

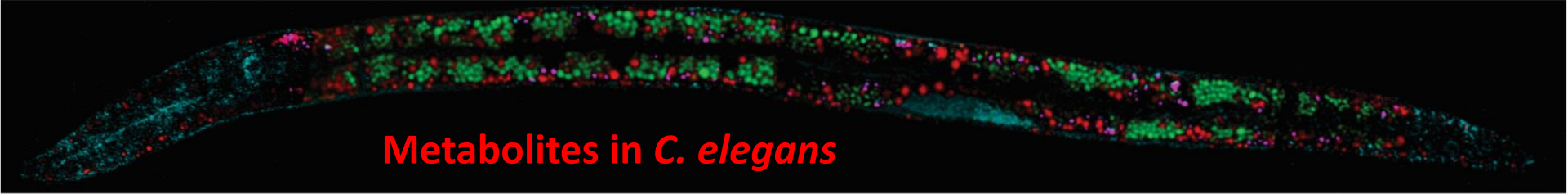


Molecular Spectroscopy of Living Systems

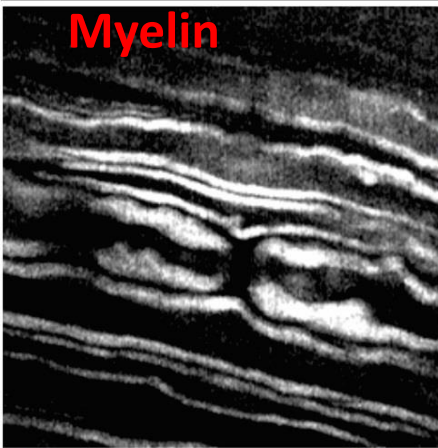
Ji-Xin Cheng

June 20, 2016

International Symposium of Molecular Spectroscopy

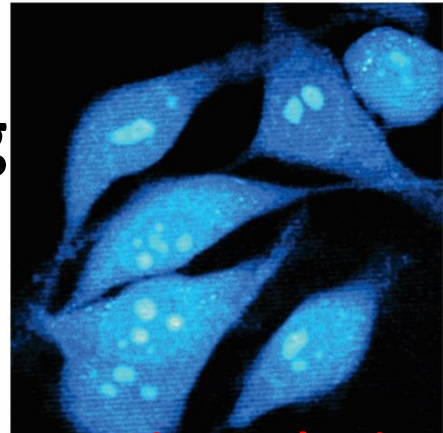
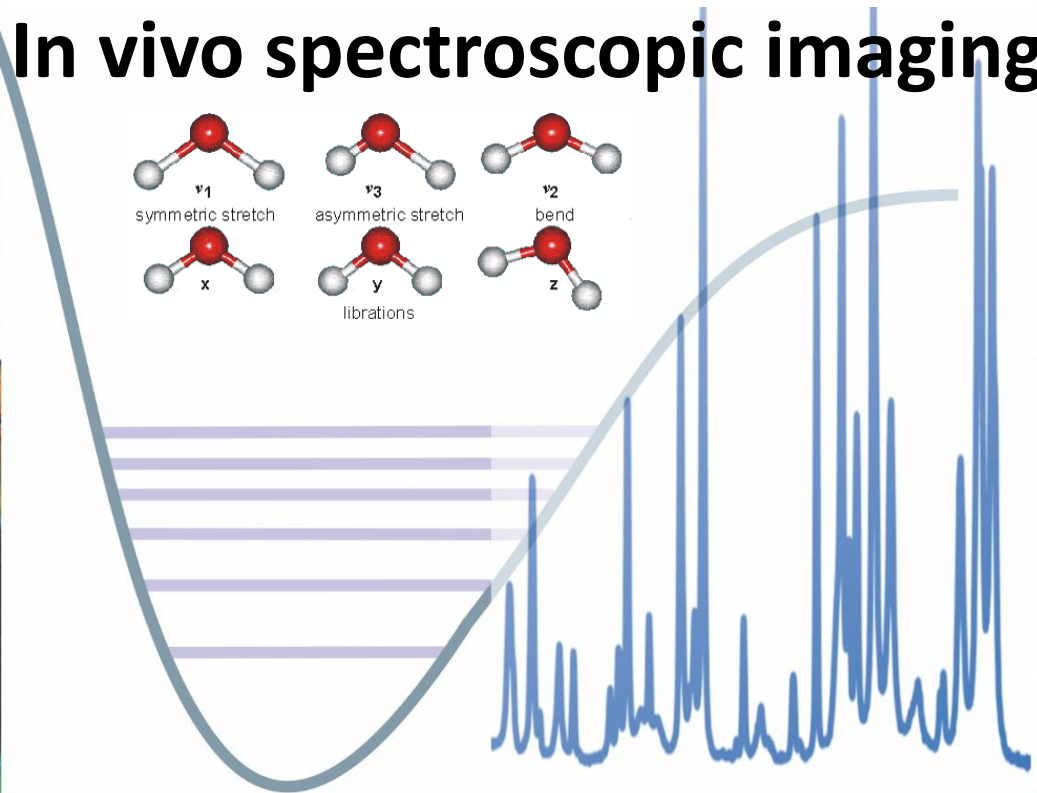
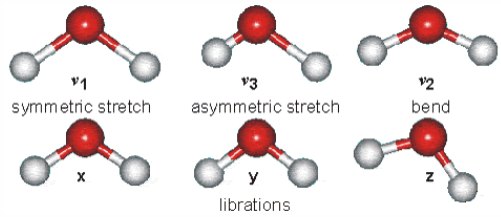


Metabolites in *C. elegans*

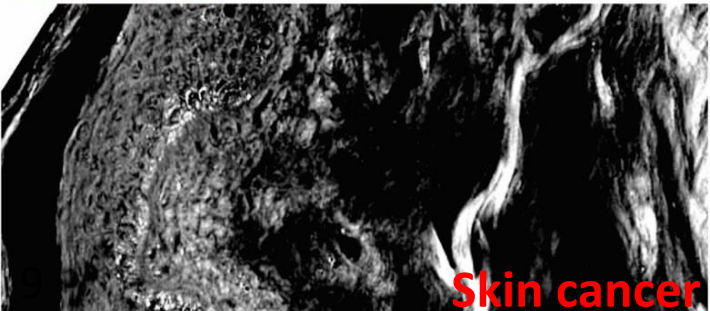
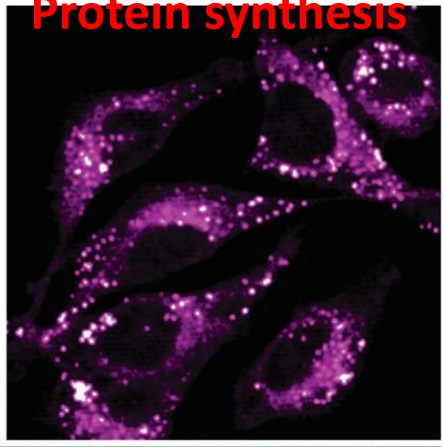
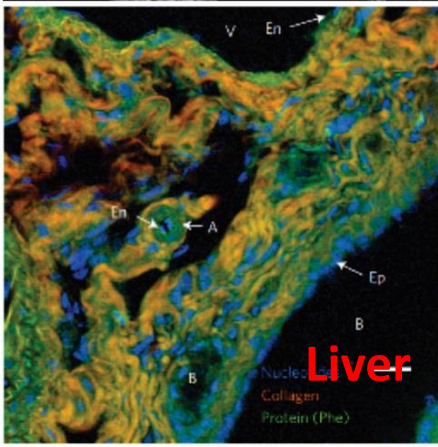


Cheng&Xie, *Science*, 2015, 350: 1054

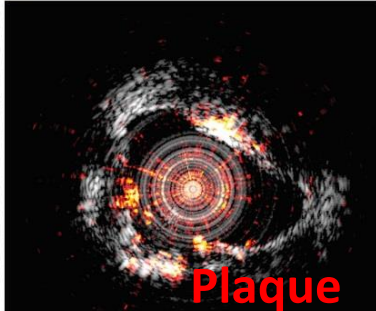
In vivo spectroscopic imaging



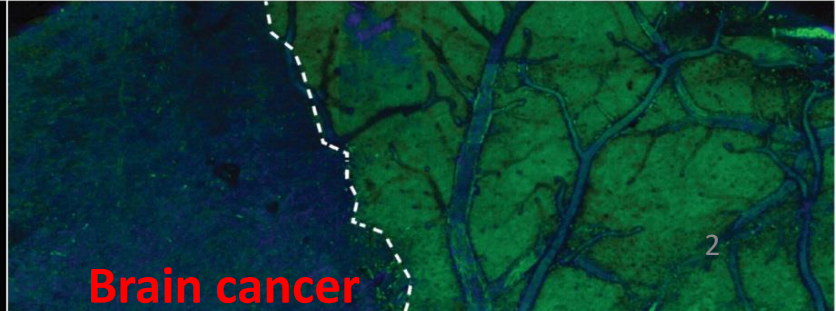
Protein synthesis



Skin cancer



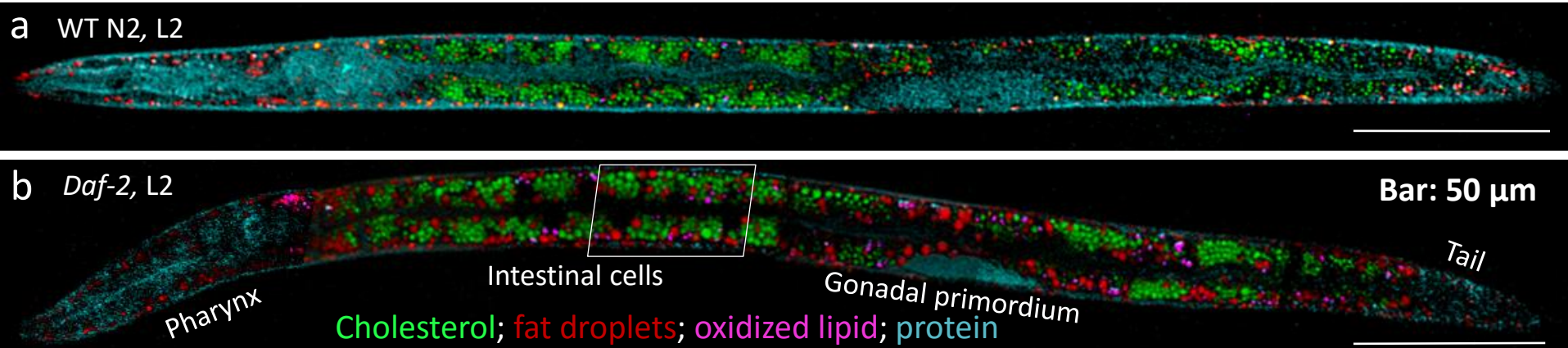
Plaque



Brain cancer

Longevity: *C. elegans* as in vivo test tube

Fingerprinting Lipid Metabolism in Live *C.elegans*



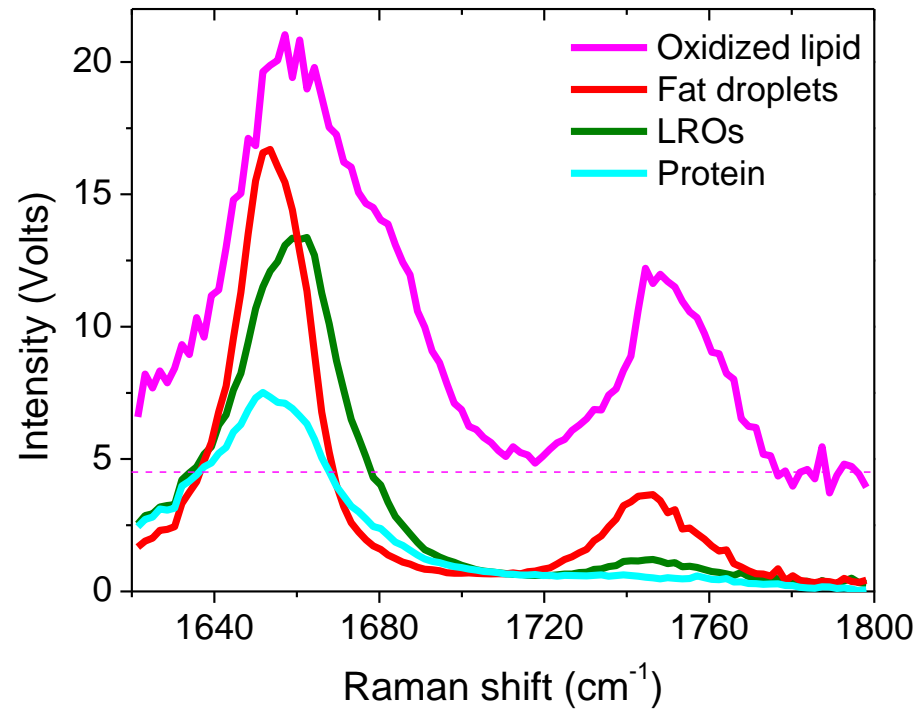
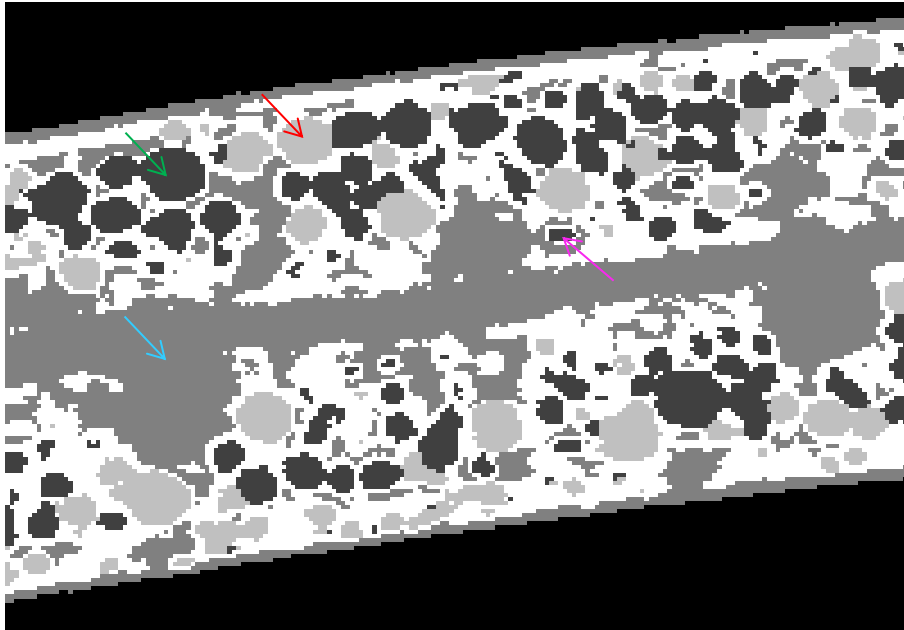
Angew Chem Int Ed, 2014, 53: 11787.
In collaboration with Heidi Tissenbaum (U Mass).



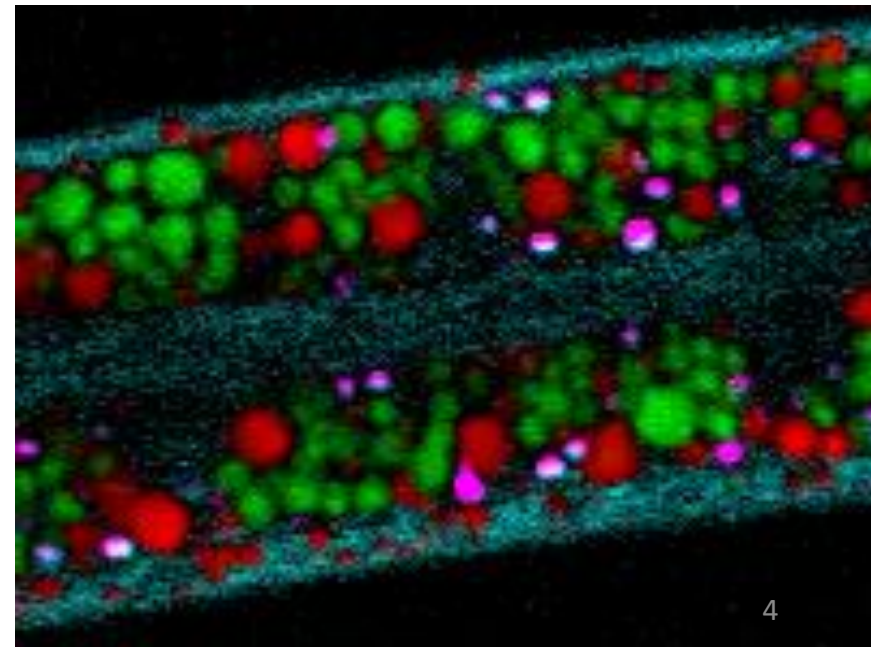
1620.0 cm^{-1}

k-means clustering

1  2  3  4 



Multivariate curve resolution



In vivo Molecular Spectroscopic Imaging

Outline

1. Pushing the physical limits of spectroscopic imaging

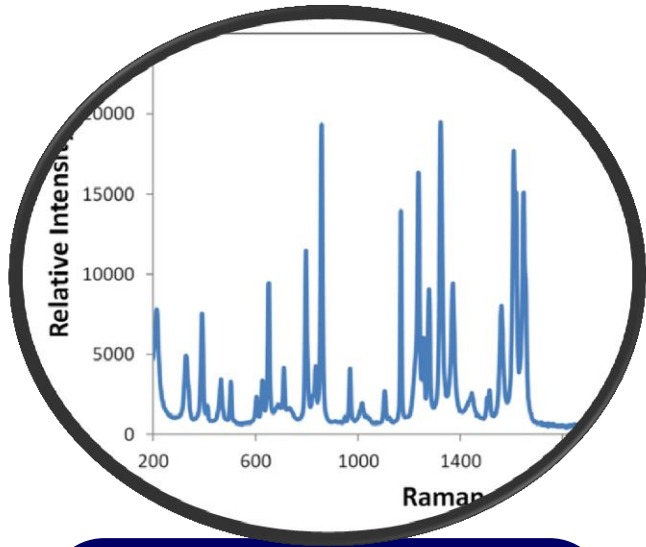
- Speed
- Sensitivity
- Depth
- Resolution
- Volume

2. Shedding new light on cellular machinery

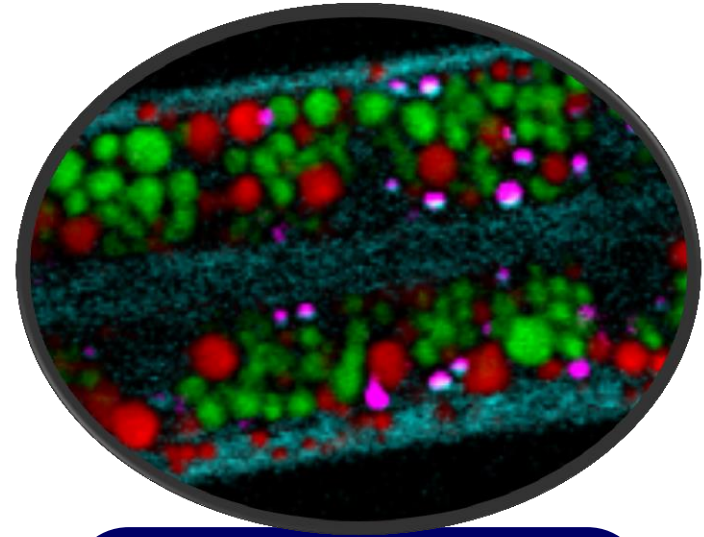
- Cell metabolism
- Stem cell marker
- Membrane voltage

3. Moving into clinic for molecule-based diagnosis

- Lipid-laden plaque
- Breast cancer margin
- precision surgery



**In vitro
spectroscopy**



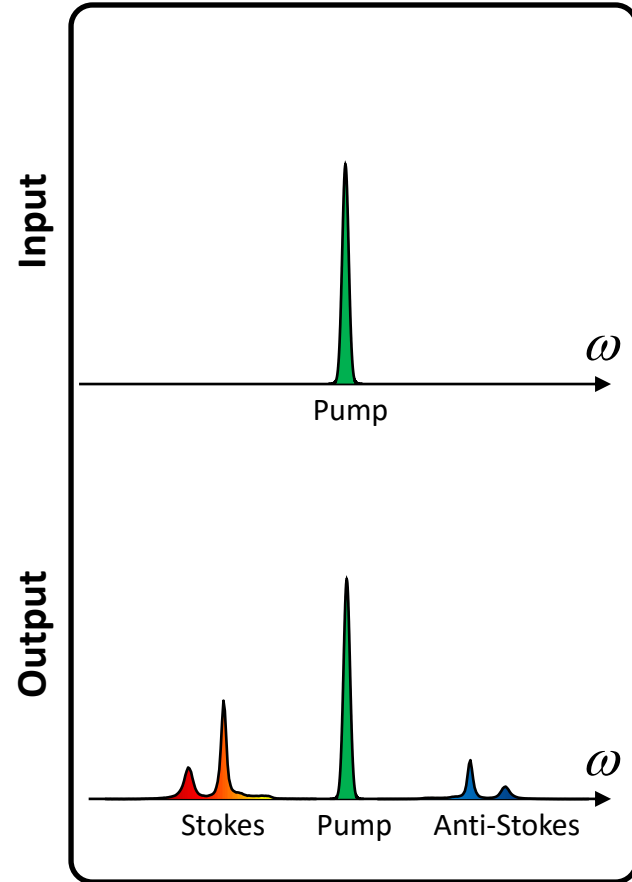
**In vivo
spectroscopic
imaging**

≠ Spectrometer + Microscope

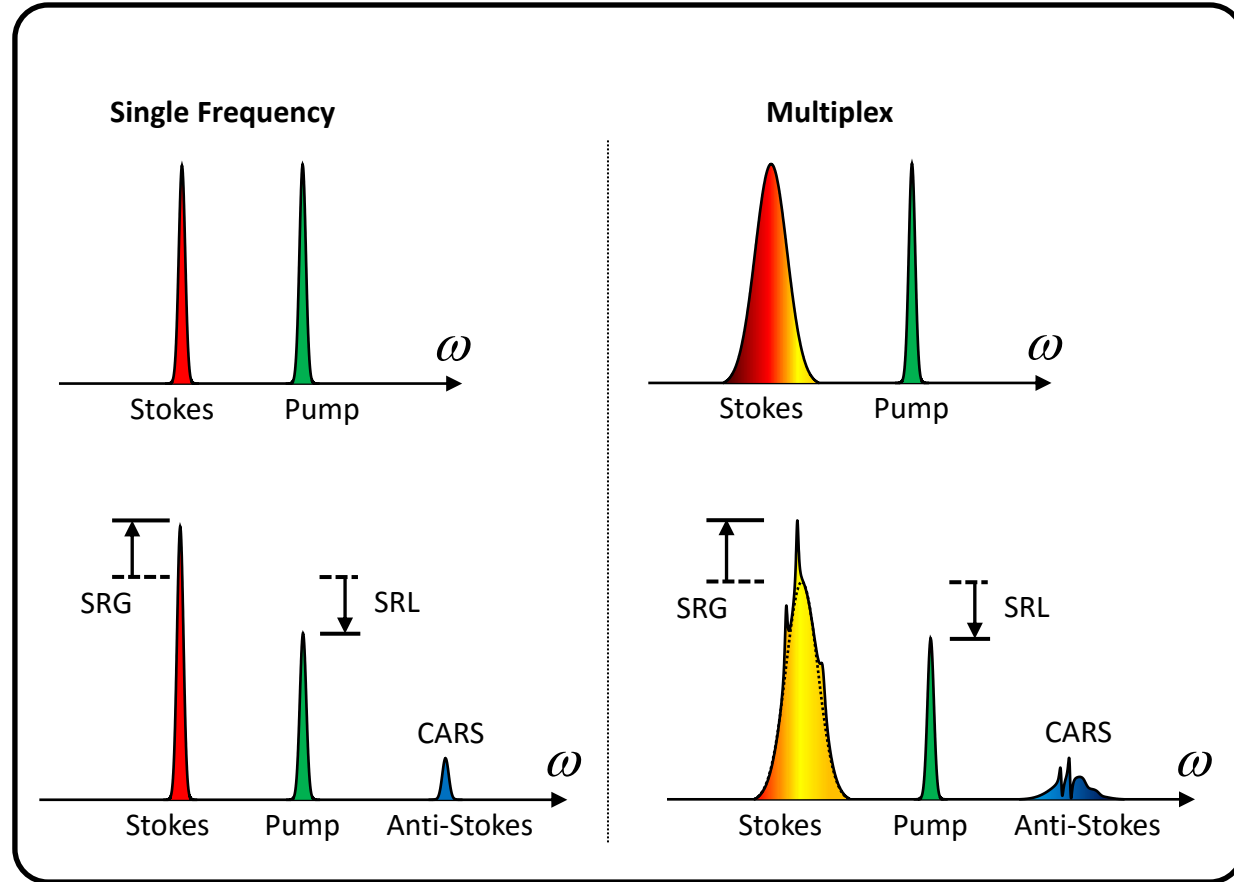
Cheng&Xie, Review, *Science*, 2015, 350: 1054

Coherent Raman Boosts the Speed

Spontaneous Raman



Coherent Raman Scattering



CARS : coherent anti-Stokes Raman scattering

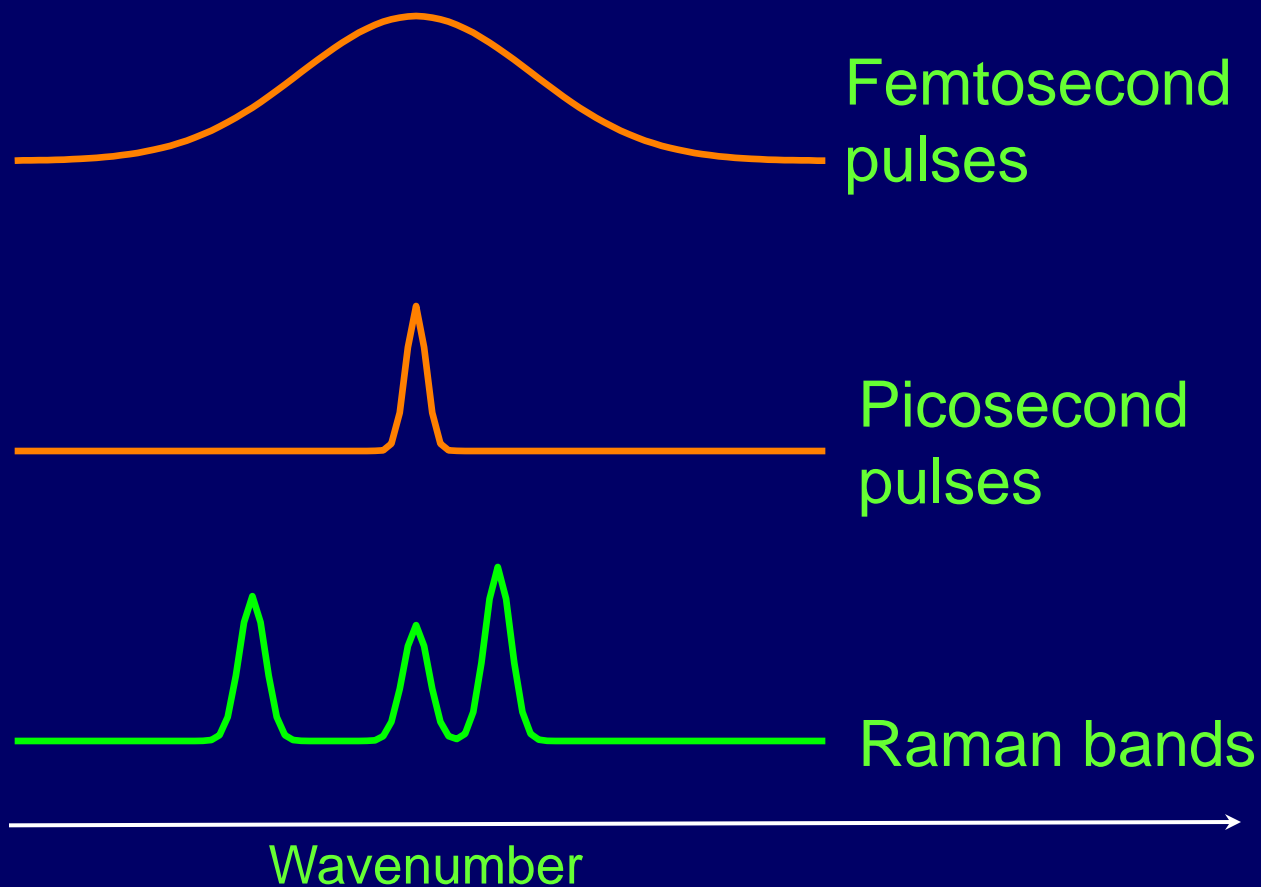
SRL: stimulated Raman loss, SRG: stimulated Raman gain

SRS: Woodbury, E. J.; Ng, W. K. *Proc. Inst. Radio Eng.* **1962**, 50, 2367.

CARS: Terhune, R. W.; Maker, P. D.; Savage, C. M. *Phys. Rev. Lett.* **1965**, 14, 681-684.

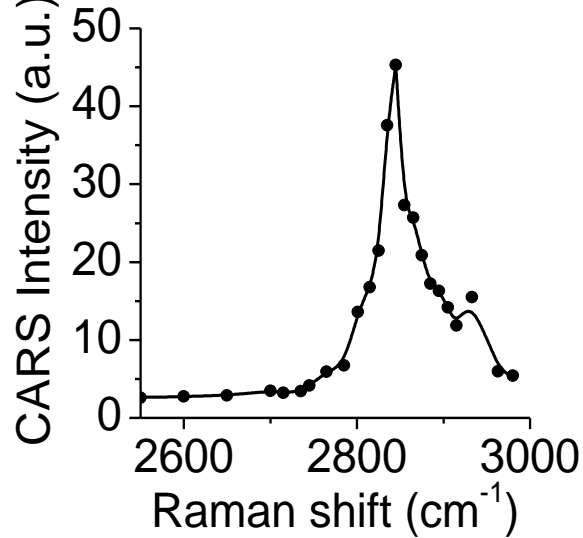
Picosecond Pulse Excitation for CARS

Focus the energy on a single Raman band; high spectral resolution

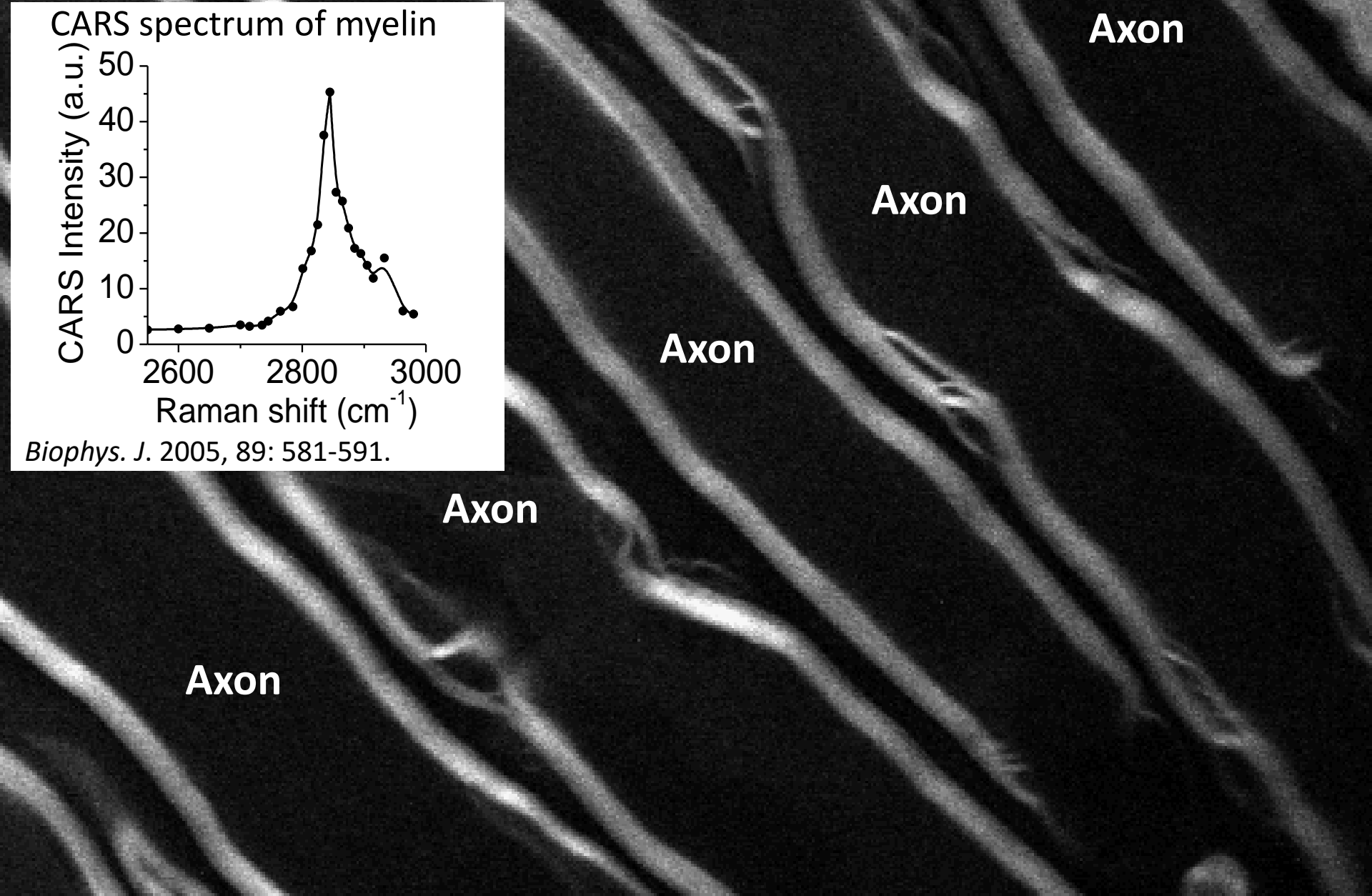


CARS image of myelin sheath

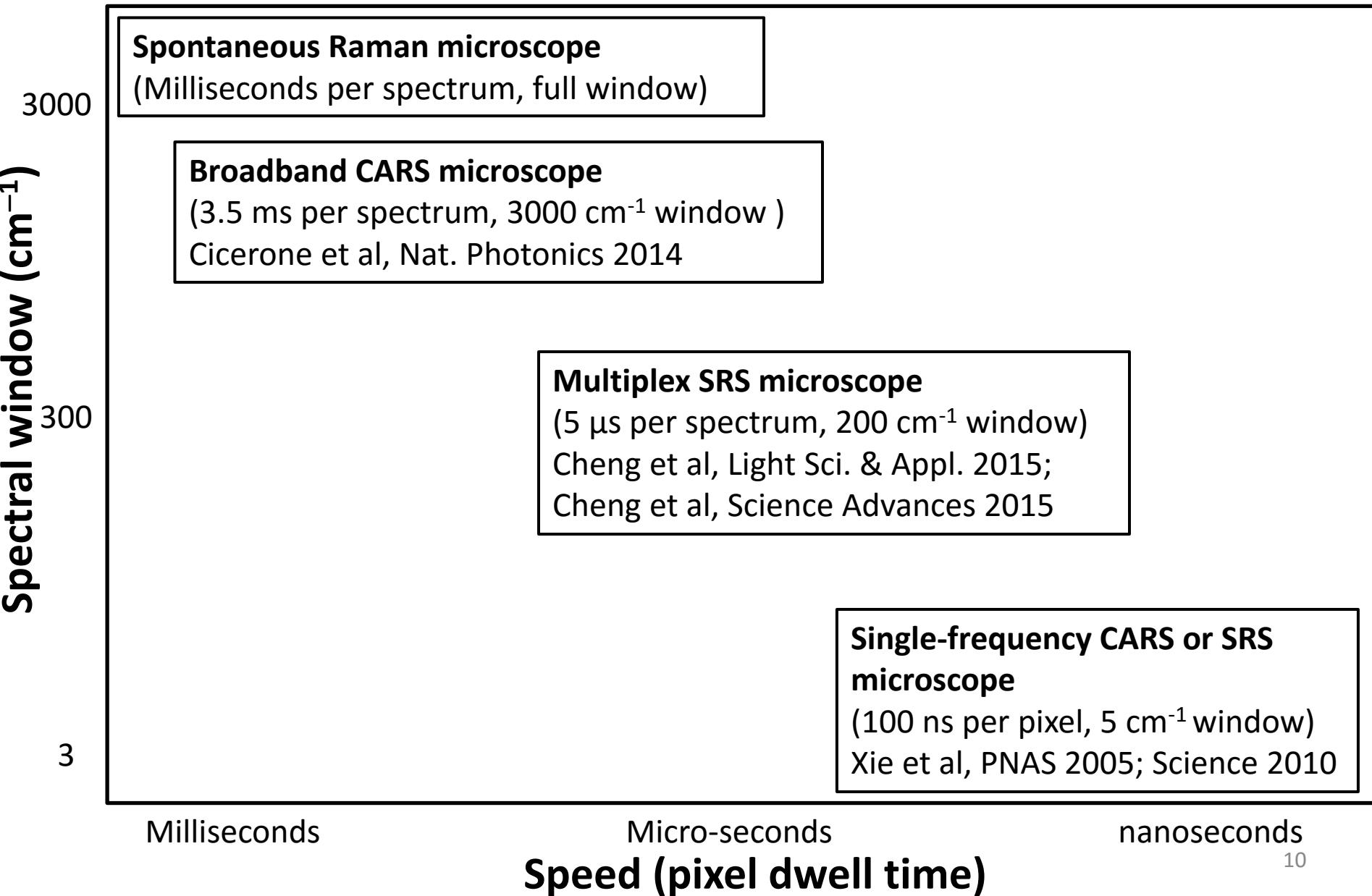
CARS spectrum of myelin



Biophys. J. 2005, 89: 581-591.

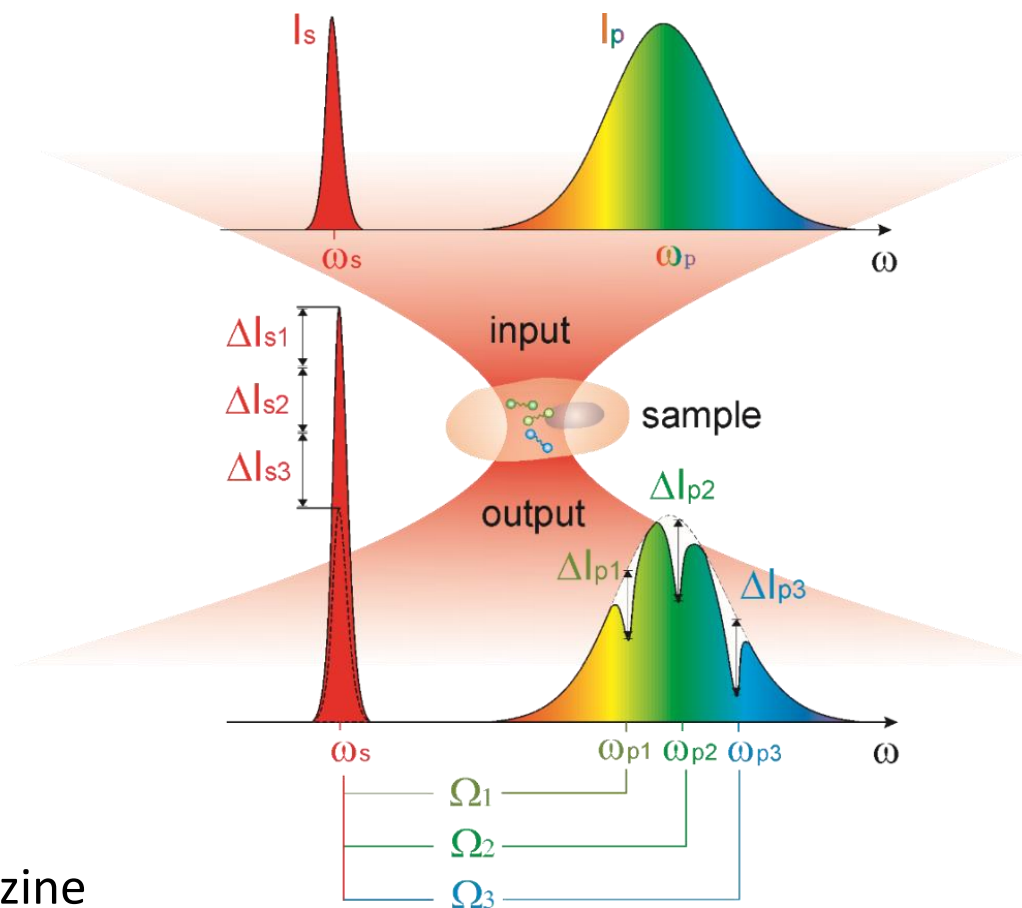


Spectral imaging speed versus spectral width



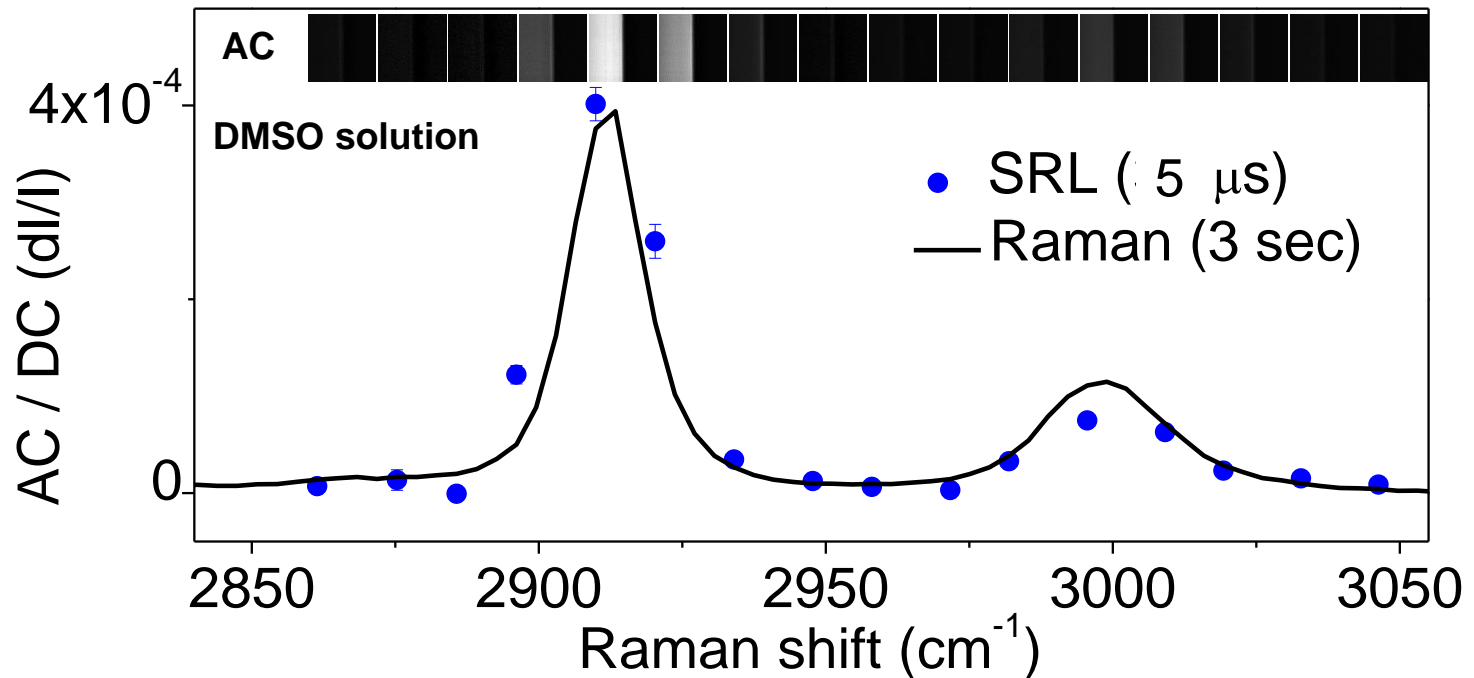
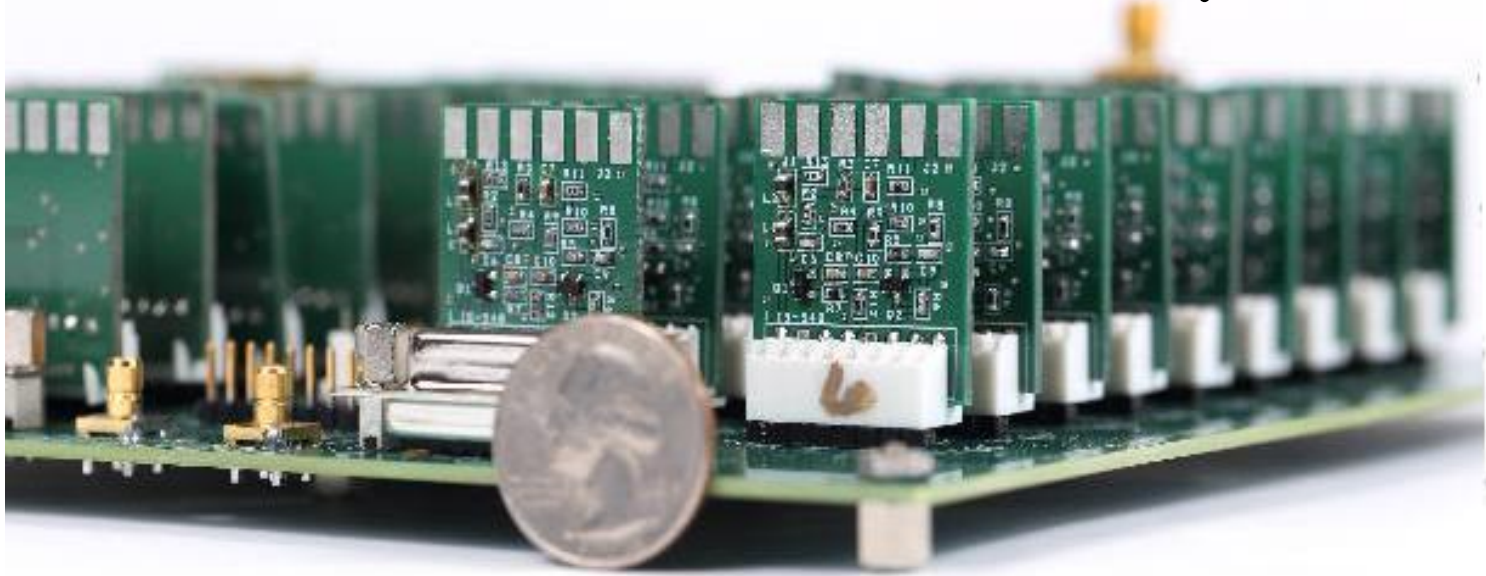
Microsecond scale vibrational spectroscopic imaging by multiplex stimulated Raman scattering microscopy

Chien-Sheng Liao^{1,*}, Mikhail N Slipchenko^{1,*}, Ping Wang^{1,*}, Junjie Li², Seung-Young Lee¹, Robert A Oglesbee³ and Ji-Xin Cheng^{1,3}

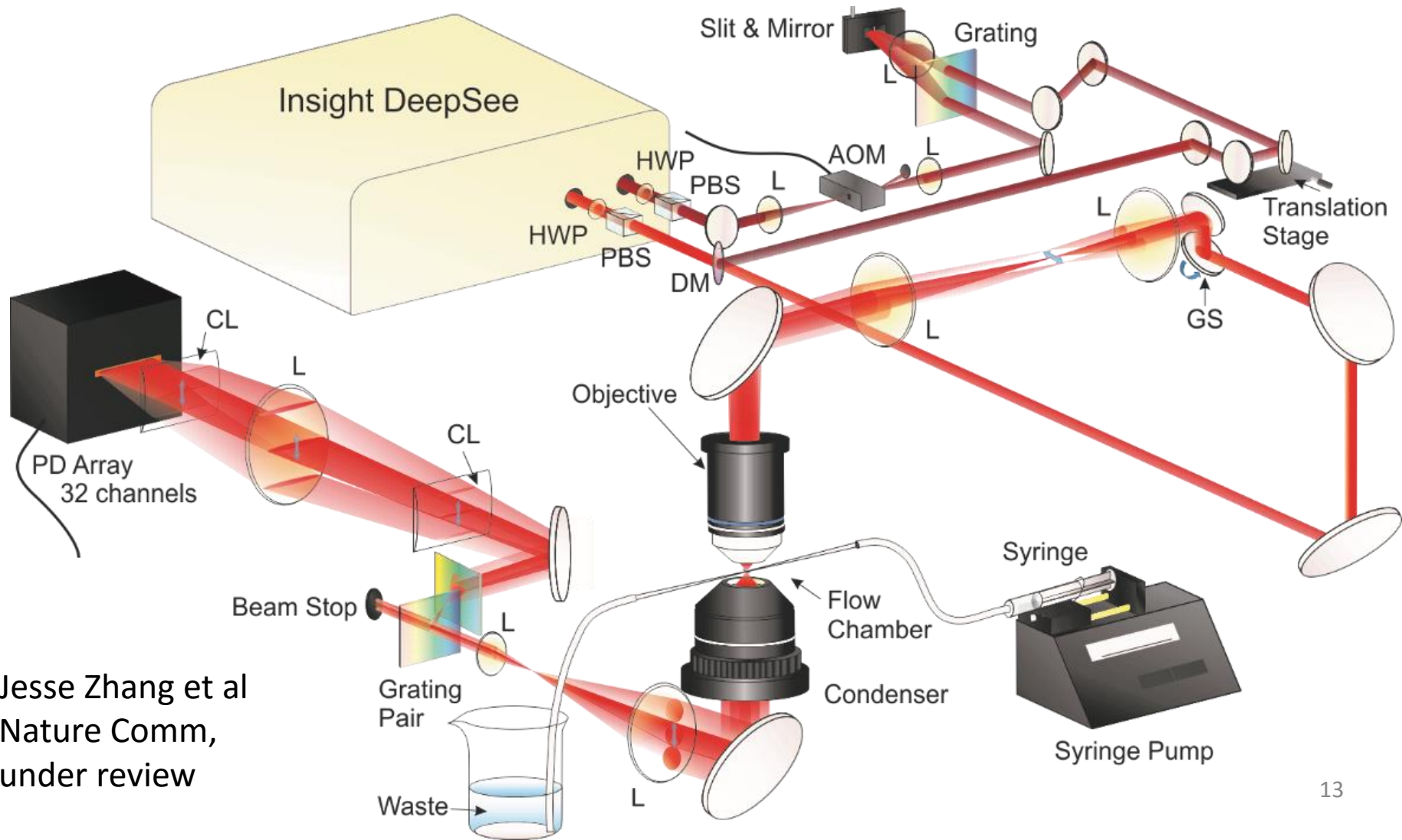


Top Story in 2015,
Biophotonics Magazine

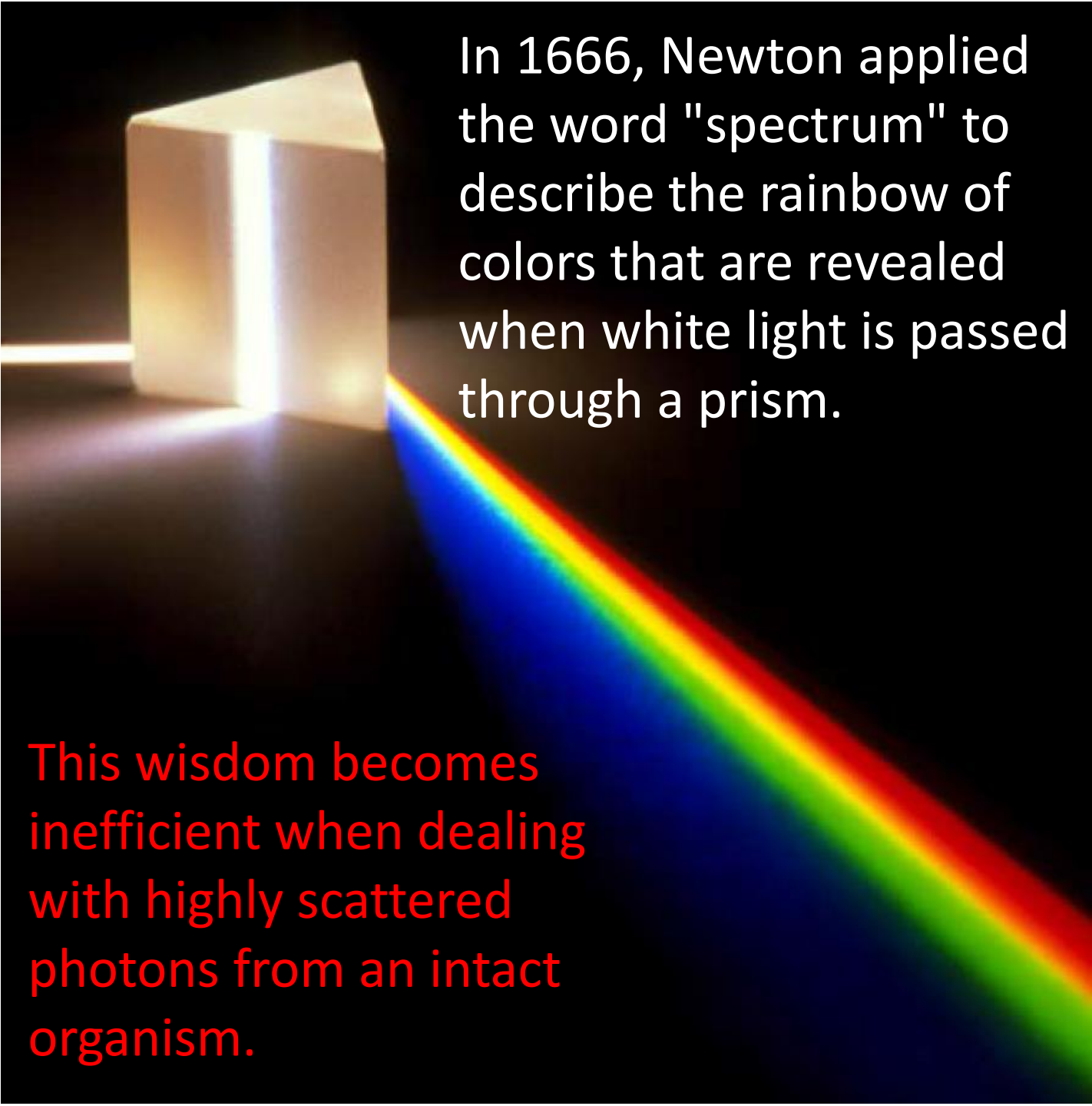
32-channel tuned amplifier for μs Raman



High-throughput single cell analysis by stimulated Raman flow cytometer ($>10,000$ particles (bacteria) /sec, $5 \mu\text{s}$ per spectrum)



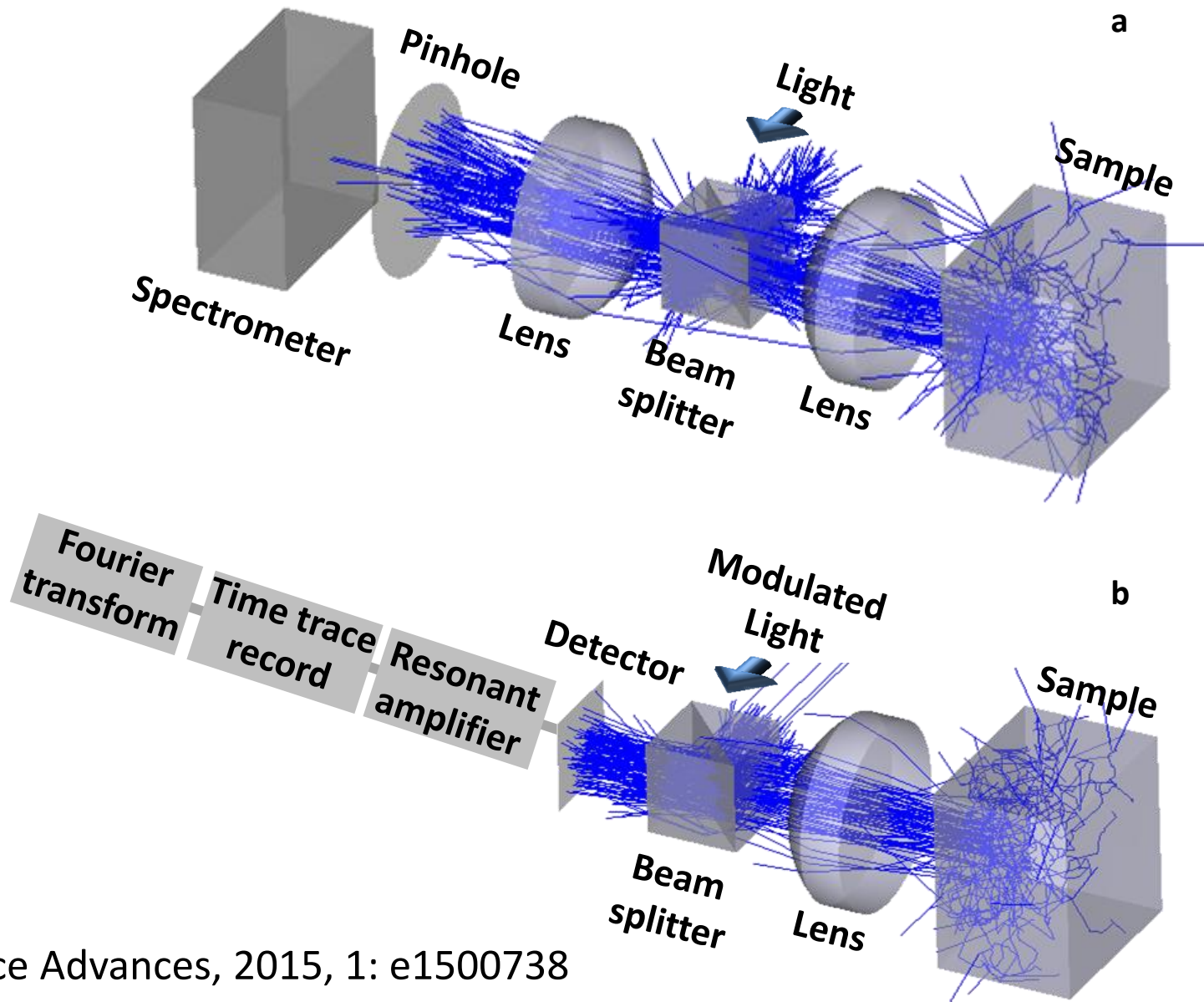
Jesse Zhang et al
Nature Comm,
under review

A photograph of a glass prism on a dark surface. A beam of white light enters the prism from the left, and a spectrum of colors (violet, blue, green, yellow, orange, red) is projected onto the surface to the right. The background is black.

In 1666, Newton applied the word "spectrum" to describe the rainbow of colors that are revealed when white light is passed through a prism.

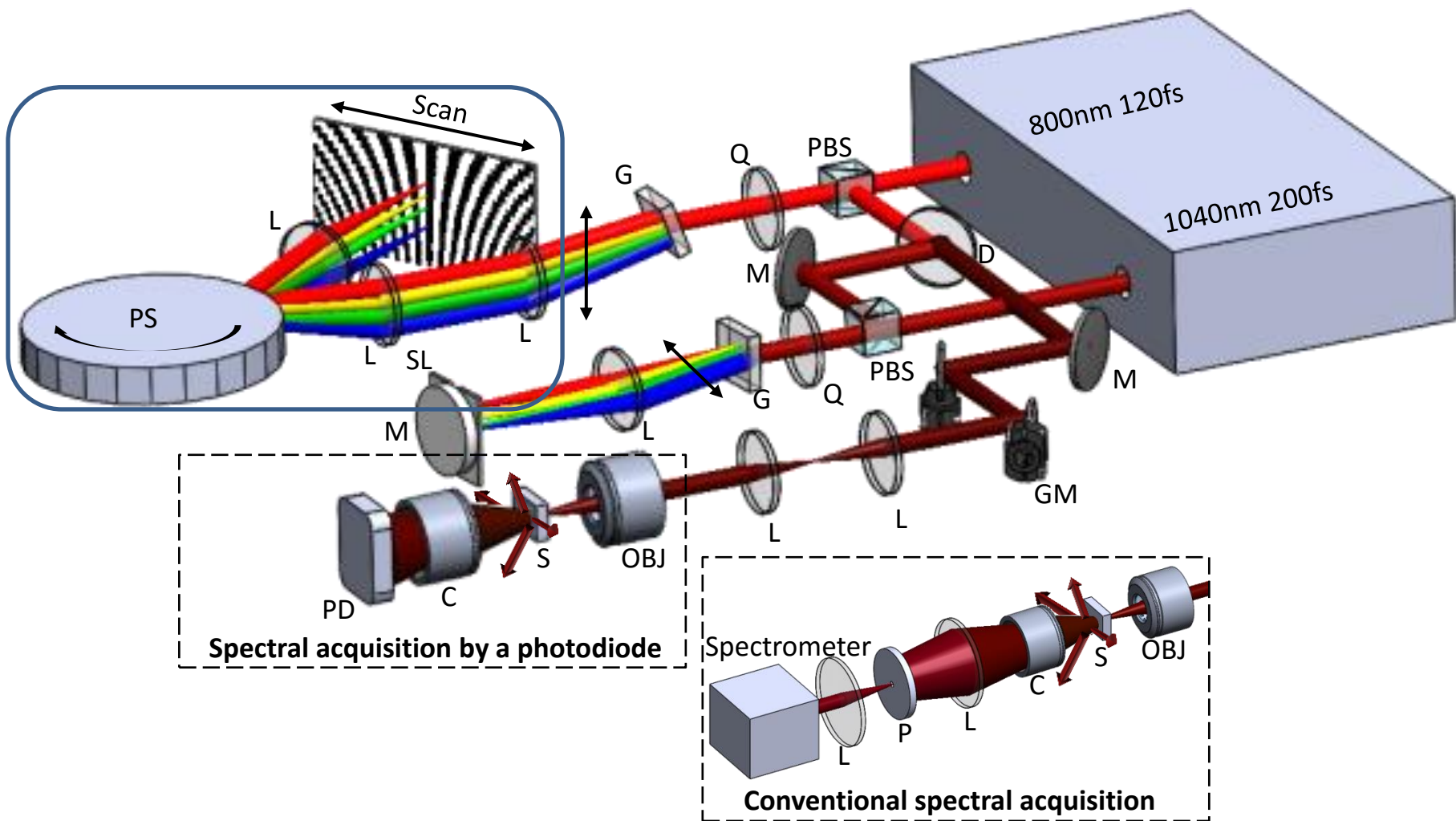
This wisdom becomes inefficient when dealing with highly scattered photons from an intact organism.

Spectral recording of a scattering specimen



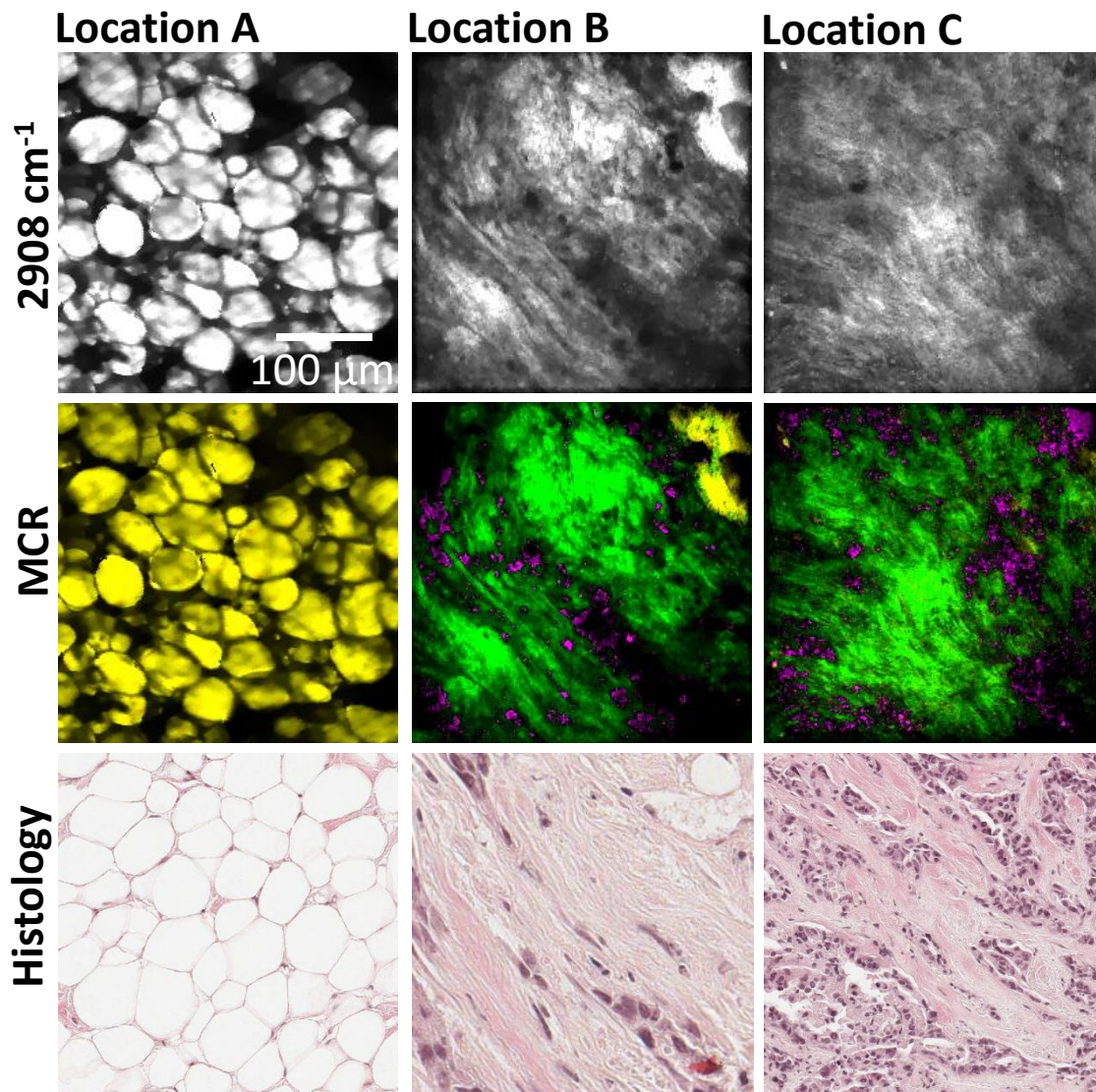
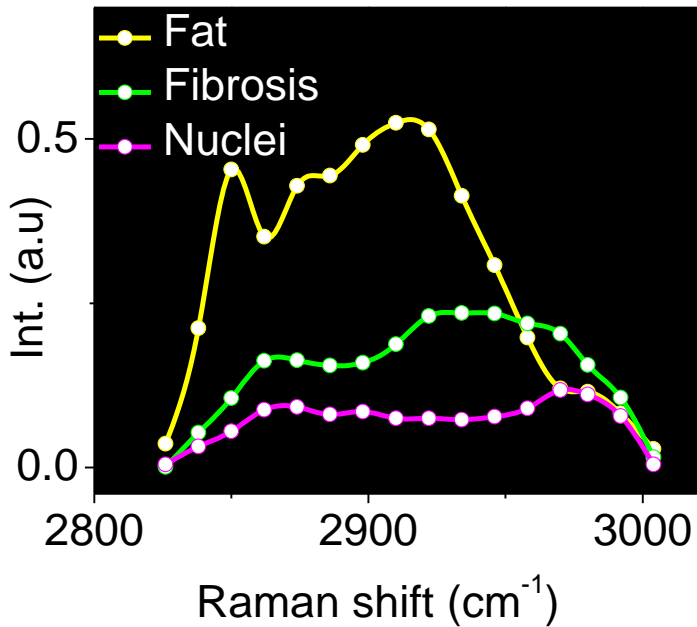
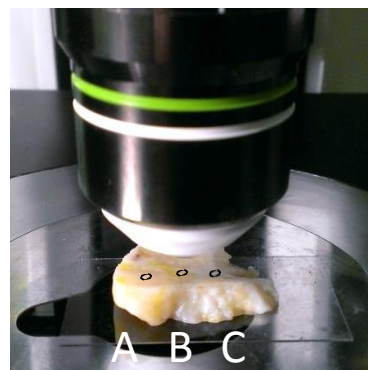
Schematic

C: Condenser, D: Dichotic mirror, G: Grating, GM: Galvo mirror, L: Lens, M: Mirror, OBJ: Objective, PBS: Polarizing beam splitter, PD: Photodiode, PS: Polygon scanner, Q: Quarter waveplate, SL: Slit,

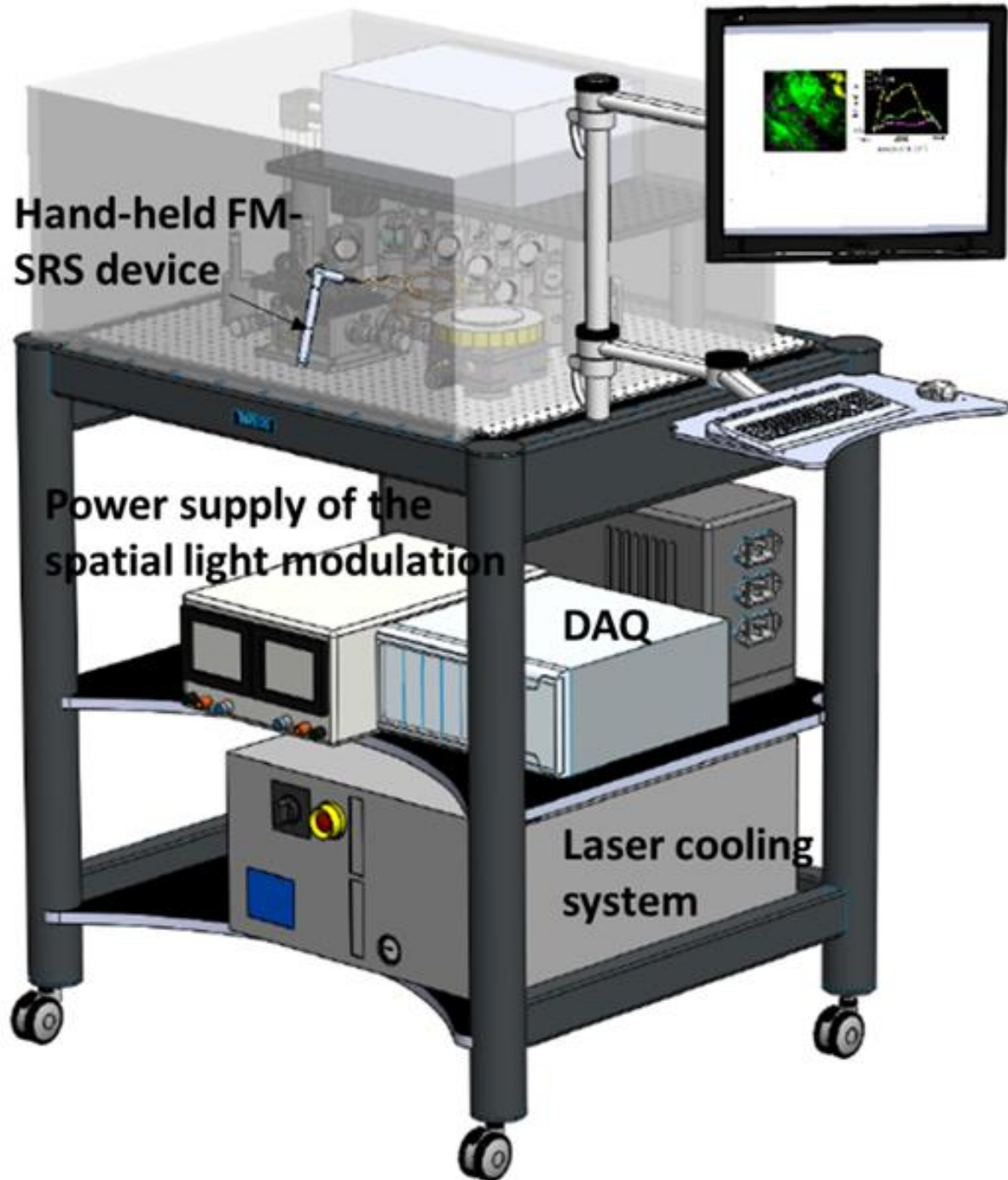


For highly scattered photons, our scheme improves detection efficacy by 2000 times!

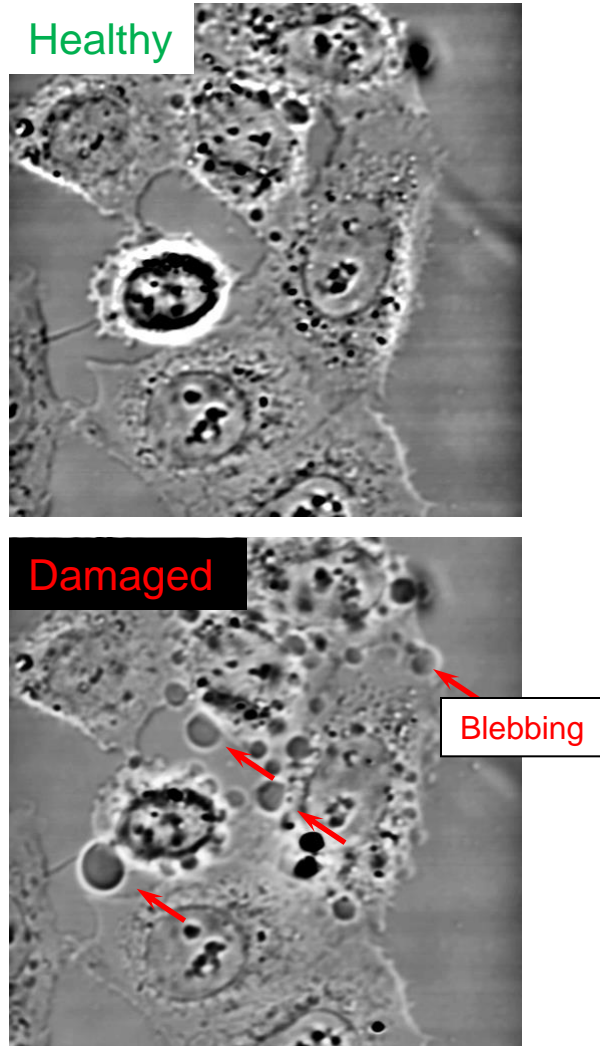
In situ histology: mapping single tumor cells and stroma in human patient breast tissue



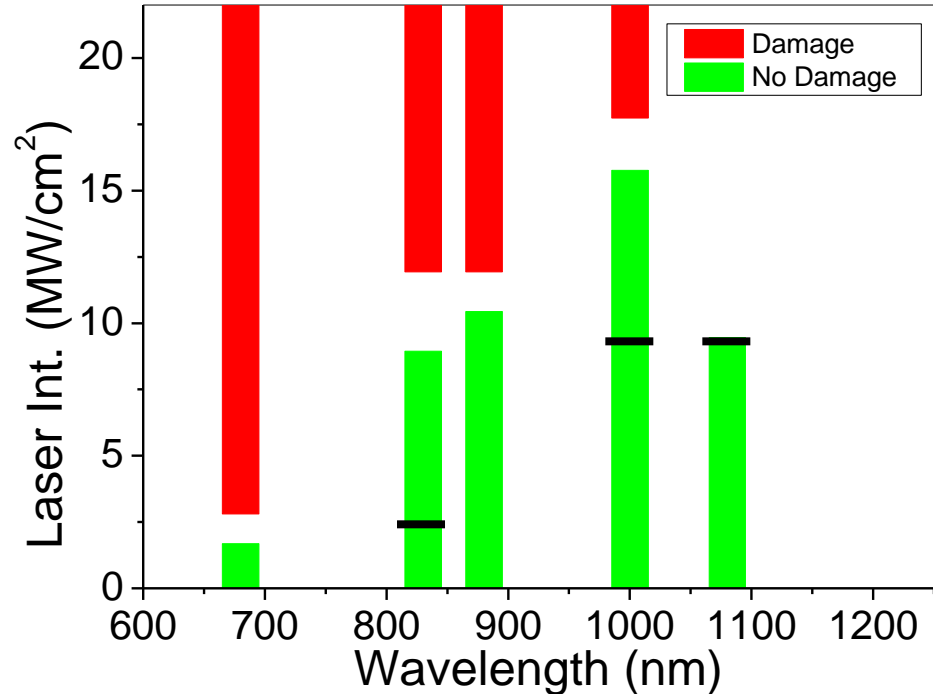
Transportable & hand-held system for in situ spectroscopic imaging



Longer-wavelength Excitation Reduces Photodamage to Cells in SRS Imaging



Cells scanned for 2 min



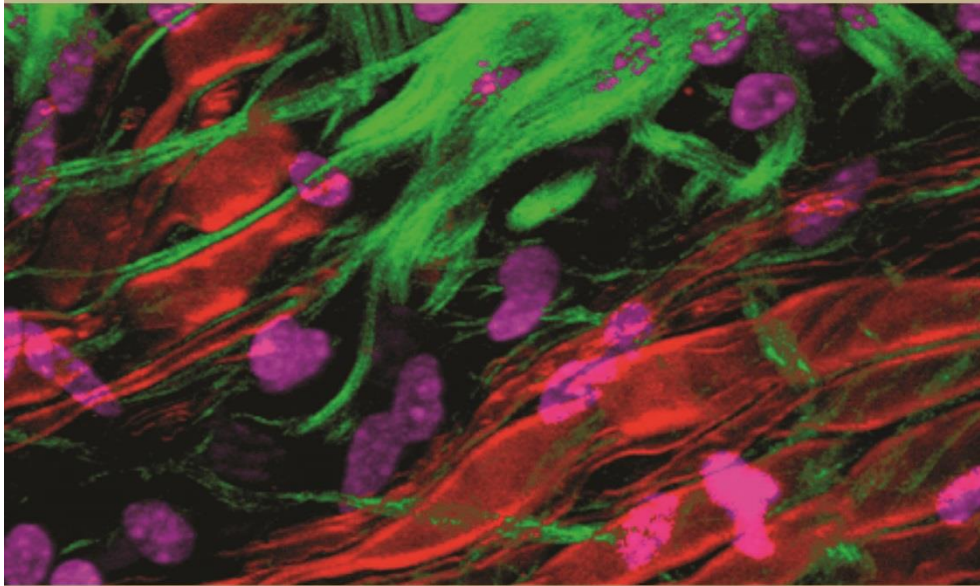
Max. power used for SRL imaging:

830 nm: 50 mW before microscope

1004 nm: 200 mW before microscope

1080 nm: 200 mW before microscope

Coherent Raman Scattering Microscopy

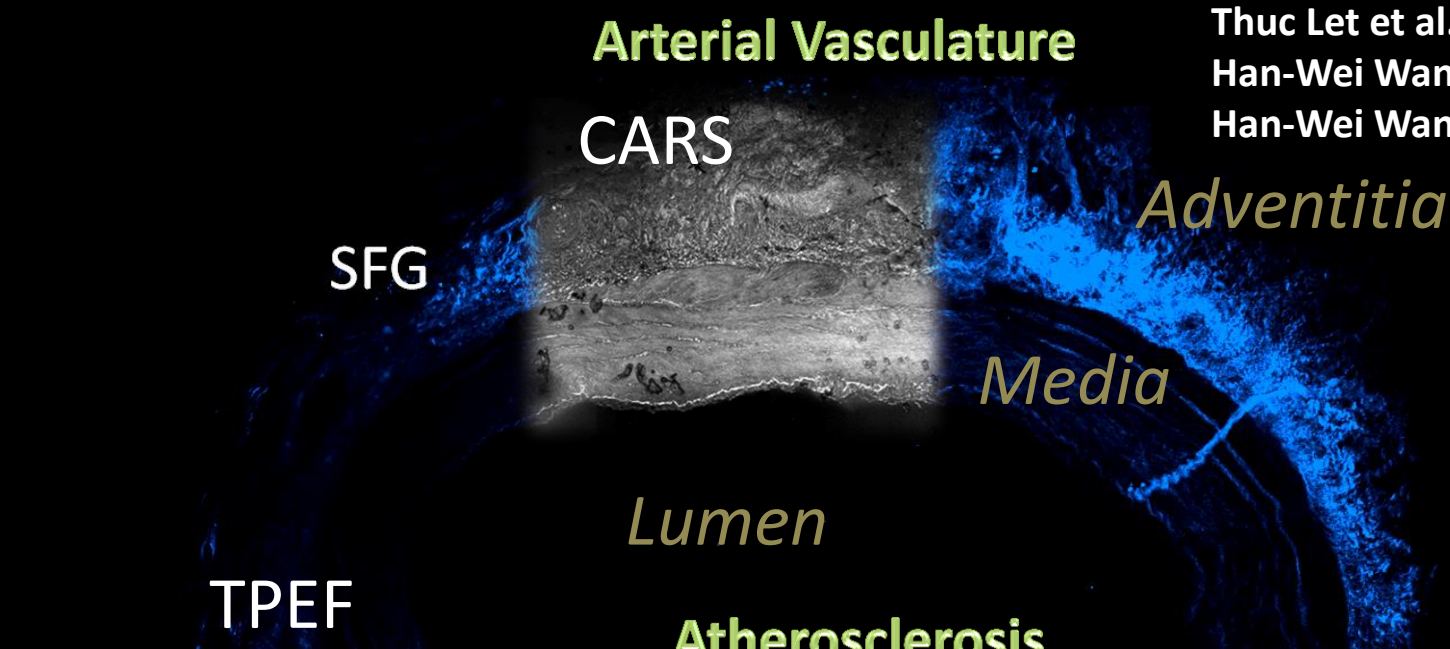


Edited by
Ji-Xin Cheng
X. Sunney Xie

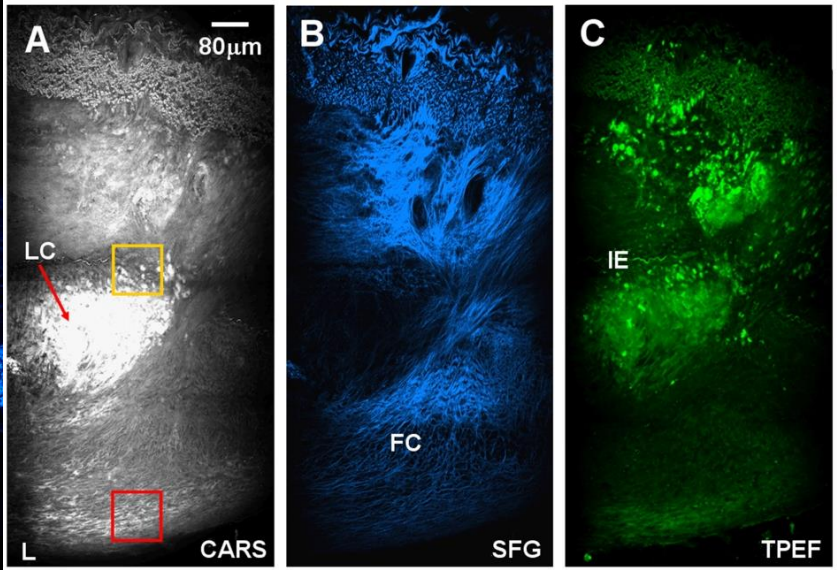
**Signals in CRS
microscopy are
generated by
ballistic photons
under the tight
focusing
condition, thus
limiting its
imaging depth
to $\sim 100 \mu\text{m}$.**

Limited Penetration Depth in CARS Microscopy

Thuc Let et al. JBO 2007
Han-Wei Wang et al. Opt Comm 2008
Han-Wei Wang et al. ATVB, 2009

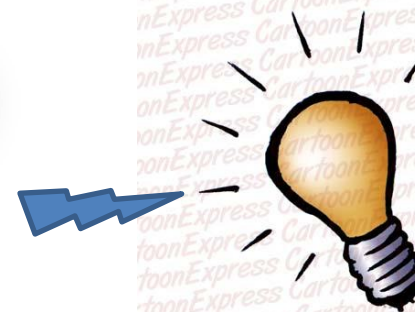
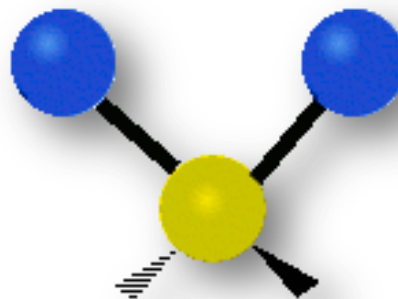
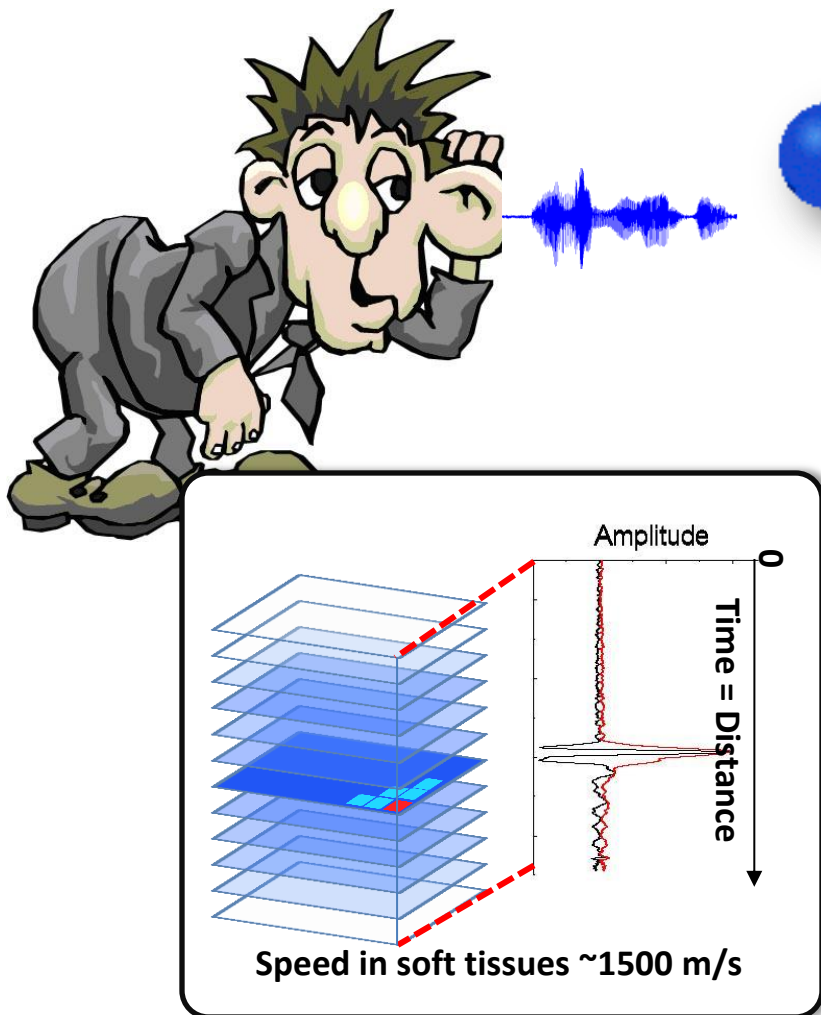


Atherosclerosis



Label-Free Bond-Selective Imaging by Listening to Vibrationally Excited Molecules

Han-Wei Wang,¹ Ning Chai,² Pu Wang,¹ Song Hu,³ Wei Dou,⁴ David Umulis,^{1,4} Lihong V. Wang,³ Michael Sturek,^{1,5} Robert Lucht,² and Ji-Xin Cheng^{1,6,*}



Boltzmann distribution

$$N_i/N_0 = \exp(\Delta E/kT)$$

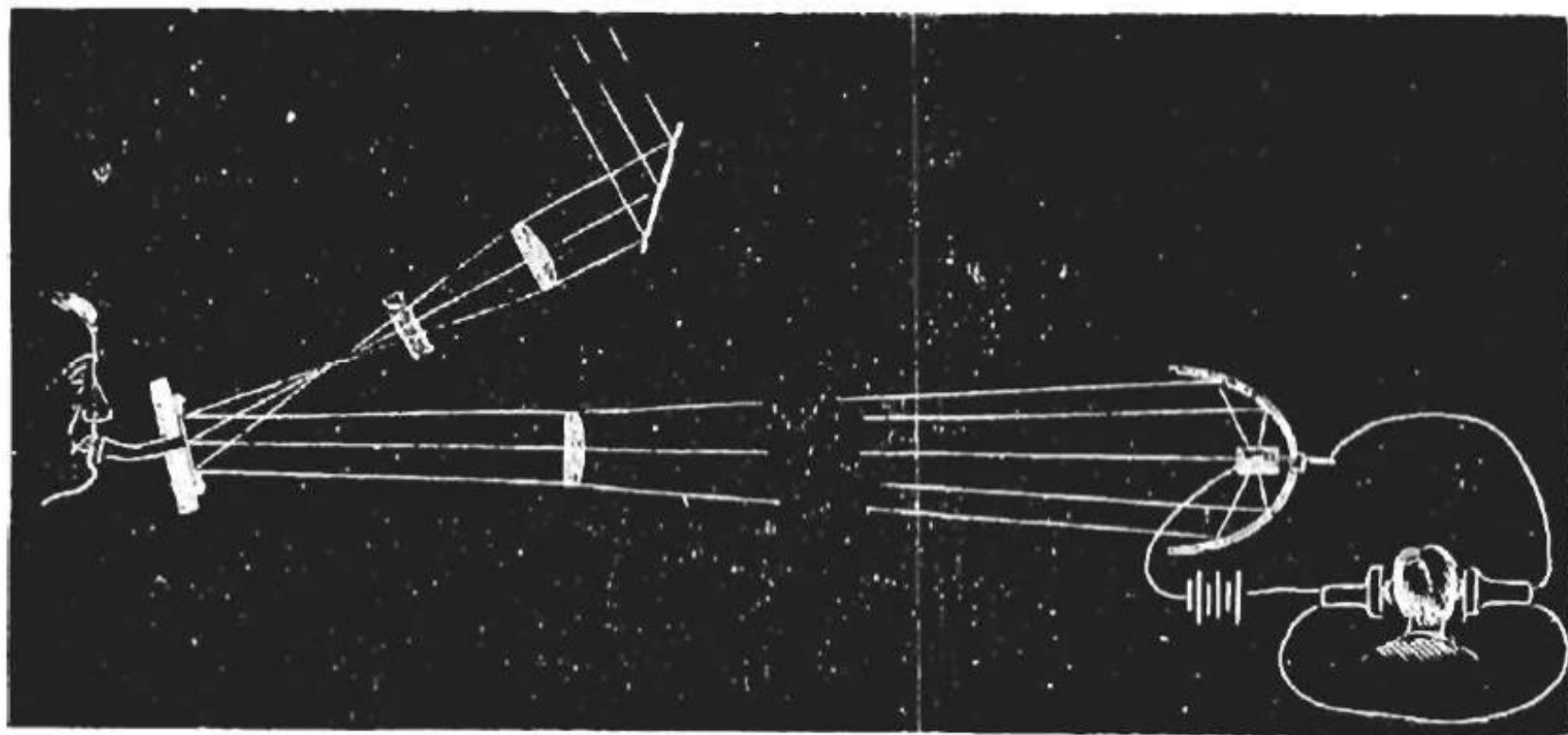
ΔE is the energy gap,

T is the temperature,

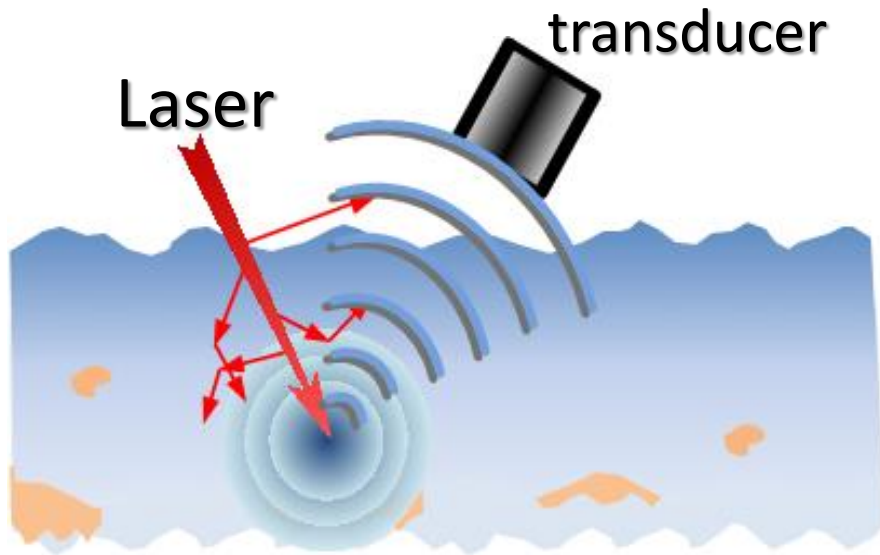
k is the Boltzmann constant.

ART. XXXIV.—*On the Production and Reproduction of Sound
by Light*; by ALEXANDER GRAHAM BELL, Ph.D.

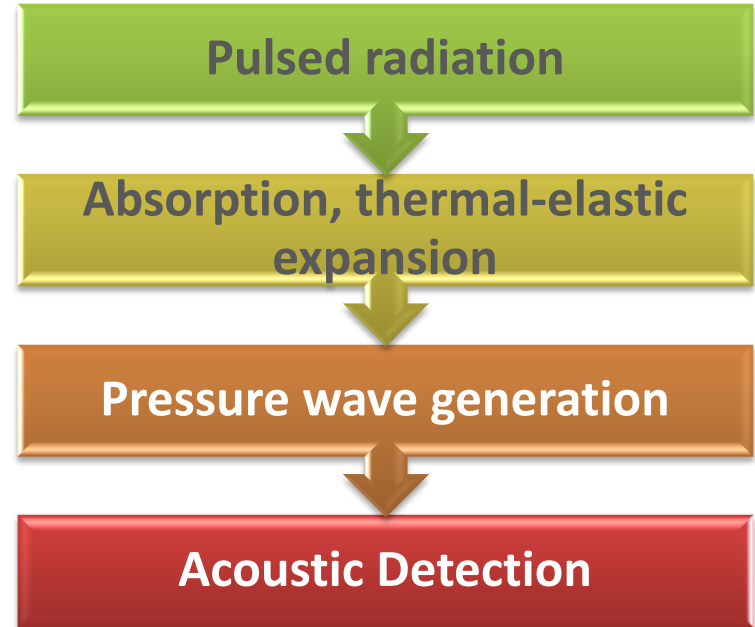
[Read before the American Association for the Advancement of Science, in Boston, August 27, 1880.]



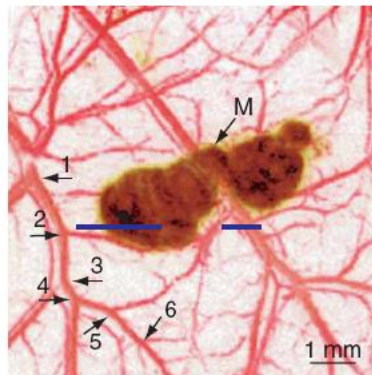
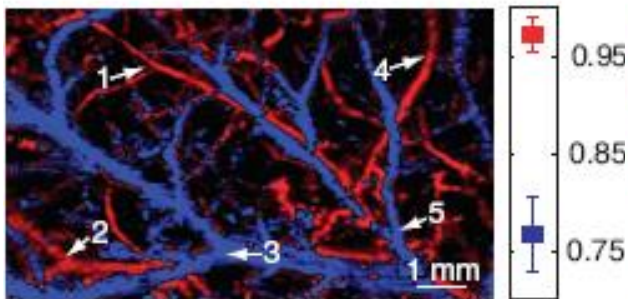
Principle of Photoacoustic Imaging



Photoacoustic Effect



Blood Vessel Plexus (Hemoglobin) & Melanoma –

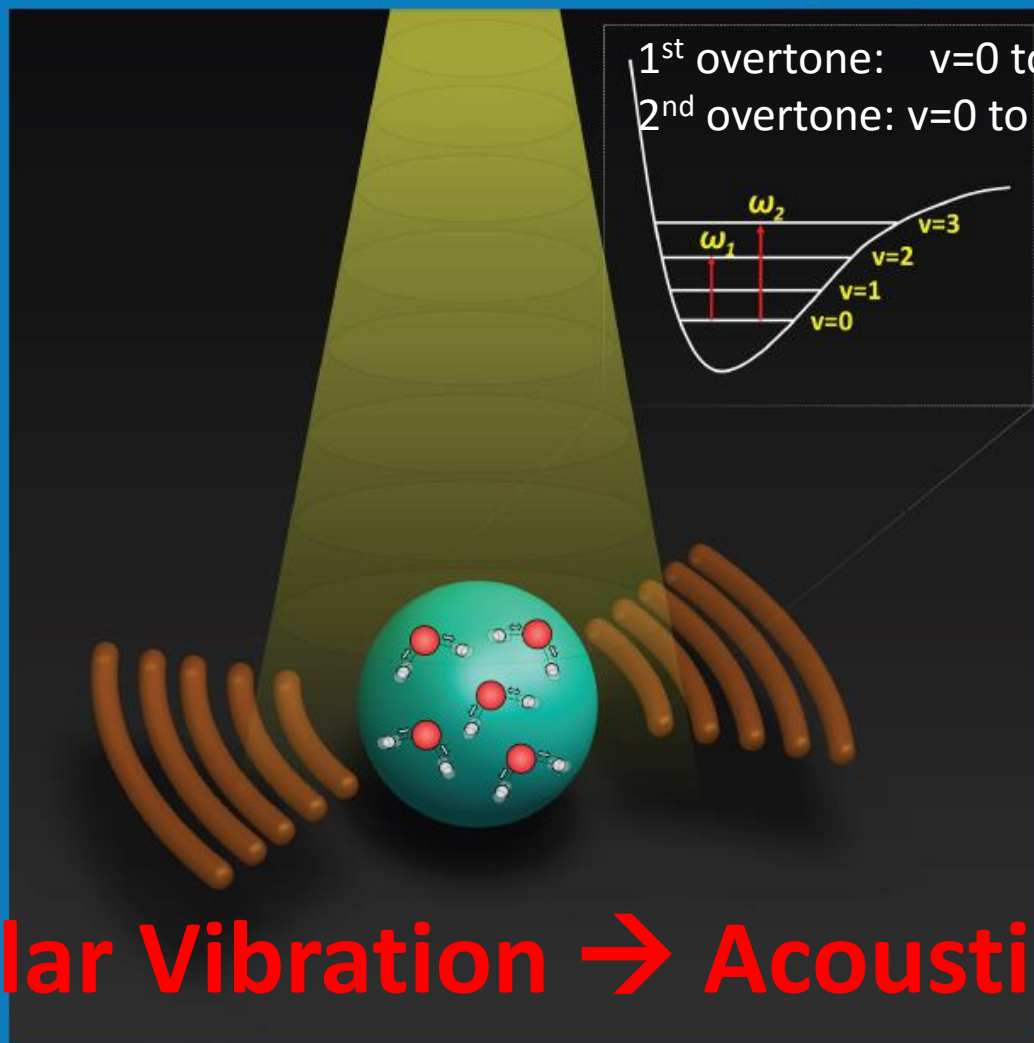


Lihong Wang and coworkers,
2006 Nat. Biotech.

JPC Letters
Perspective
2013, 4,
2177-85

THE JOURNAL OF
PHYSICAL CHEMISTRY
Letters

July 4, 2013 | Volume 4, Number 13

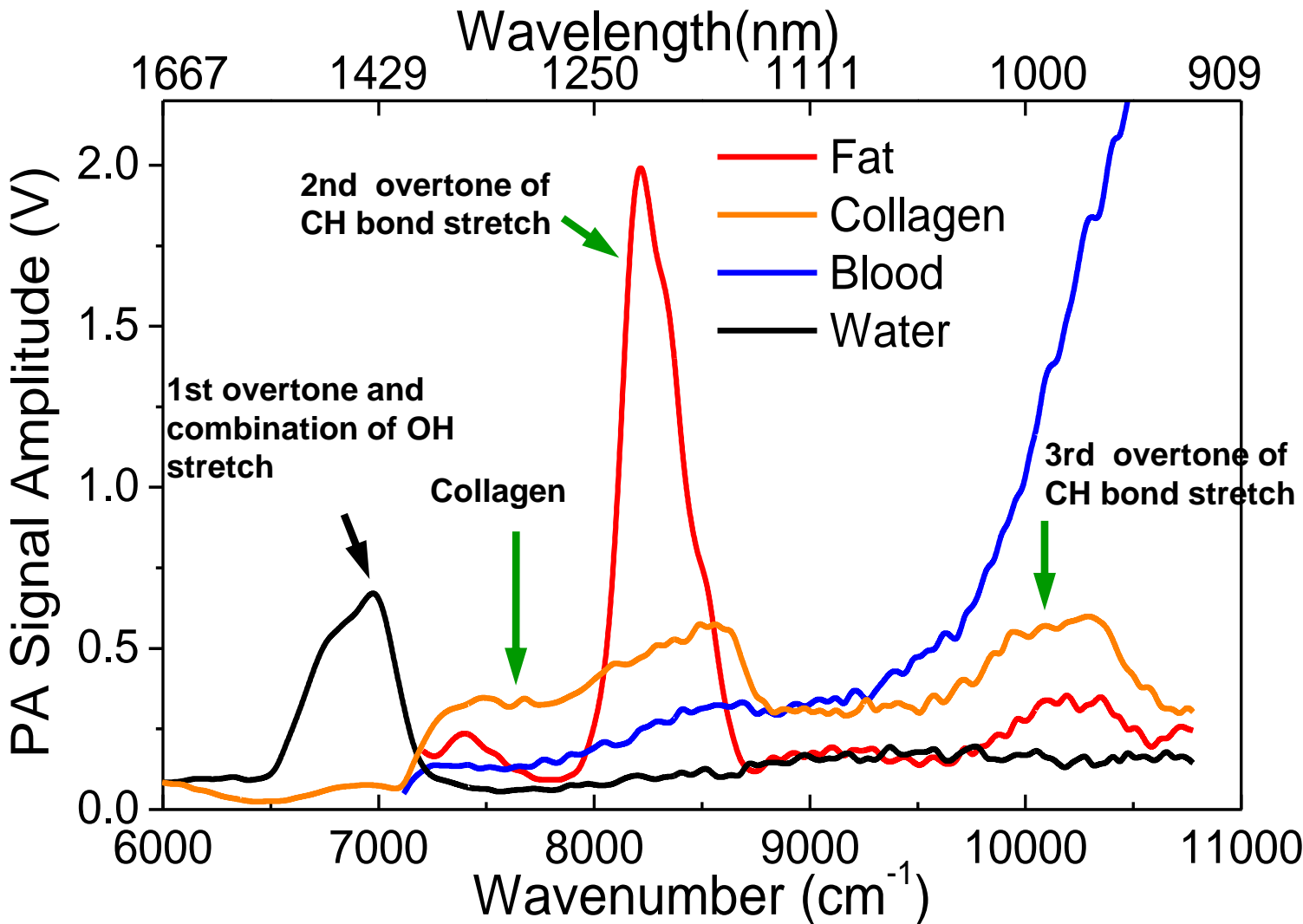


Molecular Vibration → Acoustic Wave

Deep Tissue Imaging by Acoustic Detection of Chemical Bond Vibration

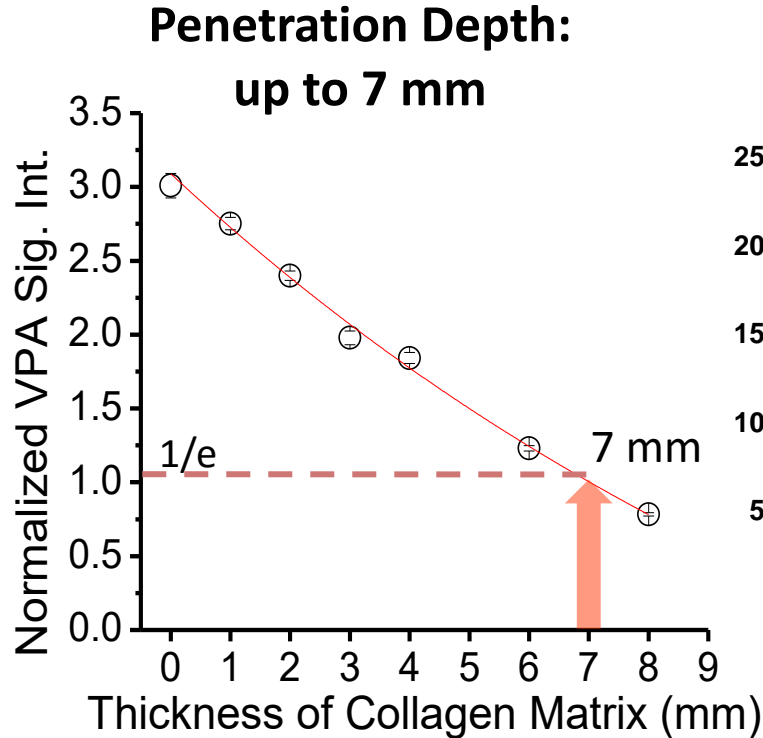
PA Spectra of Biological Molecules

Phys Rev Lett 2011, **106**, 238106.

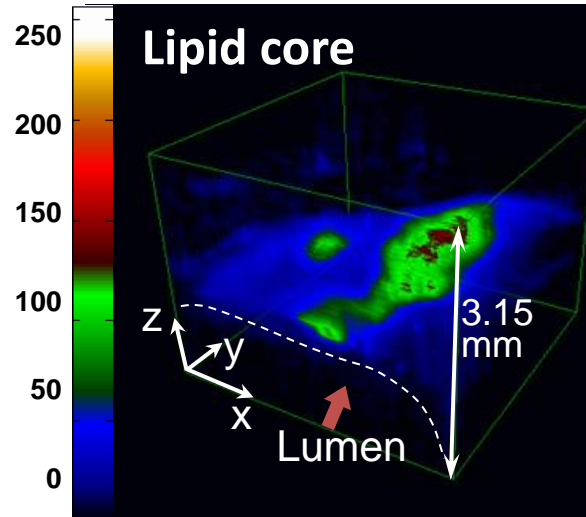


PA Imaging of Lipid-rich Plaque

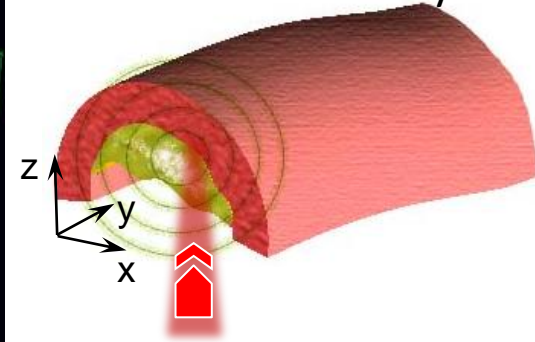
Phys Rev Lett 2011, **106**, 238106.



VPA image



Atherosclerotic Artery



**Speed: single pulse per pixel
at 10 Hz**

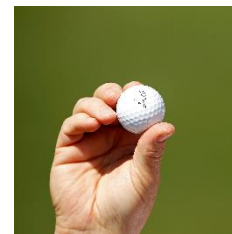
Spatial resolution:

- Lateral resolution: from 5 μm to 70 μm
- Axial resolution: $\sim 135 \mu\text{m}$; 35 μm is possible

Raman scattering cross section

$$\sim 10^{-30} \text{ cm}^2/\text{sr}$$

Golf ball



VS.

VS.

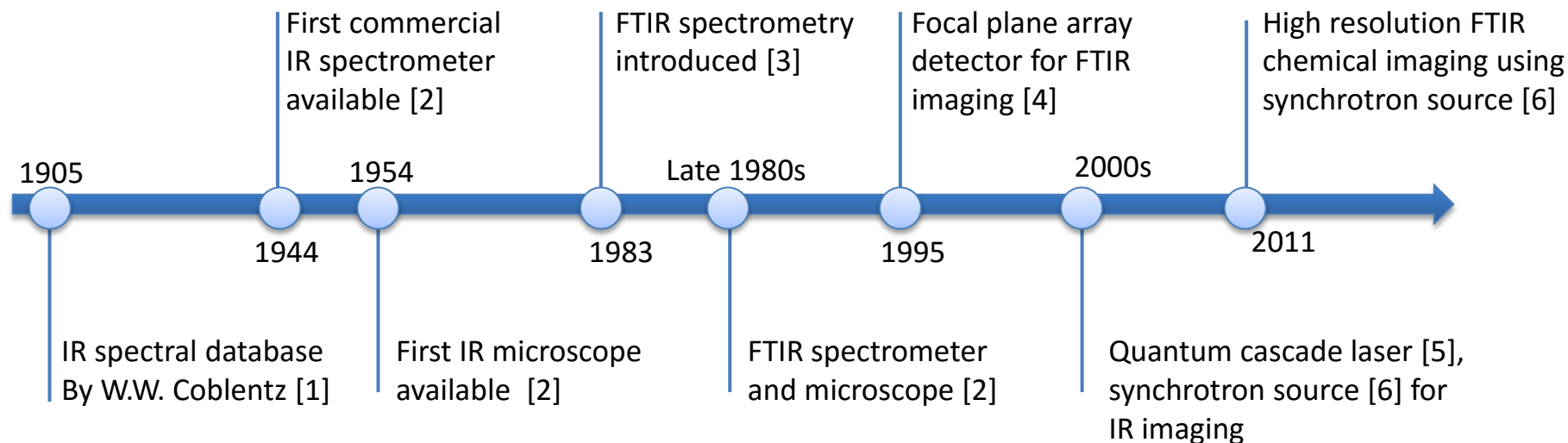
Infrared absorption
cross section

$$\sim 10^{-22} \text{ cm}^2/\text{sr}$$



Golf course

History of IR Spectroscopy and Imaging



[1] W.W. Coblentz, Investigations of Infra-red Spectra, Washington, DC: Carnegie Institution of Washington. (1905)

[2] Perkin Elmer company website <http://www.perkinelmer.com/>

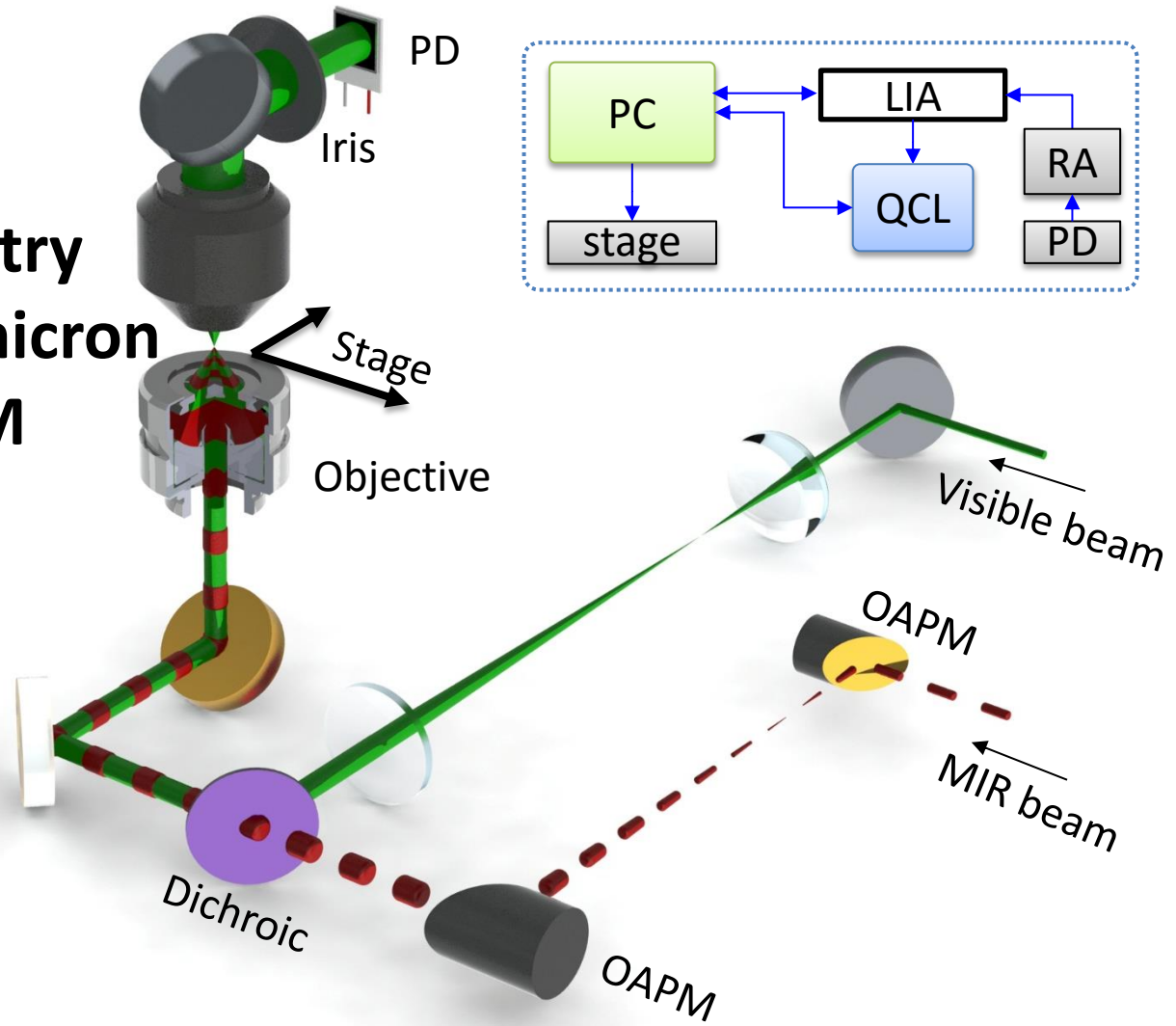
[3] P.R. Griffiths, Science, 222, 297 (1983)

[4] Lewis, Levin et al. Anal. Chem. 67, 3377 (1995)

[5] Chen, Chu et al. Anal. Chem., 84, 4118 (2012)

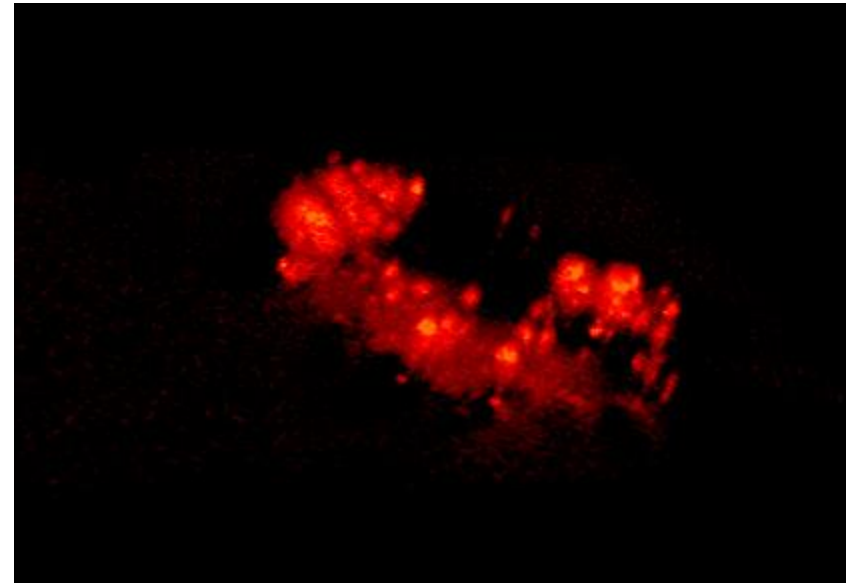
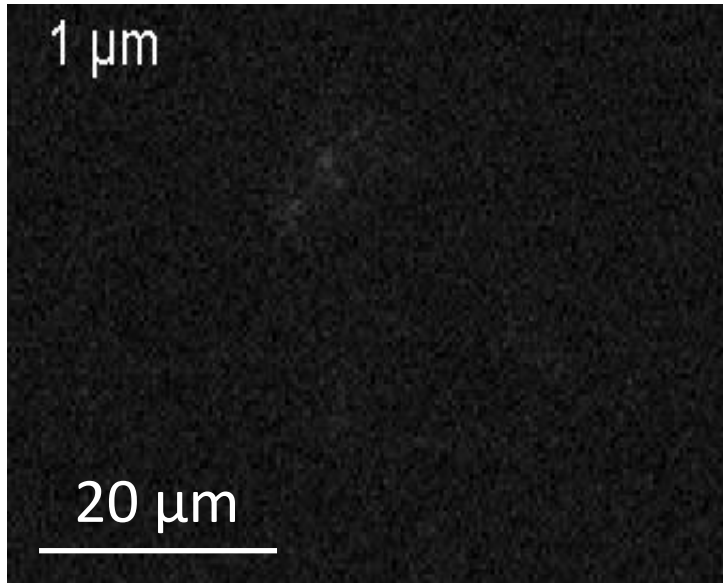
[6] Nasse, Bhargava et al. Nature Method. 8, 413 (2011)

Mid-infrared Photothermal (MIP) Imaging: Making IR Spectroscopy in vivo



Dark field geometry
Resolution: 0.6 micron
Sensitivity: 10 μ M

3D-MIP Mapping of C=O Bonds in Living Cells



- C=O band at 1750 cm^{-1}
- Sample: live PC-3 cell on CaF_2 dish
- Pump: 4 mW, Probe: 8 mW
- Pixel dwell time: 1 ms

Delong Zhang et al,
Science Advances 2016
In press

Pump-Probe: Spectroscopic Imaging in Time Domain

Second Excited State

First Excited State

First Excited State

First Excited State

Ground State

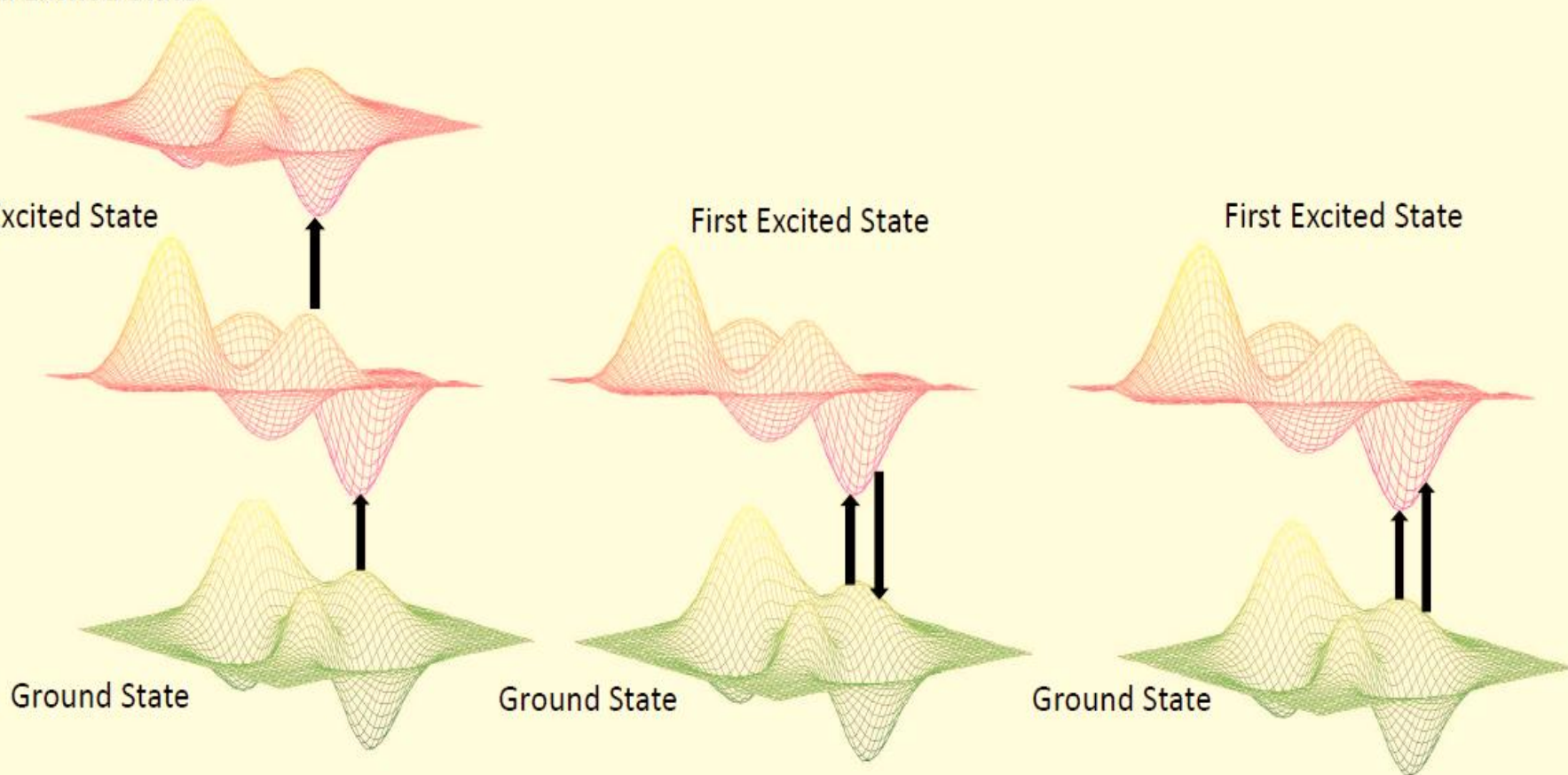
Ground State

Ground State

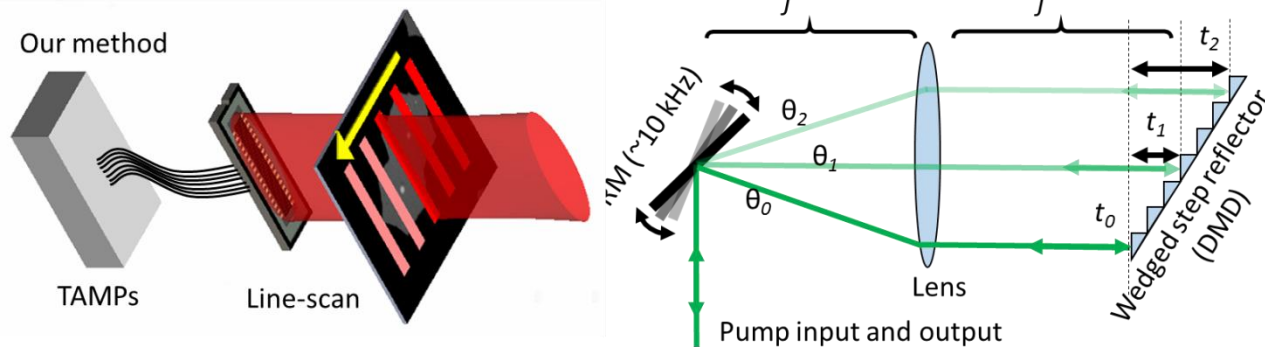
Excited State Absorption

Stimulated Emission

Ground State Depletion



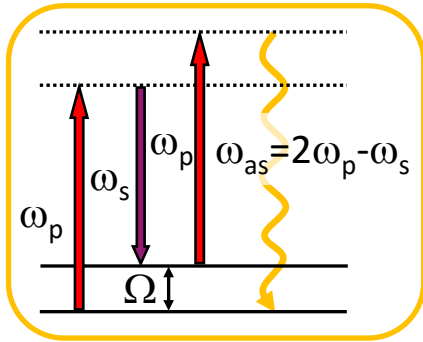
Ultrafast Pump-Probe Imaging of Nanoscale Defects in Single-layer Graphene



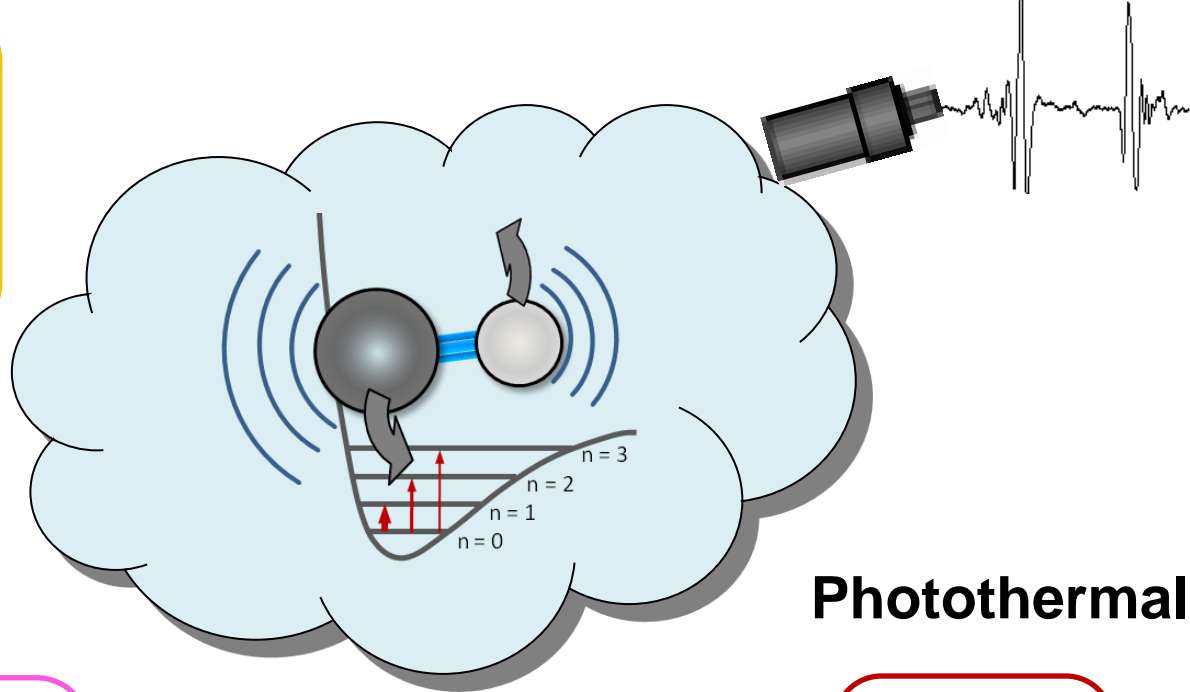
Intensity imaging 1000 frames per sec, time-resolved imaging 50 frames per sec
Nature Nanotechnology, 2016, under review

Summary of Spectroscopic Imaging Modalities

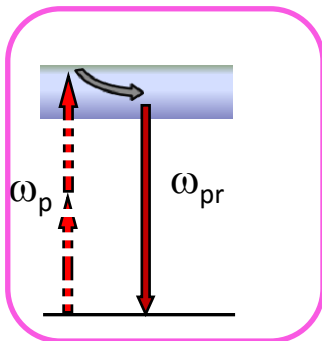
Raman/CARS/SRS



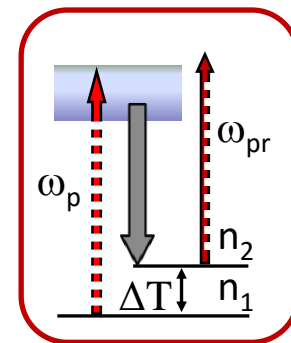
Photoacoustic

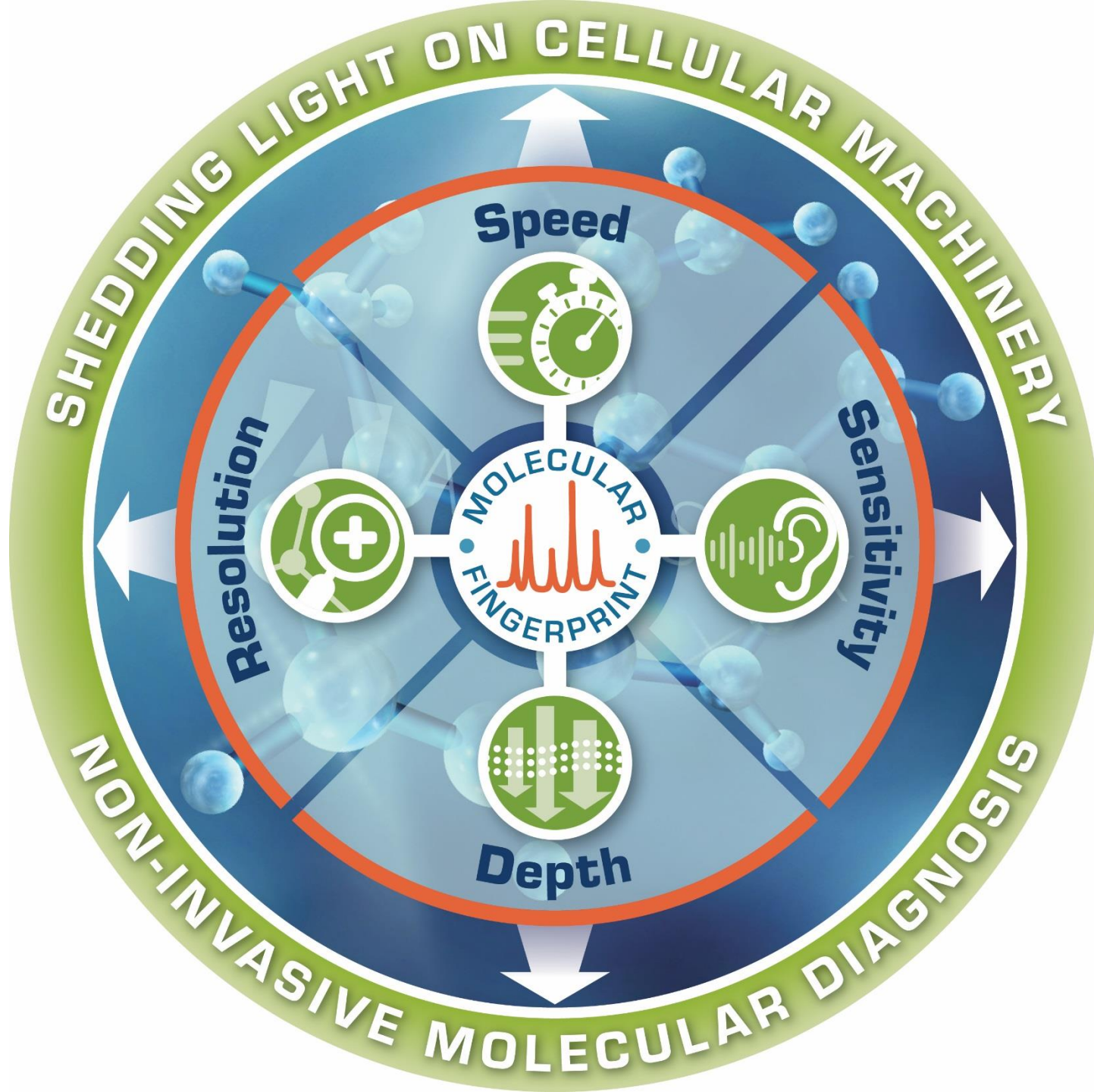


Pump-probe

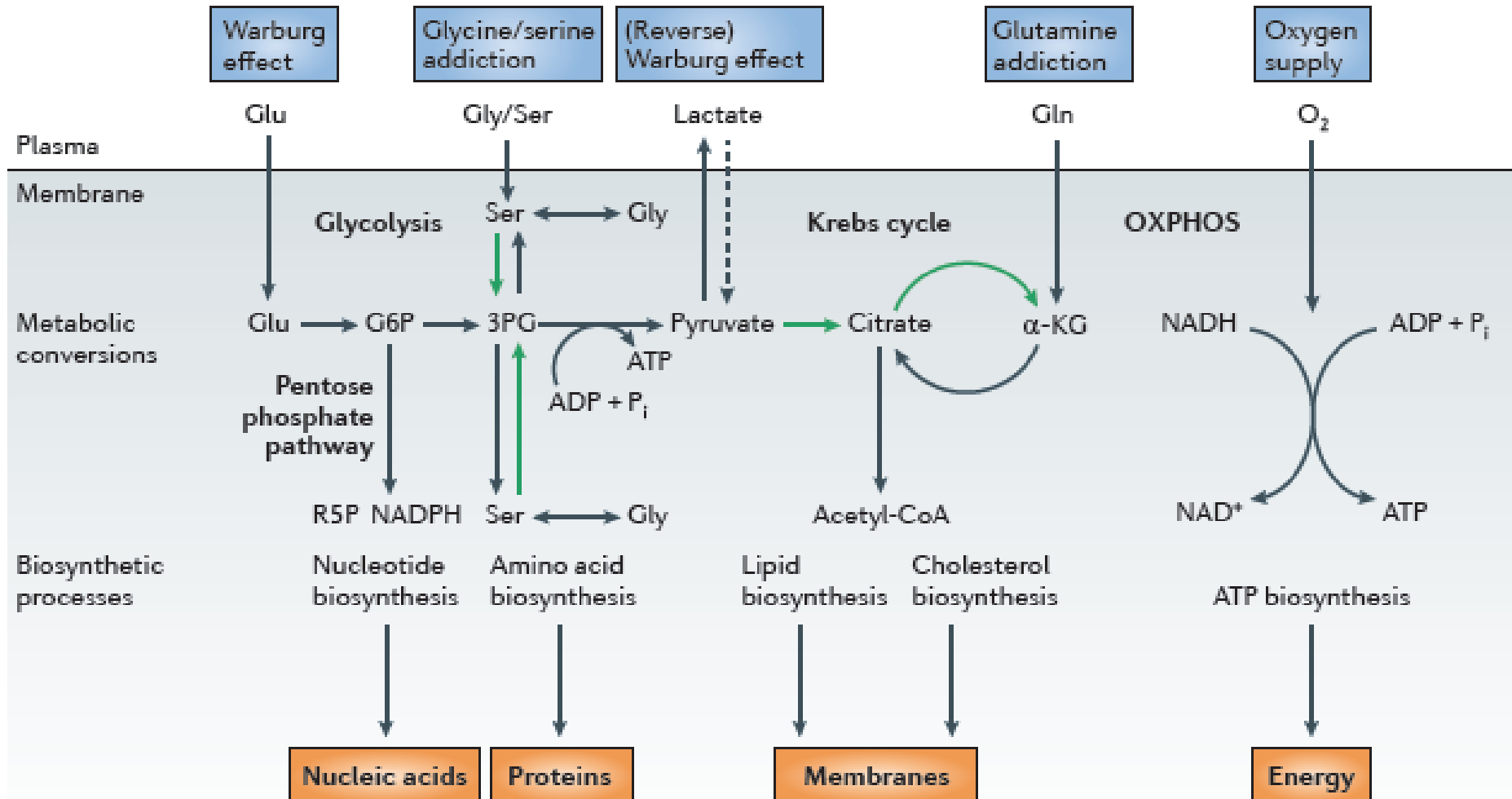


Photothermal

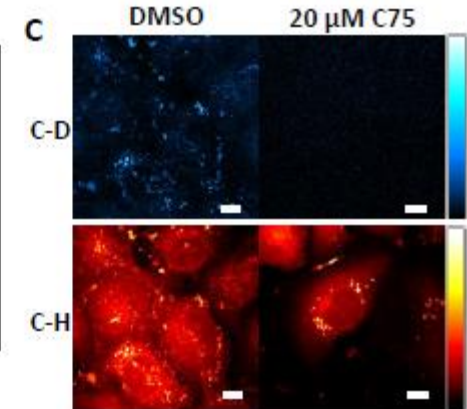
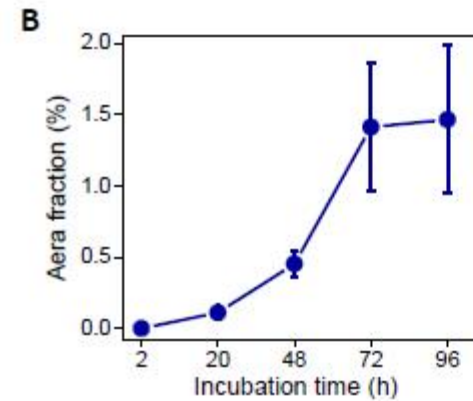
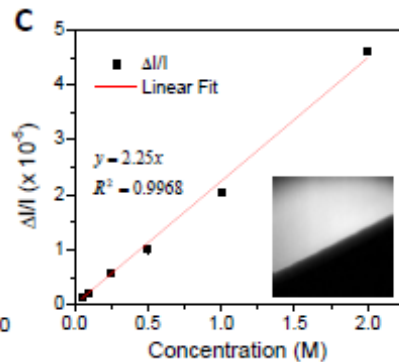
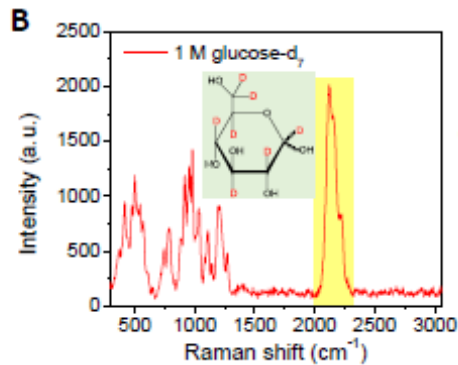
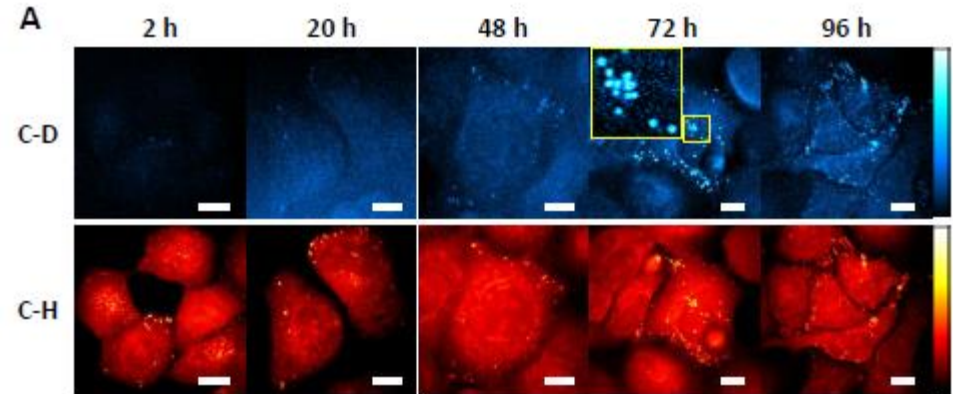
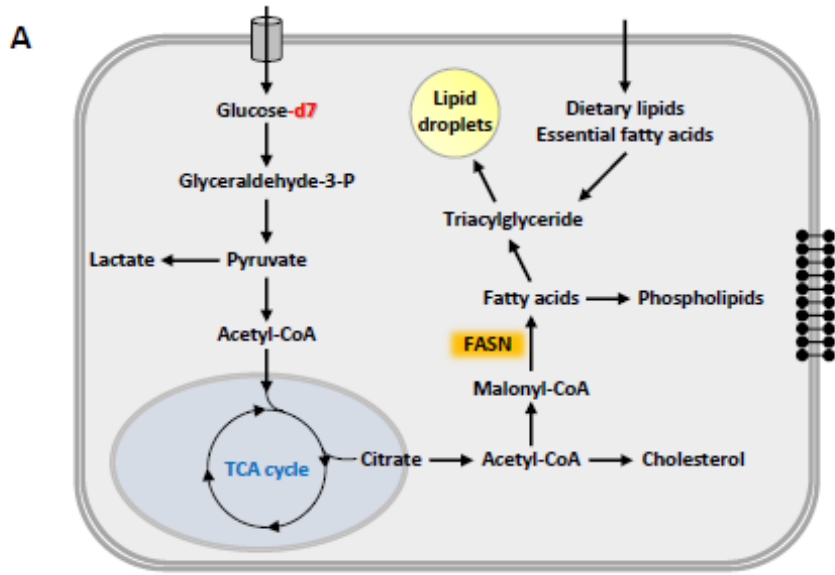




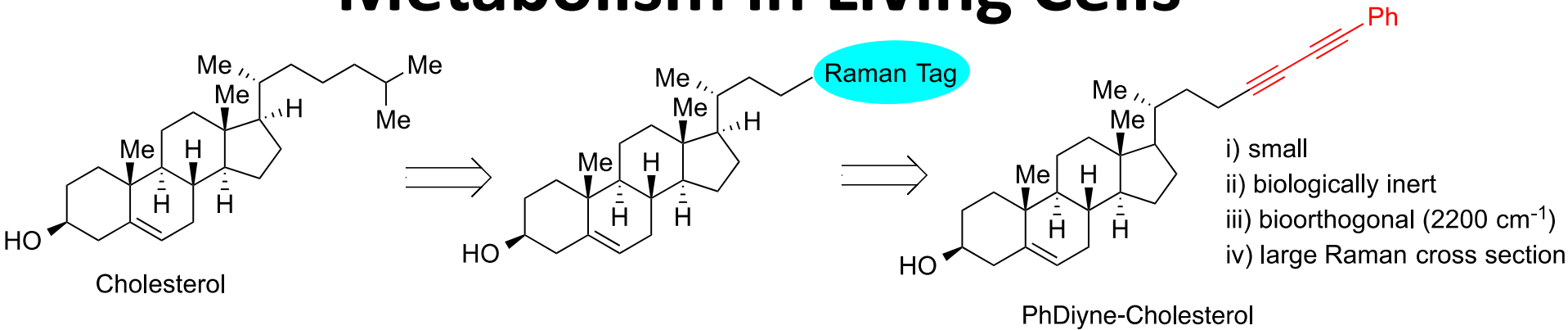
Altered Metabolism: Cancer's Achilles Heel



SRS Imaging of *de novo* Lipogenesis in Single Living Cells

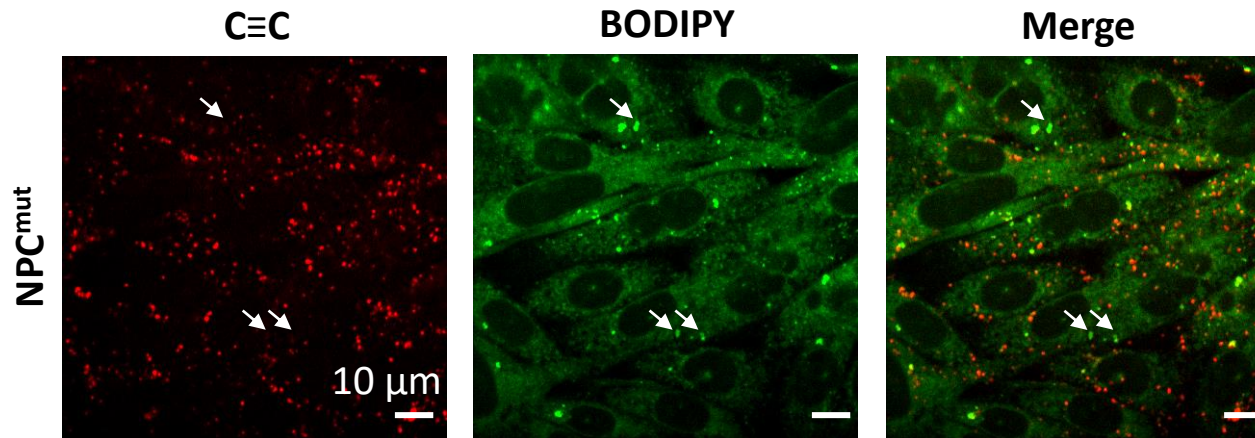


Raman Probes for Imaging Cholesterol Metabolism in Living Cells



Hyeon Jeong Lee et al. Nature Scientific Reports, 2015, 5: 7930.
Imaging detection limit: 900 molecules. Collaborator: Mingji Dai

Cholesterol storage in lysosomes in Niemann-Pick C mutant cells



Human Patient Specimen Examination by Label-free Spectroscopic Imaging

Cell Metabolism 2014, 19: 393-406

Cholesteryl Ester Accumulation Induced by PTEN Loss and PI3K/AKT Activation Underlies Human Prostate Cancer Aggressiveness

Shuhua Yue,¹ Junjie Li,² Seung-Young Lee,¹ Hyeon Jeong Lee,³ Tian Shao,⁴ Bing Song,² Liang Cheng,⁵ Timothy A. Masterson,⁶ Xiaoqi Liu,^{4,7} Timothy L. Ratliff,^{3,7} and Ji-Xin Cheng^{1,7,*}

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²Department of Biological Sciences

³Department of Comparative Pathobiology

⁴Department of Biochemistry

Purdue University, West Lafayette, IN 47907, USA

⁵Department of Pathology and Laboratory Medicine

⁶Department of Urology

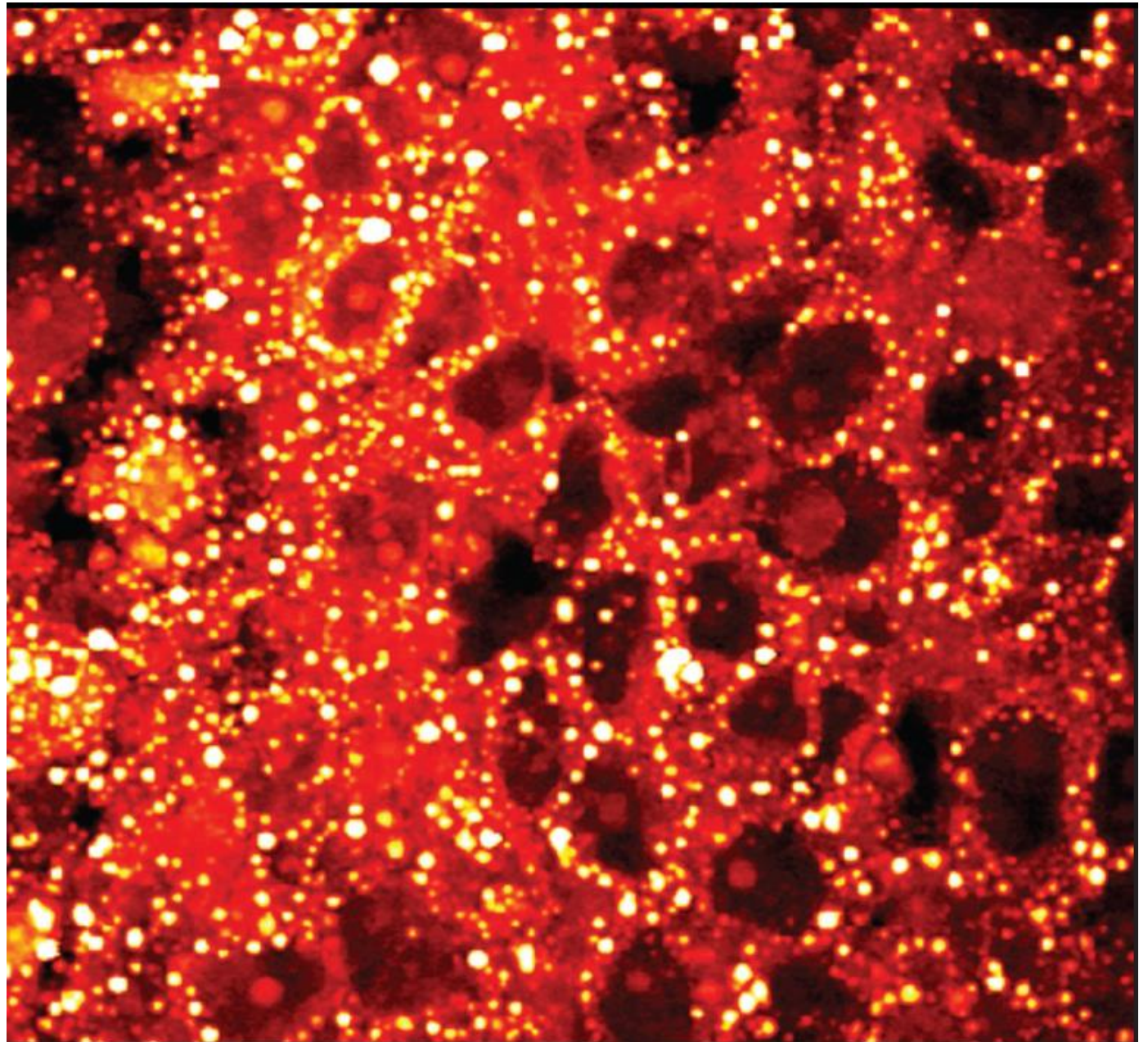
Indiana University School of Medicine, Indianapolis, IN 46202, USA

⁷Center for Cancer Research, Purdue University, West Lafayette, IN 47907, USA

*Correspondence: jcheng@purdue.edu

<http://dx.doi.org/10.1016/j.cmet.2014.01.019>

**Single-
color SRS
imaging of
human
prostate
cancer
tissue
based on
C-H
vibration**

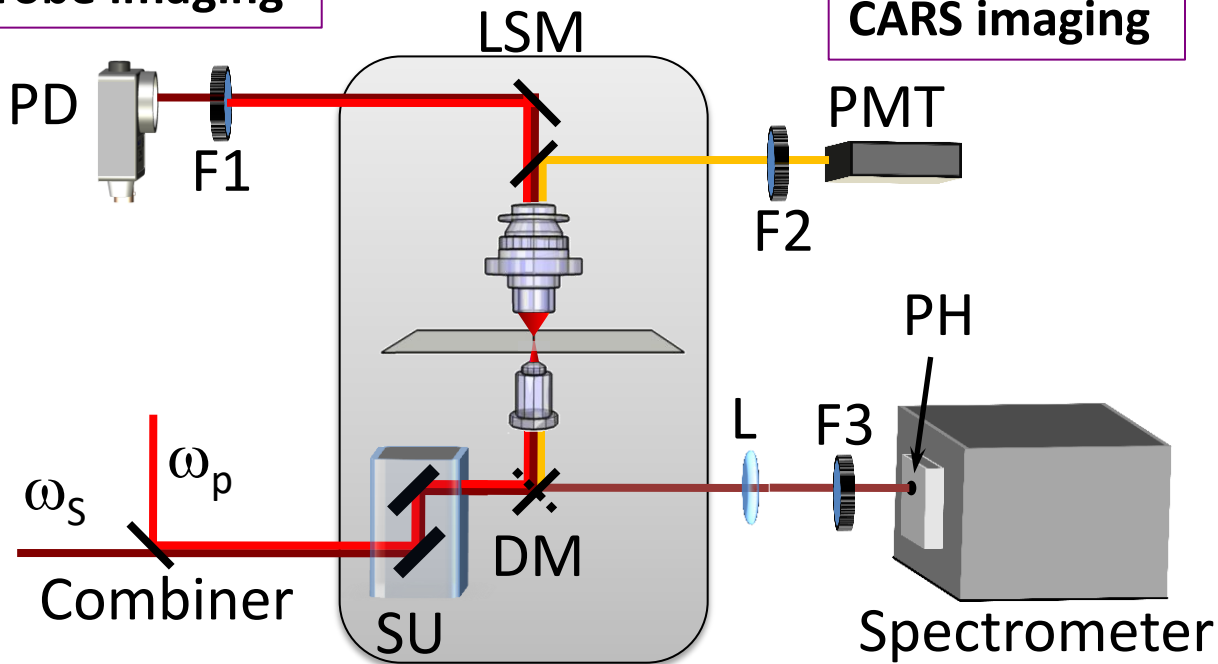


Joint Purdue-IUSM team: **Ji-Xin Cheng** (BME), **Liu** (Biochemistry) **Tim Ratliff** (Center for Cancer Research), **Tim Masterson** (Professor, Urology, IUSM), **Liang Cheng** (Professor, Pathology, IUSM)

Raman Spectromicroscopy: High-speed imaging and spectral analysis at pixels of interest

SRS or pump-probe imaging

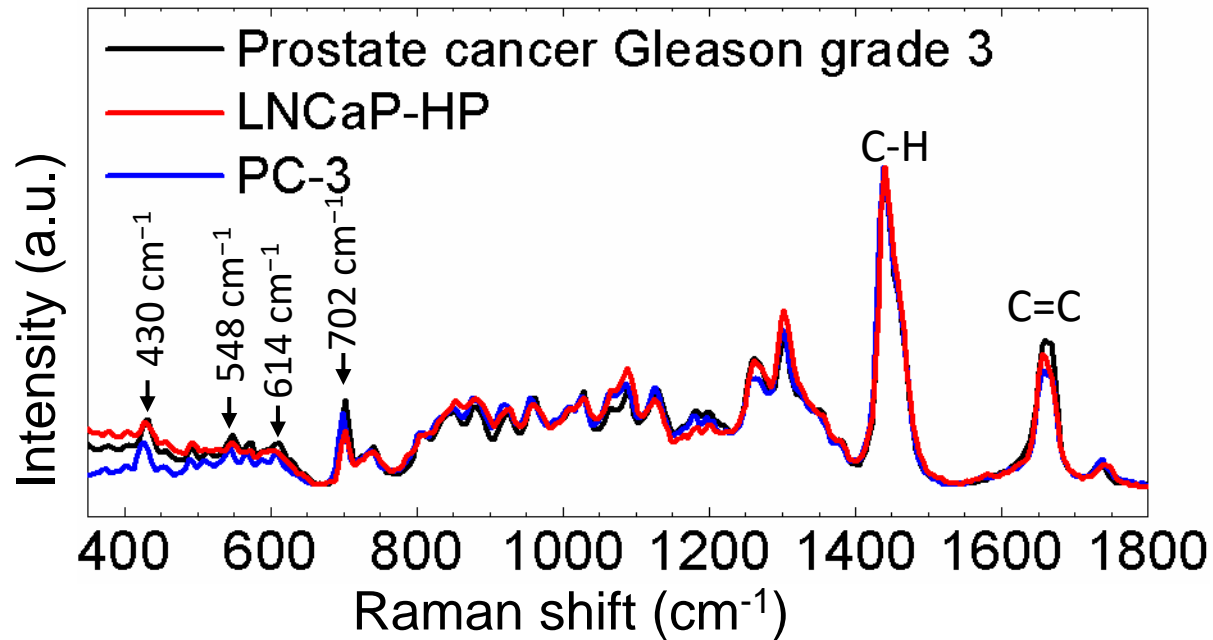
CARS imaging



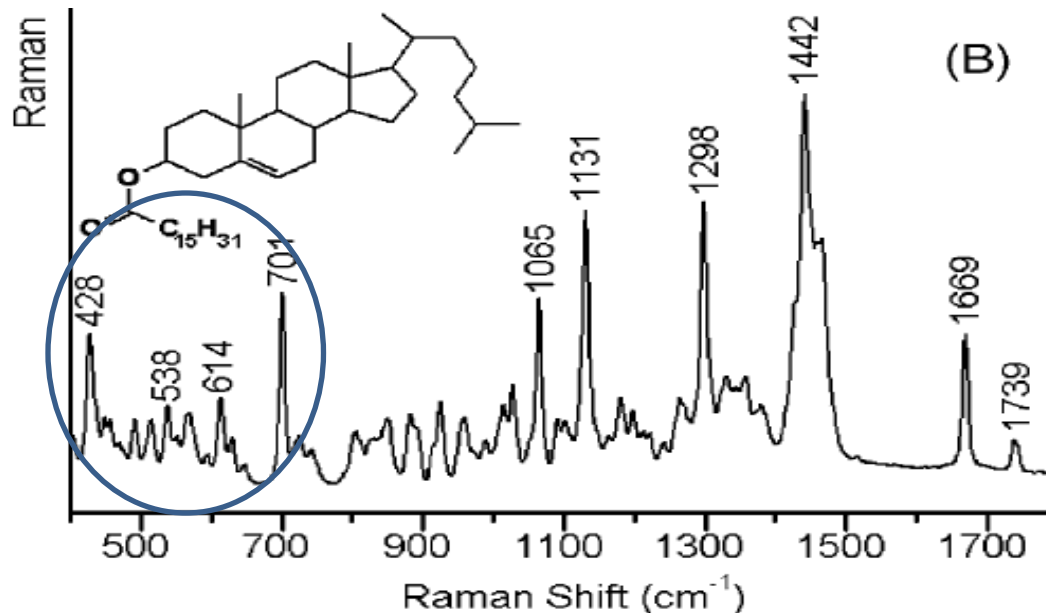
Raman spectral analysis

Spectral Profiling Identifies Cholesteryl Ester

Prostate cancer cell/tissue data:



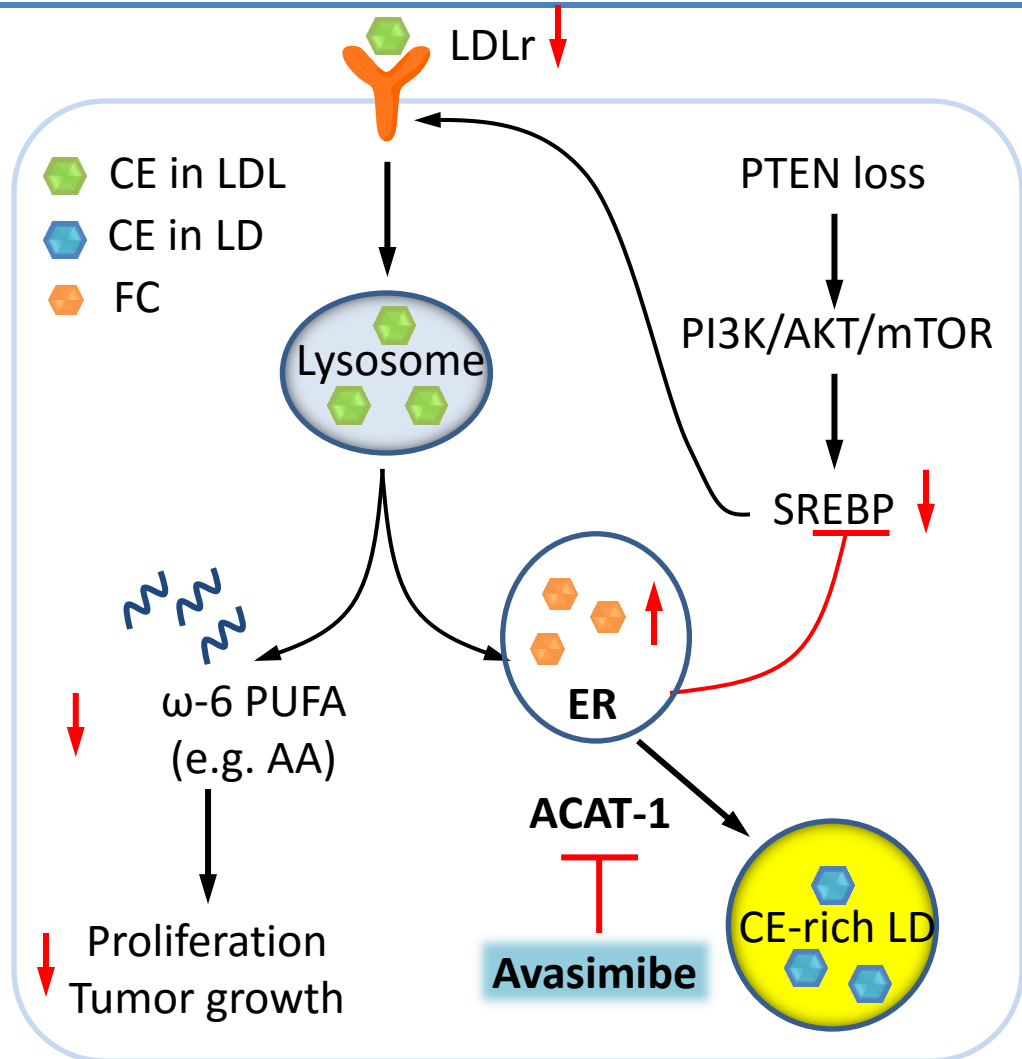
Raman spectrum of cholesteryl palmitate shows bands from 400 to 1200 cm^{-1} with the most intensive ones at 428, 538, 614 and 701 cm^{-1} .



Krafft et al.
Spectrochimica Acta Part A 61 (2005) 1529–1535

Molecular Pathways Underlying Cholesteryl Ester Accumulation

Cell Metabolism,
2014, 18: 393-406



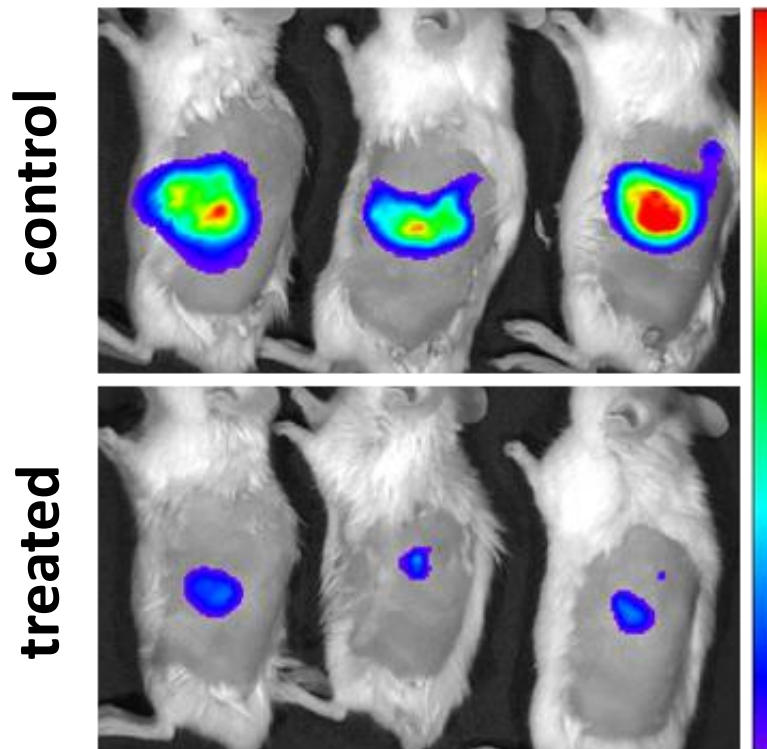
FC: free cholesterol;
PUFA: polyunsaturated
fatty acid;
AA: arachidonic acid

ORIGINAL ARTICLE (Oncogene, 2016, 1-11)

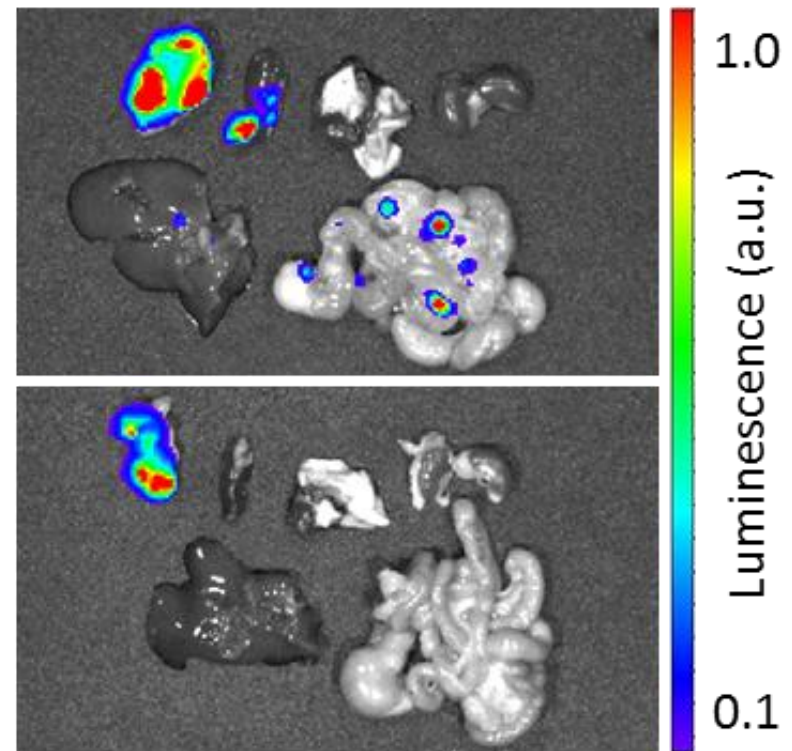
Abrogating cholesterol esterification suppresses growth and metastasis of pancreatic cancer

J Li¹, D Gu², SS-Y Lee¹, B Song¹, S Bandyopadhyay³, S Chen⁴, SF Konieczny^{3,5}, TL Ratliff^{5,6}, X Liu^{5,7}, J Xie² and J-X Cheng^{1,5}

a. growth

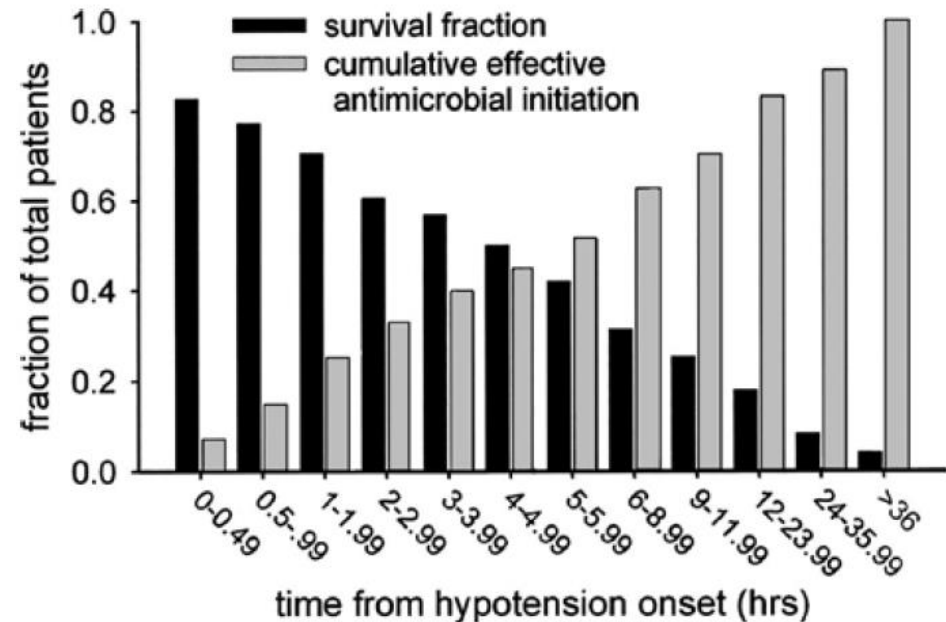


b. metastasis



Significance of Early Detection of Bacteria

Blood stream infections (bacteria, fungi or virus) or sepsis affects 18 millions people worldwide and 700,000 in United States annually, with mortality rate of 30–40 %, partly due to the inability to rapidly detect, identify and thus treat patient with antibiotics in the early stage.



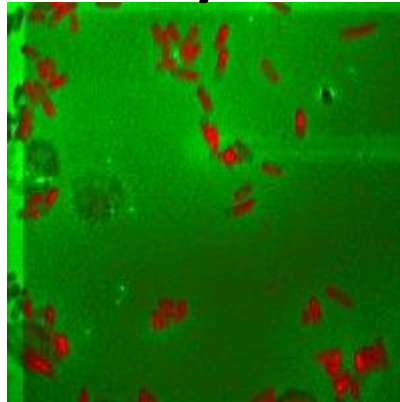
Kumar A. *et al. Crit. Care Med.* **34**, 1589–1596 (2006)

- ❖ bacteria in blood were cultured, and tested for antibiotics resistance
 - Takes 2-5 days, up to 2 weeks**
 - Some bacteria are hard to be cultured**

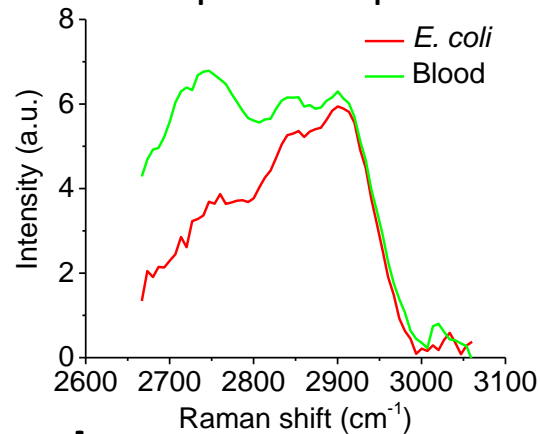
Kang DK. *et al. Nat. Commun.* **5**, 5427–5437 (2014)

In situ Detection of a Single Bacterium in complex environment (food, urine, blood)

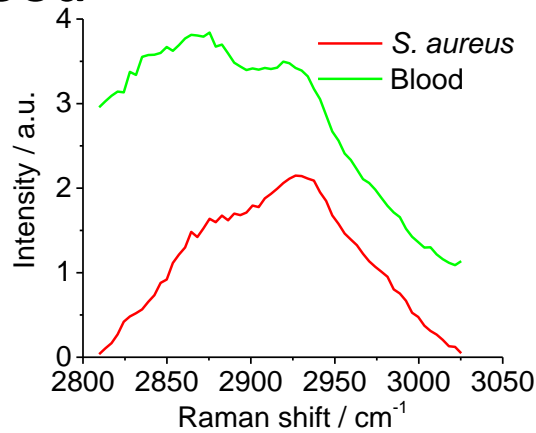
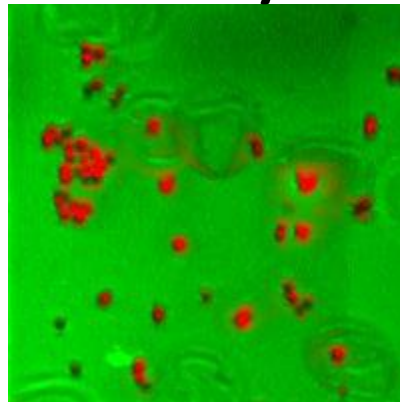
***E. Coli* in lysed blood**



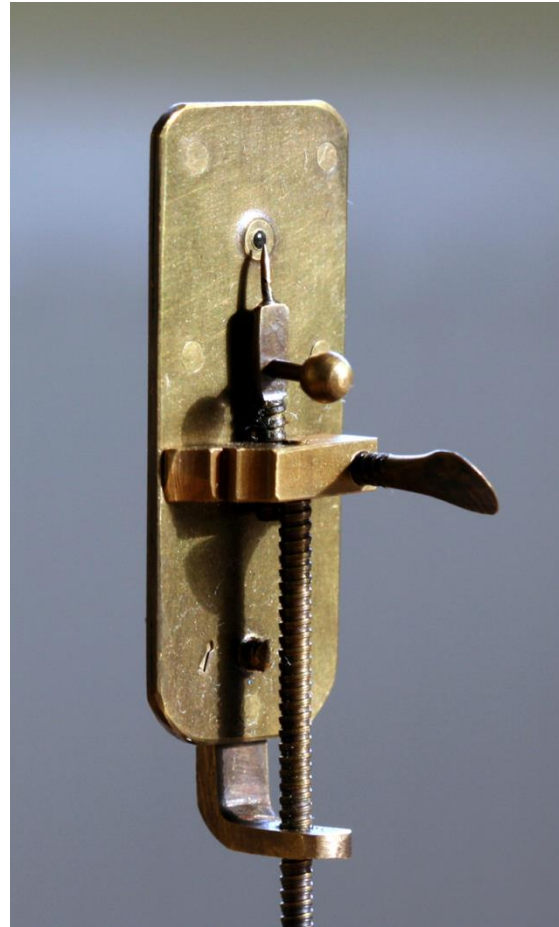
Output SRS spectra



***S. aureus* in lysed blood**

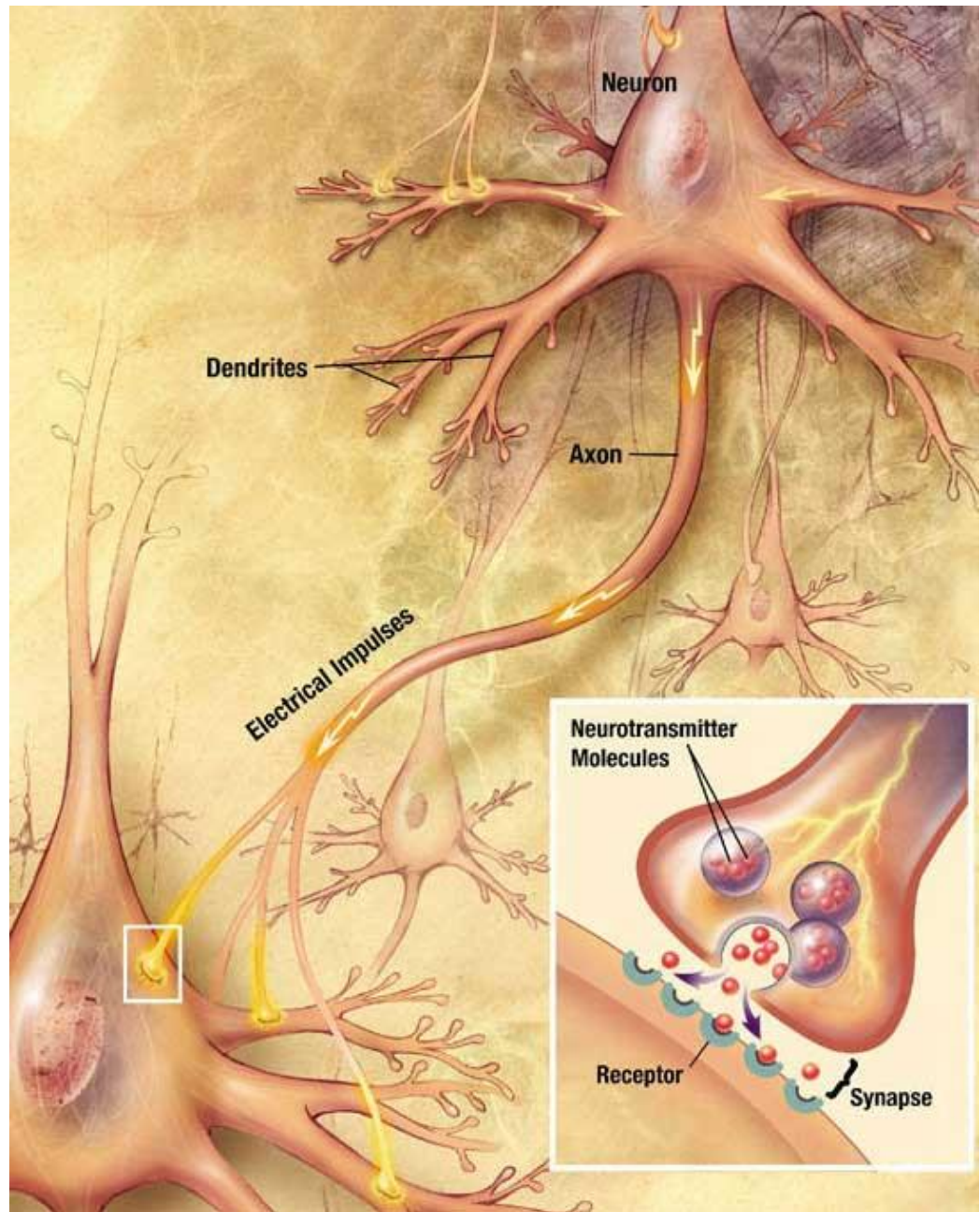


Weili Hong et al, ChemistrySelect 2016, 3: 513-517.
In collaboration with Mohamed N Seleem at Purdue



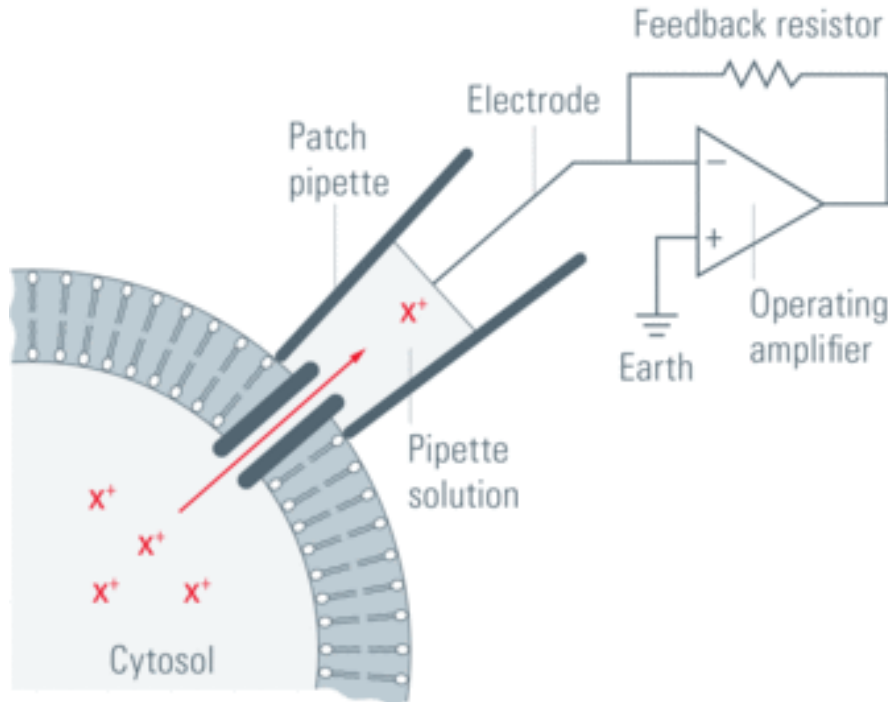
Leeuwenhoek (1632 ~ 1723)'s microscope
Father of microbiology

Visualizing neuronal communication

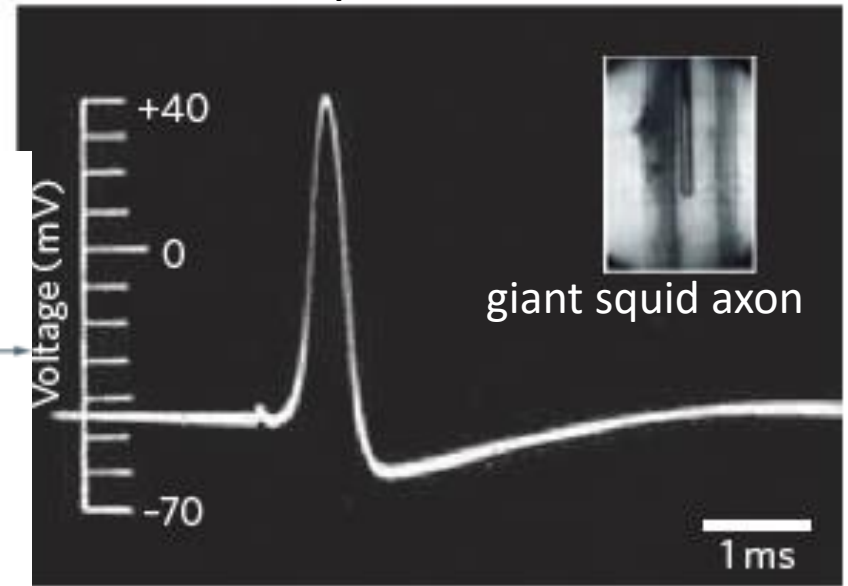


Recording electrical impulses with electrophysiology

(patch clamp)



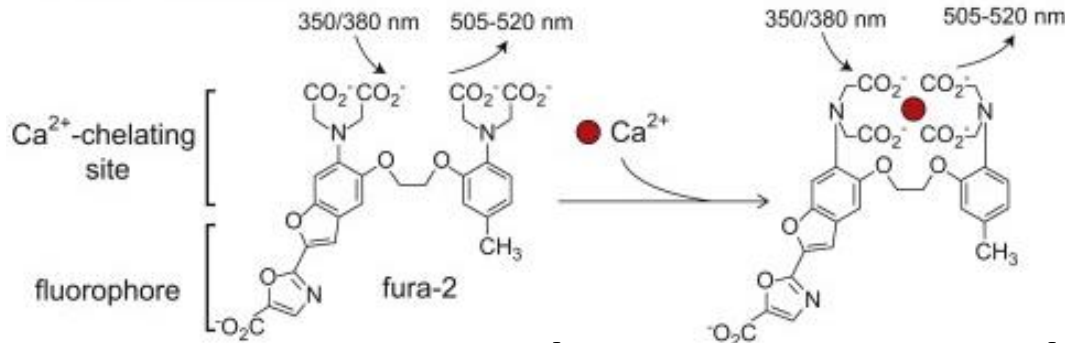
First action potential recorded:



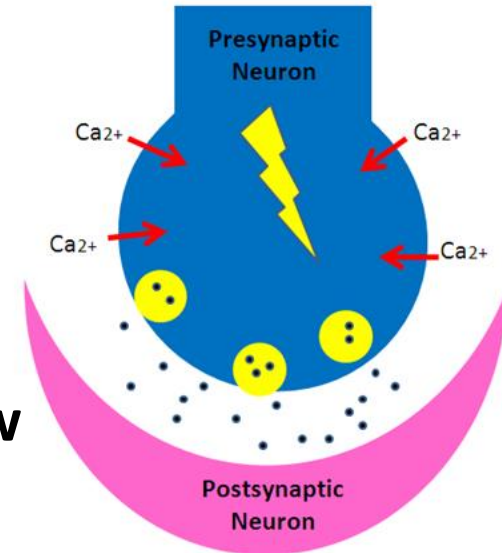
Hodgkin & Huxley. *Nature* (1939) **144**, 710

Optical recording of neural activities

Calcium indicators:

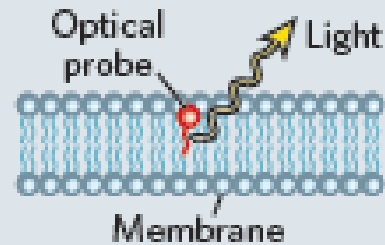


Calcium response is slow



Voltage sensitive probes:

Voltage sensors



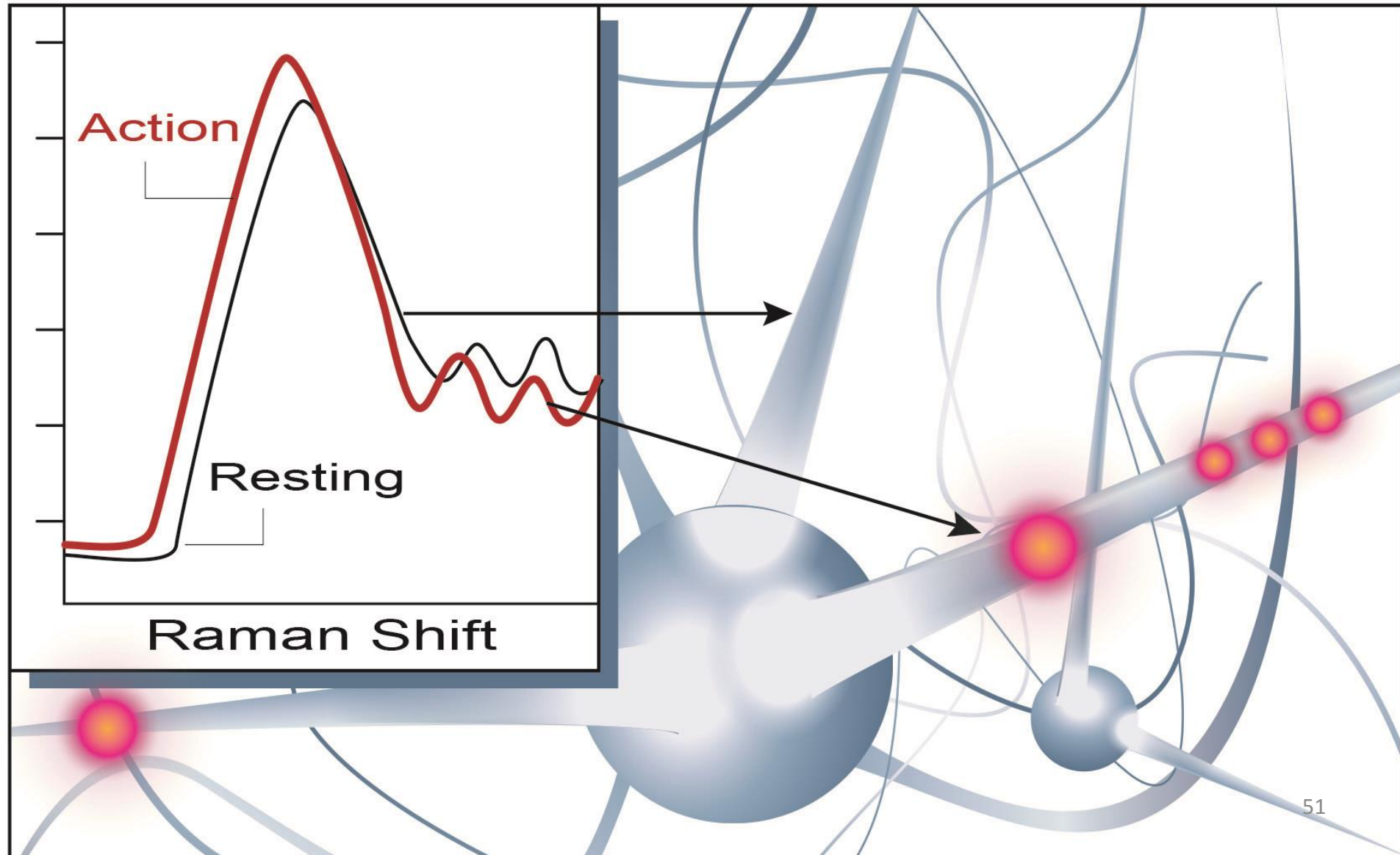
Receptor/channel reporters



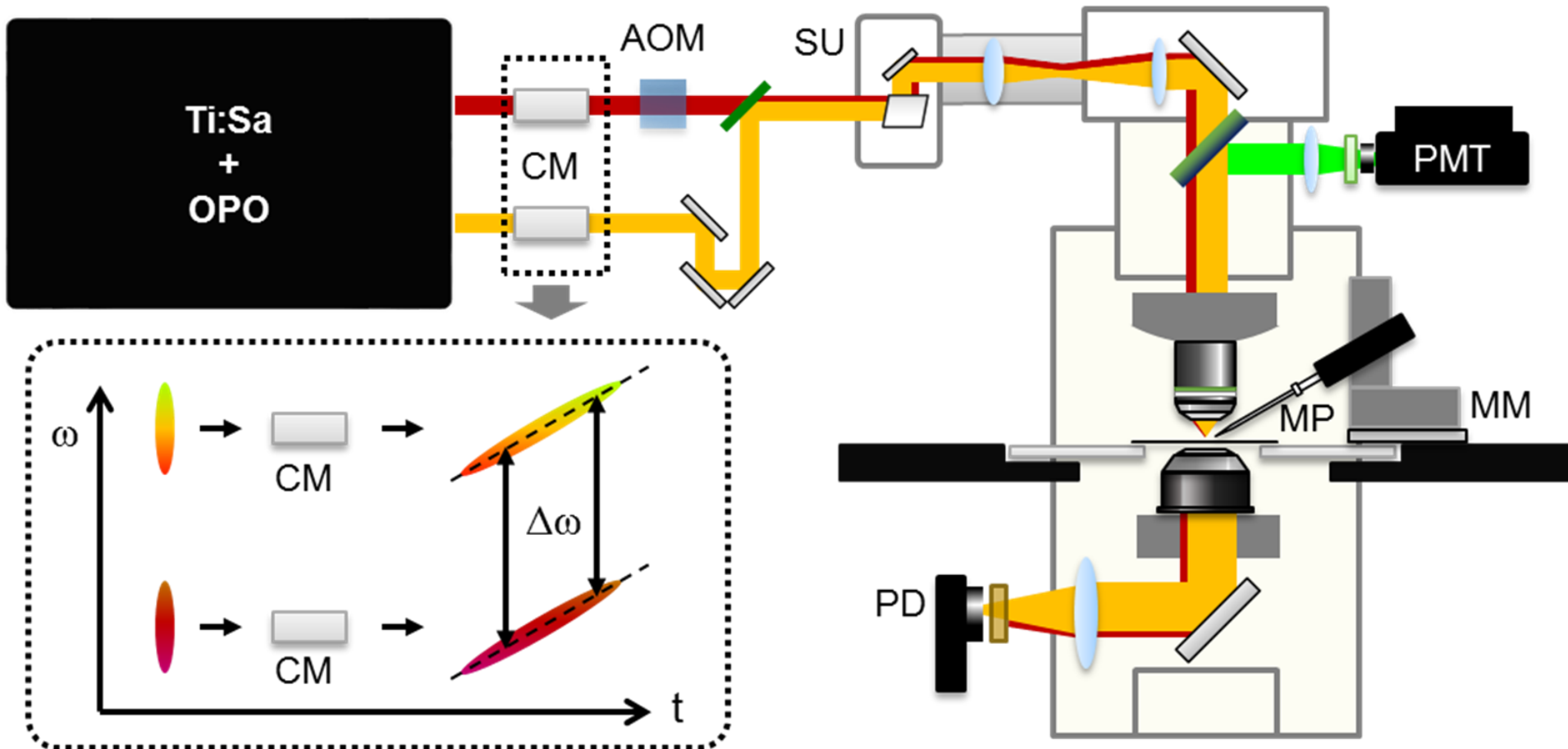
Challenges:

- Toxicity
- Photo-bleaching
- Limited physical space

Label-free spectroscopic detection of membrane potential



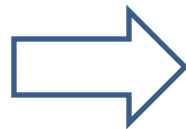
Integration of patch clamp and SRS imaging



In collaboration with
Dr. Drenan (Purdue),
Dr. Barlett (Purdue), Dr. Xu (IUSM)

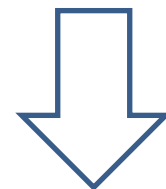
PD: photodiode
MP: micromanipulator
CM: chirping medium

**Biochemistry
information**

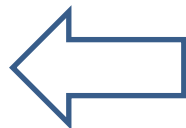


**Sufficient depth &
large field of view,**

III. Moving into Clinic



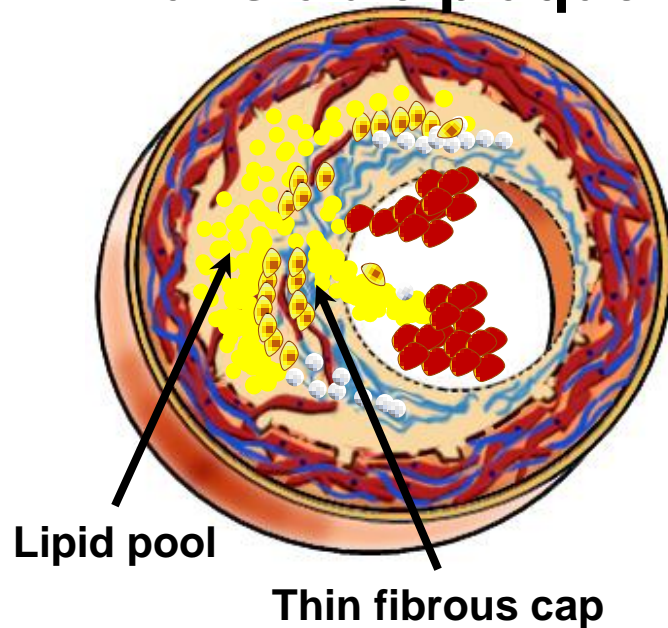
**Compact device &
commercialization**



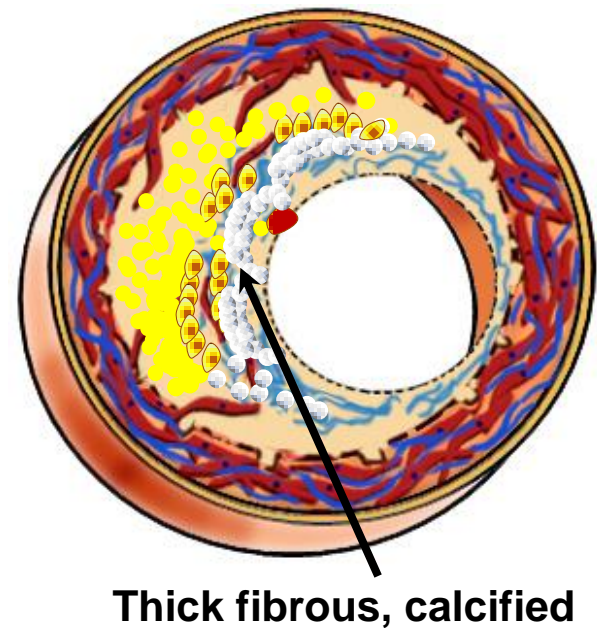
**Preclinical /clinical
studies**

Current Tools Lack the Ability to Identify Vulnerable Plaques

“Vulnerable”,
unstable plaque



“Stable” plaque

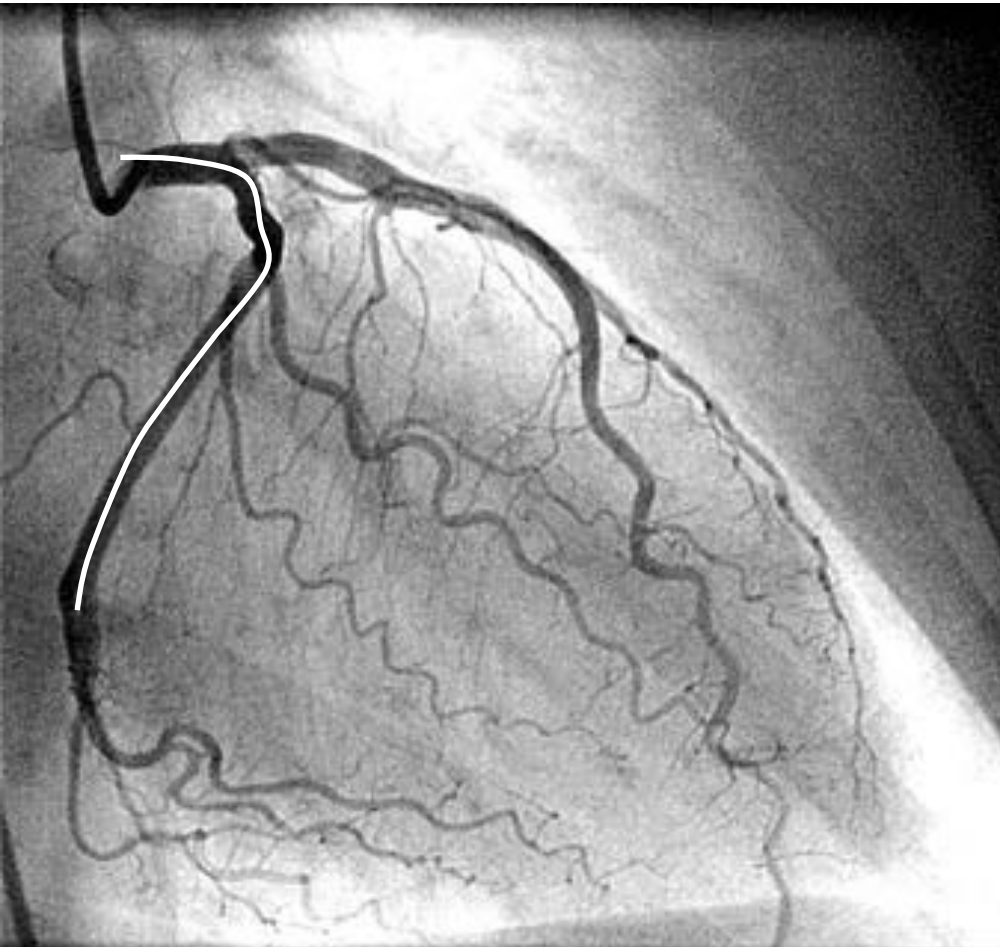


Cap erosion, thrombosis, rupture

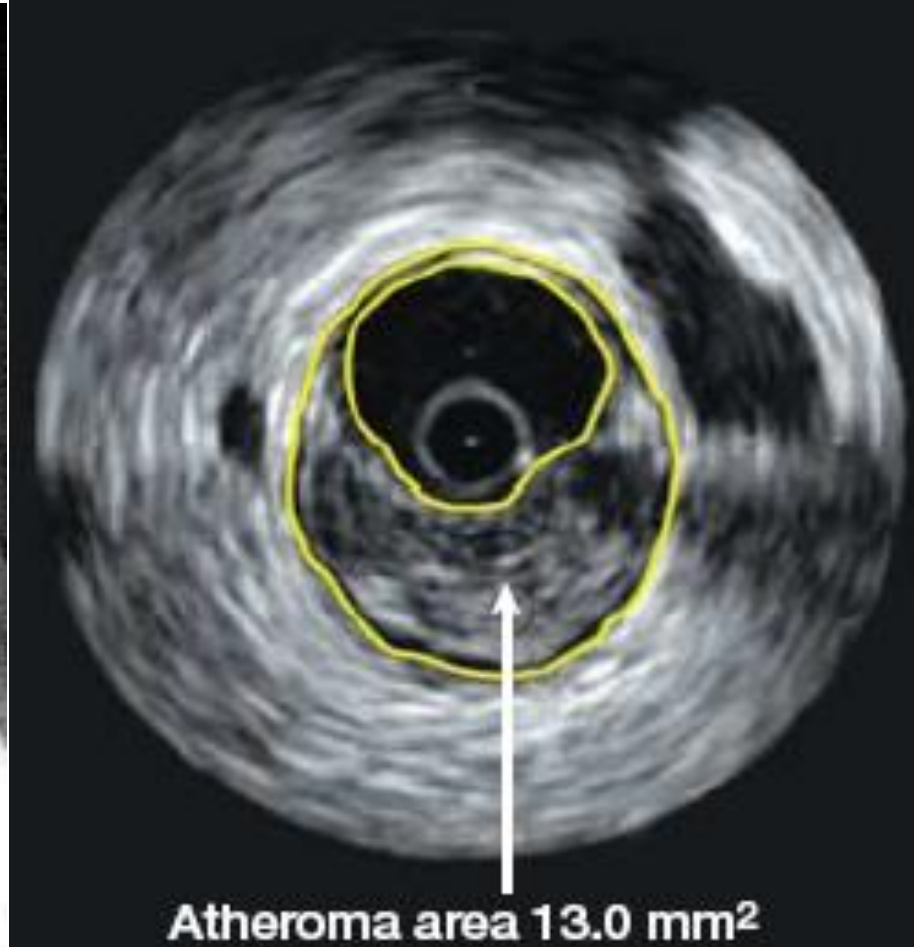
→ Heart attack

Current Medical Imaging Tools Lack the Ability to Identify Chemical Composition

Angiography

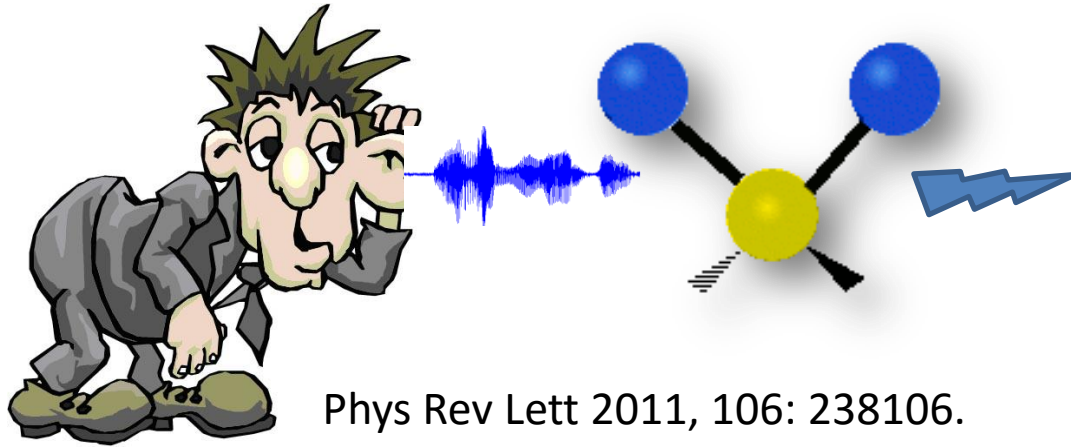


Intravascular ultrasound

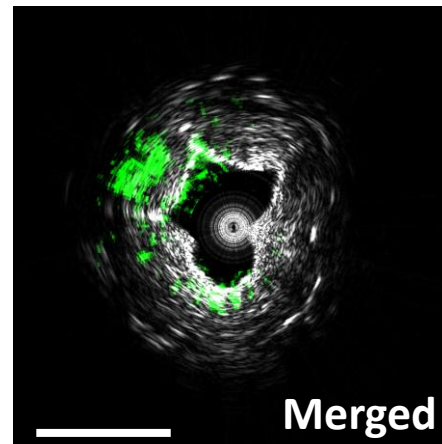
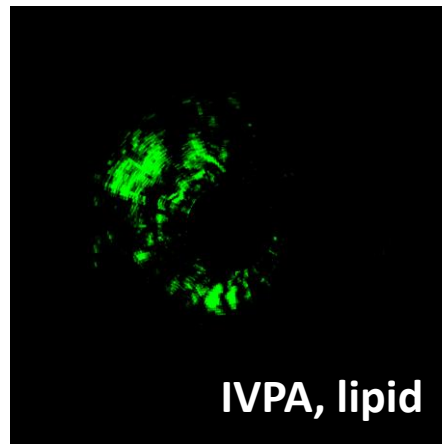
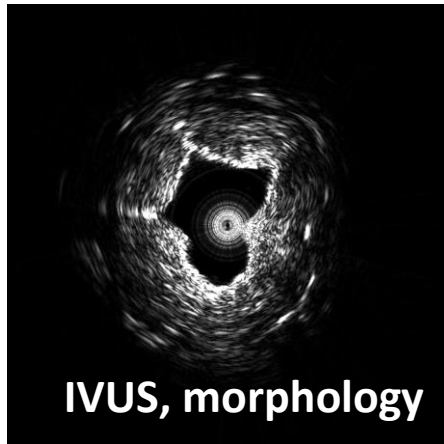
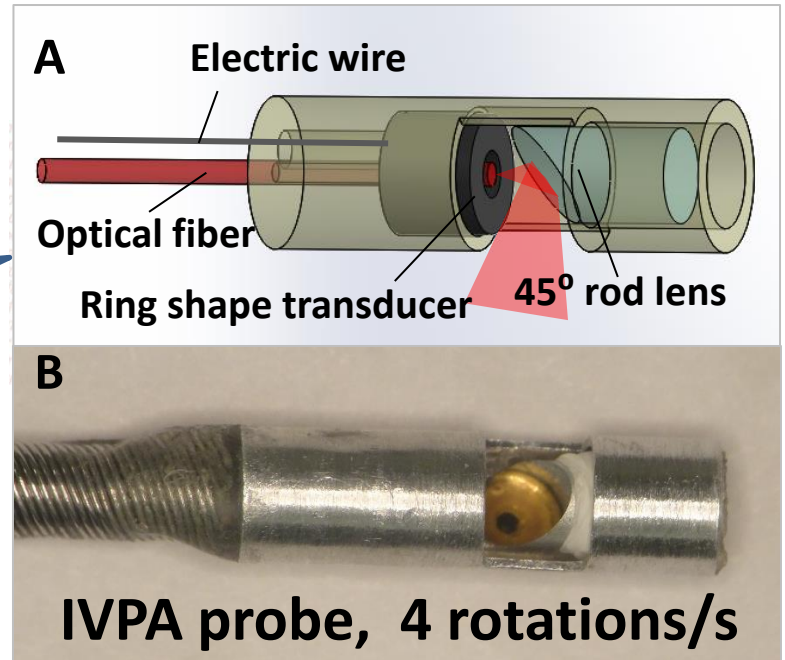


Pressing Need for Molecule-based Diagnosis!

Seeing Deep by Listening to Vibration



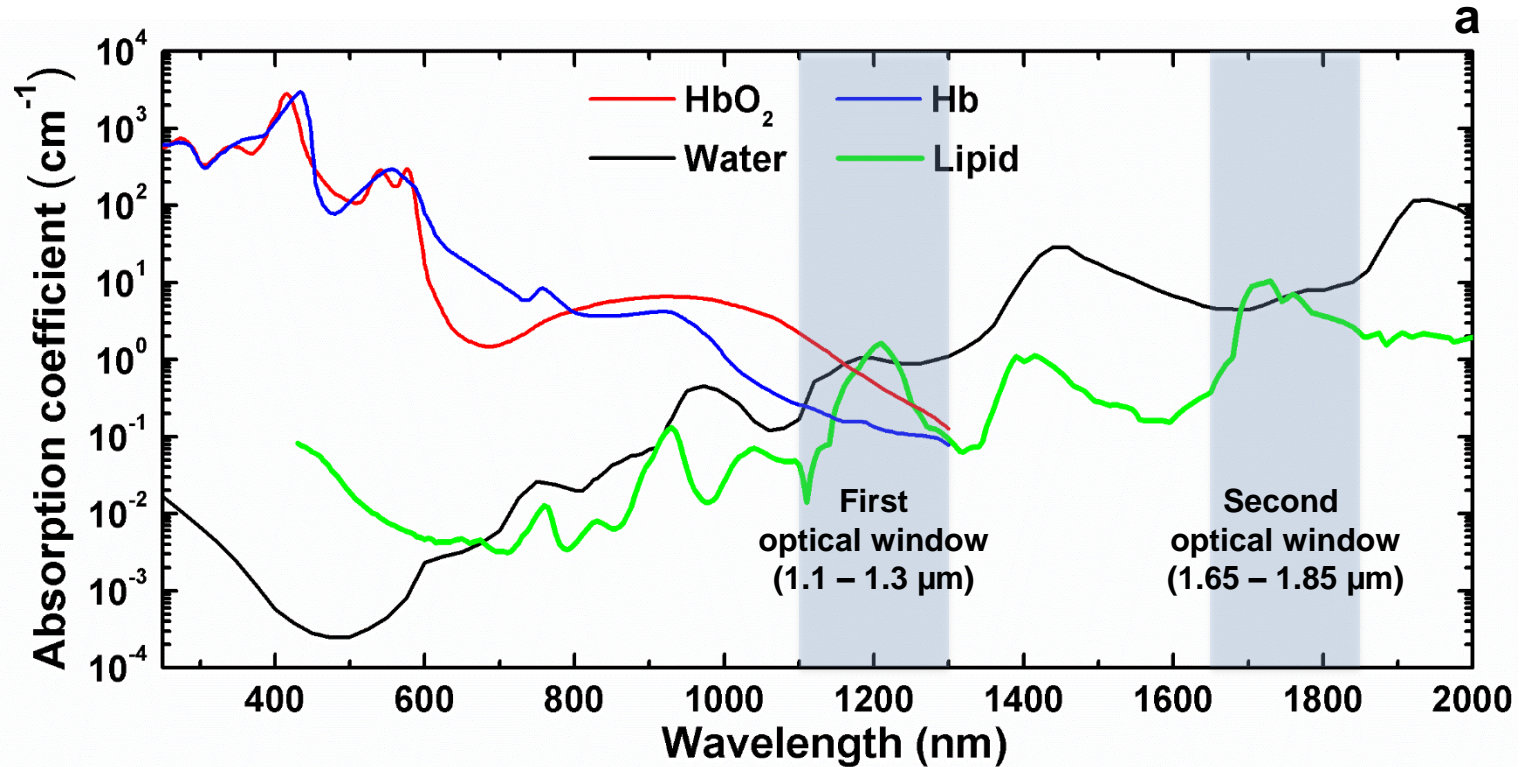
Phys Rev Lett 2011, 106: 238106.
depth, volume, and chemical info



Pu Wang et al. Nature Scientific Reports, 2014, 4: 6889.

Michael Sturek (IUSM); Ji-Xin Cheng (Purdue); Qifa Zhou (USC); Zhongping Cheng (UC Irvine)

Two Windows for Vibration-based PA Imaging



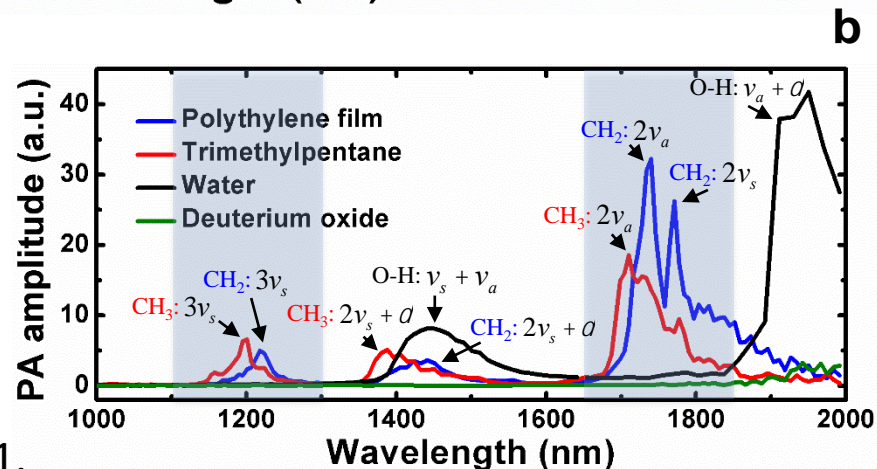
$$p_0 = \xi \Gamma \mu_a F$$

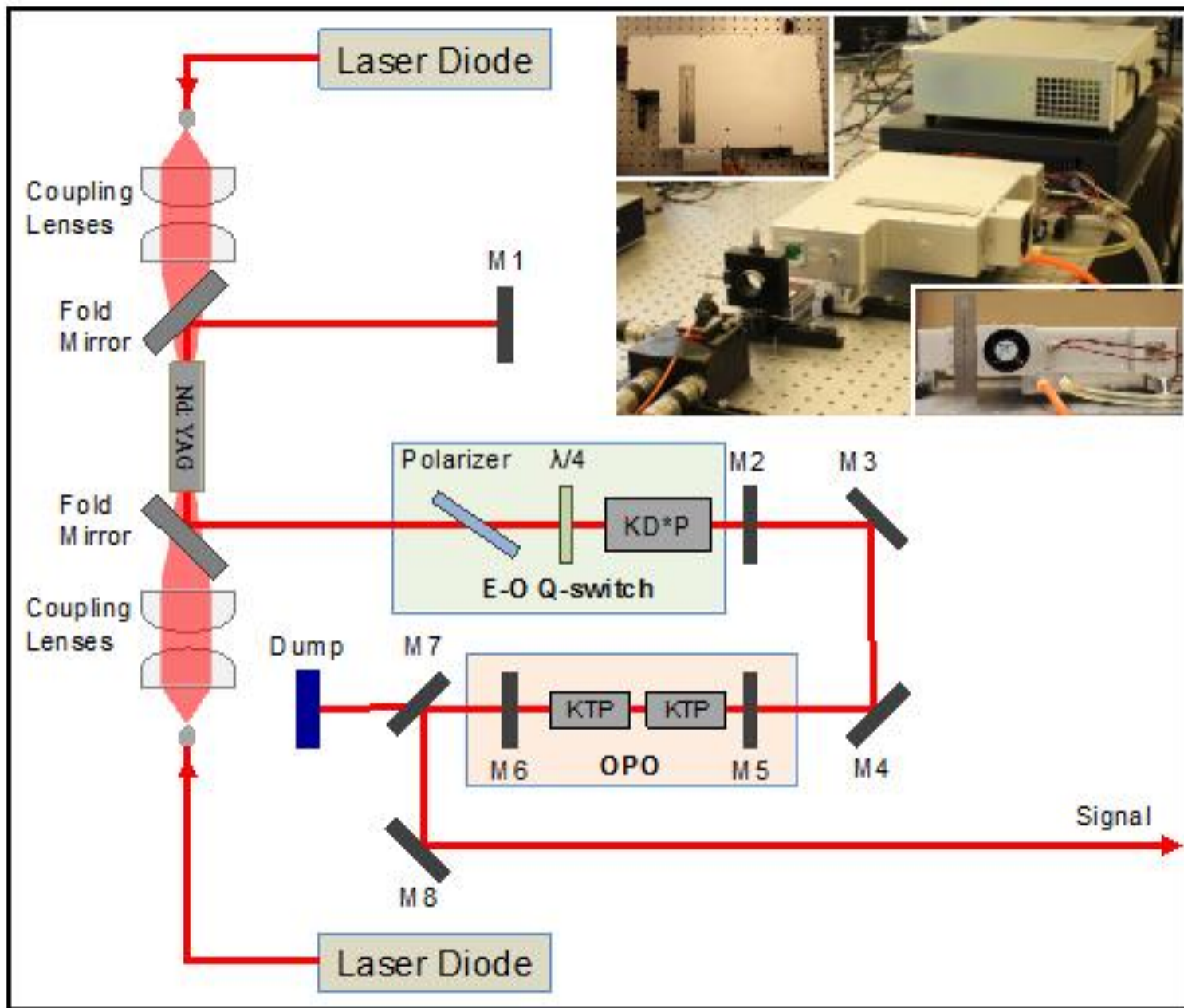
ξ is a constant,

Γ is the Gruneisen parameter,

μ_a is the absorption coefficient

F is the local light fluence.

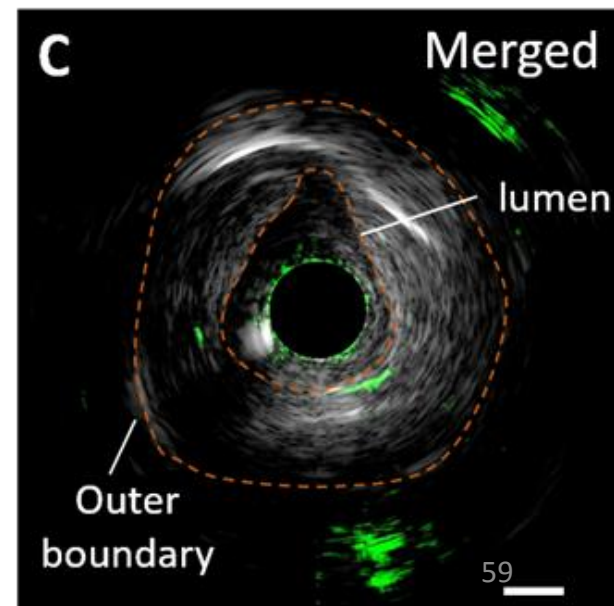
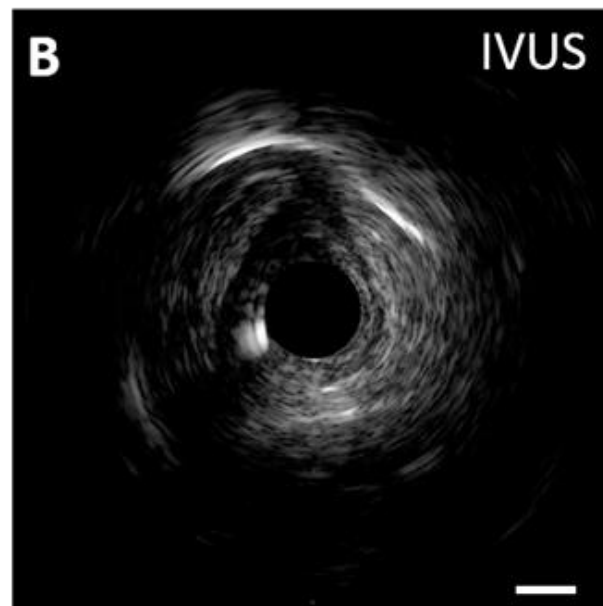
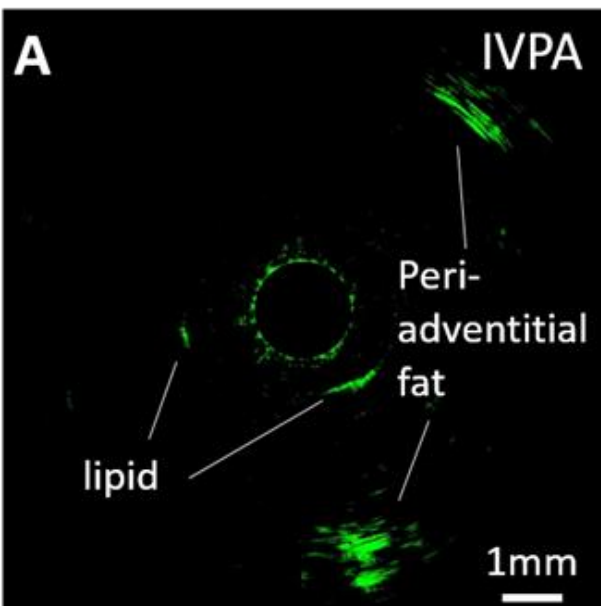
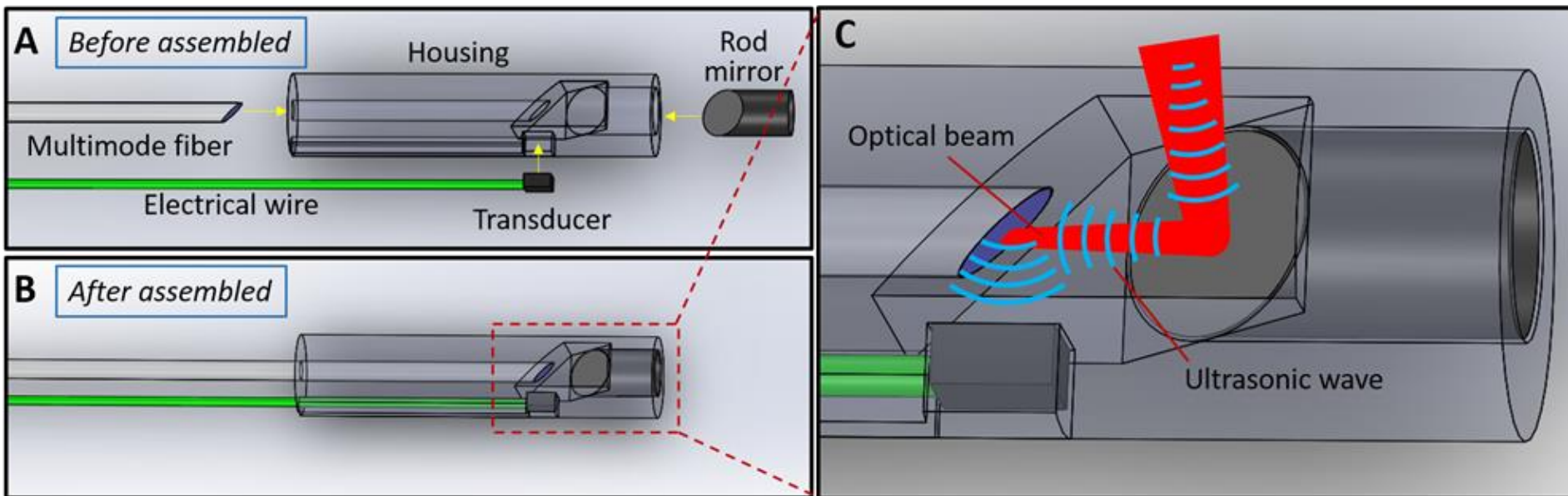




OPO, 1730 nm, 5 ns, 500 Hz, Weibiao Chen, Shanghai 58

Collinear IVPA Catheter

Yunchun Cao et al, Sci. Rep.,
2016, 6:25236



In vivo IVPA Imaging

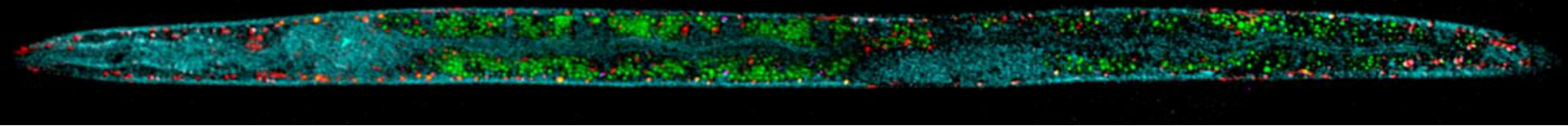




Vibronix

"Better Diagnosis, Better Treatment"

**The first company founded by Purdue Foundry
Co-founders: Ji-Xin Cheng, Pu Wang
2014**



6th Workshop “In Vivo Spectroscopic Imaging”

July 7-8, 2016, at Purdue University

www.conf.purdue.edu/spectro16

Organizer: Ji-Xin Cheng, jcheng@purdue.edu

Academic speakers:

Hui Cao, Yale University; **Ji-Xin Cheng**, Purdue
Conor Evans, Harvard; **Khanh Kieu**, U. Arizona
Gabriel Popescu, UIUC; **Adam Wax**, Duke Univ.
Hao Zhang, Northwestern.

Molecular Spectroscopic Imaging Group

Ji-Xin Cheng (Principal Investigator)
Azrielle Nunnally (Operations Manager)

Vibronix Inc

Resarci Therapeutics

1 Microscopy

Chi Zhang, Postdoc
Delong Zhang, Postdoc
Jason Liao, PhD
Kai-Chih Huang PhD
Chen Li, PhD
Haonan Lin, PhD
Peng Lin, PhD
Yin-Xin Zhang (VS)
Xueli Chen (VS)
Current Funding:
Keck Foundation
R21 GM104681
R01 on SRS Flow

2 Biology

Junjie Li, Postdoc
Weili Hong, Postdoc
Shibin Deng Postdoc
Hyeon-Jeong, PhD
Pu-Ting Dong, PhD
Andy Chen, PhD
Ying Jiang, PhD
Wei Chen (VS)
Current Funding:
R21 GM114853
(SCAP Program)
R21 CA 182608
R01 HL117990 (sub)

3 Device

Pu Wang, Postdoc
Yingchun, Postdoc
Hui Jie, PhD
Rui Li, PhD
Lu Lan, PhD
Jiayingzi Wu, PhD
Yi Zhang, PhD
Ayeeshik Kole, MD
Brittani Bungart MD
Current Funding:
R01 HL125385
R41 CA192645
AHA Innovation

4 Therapy

Junjie Li, Postdoc
Caro Karanja PhD
Shovik, UG
Jie Nee Lee, UG
Xiaochao Qu (VS)
Current Funding:
Walther Cancer
Foundation
DoD PC130964
DoD Spinal Injury