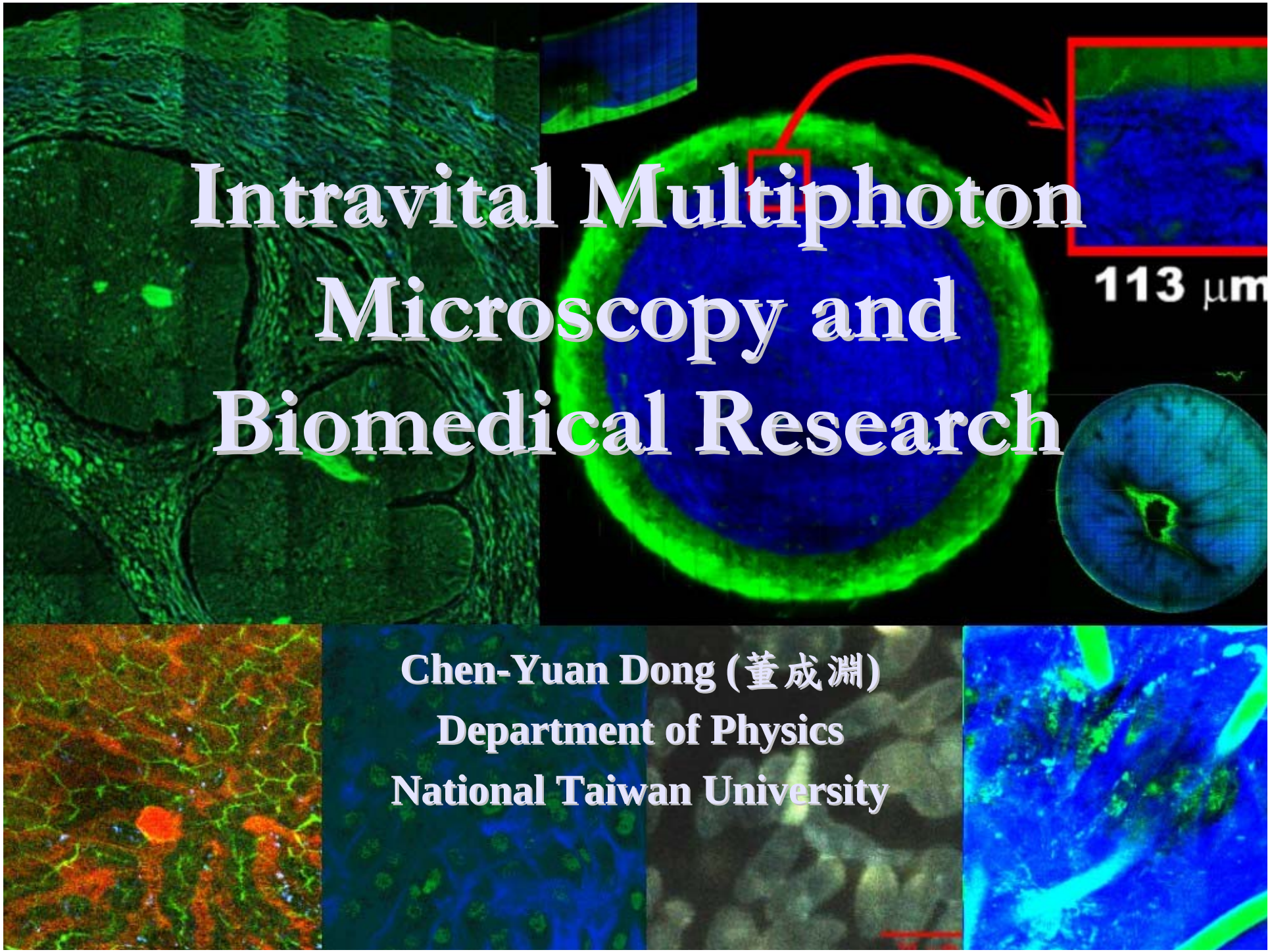
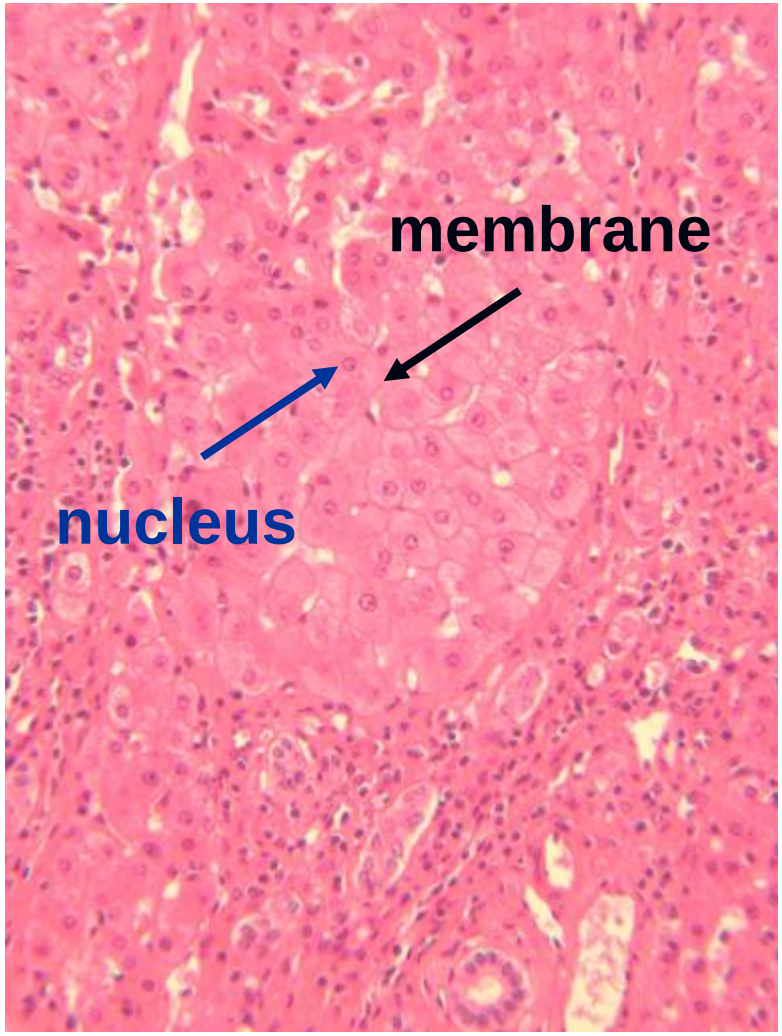


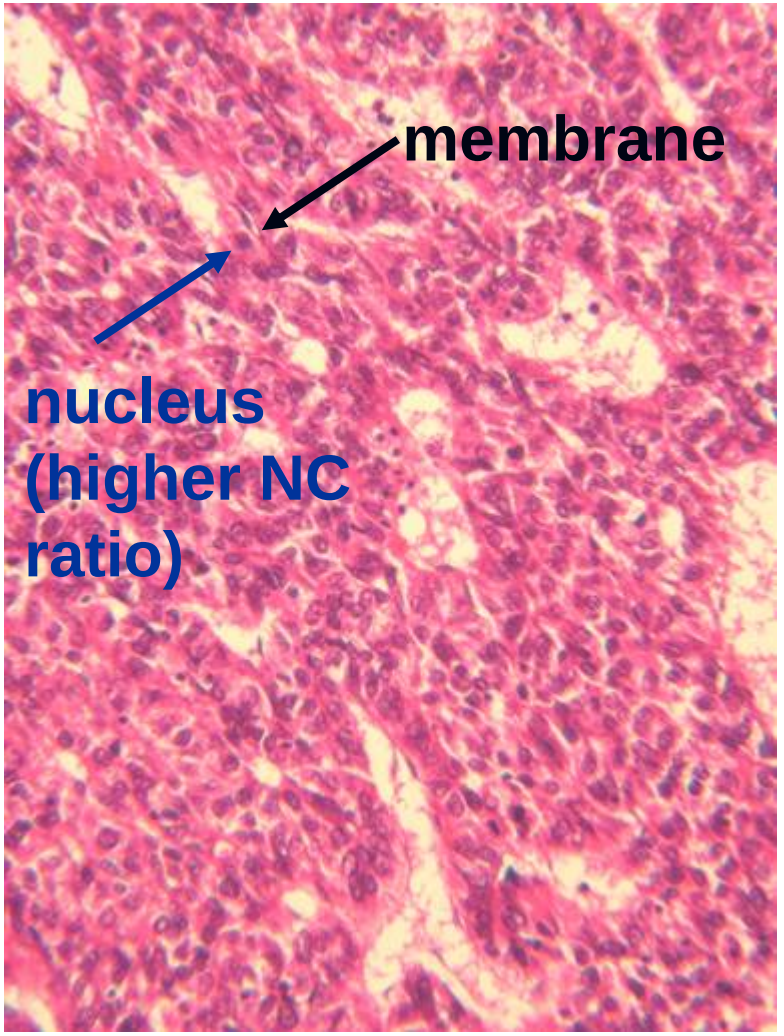
Intravital Multiphoton Microscopy and Biomedical Research

Chen-Yuan Dong (董成淵)
Department of Physics
National Taiwan University

Histological Images of Liver Tumor

(HCC: hepatocellular carcinoma)





Courtesy: Dr. Hsuan-Shu Lee, NTUH

National Research Program for Genomic Medicine (NRPGM)

Optical Microscopy Core Facility for Genomic Research

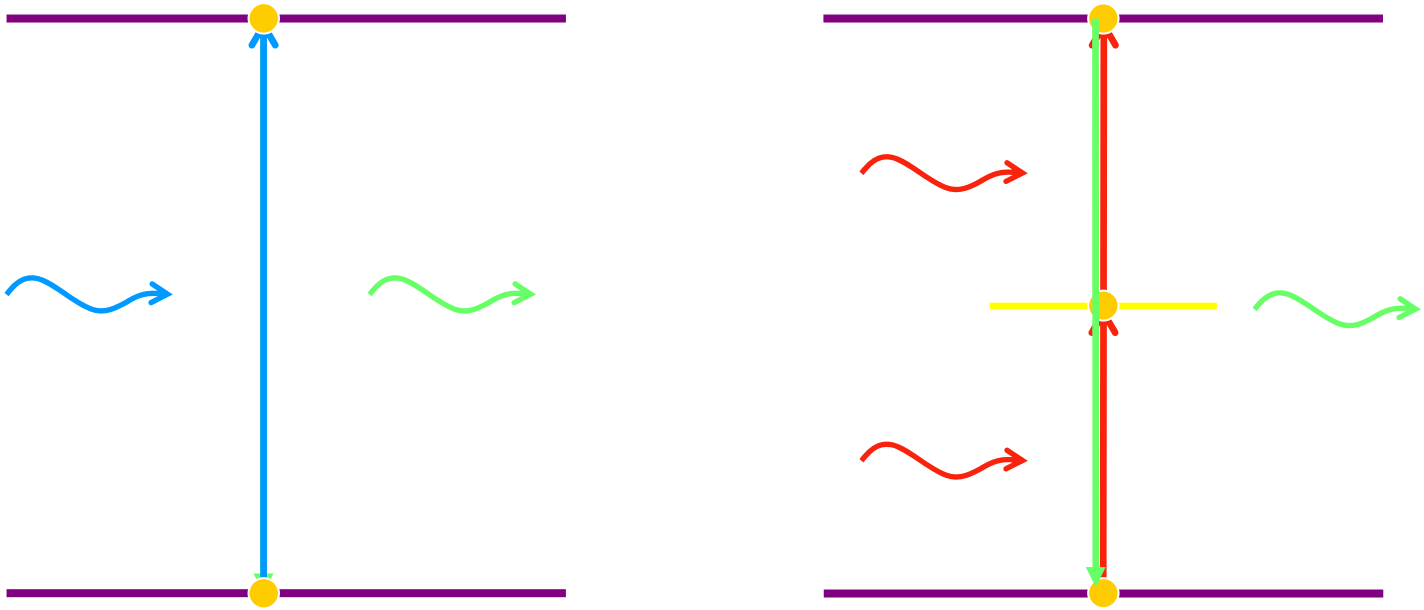
- Mission Statement:

  To establish a state-of-the-art, interdisciplinary optical microscopy core facility to help life scientists in solving biomedical problems

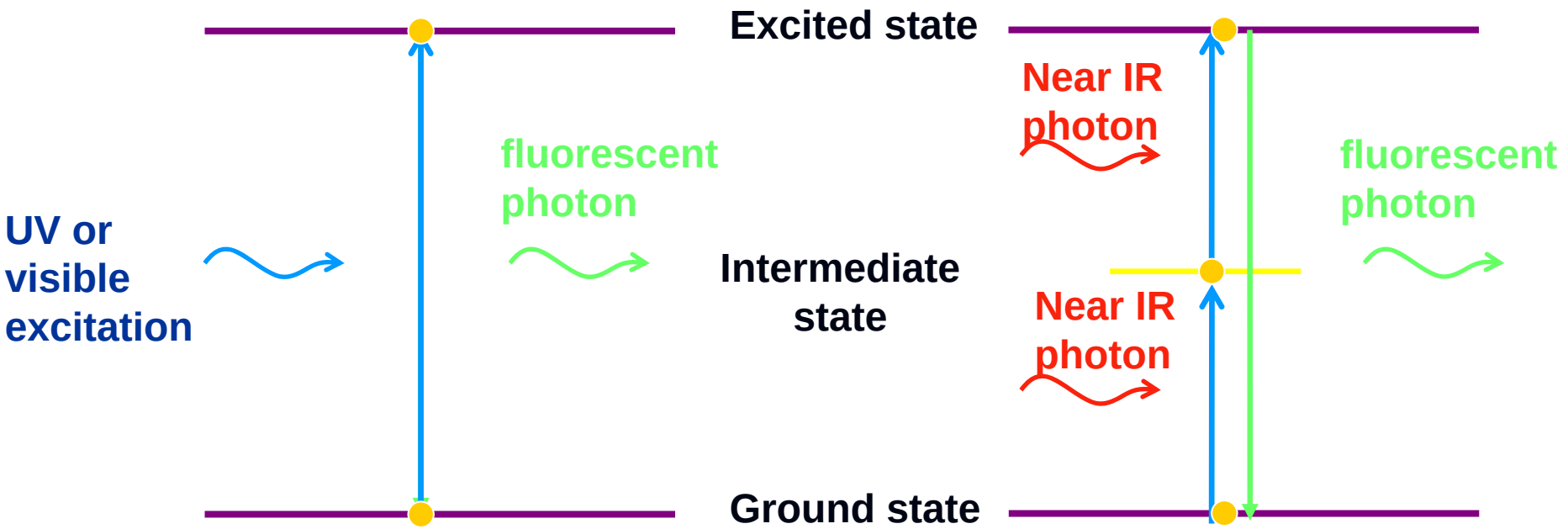
  1. **R&D**
  2. **Service**

Research Approach

1. Technical developments of imaging modalities
   - non-linear optical microscopy
2. Ex-vivo spectral characterization of tissue morphology
3. Intravital investigations of biologically and medically relevant issues
   - intravital imaging chambers



Two-Photon Fluorescence Excitation



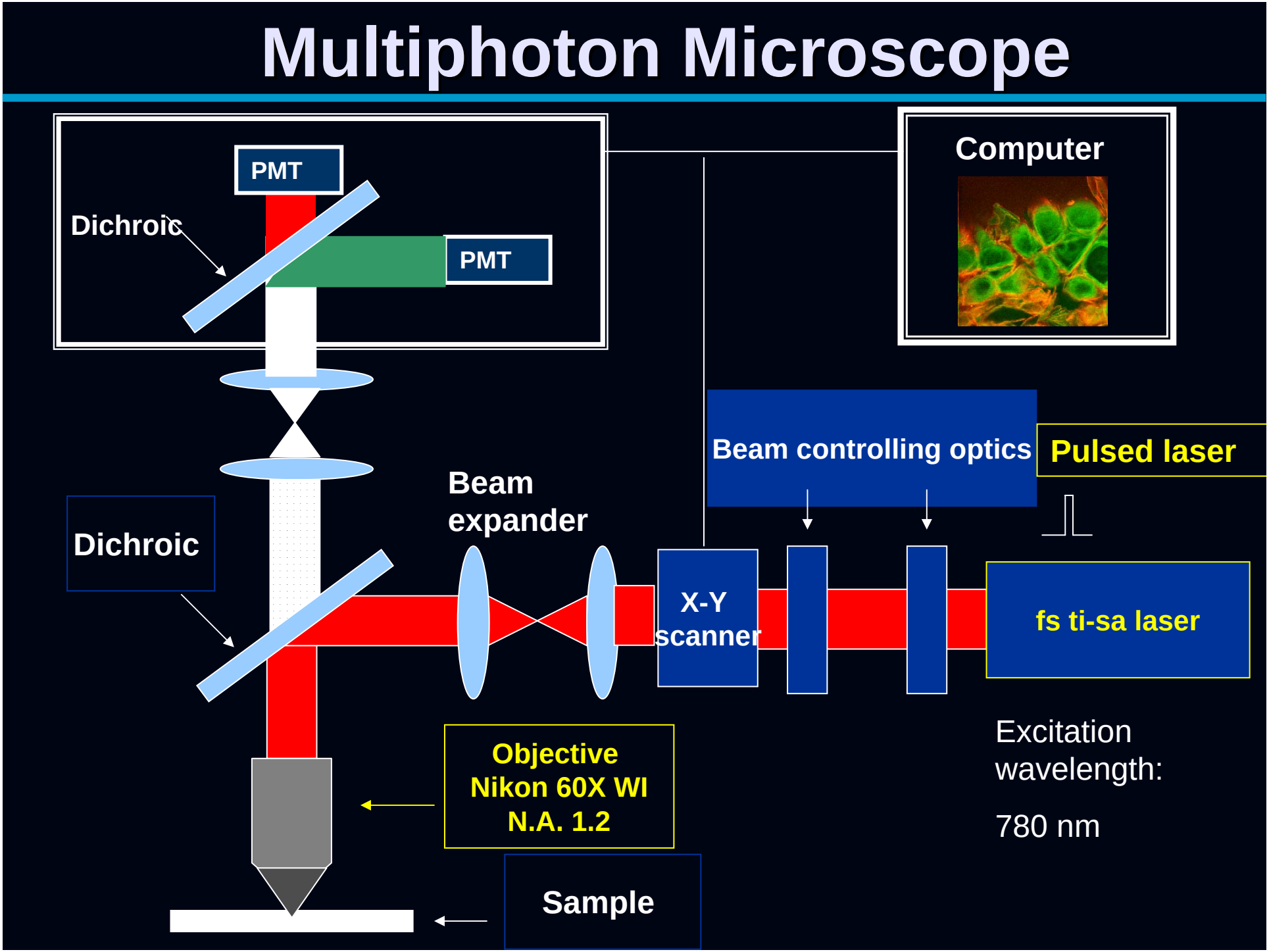
Single photon excitation probability is proportional to the excitation intensity.

Two photon excitation probability is proportional to the **square** of the excitation intensity!

Multiphoton Microscopy

- Two-Photon Microscopy: observational and manipulation technology
  - point-like, non-linear fluorescence excitation
  - deeper penetration (3-D, tissue imaging)
  - easy accessibility to emission spectrum
  - 3-D control of photochemical reactions
- Harmonic Generation Microscopy
  - $D=\varepsilon_o E+P$
  - $P_i=\chi_{ij}(1)E_j+\underline{\chi_{ijk}(2)E_jE_k}+\chi_{ijkl}(3)E_jE_kE_l+\ldots$
  - Biological harmonic generators: collagen, muscle fibers, …

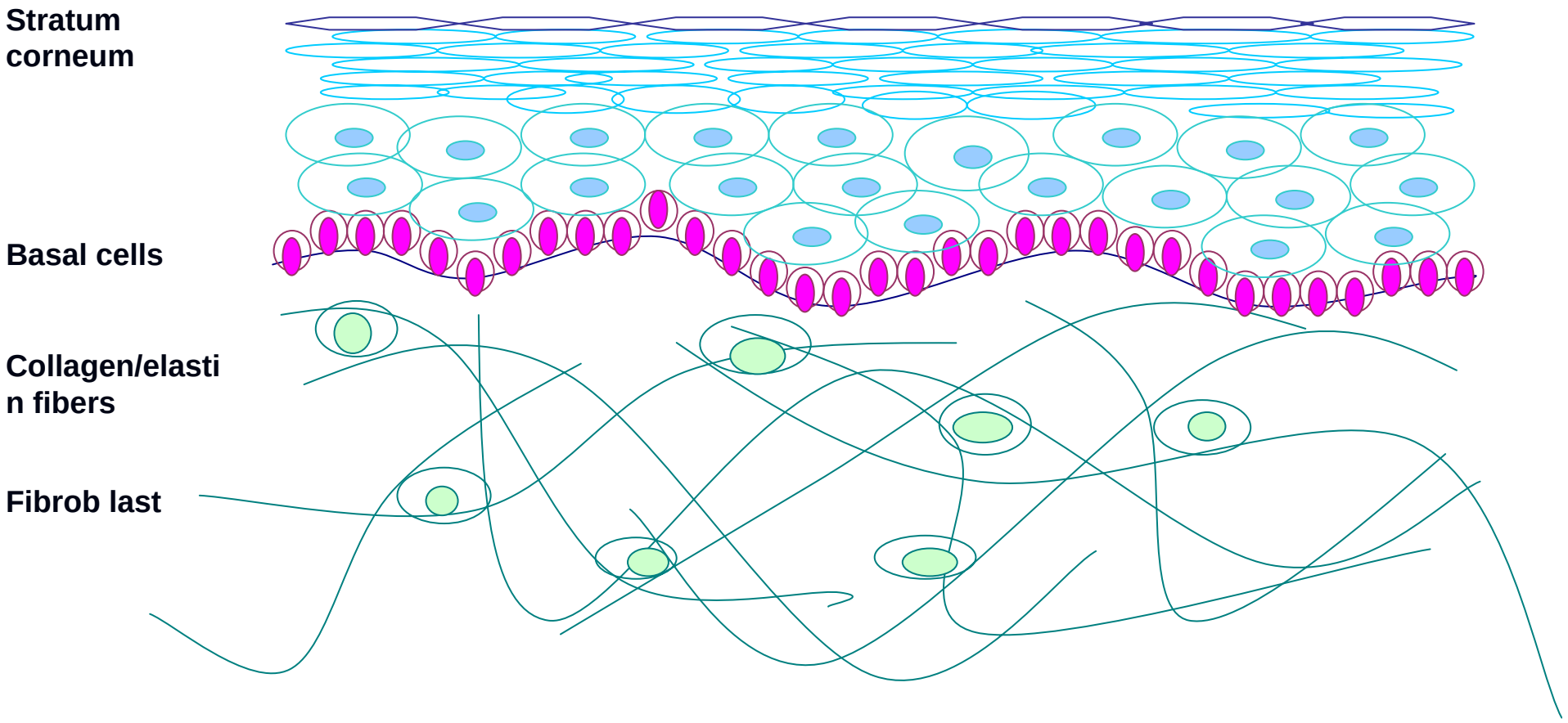
Multiphoton Microscope



Multiphoton Microscopy Applications

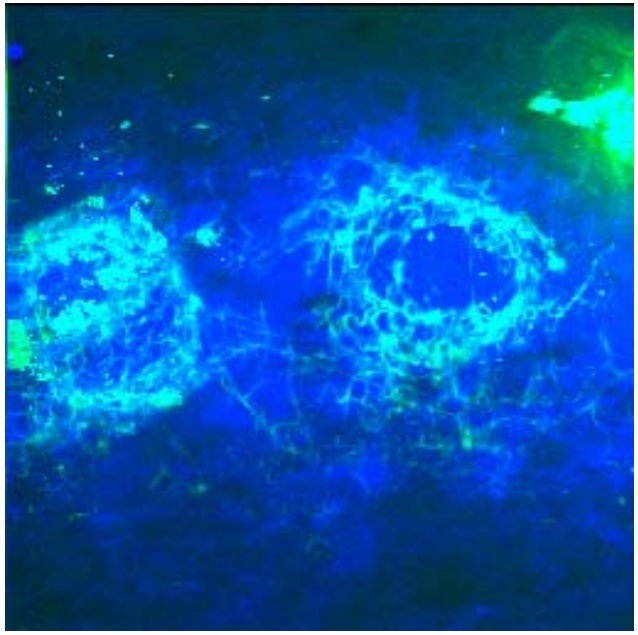
1. Dermatology
    - skin photoaging
2. Ophthalmology
    - minimally-invasive characterization of eye structures
    - corneal pathology (infection, keratoconus, scar)
    - collagen thermal denaturation
3. Tumor Physiology
    - multiphoton tissue characterization (BCC)
    - development of intravital imaging technologies
4. Hepatology
    - observation of hepatic metabolism
    - bile duct ligation
5. Tissue Engineering
    - scaffold imaging
    - chondrogenesis
6. Infectious Pathogens
    - fungi

Skin Structure (similar to eye)



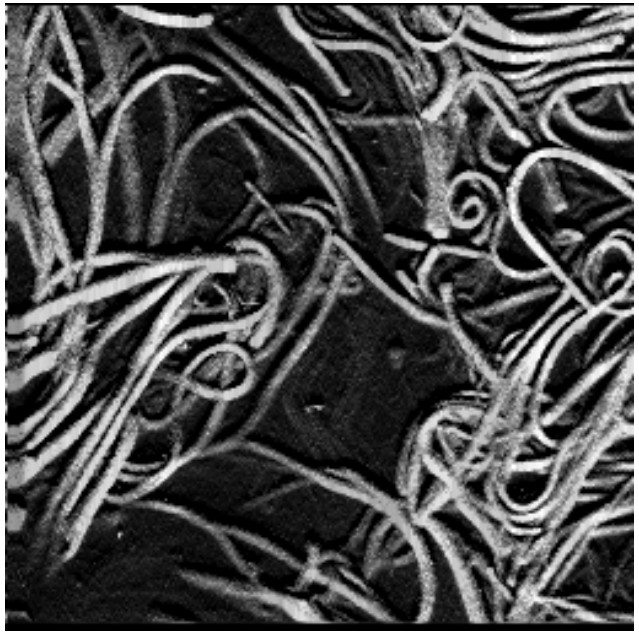
3-D Multiphoton Skin Movies

Epidermal-dermal junction



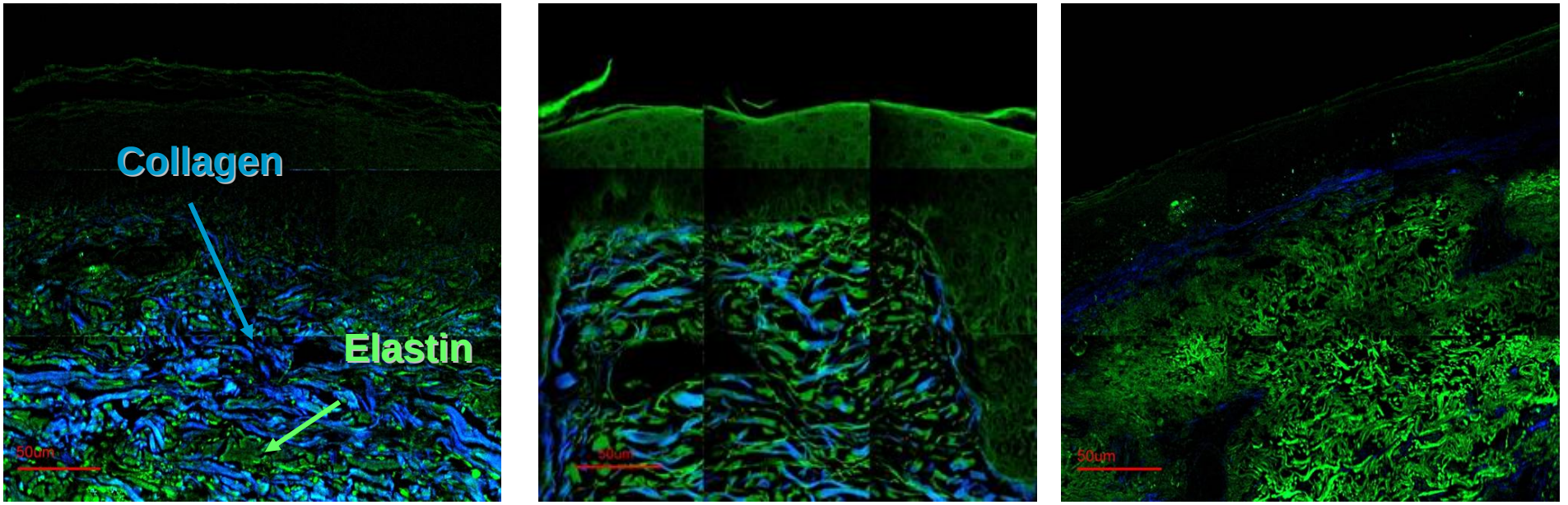
Dermal collagen and elastic fiber orientation

Elastic fiber projection



Skin Photoaging: Autofluorescence (AF) and Second Harmonic Generation (SHG) Imaging

20 years of age | **40 years of age** | **70 years of age**

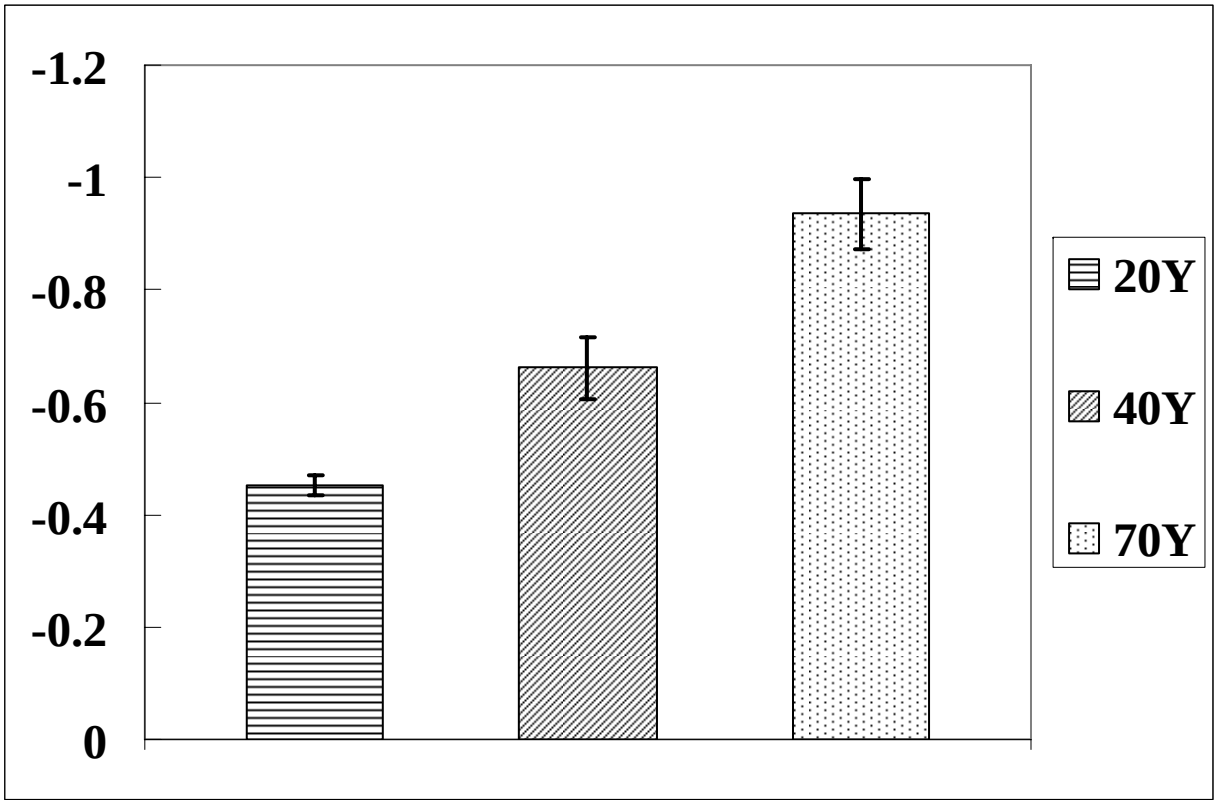


SHG: blue
AF: green

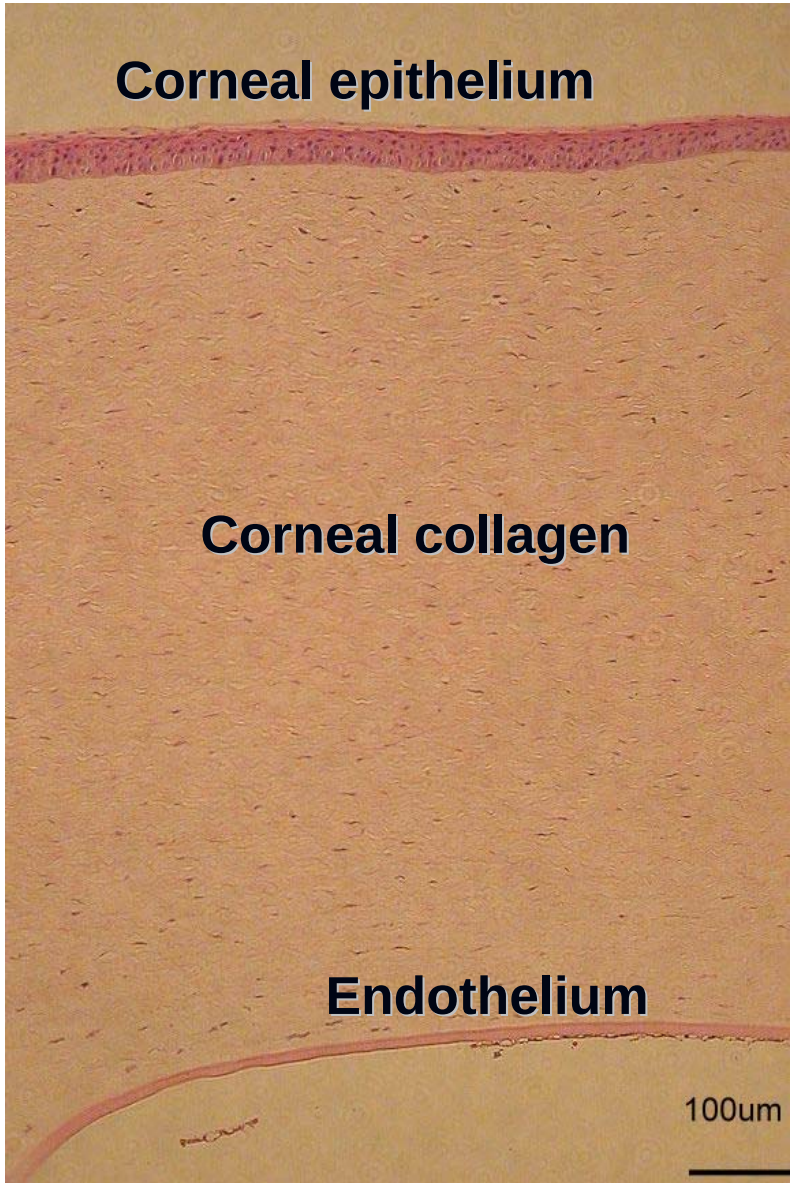
Lin et al., *Optics Letters*, 30(17): 2275-2277, (2005).

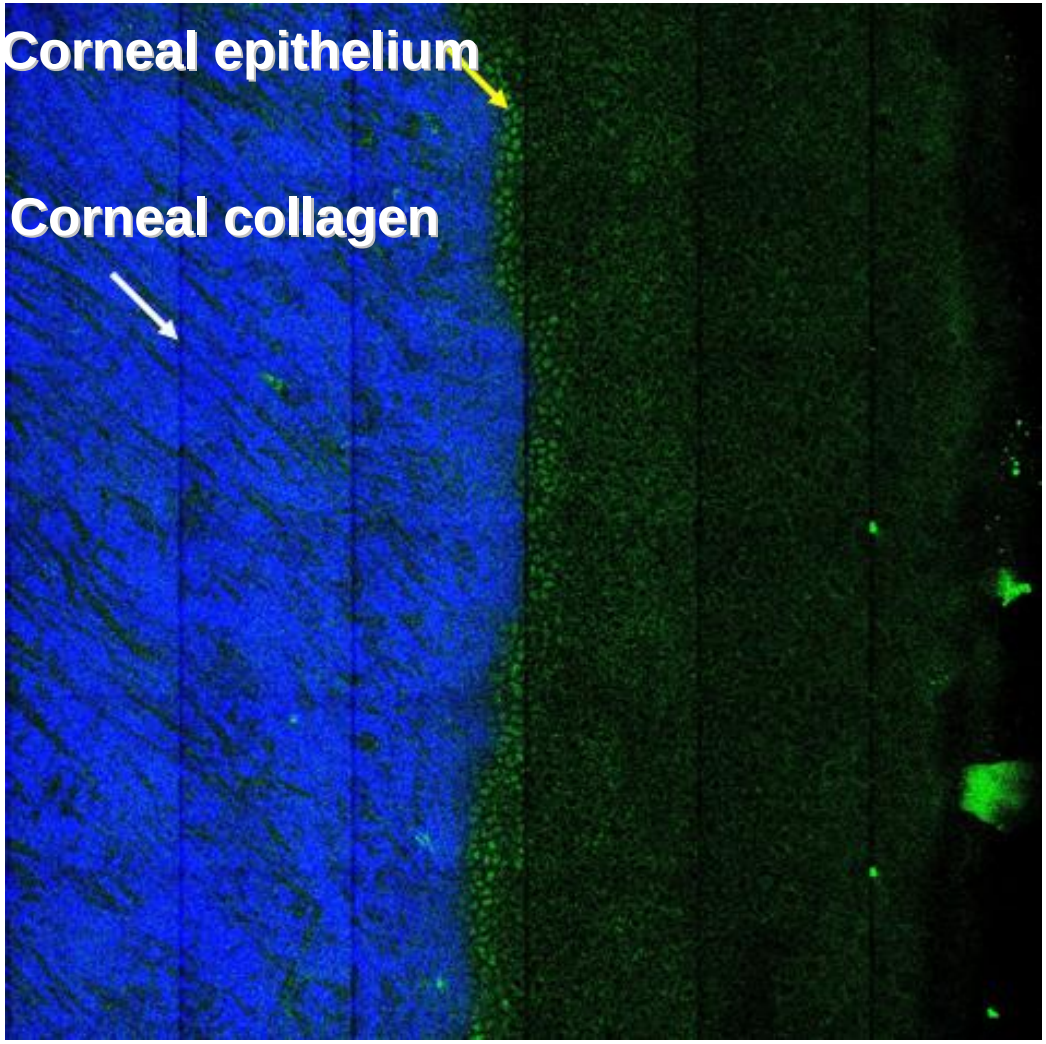
Quantitative Determination Using SHG to AF Aging Index

Skin photoaging index=
(SHG-AF)/
(SHG+AF)



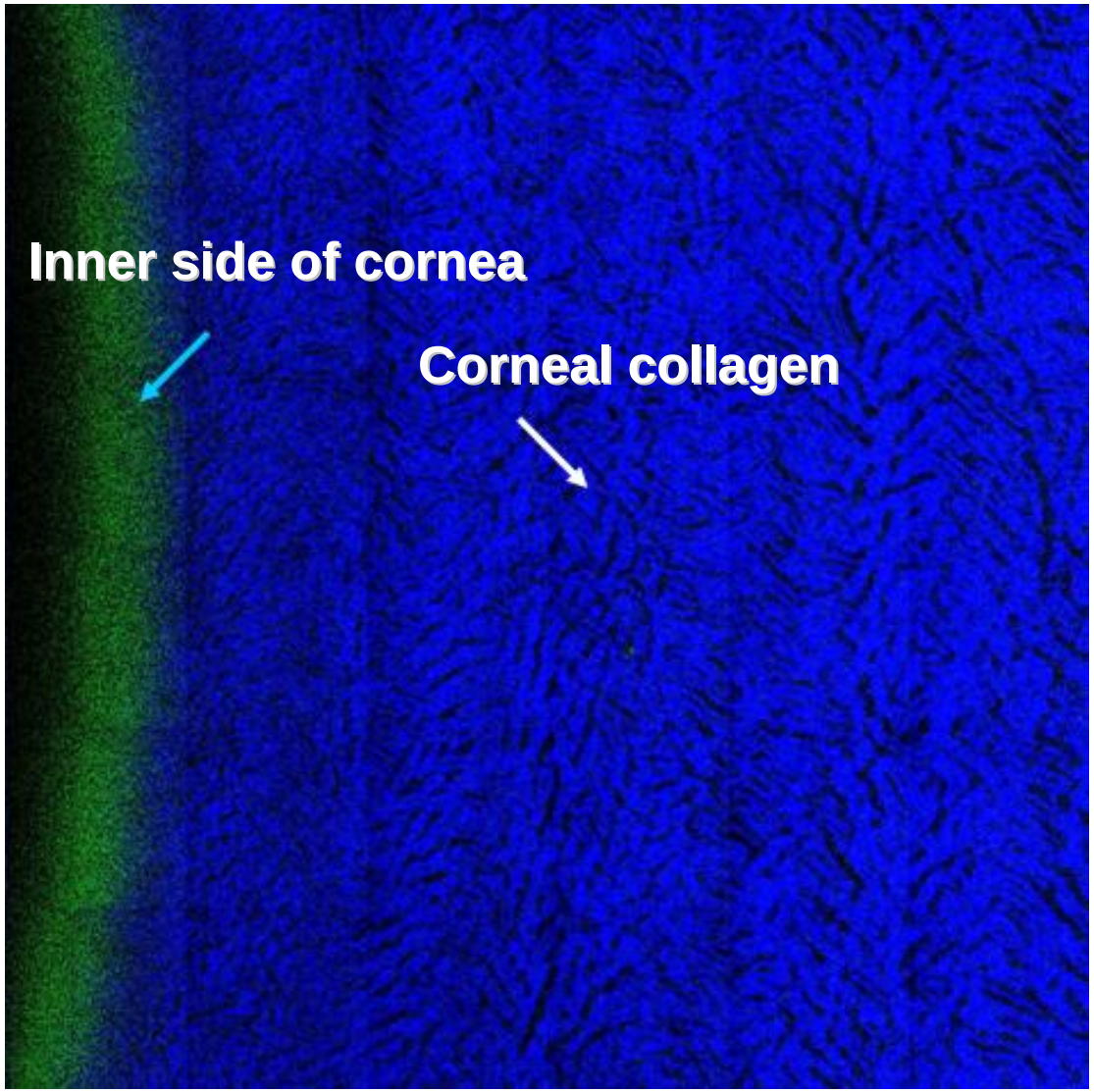
Porcine Cornea Structure





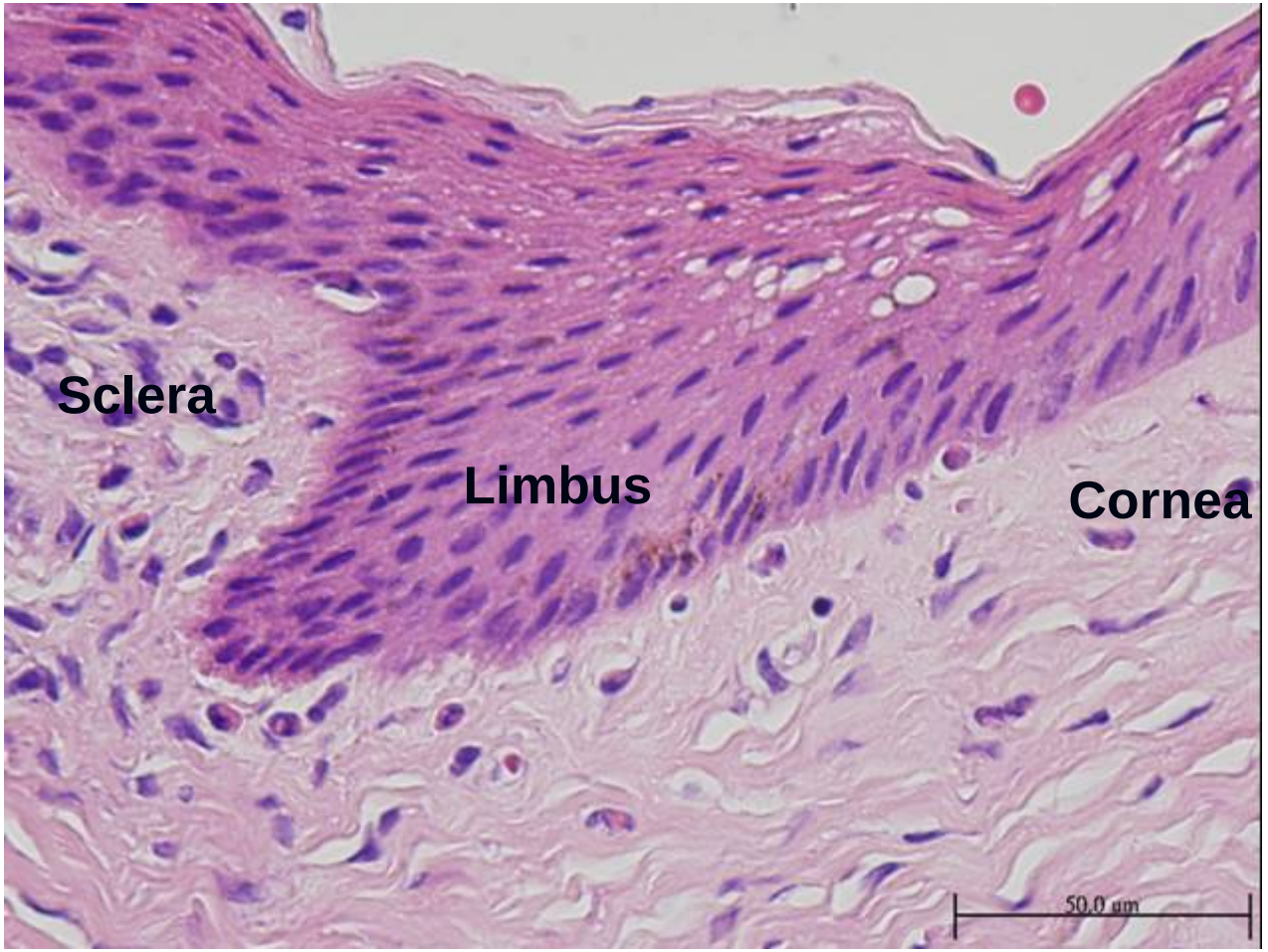
Teng et al., *IOVS*,
47: 1216-1224 (2006).

Porcine Cornea (Depth: 862μm)



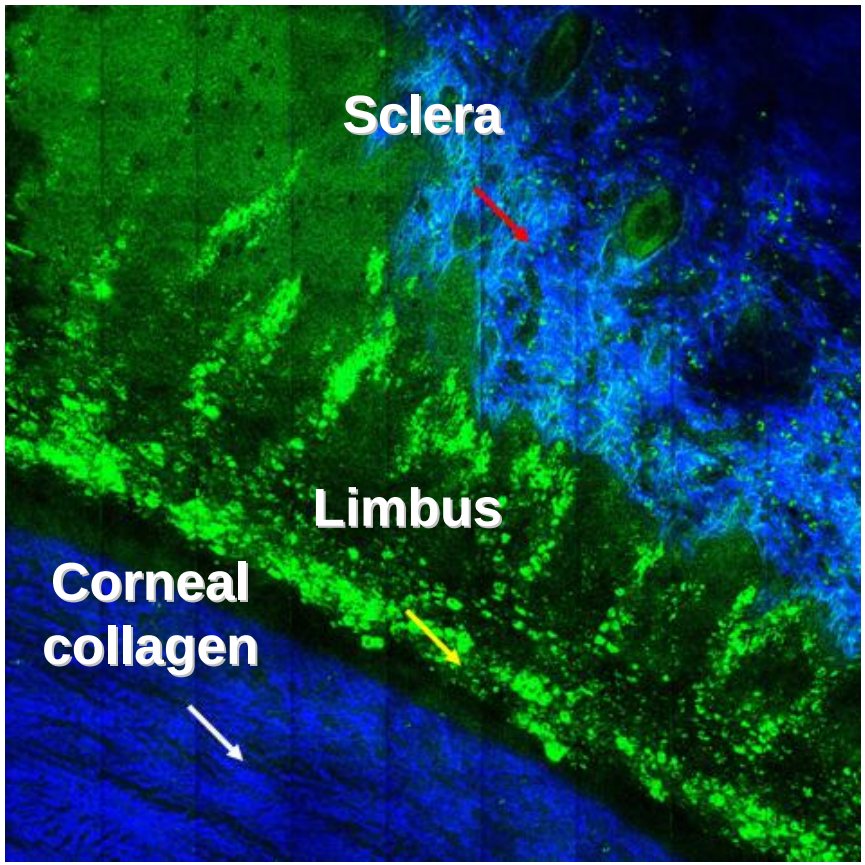
SHG: blue
AF: green

Porcine Limbus Structure

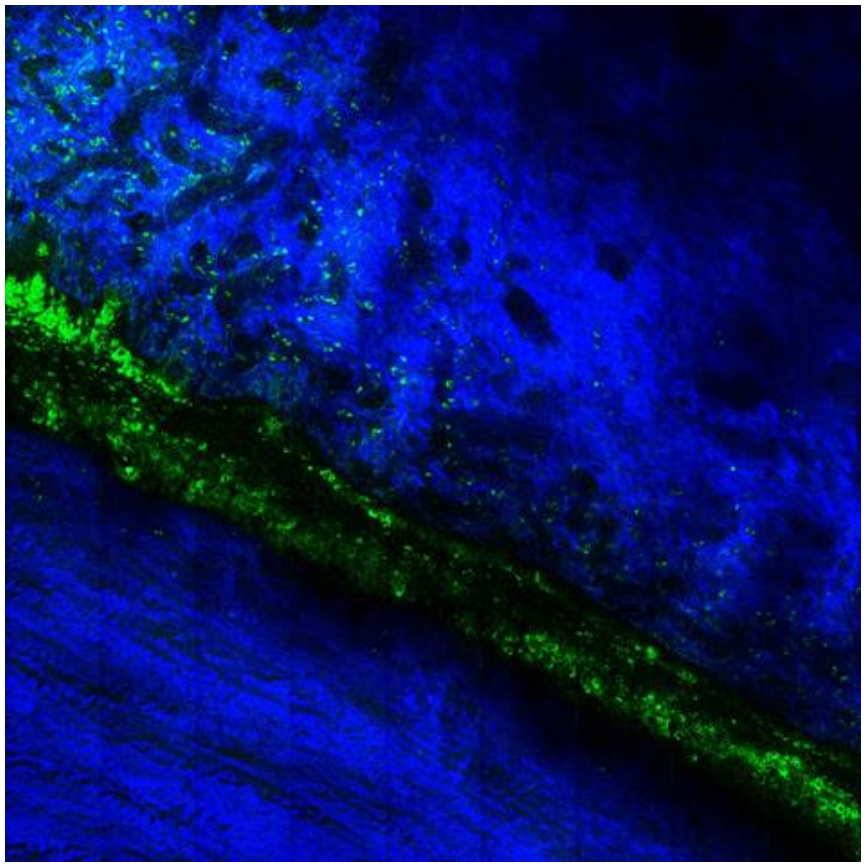


Multiphoton Scan of Porcine Limbus

Depth: surface



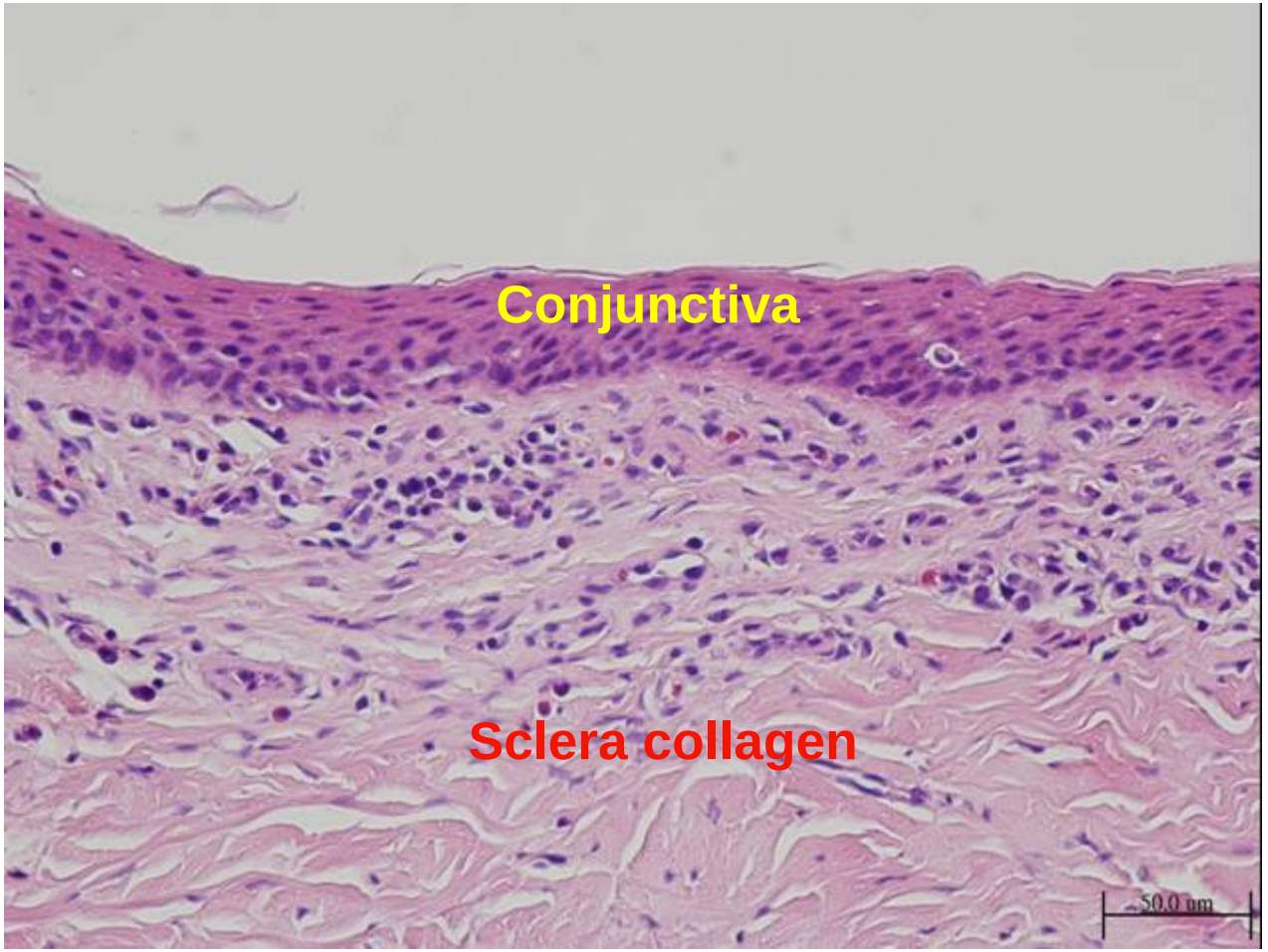
Depth: 50 μm



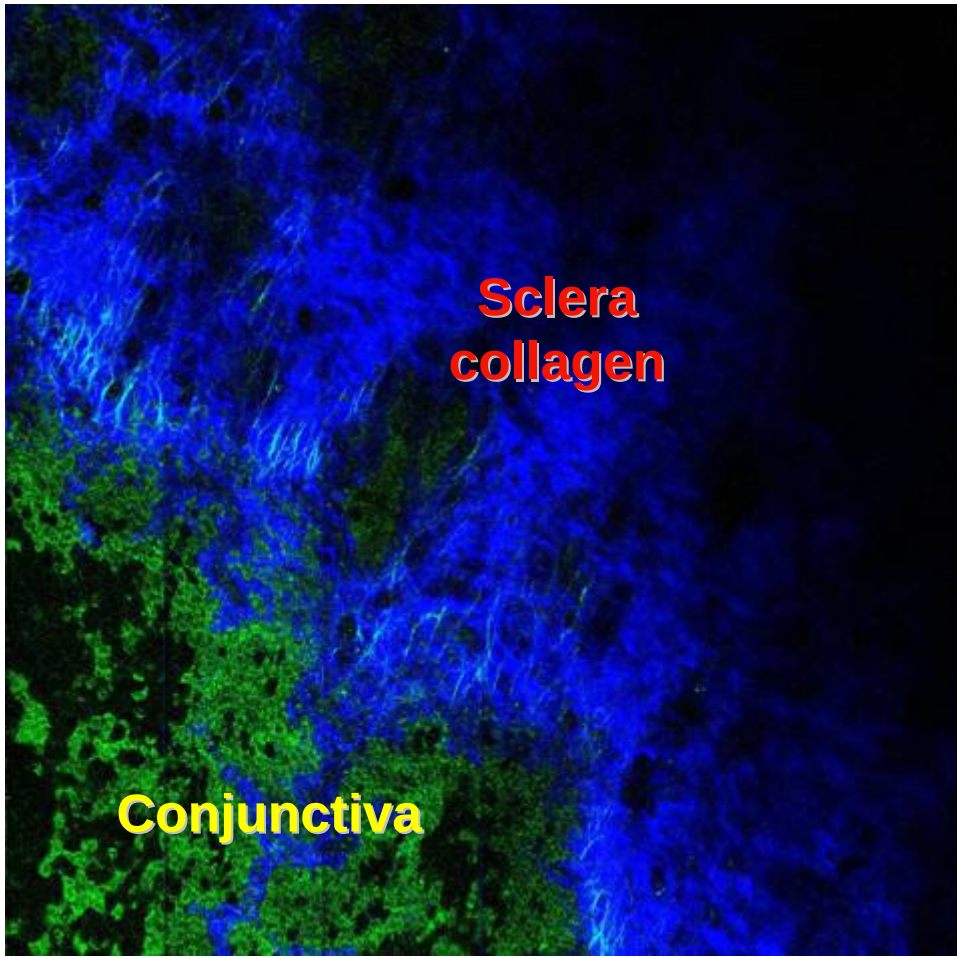
100 μm

SHG: blue
AF: green

Porcine Sclera Structure

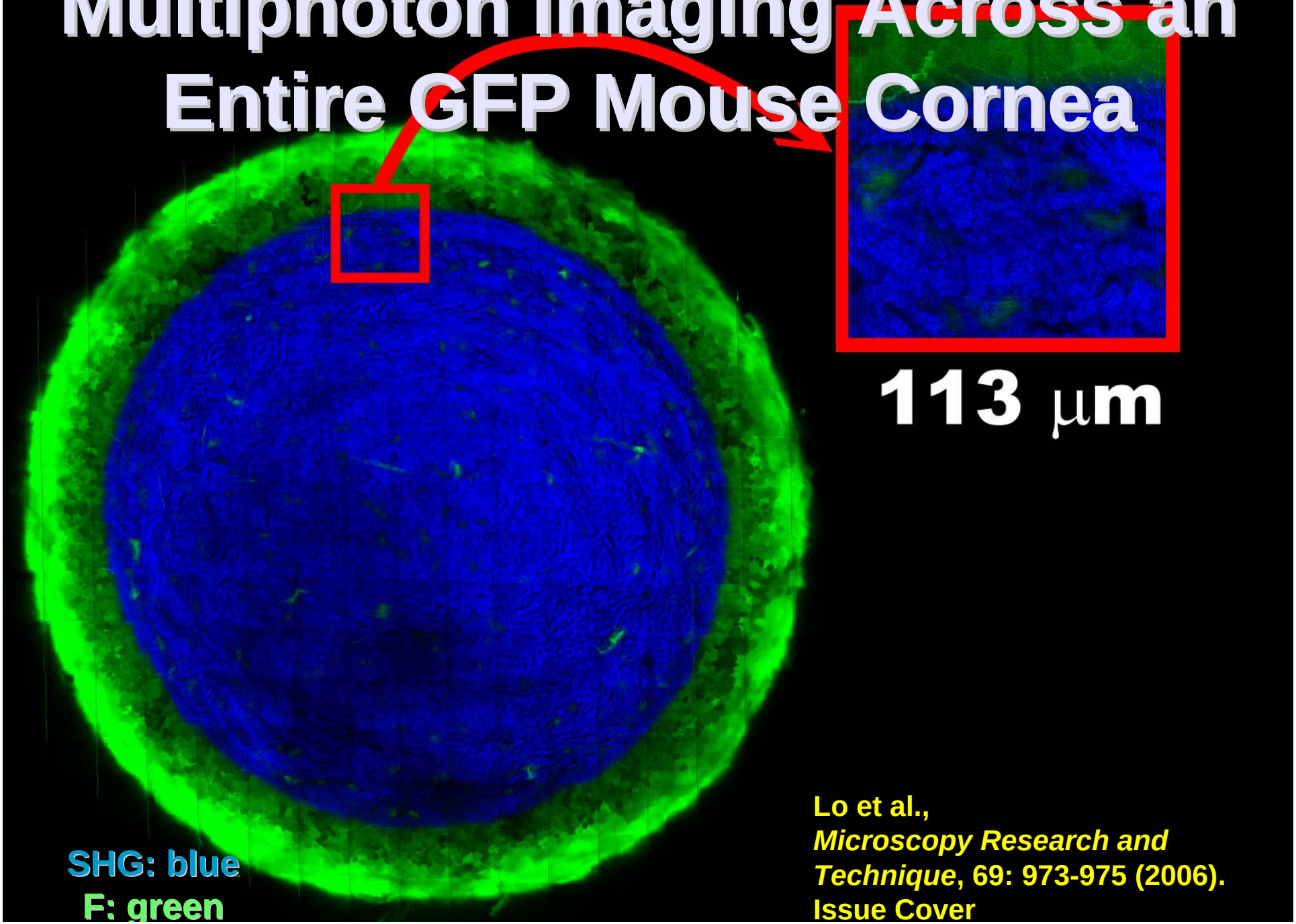


Multiphoton Porcine Sclera Imaging (Depth: 50μm)



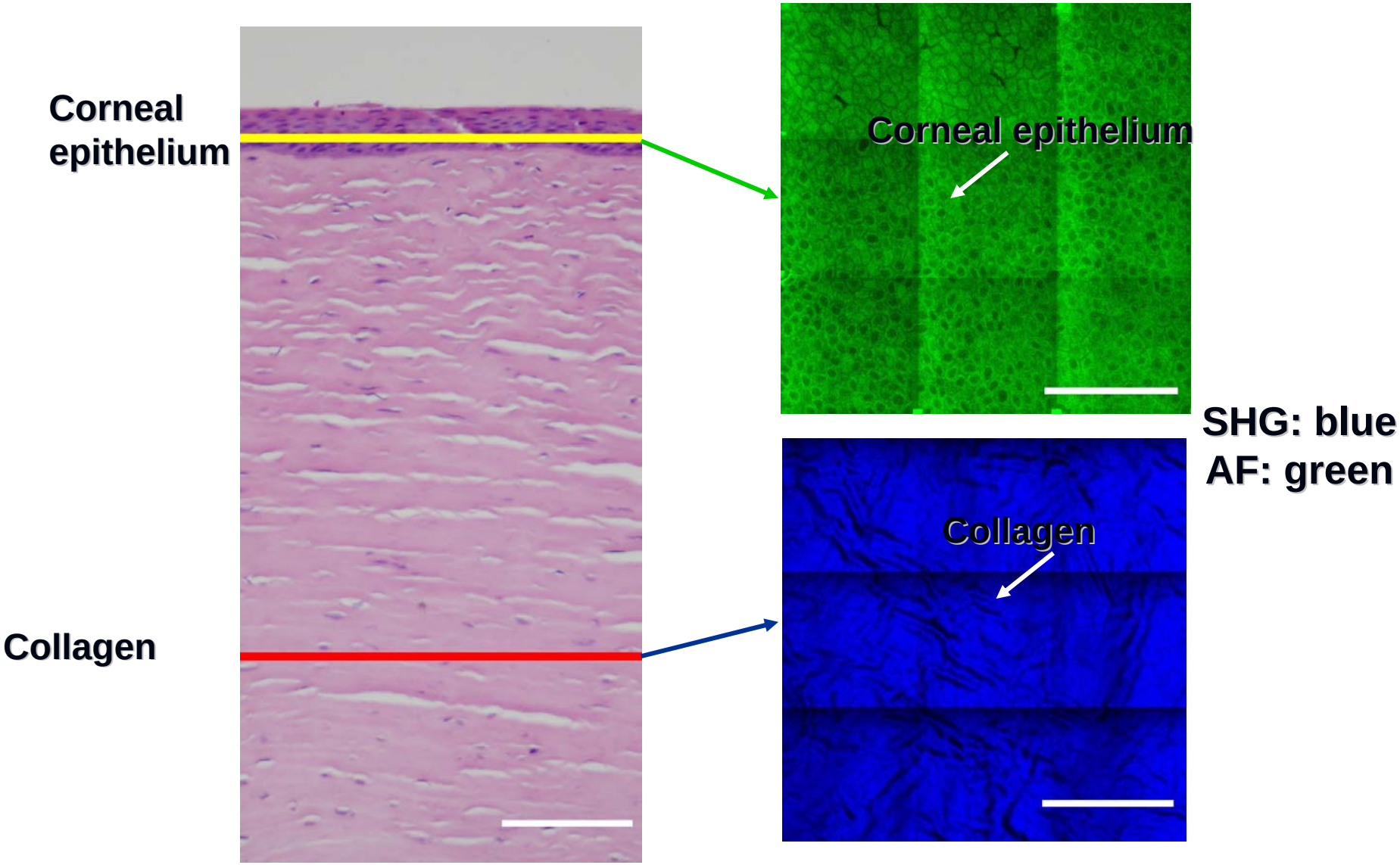
SHG: blue
AF: green

Multiphoton Imaging Across an Entire GFP Mouse Cornea

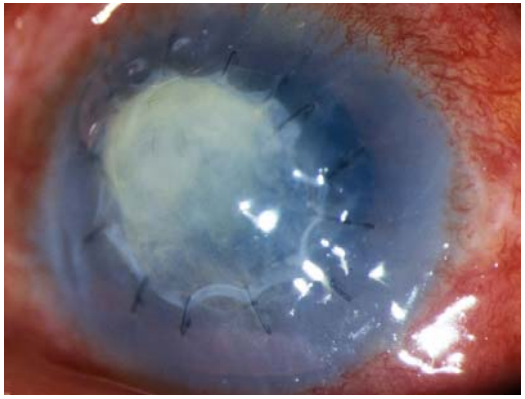


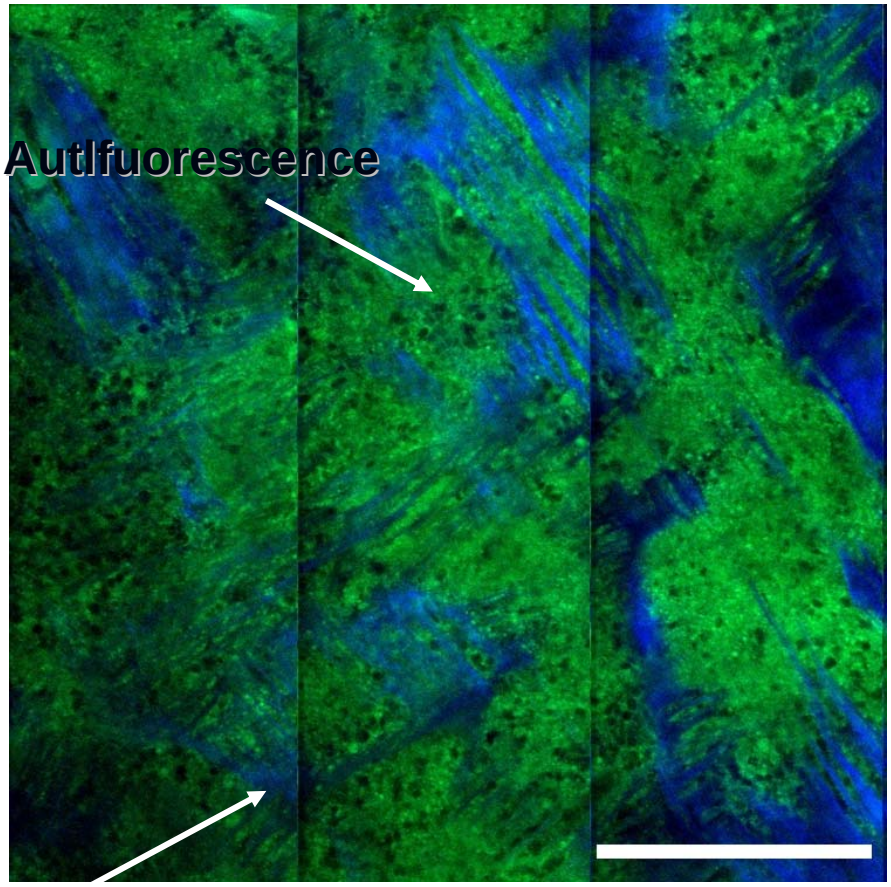
Lo et al.,
Microscopy Research and Technique, 69: 973-975 (2006).
Issue Cover

Multiphoton Imaging of Normal Human Cornea



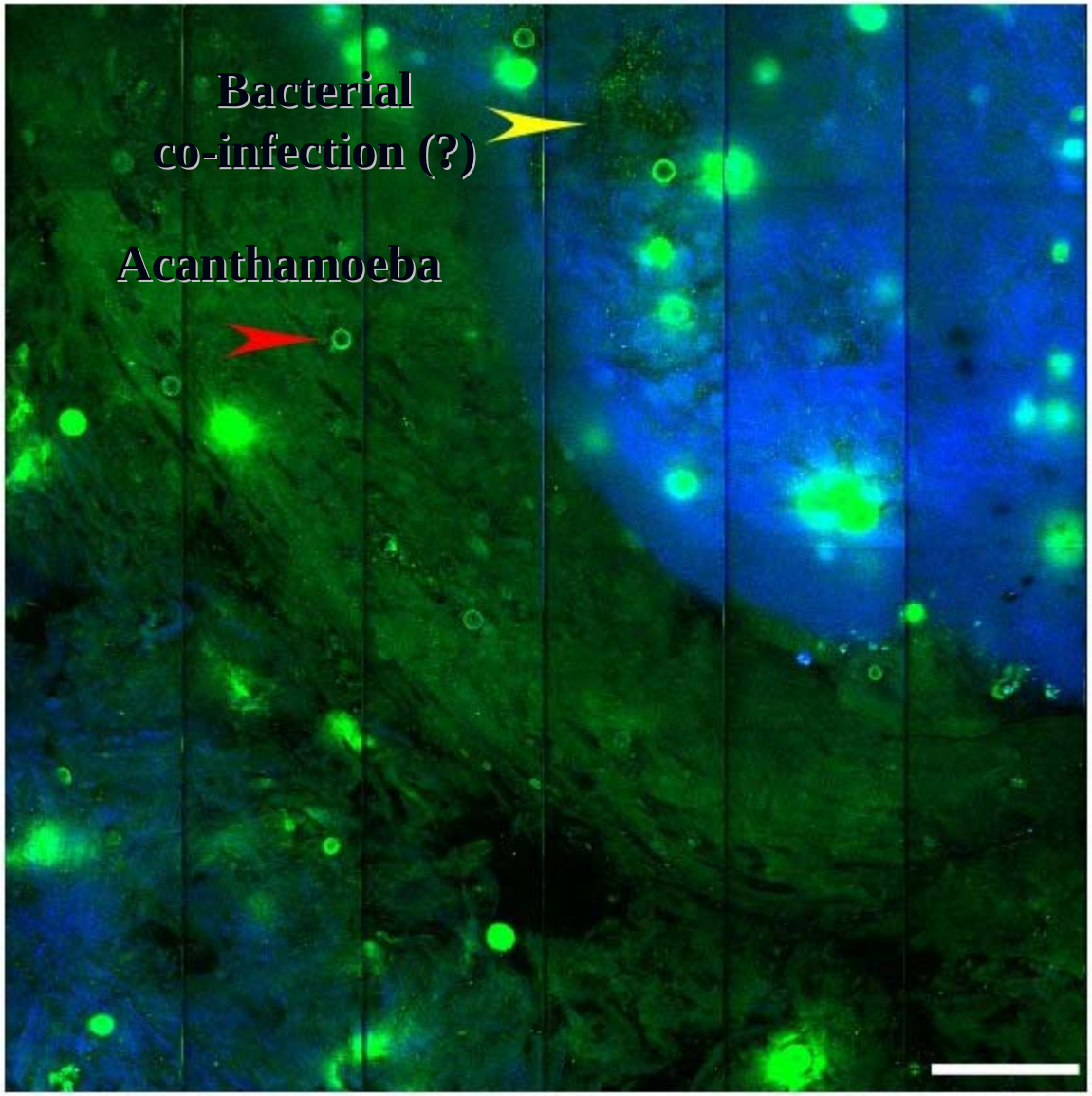
Multiphoton Imaging of Fungal (*Fusarium solani*) Infection

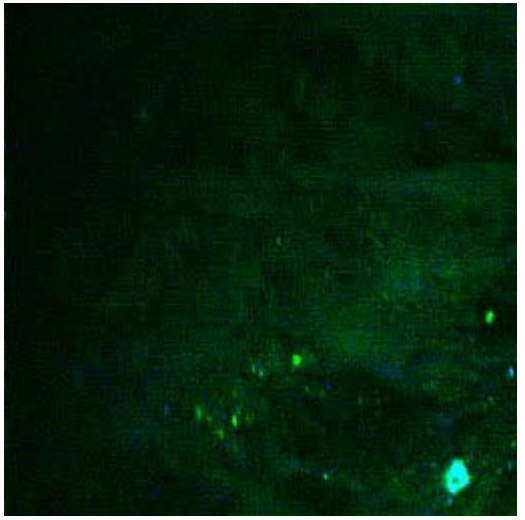




SHG: blue
AF: green

Acanthamoeba Keratitis





SHG: blue
AF: green

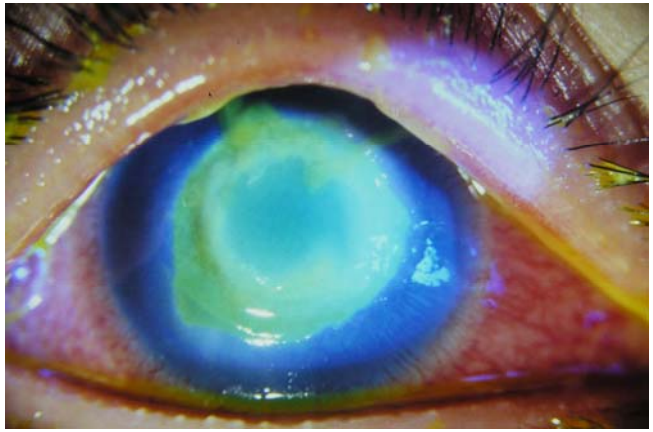
Tan et al., *Journal of Biomedical Optics*,
In press.

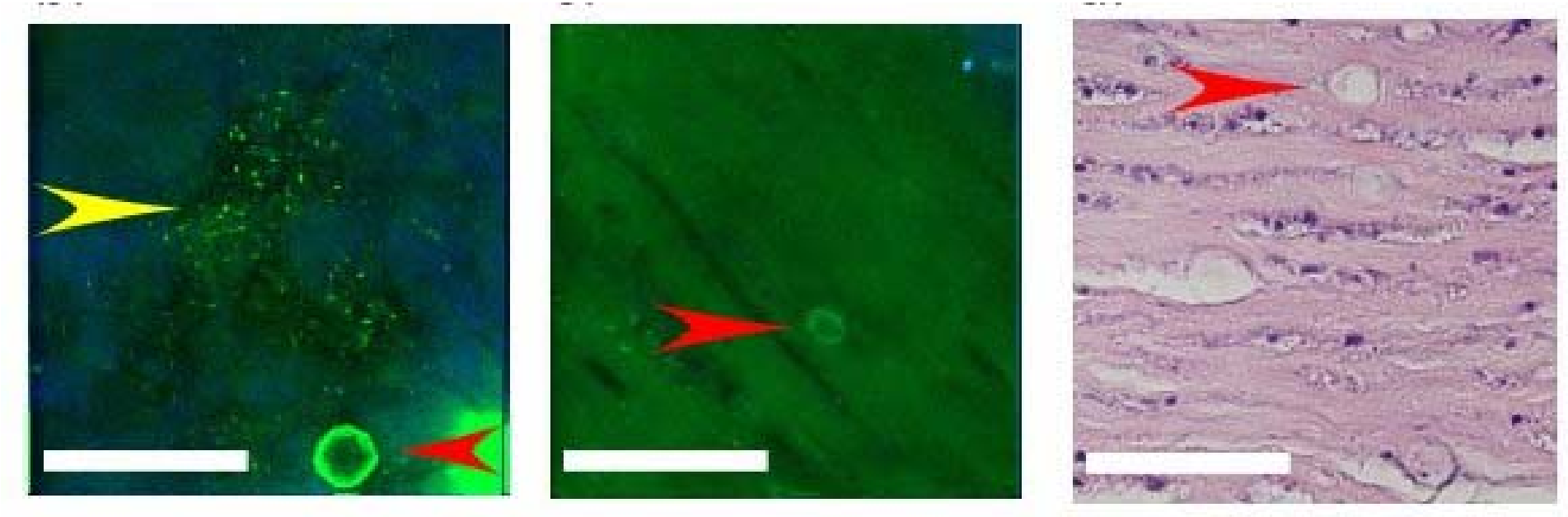
Acanthaamoeba Infection

- Culture: Acanthamoeba castellanii + Pseudomonas aeruginosa

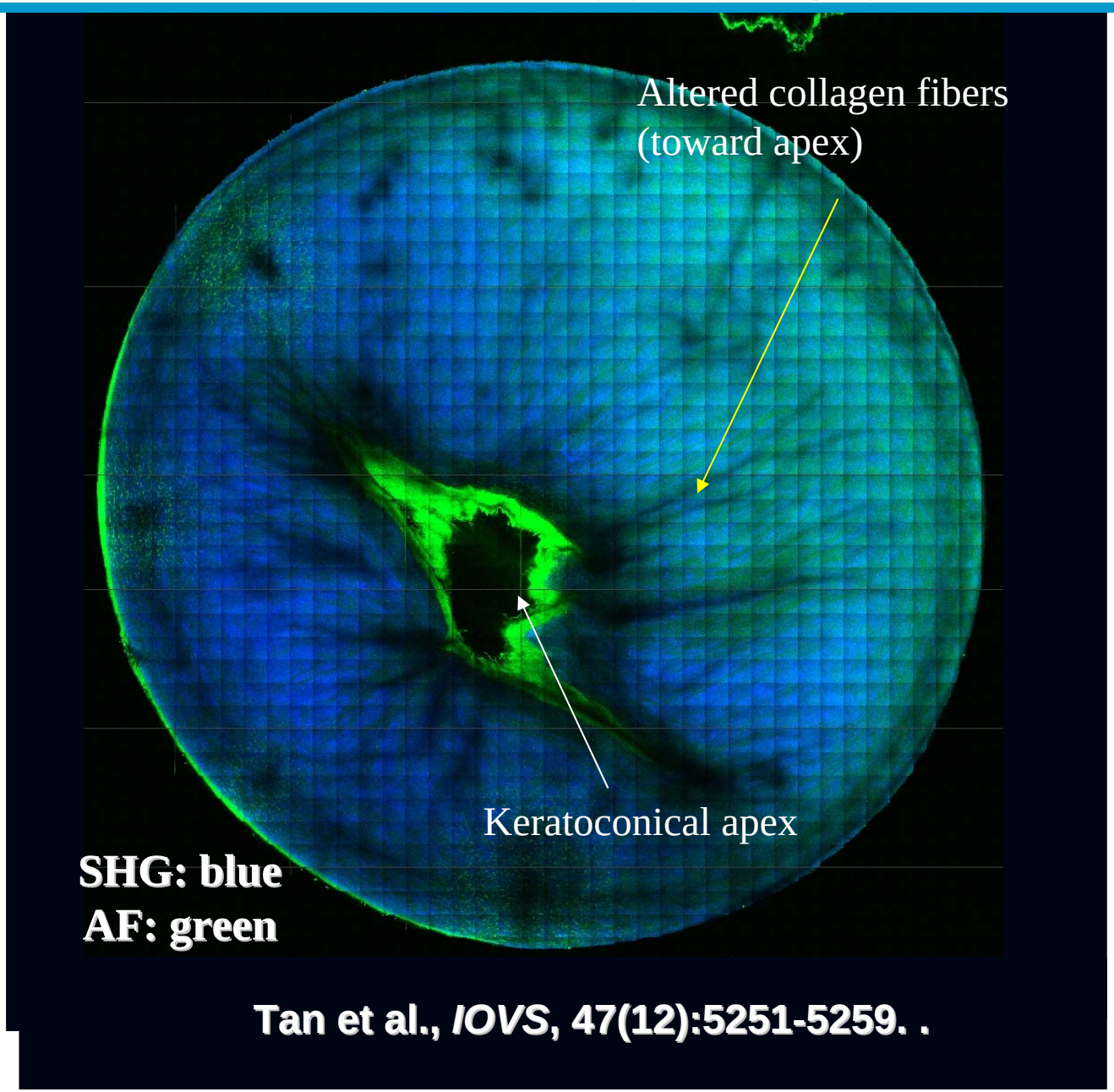
SHG: blue
AF: green

Bar: 50 μm



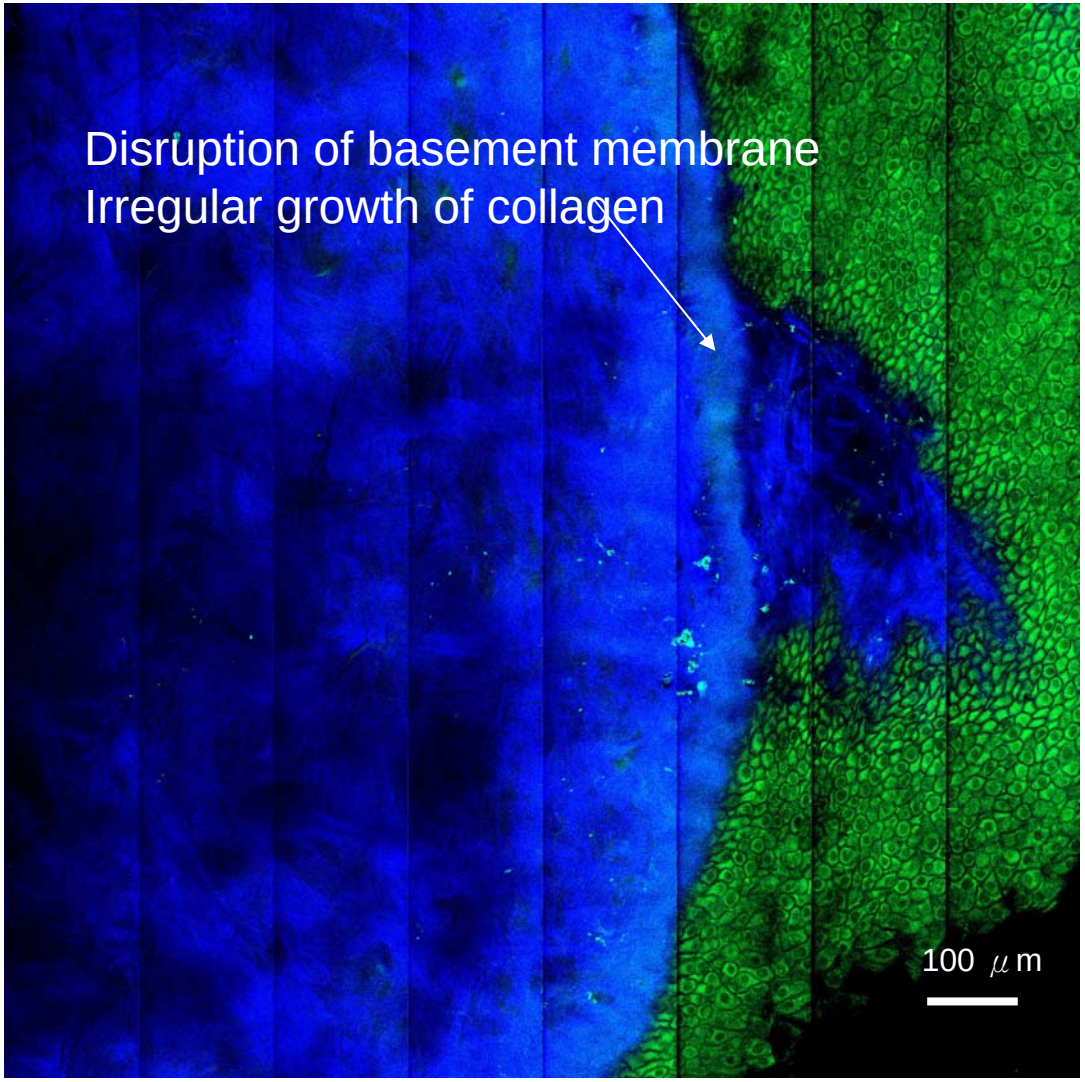


Keratotonus



Tan et al., *IOVS*, 47(12):5251-5259. .

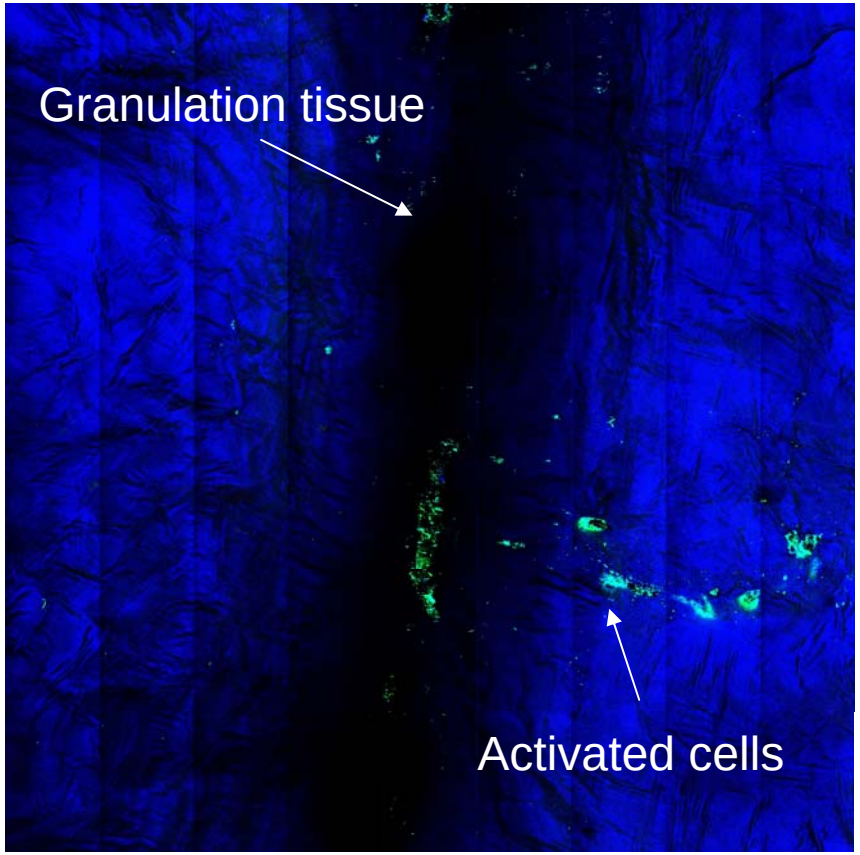
Multiphoton Imaging of Corneal Scar



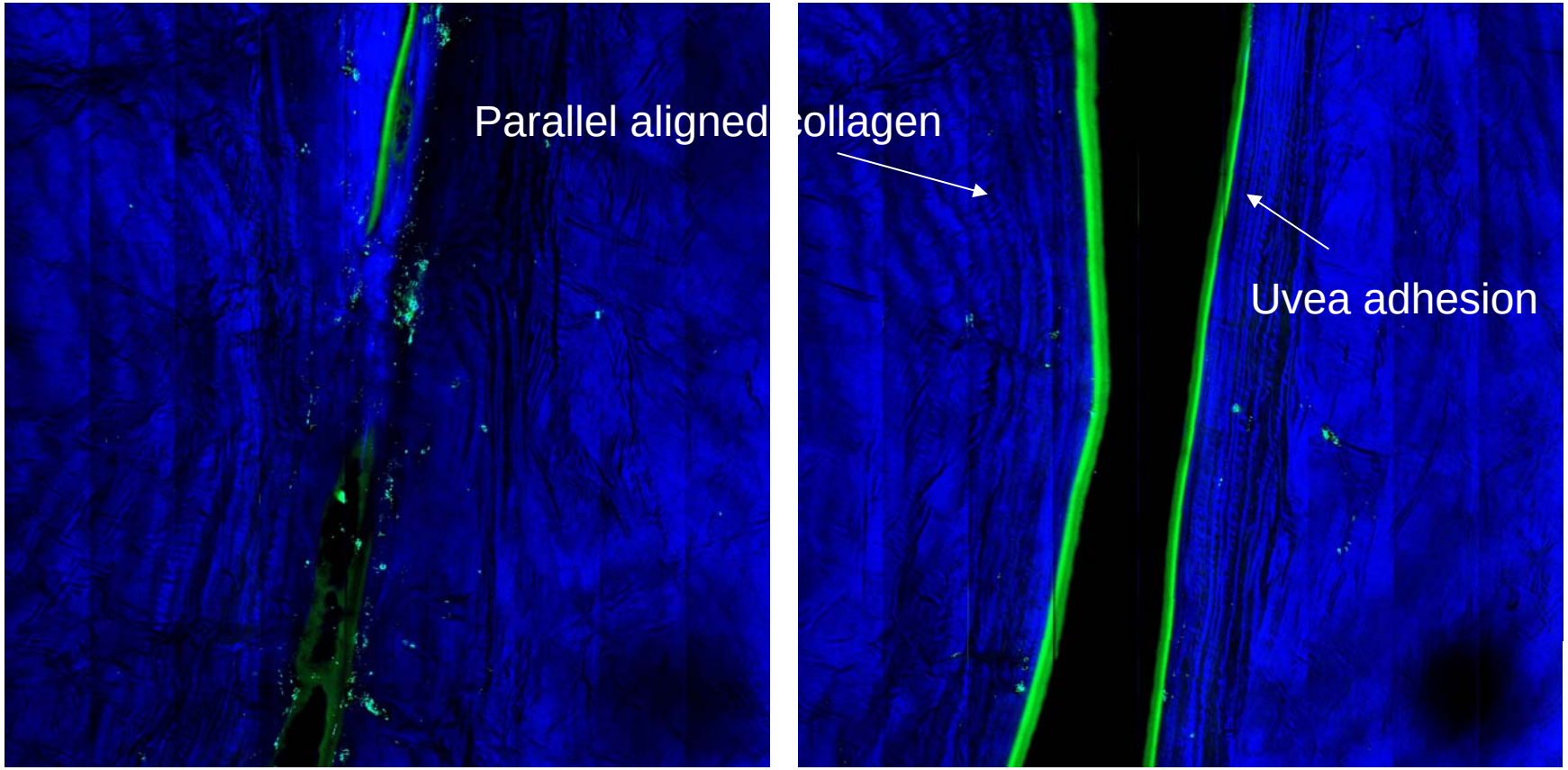
Blue: SHG; λ = 380nm; Green: Autofluorescence λ = 490nm

Teng et al., *Archives of Ophthalmology*, Accepted.

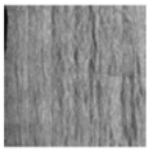
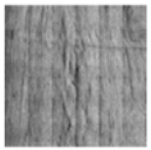
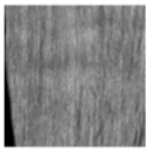
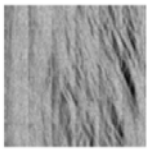
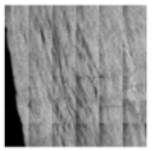
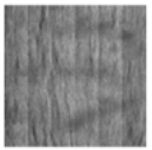
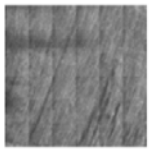
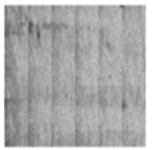
Multiphoton Imaging of Corneal Scar



Multiphoton Imaging of Corneal Scar



Collagen Thermal Denaturation (SHG)

$T = 40°C$								
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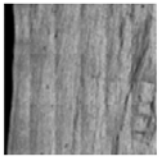
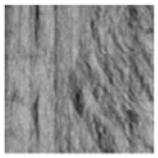
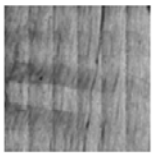
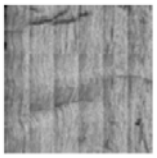
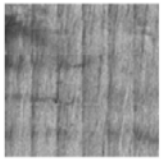
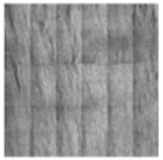
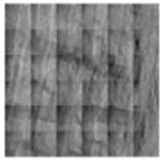
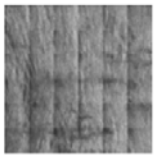
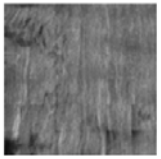
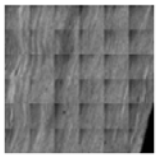
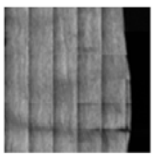
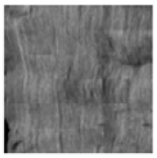
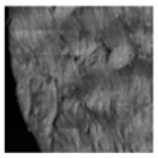
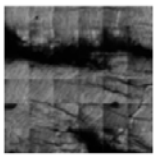
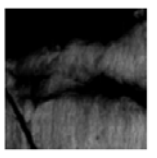
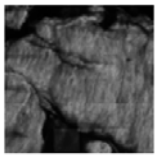
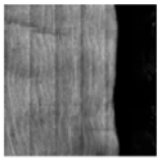
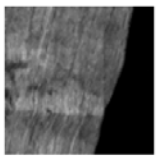
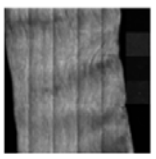
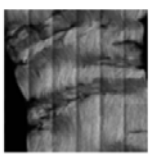
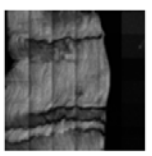
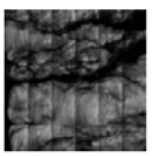
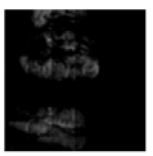
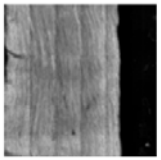
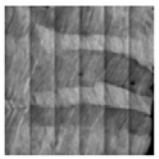
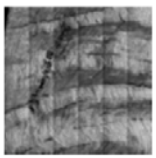
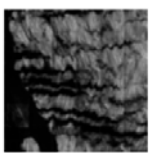
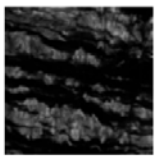
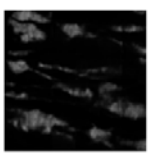
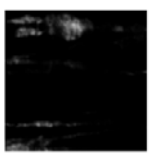
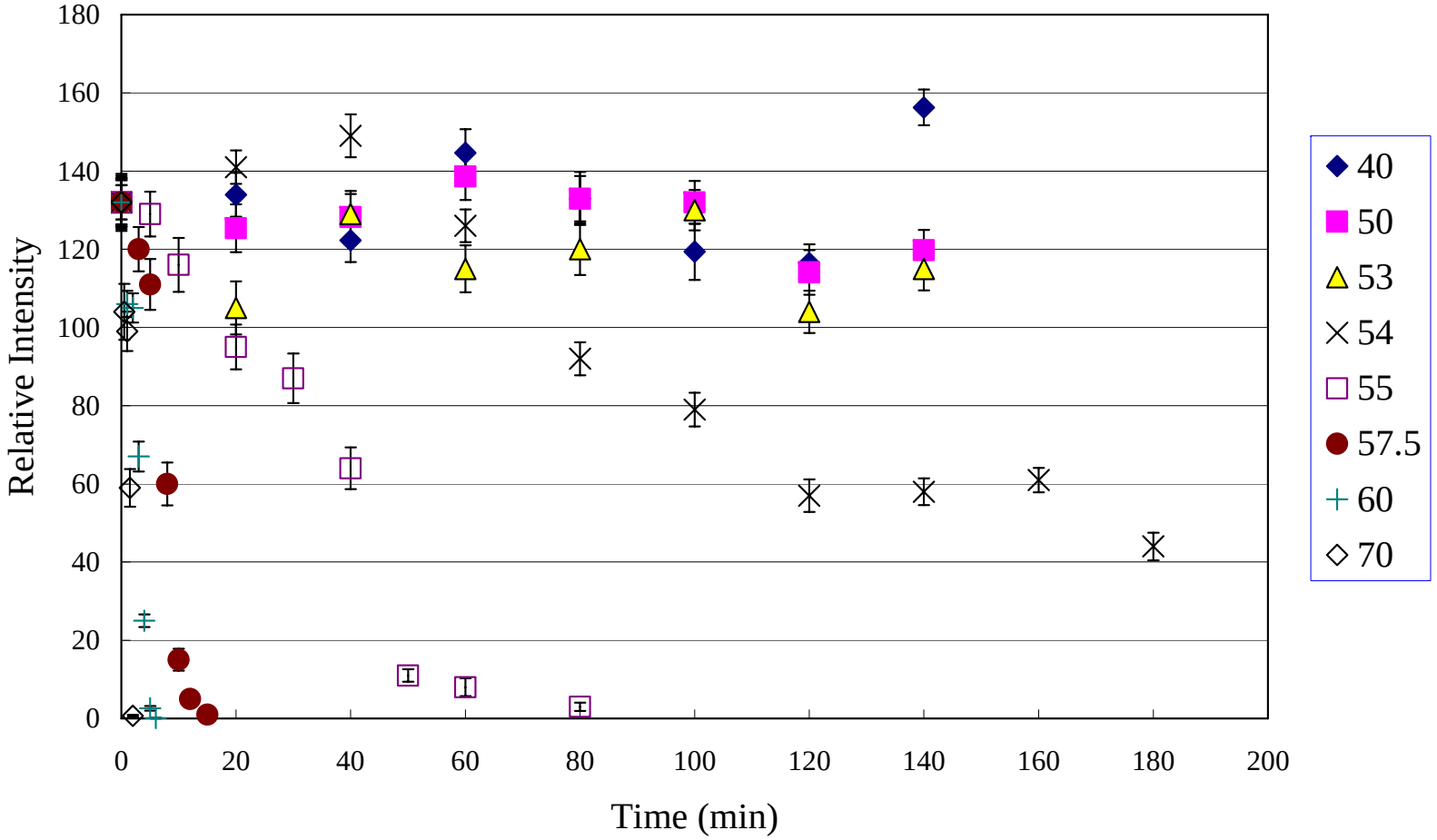
$T = 50°C$								
Time (min)	0	20	40	60	80	100	120	140
$T = 54°C$								
Time (min)	0	20	40	60	80	100	120	140
$T = 55°C$								
Time (min)	0	5	10	20	30	40	50	60
$T = 57.5°C$								
Time (min)	0	3	5	8	10	11.5	12	15

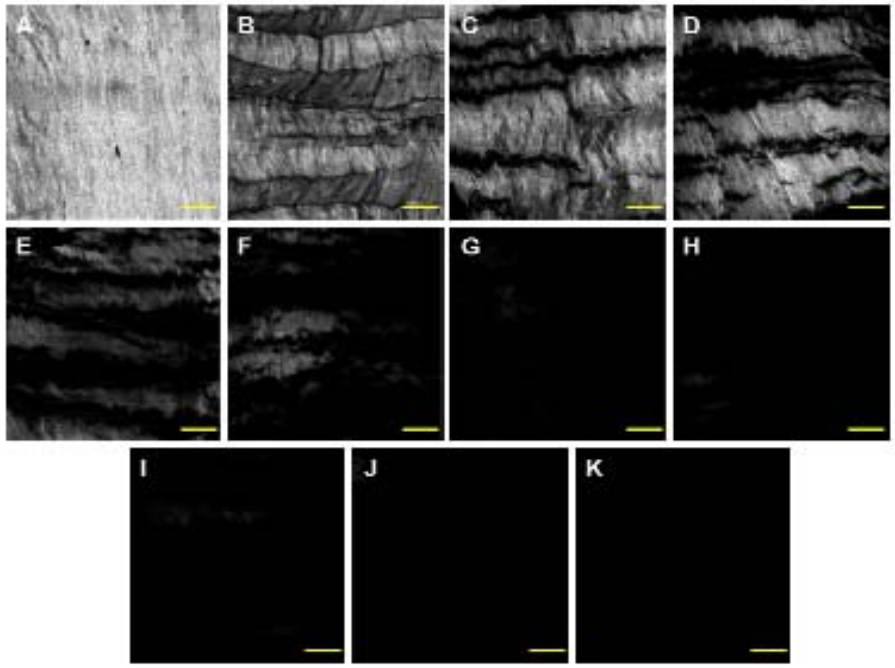
Sun et al.. *Biophysical Journal*. 91:2620-2625 (2006).

Large Area Intensity

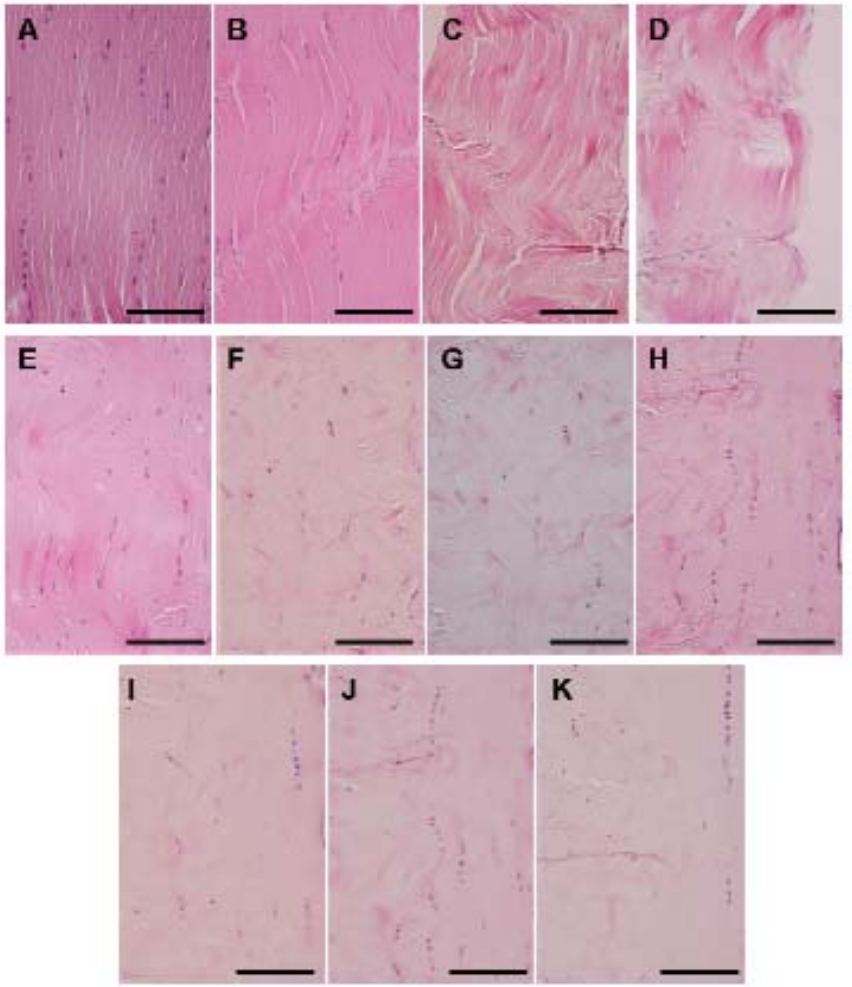


SHG and Histological Images

SHG



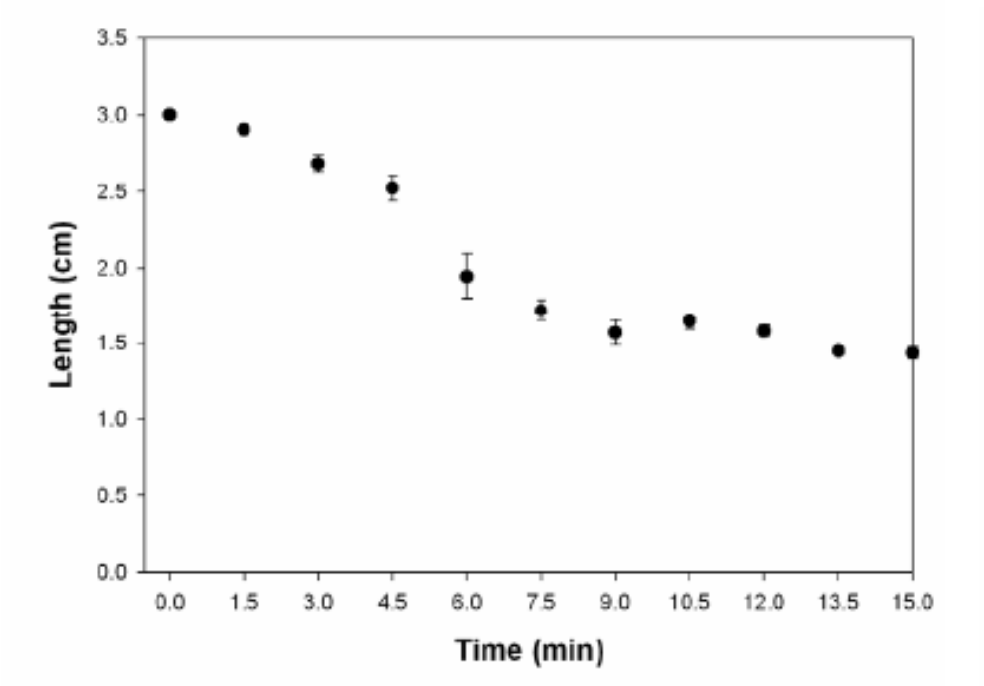
Histological Images

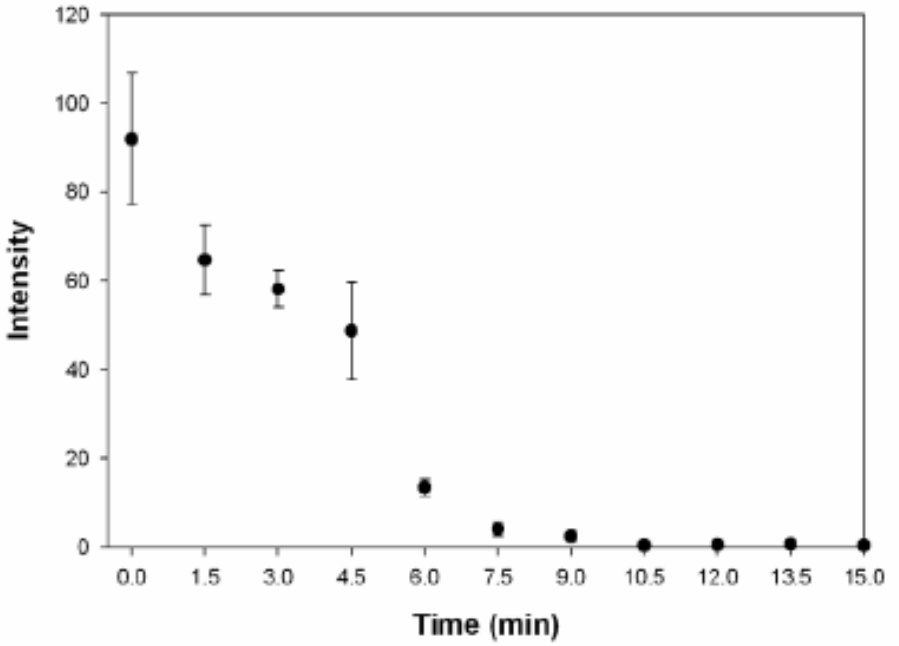


- A:0, B: 1.5, C: 3, D:4.5, E: 6, F: 7.5, G: 9, H: 10.5, I: 12, J: 13.5, K:15 min
- Scale bar: 100 μm

Collagen Shrinkage and SHG Decay

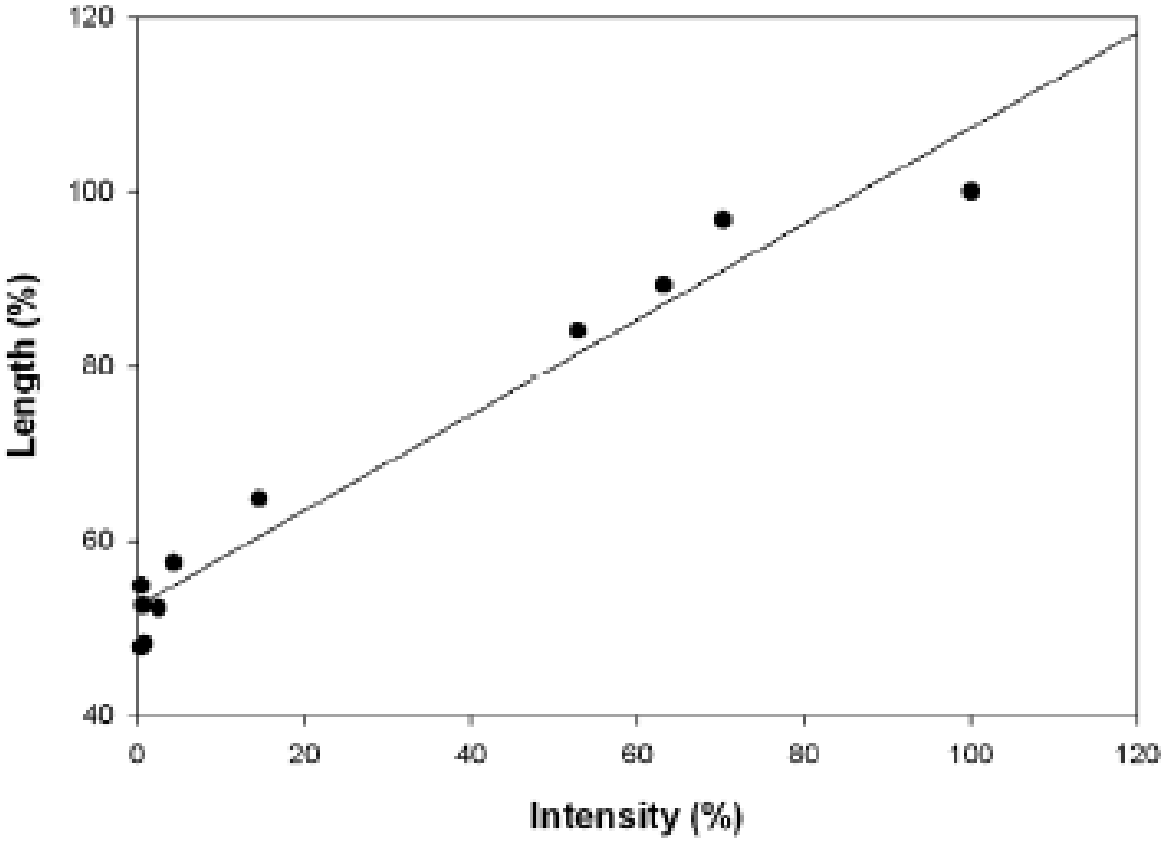
- Sample: rat tail tendon
- 58° C treatment at different times





Lin et al., *Journal of Biomedical Optics*, 11(3) 034020 (2006).

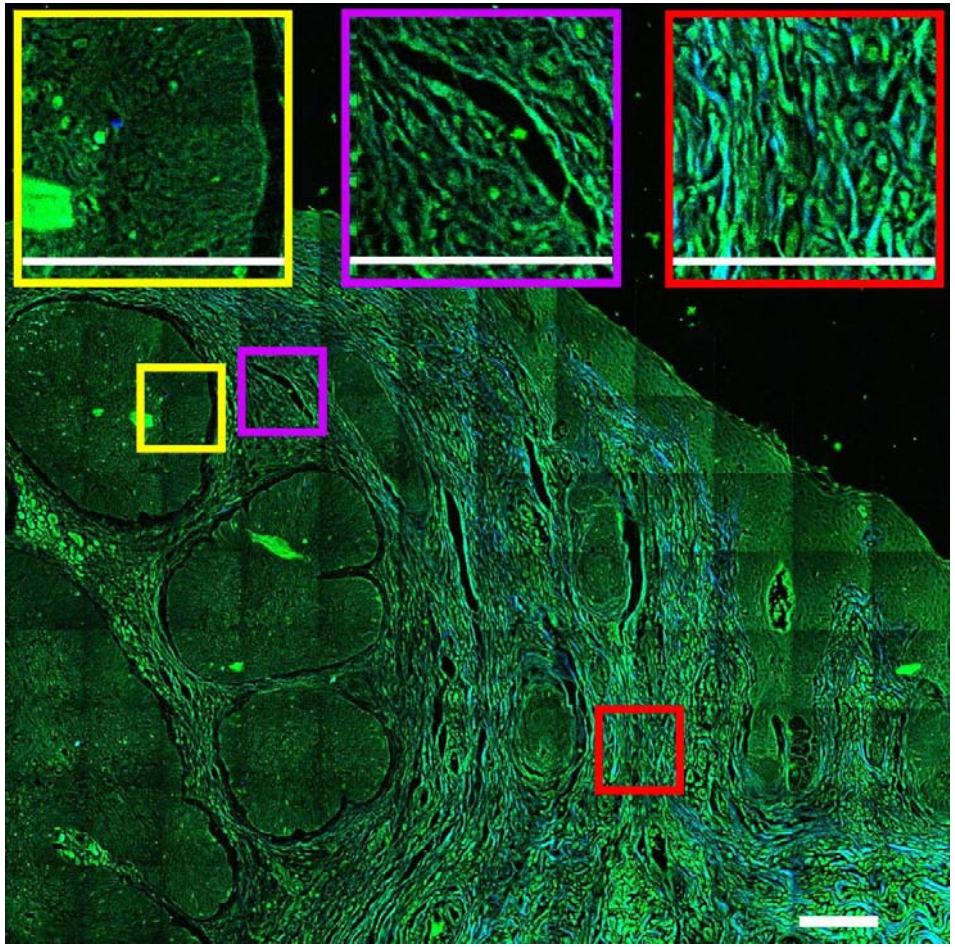
Rat Tail Tendon – SHG and Shrinkage



$$\frac{L_d}{L_0} = 0.547\,\frac{I_d}{I_0} + 0.525$$

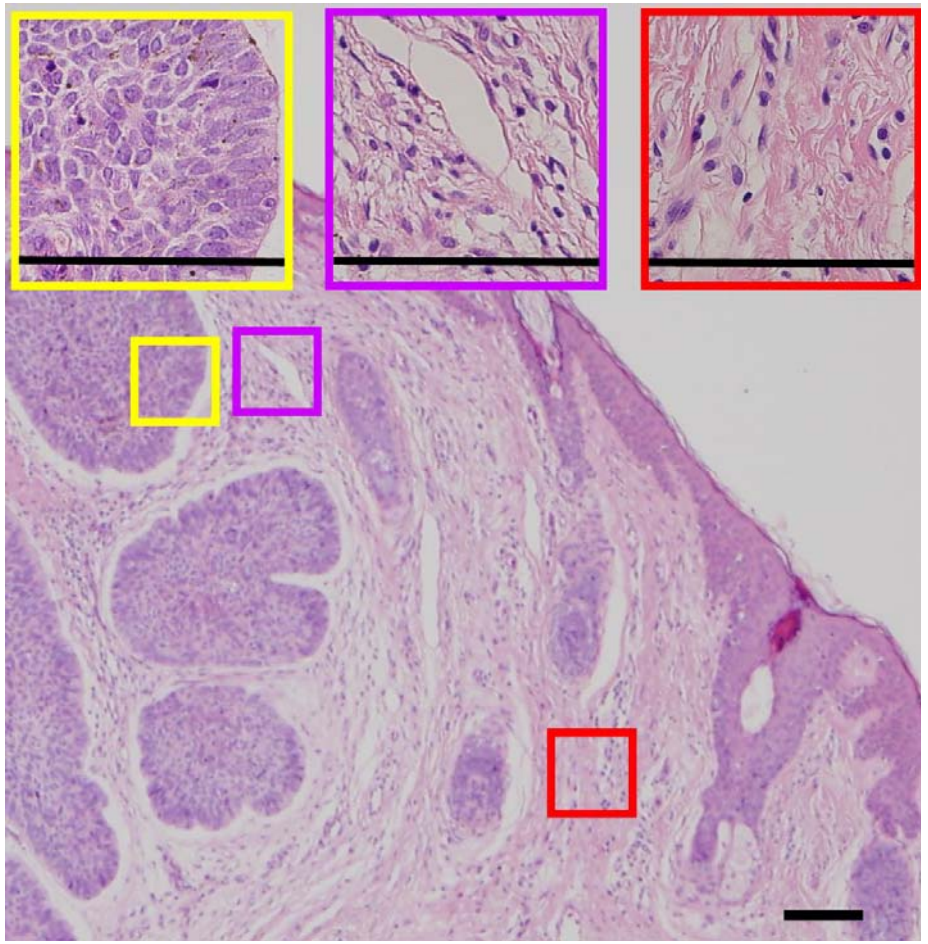
Basal Cell Carcinoma (BCC) Imaging

Histology



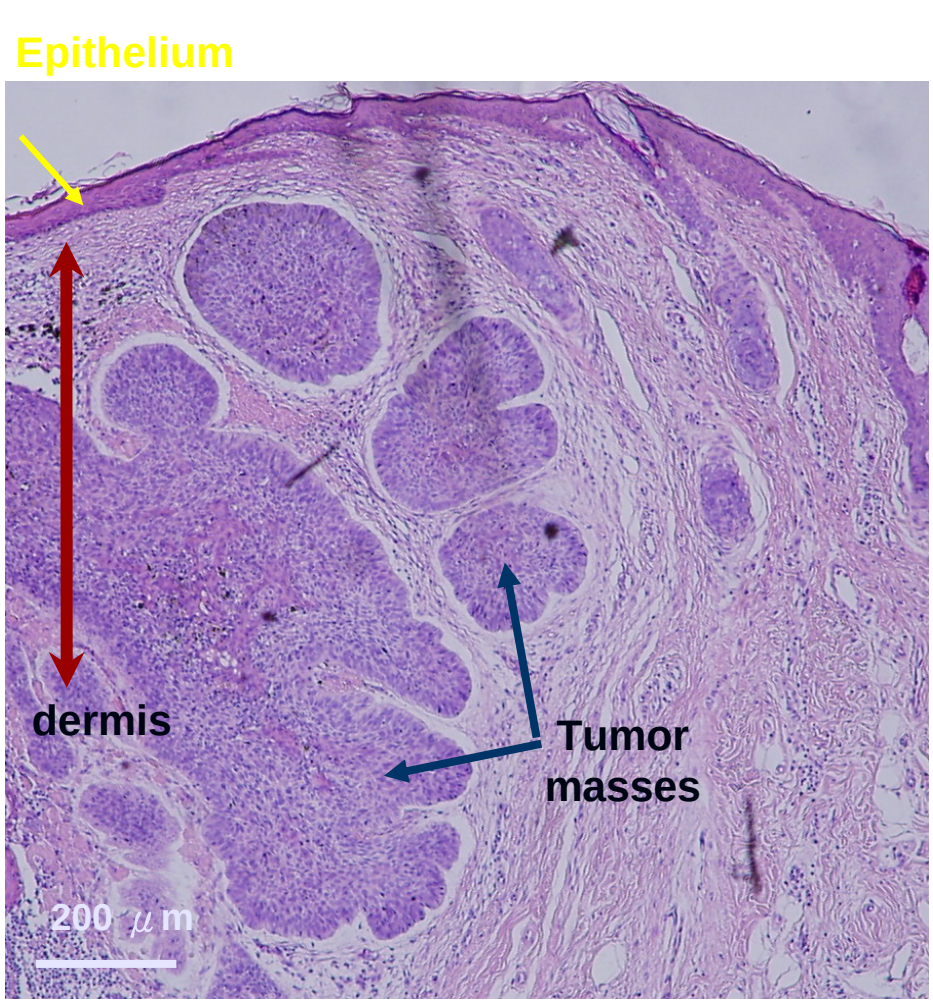
Green：AF
Blue：SHG

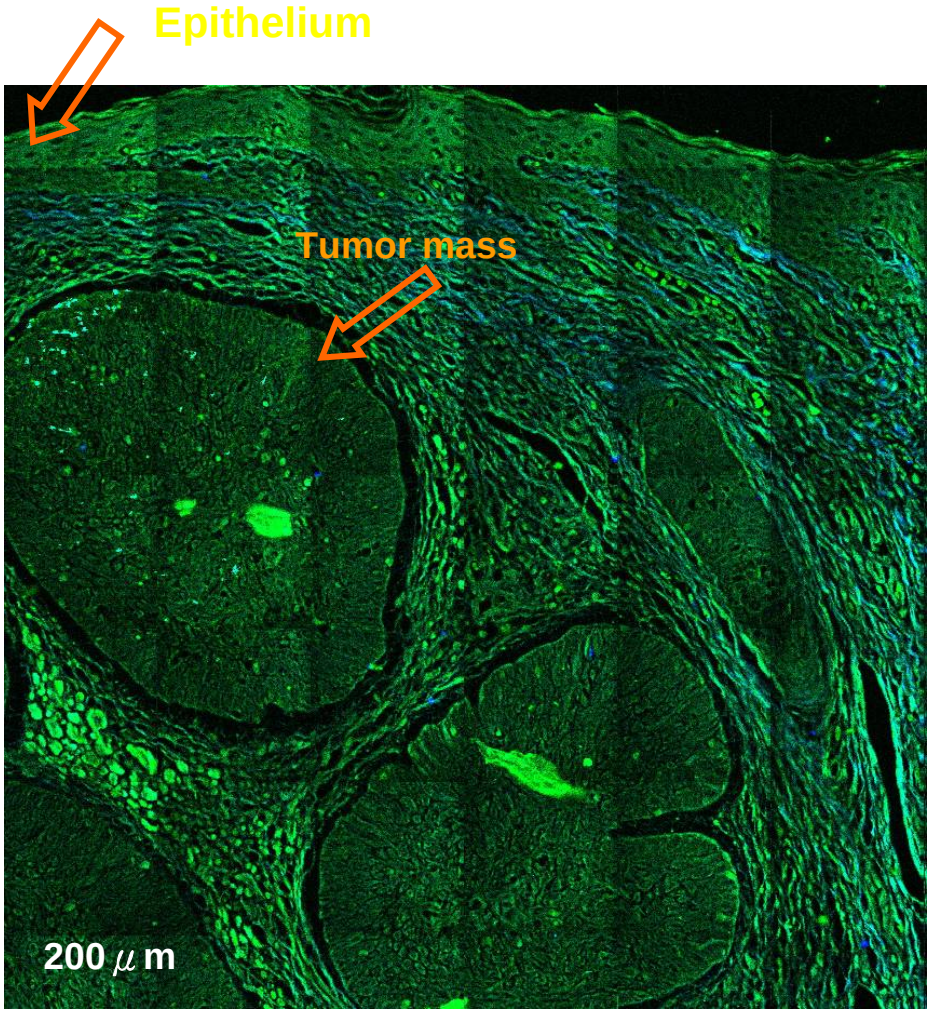
Multiphoton



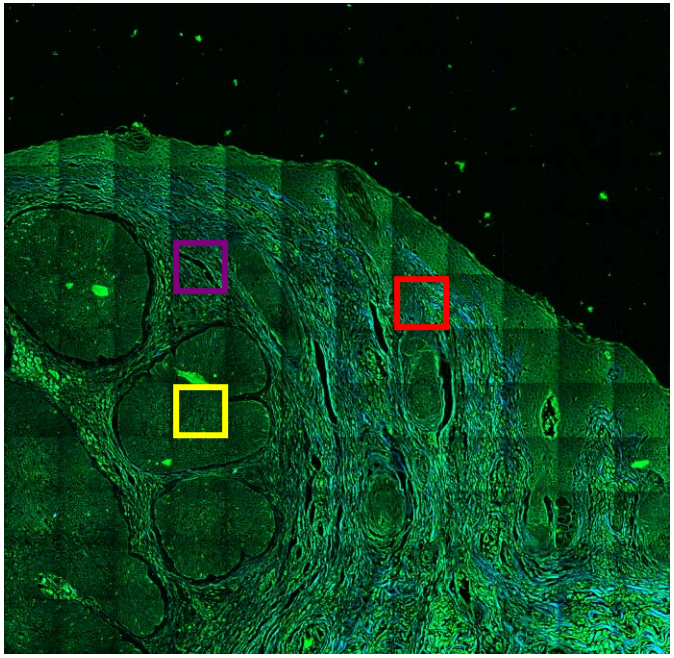
Lin et al., *Optics Letters*, 31(18):2756-2758, (2006).

Basal Cell Carcinoma (BCC) Imaging





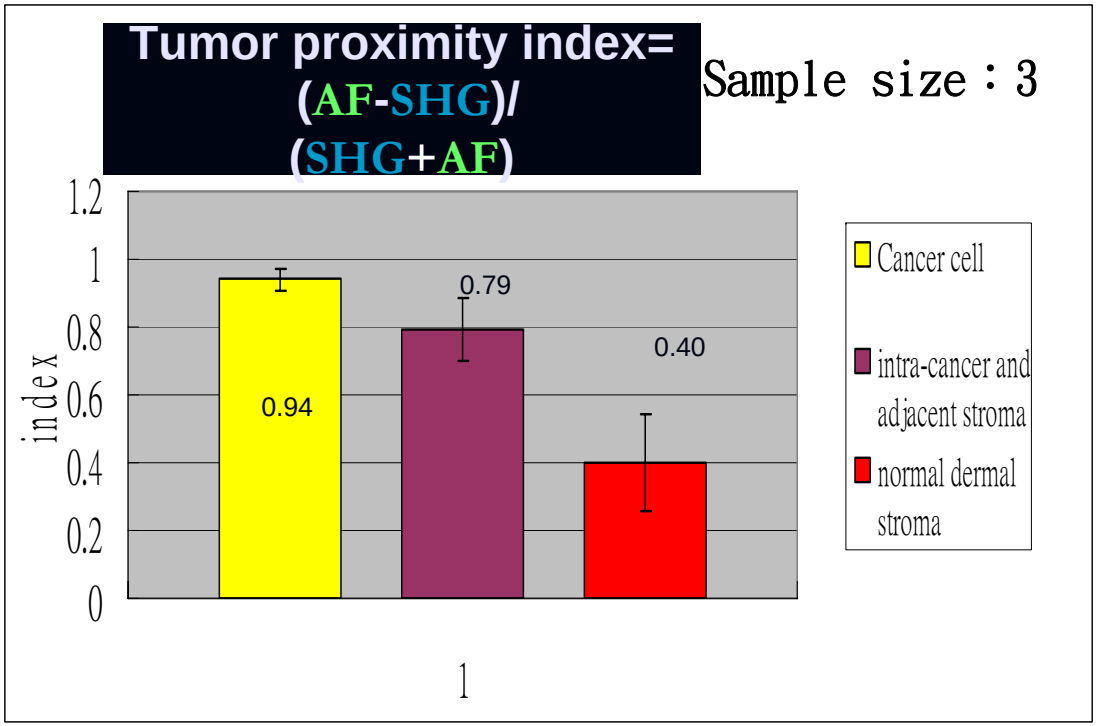
Quantitative Analysis of BCC



Cancer cell

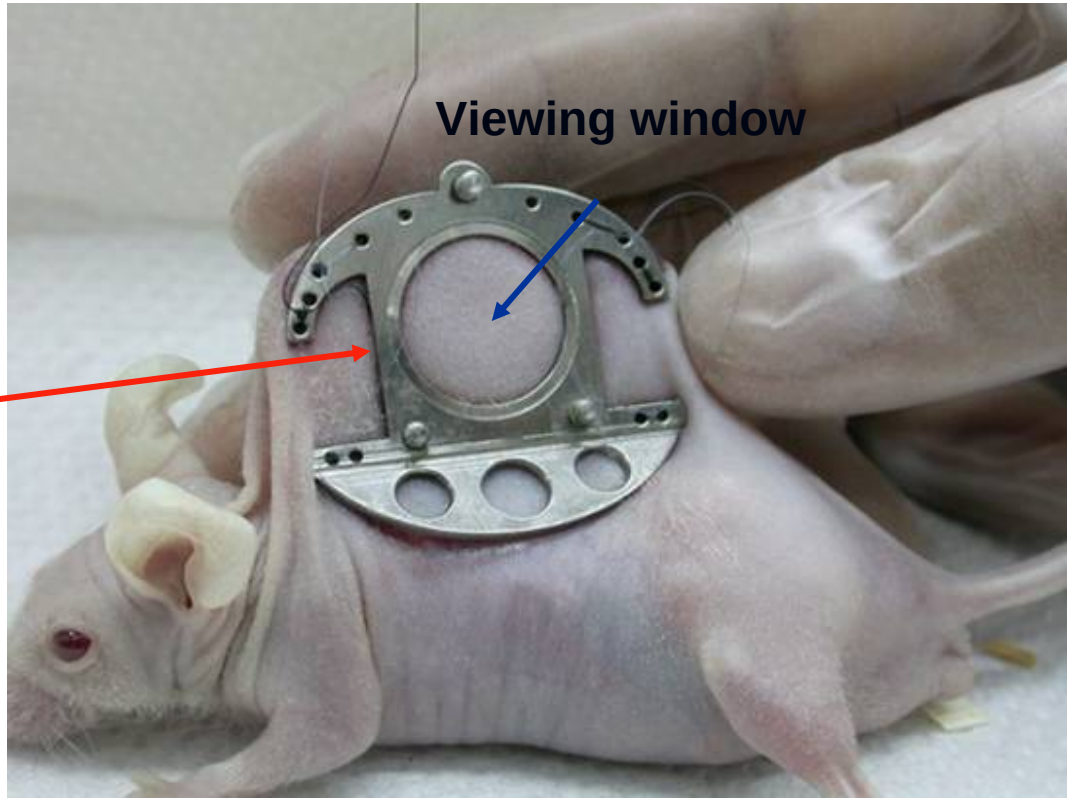
inter-tumor within dermis

normal dermal stroma



Dorsal Skin Fold Chamber for In Vivo Oncology

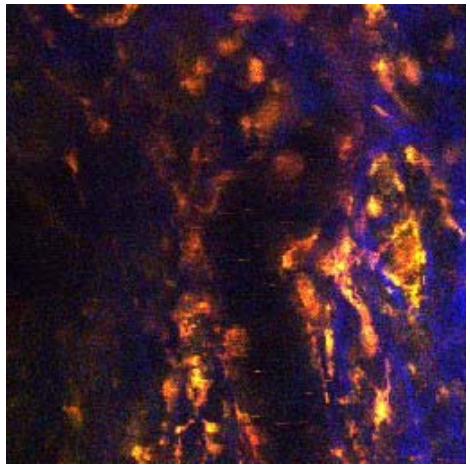
6Al/4V ELI titanium alloy



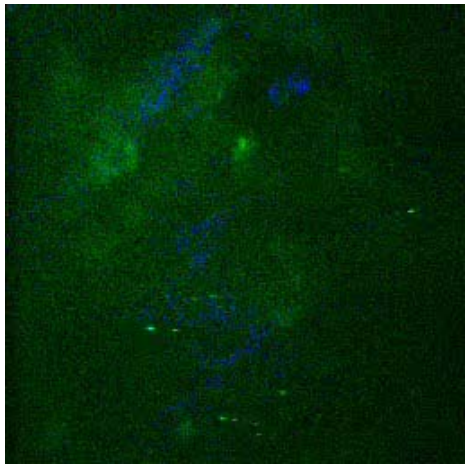
1. Angiogenesis 2. Metastasis 3. Tumor treatment

Li et al., *Optical and Quantum Electronics*. 37:1439-1445 (2005).

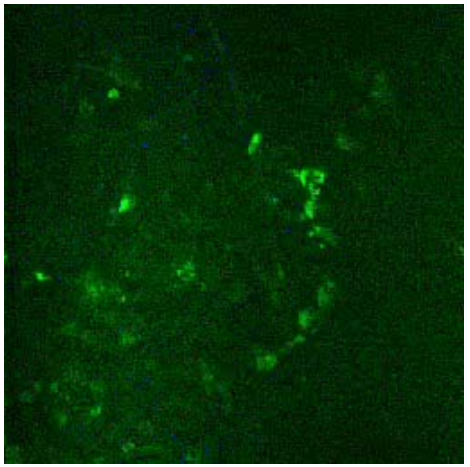
Intravital Imaging of Mouse Physiology



In vivo leukocyte movement



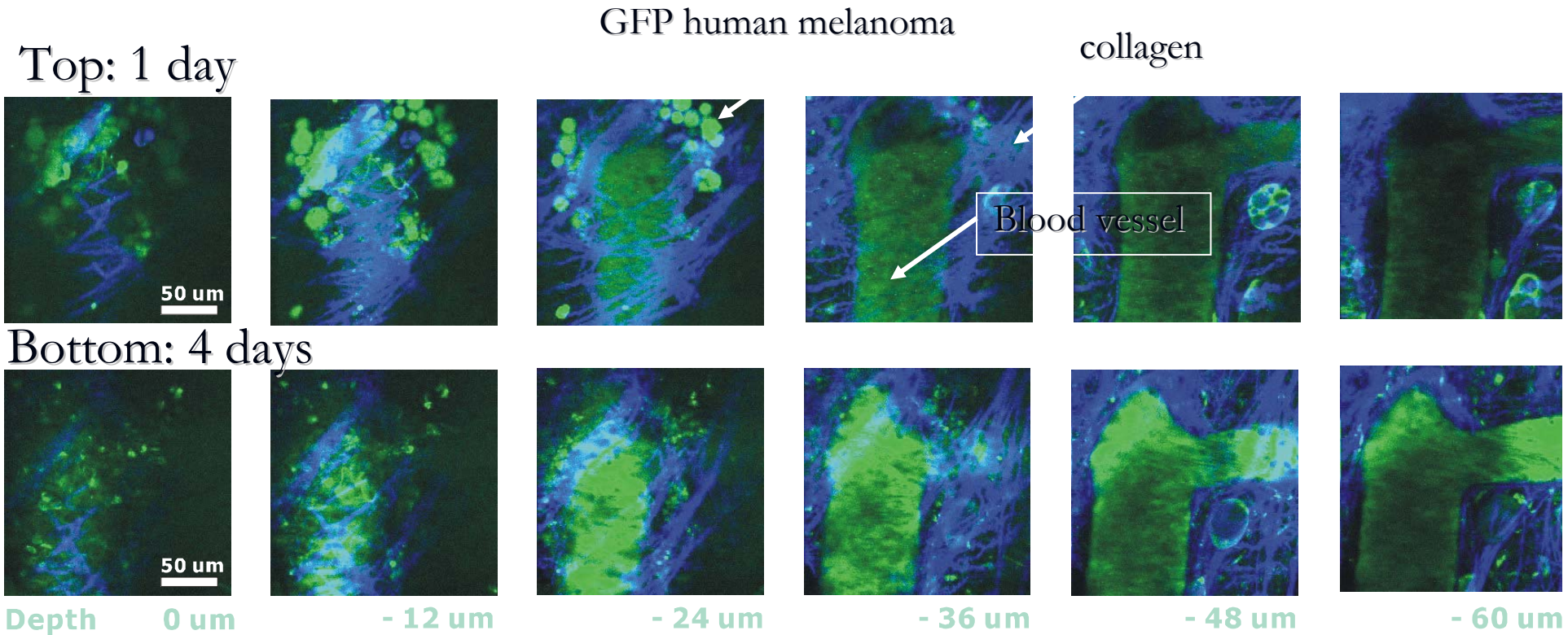
GFP melanoma injection in vivo (day 1)



GFP melanoma dispersion in vivo (day 4 later)

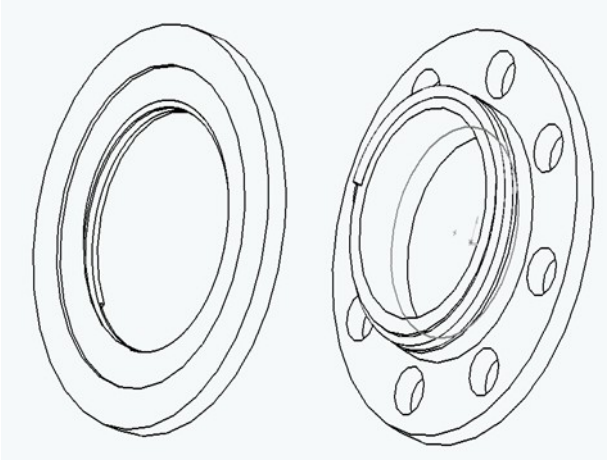
SHG: blue
AF: green

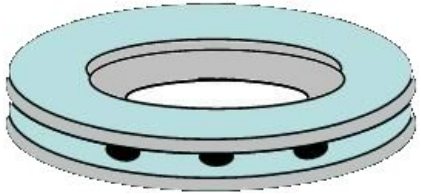
Long Term Observation of In Vivo Tumor Growth



SHG: blue
F: green

In Vivo Hepatic Imaging Chamber



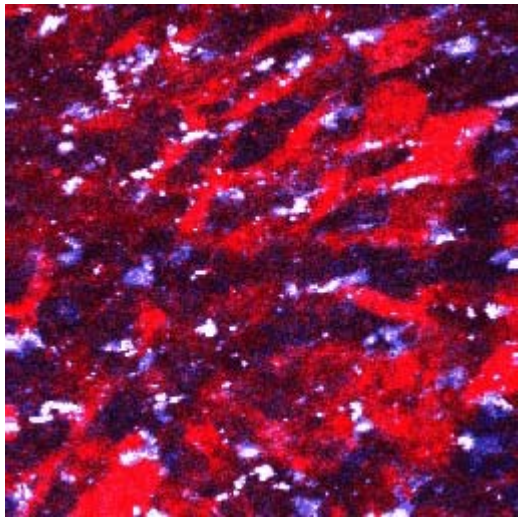


6Al/4V ELI titanium alloy

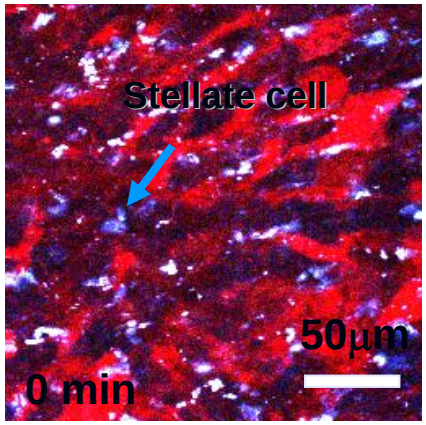
Liu et al., *Journal of Biomedical Optics*, Accepted.

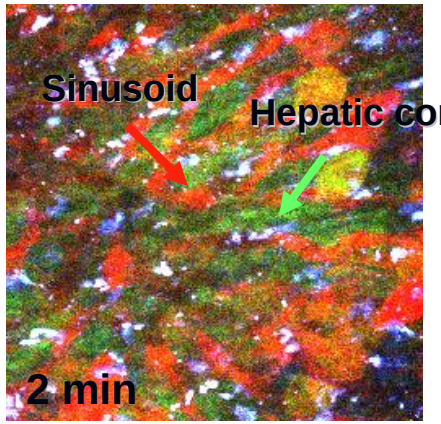
In Vivo Hepatic Metabolism

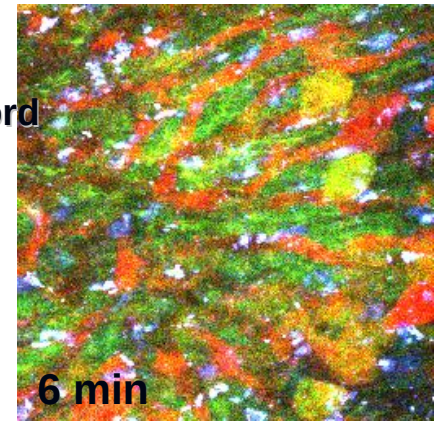
Metabolic Movie

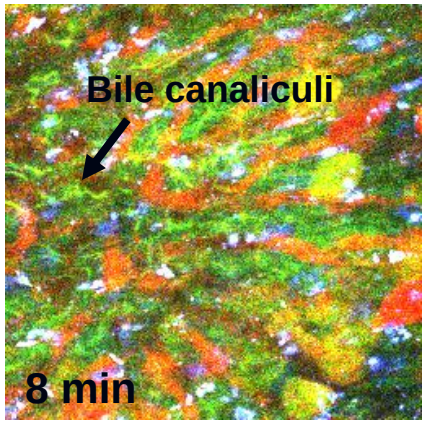


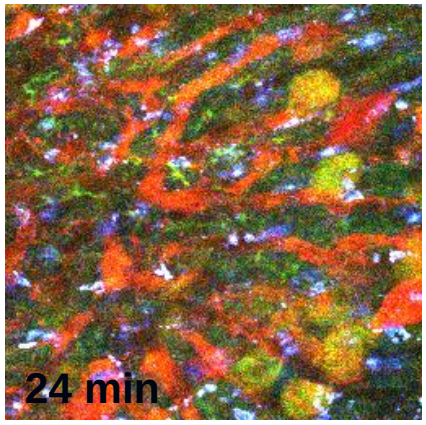
Temporal snapshots

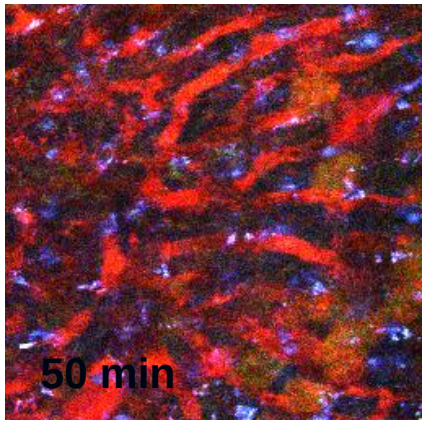












Red: Rhodamine B isothiocyanate-dextran
Green: 6-CFDA (green)

Depth Resolved, In Vivo Imaging of Mouse Liver

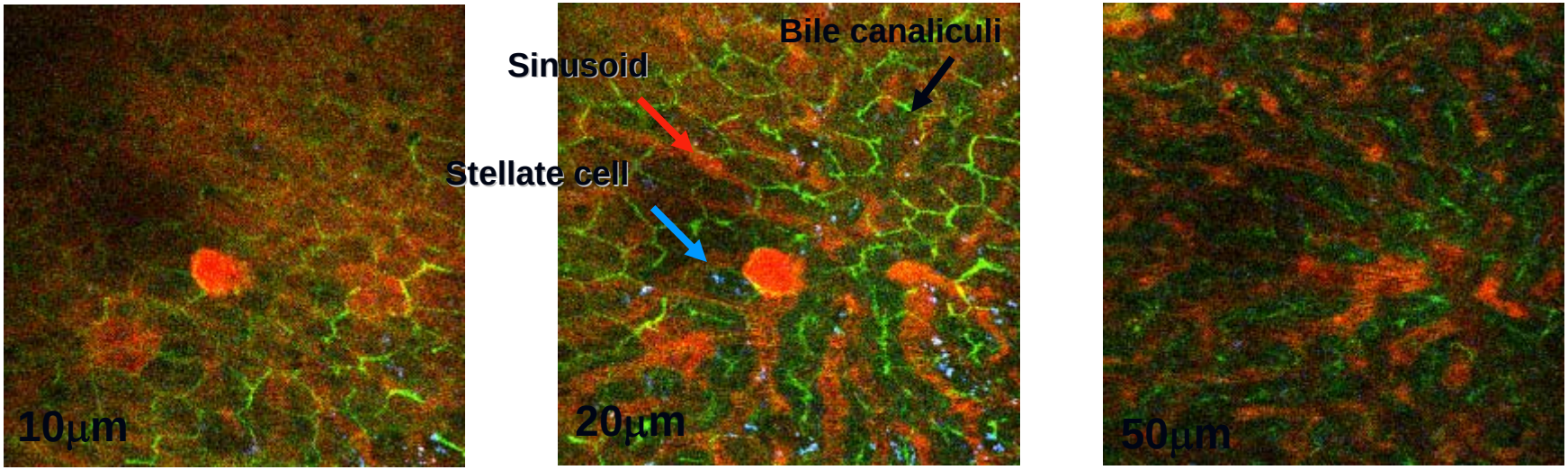
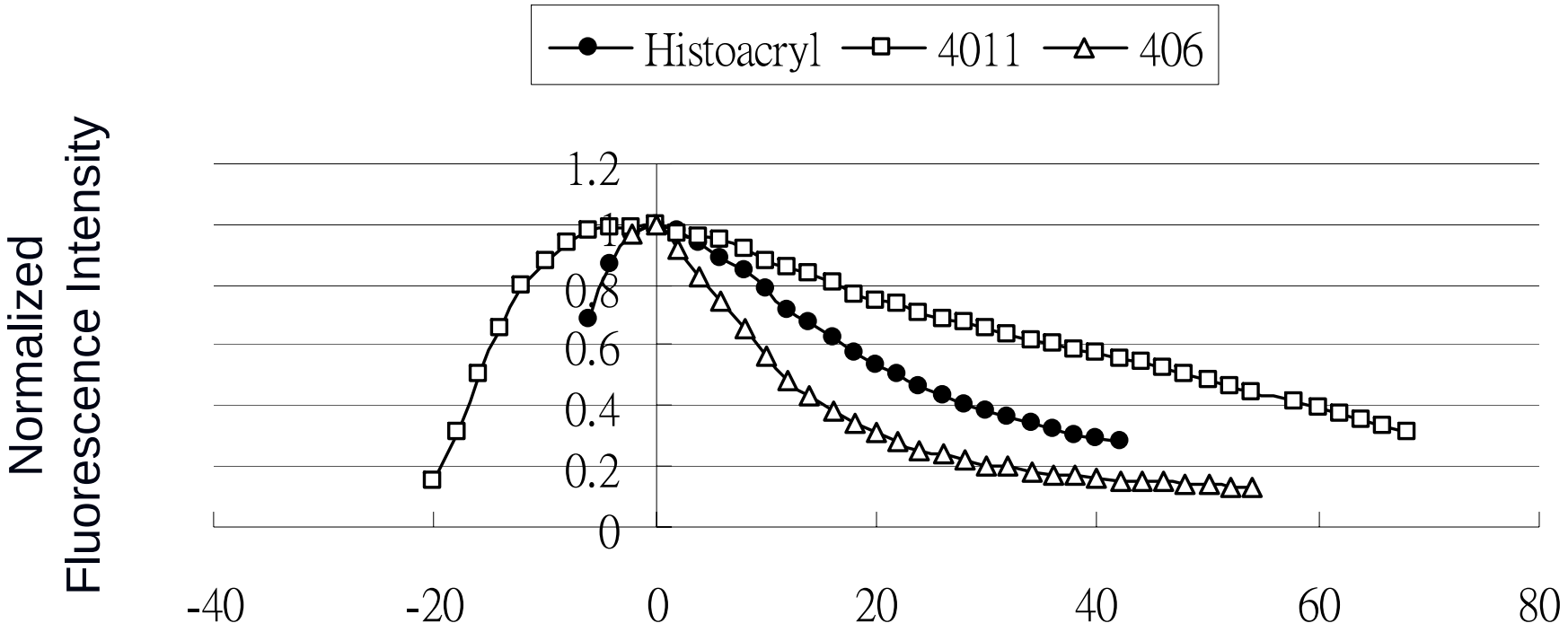


Image size: 220 μm by 220 μm

Red: Rhodamine B isothiocyanate-dextran
Green: 6-CFDA (green)

Temporal Dependence of Carboxyfluorescein Intensity Profiles



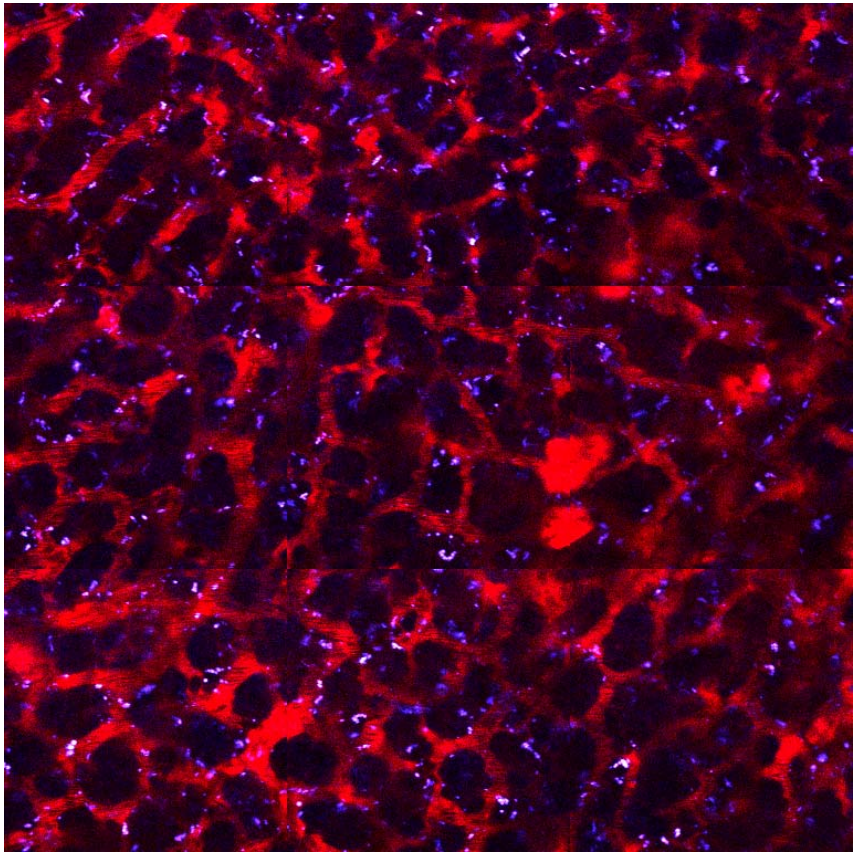
Histoacryl: tissue adhesive

4011 (Henkel Loctite): for medical device

406 (Henkel Loctite): typical instant adhesive

Bile duct Ligation

Normal liver



1 day post bile duct ligation

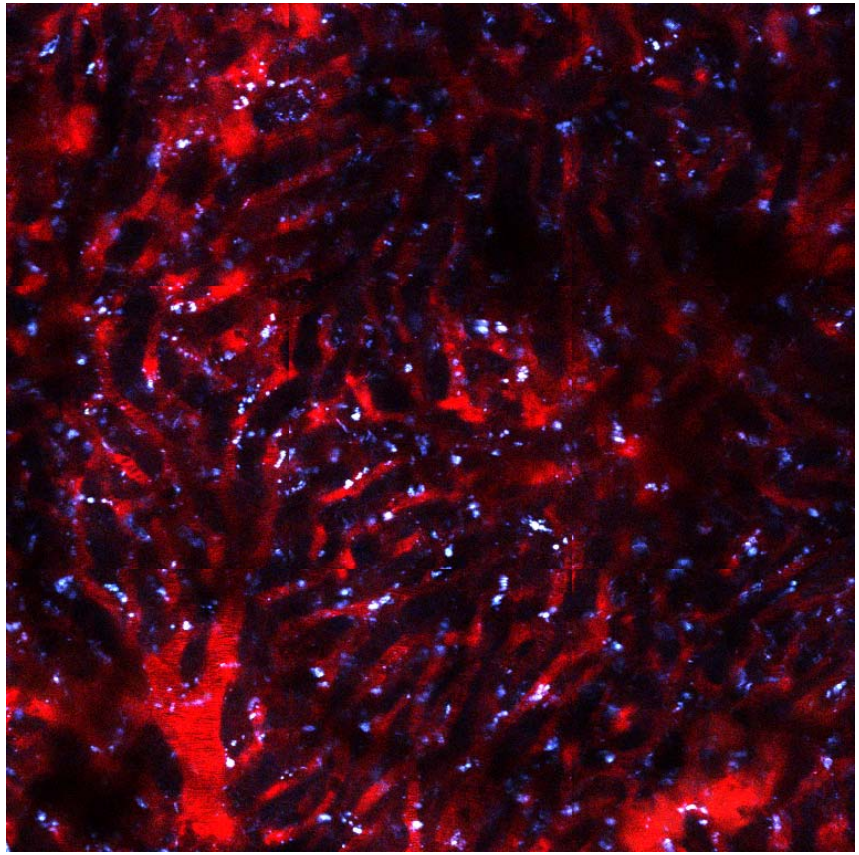
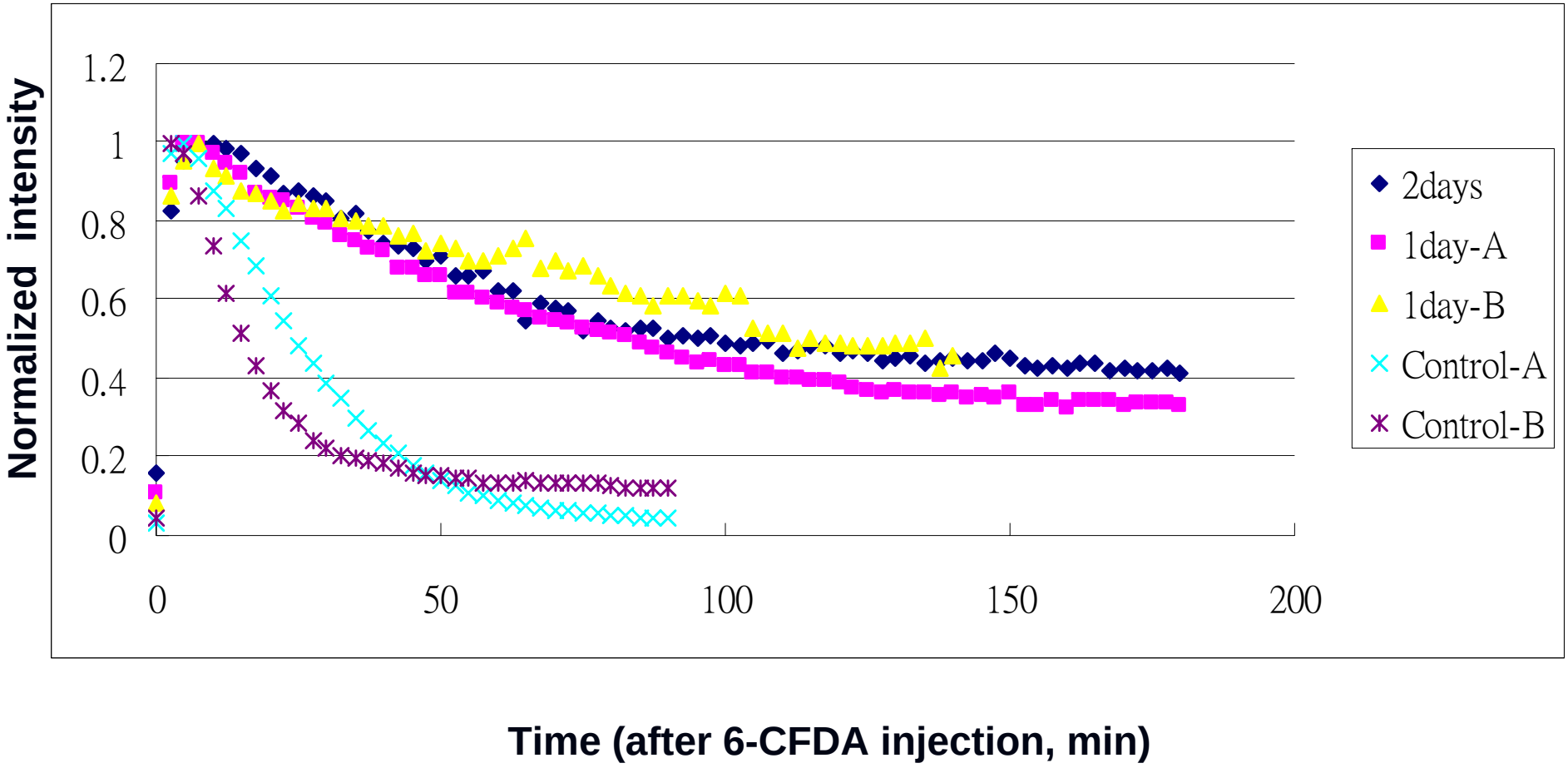
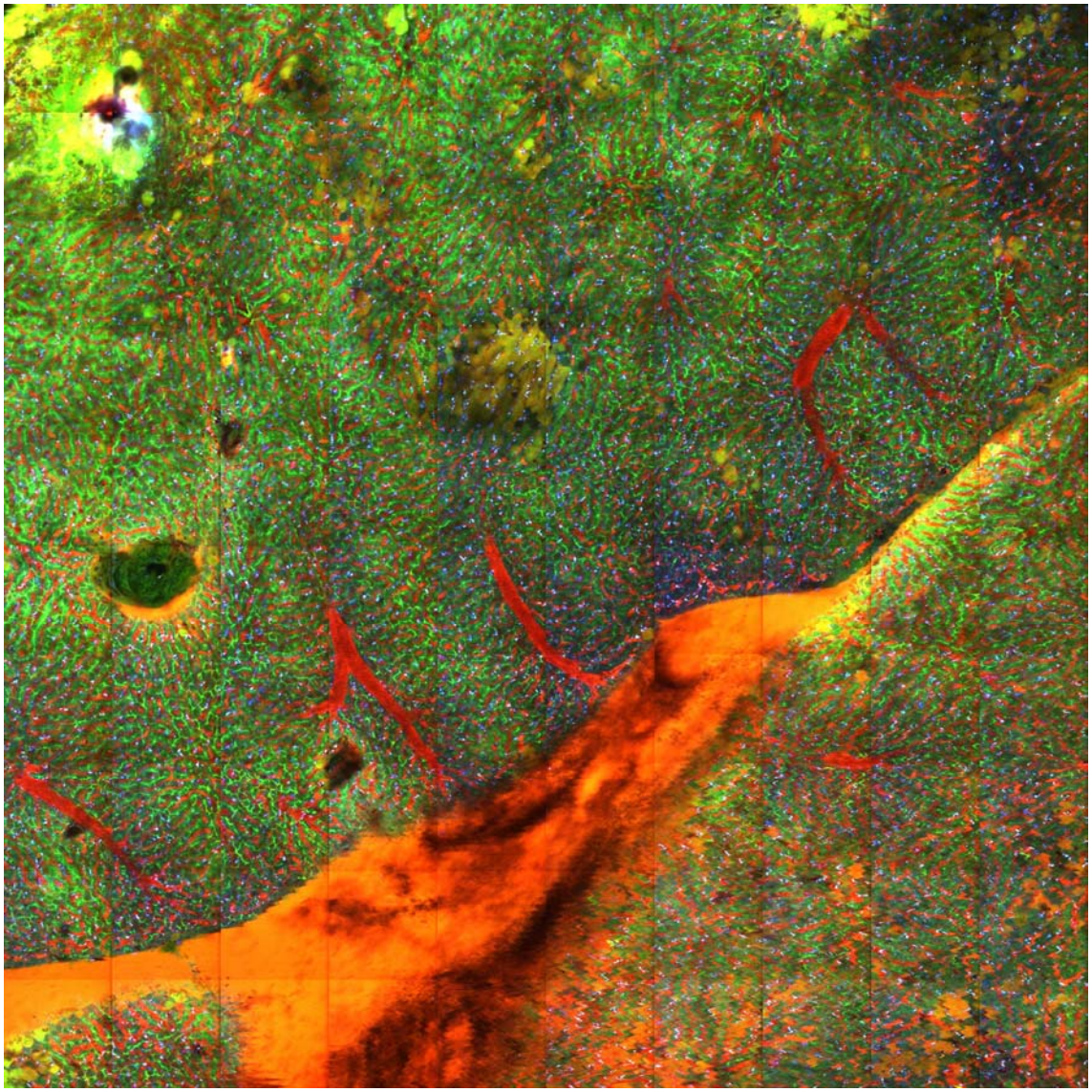


Image size: 660 μm by 660 μm

Bile duct Ligation Induced 6-CFDA Fluorescence Change

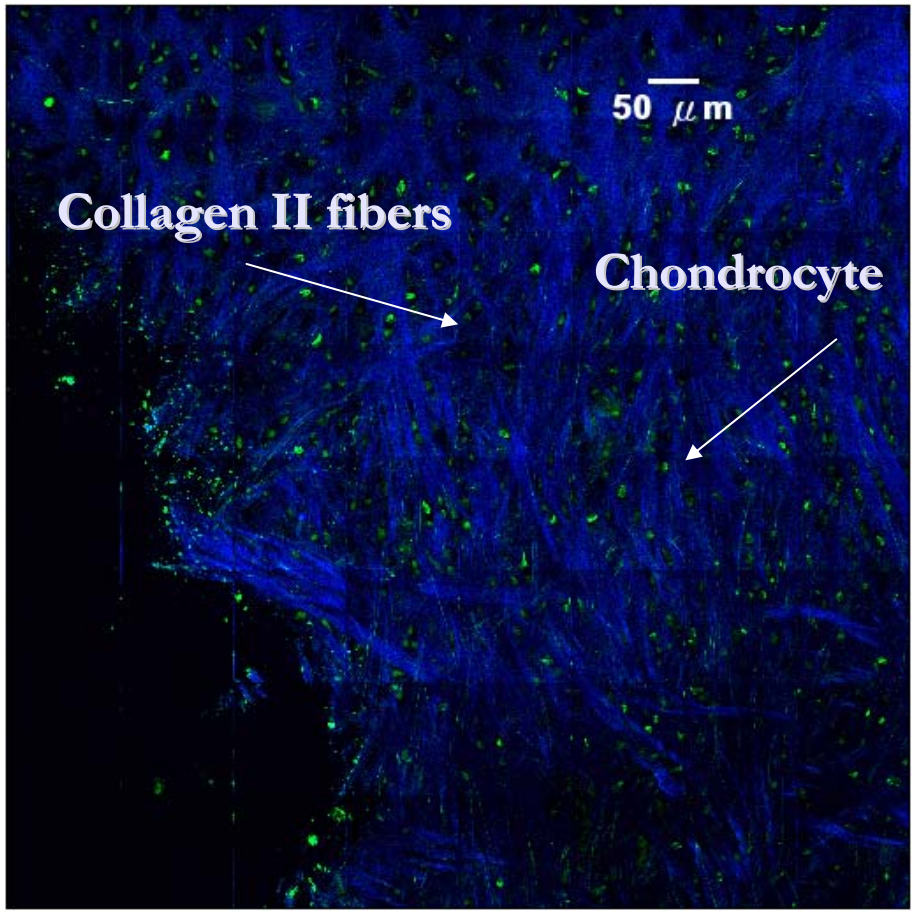


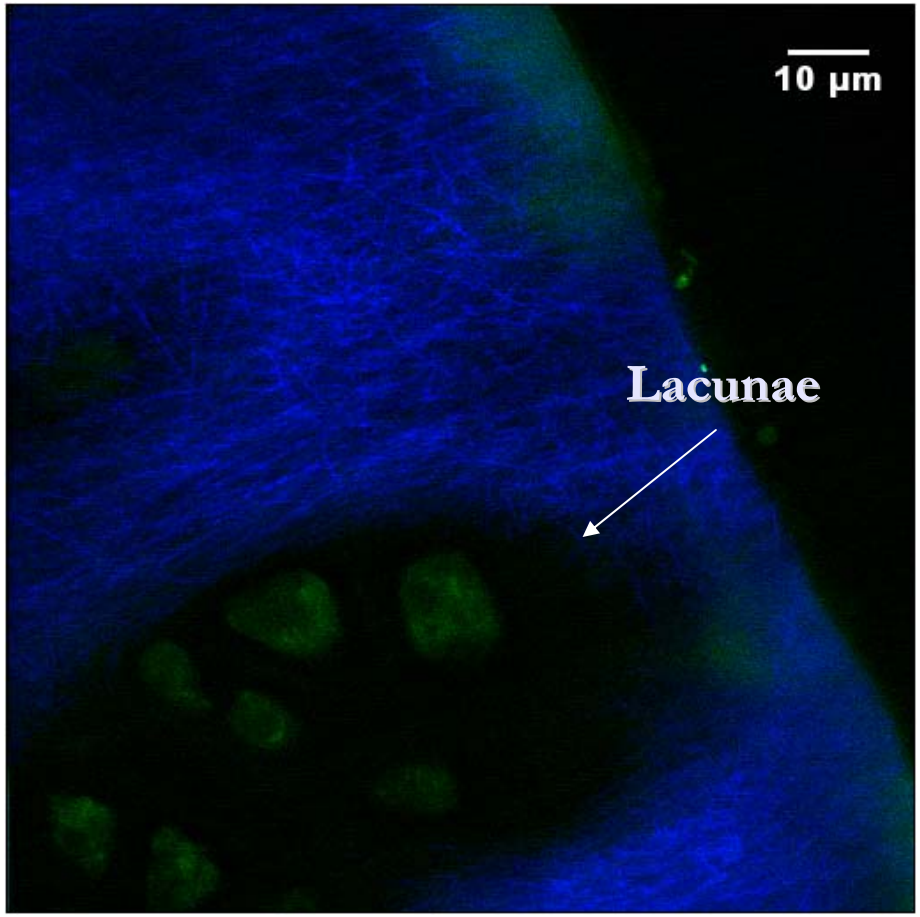
Large Area Multiphoton Imaging of Mouse Liver In Vivo



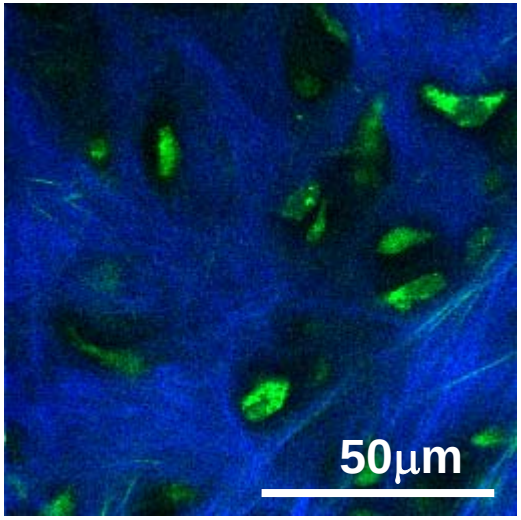
- Green: autofluorescence
- Red: rhodamine dextran

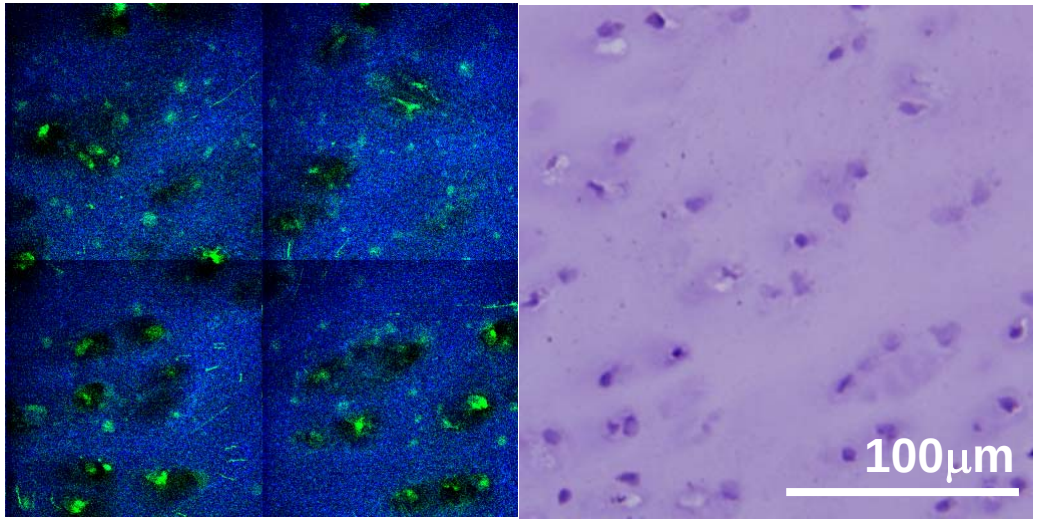
Multiphoton Porcine Cartilage Imaging



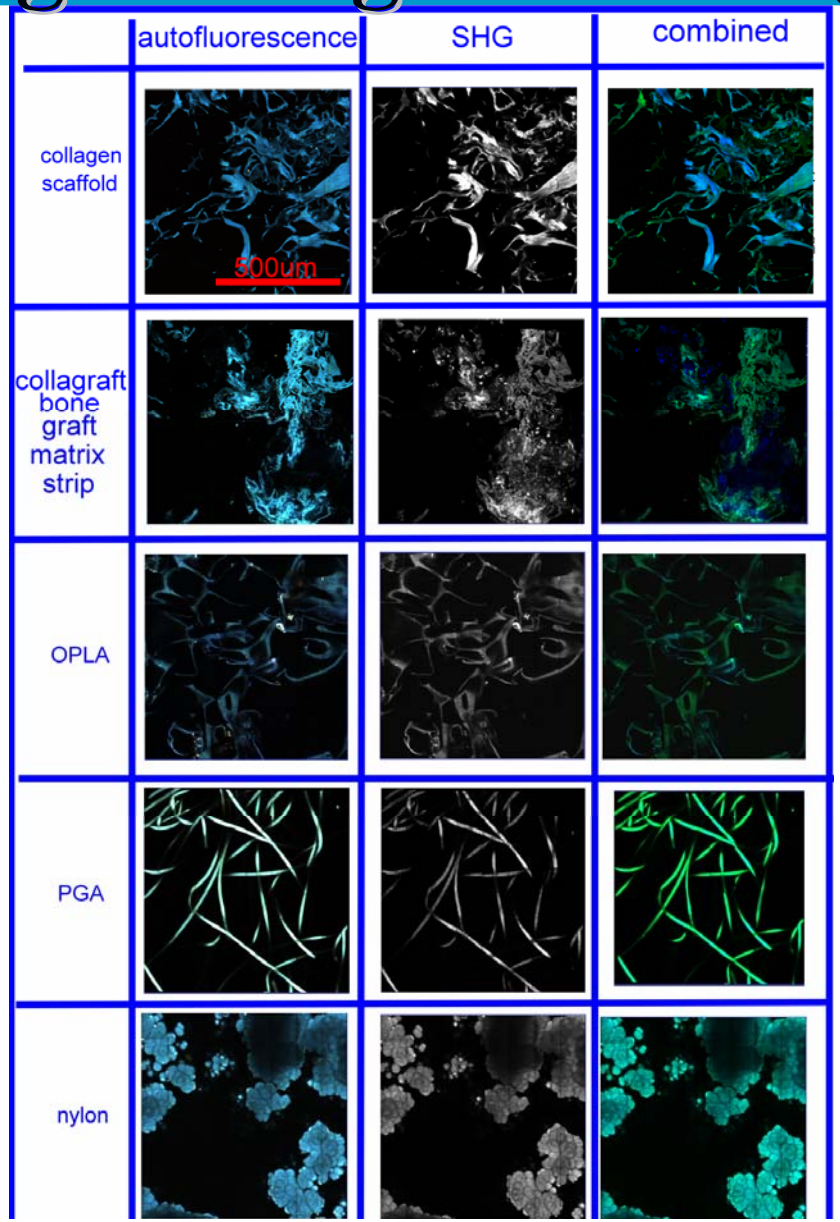


Multphoton Imaging of Porcine Cartilage

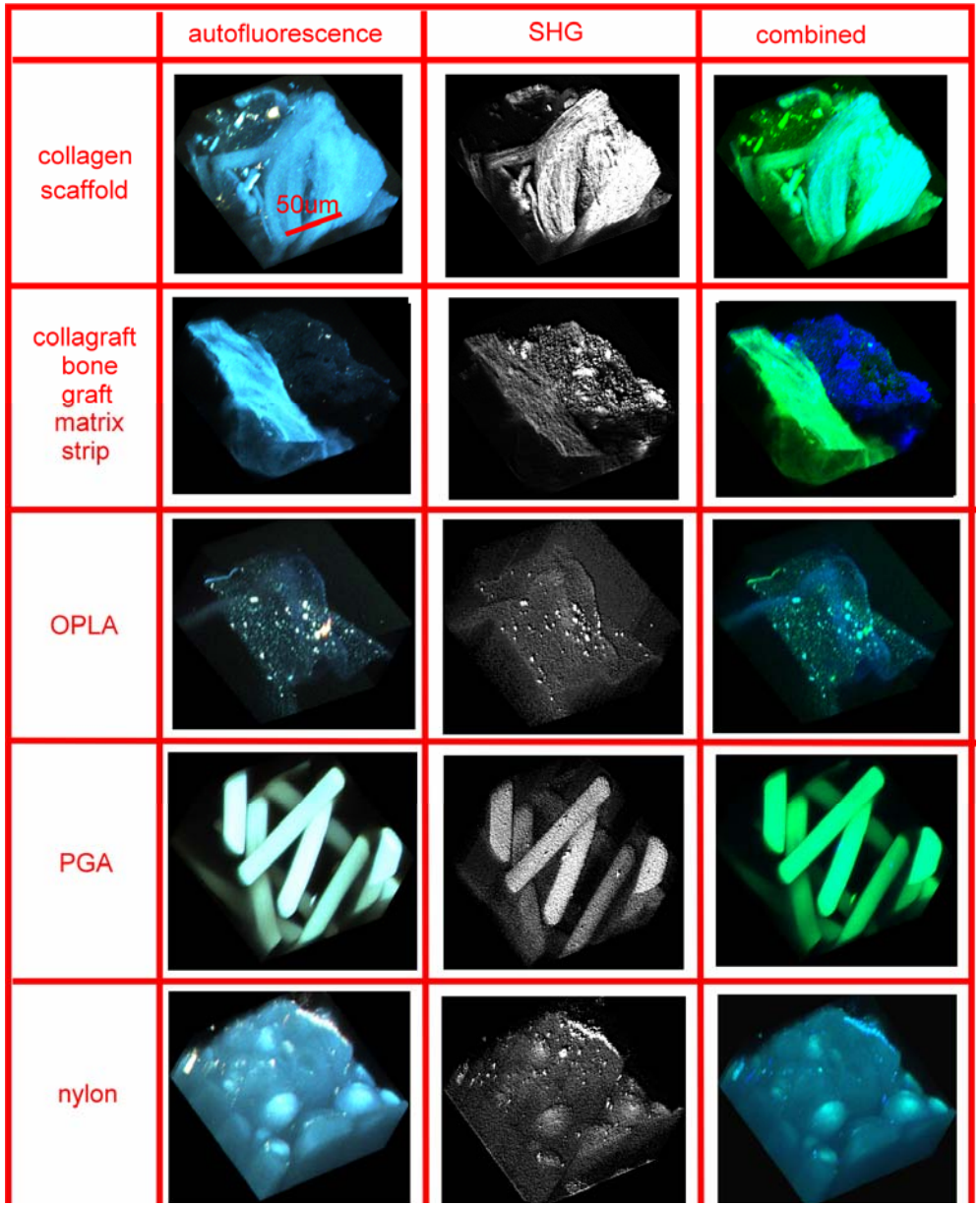




Multiphoton Imaging of Tissue Engineering Scaffolds (2-D)



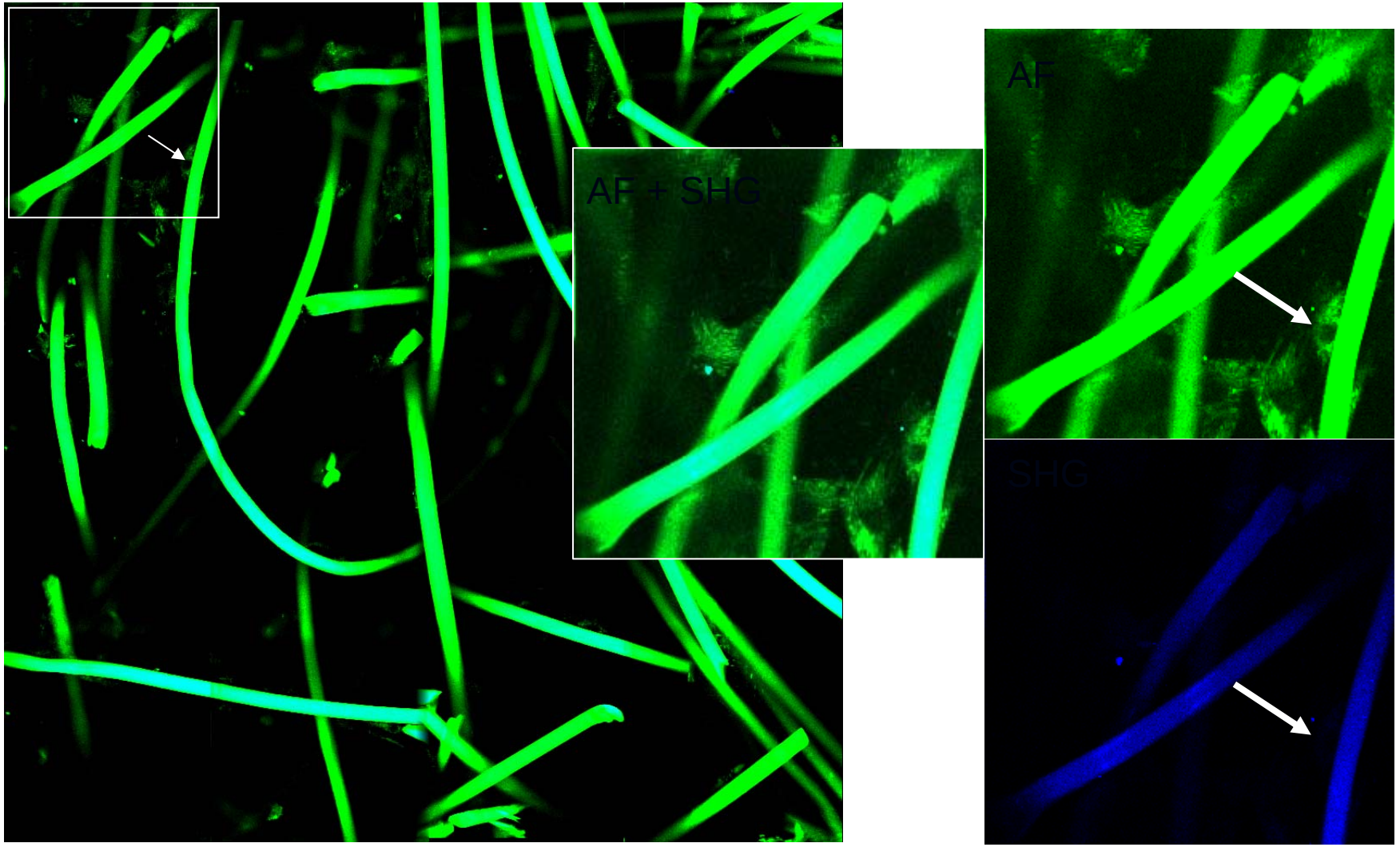
Multiphoton Imaging of Tissue Engineering Scaffolds (3-D)



Multiphoton Chondeogenesis Imaging (Tissue Engineering in PGA Scaffold)

0 week

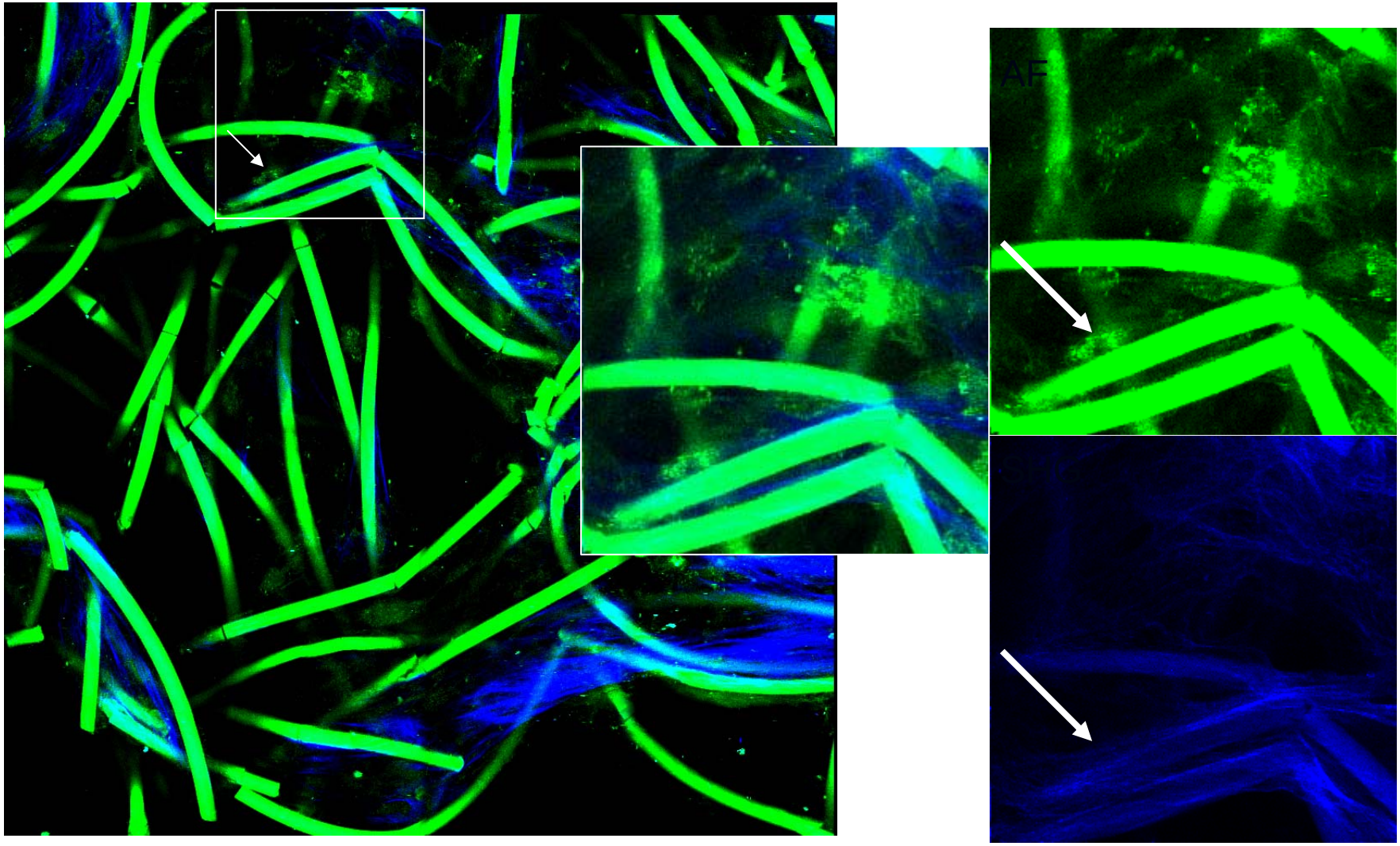
AF + SHG



Lee et al., *Tissue Engineering*, 12(10):2835-2841, (2006).

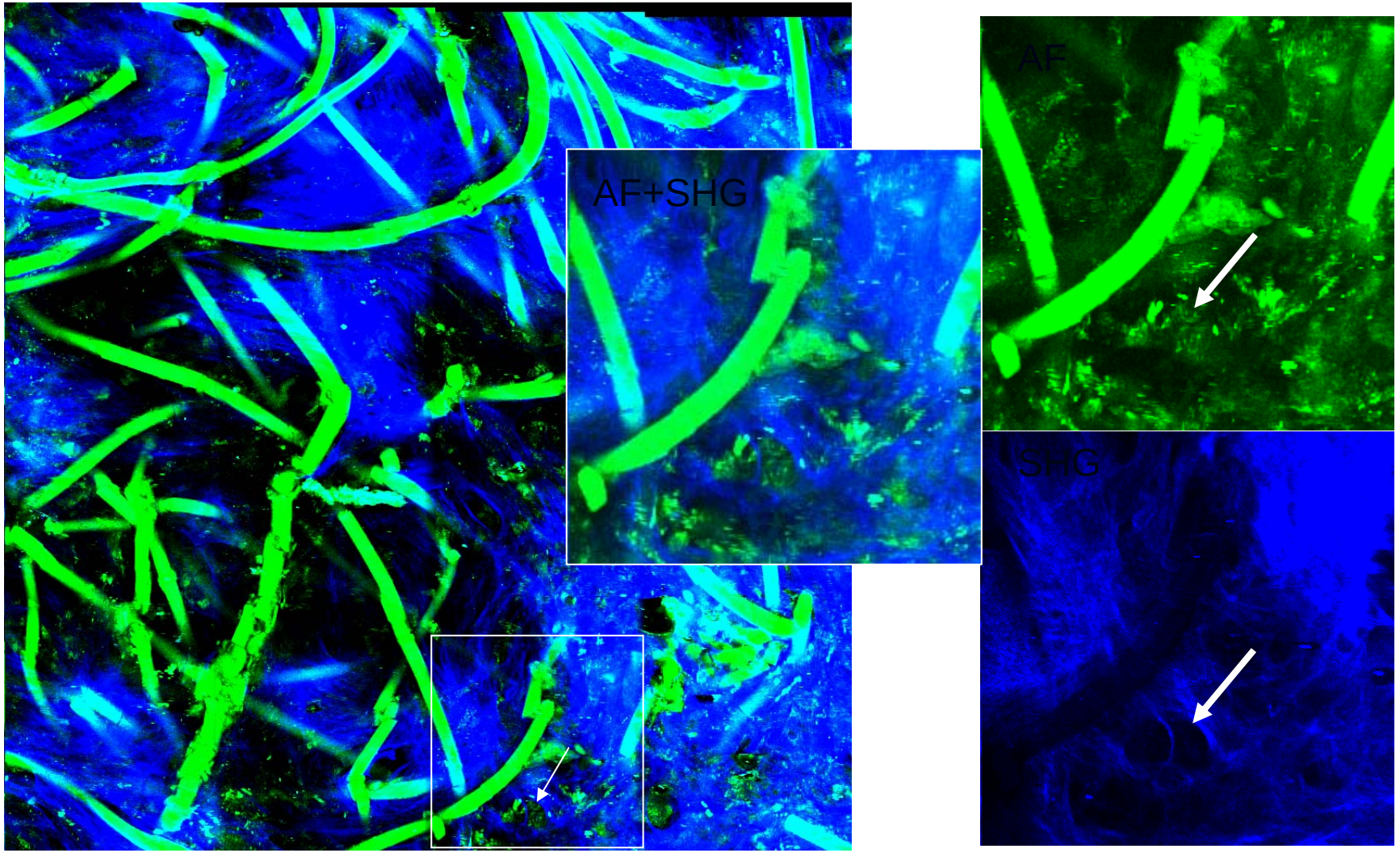
1 week

AF + SHG



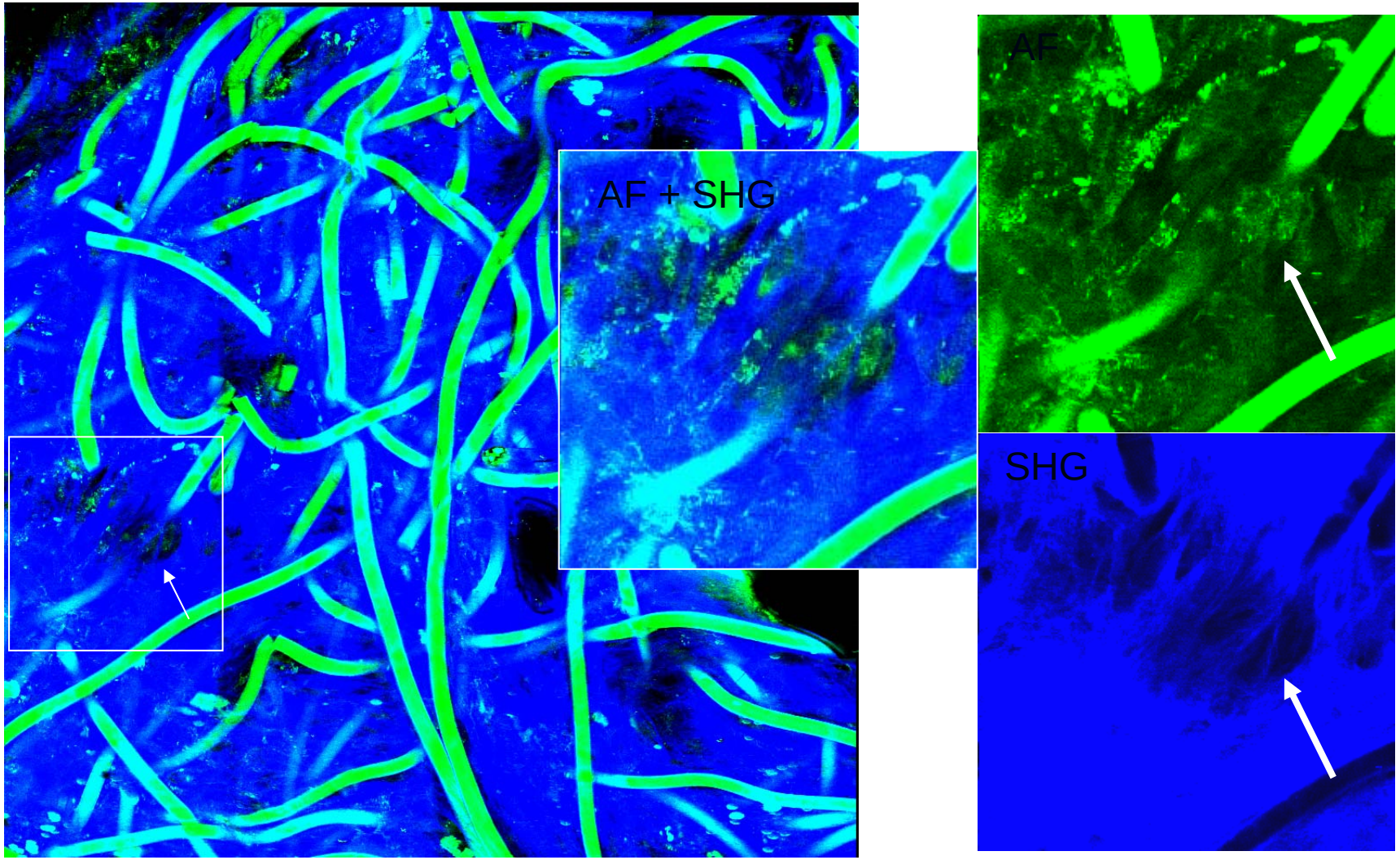
2 weeks

AF + SHG

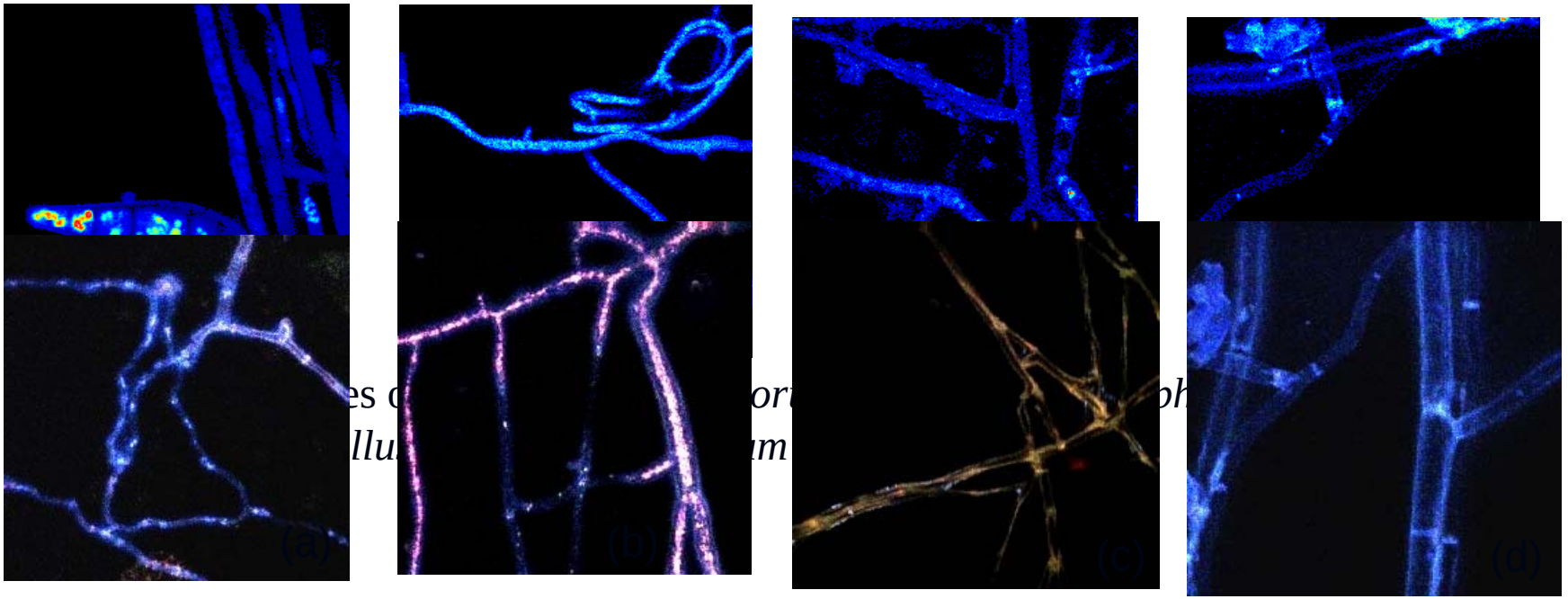


3 weeks

AF + SHG



Multiphoton Fungal Imaging



False-colored MPF images of fungi. (a) *Microsporum canis* (b) *Aspergillus flavus* (c) *Trichophyton mentagrophytes* (d) *Fusarium solani*.

What is Next?

- Multiphoton microscopy is effective for **label-free biopsy** in many tissue types
- Clinical extension

  – autofluorescence and second harmonic generation signal (**disease diagnosis**)

  – **dermatology** and **ophthalmology** may be promising for real-time diagnosis
- Applied to animal models for intravital physiological processes

Conclusion

- Multiphoton microscopy is effective for **label-free imaging** of many tissue types
  - Clinical extension
    - **disease diagnosis**
    - **dermatology and ophthalmology may be promising for real-time diagnosis**
  - Structure/function relation of tissues (3-D)
  - Investigation of intravital physiological processes

- Theoretical description/modeling
  - 3-D self-assembly of fibrous network (collagen/elastin)
  - Cell-matrix interaction (migration, differentiation, assembly)
  - Cell/tissue metabolism
  - Infection
  - Tissue reorganization/genesis and (interaction of biomechanical and biochemical pathways)

- High scientific and application values

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