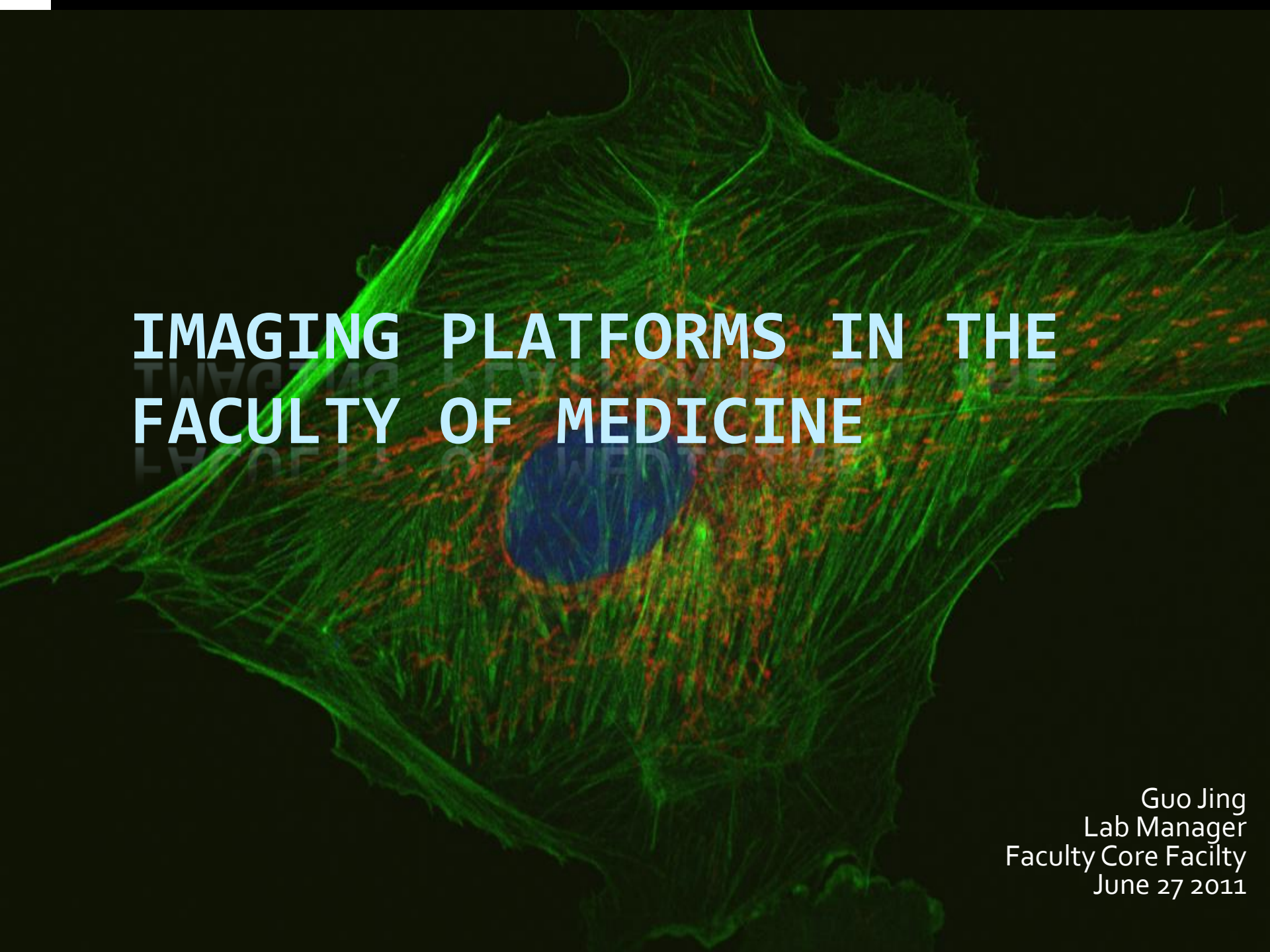


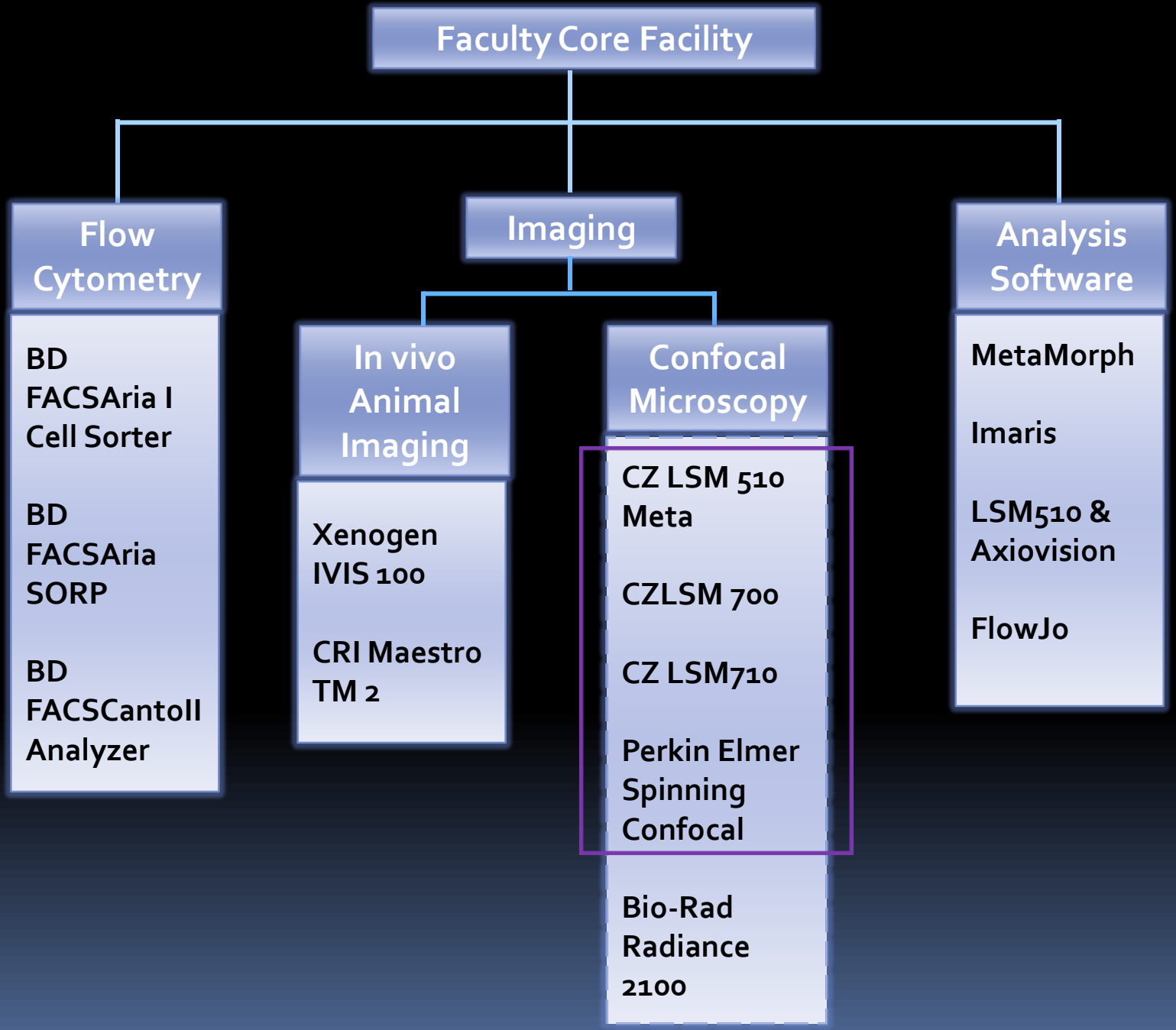
IMAGING PLATFORMS IN THE FACULTY OF MEDICINE

Guo Jing
Lab Manager
Faculty Core Facilty
June 27 2011

- 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 FACSAria I Cell Sorter
BD FACSAria SORP
BD FACSCantoII Analyzer
Imaging
In vivo Animal Imaging
Xenogen IVIS 100
CRI Maestro TM 2
Confocal Microscopy
CZ LSM 510 Meta
CZLSM 700
CZ LSM710
Perkin Elmer Spinning Confocal
Bio-Rad Radiance 2100
Analysis Software
MetaMorph
Imaris
LSM510 & Axiovision
FlowJo

Getting started to be an authorized user:

The University of Hong Kong Li Ka Shing Faculty of Medicine

Faculty Core Facility

- Home
- Contact Us

- 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

Confocal Training Guideline

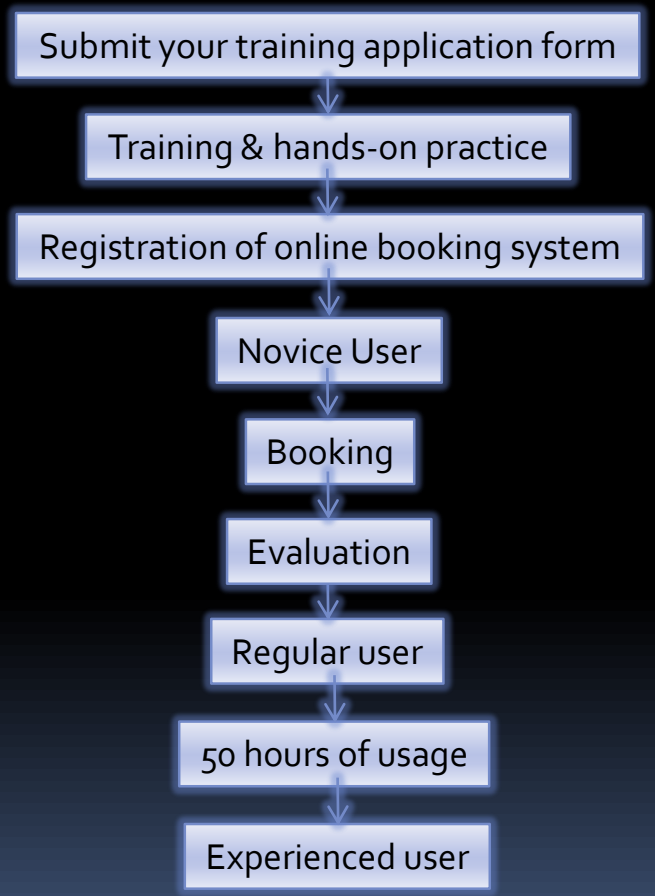
Training policy:

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:

Getting started to be an authorized user:

- 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.

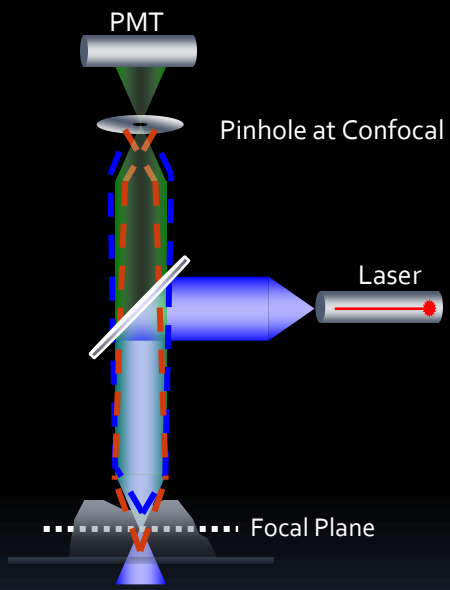


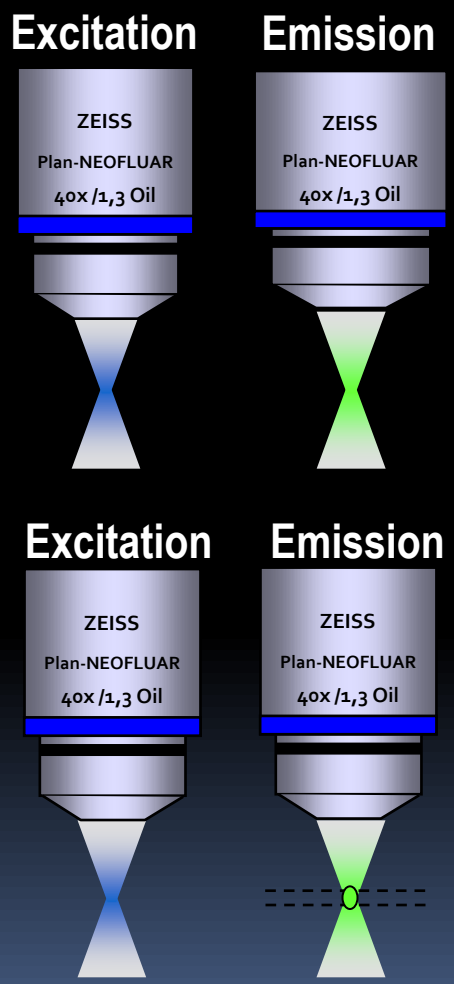
Charging policy:

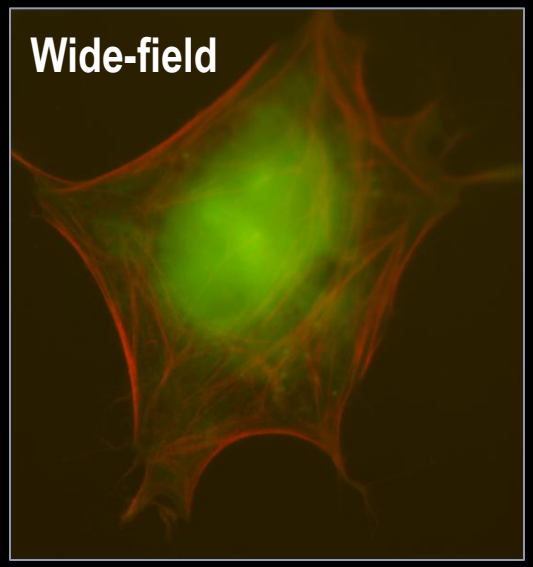
Instrument	User type	Office hour (HKD/Hour)		Non-office hour	
		w/ technical support*	w/o technical support	Booking < 5h (HKD/Hour)	Consecutive booking≥5h* (HKD/booking)
LSM 510	Novice user	220	N/A	N/A	N/A
	Regular user	220	120	N/A	N/A
	Experienced user	220	120	96	Charge for first 5 hours, 90% discount from the 6th hour
LSM 700	Novice user	200	N/A	N/A	N/A
	Regular user	200	100	N/A	N/A
	Experienced user	200	100	80	N/A
LSM710	Novice user	220	N/A	N/A	N/A
	Regular user	220	120	N/A	N/A
	Experienced user	220	120	96	Charge for first 5 hours, 90% discount from the 6th hour
PE-ERS confocal	Novice user	180	N/A	N/A	N/A
	Regular user	180	80	N/A	N/A
	Experienced user	180	80	64	Charge for first 5 hours, 90% discount from the 6th hour
PE-ERS widefield	Novice user	133	N/A	N/A	N/A
	Regular user	133	33	N/A	N/A
	Experienced user	133	33	33	Charge for first 5 hours, 90% discount from the 6th hour

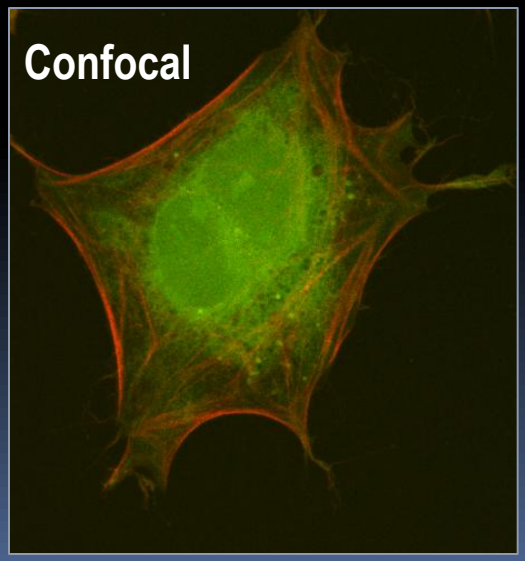
* Technical support: 100HKD/h

Why confocal microscope?







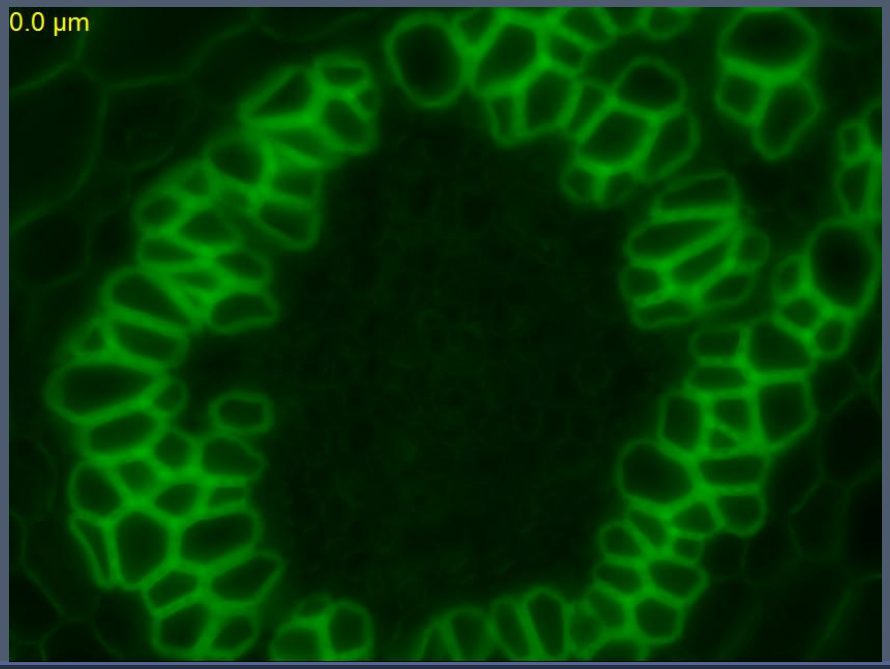


Hela cell, FITC-MT, Rh-Phalloidin

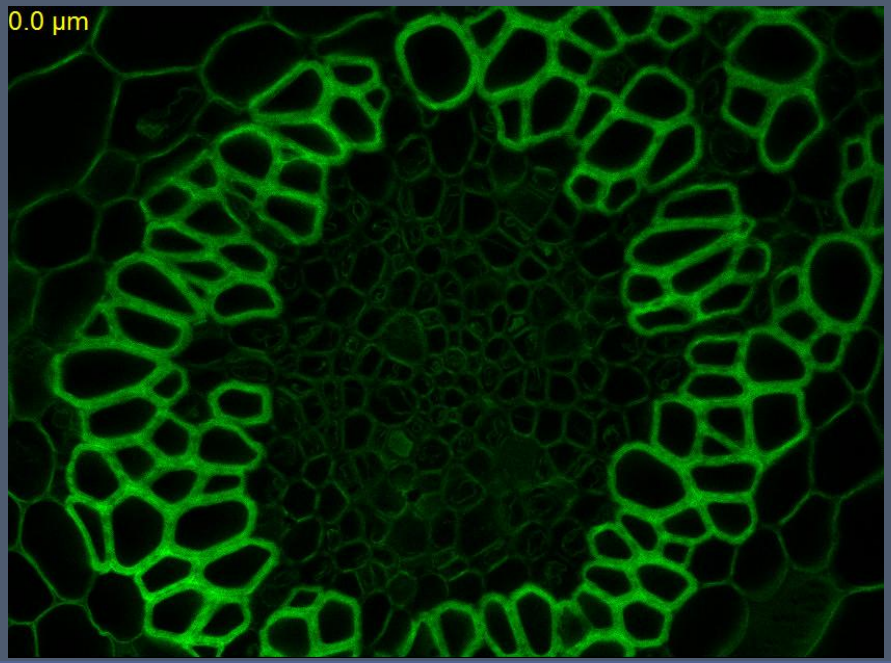
Problem:
Detecting in-focus information together with out-of-focus fluorescence signals in wide-field microscopy

Comparison of confocal and widefield microscope

Widefiled



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**

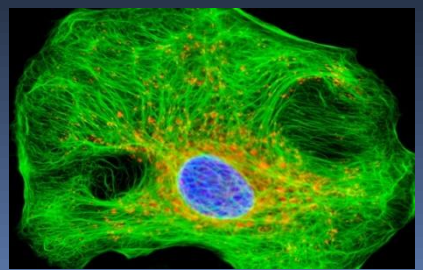
  Image generation in transmitted light: brightfield, phase and DIC images in the LSM mode with optional transmitted-light detector.

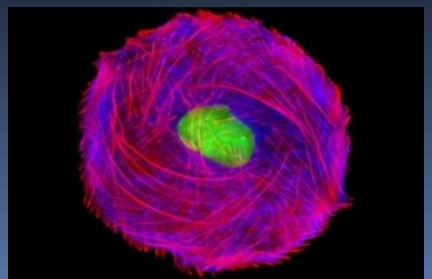
- **FRET by Sensitized Emission** (Fluorescence Resonance Energy Transfer)

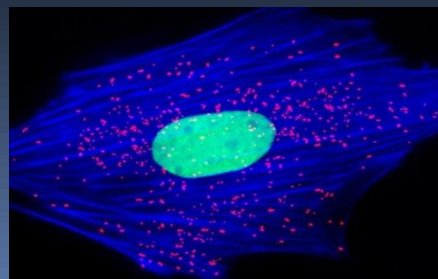
  Investigation of molecule interactions by energy transfer between fluorescence-labeled donor and acceptor molecules spaced at 1–10 nm: direct registration of FRET by detecting acceptor fluorescence intensity after donor excitation.

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

- **Photoactivation** and **Photoconversion**







Which confocal microscope?

PE Spinning Disc Confocal

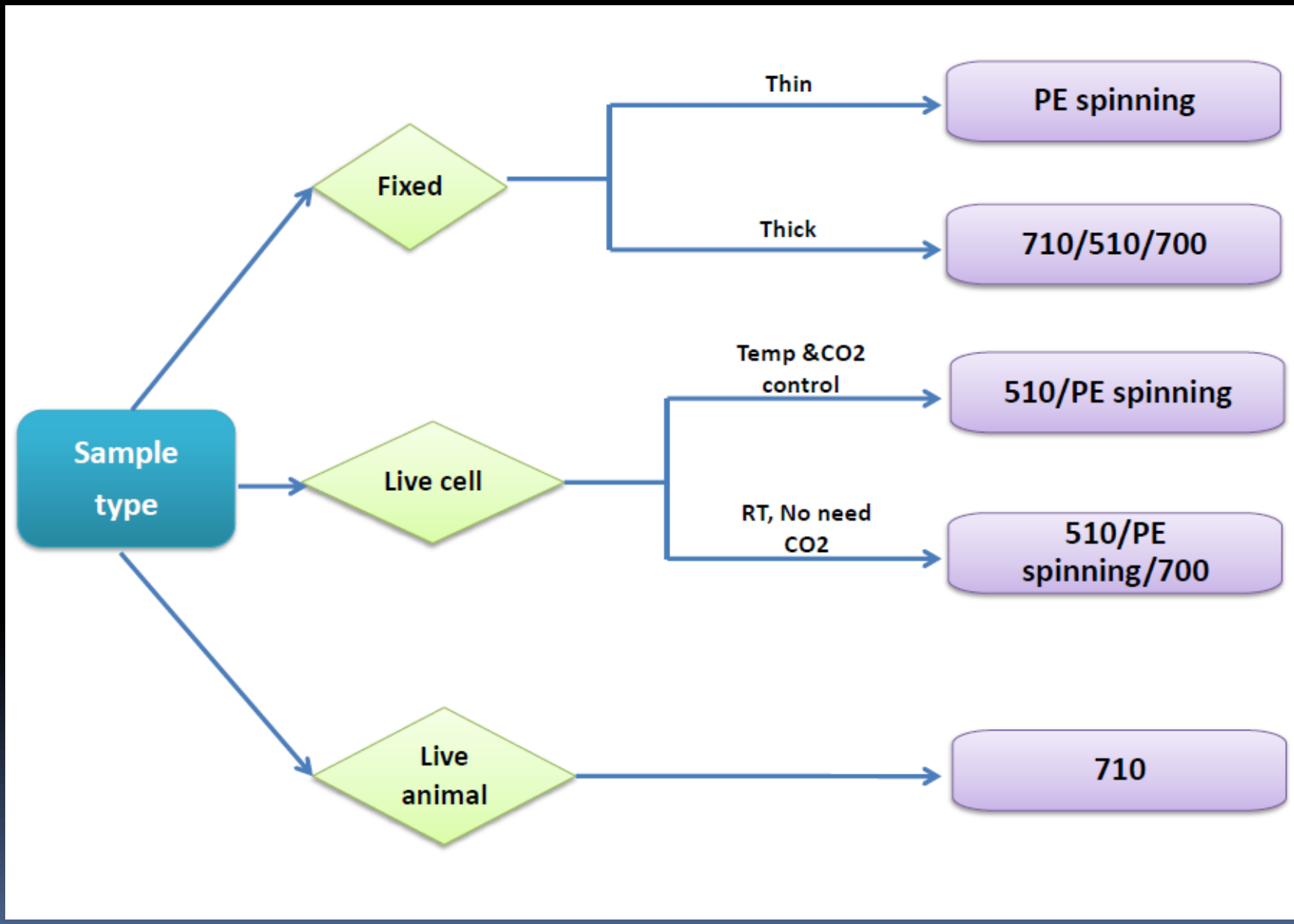
CZ LSM 710 Confocal

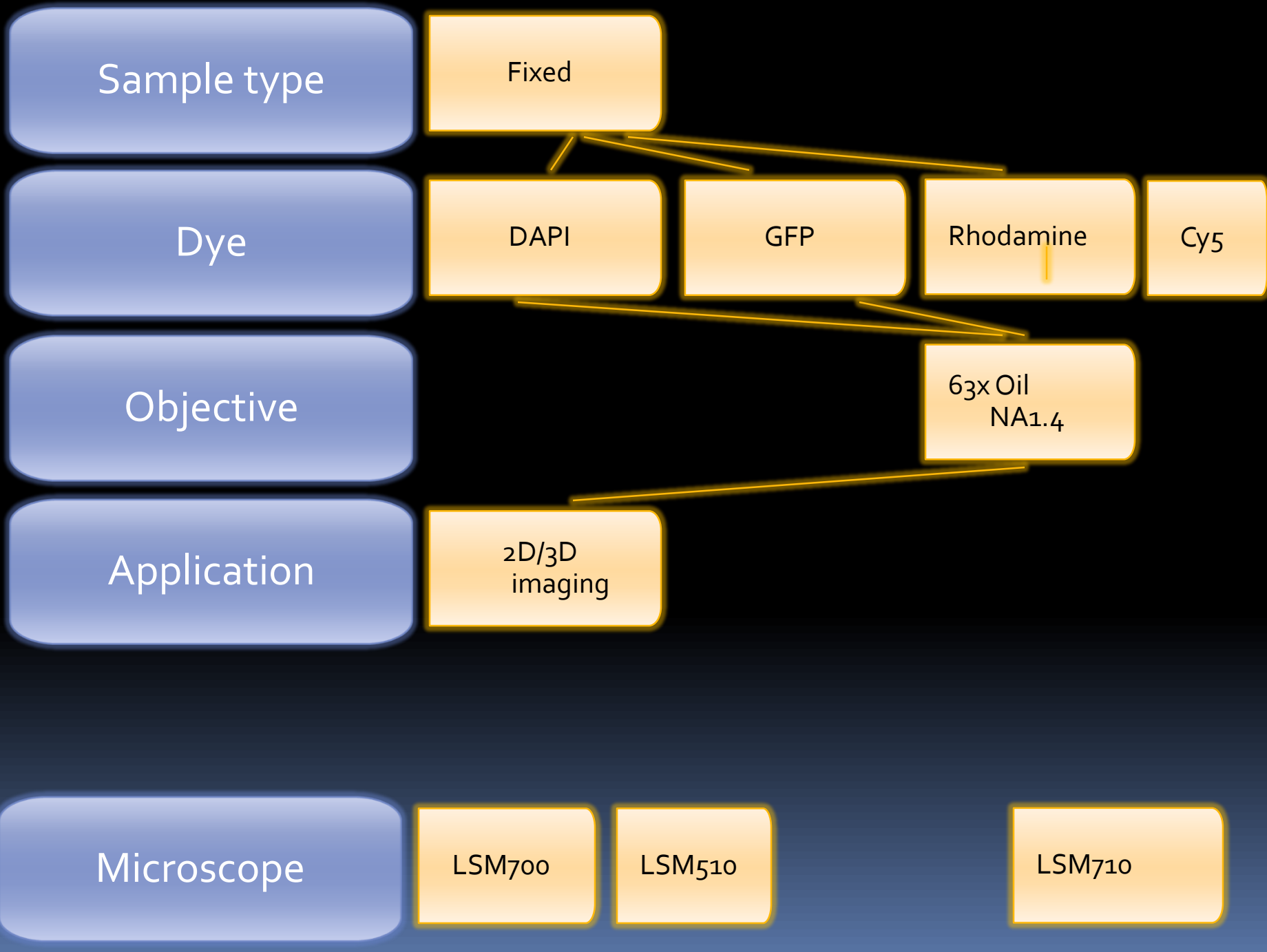
CZ LSM700 Confocal

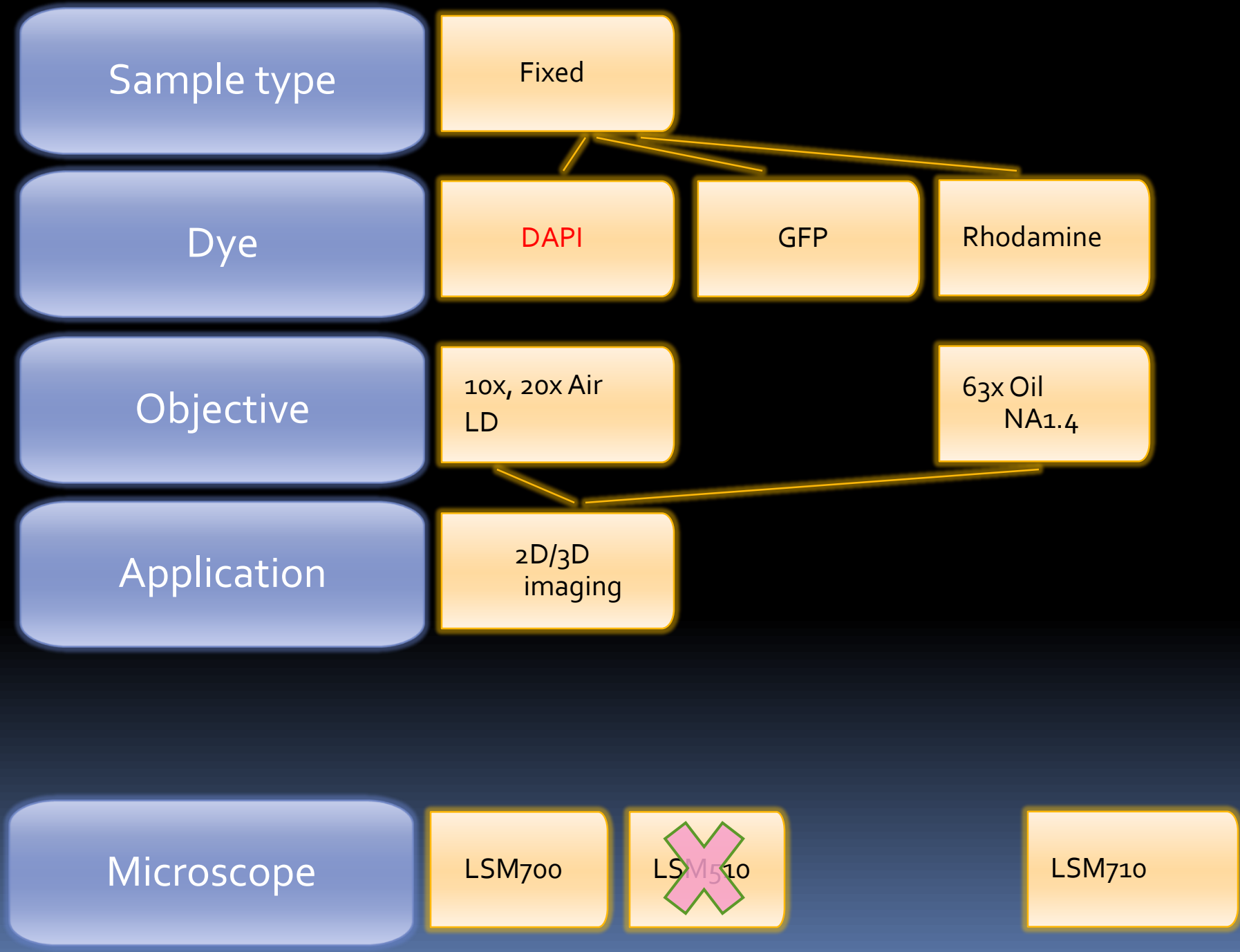
CZ LSM510 Confocal

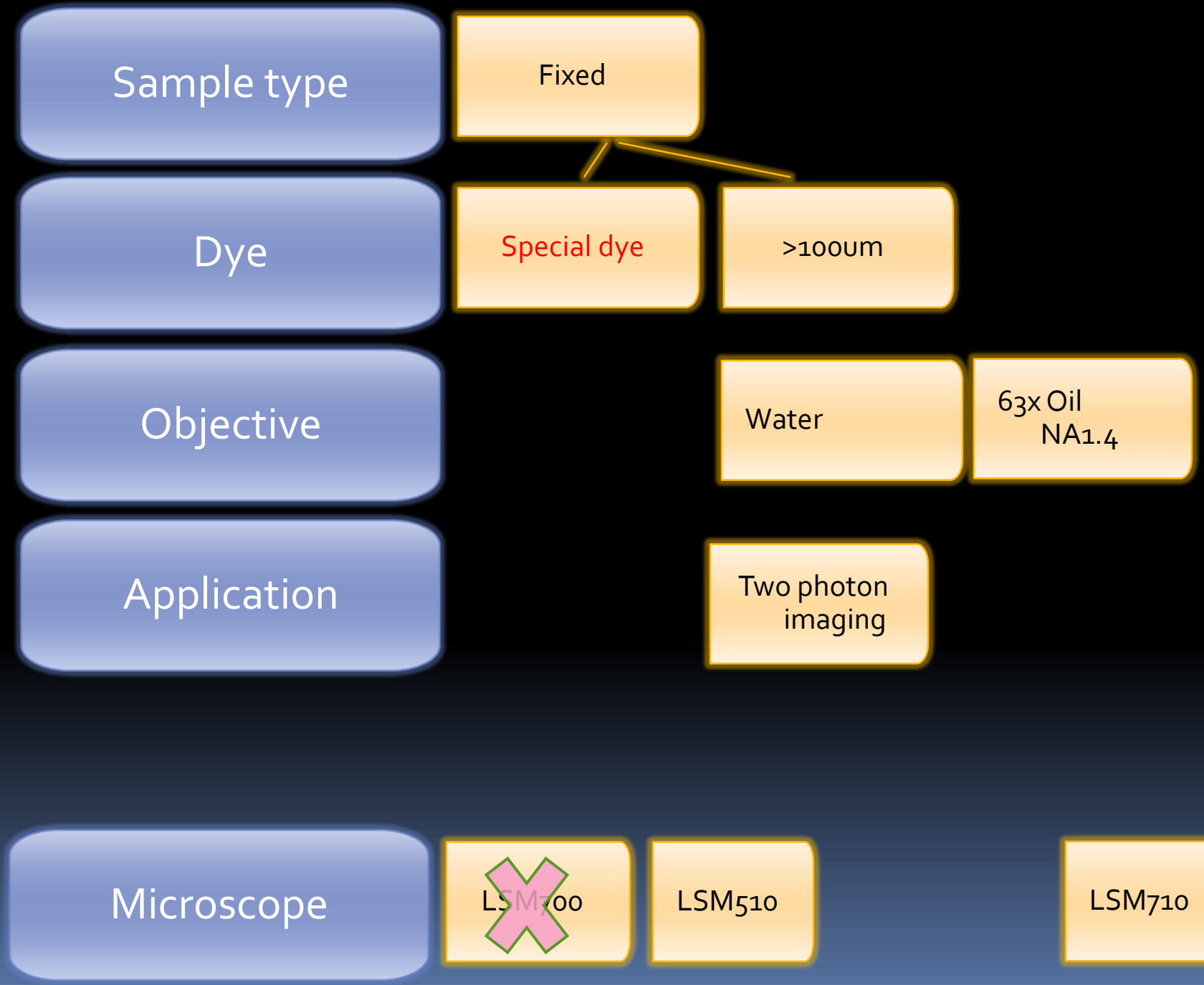
Which confocal microscope?

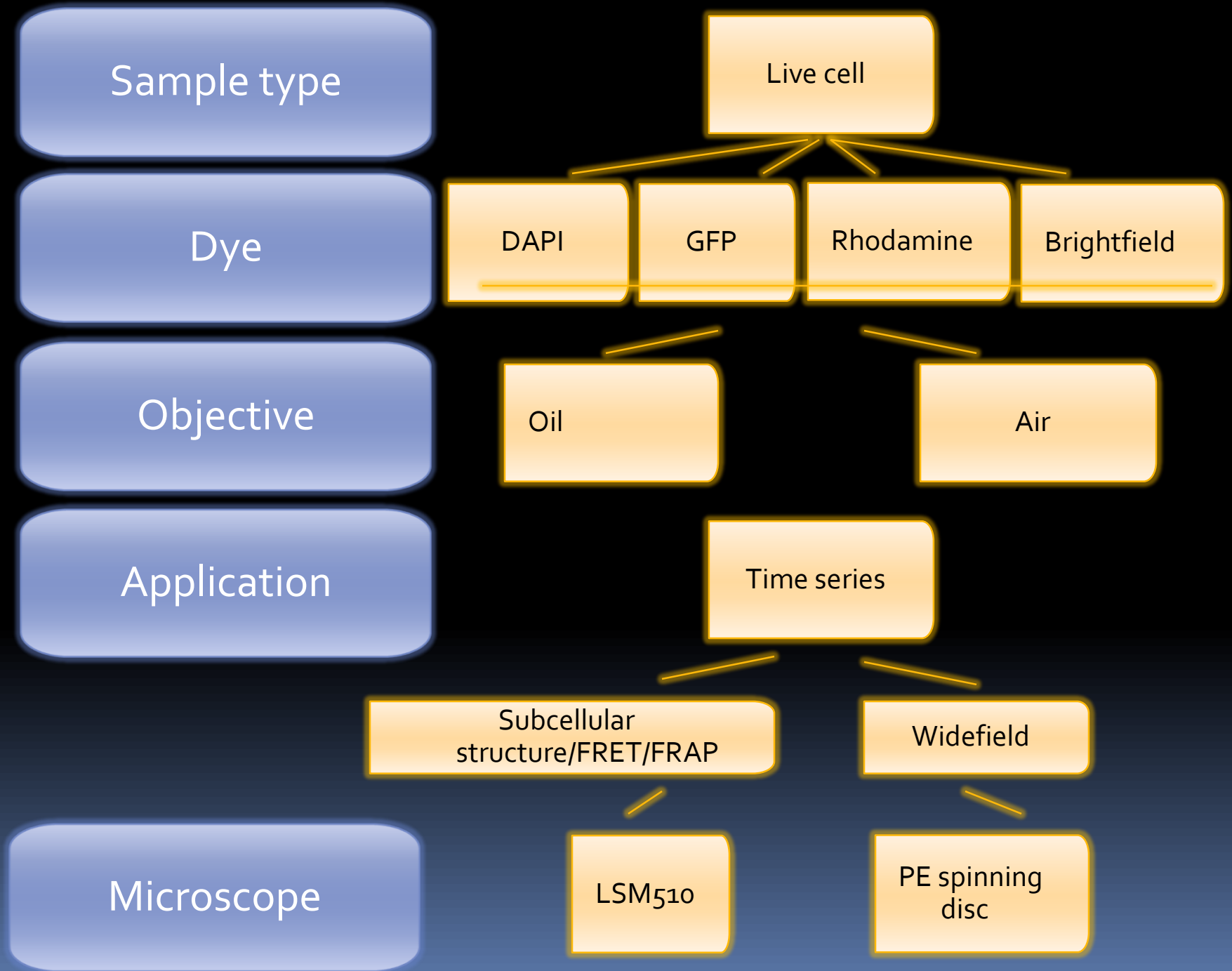
Sample type	Fixed	Live cell	Live animal
Dye	DAPI	GFP	Rhodamine
Objective	Air	Water	Oil
Application	2D/3D imaging	Time series	FRET/FRAP
	Line scan	Linear unmixing	Two photon imaging
Microscope	LSM700	LSM510	PE spinning disc	LSM710


Sample type
Fixed
Thin
PE spinning
Thick
710/510/700
Live cell
Temp &CO2 control
510/PE spinning
RT, No need CO2
510/PE spinning/700
Live animal
710


Sample type
Fixed
Dye
DAPI
GFP
Rhodamine
Cy5
Objective
63x Oil
NA1.4
Application
2D/3D
imaging
Microscope
LSM700
LSM510
LSM710


Sample type
Fixed
Dye
DAPI
GFP
Rhodamine
Objective
10x, 20x Air
LD
63x Oil
NA1.4
Application
2D/3D
imaging
Microscope
LSM700
LSM510
LSM710


Sample type
Fixed
Dye
Special dye
>100um
Objective
Water
63x Oil
NA1.4
Application
Two photon
imaging
Microscope
LSM700
LSM510
LSM710


Sample type
Dye
Objective
Application
Microscope
Live cell
DAPI
GFP
Rhodamine
Brightfield
Oil
Air
Time series
Subcellular structure/FRET/FRAP
Widefield
LSM510
PE spinning disc

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- Publications
- Image Gallery

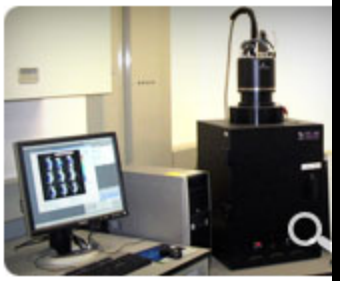
Faculty Core Facility
Address: L6-11, 6/F, Laboratory Block, 21 Sassoon Road, Pokfulam Hong Kong

Tel: 29864468
Fax: 29864297
E-mail: corefac@hku.hk

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, Masetro 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 4.6.3.0
- Detailed Configuration

Zeiss LSM 700 Inverted Confocal Microscope

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

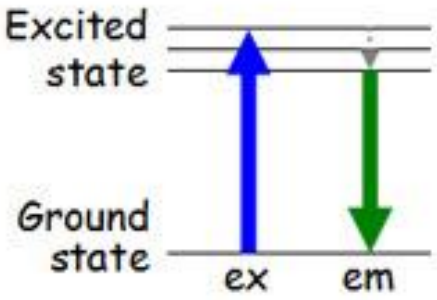
Zeiss LSM 510 Inverted Confocal Microscope

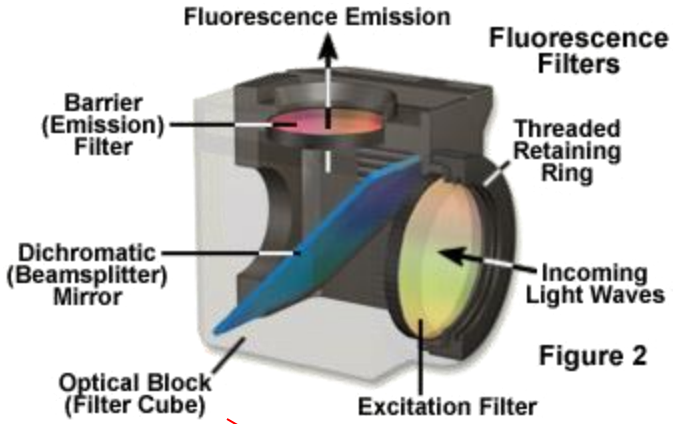
Specification	
Lasers	Argon (458,488,514 nm); HeNe laser (543 nm); HeNe-laser (633 nm); Chameleon tunable 2-photon laser (720-930nm)
Microscope	
Stand	Inverted: Axiovert 200M
XY stage	Motorized scanning stage
Filter cubes	#49 DAPI; #43 Cy3; #38 EGFP
Objectives	2.5x0.12;5x 0.15; 10x0.3; LD20x/0.4; LD40x/0.6;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

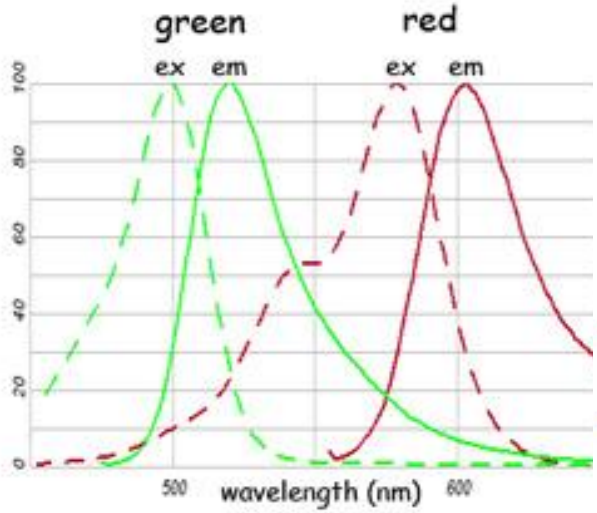
Before image acquisition:

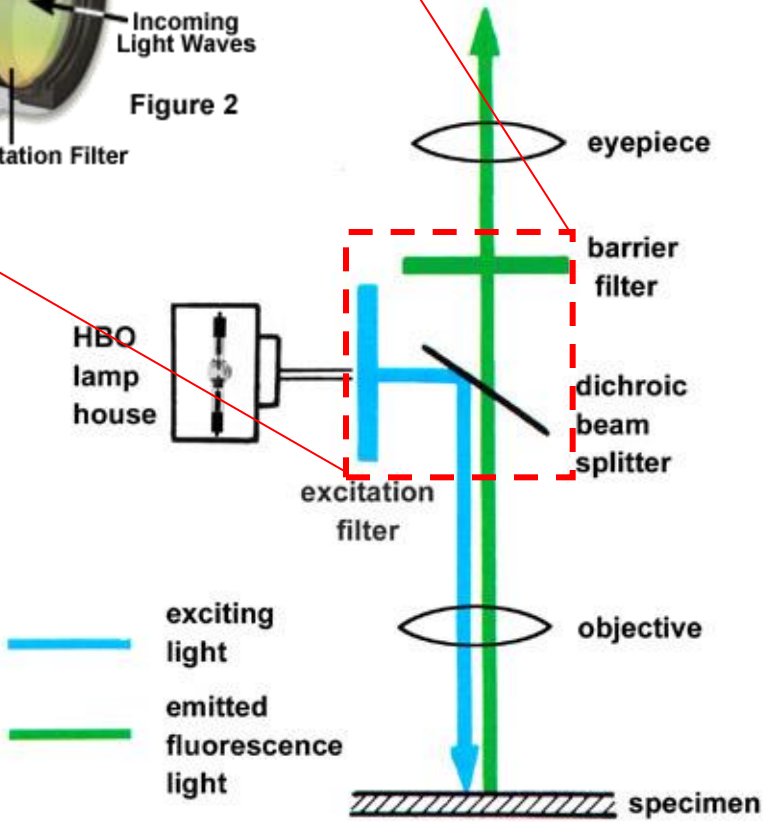
- To chose the right fluorophore
- To use the right coverslip (No. 1; No. 1.5; No. 2)
- To chose the right objective

Fluorescence

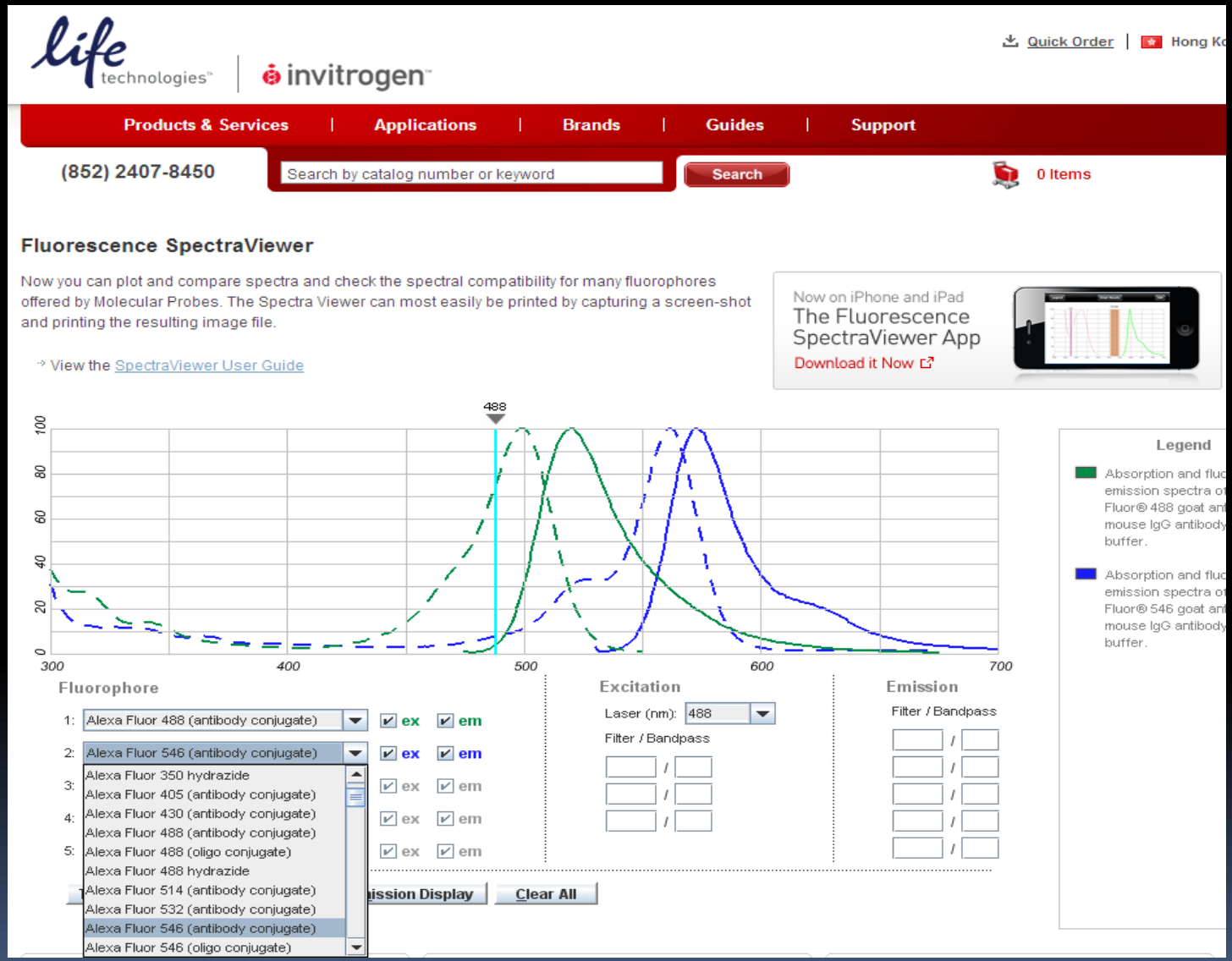








Fluorescence SpectraViewer

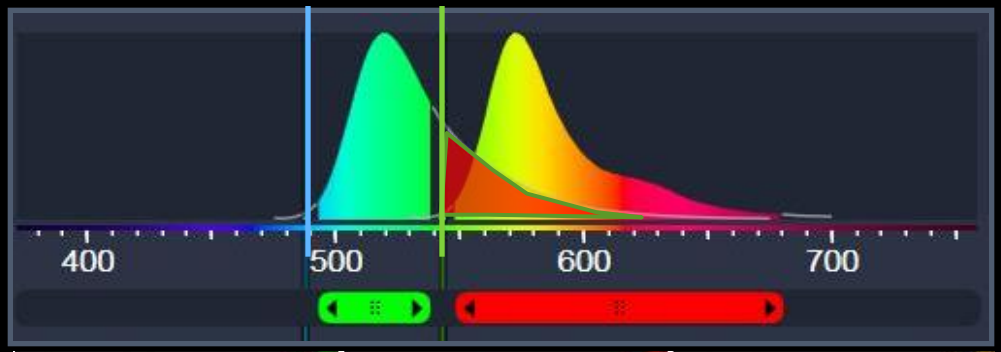


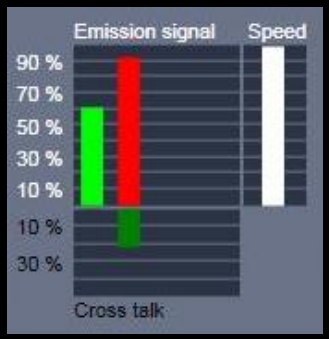
http://www.invitrogen.com/site/us/en/home/support/Research-Tools/Fluorescence-SpectraViewer.html

Multiple staining - the emission crosstalk problem

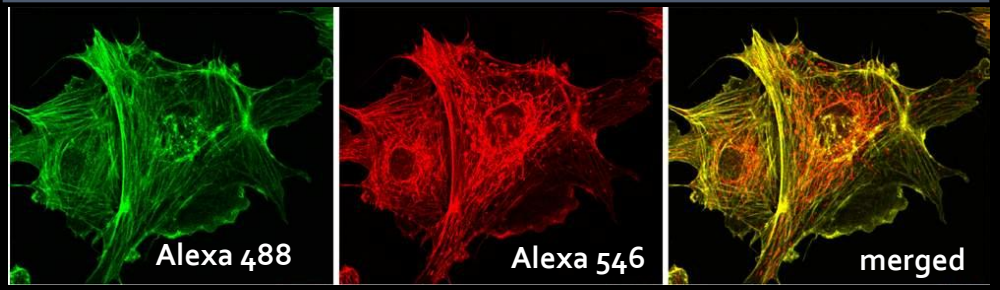
Alexa 488 em

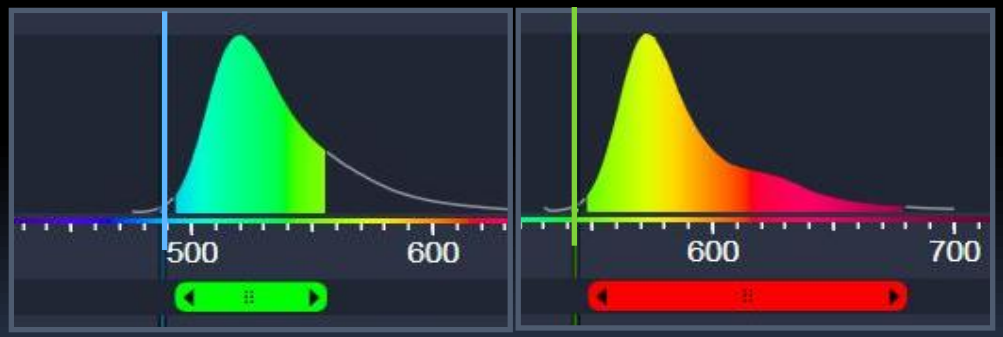
Alexa 546 em

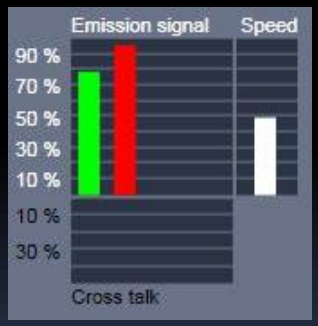




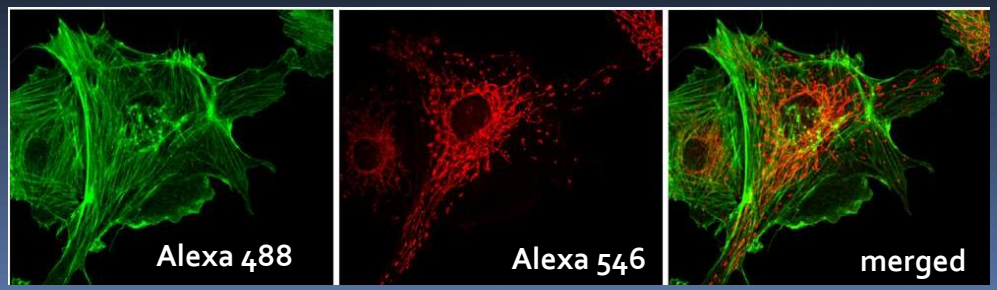
Simultaneous scan





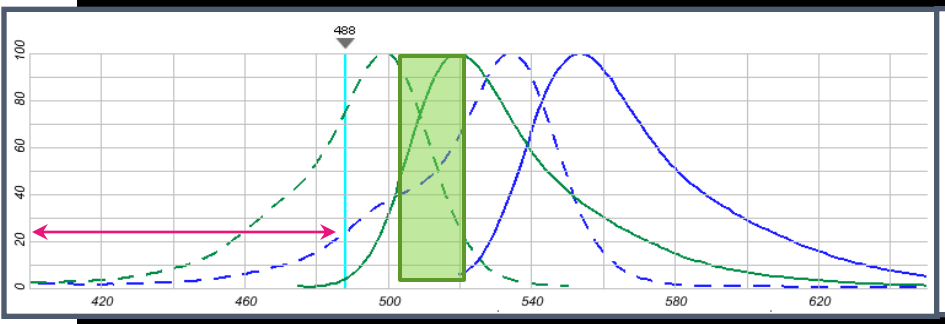


Sequential scan

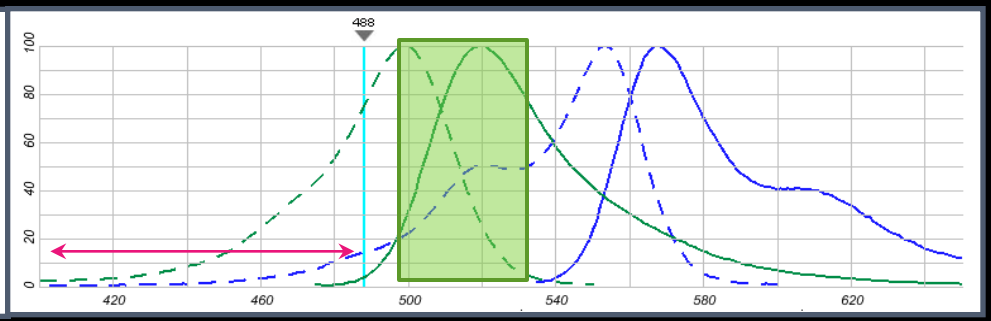


Multiple staining - the excitation crosstalk problem

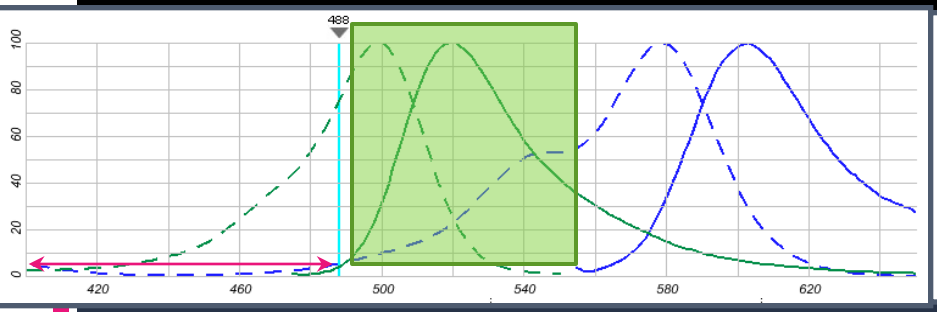
Alex fluo 488 +Alex fluo 532



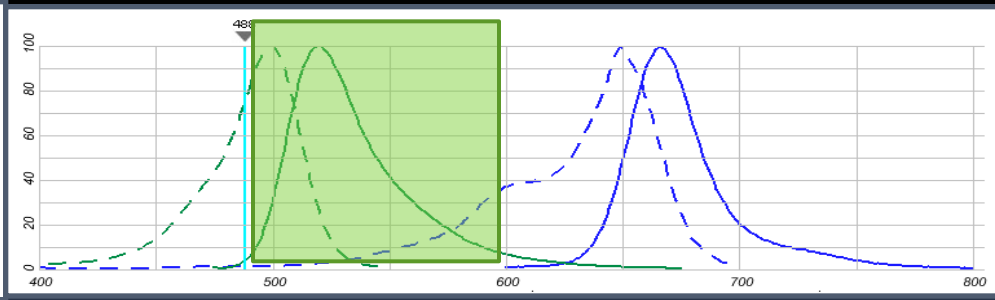
Alex fluo 488 +Alex fluo 555



Alex fluo 488 +Alex fluo 568



Alex fluo 488 +Cy5

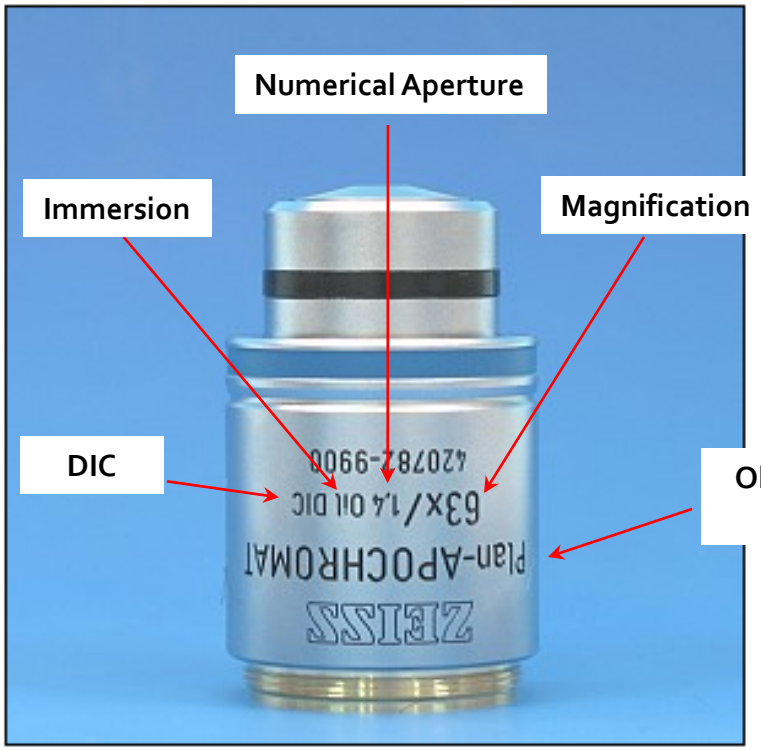


Knowing about objective

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

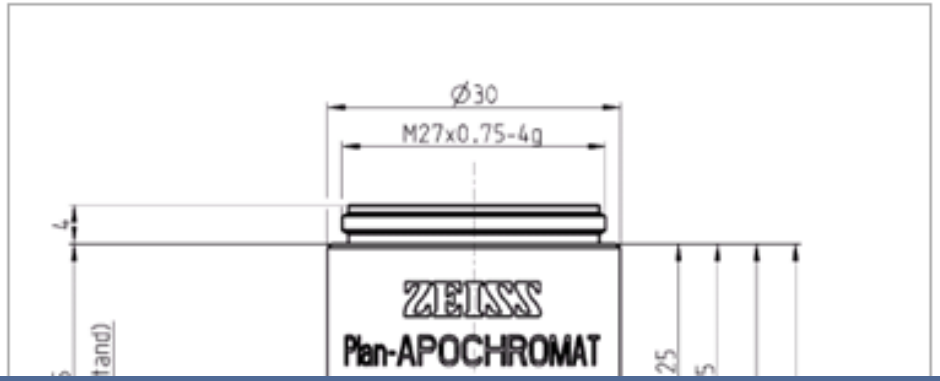
→ Transmittance curve	Objective "Plan-Apochromat" 63x/1.40 Oil DIC M27
	420782-9900-000
	Basket
Price	→ Price
Magnification	63x
Numerical Aperture	1.4
Working Distance [mm]	0.19
Coverglass Thickness [mm]	0.17
Thread Type	M27x0.75
Immersion	Oil
Field of View [mm]	25
Parfocal Length [mm]	45.06
Long Distance (LD)	
Correction Ring (Korr)	
Iris (Iris)	
Optical System	Infinity Color Corrected System (ICS)
Flatness	★★★★★
Color Correction	★★★★★
Biomedical Applications	
Fluorescence	■
- Multichannel	★★★★★
- Ultraviolet Transmission	★★★
- Infra Red Transmission	★★★★
BrightField (H)	■
Differential Interference Contrast	★★★★★

Objective "Plan-Apochromat" 63x/1.40 Oil DIC M27



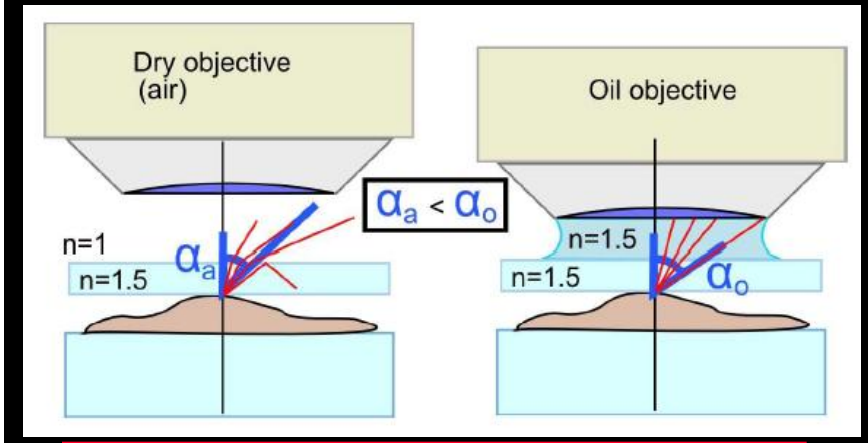
(WD=0.19mm), incl. "Immersol" 518 F, oiler 20ml and Cover glasses, high performance, D=0.17mm, box with 100 pc.

Mechanical Dimensions



Numerical aperture (NA) & resolution

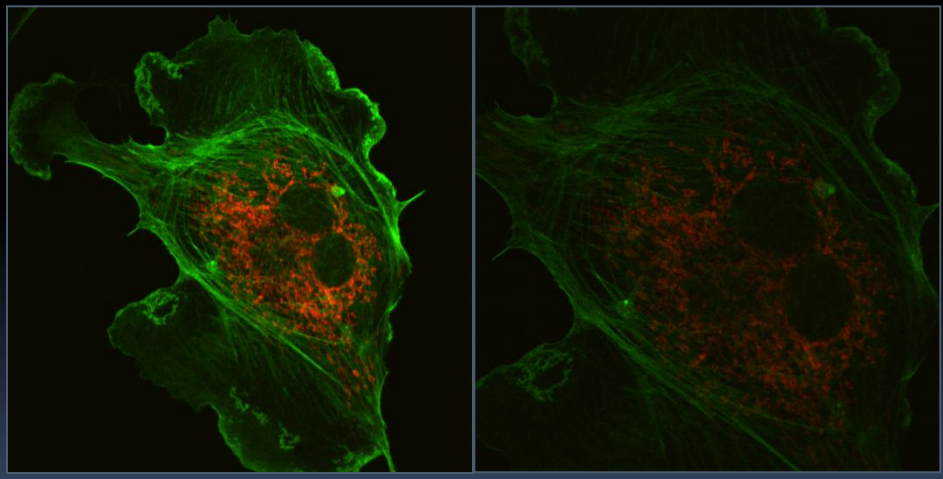
$$NA = n \sin(\alpha),$$



$$FWHM_{ill,lat} = \frac{0.51 * \lambda_{em}}{NA}$$

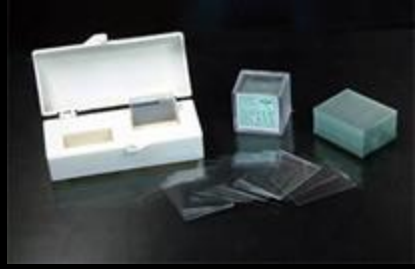
FWHM = Lateral Resolution [µm]
NA = Objective Numerical Aperture
λ_{em} = Emission Wavelength [nm]

λ = 520nm
NA = 1.4
FWHM = 189nm



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

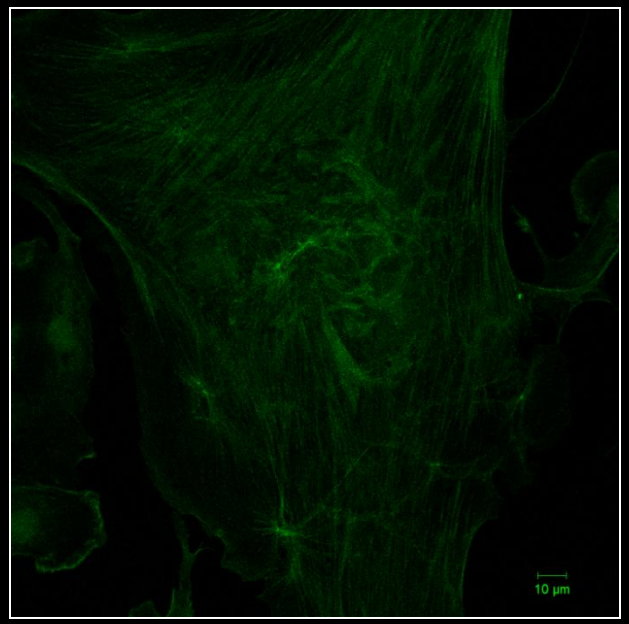
Coverslip



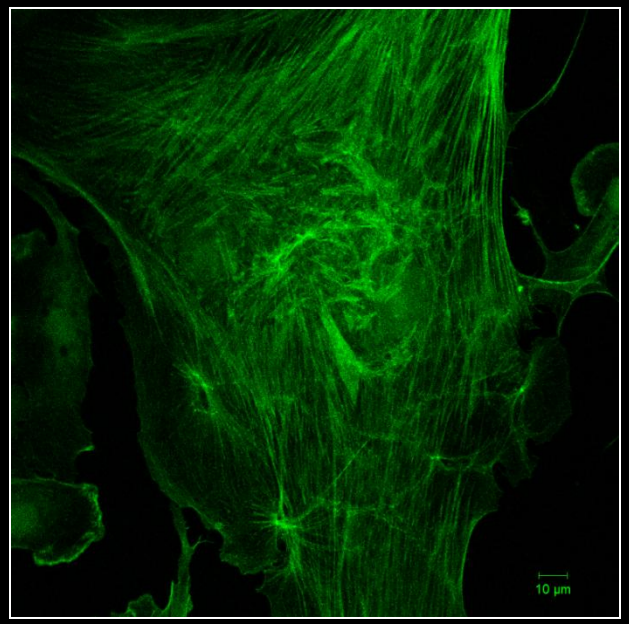
Number	Ideal thickness	Range
#0	100 µm	80-130 µm
#1	150 µm	130-170 µm
#1.5	**170 µm**	**160-190 µm**
#2.0	220 µm	190-250 µm

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 (eg 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.

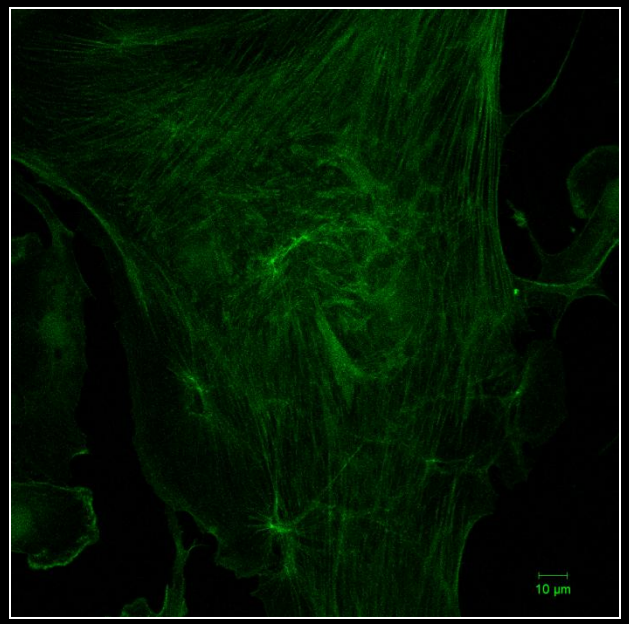
Coverslip



0.15mm



0.17mm

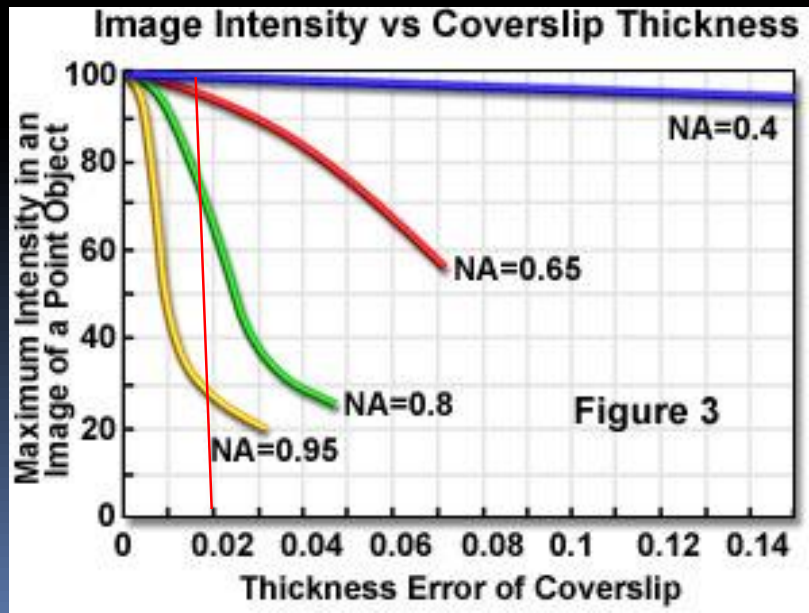


0.19mm

BPAE cells
Alexa Fluor® 488 phalloidin

Excitation: 488nm

Same settings:
Laser power
Master Gain
Offset
Digital gain



Applications

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

Acknowledgement

Faculty Core Facility Committee

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All Faculty Core Facility Users!