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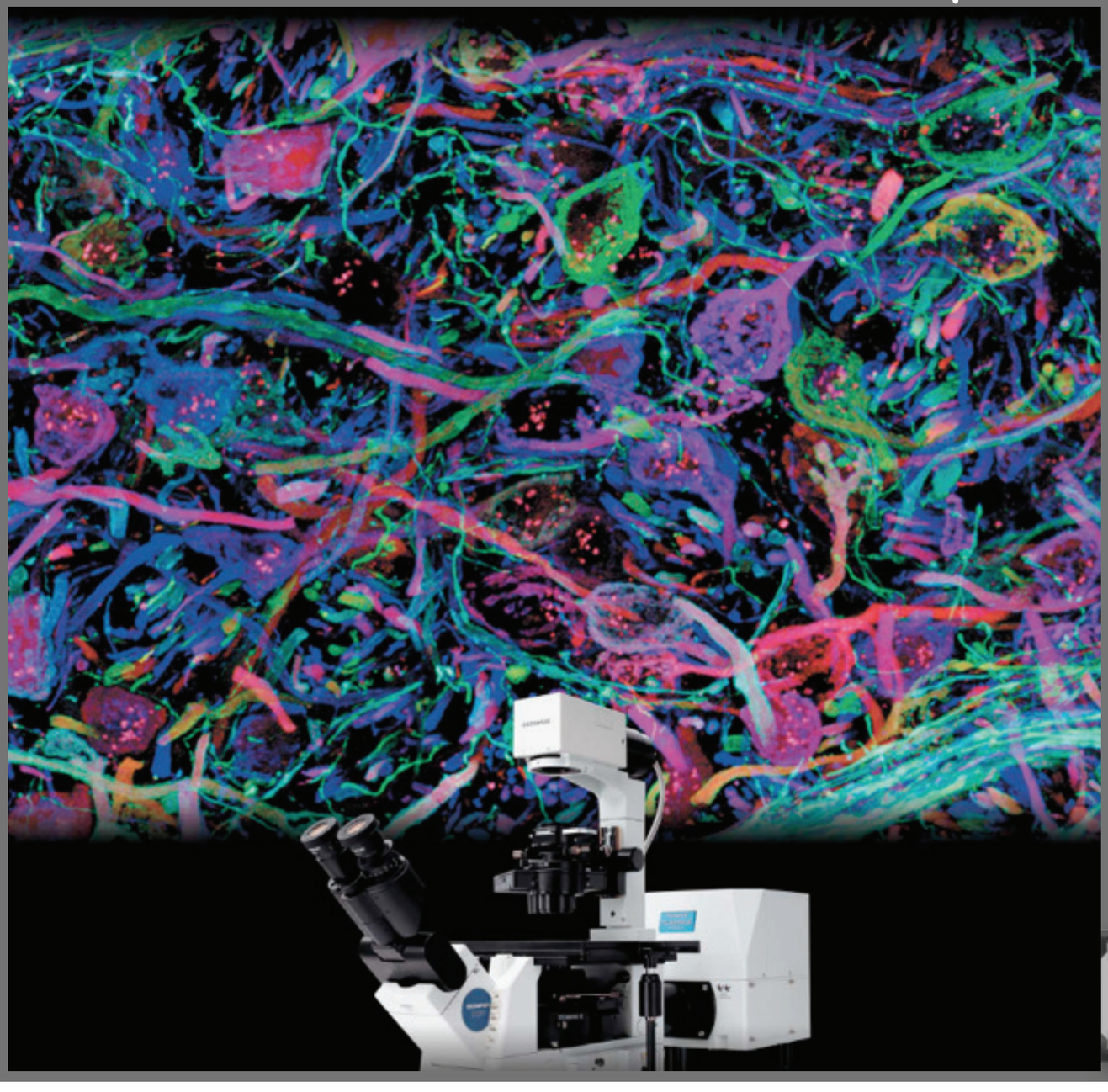
FV1000 cLSM

-concept



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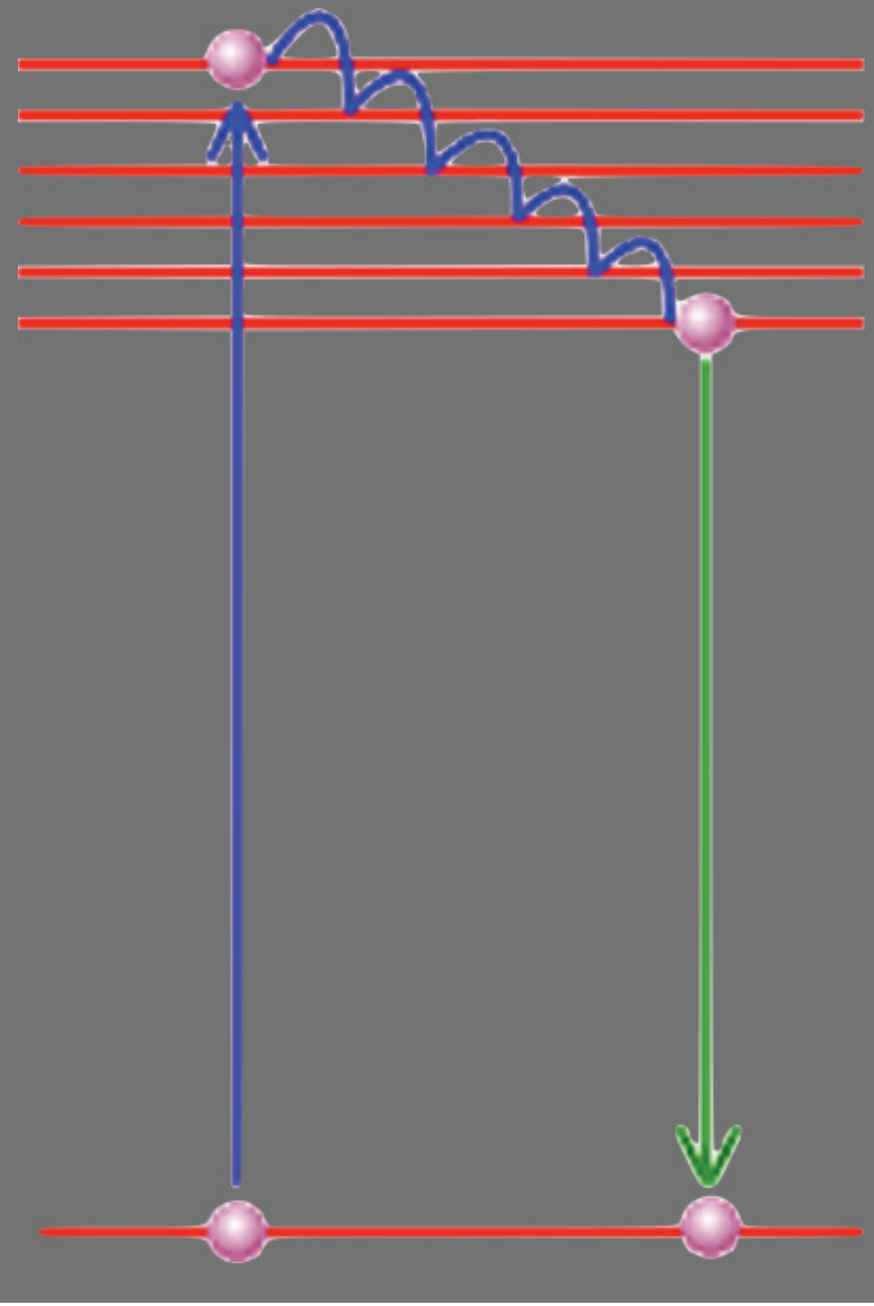
What is Fluorescence (Epi)



Excitation

Shorter WL
Stronger Energy

Emission

Longer WL
Lower Energy

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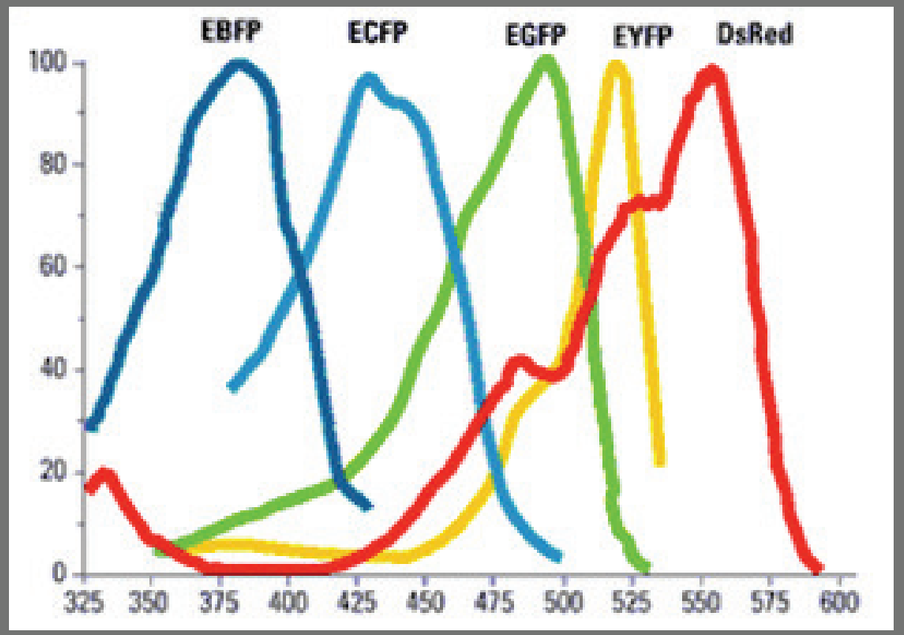
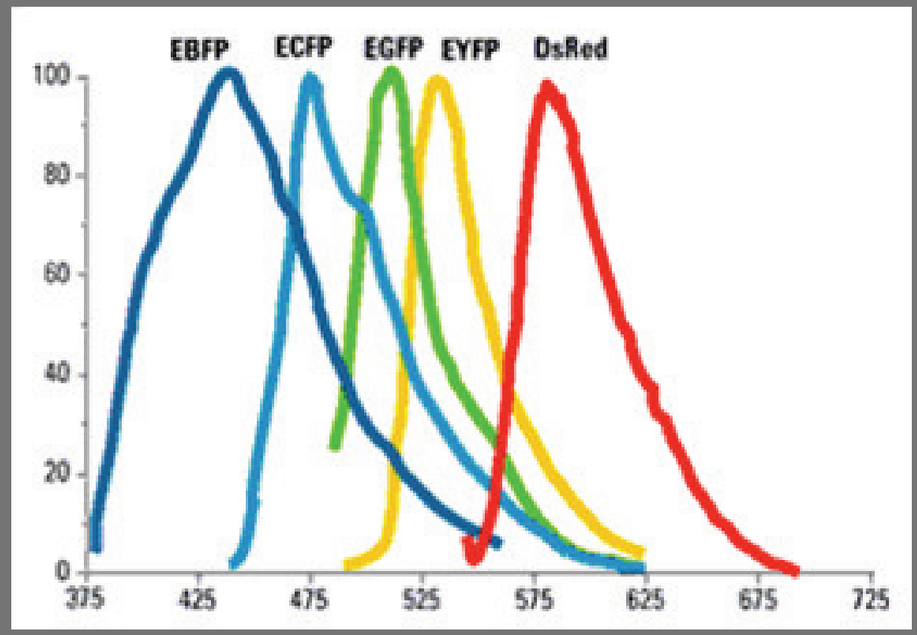
What Should We Notice!





Excitation WL

Emission WL

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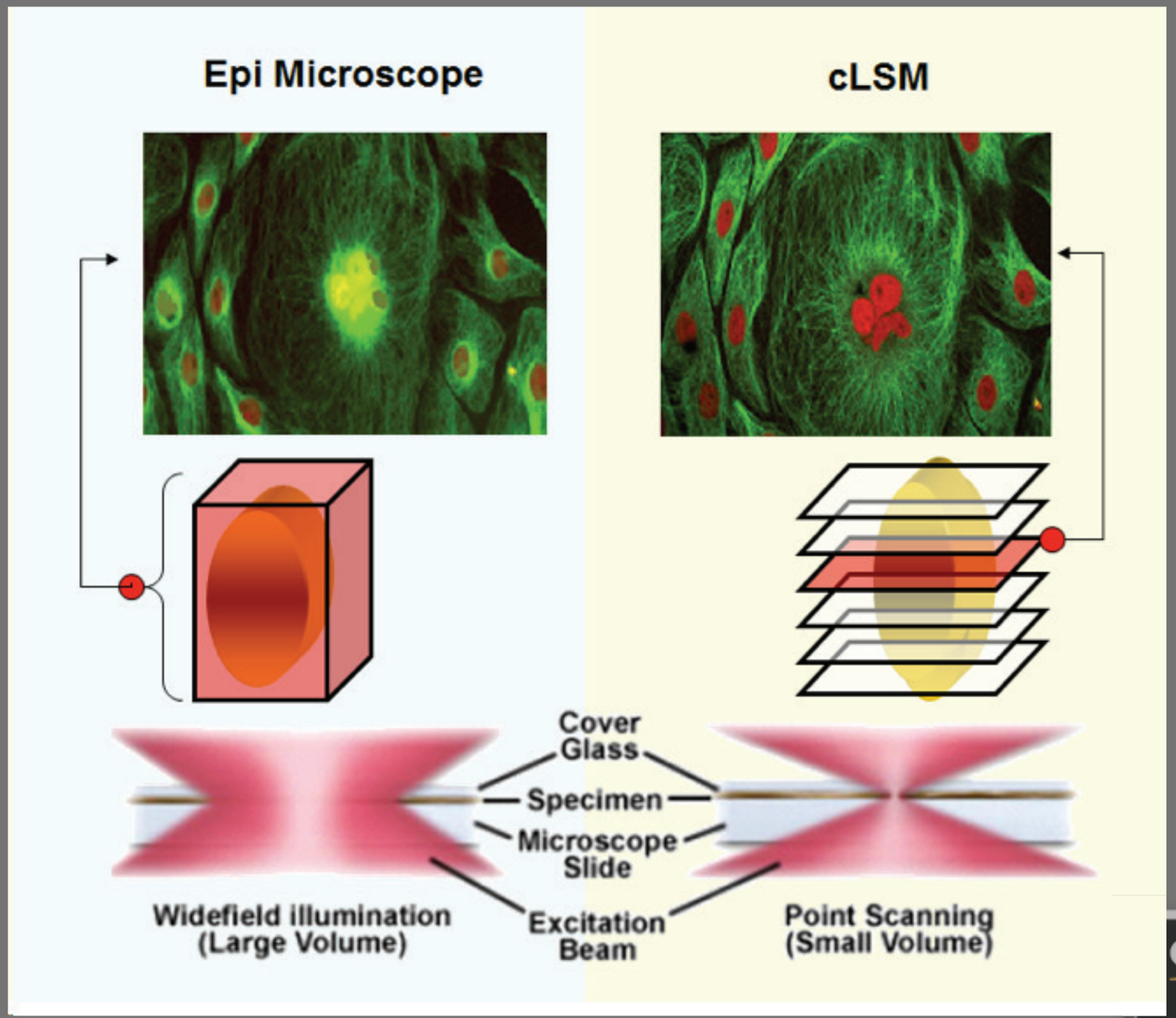
How Confocal System Reduce Blur Signal

-Laser (CW or Pulse)



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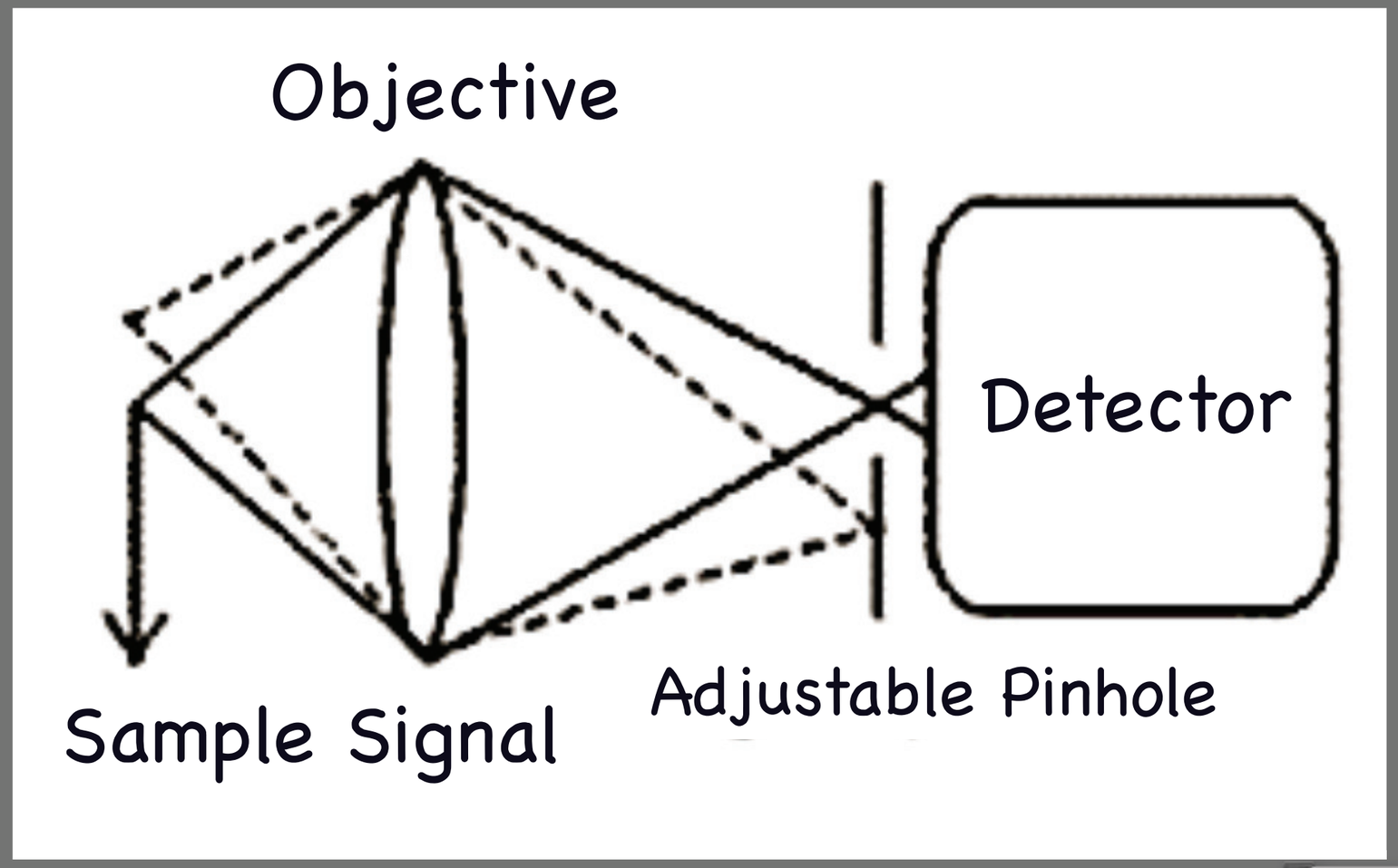
How Confocal System De-blur

-Pinhole



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Optical Resolution Under cLSM System

-XY, Z

$$FWHM_r = \frac{0.36\bar{\lambda}}{NA}\sqrt{\frac{\lambda_{ex}^2+\lambda_{em}^2}{\lambda_{em}^2+\frac{\lambda_{ex}^2}{1+\left(\frac{1.552\,NA\cdot PH_D}{\lambda_{em}Mm}\right)^2}}}$$

XY Direction

Z Direction

$$FWHM_z = \sqrt{\left(\frac{0.67\bar{\lambda}}{n-\sqrt{n^2-NA^2}}\right)^2\left(\frac{\lambda_{ex}^2+\lambda_{em}^2}{\lambda_{em}^2+\frac{\lambda_{ex}^2}{1+\left(\frac{1.55NA\cdot PH_D}{\lambda_{em}Mm}\right)^2}}\right)+\left(\frac{0.90n}{MmNA}PH_D\right)^2}$$

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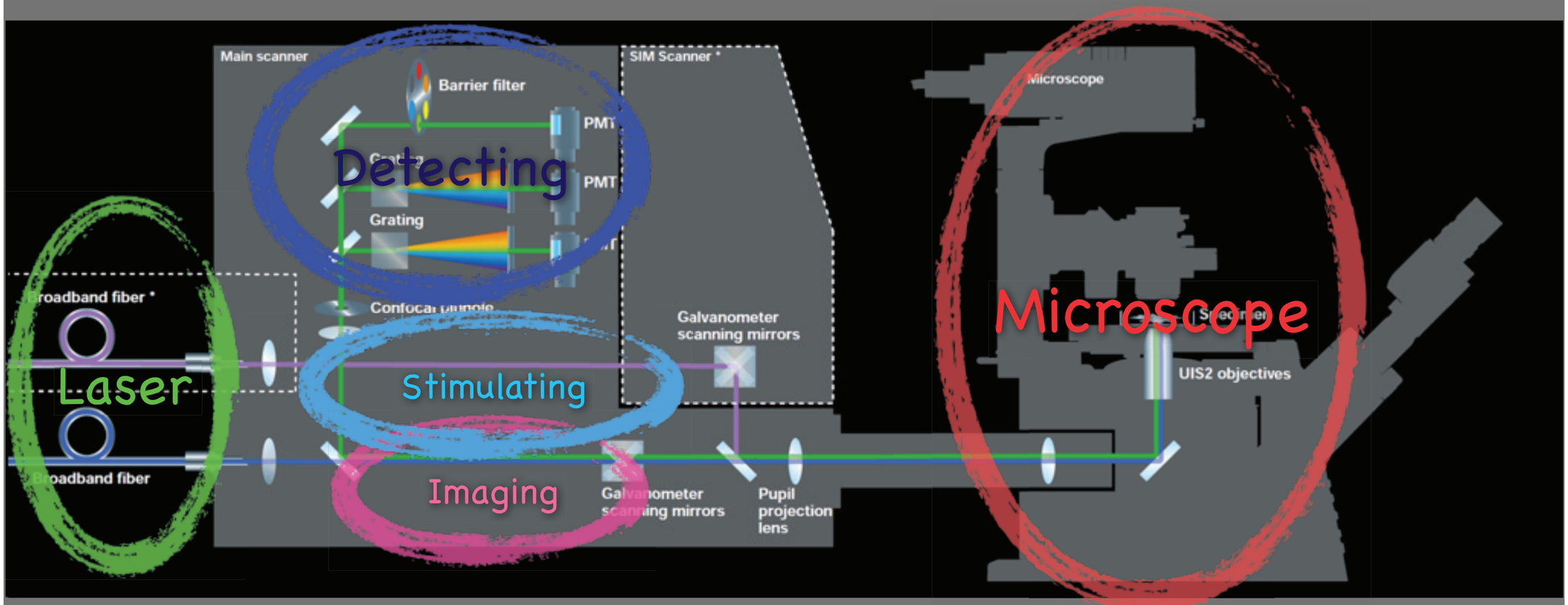

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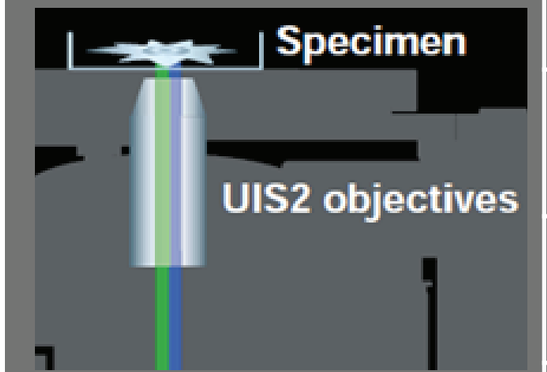
Objective



Objective	Model	Immersion Medium	N.A.	W.D.	Correction Ring
10X	UPLSAPO	Air	0.4	3.1	X
20X	UPLSAPO	Air	0.75	0.6	X
40X	UPLFLN	Oil	1.3	0.2	V
60X	UPLSAPO	Oil	1.35	0.15	V
100X	UPLSAPO	Oil	1.4	0.13	V

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-How to OBSERVE Our Sample

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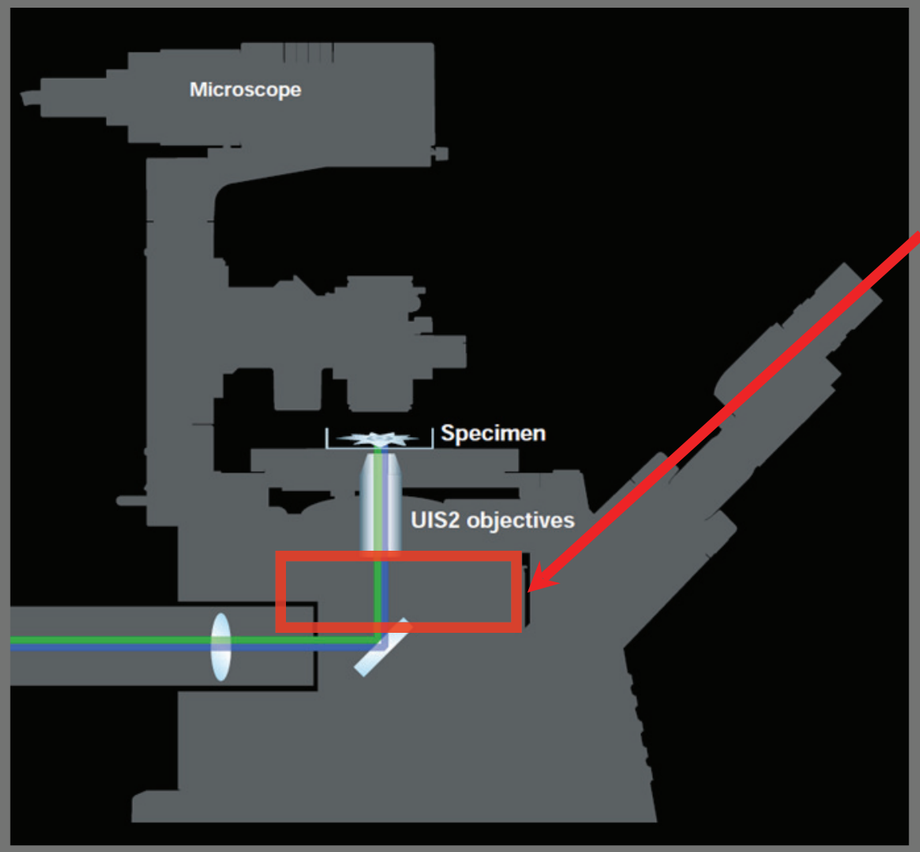
Epi filter set



	Ex.	DM	Em.
Blue	330-385	400	420
Green	470-495	505	510-550
Red	530-550	570	575
NIR	595-645	655	660-745

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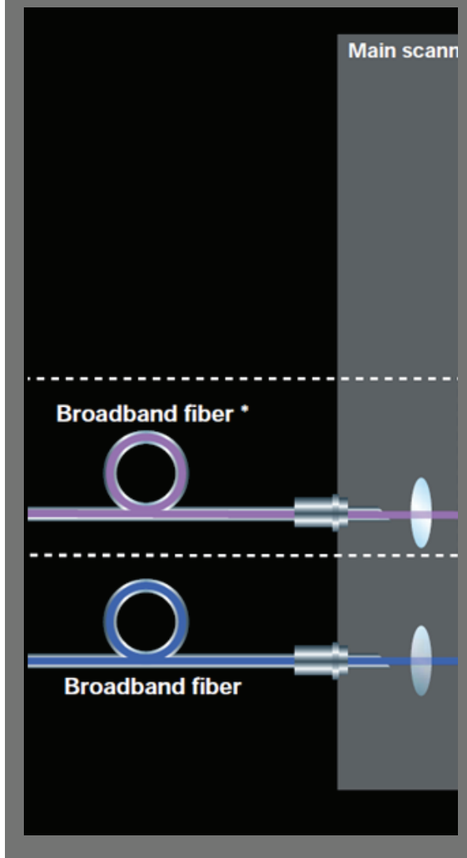
-Laser Scanning



Laser Type	Suitable Dye
LD Laser 635nm	Cy7, TOTO3
LD Laser 559nm	MitoTracker, DsRed
Multi Ar Laser 488 515nm	Alexa488, GFP
LD Laser 440nm	CFP
LD Laser 405nm	DAPI, Photobleach

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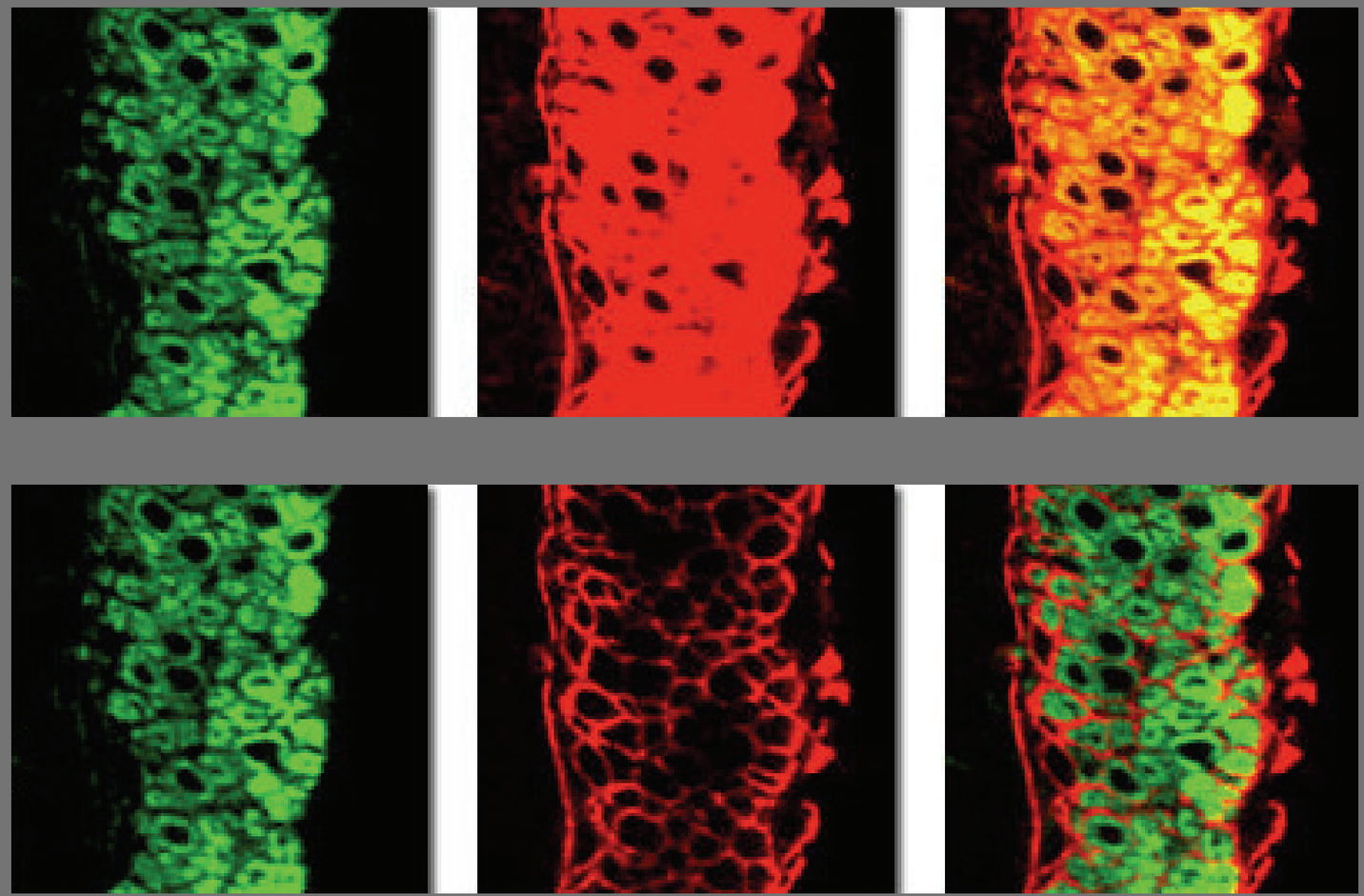
WHAT! Cross Talk

-Epi Emission Bleed-Through



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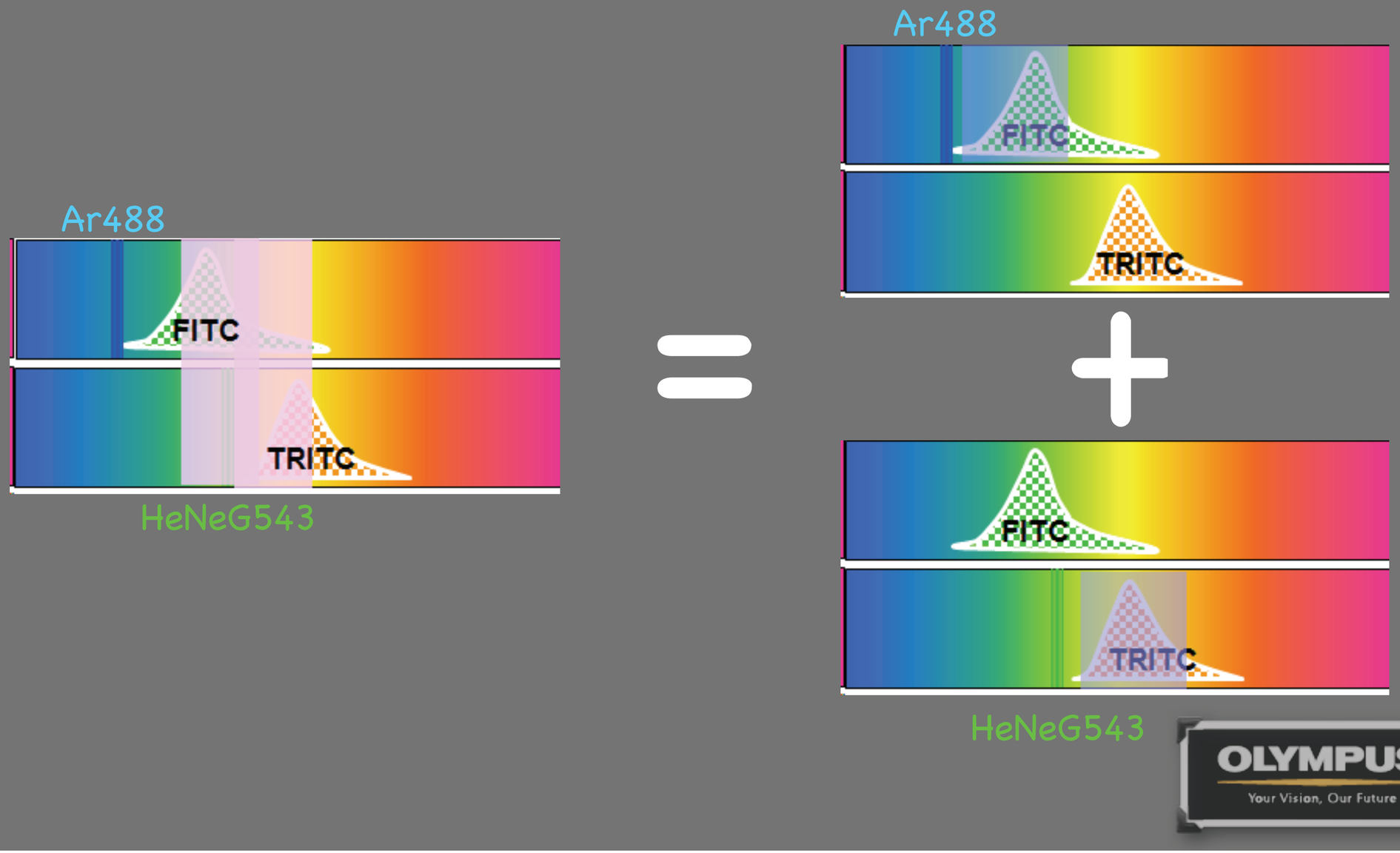
How to Solve Cross Talk

-Excitation Sequential Scanning (AOTF)



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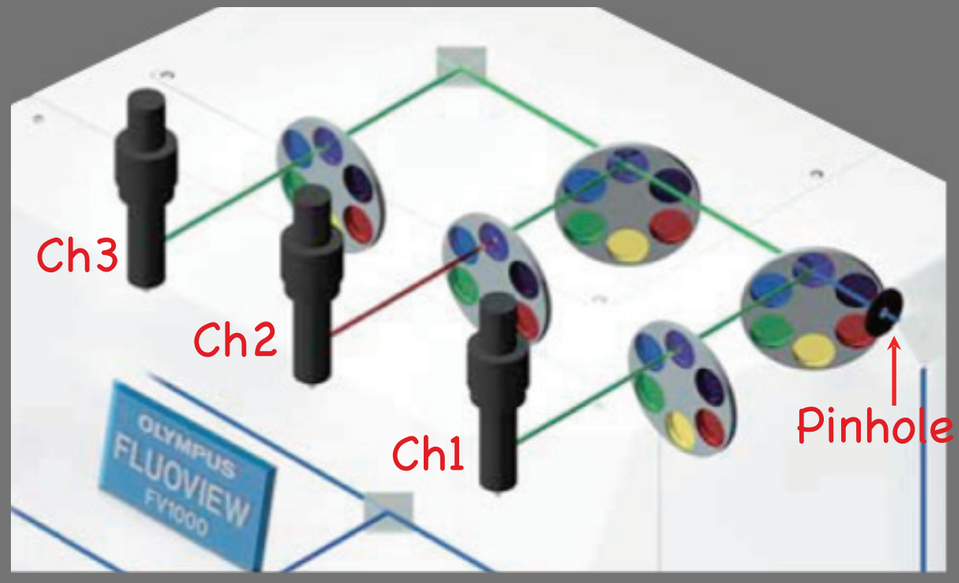
PMT Filter set



DM for PMT	1st PMT	2nd PMT	3rd PMT
Pos. 1	Mirror	Mirror	Mirror
Pos. 2	Glass	Glass	Glass
Pos. 3	SDM560	SDM640	
Pos. 4	SDM510	SDM560	
Pos. 5	SDM490		
Pos. 6			

Em for PMT	1st PMT	2nd PMT	3rd PMT
Pos. 1	465-495	505-605	655-755
Pos. 2	505-540	575-620	575-675
Pos. 3	480-495	535-565	
Pos. 4	430-470	505-540	
Pos. 5			
Pos. 6			

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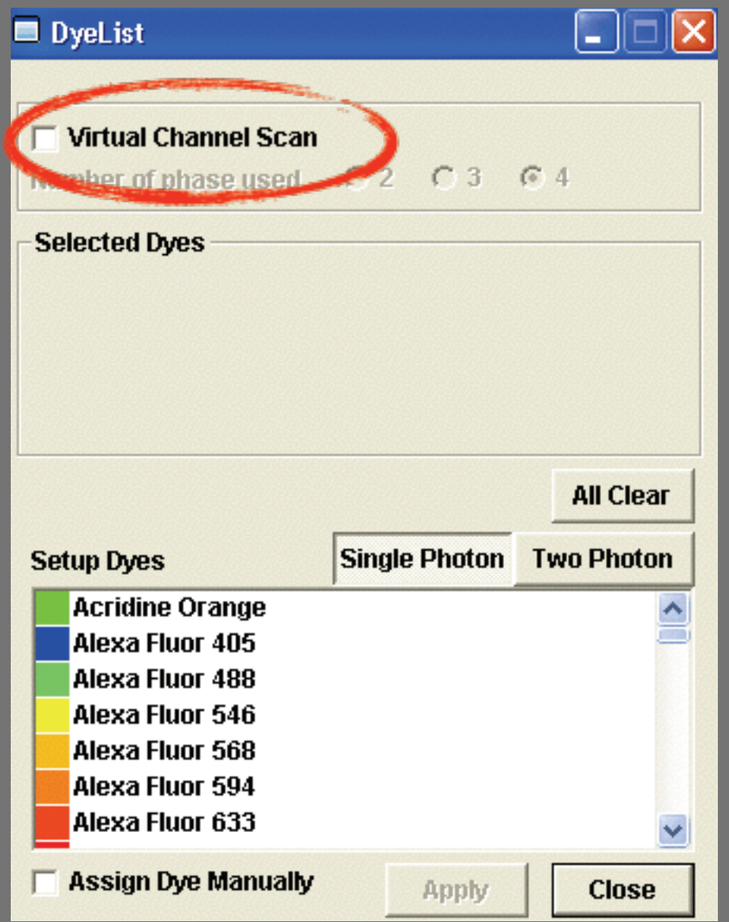
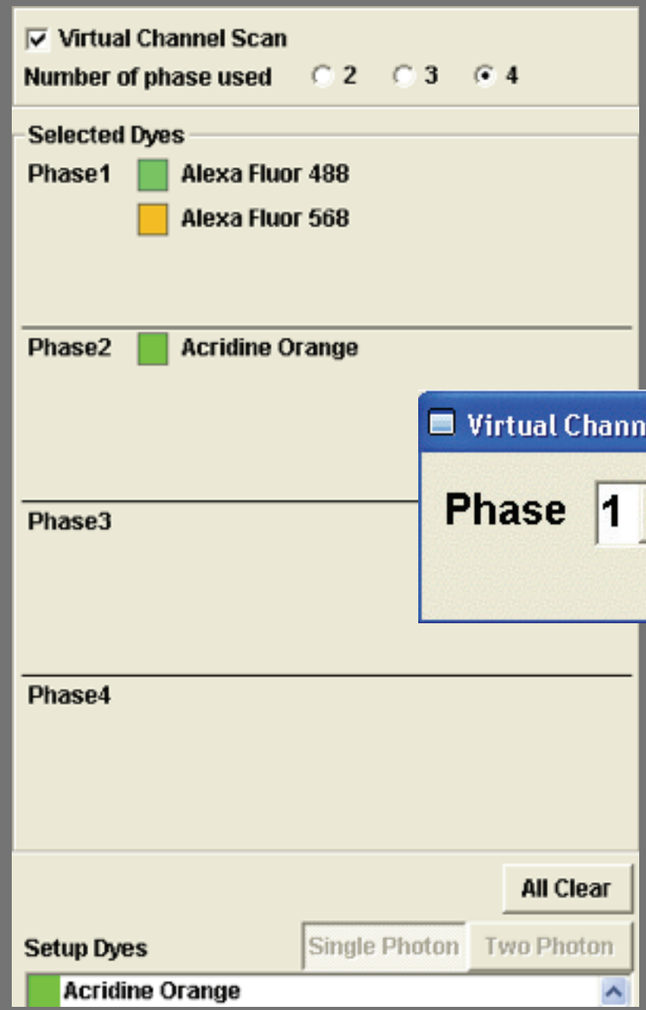
Virtual Channel

-more than 3 dye





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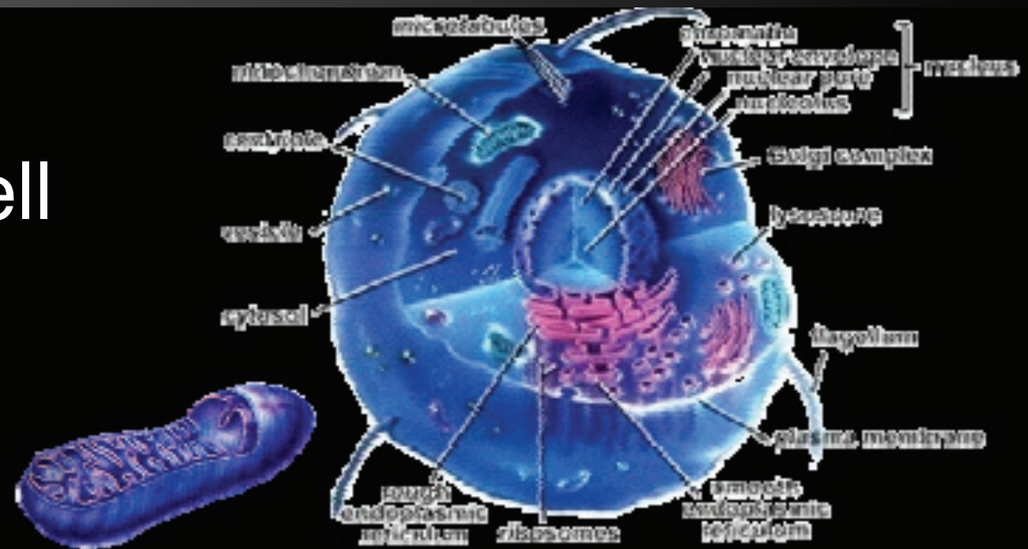
Confocal Based Super-resolution

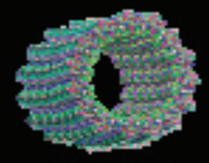
About Super-Resolution

-2014 Nobel Prize in Chemistry

Cell

Sub-cellular





1Å 1nm 10nm 100nm 1µm 10µm 0.1mm

Atomic | Molecular | Sub-cellular | Cellular

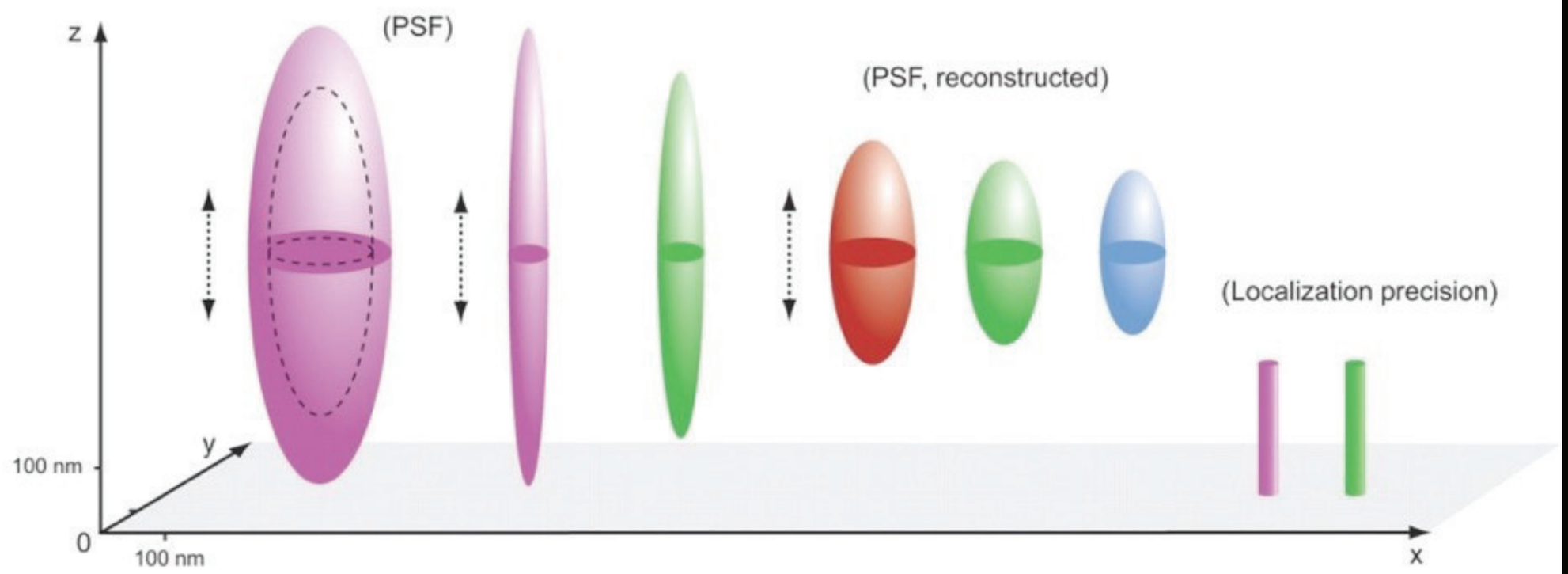
SR Microscopy

Optical Microscopy

www.3dchem.com; wikipedia.org/wiki/Kinesin; cvcweb.ices.utexas.edu; Fotin et al., *Nature* 2004; hrsbstaff.ednet.ns.ca; www.ebi.ac.uk

About Super-Resolution

	CLSM	STED	CW-STED	3D-SIM			PALM/STORM	
~λ_{em} [nm]	460-670	670	520	620	520	460	670	520



~$D_{x,y}$ [nm]	180-250	60	70	130	110	100	30
~D_z [nm]	500-700	700	560	340	280	250	140 (TIRF-range)
~$V_{x,y,z}$ [*10^{-3} µm^3]	10-23	1.3	1.5	3.0	1.8	1.3	0.1

J Cell Biol. 2010, 190(2):165-75.

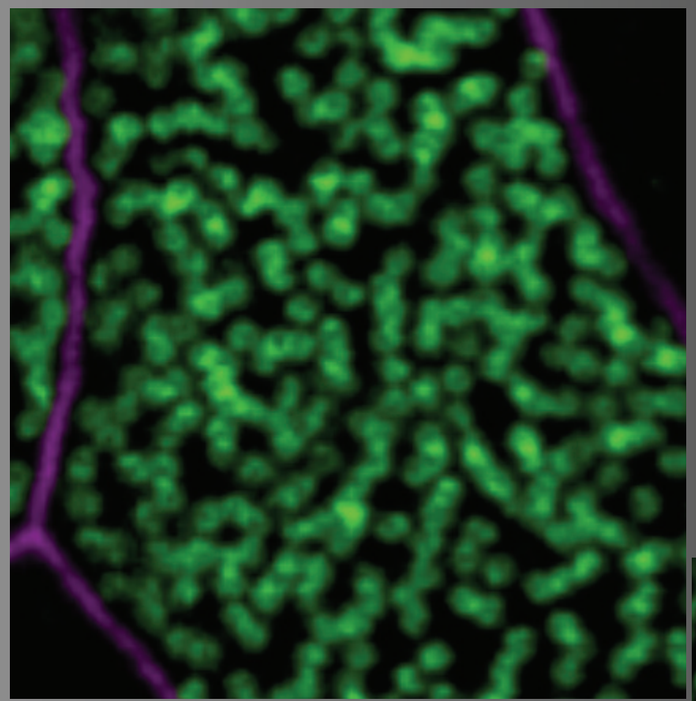
About Super-Resolution

Techniques	Strong points	Weak points
SIM	Enable to use most of dyes. Fast imaging (several fps)	No optical sectioning Not able to apply to deep plane
STED	Resolution (up to 50nm)	Not able to apply to deep plane Some restrictions in dyes Multi color imaging
PALM/STORM	Resolutions (up to 10nm)	Sampling time (>10min) Need special dyes Only for sample surface

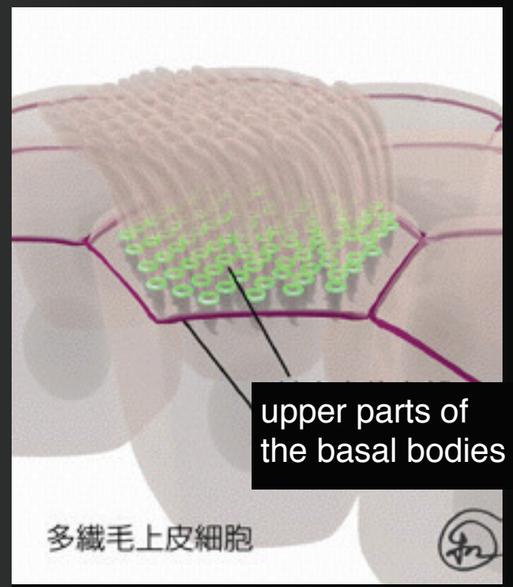
There are no perfect technique for super resolution

Confocal Based Super-Resolution

-OLYMPUS Super-Resolution (OSR)

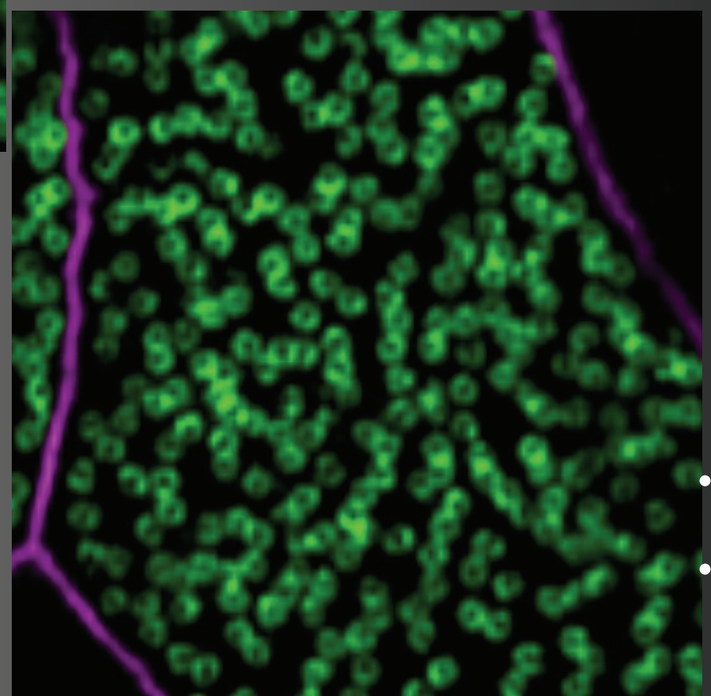


confocal



Trachea epithelial cells

Approx. 250~300nm diameter



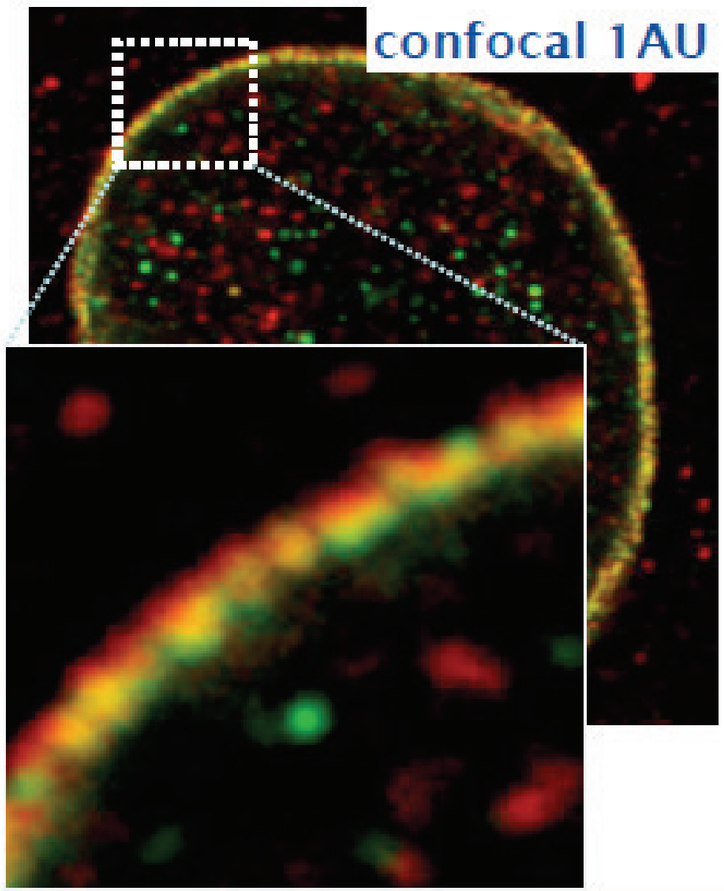
FV-OSR

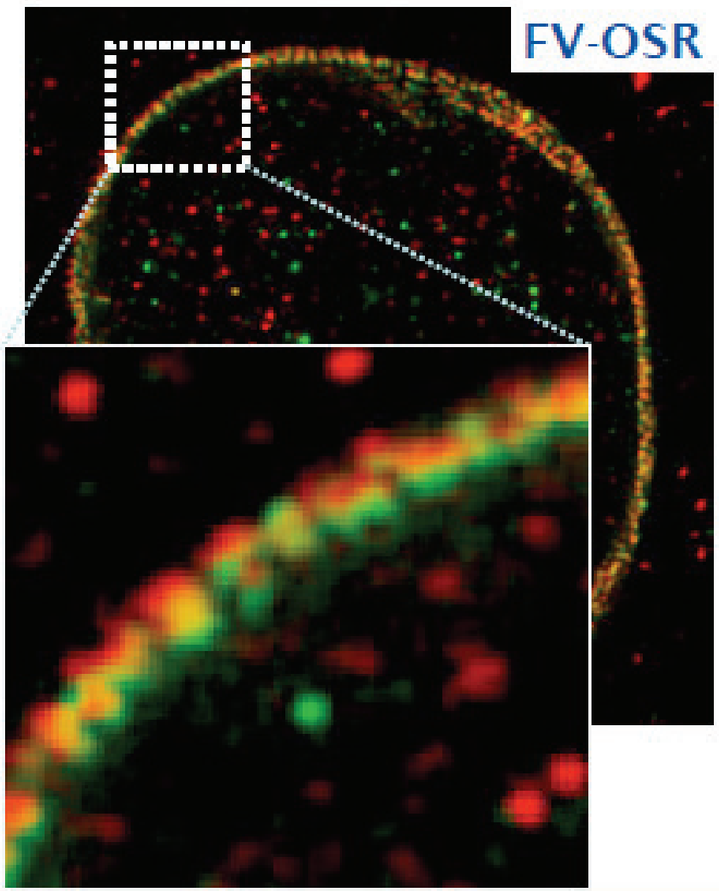
- **FV1200/UPLSAPO60XS**
- **ex 473/559 nm**

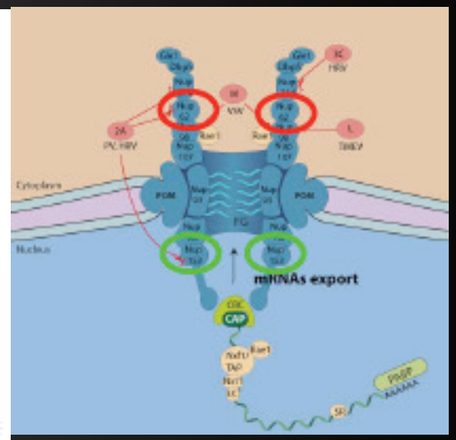
Confocal Based Super-Resolution

-OLYMPUS Super-Resolution (OSR)

Nuclear pore of HeLa cell
Green : Nup153(Alexa488)
Red: Nup62(Alexa555)
Image data courtesy of:
Prof. Kosako, H (TOKUSHIMA Univ.)







Confocal Based Super-Resolution
-FV-OSR

Techniques	Strong points	Weak points
FV-OSR	**Enable to use most of dyes.** **Enable to get the image in deep plane.** **Optical sectioning for thick samples**	**Sampling time (compare to SIM)** **Resolutions (compare to STED, PALM/STORM)**
SIM	Enable to use most of dyes. Fast imaging (several fps)	No optical sectioning Not able to apply to deep plane
STED	Resolution (up to 50nm)	Not able to apply to deep plane Some restrictions in dyes Multi color imaging
PALM/STORM	Resolutions (up to 10nm) Z resolutions	Sampling time (around 10min) Need special dyes Only able to observe surface of sample.

Confocal Based Super-Resolution
-FV-OSR

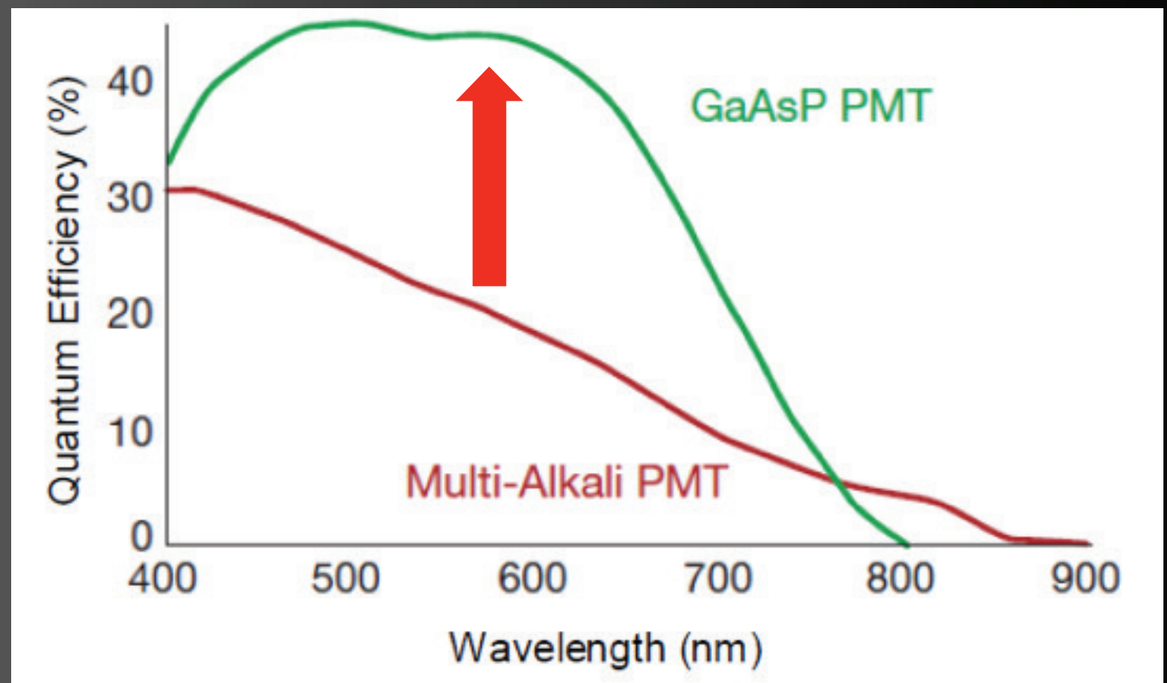
Confocal Based Super-Resolution: FV-OSR –Why Cooling GaAsP Detector?

FV12-HSD

Higher sensitivity →

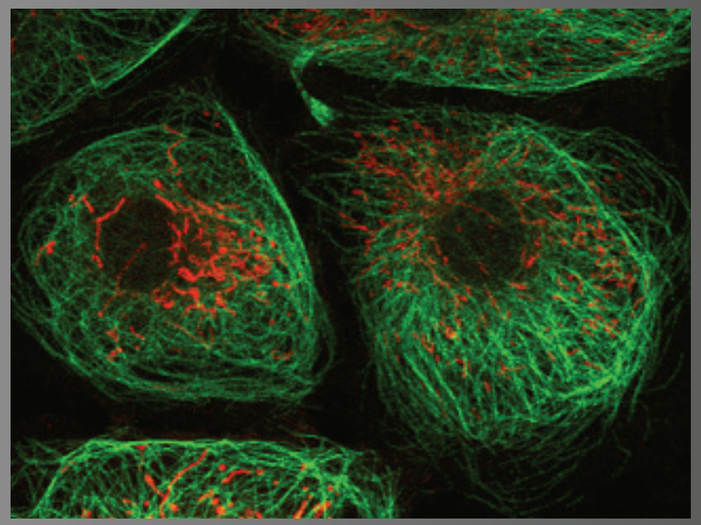
Better detail structure and less laser excitation →

Less signal bleach and photo-toxicity

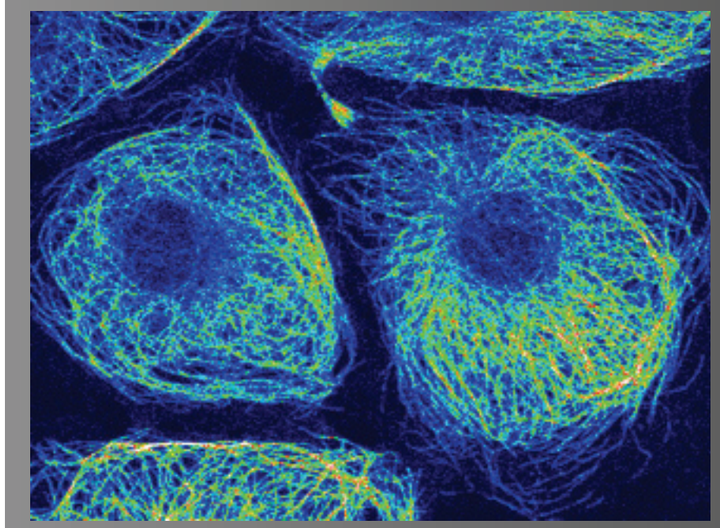


Confocal Based Super-Resolution: FV-OSR –Why Cooling GaAsP Detector?

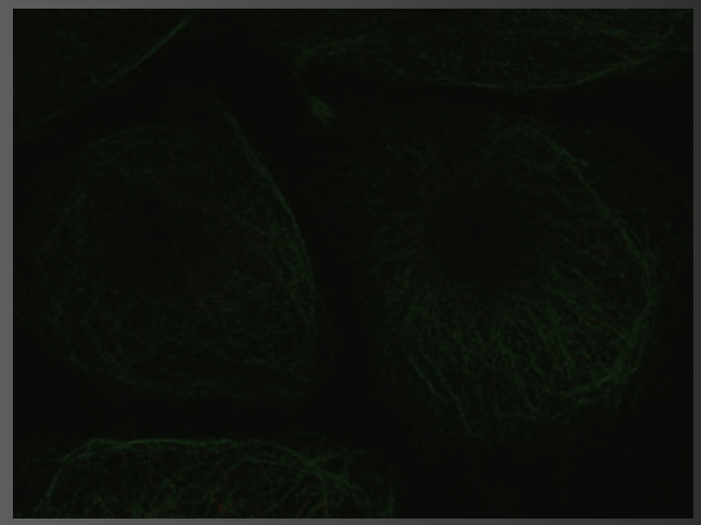
GaAsP-PMT



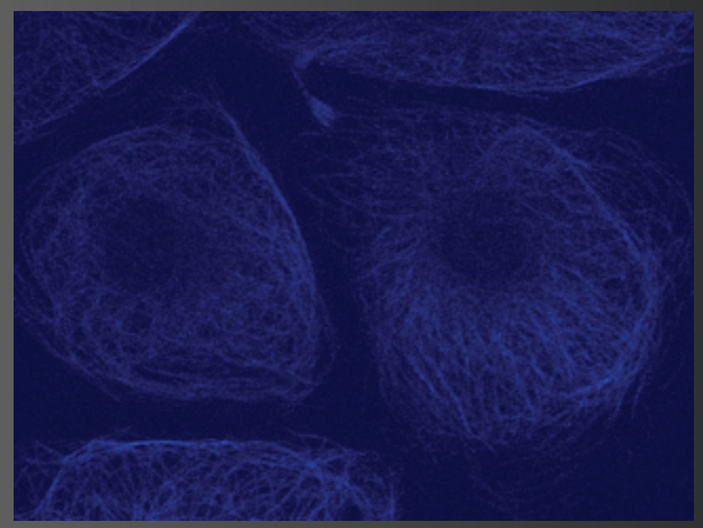
HV：600V Laser：473nm 2%, 559 1.3%



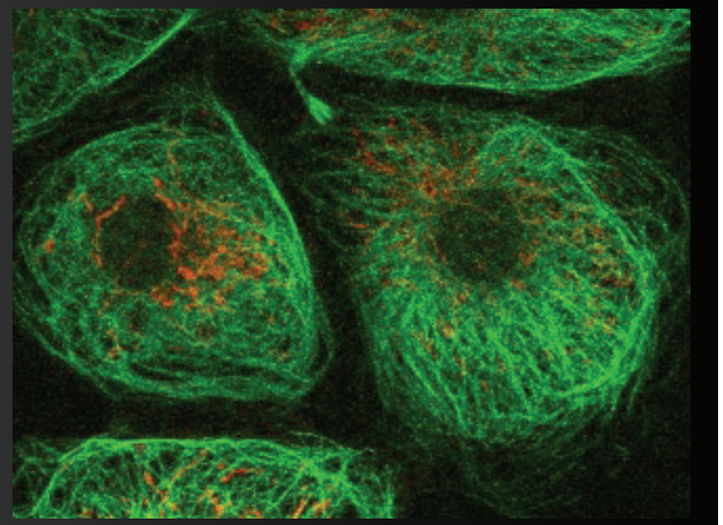
Multi Alkali-PMT



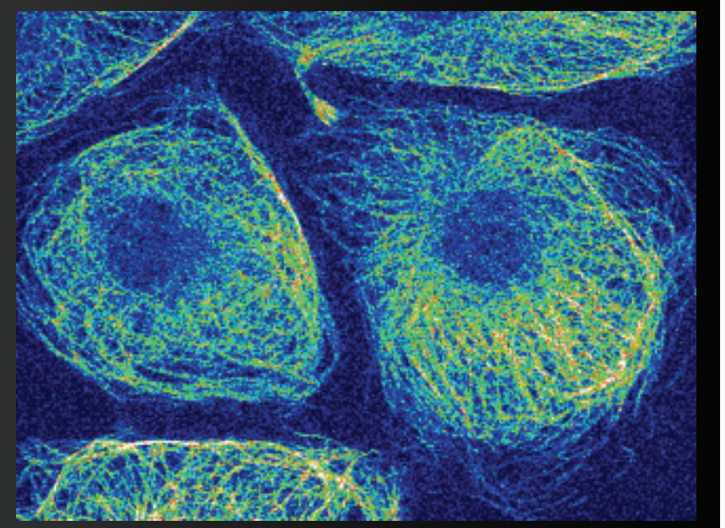
HV：600V Laser：473nm 2%, 559 1.3%



Multi Alkali-PMT



HV：900V Laser：473nm 2%, 559 1.3%



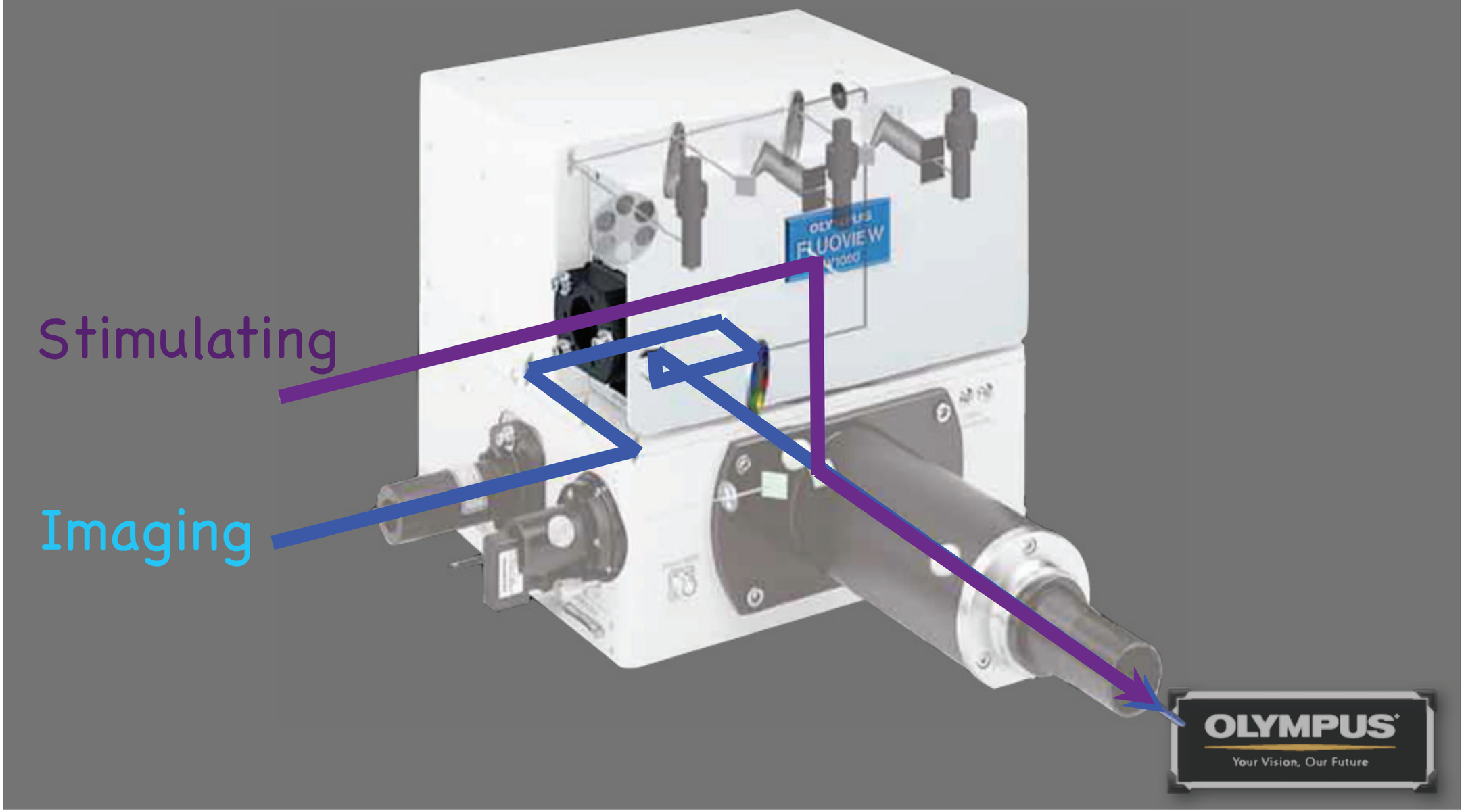
Confocal Based Super-Resolution: FV-OSR –Resolution relay on objective NA

Table : Objective lens and resolution

Objective lens	NA	Resolution(with high contrast mode) *2
UPLSAP060XW	1.2	144nm
UPLSAP060XO	1.35	128nm
UPLSAP060XS	1.3	134nm
UPLSAP060XS2	1.3	134nm
UPLSAP0100XO	1.4	121nm
UPLSAP0100XS	1.35	129nm
PLAPON60XO	1.42	120nm
PLAPON60XOSC	1.4	124nm
PLAPON60XOSC2	1.4	124nm
APON60XOTIRF *1	1.49	119nm
APON100XHOTIRF *1	1.7	111nm
UAPON100XOTIRF *1	1.49	117nm

Except Imaging??

-SIMultaneous Photobleaching



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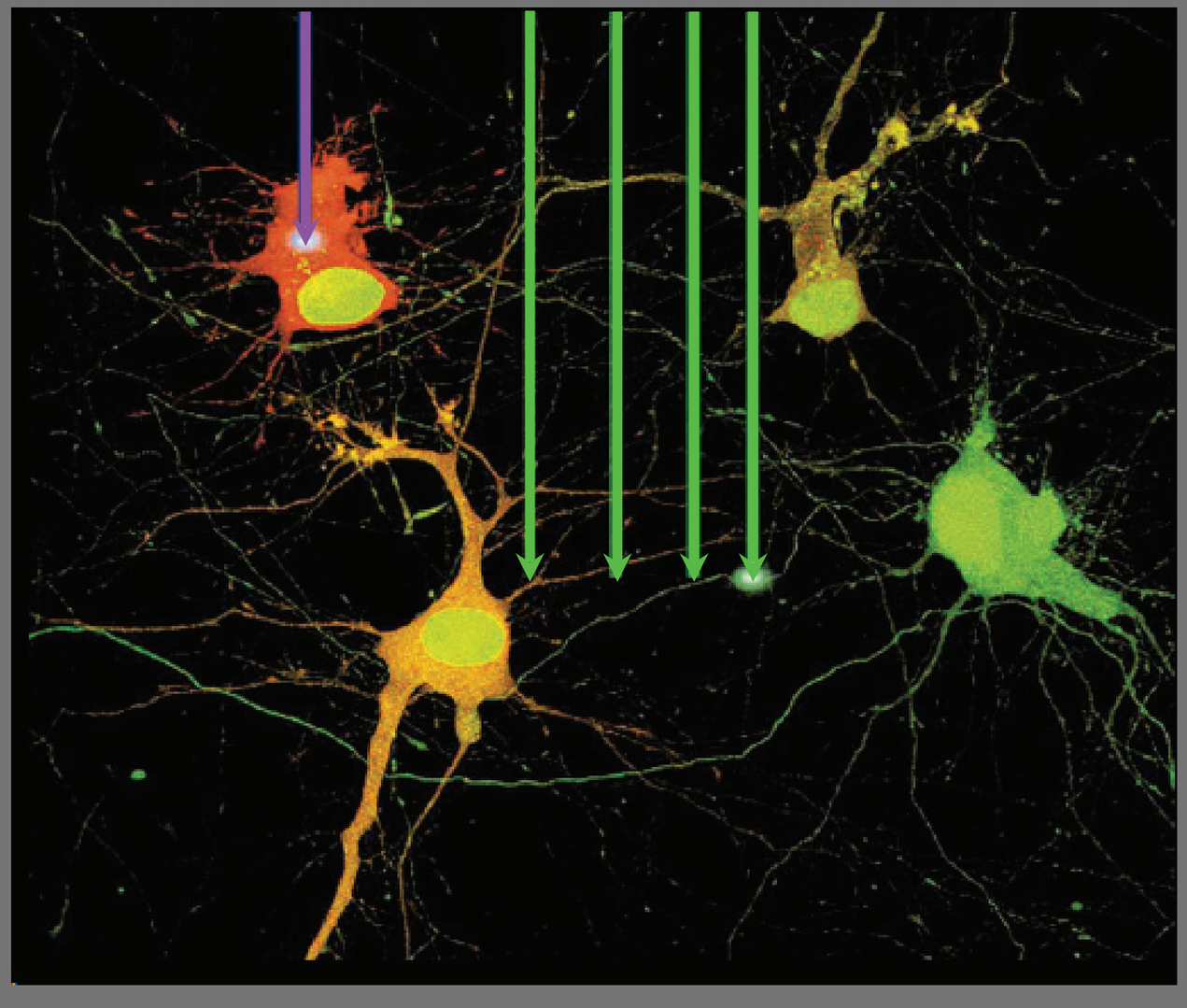
What's SIM



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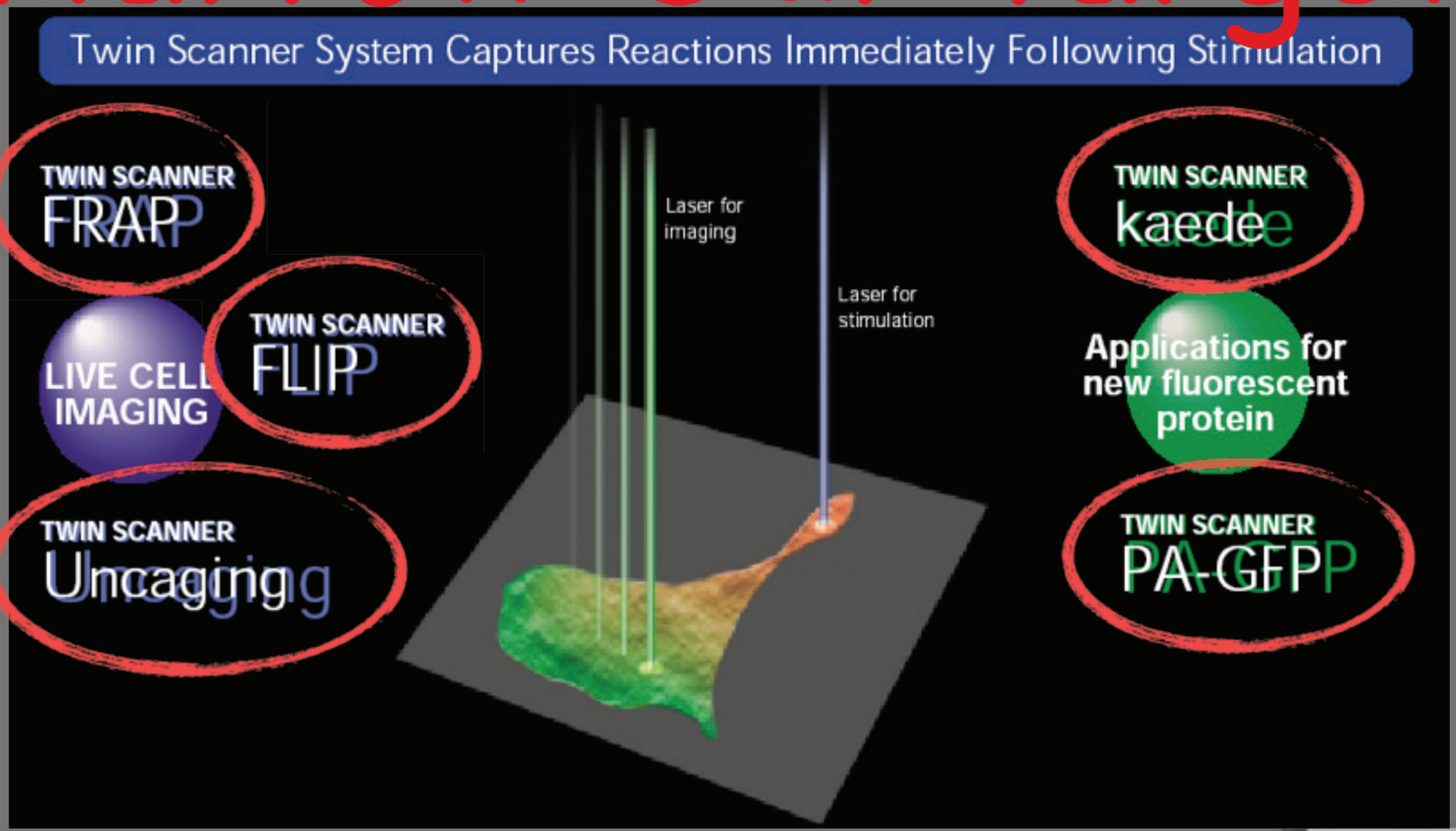
Why SIM

-Application of SIM

Narrow Our Target



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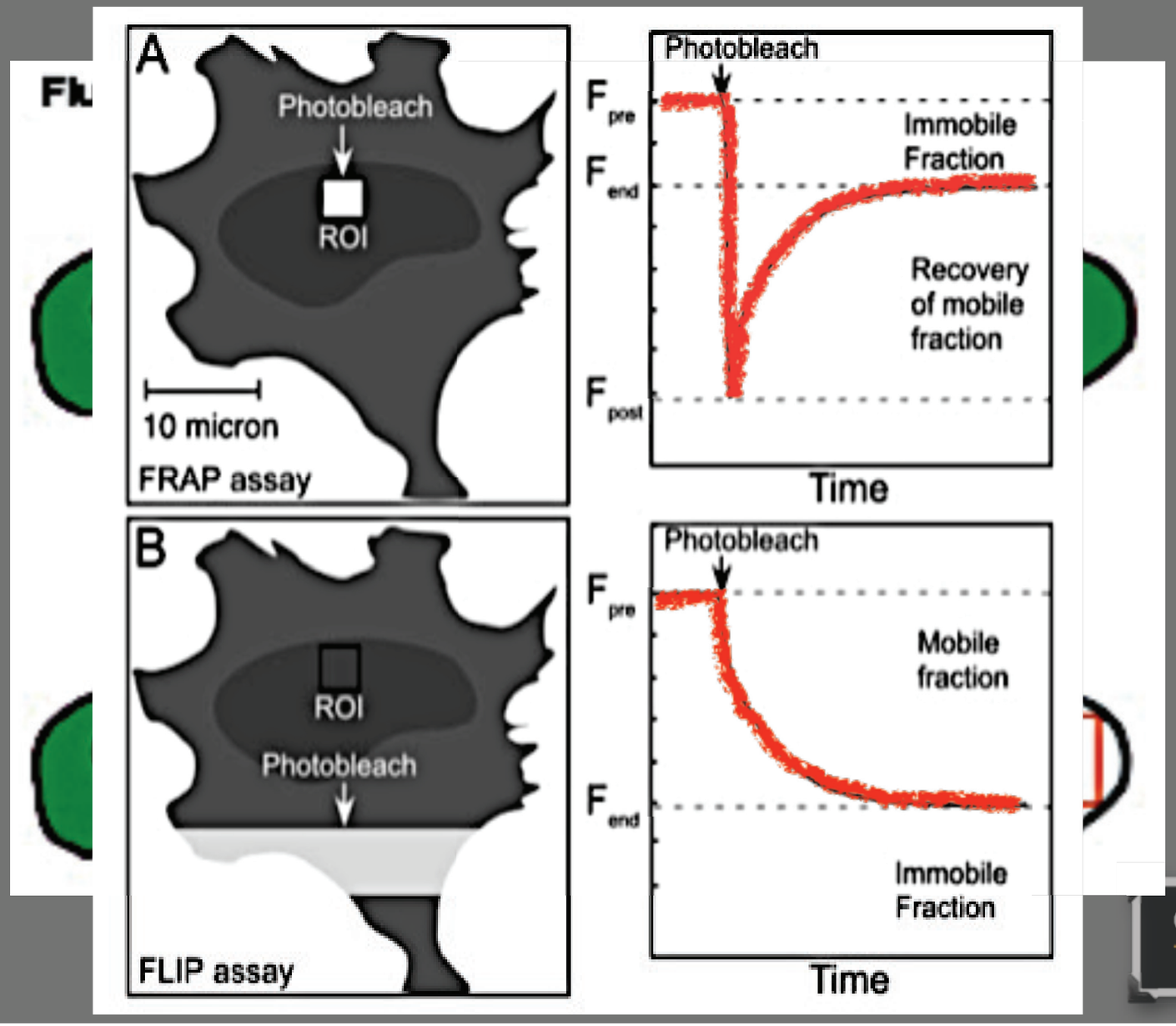
Fluorescence Recovery After Photobleach
Fluorescence Loss In Photobleach



Uncaging

-Bullseye

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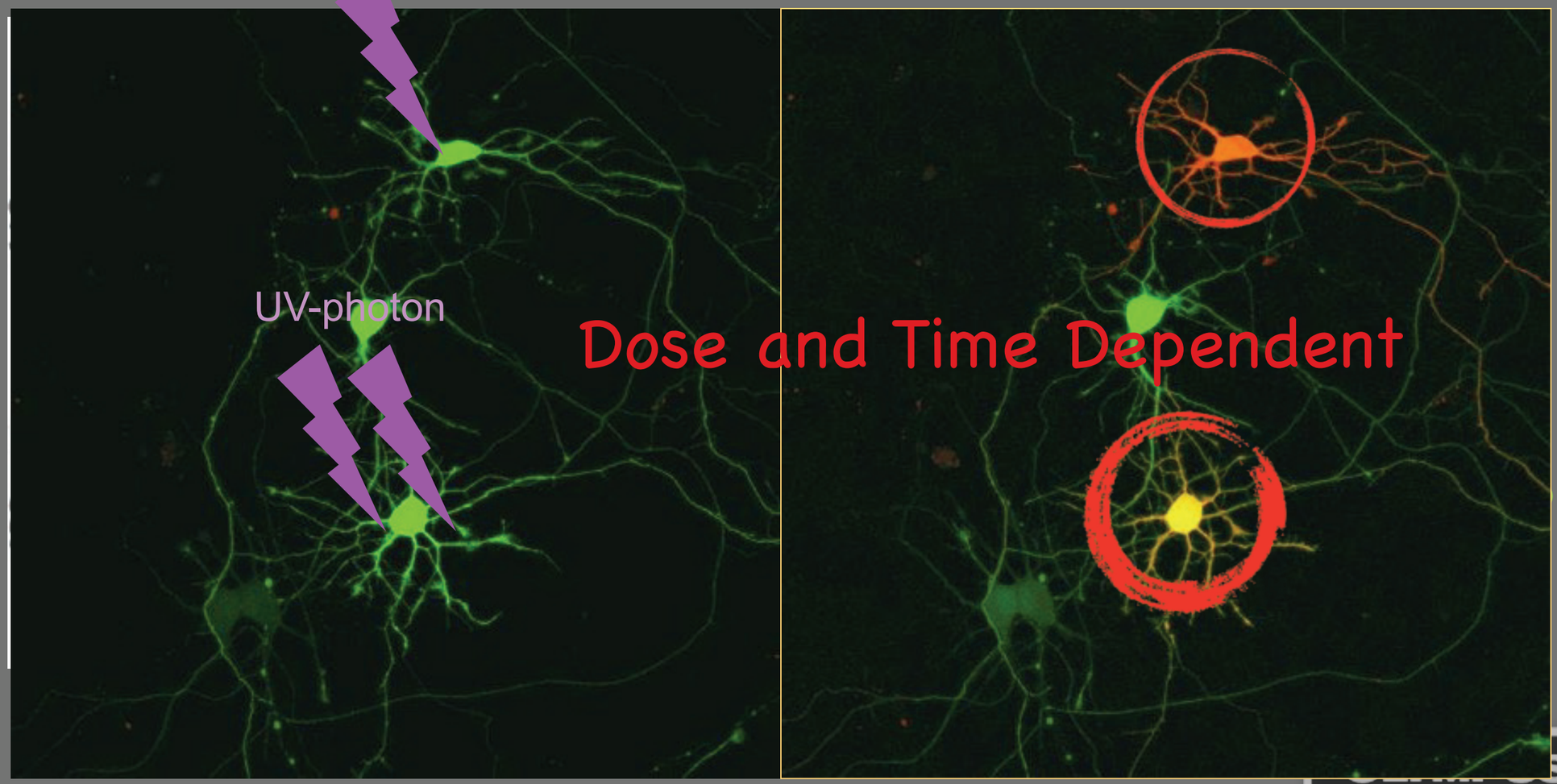
Photo-Conversion

-PA-GFP and Kaede

UV-photon



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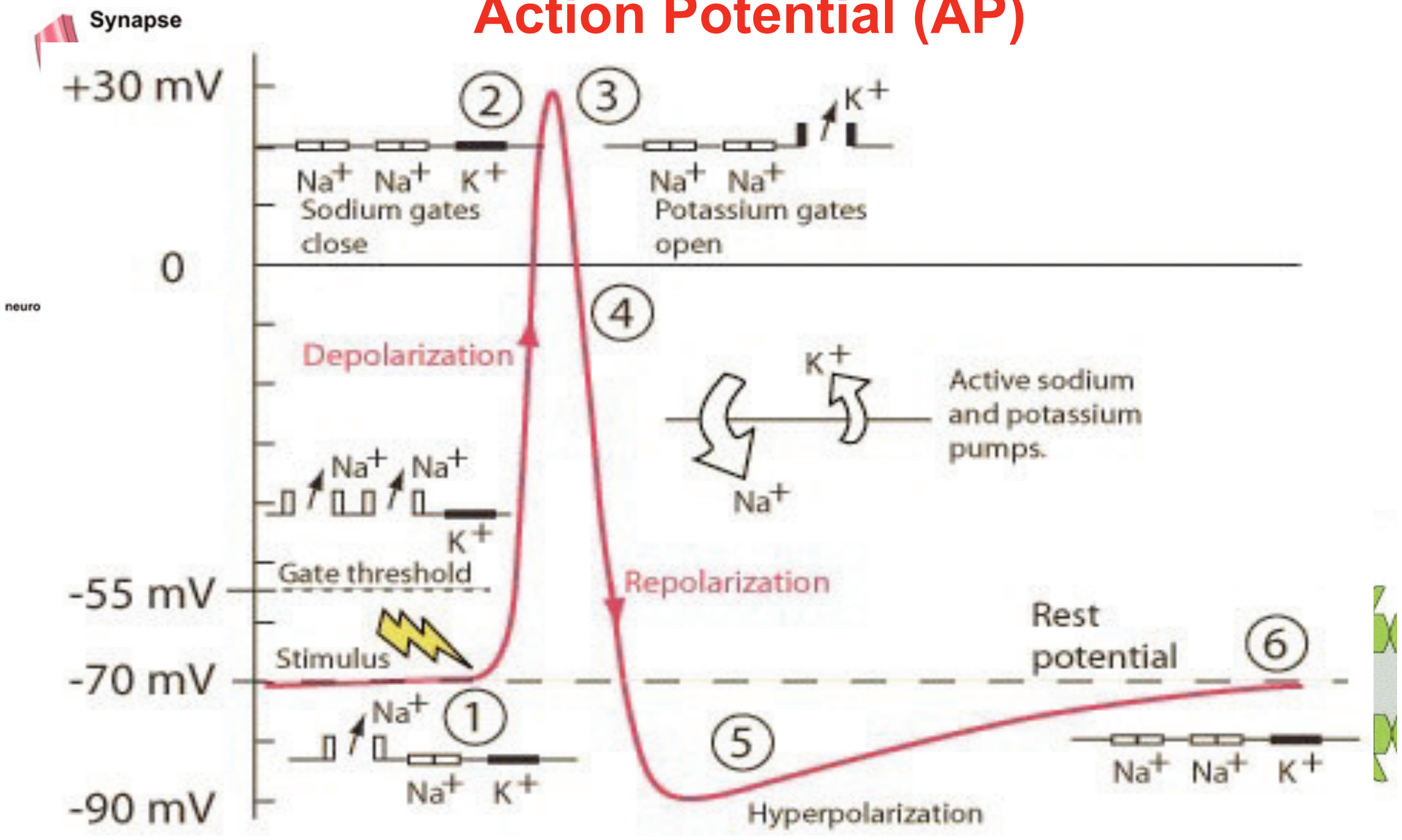
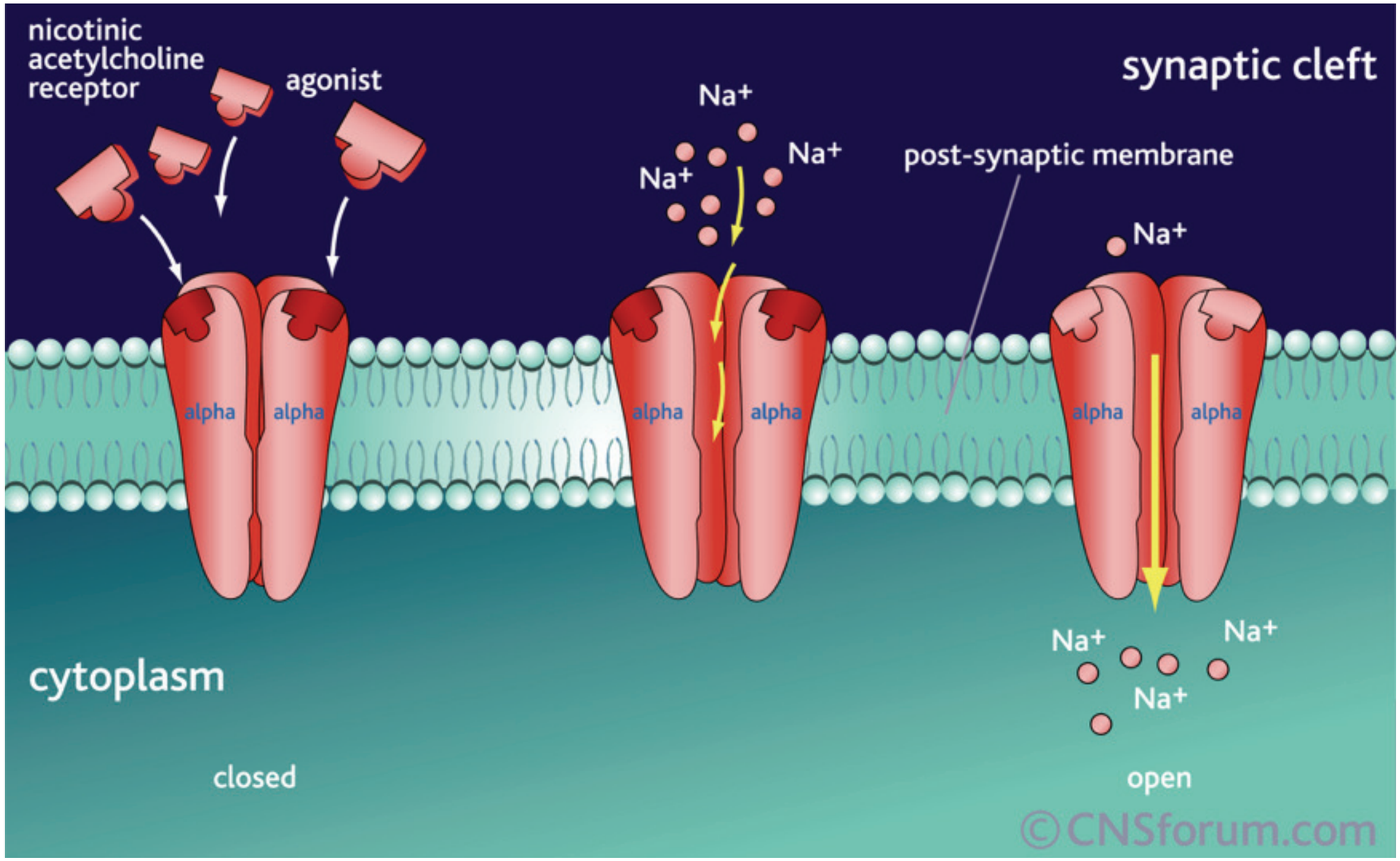
Fire or Not Fire

Action Potential (AP)



Channel Mediated Ion Concentration



Optogenetics (Optical and Genetics)-

photo-sensitive channel

- The Method of 2010- Nature Method
- Optogenetics: Breakthroughs of the Decade- Science

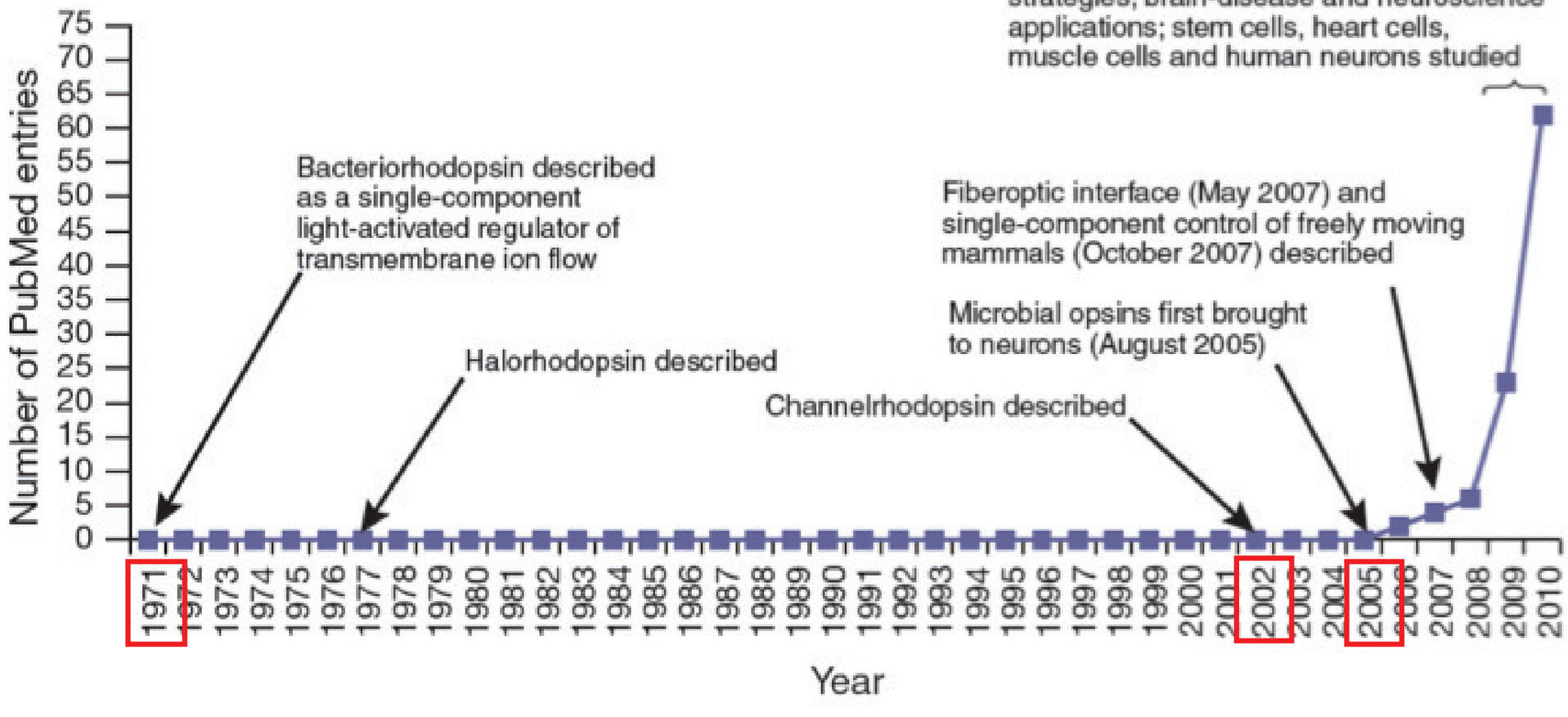
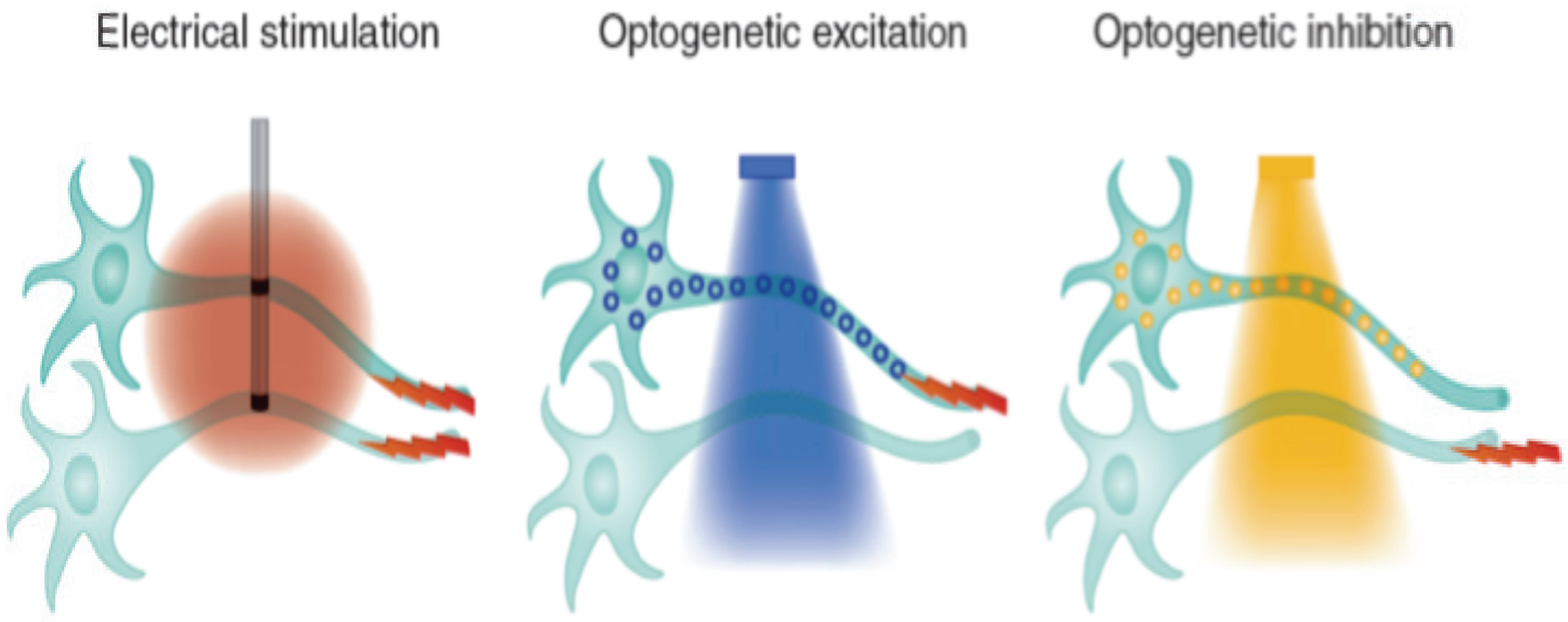
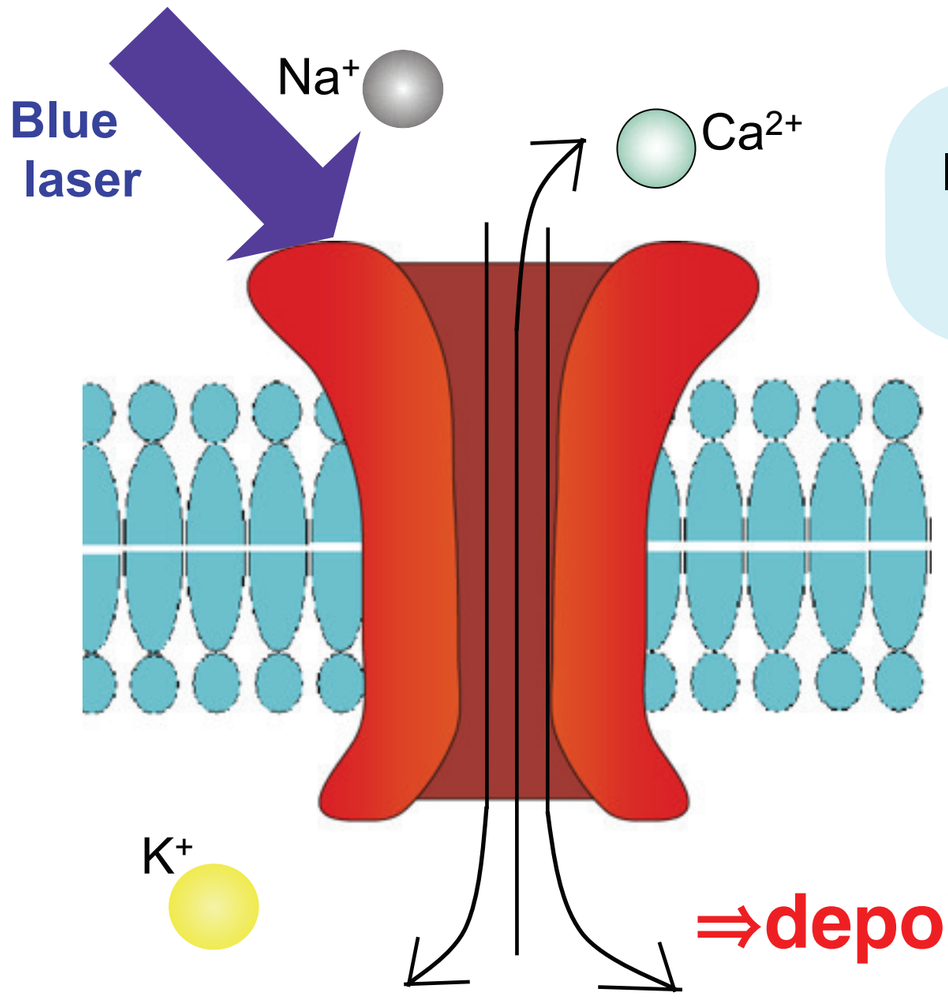


Photo- Excitatory or Inhibitory



Control cell activation using LIGHT

ChR2 — Channelrhodopsin-2 —



light-sensitive protein **Rhodopsin**

Origin of Chlamydomonas reinhardtii

+

Cation channel

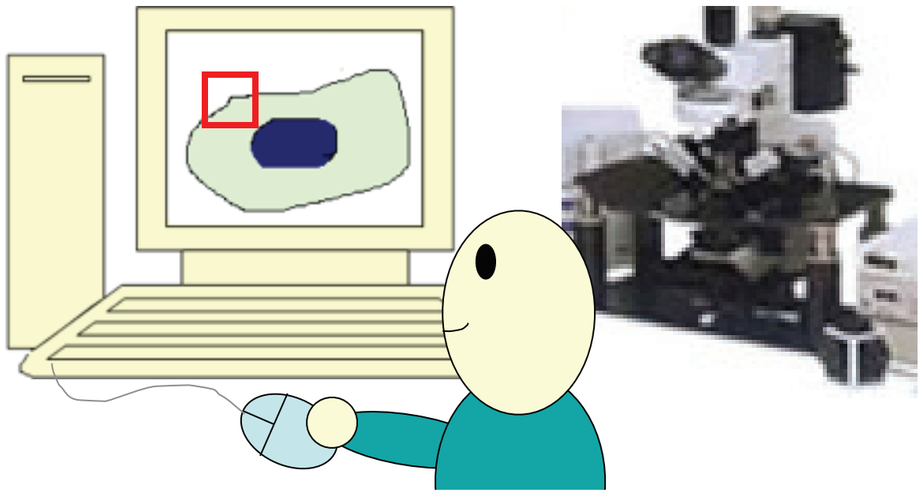
Blue laser irradiated to make Channels open, allow cation (Positive Ion) into the cell.

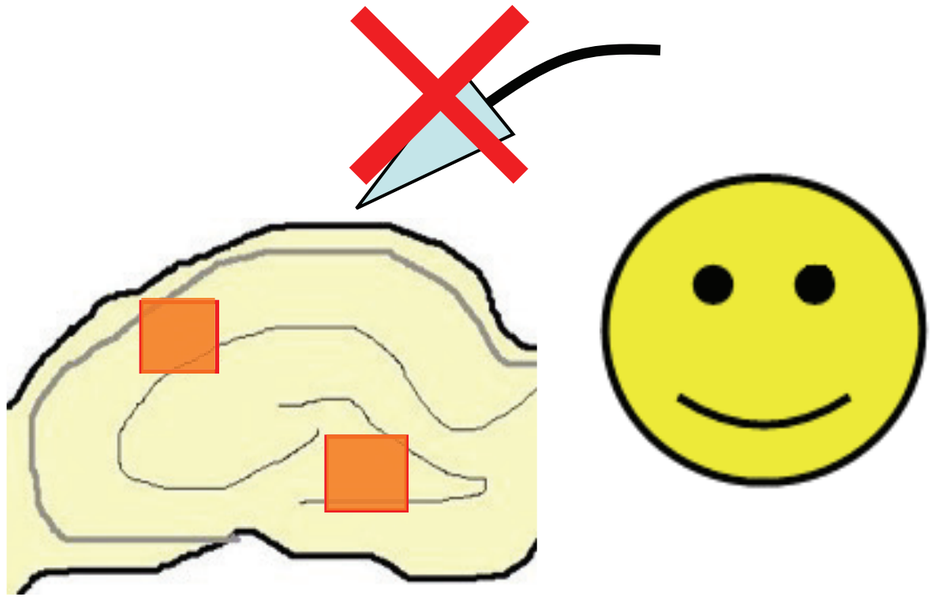
⇒depolarization ⇒Action Potential

Control cell activation

activated by
LIGHT

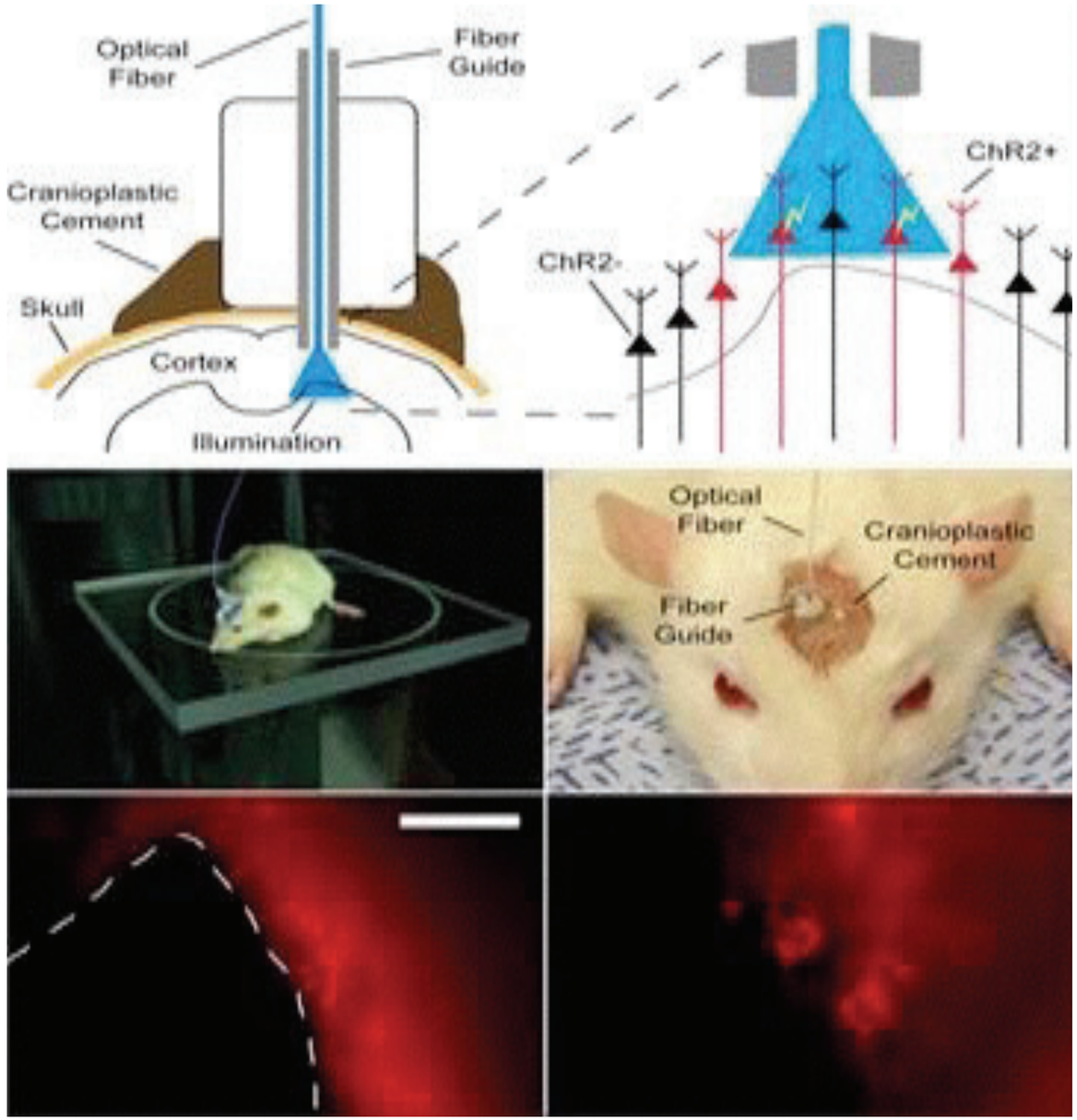
Activation in ChR2/NpHR using Blue/Yellow laser…

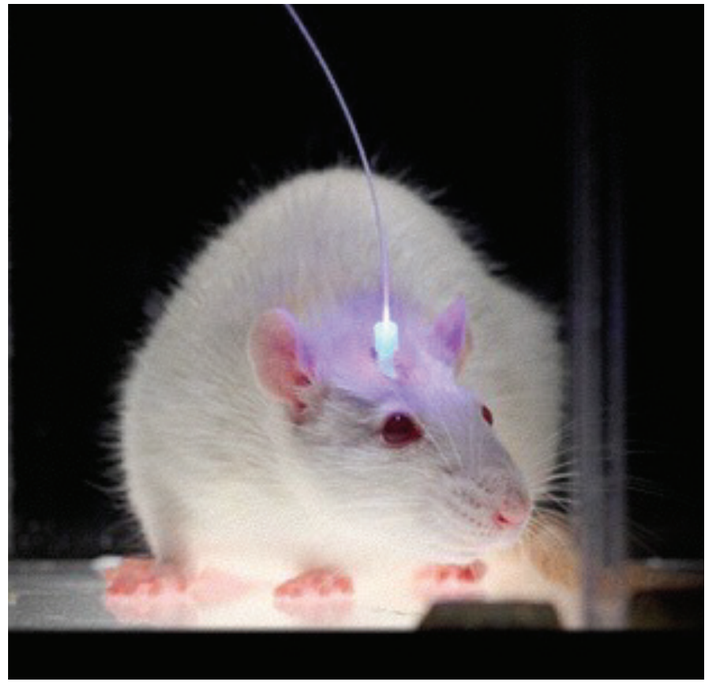




- **No need to use electrode!** Don't take time to set.
- **It is possible to select stimulus area freely.**

in vivo Model





Behavior recording