The ability of living cells to respond to mechanical cues from the microenvironment plays a vital role in physiological processes such as embryonic development and cardiovascular function. Over the past decade, the field of mechanobiology has seen major advances catalyzed by increasingly powerful strategies to measure cell-generated forces and to identify mechanosensitive molecules and cellular components. However, we know comparatively little about the nanometer-scale organization of the cellular components that underly the cell’s ability to generate and sense mechanical force. This knowledge gap reflects a lack of tools that can visualize cellular organization at the nanoscale. Cryo-electron tomography (cryo-ET) is the highest resolution tool available for structural analysis of cells, but it is limited by the throughput of data collection. In this talk, I will present an extracellular matrix (ECM) micropatterning technology developed for cellular cryo-ET. We used mask-free photo-micropatterning to functionalize electron microscopy (EM) supports to direct cell positioning at high spatial accuracy, solving an important bottleneck in sample preparation for cellular cryo-ET. We demonstrated the versatility of this technique to control cell shape across a variety of cell types and ECM geometries.

Adapting our micropatterning technology to optimally position endothelial cells (ECs) on EM supports enabled high-throughput imaging of mechanosensitive EC intercellular contacts. Here, we designed a lattice micropattern to direct intercellular contact assembly and showed that “bowtie” shaped micropatterns significantly increase the number of contacts positioned on regions of the EM supports that are accessible to the electron beam. In addition, our quantitative analysis indicates that the lattice micropattern directs the thicker cell nuclei away from the intercellular contacts such that they can be sufficiently thin for successful imaging by cryo-ET. Our cryo-tomograms revealed a diversity of sub-cellular structures at intercellular contacts, such as contacting filamentous actin rich membrane protrusions between cells, bundles of intersecting membrane protrusions, and a range of vesicle shapes and sizes within and outside of the plasma membrane.

Our micropatterning technology can be generalized to facilitate structural studies of a variety of other systems such as neuronal and immunological synapse formation. This study thus advances cellular cryo-EM by refining techniques that dramatically increase the throughput of data acquisition. In addition, by enabling direct observation of nanoscale organization in cells under morphological modulation, this technology opens the door to elucidating the nanometer-scale underpinnings of mechanobiology. I will briefly outline my lab’s future plans to implement cellular cryo-ET, together with microelectromechanical systems (MEMS) and live-cell imaging, to discover how physical cues are translated into the intracellular signals that control cell and tissue architecture.