NO NEED TO FRET: SENSITIVE IMAGING OF MOLECULAR PROCESSES BY TWO-PHOTON POLARIZATION MICROSCOPY (2PPM)

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FRET microscopy using fluorescent proteins has been a staple on the menu of optical microscopy techniques used by cell biologists. Our mathematical model indicated that linear dichroism (in contrast to fluorescence anisotropy) should be observable in most fluorescently labeled membrane protein constructs, even under non-ideal conditions. Our experiments have now shown [1] that two-photon polarization microscopy (2PPM) indeed allows observations of linear dichroism in most fluorescently labeled membrane proteins (Fig. 1), in living cells and animals, yielding insights into protein structure and function. 2PPM allows sensitive observations, for example, of G-protein activation, changes of intracellular calcium concentrations, and changes in cell membrane voltage. Conveniently, many suitable probes for 2PPM imaging of molecular processes already exist. The ability of 2PPM to yield information on protein structure will allow rational development of novel genetically encoded fluorescent probes. Due to its many advantages and potential uses, 2PPM is likely to become an indispensable tool of cell biology, systems biology, and structural biology, as well as neuroscience.

Fig. 1: Linear dichroism in a fluorescently labeled membrane protein in a living cell, observed by 2PPM.

i) An image acquired using horizontally polarized excitation light;

ii) Same as in i), but using vertically polarized excitation

iii) Linear dichroism visualized by coloring images i) and ii) red and green, respectively.

REFERENCES