

Cryo-correlative light and X-ray microscopy (cryo-CLXM) workflow for the study of mammalian cells in near-native state

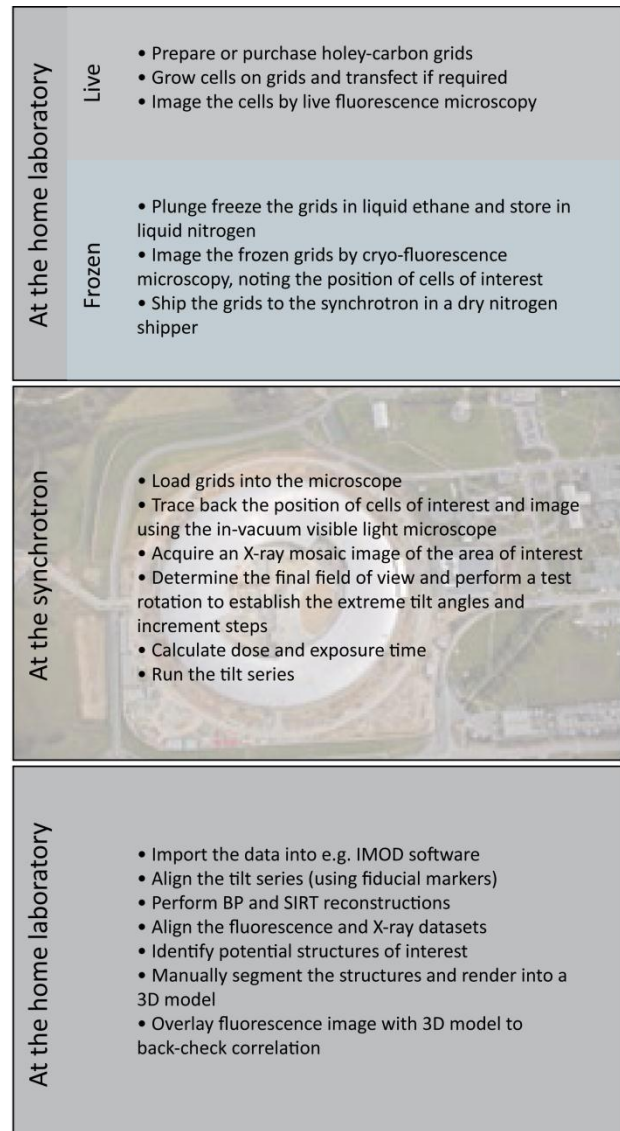
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Cryo-soft X-ray tomography (cryo-SXT) is a synchrotron-hosted imaging technique used to analyse the ultrastructure of intact, cryo-prepared cells. Correlation of cryo-fluorescence microscopy and cryo-SXT can be used to localise fluorescent proteins 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. As with any imaging technique, cryo-SXT requires excellent sample preparation for successful data collection. The cryo-CLXM workflow (see box) that we developed^{1,2} and optimised for studying adherent mammalian cells will be detailed, with a particular focus on grid preparation, cell culture, fluorescence microscopy, plunge freezing and grid storage.



References

1. Carzaniga R, Domart MC, Collinson LM, Duke E. Cryo-soft X-ray tomography: a journey into the world of the native-state cell. *Protoplasma*. **251**(2):449-58 (2014).
2. Carzaniga R, Domart MC, Duke E, Collinson LM. Correlative cryo-fluorescence and cryo-soft X-ray tomography of adherent cells at European synchrotrons. *Methods Cell Biol*. **124**:151-78 (2014).

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