User Pre-Visit Checklist

This checklist is designed to familiarise users with the workflow at B24 and to prepare them to gain the most out of their visit. If you are visiting the beamline for the first time, please complete these tasks.

STEP 1 Sample preparation

- ❑ Read the step-by-step guide for grid and fiducial preparation.
- ❑ Read the step-by-step guide for plunge freezing.
- ❑ OPTIONAL: For more detailed instructions, see this *Nature Protocols* paper

STEP 2 Cryo-structured illumination microscopy (cryoSIM)

Loading samples and acquiring data:

- ❑ Read the user manual for the cryoSIM. You may find this concise guide helpful on the day.
- ❑ Read the tutorial in the *JoVE* paper and watch its video demonstration.
- ❑ Consult the Setup menu in SpekCheck to determine which of the 'cryoSIM' laser/filter combinations would be most suitable for the fluorophores in your samples.
- ❑ Familiarise yourself with the concept of chromatic aberration, and be aware that this will cause fluorescence to focus at different Z depths in your cryoSIM sample.
- ❑ Download and install Chromagnon, a program you will use align cryoSIM data by correcting for chromatic aberration between data collected at different wavelengths.
    - Ensure that ".dv" is the output format before running the alignment in Chromagnon.

Inspecting data:

- ❑ Install Fiji and the SIMCheck plugin:
  Go to 'Help' -> 'Update…'. A window labelled 'ImageJ Updater' should appear. Select 'Manage update sites' within the ImageJ Updater window, then scroll down to SIMCheck and ensure it is ticked. Close that window and select 'Apply changes'. Restart Fiji.
- ❑ Consult the SIMCheck website and publication to learn how to use the plugin.
- ❑ View Figure 1 of this publication to familiarise yourself with common artefacts in cryoSIM data, including ghosting, hammerstroke, hatching, honeycomb, lensing, and Z-wrapping.
- ❑ OPTIONAL: Download this Fiji macro to generate maximum Z projections of your data in batches as you may find these useful when choosing regions of interests on the X-ray microscope. SIMCheck must be installed to use this.
- ❑ OPTIONAL: Consult this guide and this presentation on how to write macros in Fiji.

STEP 3 Cryo-soft-X-ray tomography (cryoSXT)

- ❑ To collect X-ray tomograms, read the step-by-step guide
- ❑ To process X-ray tomograms, view this quick start guide to using IMOD.
- ❑ Download the model datasets designed for cryo-electron tomography.
- ❑ Follow this tutorial to process the model datasets in IMOD.
- ❑ Once you receive your cryoSXT datasets, follow this tutorial to process them in IMOD.

STEP 4 Storage and transfer

Transferring data:

- ❑ Follow the steps here to use Globus or the file transfer protocol (FTP). It is best to do this as soon as possible after your visit because the data will only be available through this system for a limited time, after which it gets more difficult due to data archiving.

Organising data:

- ❑ Read the guide.
- ❑ Consult the directory structure system.

STEP 5 Correlative Light X-ray Tomography (CLXT)

- ❑ Read this quick start guide to correlation.
- ❑ Read the full standard protocol in this publication while you watch the video tutorial.

N.B. Beamline staff will be available to help answer questions and troubleshoot for the first correlation on a beamline computer.

- ❑ To carry out correlation after your visit, you should download and install Icy, a platform for bioimage analysis. Please refer to the installation guide on the Icy website for general installation, with the following specific requirements for using with our protocols:
    - Correlation is computationally intensive, so use a modern computer with at least 16 GB RAM.
    - Download Icy v2.1.3.0 (scroll down to the "previous releases" section of the downloads page), as this is the latest that is compatible with eC-CLEM v2.
    - Before opening Icy for the first time, disconnect the internet before opening for the first time to avoid auto-updates. Once open, go to 'Preferences -> General' and untick 'Enable application update', then open the 'Plugin' section and untick the 'Enable automatic update' option. Press 'Apply', then you can reconnect the internet.
    - In the 'Plugin' section of Preferences, select "Allow beta version", then open the 'Online Plugin' section. Search for 'ec-clemv2', select it and click 'Install'. If this fails, you may need to press "Reload list" due to the internet being disconnected earlier.

STEP 6 Quantitation & Segmentation (OPTIONAL)

- ❑ Consult this decision tree to determine which segmentation tool is most suitable for your data.
- ❑ To segment dark thick features like mitochondria or lipid droplets, download Contour, read the publication and follow the protocol.
- ❑ To segment other features manually, use the built-in Segmentation Editor plugin in Fiji and follow the video tutorial.
- ❑ To segment other features using machine-learning, download SuRVoS, read the publication, and follow the protocol.

Congratulations on completing the checklist! We look forward to welcoming you to the beamline.