Atelier INSERM N° 273 : Technical workshop

IVETh

Extracellular Vesicle and Nanovectors Production, Engineering, and Characterization for Therapy and Diagnosis

FRANCE 2030

Industrial Integrator
Biotherapy-Bioproduction

Advances in Therapeutic Applications of Extracellular Vesicles

2 - 6 October 2023
IVETh Industrial Integrator, Université Paris Cité
45 rue des Saints Pères 75006 Paris

FRANCE 2030 · anr · Région îledeFrance · bpifrance · INVESTIR L'AVENIR · ERGANEO

MSC · Université Paris Cité · cnrs · Inserm

INDUSTRIAL INTEGRATOR
Extracellular Vesicles and Nanovectors Production, Engineering, and Characterization for Therapy and Diagnosis

- IVETH is the first expertise and technological innovation HUB for **bioproduction**, **engineering**, and **characterization** of **extracellular vesicles** (EVs) and nanovectors for early diagnosis and personalized therapy development.

The different sites (Université Paris Cité):

MSC-Med, Campus Saint Germain – Florence GAZEAU
https://msc-med.u-paris.fr/
IPNP, Saint-Anne – David TARESTE
https://ipnp.paris5.inserm.fr/research/core-facilities/3-plateforme-biochimie-et-biophysique
CRI, Bichat – Pierre-Emmanuel RAUTOU
http://www.rautoulab.com/core-facility.html
PARCC, HEGP – Chantal BOULANGER
http://parcc.inserm.fr/research-teams/team/boulanger/

Our partners**:**

Institut Curie – Clotilde THERY
https://curie.fr/plateforme/curiecoretech-vesicules-extracellulaires
CTSA – Sébastien BANZET
https://evdg.sante.defense.gouv.fr/les-chaires-denseignement/recherche-appliquee-aux-armees/
Hôpital Paul Brousse – Philippe MAUDUIT

- Our ambition is to promote the industrialization of disruptive processes in healthcare through innovation and the creation of businesses by providing technological expertise from top research laboratories.

IVETH INNOVATION HUB / INDUSTRIAL INTEGRATOR

- Connect scientific and clinical project leaders with innovators within a large ecosystem of research labs, hospitals and companies.
- Accelerate the technological maturation, from research lab to patient bedside.

Academics | **Clinicians** | **Industrials**



IVETh

STATE-OF-THE-ART EQUIPMENT FOR PRODUCTION, ENGINEERING AND CHARACTERIZATION

MULTIDISCIPLINARY TEAM GATHERING PHYSICISTS, CHEMISTS, BIOLOGISTS, DATA SCIENTIST, PHARMACISTS AND CLINICIANS DEDICATED TO NANOMEDICINE

ARTIFICIAL INTELLIGENCE FOR ADVANCED PROCESSING OF MULTIMODAL AND MULTISCALE ANALYSES

Bio-production, Bio-engineering, and Purification / Isolation

Multimodale, Multiscale Characterization

Testing, Visualization

Multimodale Machine Learning Analyses

In Process Quality Control

THE TEAM

The IVETh team is composed of passionate researchers and engineers who work together on a daily basis, **sharing their knowledge and expertise**. They include biologists, chemists, physicists, data scientists, medical doctors, and pharmacists. It is a team of **scientific excellence** dedicated to promoting research projects on extracellular vesicles and nanovectors and **accelerating their technological maturation**.

Executive board

Florence GAZEAU, *PhD*

Co-founder

Amanda SILVA BRUN *PharmD, PhD*

Co-founder

Kelly AUBERTIN, *PhD*

Chief Scientific Officer

Sarah RAZAFINDRAKOTO

Chief Technical Officer

Fahima DI FEDERICO

Head of the Business development

SESSION 1 :

SINGLE VESICLE IMAGING TECHNIQUES : Nanoscopy of single vesicles (Abbelight), interaction with cells, cargo delivery, EV morphology, AFM

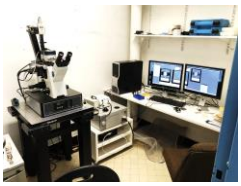
SAFe 360 (Abbelight)

Nanoscopy of EV: The aim of his workshop is to demonstrate the potential in EVs characterization using SMLM on Abbelight SAFe 360 platform. The participants will discover how to capture and characterized EVs with this system, along with a session focused on single EV analysis with Super resolution. We will go through all the steps from Sample Preparation to Data Analysis, allowing fellow researchers to deep dive into the whole process.

Stephanie Mangenot, Professor in Physics at the Université Paris Cité, Researcher at MSCmed. My goal is to use *in vitro* membrane models to understand the biophysics of cell traffic event. One of my projects is focused on understanding the uptake of EV by the host cell.

Daniele D'Arrigo, Postdoctoral Researcher at MSCmed and Abbelight (a super-resolution microscopy company in Cachan) thanks to the French National Centre for Scientific Research (CNRS – plan de relance). I am working on optimizing Super Resolution imaging to be applied specifically to EVs. The aim of my project is to use Abbelight's nanoscopy system to develop an effective EV characterization platform, by improving the imaging, as well as the multiparametric and quantitative analysis of EVs**.**

Nicolas Kuszla, ESPCI engineer, PhD student at MSCmed, trained in physics and biophysics. My thesis, supervised by Florence Gazeau and co-supervised by Laura Fouassier at Hôpital Saint Antoine, is focused on the role of extracellular vesicles in the diagnosis of cholangiocarcinoma and its resistance to treatment.



MFP 3D - Asylum

The AFM (Atomic Force Microscope): is a technique of local probe microscopy that reveals the topography of a sample with a practical lateral resolution on the order of a few tens of nanometers and a much smaller vertical resolution. The platform's AFM is coupled with an optical microscope that allows precise visualization of the probe's location (a tip attached to a cantilever). The AFM also enables the study of the mechanical properties of samples.

Jean-Marc Di Meglio, Professor in Physics at the Université Paris Cité, Researcher at MSCmed. He is interested in soap films, bubbles, foams, granular matter, polymer physics, colloids and vesicles (experimental soft matter). He now focuses his research on biomechanics (study of the locomotion of nematode worms versus age and environment, micromanipulation of biopolymers using AFM, development of frugal physics instrumentation, in particular for the behavioral analysis of mice and worms). 3D

SESSION 2 :

EV FRACTIONNATION AND CHARACTERIZATION FROM CELL SECRETOME BIOFLUIDS, BIOPRODUCTION OR ENGINEERED EVS : introduction to Tangeantial Flow Filtration (asymetric flow field flow fractionation (AF 4 Wyatt) coupled to MALS UV Vis RI fluo detection Characterization of size and concentration (Nanoparticle tracking analysis Nanosight Videodrop delivery)

Nanosight NS300 + Autosampler (Malvern Panalytical)

Tangential Flow Filtration (TFF): is an isolation and concentration technique based on a semi-conservative filtration. During the workshop, you will see how the EVs are isolated from the production media to the storage buffer. You will also learn to characterize the different time point of the TFF with NTA. Nanoparticle Tracking Analysis (NTA) enables physical characterization of the size and concentration of a nano-object suspension, including EVs.

Sarah Razafindrakoto, Biologist, Engineer at MSCmed and Chief Technical officer of IVETh. I did a Master degree in Integrative physiopathology, working on degradation of magnetic nanoparticles in mouse tissues and pancreatic tumor tissues. My projects are focused in the cell culture, as well as the production and the isolation of extracellular vesicles. I am also developing methods for EV characterization.

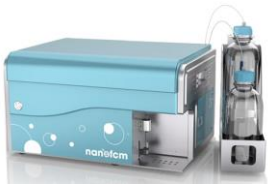
Eclipse + Dawn + Optilab + Neon (Wyatt)

AF4 fractionation: AF4 is an analytical technology which uses two orthogonal flows to perform a size based separation of nanoparticles and macromolecules. During this workshop you will see how to set up an AF4 experiment in order to separate a complex sample into its constitutive subpopulations. You will be taught the physical principles used to achieve this separation and the method to measure the size of the particles, their fluorescence, their absorbance and their concentration. You will also learn how to collect the different subpopulations to perform following characterizations (NTA will be done here as an example) and how to properly analyze and interprete the data.

Sylvain CAM, ESPCI & EPFL Engineer - 2nd year PhD student working on implementing innovative characterization of EV bioproduction and biofluids focusing by AF4. I am also focused on the encapsulation of drugs or RNA into EVs using an hydrodynamic stimulation on the producer cells.

SESSION 3 :

EV PHENOTYPING AND BIOLOGICAL IDENTITY : Introduction to Nanoparticle flow cytometry NanoFCM, Exoview platform



Flow nanoanalyzer (NanoFCM)

Characterization of surface markers on EVs using NanoFCM : NanoFCM (Nanoparticle Flow Cytometry) is a flow cytometry-based technique that allows the detection and analysis of nanoparticles, including EVs. During the workshop, you will learn how the method for analyzing particles works to measure the concentration and size of particles and how to operate and calibrate the equipment. You will also learn how to stain and analyze your samples using antibodies to characterize EVs on their surface markers.

André CRONEMBERGER ANDRADE, Postdoctoral researcher at MSCmed. I studied immunomodulatory activity of parasitic infected macrophages derived EVs during my master in Biotechnology at FIOCRUZ. I also investigated the role of parasitic EVs during infection of Chagas disease during my PhD in Biology at the Federal University of São Paulo. I worked at the Experimental Cell Therapy Laboratory at PMU in Salzburg with angiogenic potential EVs from iPSC. I am currently focused in bioproduction of therapeutic EVs and their phenotypic characterization.

Exoview (Nanoview)

Exoview R100 uses fluorescence imaging and single particle interferometry analysis to provide a complete characterization of EVs. During this workshop, you will learn how to prepare and handle the chips from sample incubation to chip scanning. You will also see how to navigate through the analysis software to extract information regarding size distribution, concentration and tetraspanin distribution.

Léa Jabbour, Biomedical engineer (BME Paris – Biomatériaux), PhD student. My PhD project is focused on the production and the characterization of EVs produced from adipose-derived stem cells for lung and intervertebral disc regeneration.

SESSION 4 :

RAMAN spectroscopy for EV and biofluids biomolecular characterization

DXR3xi (Thermofisher)

Raman spectroscopy is a vibrational technique allowing access to the global molecular fingerprint of a sample. It has the advantage of being label-free and very versatile regarding sample type (FFPE tissue, live or fixed cells, biofluids including blood plasma, EV suspension, etc.). IVETh has two different systems working at two different size scales: Raman confocal microscope, allowing bulk acquisition and Raman tweezers microscope, allowing almost single-EV acquisition. During the workshop, you will see how to prepare EV sample, how to acquire the Raman signature with both systems and how to perform pre-processing of the spectra.

Kelly Aubertin, Physicist, Research Engineer at MSC-Med and Chief scientific officer of IVETh. I have a PhD in biophysics and postdocs in cell mechanics and biomedical optics. I started to work with EVs about 10 years ago. I have specific expertise in EVs in cancer context, Raman spectroscopy and Machine Learning analysis. At IVETh, I am focused on developing multimodal EV and biofluid characterization coupled with Machine Learning methods (clustering and classification) for diagnostic applications and quality control of therapeutic EVs.

Raman Tweezers Microspectroscopy (RTM) of extracellular vesicles (EVs) : this analytical spectroscopic technique combines optical trapping with Raman probing. The main idea consists of utilizing the same laser radiation for optical trapping of single nano-bioparticles of interest in aqueous medium and excitation of Raman scattering from the particle's constituent biomolecules. All major biomolecules (proteins, lipids, nucleic acids, carotenoids, etc.) can be studied both on the surface and within the EVs volume providing global biomolecular content characterization. It can be used for EVs quality-control assessment, and for many other scientific tasks, via the quantitative analysis of EVs biomolecular composition at the single-particle level in physiological conditions. You will learn the basic principles of RTM technique, participate in real scientific RTM experiments unique in the world, and get an idea of acquired Raman data treatment and further biomolecular components analysis.

Sergei Kruglik, Research Engineer at Sorbonne University and partial member of MSCmed. I developed the RTM system for EVs characterization (Nanoscale 2019, vol. 11, pp. 1661-1679). I am expert in Raman spectroscopy with more than 30-years' experience of scientific research in leading laboratories in Japan, The Netherlands, Canada, and France.

SESSION 5 :

INTRODUCTION TO MACHINE LEARNING METHODS for quality control and biomarker discovery

Machine learning methods and workflow : you will learn how to apply machine learning, a branch of artificial intelligence that uses data-driven algorithms to make predictions or decisions without explicit programming, to the problem of discrimination analysis. Discrimination analysis is the process of finding and using features that distinguish different groups or categories from a dataset. For instance, you will learn how to use Raman spectroscopy data to differentiate EVs there were produced by different cell types. Similarly, machine learning can be used to predict if a patient is sick and how their disease will progress (asymptomatic, uncomplicated, severe symptoms, etc.) by using machine learning techniques that can handle large and complex datasets. This will include the processing steps such as features engineering/selection, model training, cross-validation and performance evaluation.

Fabien PICOT, CentraleSupélec Engineer & Polytechnique Montreal PhD eng – Postdoctoral researcher. I am working on EVs characterization for therapeutic and diagnostic applications. My work focused on developping a complete workflow for EVs characterization using the Raman microscopy platform from sample preparation to data analysis.

SESSION 6 :

POTENCY TESTS USING HIGH CONTENT SCREENING MICROSCOPY (CELL INSIGHT CX 7)

Cell Insight CX7LZR (Thermofisher)

High-Content Screening (HCS) and EV potency test: The HCS method relies on highly automated microscopic image acquisition coupled with rapid standardized image analysis. IVETh platform is equipped with a CellInsight CX7 microscopic/software system designed specifically for that task. During the workshop, you will learn how to use this system to perform a potency test of a certain variety of EVs which are supposed to have anti-inflammatory properties.

We will use a 2D culture of human endothelial cells seeded on a multi-well plate. Cells in each well will be either exposed or not to the inflammation activator. They will be also exposed to different concentrations of the EVs of interest. We will label the cells with different fluorescent markers (nuclei, live/dead, etc.) and we will scan the plate with unified acquisition parameters for all wells. In accordance with an HCS principle, we will extract multi-parametric data on a per-cell basis from our images. We will set up the software so that each cell will be individually classified as living, dead, or dying. This will allow us to rapidly estimate the percentage of cells undergoing cell death induced by pro-inflammatory factor and the protective effect of different concentrations of our EVs.

Dmitry Ayollo, Dmitry AYOLLO; Ph.D.; research engineer
Responsible for the CellInsight High Content Screening sub-platform
Specialist in optical microscopy, image processing and analysis, and cell biology

Sponsors providing coffee, lunch and networking cocktail

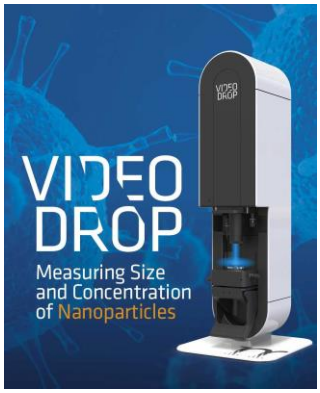
ZetaView developed by Particle Metrix GmbH and distributed by Excilone in France, is a Nanoparticle Tracking Technology (NTA) based device. It is capable to characterize biological nanoparticles, such as extracellular vesicles (EVs), lipid nanoparticles (LNP) or virus.
ZetaView delivers simultaneously information about size, particle concentration and surface charge with just one single measurement.
Depending on the configuration, ZetaView allows fluorescent studies (up to 4 layers and 10 channels), including colocalization (exclusive on NTA technology).

Bertrand DAMART, Sales Manager and application specialist for ZetaView product line.

Thermo Scientific™ DXR3xi Raman Imaging Microscope pushes Raman microscopy to the next level to unlock the power of Raman imaging.
High performance chemical imaging is now accessible to all, accelerating research for new and experienced users alike.
Optimize imaging parameters in real-time to visualize your data faster using the comprehensive Thermo Scientific™ OMNIC™xi imaging software.
With its automatic feature recognition and powerful component identification, the DXR3xi Raman Imaging Microscope will transform the way you approach life science analysis.

Maxime ALEXANDRE, Since 2003, Dr Maxime Alexandre investigated the interaction of light and living systems using mainly vibrational spectroscopy (FTIR and Raman) from single molecule to cells and from seconds down to femtoseconds. He is now a Senior application specialist at Thermo for FTIR, Raman and NIR for the EMEA region.

Sponsors providing coffee, lunch and networking cocktail



Myriade technology: VIDEODROP
Based on the principles evelops an innovative nanoscale imaging of interferometry, VIDEODROP makes it possible to measure the size and concentration of your extracellular vesicles:

- in real time (40s)
- in a single drop (5µL)
- no purification
- in a non-denaturant way without labeling
- on viscous & complex samples
- with very reproducible results and no operator bias

Marie Berger, Pharm.D specialized in biotechnologies and advanced therapies. Marie focused her studies on research and development through different positions in both academic and industrial research in biotechnology. Before joining Myriade, Marie worked for 3 years at LFB Biotechnologies, in analytical development. She joined Myriade to lead the scientific applications of Videodrop.

Abbelight The observation of extracellular vesicles (EVs) is a major challenge in the field of EV characterization, as these entities have nanometric dimensions that are well below the diffraction limit of a classical fluorescence optical microscope. Single molecule localization microscopy (SMLM) comes to the rescue by overcoming the diffraction limit and achieving resolutions of up to 20 nm. Abbelight's microscope uses the principle of direct Stochastic Optical Reconstruction Microscopy (STORM) to determine as example both the size of EVs and the presence of specific markers. Abbelight develops a cutting-edge workflow solution in microscopy for researchers, biotech labs and medical facilities in many areas that allows them to have a complete turnkey solution.

Paulina Nowak, product sales specialist at Abbelight with 11 years of experience in academic research. Paulina obtained her PhD in molecular biosciences at the University of Geneva, Switzerland, where she worked on plasma membrane biophysics using advanced microscopy techniques. She also holds an MSc in Biophysics from the Jagiellonian University in Cracow, Poland, where she specialised in super-resolution microscopy and its application to virology.

IVETh

Industrial Integrator Biotherapy-Bioproduction

FRANCE 2030

for advanced therapies & diagnosis methods

Offers

COLLABORATIONS

We accompany you at each step of your project (from project design to innovation)
Shared IP

SERVICES

We provide solution for production, engineering or characterization of your EVs

TRAINING

We offer personalized training (Technical, Theoretical, Innovation, Regulation)

Université Paris Cité

Campus Saint-Germain

45 rue des Saints-Pères
75006, Paris, FRANCE

Email
iveth@u-paris-sciences.fr

Web site
www.iveth.u-paris.fr

Linkedin
@IVETh