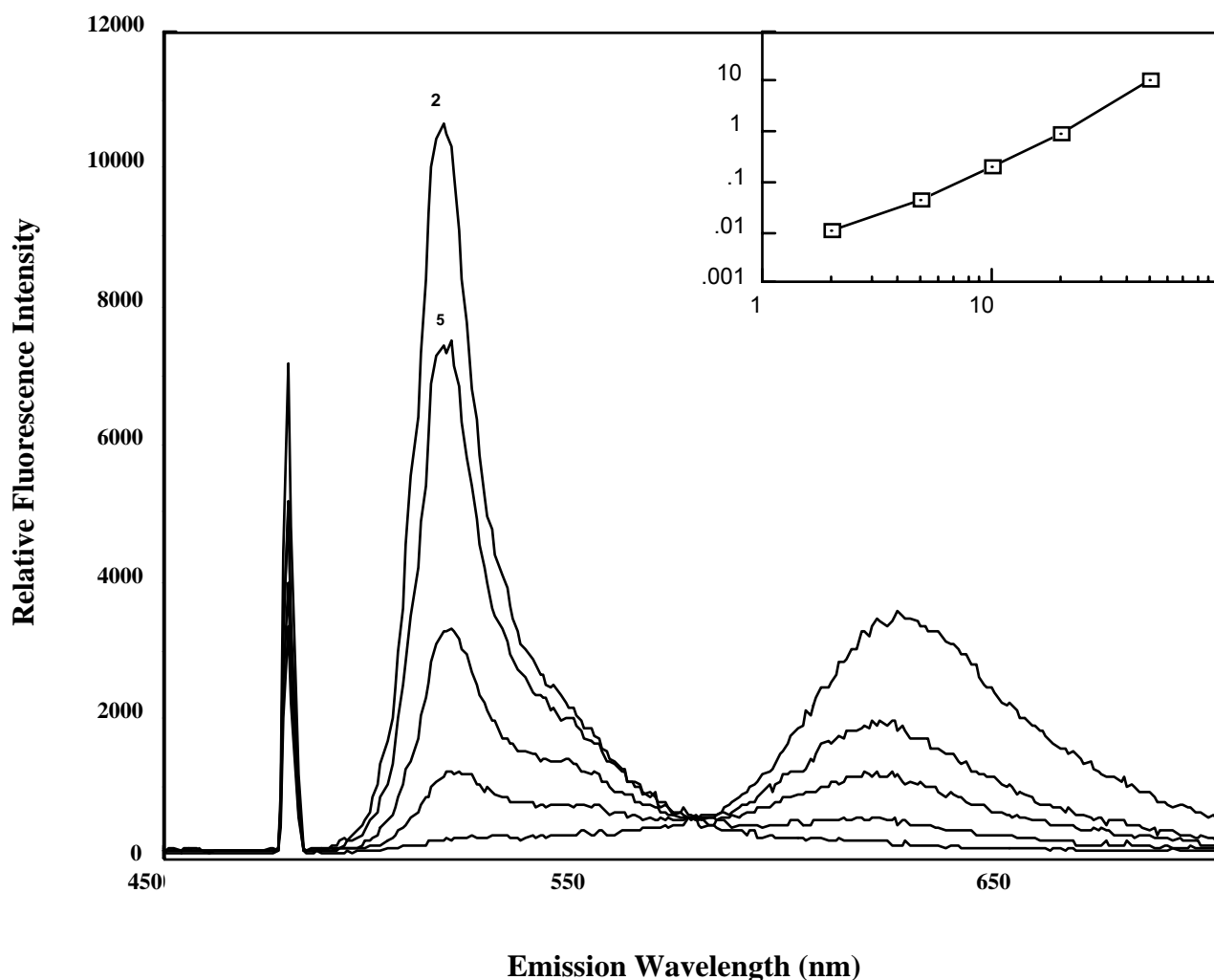


Pagano Lab – Protocol

Microscope Filters for Observing BODIPY-Fluorescence

We use lipid analogs labeled with the fluorescent fatty acid, [4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-pentanoic acid \(BODIPY\)](#). This fluorophore is optimally excited with blue light and exhibits green (monomer) or red (excimer) fluorescence, depending on its concentration in the specimen as shown in the spectra below.



Fluorescence emission spectra of lipid vesicles containing 2, 5, 10, 20, or 50 mol % BODIPY-lipid. Excitation was at 480 nm. Inset shows the ratio of relative fluorescence intensities at 620 and 515 nm vs mol % BODIPY-lipid.

Pagano Lab – Protocol

Microscope Filters for Observing BODIPY-Fluorescence

I frequently receive queries from investigators asking about the appropriate optical filters to use on their microscopes to visualize BODIPY-fluorescence. We use filters that establish three spectral windows or "channels." In each case the specimen is excited with blue light (450-490 nm). Samples are then viewed in "Green" (520-560 nm), "Green + Red" (≥ 520 nm), or "Red" (≥ 590 nm) regions of the spectrum.

Here are the filter combinations we use:

"Channel"	Exciter Filter	Dichroic Filter	Barrier Filter
Green	470/40	505	535/40
Red	470/40	505	≥ 590
Green + Red	470/40	505	≥ 515

References:

- Pagano RE, Martin OC, Kang HC, Haugland RP.
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J Cell Biol. 1991 Jun;113(6):1267-79.
- Chen CS, Martin OC, Pagano RE.
Changes in the spectral properties of a plasma membrane lipid analog during the first seconds of endocytosis in living cells.
Biophys J. 1997 Jan;72(1):37-50.

See also, [Molecular Probes Handbook](#)