

## **Forsyth Cores and Services**

Flow Cytometry

Histology

Imaging

Mass Spectrometry

Micro Computed Tomography ( $\mu$ CT)

Multiplex High Sensitivity Immunoassays

Sequencing and Bioinformatics

## Forsyth Flow Cytometry Core

The Forsyth FACS Core provides assisted use, consulting and support services associated with FACS cell sorting and analysis.

**Cytometers:** The core is equipped with a BD FACS Aria Cell Sorter which offers 13-color cell analysis and high speed cell sorting, and an Invitrogen Attune NxT with Autosampler which offers 14-color acoustic focused cell analysis. These are versatile instruments capable of a diverse range of applications, including but not limited to cell phenotype characterization, DNA cell cycle analysis, bead-based immunoassays, and cell bioprocess analysis. The machines are capable of detecting cells, platelets, bacteria, and particles down to 0.5 microns in size. Data acquisition speeds of greater than 100,000 events per second possible on the Aria and sample acquisition speeds of up to 1,000ul per minute possible on the Attune. Temperature control, culture plate/slide collection, and configurable stream nozzle diameters available for sorting. High-throughput plate sampling analysis is possible using the Autosampler. Violet side scatter filters are available for red blood cell discrimination from blood contaminated samples.



**BD FACS Aria II SORP Cell Sorter – 4 lasers, 13 colors**

Laser Color	Excitation Wavelength	Emission Filter	Common Fluorophore
Red	640	780/60 BP	APC-Cy7
		730/45 BP	Alexa Fluor 700
		670/30 BP	APC
Blue	488	710/50 BP	PerCP-Cy5-5
		530/30 BP	FITC
Yellow Green	561	780/60 BP	PE-Cy7
		660/20 BP	PE-Cy5
		610/20 BP	PE-Texas Red
		582/15 BP	PE
Violet	405	660/40 BP	Qdot 655
		605/40 BP	Qdot 605
		525/50 BP	AmCyan
		450/50 BP	Pacific Blue

**Invitrogen™ Attune™ NxT acoustic focusing cytometer w/Autosampler – 4 lasers, 14 colors**

Laser Color	Excitation Wavelength	Emission Filter	Common Fluorophore
Red	638	780/60 BP	RL3 - APC-Cy7
		720/30 BP	RL2 - AF 700
		670/14 BP	RL1 - APC
Blue	488	695/40 BP	BL3 - PerCP-Cy5-5
		530/30 BP	BL1 - FITC
Yellow	561	780/60 BP	YL4 - PE-Cy7
		695/40 BP	YL3 - PE-Cy5
		620/15 BP	YL2 - PE-Texas Red
		585/16 BP	YL1 - PE
Violet	405	710/50 BP	VL4 - Qdot 705
		603/48 BP	VL3 - Qdot 605
		512/25 BP	VL2 - Pacific Green
		440/50 BP	VL1 - Pacific Blue, AF405

**Contact:** Daniel Nguyen, Manager: 617-892-8533. [dnguyen@forsyth.org](mailto:dnguyen@forsyth.org).

## **Forsyth Histology Core**

The Histology Core provides consulting and support services for both soft tissue and precise hard tissue analysis. Services include: tissue processing, embedding, sectioning, and staining as well as guidance on protocols. The Histology Core can embed samples in standard molds or custom molds in paraffin, resin or as frozen tissue for routine histology, special stains and immunohistological studies performed either by the investigator or by Core staff. The Histology Core also houses X-Clarity tissue clearing equipment.

### **Equipment and Capabilities:**

- Fixation
- Decalcification
- Sample Embedding
- Custom sectioning can be done on one of the following microtomes:

Microtomes: The core is equipped with four microtomes for routine paraffin sectioning, one Microm HM 315 microtome and three American Optical Spencer 820 microtomes. The Microm HM 315 is a high performance microtome system designed for smooth vertical specimen movement with a precision micro feed system with fully adjustable section thickness (0.5-60 microns). The AO 820's micron selector offers 1-50 microns which can be adjusted in 1 micron increments to allow for precise sectioning. Each A820 is equipped with X-Y orientation clamps and high profile blade holders compatible with stainless steel and tungsten carbide "C" and "D" blades.

Cryotome: The core is equipped with one ThermoScientific Cryotome FSE which allows for sectioning of frozen specimens with section thickness from 1-20 microns in 1 micron increments or 20-60 microns in 5 micron increments.

- Slides and Stains

We have a wide array of histological stains in stock for both demineralized and soft tissues as well as bacteria and structural stains, please contact for more information. Unstained slides can also be prepared and returned to the investigator for further processing.

- X-Clarity

Logos Biosystems Polymerization System and Tissue Clearing systems are available to polymerize and clear samples for Clarity processing.

**Standard histology processing:** All samples will be initially screened and samples deemed unsuitable for further processing will held in the queue until further consent from the investigator. A grant number or purchase order is required prior to processing.

**Histology costs:** are per sample, based on materials and time spent for preparation, which involves the type of sample submitted and the desired downstream applications by the investigator. See next page for detailed listing.

**Additional service training:** individual training for equipment use, data interpretation or any other consulting, sample prep, and general instrument operation are available at hourly rates with advanced notice to prepare time estimates and staff availability.

**Contact:** Christine Kressirer, Manager: 617-892-8284. [ckressirer@forysth.org](mailto:ckressirer@forysth.org)

## Forsyth Imaging Core

The Forsyth Imaging Core provides consulting and support services associated with imaging analyses of both soft tissues and mineralized tissue samples and accommodates for a variety of imaging techniques including CLARITY, Fast Airy Scan, and spectral imaging as well as time lapse and live cell imaging.

### Imaging equipment:

**Zeiss Axio Observer Inverted Microscope** with Zen Blue 2012 Image Acquisition software, Colibri.2 LED light source for narrow band excitation with a reduction of cross stimulation and UV leak-through, environmental chamber and Definite Focus for temperature and focus controlled time-lapse imaging; Hamamatsu ORCA-Flash 4.0 - sCMOS camera with high quantum efficiency, low noise, 30fps at full resolution; ThorLabs camera compatible with polarized light imaging.

**Zeiss LSM 780 Confocal Microscope** with Zen Black 2012 software for acquisition, processing and analysis, simultaneous detection for three channels, 32 Channel GaAsP detector for spectral analysis and separation, linear unmixing and up to 45% quantum efficiency, 7 laser lines, environmental chamber with temperature and CO<sub>2</sub> control and Definite Focus; Shuttle & Find Software and sample holders for correlative microscopy in conjunction with Zeiss Evo LS10 SEM.

**Zeiss LSM 880 Confocal Microscope** upright microscope with a multi-alkali detector capable of capturing 3 channels simultaneously, an Airyscan detector, Fast Airyscan, 7 laser lines, environmental housing, temperature control, and 20x CLARITY objective for imaging cleared tissue specimens.

**Zeiss Evo LS10 scanning electron microscope** for imaging in high vacuum and variable pressure mode for life science application, equipped with Peltier stage for temperature control, backscattered electron detector and Oxford INCA 250 microanalysis system for EDS

**Zeiss Stemi SV11** Stereo microscope, great for larger specimens with Pentafluor and HBO light source for fluorescence imaging, Axiocam HRc camera for monochrome or color imaging

Upright light Microscopes:

Nikon SMZ1500, Leica DMRE, Leica DMLS, and Leica DMR fluorescence microscope

### Other equipment:

Denton V Sputter Coater with Tilting/Rotating Plate and Gold Target, LKB 7800 Glass knife maker, Pecon Heating insert P LabTek S1

#### Workstation for Data Analysis

Dell Precision T7610 Computer with 64 GB RAM, 16- Core: Dual Intel Xeon Processor E5-2687W v2 (Eight Core HT, 3.4GHz Turbo, 25 MB) SSDs, 4 GB NVIDIA Quadro K5000 (490-BBKM), dual 27-inch monitors

#### Software

Imaris, CellProfiler, FIJI, Confocal Standard Package, XT - Programming Interface, MATLAB, Image Processing Toolbox, Signal Processing Toolbox, Parallel Computing Toolbox, Statistics Toolbox, Computer Vision Toolbox

**Contact:** Dr. Felicitas Bidlack, Director: 617-892-8342. [fbidlack@forsyth.org](mailto:fbidlack@forsyth.org)  
Andy Kempchinsky: 617-892-8381. [akempchinsky@forsyth.org](mailto:akempchinsky@forsyth.org)

## **Mass Spectrometry Core**

The Mass Spectrometry Core at the Forsyth Center for Salivary Diagnostics provides mass spectrometry analysis and strategic consulting in proteomics for internal and external researchers. Three state-of-the-art mass spectrometers are utilized to definitively identify and quantify proteins, peptides, metabolites and lipids that may have diagnostic value. These instruments are interfaced with dedicated liquid chromatography and robotic systems.

- AB Sciex 4800 Plus MALDI TOF/TOF
- Thermo Scientific QExactive Plus
- Thermo Scientific Orbitrap Fusion

### **Capabilities**

**Protein Identification (in gels or solution):** the basic experimental workflow entails reduction/alkylation; tryptic digestion, peptide extraction, nano-LC-MS/MS or MALDI-TOF MS/MS. The peptides are fragmented in the mass spectrometer to yield diagnostic patterns that can be correlated to protein sequence databases via computer algorithms for peptide identification. Proteins in complex solution backgrounds (e.g. detergent, non-volatile salts, glycerol) require an additional cleanup-step to facilitate analysis.

**Intact Molecular Weight Determination:** Samples (intact protein, oligonucleotides, peptides) are applied to stainless steel target plate with appropriate matrix solution and air-dried. High sample concentration and omission of salts and detergents are essential for efficient ionization.

### **Additional Services**

Training, data interpretation and consulting, sample prep, and instrument operation are available at hourly rates with advance notice to prepare time estimates and staff availability.

Instrument Access: AB Sciex 4800 MALDI-TOF/TOF Proteome Analyzer: The AB Sciex 4800 offers highly accurate mass analysis (<10ppm) along with MS/MS capabilities for fragmentation analysis of peptides and other biomolecules. Researchers can be trained for walk-up use.

### **Contact**

Markus Hardt, Director: 617-892- 8350. [mhardt@forsyth.org](mailto:mhardt@forsyth.org). <http://proteomics.forsyth.org/>

## **Forsyth Micro-Computed Tomography ( $\mu$ CT) Core**

The Forsyth Micro Computed Tomography ( $\mu$ CT) Core offers micro 3D X-ray imaging of small samples in high resolution, and will provide images and quantitative analyses of internal structures of *ex vivo* samples without any destructive procedures. The Scanco  $\mu$ CT40 system allows for visualization, measurement, and quantification of the structure of mineralized specimens. Contrast reagents permit visualization of structures such as blood vessels. The non-destructive nature of this technology allows investigators to carry out complementary analyses (e.g., histology) of the same samples.

### **Capabilities**

The Core is equipped with a Scanco  $\mu$ CT 40, *ex vivo*  $\mu$ CT scanner, with a high throughput scanning option (auto sample exchanger). This system creates a three-dimensional model from a stack of two-dimensional X-ray images taken around a single axis of rotation. The high-resolution scanner can capture detail at six to 32 microns. The resolution is sample size-dependent. Scans taken of long bones have specific trabecular or cortical analysis performed on them.

The system is equipped with automatic sample changer that allows high-throughput scanning. The sample changer can hold maximum 10 sample tubes.

An additional software for advanced 3D analyses (Amira 3D Software for Life Sciences, FEI) is available for analysis.

### **Links**

Scanco  $\mu$ CT 40

<http://www.scanco.ch/en/systems-solutions/specimen/microct40.html>

Amira 3D Software for Life Sciences

<http://www.fei.com/software/amira-3d-for-life-sciences/>

**Contact:** Yang Hu, Manager: 617-892-8445. [yhu@forsyth.org](mailto:yhu@forsyth.org)

## Forsyth Multiplex Core

Bead-based flow cytometric multiplex arrays are useful for the detection of various biomarkers and proteins. This technique uses microsphere beads, coated with monoclonal antibodies against specific proteins, to **simultaneously measure multiple analyte concentrations** in various sample types including: body fluids (serum, saliva, plasma, GCF, wound fluid, etc), cell extracts and culture supernatants. Luminex® and their partners have developed many applications using the XMAP® Technology platform, including for the following fields of life science research areas:

- Cancer Markers
- Cardiovascular
- Cell Signaling
- Cellular Metabolism
- Immunology
- Genotyping
- Gene Expression Profiling
- Endocrinology
- Isotyping
- Matrix Metalloproteinases
- Metabolic Endocrinology
- Neurobiology
- Transcription Factors
- Toxicology
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Forsyth has three platforms, **Luminex® 200™**, **FLEXMAP 3D™**, and our newest addition, **AYOXXA Lunaris™ Multiplex Detection**. 96 and 384-well plate capacity are available for both Luminex and AYOXXA analysis. Plate capacity: up to 80 samples in singlet with up to 16 wells designated as standards for antigen-antibody based assays.

### Capabilities

Multiplex protein detection using antibody-based assays of up to 100 analytes or 500 analytes in a single microplate well using the Luminex® 200™ or FLEXMAP 3D™, respectively.

- Ability to custom coat beads with various mammalian and non-mammalian molecules such as nucleic acids, receptor-ligands, and siRNA. These require custom-plexing, assay development/optimization, and additional costs.
- Both systems use BioPlex Manager from Bio-Rad for computation of data.
  - Allows easy transfer of the data to Excel or other user-friendly programs.
  - In addition to the raw and computed data on concentrations and fluorescence intensity, standard curves, %CV, internal control samples, plate-to-plate variation, and standard deviation are only a few of the data output measures.
- Benefits of Multiplexing
  - Array results have compared similarly to measures from conventional techniques such as ELISA. Wide dynamic range of detection seen with all platforms (4-5 log units) with detection at pg/mL levels.
  - While an **ELISA** may use up to **100 µl** of sample, **multiplex technology** requires only **5–25 µl** of sample for multiple target detection, which offers considerable advantages when limited research sample may be available. AYOXXA technology uses **less than 5 µl** of sample.
  - Avoids the need for diluting samples multiple times or for multiple freeze-thawing of samples, each of which can affect measurement accuracy and precision.
  - Cost effective compared to performing multiple ELISAs. The more analytes plexed together, the more cost effective the assay.
  - AYOXXA technology is ideal for kinetic studies and analysis of extremely low volume samples such as GCF, tears, vitreous, aqueous, and joint fluids.

**Contact:** Alpdogan Kantarci, Director: 617-892-8530. [multiplex@forsyth.org](mailto:multiplex@forsyth.org)

See also <http://www.ayoxxa.com/products/> for more information on AYOXXA technology.

## **Forsyth Sequencing and Bioinformatics Cores**

The Forsyth Sequencing and Bioinformatics Core is under reorganization.

For more information, please contact Alexis Kokaras, Sequencing Core Coordinator: 617-892-8567,  
[AKokaras@forysth.org](mailto:AKokaras@forysth.org)