
Galene Documentation

Release 1.0

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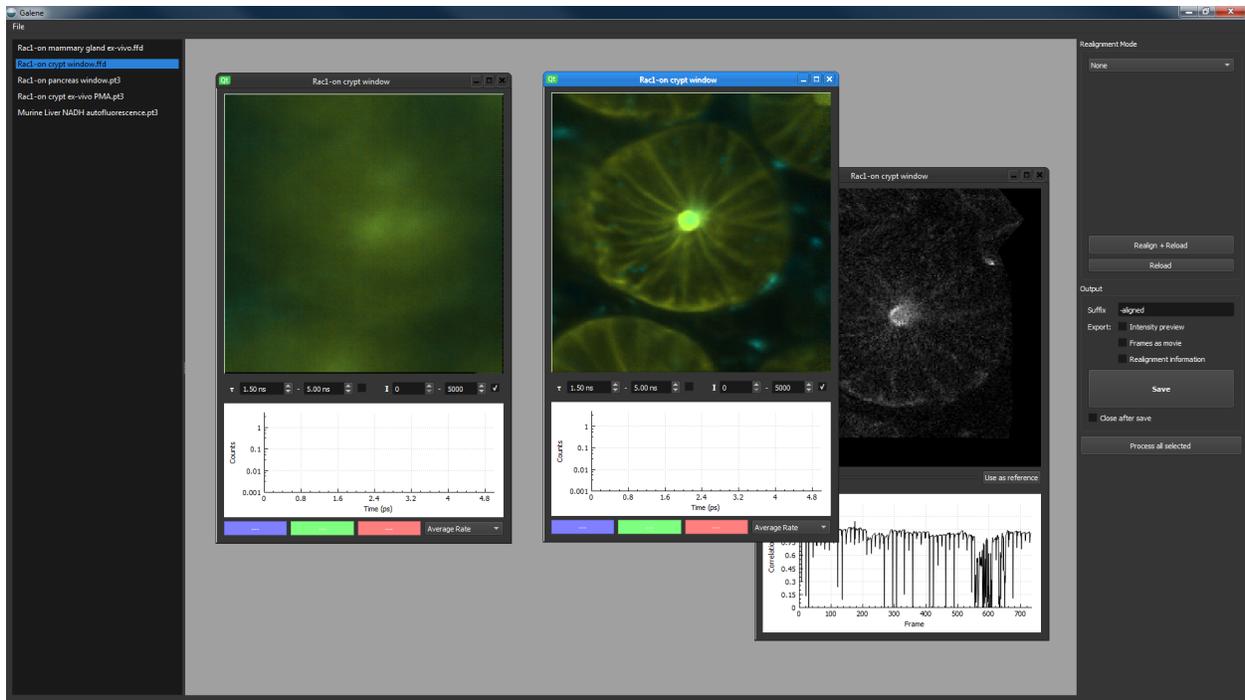
Galene is a tool to correct for sample motion in intravital FLIM. For more information please see [citation].

Download and install Galene from <http://www.flimfit.org/galene>.

Outline

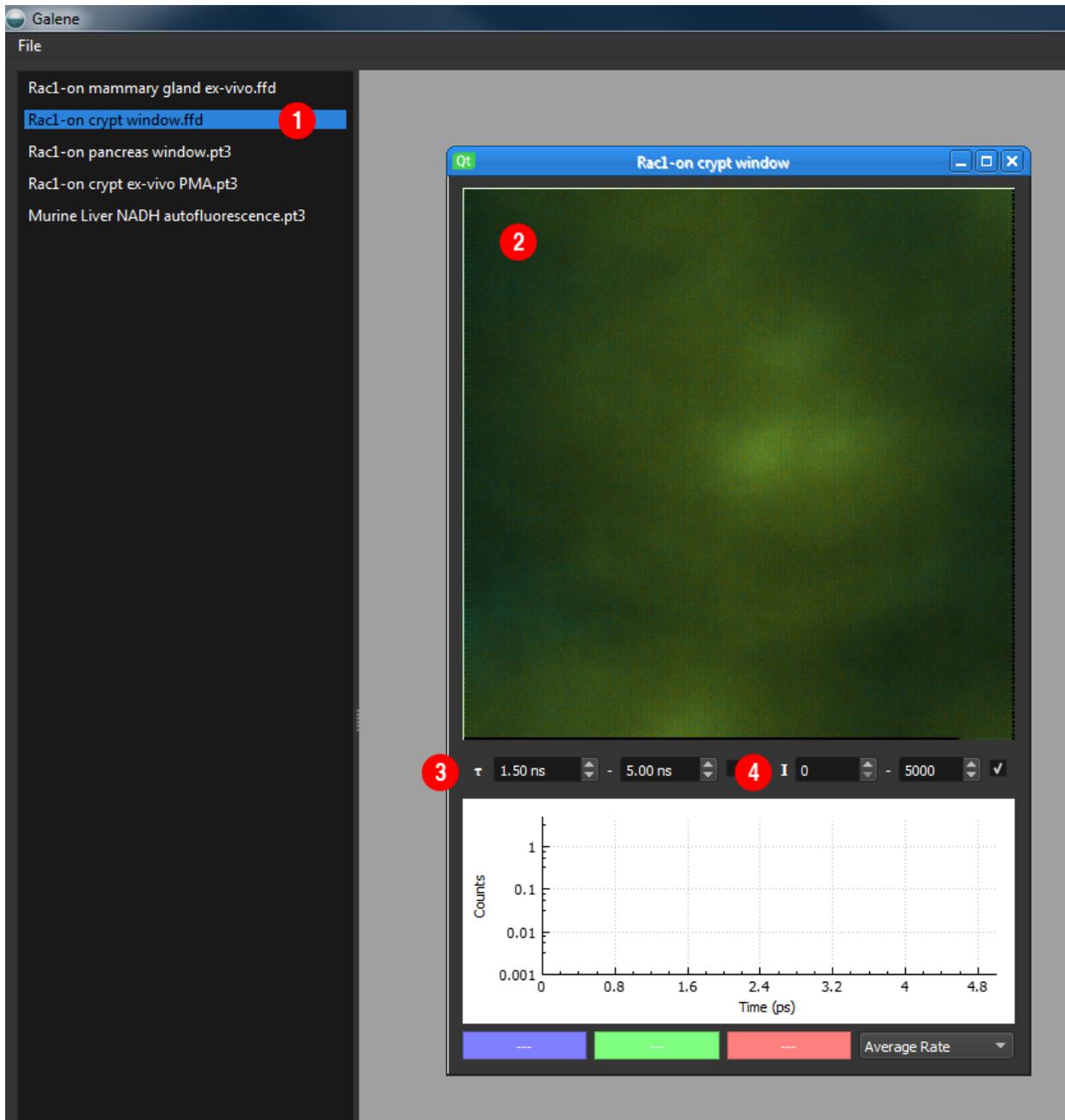
Tutorial

In this tutorial we describe how to load FLIM data, correct for motion and save the realigned data using Galene. Please also see the [video tutorial](#).



Loading and opening data

- Select a folder containing your data using *File>Open...*
- Double click on an image from the list to open
- An intensity mapped FLIM image will open the workspace
- Control the lifetime and intensity limits using the options below the image

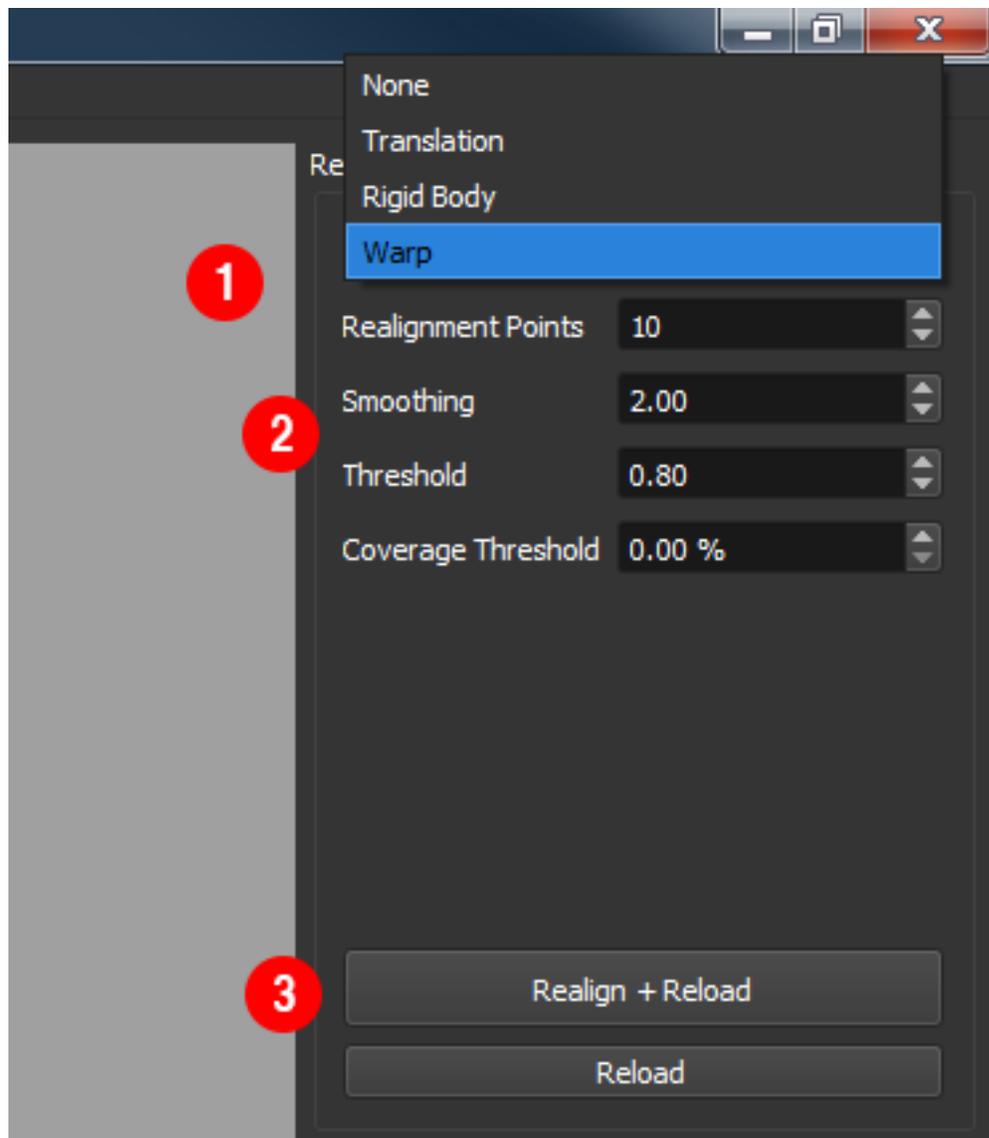


Correcting for motion

- Select the `Warp` motion compensation approach using the drop down menu on the right

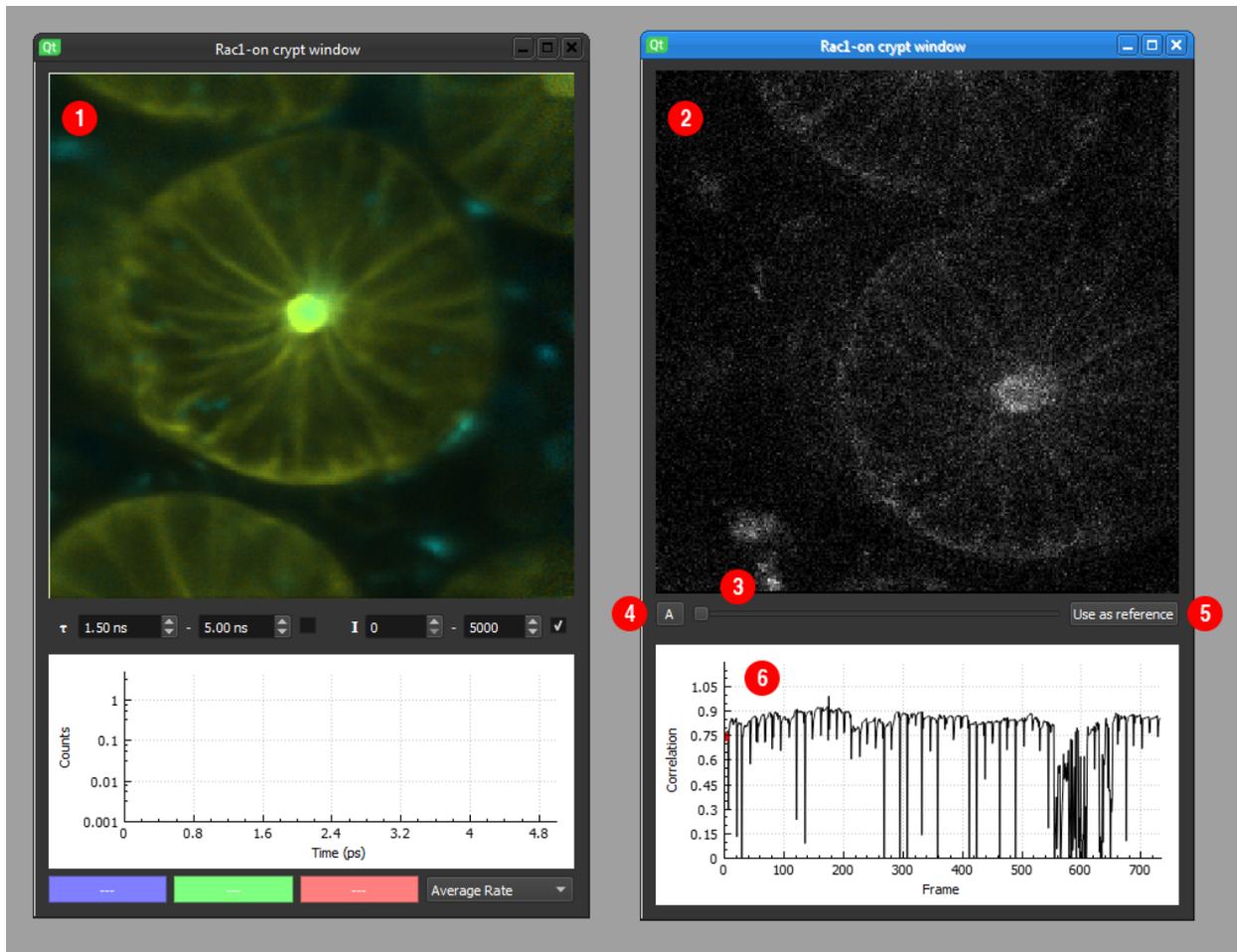
Tip: The `Warp` motion compensation mode takes the microscope scan pattern into account and in most is the best option. For more about the correction modes and the options available see [Correction Modes](#)

- Align the data using the `Realign + Reload` button



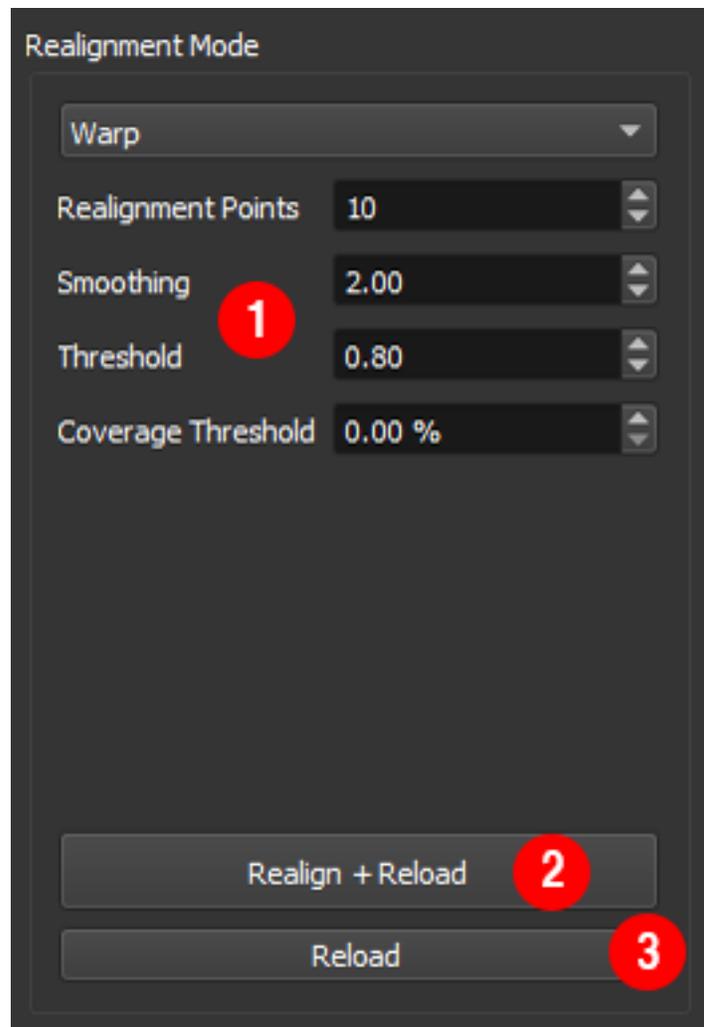
Viewing the realignment results

- The realigned FLIM image is shown alongside the individual intensity frames .
- Scroll through the aligned or unaligned frames using the **A** button in the realignment window.
- By default the first frame will be used as the reference. To select a different frame, press **Use as reference** button and realign the data using the *Realign + Reload* button.
- The graph below shows the correlation between the realigned frame and the reference frame



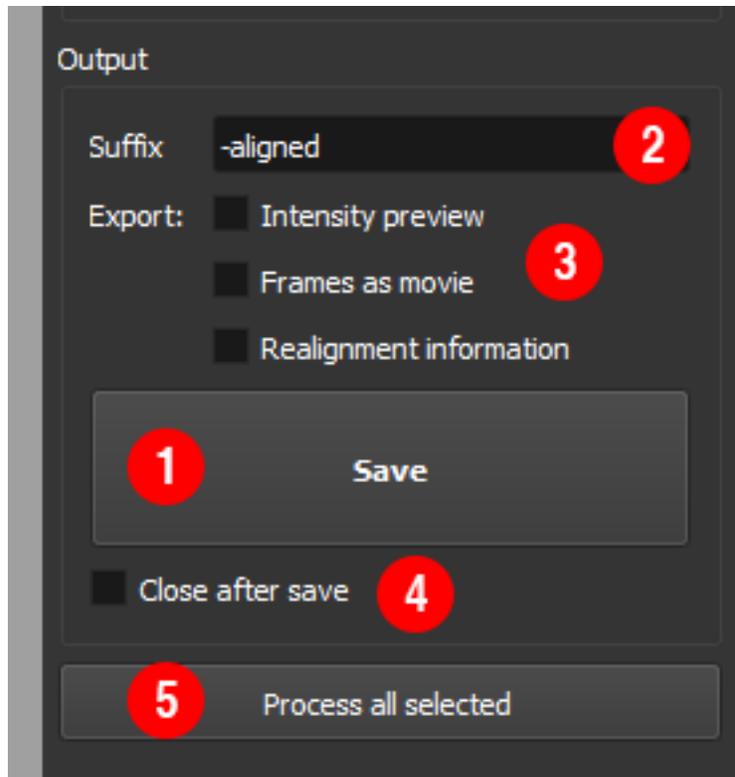
Optimising the realignment results

- If the realignment results are not satisfactory, the following parameters can be adjusted
 - `Realignment Points` controls the number of points in the image used to estimate the displacement during each frame. We have found that the default value of 10 points provides good results across a range of images. If the motion is fast relative to the frame you may need to use more points to accurately estimate the motion. If the realignment appears ‘jittery’, try using fewer points. If the displacement during each frame is relatively small as few as 4 points may be sufficient.
 - `Smoothing` controls the degree of smoothing in the x-axis applied to the image before realignment. Increasing the degree of smoothing can improve the realignment when the signal to noise in each frame is low.
 - After adjusting these settings, realign the data using `Realign + Reload`.
- Frames which are not well aligned, for example if the sample moves out of the field of view, can degrade the final image. These frames can be rejected by applying thresholds to the realignment results
 - Use `Correlation Threshold` to reject frames where the correlation with the reference frame is low.
 - Use `Coverage Threshold` to reject frames where the displacement was very large.
 - Apply the thresholds without reprocessing the data by pressing `Reload`.



Saving the realigned data

- When you are happy with the realignment, press `Save` to save the data as a histogrammed `.ffh` file. These can be read directly by FLIMfit or into Matlab using the `FlimReaderMex` file
- You can also output diagnostics about the fitting :
 - `Intensity preview`: save a `png` of the intensity of the realigned image. This is convenient for quickly assessing which files have been successfully realigned.
 - `Frames as movie`: save a `tif` stack of the aligned and unaligned frames for diagnostics
 - `Realignment information`: save a `csv` file with the estimated displacements across the frames
- To process several files using the same settings, select multiple files and click `Process all selected`.



Correction Modes

There are three correction modes with different options. In most cases *Warp* correction is the best approach

Translation

Rigid

Warp