

Current equipment list Advanced Light Microscopy Unit **(October 2016)**

Instrumentation:

Super-resolution microscopy

1. The Leica TCS SP8 STED 3X system achieves resolutions below the diffraction limit in lateral as well as in axial direction and it can be freely adjustable for best lateral resolution, best vertical resolution and smallest confocal volume.

2. Ground State Depletion (GSD) followed by Individual Molecule Return (GSDIM): GSD is a single molecule localization based, super resolution technique, with a precision of up to 25 nm lateral resolution. Furthermore, the Leica SR GSDIM is a complete widefield- and Total Internal Reflection Fluorescence (TIRF) microscope.

3. Stochastic Optical Reconstruction Microscopy (STORM) in the Nikon N-STORM 4.0 system: STORM is also a single molecule super-resolution technique that achieves a simultaneous lateral resolution of approximately 25 nanometers and an axial resolution approaching 50 nanometers in the 3D STORM regime.

Two photon microscopy platform

A tunable Mai Tai DeepSee broadband laser (710-990 nm), with automated dispersion compensation connected to the inverted and the upright Leica TCS SP5 confocal microscopes, both with non-descanned detectors. Excitation can be shared between the systems or used exclusively by one system.

Single Molecule Detection Platform (FLIM/FCS/FLCS)

A platform based on PicoQuant Hardware is connected to the inverted Leica SP5 confocal microscope for multi-parametric single molecule detection and analysis. With it, Fluorescence Correlation Spectroscopy (FCS), Fluorescence Lifetime Imaging Microscopy (FLIM) and the combination of FCS with fluorescence lifetime, Fluorescence Lifetime Correlation Spectroscopy (FLCS) and gated FCS can be performed.

Additionally, the detectors can be used for the confocal imaging of weak signals.

4 Super Resolution microscopes

a) Leica TCS SP8 STED 3X (inverted)

Stimulation Emission Depletion in 3D (X,Y,Z dimensions) with a pulsed white light laser and three STED laser depletion lines (CW 592nm, CW 660 nm and pulsed 775nm) and gated detectors for multicolor and in vivo STED imaging.

- Motorized inverted microscope with a 10x air, 40x Oil, 63x Oil, 100x/1.40 OIL STED WHITE
- Galvanometric Scanner (up to 1800 Hz)
- Galvanometric Z-stage for fast z-sectioning
- Motorized XY-stage for multiposition imaging

- White light laser ranging from 470 to 670 nm
- Gated-STED detectors: 2 super-sensitive Hybrid detectors and 2 standard PMT detectors (all with freely adjustable spectral detection ranges).
- Huygens STED deconvolution software to process 3D STED data

b) Leica TCS SP5 II CW-STED (inverted)

Super-resolution microscopy, advanced confocal imaging applications, in-vivo measurements and photobleaching experiments.

Stimulation emission depletion (STED): A 592 nm orange CW laser (> 1W) for stimulated emission depletion (STED) microscopy connected to the Leica TCS SP5 II confocal microscope provides a two to threefold resolution improvement compared to standard confocal microscopy for several fluorescence channels.

- Motorized inverted microscope. Objectives: 10x Air, 63x Oil, 63x Glycerol, 100x Oil (STED)
- Laserlines: 458 (CFP), 476, 488 (GFP, FITC), 496, 514 (YFP), 543 (mRFP, DsRED, Cy3, TRITC, TexasRed), 633 (Cy5, DRAQ5)
- Detectors: 2 super-sensitive Hybrid detectors and 2 standard PMT detectors (all with freely adjustable spectral detection ranges). Additionally 2 Avalanche Photodiodes (filter-based) for high sensitivity imaging
- Galvanometric Scanner (up to 1400 Hz) and alternatively Resonant Scanner (8000 Hz) for fast scanning
- Galvanometric Z-stage for fast z-sectioning
- Motorized XY-stage for multiposition imaging
- Scanning resolution of up to 8000 x 8000 pixels
- Environmental control (temperature, CO₂, humidity) for in-vivo imaging

c) Leica SR GSDIM (inverted)

Super-resolution microscopy, complete widefield and TIRF/HiLo imaging

- SuMo Stage, with Suppressed Motion Technology, minimizes drift for accurate localization of molecules
- Objectives: 10x Air, 20x Air, 40x Oil, 100x Oil, 100x 1.47 (GSD)
- Laserlines: 405 nm for backpumping (control of single molecule switching behavior) and 488, 532 and 642 for TIRF and EPI-Fluorescence imaging.
- Camera: EM-CCD (Andor iXon 3 897)

d) Nikon STORM (inverted)

Super resolution microscopy in 2D- and 3D-, TIRF/HiLo imaging, suitable for PAINT super resolution technique

- Inverted microscope. Objectives: 10x Air, 20x Air, 100x Oil 1.5 (TIRF)
- Laserlines: 405, 488, 561 and 647. Neutral density (ND) filters available
- Perfect Focus System (PFS) to avoid Z-drift during long acquisitions.
- Andor Ixon 897 EM-CCD camera
- Environmental chamber for CO₂
- Cylindrical lens for 3D STORM imaging

Five confocal microscopes

a) Leica TCS SP5 AOBS (inverted)

FCS, FLIM, two photon and SHG microscopy, advanced confocal imaging applications, in-vivo measurements and photobleaching experiments

- Motorized inverted microscope. Objectives: 10x Air, 20x Air, 40x Oil, 63x Oil, 63x Water with motorized coverslip correction collar for FCS measurements (63x water)
- Laserlines: 405 (DAPI, Hoechst), 458 (CFP), 476, 488 (GFP, FITC), 496, 514 (YFP), 561 (mRFP, DsRED, Cy3, TRITC, TexasRed), 633 (Cy5, DRAQ5)
- Detectors: 5 fluorescence detectors (PMT) with freely adjustable spectral detection ranges + 1 transmission detector (PMT) for DIC imaging, Additionally 2 Single Photon Counting Avalanche Photodiodes (SPAD) for FCS and FLIM and 2 non-descanned detectors for multiphoton imaging
- Galvanometric Scanner (up to 1400 Hz) and alternatively Resonant Scanner (8000 Hz) for fast scanning
- Galvanometric Z-stage for fast z-sectioning
- Motorized XY-stage for multiposition imaging
- Scanning resolution of up to 8000 x 8000 pixels
- Environmental control (temperature, CO₂, humidity) for in-vivo imaging

b) Leica TCS SP5 CFS (upright)

Two photon and SHG microscopy, advanced confocal imaging applications, in-vivo measurements using dip lenses, physiology experiments and photobleaching experiments

- Upright fixed stage microscope for physiology measurements. Objectives: 10x Air, 40x Oil, 25x and 40x Water dip lenses, 63x Oil
- Laserlines: 405, 458, 476, 488, 496, 514, 543, 633
- Detectors: 2 fluorescence detectors with freely adjustable spectral detection ranges + 1 transmission detector for DODT contrast imaging and 2 super-sensitive non-descanned Hybrid detectors for multiphoton imaging
- Electro-optical Modulator (EOM) for multiphoton intensity control and ROI bleaching
- Galvanometric Scanner (up to 1400 Hz)
- Galvanometric Z-stage for fast z-sectioning
- Scanning resolution of up to 8000 x 8000 pixels
- Digital acquisition board and triggerbox for physiology measurements

c) Leica TCS SP8 AOBS (inverted)

Advanced confocal imaging applications and photobleaching experiments.

- Motorized inverted microscope. Objectives: 10x Air, 20x Air, 63x Oil, 63x Glycerol
- Laserlines: 458 (CFP), 476, 488 (GFP, FITC), 496, 514 (YFP), 561 (mRFP, DsRED, Cy3, TRITC, TexasRed), 633 (Cy5, DRAQ5)
- Detectors: 1 super-sensitive Hybrid detector and 2 standard PMT detectors (all with freely adjustable spectral detection ranges).
- Galvanometric Scanner (up to 1800 Hz) and alternatively Resonant Scanner (8000 Hz) for fast scanning
- Motorized XY-stage for multiposition imaging
- Scanning resolution of up to 8000 x 8000 pixels

d) Leica TCS SPE

Can be used for standard confocal imaging applications.

- Inverted microscope. Objectives: 10x Air, 40x Oil, 63x Oil
- Laserlines: 405 (DAPI, Hoechst), 488 (GFP, FITC), 532 (mRFP, DsRED, Cy3, TRITC, TexasRed), 635 (Cy5, DRAQ5)
- Detectors: 1 fluorescence detector (PMT) with freely adjustable spectral detection range
- Galvanometric Scanner
- Galvanometric Z-stage for fast z-sectioning
- Scanning resolution of up to 2048x2048 pixels

e) Andor Revolution XD

Fast spinning disk confocal microscope for in-vivo imaging.

- Inverted Olympus microscope. Objectives 10x Air, 20x Air, 40x Oil, 60x Oil (TIRF), 100x Oil (TIRF)
- Laserlines: 405 (DAPI, Hoechst), 445 (CFP), 488 (GFP, FITC), 515 (YFP), 561 (mRFP, DsRED, Cy3, TRITC, TexasRed)
- Andor Ixon 897E Dual Mode EM-CCD camera
- Scanhead: Yokogawa CSU-X1
- Andor FRAPPA photobleaching/photoactivation unit
- Motorized Prior XYZ stage (ProScan II + NanoScanZ)
- Sutter filterwheel controller for 1 excitation and 2 emission filterwheels

2 Automated Widefield Fluorescence Microscopes

a) Zeiss Cell Observer HS with Eppendorf microinjector (for cells) and microdissector

Can be used for standard and advanced imaging applications and in-vivo imaging

- Motorized inverted microscope. Objectives 10x Air, 20x Air, 40x Air, 40x Oil, 63x Oil, 100x Oil
- Filtersets suited for DAPI, Hoechst, CFP, GFP, FITC, YFP, mRFP, dsRed, Cy3, TRITC, TexasRed, Cy5, DRAQ5
- Motorized Condensor for Differential Interference Contrast (DIC) and Phase contrast imaging
- Ultrafast fluorescence excitation changer (Sutter DG-4) for fast multichannel imaging
- Motorized XYZ stage for multiposition/screening and 3D imaging
- 2 CCD cameras: Zeiss AxioCam MrX for standard and high resolution imaging and Hamamatsu ImagEM EM-CCD camera for high sensitivity imaging (single molecule imaging capacity)
- Eppendorf InjectMan Microinjector
- Eppendorf MicroChisel for Microdissection
- Environmental control (temperature, CO₂, humidity) for in-vivo imaging
- Operating software (Zen) with autofocus, macro recording and programming capability

b) ImageXpress Micro Widefield High-Content Analysis System

ImageXpress[®] Micro System is a widefield automated microscope capable of fluorescent, transmitted light, and phase-contrast imaging of fixed- or live-cell assays, tissues and small organisms. Speed, flexibility, and high quality data are assured with an industry-leading stage and autofocus control, the broadest range of research-grade objective lenses (air 4x, air 10x, air 20x, air 40x, air and oil 63x) available, and multiple filter options (DAPI, GFP, TRITC, Texas Red and Cy5). Combined with the suite of MetaXpress[®] Software tools for acquisition and analysis, is a complete solution that help you interpret your images, understand your data, and explore new ideas.

Transmitted light module capable of both brightfield and phase contrast imaging

- Nikon 100W Pillar Diascopic Illuminator with TE-C ELWD Condenser
- 0.3 NA with 65 mm WD and PhL, Ph1, and Ph2 selectable phase rings
- High contrast imaging where unstained cells are easily viewed or separated from background (4x-60x)
- Ideal for non-fluorescent histochemically stained samples
- Compare fluorophore-independent morphology visualization with fluorescent imaging overlay.

1 Macro Zoom Fluorescence Microscope

Olympus MVX10

Can be used for fluorescence imaging plus stereo observation and time sequence recordings

- Objectives: 1x, 0.63x with zoom factor up to 10 times.
- Filtersets suited for CFP, GFP and RFP
- Transmitted Brightfield Illumination Base with contrast-enhancing oblique illumination.
- CCD color plus standard gray scale camera DP71 for high sensitivity and high resolution imaging.
- Snapshot resolution of up to 4080 x 3072 pixels.

2 Image Processing workstations

a) HP xw 8400

Workstation with 16 GB RAM, 584 GB SAS-controlled RAID of 10,000 rpm disks, NVIDIA Quadro FX 4600 PCI (768 MB RAM) graphics board for image processing and analysis

b) HP xw 8600

Workstation with 4 GB RAM, ATI Radeon 4870 X2 2GB graphics board for image processing and analysis

Imaris, Huygens and a selection of other image processing and analysis programs are available on these computers.

Special Equipment

- **Environmental portable device (temperature, CO₂) for in-vivo imaging**
- **Fast response mini stage temperature controller**
Portable device for control of microscope sample temperature in a range between 4 – 42°C and for rapid switches between temperatures.
- **Objective inverter**

Inverts the light path of an inverse microscope to an upright configuration (suitable for the Leica confocal microscopes).

General bench equipment for experiment preparation

- 4°C Fridge
- -20°C Freezer
- Incubator at 37°C, 5% CO₂ for transient cell storage before experiments.
- Table centrifuge
- Set of Eppendorf pipettes + tips