HUMAN TECHNOPOLE
NATIONAL FACILITY FOR
LIGHT IMAGING
CALL FOR ACCESS
26-LI-ROUND-3

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1. SUMMARY OF CHANGES TO THIS CALL FOR ACCESS

Compared to previous rounds, the following changes have been introduced in this call:

- *Specific procedures for the **resubmission** of proposals already evaluated in previous calls, and for the submission of **project continuation** (formerly known as "follow-up"), i.e. research proposals that build on the findings and results of a previously approved Access, have been implemented in the application process (section 9 and section 10).*
- ***Changes in the available services for this call (Annex III).***
- *Rules for the "Responsible use of generative Artificial Intelligence tools in proposal preparation and evaluation" (link to the document).*
- *Eligibility and admissibility (section 5).*
- *Application content and Accountability of Applicants and host institutions (section 6).*

We recommend that the applicants read the present document and the guidelines (link) carefully before applying to the current call for Access.

2. 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).

2.1 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 (Physical Access)**: 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 (Remote Access)**: 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. In this case, physical Access may be approved.

- **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. **Note that in the application, a motivation underlying the request for "Access with training" must be specified.**

3. TERMS AND DEFINITIONS

3.1 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.

3.1.1. Physical Access (or in-person Access)

"Physical Access" applies when a User accesses a NF or a service by being physically present at the selected NF. In order to obtain physical Access, the User must complete all necessary security and health, safety and environment (HSE) checks, complete all required training and be provided with all the necessary insurances. Physical Access is also required for on-site training.

3.1.2. Remote Access

"Remote Access" means Access to a NF or service without the User being physically present at the NF. Typically, NF staff perform the experimental protocols entailed by the service and return the Data and results produced to the User together with any resulting samples (when appropriate). If applicable, "remote Access" can also include remote analysis of the samples by the User after sending them to the NF.

IMPORTANT - Additional information and details about **Access** are reported in the NF Access rules available on the NFs dedicated webpage (link).

3.2 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.

3.3 Principal Investigator, Junior and Established PI

"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.

3.4 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 5.

3.5 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.

4. 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, substantiated by robust preliminary data and/or requesting a standard service based on existing, well validated experimental conditions and/or protocol(s).

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).

Additional details related to the application contents are provided in section 6.

5. ELIGIBILITY AND ADMISSIBILITY

PIs, as defined in section 3.3 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.

Applicants are highly encouraged to contact the staff of the facility for a consultation before submitting their application, to discuss any technical aspects of the project that may affect its feasibility.

All applications submitted to this call, regardless of whether a consultation has taken place, must undergo a technical feasibility assessment. Applicants choosing not to consult with the facility staff acknowledge that their application may be considered not feasible following the technical feasibility analysis.

The staff of the facility is available for consultancy until 10 working days before the call deadline. Requests received after this deadline may not be addressed in due time. To get in touch, please send an inquiry to NF.lightimaging@fht.org, indicating the name of the requested service(s) in the email subject line.

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:
  - State funded public universities, listed under the following link.
  - Specialized superior graduate schools or institutions, listed under the following link.
  - Legally recognized non-public universities, listed under the following link.
  - 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 Formazione - INVALSI;
o) Istituto Nazionale di Documentazione, Innovazione e Ricerca Educativa - INDIRE;
p) Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria - CREA;
q) Agenzia Nazionale per le Nuove Tecnologie, l'energia e lo Sviluppo Sostenibile - ENEA;
r) 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);
s) Istituto Nazionale di Statistica - ISTAT;
t) Istituto Superiore di Sanità - ISS;
u) Istituto Superiore per la Protezione e la Ricerca Ambientale - ISPRA, ferme restando le disposizioni di cui alla legge 28 giugno 2016 n.132;
v) 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/ data and relevant authorisations are available at the moment of application or no later than two (2) months** from receiving Access approval (refer to section 8). **If samples/ data 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 form.

A PI submitting an application to this call for Access **cannot request Access to other NFs** (i.e., cannot participate to other 26-ROUND-3 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, samples/ data availability, samples/ data 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 call requirements will be considered not admissible and will be rejected at the administrative review stage.

6. APPLICATION

6.1 Application content and format

All applications must be submitted through the online portal PICA (link), following carefully the guidelines (link) and filling all mandatory fields of the application form. Applicants should also consider the information provided in section 7 before initiating the submission process.

The main contents of the application form are:

1. **Applicant's general information**.
2. **Justification for requesting Access to the NF,** explaining why the project cannot be performed at the Applicant's institution.

   We recommend that the Applicant contacts the institute representative, i.e., the individual authorized to sign the Letter of Institutional Endorsement (LoE) to ensure that the most appropriate justification is provided and that it aligns with the one included in the LoE (point 6.c, below).

   If the justification provided by the Applicant does not match the one provided by the host institution through the LoE, the application will be rejected during the administrative review stage.

   The Applicant must choose the option that best applies and must provide further details supporting the choice:

   - The requested service/ technology is not available at the host institution.
   - The requested service/ technology cannot be performed at the host institution or elsewhere at an affordable cost.
   - The requested service/ technology is available at the host institution, but the necessary expertise is lacking.
   - 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.

   NOTE: The Standing Independent Evaluation Committee (SIEC), in charge of the evaluation procedure, may reserve the option to contact the host Institution and its core

facilities to confirm the justification provided. **Read carefully information in section 6.2**.

3. **Project Title, Abstract** and **Area of Research**, to be inserted in the dedicated section on the application form (Max 1500 characters including spaces).

   IMPORTANT - Do not share any confidential information: project title and abstract will be published on HT website as provided for by the Convenzione, Art 5, Comma 5 (for more information, the Convenzione is available at this link or as pdf).

4. **Project proposal**, to be uploaded in PDF format in the dedicated section on the application form, shall include the following (see also Annex II):

   - 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)

   The **use of generative AI tools** in the preparation of proposals is governed by the rules and recommendations outlined in the "Rules for the Responsible Use of Generative Artificial Intelligence in Proposal Preparation and Evaluation for Calls for Access to National Facilities" (link to the document), which is an integral part of this call. Applicants are also required to declare any use of generative AI by responding to the relevant questions in the application form.

   Below are the **mandatory formats** for the proposal, also based on the type of application (see sections 4, 9 and 10 for additional details):

   a. **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)

   b. **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)

   c. For **Resubmissions** (section 9), a **Resubmission cover page** must be included in the proposal. The total length of the application is hence increased by one page with respect to the format illustrated above. Refer to section 9 for important details on Resubmissions.

   d. For **Project Continuation** (section 10), the **Prior Results and Research Advancement cover page** must be included in the proposal. The total length of the application is hence increased by one page with respect to the format illustrated above. Refer to section 10 for important details on Project continuation.

   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 as PDF file, shall be in English and produced by using the **NIH biosketch template available in Annex**

II or at this link. For support in drafting the CV, you can still refer to the NIH website Biographical Sketch Common Form (nih.gov). Please note that having an eRA COMMONS USERNAME is NOT required.

Biosketch 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.

Accepted file format: 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:
   a. *Confirmation of the Applicant's role at their institution, and their eligibility under the category of PI (see section 3.3).*
   b. *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 (refer to section 8), in order to process samples and data through the NFs.*
   c. *Justification for requesting Access to the NF explaining why the project cannot be performed at the Applicant's institution. We recommend that the Applicant contacts the institute representative, i.e., the individual authorized to sign the Letter of Institutional Endorsement (LoE) to ensure that the most appropriate justification is provided and that it aligns with the one included in the LoE.*

      If the justification provided by the Applicant does not match the one provided by the host institution, the application will be rejected during the administrative review stage.

      *The host institution must select **only one option**, from the four listed below — please do not alter the statements:*

      - *The requested service/ technology is not available at the host institution.*
      - *The requested service/ technology cannot be performed at the host institution or elsewhere at an affordable cost.*
      - *The requested service/ technology is available at the host institution, but the necessary expertise is lacking.*
      - *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.*

      ***NOTE**: The SIEC, in charge of the evaluation procedure, may reserve the option to contact the host institution and its core facilities to confirm the justification provided.* **Read carefully information in section 6.2**.
   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* (link).

The Letter of Institutional Endorsement, to be uploaded in PDF or p7m in the dedicated section of the application form, 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).

> **IMPORTANT**: *Do not modify, add or remove any part of the Letter of Endorsement and fill in all the required information. Please, make sure that the signature of the letter is done in compliance with rules and procedures of your host institution.*

7. **Technical information**, to be filled in in the dedicated section(s) of the application form, indicatively including:
    a. *Requested service(s), as described in Annex III of this call.*
    b. *Samples technical information.*
    c. *Additional data, useful for technical feasibility analysis (if applicable).*
    d. *Whether the entire sample/ data set is already available, or will be available no later than two (2) months from receiving Access approval (refer to section 8).* ***Please note that if samples/ data 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****.*
    e. *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.*
    f. *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 form.

Information provided in points 1 to 6 (application contents) is used for the **eligibility and admissibility check.**

Information provided in point 7 (technical information of the application) 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 SIEC to assess its scientific merit, according to the evaluation process illustrated in section 8.

6.2 Accountability of Applicants and host institutions

The Applicant and the host institution are solely responsible for the accuracy, completeness, and truthfulness of all data and information provided in the application. By submitting the application, they declare that all statements and supporting documentation are true and correct, acknowledging the liability and the sanctions stated in art. 75 and 76 of the DPR 445/2000 for false affirmations and mendacious declarations, including the rejection of the application and/ or the loss of any granted benefits.

In case of Letters of Endorsement deemed not compliant or presenting inconsistencies in the submitted information (through the application), the User Access Office can conduct, on behalf of the SIEC, further verifications and/or to reject the application at the administrative review stage. This information can be also taken into consideration by the SIEC during the evaluation process and final approval decision.

Furthermore, acting on behalf of the SIEC, UAO may conduct **audits of participating institutions** to assess availability of any given or all services and ensure that these match the information submitted in their submitted applications.

In the event of violations and/or inaccurate or false declarations, the SIEC reserves the right to ban the Applicant and/or the host institution from participating in future Calls for Access for a period of up to twenty-four (24) months.

7. 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.

Application guidelines containing important information related to the submission procedure are available at this link.

This call for Access (Call ID: 26-LI-ROUND-3) will open on the 1st of October 2026 (13:00 CET) and will close on the 31st of January 2027 (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 webpages (call for Access; services).

The complete list of services offered through this call and technical requirements are available in the Annex III of this call.

Samples/ data 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** (refer to section 8). When the project foresees the analysis of more than one batch of samples/ data, similarly, the first batch should be available when the application is submitted or not later than two (2) months after Access approval.

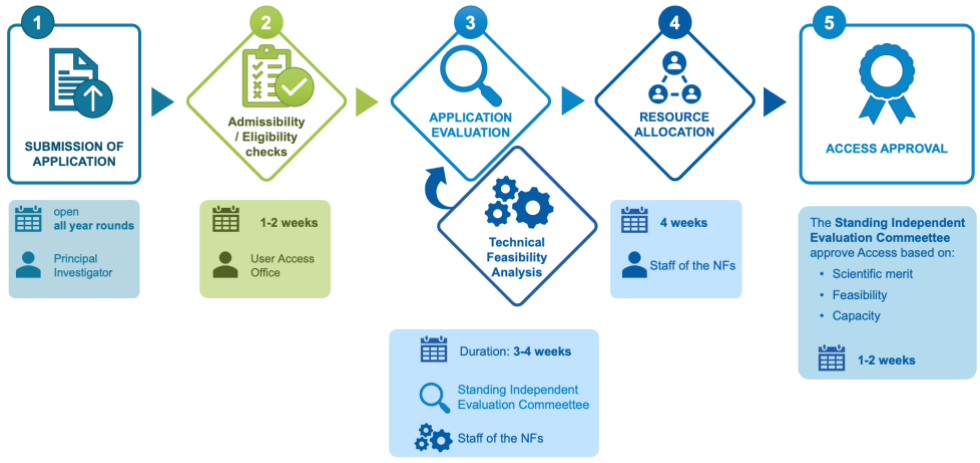
8. EVALUATION OF APPLICATIONS

Overview

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, along with a variable number of appointed external Reviewers selected according to the number of submitted proposals and their corresponding areas of expertise.

Below is a scheme describing the evaluation steps and the **indicative timeline for the process**. Evaluation results will be communicated through the PICA portal within 8 to 12 weeks after the closing of the call for Access.



The table below reports the indicative timeline for this call.

Opening	Closure	Evaluation	Access Approval
01.10.2026	31.01.2027	February – April 2027	By April 2027

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.

8.1 Triage

Based on the number of applications, if the requested services exceed 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:

1. **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 institution. For

the Triage, the Justification provided in the Letter of Endorsement (section 6, point 6.c) will be taken into consideration.

2. **Ongoing and previous support received by the NFs**: priority will be given to researchers who do not have ongoing Access to the same NF they are applying for, and/ or who have not been granted Access to it in the two calls preceding the current one *(e.g. in the call 26-LI-ROUND-3 will be given priority to researchers who do not have any ongoing Access to the NF for Genomics and/ or who have not been granted Access in the calls 26-LI-ROUND-1 and 26-LI-ROUND-2).*

Applicant career stage (Established and Junior PIs) will be considered during the triage phase to ensure alignment with the requirements of the evaluation procedure (section 8.2).

Should the number of requested services 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 institutions across Italy, proposals submitted by a single Institution/Institute that are sent for evaluation should not exceed the 10% of the total for any given career-based category.

8.2 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 (section 8.4).

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

The SIEC commits to allocating at least 50% of the available Access to applications from Junior PIs, while maintaining the Scientific merit as the primary criterion.

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.

8.3 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.

The use of **generative AI tools** in the review and evaluation of proposals is governed by the rules and recommendations outlined in the " Rules for the Responsible Use of Generative Artificial Intelligence in Proposal Preparation and Evaluation for Calls for Access to National Facilities" (link to the document), which is an integral part of this call. Reviewers are also required to declare any use of generative AI by responding to the relevant questions in the PICA portal.

8.4 Technical feasibility analysis

During the evaluation, the SIEC Chairs as well as the Reviewers will receive a report from the 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.

8.5 Evaluation results and Access approval

Applications with the highest scientific score that fulfil all technical requirements are approved for Access by the SIEC, subject to the capacity of the NF to host and execute the projects.

The SIEC commits to allocating at least 50% of the available Access to applications from Junior PIs, while maintaining the Scientific merit as the primary criterion, 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, previous Access, research outputs (e.g.,

publications) originated by previous Access, budgetary considerations and geographical distribution.

Evaluation results – Access granted, Access conditionally granted, Reserve list, Access not granted, Rejected (not eligible or not feasible proposals), Excluded during triage – are communicated to the Applicant through the portal PICA.

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 as resubmissions (see section 9).

9. RESUBMISSION OF PROPOSALS PREVIOUSLY EVALUATED AS "RESERVE LIST" OR "ACCESS NOT GRANTED"

9.1 Resubmission of a proposal evaluated as "Reserve list" in the previous round

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 this new evaluation round, maintaining the same project and review score as their initial application. Here, the application cannot be modified and is not sent out again for evaluation.

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

This option is provided only once, for the subsequent round only. An exception applies when the requested service is not available in the subsequent round. In such cases, the Reserve list application can be submitted to the first upcoming round of availability of the service *(e.g., the proposal has been submitted in the call 26-ROUND-1 and the evaluation outcome is "Reserve list"; the requested service is not available in the subsequent call 26-ROUND-2 but it is in the call 26-ROUND-3: the applicant can participate to the 26-Round-3 applying to the "Application for Reserve List Projects" call).*

b. To resubmit an updated version of the proposal (for example, including new preliminary data and/or taking into account Reviewer's comments), the Applicant should follow **the resubmission procedure described below** (section 9.2).

9.2 Resubmission

Applications that have been evaluated in a previous call, but that were not granted Access, or Reserve list projects without further Access being granted (Applicants that opt for option b above), can be submitted as a "**Resubmission**". Resubmission is not allowed for applications that were rejected (i.e., applications submitted to a previous round that were not eligible or not feasible) or excluded during triage in a previous evaluation round.

Of note, a **resubmission** is a proposal that has been **substantially improved** by adding new preliminary data, and/or by implementing Reviewers' suggestions and/or addressing concerns, etc. Applicants choosing the resubmission option shall include, in the proposal, the "Resubmission Cover page". **The Resubmission cover page must clearly describe how the proposal has been improved compared to the original version**.

IMPORTANT – The Resubmission Cover page must be included in the pdf proposal, as its cover page (Standard applications max 1+3 pages, PoC applications max 1+2 pages) and it must have the following format: *Max 1 page (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom).*

Resubmissions that do not meet these requirements will be considered not admissible and the application will be rejected at the initial administrative review stage.

Resubmissions will enter the standard competitive evaluation procedure along with all proposals submitted to the current round (section 8).

Resubmissions are allowed only once, regardless of the round. A project proposal submitted multiple times (i.e., more than one) as a resubmission will be considered not eligible and will be rejected at the initial administrative review stage.

Applicants can participate to future calls for Access submitting a new application.

10. PROJECT CONTINUATION APPLICATIONS

Applicants that would like to request Access for continuing a project that already benefited of an Access to the National Facilities, shall submit a "**Project continuation application**". These are research proposals that build on the findings and results of a previously approved Access, with the aim of further developing the research and advancing knowledge in the field.

The previously approved project has been conducted in any of the National Facilities, and it is ongoing or it has been completed (End of Access) at the time of the submission of the project continuation proposal. Please consider that, if the previously approved project is ongoing applicants cannot request **Access for the same service**. Before submitting an application requesting the same service of an ongoing Access, Applicant shall consult with the NF staff and confirm that the ongoing Access will be completed before the end of the evaluation round (refer also to section 5).

Applicants choosing to submit a project continuation application shall include, at the beginning of the proposal, the summary of **Prior Results and Research Advancement**. This summary must clearly outline the main achievements of the previously approved Access, citing any resulting publication (where applicable), and must explain how this project continuation proposal builds on and differs from the previously approved one, highlighting how it advances research knowledge.

IMPORTANT – The summary of "Prior Results and Research Advancement" must be included in the pdf proposal, as its cover page (Standard applications max 1+3 pages, PoC applications max 1+2 pages) and it must have the following format: *Max 1 page (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom).*

Project continuation applications that do not meet these requirements will be considered not admissible and the application will be rejected at the initial administrative review stage.

Project continuation applications will enter the standard competitive evaluation procedure along with all proposals submitted to the current round (section 8).

11. AFTER ACCESS HAS BEEN APPROVED

For requests where access is granted, Applicants accept the National Facilities Access rules. **Access rules** are detailed in the document: "NF Access Rules_Convenzione" (link) **which constitutes an integral part of this call**.

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/ data 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.

Of note, no biological material and/or experimental support material may be transferred from Human Technopole to the PI or from the PI to HT prior to the project having officially started (refer to the Access rules for additional details).

If samples/ data and/ or relevant ethical and legal authorisation(s) for their use are not available within two (2) months after Access approval (refer to section 8), the request for Access will be automatically rescinded and PI will need to reapply at a subsequent call.

12. AFTER ACCESS HAS BEEN COMPLETED

12.1 Reporting and feedback

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. **Refer to the Access rules document (paragraph 15) for additional details.**

12.2 Ownership of results and research outputs

Research data obtained during Access shall be made available to the scientific community following the FAIR principles. It is requested that Applicants make raw data available to the scientific community by publishing them in an appropriate public repository. Applicant must inform the NF User Access Office (via email to national.facilities@fht.org) when and how the data are made public, providing a citable identifier.

In particular, the Applicant must communicate to the NF User Access Office (via email to national.facilities@fht.org) any **publication** acknowledging the NF and/or in which any member of the NF staff is listed as a co-author. Additional details about **ownership of the data, publication of the results and intellectual property rights** are reported in the Access rules document (paragraphs 13, 14).

13. CONTACTS

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

14. REFERENCES

NF Access Workflow_Convenzione (link)

NF Access Rules_Convenzione (link)

NF Access Agreement_Convenzione (link)

NF Application Guidelines_PICA portal (link)

15. 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 - please, do not modify, add or remove any section of the letter and fill in all the required information)

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 mark the one that applies or best fits this application; only one choice is permitted***):

  ☐The requested service/ technology is not available at the Host Institution;

  ☐The requested service/ technology cannot be performed at the Host Institution or elsewhere at an affordable cost;

  ☐The requested service/ technology is available at the Host Institution, but the necessary expertise is lacking;

  ☐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 (refer to section 8 of the call text);

- 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 and/ or data 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 (refer to section 8 of the call text);
- 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;
- That the information provided in the Application is true and accurate, being aware of the accountability implications set out in section 6.2 of the call text;
- That the above data are true, being aware of the liability and the penal sanctions stated in art. 75 and 76 of the DPR 445/2000 for false affirmations and mendacious declarations that will involve the loss of the granted benefits.

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

A. Project proposal formats and template

Mandatory proposal formats

***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)

Resubmission Cover page: *must be included in the pdf proposal, as its cover page (Standard applications max 1+3 pages, PoC applications max 1+2 pages) and it must have the following format: Max 1 page (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom).*

Project Continuation Cover page: *must be included in the pdf proposal, as its cover page (Standard applications max 1+3 pages, PoC applications max 1+2 pages) and it must have the following format: Max 1 page (Page format: A4, Font type: Arial, Font size: at least 11, Line spacing: single, Margins 2 cm side/ 1.5 bottom).*

Proposal content:

- Resubmission cover page - applies to Resubmissions only (see section 9 for details)
- Project continuation cover page - applies to Project continuation applications only (see section 10 for details)
- Sections:
  1. TITLE
  2. SIGNIFICANCE
  3. INNOVATION
  4. APPROACH - including aims, preliminary data in support of the proposed experiments, experimental design and anticipated results
  5. ENVIRONMENT - including facilities and resources available to support the aspects of the project to be performed elsewhere (i.e., outside the NF)
  6. REFERENCES (Optional)

NOTE: The cover page (where required), together with Sections 1 through 6, constitutes the content of the proposal PDF to be uploaded as part of the application form.

B. Biosketch template

A **Biosketch template** can be downloaded from this link.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME:

eRA COMMONS USER NAME (credential, e.g., agency login):

POSITION TITLE:

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY

A. Personal Statement

B. Positions, Scientific Appointments, and Honors

C. Contributions to Science

ANNEX III: SERVICE LIST

HUMAN TECHNOPOLE
NATIONAL FACILITY FOR LIGHT IMAGING
CALL FOR ACCESS
26-LI-ROUND-3
SERVICE LIST

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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 (access to NF services, access to NF services including training) for each service will be discussed among the applicants and the NF staff and 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. The final decision about granting access modality will be solely taken by IU1. Images acquired using the system offered by IU1 can be optionally analyzed by the NF for Data Handling and Analysis by selecting the service: SID: NF60.001 – Light Microscopy analysis.

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 provides comprehensive flow cytometry services to support a wide range of research applications. The Unit offers full-service sorting and cloning of rare cellular populations, particle enrichment, and high-purity bulk sorting, tailored to specific experimental needs. In addition, the IU provides expert analysis services, guidance on experimental design and optimization, and hands-on training to help researchers achieve reliable, high-quality results.

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.

1. 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.

2. SERVICE LIST

IU1 - Imaging

Information to be provided in the technical details 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)

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

Description

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 live imaging with minimal phototoxicity and photobleaching.

All the systems are equipped with environmental control incubator (CO_2, temperature, humidity) for long-term live-cell imaging under physiological conditions.

The access modality (access to NF services, access to NF services including training) to this service will be discussed in advance. Access with training will be granted only at discretion of the IU1.

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.

NF50.015 - Confocal microscopy

Description

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_2, temperature, humidity) for long-term live-cell imaging under physiological conditions.

The access modality (access to NF services, access to NF services including training) to this service will be discussed in advance. Access with training will be granted only at discretion of the IU1.

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.

NF50.016 – Super-resolution microscopy

Description

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. Pease 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_2, temperature, humidity) to support live-cell super-resolution imaging under physiological conditions.

The access modality (access to NF services, access to NF services including training) to this service will be discussed in advance. Access with training will be granted only at discretion of the IU1.

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.

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 design 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

Spatial biology workflows

NF52.02.05 RNAscope

Description

Our imaging-based spatial transcriptomics and multi-omic service provides a high-throughput, automated solution for the detection of target RNA with single-molecule sensitivity. By leveraging the Leica BOND RX platform, we offer a standardized and reproducible workflow that preserves the spatial architecture of the tissue, allowing for the precise localization of gene expression within the morphological context bridging the gap between bulk transcriptomics and single-cell sequencing.

Applications

Typical applications include:

- **Biomarker validation on patient cohorts:** confirm spatial expression patterns of candidate transcripts and miRNAs across large clinically annotated cohorts.
- **Gene expression analysis when antibodies are unavailable or unreliable:** measure targets lacking validated antibodies, including studies in non-human or preclinical samples
- **Pathogen detection (viral or bacterial RNA)**: localize pathogen RNA within tissue architecture to support infection research and translational studies.
- **Co-expression studies of proteins and transcripts:** combine RNAscope and immunofluorescence to relate protein phenotypes to transcriptional states.
- **Drug response and Mechanism of Action studies:** quantify spatial pharmacodynamic transcriptional changes and link them to histopathology.

List of Services Included

Our services portfolio is composed of:

- **RNAscope Single Marker (Brightfield)**: We provide gold-standard chromogenic detection for single-marker analysis. This assay offers exceptional sensitivity and clear

morphological contrast, making it ideal for validating gene expression in a traditional pathology-like format.

- **RNAscope Duplex (Brightfield)**: This service enables the simultaneous detection of two RNA targets on a single tissue section using distinct chromogenic substrates. It is optimized for studying the co-expression of genes or the spatial relationship between two different cell populations under a standard light microscope.
- **RNAscope Multiomics (RNA and/or Proteins Multiplex Fluorescence up to 4 channels + DAPI)**: Utilizing fluorescent detection, we allow for the simultaneous visualization of up to four RNA targets with the possibility to exchange 2 RNA with 2 protein of interest. This high-resolution multiplexing is essential for characterizing complex cellular environments, such as the tumor microenvironment or neuronal sub-populations, with high signal-to-noise ratios.
- **miRNAscope Single Plex (Brightfield)**: Designed for the detection of miRNAs, siRNAs, and other oligonucleotides (17-50 nucleotides). This specialized assay provides high-resolution spatial mapping of small RNA species that are often difficult to detect with other methods.
- **RNAscope PLUS (RNA + miRNA up to 4 channels + DAPI)**: Our most comprehensive transcriptomic solution, allowing for the integrated detection of both mRNA and miRNA (or other small RNAs) on the same slide. This multi-analytical approach is perfect for investigating gene regulation and the functional impact of miRNA on target gene expression in situ.

Number of Sample per project

The following number of samples are provided as a general guideline, but custom set up may impact projects feasibility. Contact the facility to discuss about technical details.

Service	Min slides/ project	max slides/project	number of channels
RNAscope Single Marker (Brightfield)	12	50	Brightfield
RNAscope Duplex (Brightfield)	12	50	Brightfield
RNAscope Multiomic (Multiplex Fluorescent up to 4 channels + DAPI)	12	50	Fluorescence up to 5 channels
miRNAscope Single Plex (Brightfield)	12	50	Brightfield
RNAscope PLUS (RNA + miRNA up to 4 channels + DAPI)	12	50	Fluorescence up to 5 channels

Technical requirements:

- **Fixation and processing**: Provide FFPE tissue fixed in **10% neutral buffered formalin** from **16 h to 32 h at Room Temperature** with a ratio 20:1 in volume between fixative and the sample. Record and share key pre-analytical variables when possible, including **cold ischemia time**, **time in fixative**, and **processing program**.

- **Tissue blocks or sections for panel validation**: Provide **additional FFPE blocks** (or unstained sections) from **representative positive and negative control tissues** to support staining optimization and troubleshooting (e.g., known high/low express targets).
- **Slide requirements (if sending slides)**: Recommended section thickness of 3-5 µm. Using **charged slides** suitable for IHC is mandatory, recommended slides are Epredia Superfrost PLUS, Leica Apex, Dako IHC microscope slides or similar. Ensure sections are well-adhered and avoid excessive drying, changes in humidity and temperature or prolonged storage prior to staining.
- **Sample QC**: Provide one H&E and one IHC of Vimentin for each sample. Flag limited/delicate tissue clearly. Provide expected marker expression, study groups to help in the validation process.
- **Biosafety and handling**: Confirm samples are BSL1 or BSL2, include hazard declarations if relevant.
- **RNA probes:** should be chosen among available probes on Bio-Techne website.
- **Antibody selection**: Antibodies should be chosen among IVD validated antibodies, Cell Signaling Technologies or Abcam validated antibodies for Leica Bond RX.

Shipping and Storage Conditions:

- Ship FFPE blocks or slides at **ambient temperature** in robust secondary packaging, protected from shocks and excessive heat sources. Include desiccant bags for humidity control.
- Place each block in an individually labeled, sealed container or bag to prevent contamination and loss of identification.
- Include required documentation in a separate sleeve: sample list, fixation details, processing notes, and any hazard declaration.
- Avoid refrigeration or freezing during transport; minimize transit time and prevent prolonged exposure to high humidity.

Material and Data Provided:

The user will receive:

- **Validation report** for the final panels set up
- Stained tissue sections as **physical slides**
- Whole-slide **digital images** generated from fluorescence scanning when integrated with **NF52.07.07 Zeiss Axioscan Z.1**
- All the material provided for the project that have to be returned
- Data analysis when requested via **NF61.01.01 – Light Microscopy Analysis** service from NF for Data Handling

Available File Format:

- **CZI** lossy or lossless (raw)

Available Access Modalities: Access to NF service only

Slide Acquisition can be provided as a combined service with the Zeiss Axioscan Z.1. Please select: SID: NF52.07.07

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

Spatial biology platforms

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

Service Description:

The **Zeiss Axioscan Z.1** is an automated whole-slide scanner for digitizing microscopy slides. It is suitable for high-throughput widefield imaging of tissue sections and other prepared microscopy slides.

The service support:

- **Brightfield imaging**, for H&E and other histological staining
- **Fluorescence imaging**, including single- and multi-channel immunofluorescence experiments.

The scanner can process up to **100 slides per run** and generates high-resolution digital slide images that can be used for downstream image analysis.

Applications:

This service is intended for users who need digital images of microscopy slides for research-use-only of quantitative and qualitative analysis.

Typical application include:

- **Brightfield whole-slide imaging (WSI)**: High-throughput digitization of H&E, IHC and other standard histological stains. This is useful for slide review, remote sharing, archiving, and image-based quantification.
- **Immunofluorescence (IF) whole-slide imaging**: Automated acquisition for single- or multi-channel Fluorescence experiments. This can support spatial analysis and marker-based tissue characterization.
- **Standard slide imaging**: Scanning of conventional microscopy slides measuring **75 × 25 mm**
- **Macro slide imaging**: Scanning of larger slides measuring **75 × 50 mm**, useful for larger tissue sections or samples where full specimen context is important.
- **Z-Stack acquisition**: Imaging of brightfield or fluorescence imaging in z-stack, useful for acquisition of fine signals on multiple focal planes in widefield.
- **Custom application:** accepted as proof-of-concept

Equipment configuration:

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

Interactive microscope set up:

- HT Zeiss Axioscan Z.1 configuration

Light sources:

- Colibri 7 LED Light sources:
  - 385 nm
  - 430 nm
  - 475 nm
  - 555 nm
  - 590 nm
  - 630 nm
  - 735 nm
- White led lamp for transmitted light

Emission filter sets 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)

Slide racks:

- Standard microscope slides 75 x 25 mm
- Macro sections slides 75 x 50 mm

File formats:

- CZI (raw) lossless or lossy
- BigTiff after post processing
- OME.TIFF after post processing

Number of Sample per project:

The following number of samples are provided as a general guideline, but custom set up may impact projects feasibility. Contact the facility to discuss about technical details.

Service	min # of slides/ project	max # of slides/project	numbers of channels
Brightfield WSI 20X-40X	24	600	RGB
Fluorescence WSI (3ch) 20X	24	150	3
Fluorescence WSI (5ch) 20X	24	72	5

Fluorescence WSI (3ch) 40X 5 z-stack	12	40	3
Fluorescence WSI (5ch) 40X 5 z-stack	12	40	5
Proof-of-concept application	custom	custom	custom

Material and Data Provided:

The user will receive:

- Whole-slide **digital images**
- All the material provided for the project that have to be returned
- Data analysis results when requested via **NF61.01.01 – Light Microscopy Analysis** service from NF for Data Handling

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

- Fresh frozen/ Fixed frozen/ FFPE
- Section thickness
- Cell in adhesion (yes/no)
- Standard 25 mm x 75 mm glass slide/ Macro 50 mm x 75 mm glass slides
- Tissue Microarray (yes/no)
- Brightfield/Fluorescence/mixed
- List the fluorophores/channels in your experiment
- Preferred magnification
- z-stack needed (yes/no) If yes, how many z-stack you need to acquire
- List any special needs

Available Access Modalities: Access to NF as service only

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

NF53.001: Flow Cytometry Cell Sorting

Description

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

The proposal must include the list of fluorochromes to be analysed. Users should consult the instrument optical configuration links below to verify excitation and emission compatibility when designing panels:

Optical Configuration Astrios EQ

https://humantechnopole.it/wp-content/uploads/national-facilities/OPTICAL-CONFIG-Cytek-Aurora.pdf

https://humantechnopole.it/wp-content/uploads/national-facilities/OPTICAL-CONFIG-Cytoflex-LX.pdfOptical Configurations FACSDiscover S8

https://humantechnopole.it/wp-content/uploads/national-facilities/OPTICAL-CONFIG-ImageStream-MKII.pdf

This service supports high-recovery and indexed single-cell sorting for downstream applications such as sequencing. A wide range of cell types can be processed, including:

- immune cell and hematopoietic stem cell subsets
- mesenchymal stem cells
- viable cytokine producing cells
- Cell lines and primary cells isolated from tissues and organoids
- transfected cells, including iPSCs
- Nuclei purification
- Sorting of cells based on the cellular localization (eg. Sor cells with nuclear NFkB)

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 MoFLO Astrios high speed cell sorter is equipped with 6 lasers having the following emissions: 355 nm 405 nm, 488 nm, 560 nm, 592 nm, 645 nm.

The spectral BD FACSDiscover™ S8 Cell Sorter is equipped with 5 lasers having the following emissions: 355 nm 405 nm, 488 nm, 560 nm, 645 nm. This cell sorter also has sort-capable image analysis using new imaging features and combines spectral flow cytometry with real-time spatial and morphological insights.

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

- Unstained, unlabeled or other cellular controls
- Spectral overlap compensation and unmixing 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 cells
- Tested viability
- Filtered

- Sterile
- Frozen
- BSL1/BSL2
- Mycoplasma Tested
- 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, all relevant information and FCS files upon request. A purity assessment analysis will be performed on bulk sorts when sufficient cells are available.

Access modality available: Access to NF service.

NF53.002 Flow Cytometry Analysis (Autonomous)

Description: This service provides users with independent access to flow cytometry analyzers following successful completion of a ***mandatory*** training session.

Users are responsible for instrument operation and data acquisition. This service is intended for trained users requiring flexibility and autonomy in performing routine or advanced analyses.

Prior to access, the proposed staining panel must be submitted for evaluation to ensure compatibility with available instruments. Based on this review, the most appropriate analyzer will be assigned. Users are encouraged to consult instrument optical configurations during panel design.

Key Features

- Independent operation after certification
- Access to analyzers suited to panel complexity

- Support for multi-parameter and high-dimensional analyses
- Flexible scheduling

User Responsibilities

- Completion of mandatory training
- Submission and validation of staining panel
- Inclusion of appropriate controls (unstained, compensation/unmixing, FMO/FMX)
- Proper sample preparation and handling

NF53.003 Assisted Flow Cytometry Analysis (Operator-Assisted)

IMPORTANT: this service will not be offered in this call.

Description

This service provides expert-supported flow cytometry analysis, where trained facility personnel perform data acquisition on behalf of the user.

It is intended for users who require technical assistance, are working with complex experimental designs, or wish to ensure optimal data quality.

All panels must be submitted in advance for evaluation and instrument selection. Users should consult the optical configurations below when designing panels:

https://humantechnopole.it/wp-content/uploads/national-facilities/OPTICAL-CONFIG-Astrios-EQ.pdfOptical Configuration Cytek Aurora

Optical Configuration Cytoflex LX

https://humantechnopole.it/wp-content/uploads/national-facilities/OPTICAL-CONFIG-FACSDiscover_S8.pdfOptical Configuration ImageStream MKII

Applications

- 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 and apoptosis assays.
- 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
- Manual or automated acquisition modes
- 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.

- Advanced spectral and polychromatic panels.
- Controls (Required)
- 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)
- Any additional relevant information

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.

NF53.004 Flow Cytometry Consultation Session (Sample Preparation/Panel Design/Data Analysis)

IMPORTANT: this service will not be offered in this call.

Description

User consultation is a critical step in ensuring high-quality flow cytometry data. Facility staff provide guidance across all stages of the workflow, including:

- Experimental design
- Sample preparation and control selection

- Data acquisition strategies
- Data analysis approaches

This service supports projects from initial concept through to data interpretation.

Panel Design and OMIP Guidance

For high-dimensional flow cytometry (≥25–50+ parameters), users are strongly encouraged to leverage **OMIP (Optimized Multicolor Immunofluorescence Panels)** when available.

OMIP panels are peer-reviewed and experimentally validated, providing optimized marker–fluorochrome combinations, gating strategies, and control recommendations that enhance reproducibility and data quality.

Examples include:

- OMIP-047: Human T cell immunophenotyping
- OMIP-069: Comprehensive immune profiling
- OMIP-071: Myeloid and dendritic cell characterization
- OMIP-077: Leukocyte subset analysis in PBMCs

Suitability depends on the biological system, instrument configuration, and experimental objectives.

When a published OMIP panel does not fully meet the experimental needs, targeted adaptation of an existing OMIP is recommended. This may include substitution, addition, or removal of specific markers while maintaining fluorochrome compatibility, antigen density considerations, and spectral constraints of the selected platform (e.g., spectral vs. conventional cytometry). Such modifications should be evaluated in consultation with facility staff to ensure proper panel performance, including assessment of spreading error, co-expression patterns, and detector sensitivity.

Custom panel development

If no suitable OMIP framework is available, fully custom panel development may be undertaken as a proof-of-concept project in collaboration with the facility. This process involves iterative design, titration, and validation steps (e.g., antibody optimization, compensation/spectral unmixing validation, and control testing), and is therefore resource- and time-intensive. As a result, experimental samples intended for acquisition with a newly developed panel cannot be processed until panel validation is complete.

IMPORTANT - Custom panel development is time- and resource-intensive. Experimental samples for acquisition using a newly developed panel will only be accepted after successful validation. A new submission must be provided, clearly indicating the project objectives and specifying the validated panel.

A new service request must then be submitted, clearly stating the project objectives and specifying the use of the validated custom panel.

Panel Selection Workflow (Recommended Decision Path)

1. **Is there a suitable published OMIP panel for your application?**
    - **Yes:** Use the OMIP panel as published (e.g., OMIP-047, OMIP-069, OMIP-071, OMIP-077), ensuring compatibility with the selected instrument configuration.

- **No:** Proceed to Step 2.

2. **Can an existing OMIP panel be adapted to your needs with in-kind marker intensities and fluorophores?**
   - **Yes:** Modify the OMIP panel by removing or substituting markers while preserving fluorochrome compatibility and accounting for antigen density, co-expression, and spectral constraints. Consultation with facility staff is strongly recommended to evaluate panel performance (e.g., spreading error, resolution).
   - **No:** Proceed to Step 3.
3. **Is a fully custom panel required?**
   - **Yes:** Initiate a proof-of-concept panel development project in collaboration with the facility. This includes iterative optimization (antibody titration, fluorochrome assignment, compensation/spectral unmixing validation, and control design).

IU5 - Ion Imaging

NF55.003/004/010/014 – Microelectrode Arrays (MEAs) assays

Description: High-resolution, optics-free functional imaging based on high-density MEA electronic interfaces measuring extracellular voltage potentials. The assay is suitable for the characterization of functional phenotypes in cell networks and for compound screening.

The systems available:

- BioCAM DupleX and **planar MEAs** electronic chips equipped with 4,096 simultaneously recording electrodes (3BRAIN AG, Pfäffikon SZ, Switzerland). The MEAs chip is the CorePlate™ 1W38/60 (NF55.003_MEA chip testing and provision/ NF55.004_MEA chip rental) with an active sensing area of 3.8 mm x 3.8 mm; each electrode measures 21 mm x 21 mm with a 60 mm pitch. The system is particularly suitable for accessing the electrical phenotype of brain and cardiac tissue.
- BioCAM DupleX and **3D MEAs** electronic chips equipped with 4,096 simultaneously recording electrodes (3BRAIN AG, Pfäffikon SZ, Switzerland). The MEAs chip is the CorePlate™ 1W-3D 38/60/90-X (NF55.014_MEA single well 3D) with an active sensing area of 3.8 mm x 3.8 mm, each electrode height is approximately 90 mm, with a section at the tip measuring approximately 14 mm x 14 mm with a 60 mm pitch. This system is highly recommended for acute recording of tissue or organoids.
- MaxTwo (MaxWell Biosystems) and **6-Well Plate** (NF55.010_MEA multiwell system). Each well has a 3.85 x 2.10 mm^2 active sensing area equipped with 26,400 electrodes with 17.5 mm pitch. Each well can simultaneously record from 1,020 electrodes. The system is ideal for neuronal cultures and tracking changes in neuronal arborization over time.

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 point 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 planar MEAs, 10 3D MEAs, and 10 6-well chips, but the project scale should be discussed with the NF manager.

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

- Biosafety level and Mycoplasma test
- Type of sample (culture / organoids / assembloids)
- Number of samples
- Number of technical replicates
- Type of recording (Acute/ Culture / Longitudinal Recording)
- Number of Time Points to be evaluated
- Pharmacological validation of recorded activity (i.e.TTX)
- Culturing conditions for the sample (temperature, CO_2, medium composition)

Procedures: After a standard quality control to verify that the cell/organoid lines 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 processing, analysis, and interpretation. Optionally, the user may request basic processing of the raw data using the proprietary software Brainwave5 (3BRAIN AG) or MaxLab Live (MaxWell) to detect electrophysiological events of interest. When applicable, the user will be responsible for selecting processing parameters (such as high-, low- or bandpass filters and detection thresholds) which are examples and not limited to these options, depending on the software's capabilities. Processed data will be delivered to the user in a HDF5 format.

Access modality available: Access to NF service or Access with training

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_2, O_2, 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 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.

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 developmen**t: 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.