Correlative Cryogenic Super-Resolution Fluorescence and Electron Tomography

Motivation: Cryogenic electron tomography (CET) and super-resolution fluorescence (SR) are two powerful methods for the observation of subcellular organization, but both methods suffer from unique limitations. Specifically, there are no specific and non-perturbative labelling methods for CET and even high-resolution SR reconstructions lack detailed cellular context.

Result: Here we demonstrate accurate and precise single-molecule fluorescence localizations correlated with CET. This correlation identifies specifically labeled proteins of interest within a high-resolution context.

CIASM Workflow

Sample Preparation
- Mounting grid
- Place cells on grid
- Cryo freezes

Cryogenic Fluorescence
- Under cryo conditions
- Blue light

Cryogenic Electron Tomography
- Low magnification
- High magnification

Image Registration and Visualization
- Fluorescence
- Low mag SR
- High mag SR

CIASM Results For Two Proteins of Interest

McpA-PAmKate Localizations

PopZ-PAmKate Localizations

Highlights:
- Mean localization precision 9 nm
- Registration error ~30 nm
- Compatible with any protein fused to PAmKate fluorescent protein label
- Workflow “Correlative Imaging by Annotation with Single Molecules” (CIASM)