IMAGING PLATFORMS IN THE FACULTY OF MEDICINE

Guo Jing
Lab Manager
Faculty Core Facility
June 27 2011
Facility Core Facility

- Introduction
- Committee of Management
- Equipment
- Online Booking System
- Charges
- Usage Policies and Guidelines
- Workshops and Training
- Protocols
- Usage records
- Publications
- Image Gallery

Faculty Core Facility
Address: L6-11, 6/F, Laboratory Block, 21 Sassoon Road, Pokfulam, Hong Kong

- http://www.med.hku.hk/corefac/
Mission

- Training and education
  - Basic operation
  - Advanced application
  - Imaging analysis

- Consultation

- New technology development

- Host demonstrations & workshop
Faculty Core Facility

Flow Cytometry
- BD FACS Aria I Cell Sorter
- BD FACS Aria SORP
- BD FACS Cantor Analyzer

Imaging
- In vivo Animal Imaging
  - Xenogen IVIS 100
  - CRI MaestroTM 2

Confocal Microscopy
- CZ LSM 510 Meta
- CZ LSM 700
- CZ LSM 710
- Perkin Elmer Spinning Confocal
- Bio-Rad Radiance 2100

Analysis Software
- MetaMorph
- Imaris
- LSM 510 & Axiovision
- FlowJo
Getting started to be an authorized user:

Confocal Training Guideline

Training policy:
1. The training is based on the first-come first-served policy. Your name will be put on waiting list on the day you submit your application form.
2. The training course will be scheduled every month for each microscope facility. The routine training schedule will be canceled if no one is on waiting list. The extra training session may be scheduled if more than 6 people on waiting list.
3. The first time training course and hands-on practice for users are free. THE SECOND TIME TRAINING WILL BE CHARGED.

Getting started to be an authorized user:

- Submit your training application form
- Training & hands-on practice
- Registration of online booking system
The training course will be scheduled every month for each microscope facility.

The first time training course and hands-on practice for users are free.

Training is generally done in groups of no more than five. You could bring your own specimens to training session.

After the training session, you could register to be a novice user on online booking system (http://www.med.hku.hk/corefac/). Your registration will not be successful until your supervisor approves your application.
## Charging policy:

<table>
<thead>
<tr>
<th>Instrument</th>
<th>User type</th>
<th>Office hour (HKD/Hour)</th>
<th>Non-office hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>w/ technical support*</td>
<td>w/o technical</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSM 510</td>
<td>Novice user</td>
<td>220</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Regular user</td>
<td>220</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Experienced user</td>
<td>220</td>
<td>120</td>
</tr>
<tr>
<td>LSM 700</td>
<td>Novice user</td>
<td>200</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Regular user</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Experienced user</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>LSM710</td>
<td>Novice user</td>
<td>220</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Regular user</td>
<td>220</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>Experienced user</td>
<td>220</td>
<td>120</td>
</tr>
<tr>
<td>PE-ERS confocal</td>
<td>Novice user</td>
<td>180</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Regular user</td>
<td>180</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Experienced user</td>
<td>180</td>
<td>80</td>
</tr>
<tr>
<td>PE-ERS widefield</td>
<td>Novice user</td>
<td>133</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Regular user</td>
<td>133</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Experienced user</td>
<td>133</td>
<td>33</td>
</tr>
</tbody>
</table>

* Technical support: 100HKD/h
Why confocal microscope?

Problem: Detecting in-focus information together with out-of-focus fluorescence signals in wide-field microscopy

Excitation

ZEISS Plan-NEOFLUAR 40x/1.3 Oil

Emission

ZEISS Plan-NEOFLUAR 40x/1.3 Oil

PMT

Laser

Focal Plane

Pinhole at Confocal Plane

Wide-field

Confocal

Hela cell, FITC-MT, Rh-Phalloidin
Comparison of confocal and widefield microscope

Widefield

Confocal

Z scale: 1um
Z range: 11um

Sample: Rhizome of Convallaria majalis, Ruscaceae
Confocal Laser Scanning Microscopy Today
It's about more than pretty pictures ...

- **3D Reconstruction**
  Subcellular structures in three dimensions with an easy-to-use setup.

- **Time Series**
  Added information on simple dynamic processes by acquisition of image series, also in combination with local bleaching: acquisition, visualization and analysis of time series \((X, Y, t)\) or \((X, Y, Z, t)\).

- **Quantitative Colocalization**
  Detection of the coincidence of two fluorescence-labeled molecules in the confocal detection volume. Investigation of neighborhood relations and interactions: definition of parameters, image presentation and data analysis (colocalization coefficients).

- **Transmitted-Light Microscopy**
  Image generation in transmitted light: brightfield, phase and DIC images in the LSM mode with optional transmitted-light detector.

- **FRET by Sensitized Emission** (Fluorescence Resonance Energy Transfer)
  Investigation of molecule interactions by energy transfer between fluorescence-labeled donor and acceptor molecules spaced at 1–10 nm: direct registration of FRET by detecting acceptor fluorescence intensity after donor excitation.

- **FLIP** (fluorescence loss in photobleaching) and the related methodology of **FRAP** (recovery after photobleaching)

- **Photoactivation** and **Photoconversion**
Which confocal microscope?

PE Spinning Disc Confocal

CZ LSM700 Confocal

CZ LSM510 Confocal

CZ LSM 710 Confocal
Which confocal microscope?

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Fixed</th>
<th>Live cell</th>
<th>Live animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dye</td>
<td>DAPI</td>
<td>GFP</td>
<td>Rhodamine</td>
</tr>
<tr>
<td>Objective</td>
<td>Air</td>
<td>Water</td>
<td>Oil</td>
</tr>
<tr>
<td>Application</td>
<td>2D/3D imaging</td>
<td>Time series</td>
<td>FRET/FRAP</td>
</tr>
<tr>
<td></td>
<td>Line scan</td>
<td>Linear unmixing</td>
<td>Two photon imaging</td>
</tr>
<tr>
<td>Microscope</td>
<td>LSM700</td>
<td>LSM510</td>
<td>PE spinning disc</td>
</tr>
</tbody>
</table>
Sample type
- Fixed

Dye
- DAPI
- GFP
- Rhodamine
- Cy5

Objective
- 63x Oil
  - NA 1.4

Application
- 2D/3D imaging

Microscope
- LSM700
- LSM510
- LSM710
Sample type: Fixed
Dye: Special dye
Objective: >100um
Application: Water
Microscope: LSM700

Microscope options: LSM510, LSM710

Two photon imaging

63x Oil NA 1.4
Sample type
- Live cell
  - DAPI
  - GFP
  - Rhodamine
  - Brightfield

Dye
- Air
- Objective
- Oil
- Application
- Time series
  - Subcellular structure/FRET/FRAP
  - Widefield

Microscope
- LSM510
- PE spinning disc
Equipment — Imaging

**Xenogen in vivo imaging system 100 series (Xenogen IVIS100)**
- Bioluminescence tumor and non-tumor models
- OS/Software: Windows XP, Living Imaging (R), version 2.50.1

**CRI Maestro TM 2 in vivo imaging system**
- Fluorescent tumor and non-tumor models
- OS/Software: Windows XP, Maestro TM Om — Vivo Imaging System, version 2.10.0

**Carl Zeiss LSM 510 Meta/Axiocam**
- It allows complete separation within a sample of multiple fluorophores with overlapping emission spectra. It has a stage area box with temperature and CO2 regulation.
- Technical Specifications: Scope - Zeiss Inverted
  - Argon laser (458, 477, 488, 514nm)
  - HeNe laser (543nm), HeNe laser (633nm)
  - Chameleon tunable 2-photon (720-930nm)
- OS/Software: Windows XP, LSM 510 version 3.2 SP2, AxioVision version 4.6.2.0

- **Detailed Configuration**
Zeiss LSM 700 Inverted Confocal Microscope

### Specification

<table>
<thead>
<tr>
<th>Lasers</th>
<th>Solid state lasers: 405nm (5mW); 488nm (5mW); 555nm (10mW); 639nm (10mW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscope</td>
<td></td>
</tr>
<tr>
<td>Stand</td>
<td>Inverted: Axio Observer</td>
</tr>
<tr>
<td>XY stage</td>
<td>Manual stage 130x85</td>
</tr>
<tr>
<td>Filter cubes</td>
<td>#49 DAPI; #43 Cy3; #38 EGFP</td>
</tr>
<tr>
<td>Objectives</td>
<td>10x 0.3 Ph1; 20x0.8 Ph2; 40x1.3 Oil Ph3; 63x1.4 oil Ph3</td>
</tr>
<tr>
<td>Scan Module</td>
<td></td>
</tr>
<tr>
<td>Scan mode</td>
<td>xy, xyz, xz, xt, xyt, lambda</td>
</tr>
<tr>
<td>Scanning speed</td>
<td>2 frames/sec with 512 × 512 pixels</td>
</tr>
<tr>
<td>Detector</td>
<td>2 PMTs for reflection/fluorescence (R/FL) detection channels; 1T-PMT</td>
</tr>
<tr>
<td>Software</td>
<td>Windows Vista, ZEN 2010 version 6.0.0.309</td>
</tr>
<tr>
<td>Application</td>
<td>Phase contrast imaging; multi-spectrum fluorescence imaging; spot/line Scan, XY</td>
</tr>
<tr>
<td></td>
<td>2D image; Z-stack 3D imaging; colocalization; time series</td>
</tr>
</tbody>
</table>
## Zeiss LSM 510 Inverted Confocal Microscope

### Specification

<table>
<thead>
<tr>
<th>Feature</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lasers</strong></td>
<td>Argon (458, 488, 514 nm); HeNe laser (543 nm); HeNe-laser (633 nm); Chameleon tunable 2-photon laser (720-930nm)</td>
</tr>
<tr>
<td><strong>Microscope</strong></td>
<td></td>
</tr>
<tr>
<td>Stand</td>
<td>Inverted: Axiovert 200M</td>
</tr>
<tr>
<td>XY stage</td>
<td>Motorized scanning stage</td>
</tr>
<tr>
<td>Filter cubes</td>
<td>#49 DAPI; #43 Cy3; #38 EGFP</td>
</tr>
<tr>
<td>Objectives</td>
<td>2.5x0.12; 5x 0.15; 10x0.3; LD20x/0.4; LD40x/0.6; 40x1.3 oil; 63x1.4 oil DIC</td>
</tr>
<tr>
<td>Accessories</td>
<td>Digital microscope camera AxioCam</td>
</tr>
<tr>
<td></td>
<td>Incubator PM S1</td>
</tr>
<tr>
<td></td>
<td>External shutters for TL and RL</td>
</tr>
<tr>
<td><strong>Scan Module</strong></td>
<td></td>
</tr>
<tr>
<td>Scan mode</td>
<td>xy, xyz, xz, xt, xyt, lambda</td>
</tr>
<tr>
<td>Scanning speed</td>
<td>2 frames/sec with $512 \times 512$ pixels</td>
</tr>
<tr>
<td>Detector</td>
<td>Meta detector + 2 single PMTs; 1 transmitted light PMT</td>
</tr>
<tr>
<td>Software</td>
<td>Windows XP, LSM 510 version 3.2 SP2, AxioVision version 4.6.3.0</td>
</tr>
<tr>
<td><strong>Application</strong></td>
<td></td>
</tr>
<tr>
<td>DIC imaging</td>
<td></td>
</tr>
<tr>
<td>phase contrast imaging</td>
<td></td>
</tr>
<tr>
<td>spot/line Scan</td>
<td></td>
</tr>
<tr>
<td>Xy 2D imaging</td>
<td></td>
</tr>
<tr>
<td>multispectrum fluorescence imaging</td>
<td></td>
</tr>
<tr>
<td>Z-stack 3D imaging</td>
<td></td>
</tr>
<tr>
<td>lambda scan, linear unmixing</td>
<td></td>
</tr>
<tr>
<td>online fingerprinting</td>
<td></td>
</tr>
<tr>
<td>colocalization</td>
<td></td>
</tr>
<tr>
<td>time series, FRAP, FRET</td>
<td></td>
</tr>
<tr>
<td>two photon imaging</td>
<td></td>
</tr>
<tr>
<td>Multidimensional widefielded acquization</td>
<td>CCDcamera</td>
</tr>
</tbody>
</table>
Before image acquisition:

- To chose the right fluorophore
- To use the right coverslip (No. 1; No. 1.5; No. 2)
- To chose the right objective
Fluorescence SpectraViewer

Now you can plot and compare spectra and check the spectral compatibility for many fluorophores offered by Molecular Probes. The Spectra Viewer can most easily be opened by capturing a screenshot and printing the resulting image file.

View the SpectraViewer User Guide

Multiple staining - the emission crosstalk problem

Simultaneous scan

Sequential scan
Multiple staining - the excitation crosstalk problem

Alex fluo 488 + Alex fluo 532
Alex fluo 488 + Alex fluo 555
Alex fluo 488 + Alex fluo 568
Alex fluo 488 + Cy5
Knowing about objective

- https://www.micro-shop.zeiss.com/?s=38843126af7b1d&l=en&p=us&f=o
**Table:**

<table>
<thead>
<tr>
<th>Feature</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Numerical Aperture</strong></td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Magnification</strong></td>
<td>63x</td>
</tr>
<tr>
<td><strong>Working Distance [mm]</strong></td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Coverglass Thickness [mm]</strong></td>
<td>0.17</td>
</tr>
<tr>
<td><strong>Thread Type</strong></td>
<td>M27x0.75</td>
</tr>
<tr>
<td><strong>Immersion</strong></td>
<td>Oil</td>
</tr>
<tr>
<td><strong>Field of View [mm]</strong></td>
<td>25</td>
</tr>
<tr>
<td><strong>Parfocal Length [mm]</strong></td>
<td>45.06</td>
</tr>
<tr>
<td><strong>Optical System</strong></td>
<td>Infinity Color Corrected System (ICS)</td>
</tr>
<tr>
<td><strong>Flatness</strong></td>
<td>★★★</td>
</tr>
<tr>
<td><strong>Color Correction</strong></td>
<td>★★★</td>
</tr>
</tbody>
</table>

**Biomedical Applications**

- **Fluorescence**
  - Multichannel: ★★★★★
  - Ultraviolet Transmission: ★★★
  - Infra Red Transmission: ★★★★★
- **BrightField (H)**:  
- **Differential Interference Contrast**:  

**Objective Type:** DIC

**Objective “Plan-Apochromat” 63x/1.40 Oil DIC M27**

- **Numerical Aperture**: 1.4
- **Magnification**: 63x
- **Immersion**: Oil
- **Thread Type**: M27x0.75
- **Working Distance**: 0.19 mm
- **Coverglass Thickness**: 0.17 mm
- **Field of View**: 25 mm
- **Parfocal Length**: 45.06 mm
- **Optical System**: Infinity Color Corrected System (ICS)
- **Flatness**: ★★★
- **Color Correction**: ★★★

**Mechanical Dimensions**

(WD=0.19mm), incl. “Immersol” 518 F, oiler 20ml and Cover glasses, high performance, D=0.17mm, box with 100 pc.
Numerical aperture (NA) & resolution

\[ NA = n \sin(\alpha). \]

\[ FWHM_{\text{ill,lat}} = \frac{0.51 \times \lambda_{\text{em}}}{NA} \]

FWHM = Lateral Resolution [μm]  
NA = Objective Numerical Aperture  
\( \lambda_{\text{em}} \) = Emission Wavelength [nm]

\( \lambda = 520 \text{nm} \)  
NA = 1.4  
FWHM = 189nm

Sample: Bovine pulmonary artery endothelial (BPAE) cells  
Alexa Fluor® 488 phalloidin  
MitoTracker® Red
## Coverslip

<table>
<thead>
<tr>
<th>Number</th>
<th>Ideal thickness</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>#0</td>
<td>100 μm</td>
<td>80-130 μm</td>
</tr>
<tr>
<td>#1</td>
<td>150 μm</td>
<td>130-170 μm</td>
</tr>
<tr>
<td>#1.5</td>
<td>170 μm</td>
<td>160-190 μm</td>
</tr>
<tr>
<td>#2.0</td>
<td>220 μm</td>
<td>190-250 μm</td>
</tr>
</tbody>
</table>

Most objectives are designed to use #1.5 coverslips. Using the wrong one may have serious implications for image intensity and quality. This is particularly true for objectives with NA above 0.4 and when the sample is very close (e.g. adhered to) the coverslip. There is actually a surprising amount of variation in a batch of coverslips. If your application is very sensitive to coverslip thickness you can measure them and use the ones close the ideal value.
Coverslip

BPAE cells
Alexa Fluor® 488 phalloidin

Excitation: 488nm

Same settings:
- Laser power
- Master Gain
- Offset
- Digital gain

Image Intensity vs Coverslip Thickness
Applications

- 2D imaging & 3D reconstruction
- Quantification of co-localization
- FRET
- Phase contrast & DIC imaging
- Reflection imaging
- Live cell imaging
- FRAP
Acknowledgement

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Prof. George Tsao
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Mr. Benjamin Leung

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Emily Pang
Prof. Sookja K Chung
Zhang Xu
Prof. Ronald Li
Marco Kong
Harry Chen
Prof. Raymond CHANG
Ginger Wong

All Faculty Core Facility Users!