CEM Study Trip to the Human Technopole

Rho Fiera, Milano, Italy. 1st and 2nd February, 2024

Human Technopole has been designed as an innovation center on the area of the 2015 world exposition pavilions in Rho Fiera, Milan. It is a large-scale research infrastructure launched by the Italian government in 2018. From 2024, Human Technopole is opening the first national facilities that provide services and training for biomedical research with cutting-edge technology.

On February 1st, 2024, 18 students and coordinators of the Cutting Edge Microscopy (CEM) PhD program at the University of Bern visited Human Technopole. We extend our gratitude to Fabrizio Martino, Licensing Officer at the Human Technopole, for warmly welcoming us and for organizing this visit, spending two days with us to introduce the facilities and ongoing research from the source. The schedule was divided into two days: on the first day, we visited the Cryo-EM facility and on the second day continued with light microscopes, spatial omics unit, electrophysiology, and demonstrations of optical tweezers.

***Fig1**: Expo 2015 to Human Technopole source:https: //humantechnopole.it/en/about-us/. CEM PhD program group at Human Technopole, Milan*

Cryo-EM facility

At the Human Technopole's National Facility for Structural Biology, headed by Paolo Swuec, we were briefed on the intricate processes of structural characterizations that span multiple scales. Additionally, we received a comprehensive overview of how to combine the different sample preparation and imaging techniques in electron microscopy (Fig 2). The Facility specializes in tailoring electron microscopy methodologies to fit the scientific question at hand, factoring in the specific requirements of sample preparation. During our tour, the team illustrated how different samples, ranging from nanostructures like viruses to larger tissues, are prepared using techniques such as plunge freezing for vitreous ice, high-pressure freezing, and chemical fixation. The subsequent sample thinning is achieved through methods like focused ion beam milling or ultramicrotomy with a diamond knife, depending on the desired resolution and sample type.

For data acquisition, the facility boasts an impressive array of electron microscopes:

- The Thermo Scientific Titan Krios G4i 300kV TEM, with a Falcon 4i electron detector, Selectris X energy filter, CETA 16M camera, and Volta phase-plate, is a powerhouse for high-resolution imaging.
- The Spectra 300kV STEM, equipped with an enhanced CETA 16M camera, is dedicated to detailed electron tomography workflows.
- The Glacios 200kV TEM, also fitted with a Falcon 4i, Selectris X, and Volta phase-plate, provides versatility for various imaging needs.
- The Talos L120C 120kV TEM, complemented by a CETA 16M camera and an ELSA cryo-holder by Gatan, allows for both room temperature and cryogenic imaging.
- The Stellaris 5 confocal microscope and Thunder widefield microscope by Leica, both equipped with a cryo-stage, facilitate cryo-CLEM experiments that combine fluorescence microscopy with cryo-electron microscopy.

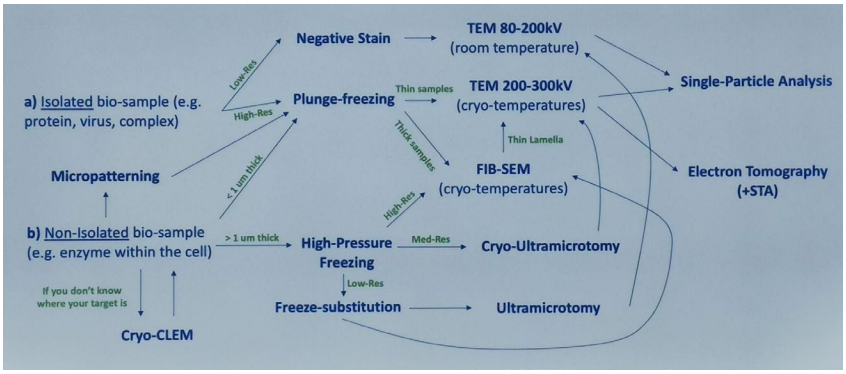


***Fig2.** The final slide of Paolo Swuec's presentation, showing currently applied sample preparation and imaging techniques in electron microscopy.*

Supporting these core instruments is a range of ancillary equipment critical for sample preparation, including plunge freezing devices, glow dischargers, plasma cleaners, carbon coaters, and high-pressure freezing and freeze substitution systems.
The tour of the facility provided a practical understanding of the workflow and capabilities of modern structural biology, showcasing Human Technopole's commitment to driving forward the understanding of biological structures at the most intricate levels.

Fig3. ***Two electron microscopes at Human Technopole.*** *Left: Spectra 300kV STEM, Right: Titan Krios G4i 300kV TEM*

Light microscopy facility

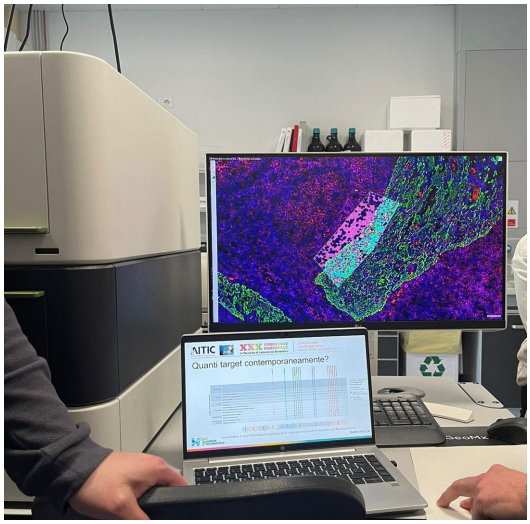
Part of our visit was a guided tour of the light microscopy facility. Our host, Nicola Maghelli, the senior manager of the facility, seamlessly transitioned from general overviews to technical details and provided valuable insights through answering our questions. The Human Technopole features an impressive collection of cutting-edge microscopes. Highlights included a Zeiss LSM980 with Airy scan system, a Spinning Disc Confocal Microscope, offering enhanced imaging speed for cellular dynamics, and a Multiphoton Microscope (***Fig4***) facilitating high-resolution imaging with minimal phototoxicity. Moreover, there is a Structural Illumination Microscope with the ability to surpass diffraction limits for high-resolution imaging (***Fig4***). In conclusion, our visit to the Light Microscopy Facility at the Human Technopole was an enriching experience and provided an insight into the light microscopy setups of a larger national imaging center.

Fig4. Light microscopes at Human Technopole. *Left: Confocal microscope LSM980, middle: Two-photon microscope system, right: Structural Illumination Microscope.*

Spatial omics unit

We visited the histology and spatial omics lab guided by Dario Ricca. We first discussed the requirements for sample preparation including different types of fixations and embedding of the tissue of interest. One of the new fixative agents they are testing is glyoxal acid-free (GAF), which is a formalin alternative without the health hazards that formalin has. Next, we discussed their novel spatial omics techniques and equipment. Spatial omics is based on combining omics (using DNA/RNA) information in reference to their native localization within the tissue. The limitation of this technique was the limited spatial resolution allowing to gather transcriptomic data with a resolution of about 55 µm. However, novel technologies, like 10 nm diameter DNA beads coating slides, reach significantly better resolutions down to 10 nm, allowing for single cell resolution.

Omics are divided into 2 categories: a biased approach in which there is no specificity to the splice version of the mRNA but knowledge about the general gene expression status and an unbiased approach, in which the sample is destroyed, and one can see the different mRNA splices for the gene of interest.



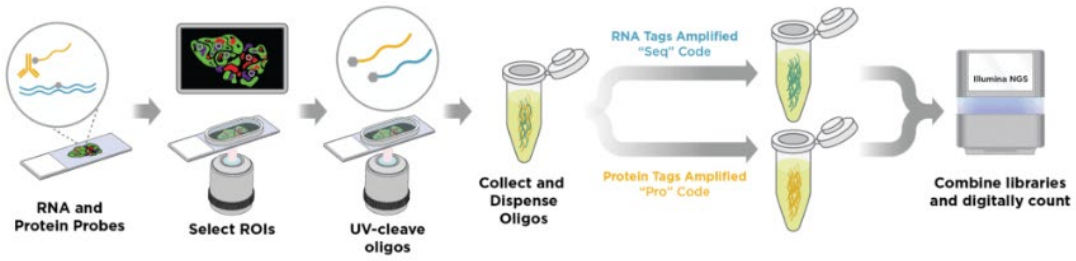


Fig5. *Above: Image from demonstration of work flow. Machine used: https://nanostring.com/products/ geomx-digital-spatial-profiler/geomx-dsp-overview/. Below: GeoMx DSP Workflow: Tissue sections are stained for simultaneous RNA and protein detection, imaged for ROI selection, followed by UV light exposure to release barcodes for NGS analysis on an Illumina system.* ***Adopted form nanostring.*** https://nanostring.com/research-focus/spatial-multiomics/

As an example of spatial omics adopted on-site, we were shown a tissue section of interest, which was incubated and hybridized with a cocktail of more than 2000 probes, as a high-end ISH technique. Those probes are tagged to unique barcodes that can be photo cleaved from the hybridized probe upon excitation with certain lasers, particularly UV light, hence why for nuclear staining they used Syto13-AF488 and not DAPI. They combined the ISH with a staining of interest (CD45 for immune cells within a tumor sample) to identify and define the regions of interest within the tissue. This way, they can direct the laser excitation and barcode release to the region of interest based on the immunofluorescence staining. With this technique, they obtain transcriptomic information for the spatial regions of interest within a tissue section. Finally, we discussed 2 types of transcriptome atlas used with GeoMx®: the human whole transcriptome atlas (WTA), in which 18 000 protein coding genes are assessed and the cancer transcriptome atlas (CTA) that involves 1800 RNA targets specific for certain cancer behavior pathways.

Electrophysiology

During our visit to the Electrophysiology Scientific Service at Human Technopole, led by Dr. Diletta Pozzi (Manager – Electrophysiology Scientific Service, National Facility for Light Imaging) we were introduced to their focus on utilizing iPSC cells and brain organoids for research. A significant aspect of our visit was the introduction to the BioCAM DupleX HD MEA system. This high-definition microelectrode array (MEA) technology allows for precise recording of neuronal activity at unparalleled resolution. The BioCAM DupleX is designed to facilitate detailed analysis of electrophysiological patterns across a wide area, making it an invaluable tool for investigating the complex interactions within neuronal networks. Its application in the field enhances the capability to study neuronal behavior and network function with high accuracy, offering insights into the mechanisms underlying neurobiological processes.

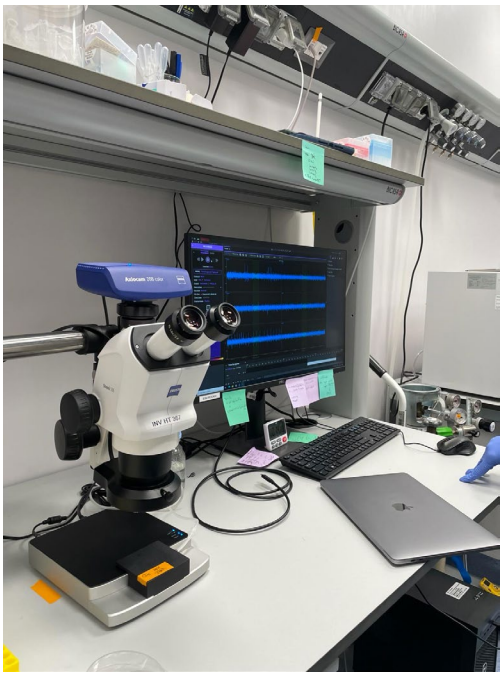


Fig6. BioCam Duplex Setup, *4096 electrodes for actuation in 2D and 3D in vitro brain models.* https://www.3brain.com/products/single-well/biocam-duplex

Optical tweezers

Single molecule methods are gaining more attention from the scientific community as they provide intrinsic information about the dynamic processes and forces involved in real-time. We had the opportunity to witness a live experiment with optical tweezers. Joanna Andrecka, from the Single Molecule Support Unit at Human Technopole, graciously conducted an experiment specifically for this demonstration. Optical traps, or optical tweezers, utilize the ability of a tightly focused beam of light to hold microscopic particles in three-dimensional space. Compared to other single molecule techniques such as atomic force microscopy and magnetic tweezers, optical tweezers offer excellent force, spatial, and temporal resolution. A combined approach of optical tweezers and fluorescence microscopy can be exploited to study the mechanical dynamics of proteins, RNA, or DNA.

In the demonstration, we used optical tweezers to trap DNA strands between polystyrene beads coated with streptavidin. The DNA was primed with biotin beforehand and allowed to flow in a laminar manner using microfluidic channels. As the trapped DNA was stretched between the beads, it continued to elongate until reaching a certain threshold of force, at which breaks in the fluorescence emitted from the molecule binding to double-stranded DNA were observed. This indicated that it could be breaking due to stretching. However, we observed that the signal from molecules binding to single-stranded DNA remained intact. This suggests that instead of breaking, beyond a certain force threshold, the DNA chose to unfold in a specific pattern, allowing for further stretching. This is called as an overstretching transition

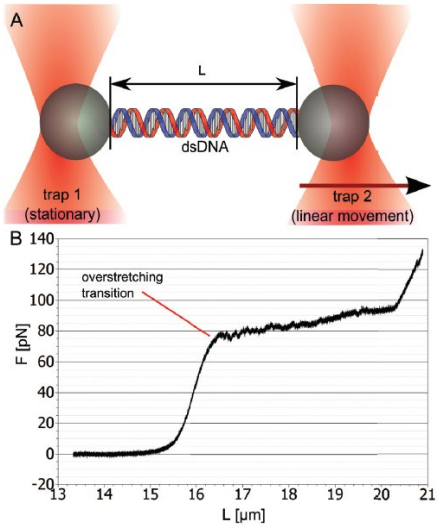


Fig7. Optical Tweezer Setup: *Above: Experimental setup for optical tweezer mediated stretch experiment on DNA. Below: Conceptual Image from [https://www.americanlaboratory.com/914-Application-Notes /180081-Optical-Tweezers-for-Single-Cell-Multicellular-Investigations-in-the-Life-Sciences](https://www.americanlaboratory.com/914-Application-Notes/180081-Optical-Tweezers-for-Single-Cell-Multicellular-Investigations-in-the-Life-Sciences)*

Acknowledgements

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This report was written in February 2024 by the students who took part in this study trip.