Cryogenic Correlated Light and Electron Microscopy (Cryo-CLEM): Call for proposals

We are pleased to announce that a special commissioning call for Cryogenic Correlated Light and Electron Microscopy (Cryo-CLEM) proposals, at eBIC, is now open.

This call will provide access to a Leica Cryo Light Microscope (cryoLM) and a Titan Krios (300 keV) electron microscope fitted with an energy filter, direct electron detector and TOMO5 data collection software. Access to this equipment will be available for a limited number of selected academic user proposals.

The CryoLM has four filter sets, for use with fluorophores compatible with the specification listed in the table below.

Filter Name	Excitation Filter	Dichroic Beamsplitter	Emission Filter
DAPI	350/50	400	460/50
GFP	470/40	495	525/50
TXR	560/40	585	630/75
Y5	620/60	660	700/75

Proposals will be evaluated with respect to scientific merit, technical feasibility, and sample suitability. Applications should include evidence that the proposed samples are suitable for CLEM experiments.

If possible, grids should be pre-screened, demonstrating that they have the appropriate ice thickness, that the majority of the EM grid support film remains intact, and that there is a reasonable distribution of samples (https://www.diamond.ac.uk/dam/jcr:e8650cd6-7862-4fbf-b331-

8f1f343f8c3d/guidance_for_cryoFIB_proposals_2022-12-14_10-49.pdf). We also require that room temperature fluorescence data be presented in the proposal, to show the presence of active fluorophores. Ideally this will be performed on an aliquot of the sample which is subsequently plunge frozen on grids; however, data of equivalent samples will be considered.

For Cryo-CLEM proposals that require CryoFIB time to achieve the desired sample thickness, please indicate this in the proposal submission.

All proposals need to be submitted through the User Administrator System (<u>UAS</u>). Once logged into the UAS you should choose the "commissioning" access route and then select the Leica CLEM instrument in your proposal submission.

For technical inquiries about proposed experiments please contact: <u>julika.radecke@diamond.ac.uk or</u> <u>craig.macgregor-chatwin@diamond.ac.uk</u>

Kind regards, The eBIC team