Molecular Spectroscopy of Living Systems

Ji-Xin Cheng

June 20, 2016
International Symposium of Molecular Spectroscopy
Metabolites in *C. elegans*


**In vivo spectroscopic imaging**

- Myelin
- Liver
- Skin cancer
- Plaque
- Brain cancer
- Protein synthesis
Longevity: *C. elegans* as in vivo test tube

Fingerprinting Lipid Metabolism in Live *C. elegans*

In collaboration with Heidi Tissenbaum (U Mass).
$k$-means clustering

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

≠ Spectrometer + Microscope

In vivo spectroscopic imaging

Coherent Raman Boosts the Speed

Spontaneous Raman

Coherent Raman Scattering

CARS: coherent anti-Stokes Raman scattering
SRL: stimulated Raman loss, SRG: stimulated Raman gain

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

Spectral imaging speed versus spectral width

- **Spontaneous Raman microscope**
  - Milliseconds per spectrum, full window

- **Broadband CARS microscope**
  - 3.5 ms per spectrum, 3000 cm$^{-1}$ window
  - Cicerone et al, Nat. Photonics 2014

- **Multiplex SRS microscope**
  - 5 µs per spectrum, 200 cm$^{-1}$ window
  - Cheng et al, Light Sci. & Appl. 2015;
  - Cheng et al, Science Advances 2015

- **Single-frequency CARS or SRS microscope**
  - 100 ns per pixel, 5 cm$^{-1}$ window
  - Xie et al, PNAS 2005; Science 2010
Microsecond scale vibrational spectroscopic imaging by multiplex stimulated Raman scattering microscopy

Chien-Sheng Liao¹,* , Mikhail N Slipchenko¹,* , Ping Wang¹,* , Junjie Li², Seung-Young Lee¹, Robert A Oglesbee³ and Ji-Xin Cheng¹,³

Top Story in 2015, Biophotonics Magazine
32-channel tuned amplifier for μs Raman shift in DMSO solution. AC / DC (dI/I) and Raman (3 sec) comparison. SRL (5 μs)
High-throughput single cell analysis by stimulated Raman flow cytometer

(>10,000 particles (bacteria) /sec, 5 μs per spectrum)

Jesse Zhang et al
Nature Comm, under review
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

Science Advances, 2015, 1: e1500738
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

Liao et al, Science Advances, 2015; chosen as a top advance in 2015 by The Scientist
Transportable & hand-held system for in situ spectroscopic imaging
Longer-wavelength Excitation Reduces Photodamage to Cells in SRS Imaging

Max. power used for SRL imaging:
- 830 nm: 50 mW before microscope
- 1004 nm: 200 mW before microscope
- 1080 nm: 200 mW before microscope

Signals in CRS microscopy are generated by ballistic photons under the tight focusing condition, thus limiting its imaging depth to ~ 100 μm.

Cover: multimodal image of central nervous system
Limited Penetration Depth in CARS Microscopy

CARS

SFG

Adventitia

Media

Lumen

Atherosclerosis

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

80 μm
Boltzmann distribution
\[ N_i / N_0 = \exp(\Delta E / kT) \]

\( \Delta E \) 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**

- Pulsed radiation
- Absorption, thermal-elastic expansion
- Pressure wave generation
- Acoustic Detection

**Blood Vessel Plexus (Hemoglobin) & Melanoma**

1st overtone: $v=0$ to $v=2$
2nd overtone: $v=0$ to $v=3$

Molecular Vibration $\rightarrow$ Acoustic Wave
PA Spectra of Biological Molecules

PA Imaging of Lipid-rich Plaque


Penetration Depth:
up to 7 mm

Speed: single pulse per pixel
at 10 Hz

Spatial resolution:
• Lateral resolution: from 5 μm to 70 μm
• Axial resolution: ~135 μm; 35 μm is possible
Raman scattering cross section
\(~ 10^{-30} \text{ cm}^2/\text{sr}~\)

\textbf{VS.}

Infrared absorption cross section
\(~ 10^{-22} \text{ cm}^2/\text{sr}~\)
History of IR Spectroscopy and Imaging

1905
IR spectral database
By W.W. Coblentz [1]

1944
First IR microscope available [2]

1954
First commercial IR spectrometer available [2]

1983
FTIR spectrometry introduced [3]

1995
Focal plane array detector for FTIR imaging [4]

2000s
FTIR spectrometer and microscope [2]

2011
High resolution FTIR chemical imaging using synchrotron source [6]

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

Dark field geometry
Resolution: 0.6 micron
Sensitivity: 10 µM

Delong Zhang et al,
Science Advances 2016
In press
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

Ground State

Excited State Absorption

Stimulated Emission

Ground State Depletion

Warren Warren, Greg Hartland, Sunney Xie, Cheng groups
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
\[ \omega_s = 2\omega_p - \omega_s \]

Pump-probe

Photoacoustic

Photothermal
Altered Metabolism: Cancer’s Achilles Heel

Glu: glucose
Gly: Glycine
Gln: Glutamine

Galluzzi et al., Nat Rev Drug Disc (2013), 12: 829
SRS Imaging of de novo Lipogenesis in Single Living Cells

Raman Probes for Imaging Cholesterol Metabolism in Living Cells

Imaging detection limit: 900 molecules. Collaborator: Mingji Dai

Cholesterol storage in lysosomes in Niemann-Pick C mutant cells

PhDiyne-Cholesterol

i) small
ii) biologically inert
iii) bioorthogonal (2200 cm\(^{-1}\))
iv) large Raman cross section
Human Patient Specimen Examination by Label-free Spectroscopic Imaging

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 Xiaqi Liu,4,7 Timothy L. Ratliff,3,7 and Ji-Xin Cheng1,7,*

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http://dx.doi.org/10.1016/j.cmet.2014.01.019
Single-color SRS imaging of human patient 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

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\(^{-1}\).

Molecular Pathways Underlying Cholesteryl Ester Accumulation

Cell Metabolism, 2014, 18: 393-406

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

Lysosome

LDLr

CE in LDL

CE in LD

FC

PI3K/AKT/mTOR

PTEN loss

SREBP

ω-6 PUFA (e.g. AA)

ER

ACAT-1

Proliferation

Tumor growth

Avasimibe

CE-rich LD

Avasimibe

43
Abrogating cholesterol esterification suppresses growth and metastasis of pancreatic cancer

J Li, D Gu, SS-Y Lee, B Song, S Bandyopadhyay, S Chen, SF Konieczny, TL Ratliff, X Liu, J Xie, and J-X Cheng

a. growth

b. metastasis

control

Luminescence (a.u.)

0.1

1.0

treated
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.

- 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


Jonas Hansson’s thesis: Microfluidic blood sample preparation for rapid sepsis diagnostics
In situ Detection of a Single Bacterium in complex environment (food, urine, blood)

**E. Coli** in lysed blood

**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

First action potential recorded: giant squid axon

Hodgkin & Huxley. Nature (1939) 144, 710
Optical recording of neural activities

Calcium indicators:

Voltage sensitive probes:

Challenges:
- Toxicity
- Photo-bleaching
- Limited physical space

Grienberger et al. *Neuron* (2012) **73**: 862
Label-free spectroscopic detection of membrane potential
Integration of patch clamp and SRS imaging

PD: photodiode  
MP: micromanipulator  
CM: chirping medium

In collaboration with  
Dr. Drenan (Purdue),  
Dr. Barlett (Purdue), Dr. Xu (IUSM)
III. Moving into Clinic

- Biochemistry information
- Sufficient depth & large field of view,
- Compact device & commercialization
- Preclinical /clinical studies
Current Tools Lack the Ability to Identify Vulnerable Plaques

“Vulnerable”, unstable plaque

Lipid pool
Thin fibrous cap
Cap erosion, thrombosis, rupture
→ Heart attack

“Stable” plaque

Thick fibrous, calcified
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


depth, volume, and chemical info

IVUS, morphology

IVPA, lipid

Merged


Michael Sturek (IUSM); Ji-Xin Cheng (Purdue); Qifa Zhou (USC); Zhongping Cheng (UC Irvine)
Two Windows for Vibration-based PA Imaging

\[ \rho_0 = \xi \Gamma \mu_a F \]

\( \xi \) is a constant,
\( \Gamma \) is the Gruneisen parameter,
\( \mu_a \) is the absorption coefficient
\( F \) is the local light fluence.

Photoacoustics (review) 2016, 4, 11-21.
OPO, 1730 nm, 5 ns, 500 Hz, Weibiao Chen, Shanghai
Collinear IVPA Catheter

In vivo IVPA Imaging
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.