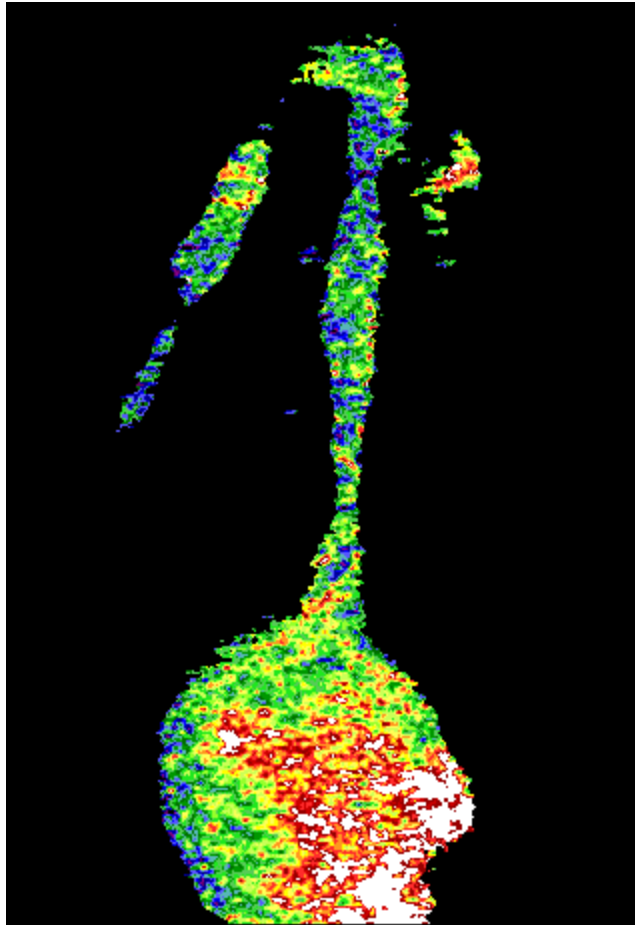


8.52

FURA Extension to PULSE



Myogenic response in afferent arteriole of hydronephrotic mouse kidney. Ca^{2+} increase is visible in vessel and glomerulum. (M. Krause, Department of Physiology, University of Heidelberg)

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1. Introduction

The FURA software is intended to perform photometric and fluorescence measurements with a photomultiplier. FURA offers the function of an interface for the hardware as an extension to PULSE. The combination with PULSE realizes the possibility to perform synchronized patch clamp and photometric measurements.

The FURA-extension supports the following photometric systems:

- a) Generic
- b) Olympus OSP3
- c) Olympus OSP100
- d) pti DeltaRam
- e) pti DeltaScan
- f) Sutter DG-4 and Lambda-10
- g) T.I.L.L. photonics monochromator

This manual describes the use of the FURA software.

Activating the FURA Extension

You will find the option *Select FURA Extension* in the *PULSE* drop-down menu. In its submenu, select the system you will use. You will have to store the configuration parameters and quit PULSE. Then restart PULSE in order to properly initialize the *FURA* extension.

Configuration and Measurements

The *FURA Configuration* window is accessible via the drop-down menu *PULSE*, option *FURA Configuration*. When the user selects that option, the *FURA-Configuration* window will be displayed. It differs depending on the selected photometric system. You only need to read the chapter which deals with the system you use.

2. Generic

The Generic system is intended for systems with control of the excitation wavelength by an analog voltage or a digital signal. If you use a system that is described in detail in this manual please refer to that section.

Hardware Connections

Connect the voltage or digital input of your system which controls the excitation wavelength with a DA-output or the digital connector on the EPC 9/ITC-16/ ITC-18. The chosen DA-output must be the same as the one set in the configuration of the software, section *DA-Filter*, when you use an analog signal to control the excitation wavelength.

Connect the output on the photomultiplier with a free AD-input on the EPC 9/ITC-16/ITC-18 with a standard BNC-cable. The selected AD-input must be the same as the one set in the configuration of the software, section *AD-Signal*.

Configuration

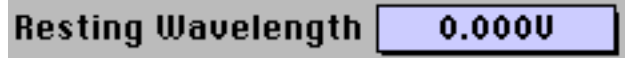
Fura Configuration (generic):

Dead Time	<input type="text" value="2.00ms"/>	AD-Signal	<input type="text" value="AD-1"/>	<input type="text" value="0.000V"/>
Resting Wavelength	<input type="text" value="0.000V"/>	DA-Filter	<input type="text" value="DA-1"/>	<input type="text" value="0.000V"/>

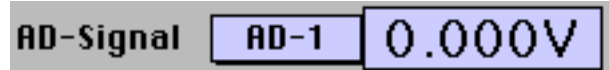
Display Settings		Signal-Ratio to [Ca]		
Min Ratio	Max Ratio	K-factor	Min Ratio	Max Ratio
<input type="text" value="-2.00E+01"/>	<input type="text" value="1.00E+01"/>	<input type="text" value="1.00E+00"/>	<input type="text" value="0.00E+00"/>	<input type="text" value="1.00E+00"/>

The *Dead Time* defines the delay between the time a voltage is sent to the monochromator and the beginning of the signal averaging. The "dead time" is intended to be the time the monochromator requires to settle at a new setting. It is advisable to measure at least once that settling time.

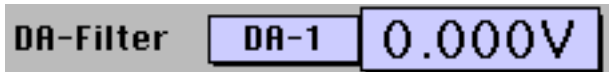
The *Resting Wavelength* control defines the value (voltage or digital value) to which the output is set while no acquisition is running.



AD-Signal: The A/D-channel from which the light intensity is monitored. This is the A/D-channel which has to be connected to the photomultiplier. The box to the right of AD-Signal continuously displays the voltage read at the A/D-input.



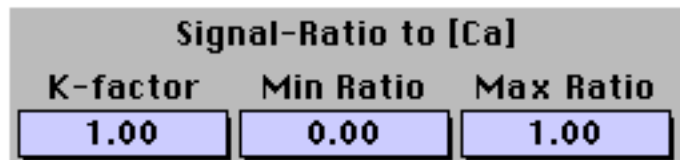
DA-Filter: This is the D/A-channel which outputs the voltage to control the monochromator wavelength ("filter" as in "diffraction filter"). The box to the right of DA-Filter displays the signal send to the output, as voltage or digital value, depending on the output channel you selected.



The *Display Settings* Min and Max Ratio define the scaling range of the FURA ratio markers when PULSE displays them in the *Oscilloscope Window*.



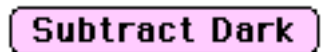
The *Signal-Ratio to [Ca]* section contains the parameters used to compute the calcium concentration, according to the formula:



$$\text{Ca-concentration} = \text{Factor} * (\text{Ratio} - \text{Rmin}) / (\text{Rmax} - \text{Ratio})$$

$$\text{Factor} = \text{scaling factor} * K_D$$

The *Subtract Dark* control allows to choose whether the light intensity during the dark period (i.e., the period during which wavelength-3 is applied) is subtracted from both, signal and reference intensity.



The *NOT Calibrated* selection should be set to *Calibration Valid* after the calibration has been successful. No FURA data acquisition will be performed as long as the calibration parameters are not declared to be valid. This is done to prevent invalid data and avoid damaging the hardware.



The *Undo* button restores the parameters to those values that were active when switching to the *FURA-Configuration* window.



Pulse Generator

To measure a FURA-signal one has to activate the FURA-option of a Series in the Pulse-Generator Window (PGF-dialog). To activate a FURA-measurement, click on the FURA control. The definitions of trigger 3 is now reserved for the FURA-parameters. Trigger 2 can still be used, but is usually hidden such that the labeling text of the FURA parameters can be displayed. The FURA parameters are:

Pulse Length		Total	1500 pts	30.00 ms
FURA		Stored	1250 pts	25.00 ms

Triggers	1	# 1(+)	FURA (⚡)		1
DAchannel	Default		epoch [ms]	20	
Segment	1		delay [ms]	5	
Time [ms]	5.00		length [ms]	5	5
Length [ms]	2.50		wavel 1 / 2	0	0
Voltage [mV]	off		wavel 3	0	

The field to the right of the *FURA* title is the total number of FURA determinations per sweep.

epoch: The total duration of one FURA-measurement

delay: The delay of the first measurement from the beginning of the sweep.

length: The length of the FURA-measurement at wavelength1 and 2, respectively

wavel 1/2: The wavelength 1 (typically the signal wavelength) and 2 (typically the reference wavelength), given in volts or digital values.

wavel 3: The "dark" wavelength (e.g., to subtract changes in the room light), given in volts or digital values.

At the end of a Sweep, the output is reset to the value defined in the *Resting Wavelength* field in the *FURA Configuration* window. It allows, e.g., to turn off the illumination of the specimen when not measuring. This will reduce bleaching of the dye.

Note: *The FURA-measurement adds one output channel and one input channel to the AD/DA-channel list. One D/A channel is used to control the monochromator, and one A/D channel is used to read the light intensity.*

No additional input channel is added, if one of the "fast" AD-input traces is acquired from the same AD-channel as the FURA signal.

Display of FURA-Measurements

There are three different options how one can display the results.

- 1) One can display each single FURA-point together with the current data on the same display, i.e., the Oscilloscope Window. For that, select the *I vs t + Fura* option in the drop-down menu *Display*.
- 2) One can display the average of all FURA-points in one sweep in the Online-analysis window, typically versus time. If *Fixed Scaling* and *Overlay Online* are selected one can have the time-course of the FURA-signal displayed continuously.
- 3) One can export the average of the FURA data of one Sweep to the X-Chart extension, and have it displayed, stored, and manipulated in the X-Chart windows.

X-Chart Extension - Traces and Parameters

Within the *Edit Traces and Param.* window the user has to select *Fura* as the *Type* to acquire FURA data.

Selecting *Fura* allows four different arguments: *F1*, *F2*, *F3* and *Ratio*. *F1* and *F2* will be acquired according to the settings in the configuration. *F3* is not available. The *Ratio* will be calculated online.

No.	Type	Arg. 1	2	3	4	Param.
1	Fura	F1	—	—	—	F1
2	Fura	F2	—	—	—	F2
3	Fura	Ratio	—	—	—	Ratio

Selecting *Ca* as the *Type* shows a calculated trace according to the formula given above. The parameters used for this calculation are set in the corresponding fields at the bottom of the window.

Calc. Param.		
Kd Sf	R min	R max
350.0000n	250.0000m	4.000000

The formula is: $Ca\text{-concentration} = KdSf * (Ratio - R_{min}) / (R_{max} - Ratio)$

An extended description and additional options are described in the X-Chart manual, chapter *Calculated Data*, section *[Ca] Calculation with Fura-2*.

3. OSP3, OSP100

Hardware Requirements, Software Requirements and Connections

For the use of the OSP systems a GPIB interface card together with the drivers from National Instruments or compatible is required. This combination works under Windows 95, Windows NT, and MacOS. Please install the card and the driver on the computer according to the instructions provided with the GPIB card.

Connect the output labeled "PMT-out" on the PMT with a free AD-input on the EPC 9/ITC-16/ITC-18 with a standard BNC-cable. The selected AD-input must be the same as the one set in the configuration of the software, section *PMT-out*. The dual emission version (2 photomultipliers) of the OSP100 is not supported.

Connect the output on the OSP system labeled "F1/F2 out" with a free AD-input on the EPC 9/ITC-16/ITC-18 with a standard BNC-cable. The selected AD-input must be the same as set in the configuration of the software, field *F1/F2-out*.

Configuration

□
OSP100 Configuration

OSP Modes			
<input checked="" type="radio"/> Standby <input type="radio"/> <input type="radio"/> F1 F2 F1/F2 <input type="radio"/> <input type="radio"/> GPIB-Settings <input type="radio"/> Offline <div style="border: 1px solid black; padding: 2px; text-align: center; margin-top: 5px;">2 excitations - 1 emission</div>			
		Integr. / Point	1
		Frequency	100 Hz
Microscope Settings			
Magnification	40 x	Wavelength 1	340 nm
Interm. Magn.	1.00 x	Wavelength 2	380 nm
Pinhole No.	1: 2.50µm	Transm. 1	100%
		Transm. 2	100%
Display Settings		Min Ratio	-20.00
		Max Ratio	10.00
Signal-Ratio to [Ca]		PMT-out	AD-1
K-factor	1.00E+00	F1/F2-out	AD-2
Min Ratio	0.00E+00	Close Shutter	Never
Max Ratio	1.00E+00		
		Param. NOT Valid	
		Undo	Done

In the *OSP Modes* section the settings of the shutters are set.

1. *Standby*: all shutters are closed
2. *Transmission Mode*: The shutter for the transmission light and for the oculars are opened. The user can inspect the specimen.

OSP Modes

<input checked="" type="radio"/> Standby <input type="radio"/> Transmission Mode <input type="radio"/> Fluorescence Mode F1 F2 F1/F2 <input type="radio"/> Photometry Mode <input type="radio"/> GPIB-Settings <input type="radio"/> Offline <div style="border: 1px solid black; padding: 2px; text-align: center; margin-top: 5px;">2 excitations - 1 emission</div>	
---	--

3. *Fluorescence Mode*: The excitation light path is opened so the user can watch the fluorescence. The excitation light used is defined by the switches F1, F2 or F1/F2, selecting one of the excitation wavelength (F1 or F2) or both (F1/F2).
4. *Photometry Mode*: The shutters for the excitation light path and the photomultiplier are opened. All others are closed to protect the photomultiplier. Selecting this mode will start measuring. This allows to inspect the settings such as PMT-voltage.
5. *GPIB-Settings*: Here the configuration of the GPIB interfaces in the computer is defined to match the requirements of the OSP system.
6. *Offline*: All shutters are closed and can be set by the controls on the OSP system. In case of a severe error (such as illegal settings) the program switches to this mode.

The system needs to be set to the mode in which the measurement will be performed. Four different modes are available although only the modes with one emission are supported.

✓ 2 excitations - 1 emission
1 excitation - 1 emission
1 excitation - 2 emissions
2 excitations - 2 emissions

In the upper right corner are controls and displays to set and inspect measurement parameters.

F1, F2, F1/F2 ratio: In Photometry Mode the actual measured values of the fluorescence intensity are displayed in the *F1* and *F2* boxes. In the *F1/F2 ratio* box the calculated ratio is displayed.

F1	0.00
F2	0.00
F1 / F2 ratio	0.00
Integr. / Point	1
Frequency	100 Hz
PMT-Voltage 1	200 V
PMT-Voltage 2	200 V

The boxes *Integr./Point* and *Frequency* determine the switching frequency of the mirror in the OSP system and the number of averaged single measurements for one data point. The frequency of ratio determinations is then $Frequency / (factor * Integr./Point)$. The "factor" is determined by the number of excitation wavelength.

PMT-Voltage 1, PMT-Voltage 2

OSP3: *PMT-Voltage 1* displays the voltage of the PMT set with the dial at the PMT turret of the OSP3 system.

OSP100: *PMT-Voltage 1 / PMT-Voltage 2* controls allow to set the voltages of the corresponding PMT. The PMT 2 control is only active when two PMT are connected to the OSP100 system.

Microscope Settings			
Magnification	40 x	Wavelength 1	340 nm
Interm. Magn.	1.00 x	Wavelength 2	380 nm
Pinhole No.	1: 2.50µm	Transm. 1	100%
		Transm. 2	100%

Microscope Settings serve as a documentation of the microscope settings. The user must select the settings according to his microscope. This part has no control function.

Display Settings	Min Ratio	Max Ratio
	-20.00	10.00

The *Display Settings Min Ratio* and *Max Ratio* define the scaling range of the FURA ratio markers when PULSE displays them in the Oscilloscope Window.

The *Signal-Ratio to [Ca]* section contains the parameters used to compute the calcium concentration, according to the formula:

Signal-Ratio to [Ca]		
K-factor	Min Ratio	Max Ratio
1.00	0.00	1.00

$$\text{Ca-concentration} = \text{Factor} * (\text{Ratio} - R_{\min}) / (R_{\max} - \text{Ratio})$$

$$\text{Factor} = \text{scaling factor} * K_D$$

PMT-out determines the AD input where the signal of the photomultiplier is acquired.

PMT-out	AD-1
---------	------

F1/F2-out (OSP100 only) determines the AD input where the voltage is measured which identifies the excitation wavelength.

F1/F2-out	AD-2
-----------	------

Adc Sync (OSP3 only) determines the AD input where the voltage is measured which identifies the excitation wavelength.

Adc Sync	AD-2
----------	------

Close Shutter sets the timing of closing the excitation shutter during measurements.

Close Shutter	Never
---------------	-------

The options are: *Never*, *After Sweep*, *After Series* and *After Acquisition*. This option can be used to protect the specimen from UV-light and minimize bleaching of the dye.

Never: shutters are kept open all the time.

After Sweep: the excitation shutter is opened before a sweep and closed immediately thereafter.

After Series: the excitation shutter is opened at the beginning of a series and closed

after the last sweep of the series is acquired.

After Acquisition: the excitation shutter is opened upon starting any acquisition and is closed when the acquisition is stopped. This can be used for linked series without closing the shutter between each series.

The *Param. NOT Valid* selection should be set to *Parameters Valid* after all parameters have been set. No FURA data acquisition will be performed as long as the parameters are not declared to be valid. This is done to prevent invalid data and avoid damaging the OSP hardware. Do not forget to **store the configuration file** before you quit PULSE. Otherwise, all the settings are forgotten the next time PULSE is started.



The *Undo* button restores the parameters to those values that were active when switching to the *FURA-Configuration* window.



Pulse Generator

To activate the OSP3 or OSP100 system during a sweep the button *OSP (+)* in the specific stimulus sequence must be selected. The FURA measurement will be performed according to the settings in the *OSP Configuration* window.

Pulse Length	Total		
OSP (+)		1500 pts	30.00 ms
	Stored	1250 pts	25.00 ms

Note: The FURA-measurement adds two input channels to the AD/DA-channel list. One A/D channel is used to read the synchronization signal and one A/D channel is used to read the light intensity.

Display of FURA-Measurements

There are four different options how one can display the results.

- 1) One can display each single FURA-point together with the current data on the same display, i.e., the Oscilloscope Window. For that, select the *I vs t + Fura* option in the drop-down menu *Display*.
- 2) One can display the average of all FURA-points in one sweep in the Online-analysis window, typically versus time. If *Fixed Scaling* and *Overlay Online* are selected one can have the time-course of the FURA-signal displayed continuously.

3) One can export the average of the FURA data of one Sweep to the X-Chart extension, and have it displayed, stored, and manipulated in X-Chart.

4) FURA-measurements acquired from the OSP3/OSP100 via the GPIB connection can be continuously displayed in the "Graph" window of the X-Chart Extension.

X-Chart Extension - Traces and Parameters

Within the *Edit Traces and Param.* window the user has to select *Fura* as the *Type* to acquire FURA data.

Selecting *Fura* allows four different arguments: *F1*, *F2*, *F3* and *Ratio*. *F1* and *F2* will be acquired according to the settings in the configuration. *F3* is not available. The *Ratio* will be calculated online.

No.	Type	Arg. 1	2	3	4	Param.
1	Fura	F1	—	—	—	F1
2	Fura	F2	—	—	—	F2
3	Fura	Ratio	—	—	—	Ratio

Selecting *Ca* as the *Type* shows a calculated trace according to the formula given above. The parameters used for this calculation are set in the corresponding fields at the bottom of the window.

Calc. Param.		
Kd Sf	R min	R max
350.0000n	250.0000m	4.000000

The formula is: $Ca\text{-concentration} = KdSf * (Ratio - Rmin) / (Rmax - Ratio)$

An extended description and additional options are described in the X-Chart manual, chapter *Calculated Data*, section *[Ca] Calculation with Fura-2*.

4. pti DeltaRam

Hardware Connections

Connect the voltage input of the DeltaRam which controls the excitation wavelength with a DA-output connector on the EPC 9/ITC-16/ITC-18. The chosen DA-output must be the same as the one set in the configuration of the software, section *DA-Filter*.

Connect the output on the photomultiplier with a free AD-input on the EPC 9/ITC-16/ITC-18 with a standard BNC-cable. The selected AD-input must be the same as the one set in the configuration of the software, section *AD-Signal*.

Configuration

Fura Configuration (pti DeltaRAM):

Dead Time	<input type="text" value="2.00ms"/>	AD-Signal	<input type="text" value="AD-1"/>	<input type="text" value="0.000V"/>
Resting Wavelength	<input type="text" value="300 nm"/>	DA-Filter	<input type="text" value="DA-1"/>	<input type="text" value="3.21V"/> <input type="text" value="300 nm"/>

Display Settings		Signal-Ratio to [Ca]		
Min Ratio	Max Ratio	K-factor	Min Ratio	Max Ratio
<input type="text" value="-2.00E+01"/>	<input type="text" value="1.00E+01"/>	<input type="text" value="1.00E+00"/>	<input type="text" value="0.00E+00"/>	<input type="text" value="1.00E+00"/>

Volts/nm	<input type="text" value="-2.1414E-02"/>	<input type="button" value="Subtract Dark"/>	<input type="button" value="NOT Calibrated"/>
Wavelength at 0 volt	<input type="text" value="450 nm"/>	<input type="button" value="Undo"/>	<input type="button" value="Done"/>

The *Dead Time* defines the delay between the time a voltage is sent to the monochromator and the beginning of the signal averaging. The "dead time" is intended to be the time the monochromator requires to settle at a new setting. It is advisable to measure at least once that settling time.

The *Resting Wavelength* control defines the wavelength to which the monochromator is set while no acquisition is running.

AD-Signal: The A/D-channel from which the light intensity is monitored. This is the A/D-channel which has to be connected to the photomultiplier. The box to the right of AD-Signal continuously displays the voltage read at the A/D-input.

AD-Signal	AD-1	0.000V
-----------	------	--------

DA-Filter: This is the D/A-channel which outputs the voltage to control the monochromator wavelength ("filter" as in "diffraction filter"). The two boxes to the right of DA-Filter display the signal sent to the monochromator as voltage and the corresponding wavelength. You can enter a new value either as voltage or wavelength. The other value will immediately be updated. This allows to easily convert between the two units. For the voltage-to-wavelength computation, the given calibration parameters are used (see below).

DA-Filter	DA-1	0.00V	348 nm
-----------	------	-------	--------

The *Display Settings Min Ratio* and *Max Ratio* define the scaling range of the FURA ratio markers when PULSE displays them in the *Oscilloscope Window*.

Display Settings	
Min Ratio	Max Ratio
-2.00E+01	1.00E+01

The *Signal-Ratio to [Ca]* section contains the parameters used to compute the calcium concentration, according to the formula:

Signal-Ratio to [Ca]		
K-factor	Min Ratio	Max Ratio
1.00	0.00	1.00

$$\text{Ca-concentration} = \text{Factor} * \text{Factor} * (\text{Ratio} - R_{\text{min}}) / (R_{\text{max}} - \text{Ratio})$$

$$\text{Factor} = \text{scaling factor} * K_D$$

The *Subtract Dark* control allows to choose whether the light intensity during the dark period (i.e., the period during which wavelength-3 is applied) is subtracted from both, signal and reference intensity.

Subtract Dark

The pti system has a linear relationship between the applied voltage and the wavelength of the excitation light. In the fields *Volts/nm* and *Wavelength at 0 Volt* the calibration parameters must be given as described in the specifications of your DeltaRAM system.

Volts/nm	2.1414E-02
Wavelength at 0 volt	450 nm

The *NOT Calibrated* selection should be set to *Calibration Valid* after the calibration has been successful. No FURA data acquisition will be performed as long as the

✓ NOT Calibrated
Calibration Valid

calibration parameters are not declared to be valid. This is done to prevent invalid data and avoid damaging the monochromator hardware. Do not forget to **store the configuration file** before you quit PULSE. Otherwise, all the settings are forgotten the next time PULSE is started.

The *Undo* button restores the parameters to those values that were active when switching to the *FURA-Configuration* window.



Pulse Generator

To measure a FURA-signal one has to activate the FURA-option of a Series in the Pulse-Generator Window (PGF-dialog). To activate a FURA-measurement, click on the FURA control. The definitions of trigger 3 is now reserved for the FURA-parameters. Trigger 2 can still be used, but is usually hidden such that the labeling text of the FURA parameters can be displayed. The FURA parameters are:

Pulse Length		Total	1500 pts	30.00 ms
FURA		Stored	1250 pts	25.00 ms
Triggers	1	# 1(+)	FURA (✦) 1	
DAchannel	Default	epoch [ms]	20	
Segment	1	delay [ms]	5	
Time [ms]	5.00	length [ms]	5	5
Length [ms]	2.50	wavel 1 / 2	0	0
Voltage [mV]	off	wavel 3	0	

The field to the right of the *FURA* title is the total number of FURA determinations per sweep.

epoch: The total duration of one FURA-measurement

delay: The delay of the first measurement from the beginning of the sweep.

length: The length of the FURA-measurement at wavelength1 and 2, respectively

wavel 1/2: The wavelength 1 (typically the signal wavelength) and 2 (typically the reference wavelength), given in nanometers.

wavel 3: The "dark" wavelength (e.g., to subtract changes in the room light), given in nanometers.

At the end of a Sweep, the monochromator returns to the *Resting Wavelength* defined in the *FURA Configuration*. It allows, e.g., to turn off the illumination of the specimen when not measuring. This will reduce bleaching of the dye.

Note: The FURA-measurement adds one output channel and one input channel to the AD/DA-channel list. One D/A channel is used to control the monochromator, and one A/D channel is used to read the light intensity.

No additional input channel is added, if one of the "fast" AD-input traces is acquired from the same AD-channel as the FURA signal.

Display of FURA-Measurements

There are three different options how one can display the results.

- 1) One can display each single FURA-point together with the current data on the same display, i.e., the Oscilloscope Window. For that, select the *I vs t + Fura* option in the drop-down menu *Display*.
- 2) One can display the average of all FURA-points in one sweep in the Online-analysis window, typically versus time. If *Fixed Scaling* and *Overlay Online* are selected one can have the time-course of the FURA-signal displayed continuously.
- 3) One can export the average of the FURA data of one Sweep to the X-Chart extension, and have it displayed, stored, and manipulated in the X-Chart windows.

X-Chart Extension - Traces and Parameters

Within the *Edit Traces and Param.* window the user has to select *Fura* as the *Type* to acquire FURA data.

Selecting *Fura* allows four different arguments: *F1*, *F2*, *F3* and *Ratio*. *F1* and *F2* will be acquired according to the settings in the configuration. *F3* is not available. The *Ratio* will be calculated online.

No.	Type	Arg. 1	2	3	4	Param.
1	Fura	F1	—	—	—	F1
2	Fura	F2	—	—	—	F2
3	Fura	Ratio	—	—	—	Ratio

Selecting *Ca* as the *Type* shows a calculated trace according to the formula given above. The parameters used for this calculation are set in the corresponding fields at the bottom of the window.

Calc. Param.		
Kd Sf	R min	R max
350.0000n	250.0000m	4.000000

The formula is: $Ca\text{-concentration} = KdSf * (Ratio - R_{min}) / (R_{max} - Ratio)$

An extended description and additional options are described in the X-Chart manual, chapter *Calculated Data*, section *[Ca] Calculation with Fura-2*.

5. pti DeltaScan

Hardware Requirements, Software Requirements and Connections

The standard control hardware and program for the DeltaScan (e.g., the program "FeliX") is required to set and control the proper switching speed, and to open and close the shutters.

The DeltaScan must be equipped with the hardware which can output the photomultiplier signal on analog D/A channel plus the synchronization signal.

Connect the output on the photomultiplier with a free AD-input on the EPC 9/ITC-16/ITC-18 with a standard BNC-cable. The selected AD-input must be the same as the one set in the configuration of the software, section *AD-Signal*.

Connect the output on the TTL-synchronization signal with a free AD-input on the EPC 9/ITC-16/ITC-18 with a standard BNC-cable. The selected AD-input must be the same as the one set in the configuration of the software, section *AD-Sync*.

Configuration

Fura Configuration (pti DeltaScan):

Dead Time	<input type="text" value="30%"/>	AD-Signal	<input type="text" value="AD-1"/>	<input type="text" value="0.000V"/>
Frequency	<input type="text" value="0.00 Hz"/>	AD-Sync	<input type="text" value="AD-2"/>	

Display Settings		Signal-Ratio to [Ca]		
Min Ratio	Max Ratio	K-factor	Min Ratio	Max Ratio
<input type="text" value="-2.00E+01"/>	<input type="text" value="1.00E+01"/>	<input type="text" value="1.00E+00"/>	<input type="text" value="0.00E+00"/>	<input type="text" value="1.00E+00"/>

The *Dead Time* defines the percentage of the illumination cycle during which the light intensity

Dead Time

should **not** be measured (see section "Adjustments" further below). A typical setting is 30%.

The *Frequency* field shows the frequency of the synchronization TTL signal as measured by PULSE. See section "Adjustments" below.

Frequency 0.00 Hz

AD-Signal: The A/D-channel from which the light intensity is monitored. This is the A/D-channel which has to be connected to the photomultiplier. The box to the right of AD-Signal continuously displays the decoded light intensity of wavelength 1.

AD-Signal AD-1 0.000V

AD-Sync: The A/D-channel from which the synchronization TTL signal is acquired. This is the A/D-channel which has to be connected to the TTL-synchronization signal output on the DeltaScan.

AD-Sync AD-2

The *Display Settings* Min and Max Ratio define the scaling range of the FURA ratio markers when PULSE displays them in the *Oscilloscope Window*.

Display Settings
Min Ratio Max Ratio
-2.00E+01 1.00E+01

The *Signal-Ratio to [Ca]* section contains the parameters used to compute the calcium concentration, according to the formula:

Signal-Ratio to [Ca]
K-factor Min Ratio Max Ratio
1.00 0.00 1.00

$$\text{Ca-concentration} = \text{Factor} * (\text{Ratio} - \text{Rmin}) / (\text{Rmax} - \text{Ratio})$$

$$\text{Factor} = \text{scaling factor} * K_D$$

The *NOT Calibrated* selection should be set to *Calibration Valid* after the calibration has been successful. No FURA data acquisition will be performed as long as the calibration parameters are not declared to be valid. This is done to prevent invalid data and avoid damaging the monochromator hardware. Do not forget to **store the configuration file** before you quit PULSE. Otherwise, all the settings are forgotten the next time PULSE is started.

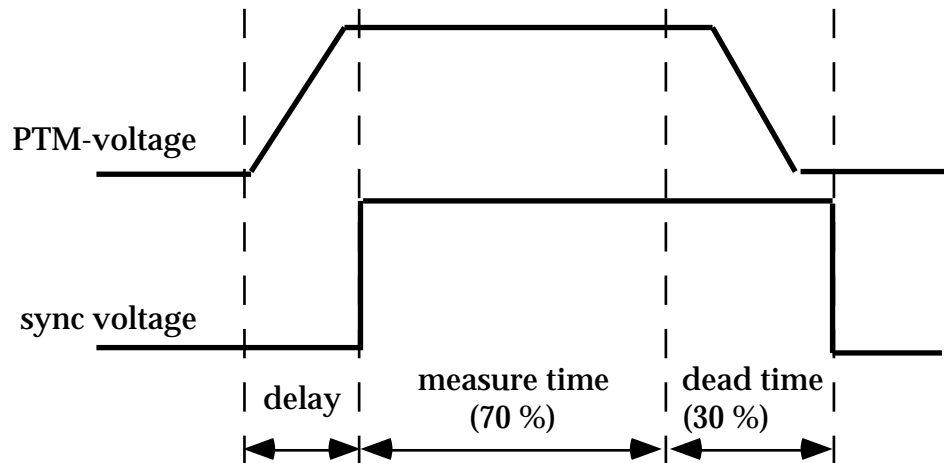
✓ NOT Calibrated
Calibration Valid

The *Undo* button restores the parameters to those values that were active when switching to the *FURA-Configuration* window.

Undo

Adjustments

One must perform two adjustments before PULSE can correctly decode the signals read from the DeltaScan. The first is to set the proper delay time of the synchronization signal on the DeltaScan itself. The second adjustment is to set the optimal *Dead Time* percentage, see the following scheme:



Please proceed as follows. Start the DeltaScan with the standard control program ("FeliX"). Set the synchronization delay time to zero. When the DeltaScan is up and running, start PULSE and activate the *FURA Configuration* window. Set the *Dead Time* percentage to 25%. PULSE should now tune in to the synchronization signal from the DeltaScan and display the correct switching frequency value in the *Frequency* field. Now apply some light to the photomultiplier and the value displayed in the top right field should start to deviate from zero. Apply enough light to get a good, constant reading. Increase now the delay time of the synchronization signal on the DeltaScan till the signal just gets to its maximum. Now, increase the *Dead Time* percentage just until the measured light value start does not start to decrease again.

Pulse Generator

To measure a FURA-signal one has to activate the FURA-option of a Series in the Pulse-Generator Window (PGF-dialog). To activate a FURA-measurement, click on the FURA control.



Note: The FURA-measurement adds two input channels to the AD/DA-channel list. One A/D channel is used to read the light intensity and one A/D channel is used to read the synchronization signal.

Display of FURA-Measurements

There are three different options how one can display the results.

- 1) One can display each single FURA-point together with the current data on the same display, i.e., the Oscilloscope Window. For that, select the *I vs t + Fura* option in the drop-down menu *Display*.
- 2) One can display the average of all FURA-points in one sweep in the Online-analysis window, typically versus time. If *Fixed Scaling* and *Overlay Online* are selected one can have the time-course of the FURA-signal displayed continuously.
- 3) One can export the average of the FURA data of one Sweep to the X-Chart extension, and have it displayed, stored, and manipulated in the X-Chart windows.

X-Chart Extension - Traces and Parameters

Within the *Edit Traces and Param.* window the user has to select *Fura* as the *Type* to acquire FURA data.

Selecting *Fura* allows four different arguments: *F1*, *F2*, *F3* and *Ratio*. *F1* and *F2* will be acquired according to the settings in the configuration. *F3* is not available. The *Ratio* will be calculated online.

No.	Type	Arg. 1	2	3	4	Param.
1	Fura	F1	—	—	—	F1
2	Fura	F2	—	—	—	F2
3	Fura	Ratio	—	—	—	Ratio

Selecting *Ca* as the *Type* shows a calculated trace according to the formula given above. The parameters used for this calculation are set in the corresponding fields at the bottom of the window.

Calc. Param.		
Kd Sf	R min	R max
350.0000n	250.0000m	4.000000

The formula is: $Ca\text{-concentration} = KdSf * (Ratio - R_{min}) / (R_{max} - Ratio)$

An extended description and additional options are described in the X-Chart manual, chapter *Calculated Data*, section *[Ca] Calculation with Fura-2*.

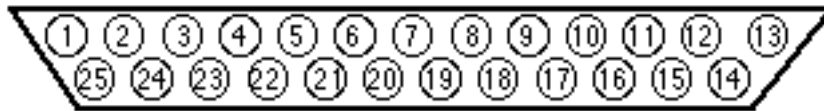
6. Sutter DG-4 Monochromator and Lambda-10 Filter Wheel

Hardware Connections

Special cables are required to connect the EPC9 or ITC-16/ITC-18 digital output connector to the Sutter DG-4 monochromator or the Lambda-10 filter wheel. The connector for the device control input has to be a female "DB-25" connector (standard parallel printer connector).

The pin assignments for the EPC9 are given in the EPC9 manual, Appendix II, Technical Data. The ITC-16 uses the same assignments for digital and ground lines.

The pin assignments for the male DB-25 are, as viewed from the back of the unit:



Connecting the Lambda-10 Filter Wheel

A TIB14 Trigger Interface Box is required to drive the Sutter Lambda-10 filter wheel with a standard EPC9. The TIB14 is not required when using an EPC9/Double, EPC9/Triple, or an ITC-16/18.

The required cable has to connect the following pins:

1. Output bits 0 to 3 to input bits 0 to 3 to control the filter wheel position
2. Output bit 7 to input pin 14 to control the shutter
3. Input bits 4 to 7 must be connected to ground
4. Output to input ground connection

Required pin connections for a TIB14

TIB14 BNC	"bit"		DB-25 pin	"bit"
0	0	->	2	0
1	1	->	3	1
2	2	->	4	2
3	3	->	5	3
7	7	->	14	SHUTTER

Required pin connections for an EPC9 Double or Triple:

EPC9 pin	"TRIGGER"		DB-25 pin	"bit"
1	0	->	2	0
2	1	->	3	1
3	2	->	4	2
4	3	->	5	3
8	7	->	14	SHUTTER
19	GND	<->	24	GND

Please, read in the EPC9 manual (Appendix II, Technical Data) about how to connect the digital output of the EP9 Double or Triple to the Lambda-10 wheel controller.

Required pin connections for an ITC-16

ITC-16 pin	"bit"		DB-25 pin	"bit"
25	0	->	2	0
27	1	->	3	1
29	2	->	4	2
31	3	->	5	3
39	7	->	14	SHUTTER
19	GND	<->	24	GND

Connecting the DG-4 Monochromator

Two connection types are possible:

1. One can use the full 8-bit wide addressing mode, when:
 - a TIB14 ("Trigger Interface Box") is connected to an EPC9, or
 - an EPC9 Double or Triple is used, or
 - an ITC-16 or ITC-18 is used.

The full 8-bit wide addressing mode is essential, when one needs to control the shutter of the DG-4 Monochromator.

2. One can use a simplified 4-bit wide addressing mode, when an EPC9 is to be used without a TIB14 ("Trigger Interface Box"). In that mode, one **cannot** control the shutter of the DG-4 Monochromator.

Full 8-bit addressing - required pin connections for a TIB14

TIB14 BNC	"bit"		DB-25 pin	"bit"
0	0	->	2	(0)
1	1	->	3	(1)
2	2	->	4	(2)
3	3	->	5	(3)
4	4	->	6	(4)
5	5	->	7	(5)
6	6	->	8	(6)
7	7	->	9	(7)

Full 8-bit addressing - required pin connections for an EPC9 Double or Triple

EPC9 pin	"TRIGGER"		DB-25 pin	"bit"
1	0	->	2	0
2	1	->	3	1
3	2	->	4	2
4	3	->	5	3
5	4	->	6	4
6	5	->	7	5
7	6	->	8	6
8	7	->	9	7
19	GND	<->	24	GND

Please, read in the EPC9 manual (Appendix II, Technical Data) about how to connect the digital output of the EP9 Double or Triple to the DG-4 Monochromator controller.

Full 8-bit addressing - required pin connections for an ITC-16

ITC-16 pin	"bit"		DB-25 pin	"bit"
25	0	->	2	0
27	1	->	3	1
29	2	->	4	2
31	3	->	5	3
33	4	->	6	4
35	5	->	7	5
37	6	->	8	6
39	7	->	9	7
19	GND	<->	24	GND

Simplified 4-bit addressing - required pin connections for an EPC9

EPC 9 pin	"bit"		DB-25 pin	"bit"
25	0	->	2	0
27	1	->	3	1
29	2	->	4	2
31	3	->	5	3
19	GND	->	6	4
19	GND	->	7	5
19	GND	->	8	6
4	15	->	9	7
19	GND	<->	24	GND

Connecting the Photomultiplier

Connect the output on the photomultiplier with a free AD-input on the EPC 9/ITC-16/ITC-18 with a standard BNC-cable. The chosen AD-input must be the same as the one set in the configuration of the software, section *AD-Signal*.

Initializing the Device Controller

The Sutter DG-4 monochromator and the Lambda-10 filter wheel must be switched to parallel port operation by pressing the on-line key on the controller box. The Lambda-10 defaults to parallel port operation at power on or at reset, while the DG-4 defaults to manual mode.

Configuration

Fura Configuration (Sutter):

Dead Time	<input type="text" value="2.00ms"/>	AD-Signal	<input type="text" value="AD-1"/>	<input type="text" value="0.000V"/>
Resting Filter	<input type="text" value="0"/>	Selected Filter	<input type="text" value="0"/>	
Close Shutter	<input type="text" value="After Acquisition"/>	Open Shutter	<input type="text" value="Lambda-10"/>	

Display Settings		Signal-Ratio to [Ca]		
Min Ratio	Max Ratio	K-factor	Min Ratio	Max Ratio
<input type="text" value="-2.00E+01"/>	<input type="text" value="1.00E+01"/>	<input type="text" value="1.00"/>	<input type="text" value="0.00"/>	<input type="text" value="1.00"/>

The *Dead Time* defines the delay between the time a voltage is sent to the monochromator and the beginning of the signal averaging. The "dead time" is intended to be the time the monochromator requires to settle at a new setting. It is advisable to measure at least once that settling time.

The *Resting Filter* control defines the index of the filter to which the system is set while no acquisition is running. The values are 0 to 7 for filter 1 to 8.

AD-Signal: The A/D-channel from which the light intensity is monitored. This is the A/D-channel which has to be connected to the photomultiplier. The box to the right of AD-Signal continuously displays the voltage read at the A/D-input.

Selected-Filter: This button allows to select the different filter. The measured intensity of the fluorescence light can be seen in the *AD-Signal* display.

Close Shutter: This control defines how the shutter of the Lambda-10 should be handled: *Inactive* will not use the shutter command at all (e.g. when using a DG-4 monochromator); *Never* will prevent the shutter to be closed by the program; *After Series* will close the shutter at the end of an acquired Series; *After SewEEP* will close the shutter at the end of each acquired Sweep.

Open Shutter: This control open and closes the shutter of the

Lambda-10, when the control *Close Shutter* (see just above) is not set to *Inactive* .

Note: Both controls, *Close Shutter* as well as *Open Shutter*, will be disabled, when an EPC9 is used without an attached TIB14.

The *Device Selection* allows to choose whether the DG-4 monochromator or the Lambda-10 filter wheel is to be used.

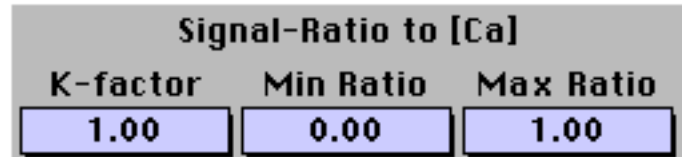


Note: It is very important to specify the correct device, since the two devices require different controlling protocols.

The *Display Settings* *Min Ratio* and *Max Ratio* define the scaling range of the FURA ratio markers when PULSE displays them in the *Oscilloscope Window*.

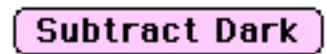


The *Signal-Ratio to [Ca]* section contains the parameters used to compute the calcium concentration, according to the formula:



$$\text{Ca-concentration} = \text{Factor} * (\text{Ratio} - \text{Min}) / (\text{Max} - \text{Ratio})$$
$$\text{Factor} = \text{scaling factor} * K_D$$

The *Subtract Dark* control allows to choose whether the light intensity during the dark period (i.e., the period during which wavelength-3 is applied) is subtracted from both, signal and reference intensity.



The *NOT Calibrated* selection should be set to *Calibration Valid* after the calibration has been successful. No FURA data acquisition will be performed as long as the calibration parameters are not declared to be valid. This is done to prevent invalid data and avoid damaging the monochromator hardware.



The *Undo* button restores the parameters to those values that were active when switching to the *FURA-Configuration* window.



Pulse Generator

To measure a FURA-signal one has to activate the FURA-option of a Series in the Pulse-Generator Window (PGF-dialog). To activate a FURA-measurement, click on the FURA control. The definitions of trigger 3 is now reserved for the FURA-parameters. Trigger 2 can still be used, but is usually hidden such that the labeling text of the FURA parameters can be displayed. The FURA parameters are:

Pulse Length		Total	1500 pts	30.00 ms
FURA		Stored	1250 pts	25.00 ms

Triggers	1	# 1(+)	FURA (⚡)		1
DAchannel	Default		epoch [ms]	20	
Segment	1		delay [ms]	5	
Time [ms]	5.00		length [ms]	5	5
Length [ms]	2.50		wavel 1 / 2	0	0
Voltage [mV]	off		wavel 3	0	

The field to the right of the *FURA* title is the total number of FURA determinations per sweep.

epoch: The total duration of one FURA-measurement

delay: The delay of the first measurement from the beginning of the sweep.

length: The length of the FURA-measurement at wavelength1 and 2, respectively

wavel 1/2: The wavelength 1 (typically the signal wavelength) and 2 (typically the reference wavelength), given as filter index (0 to 7).

wavel 3: The "dark" wavelength (e.g., to subtract changes in the room light), given as filter indexes (0 to 7).

At the end of a Sweep, the monochromator returns to the *Resting Wavelength* defined in the *FURA Configuration*. It allows, e.g., to turn off the illumination of the specimen when not measuring. This will reduce bleaching of the dye.

Note: *The FURA-measurement adds one output channel and one input channel to the AD/DA-channel list. One D/A channel is used to control the monochromator, and one A/D channel is used to read the light intensity.*

No additional input channel is added, if one of the "fast" AD-input traces is acquired from the same AD-channel as the FURA signal.

Display of FURA-Measurements

There are three different options how one can display the results.

- 1) One can display each single FURA-point together with the current data on the same display, i.e., the Oscilloscope Window. For that, select the *I vs t + Fura* option in the drop-down menu *Display*.
- 2) One can display the average of all FURA-points in one sweep in the Online-analysis window, typically versus time. If *Fixed Scaling* and *Overlay Online* are selected one can have the time-course of the FURA-signal displayed continuously.
- 3) One can export the average of the FURA data of one Sweep to the X-Chart extension, and have it displayed, stored, and manipulated in the X-Chart windows.

X-Chart Extension - Traces and Parameters

Within the *Edit Traces and Param.* window the user has to select *Fura* as the *Type* to acquire FURA data.

Selecting *Fura* allows four different arguments: *F1*, *F2*, *F3* and *Ratio*. *F1* and *F2* will be acquired according to the settings in the configuration. *F3* is not available. The *Ratio* will be calculated online.

No.	Type	Arg. 1	2	3	4	Param.
1	Fura	F1	—	—	—	F1
2	Fura	F2	—	—	—	F2
3	Fura	Ratio	—	—	—	Ratio

Selecting *Ca* as the *Type* shows a calculated trace according to the formula given above. The parameters used for this calculation are set in the corresponding fields at the bottom of the window.

Calc. Param.		
Kd Sf	R min	R max
350.0000n	250.0000m	4.000000

The formula is: $Ca\text{-concentration} = KdSf * (Ratio - R_{min}) / (R_{max} - Ratio)$

An extended description and additional options are described in the X-Chart manual, chapter *Calculated Data*, section *[Ca] Calculation with Fura-2*.

7. T.I.L.L. photonics Monochromator

Hardware Connections

Connect the input on the rear panel of the monochromator unit labeled " " with a free DA-output on the EPC 9/ITC-16/ITC-18 with a standard BNC-cable. This DA-output must be the same as set in the configuration of the software in the section *DA-Filter*.

Connect the output on the photomultiplier labeled "Signal" or "Sgn" with a free AD-input on the EPC 9/ITC-16/ITC-18 with a standard BNC-cable. This AD-input must be the same as set in the configuration of the software in the section *AD-Signal*.

Configuration

☐
Fura Configuration (T.I.L.L.):

Light Adjustment

Sound

Resting Wavelength

AD-Signal

DA-Filter

Dead Time

Wavelength to Volts

Slope	Offset	Angle β
<input type="text" value="1.5433E-02"/>	<input type="text" value="1.3678E+00"/>	<input type="text" value="30°"/>

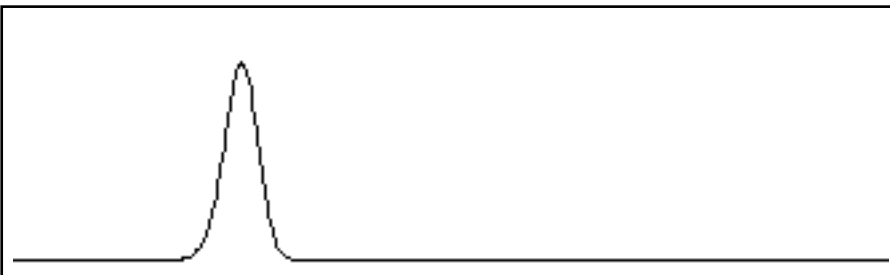
Signal-Ratio to [Ca]

K-factor	Min Ratio	Max Ratio
<input type="text" value="1.00E+00"/>	<input type="text" value="0.00E+00"/>	<input type="text" value="1.00E+00"/>

Calibration Wavelengths from to

wavelength - lower -	volt	wavelength - upper -	volt
<input type="text" value="340 nm"/>	<input type="button" value="Scan"/> <input type="text" value="-5.00V"/>	<input type="text" value="380 nm"/>	<input type="button" value="Scan"/> <input type="text" value="5.00V"/>

Display Settings Min Ratio Max Ratio



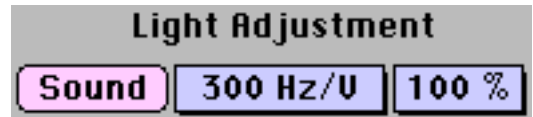
AD-Signal: The A/D-channel from which the light intensity is monitored. This is the A/D-channel which has to be connected to the photomultiplier. The box to the right of AD-Signal continuously displays the voltage read at the A/D-input.

AD-Signal

DA-Filter: This is the D/A-channel which outputs the voltage to control the monochromator wavelength ("filter" as in "diffraction filter"). The two boxes to the right of DA-Filter display the signal sent to the monochromator as voltage and the corresponding wavelength. You can enter a new value either as voltage or wavelength. The other value will immediately be updated. This allows to easily convert between the two units. For the voltage-to-wavelength computation, the given calibration parameters are used (see below).

DA-Filter

Light Adjustment: This are the controls which allow to optimize the light yield. The voltage is continuously read from the selected AD-channel and displayed. If the *Sound* option is selected, there will be sound output proportional to this voltage. Its base frequency and sound volume can be adjusted with the two controls below the *Sound* button.



The *Resting Wavelength* control defines the resting wavelength to which the monochromator is set while no acquisition is running.

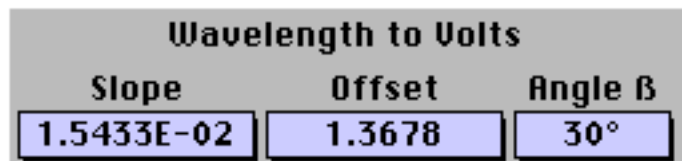


The *Dead Time* defines the delay between the time a voltage is sent to the monochromator and the beginning of the signal averaging. The "dead time" is intended to be the time the monochromator requires to settle at a new setting. It is advisable to measure at least once that settling time. Typical values are around 3ms.



Below the controls are described which are used to define and determine the monochromator calibration parameters required to convert from voltage to wavelength and back:

The *Wavelength to Volts* section contains all parameters used to compute the voltage needed for a specific wavelength, according to the following formulas:



$$1) \quad \lambda = \frac{2 \cdot \sin(\theta)}{n} \cdot 833.33$$

$$\Rightarrow \theta = \arcsin\left\{ \frac{\lambda}{[1666.67 \cdot \cos(\theta/2)]} \right\}$$

$$= F(\lambda)$$

$$2) \quad V = \text{slope} \cdot \lambda + \text{offset}$$

θ grid angle
 V DA-voltage to be set
 λ requested wavelength
 n reflection increment
 (typically 30 degrees, see your hardware manual)
 slope from wavelength calibration
 offset from wavelength calibration

$$3) \quad \text{slope} = \frac{F(\lambda_1) - F(\lambda_2)}{V_1 - V_2}$$

$$\text{offset} = F(\lambda_1) - \text{slope} \cdot V_1$$

The *Signal-Ratio to [Ca]* section contains the parameters used to compute the calcium concentration, according to the formula:

Signal-Ratio to [Ca]		
K-factor	Min Ratio	Max Ratio
1.00	0.00	1.00

$$\text{Ca-concentration} = \text{Factor} * (\text{Ratio} - R_{\min}) / (R_{\max} - \text{Ratio})$$

$$\text{Factor} = \text{scaling factor} * K_D$$

Calibration

Wavelength: The

Calibration Wavelengths	from	-10.00U	to	10.00U
-------------------------	------	---------	----	--------

lower and the upper voltage limits of the monochromator must be entered here.

The *Scan* buttons start an automatic scanning to acquire a calibration spectrum. The filter is stepped through the full voltage range (at present: 400 steps of 50 mV each, from V_{\min} to V_{\max}). During each step the voltage is kept constant for 5ms and the trigger is set high for 4 ms. 0.5 ms after the trigger output goes low the selected AD-channel (left, top corner) is read out. Then the next step is delivered.

Scan

At the end, the acquired spectrum is auto-scaled and displayed and the voltage at which the maximum is found is copied to the corresponding *volt* field.

volt
-5.00U

The *wavelength* fields should be filled with the two calibration wavelengths.

wavelength
340 nm

The *Compute Factors* button initializes the computation of the parameters *Slope* and *Offset* from the given calibration wavelengths and voltages.

Compute Factors

Display Settings	Min Ratio	-2.00E+01	Max Ratio	1.00E+01
------------------	-----------	-----------	-----------	----------

The *Display Settings* *Min Ratio* and *Max Ratio* define the scaling range of the FURA ratio markers when PULSE displays them in the *Oscilloscope Window*.

The *Subtract Dark* control allows to choose whether the light intensity during the dark period (i.e., the period during which wavelength-3 is applied) is subtracted from both, signal and reference intensity.

Subtract Dark

The *NOT Calibrated* selection should be set to *Calibration Valid* after the calibration has been successful. No FURA

✓ NOT Calibrated
Calibration Valid

data acquisition will be performed as long as the calibration parameters are not declared to be valid. This is done to prevent invalid data and avoid damaging the monochromator hardware.

The *Undo* button restores the parameters to those values that were active when switching to the *FURA-Configuration* window.



Performing a Calibration

To determine the calibration parameters, proceed as follows:

- a) Define the A/D and D/A channels used (AD-Signal and DA-Filters). Then connect the chosen D/A output connector to the monochromator input, and the selected A/D input connector to the photomultiplier output.
- b) Enter the lower and upper voltage limits of the monochromator in the *from* and *to* boxes. These values are supplied with the hardware.
- c) Insert one of the small bandpass filters (supplied with the hardware) between the optical fiber and the photomultiplier.
- d) Enter the wavelength of the inserted bandpass filter in the *wavelength - lower* field.
- e) Click on *scan - lower*. PULSE will now deliver a voltage ramp while measuring the transmitted light intensity. When the scan is done, the measured response is displayed, and PULSE will automatically find the voltage at which the light intensity was at its peak.
- f) Insert the second small bandpass filter between the optical fiber and the photomultiplier.
- g) Enter the wavelength of the inserted bandpass filter in the *wavelength - upper* field.
- h) Click on *scan - upper*. PULSE will now deliver a second voltage ramp while measuring the transmitted light intensity. When the scan is done, the measured response is displayed, and PULSE will automatically find the second voltage at which the light intensity was at its peak.
- i) Click now on *Compute Factors* and PULSE will compute and update the calibration parameters.

j) Do not forget to select *Calibration Valid* and to **store the configuration file** before you quit PULSE. Otherwise, all the settings are forgotten the next time PULSE is started.

Pulse Generator

To measure a FURA-signal one has to activate the FURA-option of a Series in the Pulse-Generator Window (PGF-dialog). To activate a FURA-measurement, click on the FURA control. The definitions of trigger 3 is now reserved for the FURA-parameters. Trigger 2 can still be used, but is usually hidden such that the labeling text of the FURA parameters can be displayed. The FURA parameters are:

Pulse Length		Total	1500 pts	30.00 ms
FURA		Stored	1250 pts	25.00 ms

Triggers		# 1(+)	FURA (⚡)		1
D/A channel	Default	epoch [ms]	20		
Segment	1	delay [ms]	5		
Time [ms]	5.00	length [ms]	5	5	
Length [ms]	2.50	wavel 1 / 2	0	0	
Voltage [mV]	off	wavel 3	0		

The field to the right of the *FURA* title is the total number of FURA determinations per sweep.

epoch: The total duration of one FURA-measurement

delay: The delay of the first measurement from the beginning of the sweep.

length: The length of the FURA-measurement at wavelength1 and 2, respectively

wavel 1/2: The wavelength 1 (typically the signal wavelength) and 2 (typically the reference wavelength), given in nanometers.

wavel 3: The "dark" wavelength (e.g., to subtract changes in the room light), given in nanometers.

At the end of a Sweep, the monochromator returns to the *Resting Wavelength* defined in the *FURA Configuration*. It allows, e.g., to turn off the illumination of the specimen when not measuring. This will reduce bleaching of the dye.

Note: *The FURA-measurement adds one output channel and one input channel to the AD/DA-channel list. One D/A channel is used to control the monochromator, and one A/D channel is used to read the light intensity.*

No additional input channel is added, if one of the "fast" AD-input traces is acquired from the same AD-channel as the FURA signal.

Display of FURA-Measurements

There are three different options how one can display the results.

- 1) One can display each single FURA-point together with the current data on the same display, i.e., the Oscilloscope Window. For that, select the *I vs t + Fura* option in the drop-down menu *Display*.
- 2) One can display the average of all FURA-points in one sweep in the Online-analysis window, typically versus time. If *Fixed Scaling* and *Overlay Online* are selected one can have the time-course of the FURA-signal displayed continuously.
- 3) One can export the average of the FURA data of one Sweep to the X-Chart extension, and have it displayed, stored, and manipulated in the X-Chart windows.

X-Chart Extension - Traces and Parameters

Within the *Edit Traces and Param.* window the user has to select *Fura* as the *Type* to acquire FURA data.

Selecting *Fura* allows four different arguments: *F1*, *F2*, *F3* and *Ratio*. *F1* and *F2* will be acquired according to the settings in the configuration. *F3* is not available. The *Ratio* will be calculated online.

No.	Type	Arg. 1	2	3	4	Param.
1	Fura	F1	—	—	—	F1
2	Fura	F2	—	—	—	F2
3	Fura	Ratio	—	—	—	Ratio

Selecting *Ca* as the *Type* shows a calculated trace according to the formula given above. The parameters used for this calculation are set in the corresponding fields at the bottom of the window.

Calc. Param.		
Kd Sf	R min	R max
350.0000n	250.0000m	4.000000

The formula is: $Ca\text{-concentration} = KdSf * (Ratio - R_{min}) / (R_{max} - Ratio)$

An extended description and additional options are described in the X-Chart manual, chapter *Calculated Data*, section *[Ca] Calculation with Fura-2*.