Shedding new light on cells with Coherent Raman Scattering microscopy

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Optical microscopy is an indispensable tool that is driving progress in cell biology, and is still the only practical means of obtaining spatial and temporal resolution within living cells and tissues. Coherent Raman Scattering (CRS) microscopy has attracted increasing attention as a powerful multiphoton microscopy technique which overcomes the need of fluorescent labelling and yet retains biomolecular specificity and intrinsic 3D resolution [1]. Over the past 10 years, our laboratory has developed and demonstrated a range of label-free CRS microscope set-ups featuring innovative excitation/detection schemes and quantitative image analysis.

Our second-generation Coherent anti-Stokes Raman Scattering (CARS) instrument is based on a single 5fs Ti:Sa laser source with 350nm bandwidth capable of exciting a wide vibrational range from 1000/cm to 3500/cm [2], thus enabling hyperspectral microscopy and associated quantitative chemical imaging algorithms and unsupervised analysis [3]. With this system, we have determined the lipid uptake of fixed and living adipose derived human stem cells differentiating into pre-adipocytes [4], the lipid content and spatial distribution in live mammalian oocytes and early embryos [5], addressed a critical side effect in drug screens, namely, drug-induced lipid storage within hepatic tissue [6], and more recently we have quantitatively measured masses of lipids, proteins and DNA during cell division [7]. We have also shown that CARS can be used to visualise single non-fluorescing nanodiamonds in cells for the first time [8].

We recently developed a new CRS set-up, which offers background-free chemically-specific image contrast, shot-noise limited detection, and phase sensitivity enabling topographic imaging of interfaces [9]. The technique features interferometric heterodyne detection of CARS in epi-geometry, as well as multi-modal acquisition of stimulated Raman scattering (SRS) and forward-emitted CARS intensity in the same instrument. As an important biologically-relevant application, epi-detected heterodyne CARS (eH-CARS) imaging of individual lipid bilayers is demonstrated.

I will present our latest progress with these techniques and their applications to bio-imaging.

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