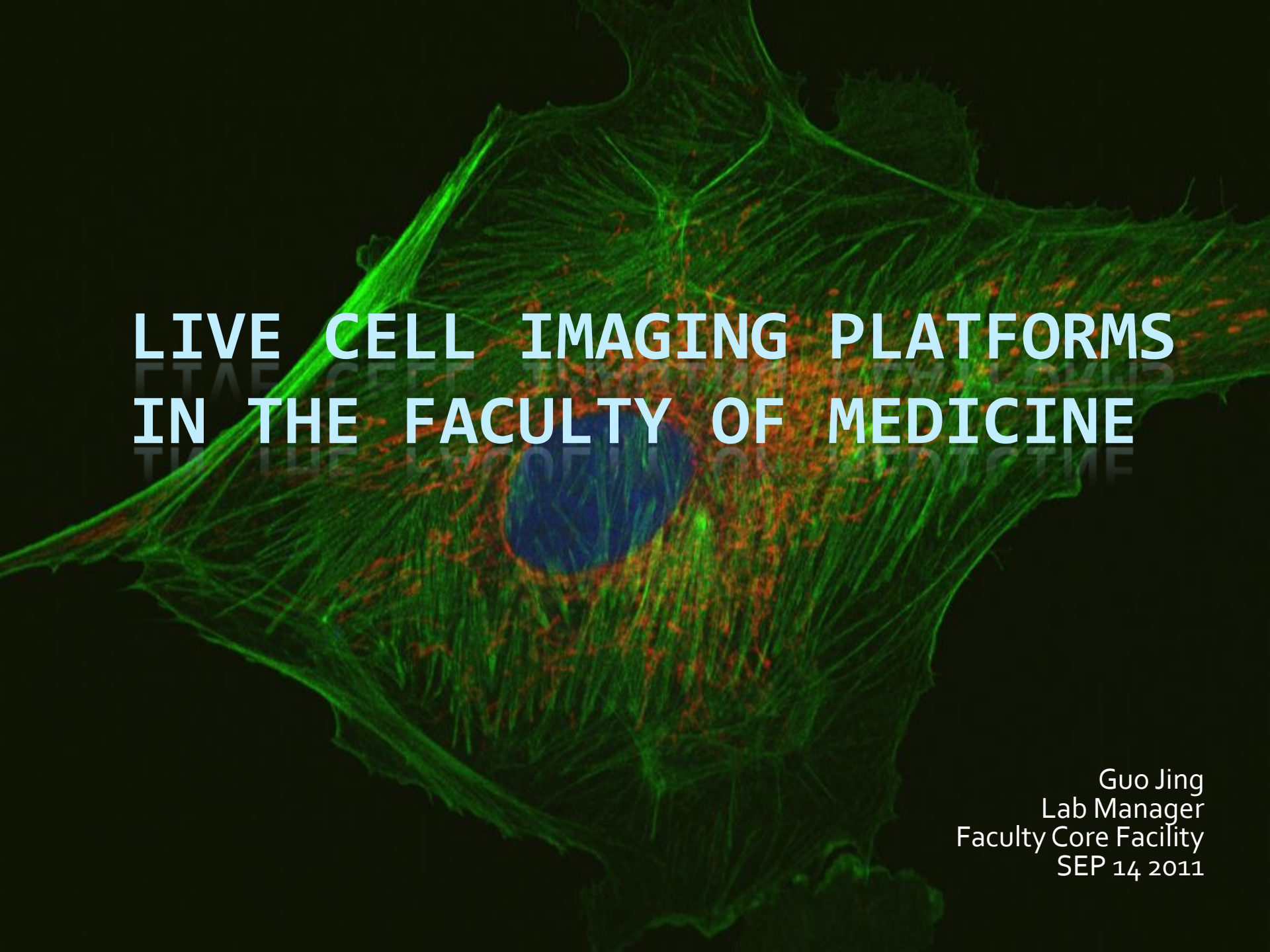


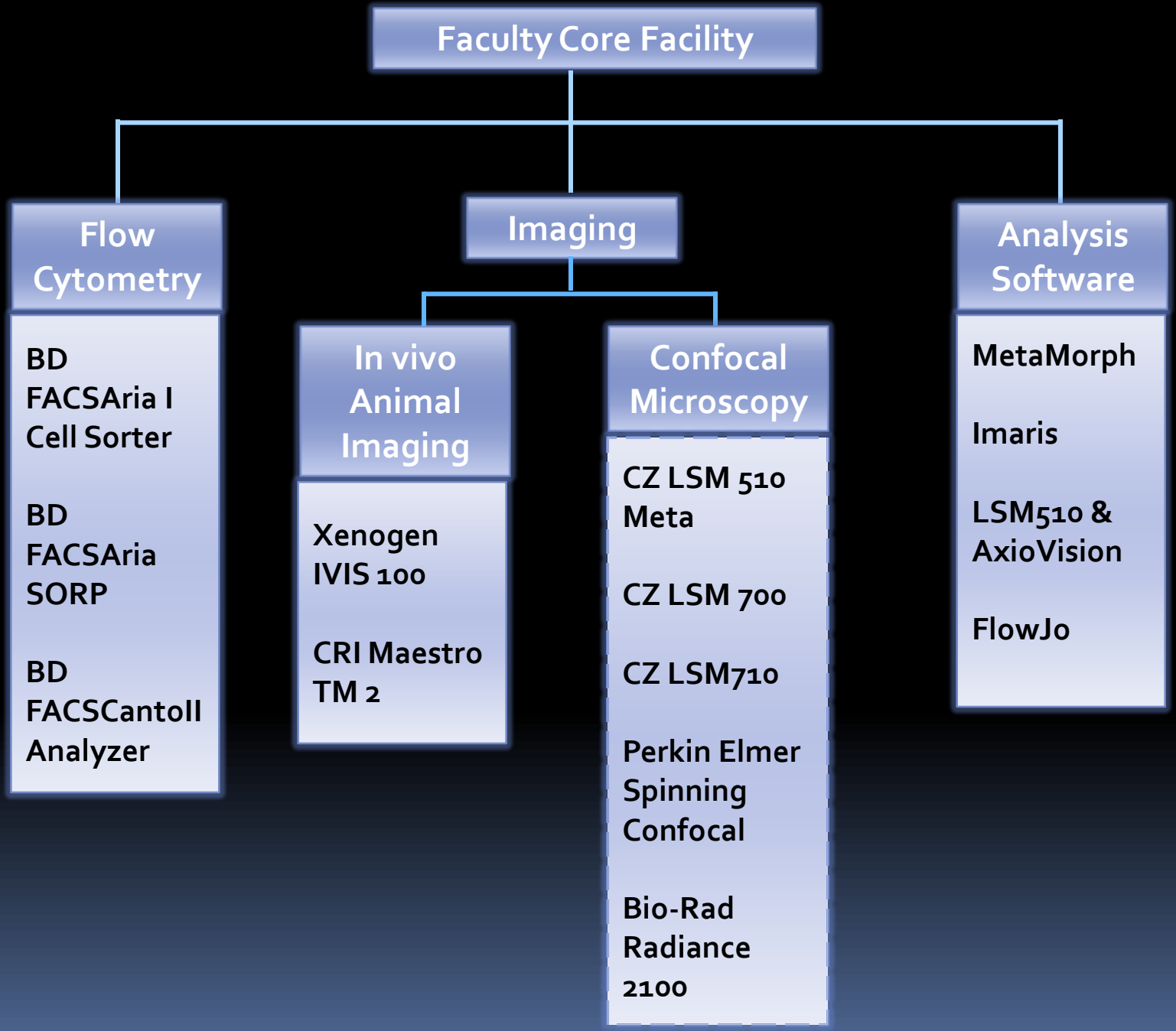
LIVE CELL IMAGING PLATFORMS IN THE FACULTY OF MEDICINE

Guo Jing
Lab Manager
Faculty Core Facility
SEP 14 2011

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

Mission

- Training and education
  - Basic operation
  - Advanced applications
  - Imaging analysis
- Advice & consultation
- New technology development and collaboration
- 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
CZ LSM 700
CZ LSM710
Perkin Elmer Spinning Confocal
Bio-Rad Radiance 2100
Analysis Software
MetaMorph
Imaris
LSM510 & AxioVision
FlowJo

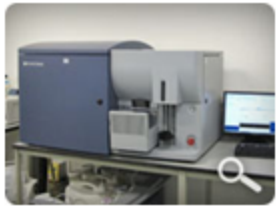
- Introduction
- Committee of Management
- Equipment
- Online Booking System
- Charges
- Usage Policies and Guidelines
- Workshops and Training
- Protocols
- Usage records
- Publications
- Image Gallery

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

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

Equipment — Flow Cytometry

BD FACSAria I Cell Sorter:



- 488nm blue laser:
  - FITC (530/30nm)
  - PE (585/42nm)
  - iii. PE-Texas Red/PI (616/23nm)
  - iv. PerCP-Cy5.5(695/40nm)
  - v. PE-Cy7 (780/60nm)
- 633nm red laser:
  - APC (660/20nm)
  - ii. APC-Cy7(780/60nm)
- 407nm violet laser:
  - Pacific Blue/Horizon V450 (450/50nm)
  - ii. Pacific Orange/Horizon V500 (530/30nm)

BD FA
- 488
  - •
  - ii.
- 640
  - •
  - ii.
- 561
  - •
  - ii.
  - iii.
  - iv.
- 405
  - •
  - ii.

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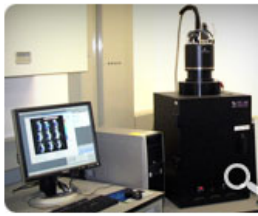
Equipment — Imaging

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

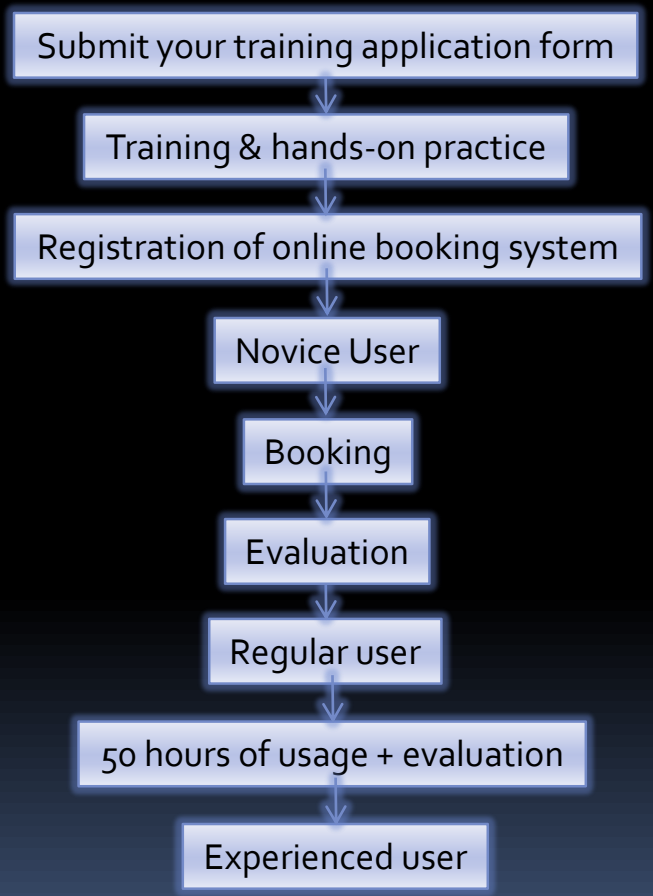
- Fluorescent tumor and non-tumor models
- OS/Software: Windows XP, Masetro TM Om — Vivo Imaging System, version 2.10.0

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

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

Instrument	Office hour (HKD/Hour)	Non-office hour
BR Radiance 2100	20	16
LSM 510	120	96
LSM 700	100	80
LSM710	120	96
PE-ERS confocal	80	64
PE-ERS widefield	33	33
FACSCanto II	40	40
FACSAria I	100	80
FACSAria SORP	130	100
Xenogen IVIS100	40/session	N/A
CRI Maestro TM 2	N/A	N/A
Imaris	0	0
MetaMorph	0	0
LSM510	0	0
FlowJo	0	0

*Technical support: 100HKD/Hour
*Technical support is mandatory for Novice users.

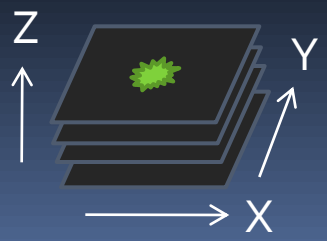
- Charges will apply based on the time reserved and/or time used, whichever is longer. No booking of partial session is allowed.
- Same charges will apply for no-show/ failure to cancel booking/late-arrival.
- Same charges will apply for overrun of experiment beyond the booking time. Overrun will only be allowed if there is no overlapped booking.

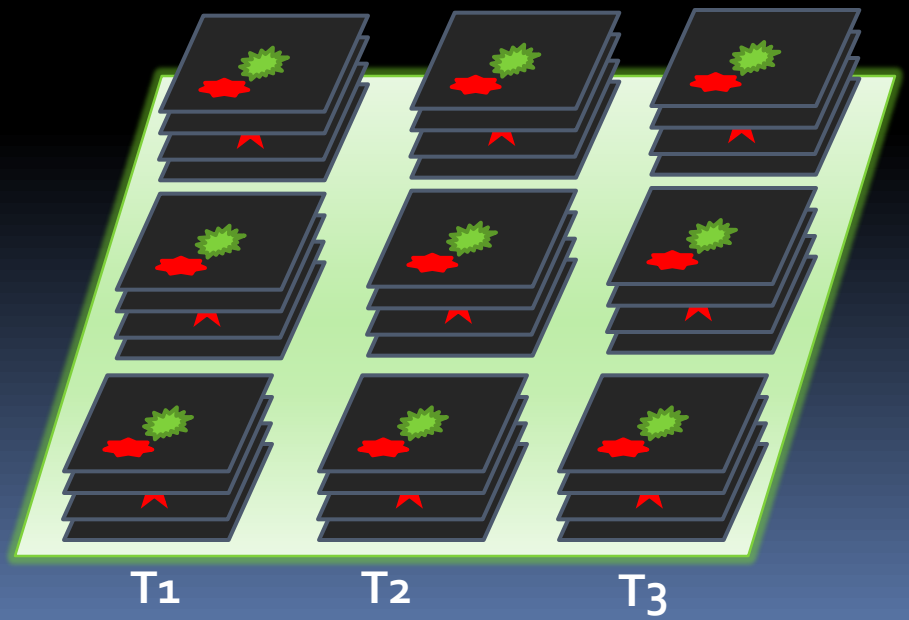
Why Live cell imaging?

- Physiological condition
- Single cell based
- Better time resolution
- Cellular dynamics
- Multi-dimensional imaging acquisition

- 2D: XY
- 3D: XY+Z
- 4D: W+ XY + Z
- 5D: T + W + XY + Z
- 6D: P+ T+ W + XY + Z

W= Wavelength
T=Time
P= Position

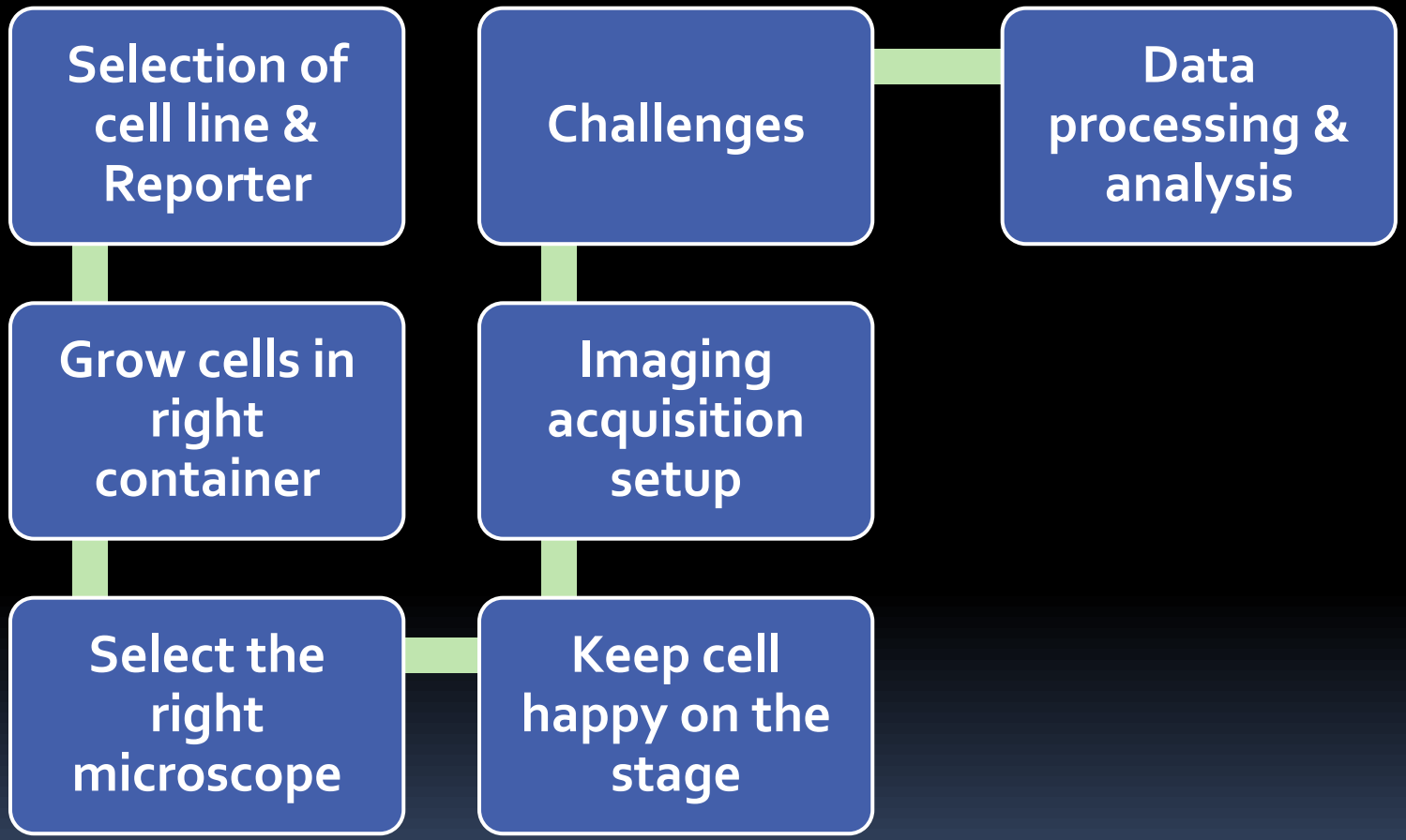




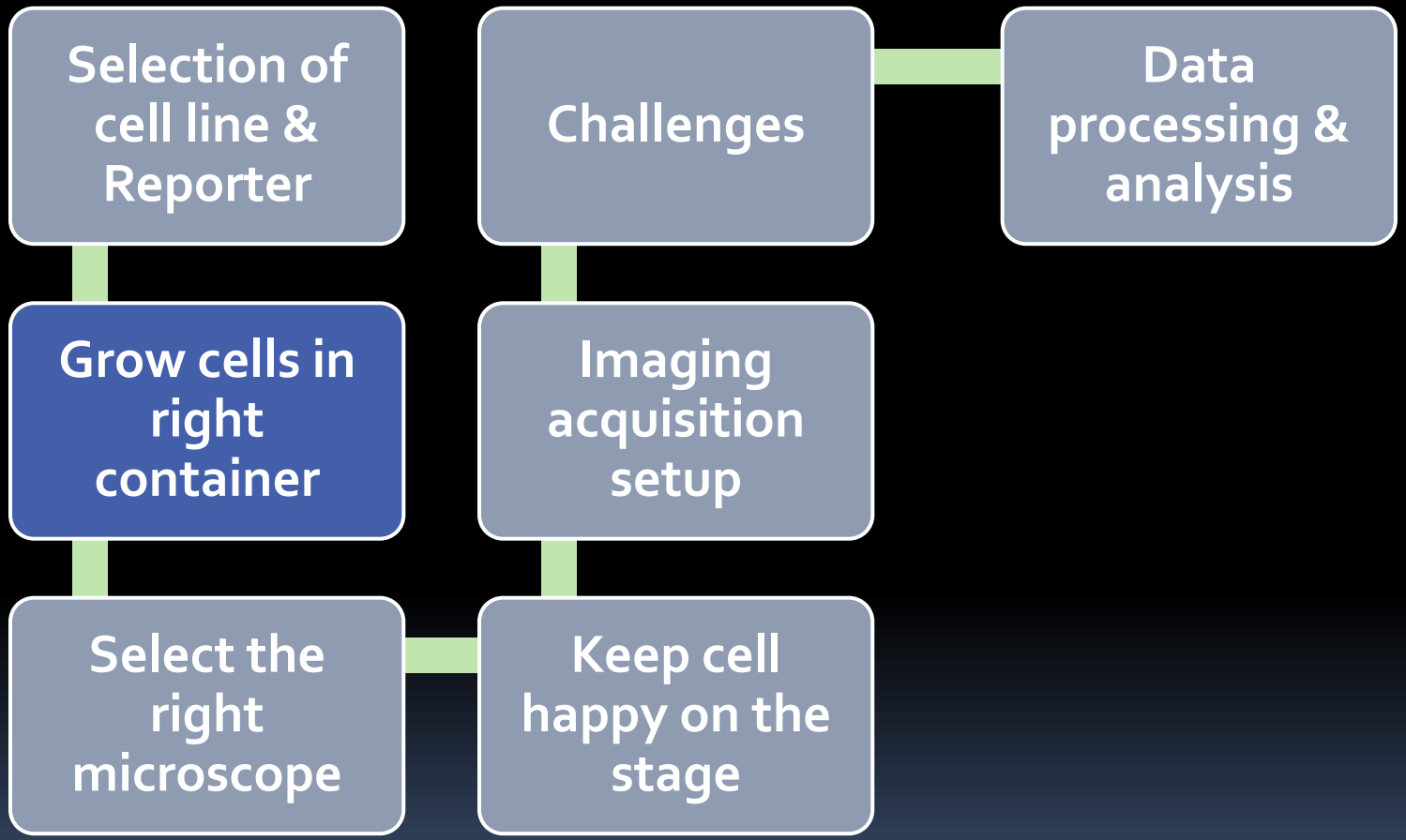
Live Cell Imaging Applications

- Dynamic Fluorescence - Calcium Signaling
- Cell Motility - Cellular movement and differentiation, morphological response to stress and environment
- Neurobiology - Interaction of neurons and neuroglial cells, the growth of axons/dendrites
- Elucidation of cellular signaling pathways
- Protein & vesicle tracking
- Cell cycle and development studies
- FRET/FLIM analysis of molecular interaction
- ……..

Workflow of Live Cell Imaging

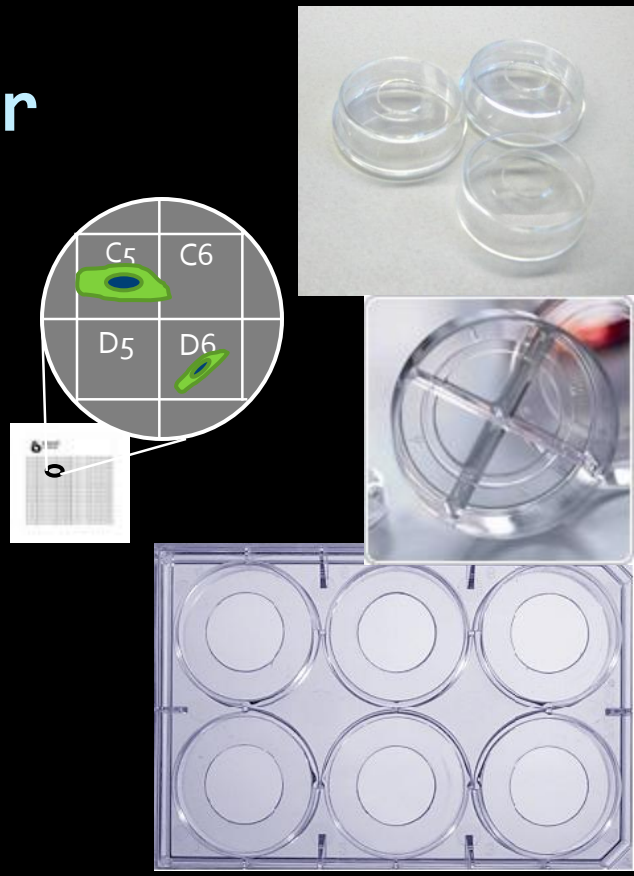


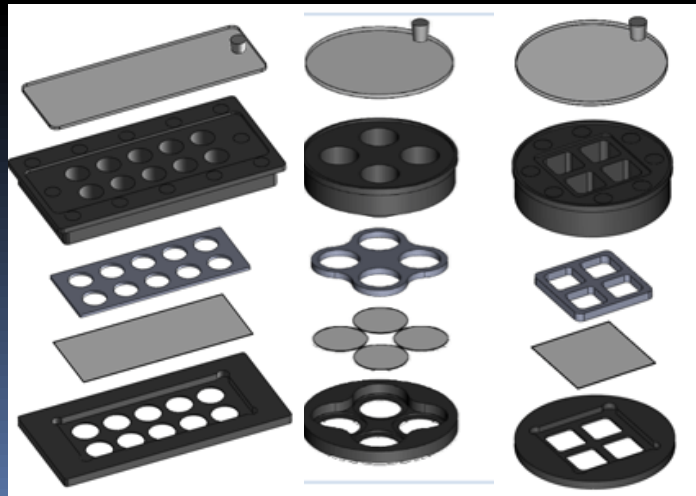
Workflow of Live Cell Imaging



Grow cells in right container

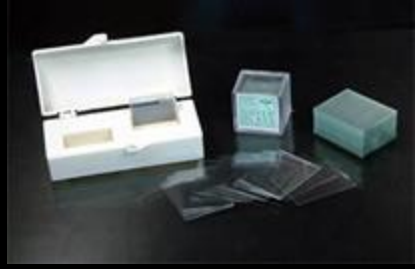
- Coverslip bottom dish; No. 1.5 coverslip; coating
- Cell chamber for coverslip (convenient for fixation after live cell imaging)
- Grid coverslip – locate individual cell during & after live cell imaging (The coverslip has 200 alphanumeric locations in a diamond pattern and each measures 0.6 x 0.6mm with line thickness of .02mm)
- Plastic is not transparent enough for fluorescence and is frequently itself autofluorescent. Additionally, plastics polarize light and are not suitable for DIC imaging
- Both plastic and glass slides are too thick for use with high numerical aperture objectives





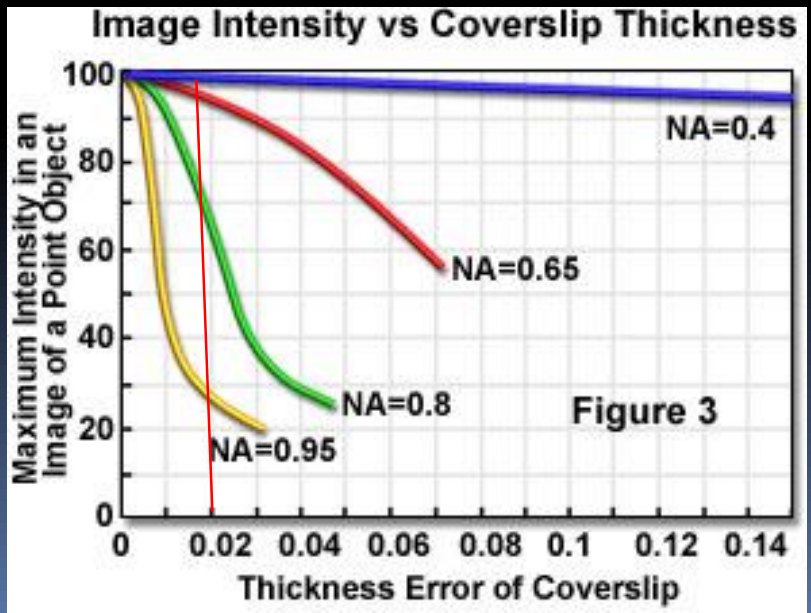
http://www.glass-bottom-dishes.com/product.html
http://www.chamlide.com/

Coverslip

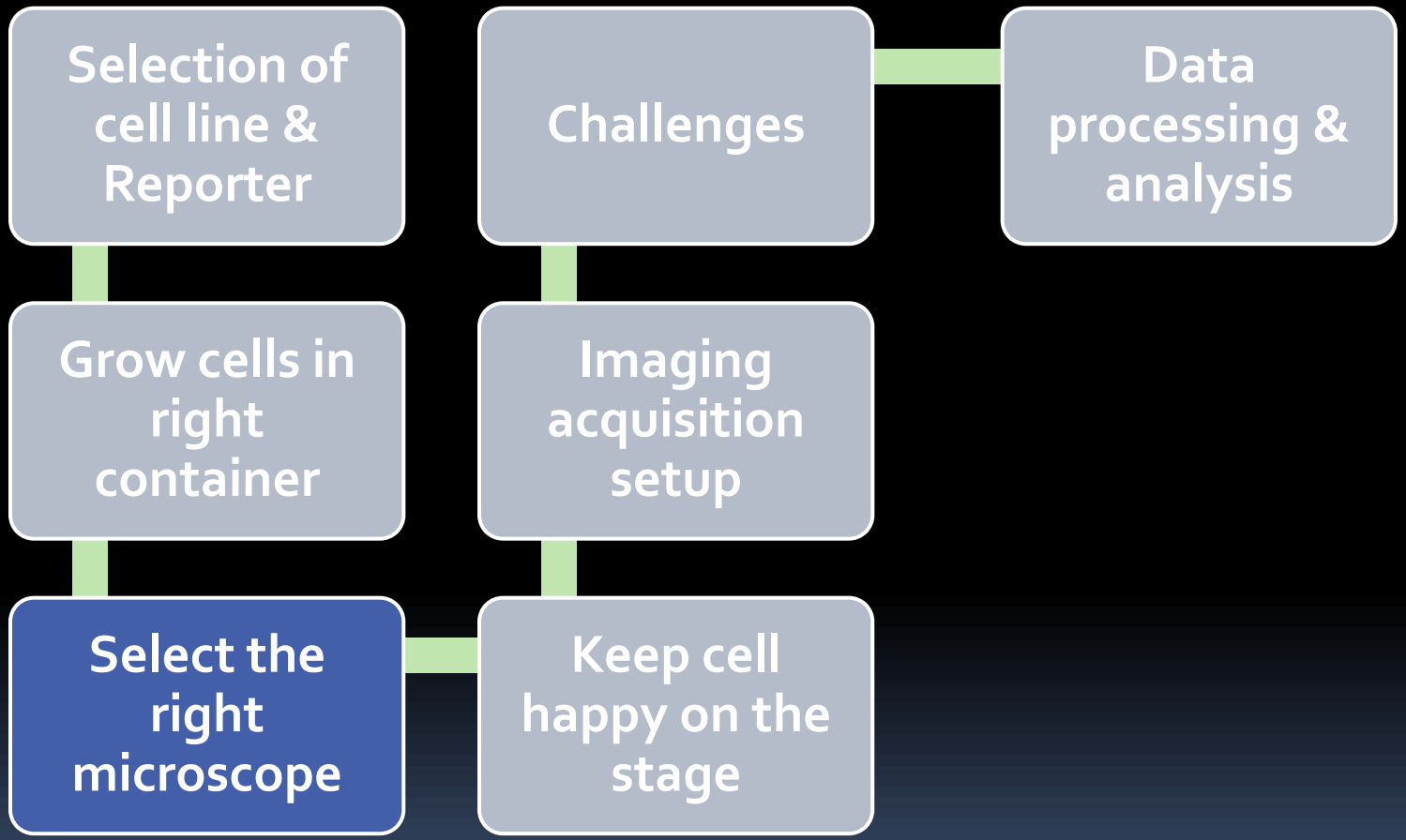


Number	Ideal thickness	Range
#0	100 µm	80-130 µm
#1	150 µm	130-170 µm
#1.5	**170 µm**	**160-190 µm**
#2.0	220 µm	190-250 µm

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.



Workflow of Live Cell Imaging



Which confocal microscope?

PE Spinning Disc Confocal

CZ LSM 710 Confocal

CZ LSM700 Confocal

CZ LSM510 Confocal

Which microscope do I need to use for live cell microscopy?

Sample type	Thin	Thick	Live animal
Dye	DAPI	GFP	Rhodamine
Objective	Air	Water	Oil
Environment	PH	Temperature	Humidity
Application	2D/3D imaging	**Time series**	FRET/FRAP
	Line scan	Linear unmixing	Two photon imaging
Microscope	LSM700	LSM510	PE spinning disc	LSM710

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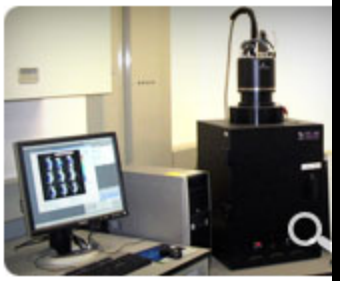
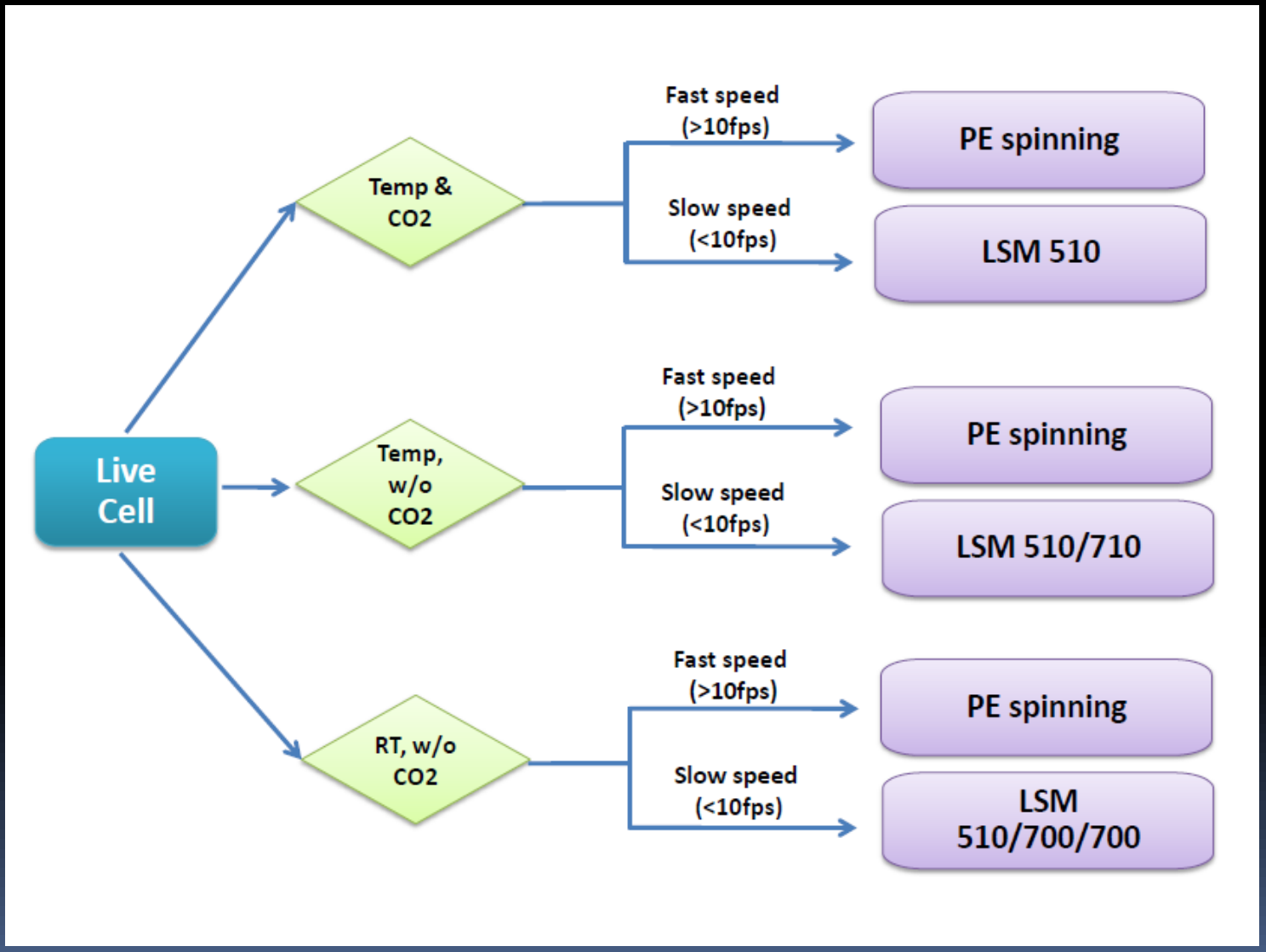


CRI Maestro TM 2 in vivo imaging system

- 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


Live Cell
Temp & CO2
Temp, w/o CO2
RT, w/o CO2
Fast speed (>10fps)
Slow speed (<10fps)
PE spinning
LSM 510
Fast speed (>10fps)
Slow speed (<10fps)
PE spinning
LSM 510/710
Fast speed (>10fps)
Slow speed (<10fps)
PE spinning
LSM 510/700/700

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	5 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

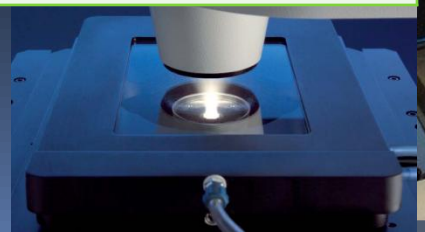
Temp √
CO_2 √

DAPI √
GFP √
Rhodamine √
Cy5 √

DAPI : LD objectives

Temporal resolution : low

Laser manipulation √
2p imaging √
5D: T + W + XY + Z



Perkin Elmer Spinning Confocal Microscope

Specification	
Lasers	Argon/Krypton laser (488, 568nm)
Microscope	
Stand	Inverted: Axio Obsever
XY stage	Motorized scanning stage
Filter cubes	#49 DAPI; #10 Alexa 488; #15 Alexa 546
Objectives	10x 0.3 Ph1; 20x 0.4 LD Ph2; 40x 0.6 LD Ph2; 63x1.4 Oil Ph3; 100x 1.4 Oil Ph3
Accessories	CCD camera Live cell Incubator Piezo objective focus
Scan Module	
Scan mode	xy, xyz, xyt,
Scanning speed	30f/s
Detector	EM CCD camera
Software	Windows XP, Metamorph Version 7.7.2.0
Application	Phase contrast imaging; Xy 2D imgaging; multi-spectrum fluorecence imaging; Z-stack 3D imaging; live cell imaging Multidimentional widefiled acquization with CCDcamera

Widefiled √

Confocal √

Temp √
CO_2 √

DAPI √
GFP √
Rhodamine √

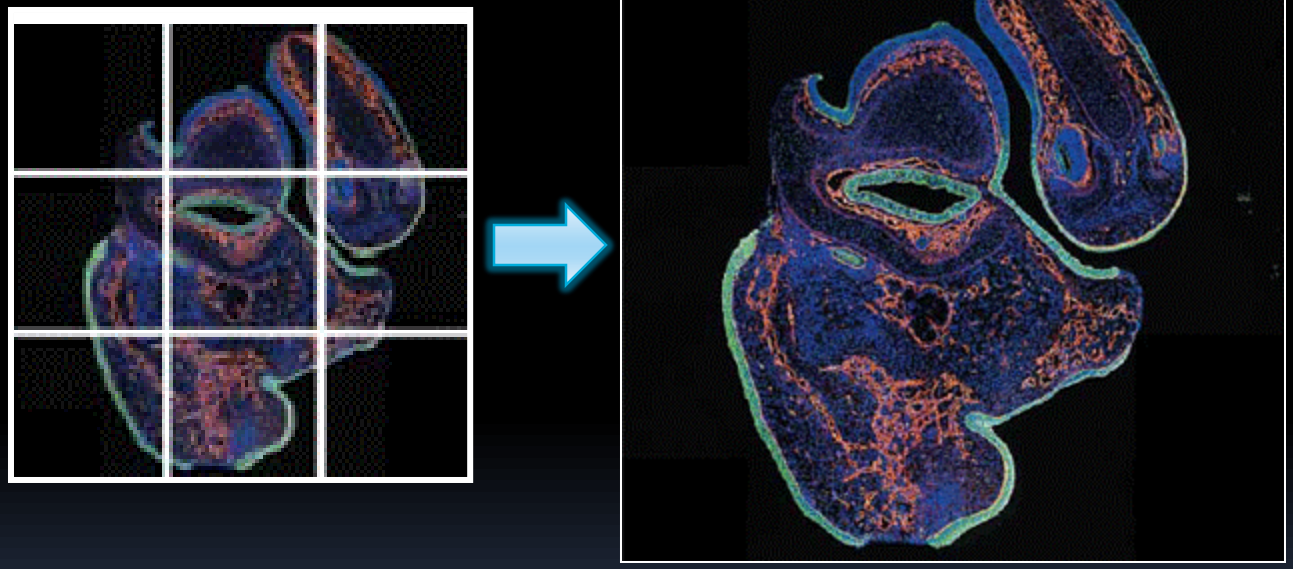
GFP √
Rhodamine √

TR: low
Up to 7fps

T R: High
30 fps

6D: P + T + W + XY + Z
Tile scan + Stitch

Tile scan + stitching



Zeiss LSM 710 Upright Confocal Microscope

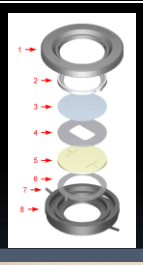
Specification	
Lasers	Argon (458,488,514 nm); HeNe laser (543 nm); HeNe-laser (633 nm); Spectra Physics MaiTai HP tunable 2-photon (690-1040nm)
Microscope	
Stand	Upright: Axio Examiner
XY stage	Mechanical stage for small animal imaging
Filter cubes	#49 DAPI; #43 Cy3; #38 EGFP
Objectives	5x0.16;10x 0.3; 20x0.5; 40x0.95; 63x1.4 oil DIC; 10x0.3W; 20x1.0WDIC; 63x1.0W
Accessories	Digital microscope camera AxioCam FCS3 closed chamber
Scan Module	
Scan mode	xy, xyz, xz, xt, xyt, lambda
Scanning speed	5 frames/sec with 512 × 512 pixels
Detector	32-array PMTs + 2 single PMTs (spectral detection resolution); 1 transmitted light PMT 2 channels NDD module for reflected fluorescence
Filter set for NDD	Red/green (BP 565-610 /BP 500-550); CFP/YFP (BP455-500/ BP 520-560)
Software	Windows Vista, ZEN 2009 version 5.5 SP1; Physiology for ZEN 2009
Application	DIC imaging; Spot//line Scan; Xy 2D imgaging; multi-spectrum fluorecence imaging; Z-stack 3D imaging; lambda scan, linear unmixing; online fingerprinting, colocalization, live cell imaging; FRAP, FRET, two photon imaging, small animal imaging

Temp √
CO_2 X

DAPI √
GFP √
Rhodamine √
Cy5 √

Temporal resolution : low

Laser manipulation √
2p imaging √
5D: T + W + XY + Z



Zeiss LSM 700 Inverted Confocal Microscope

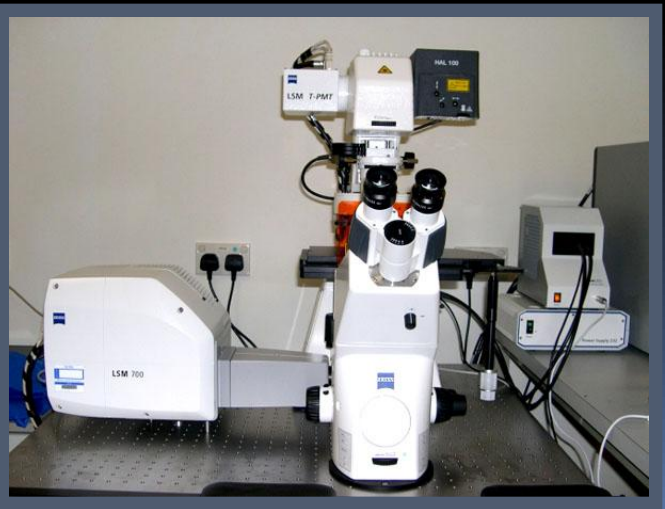
Specification	
Lasers	Solid state lasers: 405nm (5mW); 488nm (5mW); 555nm (10mW); 639nm (10mW)
Microscope	
Stand	Inverted: Axio Observer
XY stage	Manual stage 130x85
Filter cubes	#49 DAPI; #43 Cy3; #38 EGFP
Objectives	10x 0.3 Ph1; 20x0.8 Ph2; 40x1.3 Oil Ph3; 63x1.4 oil Ph3
Scan Module	
Scan mode	xy, xyz, xz, xt, xyt, lambda
Scanning speed	5 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; FRAP/FRET

Temp X
CO_2 X

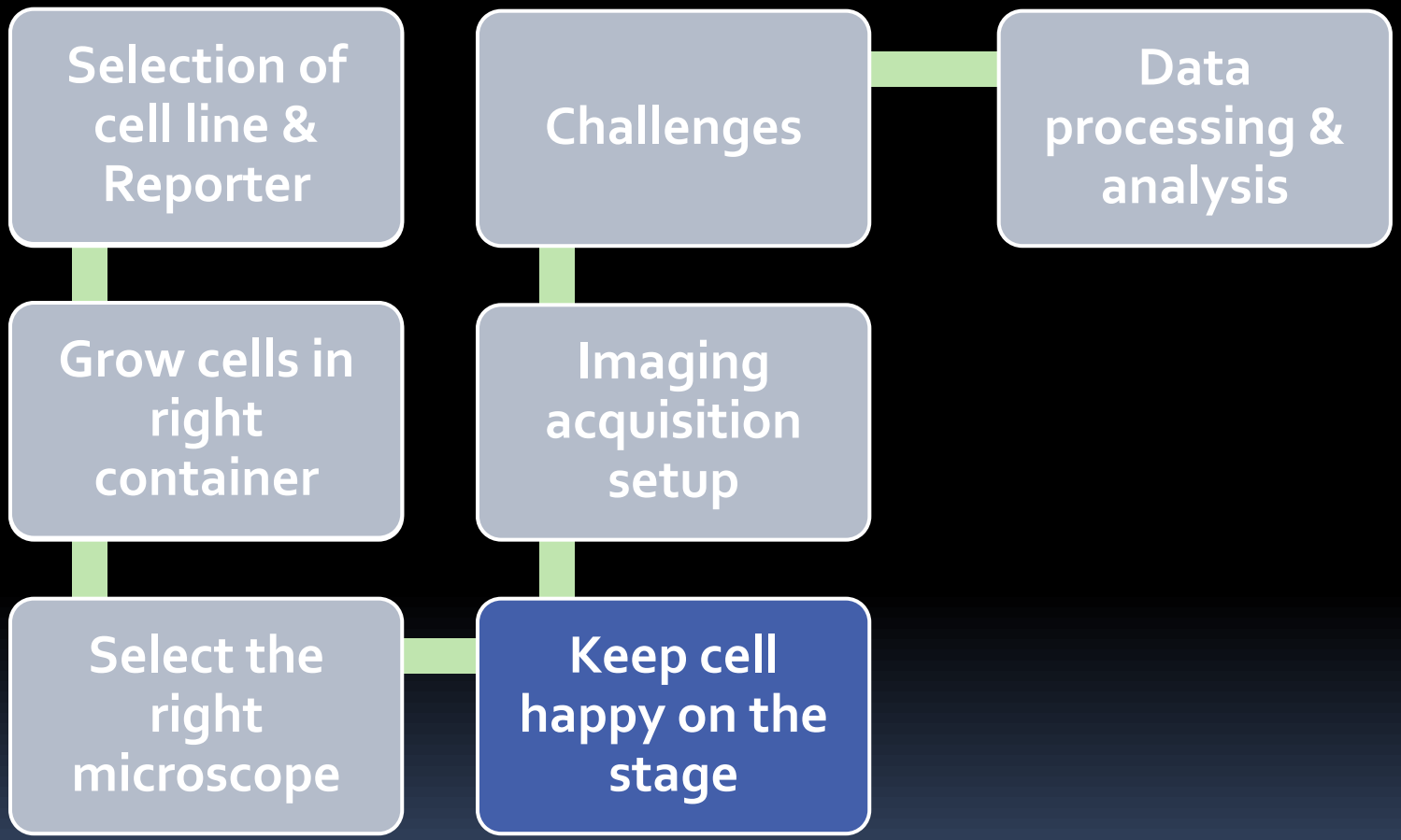
DAPI √
GFP √
Rhodamine √
Cy5 √

Temporal resolution : low

Laser manipulation √
5D: T + W + XY + Z



Workflow of Live Cell Imaging



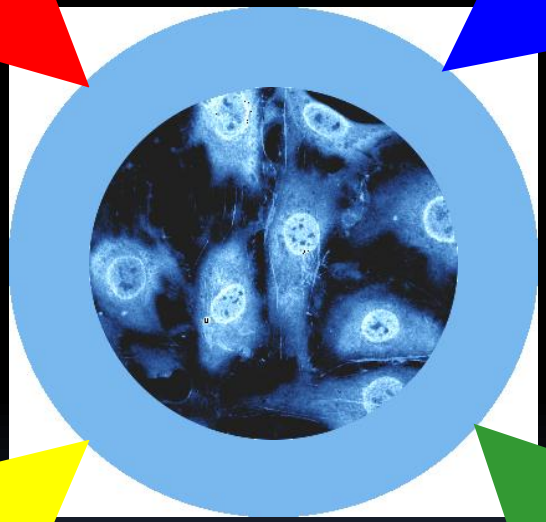
Live cell imaging: Enviromental Controls

Temperature

Heating/Temperable Stages, Inserts, Mounting Frames, Incubators, Objective Heaters

Humidity

Humidifier System
Humidifier Modules



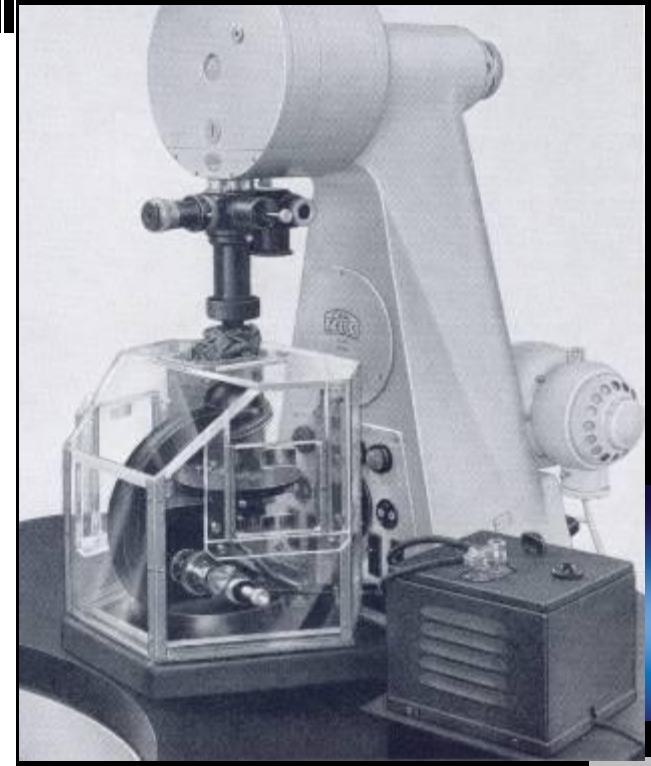
CO_2 control with Incubators
Carbonate buffer for stabilization

pH-Value

Incubator O_2 control
Displacement of O_2 by Nitrogen

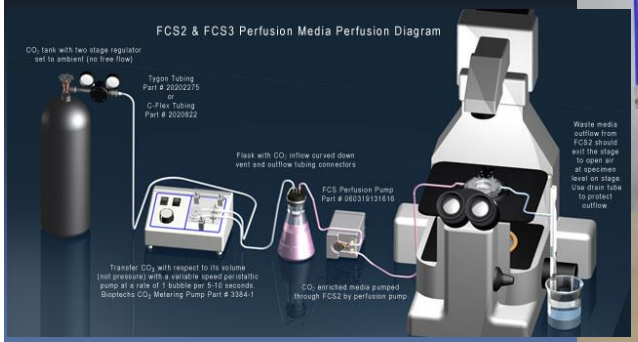
Oxygen

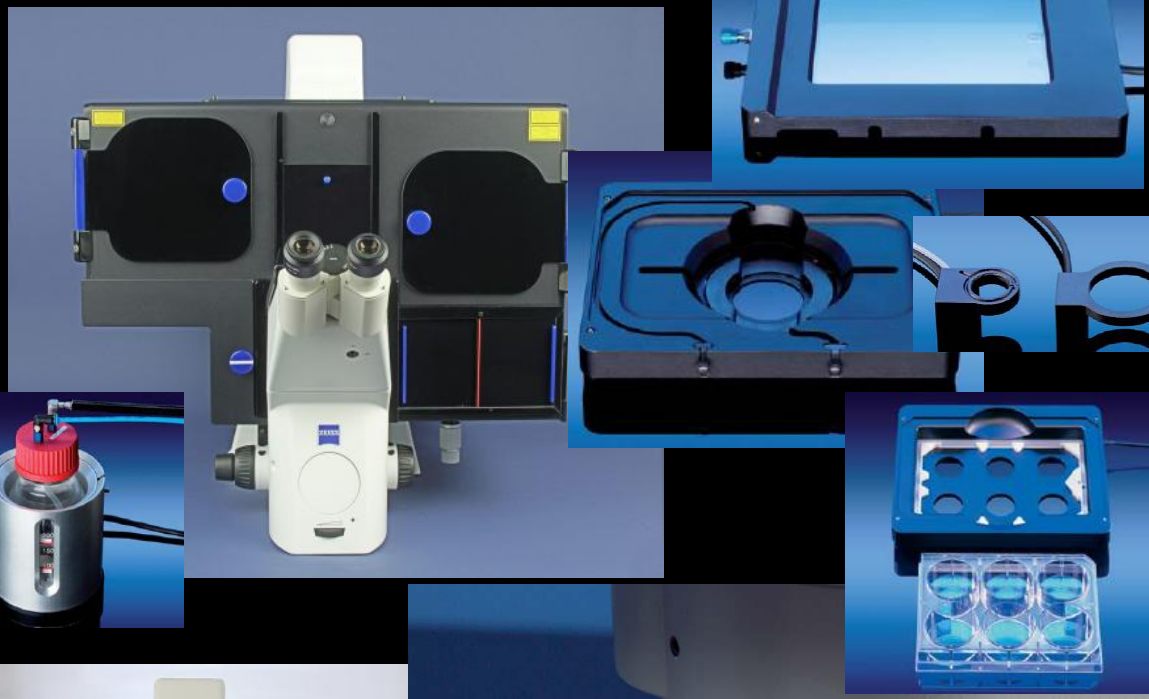
Live-Cell Imaging Chamber

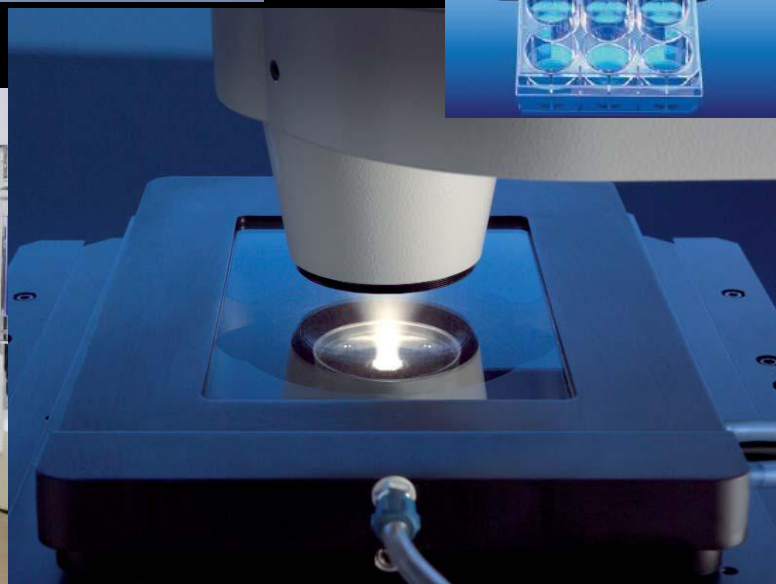


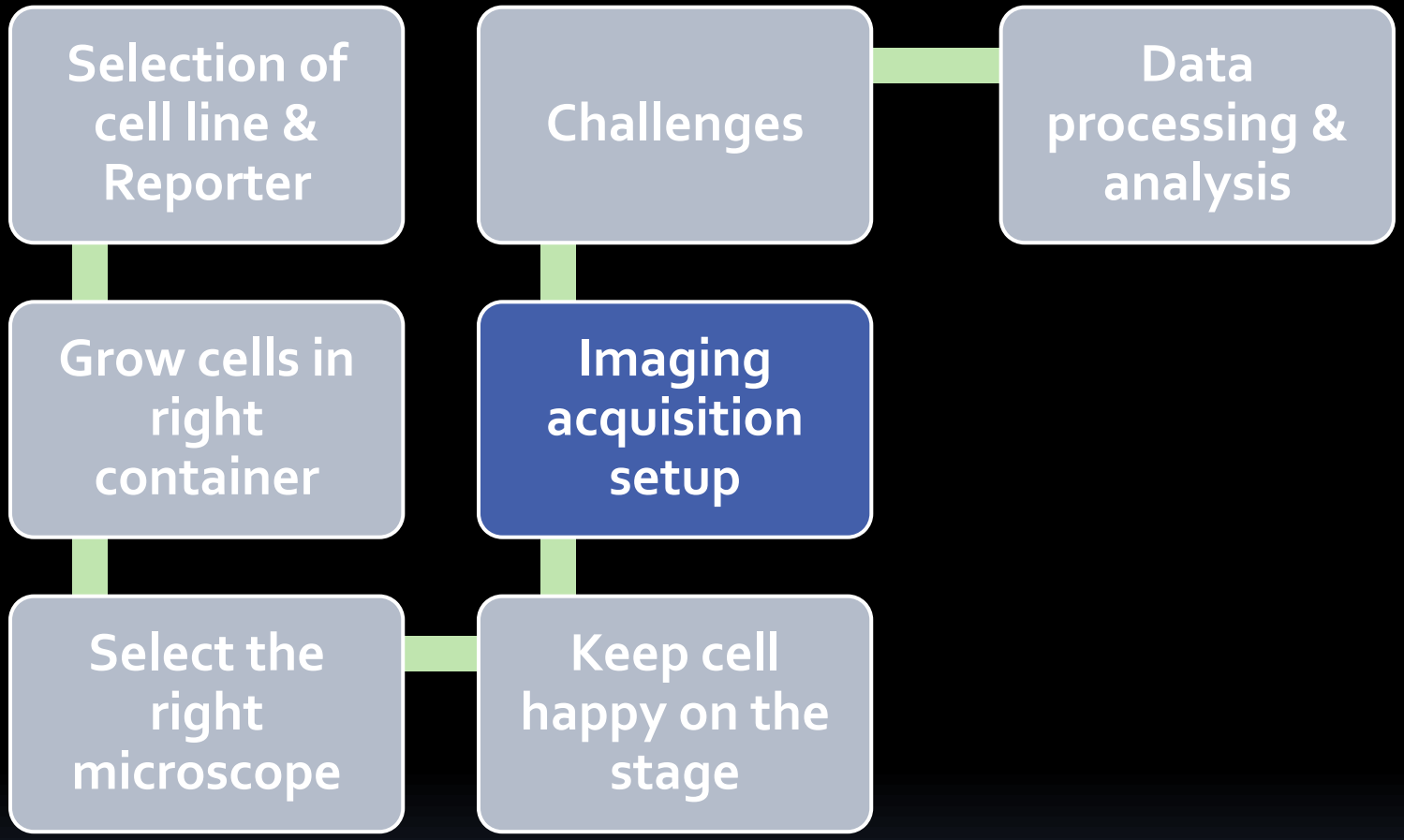
Carl Zeiss Microcinematograph

Göttingen, 1955







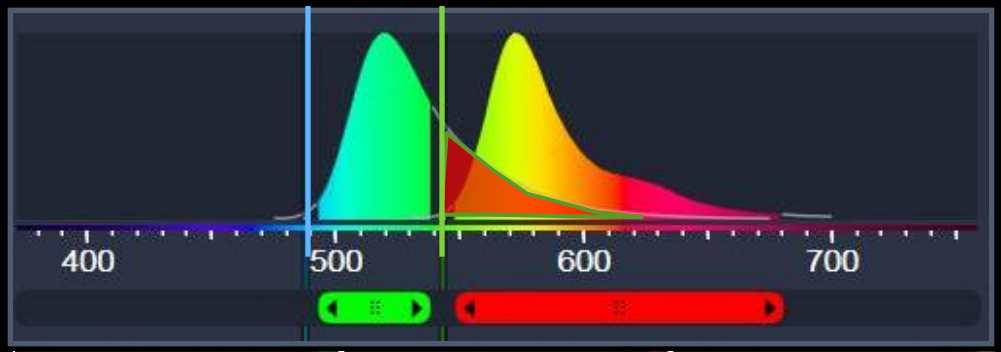


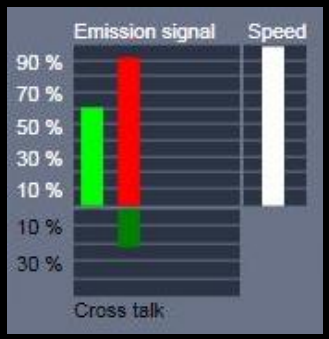
- Multi-wavelength light path configuration
- Spatial resolution *VS* temporal resolution

Multiple staining - the emission crosstalk problem

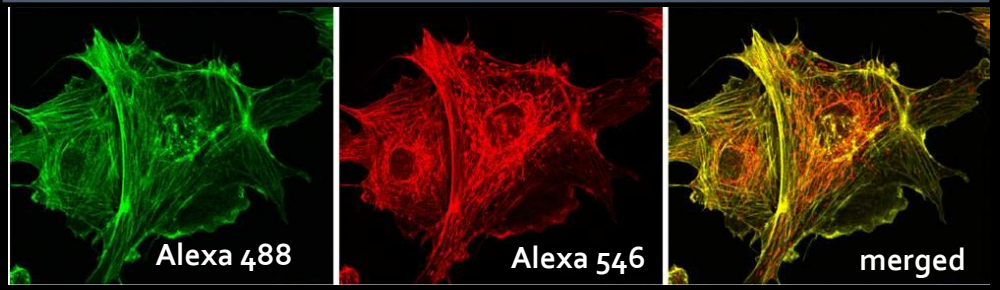
Alexa 488 em

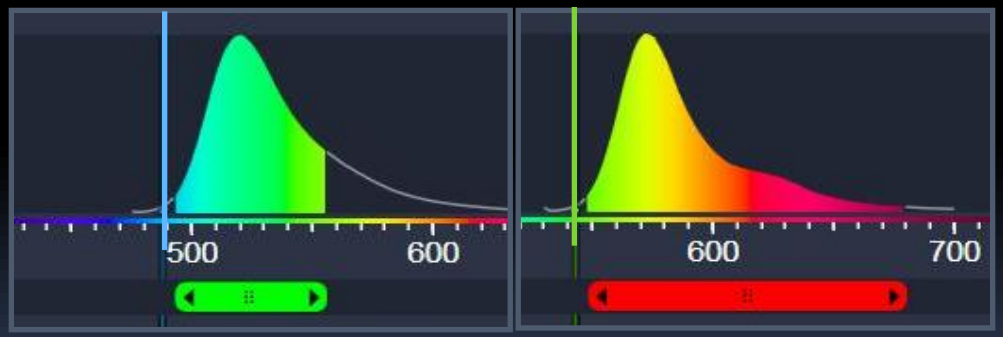
Alexa 546 em

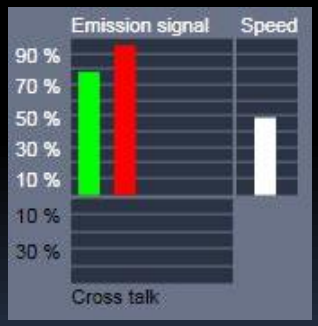




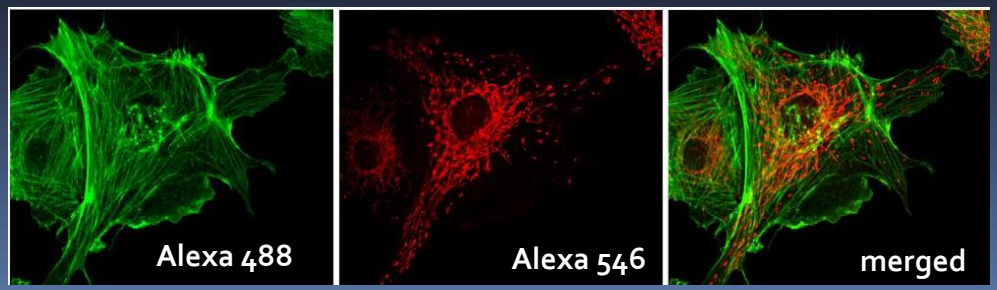
Simultaneous scan



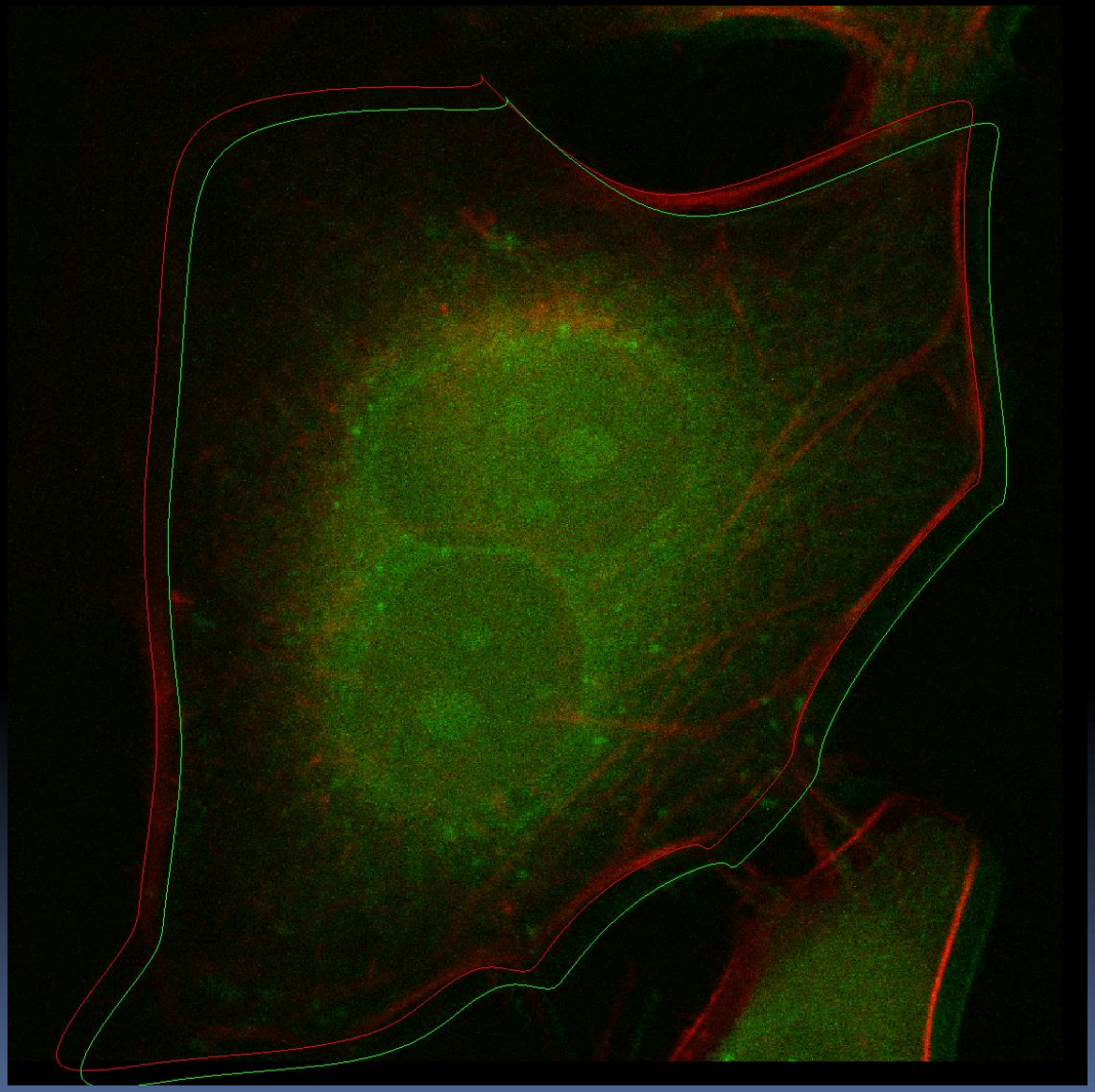




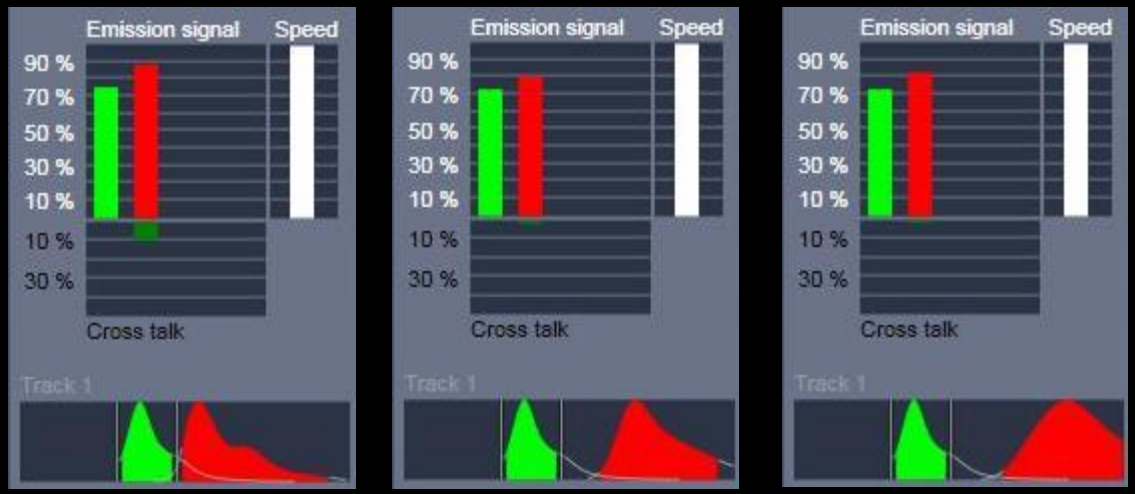
Sequential scan



Spatial Shift in Sequential Mode



Multi-Colour Live Cell Imaging Acquisition Strategy



GFP + CY_3 | GFP + mCherry | GFP + mPlum

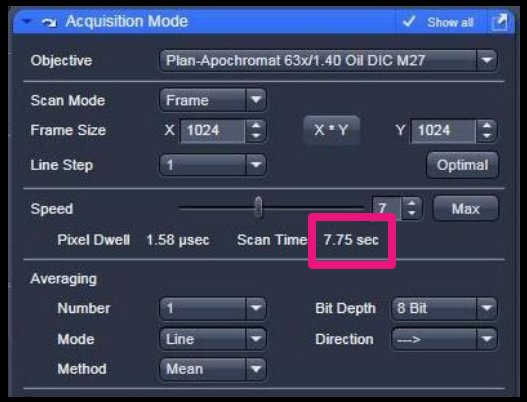
Strategy 1:

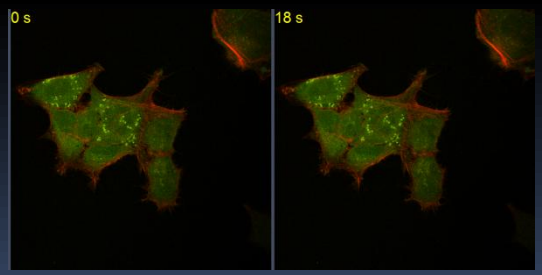
Seletion of fluophores with more sperated spectrum

Simultaneous data acquisition in *Channel Mode*

Multi-Colour Live Cell Imaging
Sequential Acquisition in Channel Mode

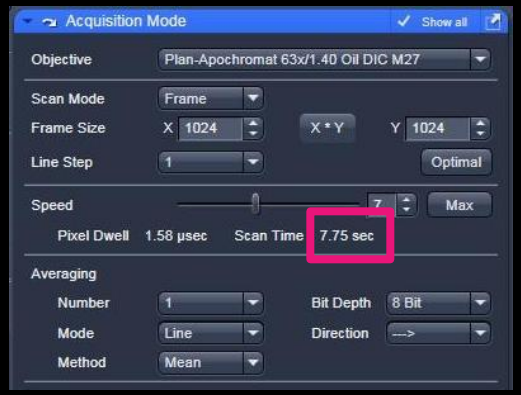
Framewise

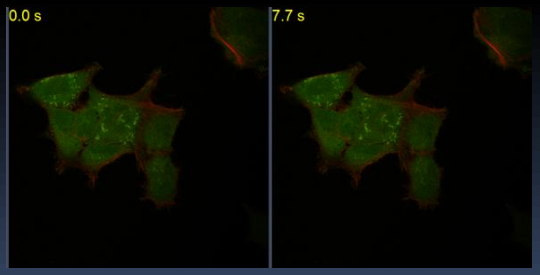




Mechanical time = 18-7.75 s

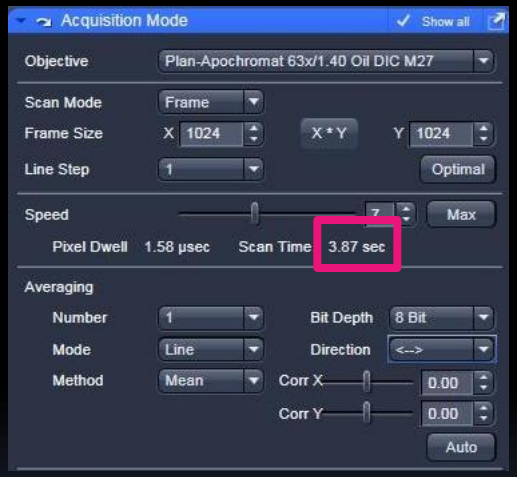
Linewise





Mechanical time = 0 s

Bi-directional Linewise



Fastest!

Strategy 2:

Sequential data acquisition in *Channel Mode*

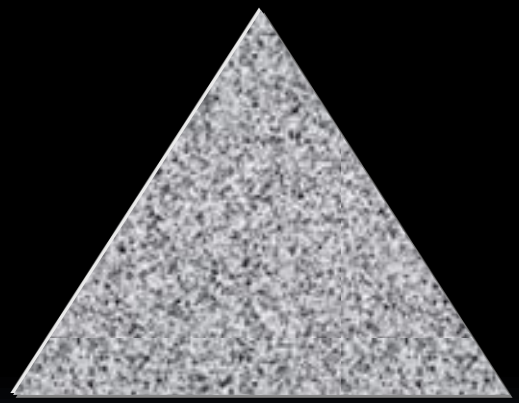
Note: line-wise switching between tracks should be chosen in live cell imaging.

Visualizing Dynamic Processes
The Eternal Triangle of Compromise

„Non-invasive" Data Recording

- low photobleaching
- low cytotoxicity (from laser irradiation)

Note: a good compromise has to be found for every live cell imaging task!



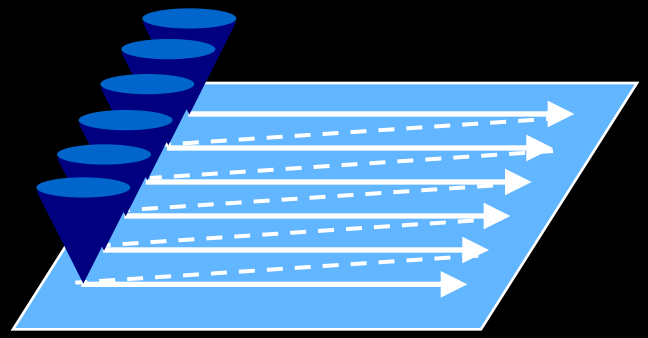
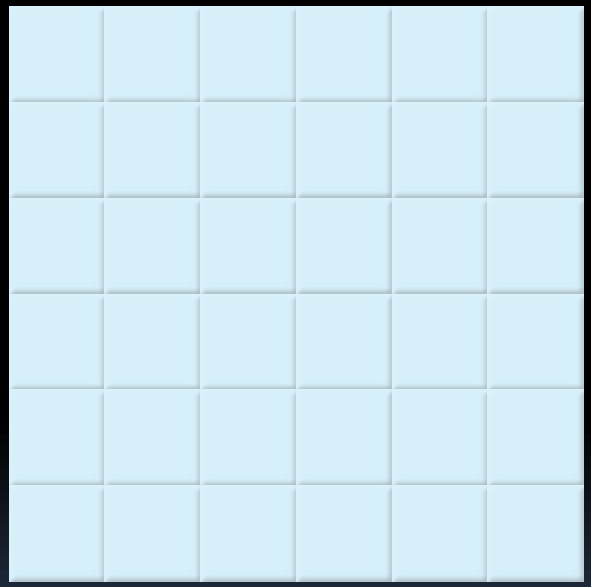
High temporal resolution

- fast acquisition speed
- high number of different time points

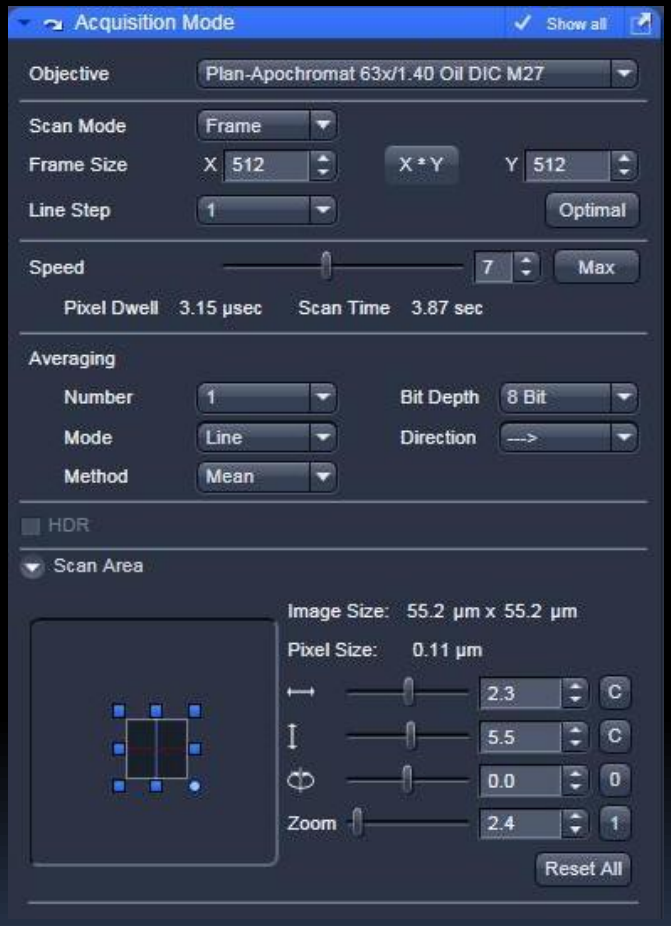
High spatial resolution

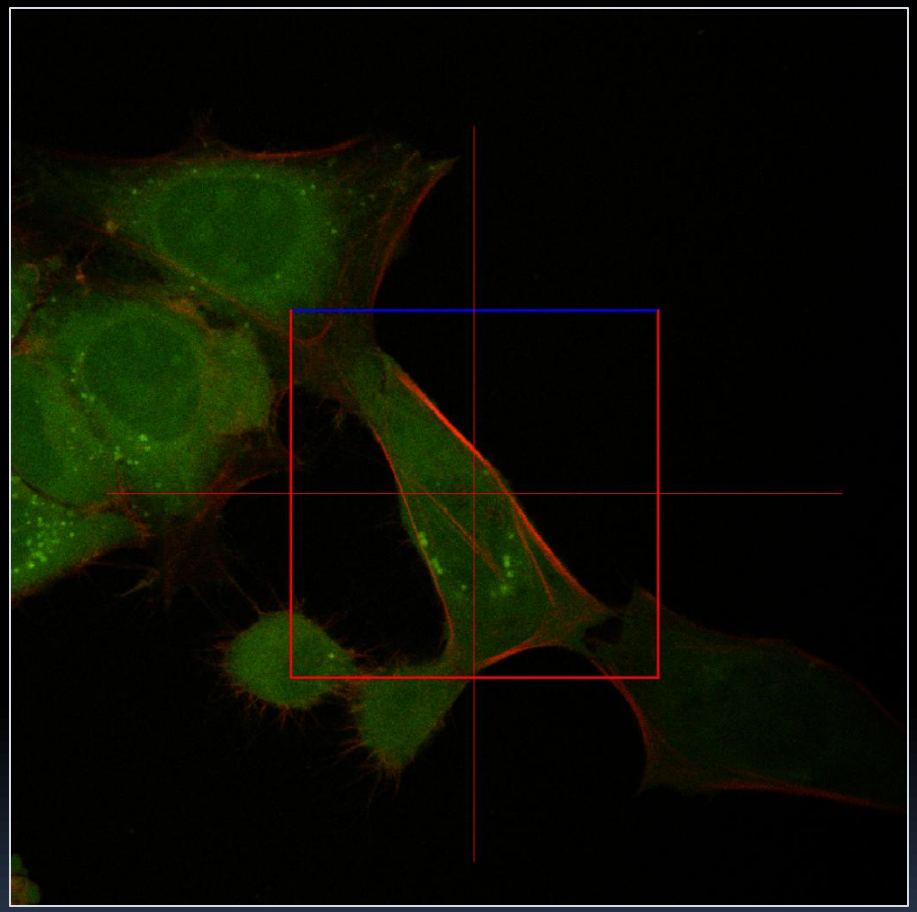
- high resolution image formats
- high resolution Z-Stacks
- optimal S/N

Visualizing Dynamic Processes
Point Scanners vs. CCD Camera

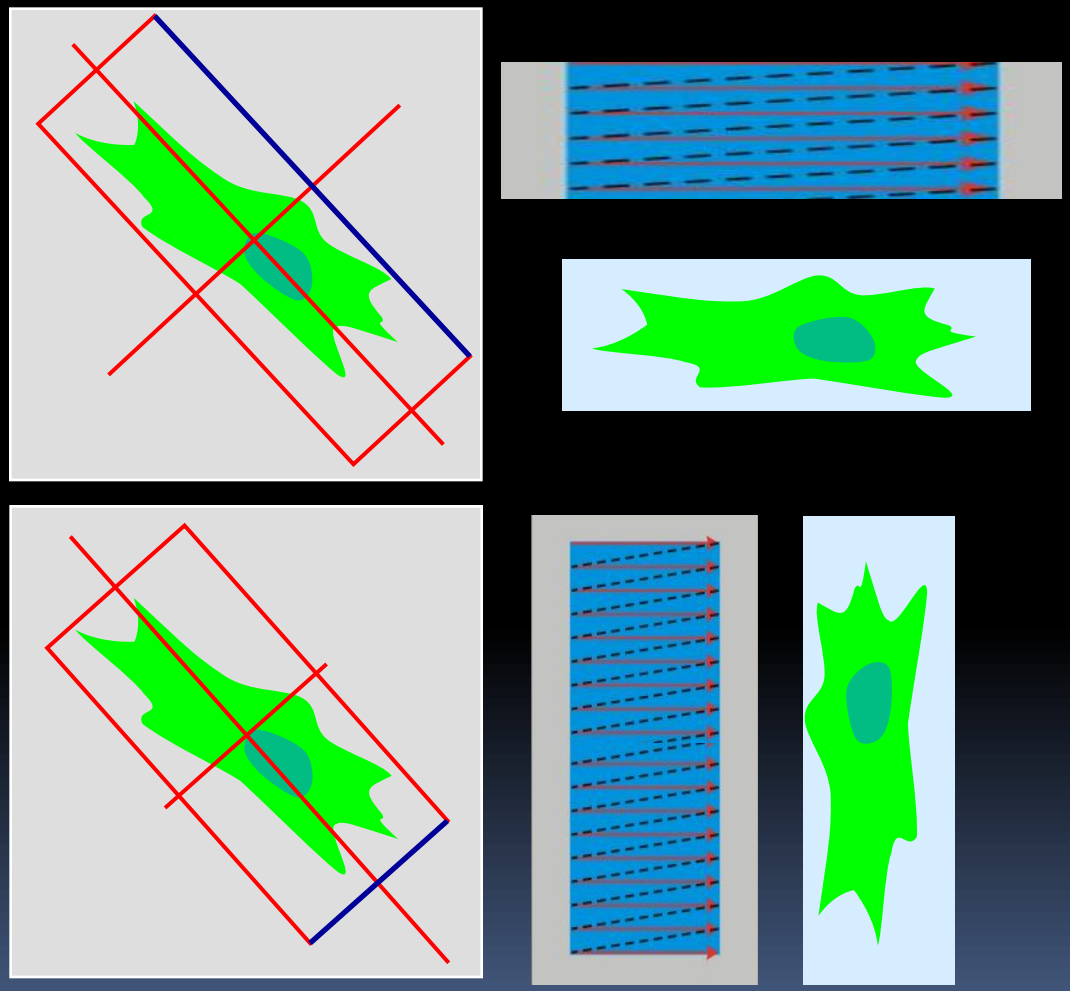
Point scanning confocal	CCD Camera
	
XY scanning	
LSM 710/510/700	PE-ERS spinning disc

Crop function



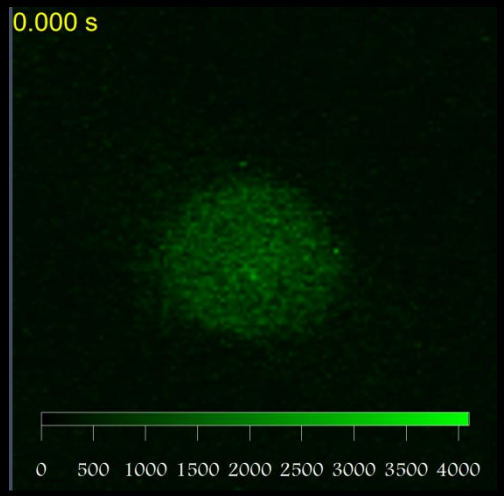


Confocal Laser Scanning Microscopy
Scanning Modes: Crop Tool

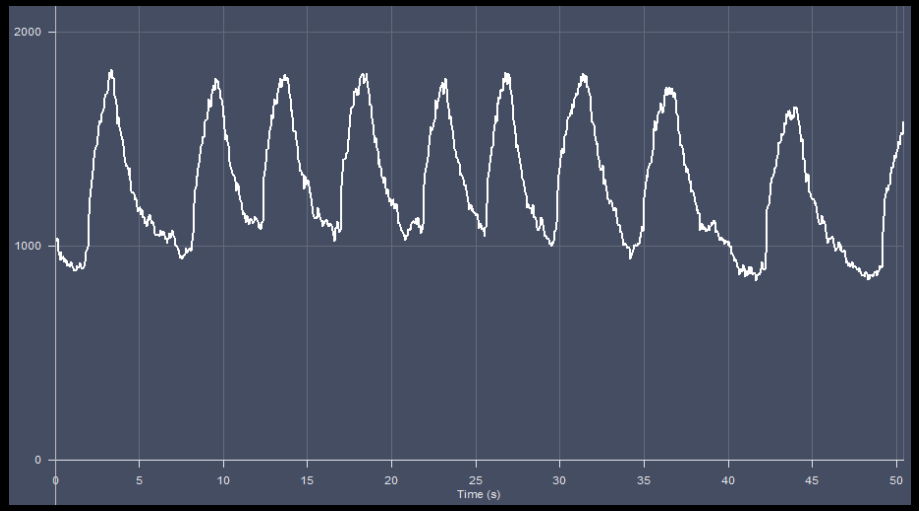


Both images are the same size – but due to the scanner movement the image below takes longer

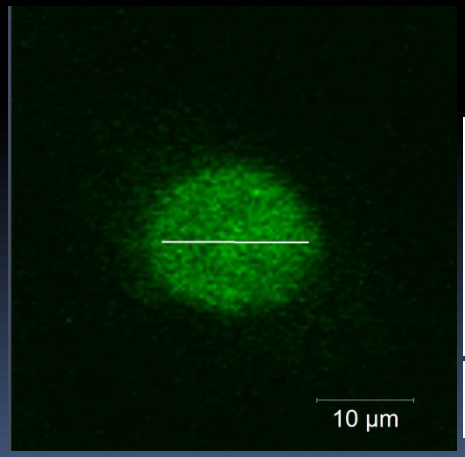
Visualizing Dynamic Processes
Temporal Resolution: Line scan

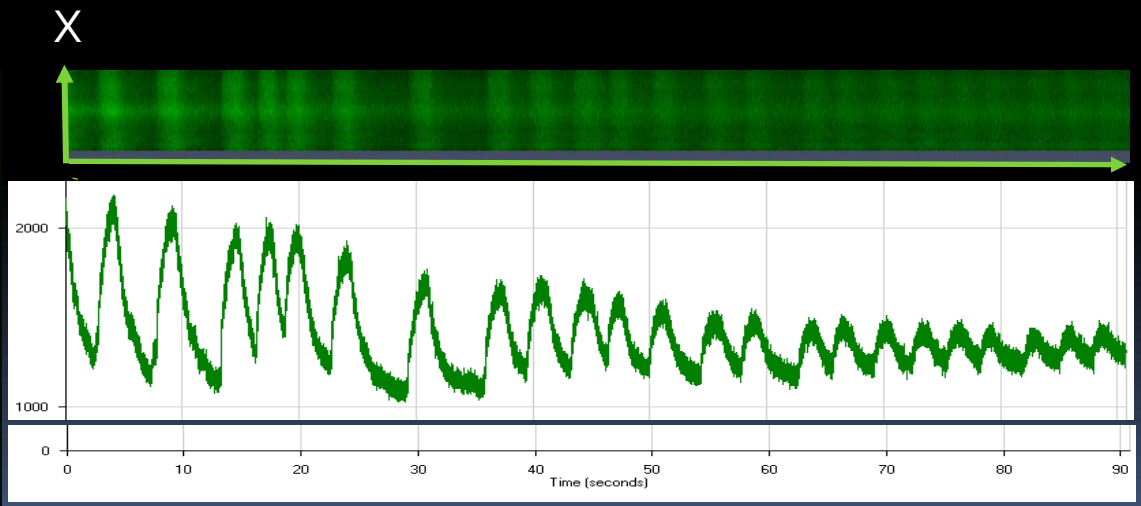


Frame mode; 49ms/frame



Line mode; 6oous/line



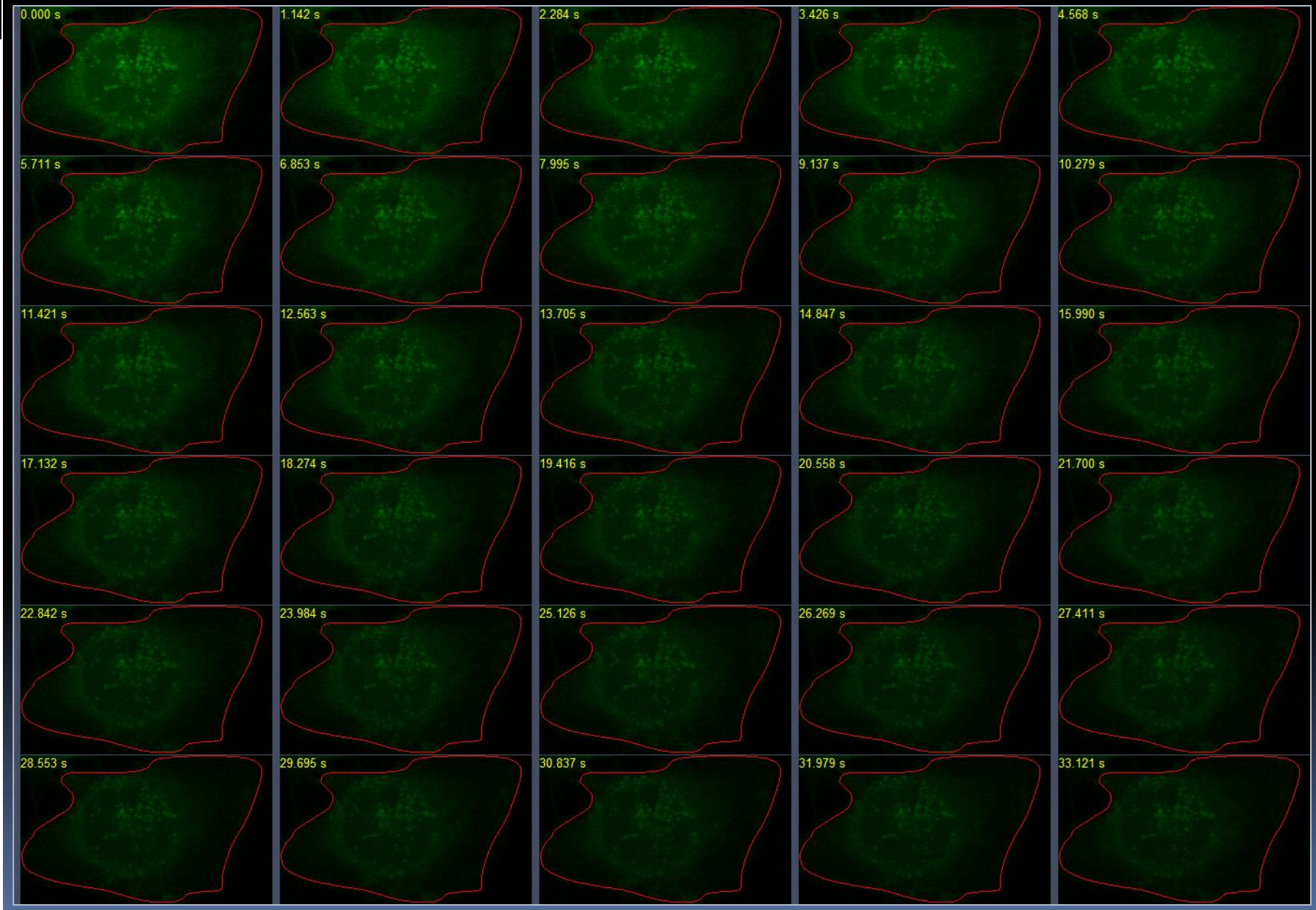


Human embryonic stem cell (hES2) derived cardiomyocyte (hESC-CM), Fluo-3 staining
Image courtesy of Prof. Ronald LI, Stem Cell & Regenerative Medicine Consortium

Challenges

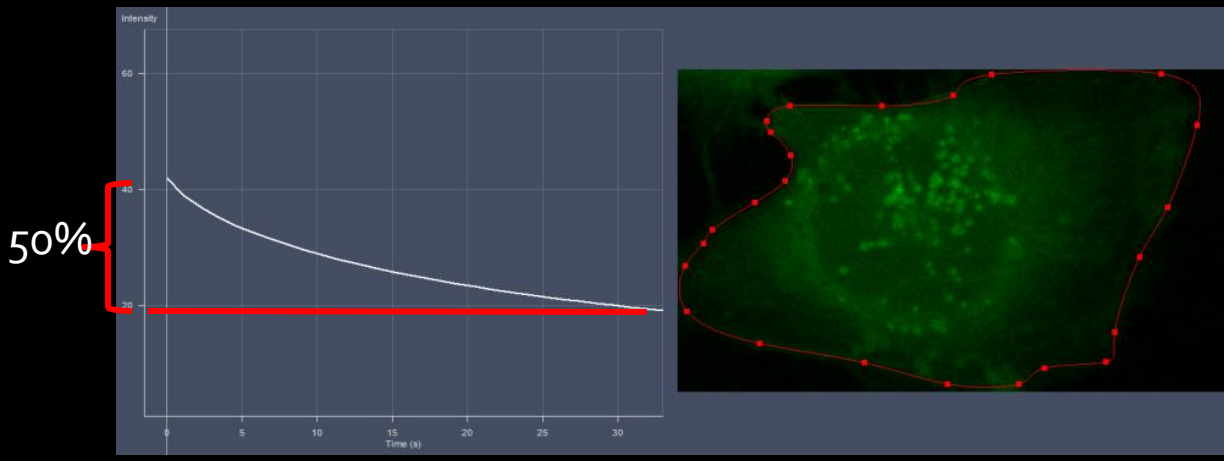
- Photo bleaching
- Photo cytotoxicity

Photo bleaching

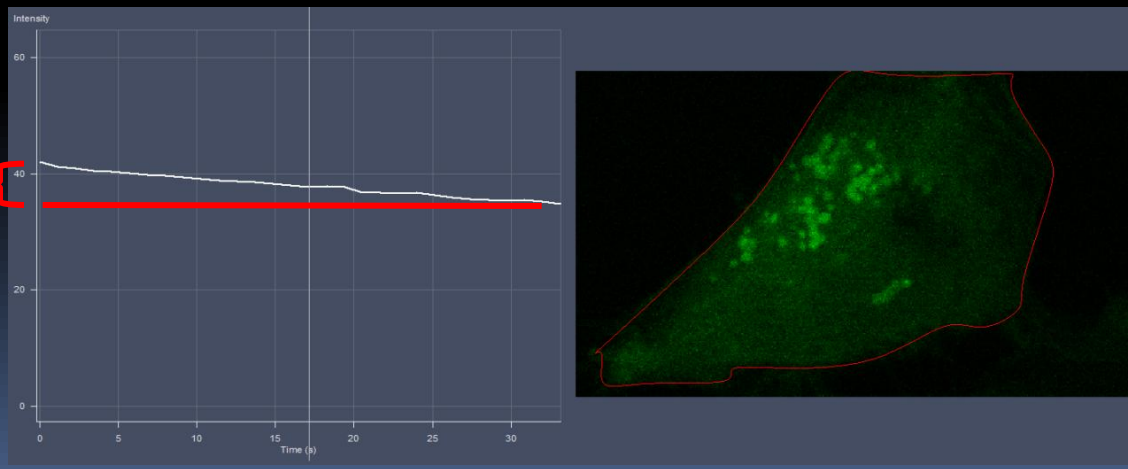


Imaging Strategy:

- Time series of images acquired at low laser power but relatively high gain setting.
- Focus with brightfield.



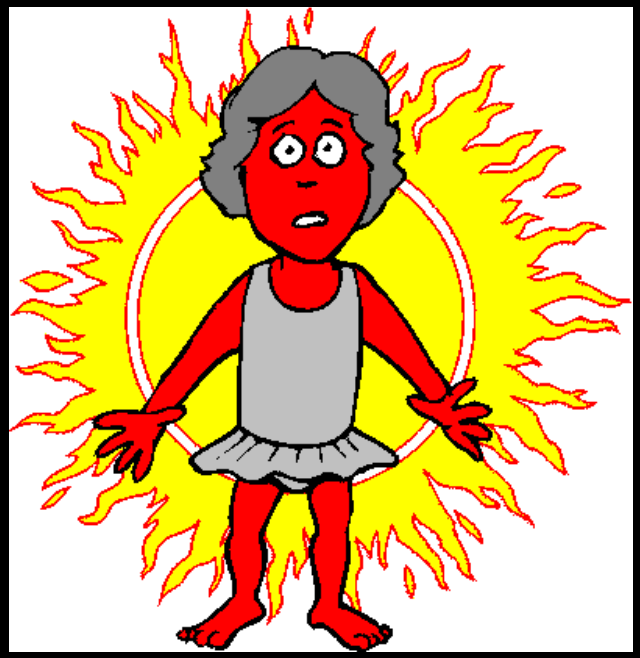
50%

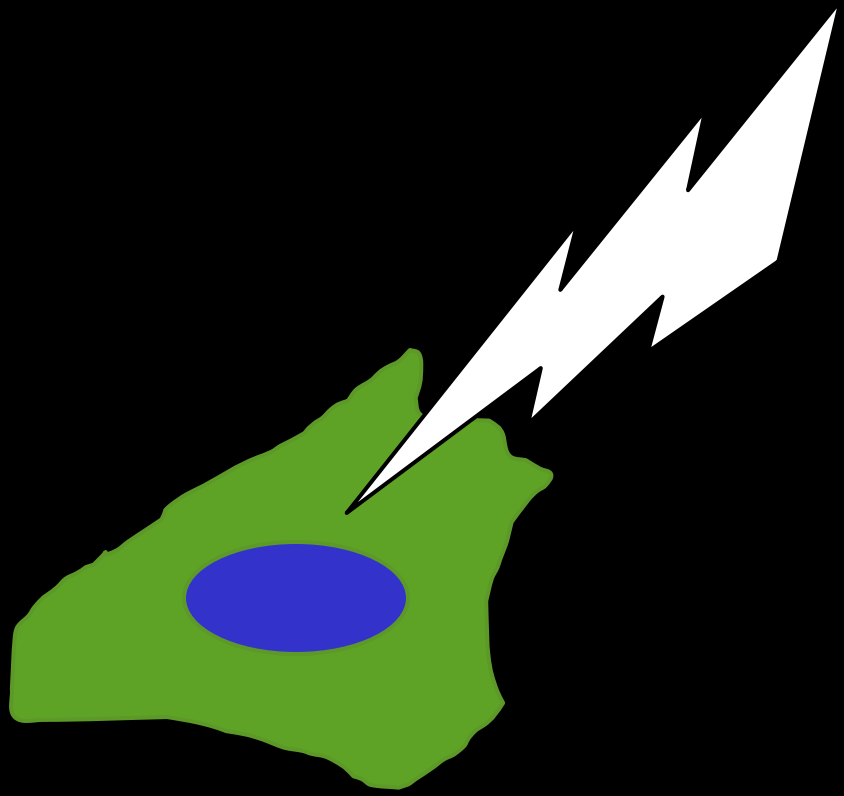


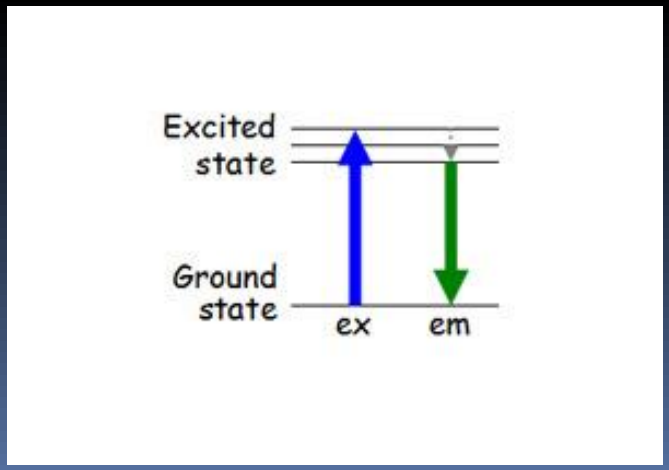
10%

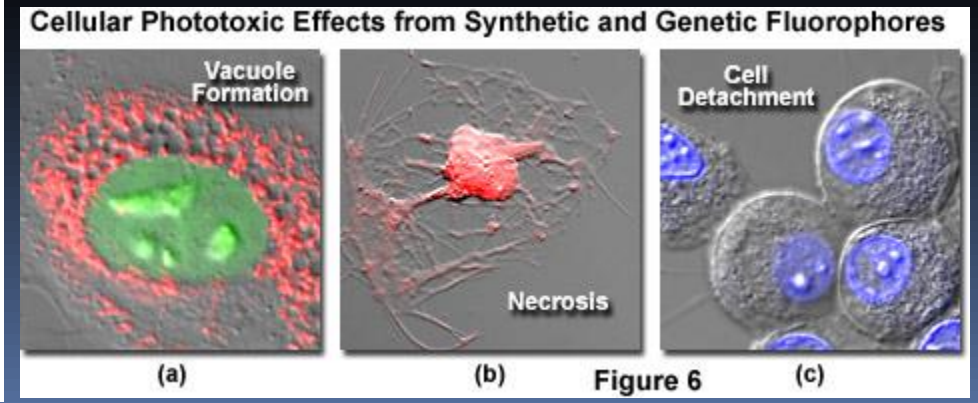
Intensity Profile of time series

Phototoxicity









Strategies: Minimize light exposure

- Avoid blue dyes
- Reduce concentration of fluorescence probes
- Phenol red free medium
- Dye specific filters
- EM CCD camera
- High NA objective
- Avoid Phase objective for fluorescence detection
- Reduce Illumination
- Less frame size/or binning
- Minimal lateral (*x and y axes) and axial (z axis) resolution should* be used to see the structures of interest.
- Proper temporal resolution to reconstruct dynamic events of interest.

- Antioxidant

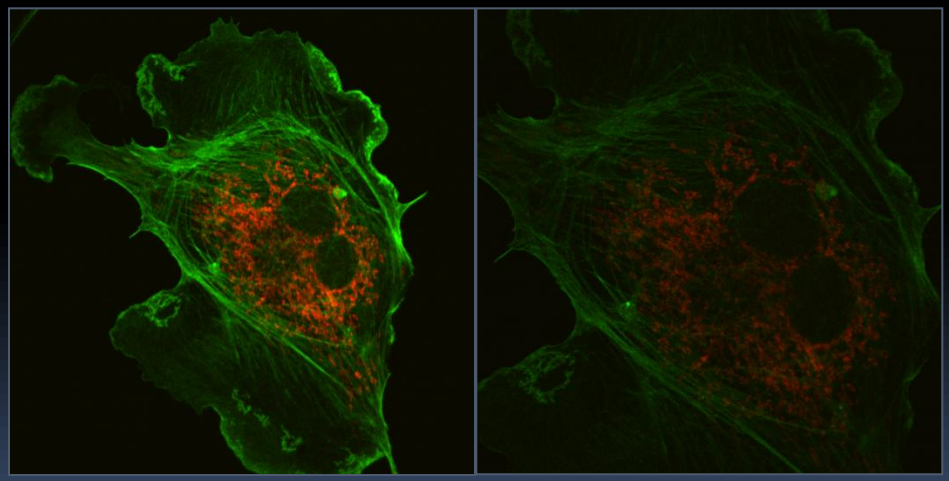
$$FWHM_{ill,lat} = \frac{0.51 * \lambda_{em}}{NA}$$

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

λ = 520nm
NA = 1.4
FWHM = 189nm

$$E \propto \frac{NA^4}{Mag^2}$$

E = Efficiency of light collection
Mag = Magnification of lens



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

Acknowledgement

Faculty Core Facility Committee

Prof. George Tsao

Dr. Camie Chan

Mr. Benjamin Leung

Prof. Ronald Li

Dr. Marco Kong

Mr. Harry Chen

All Faculty Core Facility Users!