Fluorescence-free quantification of vesicle loading and microviscosity

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Lipid bilayers are found in all life on Earth – they are the semipermeable barriers that delineate all cells. Consequently, understanding the compartmentalisation properties of lipid bilayer vesicles/liposomes is critical in fields as diverse as drug delivery and astrobiology.

Many analysis techniques rely on using fluorescently-labelled molecules. Fluorescent molecules, however, can often interact undesirably with the lipid bilayers[1]. In this talk, we discuss how we use digital holographic microscopy[2] to quantify various lipid vesicle/liposome properties, without using fluorophores. By fitting light scattering models to holograms of vesicles (Fig. 1), we are able to recover the best-fit parameters for the model, and thus measure the refractive index to quantify vesicle loading. We also perform microrheology inside the vesicles to investigate whether confinement inside lipid vesicles can alter the effects of macromolecular crowding.

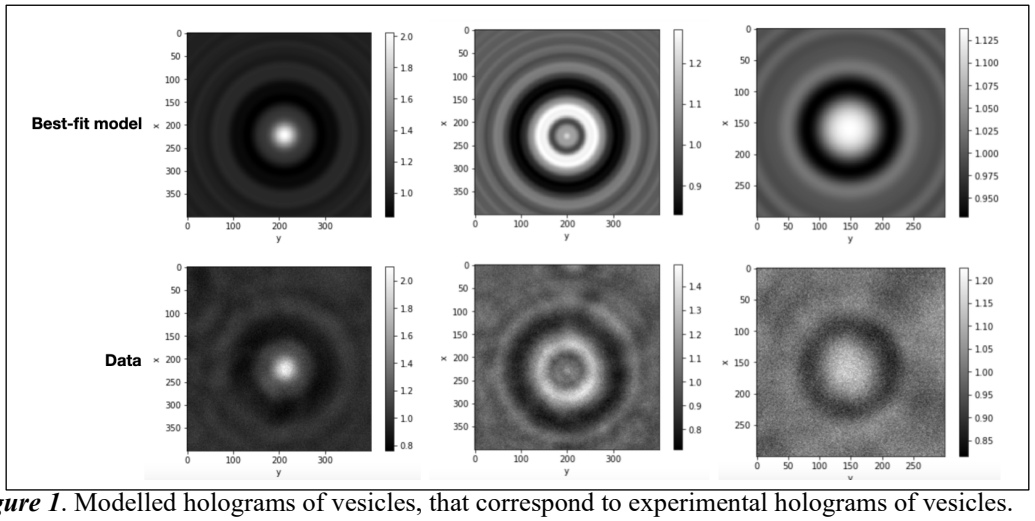

Figure 1. Modelled holograms of vesicles, that correspond to experimental holograms of vesicles.

References

[1] L. D. Hughes, R. J. Rawle, S. G. Boxer, Choose Your Label Wisely: Water-Soluble Fluorophores Often Interact with Lipid Bilayers. *PLOS ONE*. **9**, e87649 (2014).

[2] C. Martin, L. E. Altman, S. Rawat, A. Wang, D. G. Grier, V. N. Manoharan, In-line holographic microscopy with model-based analysis. *Nat Rev Methods Primers*. **2**, 1–17 (2022).