An interactive ImageJ tool macro to visualise Leica Stellaris Tau contrast images from raw image data

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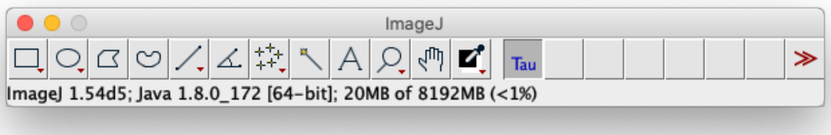
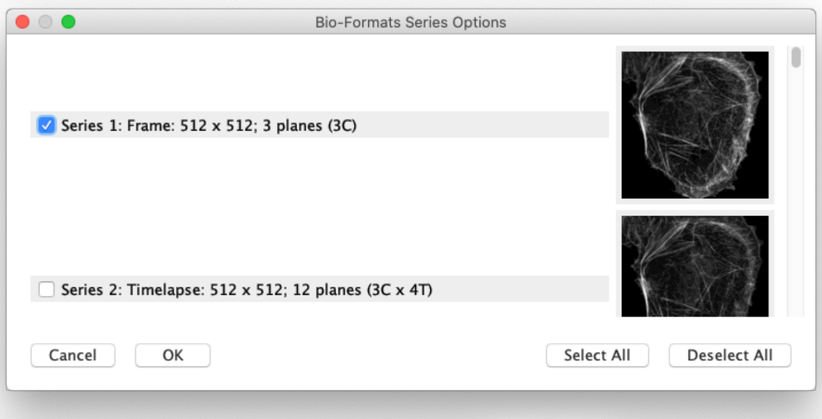
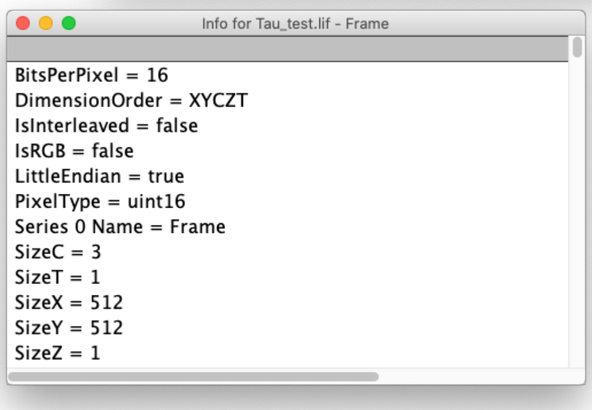
Goal

Leica Stellaris "Tau STED" imaging mode allows fast measurement of fluorescent lifetime in living cells. Point and click or areas of interest tau measurements are available in the proprietary acquisition software, but the Tau calculated image is missing from datasets saved in the proprietary *.lif file format. Here we provide a simple ImageJ tool to address this limitation.







Tau contrast image raw data

*.lif files containing raw data can be imported as a single dataset into ImageJ using the BioFormats library by chosing the Hyperstack stack viewing option. A single frame tau contrast image is imported as a 3 channels, 16-bit image.

Anatomy of an ImageJ macro tool

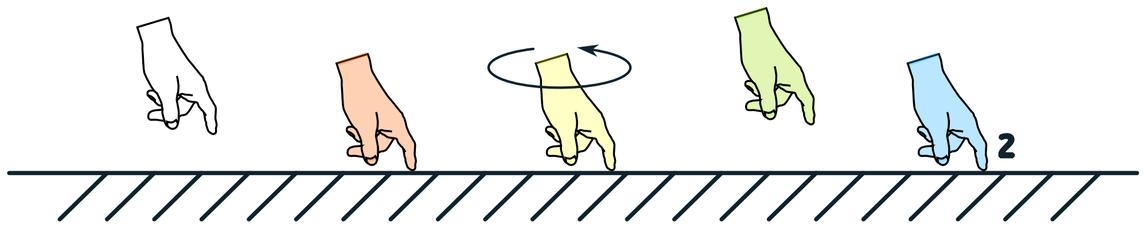
Standard macro tools will be run by clicking with the mouse inside image windows.

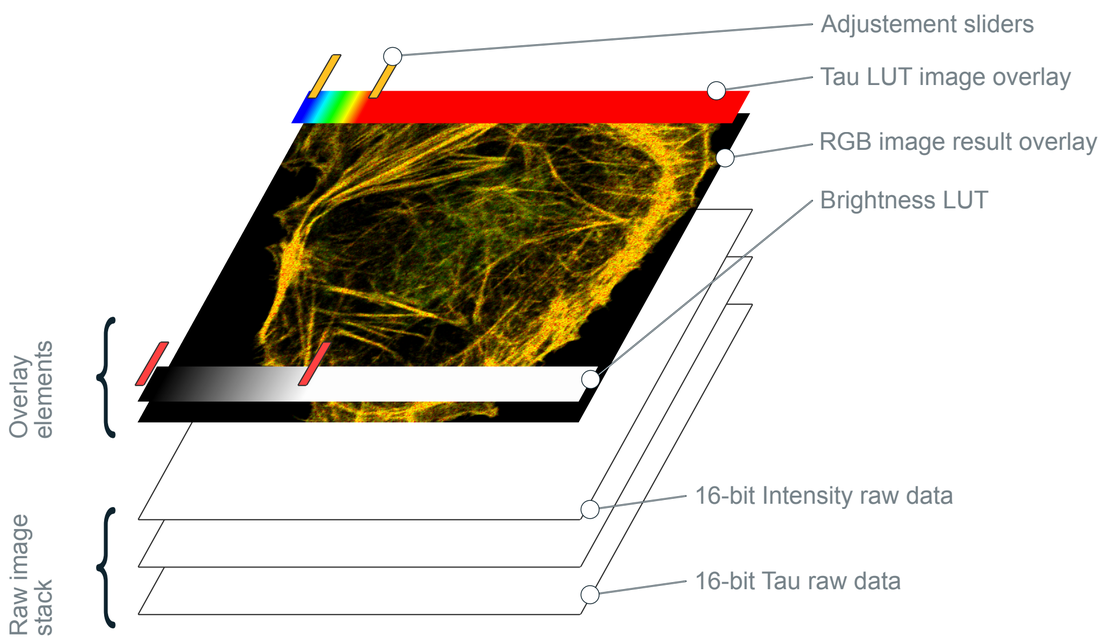
```
// An ImageJ macro tool to visualise Leica Stellaris
// Tau contrast images

var params = newArray(0.1,0.2,0.0,0.8);
...

macro "TauDisplay Tool - C00cT0f12TT6f10aTcf10u" {
  // code run once when mouse is pressed
  getCursorLoc(x, y, z, flags);
  // the flags variable holds events informations
  while (flags&16>0) {
    getCursorLoc(x, y, z, flags);
    // code looped while mouse is pressed
  }
  // code run when mouse button is released
}

macro "TauDisplay Tool Options" {
  // this code is run when the tool icon is right clicked
}
```





Average Arrival Time estimation

Tau channel raw data in encoded as 16-bit integers that need to be scaled to match real world lifetime data, which Leica defines as "Average Arrival Time". The AAT value can be computed using the formula shown in the dialog below, using a fixed 0.097ns per grayscale level and a 1ns offset to match LASX [-1..11.5ns] AAT interval. ATT estimates are subject to a session-specific uncertainty or "variation of zero point calibration" that has to be measured and updated for each session. The image calibration itself is done with the built-in "Calibrate..." command to account for the linear relation ship between grayscale and AAT values.

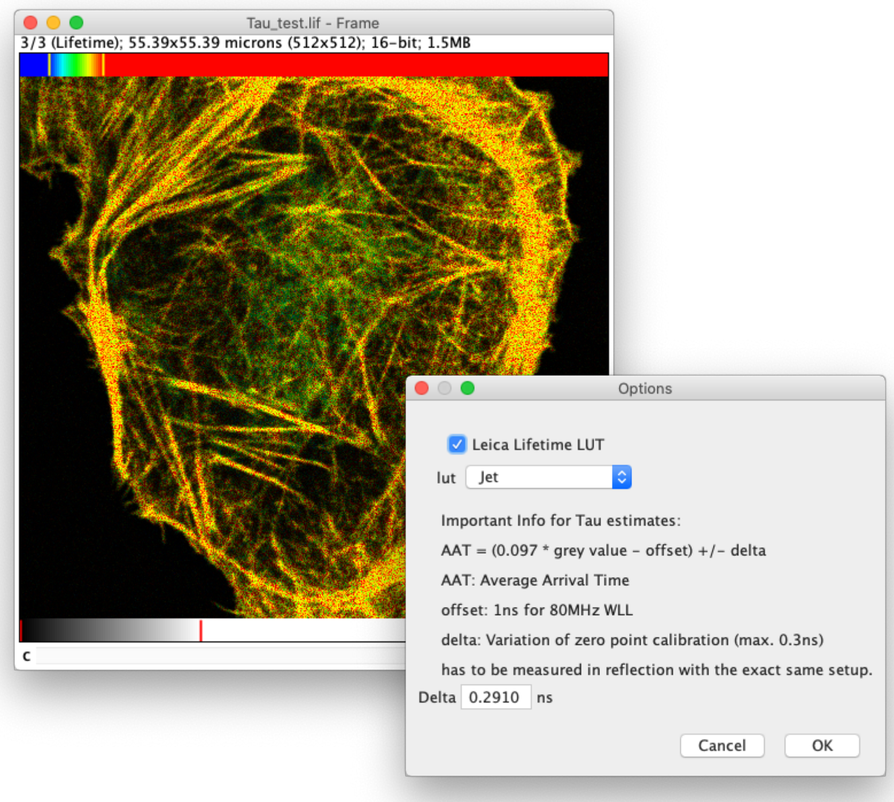


Image window components

Controls and visualisation preview are added to the raw image data as overlay elements, stacked as shown. The Tau-colorised result is an image-type overlay item. Brightness contrast and Tau LUT contrast color scales are aslo image-type overlay elements. Contrast in the brightness space or Tau space are adjusted using four rectangle ROI overlay elements whose positions can be adjusted by dragging with the mouse and recomputing the corresponding LUT and preview Tau image.

Tool availability and useful links to key components

About tool macros: https://imagej.net/ij/developer/macro/macros.html#tools
Bioformats library: http://www.openmicroscopy.org/bio-formats/
Creating and managing overlay elements: https://wsr.imagej.net/developer/macro/functions.html#Overlay
Handling mouse events using the flags variable: https://wsr.imagej.net/developer/macro/functions.html#getCursorLoc
Mapping image raw values to real world values: https://imagej.net/ij/docs/guide/146-30.html#toc-Subsection-30.9

Source code for this tool: https://github.com/mutterer/stellaris