

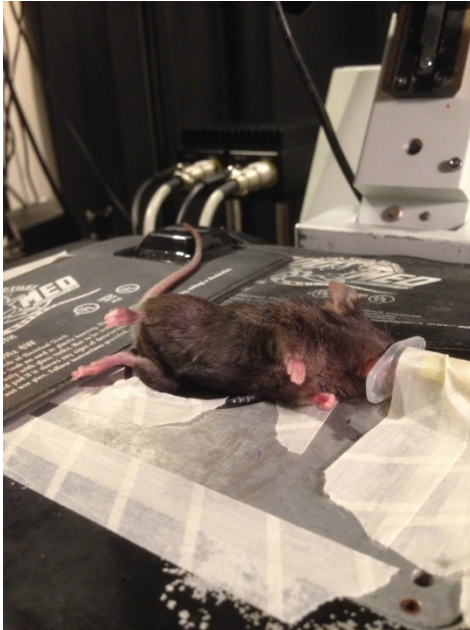
Intravital fluorescence lifetime imaging

Takashi Hato, MD

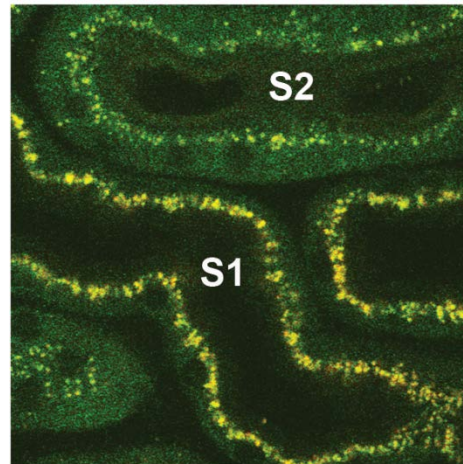
Indiana University School of Medicine

Division of Nephrology

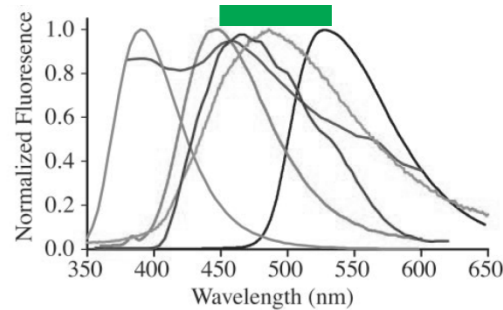
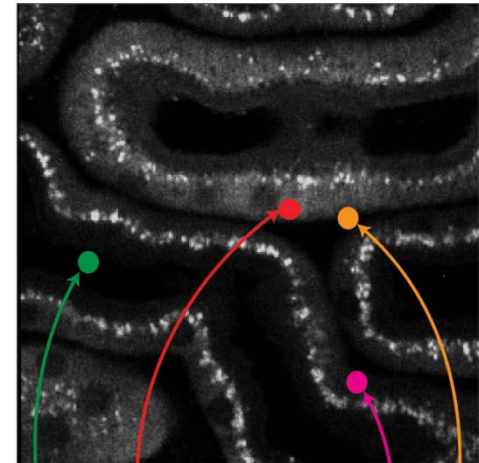
Autofluorescence Lifetime Imaging



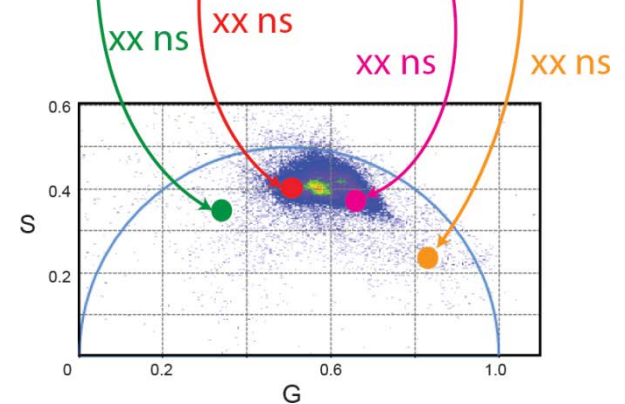
Conventional 2 photon
(RBG Intensity Image)



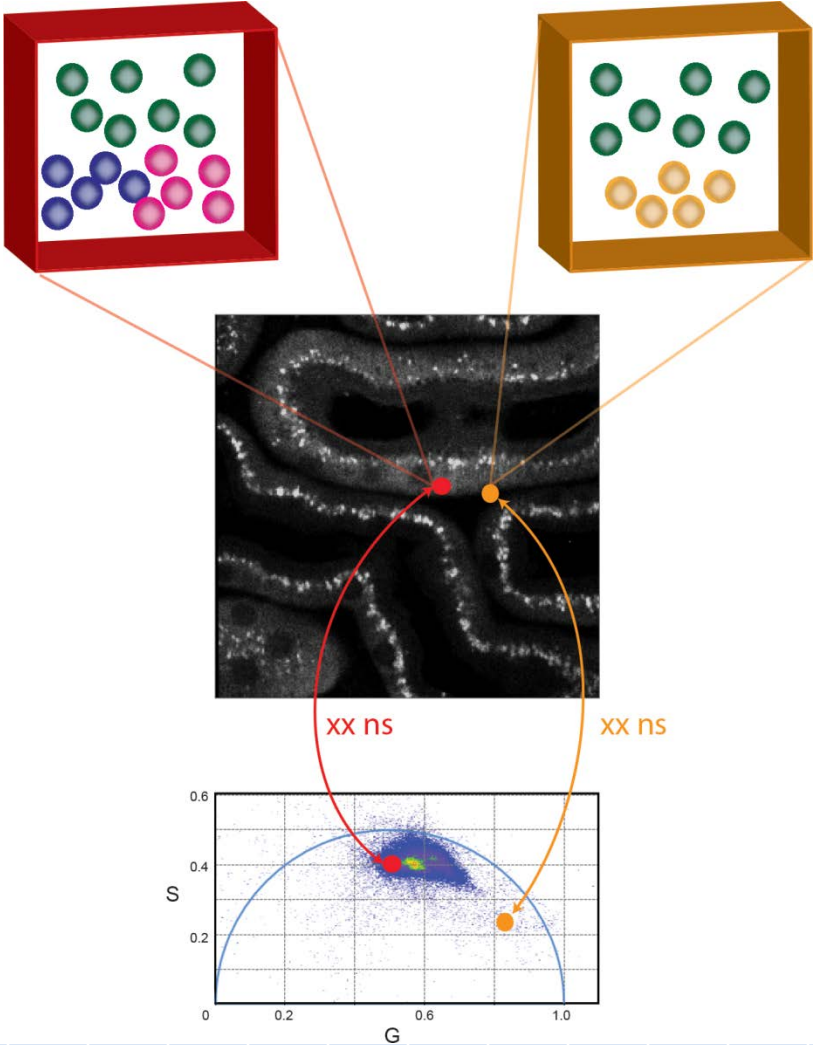
FLIM
(Blue channel Intensity Image)

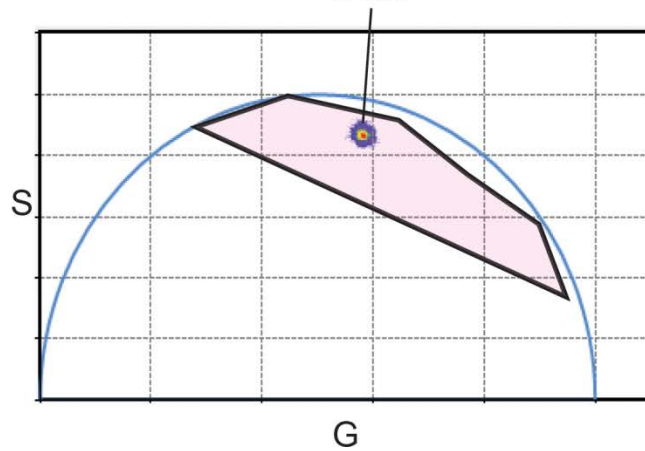
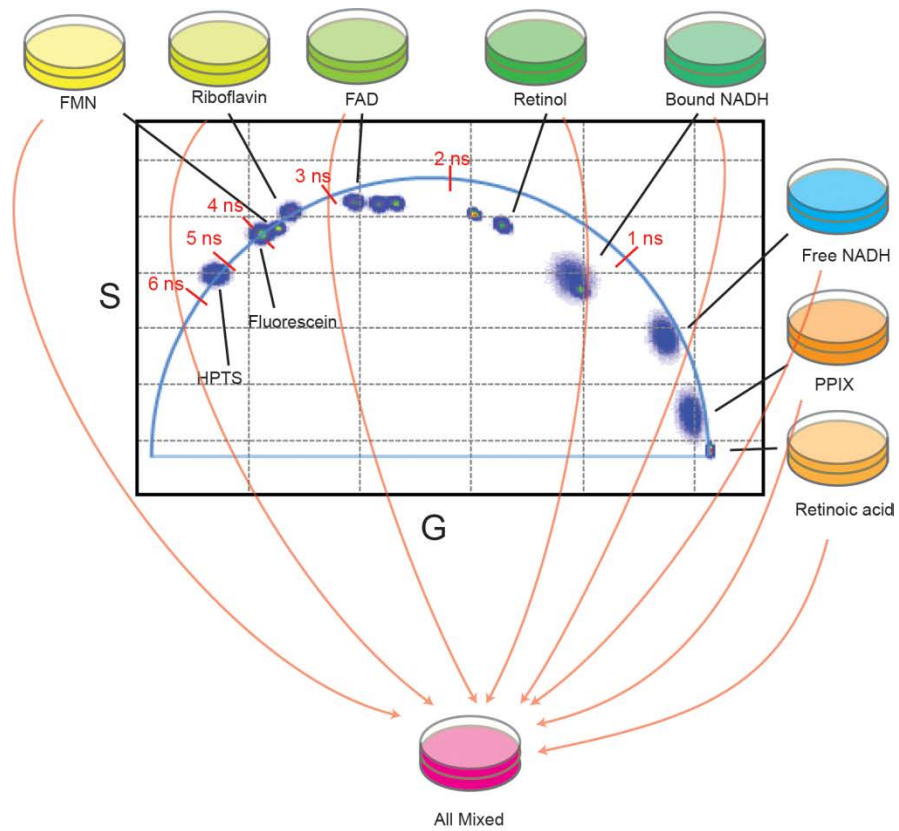


Zipfel PNAS 2003

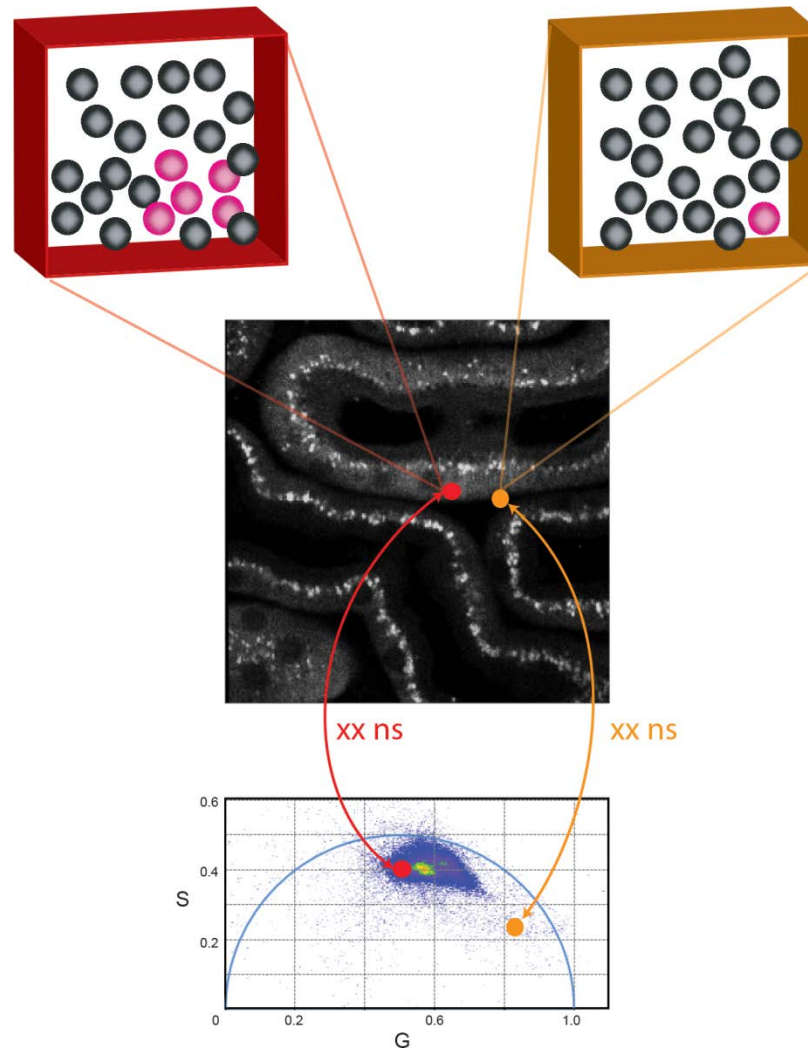


The lifetime in each pixel: an average lifetime for all photons



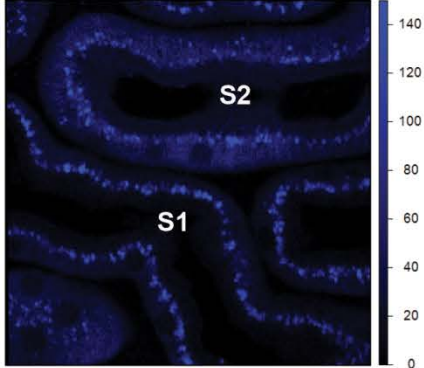


An average lifetime is determined partly by: abundance and inherent “brightness” of the species

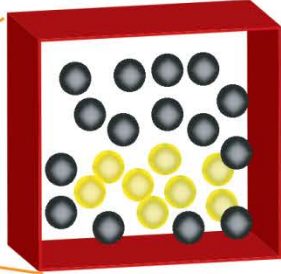
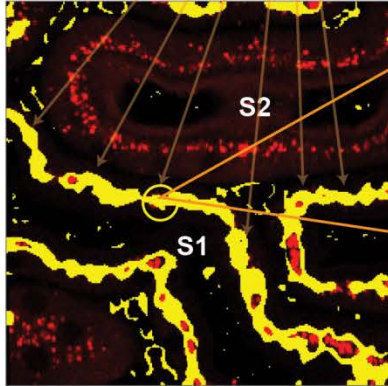
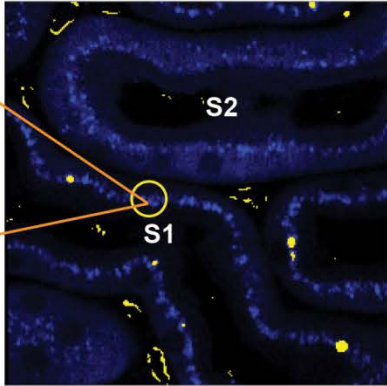
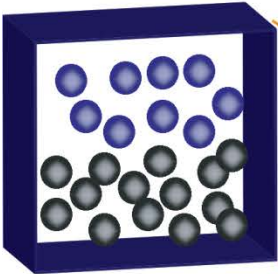
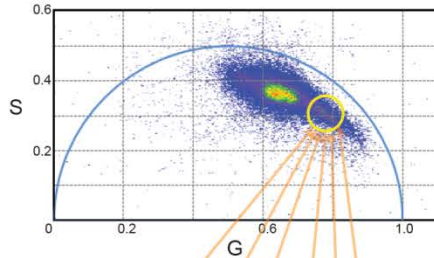
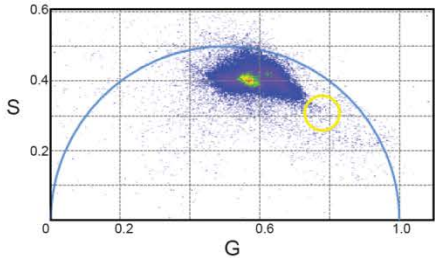
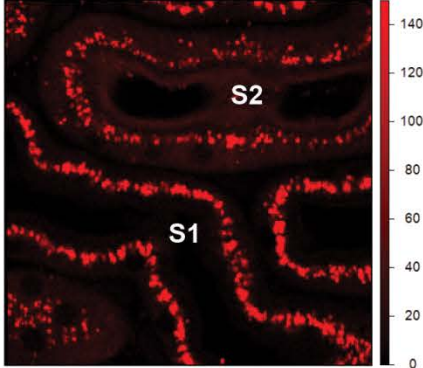


Excitation and emission

Blue channel photon counts

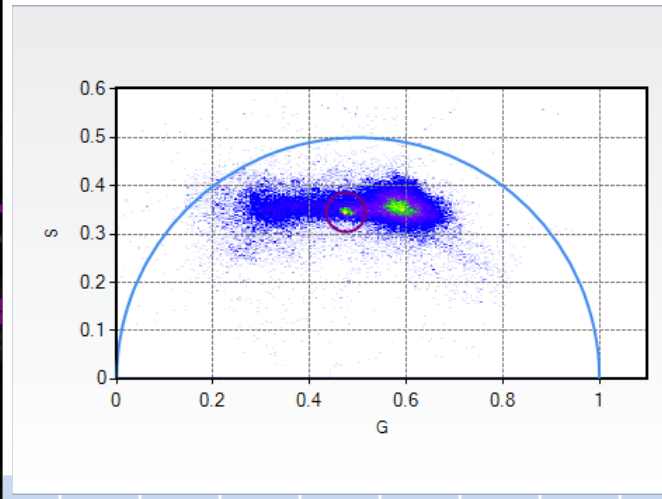
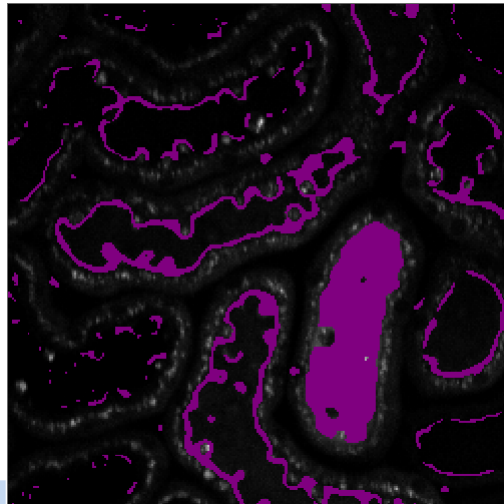
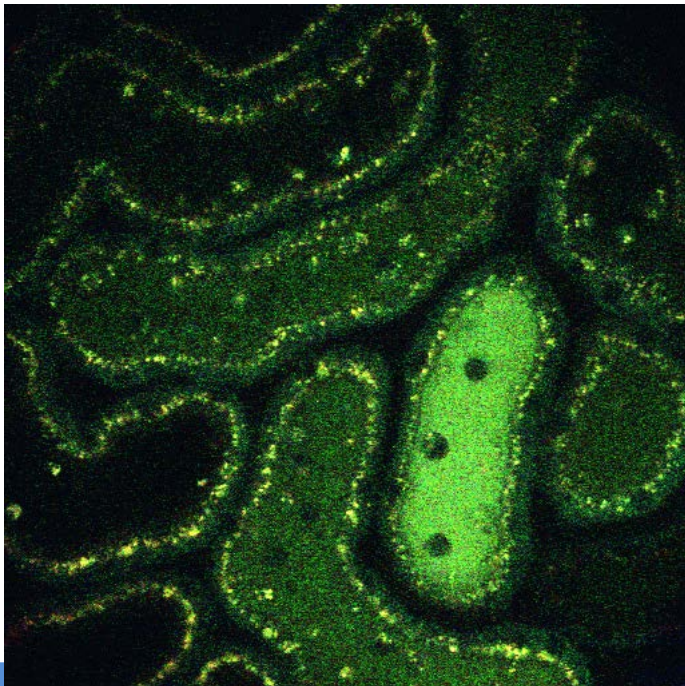
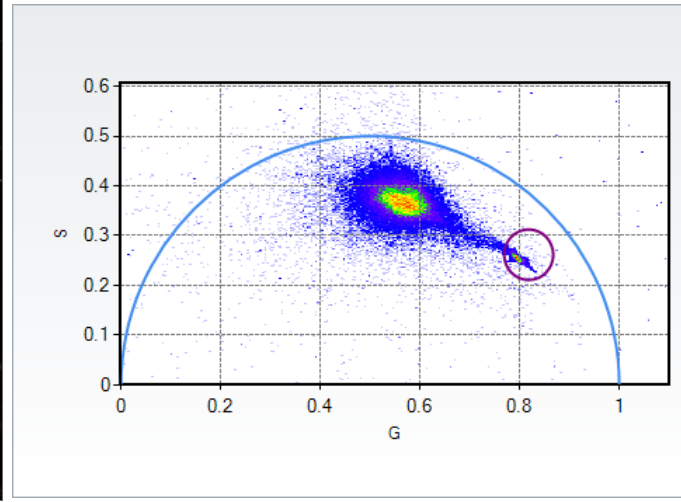
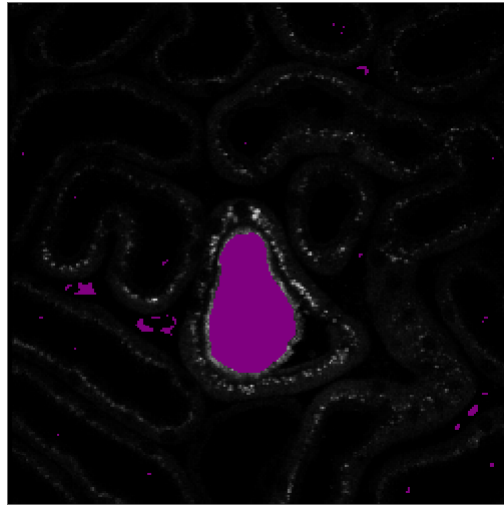
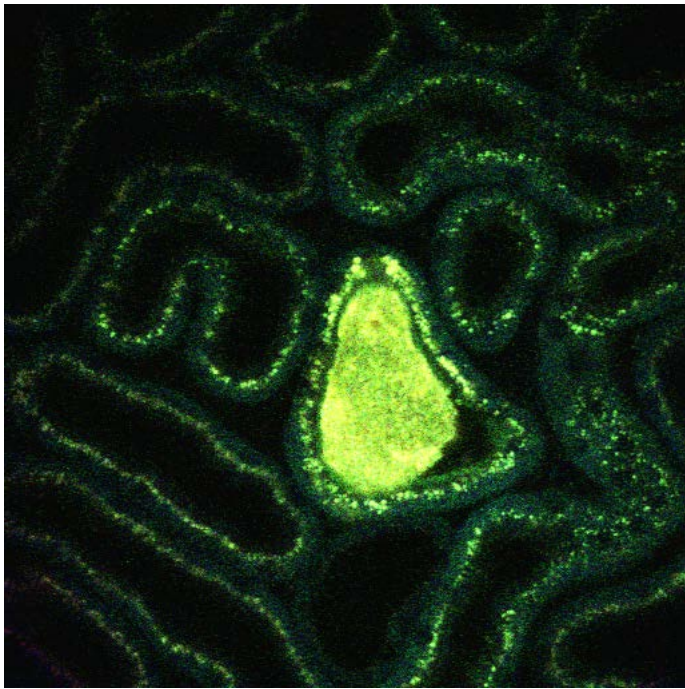


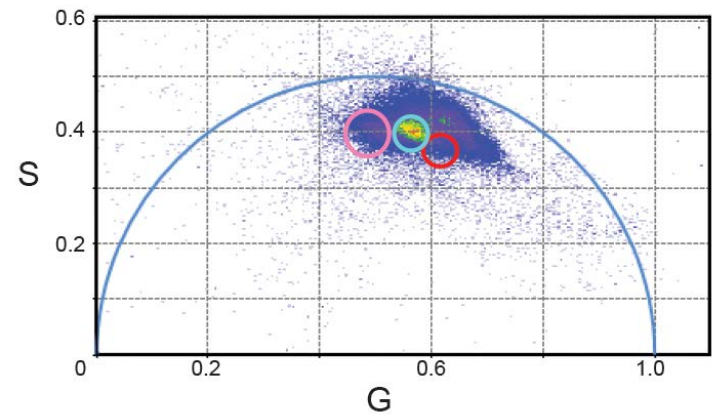
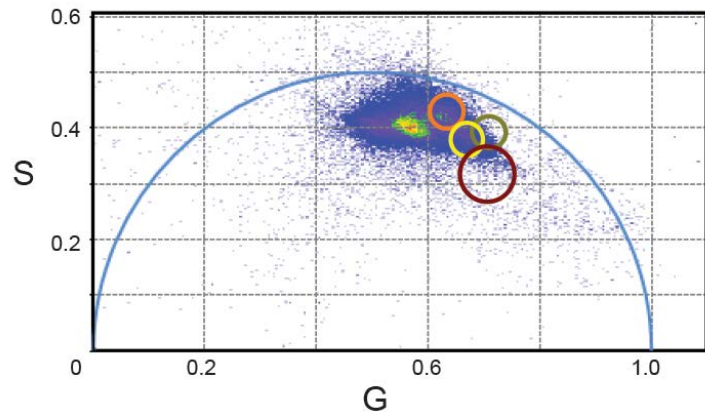
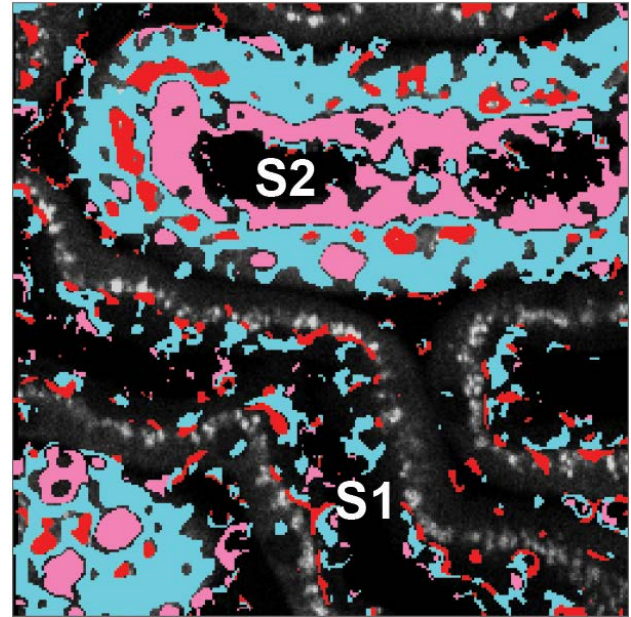
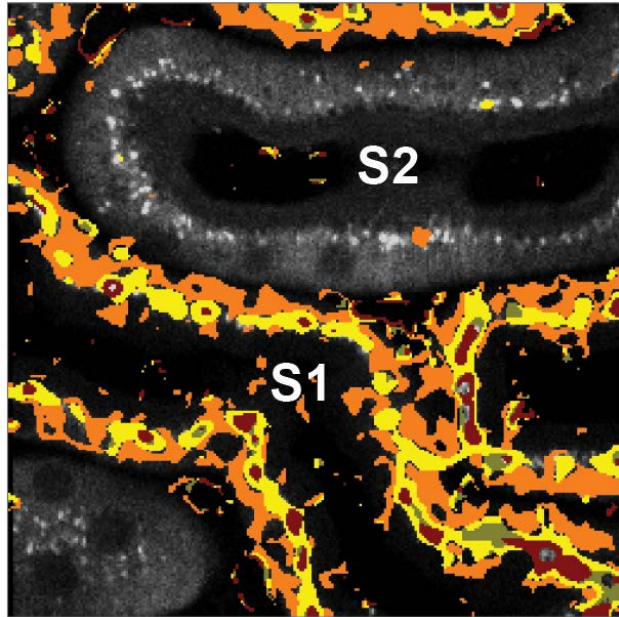
Red channel photon counts

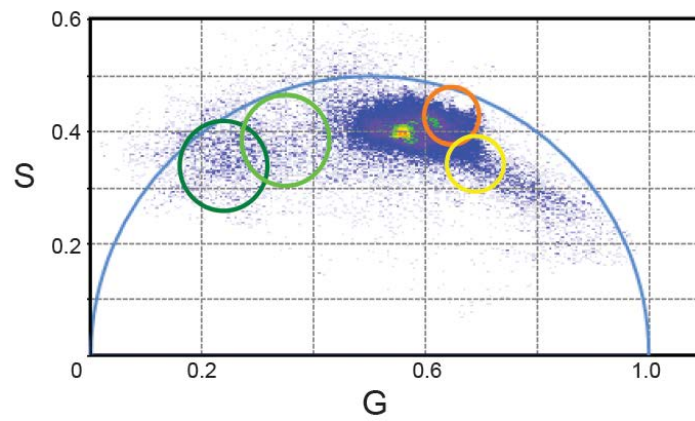
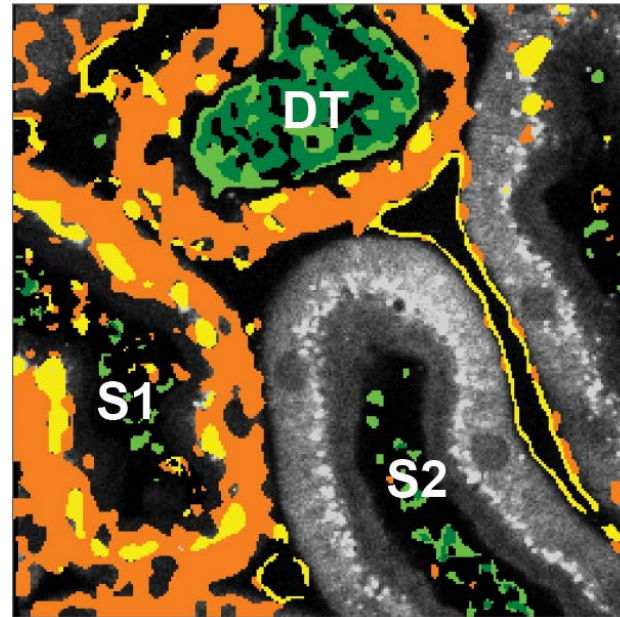
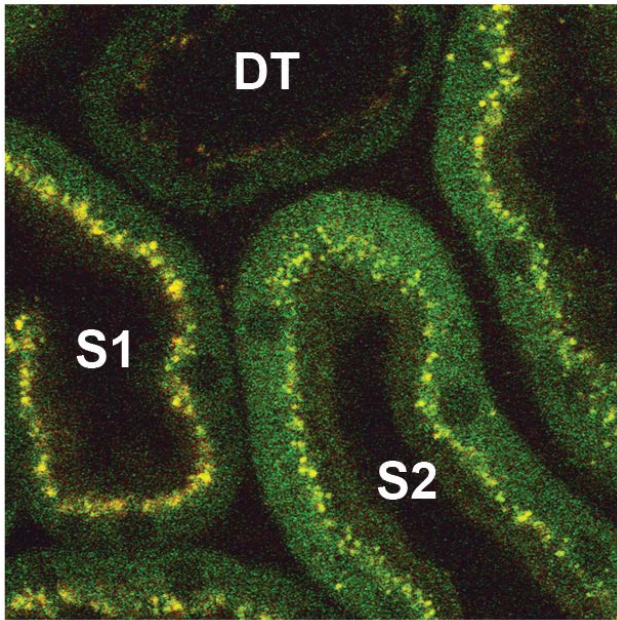


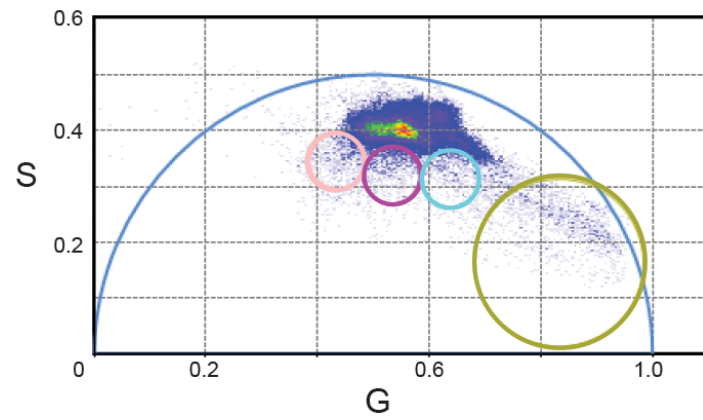
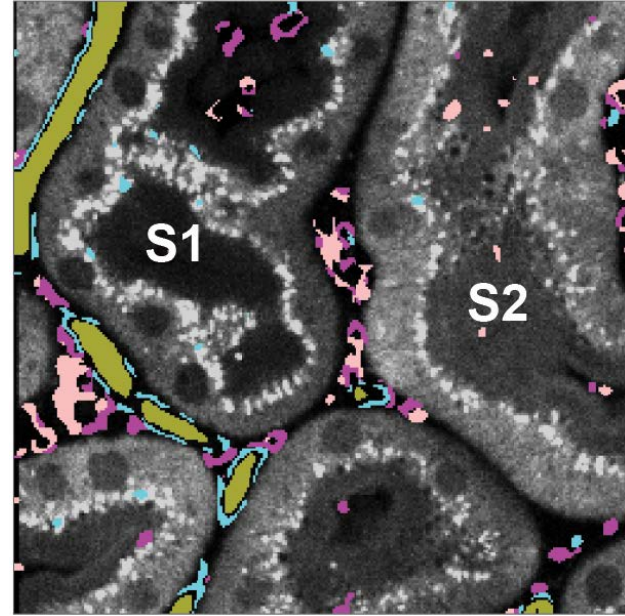
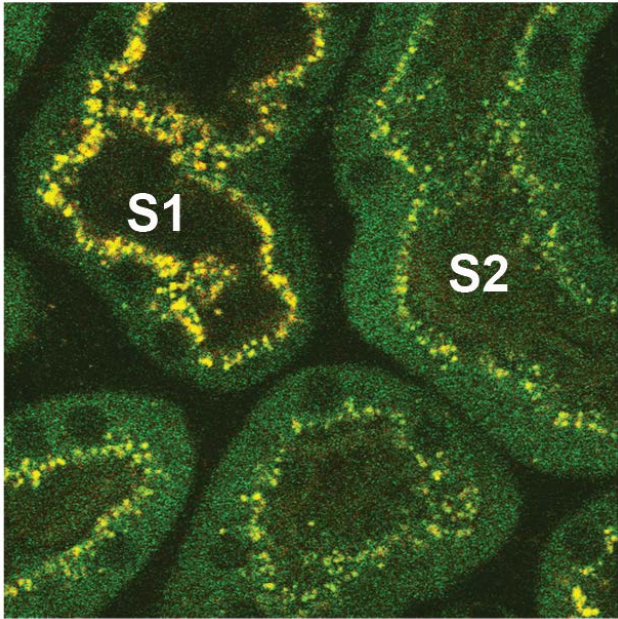
Local microenvironment factors

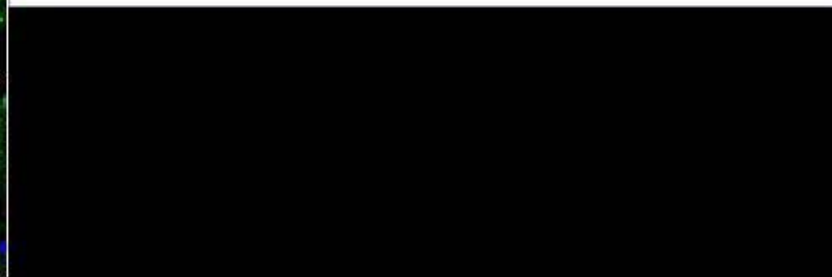
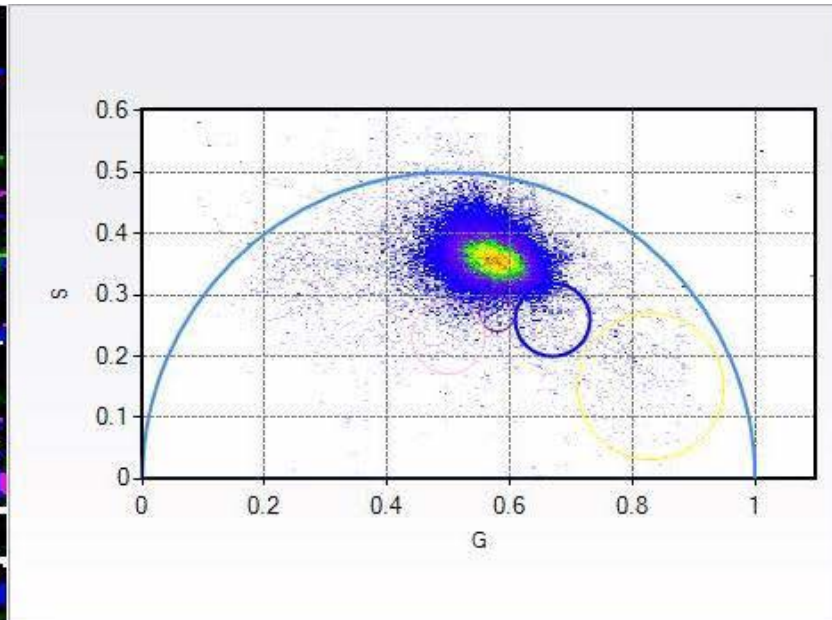
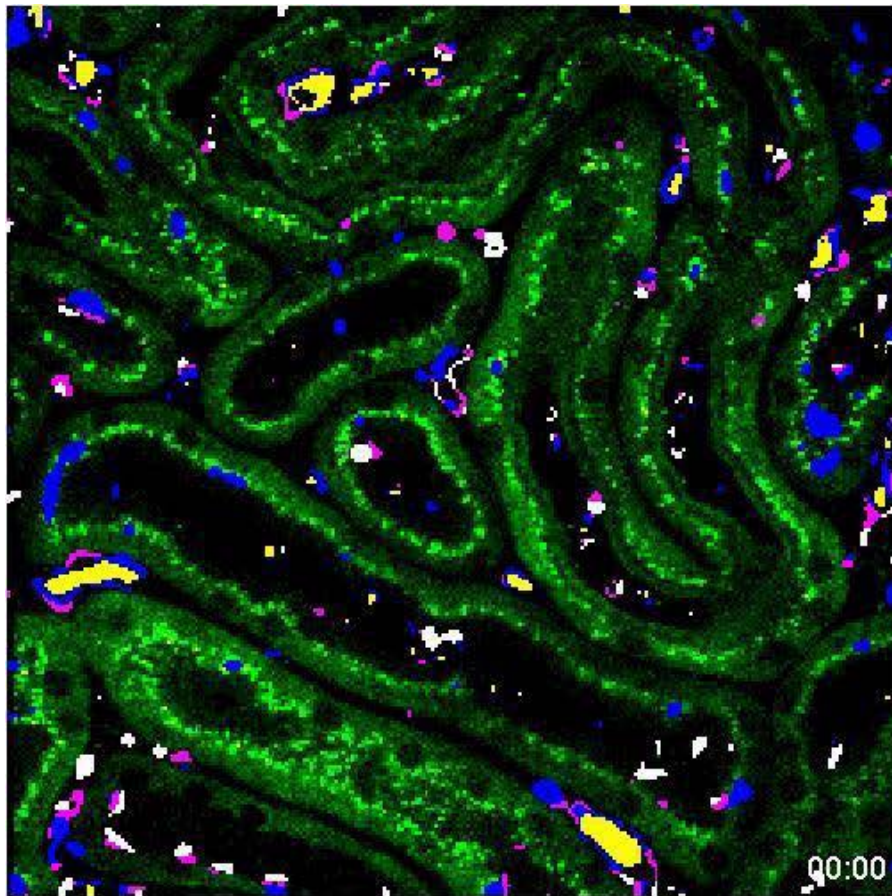
- pH
- Ionic changes
- Protein-bound or unbound state
- ...

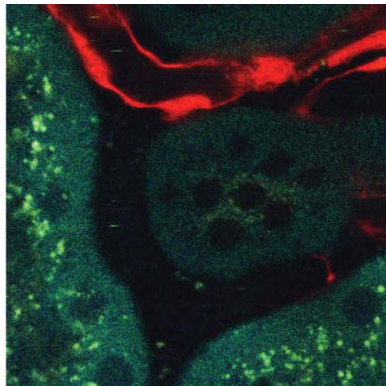










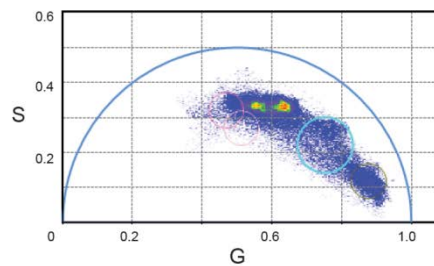
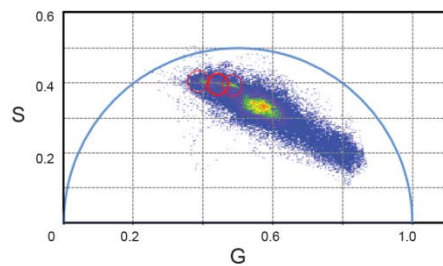
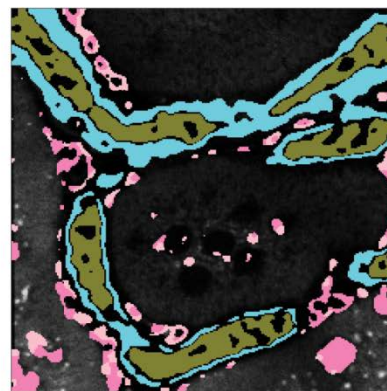
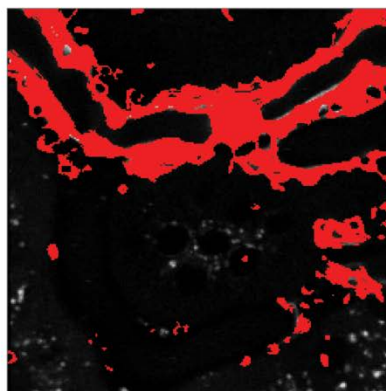


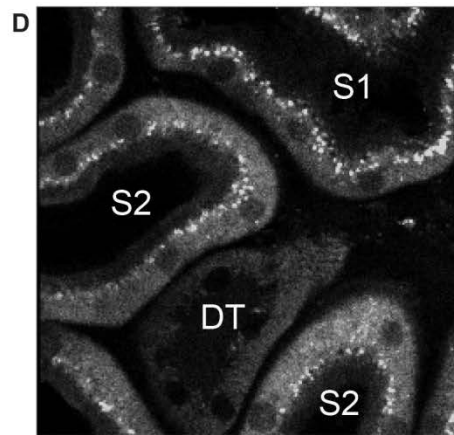
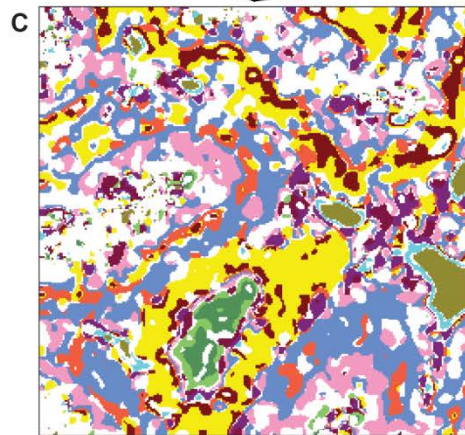
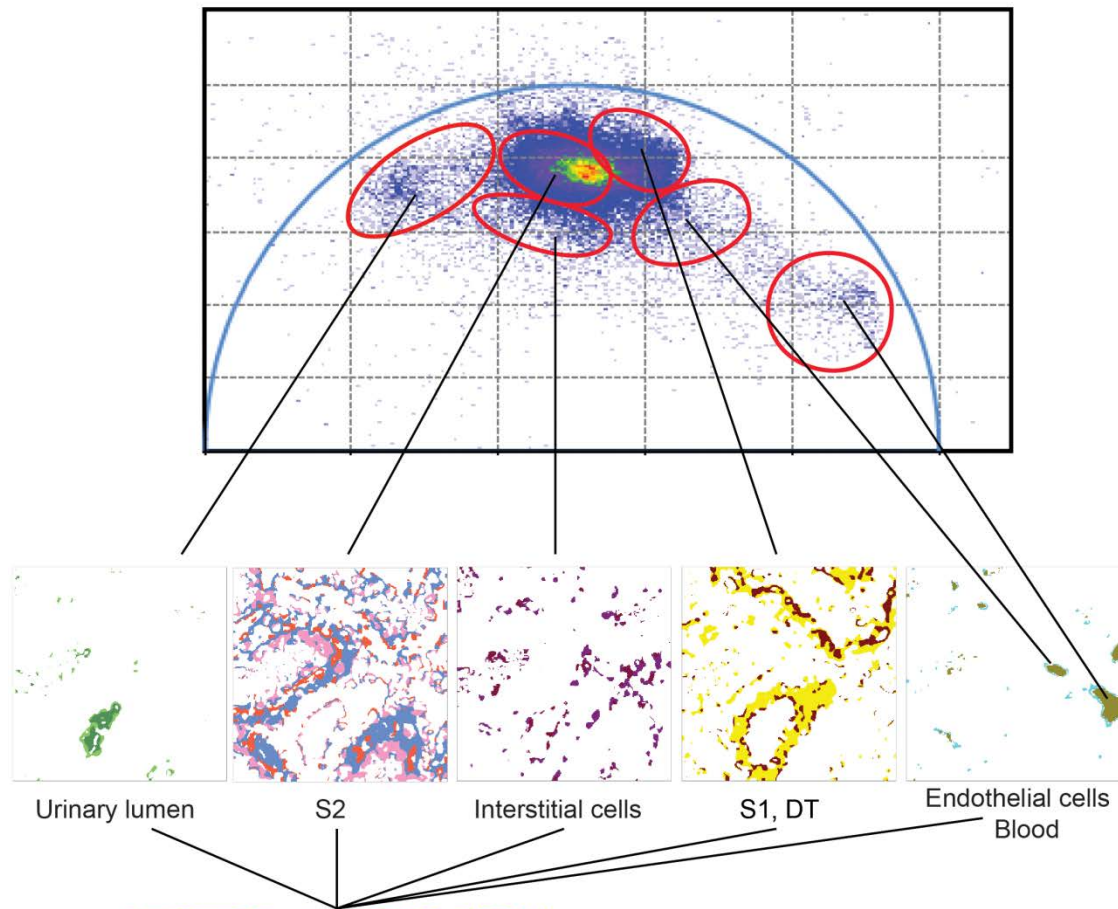
Conventional 2 photon (TdTomato-endothelium)

FLIM

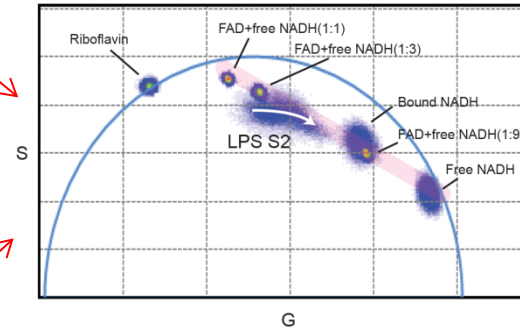
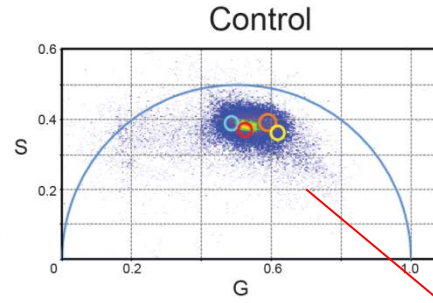
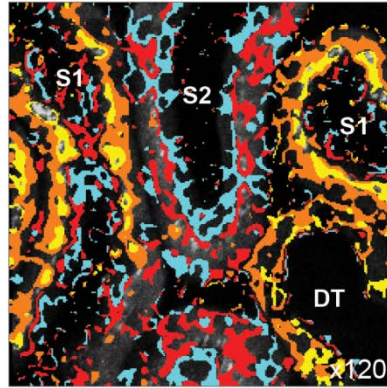
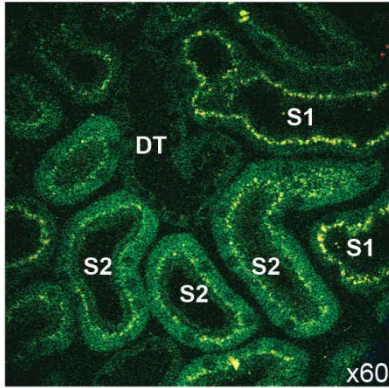
Red channel

Blue channel

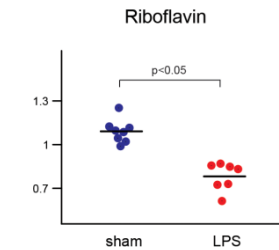
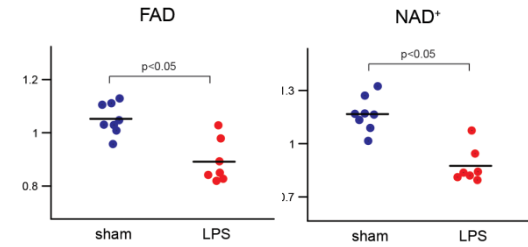
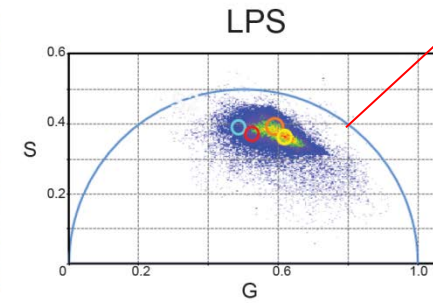
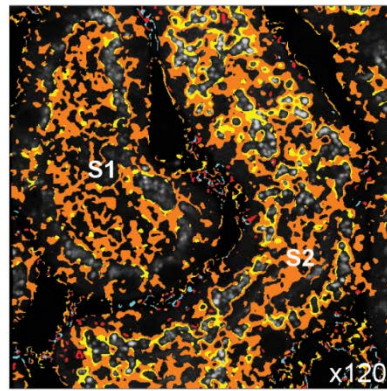
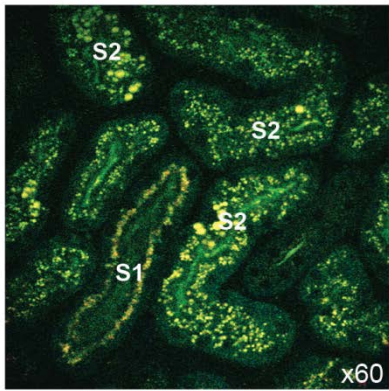




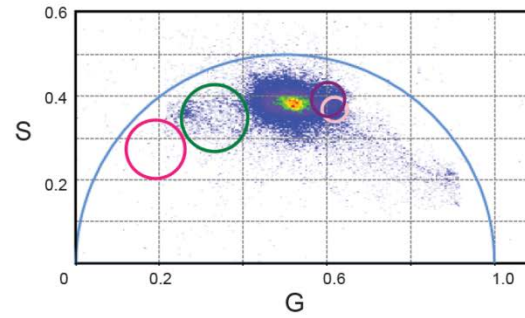
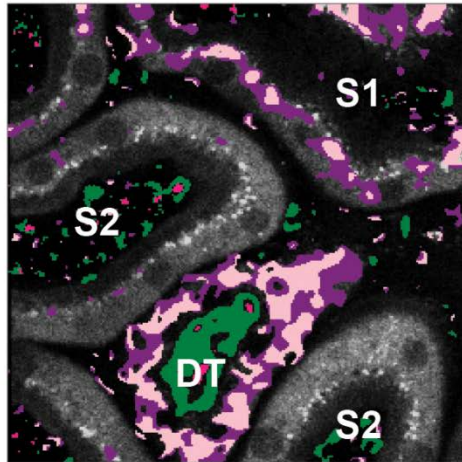
Control



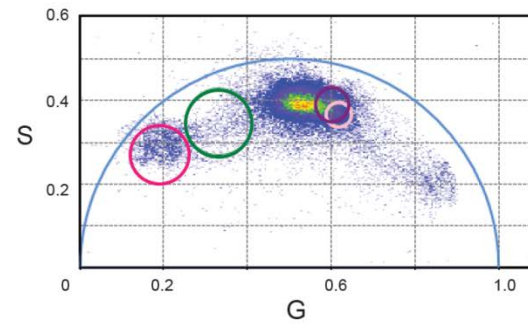
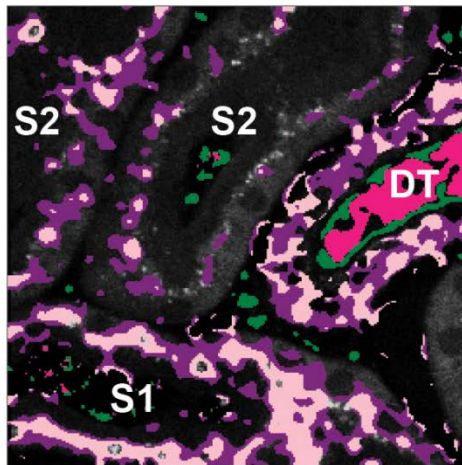
LPS treated



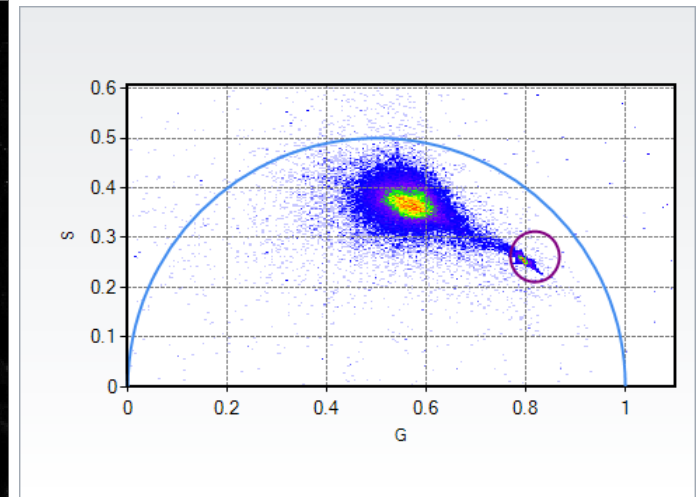
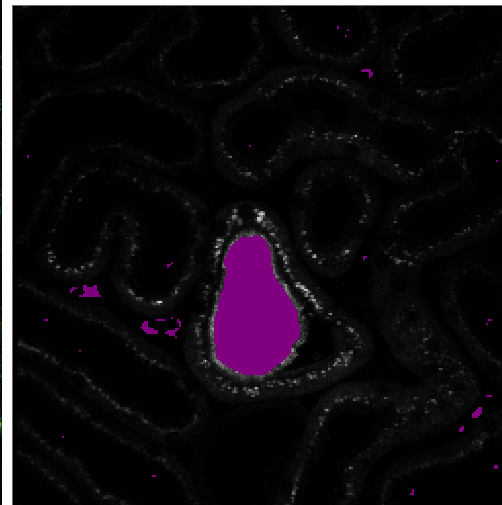
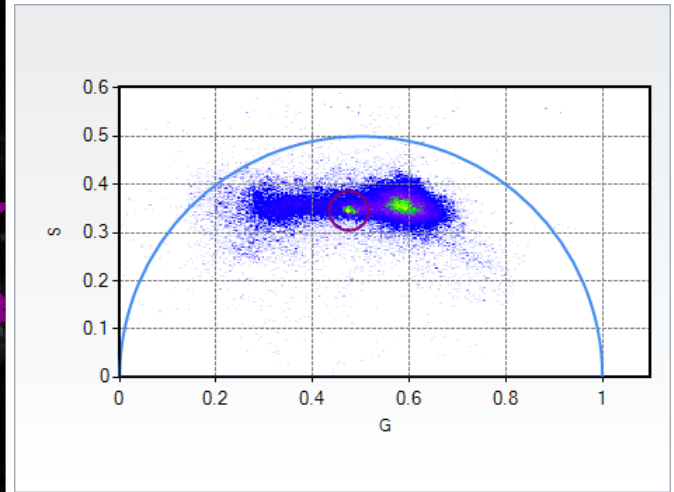
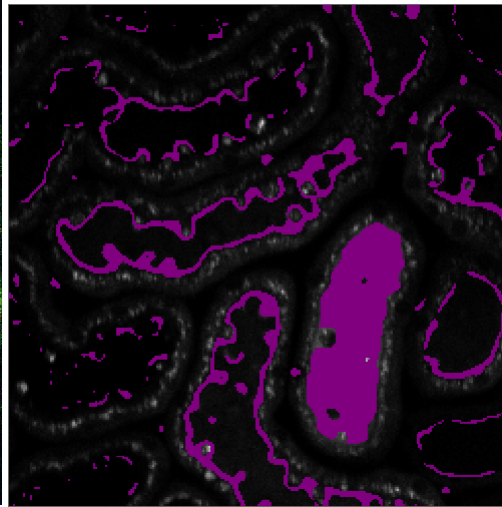
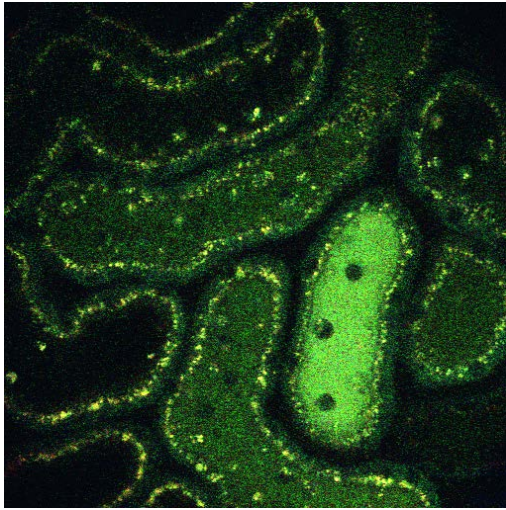
Control

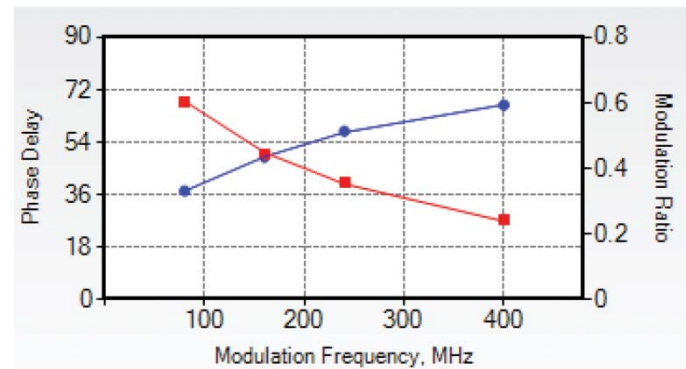
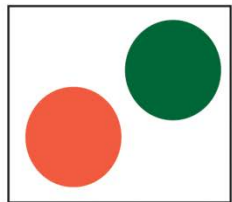
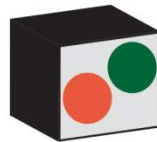
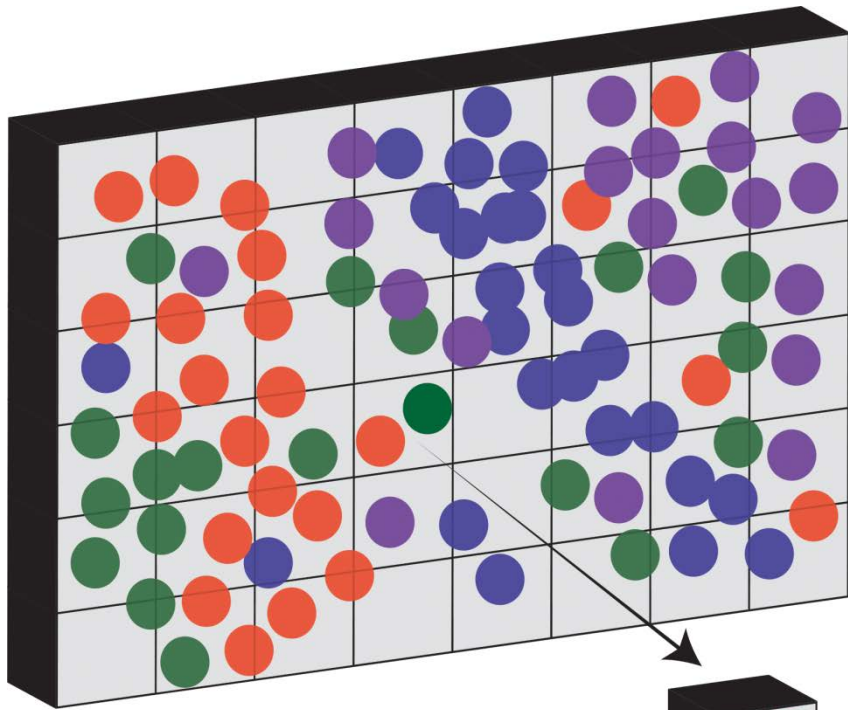


LPS treated (4hrs)



What exactly is it?





	Tau (ns)	Fraction	Alpha
▶	0.649 <input type="checkbox"/> Fix	0.380 <input type="checkbox"/> Fix	0.774
	3.626 <input type="checkbox"/> Fix	0.620 <input type="checkbox"/> Fix	0.226

$\langle \tau(f) \rangle$: 2.494 $\langle \tau(\alpha) \rangle$: 1.322

χ^2 : 42

τ Lifetime Fitting