Euro-BioImaging

European Research Infrastructure for Imaging Technologies in Biological and Biomedical Sciences

WP7 Access to innovative light microscopy technologies

Guidelines for Proof-of-Concept Studies –
Correlative Light Electron Microscopy (CLEM)

UMC UTRECHT Utrecht

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The Cell Microscopy Centre (CMC) of the Department of Cell Biology of the University Medical Centre (UMC) Utrecht, The Netherlands, provides advanced light microscopy, (immuno)-electron microscopy and correlative light electron microscopy (CLEM) service for all UMC UTRECHT groups and external visitors. Per year, we host circa 80 people that use our microscopy set up or participate in our annual Cryomethods, Ultracryotomy and Immunolabeling workshop. See our homepage at: http://www. www.cmc-utrecht.nl for more detailed information.

The enclosed report contains the guidelines used at the CMC to evaluate and run visits by external scientists who wish to use our microscopy possibilities

1 Guidelines for scientific visits to the CMC Utrecht conducting correlative light – electron microscopy projects

1.1 General outline of the project schedule

1. The scientist interested to conduct a CLEM project in the CMC contacts informally the head of the facility (j.klumperman@umcutrecht.nl) to enquire the feasibility to conduct the project in the CMC, to explore the possibilities for a scientific host and to estimate the approximate project costs. After positive response from the CMC head, the visiting scientist, scientific host and CMC staff establishes a project milestone plan (see Appendix 1).
2. The scientist submits a formal but concise application to the CMC including the formal agreement of the scientific host and CMC head and the developed project milestone plan (see 1.2. for an application template).
3. The project application is evaluated on a scale from 1-10 by written procedure (email) by the CMC-scientific committee, the head of the CMC and the proposed scientific host at UMC UTRECHT. (see 1.3. for an evaluation template)
4. After positive project evaluation the scientist is invited to visit the CMC and conduct the project work. Timelines are arranged according to the project ranking (average score of the board evaluation). Highly ranked projects may be considered to start earlier than lower ranked ones. Due to space and personnel constraints no more than three external visitors are accepted to work in the CMC at any time and scientific hosts (except for the head of the CMC) typically accept only one external visitor at a time. The CMC makes every effort to host the scientist as soon as possible after application to maintain scientific competitiveness.The time spent at the CMC depends on the type of access that is required. The CMC offers different kinds of access:
    - Experimental work conducted by users. When the pilot experiments are conducted successfully the CMC will train a person from the requesting group to perform the follow-up experiments.
    - Experimental work conducted by CMC staff members. In cases that the requesting groups have no time or available personnel to be trained in the CLEM techniques they can hire CMC staff. This is time effective since it reduces training time, but FTE intensive and therefore with limited capacity.
    - Annual course. In collaboration with Leica the CMC organizes an annual 10-days international, hands-on course.
5. The logistics of the visit (e.g. accommodation, travel, shipment of reagents) should be arranged with CMC staff, scientific host and the administrative assistant of the UMC UTRECHT's visitor programme.
6. The project work is conducted in the CMC supported by the scientific host's laboratory.

7. After project completion, the scientist summarizes the project results and in a short report (typically one page) and provides standardized feedback on various issues of his/her stay (see 1.4 for a report template).
8. In the post visit period the scientist will inform the CMC when the results obtained at UMC UTRECHT are published in scientific journal(s) with appropriate mention of CMC support in the acknowledgement section of the article. This is included in CMC visitor reports.

1.2 Application Guidelines

The formal application of the scientist to the CMC should be concise and typically not exceed two pages and include 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 CMC

Please answer the following:

- **What is the nature of the CLEM work you need doing?** Please provide a very brief project description and justify why 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).

- **What is the most critical question that you need to be answered by EM?** Eg "Does my antibody internalize at all?" as opposed to "Which compartment does it internalize to?".

- **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 scientist
- 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
    - Approximate costs of the project (e.g. based on equipment usage hours and reagents; needs to be estimated consulting the head of CMC and scientific host)
    - Previous experience of the applicant in light and electron microscopy techniques (in particular the one that he/she intents to use at the CMC)
    - Biological hazards associated with the project
    - Approval of the scientific host at UMC UTRECHT (could be head of CMC)
    - Estimation of the time to be spent at UMC UTRECHT (preferred starting and ending dates should be proposed according to the milestone plan).

There are two types of services offered by the CMC:

1. Experimental work conducted by users. This involves a full training on our biological specimen preparation methods and microscopes types. The length

of the stay that is required depends on the level of experience of the visiting scientist. In case of a non-experienced person the minimum period in to be spent n the lab is 6 months.

2. Experimental work conducted by CMC members. In cases that the requesting groups have no time or available personnel to conduct their own research in the CMC they can hire CMC staff. This is time effective since it reduces training time and the time to be spent in the lab, but FTE intensive and therefore more expensive and with a limited capacity.

- Agreement to acknowledge the CMC in publications resulting from data obtained during the visit or, depending on the project, include CMC staff as co-author.
- Approval of the scientists home institution supporting the visit to the CMC

1.3 Evaluation Guidelines

The project application will be evaluated according to the following criteria (scale 1 to 10, 1=lowest, 10=best mark). Evaluations should be concise and typically not exceed one page:

1. Scientific excellence

- What is the significance/importance of the project in comparison with international standards in the field?
- What is the relevance/contribution of the project to the scientist's overall scientific work/interests?
- Are the project's results likely to be included in future scientific publications?
- What is the scientific quality of the scientist or home laboratory?

2. Feasibility of the project

- Is the project feasible to be successfully conducted in the CMC?
- Is the milestone plan and project exit routes realistic?
- Are the technologies available at the CMC appropriate to address the posed question(s)?
- Does the applicant have sufficient specific training for the experiments to be conducted, or will he/she be able to acquire the skills in the timeframe of the proposed project?
- Are the estimated project costs reasonable and can they be covered by the scientist?

If any of the three questions above are evaluated as not feasible or insufficient (ranking as "1") the project will be rejected.

3. Others

- Will the applicant benefit in the post-visit period from the project (e.g. by the training received, results obtained, scientific networking started, be able to apply for his/her own grant)?
- Does the applicant need to conduct the research at the CMC (or could he/she conduct the work in another place that is closer by his home laboratory, or more qualified for the specific application)?

1.4 Reporting Guidelines

1.4.1 Reporting and management during the project

Project meetings will be held according to the milestone plan to discuss whether the respective milestone could be achieved. If necessary, the timelines of the project and 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, the scientific host at UMC UTRECHT, and the CMC staff involved in the project. 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 project completion the scientist is asked to report on the scientific results obtained, the impact the results have on his/her future work, the quality of the scientific, technical and logistic support from the CMC and UMC UTRECHT (if feasible scale 1 to 10, 1=lowest, 10=best mark). Reporting should be concise and typically not exceed one page:

- 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
- Summary on project results which were achieved by using CMC instrument(s)
- List of publication(s) containing project results based on using CMC 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 CMC 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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