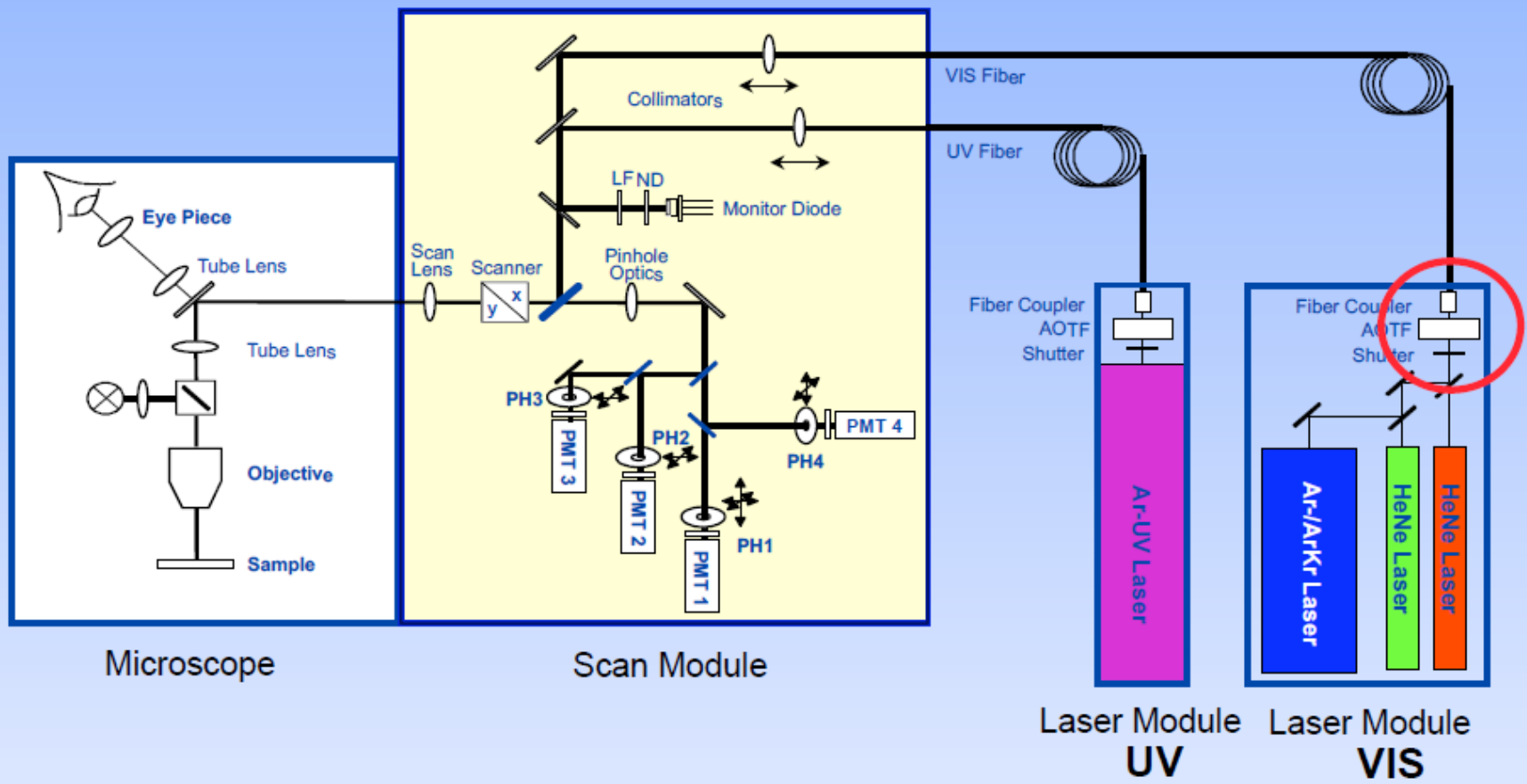
Confocal Laser Scanning Microscopy

How Does a CLSM Work?

ZEISS

Optical Beam Path

AOTF	Acousto Optical Tunable Filter
PMT	Photomultiplier
PH	Variable Pinhole

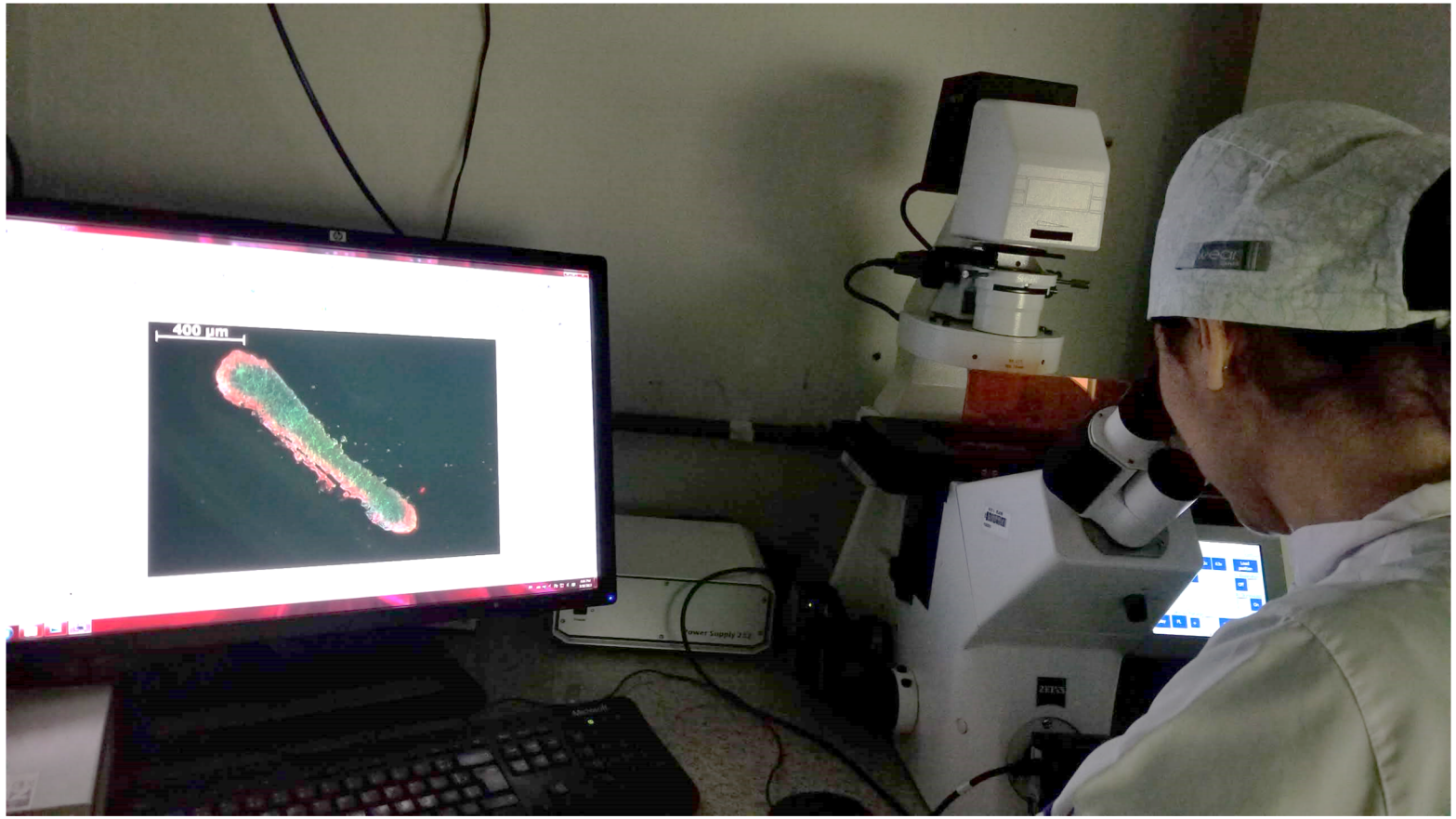


Laboratório multiusuários - IFSC

LSM 780 (Zeiss) – Confocal laser scanning

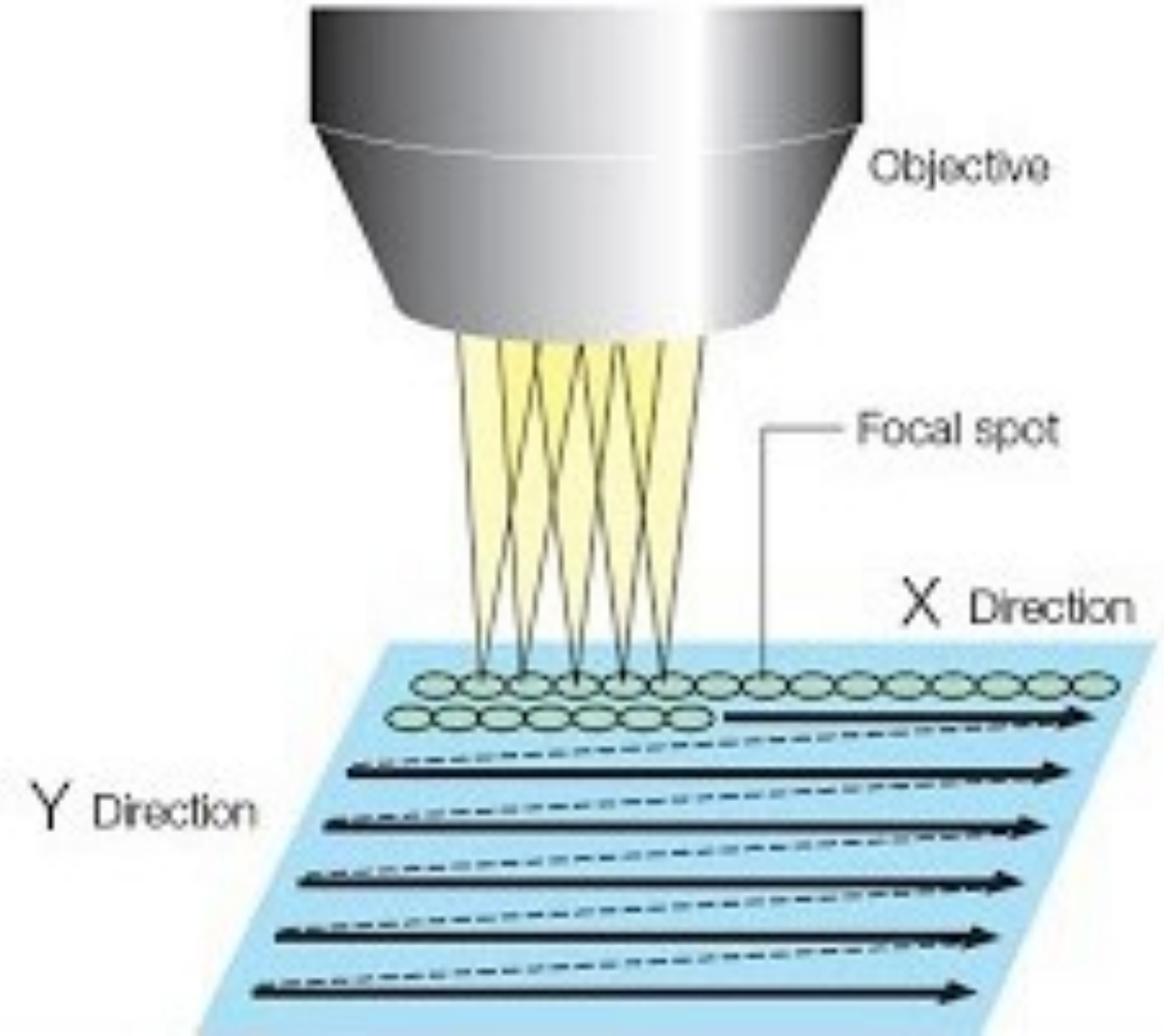
- Microscopia confocal (fluorescência)
- Microscopia multifótons (SHG e THG)
- FLIM (Fluorescence Lifetime Imaging)
- FRET (Förster Ressonance Energy Transfer)
- FCS (Fluorescence Correlation Spectroscopy)

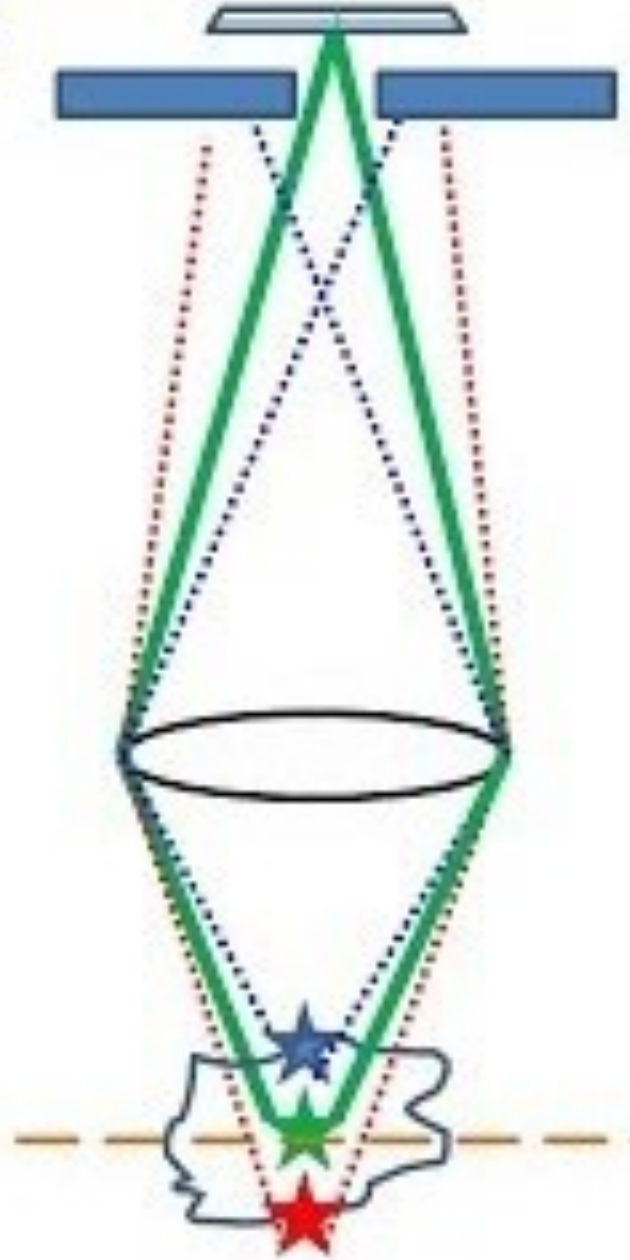
Introdução a Microscopia confocal



https://www.youtube.com/watch?v=QFtZFbug1SA

Confocal Microscopy





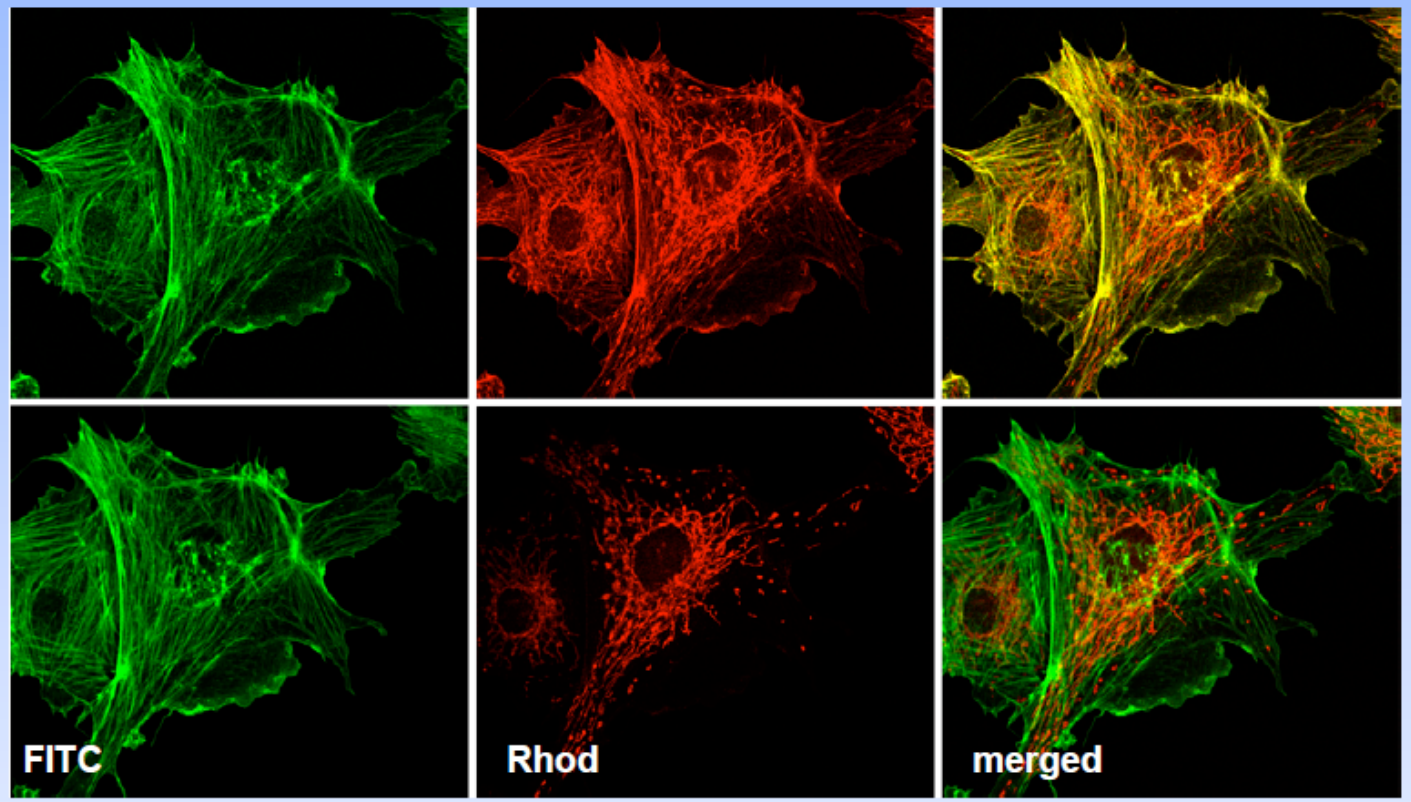
Confocal Laser Scanning Microscopy
LSM510 – Features and Highlights

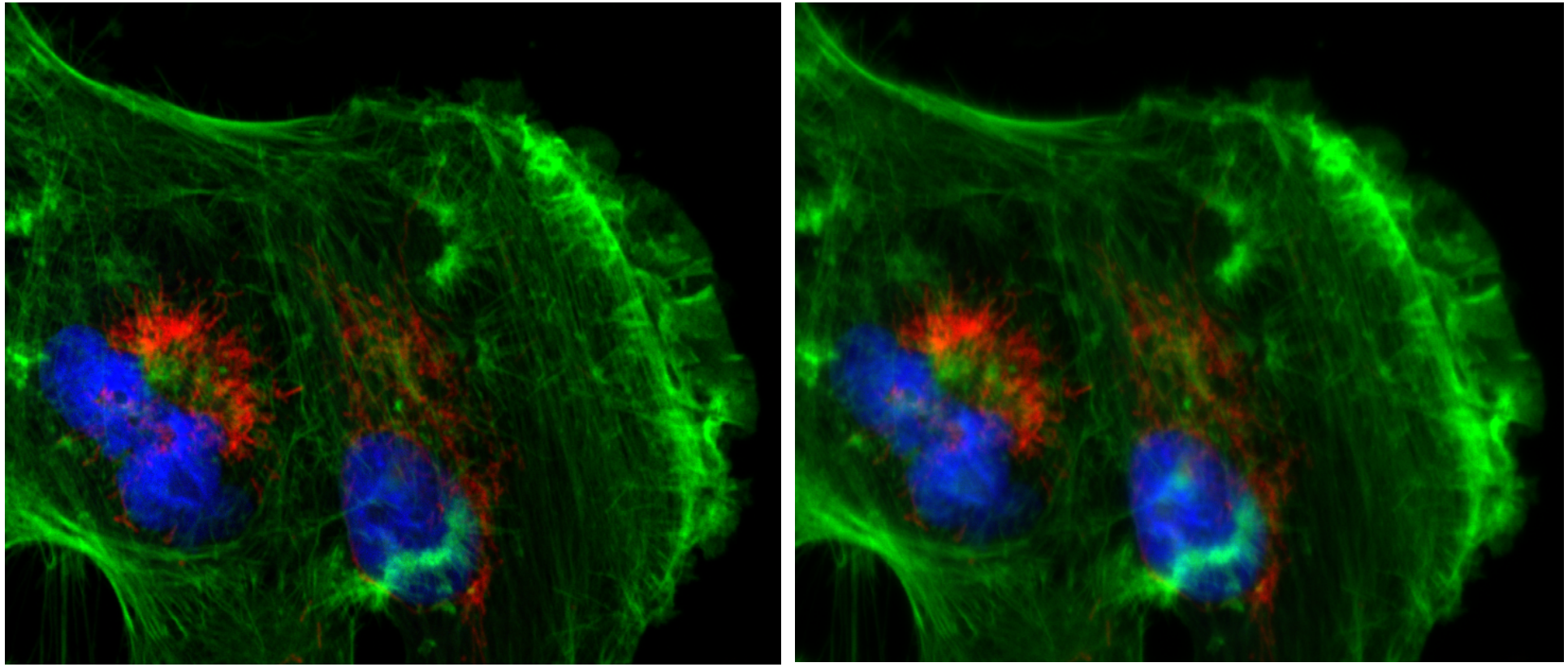
MultiTracking

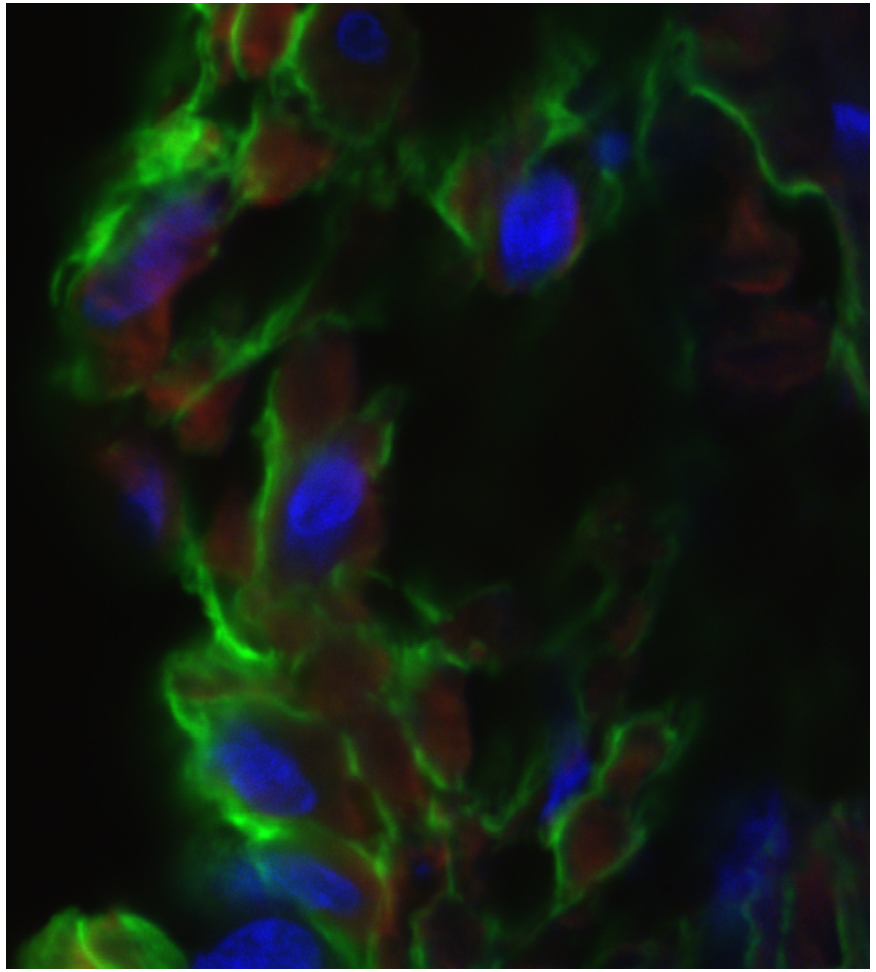
- Effective elimination of emission crosstalk
- Improved signal/noise by using long pass - instead of band-detection
- Fast Switching between complete configurations (laser lines)

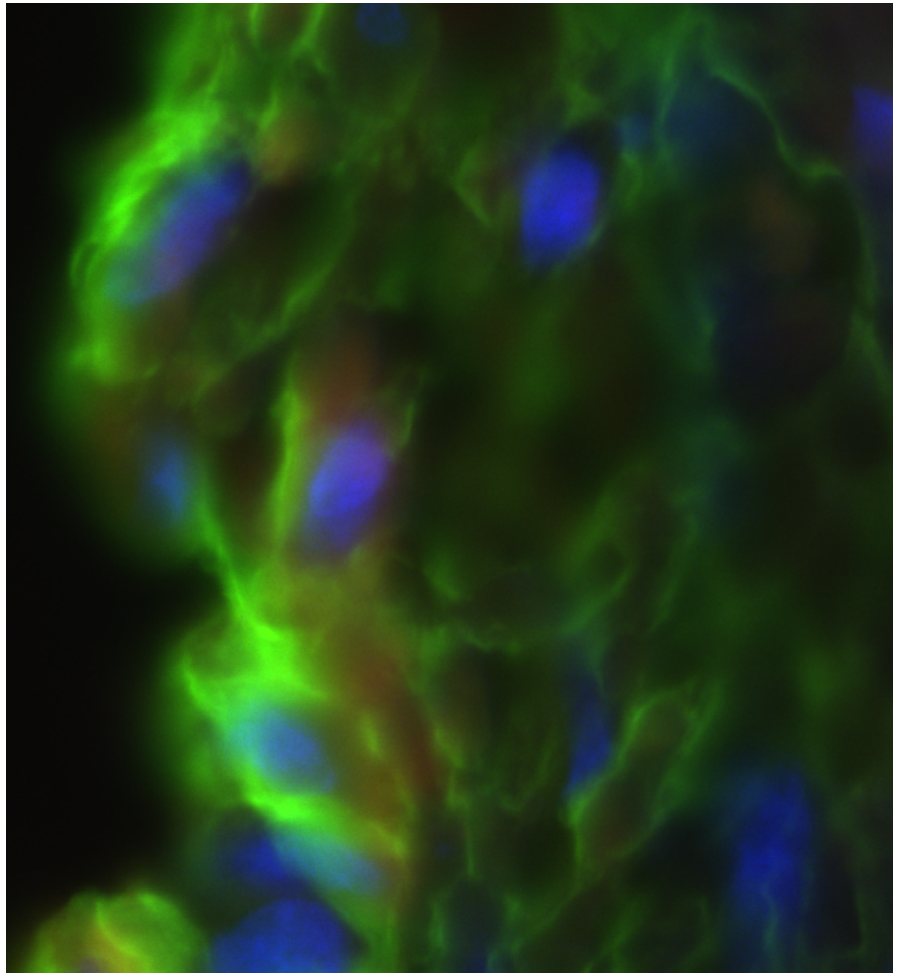
Simultaneous

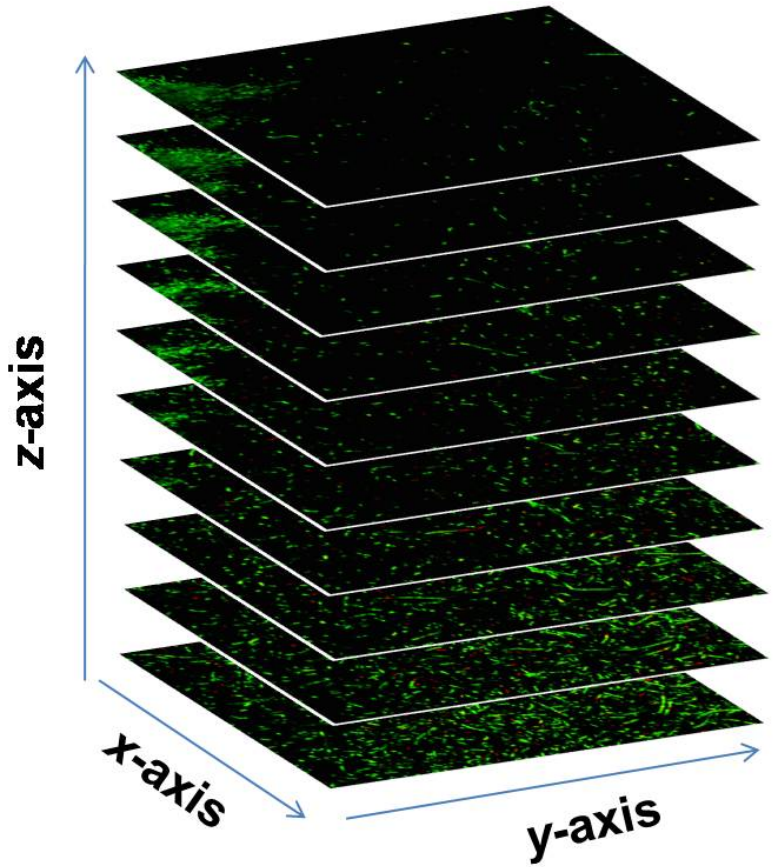
Multitracking

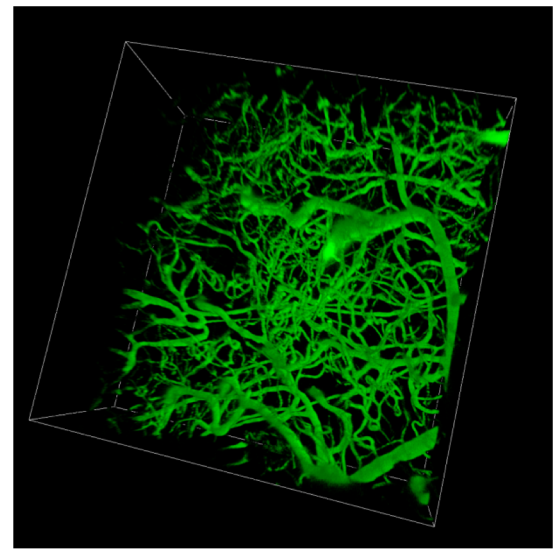


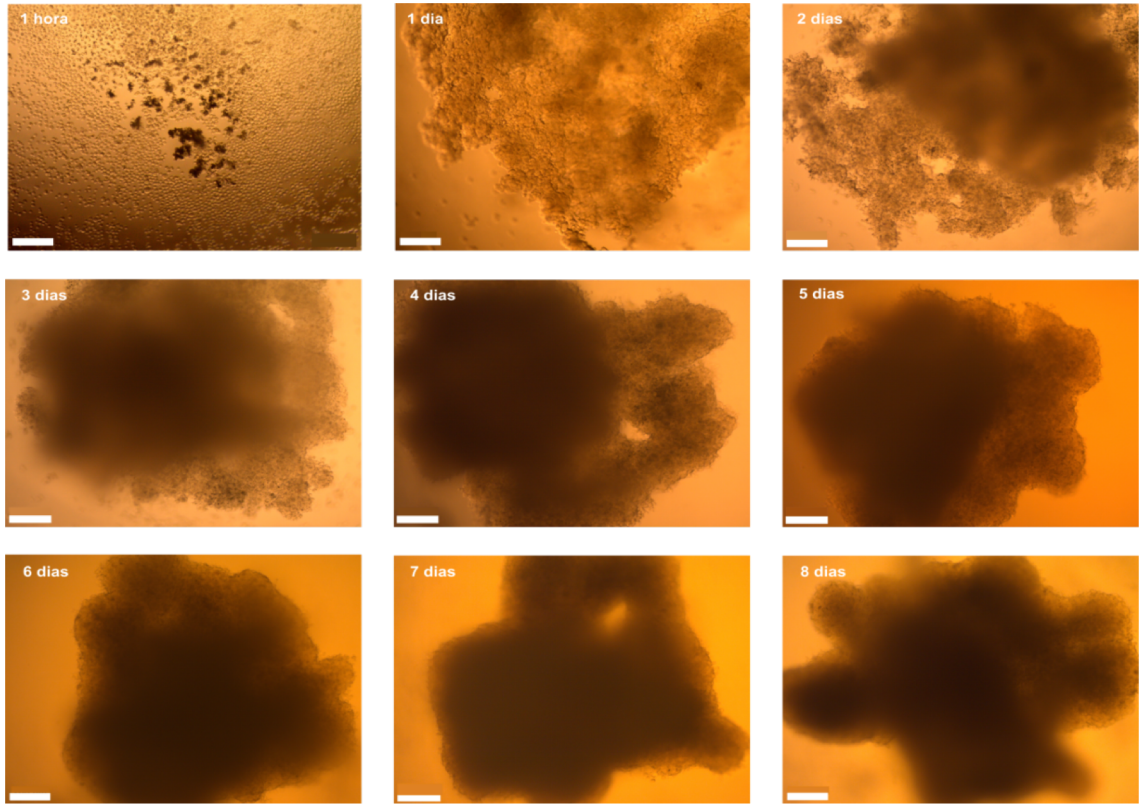


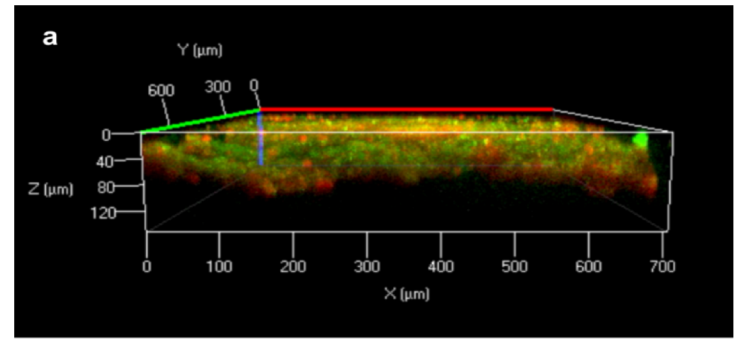


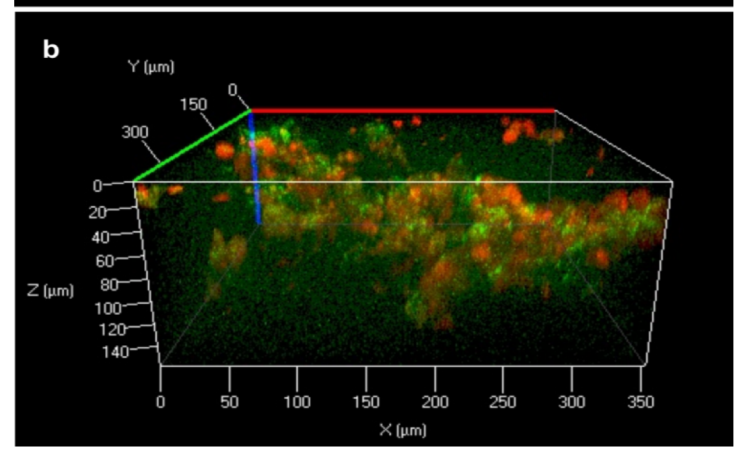



z-axis
x-axis
y-axis

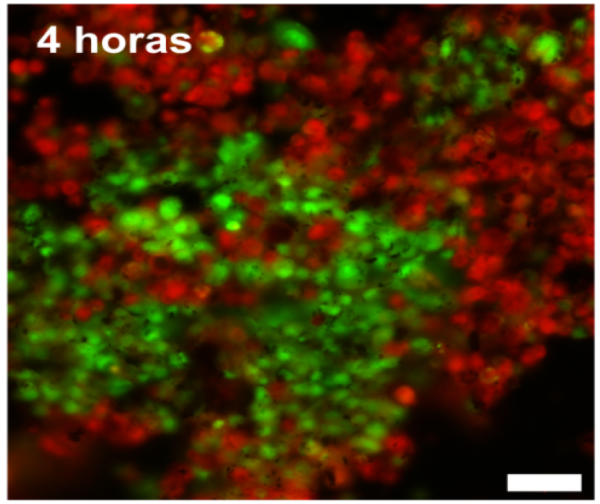


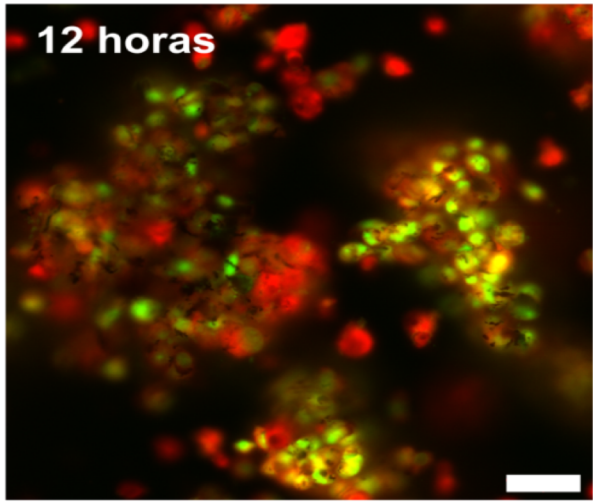

1 hora
1 dia
2 dias
3 dias
4 dias
5 dias
6 dias
7 dias
8 dias


a
Y (µm)
600
300
0
0
40
80
120
Z (µm)
0
100
200
300
400
500
600
700
X (µm)


b
Y (µm)
0
150
300
0
20
40
60
80
100
120
140
Z (µm)
0
50
100
150
200
250
300
350
X (µm)

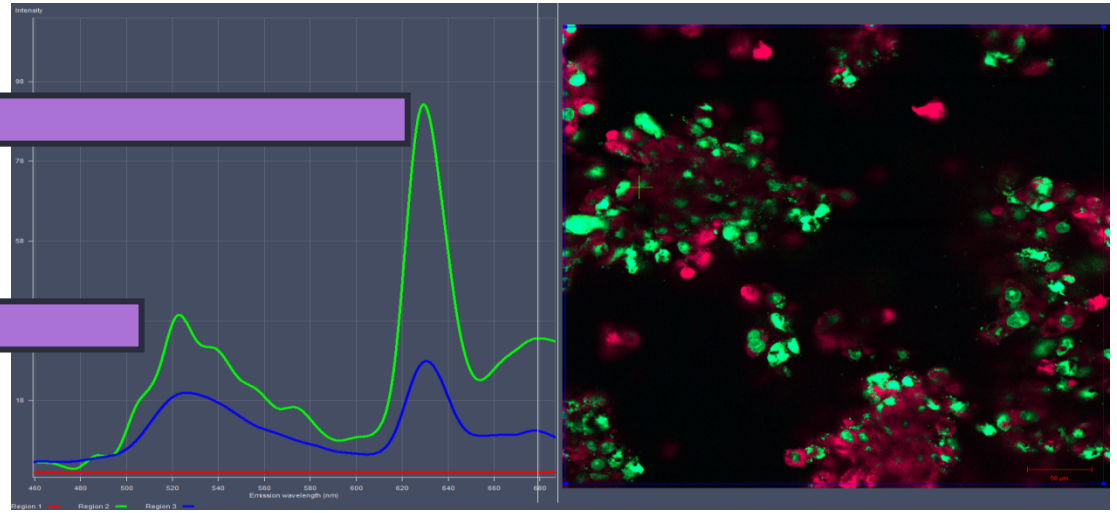
Incubação com Photogem 50 µg/mL (Escala: 50 µm). Aumento de 40 vezes.



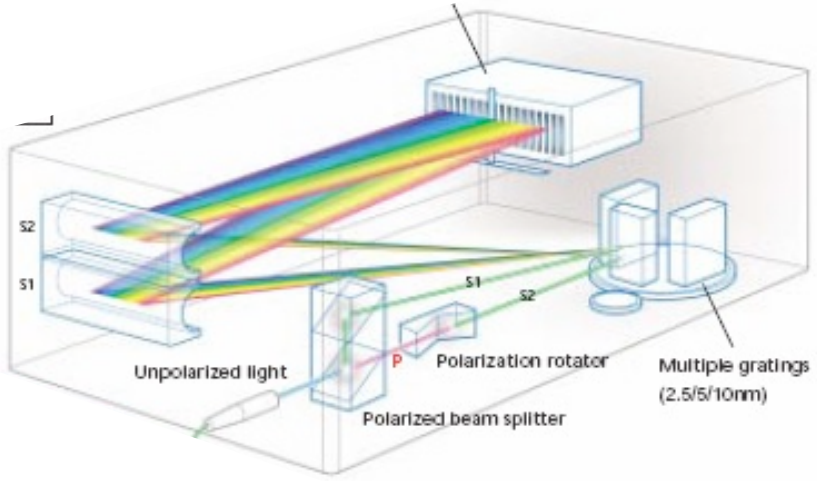


Pico 620nm:
Photogem

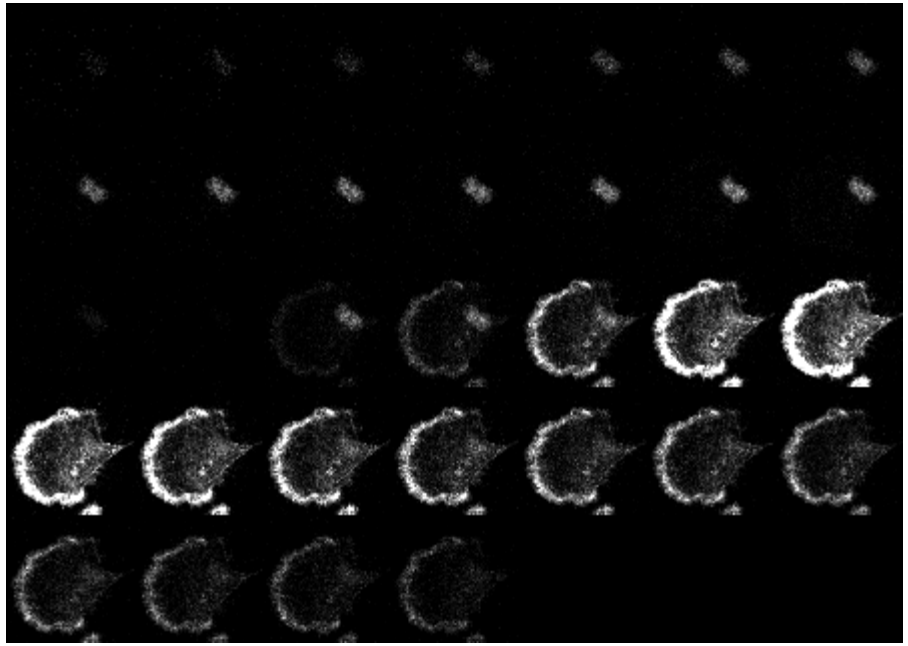
Pico 530nm:
Laranja de acridina

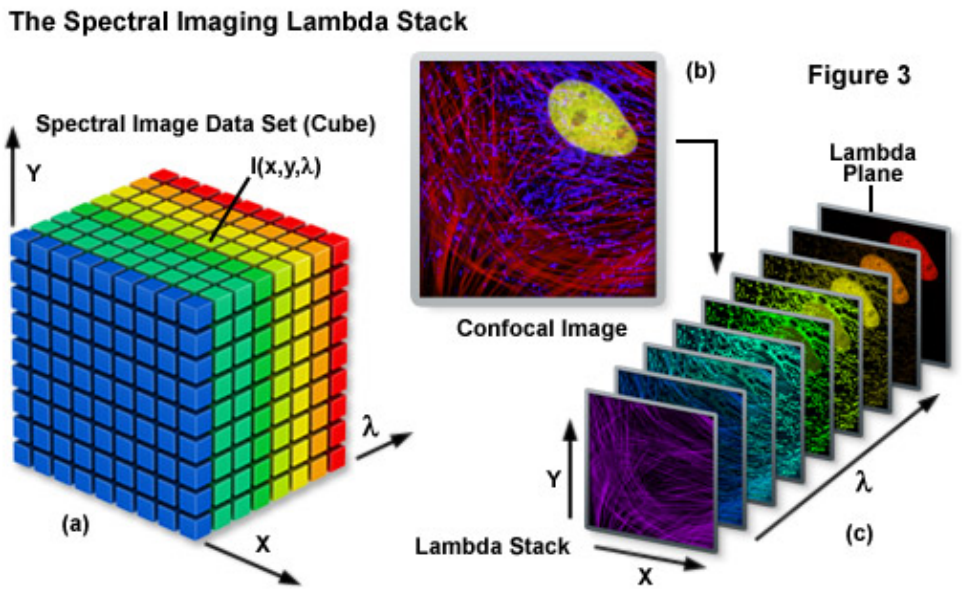


Spectral Detection



Montage of 32 channels of sample stained with DAPI and Alexa 488





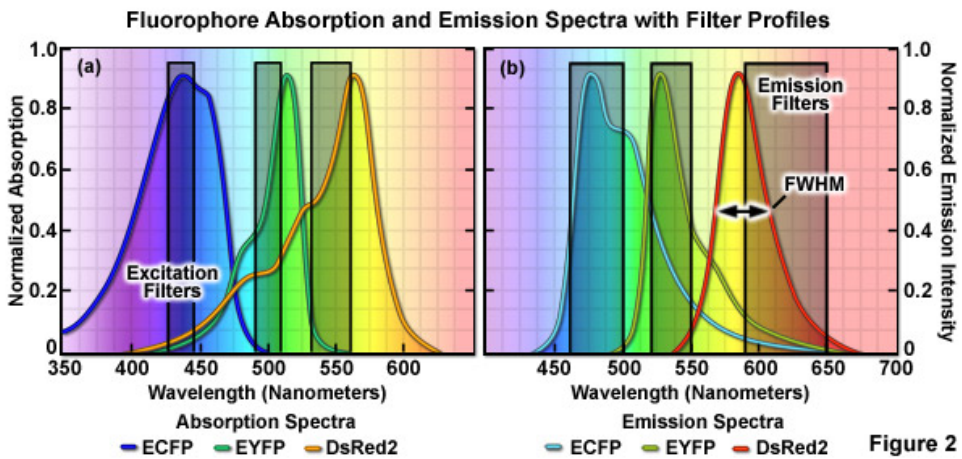
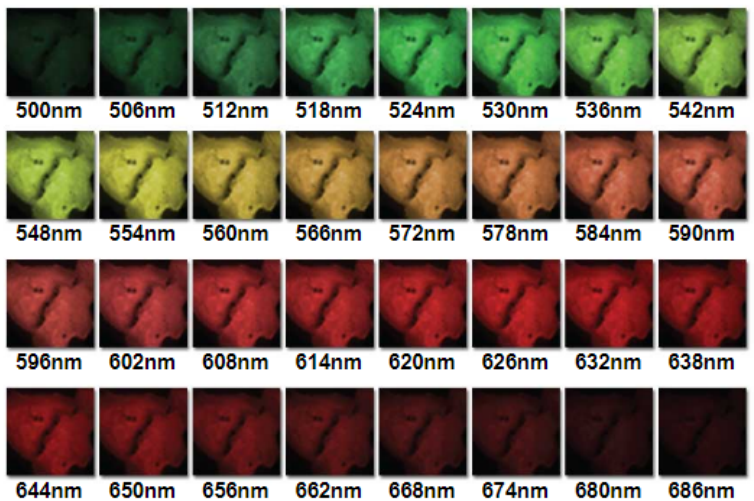
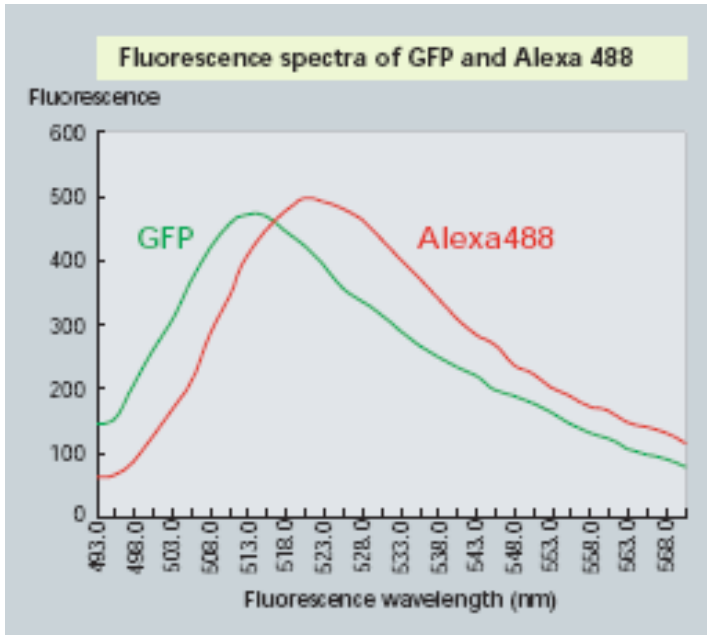


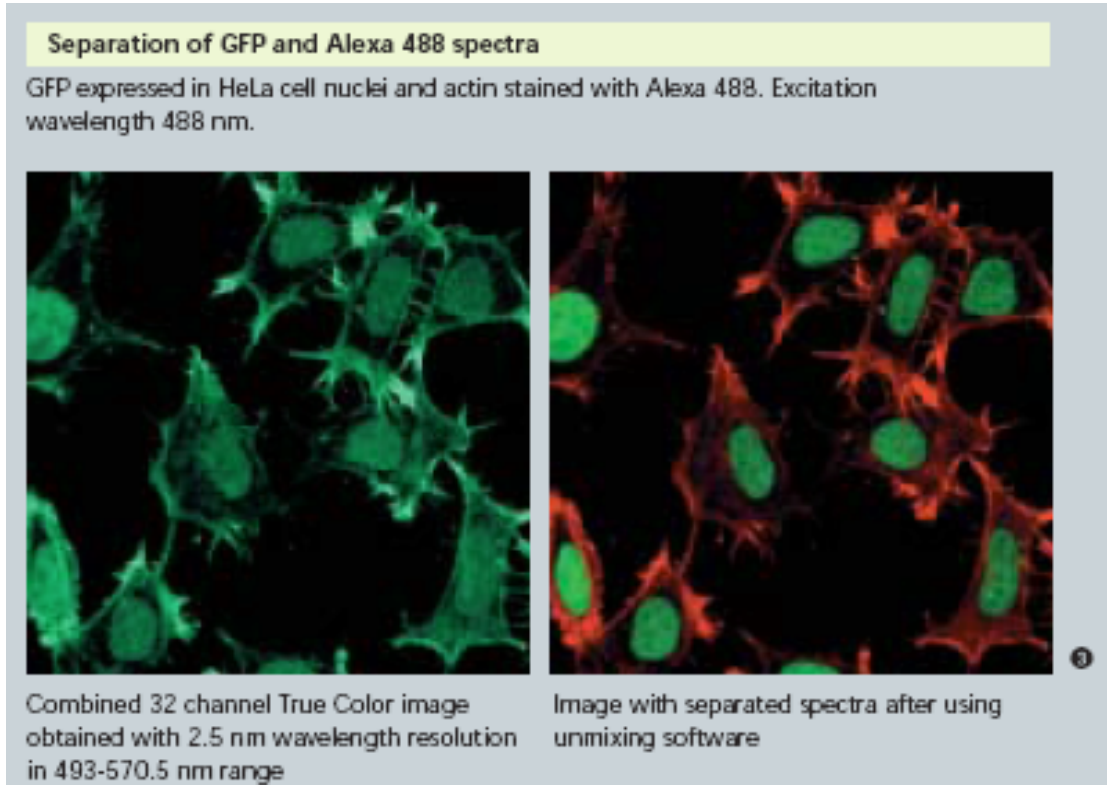
Figure 2

Figure 4 - 32-Channel Spectral Image Lambda Stack Acquisition



Spectral Confocal



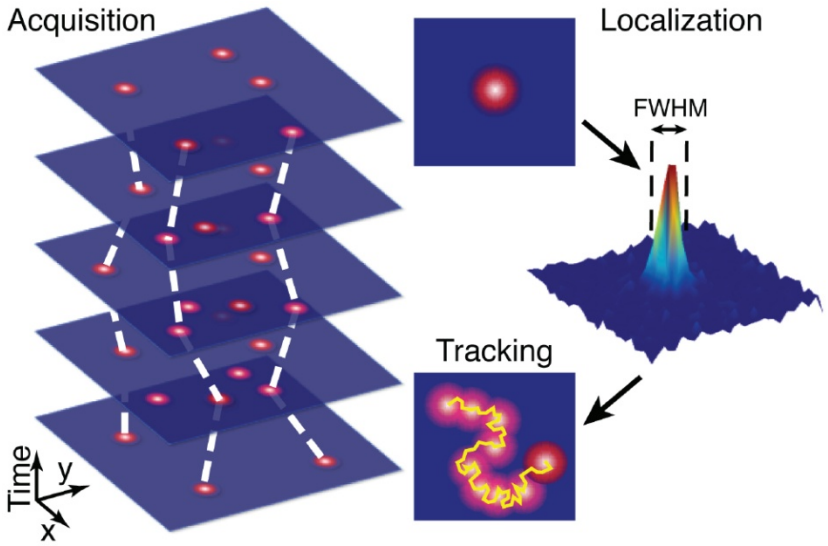


Channel	Band	Excitation laser (nm)				
		405	488	561	642	785
1	435-505	DAPI, BV421, Hoechst, PacBlue, CascadeBlue, eFluor450, DyLight405, CFP, LIVE/DEAD Violet				
2	505-560	BV510, PacOrange, Cascade Yellow, AF430, eFluor525, QD525	FITC, AF488, GFP, YFP, DyLight488, PKH67, Syto13, LysoTrackerGreen, MitoTrackerGreen			
3	560-595	QD565, QD585, eFluor565	PE, PKH26, DSRed, mOrange, Sytox Orange, Cy3	PE, AF546, Cy3, DyLight550, PKH25, DSRed		
4	595-642	QD625, eFluor625, BV605	PE-TexRed, PI, RFP, QD625, eFluor625	AF568, Cy3, PE-TexRed, TexRed, AF610, RFP, mCherry, PI		
5	642-745	QD705, eFluor700, BV711	PE-Cy5, PE-AF647, 7AAD, PerCP, PerCP-Cy5.5, DRAQ5, QD705	PE-Cy5, PE-AF647, DRAQ5, 7AAD	APC, AF647, AF660, AF680, APC, Cy5, DyLight649, PE-AF647, PE-Cy5, DRAQ5, PerCP, , PerCP-Cy5.5	
6	745-780	QD800, BV786	PE-Cy7, PE-AF750, QD800	PE-Cy7, PE-AF750	APC-Cy7, APC-AF750, APC-H7, Cy7, AF750, PE-Cy7, PE-AF750	SSC

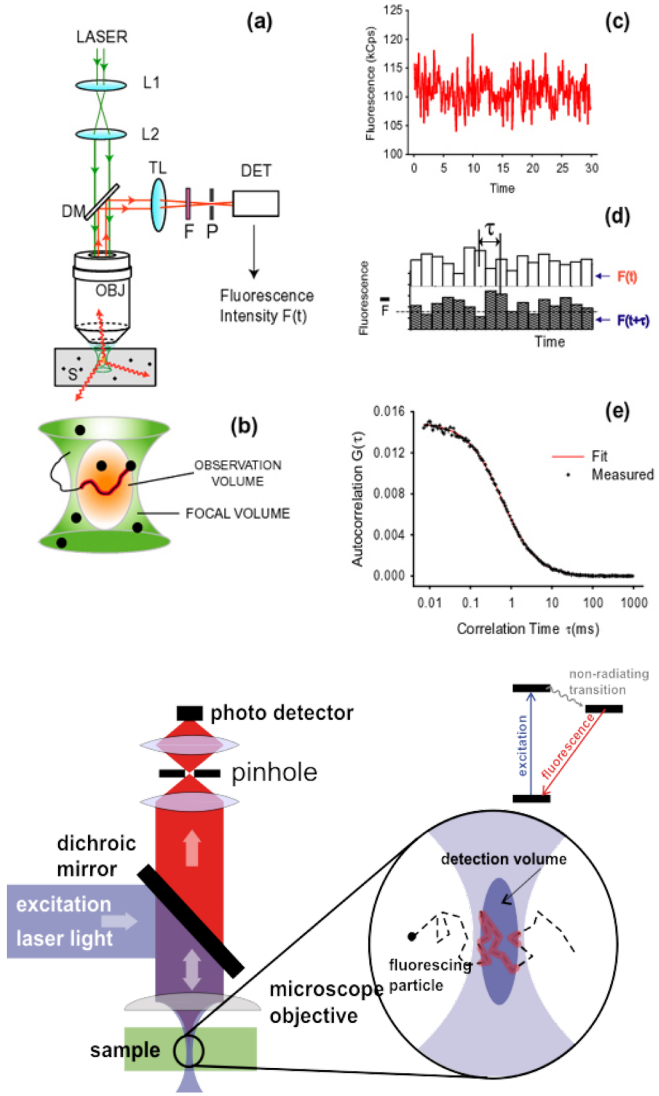
https://www.youtube.com/watch?v=QXGqA2IsivM

Fluorescence Correlation Spectroscopy

Single molecule tracking



Manzo and Garcia-Parajo, Rep Prog Phys 78(12), 2015



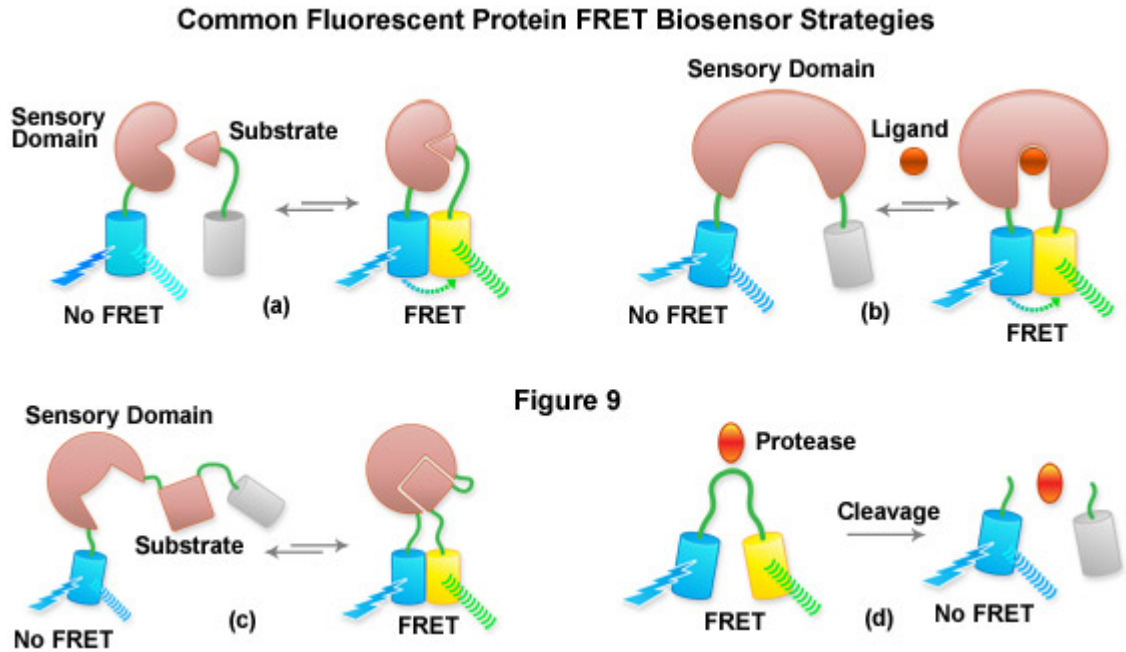

Common Fluorescent Protein FRET Biosensor Strategies
Sensory Domain
Substrate
No FRET
(a)
FRET
Sensory Domain
Ligand
No FRET
(b)
FRET
Sensory Domain
Substrate
No FRET
(c)
FRET
Protease
FRET
Cleavage
(d)
No FRET

Figure 9

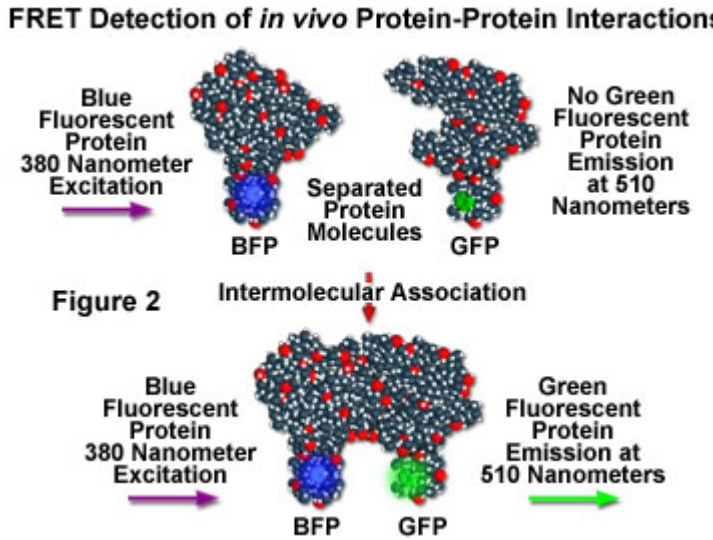


Figure 2

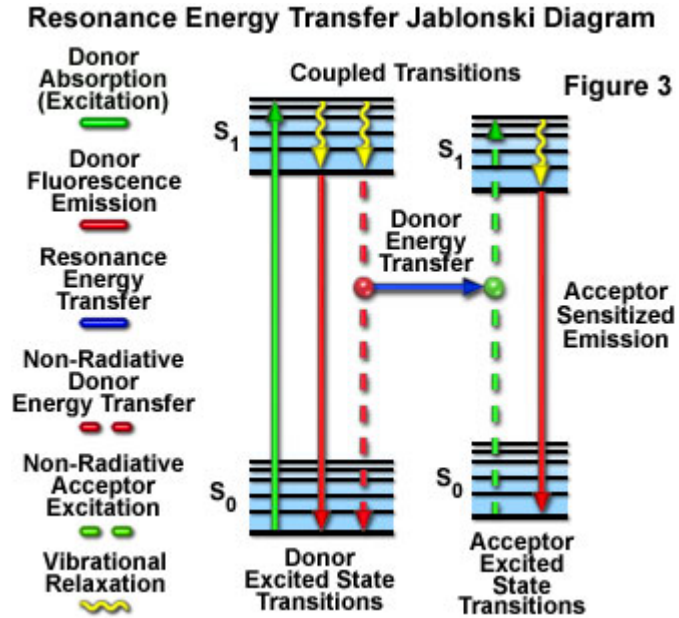


Figure 3

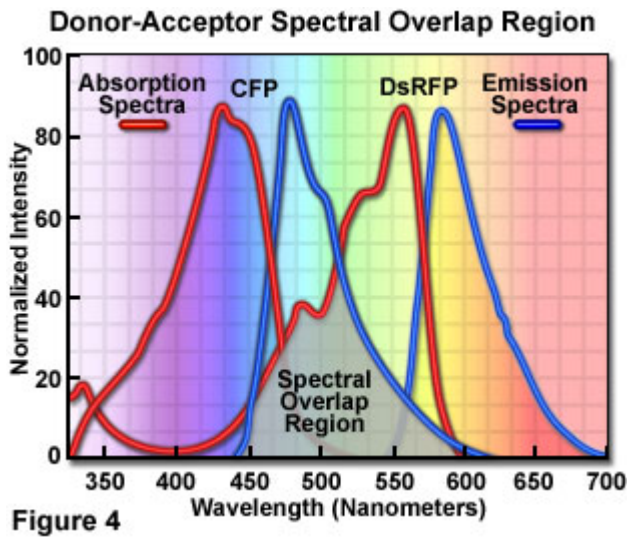


Figure 4

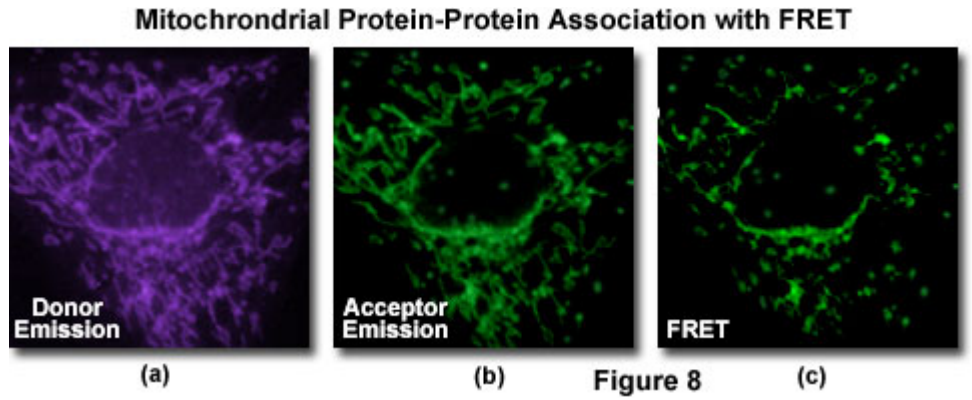


Figure 8

https://www.olympus-lifescience.com/en/microscope-resource/primer/techniques/fluorescence/fret

MICROSCOPIA MULTIFÓTONS

K. Konig, 2008