

## **FLIM fitting software and OMERO at Imperial College London in the Photonics Group**

The Imperial/Photonics Group's main FLIM analysis software is internally named "*GlobalProcessing*". It is written in MATLAB and provides state of the art fitting of time domain data for both laser-scanning time-correlated single photon (TCSPC) and wide-field time-gated FLIM images. It includes an advanced GUI for data loading, visualization, and reporting.

We have developed a version of this as an OMERO client that was provided to Dundee in August 2012.

When interfaced to an OMERO server, this client can:

- (a) process (TCSPC) images that have previously been imported into OMERO (via an OMERO client e.g. OMERO.insight) from either of two proprietary file formats (Becker & Hickl .sdt files or OME-tiffs generated by LaVision BioTec's TCSPC system)
- (b) write the results of a fit back into OMERO as a multi-channel floating point image of fluorescence lifetime parameters (in a new dataset)

When interfaced to a local disk, this client can:

- a) process data stored in the above proprietary TCSPC file formats.
- b) process wide-field time-gated image stored on the disk as set of TIFF files
- c) process gated time-resolved multi-well plate data stored on the disk as many sets of .tiff files (one sub-directory per FOV)
- d) write the results of a fitting procedure back to the disk as multi-channel floating point images of fluorescence lifetime parameter (in a new data directory)

NB b) and c) require that Imperial in-house file naming and directory structure conventions are followed.

Since August 2012, we have been working to add the capability to upload wide-field time-gated image data (including multiwell plate data) to the OMERO database, ensuring that the directory and sub-directory structure and filenames are suitably encoded such that they can be handled by our FLIM fitting client.

We are currently working on importing our multiwell plate FLIM data to OMERO in the format used by the wider OMERO community, i.e. SPW (which is used by *Columbus* and *JCB*)

To this end, we have written (in MATLAB) a programme called "*IC\_importer*" to import FLIM data into OMERO. This client can import our in-house wide-field time-gated FLIM data and the two proprietary TCSPC formats described above and encodes lifetime data along the C axis (as is currently the case for Bio-Formats importing Becker & Hickl .sdt files). We are now going to change this to encode lifetime data using *ModuloAlongT*, and will leave C and *ModuloAlongC* for spectral and polarisation channels.

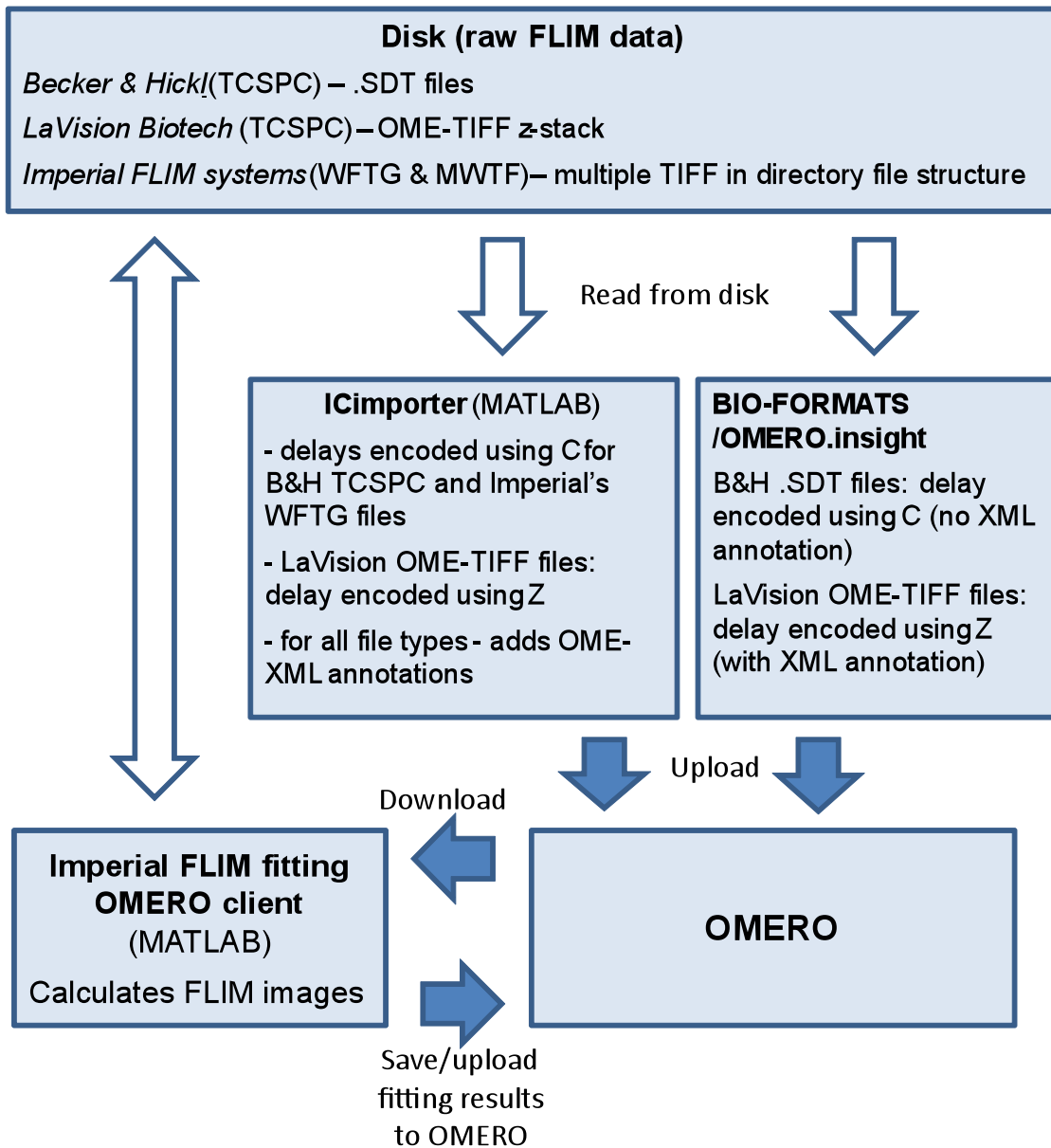
Figure 1 shows a schematic of the capabilities of our FLIM OMERO clients.

### **Key issues going forward:**

1) We intend to standardise on encoding FLIM data using *ModuloAlongT*. Currently Bio-Formats uses C for .sdt files while LaVision Biotec uses Z for lifetime data. Ideally this should be changed but we can include translators for this "legacy data".

- 2) Where should the functionality currently in IC-importer fit into the OMERO eco-system?  
 - in Bio-Formats? - Who should write this (in Java?)
- 3) When importing multiwell plate lifetime data into OMERO:  
 - will the way we standardise metadata with SPW for FLIM and other spectroscopic parameters impact anyone else, e.g. create future incompatibilities?

Please answer these queries via the ome-devel list!



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

Fig. 1. The Imperial College London OMERO FLIM clients

## Appendix

The “*GlobalProcessing*” software operates as an OMERO client (Fig.1).

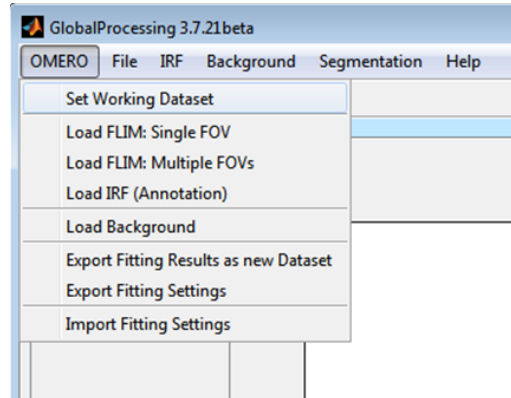


Fig.1. IC FLIM analysis software’s OMERO menu

Currently, the following image and data I/O options are supported in “*GlobalProcessing*” for the OMERO-Dataset based analysis:

### Input FLIM images

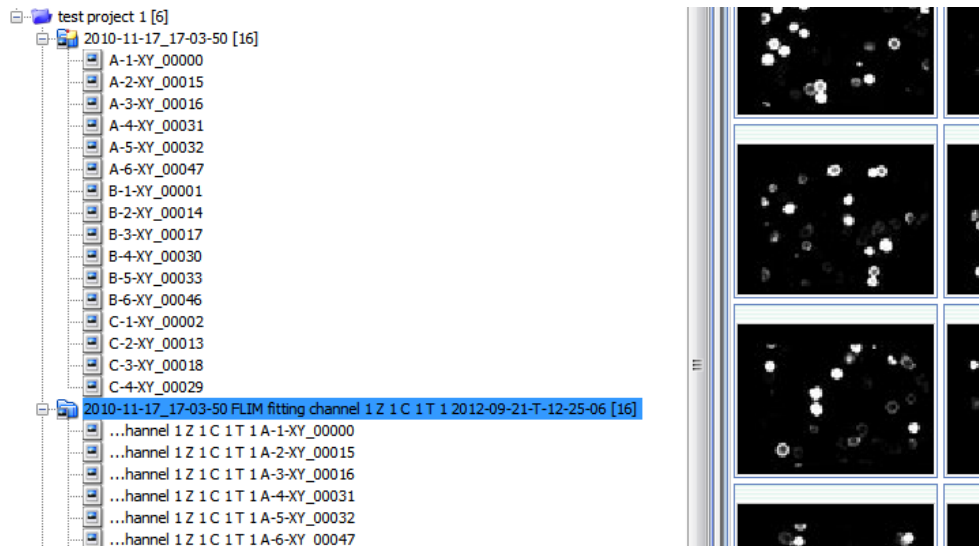
- 1) B&H TCSPC (.sdt) images imported to OMERO by “native” OMERO-clients (which do not use OME metadata convention) encode lifetime data using C as default.
- 2) B&H images imported by *IC\_importer* are handled the same as above in (1) but include the OME metadata convention.
- 3) OME-tiffs imported by *IC\_importer* could encode FLIM data using *ModuloAlongC, T, Z*, and could contain TCSPC or wide-field time-gated FLIM data. We are choosing *ModuloAlongT* as a matter of policy.
- 4) A version importing multiwell plate data using the SPW-format is currently implemented (working with SPW data transferred to OMERO by *IC\_importer*). For all of the above, single or multiple images (Fields of Views, FOVs) can be loaded for the analysis from Datasets

### Auxiliary data and images

- 1) Single-channel text-formatted IRFs (Instrument Response Functions) are loaded as Dataset annotations
- 2) Original B&H IRFs, including multi-channel ones are also kept as Dataset annotations
- 3) Background reference images are stored as Dataset images
- 4) *GlobalProcessing* fitting options and set-up files, xmls, are stored as Dataset annotations)
- 5) *Processing set-up option files also might be stored as Dataset annotations*
- 6) The above are true for SPW Plate but it is not currently possible to attach auxiliary images to SPW Plate

## Analysis results

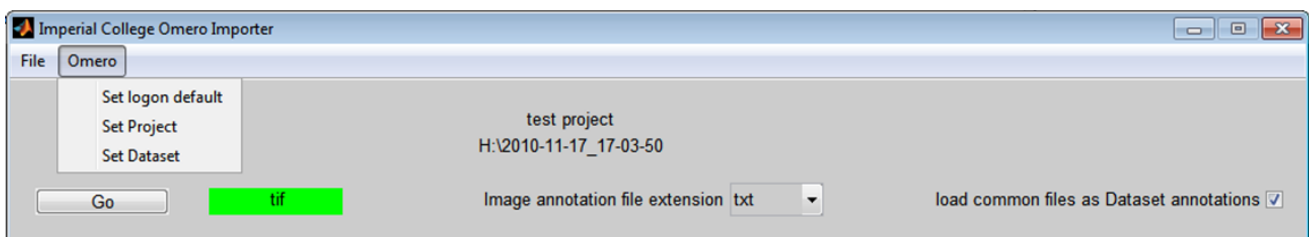
The results of FLIM fittings can be imported to OMERO as new Datasets containing, for each input FOV image, the corresponding multi-plane output image where fitted parameters are stored as double-precision XY-planes along C dimension. The names of results Datasets and images contain “FLIM fitting” postfix, information about spectral channel, Z,C,T and the time when analysis was performed (Fig.2).



**Fig.2.** Snapshot of the Omero.Insight client showing the original input Dataset (Gated FLIM, plate reader data – the Dataset above), and the corresponding Dataset with fitting results (highlighted).

## IC-importer utility

The “IC\_importer” utility is based on the same MATLAB client functions as for “GlobalProcessing” client (Fig.3). It allows transferring 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. “IC\_importer” also can attach any auxiliary files with relevant extensions (doc, txt, xml, pdf, ppt xls) as OMERO annotations to that new Dataset, if such files are located in the source directory. Also, single auxiliary images (e.g. background images) might be imported to Datasets, tagged according to convention, and then loaded from OMERO into analysis software.



**Fig.3.** IC Omero importer utility.