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Improved Environmental Control and Experimental Repeatability with New In-Situ Devices

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Abstract: As liquid cell experiments in electron microscopy have increased in popularity, a number of challenges have emerged surrounding current microfluidic platform limitations. Commercially available liquid flow cell holders trap a small volume of liquid between two thin electron transparent membranes, most commonly silicon nitride supported by a thicker silicon substrate [1]. Often a spacer material, usually either an inert metal or polymer thin film, is used in an attempt to define the fluid path length. This assembly is then loaded within a chamber at the tip of an instrument compatible holder and sealed hermetically with a series of O-rings. Liquid or gas is delivered to the viewing area via microfluidic tubing that travels the length of the holder and empties into wells surrounding the silicon devices without exposure to the vacuum of the instrument. Sample inflow/outflow wells are arranged such that the silicon devices containing the electron transparent membranes are located between wells, and any sample introduced into the hermetic chamber must flow either between or around the silicon devices before exiting through the outflow well. In this way, liquid samples can be imaged while protected from the high vacuum environment of an electron microscope, and exchange/introduction of sample material can be presented to the imaging area with flow capabilities.

While these designs have proven effective, a number of limitations have been identified in recent years that continue to limit experimental reproducibility of liquid cell experiments. The dimensions and design of current electron transparent membranes and support geometries confine the effective viewing area to a maximum of $0.01mm^2$, with many experiments restricted to $0.0025mm^2$ or less. Additionally, the pressure differential between the vacuum of the instrument and the environmental sample results in significant outward bulging of the electron transparent membranes, increasing the thickness of the liquid cell and further limiting the overall effective imaging areas [2]. For example, many dose related experiments studying water hydrolysis and reaction kinetics have been shown to be volume sensitive, and membrane bulging complicates attempts to quantify beam related damage and reproduce growth, or other dynamic experiments [3]. Finally, the thickness of the liquid layer is also difficult to reproduce even when using patterned spacers of known thickness. Environmental contaminants or the sample itself can function as unintended spacers which dictate the experimental thickness if they become trapped between the surface of one device and the spacer of another, or are themselves larger than the intended nominal spacing. Thickness increases due to sample size or entrapment can be mitigated somewhat by assembling the device and flowing sample particles between the windows once within the instrument. However, holder geometries allow for significant bypass of flow around the chips, which in many cases can be 2-3 orders of magnitude greater than the intended fluid thickness between the membranes, hindering the amount of sample that can be detected in the already very limited viewing area.

We will present a new platform for overcoming the limitations listed above, in an attempt to increase the usability of in-situ holders and simplify interpretation and repeatability of liquid cell results. We will

highlight results from using this platform to study both soft/biological samples and materials/chemistry systems to demonstrate the versatility of the approach.

References:

[1] N. de Jonge and F. Ross Nature Nanotechnology **6** (2011) p.695
[2] K. Jungjohann, *et al* Microscopy and Microanalysis **18** (2012) p. 621
[3] T. Woehl, *et al* ACS Nano **6** (2012) p. 8599
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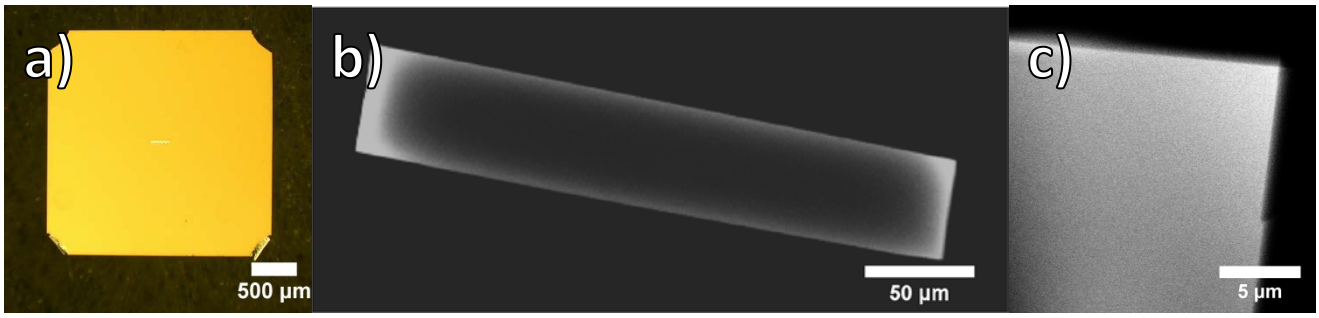

Figure 1: a) A standard silicon device for use in commercial in-situ holders. A single silicon nitride window can be seen centered on the device. b) Low-magnification bright field STEM image of an assembled liquid cell. Significant bulging of the windows is apparent by the contrast gradient seen across the window. Imaging is often limited to corners where electron transmission is maximized. c) Bright field STEM image of the corner of a silicon nitride window similar to that seen in (b). The contrast gradient indicates thickness changes as a result of window bulging, and highlights the lack of equivalent imaging areas within a typical liquid cell.

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