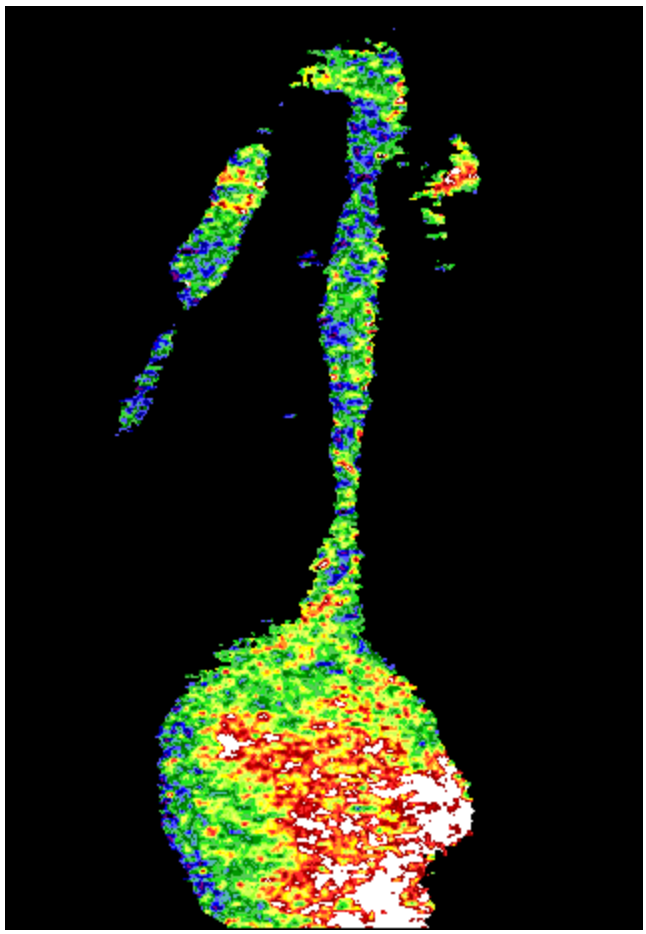
8.52
FURA Extension to X-Chart



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 X-Chart.

The FURA-extension supports the following photometric systems:

a) Generic

b) Olympus OSP3

c) Olympus OSP100

d) pti DeltaRam

e) Sutter DG-4 and Lambda-10

f) 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 *FURA* drop-down menu. In its sub-menu, select the system you will use. You will have to quit X-Chart and restart it in order to properly initialize the *FURA* extension.

Configuration and Measurements

The *FURA Configuration* window is accessible via the drop-down menu *FURA*, 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 section which deals with the system you use.

2. Generic

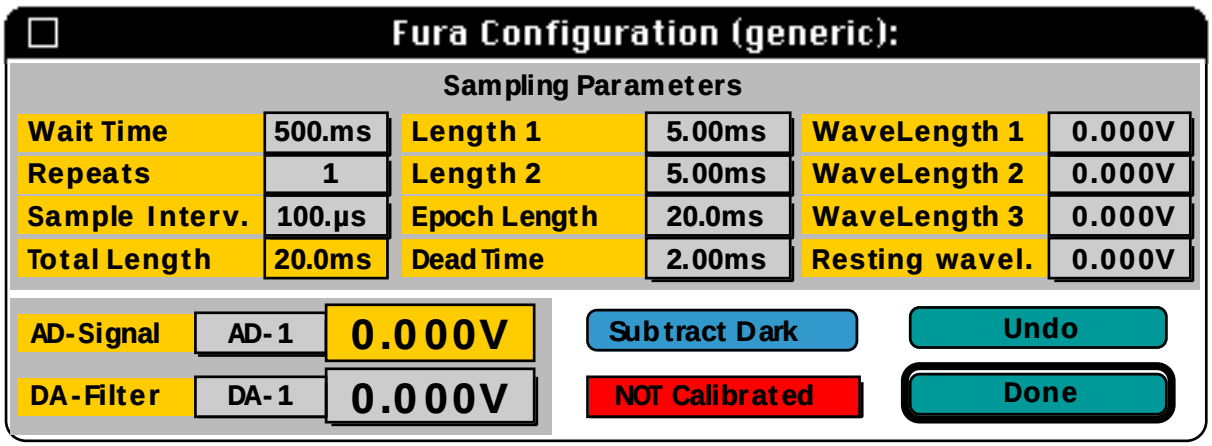
The Generic system is intended for systems with control of the excitation wavelength by an analog voltage or a digital signal. If you use a system that is described in detail in this manual please refer to that section.

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



Wait Time: The time period between the start of two following measurements.

Repeats: The number of single photometric measurements which are accumulated to one mea-

surement.

Sample Interv.: The sampling interval of the A/D converter.

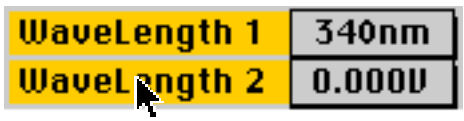
Total Length: The time needed for acquiring one data point. This time is calculated (*Repeats* * *Epoch Length*) and only displayed for information. No input is possible in this field.

Length 1/2: The length of the FURA-measurement at wavelength1 and 2, respectively.

Epoch length: The length of a single measurement.

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.

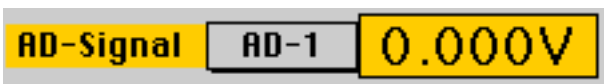
Wavelength 1/2: The wavelength 1 (typically the signal wavelength) and 2 (typically the reference wavelength), given as voltage or digital value, depending on the output channel you selected.



Wavelength 3: The "dark" wavelength (e.g., to subtract changes in the room light), given as voltage or digital value, depending on the output channel you selected.

Resting wavel.: This control defines the value (voltage or digital value) to which the output is set while no acquisition is running. At the end of a measurement sequence, the monochromator returns to the *Resting Wavelength*. It allows, e.g., to turn off the illumination of the specimen when not measuring. This will reduce bleaching of the dye.

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.

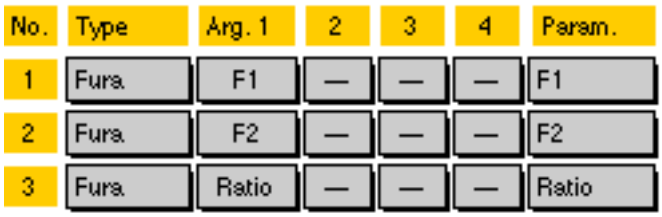
The *Subtract Dark* control allows to choose whether the light intensity during the dark period (i.e., the period during which wavelength-3 is applied) is subtracted from both, signal and reference intensity.

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.

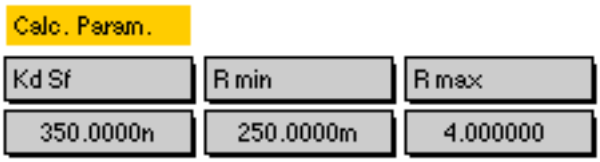
The *Undo* button restores the parameters to those values that were active when switching to the *FURA-Configuration* window.

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.



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

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

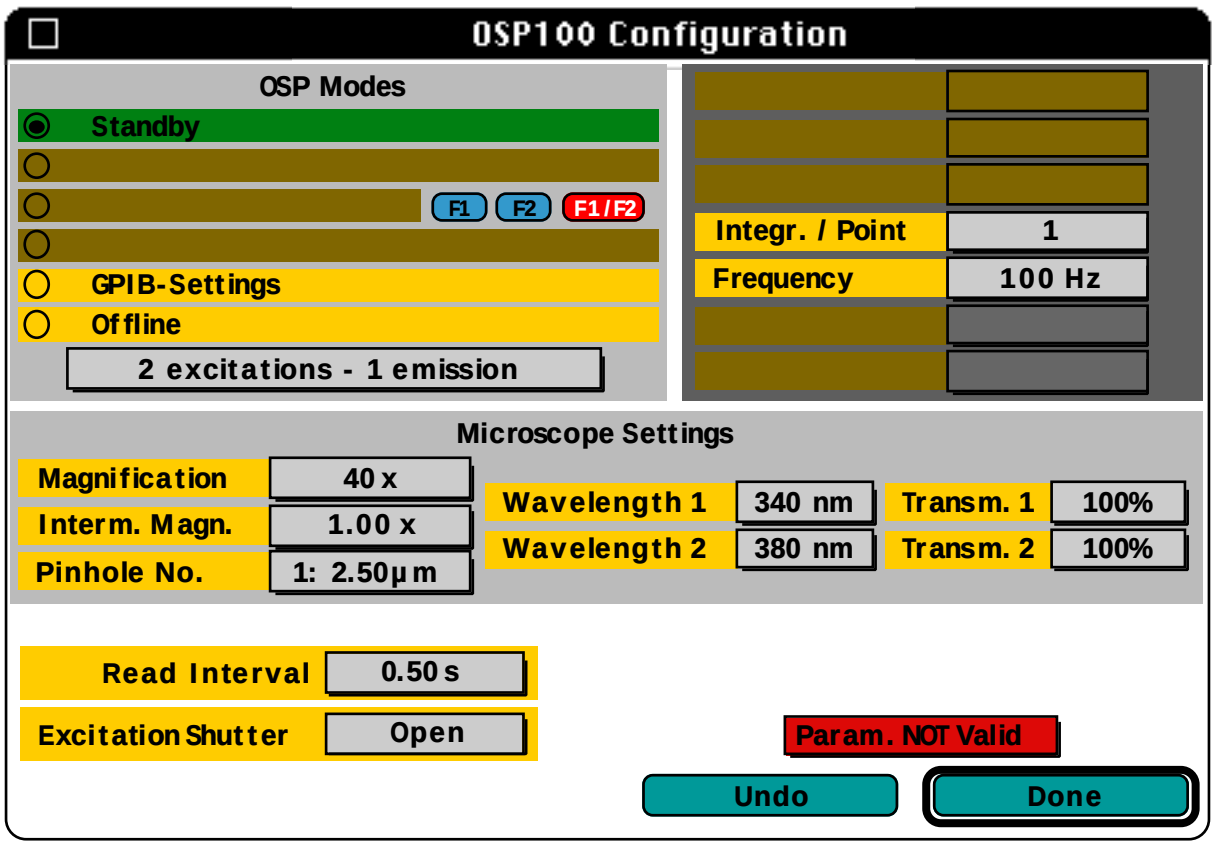
An extended description and additional options are described in the X-Chart manual, chapter *Calculated Data*, section *[Ca] Calculation with Fura-2.*

3. OSP3, OSP100

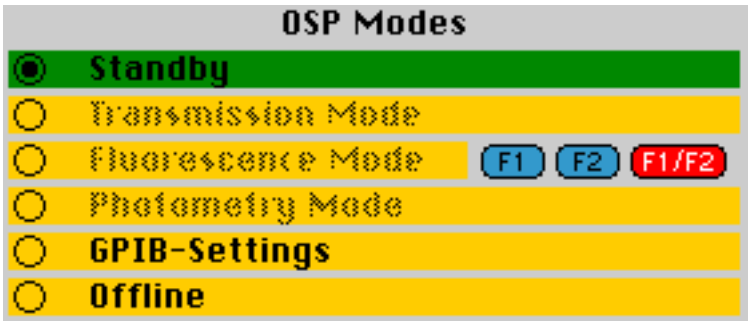
Hardware Requirements, Software Requirements and Connections

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. Data acquisition will be done via the GPIB interface card.

Configuration



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.

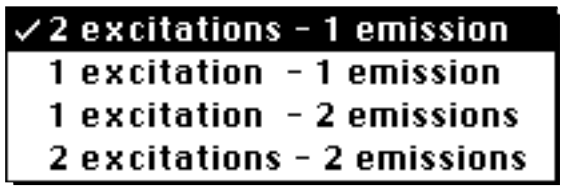
3. *Fluorescence Mode*: The excitation light path is opened so the user can watch the fluorescence. The excitation light used is defined by the switches F1, F2 or F1/F2, selecting one of the excitation wavelength (F1 or F2) or both (F1/F2).

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.

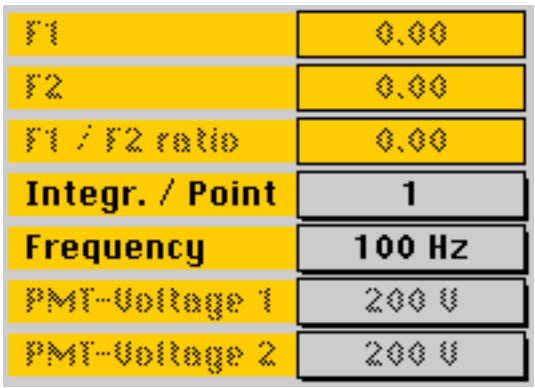
5. *GPIB-Settings*: Here the configuration of the GPIB interfaces in the computer is defined to match the requirements of the OSP system.

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 for the use with the commonly used fluorescence dyes.



The following controls and displays are used 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.

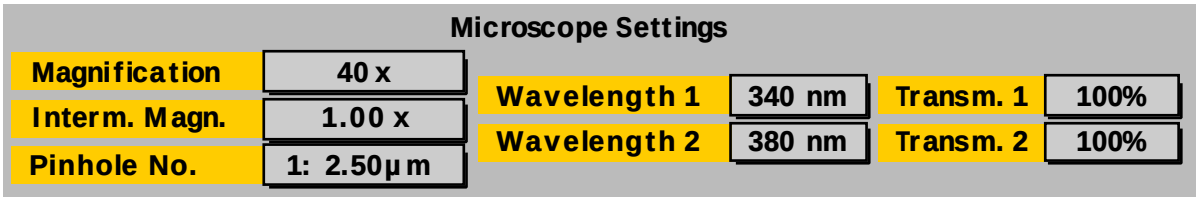
The boxes *Integr./Point* and *Frequency* determine the switching frequency of the mirror in the OSP system and the number of averaged single measurements for one data

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.)

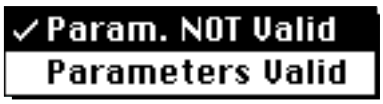


Microscope Settings serve as a documentation of the microscope settings. The user must select the settings according to his microscope. This part has no control function.

Read Interval: X-Chart will acquire at the given interval photometric measurements from the OSP system through the GPIB card.

Excitation Shutter: If set to *Close*, the excitation shutter will be opened before acquiring a single data pair and closed thereafter. If set to *Open* the shutter is opened at the start of acquisition (Run) and closed when acquisition is stopped (Stop).

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.



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

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 below. 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-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.*

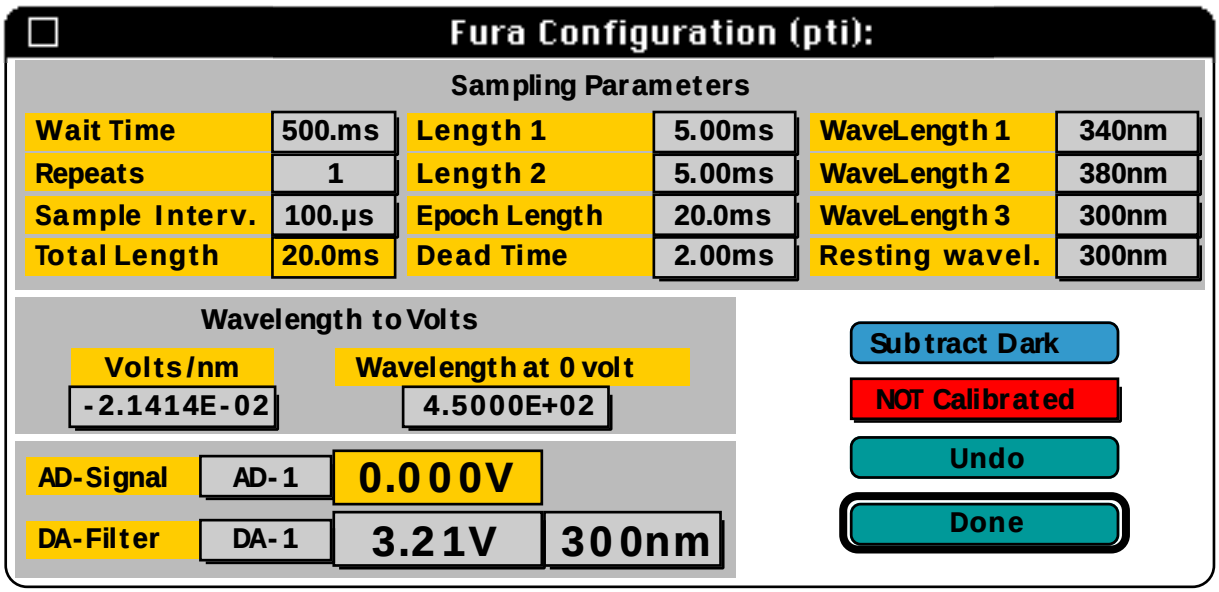
4. pti DeltaRam

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



Wait Time: the time period between the start of two following measurements.

Repeats: number of single photometric measurements which are accumulated to one measurement.

Sample Interv.: sampling interval of the A/D converter.

Total Length: The time needed for acquiring one data point. This time is calculated (*Repeats* * *Epoch Length*) and only displayed for information. No input is possible in this field.

Length 1/2: The length of the FURA-measurement at wavelength1 and 2, respectively.

Epoch length: The length of a single measurement.

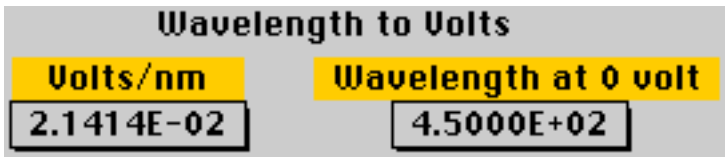
Dead Time: Defines the delay between the time a voltage is sent to the monochromator and the beginning of the signal averaging. The "dead time" is intended to be the time the monochromator requires to settle at a new setting. It is advisable to measure at least once that settling time.

Wavelength 1/2: The wavelength 1 (typically the signal wavelength) and 2 (typically the reference wavelength)

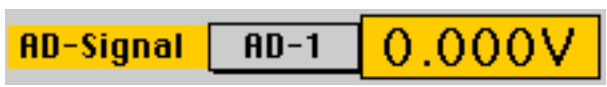
Wavelength 3: The "dark" wavelength (e.g., to subtract changes in the room light).

Resting wavel.: This control defines the resting wavelength to which the monochromator is set while no acquisition is running. At the end of a measurement sequence, the monochromator returns to the *Resting Wavelength.* This allows, e.g., to turn off the illumination of the specimen when not measuring. This will reduce bleaching of the dye.

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.



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 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.

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.

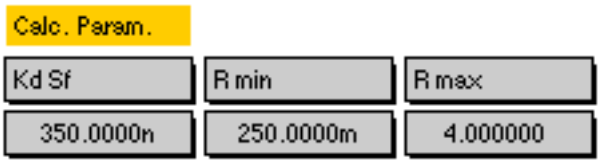
Traces and Parameters

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.



The formula is: Ca-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.*

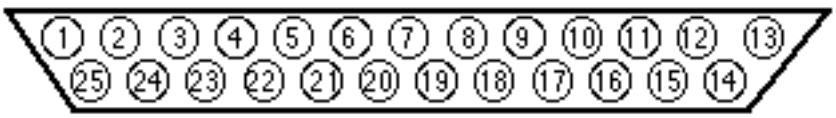
5. Sutter DG-4 Monochromator and Lambda-10 Filter Wheel

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

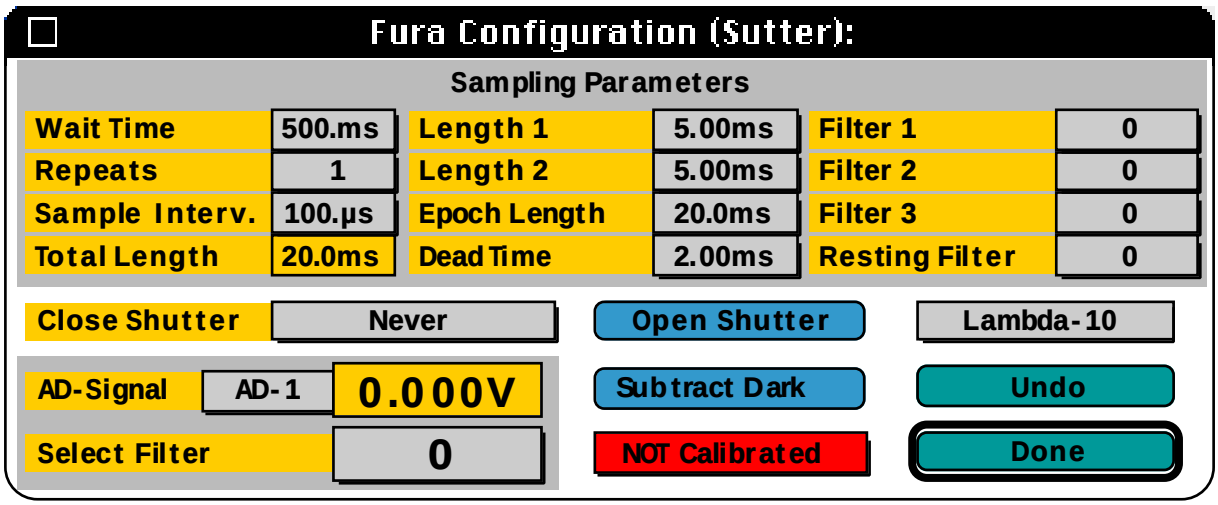
Connecting the Photomultiplier

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



Wait Time: the time period between the start of two following measurements.

Repeats: number of single photometric measurements which are accumulated to one measurement.

Sample Interv.: sampling interval of the A/D converter.

Total Length: The time needed for acquiring one data point. This time is calculated (*Repeats* * *Epoch Length*) and only displayed for information. No input is possible in this field.

Length1/2: The length of the FURA measurement at wavelength1 and 2, respectively.

Epoch length: The length of a single measurement.

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.

Filter 1/2: The index of the filter 1 (typically the signal wavelength) and 2 (typically the reference wavelength). The values are 0 to 7 for filter 1 to 8.

Filter 3: The index of the filter setting for the "dark" wavelength (e.g., to subtract changes in the room light). The values are 0 to 7 for filter 1 to 8.

Resting filter: This control defines the filter index for the resting wavelength to which the system is set while no acquisition is running. At the end of a measurement sequence, the system returns to the *Resting Wavelength*. This allows, e.g., to turn off the illumination of the specimen when not measuring and will reduce bleaching of the dye.

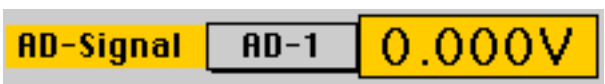
Close Shutter: This control defines how the shutter of the Lambda-10 should be handled: *Inactive* will not use the shutter command at all (e.g. when using a DG-4 monochromator); *Never* will prevent the shutter to be closed by the program; and *After Acquisition* will automatically close the shutter after acquisition has been stopped.

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* .

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

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

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.



Select-Filter: This button allows to select the different filters from this command window. The measured intensity of the fluorescence light can be seen in the *AD-Signal* display.

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.

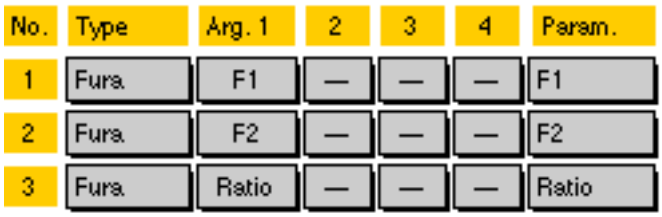
The *NOT Calibrated* selection should be set to *Calibration Valid* after the calibration has been successful. No FURA data acquisition will be performed as long as the calibration parameters are not declared to be valid. This is done to prevent invalid data and avoid damaging the hardware.

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

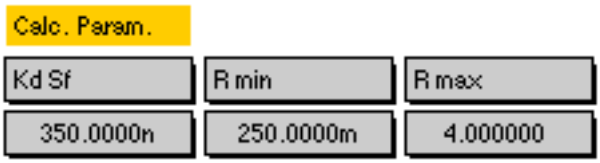
Traces and Parameters

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.



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



The formula is: Ca-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.*

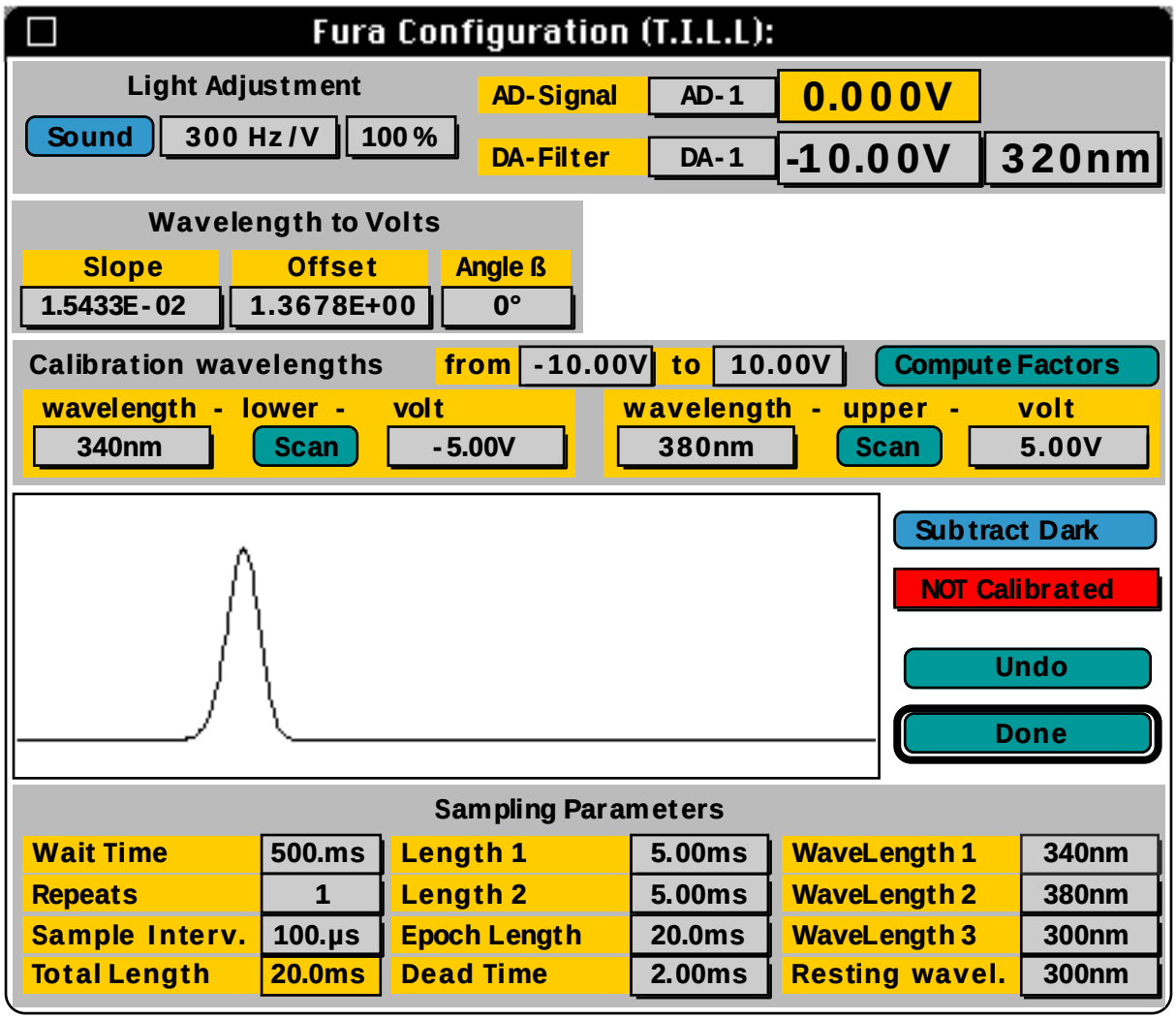
6. T.I.L.L. photonics Monochromator

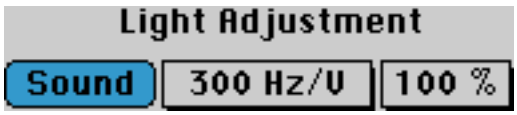
Hardware Connections

Connect the input on the rear panel of the monochromator unit labeled "λ" 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





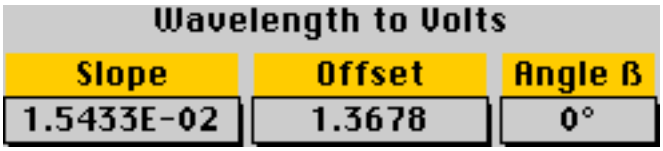
Light Adjustment: This are the controls which allow to optimize the light yield. The voltage is continuously read from the selected AD-channel and displayed. If the *Sound* option is selected, there will be sound output proportional to this voltage. Its base frequency and sound volume can be adjusted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

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 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).

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



1) $2 * \sin \alpha = \lambda / 833.33$
   $\Rightarrow \alpha = \arcsin\{\lambda / [1666.67 * \cos(\beta/2)]\}$
   $= F(\lambda)$

2) α = slope * V + offset

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

3) slope $= [F(\lambda_1) - F(\lambda_2)] / (V_1 - V_2)$
   offset $= F(\lambda_1)$ - slope * V_1

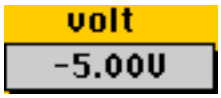
Calibration Wavelength:

The 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.

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.



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

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

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!

Wait Time: the time period between the start of two following measurements.

Repeats: number of single photometric measurements which are accumulated to one measurement.

Sample Interv.: sampling interval of the A/D converter.

Total Length: The time needed for acquiring one data point. This time is calculated (*Repeats* * *Epoch Length*) and only displayed for information. No input is possible in this field.

Length 1/2: The length of the FURA-measurement at wavelength1 and 2, respectively.

Epoch length: The length of a single measurement.

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.

Wavelength 1/2: The wavelength 1 (typically the signal wavelength) and 2 (typically the reference wavelength)

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

Resting wavel.: This control defines the resting wavelength to which the monochromator is set while no acquisition is running. At the end of a measurement sequence, 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.

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* .

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 below. 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-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.*