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Temperature-Controlled Fluidic-Cell Scanning Electron Microscopy

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Fluidic-cell electron microscopy has generated a great deal of interest due to the potential for real-space and real-time imaging of nanometer-scale objects in a fluidic environment [1]. While the possibilities of this technique have motivated a number of interesting studies from nanoparticle growth to electrochemical processes, there remain a number of significant challenges to achieving true *in situ* microscopy on technologically relevant samples [2, 3]. One of these challenges is the lack of temperature control in commercially available systems for fluidic-cell transmission electron microscopy (TEM). Even in newer TEM fluidic-cell systems with integrated resistive heating and temperature sensing elements, there are significant unresolved questions of temperature control and uniformity. Clearly, achieving temperature control inside the geometrically restricted environment of the TEM sample holder is a significant challenge. However, the scanning electron microscope (SEM) chamber does not have the same restrictions on sample size. Here, we demonstrate the use of a custom built MEMS-based SEM fluidic cell with temperature control to address this limitation.

While much of the effort in fluidic-cell electron microscopy has focused on the TEM platform, there are several significant advantages for an SEM-based fluidic cell, assuming experiments can be designed around the concomitant constraints. First, the nanofluidic environment of the TEM fluidic cell is not truly representative of "beaker-scale" experiments as the fluid is confined to a thin layer in close proximity to two membranes. As a result, imaging in fluidic-cell experiments is rarely carried out in a homogeneous fluidic environment, but rather one images processes at the fluid/membrane interface. The situation in the SEM fluidic cell is similar, however only one electron transparent membrane is required allowing a relatively large fluid reservoir to better represent bulk fluidic processes. Second, due to the limited penetration depth of the lower energy electron beam in the SEM, imaging is limited to features on or near the membrane. While this may be a disadvantage in some experiments, for processes where one is interested in surface phenomena (e.g. particle adsorption/desorption), this is actually an advantage as only particles on the membrane are visible and quickly blur as the particle-membrane distance increases. Third, due to the large SEM sample chamber it is possible to use temperature control systems that are more robust and provide better uniformity. Fourth, by using a variable-pressure SEM, one can mitigate the effects of membrane charging which can significantly impact experimental results due to electrostatic interactions with particles.

In our SEM fluidic-cell implementation, we use a custom-fabricated MEMS chip with a 50 nm thick silicon nitride membrane mounted over an o-ring which surrounds a fluid reservoir in an aluminum sample holder. Resistive heating elements are attached to either side of the holder which is mounted on a Peltier stage for active cooling. The large size of the heaters and sample stage provides for a uniform temperature distribution and temperature control of within approximately 0.2 °C using an automated PID temperature controller. Temperature measurement is made possible by mounting a platinum resistance temperature detector (RTD) on top of the aluminum lid near the MEMS chip. To demonstrate the temperature control capabilities, we investigated the association and dissociation of DNA-

functionalized gold nanoprisms to a silicon nitride membrane functionalized with complementary DNA sequences [4]. Two separate sets of experiments were performed: 1) the nanoparticles were first adsorbed on the membrane *ex situ* and then loaded in to the fluidic cell with a buffer solution for melting *in situ* and 2) a nanoparticle suspension was loaded in the fluidic cell and a functionalized membrane was mounted for *in situ* adsorption by DNA hybridization. These samples were monitored by SEM imaging by backscattered electrons as a function of temperature and time to visualize the adsorption and desorption of particles. However, since the electron beam effects during imaging disrupt the process, each image was captured in a new and undisturbed area. By this method, we can develop a statistical understanding of the dynamics of nanoparticle adsorption and desorption in this complex and strongly temperature-dependent system. Our results show hybridization (Figure 1) and melting behaviour that corresponds closely to results from spectrophotometry experiments and offers the potential for new and critical insight at the single-particle level [5].

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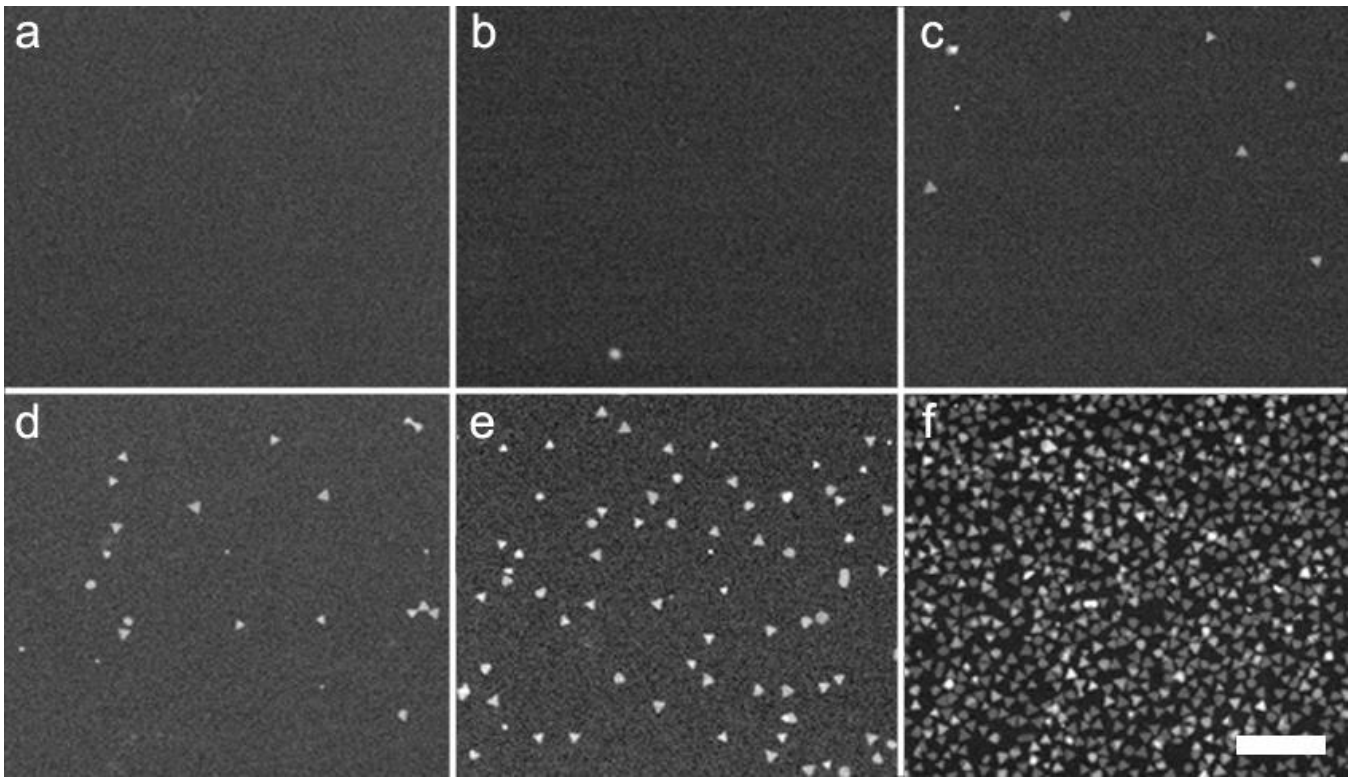

Figure 1. Series of backscattered electron images from SEM fluidic-cell studies showing the temperature-controlled adsorption of DNA-functionalized nanoprisms on a silicon nitride membrane functionalized with complimentary DNA at a) 10 minutes - 22 °C, b) 60 minutes - 37 °C, c) 75 minutes - 38.5 °C, d) 80 minutes - 40 °C, e) 120 minutes - 40 °C, f) 250 minutes - 40 °C. (Scale is 1 µm)

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