FRET ratiometric tool: imageJ script

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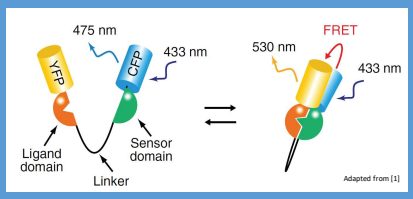
FORSTER RESONANCE ENERGY TRANSFER - FRET

The central mechanism of FRET comprises a donor fluorophore that is in an excited electronic state and may transfer its excitation energy to an acceptor fluorophore that is closed enough. There are several ways of measuring FRET efficiency. However, here we are going to focus on single-chain sensitised emission FRET system to follow protein interaction signalling dynamics in real-time.



We will cover the processing of raw image data sets into ratiometric measurements, capable of illustrating relative differences in the protein activation states within a single cell.

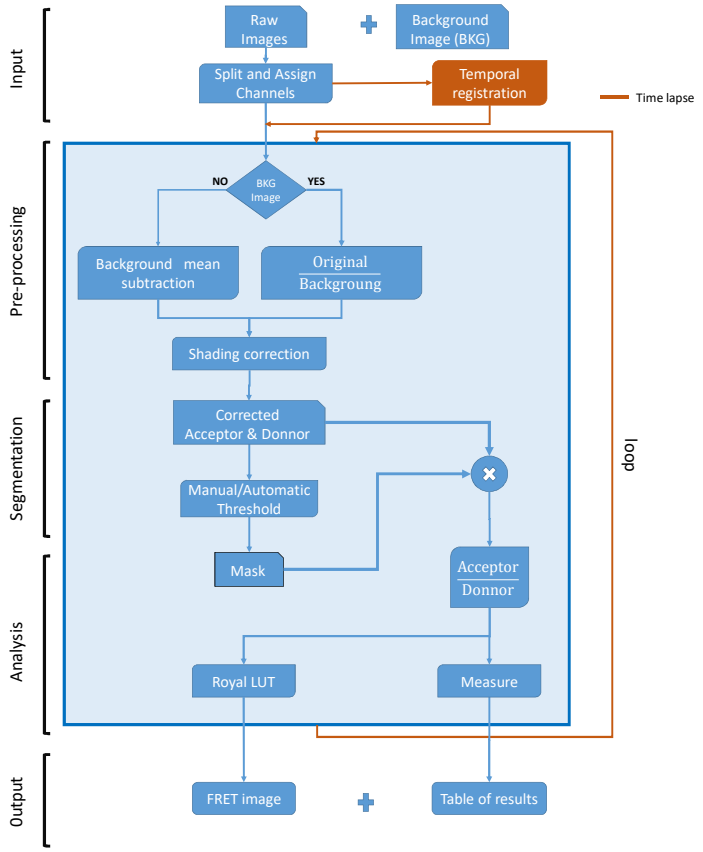
Ratiometric FRET :

If the acceptor (A) is florescent, FRET is most easily characterized using ratio between the donor (D) and acceptor (A) emission intensities which is known as ratiometric method. The relative FRET efficiency, also known as the proximity ration is given by:

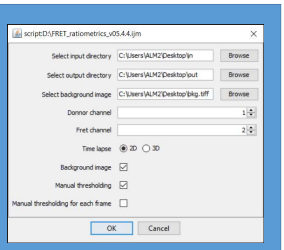
$$E_{rel} = \frac{I_A}{I_A + I_D}$$

Where I_A and I_D are the total A and D fluorescence intensities but both following excitation.

WORKFLOW



SCRIPT FEATURES

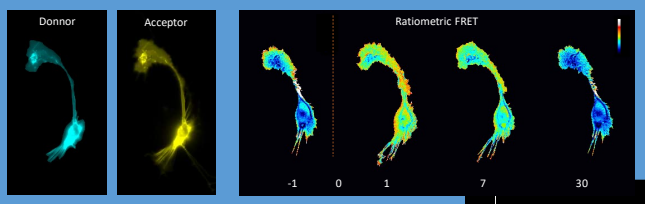


- Open solution developed in ImageJ/Fiji macro scripting
- Graphical User Interface for input parameters
- Input of optical fluorescence (.tiff) images with multiple dimensions: xyzct
- Multiple combinations of input options:
  - 2D/3D; background subtraction with/without background image; automatic/manual thresholding; specify which channels are donor and acceptor
- Ratiometric FRET Analysis with: background subtraction, uneven illumination correction, temporal registration, segmentation and the ratiometric measurements.
- Batch mode for single time point images or in time-lapse series
- Output of FRET image with royal LUT normalization and the result table with Ratio fret intensity values and cell area for each time frame or each image (batch analysis).
- TODO: convert macro to FIJI plugin

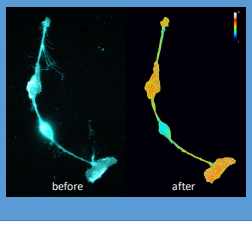
APPLICATION: mouse Schwann cells

Rho GTPases are molecular switches that coordinate intracellular signaling cascades in response to extrinsic factors such as extracellular matrix proteins and growth factors. In broad terms, Rho GTPase-dependent signaling regulates many different cellular processes important for tissue morphogenesis and organism biology. These signaling molecules cycle between inactive and active states by the action of hundreds of regulatory proteins, which define activity of Rho GTPases in a very locally and temporally specific manner in the cell. Observing and analyzing this activity in live systems is therefore of great importance and the development of FRET biosensors allowed us to follow Rho GTPase signaling dynamics in real time. Images are shown just for illustrating proposes of the tool's workflow and output.

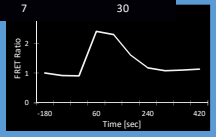
3D Ratiometric Analysis



2D Ratiometric Analysis



Images acquired at Leica DMI6000 microscope, HCX PL APO CS 63x/1.30 GLYC 21°C, with temperature control



Case	Label	Area	Fret Ratio Mean	Fret Ratio Min	Fret Ratio Max
1	Position016.tif	1768.129	3.468	1.445	5.307
2	Position002.tif	893.198	2.527	1.375	4.538
3	Position006.tif	1608.038	2.124	1.111	4.100
4	Position012.tif	789.141	2.247	1.203	3.951
5	Position021.tif	293.071	1.846	1.050	3.394
6	Position036.tif	1053.541	2.102	1.249	3.382

The proposed tool is available for testing @

https://github.com/mafsousa/FretRatiometric

References

[1] Naoki Komatsua, et al. (2011), *Development of an optimized backbone of FRET biosensors for kinases and GTPases*, vol 22, Mol. Biol. Cell

Acknowledgments

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