

**HUMAN TECHNOPOLE  
NATIONAL FACILITY FOR LIGHT IMAGING  
CALL FOR ACCESS  
25 – LI – ROUND 2**

## **Table of Contents**

<b>1. INTRODUCTION .....</b>	<b>3</b>
<b>2. TERMS AND DEFINITIONS.....</b>	<b>4</b>
<b>3. APPLICATION TYPE .....</b>	<b>5</b>
<b>4. ELIGIBILITY AND ADMISSIBILITY .....</b>	<b>5</b>
<b>5. APPLICATION CONTENT AND FORMAT .....</b>	<b>7</b>
<b>6. APPLICATION SUBMISSION METHODS, CALL DEADLINE AND EVALUATION PERIODS.....</b>	<b>10</b>
<b>7. EVALUATION OF APPLICATION .....</b>	<b>10</b>
<b>8. RESUBMISSION OF RESERVE LIST PROJECTS.....</b>	<b>13</b>
<b>9. AFTER ACCESS HAS BEEN APPROVED .....</b>	<b>14</b>
<b>10. AFTER ACCESS HAS BEEN COMPLETED.....</b>	<b>14</b>
<b>11. CONTACTS.....</b>	<b>14</b>
<b>11. REFERENCES .....</b>	<b>14</b>
<b>12. CHANGES TO THE CALL .....</b>	<b>15</b>
<b>ANNEX I: LETTER OF INSTITUTIONAL ENDORSEMENT TEMPLATE .....</b>	<b>16</b>
<b>ANNEX II: PROJECT PROPOSAL TEMPLATE .....</b>	<b>18</b>
<b>ANNEX III: SERVICE LIST .....</b>	<b>19</b>

## 1. INTRODUCTION

The Access of Researchers affiliated with Universities, *Istituti di Ricovero e Cura a Carattere Scientifico* (IRCCS), and Public Research Entities to Fondazione Human Technopole (HT) National Facilities (NFs) is regulated by the NF Access rules available on the NFs dedicated webpage ([link](#)).

Services offered by the NFs are available through regular open calls for Access that are published yearly on the HT website ([link](#)) and are entirely subsidized by HT through an indirect financing system for the project (or aspects of the project) approved for Access.

The open call for Access is aimed at supporting Access to the technologies offered by the NFs and it is not meant to provide direct funding to the Applicant. The costs for the activities to be performed at the NFs will be fully subsidized. This includes shipment of relevant material from and to the Applicant's laboratory as well as travel and accommodation for the Applicant and/or Applicant's team member(s) (User) while accessing the NF. Project-related costs (personnel, consumables, and other costs) at the Applicant's laboratory are not funded.

The User Access workflow comprises different steps, spanning from the initial submission of the application to evaluation and Access approval, Access to the performance of the service(s) and Access conclusion. A detailed description of the workflow is available on the NFs dedicated webpage ([link](#)).

### 1.1 Access modalities

Three different Access modalities can be requested. Their availability will vary, based on the service specifics of each NF:

- **“Simple” Access to NF or individual instruments thereof:** This modality is intended for Users involved in projects requiring technologies that are available at the NF for **direct Access by User**. This Access modality requires prior expertise with the technology of interest. After an initial introductory training aimed at defining the level of expertise of the User, **the use of the instrument with limited supervision by NF staff is authorised**. For defined NFs/ instruments/ services this Access modality may be restricted or not available.
- **Access to NF services:** This procedure entails the provision of **services performed by NF staff on behalf of the User**. NF services may include both standard services as well as, when foreseen by the technology development specifics of each NF, bespoke services conceived and discussed with the User. To allow the NF staff to best align the experimental activity to the research objective, the User may be invited, if needed, to assist the NF staff while performing the project or aspects of it.
- **Access to NF services including training:** This procedure **entails training by NF staff** to provide Users, in addition to or alternatively to the services described in the previous modality, with training courses and/or programs, aimed at transferring the expertise necessary for the independent use of the specific technology. In this case, technical and/or experimental activities are conducted with the active participation of the User. Training can be provided by NF staff while performing the service(s) or in a dedicated session. This type of Access is also aimed at researchers who want to acquire expertise for subsequent independent use of a specific technology in other laboratories.

## 2. TERMS AND DEFINITIONS

### 2.1 Access

“Access” refers to the authorised use of the NF and of the services offered. Such Access can be granted for sample preparation, set-up, execution and dismantling of experiments, education and training, expert support and analytical services, among others. Access to the NFs includes all infrastructural, logistical, technical and scientific support (including training) that is necessary to perform the aspects of the project approved for Access.

### 2.2 Researcher

“Researcher” is a professional engaged in the conception or creation of scientific knowledge. They conduct research and improve or develop concepts, theories, models, techniques, instrumentation, software or operational methods.

### 2.3 Principal Investigator

“Principal Investigator” (PI) is the Researcher affiliated with an eligible Institution with the role of independent Group Leader, who is responsible for coordinating the research activities conducted within the framework of the submitted project.

The PI shall hold a primary appointment as Group Leader at an eligible Institution, with the following requisites:

- Coordinate an independent research team.
- Have a supervisory role towards junior and/ or senior Researchers.
- Their Group has an autonomous budget sufficient to cover their current research expenses.
- Be the recipient of independent research funding as PI or co-PI.

Junior PI: Up to 6 years from their first appointment in an independent Group Leader position.

The period specified above may be extended beyond 6 years in the event of adequately documented career breaks, occurring before the submission of the application and resulting from:

- i.* Maternity leave: The time limit is increased by 18 months for each child born after their first appointment in an independent group leader position; if the Applicant is able to document a longer total maternity leave, the period of eligibility will be extended by a period equal to the documented leave, taken before the submission of the application. Maternity status must be documented by submitting the birth certificate of the child or children.
- ii.* Paternity leave: The time limit is increased by the actual amount of paternity leave taken before the application submission deadline for each child born after their first appointment in an independent group leader position. Paternity status must be documented by submitting the birth certificate of the child or children.
- iii.* Long-term illness of more than 90 days, or national service: The time limit is increased, for each eligible event occurring after their first appointment in an independent group leader position, by the actual amount of leave from which the Applicant has benefited prior to the application submission deadline.

Established PI: More than 6 years from their first appointment in an independent group leader position.

## 2.4 Applicant

“Applicant” is the Principal Investigator who applies to a NF open call for Access and who is responsible for the submitted project. They can be of any nationality and must be affiliated with an eligible Italian Institution, as detailed in [section 4](#).

## 2.5 User

A “User” is intended as a Researcher affiliated with an eligible Institution who accesses, physically or remotely, the NFs to perform the approved activities or to support the National Facility staff while performing the approved service.

If requested by the Applicant, the User of the NF can also be a separate member of their research team.

# 3. APPLICATION TYPE

Applicants shall select the type of application they want to submit, choosing between two options:

- a. **Standard** application for projects that are technically mature.
- b. **Proof-of-concept** application for:
  - i. Projects with high scientific potential but with insufficient technical maturity or preliminary data.
  - ii. Projects aimed at setting up the experimental conditions required for a standard project, including methods or technology development projects.
  - iii. Time-limited Access projects (e.g., to acquire data to complete a manuscript, or preliminary data needed for a grant application, or single microscopy session).

# 4. ELIGIBILITY AND ADMISSIBILITY

PIs, as defined in [section 2.3](#) of this call, affiliated with an eligible Institution are eligible to apply. The Applicant’s role as a PI shall be confirmed by their Institution in a mandatory letter of Institutional endorsement (Template available in [Annex I](#)).

**Applications from Researchers who are not independent should be submitted by their Group Leader.** Applicants are strongly encouraged to support NF Access by young Researchers (R1 and R2 profiles of the European Framework for Research Careers, [link](#)) who are part of their group. In this case, the Applicant shall indicate in the application form that the NF User is a member of their group, specifying User’s career stage.

Below are the links to the relevant lists of eligible Institutions:

**Universities:** This category includes Institutions recognized by the Ministry of University and Research ([link](#)). In detail:

- i. State funded public universities, listed under the following [link](#).
- ii. Specialized superior graduate schools or Institutions, listed under the following [link](#).
- iii. Legally recognized non-public universities, listed under the following [link](#).
- iv. On-line universities, listed under the following [link](#).

**Istituti di Ricerca e Cura a Carattere Scientifico (IRCCS):** this category includes Institutions recognized by the Ministry of Health and listed at the following [link](#).

**Public research entities:** this category includes:

- a) Institutions recognized by the Ministry of University and Research and listed at the following [link](#);
- b) Area di Ricerca Scientifica e Tecnologica di Trieste - Area Science Park;
- c) Agenzia Spaziale Italiana - ASI;
- d) Consiglio Nazionale delle Ricerche - CNR;
- e) Istituto Italiano di Studi Germanici;
- f) Istituto Nazionale di Astrofisica - INAF;
- g) Istituto Nazionale di Alta Matematica "Francesco Severi" - INDAM;
- h) Istituto Nazionale di Fisica Nucleare - INFN;
- i) Istituto Nazionale di Geofisica e Vulcanologia - INGV;
- j) Istituto Nazionale di Oceanografia e di Geofisica Sperimentale - OGS;
- k) Istituto Nazionale di Ricerca Metrologica - INRIM;
- l) Museo Storico della Fisica e Centro Studi e Ricerche "Enrico Fermi";
- m) Stazione Zoologica "Anton Dohrn";
- n) Istituto Nazionale per la Valutazione del Sistema Educativo di Istruzione e di
- o) Formazione - INVALSI;
- p) Istituto Nazionale di Documentazione, Innovazione e Ricerca Educativa - INDIRE;
- q) Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - CREA;
- r) Agenzia Nazionale per le Nuove Tecnologie, l'energia e lo Sviluppo Sostenibile - ENEA;
- s) Istituto per lo Sviluppo della Formazione Professionale dei Lavoratori - ISFOL (a decorrere dal 1° dicembre 2016 denominato Istituto nazionale per l'analisi delle politiche pubbliche - INAPP);
- t) Istituto Nazionale di Statistica - ISTAT;
- u) Istituto Superiore di Sanità - ISS;
- v) Istituto Superiore per la Protezione e la Ricerca Ambientale - ISPRA, ferme restando le disposizioni di cui alla legge 28 giugno 2016 n.132;
- w) Istituto nazionale per l'assicurazione contro gli infortuni sul lavoro – INAIL.

Applicants shall declare that they have **not received funding to perform the submitted project (limited to the aspects included for Access to the NF)** in their own laboratory, host Institution or elsewhere.

Applicants shall confirm the **economic and scientific feasibility** for the aspects of the project to be performed outside the NFs.

Applicants will need to certify that **samples and relevant authorisations are available at the moment of application or no later than two (2) months** from receiving Access approval. **If samples and/ or relevant ethical and legal authorisation(s) for their use will not be**

**provided within this time frame, the request for Access will be automatically rescinded and PI will need to reapply at a subsequent call.**

Applicants **cannot request Access for the same service** if an approved Access is ongoing (i.e., Access that has been granted in a previous call for Access and is not yet completed). Before submitting a new application for the same service, Applicant shall consult with the NF staff and confirm that the ongoing Access will be completed before the end of the next evaluation round. **A clear motivation for the request must be provided** in the dedicated section of the application portal.

A PI submitting an application to this call for Access **cannot request access to other NFs** (i.e., cannot participate to other 2025 - ROUND 2 calls for Access). If more than one application is submitted, **ALL will be rejected** during administrative review.

Applicants who have an **application under evaluation** are not allowed to submit another one before receiving notification of the results. If an application is erroneously submitted, this will be rejected at the administrative review stage.

Applications must be **written in English**, they must be **complete** (i.e., consist of all the requested elements and information) and **abide to all administrative and technical requirements** (e.g., proposal and/or CV format, mandatory declarations, technical requirements for the services, sample availability, sample requirements, including but not limited to number of samples to be analysed, and research data management plan).

**Incomplete applications or applications that do not meet the requirements will be considered not admissible and will be rejected at the administrative review stage.**

## 5. APPLICATION CONTENT AND FORMAT

The application, to be submitted through the online portal PICA ([link](#)) consists of six components:

1. **Applicant's general information.**
2. **Justification for requesting Access to the NF.** The Applicant is requested to select the one that best applies from these four options:
  1. The requested service/ technology is not available at the host Institution;
  2. The requested service/ technology cannot be performed at the host Institution or elsewhere at an affordable cost;
  3. The requested service/ technology is available at the host Institution but the necessary expertise is lacking;
  4. The requested service/ technology is available at the host Institution but the service cannot be performed in a timeframe or scale compatible with the experimental requirements.

The Standing Independent Evaluation Committee, in charge of the evaluation procedure, may reserve the option to contact the host Institution and its core facilities to confirm the justification provided.

3. **Abstract** to be inserted in the dedicated section on the application portal (Max 1500 characters including spaces).

4. **Project proposal**, to be uploaded in PDF format in the dedicated section on the application portal, shall include the following sections:
- Title*
  - Significance.*
  - Innovation.*
  - Approach, including aims, preliminary data in support of the proposed experiments, experimental design and anticipated results.*
  - Environment, including facilities and resources available to support the aspects of the project to be performed elsewhere (i.e., outside the NF).*

Below, the mandatory format for the proposal:

**Standard application:** Max 3 pages (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom) figures included, references excluded. Accepted file formats: PDF. Max size: 30MB - Name the file as APPLICATION ID\_PROPOSAL\_Surname (e.g., ID123456\_PROPOSAL\_Rossi)

**Proof-of-Concept application:** Max 2 pages (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom) figures included, references excluded. Accepted file formats: PDF. Max size: 30MB - Name the file as APPLICATION ID\_PROPOSAL\_Surname (e.g., ID123456\_PROPOSAL\_Rossi)

Proposal template is available in [Annex II](#) of this call.

**Applications that do not meet the format requirements will be considered not admissible and will be rejected at the initial administrative review stage.**

5. **Applicant's CV in NIH biosketch format.** The CV, to be uploaded in PDF, shall be drafted in English, using the template available at this [link](#) and following the mandatory format: max 4-5 pages, page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom. For support in drafting the CV, please refer to NIH website: [Create Biosketches | NIAID: National Institute of Allergy and Infectious Diseases \(nih.gov\)](#). Please note that providing the eRA COMMONS USER NAME is NOT mandatory.

Accepted file formats: PDF. Max size: 30MB - Name the file as APPLICATION ID\_CV\_Surname (e.g., ID123456\_CV\_Rossi).

**Applications that do not meet the format requirements will be considered not admissible and will be rejected at the administrative review stage.**

6. **Letter of Institutional Endorsement**, addressing the following points:
- Confirmation of the Applicant's role at their Institution, and their eligibility under the category of PI (see section 2.3).*
  - Confirmation that relevant authorisations, declarations and accreditation from the competent authority(ies) have been obtained or will be obtained no later than two (2) months after Access approval, in order to process samples and data through the NFs.*
  - Justification of the request for Access – include a statement on why the project cannot be performed at the Applicant's Institution. Such statement shall confirm Applicant's justification provided (see point 2 above).*



- d. *Confirmation that the Applicant has not received funding for performing the submitted project, for the aspects to be performed at the NFs, in their own laboratory, host Institution, or elsewhere.*
- e. *Confirmation of the project's economic and scientific feasibility for the aspects to be performed at the host Institution.*
- f. *Acceptance of NF Access Rules.*

The Letter of Institutional Endorsement, to be uploaded in PDF or p7m in the dedicated section on the application portal, shall be drafted using the facsimile available as [Annex I](#) of this call. Name the file as APPLICATION ID\_ENDORSEMENT\_Surname (e.g., ID123456\_ENDORSEMENT\_Rossi).

7. **Technical information**, to be filled in in the dedicated section(s) of the application portal, indicatively including:

- a. *Requested service(s), as described in [Annex III](#) of this call.*
- b. *Sample technical information.*
- c. *Further analysis to be conducted in case additional capacity becomes available, once the proposed project is completed. Please consider that project's scope must remain unchanged. Follow-up activities are granted only as an exception and solely in case additional capacity becomes available after project completion. The allocation of additional capacity among projects is prioritized based on scientific merit.*
- d. *Requested preliminary data for technical feasibility analysis (if applicable).*
- e. *Whether the entire sample set is already available, or will be available no later than two (2) months from receiving Access approval. **Please note that if samples and/ or relevant ethical and legal authorisation(s) for their use will not be provided within this time frame, the request for Access will be automatically rescinded and PI will need to reapply at a subsequent call.***
- f. *Resources and expertise to receive and process the output – data (e.g. Cryo-EM micrographs) or reagents (e.g. human iPSCs) – generated by the NF.*
- g. *Research data management plan and bioinformatics support for data analysis, specifying (**mandatory when the project output includes research data** - e.g., genomics or proteomics data, bioimages from microscopy services, among other):*
  - i. *How the bioinformatics analysis of the data generated by the NF will be performed (if such analysis is not provided by the NF for Data Handling and Analysis).*
  - ii. *How the data generated by the NF will be handled during and after the end of the project.*
  - iii. *Whether and how the data will be shared/ made Open Access.*
  - iv. *How data will be curated and preserved, including after the end of the project.*

Details and format of the technical information to be provided are available in the dedicated section of the application portal.

Information provided in sections 1 and 6 are used for the eligibility and admissibility check.

Information provided in section 7 is used for assessing the technical feasibility of the aspects of the project to be performed at the NF.

The entire application is evaluated by the Standing Independent Evaluation Committee (SIEC) to assess its scientific merit.

## 6. APPLICATION SUBMISSION METHODS, CALL DEADLINE AND EVALUATION PERIODS

Applications shall be submitted exclusively through the application portal PICA managed by CINECA and accessible at this [link](#), according to the indicated terms and methods.

**This call for Access (Call ID: 25-LI-ROUND2) will open on the 1<sup>st</sup> of June 2025 (13:00 CET) and will close on the 30<sup>th</sup> of September 2025 (13:00 CET).**

A comprehensive list of services, available equipment and the technical requirements for Access as well as terms and conditions are available on the dedicated NFs webpage ([link](#)).

The complete list of offered services and technical requirements are available in the [Annex III](#) of this call.

**Samples as well as relevant authorisation** for their use, **shall ideally be available by when the application is submitted, but categorically not later than two (2) months after Access approval.** When the project foresees the analysis of more than one batch of samples, similarly, the first batch should be available when the application is submitted or not later than two (2) months after Access approval.

## 7. EVALUATION OF APPLICATION

The evaluation procedure is conducted by the SIEC that is supported by a Panel of independent external Reviewers (Review Panel) selected by the SIEC on the basis of their scientific expertise.

Each Review Panel is composed of two (2) SIEC members, who will act as Chairs, plus 10 appointed external Reviewers, with the relevant expertise.

Below is a scheme describing the evaluation steps and the **indicative timeline for the process.**



There are four application categories that are evaluated and ranked separately:

- Junior PI – Standard application
- Established PI – Standard application
- Junior PI – Proof of Concept application
- Established PI – Proof of Concept application

The NF User Access Office first performs an administrative review of the application to ensure that all the requested components have been provided, and that all eligibility criteria have been met. Incomplete applications or applications that do not meet all the requirements will be considered not admissible and will be rejected at the administrative review stage.

### 7.1 Triage

If the number of applications exceeds by a factor of three (3) the estimated capacity of the NF, a triage will be applied within each application category.

Triage criteria will include:

- a. Justification for requesting Access to the NF: priority will be given to researchers who do not have direct access to the service/ technology at their home institute.
- b. Ongoing and previous support received by the NFs: priority will be giving to researchers who do not have any ongoing Access to the NFs or who have never benefit form NF Access.

Should the number of proposals still exceed the allowable estimated limit after having applied the triage, as a tool of last resort, a lottery will be applied.

To ensure broader access for all institutes across Italy, proposals submitted by a single Institution that are sent for evaluation should not exceed the 10% of the total for any given career-based category.

### 7.2 Evaluation procedure and criteria

The application is then sent to the Review Panel for assessing technical feasibility and scientific merit. A comprehensive analysis of the technical feasibility of the project, which is performed by the NF staff, is provided as supporting documentation.

The application will remain confidential throughout the entire evaluation process. Reviewers will be asked to declare that they do not have any conflict of interest, and they will be bound by a Confidentiality Agreement.

The application will be individually evaluated by three Reviewers who are part of the relevant Review Panel.

Proposals will be evaluated and ranked based on their average score, within each category.

An on-line meeting of the Review Panel may be requested by the Chairs if deemed necessary (for example to discuss proposals with highly discrepant scores).

**At least 50% of the available Access will be allocated to applications from Junior PIs.**

The scientific merit of the project is assessed based on the following criteria:

- **Significance:** Overall scientific merit of the proposed research. If all the experiments proposed are successful, how will the resulting knowledge advance the field?
- **Innovation:** Degree of innovation (conceptual and/ or technological), and ambition of the proposed study compared to the state-of-the-art in the relevant field.
- **Approach:** Appropriateness of proposed methodology, preliminary data in support of proposed experiments, and project feasibility.
- **Environment:** Facilities and resources available to support the aspects of the project to be performed elsewhere (i.e., outside the NF).
- **Justification for requesting Access to the NF:** Explanation on why the service cannot be performed at the host Institution, at a cost which is deemed affordable for the applicant.
- **Applicant:** PI's scientific background and expertise.

### 7.3 Scoring system

A numeric score between 1 (exceptional) and 9 (poor) is provided for each of the six evaluation criteria. Moreover, an overall project score including a short descriptive comment is provided as feedback to the Applicant.

- **HIGH:**
  - **Score 1 (Outstanding)** – The proposal successfully addresses all relevant aspects of the criterion. There are no weaknesses.
  - **Score 2-3 (Excellent - Very Good)** – The proposal addresses the criterion exceptionally well, aside from a small number of minor weaknesses.
- **MEDIUM:**
  - **Score 4-6 (Very good - Good)** – The proposal addresses the criterion well, but a number of weaknesses are present.
- **LOW:**
  - **Score 7-8 (Fair - Poor)** – The proposal broadly addresses the criterion, but there are significant weaknesses.
  - **Score 9 (Poor)** – The criterion is inadequately addressed, or there are serious inherent weaknesses.

#### 7.4 Technical feasibility analysis

During the evaluation, the SIEC Chairs as well as the Reviewers will receive a report from NF staff who will perform a comprehensive analysis of the proposed project's technical feasibility. Technical feasibility also includes an evaluation of the fulfilment of the technical requirements in terms of capacity to receive and process the research data generated by the NF, as described in the research data management plan. This latter evaluation is performed in consultation with the NF for Data Handling and Analysis.

Based on the technical maturity of the project, the application can be assessed as Feasible/ Not Feasible/ Proof-of-Concept study required.

At this stage, the NF staff provides the SIEC Chairs with information on the resources needed (cost and time) to perform the proposed projects.

#### 7.5 Evaluation results and Access approval

Applications with the highest scientific score that fulfil all technical requirements are approved for Access by the SIEC, based on the capacity of the NF.

At least 50% of the overall capacity of each requested service is guaranteed for projects submitted by Junior PIs, provided that a sufficient number of qualified proposals are received.

In case of comparable scores, for applicants that are at the same career level category, the SIEC Chairs will have the authority to rank the applications based on secondary parameters such as number of applications per Institution, budgetary considerations and geographical distribution.

Evaluation results – Access granted, Access conditionally granted, Reserve list, Access not granted – are communicated to the Applicant through the Access portal.

A selected number of applications may be placed on a reserve list (i.e., waiting list in case of cancellations of Access granted projects). Applicants whose applications are placed on this list will receive additional information advising whether the project can be Access approved or should be resubmitted within the subsequent application window (see below).

## 8. RESUBMISSION OF RESERVE LIST PROJECTS

Reserve list projects that are not accommodated in the round they have been evaluated for will be granted two options:

- a. To be included in the new evaluation round maintaining the same project and review score as their initial application. Here, the application cannot be updated and is not sent out again for evaluation. **This option is provided only once, for the subsequent round only.**

To choose this option, the Applicant shall submit the request through the dedicated application dedicated call (Application for Reserve List Projects) accessible at this [link](#), indicating the call and the ID of the reserve list project.

- b. To resubmit an updated version of the proposal (for example including new preliminary data and/or taking into account Reviewer's comments).

To choose this option, the Applicant shall submit a new application which will be sent out for evaluation, indicating that it is a resubmission of a reserve list project and specifying the project ID.

## **9. AFTER ACCESS HAS BEEN APPROVED**

A kick-off meeting is organised after Access approval, in which the Applicant is invited to meet NF staff to discuss the experimental design of the project and to finalize the project plan.

Once the project plan has been agreed and the relevant ethical and legal authorisation(s) for the use of the samples has(have) been provided, the NF User Access Office coordinates the signature of the required formal Agreements (e.g., Access Agreement, Collaboration Agreement, other), when required, and the project can commence.

If samples and/ or relevant ethical and legal authorisation(s) for their use are not available within two (2) months after Access approval, the request for Access will be automatically rescinded and PI will need to reapply at a subsequent call.

## **10. AFTER ACCESS HAS BEEN COMPLETED**

At the end of the activities carried out at the NF, and not later than three (3) months thereafter, if not differently agreed with the NF User Access Office, the Applicant must submit a short report to be published on the NFs website on the results obtained and the impact of the service on their research. Moreover, a final report describing the impact of the Access to the NF on the research project for which the service has been requested, shall be provided upon publication of the relevant results. The NF User Access Office will provide a template for the requested reports including the information required (activities performed, outcomes, impact on PI's research, plan for data sharing with scientific community, among others). Applicants who will not be able to demonstrate the consistency and relevance of the activities carried out at the NF with the research project for which Access was requested will be considered not eligible to participate in the subsequent calls for Access.

Moreover, the Applicant will be asked to fill in a brief, mandatory survey regarding their experience, providing feedback and suggestions for further service improvement.

The Applicant must communicate to the NF User Access Office (via email to [national.facilities@fht.org](mailto:national.facilities@fht.org)) any publication acknowledging the NF.

Research data obtained during Access shall be made available to the scientific community following the FAIR principles. Applicant must inform the NF User Access Office (via email to [national.facilities@fht.org](mailto:national.facilities@fht.org)) when and how the data are made public.

## **11. CONTACTS**

Requests for information and/or clarifications concerning the application procedure may be sent to the dedicated e-mail address [national.facilities@fht.org](mailto:national.facilities@fht.org), indicating the call ID in the subject line.

## **11. REFERENCES**

NF Access Workflow\_Convenzione ([link](#))

NF Access Rules\_Convenzione ([link](#))

NF Access Agreement\_Convenzione ([link](#))

## **12. CHANGES TO THE CALL**

Any changes or additions to this notice will be communicated through publication on the NFs website ([link](#)).



## ANNEX I: LETTER OF INSTITUTIONAL ENDORSEMENT TEMPLATE

*(Print on paper bearing the official letterhead of the host Institution)*

### Endorsement letter of the host Institution

To whom it may concern:

I, the undersigned, ..... (*name of legal representative or special attorney*), born in ..... (*city*) on .....(*date*), as legal representative (*or special attorney, by means of special power of attorney identified by* ..... ) and on behalf of .....(*name of the host Institution*), legal residence in (*referred to the host Institution*) .....(*city*), address ....., regarding the project (*Title*) ..... presented by .....(*Applicants's first name and surname*), as Principal Investigator on the call for Access to Human Technopole National Facilities.....(*ID of the call*),

### Declare

- That the host Institution is among those eligible to participate in the call for Access as it belongs to the following eligible category: **(please select the one that applies: University, IRCSS, Public Research Entities);**
- That the Applicant, Dr ..... (*Applicant's first name and surname*) is an independent group leader (Principal Investigator) affiliated with a primary appointment at the host Institution and that they meet the eligibility criteria as indicated in the call;
- That the Applicant has not received funding for performing elsewhere, the aspects of the project for which they are seeking here support from or Access to Human Technopole National Facilities;
- That Applicant's request to Access the National Facilities is justified for the following reason **(please select the one that applies or best fits this application):**
  1. The requested service/ technology is not available at the Host Institution;
  2. The requested service/ technology cannot be performed at the Host Institution or elsewhere at an affordable cost;
  3. The requested service/ technology is available at the Host Institution but the necessary expertise is lacking;
  4. The requested service/ technology is available at the Host Institution but the service cannot be performed in a timeframe or scale compatible with the experimental requirements.
- That relevant authorisations, declarations and accreditations from the competent authority(ies) have been obtained in order to process samples and data through Human Technopole OR that, if relevant authorisations, declarations and accreditations



from the competent authority(ies) have not been obtained yet, they will be available before the starting date of the project, and not later than 2 months after Access approval;

- That, if applicable, biological specimens have been obtained or will be obtained with the corresponding approval of the Bioethics Committee and appropriately signed 'informed consent', both for their collection and their use, including conservation, manipulation, derivation and processing to be carried out by Human Technopole National Facilities;
- That, if samples were obtained or will be obtained from subjects who signed an 'informed consent', said informed consent allows or will allow that sequencing data and results are included in secure controlled Access databases and accessed/ used by authorized third parties;
- That, if applicable, copy of the relevant authorisations, declarations and accreditations will be provided at the moment of the application or not later than 2 months after Access approval;
- That, in case Physical Access to the National Facilities is requested, Applicant and/ or the team member(s) who will Access the National Facility have comprehensive insurance coverage for accidents and third-party liability, encompassing all their activities during their stay at HT and ensure that HT is recognized as third party. Name of the insurance company, insurance policy number and expiration date will be provided to HT before physical Access.

**and is committed**

To accept the terms and conditions to Access Human Technopole National Facilities as described in the National Facilities Access Rules ([link](#)) and, when applicable, the Access Agreement and its annexes ([link](#)).

For the host Institution (Applicant legal entity/beneficiary):

Date .....

Name and Title ..... ; .....

Email and Signature of **legal representative or delegated person (e.g., Head of Department)**

..... ; .....

## ANNEX II: PROJECT PROPOSAL TEMPLATE

### ***Mandatory proposal format***

**Standard application:** Max 3 pages (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom) figures included, references excluded. Accepted file formats: PDF. Max size: 30MB - Name the file as APPLICATION ID\_PROPOSAL\_Surname (e.g., ID123456\_PROPOSAL\_Rossi)

**Proof-of-Concept application:** Max 2 pages (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom) figures included, references excluded. Accepted file formats: PDF. Max size: 30MB - Name the file as APPLICATION ID\_PROPOSAL\_Surname (e.g., ID123456\_PROPOSAL\_Rossi)

**PLEASE REMOVE THE INFORMATION ABOVE BEFORE SUBMITTING**

### ***Proposal content:***

1. TITLE
2. SIGNIFICANCE
3. INNOVATION
4. APPROACH
5. ENVIRONMENT
6. REFERENCES (Optional)

**ANNEX III: SERVICE LIST**

**HUMAN TECHNOPOLE**

**NATIONAL FACILITY FOR LIGHT IMAGING**

**CALL FOR ACCESS**

**25-LI-ROUND2**

**SERVICE LIST**

## Table of contents

1. INTRODUCTION.....	21
2. GENERAL TECHNICAL REQUIREMENTS.....	22
3. SERVICE LIST.....	22
(IU1) Imaging.....	22
SID: NF50.014 - Widefield microscopy, including TIRF and Lattice Lightsheet .....	22
SID: NF50.015 - Confocal microscopy.....	23
SID: NF50.016 – Super-resolution microscopy.....	24
SID: NF50.017 – Microscopy Consultation Session: Sample Preparation/ Experimental Design/ Basic Image Processing.....	25
(IU2) Tissue Processing .....	25
SID: NF52.07.07- Zeiss Axioscan Z.1 automated slide scanner .....	25
(IU3) Flow Cytometry .....	27
SID: NF53.001: Flow Cytometry Cell Sorting.....	27
SID: NF53.002/003 Flow Cytometry Analysis/ Assisted Flow Cytometry Analysis....	28
SID: NF53.004 Flow Cytometry Consultation Session: Sample Preparation/Panel Design/Data Analysis.....	30
(IU5) Ion Imaging.....	30
SID: NF55.003/004 – Microelectrode Arrays (MEAs) assays .....	30
SID: NF55.006 - Ion imaging assisted experiment .....	32
Appendix 1: Description of the Data analysis service available in combination with the NF for Light Imaging services .....	34

## 1. INTRODUCTION

The NF for Light Imaging offers services related to imaging, sample preparation and cell sorting. Currently, four Infrastructural Units (IU) within the NF are active and available to external applicants:

### IU1: Imaging

The Imaging IU offers Access to several high-end microscopy systems. Microscopes can be used to image fixed or living samples. The Access modality (simple access, access to NF services, access to NF services including training) for each service will consider the complexity of the experiment and the system being used, the user's knowledge of the microscopy technique, and the time required to complete the experiment. Images acquired using the systems offered by IU1 can optionally be analyzed by the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis (please refer to Appendix 1 for more details).

### IU2: Tissue Processing

The Tissue Processing IU2 is dedicated to advancing biomedical research by providing platforms, services and specialized training. Our focus areas include Spatial Biology, Digital Pathology and 3D imaging, enabling researchers to achieve spatially resolved insights into complex biological systems

### IU3: Flow cytometry applications

The Flow Cytometry IU offers full-service sorting & cloning of rare cellular populations, particle enrichment, and high purity bulk sorts. The Unit also offers Analysis Services, advice in experimental design and training.

### IU5: Ion Imaging

The Ion Imaging IU offers functional cell imaging using fluorescence-based time-lapse recordings of intracellular ion oscillations. Optical imaging is primarily based on confocal or epifluorescence microscopy.

Samples are required to have biosafety containment level (BSL) 1 or 2. Living samples must be delivered according to the guidelines available on request from the NF. Successful applicants will be responsible for maintaining living samples using the infrastructures (incubators, hoods, etc.) available on-site. Applicants and NF staff will prepare and mount the samples according to the experimental protocols agreed in advance. NF will ultimately decide whether an experiment should be performed using NF microscopes.

## 2. GENERAL TECHNICAL REQUIREMENTS

The NF for Light Imaging can accept biological samples of biosafety containment level (BSL) 1 and biosafety containment level (BSL) 2.

Applicants must ensure that the samples are available in sufficient quantity and quality before the closing date of the application period.

The NF for Light Imaging shall reserve the right to perform a pilot experiment on samples provided by the Applicants to assess the technical feasibility of the submitted project.

## 3. SERVICE LIST

### (IU1) Imaging

Information to be provided in the technical information section of the application:

- Number and type of samples (e.g., cell lines, organoids, tissue sections, etc.)
- Number of and sample conditions (e.g., control, wild-type, mutant, treatments, etc.)
- Number of replicates (e.g., number of technical or biological replicates, etc.)
- Number of images to be acquired per sample and/or condition
- Sample information: Fixed/ live samples, BSL1 or 2
- Imaging support: Coverslip/ Petri dish/ Multiwell/ Other (specify)

### SID: NF50.014 - Widefield microscopy, including TIRF and Lattice Lightsheet

Widefield microscopy is a versatile imaging technique where the entire imaged volume is illuminated simultaneously, making it ideal for capturing fast dynamics in live cells or for imaging a large area quickly. It is commonly used for fluorescence and non-fluorescent imaging, high-throughput acquisition, and time-lapse experiments in cell biology and related fields. This imaging technique includes advanced configurations:

- Bright field, Phase contrast, Differential Interference Contrast (DIC) for label-free visualization of cells and tissues;
- Fluorescence imaging, with motorized epifluorescence and integrated digital clearing, for improved contrast and signal-to-noise ratio;
- Total internal reflection (TIRF), for high-resolution imaging of events near the cell membrane;
- Lattice Lightsheet with dual cameras for fast, volumetric imaging with minimal phototoxicity and photobleaching.

All the systems are equipped with environmental control incubator (CO<sub>2</sub>, temperature, humidity) for long-term live-cell imaging under physiological conditions.

Available systems:

- Leica Thunder;
- Zeiss Lattice Lightsheet;
- Zeiss Elyra 7;
- Nikon-Crest spinning disk.

The choice of the most suitable microscope will be made by facility staff based on the scientific, technical, and experimental requirements outlined in the user's application.

**Access modality available:** Simple Access, Access to Facility services or Access with Training.

**Image Analysis can be provided as a combined service by the National Facility for Data Handling and Analysis. Please select: SID: NF61.01.01 – Light Microscopy Analysis.** For more details, please refer to Appendix 1.

#### SID: NF50.015 - Confocal microscopy

Confocal microscopy is a laser-scanning fluorescence imaging technique that allows for optical sectioning and high-resolution imaging of thick or complex biological samples. By rejecting out-of-focus light through pinhole-based detection, confocal imaging enables detailed three-dimensional reconstruction of structures in both live and fixed specimens. This service includes:

- Confocal imaging with resonant and conventional galvanometer scanner;
- Spectral imaging;
- Spinning disk imaging with four high-speed, back sided illuminated cameras and photomanipulation module (e.g FRAP, optogenetics);
- Fluorescence lifetime imaging (FLIM);
- Airyscan and MATRIX detection (enhanced resolution and sensitivity);
- Multiphoton imaging for deep tissue imaging and nonlinear techniques (second-harmonic generation).

All the systems are equipped with environmental control incubator (CO<sub>2</sub>, temperature, humidity) for long-term live-cell imaging under physiological conditions.

Available systems:

- Leica Stellaris 8;
- Zeiss LSM980;
- Zeiss LSM980-NLO;
- Nikon-Crest spinning disk;
- Abberior Facility Line.

The most suitable system will be selected by facility staff based on the experimental, scientific, and technical requirements provided in the user's application.

**Access modality available:** Simple Access, Access to Facility services or Access with Training.

**Image Analysis can be provided as a combined service by the National Facility for Data Handling and Analysis. Please select: SID: NF61.01.01 – Light Microscopy Analysis.** For more details, please refer to Appendix 1.

#### SID: NF50.016 – Super-resolution microscopy

Super-resolution microscopy allows for acquiring images at a resolution below the diffraction limit. It is possible to achieve super-resolution imaging using several technologies, such as STED microscopy, structured illumination (SIM) or localization-based (PALM, SOTRM) approaches. These techniques are essential for studying fine molecular details, protein organization, and complex intracellular dynamics that are not resolvable with conventional light microscopy.

**NOTE:** super-resolution requires high-precision coverslips. Please contact the facility if in doubt.

The IU1 offers several super-resolution techniques tailored for a wide range of biological applications:

- 2D and 3D STED (Stimulated Emission Depletion) microscopy for super-resolution imaging in live or fixed samples;
- 2D and 3D Single Molecule Localization Microscopy (SMLM) techniques including STORM (Stochastic Optical Reconstruction Microscopy) and PALM (Photoactivated Localization Microscopy) for precise localization of individual fluorophores;
- Structured Illumination Microscopy (SIM) for super-resolution imaging using standard fluorescent proteins.

All the systems are equipped with environmental control (CO<sub>2</sub>, temperature, humidity) to support live-cell super-resolution imaging under physiological conditions.

Available systems:

- Abberior Facility Line;
- Leica Stellaris 8 STED;
- Zeiss Elyra7.

Facility staff will advise and assign the most appropriate system based on the scientific goals, sample characteristics, and technical requirements outlined in the user's application.

**Access modality available:** Simple Access, Access to Facility services or Access with Training.



**Image Analysis can be provided as a combined service by the National Facility for Data Handling and Analysis. Please select: SID: NF61.01.01 – Light Microscopy Analysis.** For more details, please refer to Appendix 1.

SID: NF50.017 – Microscopy Consultation Session: Sample Preparation / Experimental Design / Basic Image Processing

**NOTE:** This service can be only requested in association with the services NF50.014, NF50.015 and NF50.016.

The service provides personalized consultation sessions to support users at all stages of their microscopy-based experiments. Our expert staff will help designing robust, reproducible, and technically sound workflows tailored to the scientific goals of the project.

Consultations may include:

- Sample preparation guidance, including fixation protocols, mounting techniques, and fluorophore selection based on imaging modality;
- Basic image processing;
- For advanced image analysis refer to service NF60.001 – Light Microscopy Analysis.

## (IU2) Tissue Processing

SID: NF52.07.07 - Zeiss Axioscan Z.1 automated slide scanner

**Description:** The Zeiss Axioscan Z.1 is an automated slide scanner designed to capture whole slide images in both brightfield and epifluorescence modes, accommodating up to 100 slides per session.

**Configuration:**

- Cameras:
  - Hamamatsu Orca-Flash 4.0 v3 sCMOS mono camera
  - Hitachi HV-F203SCL 3CCD color camera
- Light sources:
  - Colibri 7 for epifluorescence (385 nm, 430 nm, 475 nm, 555 nm, 590 nm, 630 nm, 735 nm)
  - White led lamp for transmitted light
- Filter sets optimised for:
  - DAPI (Zeiss BP 450/40)
  - GFP / Alexa Fluor 488 / FITC (Zeiss BP 525/50)
  - Cy3 / Alexa Fluor 555 / TRITC (Zeiss BP 605/70)
  - Cy5 / Alexa Fluor 647 / APC (Zeiss BP 690/50)

- DAPI/GFP/Cy3/Cy5/Cy7 (Zeiss PBP 425/30+514/31+592/25+681/45+785/38)
- Six (6) objectives:
  - 2,5x / 0.12 NA Fluar M27
  - 5x / 0.25 NA Fluar M27
  - 10x / 0.45 NA Plan-Apochromat M27
  - 20x / 0.45 NA N-Achroplan Pol M27
  - 20x / 0.8 NA Plan-Apochromat M27
  - 40x / 0.95 NA Plan-Apochromat M27
- Slide racks:
  - Standard microscope slides 75 x 25 mm
  - Macro sections slides 75 x 50 mm
- File formats:
  - “.czi” native lossless or lossy file format with JpegXR compression
  - “BigTiff” after post processing
  - “OME.TIFF” after post processing

**Applications:** High-speed, automated whole slide imaging in brightfield and fluorescence.

**Information to be provided in the technical information section of the application:**

- Fresh frozen/ Fixed frozen/ FFPRE
- Cell in adhesion (yes/no)
- Small biopsies (yes/no)
- Classical sections (fit a 25 mm x75 mm glass slide)/ Macro sections (fit a macro glass slide 50 mm x 75 mm)
- Tissue Microarray (yes/no)
- Brightfield (yes/no)
- Fluorescence (yes/no)
- List the fluorochrome(s) in your experiment
- Magnification needed
- z-stack needed (yes/no) If yes, how many z-stack you need to acquire
- List any special needs
- Estimation of time (hours) needed to complete the Access

**Access modality available:** Simple Access or Access to NF service (to be discussed with the NF manager).

**Image Analysis can be provided as a combined service by the National Facility for Data Handling and Analysis. Please select: SID: NF61.01.01 – Light Microscopy Analysis.** For more details please refer to Appendix 1

### (IU3) Flow Cytometry

#### SID: NF53.001: Flow Cytometry Cell Sorting

**Description:** Full-service sorting of rare populations from heterogeneous samples, cell cloning (single cell deposition into multi-well plates), particle enrichment, and high purity bulk sorts.

The proposal must include the list of fluorochromes to be analysed. Please use the links below to find the excitation and emission wavelength details for each instrument, as a reference to help you design your panel.

[Optical Configuration Astrios EQ](#)

[Optical Configuration Cytex Aurora](#)

[Optical Configuration Cytoflex LX](#)

[Optical Configurations FACSDiscover S8](#)

[Optical Configuration ImageStream MKII](#)

High-recovery and indexed single-cell sorting for sequencing. Cell sorting of many cell types including:

- immune cell and hematopoietic stem cell subsets
- mesenchymal stem cells
- viable cytokine producing cells
- general cell sorting approaches for cell lines
- transfected cells, including iPSCs

#### Sorting Technical Details

Capable of standard and high-speed sorting of up to 6 populations simultaneously. Sorted cells may be recovered in numerous devices including:

- 6, 12, 24, 28, 96, 384, 1536 well plates & 96 deep well plates
- 0.2, 0.5, 1.2, 1.5, 2, 5 mL tubes for 6-way sorting
- 15, 50 mL tubes for 2- or 3-way sorting
- Slides or Ibidi vessels
- Custom vessels may also be programmed.

The sorter is equipped with 6 lasers having the following emissions: 355 nm 405 nm, 488 nm, 560 nm, 592 nm, 645 nm.

Proper controls for each session must be included with the analysis samples. For example:

- Unstained, unlabeled or other cellular controls
- Spectral overlap compensation controls
- FMO/FMX controls when appropriate

**Information to be provided in the application:**

For samples submitted for flow cytometry cell sorting, please specify the following characteristics or conditions:

- Fresh/Fixed
- Tested viability (Yes / No)
- Filtered (Yes / No)
- Sterile (Yes / No)
- Frozen (Yes / No)
- BSL1/BSL2
- Mycoplasma Tested (Yes / No)
- Collection support
  - Tube
  - Eppendorf
  - Multiwell
  - Slide
  - Other
- Application after sorting
  - Cell culture
  - DNA
  - RNA

**Shipping and storage conditions:** The cell type and the distance to the flow cytometry lab both dictate the type of shipping conditions that you should use. For example, most lymphocytes can be isolated from whole blood and can be shipped and stored as frozen samples, but certain cell types like dendritic cells lose viability when frozen and can only be shipped and stored under cold conditions. You will need to evaluate the viability of your cells of interest under different storing and shipping conditions to determine which method provides the greatest viability.

**Data provided:** the NF staff will provide a report of the cell sorting session indicating the gating strategy, the sorting efficiency, the final number of sorted cells and all relevant information. A purity assessment analysis will be performed on bulk sorts when sufficient cells are available.

**Access modality available:** Access to NF service.

[SID: NF53.002/003 Flow Cytometry Analysis/ Assisted Flow Cytometry Analysis](#)

**Description:** Autonomous Flow Cytometry Analysis (after completion of a training session) or Operator Assisted Flow Cytometry Analysis.

The proposal must include the list of fluorochromes to be analysed. Please use the links below to find the excitation and emission wavelength details for each instrument, as a

reference to help you design your panel.

[Optical Configuration Astrios EQ](#)

[Optical Configuration Cytex Aurora](#)

[Optical Configuration Cytoflex LX](#)

[Optical Configurations FACSDiscover S8](#)

[Optical Configuration ImageStream MKII](#)

The following are examples of analyses we offer:

- Prepared immune cell and hematopoietic stem cell subsets.
- Prepared and labelled samples of mesenchymal stem cells.
- Analysis of cytokine producing cells.
- Multi-color extracellular and intracellular stained samples.
- DNA content analysis (single color or multi-color).
- Cell proliferation analysis.
- Apoptosis analysis.
- Functional and metabolic assays (mitochondrial function, ROS production, lipid metabolism).
- Analysis of bead assays (e.g., Cytokine/chemokine bead assays).

Analyzer Technical Details:

- Integrated absolute count
- Plate loader supporting 96 wells plate and 96 deep wells plate. U, V or Flat bottom
- Different sample injection modes, manual or automatic
- Wide sample flow rate and minimal dead sample volume (about 20 µl)

Analyzers are equipped with 5 or 6 lasers having the following emissions: 355 nm, 405 nm, 488 nm, 561 nm, 640 nm and 808 nm. SSC parameter is available for the 488 and the 405 nm lasers lines.

- Capable of complex polychromatic panels.
  - Advice in experimental design and dedicated training sessions are also available. Proper controls for each session must be included with the analysis samples.
- For example:

- Unstained, unlabeled or other cellular controls.
- Spectral overlap compensation controls.
- FMO/FMX controls when appropriate.

**Information to be provided in the application:**

For samples submitted for flow cytometry analysis services, please specify the following characteristics or conditions:

- Fresh/Fixed

- Filtered (Yes / No)
- Frozen (Yes / No)
- BSL1/ BSL2
- Mycoplasma Tested (Yes / No)
- Other

**Shipping and storage conditions:**

The cell type and distance to the flow cytometry lab dictate the type of shipping conditions you will use. For example, most lymphocytes can be isolated from whole blood and can be shipped and stored as frozen samples, but certain cell types like dendritic cells lose viability when frozen and can only be shipped and stored under cold conditions. You will need to evaluate the viability of your cells of interest under different storing and shipping conditions to determine which method provides the greatest viability.

**Staining or labeling stability:**

Some cell samples can be stained as fresh samples and then shipped for flow cytometry analysis but the staining panel and fluorochromes dictate the stability of staining and ability to ship pre-stained samples for analysis. Fixing the samples after staining if appropriate for your experiment will provide longer transit times.

**Access modality available:** Access to NF service or Access with training. A consultation session (SID: NF53.004) can be added to the service.

#### [SID: NF53.004 Flow Cytometry Consultation Session: Sample Preparation/Panel Design/Data Analysis](#)

User consultation is an essential first step in assuring high-quality flow cytometric data. Our staff are available to guide new and current Users to meet each projects' needs, addressing all critical steps between the conception of the flow cytometry experiment, the appropriate steps for sample preparation including the required experimental controls, the acquisition of samples and the analysis of the data generated.

#### **(IU5) Ion Imaging**

##### [SID: NF55.003/004 – Microelectrode Arrays \(MEAs\) assays](#)

**Description:** High resolution, functional imaging based on high density MEA electronic interfaces measuring extracellular voltage potentials. The system available is the multichannel amplifier BioCAM Duplex and planar MEAs electronic chips equipped with 4,096 electrodes (3BRAIN AG, Pfäffikon SZ, Switzerland). The MEAs chip is the CorePlate™ 1W38/60 (NF55.004\_MEAs chip testing and provision) with a recording area of 3.8 mm x 3.8 mm, each electrode is 21 mm x 21 mm with a 60 mm pitch. The assay is suitable for the characterization of functional phenotypes in cell networks, and for compound screening. The high density of electrodes allows to collect a large amount of data within a single recording, and it is particularly suitable for accessing the electrical phenotype of brain and cardiac organoids.

**Applications:** brain and cardiac cell models in 2- and 3D. Different biological models will be evaluated as custom services during the feasibility study of the project

Typical investigations include:

- Longitudinal recording, by means of multiple recording at different time points to follow the development of the electrical activity. It is applicable on 2- and 3D models cultured onto the MEA chip.
- Acute recording (only for 3D models)
- Characterization of spontaneous or pharmacologically triggered activity. The assay can also be used to investigate the cellular network and for compound screening

**Limit:** MEA services are available for proof-of-concept or small-scale projects. The maximum capacity per batch is 30 MEA chips, but the project scale should be discussed with the NF manager.

**Information to be provided in the technical information section of the application:**

- Type of sample (2D culture / 3D culture) and number of samples
- Number of technical replicates
- Recording conditions (Basal only, One drug/ compound, More than one drug/compound)
- Number of Time Points to evaluate
- BSL1/ BSL2
- Viability tested
- Mycoplasma tested
- Estimation of time (Hours) needed to complete the Access
- Estimation number of chip (NF55.004) to complete the Access
- Type of recording (Acute/ Culture / Longitudinal Recording)
- Pharmacological validation of recorded activity
- Culturing conditions for the sample (temperature, CO<sub>2</sub>, medium composition)

**Procedures:** After a standard quality control to verify that the cultures/ organoids express the cell types of interest, samples will be prepared as cell/organoid cultures on MEAs electronic chips by the Facility staff when they reach the proper developmental stage for functional characterization (this timing depends on the biological model and will be discussed during the feasibility study of the project). Recordings from MEA cultures will be acquired with the parameters (i.e. sampling rate, filters) established by the user and within the range of the instrument's capability.

**Data provided:** The user will receive raw data from MEA recordings in a hdf5 format, and will be responsible for their processing, analysis, and interpretation. Optionally, the user can ask to perform a basic processing of raw data using the 3BRAIN AG proprietary software (Brainwave) for the detection of the electrophysiological events of interest. The user will be responsible for the choice of the parameters used in data processing (such as high-, low- or bandpass filters and threshold for event detection) within the capability of the software. Processed data will be delivered to the user in a hdf5 format.

**Access modality available:** Access to NF service.

**SID:** NF55.006 - Ion imaging assisted experiment

**Description:** Fluorescence-based time-lapse recordings of intracellular ion oscillations, based on confocal or epifluorescence microscopy.

- Imaging on Nikon Ti2 spinning disk with four cameras, TIRF and FRAP module (SID: NF50.007 or equivalent system suggested by the NF staff).

**Applications:** Calcium imaging based on chemical or genetically encoded sensors. Available and ready-to-use sensors are the chemical, cell permeable calcium dyes such as Fluo-4 AM or Rhod-3. Genetically encoded sensors should be expressed and tested in the biological model of interest before entering the NF (the applicant is responsible for this task). Different ions (e.g., chloride) and voltage imaging protocols can be developed upon request.

Typical investigations include:

- Characterization of spontaneous or pharmacologically induced ion oscillations across cell networks, in 2- and 3-dimensional cell culture models.
- Characterization of functional connectivity and signaling within cell networks, in 2- and 3-dimensional cell culture models.

**Technical requirements:**

- Only BSL 1 or BSL 2 samples.
- The method of shipment of the living sample will be discussed with the NF manager during the kick-off meeting.
- Applicants will prepare the samples in accordance with the technical requirements of the microscope and of time-lapse imaging approaches (e.g. using microscopy-grade dishes for adherent cell cultures).
- Applicants will provide the NF staff with the protocols for culturing and the media culture recipe to maintain living samples within the NF for the total duration of the experiments.
- The samples tested with this approach must be in a developmental stage (i.e., days in vitro) sufficient to express the phenomena of interest. This aspect strongly depends on the biology of the model. Therefore, the timing of experimental procedures will be discussed within the context of the specific project. The Applicant will deliver the samples to the Light Imaging NF at their estimated time of maturity for ion imaging experiments.

**Information to be provided in the technical information section of the application:**

For samples submitted for ion imaging services, please specify the following characteristics or conditions:

- Type of sample (2D culture / 3D culture) and number of samples
- Number of technical replicates
- Recording conditions (Basal only, One drug/ compound, More than one drug/compound)



- Number of Time Points
- BSL1/ BSL2
- Viability tested
- Mycoplasma tested
- Estimation of time (Hours) needed to complete the Access
- For adherent cells (2D culture), sample already tested for attachment on microscopy-grade dishes/glass coverslips (Yes/No)
- Culturing conditions for the sample (temperature, CO<sub>2</sub>, O<sub>2</sub>, medium composition)

**Data provided:** The Applicant will receive raw data from ion imaging recording in the format of the acquisition software used.

**Access modality available:** Access to NF service.

**Image Analysis can be provided as a combined service by the National Facility for Data Handling and Analysis. Please select: SID: NF61.01.01 – Light Microscopy Analysis.** For more details please refer to Appendix 1.

## Appendix 1: Description of the Data analysis service available in combination with the NF for Light Imaging services

### NF61.01.01 Light Microscopy Analysis

#### Service description

Light microscopy analysis encompasses the analysis of data generated by any light microscopy modality (ie, brightfield, phase contrast, widefield epi-fluorescence, confocal, lightsheet, etc) and across any sample type.

The services we provide include, but are not necessarily limited to, the following use-cases:

- **Image restoration and denoising:** Removal of pixel-independent noise from images to increase signal-to-noise ratio (SNR).
- **Semantic and Instance segmentation:** Identification and segmentation of objects in an image, generation of image masks.
- **Quantitative Image Analysis:** Quantification of intensity levels in images or segmented objects.
- **Morphometric Analysis:** Analysis of shape and morphology of segmented objects.
- **Custom pipeline development:** Construction of an analysis pipeline combining two or more individual steps.

While these are examples of the services we can provide, we anticipate that most projects will require some combination of tools and services and so we will work with users to craft pipelines that fulfil their analysis needs, as well as provide training and support in their future use. Our ethos is to work openly and transparently with our users in the spirit of scientific collaboration. During the application phase, it will only be necessary to describe the analysis goals; the precise details of the analysis will be discussed with the users upon selection of the project.

#### Access modality available

- Access to facility service
- Access to facility service including training

#### Requested inputs from users

For this service, we require a detailed project description outlining the analysis goals, and the expected data to be analyzed. A full analysis plan will be developed in collaboration with the successful applicants and the National Facility for Light Imaging as the project proceeds. The data will be transferred directly from the National Facility for Light Imaging to the National Facility for Data Handling and Analysis upon successful completion of the data acquisition phase of the project.

#### Technical requirements

Applicants must ensure that the samples meet the quality standards of the National Facility for Light Imaging, and that a sufficient number of samples are available to achieve the desired analysis goals.

## Results

Upon successful completion of the selected project, results will be delivered in a format of the Users' choosing and depending on the project needs. In addition, we will provide whatever software, code, and support is required for the User to reproduce the analysis at their home institute. The form will depend on the specifics of the project and the needs of the Users, but we anticipate delivery in the form of Python scripts and/or ImageJ macros. To reduce the burden of Access for our Users, we will use open-source software tools during the NF projects.

The facility will also assist the user in submitting raw data to public repositories, as stipulated in the *National Facilities Access Rules*.

## Combined services

This service can be combined with the following services offered by the National Facility for Light Imaging:

NF50 – All services

NF55.006 - Ion imaging assisted experiment

To access the combined services, please submit an application to the National Facility for Light Imaging [requesting data analysis](#).