2P, OR NOT 2P:
INSIGHTS INTO MEMBRANE PROTEIN STRUCTURE FROM SINGLE- AND TWO-PHOTON POLARIZATION MICROSCOPY

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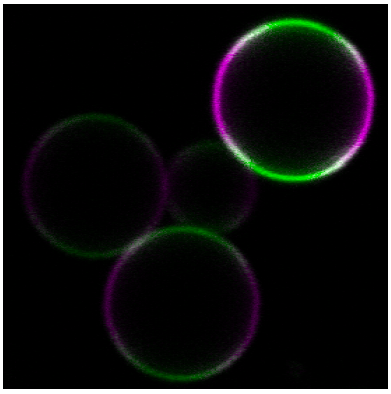
KEY WORDS: Membrane proteins, polarization microscopy, linear dichroism, two-photon fluorescence microscopy, genetically encoded fluorescent probes, cell signaling

Polarization fluorescence microscopy, also called fluorescence resolved linear dichroism microscopy, has in recent years been applied to a growing range of biological and models systems[1]. It has been used for observations of molecular events in living cells[2], as well as for gaining insights into molecular organization[2, 3]. The two main implementations, utilizing single-photon and two-photon excitation, possess distinct advantages and disadvantages. With our current software tools and experimental methods, it has now become extremely simple to obtain quantitative structural information even from images acquired by regular single-photon confocal or two-photon fluorescence microscopy. Our results demonstrate the simplicity of use, and the utility of polarization microscopy for rational development of genetically encoded fluorescent probes, for observing a wide range of molecular processes, and for gaining structural insights into the mechanisms of these processes.

a)



b)

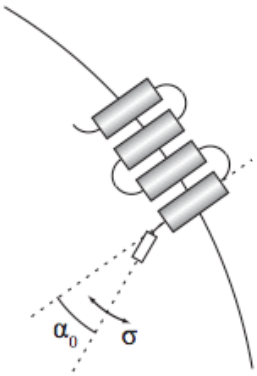


Figure: (a) Giant plasma membrane vesicles derived from mammalian cells expressing a construct containing a fluorescent protein, imaged by polarization fluorescence microscopy. (b) Examples of some of the structural parameters obtainable, along with more detailed information, by polarization fluorescence microscopy.

References:

[1] J. Lazar, A. Bondar, S. Timr, and S. Firestein, "Two-photon polarization microscopy reveals protein structure and function", *Nature Methods* **8(8)**:684-7 (2011).
[2] A. Bondar, J. Lazar, "Dissociated GαGTP and Gβγ protein subunits are the major activated form of heterotrimeric Gi/o proteins", *J. Biol Chem*. **289(3)**:1271-81 (2014).
[3] M. Mavrakis, Y. Azou-Gros, F.C. Tsai, J. Alvarado, A. Bertin, A. Kress, S. Brasselet, G.H. Koenderink, T. Lecuit, "Septins promote F-actin ring formation by crosslinking actin filaments into curved bundles". *Nature Cell Biology*. **16(4):**322 (2014).