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Development of Reinforced Silicon Nitride Membranes for *in situ* Liquid Electron Microscopy

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In situ transmission electron microscopy (TEM) of samples in liquid environments is a rapidly growing field in both material and biological research [1, 2]. Samples are commonly isolated from the vacuum of the microscope column by confining them between a pair of silicon microchips that support a thin, electron transparent silicon nitride (SiN_x) membrane [3]. However, elastic deformation (bulging) of the SiN_x membrane under the pressure differential from the liquid-filled cell to the vacuum results in an increase in the liquid thickness as the distance from the edge of the silicon nitride window increases, thus compromising resolution [4, 5]. The maximum deformation in the window or bowing displacement is proportional to the width of the window and inversely proportional to membrane thickness [6, 7]. The liquid cell microchip design aims to maximize resolution by limiting the amount of membrane bowing that occurs due to the vacuum of the electron microscope. Increasing membrane thickness reduces the amount of bowing according to a load-deflection relationship, as shown in Figure 1, but negatively impacts image resolution due to increased scattering. Additionally, reducing membrane window dimensions also reduces deformation but severely limits the area available for observing the sample. Previously, we have shown how using a cross-window geometry can reduce the bulge effect and enable a more uniform thickness profile [4]. Here, we explore an additional strategy to reduce membrane bowing while maintaining a large imaging area by reinforcing the nitride membrane itself.

We have established two methods for reinforcing the SiN_x membrane. In the first technique, shown in Figure 2, selected regions of a 200-nm-thick silicon nitride membrane are etched to achieve imaging regions with 50-nm thickness. Here, smaller squares of 50-nm nitride are patterned into a larger 200-nm thick nitride frame. This helps to minimize deflection because the slope of the deflection is largest at the membrane edges and the overall size of the thinned membrane area is minimized. The second technique involves reducing membrane deflection by reinforcing the nitride windows with Si struts across the entire window. The Si regions that are to be retained for the struts are heavily doped to create an etch-stop during the bulk etch during formation of the membrane. The Si struts are thicker than the nitride and have a larger moment of inertia and therefore should deflect less than the nitride membrane without any reinforcement.

The amount of bowing undergone by the reinforced windows was determined by measuring the liquid thickness using mass-thickness maps of liquid chambers assembled from both standard and reinforced silicon nitride windows. These maps were generated by the log-ratio method: $t / \lambda = \ln(I_t/I_0)$ [8], where ratio of specimen thickness (t) to the inelastic scattering mean-free-path (λ) is calculated from the intensity of the zero-loss energy-filtered image (I_0) and unfiltered image (I_t). For fluid thicknesses above 1.5 μm, Klein and Anderson developed a second method using the mode (energy of peak intensity) of the electron energy-loss spectrum, which provides a linear correlation with fluid thickness [4]. The effectiveness of reinforcement to reduce membrane bulging will be presented.

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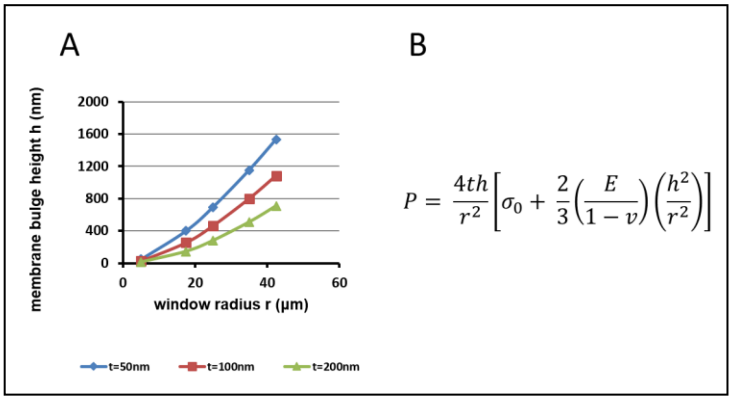

$$P = \frac{4th}{r^2}\left[\sigma_0 + \frac{2}{3}\left(\frac{E}{1-v}\right)\left(\frac{h^2}{r^2}\right)\right]$$

Figure 1. (A) Plot of membrane bulging as a function of window width and membrane thickness. (B) Theoretical pressure differential (P) dependence and calculated bulge height h as a function of radius r of electron-transparent SiN_x window of thickness t. Here, σ_0 is the residual ($P = 0$) stress, E is Young's modulus, and v is Poisson's ratio for the SiN_x membrane [9].

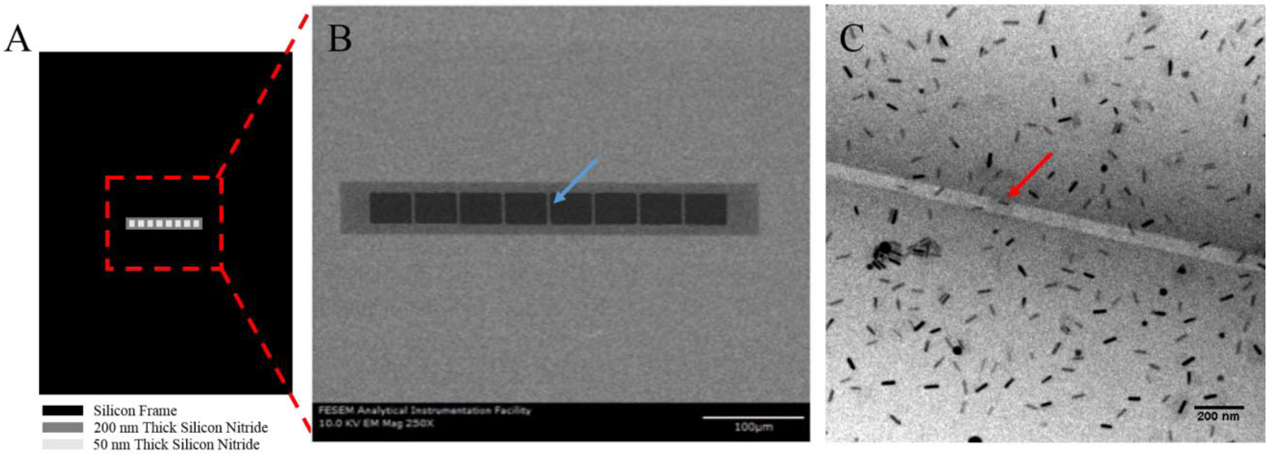

Figure 2. Reinforced SiN_x microchips for liquid TEM imaging. (A). Schematic (not to scale) of a liquid cell microchip containing a reinforced SiN_x membrane. (B) Scanning electron microscopy (SEM) image of the reinforced window. An example of the SiN_x strut is highlighted by the blue arrow. (C) Energy-filtered TEM image of gold nanorods in water. The 200 nm thick SiN_x strut is indicated by the red arrow.

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