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# **An encapsulated Droplet Interface Bilayer Array for the High-Throughput Optical Measurement of Lipid Membranes with Single Bilayer Resolution.**

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Droplet interface bilayers (DIBs) represent a recently developed method of generating stable artificial lipid membranes suitable for electrophysiology and optical interrogation. Prepared from the contacting of two aqueous droplets in oil, in the presence of dissolved lipid, DIBs are usually confined to a hydrophobic medium. Recent advances have created freestanding, hydrogel-encapsulated droplet interface bilayers (eDIBs) that are aqueous compatible, self-supporting and can withstand mechanical handling. Microfluidic methods allow for their rapid generation, paving the way for high throughput measurements on individually addressable lipid bilayers.

Here we report on the mass preparation of eDIBs using a 3D-printed microfluidic device and the proof-of-principle demonstration of high-throughput optical membrane screening with single bilayer resolution. Individual eDIBs are produced and output into wells of a 96-well plate. Optical measurements are made reporting on membrane leakage to monitor membrane integrity by fluorescent measurement. Addition of bilayer disrupting agents, such as detergents, to individual wells enables parallel measurement of membrane activity using a standard fluorescent plate reader. This platform provides sufficient sensitivity to measure the leakage of dye through membrane spanning pores in otherwise intact bilayers, as well as direct detection of bilayer failure.

This high-throughput, scalable and automatable approach to arrayed bilayer measurements offers exciting opportunities for application with further fluorescent-based membrane, or protein assays. The segregated nature of each droplet allows for the separation of contents either side of the bilayer. Individual bilayers can be independently addressed, affording the opportunity to screen a wide parameter space with single bilayer resolution. The ability to reconstitute membrane proteins into DIBs and eDIBs creates many exciting opportunities for the optical screening of membrane proteins and high throughput biophysical measurements.