

# HYPERSPECTRAL COHERENT RAMAN IMAGING OF COEXISTING DOMAINS IN SINGLE LIPID BILAYERS

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Coherent Raman Scattering (CRS) microscopy has emerged in the last decade as a chemically specific, label-free technique which overcomes the speed limit of spontaneous Raman microscopy by coherently exciting identical vibrational modes in the sample, which results in constructive interference of Raman-scattered light [1]. To achieve the full potential of CRS chemical sensitivity, a multiplex or hyperspectral approach, where for each spatial position a wide spectrum of several vibrational resonances is acquired, must be applied. This also enables a quantitative determination of the concentration of chemical components [2]. The application of this technique to study lipids has proven to be particularly successful [1], yet single lipid bilayers push CRS microspectroscopy to its detection limits.

In this work, we have imaged supported unilamellar lipid bilayers using a home-built hyperspectral stimulated Raman scattering (SRS) microscope with high spatial and spectral resolution [3]. Synthetic lipid bilayers were prepared using different ratios of chicken egg sphingomyelin, dioleoylphosphatidylcholine (DOPC) and cholesterol, a mixture which exhibits phase-separated lipid domains. Despite the low SRS signal generated from the bilayers, which are only about 4 nm thick, we clearly observed the coexistence of different lipid domains — the liquid-ordered sphingomyelin-cholesterol mixture and the liquid-disordered DOPC. Images were quantitatively analysed using our in-house algorithms [2], which resulted in the factorisation of the hyperspectral data into separate chemical components, namely water and the two types of lipid domains.

To our knowledge, this is the first time single bilayers have been imaged with hyperspectral SRS with high enough spatial and spectral resolution to distinguish the domains

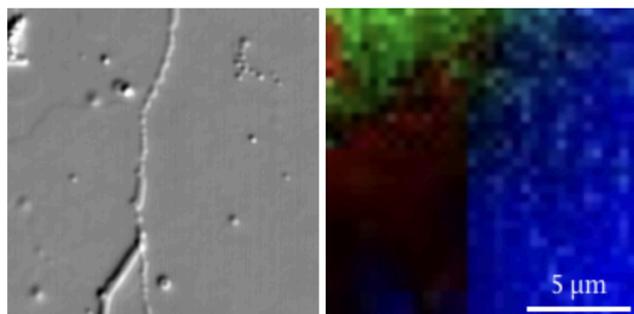


Figure 1: DIC image (left) and SRS image (right) of a supported lipid bilayer exhibiting different domains (red and green).

and their chemical composition. Our results demonstrate the thermodynamically stable coexistence of different lipid phases at room temperature for different ratios of the three components. They are also in agreement with complementary findings using quantitative differential interference contrast microscopy, with which we have been able to distinguish between lipid domains in ternary mixtures by virtue of their optical thickness difference.

[1] A. Zumbusch, W. Langbein and P. Borri, “Nonlinear vibrational microscopy applied to lipid biology”, *Prog. Lipid Res.*, **52**, 615–632 (2013).

[2] F. Masia, A. Glen, P. Stephens, P. Borri and W. Langbein, “Quantitative chemical imaging and unsupervised analysis using hyperspectral coherent anti-Stokes Raman scattering microscopy”, *Anal. Chem.*, **85**, 10820–10828 (2013).

[3] W. Langbein, D. Regan, I. Pope and P. Borri, “Heterodyne dual-polarization epi-detected CARS microscopy for chemical and topographic imaging of interfaces”, *APL Photonics*, **3**, 092402 (2018).