Mapping intracellular polarity and viscosity simultaneously in live cells using fluorescence lifetime imaging (FLIM)

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Knowledge of local physical properties of intracellular environments and organelles can improve our understanding of the mechanisms which are responsible for correct cell function including signalling and trafficking.

Lipid droplets are cellular organelles comprised of a neutral lipid core surrounded by a phospholipid monolayer. They function as energy storage for cells with droplet irregularities having implications for diseases including obesity and diabetes.[1,2] We have used spectrally-resolved confocal fluorescence microscopy and fluorescence lifetime imaging (FLIM) to map polarity and viscosity sensed by the polarity-sensitive probe Nile Red, and the viscosity sensitive probe, BODIPY-C₁₂ – a fluorescent molecular rotor.[3,4] Both of these dyes are selective stains for hydrophobic, lipid-rich regions, and in particular lipid droplets.

Nile Red is a solvatochromic dye which exhibits redshifting of its emission spectrum in increasingly polar media. We have measured spectrally-resolved confocal fluorescence images of living HeLa cells stained with Nile Red which reveal emission centred around 580 nm for (neutral) lipid droplets and emission at around 630 nm for other, more polar, stained regions of the cell. Furthermore, we have measured FLIM of Nile Red in HeLa cells at 550 nm, 600 nm and 650 nm. Our FLIM measurements reveal longer lifetimes in lipid droplets compared to other stained regions at all wavelengths with lifetimes of the lipid droplets ranging from 3.2 ns at 550 nm to 3.85 ns at 650 nm. Longer fluorescence lifetimes are associated with lower polarity environments and our results agree with the premise that lipid droplets are non-polar. The fluorescence decay profiles vary markedly as a function of emission wavelength, with biexponential fitting required at shorter emission wavelengths. We discuss the spectral dependence of the fluorescence decay profiles in terms of intramolecular charge transfer and emission from both relaxed and unrelaxed excited states of Nile Red.

By counterstaining the cells with BODIPY-C₁₂ we show that the two dyes can be seen to colocalise (Figure 1). The fluorescence lifetime and quantum yield of BODIPY-C₁₂ increases with increasing viscosity of the medium in a quantifiable way in accordance with equations first presented by Förster and Hoffmann.[5] The intracellular emission peaks of the two dyes are sufficiently spectrally separated to allow for identification of both dyes in a given spatial position. Furthermore, unlike Nile Red the position of the BODIPY-C₁₂ emission peak is independent of solvent polarity and viscosity.

Moreover, we have exploited the spectrally distinct emission to measure FLIM in two wavelength regions simultaneously, 514 nm and 600 nm, corresponding to emission from BODIPY-C₁₂ and Nile Red, respectively (Figure 2). Due to the concentration independence of the fluorescence lifetime measurements it is possible to measure the map the intracellular viscosity using BODIPY-C₁₂ and the spatially variant polarity using Nile Red. For BODIPY-C₁₂ we find that the neutral lipid droplets exhibit shorter fluorescence lifetimes than other stained regions, and that the reverse is true for Nile Red. This shows that lipid droplets have a lower viscosity and are less polar than the other stained cellular regions.

This study highlights the potential of small organic fluorophores for reporting on local physical properties of intracellular environments, and the power of spectrally-resolved FLIM for mapping these properties in living cells.
References


**Figure 1.** (a) Transmitted light and (b) confocal fluorescence images of living HeLa cells stained with Nile Red and BODIPY-C12. (c) Normalised emission spectra recorded from all pixels (filled black squares), lipid droplets (open circles) and regions of diffuse staining (open squares). Scale bars 10 μm.

**Figure 2.** Images of HeLa cells co-stained using BODIPY-C12 and Nile Red. (a) Confocal fluorescence intensity and (c) FLIM images of HeLa cells measured at 514 nm showing staining by BODIPY-C12. Images (b) and (d) show the confocal fluorescence intensity and FLIM images, respectively of measured at 600 nm showing staining by Nile Red. Scale bar 10 μm.