Interferometric Gated Off-Axis Reflectometry (iGOR) - A Label Free Method to Measure Lipid Membrane Dynamics and Deduce Biophysical Properties

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The function and organisation of proteins within the cell membrane is a fundamental area within cell biology, underpinning key processes. When investigating the interaction between proteins and lipid membranes, it is important to consider a dynamic system, as proteins and membrane phase behaviour are known to modulate the function of each other [1]. However, due to limits in technology, the study of membrane dynamics often falls short, especially when evaluating thickness and curvature. Traditional fluorescence methods suffer from photobleaching and phototoxicity [2]. Label-free techniques like iSCAT and mass photometry allow the observation of membrane components on a supported lipid bilayer which inevitably dampens fluctuations of the membrane [3,4]. We have developed a technique, Interferometric gated off-axis reflectometry (iGOR), to alleviate these limitations by facilitating precise observation of a section of suspended lipid membrane. iGOR is a form of digital holography (see Fig.1a,c) in which we combine measurements of membrane thickness and height to analyse membrane stiffness and phase transitions using thermal fluctuations. To maximise the temporal resolution our system acquires at 327Hz with an exposure time of 3ms.

To observe a small biologically relevant section of a suspended membrane we are using giant unilamellar vesicles (GUVs) with diameters greater than 30µm. Our method of GUV production without small vesicles inside the GUV or in the surrounding is an optimised wire electroformation protocol. By improving the preparation steps prior to electroformation, and filtration afterwards, we can create a clean sample with minimal debris reducing background scattering. As this has been optimised for a binary composition, provided the phase transition temperature is considered, this is a potentially widely applicable way of producing vesicles. The filtration method concentrates the GUVs in the sample as well as exchanges the external medium, enabling control of both the interior and exterior medium. Using quantitative differential interference contrast microscopy (qDIC) developed in house (see Fig.1b), we investigated the effect of sucrose concentration gradients on GUV lamellarity. Concentration differences of sucrose <0.2mM establishes an internal pressure which GUVs can withstand while stabilising their shape and controlling their tension. We have developed a numerical simulation model to fit the experimental qDIC data to accurately characterise vesicles.

Combining this sample preparation with iGOR we have a method for the analysis of biophysical properties of model membranes with both a temporal and spatial resolution that could allow the observation of membrane phases and protein insertion into the membrane which will be discussed in the presentation.

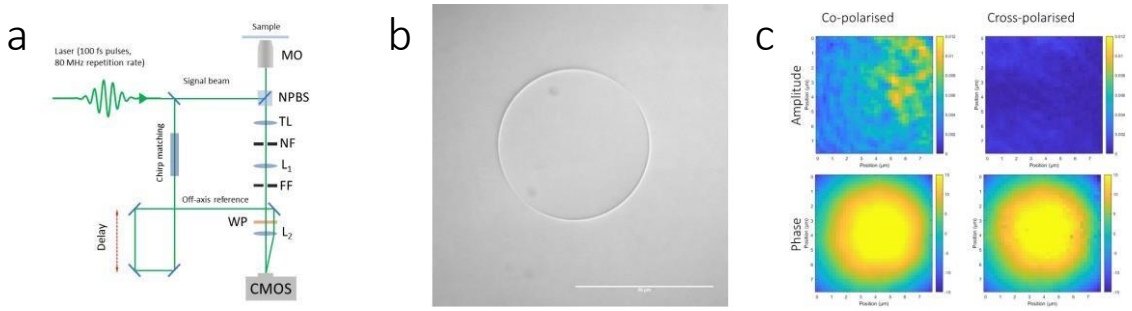

Fig. 1. a. Schematic showing the iGOR set-up. MO: microscope objective. NPBS: non-polarising beam splitter. TL: Tube lens. L1,2: lenses. NF&FF: apertures in the near-field (NF) and far-field (FF) planes of the sample. WP: Wollaston prism [5]. **b, c.** 33µm POPC:POPE vesicle imaged in the equatorial plane using DIC (**b**) and close to the on the top surface with iGOR (**c**), showing co and cross-linearly polarized reflection amplitude and phase measured over 3ms.

References

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