ADVANCED LIGHT MICROSCOPY

TECHNOLOGY SPECIFIC REVIEW CRITERIA FOR EURO-BIOIMAGING NODE

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Introduction

Applications for the Euro-BioImaging Nodes will be evaluated based on the general and technology specific review criteria.

Technology Specific Criteria and Resources:

Technology specific review criteria refer to the resources that are either required or desirable to enable user access to advanced light microscopy technologies in Euro-BioImaging Nodes**.**

In the moment of application for Euro-BioImaging Node, resources described here can be already provided by the applying technology provider, or planned to be built up as a part of Euro-BioImaging Node application. In order to submit a successful application, each Node applicant is invited to provide as many resources listed here as possible. However, applications by the technology providers who can only offer a selection of resources will also be considered.

"Common Technology Review Criteria" chapter provides detailed description of each resource required from the future ALM Euro-BioImaging Nodes. Resources are defined as High Priority/Medium Priority/Not applicable for each technology individually in the Table 1. Specific additional requirements for selected technologies are described in the chapter **"Specific Technology Review Criteria".**

In addition to individual resources listed here, future Nodes are expected to have capacities available to handle the often complex administrative matters associated, in particular, with animal facilities, cell culture and model organisms.

In the framework of Euro-BioImaging, one expects to have considerable diversity between ALM users, depending on their expertise and availability of certain technology at their home institution. In order to successfully host all users, a node should provide an infrastructure together with **specially trained and experienced staff** who will support the user with project planning, sample preparation, data acquisition & storage, data processing, analysis & interpretation and who will enable users to utilize the facility in the best possible way. This mandatory comprehensive support will also ensure that data is recorded under optimal technical conditions.

Types of Nodes:

Technology provider can apply to become a "Multi-Modal Technology Node" or "Single Technology Flagship" Euro-BioImaging Node.

Single Technology Flagship Nodes would offer an innovative technology at European leading level. Following ALM technologies can be offered by the future flagship Nodes in the first call for Node applications in 2013:

- Super-resolution microscopy
- High-throughput microscopy
- Functional Imaging
- Correlative Light and Electron Microscopy
- Mesoscopic Imaging

If a Node-applicant wants to offer more than one innovative technology as flagship capabilities, it would have to submit a single application, which contains detailed documentation for each technology to allow reviewing each technology by the suitable expert groups of the Independent Evaluation Board.

Multimodal Technology Nodes would provide excellence by the integration of multiple imaging technologies at one site. This may be an especially suitable node type to consider for new member states that wish to build up their imaging infrastructure. Multimodal Advanced Light Microscopy Nodes could offer for example: Laser scanning confocal systems, Spinning disc confocal systems, Deconvolution widefield microscopy, Multiphoton systems, TIRF, Fourie Transform Infrared Imaging, Electron Microscopy. A Multi-modal node can include one of the Flagship technologies, in which case the applicant has two options to apply: a) flagship technology is included under the multi-modal umbrella (flagship technology service is not at European leading level) or b) the applicant submits a separate application for the Flagship node for this technology (flagship technology service is at European leading level).

Common Technology Review Criteria for Advanced Light Microscopy Euro-BioImaging Nodes

This chapter provides detailed description of resources that are considered either required or desirable to enable user access to advanced light microscopy technologies in Euro-BioImaging Nodes. Please refer to Table 1 for specific requirements applying to selected/individual technologies.

1. Wet lab

Wet lab facilities will be necessary for most user access projects. The future Node should provide approved H&S bio-lab infrastructure and capacity to provide bench space and use of basic equipment and resources.

Basic equipment: Common glassware, common laboratory solvents and reagents including the necessary sample mounting equipment and tools.

Reagent supplies: The wet lab should have defined procedures and personnel ready to order and provide specific reagents required by users. In most cases, special reagents (specific cDNA clones, small molecules, etc.) will be provided by the users of the infrastructure. They may be shipped directly to the Node, although in rare cases they may need to be ordered by the Node. Provision for storage (4°C, -20°C, -80°C) will be supplied by the Node.

Location: Within the same building and immediate proximity of the ALM facility.

2. Cell culture facilities

Use of ALM for cellular and sub-cellular imaging will require the use of cell culture facilities. These facilities should have necessary approvals for Biosafety Level 1 (BSL-1).

Basic equipment:

Cell culture hood (i.e., laminar-flow hood or biosafety cabinet); Incubator (humid CO2 incubator recommended); Water bath; Centrifuge; Refrigerator and freezer (–20°C); Cell counter (e.g., Countess® Automated Cell Counter or hemacytometer); Inverted microscope; Liquid nitrogen (N_2) freezer or cryostorage container; Sterilizer (i.e., autoclave); Cell culture vessels (e.g., flasks, Petri dishes, roller bottles, multiwell plates); pH metre.

Location: Within the same building and immediate proximity of the ALM facility.

3. Probes

A bank of fluorescently-conjugated secondary antibodies, especially if specific fluorophores are required, small molecule fluorophores (e.g., DAPI, Hoechst) should be available. If specific probes are required for imaging that are not commercially available, these should be made available by the Node, along with expertise in their use. Support in the use of probes and set-ups appropriate to the ALM technology should be provided. Please see specific requirements for probes availability in the technology specific sections.

4. Animal facilities

Facilities for the handling and preparation of small animals are often necessary for users to house specific animal models required for experiments. Animal facilities should formally be approved to the national and EU standards required for the organisms involved.

All experiments carried out using experimental animals should be approved by the local (national, institutional) regulatory bodies

These approvals should be secured by the Node and in place, prior to the start of the user access.

Organisms: Mice; Rats, Zebrafish, etc.

Location: The animal facilities should be located within easy working access to the ALM facility. In the case of Intravital Microscopy, the Imaging facility should itself be located within the "clean space".

Transport and shipping: The animal facility should be staffed by appropriately trained personnel who have the capacity to plan for and deal with incoming animal models from, and their return to, international locations. All such processes will be carried out with close adherence to the local regulations at Node concerning animal experiments. Transport and shipping itself will be organized by users.

5. Other Model organism

Some users will require model organisms for imaging such as: yeast, C. elegans, Drosophila etc. Where appropriate, facilities for growth, culture, propagation and resources for media production will be required.

Organisms: yeast, C. elegans, Drosophila, bacteria, Dictyostelium, fungi, Aplysia, Medaka, plants, chick embryos, etc.

Location: The facilities should be located within easy working access and the same building as the ALM facility.

6. High Biological Safety Level

Facilities that can handle BSL 2 samples are required by some users. Nodes offering BSL 2 or above should have the necessary national licenses and approvals to handle GM samples as well as bio-containment of pathogens. It is not expected that access to P3 or P4 laboratories will be required by Euro-BioImaging users.

7. Workstations

Image processing and analysis workstations are necessary for most users particularly those who are accessing a Node for more than a few days. Nodes should have the capacity to offer a basic workstation to all users accessing their facility, and where necessary, high-powered workstations with sufficient processing, memory and storage capacity to enable users to efficiently achieve their experimental aims. Please see detailed requirements for specific techniques.

Basic equipment: Desk space, ICT access.

Location: Within the same building and immediate proximity of the ALM facility.

8. Data Storage

Data storage is necessary for most users during access. Nodes should have the capacity to offer server space to users for storage of images and results. Data storage requirements vary greatly depending on the imaging technique used, but in general, capacity to store several tens to hundreds of Gigabytes is routine, and in certain cases, larger capacities will be necessary. In general, sufficient capacity to store both original and processed datasets from several users will be necessary. Please see detailed requirements for specific techniques.

Once data is acquired and processed, users will likely need to return home with their data. Depending on data size and complexity, transporting it may require various mobile media (e.g., USB sticks, external hard drives) or download via internet. An appropriate provision and capacity for data

transfer should be stated in the project plan and then be part of the proper execution and completion of the project

9. Accommodation

Accommodation may be required by some users, particularly those who have travelled internationally to access a facility. Whether a node can offer accommodation on site should be made clear to the user during the pre-visit preparation phase. If no accommodation is available on site, the Node should have the capacity to offer assistance in finding affordable accommodation

10. Methodological Set Up

Nodes should offer the capacity for planning on-site experiments with the applicant prior to physical access by the user. Assistance should include guidance on methodological set up required to obtain appropriate images, including guidance on the technology requirements for the preparation of samples, provision/procurement of materials required for image acquisition (appropriate stains, mounts etc). For some technologies more detailed project planning should be provided (Please see detailed requirements for specific techniques).

11. Facility Induction

Nodes should offer a general induction programme for all visiting researchers. The induction should include documented safety and quality management procedures for use of the technology and the supporting infrastructure to be accessed by the user.

12. Technical Assistance to Run Instrument

Nodes should have the capacity to support user access and provide expert support for operation of the imaging technologies being accessed. Facilities should offer documented procedures and expert assistance for operation of the technology and image acquisition. All staff offering support should be fully trained in the operation of the instrument.

13. Image Acquisition

Nodes should have the capacity to support users in image processing and interpreting results. Documented guidance and expert advice on image processing and analysis using offered technologies is essential.

14. Image Processing and Analysis

Nodes should have the capacity to support users in acquiring suitable images for their research. Fully documented procedures on image acquisition using offered technologies should be available as well as expert assistance from support staff/technicians.

15. Project Planning

To ensure that users achieve the objective of the requested access, thorough project planning by the Node staff and a user will be required. It is essential to have in place a project assessment and planning process that includes input from experienced technical and scientific staff. These staff should have the time and resources to contribute to this process. Planning should also include safety and regulatory considerations, e.g. having facilities to rapidly obtain permissions for hosting/handling different cell lines etc.

Some user projects, e.g. for HTM, CLEM, Functional Imaging, Super-resolution Microscopy, Mesoscopic Imaging, could last several months and sometimes even beyond one year. This may require repeated visits of the users to the infrastructure at different stages of the project or for multiple iterations as users gain experience and redesign or optimise their experiments. The feasibility of longer term projects needs to be regularly reviewed, particularly during early stages, and projects could be refined or postponed until required preparatory work has been carried out or gaps in user training filled by Euro-BioImaging measures. It is therefore essential to have in place a **project management planning procedure and experienced staff able to over-see the project through all stages to support users**.

Nodes need to be able to support multiple users over extended periods and need **dedicated flexible staff to support projects with diverse aims**. **Node staff needs to be knowledgeable about the biological applications** as well as their range of imaging techniques in order to be able to judge the capabilities and commitment of potential users.

Table 1: Technology Review Criteria for ALM Euro-BioImaging Nodes

Type of Node		Technology	Facilities									Training					
Multi-modal	Flagship		1. Wet Lab	2. Cell Culture Facility	3. Probes	4. Animal Facility	5. Model Organisms	6. High Biological Safety Level	7. Workstations - Desk, ICT access	8. Data Storage - images	9. Accommodation	10. Methodological set up	11. Facility Induction	12. Technical assistance to run instrument	13. Image acquisition	14. Image processing and analysis	15. Project Planning
			Priority														
Yes		**General ALM technologies**															
Yes		*Spinning Disc Confocal Systems*	High	High	High	Med.	Med.	Med.	High	High	Med.	High	High	High	High	High	Med.
Yes		*Laser Scanning Confocal Systems*	High	High	High	Med.	Med.	Med.	High	High	Med.	High	High	High	High	High	Med.
Yes		*Deconvolution widefield microscopy*	High	High	High	Med.	Med.	Med.	High	High	Med.	High	High	High	High	High	Med.
Yes		*Multiphoton Systems*	High	High	High	Med.	Med.	Med.	High	High	Med.	High	High	High	High	High	Med.
Yes		*Total Internal Reflection Fluorescence Microscopy (TIRF)*	High	High	High	Med.	Med.	Med.	High	High	Med.	High	High	High	High	High	Med.
Yes		*Fourier Transform Infrared imaging*	High	High	High	N.A.	N.A.	N.A.	High	High	Med.	High	High	High	High	High	Med.
Yes		*Electron Microscopy*	High	High	Med.	N.A.	N.A.	N.A.	High	High	Med.	High	High	High	High	High	Med.
*	Yes	**Functional Imaging**	High	High	High	Med.	Med.	Med.	High	High	Med.	High	High	High	High	High	High
	Yes	**High-throughput Microscopy**	High	High	Med.	N.A.	Med.	Med.	High	High	Med.	High	High	High	High	High	High
	Yes	**Correlative Light and Electron Microscopy**	High	High	High	Med.	Med.	Med.	High	High	Med.	High	High	High	High	High	High
*	Yes	**Super-resolution Microscopy**	High	High	High	Med.	Med.	Med.	High	High	Med.	High	High	High	High	High	High
*	Yes	**Mesoscopic Imaging**	High	High	Med.	High	High	Med.	High	High	Med.	High	High	High	High	High	High

Requirements for Functional Imaging apply to following techniques: FRAP, FRET, FLIM, FCS.
Requirements for Super-resolution imaging apply to following techniques: Structured Illumination Microscopy, Stimulated Emission Depletion Microscopy (STED), Stochastic Optical Reconstruction Microscopy (STORM), Photo-activated Localisation Microscopy (PALM, SPT-PALM), Ground State Depletion Imaging and 4PI Microscopy.
Mesoscopic Imaging resources requirements apply to Optical Projection Tomography and Single Plane Illumination Microscopy.
* Flagship technologies which can be included in the Multi-Modal nodes.
Priority level can be high, medium (Med.) or not applicable (N.A.)

Specific Technology Review Criteria for selected techniques offered by Euro-BioImaging Nodes

In addition to the general eligibility and review criteria and the above listed resources for Euro-BioImaging Nodes, additional specific criteria are considered critical for providing efficient access to **multi-modal technology nodes, high throughput microscopy for systems biology, correlative light and electron microscopy, super-resolution microscopy and functional imaging.**

Multi-modal Technology Nodes

Multimodal Technology Nodes integrate multiple imaging technologies within one site. They must provide excellence across the offered technologies**.**

In addition to the Common Technology Review Criteria defined above, the Multi-modal Technology Node should offer:

- The expertise to advise users on the most appropriate technology, among their portfolio of imaging modalities, to deliver the research objectives for all project types that a Multimodal Node will accept. In particular, Node Staff should help decide which imaging modalities they offer are best-suited for a particular experiment or sample type or help the user to test different modalities in a comparative manner at the beginning of the project.
- There may be cases where a Multi-modal Technology Node does not contain technology or expertise appropriate for a given proposed project. In these cases, the proposed project should be forwarded to a more appropriate resource and where possible, another Euro-BioImaging Node.
- The capacity and expertise to maintain the different devices, guide measurements and help in interpreting data recorded across the range of imaging modalities offered. Tools for performing these analyses should be installed at the Node and/or be easily accessible via on-line resources.
- As the duration of projects may vary (from ½ day to 1 year) nodes should be able to facilitate repeated visits by the users to the infrastructure at different stages of the project.

High Throughput Microscopy for Systems Biology

High throughput microscopy (HTM) for systems biology comprises automation of all aspects of microscopy-based experiments including sample preparation, image acquisition, image analysis, data handling and mining. Through the automation, image data can be acquired and analysed at a large scale up to whole genome levels. The data generated by the technology is highly quantitative and thus suitable for systems biology applications.

In addition to common technology requirements, the HTM infrastructure should be able to support users at all levels of a high throughput microscopy-based project, and should provide in particular:

- Large-scale reagent libraries for cell or organism perturbations (e.g. siRNA , cDNA or chemical libraries)
- Support in assay development and adaptation of low throughput laboratory assays to automation
- Support in high-throughput sample preparation (e.g. cell seeding devices, liquid handling, robots)
- High-throughput image acquisition technology and live cell imaging
- Support in large-scale data management and handling (e.g. data storage capacities in the tens of Tbyte range, LIMS system to retrieve samples, corresponding image data)
- Tools and support for automated image analysis of large data sets
- Tools and support for data mining and evaluation tools (e.g. for statistical analyses and presentation of results or correction of systematic errors during image acquisition)
- Large-scale computing ("cluster") to enable data analysis of large data sets in reasonable time

Correlative Light and Electron Microscopy

Correlative light electron microscopy (CLEM) combines the resolution of electron microscopy (EM) with the high sensitivity and large field of view of light microscopy (LM). CLEM is not one microscopy method, but encompasses a variety of approaches that enable the researcher to visualize the same region of a specimen first by LM and next by EM. The LM, usually a fluorescence microscope, can be used as a navigation tool to identify regions of interest in large specimens (e.g. a typical staining pattern induced by drugs or gene knockdown) or to identify sparse cells in a complex tissue (e.g. stem cells, cancer cells), which then are selected for studying by EM to reveal the ultrastructural context. In addition, LM can be used for live cell imaging, thereby adding kinetics and directionality to the otherwise static EM pictures.

The CLEM Node should be able to support users at all levels of a CLEM-based project. In addition to the common technology review criteria, a CLEM Node should in particular be able to provide:

- specialized sample preparation technology in addition to conventional EM techniques, e.g. cryo sectioning, cryo fixation, high pressure freezing, freeze-substitution, ultrathin-sectioning, electron tomography, immunoEM etc.
- Sample preparation for correlative light and electron-microscopy
- Robust methods to correlate sample coordinates between light and electron microscopy (via hardware and/or software)
- Bifunctional probes that allow correlation between light and electron microscopy
- Correlative signal and/or signal structure detection technology

Super-resolution Microscopy

Super-resolution imaging denotes all techniques that provide an optical resolution or a localization ability which is fundamentally below the diffraction limit. A typically achieved resolution is in the order of 20-50nm. These include methods like STED[1], RESOLFT[2], PALM[3], STORM[4], GSD[5] and GSDIM[6] microscopy and all related methods thereof. Super-resolution imaging experiments include sample preparation with dedicated labels and is typically supported by data analysis.

In addition to common technology review criteria, a super-resolution Node should provide in particular:

- Support of users in all aspects during an entire super-resolution microscopy based project
- Access to different technical approaches to super-resolution microscopy, e.g. structured illumination, stochastic and targeted fluorophore switching approaches, etc.
- Experienced staff advising on method selection
- Competence in application development
- Algorithms for super-resolution data analysis

Since the fluorescent markers are key to enable super-resolution imaging, the Node should offer a clear guidance on the specific markers to be used in order to fully exploit the potential of the selected super-resolution technique. In this regard, it is strongly recommendable that the Node provides a broad selection and supply of dedicated fluorescent markers as well as staining and embedding protocols and has labelling optimization knowledge.

[1] STED: Stimulated Emission Depletion Microscopy
[2] RESOLFT: Reversible Saturable Optical Fluorescence Transitions
[3] PALM: Photo-Activated Localization Microscopy
[4] STORM: Stochastic Optical Reconstruction Microscopy
[5] GSD: Ground State Depletion Imaging
[6] GSDIM: Ground State Depletion Microscopy followed by individual molecule return

Functional Imaging

Functional imaging describes techniques aimed at elucidating biomolecular interactions and processes including cell signalling mechanisms. These include fluorescence correlation spectroscopies (FCS), fluorescence lifetime imaging (FLIM), polarisation-resolved imaging, FRET[7], FRAP[8], optogenetics and related technologies as well as vibrational spectroscopic techniques such as Raman scattering and CARS[9]. Functional imaging experiments could include combinations of different modalities and could be supported by model based data analysis.

In addition to common technology requirements, Nodes providing Functional Imaging should in particular have:

- Several functional imaging modalities with diverse equipment, e.g. FLIM[10] (time and/or frequency domain), FRAP/photoactivation, FCS[11], etc.
- Expertise to advise users on choosing the appropriate imaging modality or compare them in preliminary experiments
- In-house expertise and facilities for handling and labelling samples
- Expertise with functional reporters (e.g. probes and standards for FLIM, FRET probes and biosensors, photo-switchable probes, photo-activatable dyes, caged dyes, optogenetic tools, etc.)
- Expertise to guide users on choosing functional reporters and preparing samples
- Comprehensive and flexible data analysis capabilities
- Expertise in customised and model-based data analysis

Nodes need to be able to advise users on how to label and prepare samples. They do not need to maintain a complete repository of probes but they need to know from where probes can be obtained when necessary (from repositories or other sources).

Functional Imaging nodes may provide access to different kinds of experiment that require different types of data analysis. Data analysis capabilities may need to be optimised for specific user applications and so infrastructures should be able to develop or adapt customised data analysis. Expertise in model based data analysis (modelling) is also desirable.

It is desirable that Functional Imaging Nodes can provide access to a range of different imaging modalities, particularly those that can provide commentary information for user projects.

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[7] FRET: Fluorescence Resonance energy Transfer
[8] FRAP: Fluorescence Recovery After Photobleaching
[9] CARS: Coherent anti-Stokes Raman Spectroscopy
[10] FLIM: Fluorescence Life Time Imaging
[11] FCS: Fluorescence Correlation Spectroscopy

Mesoscopic Imaging

Mesoscopic imaging refers to technologies, which are able to capture 3D data-sets of biological samples in the mm size range (~1-10mm). It focuses on embryos and tissues and other large multicellular structures, capturing both the 3D geometry and molecular distributions (such as spatial distributions of gene and protein expression patterns) as well as their dynamic changes if in vivo reporters are available. Technologies in this category include optical projection tomography (OPT) and related methods as well light-sheet based imaging (e.g. SPIM [12], DSLM [13] etc.). The primary areas of application are studying embryo development, organogenesis, mouse models of disease, tissue regeneration and human biopsies.

The infrastructure should be able to **support users at all levels of a mesoscopic imaging project** and particularly should have:

- Experience in the biological model systems the users study (a) <1mm, Drosophila, zebrafish, preimplantation mammalian embryos, cell culture cysts, etc. and/or b) >1mm, mouse organs, human biopsies, vertebrate embryos, etc.)
- Portfolio of several modalities (e.g. SPIM, DSLM, ultramicroscopy, OPFOS[14], OCPI[15] or equivalent; OPT[16] or equivalent)
- Large-scale IT infrastructure for data storage and analysis in the multiple tens of terabytes range
- Experience in analysis of large 3D data and 4D data sets
- Close access to space and equipment for maintaining live stocks of the biological model systems, e.g. Drosophila, zebrafish, mouse and general tissue culture (incubators, fish tanks, animal facilities etc.).

[12] SPIM: Selective Plane Illumination Microscopy

[13] DSLM: Digital Scanned Laser Light-Sheet Fluorescence Microscopy

[14] OPFOS: Orthogonal-plane Fluorescence Optical Sectioning Microscopy

[15] OCPI: Objective-coupled Planar Illumination Microscopy

[16] OPT : Optical Projection Tomography