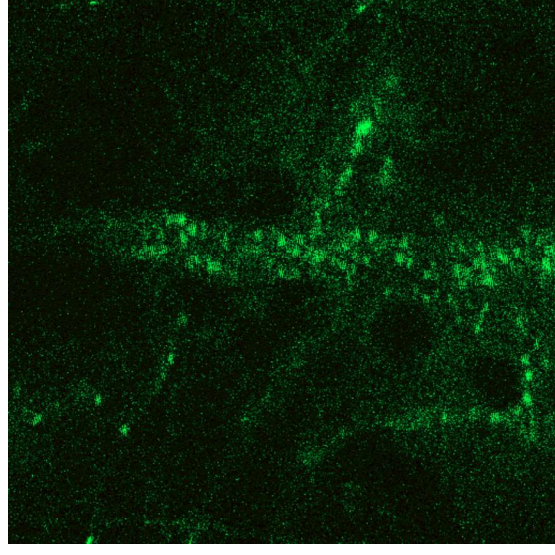
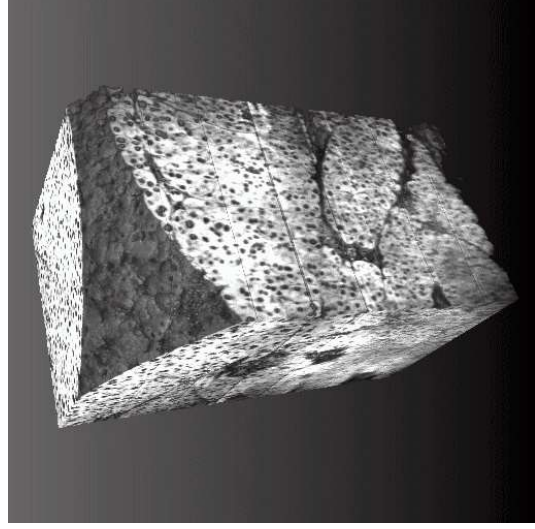
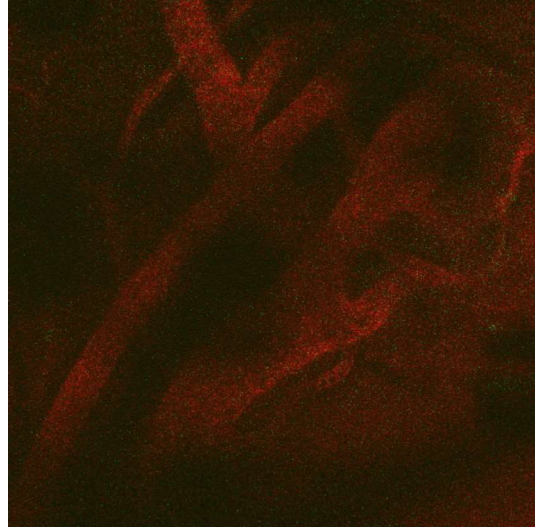
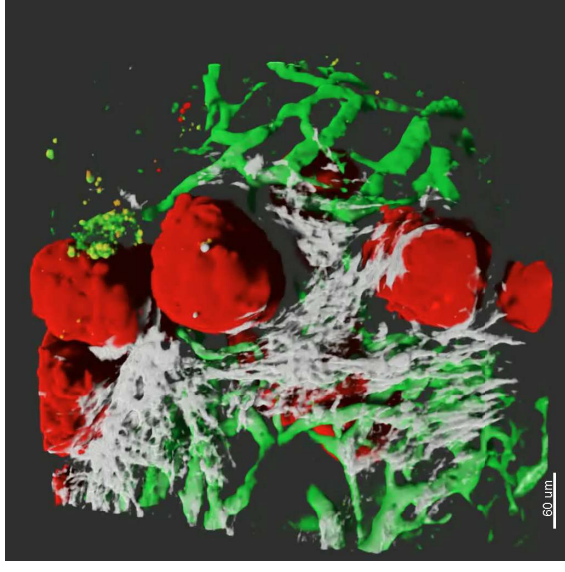
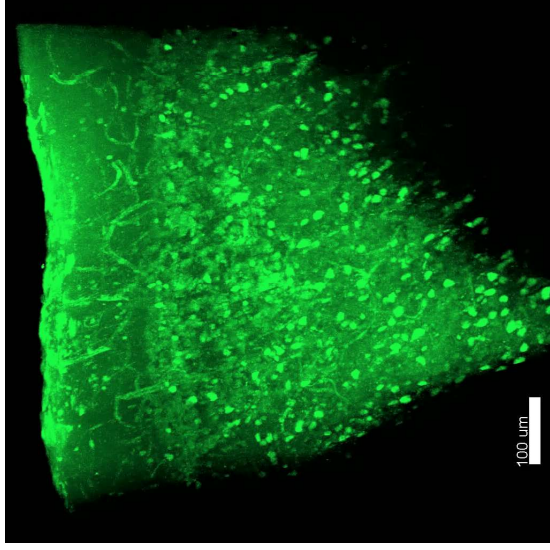
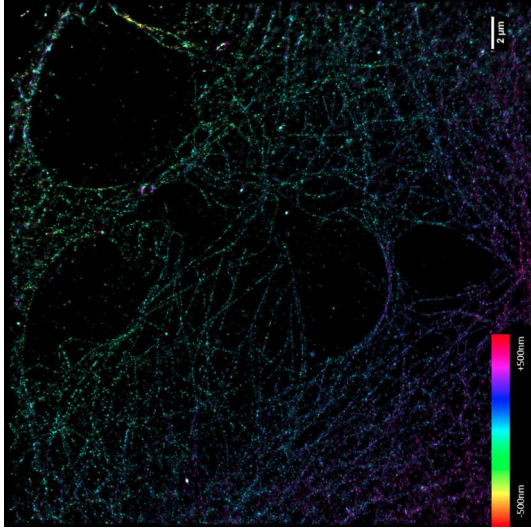


Applications of Two-Photon Microscopy

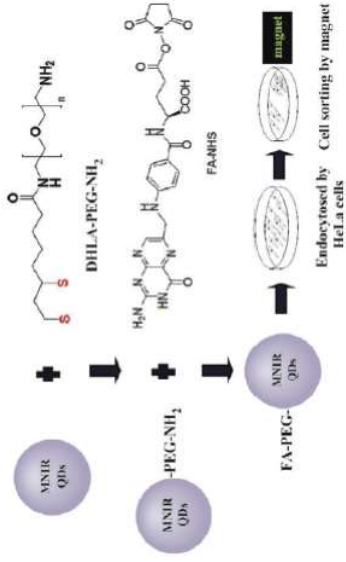
Peilin Chen

Research Center for Applied Sciences



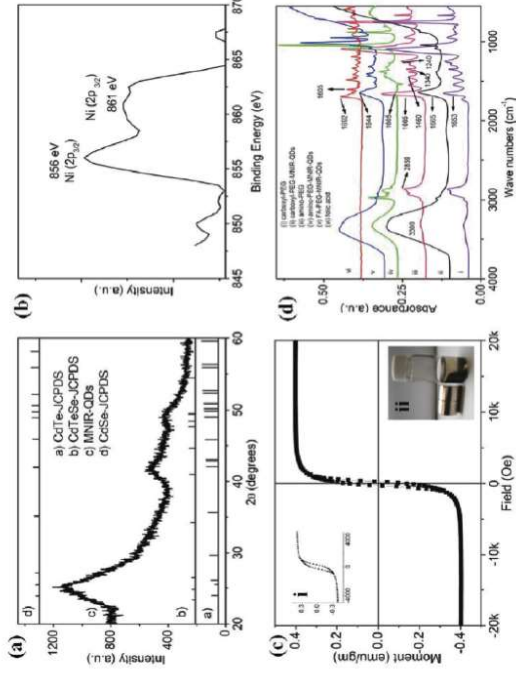
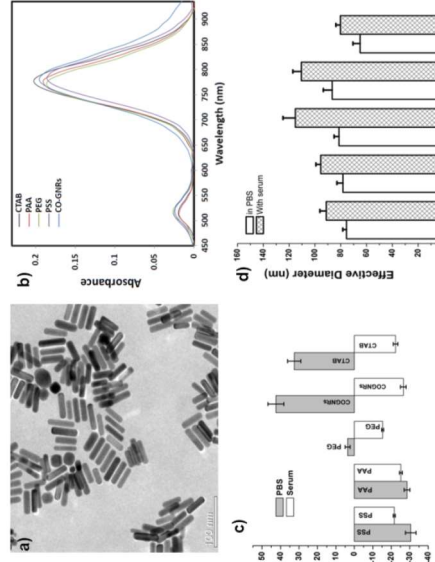
Nanoparticles for Bioimaging

Scheme 1. Schematic Diagram for Surface Modification of MNIR-QDs



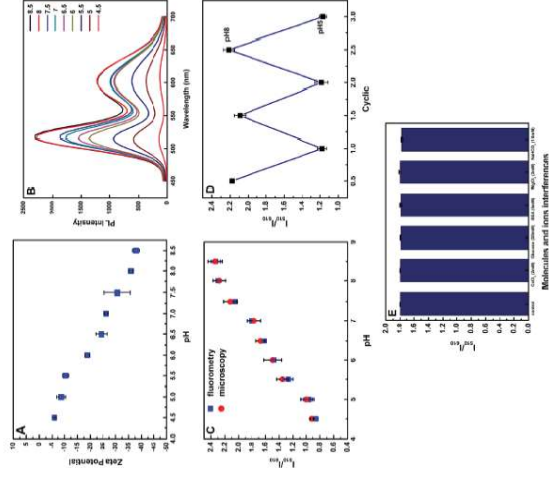
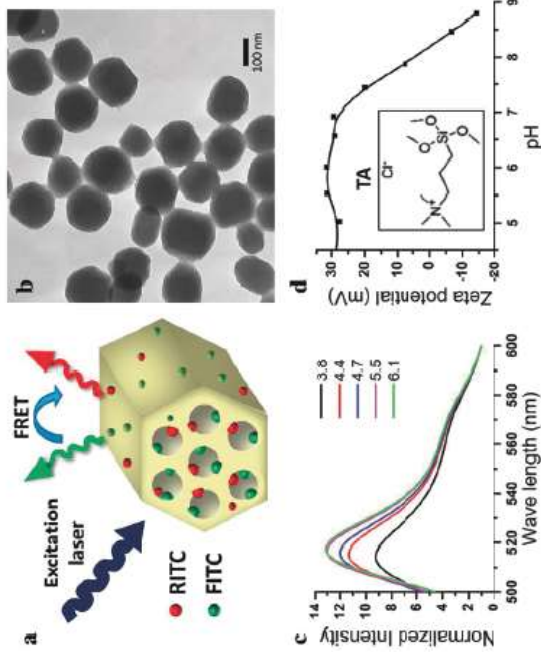
Quantum Dots

Nanorods



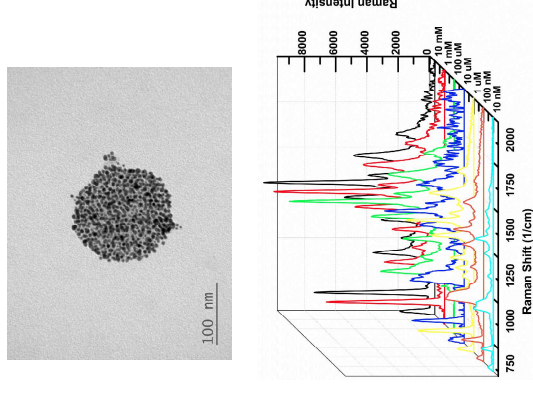
Magnetic quantum dots

MSNs for pH sensing

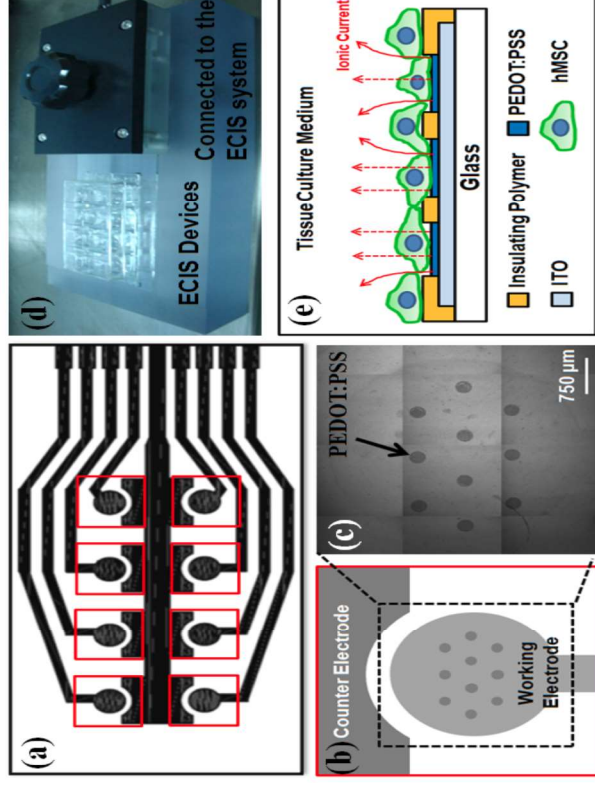
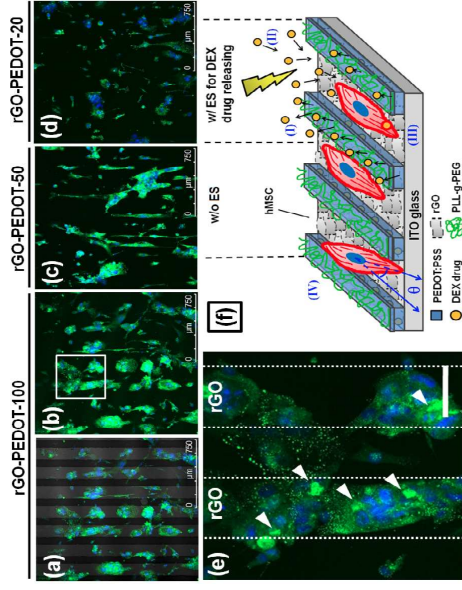
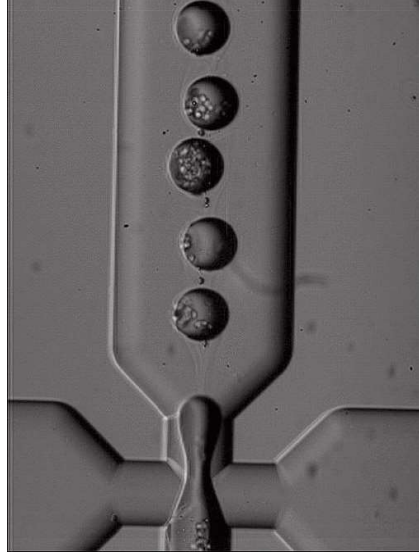
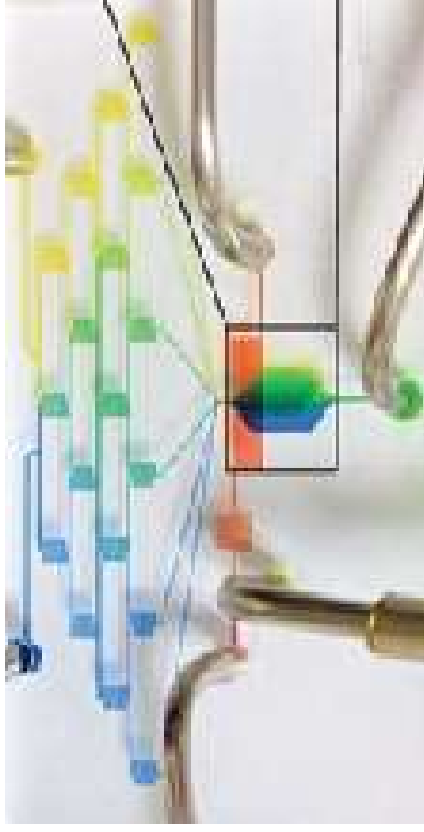
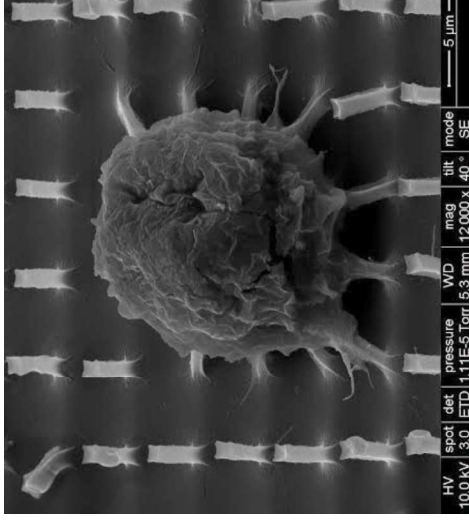


Quantum dots for pH sensing

Raman Probes

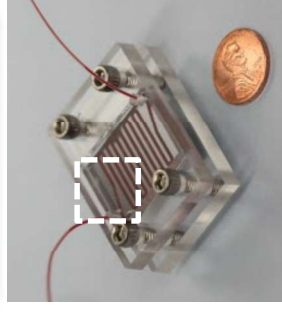


Bioelectronics and Microfluidics

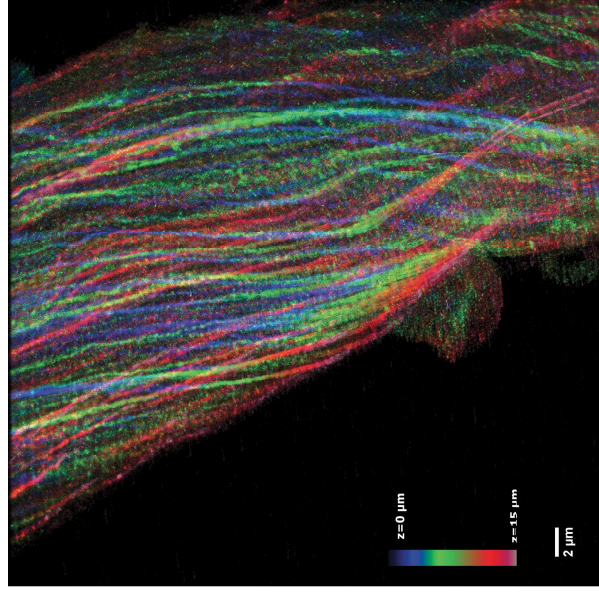
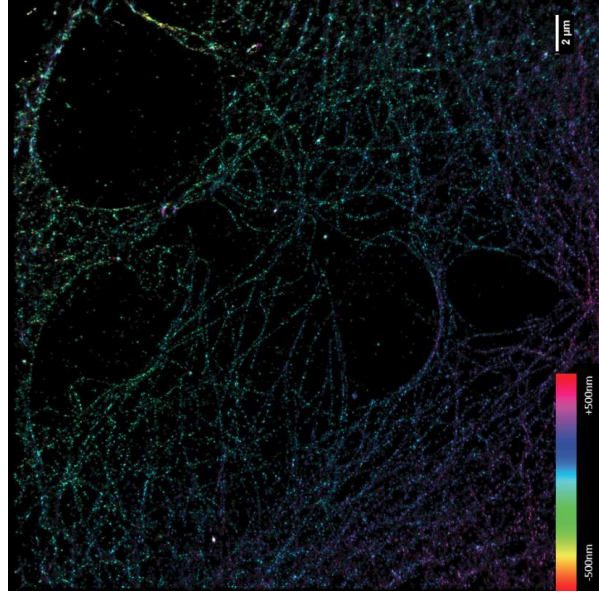
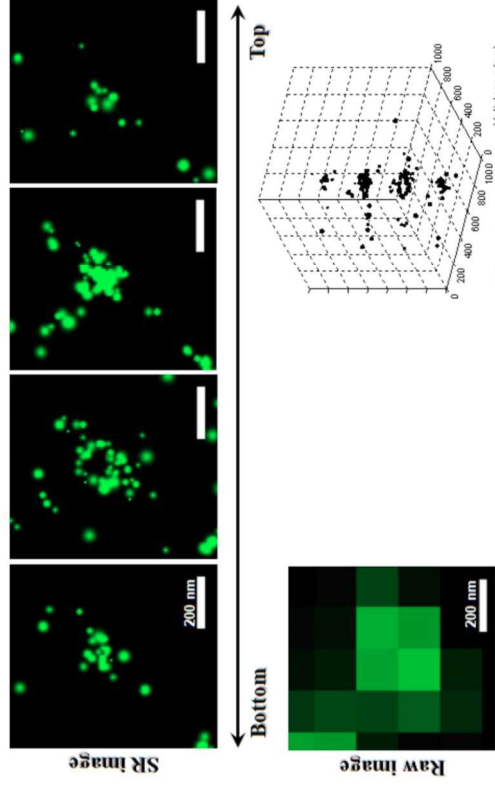
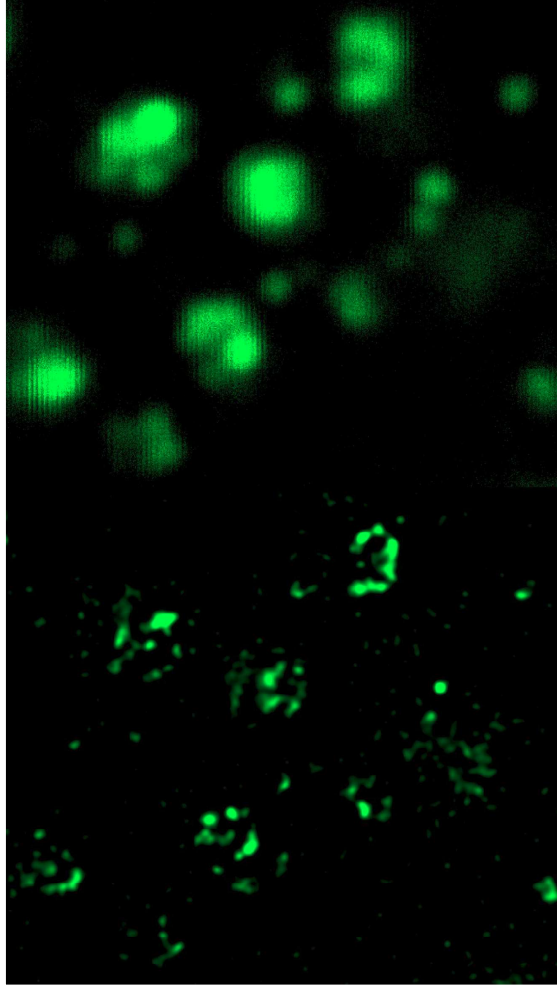
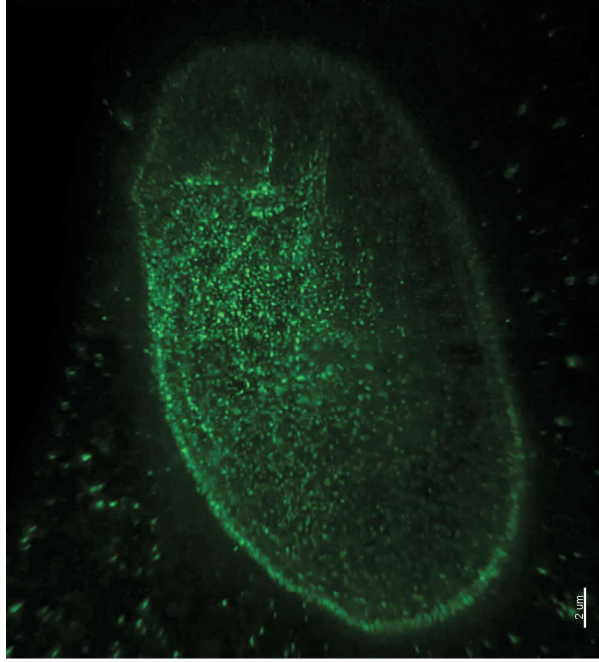


Ten circular 250 μm diameter active electrodes connected.

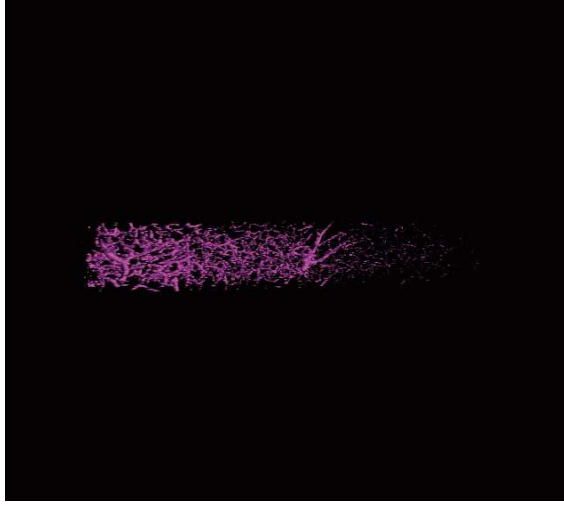
Recording and Stimulation



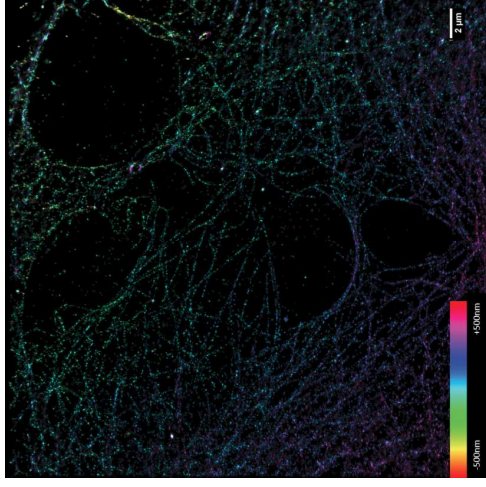
Super-resolution Imaging



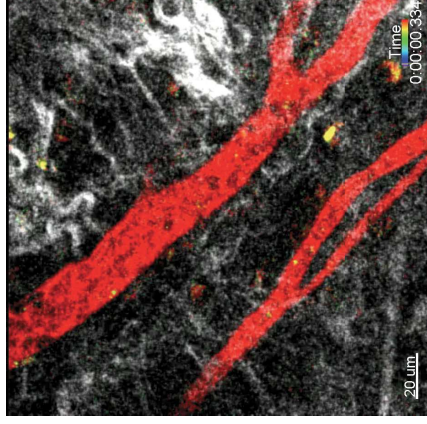
Bioimaging



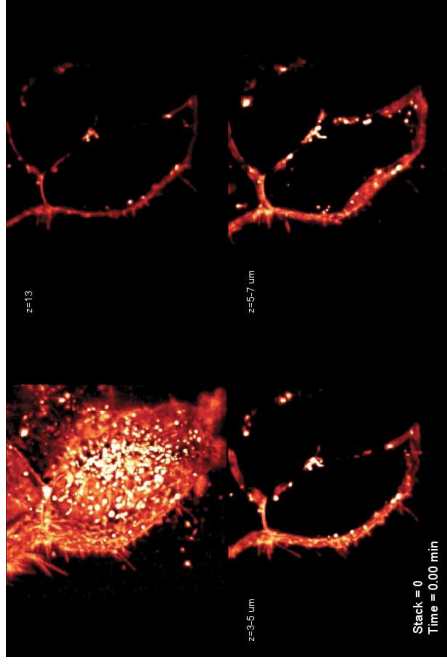
Deeper



Smaller

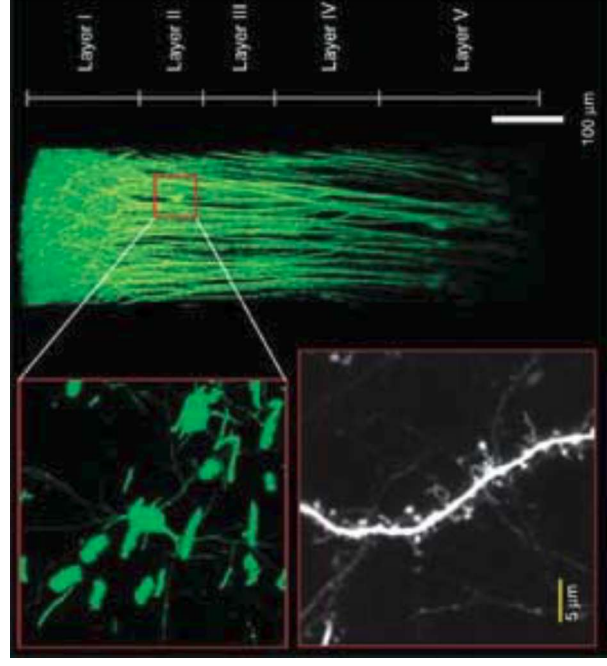
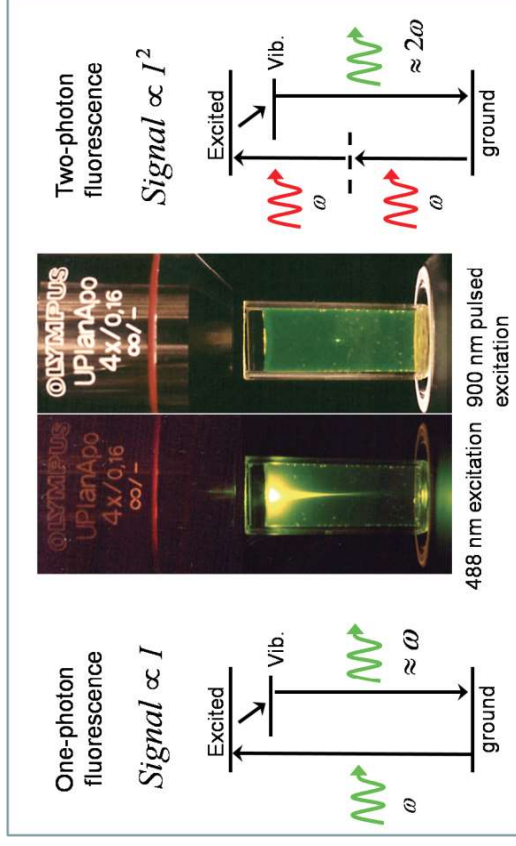


Live

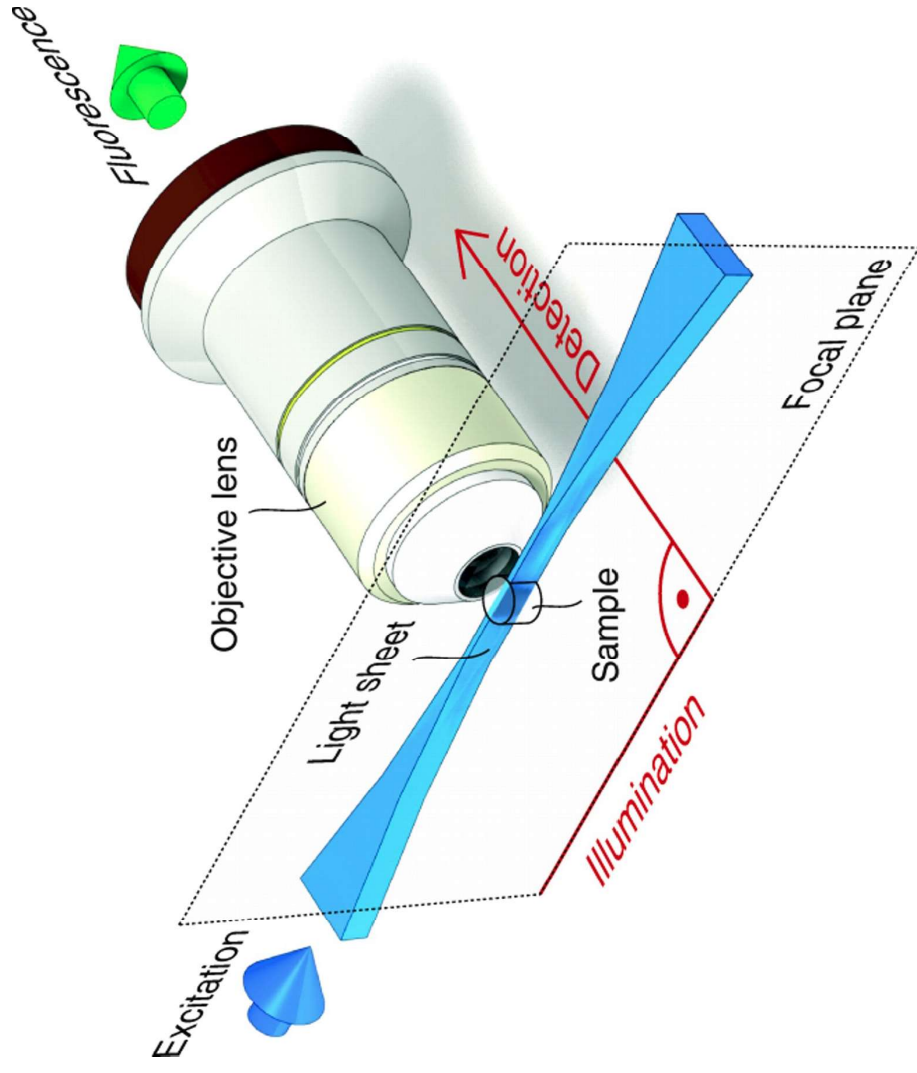


Faster

Two Photon Microscopy



Light Sheet Microscopy



Comparison Between Different Techniques

Performance comparison of three-dimensional fluorescence microscopy in the context of long-term <i>in vivo</i> imaging of entire embryos.				
	DSLIM		Confocal	Two-photon
Sectioning efficiency ^a	In-focus plane		Entire specimen	In-focus plane + surface contribution
Energy load ^b	E		$\sim 10^3 \cdot E$	$\sim 10^6 \cdot E$
Lateral resolution ^c	r		$r/\sqrt{2}$	$r \cdot \sqrt{2}$
Axial resolution ^d	++ (equiv. to lat. res. in multi-view)		+	–
Imaging speed ^e	63 Mpixel/s		2–6 Mpixel/s	1 Mpixel/s
Signal-to-noise ratio ^f	1000:1		60:1	10:1
Cost efficiency ^g	$\geq 50k \text{ €}$		$> 100k \text{ €}$	$> 200k \text{ €}$

Exposure time per pixel:

Confocal: $1\text{-}10 \times 10^{-6} \text{ s}$

Bright field: $0.1\text{-}1 \text{ s}$

Z-stacking $10\text{-}1000X$

Energy:

DSLIM $1.7 \times 10^{-6} \text{ J @ } 488 \text{ nm}$

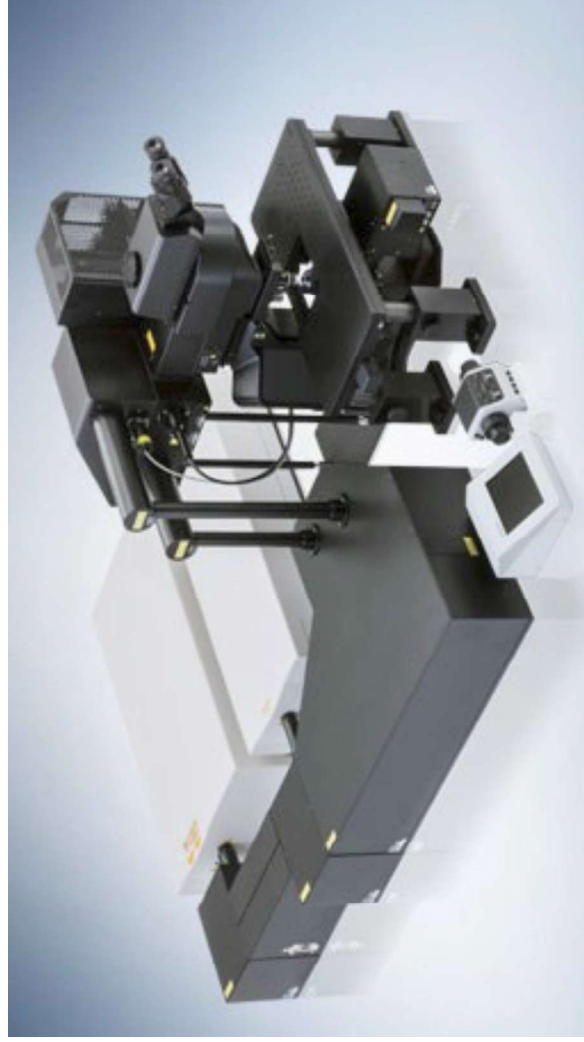
Confocal $9.6 \times 10^{-3} \text{ J @ } 488 \text{ nm}$

Two photon $1.7 \text{ J @ } 930 \text{ nm}$

Confocal Systems



FV1200 MPE

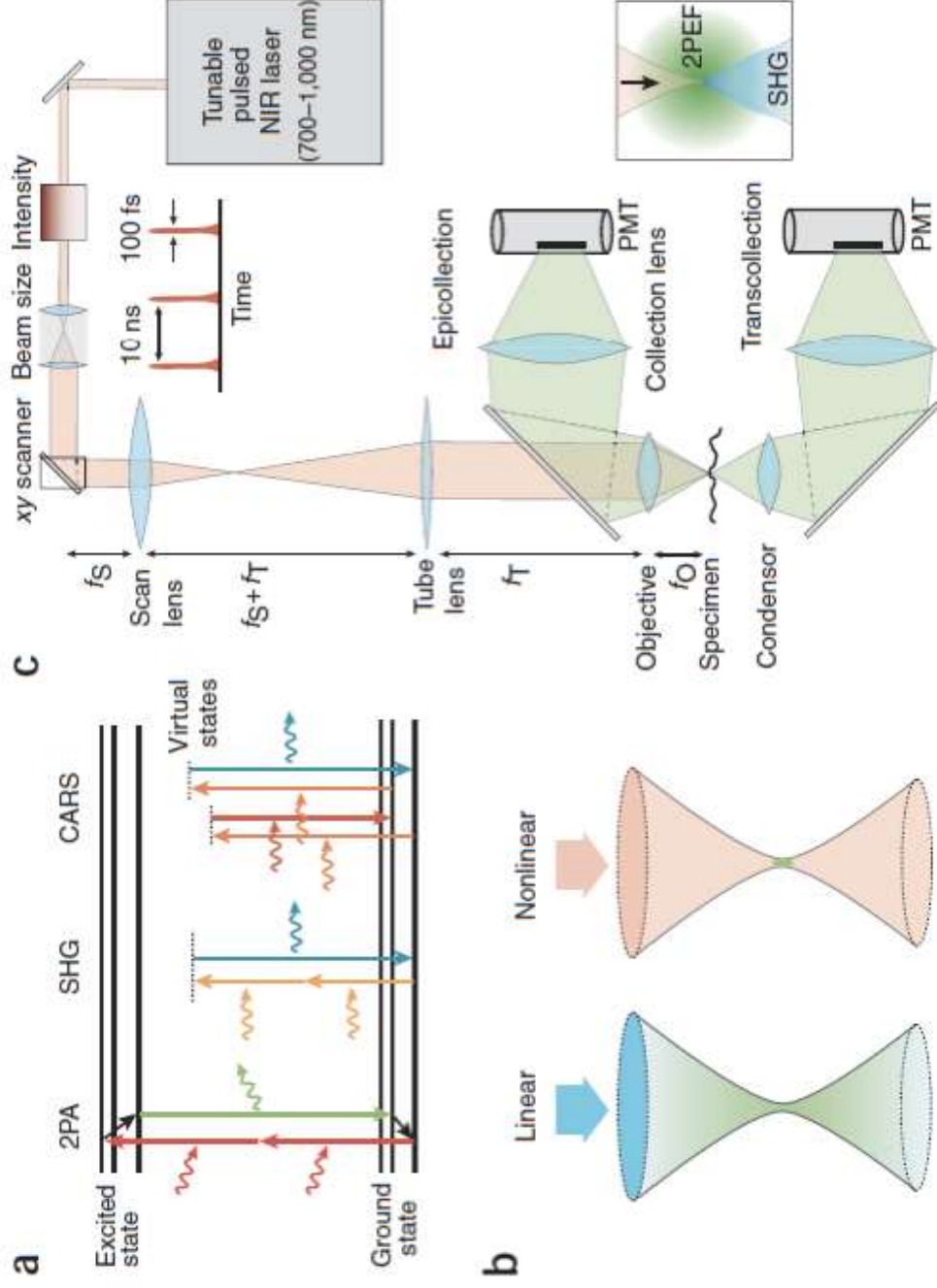


FVMPE-RS

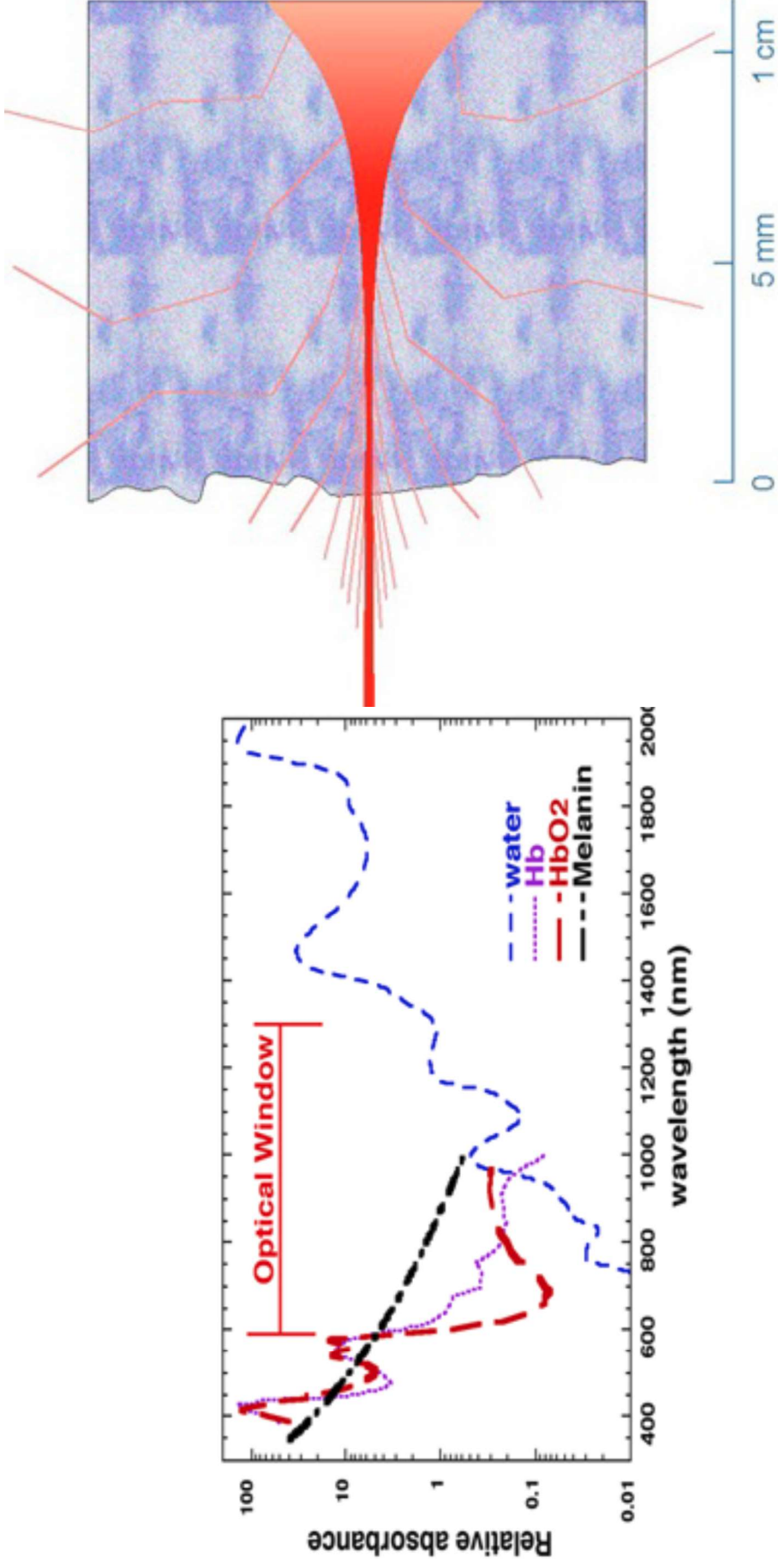
Deep tissue two-photon microscopy

Fritjof Helmchen¹ & Winfried Denk²

932 | VOL.2 NO.12 | DECEMBER 2005 | **NATURE METHODS**



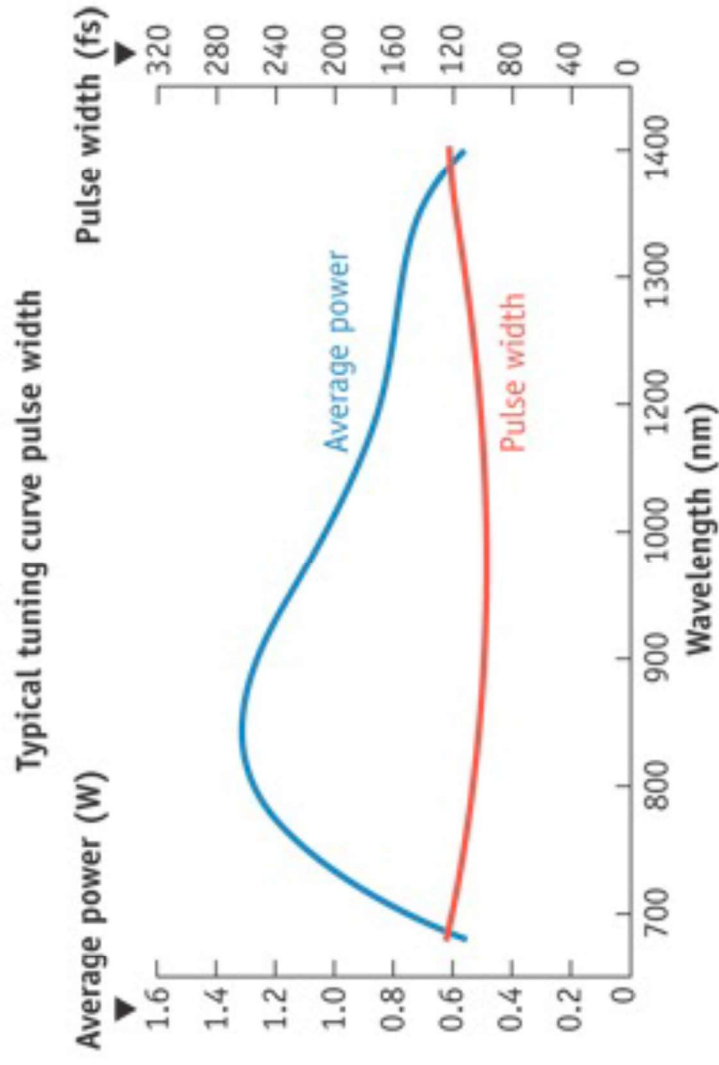
Challenges in Deep Tissue Imaging



Absorption

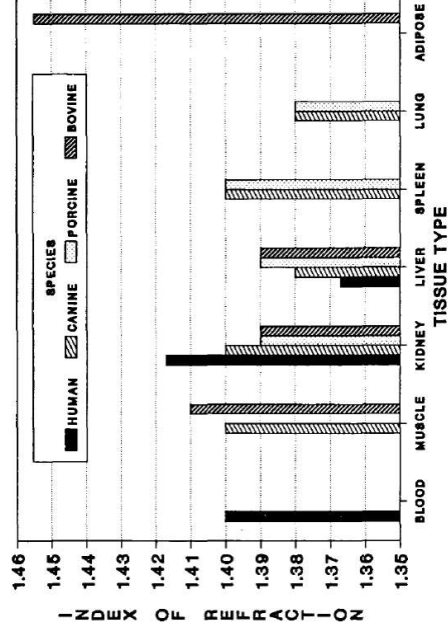
Scattering

Tunable Laser



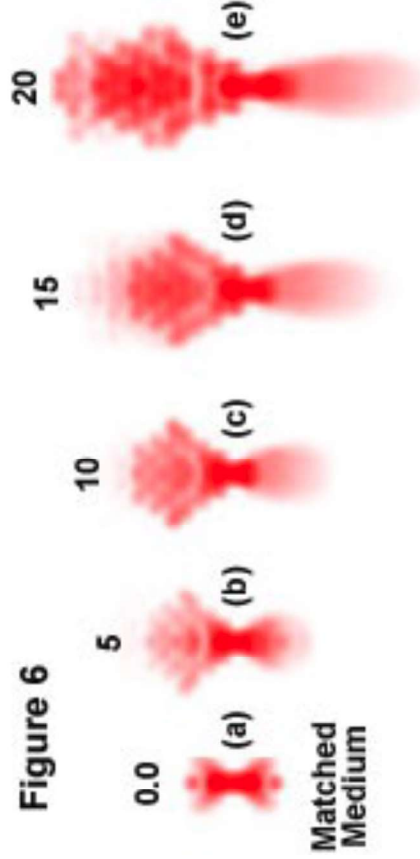
Refraction Index Matching

Refraction Index
Air: 1.00029
Water: 1.333
Fused silica: 1.458
Glycerol: 1.4729
Scale: 1.38
Urea: 1.48-1.49

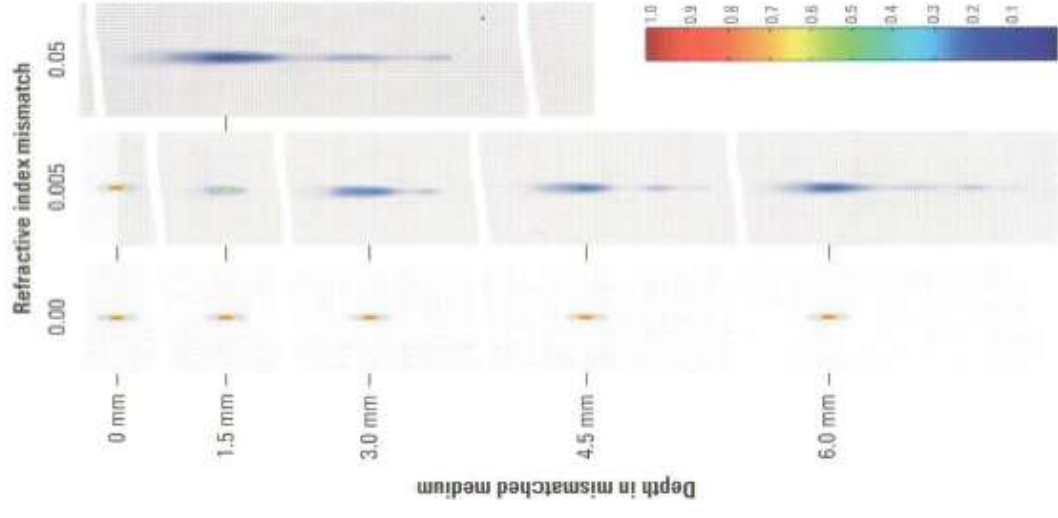


Refraction Index Matching

Figure 6 Confocal Point Spread Function Contour Plots

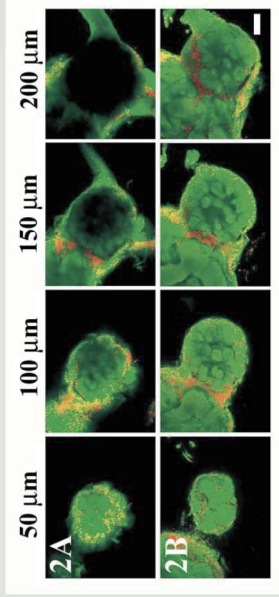


Penetration Depth in Aqueous Medium (Micrometers)



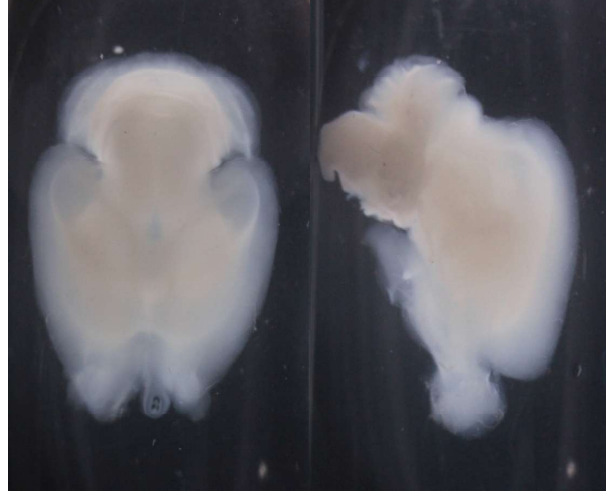
FocusClear 澄清液

讓您的螢光顯微觀察更深入清晰；更



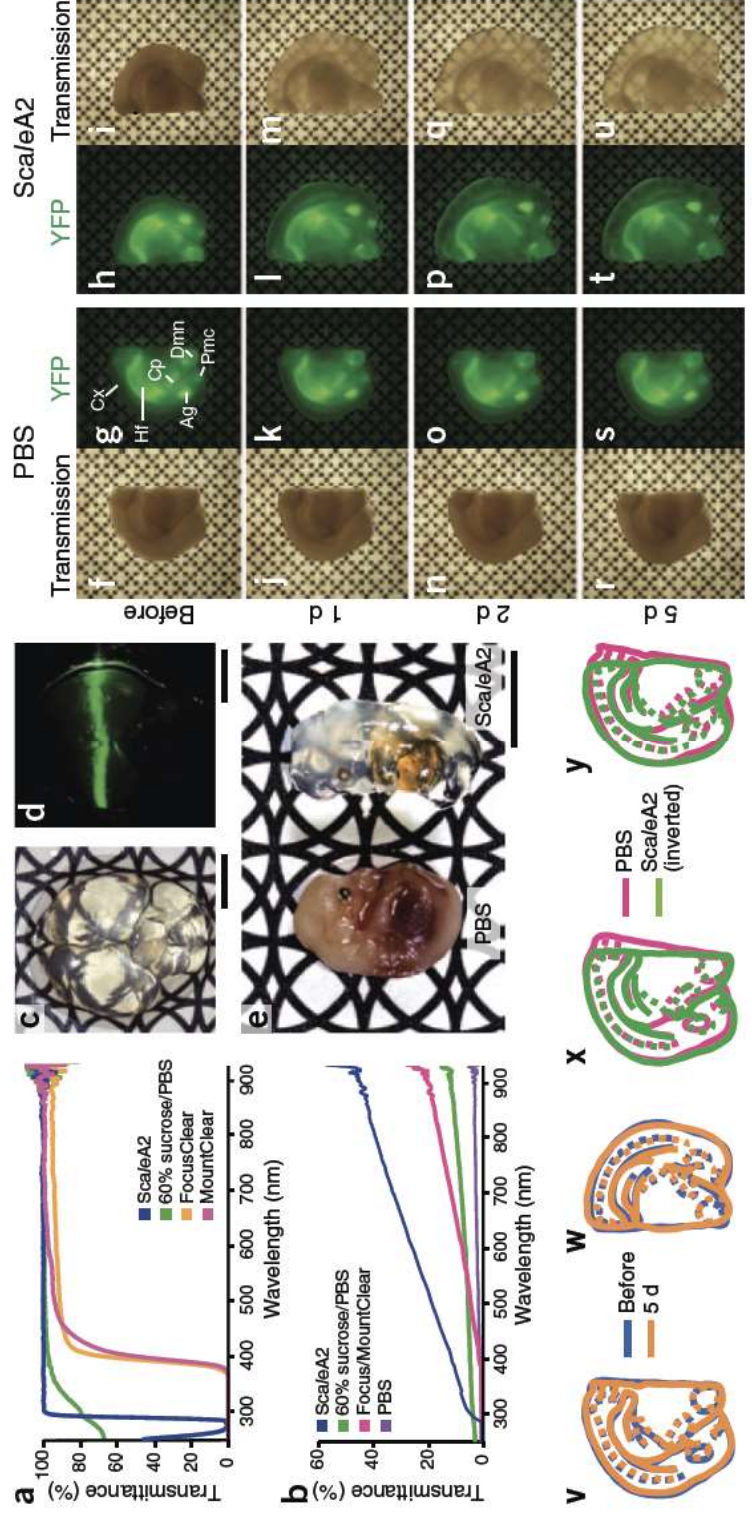
OLYMPUS
SCALEVIEW-A2
 Optical Imaging Agent
 and
 Immersion Liquid for
 SCALEVIEW immersion objectives
 ref#1-390 3 g/51 23°C
 Store at 2~25°C protected from sunlight
 For laboratory use only
 1000ml

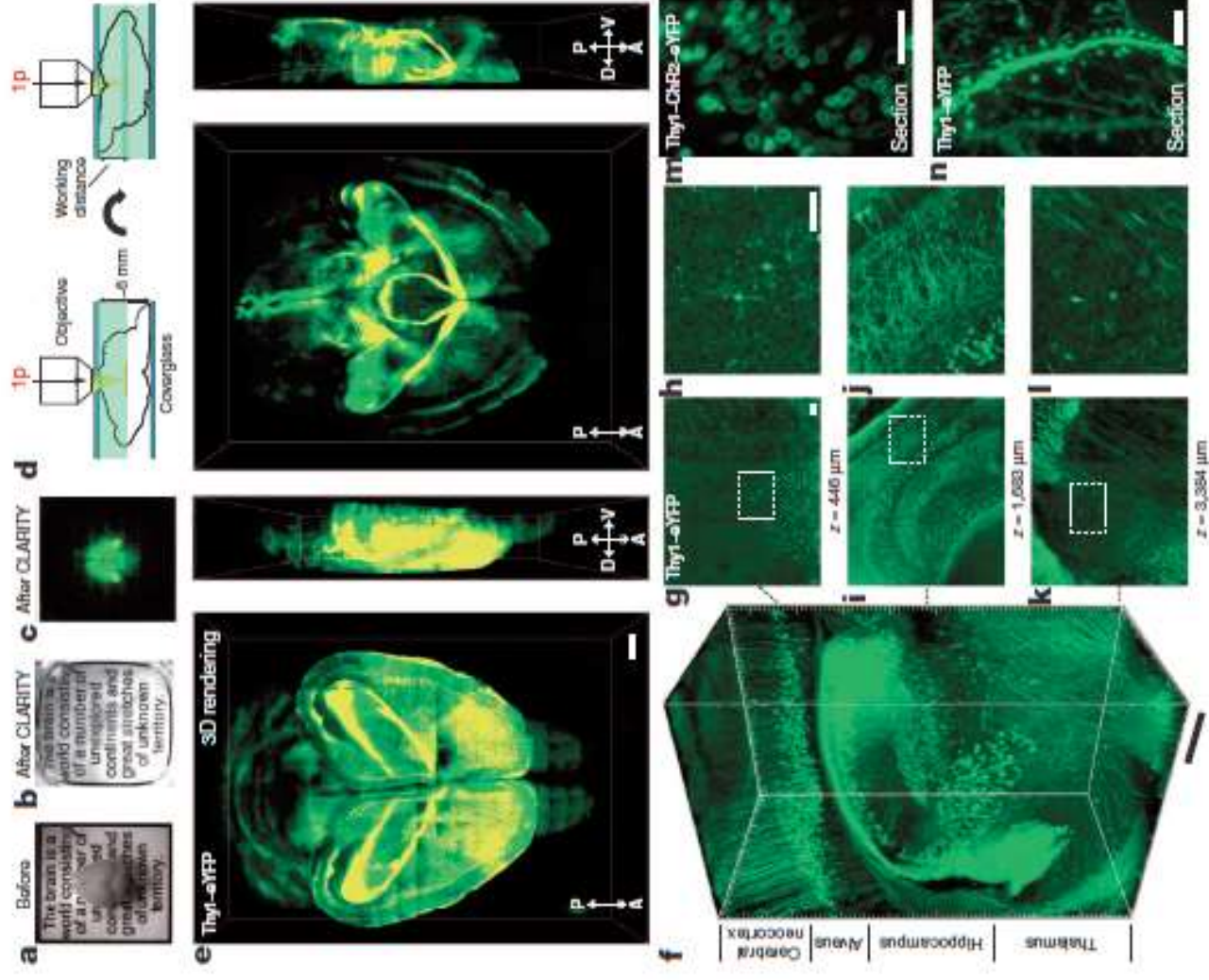
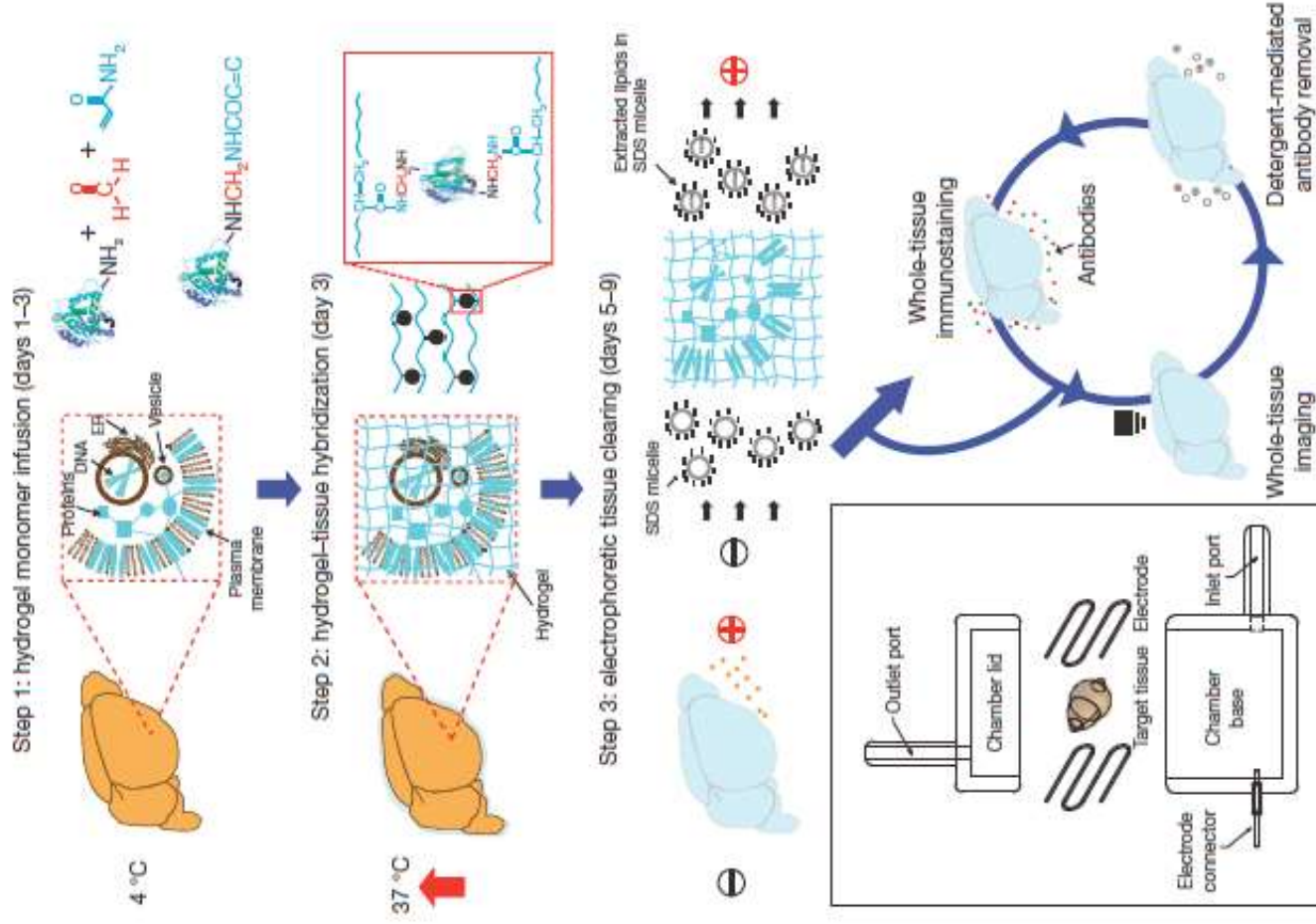
⚠ 使用前に必ず取扱説明書をお読みください。
 ⚠ 使用前に必ず目視検査を行ってください。
 ⚠ 使用後は必ず洗浄してください。
 ⚠ 使用後は必ず廃棄してください。
 ⚠ 使用後は必ず廃棄してください。



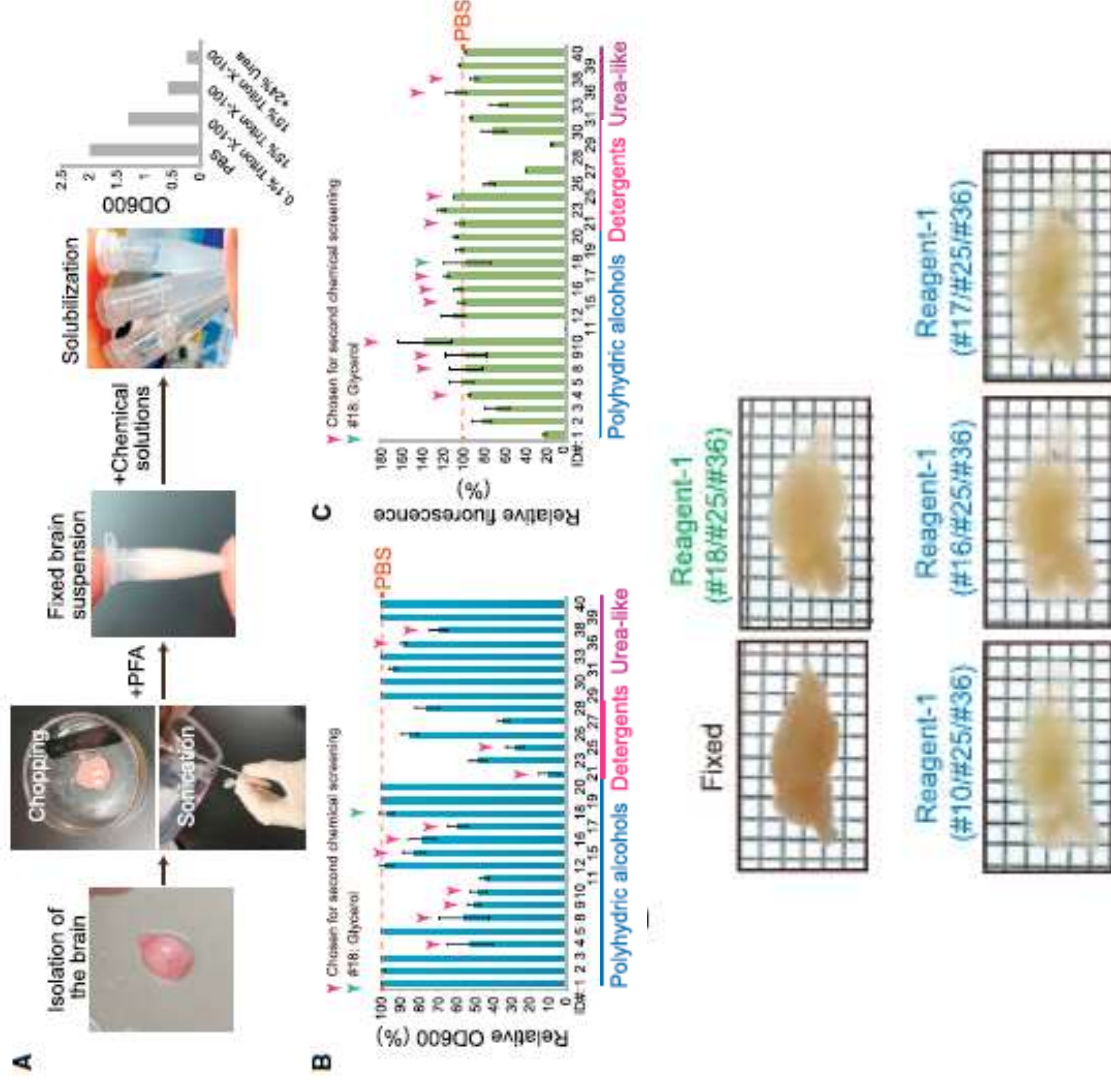
Scale: a chemical approach for fluorescence imaging and reconstruction of transparent mouse brain

Hiroshi Hama¹, Hiroshi Kurokawa^{1,2}, Hiroyuki Kawano^{1,3}, Ryoko Ando¹, Tomomi Shimogori¹, Hisayori Noda^{1,4}, Kiyoko Fukami², Asako Sakaue-Sawano^{1,3} & Atsushi Miyawaki^{1,3}

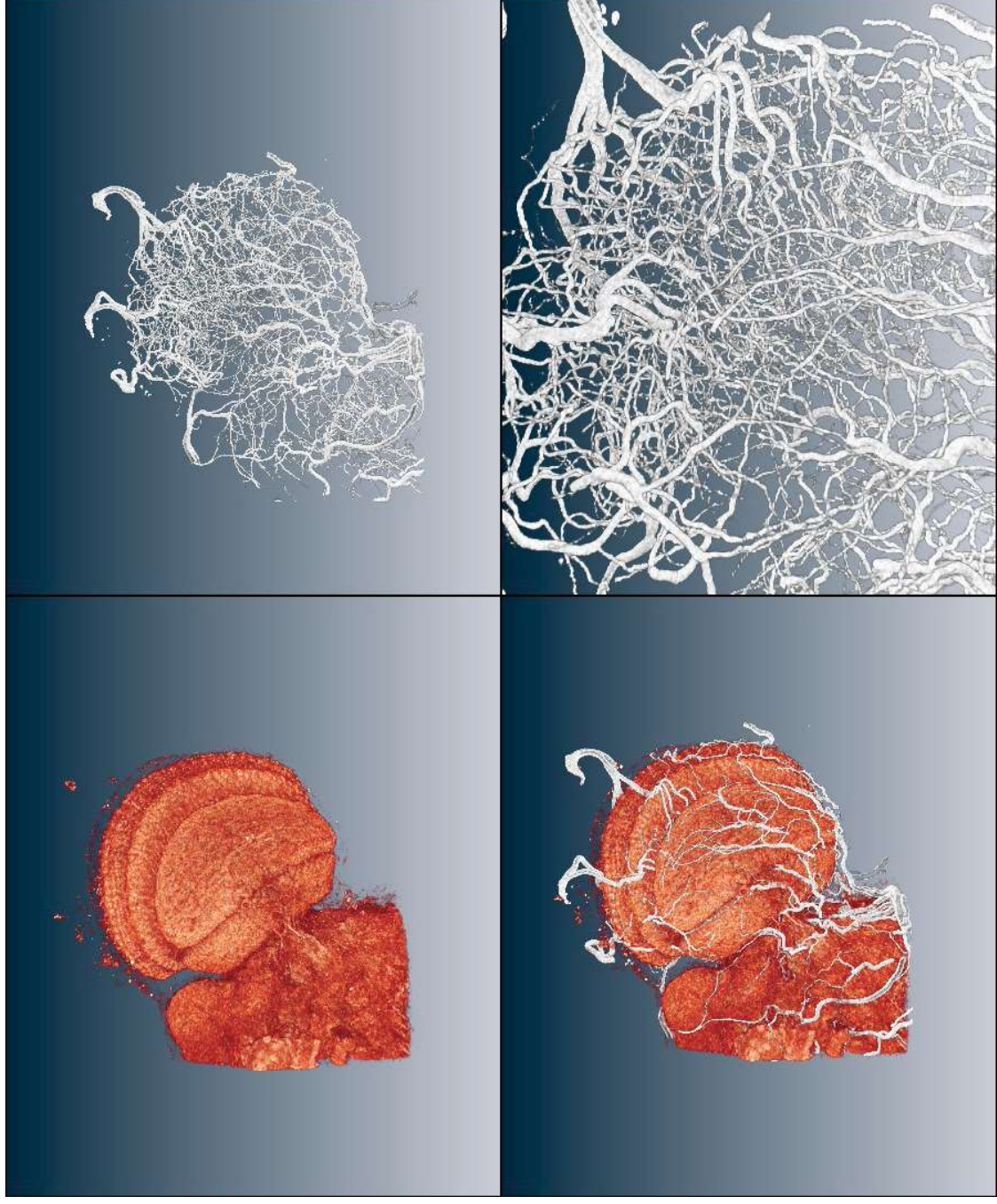




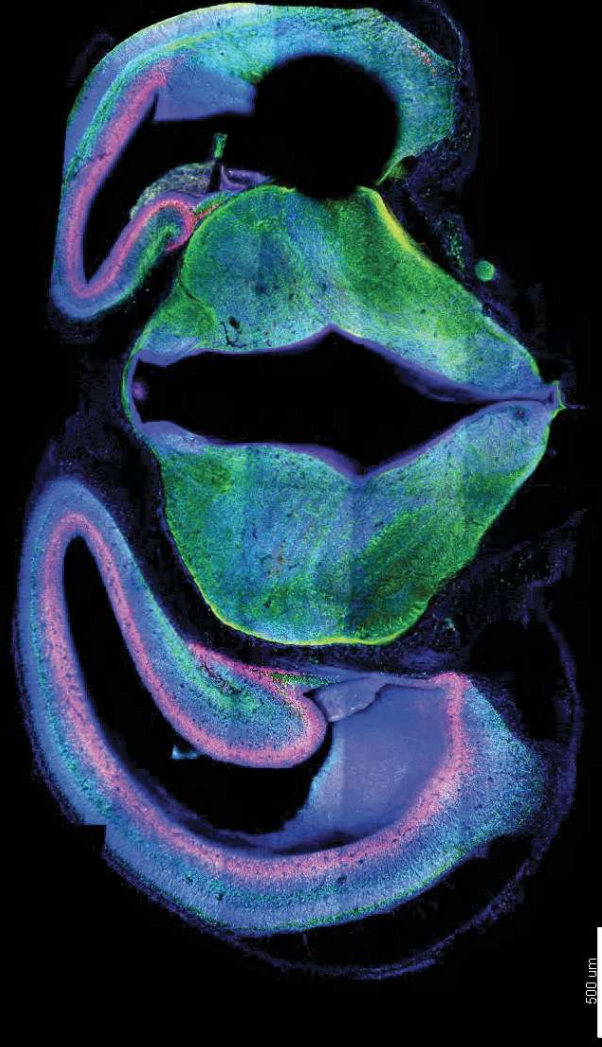
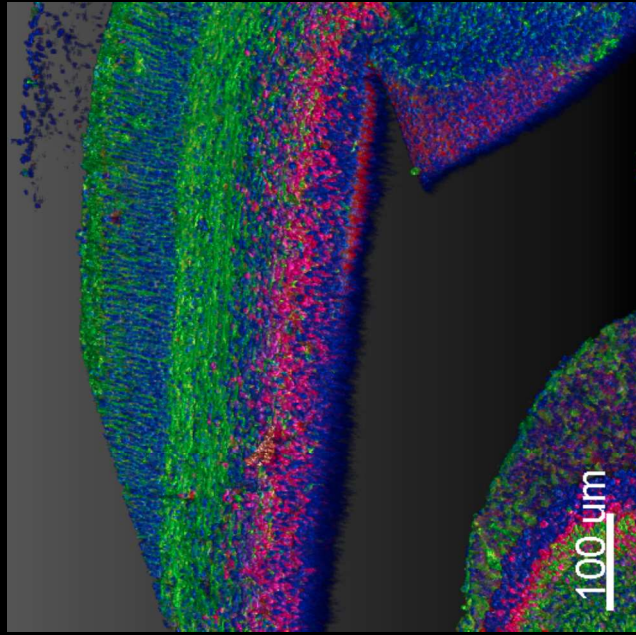
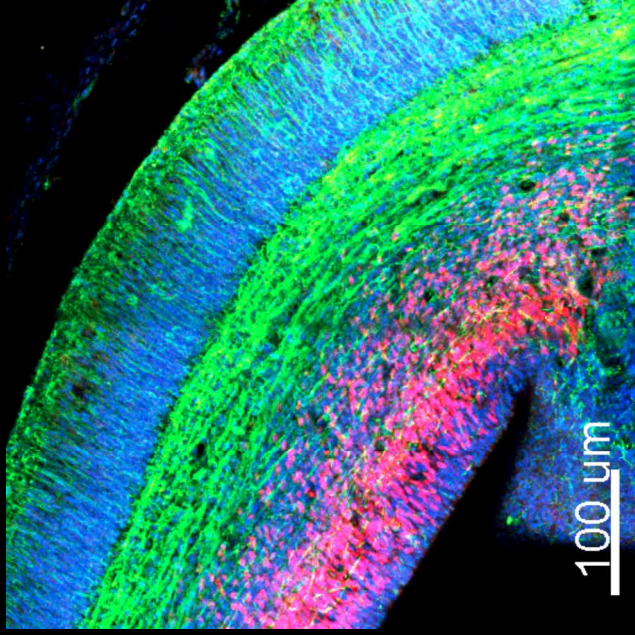
Whole-Brain Imaging with Single-Cell Resolution Using Chemical Cocktails and Computational Analysis



Fly Brain



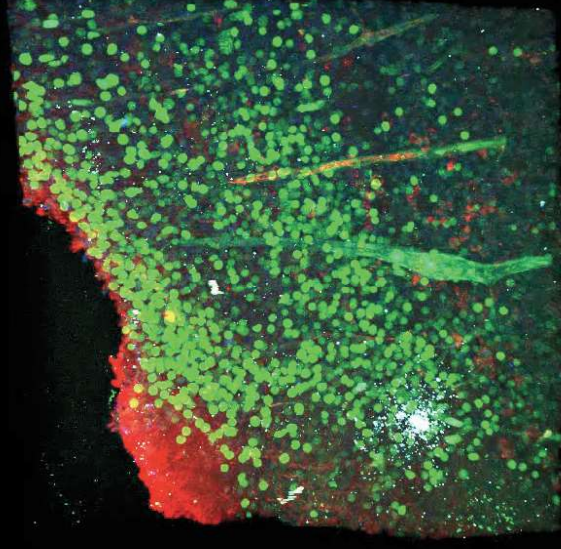
Without clearing reagent (<100 μm depth)



TBR2 TUJ1 DAPI

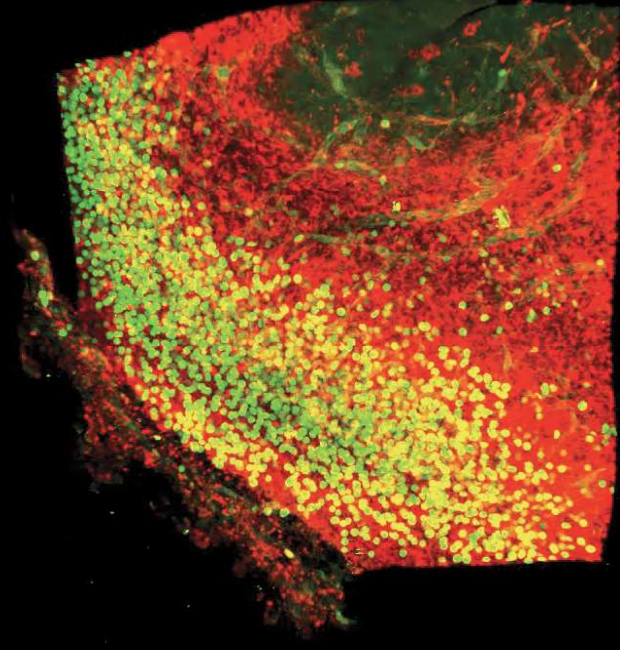
With clearing reagent (0.5 mm depth)

Olympus scaleview
1.38
(> 0.5 mm depth)



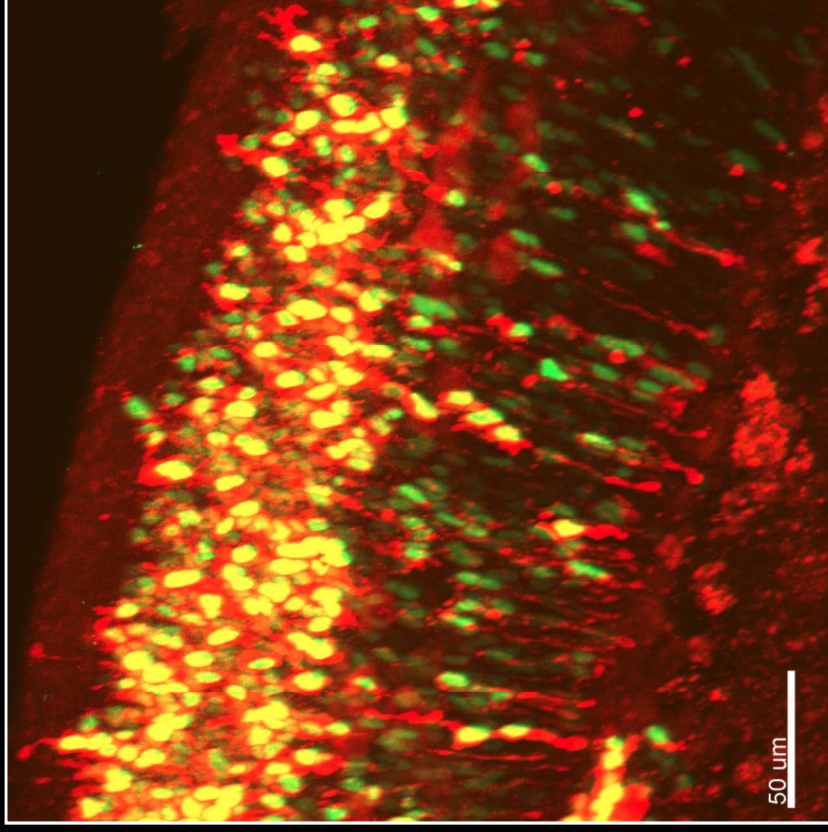
150 μ m

SunJin lab Rapiclear
1.47
(< 0.5 mm depth)

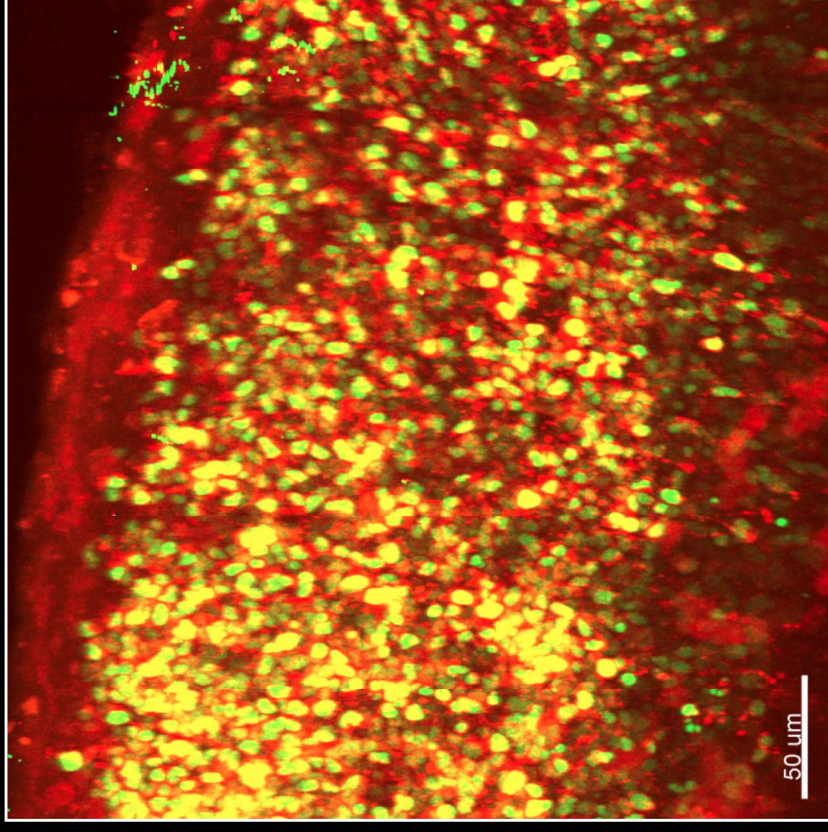


40 μ m

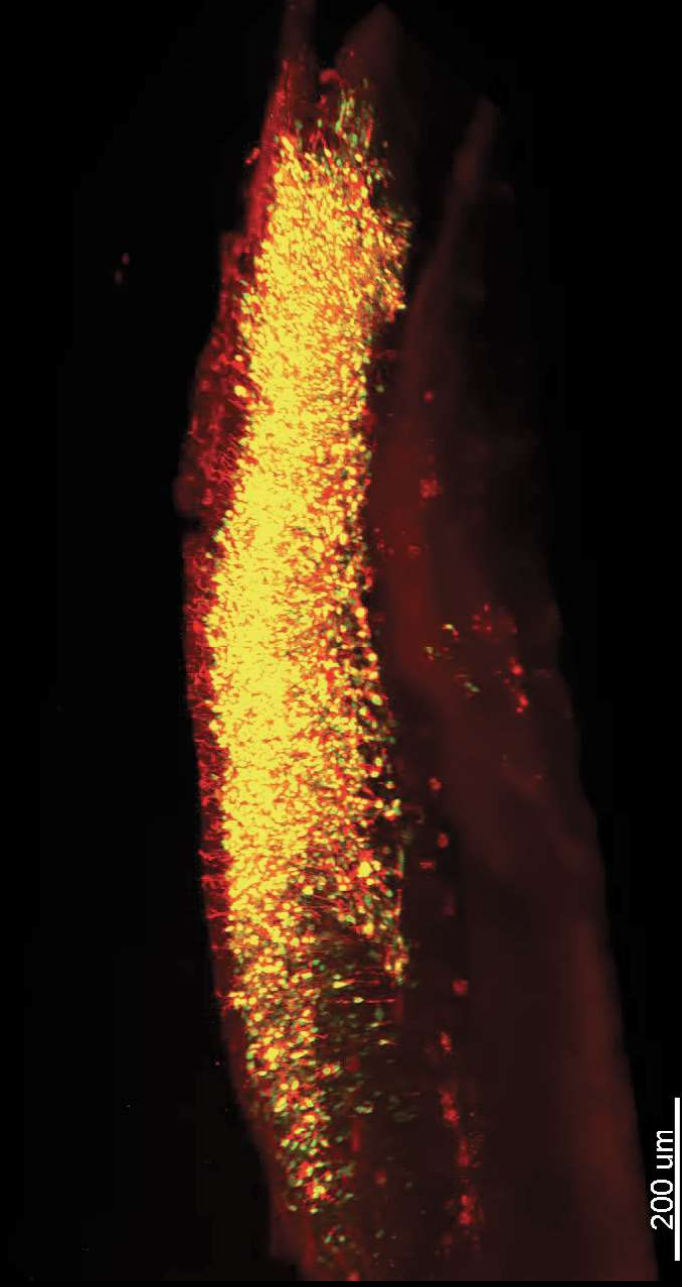
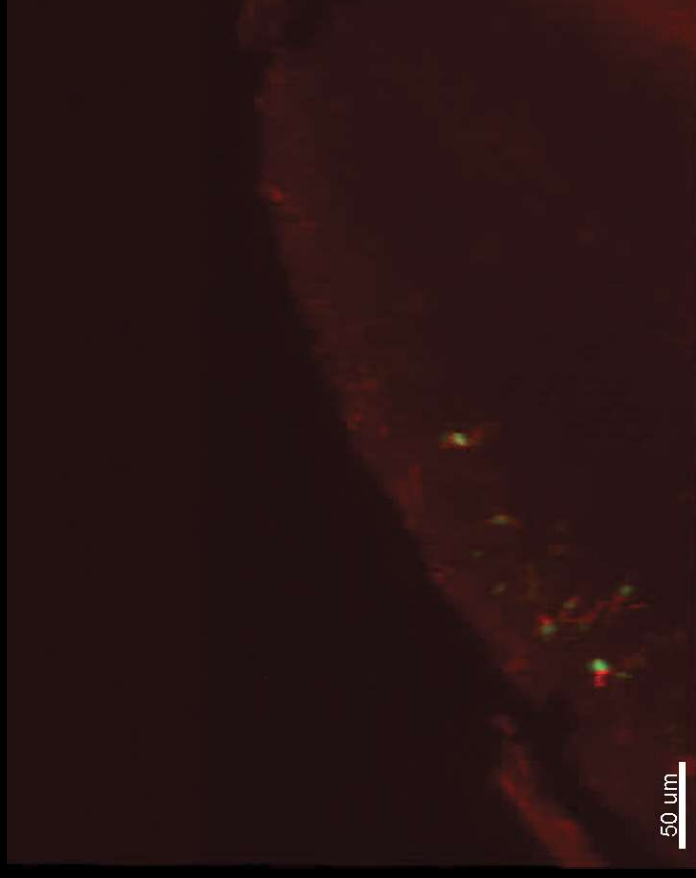
Control
p53^{WT}: Cpap^{flox/Δ}; Nes-Cre+



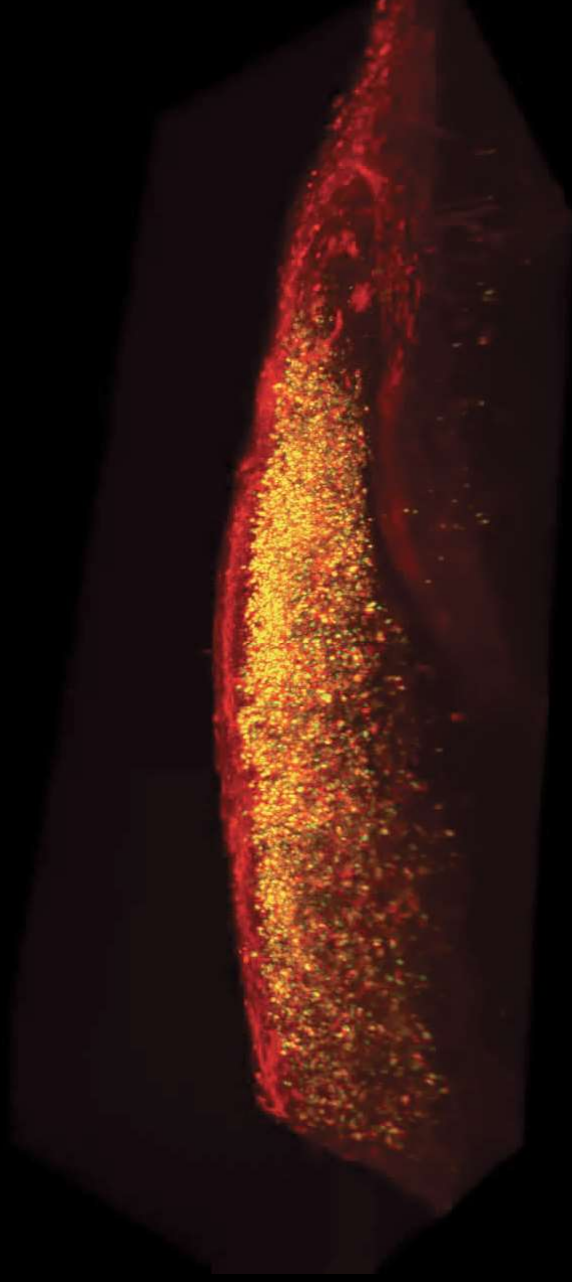
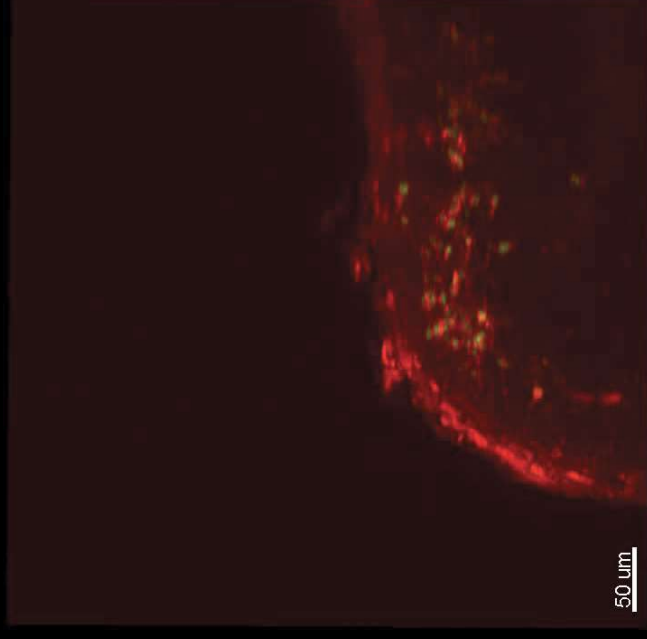
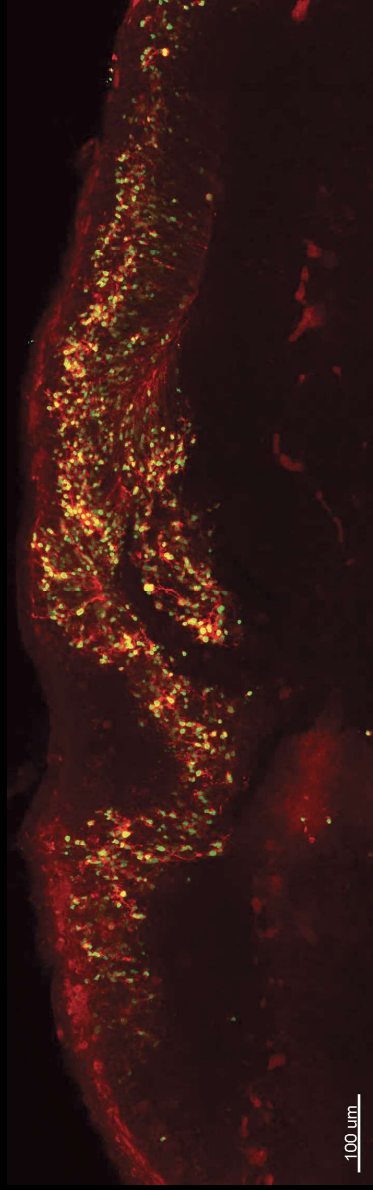
Cko
p53^{KO}: Cpap^{+ / flox}; Cre-



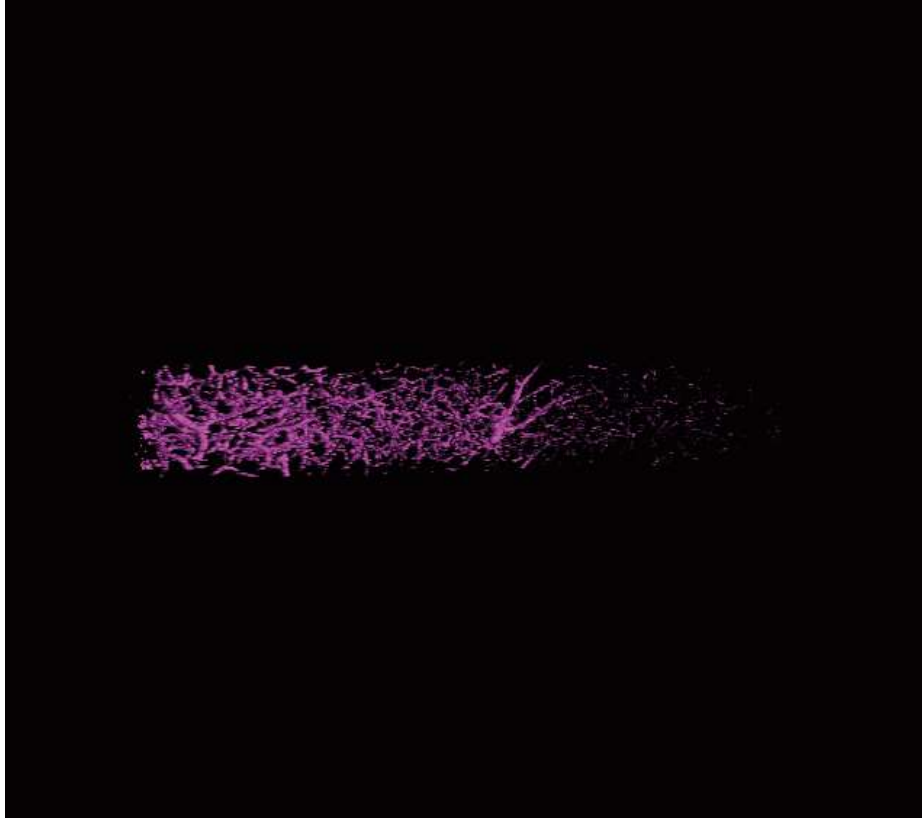
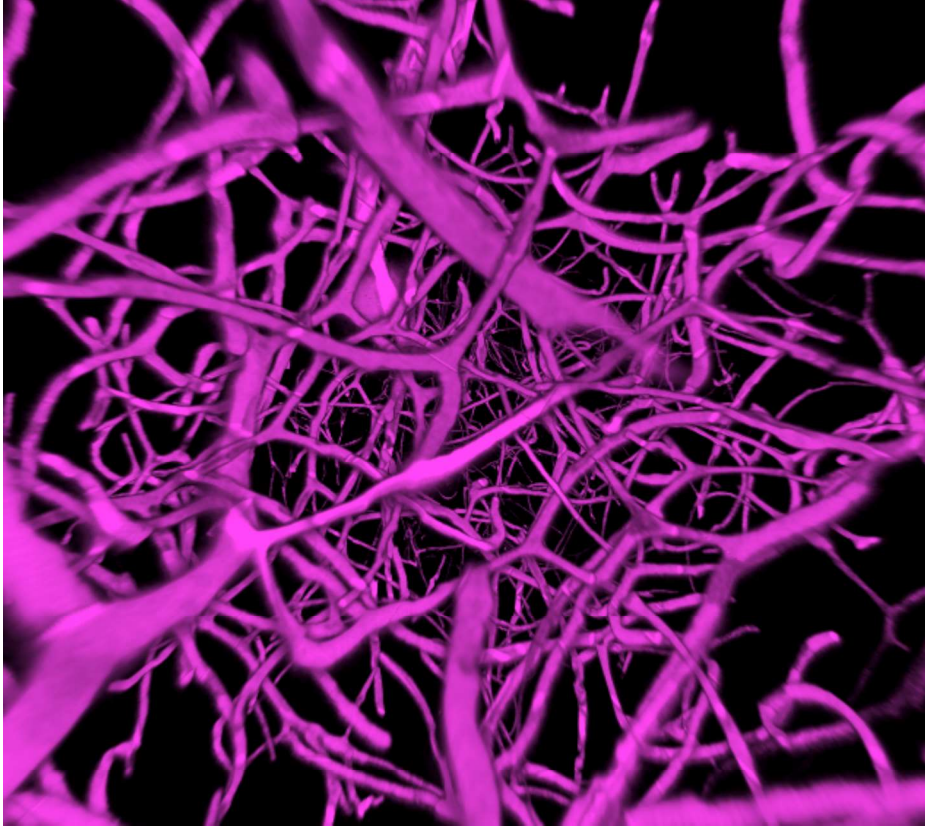
Control

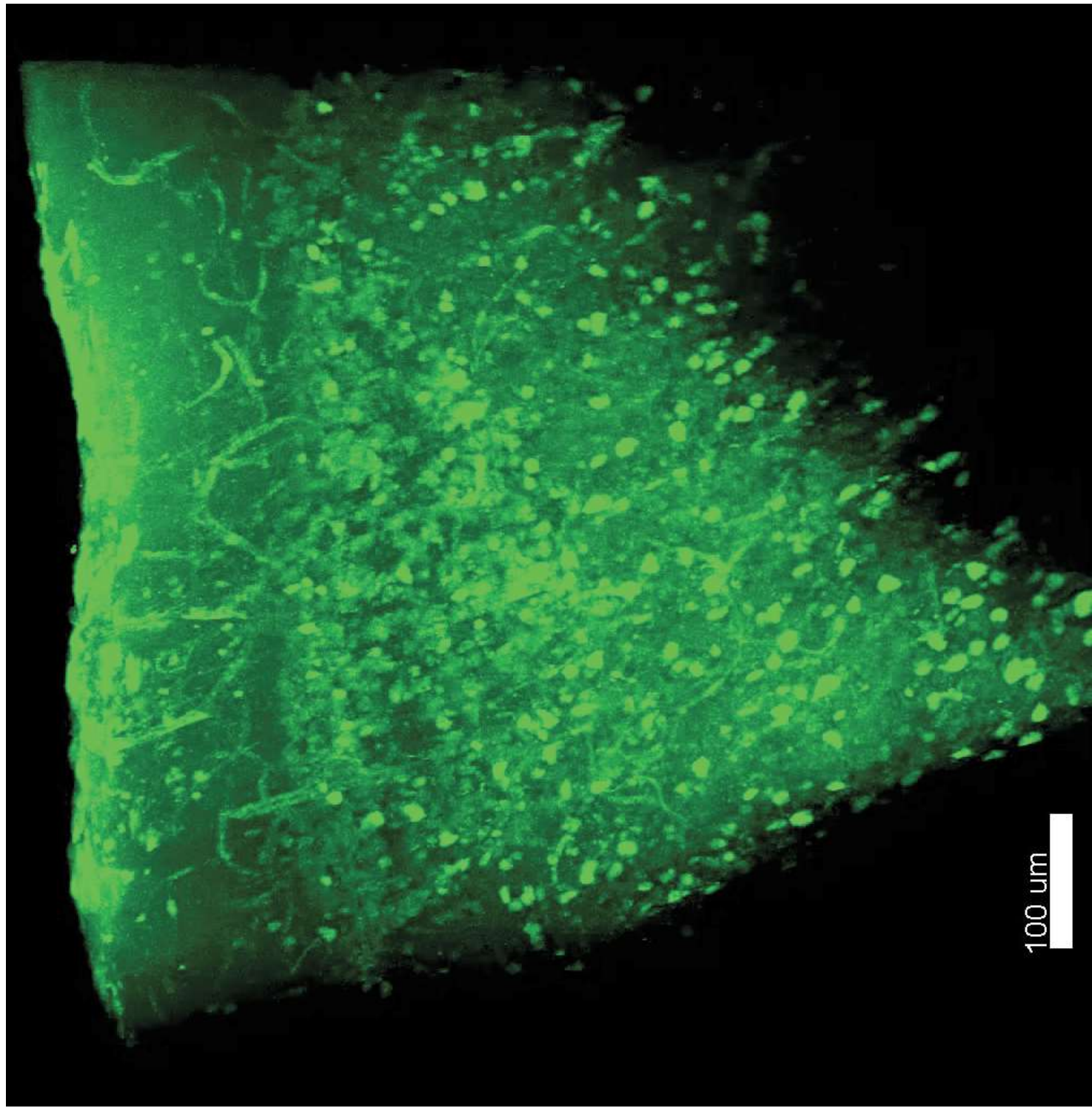


CKO

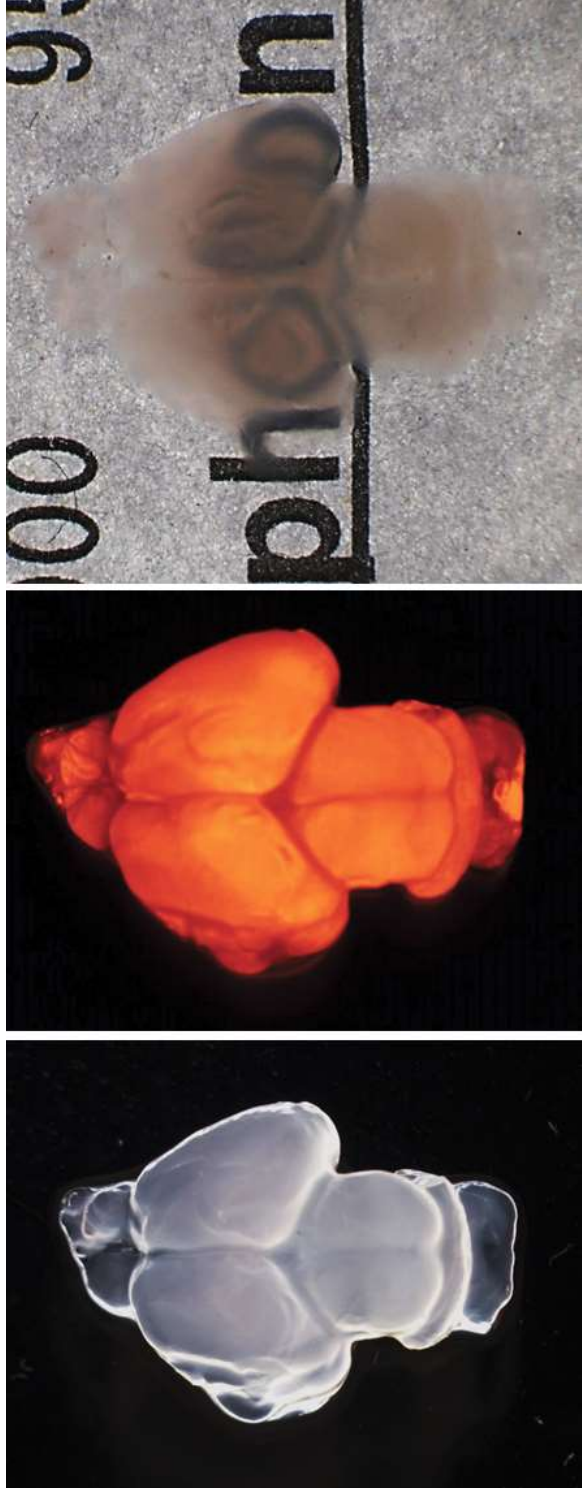


Blood Vessels in Mouse Brain

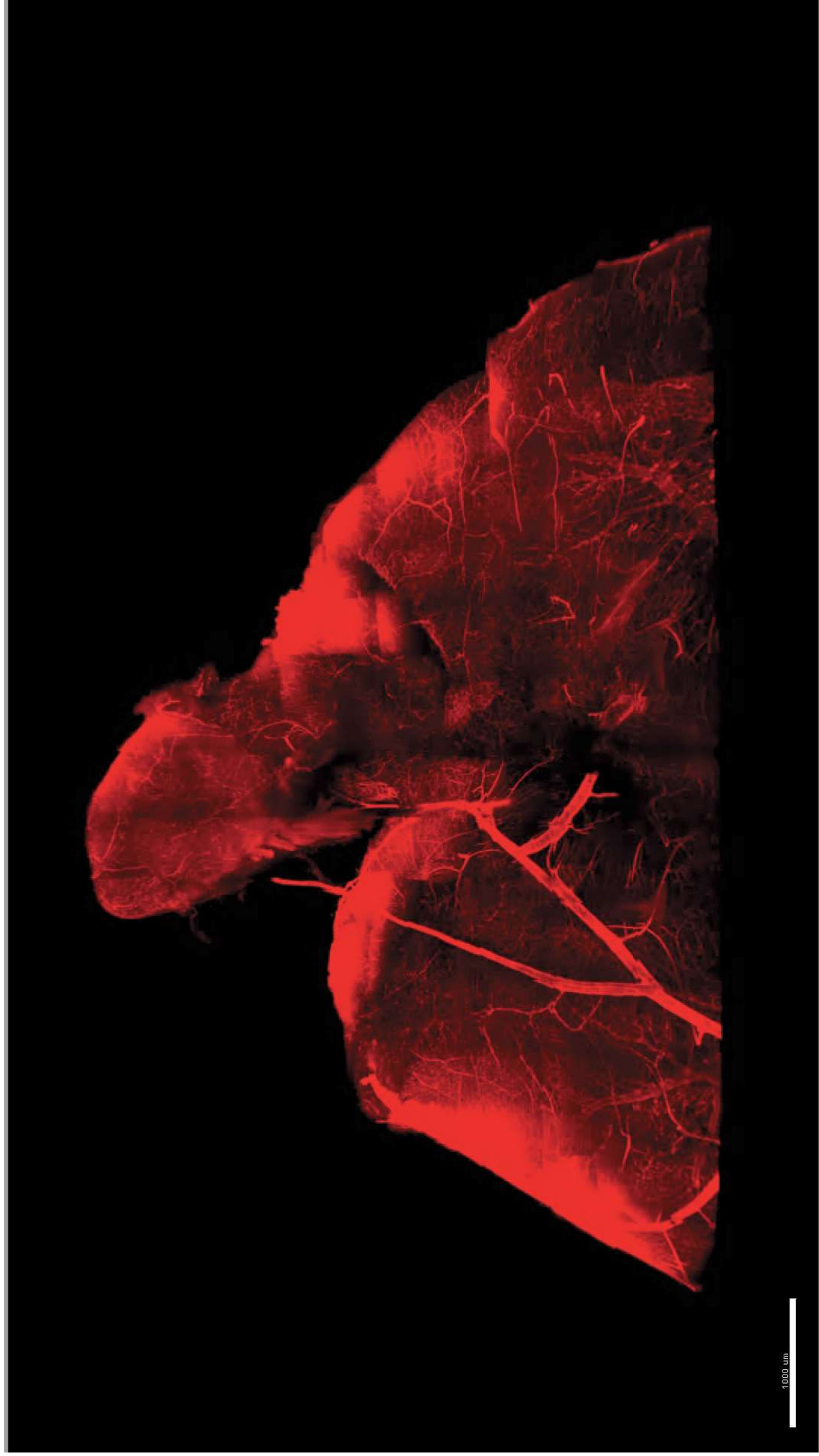




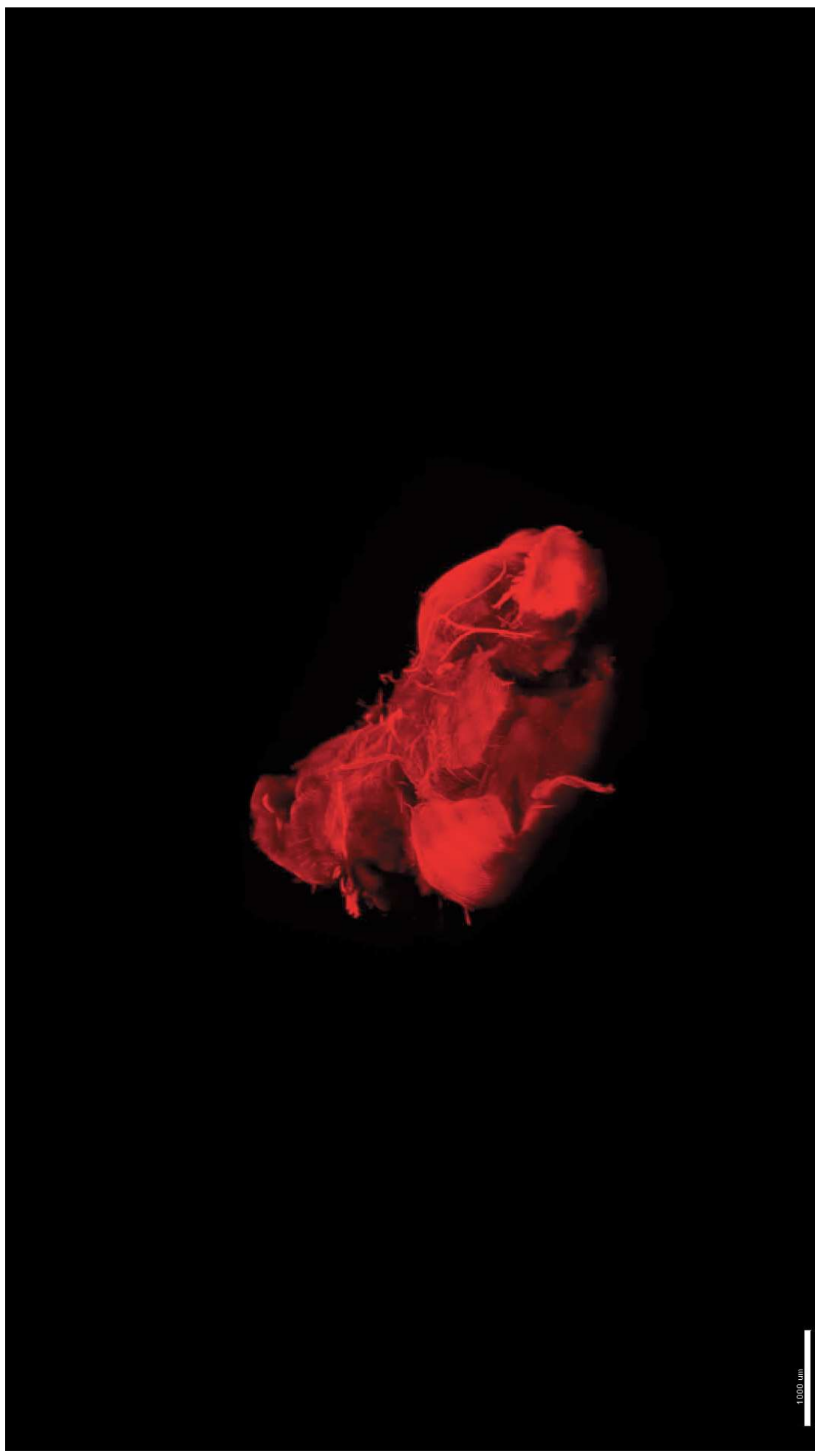
Whole Mouse Brain



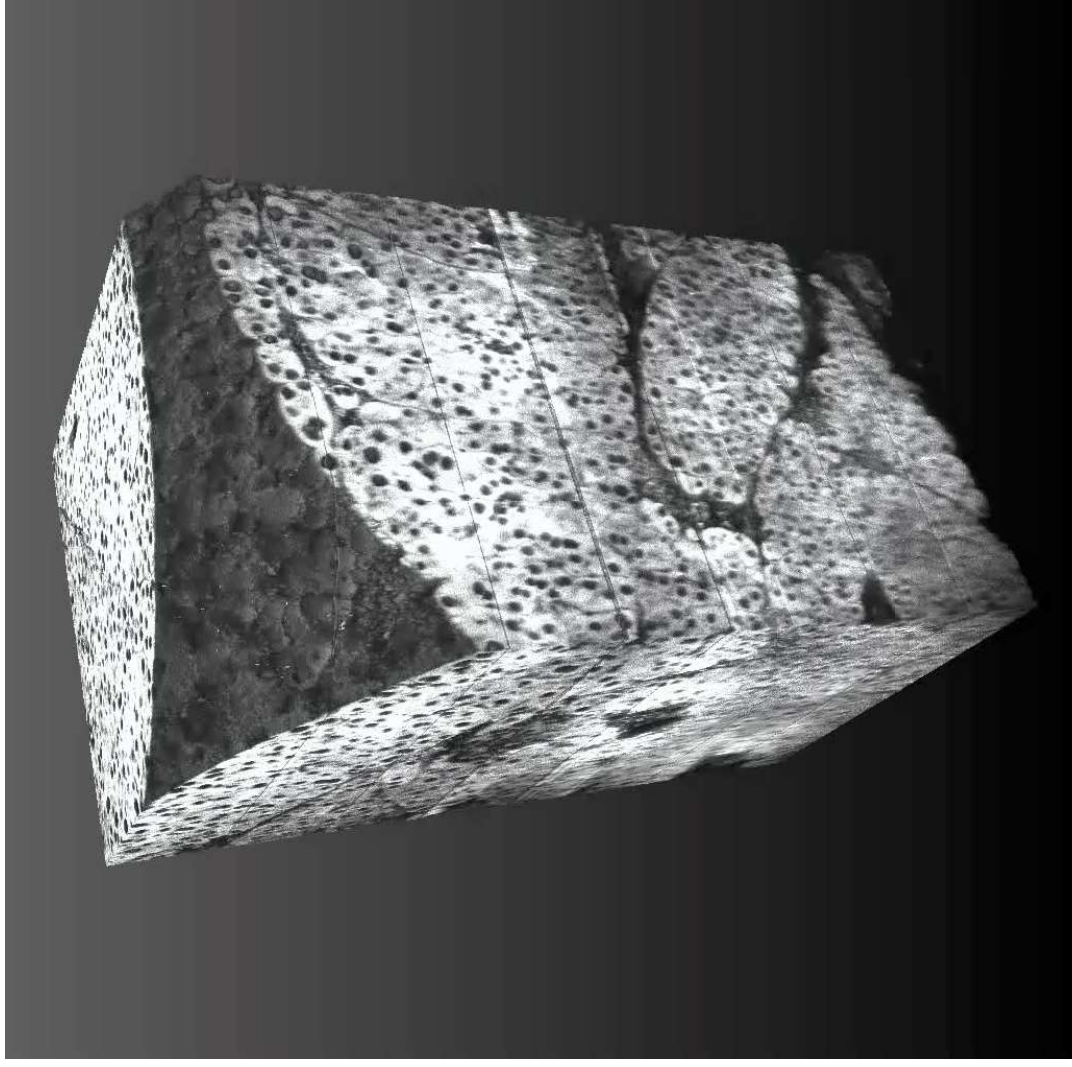
Whole Mouse Brain



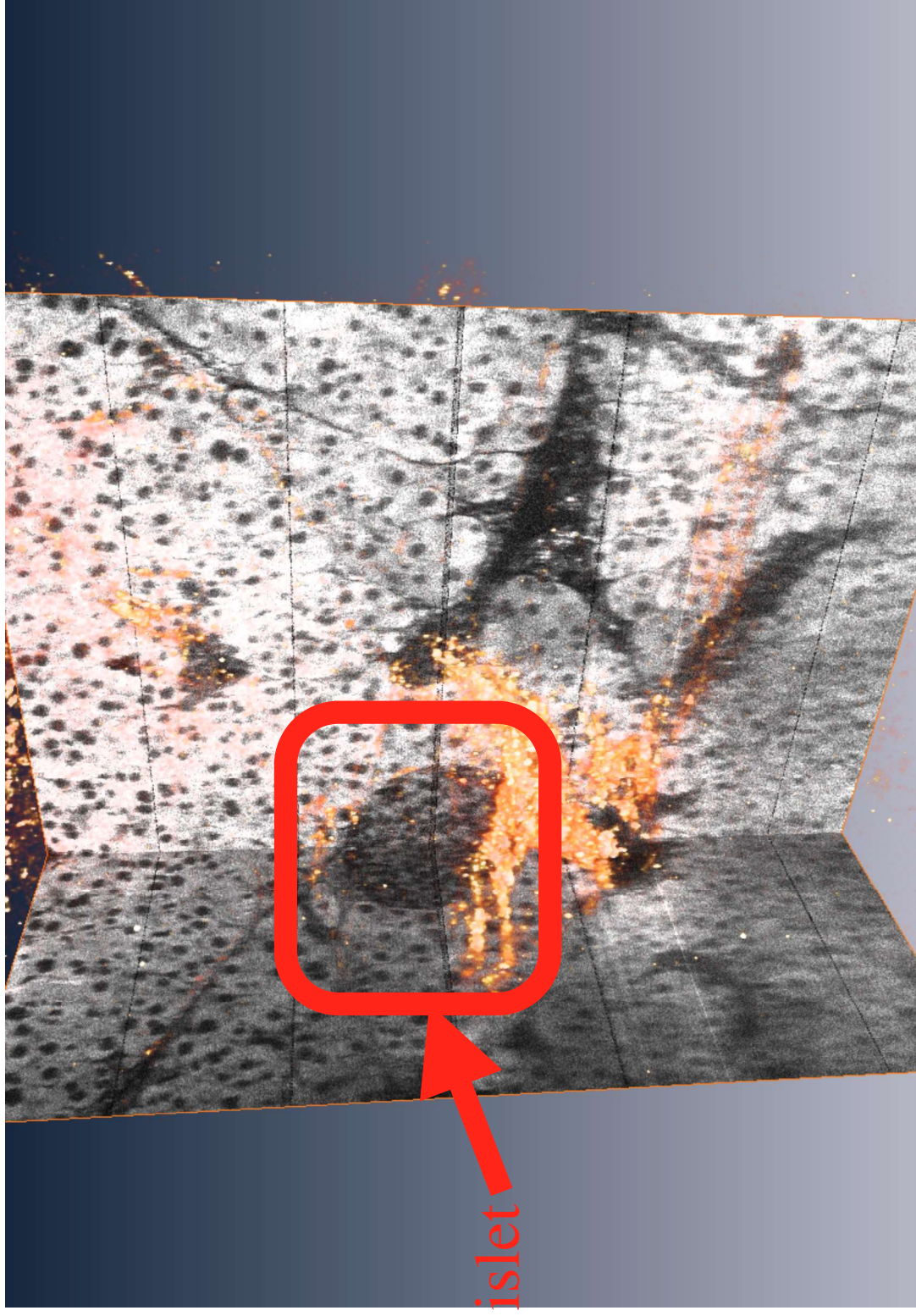
Whole Mouse Brain



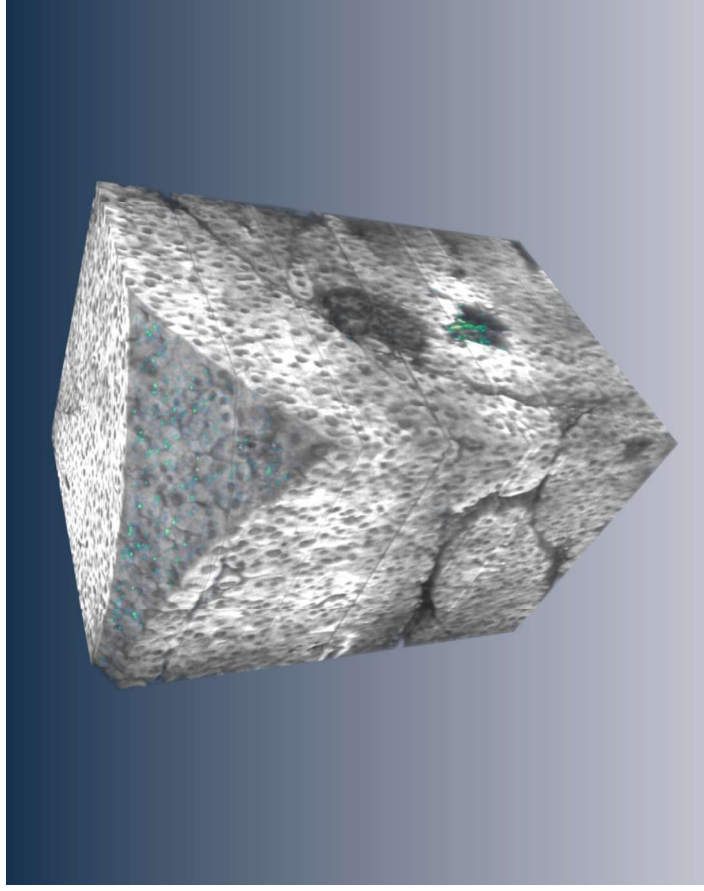
Pancreas



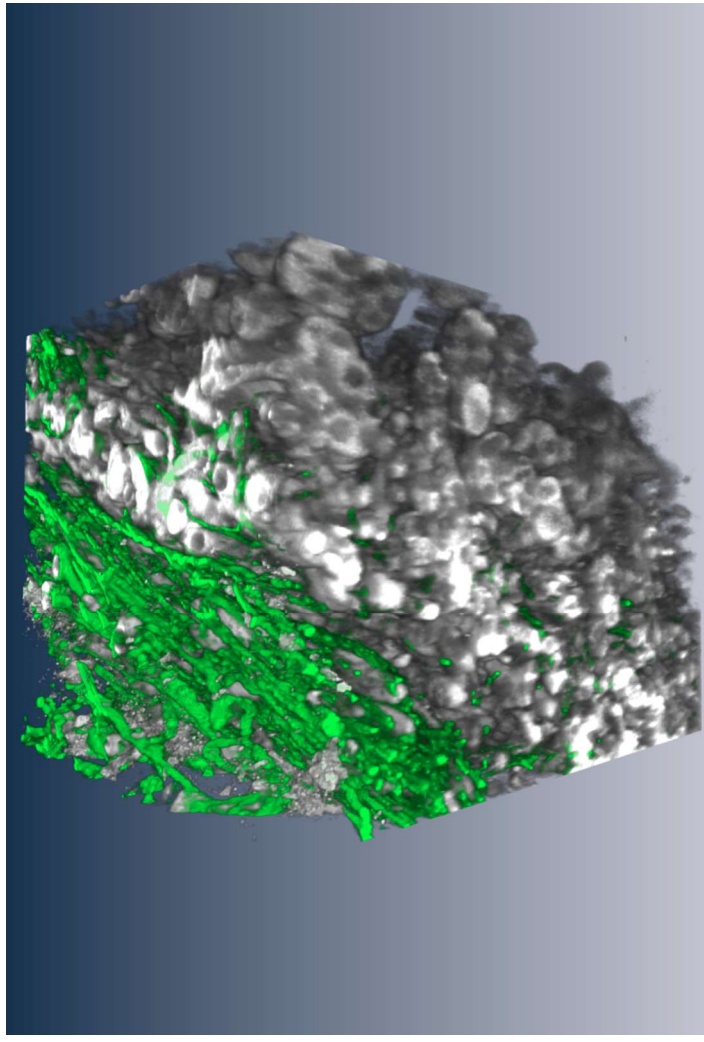
Normal Pancreas



Second Harmonic and Two Photon Photon Imaging

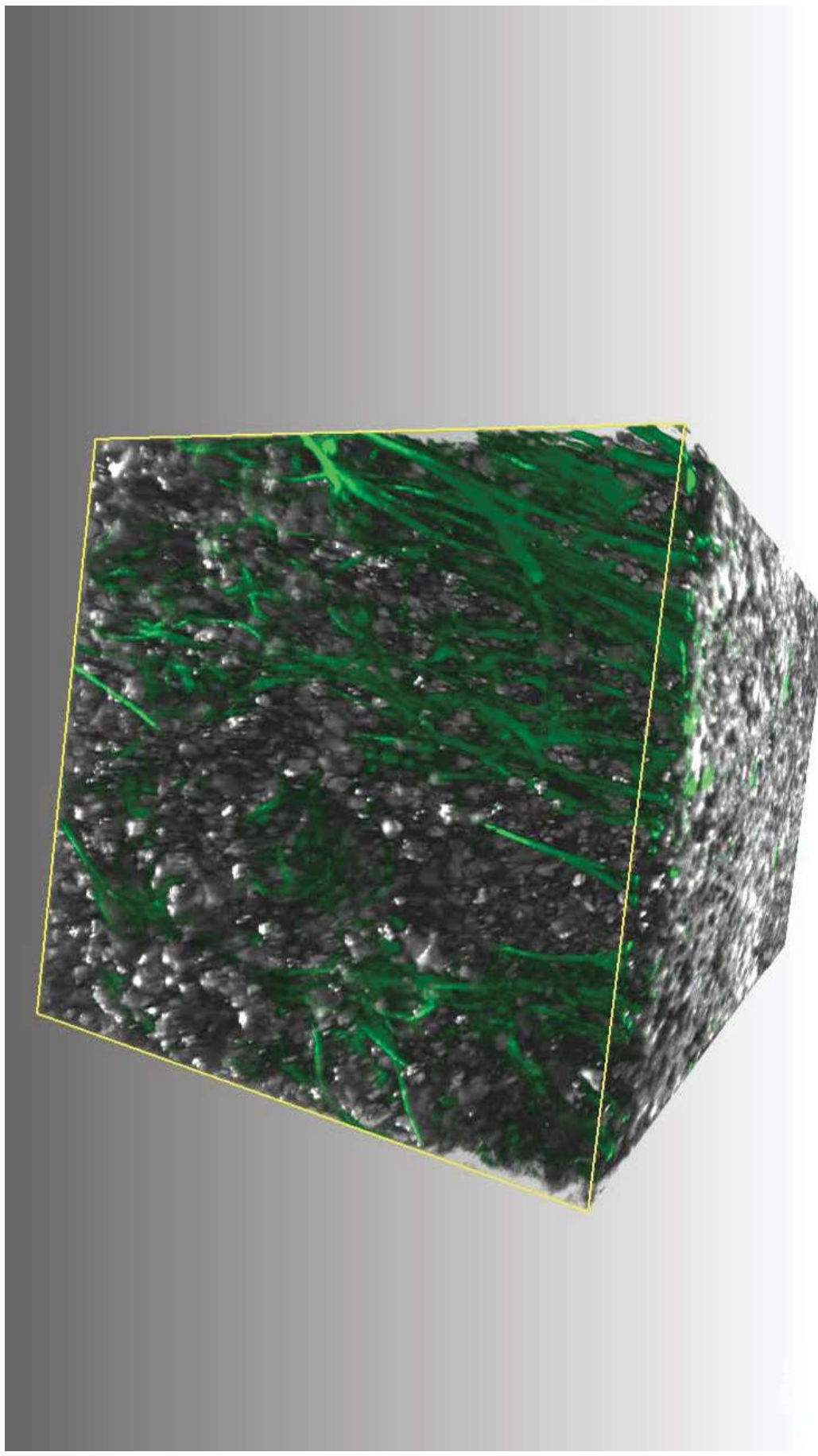


Normal



Cancer

Second Harmonic and Two Photon Imaging



Tracking cells in their native habitat: lineage tracing in epithelial neoplasia

Maria P. Alcolea and Philip H. Jones

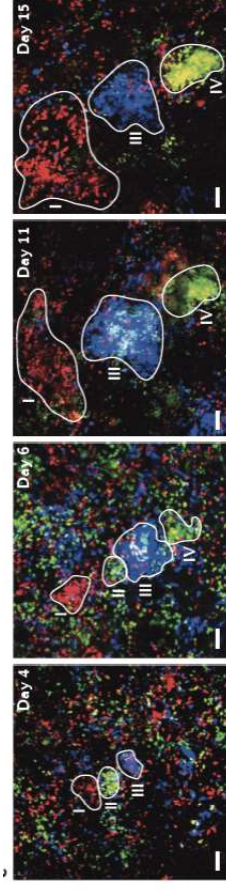
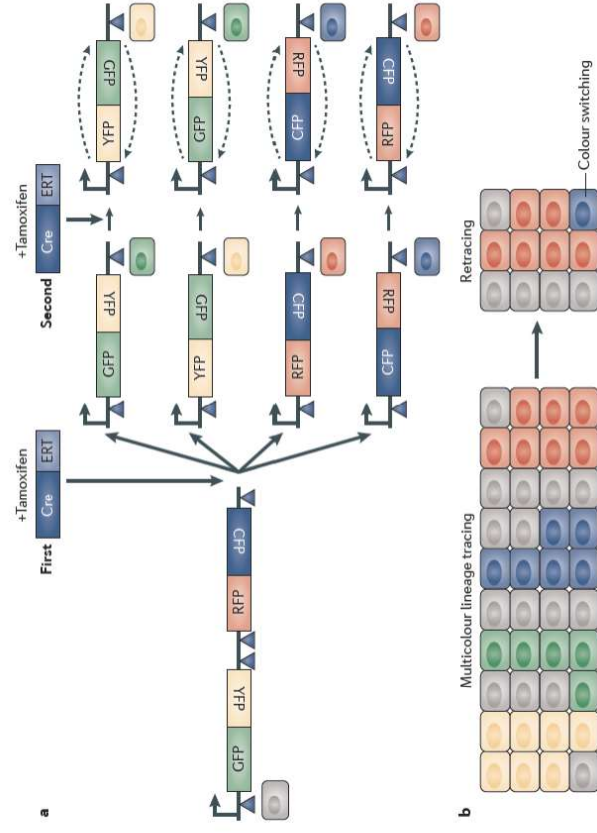
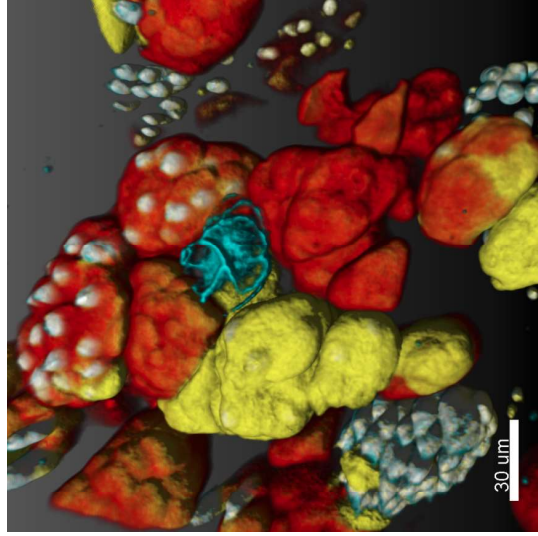
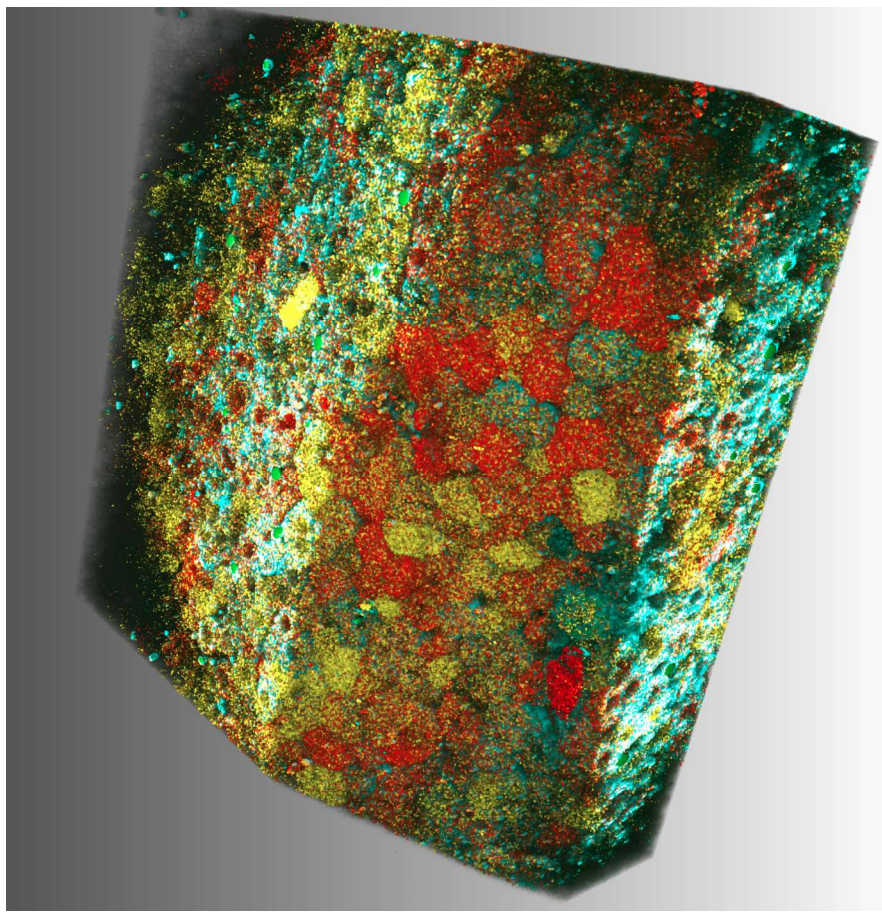
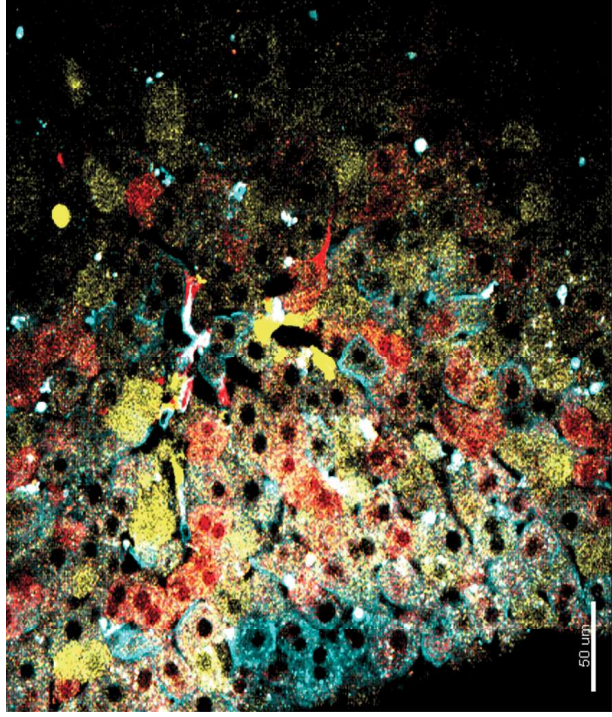
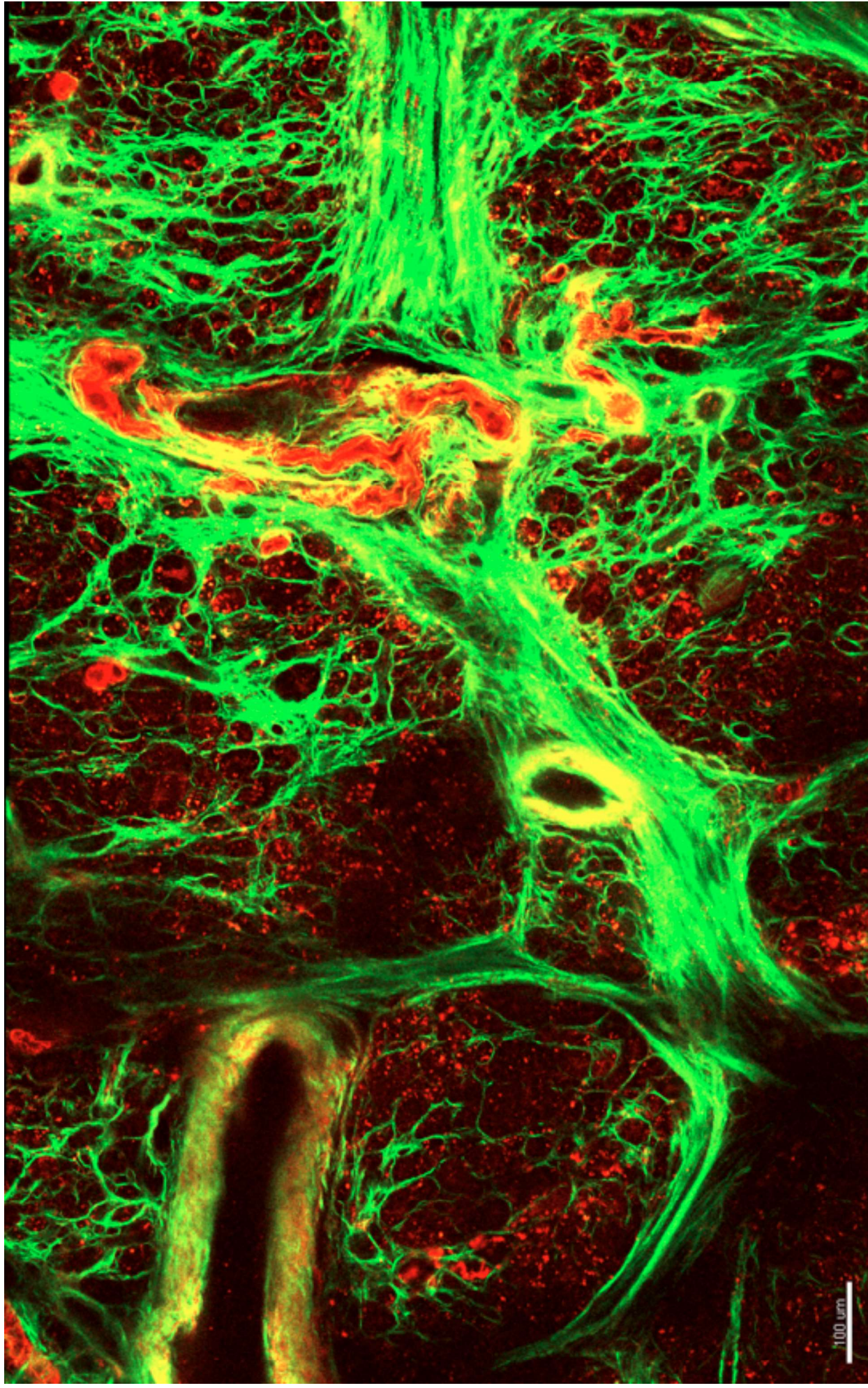


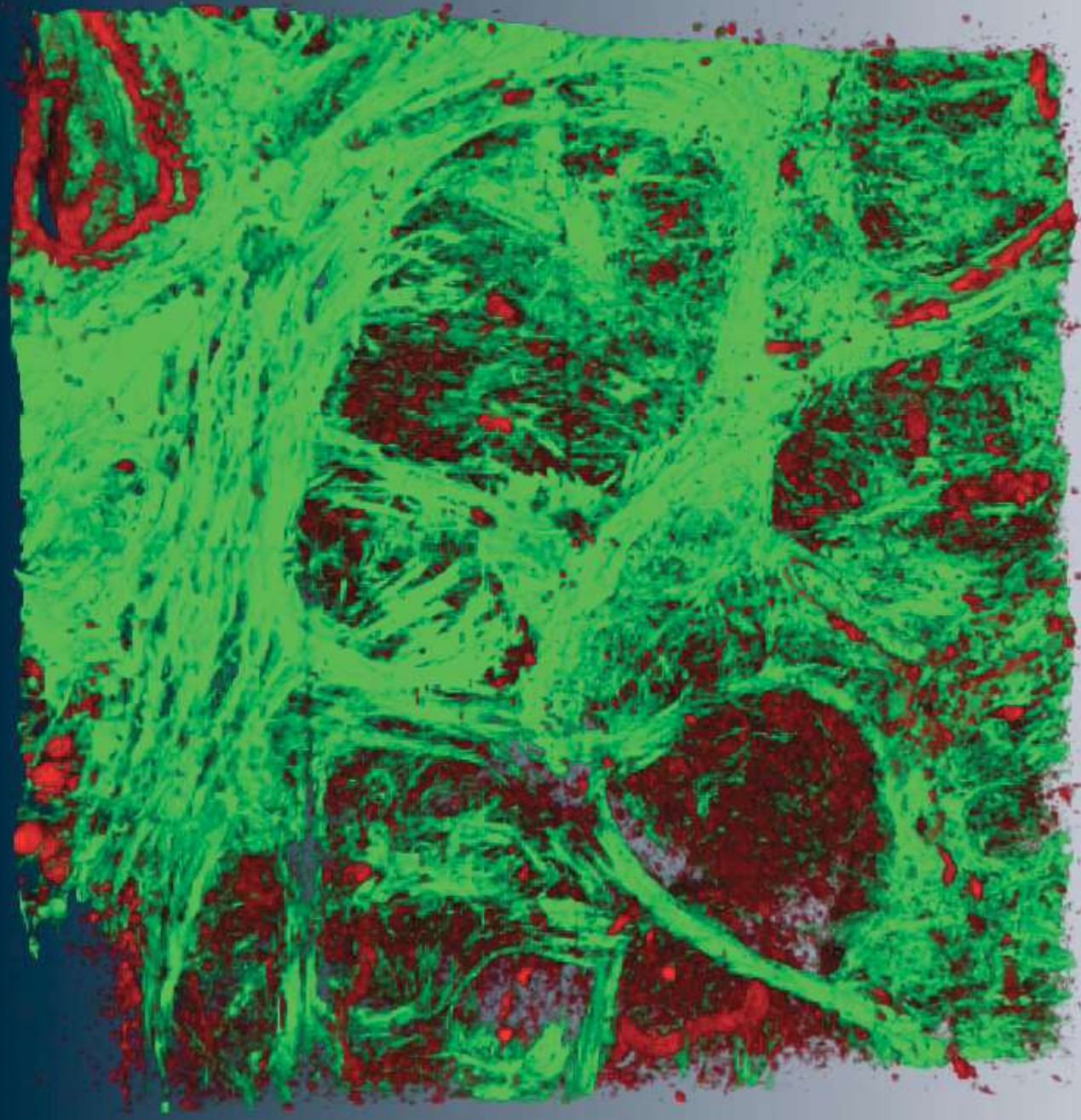
Figure 3 | Intravital lineage tracing using the Confetti construct to study cancer stem cell plasticity. a | The expression of four differentially coloured fluorescent proteins is blocked by a neomycin resistance cassette ('road block') flanked by LoxP sites. The Cre recombinase, upon activation, recombines the construct in a random manner, which results in the excision of the road block and the expression of one of the four fluorescent proteins (cyan fluorescent protein (CFP), green fluorescent protein (GFP), yellow fluorescent protein (YFP) or red fluorescent protein (RFP)). **b** | An illustration of a mammary tumour in which cells are randomly labelled with Confetti labels and grow in a hierarchically organized manner. **c** | A series of intravital images of the same region in a growing mammary carcinoma at the indicated time points after induction of the Confetti label randomizer. In the images, only four Confetti-labelled cells were able to form small clones (white outline, regions I–IV), indicating hierarchical growth. Different clonal growth patterns were observed, indicating plasticity of the lineages. Scale bars represent 50 μ m. Part b is adapted and Part c is reproduced with permission from REF 81, Wiley. Copyright © 2012 AlphaMed Press.

Multi-Color Imaging

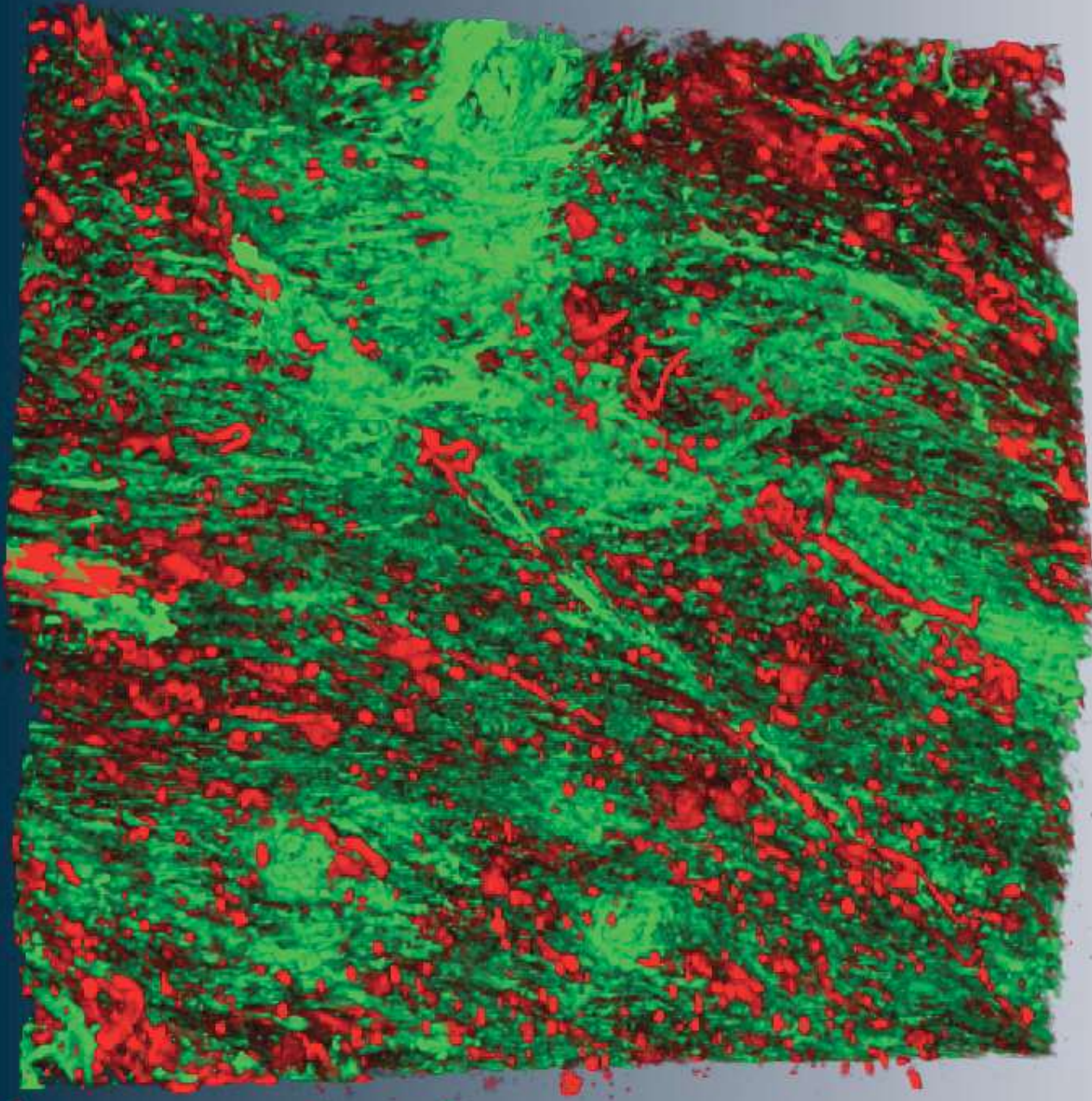


Human Pancreas



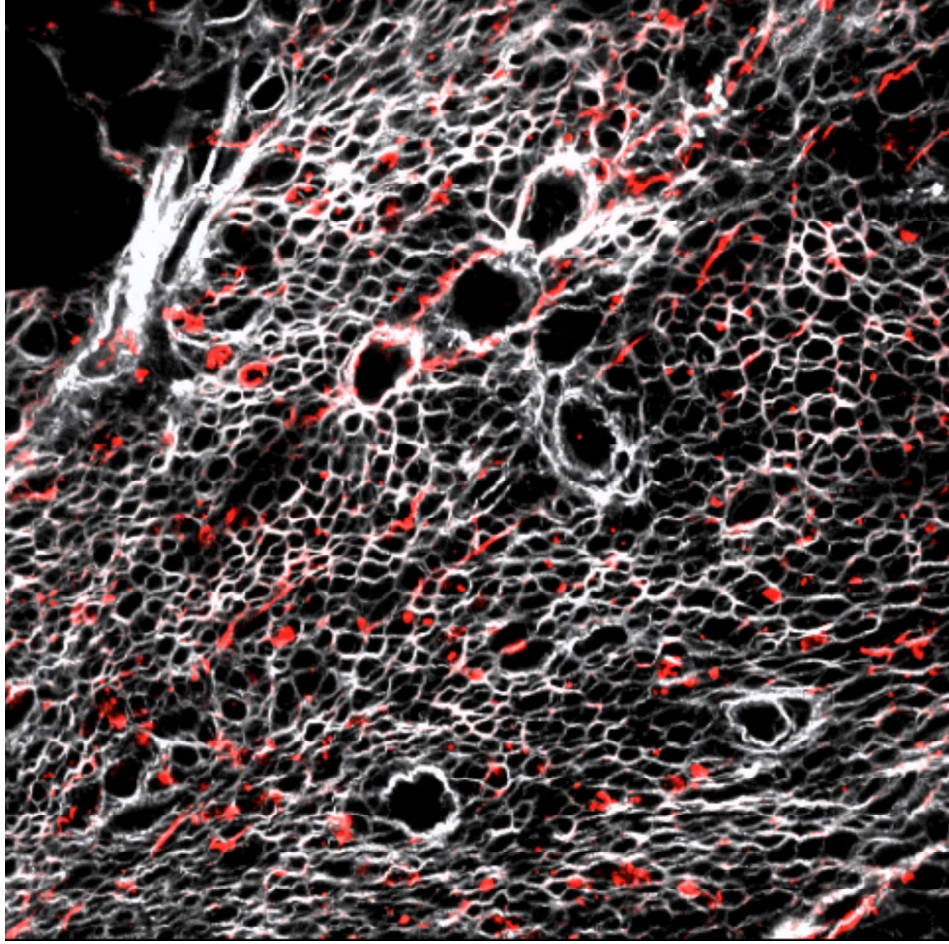


Normal



Tumor

Human Tumor Sample

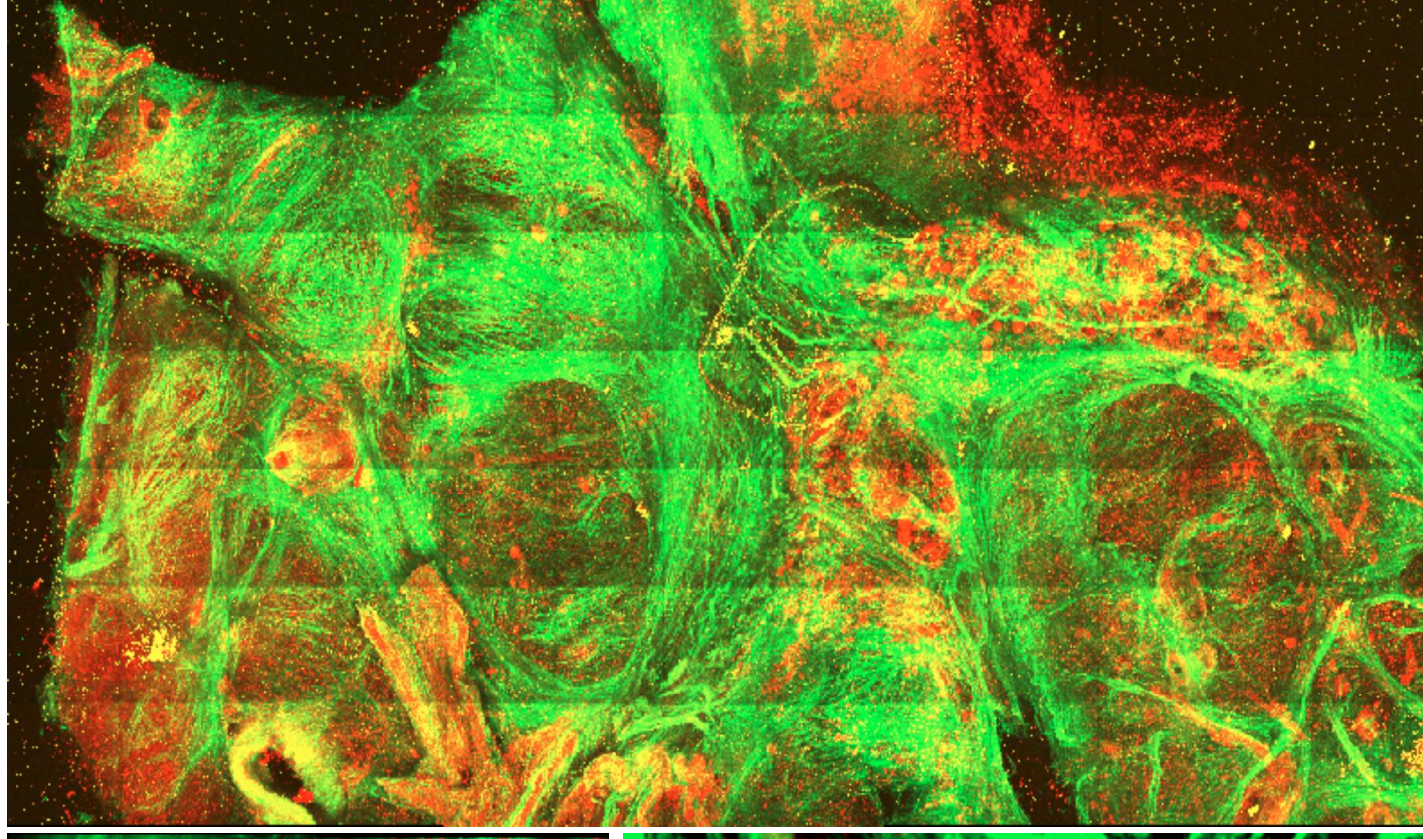
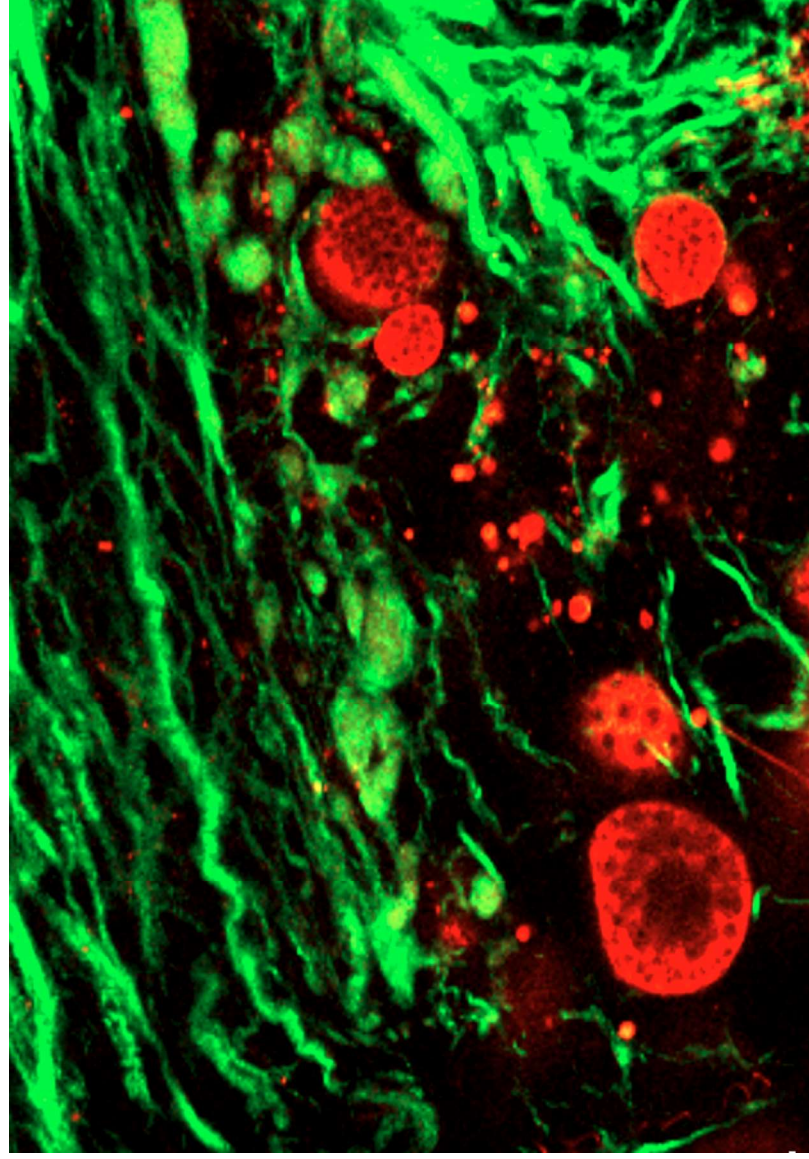
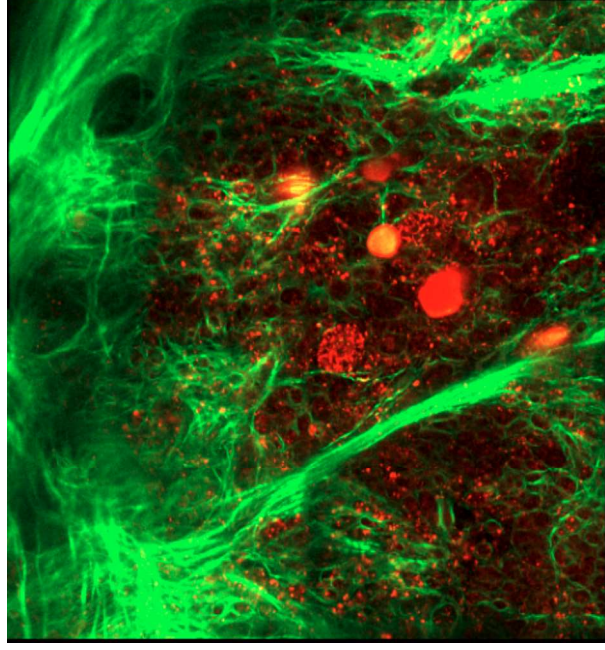


Human 204 normal

Laser wavelength : 900nm

Red: auto fluorescent

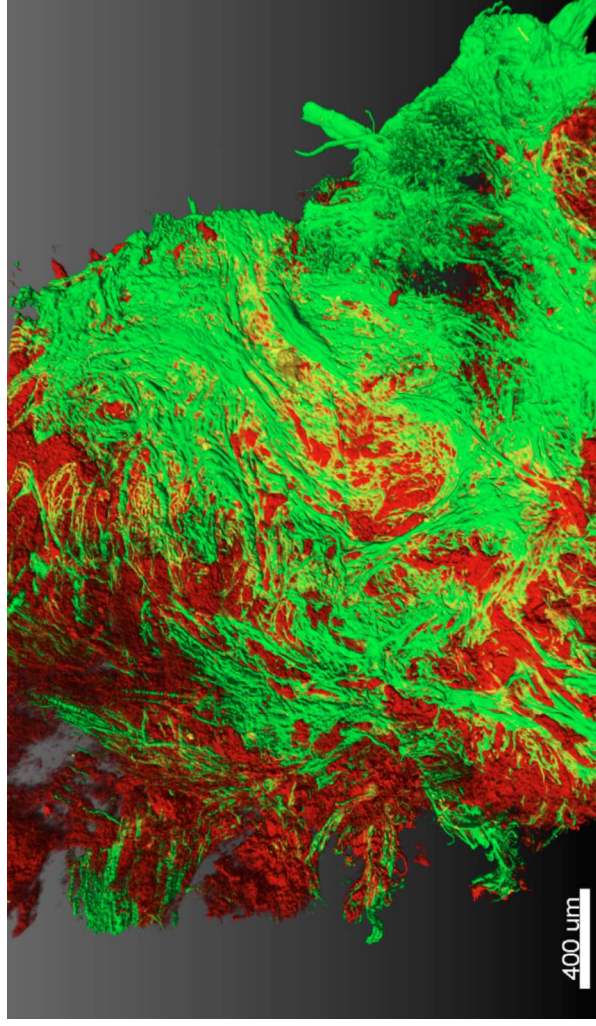
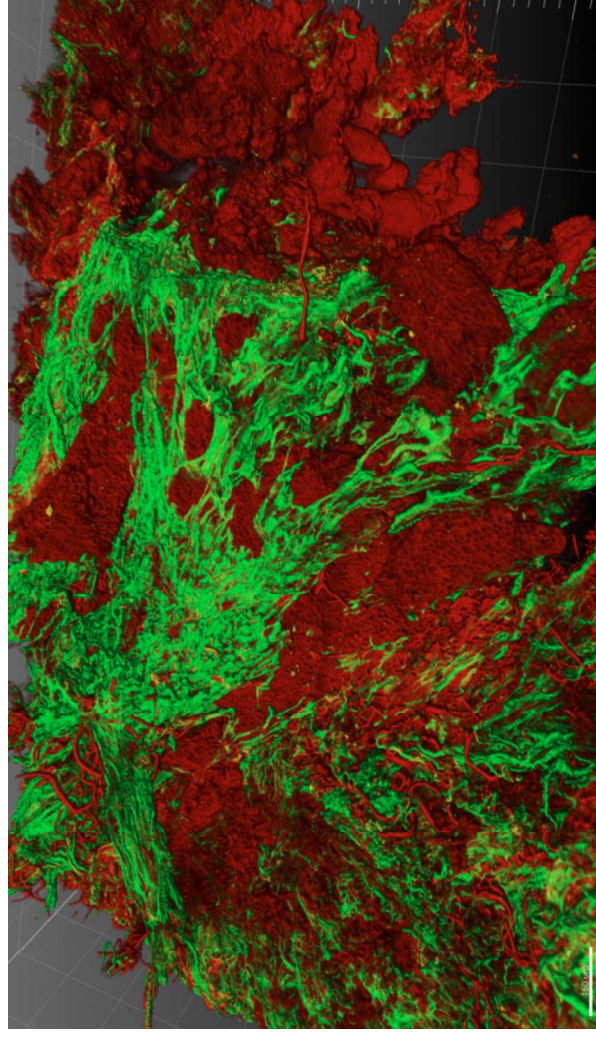
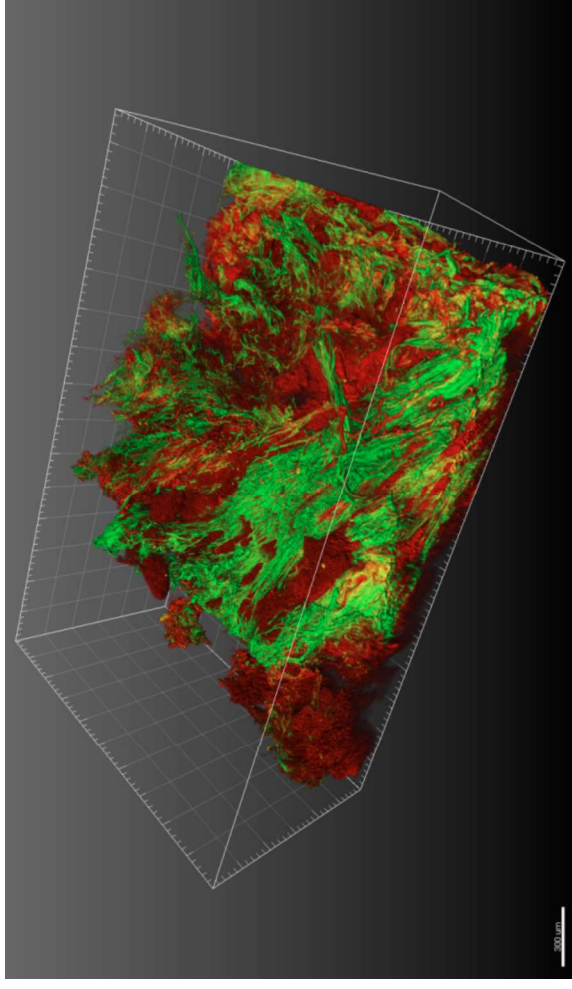
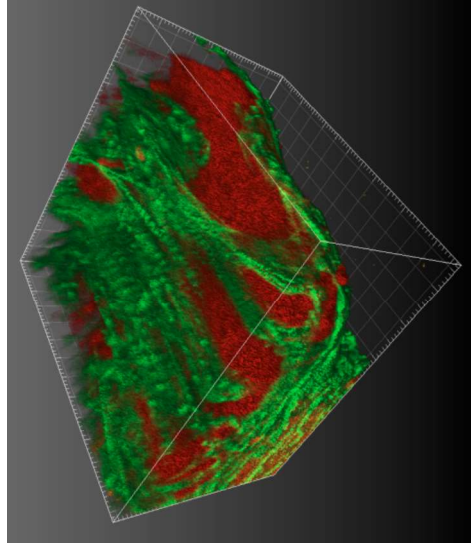
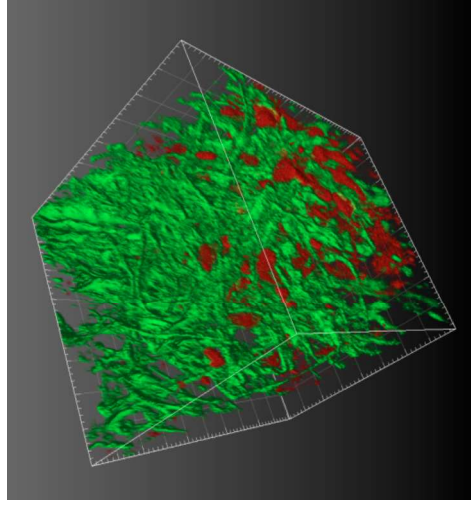
Green : SHG

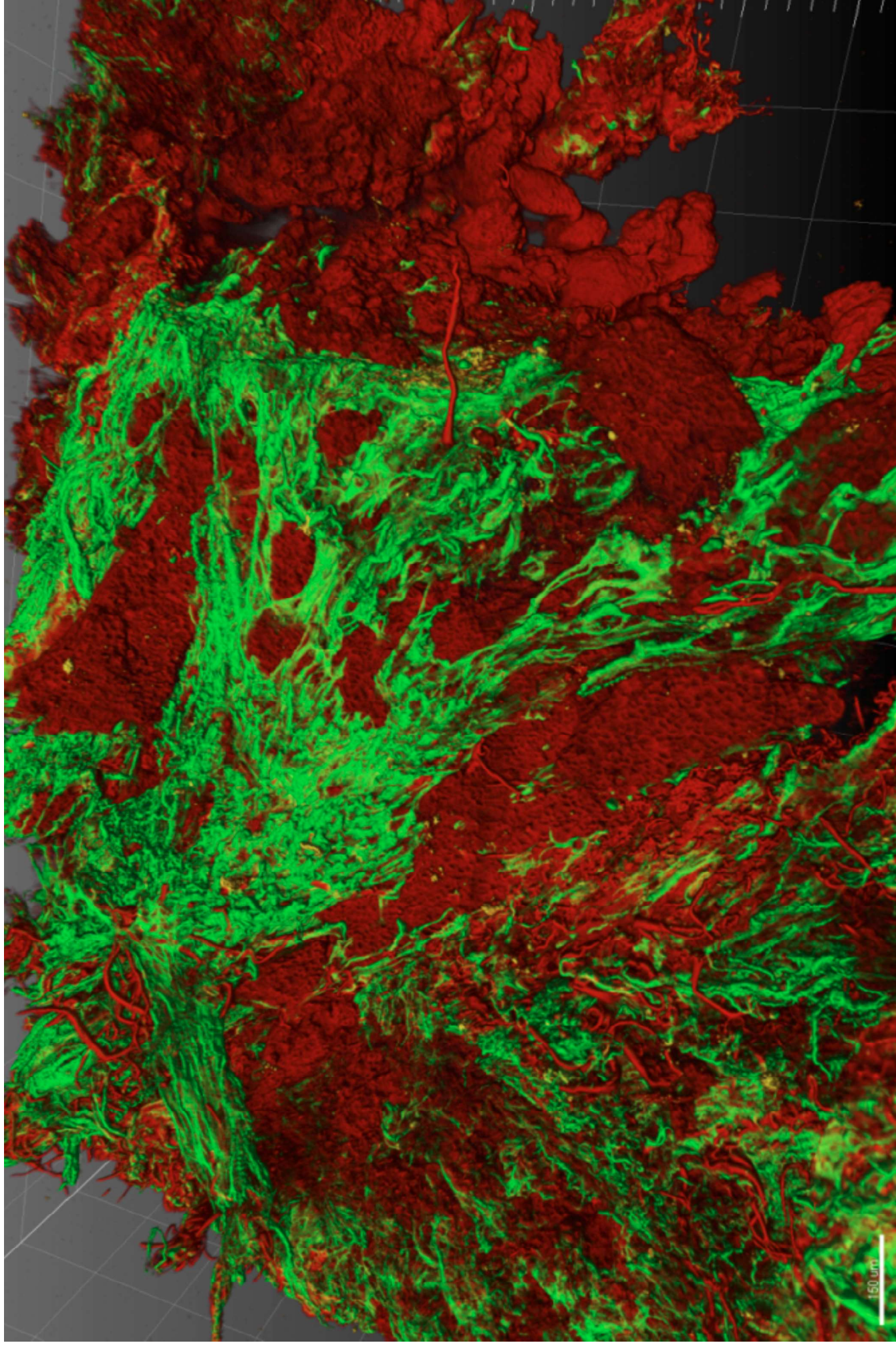


Human 205 tumor
Laser wavelength : 900nm

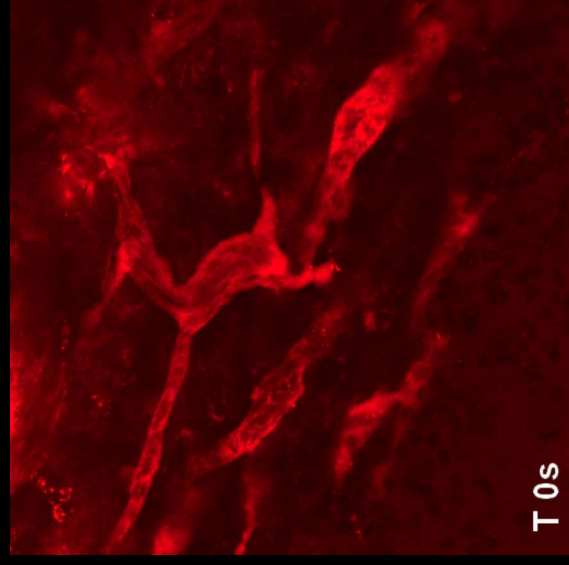
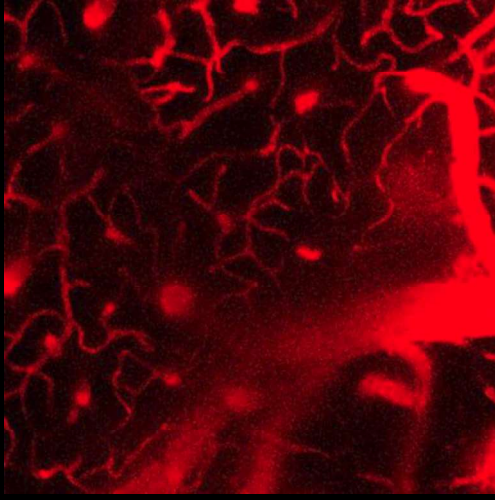
Red: auto fluorescent

Green : SHG

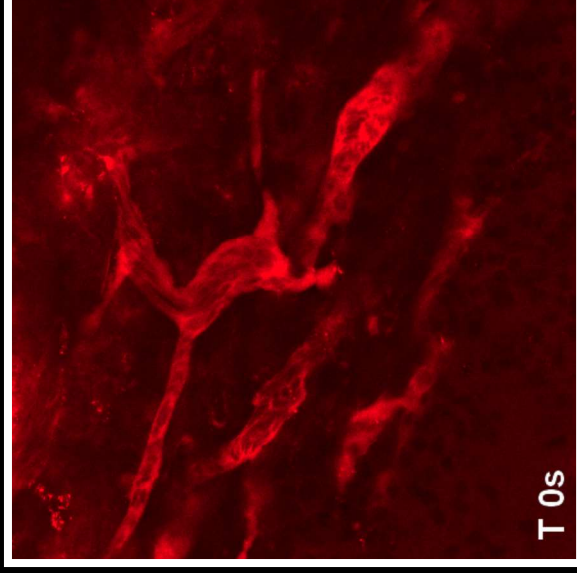
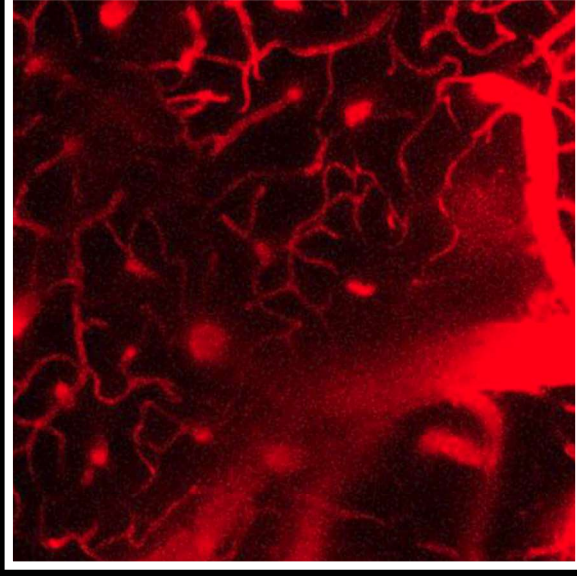
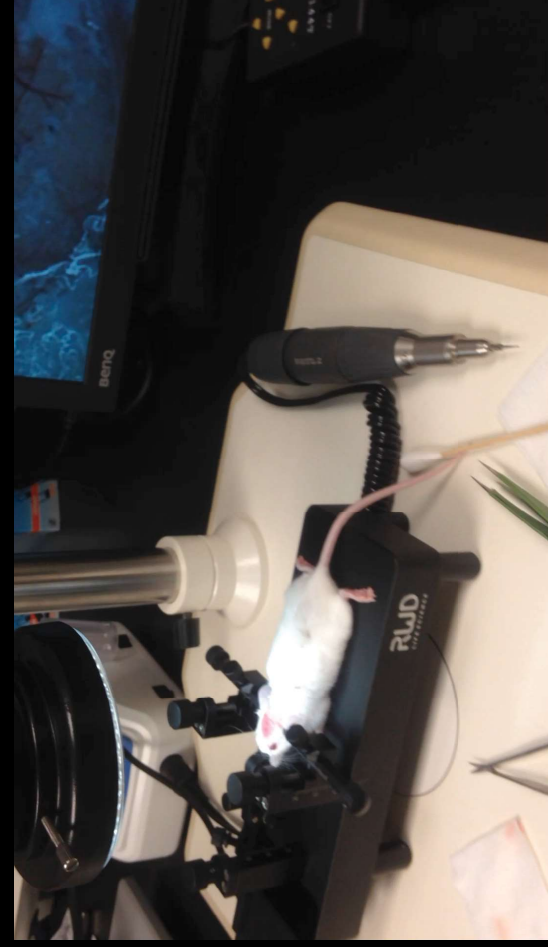




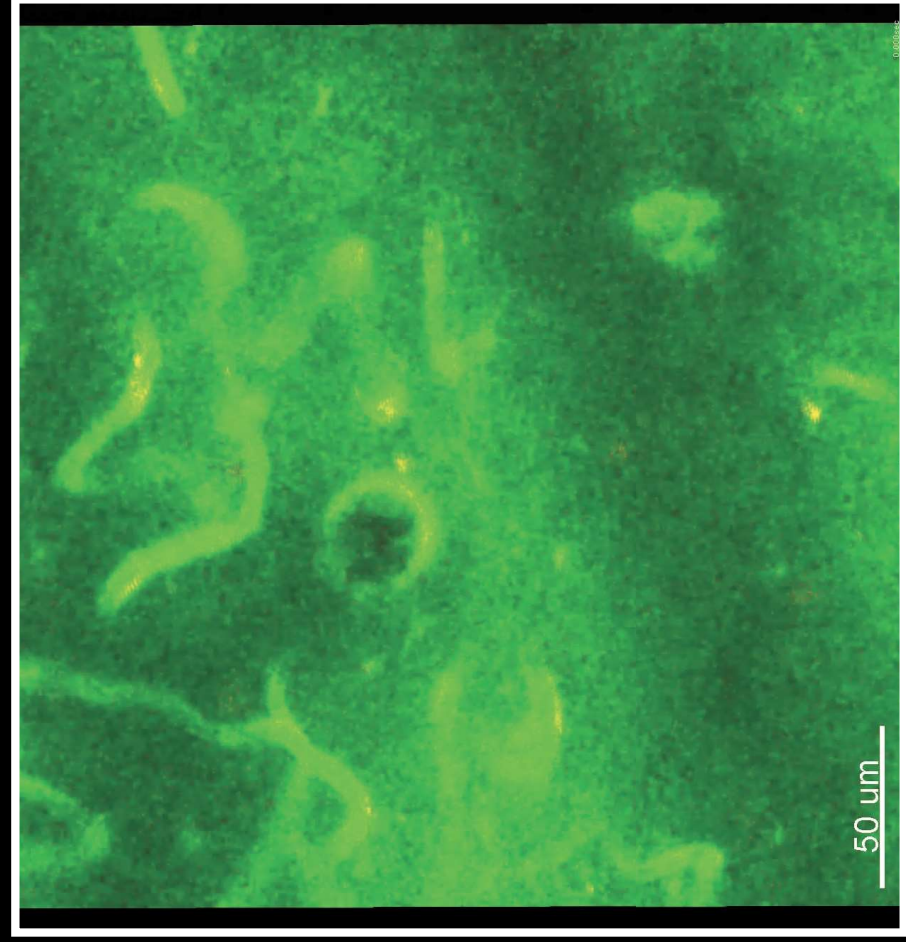
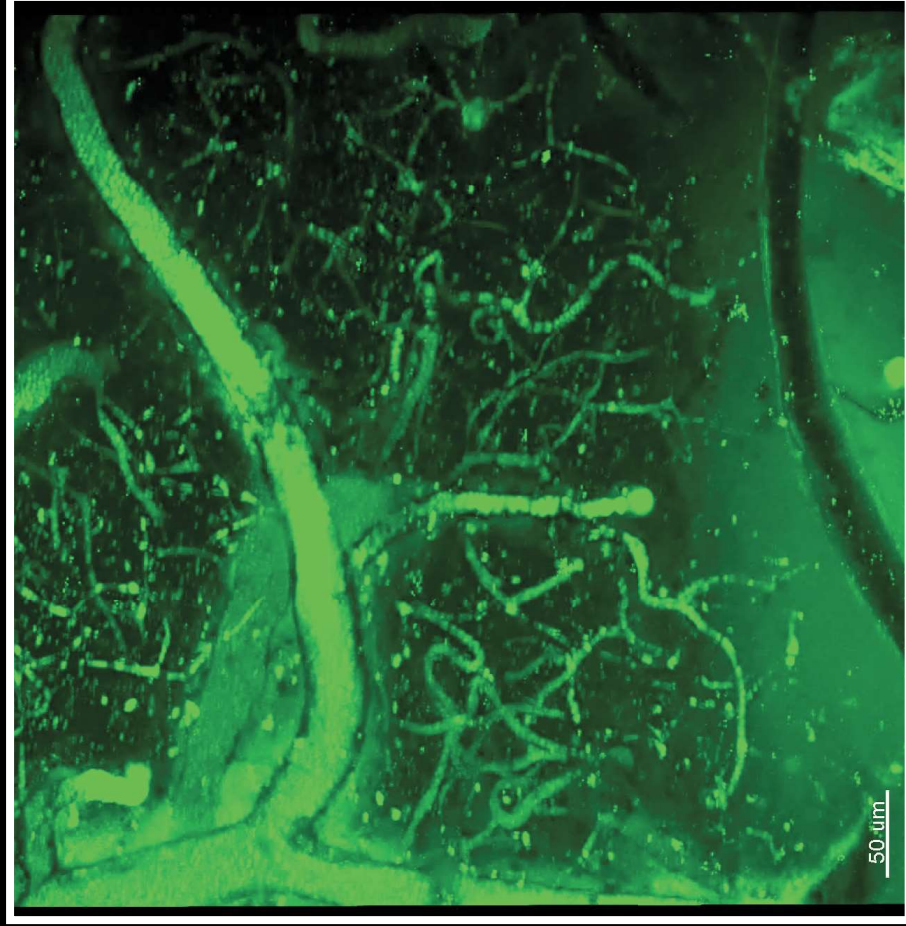
Intravital Imaging



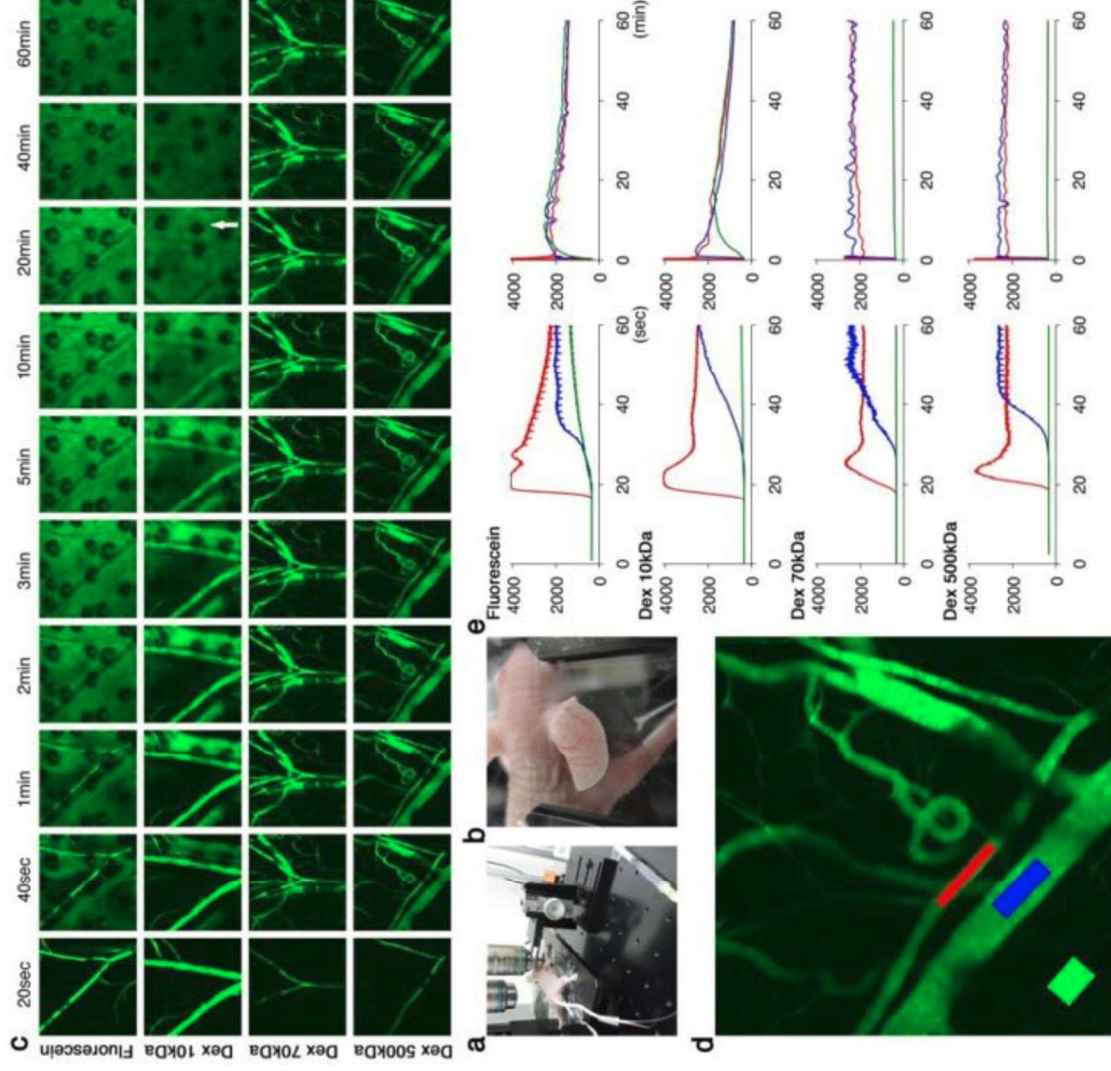
Imaging Mouse Brain



Imaging Mouse Brain

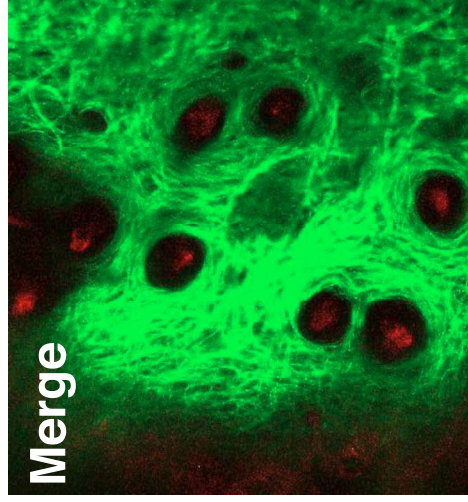
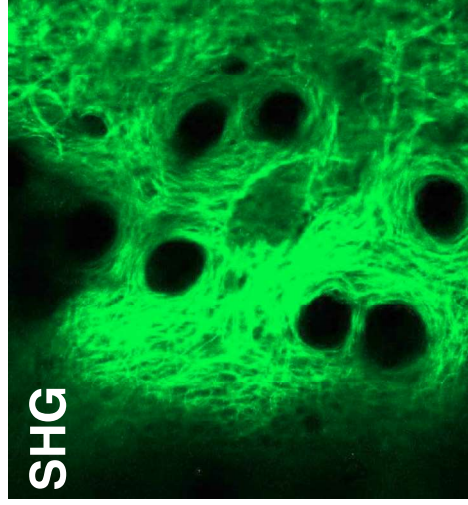
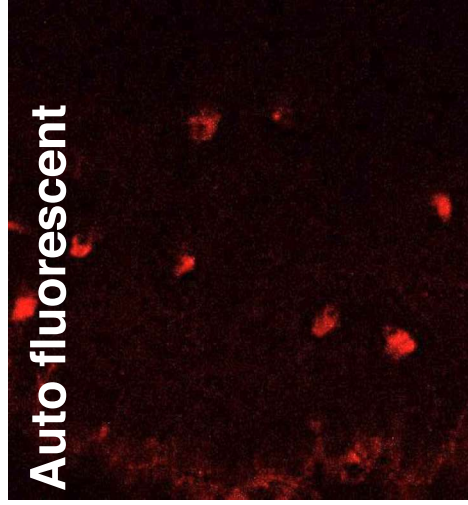


Mouse Earlobe Imaging

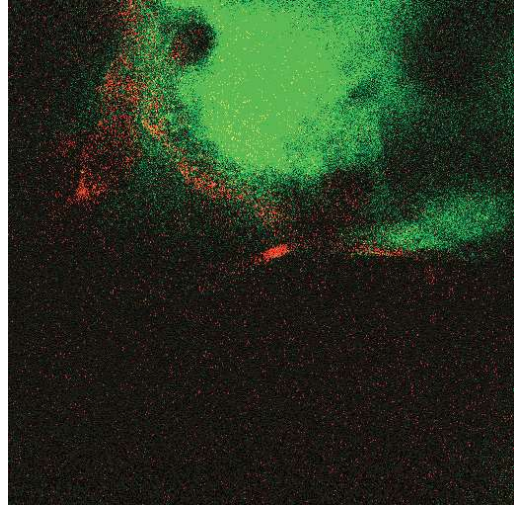
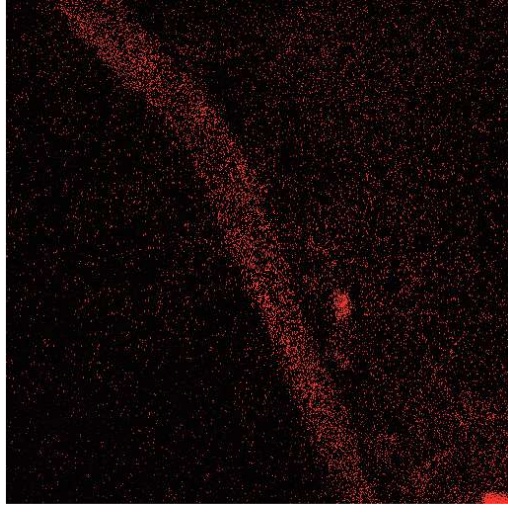
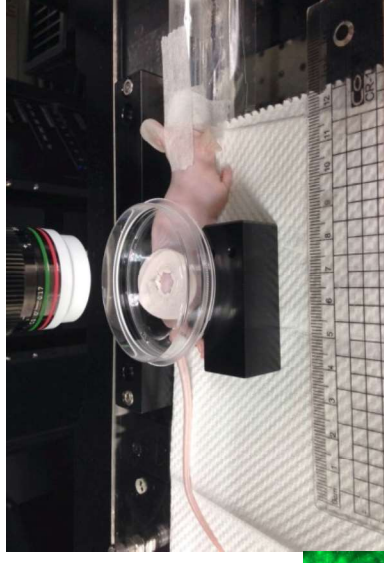
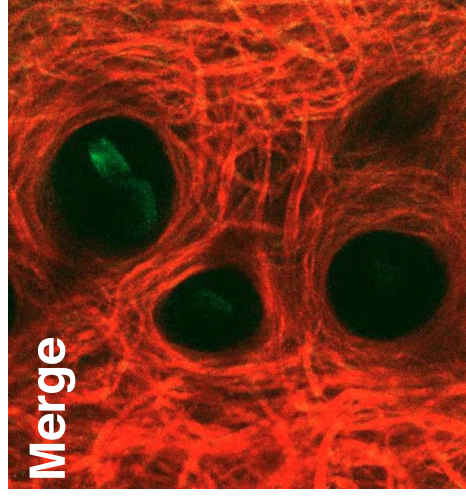
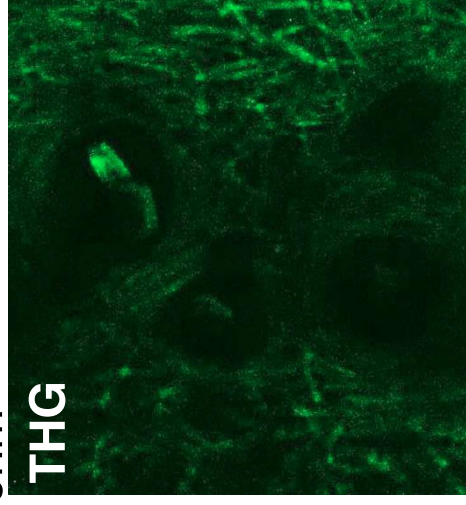
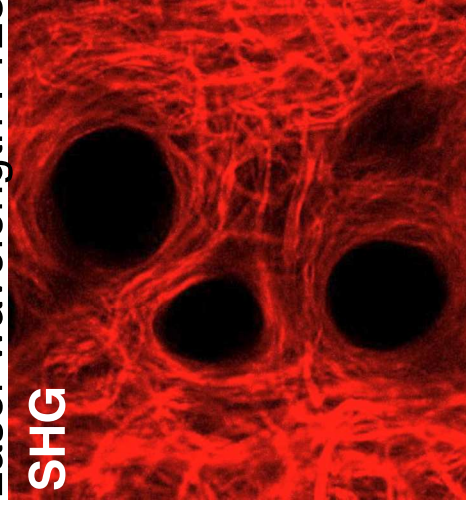


Living Mouse Imaging by Multi-Photon Microscope

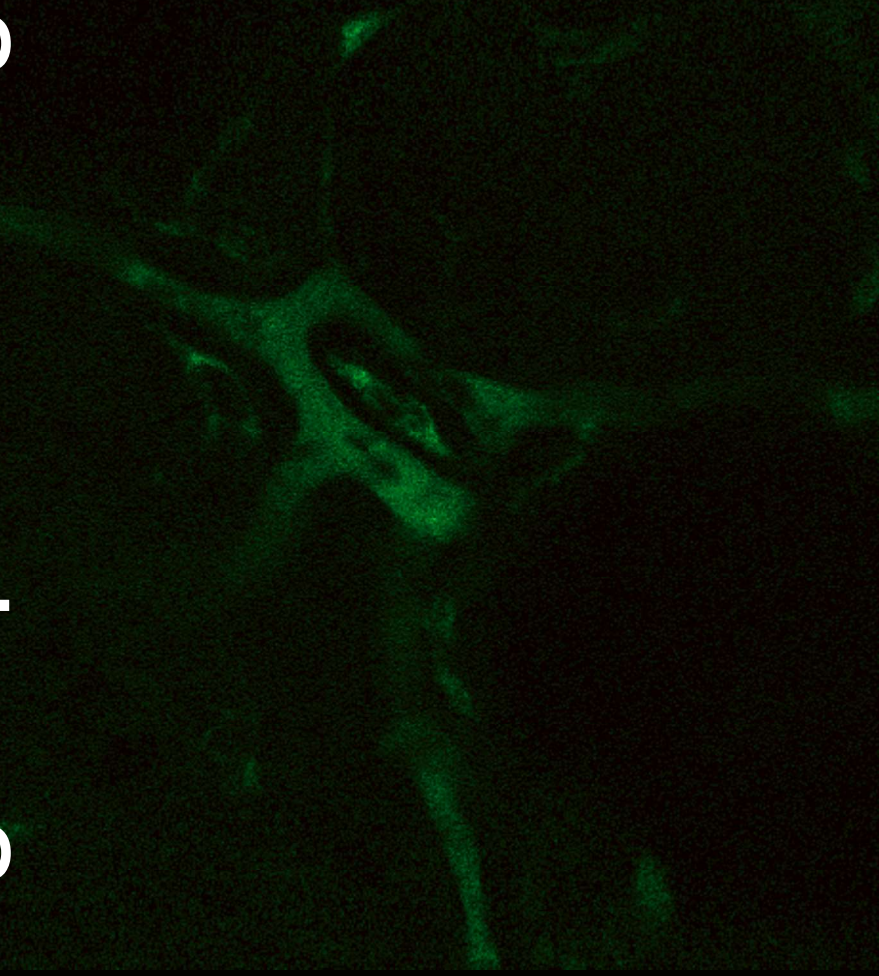
Laser wavelength : 900nm



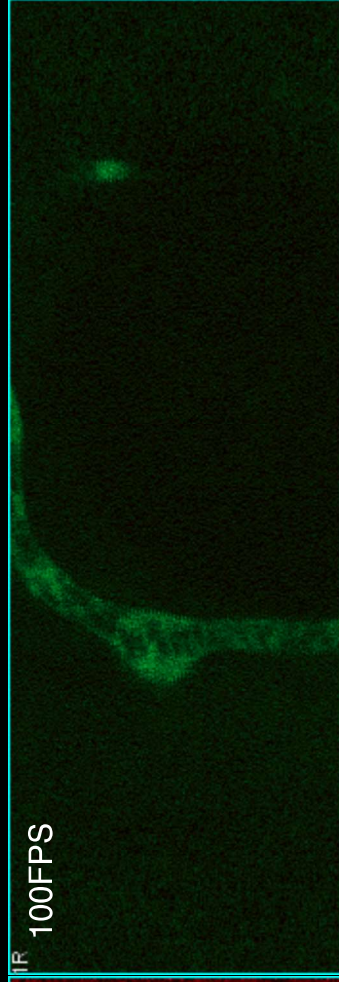
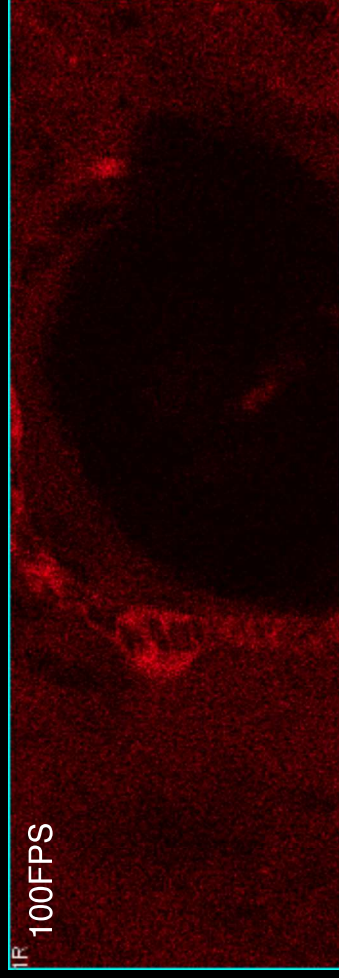
Laser wavelength : 1280nm



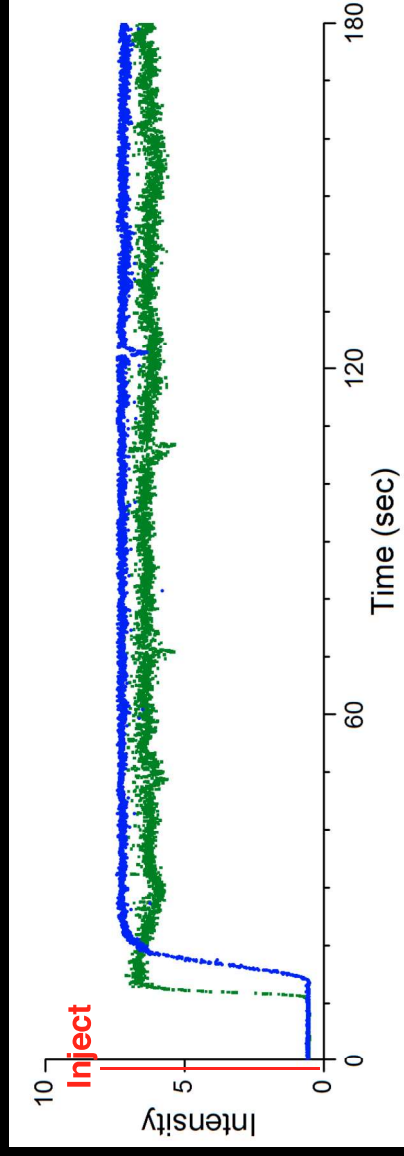
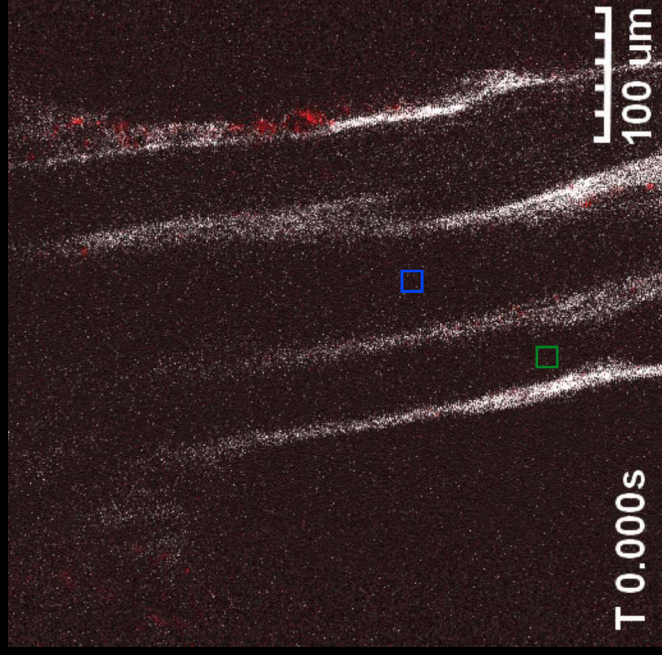
High Speed Imaging



30FPS



Real Time Drug Distribution



Blood Cells in Mice

Table 17-1. MEANS AND STANDARD ERRORS OF MEANS FOR ERYTHROCYTE CHARACTERISTICS OF 18 INBRED STRAINS. ^a (After Russell et al., 195L , with permission of the authors and of the Society for Experimental Biology and Medicine.)										
Strain	RBC ^b		Hematocrit		Mean cell		Hemoglobin,		Reticulocytes, per cent (mean)	
	(mean and S.E.)		percentage (mean and S.E.)		volume μ ³ (mean and S.E.)		g/100 cc blood (mean and S.E.)	g/cc cells (mean)		
A/J	9.42	+/- 0.28	42.5	+/- 0.4	45.1	+/- 1.4	12.9	+/- 0.2	0.30	3.5
A/HeJ	9.48	+/- 0.18	42.5	+/- 0.5	44.8	+/- 1.0	12.7	+/- 0.2	0.30	2.9
AKR/J	9.38	+/- 0.24	45.6	+/- 1.0	48.5	+/- 1.6	13.9	+/- 0.2	0.30	2.3
BALB/cAnJ	10.14	+/- 0.15	46.5	+/- 0.8	45.9	+/- 1.1	14.5	+/- 0.2	0.31	3.3
BALB/cJ	10.51	+/- 0.16	48.0	+/- 0.7	45.7	+/- 1.0	15.0	+/- 0.2	0.31	2.9
CBA/J	10.04	+/- 0.27	45.0	+/- 1.3	44.8	+/- 1.8	13.5	+/- 0.2	0.31	2.6
C3H/J	8.79	+/- 0.24	39.5	+/- 0.7	44.9	+/- 1.4	12.2	+/- 0.4	0.31	2.8
C3H/SeJ	9.63	+/- 0.26	43.0	+/- 1.0	44.7	+/- 1.6	13.2	+/- 0.3	0.30	2.2
C57BL/6pJ	9.70	+/- 0.15	43.4	+/- 0.8	44.7	+/- 1.0	13.0	+/- 0.3	0.30	2.5
C57BL/6J	9.66	+/- 0.09	44.0	+/- 0.4	45.5	+/- 0.6	13.3	+/- 0.2	0.30	2.6
C57BL/cdJ	10.54	+/- 0.17	50.0	+/- 0.5	47.4	+/- 0.9	14.6	+/- 0.2	0.29	2.4
C57BL/HeJ	9.82	+/- 0.20	50.6	+/- 0.5	51.5	+/- 1.1	14.9	+/- 0.2	0.29	2.6
DBA/1J	10.52	+/- 0.27	43.8	+/- 0.6	41.6	+/- 1.2	13.2	+/- 0.2	0.30	1.5
DBA/WaJ	9.93	+/- 0.27	43.0	+/- 0.6	43.3	+/- 1.1	12.5	+/- 0.2	0.29	2.6
DBA/2J	10.30	+/- 0.25	42.6	+/- 0.5	41.4	+/- 1.1	12.7	+/- 0.1	0.30	3.1
I/J	10.27	+/- 0.27	46.8	+/- 0.7	45.6	+/- 1.5	13.5	+/- 0.1	0.29	2.4
RIII/J	9.63	+/- 0.25	44.5	+/- 0.6	46.2	+/- 1.3	13.7	+/- 0.2	0.31	2.8
ST/J	9.88	+/- 0.19	44.1	+/- 1.1	44.6	+/- 1.4	12.1	+/- 0.2	0.31	2.1

^aAll means are based on 10 animals, except that those marked * are based on 20 animals.

^bRed blood count in millions per cubic millimeter.

^aAll means are based on 10 animals, except that those marked * are based on 20 animals.

^bRed blood count in millions per cubic millimeter.

Red blood cell: 10^10/ml
Platelet: 10^9/ml

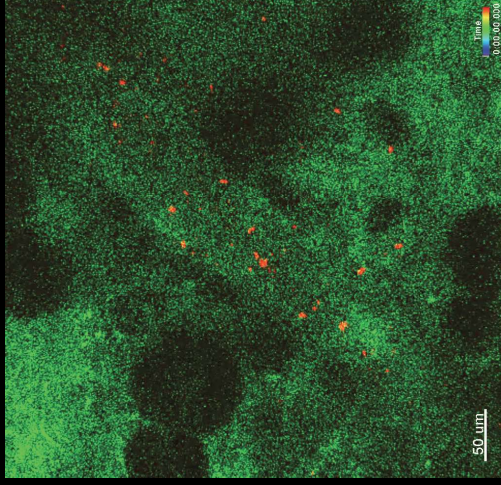
Table 17-2. MEANS AND STANDARD ERRORS OF MEANS FOR LEUKOCYTE CHARACTERISTICS OF 18 INBRED STRAINS. ALL MEANS ARE BASED ON 10 ANIMALS. (After Russell et al., 195L, with permission of the authors and of the Society for Experimental Biology and Medicine.)									
Strain	WBC ^a		Percent granulocytes		females		males		S.E./mean
	Mean and S.E.		Mean and S.E.		Mean and S.E.		Mean and S.E.		
A/J	8.71	+/- 1.37	10.7	+/- 1.1	0.10		14.5	+/- 5.0	0.35
A/HeJ	6.14	+/- 0.68	19.8	+/- 2.3	0.12		37.2	+/- 7.5	0.20
AKR/J	6.80	+/- 0.65	21.6	+/- 4.5	0.21		23.8	+/- 5.2	0.22
BALB/cAnJ	8.70	+/- 1.03	15.4	+/- 2.3	0.15		29.6	+/- 5.0	0.17
BALB/cJ	8.54	+/- 1.04	14.9	+/- 2.1	0.14		15.3	+/- 3.8	0.25
CBA/J	6.44	+/- 0.33	26.8	+/- 4.5	0.17		24.2	+/- 2.5	0.10
C3H/J	7.34	+/- 0.80	21.4	+/- 4.3	0.20		27.5	+/- 5.1	0.19
C3H/SeJ	5.07	+/- 0.30	19.0	+/- 3.9	0.21		22.0	+/- 2.2	0.10
C57BL/6pJ	10.61	+/- 0.64	12.1	+/- 3.8	0.31		15.3	+/- 5.1	0.33
C57BL/6J	11.43	+/- 1.04	8.2	+/- 1.2	0.15		10.4	+/- 3.5	0.34
C57BL/cdJ	9.43	+/- 0.78	10.6	+/- 1.1	0.10		11.0	+/- 2.4	0.22
C57L/HeJ	10.82	+/- 1.13	8.5	+/- 1.3	0.15		6.7	+/- 1.4	0.21
DBA/1J	8.60	+/- 1.60	16.5	+/- 1.5	0.09		20.4	+/- 4.2	0.21
DBA/WaJ	8.32	+/- 0.96	19.6	+/- 0.9	0.05		17.2	+/- 1.5	0.09
DBA/2J	9.28	+/- 0.29	16.7	+/- 2.3	0.14		18.1	+/- 1.3	0.07
I/J	11.62	+/- 1.41	13.2	+/- 3.2	0.24		18.1	+/- 2.9	0.16
RIII/J	5.87	+/- 0.64	26.2	+/- 2.8	0.11		18.6	+/- 3.7	0.20
ST/J	7.74	+/- 1.23	14.9	+/- 2.4	0.16		19.4	+/- 3.7	0.19

^aWhite blood count in thousands per cubic millimeter.

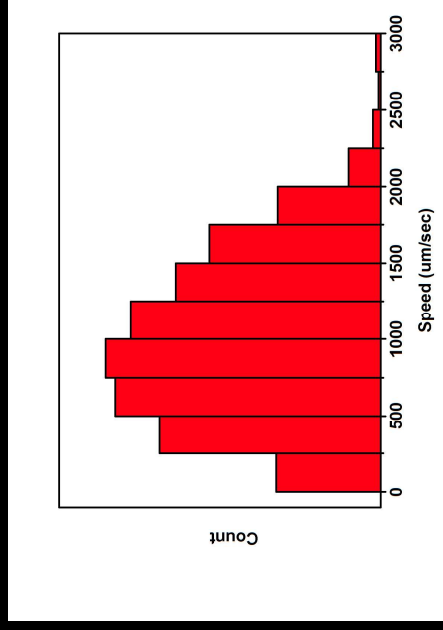
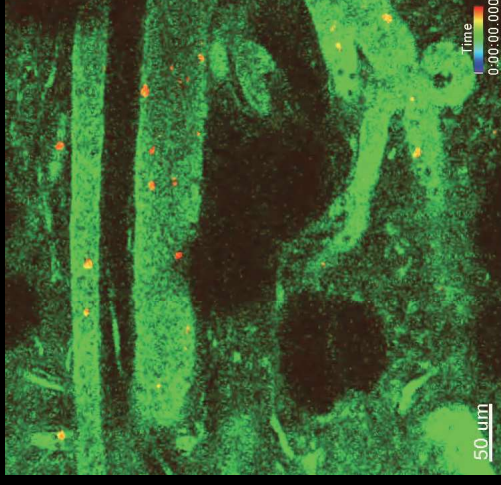
White blood cell: 10^7/ml
25 g mouse = 1.5 ml blood

Red Blood Cells in Mice

10^9 RBC

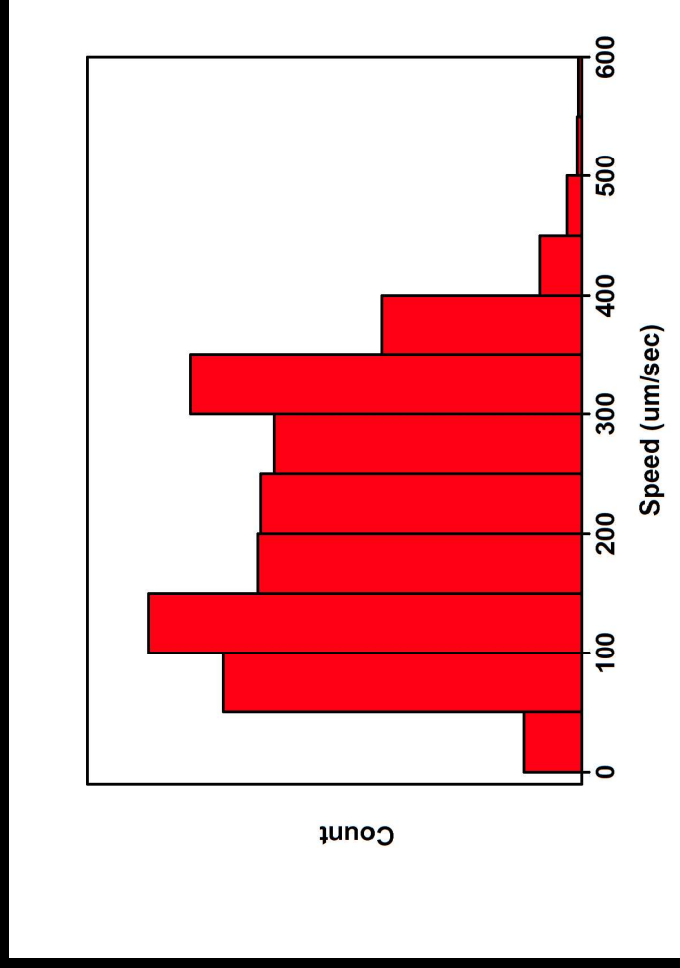
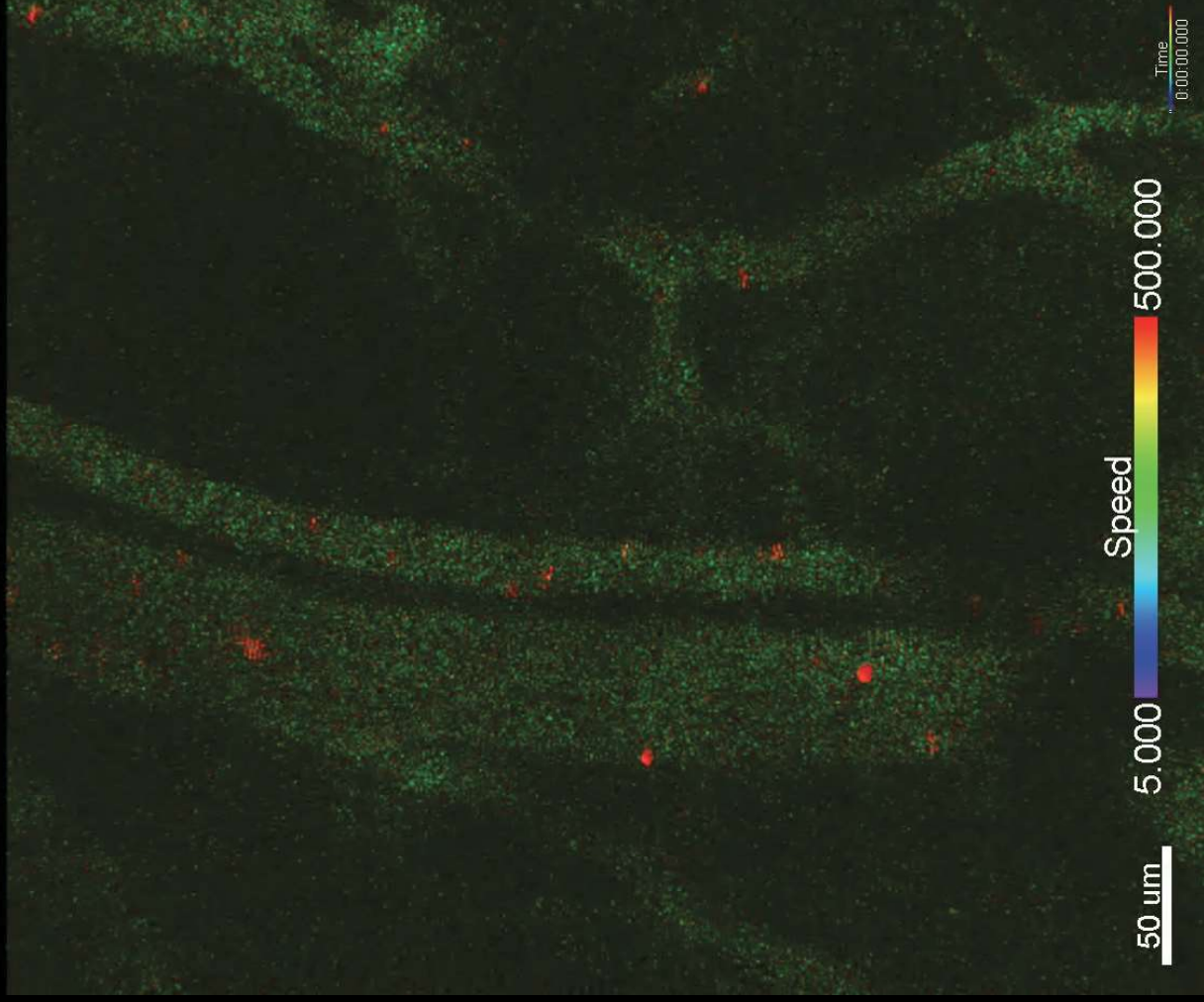


10^8 RBC

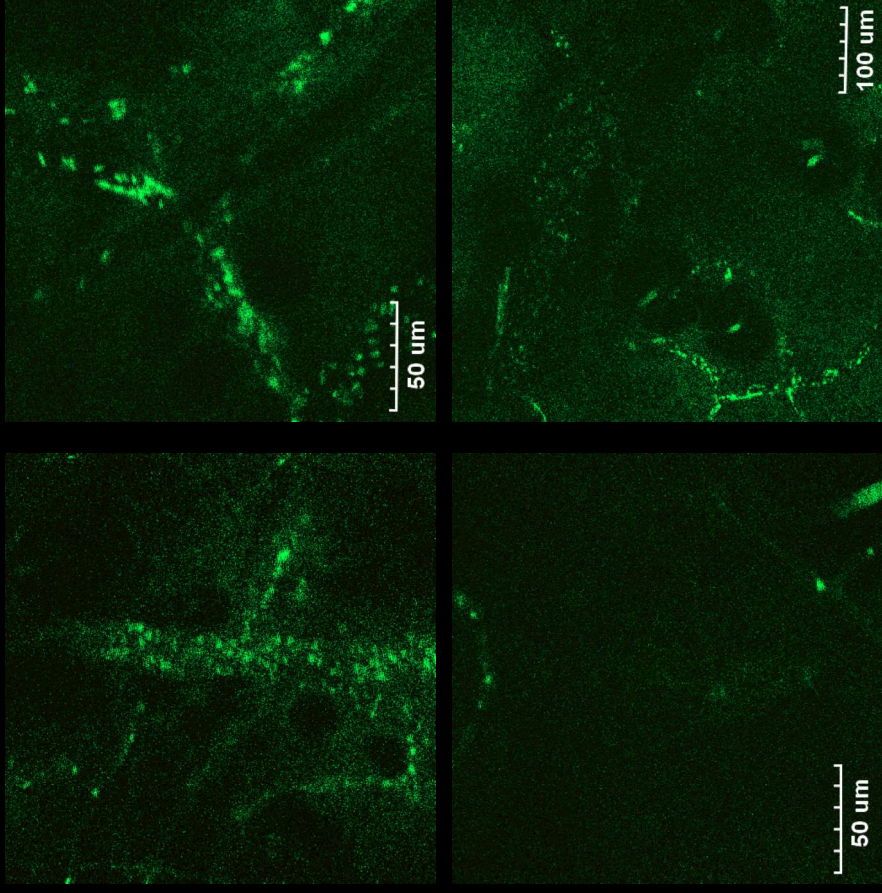


~ 1 mm/s

Nanoparticle Labeled Red Blood Cells

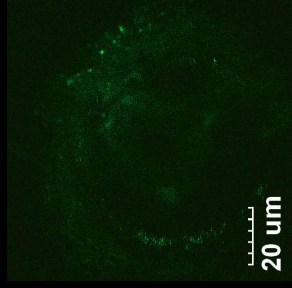
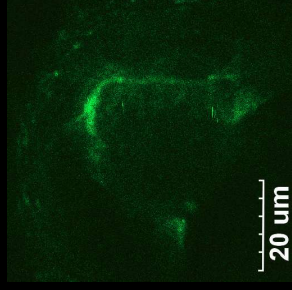
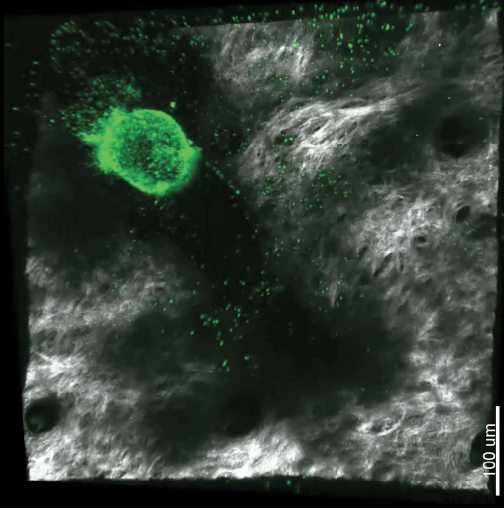
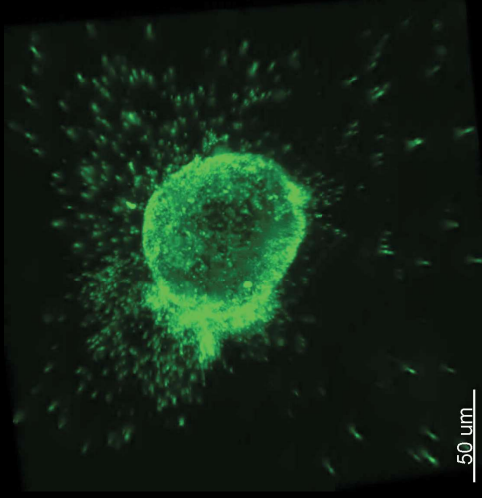


Imaging Platelets

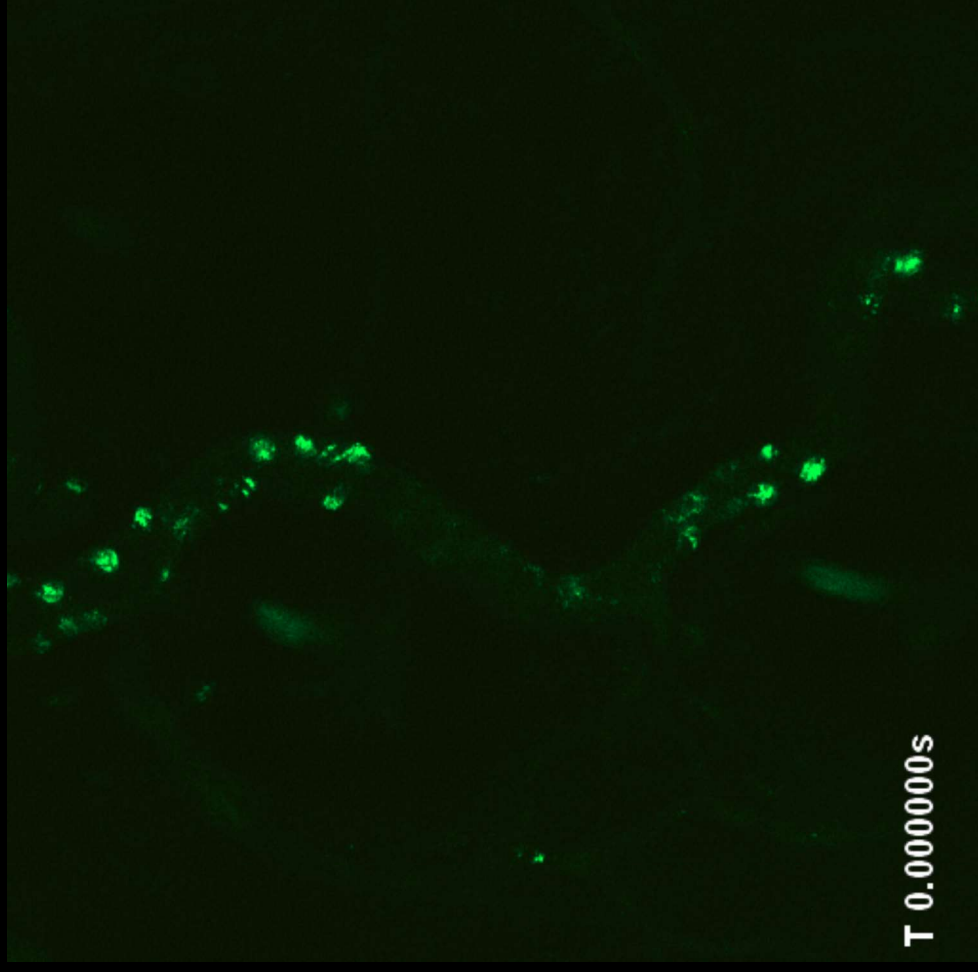


1. inject CD41-biotin antibody
2. after 5 mins, inject Streptavidin QDots
3. Healthy mice ear vasculature

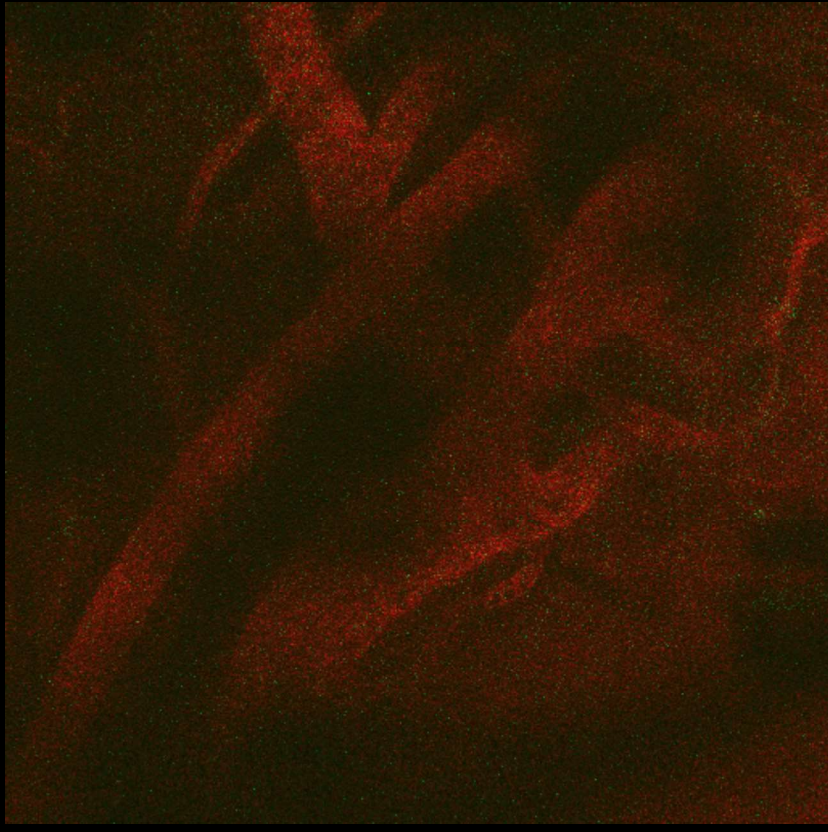
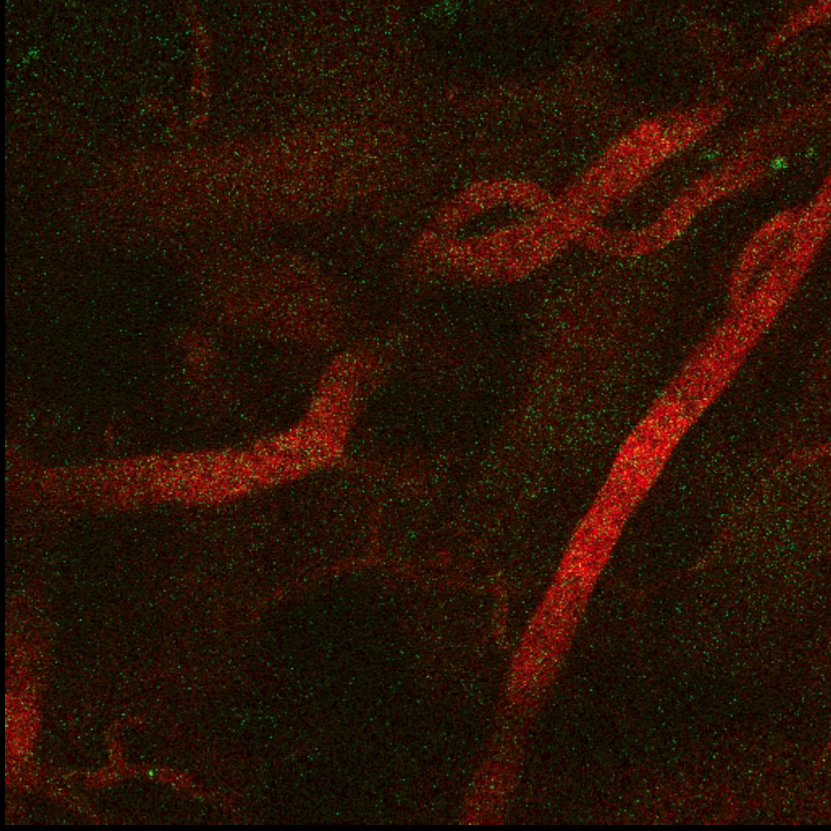
Laser Injury



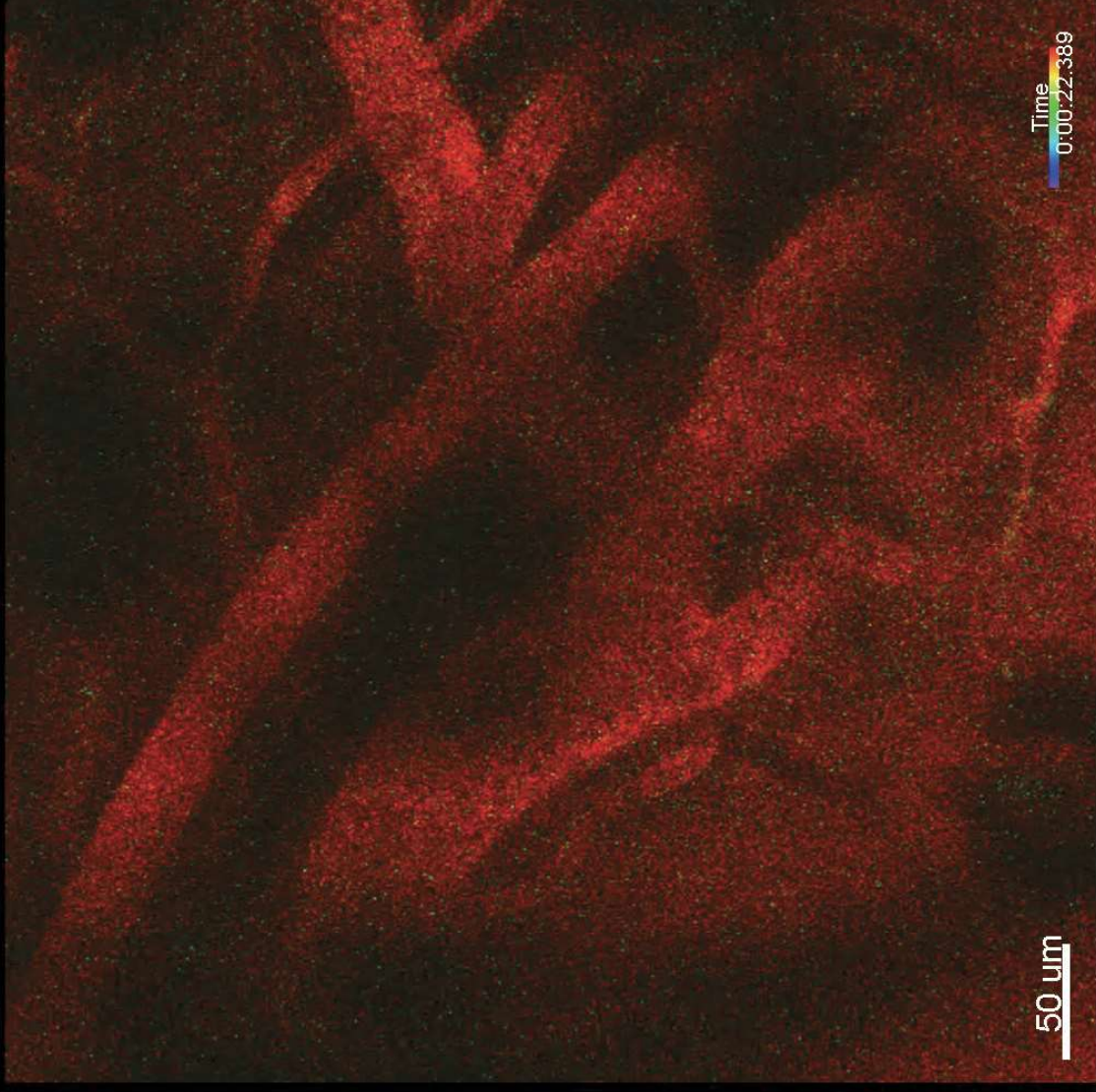
Neutrophil



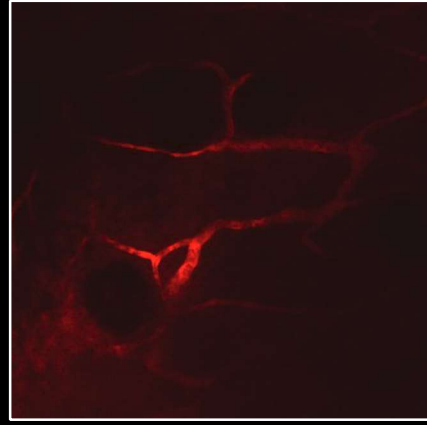
Neutrophil



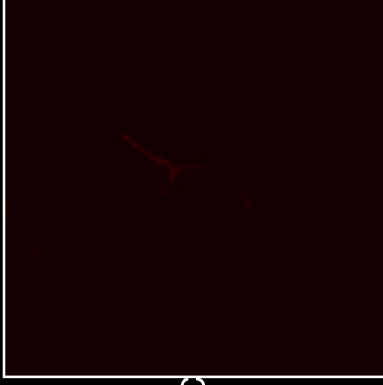
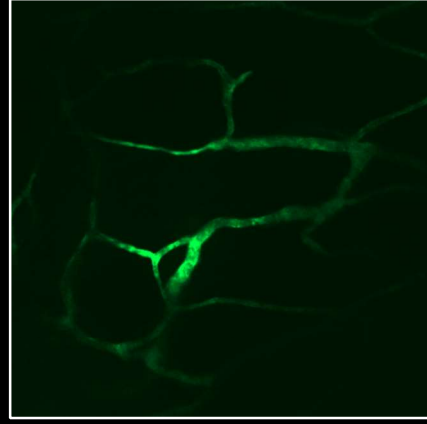
Tracking CD19 Positive Cells (B cell marker)



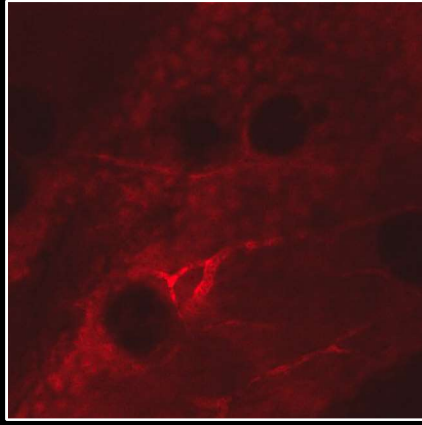
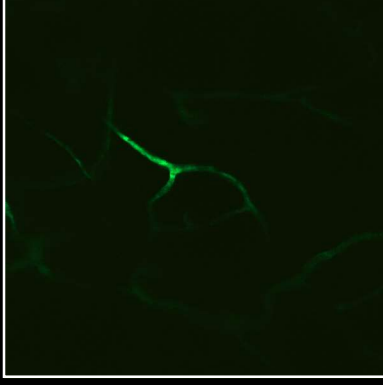
Rhodamine-PEG injected for 4hrs



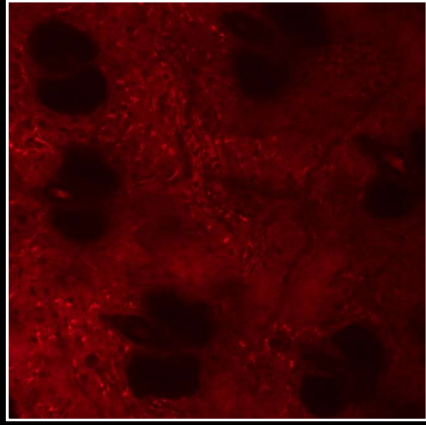
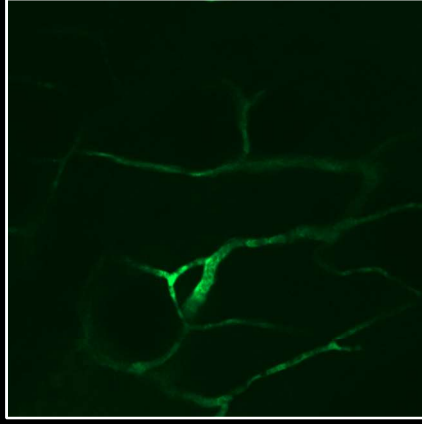
Rho
1min



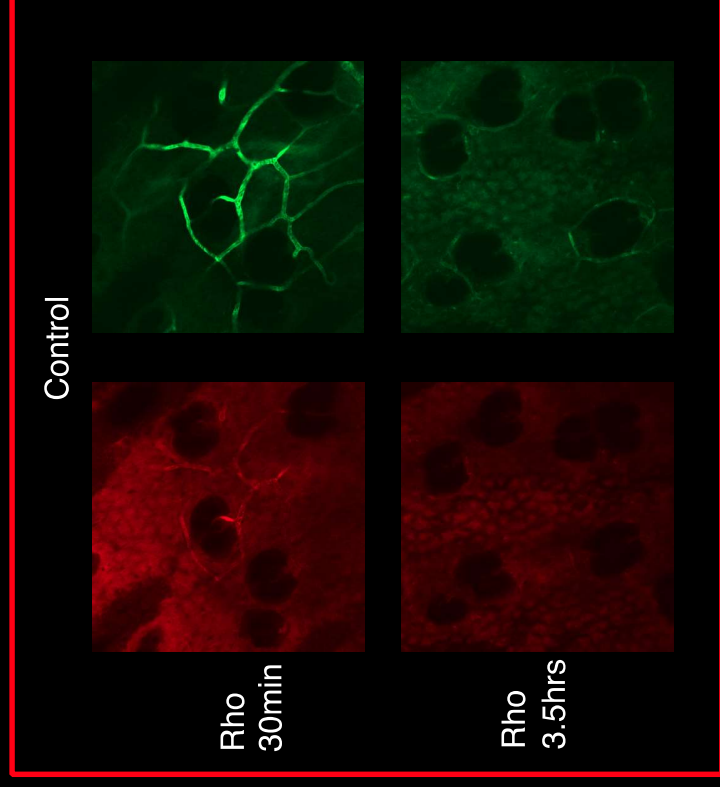
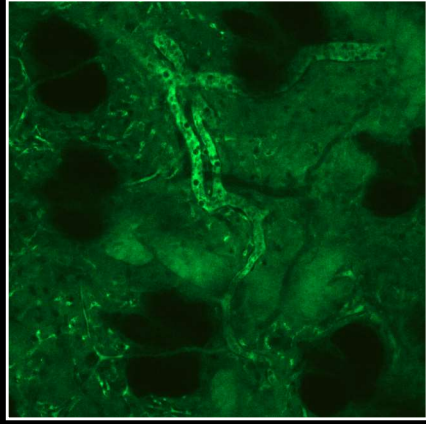
FITC



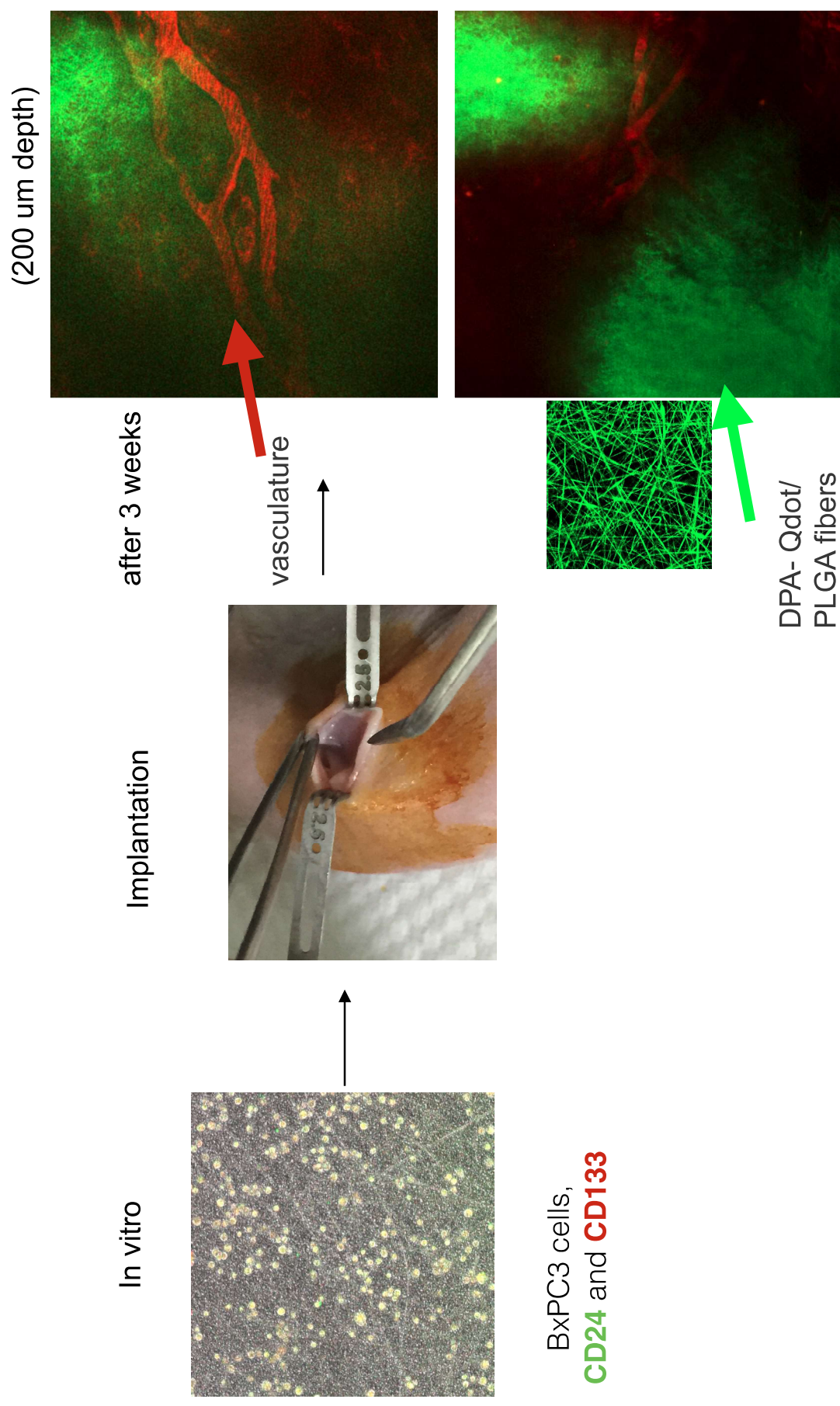
Rho
30min



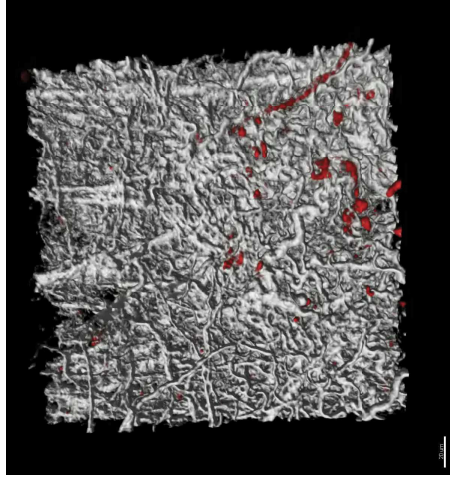
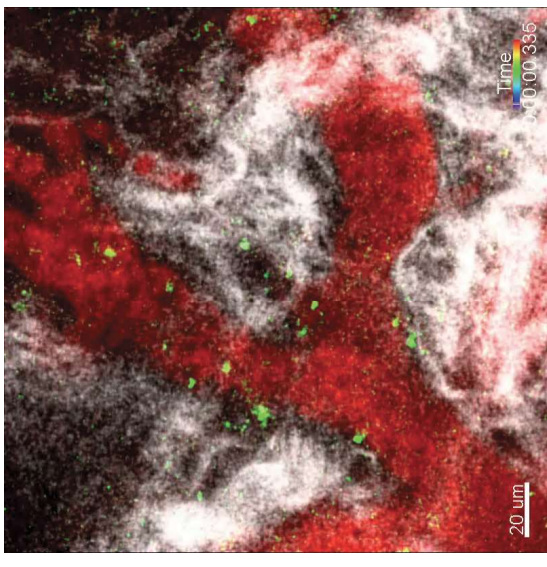
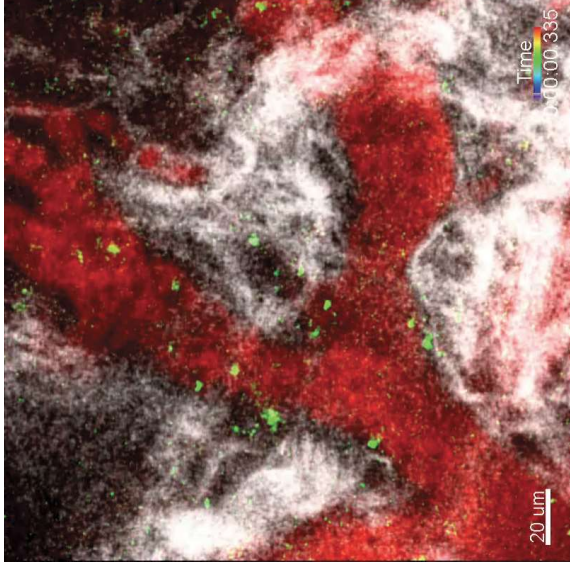
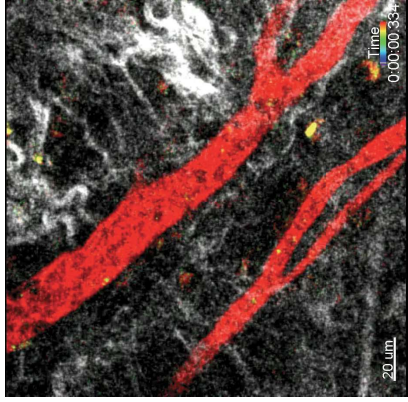
Rho
4hrs

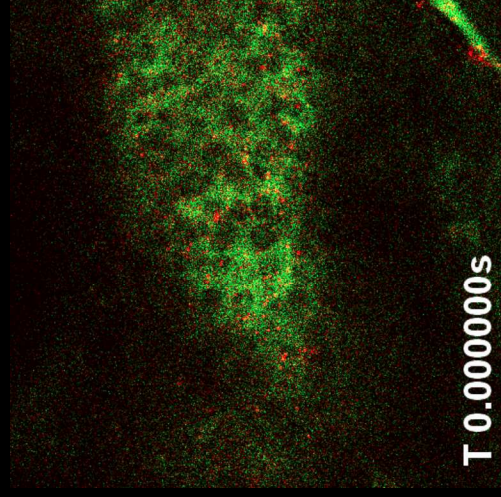


Cancer Model Using DPA- Qdot/ PLGA Fibers

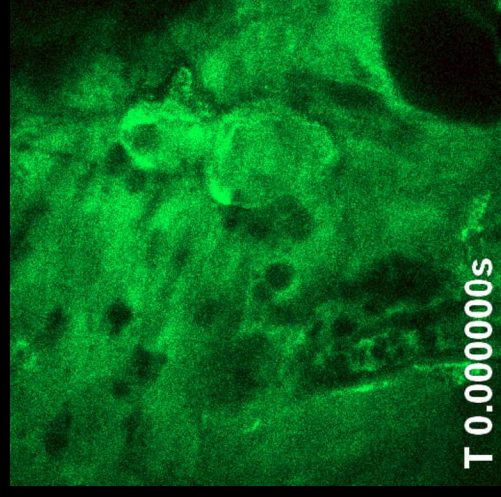


Cancer Model Using DPA- Qdot/ PLGA Fibers



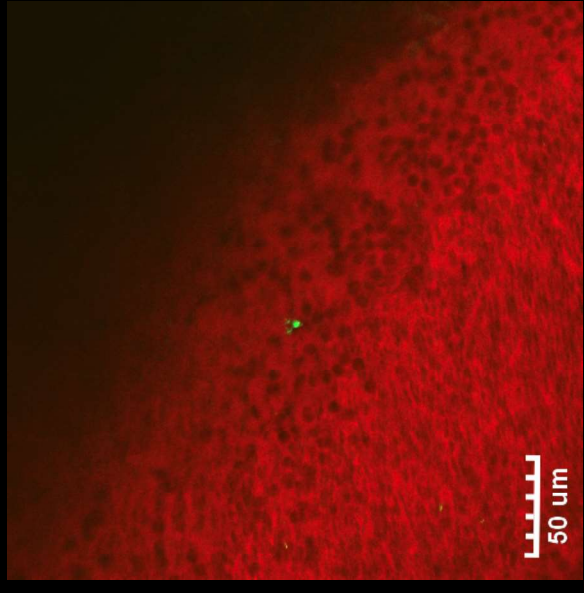
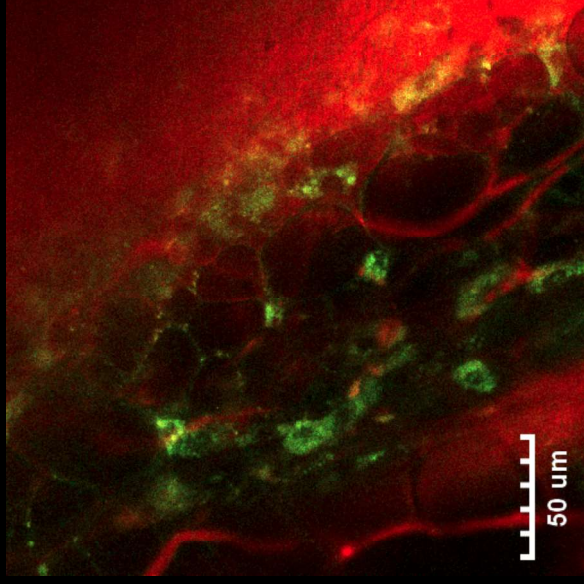
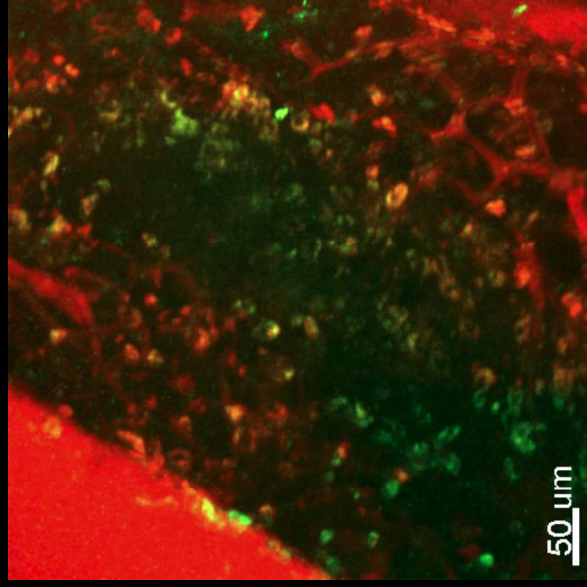
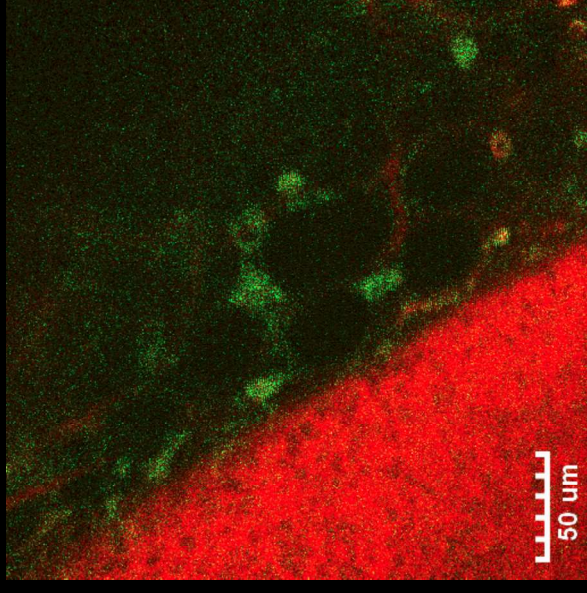
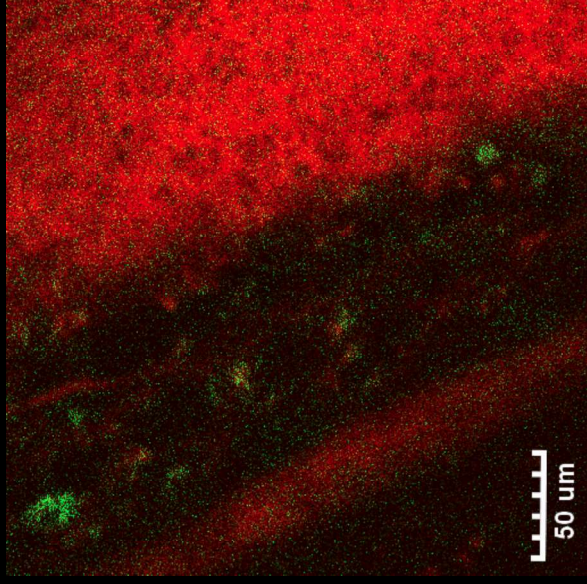
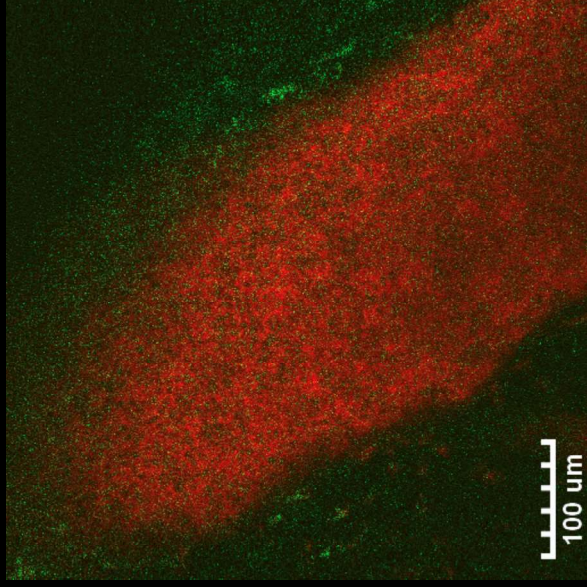


T 0.000000s

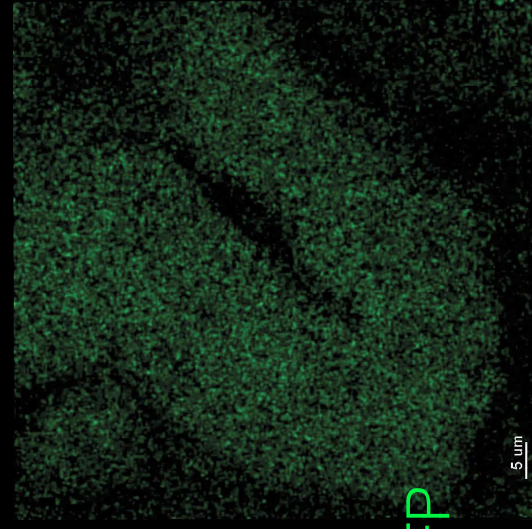


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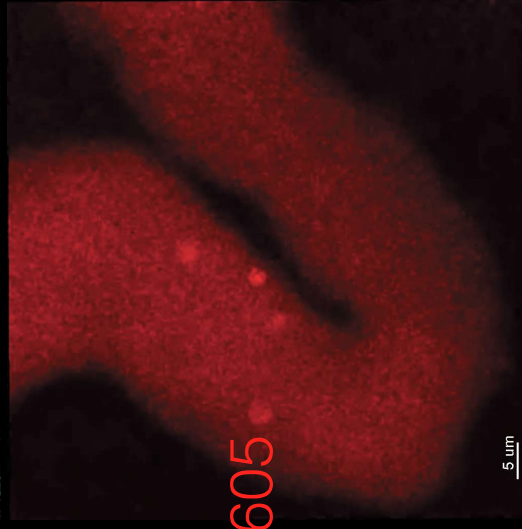
Observing CD24 Positive Cells in Tumor (CSC marker)



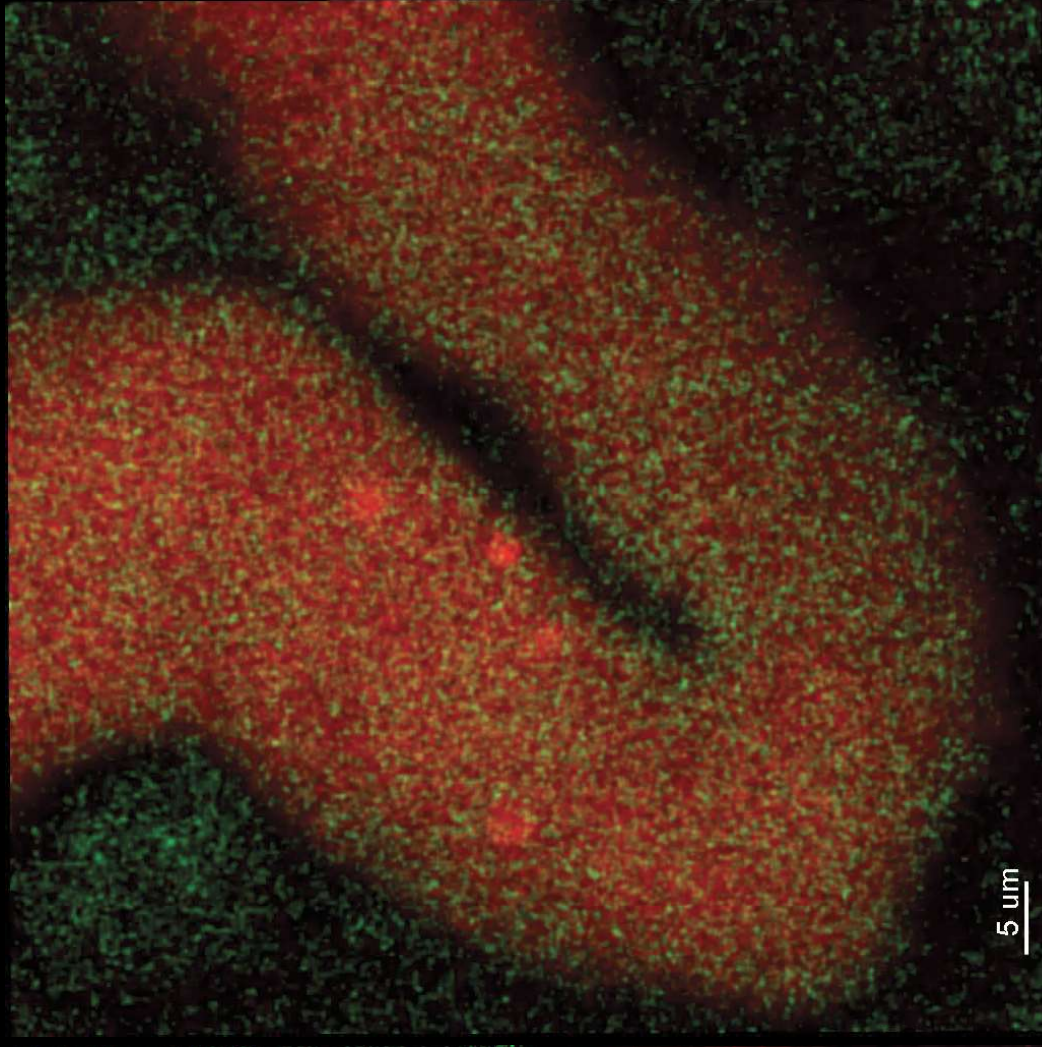
Observing Circulating Tumor Cells



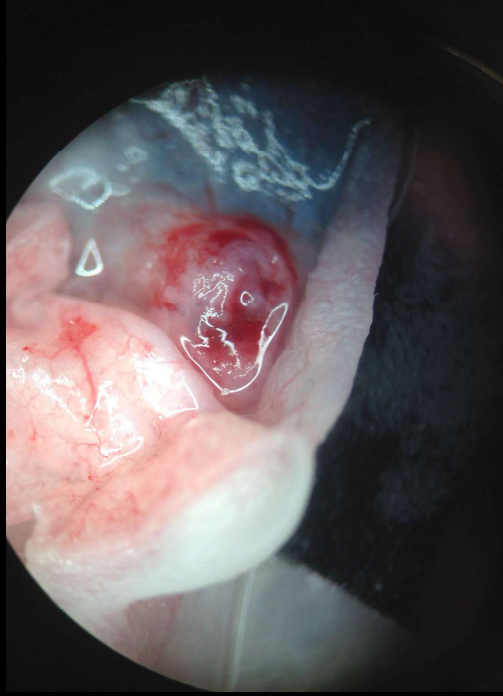
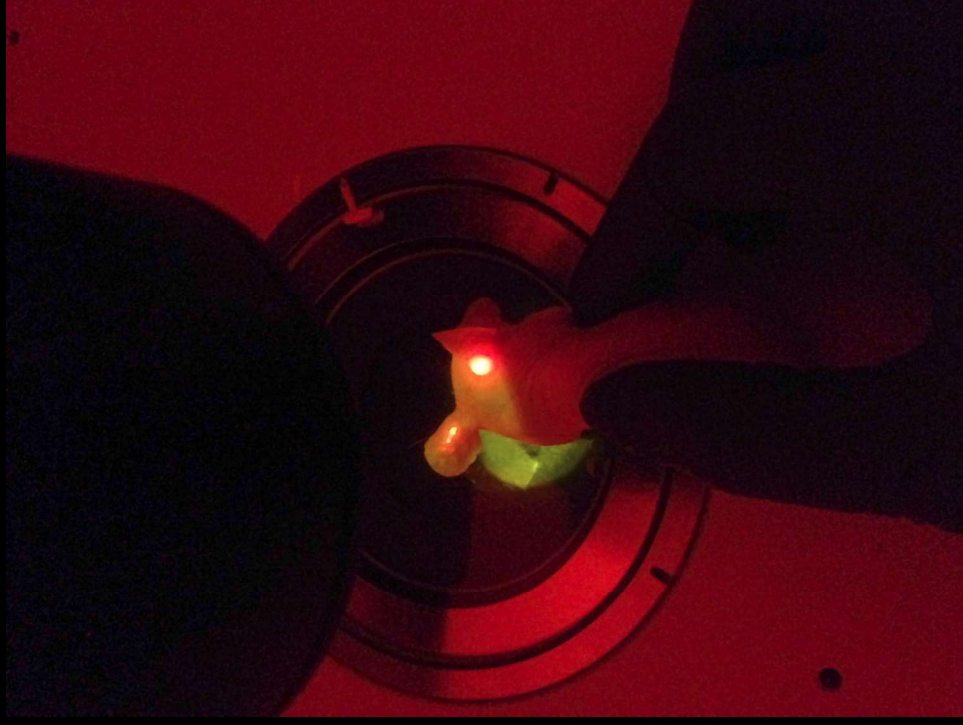
GL261-GFP



CD44-QD605



Tumor on Earlobe



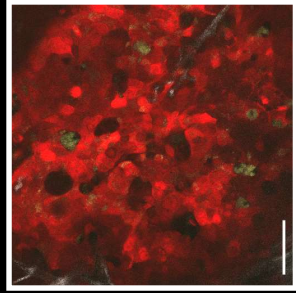
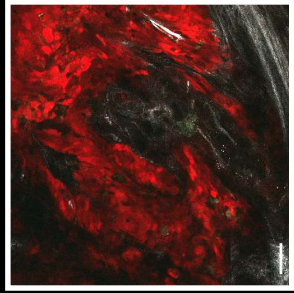
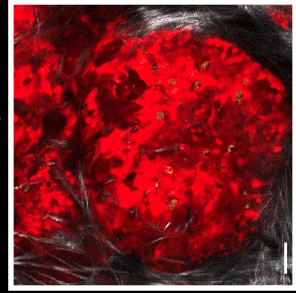
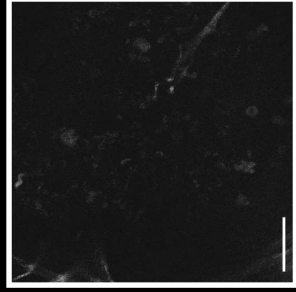
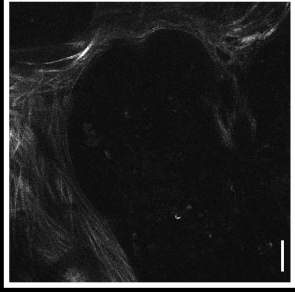
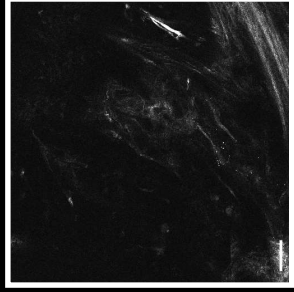
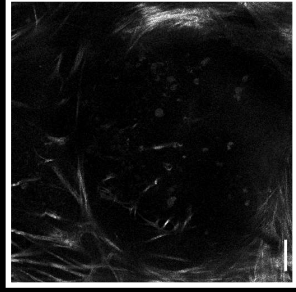
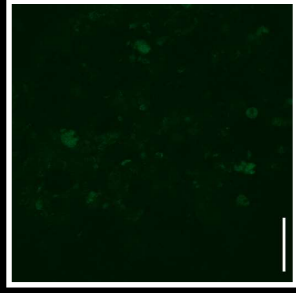
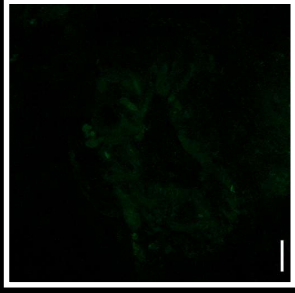
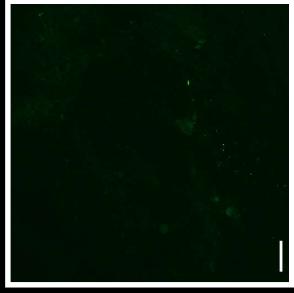
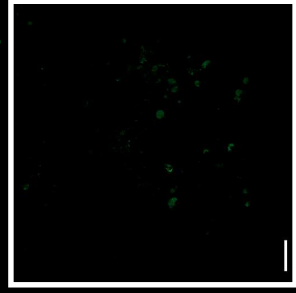
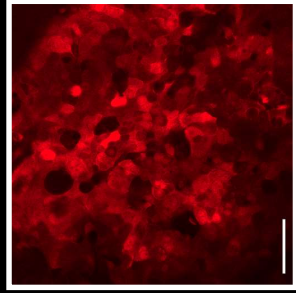
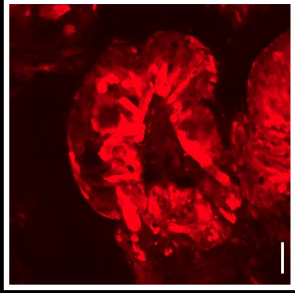
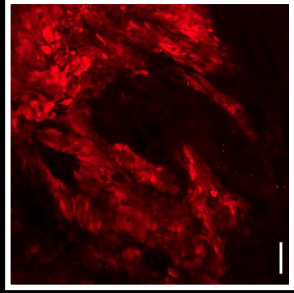
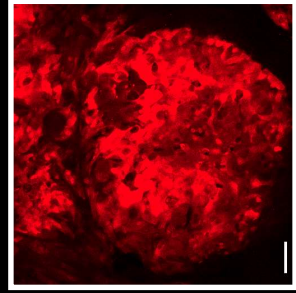
10min

BXPC3-RFP

QD525 only

SHG

Merge



Scale bar : 50um

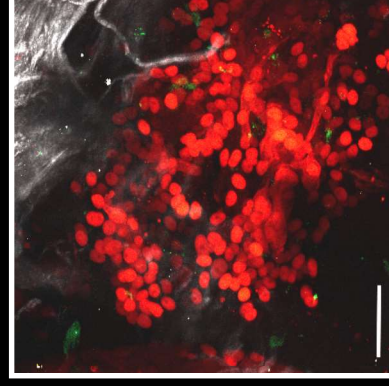
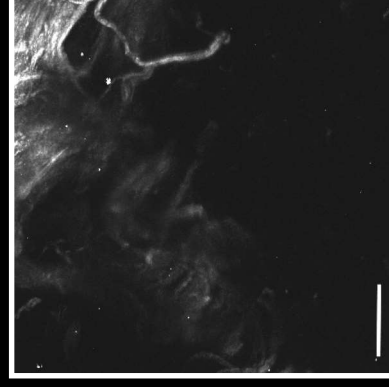
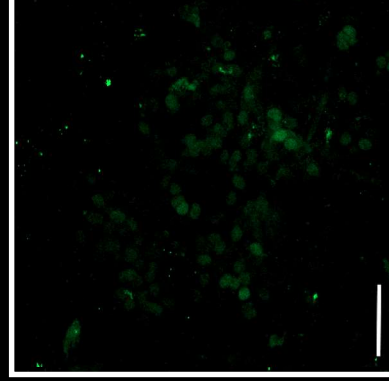
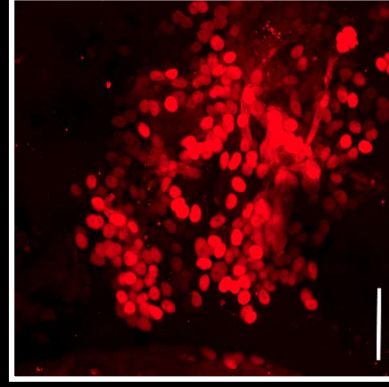
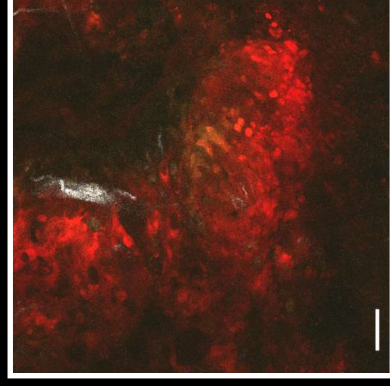
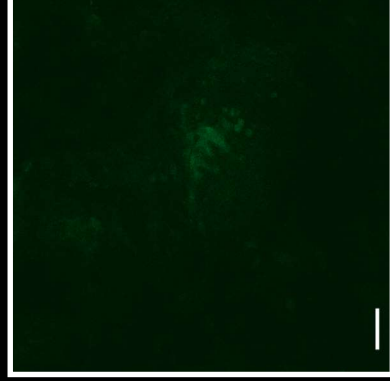
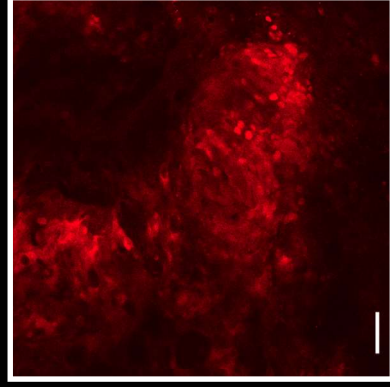
1hr

BXPC3-RFP

QD525 only

SHG

Merge



Scale bar : 50um

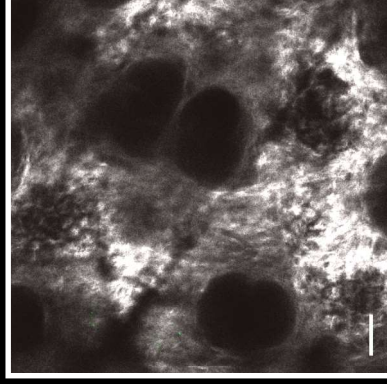
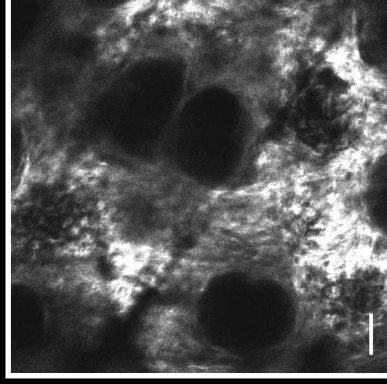
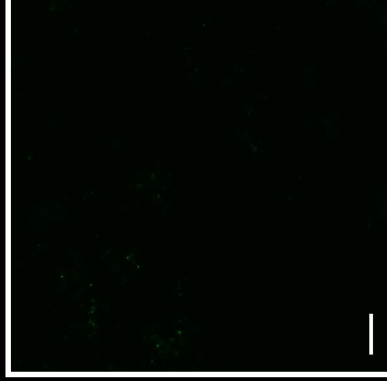
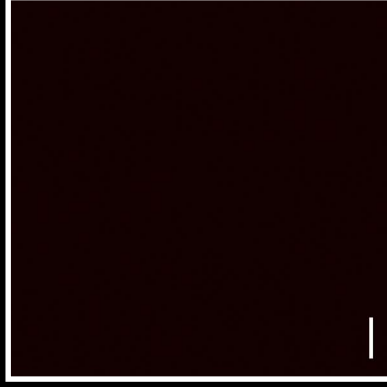
BXPC3-RFP

VK9-QD525

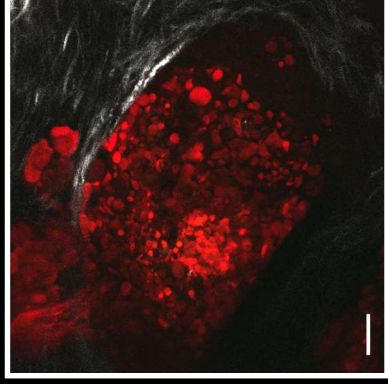
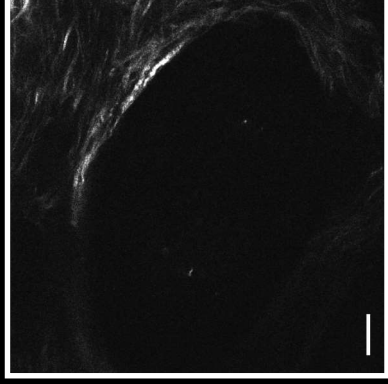
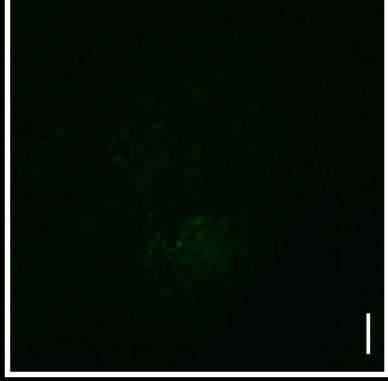
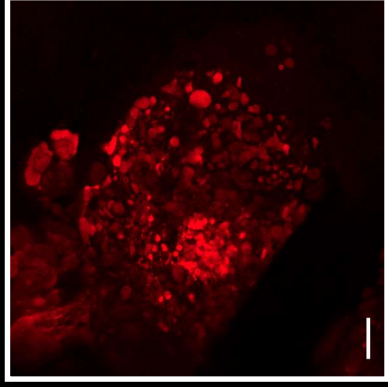
SHG

Merge

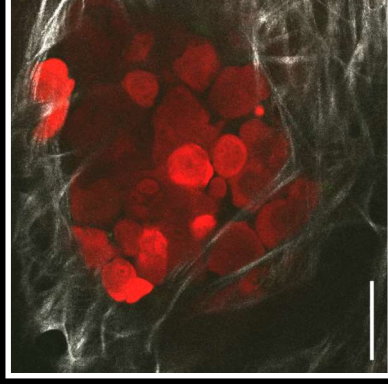
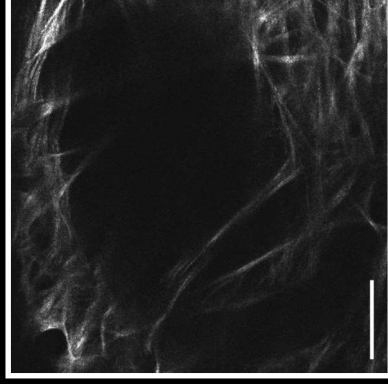
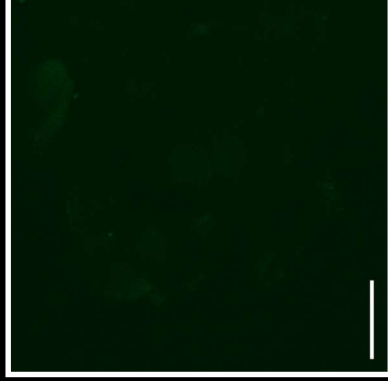
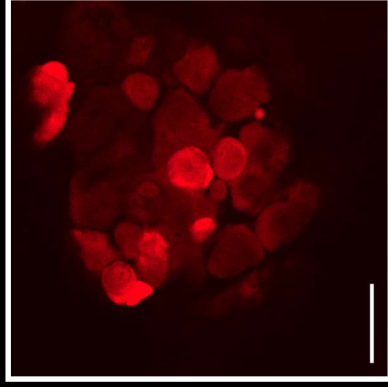
**Control
(skin)**



**Control
(Tumor)**



**Control
(Tumor)**



Scale bar : 50um

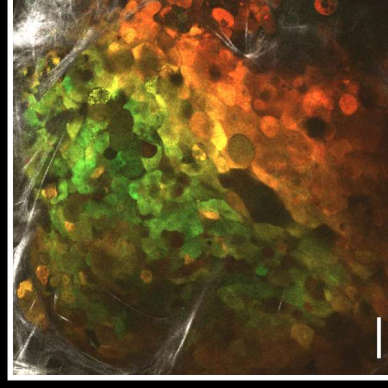
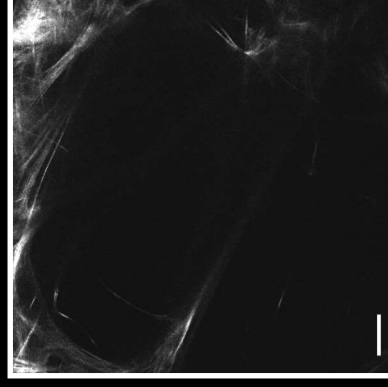
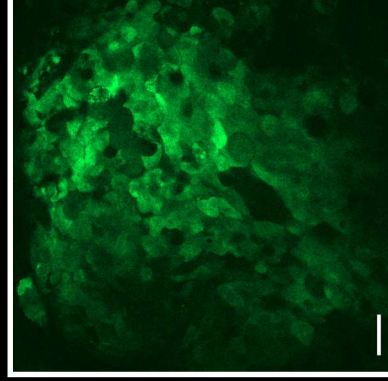
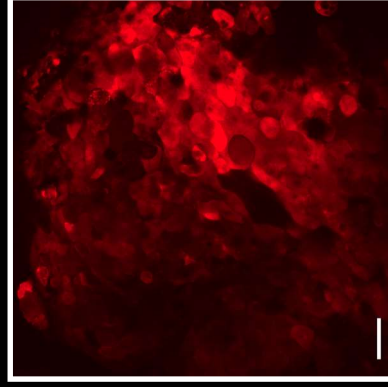
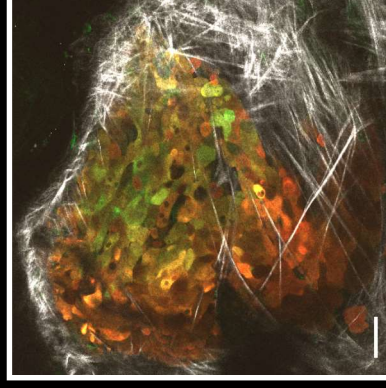
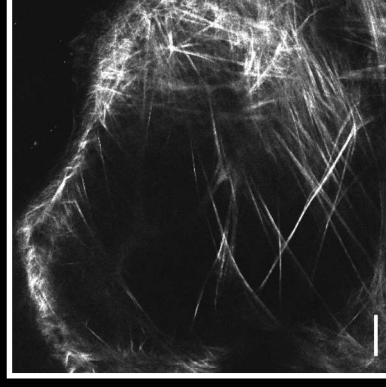
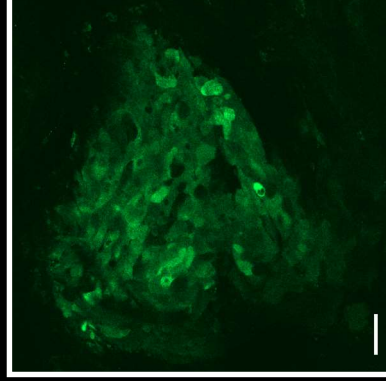
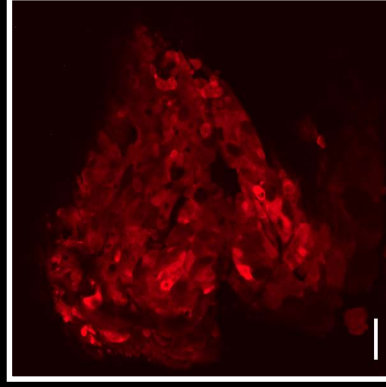
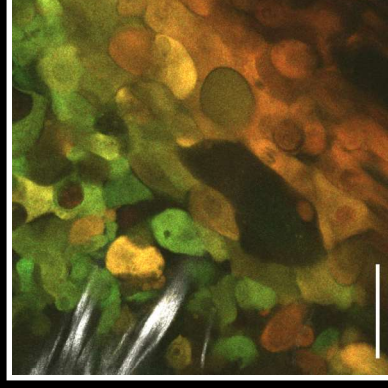
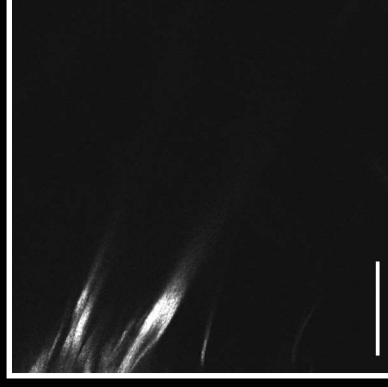
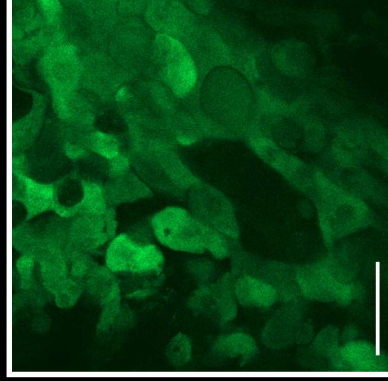
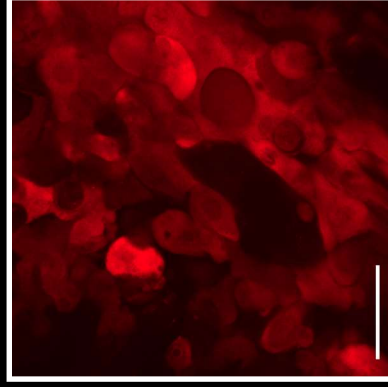
10min

BXPC3-RFP

VK9-QD525

SHG

Merge



Scale bar : 50um

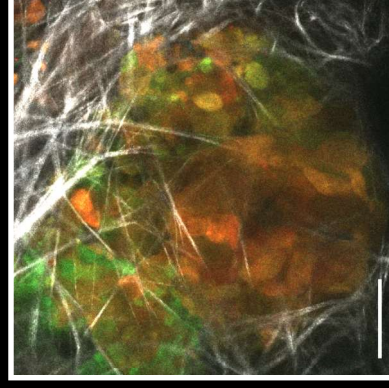
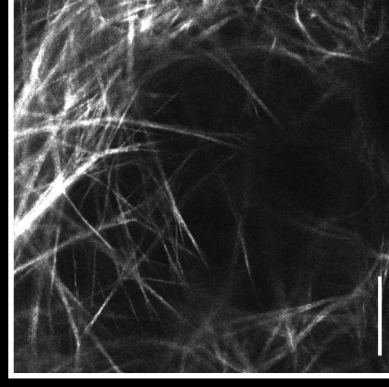
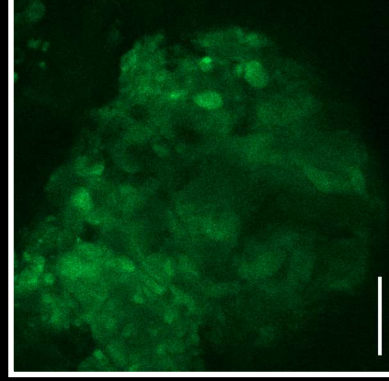
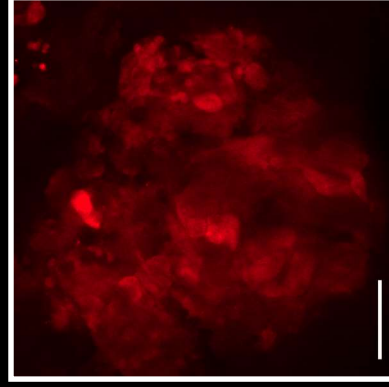
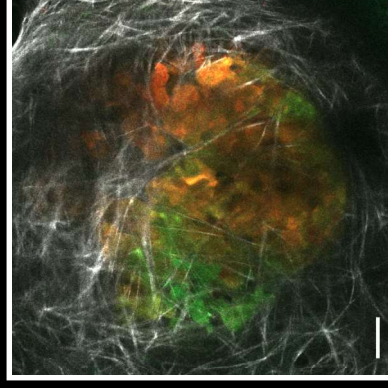
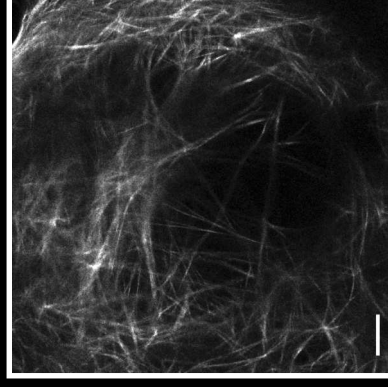
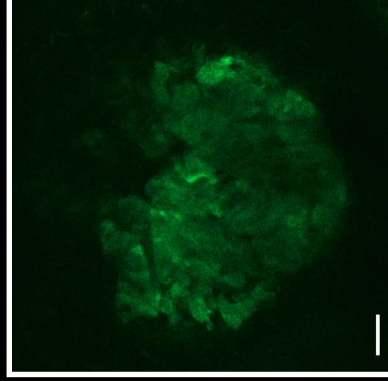
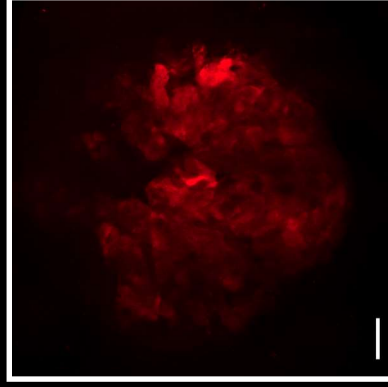
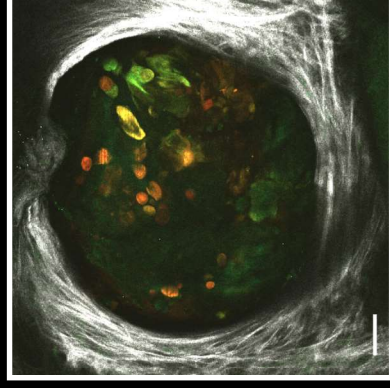
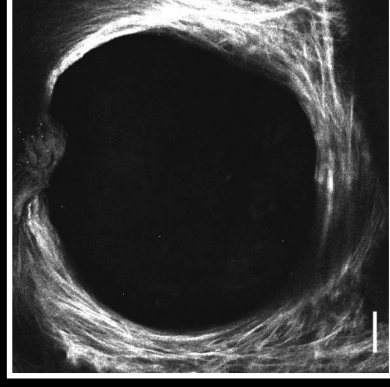
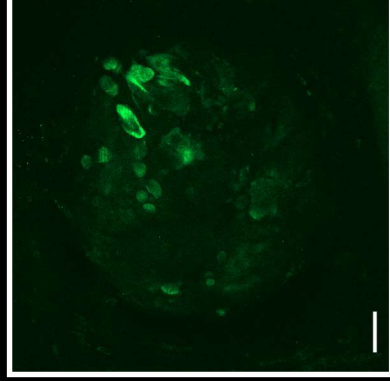
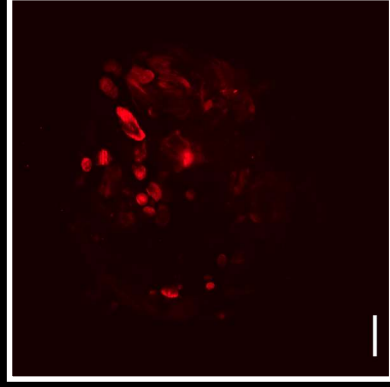
10min

BXPC3-RFP

VK9-QD525

SHG

Merge



Scale bar : 50um

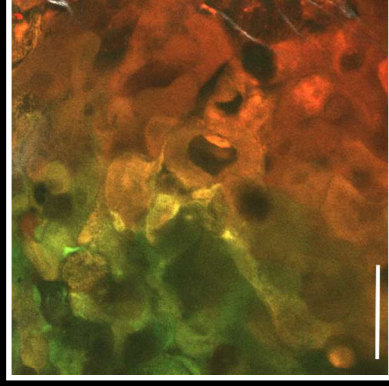
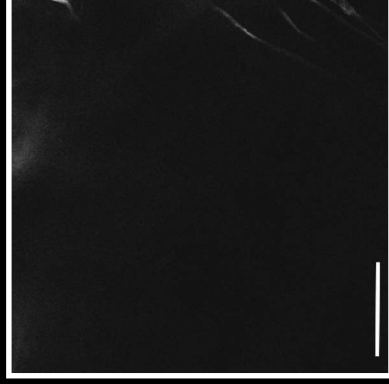
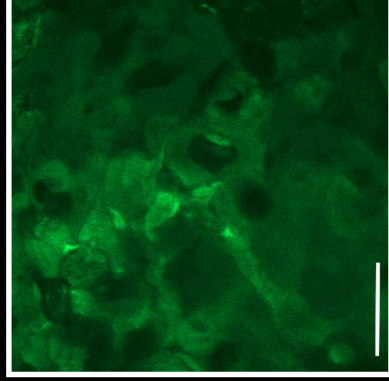
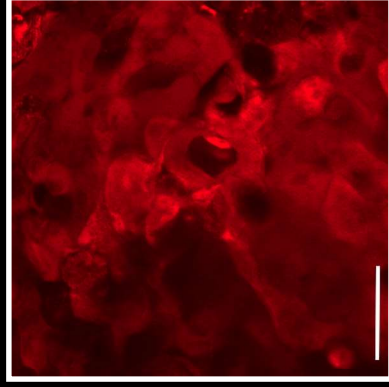
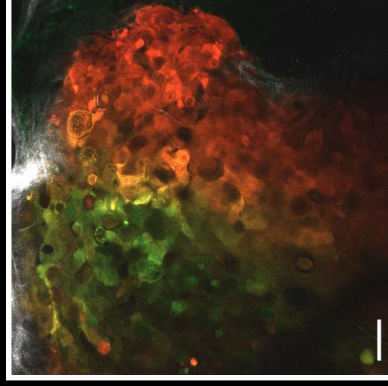
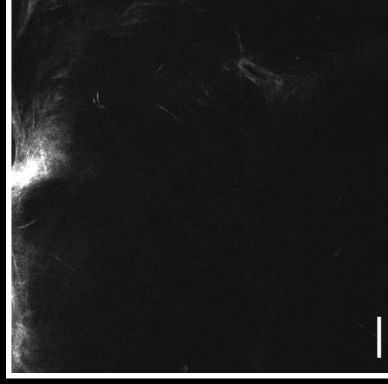
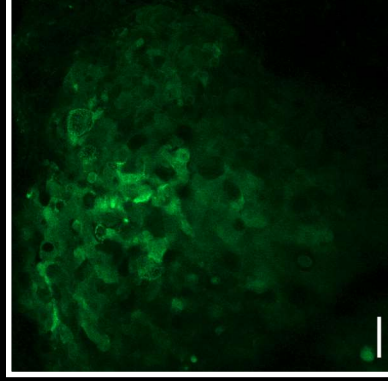
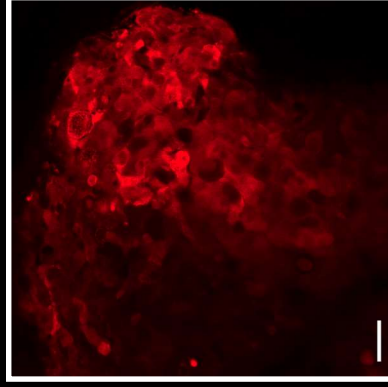
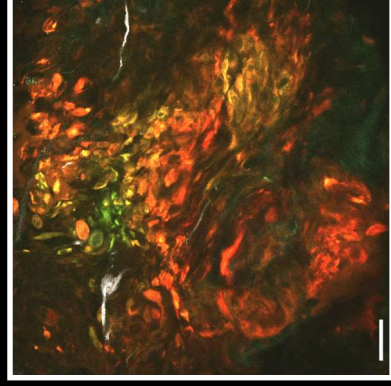
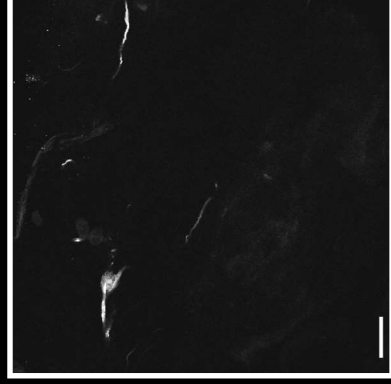
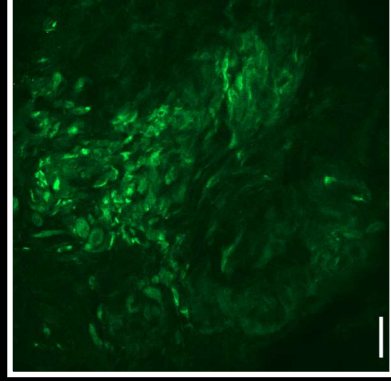
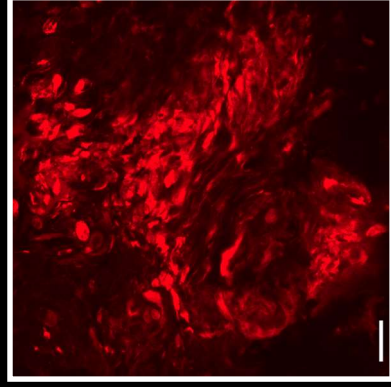
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BXPC3-RFP

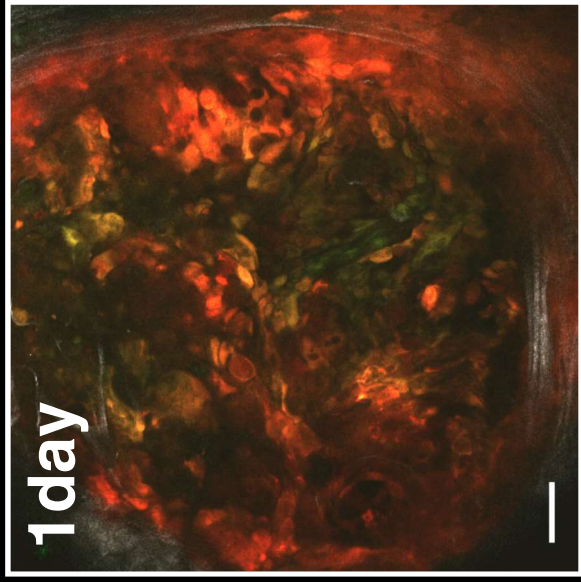
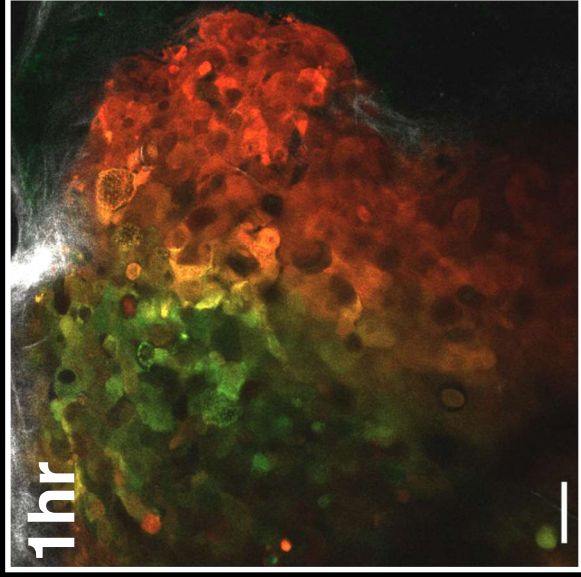
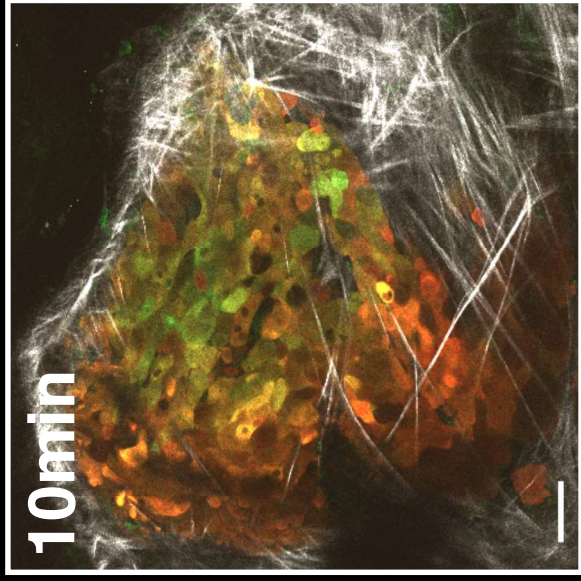
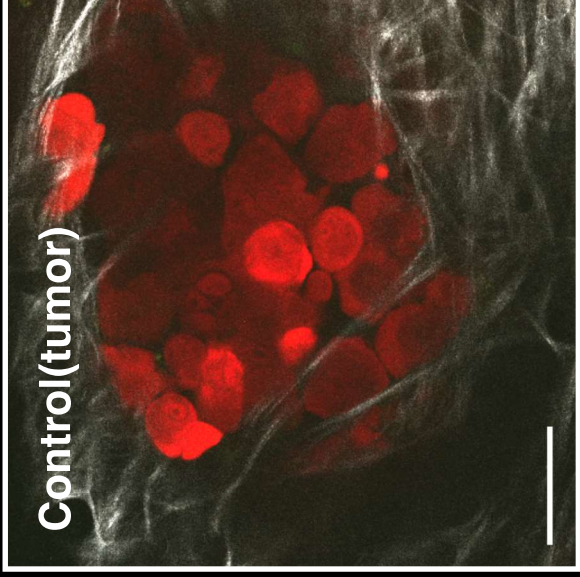
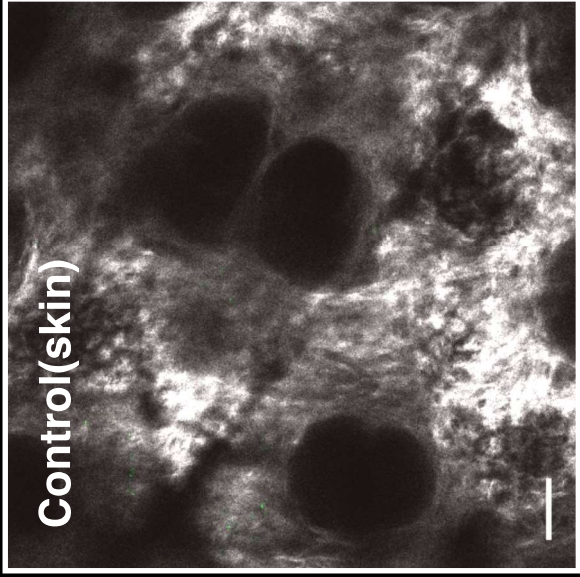
VK9-QD525

SHG

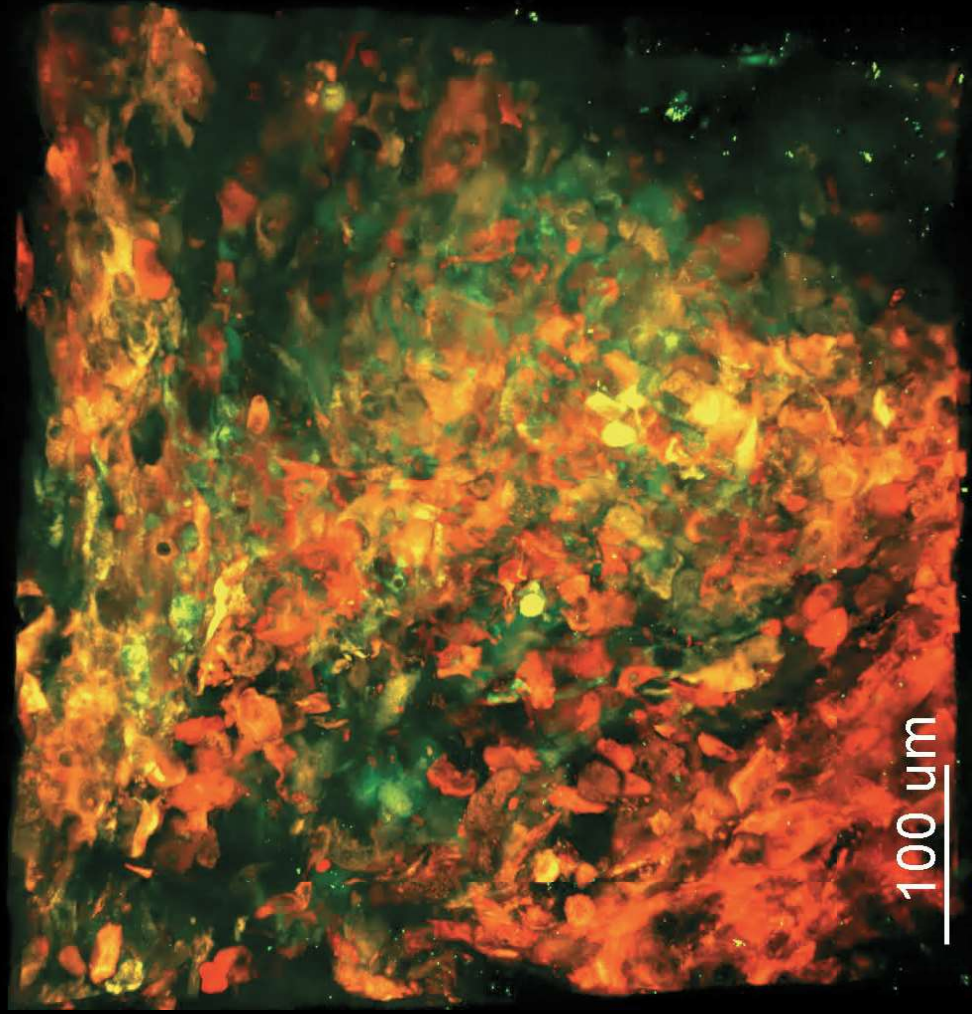
Merge



Scale bar : 50um

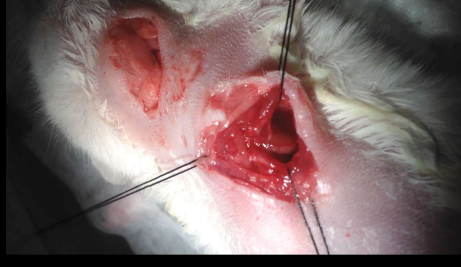
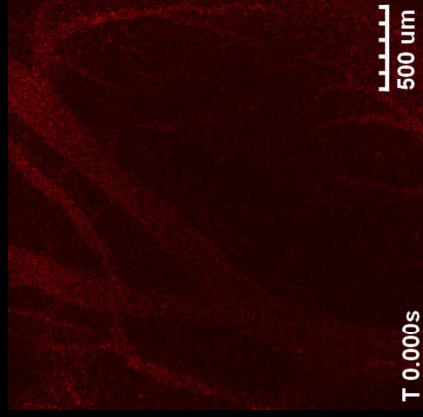
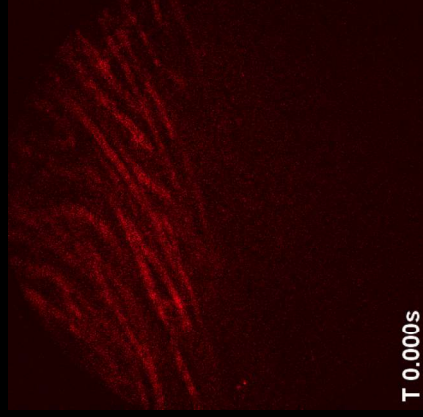


Scale bar : 50um

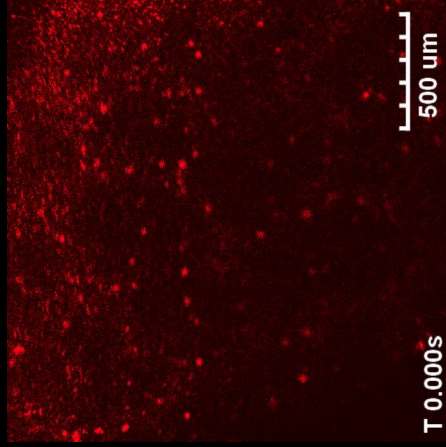


Imaging Moving Organs

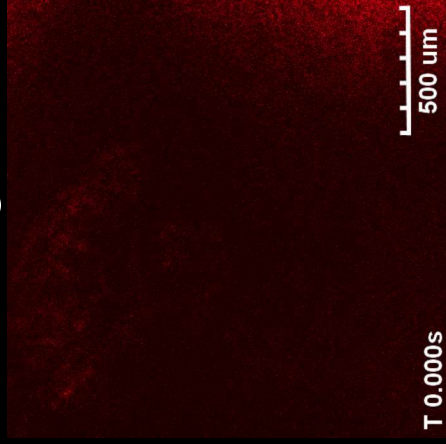
Heart



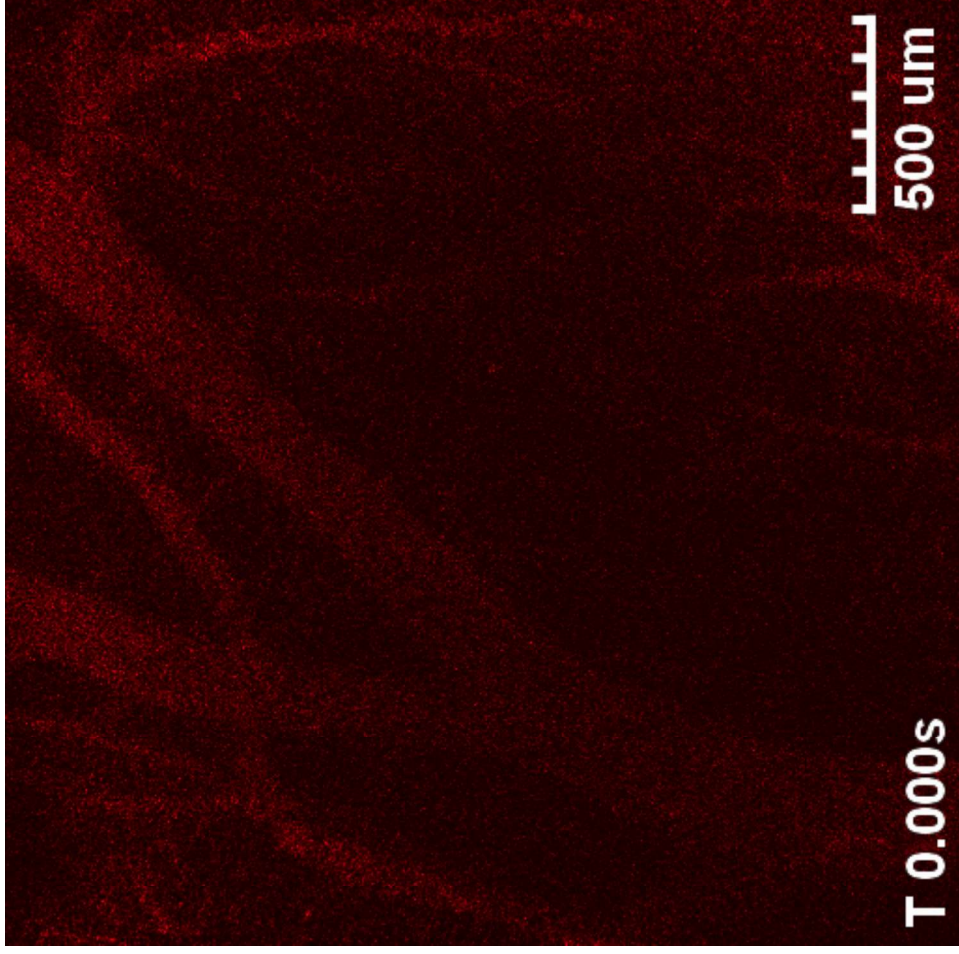
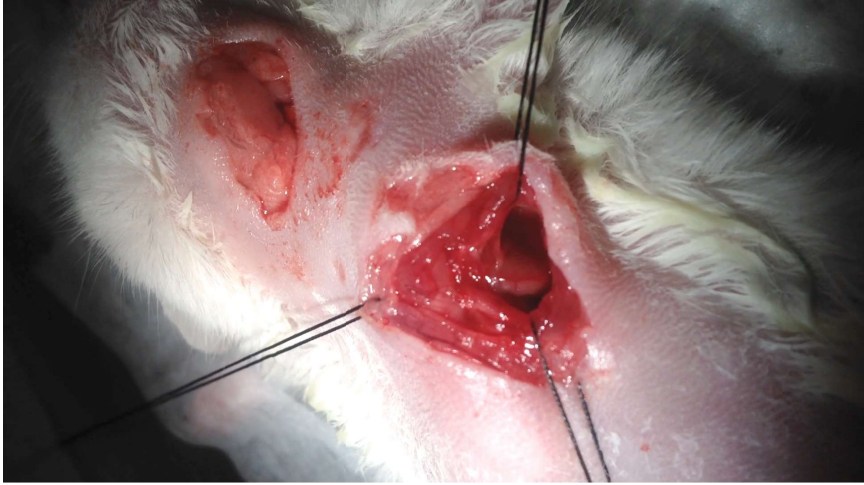
Liver



Lung



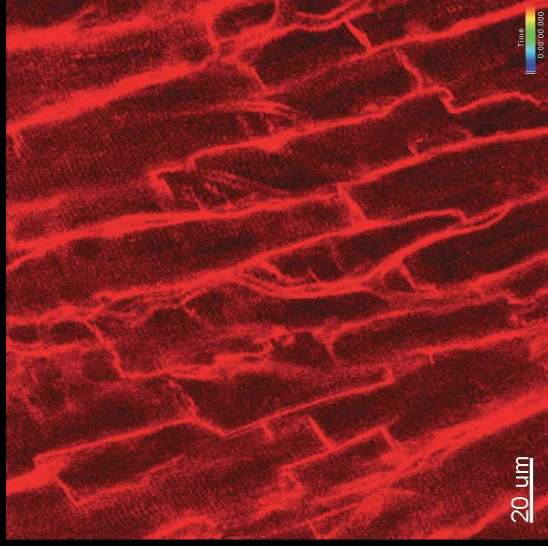
Images of blood vessels on the anterior surface of the heart in vivo.



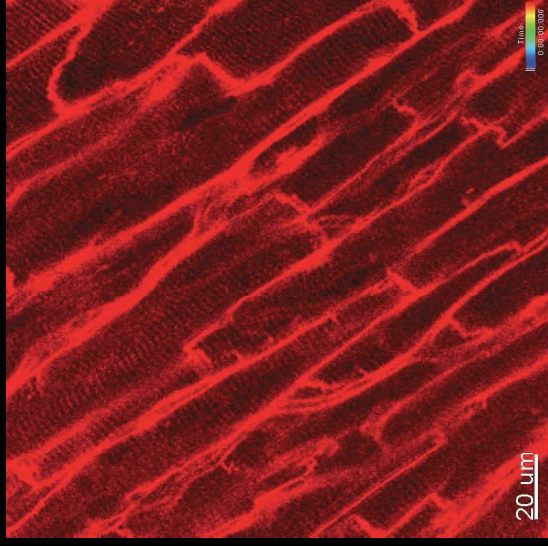
The vessels are visualized by intravenously injected Evans blue. (M plan N 5x N.A. 0.1 ; Olympus) very-low-resolution cardiac imaging (e.g., microvasculature, cellular recruitment, and flow), frames were acquired consecutively at a constant speed, times is 30 frame/sec.)

Reconstructed Images

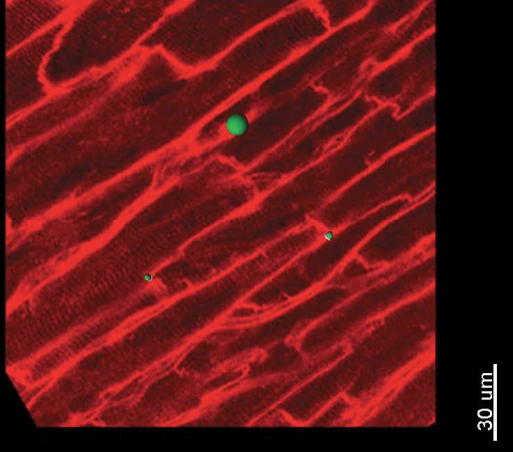
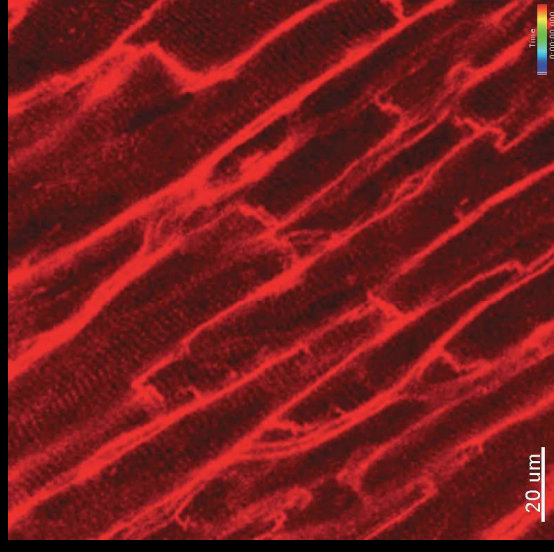
RAW Images



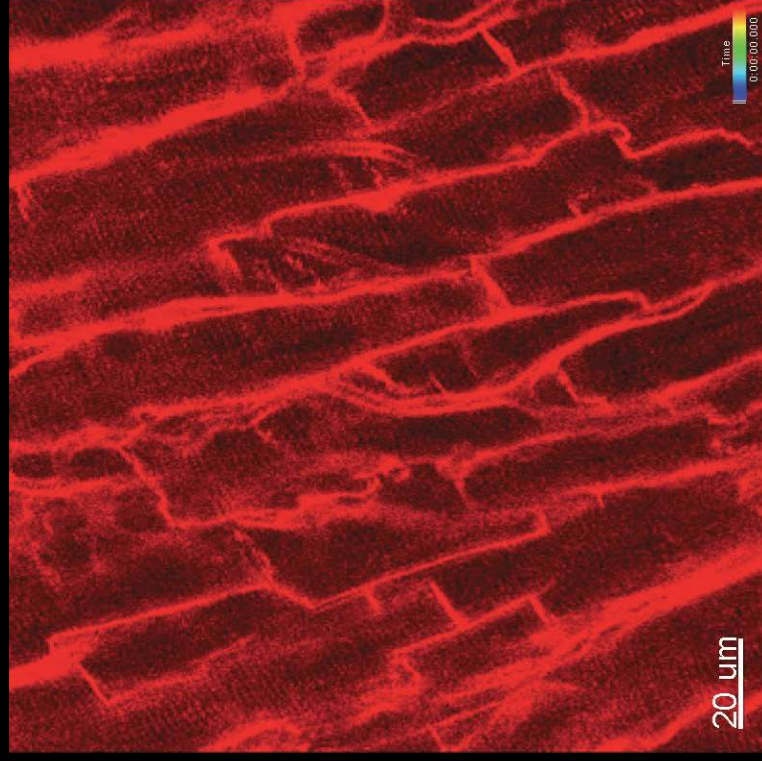
Cropped Images



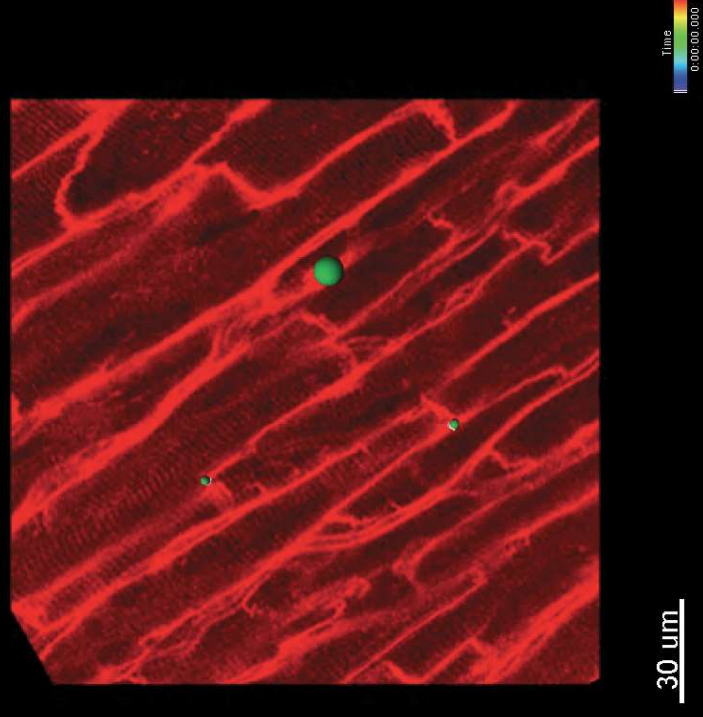
Cropped Images

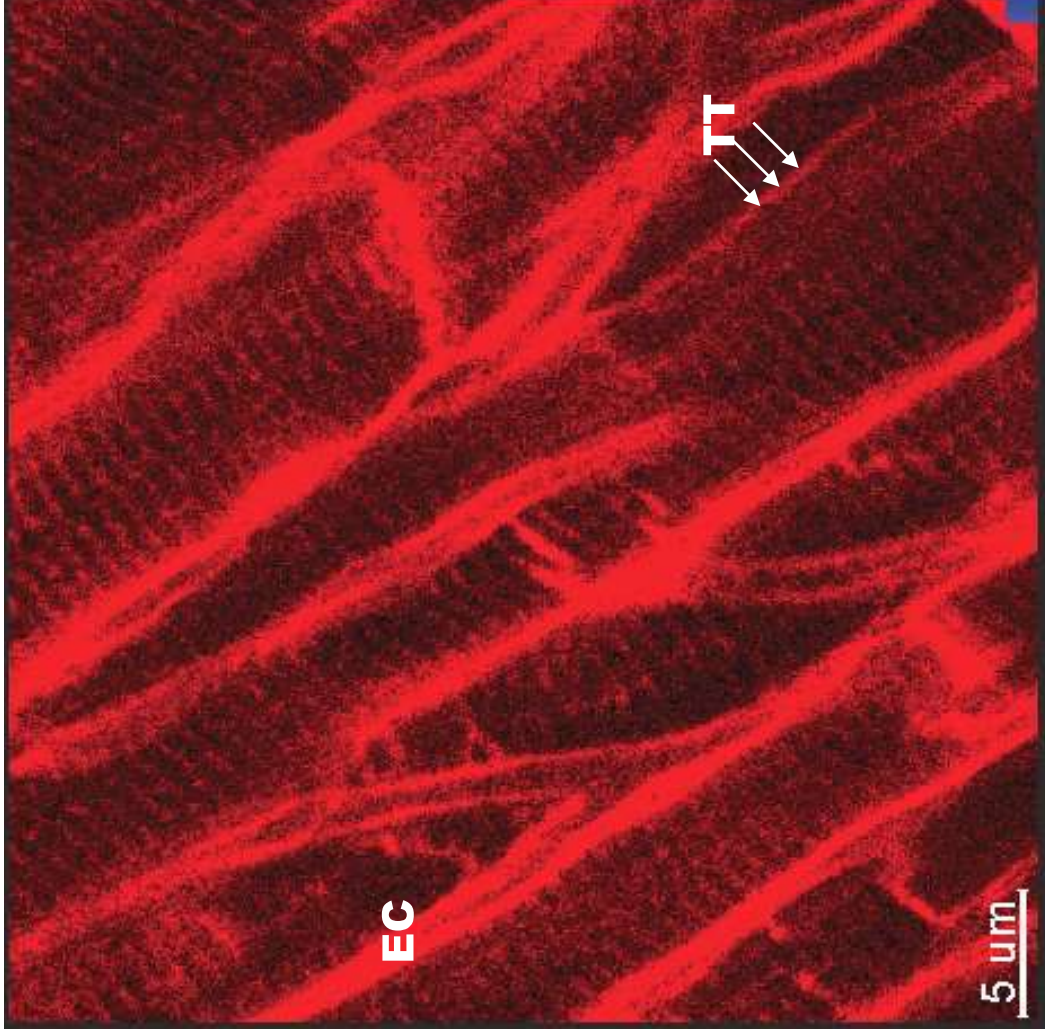


RAW Images



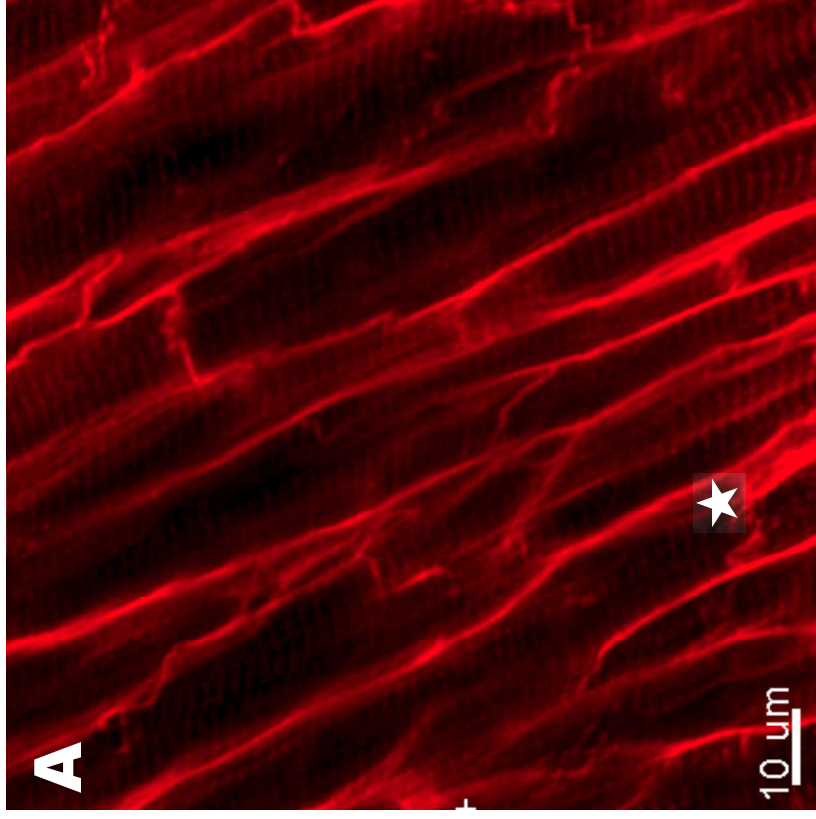
Reconstructed Images



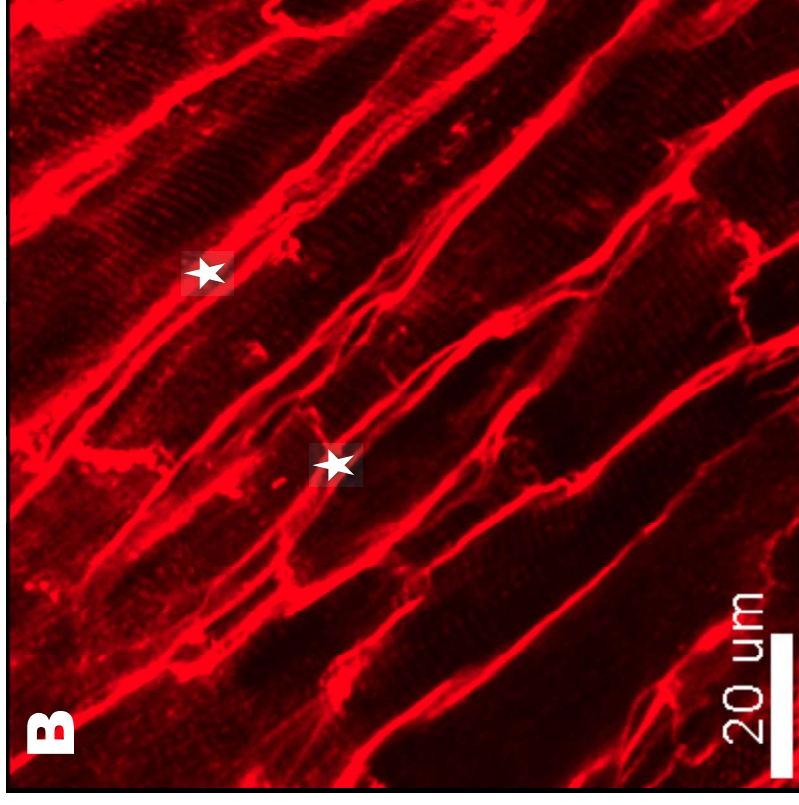


Endothelial cells ,EC
Transverse tubules, TT,
The resolution of transverse tubule structure measured <2μm.

Cells in a densely packed tissue assembly



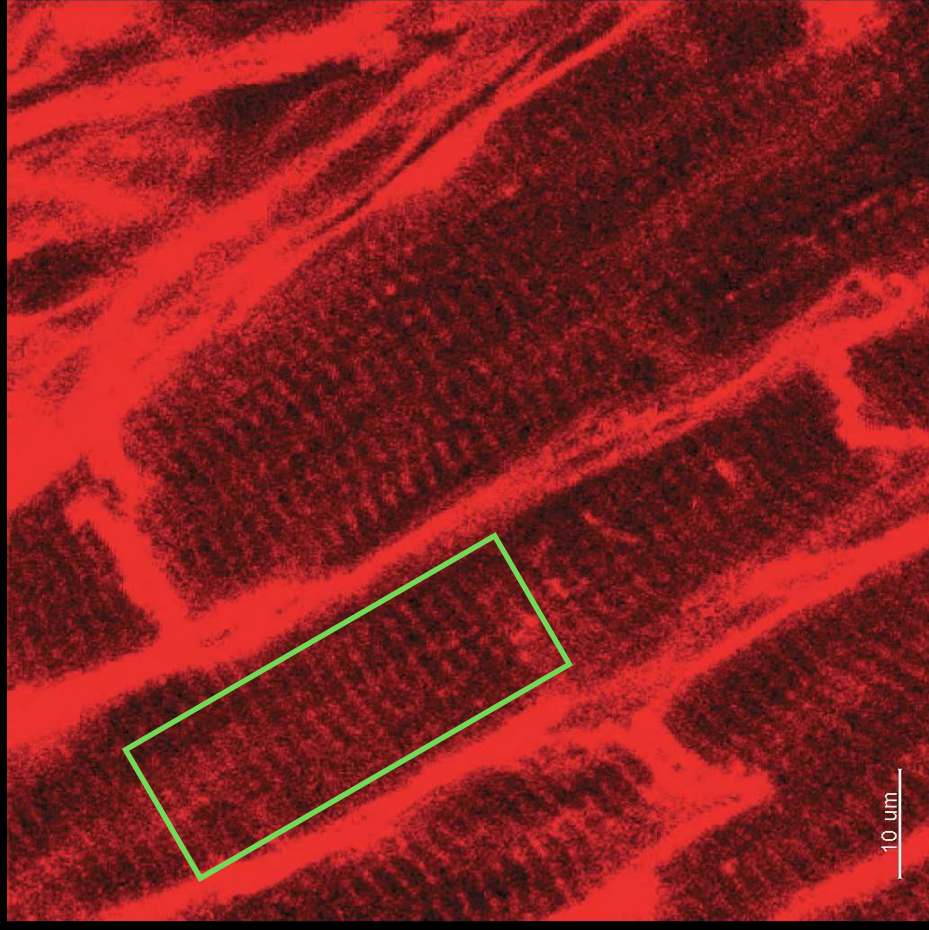
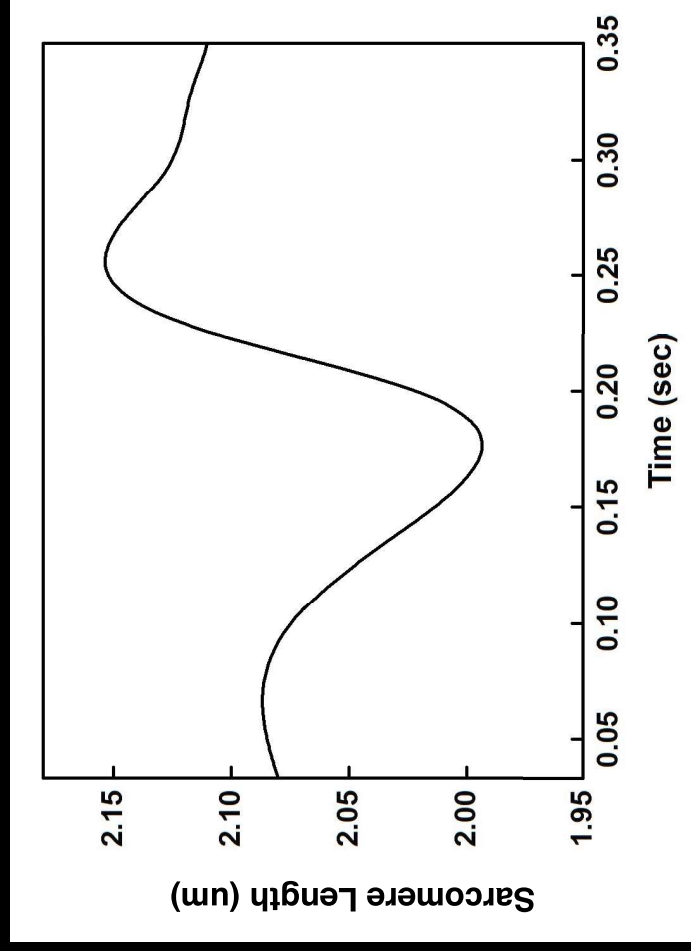
cells in a loosely packed tissue assembly.



two-photon fluorescence images of di-2-anepeq stained cardiomyocyte cell membranes of mouse heart showing two different morphologies. A: cells in a densely packed tissue assembly. B. cells in a loosely packed tissue assembly.

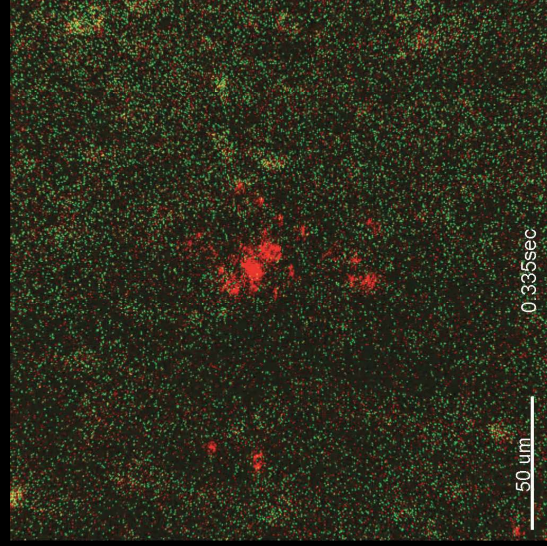
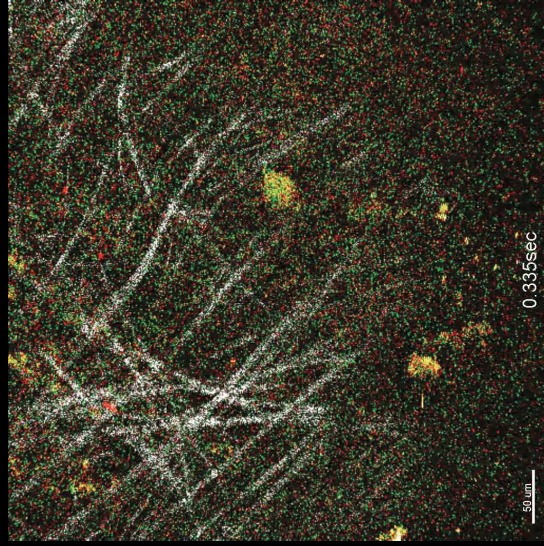
Capillaries (*) are more frequently visualized in loosely packed tissue.

Measuring Sarcomere Length in a Beating Heart



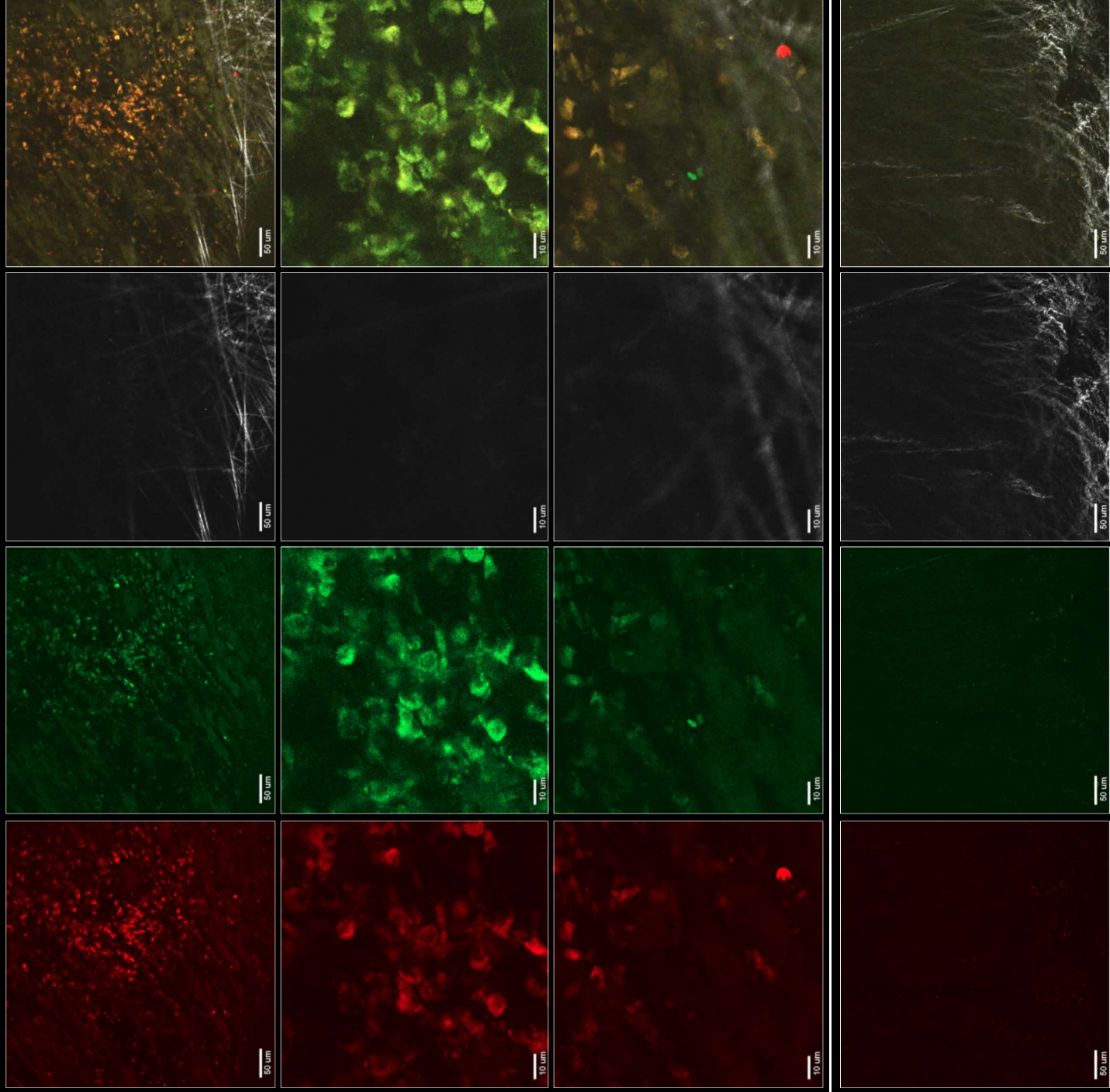
1. measure average sarcomere length at each point
2. measure sarcomere spacing in the beating heart with microscopy. the cell orientation during contraction, (use di-2 and Hoechst 33258), the intrinsic distribution of sarcomere length within and between myocytes, nonmyocyte components.
3. tracking of single myocytes.
4. microvascular flow analysis.

Live images



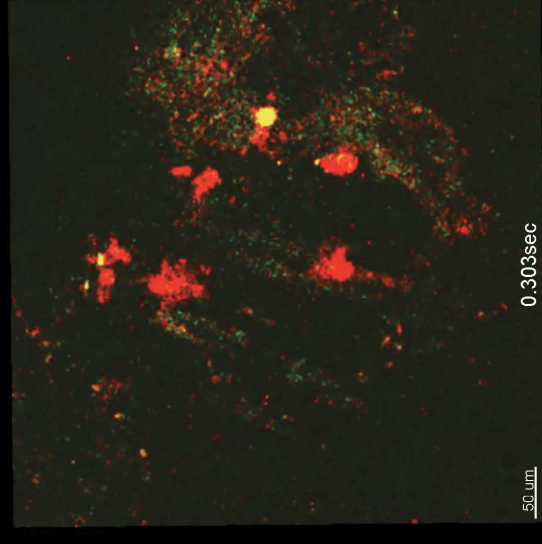
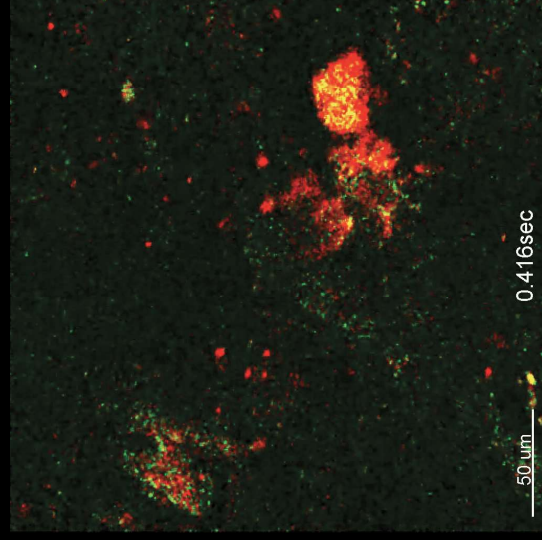
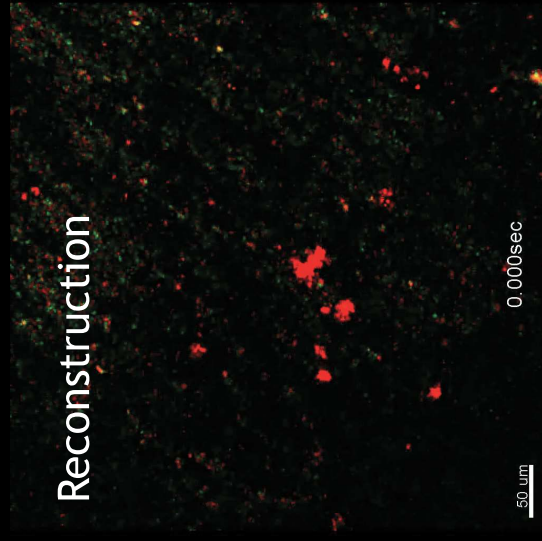
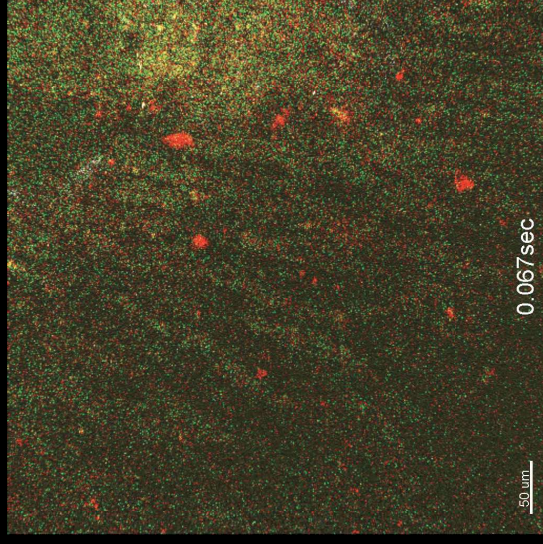
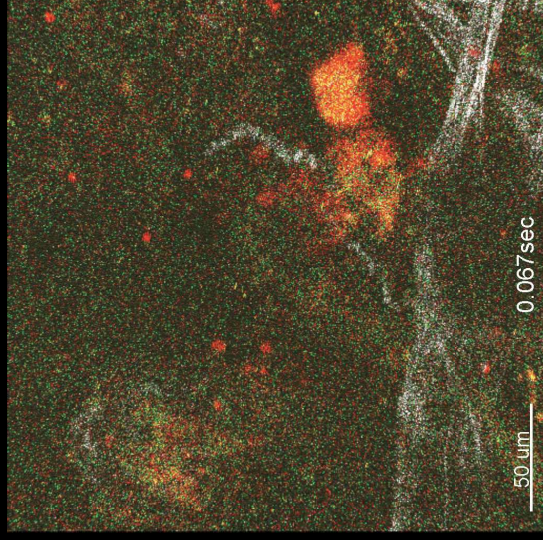
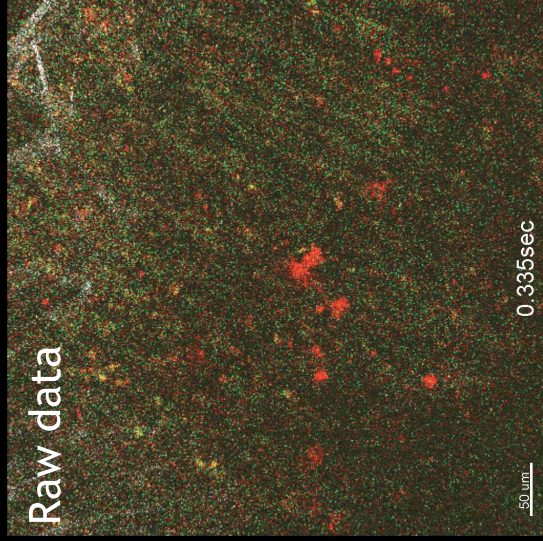
Z-section images





Control

Live images



3D images

