

# Correlative cryo-fluorescence and cryo-soft X-ray tomography of autophagy in whole, unstained mammalian cells

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Cryo-soft X-ray tomography (cryo-SXT) is a synchrotron-hosted imaging technique used to analyse the ultrastructure of intact, cryo-prepared cells. Correlation of cryo-fluorescence microscopy and cryo-SXT can be used to localise fluorescent proteins to organelles preserved close to native-state. Cryo-correlative light and X-ray microscopy (cryo-CLXM) is particularly useful for the study of organelles that are susceptible to chemical fixation artefacts during sample preparation for electron microscopy. In our recent work, we used cryo-CLXM to characterise GFP-LC3-positive early autophagosomes in nutrient-starved HEK-293A cells (Duke et al., 2013). Cup-shaped omegasomes were found to form at 'hot-spots' on the endoplasmic reticulum. Furthermore, cryo-SXT image stacks revealed the presence of large complex networks of tubulated mitochondria in the starved cells, which would be challenging to model at this scale and resolution using light or electron microscopy.

Other biological application examples will be discussed with intent to give a flavour of the kind of study that would benefit from this novel imaging platform, though applications will undoubtedly expand as cell biologists adopt the technique.

## Reference

Duke EM, Razi M, Weston A, Guttman P, Werner S, Henzler K, Schneider G, Tooze SA, Collinson LM. Imaging endosomes and autophagosomes in whole mammalian cells using correlative cryo-fluorescence and cryo-soft X-ray microscopy (cryo-CLXM). *Ultramicroscopy*. **143**:77-87 (2014).

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