FLIMX User Guide

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Abbreviations

Amp	amplitude of fluorescence α
ASCII	American Standard Code for Information Interchange
Ch.	channel
Chi²	error of model curve
CI high	upper boundary of the confidence interval
CI low	lower boundary of the confidence interval
Ctrl	control
CV	coefficient of variation
Desc.	descriptive
diff.	difference
ETDRS	early treatment diabetic retinopathy study
FLIM	fluorescence lifetime imaging microscopy
FLIMX	fluorescence lifetime imaging explorer
FLIO	fluorescence lifetime imaging ophthalmology
FuncEvals	function evaluations
GUI	graphical user interface
h	hour
horiz	horizontal
Init.	initialization
IRF	instrument response function
Kurt	kurtosis
log10	logarithm to the base of 10
MHz	megahertz
min	minute
Misc	miscellaneous
MSB	MSimplexBnd (optimization algorithm)
Norm.	normalized
ns	nano second
ps	pico second
Q	equivalent of area under curve
Quant.	quantization
RAUC	relative area under curve
RAUCIS	relative area under curve including scatter contribution
RGB	red green blue
ROI	region of interest
SD	standard deviation
SDT	Becker&Hickl data file format
sec	second
Skew	skewness
Stats	statistics
t	time
TAC	time to amplitude conversion
Tau	fluorescence lifetime τ
tci	time shift of fluorescence
tol.	tolerance
Var	variance
vert	vertical

Introduction

About FLIMX

FLIMX (Fluorescence Lifetime Imaging eXplorer) is a MATLAB Software Package to Determine and Analyze the Fluorescence Lifetime in Time-Resolved Fluorescence Data available at www.flimx.de.

Open FLIMX

To open FLIMX follow the instructions below:

1. Start MATLAB
2. Go to the FLIMX folder
3. Do NOT add the FLIMX folder to your MATLAB path, this will be done automatically
4. Type "FLIMXLauncher" in the command window and press "Enter"

How to Cite

Data analysis was performed with FLIMX (Klemm et al. 2015), which is documented and freely available for download online under the open source BSD–license (http://www.flimx.de).

Please cite the following reference in your publications if you have used our software for your data analyses: Klemm M, Schweitzer D, Peters S, Sauer L, Hammer M, Haueisen J (2015) FLIMX: A Software Package to Determine and Analyze the Fluorescence Lifetime in Time-Resolved Fluorescence Data from the Human Eye. PLoS ONE 10(7): e0131640. doi:10.1371/journal.pone.0131640

Study Manager

The Study Manager helps to organize and maintain the personal information of the study participants. When opening the Study Manager a window appears with seven sections.

1 - The Menu Bar

2 - The Study Management

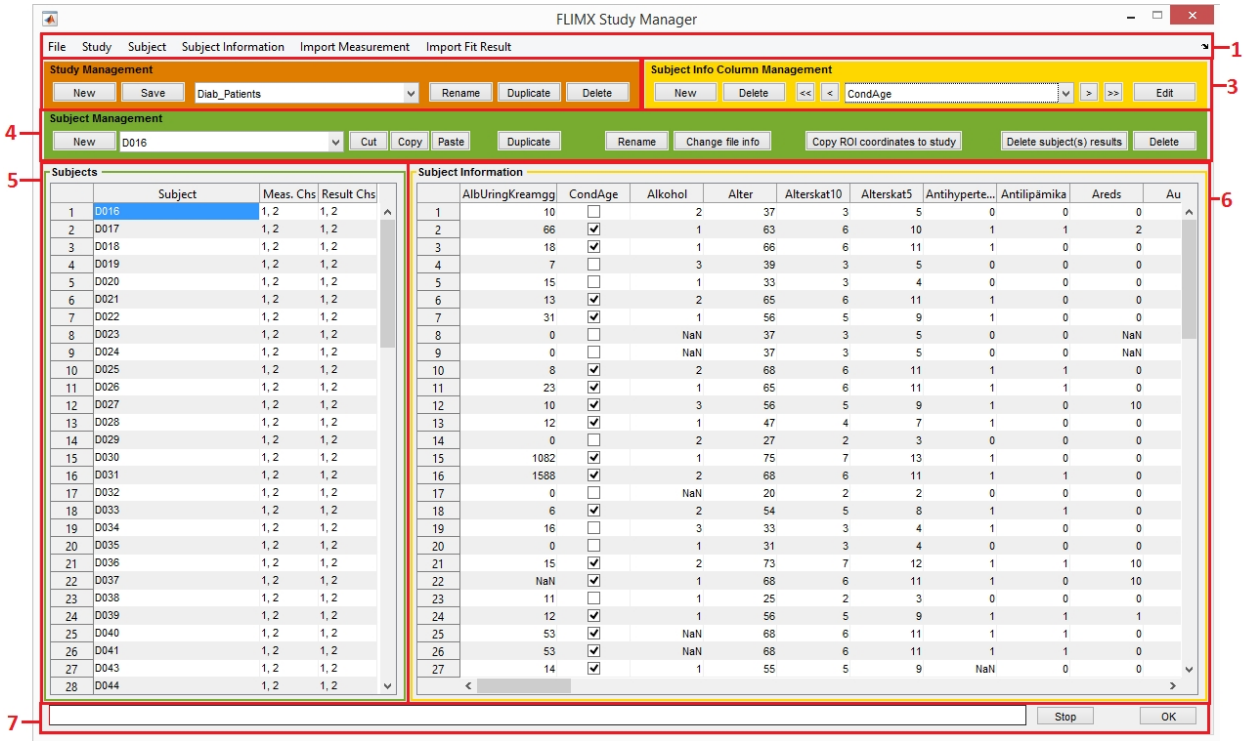
3 - The Subject Info Column Management

4 - The Subject Management

5 - The Subjects

6 - The Subject Information and

7 - Status Bar



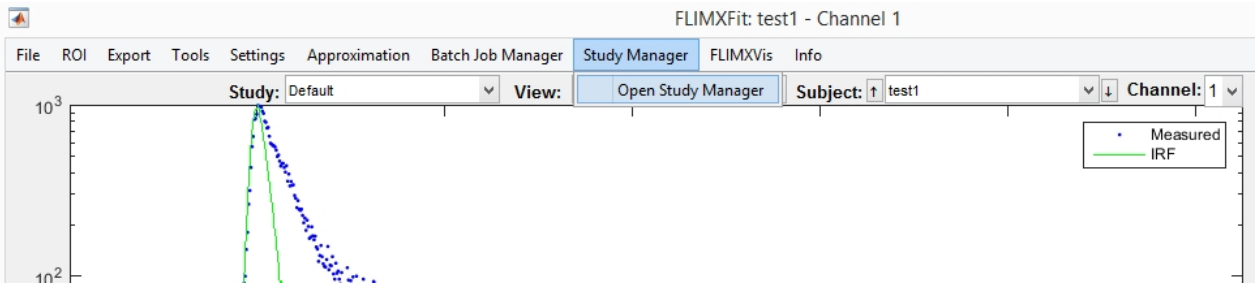
To learn how to open Study Manager, see below.

Open Study Manager

To open FLIMX Study Manager from FLIMXFit or FLIMXVis,

Open menu item **Study Manager** and select **Open Study Manager**

As of now, we will use the short command Menu Item > Submenu Item to refer to selecting a menu choice. For the instruction above, the corresponding short command is **Study Manager** > **Open Study Manager**.



The FLIMX Study Manager shown above will pop up.

Go back to top

Menu Bar

The Menu Bar is divided into six main functions.

File, Study, Subject, Subject Information, Import Measurement, Import Fit Result

Docking

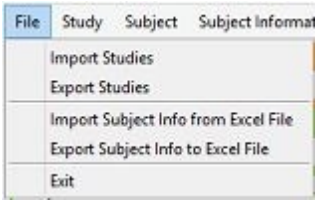
With clicking on the docking arrow the Study Manager is docked to the command window of MATLAB.

Back to Study Manager

File

File

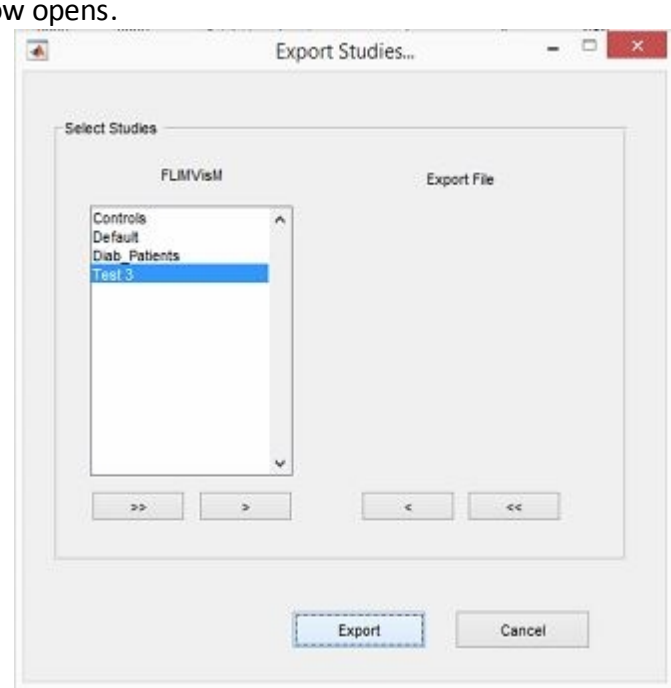
A click on File opens a window with five options: Import Studies, Export Studies, Import Subject Info from Excel File, Export Subject Info to Excel File and Exit.



Import Studies loads whole studies in form of a MATLAB file with a certain structure. This

helps to share studies between different users of this software. With importing a study the study participants and their information is loaded. With clicking on Import Studies another window opens. It serves to browse through the computer. The selected file needs to be confirmed by clicking on the "Open" button. If the file is different to the MATLAB file an error report occurs.

Export Studies saves studies as a MATLAB file in a selected folder of the computer. The export concludes the study participants and their information. With clicking on Export Studies a window opens.



The two boxes in the window show studies which can be exported (left box) and the studies which are currently selected to be exported (right box). The buttons with the double arrow underneath the boxes move all the studies into the other box. The buttons with the single arrow move only the selected file or study. Cancel aborts the exporting. With clicking on export a window opens to select the destination folder and the file name for the export. The "Save" button is the confirmation of the process. The file is saved as *.mat-type

Import Subject Info from Excel File allows the user to import subject information to the current study from an excel file. Only the content of a sheet called "subject-info" is used! The subject names must be in the first column! If the current study already contains subject information, there are three options: (1) update the existing information and add new information, (2) delete all existing information and load the new information or (3) abort the process.

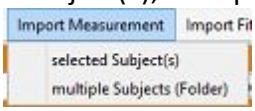
Export Subject Info to Excel File saves all the subject information in an excel file on the computer. With clicking on this function a window opens. It serves to browse through the computer. A file name has to be entered. The "Save" button is the confirmation of the process. The file is saved as *.xls-type.

Exit closes the *Study Manager*. If any unsaved changes are recognized the user is asked if changes to studies should be saved before quitting. If changes are not saved, all changes since the last saving of the study are discarded.

Import Measurement Data

Import Measurements

Measurements can only be possible in Becker&Hickl format (*.sdt) or as single decay (ASCII) files (*.txt, *.dat, *.asc). When clicking on this menu item a window opens with two options: selected Subject(s), multiple Subjects (Folder)



selected Subject(s): With clicking on this option the measurements for one or more selected subject can be imported. Therefore a window opens to browse your computer.

multiple Subjects (Folder) follows the same principle as selected Subject(s). The selected folder provides measurements for all the study participants.

Tutorial

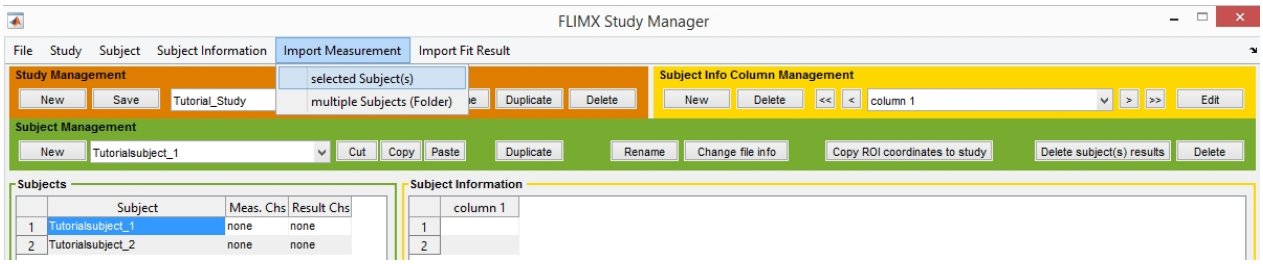
Within this section, you will learn to import measurement data for

- Single subject or
- Multiple subjects.

Import data for a single subject

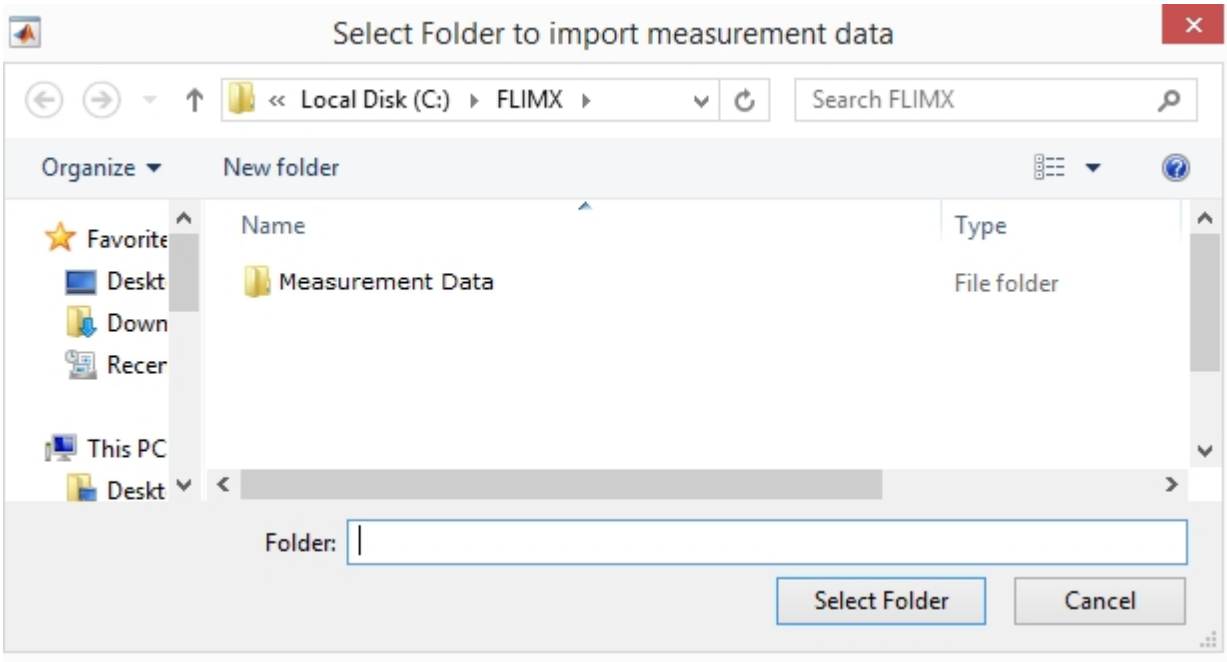
Open FLIMX Study Manager and select a study. To add measurement data for a single subject, select this subject. To add multiple measurements for large studies, see below. Then,

Open menu item **Import Measurement** > **Selected Subject(s)**

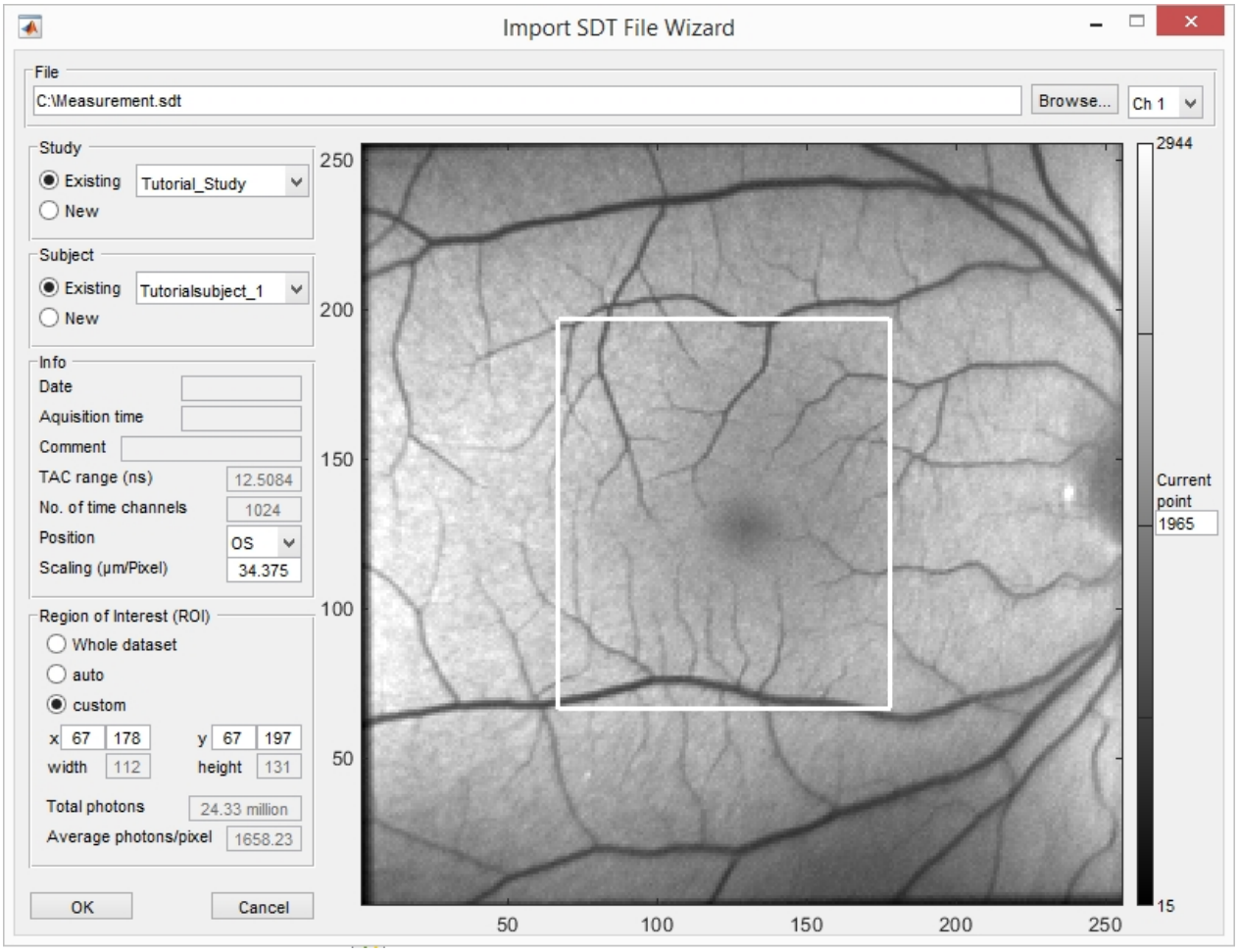


The following window will pop up, allowing you to browse your files. Select your measurement data and use **Open** to load the file or **Cancel** to close without importing data.

FLIMX uses *.sdt data format for measurement data.



The Import File SDT Wizard GUI (graphical user interface) will open as seen below.



Import File SDT Wizard is separated into the following sections:

- Study
- Subject

- Info
- ROI (region of interest)

See below for more information.

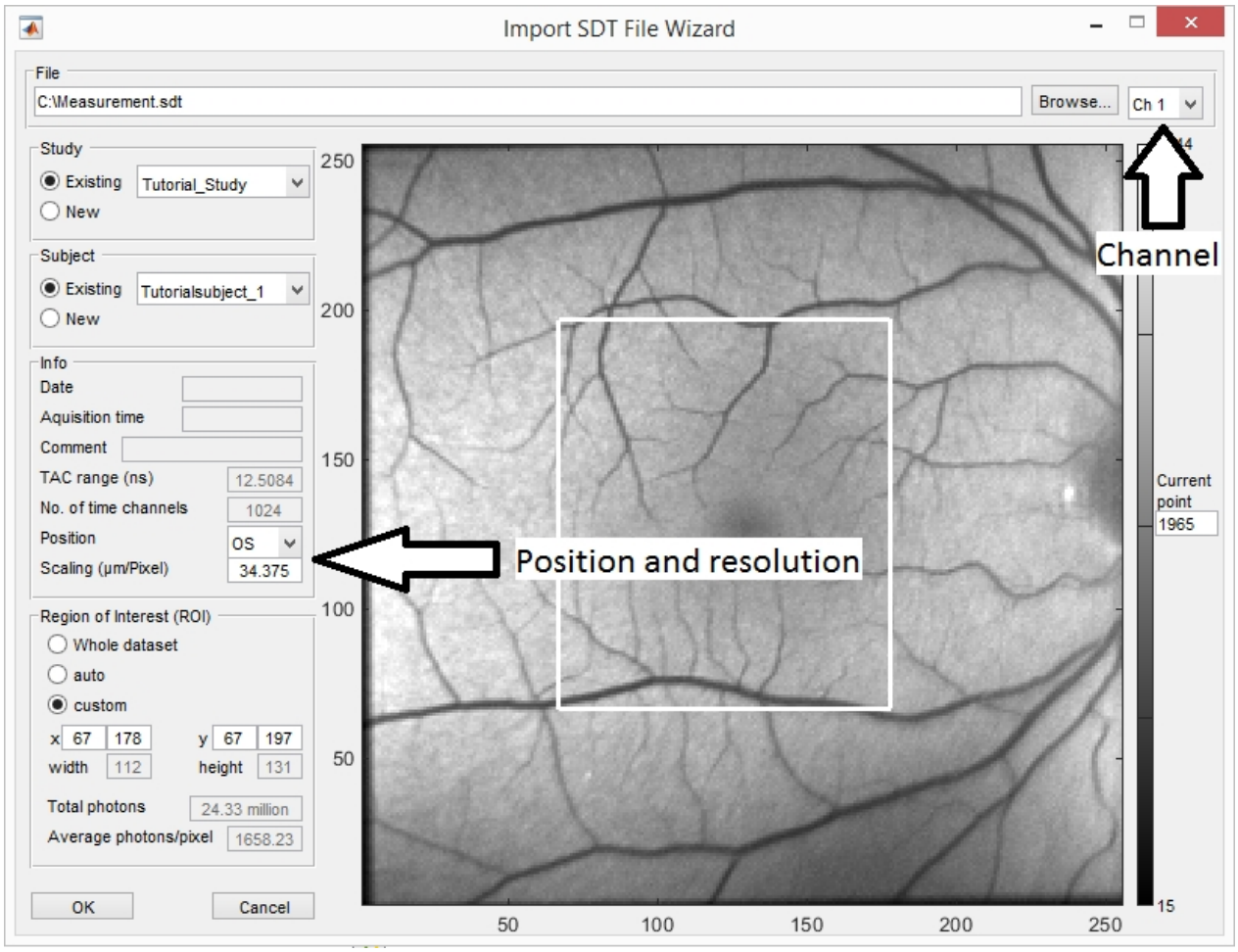
Study and Subject
The study name (here: Tutorial_Study) and subject name (here: Tutorialsubject_1) are displayed on the top left.

- **Select study (subject):** Click on the arrow next to your current study (subject). A list showing all of your studies (subjects) will be shown. Click on a study (subject) to select it.
- **New study (subject):** To assign the imported measurement to a new study (subject), click on the **New** button and type a name in the field.

Info

- **Date, Aquisition Time, Comment, Number of Time Channels:** If your measurement data contains further information, this information will be shown.
- **Photons:** On default, the total and average number of photons for the whole picture are shown in the info subsection. The average number indicates the average amount of photons per pixel.
- **TAC range:** TAC (time to amplitude conversion) range in ns represents the reciprocal of the stimulus laser repetition rate. The 12.5 ns seen in the window below correspond to a stimulus laser repetition rate of ~80 MHz.
- **Position:** Specify position of the eye by choosing OD (right eye) or OS (left eye).
- **Resolution/Scaling:** It describes the relationship between µm and pixel. In the picture below, one pixel consists of 34x34 µm². This parameter may be manually acquired from your measurement device.
- **Time channel:** Select the correct channel in the upper left corner of the File Import Wizard window

Options for position, resolution and channel are indicated by arrows in the picture below.



ROI

ROI options are indicated by a red frame in the picture below. Use this option to compute the total and average number of photons within a region of interest (ROI). The width and height of the ROI in pixel is shown at the bottom.

For manual selection,

Select **custom** in the ROI section. Type in the x and y coordinates in the lower part of the window or create a ROI by moving your cursor over the image and selecting a rectangle.

For automatic selection,

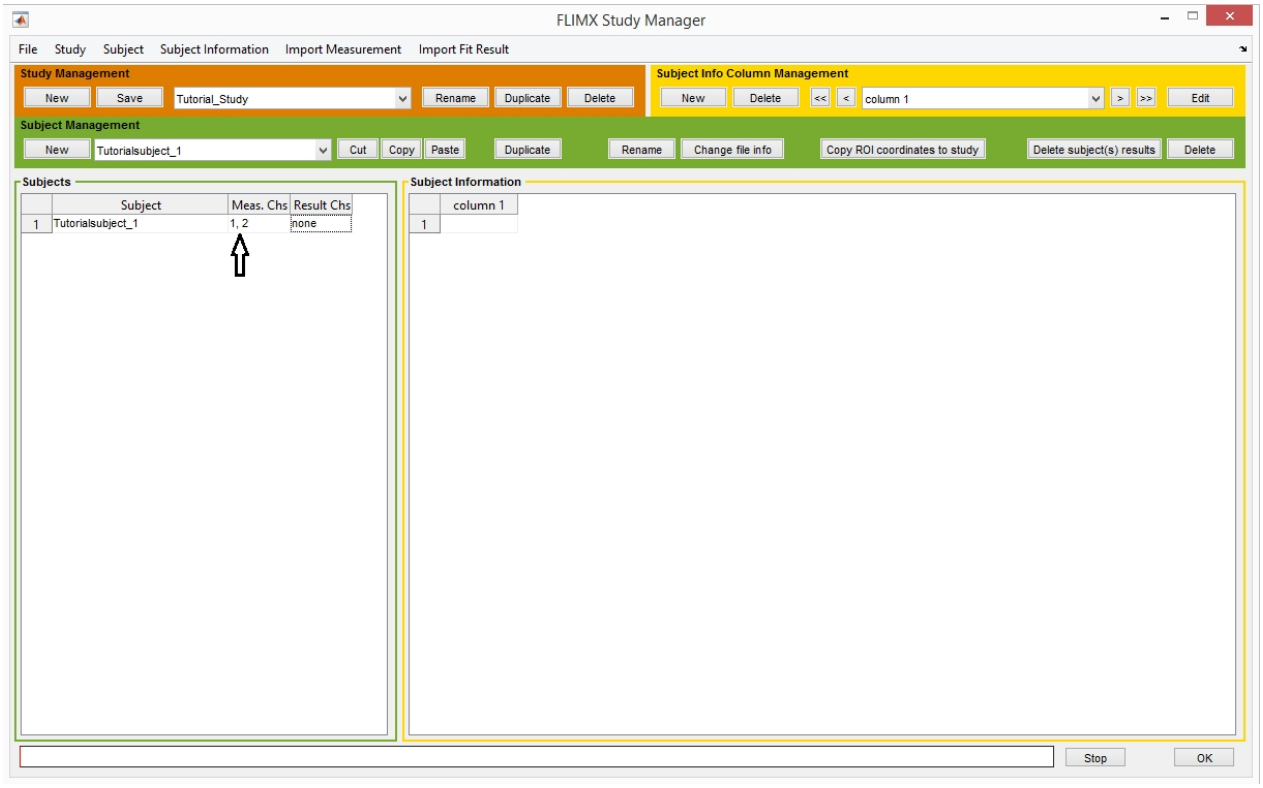
Select **auto** in the ROI section

The automatic ROI selection ensures that only pixels which do contain information will be selected. In the picture seen below, automatic ROI selection will exclude the black frame which can be seen on the left and lower edge.

After choosing a ROI, select **OK** to close the Import Wizard.

Import

After finishing import, the measurement data will be listed in your study manager as indicated by the arrow in the picture below.



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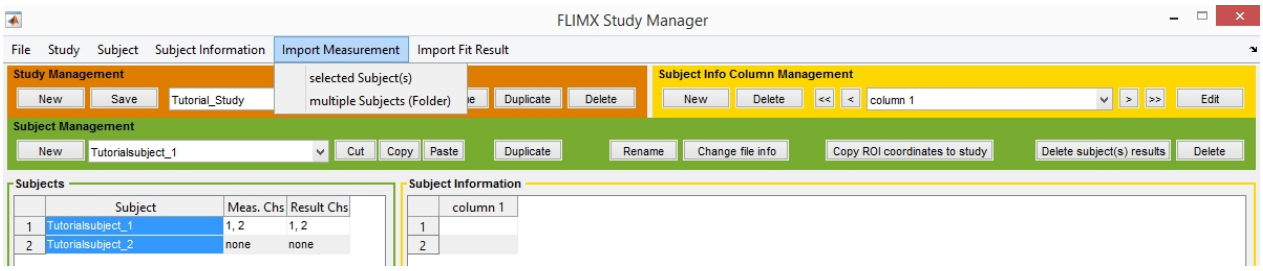
Import data for multiple subjects

If you need to import data for a large study, it might be more convenient to import the whole folder instead of importing data separately for each subject. However, if your images contain borders or defects that you wish to remove, you should use single import. Import of multiple subjects applied automatic ROI definition to each subject.

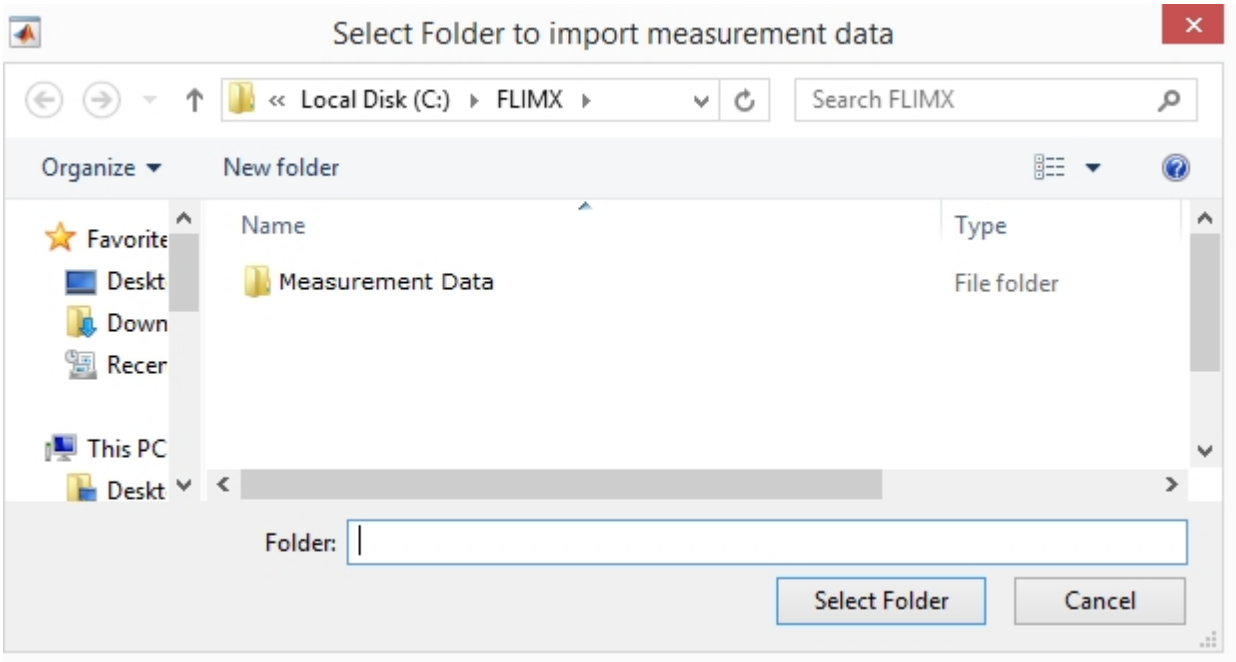
For multiple import, open FLIMX Study Manager and select a study. Then

Open menu item **Import Measurement** > **Multiple Subjects**

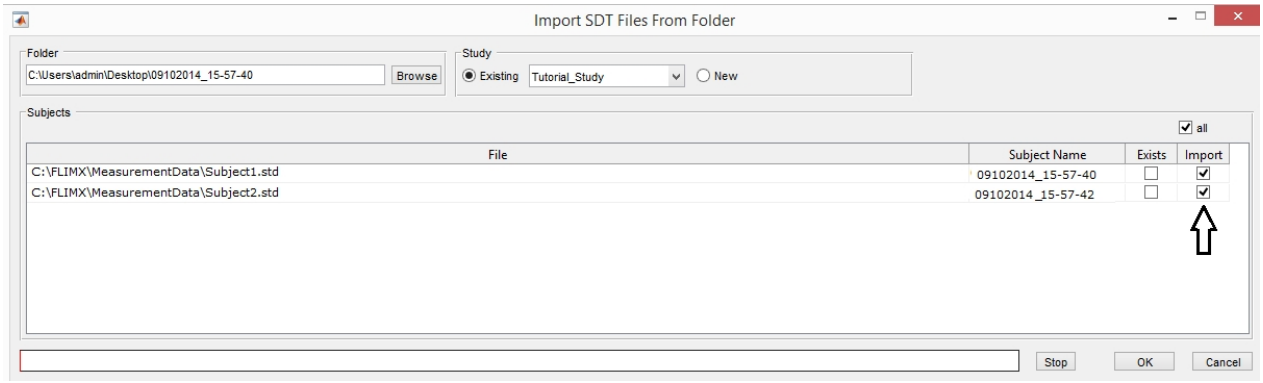
as seen in the picture below.



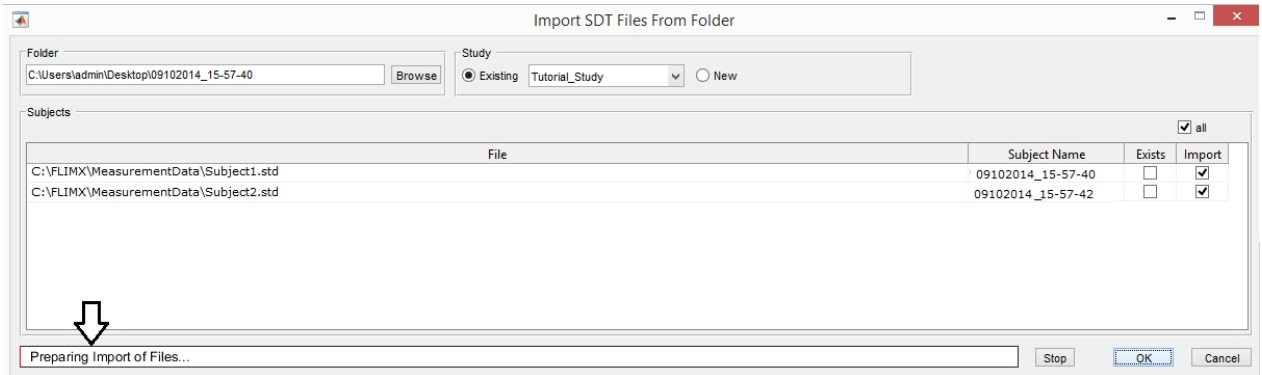
The following window will pop up, allowing you to browse your files. Select the folder containing your measurement data and use **Open** to load the folder or **Cancel** to close without importing data.



The "Import SDT Files From Folder" window will open. Tick the **import** boxes on the right to select files that you wish to import. In the picture below, these boxes are marked by an arrow. If you want to import all files within this folder, tick the box next to **all** on the top right corner of the window. Click **OK** to import these files or **Cancel** to close the window.

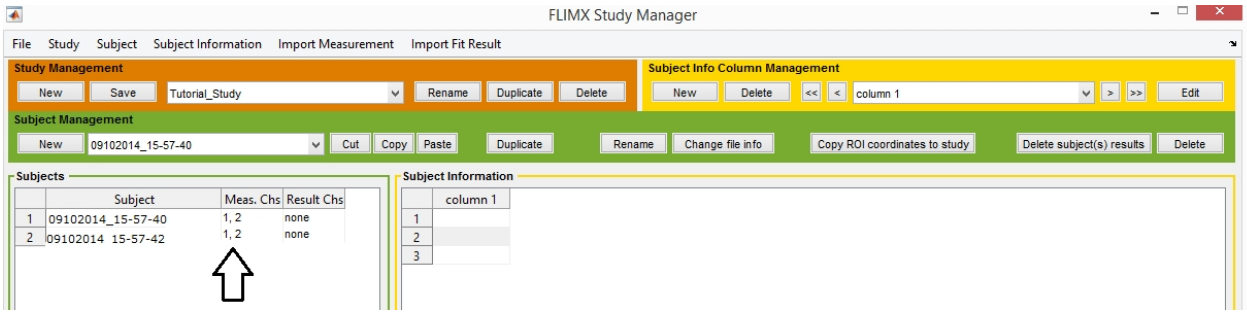


While importing the files, progress in shown on the bottom of the window as indicated in the picture below. Wait for this progress to finish.



The import window will close automatically and measurement data will be listed in your study

manager as indicated by the arrow in the picture below.



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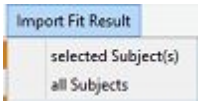
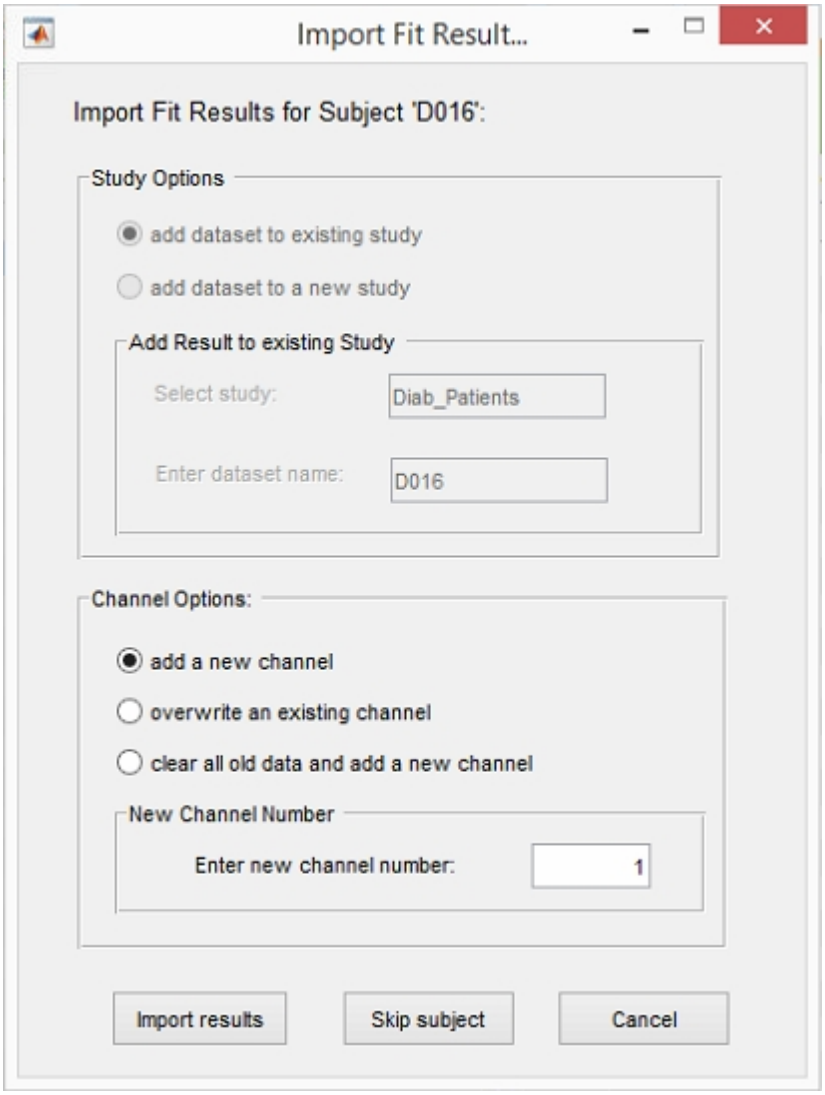
Import Fit Result

Import Fit Results

When clicking on this menu item, a window opens with two options: selected Subject(s), all Subjects



selected Subject(s): the FLIMXVis GUI opens. See also: FLIMXVis
The Import Fit Results window opens:



This window shows that the Fit Results are imported for the selected subject. The Study Options organizes where to import the data. The Channel Option is more important for existing studies with compiled data. Add a new channel for new data beside the already compiled data. Overwrite an existing channel erases the old data and writes the new data into this channel. Clear the old data and add a new channel erases every data and channel. All channels are required to have identical image sizes.

all Subjects follows the same principle as selected Subject(s) but for all subjects at once.

Tutorial

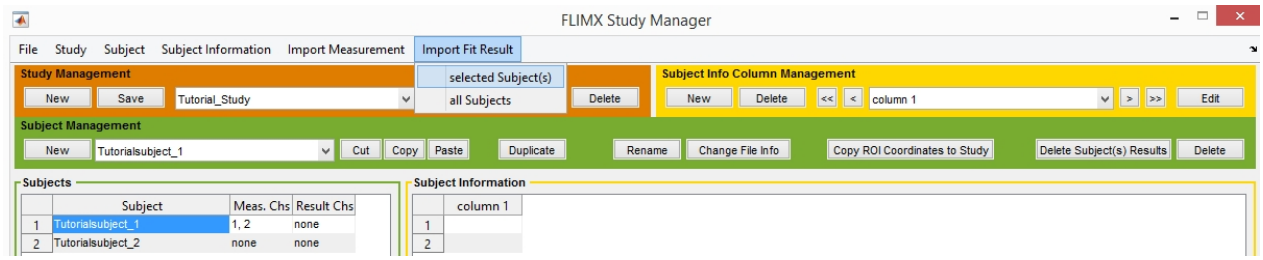
Within this section, you will learn to import fit data for

- Single subject or
- All subjects.

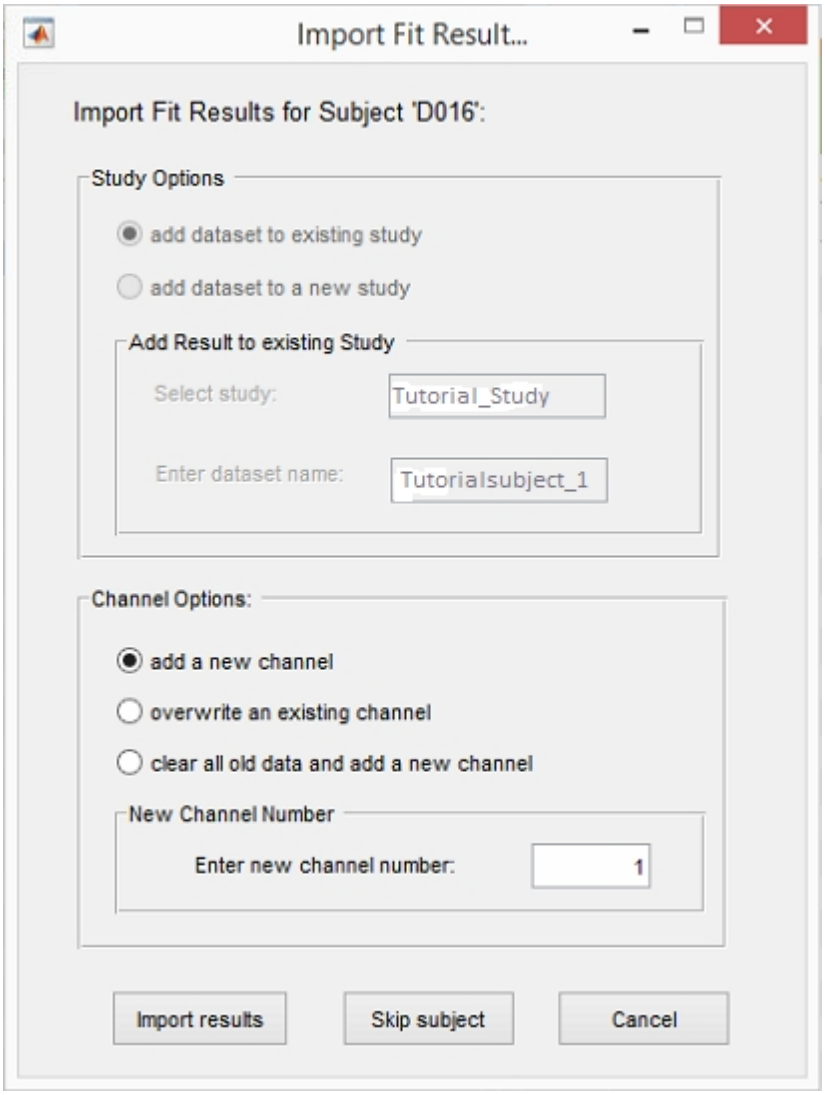
Import fit data for a single subject

Open FLIMX Study Manager and select a study. To add fit results for a single subject, select this subject. To add multiple results from large studies, see below. Then,

Open menu item **Import Fit Result** > **Selected Subject(s)** .



The following window will pop up:



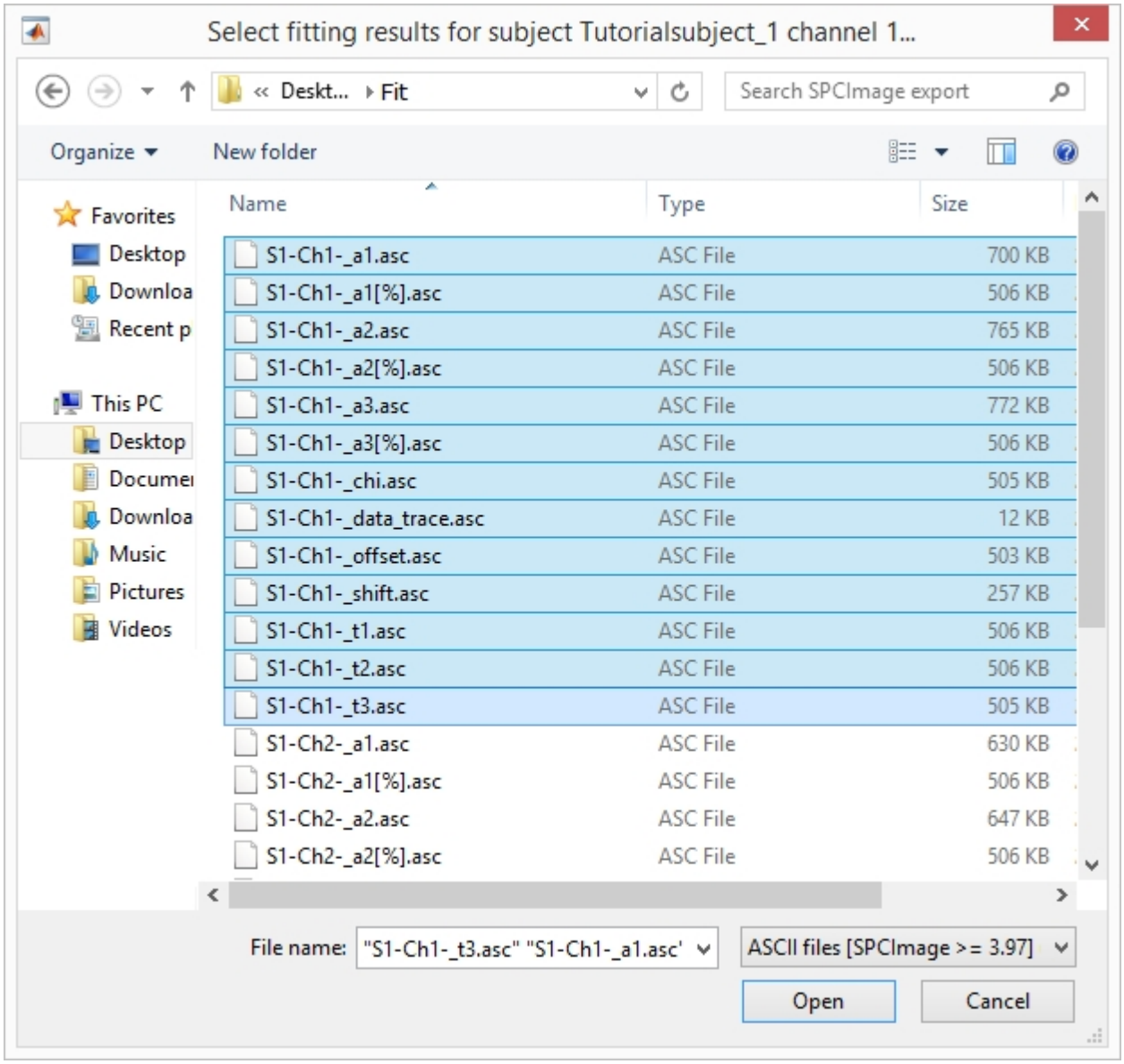
If your subject data contains more than one channel, enter the number of the current channel at the bottom of the window and click **Import Results**.

A window will open allowing you to browse your files. FLIMX uses ASCII files for fitting results. As seen below, the fitting results consist of amplitude (a) and time (t) parameters. Corresponding amplitude and time parameters must be imported simultaneously, otherwise an

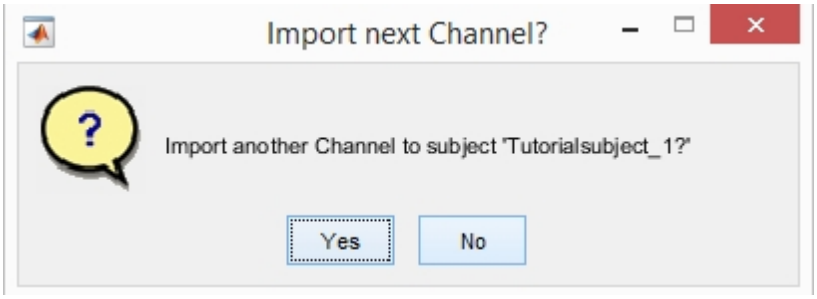
error will occur.

In the picture seen below for example, S1_Ch1-_a1.asc (Channel 1, Amplitude 1) and S1_Ch1-_t1.asc (Channel 1, Time 1) must be imported simultaneously. Additional FLIMX parameters, such as offset and shift, might be selected.

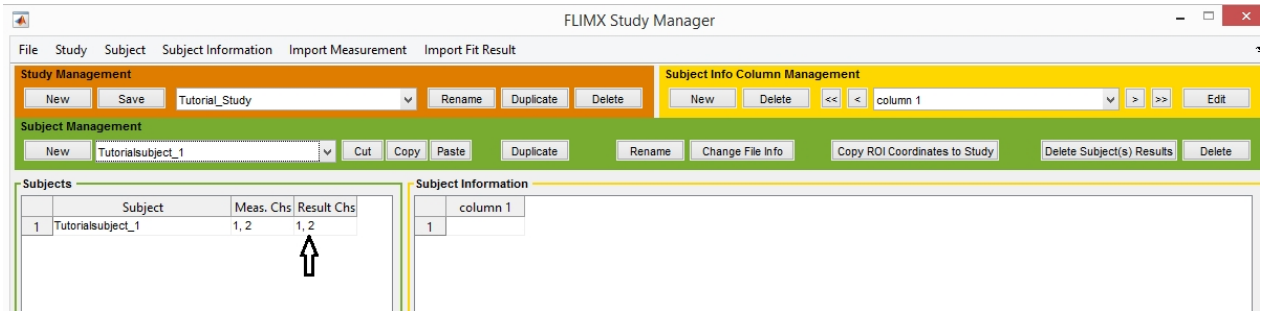
After selecting your fitting results for the current channel use **Open** to load the file.



A window asking you to import another channel will pop up. For multiple channels, click **Yes** and repeat the import routine. For a single channel or after importing all your channels, click **No**.



After finishing import, the fit results will be listed in your Study Manager as indicated by the arrow in the picture below.



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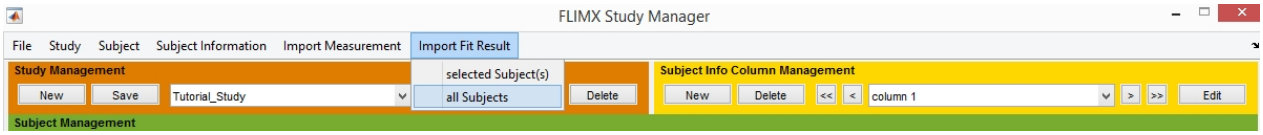
Import fit data for all subjects

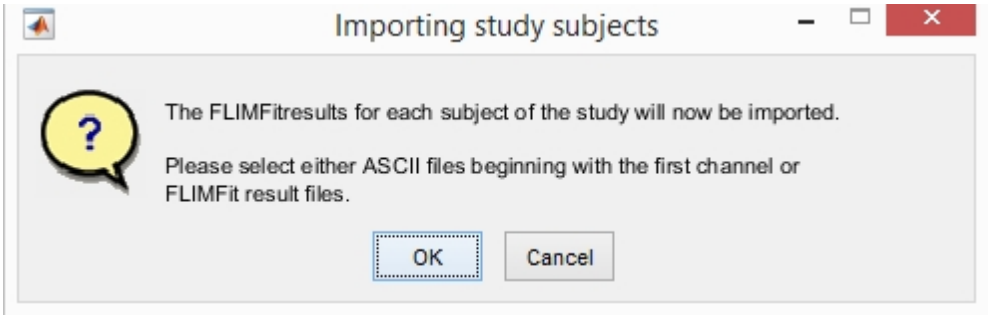
If you need to import data for a large study, it might be more convenient to import the whole folder instead of importing data for each subject. To do this, open FLIMX Study Manager and select a study. Then,

Open menu item **Import Fit Result** > **all Subjects**

as seen in the picture below.

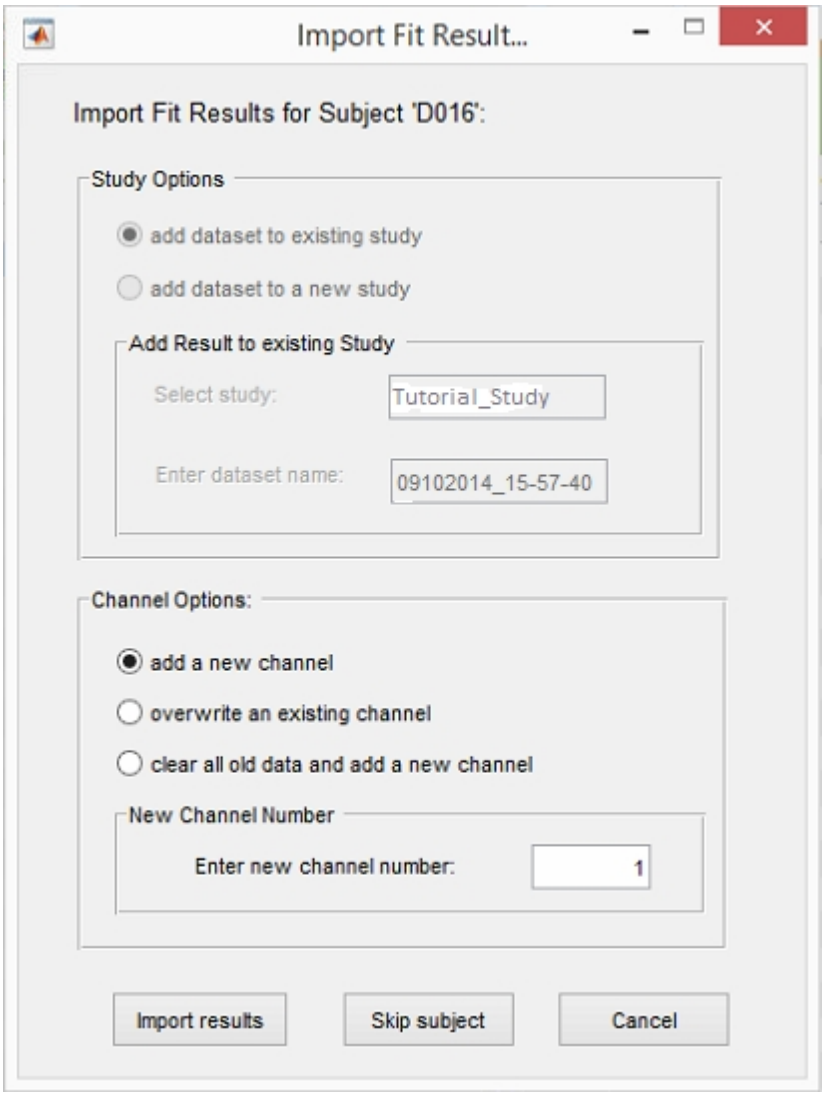


The following window will pop up. Click **OK** to continue.



The following window will open up. In this view, you can select fit results for each subject. If

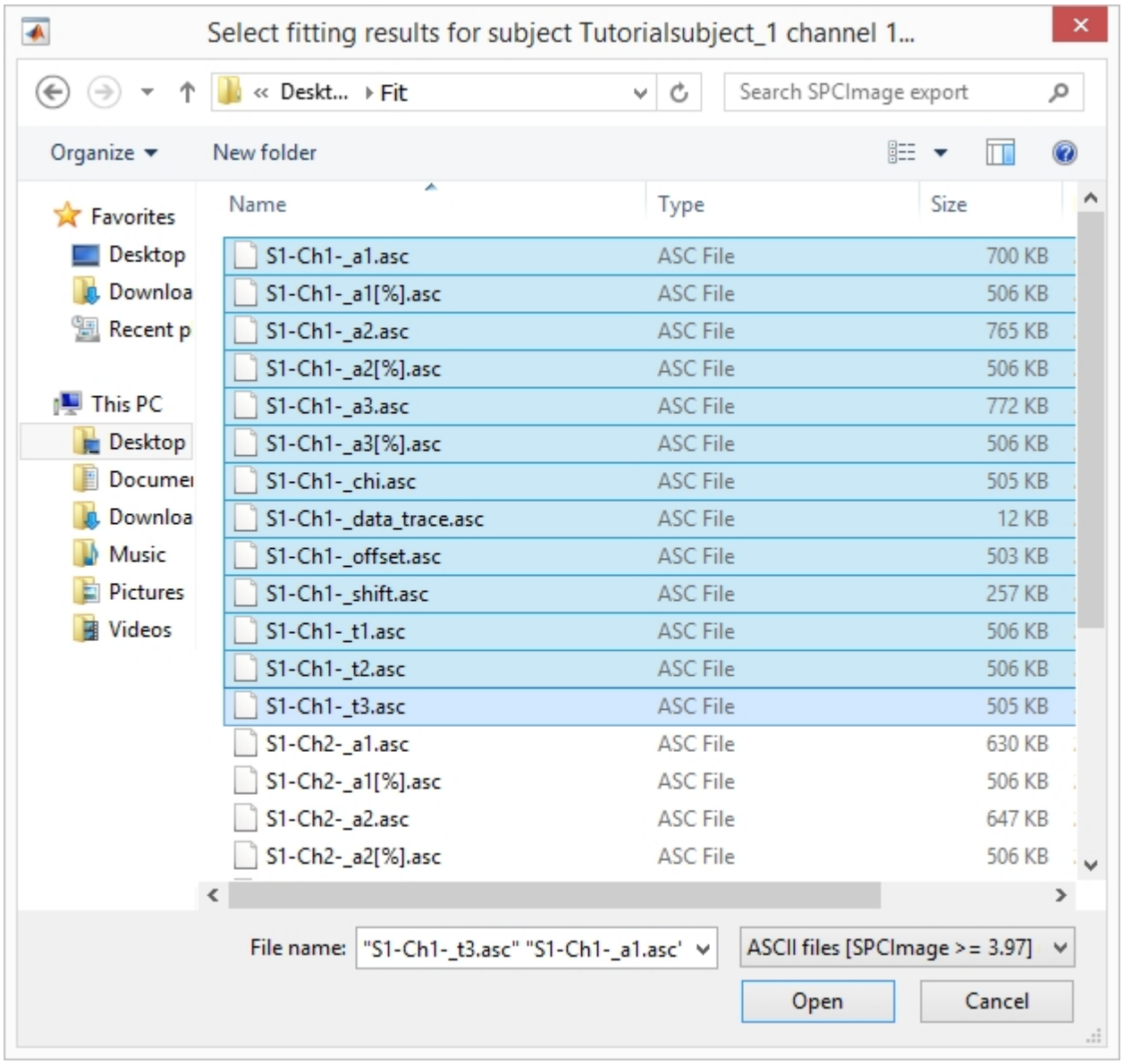
your subject data contains more than one channel, enter the number of the current channel at the bottom of the window and click **Import Results**.



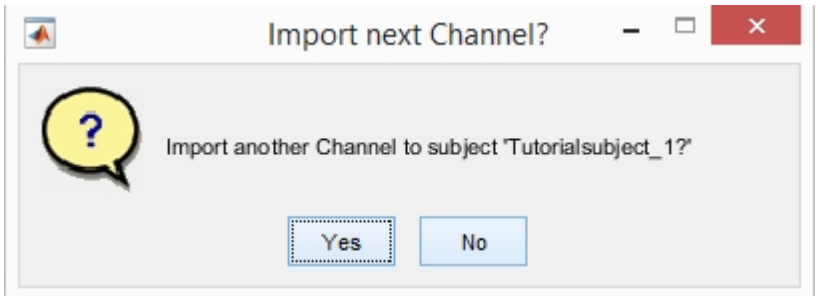
A window will open allowing you to browse your files. FLIMX uses ASCII files for fitting results. As seen below, the fitting results consist of amplitude (a) and time (t) parameters. Corresponding amplitude and time parameters must be imported simultaneously, otherwise an error will occur.

In the picture seen below for example, S1_Ch1-_a1.asc (Channel 1, Amplitude 1) and S1_Ch1-_t1.asc (Channel 1, Time 1) must be imported simultaneously. Additional FLIM parameters, such as offset and shift, might be selected.

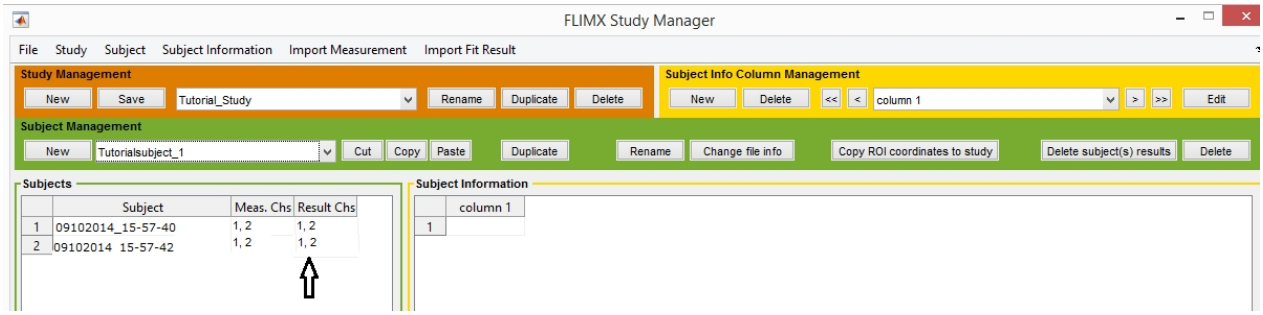
After selecting your fitting results for the current channel use **Open** to load the file.



A window asking you to import another channel will pop up. For multiple channels, click **Yes** and repeat the import routine. For a single channel or after importing all your channels, click **No**.



Repeat this routine until results for all of your subjects have been imported. After finishing import, the fit results will be listed in your Study Manager as indicated by the arrow in the picture below.



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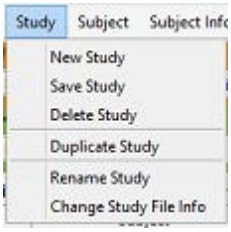
Study Management

Study Management

With one click on Study a window opens with six options: New Study, Save Study, Delete Study, Duplicate Study, Rename Study and Change Study File Info.



New Study inserts a new study into the study manager. The name for the new study can be written into the text box and confirmed. It is not possible to enter the same name for different studies, each study must have a unique name.

Save Study saves the changes of study to the hard disk.

Delete Study deletes the current study permanently. A confirmation of the deletion is needed.

Duplicate Study duplicates the opened study. This is helpful e.g. to apply different approximation methods to the same measurements. Like in New Study a unique study name has to be entered.

Rename Study sets a new name for the current study. Like in New Study a unique name has to be entered.

As explained above, the Study Management provides functions to organize the studies. Different to the tab "Study" in the Menu Bar the Study Management window only provides five options and one additional function:

The options "New", "Save", "Rename", "Duplicate" and "Delete" are equivalent to the options explained in above.

With the box in the middle of the Study Management the different applied studies can be chosen. With clicking on the arrow these studies are shown and the suitable one can be chosen by clicking on the name of the study.

If there are no other studies to choose the arrow becomes gray and the function is unusable.

Tutorial

In this section, you will learn how to

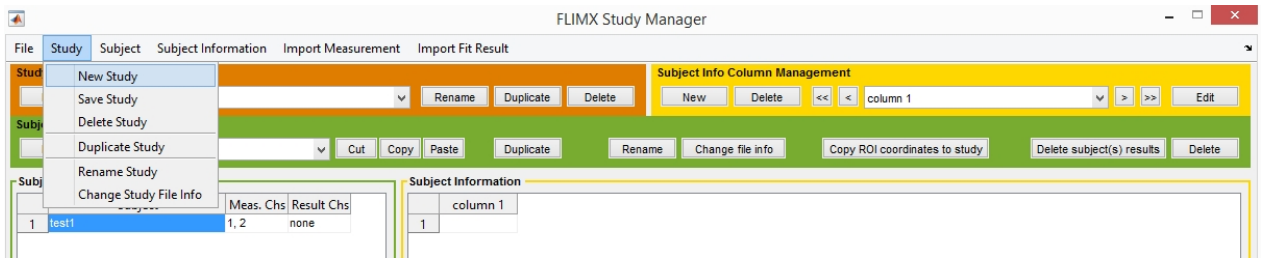
- Create a new study or
- Select a study from your existing studies

using FLIMX Study Manager.

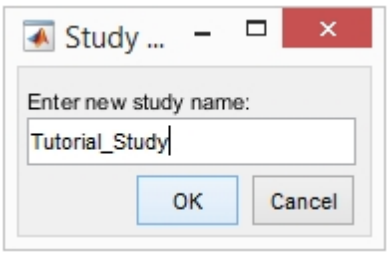
Create a new study

To create a new study,

Open menu item **Study** > **New Study**

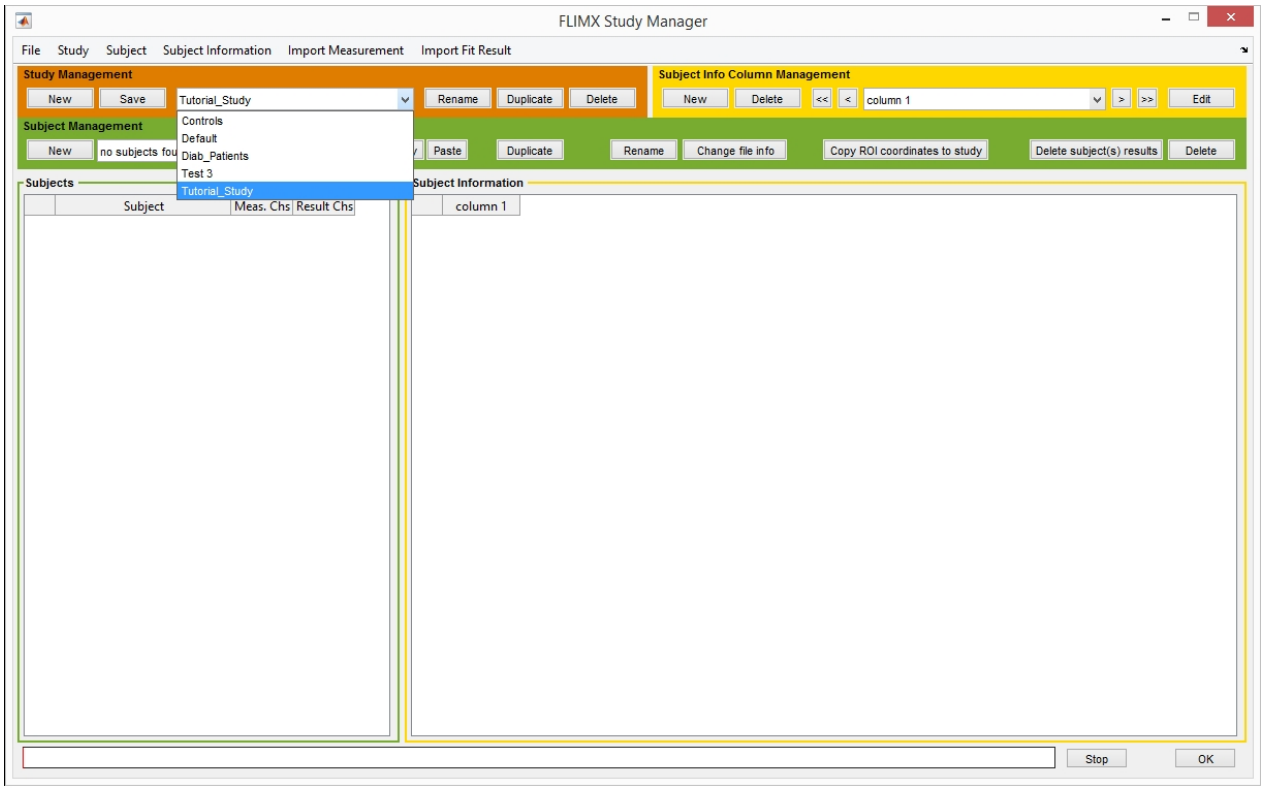


The following window asking you for the name of the new study will pop up:



Type in the name and press **OK**. Here, we named our study "Tutorial_Study". The new study will appear under the section Study Management (orange bar) as seen below.

You may rename, duplicate and delete the study by pressing the buttons arranged on the left of your current study or via menu item **Study**.



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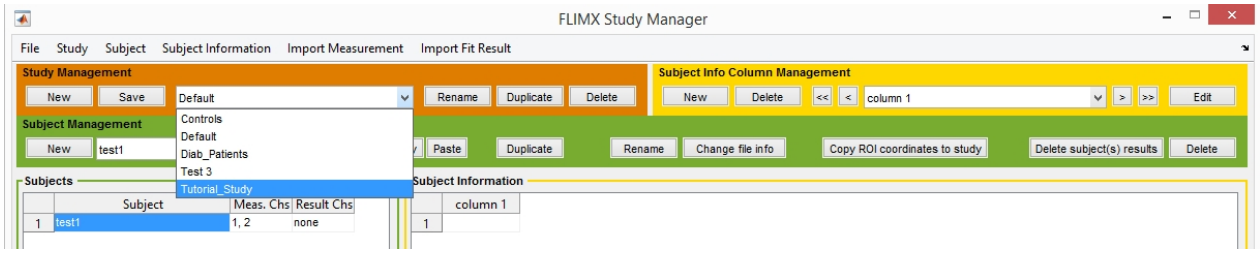
Select an existing study

To select a study, click on the arrow next to your current study. The names of your existing studies will show up. Select a study by clicking on it.



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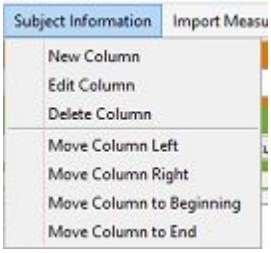
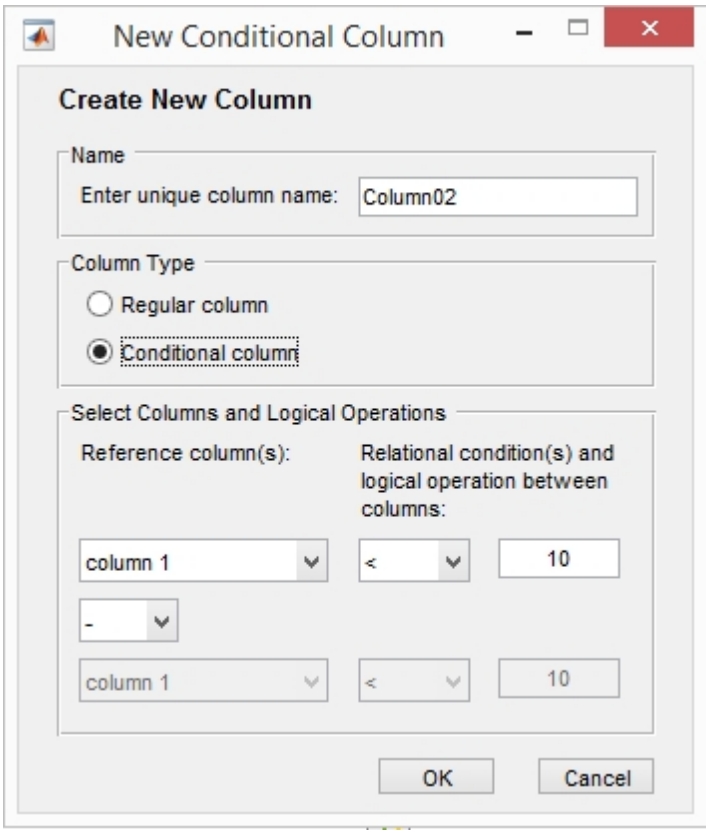
Subject Info Column Management

Subject Info Column Management (Subject Information)
With clicking on this menu item a window opens with seven options: New Column, Edit Column,

Delete Column, Move Column Left, Move Column Right, Move Column to Beginning and Move Column to End.



New Column creates a new column in the *Subject Information* window. The following window opens:



The name of the column can be written in the text box. It is advisable to give a descriptive name for each column.
A regular column has no special conditions or any connection to other columns, it just stores data. A conditional column evaluates a relational operation (<, ≤, =, ≠, ≥, >) on a specific column. The relational operation can be combined with a relational operation on another column using a logical operation (AND, NOT AND, OR, NOT OR). Both relational operations are independent. The targeted columns are independent and can be regular columns with numerical data (no strings!) or conditional columns. Thus, conditional columns can be nested with an infinite number of conditional columns.
New columns always appear at the right end of the *Subject Information* window. A more detailed tutorial is given below.

Edit Column opens the same window as explained in New Column. The selected and already existing column can be changed and adapted.

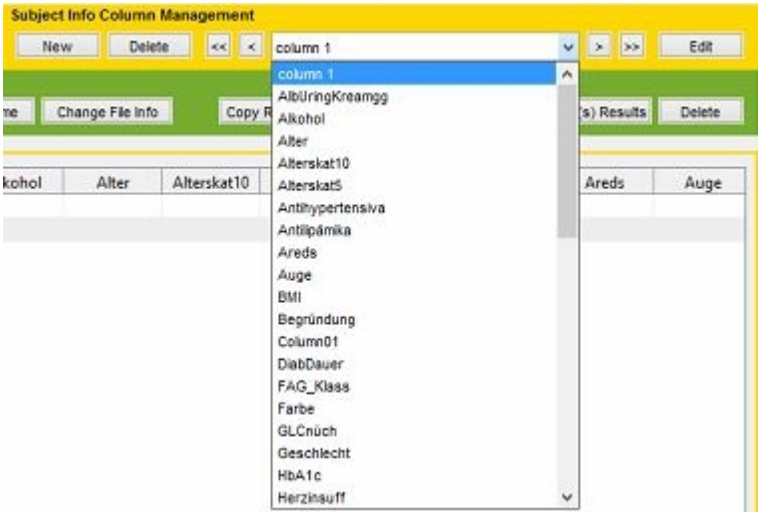
Delete Column deletes the selected column with all entries of all subjects at once.

Move Column helps to arrange the columns. Four different moves are available: Left, Right, to Beginning, to End. Left and Right moves the selected column only one position in the respective direction. If there is another column this will be moved to the place from which the selected column comes from. To Beginning / to End moves the selected column to the left end side / the right end side of the Subject Information window. The following columns are placed one position to the right / left.

As explained above the Subject Info Column Management provides functions to organize the information of the subjects/study participants. Different to the tab "Subject Information" in the Menu Bar the Subject Info Column Management window provides all seven options and one additional function:

The options "New", "Delete", "Edit" and "Move Column" are equivalent to the options explained above.
The arrows represent the Move Column option. Thereby the selected column is the column shown in the box between the arrows.
"<<": Move to Beginning
"<": Move Left
">": Move Right
">>": Move to End

With the box in the middle of the Subject Info Column Management the different applied columns can be chosen. With clicking on the arrow these columns are shown and the suitable one can be chosen by clicking on the name of the column.



If there are no other columns to choose the arrow becomes gray and the function is unusable.

Tutorial

FLIMX offers you the possibility to add additional informations to your subjects, such as age, weight or diseases. After adding such information, you are able to select subjects from your

group which meet certain criteria. For example, you may select a subgroup which contains all subjects with an age above 60 and compare this subgroup to the whole study.
In this section you will learn how to

- Create a regular column
- Create a conditional column
- Create a linked conditional column

Study manager distinguishes regular columns and conditional columns. While information itself is stored in regular columns, conditional columns define the ranges for subgroups.

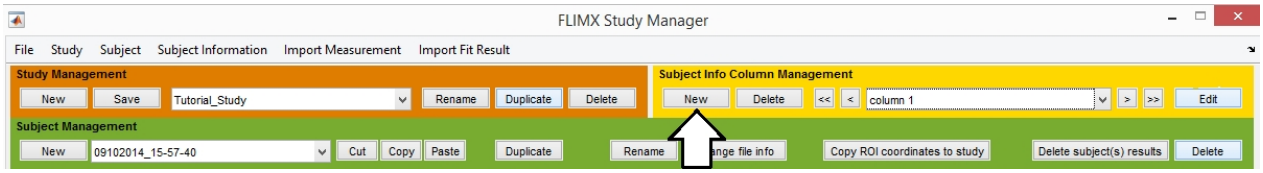
For example, a regular column may store the BMI while a conditional column defines all subjects with a BMI above 25 as adiposis group. Linked conditional columns help you to combine several conditions in one column.

In order to use conditional columns, you must first define the corresponding regular columns.

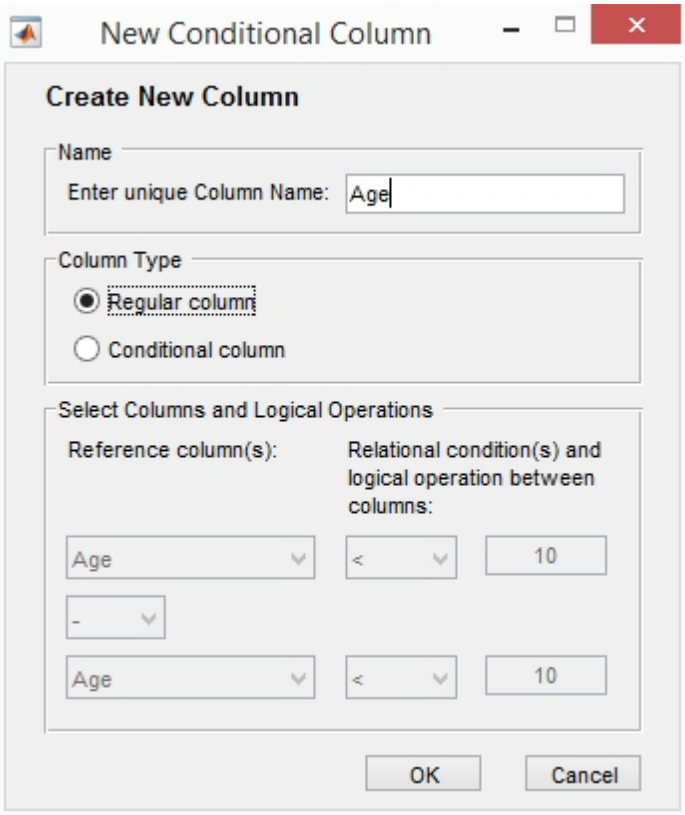
The options for information management are located within Subject Info Column Management (yellow bar) of your Study Manager.

Create a regular column

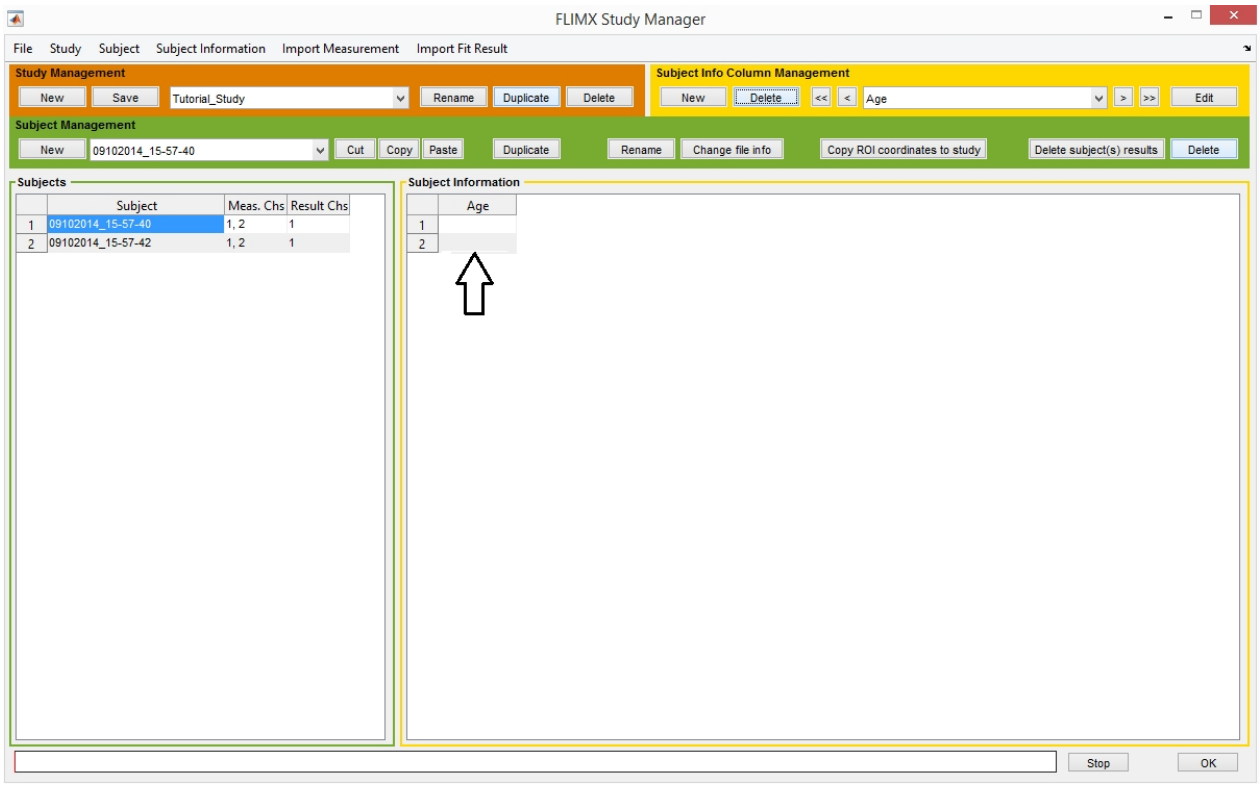
After adding subjects to your study, click on the **New** button in the Subject Info Column Management as indicated by an arrow in the picture below.



The following window will pop up. Type in a unique name for the column (here: Age) and click on **OK** to confirm or **Cancel** to close the window without saving. Here, we chose regular column. To define conditional columns, see below.

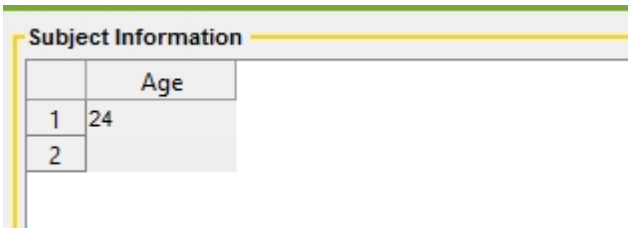


The column will now be displayed in the informations list as indicated by an arrow in the picture below.



The numbers correspond to the number of the subjects in the subjects list on the left. To add information for a subject, simply click on the corresponding column and type in the information.

In the example below, age 24 has been assigned to subject 1 which is labeled 09102014_15-57-40.



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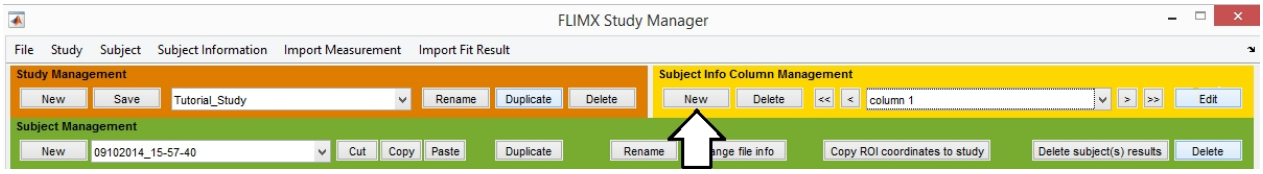
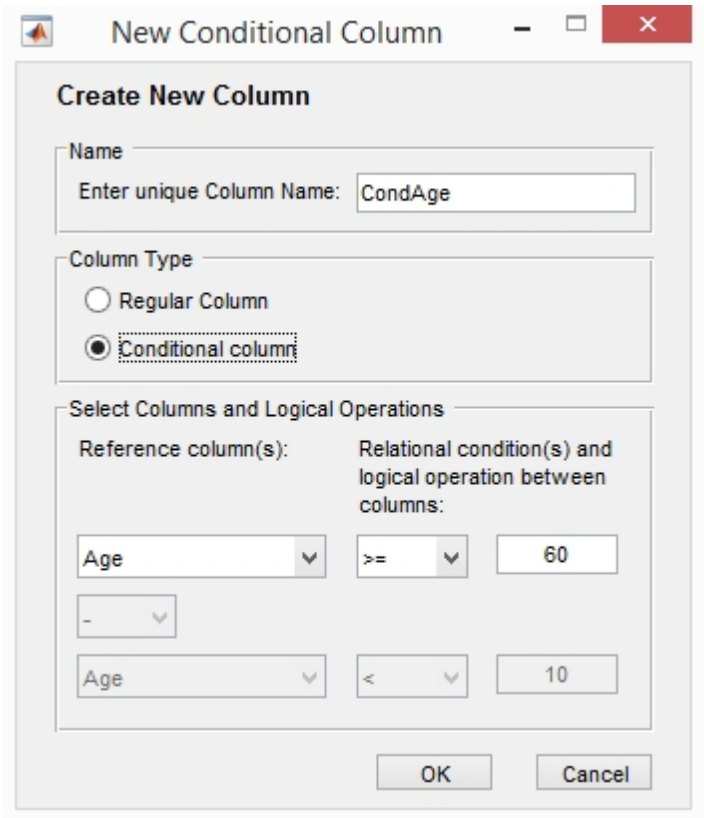
Create a conditional column

To define a new conditional column, click on the **New** button in the Subject Info Column Management as indicated by an arrow in the picture below. Note that you must first define a corresponding regular column. For the difference between regular and conditional columns, see above.



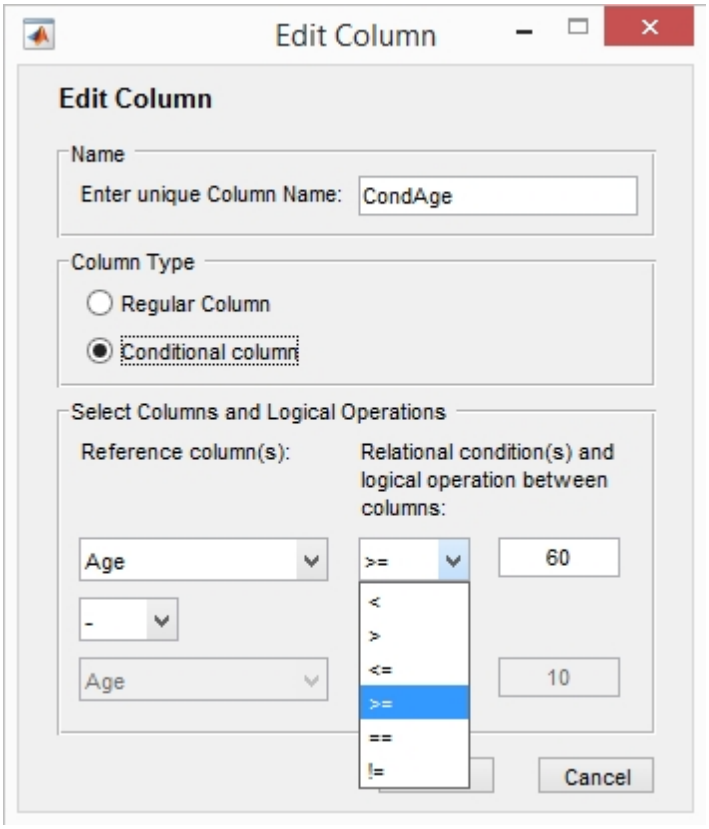
The following window will pop up. Type in a unique name for the column (here: CondAge) and select **Conditional Column** by ticking the corresponding box in the column type bar.



You can now select a reference column and logical operations for the relational condition. In the example, **Age >= 60** has been selected. All subjects with an age equal to or greater than sixty are now part of the subgroup **CondAge**.

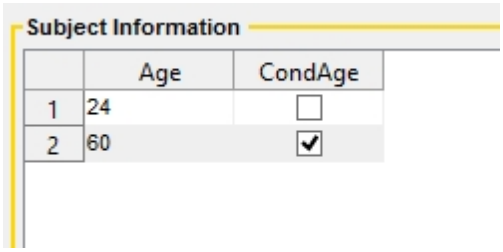
To select another regular column as reference column, click on the arrow next to your current column. A list with all your regular columns will show. Select a column by clicking on it. The same procedure applies for selecting a logical condition.
The logical conditions are:

- < : less than
- > : greater than
- <=: equal to or less than
- >=: equal to or greater than
- ==: equal to
- != : not equal to



Click on **OK** to confirm or **Cancel** to close the window without saving.

The new conditional column will now be displayed as seen in the picture below. Because subject 2 meets the criterion ">= 60", the corresponding box in the **CondAge** column has been automatically ticked. Repeat this routine to add more information to your subjects. To create linked or nested conditional columns, see below.



To edit or delete columns, click on the corresponding boxes within the Subject Info Column Management (yellow bar) of your Study Manager.

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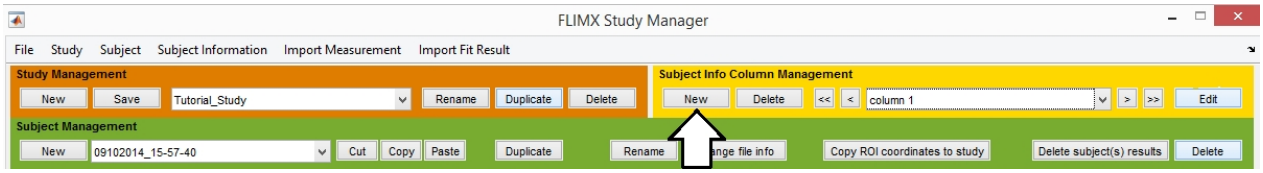
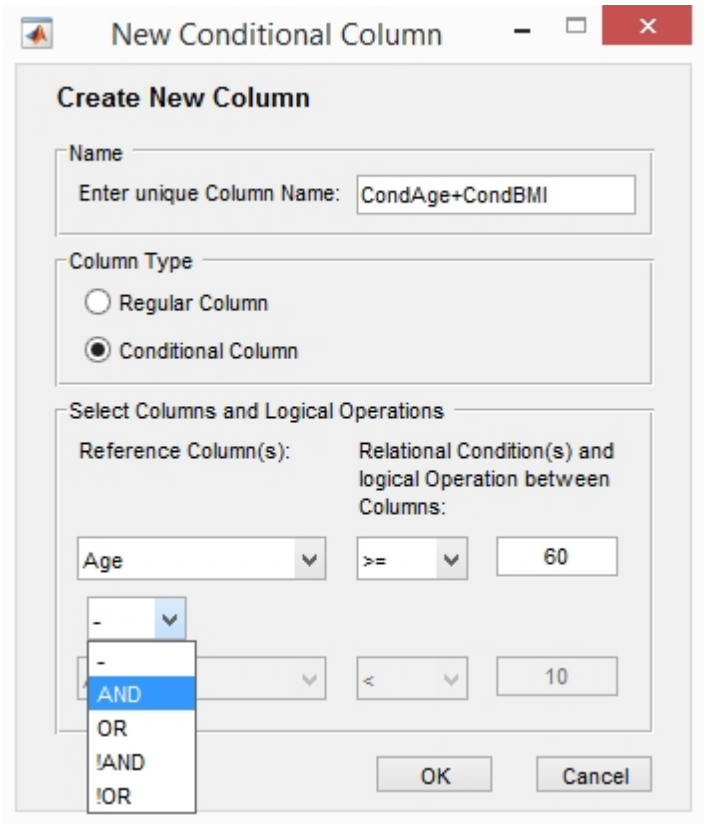
Create a linked conditional column

To define a linked conditional column, click on the **New** button in the Subject Info Column Management as indicated by an arrow in the picture below. Note that you must first define a corresponding regular column. For the difference between regular and conditional columns, see above.



The following window will pop up. Type in a unique name for the column (here: CondAge +CondBMI) and select **Conditional Column** by ticking the corresponding box in the column type bar.

In our example, we want to link the condition age greater than sixty with the condition BMI above 25. Select the condition **Age >= 60** as described above.



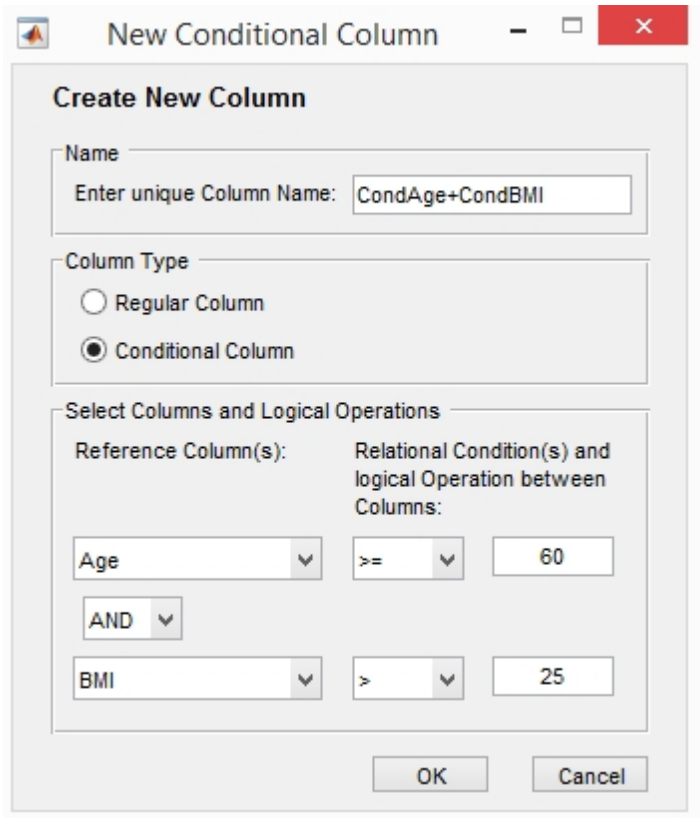
To link this condition with another condition, click on the arrow next to - . A list showing the logical operators will show. Select the appropriate operator by clicking on it.

The logical operators are:

- AND : logical and (condition A and B must be true)
- OR : logical or (condition A or condition B or both must be true)
- !AND : logical not and (condition A must be true, condition b not true)
- !OR : logical not or (condition A or condition B must be true)

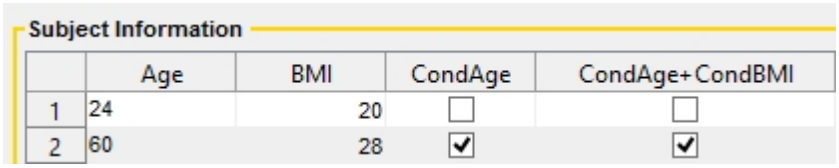
For the example CondAge+CondBMI, **And** is chosen. The lower bars will become visible, allowing you to chose a reference column and relational condition. You might as well define

upper and lower boundaries for your conditional column by using a AND condition and the same reference column twice. To nest linked conditional columns, use conditional columns as reference columns.



Click on **OK** to confirm or **Cancel** to close the window without saving.

The new conditional column will now be displayed as seen in the picture below. Because subject 2 meets the criterion "Age>= 60 AND BMI >25", the corresponding box in the **CondAge +CondBMI** column has been automatically ticked. Repeat this routine to add more information to your subjects.



To edit or delete columns, click on the corresponding boxes within the Subject Info Column Management (yellow bar) of your Study Manager.

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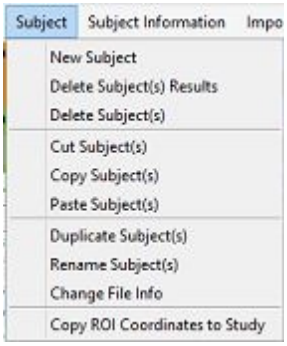
Subject Management

Subject Management

With clicking on Subject a window opens with ten options: New Subject, Delete Subject(s) Results, Delete Subject(s), Cut Subject(s), Copy Subject(s), Paste Subject(s), Duplicate Subject(s), Rename Subject(s), Change File Info and Copy ROI Coordinates to Study.



New Subject creates a new subject in the *Subjects* window. With clicking on New Subject a window opens. With entering a unique name the subject will be created. The numbering of the new subject is in alphabetical order. If using numbers instead of letters the numbers will appear above the letters. The length of the name is unlimited. The new subject has no information in the Measurement Channels or the Result Channels. Because of this the entry is "none". The subject does also appear in the *Subject Information* but without any entries.

Delete Subject(s) Result deletes all the entries of the column Result Channels. More subjects can be chosen by clicking on more subjects while holding the CTRL-button.

Delete Subject(s) follows the same principle as Delete Subject(s) Result. But this function deletes the whole subject with all entries and additionally deletes all subject information.

Cut Subject(s) allows to remove unwanted subjects out of the current study. The subject(s) will be saved in the buffer memory. They can be inserted into other studies and will be removed from the original study.

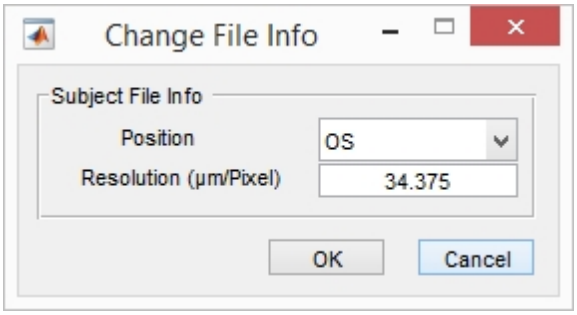
Copy Subject(s) allows to copy subjects. In this way the subject can be part of more than one study. The subject will be saved in the buffer memory.

Paste Subject(s) inserts all the subjects in the buffer memory into the selected study. The subject and its information will be transmitted to the new location.

Duplicate Subject(s) duplicates the selected subject. A different and unique name for the current study has to be entered. The rest of the subject's information are identical.

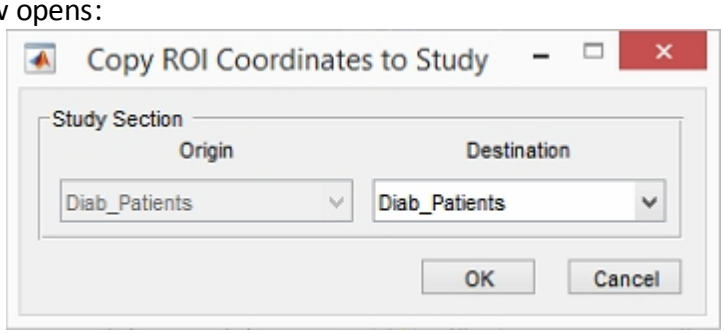
Rename Subject(s) gives the opportunity to set a new name for an existing subject. The new name has to be unique for the current study.

Change File Info opens the following window:



The Subject File Info provides two options. The position can be selected. OS stands for oculus sinister (left eye) and OD is selectable (oculus dexter = right eye).
The Resolution refers to the image shown in FLIMXFit. The scaling of a pixel in µm can be set here.

Copy ROI Coordinates to Study copies all ROI (Region of Interest) coordinates for all ROI types from the selected study into another. Existing ROI coordinates are overwritten! The following window opens:

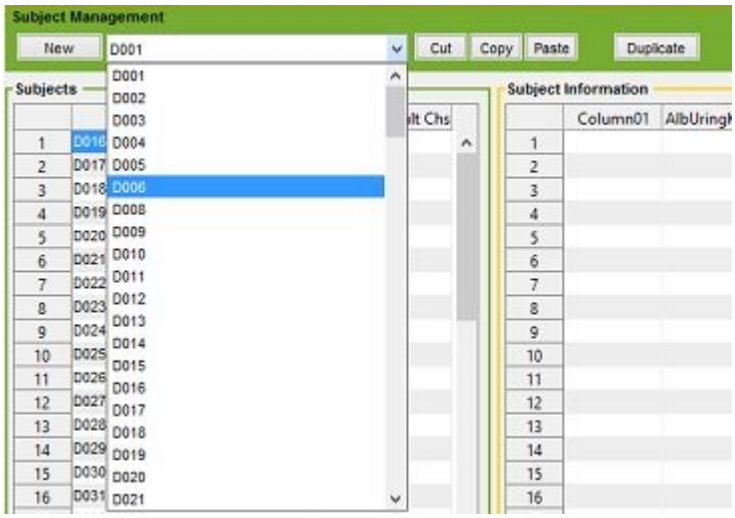


The Origin is the selected study which provides the coordinates. The destination is the study in which the coordinates shall be copied. The process needs a confirmation.

As explained above the Subject Management provides functions to organize the subjects/study participants. Different to the tab "Subject" in the Menu Bar the Subject Management window provides all the ten options and the additional function of the pop-up menu.

All options are equivalent to the options explained above.

With the box in the middle of the Subject Management the different applied subjects/study participants can be chosen. With clicking on the arrow these subjects are shown and the suitable one can be chosen by clicking on the name of the subject.



If there are no other subjects to choose the arrow becomes gray and the function is unusable.

Tutorial

In this section, you will learn how to

- Add a new subject to your study or
- Select a subject from your existing subjects

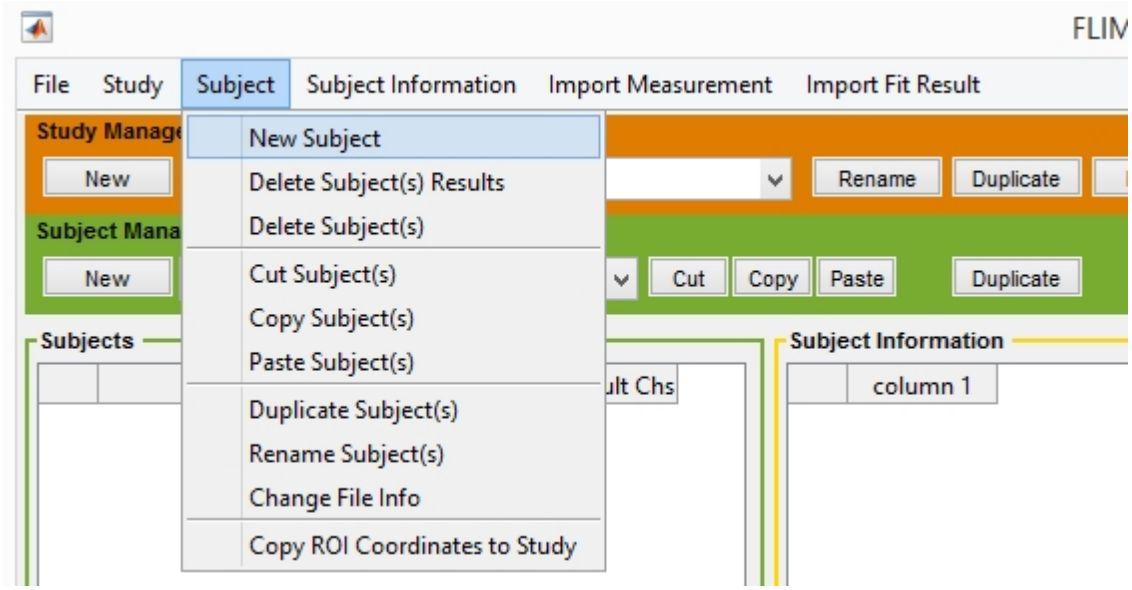
using FLIMX Study Manager.

Create a new subject

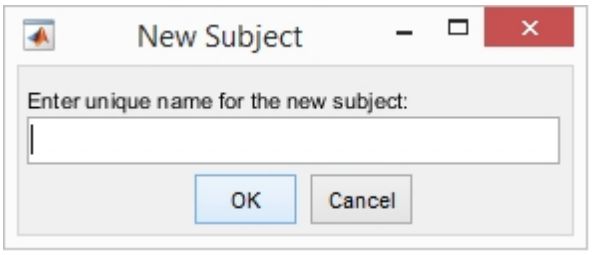
To create a new subject,

Open menu item **Subject** > **New Subject**

as seen below.



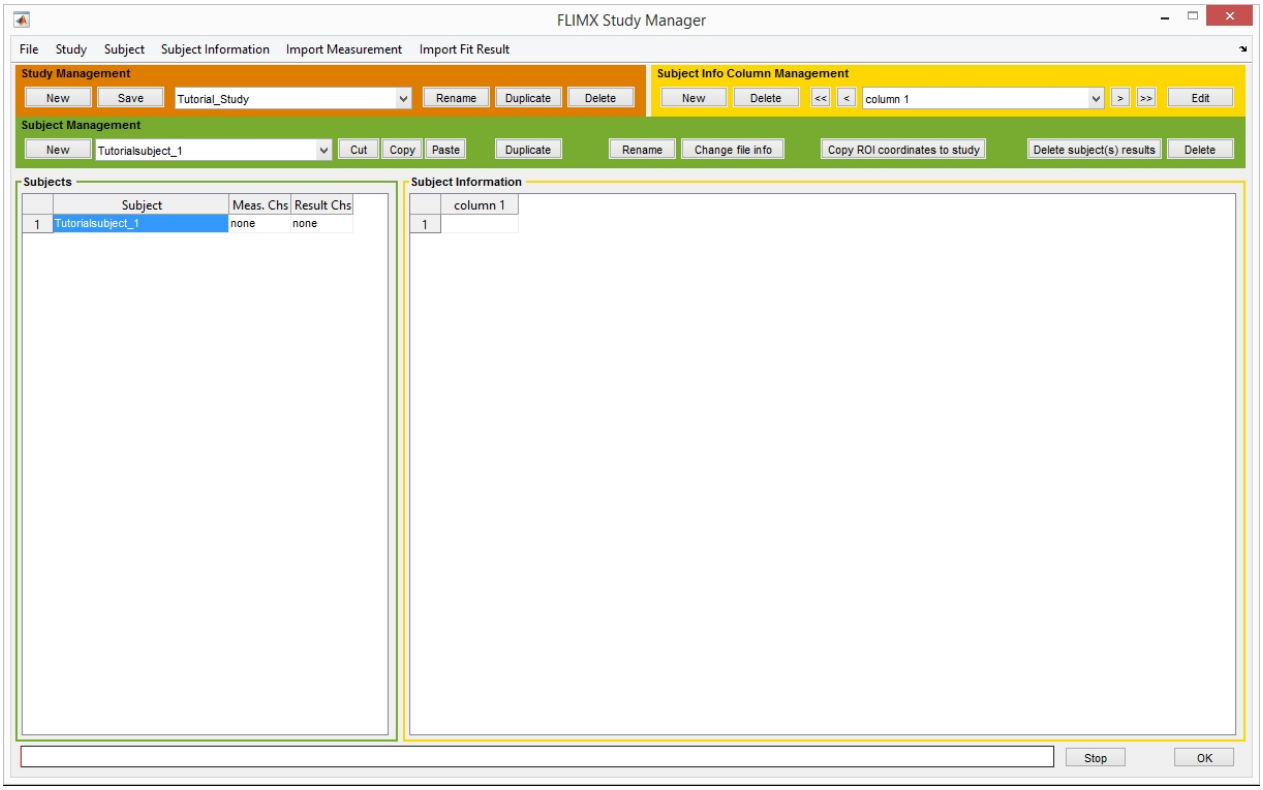
The following window asking you for the name of the new subject will pop up.



Type in the name. Here, we named our subject "Tutorialsubject_1". Click **OK** to save the name and close the window or **Cancel** to exit without saving.

The new subject will appear in the section Subject Management (green bar) as seen below as well as in the list of subjects.

You may cut, copy, paste, duplicate and rename the subject by pressing the buttons arranged on the left of your current subject or via menu item **Study**.



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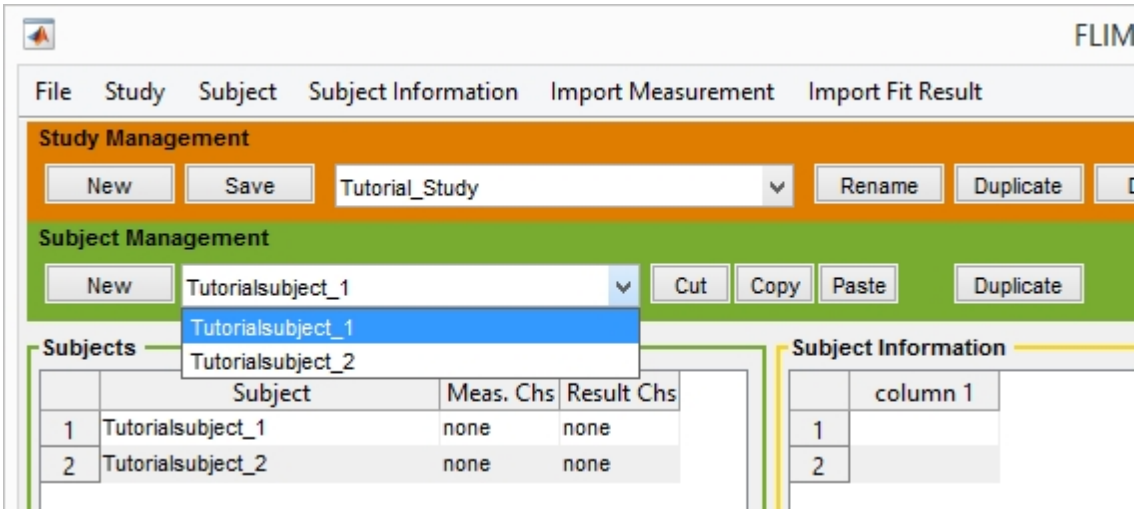
Select an existing subject

To select an existing subject from your current study, click on the arrow next to your current subject. The names of your existing subjects within the current study will show. Select a subject by clicking on it.



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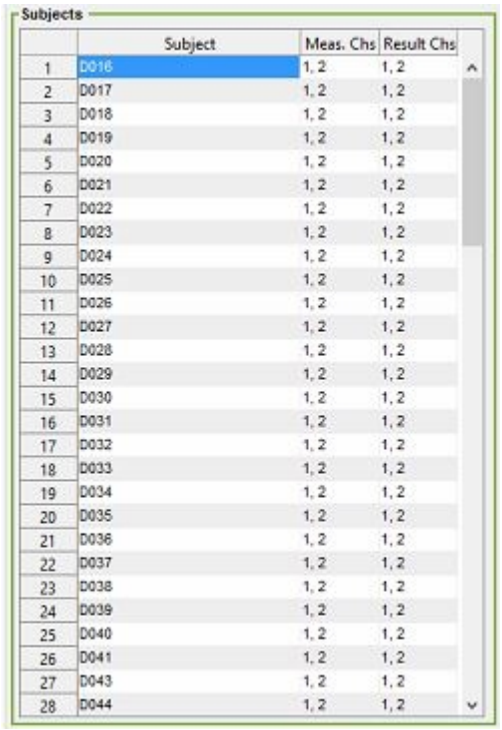
Subjects

The box Subjects gives an overview of all the subjects/study participants with their names in the column Subject, the measurement channels and the result channels.



The subjects are sorted in an alphanumerical order, distinguishing between lower and upper case. To create an entry in the measurement channel column the measurements have to be imported with the function Import Measurements as explained in *Menu Bar.* The same applies for entries in the result channel column. See also: Import Fit Results

With a right click within the box the window explained and shown in Subject Management opens.

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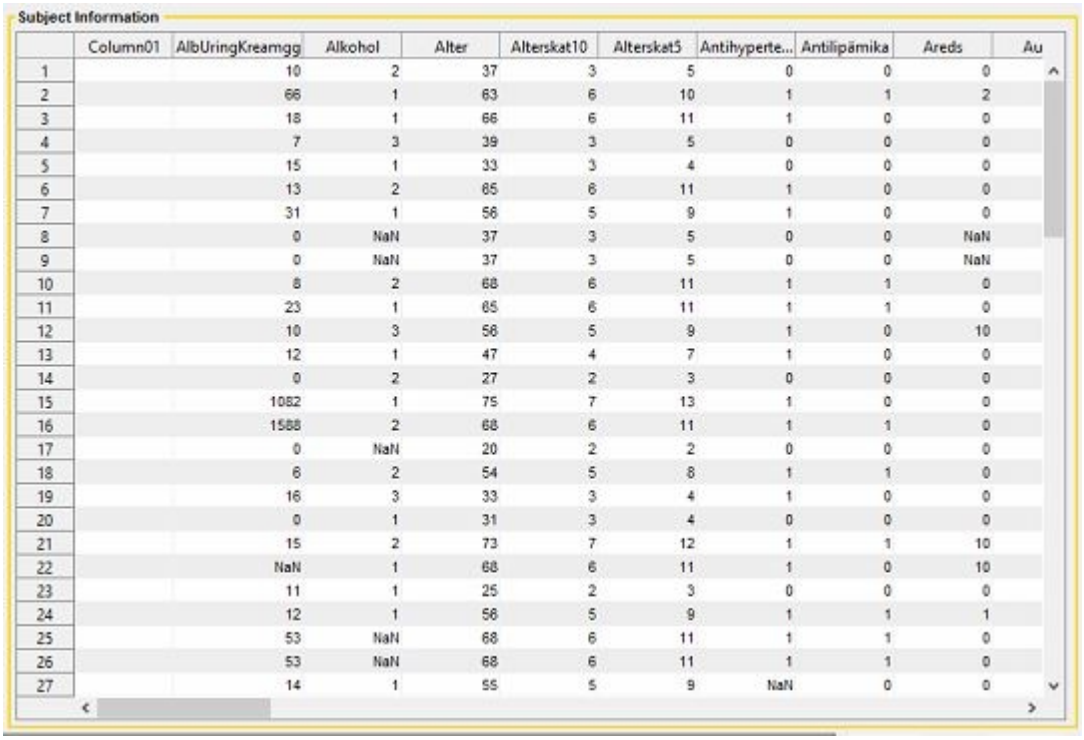
Subject Information

The box Subject Information gives an overview of all the information of the subjects/study participants.


Subject Information

	Column01	AlbUringKreamgg	Alkohol	Alter	Alterskat10	Alterskat5	Antihyperte...	Antilipämika	Areds	Au
1		10	2	37	3	5	0	0	0	
2		66	1	63	6	10	1	1	2	
3		18	1	66	6	11	1	0	0	
4		7	3	39	3	5	0	0	0	
5		15	1	33	3	4	0	0	0	
6		13	2	65	6	11	1	0	0	
7		31	1	56	5	9	1	0	0	
8		0	NaN	37	3	5	0	0	NaN	
9		0	NaN	37	3	5	0	0	NaN	
10		8	2	68	6	11	1	1	0	
11		23	1	65	6	11	1	1	0	
12		10	3	58	5	9	1	0	10	
13		12	1	47	4	7	1	0	0	
14		0	2	27	2	3	0	0	0	
15		1082	1	75	7	13	1	0	0	
16		1588	2	68	6	11	1	1	0	
17		0	NaN	20	2	2	0	0	0	
18		6	2	54	5	8	1	1	0	
19		16	3	33	3	4	1	0	0	
20		0	1	31	3	4	0	0	0	
21		15	2	73	7	12	1	1	10	
22		NaN	1	68	6	11	1	0	10	
23		11	1	25	2	3	0	0	0	
24		12	1	56	5	9	1	1	1	
25		53	NaN	68	6	11	1	1	0	
26		53	NaN	68	6	11	1	1	0	
27		14	1	55	5	9	NaN	0	0	

The order of the subjects is taken from the box *Subjects*. So number 1 in the Subject Information is the subject number 1 in the *Subjects*.

With a right click within the box the window explained and shown in Subject Information Management opens.
To create or change the entry of one row in a regular column double click the row. The row is now selected and an entry can be made. After changing a value in a regular column, the conditional columns are updated. The values of conditional columns cannot be edited manually.

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Status Bar

The Status Bar shows the progress of a prolonged action and the estimated remaining time. Most of these actions are duplicating or copying whole studies or duplicating and copying a large amount of subjects at the same time.

The red bar represents the achieved status. This is also as a value in percentage. In this case

3.9%. The remaining time is exemplary depicted as "Time left: 0h 2min 20sec".
The button "Stop" aborts the progress. The button "OK" closes the Study Manager. If changes have made the program asks to save these changes before closing the program.

Back to Study Manager

FLIMXFit

The subprogram FLIMXFit of the FLIMX software focuses on the approximation of the fluorescence lifetimes. This subprogram may use different approximation methods to compute a resulting graph. This graph gives shows the Photon-Frequency (or Light-Intensity) in relation to time. In the following the interface of this software is explained to give an overview of the possibilities.
The Description is divided into:

1 - Photon-Frequency Time Graphic

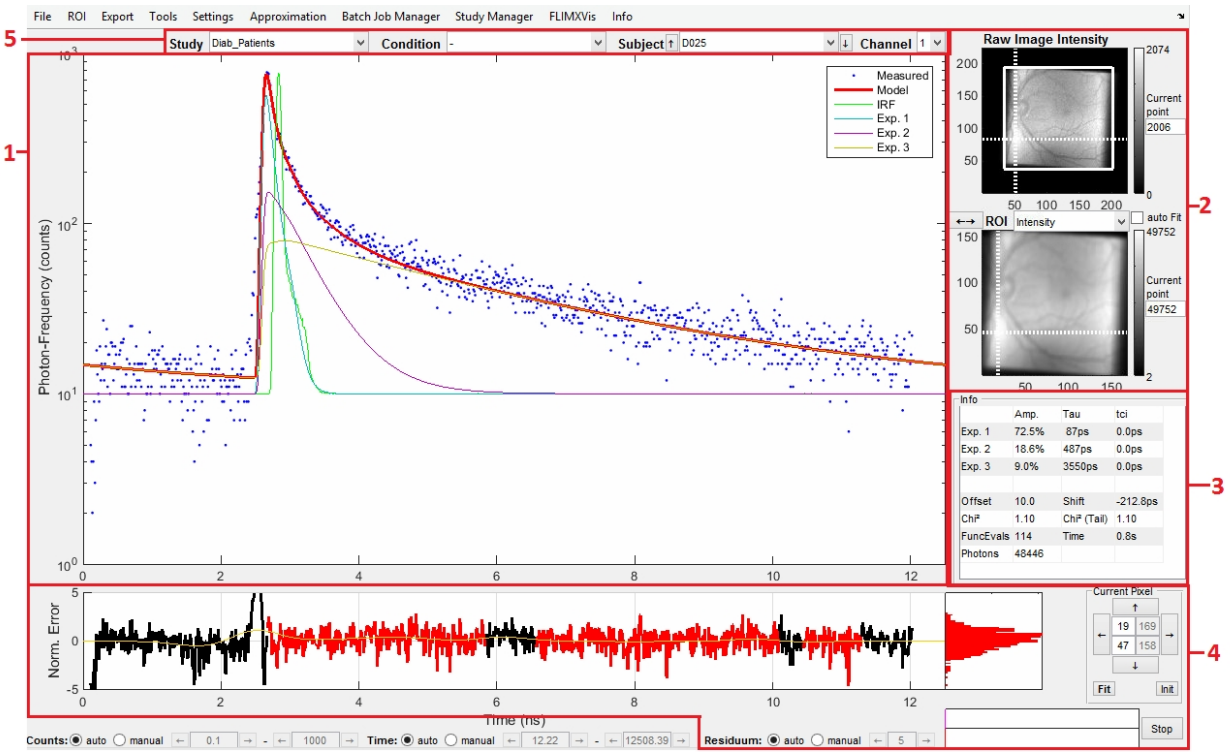
2 - Raw Image Intensity and ROI

3 - Info box

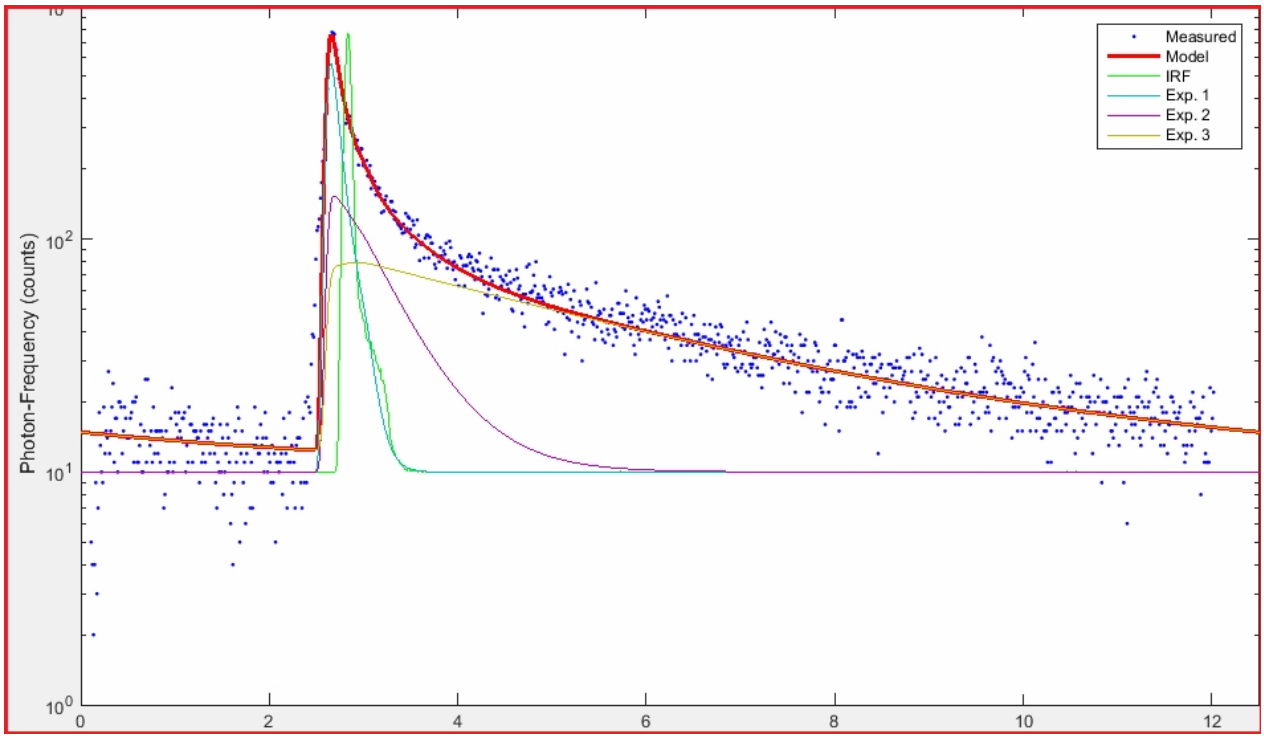
4 - Norm. Error, Current Pixel and Status Bar and

5 - Study, Condition, Subject and Channel

The Menu Bar is explained separately in the subtopic Menu Bar.



Photon-Frequency over Time Plot



Photon-Frequency Time Graphic

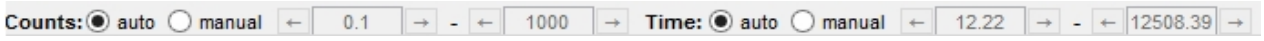
The greater part of the window is used to show the Photon-Frequency over Time Plot. The y-axis (vertical scale) is the Photon-Frequency (Light-Intensity) in counts. The frequency is the number of photons counted in a specific time channel by the measurement device. The scale is logarithmic (base 10). The x-axis (horizontal scale) is the time in ns (nano seconds).

The points in the graphic (**Measured**) represent the measured Photon Frequency for one time channel. In the example above, the time channel width is 12.22 ps (pico seconds). The maximum period of time is 12.5 ns (80 MHz repetition rate of the excitation laser) and the number of time channels is 2^{10} (1024). The 12.5 ns divided by the 1024 time channels results in the 12.22 ps per time channel. The laser repetition rate and the number of time channels are automatically determined from the measurement.

The **IRF** (instrument response function) curve represents the transfer function of optical pathway of the measurement device. The IRF has to be supplied to FLIMX by the user (IRF Manager), it cannot be determined automatically.

The **Model** curve is the approximated curve according to the measured points. In the example above, it is a summation of the three exponential functions **Exp. 1**, **Exp. 2** and **Exp. 3**.

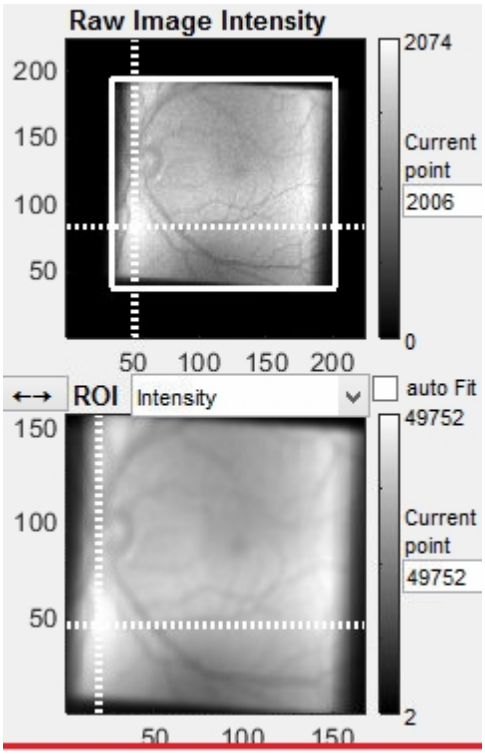
At the bottom of the window the **Counts** and **Time** options vary the depiction of the graphic.



It can be selected between auto and manual mode. In the auto mode, FLIMX selects the maximum range of the Photon-Frequency-axis (counts). In the manual mode the range can be selected by the user. The two boxes next to the manual mode show the minimum and maximum range. It can be adapted by using the arrow keys beside the boxes or by entering a value. Only positive values are possible.
Similar restrictions apply to the *Time* options. The minimum value is the time channel width. The maximum is the reciprocal of the laser repetition rate. Only multiples of the time channel width are possible. If an invalid value is entered, FLIMX rounds it to the closed suitable value.

Back to FLIMXFit

Raw Image Intensity and ROI



Raw Image Intensity and ROI

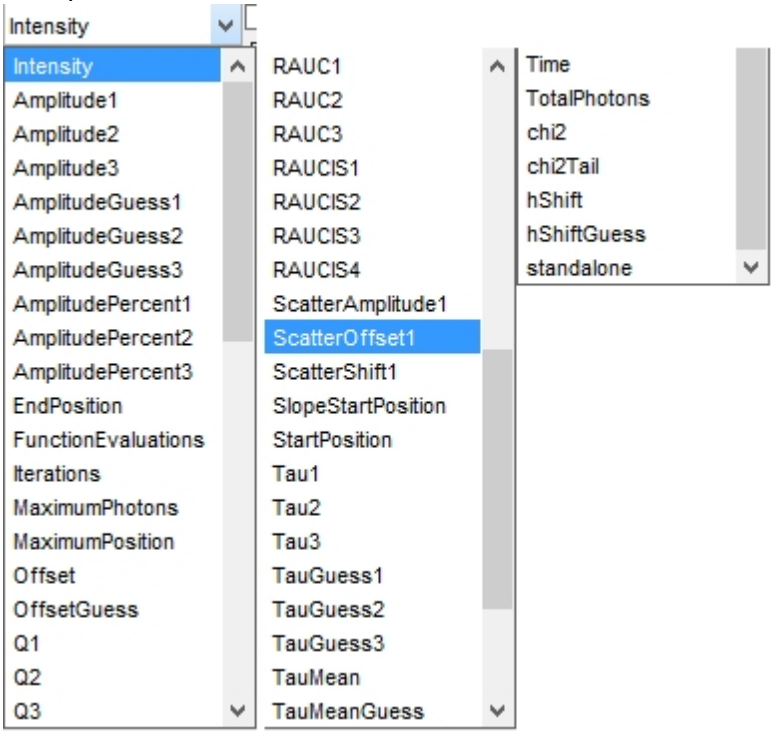
The **Raw Image Intensity** is the integral over the time axis for each pixel. The different gray-scale values represent the Photon Frequency or Light Intensity. A very low Photon Frequency is recorded in black or dark areas. A very high Frequency is recorded in white or bright areas. The vertical bar on the right hand side of the window shows the photon frequency with a minimum and maximum value. The value in the box underneath the information current point states the fluorescence intensity of the currently selected pixel. The currently selected pixel is marked with a white cross hair . On the picture above the white box around the output image of the Raw Image Intensity is the Region of Interest defined by the Import Wizard.

The **ROI** is shown in detail in the image underneath. In addition to the fluorescence intensity, different FLIM Items can be selected (more details under ROI Visualization). The different pixels can be selected within the image by simply clicking on the region of interest. The vertical bar on the right hand side of the window shows the color scaling with a minimum and maximum value. There are three additional options above the ROI window. A button with a bidirectional arrow to switch the places of the Photon-Frequency over Time plot and the ROI window to obtain a larger view of the ROI. The Auto Fit function can be selected to automatically calculate the fluorescence lifetime for the current pixel (if it was not calculated before). Caution: depending on the Approximation Settings this may take some time!

Back to FLIMXFit

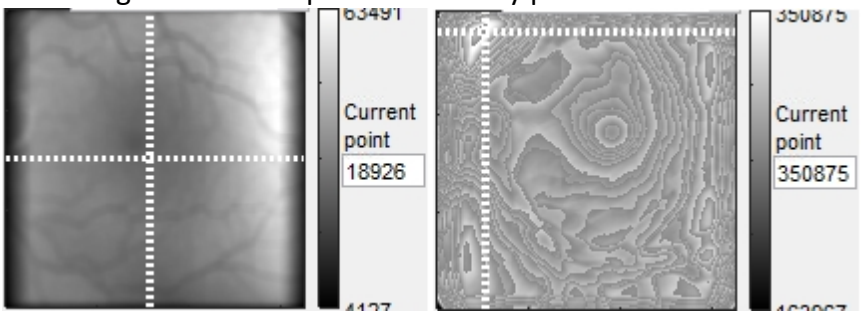
ROI Visualization

Various interpretations can be selected for the ROI window. According to the selected *FLIM items view* (Visualization -> General) some of the following items are not available. These items allow to observe other parameters of the approximation. The figure below shows exemplary some of the possibilities. This list is not intended to be exhaustive.



Only the most important ones are explained here. Others are explained in section Glossary.

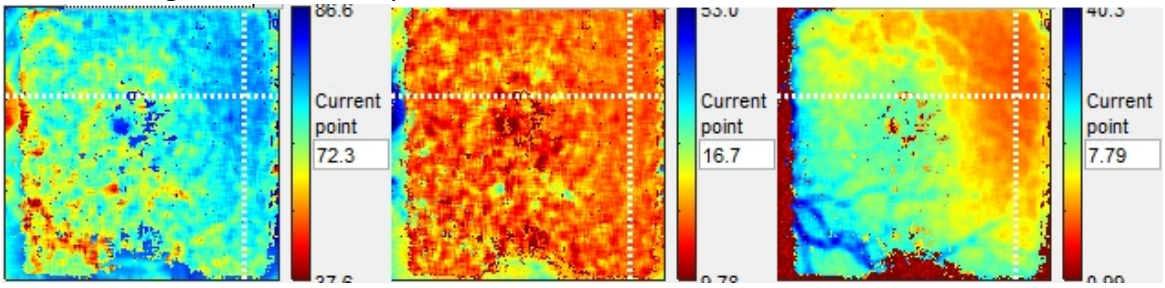
Intensity shows the number of photons per pixel. This refers to the info box (current Point) to the right of the color-bar. This info box contains the number of photons of the currently selected pixel. A static (left figure) binning leads to a picture similar to the original measured one but blurred. An adaptive binning (right figure) leads to a more homogeneous distribution of the photons. The target number of photons of every pixel is set to 30000.



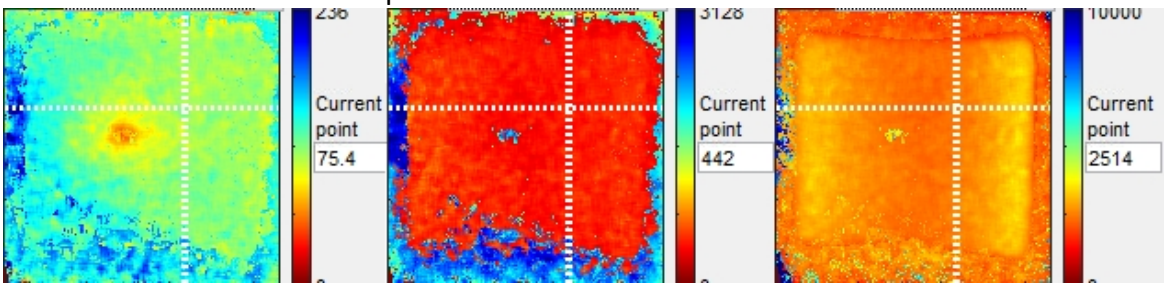
For the following options the general setting **Jet** is selected for the Colormap and the Colormap is **inverted**. For more information see also: Settings -> Visualization.

AmplitudePercent1, **AmplitudePercent2** and **AmplitudePercent3** refer to the relative height

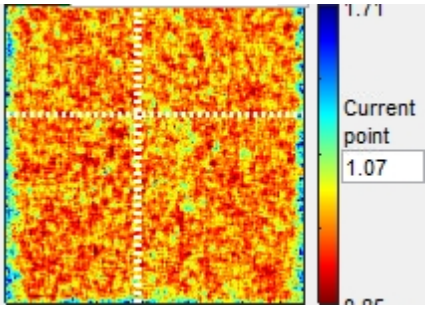
of the exponential function in comparison to the Model curve. For example the Model curve has an amplitude of 1000 counts on the Photon-Frequency axis and exponential function 1 has a relative height (AmplitudePercentage) of 70% then the exponential function has an amplitude of 700 counts on the Photon-Frequency axis. The picture shows the three different AmplitudesPercentages for the same pixel.



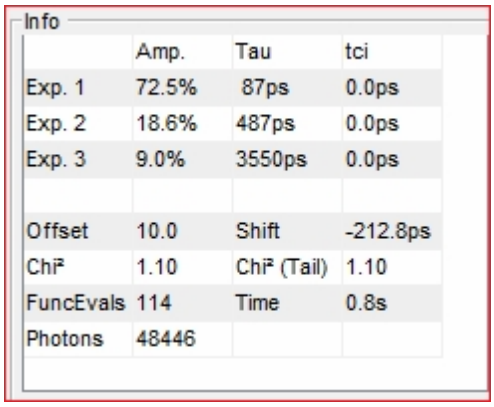
Tau1, **Tau2** and **Tau3** refer to the lifetime of the exponentials in picoseconds. The picture below shows three tau values for the same pixel.



chi² and **chi²Tail** describe the squared error. For further information see also Info Box. These two values are commonly the same when only selecting the Tail Fit. For further information see also: Settings -> Approximation -> Temporal Parameters. The picture below shows the chi² and the chi²Tail distribution.



Info box



Info box

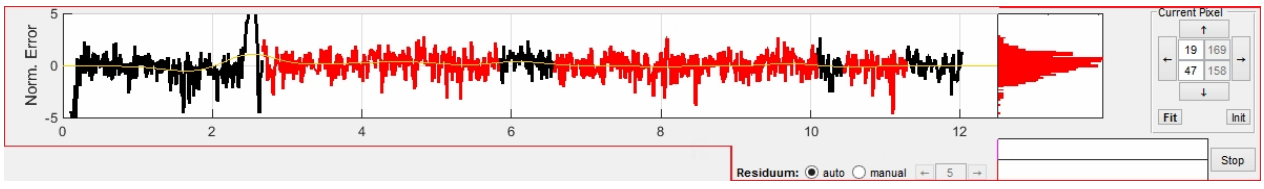
The Info-Box provides numerical values for the currently selected pixel. It can be divided into two parts. The upper part hosts information about the exponential functions:
The abbreviation **Amp.** stands for Amplitude and states the relative height in percent of the exponential function to the model curve. **Tau** represents the fluorescence lifetime of the specific exponential function. The **tci** value represents the shift of the exponential function on the time axis in pico seconds. The measuring point is the starting point of the ascending part of the model curve. Only negative values (shift to earlier points in time) are possible.

The lower part of the Info-Box hosts general information about the model curve and the approximation algorithm:
hShift shows the shift of the model curve on the time axis in relation to the IRF in pico seconds. **Chi²** represents the error of the model curve. The ideal value is 1. **Chi²(Tail)** describes the same error but only for the descending part of the model curve. The value of **FuncEval** states the number of steps (function evaluation) the approximation algorithm required to calculate the model curve. The computation time for the approximation is given after **Time** in seconds. **Photons** states the number of photons used for one pixel to create the Photon-Frequency. All the curves start at around 10 counts on the Photon-Frequency axis. Due to e.g. thermal noise of the detectors, surrounding light in the room, etc. the exponential functions are elevated. This is the **Offset**.

See Glossary for more details.

Back to FLIMXFit

Norm. Error, Current Pixel and Status Bar



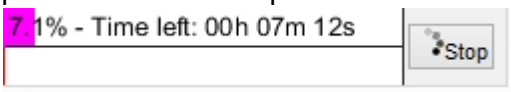
Norm. Error, Current Pixel and Status Bar

The Norm. Error (normalized error) visualizes the difference between the approximated model curve and the measured data, which is also called residuum. The red parts of the residuum mark the time channels, which are used for the chi² calculation. A histogram of the residuum is shown next to it on the right hand side. The scaling of the **Residuum** can be set to auto or

manual mode. In the auto mode FLIMX chooses the scaling. In the manual mode the residuum scaling can be set by the user. It can be adapted using the arrow keys beside the box or by entering a value directly.

In the bottom right corner, the **Current Pixel** is placed. It provides the coordinates of the currently selected pixel (left two boxes) and the size of the ROI (right two boxes) in pixels. The upper boxes represent the x-axis position and the lower boxes represent the y-axis position of the selected pixel. The current pixel can be changed by a click inside the ROI axes or by using the arrow keys around the boxes or by entering a new value directly. The button "Init" shows the data for the Initialization Fit (if available). For further information see also Settings -> Approximation. It it a toggle switch. The switch keeps selected until clicked on again. The button "Fit" approximates the currently selected pixel with the settings of the approximation. For further information see also Approximation -> Fit Current Pixel.

The **Status Bar** in the lower right corner of the FLIMXFit window shows the process of all actions that take place. It is divided into two parts. The upper part shows the process of the currently conducted action in percent with an information about the remaining time. The lower part shows the total process of the action which is often divided into several parts



Back to FLIMXFit

Study, Condition, Subject and Channel

Study, Condition, Subject and Channel

At the top of the window underneath the Menu Bar the functions **Study**, **Condition**, **Subject** and **Channel** are placed. **Study** shows the selected study. Other studies can be selected with clicking on the arrow key. **Condition** accesses the information of conditional columns of the selected study. See also: Subject Information. If a condition is selected, the available **Subject** are updated. Only subjects that fulfill the condition are shown. If no conditional column is chosen ("-") all the subjects are shown. With the arrow keys to the right and the left of the subject box, it possible to switch to the next (arrow down) or previous (arrow up) subject. **Channel** shows the currently chosen spectral channel.

Back to FLIMXFit

Menu Bar

The Menu Bar is divided into eleven main functions: File ROI Export Tools Settings Approximation Batch Job Manager Study Manager FLIMXVis Info

Docking

With clicking on the docking arrow the Study Manager is docked to the command window of MATLAB.

Back to FLIMXFit

File

File

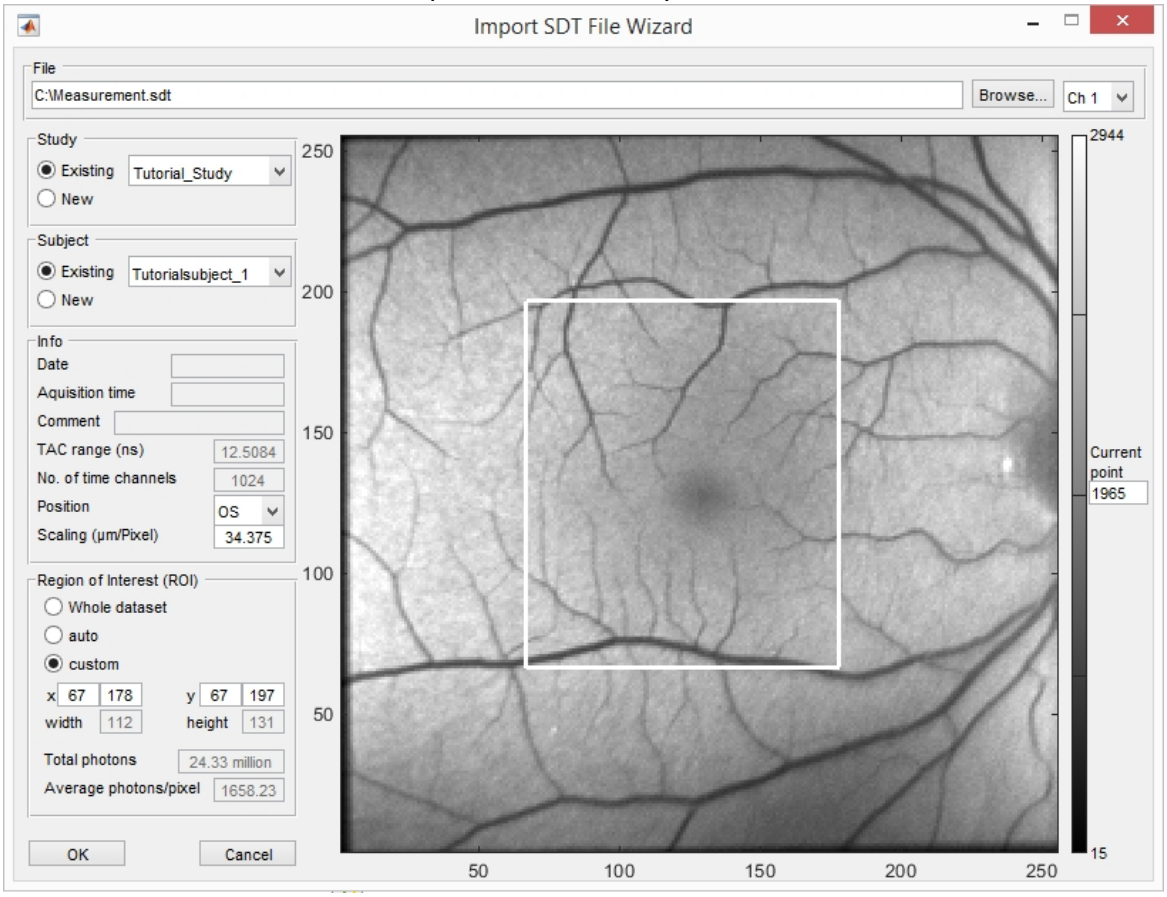
By clicking on File, a window opens with one option. The Exit option closes the Fit GUI. This is also possible with the key combination CTRL(Control)+X. If there are unsaved changes the program asked to save these before quitting the GUI.

Back to Menu Bar

ROI

ROI

With clicking on ROI the option to redefine the ROI is selectable. This option opens the Import SDT File Wizard window. It can also be opened with the key combination CTRL+I.



In the **File** box the measurement channel can be chosen.

The **Study** and **Subject** box refer to the study and subject where the measurement is located.

The **Info** box hosts information about the subject and the measurement. The TAC range

(reciprocal of the excitation laser repetition rate) and the number of time channels are read from SDT file. Position states which eye was measured (only relevant for the ETDRS grid). OS stands for oculus sinister (Latin for left eye) and OD stands for oculus dexter (Latin for right eye).

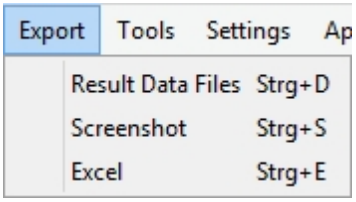
The **ROI** box enables the redefine of the Region of Interest. The option **Whole dataset** sets the ROI to the full image size. The **auto** option automatically sets a ROI window based on the fluorescence intensity image. The **custom** option allows the user to set the desired ROI. The ROI can be defined by entering the ROI-coordinates directly or by mouse. Within the Dataset visualization, the mouse pointer changes to a cross hair. By holding the left mouse button, the ROI can be drawn. The width and height of the ROI are calculated automatically. Further, the total and average numbers of photons inside the ROI are calculated.

Clicking on "OK", the new ROI is saved in FLIMX, "Cancel" aborts the process. The vertical bar on the right hand side of the window shows the photon frequency with a minimum and maximum value. The value in the box underneath the information current Point states the intensity of the currently selected pixel.

Back to Menu Bar

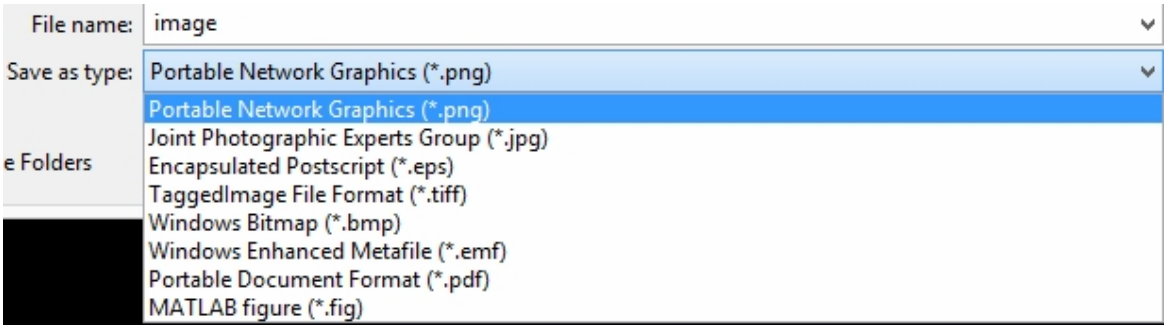
Export

Export
By clicking on this option, a window opens.



Result Data Files exports all the data files of the current subject as MATLAB files. With clicking on this option the Select Directory to Open window is opened, which allows the user to select the folder in which the data is stored. If both channels are used for the measurement the program stores four different MATLAB files. Two files for each channel, one measurement file and one result file per channel. The window can also be opened with the key combination CTRL +D.

Screenshot saves a picture of the whole FLIMXFit window except from Menu bar. With clicking on this option the Export as window is opened. In the lower part of the window the name of the picture can be set. When the window opens this input field is automatically selected to enter a name. Underneath the file name input field the type of file can be selected.



There are various types to choose. The file is saved as the selected type in the selected folder.

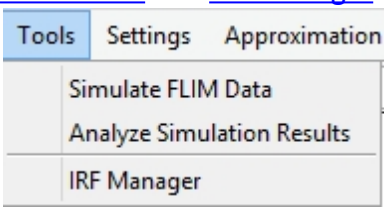
Excel saves the measured data, model curve, exponential functions and IRF of the selected pixel and the information in the Info box into an excel file.

Back to Menu Bar

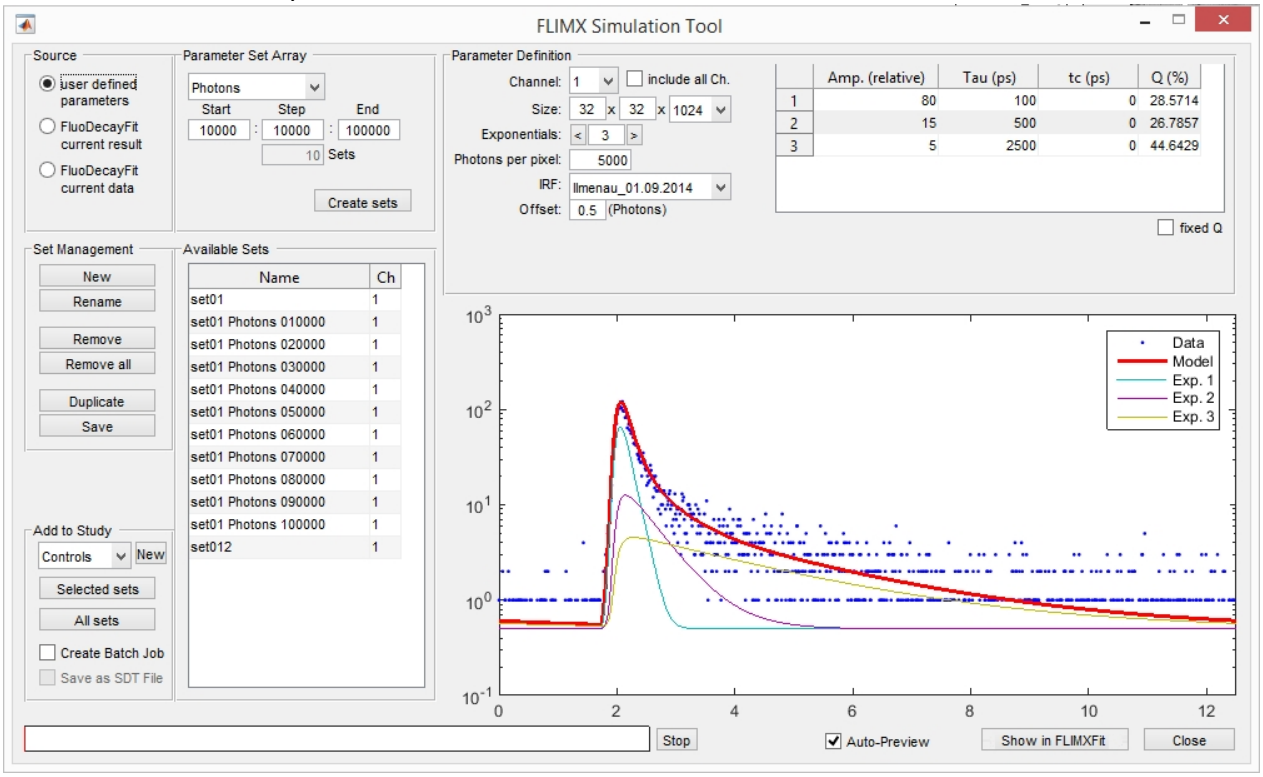
Tools

Tools

By clicking on this option, a window opens with three functions: Simulate FLIM Data, Analyze Simulation Results and IRF Manager



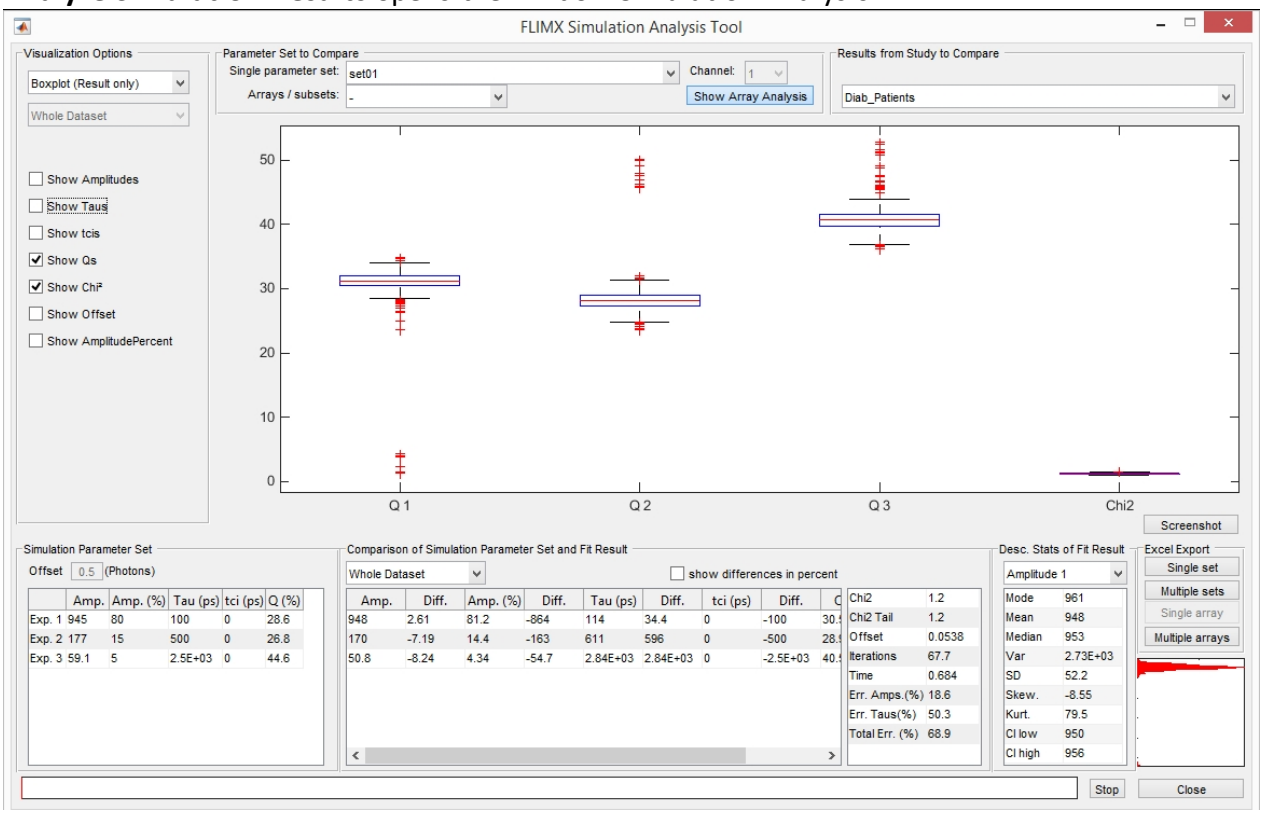
Simulate FLIM Data opens the FLIMX Simulation Tool.



The window is divided into different parts: Source, Set Management, Available Sets, Add to

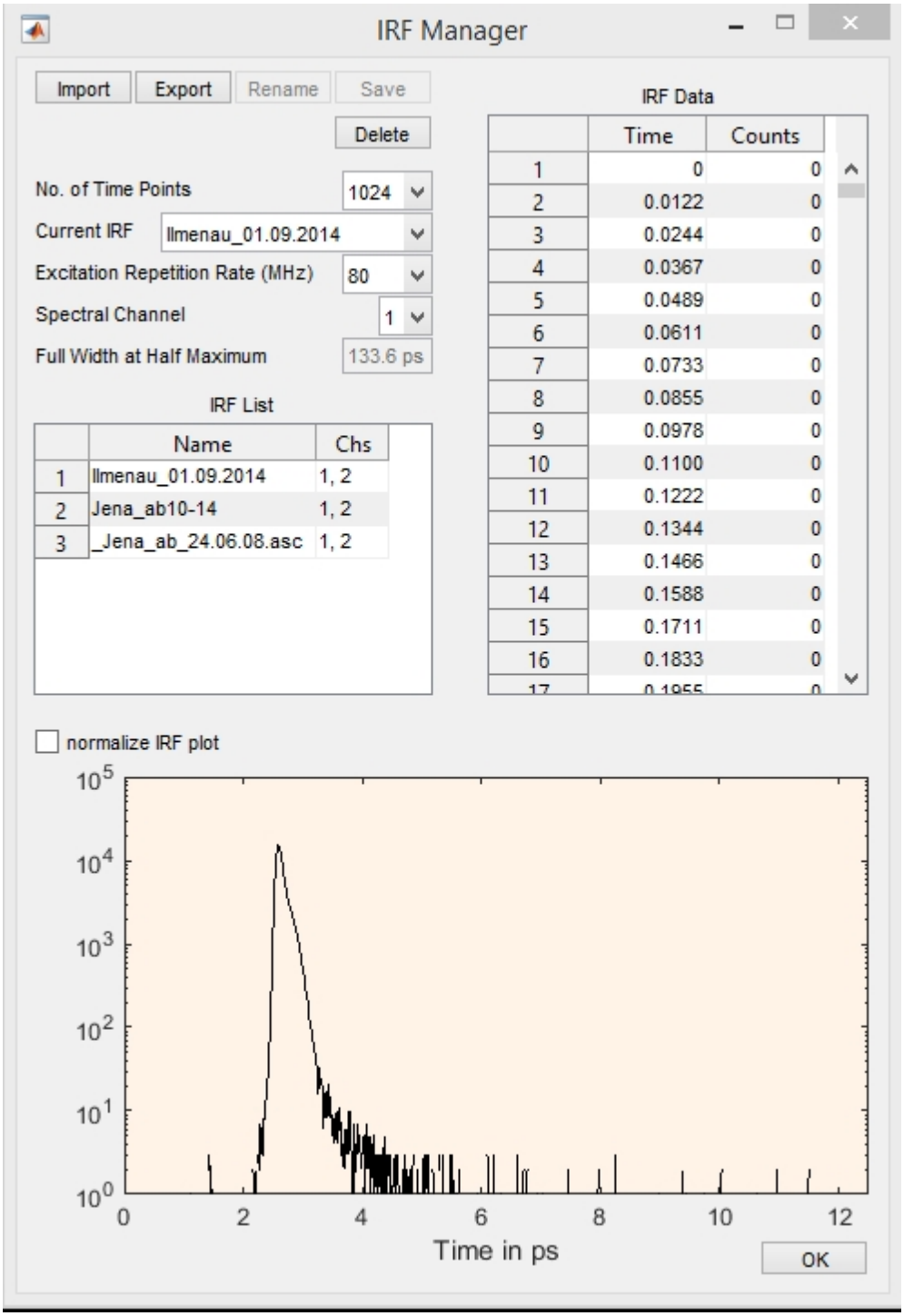
Study, Parameter Set Array, Parameter Definition and Preview and status bar.

Analyze Simulation Results opens the window Simulation Analysis.



The window is divided into different parts: Visualization Options and display, Parameter Set to Compare, Results from Study to compare, Simulation Parameter Set, Comparison of Simulation Parameter Set and Fit Results, Desc.(descriptive) Stats(Statistics) of Fit Results, Excel Export and Status Bar.

IRF Manager opens the IRF Manager window.

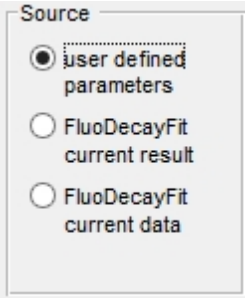


This window is divided into different parts: IRF settings and list, IRF Data and IRF Plot

Back to Menu Bar

Simulate FLIM Data

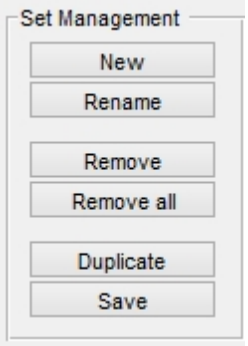
Source



The Source selection allows to choose where to get the information for the simulation. The user defined parameters are explained below under Parameter Definition. The FluoDecayFit current result and data refer to the results and data of analyzed subjects.

Back to Tools

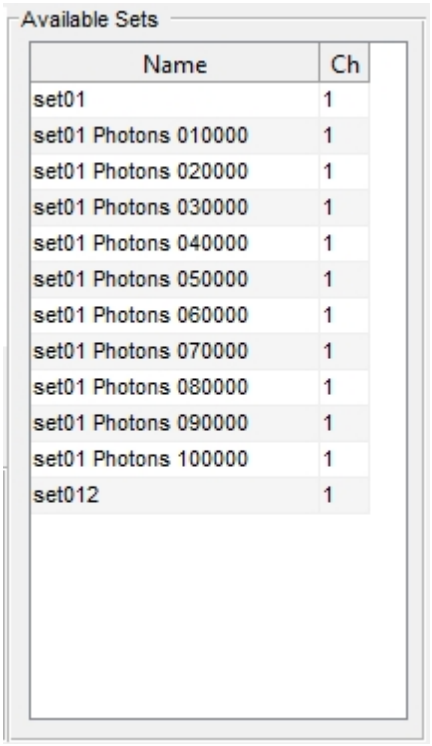
Set Management



The Set Management helps to organize the simulation sets. With "New" a set can be created. The name must be unique. With "Rename" the selected set can be adapted if the already given name is unsuitable. "Remove" deletes the currently selected set. "Remove All" deletes all sets of the list "Available Sets". "Duplicate" copies the selected set. The name for the copy must be changed or adapted to create a unique name. "Save" stores the list of sets to restore it after a system shut down.

Back to Tools

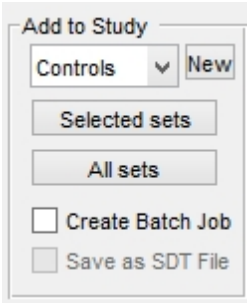
Available Sets



Available sets is a list of all created sets from the Set Management. The set01 is the initial set with the default parameters. The set01 Photons 010000 to set01 Photons 100000 are subsets with the same settings of set01 but different number of Photons per pixel. This creates a set array and will be explained under Parameter Set Array.

Back to Tools

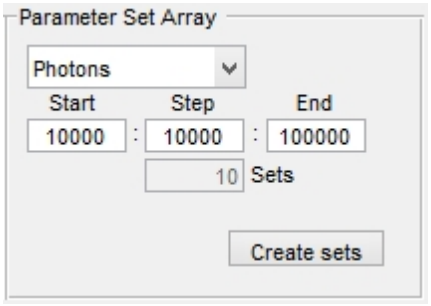
Add to Study



This enables the transfer the simulated sets to a target study. A new Study can also be created with "New". The name of the study must be unique. The buttons "Selected Sets" and "All Sets" add the appropriate sets to the selected study. If choosing "Create Batch Job" the added sets will be automatically transferred to the Batch Job Manager using the currently set approximation settings.

Back to Tools

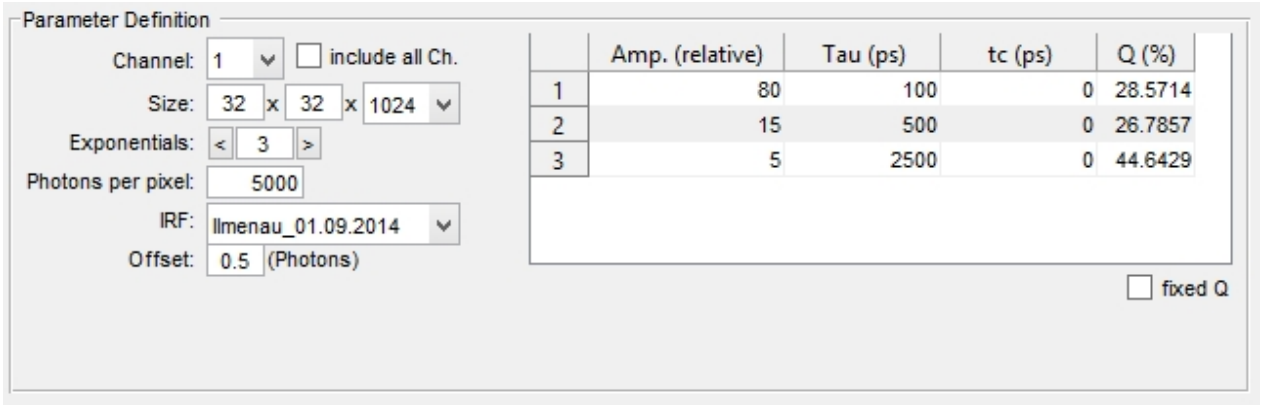
Parameter Set Array



The parameter set array helps to create different sets with the same parameter and one variable. The default variable is Photons. Other variables can be chosen (Offset, Amplitude, Tau, tc). The chosen variable is given a initial or start point and a step range and an end point. For example the start point is 10000 and the step is set to 10000 with an end point of 100000. The created array has 10 sets each 10000 photons more than the prior one. The results for this array are shown under "Available Sets"

Back to Tools

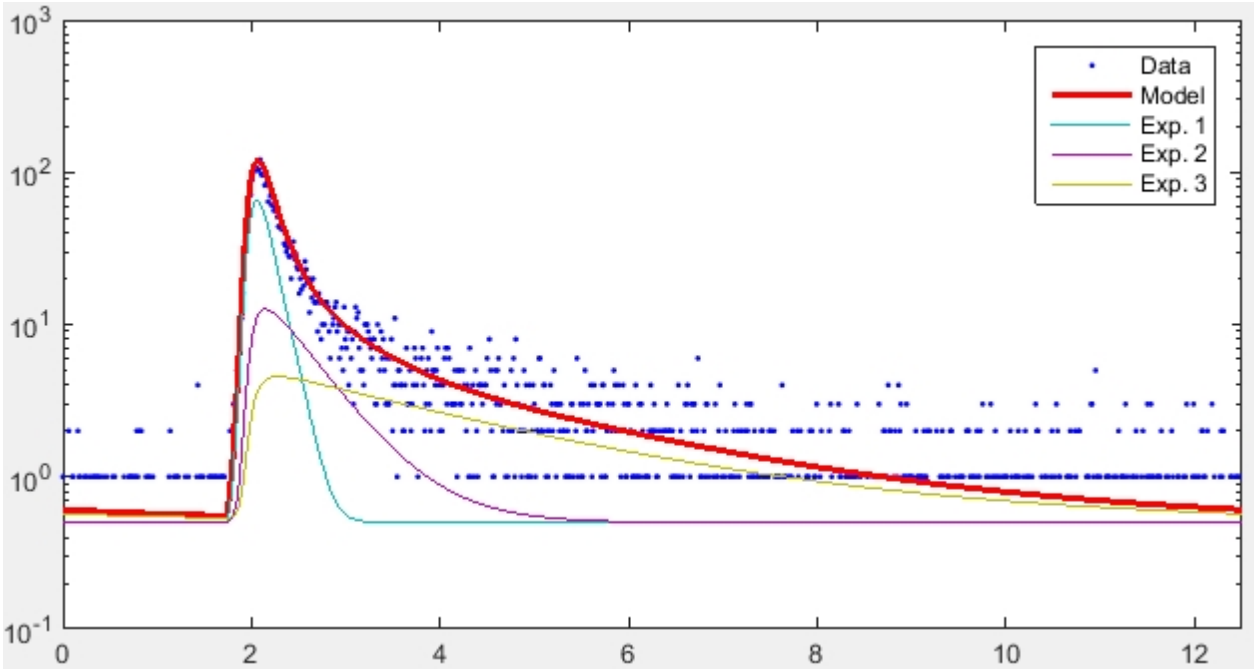
Parameter Definition



The parameter definition allows to organize the simulation.
The **channel** selection allows to choose the channel which is simulated. If choosing "include all Ch." both of the channels will be simulated.
The **Size** refers to the size of the RAW image window. The default settings are 32x32x1024. The resulting window will be 32x32 pixels with a time resolution of 1024 time channels. The **exponentials** refer to the number of exponentials used to create the simulation. It can be adapted with using the arrow keys to the left and right of the input field.
The 50000 **Photons per Pixel** describe the default settings. The number of Photons can be adapted even for already created sets by clicking into the field and entering a new number of photons. A constant number of photons always leads to a white RAW image because every pixel has 50000 photons.
The **IRF** selection provides all the selectable IRFs.
The **Offset** defines an offset for the photons. There will be no photon counts underneath this threshold.
The table on the right-hand side shows the settings for amplitude, tau, tc and Q. All these settings can be adapted by clicking into the field and entering a new value. The Q (area under curve) can be fixed for all simulations of the same set.
The simulation data can be adapted to reach a high correlation between the a study to compare and the simulation parameters. Because of that the Simulation tool serves as preparation for measurements.

Back to Tools

Preview and status bar



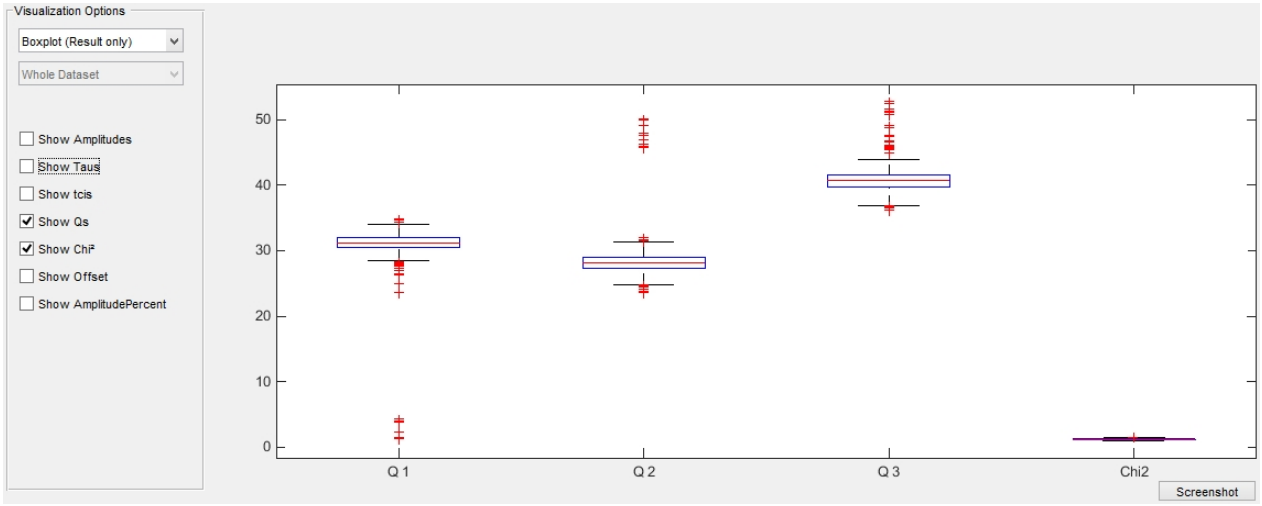
The preview shows a rough approximation of the simulation results.

The status bar shows the progress of all the functions selectable for the simulations. If choosing the Auto-Preview the simulation results will automatically be shown in the preview window. "Show in FluoDecayFit" transfers the simulation results to the FLIMXFit window. "Close" closes the Simulate FLIM Data tool.

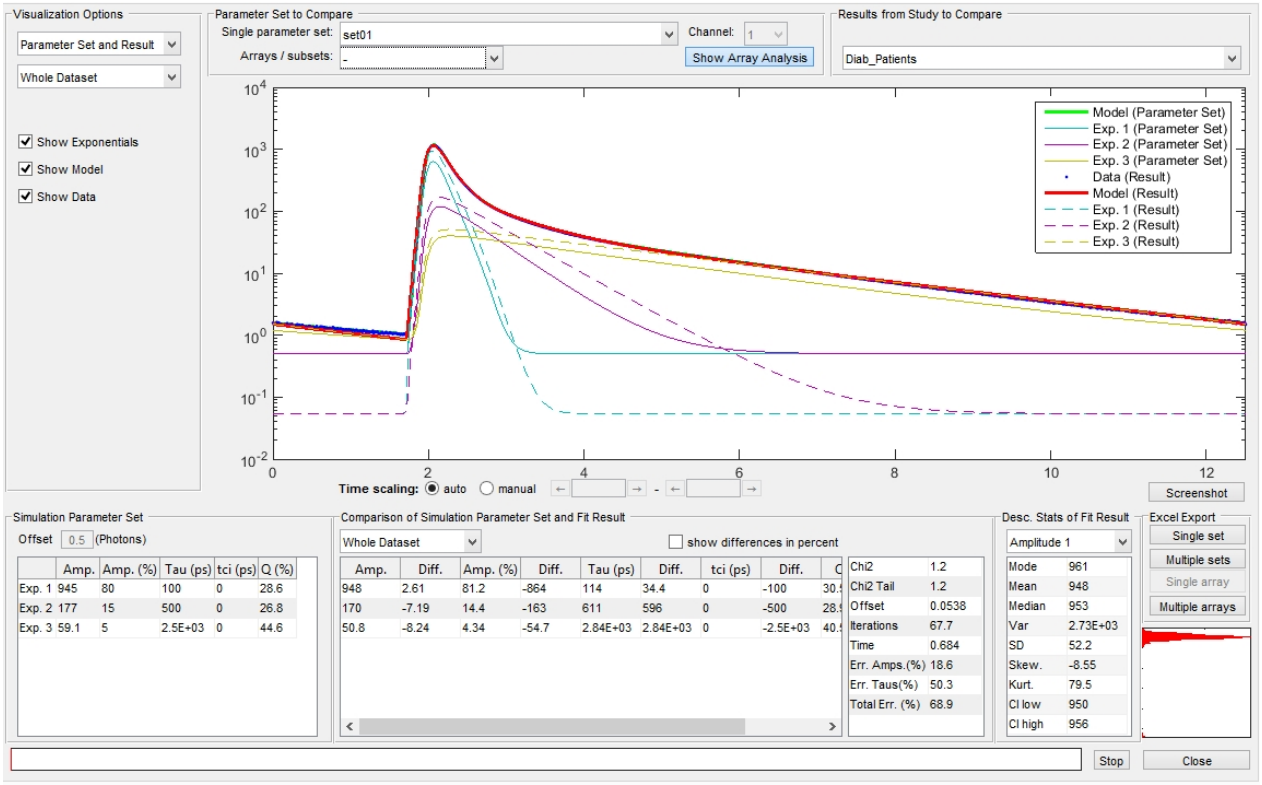
Back to Tools

Analyze Simulation Results

Visualization Options and display



The **Visualization Options** allow to choose how the results are displayed. The first select field has four options: Boxplot (Results only), Parameter Set and Results, Parameter Set only and Results only. The Boxplot is shown in the picture above. It shows the selected parameters. Selectable parameters are: Amplitude, Tau, tc, Q, Chi², Offset and AmpPercent. The box contains more information about one parameter. The average (middle line within box), the standard deviation (box), the interval of all occurring results for the parameter (T-shaped extension) and outliers (lines above oder underneath the interval). Underneath the display the button "Screenshot" allows to take a picture of the currently shown boxplot. The window "Export as" opens. Chosse the destination folder and save the file as the desired type.
If choosing the Parameter Set and Results visualization the display changes. Instead of the parameter display the curves are shown. The picture below shows the difference.



The second select field is now available. The whole dataset refers to all points that are measured. Other options are selectable: Whole Dataset (sdt), Whole Dataset (Max-Min), Best Pixel and Worst Pixel. The Whole Dataset (sdt) refers to the regions which are used for the approximation. The ascending part and the parts with outliers are not included. The Whole Dataset (Max-Min) contains all the data between the amplitude and the lowest point of the

descending part. The display options are reduced to three: Exponentials, Model and data. These refer to the shown exponentials used to form the model curve, the model curve itself and the measured data. Thereby the description in the legends "Parameter Set" means the simulated information and the "Result" means the measured and approximated results. Additional to that underneath the display a Time Scaling function appears. This allows to zoom in a desired area. The auto function scales the time line to the maximum range. The manual function allows to scale the time line individually. If choosing the manual function the two boxes to the right are available. The left box is for the left end and the right box for the right end of the time line. To set the start and end position use the arrow keys or click into the input field and enter the desired time.
The two other option of the first select field show the Parameter Set and the Results individually.

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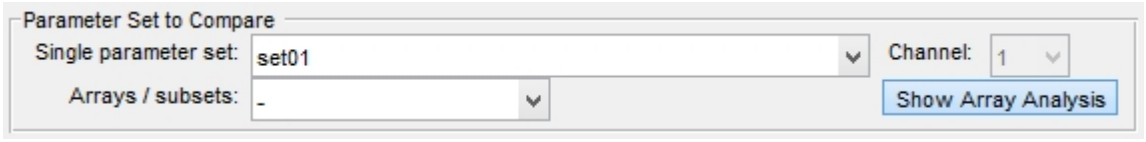
Parameter Set to Compare



The selected Parameter Set is the set of simulated data which is used to compare it with real compiled data. The sets can be created with the FLIMX Simulation Tool. The Analysis tool recognizes created subsets automatically which can be selected in the select field "Arrays/ Subsets" The Channel can be selected with the select field on the right-hand side. It is only available if more than one channel was simulated.

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Results from Study to Compare

Results from Study to Compare provides the fit results from the selected study. These results are compared to the simulated data and the differences are shown in the boxplot. The simulation data can be adapted to reach a high correlation between the selected study and the simulation parameters. Because of that the Analysis tool serves as plausibility check or preparation for measurements.

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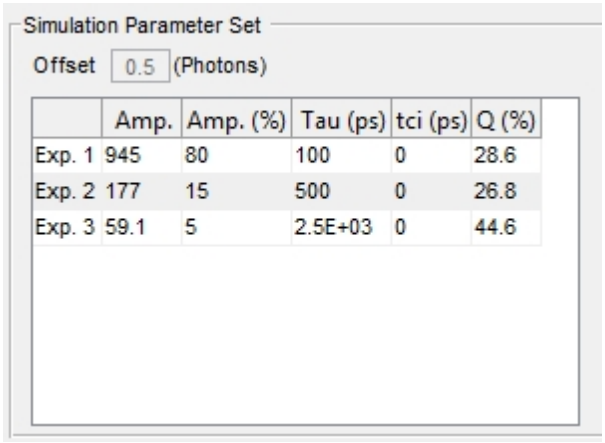
Simulation Parameter Set



The data provided in this box show the parameters of the simulation's exponentials. For further information to the meaning of the abbreviations see: Glossary

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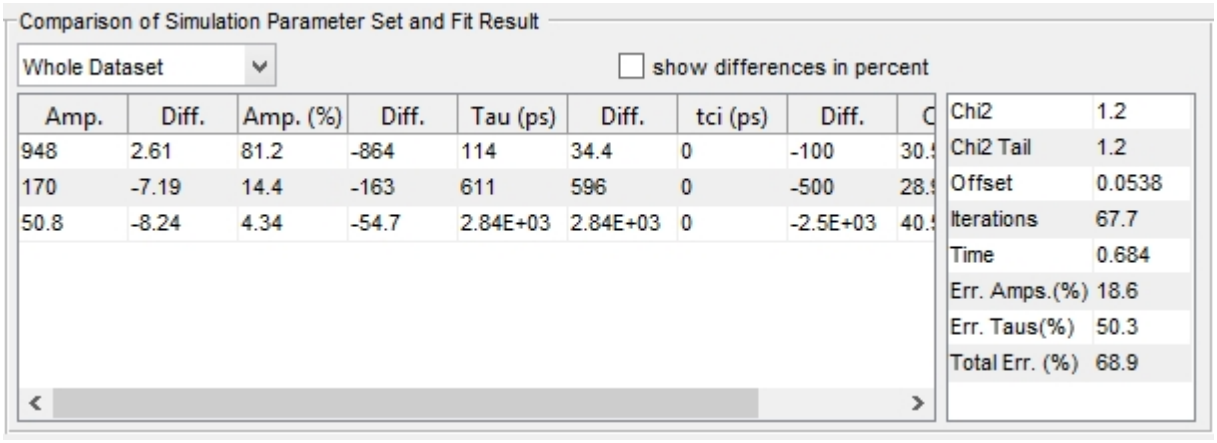
Comparison of Simulation Parameter Set and Fit Results



This window shows the information of the fit results of the selected study. The abbreviations mean the same as in Simulation Parameter Set. An additional abbreviation "Diff." provides the difference between the simulation parameter and the fit results. The box on the right-hand side provides statistical information about the fit results. For further information see: Info box
The select field above the data comparison allows three options: Whole Dataset, Best Pixel and Worst Pixel. These refer to the all measured points (Whole Dataset), to the Pixel with the lowest chi² (Best Pixel) and the Pixel with the highest chi² (Worst Pixel).

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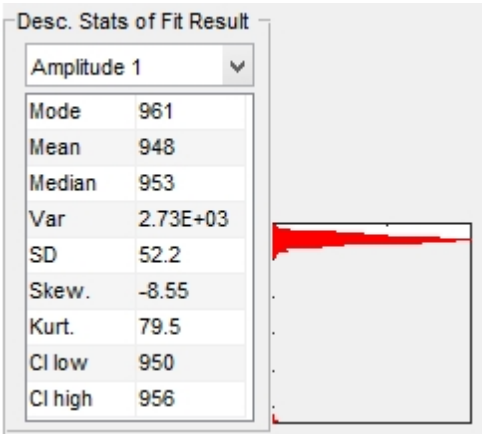
Desc.(descriptive) Stats(Statistics) of Fit Results



The descriptive Statistics of Fit Results provide statistical evaluation for the options of the select field (Amplitude, AmplitudePer, Tau, tc, Q, chi², Offset, Iterations, Time). The histogram on the right-hand side refers to the Error (Err.) values given in Comparison of Simulation Parameter Set and Fit Results.

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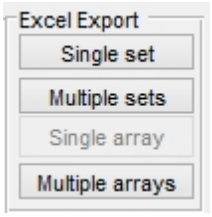
Excel Export



The export function allows to store the data on your PC. The options Single Set or Multiple Sets refer to the number of simulation sets that are exported. Single Set only exports one selected set. Multiple Sets exports all sets that are available. The same applies for Single and Multiple Arrays but the exported data is subsets or arrays.

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Status Bar

The Status Bar shows the progress of an action. The button "Stop" allows to abort the process while compiling. "Close" closes the Analysis tool.

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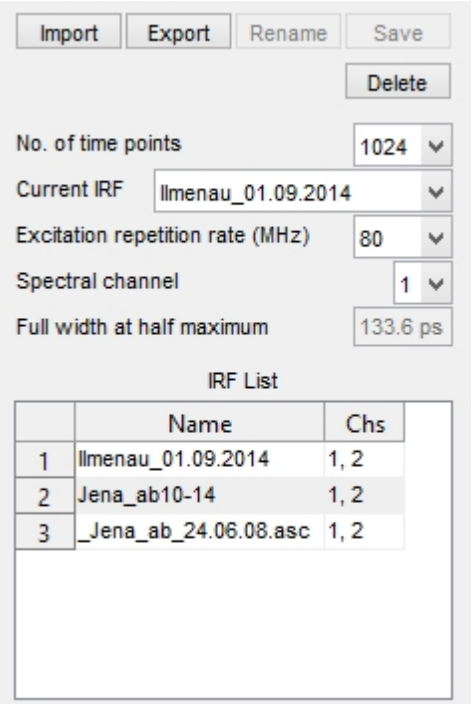
IRF Manager

IRF settings and list



The IRF settings and list helps to find the suitable IRF function for the needed approximation. In the upper left corner new IRF data can be imported with the button "Import". A new window opens to browse the computer. The new data can be renamed and internally saved with the appropriate buttons. Already stored function data can be deleted with the "Delete" button. With "Export" the data of the selected IRF function can be saved on the computer to extract the data and load it onto another computer. **No. of Time Points** is referring to the number of measured points across the time axis that can be measured with the resolution of the measurement device. The common time axis range is 12.5 ns due to the selected 80 MHz (Megahertz) excitation **Repetition rate** of the laser pulse. **Current IRF** shows the IRF curve, which is shown in table and the diagram below. With **Spectral Channel** the desired channel for the measurement can be selected. **Full Width at Half Maximum** describes the distance between the ascending and the descending part of the IRF function measured at half the maximum of the IRF. The **IRF List** shows the currently available IRF functions and the channels they can be used for.

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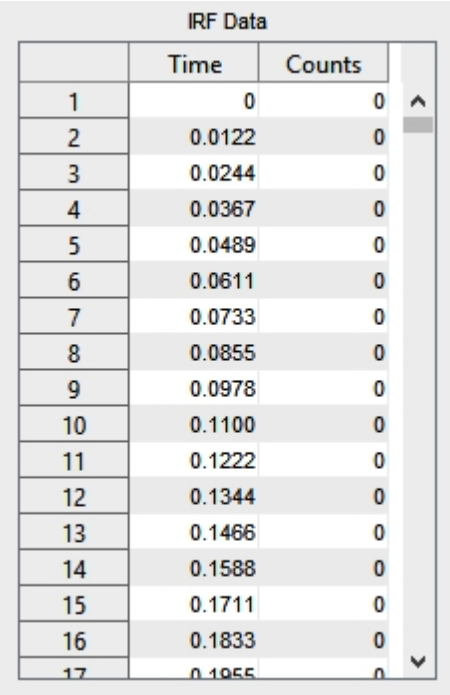
IRF Data


IRF Data

	Time	Counts
1	0	0
2	0.0122	0
3	0.0244	0
4	0.0367	0
5	0.0489	0
6	0.0611	0
7	0.0733	0
8	0.0855	0
9	0.0978	0
10	0.1100	0
11	0.1222	0
12	0.1344	0
13	0.1466	0
14	0.1588	0
15	0.1711	0
16	0.1833	0
17	0.1955	0

On the right hand side of the window the **IRF Data** is listed up. It is divided into three parts. The first part on the left hand side is the number of the time channel. The maximum number of time channels is referring to the number of points selected (e.g. 1024). The row in the middle lists up the starting time of the associated time channel in nano seconds (e.g 0.9266). The row on the right hand side lists up the counts measured in the appropriate time channel. With clicking on the "OK" button the values and settings are stored and the IRF Manager closes.

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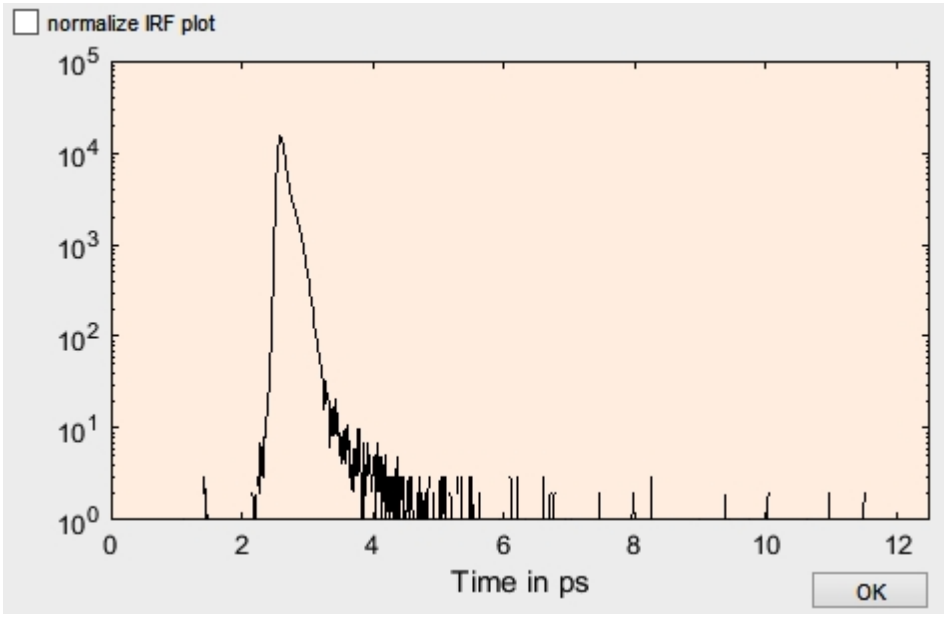
IRF Plot



The graph at the bottom of the window depicts the course of the IRF curve. The x-axis is the time axis in ps (pico seconds). The y-axis is the Photon-Frequency-axis given in counts and in logarithmic scale. The option **normalize IRF plot** shifts the graph on the y-axis.

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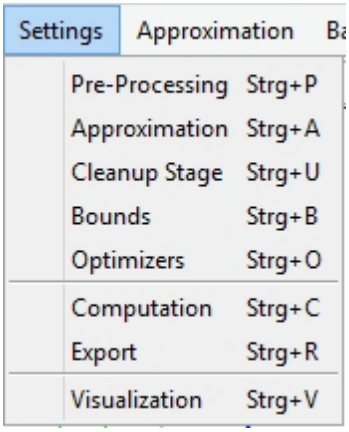
Settings

Settings

By clicking on this option, a window opens with eight options: Pre-Processing, Approximation, Cleanup Stage, Bounds, Optimizers, Computation, Export, Visualization.



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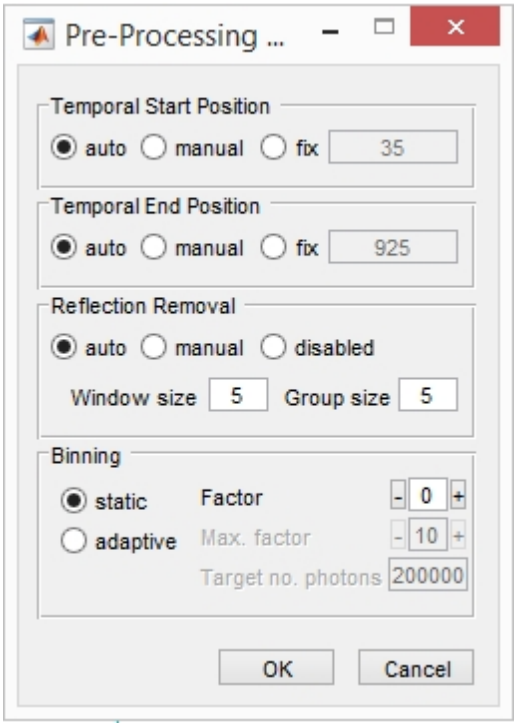
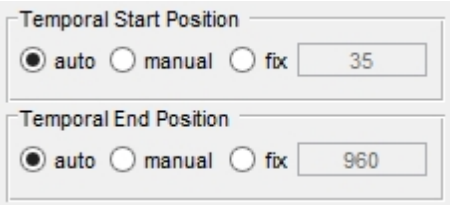
Pre-Processing

By clicking on Pre-Processing, the following window opens, which can also be opened with the key combination CTRL+P:



The window is divided into different parts: Temporal Start and End Position, Reflection Removal and Binning.

Temporal Start and End Position



The Temporal Start and End Position determine the starting and ending point of an interval on the time axis which is used for the approximation. Three functions can be selected. The "auto" function allows the program to choose these points automatically. The "manual" function allows the user to choose these points. Therefore the Manual Start and End Position Wizard window opens. This will be explained later. The "fix" function allows to choose these points with fixed values. This is quite good for a rough approximation or if these positions can be seen as empirical values. The value itself is the number of the appropriate time channel. It is NOT the time in pico seconds. In this case the fixed values are 35 and 960. With the resolution of 1024 of the measurement device the fixed values have to be multiplied with the 12.2 ps as explained in section Tools -> IRF Manager. The time channel number 35 equals the time of 427 ps on the time axis and 960 equals 11712 ps or 11.712 ns.

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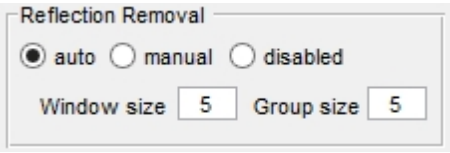
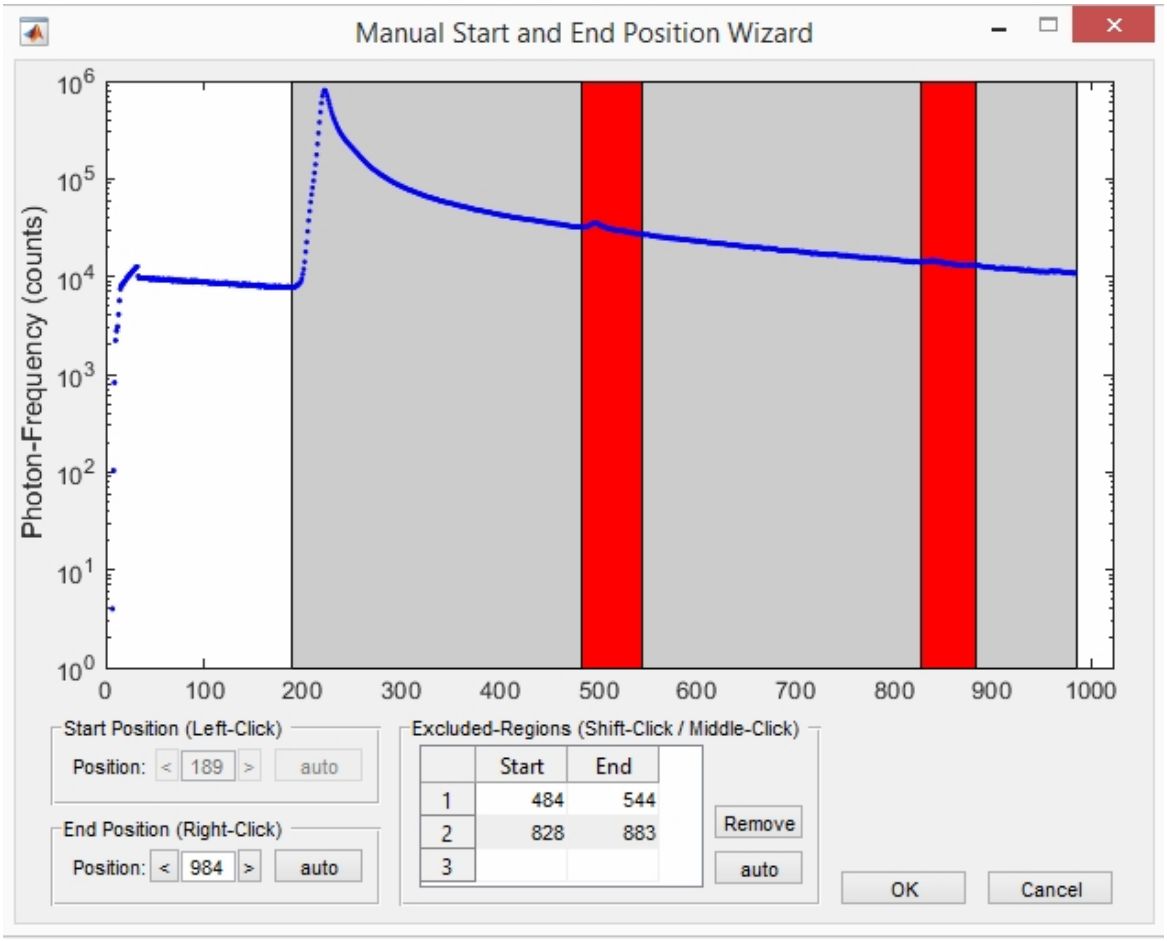
Reflection Removal



The Reflection Removal excludes all the reflections caused by the surface of the eye lens or the surface of the measurement device. The reflected light travels back into the eye and again back to the measurement device. This causes a peak on the descending part of the measured points. If the ascending part of this peak is steeper than 30% (16.7° to the time axis) the "auto" function recognizes the region and excludes it. This region is not used for the approximation. The window size and the group size of the "auto" Reflection removal have to be chosen. The window size describes the size of the window in pixel used to remove the reflections.



This is the Manual Start and End Position Wizard window for determination of the Start and End Position for the approximation. The graph shows an exemplary course of a model curve. the x-axis is the time in pico seconds and the y-axis is the Photon-Frequency in counts and logarithmic scale. At the bottom of the window the Start and End Position can be set with values by clicking into the Position field and entering a value. The value can also be changed with the arrow keys to the right and left of the Position field. In this case the Start Position can be changed but the End Position can't. This is because the "auto" function for the End Position was selected in Pre-Processing window. To enable the "auto" function the "auto" button is installed. The Start and End Points can be set with the mouse within the graph. Place the mouse pointer on the desired Start Position and set it with a left click. This also applies for the End Position but this is set with a right click of the mouse. The region within the Start and End Point is colored gray. The box for the Excluded-Regions is the manually controlled Reflection Removal. To exclude regions place the mouse pointer on the desired position and press shift and left click or just the middle click of the mouse and drag the mouse to the desired end position. The region is red colored and thus excluded. The excluded regions are listed up in the box and can be removed with the "remove" button. To enable the auto function the "auto" button is installed. The "OK" button stores the settings and closes the window. "Cancel" aborts the process and closes the window.

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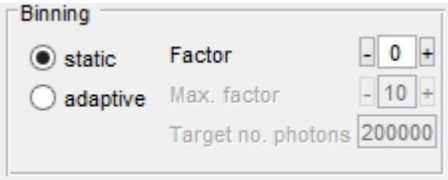
Binning



The Binning function is an image processing tool to reach a more homogeneous distribution of photons per pixel. The reason for this is that the raw image is taken with approximately 1000 photons per pixel but the various tissues absorb or reflect the light differently. A higher number of photons increases the spatial and time resolution but also the acquisition time. This is a negative aspect for the patient. The binning function combines the photons of neighboring pixels to receive a higher number of photons to start a multi-exponential approximation. There are two different binnings.

Static binning. The static binning utilizes a pre-defined window which is moved across the raw image and combines the neighboring photons for every pixel. The binning factor is the window size which correlates with the planned approximation. Thus the factor is an empirical value. For a one exponential approximation the factor is 0. For a two exponential approximation the binning factor is one. For a three exponential approximation the binning factor is two. The binning factor can be set with the arrow keys to the left and right of the factor. According to the equation **l=2*f+1,** where l is the length or edge of the binning window and f the binning factor, the length of the window for e.g f=1 is 3. This means that the binning window has a length of 3 pixels around the currently binned pixel. Thus the number of photons of nine pixels are combined, inclusive the currently binned pixel, to increase the number of photons. The disadvantage of the static binning is that darker regions will not exceed a desired threshold whereas brighter regions will. In the middle of the raw image this leads to acceptable results as very thin vessels will be more homogeneously distributed but the outer regions of the image are basically darker than inner regions. Consequently, the outer regions will again be darker than the inner regions. Even with an increase of the factor the outer regions will have a sufficient number of photons but the brighter regions lose spatial resolution.

Adaptive binning. Instead of using a squared and pre-defined window the adaptive binning utilizes a circular window which iteratively grows until a pre-defined threshold of photons is reached. If choosing the adaptive binning the maximum factor can be set quite high without losing spatial resolution because the second condition (target no. of photons) will stop the process. The target number of photons can be set by entering the desired number of photons into the input field. The adaptive binning results in a more homogeneously distributed Photon-Frequency. Even if the anatomy (vessels, macula, papilla) can not be identified the life time information is still available.

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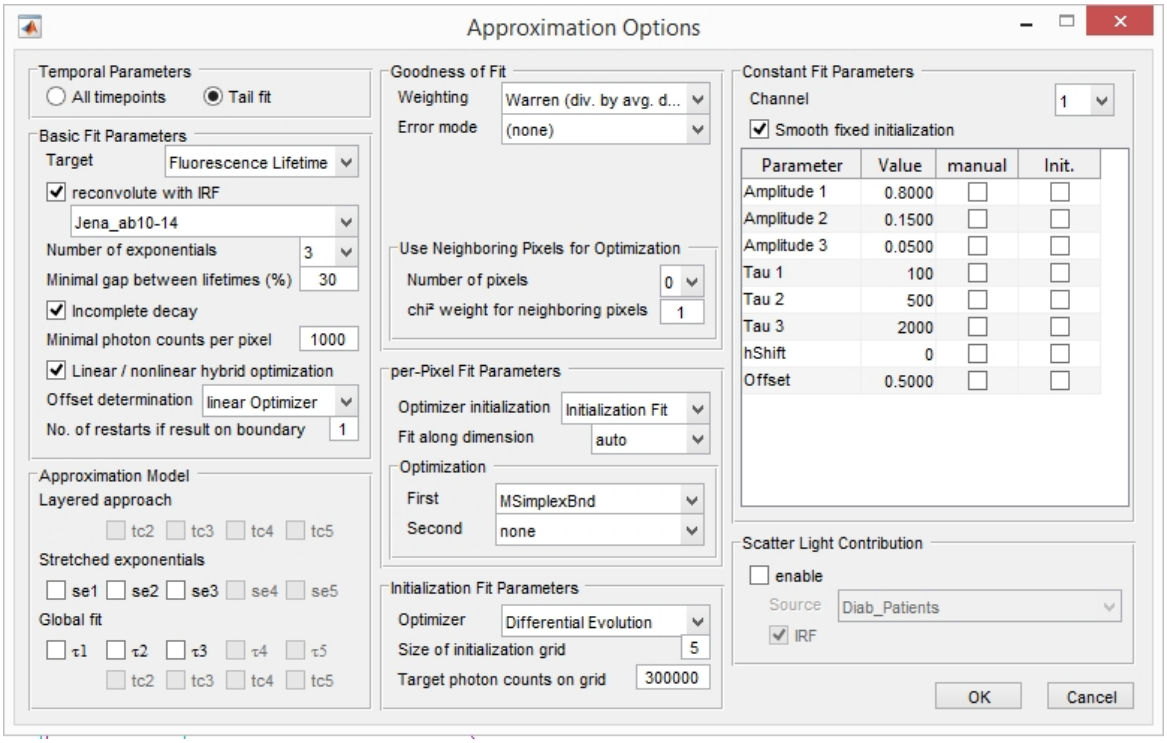
Approximation

By clicking on Approximation, the following window opens, which can also be opened with the key combination CTRL+A:



The window is divided into different parts: Temporal Parameters, Basic Fit Parameters, Approximation Model, Goodness of Fit, per-Pixel Fit Parameters, Initialization Fit Parameters, Constant Fit Parameters and Scatter Light Contribution.

Temporal Parameters

The Temporal Parameter limits the time points used for the approximation. The setting **All Timepoints** uses all points available on the whole time range. **Tail Fit** uses all the time points in the descending part of the curve.

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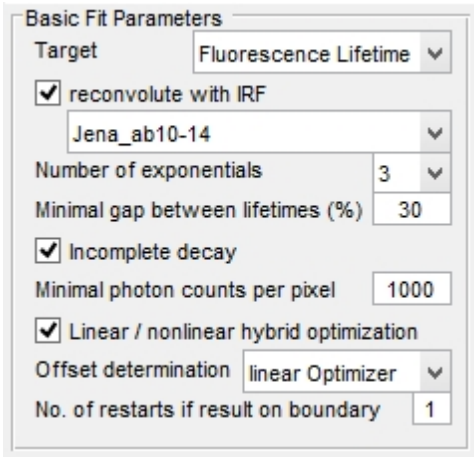
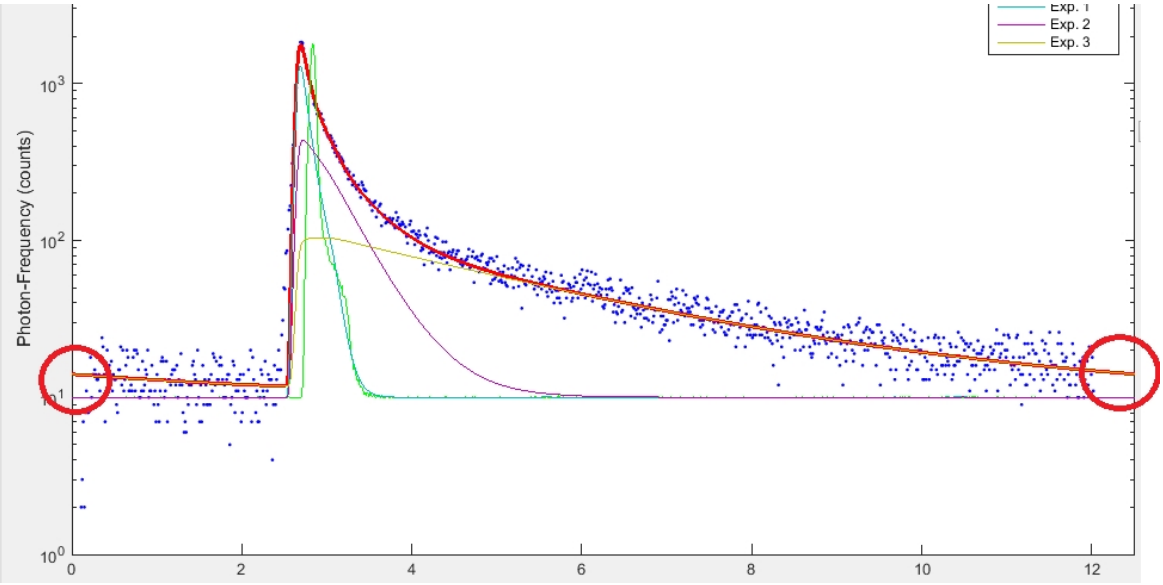
Basic Fit Parameters



In the box Basic Fit Parameters the target can be chosen. Two options are possible: The fluorescence lifetime or the anisotropy. Furthermore the IRF function can be chosen. For more details to the IRF function see also: Tools -> IRF Manager. The target and the IRF can be

convoluted with selecting the function *reconvolute with IRF.* The number of exponentials determines how many exponential functions are used to approximate the Model curve. The here selected number of exponentials refers to the settings in Bounds. The exponential function must have different lifetimes. To realize that the minimal gap between the lifetimes of the exponentials can be set to a certain threshold in percent. To create an order the lifetimes are always matched like this: $\tau_1 < \tau_2 < \tau_3$. If the Model curve does not reach the offset again because of missing measured points, the model curve can be cut and the rest of the descending part will appear at the beginning in the pre-excitation time. This option is called the incomplete decay.



This picture shows the incomplete decay. The red circles mark the Photon-Frequency, which is the same for both sides. The descending part of the model curve on the right-hand side is cut and inserted at the beginning. The program automatically considers this cut in the modulation. The binning process sums up the photons of neighboring pixels to create a homogeneous distribution of photons. The minimal Photons Counts per Pixel is an additional setting to determine the minimum number of photons per pixel after the binning. This means that after the binning process every pixel possesses at least the entered number of photons. The linear or non-linear Hybrid Optimization is an optimization process that supports the approximation. If selecting this option the linear optimization runs. If not selected the non-linear optimization runs which increases the complexity of the approximation but also increases the accuracy.

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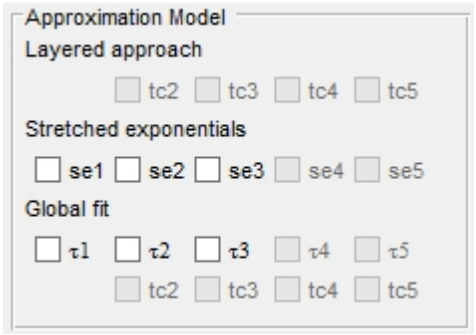
Approximation Model



If All Timepoints is selected in Temporal Parameters the layered Approach in Approximation Model can be selected. This option enables a time shift of the exponential functions in relation

to the first exponential. If selecting the exponentials for the layered approach the appropriate tc appears on the right hand side in Constant Fit Parameters with a fixed value. The stretched Exponentials describe factors which can stretch or compress the appropriate exponential. If selecting an exponential the factor appears on the right-hand side in Constant Fit Parameters with a fixed value. The factors are named Ņ . The option global fit equals the Ą -values for every spectral channel if the fluorescence remedy is the same. The tc-values are only selectable if the appropriate tc-value under layered approach is selected, too. The here selected settings refer to the settings in Bounds.

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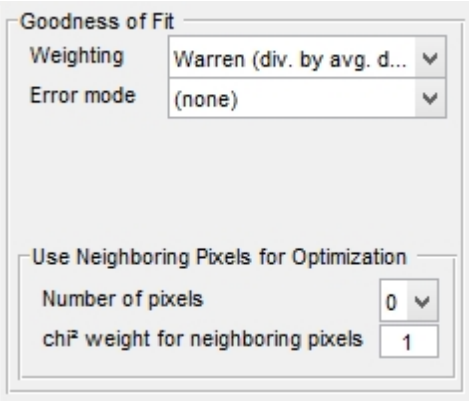
Goodness of Fit



The Goodness of Fit describes how well the Model (expectation) curve approximates the measured points (observation). The difference between the the measured points and the Model curve is the error. In statistical analysis this error is squared. The squared error is chi². It is calculated as the sum of all squared differences between Model (E) and measured points (O) divided by the variance Å². So chi² is a measure for the goodness of fit.

$$\chi^2 = \sum \frac{(O-E)^2}{\sigma^2}$$

This variance can assume different values according to the chosen weighting. The selectable weighting are the Neyman, Pearson, fitted and Warren weighting. These different weightings generate different outcomes which are all weighted on different aspects of the modulation. The variance of Neyman is formed by the data. So it equals the measured points (O). The variance of Pearson is formed by the model (E). The fitted weighting uses an initial model so the result of a previous approximation is used for the variance. The variance of Warren is formed by the average of the data (measured points). Two options are selectable for the error mode. None or chi² + Peak boost. The chi² + Peak boost emphasizes the peak of the model curve. The highest measured point is used as initial point and 5 neighboring measured points to both sides including the initial point are multiplied with the boost factor.
The Use of Neighboring Pixel for Optimization smooths the chi² for neighboring pixel. The chi² is expected to be quite similar to the chi² of neighboring pixel. The number of pixels is the range of the smoothing. 0 pixel refers to the selected pixel (no smoothing process), 4 refers to four pixels in direct contact to the selected one and 8 to eight pixels around the selected pixel. The chi² weight for neighboring pixels is a multiplication factor and describes how much the chi² are smoothed according to the selected pixel. The factor 1 describes that one part divided by the number of pixels is used for the weighting.
The approximation can be divided into two steps. The first step is to determine an initial point where to start the approximation and the second step is to approximate all pixels using this

initial point. The initial point is the pixel with the lowest chi^2.

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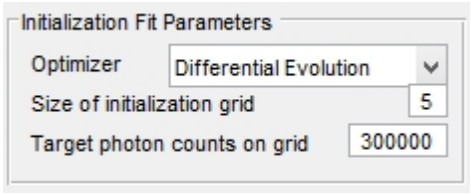
Initialization Fit Parameters



To find the lowest chi² point the Initialization Fit Parameters have to be defined. Four different Optimizers are selectable. For more detailed information about the optimizers see also "**Optimizers**" below. The Size of the Initialization grid refers to the window that is build to find the lowest chi^2. The target photo counts on grid describes the desired number of photons per pixel after the binning. If choosing the Differential Evolution avoid selecting the same optimizer under the Per-Pixel Fit Parameters.

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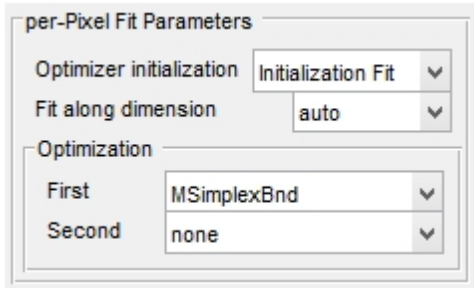
Per-Pixel Fit Parameters



The per Pixel Fit Parameters describe how the per pixel approximation is done. The initial point can be set under Optimizer Initialization. Two options are selectable there. If choosing Initialization Fit the values determined in Initialization Fit Parameters are used for the initial point. If choosing guess Values the values of a very rough estimation are used for the initial point. This can speed up the calculation and saves computational power. For determination of the guess values use the command **Determine Guess Values** under Approximation. The per pixel fit can't deal with all pixels at the same time. Therefore the pixels are fitted step by step. It can be selected along which dimension the pixels are fitted. The auto function chooses the dimension automatically. Otherwise the pixel fit can be done for each row or column. The sub-box Optimization hosts the actual fit parameters. The first optimizers generates a rough result which can be smoothed or improved with the second optimizer. Based on experiments the best setting is to use a non-linear optimizer for the first one (Differential Evolution or Particle Swarm) smoothed with a linear one (MSimplexBnd or FMinSearchBnd) as the second optimizer. The second optimizer can be disabled.

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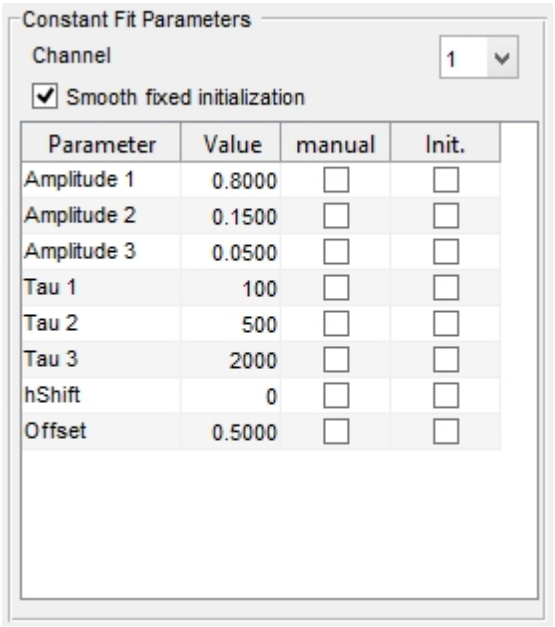
Constant Fit Parameters



The box Constant Fit Parameters on the right-hand side gives information about the currently selected channel and lists up the chosen parameters with their values. The values can't be changed as long as no check mark is in the boxes under manual. To receive values calculated by the program the boxes under init must be given a check mark.
The scatter light contribution can be enabled for measuring the excitation light in a selected study. An additional curve is plotted. As information the amplitude, the shift and the offset are given in the Constant Fit Parameter window. The IRF variety weights the scatter light curve differently. If choosing the IRF weighting additional information occur like the amplitude 2, shift 2 and offset 2.

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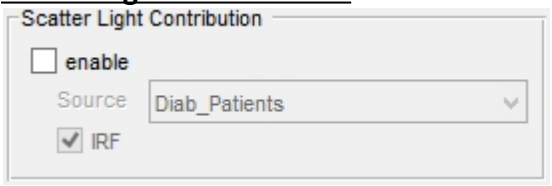
Scatter light contribution



The scatter light contribution rectifies the error arising from the lens. The lens scatters the light and a part of the excitation light gets lost. Additionally the Intensity distribution is more homogeneous. As a result of the scatter light contribution with the IRF function enabled the Model curve is equal to the scatter light curve and does not approximate the measured points. If the IRF function is disabled the scatter light curve is not folded with the IRF curve and the Model curve is approximating the measured points.

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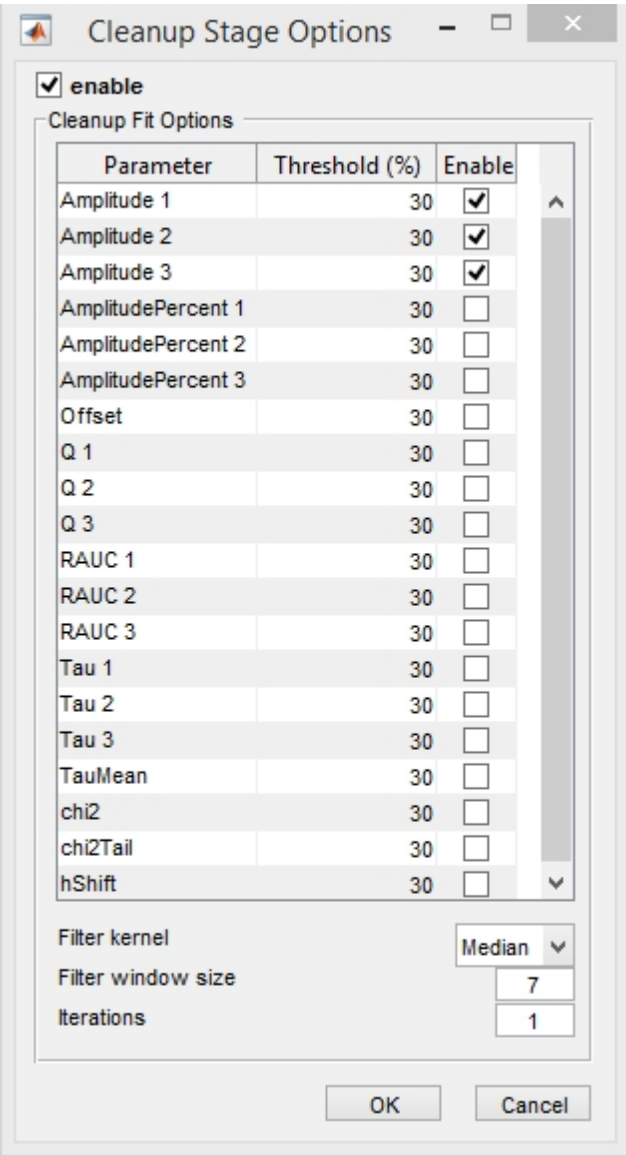
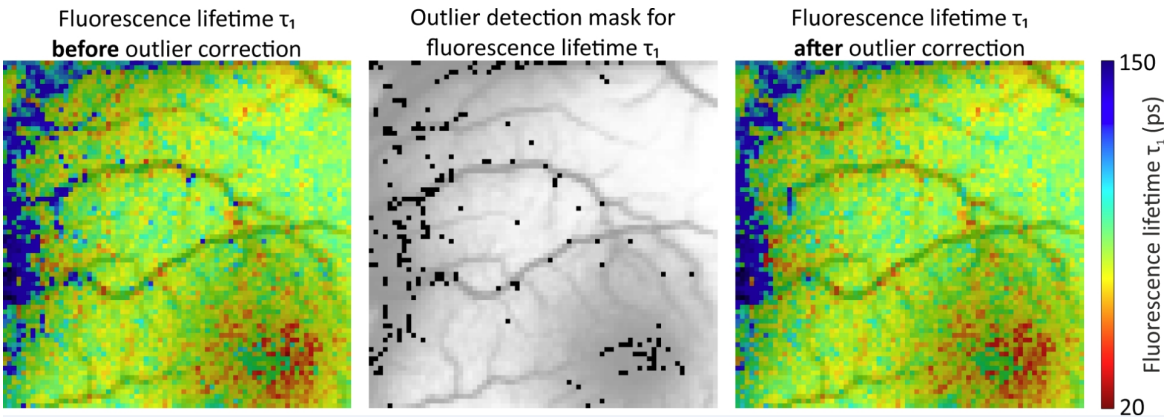
Cleanup Stage

By clicking on Cleanup Stage, the following window opens, which can also be opened with the key combination CTRL+U:



The Cleanup Stage is a process for detecting and correcting outliers. The settings of the cleanup stage allow to set the parameters which are conducted in the **Run Cleanup Stage**. For further information see also: Approximation -> Run Cleanup Stage. The Cleanup Stage can be enabled or disabled. The outlier search utilizes a pre-defined value of more than 30% difference to the values in direct neighborhood. The percentage value can be adapted as desired. Double left click on the value marks the value blue. The value can now be overwritten. When the Cleanup Stage is enabled the various parameters, where outliers have to be removed, can be selected individually. The meanings of the parameters are explained in detail in section ROI Visualization. Underneath the parameter selection the filter kernel can be chosen. Two options are selectable: Median and Mean. The Median is the middle value of a data set. As a result a boundary appears with the same amount of measured points on both sides. This value is used to correct the outliers. The Mean is the arithmetic average of the values of a data set. All values are summarized and divided by the number of values. The resulting value is used for the correction. The filter window size describes the amount of values used to find and correct an outlier. The given example with a window size of 7 means 7*7 pixels surrounding the outlier. Iterations gives the number of correction cycles. If the difference of an outlier is still higher than 30% after the first correction a second or third iteration can be initialized. How the outlier correction works is explained in section Run Cleanup Stage.
The following picture shows the idea of outlier correction.



The picture on the left-hand side shows the fluorescence lifetime as measured. Some dark blue points can be found in the middle and on the right-hand side of the left picture. These blue points are the outliers because the fluorescence lifetime distribution should be nearly the same for neighboring pixels. The picture in the middle shows the detection mask. The black points were recognized as outliers. The black points match with the blue points of the left picture stated as outliers. the picture on the right-hand side shows the result. Most of the blue points are gone and replaced with red or yellow colored pixels.

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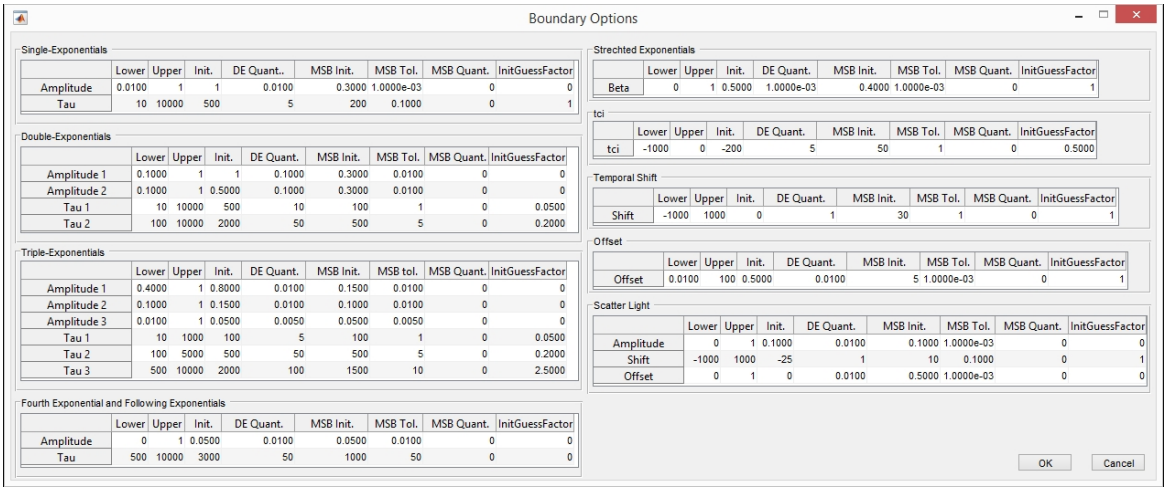
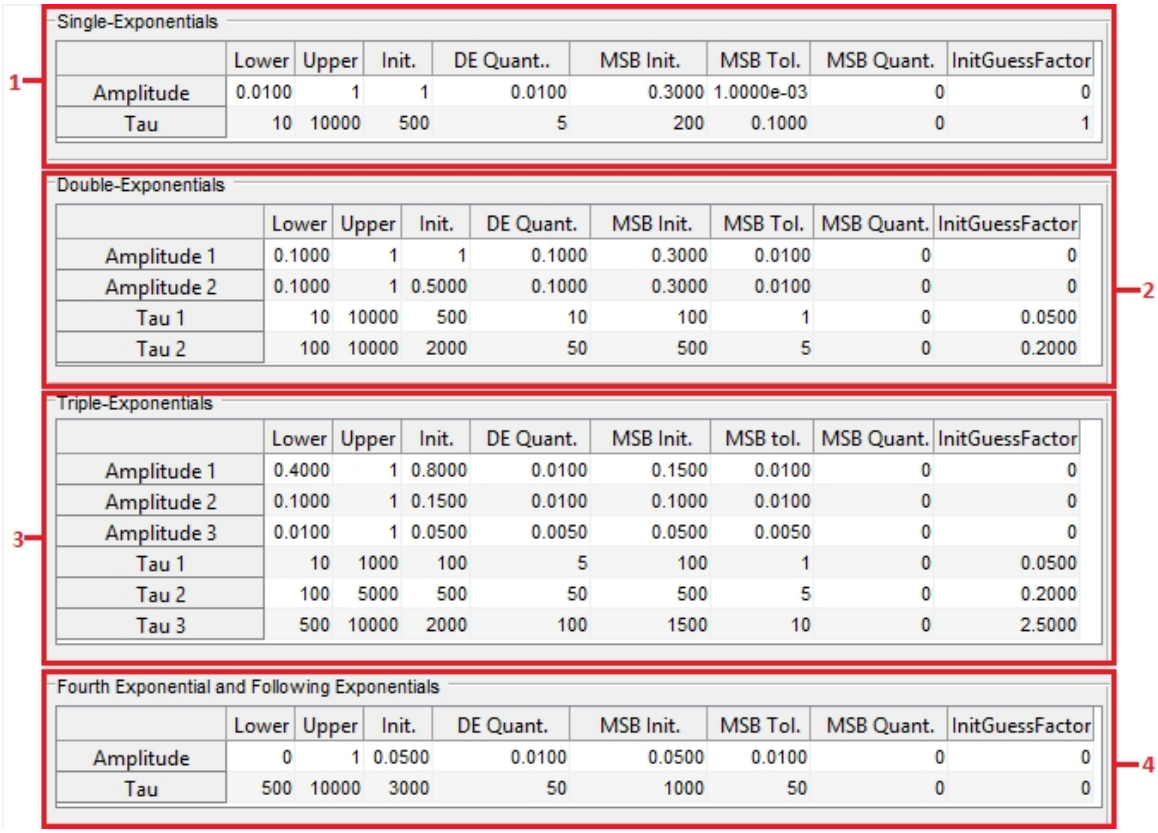
Bounds

By clicking on Bounds, the following window opens, which can also be opened with the key combination CTRL+B:



This window hosts all the settings that can be made for the maximum ranges of all shown curves. The window can be divided into two parts. The first part on the left-hand side is for the exponentials used to form the Model curve.



Single-Exponentials

	Lower	Upper	Init.	DE Quant..	MSB Init.	MSB Tol.	MSB Quant.	InitGuessFactor
Amplitude	0.0100	1	1	0.0100	0.3000	1.0000e-03	0	0
Tau	10	10000	500	5	200	0.1000	0	1

Double-Exponentials

	Lower	Upper	Init.	DE Quant.	MSB Init.	MSB Tol.	MSB Quant.	InitGuessFactor
Amplitude 1	0.1000	1	1	0.1000	0.3000	0.0100	0	0
Amplitude 2	0.1000	1	0.5000	0.1000	0.3000	0.0100	0	0
Tau 1	10	10000	500	10	100	1	0	0.0500
Tau 2	100	10000	2000	50	500	5	0	0.2000

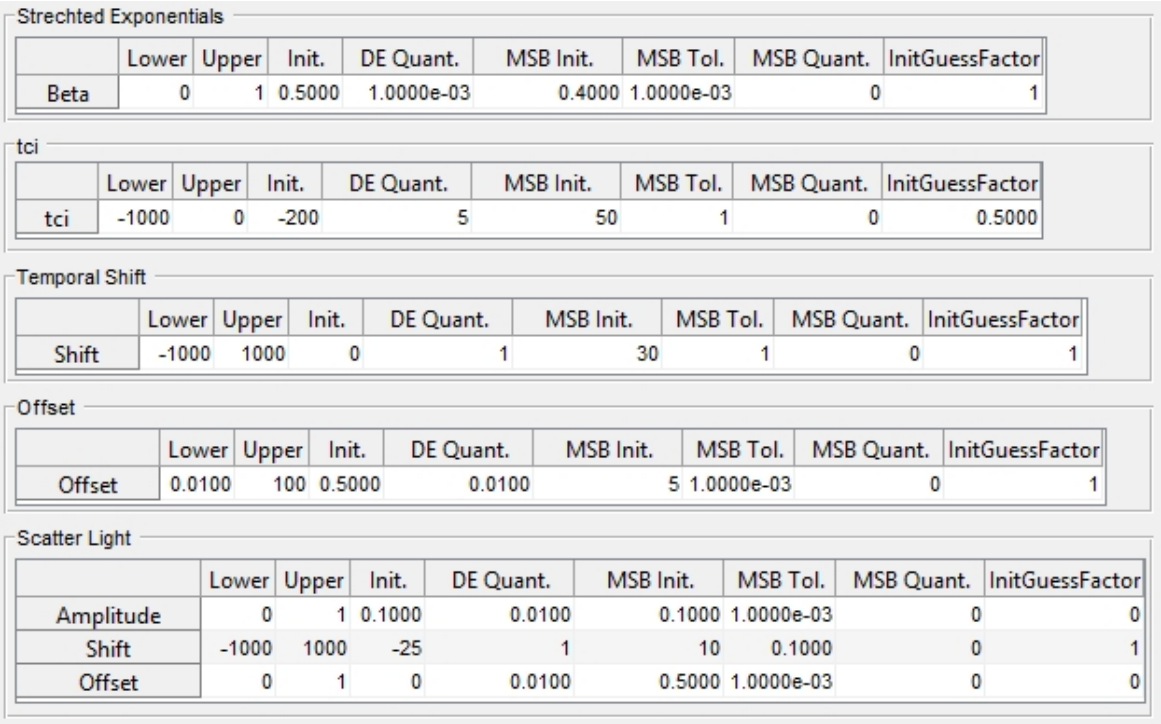
Triple-Exponentials

	Lower	Upper	Init.	DE Quant.	MSB Init.	MSB tol.	MSB Quant.	InitGuessFactor
Amplitude 1	0.4000	1	0.8000	0.0100	0.1500	0.0100	0	0
Amplitude 2	0.1000	1	0.1500	0.0100	0.1000	0.0100	0	0
Amplitude 3	0.0100	1	0.0500	0.0050	0.0500	0.0050	0	0
Tau 1	10	1000	100	5	100	1	0	0.0500
Tau 2	100	5000	500	50	500	5	0	0.2000
Tau 3	500	10000	2000	100	1500	10	0	2.5000

Fourth Exponential and Following Exponentials

	Lower	Upper	Init.	DE Quant.	MSB Init.	MSB Tol.	MSB Quant.	InitGuessFactor
Amplitude	0	1	0.0500	0.0100	0.0500	0.0100	0	0
Tau	500	10000	3000	50	1000	50	0	0

The parameter shown are the same for every box. Which box is to use for the approximation refers to the selected settings in Approximation -> Basic Fit Parameters. The selected number of exponentials there indicates which box is to use here. The Single-Exponential uses only one exponential for the approximation. This can be increased to a maximum of five. For every situation the settings can be adapted for the approximation. Usually the values are determined automatically but the boundaries can be set manually if a more special view is needed or the approximation can be improved because of a priori knowledge. The lower and upper boundary describe the minimum and maximum value in which the approximation is made. The Initialization value defines a initial point for the approximation if an empirical value is available. The DE Quantization describes the sampling distance of the Differential Evolution. The same applies for the MSB (Most significant bit) Init. (Initialization) It describes the size of the MSB window. For further information to DE and MSB see also Optimizers below. A high Init. value lowers the computational power needed but also lowers the chance to find the global (whole RAW image included) minimum of chi^2. A very low Init. value increases the computational power needed and thus the approximation time but the global minimum will be found. The MSB tol. (tolerance) describes the function's exit criteria. The MSB function is a window which literally moves across the RAW image to find the lowest chi^2. If the movement nearly stops this point is found. So if the movement per iteration step is under the defined threshold the process is stopped. MSB Quant. (Quantization) is an additional setting for the speed of the MSB function. Commonly this value is 0 but a higher value results in a faster movement of the window but lowers the precision of the search. The global minimum could be skipped. The InitGuessFactor describes a multiplication factor to weight the exponentials. The factor is calculated as average out of the measured points of the descending part of the Model curve. Commonly the InitGuessFactor for the exponentials is 0.05 for the first, 0.2 for the second and 2.5 for the third exponential ind a triple exponential approximation.



Strechted Exponentials

	Lower	Upper	Init.	DE Quant.	MSB Init.	MSB Tol.	MSB Quant.	InitGuessFactor
Beta	0	1	0.5000	1.0000e-03	0.4000	1.0000e-03	0	1

tci

	Lower	Upper	Init.	DE Quant.	MSB Init.	MSB Tol.	MSB Quant.	InitGuessFactor
tci	-1000	0	-200	5	50	1	0	0.5000

Temporal Shift

	Lower	Upper	Init.	DE Quant.	MSB Init.	MSB Tol.	MSB Quant.	InitGuessFactor
Shift	-1000	1000	0	1	30	1	0	1

Offset

	Lower	Upper	Init.	DE Quant.	MSB Init.	MSB Tol.	MSB Quant.	InitGuessFactor
Offset	0.0100	100	0.5000	0.0100	5	1.0000e-03	0	1

Scatter Light

	Lower	Upper	Init.	DE Quant.	MSB Init.	MSB Tol.	MSB Quant.	InitGuessFactor
Amplitude	0	1	0.1000	0.0100	0.1000	1.0000e-03	0	0
Shift	-1000	1000	-25	1	10	0.1000	0	1
Offset	0	1	0	0.0100	0.5000	1.0000e-03	0	0

The parameters on the right-hand side can only be adapted if the appropriate settings are selected in Approximation -> Approximation Model and Constant Fit Parameters. Usually the values are determined automatically but the boundaries can be set manually if a more special view is needed or the approximation can be improved because of a priori knowledge. The lower and upper boundary describe the minimum and maximum value in which the approximation is made. The Initialization value defines a initial point for the approximation if an empirical value is available. The DE Quantization describes the sampling distance of the Differential Evolution. The same applies for the MSB Init. It describes the size of the MSB window. For further information to DE and MSB see also Optimizers below. A high Init. value lowers the computational power needed but also lowers the chance to find the global (whole RAW image included) minimum of chi^2. A very low Init. value increases the computational power needed and thus the approximation time but the global minimum will be found. The MSB tol. (tolerance) describes the function's exit criteria. The MSB function is a window which literally moves across the RAW image to find the lowest chi^2. If the movement nearly stops this point is found. So if the movement per iteration step is under the defined threshold the process is stopped. MSB Quant. (Quantization) is an additional setting for the speed of the MSB function. Commonly this value is 0 but a higher value results in a faster movement of the window but lowers the precision of the search. The global minimum could be skipped. The InitGuessFactor describes a multiplication factor to weight the exponentials. The factor is calculated as average out of the measured points of the descending part of the Model curve.

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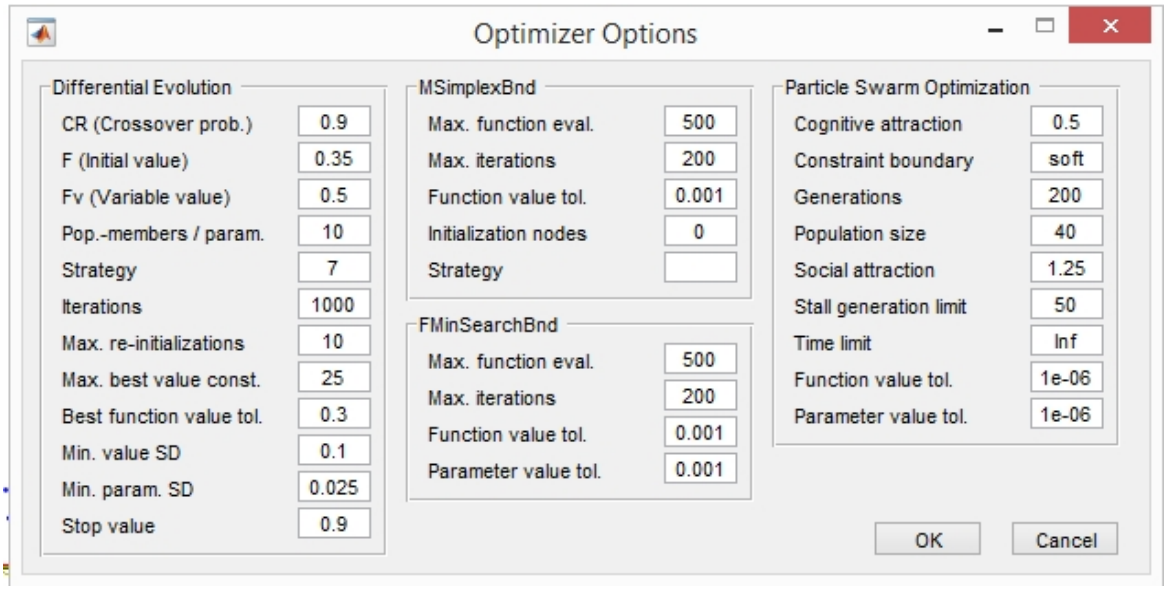
Optimizers

By clicking on Optimizers, the following window opens, which can can also be opened with the key combination CTRL+O:



There is a separate box for each optimization algorithm:

Differential Evolution

The Differential Evolution is described by Rainer Storn and Kenneth Price in an article for the *Journal of Global Optimization* volume 11 from 1997 printed in the Netherlands. It is a heuristic parallel direct search method. A randomly chosen number of initial vectors of the first generation is defined. The algorithm generates new vectors of the second generation.
The main control variables are CR, F and Pop.-members / Param.. CR describes the probability of the crossover process which forms the new vectors of the consecutive generations. A high CR makes it more likely to change most parameters of the vector. F describes the weight of the difference between two vectors which is used for generating the a vector which is used for the new vectors. Pop.-members/Param are the number of initial vectors of the first generation. Iterations are the number of generations that are formed during the process. The max Re-Initializations describes the number of repetitions that can be performed if the result of the last approximation is unsatisfactory.

For more information see also: Storn_1997_Differential evolution
and: Differential Evolution MATLAB

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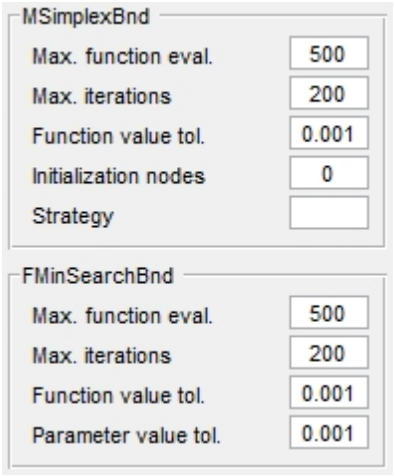
MSimplexBnd and FMinSearchBnd



The two linear algorithms MSimplexBnd and FMinSearchBnd are based on the algorithm described by J. A. Nelder and R.Mead in an article for *The Computer Journal* volume 7 from 1965 printed in Oxford. The algorithm utilizes an object which is moved across the space. The object's dimension is one higher than the space. In this case the RAW image has two dimensions and the object three. Because of the higher dimension the search algorithm is much more stable.

For more information see also: Nelder_1965_A simplex method for function minimization and: MSimplexBnd FMinSearchBnd

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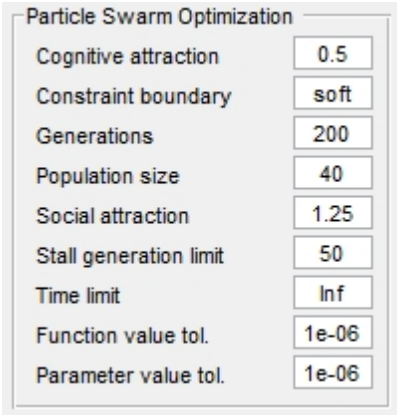
Particle Swarm Optimization (PSO)



The **Particle Swarm Optimization (PSO)** utilizes randomly chosen points in the space (particles) which move around the space to find the optimum (lowest chi^2). The algorithm is described by Dr. Eberhart and Dr. Kennedy in 1995. This moving swarm is directed by two best solutions. One best solution is the best solution of any particle it has achieved so far (pbest) and the other best is the best solution of all particles, a global best (gbest). The gbest is the particle which is tracked by all the other particles. The cognitive and the social attraction describe the learing factors of the swarm patricles. The particle with the gbest value is moving towards the optimum with a velocity in cognitive attraction. The other particles follow at the speed of the social attraction. Every movement is a new generation thus the number of generations limits the optimization process. The population size describes the number of

particles that are used. The time limit is only set if the adaption of a new generation takes longer. In that case the time limit can be set to abort the process although the maximum number of generations is not reached.

For more information see also: Particle Swarm Optimization
and: Particle Swarm MATLAB

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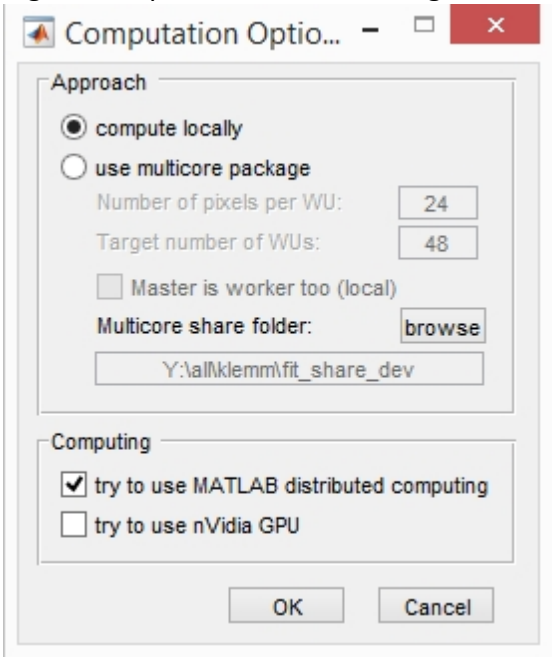
Computation

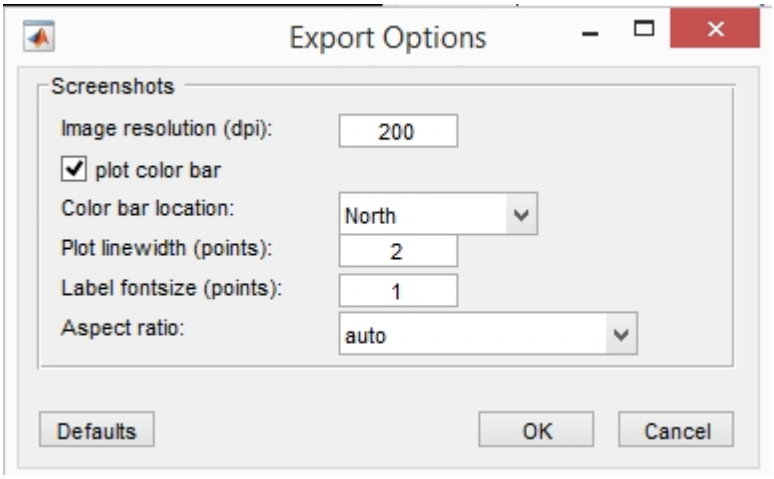
By clicking on Computation, the following window opens:



Under **Approach** means the approximation. Compute locally means that only the processor(s) of the local computer calculate the fluorescence approximation. This takes more time but for smaller amounts of calculations this setting is sufficient. The Multicore package utilizes the computational power of servers, which have to be setup and are synchronized using a shared folder (please refer to the documentation of the multicore package). The local computer is the master. If "Master is Worker too" is chosen, the local computer also joins the calculation. Under **Computing**, the user can choose if the Matlab parallel computing toolbox should be used (if available). GPU utilization is currently not supported.

Export

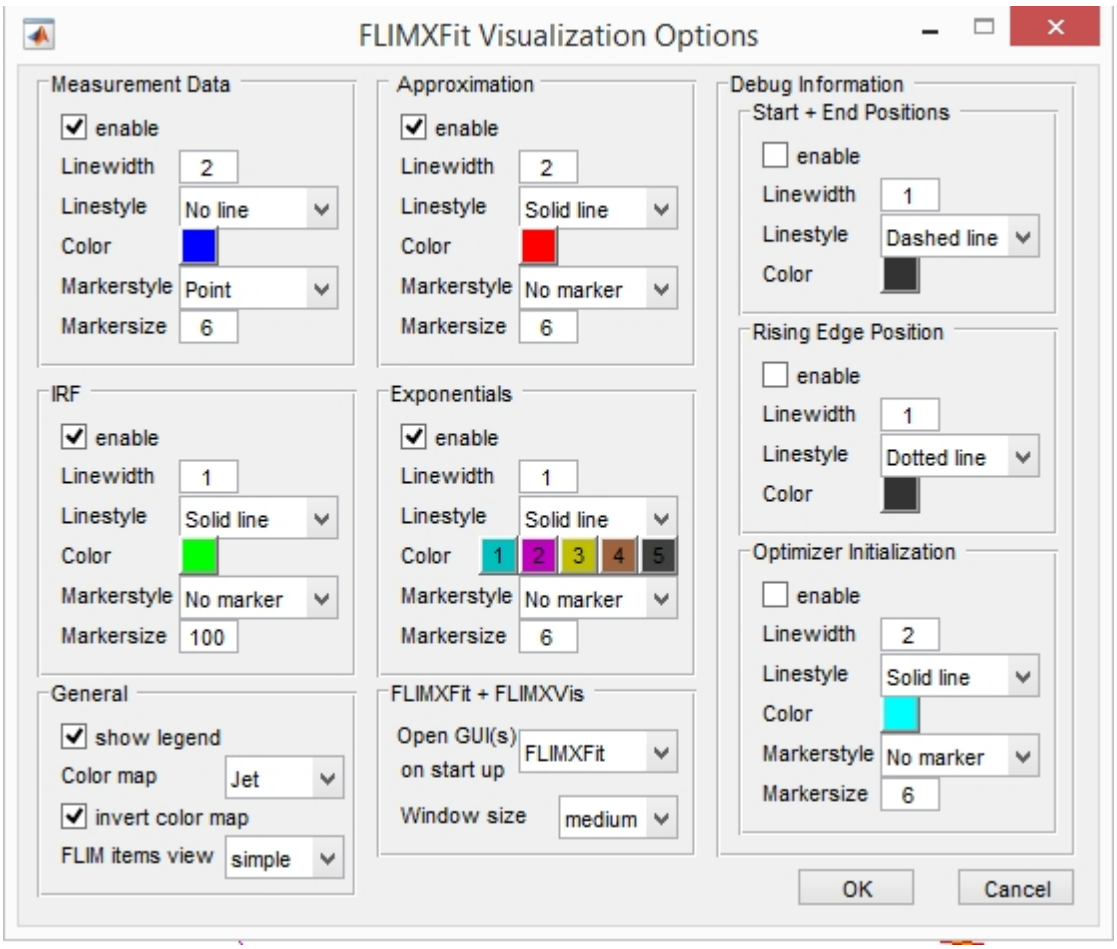
Clicking on Export, the following window opens:



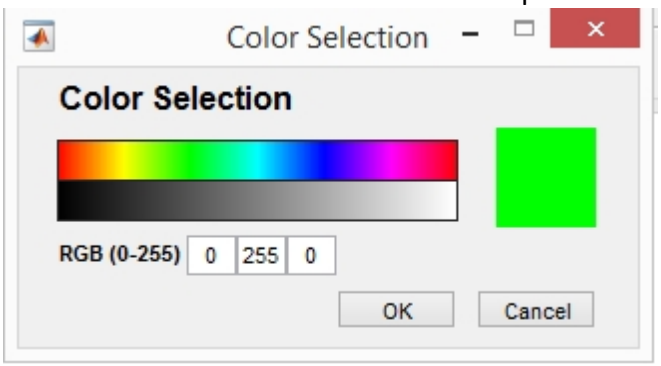
The Export Options refer to the Screenshots that can be made under Export -> Screenshot. The Image resolution is commonly set to 200 dpi. The color bar can be plotted if enabling plot color bar. The color bar can be placed within the main window of the Photon-Frequency graph or outside. Additionally the linewidth and the fontsize can be adapted. The picture above shows the default settings. To return to these settings the "Default" button is installed. The "OK" button saves the settings. "Cancel" aborts the process and the Export Options window is closed.

Visualization

Clicking on Visualization, the following window opens, which can also be opened with the key combination CTRL+V:



This hosts options for the depiction of the Photon-Frequency graph. All curves can be enabled to show them in the graph and disabled to hide them. On the left hand side the Measurement Data, the IRF curve general options can be adapted. The Measurement Data describe the measured points in the graph. Commonly there is no linestyle because this directly connects the points. The Measurement Data is only displayed as points (Markerstyle) and the Markersize is 6. The minimum linewidth and markersize is one and a maximum is not given. Commonly the best visualization is between one and ten. This applies for all settings. The Start + End Position and the Slope Start Position are commonly disabled. The color can be changed by clicking on the colored button. The window Color Selection is opened.



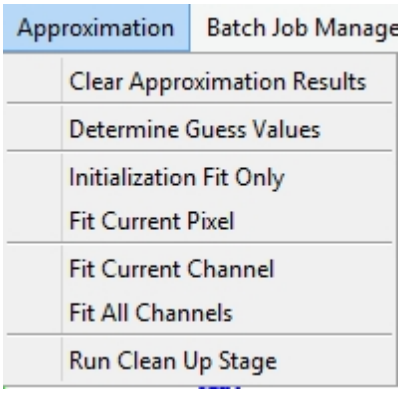
The two bars on the left-hand side show the spectrum of colors and contrasts that are selectable. The RGB (Red Green Blue) input fields go from 0 to 255. The figure shows a green color. The RGB value is 0-255-0. All colors can be mixed by adding a red or blue value different to zero. If the three values are 0 the color is black, if 255 the color is white. The colors can also be selected by clicking on the color bars at the desired color. On the right-hand side the currently color is displayed. With clicking the "OK" button the color is assumed with "Cancel" the initial color is kept. The color selection applies for all colors.

FLIMXFit + FLIMXVis
With the selection FLIMXFit + FlLIMXVis the start window can be chosen and in which size the window appears.

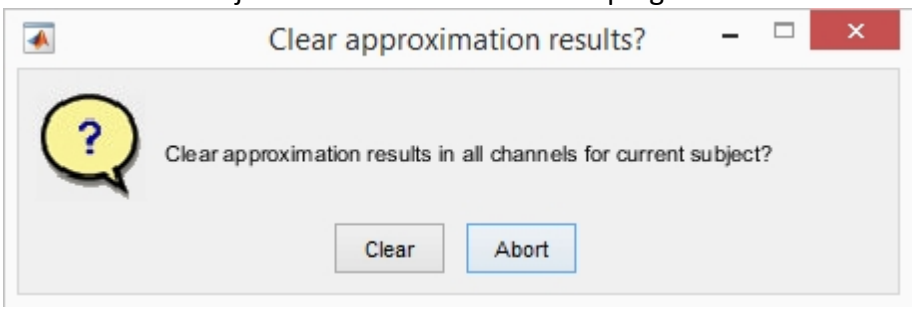
General
The box General hosts settings about the legend and the view of the FLIM items. The legend can be enabled or disabled. The visualization of the color map can be chosen from a list and inverted if desired. The FLIM items view refers to the selectable items in the ROI visualization pop up menu.
There are three options:
simple 'Tau','AmplitudePercent','Offset','TauMean','Q','hShift','chi2' are selectable,
expert:
'Tau','AmplitudePercent','Offset','TauMean','Q','hShift','chi2','RAUC','RAUCIS','Amplitude','MaximumPosition','MaximumPhotons','chi2Tail' are selectable,
all all items are selectable.

Approximation

Approximation
With clicking on this option a window opens with seven functions.



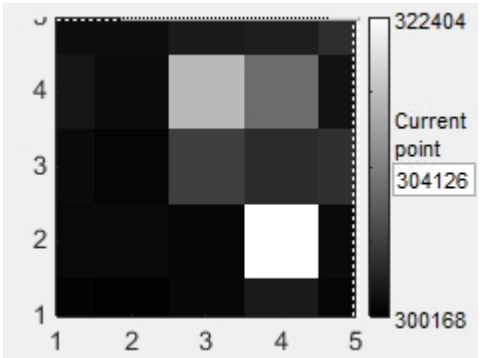
Clear Approximation Results deletes the approximations results for all pixels and channels done for the selected subject. Before the deletion the program asks to delete the data.



With the button "Clear" the program deletes the data. "Abort" stops the process.

Determine Guess Values is needed for the per-Pixel Fit Parameters (see also: Settings -> Approximation). Every pixel has its own Optimizer Initialization. The guess values lower the computational effort because the initialization is guessed. The accuracy of the Initialization and because of that the accuracy of the approximation is lowered. The guess values are good for a rough approximation.

Initialization Fit Only calculates the initial point for further approximations. The settings for this function can be adapted in Settings -> Approximation -> Initialization Fit Parameters. The picture below shows the result for the Differential Evolution, a grid size of 5 pixels and a target number of photons of 300000.



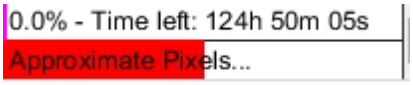
Fit Current Pixel runs an approximation for the currently selected pixel and channel.

Fit Current Channel runs an for all pixels of the currently selected channel and subject.

Fit All Channels runs an approximation for all pixels of all channels of the selected subject.

Run Clean Up Stage runs a additional clean up stage.

The progress and estimated time of competition of each operation is shown in the progress bars:



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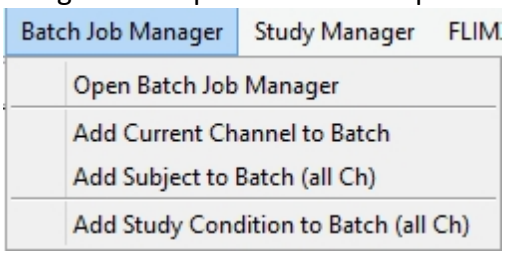
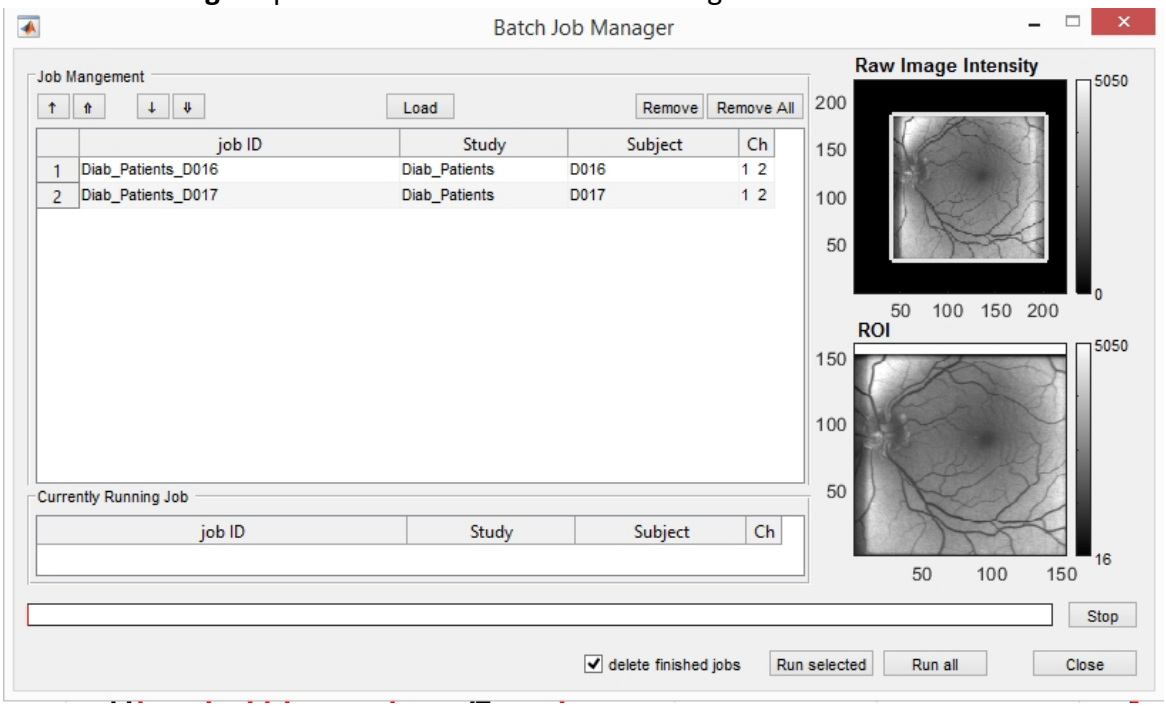
Batch Job Manager

Batch Job Manager

With clicking on this option a window opens with four functions.



Open Batch Job Manager opens the window Batch Job Manager.



This window hosts all the selected subjects which data have to be approximated. To insert jobs into the Manager the functions **Add Current Channel to Batch**, **Add Subject to Batch (all Ch)** and **Add Study Condition to Batch (all Ch)** can be selected. With Add Current Channel to Batch the currently in the FLIMXFit window selected channel of the subject is inserted. Add Subject to Batch (all Ch) inserts the currently in the FLIMXFit window selected Subject to the Manager with all measured Channels. The function Add Study Condition to Batch (all Ch) inserts

all Subjects of the selected Study into the Manager under the condition that is selected. The Manager only assumes Subjects which fulfill the condition. If no condition is selected all Subjects of the Study are inserted.

In the **Job Management** box the jobs are listed. According to the insertion order of the subjects the numbers are given. To change the number of a subject in this order the arrow keys can be used. The single arrows move the selected subject one number up or down. The double arrows move the selected subject to the end or the beginning of the list. The button "Load" loads the data of the selected subject into the FLIMXFit window. With the buttons "Remove" and "Remove All" the subjects can be removed individually or as a whole. The list of subjects consists of the job ID which is formed by the name of the study followed by the name of the subject out of this study. This job ID has to be unique otherwise the program can't match the subject and its study. The study name and the subject name are also given in. The line Ch shows the Channel(s) that are selected to be approximated. The RAW image intensity and the ROI picture are taken from the currently selected subject. This is to check whether the ROI is right and the selected subject belongs to the shown picture.

The box **Currently Running Job** shows the subject and the channel of the subject that is currently been working on.

The white bar at the bottom of the window is the **status bar** for the currently running job. The "Stop" button aborts the process and the job returns to the batch. The function delete finished Jobs is commonly enabled to create free space when the approximation is completed. With the buttons "Run Selected" and "Run All" the subjects can be approximated individually or as a whole. The Run All function treats the subjects sequentially. The button "Close" closes the Batch Job Manager. If choosing "delete finished Jobs" the Batch Job Manager automatically removes the job from the list.

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Study Manager

Study Manager
With clicking on this option the subprogram Study Manager can be opened. See also: Study Manager

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FLIMXVis

FLIMXVis
With clicking on this option the subprogram FLIMXVis can be opened. See also: FLIMXVis

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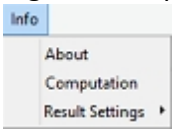
Info

Info

By clicking on this option, a window with three options opens:



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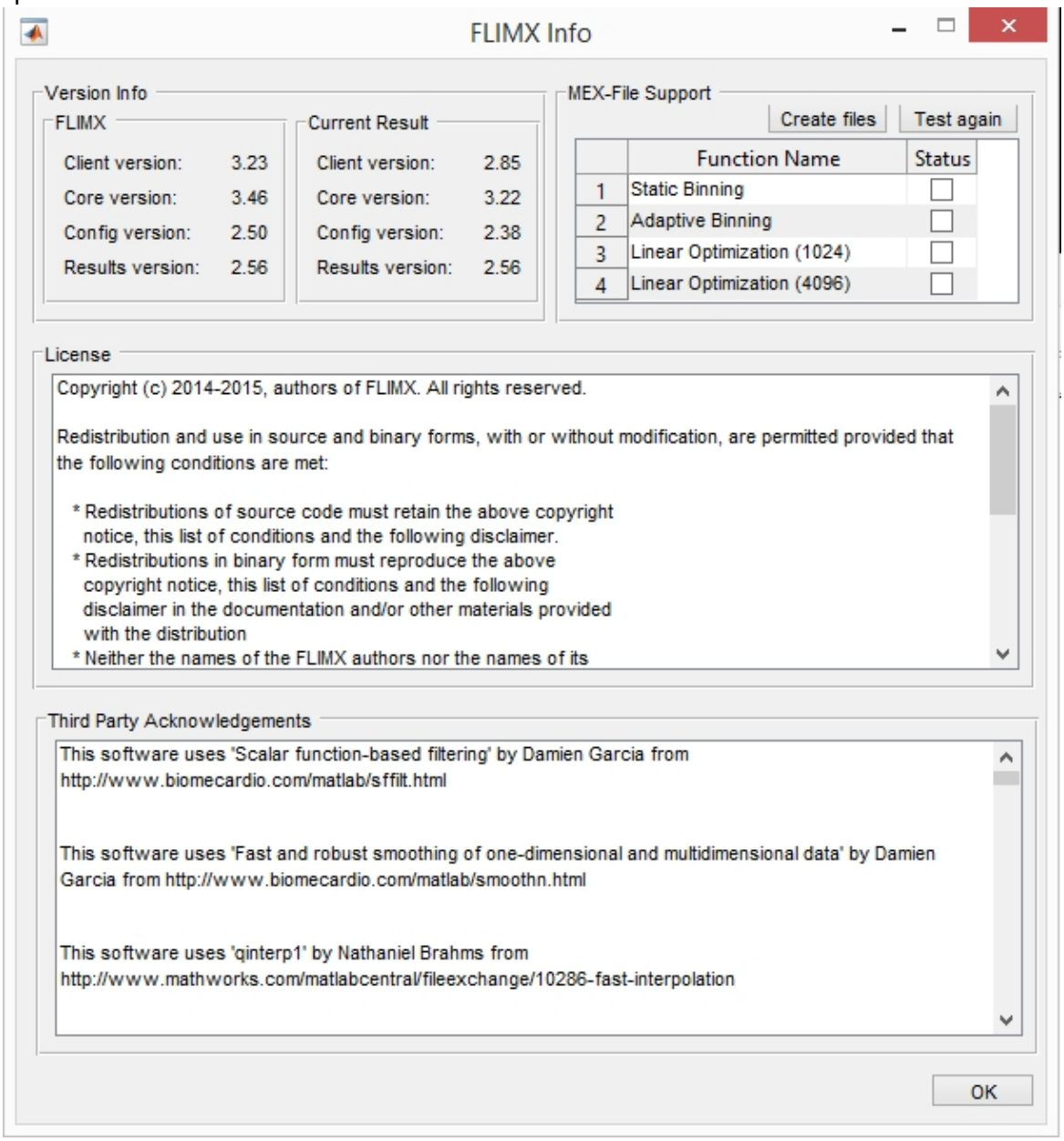
About

About opens the window FLIMX Info.



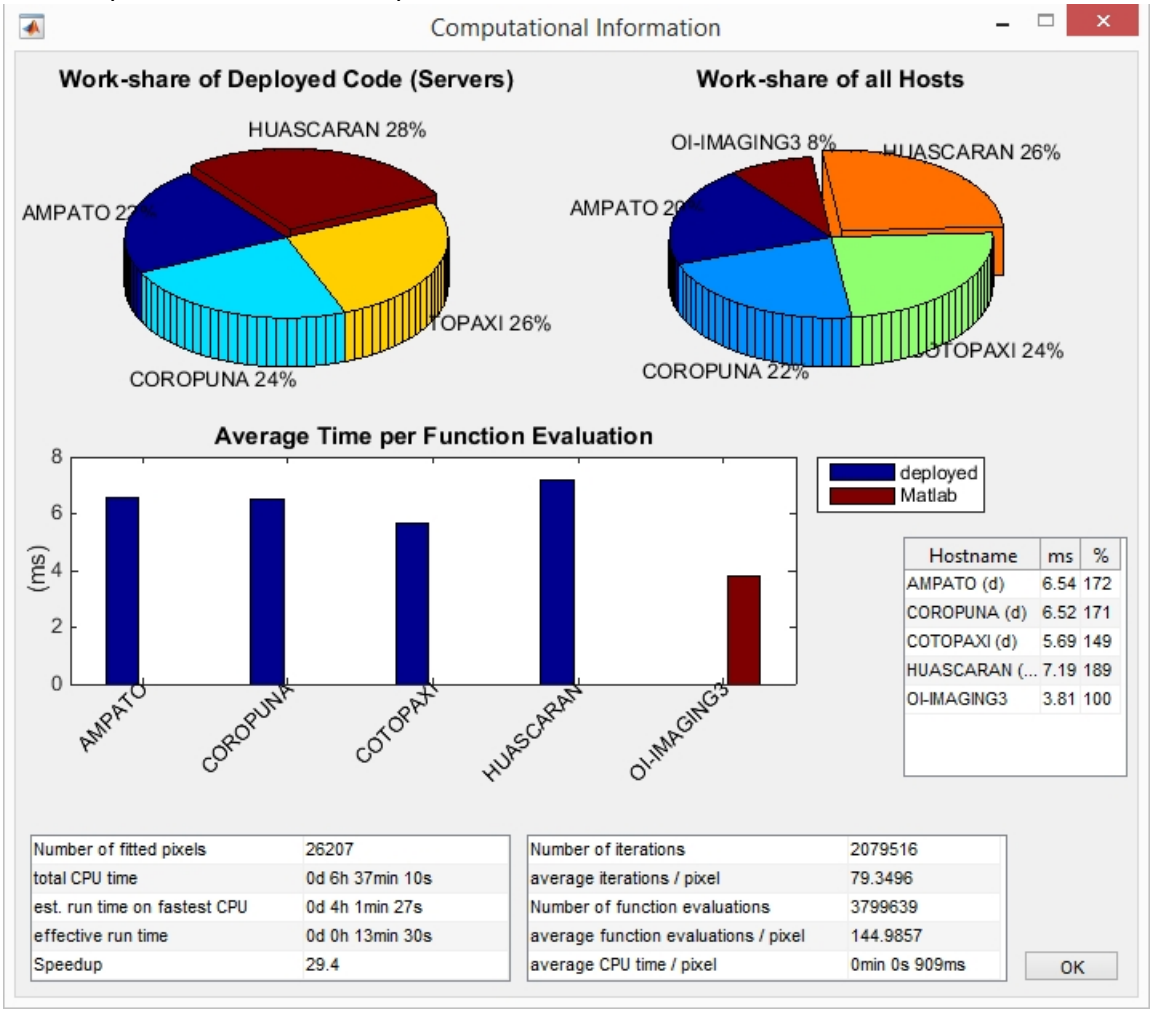
This window hosts information about the FLIMX version. The MEX-file Support shows whether a MEX-file for the specific function is available. MEX-files are MATLAB code translated to C/C++ and provide a significant speedup. The MEX-files can be created using the button "Create files" which requires a C/C++ compiler supported by MATLAB.

License shows the license agreement. The Third Party Acknowledgements lists up the functions

or subroutines for MATLAB that are used for this software.

Computation

Computation opens the window Computational Information.



This window shows the distribution of the computational power in percent. The servers (left pie chart) are deployed for the multicore process. If the master is worker too, an additional MATLAB host is deployed. The average time per function evaluation is given as bar chart and table. In addition, the table gives the function evaluation time relative to the fastest computer. The boxes below give statistic information about the fitting process.

Result Settings

Result Settings opens a new window with five options of the Settings tab: Pre-Processing, Approximation, Bounds, Optimizers and Computation. The settings which have been used to compute the current approximation result are shown.

FLIMXVis

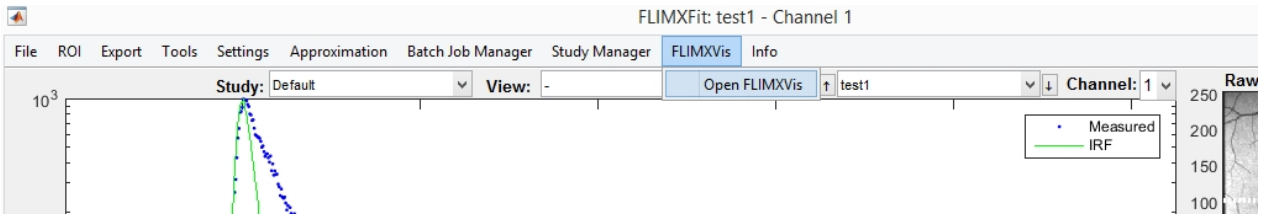
FLIMXVis is a powerful tool to visualize your FLIMXFit results, analyze these results statistically and export your data.
Before analyzing data in FLIMXVis, you must create or import this data using Study Manager.
This guide helps you to understand and use the following functionalities:

- Open FLIMXVis
- Dataset Options: Select study and subject, parameters and views
- Region Of Interest: Define a region of interest and use the ETDRS (Early Treatment Diabetic Retinopathy Study) grid
- Supplemental Plots: Visualize a histogram for your data or cross-sections of your image
- Descriptive Statistics: Show descriptive statistics of your data
- Scatter Plots: Create scatter plots from your data
- Group Comparison: Analyze difference between pathologic and control groups and find a classifier
- Export Data : Export Screenshots, Excel data or movies

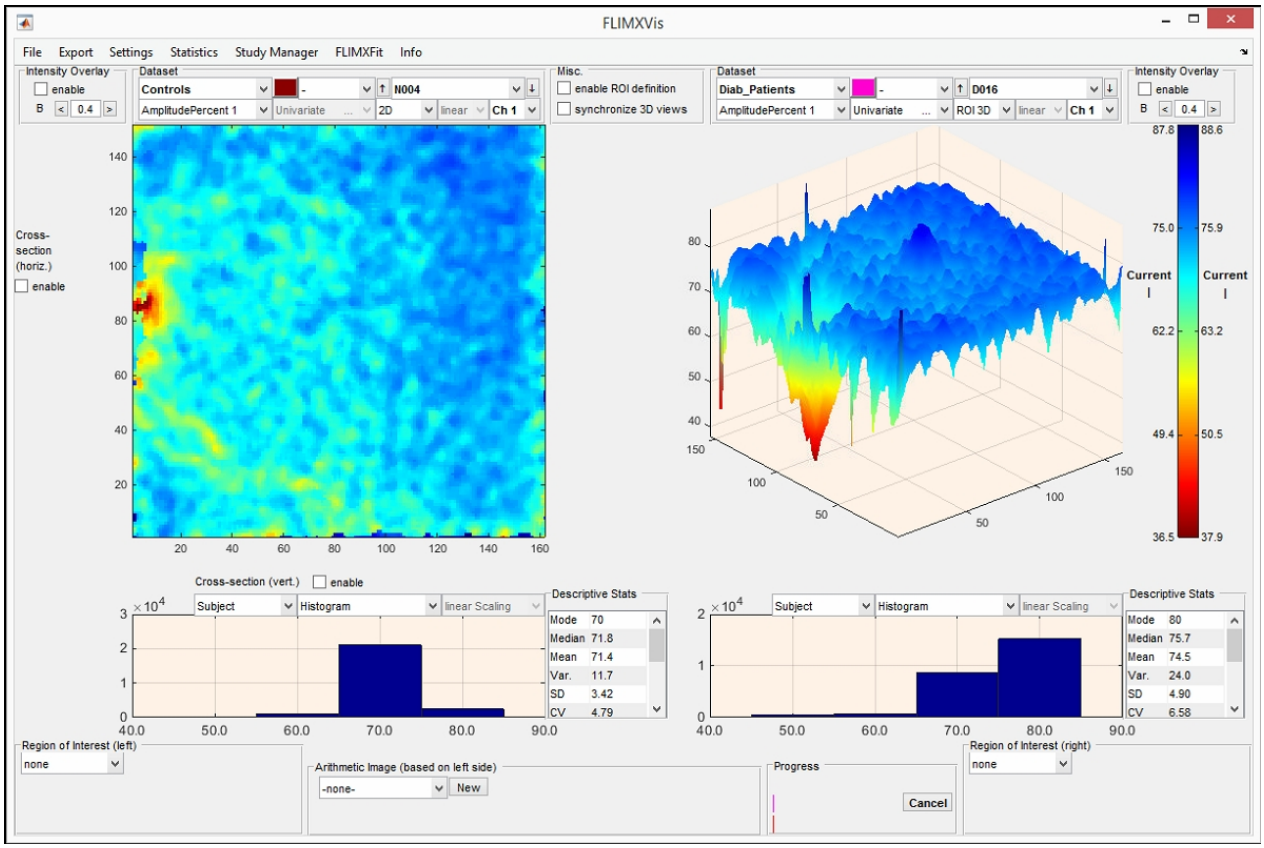
Open FLIMXVis

To open FLIMXVis from FlimXFit,

Open menu item **FLIMXVis** > **Open FLIMXVis**



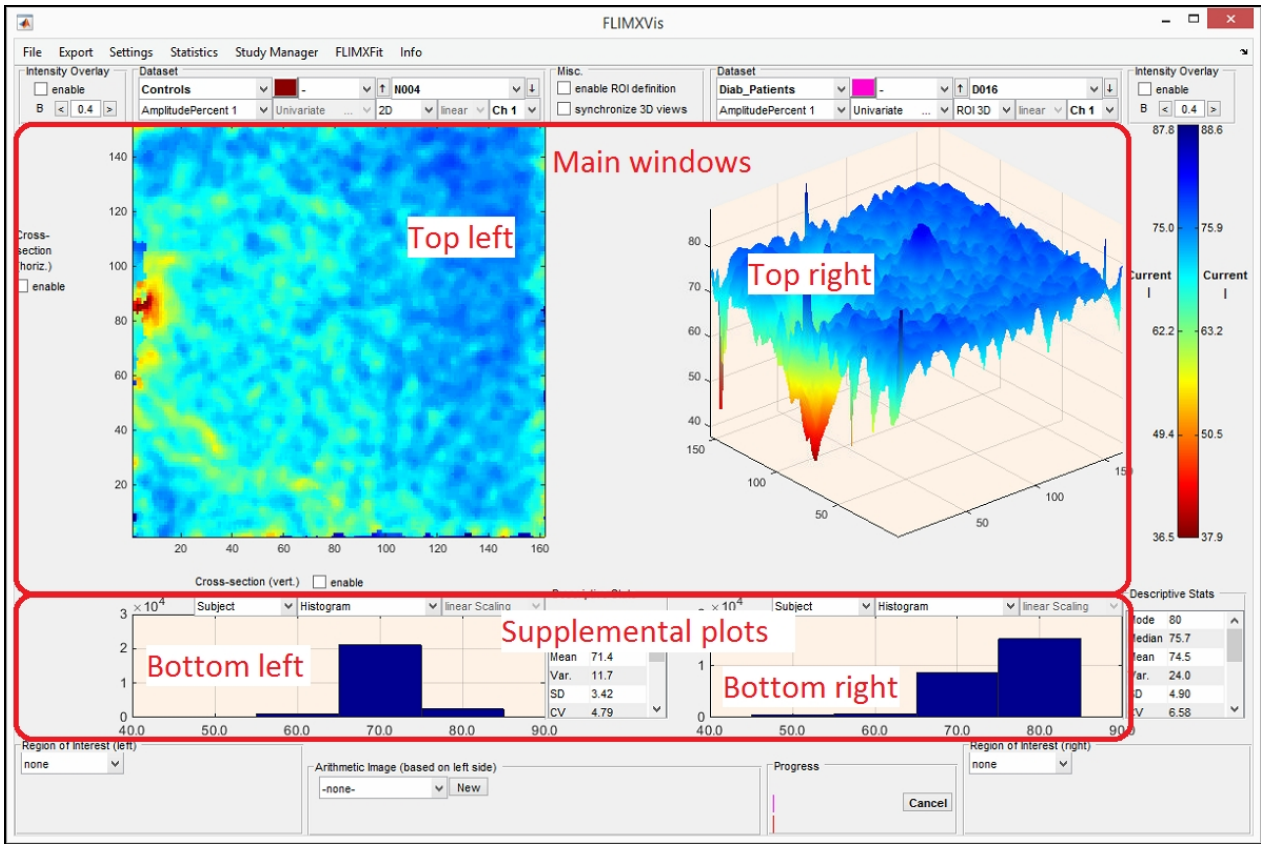
A window with FLIMXVis will pop up:



To set FLIMXVis as default when starting the GUI or change window size, see FluoDecayFitGUI + FLIMVis.

FLIMXVis contains four independent windows which are Top Left, Top Right, Bottom Left and Bottom Right.
The top windows are called main plots. The bottom windows are called supplemental plots.

The allocation is shown in the following picture:



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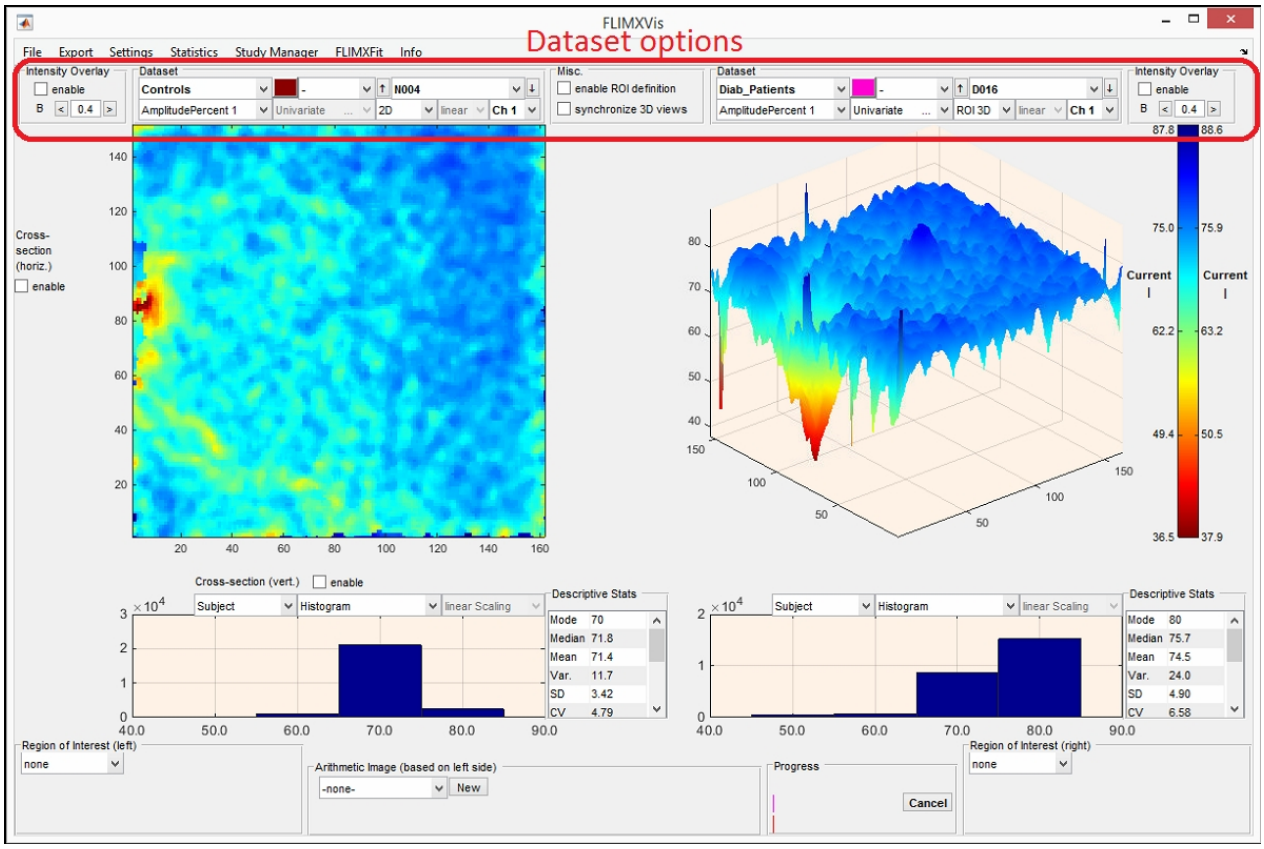
Dataset Options

Within this section, Dataset Options are explained. The dataset options within FLIMXVis GUI are marked by a red frame in the picture below.

- Selecting Data: Select study, subject, channel and parameter
- Dimension: Show 2D, 2D (ROI) or 3D (ROI) view of your image
- Views: Display data with different views
- Linear and log10 Colormap: Use different colormaps for the presentation of your data
- Intensity Overlay: Enable intensity overlay for better contrast and orientation
- Miscellaneous: Enable ROI definition and synchronize 3D views



Selecting Data

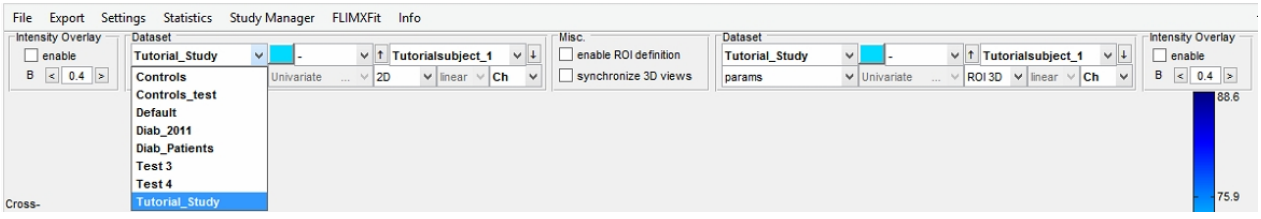
Within this section, options for selecting

- Study
- Subject
- Channel and
- FLIM parameter

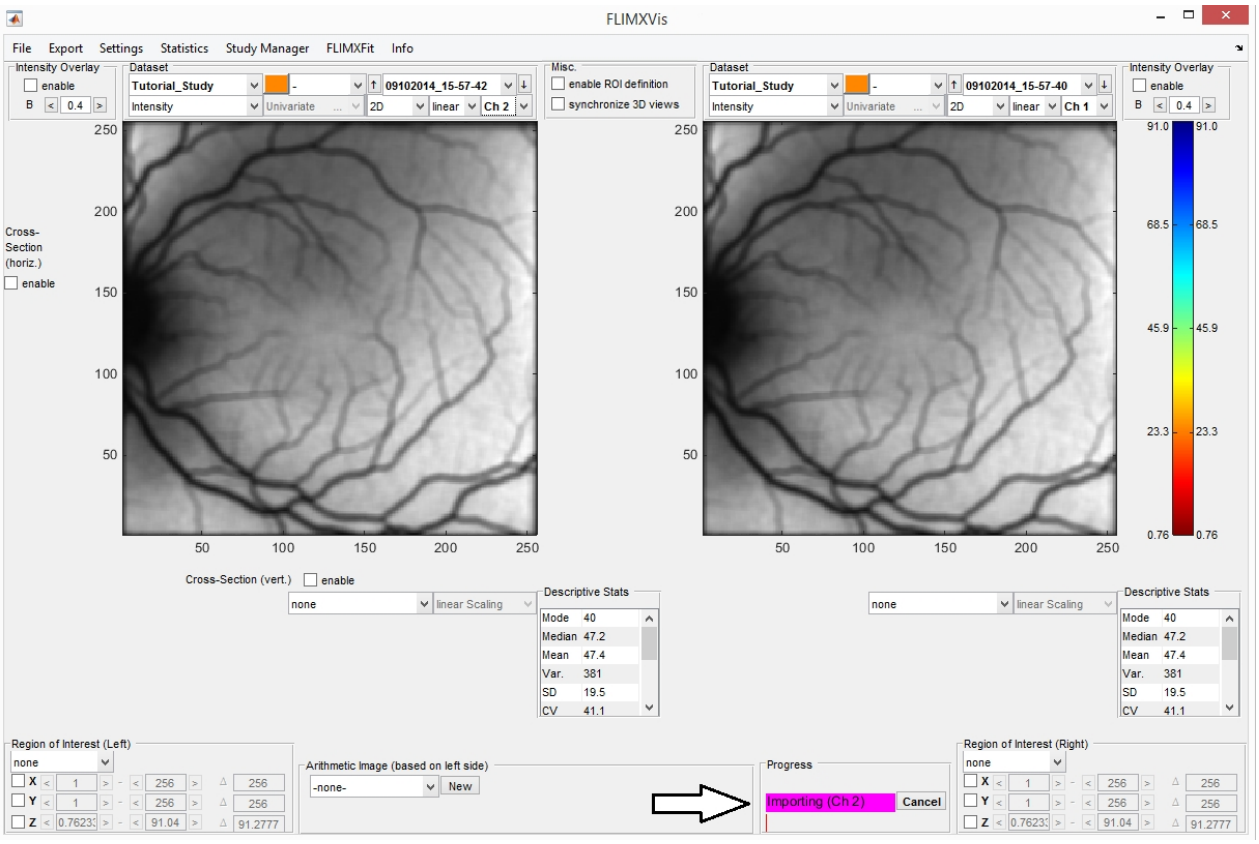
which you want to display are explained. Note that you must first add studies and data using Study Manager.

Select Study

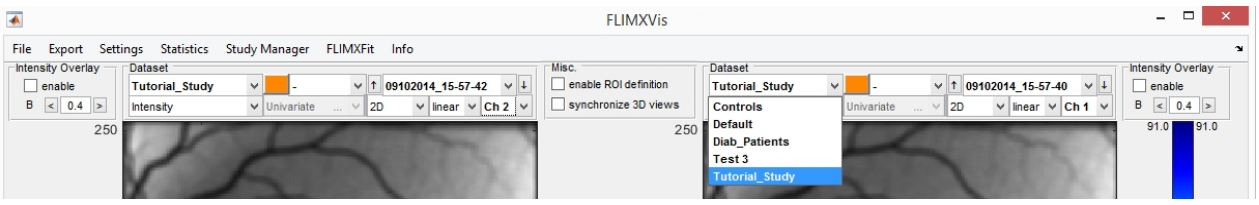
To visualize data from a study in FLIMXVis, click on the arrow in the dataset section as seen below. The names of your existing studies will show up. Select a study by clicking on it.



While loading data, the progress will be seen in the lower part of FLIMXVis as indicated by the arrow in the picture below. Wait for this progress to finish before continuing.



Repeat this process on the top right window of FLIMXVis to select data for visualization in the top right window.



Note:
Only your currently chosen subjects and channels are imported. This data will remain saved in your working memory during one session, e.g. until you close FLIMX. Accordingly, the size of working memory in use is dependent on the amount of subjects and channels imported during one session. Depending in the amount of pixels, one subject needs about 16 MB working memory per channel.

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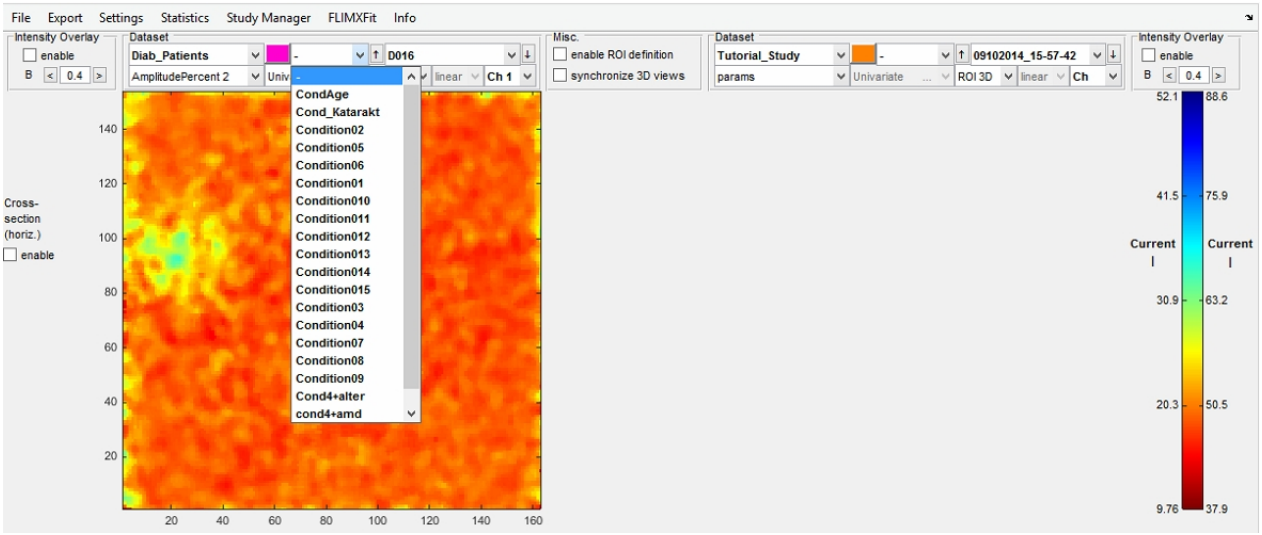
Select Condition

To select only study data which match a certain condition in FLIMXVis, for example all subjects with age >=60, click on the arrow in the dataset section as seen below. The names of all conditions for your current study will show up. Select a condition by clicking on it.

Repeat this process on the top right window of FLIMXVis to select a condition for visualization in the top right window.

Note that after selecting a condition your subjects list will contain only those subjects which match the condition. To show all subjects, select -.



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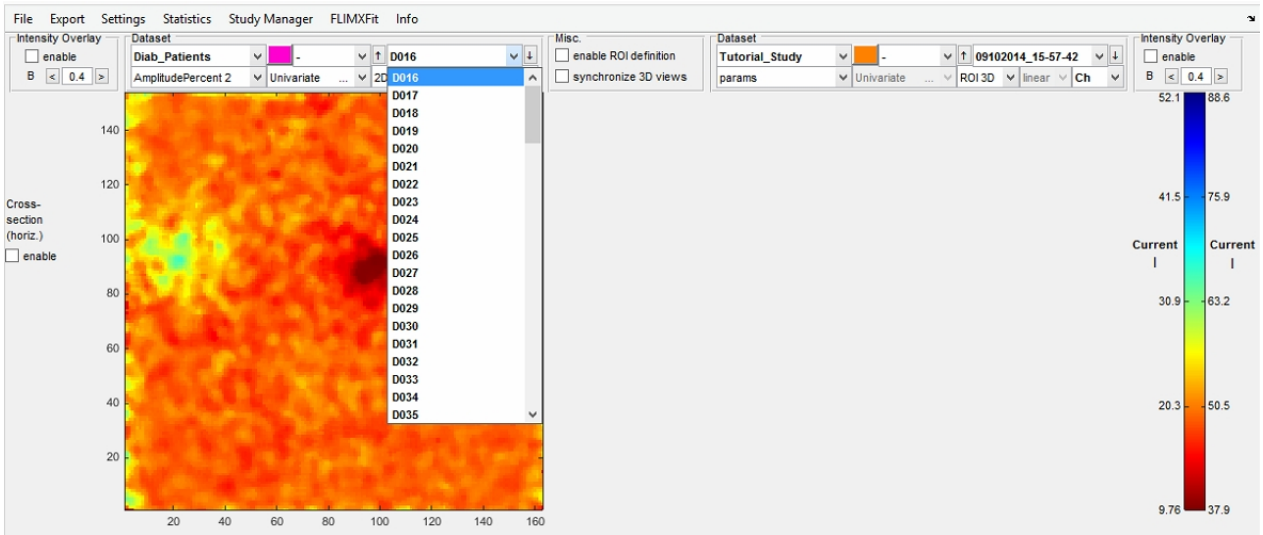
Select subject

To visualize a subject from your current study in FLIMXVis, click on the arrow in the dataset section as seen below. The names of all subjects in your current study will show up. Select a subject by clicking on it. Repeat this process on the top right window of FLIMXVis to select data for visualization in the top right window.



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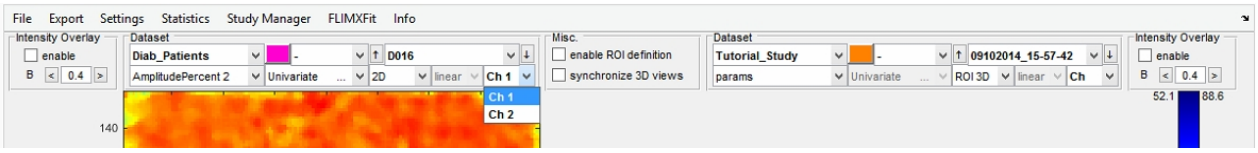
Select Channel

In case of multiple channels, select a channel by clicking on the current channel as seen below. The available channel numbers for your current subject will show up. Select a channel by clicking on it. Repeat this process on the top right window of FLIMXVis to select a channel in the top right window.



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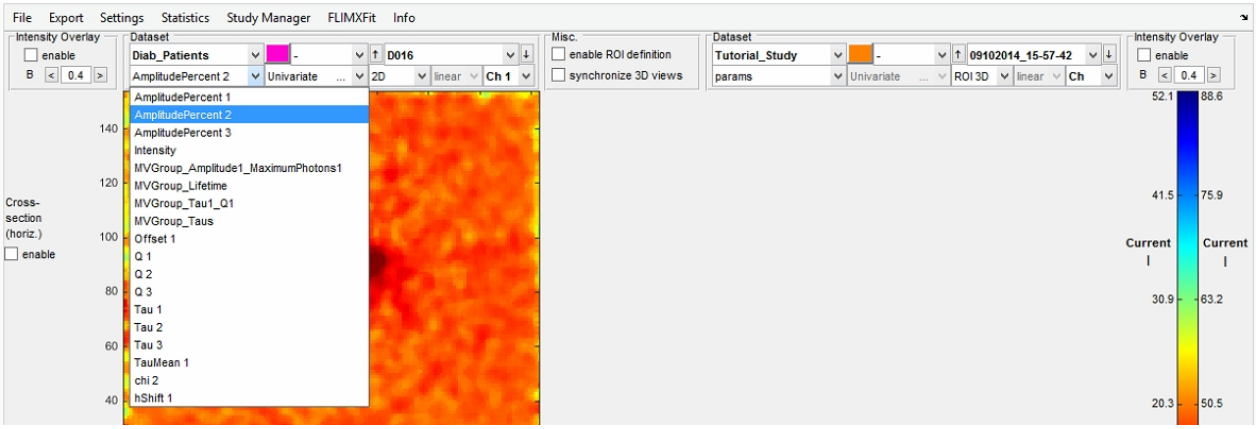
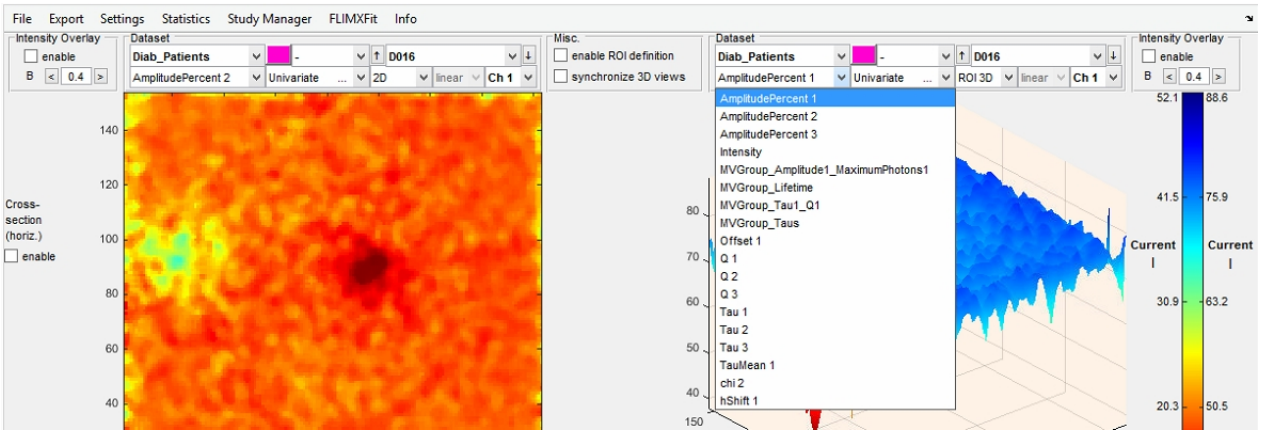
Select FLIM Parameter

To visualize one FLIM parameter, click on the arrow in the parameter section as seen below. The FLIM parameters will show up. Select a parameter by clicking on it. Note that for parameter visualization, you must chose univariate view (which is set on default).



The same routine applies for visualizing a parameter in the top right window.



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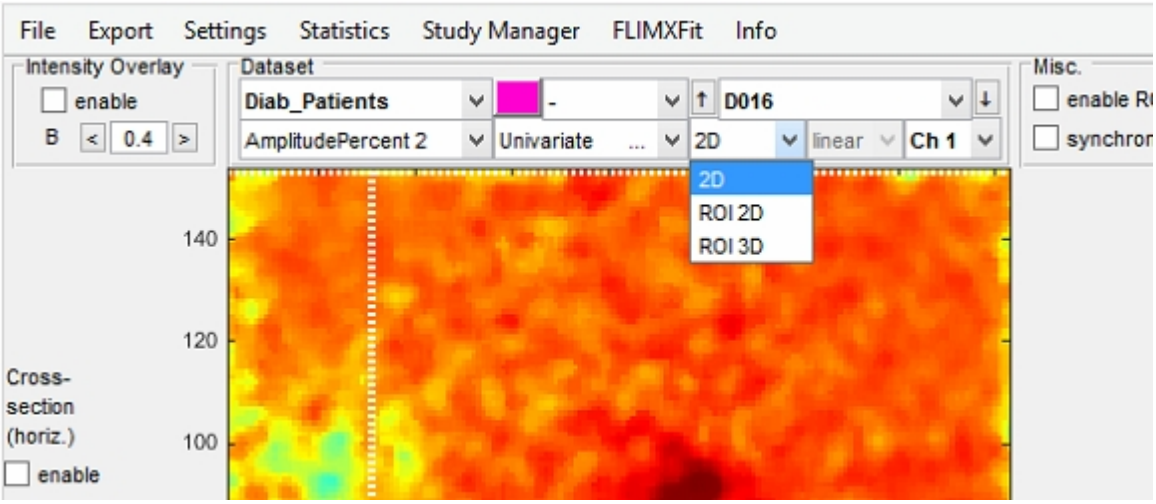
Dimensions

FLIMXVis offers three different dimensions:

- 2D: 2D Overview. The whole image is displayed. Use this view to define your region of interest (ROI).
- ROI 2D: Shows a 2D image of your region of interest (ROI)
- ROI 3D: Shows a 3D image of your region of interest (ROI)

To select a dimension, click on the arrow next to your current dimension as seen below. A list containing the three dimensions will show up. Select a dimension by clicking on it. The same routine applies for selecting a dimension in the top right window. Dimension can be selected separately for each window.



2D

2D is the default setting for viewing an image. Use this view to define your region of interest (ROI). The whole image is displayed in a 2D view. To change from ROI view to 2D, see above.

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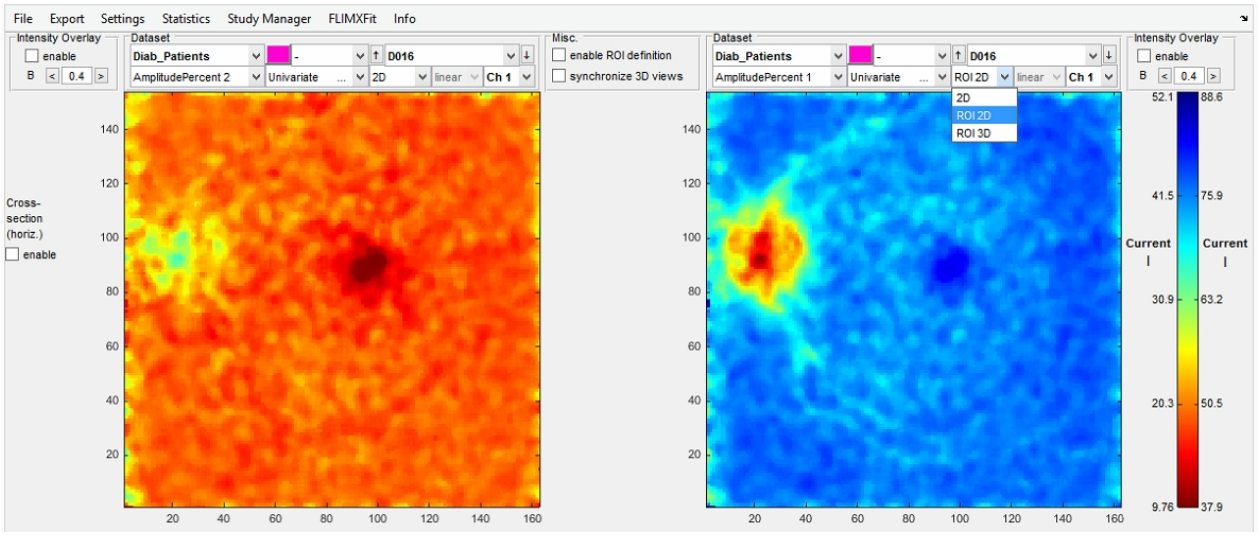
ROI 2D

ROi 2D shows a 2D image of your region of interest (ROI). In order to use this view, you must first define a ROI in 2D.

In the example below, this view has been chosen for the top right window. The whole picture as well as ROI are seen in the top left window while a close-up of the selected ROI is displayed in the top right window.



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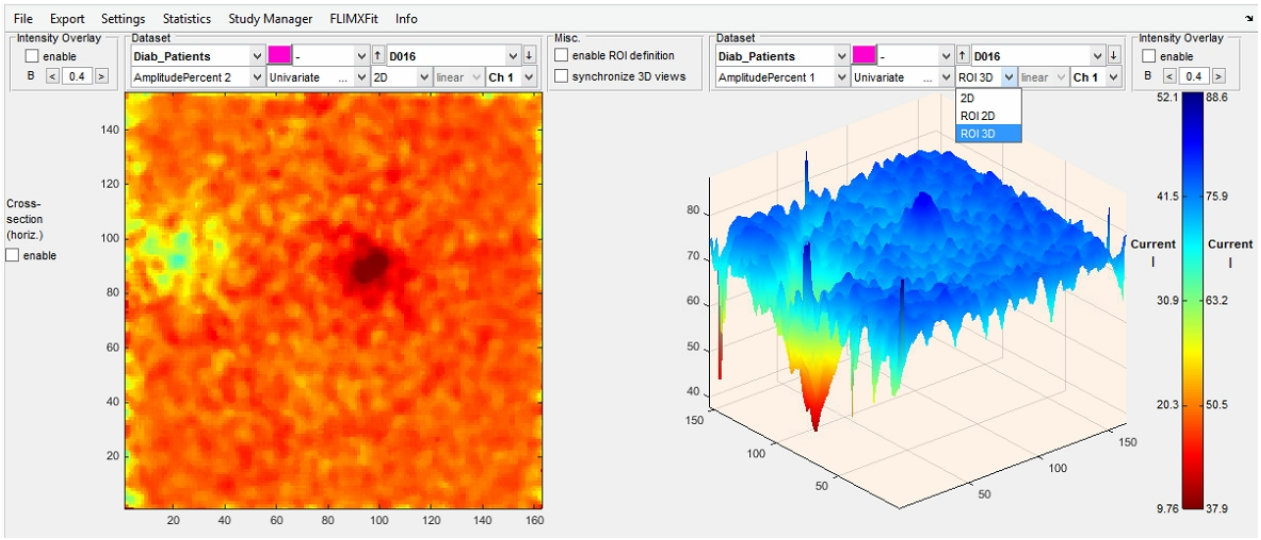
ROI 3D

In order to use 3D view, you should first define a ROI in 2D.

The whole picture as well as ROI are seen in the top left window (2D view) while a close-up of the selected ROI in 3D is displayed in the top right window.

Rotate the 3D image by clicking on the image and simultaneously moving your cursor.

To synchronize 3D views, see Miscellaneous. For 3D settings, see 3D Plots.



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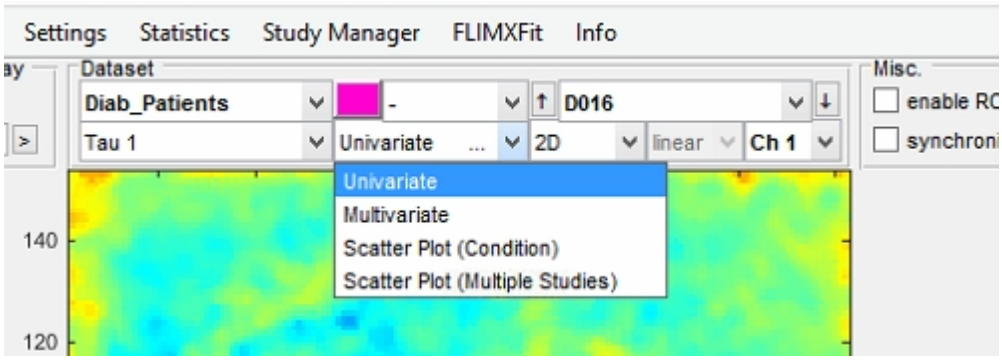
Views

View options provide different views in which you can display single or multiple parameters. This section explains the following four options:

- Univariate: display one subject and one parameter
- Multivariate: display multiple parameters in 3D view
- Scatter Plot (Condition): display scatter plot for all subjects from one study/condition
- Scatter Plot (Multiple Studies): display global cluster for multiple studies

To select an option, click on the arrow in the dataset section as seen below. Select an option by clicking on it.



Univariate

Univariate view is the default option. Within this view, one FLIM parameter or scatter plot for one single subject is displayed. To select a study, subject and parameter, see here.

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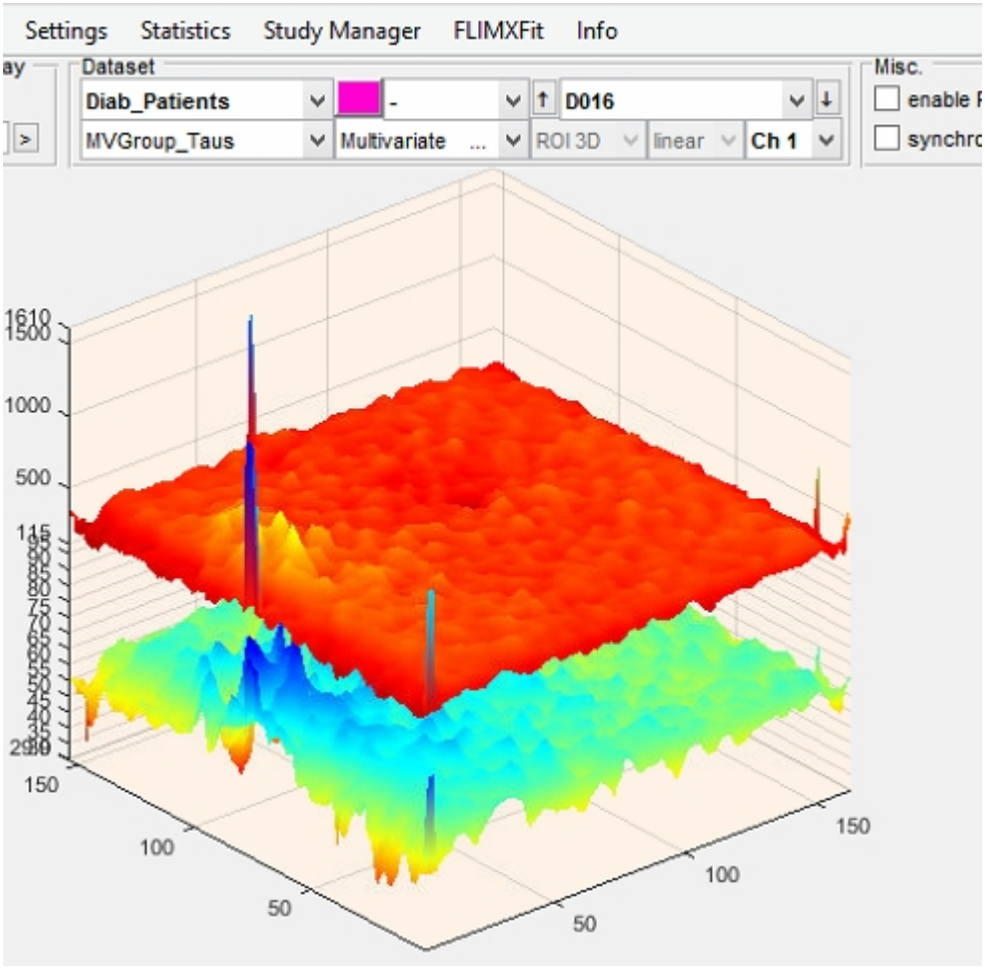
Multivariate

In this view, FLIMXVis displays multiple parameters on top of each other in one single view. Note that you must first define a cluster before using this view. For more information on clusters and scatter plots, see Scatter Plots.

To use this view, first chose **Multivariate** from the view section. After this, the parameter section will include only your clusters. Chose a cluster. In this view, only 3D dimension is available. The necessary settings are indicated by arrows in the picture below.

After making the appropriate selections, the cluster parameters will be displayed on top of each other as seen in the example. Rotate the 3D view by clicking on the image and simultaneously moving your cursor.

For options on scaling of multivariate views, see Visualization Settings.



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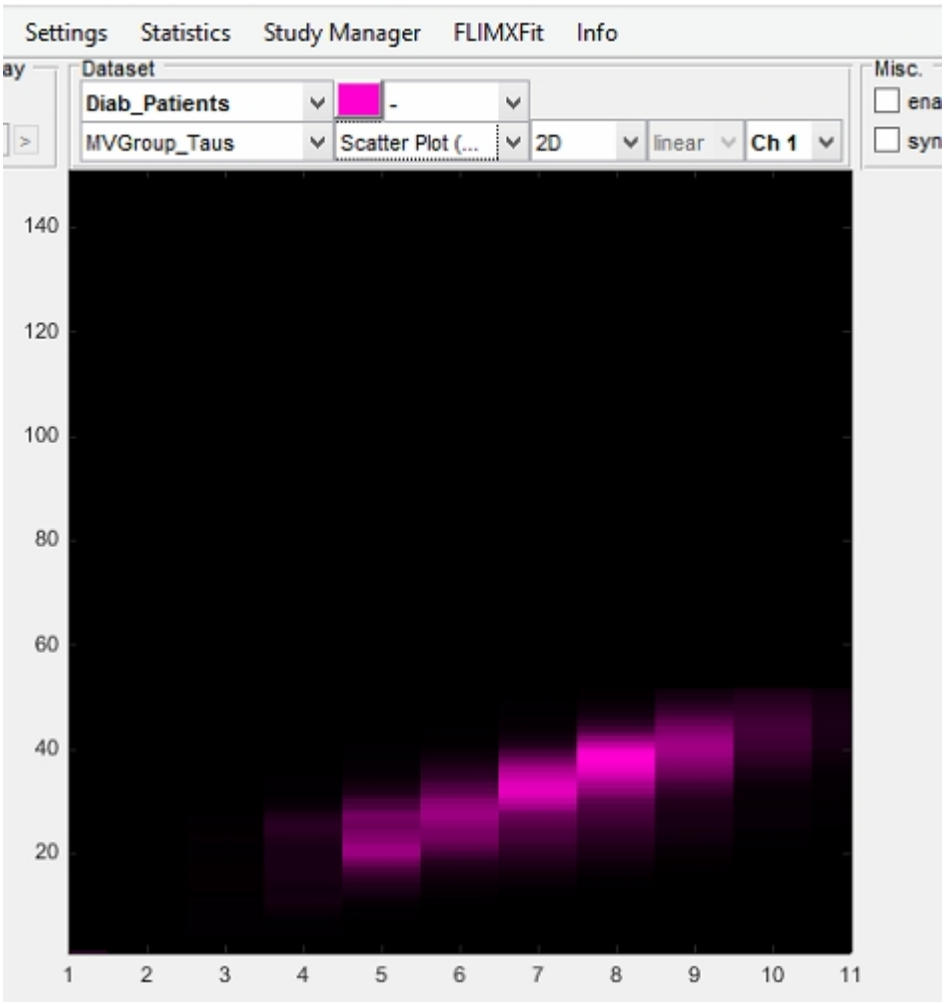
Scatter Plot (Condition)

In this view, FLIMXVis displays a sum scatter plot over all subjects from one study/condition. Note that you must first define a cluster before using this view. For more information on scatter plots and clusters, see Scatter Plots.

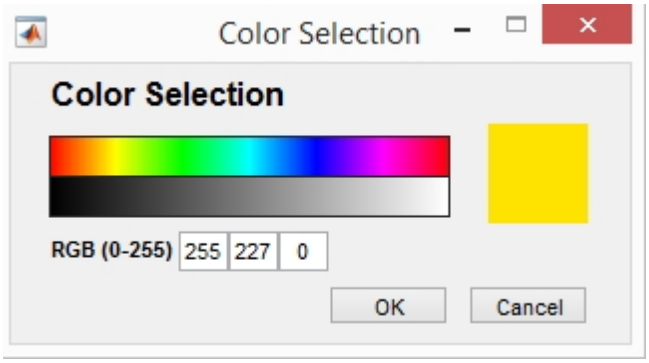
To use this view, first chose **View Clusters** from the view section. After this, the parameter section will include only your clusters. Chose a cluster. You may select study, condition, cluster and channel in the dataset section.

After making the appropriate selections, the sum scatter plot over all subjects from your selected study will be shown as seen in the example below. You may use different dimensions. The necessary settings are indicated by arrows in the picture below.

For better visualization of small changes, use log10 (logarithm to the base of 10) colormap.



To change color of the clusters, click on the buttons on the top showing your current color (here: red). The following window will pop up. Select a color by clicking on it or type in three RGB values. Click **OK** to save your settings and close the window or **Cancel** to exit without saving.



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Scatter Plot (Multiple Studies)

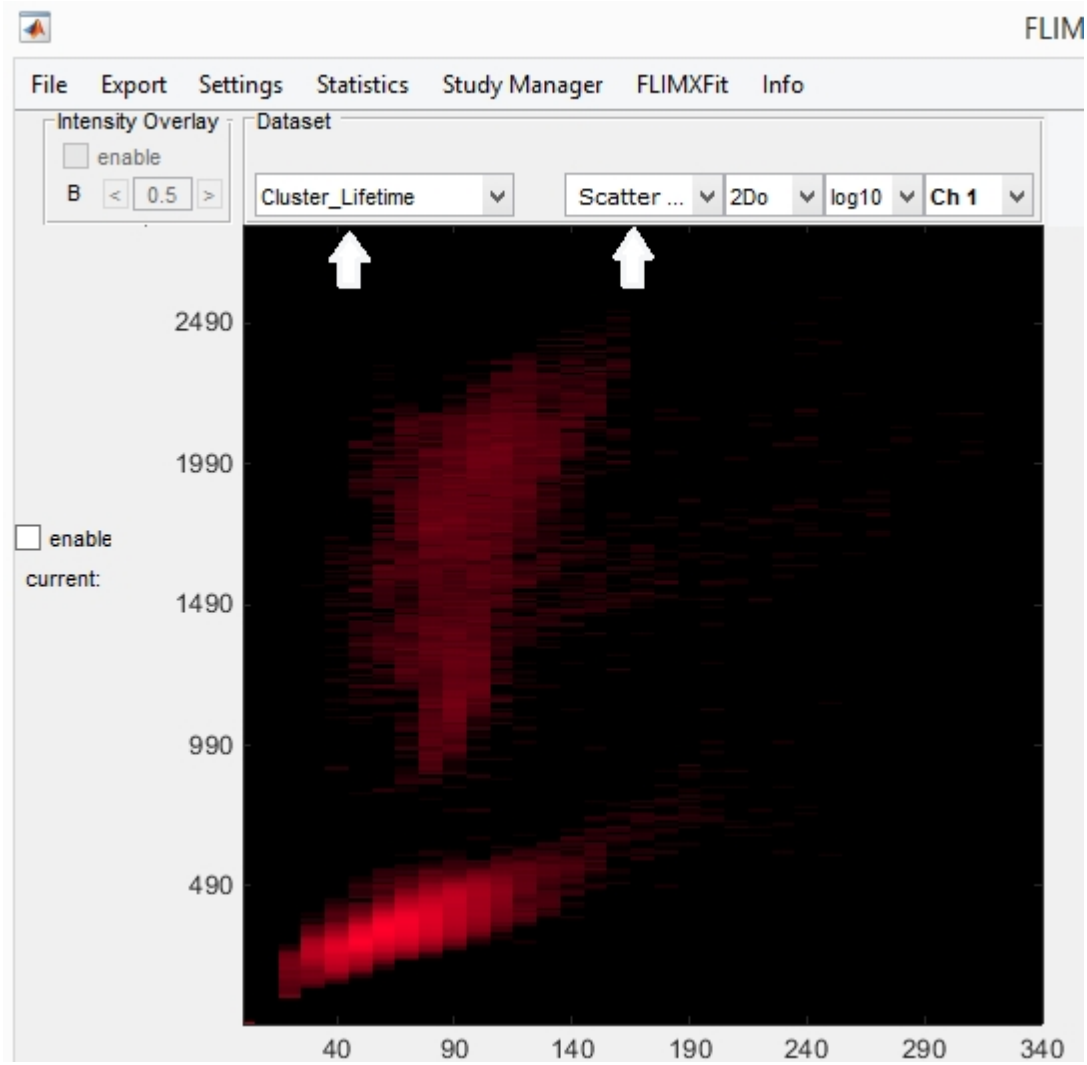
In this view, FLIMXVis displays the scatter plots over all subjects from all studies which belong to one global cluster. Note that you must first define a global cluster before using this view. For

more information on group clusters, see Scatter Plots.

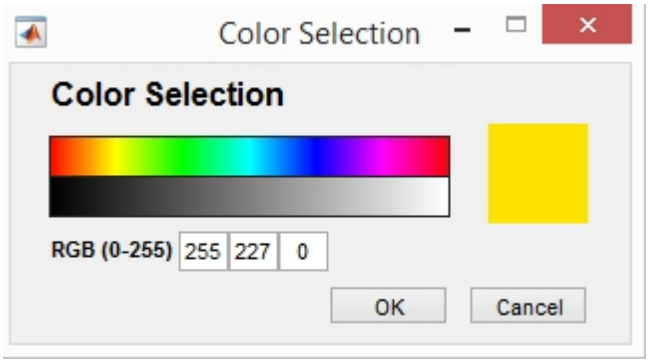
To use this view, first chose **Global Clusters** from the view section. After this, the parameter section will include only your global clusters. Chose a cluster.

After making the appropriate selections, the scatter plots for your global cluster will be shown in one picture as seen in the example below. The necessary settings are indicated by arrows in the picture below.

For better visualization of small changes, use log10 colormap.



To change color of the clusters, click on the buttons on the top showing your current color (here: red). The following window will pop up. Select a color by clicking on it or type in three RGB values. Click **OK** to save your settings and close the window or **Cancel** to exit without saving.



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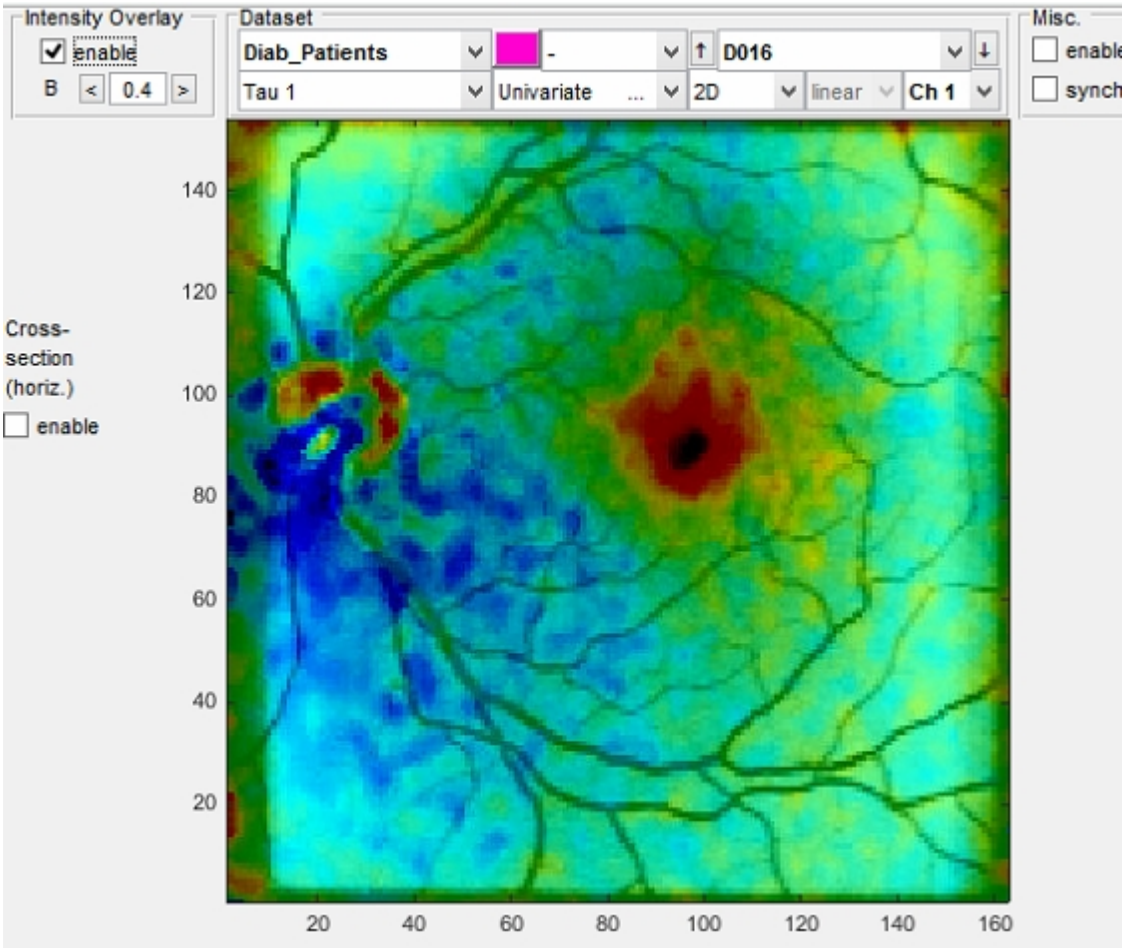
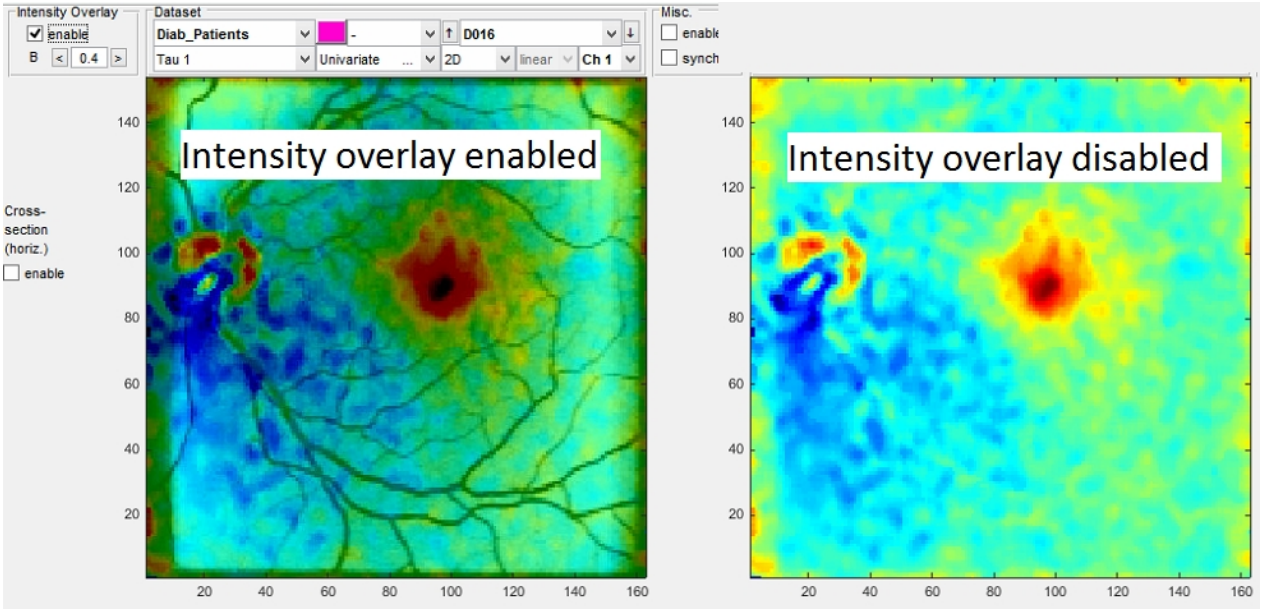
Intensity Overlay

When displaying FLIM parameters, intensity overlay may be enabled for better contrast and orientation.

These options are marked by two arrows in the picture below. To enable intensity overlay, tick the **enable** box. Use the arrows next to B to set brightness (B) of the intensity overlay. B lies within 0 and 1. Intensity overlay and brightness are chosen independently for each window.



In the example below, intensity overlay has been enabled for the left picture. In the right picture, intensity overlay has been disabled.



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Linear and log10 Colormap

FLIMXVis offers the possibility to visualize data using a

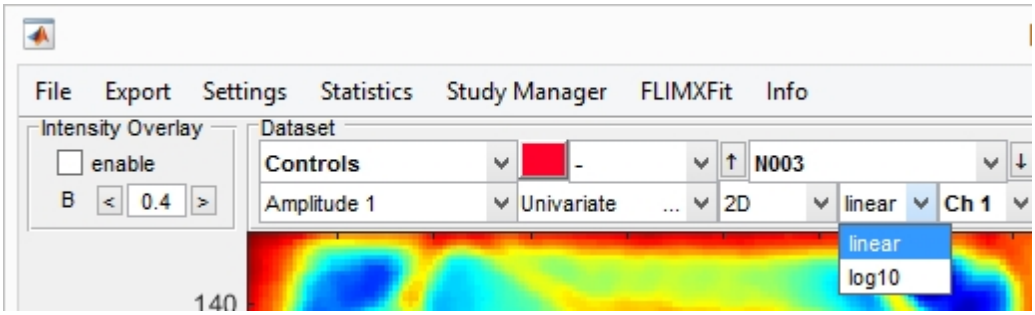
- Linear or
- log10

colormap.

Since log10 colormap converts numerical values to logarithmic scale, small numerical differences will result in larger logarithmic differences and are therefore easier to spot.

To select linear or log10, click on the arrow in the view section as seen below. Select a colormap by clicking on it.

Note: log10 colormap is especially suitable for displaying scatter plots.



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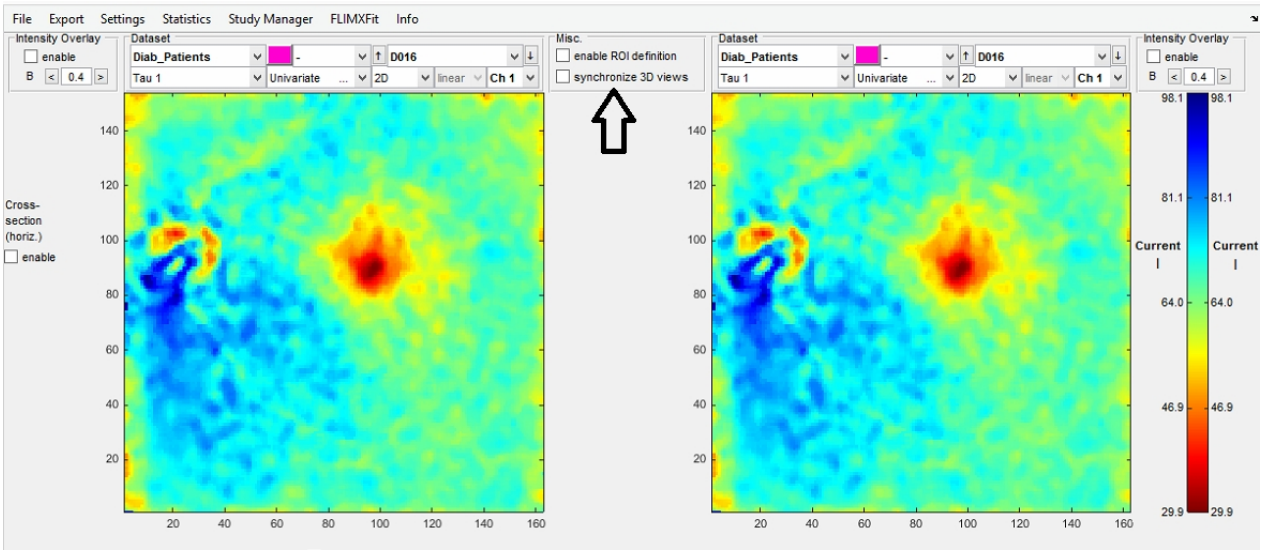
Miscellaneous

The miscellaneous options (labeled Misc.) are located in between the left and right Dataset Options window as indicated by the arrow on the picture below. The two options are

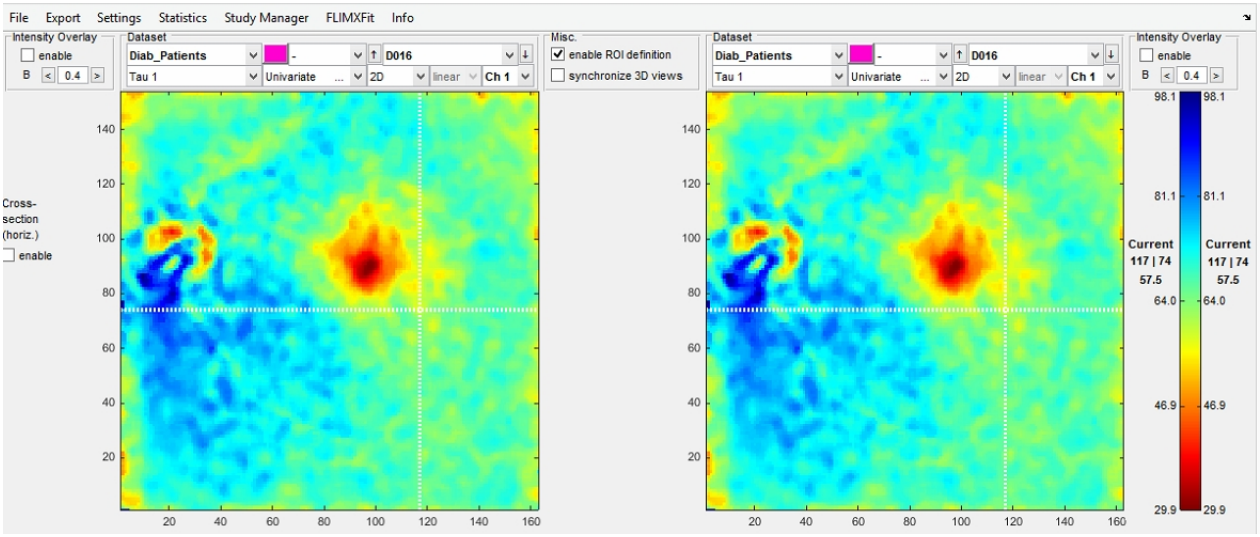
- Enable ROI definition and
- Synchronize 3D views.



Enable ROI Definition

To enable the ROI definition through a 2D Mouse Pointer in 2D view, tick the box **Enable ROI definition**. Hovering your cursor over the windows results a cross hair which can be used for better positioning of the ROI. The current mouse pointer position on the windows is shown on the right side next to the color bar. In the example below, the current position is 156|151|122. The coordinates correspond to x|y|z. To define the ROI on the left window hold the left mouse button and draw the desired ROI. While *enable ROI definition* is enabled the rotation of the right window (3D view of one window) is not available.

Enabling the ROI definition disables the ability to rotate 3D plots. Disabling the ROI definition allows 3D rotation again.



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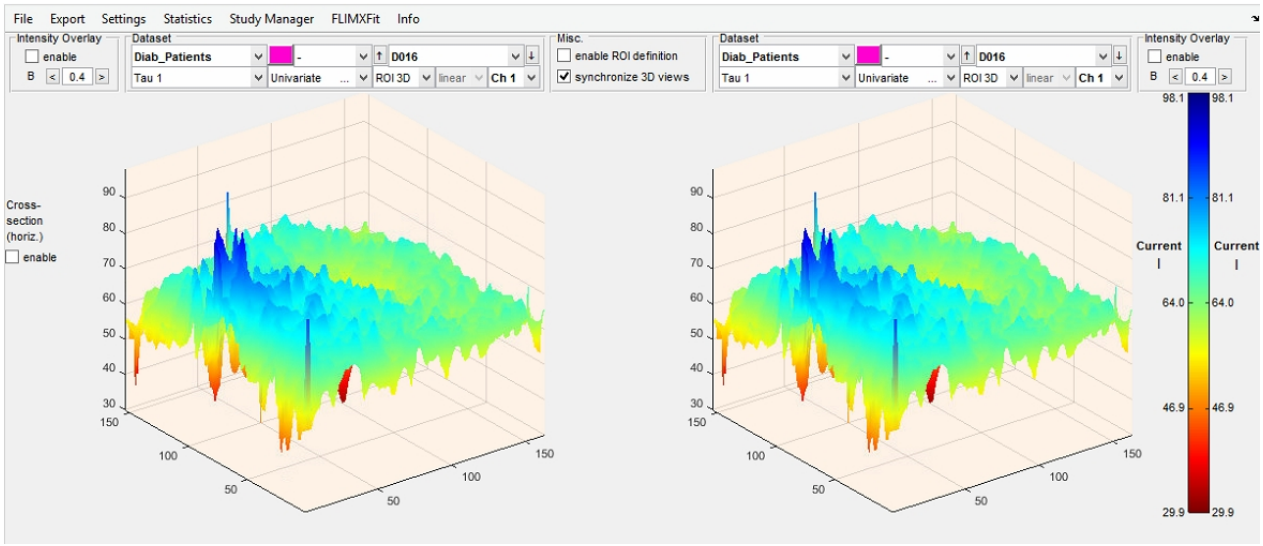
Synchronize 3D Views

To synchronize the views in the top left and right window in 3D view, tick the box **Synchronize 3D Views** as indicated by an arrow in the picture below. By clicking on one image and simultaneously moving your cursor, both 3D windows will now rotate synchronously.



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Supplemental Plots

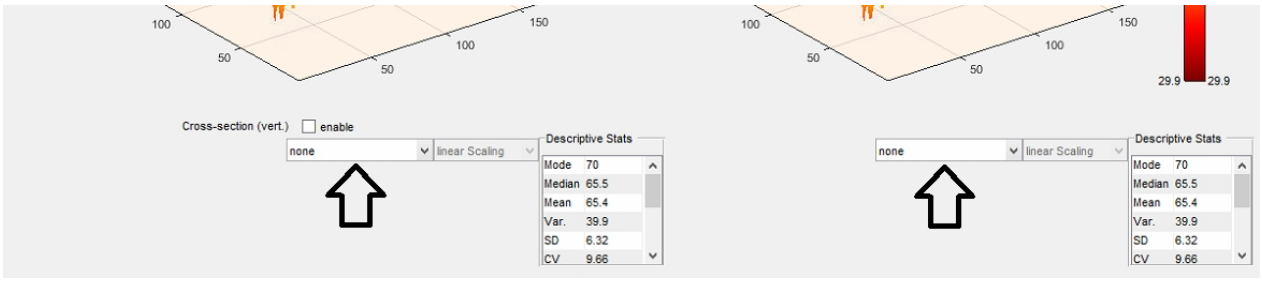
FLIMXVis offers the possibility to show a Histogram, Horizontal Cross-Section or Vertical Cross-Section of your image. These plots are called supplemental plots.

The options are located underneath the top left and right window as indicated by the arrows in the picture below.

Within this section, the following functionalities are explained:

- Select a supplemental plot
- Histogram
- Horizontal cross-section
- Vertical cross-section
- Show cross-section in 3D

For supplemental plot visualization options, see Settings.



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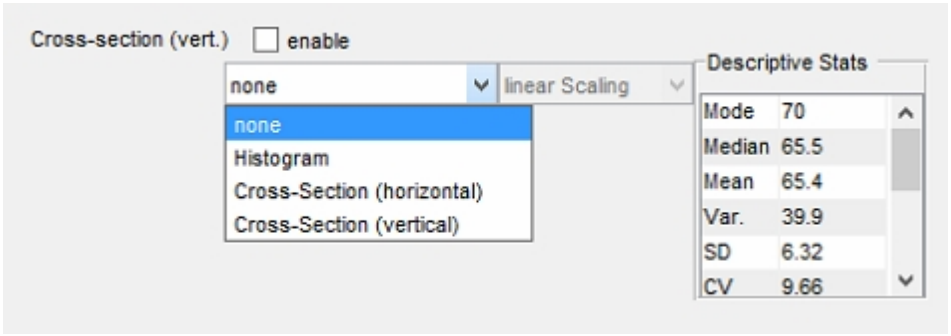
Select a supplemental plot

To select histogram, horizontal or vertical cross-section in FLIMXVis, click on the arrow next to **none** underneath your main window as indicated in the example below. A list with the three options will pop up. Select an option by clicking on it.



The same procedure applies to selecting an option for the image on the right. Histogram, horizontal cross-section and vertical cross-section can be chosen separately for both windows.

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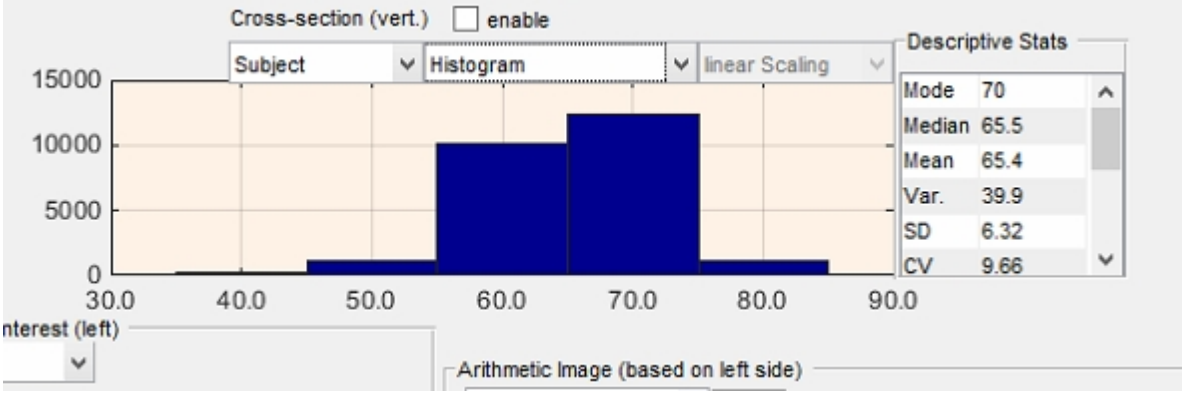
Histogram

The histogram shows the frequency distribution of a certain parameter within your current picture.

In the example below, the distribution for Tau 1 is shown in the bottom left window. The numerical values of your parameter are seen on the x-axis while the amount of pixels for each parameter value is shown on the y-axis. The unity corresponds to the FLIM parameter.

From the histogram in our example, it can be seen that for example at the time constant of 120

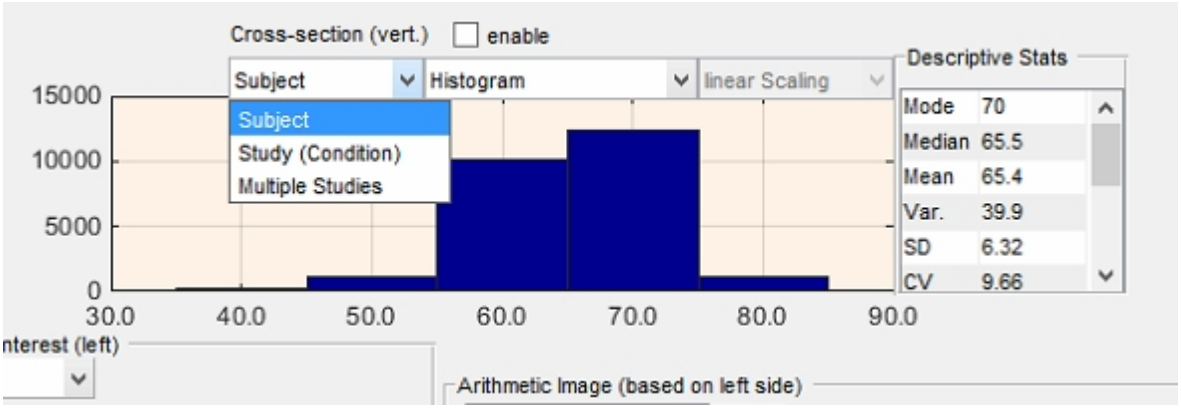
ns, the picture contains ~ 1.5 x 10^4 pixels.



Histogram FLIMX offers the possibility to show three different histograms:

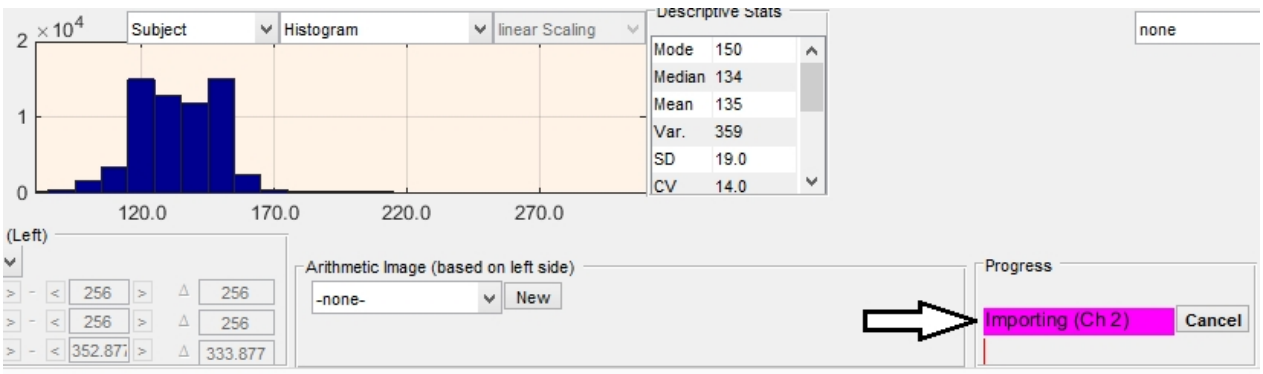
- **Subject**: Shows a histogram for a single subject. The current parameter, subject, channel and, if selected, ROI (default) are shown.
- **Study (Condition)**: Shows a histogram over all subjects within your current study and, if chosen, condition.
- **Multiple Studies**: Shows a histogram over more than one study. To use this option, you must first define a group.

To select an option, click on the arrow next to your current option. A list will show up. Select one of the three options by clicking on it as seen in the example below.



While loading histogram data, the progress of importing this data will be seen in the lower part of FLIMXVis as indicated by the arrow in the picture below. The red bar shows the progress and approximated time remaining. Wait for this progress to finish before continuing.

Note:
Only your currently chosen subjects and channels are imported and calculated. This data will remain saved in your working memory during one session, e.g. until you close FLIMX. Accordingly, the size of working memory in use is dependent on the amount of subjects and channels imported during one session.



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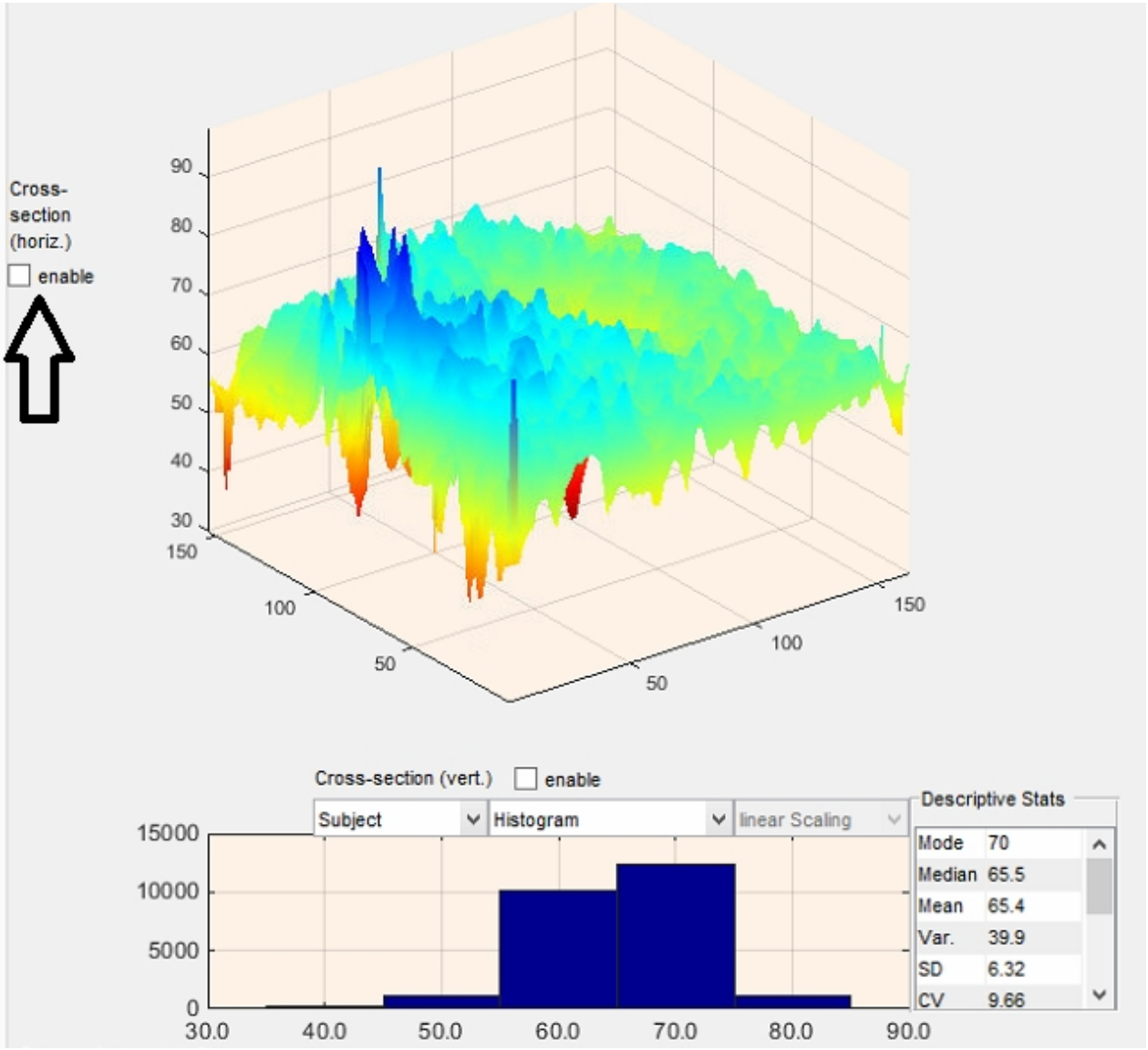
Horizontal Cross-Section

In order to show the horizontal cut, you must tick the box **enable** on the left side of your image. In the picture below, this box is indicated by an arrow.

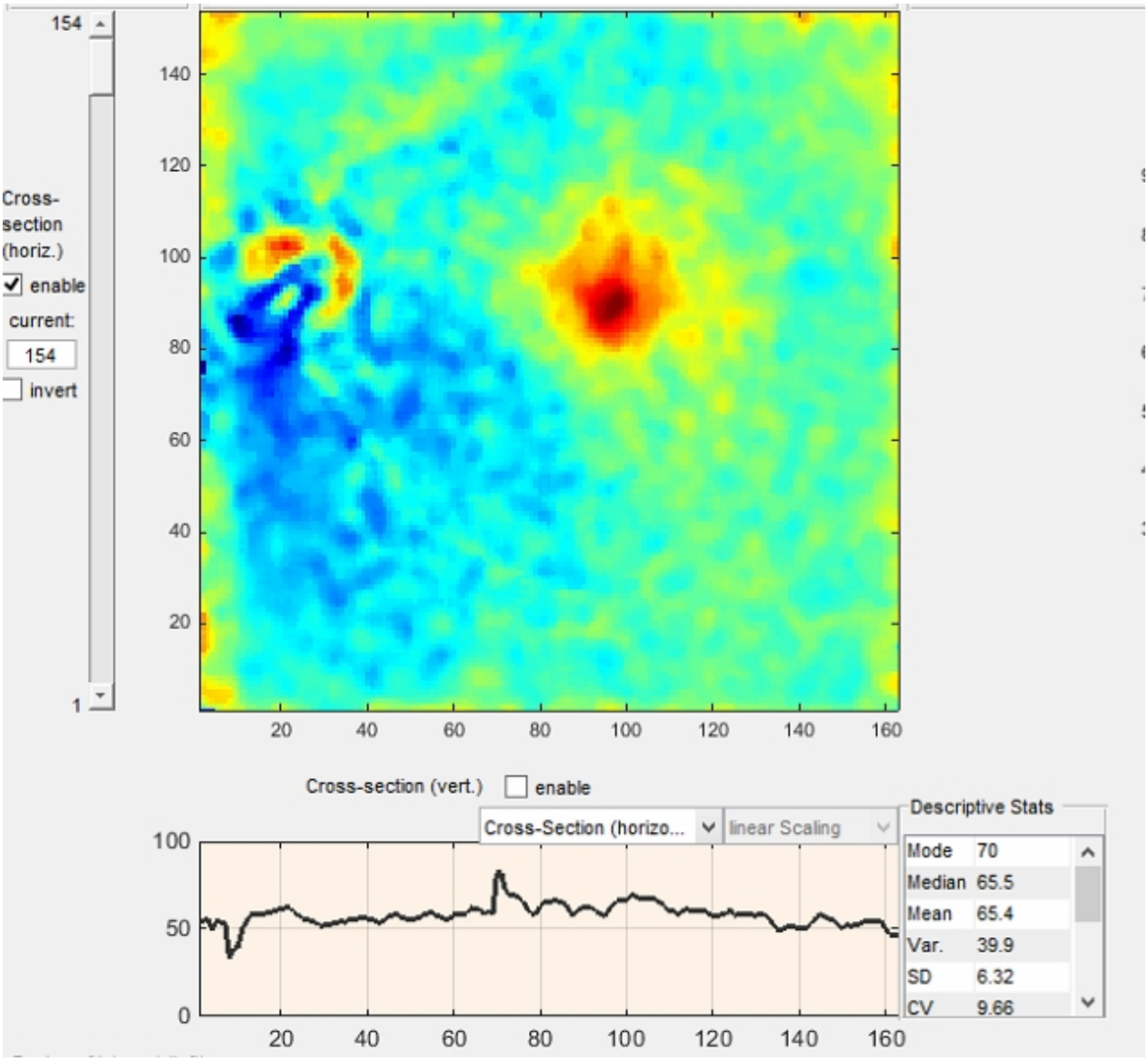


After ticking enable and selecting horizontal cross-section from the drop-down menu, a dashed

line will appear horizontally across your image. This line represents the location of the cross-section. In the bottom window, the z-axis at the location of the cross-section will be displayed.

To change the position of the cross-section, type in a coordinate or move the bar on the left of your image. In the example seen below, y-coordinates 154 have been selected for horizontal cross-section. Coordinates are given in pixel.

For cross-section visibility in 3D view, see below.



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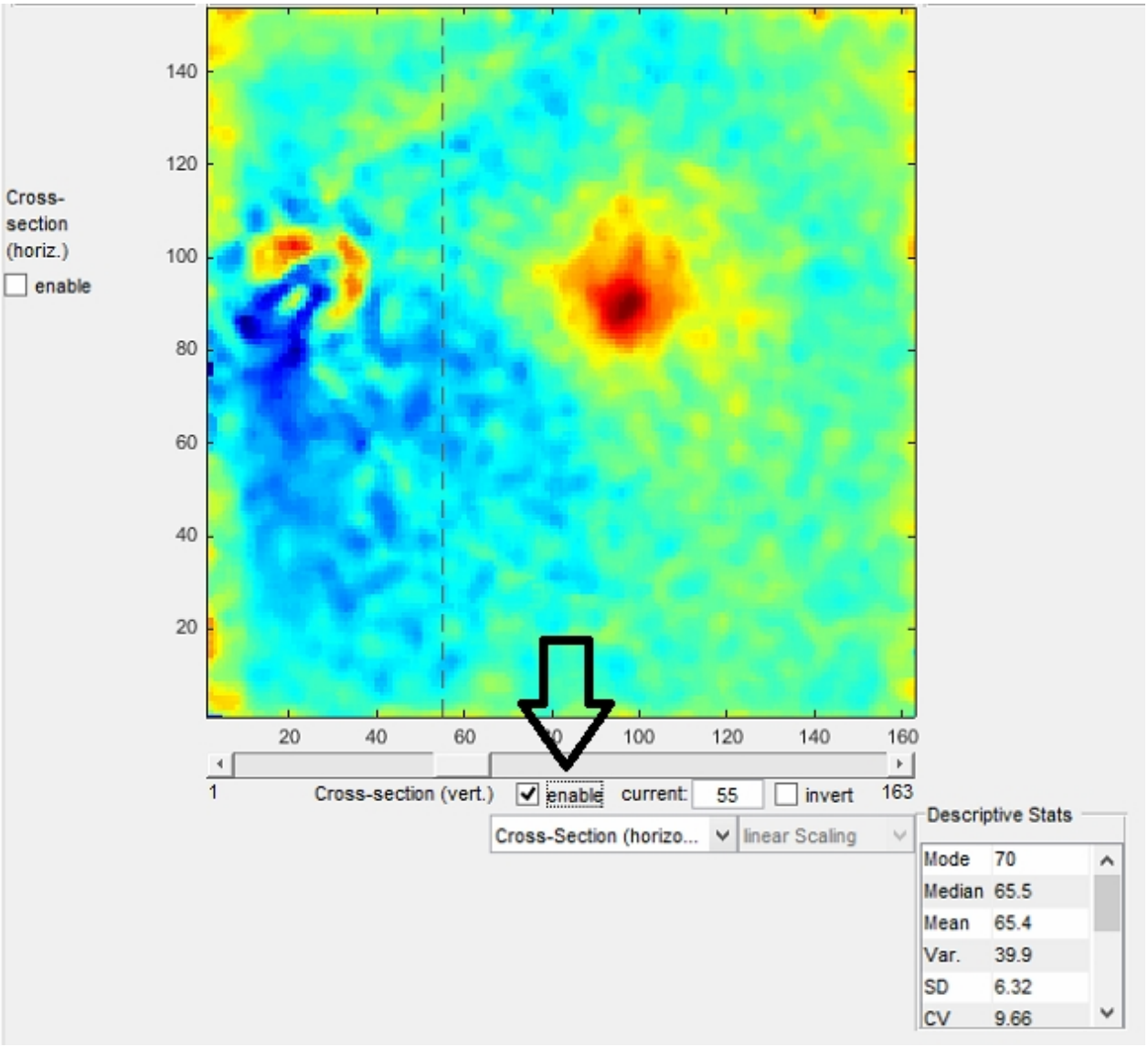
Vertical Cross-Section

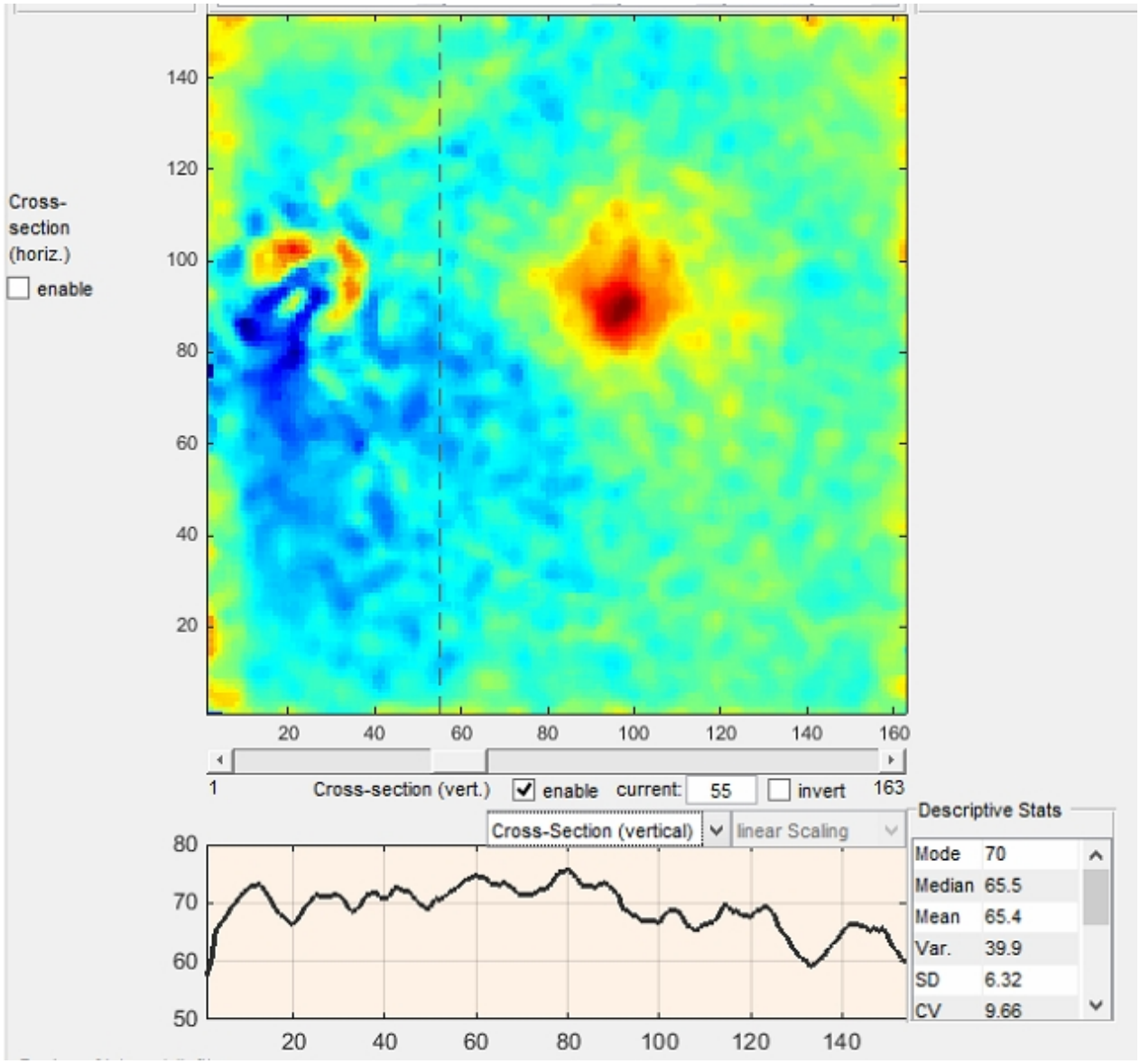
In order to show the vertical cross-section, you must tick the box **enable** underneath your top window. In the picture below, this box is indicated by an arrow.



After enabling vertical cross-section and selecting the option from the drop-down menu, a dashed line will appear vertically across your image. This line represents the location of the cross-section. In the bottom window, the z-axis at the location of the cross-section will be displayed.

To change the position of the cross-section, type in a coordinate or move the bar at the bottom of your image. In the example seen below, x-coordinates 55 have been selected for vertical cross-section. Coordinates are given in pixel.

For cross-section visibility in 3D view, see below.



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3D Cross-Sections

In this section, 3D options for vertical cross-section are shown, but same routine applies for horizontal cross-sections. The following options will be explained:

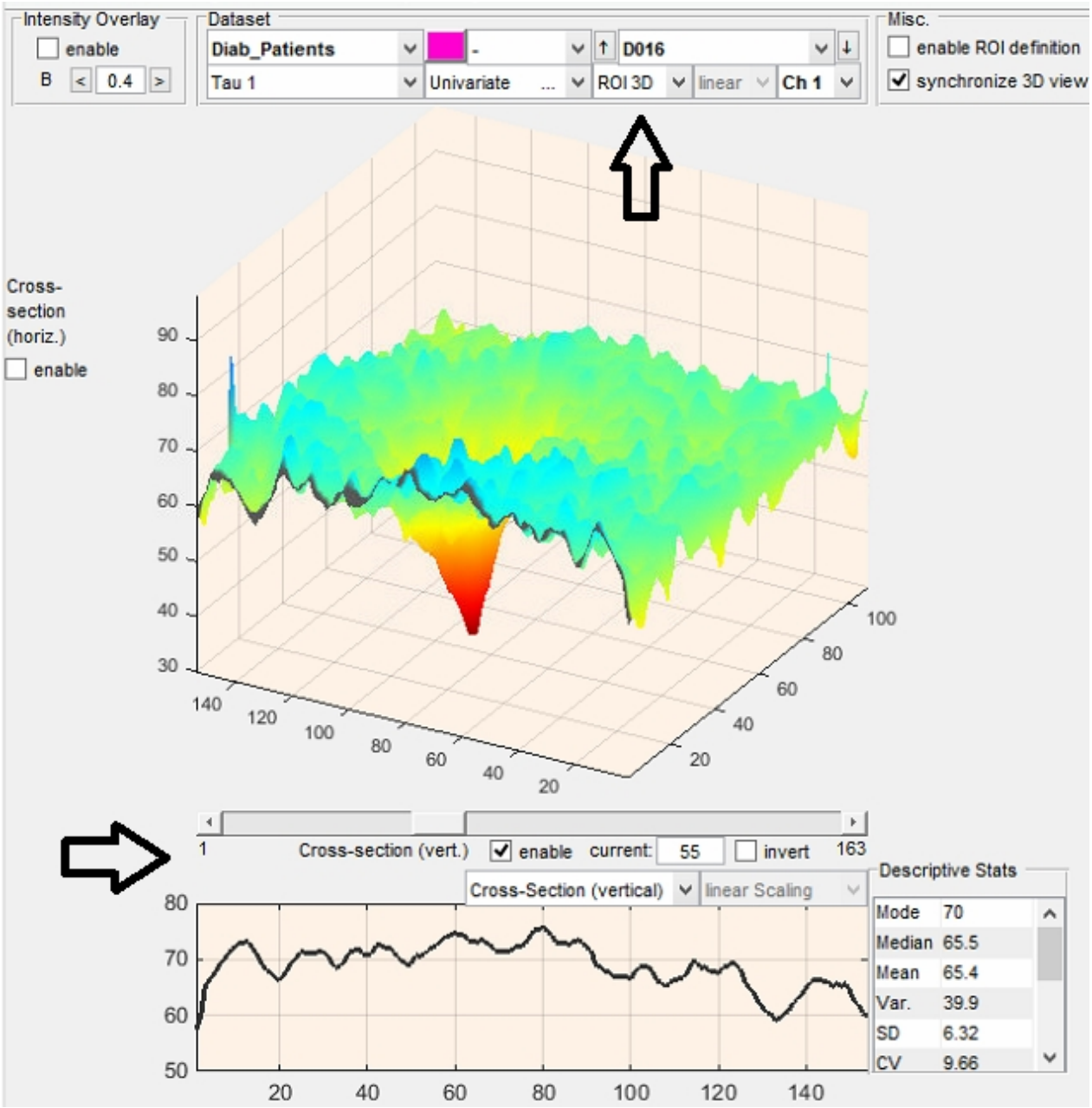
- Show 3D view of cross-section
- Invert 3D view of cross-section
- Pad cross-section space.

Show 3D view of cross-section

To show a 3D view of your cross-section, you must first tick enable and select the option from your drop-down menu. Then, chose 3D dimension from the menu in your dataset section. The corresponding options are indicated by arrows in the picture below.

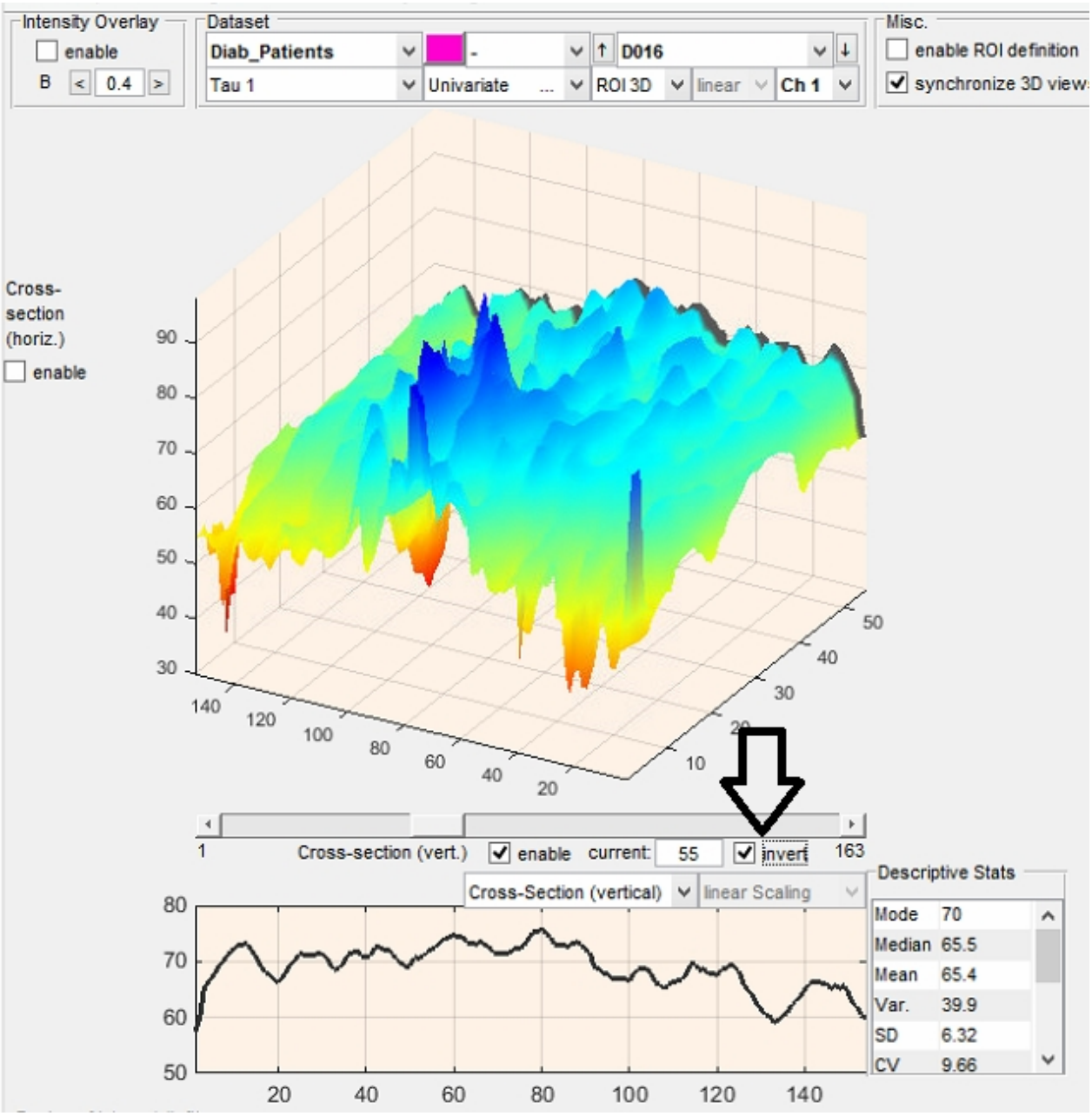
The 3D view now only shows the part of the image which is located on the right side of your

cross-section. For better comprehension, a 2D image of the cross-section has been selected in the right window, while a 3D view is shown on the left.



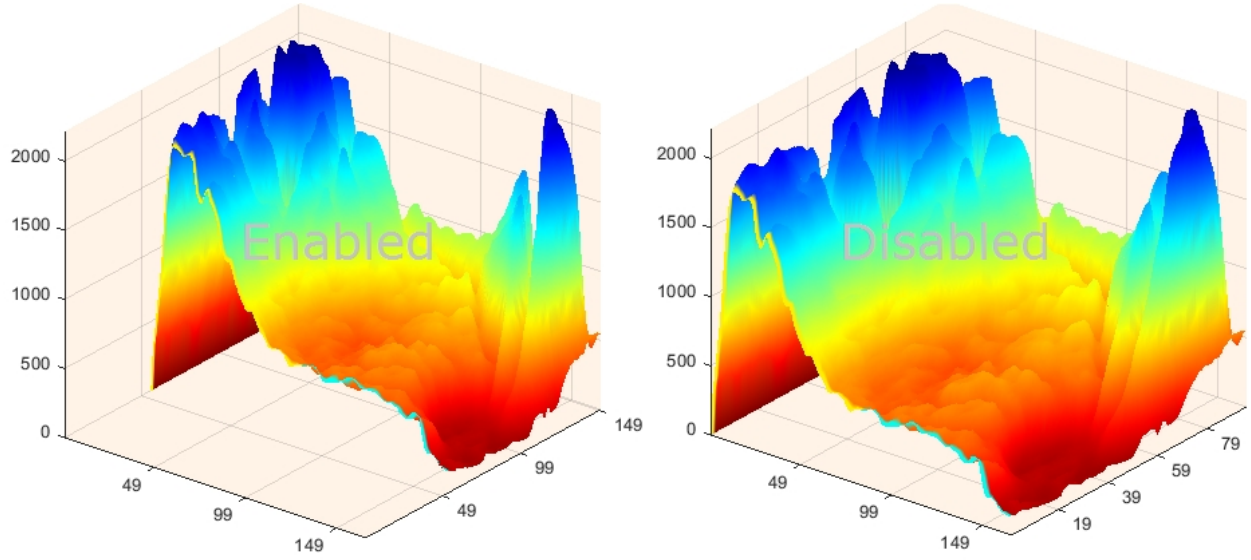
Invert 3D view of cross-section

To see a 3D view of the left side of your cross-section, simply tick the box **invert** above your supplemental plot as indicated by an arrow in the picture below.



Pad cross-section space

To show the whole image in 3D, and not only the part on the right or left of your cross-section, tick Pad Cross-Section Space within the visualization settings. For more information, see Visualization.



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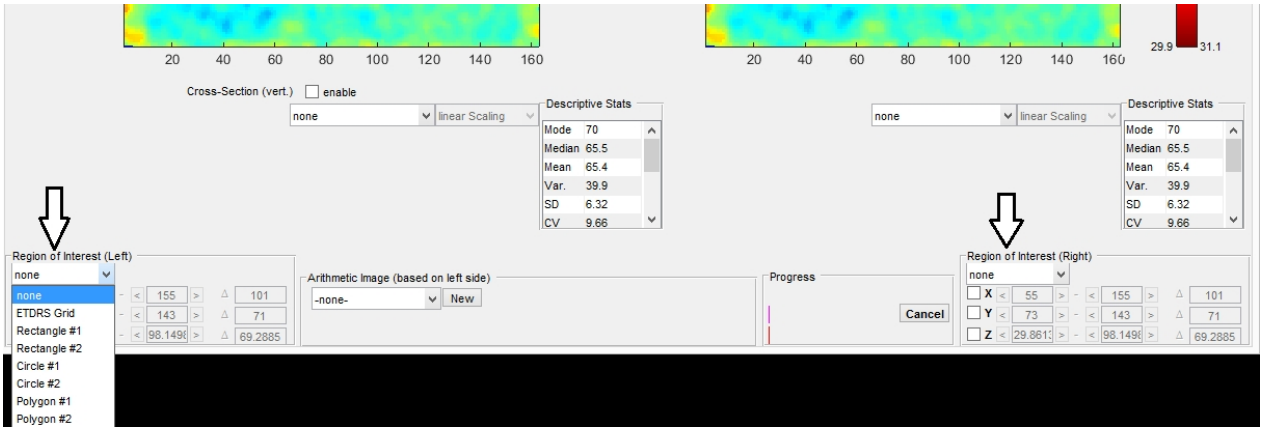
Select A Region Of Interest (ROI)

Options for selecting a region of interest (ROI) are located in the lower part of FLIMXVis as indicated by the arrows in the picture below. On default, none is selected.

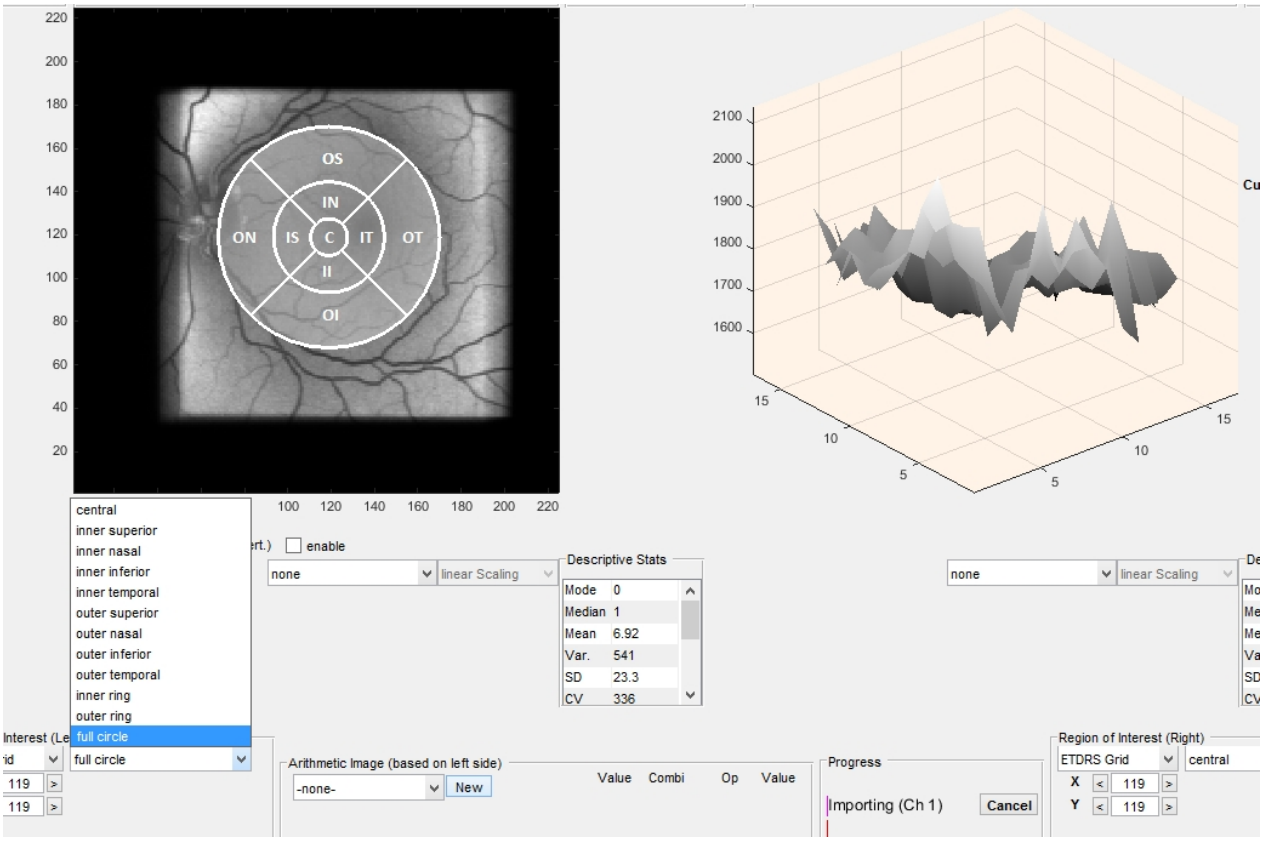
FLIMXVis offers the possibility to use

- ETDRS grid
- Rectangular ROI
- Circular ROI
- Polygonal ROI



ETDRS Grid

To select an ETDRS Grid, click on **ETDRS Grid** within the ROI section. Then type in the x and y coordinates or click on a point in the picture with your cursor to define the center of the circle. The size of the ETDRS grid is fixed. Optionally, z coordinates might be defined. The ETDRS Grid will be displayed in the left window and the content will be displayed in the right window.
To choose which area of the ETDRS Grid is displayed, click on the arrow next to your current area (here: full circle) as seen below. A list showing all available areas will pop up. Select an area by clicking on it.



For ETDRS Grid options, see settings.

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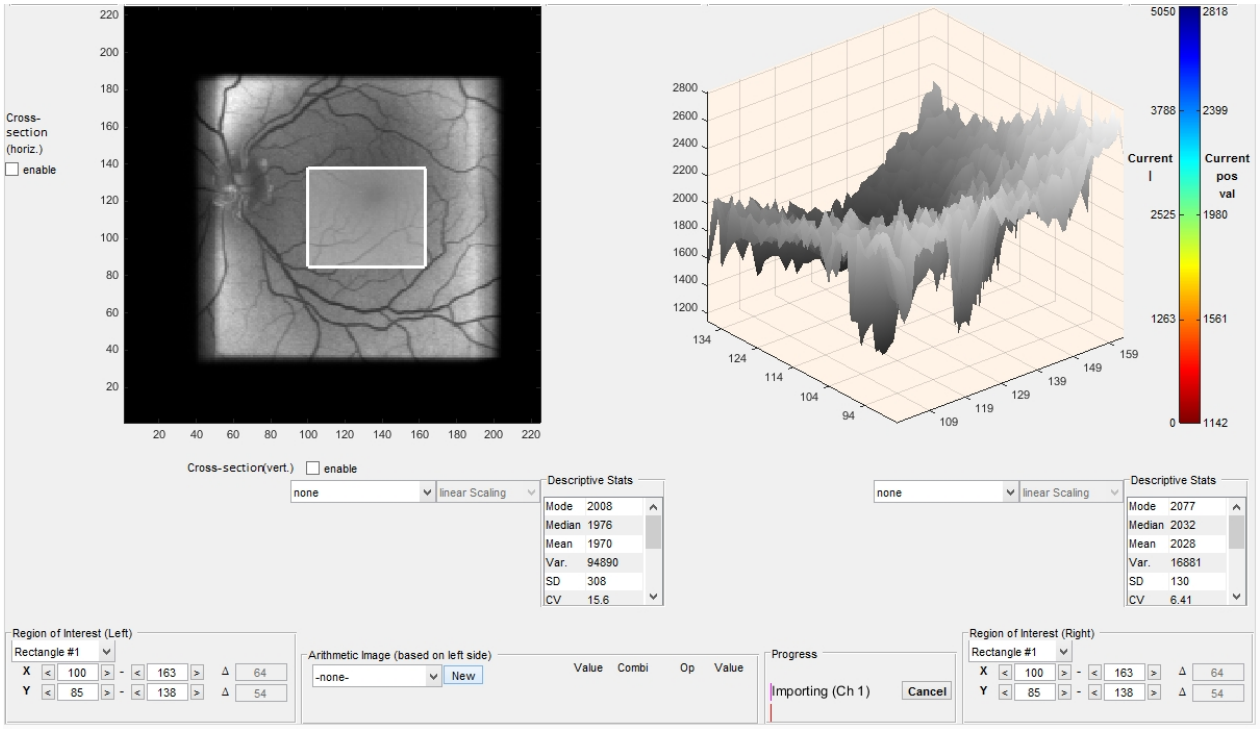
Rectangle ROI

To select a rectangular region of interest, select **Rectangle #1** or **Rectangle #2** within the ROI section. Type in the basic points of your rectangle. The rectangle will be displayed simultaneously in both windows. The size of the rectangle is indicated in the box with Δ. You can also draw the rectangle by holding the left mouse button within the ROI section and drag the pointer to the desired place.



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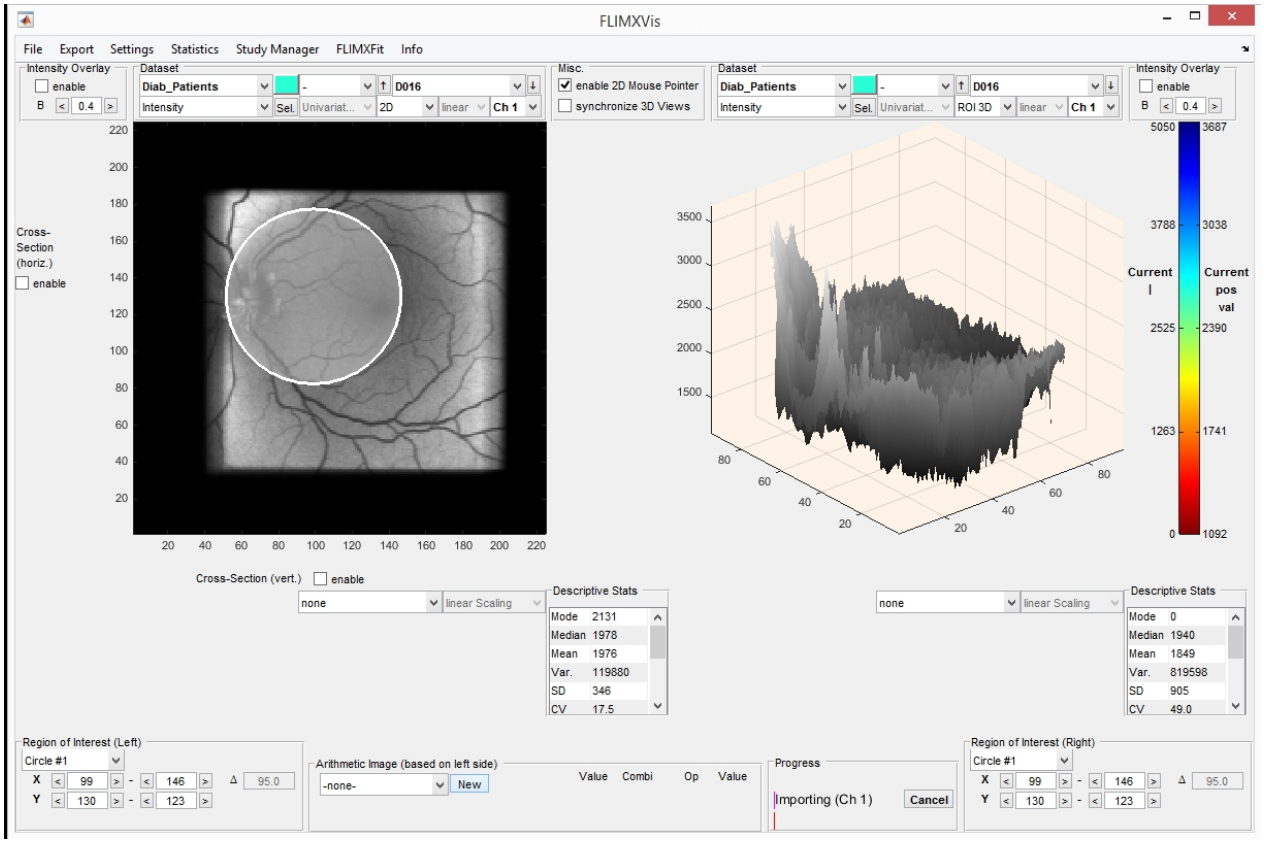
Circular ROI

To select a circular region of interest, select **Circle #1** or **Circle #2** within the ROI section. Type in the basic points of your circle where the first value stands for the center and the second for the spread from the center. The circle will be displayed in the left window and the content will be displayed in the right window. The diameter of the circle is indicated in the box marked with Δ. You can also draw the circle by holding the left mouse button within the left picture of the dataset and drag the pointer to the desired place.



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Polygonal ROI

To select a polygonal region of interest, select **Polygon #1** or **Polygon #2** within the ROI section.

Polygons are not yet implemented.

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Descriptive Statistics

FLIMXVis descriptive statistics helps you to analyze your study data based on descriptive statistics such as median, standard deviation or skewness. This section helps you to understand and use the following functionalities:

- Open Descriptive Statistics
- Graphic Interface
- Select study, condition, channel, FLIM parameter and ROI
- Show Histogram
- Export statistics data

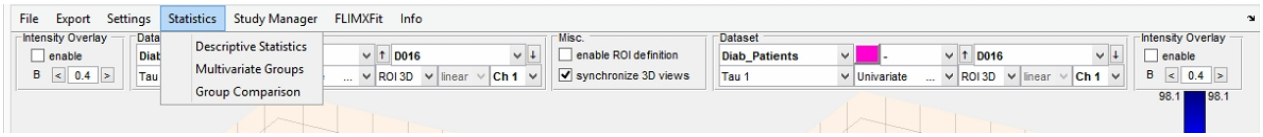
For statistics options, see Settings. Note that you must first create a study using Study Manager.

Open Descriptive Statistics

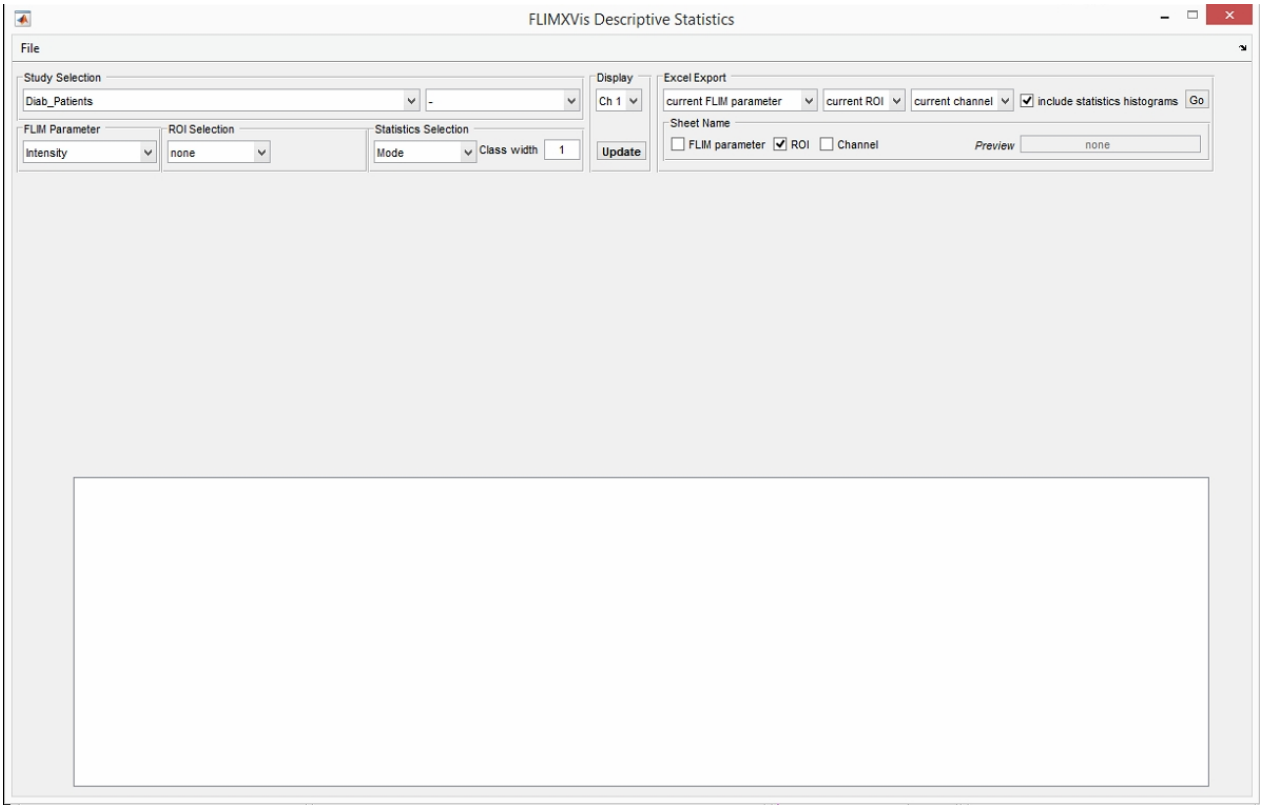
To show descriptive statistics for your images in FLIMXVis,

Open menu item **Statistics** > **Descriptive Statistics**

as seen in the picture below.



The following window will pop up:



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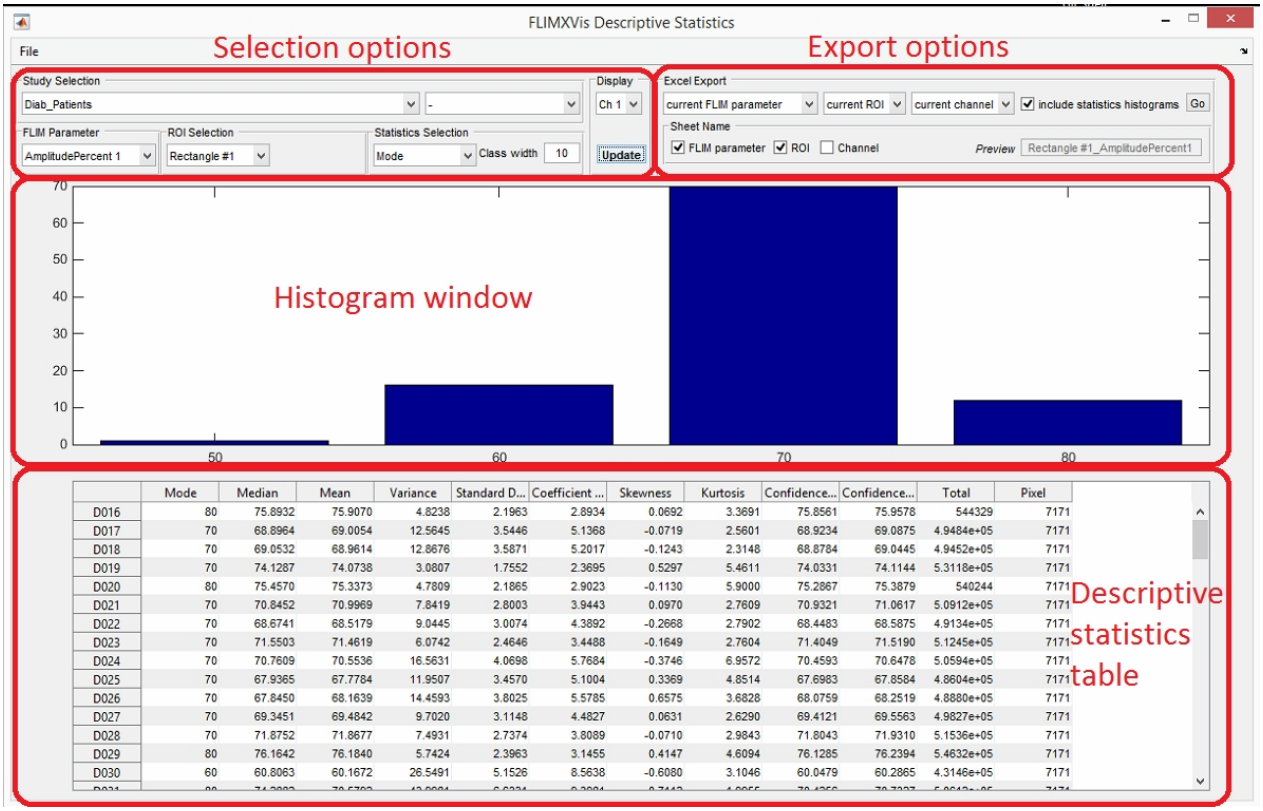
FLIMXVis Descriptive Statistics Graphic Interface

The FLIMXVis descriptive statistics graphic interface can be divided into four functional sections. These sections are marked and labeled in the picture below:

- Selection Options
- Export Options
- Histogram Window
- Descriptive Statistics Table



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Selection

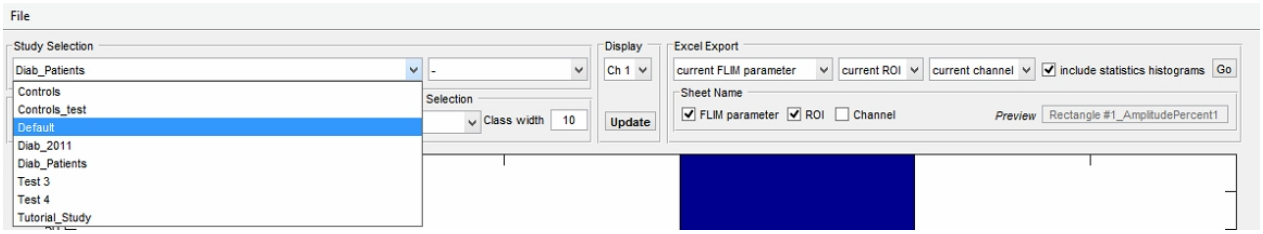
Options for selecting a

- Study
- Condition
- Channel
- Parameter and
- ROI

are located in the selection window of FLIMXVis descriptive statistics.

Study

To select a study, click on the arrow next to your current study. A list containing all of your existing studies will show up. Click on the name to select a study.



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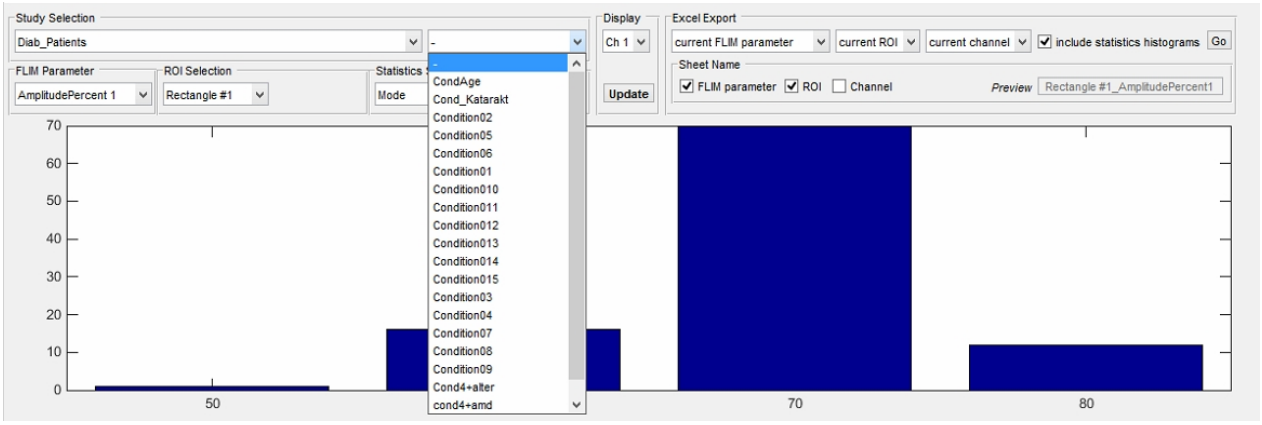
Condition

To select a condition, click on the arrow in the field left to your current study. A list containing all conditions within this study will show up. Click on the name to select a condition.



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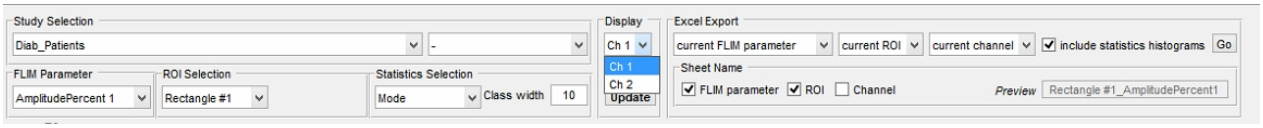
Channel

In case of multiple channels, click on the arrow next to your current channel as seen in the picture. A list containing all channel numbers within this study will show up. Click on the name to select a channel.



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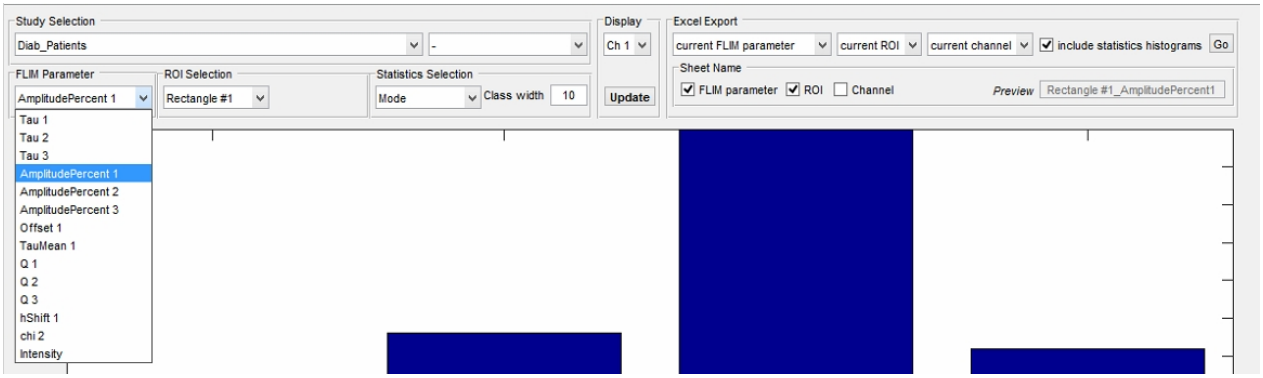
Parameter

To select a FLIM parameter such as amplitude or time constant, click on the arrow next to your current parameter as seen in the picture below. A list containing your parameters will show up. Click on the name to select a parameter.



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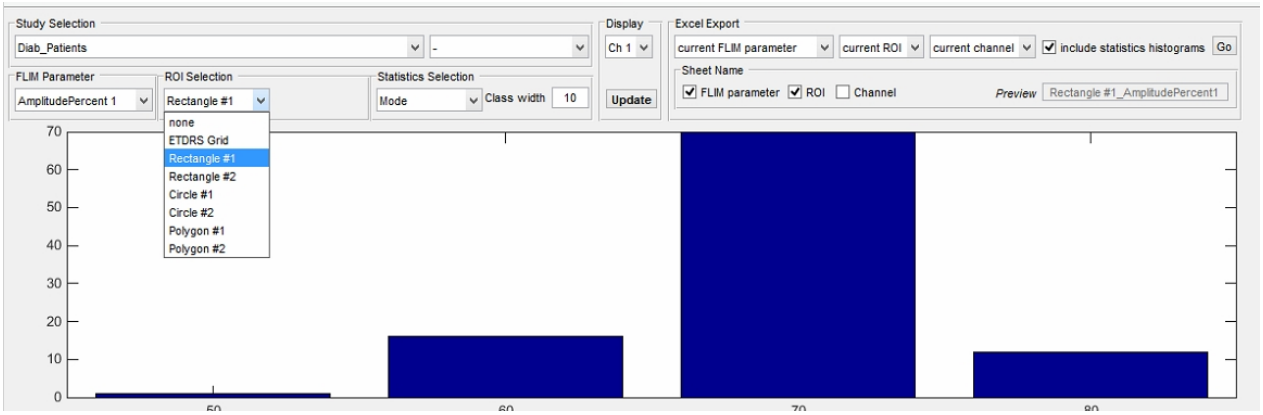
ROI

To select a Region Of Interest (ROI) within which descriptive statistics are calculated, you must first define this ROI. To select a ROI, click on the arrow in the **ROI Selection** box and chose custom or ETDRS grid by clicking on it.



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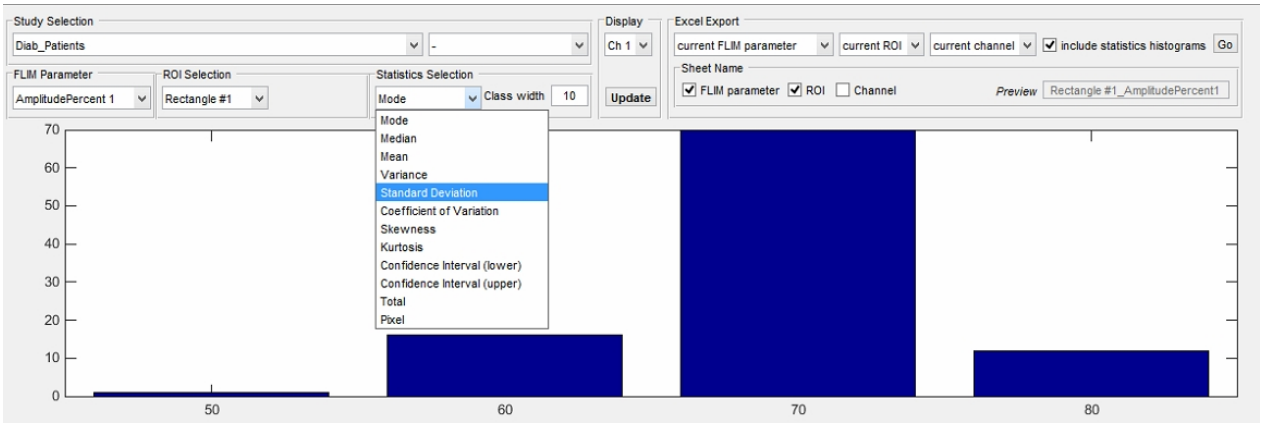
Histogram and Statistics

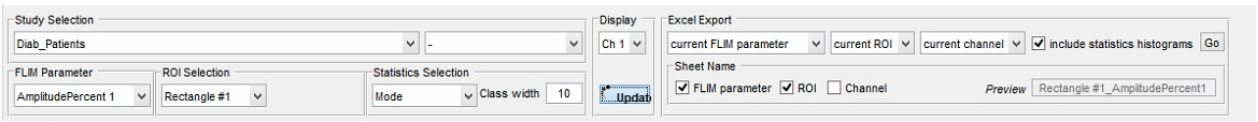
Histrogram for one statistic parameter is shown in the histogram window. Descriptive statistics for each subject within your current study are shown in the descriptive statistics window at the bottom of FLIMVis Descriptive Statistics.

To select which statistic parameter to show in the histogram window, click on the arrow next to your current statistic parameter as seen in the picture below. A list containing all statistic parameters will show up. Click on the name to select a parameter.

The class width defines the amount of values contained in each step. To select a class width, type in a number between one and your maximum number of individuals within your current study. In the example below, a class width of 10 has been chosen. To set a global class width, see Statistics Settings.



To confirm your changes and show descriptive statistics for your current options, click **Update** as indicated by the arrow in the example below. While FLIMXVis updates your data, a moving circle will show on the update button. Wait for this process to finish before continuing.



In the example below, the study Diab_Patients is displayed.

Since no condition has been selected, all subjects within the study will be compared statistically. For each subject, descriptive statistics for Channel 1, Amplitude 1 and custom ROI are computed. These statistics (mode, median, mean, ...) are shown in the descriptive statistics window at the bottom. Use the bar on the left to scroll through your subjects.

In the histogram window, the histogram for median with a class width of 10 is displayed. On the x-axis, the median values are shown. On the x-axis, the amount of subjects within each median value is shown. When decreasing or increasing class width, bars will get finer or less fine.

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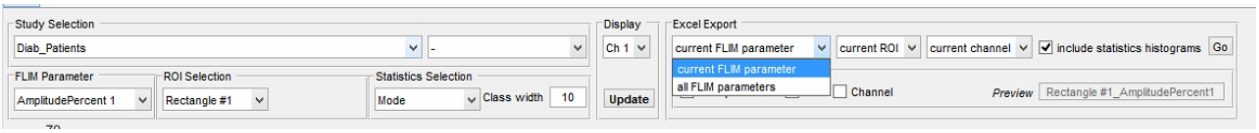
Export

Options for excel export are located in the upper left window within the descriptive statistics graphic interface. FLIMXVis offers you the possibility to export current or all FLIM Parameters, ROI and channel. Chose **current** or **all** by clicking on each arrow and selecting the appropriate option as seen in the picture below.

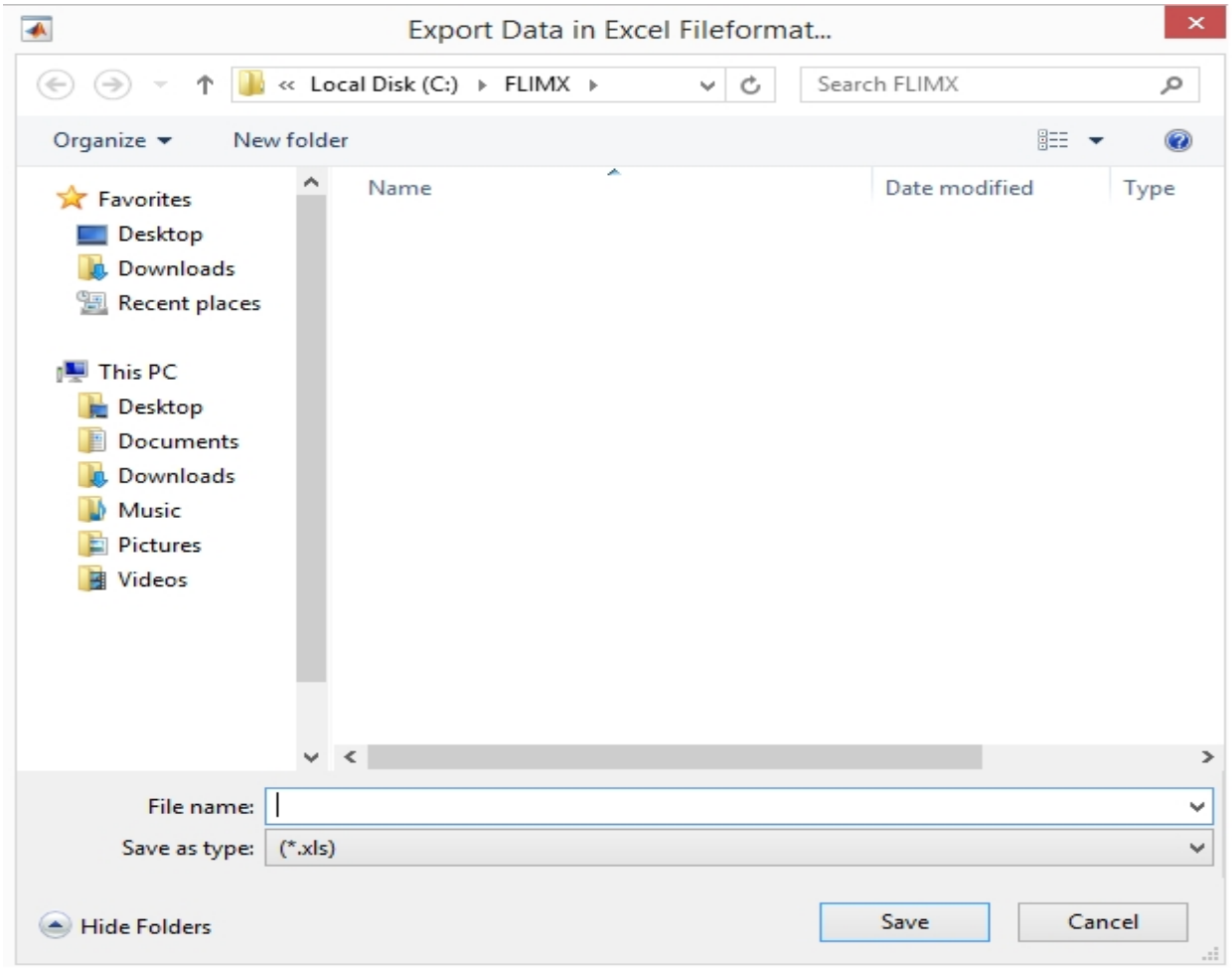
To include statistics histogram in your excel sheet, tick the corresponding box.

Data name is previewed in the export window. To change labeling, tick or untick the boxes on the left of labeling preview.

After selecting all options, confirm by clicking on **Go**.



The following window will pop up allowing you to browse your files and select a location to save your file. Type in a name for your file. Click **Save** to save your data in excel format (.xls) or **Cancel** to close the window without saving.



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Multivariate Groups

Multivariate Groups plots help you to analyze linear correlations between different FLIM Parameters. This section deals with the following functionalities:

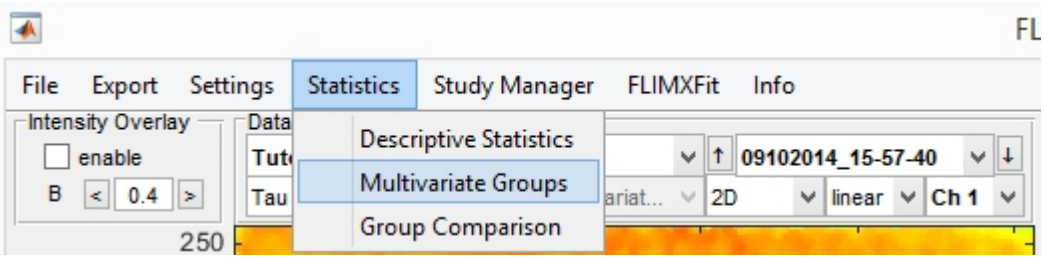
- Open Multivariate Groups
- Multivariate Selection
- Multivariate Group Definition
- Multivariate Group in Multiple Studies
- Visualize Multiple Groups

Open Multivariate Groups

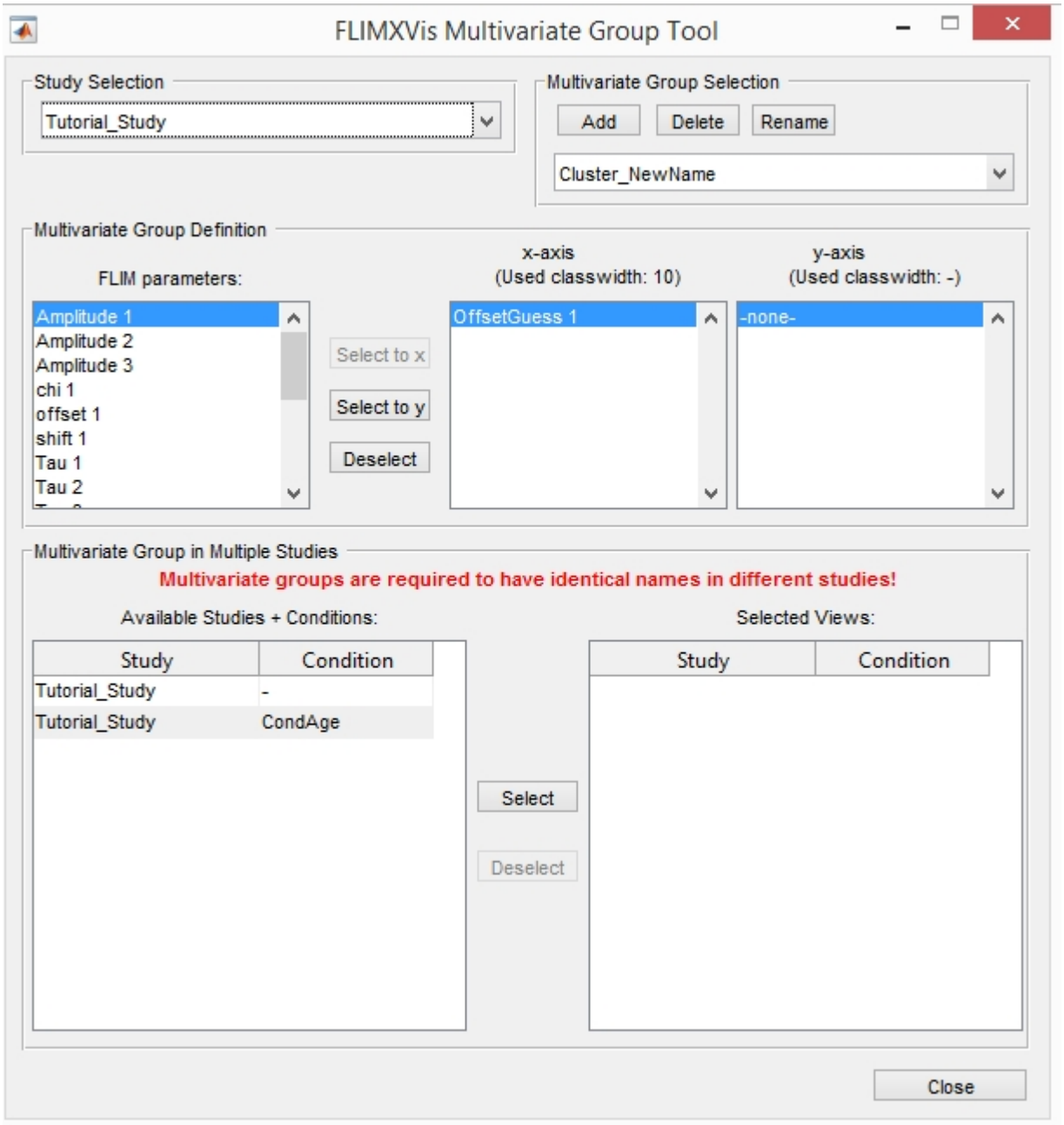
To create multivariate groups for your parameters in FLIMXVis,

Open menu item **Statistics** > **Multivariate Groups**

as seen in the picture below.



The following window will pop up:



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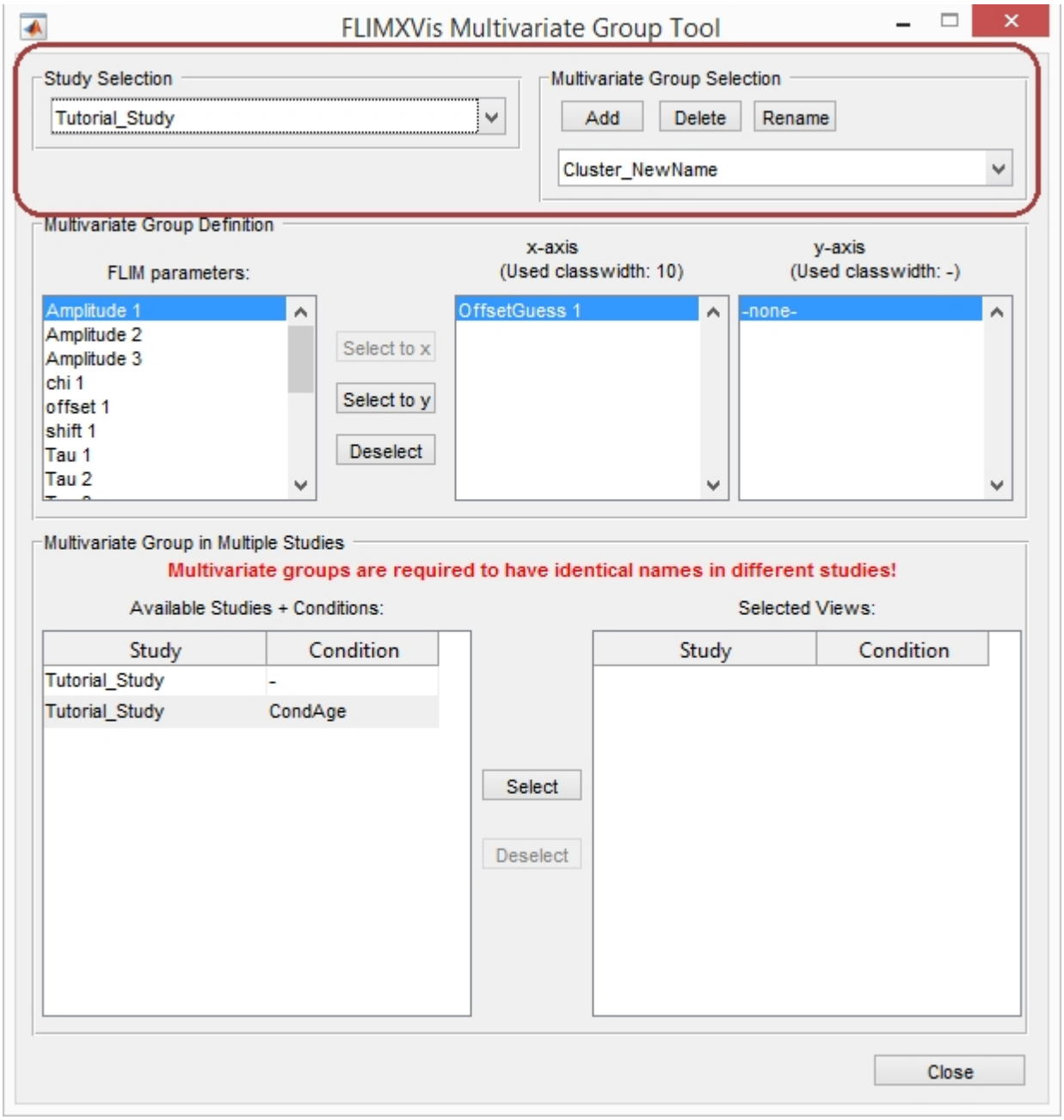
Multivariate Selection

Within Multivariate Selection, you can add, delete or rename multivariate groups. This section

is marked by a red frame in the picture below.



- **Add :** First select a study from the **Study Selection** list by clicking on it. Then type in the name of your multivariate group in the box on the right (here: Cluster_NewName) and confirm by clicking on the button labeled **Add**.
- **Delete:** Click on the group you wish to delete. Then confirm by clicking on the button labeled **Delete**.
- **Rename:** Click on the group you wish to rename. Then confirm by clicking on the button labeled **Rename**. Type in a new name in the box on the top right.

In order to visualize a scatter plot, you must define x and y axis parameters for your group. To do this, see Multivariate Group Definition. After creating a group, this group will be available in the FLIMXVis parameter section.

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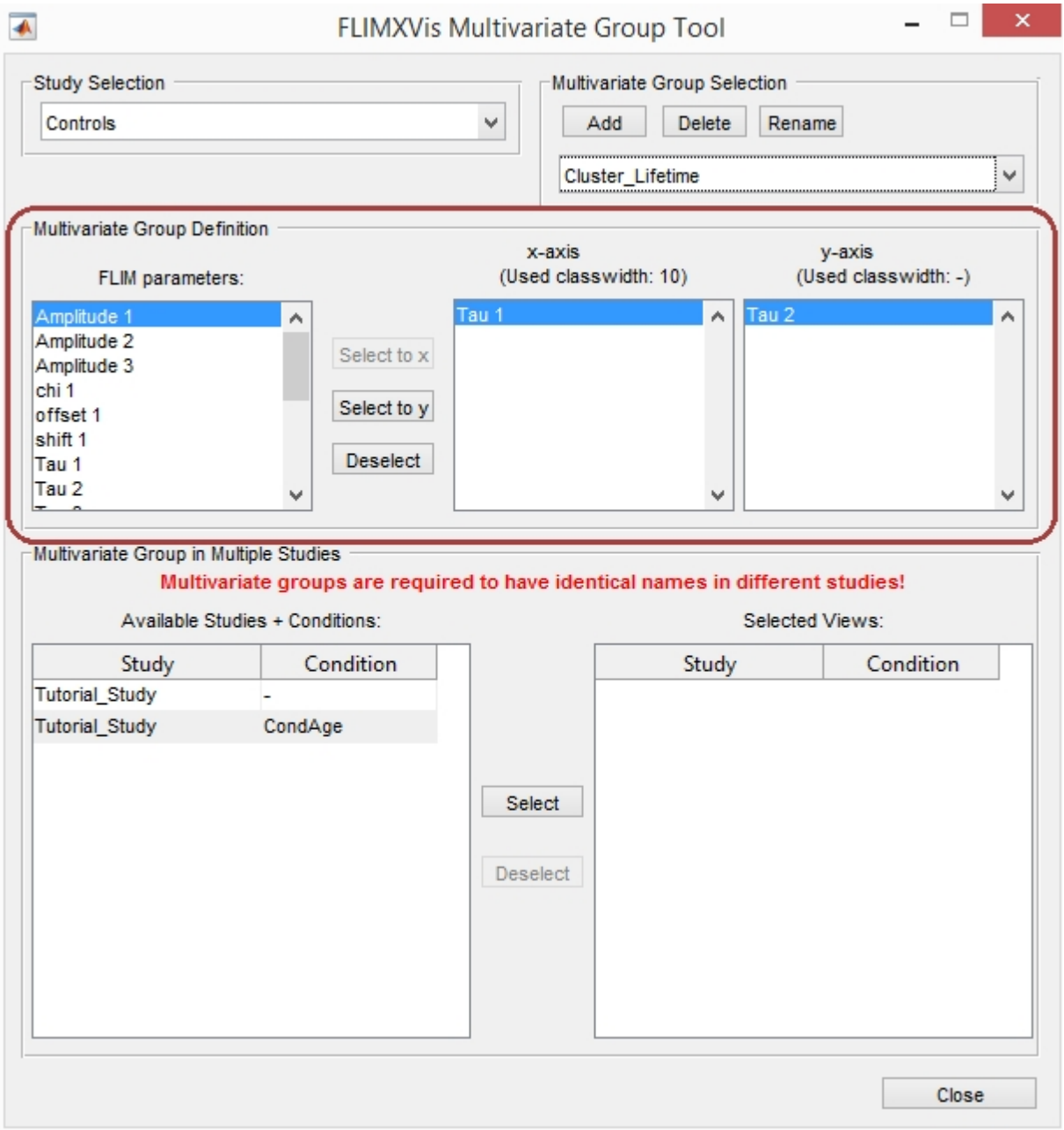
Multivariate Group Definition

Use Multivariate Selection to define x and y axis parameters for visualization of a scatter plot. These options are marked by a red frame in the picture below.



- **Select a parameter to x-axis:** Click on a item in the FLIM items list. Then, confirm by clicking on **Select to x**. The parameter will now appear in the left list.
- **Select a parameter to y-axis:** Click on a item in the FLIM items list and confirm by clicking on **Select to y**. The parameter will now appear in the right list.

In the example above, Tau1 has been selected for x-axis and Tau2 for y-axis. See below to learn

how to visualize the cluster which you just defined.

To create multivariate groups with the same parameters for different groups, see Multivariate Groups in Multiple Studies.

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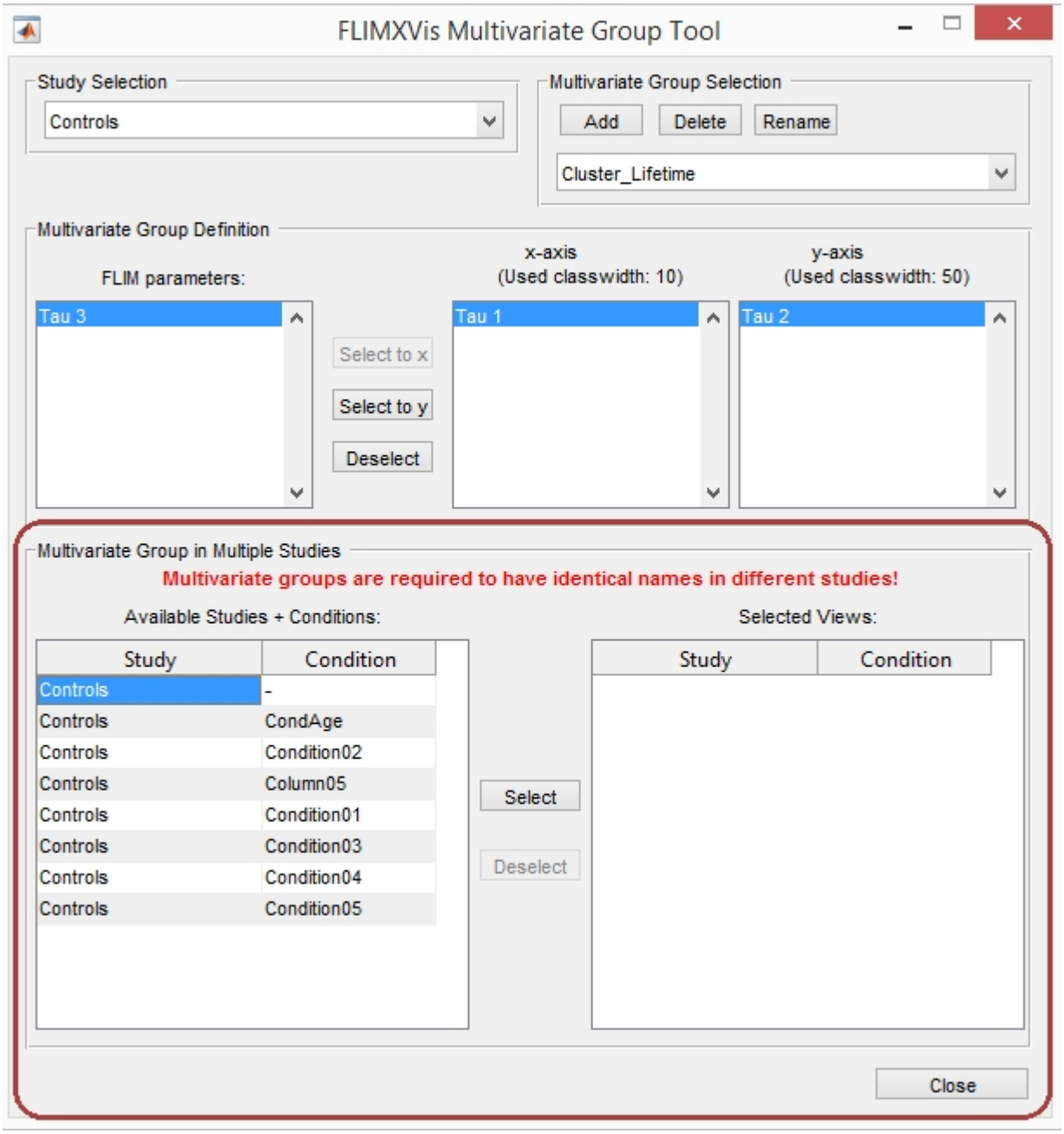
Multivariate Groups in Multiple Studies

A multivariate group in multiple studies is a group which contains the same x and y axis parameters for different studies.

By defining these, you can compare multivariate groups from different studies, for example pathologic and control groups. These options are located at the bottom of the window as marked by a red frame in the picture below.



To define a multivariate group in multiple studies, select a study and group at the top of the window by clicking on it. If you have not defined any groups yet, see above to create these first

In the example below, the study Controls and the group Lifetime (shaded gray) have been selected. Tau1 and Tau2 are defined on the x and y axis.

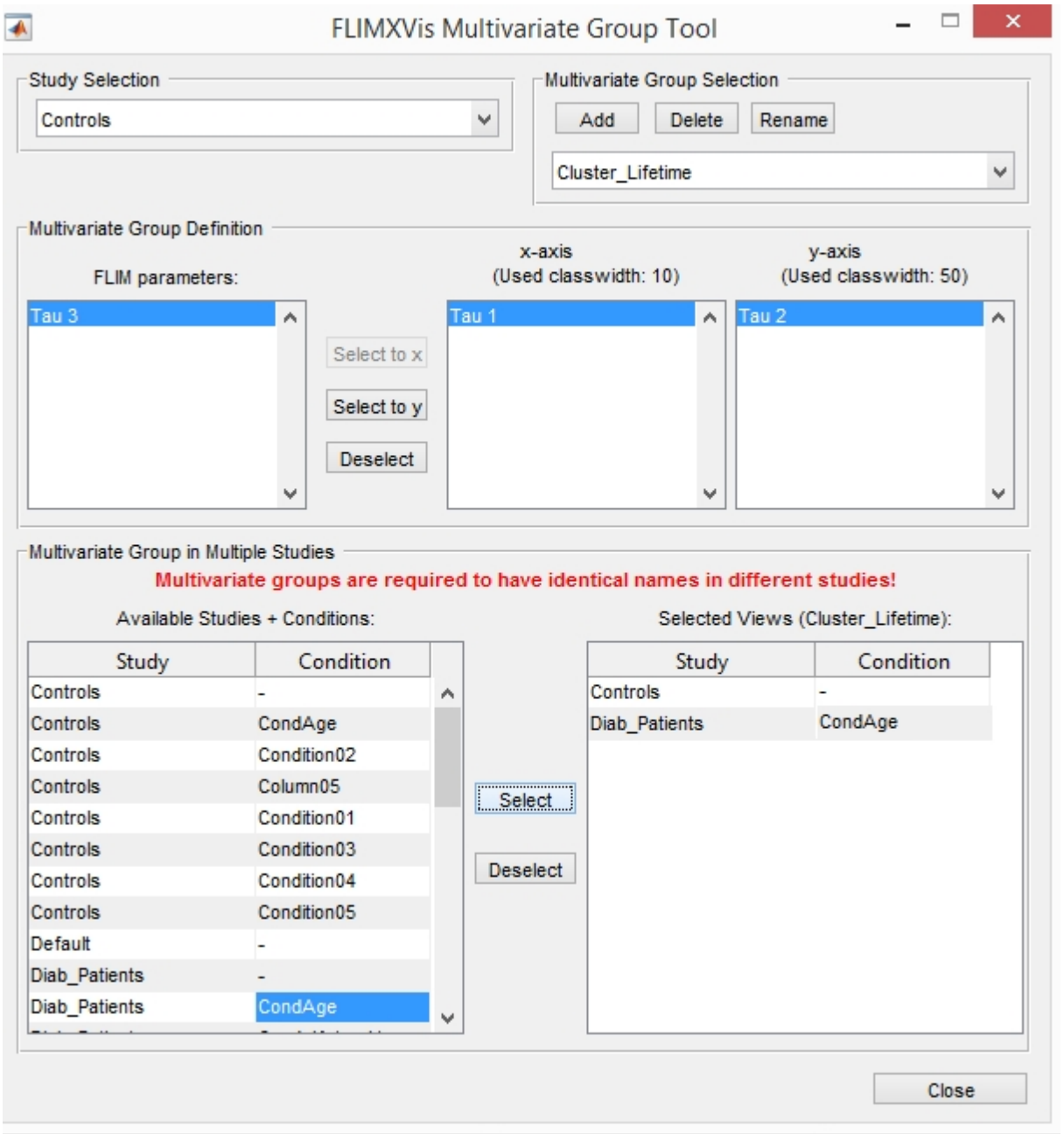
At the bottom of your window, this study will now appear. To select subjects with a certain condition, click on this condition in the Study Views list. To select all subjects from this study, click on - . To confirm your selection, click on the **Select** button. Your choice will now appear in the list on the right (Selected Views) as marked by an arrow in the example below. In the example below, CondAge has been chosen.

To delete your choice, click on the item in the selected views list and then click on the **Deselect** button. It will be removed from the list.

You might have noticed that all your studies now appear in the Study Views list on the bottom. By choosing a study/condition in this list and then clicking the select button, your group will automatically be created within this study. This option enables you to easily define multivariate groups for healthy and pathologic group and then compare these.

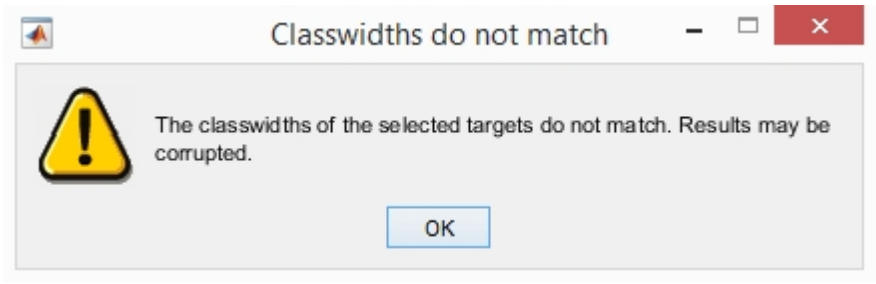
If you want to create more multiple groups, repeat this routine.

In the example below, the study Diab_Patients with CondAge has been chosen as second group.



Click on **Close** to close the window. For visualization, see below.

If the following error window occurs, you might need to adjust class widths for your parameters.



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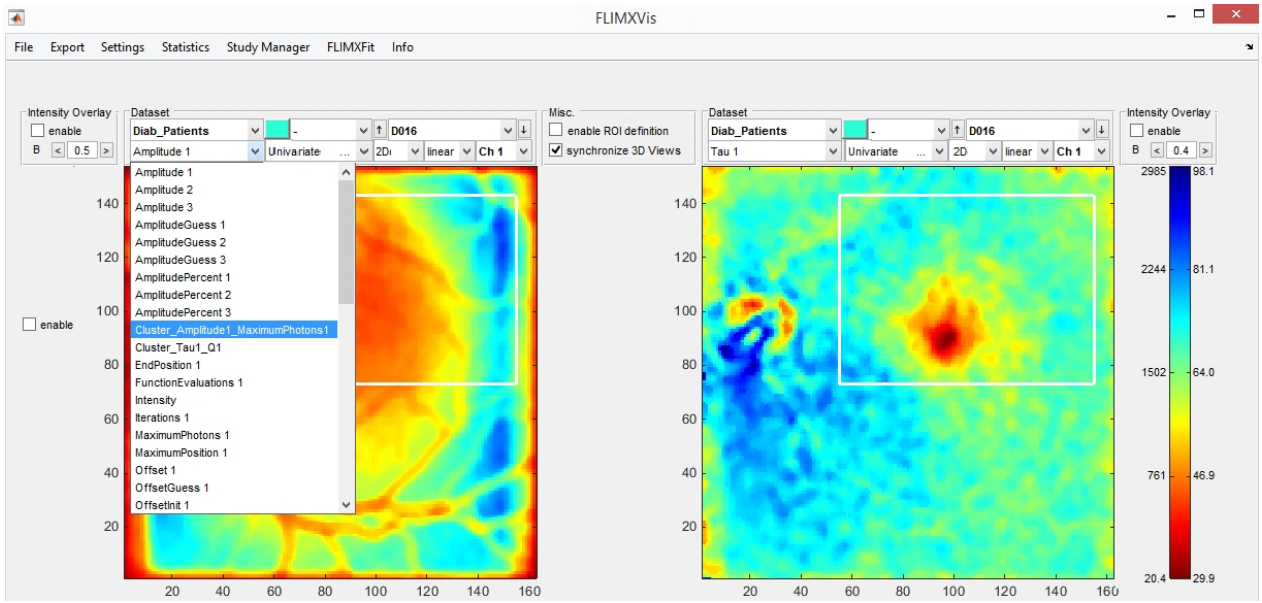
Visualize Multivariate Groups

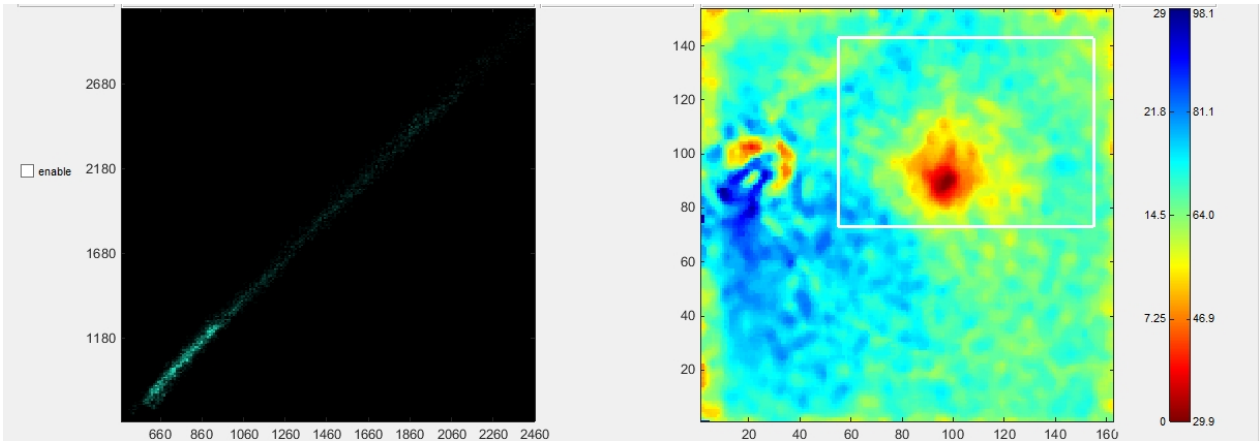
To visualize your scatter plot, go back to FLIMXVis.

Within FLIMXVis, your multivariate group will now be available as a parameter for visualization within FLIMXVis Selection Options. Click on the arrow next to your current parameter as seen in the picture below. A list containing all your parameters plus the multivariate groups you defined will show. Click on your group to select it for visualization.



Your scatter plot will now be displayed in the left window as seen in the picture below. To select other parameters, see above. To show the scatter plot for a different subject (here: D016), see Selection.

For more visualization options, see Views.



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Group Comparison

Group Comparison helps you to compare data from pathologic and healthy subjects using test statistics. You can

- Analyze measurement data and search for statistically different values between a pathologic and a control group
- Find a classifier that helps you to distinguish pathologic subjects from a control group

Within this section, the following functionalities are explained

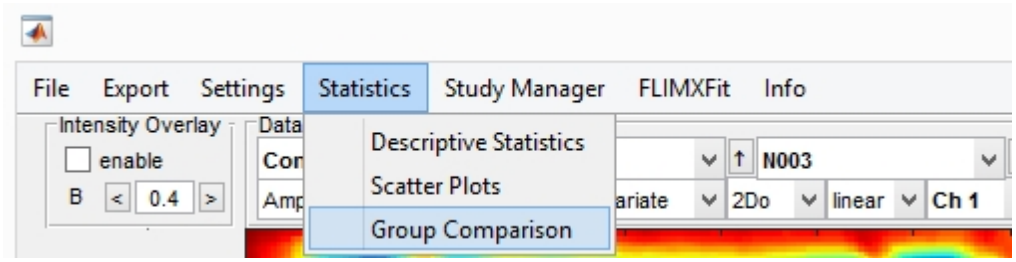
- Open group comparison
- Define pathologic and control group, select parameters and statistic method
- Display group comparison
    - T-Tests
    - Wilcoxon Tests
    - Holm-Bonferroni Test (classifier)
- Export data

Open Group Comparison

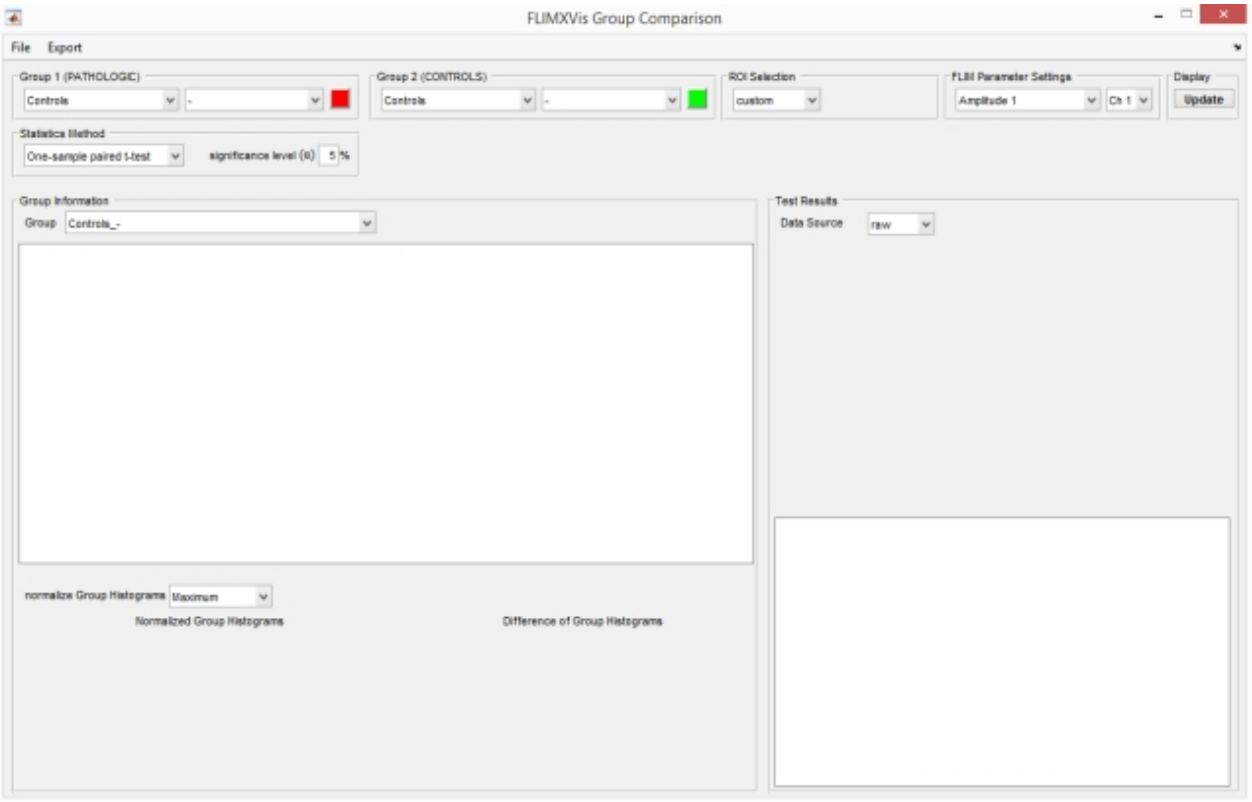
To create group comparison options in FLIMXVis,

Open menu item **Statistics** > **Group Comparison**

as seen in the picture below.



The following window will pop up:



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Selection

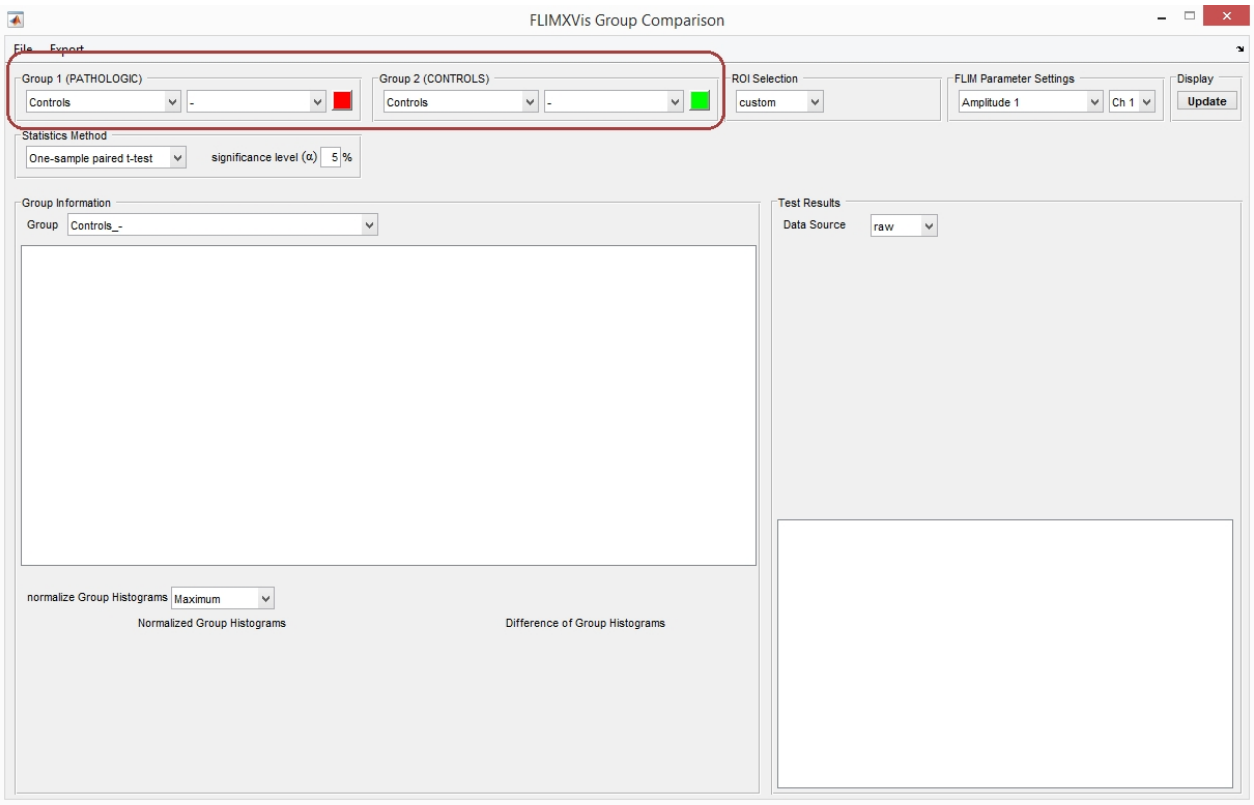
Within this section, the following functionalities are explained

- Define pathologic and control group
- Chose ROI, channel and parameter
- Select statistic method

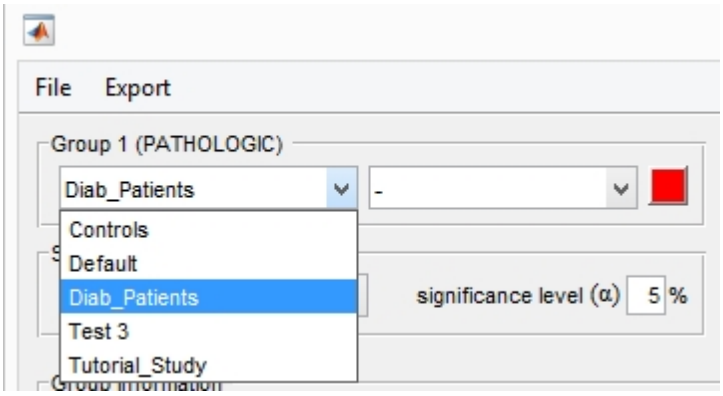
Define pathologic and control group

The options for defining a pathologic and a control group are located in the upper left part of

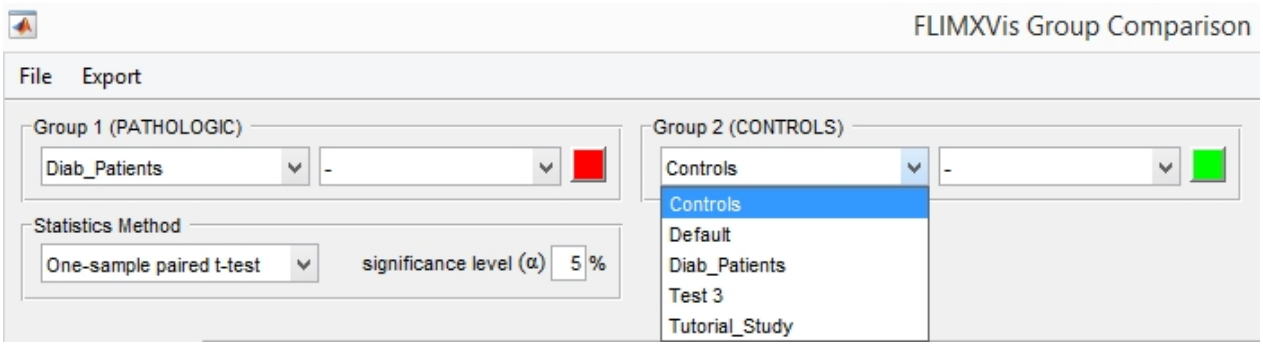
the group comparison interface as indicated by the red box in the picture below.



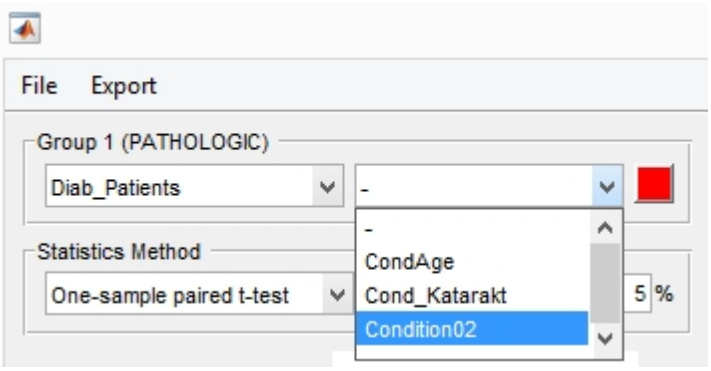
For each group (pathologic and control) you must select a study. Note that studies and subjects must first be imported using Study Manager. To select a study, click on the arrow next to your current study as seen in the picture below. A list containing all of your existing studies will show up. Click on a study to select it (here: Diab_Patients).



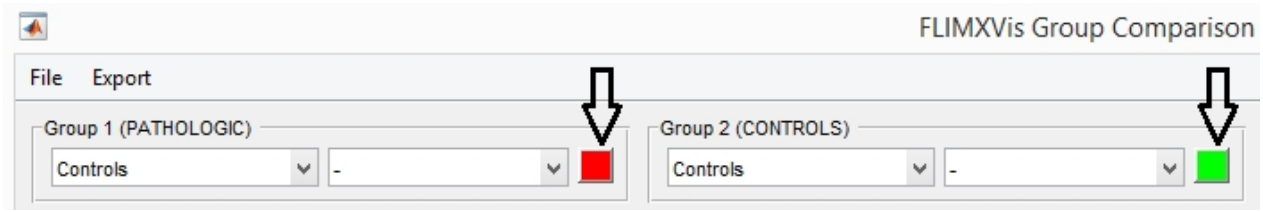
Do the same for the Group 2 (CONTROLS) on the left.



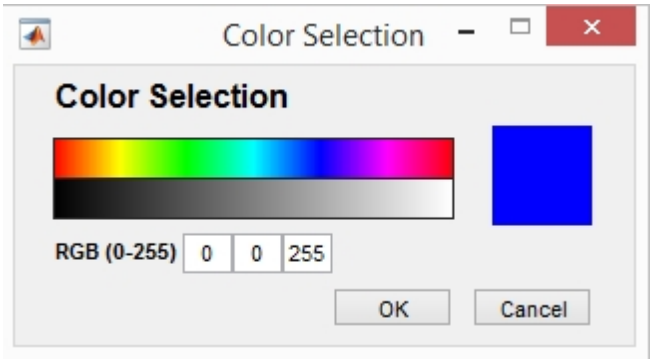
If you wish to include only subjects which are matching a certain condition, click on the arrow next to -. A list with all conditions for your selected study will show up. Click on a condition to select it. Selecting - will include all subjects within this study.



Data from both groups is displayed with different colors. To change these colors, click on the color buttons next to study selection as indicated by the arrows in the picture below.



After clicking on a color button, the following window will pop up:



The current color is shown on the left. To select another color, click on a color in the colorbar or

type in three RGB values (here: 0 0 255). To confirm your color selection and close the window, click **OK**. To close the window without saving, click **Cancel**.

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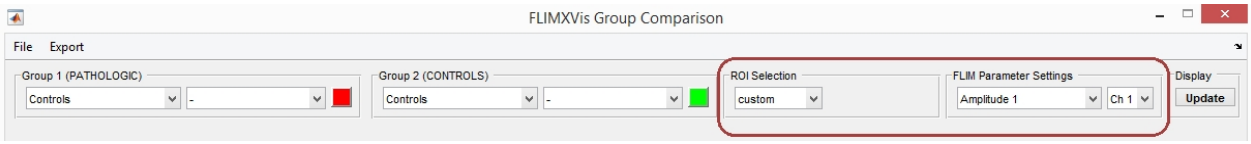
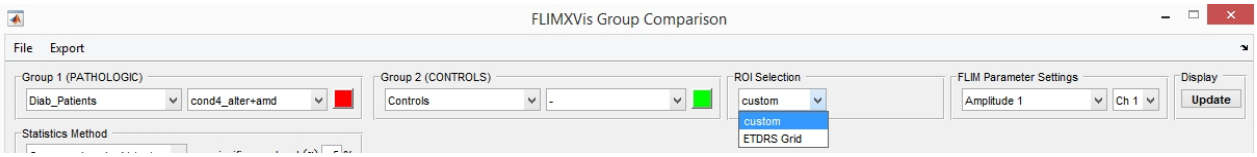
ROI, Channel and Parameter Settings

The options for selecting FLIM parameter, channel and ROI are located on the top right as indiacted by the red box in the picture below.



To select a ROI, click on the arrow next to your current ROI as seen in the picture below. Note that you must first define a custom ROI or ETDRS grid FLIXVis. A list will show up. Click on custom or ETDRS to select it. Proceed in the same way for parameter (here: Amplitude 1) and channel selection.



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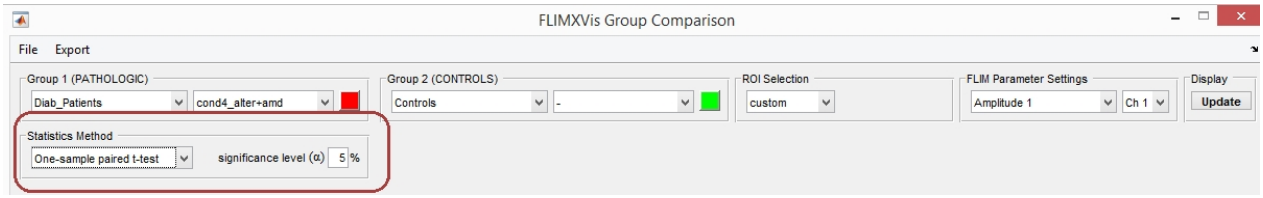
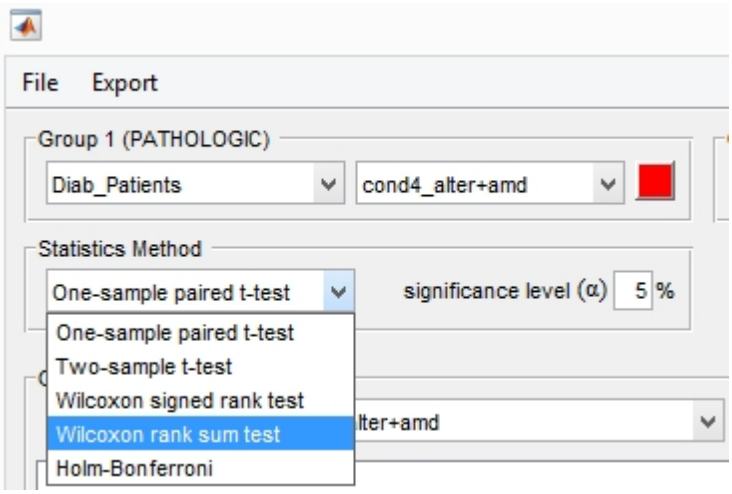
Statistics Method

The statistics section is indicated by a red box in the picture below. These options allow you to select a test method and a significance level.



To select a test, click on the arrow next to your current test (here: One-sample paired t-test). A list containing the different tests will show up. Click on a test to select it. When selecting Holm-Bonferroni, additional options will pop up. See Holm-Bonferroni preferences for further information. To change the significance level α type in a value in the box on the right.



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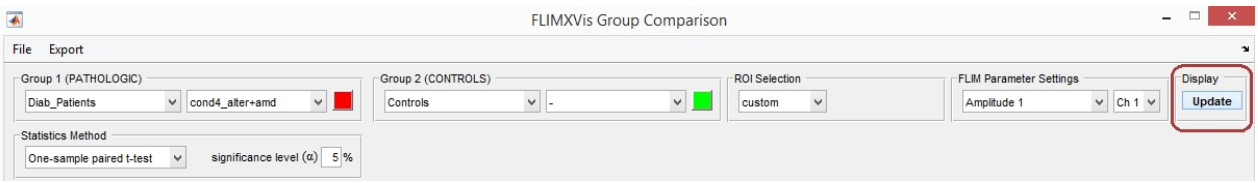
Display Group Comparison

After defining pathologic and control groups and selecting parameter and statistics method options, group comparison can be computed.

To display group comparison, click on the **Update** button on the left side of your FLIMXVis Group Comparison window. The progress is indicated by a moving circle of dots on the update button. Wait for this progress to finish.



Depending on the test method you have chosen, different windows and parameters will appear. To learn more about group comparison and what's displayed when using

- One-sampled paired t-test
- Two-sampled t-test
- Wilcoxon signed rank test and
- Wilcoxon rank-sum test
- Holm-Bonferroni

see the sections below.

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T-Test

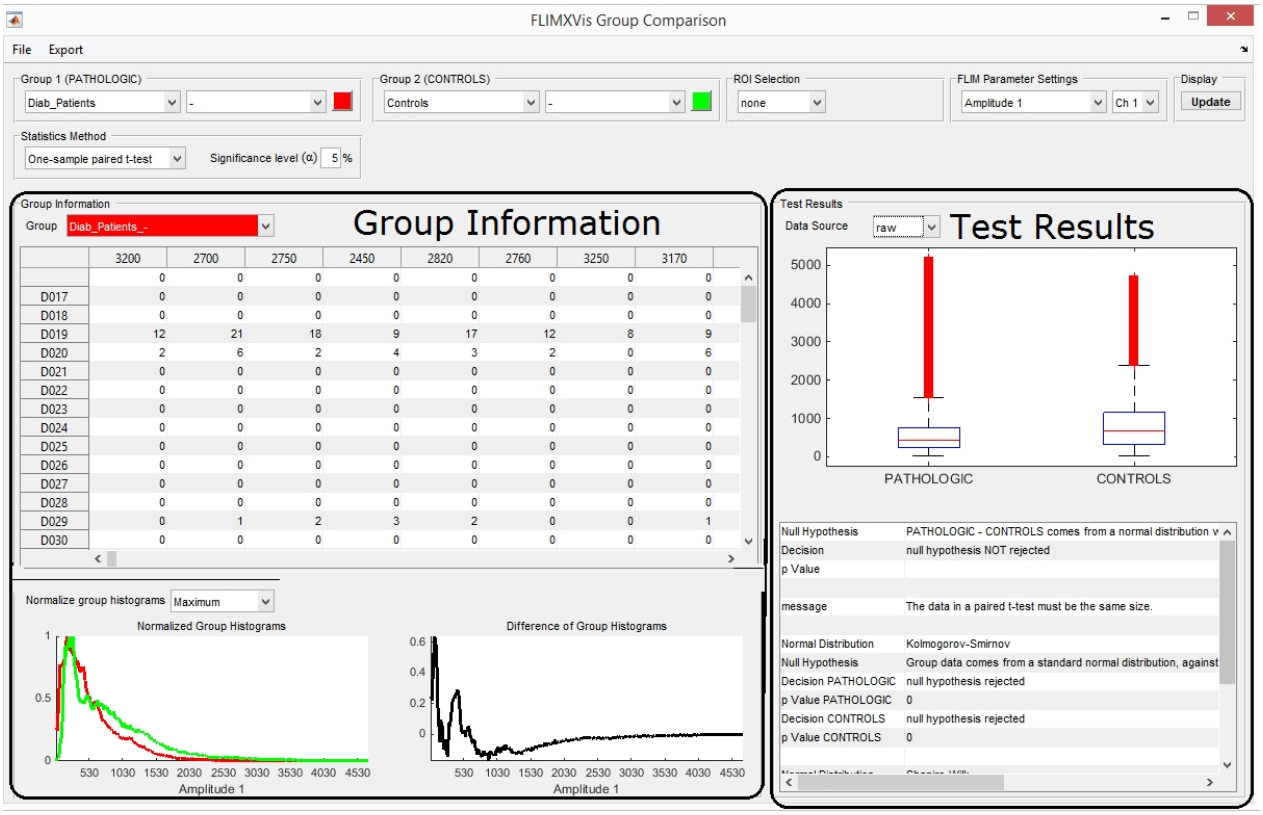
Using the t-test, you can compare the statistics of pathologic and control group and find a suitable classifier to distinguish these groups. The two options are:

- One-sample paired t-test: Test whether your data comes from a standard normal distribution
- Two-sample t-test: Test the relation of the mean values from two independent samples. Use Wilcoxon-test if your data does not come from a normal distribution.

To use a t-test, chose this option in the test section and select parameters as described above. After clicking on update, the FLIMXVis Group Comparison window will look like the example seen below.

For both options, the following two boxes which are marked in the picture below will be shown:

- Group Information: Table and visualization of histogram data. This is the same for all tests.
- Test Results: Test Results. See one-sample paired t-test and two-sample t-test for more information.

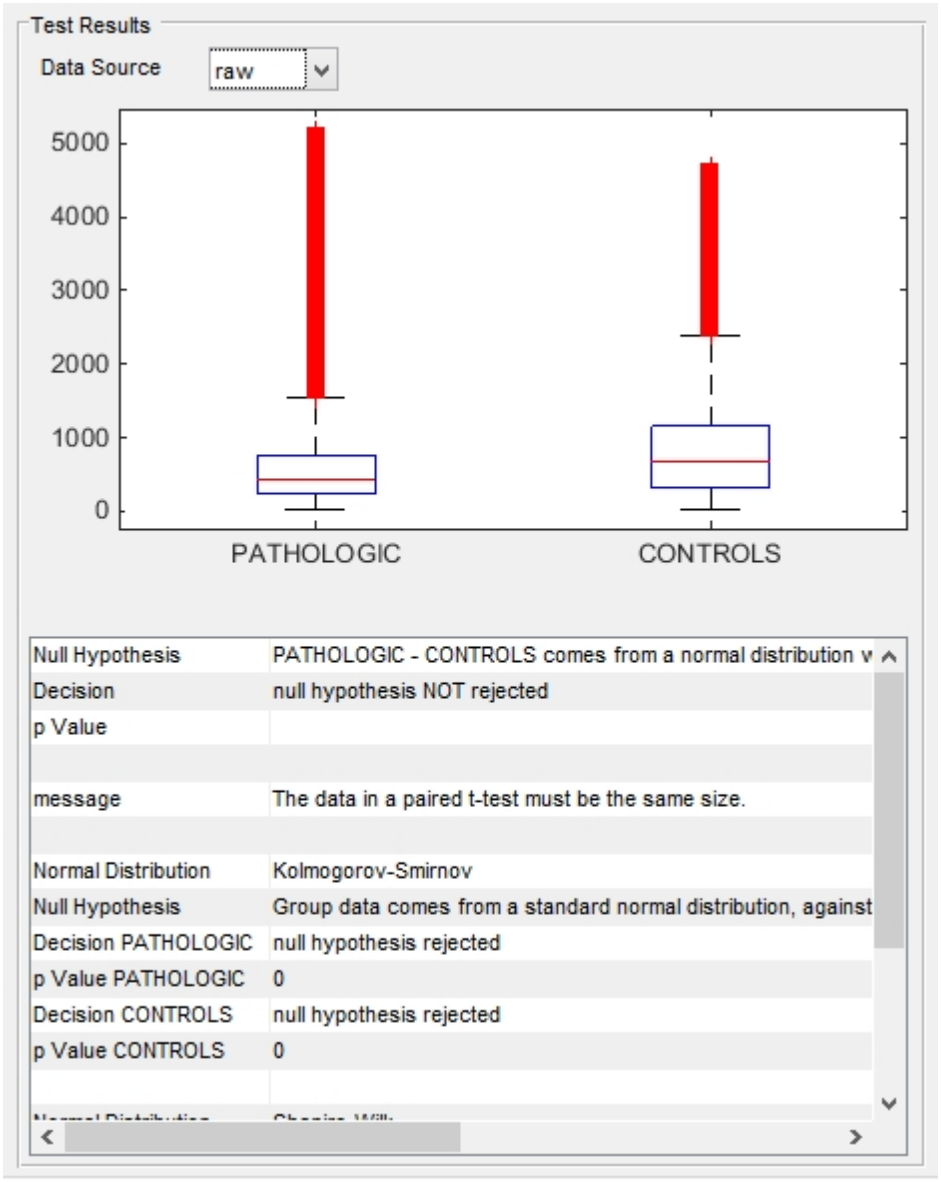


One-sample paired t-test

When using one-sample paired t-test, the algorithm compares whether the groups come from a normal distribution. The results are shown in a box plot and in a information window.

In the example below, the null hypothesis (Pathologic-Controls comes from a normal distribution) is not rejected. Additional test results (Kolmogorov-Smirnov and Shapiro-Wilk) are displayed when scrolling down.

For more information on the results, see here.



Two-sample t-test

When using two-sample t-test, the algorithm tests the relation of the mean values from two independent samples. The results are shown in a box plot and in a information window.

For more information on the results, see here.

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Wilcoxon Test

Using Wilcoxon Tests, you can compare the statistics of pathologic and control group and find a

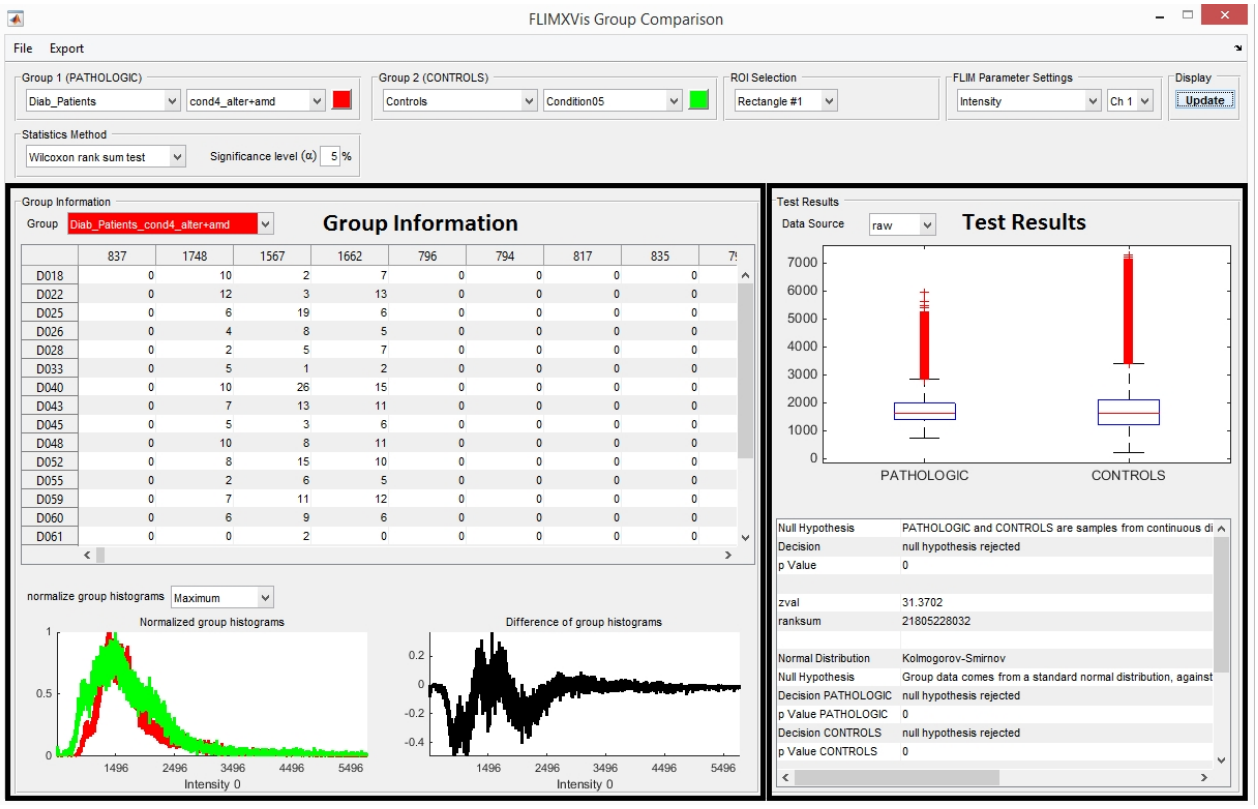
suitable classifier to distinguish these groups. The two options are:

- Wilcoxon signed rank test: Test whether the population mean ranks on your data differ
- Wilcoxon rank-sum test:Test whether your groups belong to the same population

To use a Wilcoxon test, chose this option in the test section and select parameters as described above. After clicking on update, the FLIMXVis Group Comparison window will look like the example seen below.

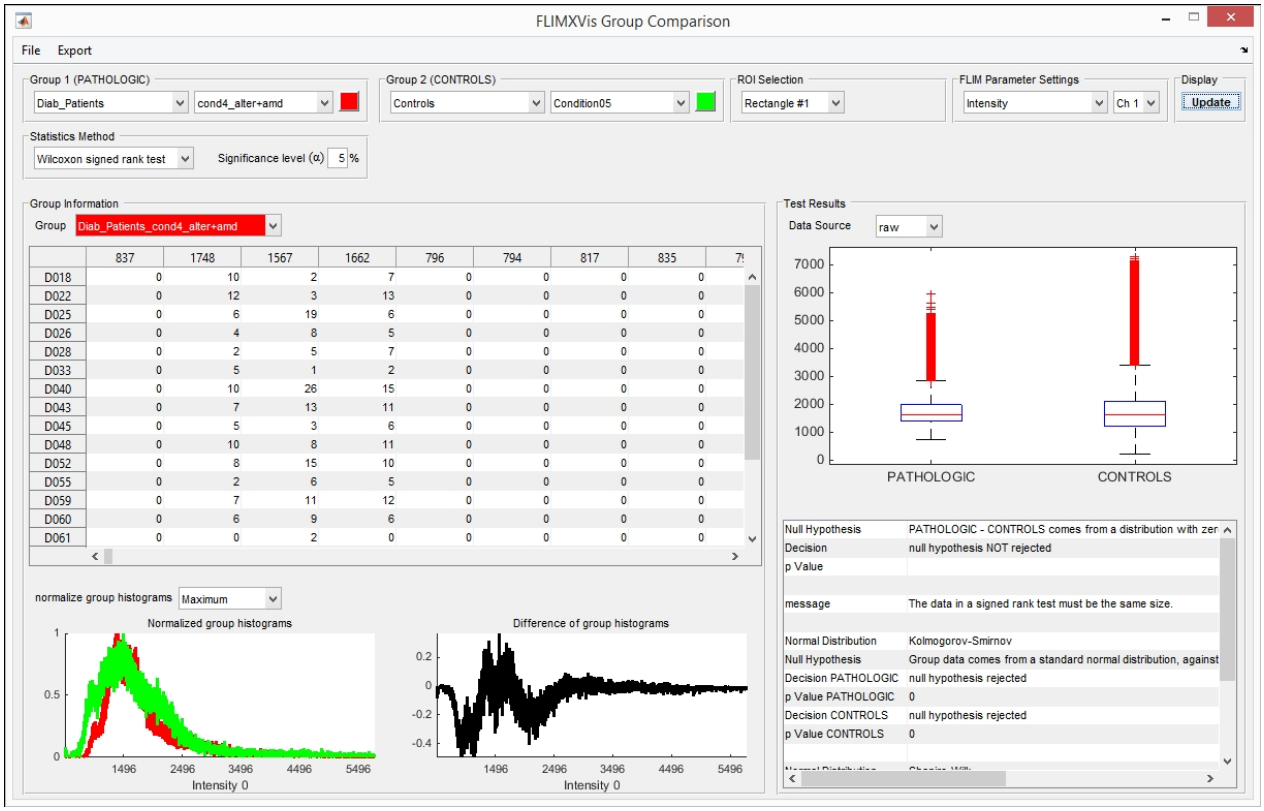
For both options, the following two boxes which are marked in the picture below will be shown:

- Group Information: Table and visualization of histogram data. This is the same for all tests.
- Test Results: Test Results. See Wilcoxon signed rank test and Wilcoxon rank-sum test for more information.

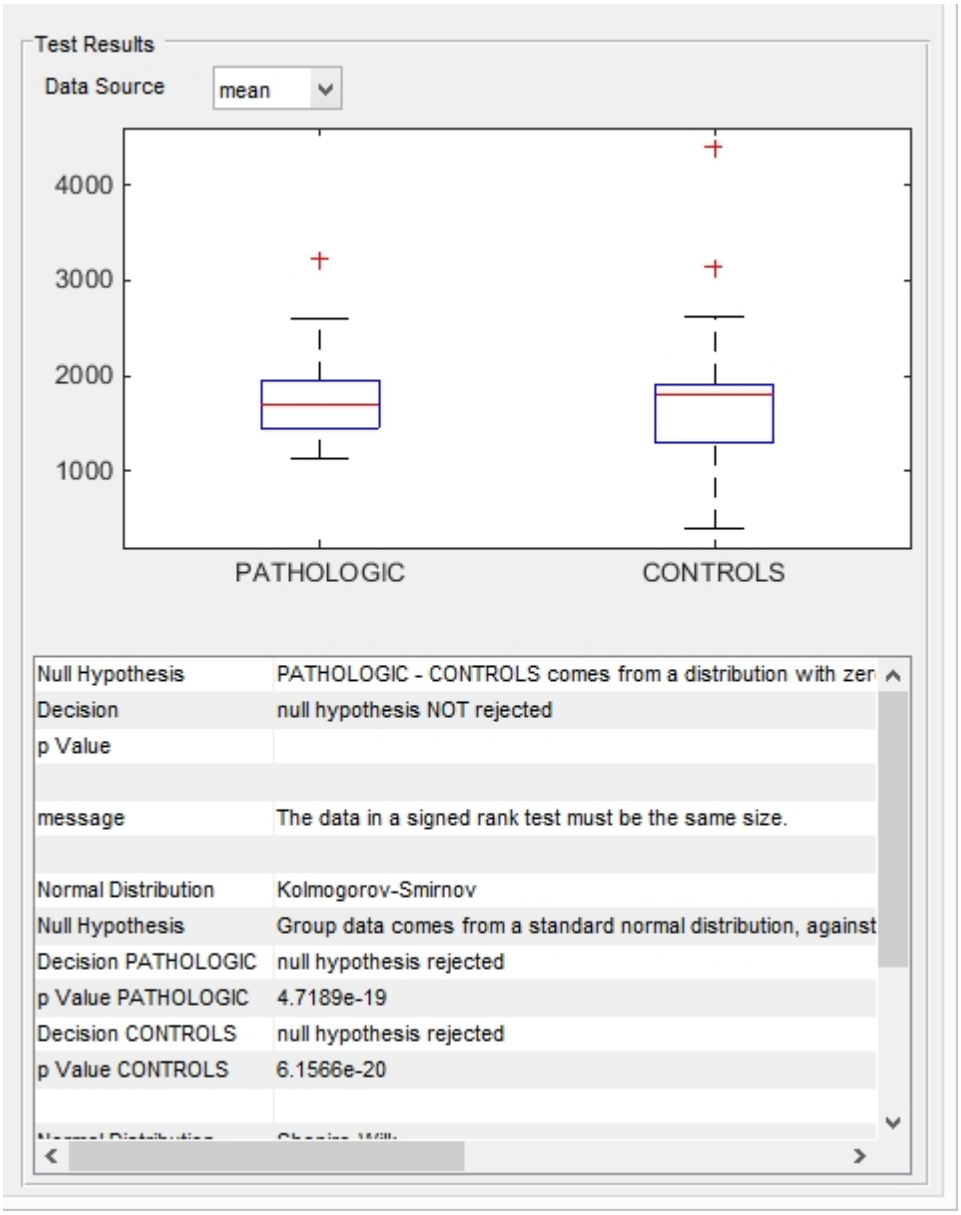


Wilcoxon signed rank test

Use Wilcoxon signed rank test instead of a t-test when your data does not come from a normal distribution. It tests whether the population mean ranks on your data differ.



The use of mean as data source leads to more comparable box plots. Raw data includes all data points of all subjects. This smallers the boxes and leads to more outlying results (red bars above boxes).

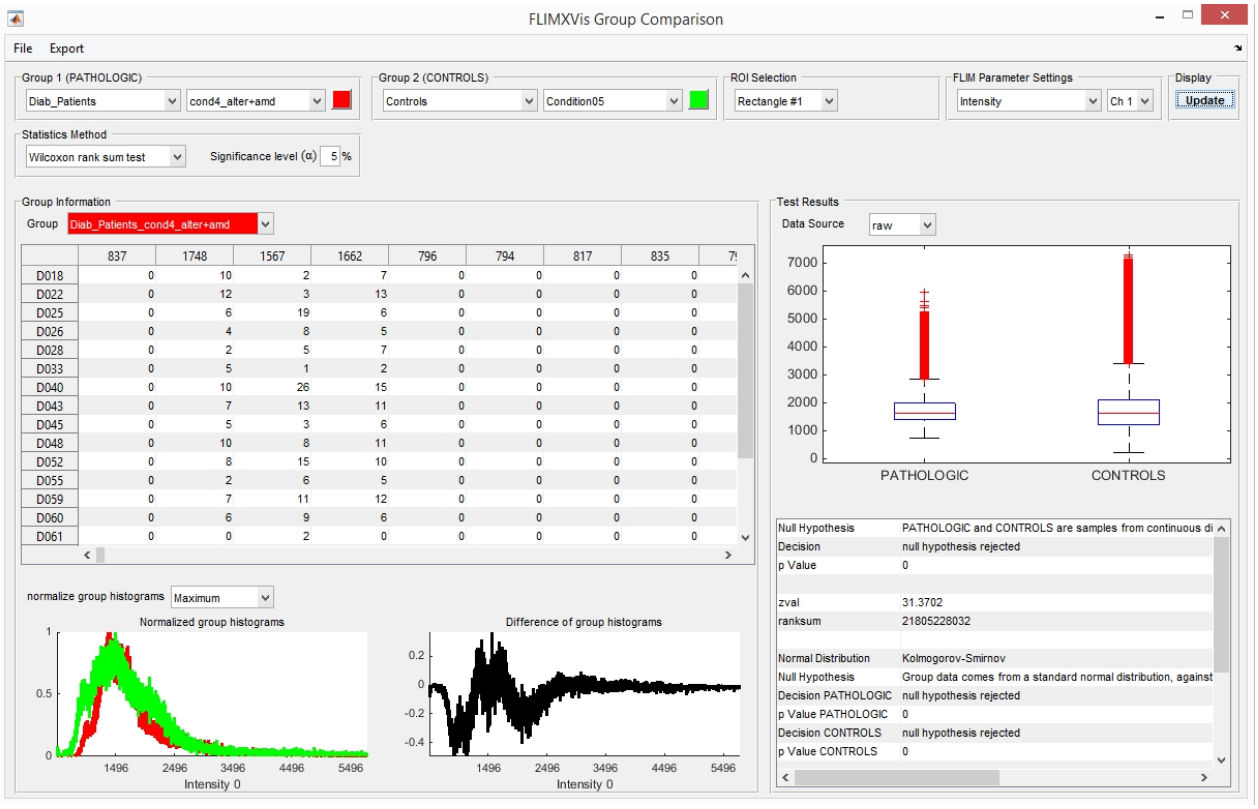


Additional test results (Kolmogorov-Smirnov and Shapiro-Wilk) are displayed when scrolling down

For more information on the results, see here.

Wilcoxon rank-sum test

Wilcoxon rank-sum tests a null hypothesis (Two samples come from the same population) on your data sets.



For more information on the results, see here.

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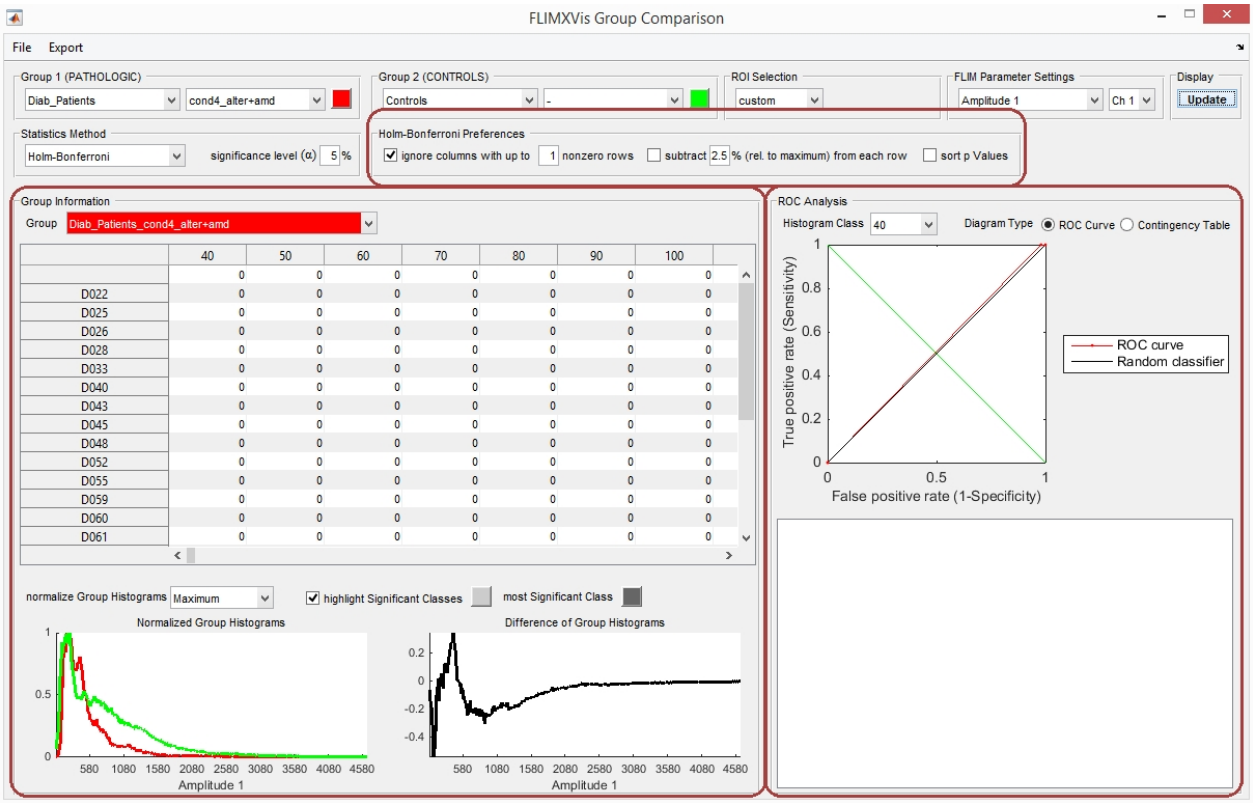
Holm-Bonferroni Method

With Holm-Bonferroni Method, you can compare the statistics of pathologic and control group and find a suitable classifier to distinguish these groups. To use Holm-Bonferroni Method, chose this option in the test section and select parameters as described above. After clicking on update, the FLIMXVis Group Comparison window will look like the example seen below.

The three boxes which are marked in the picture below are described in the following section:

- Holm-Bonferroni Preferences: Settings for the Holm-Bonferroni Method
- Group Information: Table and visualization of histogram data
- ROC Analysis: See characteristics of the classifier



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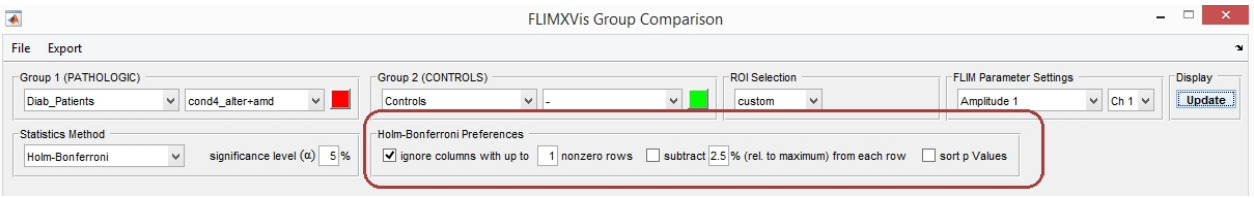
Holm-Bonferroni Preferences

The Holm-Bonferroni preferences show up after selecting Holm-Bonferroni as test method. In the picture below, these options are marked by a red frame.

There are three options:

- Ignore columns with nonzero rows
- Substract offset
- Sort p values



The test methods use histogram data to compare pathologic and control groups. Rows correspond to single subjects while each class is assigned to a column. When ticking the box **ignore columns with up to**, columns containing zeros will be ignored. The number in the subsequent box specifies the minimum number of non-zero rows a column must contain in order not to be ignored. Here, columns containig at least one non-zero row will included in your test statistics.
The purpose of this options is to increase the informative value of your test. For example, your histogram might be calculated for values from 0 to 100, but your actual measurement contains only values in the range from 0 to 50. The columns 51 to 100 will contain only zeros for all subjects since these values are not part of the actual measurement. When including these zero columns in the group comparison calculations, holm-bonferroni test will state that your to groups are highly similar since most values are the same even though both groups might be statistically significant different in the actual measurement range from 0 to 50. By omitting those columns containing only zeros, this falsely assumed similarity will not occur.

To substract offset or noise from your measurement, tick the box next to **substract** and type in a value (here: 2.5).This value specifies how much percent relative to maximum amplitude will be substracted from each row. Note that chosing this option might result in a higher number of empty columns.

Ticking the box **sort p Values** will result in sorting the rows in the group information section not according to numerical order, but according to computed p values in ascending order.

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Group Information

The table and histograms within group information are explained in an extra section since they are basically the same for all test statistics. For general information about this, see here. Special characteristics within group information that appear only with Holm-Bonferroni Method are explained below.

To see more information about your data, scroll down within the table by clicking on the arrow or move the bar on the left. The following rows will show:

- **ignored:** If this box is ticked, the corresponding column contains too many zeros and is ignored during calculation. See above for more information.
- **pValue:** This row contains the p value for each column. FLIMX ticks boxes automatically. To sort columns by p value, see above
- **Threshold:** The threshold specifies the value with which p value will be compared. Threshold calculation is based on Holm-Bonferroni Method. This value depends on the selected significance level
- **significant:** If this box is ticked, the p-value is smaller than the computed threshold and therefor, the column shows a significant difference between pathologic and health group. FLIMX ticks boxes automatically
- **sum (norm.):** This row contains the normalized group histogram for each column in your current group
- **diff (difference) (norm.):** This row contins the normalized difference between the normalized group histograms for pathologic and health group for each column

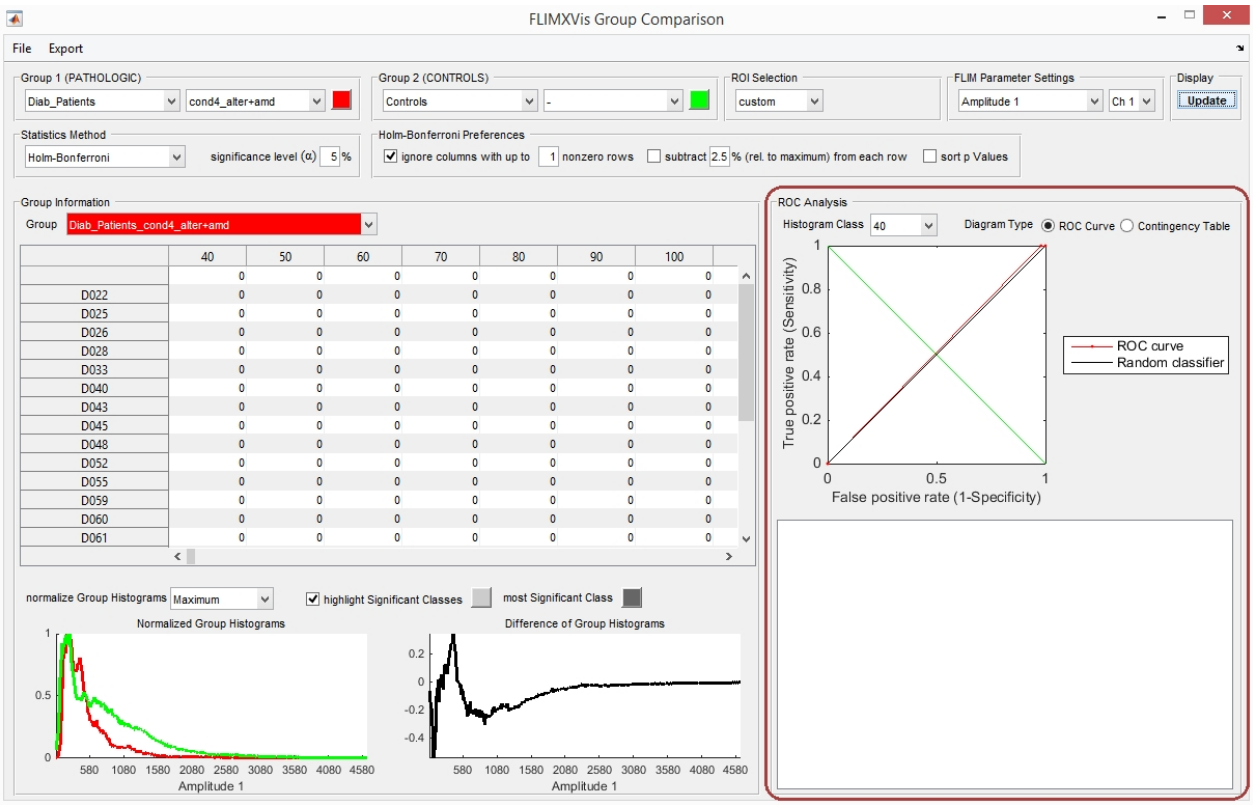

Group Information
Group
Diab_Patients_CondAge
10
20
30
40
50
60
70
D117
D120
D121
D126
D127
D128
D131
D132
ignored
pValue
Threshold
significant
sum (norm.)
diff. (norm.)
normalize Group Histograms
Maximum
highlight Significant Classes
most Significant Class
Normalized Group Histograms
Tau 1
Difference of Group Histograms
Tau 1

ROC Analysis

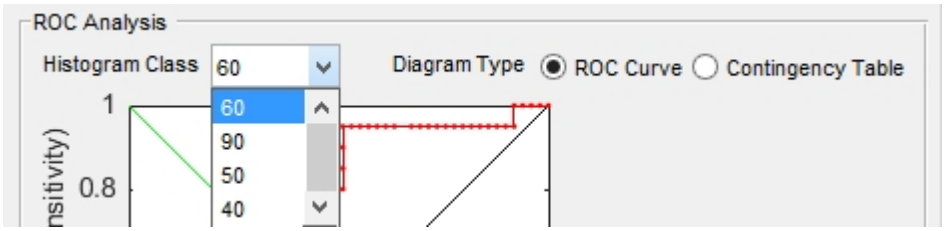
Information about ROC analysis is located on the left as indicated by the red frame in the picture below. ROC analysis options are only available when using Holm-Bonferroni method. A

- ROC curve or
- Contigency table

may be displayed.



In the example above, ROC analysis is displayed for the histogram class 60. To see ROC analysis for a different class, click on the arrow next to your current class. A list containing all classes will show. Select a class by clicking on it.



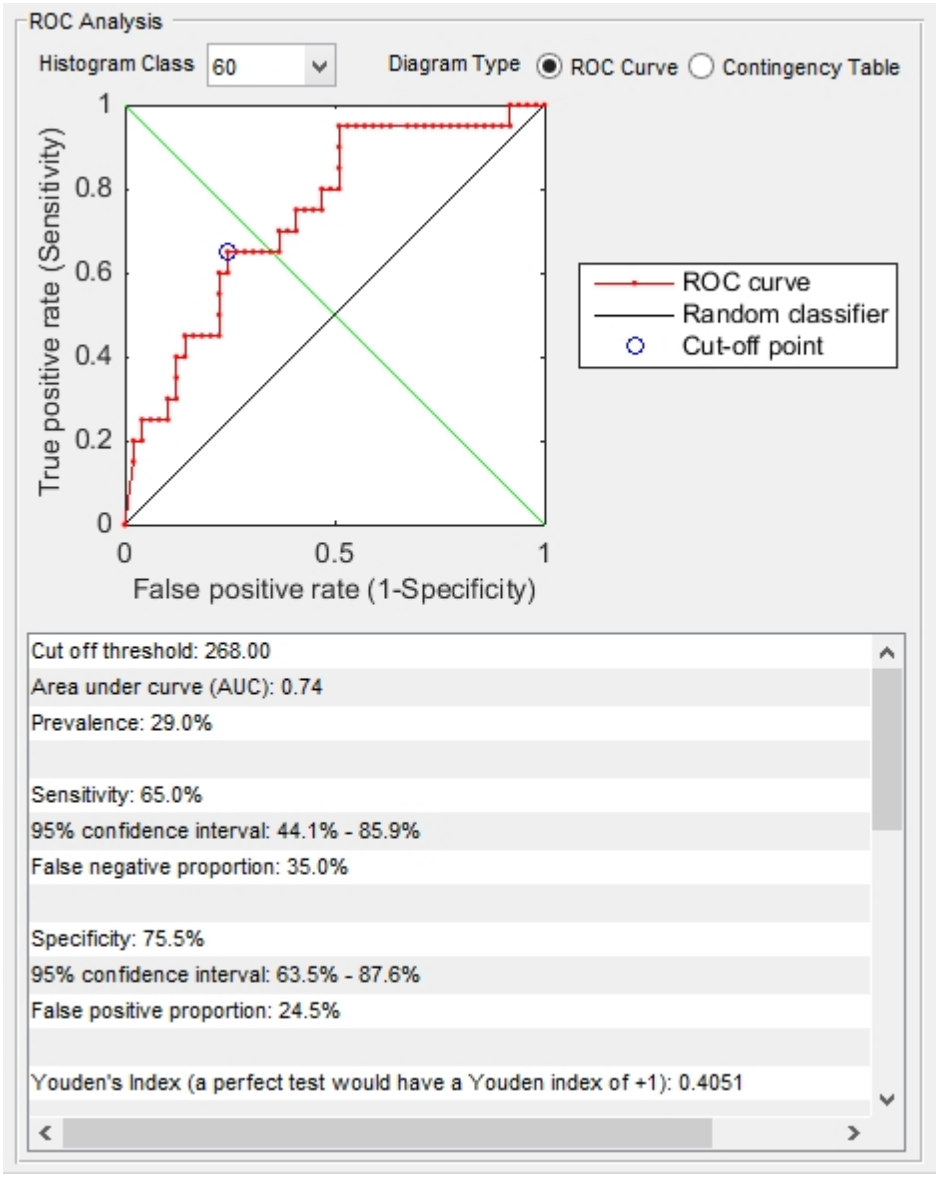
ROC Curve

The ROC (receiver operating characteristics) curve displays the relationhip between true postive rate and false positive rate for a classifier. An ideal classifier would be located in the upper left corner with a Sensitivity of 1 and a 1-Specificity of 0. A random classifier is indicated by the black graph in the ROC curve.The red graph in the picture below is the ROC curve for your current classifier.

FLIMXVis calculates the cut-off point which is marked by a blue dot in the ROC. At the cut-off point, the classifier provides the best compromise between high sensitivity and high specificity.

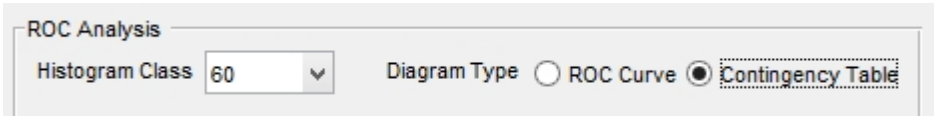
The cut-off threshold as well as other ROC statistics such as area under curve and sensitivity for the cut-off point are displayed in the lower part of ROC Analysis. ROC statistics calculation is based on the Clinical Test Performance Toolbox. For more information on the statistical parameters, see here.

To show the ROC curve for a different histogramm class, see here.



Contigency Table

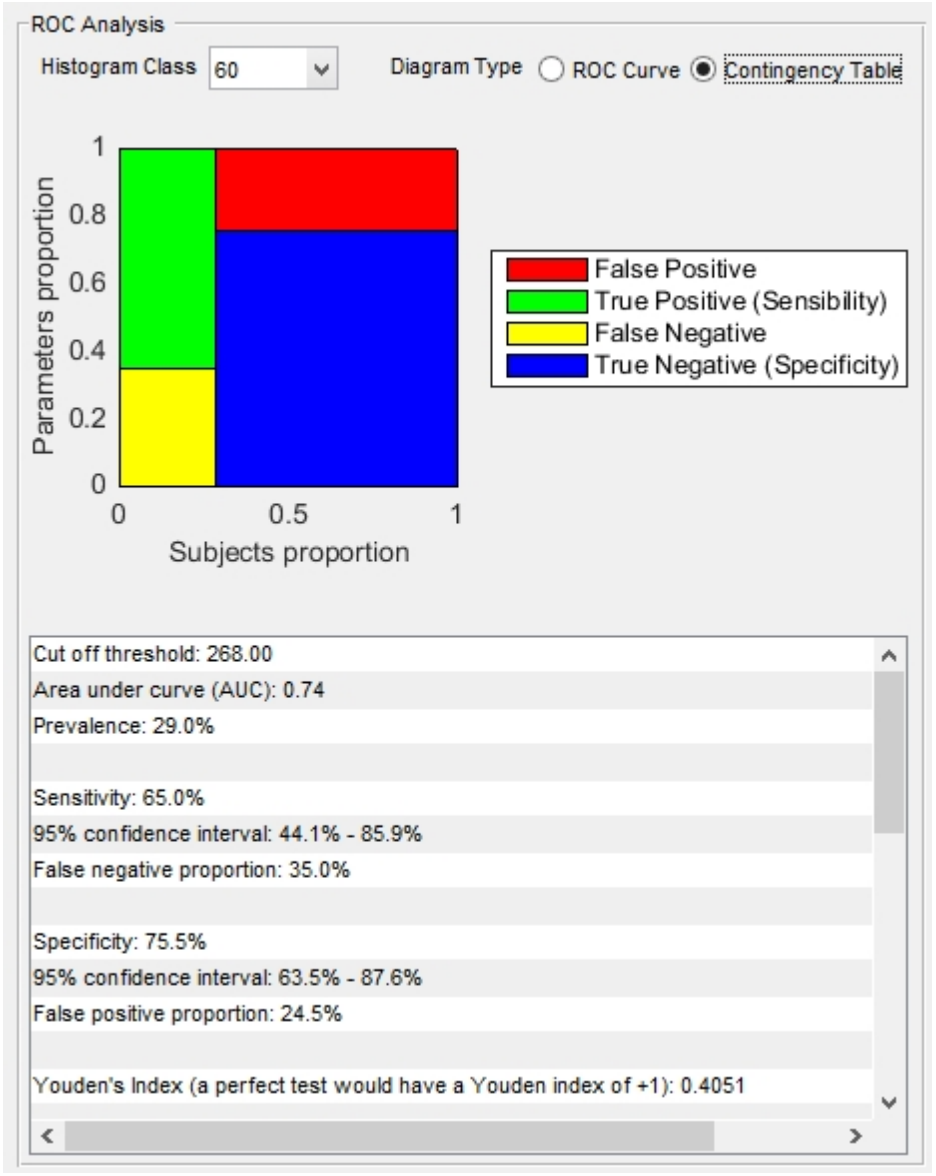
To show a contigency table, tick the corresponding dot in the ROC analysis window as seen in the picture below.



The window will now show a contigency table. This table displays the amount of false positives, true positives, false negatives and true negatives for the classificator at the cut off threshold. The true positives and false negatives correspond to the pathologic group while the false positives and true negatives belong to the control group. The x axis shows the proportion of pathologic subjects to control subjects. The proportion between true and false results can be read from the y axis.

The cut-off threshold as well as other ROC statistics such as area under curve and sensitivity for the cut-off point are displayed in the lower part of ROC Analysis. ROC statistics calculation is based on the Clinical Test Performance Toolbox. For more information on the statistical parameters, see above.

To show the contigency table for a different histogramm class, see here.



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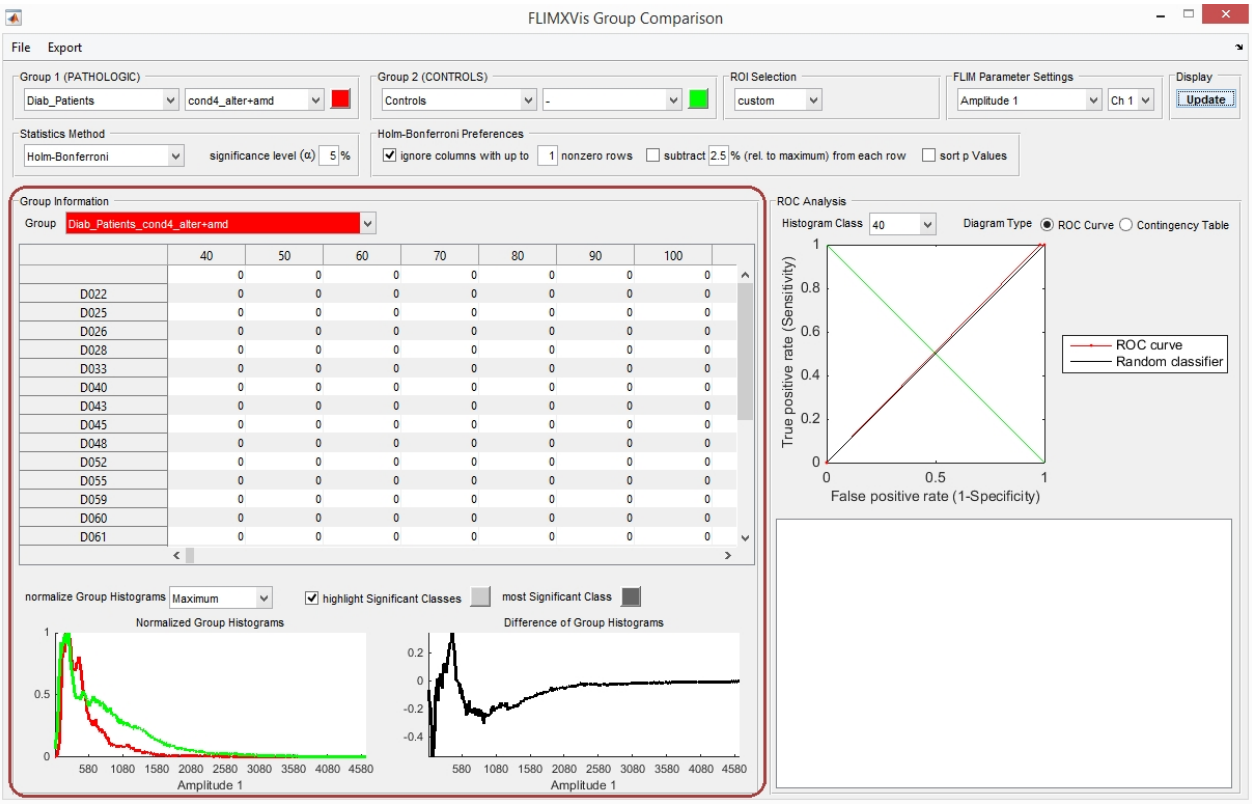
Group Information

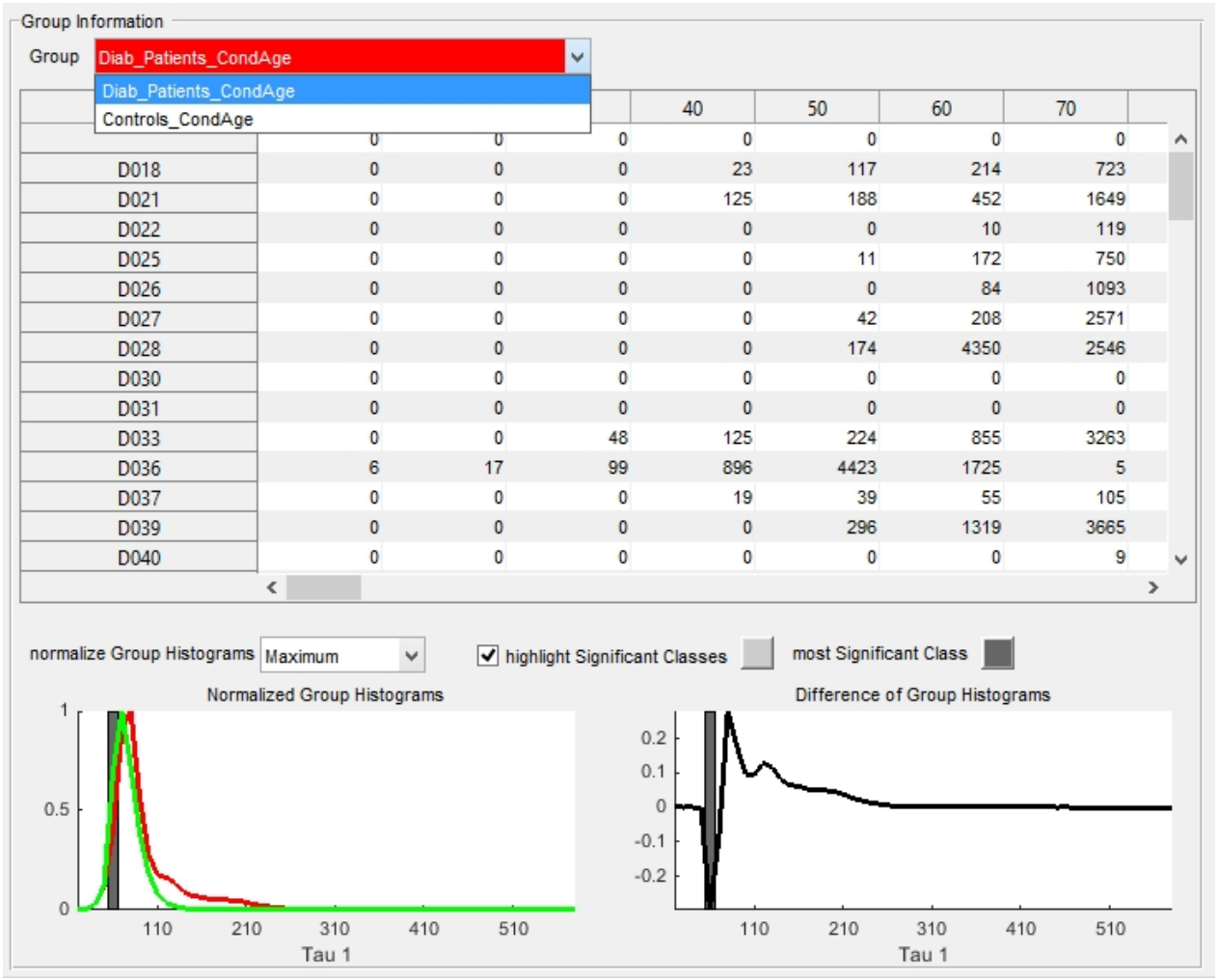
The section group information contains

- Group information table
- Normalized Group Histograms
- Difference of Group Histograms

of the histogram of your group. In the picture below, group information section is marked by a red frame.

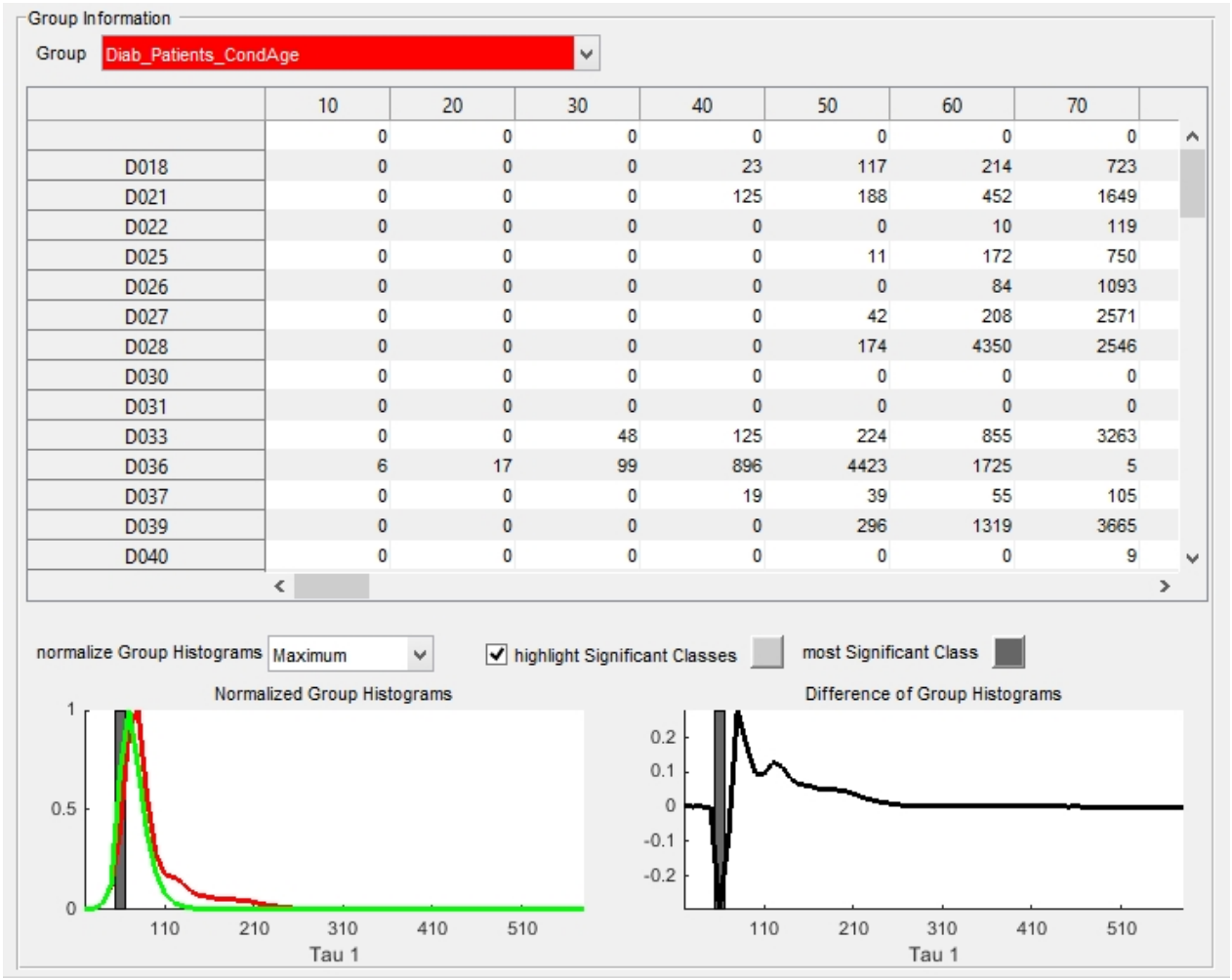


Information is displayed for one group (pathologic or control). To select a group, click on the arrow next to your current group and select pathologic or control group by clicking on it.



Table

Within the group information section, a table is displayed. This table shows histogram values for your specific parameter for each subject within the current group. Rows correspond to single subjects while each class is assigned to a column. The table entries specify frequency values. In the example below, the histogram starts at 25 and has a class width of 10. The central value within this range is displayed. To change options for histogram range and class width, see Statistics Settings.

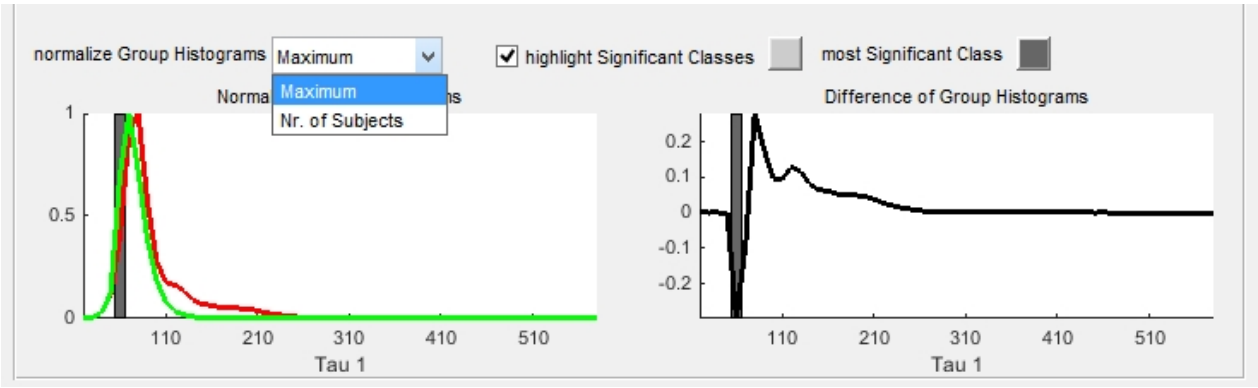


Visualization

A visualization of the histogram data is shown at the left and right bottom of the group information window. If classes with significantly different values between pathologic and control group exist within your current dataset, a gray bar will mark these classes within your plot. In case of several classes, the most significant class is marked with a dark grey bar.

Normalized Group Histogram

On the left, normalized group histograms for pathologic and health groups are displayed. The group histogram is the sum of all subjects within each class normalized by maximum value or number of subjects. To change normalization, click on the arrow next to your current normalization above the histogram plot. Select number of subjects or maximum by clicking on it.



The colors of the graphs correspond to the groups (here: pathologic group red, control group green). To change these colors, see above.

Difference of Group Histograms

On the right, the difference of group histograms is displayed, e.g. the difference between both group histograms for each class.

If classes with significantly different values between pathologic and control group exist, a gray bar will mark these classes within your plot. In case of several classes, the most significant class is marked with a dark grey bar. To mask this bars, untick the box next to **highlight Significant Classes**.

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Export

FLIMXVis offers you the possibility to export

- Screenshots or
- Excel data

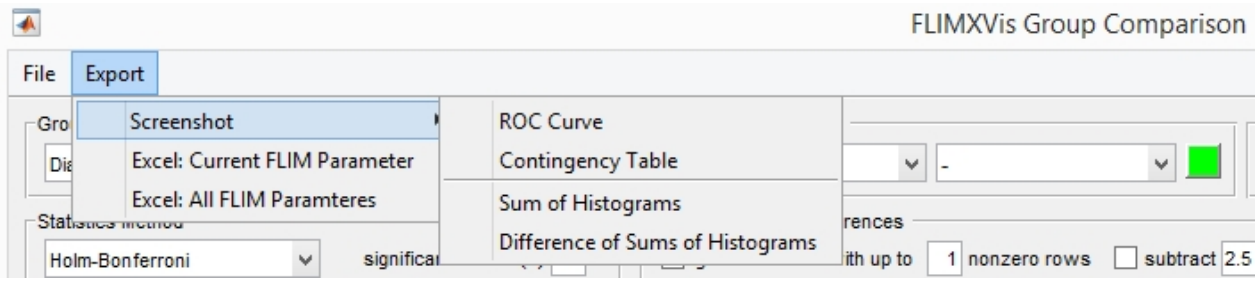
from your group comparison. To directly export screenshots or excel data from your images, see Export.

Export Screenshot

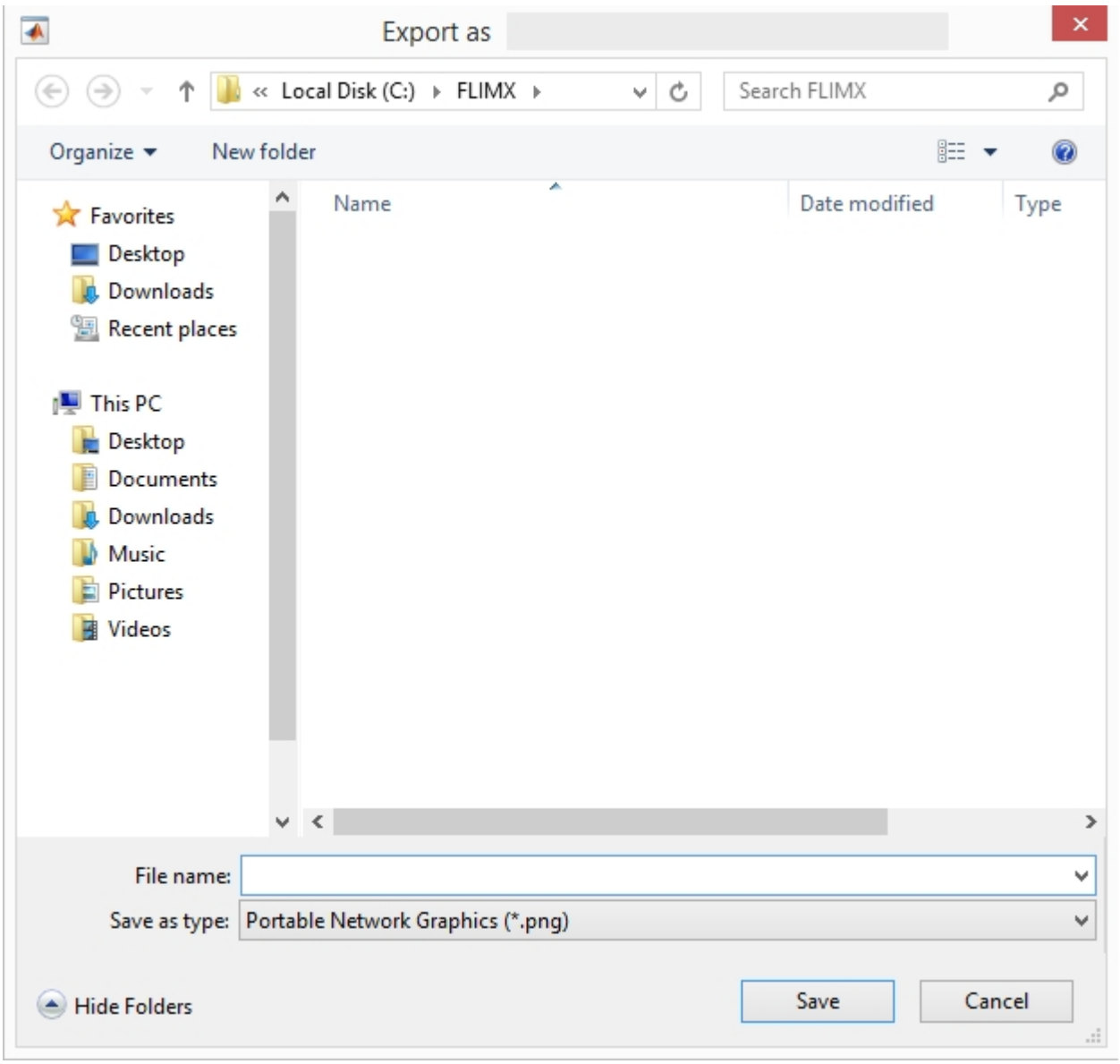
To export a screenshot within group comparison,

Open menu item **Export** > **Screenshot**

as seen in the picture below. Chose the graphic which you would like to export by clicking on the corresponding name in the list.



After selecting one option, the following window will pop up allowing you to browse your files. Select a location and type in a name for your screenshot. Click **Save** to save your file and close the window or **Cancel** to close without saving.



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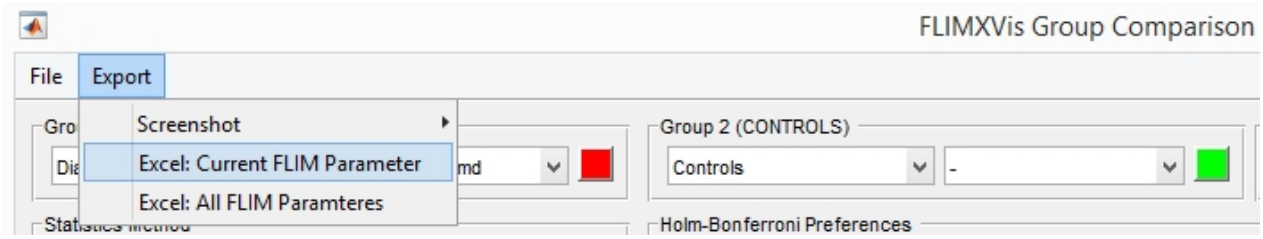
Export Excel Data

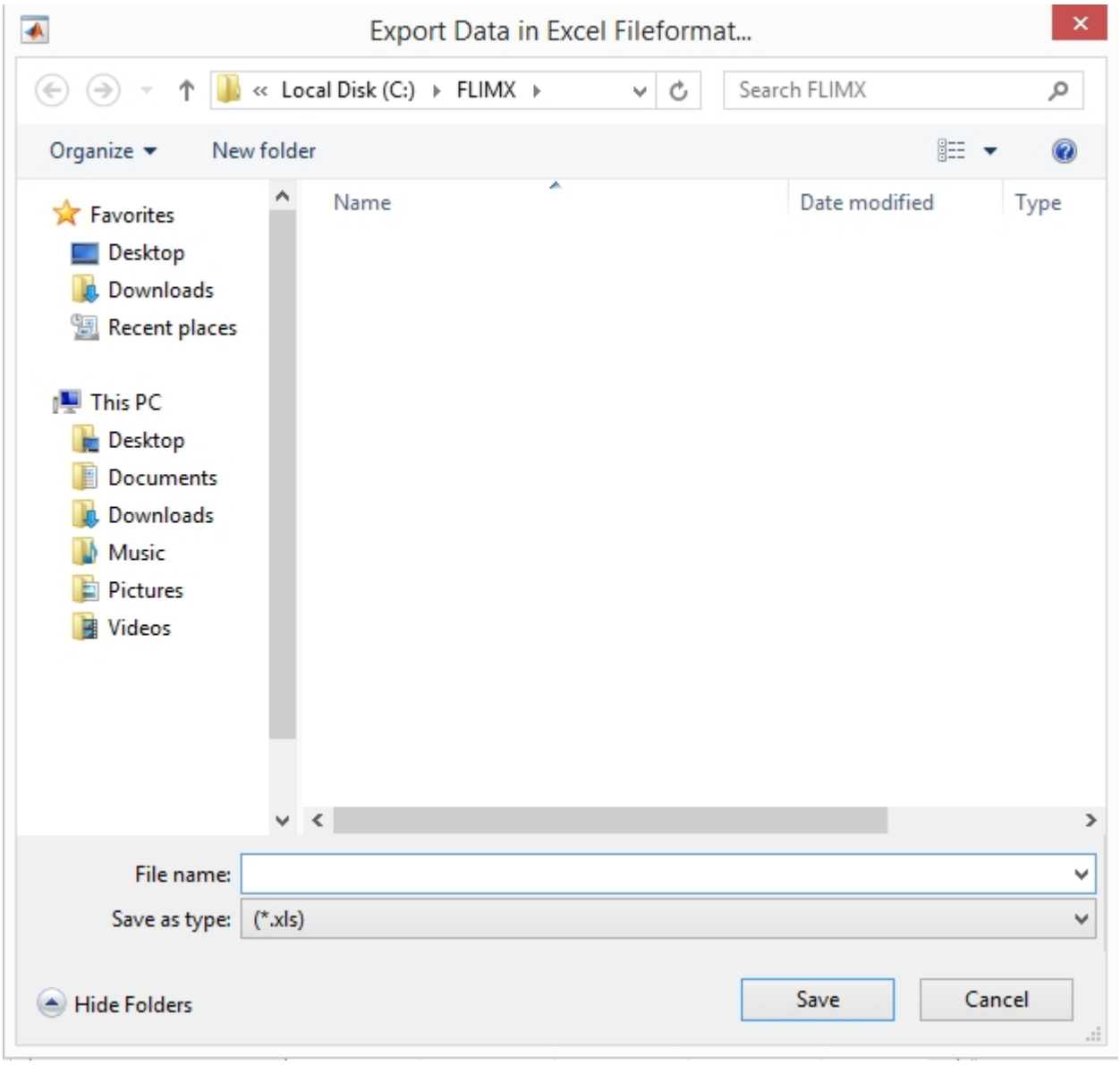
To export excel data within group comparison,

Open menu item **Export**

as seen in the picture below. Select **Excel: Current FLIM Parameter** to export only data from your current parameter or **Excel: All FLIM Parameters**.



The following window will pop up allowing you to browse your files. Select a location and type in a name for your excel file. Click **Save** to save your file and close the window or **Cancel** to close without saving.



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Export

FLIMXVis offers you the possibility to export

- Screenshots
- Excel data or
- Movies.

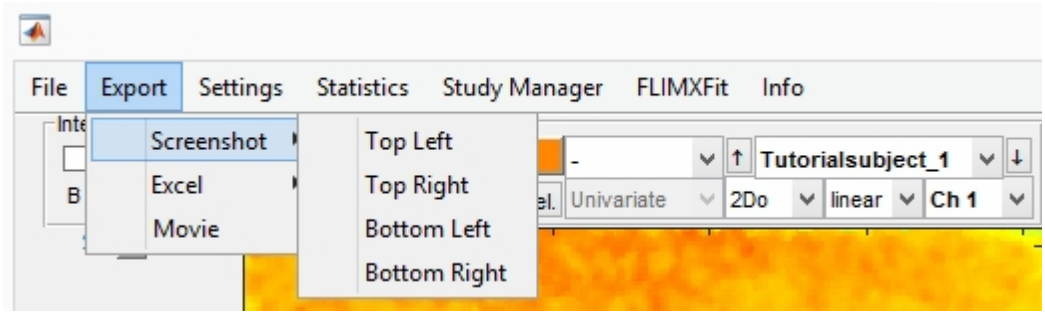
To export ROC data, see Group Comparison.

Export Screenshot

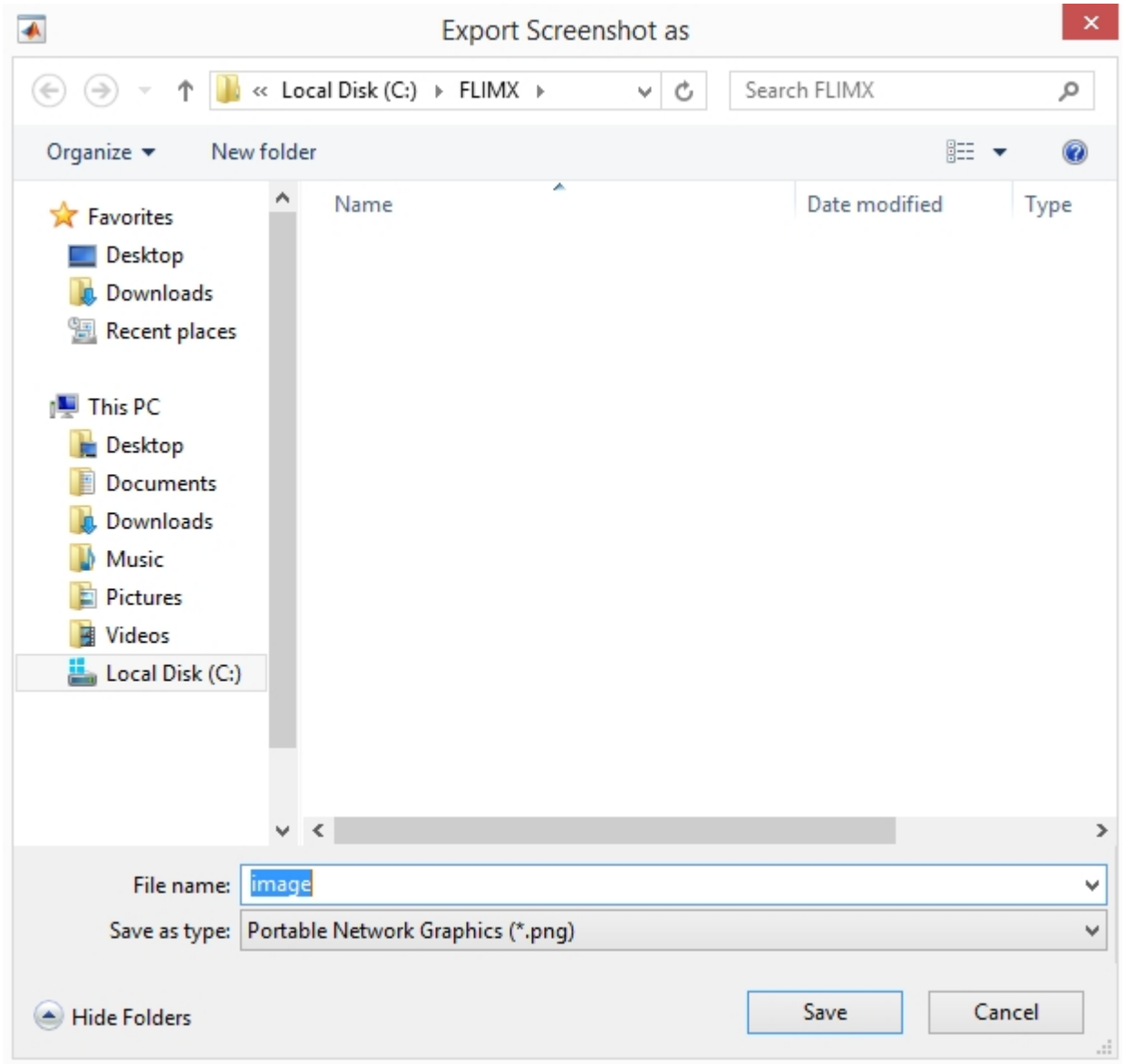
To export an image from FLIMXVis,

Open menu item **Export** > **Screenshot**

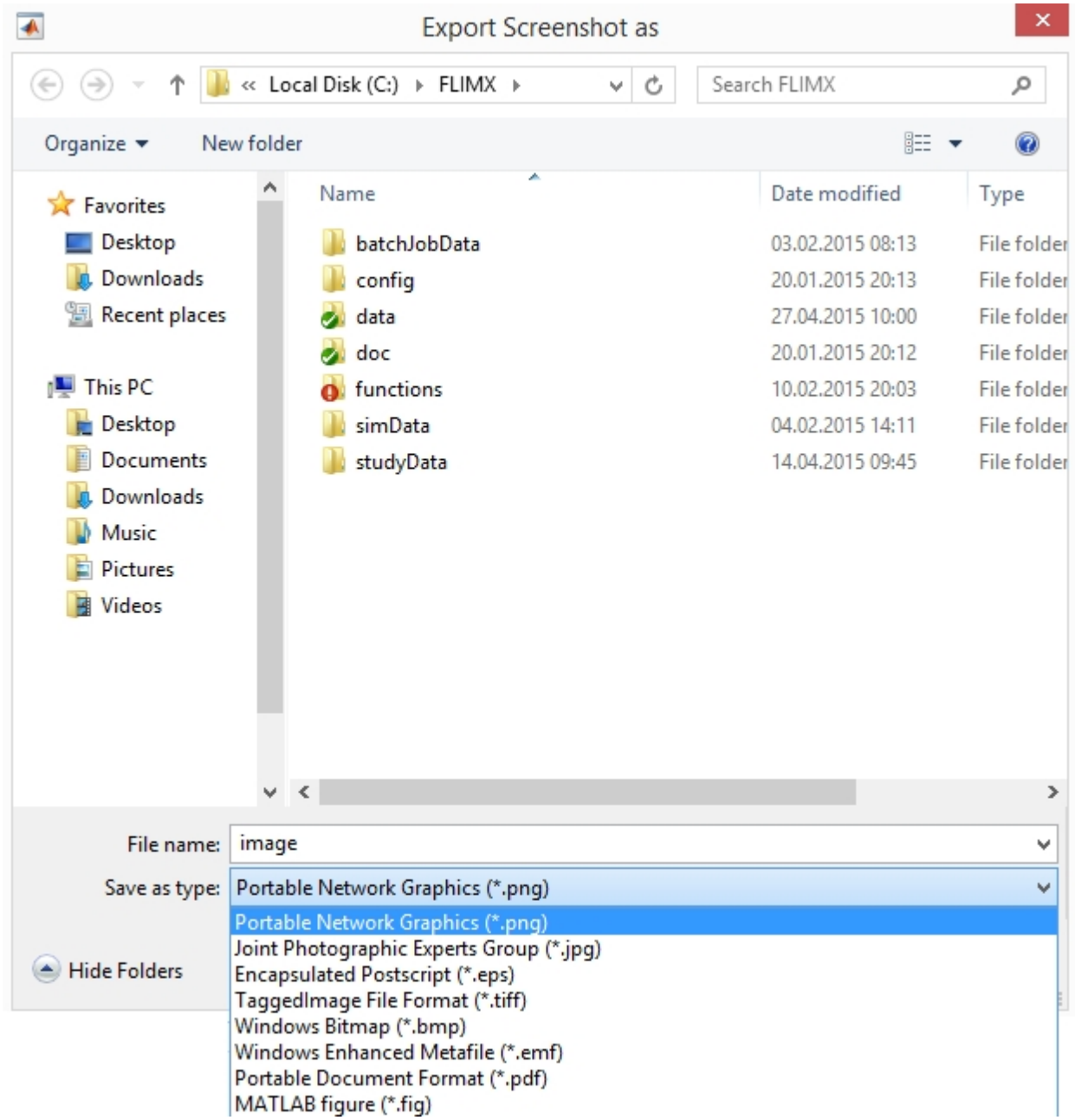
as seen in the picture below. For screenshot export options, see here.



Select one window for export. After selecting one image to export, the following window will pop up allowing you to browse your files and select a location to save your file.



Type in a name for your file (here: image) and select a data type as shown in the example below. Click **Save** to save your image or **Cancel** to close the window without saving.



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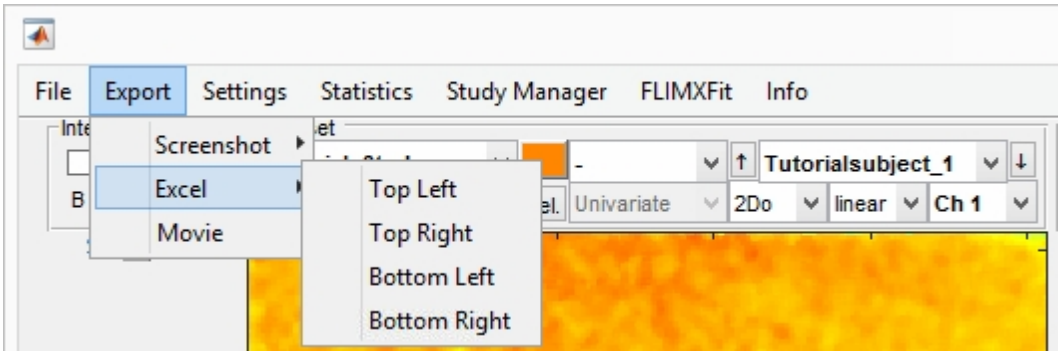
Export Excel Data

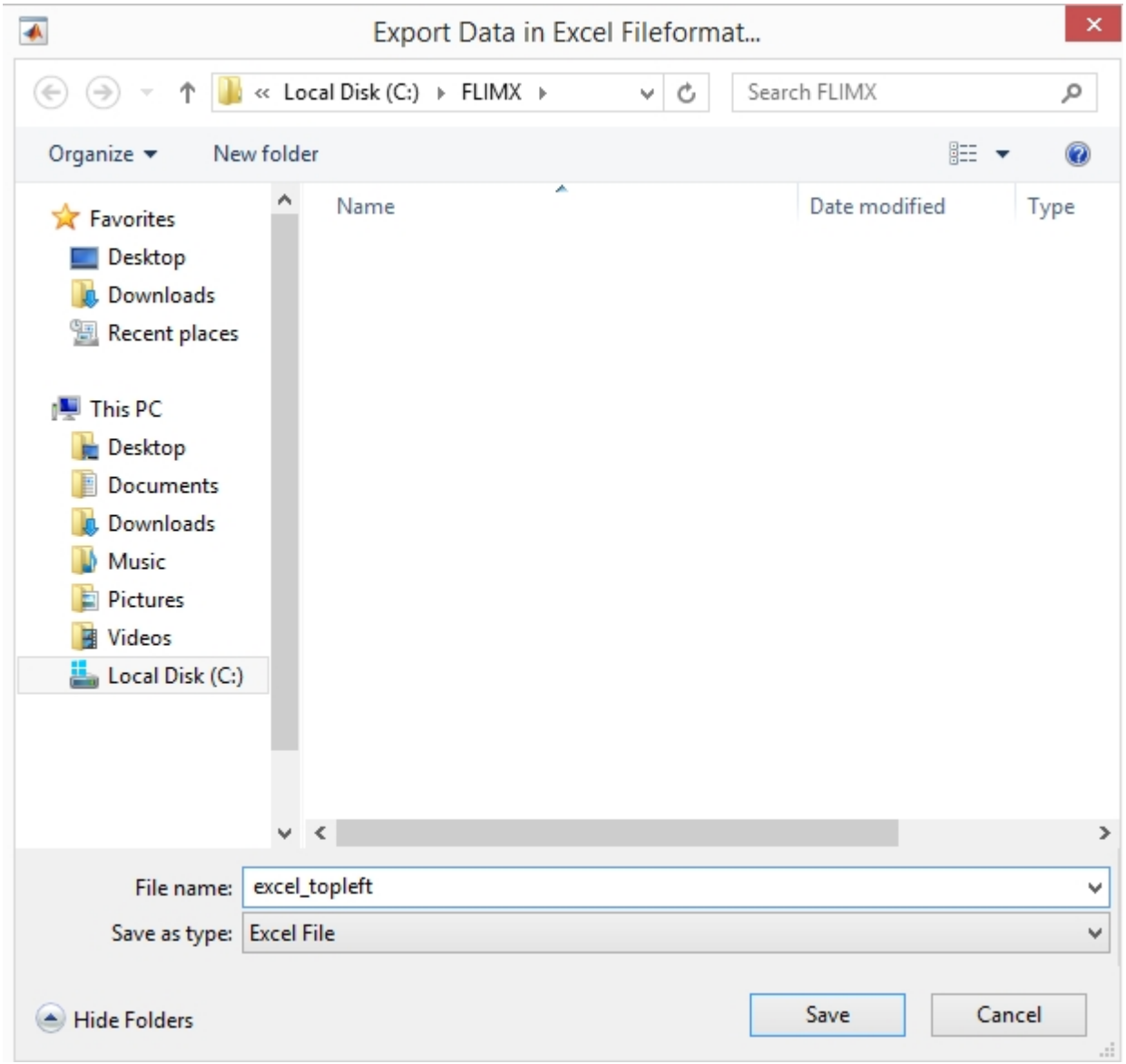
To export data in Excel format from FLIMXVis,

Open menu item **Export** > **Excel**

as seen in the picture below.



Select one window for export. After selecting one image to export, the following window will pop up allowing you to browse your files and select a location to save your file.



Type in a name for your file (here: excel_topleft). Click **Save** to save your image or **Cancel** to close the window without saving.

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Export Movie

To export a movie from FLIMXVis,

Open menu item **Export** > **Movie**

as seen in the picture below.

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Settings

FLIMXVis settings are divided into five subgroups:

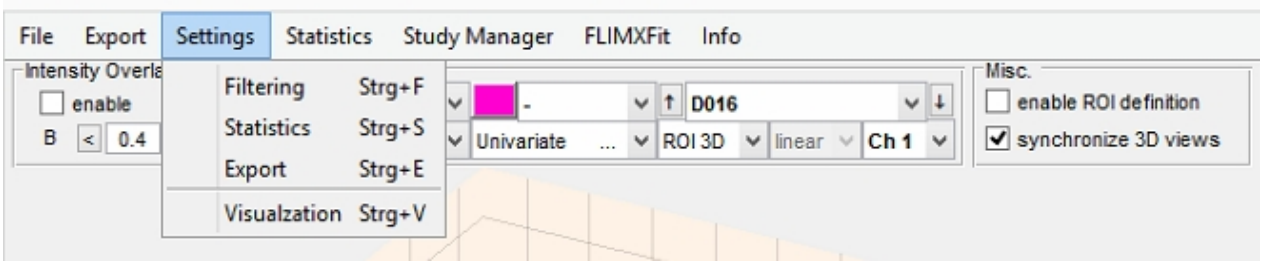
- Filtering
- Statistics
- Export
- Visualization Options

Filtering

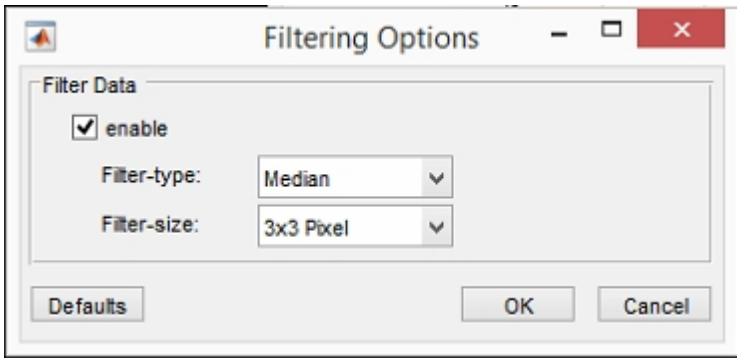
To open FLIMX Filtering Options from FLIMXVIS,

Open menu item **Settings** and select **Filtering**

as seen below or type **CTRL+F**.



A window with FLIMXVis Filtering Options will pop up.



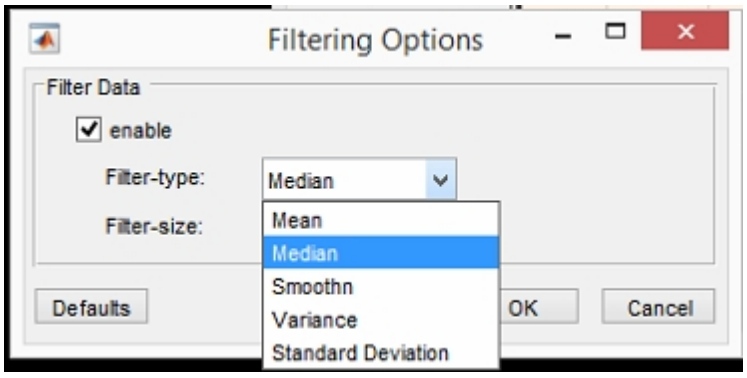
To enable filtering, tick **enable**. A filter window will be moved over your picture. The amount of pixels before and after filtering remains the same.

To chose a filter type, click on the arrow next to your current filter type. A list showing all available filters will show up. Click on a filter to select.

The filter types are:

- **Mean:** The center value of the filter window will be replace by the filter window mean value. Also known as Moving Average Filter.
- **Median:** The center value of the filter window will be replace by the filter window median value. More robust to outliers than mean filter.
- **Smoothn:** Smooths your data. For more information, see http://www.biomecardio.com/matlab/smoothn.html .
- **Variance:** Highlights edges. Values will be replaced by the neighbouring variances within the filter window.
- **Standard Deviation:** Highlights edges. Values will be replaced by the neighbouring standard deviations within the filter window.

To reset filter settings, click on **Default**. To apply your filtering options, click **OK**. To close the window without saving, click **Cancel**.



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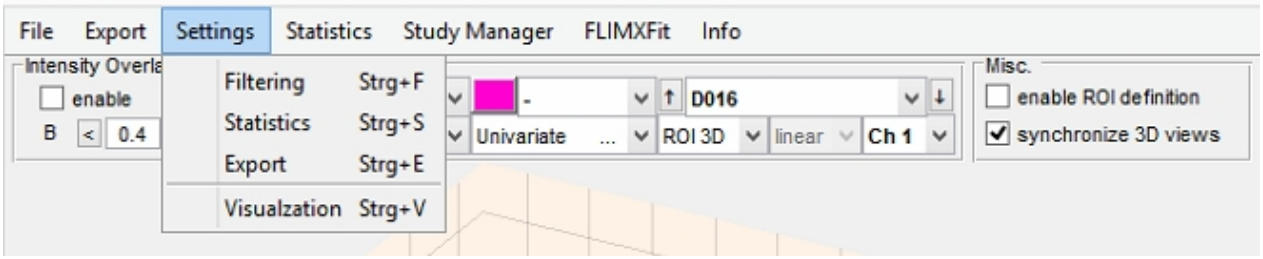
Statistics

To open FLIMX Statistics Options from FLIMXVIS,

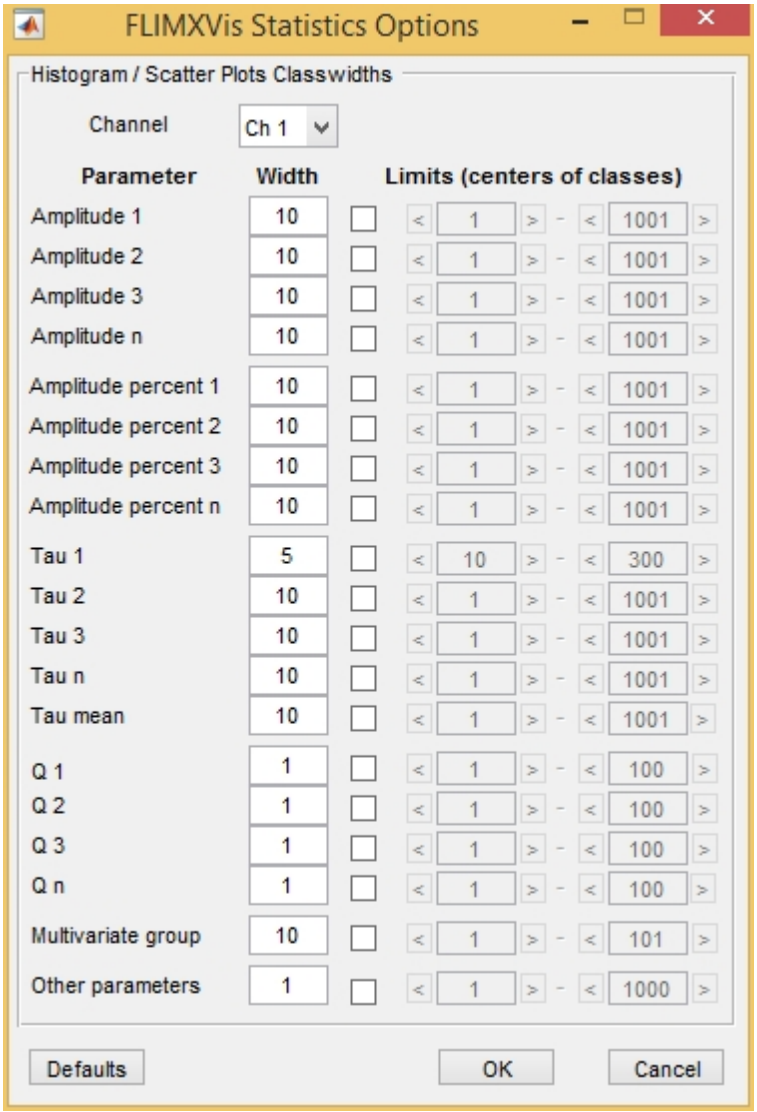
Open menu item **Settings** and select **Statistics**

as seen below or type **CTRL+S**.

Within this window, you can set class width for each FLIM Parameter for each spectral channel. This is important for Scatter Plots and Group Comparison.



The following window will pop up:



Select a channel from the drop down menu.

To set a class width, type in a number in the **Width** window.

To set a lower boundary, first tick the box next to the parameter you wish to modify. Then, type in the lower values in the corresponding boxes. Upper boundaries are calculated automatically based on class width.

Click on **Save** to confirm your changes or **Cancel** to close the window. To reset class width values, click on the **Default** button.

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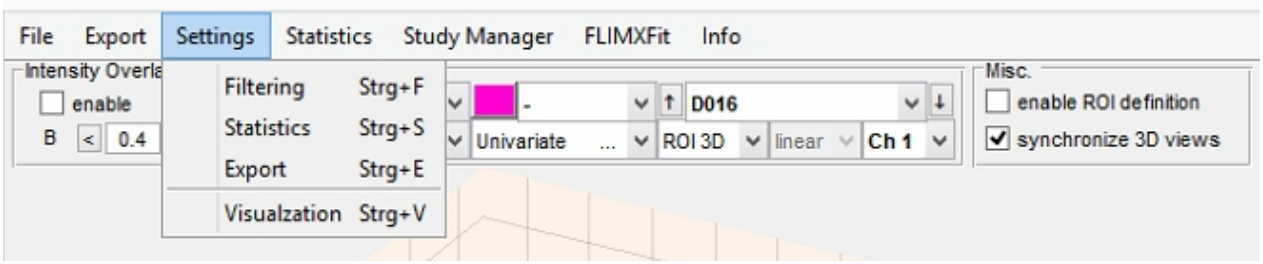
Export

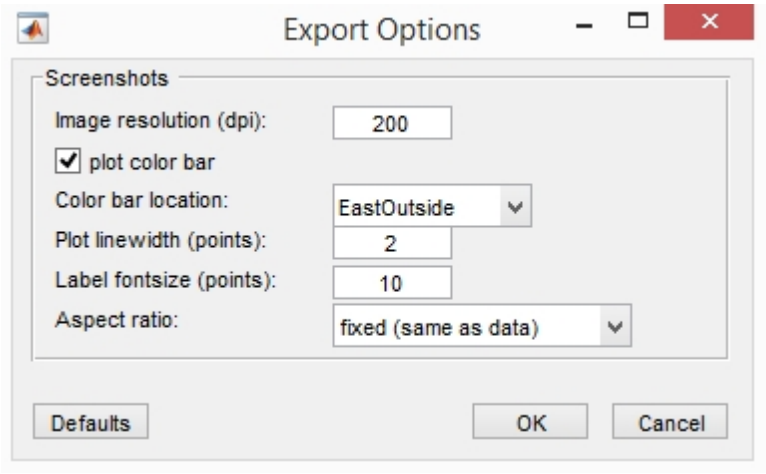
To open FLIMX Export Options from FLIMXVIS,

Open menu item **Settings** and select **Export**

as seen below or type **CTRL+E**.



The following window will pop up.



- **Image Resolution:** To change image resolution in dpi, type in a numerical value in the box.
- **Plot Colorbar:** To plot a color bar within your screenshot export, tick the corresponding box. To turn off a colorbar, untick the box. To select a colorbar location, click on the arrow next to your current location (here: east outside). A list with all possible locations will show. Click

on a location to select.

- **Plot linewidth and label fontsize:** It can be defined by typing a numerical value in the corresponding box. Unity is points.
- **Aspect Ratio:** To change aspect ratio to auto or fixed, click on the arrow next to your current aspect ratio and select one of the options by clicking on it. Auto: MATLAB calculates aspect ratio. Fixed: Aspect ratio calculated based on data.

Click on **OK** to confirm your changes and close the window or **Cancel** to close the window without saving. To reset export settings, click on the **Default** button.

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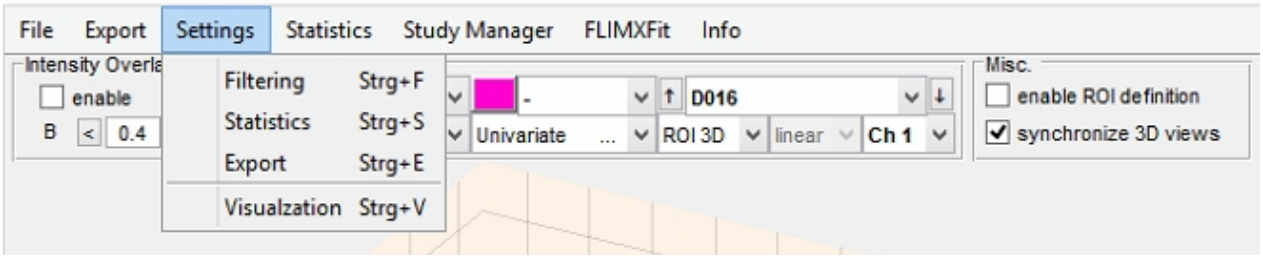
Visualization

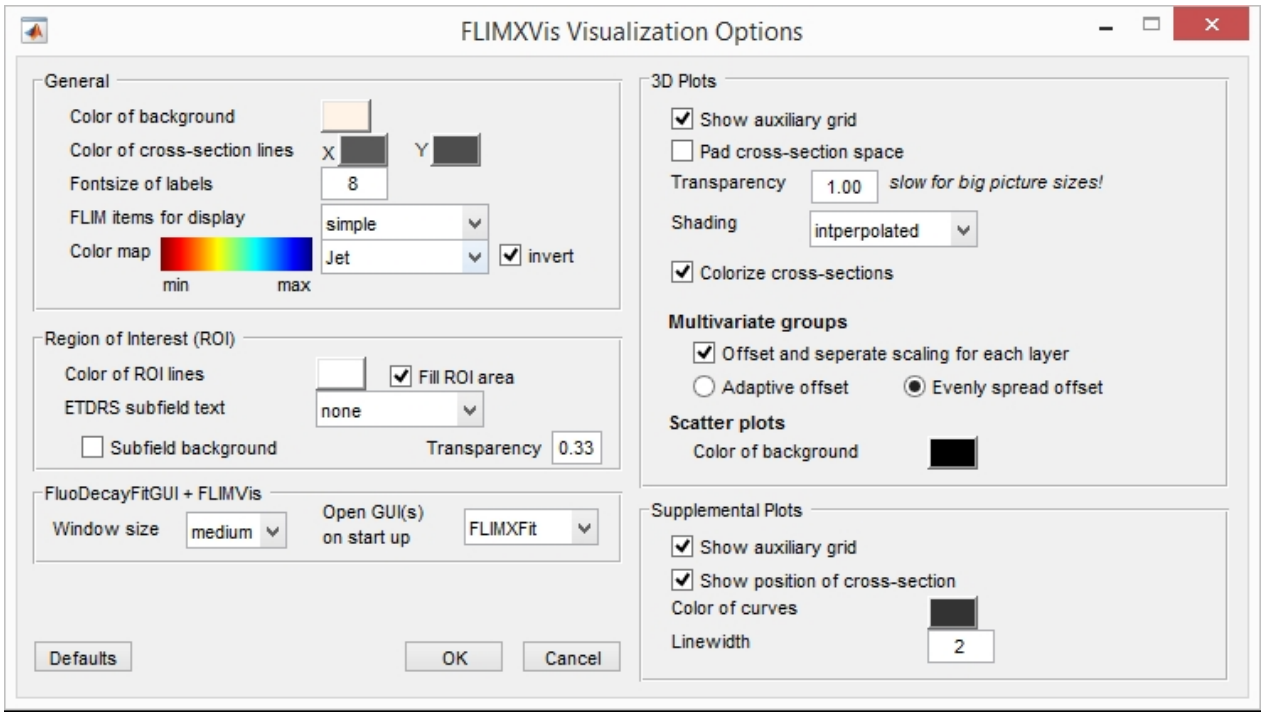
To open FLIMX Visualization Options from FLIMXVIS,

Open menu item **Settings** > **Visualization**

as seen below or type **CTRL+D**.



A window with FLIMXVis Visualization Options will pop up.

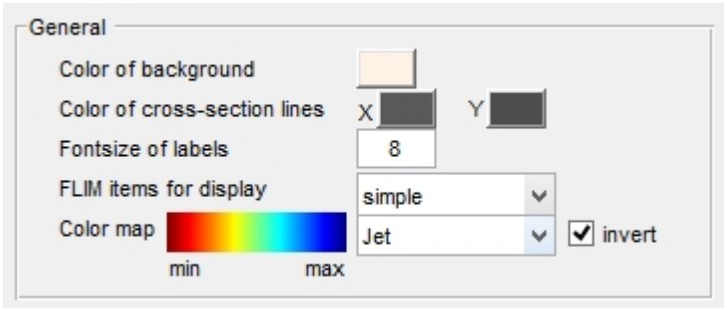


The Visualization Options are divided into the following items:

- General
- Region of Interest (ROI)
- 3D Plots
- Supplemental Plots
- FluoDecayFitGUI + FLIMVis

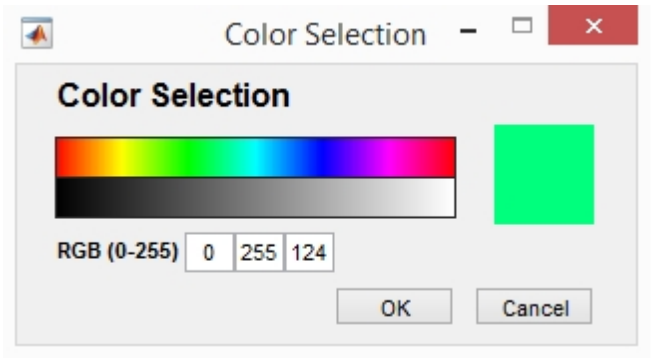
General

Within General Options, you can change the Color of Background and Cross-Section Lines, Fontsize of Labels and Colormap. To open FLIMX Visualization Options from FLIMXVis, see above. General Options are located in the uppermost box as indicated by an arrow in the picture below.



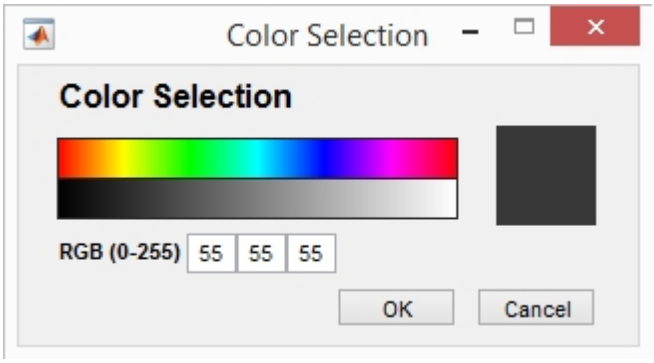
Color of Background

To change the background color of your images, click on the box showing your current color in the General Options section. The following window will pop up. Select a color by clicking on it or type in three RGB values. Click **OK** to save your settings and close the window or **Cancel** to exit without saving.



Color of Cross-Section Lines

To change the color of cross-section lines, click on the box showing your current color for x and y in the General Options section. The following window will pop up. Select a color by clicking on it or type in three RGB values. Click **OK** to save your settings and close the window or **Cancel** to exit without saving.

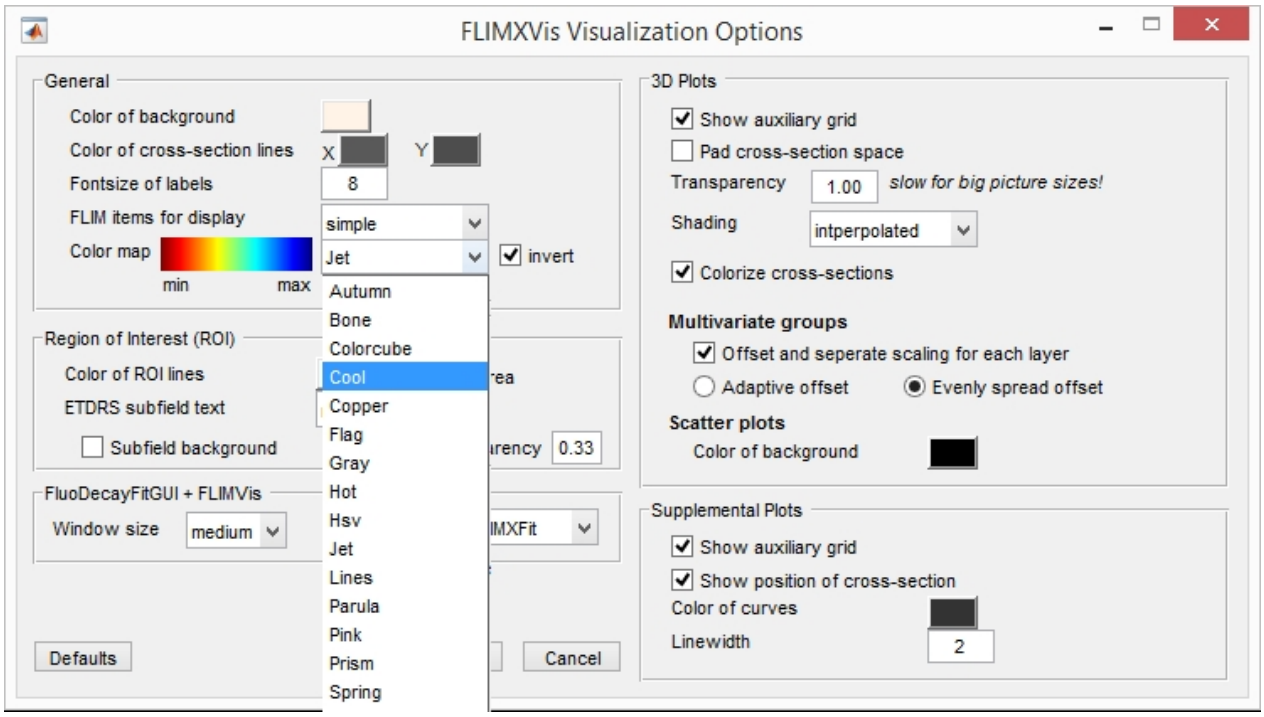


Fontsize of Labels

To change the fontsize of axis labeling, type in a fontsize value in the box.

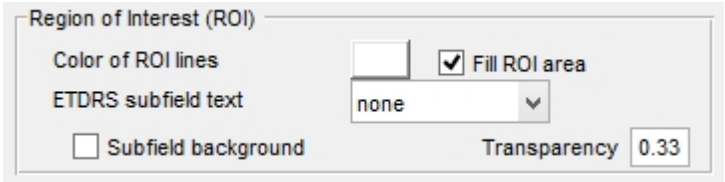
Colormap

To change the colormap, click on the arrow next to your current colormap as seen below. The names of all possible colormaps will show. Select a colormap by clicking on it. By enabling "Invert", the colorbar will be inverted.



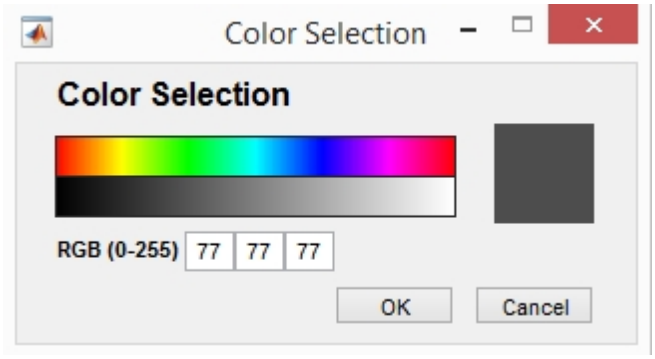
ROI

Within ROI Options, you can change the color of the ROI lines. The additional option "Fill ROI Area" enables an area-covering coloring of the ROI with a transparency value of 2. You can also change the ETDRS grid labeling. To open FLIMX Visualization Options from FLIMXVis, see above. ROI options are located in the second box from top to bottom as indicated by an arrow in the picture below.



Color of ROI lines

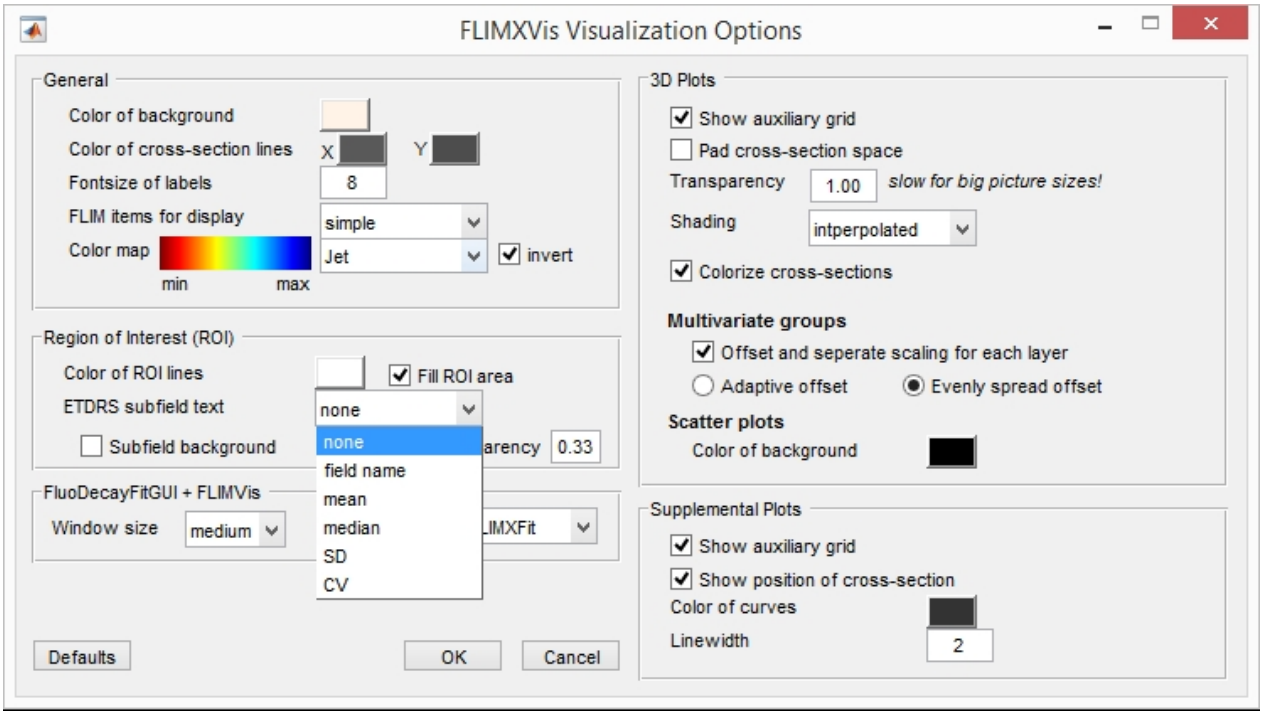
To change the color of ROI lines, click on the color field which shows your current ROI line color. A window will pop up as seen below. To select a color, enter RGB values or click on a color in the colorbar.



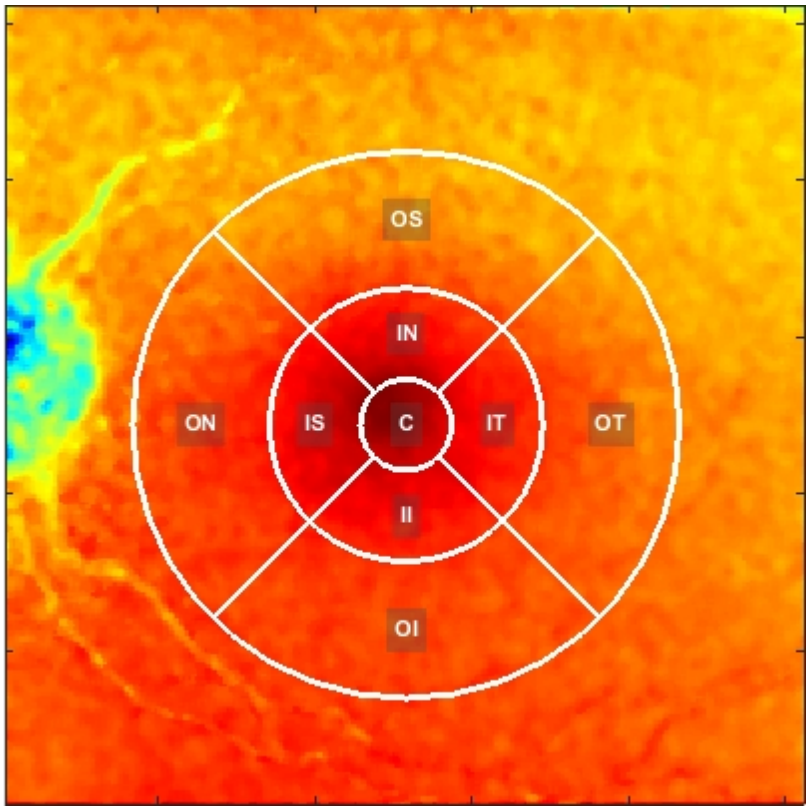
ETDRS Grid labeling

To change ETDRS Grid labeling, click on the arrow next to ETDRS Subfield Text. A list will show up. Select a labeling option by clicking on it.

- none: no ETDRS Grid labeling
- field name: show field names
- mean: show mean value for each field
- median: show median value for each field
- SD: show standard deviation for each field
- CV: show coefficient of variation for each field

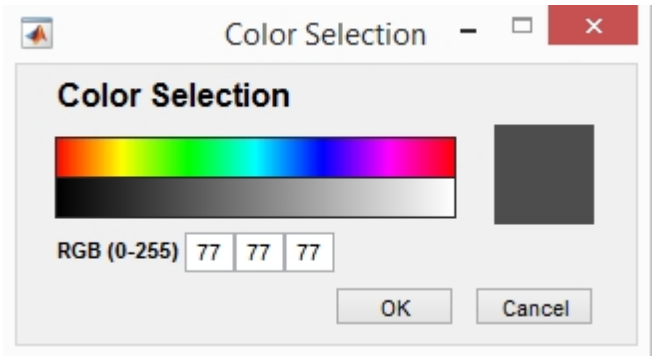


When choosing labeling option **field name**, the ETDRS Grid will be labeled as seen in the example below.

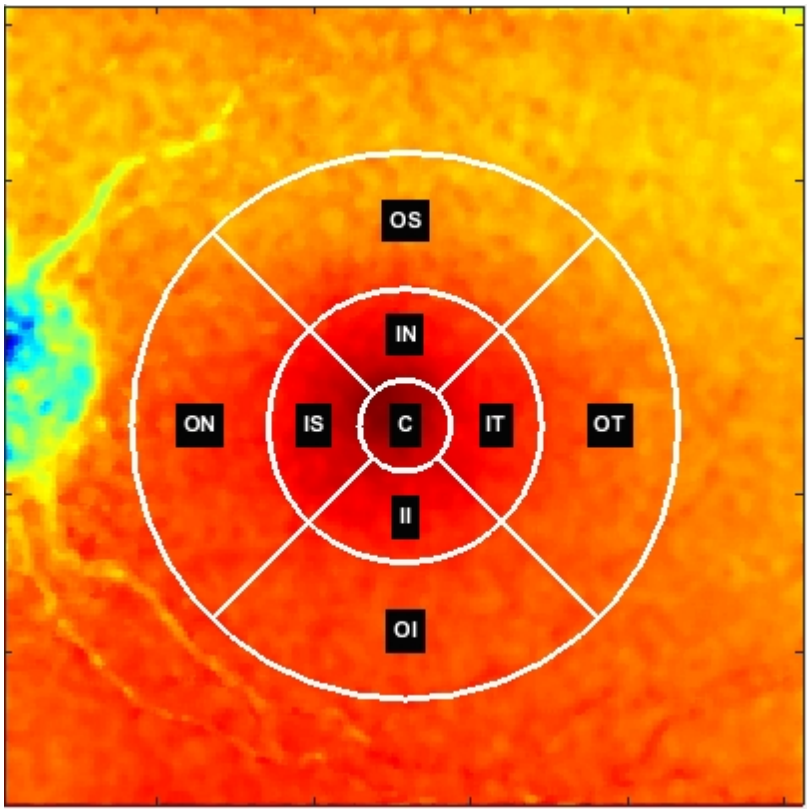


For better visibility of ETDRS Grid labeling, a subfield background may be applied. Tick **Subfield Background** to enable.

To change the subfield background color, click on the color field which shows your current subfield background color. A window will pop up as seen below. To select a color, enter RGB values or click on a color in the colorbar. To change transparency of the subfield background, enter a transparency value within 0 an 1.

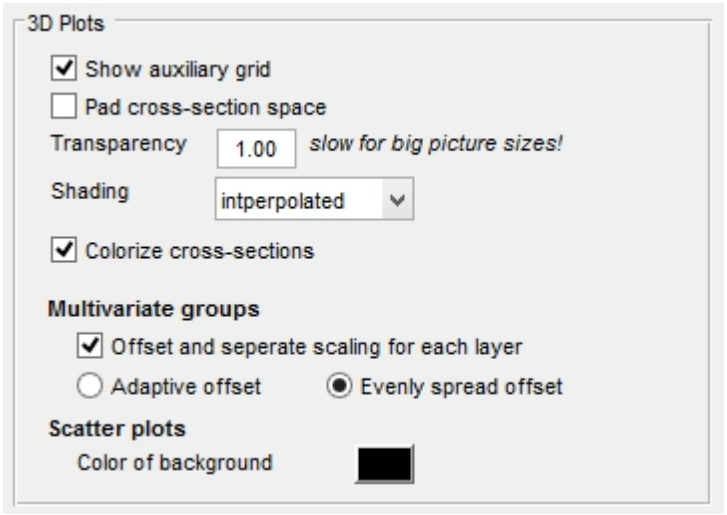


In the example seen below, RGB values 4-4-4 and a transparency of 1 has been chosen.



3D Plots

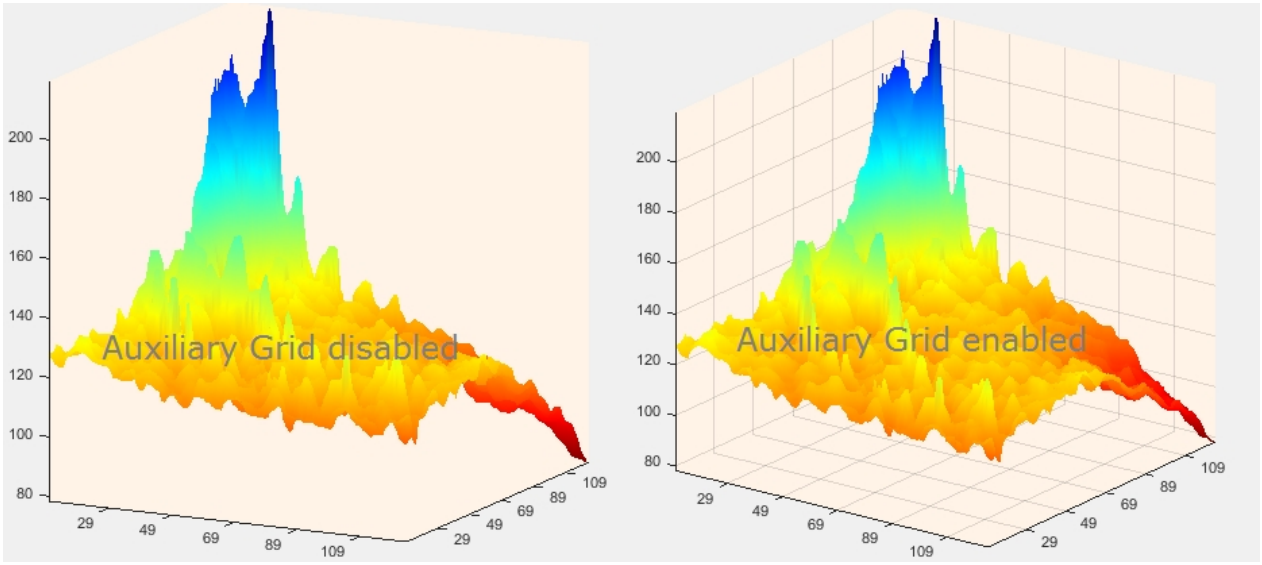
Within 3D Plot Visualization Options, there are various options for modifying your 3D Plot. To open FLIMX Visualization Options from FLIMXVis, see above. 3D plot options are located in the third box from top to bottom as indicated by an arrow in the picture below.



Auxiliary Grid

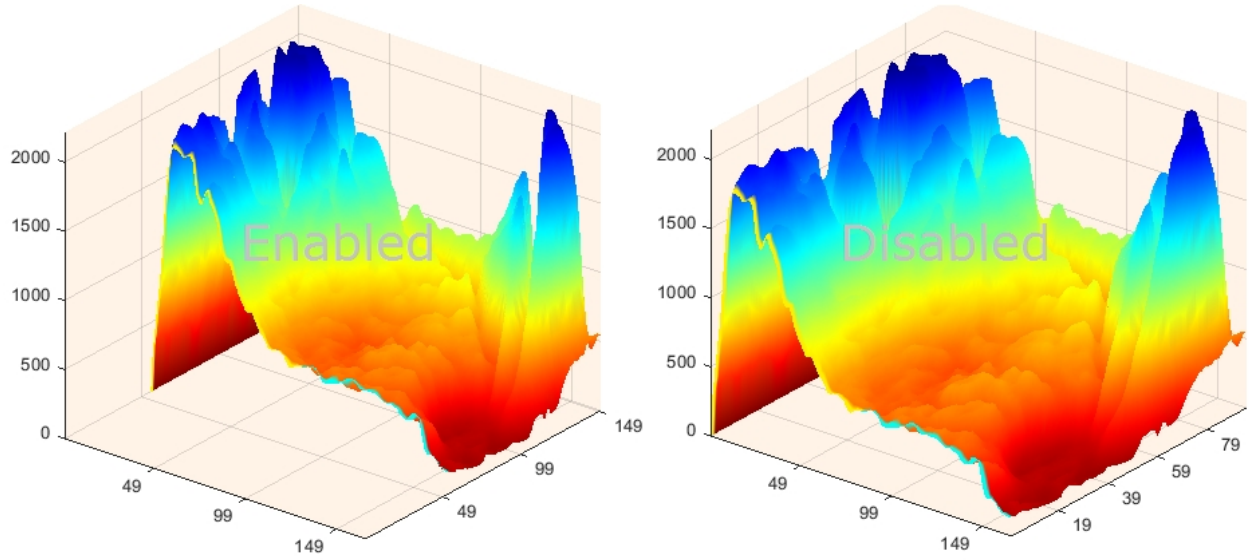
Ticking the box **Show Auxiliary Grid** will enable a grid in your 3D Plot. In the example below, a 3D plot with and without auxiliary grid are shown. Enabling or disabling the Auxiliary Grid will

automatically adjust auxiliary grid options within the Supplemental Plots menu.



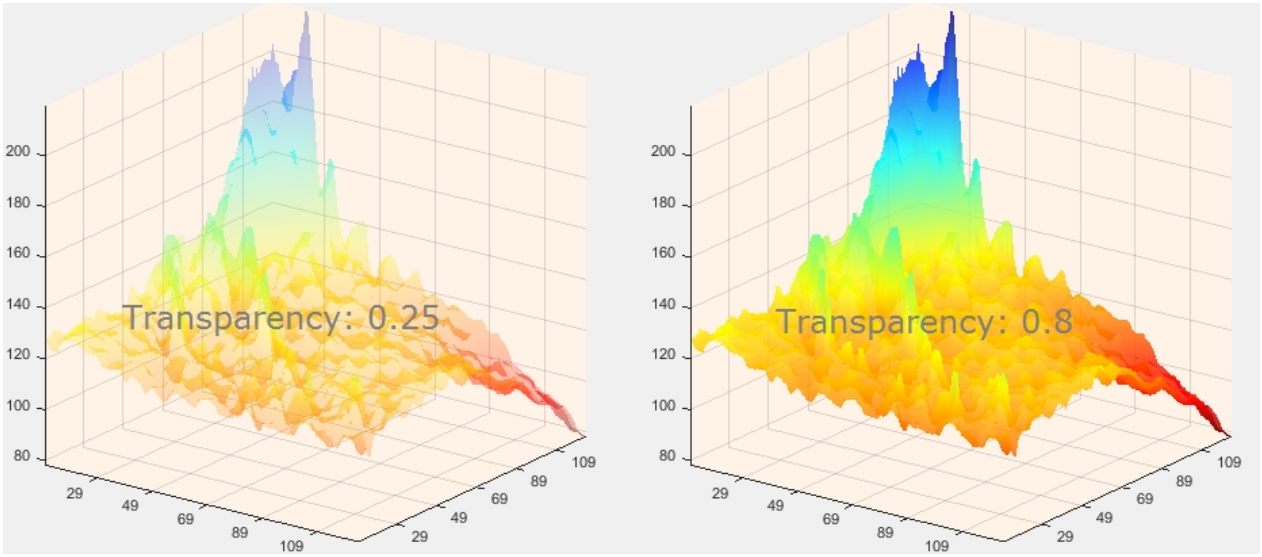
Zero Padding

Ticking the box **Pad Cut Space With Zeros** will show an actual cut in 3D view when using horizontal or vertical cross-section. In the example below, a 3D plot with and without zero padding are shown.



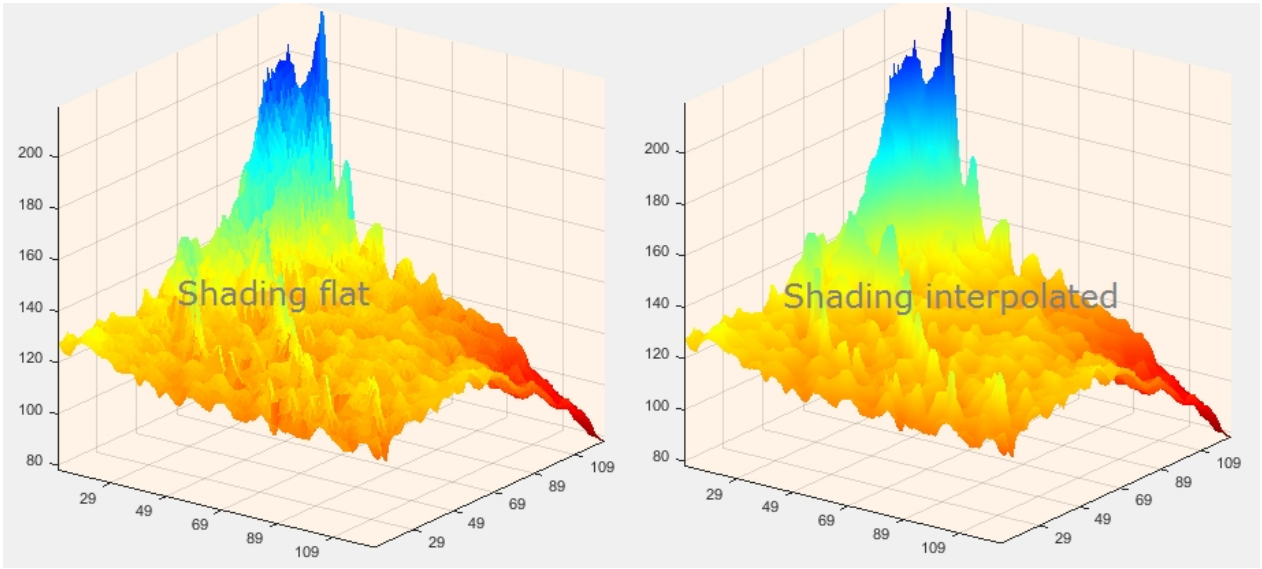
Transparency

Transparency options allow you to enter a transparency value between 0.00 (plot not visible) and 1.00 (plot non transparent). In the example below, transparency values of 0.25 and 0.8 have been chosen. Note that a high transparency, which corresponds to a low transparency value, will slow down your computer when using big pictures.



Shading

FLIMXVis offers you two options for shading your plot. Choose **Flat** to show colors per segment and **Interpolated** to show a smoother, interpolated version of your plot. In the example below, a 3D plot with both shading options is shown.



Multivariate Groups

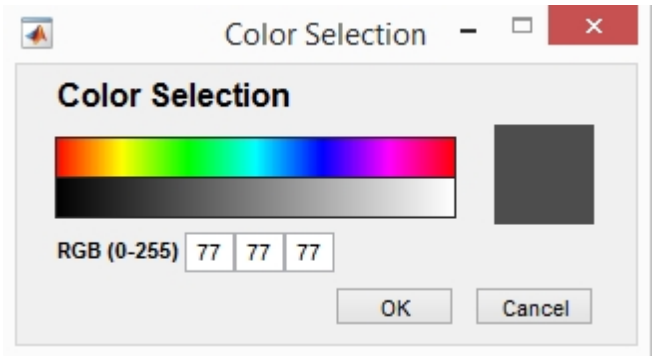
When using multivariate view, FLIMXVis allows you to use separate offset and scaling parameters for each stacked plot. This might be useful when visualizing two FLIM Parameters with different properties.

Tick the corresponding box to enable offset and separate scaling for each plot.

Group Clusters

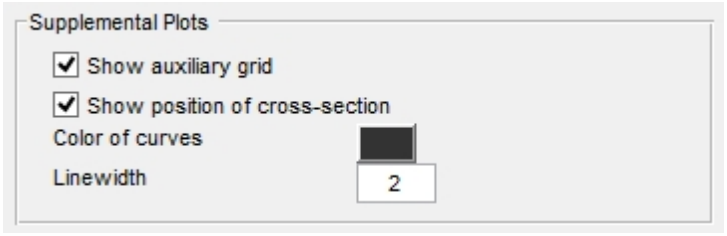
To change to background of scatter plots, click on the color field which shows your current ROI line color. A window will pop up as seen below. To select a color, enter RGB values or click on a

color in the colorbar.



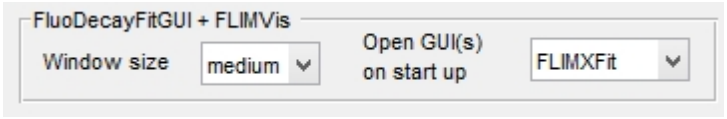
Supplemental Plots

Histogram and cuts are shown in supplemental plots. Supplemental plot options are located in the second box from bottom to top as indicated by an arrow in the picture below. You can chose to show or not show auxiliary grid and cross-section position as well as color and width of curves.



FluoDecayFitGUI + FLIMVis

To set window size and which GUI to open on start up, open FLIMX Visualization Options from FLIMXVis. Window size and GUI options for FLIMX Launcher are located in the lowermost box as indicated by an arrow in the picture below.



Window Size

To set a Window size for FLIMXVis, click on the arrow next to your current window size as demonstrated in the picture below. Select which window size you wish to use by clicking on it. Select **OK** to save your settings and close the window or **Cancel** to exit without saving.

- **Small** : Optimized for a resolution of 1366x768 pixels
- **Medium** : Any resolution in between
- **Large** : Optimized for Full HD resolution (1920x1080)

Open GUI(s) on Start Up

To select which GUI to open on start up, click on the arrow next to your current GUI as demonstrated in the picture below. A list containing FLIMXFit, FLIMXVis and both will show up. Select which GUI you want to show on start up by clicking on it. Select **OK** to save your settings and close the window or **Cancel** to exit without saving.

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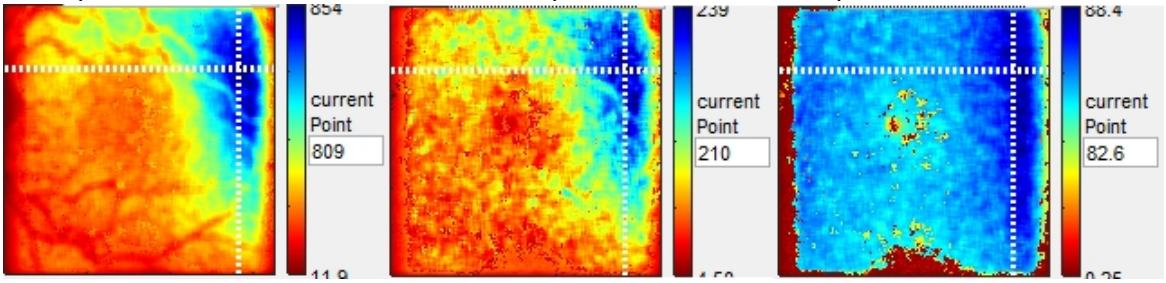
Glossary

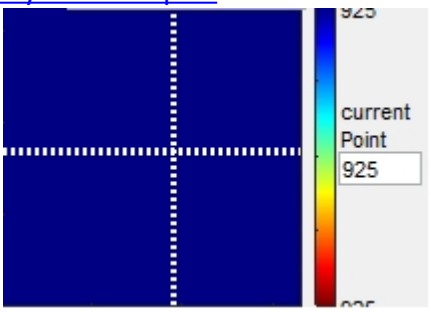
FLIM Items

Amplitude1, **Amplitude2** and **Amplitude3** refer to the highest value (Maximum/Amplitude) of the appropriate exponential function. If for example the current Point has 800, the exponential function has its maximum at 800 counts on the Photon-Frequency axis. Commonly Amplitude1 (left picture) has the highest values followed by Amplitude2 (middle) and then Amplitude3 (right picture). The picture shows the three different amplitudes for the same pixel.



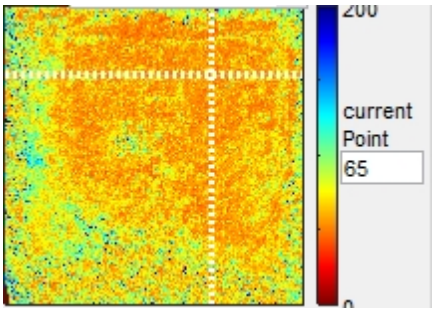
AmplitudeGuess1, AmplitudeGuess2 and **AmplitudeGuess3** describe guessed amplitudes for the exponentials as initial point for the approximation.

EndPosition describes the time channels between the start and the end position of the measured points. The interval is used for the approximation. For further information see also: Settings -> Pre-Processing. Commonly the auto function is selected. In this case the EndPosition distribution is the same for every pixel. The picture below shows the distribution. The value next to the Roi window is the number of time channels and NOT the time. The time channels occur due to the resolution of the measurement device. For further information see also: Photon-Frequency Time Graphic.

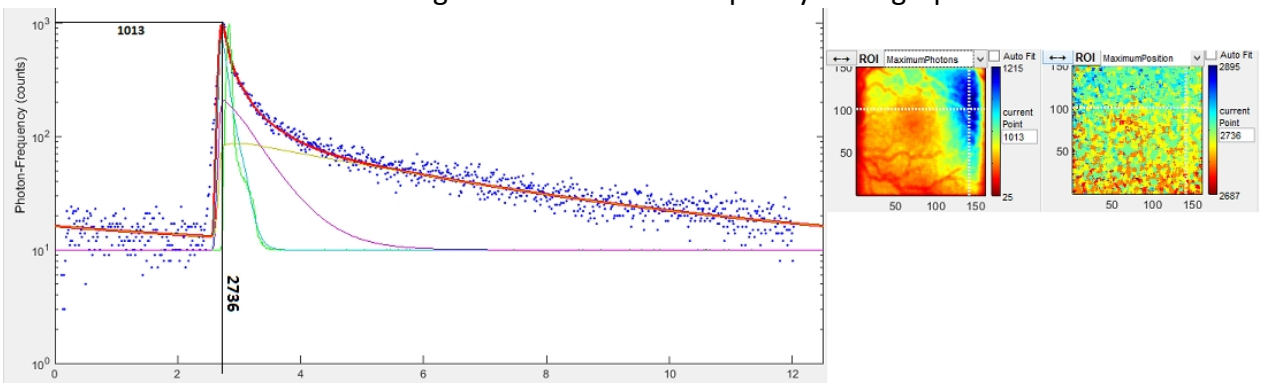


Iterations are the number of steps the approximation algorithm needs to get to the shown

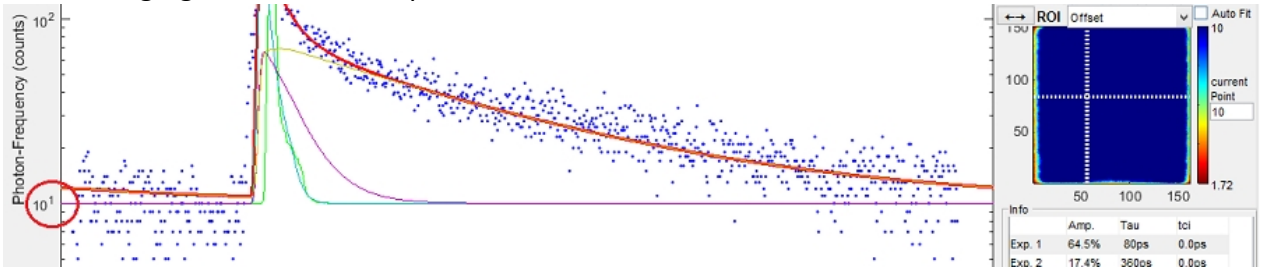
result. Typically the Iterations-value is between 0 and a view hundred. The picture below shows the Iterations distribution. The strong variation in the number of iterations refers to the binning process.



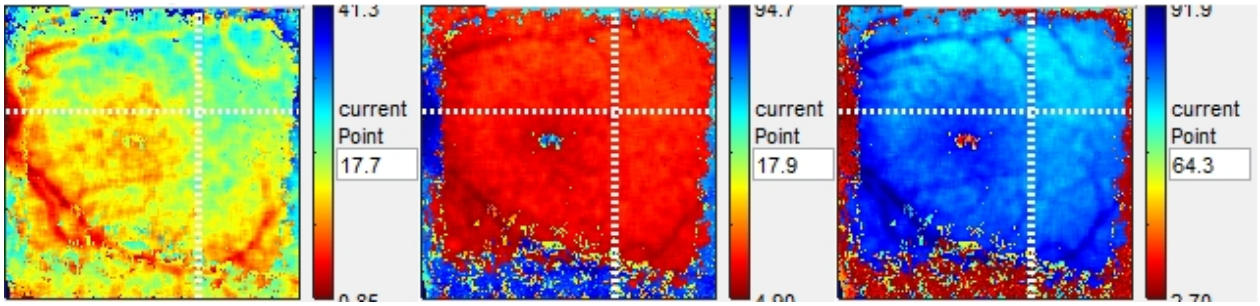
MaximumPhotons and **MaximumPosition** describe the number of counts on the Photon-Frequency axis or the time in pico seconds at the highest measured point. The picture shows the relation between the ROI image and the Photon-Frequency Time graph.



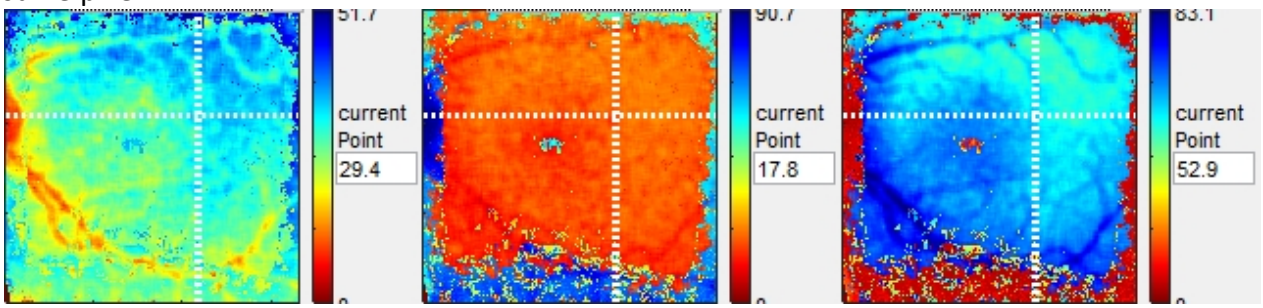
Offset refers to the Offset of the currently selected pixel. The Offset appears because of surrounding light as noise. The picture shows where to read the Offset.



Q1, **Q2** and **Q3** describe the area under the curve. Pixels with high Q-values have longer lifetimes. The three pictures below show the different Q-values for the same pixel.

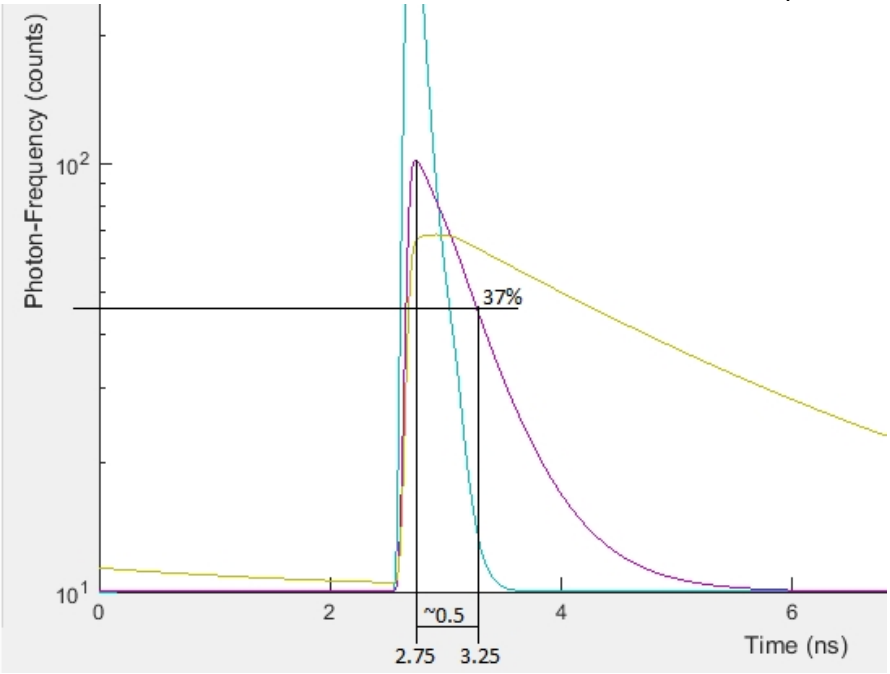


RAUC1, **RAUC2** and **RAUC3** describe the relative area under curve. It is calculated differently to the Q-values. The lifetimes are multiplied with the appropriate amplitudes and scaled to the total area under the curve. The pictures below show the three different RAUC-values for the same pixel.



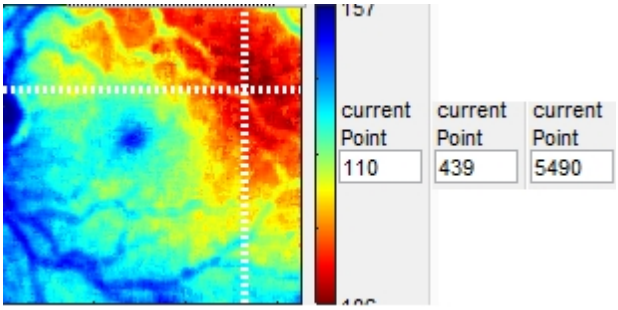
The Q and RAUC-values look very similar. This is due to the scaling as the only difference between them.

TAU: The lifetime describes the time in which the exponential function drops from the value of the amplitude to the value of 1/e of the amplitude (approximately 37%, eĒ 2,718). The picture below shows the time distance as a horizontal bar for the second exponential.



The real value of tau2 for this example is 0.442 ns (442 ps).

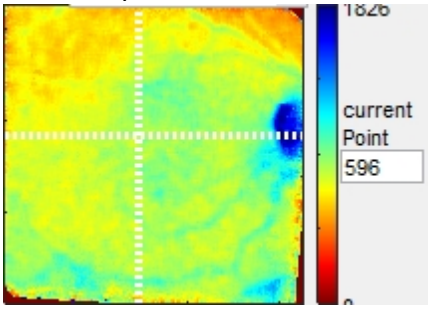
TauGuess1, **TauGuess2** and **TauGuess3** refer to the result of a rough estimation. The program guesses the lifetime of the exponentials by use of the method of moments and uses this result as initial point for the per pixel fit. The picture below shows the different guessed lifetimes for the same pixel. The difference to the calculated lifetimes is that the program automatically assigns a longer lifetime to a lower intensity. Because of that the lifetime distribution looks like a negative intensity picture for all three lifetimes. The resulting lifetimes arise from the multiplication factor given for the exponentials. This factor can be adapted under Settings -> Approximation -> Constant Fit Parameters.



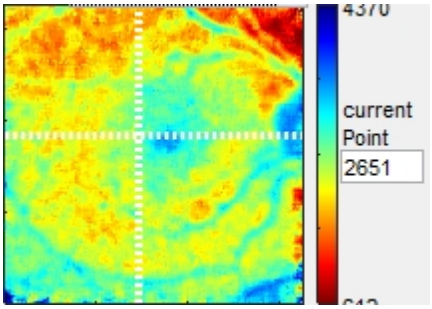
TauMean is calculated with the equation below.

$$\tau_m = \frac{\sum_i \alpha_i \cdot \tau_i}{\sum_i \alpha_i}$$

It is a weighted average of the tau-values. The weight is the amplitude of the exponentials. Thereby the sum of the arithmetic products of amplitude times tau is divided by the sum of the amplitudes. The picture below shows the tau-mean distribution.

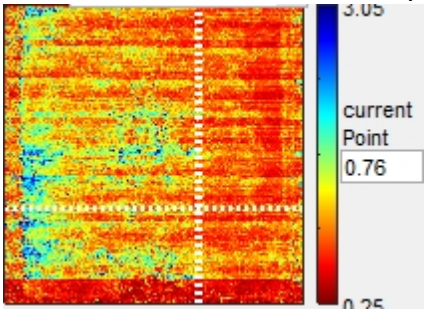


TauMeanGuess gives a rough approximated value for TauMean. The picture below shows the distribution of TauMeanGuess for the same pixel as TauMean.

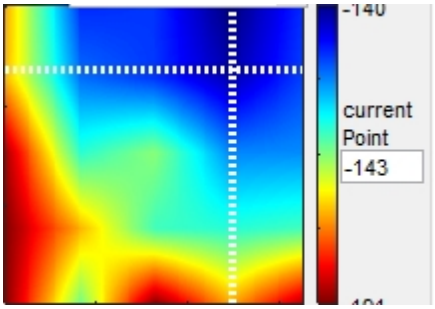


The value is higher and the distribution is less homogeneous.

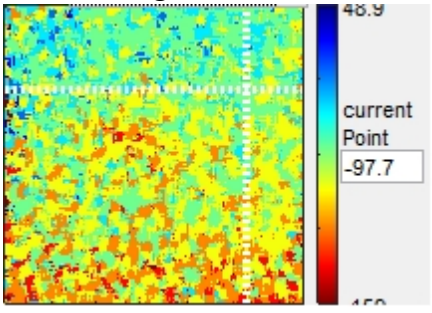
Time determines the time in seconds that is needed to approximate the pixel. The picture shows that the time difference can vary vastly.



hShift describes the left shift of the Model curve in relation to the IRF curve. The picture below shows the distribution of the shifts.



hShiftGuess is the guessed shift of the Model curve.



standalone refers to the Computational Information. It shows where the calculation is done. 0 stands for a MATLAB related calculation. 1 stands for a deployed calculation. A deployed calculation is done with a deployed code. This code can be used by every PC without having a licensed MATLAB software. This allows to share the work. The figure below shows the distribution of MATLAB related calculations and deployed calculations.