UAT SERVICES PORTFOLIO

1. APPROVAL:

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2. REVIEW RECORD:

REVISION	DATE	DESCRIPTION
1	05/08/2014	Document creation
2	03/09/2015	Document review in order to update UAT services
3	24/01/2017	Document review in order to update UAT services
4	13/06/2019	Document review in order to update UAT services
5	21/10/2019	Document review in order to update UAT services
6	30/11/2020	Document review in order to update UAT services: 1. New services and equipment have been included (mouse genotyping, Thunder multidimensional widefield microscope, Luminex Magpix multiplexing technology) 2. Molecular diagnosis platform: an update of different array types has been included 3. Pictures of every machine have been added to the "Equipment" section of each Platform
7	28/07/2021	Document review in order to update UAT services: 1. New service included: Mycoplasma testing (Genomics Platform) 2. The equipment ABI7900HT, belonging to the Genomics Platform, has definitively become out of service due to an irreparable fault. In consequence, this machine gets excluded from this document. 3. The colour camera Hitachi coupled to the laser microdissector (Microscopy Platform) has definitely become out of service due to an irreparable fault. In consequence, this feature gets excluded from this document.
8	27/06/2022	Document review in order to update UAT services: 1. Comment on Genotyping service: C. bovis detection. 2. New equipment included (sorter Aurora CS and associated workstation; qPCR equipment QuantStudio 6 Pro, as a temporary loan). 3. Red laser from the FacsCalibur cytometer out of service. 4. Cytometry software update.

9	23/12/2022	Document review in order to update UAT services: 1. Description of full RT-qPCR services. 2. New independent service: C. bovis detection by conventional PCR and quantitative PCR. 3. qPCR machines updated (7000SDS and QuantStudio 6 out of service. QuantStudio 5 and QuantStudio 7 Pro incorporated). 4. References to auxiliary, common equipment removed. 5. Reference to available expression arrays.
10	02/11/2023	Document review in order to update UAT services: 1. Generic contact addresses for each platform have been included at the beginning of each section. 2. Update of the Axiom genotyping arrays, including a reference to the new Axiom PangenomiX Array (Applied Biosystems ®) for human genotyping. 3. Update of the cytometry platform-analyzers (FacsCalibur out of service). Incorporation of the Aurora analyser. General introduction to full-spectrum cytometry. 4. Update of available workstations and softwares for data analysis.
11	29/05/2024	Document review in order to update UAT services: 1. Genomics Platform: new equipment and service for single cell multiomics analysis (Rhapsody HT Xpress System), currently available upon temporary assignment. New equipment: Qubit system and assays for fluorimetry. 2. Metabolomics Platform: services that require technical support are temporarily unavailable due to changes in the UAT staff. 3. Cytometry Platform: FacsAria sorter out of service. Cytometry software updated. 4. Microscopy Platform: confocal microscope FV1000: violet diode laser (405 nm) out of service.

12	16/05/2025	Document review in order to update UAT services: 1. Genomics platform: new equipment TapeStation for measuring concentration and QC of nucleic acids. Information about the single cell system Rhapsody has been extended. 2. Metabolomics Platform: technician-assisted services are available again. 3. Cytometry Platform: upgrade of the Aurora analyser with a plate loader. Revision of the information about workstations and software. 4. Microscopy Platform: 2 Nikon inverted fluorescence microscopes transferred from Laboratory coordination. Upgrade of the Thunder microscope software with the "Live mode" option.

3. INTRODUCTION

The High Technology Unit (UAT, from *Unitat d'Alta Tecnologia*) is a preclinical core facility at VHIR that offers researchers access to cutting-edge equipment and expert technical support. It delivers a range of services through its five specialized platforms: Genomics, Cytometry, Molecular Diagnosis, Metabolomics, and Microscopy.

UAT provides comprehensive support throughout the research process, including experimental design, assay development and execution, as well as assistance with data analysis and interpretation.

UAT offers:

- Sample processing using different experimental techniques.
- Technical support and training for self-service usage of a variety of equipment.
- Development of protocols and bioanalytical methods based on mass spectrometry.
- Customized services related to any of the Platforms.
- Advice on experimental design, technical execution and data analysis.
- Technical support in the elaboration of scientific and technical documents (budgets, grant proposals, articles, protocols…).
- Training activities related to different technologies.

UAT is located in VHIR Central Building, floor -1.

4. UAT PLATFORMS AND SERVICE PORTFOLIO DESCRIPTION

GENOMICS PLATFORM

Contact: genomica@vhir.org

This Platform provides essential technologies dedicated to the quantitative and qualitative analysis of the nucleic acids in terms of base sequence, quality and expression levels.

➢ **Services and applications:**

1. **Real-time qPCR:**
   - Relative and absolute quantification studies for transcriptional profiling
   - Analysis of gene copy number of genomic and viral DNA
   - Allelic discrimination studies (SNP detection)
   - Pathogen detection and identification trials
   - High Resolution Melting (HRM) for gene scanning

   Assistance with experimental design, selection of probes and data analysis is also available. Comprehensive services fully tailored to the client's needs, including RT-qPCR design and performance, are available upon request. All necessary steps are integrated into a single circuit to offer an integrated service, including sample processing and analysis (if required, in collaboration with the Biobank and the Statistics and Bioinformatics Unit).

2. **Quantification and quality control of nucleic acids using microfluidic chips:**
   - RNA quality check (RIN, RINe) and quantification
   - DNA quality check (DIN) and quantification
   - QC of fragmented RNA (DV200) and genomic DNA
   - QC of cell-free DNA with qualification based of the calculation of %cfDNA
   - QC of different steps along NGS library generation protocols
   - Automatic separation, sizing and quantification of dsDNA fragments

3. **Fluorimetric quantification of nucleic acids: Picogreen ® for DNA and Ribogreen ® for RNA / Qubit assays for DNA and RNA (*).** Highly specific and sensitive method, useful when:
   - Only a few cells are available for nucleic acid extraction
   - An extremely precise quantification is needed (i.e. for library construction in NGS)

   (*) Kits for protein quantification may be available upon request.

4. **Mouse genotyping.** Customized testing will be adapted to each client's requirements for individual projects. We offer:
   - DNA extraction from different types of samples (tail, ear…).

- PCR: specific PCR conditions will be set up in the UAT for each project, starting from DNA extracted in the UAT or brought by user. Each client will always provide the specific primers.
- Analysis by gel electrophoresis.

A genotyping report will be delivered for each set of analysed samples.

Please contact us if you are interested in setting up your personalized genotyping protocol.

5. **Corynebacterium bovis detection:** contamination by these bacteria is rather common in animal house facilities, so assays for detection by conventional PCR and quantitative PCR have been developed. Theses assays works for both environmental samples and animal swabs, and can complement clinical signs and other tests to confirm the presence and infectivity of this pathogen.
   Other frequent pathogens can be detected using PCR strategies. Users interested in developing specific analysis protocols can contact UAT for further information.

6. **Mycoplasma testing in cell cultures:** test based on PCR, starting form cell-culture supernatants. A report will be delivered, including the agarose gel picture showing the negative and positive controls, as well as the results obtained for each sample. For further instructions about sample collecting conditions, please contact UAT.

6. **Automated DNA sequencing by capillary electrophoresis**, including the following applications:
   - De novo sequencing and resequencing
   - Detection of SNPs, mutations and CNV
   - Confirmation of clone constructs
   - Microsatellite analysis
   - Allele discrimination

   The service is available in different modalities (pre-mixed or separated primers and samples) and formats (tubes, plates). Universal primers are freely provided in some modalities (if interested, contact UAT prior to bring your samples).
   Sequencing is outsourced through an external partner, but UAT is in charge of sample handling and results delivery.

7. **Human cell line authentication service:** identification and detection of cross-contamination of human cell lines based on STR profiling, according to the ANSI/ATCC standards. STR analysis is performed using the kit GenePrint 10 System (Promega) to analyse the following microsatellites: D21S11, TH01, TPOX, vWA, CSF1PO, D16S539, D7S820, D13S317, D5S818 and amelogenin (X,Y). A mouse marker can be added to check cross-contaminations.
   This service is outsourced through an external provider, but UAT is in charge of sample handling and results delivery.

8. **Single cell multiomics analysis:** service provided using equipment in temporary assignment provided by the BD company (it will be available until the end of the contract). The Rhapsody HT Xpress System allows the capture of multimodal information from thousands of single cells in parallel ("Multiomics"), covering: mRNA expression levels, extracellular and intracellular protein expression levels, the immune repertoire for T-cell receptors (TCR) and B-cell receptors (BCR), open chromatin regions landscape (ATAC-Seq). The system utilizes microwell-based cartridges that allow capturing a broad range of single cells, and an imaging device for sample quality control and workflow quality control (including viability and multiplets). The power of multiomics relies on simultaneously measuring several aspects of single cells, using next generation sequencing (NGS) as a single readout. This platform allows sample multiplexing in a same lane or cartridge, in order to maximize its capacity while reducing the cost per sample/cell.

➢ **Equipment:**

✓ Real-time PCR:

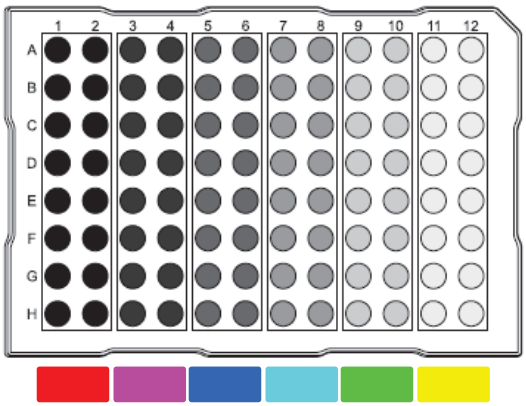
- **QuantStudio 5** (Applied Biosystems-ThermoFisher Scientific): equipped with a fixed 96-Well, VeriFlex™ 0.2-mL Block. Standard or fast operation modes.

- **QuantStudio 7 Pro** (Applied Biosystems, ThermoFisher): 3 interchangeable blocks:

  - Fast, 0.1 ml, 96-wells block, Veriflex technology with 6 independent, programmable Peltier zones
  - Standard, 384-wells block
  - Taqman Low Density Array Cards (TLDA) block

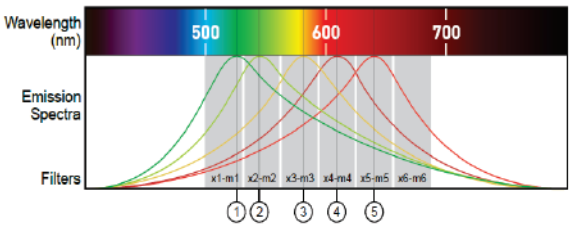
For both QuantStudio instruments:

✓ 96-well blocks have six independent Peltier programmable zones, so multiple experiments can be performed in a same run, for example for qPCR optimization:



✓ Instrument filters and supported dyes: 6 excitation + 6 emission filters, which allow the _ simultaneous detection of 6 different fluorochromes. Multiplexing capabilities: up to 21 wavelength combinations in a single run._Custom dyes excited between 455 – 672 nm and emitting between 505-723 nm can also be used with this instrument.

Peak channel	Color	Filter wavelength (nm)[1]		Pre-calibrated dyes	Example custom dyes
		Excitation	Emission		
x1-m1	Blue	470 ± 15	520 ± 15	FAM™ and SYBR™ Green	SYT09
x2-m2	Green	520 ± 10	558 ± 12	VIC™	HEX™, TET™, and JOE™[2]
x3-m3	Yellow	550 ± 10	587 ± 10	ABY™, NED™, and TAMRA™	Cy® 3
x4-m4	Orange	580 ± 10	623 ± 14	JUN™ and ROX™	Texas Red™
x5-m5	Red	640 ± 10	682 ± 14	Cy® 5 and MUSTANG PURPLE™	LIZ™
x6-m6	Deep-Red	662 ± 10	711 ± 12	None[3]	Cy® 5.5



① x1-m1 — FAM™, SYBR™ Green
② x2-m2 — VIC™
③ x3-m3 — ABY™, NED™, Cy® 3, and TAMRA™
④ x4-m4 — JUN™, ROX™, and Texas Red™
⑤ x5-m5 — Cy® 5 and MUSTANG PURPLE™

- **LightCycler480** (Roche): interchangeable 96 and 384-well blocks.

- **Agilent 2100 Bioanalyzer microfluidics-based platform**:
  - DNA chips: DNA1000, High Sensitivity DNA (check availability first).
  - RNA chips: Nanochip, Picochip.
  - Users can bring their own chips to be processed (i.e. High Sensitivity DNA or small RNA chips).

- **TapeStation 4150 microfluidics-based platform:** individual sample loading to two 8-tube strips.

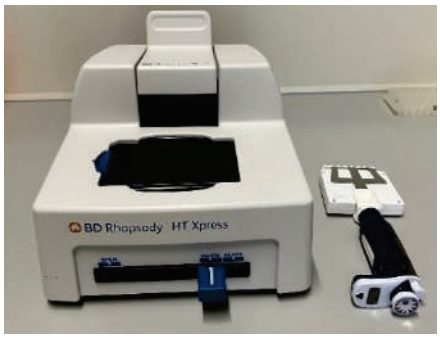
- **Qubit 4.** Qubit instruments are accurate, sensitive, fast and simple fluorimeters that use target-specific fluorescent fluorochromes in Qubit assays to quantify DNA, RNA or proteins. The Qubit 4 Fluorometer is designed to offer an accurate measure of a single sample at a time. *For a higher number of samples, the VarioSkan microplate reader (shared equipment managed by the General Services and Infraestructures Unit) can be used with the PicoGreen and RiboGreen kits.*

- **Rhapsody HT Xpress system:** The BD Rhapsody™ HT Single-Cell Analysis System allows flexible sample processing and cell capture from hundreds to hundreds of thousands of single-cells using a gentle and robust micro well-based cartridge technology and multitier barcoding system. Multiple samples can be processed in a single run when utilizing BD multiplexing antibodies. The captured cellular information is utilized to generate various types of libraries for multiomic analysis: extracellular and

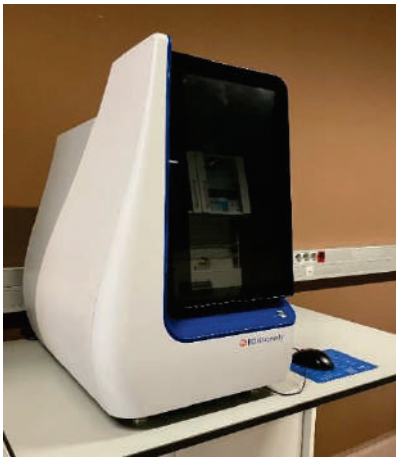
intracellular proteins (AbSeq), targeted gene expression, whole transcriptome, TCR/BCR seq, ATACseq.

The system consists of:

- HT Xpress package: this device allows isolating single cells using a micro-well cartridge divided in 8 lanes, where barcoded, capture beads conjugated to the specific probes for library generation are also loaded. After single-cell, bead-based capture, isolation and bead retrieval, the protocol continues with the library generation steps for further sequencing.



- Scanner: designed to check the viability of the input cell sample and the success of each step of the cartridge workflow.



Experimental design of single-cell experiments involves optimizing cell isolation methods, ensuring sufficient cell numbers for statistical power, and accounting for batch effects and technical variability. Given that single-cell experiments have to be carefully planned in order to optimize conditions, cost and avoid batch-effects, a previous meeting with the UAT staff and the Statistics and Bioinformatics Unit (UEB) is highly recommended. Support on high-dimensional data-analysis and multiomic integration is also provided in collaboration with the UEB.

METABOLOMICS PLATFORM

Contact: metabolomica@vhir.org

UAT offers to the research community a Metabolomics Platform including mass spectrometry and cromatography equipment. These techniques, combined with others as the MRI, allow obtaining quantitative and qualitative data of metabolites and small peptides that are present in biological substrates as serum, plasma, urine, cell tissues or cell cultures.

➢ Services:

- Development of analytical methods for detection and quantification of both endogenous and exogenous analytes in biological substrates (i.e. serum, urine, plasma, cerebrospinal fluid, cell tissues and cultures).
- Methodological support of self-service users.
- Self-service usage of the Platform equipment by trained users.

Assistance with experimental design, implementation of new protocols and help with data analysis and interpretation are also available.

➢ Applications:

The chromatographic and spectroscopic techniques provide with high sensibility compared to MRI (detection limit of femtomols) and allow detecting about 4000 different substances.

Targeted metabolomics is used in biomarker discovery, disease diagnosis, and understanding disease mechanisms. It supports drug development, therapeutic monitoring, and studies on nutrition and metabolic health. Additionally, it plays a key role in personalized medicine and clinical trials, helping translate research into clinical applications and improving patient outcomes.

Some examples of determinations than can be made are:

- Vitamins and phytoestrogens in serum
- Determination of intermediate and final metabolites of different biological pathways
- Pharmacokinetic studies and determination of drugs in biological fluids and tissues
- Amino acids in urine, serum and cerebrospinal fluid
- Lipid compounds in plasma or serum
- Determination of drugs in nanoparticle-release studies
- Drug encapsulation efficiency in nanoparticle formation
- Determination of endogenous metabolites and drugs in Dried Blood Spot (DBS) samples
- Stability studies of biological drugs

Development of custom, specific methods for the detection of one or more analytes (metabolites and peptides) is offered upon request.

➢ Equipment:

- **ACQUITY UPLC coupled to Xevo TQ (Waters)**
  - This Ultra Performance Liquid Chromatographic (UPLC) system equipped with a binary pump and one column oven supports the use of sub-2-µm particle column technology.
  - The Triple Quadrupole mass spectrometer detector is equipped with Electrospray ionization source (ESI). It also allows working with the IntelliStart function, which automatically search for ion transitions, optimal cone voltages, and collision energies.
  - Software packages for equipment operation and analysis: MassLynx and TargetLynx.
  - The system is also equipped with two additional detectors: ultraviolet/visible and fluorescence. *The fluorescence detector has operating limitations, so it is highly recommended consulting UAT before using it.*

MOLECULAR DIAGNOSIS PLATFORM

Contact: genomica@vhir.org

This platform offers to the scientific community a variety of microarray solutions.
Due to the complexity of these techniques, we strongly recommend to contact the UAT staff before requesting any service. VHIR's Statistics and Bioinformatics Unit (UEB) provides assistance for experimental design and data analysis.

➢ Services and applications:

1. **Microarrays:** suitable for high-throughput expression and genotyping analysis. Based on Applied Biosystems ® (former Affymetrix) arrays.
   Applications:
   - Differential analysis of gene expression in model species (human, mouse, rat). Types of arrays:
     - Clariom S ®: for gene-level expression analysis across the transcriptome. Extensive coverage of >20.000 well-annotated genes.
     - Clariom D ®: transcriptome-wide, exon-level expression analysis of >134.000 genes and >540.000 transcripts (data for human arrays). These arrays allow the detection of genes, exons, alternative splicing events and lncRNAs, including low-expression transcripts.
     - Older versions of expression arrays (i.e. Gene Arrays).

     The protocol can be started using RNA from different sample types (whole blood, cell cultures, fresh/fresh-frozen or formalin-fixed, paraffin-embedded (FFPE) tissues). Robust expression profiles can be generated starting from 100 pg-500 ng, in only a few days (depending on the number of samples to process).

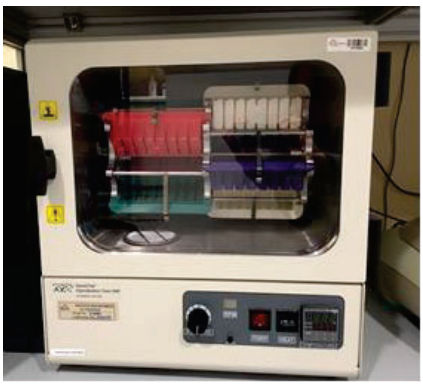
   - miRNA screening: mature and pre-miRNA can be analysed using the miRNA4.1 arrays, starting from 130 pg total RNA. These arrays are complementary to expression studies, since it has been estimated that more than 30% of protein translation of coding genes is regulated by miRNAs.

   - Axiom ® Genotyping arrays: these arrays are available for human studies, and for a great variety of animal and plant species, allowing comprehensive, high-throughput analysis of variants (CNV, SNPs, indels) of large cohorts, using a rapid and robust protocol by processing the samples in 24x or 96x array plates. Different array designs are available, that may include customized designs. Some examples of applications are:
     - Whole Genome imputation with diverse and global, or more specific population coverage, for applications like GWAS studies or population-level health initiatives.

- Assessing of the PRS (Polygenic Risk Score) for downstream applications, like patient stratification for clinical assays, or to design personalized preventive or therapeutic strategies for individual patients.
- Pharmacogenomics research.
- High-resolution HLA typing.
- The Axiom ® Microbiome arrays enable the detection of over 11,000 organisms and five microbial domains: archaea, bacteria, fungi, protozoa, and viruses. Human and animal microbiomes can be analysed, achieving identification of species, at strain and sequence level.
- The Axiom ® SARS-CoV-2 research array contains a comprehensive selection of markers related to different aspects of COVID infection.

➢ Equipment:

- **Affymetrix GeneChip System for cartridge arrays**. Includes: 1) two Fluidic Stations, 2) two Hybridization Ovens (640 and 645), and 3) a Scanner 3000 7G coupled to a computer workstation loaded with Affymetrix® GeneChip® Command Console® (AGCC) Software.


- **Affymetrix GeneTitan Multi-Channel Instrument for array plates**. It supports gene expression and genotyping studies on 16, 24 and 96 format array plates, and combines a hybridization oven, fluidics processing and state-of-the art imaging device into a single bench-top instrument.

CYTOMETRY PLATFORM

Contact: citometria@vhir.org

The Cytometry Platform integrates the latest technologies in the field of flow cytometry, applied to both fluorescence-activated cell analysis and sorting. The cytometry techniques allow to analyse and to sort easily and quickly a significant number of cells with high sensitivity and objectivity. It is also possible to obtain both qualitative and quantitative measurements and to define complex cell subpopulations based on the study of a set of parameters defined for each individual cell.

In recent years, the launching of "full-spectrum" cytometry has revolutionized the field compared to conventional techniques. Full-spectrum cytometry relies on the detection of the entire emission spectrum of each fluorochrome, rather than on discrete emission peaks. This allows a considerable increase in the number of fluorochromes that can be detected simultaneously (more than 40), even those that have an overlapping emission spectrum. This technology makes possible to perform immunophenotyping studies with high resolution, as well as the extraction of auto-fluorescence, which is analysed as an independent parameter.

The two spectral cytometers installed in the UAT offer high sensitivity and resolution across the spectrum range from 365 to 829 nm, which facilitates the detection of minority populations, being useful even when not working with a high number of fluorochromes.

Regarding high-throughput, multiplexed, bead-based immunoassays, Luminex® assays (driven by xMAP technology) are designed to simultaneously detect and quantitate multiple secreted proteins (e.g., cytokines, chemokines, and growth factors) or expressed genes. Results are comparable to conventional assays such as ELISA, with higher throughput.

➢ Services:

1. **Cell analysis by flow cytometry**
2. **Fluorescence-activated cell sorting**
3. **Multiplexed Genomic and Proteomic Biomarkers Analysis**

Training on self-service use of the equipment, advice on cytometry techniques, experimental set-up, complex panel design and assistance in data analysis are also available.

➢ Applications:

✓ Flow cytometry: main applications are:

- Multicolour cell surface immunophenotyping and intracellular antigen detection.
- Deep immunoprofiling (40-color demonstrated assays using 5 lasers and 64 fluorescence detectors in the Aurora cytometers)
- Studies based in nucleic acid analysis (ploidy, cell cycle…)

- Functional studies (ex. membrane potential changes, apoptosis, intracellular calcium fluxes, proliferation assays)
- Transfection efficiency evaluation using a fluorescent marker
- Live and dead bacteria discrimination

✓ FACS (fluorescence-activated cell sorting):

- Identification and cell sorting of unique and well-defined cell populations from heterogeneous samples, in tubes (up to 6-way sorting) or in plates (96-well plates). High or low-frequency populations can be sorted, including rare cell types or populations (i.e. circulating tumour cells, minimal residual disease).
- Single-cell sorting in 96-well plates (index sorting)
- Isolation of fluorescently labelled cells for down-stream applications, such as functional assays, clonal expansion of modified cells, preclinical models generation.

✓ Multiplexed Genomic and Proteomic Biomarkers Analysis

- Genomic and proteomic detection up to 50 different analytes per sample, using a small volume of sample (25 ul of plasma or serum/50 ul of cell culture supernatant) in 96-well-plate format.

- Compatible assays:

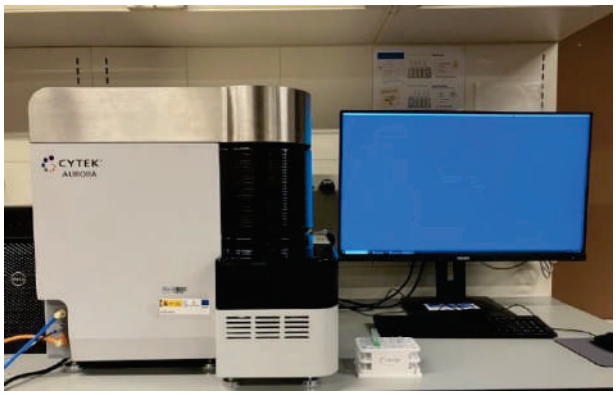
    - **ProcartaPlex assays**: assays are provided in multiple formats across six species (human, mouse, rat, nonhuman primate, porcine, and canine):
        - Procartaplex preconfigured panels (i.e. immunoassays for cytokines, chemokines, growth factors, or specific pathway panels for coagulation, inflammation, immune-oncology, etc).
        - Simplex kits detect individual analytes and are designed to be added to ProcartaPlex panels to increase customization or to create new panels.
        - ProcartaPlex Mix & Match panels (custom mixed)

    - **QuantiGene Plex multiplex gene expression assays.** Simultaneous measurement of up to 80 genes of interest. The assays are hybridization-based and incorporate branched DNA (bDNA) technology, which uses signal amplification rather than target amplification for direct measurement of RNA transcripts. The sensitivity (LOD) is 1,000–2,000 transcripts/assay well. Many panels for different pathways are already predesigned, and any combination of existing genes (17.000) can be used to create a new panel.

➢ Equipment:

✓ Flow cytometry analyzers:

- **Full-spectrum analyser-Aurora** (Cytek): it is equipped with 5 lasers (UV 355nm, Violet 405nm, Blue 488nm, Yellow-Green 561nm and Red 640nm) and 67 detection channels (64 fluorescence channels, FSC, blue laser SSC, and violet laser SSC). Its semiconductor detector arrays provide a more efficient spectrum capture for dyes emitting in the 365-829 nm range. Previously incompatible dyes and/or fluorescent proteins, such as APC and AF647 or GFP and FITC, can be combined together in the same sample. Small particle detection is enabled by violet laser scatter, narrow vertical beam height, and proprietary flat top laser design.
  This cytometer has been upgraded with a plate loader for 40-tube racks and 96-well plates.



- **LSR Fortessa Cell analyzer** (BD): 4 lasers (blue, red, violet and yellow-green) for in parallel detection of up to 16 fluorochromes (plus FSC and SSC). High-throughput (96 and 384 well-plates) sample acquisition.

✓ Fluorescence-activated cell sorter:

- **Full-spectrum Aurora Cell Sorter (Cytek):** this sorter combines full-spectrum technology and high-end sorting functionality. It has the same configuration than the Aurora analyser: 5 lasers and 67 detection channels (64 fluorescence + FSC + Blue laser SSC + Violet laser SSC), so experiment transfer between both machines is possible. In fact, the sorter can also be used as an spectral analyser.
  Output sorting formats are up to 6 ways in tubes or indexed single cell sorting in 96-well plates.
  The sorter is placed inside a biosafety cabinet Class II, Type 2A, which increases the biosecurity level to avoid sample contamination and to safeguard the operator.

- **Workstations with specific software for cytometry data analysis, available for internal users.**
  Software licenses:
  - FlowJo: general cytometry data analysis software
  - SpectroFlo: pre-analysis of spectral data coming from the Auroras.
  - OMIQ: cloud based software for high-dimensional data analysis, accessible from any computer.

  Available software in each workstation is detailed in the document VHIR-UAT-DOC-012-Llistat de programes d'anàlisi de dades.

✓ Multiplexed Genomic and Proteomic Biomarkers Analysis

- **Luminex MagPix:** This compact multiplexing unit performs up to 50 different tests in a single reaction volume and reads a 96-well-plate in ≤ 60 minutes.
  Sensitivity: approximately 10^6 copies of DNA or single-digit picogram levels of protein.

  The **ProcartaPlex Analyst Software** provides:
  - 4-parameter logistic (4PL) or 5PL standard curve
  - Linear, logarithmic, and point-to-point fit
  - Predefined dilution factor and standard range
  - Export settings for fast and easy reanalysis

MICROSCOPY PLATFORM

Contact: microscopia@vhir.org

The Microscopy Platform is dedicated to sample preparation (sectioning) and subsequent observation (both fixed cells/tissues and live samples), by using brightfield and/or fluorescence microscopy ("widefield" and confocal). The morphological and structural characteristics of cells, tissues, subcellular organelles and non-biological samples (for example, nanoparticles) can be analysed, and it is possible to develop different types of functional tests (for example, protein-protein interaction, endocytic processes, calcium flows, etc.)

➢ Services:

- Widefield microscopy.
- Confocal microscopy (also possible for living cells imaging)
- High-speed multidimensional microscopy, including living-cell and tissue image acquisition.
- Laser microdissection *(limited functionality due to obsolescence of equipment)*
- Cryostat for fine sectioning of frozen samples.

UAT staff offers training in self-service usage of the equipment, support during the realization of experimental techniques, as well as advice in the development of new applications, experimental design and image analysis and interpretation.
The UAT Microscopy Platform also offers image analysis services, customized to user necessities, upon demand.

➢ Applications:

✓ **SAMPLE PREPARATION:**

- Frozen tissue sectioning (cryostat) for subsequent staining and observation.
- Isolation and recovery of cellular and subcellular structures by laser microdissection.

✓ **GENERAL APPLICATIONS OF WIDEFIELD MICROSCOPY:**

- Analysis of the architecture and morphological characteristics of cells and tissues.

Specifically, **the multidimensional microscope Thunder allows:**

- Time-lapse acquisition for live-cell imaging (slow and fast dynamics).
- Multi-dimensional acquisition: multi-channel, [XYZ tλn] serial acquisitions.
- Fast acquisition and reconstruction of mosaics, useful to image large samples.
- Computational Clearing method: generation of high resolution/high contrast images of thick samples, removing instantaneously the out-of-focus blur.

✓ **SPECIFIC APPLICATIONS OF CONFOCAL MICROSCOPY, for both in vitro and in vivo studies:**

- Spectral unmixing in samples labelled with several fluorochromes.
- Functional studies of cell dynamics (in vivo): endocytosis, calcium fluxes, cell contraction, cell cycle, etc.
- Colocalization analysis, being especially useful the high resolution imaging option (up to 120 nm lateral resolution).
- Reconstruction of three-dimensional structures (thick cuts of tissue, spheroids, organoids) with high resolution, acquiring mosaic images (XY) and Z-stacks.
- "High Screening count", for quantitative or colocalization studies where statistical significance and high resolution are required.
- High resolution analysis of subcellular structures, exosomes.
- Nanoparticle imaging.

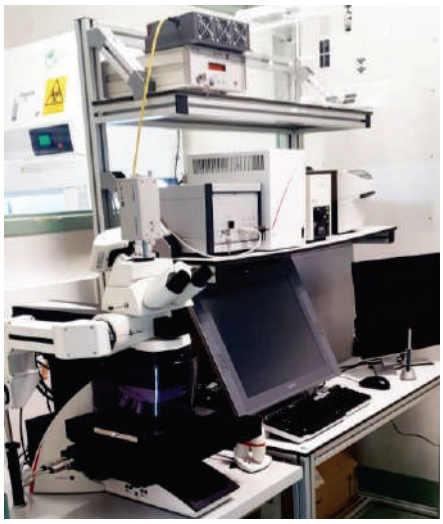
➢ Equipment:

✓ Sample preparation:

- **Leica CM3050 Cryostat.** Fine sectioning (0.5 – 300 µm) of frozen samples.

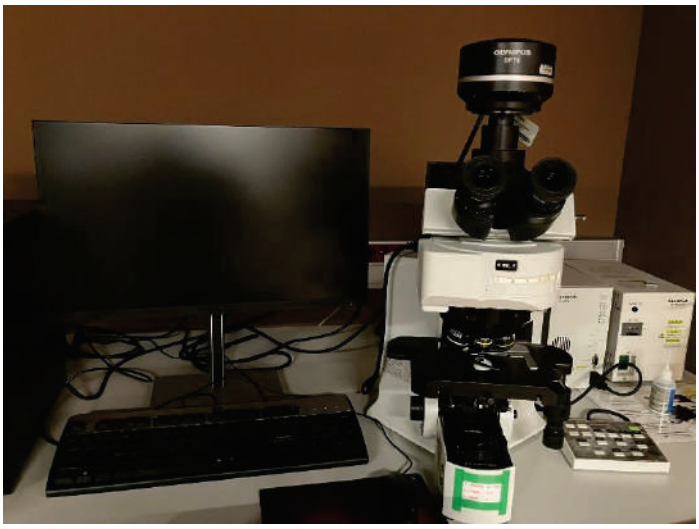
- **Leica LMD 6000 laser microdissection system**.
  - Inverted, motorized microscope, equipped with the following objectives: 1.25x, 5x, 10x, 20x, 40x, 63x (oil), 100x (oil) and 150x (for chromosome microdissection).
  - Contrast techniques: bright field, phase contrast, DIC, polarization, darkfield.
  - Diode laser for cutting (355 nm).
  - Camera DFC360FX (1.4 Mp digital camera, high sensitive, cooled, monochrome for fluorescence).

- External fluorescence source and BGR (blue-green-red) filter cube.
- Control software for high precision cutting, including the AVC automatic feature recognition module.
  Due to equipment obsolescence, the capabilities of this equipment are limited. Please contact UAT before booking.

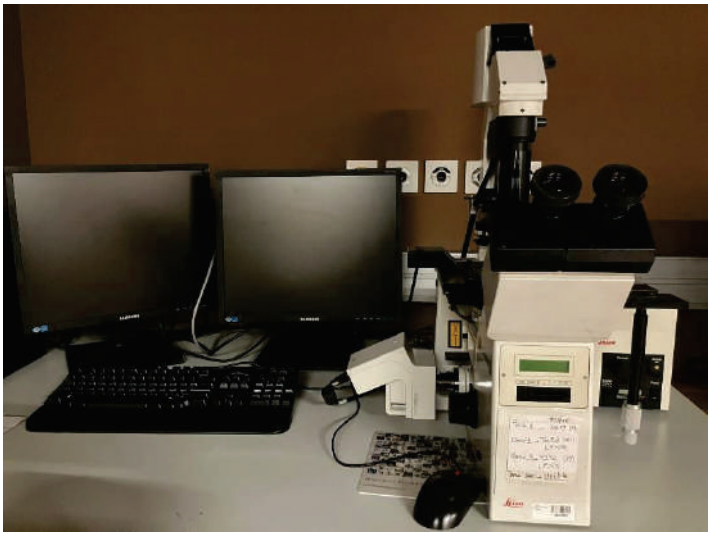


✓ Widefield microscopes: different fluorescence microscopes, equipped with filters for the most common fluorochromes. Image capture using digital cameras controlled by Olympus/Leica acquisition software.

- **Olympus BX61 straight microscope (for slides).**
  - Illumination by mercury lamp.
  - Filters: GFP/TRITC/DAPI/Cy5 ranges
  - Objectives:
    - PLAN N 4X/0.1 Plan Neofluotar A.N 0,1 WD 18.5mm
    - UPLFLN 10x/0.3 Plan Fluorita, A.N 0,3 WD 10.0mm
    - UPLFLN 20x/0.5 Plan Fluorita, A.N 0,5 WD 1.6mm
    - UPLFL 40x/0.75 Plan Fluorita, A.N 0,75 0.49mm
    - PLAPON 60x/1.42 WD 0.15 mm (oil)
    - UPLSAPO 100xO/1.40 Plan Super Apocromatic 100x, NA 1.40, (oil), WD 0.12mm
  - Camera DP72.
  - Filters: GFP/TRITC/DAPI/Cy5 ranges
  - Software: CellSens.



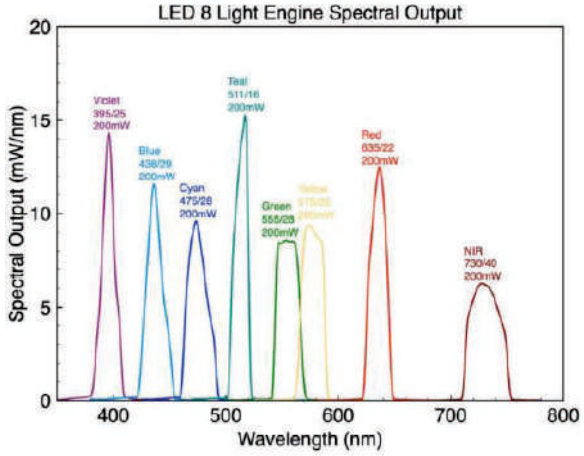
- **Leica DM-IRB inverted microscope (for cell cultures).**
  - Stage for slides, 60 mm plates, multi-well plates and falcons.
  - Illumination by LEDs (EL6000: UV, blue and green light).
  - Filters: GFP, TRITC, DAPI ranges
  - Objectives:
    - HC FL PLAN 2.5x/0.07
    - UPLFLN 10x/0.3 Plan Fluorita, AN 0.3 WD 10.0mm
    - HC PL FL 20x/0.40 CORR 02/C,6.9
    - PLAPON 63x / NA 1.32 WD 0.15 (oil)
  - Camera DFC550.
  - Software: LAS/LAS X.



- **Nikon Ts2R-FL inverted microscope.** Two microscopes of the same model, located respectively in the UAT-Central Building and in Collserola Building.

    - LED illumination: Internal LED illumination (Central Building) / pE300-white CoolLED LED illumination (Collserola Building)
    - Filters: GFP/TRITC/DAPI ranges (Band Pass)
    - Objectives:
        - Plan Fluor 4X/0.13 A.N
        - Plan Fluor 10x/0.3 A.N
        - S Plan Fluor ELWD 20x/0.45 A.N, ∞/0-2 W.D. 8.2-6.9
        - S Plan Fluor LWD 20x/0.70 A.N, ∞/0-1.8 W.D. 2.3-1.3 (only in the Collserola microscope)
        - S Plan Fluor ELWD 40x/0.6 A.N
        - Plan Fluor 60x/0.7 A.N W.D. 2.6-1.8
        - Plan Fluor 100x/1.30 A.N, Oil
    - Camera: Nikon DS-Fi3
    - Software: NIS-Elements.

- **Leica Thunder Imager 3D Cell Culture:** multidimensional microscope adapted for in vivo experiments, completely automatized. Its main features are:
    - Inverted stand, fully motorized, including real-time control of all the elements (condenser, lighting, filter blocks, plate ...)
    - Motorized focus, with focus correction system AFC (Adaptative Focus Control) using an infrared diode.



- White light LED lighting, plus 8 independent fluorescent LEDs. Motorized filter block turret and emission filter wheel to prevent signal crossing when using multiband filters.

- 8 objectives with the following characteristics:

Tipo de objetivo	*Aumento / A.N.*	*Medio*	*Grosor cubre*	*Distancia de trabajo (mm)*	*Técnicas*[1]
N Plan	*5x/0.12*	*Aire*	*cualquiera*	*14*	*PH*
HC PL FLUOTAR	*10x/0.32*	*Aire*	*0.17*	*11.13*	*PH*
HC PL FLUOTAR L	*20x/0.40*	*Aire*	*0-2 corr*[2]	*6.9*	*PH*
PL APO CS	*20x/0.75*	*IMM*[3]	*0.17 corr*[2]	*0.67*[4]	*DIC*
PL APO	*20x/0.80*	*Aire*	*0.17*	*0.4*	*DIC*
PL APO	*40x/0.95*	*Aire*	*0.11-0.23 corr*[2]	*0.17*	*DIC*
PL APO	*40x/1.25*	*Gly*	*0.14-0.19 corr*[2]	*0.35*	*DIC*
PL APO	*63x/1.30*	*Gly*	*0.14-0.19 corr*[2]	*0.3*	*DIC*

- High speed Quantum LMT200 motorized stage (max v: 500 mm / sec). Inserts for multi-well plates, 35 mm plates and slides.
- DFC9000GTC digital camera, with high sensitivity and dynamic range. High-speed acquisition (up to 100 fps in "streaming" at full resolution).
- Thunder Live Software, including the Live-Computational Clearing option, that improves visualization by extracting out-of-focus blur, directly in the “live mode”. Especially useful for thick samples (tissues, spheroids, organoids…).
- Black opaque incubation system, with temperature, CO2 and humidity control, fully controlled from the microscope software.

✓ Confocal microscopes:

- **FV1000 spectral confocal microscope (Olympus)**, with the following characteristics:
  - Inverted microscope (model IX81). Excitation filters for the most common fluorochromes (DAPI, GFP, Cy3, Cy5). DIC Objectives:

Objectius	Immersió
UPLSAPO 10x / NA 0.40 WD 3.10 mm	Sec
UPLSAPO 20x / NA 0.75 WD 0.60 mm	Sec
UPLSAPO 40x / NA 0.90 WD 0.18 mm	Sec
PLAPON 60x / NA 1.42 WD 0.15 mm	Oli
UPLSAPO 100XO / NA 1.4 WD 0.13 mm	Oli

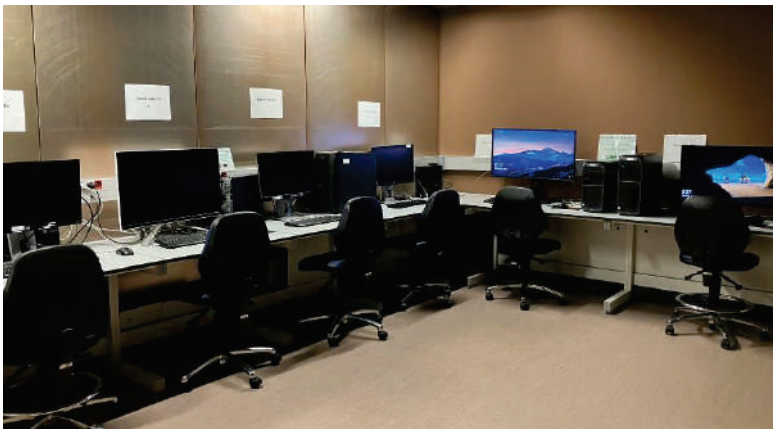
  - Excitation: green laser (488 nm), green diode laser (561 nm) and red He / Ne laser (633 nm). *Violet diode laser (405 nm) currently non-functional.*
  - Detection: 3 PMT-type detectors (2 spectral and 1 filter detector), plus 1 PMT-T detector for transmitted light.
  - Portable incubation device, incorporating Tª and CO_2 control, for short experiments with living cells.
  - Software for image acquisition (FluoView).

- **Zeiss LSM980 fully spectral confocal microscope amb AiryScan 2 confocal super-resolution detector.**
  - Fully motorized system, controlled by software / TFT display.
  - Inverted microscope (AxioObserver 7), with the following objectives ("Plan-Apochromat") equipped with inter-differential contrast (DIC):

Objectius	Distància de treball	Medi Immersió	DICT
Plan-Apochromat 10x/ NA 0,45	2,1mm	Sec	SI
LD LCI Plan-Apochromat 25x / NA 0,8 Imm	0,57mm	Oli, Aigua, Glicerol	SI
Plan-Apochromat 40x/ NA 1,3 Oil	0,2mm	Oli	SI
Plan-Apochromat 63x/ NA 1,4 Oil	0,19mm	Oli	SI
Plan-Apochromat 63x/ NA 1,2 Imm	0,49mm	Aigua, Glicerol	SI
Alpha Plan-Apochromat 100x/ NA 1,46 Oil	0,11mm	Oli	SI

  - LED lighting system (Colibri 7). Motorized turret of fluorescence cubes.
  - Motorized stage.
  - Auto-focus system based on software / hardware.
  - Full-size incubation system, with controlled temperature, O_2, CO_2 and humidity.
  - Excitation: solid state lasers (405, 445, 488, 514, 561 and 639 nm).
  - Detection: Quasar detection unit, consisting of a central detector composed by a 32-GaAsP elements array, plus 2 lateral PMTs (MA). Detection range between 370 and 760 nm (allows simultaneous acquisition of all fluorescent signals for further spectral unmixing). PMT for transmitted light.
  - AiryScan 2 Detector, which allows to work in super-resolution mode (120 nm in xy, 350 nm in z), or in multiplex mode (acquisition of several lines in a single sweep). The super-resolution mode is useful for structures that cannot be clearly uncovered with the standard confocal mode. Multiplexing allows increasing the acquisition speed 4-8x, with a better SNR (signal-to-noise ratio) than the confocal mode. This is especially interesting for dim labeling, ultra-fast imaging (tiling, Z series) and in vivo experiments.
  - Software for image acquisition (ZEN with several additional modules).

- **Workstations with specific software for image visualization and processing.** Available for internal users. Software:
  - Commercial software: Imaris (workstation 1), Zeiss-ZEN Blue software with additional analysis modules (workstation 2), Leica LAS X with 3D visualization and analysis modules (workstation 3).
  - Freeware: ImageJ-FiJi and other free applications. Installed in the three computers.



Available software is detailed in the document VHIR-UAT-DOC-012-Llistat de programes d'anàlisi de dades.