Optimized procedures for subcellular spatial alignment during live-cell CLEM

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Abstract
Live-cell correlative light and electron microscopy (CLEM) offers unique insights into the ultrastructure of dynamic cellular processes. A critical and technically challenging part of CLEM is the 3-dimensional relocation of the intracellular region of interest during sample processing. We have developed a simple CLEM procedure that uses toner particles from a laser printer as orientation marks. This facilitates easy tracking of a region of interest even by eye throughout the whole procedure. Combined with subcellular fluorescence markers for the plasma membrane and nucleus, the toner particles allow for precise subcellular spatial alignment of the optical and electron microscopy data sets. The toner-based reference grid is printed and transferred onto a polymer film using a standard office printer and laminator. We have also designed a polymer film holder that is compatible with most inverted microscopes, and have validated our strategy by following the ultrastructure of mitochondria that were selectively photo-irradiated during live-cell microscopy. In summary, our inexpensive and robust CLEM procedure simplifies optical imaging, without limiting the choice of optical microscope.