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Multimodal Coherent Raman Spectroscopy & 3D Microscopy:

Nonlinear Optical Tools for Geoscience Research

Albert Stolow, A.D. Slepkov, A. Pegoraro, **Robert C. Burruss**
National Research Council Canada
& USGS

Microscopy of Organic Molecules in Rock

In order to image the micro-world, we will need two things:

(1) Spatial resolution
(2) Contrast

Problems:

(1) Prefer to use visible light, UV absorbed and scattered

(2) Features may be small: <1μm. IR wavelength too long.

(3) Organic inclusions do not come colour-coded for our viewing pleasure….

(4) How can we get molecule-specific contrast?

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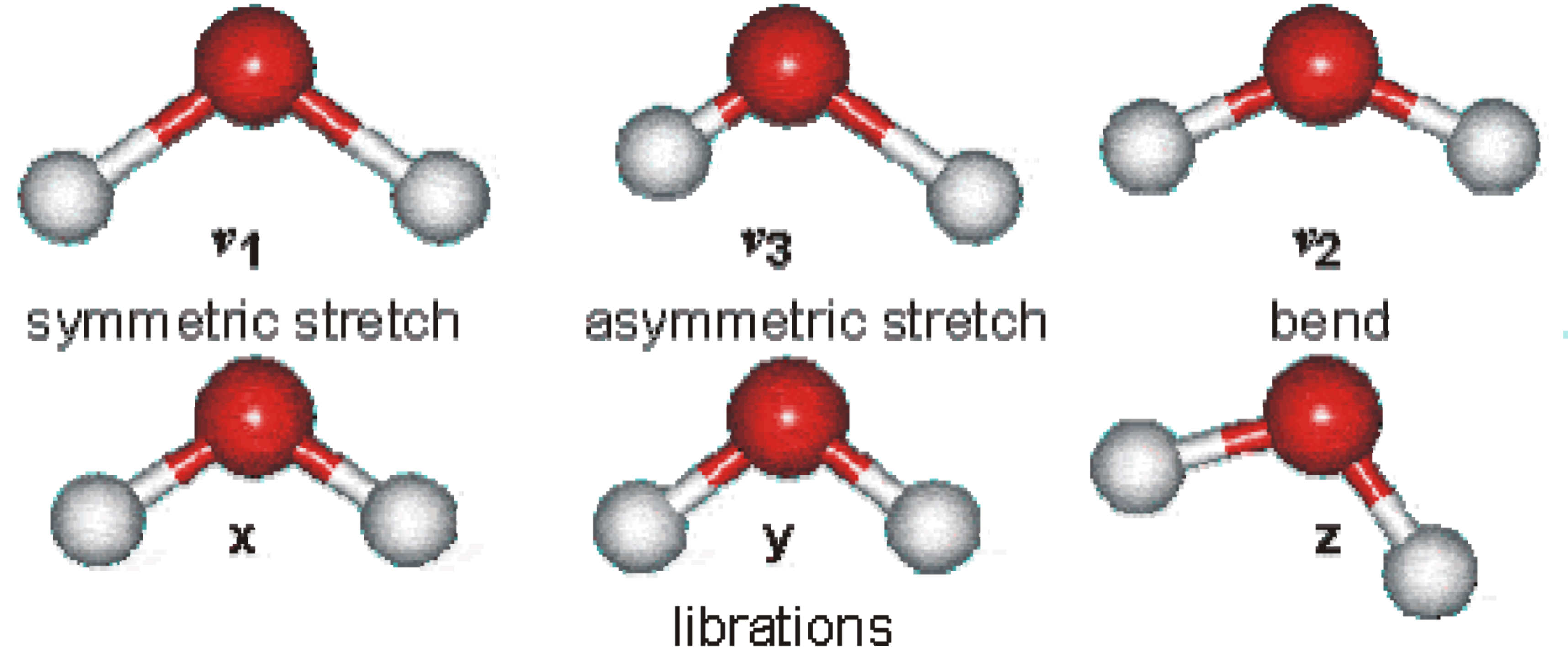
How can we achieve Molecule Specific Imaging?

- Want to avoid exogenous labels (dyes, stains, markers)
- Fluorescence (electronic, X-ray) spectroscopy of organic molecules in condensed phases is <u>NOT</u> specific enough
- Magnetic Resonance does <u>NOT</u> have required spatial resolution

Molecular Vibrations
are
Molecular Fingerprints

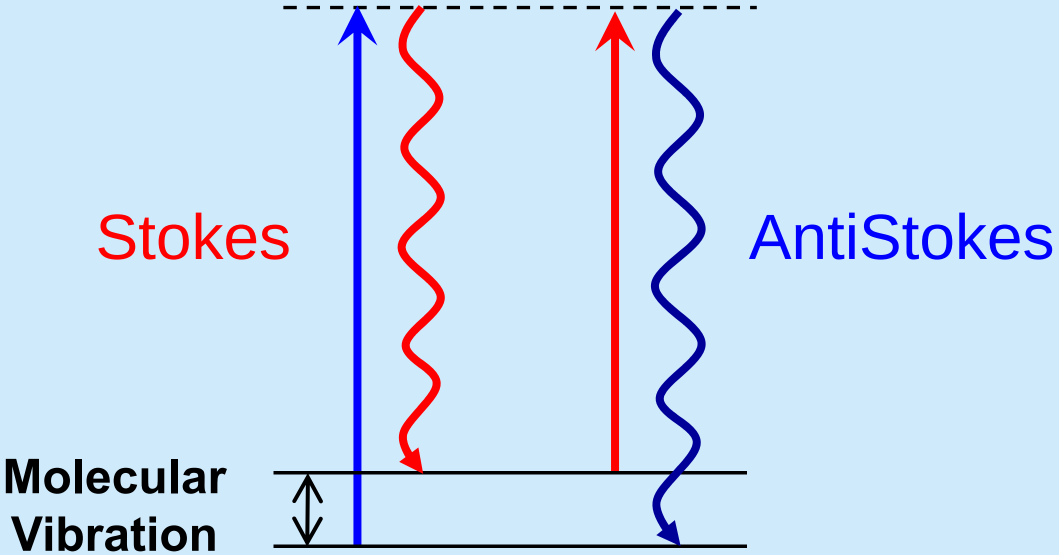


The Raman Effect

C.V. Raman
1928

The Inelastic Scattering of Light

**Spontaneous
Raman Scattering**



Raman Spectroscopy probes Molecular Vibrations

Challenges with Raman Microscopy

- The Raman effect is famously insensitive
- Any fluorescence background can completely overwhelm the Raman signal
- Raman microscopy generally insensitive to crystallographic features: edges, cracks, grain boundaries

A New Tool:
Non-linear Optics

Apply an intense laser electric field to matter.
Expand the material response in powers of the electric field *E*

$$P = \varepsilon_0(\underbrace{\chi^{(1)}E}_{\text{linear response}} + \underbrace{\chi^{(2)}E^2 + \chi^{(3)}E^3 + \cdots}_{\text{nonlinear response}})$$

The linear response gives us things we know well.

refractive index and the **absorption coefficient**

When *E* is small (weak laser field), ***E***^{2} , ***E***^{3} etc. are negligible

The nonlinear response brings many new opportunities.

Important (useful) when ***E*** gets large, as in an ultrashort laser pulse

2nd Order Nonlinearities

- The incident optical field

$$\tilde{E}(t) = E_1(t) + E_2(t)$$

- Nonlinear polarization contains the following terms

$$P(2\omega_1) = \chi^{(2)} E_1^2 \qquad \text{(SHG)}$$

$$P(2\omega_2) = \chi^{(2)} E_2^2 \qquad \text{(SHG)}$$

$$P(\omega_1 + \omega_2) = 2\chi^{(2)} E_1 E_2 \qquad \text{(SFG)}$$

$$P(\omega_1 - \omega_2) = 2\chi^{(2)} E_1 E_2^* \qquad \text{(DFG)}$$

$$P(0) = 2\chi^{(2)} (E_1 E_1^* + E_2 E_2^*) \qquad \text{(OR)}$$

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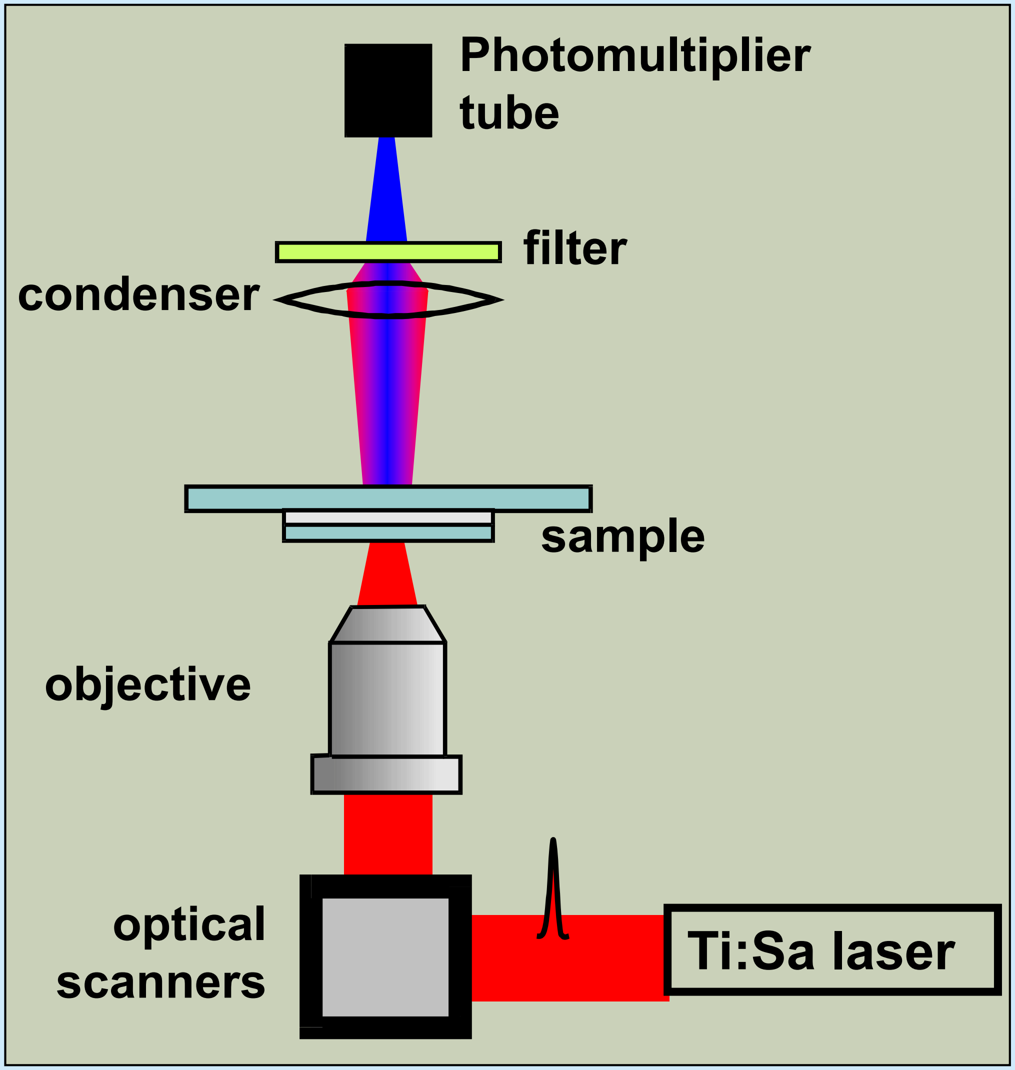
Two Photon Microscopy

- Confocal scanning microscope
- Femtosecond Ti:Sa oscillator

Detect Signals
2nd Harmonic (SHG)
2*hv* fluorescence (TPEF)



SHG: Sensitive to cracks, grain boundaries etc.
TPEF: Sensitive to higher organics

3rd Order Nonlinearities

When the general form of the incident electric field is

$$\tilde{E}(t) = E_1 e^{-i\omega_1 t} + E_2 e^{-i\omega_2 t} + E_3 e^{-i\omega_3 t}$$

The third order polarization can have 22 components containing the following frequencies:

$$\omega_i, 3\omega_i, (\omega_i + \omega_j + \omega_k), (\omega_i + \omega_j - \omega_k)$$
$$(2\omega_i + \omega_j), \boxed{(2\omega_i - \omega_j)}, i, j, k = 1,2,3$$

CARS

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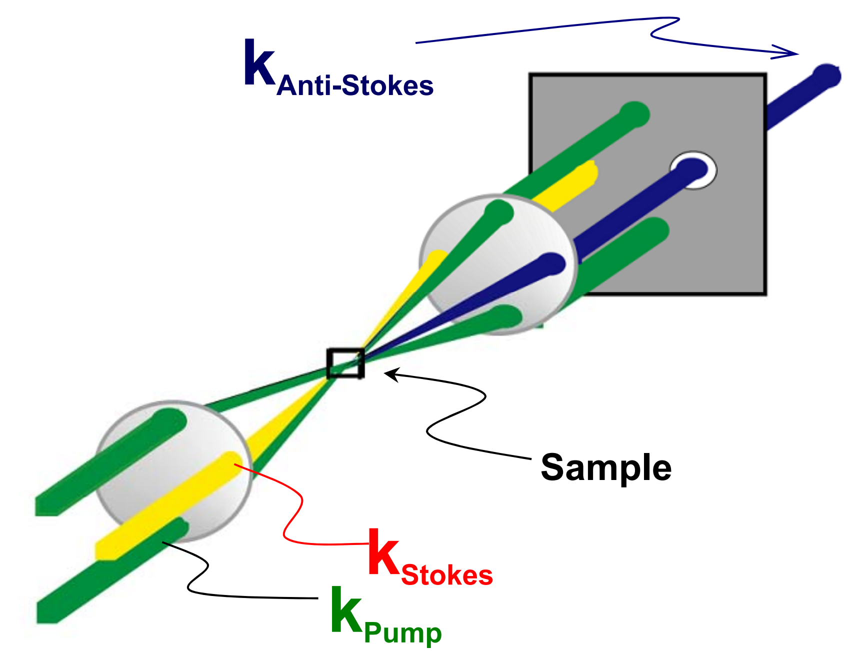
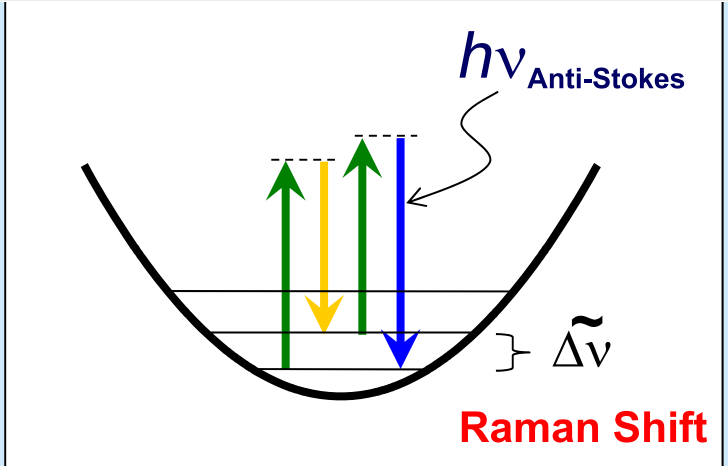
Coherent Anti-Stokes Raman Scattering

- A 3rd order non-linear optical version of Raman Spectroscopy
- Optimally used with *ultrashort* laser pulses
- CARS signal is a coherent laser pulse, blue-shifted and spatially distinct from all other light sources.



2 Input Colours: Pump & Stokes



2x Pump – Stokes = AntiStokes

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CARS Microscopy

- Non-linear Optical Microscopy. □ Enhanced 3D spatial resolution
- CARS Microscopy has *chemical selectivity* via Raman mode resolution
- CARS Microscopy is >10^4 *times more sensitive* than Raman microscopy

LABEL FREE. Vibrational contrast means fluorescent probes unnecessary.

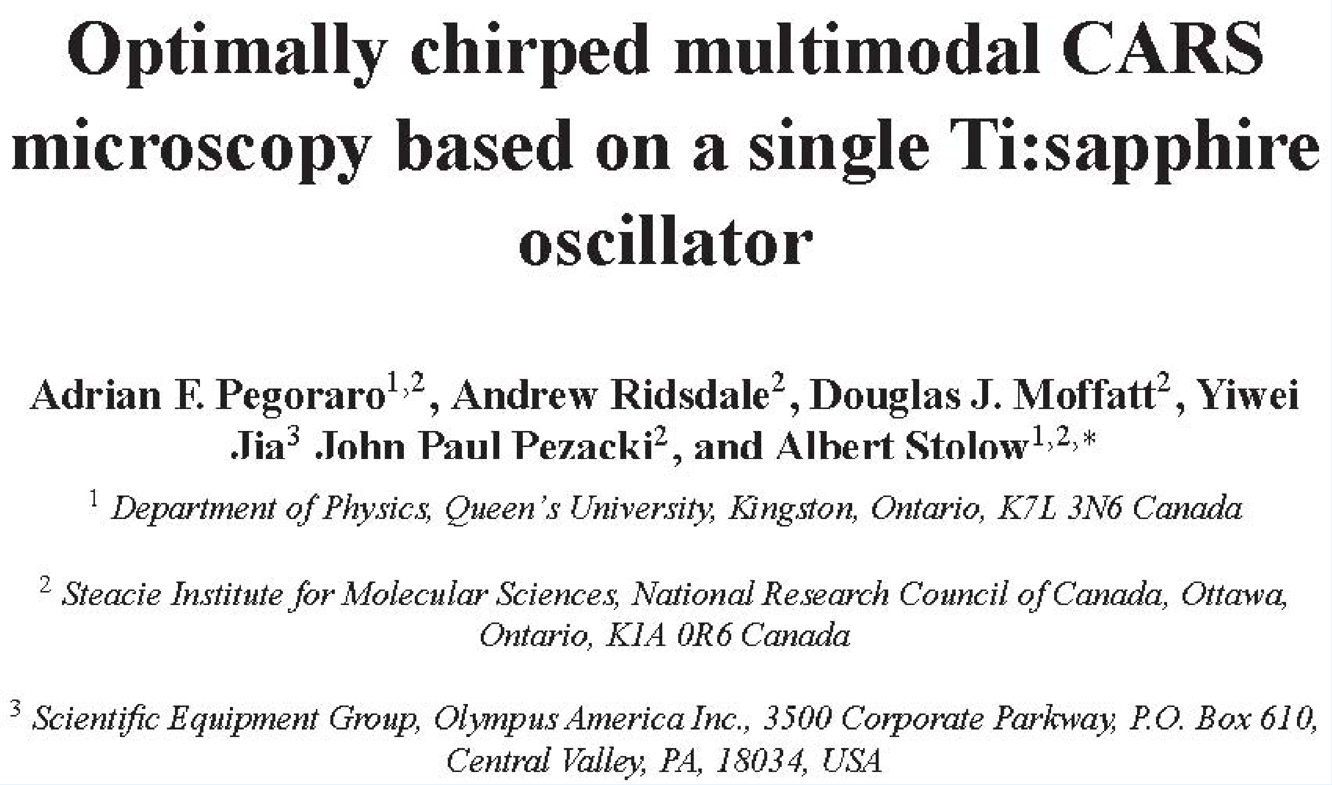
MINIMAL DAMAGE. No linear absorption. Photobleaching is circumvented: *no heating effects.*

FLUORESCENCE FREE. CARS signal is higher in frequency than one-photon fluorescence, so it can be detected in the presence of a strong fluorescent background.

DEEPER PENETRATION. Reduced absorption, scattering of the near-infrared excitation beams.

CARS Microscopy Made Simple

Optics Express 17, 2984 (2009)



Optimally chirped multimodal CARS microscopy based on a single Ti:sapphire oscillator

Adrian F. Pegoraro[1,2], **Andrew Ridsdale**[2], **Douglas J. Moffatt**[2], **Yiwei Jia**[3] **John Paul Pezacki**[2], **and Albert Stolow**[1,2,*]

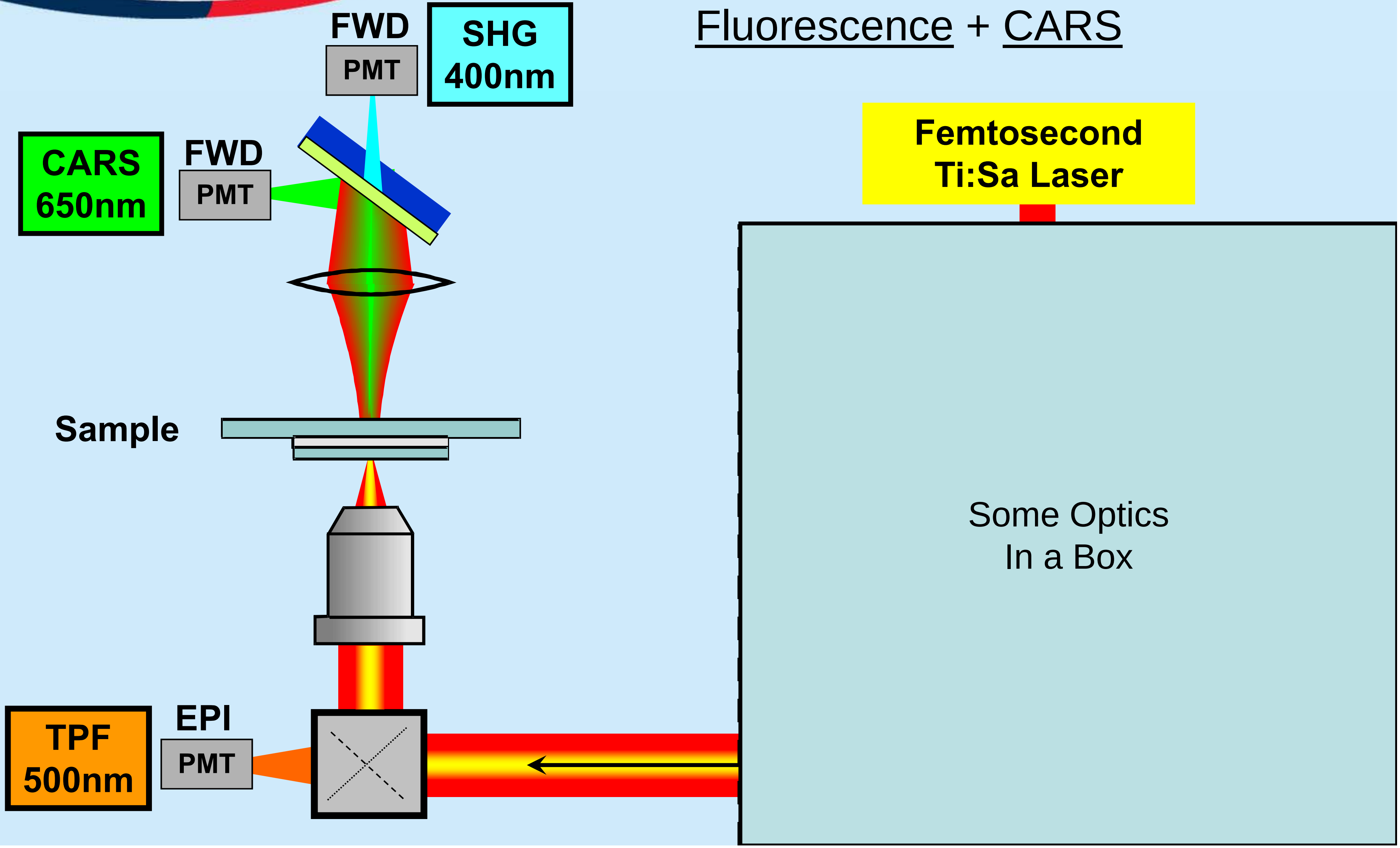
[1] *Department of Physics, Queen's University, Kingston, Ontario, K7L 3N6 Canada*

[2] *Steacie Institute for Molecular Sciences, National Research Council of Canada, Ottawa, Ontario, K1A 0R6 Canada*

[3] *Scientific Equipment Group, Olympus America Inc., 3500 Corporate Parkway, P.O. Box 610, Central Valley, PA, 18034, USA*

Multimodal Non-linear Microscopy

Second Harmonic + 2-Photon Fluorescence + CARS



FWD
PMT
SHG
400nm

CARS
650nm
FWD
PMT

Femtosecond
Ti:Sa Laser

Sample

Some Optics
In a Box

TPF
500nm
EPI
PMT

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First Commercially Available CARS Microscope

Released October 2009

Fluoview® FV1000MPE femtoCARS Add-on

A low-cost high-performance accessory for multimodal in vivo imaging without the need for staining protocols or fluorescent protein expression.

The femtoCARS equipped FV1000MPE provides a label-free lipid imaging capability based on molecular vibrations using the Coherent Anti-Stokes Raman Scattering (CARS) microscopy method. CARS is a non-linear optical method with high chemical selectivity and sensitivity that is improved orders of magnitude relative to conventional Raman detection. In Olympus's design, the CARS imaging mode can be combined with two photon excitation fluorescence (TPEF) and second harmonic generation (SHG) imaging modes as a multimodal femtosecond laser imaging station. The femtoCARS add-on provides an entirely new commercial solution for investigating structures such as the myelin sheath, intracelluar lipid bodies, mechanisms underlying fat storage and membrane organization, as well as tissue and skin reorganization *in vivo*.

Locate your Olympus sales representative/dealer by entering your zip code:

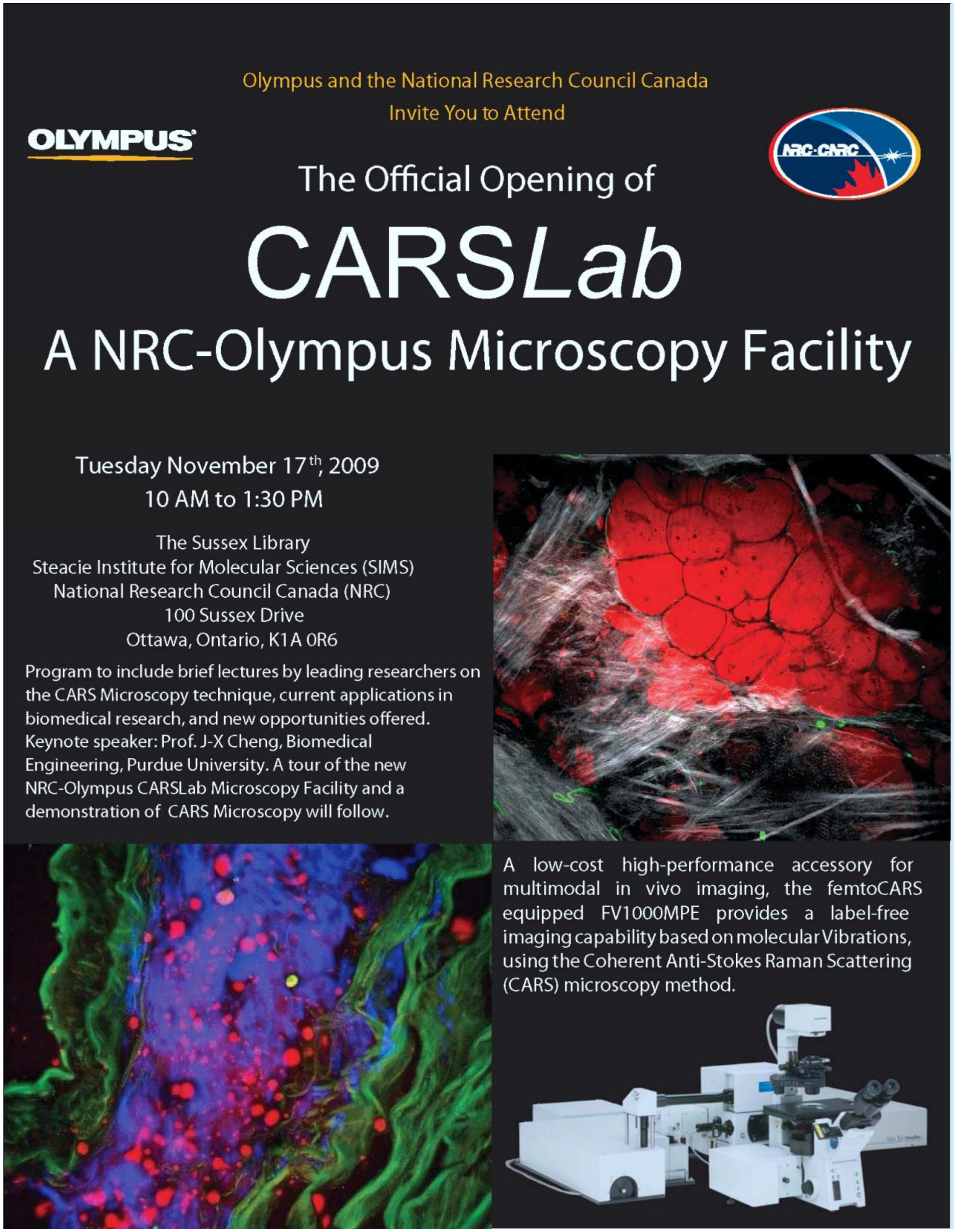
Feature & Benefits:

- Simultaneous Lipid (CH2), TPEF, and SHG imaging. The lipid filter set is included and TPEF and SHG filter sets are optional.
- Uses the standard Olympus DeepSee lasers which are specifically tuned to match Olympus' optical requirements. No additional laser is required.
- The output of the femtoCARS is pre-set to lipid detection.
- Standard MPE detection system, up to two forward (transmitted) and four backward (epi) Non-Descanned Detection channels.
- Standard MPE imaging (by-pass CARS add-on) and single photon imaging are available.
- The optical delivery system including a laser-safe enclosure is provided and serviced by Olympus America, Inc.
- The overall footprints for MPE with femtoCARS remain small, but different from regular MPE systems.
- Upright platforms are standard. For inverted platform, please contact Olympus America, Inc.
- Acousto-optical modulation (AOM) standard for MPE and manual power adjustment operation for the Stokes' beam.
- Dispersion compensation standard for MPE and for efficient Stokes' beam generation.

The femtoCARS add-on unit is only available in US and Canada currently.

Return to FluoView MPE Multiphoton Microscopes Summary Page.

CARS User Facility



Olympus and the National Research Council Canada
Invite You to Attend

The Official Opening of

CARS*Lab*

A NRC-Olympus Microscopy Facility

Tuesday November 17th, 2009
10 AM to 1:30 PM

The Sussex Library
Steacie Institute for Molecular Sciences (SIMS)
National Research Council Canada (NRC)
100 Sussex Drive
Ottawa, Ontario, K1A 0R6

Program to include brief lectures by leading researchers on the CARS Microscopy technique, current applications in biomedical research, and new opportunities offered. Keynote speaker: Prof. J-X Cheng, Biomedical Engineering, Purdue University. A tour of the new NRC-Olympus CARSLab Microscopy Facility and a demonstration of CARS Microscopy will follow.

A low-cost high-performance accessory for multimodal in vivo imaging, the femtoCARS equipped FV1000MPE provides a label-free imaging capability based on molecular Vibrations, using the Coherent Anti-Stokes Raman Scattering (CARS) microscopy method.

www.CARS*Lab*.ca

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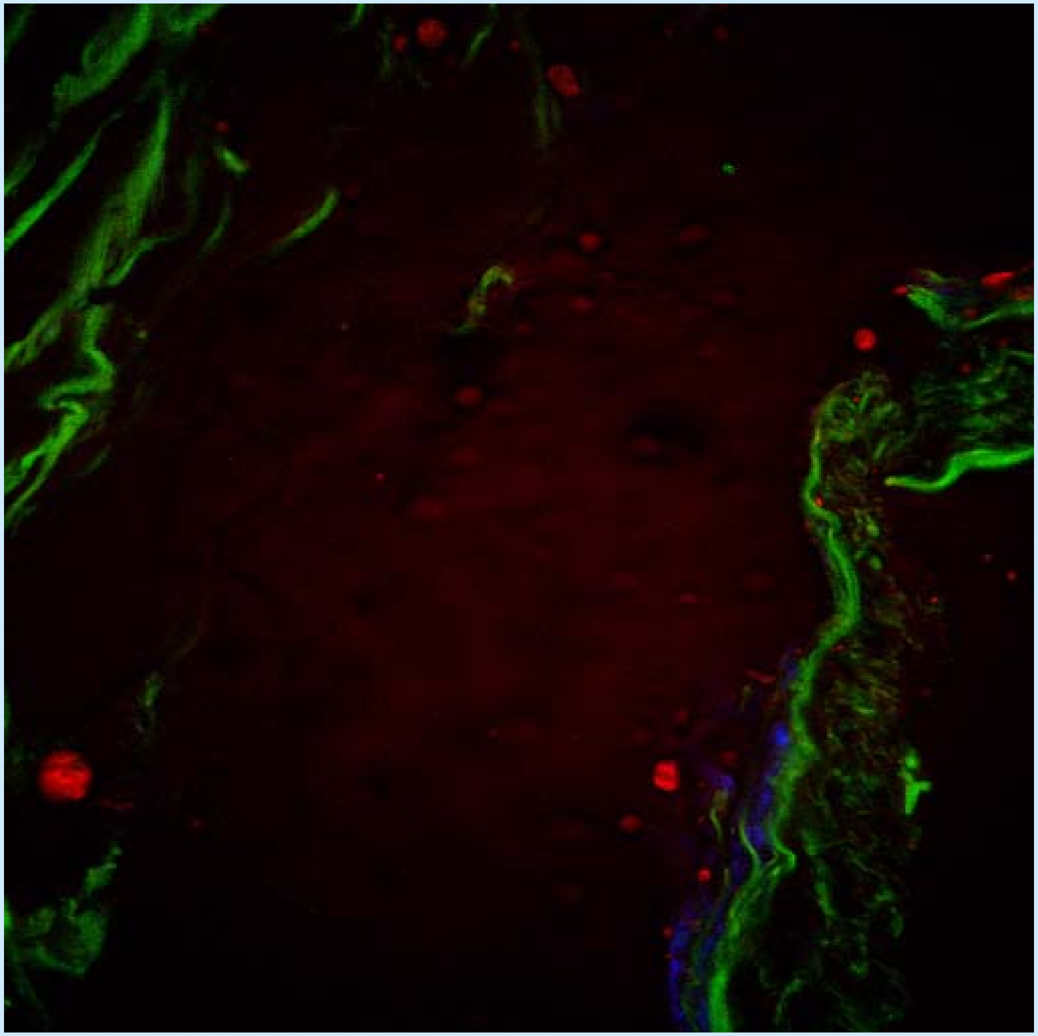
Biomedical Applications of Multimodal CARS

Atherosclerosis

Real Time Z-scan
movie: 50μm



Red is CARS
(2845 cm^{-1})
(lipids droplets)

Blue is SHG
(collagen)

Green is epi TPF
(elastin)

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Geoscience Applications?
Fluid Inclusions?

- Fluid inclusions provides information about the conditions existing during formation

- Host matrices can be amber, ice, quartz, calcite, feldspar, and many other “rock” materials.

- Formation occur at various conditions:
  Temperature can be 10^2 - 10^3 C
  Pressure can be > 10^4 atm

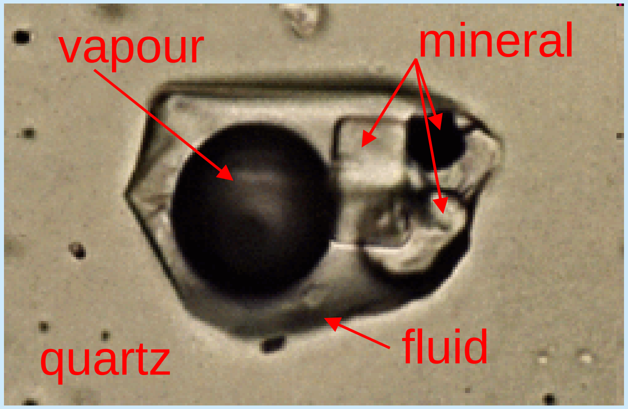
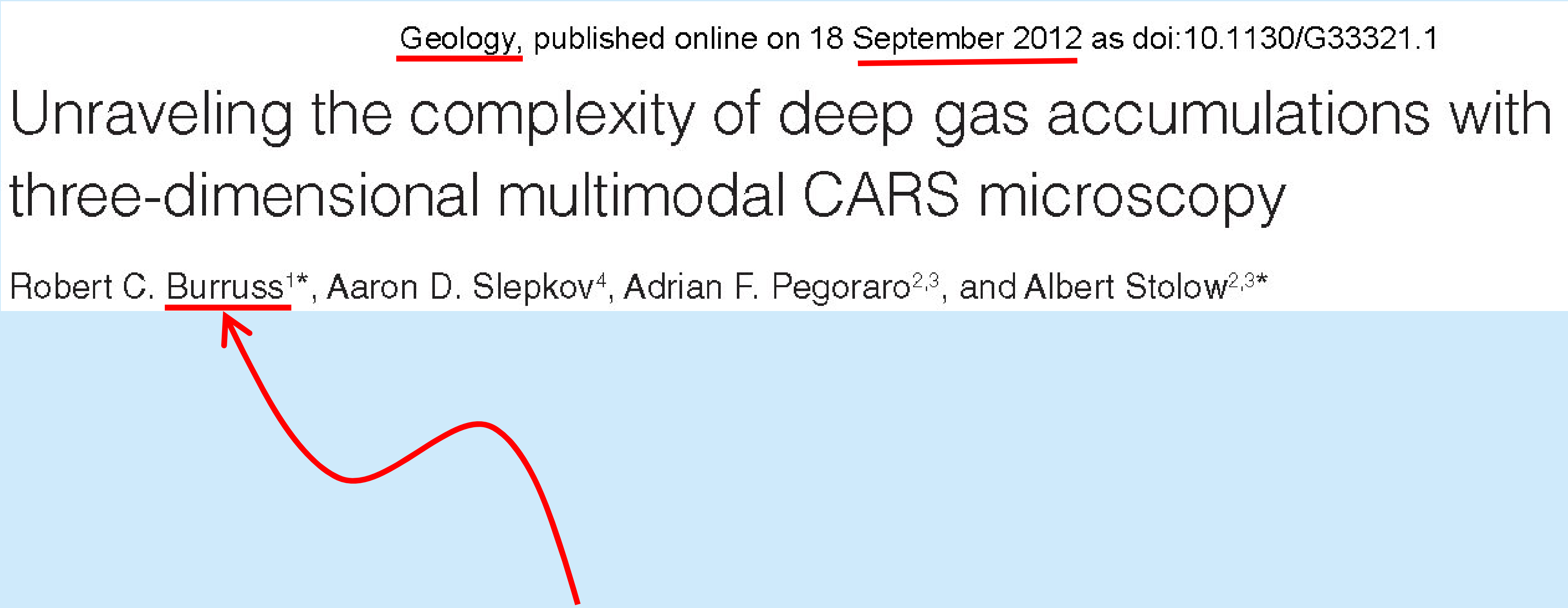


Image: wikipedia

Yes we can.

First Application of CARS Microscopy to Geosciences



Geology, published online on 18 September 2012 as doi:10.1130/G33321.1

Unraveling the complexity of deep gas accumulations with three-dimensional multimodal CARS microscopy

Robert C. Burruss[1]*, Aaron D. Slepkov[4], Adrian F. Pegoraro[2,3], and Albert Stolow[2,3]*

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Multimodal CARS Microscopy of Rocks

Using CARS we demonstrate four “firsts” in Geology:

1. CARS allows for high-throughput screening of fluid distribution in 3D irrespective of fluorescence.

2. CARS + TPEF confirm previously controversial presence of higher organic matter in deep-mantle samples

3. CARS Spectro-microscopy of methane-rich crude oil inclusions in a highly-fluorescing environment

4. CARS + SHG correlates inclusion distribution and migration with hidden crystallographic history of host mineral.

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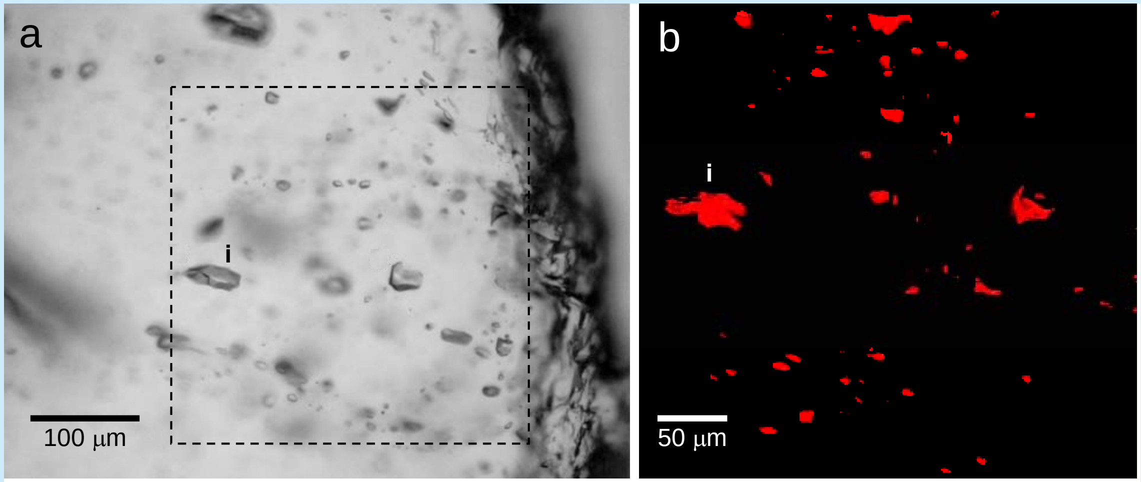
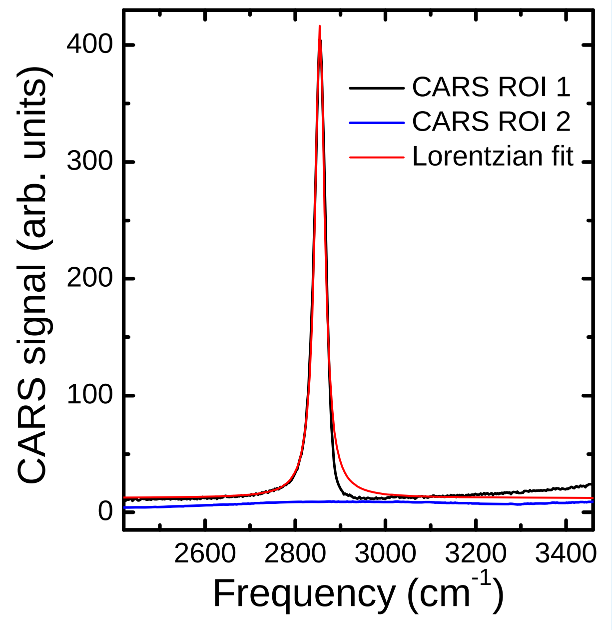
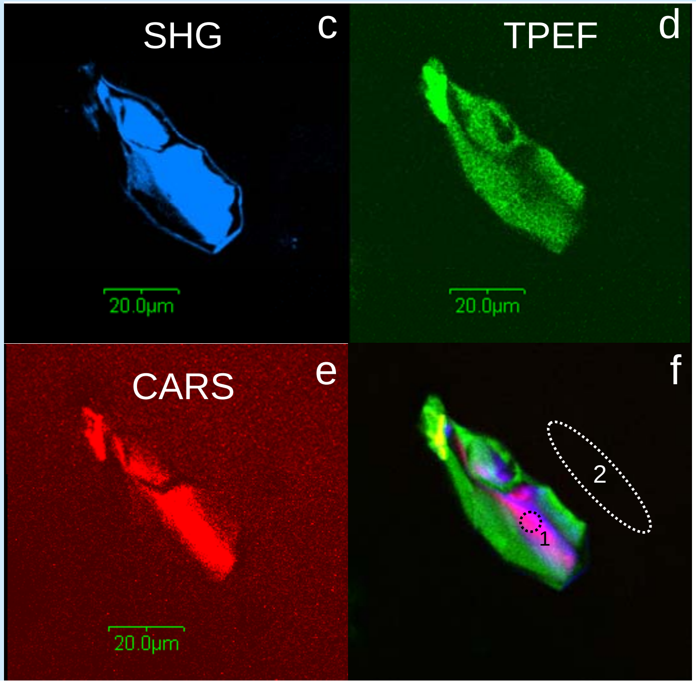
1. High Throughput 3D screening of **Shale Gas**

Methane inclusions in quartz

Paleozoic sedimentary rock
(250 to 550 million years old)







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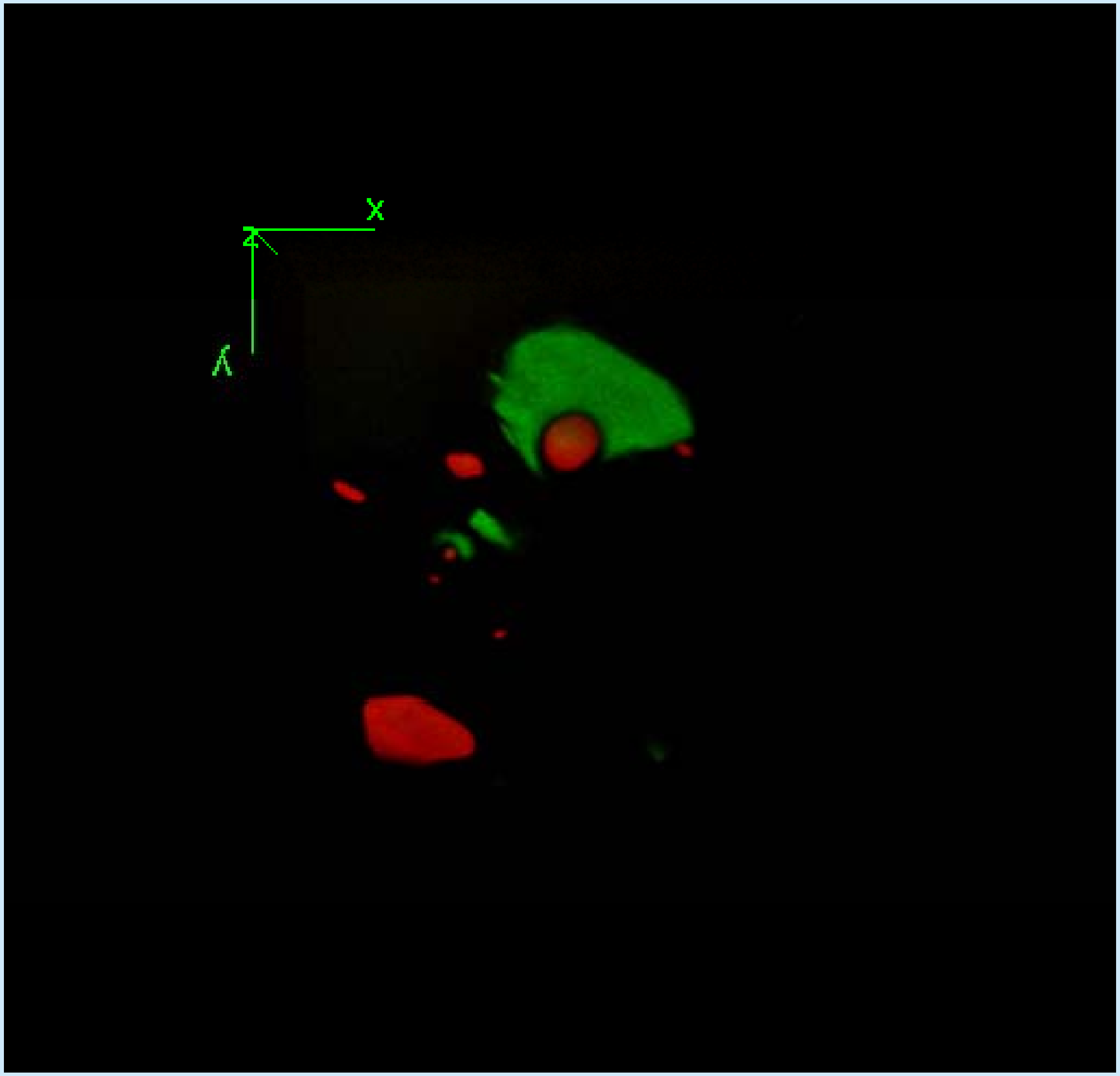
1. High Throughput 3D screening of **Shale Gas**

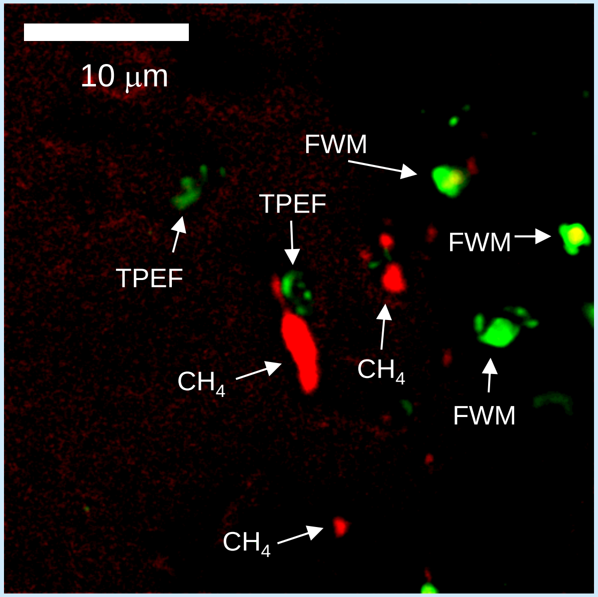


Red: Methane imaged at 2905 cm^{-1}
Green: Water imaged at 3080 cm^{-1}

free-hand rotation
(78 x 78 x 44 μm)

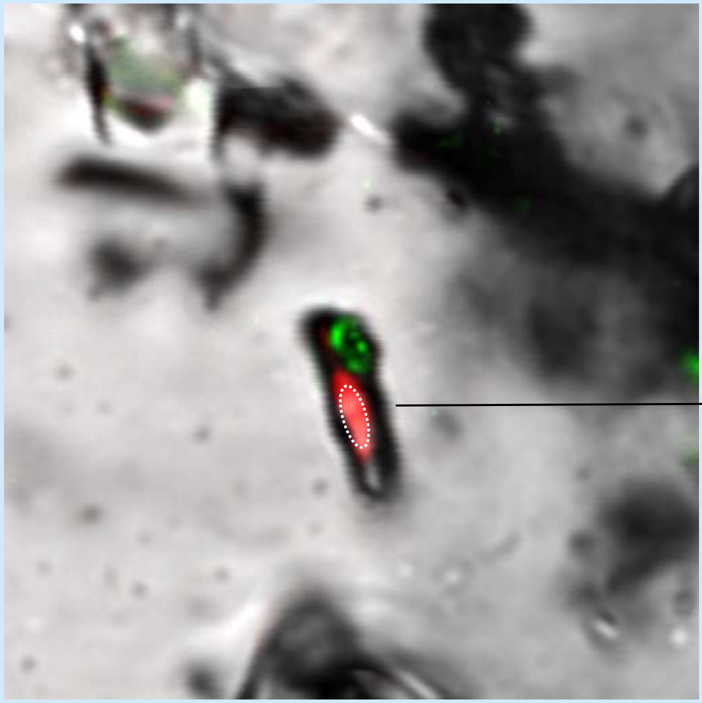
2-phase and 1-phase inclusions are seen in same FOV

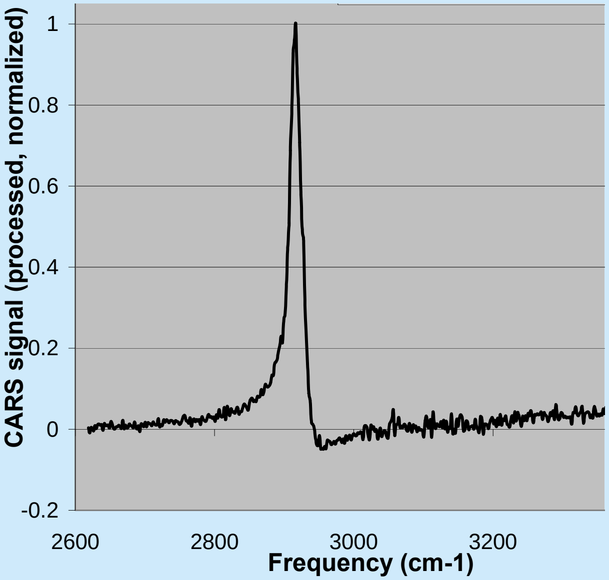
2. Organic Matter co-localized with methane



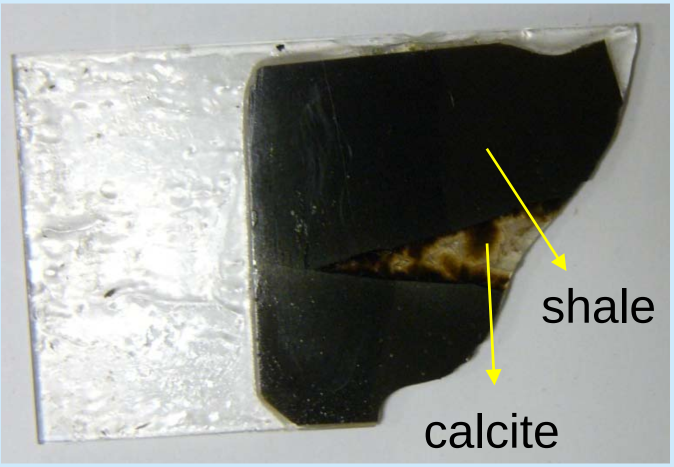
Methane and higher hydrocarbons

- Basaltic ocean crust (feldspar plagioclase)
- Source of the carbon is unknown!
- **Fluorescence from higher hydrocarbons is an important new observation**



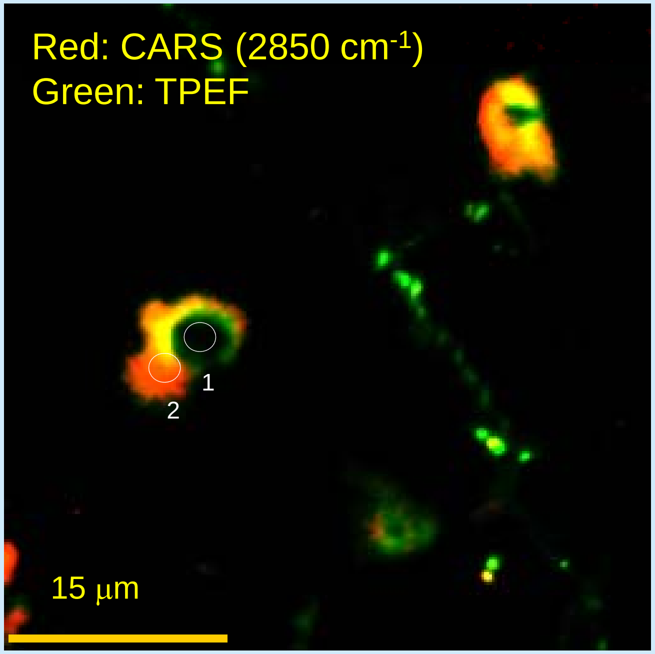


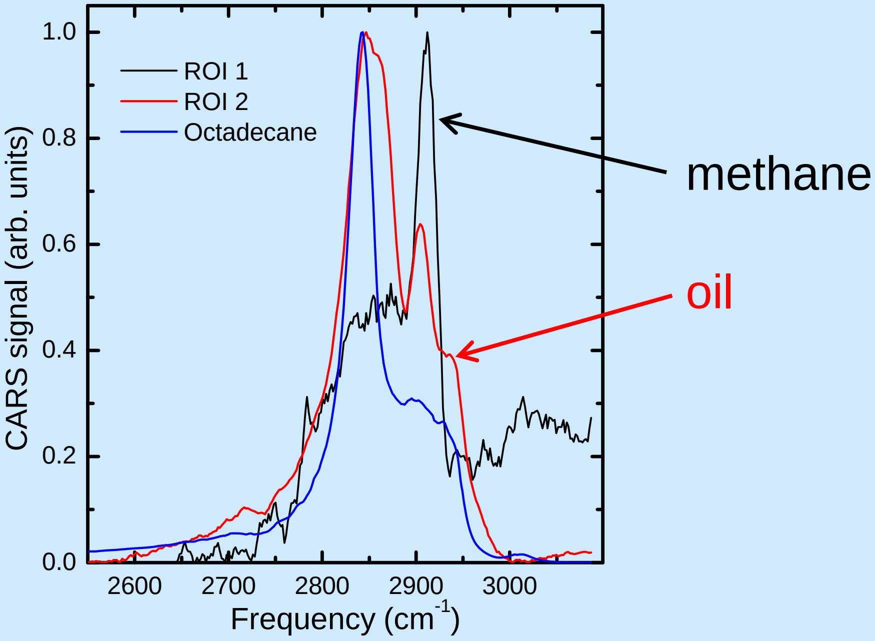
3. Micro-spectroscopy of crude oil in rock



Crude oil fluid inclusions (hydrocarbons + CH_4)

- Calcite grain in black shale fracture
- CARS detect crude oil & methane bubble
- Conventional Raman overwhelmed by large background fluorescence





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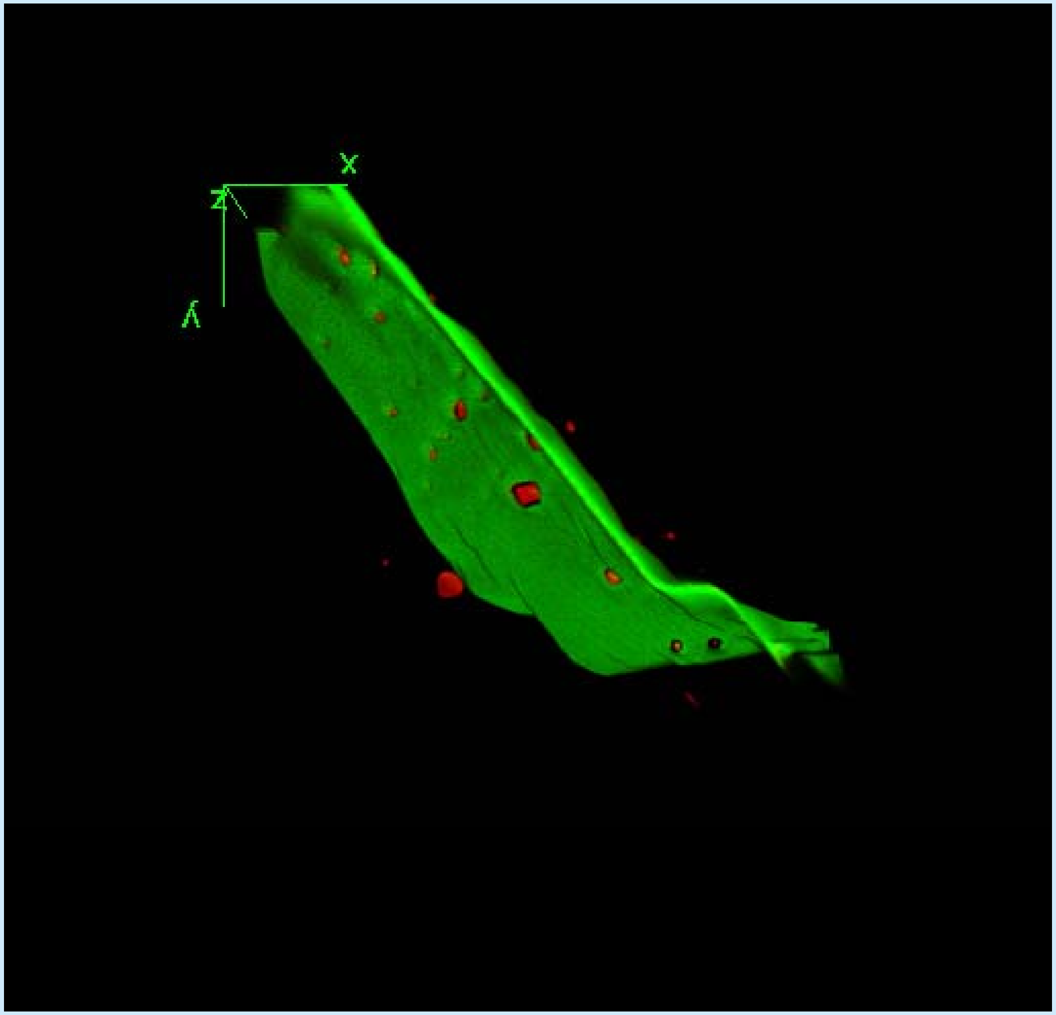
4. SHG
Grain Boundaries and Fluid History

Red: Methane imaged at 2905 cm^{-1}
Green: SHG (grain boundary)

free-hand rotation
(175 x 175 x 60 μm)

A veil of methane-rich inclusions formed around a grain boundary in shale



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GeoCARS Summary

CARS+SHG+TPEF provides unique information on:
shape, volume, distribution, composition, history
of fluid inclusions in the earth.

- The applications of multimodal CARS microscopy extend well beyond fluid inclusions: petroleum science, mineralogy, paleobiology, mining etc.

Geology questions? Ask Bob Burruss!

Acknowledgements

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