

Euro-BioImaging
European Research Infrastructure for Imaging Technologies in Biological
and Biomedical Sciences

Interim Operation

**Guidelines for User Access at
Correlative Light Electron Microscopy (CLEM)
EuBI Node Candidates**

March 2016

The following guidelines are based on the principles that have been developed by the EuBI Preparatory Phase I consortium and guided successfully visiting scientists during the Proof-of-Concept Studies at participating CLEM facilities of several research institutions, such as e.g. the University Medical Centre Utrecht.

1 Guidelines for user access to a CLEM Euro-Biolmaging (EuBI) Node Candidate

1.1 General outline of the project schedule

1. The user interested to conduct a project at a CLEM EuBI Node Candidate can learn about the respective facilities on the EuBI web access portal. **It is strongly recommended that the user contacts informally the Node Candidate of interest** to enquire about the feasibility to conduct the project in this place, to explore the possibilities also for scientific support and to estimate together the approximate user access costs.
2. For entering the formal procedure to apply for access, the user submits a concise application via the online EuBI web access portal, including a project milestone plan to the EuBI Hub. The application can include the specific request for accessing a pre-identified Node Candidate.
3. In a first step, the user application is then scientifically evaluated by an independent board of senior scientists representing a broad scope of scientific disciplines and imaging technologies offered by EuBI.
4. After positive evaluation the user application is then forwarded to the Node Candidate for their technical evaluation and feasibility check. The Node Candidate will contact the applicant, e.g. via Skype, to clarify potentially unclear issues. If the Node Candidate approves the application in this second step, the user is invited to visit this CLEM EuBI Node Candidate and conduct the project work.
5. Due to space and personnel constraints each Node Candidate defines the number of external visitors who can be accepted for user access in a given period of time. The CLEM EuBI Node Candidate makes every effort to host the scientist as soon as possible after application to maintain scientific competitiveness. The time spent at the CLEM EuBI Node Candidate depends on the type of access that is required. The CLEM EuBI Node Candidate offers different kinds of access, which are further explained in the description of access mode (see below 1.3).
6. The logistics of the visit (e.g. accommodation, travel, shipment of reagents) should be arranged in communication with facility staff of the Node Candidate.
7. The access to CLEM instrumentation is conducted in the facility of the CLEM EuBI Node Candidate supported by the scientific environment when feasible.
8. After completion, the user will be asked to provide standardized feedback on various issues of his/her stay.
9. In the post visit period the scientist will inform the EuBI Hub when the results obtained at the EuBI Node Candidate are published in scientific journal(s) with appropriate mentioning of the support provided by the CLEM EuBI Node Candidate in the acknowledgement section of the article. Such outputs will be recorded in EuBI annual reports.

1.2 Application Guidelines

The user will send the brief application for access via a standardized template on the EuBI Web Access Portal. The application includes the following items:

1. A short CV of the applicant.
2. A short scientific project description containing the following information:
 - Project title
 - Scientific background of the project
Description of work proposed to be conducted at the CLEM EuBI Node Candidate. Address in your answer the **following topics**:
 - i. **What is the nature of the CLEM work you need doing?** Please provide a very brief project description and indicate whether the project needs both LM and EM. Also indicate which LM and EM technologies you require (e.g. live cell imaging, thin section LM, 'morphological' EM, immunoEM).
 - ii. **What is the most critical question that you need to be answered by EM?** (E.g. "What is the cellular structure that underlies the fluorescent spot that I am seeing? Which non-fluorescent labelled cellular structures are present in a region of interest identified by LM?")
 - iii. **If immunoEM of your specific protein of interest is required as part of the CLEM procedure, have you demonstrated that your antibodies work by immunofluorescence?**
 - Importance of the project for the overall research of the user
 - Expected results
 - A milestone plan of the project with clear deliverables and routes for exit, if the milestones are not achieved.
3. Further information requested
 - Equipment/technology that is envisaged to be used; needs to be estimated consulting the Node Candidate
 - Consent to cover part of the access costs, which will be charged to the application's institution. This amount will be negotiated with the EuBI Node Candidate granting access, after the full technical details of the project are defined and agreed
 - Previous experience of the applicant in light and electron microscopy techniques (in particular the one that he/she intends to use at the CLEM EuBI Node Candidate)
 - Biological hazards associated with the project
 - Estimation of the time to be spent at the CLEM EuBI Node Candidate; needs to be estimated consulting the Node Candidate (preferred starting and ending dates should be proposed according to the milestone plan and the access mode as described in 1.3).
 - Agreement to acknowledge the CLEM EuBI Node Candidate in publications resulting from data obtained during the visit
 - In case the applicant is not a principal investigator: approval of the scientist's PI supporting the visit to the CLEM EuBI Node Candidate

1.3 Access mode to the CLEM EuBI Node Candidate

CLEM studies require intensive support by local staff, since many users lack a basic training in EM sample preparation and interpretation of EM images. In addition, the execution of EM and CLEM techniques requires assistance of highly qualified and trained personnel for correlation and processing of the images. Therefore, it can be an advantage that a major part of the practical work is carried out by the staff of the CLEM EuBI Node Candidate. However, there is also the option that users get a professional training by staff of the CLEM EuBI Node Candidate, including documentation on use of the instruments, image acquisition and image processing and analysis. Only when the staff approves that users are trained to perform independently, they will be granted independent access. Note that this will commonly be the case for most of the optical microscopes, whereas training for EM-procedures (e.g. ultrathin-cutting) and analysis typically needs full staff support for projects with shorter duration. The balance between support and independent experimental work varies depending on the challenging aspects of the studies and the prior training of users. To find the right balance will be an essential part of the initial project planning between the user and the staff of the CLEM EuBI Node Candidate.

1.4 Evaluation Guidelines

The project application will be evaluated independently by scientific experts according to the following criteria:

1. *Scientific excellence*

- Significance/importance of the project in the context of international research and standards in the field
- Relevance/contribution of the project to the scientist's overall scientific work/interests
- Progress beyond state-of-the-art
- Relevance of the project's results for inclusion in future scientific publications
- Scientific quality of the research and study concept
- Benefit for applicant (e.g. training received, results obtained, scientific networking started, being able to apply for his/her own grant)
- Impact of project on field of science, economy and society

2. *Feasibility of the project*

- Feasibility of the project to be successfully conducted at the EuBI Node Candidate
- Availability of required technologies and expertise at the EuBI Node Candidate
- Availability of possible required supporting laboratory or animal facilities for the project
- Technical ability of the applicant to conduct the planned experiments, or the possibility to acquire the required skills in the time frame of the proposed project
- Reasonable estimation of project duration, and availability of the EuBI Node Candidate during the proposed time frame
- Reasonable estimation of project costs and coverage by the scientist
- Necessity to conduct the research at the requested EuBI Node Candidate (or could the applicant conduct the work at another EuBI Node Candidate that would be closer to his/her home laboratory, or that would be more qualified for the specified application)

If any of the questions above are evaluated as not feasible or insufficient, the application will be rejected.

1.5 Specific Guidelines for user project management at CLEM EuBI Node Candidates

1.4.1 Reporting and management during the CLEM-based project

Project meetings will be held according to the milestone plan to discuss whether the respective milestone could be achieved (please see Appendix for exemplary milestone plan). If necessary, the timelines of the project and the milestone plan will be adapted accordingly, or the project will be terminated. Participants (also via teleconference) of these project meetings are: The scientist(s) conducting the project, the project supervisor at the home institution, and the facility staff of the CLEM EuBI Node Candidate. A brief meeting report is generated by the meeting participants for documentation of the progress of the project.

1.4.2 Reporting after project completion

After access completion, the user is asked to report on the visit, the impact the results have on his/her future work, the quality of the scientific, technical and logistic support from the CLEM EuBI Node Candidate and the respective research institution. The survey will be provided online by the EuBI Hub.

- Type of instruments used
- Satisfaction concerning given advice and information on usage of most appropriate imaging instrument(s)
- Satisfaction concerning logistic support at the facility (office space, computing, libraries, accommodation)
- Satisfaction concerning technical support to make best use of the imaging instrument(s)
- Satisfaction concerning scientific support to set up the experiments and interpretation of results
- Rating of scientific impact of imaging infrastructure usage on the project
- Satisfaction concerning administrative support

- In the future: List of publication(s) containing project results based on using CLEM EuBI Node Candidate instrument(s)

APPENDIX 1**Example for a CLEM project milestone plan****Project title:**

Establish the cellular phenotype for gene X by CLEM

Aims of the project:

Gene X is involved in endosomal maturation, however, its precise function has remained unknown. HeLa cells will be silenced for gene X for 3 days after which the endosomal system will be imaged in live cells and by thin section CLEM.

Estimated project duration:

In total the project is expected to last for 9 months. The total time spent in the facility of the CLEM EuBI Node Candidate is estimated to be around 6 months.

Milestone plan:

MS1 (M6): Reagents

Establishment of a >90% knockdown for gene X in a HeLa cell line that expresses GFP. LAMP1. Perform control experiments. Establish phenotype by fluorescence imaging of whole cells. Select and purchase appropriate markers (antibodies, reagents) to conduct a full phenotypic analysis

This work will be conducted at the home institution.

Risk assessment and exit routes

If the knockdown fails, the project will be terminated. If it is delayed, following milestones will be delayed accordingly.

MS2 (M7): Laboratory assay

Prepare ultrathin (cryo)sections for fluorescent analysis of the phenotype on thin sections.

Risk assessment and exit routes

If the phenotype is not apparent in ultrathin sections or the key fluorescent signal(s) below detection, the project will be terminated. If it is delayed, following milestones will be delayed accordingly.

MS3 (M10): CLEM assay

Prepare ultrathin (cryo)sections for CLEM. Analyse phenotype by electron microscopy. Repeat procedure by adding additional markers for immunoEM analysis.

Risk assessment and exit routes

If the establishment fails, the project will be terminated. If it is delayed, following milestones will be delayed accordingly.

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