

Subcellular Microdissection for the Identification of Organelle Proteins

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Abstract:

Laser capture microdissection (LCM) and expression microdissection (xMD), a related technology, are being explored to allow unsupervised capture of subcellular compartments in a high-throughput fashion. Our objective is to establish a means of dissecting these chemical components of cells while preserving spatially relevant associations of proteins and other biomolecules. LCM, as originally developed at NIH, exhibits a limitation in that lateral resolution is constrained by the IR laser beam size and the volume expansion of the melted polymer. A team of engineers, histologists, and analytical chemists are testing materials and methods to enhance the lateral and depth resolution of these microdissection techniques, in order to procure subcellular organelles, such as nuclei, cilia, mitochondria, and the Golgi apparatus. The subcellular targets of interest can be selectively stained with either metal-containing or darkly staining dyes (e.g., immunohistochemical) so that they can be identified and transferred to film surfaces in an unsupervised manner for chemical proteomic analyses using mass spectrometry. In the first four months of this award, we have been able 1) to routinely make new thermoplastic films of varying thickness, absorbance, and polymer composition, 2) bond to the apical surface of epithelial membranes and transfer fluorescence-labeled elements selectively where the film was activated with these thin transfer films; and 3) began optimizing procedures for direct digestion of proteins on the thermoplastic films for introduction into an electrospray ionization mass spectrometer (MS) for proteomic analysis.

