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Microsystems for cryo-CLEM



Correlative Light and Electron Microscopy (CLEM) is a powerful tool for investigating the connections between cellular structure and function. Performing CLEM on vitrified cells at cryogenic temperature (cryo-CLEM) is particularly interesting, as sub-cellular structures can be preserved very close to the native state and radiation damage is significantly reduced. However, cryo-CLEM technology still has several important limitations today. First, conventional cryofixation by high-pressure freezing or plunge freezing interrupts live-cell imaging, as it requires sample preparation and transfer between instruments. Second, only air objectives are available in cryogenic light microscopy, limiting the numerical aperture to less than one. To overcome the first limitation, our group has introduced a microsystems-based cryofixation technology that enables cryofixation of thin samples ($\leq 20 \mu\text{m}$) within fully operating microfluidic devices directly in the light microscope. Recently, we have been investigating applications of this approach to studies of cell biomechanics and cryopreservation. To address the second limitation, we designed a new type of light microscope and cryo-immersion objectives. I will describe recent advances towards integrating this technology into a new immersion-based correlative light and electron microscopy workflow and enabling cryo-STED microscopy with immersion objectives. We expect that, together, microfluidic cryofixation and immersion-based cryo-CLEM will be of particular interest for studying dynamic relationships between cell stimulation, function, and structure at the nanometer scale.

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