The Structural Cell and Tissue Biology Program at ALBA

2021-11-22

The mission, instruments and services of the Structural Cell and Tissue Biology (SCTB) Program at ALBA are reviewed, and a strategy to be implemented within the ALBA-II upgrade program is proposed, including new and upgraded equipments and services. The corresponding topics for the Macromolecular Biology Program are discussed and summarized in another review and report.

Table of Contents

Contributed by	Klaus Attenkofer	Ana J. Pérez-Berná
	Javier Conesa (CNB-CSIC)	Nicolas Soler
	Tanja Dučić	Andrea Sorrentino
	Judith Juanhuix	Núria Valls
	Barbara Machado Calisto	Ibraheem Yousef
	Eva Pereiro	

1. Program and Upgrade Overview

1.1. Upgrade of ALBA and context of the review

The Structural Cellular and Tissue Biology Review covers all tools necessary for imaging of cell systems, cells, up to the tissue level. The Review, together with other three –one of them dedicated to Structural Molecular Biology–, aims to refine our mission, baseline our current programs, create a gap analysis and ultimately develop a strategy by blending upgraded beamlines, new beamlines, supporting laboratory infrastructure, and computing tools to create a state-of-the-art research infrastructure which is dedicated to the needs of its user community. The deliverable of the full process is a reviewed *White Paper* which describes the present programs at ALBA, point out the growth potential and unique opportunities, develops the scientific case for these opportunities, identifies tools, instruments, and any other enabler technologies necessary, and spells out at least one new beamline to be built which will fully benefit by ALBA II. This will also include the upgrade program of the existing instruments and services.

The goal of the reviews is an assessment of the current capabilities, their effectivity as an infrastructure for the Spanish community, and its competitiveness in comparison to the European and international community. Based on this assessment, the review will also evaluate the strategy of the program and its implementation plan for ALBA II. The review with its documentation and review report will be used to define the mission of the future program and will be the guideline for completing the instrument and support infrastructure allowing to fulfill this mission.

1.2. Current status of the tools in Cell and Tissue Biology

The Structural Cell and Tissue Biology (SCTB) section aims at fulfilling the ALBA mission as stated in the current strategic plan in the field of life sciences. The specific mission of the section is:

- Make accessible effective, state-of-the-art scientific services and instruments dedicated to solving societal challenges related to life sciences such as health and environment.
- Act as a catalyst for regional and national collaborations addressing societal challenges for which life sciences may provide solutions.

Remarkably, the mission is not limiting the techniques or probes to employ, nor the science to address within life sciences, but has a holistic approach focusing on the societal challenges and the collaboration with other relevant actors. In particular, the section shall not be restricted to provide access to synchrotron beamlines but also aims at making accessible a set of tools to address challenges in life sciences through collaborations or open peer-reviewed access. This drives ALBA to focus on a multi-scale, multi-technique approach to understand the behavior of biological systems.

This approach implies a number of challenges for ALBA: the diagnose and elaboration of a scientific strategy including priorities, the implementation of more complex scientific projects, the design and construction of state-or-the-art sometimes out of the *comfort* (photon) zone, the operation of the instrument in the *experiment* (custom) and *measurement* (routine, automated) modes, the provision of

integrated services to scientific and proprietary research, the integration of ALBA into European networks of excellence, the effective dissemination of the ALBA capabilities and the development of career paths for ALBA staff.

Branding is essential to implement at ALBA this multi-scale, multi-technique approach. Without neglecting other general life sciences studies, the specific topics in which ALBA is committed in this section are Pathogen Infections, Cancer and Diseases, Drug Delivery, and Cell and Tissue ultrastructure. The techniques considered to address these topics evaluated in this review are full field tomography (cryo-SXT, phase contrast μCT), sub-μm spectroscopy, nanoXRF, nanoPCXT, cryo-3DSIM, μFTIR, and electron-based techniques cryo-FIB-SEM and cryo-ET.

ALBA has currently instruments in operation and in construction phases in the SCTB field (Figure 1.1). Operating tools are a soft x-ray microscope with cryogenic capabilities (cryo-SXT, MISTRAL beamline) and a spectroscopy and microscopy infrared beamline (μFTIR, MIRAS beamline). In addition, two P2 biosafety laboratories for cell culture are fully equipped and available to users. The instruments in construction are a micro-tomography beamline (μCT, FAXTOR beamline) and a cryo 3D Structured Illumination Microscope (SIM). These instruments are complemented with the ones dedicated to macromolecular biology. The current and proposed instruments and services in Structural Cell and Tissue Biology are reviewed in sections **Error! Reference source not found.** and 3. The actual instruments proposed for the ALBA-II upgrade are listed in section 4.

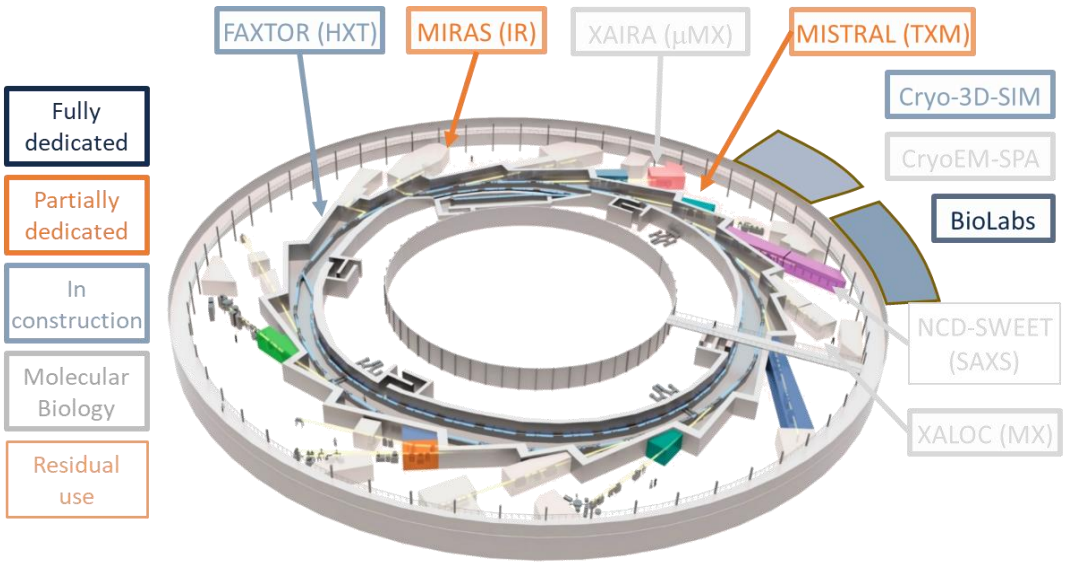


Figure 1.1. *Existing or in construction tools in Structural Cell and Tissue Biology at ALBA. Tools in Structural Macromolecular Biology are also marked in light grey*

1.3. Perspectives on imaging of biological samples

Biological techniques today span from the highest resolution techniques such as MX, NMR and cryoEM-SPA, which aim at resolving macromolecular structures (Angstrom resolution), to the lower imaging resolution techniques which aim at solving the 3D organization of organs or organisms (micron resolution). Each of these techniques have specific constraints in terms of the sample dimensions they can tackle as this is inversely proportional to the spatial resolution achievable. This is the reason why understanding the complexity of biological organization requires multimodal imaging across resolution scales.

According to the type of information obtained, the synchrotron-based techniques in the field of cell and tissue biology can be grouped in three categories:

1. **3D structural techniques**
   For imaging isolated cells we can find cryo soft X-ray tomography (cryo-SXT) with a spatial resolution of 30 nm half pitch and acquisition times of few minutes on a field of view (FoV) up to 16×16×10 μm^3; holo-nanotomography (such as ID16A-ESRF) also imaging isolated cells but excelling at the tissue level with sub-100 nm resolution half pitch on a large FoV (few hundreds of μm^3) and few hours of acquisition time (~4h/sample); finally ptycho-tomography (cSAXS-SLS), which has also demonstrated sub-100 nm resolution half pitch although on smaller FoV and with acquisition times that are clearly, as of today, too slow to attract a wide life sciences community (1day/sample). At the micron-range scale, phase contrast-based tomography techniques are used on tissue and small organisms with very fast acquisition times.
   ALBA is currently missing techniques to cover the full resolution range. There is currently a resolution gap at the sub-micron level, that is, the range between μCT technique (FAXTOR beamline) and cryo-SXT (MISTRAL beamline).
2. **chemical information techniques**
   2D nano XANES at the level of single cell or edge-enhanced nanotomography all in cryogenic conditions (pre-edge/edge tomography) can reveal speciation or locate specific elements at the sub-cellular level. Currently this is only available in the soft X-ray regime. 2D μXANES, μXRF and μFTIR are also used at the cellular and tissue level for elemental speciation, obtaining chemical information of specific cellular components. ALBA is currently lacking techniques at the micron or sub-micron level except for μFTIR.
3. **elemental quantification with ppm sensitivity techniques**
   Cryo nano-XRF in 2D and 3D although currently with slow acquisition times (such as ID16A-ESRF or Bionanoprobe-APS), allow for very high sensitivity at the cellular and sub-cellular level. These capabilities are currently missing in the ALBA portfolio.

Correlative approaches are required to face the complexity of biological systems and ultimately to link structure with function, chemical information and elemental quantification in whole single cells in 3D. Recent publications using X-ray imaging techniques illustrate very well this need (Domart et al. eLife 9, e62334, 2020; Conesa et al. Angew. Chem. 59, 1270-1278, 2020; Groen et al. Chem. Sci. 2021). The goal of correlative microscopy is to exploit all possible complementary information on the same sample from different techniques. Ideally, the techniques used in this multimodal approach should be non-destructive

and provide similar spatial resolution at similar conditions, in particular at cryogenic temperatures. This approach inherently requires registration or fiducialisation strategies to allow for 3D correlation of datasets, as well as automated pipelines.

MISTRAL and MIRAS scientists have been exploring correlative approaches and have published demonstrations on model systems that can act as seeds for users to follow equivalent strategies applied to their biological systems. These approaches link 3D structures with elemental quantification at ppm sensitivity, chemical information, conformational changes or specific localization of particular structures or macromolecules, using techniques not always available currently at the facility. Cryo-SXT (MISTRAL) has been combined with 3D cryo-nano-XRF (ID16A-ESRF), cryo nano-XANES (MISTRAL) and μFTIR (MIRAS), in addition to cryo-3D-SIM (B24-Diamond). Cryo-XRF (Bionanoprobe-APS) has also been combined with μFTIR (MIRAS). Currently, MISTRAL is developing a cryo-3D-SIM that should be available to users in 2022. Other correlative approaches could be exploited in the future, such as cryo-SXT and cryo-ET, provided that the dose is well controlled.

In summary, our vision is to grow from these already explored correlative approaches adding new tools, which will be in operation at ALBA in the near future, such as phase contrast μ-tomography (μCT), but also proposing new instruments to complete ALBA/ALBA-II portfolio with the goal to bring to the user community the possibility to access these powerful capabilities. Undoubtedly, existing and future generation facilities should provide correlative microscopies as a day to day basis and therefore, efforts are required to develop these into user friendly pipelines from the administration level (e.g. new type of proposals) to the sample preparation, experiment and data analysis support.

2. Status and Vision of the Existing and Proposed Tools

2.1. MISTRAL: status and current user program

MISTRAL is the full-field soft X-ray microscopy beamline with cryogenic capabilities at ALBA. Its role within the Life Science section is the visualization of the near-native cellular structure to understand cellular processes by providing cryo-tomography (cryo-SXT) and eventually cryo-spectromicroscopy on frozen hydrated whole biological cells, although the use of the beamline is shared between materials sciences and life sciences projects. The beamline staff comprises four scientists, plus two part-time engineers and one part-time technician. The beamline has one scientist and one PhD student for CLXM methods and instrumentation development, respectively, both externally funded.

The beamline is optimized for cryo-tomography of cells in the water window energy range and was designed to provide full illumination of the condenser lens for the whole energy range with maximum photon flux. 2D measured spatial resolution is ~30nm half pitch, and the FoV is up to 16×16 μm^2. It is equipped with a cryogenic environment (~110K) to manipulate vitrified samples and an on-line visible light epifluorescence microscope for correlative, low-resolution 2D imaging.

Available techniques for bio-samples are cryo-tomography (±70deg max angular range, typical acquisition time ~5-10 min) and cryo-spectromicroscopy (from Ca L-edge), both at 30nm spatial resolution. MISTRAL

bio-shifts over-subscription is stable from 2018 and, lately, about 70% of total beamtime is dedicated to bio-science. The international user community amounts to 45% of the total, and is still growing. More than half of the experiments are dedicated to health (pathogenic infections, cancer and diseases), and overall publications have a high average impact factor of 7.6. Biomineralization and nanoparticles (NP) internalization experiments are awarded 10% of the beamtime each. In this case, chemical characterization enabled by cryo-spectromicroscopy at MISTRAL is correlated with the morphological information delivered by cryo-SXT. Finally, about 20 % of the experiments aim at characterizing particular organelles or structures inside the cell.

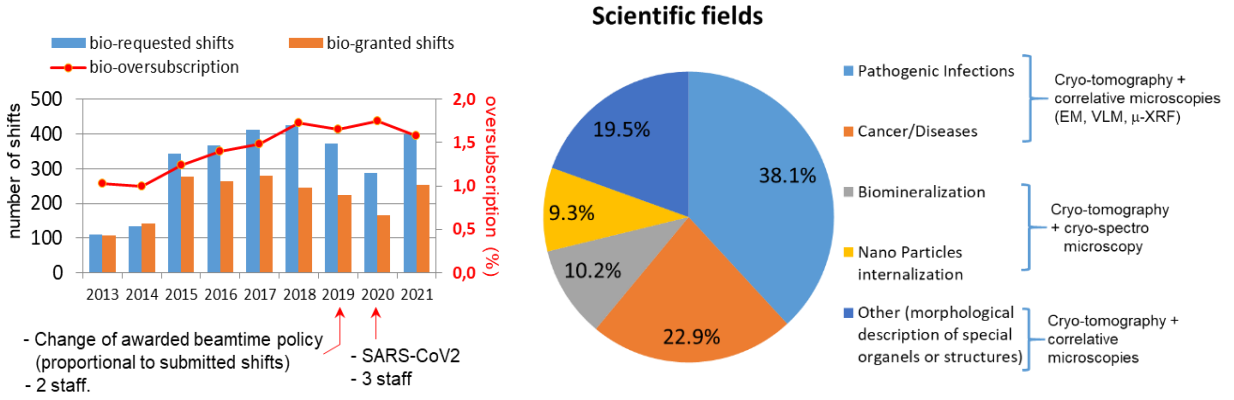


Figure 2.1. *(left) Evolution of the requested and awarded shifts in life sciences at MISTRAL beamline. (right) Scientific fields of the awarded proposals, and associated techniques.*

The beamline and the microscope have separated control systems. The acquired data is pre-processed with homemade automatic online pipelines to produce a normalized and deconvolved absorbance tilt stack. An automatic alignment is then performed using Au nanoparticles as fiducials. If successful, the tomographic reconstruction process will produce the reconstructed volume, which is the final deliverable to the users. If not successful (e.g. due to the absence or a defective distribution of the fiducial beads), a manual alignment is required, for which tutorials are given to non-expert users. Finally, the proper data analysis consists in the segmentation, i.e. the 3D rendering, and statistical of the reconstructed volume (in the case of the tomography), or in the extraction, classification and normalization of the spectra in the case of spectro-microscopy. Non-expert users are assisted during the data analysis.

The operation of MISTRAL is currently limited by the sensitivity to the perfect optical alignment, the axial rotation run-out induced by the cooling braids (which is not reproducible and similar to the zone plate depth of focus, ~1.5µm), the absence of automated fiducialess alignment and segmentation pipelines and finally the lack of a technician for sample preparation. In a broader view, the *SWOT analysis* of MISTRAL shows the following conclusions:

Strengths:	**Weaknesses:**
• *Few competitors.* • *Complementarity to other microscopies.*	• *Major BL upgrades required because of aging.* • *No reproducible sample preparation.*

• *High throughput (cell population is described with statistical meaning).* • *Staff dedication.* • *Good publications IF.* • *New user groups accessing the technique.*	• *Difficult sample quality control.* • *Manual data alignment required.* • *Lack of bio-users expertise in tomography*
Threats: • *Cryo 3D CLXT (by implementing cryo-3DSIM) is mandatory to keep competitiveness.* • *Productivity loss if delayed BL upgrades.* • *Proprietary TXM software.* • *Lack of support staff for sample preparation and cryo-3D-SIM.* • *Arising of competing laboratory techniques.*	**Opportunities:** • *Growth of the local community of users with expertise in μFTIR* • *Integrating cryo 3D CLXT into the program.* • *BL upgrades can improve significantly experiment conditions.* • *Higher data throughput by implementing reliable fiducialess alignment.* • *Growing user community*

2.2. MISTRAL user support and inhouse research

The user support at MISTRAL from scientific staff includes the sample preparation, sample screening, the beamtime and the data analysis:

- **Cell culture Platform**: Complete service for sample preparation when *in situ* cell culture is required for the experiment. The culture can take from 3 to 10 days, during which the MISTRAL staff manages the thawing of the cells, supervises the cell culture or subculture and the required treatments, assists on the correct seeding of the cells into the grids and selects the best timing for the vitrification.
- **Vitrification Platform**: Preparation of the fluorophore and fiducialization for the samples, vitrification of the grids and storage in the specific dewars. This step usually takes half a day.
- **Sample screening in cryo conditions**: Assistance on the screening and mapping of the grids by epifluorescence in cryo conditions using a cryo-CLEM instrument adapted for cryo-CLXM. The screening is made for all life sciences proposals to select the best grids and optimize the beamtime. This step typically takes half a day.
- **Beamtime**: The users receive local contact assistance from scientific staff from 8.00 to 20:00.
- **Data Processing support**: All the datasets obtained during the beamtime are automatically reconstructed with the support of the local contact to select the best tomograms for the manual data analysis. The users usually have no experience in the software data analysis, so MISTRAL staff offer them webinars on alignment and segmentation at least twice a year. Additionally, the users are provided with detailed instructions for auto-training on manual alignment and segmentation of the data. More than half of the users find difficulties and problems during the data analysis and need personalized guidance, advice or support.

The sample preparation is a highly time-consuming task which is done by MISTRAL scientists, as no technical staff is assigned to the biolab. The vitrification of the sample is done at ALBA for 60% of the bio proposals since users lack experience in growing cells on grids and do not have access or are not familiar with the required tools (plunge-freezing and linkam cryostage).

MISTRAL team has developed an intense in-house program with two clear goals:

- To stablish advanced protocols to be implemented in the user program.
- To implement the proof of concept of multimodal and/or correlative experiments.

In particular, one of the ultimate aims of bioimaging is to localize specific macromolecules in cells in near-to-native conditions, usually achieved by combining different techniques. As such, cryo-SXT provides nanoscale 3D information from cryo-preserved unstained whole cells, which can be combined with functional information or specificity by CLXM and with elemental localization and quantification using nano-XRF. Moreover, µFTIR microscopy and nano-XANES can add functional and chemical information, enabling studies of cellular events that cannot be captured using the techniques separately. The MISTRAL in-house program has three legs:

1. Structural studies
   - Structural studies of infectious diseases causing intracellular membrane alterations and their therapies: HCV (Perez-Berná *et al.*, 2016) and SARS-Cov-2 (in preparation).
   - Multi-scale collaboration with MX**:** Studies of Mycoplasma and Zika virus integrating 3D structural information from cryo-SXT with MX, in collaboration with the MX beamlines at ALBA (Garriga *et al.*, 2021).

2. Correlation with chemical information
   - **Correlation with nano-XRF:** Cryo-SXT at MISTRAL (which provides high contrast for carbon-dense structures as cellular membranes at 50 nm half pitch resolution) was correlated with cryo-XRF done at ID16@ESRF (which provides the elemental sensitivity with a 70nm step size) to measure ion concentrations at different locations (Conesa *et al.*, 2020).
   - **Correlation with cryo-µXANES microscopy:** Cryo nano-spectroscopy capabilities at MISTRAL have been used combined with cryo-SXT, allowing the study of the biomineralization at intracellular level in frozen hydrated whole cells and with a spatial resolution of few tens of nanometers. (Sorrentino, Malucelli et al. 2021)
   - **Correlation with µFTIR spectroscopy**: The correlation of the SXT volumes with SR-µFTIR microscopy allows setting up the chemical nature of the viral structures during the Hepatitis C virus replication and also during the healing process. (Perez-Berná *et al.*, 2021)

3. Correlative light and X-ray tomography (CLXT)
   - Given the constant demand for easily accessible and user-friendly methodologies, MISTRAL scientists are developing new instruments and methods in CLXT on whole vitrified cells. A new cryo 3D Structured Illumination Microscope (cryo-3DSIM) is being developed in-house. This new approach allows non-destructive correlative 3D cryo-imaging as the sample is preserved without process-induced damage.
   - Automatic registration methods are being developed to correlate the data from cryo-3DSIM and cryo-SXT. An externally funded FTE from the EU iNext-Discovery project is dedicated to this.

The inclusion of the cryo-3DSIM in the user program as a service complementary to the beamline, is key to attract top-level research in bioimaging and new advanced users. Once this microscope is made routinely available, the MISTRAL users would image the samples with the cryo-3DSIM before the beamtime at the beamline. Automatic correlation methods will allow processing the 3D data sets to deliver reconstructed CLXM 3D data sets to the users. The inclusion of cryo 3D-SIM in the user program will require extra human resources to support users in parallel to the beamtimes.

2.3. MISTRAL vision

The role of MISTRAL is to enable the 3D visualization of the cellular environment in near-to-native conditions to reveal e.g. pathogen infections, morphological changes in genetic recessive diseases, intercellular structures or to evaluate drug treatments at the cellular level and locate specific structures or molecules. Ultimately, the goal of biological imaging is to link *structure* with *function*, *chemical information* and *elemental quantification*.

From the SWOT analysis (section 2.1), several opportunities have been identified, which are specified in the *gap analysis* below.

1. The aging instrumentation requires a beamline optics major upgrade (new mirror benders to reach optimum beamline stability).
2. The current irreproducible run-out of the rotation due to the Cu braids providing the cryogenic temperature needs to be upgraded to achieve sub-100 nm run-out.
3. Automated data throughput needs to be achieved by implementing reliable fiducialess alignment at sub-pixel accuracy.
4. Several methodological improvements need to be considered for a more realistic tomographic approach; among them, cone beam geometry and a partial coherent image formation model.
5. 3D cryo correlative light X-ray tomography (cryo-CLXT) is mandatory to achieve location specificity. Without this, the results are incomplete and Mistral will lose competitiveness.

Currently, MISTRAL offers in the user program correlative 2D cryo-epifluorescence images to choose a specific region of interest within cells for which cryo-SXT images will be collected, or to select specific cells among those on the grid. The correlation achieved (2D only and low resolution) is poor. To enable higher resolution in 3D, MISTRAL staff is developing the cryo 3D-SIM instrument, which doubles the resolution in all 3 directions. To support the use of cryo 3D-SIM by the next-in-line users in parallel to the allocated MISTRAL beamtime, an extra FTE is required.

The upgrade of the beamline can be performed in two phases. The first phase (2022-24) will focus on instrumentation and methodology. Concerning instrumentation, two actions are required:

- cryo 3D-SIM operational within 2022 for users
- reproducible run-out below 1 μm.

Concerning methodology, four developments should be made:

- Automatic 3D CLXT (there is currently available 1 FTE from iNEXT-Discovery EU project)
- Cone beam tomography

- Reliable fiducialess alignment
- Partially coherent image formation model

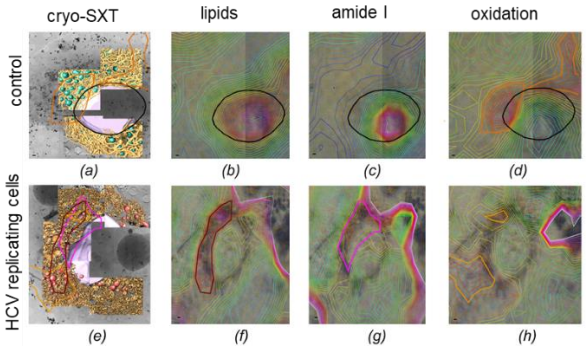
The second phase (2024-2028) will focus exclusively on instrumentation:

- 3D run-out below 100nm with active correction
- upgrade of all elliptical cylinder mirrors with nanobenders
- towards higher resolution 3D CLXT with sub-100nm super-resolution in cryo.

To fully exploit all possible information on a specific cell and condition, correlative approaches have been developed by MISTRAL staff on model systems which are of benefit for the user community. In addition to the correlation with cryo 3D-SIM (Okolo et al. *Nature Protocol* 2021) already mentioned, which enabled locating intracellularly a specific novel therapeutic agent (Groen et al. *Chemical Science* 2021), these approaches include cryo 3D X-ray fluorescence using the cryo nanoprobe beamline at the ESRF (Conesa et al. *Angewandte Chemie* 2020) and µFTIR at MIRAS (Pérez-Berná et al. *Acta Cryst. D*, 2021) (Fig. 2.2). Additional correlative approaches are planned to be explored such as cryo-SXT with consecutive lamellae production by cryo-FIB milling and cryo-ET to achieve the highest possible resolution at a specific sub-cellular region. These demonstrations producing high quality data should enable considering new capabilities at ALBA to expand the portfolio of biological imaging tools available to users. This is particularly important within the ALBA-II frame for which a nanofocusing beamline should be considered.

Cryo-SXT correlated to 3D cryo XRF
(J.J Conesa *et al.* Angew. Chem. 59, 1270-1278, 2020)

Cryo-SXT correlated to SR-µFTIR
(Pérez-Berná *et al.*, *Acta Cryst. D*, 2021)



Cryo-SXT correlated to cryo-3DSIM
(Groen *et al.* Chem. Sc., 2021)

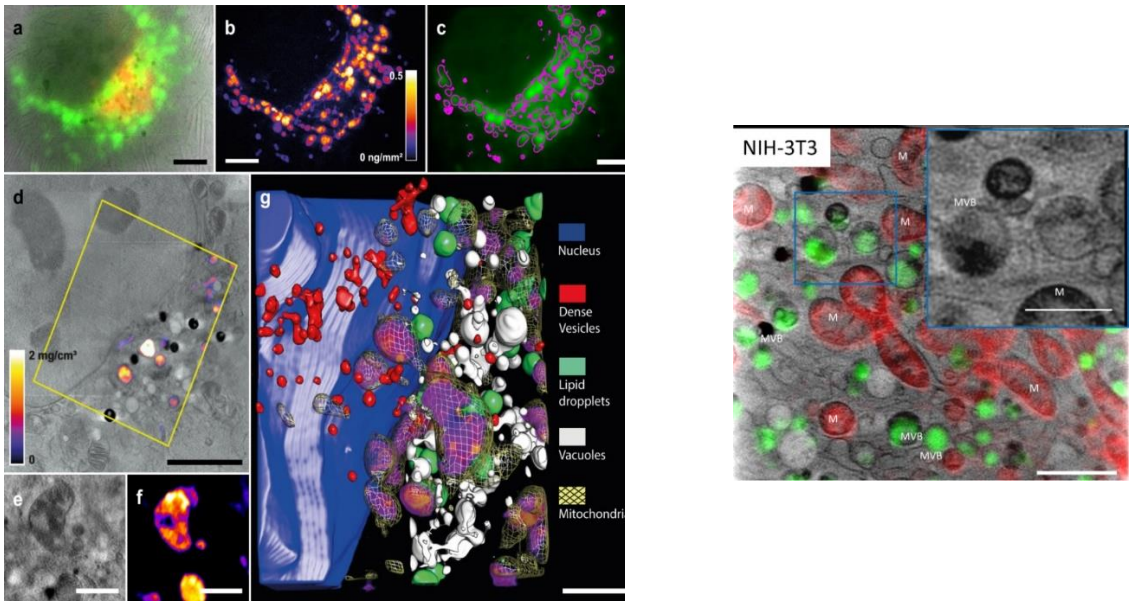


Figure 2.2. *Examples of Cryo-SXT correlated to cryo-XRF, SR-μFTIR and cryo-3DSIM*

In summary, the MISTRAL upgrade and the addition of new instrumentation to exploit multimodal approaches on the same sample will produce a more complete information, which will enable growing the user community.

2.4. MIRAS: status and current user program

The MIRAS end station is devoted to Fourier Transform Infrared (μFTIR) micro-spectroscopy. The beamline is staffed with three scientists and one postdoctoral researcher. In addition, the beamline receives technical support through a matrix staff of 3 engineers (mechanical, electrical and control) and one technician shared with other beamlines and general services.

The main roles of MIRAS within the life science section are:

- To map the state of the bio macro molecules non-destructively in their natural environment.
- To develop new methods adapted to IR-based biological and biomedical applications for bio-multidisciplinary research projects (connecting groups with diverse expertise to solve a specific biological problem) and for projects employing multimodal approaches (offering μFTIR as a complementary tool to other techniques).

The beamline design and the availability of specific detectors with different detection spectral ranges allows optimizing the performance in the Mid-IR and Far-IR regions to cover a broad wavelength range from ~1 μm to ~100μm. The optical layout of MIRAS includes an option for splitting the extracted infrared beam into two parts, with one containing the edge radiation of the beam. The experimental cabin and the transport optics are designed to accommodate two additional end stations as a future upgrade of the MIRAS beamline.

The beamline started user operation in October 2016. The number of submitted proposal and new users of the beamline have been growing since then, and is stable since 2019. The average beamtime oversubscription factor is 1.6.

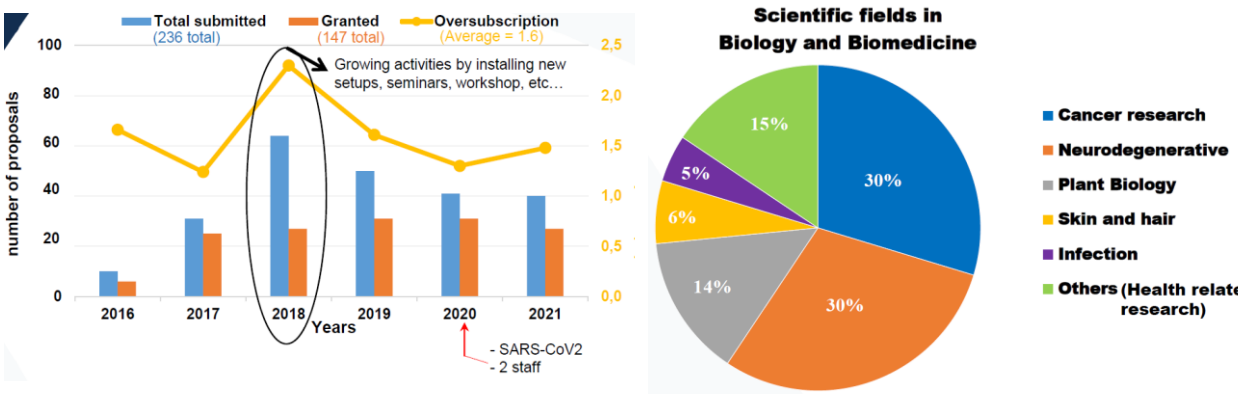


Figure 2.3. *(left) Evolution of the requested and awarded proposals at MIRAS beamline. (right) Scientific fields of the awarded proposals.*

The applications of this beamline are covering a wide range of research fields in life science and material sciences. The life sciences are representing the main body of research with 65% of submitted proposal, with direct impact in the study of different disease states. The scientific record of the beamline is also growing according to the number of publications every year, with around 72% of these publications emerging from the biology and biomedical applications. In parallel, the material science field has grown significantly in recent years. The beamtime allocated to Spanish community amounts to 75% of the total.

The users of MIRAS can deal with their output data from the end station as spectral analysis (spectroscopy) or as imaging (microscopy) depends on the demand of the project. The beamline includes user friendly control system and provides a service of remote access of users to data analysis commercial software.

Single cells and tissue (analysis and imaging) are the main measurement techniques performed in biological and biomedical applications at the BL through the transmission operation mode of the μFTIR microscope. Thanks to the sample preparation tools for cell culture and tissue cryo-sectioning available at ALBA biolab, it is possible to combine multimodal approach of different techniques. For example, the multimodal approach combines μFTIR, and soft X-ray microscopy through the established collaboration between the MIRAS and MISTRAL beamlines has led to two publications in neurodegenerative disease and cells infection. It will be possible to enlarge the collaborative partners to FAXTOR (for the X-ray 2D imaging of thin tissue).

Recently, live cells spectroscopy and imaging has been successfully implemented at the beamline thanks to the support and collaboration of the infrared group at Elettra synchrotron facility.

The limitation of the current technique at MIRAS beamline can be summarized as the following:

- Spatial resolution is limited by diffraction limit.

- Energy range in the low frequency range is limited (in air spectrometer) 60cm-1.
- Rapid scan is not implemented (currently 1s/scan).
- Working distance under the microscope limiting the sample environment with external setups.
- Extracting IR from 4th Generation source is not clear: However, the machine group at ALBA is looking for different solutions for the extraction angle. The SRW simulations and expected performances will be carried out after defining the extraction angle.

The *SWOT analysis* of the MIRAS beamline shows the following conclusions:

Strengths: • *Multidisciplinary scientific applications.* • *Diversity of sample types and measurements modes* • *Spectroscopic and microscopic analysis in the (Far/Mid-IR) ranges* • *Collaborations and Synergies with other beamlines/techniques and resources available in ALBA*	**Weaknesses:** • *Dependency on basic instruments limits throughput and growth of the program* • *Unstable users demand and long publication cycle* • *Lack of complementary vibrational analysis* • *Kinetics experiments not optimum* • *Sophisticated sample preparation*
Threats: • *Extraction of IR light from 4th generation machine is not clear yet and can be under risk* • *Rapid and significant development of conventional IR sources that compete with the IR synchrotron source.* • *Competitive European environment.*	**Opportunities:** • *Growth of the local community of users with expertise in μFTIR.* • *Growth of the demand from upgrades and new instruments. (Nano-FTIR, Raman, …)* • *ALBA–II upgrade: enhanced brilliance of the source and performances in the Far-IR frequency* • *New instruments will attract industrial projects*

2.5. MIRAS: inhouse research and perspectives

The biological in-house research at MIRAS started in 2017 and currently receives ~12% of total allocated beamtime per cycle. The scientific staff (3 beamline scientists, one post-doc and one externally-funded post-doc) have submitted 2 in-house projects per year in average since the beginning of the program in 2017. The scientific output of the in-house program is 27% of total publications of the beamline in the past five years, with an average impact factor of 4,7. In-house collaborations include Universities (UAB, North Western, Belgrade, Paris, Goettingen, Hamburg), Hospitals (Bellvitge, Hospital del Mar, Tortosa, Himac, Goettingen) and other synchrotrons (Elettra, APS, Diamond, ESRF). Some of the in-house programs were funded through several EU projects (Twinning, COST project, International Cancer Control, Horizon 2020).

The main tasks of the in-house research consist in:

1. Sample preparation.

2. Follow the bio-macromolecular changes (as protein aggregation, lipids peroxidation, carbonylation etc.).
3. Analysis of spectra including the statistical evaluating of cellular chemical changes.
4. Searching for biomarkers in cells/tissues in different diseases.

The in-house program focuses on three main topics: cancer, Alzheimer and Amyotrophic Lateral Sclerosis (ALS), including the possible treatment at the cellular and tissue levels. Multimodal synergetic synchrotron-based techniques are applied in most of the projects, combining μFTIR spectroscopy and microscopy with visible microscopy (including super-resolution techniques), nano-XRF and nano-XANES, and cryo-SXT.

First highlighted topic is the development of innovative radiotherapy approaches and the study of the tumor response at the cellular and molecular levels through μFTIR (Martínez-Rovira et al., Analyst, 2019). The intracellular NP-based radiosensitization mechanisms in F98 glioma cells treated with charged particle therapy were also studied (Martínez-Rovira et al., Analyst, 2020). Conclusion was that SR-μFTIR is able to assess cell response to innovative radiotherapy approaches, and that NPs induce conformational changes in proteins, lipids and DNA.

The effects of Alzheimer's Aβ amorphous and fibrillar aggregates on PC12 Cells were also studied (Benseny-Cases et al., Anal. Chem. 2018). Fibrillary and non-fibrillary plaques in-situ were distinguished by SR- μFTIR, while the oxidative state of iron was identified by nano-XANES (Álvarez-Marimon *et al.*, ACS Chem. Neurosci. 2021) on the same tissue.

A multimodal approach combining μFTIR and hard and soft X-ray microscopy was used to characterize the intact neurons of the hSOD1 G93A rat model for ALS (Dučić et al., Anal. Chem. 2019). The lipids status and copper were evaluated and mapped in a single astrocyte (Kreuzer et al., J. Biophotonics, 2020). It was found that ALS cells contained more copper, colocalized with total lipids, carbonyl groups and oxidized lipids.

The in-house projects were also instrumental to develop two sample preparation methods for multimodal experiments: the *freeze-dryer* for the water sublimation on low temperature (in-house Dučić and Kreuzer, 2018) and the *live-cells setup* for the live cells metabolic evaluation and for the drug treatment assessment (in-house Dučić 2019). These developments are foreseen to be integrated into the user program in near future.

2.6. Vision of the μFTIR program at ALBA

The mission of the MIRAS beamline is to visualize the macromolecular composition in diseases and treatments at cellular, tissue, and small organism (model system) levels, and to characterize and link the chemical and the structural informations. Ultimately the vision is to contribute to understand the macromolecular conformational changes in synergetic multimodal approaches to fully connect and exploit all available information of biological processes and health challenges.

These ambitious vision and mission lead to several gaps with respect to the current situation. We propose several actions to meet the high standards of the aimed approaches. First, we propose to build dedicated end-stations, which is allowed by the optical design of MIRAS, to implement additional experimental setups. The availability of different techniques in dedicated ports is expected to ease access and increase the throughput.

Second, concerning the samples, it is expected to offer the live-cells μFTIR setup to users. MIRAS would integrate *3D cell culture* and *tissue on-chip* sample preparations and methods (Raman, Mass-spectrometry) and *live-cell microfluidic* setup. The inclusion of the sample preparation services and methods in the user program may require extra technical human resources at the biolab.

Third action would be to extend Terahertz (THz) spectroscopy to life sciences projects, and to implement nano-FTIR for spectroscopy and imaging. Regarding the THz spectroscope, the implementation at the beamline would be in collaboration with the Materials Science program, which is the main scientific case for THz. However, the THz range would also be important for life sciences, as it provides information on hydrophobic interactions, van der Waals interactions, hydrogen bonds or DNA epigenetic changes without inducing radiation damage on the samples. However, the instrument and the sample preparation are very demanding, mostly due to the required in-vacuum sample environment.

Also, the possibility to include in the ALBA user program the nano-FTIR technique, already enabled by the development of new laser sources, is to be explored. An increasing number of studies on single fibrils, viruses, individual protein complexes, membranes and protein aggregates are published. The technique allows the their mapping with ~10-30 nm lateral resolution. Still, the technique is also challenging and requires successful pilot projects before including it to the user program.

The instrumentation upgrades, although ambitious, would strongly benefit from the long-term experience in the multimodal approach of the MIRAS beamline staff. During the last decade (from Dučić et al., *J. Structural Biology*, 2010), we have combined the SR-μFTIR with cryo-SXT, cryo-XF, cryo-XANES, cryo-FE-TEM, *in vivo* and *ex vivo* Magnetic Resonance Imaging (MRI), *in vivo* AFM, confocal fluorescence microscopy (fixed cells), and classical histological analysis. Examples of multimodal studies are the research on intact astrocytes from the rat model of ALS (combining μFTIR, hard and soft X-ray microscopy on single-cell, Dučić *et al.*, 2019, Kreuzer *et al.*, 2020), and that on the single glioblastoma cells (the live-cell spectroscopy and imaging, Dučić *et al.*, *Anal. Chem* 2021). The beginning of operation of the FAXTOR beamline will open a new opportunity for the multimodal study of organoids with μFTIR and μCT). Other possible future beamlines with common, synergetic scientific cases would be bio-SAXS (to study macromolecular complexes), nano-XRF, and sub-μXANES).

In summary, the proposed upgrade of the μFTIR program would allow live-cells multimodal approach and new sample preparation techniques to collect information in close-to-native (physiological) conditions. Also, additional techniques (THz, Raman and nano-FTIR) would provide new chemical information and increase the collaboration with the life-sciences user community. Concerning the choice of the IR sources, it is preliminarily proposed a dedicated station of the synchrotron beamline for current mid-IR applications

including live-cells setup, a second dedicated station of the beamline for the THz, in-vacuum spectrometer, and laser sources for nano-FTIR.

2.7. uCT at ALBA: status and vision

ALBA, with the construction and operation of the Fast X-ray Tomography and Radiography beamline (FaXToR), will provide to the user community a new instrument dedicated to µ-Computed Tomography (µCT). The beamline is expected to start user operation in 2024. Following the ALBA scheme, the FaXToR scientific staff consists in three beamline scientists and one post-doc, of which only the BL responsible is appointed since the beginning of the project and another beamline scientist will start in January 2022. The beamline relies as well on the so-called matrix staff, which includes one mechanical, one electrical, and one controls engineer. Joint efforts with the microfocus MX beamline (XAIRA) are in place to set up a high throughput data analysis infrastructure.

FaXToR will be fed by an in-vacuum wiggler, currently in the production phase, that will provide a beam with usable energy range 10-70 keV and a maximum size of 35×12 mm^2 at the sample. A double multilayer monochromator will filter the beam in the 10-50 keV energy range with a bandwidth of 2-5%. The possibility of working with filtered white beams in the end-station is foreseen. In terms of temporal resolution, FaXToR aims at maximum of 10 tomograms per second and 100 µs/image in radiography. The beamline will be equipped with two different detection stages allowing a spatial resolution, in terms of pixel size, between 1 and 10 µm, with a FoV varying between several mm to several cm.

Phase-contrast imaging will be feasible at FaXToR using the propagation-based imaging technique and in absorption-based mode, to be used in radiography and in static and time-resolved µCT studies. Some examples of applications are the morphological dynamics in human cartilage plugs under external loads; the morphological changes in pathologic liver biopsies; the multi-modal visualization of the effects of microbeam radiation therapy, and the 3D distribution of motor neurons in spinal cords.

The *SWOT analysis* of the FaXToR beamline shows the following conclusions:

Strengths: • *Flexible End-station (in-situ/in-operando capabilities).* • *Broad energy range with Tunable spectrum.* • *Broad time resolution*	**Weaknesses:** • *No in-vivo measurements capabilities (small animals).* • *Limited sensitivity in phase-contrast (Spatial coherence).* • *Limited spatial multi-scale capabilities.* • *Not yet established user community.*
Threats: • *ALBA2 long shutdown during possible ramping up of user community.* • *Competing communities at the beamline (Bio / Mat. science).* • *Competitive environment in Europe.*	**Opportunities:** • *Reduced horizontal electron beam size with ALBA2.* • *IT Infrastructure upgrade, creation of computing and data analysis unit.* • *Long shutdown (machines upgrade) in other facilities.*

A number of mitigation actions follow after the gap analysis. The requirement on heavy computation services will be mitigated by coupling the beamline with the IT infrastructure upgrade and new data analysis services. The sensitivity of the phase-contrast imaging can be increased by implementing a mini-beta section and by the ALBA2 machine upgrade. Increasing the multimodal capabilities requires the implementation of new instruments and the development of pilot projects in the in-house program.

In a multimodal context, the morphological information at the micron scale in large volumes provided by μCT is to be correlated with other techniques already available at ALBA like μFTIR and cryo-SXT. However, in spite of FaXToR coming into user operation and the ALBA-II upgrade, two gaps will still remain, namely one at low spatial resolution, large FoV and very high energies, and the second at the sub-micron spatial resolution scale, that is, between the resolutions given by μCT and the cryo-SXT techniques.

Imaging of larger and denser samples, e.g. full organs, and low dose/in-vivo experiments, e.g. breast imaging and in-vivo lung imaging is achieved by an unfocused beam at very high energy (50-120 keV). Even though ALBA is in a non optimal position compared to high-energy facilities, hosting such a beamline should be considered in case of a substancial growth of the in-vivo user community.

Filling the sub-micron spatial resolution gap is prioritary in the multimodal approach, as will allow covering the ALBA multi-scale capabilities from the cellular up to the tissue level. Scientific trends show a strong push towards this direction. Applications include studies of the vascularization of spinal cords; multiscale visualization of the effect of osteoarthritis on cartilage, and quantitative investigations of neural networks in animal models. This range of applications require a dedicated beamline or, alternatively, a new dedicated branch of the FaXToR beamline. In either of the alternatives, the end station can host both phase-contrast techniques and sub-μXANES in the few-hundreds-microns range. While FaXToR will benefit from higher spatial coherence with ALBA-II, there will be still a need to cover the gap towards sub-micron CT with a new instrument, which considered first priority.

2.8. Status and perspectives of cryo-electron microscopy tools in bioimaging

Cryo-FIB-SEM and cryo-ET are major contributors in the field of cell imaging. Cryo-ET on thin lamellae of about 100 nm, is able to visualize the structure of protein complexes with high resolution by subtomogram averaging. Cryo-FIB SEM is able to reveal the whole cell architecture at the level of organelles by producing a stack of SEM images of the cell at different heights after recurrently milling the sample using a focused ion (gallium) beam (FIB). Lamellae of cells can also be produced for cryo-ET, in which a set images at different sample rotation angles are acquired.

Both cryo-FIB-SEM and cryo-ET are destructive techniques. These techniques require for sample quality control and targetting of specific features cryo visible light fluorescence microscopy. The results obtained are highly dependent on the computing methods to analyse and reconstruct the 3D structure from the set of images. Advanced imaging methods, high performance computing hardware and expertise are crucial for the outcome of the experiment.

In response to the growing demand of cryo-FIB-SEM and cryo-ET, the Centro Nacional de Biología (CNB-CSIC) has established the Severo Ochoa Bioimaging Initiative to service the Spanish and worldwide groups with a number of visible-light and electron-based techniques at cryogenic conditions, as well as data analysis and reconstruction methods. The access is either via European projects (iNext-Discovery, Instruct-ERIC) or by fee charge per session.

Cryo-FIB-SEM and cryo-ET can be correlated with cryo-SXT since the sample preparation and sample supports are identical and cryo-SXT is a non-destructive technique. Still, this correlative approach has not yet been explored and remains a very promising method development. Other approaches can also be studied in the future, such as the correlation of cryo-ET and nano-XRF. In any case the synergies between these electron microscopy techniques and synchrotron imaging techniques are obvious as well as with macromolecular crystallography, as revealed by the growing number of these facilities which associated electron microscopy centers.

3. New proposed services

3.1 Computing and data analysis vision

The Scientific Data Management (SDM) section has been created in 2021 to provide a bridge between computing and science and to ease the adoption of modern computational approaches in data processing pipelines. On the one hand, SDM is to provide the necessary scientific computing framework for data processing and analysis to the beamlines in collaboration with domain-expert method developers in the Experiments division in order to translate the methods into concrete data analytics tools. On the other hand, SDM coordinates the transition to FAIR data integrating into European projects (ExPaNDs, LEAPS-INNOV), and other European synchrotron facilities.

Within the Structural Cell and Tissue section, the first priority in terms of data processing and analysis for MISTRAL is the implementation of a reliable solution for fiducialess tilt-series alignment. The software developments in the field are to be monitored to automate steps which currently require manual intervention so far (i.e. segmentation, correlative microscopy) and to smoothen the user-experience (workflow managers, GUIs). These points are also valid for the future µCT beamline FAXTOR, which will have, in addition, a very high data production rate. The issues arising from manipulating and storing very large files (fast bandwidth, data selection, compression, GPU-based calculations, etc.) will need to be tackled. Finally, for the µFTIR beamline MIRAS, the use of recent open-source spectroscopy-dedicated tools such as Quasar will progressively replace current proprietary solutions.

3.2 Industrial program: overview

The ALBA industrial program aims at promoting and making available to the industrial sector the potential of the synchrotron services to boost competitiveness of the industry and benefit the society. In particular, the mission of the SCTB industrial section is to expand the portfolio of industry clients by offering high end services and advanced synchrotron techniques and by rising awareness of the industrial applications of those techniques for boosting innovation and competitiveness of the companies

With the aim of supporting the innovation and engagement with industry, ALBA has created an Industrial Liaison Office to promote the ALBA services to the industry, actively look for external funds and to be the one-entry contact and support point to industries during the service. There is a dedicated access mode for companies which ensures the property confidentiality of the results at a cost-covering fee.

The industrial services on cellular and soft tissue studies started in 2017 and comprises 7.5 % of the total industrial beamtime. So far, 5 different companies have used these services, covering cosmetics, textile and pharmaceutical industrial sectors. MISTRAL and MIRAS are the most used beamlines by industry in this sector, followed by NCD and CLAESS (Figure 1). The services offered are diverse, comprising studies on structural effects of drugs at the cellular level, collagen orientation in leather samples upon different treatments, biochemical and structural effects of cosmetic products on skin and hair, among others.

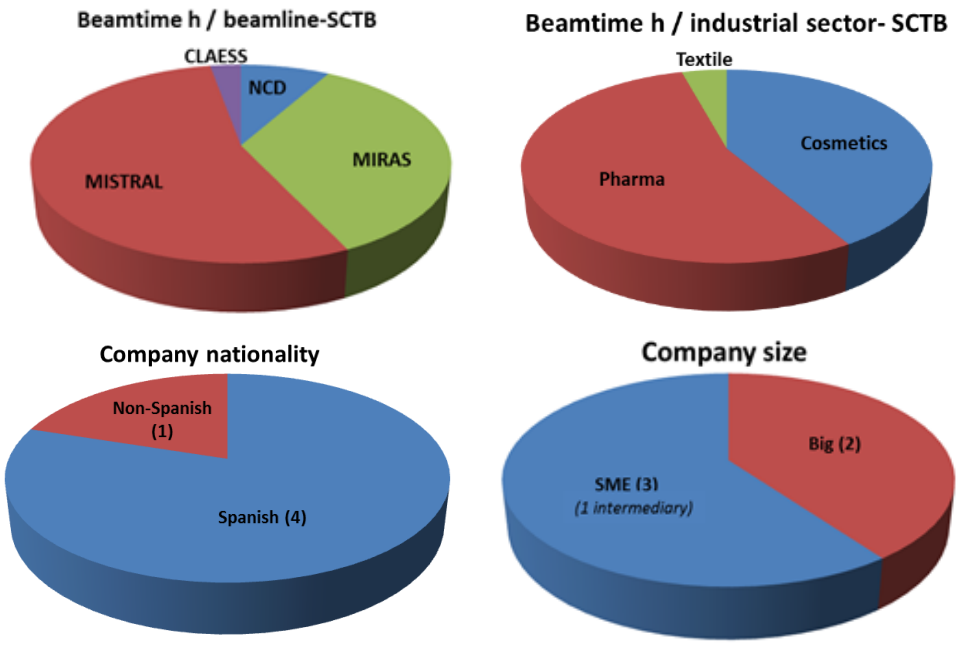


Fig 3.1. *SCTB industrial services. From left to right: beamlines used, industrial sectors addressed, company nationality and company size.*

Most of the companies requiring the ALBA services are Spanish. Profiles are diverse, ranging from Small and Medium Enterprises (SMEs) to big companies. Despite the differences in the company size and sectors

covered, most of the companies have similar needs, requiring full service, from sample preparation to beamtime and data analysis. Pharmaceutical and cosmetic sectors are recognized to be very important in the local area. The cosmetic sector is particularly promising as companies are looking for more scientific evidence for the efficacy of their products due to new regulations. Synthetic skin, now in development, may also play an important role in product characterization using synchrotron facilities.

Specific dissemination and outreach initiatives will be developed to stimulate the use of the synchrotron light by the SCTB industrial sector by fostering the use of the EU funding for SMEs and by offering more consolidated and new services to the companies.

3.3 Vision of industrial activities

The industrial program in cellular and soft tissues at ALBA is maturing, especially in the pharma (with one experiment at MISTRAL) and cosmetics (two experiments at MIRAS). The potential of the industrial applications in this area can be further exploited with the growing interest on bioimaging tools and with the increasing need for science-driven evidence of products effectiveness. In this context three objectives have been defined to the future industrial program in the area: i) boosting client satisfaction, ii) attracting more companies, mainly local, and enabling them to build-up their technological competences, and iii) supporting the companies towards innovation with our expertise on X-ray methods.

The strategy to accomplish these objectives is based on the dissemination of the industrial services, the enhancement of the current services to cosmetic companies, and the implementation and diversification of the industrial services:

- The dissemination of the portfolio of techniques and industrial services offered by ALBA to new companies and companies from new industrial sectors will be reached out by marketing actions and campaigns. Workshops and webinars will continue to be organized mostly jointly with industrial clusters. In the local area there it is planned to participate actively with the (CataloniaBIO and HealthTech cluster. The dissemination tasks depend on human resources from the ALBA Industrial Office, which will count from 2022 on 3.5 FTEs.
- The improvement and enhancement of the current services to cosmetic companies will require a closer follow-up of the different experiments by the staff of the Industrial Office to promote engagement in productive relationships between customers and ALBA scientists.
- The diversification of the industrial services on cell and soft tissue biotechnology is intimately linked to the upgrade of the present beamlines towards faster data acquisition, higher data quality and new methods which make the present industrial beamtime more competitive and increase the interest of new companies. The ALBA-II upgrade plays a capital role in achieving these upgrades. In addition, the automation of data acquisition and data treatment will partially relief the scientific staff workload. The availability of complementary analytical techniques is also very appealing to companies.

The successful implementation of the future industrial program depends on transversal support and services such as extensive mail-in services, remote access, technical support in sample preparation,

sample screening and selection for the offered methodologies, availability of a sample tracking system, fast and as much automated as possible standard data collection, and of data analysis pipelines.

The industrial service to be offered at MIRAS points to full service, from sample preparation to data analysis. MIRAS is particularly interesting for pharma and cosmetic companies to verify the diffusion, efficacy and safety of active ingredients by analyzing the biochemical composition of the cells and tissues upon treatment. Several industrial experiments on skin at MIRAS beamline have been nicely complemented with small angle scattering data measured at NCD beamline at ALBA. This a nice example of the future services to be offered in the future.

For tomography beamlines, MISTRAL and FaXToR, main applications are the structural effects of drugs or excipients at the cellular level and the evaluation of the morphological changes upon treatment on tissues or small organs, respectively. This is particularly interesting to pharma, implants and agri-food sectors, and in general to healthcare sector. The techniques at the current beamlines are to be complemented by other techniques like cryo-3D SIM and cryo-nano XRF to be made available in the ALBA-II upgrade, for instance to characterize infection mechanisms and drugs efficiency and location and quantification of drugs.

The strategy towards the effective implementation of industrial services for Cell and Tissue Biology section comprises support from beamline staff for established services as for μFTIR and SAXS (currently done at NCD beamline). For bioimaging techniques, we will offer short feasibility experiments to proof the techniques for particular industrial applications with support from beamline staff. As these activities are time-consuming because of sample preparation and, mainly, data analysis, different solutions are foreseen to find additional resources. These include setting-up collaborations with research groups or technical platforms from research institutes for sample preparation, using data analysis services from intermediary companies and establishing long-term agreements with companies.

In summary, the future ALBA industrial program for this section strongly relies on the ALBA-II project as it is crucial for the improvement of the technical performance of the current beamlines but also for its generated momentum to boost the ancillary laboratories and the human resources associated.

4. Overview and Policy Issues

4.1 Planned capabilities in Cell and Tissue Biology at ALBA

The Structural Cell and Tissue Biology (SCTB) section envisions to offer to the life sciences community a set of instruments and services to solve societal health challenges in a multi-scale, multi-technique approach, as described in the mission. Some techniques such as cryo-SXT and μFTIR will undergo an upgrade in instrumentation and services, while others such as hard X-ray nanoprobe for nanoXRF and phase contrast based techniques, sub-μm CT and sub-μm spectroscopy are to be newly built.

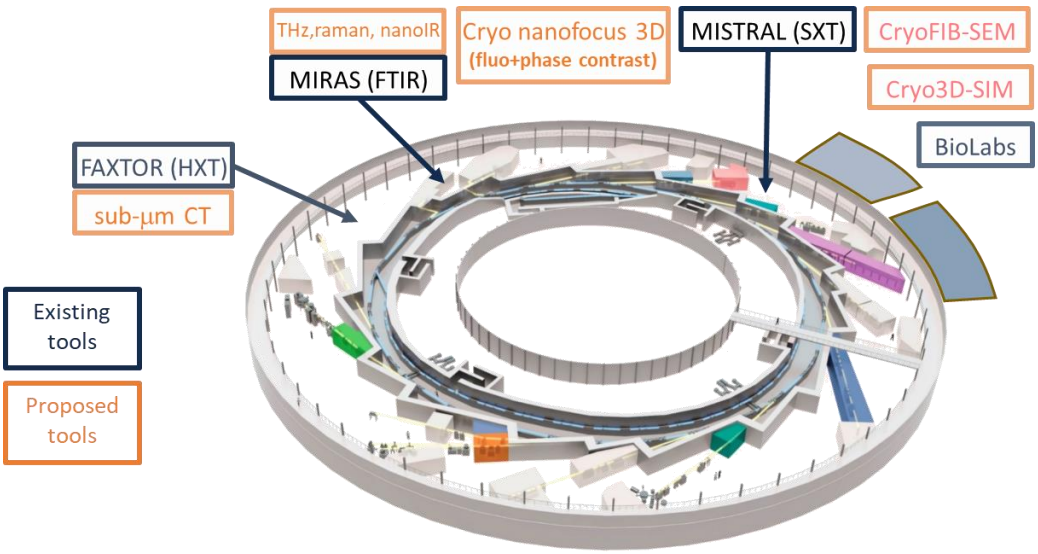
To achieve the mission of the SCTB section the following tools are proposed (figure 4.1):

1. **Upgrade of the cryo-TXM beamline (MISTRAL)**

   The upgrade of MISTRAL focuses on instrumentation, computing methods and correlative microscopies. A complete refurbishment of the beamline to reach the limits of the technique is required, including end station and optics. Development of computing methods are key to increase quality and throughput of MISTRAL and to access non-expert users. Methods to be developed are fiducialess and automated data alignment, cone beam tomography and partially coherent image formation models. Correlative microscopies (in particular Cryo-3DSIM) need to be included in the user program of the beamline to keep competitivity and link with a wider community on bioimaging. This CLXT method is to be upgraded to reach sub-100nm super-resolution in cryo to enable new methods towards multimodal approaches

2. **Upgrade of the µFTIR beamline (MIRAS) with a dedicated station on biology and new capabilities**

   New live-cells setups, such as tissue-on-chip and microfluidic devices, and the availability of new techniques (Raman, mass spec) are essential in the µFTIR user program. In parallel, the extension of the beamline towards THz range, also requested by the materials science program, will enable the access to additional secondary structure signatures. Dedicated life-science stations are instrumental to implement these upgrades and increase productivity. Finally, feasibility of the nano-FTIR is also to be studied in the program. The optimal photon source (laser, beamline) for every technique and frequency range is to be studied



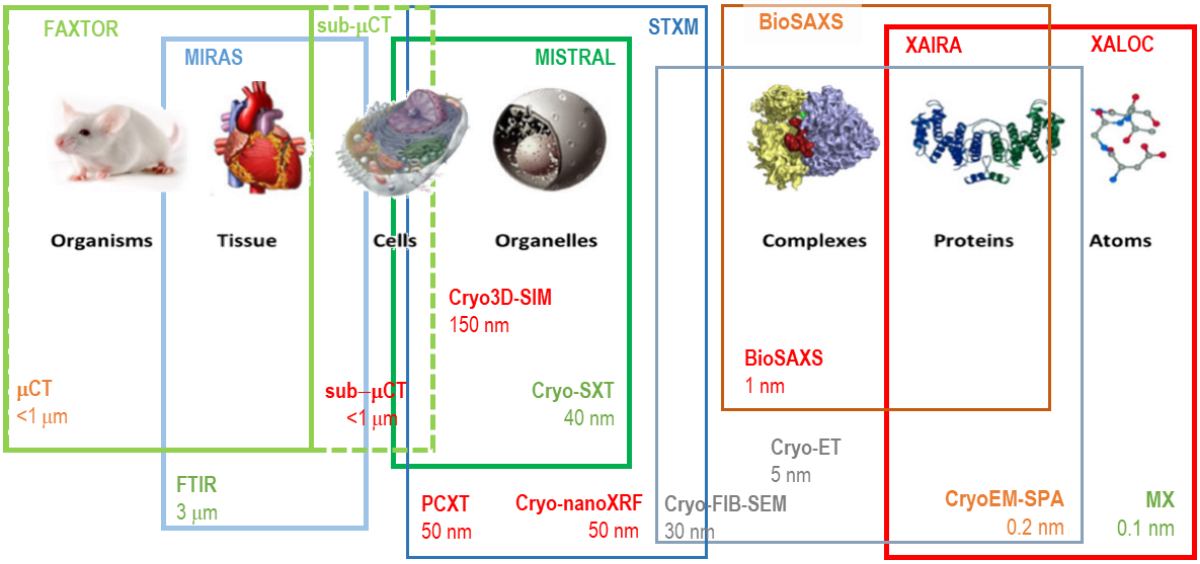


Figure 4.1. *(top) Existing and proposed tools in Structural Cell and Tissue Biology at ALBA. (bottom) Existing and in proposed tools in life sciences at ALBA distributed according to the studied organization level. Color code of the techniques: green-operating, orange-in construction, red-proposed, grey-studied..*

3. A new cryo-nanoprobe (cryo-nanoXRF and nanoPCXT) undulator beamline

A new hard X-ray nanofocus scanning transmission x-ray microscopy (STXM) beamline dedicated to life sciences is proposed to map and image biological material in cryo conditions at high resolution (~50 nm beam size). Nano-XRF in 2D and 3D maps the quantification of trace endo- and exogenous elements at cellular and sub-cellular levels with ppm sensitivity. The station will also allow phase contrast-based imaging of near-to-native cells in 2D and tomography (nanoPCXT). Special attention is to be paid to beamline stability and built-in cryogenic sample environment to reach the limit of the techniques.

The beamline will fully profit from the improved performance of ALBA-II in terms of beam size, stability and coherence. The covered techniques are key in the integrative approach in life sciences and will address a wide community encompassing cell biology, metallobiology, drug delivery, nanomedicine, applied nanomaterials, new therapeutic agents, genetic recessive diseases, viral infections and biocatalysis, among others.

4. A new sub-µm cryo CT (sub-µPCXT with sub-µXANES) station

Filling the sub-micron spatial resolution gap in bioimaging, left between FaXToR beamline and the cryo-nanoprobe and MISTRAL beamlines, is prioritary to cover structural studies from sub-cellular level up to the tissue. In addition, chemical information at organelle level, such as oxidation states of relevant metals or lipids peroxidation, should be provided to complement structural and functional informations. These two domains can be covered by a dedicated end-station with built-cryogenic sample conditioning either on a new beamline or, alternatively, on a new branch of the FaXToR beamline. The new instrument and techniques therein will benefit from the enhanced performance of

the ALBA-II upgrade and will significantly increase multimodal capabilities by linking to to MIRAS, MISTRAL, and cryo-nanoprobe beamlines.

5. **Correlative microscopy (CLXT) and advanced sample preparation methods (cryoFIB-SEM)**
   Non-synchrotron-based imaging techniques and advanced sample preparation methods are essential to the development of the cell and tissue program at ALBA. Cryo-3DSIM is set as the preferred correlative microscopy to link the visible light techniques from external research groups and companies with the bioimaging techniques at ALBA. Concerning the advanced sample preparation methods, cryoFIB-SEM is the best technique to prepare and characterize samples to be studied using the bioimaging beamlines at ALBA. Both instruments require to be included in a biolab certified at least BSL2 to develop the scientific program towards relevant societal challenges.
   Interestingly, the cryoFIB-SEM is also a powerful imaging tool for structural studies on cryo-sectioned cells and tissues at high resolution. In spite of its long acquisition time, the instrument can develop towards imaging, in collaboration with the relevant Spanish Microscopy National Center CNB, as the scientific and industrial community grows.

6. **Electron-based bioimaging techniques (Cryo-ET and cryoFIB-SEM)**
   Cryo-ET is a fundamental tool in bioimaging, able to visualize organelles and cell ultrastructure in situ, including mixed populations, due to its high resolution and FoV. Importantly, cryo-ET bridges the gap between structural molecular and cellular biology. Cryo-FIB-SEM complements with Cryo-ET as it can be used as a microscopy technique or as instrument to prepare the lamellae of cells to be user in both instruments.
   Cryo-FIB-SEM and cryo-ET instruments are often associated with synchrotron facilities for technical, operational and scientific reasons. The equipment required, namely a sample preparation lab, screening electron microscopes and correlative cryo super-resolution microscopies (CLEM) for best results, is common to that required by the SR-based techniques. The scientific projects often benefit from synergistic effects between electron- and SR-based techniques. Finally, the facility approach established for synchrotron experiments is beneficial for the productivity of the microscopies.
   Still, the above considerations need to be accomodated in the Spanish context, where the Centro Nacional de Biología (CNB-CSIC) in Madrid has established a potent national electron microscopy center. Moreover the Basque Resource for Electron Microscopy (Fundación Biofísica Bizkaia, UPV/EHU and CSIC consortium) will start operation soon. In this situation, as a first phase, a close partnership between ALBA and CNB is proposed to take full advantage of the synergies between electron and SR techniques. Once the scientific community widens and the ALBA-II program develops, notably with cryo-EM-SPA, cryo-FIB-SEM for sample preparation and upgraded beamlines, the pressure for on-site electron-based techniques will presumably increase. In this scenario, it is envisaged to enlarge the user program instruments to include cryo-ET and cryo-FIB-SEM, always in collaboration whin CNB partners.

The capabilities of the upgraded and newly built tools are summarized in Figure 4.2, together with a coarse schedule of the implementation of these tools.

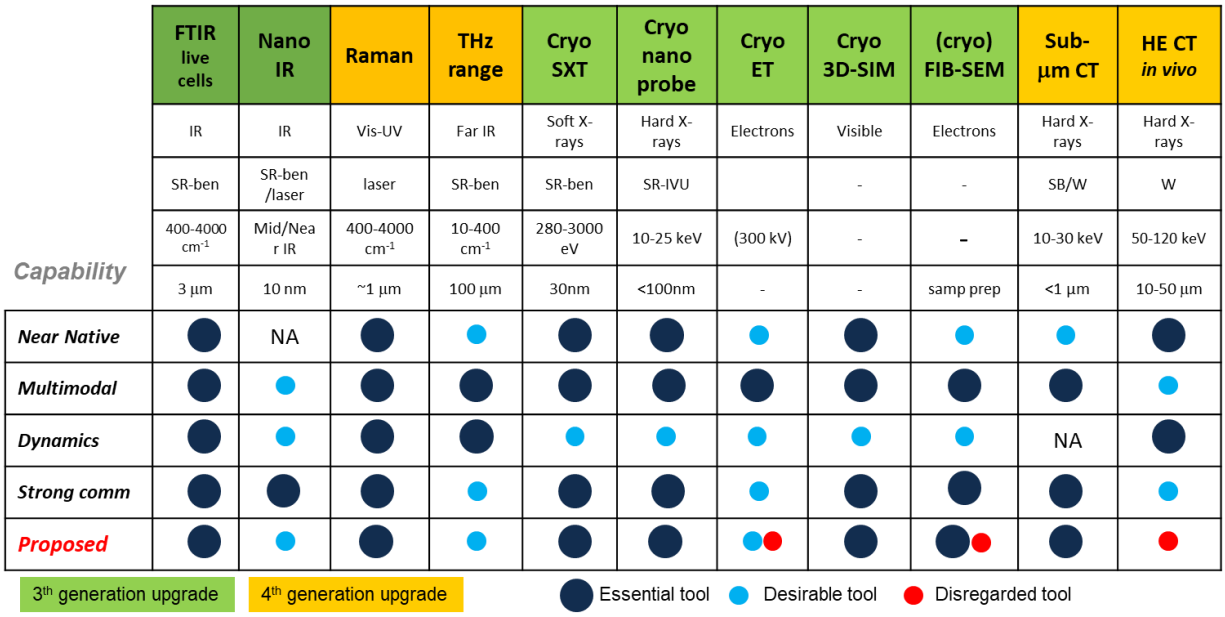


Figure 4.2. *Prioritization of the proposed tools for the Structural Cell and Tissue Biology program at ALBA. A coarse estimation of the implementation schedule, before (green) and after (orange) ALBA dark period is given.*

4.2 Strategic partnerships

ALBA has practically no experiences in implementing strategic partnerships, even if a policy for partner beamlines was approved already in 2014. However, the scope of the required changes and investments for providing the Spanish community with tool sets, appropriate to solve the societal challenges as expected from our funding agencies, is pushing a new strategy of teaming up with partners. It is important to stress, that the partnerships not only provide additional welcome resources, but also provide essential and hard to come by know-how, and that they open a new often specialized research network which helps to disseminate successful methodologies, creates the "golden standards" for the field and even more importantly, helps in prioritizing the individual milestones for defining a realistic roadmap within a resource limited environment.

As in any partnership, it is essential to understand the needs, structure, roles and responsibilities of each partner. In the case of research institutes and research infrastructures, there is a large overlap but there are also a few essential differences: I.) Goals; whereas institutes are focused to develop and execute research programs to tackle specific scientific challenges, the infrastructure has to provide services for the full national and international community for a diverse set of cases. II.) Resources; whereas institutes are typically project-oriented and therefore limited to long-term commitment, facilities are less flexible in

short term investments but are more focused on long term commitments. This creates for both partners different needs which have to be respected within the partnership agreement.

Accordingly, the advantages, an institute typically wants to get out of the partnership, are: I.) services which correlate with long-term commitment like engineering or access time at instruments. II.) benefitting from the large network of the facility for dissemination. At the same time, the facility benefits by the know-how provided by the institute to develop new or complex services often required by the community to address grand challenges. Both, institute and infrastructure benefit from the increased political and research network.

Specifically, ALBA has translated these in the following requirements/criteria for strategic partnerships: I.) Any developed service has to be available to the user community. II.) Available *in-house* time will depend on investment. III.) Any granted instrument time to third parties has to be peer reviewed. IV.) Any suggested partnership selection is performed by ALBA management under guidance of SAC.

Currently there are three different partnerships with moderate scope developed, being one of these, the Advanced Microscope Platform for cryo-EM and TEM, related to Life Sciences. This platform includes a 200 kV full-equipped Glacios microscope for cryoEM-Single Particle Analysis open to public user operation, starting second half of 2022.

Another project of much larger scale is currently in the preproposal phase. Called ALBA Science, Technology and Innovation Park (ASTIP), the center will be an interdisciplinary hub for complex materials and biological systems that blends a unique combination of imaging and characterization tools, big-data, material growth and device fabrication facilities. ASTIP revolves around ALBA II, and includes three new collaborative centers: The *Complex Materials and Technologies Center* (COMTEC), the *Advanced Multiscale Bio Imaging Center* (AMBIC), and the *Innovation Hub* (SYNDUSTRY). The *Universitat Autònoma de Barcelona*, several leading research institutions, and the *Eurecat* innovation center participate in ASTIP, which will include a service complex with an auditorium and a guest house.

AMBIC will build on the expertise and infrastructure of its partners and the X-ray and cryo-electron microscopy imaging capabilities of ALBA II, to expand the research to a multiscale and holistic approach that would otherwise not be possible. The center would include sample preparation areas, imaging and bio-computing facilities connected to the synchrotron beamlines. A biosafety level 3 (BSL-3) laboratory is under study to empower clinical researchers to provide a fast response to health threats and crises.

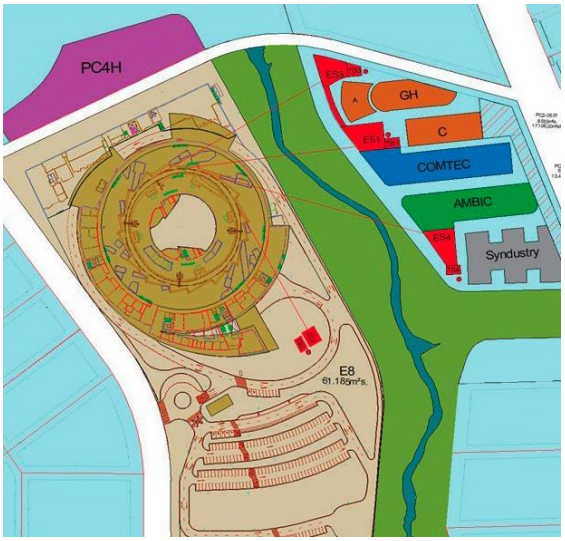


Fig. 4.3. *ASTIP layout, including ALBA Synchrotron*

Syllabus

CLEM –Correlative Light Electron Microscopy
cryo-CLXM – cryo Correlative Light X-ray Microscopy
cryo-CLXT – cryo Correlative Light X-ray Tomography
cryoEM-SPA – cryo-Electron Microscopy – Single Particle Analysis
cryo-ET – cryo-Electron Tomography
cryo-FIB-SEM – cryo-Focused Ion Beam - Scanning Electron microscope
cryo-SXT – cryo-Soft X-ray Tomography
cryo-STM – cryo-Soft Transmission Microscope (i.e. the instrument to perform cryo-SXT)
FoV – Field of view
MX – Macromolecular Crystallography
μCT – Computed microTomography
μFTIR – micro Fourier Transform InfraRed
(nano)μXANES – (nano)micro X-ray Absorption Near Edge Structure
(nano)μXRF – (nano)micro X-ray Fluorescence
MRI –Magnetic Resonance Imaging
NMR – Nuclear Magnetic Resonance
SCTB – Structural Cell and Tissue Biology
STXM – Scanning Transmission X-ray Microscopy