Investigating the Preparation Methods for Super-Resolution Imaging of Tunneling Nanotubes

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Tunneling nanotubes are long-range, membrane enclosed tubular structures spanning from cell-to-cell that can mediate intercellular communications and transfer of organelles, viruses, and protein aggregates across many microns. The major cytoskeleton component responsible for this transfer are linear actin bundles that span the entire length of the nanotube, connecting the cytoplasm of each cell. Despite the near two decades of study into nanotubes, the understanding of their formation and organization is limited. Tunneling nanotubes regularly measure under 700 nm in diameter but can measure up to 200 µm in length; the thin, fragile nature of these structures cause them to easily become disrupted during fixation processes and inhibit their study. The goal of this study was to establish methods that allow us to observe organizations of actin within tunneling nanotubes at nanoscale using super-resolution microscopy. The study tested a range of cell confluency, fixation and permeabilization conditions for establishing and preserving nanotubes between U2OS cells and used Alexa-647 phalloidin to label actin filaments for stochastic optical reconstruction microscopy (STORM) imaging. We found that gentle, multi-step fixation and low-degree permeabilization gave the best results at preserving long and thin cellular protrusions, such as filopodia, which are considered potential precursors of nanotubes, as well as nanotube-like structures connecting between cells.