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Electron Microscopy in Air: Transparent Atomic Membranes and Imaging Modes

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Environmental Scanning Electron Microscopy (ESEM) where differential pumping and a pressure-limiting aperture [1] has enabled electron imaging in partial atmosphere environments. The typical imaging gas path length (GPL) is 2~5 electron mean free paths (mfp), resulting in lower contrast than a pure vacuum SEM. More recently, the use of thick (100nm) SiN_x membranes to separate the electron optics in vacuum and the specimens in atmosphere has led to Atmospheric Scanning Electron Microscopy (ASEM) [2] where imaging in liquid or air without a specimen chamber is made possible by placing specimens in contact with the membrane. However, the resolution and contrast in both ESEM and ASEM are compromised by the multiple electron scattering. By keeping the sample away from the electron transparent membrane, thinner membranes can be used (and reused). This has led to the airSEM [3,4], where a thin SiN_x window is used to separate electron optic and air from an optically-aligned sample (Fig 1(a)). The airSEM is able to image specimens in air with high throughput – a few minutes per sample at low magnification [3].

The key to high quality imaging is minimizing the multiple scattering. The mfp of low energy electrons (lower than 10 keV) in SiN_x is a few nanometers, much shorter than that in air (tens of microns), indicating the electron scattering within the SiN_x window is the main limitation on image contrast for the GPL at which the airSEM operates. Graphene is an ideal window material due to its high mechanical strength, single atomic layer thickness and long electron mfp, which keeps the combined path length to within one mfp, a single-scattering regime comparable in resolution and contrast to vacuum SEM. Monte Carlo simulations (Fig 1 (b)) show the image contrast of gold nano particles imaged through a 2-layer graphene window is 2.1 times greater than the contrast through the thinnest commercially available (5 nm) SiN_x windows. We have fabricated and tested bilayer graphene windows. The experimental airSEM images at 7 keV of gold nanoparticles in Fig 1 (c) and (d), the signal-to-noise ratio (SNR) for our graphene window are 2.85 times greater rather than for a 10 nm SiN_x window.

In addition to preserving a smaller probe in airSEM, different imaging modes have been developed to improve the resolution and contrast. A secondary electron detector will not work in air, but by collecting the ion current in air generated mainly by secondary electrons, a surface detector can show better resolution and contrast than a standard backscatter electron (BSE) detector. With the surface detector, we observed single-layer graphene on a copper foil in Fig 2 (b), where the contrast follows that of the secondary electron coefficients of Cu and carbon. We have taken in-air images of untreated biological samples, such as e.coli bacteria in Fig 2 (d), which were prepared simply by drop casting living e.coli bacteria on a conductive optical microscope slide. The surface detector is sensitive to surface features, and shows typical secondary electron edge contrast. We built high-resolution bright field (BF) [4] and dark field (DF) airSTEM detectors for imaging the internal structure of very thin samples. Images of e.coli bacteria in air using BF and DF airSTEM detectors, show their internal structure clearly (Fig 3 (a) and (b)). In addition, spectroscopic detectors, such as EDS and cathodo luminescence (CL), were combined with airSEM without the need for special sample preparation. Fig 3 (c) is an X-ray map taken from an adobe plaster from the Jordan Valley, showing the elemental composition of the grains.

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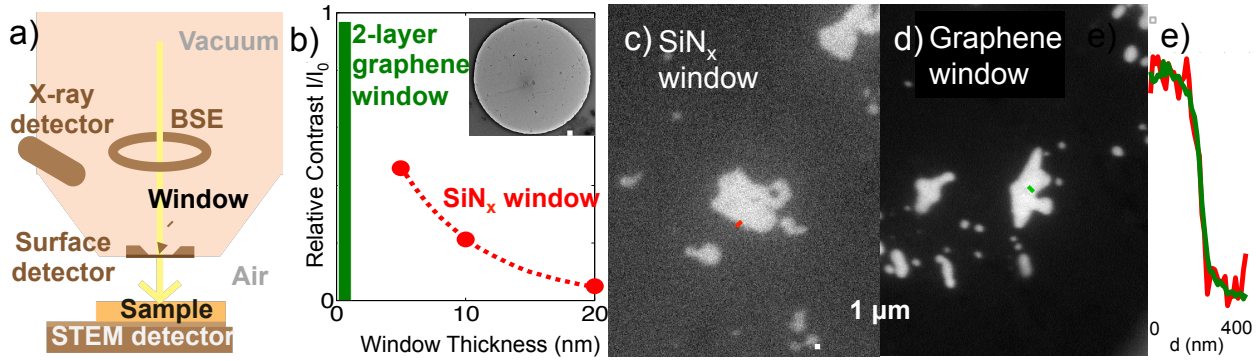

Figure 1. (a) Schematic of airSEM; (b) Monte Carlo simulation for 7 keV electrons of the relative contrast for a 2-layer graphene window (Green bar) and SiN_x windows with different thickness (Red dots). The contrast has been normalized to that of the gas path without a window, I_0. Inset is the TEM image of a 2-layer graphene window, scale bar: 1 μm. AirSEM BSE images of gold nanoparticles at 7 keV electron beam energy with (c) 10 nm SiN_x window and (d) 2-layer graphene window; (e) Line profiles of gold particle edges, which are indicated by red line in (c) and green line in (d).

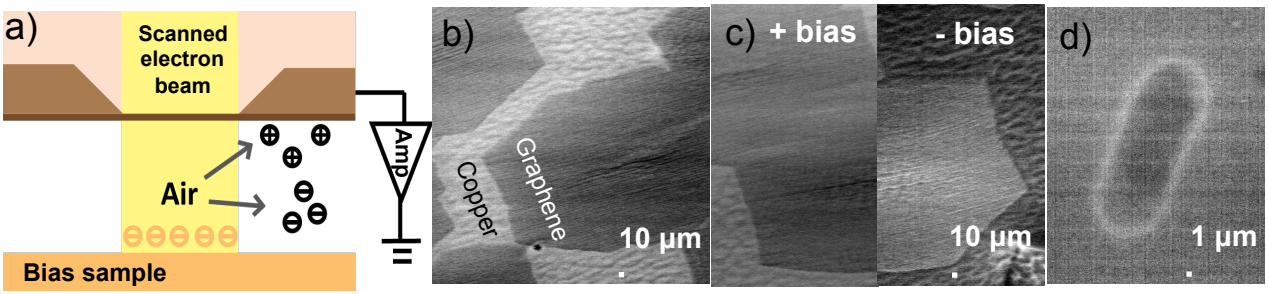

Figure 2. a) Schematic of the surface detector b) Surface detector image of single-layer graphene grown on Cu foil; c) Single-layer graphene images with positive and negative specimen bias voltage d) Unstained drop-cast living e. coli bacteria image by surface detector.

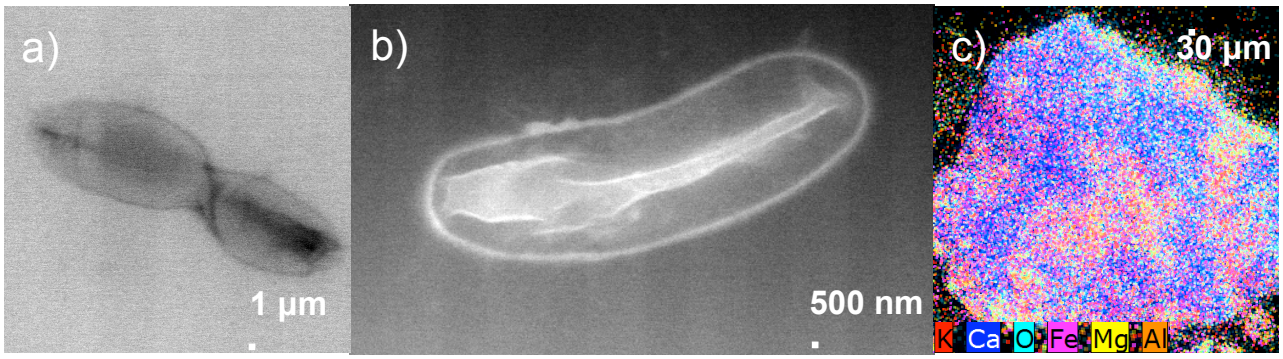

Figure 3. High-resolution a) BF airSTEM image and b) DF airSTEM image of uranium-stained e.coli bacteria; c) X-ray compositional map of an adobe plaster from Jordan Valley.

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