label, 108 Set Value, 116 Switch Window, 106 Trace Buffer, 114 Value/Parameter, 115 WAIT, 106 Write Icon Value, 118 Export Filename, 112 Full Sweep, 112 Overwrite, 112 Target, 112 File Operation Close File, 113 File Status, 113 Label, 113 New Experiment, 113 New File, 113 New Group, 113 Save after Break, 113 Store Data, 113 Update File, 113 GOTO Mark, 103 GOTO_MARK Mark. 103 IF...THEN Left Source, 103 Logical operators, 104 Right Source, 104 Macro Command Command, 105 **PGF** Parameters 1...10, 115Operation, 115 Value, 115 Photometry New Resting Wavelength, 118 Open Shutter / Close Shutter, 118 Set New Speed, 118 Set New Wavelength, 118 Set Resting Wavelength, 118 Protocol Handling, 99 Delete, 100 List, 100 Load, 99 Move, 100 Name, 100 New, 100 Protocol Pool, 99 Record Macros, 100 Relative Value, 100 Save, 99 Step, 100 To End, 100 Write, 100

Recurring Functions, 101 Delay, 101 If etc. Result, 101 Repeat Status, 101 Skip, 101 REPEAT Duration, 105 Increment, 105 Serial Output String, 109 Set DAC Channel, 109 Voltage, 109 Set Digital Bit Channel, 109 low, 109 Set Digital Word Digital Bits, 109 Digital Word, 109 Set Solution Changer Set Marker, 110 Solution. 109 Set Solutions Set External, 115 Set Internal, 115 Set Marker, 115 Set Sweep Label Sweep Label, 108 Sweep Label Target, 108 Set Value Copy, 117 Operation, 116 Value, 117 Value 1...16, User 1 - 2, 116 Trace Buffer Clear Buffer, 114 Operation, 114 Replace, 114 Scaling, 114 Target, 114 Trace Buffer Source, 114 Update Analysis, 114 Update Display, 114 WAIT Wait type, 106 Write Icon Value Alertbox, 118 Icon Text / Icon Value, 118 Item ID, 118 LF, 118 Notebook, 118 Title, 118 Pulse Generator Check and Execute Execute, 77 Segment Classes Delete, 93 Pulse Generator Window, 73

Acquisition Modes, 76 Gap Free Mode, 76 Interactive Mode, 76 AD Input Channel AD, 89 Build Instructions, 91 Chirp. 89 Compression Modes, 90 Defaults, 90 Dig-0...15, 89 Dig-in (word), 89 Digital Filter, 90 Imaging, 90 Link, 90 LockIn, 90 Off, 89 Photometry, 90 Set Offset, 90 Unit, 90 Virtual, 89 Zero Offset, 92 Channel Settings DA - Output/AD Input, 87 AD Input Channel, 89 Channels, 87 DA Output Channel, 87 Use of Digital Outputs, 88 Check and Execute, 77 Checking, 77 Not Checking, 77 Chirp Wave Parameters Amplitude, 84 Chirps to Skip, 85 End Frequency, 84 Exponential Chirp, 84 Linear Chirp, 83 Points/Cycle, 84 Pre-Chirp, 85 Segment Points, 84, 85 Spectroscopy Chirp, 84 Start Frequency, 84 Value/Parameter, 84 DA Output Channel Analog output, 87 Dig-0...15, 87 Dig-out (word), 87 load from file template, 88 Off, 87 relative to hold, 88 Stimulus - DA, 88 Unit. 88 use DA-Scaling, 88 use for Imaging, 88 use for LockIn, 88 use for Spectroscopy, 88 use for Wavelength, 88 Error Handling, 98 Increment Modes Alternate, 94

Decrease, 94 Increase, 94 Interleave +, 94Interleave -, 94 Toggle, 94 Logarithmic Increment Modes dt*Factor. 95 dV*Factor, 95 t*Factor, 95 V*Factor, 94 Miscellaneous, 96 Analysis Method, 96 Break Condition, 96 Break if >, 96 Break if <, 96Break if abs <, 96Break on next, 96 Common Timing, 96 Filter Factor, 96 No Break, 96 Relevant Segments, 96 Separate Timing, 96 PGF Parameters, 98 Photometry Wave Parameters Adapt to Maximal Sweep Length, 85 Expand, 85 Number of Cycles, 85 Number of Segments, 85 Trunc. 85 Segment Classes Chirp, 93 Constant Segment, 93 Continuous, 93 Delete, 93 Duplicate, 93 Duration, 93 Insert, 93 Ramp, 93 Segment Class, 93 Sine, 93 Square, 93 Store, 92 Value setting, 93 Voltage, 93 Segments, 92 Increment Modes, 94 Logarithmic Increment Modes, 94 Scan Rates, 94 Segment Classes, 92 Sequence Pool, 75 Copy, 75 Delete, 76 List, 75 Load, 75 Merge, 75 Move, 76 Name, 75 Save, 75

Undo, 76 Sequence Selector, 75 Sequence Buttons, 75 Sine Wave Parameters, 78 Actual Frequency, 79 Common Frequency, 78 Cycles to Average, 80 Cycles to Skip, 80 LockIn, 79 Peak Amplitude, 78 Points / Cycle, 79 Requested Frequency, 79 Separate Frequency, 79 Total Cycles, 80 Use Theoretical Stimulus, 80 Value/Parameter, 79 Square Wave Parameters Actual Frequency, 81 Amplitude Increment, 81 Common Frequency, 80 Cycles to Average, 83 Cycles to Skip, 82 LockIn, 82 Negative Amplitude, 81 Peak Amplitude, 81 Points/Cycle, 82 Positive Duration Factor, 81 Requested Frequency, 81 Separate Frequency, 82 Total Cycles, 83 Use Theoretical Stimulus, 82 V-reversal, 83 Value/Parameter, 81 Stimulus Template, 97 Delay, 97 Preview Mode, 97 Sweep and Channel Length, 78 Channel Length, 78 Stimulus, 78 Stored Sweep Duration, 78 Sweep Duration, 78 Sweep Length, 78 Timing, 76 Allow Continuous Redraw, 76 No Wait before 1. Sweep, 76 No. of Sweeps, 76 Not Triggered, 77 Sample Interval, 76 Start Segment, 77 Start Time, 77 Sweep Interval, 76 Trigger Mode, 77 Trigger Series, 77 Trigger Sweeps, 77 Use Durations, 77 Use Scan Rates, 77 Wait before 1. Sweep, 76 V-Membrane, 97

Set Last Seg. Amplitude, 97 Views, 74 Cartoon View, 74 Condensed View, 74 Full View, 74 Wave Parameters, 78 Chirp Wave Parameters, 83 Imaging Wave Parameters, 86 Photometry Wave Parameters, 85 Square Wave Parameters, 80 Replay Window, 69 Main window functions, 69 Apply, 70 Arrows, 70 Interpreting Tree objects, 70 Label, 69 Maneuvering, 71 Mark, 70 Mark a Target and its Children, 71 Mark All, 70 Repeat Rate, 70 Selecting/Marking multiple objects, 71 Show, 70 Text, 69 Tracing, 70 Tree Handling, 70 Unmark, 70 Visible, 70 Window Scroll Arrows, 70 Software LockIn Extension, 181 Capacitance Measurements, 183 Activating the LockIn Extension, 184 Amplifier Settings, 187 Analysis, 188 Creating a PGF sequence, 185 LockIn Configuration, 184 Running the PGF sequence, 187 LockIn Configuration Points to Average, 182 LockIn Configuration Window, 181 Attenuation, 182 Compute LockIn of Marked Targets, 182 Default Y-ranges, 182 LockIn Mode, 181 Offline Computation - Trace to create, 182 Parent Trace, 182 Phase Shift, 181 SetDefaults, 182 Skip, 182 Write to Notebook, 182 LockIn Mode On Cell. 181 Phase Range: 0..180, 181 Phase Range: 0..360, 181 Piecewise Linear, 181 Sine + DC, 181 LockIn Traces, 182

LockIn_Admit(Y), 183 $LockIn_Imag(Y), 182$ $LockIn_Imag(Z), 183$ $LockIn_Imp|Z|$, 183 LockIn_Phase, 182 LockIn_Real(Y), 182 LockIn_Real(Z), 183 Solution Changer, 151 Solutions, 147 Solution Base, 147 Solution Data Base, 148 Append, 148 Concentration, 148 Create Entry, 149 Delete, 148 Delete Entry, 149 Duplicate Entry, 149 Entries, 149 Export Label, 149 Export Listing, 149 Ingredient, 148 Insert, 148 Last Entry, 149 Name, 148 Next Entry, 149 Numeric Name, 148 Numeric Name Value, 148 Osmolarity, 148 pH. 148 Solution Index, 148 Solution Indices, 148 Spectroscopy Extension, 191 Display of Spectra, 198 Introduction, 191 Chirp Wave Forms, 191 Exponential Chirp, 191 Linear Chirp, 191 Spectroscopy Chirp, 191 Transfer Function Ratio, 191 Spectroscopy Acquisition, 195 Adding Chirp Analysis Traces, 196 Minimizing Swing-In Effects, 196 Parametrization of the Chirp Stimuli, 195 Possible Frequency Range, 195 The Chirp Wave Dialog, 195 Useful Frequency Range, 195 Spectroscopy Extension, 192 Chirp_Admit(Y), 194 Chirp_Avg, 194 $Chirp_Imag(Y), 194$ $Chirp_Imag(Z), 194$ $Chirp_Imp(Z), 194$ Chirp_Phase, 194 $Chirp_Real(Y), 194$ $Chirp_Real(Z), 194$ Compute Spectroscopy of Marked Targets, 194 Correction Mode, 193 Default Ranges, 194

Input Source, 193 Log Compression, 193 Offline Computation - Traces to create, 193 Parent AD-Trace, 194 Reference Element Correction, 193 The Spectroscopy Traces, 194 Supported Interfaces, 4 User Inferface Dialog Controls, 9 Background Color, 9 Boolean, 9 Button, 9 Checkbox, 9 Drag, 9 Edit Text, 9Enter, 9 Framed Text, 9 List, 9 Number, 9 Radio Button, 9 Switch, 9 User Interface, 9 Copy and Paste Functions, 15 **Dialog Controls** Numerical Input, 10 SI Units, 10 String Buffer, 10 Modifying Dialogs and Controls, 10 Deleting Controls, 13 Dialog Control Window, 11 Hiding Controls, 13 Saving Dialogs and Controls, 14 Switching Windows, 14