Measuring vesicle loading with holographic microscopy

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Abstract

We report a label-free method for quantifying the loading of lipid vesicles. Using an inline holographic microscopy setup, we capture holograms of vesicles in a single shot, and use a Lorenz-Mie scattering model to extract the vesicle refractive index and thus loading. In contrast to existing methods, no fluorescent reporters, fluorescent tracers, or radioactive tracers are necessary.

I. INTRODUCTION

The semi-permeable lipid bilayer membrane is a core feature of all life on Earth [1]. As a result, entire fields of research are dedicated to lipid bilayer assemblies: to use them as models for plasma membranes, bio-mimicking artificial cells, and vessels for drug delivery. Consequently, having methods to quantify their loading i.e. the amount of material they encapsulate, and how that changes as a function of time, is critical to understanding their key function as a biological container.

Presently, encapsulated solutes are typically labelled to provide a means of tracking their whereabouts. Radio-labelling or fluorescently-labelling solutes, however, can be expensive. Moreover, the hydrophobic moieties in fluorescent tags can often interact with the hydrophobic membrane. A label-free technique is thus preferable.

In previous work, we demonstrated that a core-shell light scattering model could be used to measure the thickness of lipid bilayer membranes to within the accuracy of cryo-EM measurements [2]. This approach required pre-processing the vesicle samples with extrusion through nanometre-sized pores to create a sample of a narrow size distribution.

Here we demonstrate how the solute loading of single cell-sized vesicles (giant unilamellar vesicles, GUVs) can be quantified from a digital image without sample processing. We use digital holographic microscopy to capture light that is scattered from lipid vesicles on a microscope, and use a light scattering model to extract the vesicle’s refractive index, radius, and location in three-dimensional space.

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II. RESULTS AND DISCUSSIONS

We opted to use a self-assembly method to encapsulate a model solute, sucrose. This is because methods that are used to make GUVs that encapsulate a known concentration of solute often requires the presence of oil, which often remains as contaminants in the bilayer. Furthermore, emulsion-transfer methods can also lead to vesicles catastrophically rupturing and thus losing contents. To avoid these complications, we followed the protocol for making oleic acid giant unilamellar vesicles from micelles as described in detail in Lowe et al. \cite{Lowe}, in the presence of different concentrations of sucrose. This method has been previously shown to encapsulate a range of solutes including small molecule dyes and even colloidal particles \cite{Liu}. We then diluted the samples 1 part in 10 into a equiosmolar solution containing glucose, resulting in vesicles that encapsulate and maintain a sucrose gradient (Fig. 1A).

Holograms of the same vesicles are shown in Figure. When in focus, the vesicles are almost invisible. As the focus is shifted, however, interference fringes appear, containing information about the contents of the vesicles.

We then fit a Lorenz-Mie model for how spheres scatter light to the holograms \cite{Liu}, using an implementation within the Python package HoloPy \cite{HoloPy} that takes the objective lens into account \cite{Gaussian}. The input parameters for the model are the vesicle’s refractive index $n$, radius
To correlate a refractive index measurement with vesicle loading, we needed to know the refractive index of sucrose as a function of concentration. We used an Abbe refractometer to measure standard curves for sucrose and glucose solutions in the presence of 100 mM bicine buffer at the sodium line ($\lambda = 589$ nm).

We found that a sample initially measured $n = 1.3497 \pm 0.0004$, corresponding to an encapsulated sucrose concentration of 450 mM. Over one week, the same sample had vesicles measuring $n = 1.3476 \pm 0.0003$, corresponding to encapsulation of 350 mM sucrose. Given the timescale of a week and the average flux across the membrane, this corresponds to a sucrose permeability of $2 \times 10^{-11}$ cm/s.

III. CONCLUSIONS

In summary, we have shown that holographic images of vesicles can be analysed against a Lorenz-Mie light scattering model to quantify the refractive index of the vesicles. The measurement is non-invasive, and requires only 1 $\mu$L of sample. In future, this work can be expanded to analysing different solutes, and the effect of pores and toxins on the ability of
FIG. 3. Vesicle loading was tracked over time ($N = 10$) to determine sucrose leakage over time.

lipid membranes to retain solutes.

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