EURO-BIOIMAGING ERIC:
General and Technology-Specific Review Criteria for Euro-BioImaging Nodes

Biological Imaging Technologies

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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**.**

At 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 the 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.

The "Common Technology Review Criteria" chapter provides a detailed description of each resource required from the Euro-BioImaging Nodes for biological imaging. 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 ERIC, one expects to have considerable diversity between Euro-BioImaging 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 the 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.

Common Technology Review Criteria for Euro-BioImaging Nodes for Biological Imaging Technologies

This chapter provides a detailed description of resources that are considered either required or desirable to enable user access to advanced microscopy **technologies in Euro-BioImaging Nodes**.

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 imaging facility.

2. Cell culture facilities

Cellular and sub-cellular imaging will require the use of cell culture facilities. These facilities should have necessary approvals for at least 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 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 imaging technology should be provided.

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 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 where possible their return to, international locations. All such processes will be carried out with close adherence to the local regulations at Node concerning animal experiments.

5. Other Model organism

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

Location: The facilities should be located within easy working access and the same building as the 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 licences and approvals to handle GM samples as well as bio-containment of pathogens.

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 all users accessing their facility sufficiently high-powered workstations meeting the processing, memory and storage capacity needs to enable users to efficiently achieve their experimental aims. Please see detailed requirements for specific techniques.
Basic equipment: Desk space, ICT access.

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 the 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.

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 and be able to provide at least basic guidance on the analysis of their data. The extent to which image data analysis can be offered by the Node should be discussed with the user ahead of the visit and image data analysis should be included in the project plan. For

providing image processing and analysis support, Nodes should be able to provide access to the required computational capacity, specialist software and have the expert trained staff to support the users.

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 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 oversee 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 need 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.

16. Remote Access

Where Nodes are able to provide remote access to imaging services, these should be clearly communicated to the users, including the specifics of the remote access modality, such as whether the Node is providing full service on mail-in samples or can allow users remote control of instruments to remotely run the experiments themselves. The Nodes offering this service need to have the required technical setups in place, such as secure access modalities through VPNs, policies and processes for international sample shipment, and trained staff to support users in remote experimental procedures and sample handling.

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 specific technologies.

High Throughput Microscopy

High throughput microscopy (HTM) 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.
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 to enable data analysis of large data sets in reasonable time

Correlative Light and Electron Microscopy and Correlative X-ray and EM

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). Correlative X-ray imaging and EM (CXEM), additionally brings in X-ray imaging as a correlative method, allowing the inclusion of another scale of imaging. 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 Node offering CLEM and CXEM should be able to support users at all levels of a CLEM/CXEM-based project. In addition to the common technology review criteria, such a Node should in particular be able to provide:

- Specialised sample preparation technology, e.g. cryo-sectioning, cryo-fixation, high pressure freezing, freeze-substitution, ultrathin-sectioning, electron tomography, immunoEM etc.
- Sample preparation for correlative light, X-ray and electron-microscopy, that is compatible across methods
- Robust methods to correlate sample coordinates between light, X-ray and electron microscopy (via hardware and/or software)
- Bifunctional probes that allow correlation between light and electron microscopy

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, RESOLFT, SMLM, SRRF, and MINFLUX 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 recommended 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.

Functional Imaging

Functional imaging describes techniques aimed at elucidating biomolecular interactions and processes including cell signalling mechanisms. These include FCS/FCCS, FLIM, FRET, FRAP, optogenetics and related technologies as well as (stimulated) Raman scattering and CARS. 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
- 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).
Nodes offering Functional Imaging may provide access to different kinds of experiments 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.

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, photoacoustic imaging, serial-blockface fluorescence imaging/HREM as well light-sheet based imaging. 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, such as Drosophila, zebrafish and preimplantation mammalian embryos, organoids, organs, human biopsies etc., and the required sample preparation methods for maintaining the system during long term imaging
- Portfolio of several modalities
- 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.).

Volume EM

Volume electron microscopy or volume EM (vEM) refers to a group of recently developed imaging approaches that use scanning and transmission electron microscopy (SEM and TEM) to allow the interrogation of cell and tissue ultrastructure in 3D, at µm to mm volume scales and nm resolutions. These methods include serial section TEM (ssTEM), serial TEM tomography, Focussed Ion Beam SEM (FIB-SEM), Serial Blockface TEM (SB-TEM), Array Tomography (AT), etc. vEM workflows encompass the use of a variety of ancillary techniques both during the preparatory stages and identifying regions of interest, prior to the final electron imaging and can be part of CLEM workflows.

Nodes offering volume EM should be able to support users during the entirety of a volume EM project and particularly should have:

- Experience and tools necessary for the sample preparation for the specific vEM method, such as serial sectioning for ssTEM and AT
- Expertise to support users in choosing the most appropriate vEM technique for their question. If the technique is not available at the specific Node, the user should be advised on alternative support sources, ideally at another Euro-BioImaging Node.
- Access to the required IT infrastructure for data storage and analysis and expertise to provide at least basic guidance to users on data analysis.

Cryo-EM

Cryo-EM refers to technologies in which samples are fixed and/or imaged at cryogenic temperatures, preserving them in a close-to-native state through cryo-fixation. A number of methods in this range are included in the Euro-BioImaging technology portfolio, including Cryo-ET, Cryo-FIB, Cryo-SEM, and Cryo-TEM.

Nodes offering cryo-EM methods should be able to support users during the whole sample preparation and imaging workflow and particularly should have:

- Experience and machinery for cryo-fixation, such as plunge-freezing, high-pressure freezing or others
- Instrumentation and expertise to support sample preparation, sample transfer and imaging adapted to cryo-samples
- Potentially capacity to receive shipped cryo-samples

Image Analysis

Image Analysis covers a wide range of different approaches to extract quantitative data from images. These services are available in conjunction with access to imaging instruments at a Node or Nodes may offer Image Analysis as a standalone analysis service requested on image data irrespective of where it was acquired.
Nodes offering Image Analysis services should have:

- Compute and IT hardware resources, and required licences for analysis and visualisation software, dependent on the specific analysis service offered
- Expertise and staff capacity in supporting researchers with choosing the most suitable analysis pipeline and providing support to researcher on image analysis
- Workstations, ideally including remotely accessible ones, for performing image analysis

Further specialised imaging methods and support technologies

Euro-BioImaging Nodes may offer a wide range of other further specialised imaging methods and support technologies, including but not limited to:

- Expansion Microscopy and Tissue Clearing
- Feedback Microscopy
- Multiplex Imaging
- Spatial transcriptomics
- Imaging at Biosafety Levels >1
- High-speed Imaging
- Laser microdissection
- Voltage/pH/Ion Imaging

Nodes that offer such methods and technologies should have, dependent on the specific technology:

- Required expertise, instrumentation, tools, chemicals, and probes for sample preparation (such as in Expansion Microscopy, Tissue Clearing, Multiples Imaging, Spatial Transcriptomics, Voltage/pH/Ion Imaging)
- Required instrumentation for image acquisition, including adaptive and add-on tools (such as for multiplex imaging and laser microdissection)
- Certification of Biosafety status, expertise and tools for maintaining safety standards for Imaging at Biosafety Levels >1
- Large-scale IT infrastructure for data storage and analysis

Physical and chemical sample characterisation methods

Many imaging methods exist to characterise the physical and chemical composition and state of biological samples, such as Atomic Force (AFM) and Traction Force Microscopy (TFM) for physical characterisation, Mass Spec Imaging (MSI), Micro X-ray Fluorescence Spectrometry (XRF), Micro-Particle Induced X-ray Emission (μ-PIXE), Energy-dispersive X-ray spectroscopy (EDX) for chemical and elemental analysis of samples.

In addition to common technology requirements, Nodes providing such sample characterisation methods should in particular have:

- Expertise to advise users on choosing the appropriate imaging modality
- In-house expertise, facilities, tools, and access to materials for handling, preparing, and labelling samples as required by the specific technique
- Comprehensive and flexible data analysis capabilities
- Potentially access to correlative and additional imaging modalities to complement the information gathered via the listed methods