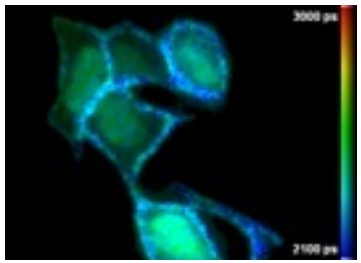
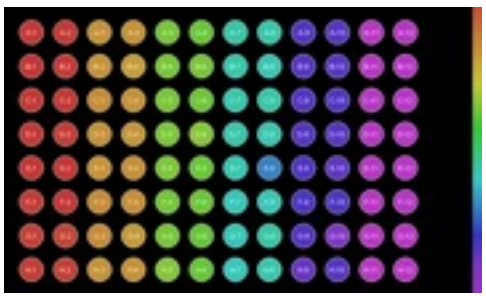
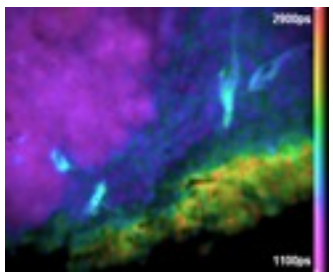
Imperial College London

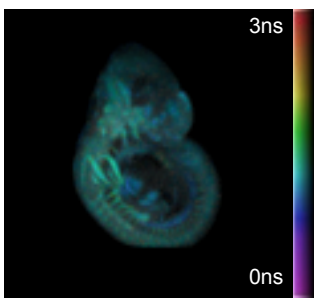
Fluorescence Lifetime Imaging (FLIM) and OMERO.



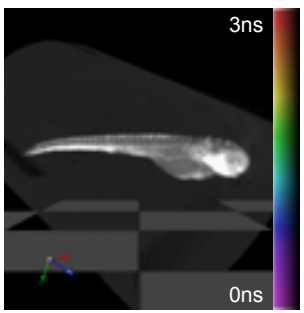


Yuriy Alexandrov
Sean Warren
Ian Munro
Chris Dunsby
Paul French



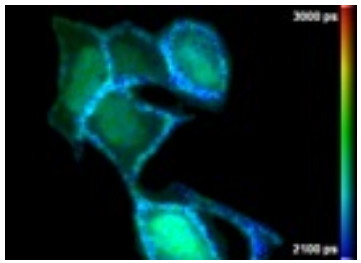


Photonics Group,
Physics Department
Imperial College London

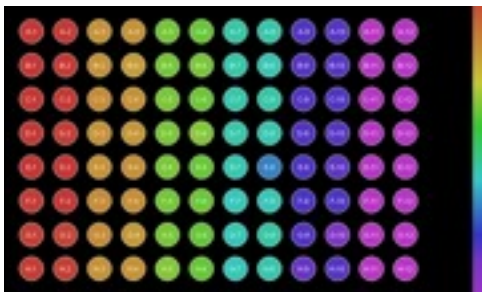


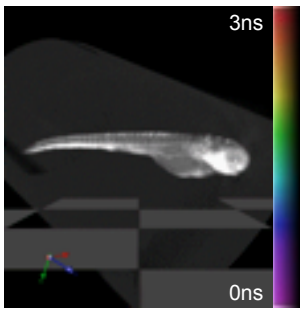
Imperial College London

- **Introduction to our data**
  - Time-Domain FLIM (TCSPC & time-gated)
  - Förster Resonant Energy Transfer (FRET)
  - *Multi-dimensional data (x y Z C T relative-time)*
  - *Time-lapse, multiwell plate, z-stack*

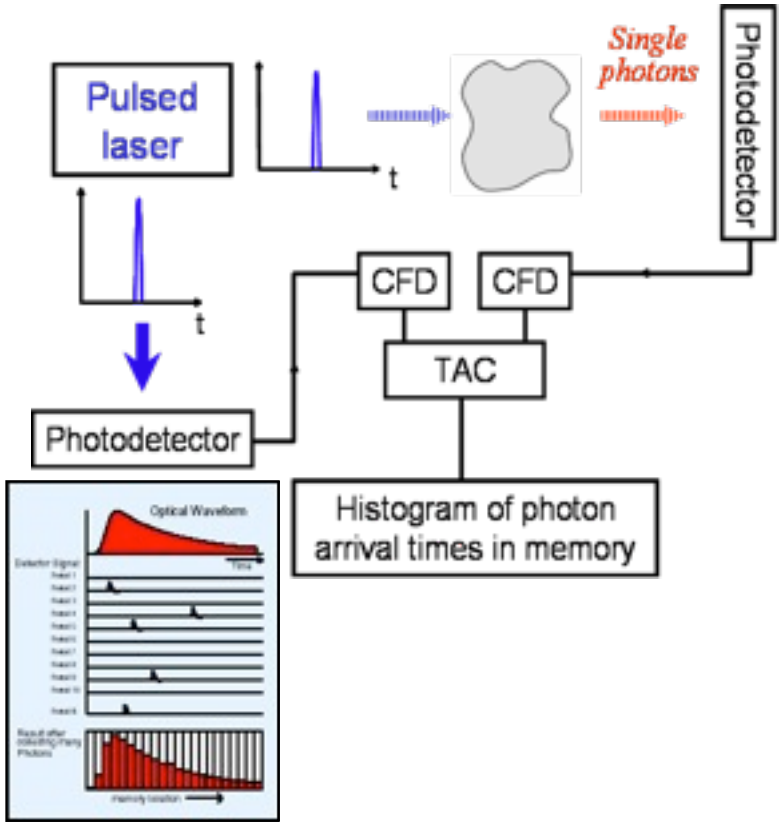


- **FLIMfit**
  - *Open source tool to Fit TCSPC & time-gated FLIM data*
  - *Integrated with OMERO*

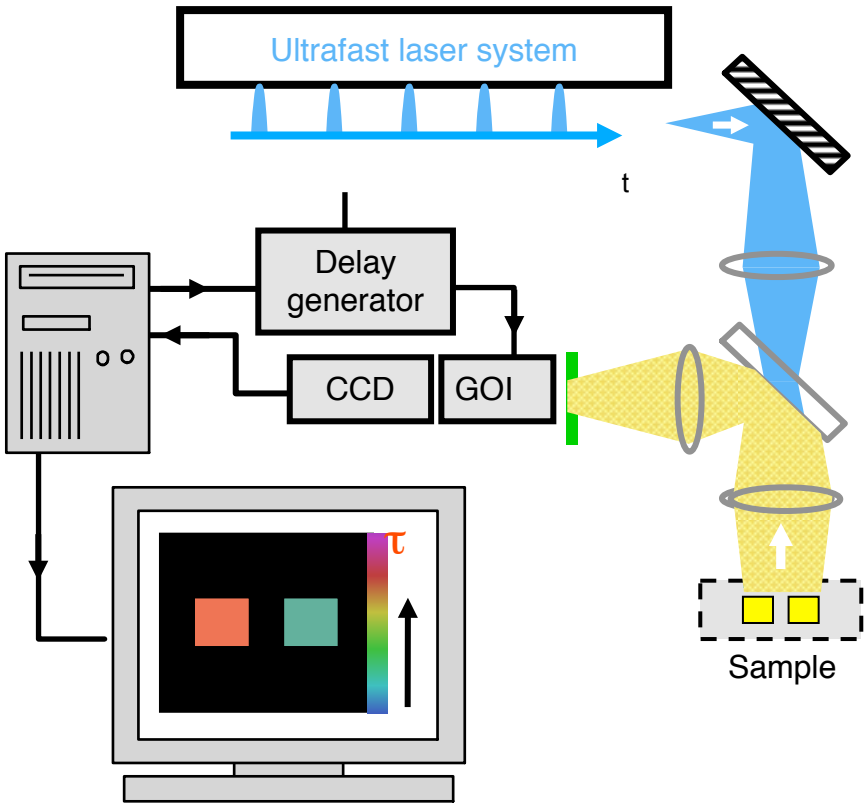




(Time domain) FLIM technology

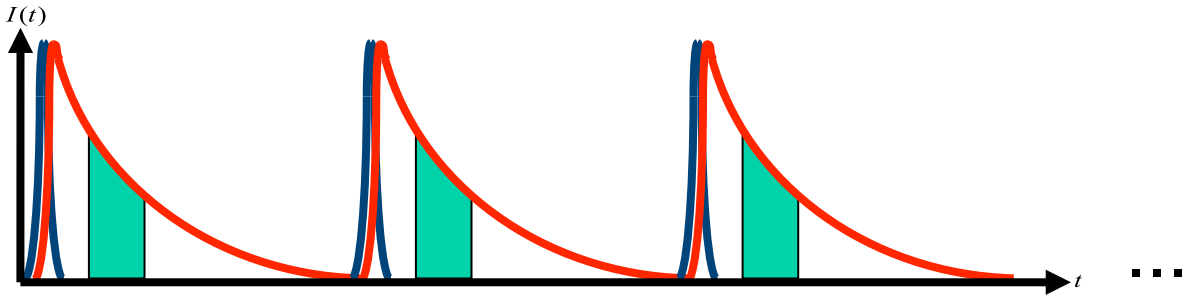


Confocal TCSPC



Wide-field time-gated imaging

Time-gated FLIM: Gated optical intensifier



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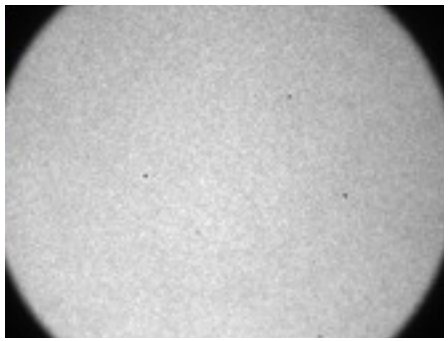

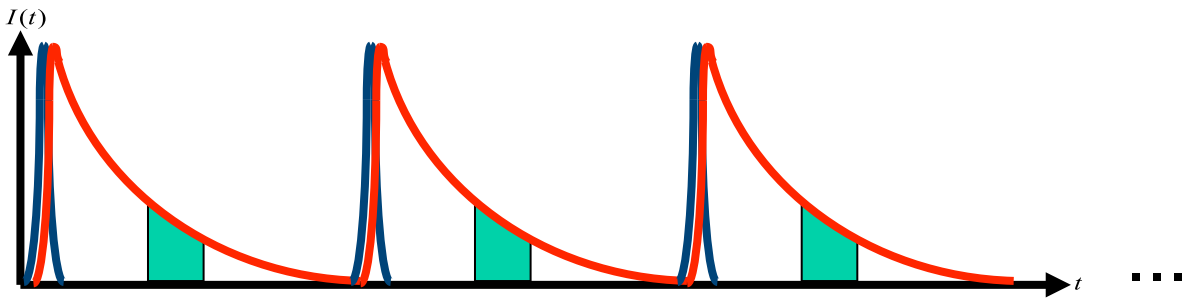

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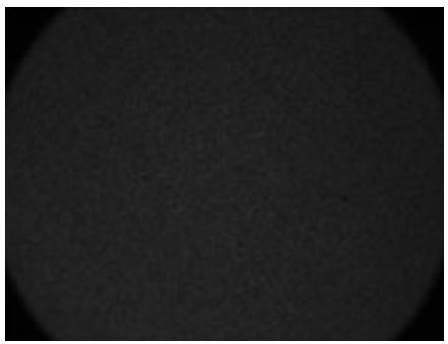
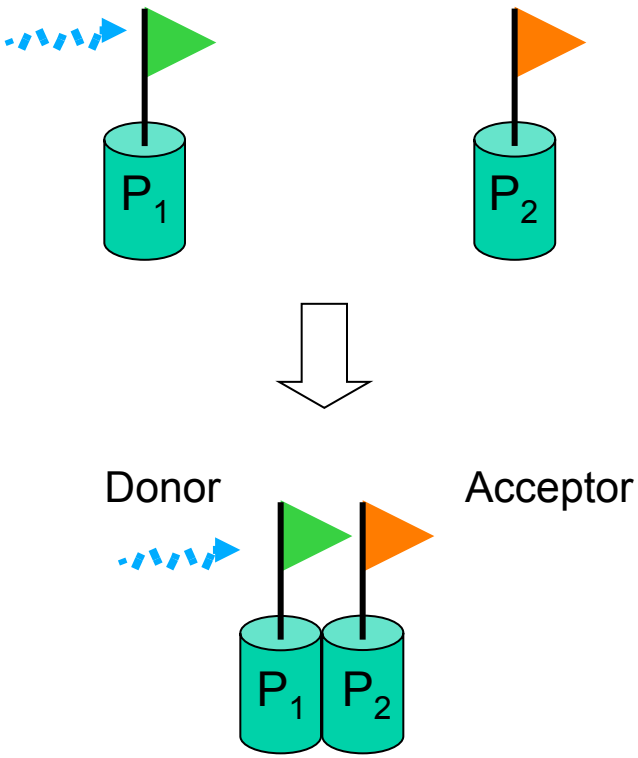


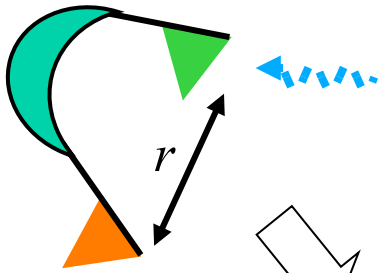
FLIM FRET to read out interactions & dynamics

Förster Resonant Energy Transfer between fluorescent molecules over short (< 20 nm) distances : *dipole-dipole interaction (NO PHOTONS)*

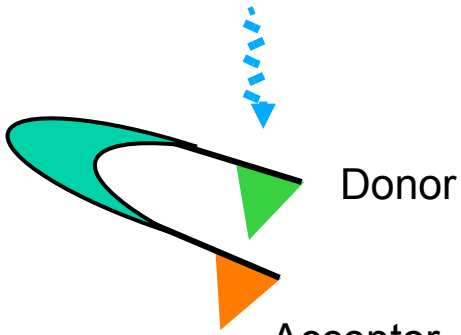
e.g. protein binding

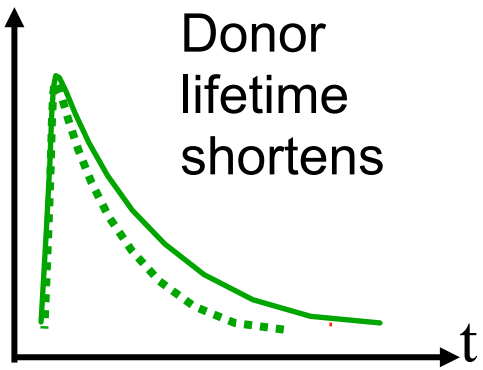


e.g. change in conformation



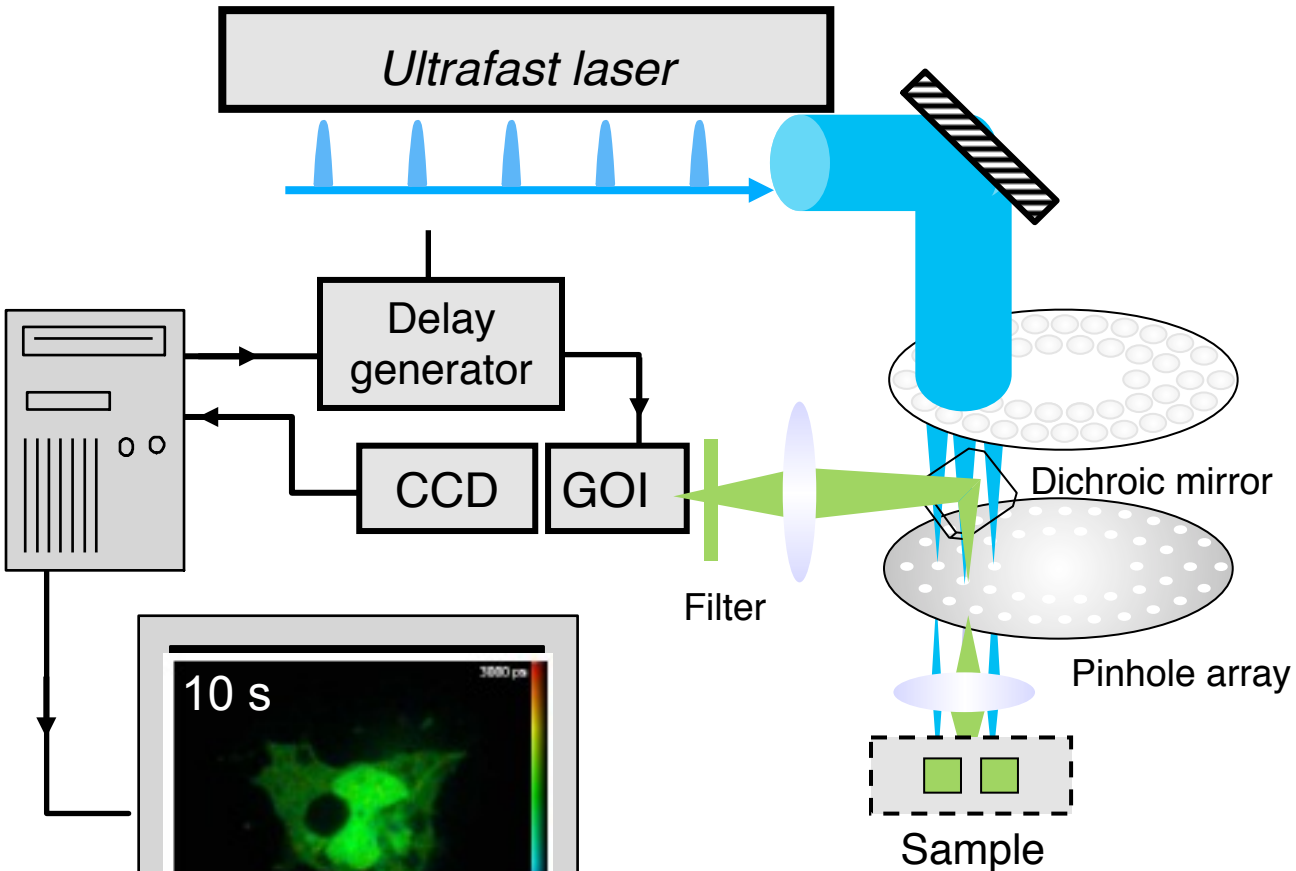
$$r = R_0 \left({}^{1}\!/\!_{\eta_{FRET}} - 1 \right)^{1/6}$$



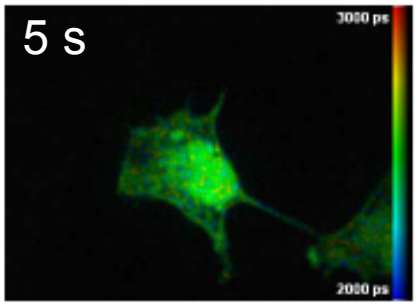


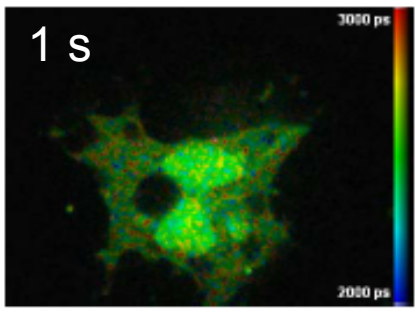
$$I = I_0 \left(\alpha e^{-{}^{t}\!/\!_{\tau_{DA}}} + (1-\alpha) e^{-{}^{t}\!/\!_{\tau_D}} \right) \qquad \eta_{FRET} = 1 - {}^{\tau_{DA}}\!/\!_{\tau_D}$$

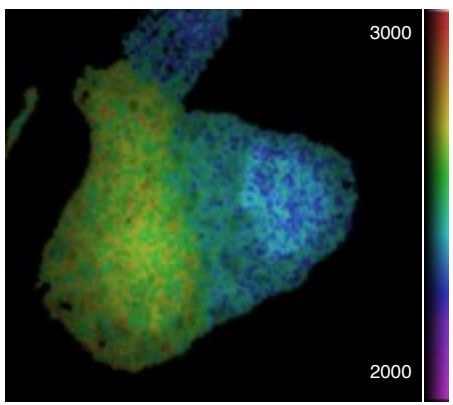
Wide-field optically-sectioned FLIM



FLIM of live COS cells with EGFP

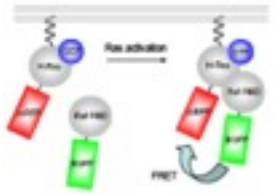






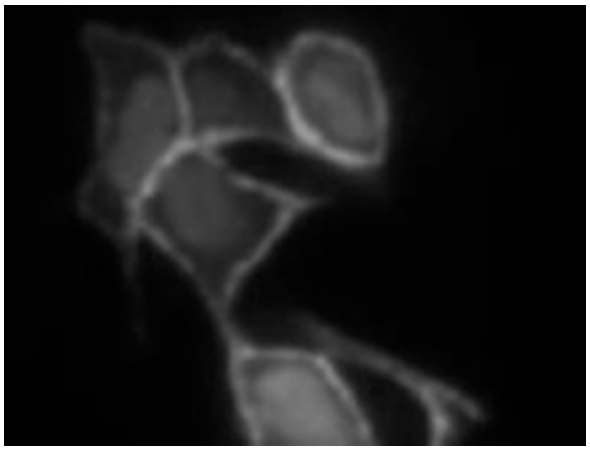
Live cell FLIM/FRET of EGFP and EGFP-mRFP imaged at 5 frames/s:

Nipkow FLIM-FRET of Raf RBD/Ras-mRFP

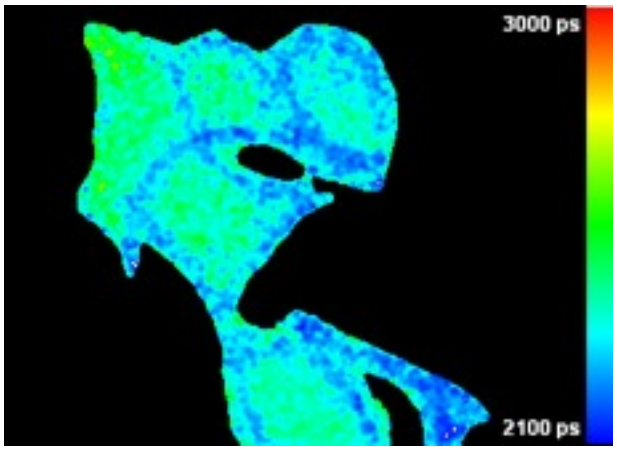


Imaged after 10 minutes stimulation with EGF (5 s FLIM acquisition)

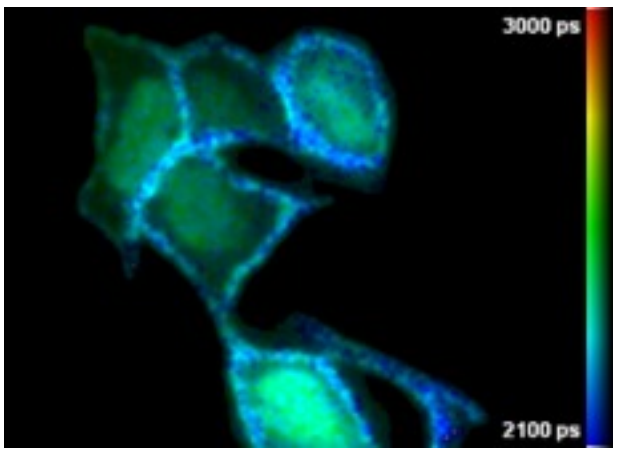
Raf-RBD-EGFP



Intensity image



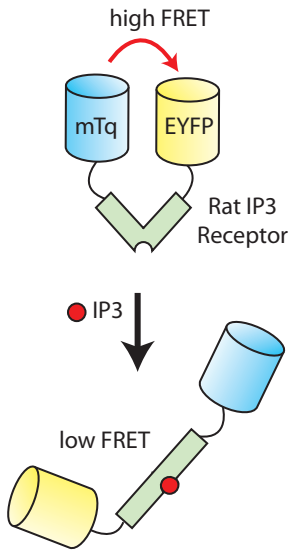
FLIM of Raf-RBD-EGFP



(RBD = Ras Binding Domain)

Grant et al. Opt. Exp. (2007)

Time-lapse FLIM-FRET of [IP_3] in MEFs experiencing a PDGF gradient



MEF expressing mTqFP-YFP "LIBRA" biosensor

⇒ IP_3 oscillations observed

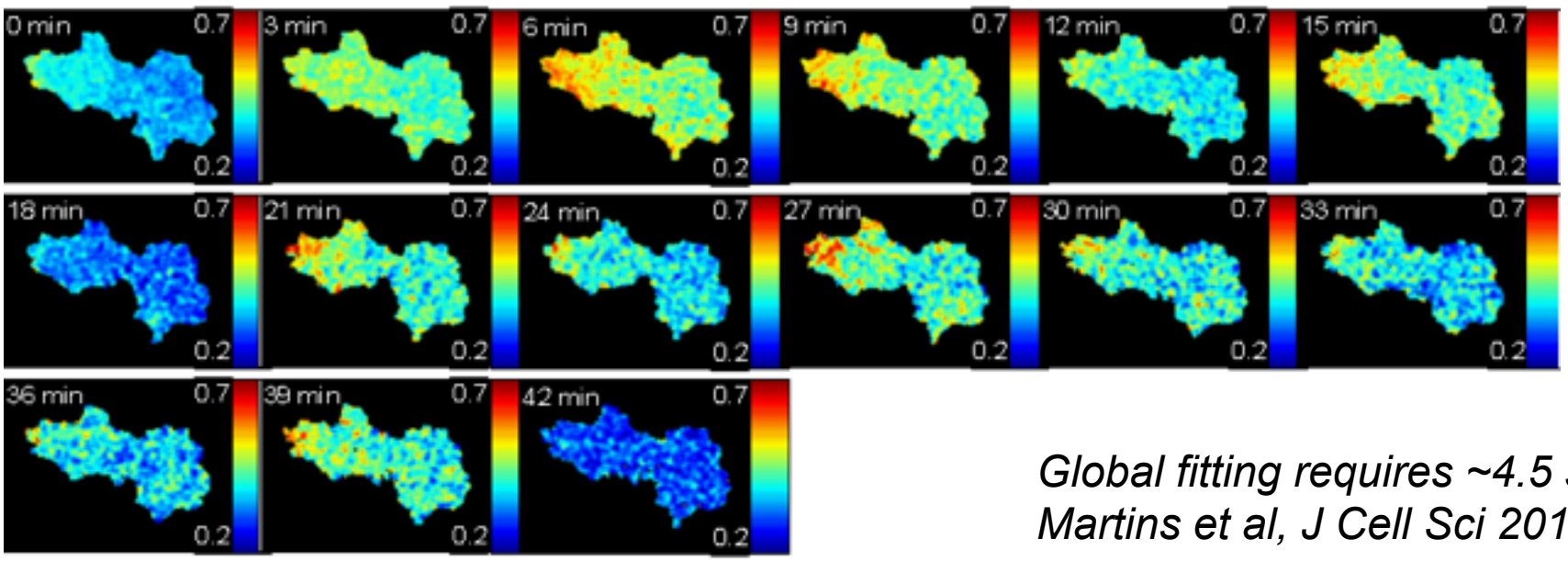
⇒ higher IP_3 concentration in direction of stimulation

Use global fitting across series to biexponential decay model

$$I_{GFP} = I_0\left(\alpha e^{-t/\tau_1} + (1-\alpha)e^{-t/\tau_2}\right)$$

$\tau_{1\ (\text{low FRET})} = 3.2$ ns

$\tau_{2\ (\textit{high FRET})} = 0.78$ ns



Global fitting requires ~4.5 s
Martins et al, J Cell Sci 2012

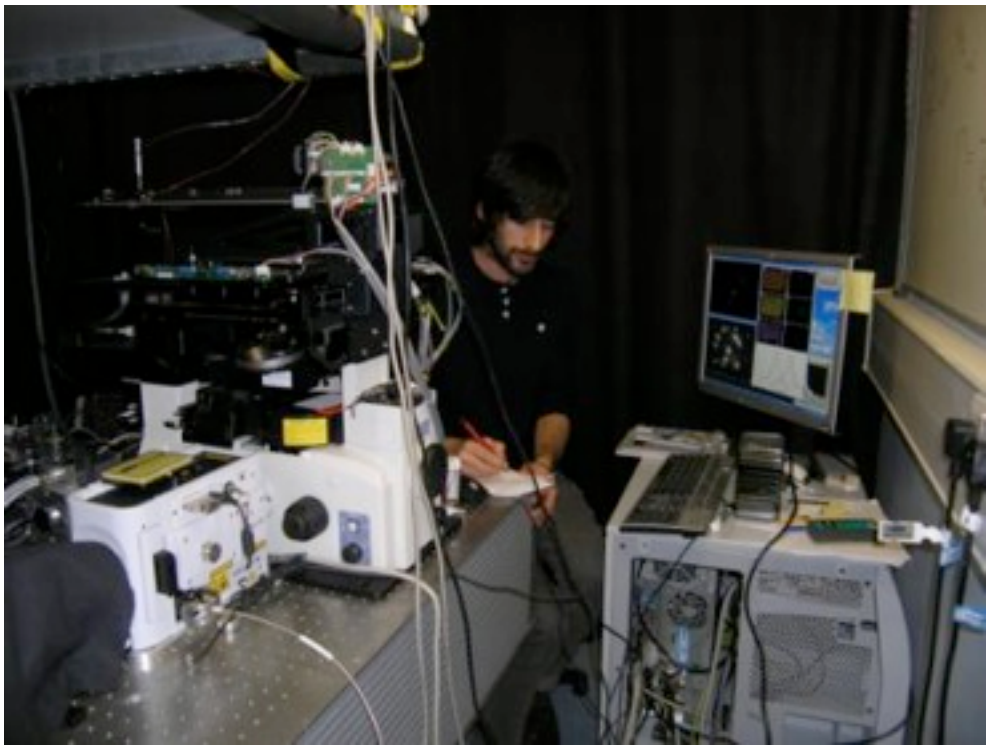
TSB project: prototype FLIM multiwell plate reader

(based on GE Healthcare IN Cell 1000)

Established wide-field multiwell plate reader

+ *Yokogawa CSU-X (more efficient)*
+ *wide-field time-gating*
+ *supercontinuum excitation source*
+ *FLIM/segmentation analysis*
+ *prescan mode*

⇒ <~15 min/96 well plate for FLIM of FP-labelled live cells

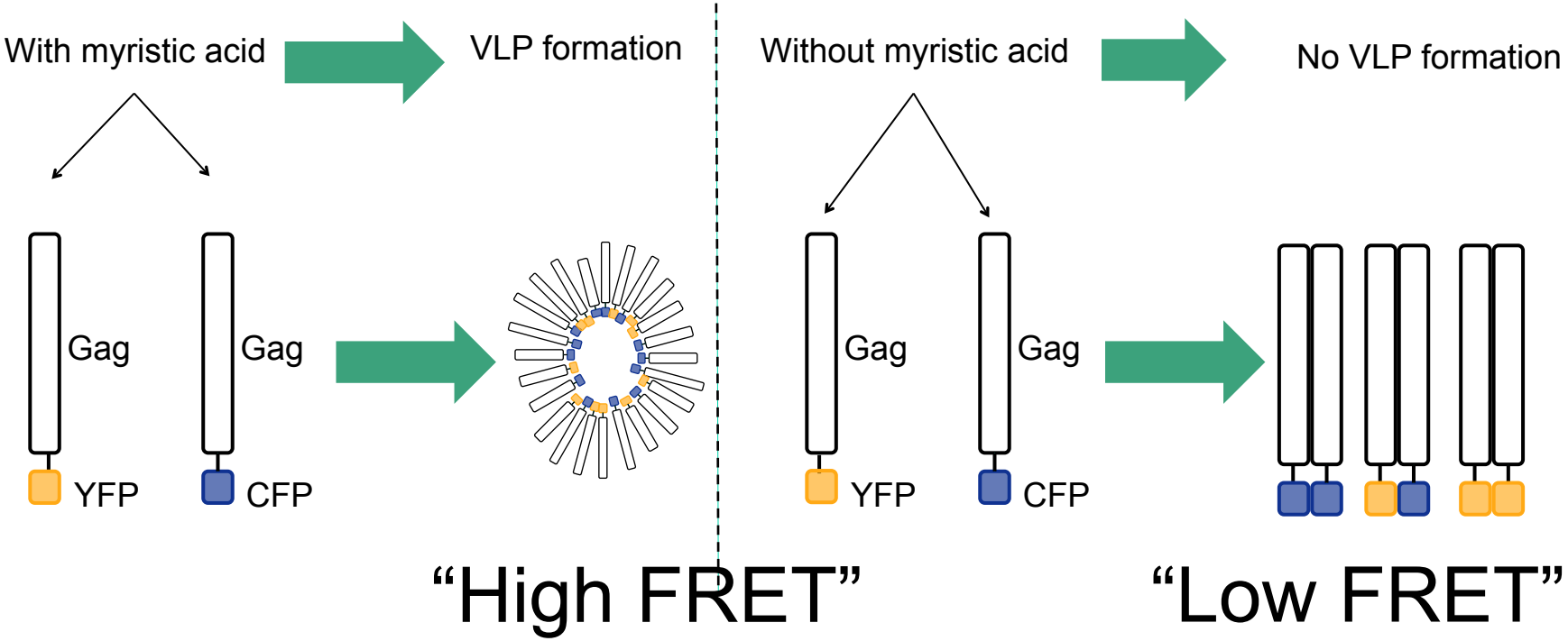


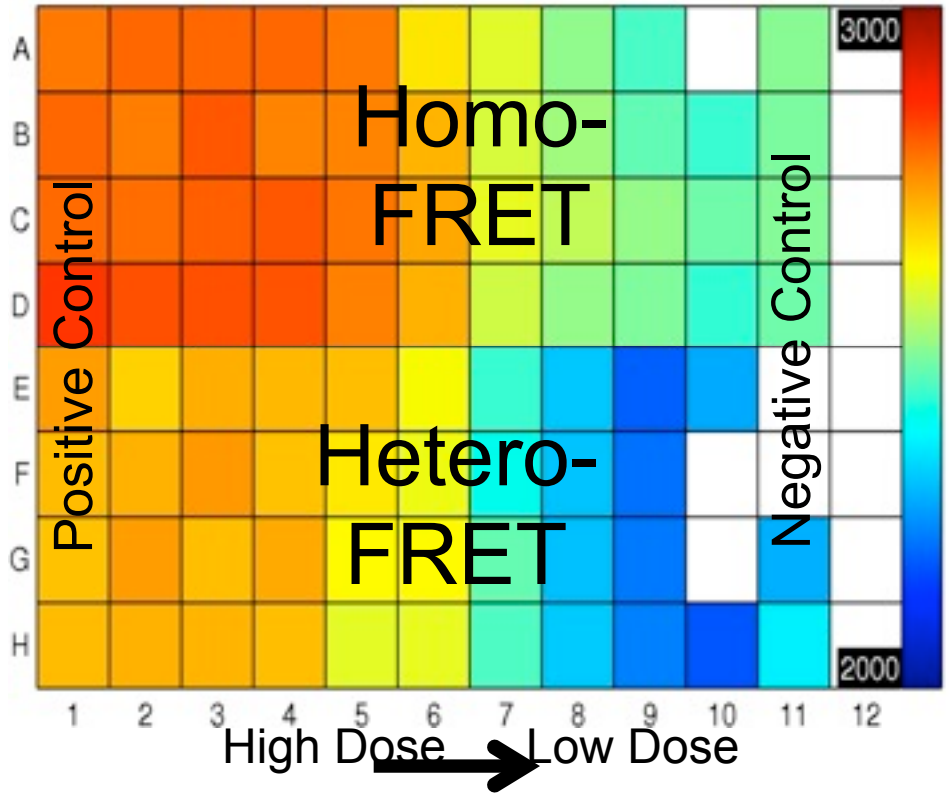
Kumar et al. ChemPhysChem (2011)

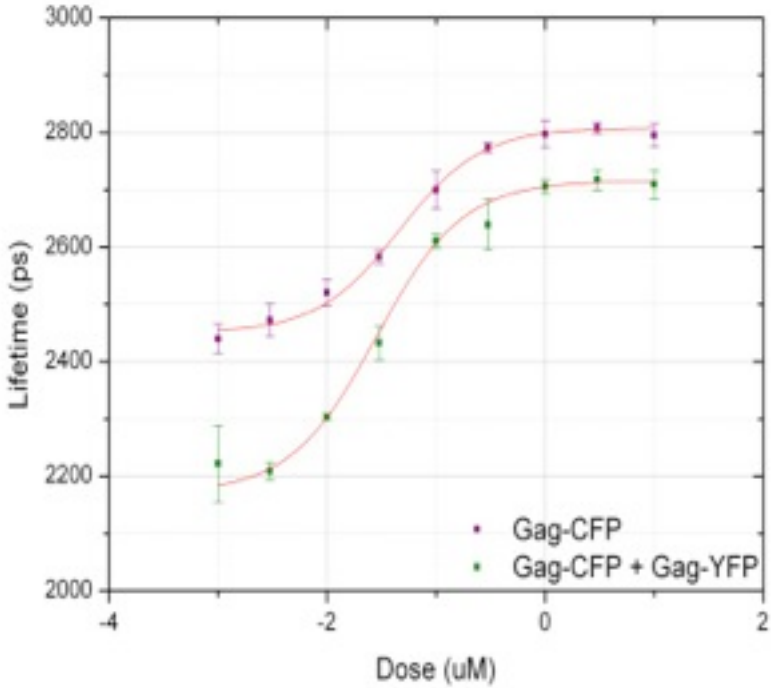
HIV-1 Gag Assay: Fluorescently labelled Gag protein

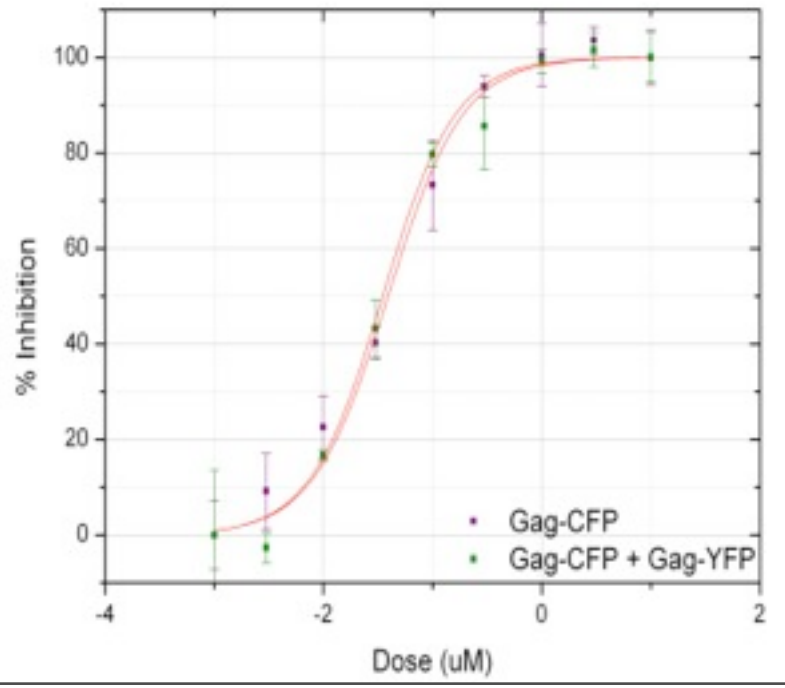
- HIV-1 Gag proteins are dependent on a 'myristic switch' mechanism for membrane binding

- Removal of the first N-terminal glycine residue removes the ability for myristoylation → no VLP formation should take place









- Assay performance measured by Z' parameter:

$$Z' = 1 - \left(\frac{3 * [\sigma_{pos} + \sigma_{neg}]}{\mu_{pos} - \mu_{neg}} \right)$$

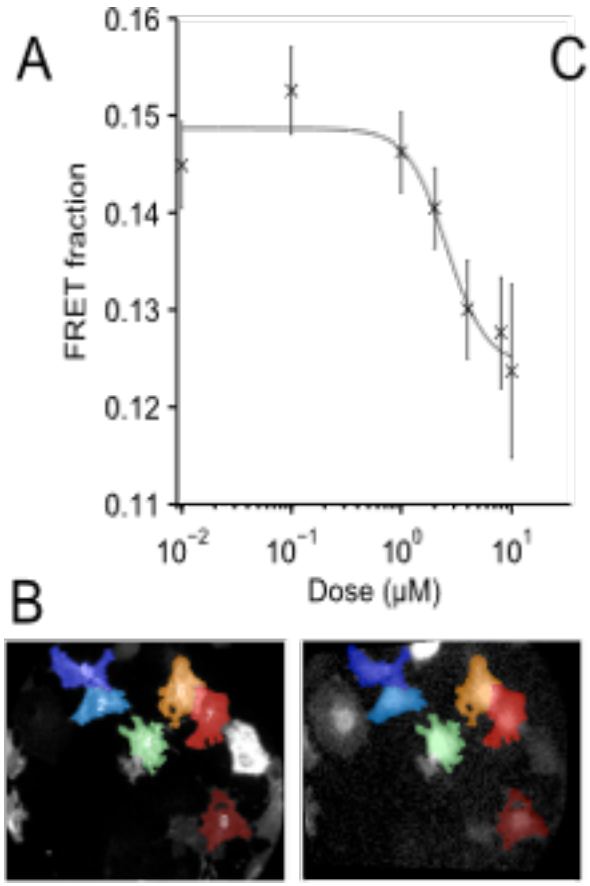
$Z'_{\text{Homo-FRET}} = 0.63$

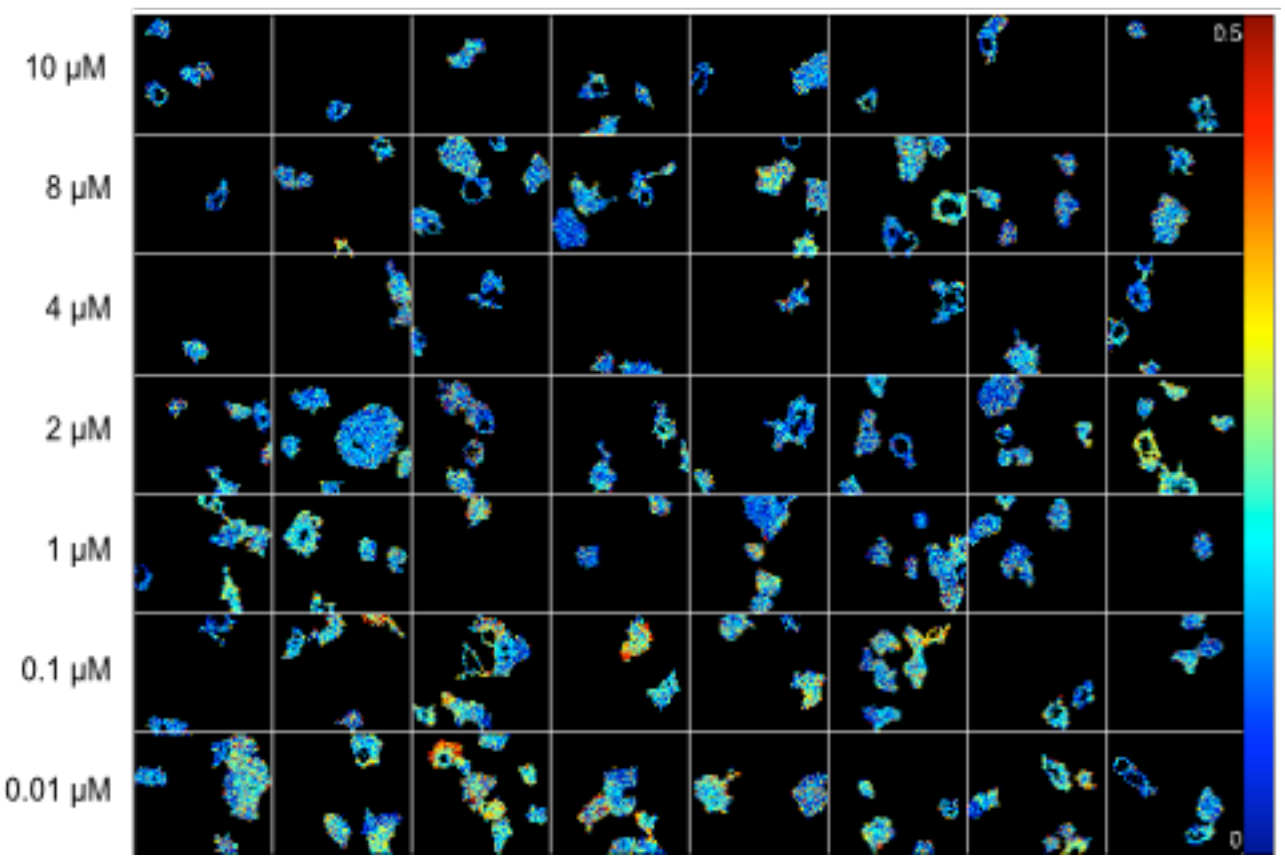
$Z'_{\text{Hetero-FRET}} = 0.45$

Global a**nalysis of FLIM data**

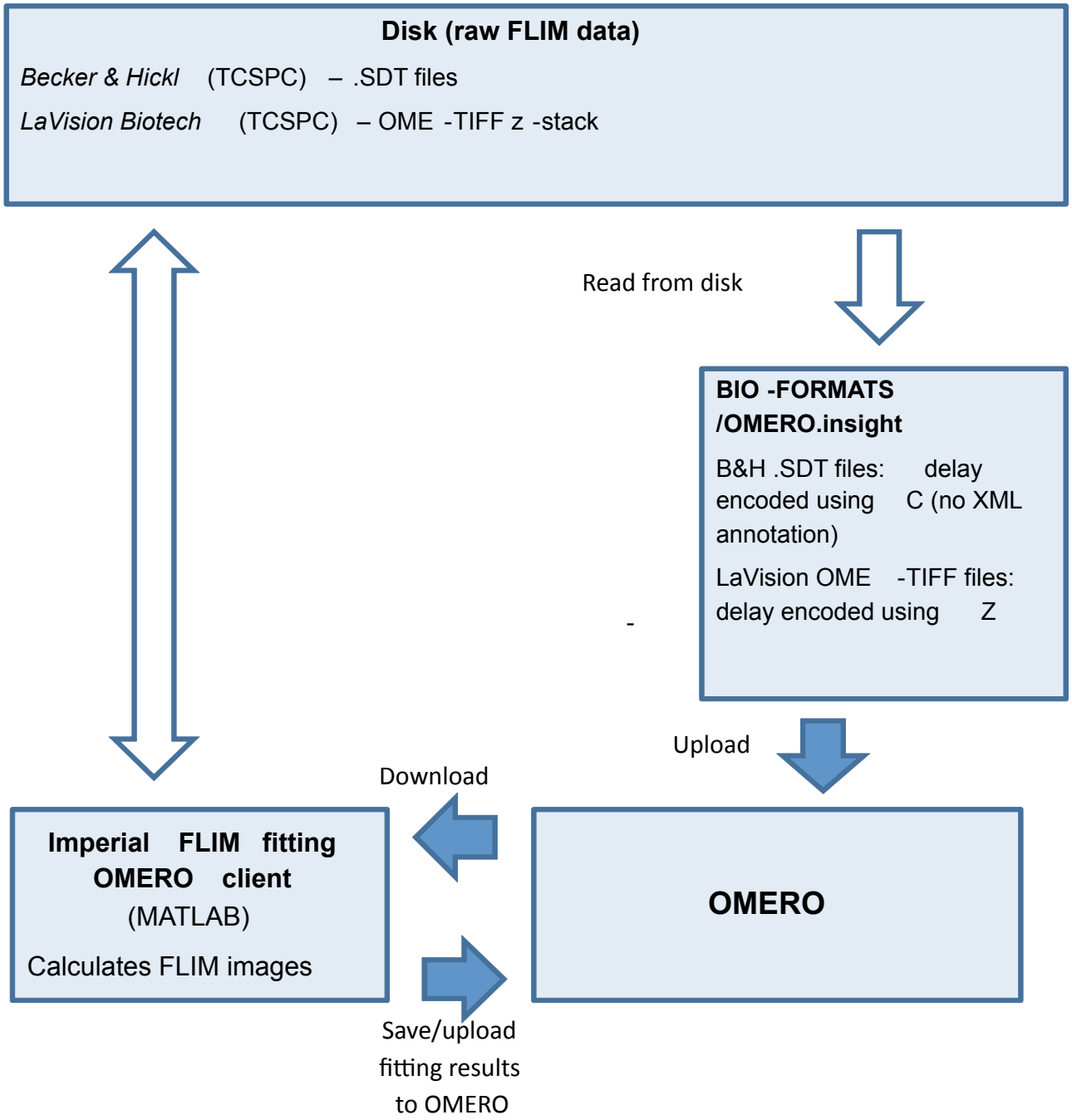
Global fitting enables complex decay models (e.g. for FRET) with modest (100's) photon numbers/pixel

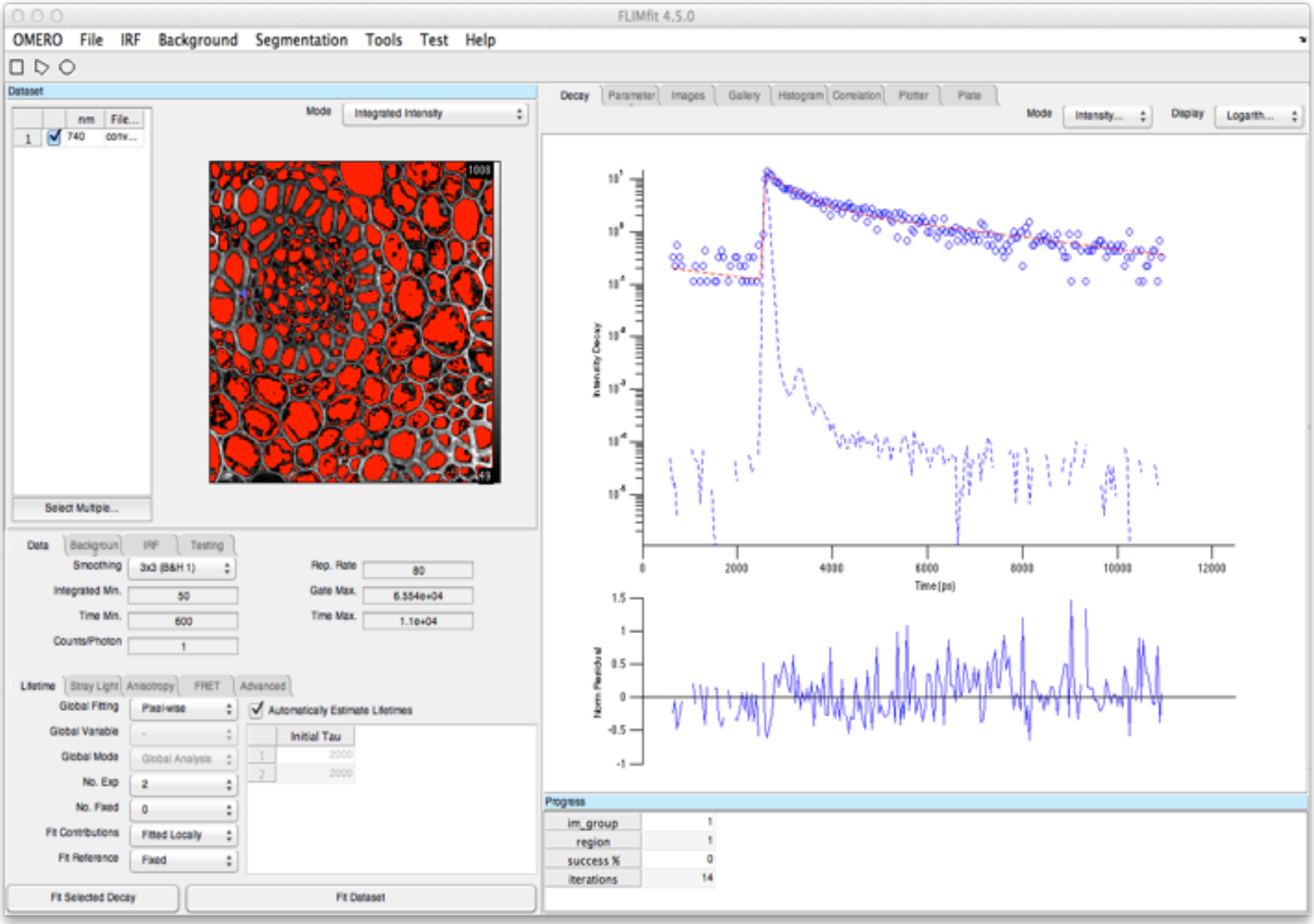
e.g. assay of inhibitor IPA-3 on interaction between Rac1 and p21-activated kinase read out with mTurquoise FLAIR biosensor in COS-7 cells stimulated with EGF





FLIMfit: an OMERO client in MATLAB/C++




FLIMfit 4.5.0
OMERO File IRF Background Segmentation Tools Test Help
Dataset
nm File...
1 740 conv...
Mode Integrated Intensity
Select Multiple...
Data Background IRF Testing
Smoothing 3x3 (B&H 1)
Integrated Min. 50
Time Min. 600
Counts/Photon 1
Rep. Rate 80
Gate Max. 6.554e+04
Time Max. 1.1e+04
Lifetime Stray Light Anisotropy FRET Advanced
Global Fitting Pixel-wise
Global Variable -
Global Mode Global Analysis
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No. Fixed 0
Fit Contributions Fitted Locally
Fit Reference Fixed
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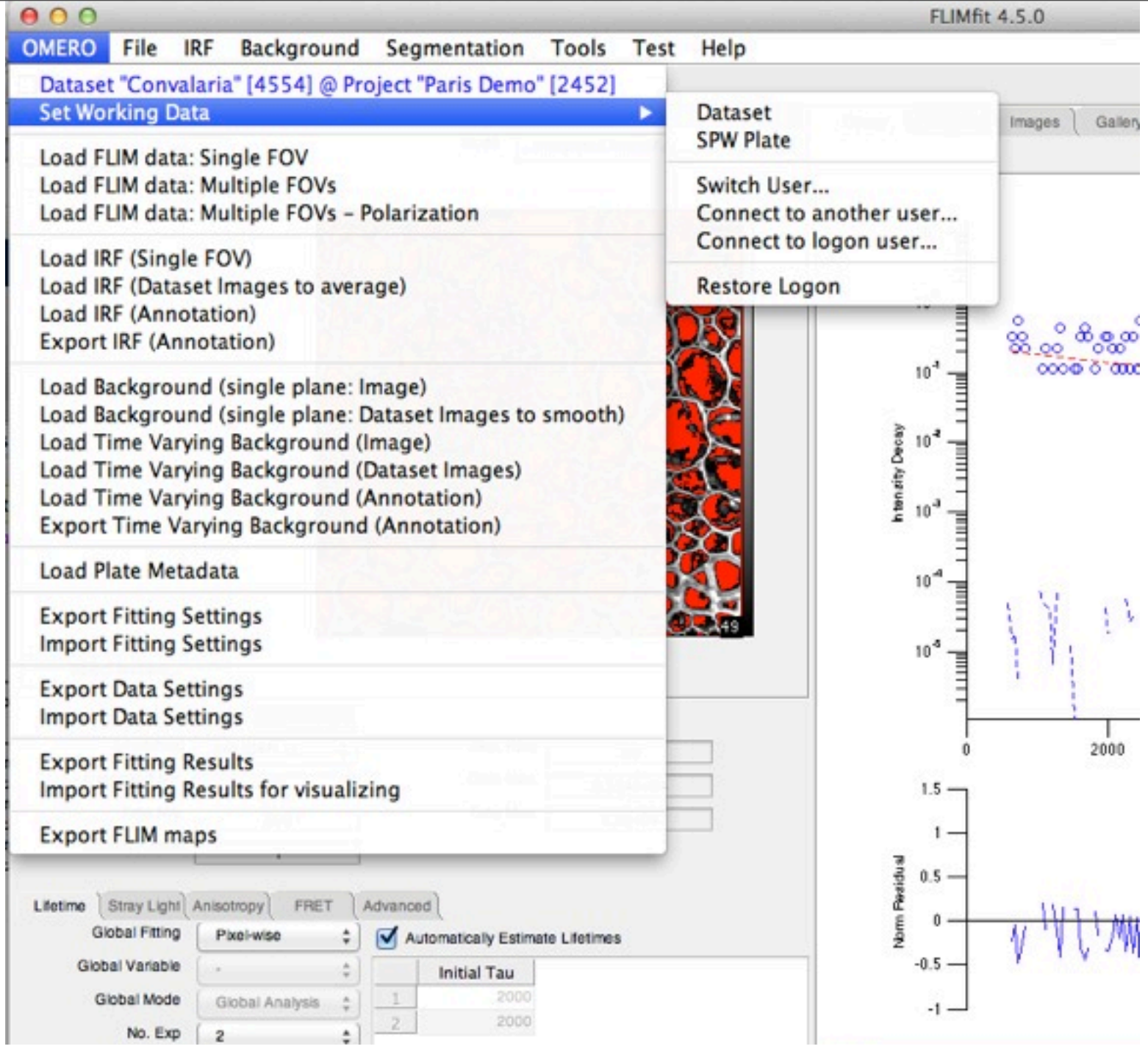
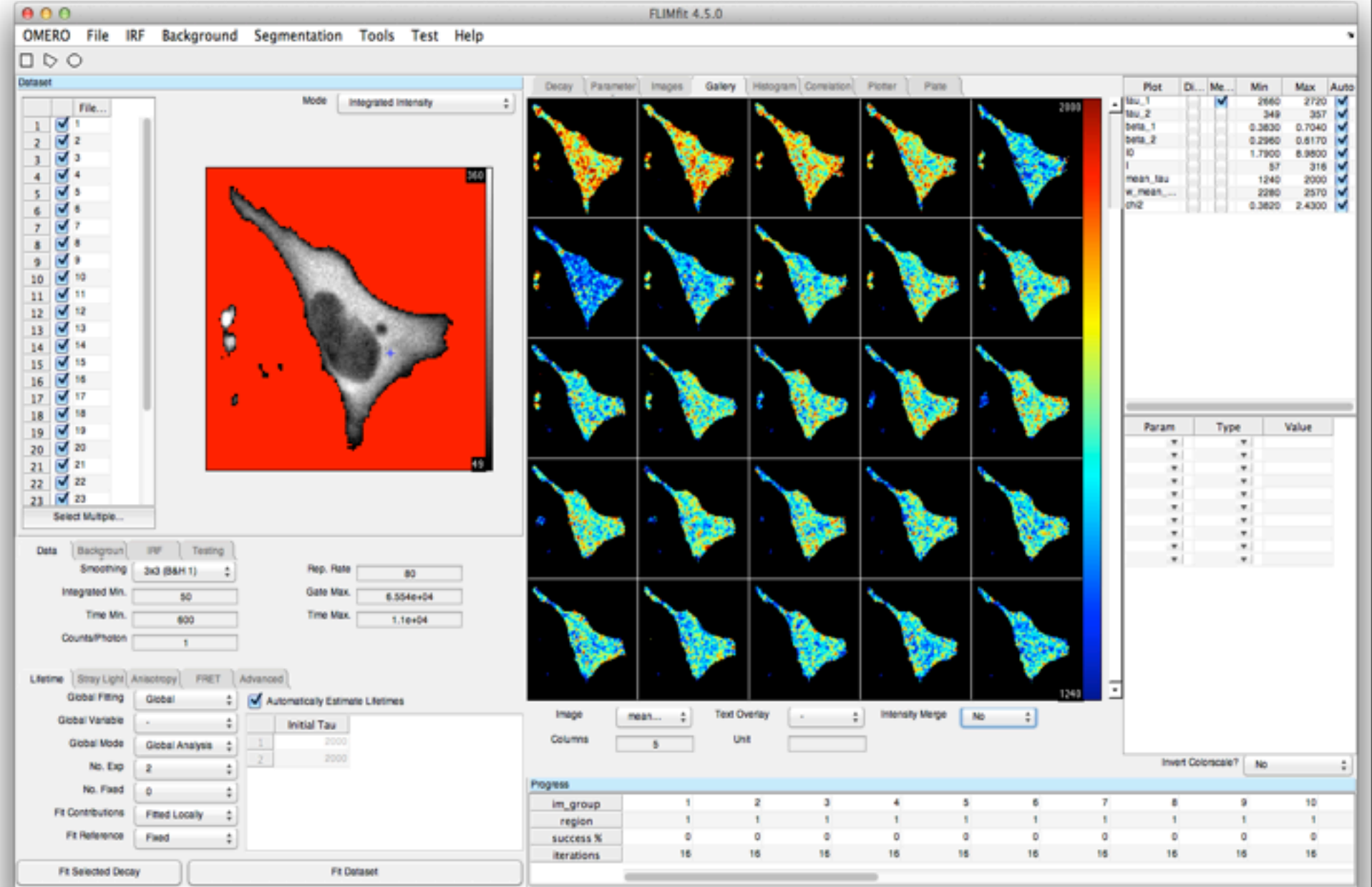
iterations 14


FLIMfit 4.5.0
OMERO
File
IRF
Background
Segmentation
Tools
Test
Help
Dataset "Convalaria" [4554] @ Project "Paris Demo" [2452]
Set Working Data
Load FLIM data: Single FOV
Load FLIM data: Multiple FOVs
Load FLIM data: Multiple FOVs - Polarization
Load IRF (Single FOV)
Load IRF (Dataset Images to average)
Load IRF (Annotation)
Export IRF (Annotation)
Load Background (single plane: Image)
Load Background (single plane: Dataset Images to smooth)
Load Time Varying Background (Image)
Load Time Varying Background (Dataset Images)
Load Time Varying Background (Annotation)
Export Time Varying Background (Annotation)
Load Plate Metadata
Export Fitting Settings
Import Fitting Settings
Export Data Settings
Import Data Settings
Export Fitting Results
Import Fitting Results for visualizing
Export FLIM maps
Dataset
SPW Plate
Switch User...
Connect to another user...
Connect to logon user...
Restore Logon
Images
Gallery
Intensity Decay
Norm Residual
Lifetime
Stray Light
Anisotropy
FRET
Advanced
Global Fitting
Pixel-wise
Automatically Estimate Lifetimes
Global Variable
Global Mode
Global Analysis
No. Exp
Initial Tau


FLIMfit 4.5.0
OMERO File IRF Background Segmentation Tools Test Help
Dataset
Mode
Integrated Intensity
Select Multiple...
Data Background IRF Testing
Smoothing
3x3 (B&H 1)
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Time Min.
600
Counts/Photon
1
Rep. Rate
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Gate Max.
6.554e+04
Time Max.
1.1e+04
Lifetime Stray Light Anisotropy FRET Advanced
Global Fitting
Global
Global Variable
Global Mode
Global Analysis
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Fit Contributions
Fitted Locally
Fit Reference
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Automatically Estimate Lifetimes
Initial Tau
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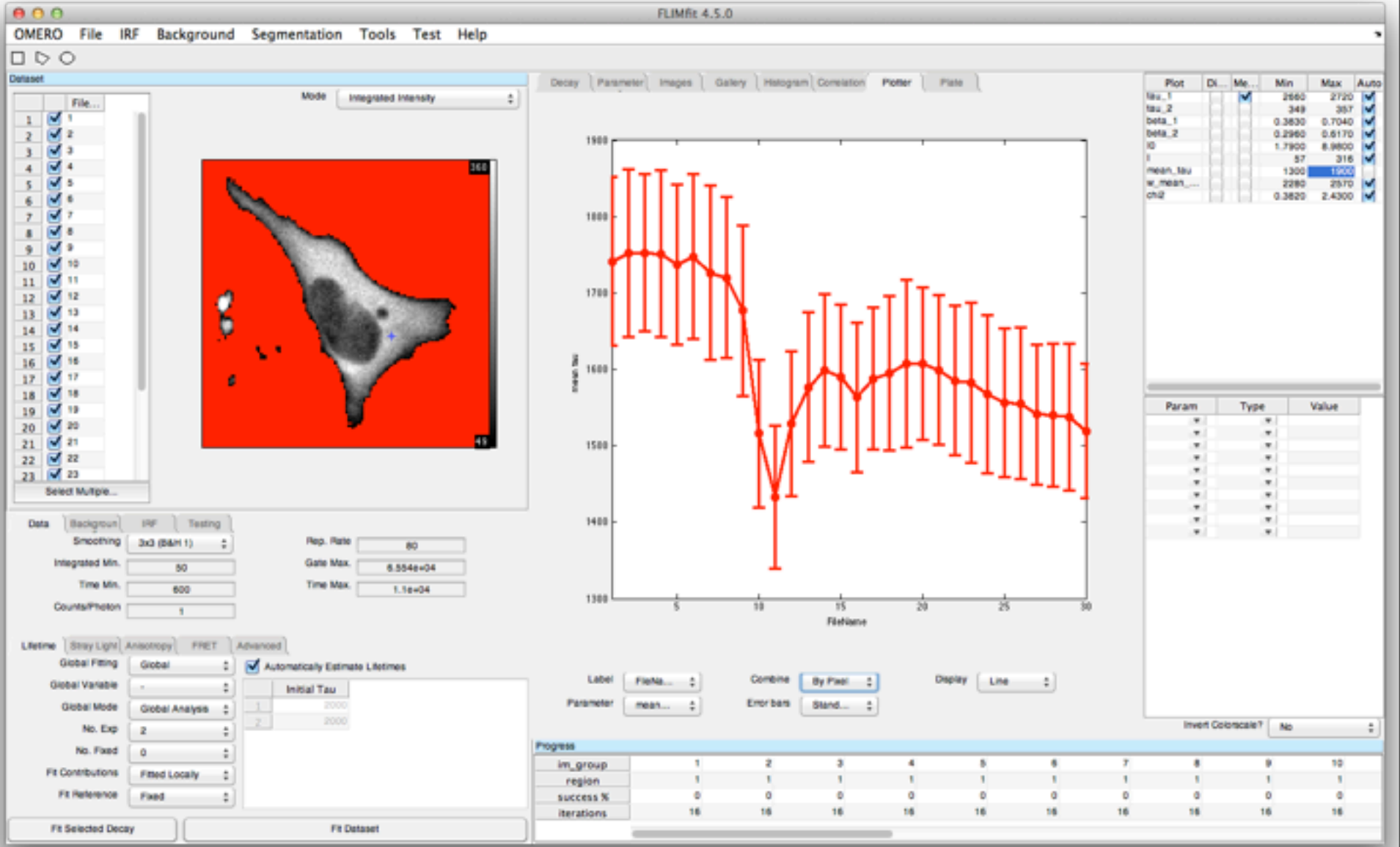
tau_1 2660 2720
tau_2 349 357
beta_1 0.3830 0.7040
beta_2 0.2960 0.6170
I0 1.7900 6.9800
I 57 316
mean_tau 1240 2000
w_mean_... 2280 2570
chi2 0.3620 2.4300
Param Type Value
Invert Colorscale?
No
Progress
im_group 1 2 3 4 5 6 7 8 9 10
region 1 1 1 1 1 1 1 1 1 1
success % 0 0 0 0 0 0 0 0 0 0
iterations 16 16 16 16 16 16 16 16 16 16


FLIMfit 4.5.0
OMERO File IRF Background Segmentation Tools Test Help
Dataset
Mode
Integrated Intensity
Select Multiple...
Data Background IRF Testing
Smoothing
3x3 (B&H 1)
Integrated Min.
50
Time Min.
600
Counts/Photon
1
Rep. Rate
80
Gate Max.
6.554e+04
Time Max.
1.1e+04
Lifetime Stray Light Anisotropy FRET Advanced
Global Fitting
Global
Global Variable
Global Mode
Global Analysis
No. Exp
2
No. Fixed
0
Fit Contributions
Fitted Locally
Fit Reference
Fixed
Automatically Estimate Lifetimes
Initial Tau
Fit Selected Decay
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Decay Parameter Images Gallery Histogram Correlation Plotter Plate
mean tau
FileName
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Parameter
Combine
By Pixel
Error bars
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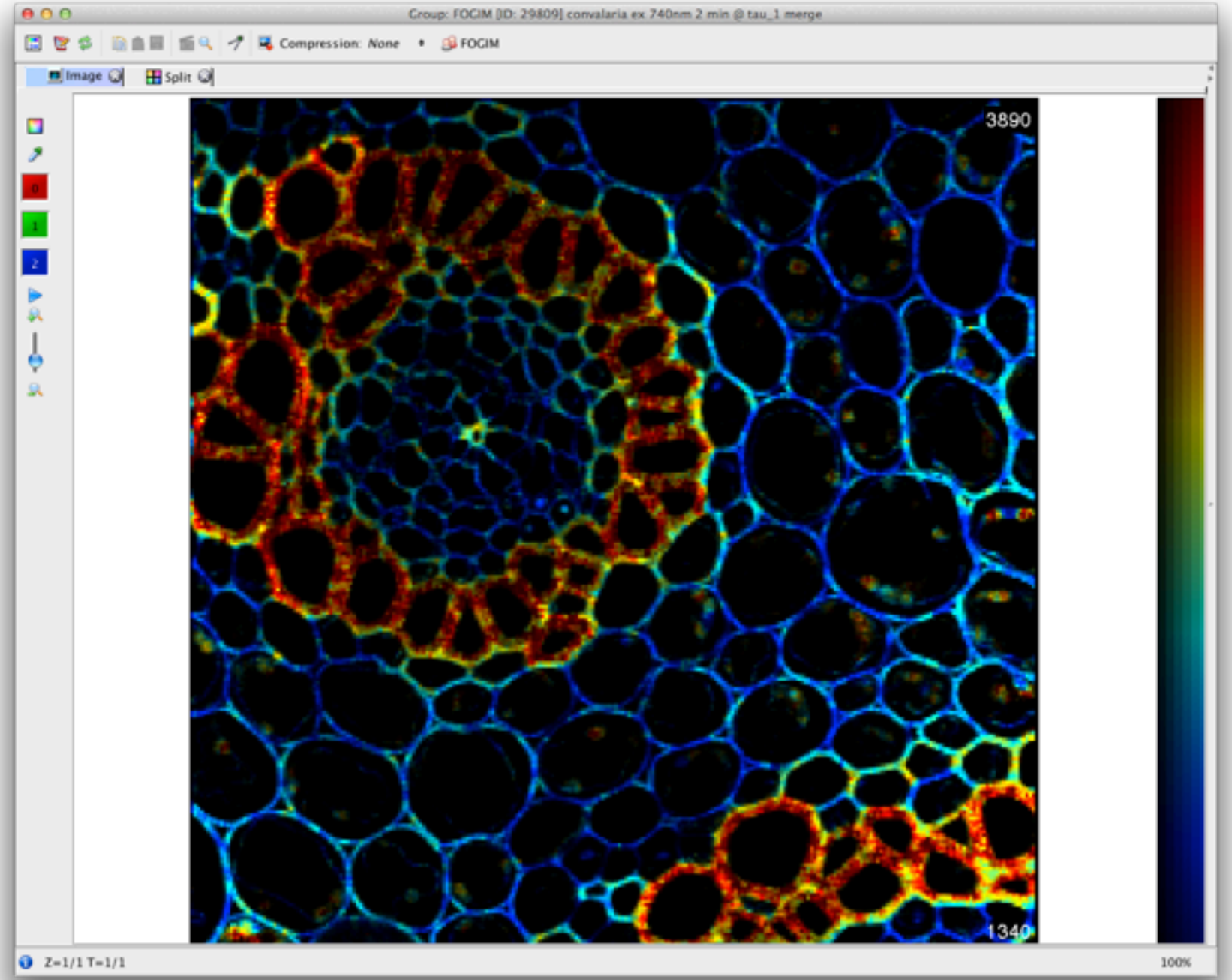
tau_1 2660 2720
tau_2 349 357
beta_1 0.3830 0.7040
beta_2 0.2960 0.6170
I0 1.7900 8.9800
I 57 316
mean_tau 1300 1900
w_mean_... 2280 2570
chi2 0.3820 2.4300
Param Type Value
Invert Colorscale?
No
Progress
im_group 1 2 3 4 5 6 7 8 9 10
region 1 1 1 1 1 1 1 1 1 1
success % 0 0 0 0 0 0 0 0 0 0
iterations 16 16 16 16 16 16 16 16 16 16


Group: FOGIM [ID: 29809] convalaria ex 740nm 2 min @ tau_1 merge
Compression: None
FOGIM
Image
Split
3890
1340
Z=1/1 T=1/1
100%

FLIMfit: an OMERO client

Sean Warren + …

Open Source, cross-platform

Two time-domain FLIM modalities (TCSPC, Gated)

Multi-threaded

Global Analysis

Two forms of IRF (scatterer & reference fluorophore)

Support for simultaneous analysis of large datasets (100's images)

Supports time-varying background (TVB)

Maximum likelihood option

PLoS One : Rapid global fitting of large fluorescence lifetime imaging microscopy datasets S.C. Warren, A. Margineanu, D. Alibhai, D.J. Kelly, C. Talbot, Y. Alexandrov, I. Munro, M. Katan, C. Dunsby*, P.M.W. French*

http://www.openmicroscopy.org/site/products/partner/flimfit

https://github.com/openmicroscopy/Imperial-FLIMfit

Multidimensional fluorescence imaging

Imperial College London

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