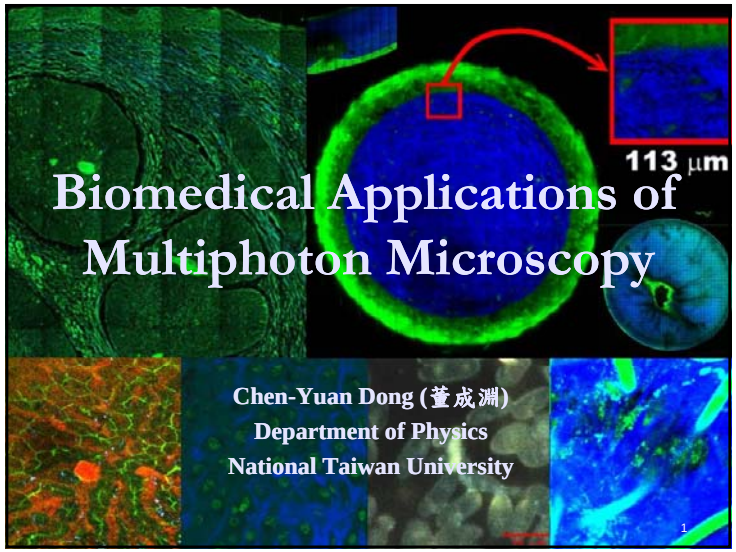
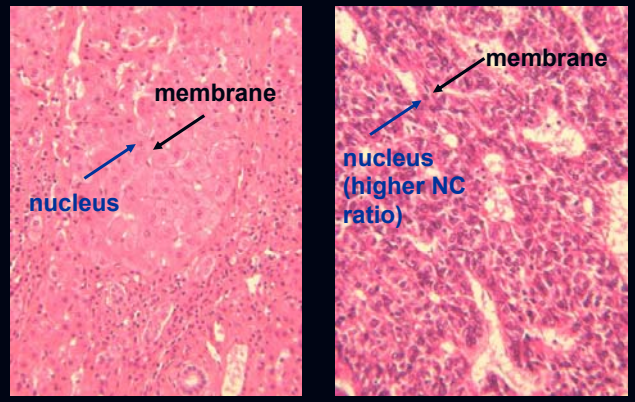


Histological Images of Liver Tumor
(HCC: hepatocellular carcinoma)



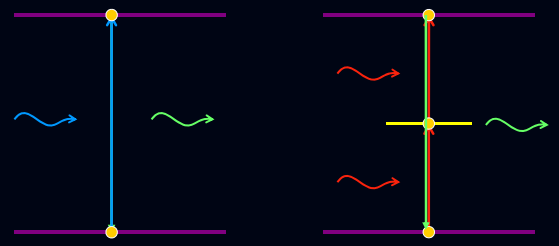
Non-tumor Tumor

2

Research Approach

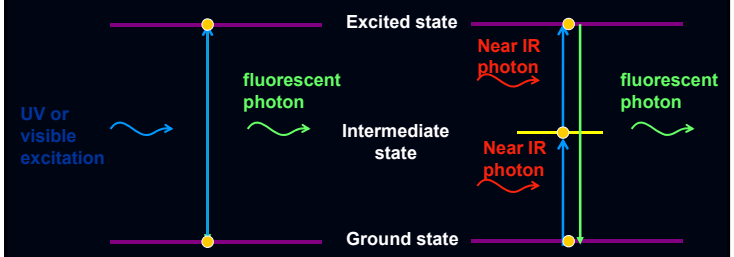
1. Technical developments of imaging modalities
   - non-linear optical microscopy (fluorescence/harmonic generation)
   - label-free, *in vivo* imaging
   - quantify physiological changes in tissues
2. *Ex-vivo* spectral characterization of tissue
3. Intravital investigations of biologically and medically relevant issues
   - intravital imaging chambers (dorsal skin fold, hepatic)
   - characterization of synthesized tissues (tissue engineering)

3



4

Two-Photon Fluorescence Excitation



- Theoretical prediction – Göppert-Mayer (1931)
- Experimental verification – Kaiser and Garrett (1961)
- Harmonic generation microscopy – Sheppard (1978)
- Multiphoton microscopy – Webb (1990)

5

Multiphoton Microscopy

- Two- (Multi-) 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 (uncaging, femtosecond laser surgery)
- Harmonic Generation Microscopy
  - $D=\varepsilon_0 E+P$
  - $P_i=\chi_{ij}(1)E_j+\chi_{ijk}(2)E_jE_k+\chi_{ijkl}(3)E_jE_kE_l+\ldots$
  - Biological harmonic generators: collagen, muscle fibers, …

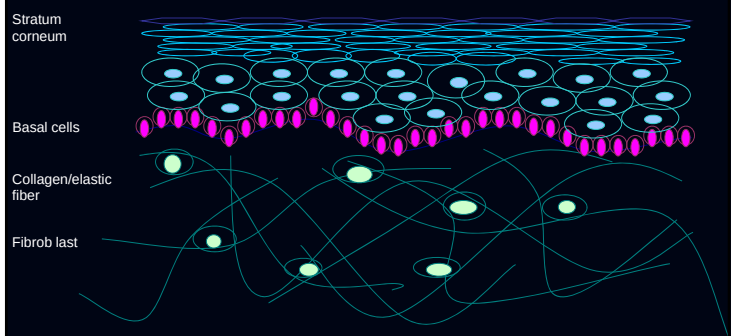
6

Biological Applications

1. Dermatology
   - skin photoaging, skin cancer (BCC), transdermal drug delivery
2. Ophthalmology
   - minimally-invasive characterization of eye structures
   - corneal pathology
   - corneal infection and wound healing (CK, etc.)
3. Infectious Pathogens
   - fungi, bacteria
4. Tumor Physiology
   - multiphoton tissue characterization
   - development of intravital imaging technologies
5. Hepatology
   - *in vivo* liver imaging (metabolic activities)
   - infection
6. Tissue Engineering
   - chondrogenesis
7. Neurobiology/Neuropathology
   - cranial window
8. Developmental Biology
   - *Drosophila*, zebra fish
10. Dentistry

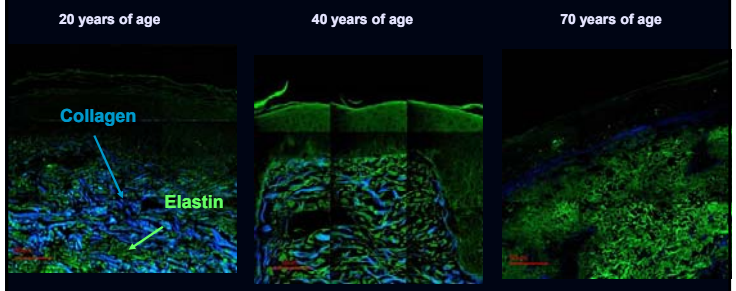
7

Skin Structure (similar to eye)



8

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

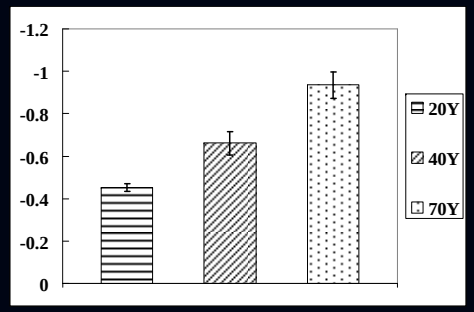


SHG: blue
AF: green

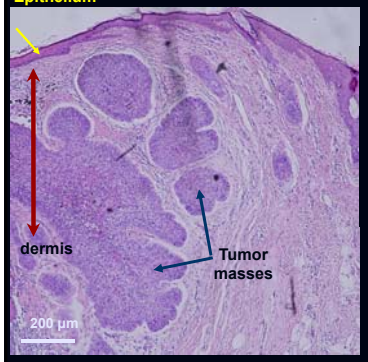
Lin et al., *Optics Letters*, 30(17): 2275-2277, (2005).

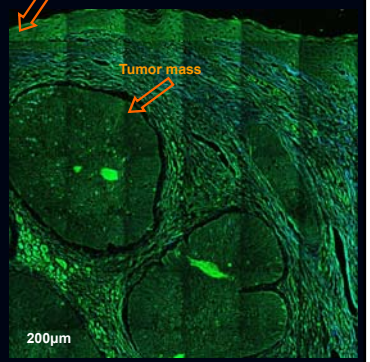
Quantitative Determination Using SHG to AF Aging Index

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

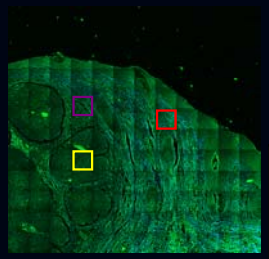


Basal Cell Carcinoma (BCC) Imaging





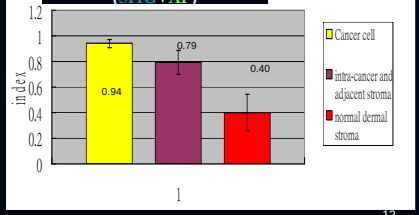
Quantitative Analysis of BCC



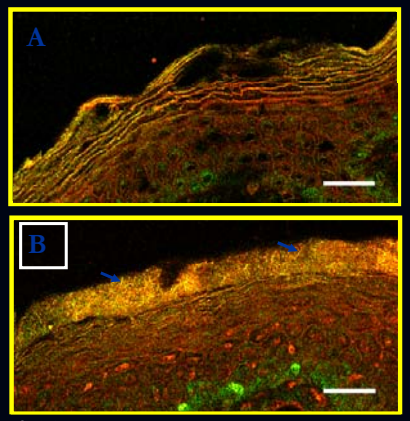
- Cancer cell
- inter-tumor within dermis
- normal dermal stroma

Tumor proximity index= (AF-SHG)/ (SHG+AF)

Sample size：3



Nile red detection of alterations of intercellular lipid organization



Control

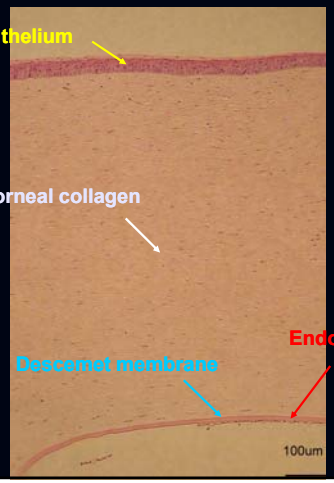
Lipid: yellow-orange
Green: AF

Depilatory

Scale bar : 30μm

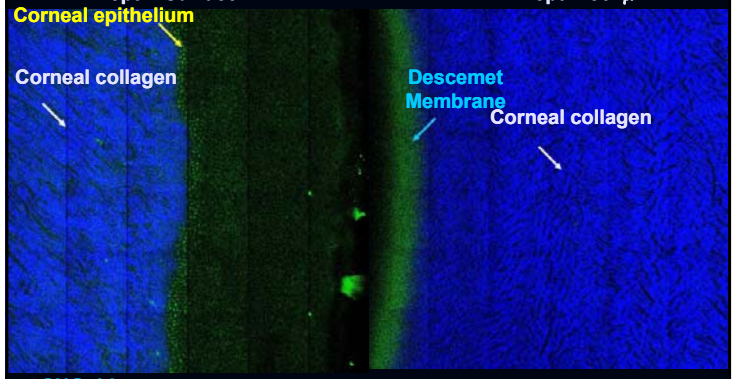
13

Porcine Cornea Structure



14

Large Area Multiphoton Scan of Porcine Cornea

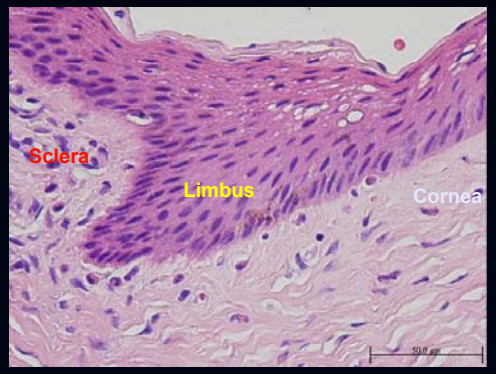


SHG: blue
AF: green

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

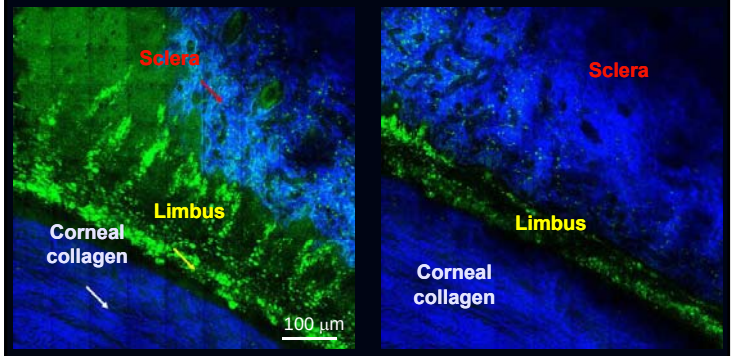
15

Porcine Limbus Structure



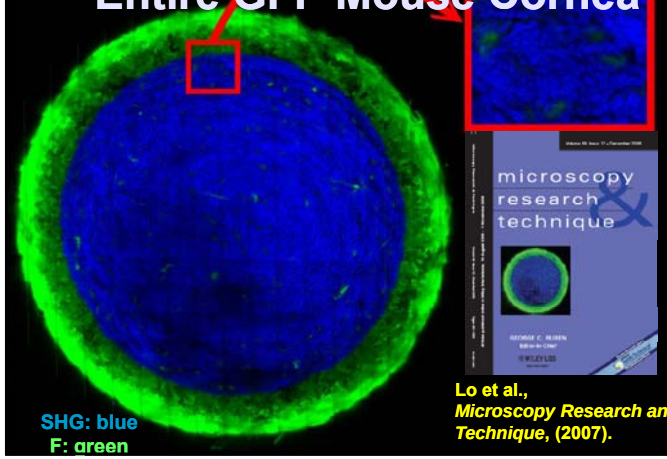
16

Multiphoton Scan of Porcine Limbus



17

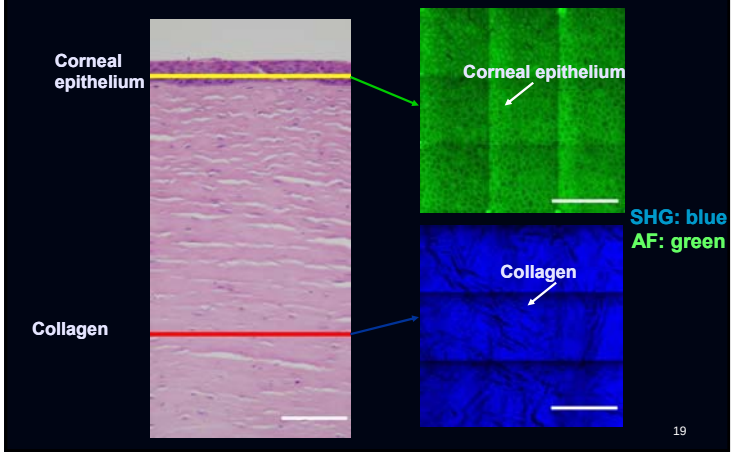
Multiphoton Imaging Across an Entire GFP Mouse Cornea



Lo et al., *Microscopy Research and Technique*, (2007).

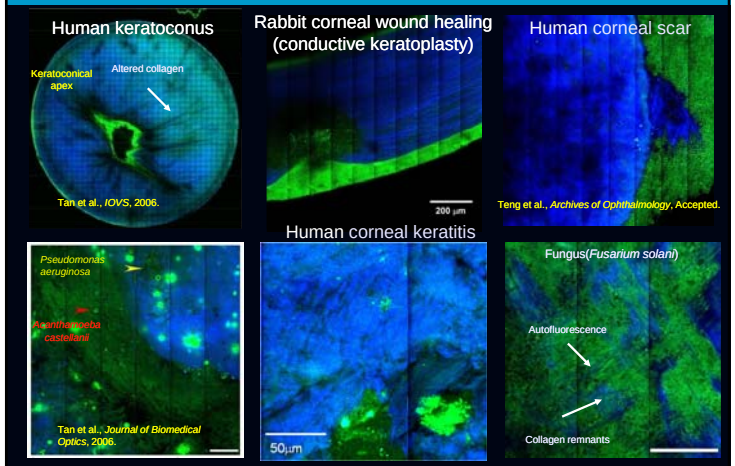
18

Multiphoton Imaging of Normal Human Cornea



19

Ophthalmology (cornea pathology)

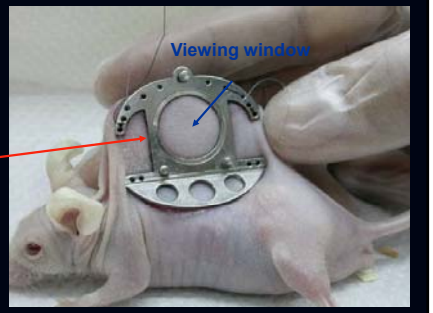


SHG: blue; Fluorescence, Autofluorescence : green

20

Dorsal Skin Fold Chamber for In Vivo Oncology

6Al/4V ELI titanium alloy

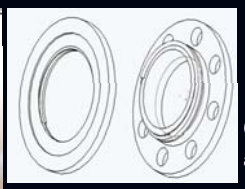


1. Angiogenesis 2. Metastasis 3. Tumor treatment etc.

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

21

In Vivo Hepatic Imaging Chamber



6Al/4V ELI titanium alloy

Liu et al., *Journal of Biomedical Optics*, (2007).

22

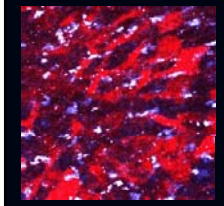
Commonly Encountered Liver Pathologies

- Hepatocellular carcinoma (HCC)
  - No. 1 or 2 leading causes of death in Taiwanese cancer patients
- Liver fibrosis (response to liver injury)
  - Chronic hepatitis B infection
  - Toxin (CCl_4, alcohol, …)
- Fatty liver (excessive lipid or sugar uptake)
- Infection (virus, amoeba, …)
- Traditional diagnostic methods
  - Biochemical
    - Albumin secretion, indocyanine green , …
  - Structural
    - Ultrasound, CT
    - EM, optical histological examination (H&E)

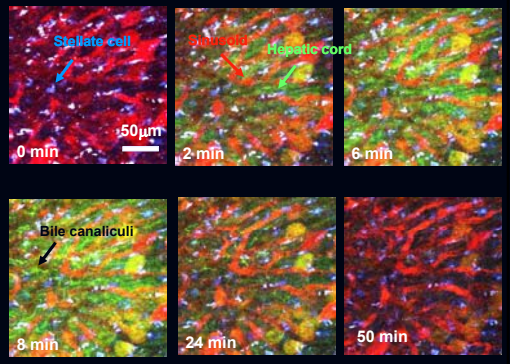
23

In Vivo Hepatic Metabolism

Metabolic Movie



Temporal snapshots



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

24

Depth Resolved, In Vivo Imaging of Mouse Liver

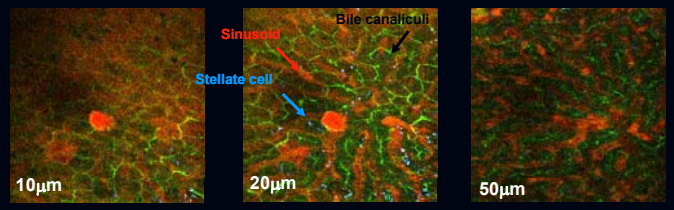
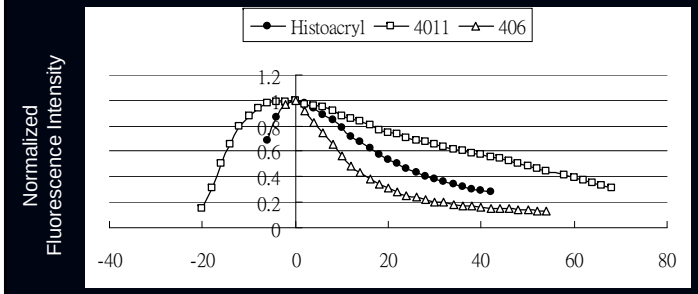


Image size: 220 μm by 220 μm

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

25

Temporal Dependence of Carboxyfluorescein Intensity Profiles



Minutes Relative to Maximum Fluorescence

Histoacryl: tissue adhesive

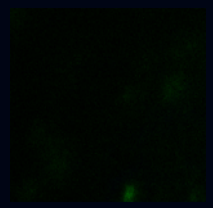
4011 (Henkel Loctite): for medical device

406 (Henkel Loctite): typical instant adhesive

26

High Resolution *In Vivo* Hepatic Imaging (axial)

Label-free

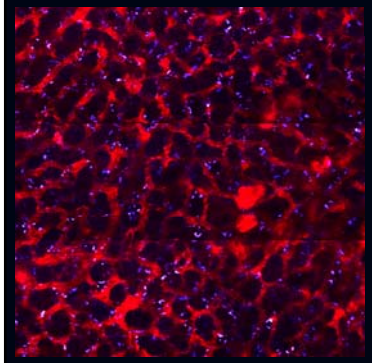


Rhodamine dextran/CFDA-6

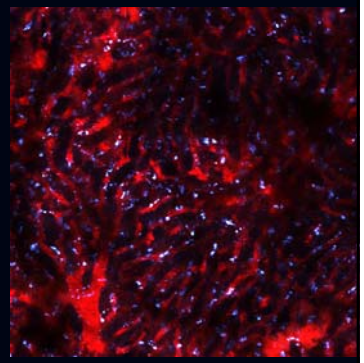
27

Bile Duct Ligation

Normal liver

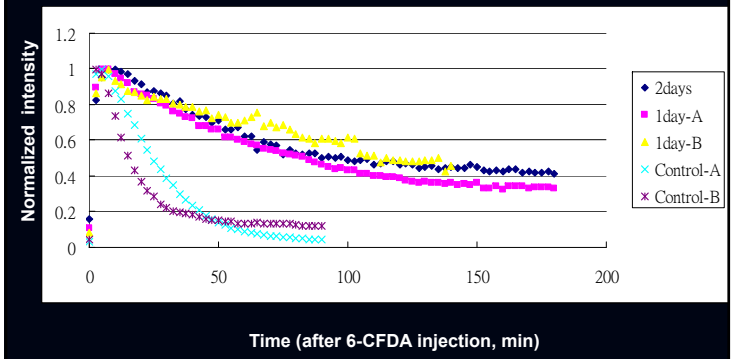


1 day post bile duct ligation



28

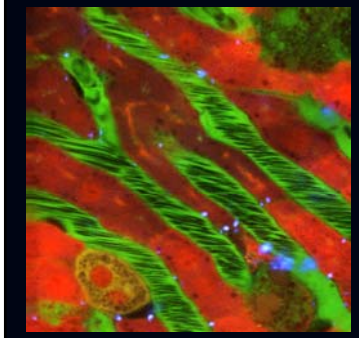
Bile Duct Ligation Induced 6-CFDA Fluorescence Change

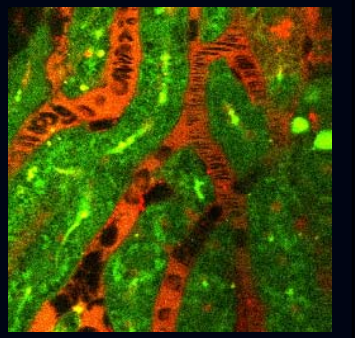


29

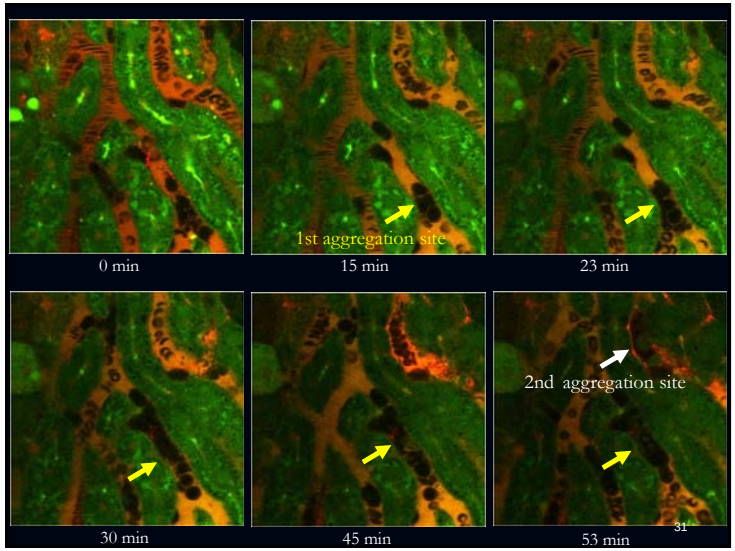
Mouse Liver Response to Lipopolysaccharide (LPS) Injection

Normal mouse **Mouse with LPS**





30



31

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
  - Quantifying physiological parameters in vivo
- Theoretical description/modeling
  - Cell-matrix interaction (migration, differentiation, assembly)
  - Cell/tissue metabolism
  - Tissue reorganization/genesis (interaction of biomechanical and biochemical pathways)
  - Infection
- High scientific and application values

32