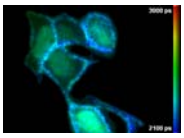
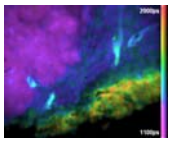
OMERO →

Imperial College London

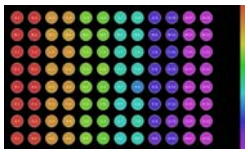
Fitting FLIM data in OMERO and sharing it

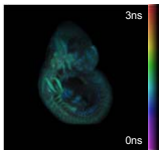


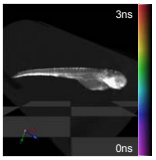


Yuriy Alexandrov
Ian Munro
Chris Dunsby
Paul French

Photonics Group,
Physics Department
Imperial College London



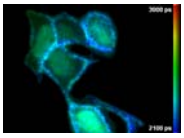


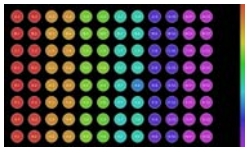


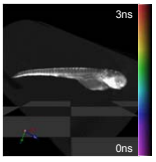
Fitting FLIM data in OMERO and sharing it

Imperial College London

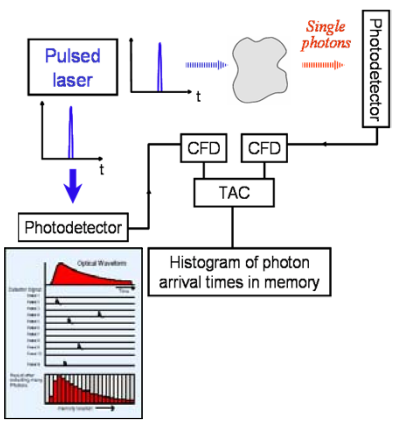
- **Introduction to our data**
  - *FLIM & FRET*
  - *Time-lapse, multiwell plate, OPT, DFT, clinical ..*
- ***FLIMfit***
  - *Fit TCSPC & time-gated FLIM data (Sean Warren)*
  - *Integrated with OMERO*
  - *ICimporter for time-gated multiwell plate FLIM*
- **Public sharing of OMERO (FLIM) data**
  - *How?*
  - *Imperial proposal …*

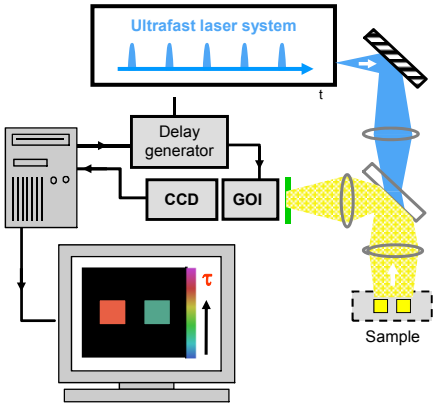






(Time domain) FLIM technology





Confocal/MP TCSPC

Highest accuracy/photon

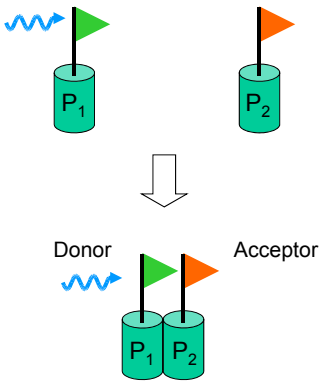
Wide-field time-gated imaging

Highest accuracy/second

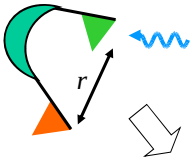
FLIM FRET to read out interactions & dynamics

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

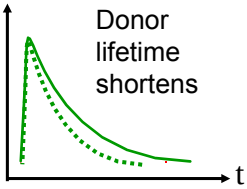
e.g. protein binding

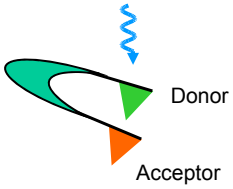


e.g. change in conformation



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

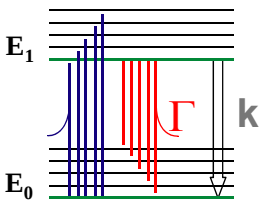




$$I = I_0\left(\alpha e^{-t/\tau_{DA}} + (1-\alpha)e^{-t/\tau_D}\right) \qquad \eta_{FRET} = 1 - \tau_{DA}/\tau_D$$

Multidimensional fluorescence imaging

Imperial College London



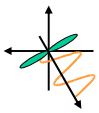
Intensity ~ f{η}, $\eta = \Gamma/(\Gamma+k)$

Wavelength, $\lambda \sim hc/(E_1-E_0)$

Lifetime, $\tau = 1/(\Gamma+k)$

Polarisation (θ)

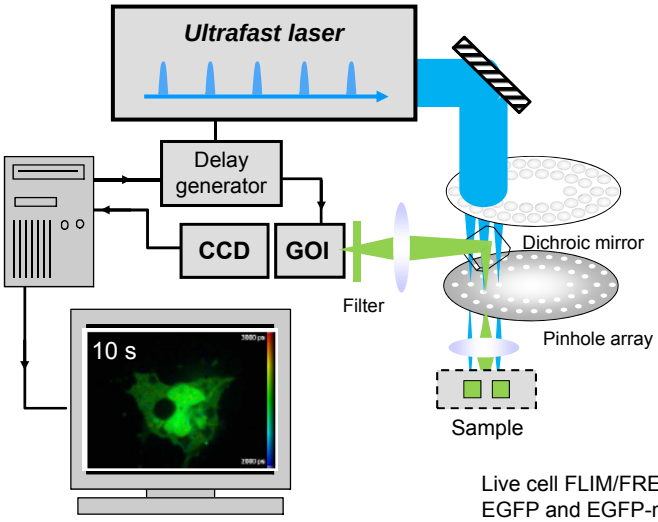
Elapsed time



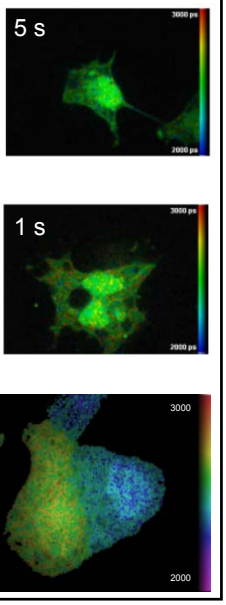
optical molecular readouts

Molecular Biology **Drug Discovery** **Clinical imaging**

Wide-field optically-sectioned FLIM

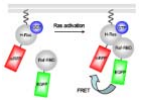


FLIM of live COS cells with EGFP



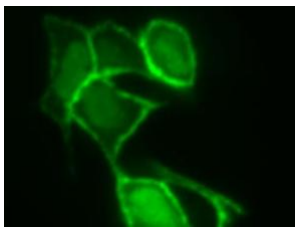
Live cell FLIM/FRET of EGFP and EGFP-mRFP imaged at 5 frames/s:

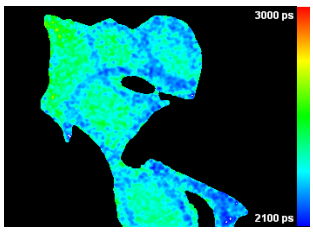
Nipkow FLIM-FRET of Raf RBD/Ras-mRFP



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

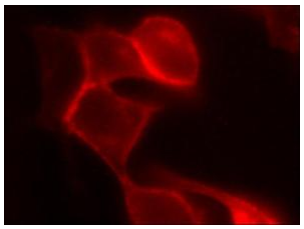
Raf-RBD-EGFP

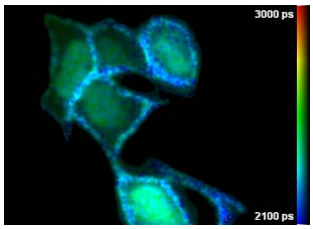




Intensity images **FLIM of Raf-RBD-EGFP**

HRas-mRFP

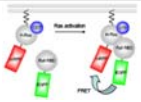




(RBD = Ras Binding Domain)

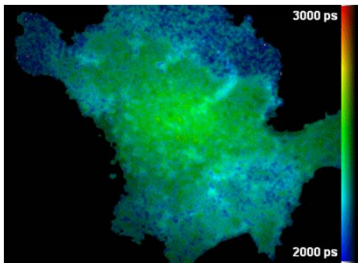
Grant et al. Opt. Exp. (2007)

Nipkow FLIM-FRET of Raf RBD/Ras FRET



High-speed *⇒ 3-D imaging and/or time lapse imaging*

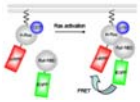
COS 7 cell expressing H-Ras-mRFP and Raf-RBD-EGFP imaged in 100 s after EGF stimulation



Z stack

Grant et al. Opt. Exp. (2007)

Nipkow FLIM-FRET of Raf RBD/Ras FRET



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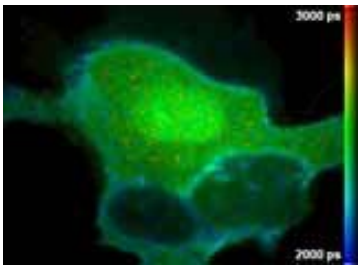
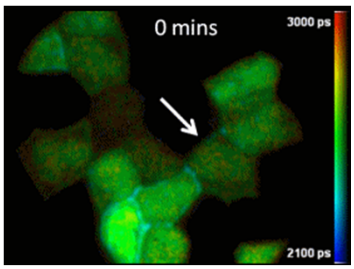


Z stack

HRas-mRFP with Raf-RBD-EGFP in MDCK cells following EGF stimulation

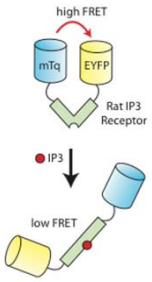


Time lapse

6 s per FLIM image

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

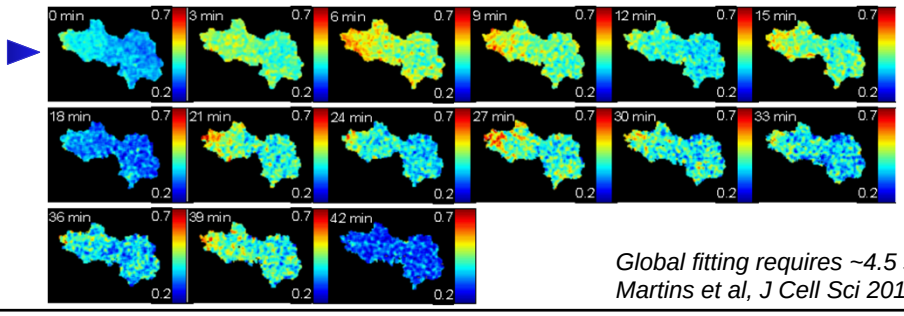
- ⇒ IP_3 oscillations observed
- ⇒ higher IP_3 concentration in direction of stimulation

Use global fitting across series to biexponential decay model

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

τ_1 (low FRET) = 3.2 ns

τ_2 *(high FRET)* = 0.78 ns



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

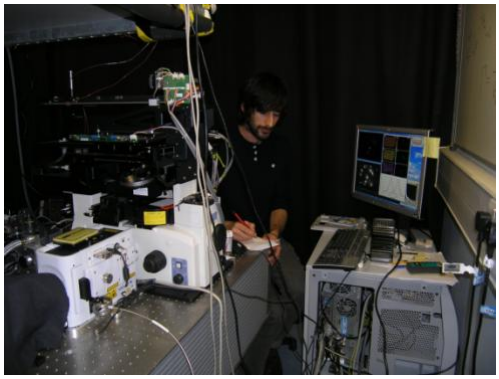
TSB project: prototype FLIM multiwell plate reader

(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***

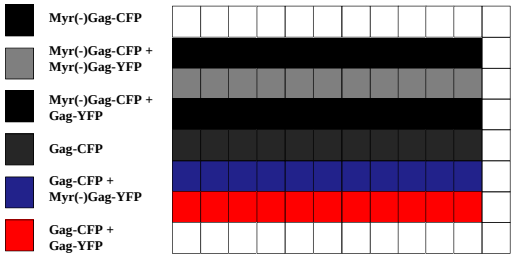
⇒ <~15 min/96 well plate for FLIM of FP-labelled live cells

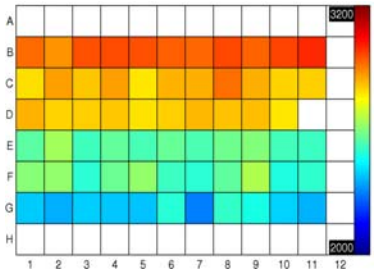


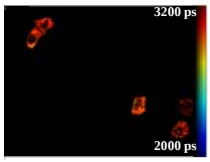
Kumar et al. ChemPhysChem (2011)

Automated FLIM-FRET of Gag oligomerisation

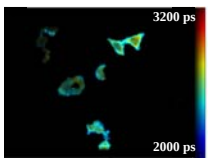
HIV-1 Gag-CFP and Gag-YFP in VLPs in fixed HeLa cells



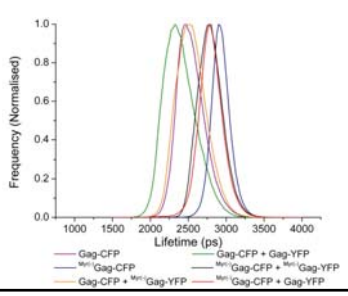




Myr(-)Gag-CFP



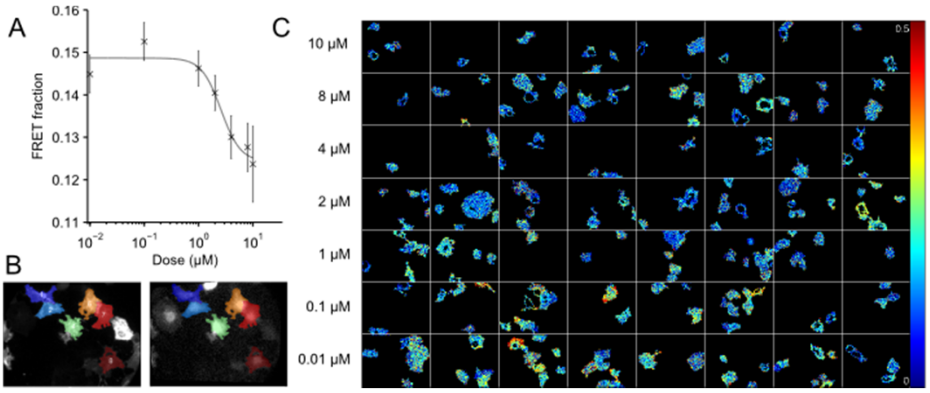
Gag-CFP



Global analysis of FLIM data

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

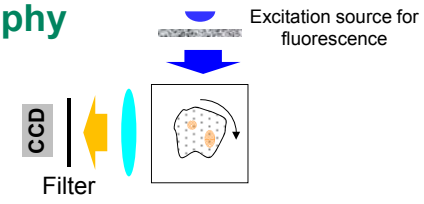
e.g. assay of inhibitor IPA-3 on interaction between Rac1 and p21-activated kinase read out with mTurquoise FLAIR biosensor in COS-7 cells stimulated with EGF



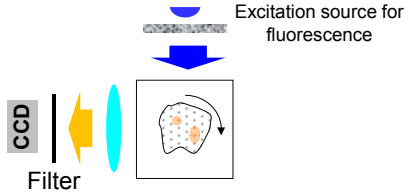
Analysis of 394 FOV took 93 seconds and required 2 GB of memory

Optical Projection Tomography

Sharpe et al, Science (2002)
Optical analogue of X-ray CT
- transparent samples (chemically cleared)
- size <1cm diameter

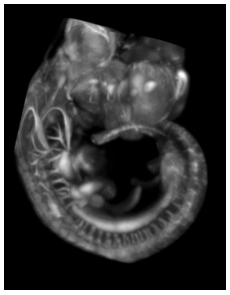


Fluorescence Lifetime OPT

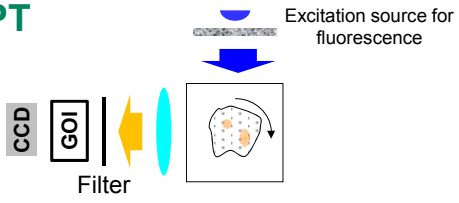


Mouse embryo
neurofilament labelled with
Alexa-488 conjugated antibody

fixed and cleared in BABB



Fluorescence Lifetime OPT



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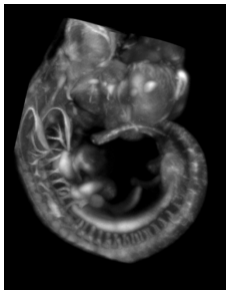
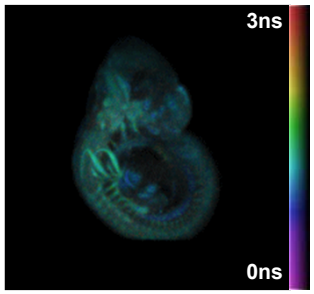
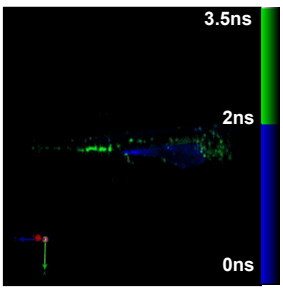




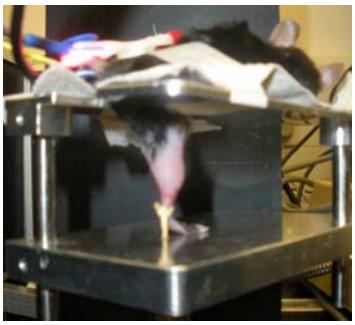
Live Fli-GFP zebrafish



McGinty et al., J. Biophotonics (2008)

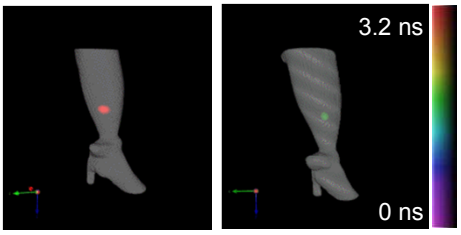
McGinty et al. Biomed Opt Exp (2011)

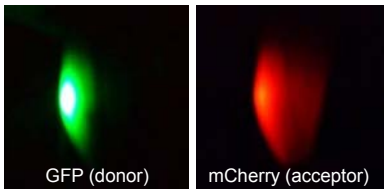
In vivo FLIM FRET tomography



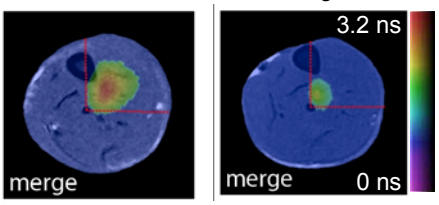
Control
(EGFP, mCherry unlinked)

FRET
(EGFP-mCherry linked)





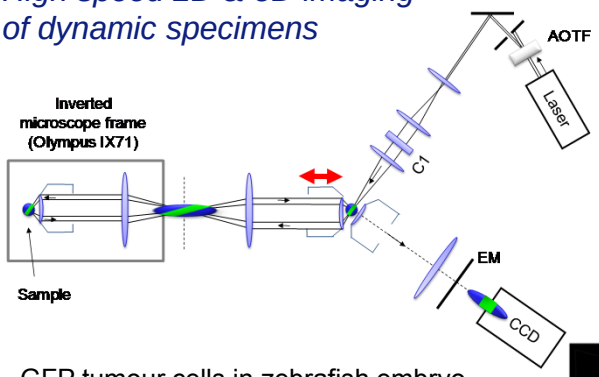
Combined with MRI image



McGinty et al., Biomed Opt Express (2011)

Oblique Plane Microscopy

High speed 2D & 3D imaging of dynamic specimens

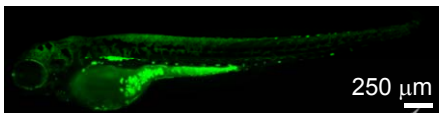


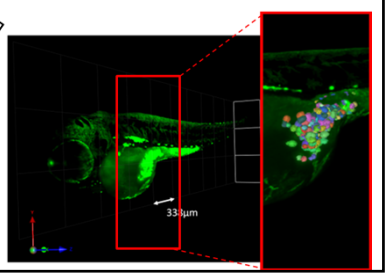
Oblique incidence SPIM with remote focussing

Works with "normal" inverted microscope

Rapid – can image 30 volumes/second

GFP tumour cells in zebrafish embryo





with J. Castle, H. Taylor and M. Dallman

4 Channels
– *ex vivo* images

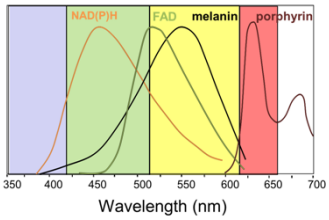
<415nm(ch3)
collagen, **SHG**

415-515nm(ch2)
collagen, elastin, FAD, keratin, melanin
NAD(P)H,

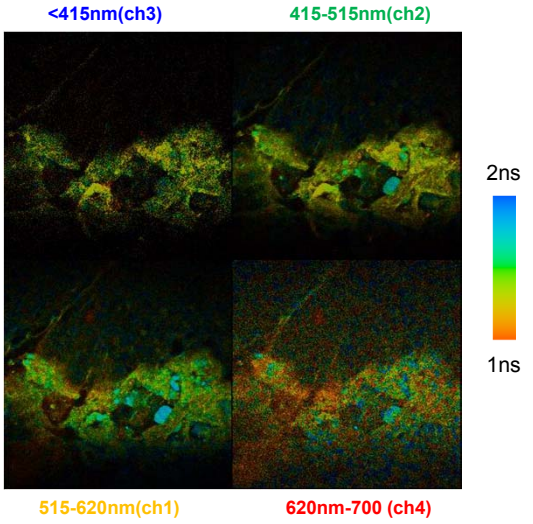
515-620nm(ch1)
elastin FAD, keratin, **melanin**

620nm-700 (ch4)
melanin, porphyrins

Intracellular fluorophores



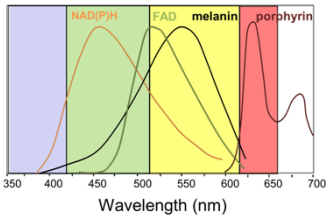
Dysplastic naevus imaged with the multichannel FLIM detector unit

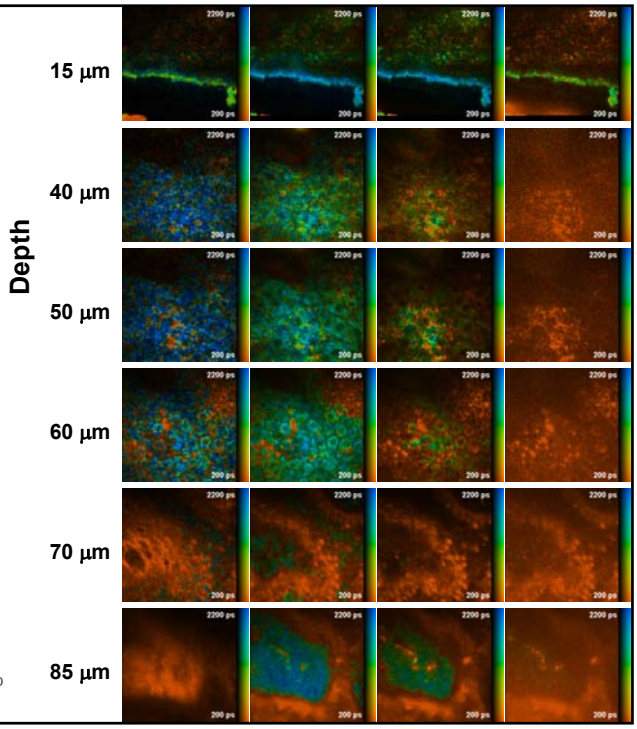


4 channel FLIM
– *in vivo* imaging

Normal skin - medial forearm

Acquisition Time 25.5s/depth
Excitation @ 760nm
Images 177µm x 177µm





Multidimensional fluorescence imaging

Dominic Alibhai, **Natalie Andrews**, Lingling Chen, Sergio Coda, *Pieter de Beule*, *David Grant*, Douglas Kelly, Romain Laine, *Hugh Manning*, *Dylan Owen*, *Stephane Oddos*, Rakesh Patalay, *Tom Robinson*, Hugo Sinclair, Hugh Sparks, Sean Warren, *Neil Galletly*, Yuriy Alexandrov, *Egidijus Auksorius*, *Alice Brown*, Sunil Kumar, *Peter Lanigan*, *Martin Lenz*, Anca Margineanu, *Ewan McGhee*, Ian Munro, *Jose Requejo-Isidro*, Gordon Kennedy, *Daniel Stuckey*, *Paul Tadrous*, *Harriet Taylor*, *Khadija Tahir*, *Clifford Talbot*, **James McGinty, Chris Dunsby, Mark Neil, Paul French**

Imperial College London
Biology, Chemistry, ICB, Medicine, Physics

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Tony Magee
Ken MacLeod
Nicholas Peters
Guy Rutter
Ann Sandison
Alex Sardini
Gordon Stamp
Ed Tate
Andrew Thillainayagam..

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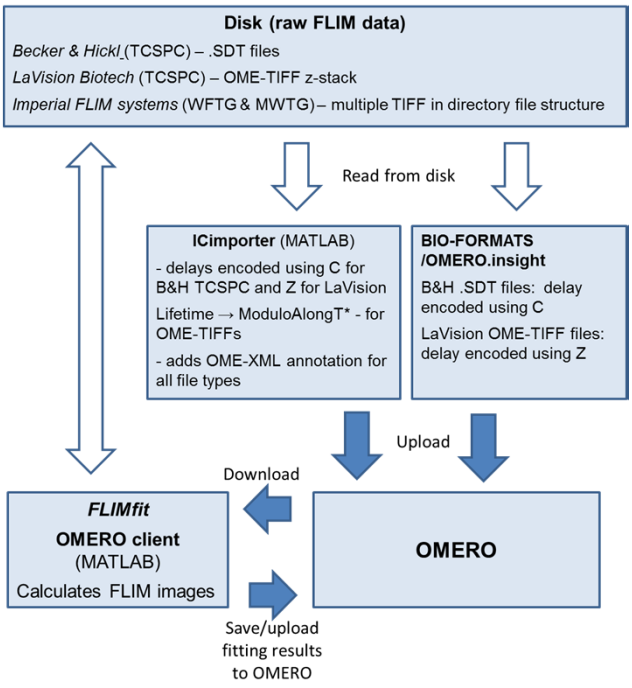
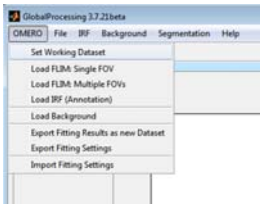
FLIMfit: an OMERO client in MATLAB



TCSPC: Time-correlated single photon counting
WFTG: Wide-field time-gated
MWTG: Multiwell plate time-gated

FLIMfit: an OMERO client

Sean Warren + …



Two (time domain FLIM modalities

- TCSPC
- Time-gated wide-field

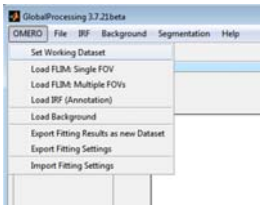
Many potential FLIM applications:

Multiwell plate readers (FLIM/FRET)
Endoscopes
SPIM
OPT
Skin autofluorescence studies
....

Large data.. need effective analysis and storage

FLIMfit: an OMERO client

Sean Warren + …

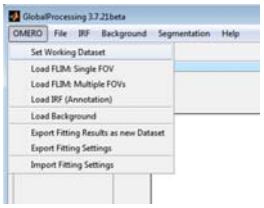


MatLab based with calls to C++
Fits complex decay models
Advanced artefacts compensation solutions
Data reduction
Accepts different file storage formats (TCSPC & time-gated data)
Visualization, assessment, import/export of fitting data
Special visualizations tailored for multi-well plates
Global analysis
.... → feature list/user manual

- will benefit from OMERO capabilities for data sharing, public access, archiving, and unification...

FLIMfit: an OMERO client

Sean Warren + …



MatLab OMERO bindings

Cross platform

Additional dimension challenge for lifetime data:
Presently used FLIM formats: channel C for B&H, Z for LaVision OME-TIFF)
⇒ standardizing to "**ModuloAlongT**" for lifetime data

Images from **OMERO Datasets and SPW Plates** are loaded to *FLIMfit*

Option to **transfer fitting results to OMERO** as new Datasets or Plates

Auxiliary data: different options for loading IRFs, Backgrounds etc. as OMERO Images, Datasets and file Annotations

(***FLIMfit* user manuals** – work in progress..)

"*Importer*" utility to handle internal plate reader formats with "ModuloAlong"
- can also attach user file annotations

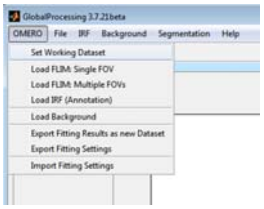
For OME-TIFF - attaches the whole xml description as metadata file

Disk data transfers to OMERO as Datasets or SPW Plates

now ~online ..

FLIMfit: an OMERO client

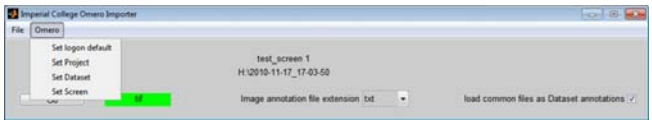
Ian Munro



1. Illustrate OMERO display limitations with time-resolved data.
2. Show the nature of time-resolved data
3. Demonstrate that our code can read & interpret 6D data with the "moduloAlong" annotation & offer a solution to 1)
4. Show FLIM workflow (including loading IRF from OMERO) for the vast majority of the audience who are unfamiliar.
5. Demonstrate the ability to store results (parameter images) in OMERO.

IC Importer

Yuriy Alexandrov



The “IC_importer” utility is based on the same MATLAB client functions as for “FLIMfit” client

It can transfer to OMERO the data from disk directories, as well as images in other formats (B&H, OME-tiff) - according to the OME conventions. Directories with images are transferred as new Datasets (Plates).

“IC_importer” also can attach any auxiliary files with relevant extensions (doc, txt, xml, pdf, ppt, xls) as OMERO annotations to that new Dataset (Plate) if such files are located in the source directory.

Single auxiliary images (e.g. background images) can be imported to Datasets, tagged according to convention, and then loaded from OMERO into analysis software.

Sharing OMERO data

Mark Woodbridge

How to enable Imperial College researchers to allow anonymous, public access to a subset of their imaging data?

Motivation

There is currently no public repository for imaging data analogous to ArrayExpress, ENA etc.

Researchers require a facility to store supplementary information directly related to publications, and, subject to funders' requirements, to archive and share all data generated in the course of a long-term investigation.

Third-party services fulfil the first requirement (e.g. Dryad, Figshare) but are generic and do not provide visualisation or metadata browsing.

They do provide persistent identifiers (DOIs) which we are unable to do at no cost.

Some journals (e.g. JCB) do provide bespoke repositories for images but these are rare.

Sharing OMERO data

Mark Woodbridge

How to enable Imperial College researchers to allow anonymous, public access to a subset of their imaging data?

Requirements

- Security
- Anonymous access
- Imperial College branding
- Project/dataset/image hierarchy
- Provide access to images in original format
- Masking of selected metadata
- Direct web linking to individual projects/datasets

Sharing OMERO data

1. OMERO.insight

Projects, Datasets or Images can be shared using OMERO.insight (desktop OMERO client). This is performed by moving them to the 'Public' group. OMERO only allows images to appear in one group at a one time, so once moved they no longer appear in your personal or lab group. They remain editable/deletable only by the owner.

Pros

- Preserves project/dataset/image hierarchy
- Image membership of public group makes it clear to user that they have been published
- Can deep-link (i.e. send someone a URL that points directly at share)

Cons

- Moving files between groups can lead to fragmented datasets (if attempting sharing selected files)

Sharing OMERO data

2. OMERO.web

A 'basket' of images can be shared using OMERO.web (online OMERO client). These images remain in their original group but are visible online to anonymous users.

Pros

- Does not affect which groups original files are stored in

Cons

- No hierarchy?
- Bug means that acquisition metadata cannot be viewed? (needs confirmation)
- Not clear which files are shared - files can accidentally be deleted, breaking share.
- No deep-linking? (needs confirmation)

Sharing OMERO data

3. Custom solution (based on OMERO web?)

Users just add a tag (e.g. 'public') to arbitrary images to be shared

- would only work with a bespoke version of OMERO.web.
- web application would require root access to OMERO - and would effectively involve building a parallel permissions system in OMERO, which is undesirable.

Public shares are intended to be long-term fixtures, with URLs

- Shared data needs to be isolated to avoid deletion, moving, changing access rights ..
- leads to data duplication

Shared data needs metadata (context) to be useful

- Needs standardised (local?) validation of data to be shared