HUMAN TECHNOPOLE

NATIONAL FACILITY FOR LIGHT IMAGING

CALL FOR ACCESS

24-LI-PILOT

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1. INTRODUCTION

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

Services offered by NFs are available through regular open calls for Access that are published yearly on the HT website (link) and are free of charge 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 User. The costs for the activities to be performed at the NFs will be fully covered, including shipment of relevant material from and to the User's laboratory as well as travel and accommodation for the User while accessing the NF. Project-related costs (personnel, consumables, and other costs) at the User laboratory are not funded.

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

1.1 Access modalities

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

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

2. TERMS AND DEFINITIONS

2.1 Access

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

2.2 Researcher

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

2.3 Principal Investigator

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

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

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

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

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

i. Maternity leave: The time limit is increased by 18 months for each child born after their first appointment in an independent group leader position; if the Applicant is able to document a longer total maternity leave, the period of eligibility will be extended by a period equal to the documented leave, taken before the submission of the application. Maternity status must be documented by submitting the birth certificate of the child or children;

ii. Paternity leave: The time limit is increased by the actual amount of paternity leave taken before the application submission deadline for each child born after their first appointment in an independent group leader position. Paternity status must be documented by submitting the birth certificate of the child or children;

iii. Long-term illness of more than 90 days, or national service: The time limit is increased, for each eligible event occurring after their first appointment in an independent group leader position, by the actual amount of leave from which the Applicant has benefited prior to the application submission deadline.

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

2.4 Applicant

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

2.5 User

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

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

3. APPLICATION TYPE

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

a. **Standard** application for projects that are technically mature.

b. **Proof-of-concept** application for:

i. Projects with high scientific potential but with insufficient technical maturity or preliminary data.

ii. Projects aimed at setting up the experimental conditions required for a standard project, including methods or technology development projects.

iii. Time-limited Access projects (e.g., to acquire data to complete a manuscript, or preliminary data needed for a grant application, or single microscopy session).

4. ELIGIBILITY AND ADMISSIBILITY

PIs, as defined in section 2.3 of this call, affiliated with an eligible Institution are eligible to apply. The Applicant's role as a PI shall be confirmed by their Institution in a mandatory letter of Institutional endorsement.

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

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

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

i. State funded public universities, listed under the following link.

ii. Specialized superior graduate schools or Institutions, listed under the following link.

iii. Legally recognized non-public universities, listed under the following link.

iv. On-line universities, listed under the following link.

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

Public research entities: this category includes Institutions recognized by the Ministry of University and Research and listed at the following link.

Eligible Institutions are strongly encouraged to limit the number of applications to the very best 2/ NF, with at least 50% coming from Junior PIs.

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, home Institution or elsewhere. Applicants shall confirm the economic and scientific feasibility for the aspects of the project to be performed outside the NFs.

Applicants cannot request Access for the same service if an approved Access is ongoing. 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.

During the same application window, a PI can submit only one application to the NFs (i.e., participate to only one call 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 application before receiving notification of the results.

Applications must be written in English and must be complete (i.e., consist of all the requested elements and information). Incomplete applications will be considered not eligible and will be rejected at the administrative review stage.

The application form consists of six components:

1. Applicant's general information.
2. Justification for requesting Access to the NF.
3. Abstract.
4. Project proposal, including:
    a. *Significance.*
    b. *Innovation.*
    c. *Approach, including aims, preliminary data in support of the proposed experiments, experimental design and anticipated results.*
    d. *Environment, including facilities and resources available to support the aspects of the project to be performed elsewhere (i.e., outside the NF).*

5. Applicant's CV in NIH biosketch format.
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 2.3).*
    b. *Confirmation that relevant authorisations, declarations and accreditation from the competent authority(ies) have been obtained in order to process samples and data through the NFs.*
    c. *Justification of the request for Access – including a statement on why the project cannot be performed at the Applicant's Institution.*
    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, home 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 terms and conditions.*

    The facsimile available as Annex II of this call shall be used as template.
7. Technical information, including:
    a. *Requested service(s), as described in Annex I of this call.*
    b. *Sample technical information.*
    c. *Requested preliminary data for technical feasibility analysis (if applicable).*
    d. *Whether the entire sample set is already available (otherwise indicate the date of availability of the entire sample set).*
    e. *Resources and expertise to receive and process the products – 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:*
        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.*

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

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

The entire application is evaluated by the Standing Independent Evaluation Committee (SIEC) for assessing scientific merit.

5. APPLICATION SUBMISSION METHODS, CALL DEADLINE AND EVALUATION PERIODS

Applications shall be submitted exclusively through the web-based procedure managed by CINECA and accessible at this link, according to the terms and methods there indicated.

Applications are accepted throughout the year and assessed in three evaluation rounds per year. The time between one evaluation round and the next one is considered an application window.

- Evaluation round 1: applications submitted between the 1st of January and the 30th of April will enter the first evaluation round (May/ June).
- Evaluation round 2: applications submitted between the 1st of May and the 31st of August will enter the second evaluation round (September/ October).
- Evaluation round 3: applications submitted between the 1st of September and the 31st of December will enter the third evaluation round (January/ February).

This first call for Access (Call ID: 2024-LI-Pilot) will be **open from the 10th of June 2024 (1 pm) to the 31st of December 2024 (1 pm)** and will involve 2 rounds of evaluation (September 2024 and January 2025).

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

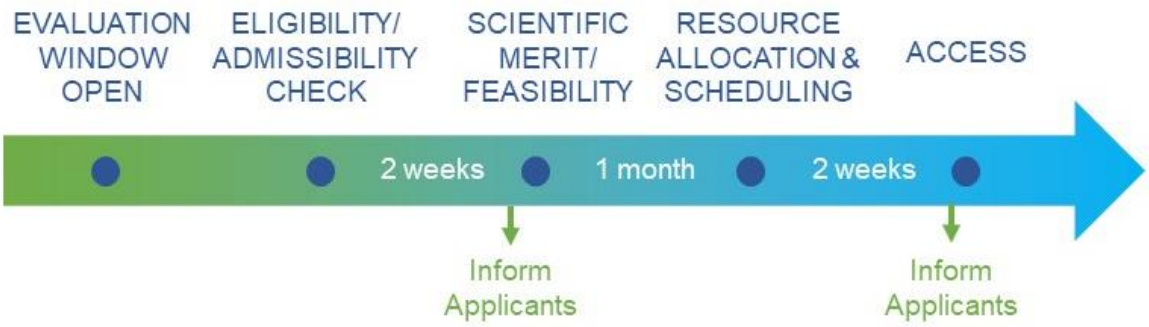
The complete list of offered services and technical requirements are available in the Annex I of this call.

6. EVALUATION OF APPLICATION

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

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

Below is a scheme describing evaluation steps and timeline.



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.

The application is then sent to the Review Panel for assessing scientific merit and technical feasibility.

If the number of applications exceeds by a factor of 4 the estimated capacity of the NF, a triage will be applied within each application category by the relevant Review Panel.

Triage criteria will include:

a. Justification for requesting Access to the NF.
b. Field-Weighted Citation Impact (FWCI).
c. Track record in securing research funding.

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

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

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

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

At least 50% of the available Access will be allocated to applications from the two Junior PI categories.

6.1 Evaluation criteria

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.

6.2 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.

6.3 Technical feasibility analysis

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

Based on the technical maturity of the project, the application can be assessed as Feasible/ Not Feasible/ Pilot study required (switch from Standard to "Proof-of-Concept application track).

6.4 Evaluation results and Access approval

NF staff provides the SIEC with information on the resources needed (cost and time) to perform the highest ranked projects. The most positively evaluated applications that fulfil all technical requirements are approved for Access by SIEC, based on the capacity of the NF. NF staff schedules Access. A selected number of applications may be placed on a waiting list (in case of cancellations).

Evaluation results – Access approved, Access waitlisted, Access not approved – are communicated to the Applicant through the Access portal.

Applicants whose applications are placed on the waiting list will receive additional information advising whether the project can be Access approved or should be resubmitted within the subsequent application window.

7. AFTER ACCESS HAS BEEN APPROVED

After Access approval, a kick-off meeting is organized by the NF User Access Office and 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, the NF User Access Office coordinates the signature of the required formal Agreements (e.g., Access Agreement, Collaboration Agreement, other) and the project can start.

8. AFTER ACCESS HAS BEEN COMPLETED

At the end of the activities carried out at the NF, and not later than 3 months thereafter if not differently agreed with the NF User Access Office, the User must submit a short report on the results obtained and the impact of the service on their research. Moreover, a final report to be published on the NFs website and 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. Users 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 User will be asked to fill in a brief, mandatory survey regarding their experience, providing feedback and suggestions for further service improvement.

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

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

9. CONTACTS

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

10. REFERENCES

NF Access workflow_Convenzione (link)

NF Access rules_Convenzione (link)

NF Access Agreement_Convenzione (link)

11. CHANGES TO THE CALL

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

ANNEX I: NATIONAL FACILITY FOR LIGHT IMAGING: SERVICES LIST

HUMAN TECHNOPOLE

NATIONAL NF FOR LIGHT IMAGING

CALL FOR ACCESS

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SERVICE LIST

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1. INTRODUCTION

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

- IU1: Imaging
  The Imaging IU offers Access to several high-end microscopy systems. Microscopes can be used to image fixed or living samples. After mandatory training, Users can Access the microscopes autonomously and request supervision from NF staff. For selected applications and only in agreement with the IU manager, Users can delegate image acquisition entirely to the NF staff. Detailed descriptions of each available system are reported below. Images acquired in IU 1 can optionally be analyzed by the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis.
- IU2: Tissue Processing
  The Tissue Processing IU provides platforms, technical support and training for optimally preparing samples for downstream analysis, such as light imaging or spatial transcriptomics.
- IU3: Flow cytometry applications
  The Flow Cytometry IU offers full-service sorting & cloning of rare cellular populations, particle enrichment, and high purity bulk sorts. The Unit also offers Analysis Services, advice in experimental design and training.
- IU5: Ion Imaging
  The Ion Imaging IU offers functional cell imaging using fluorescence-based time-lapse recordings of intracellular ion oscillations. Optical imaging is primarily based on confocal or epifluorescence microscopy.

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

2. GENERAL TECHNICAL REQUIREMENTS

- The NF for Light Imaging can accept biological samples of biosafety containment level (BSL) 1.
- The NF for Light Imaging can accept biological samples of biosafety containment level (BSL) 2 only for selected services.
- Applicants must ensure that the samples are available in sufficient quantity and quality before the closing date of the application period.
- The NF for Light Imaging shall reserve the right to perform a pilot experiment on samples provided by the Applicants to assess the technical feasibility of the submitted project.

3. SERVICE LIST

(IU1) Imaging

SID: NF50.001 - Zeiss LSM980-NLO confocal microscope with multiphoton excitation

Service description: Zeiss LSM980-NLO confocal microscope with multiphoton excitation and Airyscan2 detector.

- Multiphoton excitation: Coherent Discovery NX (dual line, tunable 700-1300 nm and fixed 1040 nm)
- Visible laser sources: 405 nm, 488 nm, 561 nm, 594 nm, 639 nm
- Non-descanned detectors
- Airyscan2 detector
- Incubator (CO_2, temperature)
- Epifluorescence (sCMOS camera Zeiss 702)
- Zen Blue version 3.7
- Available objectives:
  2.5x/0.085 EC Plan-Neofluar
  20x/0.45 PlanApo DIC II
  40x/0.8 PlanApo DIC II
  40x/1.4 Oil PlanApo DIC II
  40x/1.1 W CApo
  63x/1.4 Oil PlanApo DIC II

Applications: thick sample imaging (up to several hundreds of µm), long-term time lapses, live cell imaging, second-harmonic generation.

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

- Estimation of time (hours) needed to complete the Access.

Access modality available: Simple Access.

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis.

SID: NF50.002 - Zeiss LSM980 confocal microscope

Description: Zeiss LSM980 confocal microscope with Airyscan2 detector.

- Visible laser sources: 405 nm, 488 nm, 561 nm, 594 nm. 639 nm
- Airyscan2 detector
- Sample finder
- Incubator (CO_2, temperature)
- Epifluorescence (sCMOS camera Zeiss 702)
- Zen Blue version 3.7

- Available objectives:
  2.5x/0.12 EC Plan-Neofluar
  10x/0.45 PlanApo
  20x/0.8 PlanApo
  40x/1.4 Oil PlanApo
  63x/1.4 Oil PlanApo

Applications: high resolution imaging, long-term time lapses, live cell imaging.

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

- Estimation of time (hours) needed to complete the Access.

Access modality available: Simple Access.

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis.

SID: NF50.003 - Zeiss Elyra7 super resolution microscope

Description: Zeiss Elyra7 lattice structured illumination and localization microscopy system.

- Visible laser sources: 405 nm, 488 nm, 561 nm, 638 nm
- Dual camera (PCO.EDGE)
- Incubator (CO_2, temperature)
- Dual computer with Zen black 3.0 SR for image acquisition and Zen Blue 3.7 for processing
- Available objectives:
  10x/0.3 EcPlan DIC I
  20x/0.8 PlanApo DIC II
  40x/1.2 W Apo DIC III
  63x/1.4 Oil PlanApo DIC II
  63x/1.46 αPlanApo DIC III
  100x/1.57 αPlanApo DIC III

Applications: super-resolution imaging, live cell imaging, single molecule localization microscopy, total internal refraction microscopy (TIRF).

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

- Estimation of time (hours) needed to complete the Access.

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

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis.

SID: NF50.005 - Zeiss Axiozoom V16

Description: Zeiss Axiozoom V16 large field of view epifluorescence microscope with apotome.

- High-sensitivity back-side illuminated sCMOS camera (Prime BSI)
- Apotome module
- Motorized sample holder
- Zen blue version 3.7

Applications: large field of view acquisitions of fixed samples with enhanced optical sectioning.

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

- Estimation of time (hours) needed to complete the Access.

Access modality available: Simple Access.

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis.

SID: NF50.006 - Zeiss Axioscope 5

Description: Zeiss Axioscope 5 upright epifluorescence microscope with monochrome sCMOS camera.

- Monochromatic sCMOS camera
- Zen blue version 3.7
- Available objectives:
  2.5x/0.16
  10x/0.3 EC Plan-Neofluar
  20x/0.5 EC Plan-Neofluar
  40x/0.65 N-Achroplan Ph2
  63x/0.95 Korr EC Plan-Neofluar
  100x/1.3 Oil EC Plan-Neofluar

Applications: routine analysis of samples in epifluorescence, transmitted light, phase and darkfield contrast.

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

- Estimation of time (hours) needed to complete the Access.

Access modality available: Simple Access.

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis.

SID: NF50.007 - Nikon Ti2 spinning disk with four cameras, TIRF condenser and FRAP module

Description: Nikon Ti2 inverted microscope with CrestOptics X-light V3 spinning disk scan head.

- Visible lasers sources: 405 nm, 446 nm, 477 nm, 520 nm, 547 nm, 638 nm, 749 nm
- Visible laser sources (TIRF and FRAP): 405 nm, 488 nm, 561 nm, 640 nm

- Four high-speed, back sided illuminated sCMOS cameras (Prime 95B, 25 mm FOV)
- Incubator (CO_2, temperature)
- TIRF condenser
- FRAP module
- NIS elements
- Available objectives:
  10x/0.45 λD CFI Plan Apochromat
  20x/0.8 λD CFI Plan Apochromat
  40x/1.25 Sil λD CFI Plan Apochromat
  100x/1.35 Sil λS CFI SR HP Plan Apochromat
  100x/1.49 Oil CFI Apo TIRF

Applications: high-speed imaging, live cell imaging, long term time lapses, photomanipulation, TIRF.

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

- Estimation of time (hours) needed to complete the Access.

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

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis.

SID: NF50.009 - Leica Stellaris 8 for super-resolution imaging

Description: Leica Stellaris 8 confocal microscope with tandem scanners (resonant and conventional galvanometer scanner), STED and FALCON modules.

- Visible lasers sources: 405 nm laser and tunable white laser with up to 8 simultaneous lines between 440 nm and 790 nm
- AOBS tunable dichroic
- 4 hybrid detectors
- STED module wit 775 nm depletion laser
- FALCON module for fluorescence lifetime imaging
- Incubator for live-cell imaging
- Leica LAX software
- Leica Navigator
- Available objectives:
  10x/0.40 HC PL APO CS2
  20x/0.75 HC PL APO CS2
  40x/1.30 HC PL APO Oil CS2
  63x/1.40 HC PL APO Oil CS2
  100x/1.40 Oil STED

Applications: super-resolution imaging, live-cell imaging, fluorescence lifetime imaging.

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

- Estimation of time (hours) needed to complete the Access.

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

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis.

SID: NF50.010 - Leica Stellaris 8 for live-cell imaging

Description: Leica Stellaris 8 confocal microscope with tandem scanners (resonant and conventional galvanometer scanner), incubator and FALCON module.

- Visible lasers sources: 405 nm laser and tunable white laser with up to 8 simultaneous lines between 440 nm and 790 nm
- AOBS tunable dichroic
- 4 hybrid detectors
- Incubator for live-cell imaging
- FALCON module for fluorescence lifetime imaging
- Leica LAX software
- Leica Navigator
- Available objectives:
  10x/0.40 HC PL APO CS2
  20x/0.75 HC PL APO CS2
  40x/1.30 HC PL APO Oil CS2
  63x/1.40 HC PL APO Oil CS2

Applications: live-cell imaging, fluorescence lifetime imaging.

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

- Estimation of time (hours) needed to complete the Access.

Access modality available: Simple Access.

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis.

SID: NF50.011 - Leica Thunder imager Live Cell

Description: Leica Thunder motorized epifluorescence microscope with digital clearing.

- LED illuminator with 8 single LEDs (395 nm, 438 nm, 475 nm, 551 nm, 555 nm, 575 nm, 635 nm and 730 nm)
- Leica K8 sCMOS camera
- Leica LAX software

- Leica Navigator
- Available objectives:
  4x/0.10 HI PLAN
  10x/0.32 HC PL FLUOTAR PH1
  20x/0.40 HC PL FL L PH1
  63x/1.40-0.60 HC PL APO Oil

Applications: live-cell imaging.

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

- Estimation of time (hours) needed to complete the Access.

Access modality available: Simple Access.

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis.

NOTE: services NF50.009, NF50.010 and NF50.011 are integrated and the same sample can be imaged using the three microscopes retaining its context using Leica Navigator

SID: NF50.012 - Abberior STED with adaptive optics

Description: Abberior NF Line STED with adaptive optics for deep-tissue super-resolution imaging.

- Visible laser sources: 405 nm, 561 nm and 640 nm
- Depletion laser: 775 nm
- Adaptive optics system
- Incubator

Applications: Super-resolution imaging of thick samples, live-cell imaging.

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

- Estimation of time (hours) needed to complete the Access.

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

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis.

SID: NF50.013 - Zeiss Lattice Lightsheet

Description: Zeiss Lattice Lightsheet 7 with dual cameras.

Visible lasers sources: 488 nm, 561 nm and 640 nm

- Dual cameras (Hamamatsu ORCA Fusion)
- Integrated incubator
- Zeiss Zen software

Applications: low-phototoxicity, long-term live-cell imaging.

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

- Estimation of time (hours) needed to complete the Access.

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

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis.

(IU2) Tissue Processing

SID: NF50.014 - Zeiss Axioscan Z.1 automated slide scanner

Description: Zeiss Axioscan Z.1 for the acquisition of whole slide images both in brightfield or in epifluorescence up to 100 slides per session.

Configuration:

- Cameras:
  - Orca-Flash4.0 v3 sCMOS mono camera
  - Hitachi HV-F203SCL 3CCD color camera
- Light sources:
  - Colibri 7 for epifluorescence (385 nm, 430 nm, 475 nm, 555 nm, 590 nm, 630 nm, 735 nm)
  - White led lamp for transmitted light
- Filter sets optimised for:
  - DAPI (Zeiss BP 450/40)
  - GFP / Alexa Fluor 488 / FITC (Zeiss BP 525/50)
  - Cy3 / Alexa Fluor 555 / TRITC (Zeiss BP 605/70)
  - Cy5 / Alexa Fluor 647 / APC (Zeiss BP 690/50)
  - DAPI/GFP/Cy3/Cy5/Cy7 (Zeiss PBP 425/30+514/31+592/25+681/45+785/38)
- 6 objectives:
  - 2,5x / 0.12 NA Fluar M27
  - 5x / 0.25 NA Fluar M27
  - 10x / 0.45 NA Plan-Apochromat M27
  - 20x / 0.45 NA N-Achroplan Pol M27
  - 20x / 0.8 NA Plan-Apochromat M27
  - 40x / 0.95 NA Plan-Apochromat M27
- Slide racks:
  - Standard microscope slides 75 x 25 mm

  - Macro sections slides 75 x 50 mm
- File formats:
  - ".czi" native lossless or lossy file format with JpegXR compression
  - "BigTiff" after post processing
  - "OME.TIFF" after post processing

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

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

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

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

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis.

(IU3) Flow Cytometry

SID: NF53.001: Flow Cytometry Cell Sorting

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

High-recovery and indexed single-cell sorting for sequencing.

Cell sorting of many cell types including:

- immune cell and hematopoietic stem cell subsets

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

Sorting Technical Details

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

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

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

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

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

Information to be provided in the application:

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

- Fresh (Yes / No)
- Fixed (Yes / No)
- Tested viability (Yes / No) and percentage of viable cells
- Filtered (Yes / No)
- Sterile (Yes / No)
- Frozen (Yes / No)
- BSL1 (Yes / No)
- BSL2 (Yes / No)
- Mycoplasma Tested (Yes / No)
- Collection support (Check those that apply)
    - Tube
    - Eppendorf
    - Multiwell
    - Slide

  - Other
- Application after sorting
  - Cell culture
  - DNA
  - RNA
- List the fluorochrome(s) in your experiment (Please use the panel template available at this link)

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

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

Access modality available: Access to NF service.

SID: NF53.002/003 Flow Cytometry Analysis/ Assisted Flow Cytometry Analysis

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

The following are examples of analyses we offer:

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

Analyzer Technical Details:

- Integrated absolute count
- Plate loader supporting 96 wells plate and 96 deep wells plate. U, V or Flat bottom
- Different sample injection modes, manual or automatic

- Wide sample flow rate and minimal dead sample volume (about 20 µl)

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

- Capable of complex polychromatic panels.
- Advice in experimental design and dedicated training sessions are also available.

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

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

Information to be provided in the application:

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

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

Shipping and storage conditions:

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

Staining or labeling stability:

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

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

SID: NF53.004 Consultation session: Sample Preparation/Panel Design/Data Analysis

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

(IU5) Ion Imaging

SID: NF55.006 - 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 condenser 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 samples.
- Living samples must be delivered using portable incubators provided by the NF.
- Applicants will be responsible for maintaining living samples using the infrastructures (incubators, hoods, etc.) available on-site.
- The samples analyzed with this approach must be at a stage of differentiation (i.e., days in vitro) sufficient to manifest the phenomena of interest. This aspect strongly depends on the intrinsic biological features of the model. Therefore, the precise timeline of the experiment will be discussed within the context of specific projects. The Applicant will deliver the samples to the Light Imaging NF at their estimated time of maturity/differentiation for ion imaging experiments.
- 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/glass coverslips for adherent cell cultures and plating cells at sufficient densities to allow the formation of syncytia/synaptic networks).

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 samples
- Number of samples
- BSL1 (Yes/No)
- Viability tested (Yes / No)
- Mycoplasma Tested (Yes / No)
- For adherent cells, sample already tested for attachment on microscopy-grade dishes/glass coverslips (Yes/No)
- Culturing conditions for the sample (temperature, CO2, 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.

Services available in combination with the NF for Data Handling and Analysis: SID: NF60.001 – Light Microscopy analysis.

ANNEX II: LETTER OF INSTITUTIONAL ENDORSEMENT TEMPLATE

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

Endorsement letter of the host Institution

To whom it may concern:

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

Declare

- That the host Institution is among those eligible to participate in the call for Access as it belongs to the following eligible category: (select among 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 the services requested here cannot be performed by the Applicant at the host Institution, at a cost which is deemed affordable for them.
- That relevant authorisations, declarations and accreditation from the competent authority(ies) have been obtained in order to process samples and data through Human Technopole;
- That, if applicable, biological specimens have been obtained with the corresponding approval of the Bioethics Committee and appropriately signed 'informed consent', both for their collection and their use, including conservation, manipulation, derivation and processing to be carried out by Human Technopole National Facilities;
- That, if samples were obtained from subjects who signed an 'informed consent', said informed consent allows that sequencing data and results are included in secure controlled Access databases and accessed/ used by authorized third parties;

and is committed

- To accept the terms and conditions to Access Human Technopole National Facilities as described in the National Facilities Access rules (link);
- To sign the Access Agreement should the project be approved (link)

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

Date …….

Name and Title ……. ; …….

Email and Signature of legal representative or delegated person

……. ; …….