

Usage of the Phasor Plot Software "SimFCS" developed by Enrico Gratton for FLIM analysis

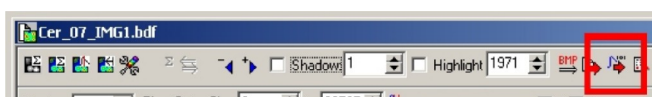


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Procedure

1. Export PQ FLIM Data to be read for SimFCS import

- SymPhoTime software version must be 5.0 or higher
- Select the desired detection channels and recalculate the image ("FastFLIM button")
- Export to binary file

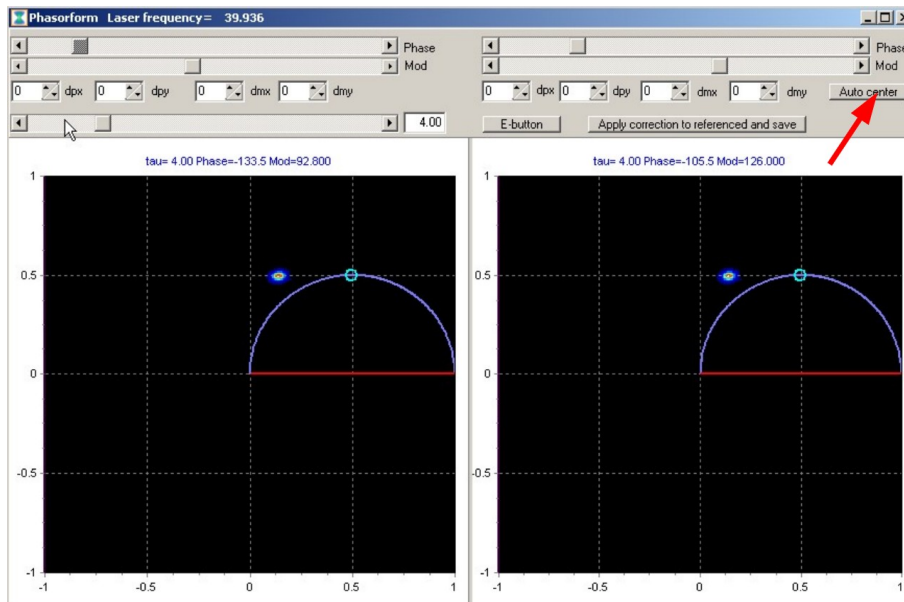


2. Install SimFCS

- Install the software locally on the PC
- The software version should be ≥ 2.0 and from 05.04.2010 or newer
- SimFCS is only working when the number format on the local PC is US style (Regional and Language Options \rightarrow "English (United States)")

3. Import, Convert and Calibrate PQ Data

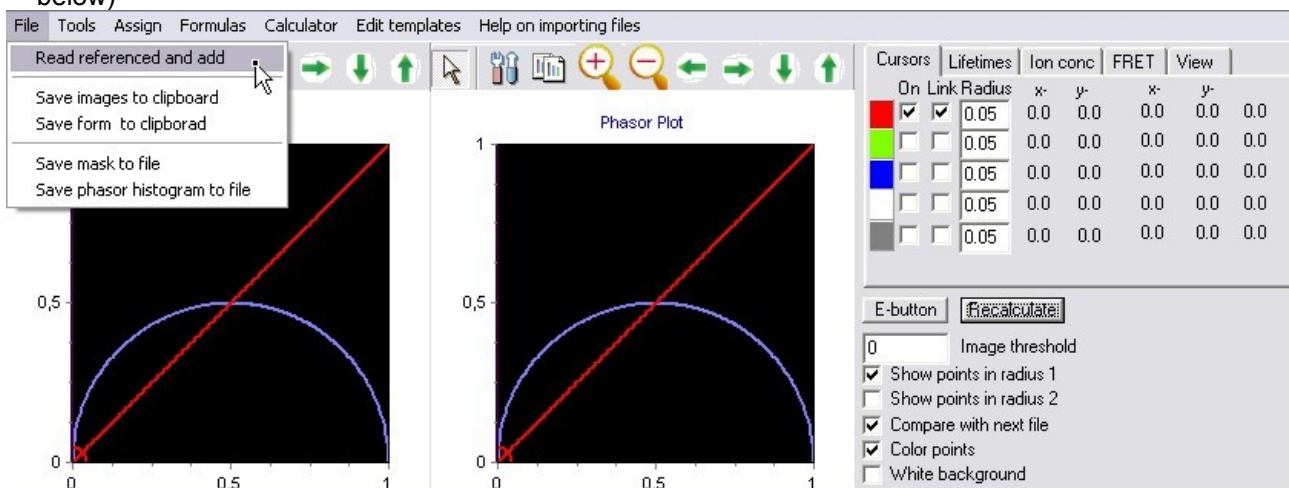
- All bin-files must be stored locally on the same PC where the Sim-FCS software is installed
- Start "simfcs.exe"
- Press "FLIM"
- Use the 'Lifetime image calculation window' \rightarrow File \rightarrow Open sample
- Open first the image of a reference dye with known lifetime
It is important that the reference measurement has a signal to noise ratio which is higher compared to the measurement that should be analysed.
- Press in the 'Lifetime image calculation window' \rightarrow Tools \rightarrow "Calculate Phasor Absolute"
- Press "Auto center". The software will smoothen the data and adjust phase and modulation, so that the measurement points are in the small blue circle.



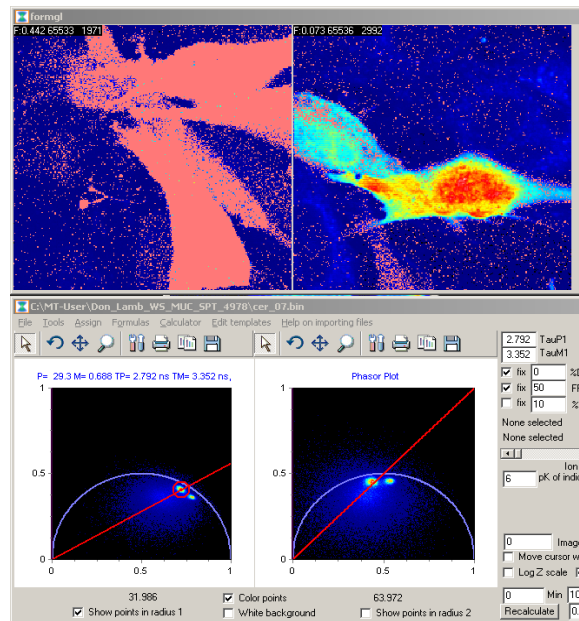
- Press “Apply Correction to reference and save”
- Again use the 'Lifetime image calculation window' → File → Open sample” to load the bin-file of your sample
- In the "Lifetime image calculation window" → File → Save as referenced (*.ref is created)
- Close SimFCS

4. Run Phasor Analysis

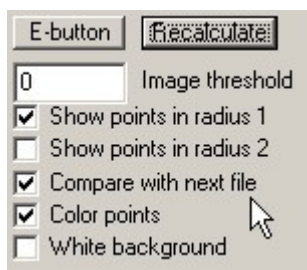
- Start SimFCS
- Start "FLIM"
- Open ref-files via File → Read referenced and add (in the “phasor plot analysis window”, see image below)



- Use the left plot (Phasor first order) and identify the different lifetime components in the image. The left phasor plot graph shows the first order, for example 40MHz (repetition rate of the laser). The right phasor plot graph shows the second order, in this example 80MHz. Two different measurements are loaded in the example below.



Some useful functions



- Image threshold settings in order to get rid of unspecific background signal.
- White background can be applied to get a better contrast.
- E-button smoothes the data by calculating the moving average.

Cursors	Lifetimes	Ion conc	FRET	View
<input checked="" type="checkbox"/> In Link	<input checked="" type="checkbox"/> Radius	0.05	2.509 3.532	4.295 6.159 0.457
<input type="checkbox"/>	<input type="checkbox"/>	0.05	0.000 0.000	0.000 0.000 0
<input type="checkbox"/>	<input type="checkbox"/>	0.05	0.000 0.000	0.000 0.000 0
<input type="checkbox"/>	<input type="checkbox"/>	0.05	0.000 0.000	0.000 0.000 0
<input type="checkbox"/>	<input type="checkbox"/>	0.05	0.000 0.000	0.000 0.000 0

- Possibility to select multiple cursors and adjust the radius of the displayed circle.

Cursors	Lifetimes	Ion conc	FRET	View
2.509	TauP1	4.402	TauP2	3.3 T1
3.532	TauM1	5.948	TauM2	1.8 T2
				0.9 F1
Solve T1,T2				

- Possibility to calculate the average lifetime corresponding to the selected phasors

The image threshold can be applied to get rid of unspecific background signal. If the background is very large compared to the signal, the measurement points in the phasor plot are shifted in the direction of the background.

Image threshold = 0

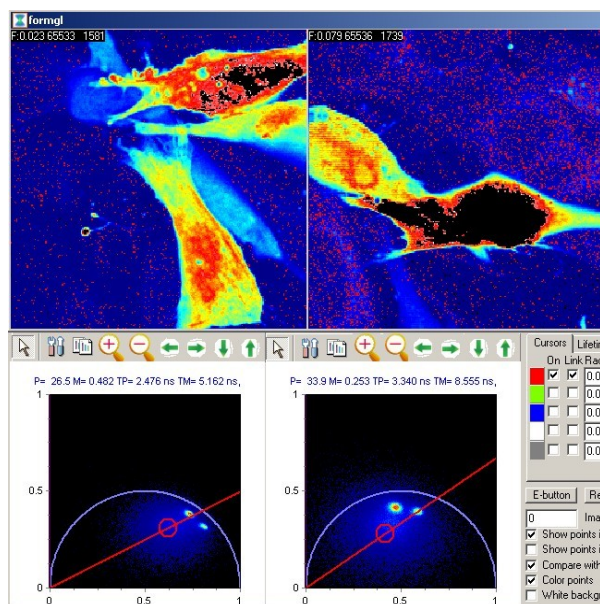
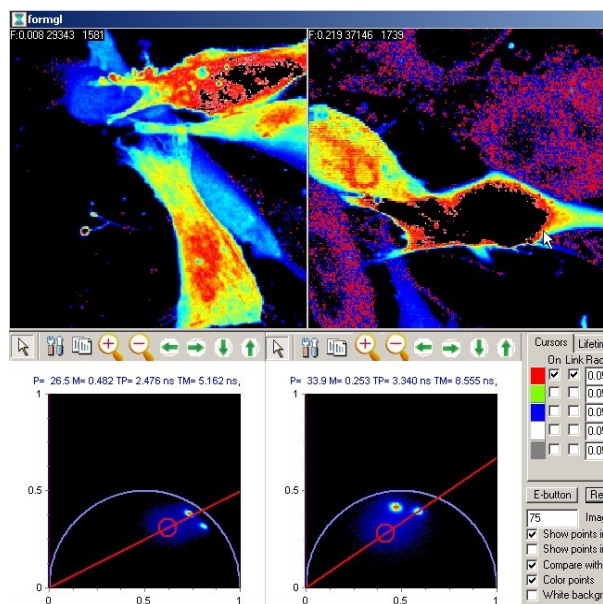


Image threshold = 75



General comments

- Be aware that the reference sample should have a good signal to background, signal to autofluorescence and signal to noise ratio.
- Record the reference with exactly the same settings as the image to be analysed, best just before or after the final measurement. Laser intensity settings may be adapted in order to achieve a suitable count rate, but they should not be changed using the laser driver (PDL) power setting.
- General information about the SimFCS usage can be found on the "Globals" website <http://www.lfd.uci.edu/globals/>

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