

Current equipment list Advanced Light Microscopy Unit **(February 2015)**

Instrumentation:

Super-resolution microscopy

1. 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.

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. Furthermore, the Leica SR GSDIM is a complete widefield- and Total Internal Reflection Fluorescence (TIRF) microscope.

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.

6 Confocal Microscopes

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

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

- 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

b) Leica SR GSDIM (inverted)

Super-resolution microscopy, complete widefield and TIRF imaging

- SuMo Stage, with Supressed 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)

c) 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

d) 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 (DAPI, Hoechst), 458 (CFP), 476, 488 (GFP, FITC), 496, 514 (YFP), 543 (mRFP, DsRED, Cy3, TRITC, TexasRed), 633 (Cy5, DRAQ5)
- 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
- Acousto-optical Modulator (AOM) 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

e) 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

f) Leica TCS SP2

Can be used for standard confocal imaging applications.

- Motorized inverted microscope. Objectives: 10x Air, 20x Air, 40x Oil, 63x Oil, 100x Oil
- Laserlines: 458 (CFP), 488 (GFP, FITC), 514 (YFP), 543 (mRFP, DsRED, Cy3, TRITC, TexasRed), 633 (Cy5, DRAQ5)
- Detectors: 3 fluorescence detectors (PMT) with freely adjustable spectral detection range + 1 transmission detector (PMT) for DIC and Phase imaging
- Galvanometric Scanner
- Galvanometric Z-stage for fast z-sectioning
- Scanning resolution of up to 2048x2048 pixels
- Environmental portable device (temperature, CO2) for in-vivo imaging

g) Andor Revolution XD

Fast spinning disk confocal microscope for in-vivo imaging. Additional FRAP, TIRF and PALM/STORM capability

- 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)
- 3-fiber-output lasercombiner for confocal unit, photobleach unit and TIRF illumination
- 2 Andor Ixon 897E Dual Mode EM-CCD cameras (for confocal/TIRF imaging)
- Scanhead: Yokogawa CSU-X1
- Andor FRAPPA photobleaching/photoactivation unit
- Olympus TIRF coupling
- 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 phasecontrast 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

- **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