



## Supplementary Materials

## Cell Volume (3D) Correlative Microscopy Facilitated by Intracellular Fluorescent Nanodiamonds as Multi-Modal Probes

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**Figure S1.** A thin 100 nm cell section imaged with SEM. The Zeiss LEO 1530 (Zeiss, Germany) SEM instrument used was for imaging. FNDs were aggregated in vesicles of different sizes ranging from 0.5–2µm. Vesicular localization of FNDs provides high contrast for imaging.



**Figure S2.** FNDs landmarks facilitated the alignment of the MitoTracker channel over SB-EM stacks. We have demonstrated here the image alignment across different Z planes. **A)** FNDs (red) and Mitotracker (green) are shown overlaid over SB-EM images and vesicle aggregated FNDs can be seen as well. **B)** A large FNDs vesicle correlated with complementary fluorescence data from same planes. **C)** Corresponding SEM and LM sections alignment.



SB-EM slice no: 37 LM slice no: 9

SB-EM slice no: 73 LM slice no: 23

**Figure S3.** The major challenge encountered in the CLEM experiment was inherently low axial (600 nm) and lateral (250 nm) resolution provided by confocal microscopes compared to the nanometer scale resolution provided by EM. Currently, the limited resolution of confocal microscopy can result in the misalignment of details within large scale datasets, especially across the Z-axis.

**Supplementary Video Files 1–6**. The video files referred to in the article as **Video 1–6** are available on Zenodo at <u>https://zenodo.org/record/4279702</u> (DOI: 10.5281/zenodo.4279702)



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