Microscopy FAQ – Quantifying Fluorescence Intensity.

Fluorescent probes provide a form of contrast staining, enhancing the visibility and hence detection of labelled compounds and stuctures.

Experiments utilizing fluorescent probes can readily supply descriptive information on aspects of size and morphology, and on spatial information such as area and distance (the latter, for example, by FRET). They can provide information on changes in the number, distribution, transport and diffusion (FLIM & FRAP) of labelled bodies, for example, in response to experimental manipulation.

A good treatment of the practice and potential problems of techniques such as FRET, FLIM and FRAP is provided by:

> *Esposito, A., et al. (2009) Quantitative Fluorescence Microscopy Techniques. pp117-143* in Gavin, R.H. (ed) *Cytoskeleton Methods and Protocols*. 2nd ed. Humana Press.

There is one aspect of characterization, however, that is not straightforward. That is the meaningful quantification of fluorescence intensity, and the relation of this with substance concentration.

Unfortunately, many of the available review papers either gloss over difficulties or omit mentioning them altogether.

Merely taking "control" and "treatment" images at the same microscope and camera settings and then comparing brightness values will NOT provide a useful measure of change, or yield reliable information on concentration. Variation in an array of equipment parameters occurs during normal operation, and any experimental manipulation of the specimen is likely to influence the fluorescent response and output of the fluorophore itself.

Some of the difficulties of fluorescence intensity quantitation are discussed in:

> *Pawley, J. ( ) The 39 Steps: A Cautionary Tale about "Quantitative" 3D Fluorescence Microscopy.*

Both papers mentioned above may be downloaded from the "Documents" menu of your PPMS page.