Integrated wafer-scale process for batch-fabricating electron microscopy grids with tunable foils for cell guidance

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Cryogenic electron microscopy and tomography (cryo-EM and cryo-ET) are high resolution transmission electron microscopy techniques that are revolutionizing biological imaging. Specimen supports, known as EM grids, directly influence image quality, with all-gold grids minimizing beam-induced motion relative to grids with carbon foil. We have developed a fully integrated, wafer-scale batch-fabrication process for manufacturing biocompatible all-gold EM grids using a 4″ fused silica sacrificial substrate, photolithography, lift-off, gold electroplating, and wet etching. This process yields ~600 gold EM grids per wafer with performance that matches or exceeds commercial alternatives, while providing cost efficiency and design flexibility in both mesh and foil patterning.

To enable reliable grid thickness and optimize the trade-off between mechanical robustness and tilt-series transparency, we established a quantitative simulation framework for gold electroplating. The model captures time-dependent changes in effective plating area as foil holes progressively fill, providing predictive control of deposition dynamics and final thickness. Using this approach, we reproducibly fabricated grids with electroplated rims and bars on the order of several micrometers (e.g., ~8 μm) and demonstrated their compatibility with routine vitrification and cryo-EM handling workflows.

Beyond scalable manufacturing, our grid manufacturing process provides design freedom in foil and mesh geometry. We demonstrate a second-generation grid design featuring anisotropic (2×6 μm oval) foil perforations that passively bias cytoskeletal filament alignment in endothelial cells (Fig. 1), yielding substantially higher directional anisotropy compared to circular-hole foils (Fig. 2). In addition, we show compatibility with established grid micropatterning methods for controlled cell adhesion and organization. Together, these results provide an accessible route to high-performance, customizable all-gold EM grids that combine wafer-scale manufacturing with programmable cell–substrate interactions, enabling unprecedented cryo-ET studies in mechanobiology.

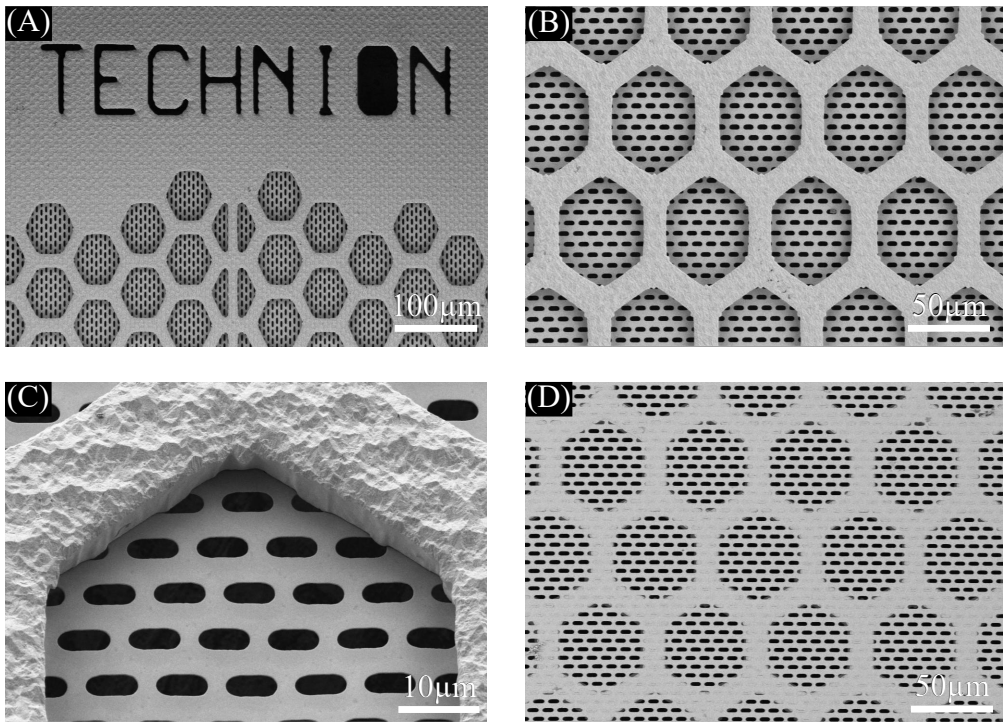

Figure 1: Scanning electron micrographs of EM grids with oval foil. (A) Rim of the grid. (B) Back side of the grid. (C) Close-up of a single grid bar hexagon imaged at a 35◦ tilt angle. (D) Front side of the grid on which cells are grown

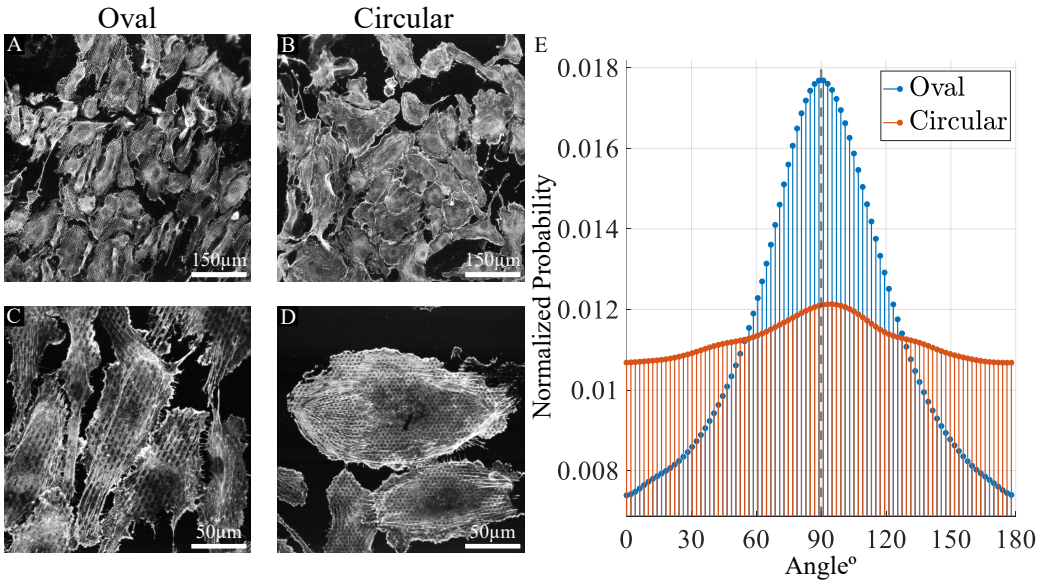

Figure 2: Cells cultured on EM grids with (A,C) oval vs. (B,D) circular holes in foil. (E) Normalized probability of local filament orientations for cells grown on oval (blue) and circular (orange) holey foil grids. The pronounced peak near 90◦ for the oval grids indicates strong alignment of the filaments. In contrast, the circular grids show a broader, lower-amplitude distribution without a distinct alignment to a specific angle.