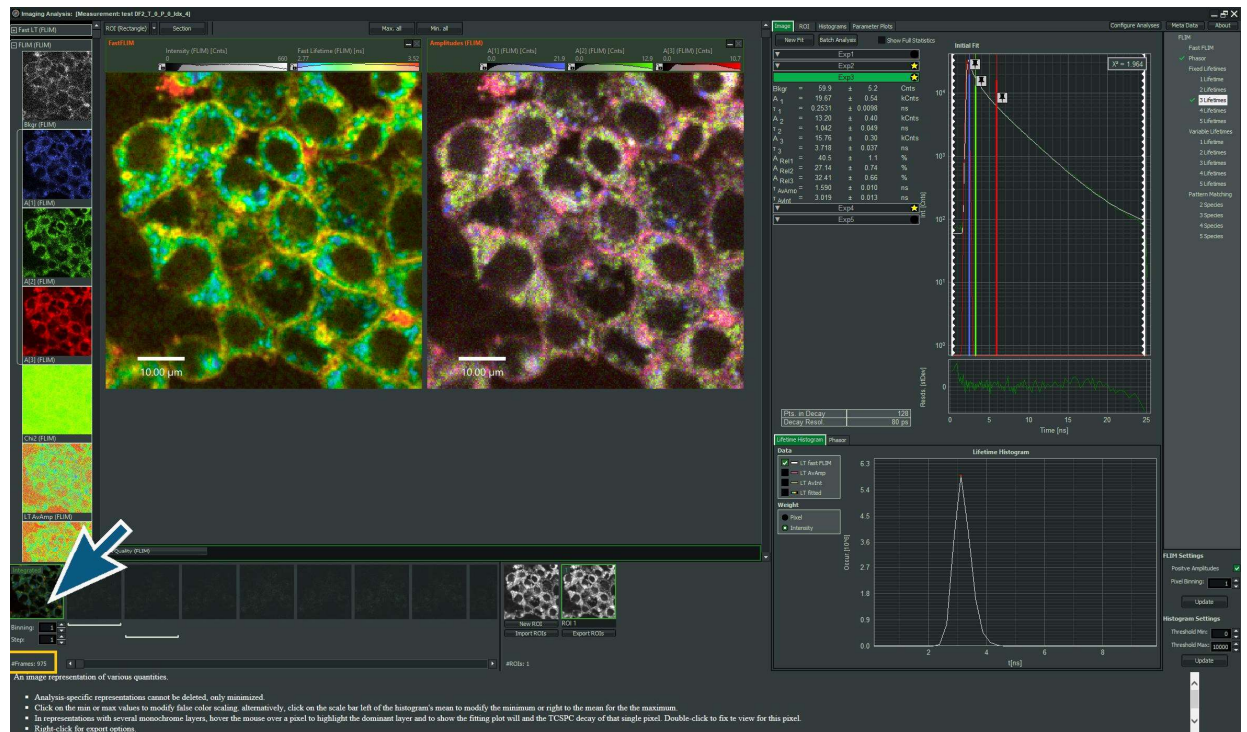


# **How to Perform Batch Analysis over Multiple Frames**

In this “How to” we work with a multi-frame acquisition. See the total number of frames. We have 975 frames. When the Analysis Opens, you see the integrated frame, i.e. all frames are summed together.



2

By double-clicking on individual frames one can see a single frame.

Image Analysis [Measurement: Test MP\_1\_1\_3\_P\_3\_36\_4]

Frame Selector: Display of the single recorded frames (IntLM). Integrating image (left). Sum of all frames from a stack of recorded images (SumLM).

- Run and/or Stop: integrate of frames
- Double-click on individual/stacked frames to load and display data from the frame selection. Currently active frame is marked by a green edge.
- Selection for Histograms: Select frames by left-click. <CTRL>+ add frames to selection. <SHIFT> select range. Selected frames will be marked green. Drag and drop selection onto histogram to apply as filter.

3

By double-clicking on individual frames one can see a single frame. The active frame gets a green square for highlighting the selection.

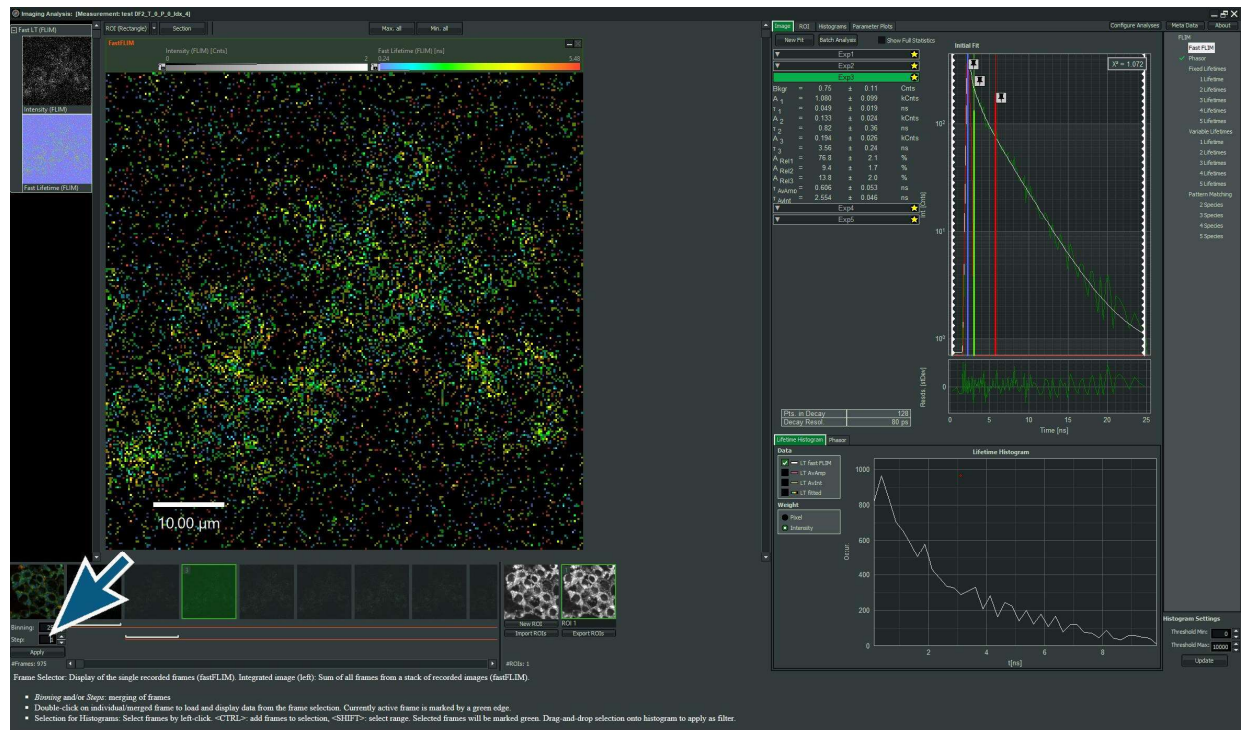
The screenshot displays the PicoQuant Imaging Analysis software interface. The main window is titled "Imaging Analysis [Measurement: Test MP\_1\_1\_3\_P\_3\_36\_4]". The central area shows a large, multi-colored image of a sample, with a scale bar indicating 10.00 μm. A blue arrow points to a small green square on the bottom frame selector, indicating the active frame. The bottom frame selector shows a sequence of frames, with the active frame highlighted in green. The right side of the interface features a "Histogram" plot showing "Count" versus "Time [ns]". The plot shows a decay curve with several peaks. The "Histogram Settings" panel on the right includes options for "Threshold [cts]", "Threshold [ns]", and "Update".

Frame Selector: Display of the single recorded frames (fastFLIM). Integrated image (left). Sum of all frames from a stack of recorded images (fastFLM).

- **Rolling and/or Stop:** integration of frames
- **Double-click** on individual integrated frames to load and display data from the frame selection. Currently active frame is marked by a green edge.
- **Selection for Histograms:** Select frames by left-click. <CTRL>- add frames to selection. <SHIFT>- select range. Selected frames will be marked green. Drag-and-drop selection onto histogram to apply as filter.

4

One can change the binning of the frames. Here for example we chose a 25-frame-binning i.e. 25 frames are summed together.

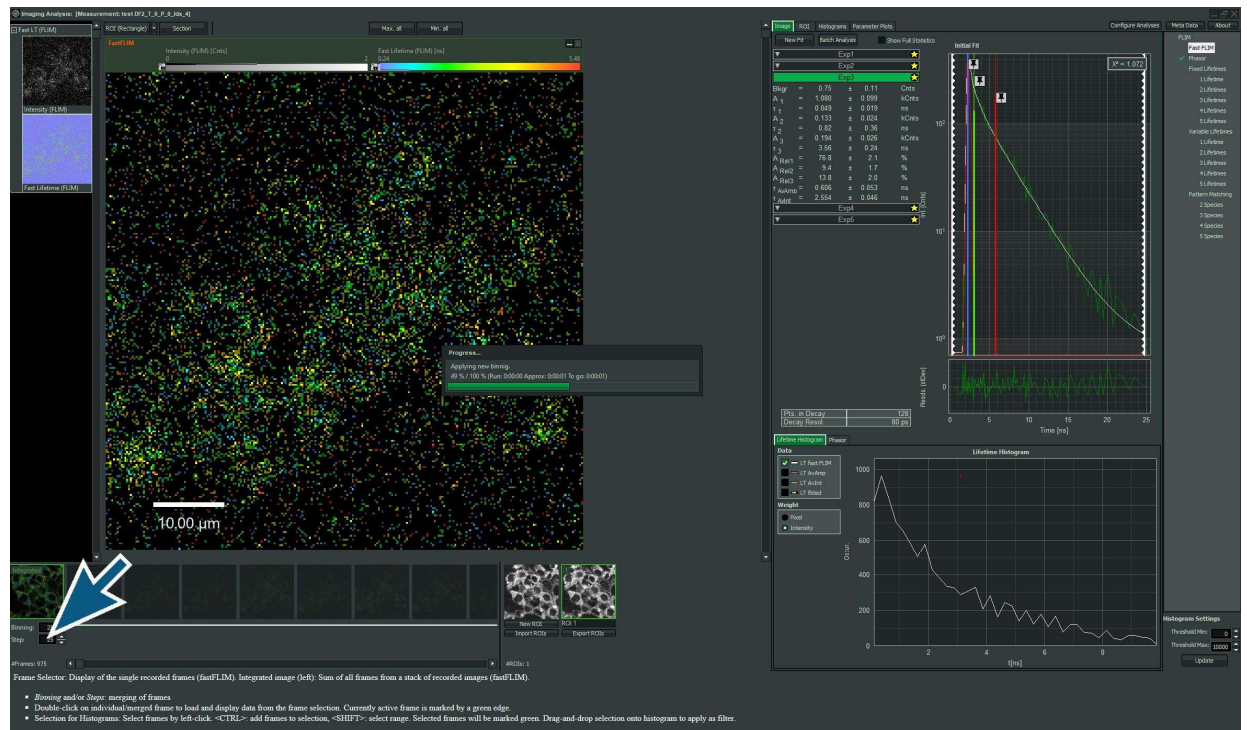




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One can also set independently the step. With this option, the user can even create new frames which are “sliding averages” of the original frames.

If the step is equal to the binning then there is no overlap between the newly created frames.





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The newly created binned frames can be also selected and inspected individually.  
 In each frame the user can try all the available analysis and fitting options.

The screenshot displays the PicoQuant Imaging Analysis software interface. The main window is titled "Imaging Analysis [Measurement: Test MP\_1\_1\_3\_P\_3\_36\_4]". It features a central panel showing a fluorescence image of a sample with a 10.00 μm scale bar. To the left, there are thumbnails for "Fast LT (FLIM)", "Intensity (FLIM)", and "Fast Lifetime (FLIM)". Below the main image is a row of binned frames. The right side of the interface contains several panels: "Image" with a "Fast Lifetime (FLIM)" plot showing intensity vs. time with a fitted curve and a yellow stop watch icon; "Histogram" with a "Lifetime Histogram" plot showing count vs. time; and "Parameter Lists" with a table of fit parameters. A blue arrow points to the "Fast Lifetime (FLIM)" plot.

Parameter	Value	Unit
Offset	22.29	Counts
A <sub>1</sub>	0.9177	ns/Counts
t <sub>1</sub>	0.980	ns
A <sub>2</sub>	4.852	ns/Counts
t <sub>2</sub>	3.288	ns
A <sub>Fast</sub>	58.57	%
A <sub>Fast2</sub>	41.43	%
τ <sub>Fast</sub>	1.65	ns
τ <sub>Fast2</sub>	3.028	ns
τ <sub>Fast</sub>	Exp	
τ <sub>Fast2</sub>	Exp	

**Fast Lifetime (FLIM) Parameters:**

- Offset: 22.29 ± 0.92 Counts
- A<sub>1</sub>: 0.9177 ± 0.008 ns/Counts
- t<sub>1</sub>: 0.980 ± 0.015 ns
- A<sub>2</sub>: 4.852 ± 0.050 ns/Counts
- t<sub>2</sub>: 3.288 ± 0.200 ns
- A<sub>Fast</sub>: 58.57 ± 0.46 %
- A<sub>Fast2</sub>: 41.43 ± 0.46 %
- τ<sub>Fast</sub>: 1.65 ± 0.195 ns
- τ<sub>Fast2</sub>: 3.028 ± 0.010 ns
- τ<sub>Fast</sub>: Exp
- τ<sub>Fast2</sub>: Exp

**Fast Lifetime (FLIM) Histogram:**

- Y-axis: Count
- X-axis: [ns]
- Peak at approximately 1.65 ns

**Fast Lifetime (FLIM) Plot:**

- Y-axis: Intensity [Counts]
- X-axis: Time [ns]
- Fit equation:  $I(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + \text{Offset}$
- χ<sup>2</sup> = 1.050

**Fast Lifetime (FLIM) Settings:**

- Fast Lifetime: 1.65 ns
- Fast Lifetime: 3.028 ns
- Fast Lifetime: 1.65 ns
- Fast Lifetime: 3.028 ns
- Fast Lifetime: 1.65 ns
- Fast Lifetime: 3.028 ns
- Fast Lifetime: 1.65 ns
- Fast Lifetime: 3.028 ns
- Fast Lifetime: 1.65 ns
- Fast Lifetime: 3.028 ns

**Fast Lifetime (FLIM) Histogram Settings:**

- Threshold: 1000
- Threshold: 1000
- Update

**Fast Lifetime (FLIM) Plot Settings:**

- Update

**Fast Lifetime (FLIM) Overview:**

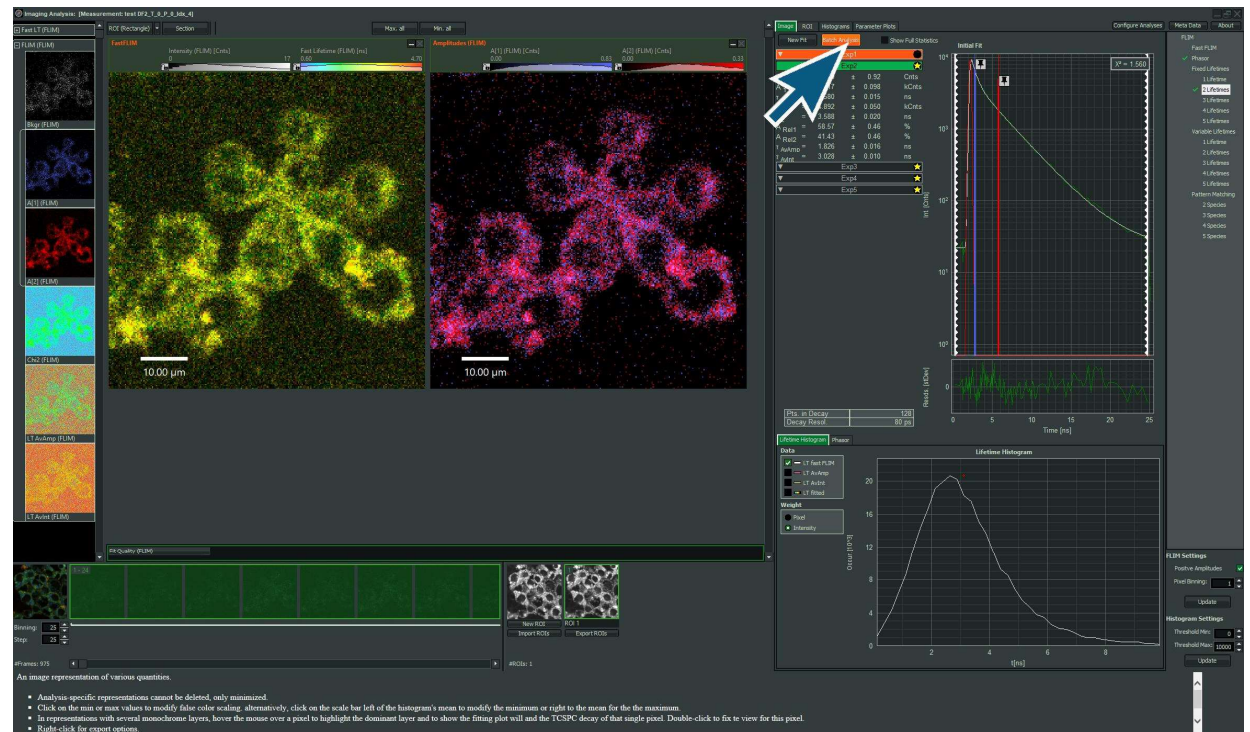
Fit model selection/analysis overview. Highest level nodes switch between different imaging analyses. Note: This will reload the photon data depending on the configured analysis. Each imaging analysis provides a distinct set of analysis options.

- Each category has a separate settings panel, which appears at the bottom of the panel. More detailed information will be shown in the individual help for the different models.
- Select a fit model marked with a green checkmark to show the settings panel below and/or start a new analysis, discarding previous results. Some analyses (like steady state microscopy) do not offer TCSPC image fitting.
- Green checkmark: fit models that are currently used in the image fit. Line green checkmark in brackets: fit model used to extract patterns for the pattern matching analysis. Yellow stop watch: analysis is running.



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FOR the Batch Analysis of all pixel fits over all frames. Batch Analysis of all pixel fits over all frames. Select 'Batch Pixel Fits' from the menu.

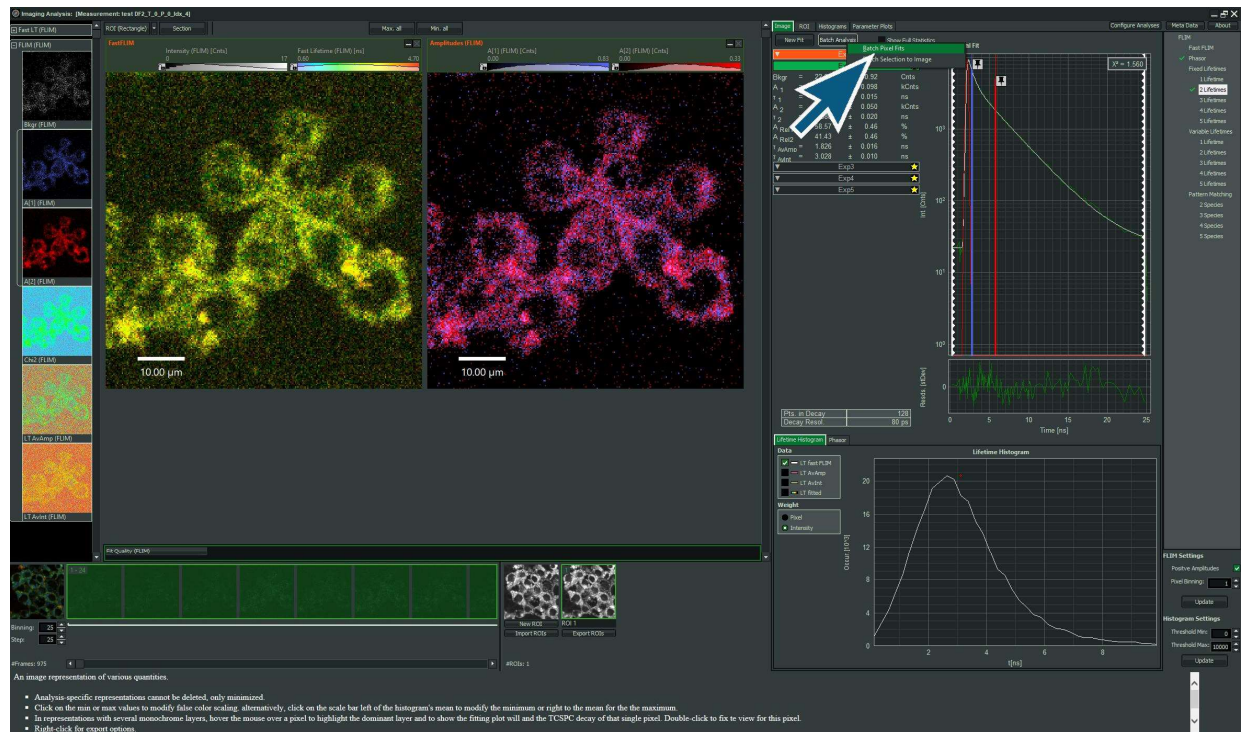


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The user has two options. Either “Batch Pixel Fits” or “Batch Selection to Image”.

“Batch Pixel Fits” : Multi exponential fits over all frames,

“Batch Selection to Image” : If the Phasor Selection to Image is already done for one frame. The analysis is applied over the whole frames.







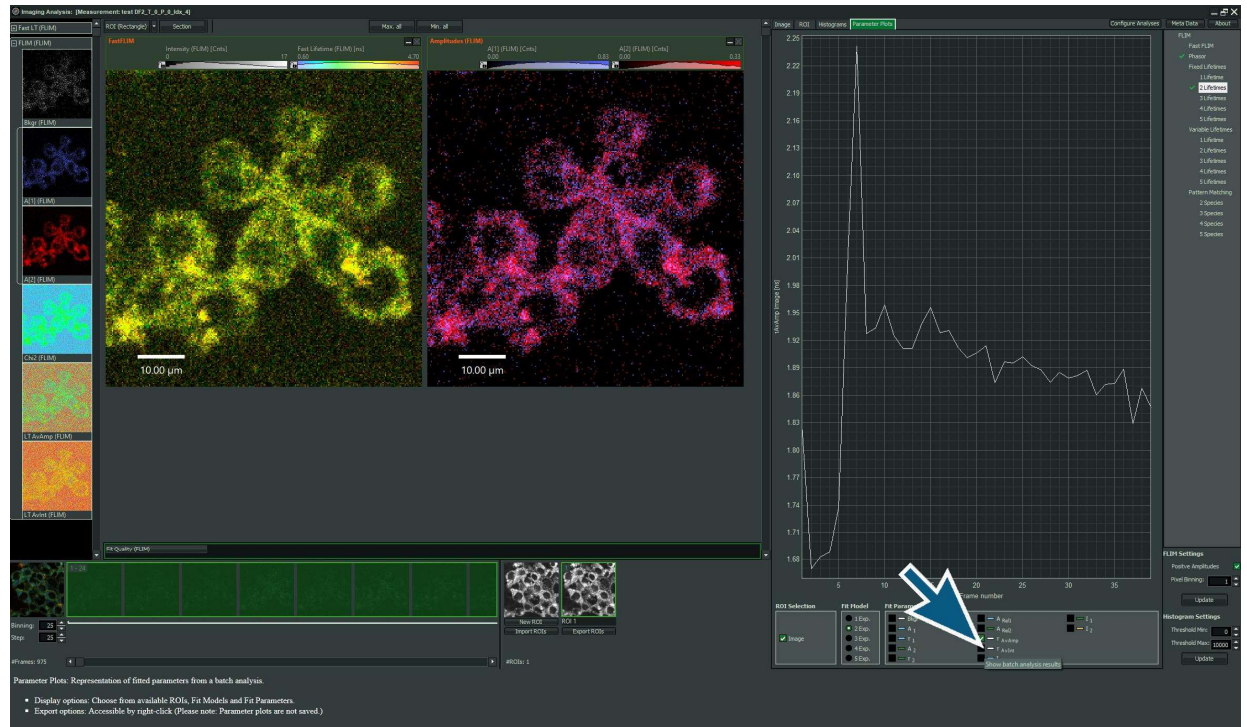


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ROI Selection : By default the batch Analysis runs over the whole image. If ROIs are set before the batch Analysis the the batch analysis will run also for each individual ROI separately.

Fit Model: The user can view the results of many multi-exponential models.

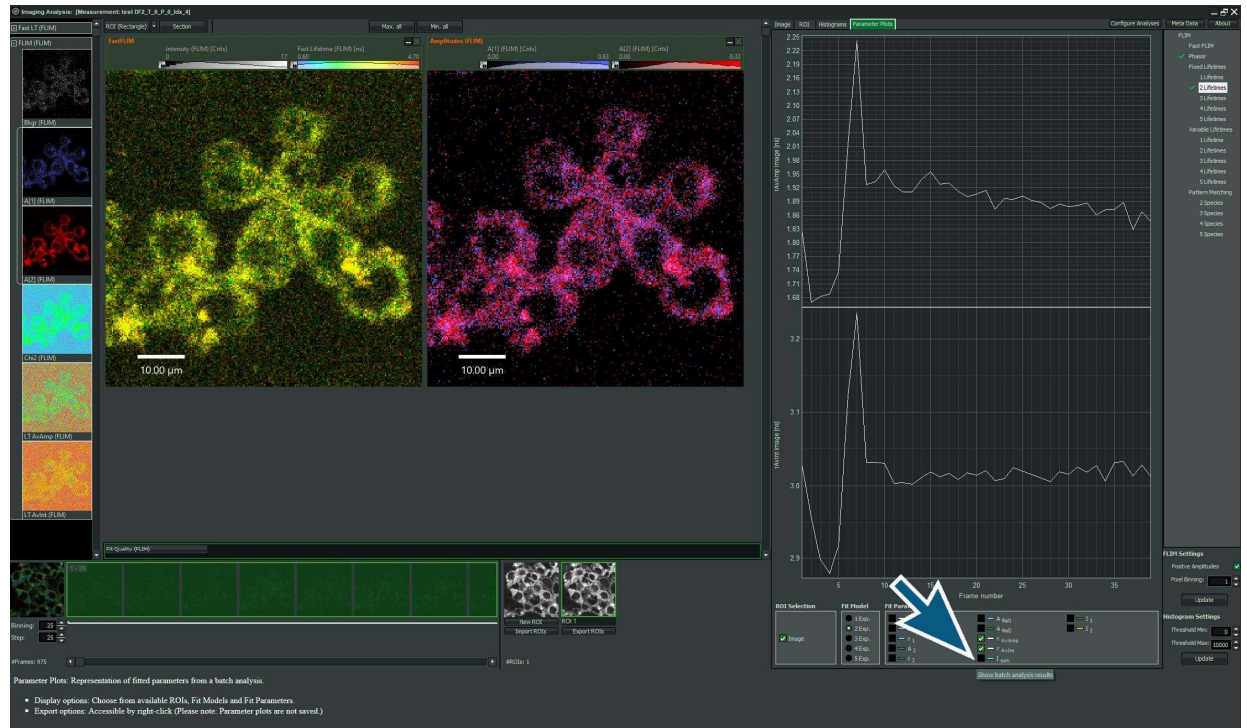
Fit Parameters: The user can select the parameter to be plotted.





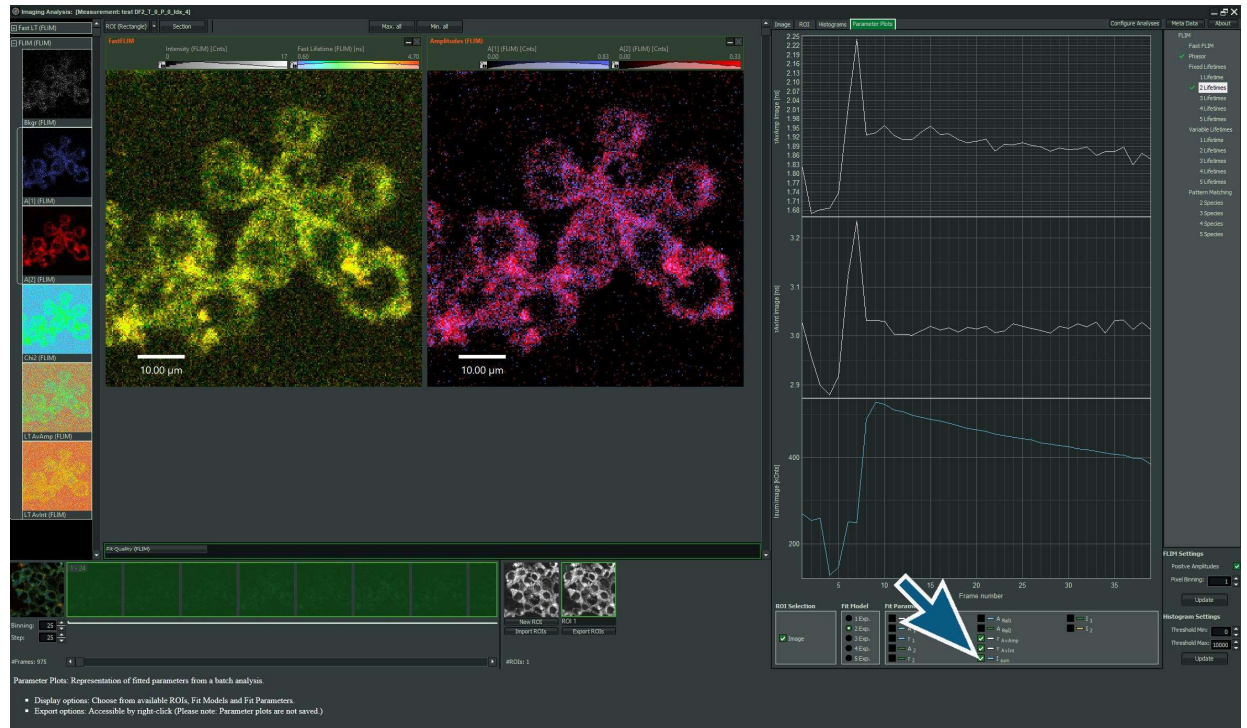
13

Multiple Selection of fitted parameters are allowed.



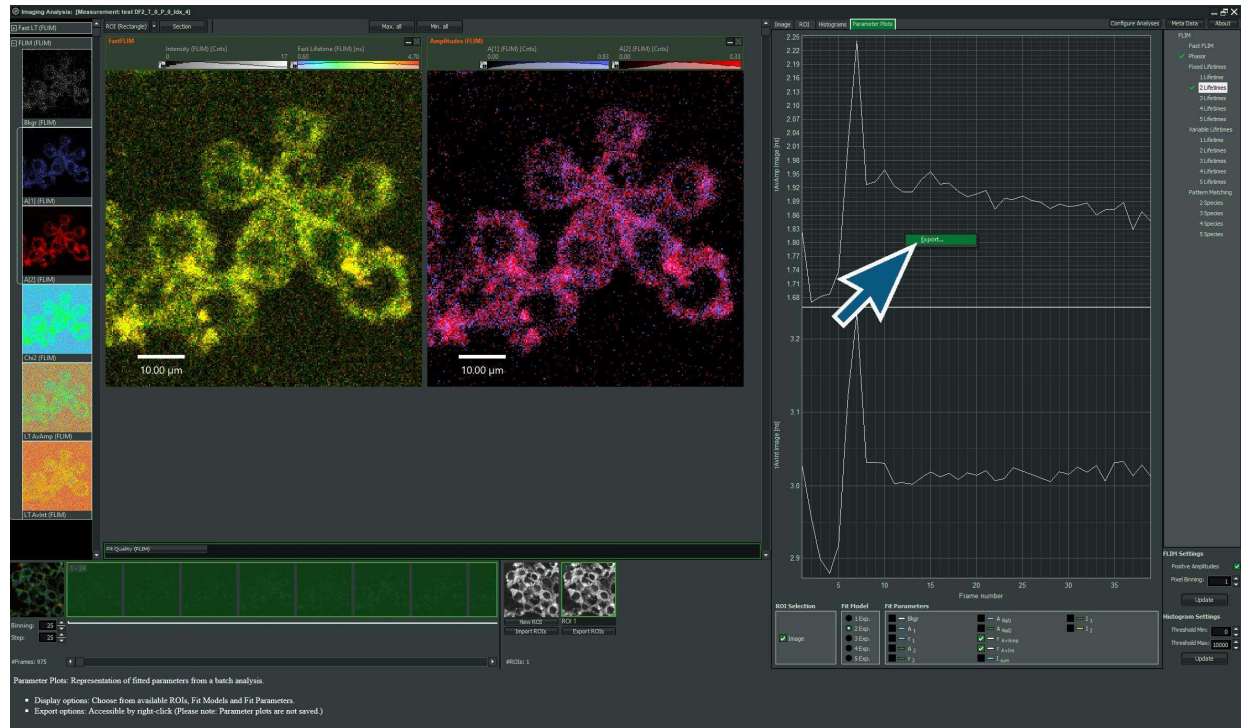
14

I\_sum gives you the overall intensity in terms of total photons counts included per frame .



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You can right-click on any active plot.  
By selecting export one can export all active graphs as ASCII or bmp.



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The user can select the path.

The screenshot displays the PicoQuant software interface. The main window shows a microscopy image with a color scale and various analysis tools. A 'Save As' dialog box is open, showing the file path 'Documents > exports'. The 'Save as type' dropdown menu is highlighted with a blue arrow. The background interface includes a 'Parameter Plots' window with two line graphs showing intensity over time, and a 'Histogram' window. The bottom of the interface contains a 'Parameter Plots' section with a legend and a 'Histogram Settings' section.

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Example of the exported data.

The screenshot displays the PicoQuant Image Analysis software interface. A central window shows a table of exported data with columns for 'Frame number', 't0vMap Image', and 't0vInt Image'. A blue arrow points to the table. To the right, a histogram plot shows intensity distribution. The software interface includes various toolbars and panels for image processing and analysis.

Frame number	t0vMap Image	t0vInt Image
1	1.826444077294160	3.02039917779619
2	1.66990813604657	2.9561416373251
3	1.6819848995119	2.8984029072306
4	1.68757534963183	2.879406246018621
5	1.73666363982854	2.91793175658715
6	2.0155900650729	2.1230504054566
7	2.245160829576431	3.2355493458844
8	1.9272159689315	3.0318095588968
9	1.93170040611	3.03160682915065
10	1.9584940015872	3.03201806472612
11	1.9349163008549	3.0035172956679
12	1.9118228339798	3.00401821432168
13	1.9111079926179	3.0019703400214
14	1.93789772131625	3.01244646020835
15	1.95591770518211	3.01314279899889
16	1.9282136413113	3.01161780792516
17	1.93071830654569	3.01674640155477
18	1.91178349912819	3.00813760193374
19	1.90092524879266	3.01727696292643
20	1.90619128792	3.014048951283
21	1.9138080955160	3.0200801926568
22	1.87363396767488	3.0066489887523
23	1.89534809595973	3.01008027766786
24	1.8953159578244	3.02498598294275
25	1.90195975463626	3.01393894391
26	1.89108544006392	3.01577482633613
27	1.88758214401614	3.01862028273362
28	1.87427095494087	3.00557433949499