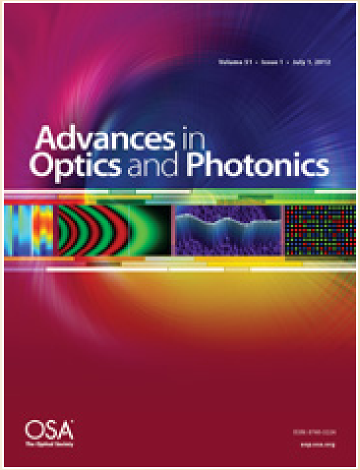
What is Next for Brillouin Spectroscopy in Biology and Medicine?

Vladislav Yakovlev
February 7, 2017

BIOMEDICAL ENGINEERING
TEXAS A&M UNIVERSITY

Outline

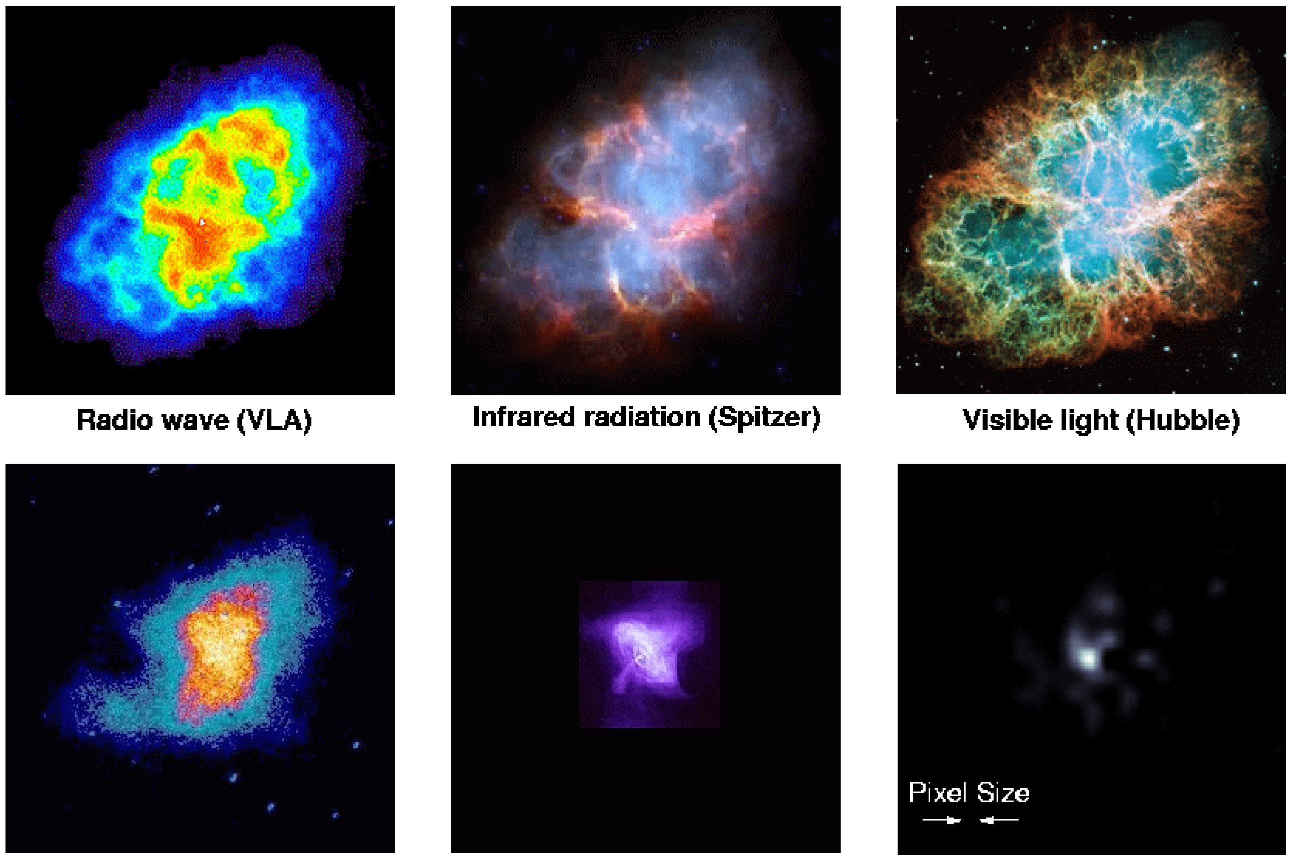
- Introduction
- Motivation
- Mechanobiology
  - Important biomedical questions
  - Technology gaps
- Brillouin spectroscopy
  - History of Brillouin spectroscopy
  - Recent renaissance
- Applications
- Advanced instrumentation
- Summary and Future outlook



Zh. Meng, et al, *Advances in Optics and Photonics* **8**(2), 300- 327 (2016).

Seeing things in a new light

Crab Nebula: Remnant of an Exploded Star (Supernova)

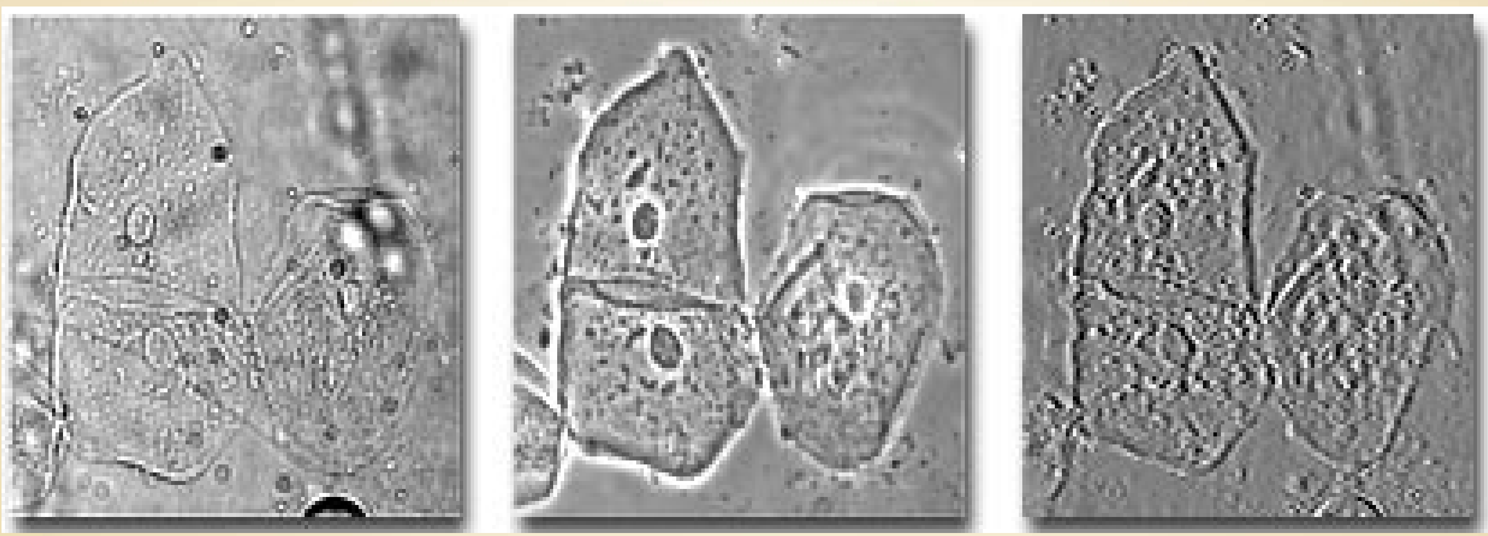


Radio wave (VLA) | Infrared radiation (Spitzer) | Visible light (Hubble)

Ultraviolet radiation (Astro–1) | Low–energy X–ray (Chandra) | High–energy X–ray (HEFT) *** 15 min exposure ***

Seeing life in a new light

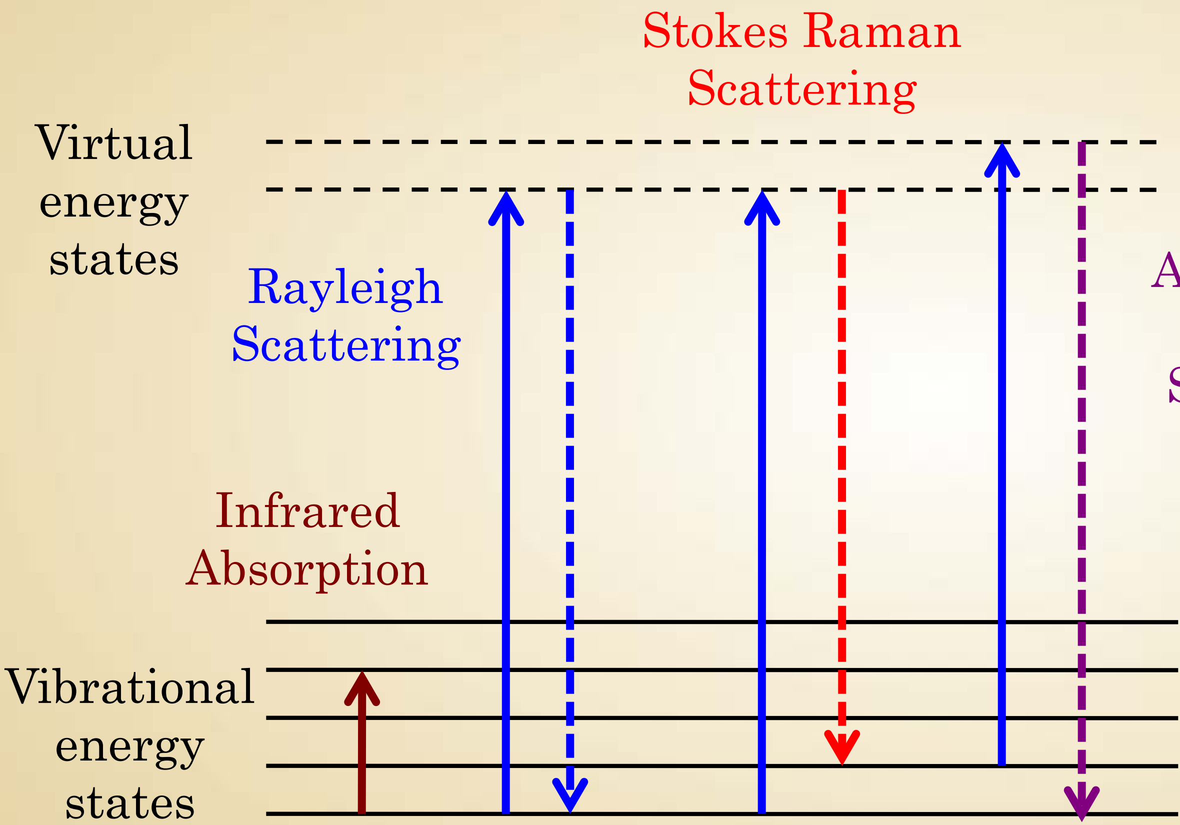
Optical range of the spectrum provides a better contrast, but extracting the necessary chemical, physical, and physiological information is not always straightforward. **New contrast mechanisms are needed**.

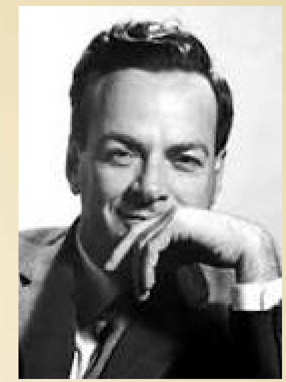


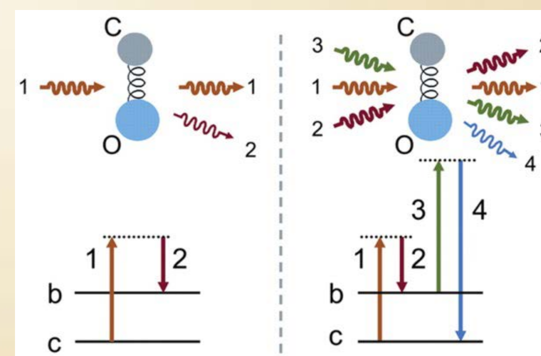
Transmission | Phase Contrast | Hoffman Modulation Contrast

Vibrational spectroscopy

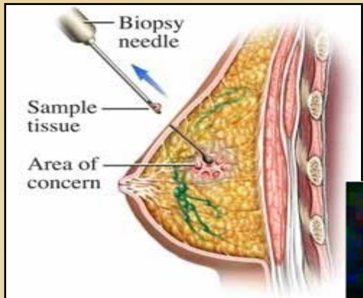
"Everything that living things do …
…can be reduced to the wiggling and jiggling of atoms." R. Feynman



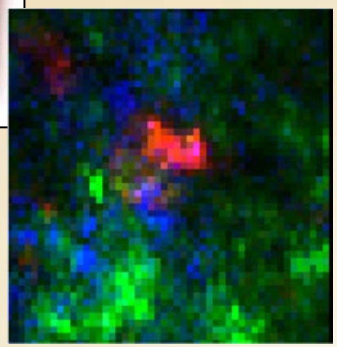




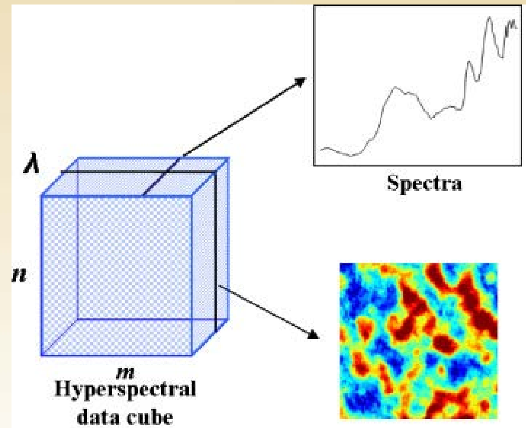
Chemical imaging

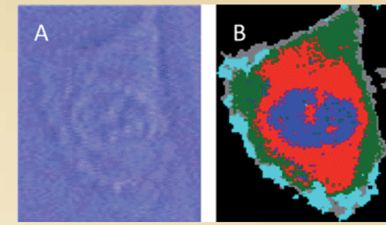


Calcium hydroxyapatite
Calcium oxalate Skin



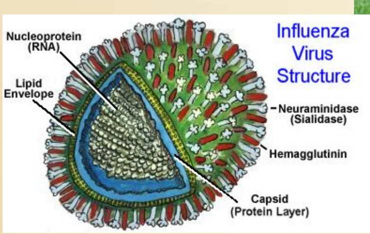
Tissue level



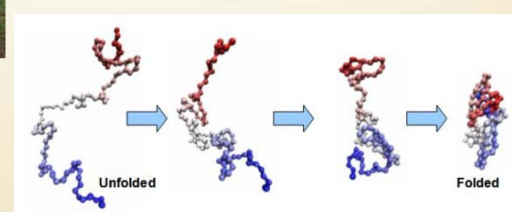


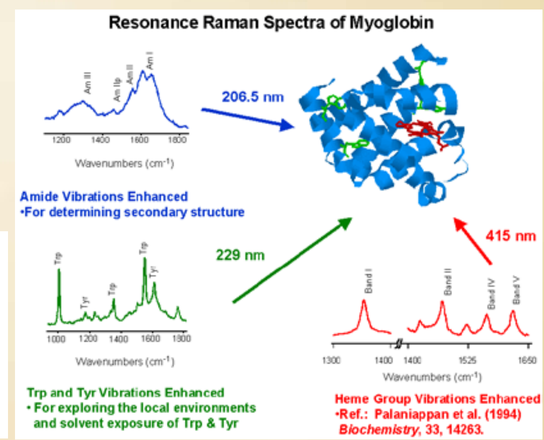
Sub-cellular level

Nanoscopic optical imaging



Molecular-level (protein folding, protein-drug, etc.) interactions





Biomechanics

Biomechanics: the study of the structure and function of biological systems. Usually, it studies responses of the living organisms under external mechanical perturbations. **Mechanobiology** = Biomechanics on a **microscale**.

Elastic modulus: the mathematical description of mechanical properties.

$$\lambda \stackrel{def}{=} \frac{stress}{strain}$$

How to measure elastic properties on a microscopic scale?

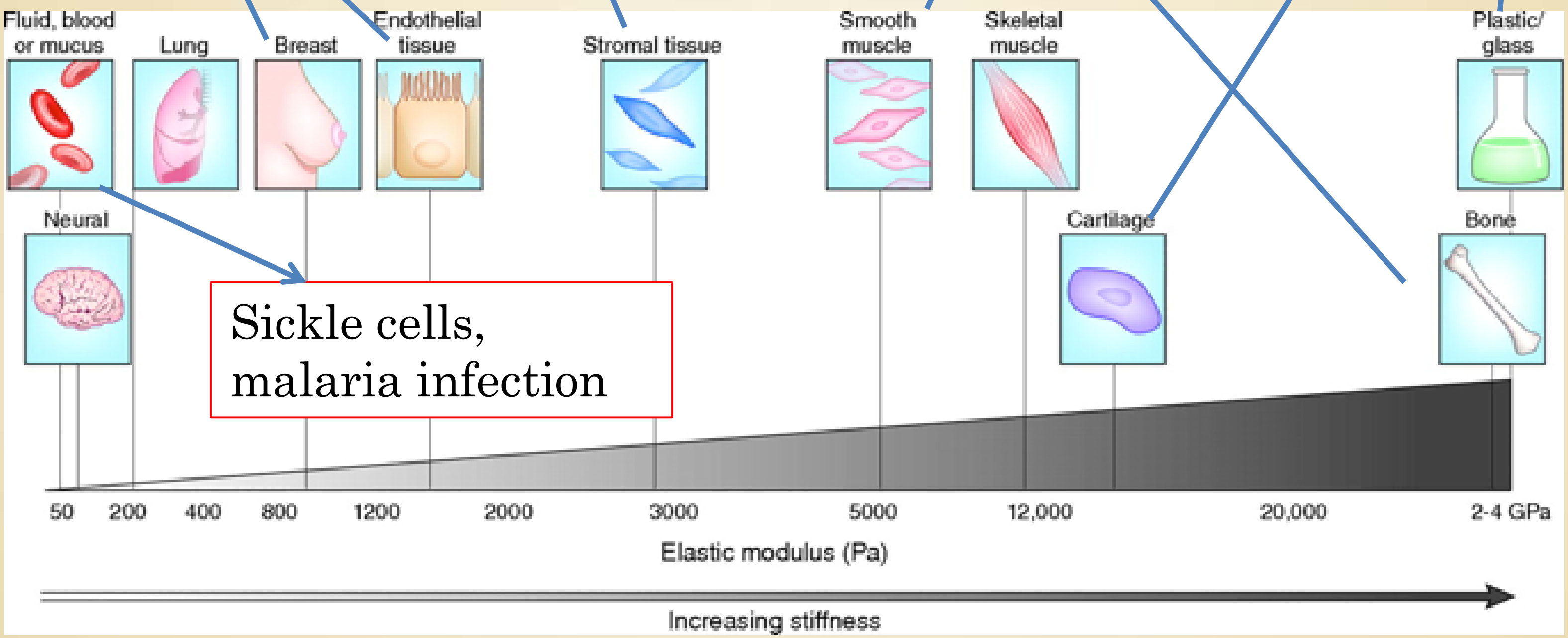
Who cares?

Cancer diagnosis and treatment

Cardiovascular diseases characterization

Kinematics studies
Sport biomechanics

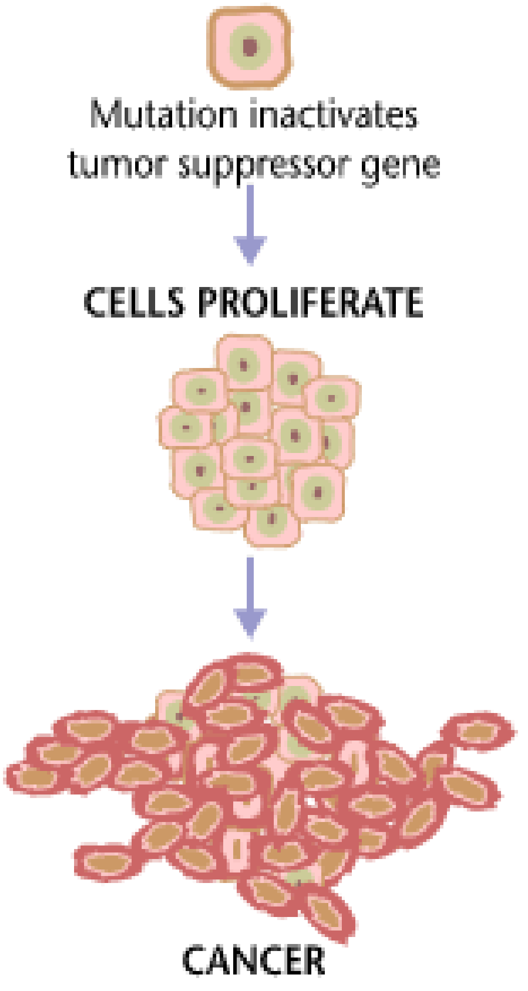
Tissue engineering
Biomaterials



Sickle cells,
malaria infection

Cox TR, Erler JT. *Disease Models & Mechanisms.* 2011; 4(2):165–178.

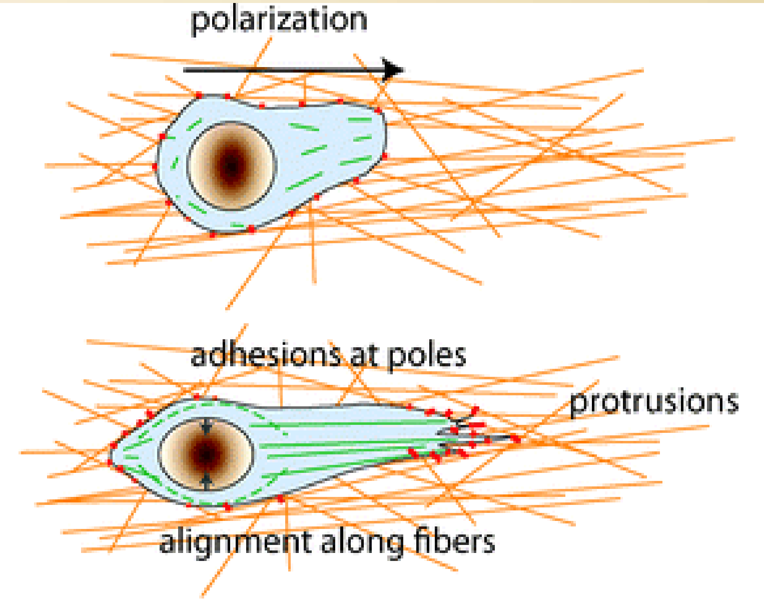
Carcinogenesis



Cells are growing in an environment, which, in its turn, affects physical properties of those cells.

How does the local stiffness influence cells' development?

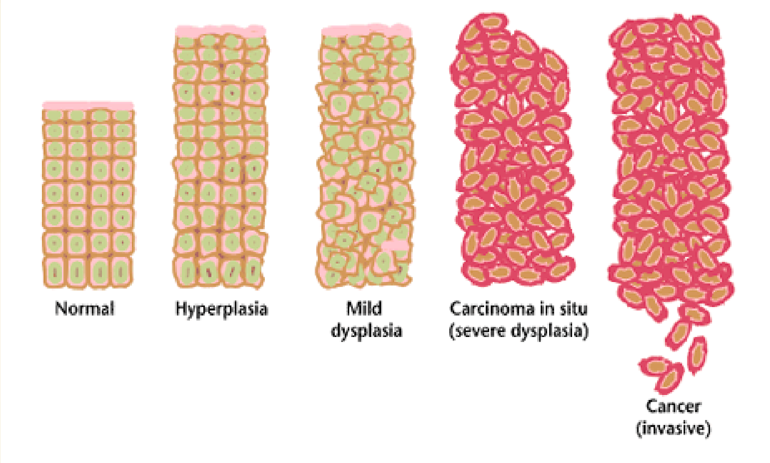
Cellular microenvironment (extra cellular matrix; ECM) is important

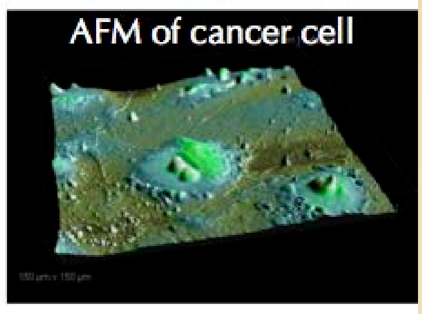


P. M. Comoglio, L. Trusolino, Cancer: the matrix is now in control. *Nat Med.* **11**, 1156–1159 (2005).

Macroscopic ≠ Microscopic

Living organisms show different mechanical properties on different **spatial scales**. This is mainly due to **structural** differences.



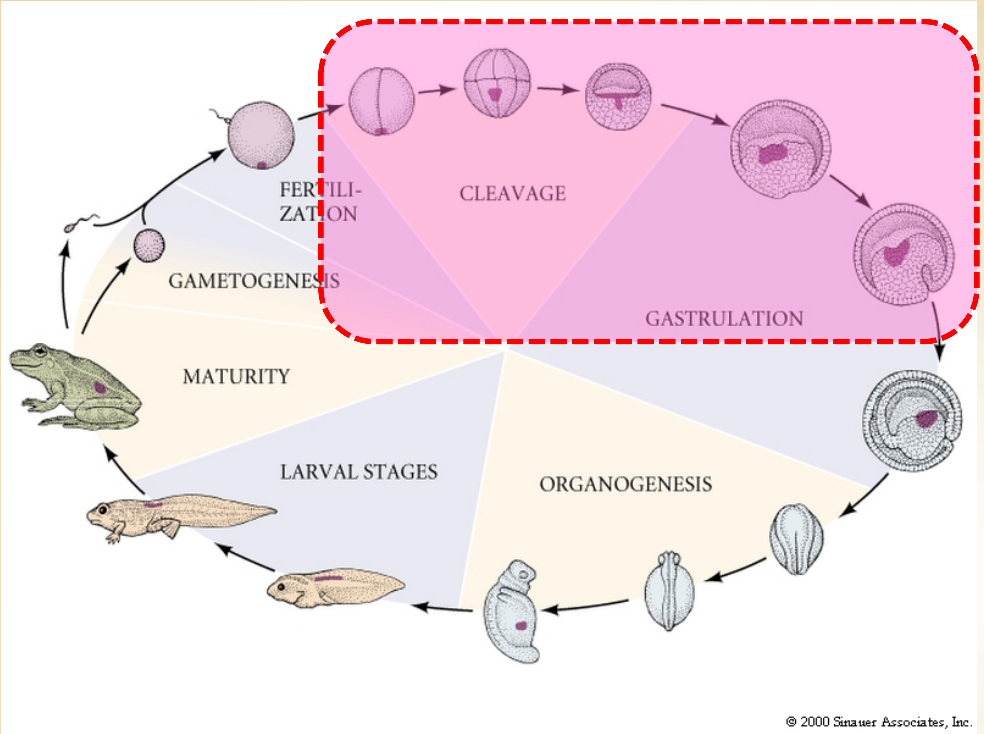


Microscopic:
Cancer cells are **softer**

Macroscopic:
Cancerous tissues are **stiffer**

Left: Nancy Shute, *Scientific American*, May, 2011
Middle: en.wikipedia.org
Right: http://www.dme-spm.dk/anwendungen_topographie.html

Morphogenesis



Morphogenesis, the biological process of developing shape, is fundamentally a biomechanical process. Cells bring about changes in embryonic form by generating patterned forces and by differentiating the tissue mechanical properties that harness these forces in specific ways.

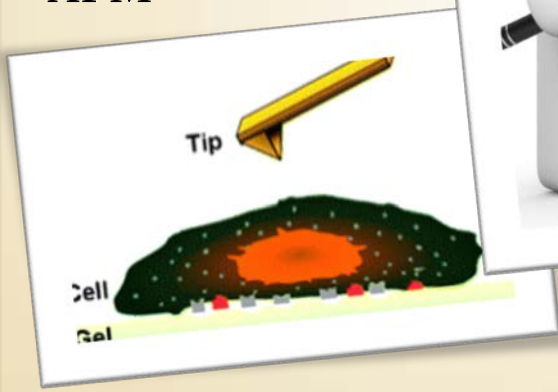
Microscopic variations of viscoelastic properties drive the process of cells and tissues differentiation

C. J. Miller, L. A. Davidson, "The interplay between cell signaling and mechanics in developmental processes," *Nature Reviews Genetics* **14**, 733–744 (2013).

Technology gap

Speed

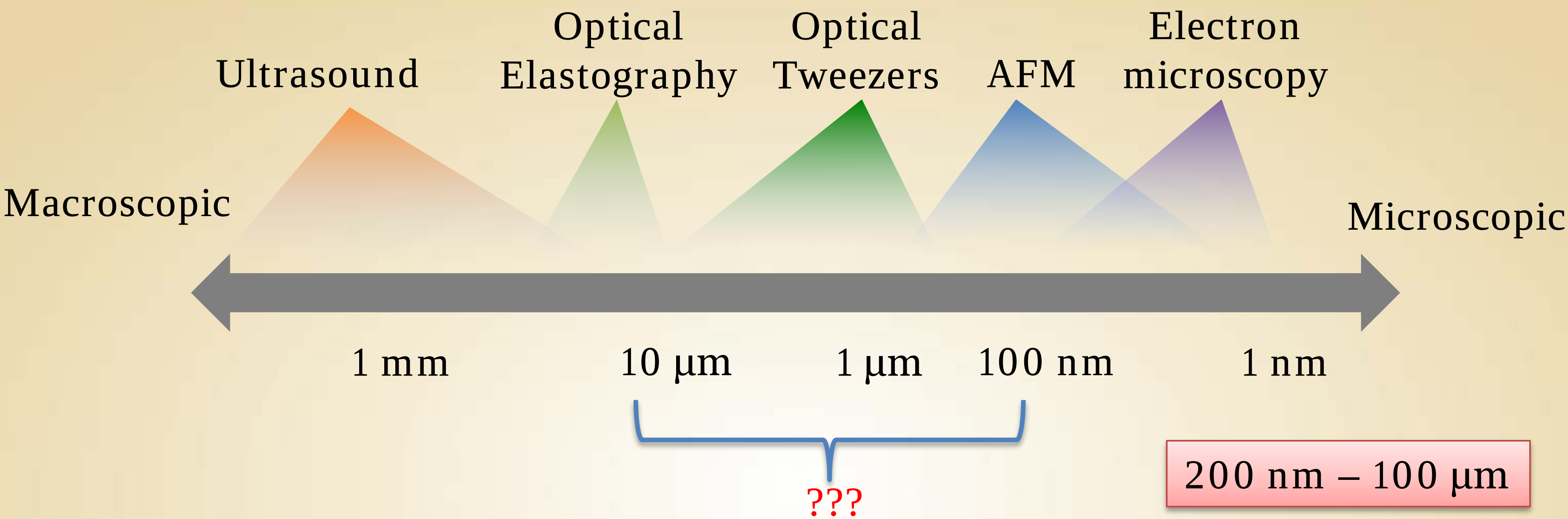
Optical tweezers
AFM



Ultrasound imaging

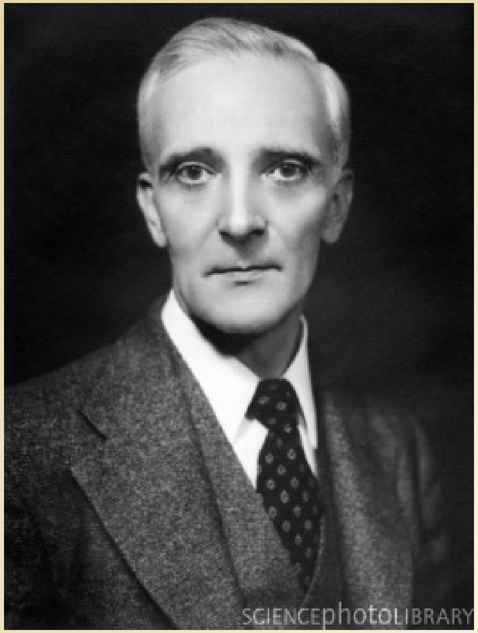
Scale

Needs assessment



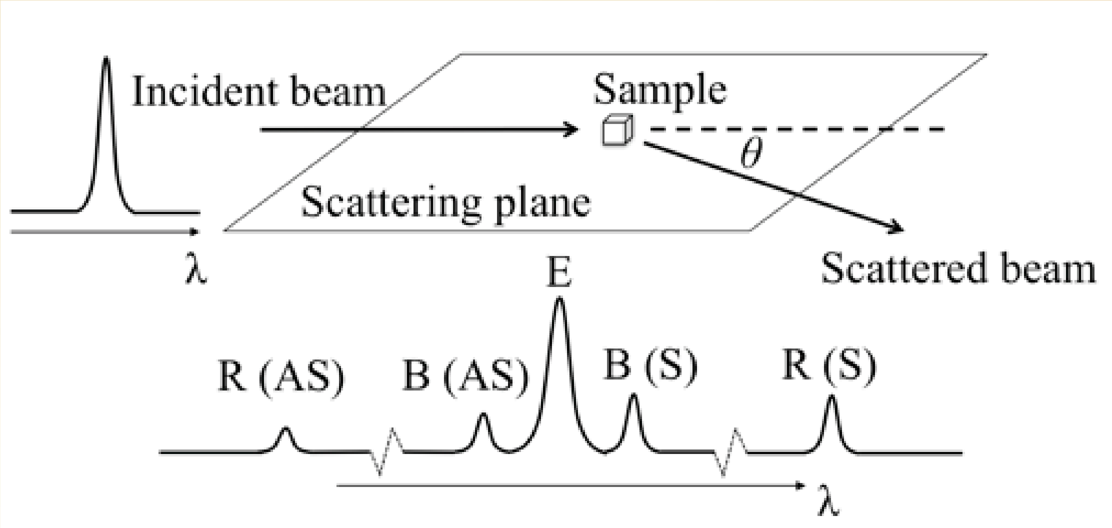
Fast:	**~1-10 s** for a 100 x 100 pixel image.
Non-contact and non-invasive:	No break in skin or mucosa, no damage.
Mechanically-specific:	Probing **elastic modulus**.
Adequate chemical contrast:	Information for lipid membrane, protein, etc.
Microscopic spatial resolution:	**Sub-micron resolution** with > **100 μm^2** FOV.

Brillouin spectroscopy



Leon Brillouin: 1922

Predicted inelastic light scattering off the induced acoustic waves (essentially, stimulated scattering)

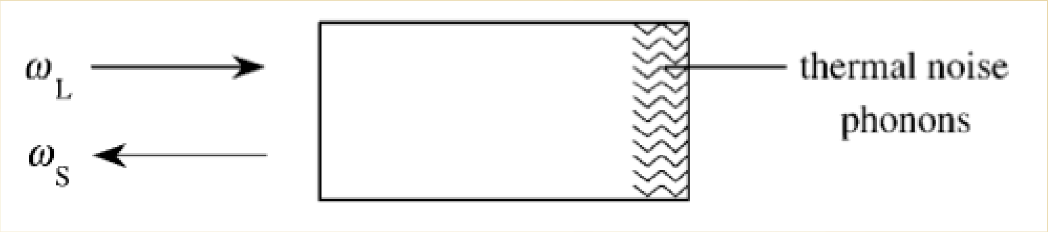


Inelastic light scatterings, including Brillouin scattering and Raman scattering, are usually associated with **specific characteristic information** for the sample. Brillouin spectroscopy is associated with acoustic vibrations of the media,

Mandelshtam-Brillouin spectroscopy

Leonid Mandelshtam

First reported: 1918
First published: 1926

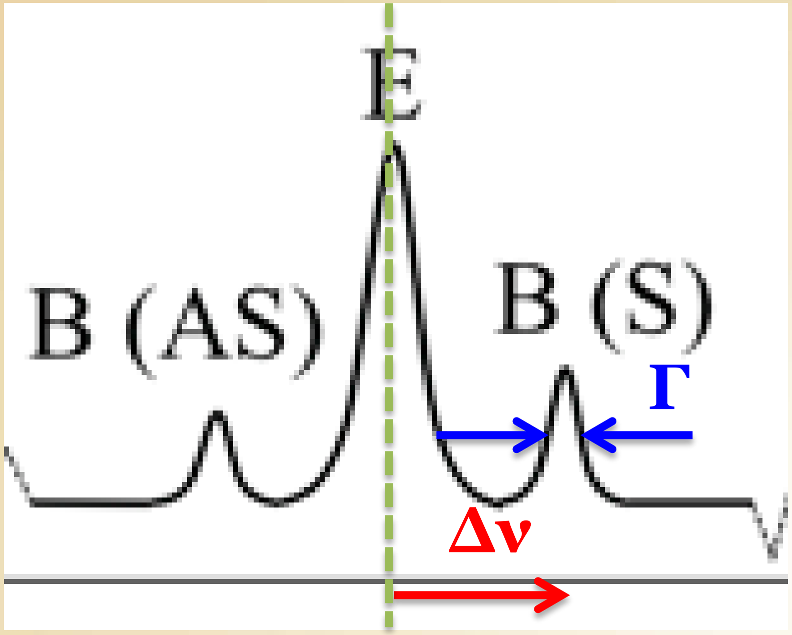


Inelastic scattering can be caused by density (entropy) fluctuation of the media, i.e. incident light will experience spontaneous inelastic scattering.

$$\Delta\nu = 2\nu_0 \frac{v}{c/n} \sin\left(\frac{\theta}{2}\right) = 2\nu_0 \frac{n}{c} \sqrt{\frac{K}{\rho}} \sin\left(\frac{\theta}{2}\right)$$

$\Delta\nu$:	Brillouin shift
ν_0:	optical frequency
c:	speed of light in vacuum
n:	refractive index of the medium
θ:	Scattering angle
***v*:**	**sound speed**
***K*:**	**longitudinal modulus**

Brillouin elastic modulus



$$G^* = G' + i\,G'' =$$
$$= \rho v^2 + i\,(2\rho v^3 \alpha),$$

Velocity which is proportional to the **frequency shift**

Attenuation which is proportional to the **line-width**)

Viscoelastic modulus is the function of frequency of the acoustic wave, and, in Brillouin spectroscopy, we are dealing with GHz waves, which do not propagate far.

Applications of Brillouin spectroscopy

nature COMMUNICATIONS

ARTICLE

Received 5 Nov 2013 | Accepted 9 Jan 2014 | Published 30 Jan 2014 DOI: 10.1038/ncomms4225 OPEN

Photonic Aharonov-Bohm effect in photon-phonon interactions

Enbang Li[1,2,3], Benjamin J. Eggleton[3], Kejie Fang[4,5] & Shanhui Fan[6]

nature nanotechnology LETTERS

PUBLISHED ONLINE: 28 AUGUST 2011 | DOI: 10.1038/NNANO.2011.140

Direct observation of a propagating spin wave induced by spin-transfer torque

M. Madami[1†*], S. Bonetti[2†*], G. Consolo[3,4], S. Tacchi[1], G. Carlotti[1,5], G. Gubbiotti[1,6], F. B. Mancoff[7], M. A. Yar[8] and J. Åkerman[2,9]

nature physics ARTICLES

PUBLISHED ONLINE: 26 JANUARY 2015 | DOI: 10.1038/NPHYS3236

Non-reciprocal Brillouin scattering induced transparency

JunHwan Kim[1], Mark C. Kuzyk[2], Kewen Han[1], Hailin Wang[2] and Gaurav Bahl[1*]

NATURE | Vol 442 | 3 August 2006 BRIEF COMMUNICATIONS ARISING

GLASS BEHAVIOUR

Poisson's ratio and liquid's fragility

Arising from: V. N. Novikov & A. P. Sokolov *Nature* **431**, 961–963 (2004)

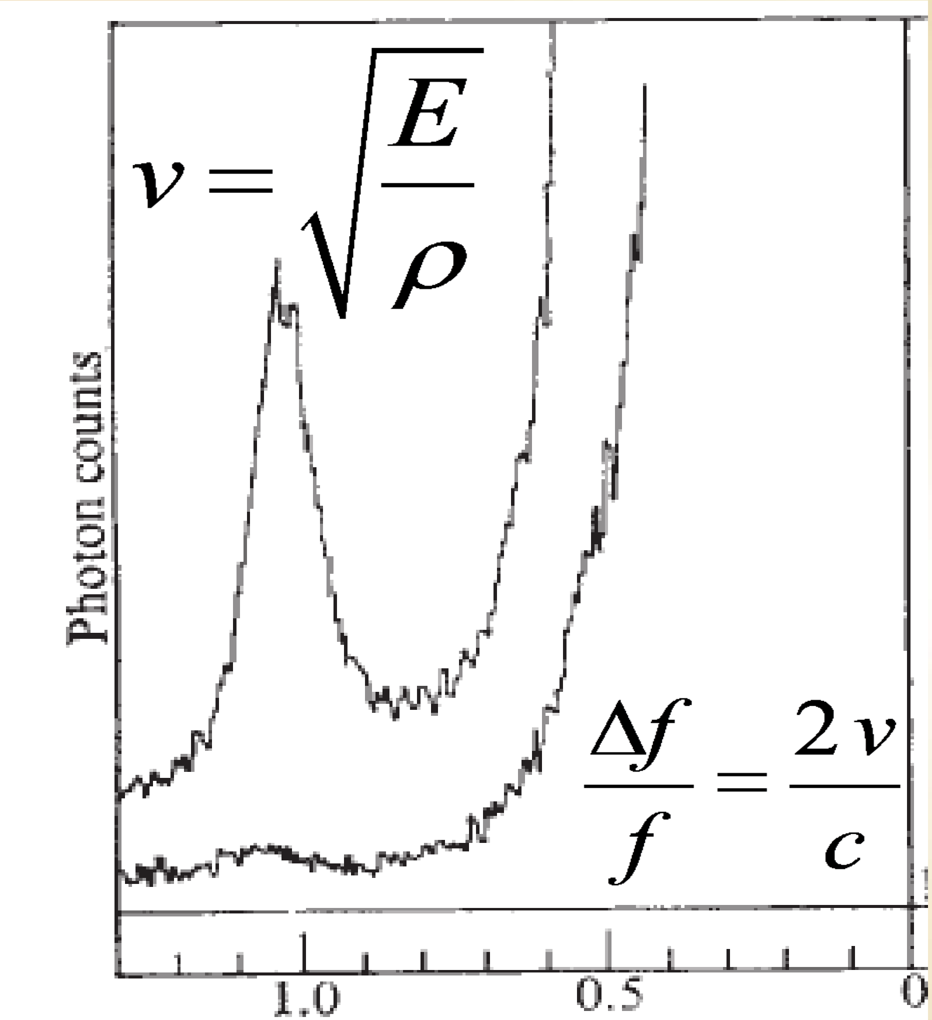
ARTICLES nature materials

PUBLISHED ONLINE: 27 JANUARY 2013 | DOI: 10.1038/NMAT3549

Non-invasive determination of the complete elastic moduli of spider silks

Kristie J. Koski[1], Paul Akhenblit[2], Keri McKiernan[2] and Jeffery L. Yarger[2*]

First biomedical applications



Nature **267**, 285 - 287 (1977)

Phonons and the elastic moduli of collagen and muscle

R. Harley, D. James, A. Miller, and J. W. White

Nature **284**, 489 - 491 (1980)

Brillouin scattering, density and elastic properties of the lens and cornea of the eye

J. M. Vaughan and J. T. Randall

Brillouin scattering

This review was completed in November 1974.

"***Brillouin spectroscopy has not been as useful*** in the study of polymers as the other light scattering methods have. Nor has it, as far as the author is aware, been used at all in the study of biological molecules. ***The reason for this is largely experimental***. Because of the problems of large random scattering, ***samples have been chosen for their excellent optical quality rather than for their intrinsic interest*** or technological importance. "

Brillouin and Raman scattering

	Brillouin	Raman
Typical shift	~ 1 – 10 GHz (0.03 – 0.3 cm^{-1})	100 – 4000 cm^{-1}
In wavelength	~ 1 – 10 pm	~ 10 – 150 nm (for 532 nm source)
Source of contrast	Sound velocity	Chemical bonds

Need a narrow band laser

Need a high-resolution spectrometer

Need a narrow band notch filter for elastic scattering

Narrow band laser

Linewidth **1 MHz or less**

Stable over a long time

Low ASE background

Our solution:

1064 nm or 1560 nm diode laser
1 MHz linewidth
Tunable over 30 GHz

→ Fiber amplifier → SHG

Frequency standard:
molecular or atomic
absorber

Output: 200 mW; 1MHz linewidth, TEM_{00} mode, low-noise, <1 MHz frequency drift

High resolution spectrometry

Chlorbenzene . .	0·047	0·042

In Fig. 1 microphotometer curves of spectrograms

photographed simultaneously side by side on the same photographic plate. It was found that the central component of benzene coincides with the single line

iron are as yet incomplete.

The factors which determine the results are temperature and time of treatment, pressure of hydrogen, and thickness of the metal. These are the factors which enter into Richardson's equation for diffusion and absorption of hydrogen by metals.[15] If the large magnetic improvements are dependent upon the absorption of an optimum quantity of hydrogen, it should be possible to obtain the same results by any suitable combination of the factors satisfying Richardson's equation. Experiments indicate that this is so.

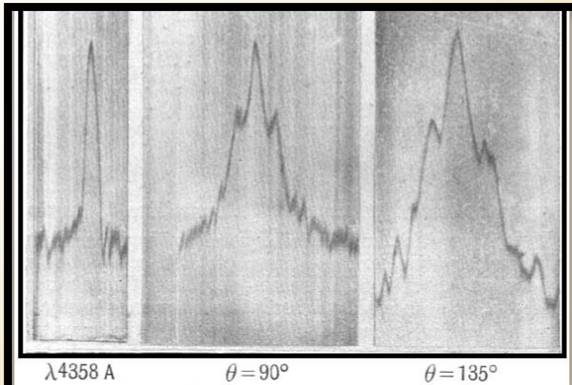
P. P. Cioffi.

Bell Telephone Laboratories, New York, June 16.

the components of hyperfine structure of line 4358 A. did not hinder the observations.)

These results were obtained last summer but their interpretation remained for some time not clear. Some considerations and further experiments (which will be published elsewhere) have led me to the conclusion that it is scarcely possible to regard the displaced components as Raman lines due to the rotational quanta.

Another explanation of the observed splitting of the scattered light is that this splitting is due to acoustic oscillations like those used by P. Debye (*Ann. d. Phys.*, **39**, p. 789; 1912) for explaining the



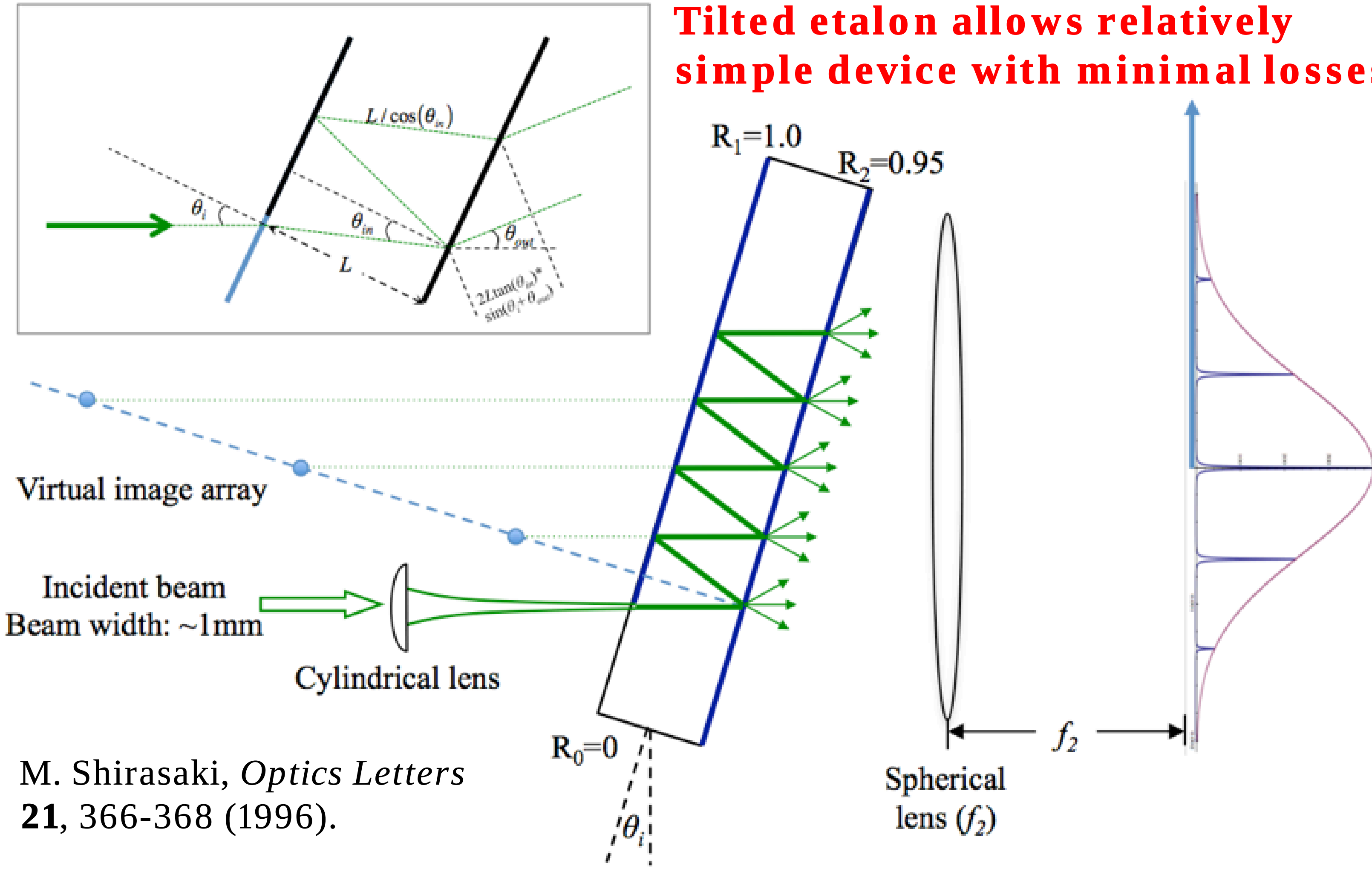
Evgeniy Gross

Used a system of etalons
First reported: 1930

Interferometer is the proper tool for looking at Brillouin spectra

Virtual Image Phase Array (VIPA)

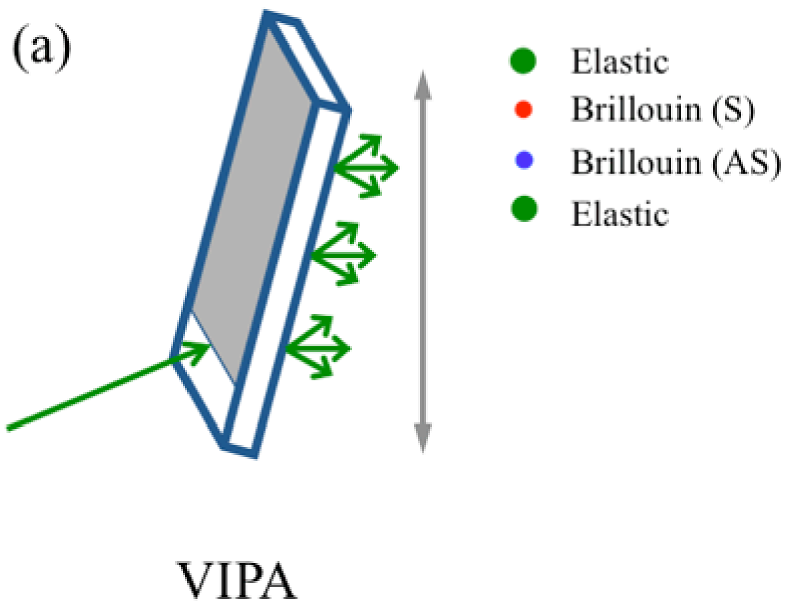
Tilted etalon allows relatively simple device with minimal losses



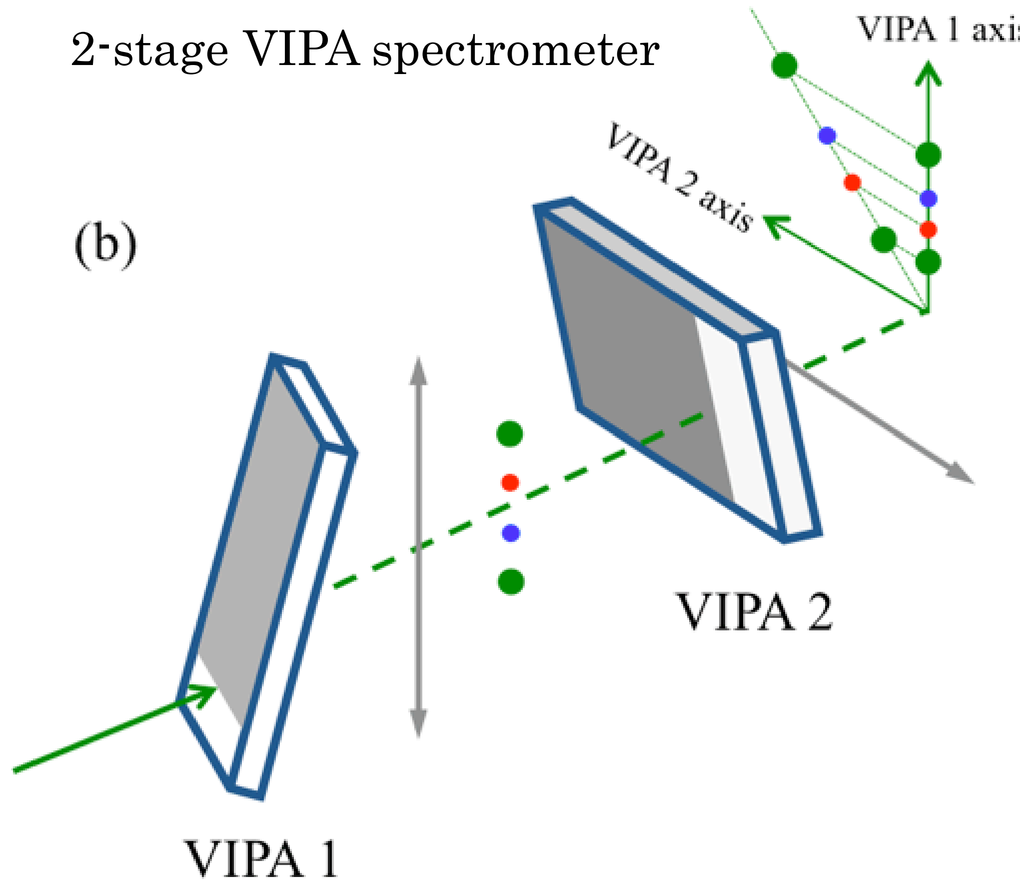
M. Shirasaki, *Optics Letters* **21**, 366-368 (1996).

VIPA spectrometer

1-stage VIPA spectrometer



2-stage VIPA spectrometer



A. Vega, C. Lin, and A. M. Weiner, *Applied Optics* **42**, 4152-4155 (2003).

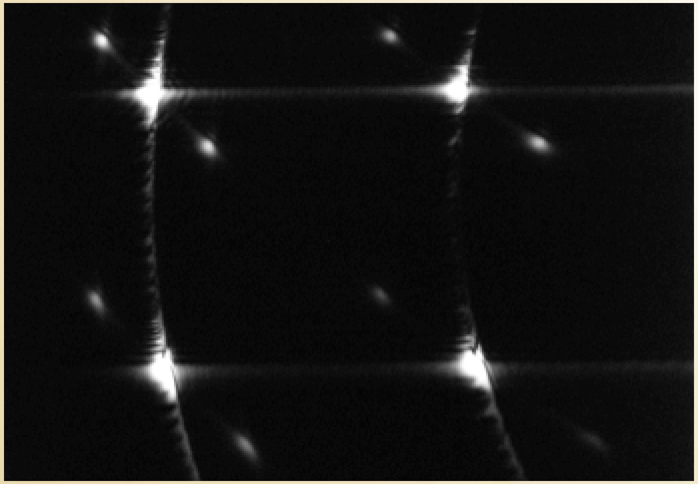
G. Scarcelli, S. H. Yun, *Optics Express* **19**, 10913-10922 (2011).

Notch filter

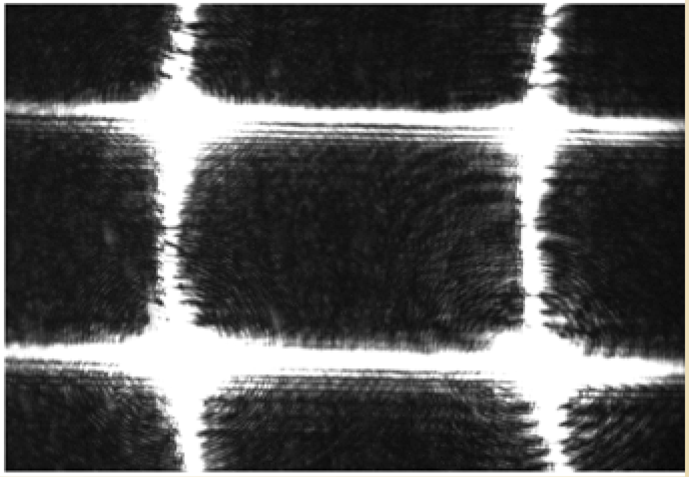
The Brillouin scattering is usually contaminated by the Rayleigh and/ or Mie scattering.

For biological samples, elastic scattering is extremely strong. The CCD blooming effect makes it difficult to quantify the Brillouin shift.

Pure DMSO DMSO+ coffee cream

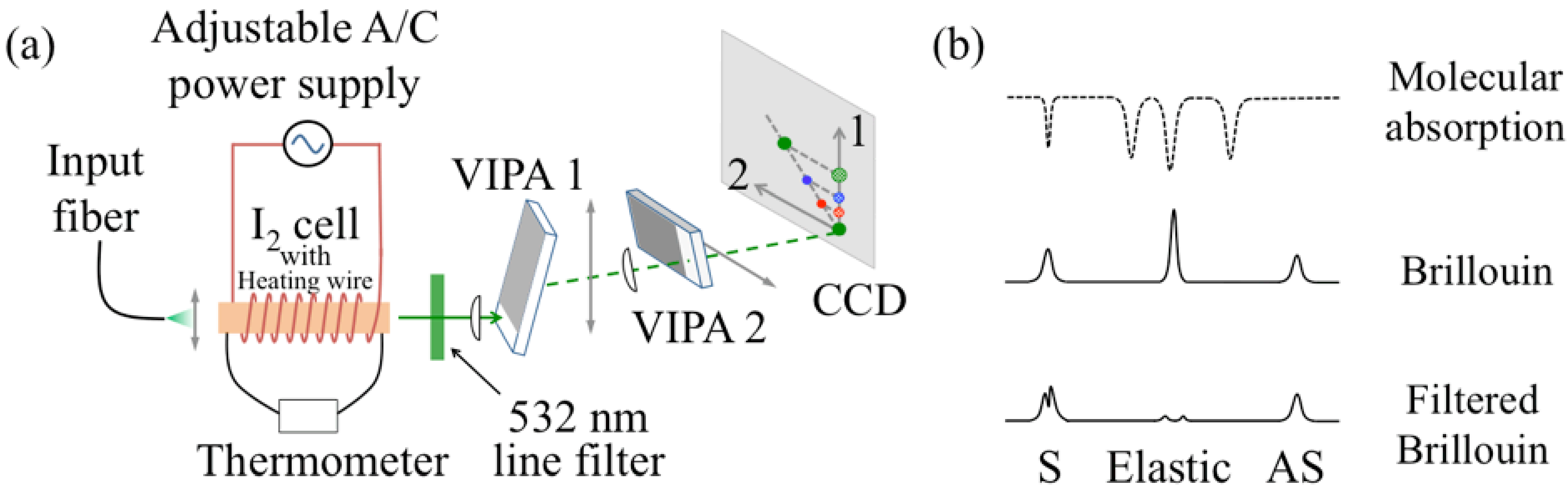


Pure DMSO



DMSO + coffee cream

Atomic / molecular filter



Zh Meng, A Traverso, V Yakovlev, *Optics Express* 22, 5410 (2014)

532 nm laser line is near several vibrational absorption lines of iodine

780 nm, **795 nm** are near atomic transitions of **rubidium**

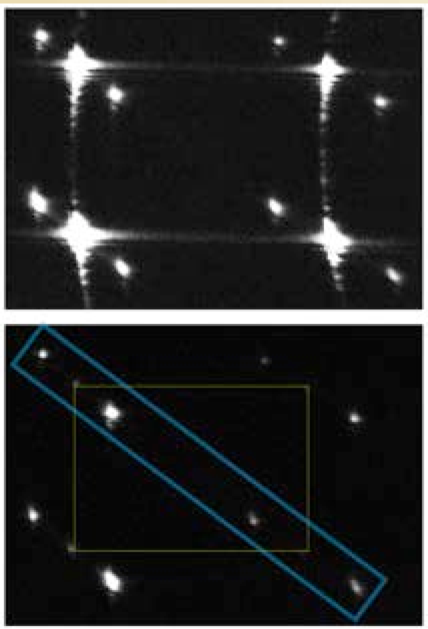
Hg – good in UV, Cs – 852 nm, etc.

RB Miles et al, *Meas. Sci. Techn.* 12(4), 442-451 (2001)

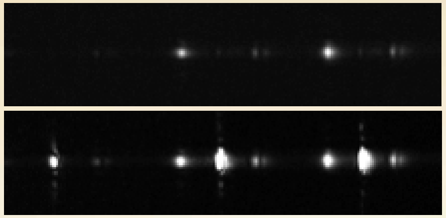
The same absorption filter is good for wavelength stabilization

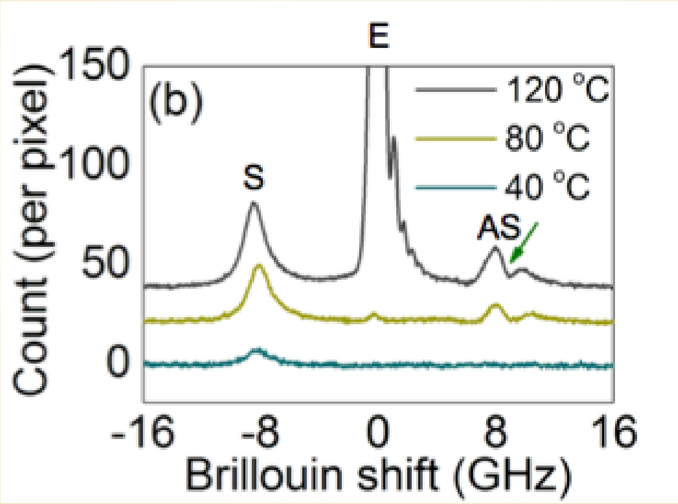
Brillouin spectroscopy with I_2 filter

2 stage VIPA spectrometer
acetone
35 mW, **20 sec**



1 stage VIPA spectrometer
DMSO, 35 mW, **2 sec**





$\Delta\nu$=8.320±0.008 GHz
v= 1496.360±1.438 m/s

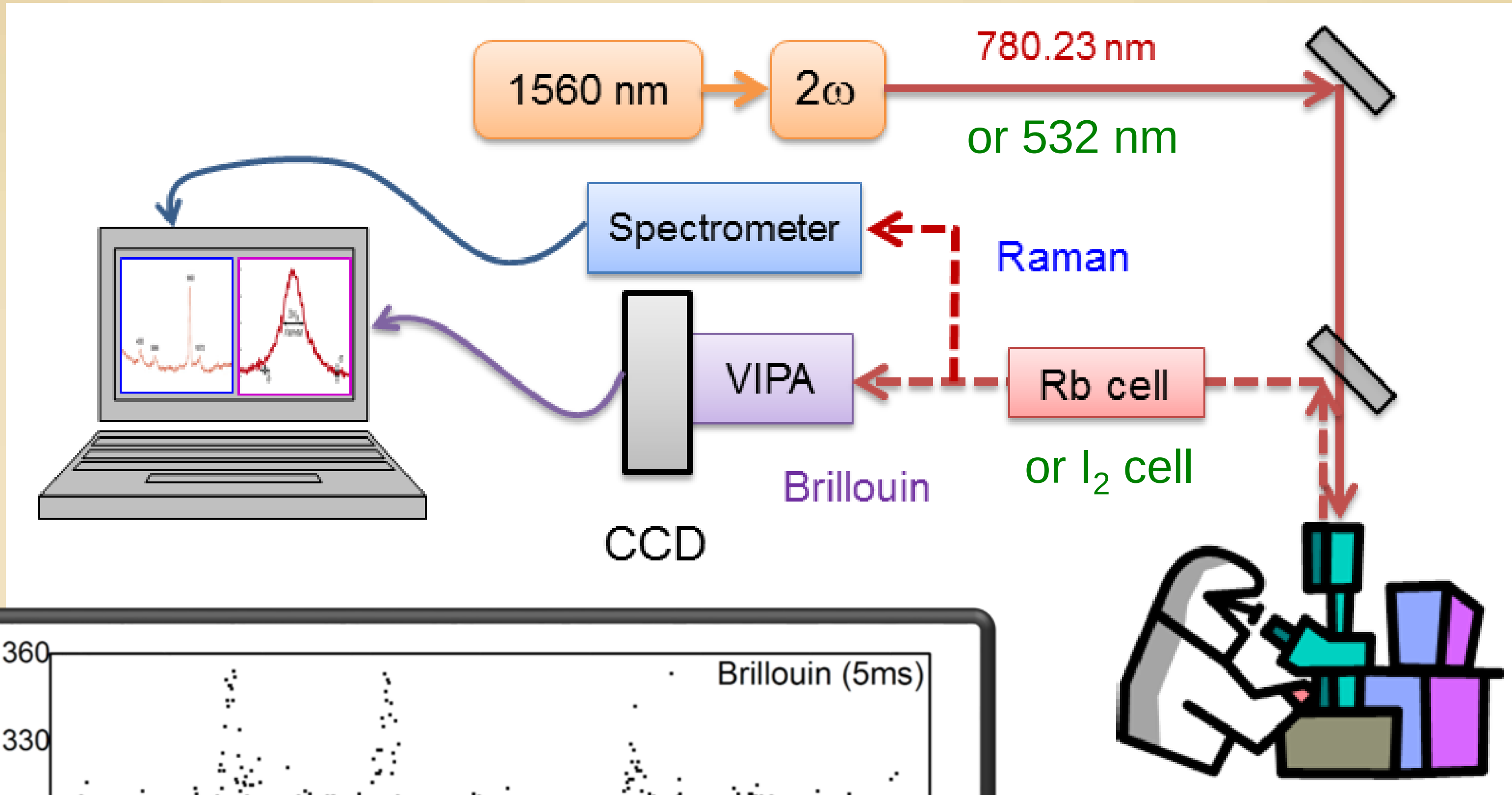
Previous studies:
v_{DMSO}=1494.0 m/s @ 1MHz

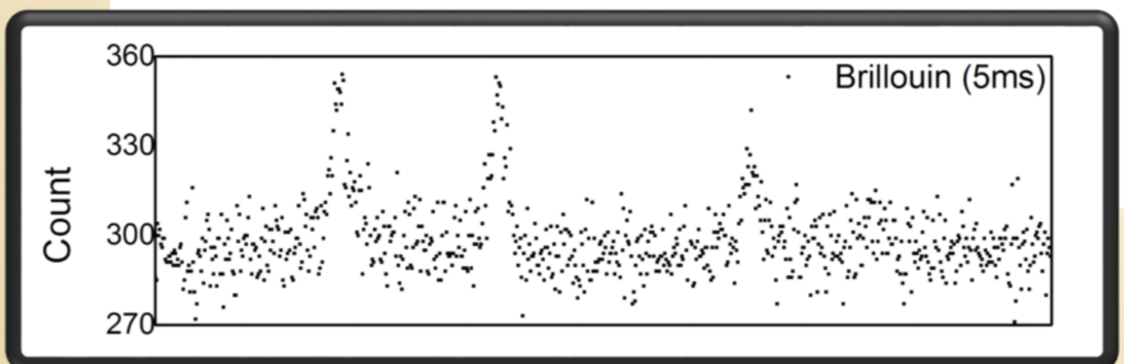
Mass density:
ρ=1,100 kg/m^3

Longitudinal modulus:
K=2.463±0.004 GPa

Zh Meng, A Traverso, and V Yakovlev, *Optics Express* 22, 5410 (2014)

Brillouin microspectroscopy





AJ Traverso, et al
Anal Chem **87**, 7519-7523 (2015)

Applications

- **Biomaterials**
- **Muscular dystrophy**
- **Developmental biology**
- **Cancer**
- **Cardiovascular diseases**

Methacrylated gelatine (GelMA)

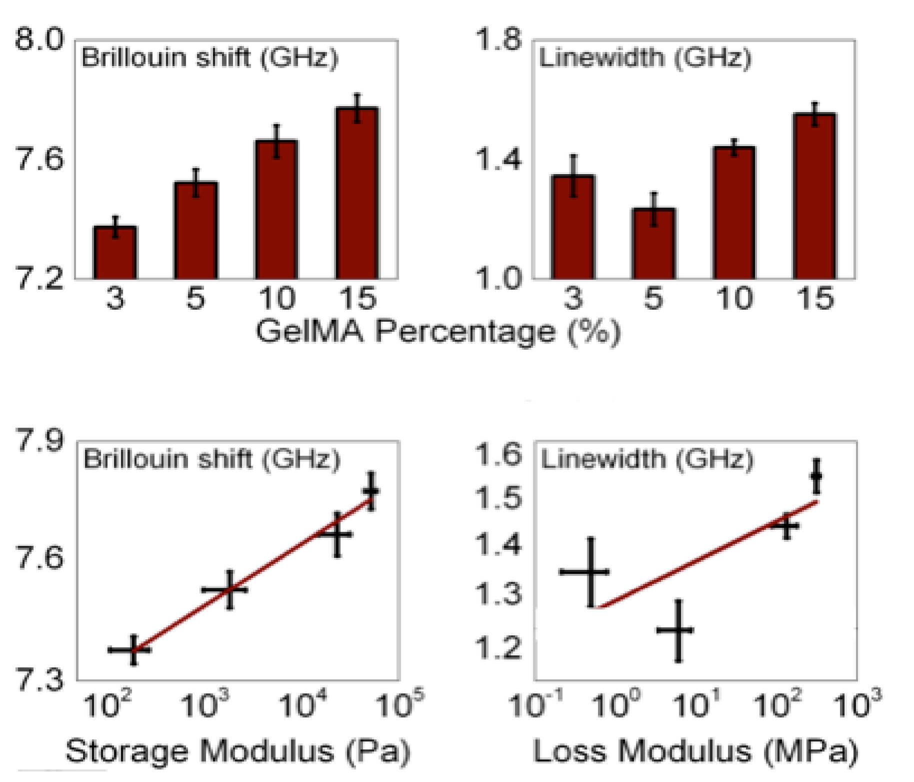
Motivation:

Methacrylated gelatin (GelMA) is an important bio-compatible hydrogel for bone repairing and hemorrhage prevention.

Summary of Results:

In both Brillouin measurements and mechanical compression tests, larger elastic modulus was achieved for the hydrogels with higher GelMA concentration.

Z Meng, T Thakur, C Chitrakar, MK Jaiswal, AK Gaharwar, VV Yakovlev, *ACS Nano* (2016) *Under Review*



GelMA + nHAP ?

Motivation:

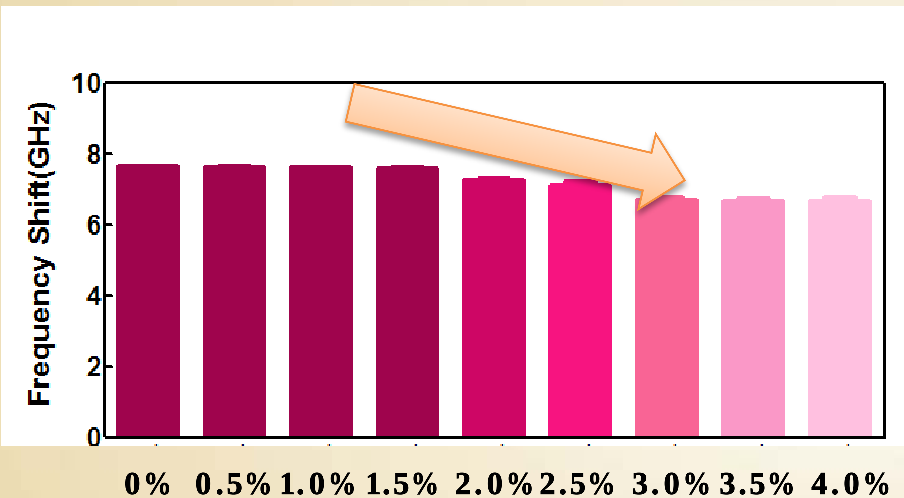
Bone contains calcium. Can we mimic biological structure of a bone ?Methacrylated gelatin (GelMA) and Hydroxyapatite (HAP) nanoparticles nanocomposite may be beneficial in **bone repair** due to its superior biodegradability. However, the corresponding relationship between HAP level and microscopic elasticity is unknown.

Flexible hydrogel-nHAP composite

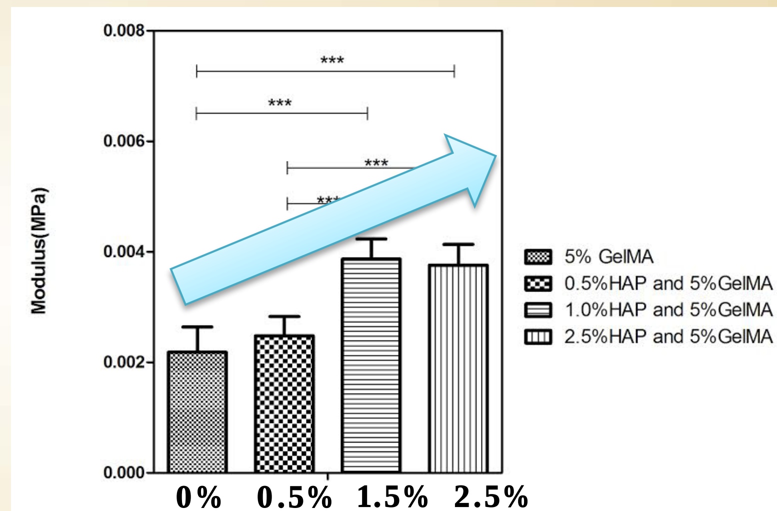
GelMA + nHAP

Experimental Results:

The Brillouin modulus **negatively** correlates with the HAP concentration. This may indicate that HAP helps enhancing the material stiffness on a **structural level**, not on **molecular level**.



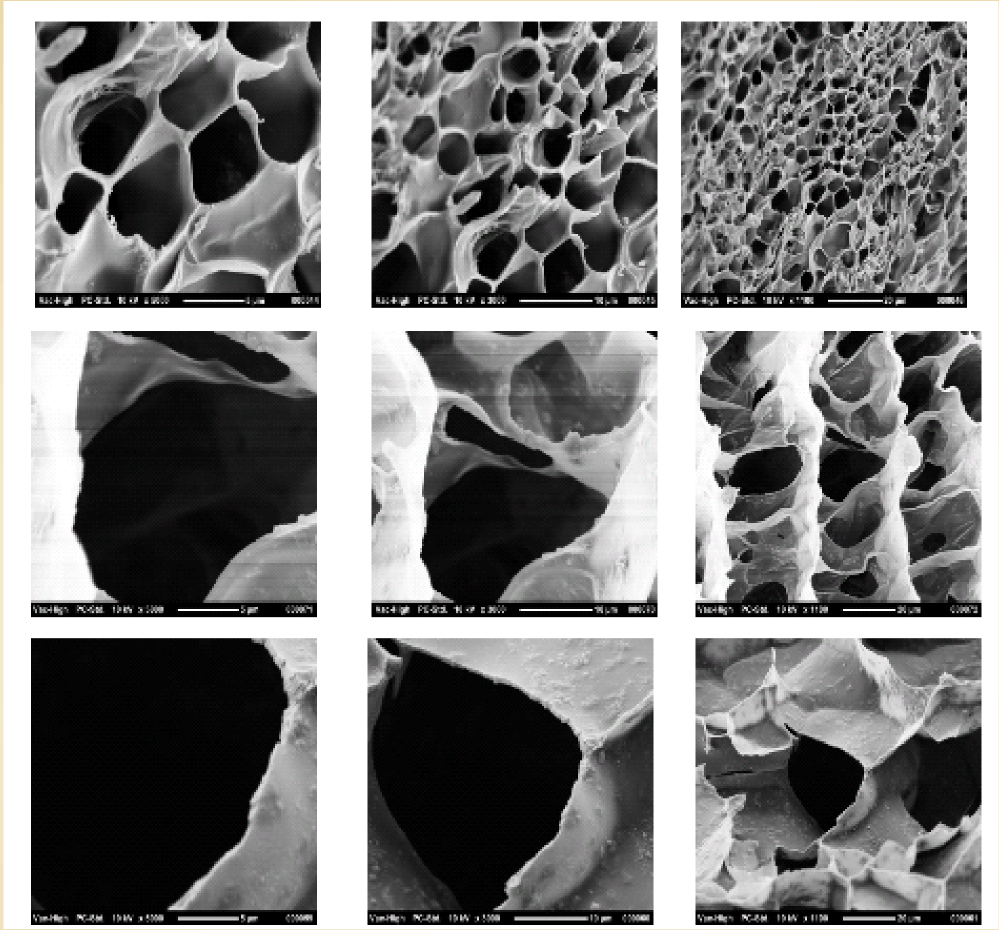
HAP concentration



HAP concentration

Z Meng, T Thakur, C Chitrakar, MK Jaiswal, AK Gaharwar, VV Yakovlev,
ACS Nano (2016) *Under Review*

GelMA + nHAP microscopy



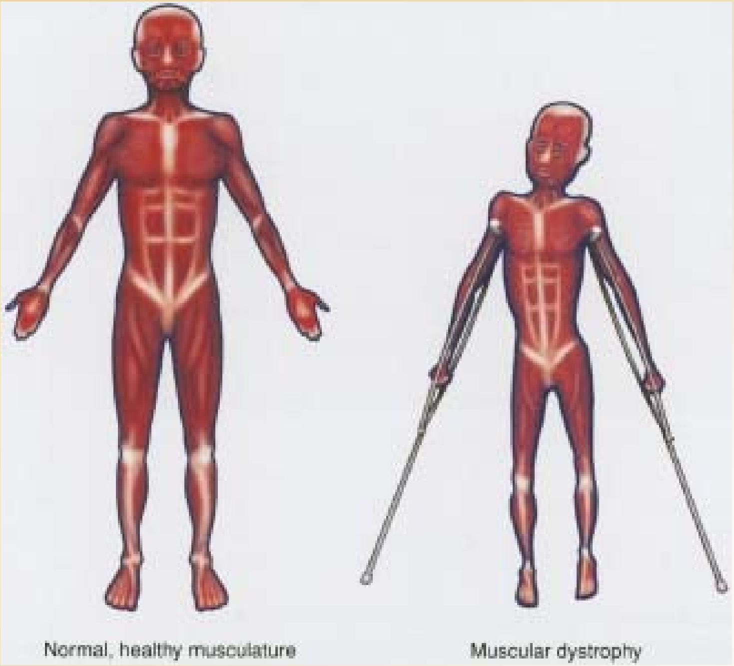
0.5% HAP

1.0% HAP

2.5% HAP

Structural heterogeneity drives local viscoelastic properties

Muscular Dystrophy

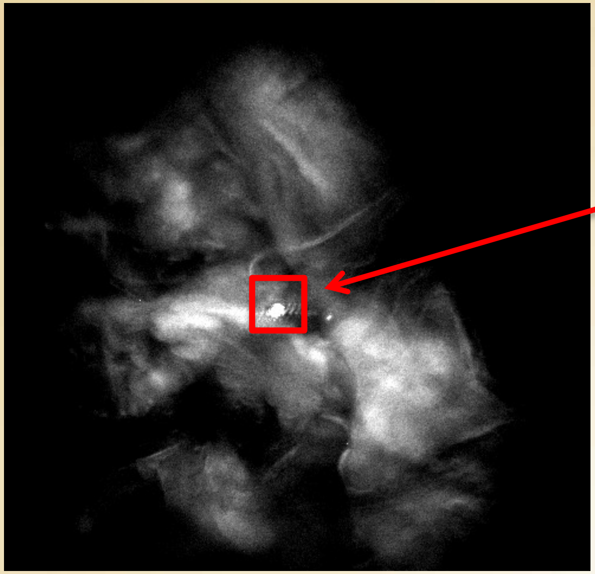


www.humanillnesses.com

Muscular dystrophy is a group of muscle diseases that weaken musculoskeletal system.
Mostly due to genetics.

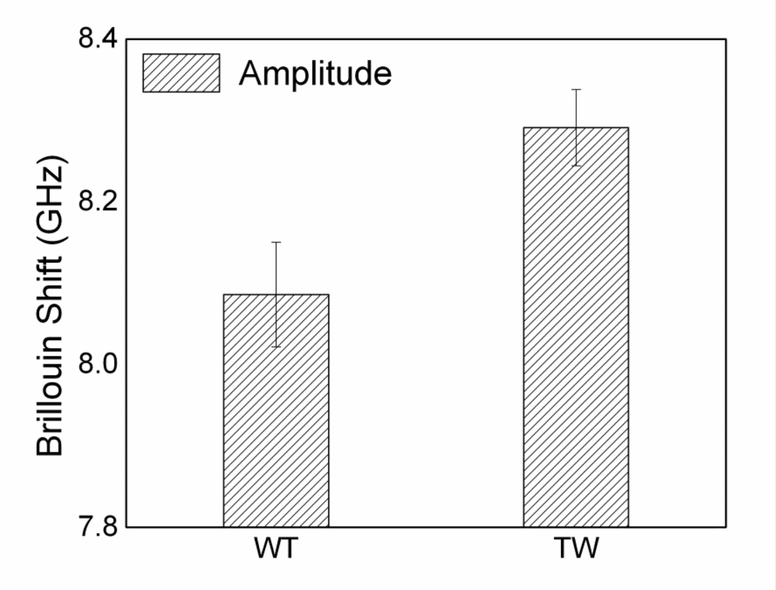
Model system: abdominal tissue in generically modified larvaes (*Drosophilae*)

Muscular Dystrophy: Results



Objective: N.A. = 1.0; Magnification: 1000x

GFP (green fluorescent protein) image of muscular tissue
GFP labels myosin fibrils

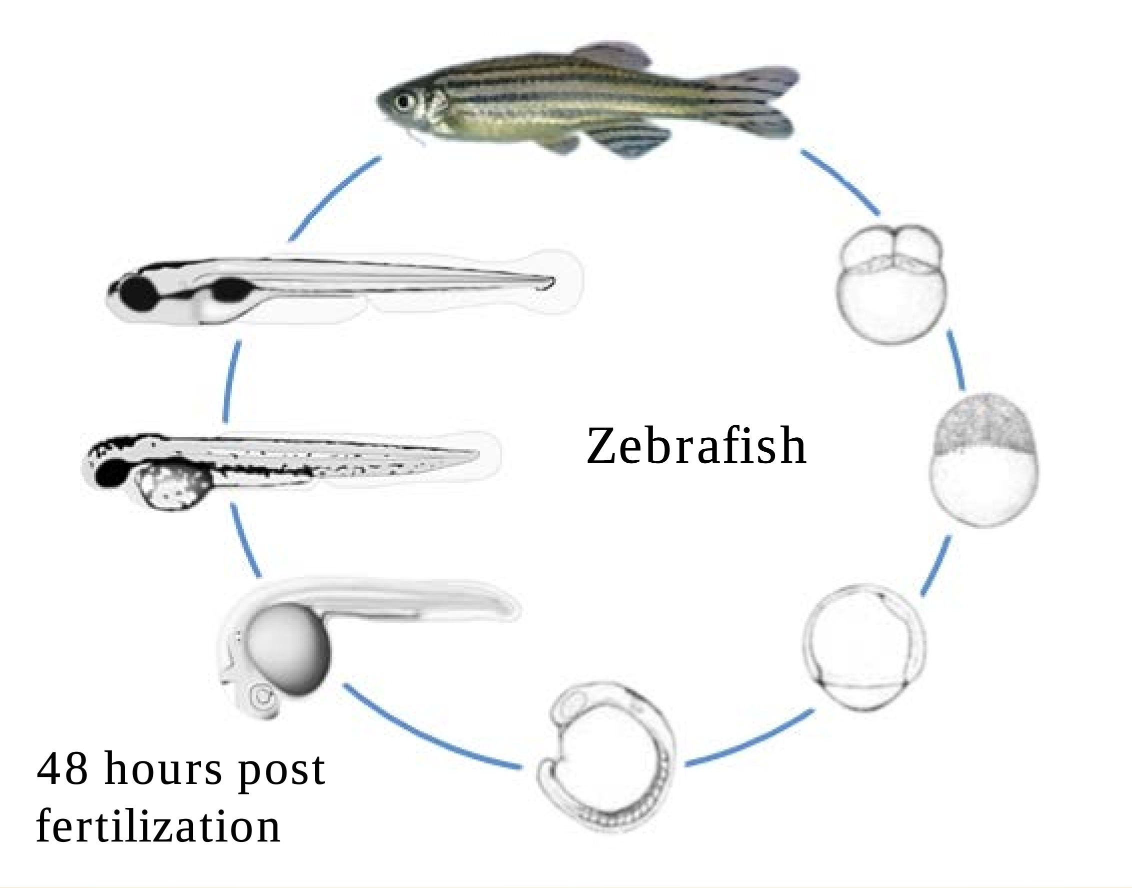


Wild **T**ype (normal)

"**Tw**isted" type Muscular dystrophy

Muscle stiffness increases in muscular dystrophy: first in vivo demonstration

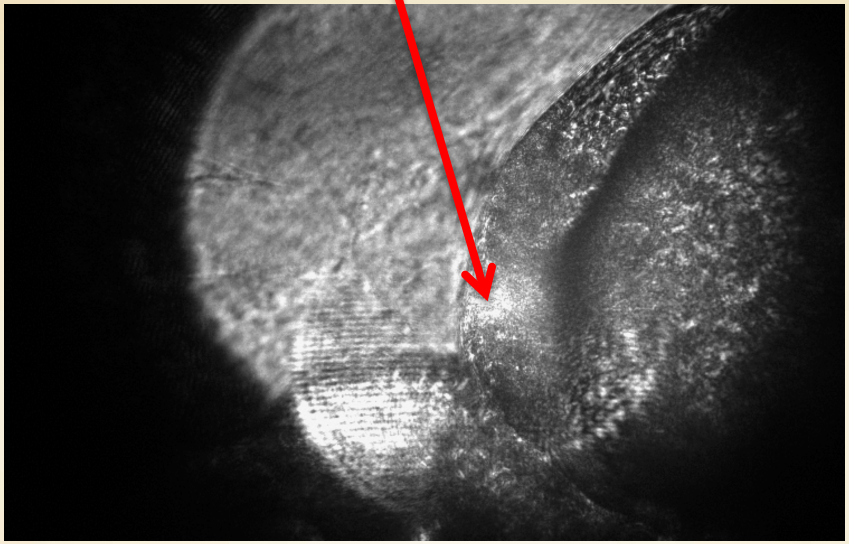
Morphogenesis



Viscoelasticity measurements are critical for understanding formation of heart and brain

Zebrafish embryo

Laser spot



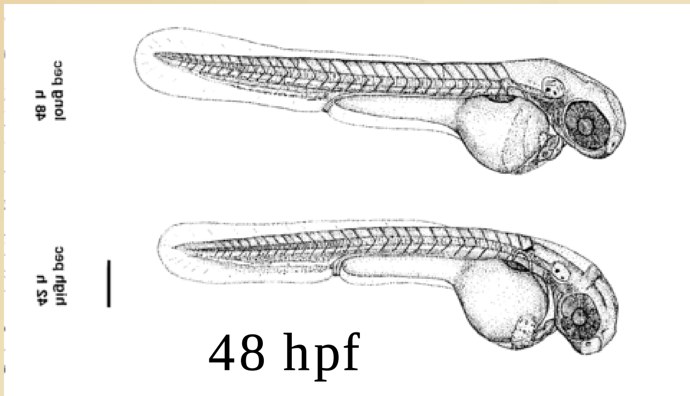
Heart is beating

Spot diameter: ~ 1 μm

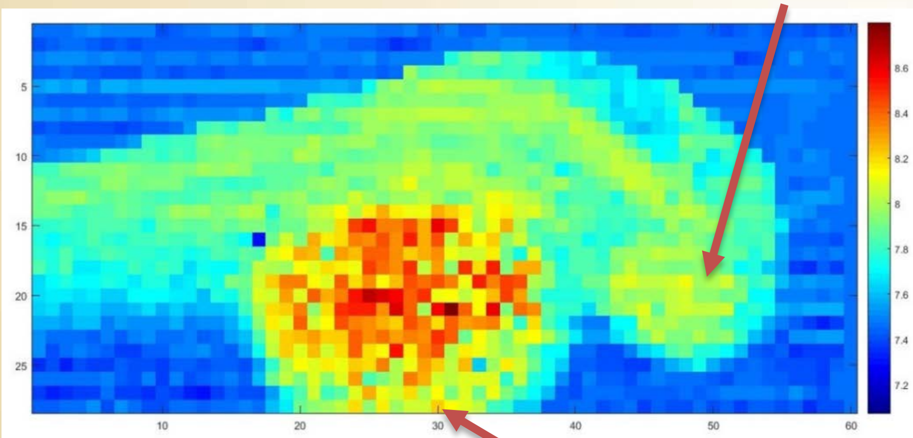
Brillouin microspectroscopy allows simultaneous fluorescent, Raman, DIC microscopy

Zebra-fish development

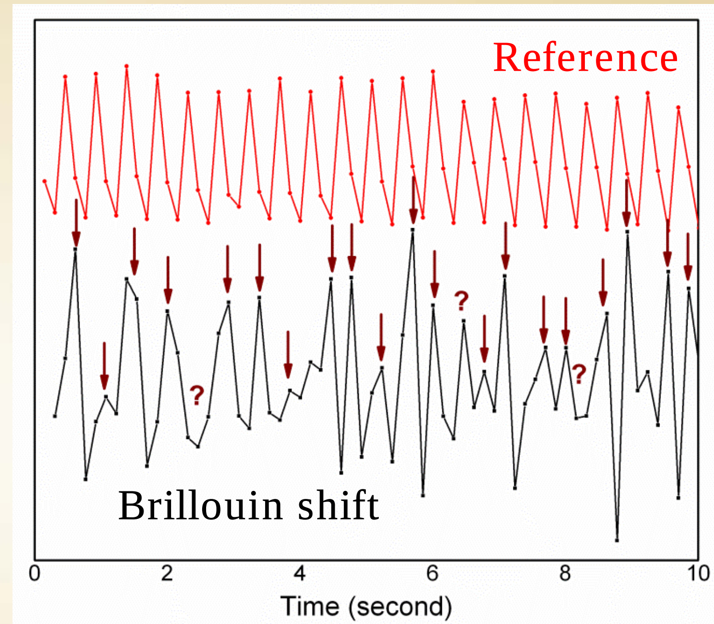
First in vivo elasticity map is recorded



Eye



Yolk Sac



Heart muscles stretch and compress during heart beating. Those variations in elasticity can be seen in Brillouin measurements

Cancer (melanoma) imaging

Melanoma is one of the most serious skin cancers, since it tends to develop and spread to other organs very fast.

A – Asymmetrical Shape: Melanoma lesions are often irregular in shape

B – Border: Melanoma lesions usually have irregular borders that are difficult to define.

C – Color: The presence of more than one color (blue, black, brown, tan, etc.) or the uneven distribution of color is a warning sign of melanoma.

D – Diameter: Melanoma lesions are often greater than 6 mm in diameter.

E – Evolution.

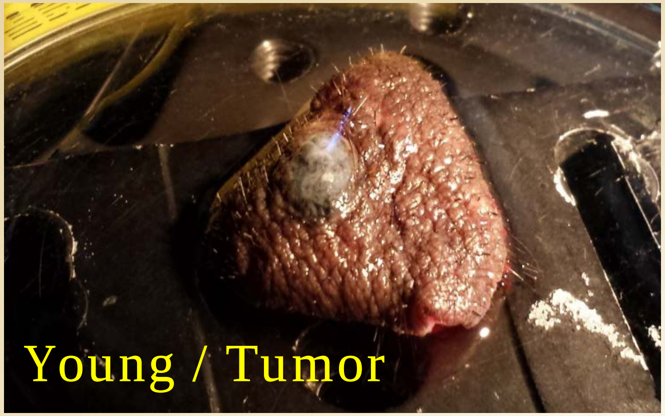
Assessing mechanic properties, which are believed to be linked to cancer cells' ability to spread quickly, might provide additional ways to evaluate the potential risk.

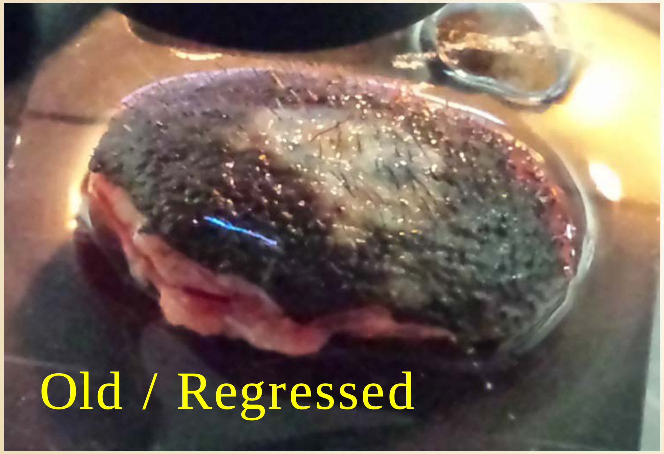
Melanoma imaging

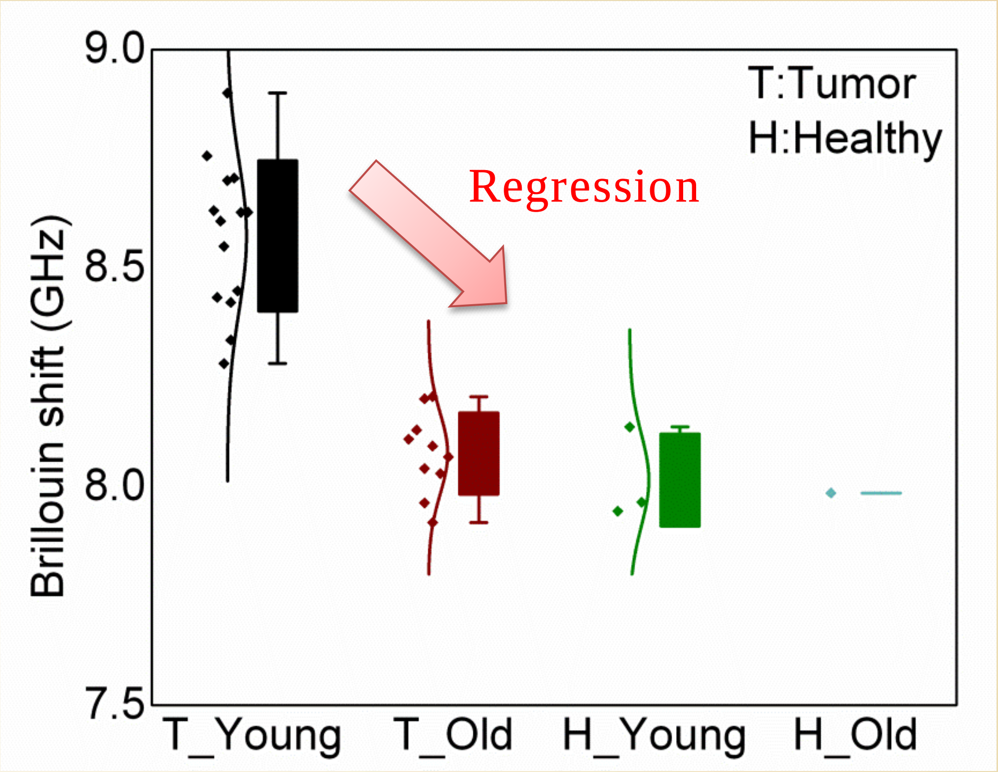
A strain of miniature swine has genetically determined tumors similar to many types of human tumors. Some tumors regress spontaneously, making those animals ideal for study malignant degeneration and the role of immunity and growth control factors in regression and regulation of tumor growth.

LE Millikan, JL Boylon, RR Hook, PJ Manning, *J. Invest. Dermat.* **62**(1), 20–30 (1974)

Melanoma imaging





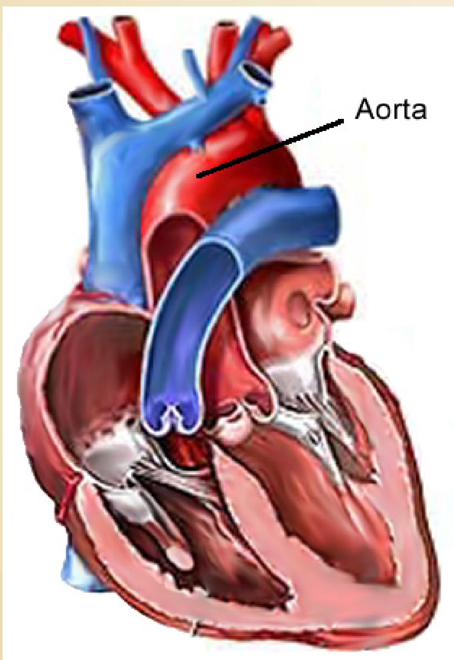


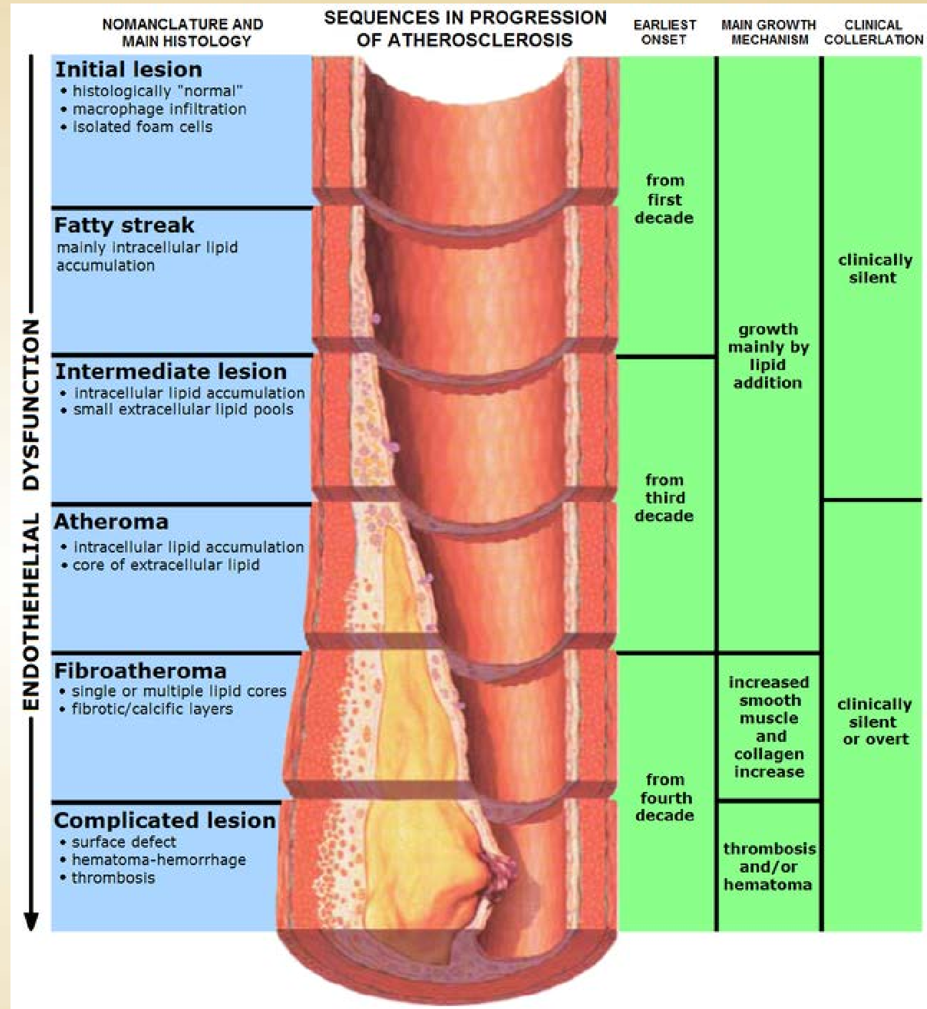
In collaboration with Dr. Duane Kraemer,
College of Veterinary Medicine, TAMU

M Troyanova-Wood et al,
To Be Published

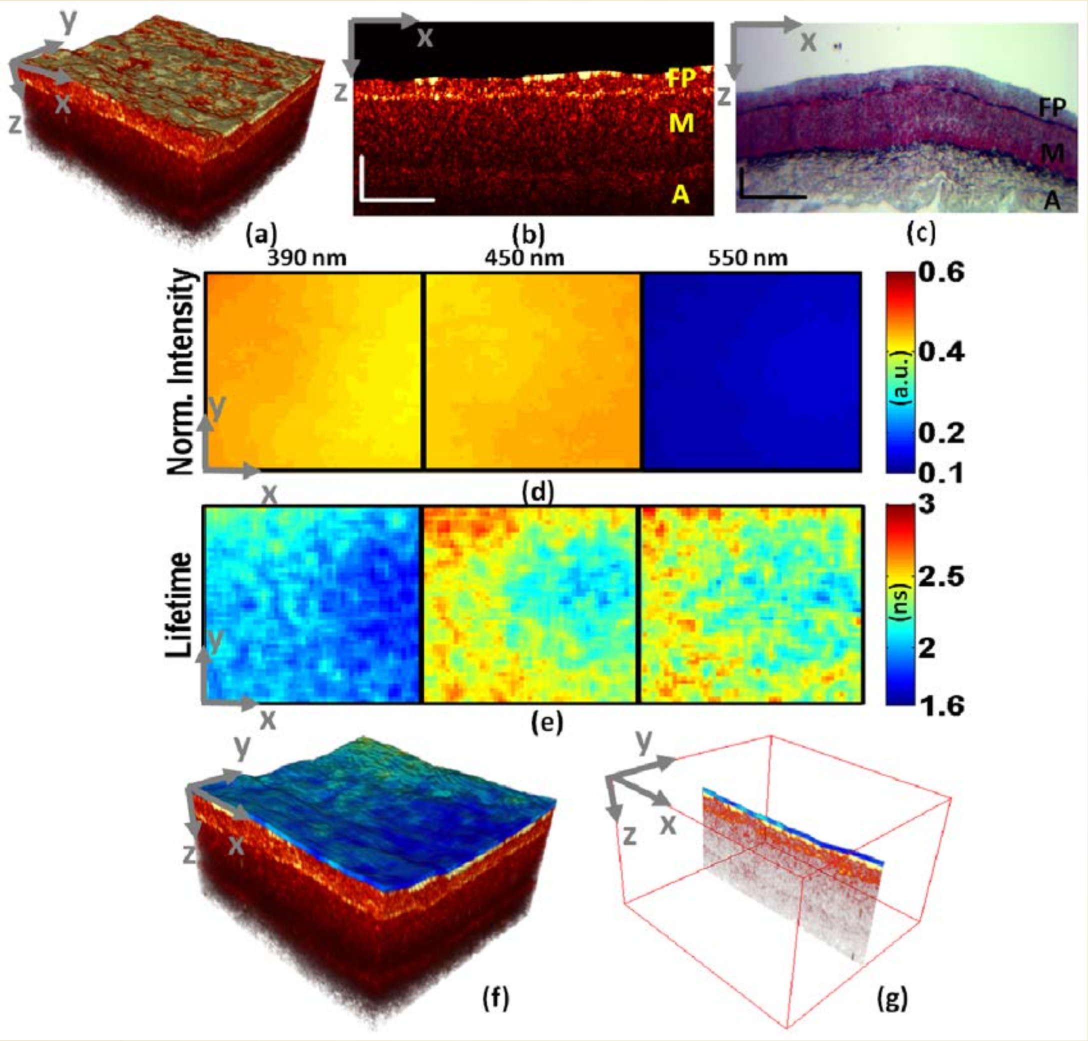
Atherosclerosis

Atherosclerosis: vascular disease in which artery-wall thickens and plaque is formed. Mechanical properties get substantially affected.





Optical imaging of atherosclerosis

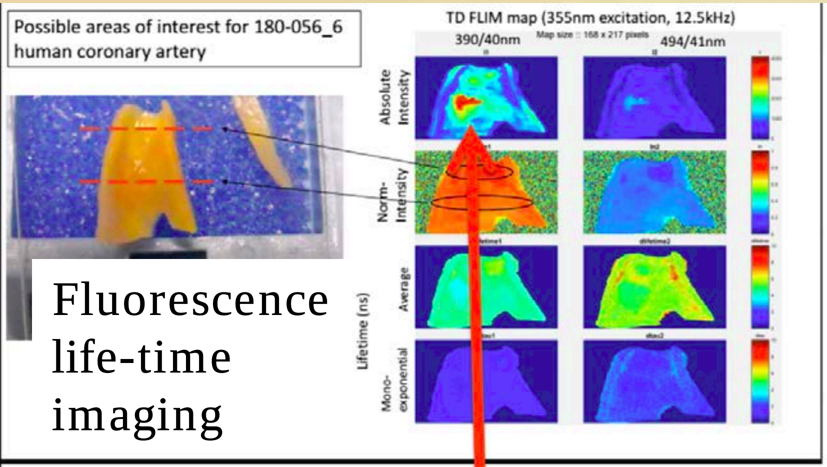


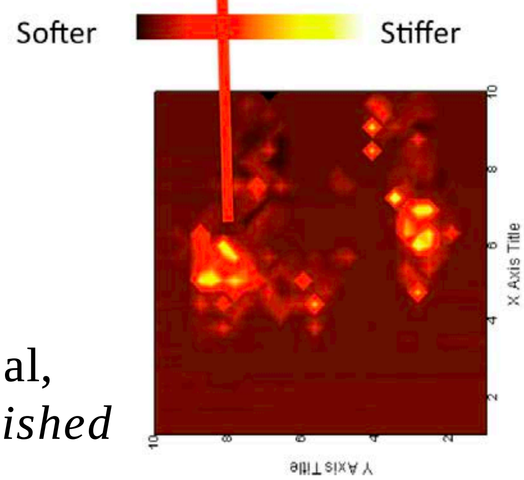
OCT and FLIM images of an *ex vivo* human atherosclerotic artery tissue with thin fibrotic plaque:

(a) 3-D OCT volume,
(b) 2-D OCT B-scan (**FB**: Fibrotic plaque in Intima, M: Media and A: Adventitia),
(c) H&E histology corresponding to (b)
(d) Normalized fluorescence intensity maps,
(e) Fluorescence lifetime maps,
(f) 3-D OCT/FLIM volume with fluorescence lifetime in 390 nm band, and
(g) Ortho- sliced image from (f).

J Park, JA Jo, S Shrestha, P Pande, Q Wan, BE Applegate, *Biomed. Opt. Express* **1**, 186-200 (2010).

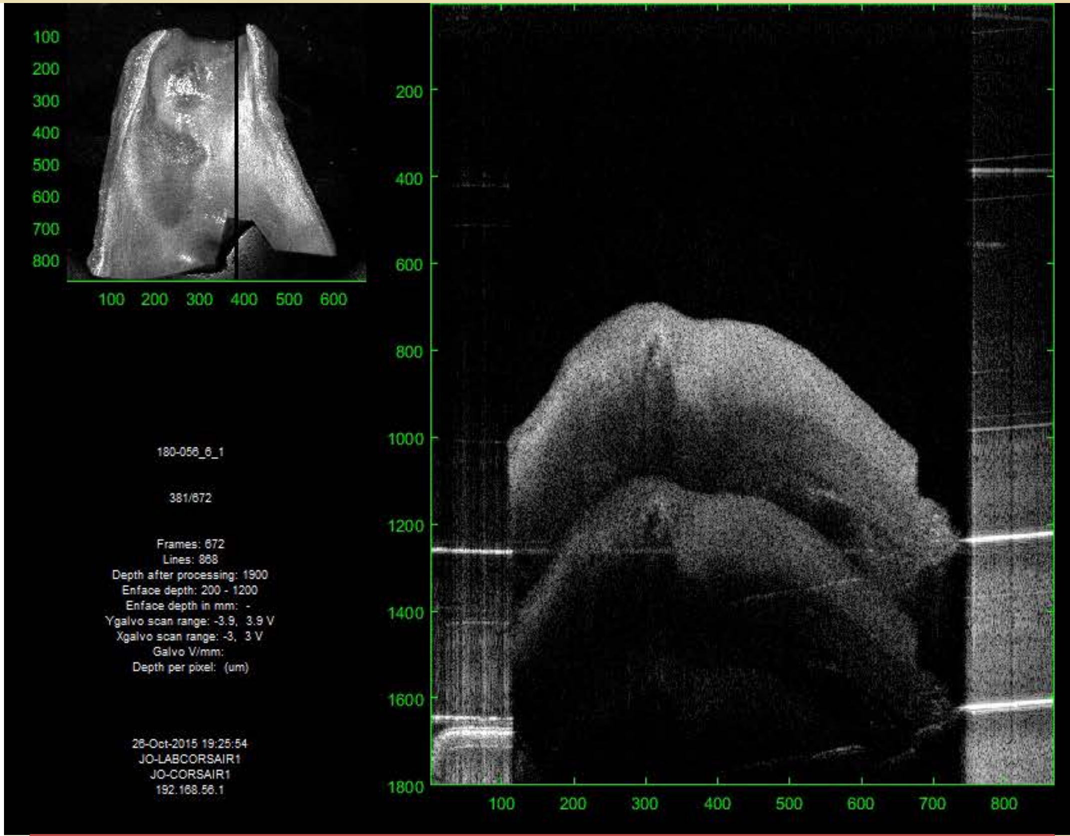
Brillouin imaging of atherosclerosis





Brillouin image

Z Meng et al,
To be published



Fluorescence and OCT images correlate with Brillouin assessment and histopathology

Advanced Instrumentation

Motivation:

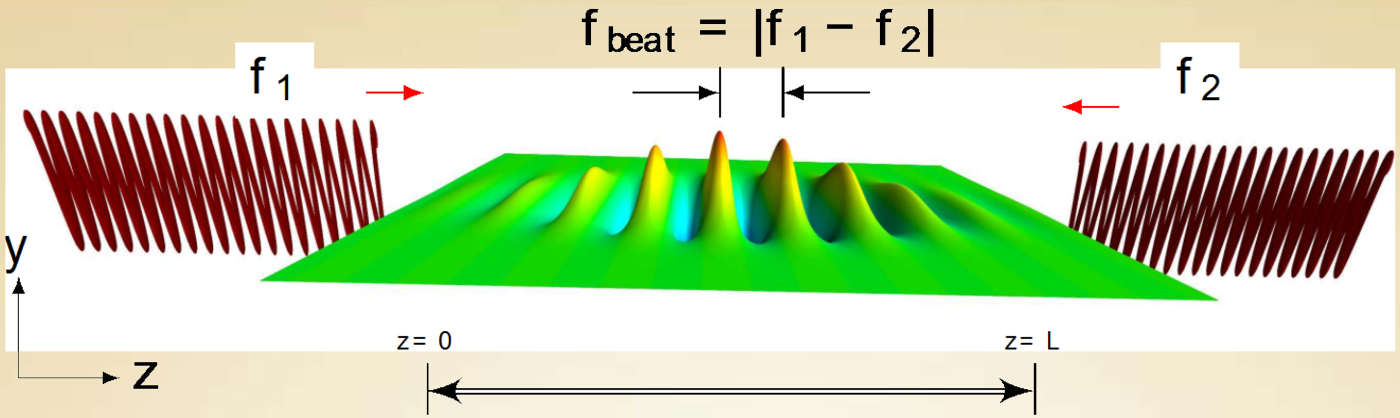
Our best results with **spontaneous** Brillouin spectroscopy provide us with 100-ms acquisition time per data point. To make a 1000 x 1000 image (250μm x 250μm with 0.25μm spatial resolution), 2 hours are needed. Acquisition rate needs to be improved by, at least, a factor 0f 1000.

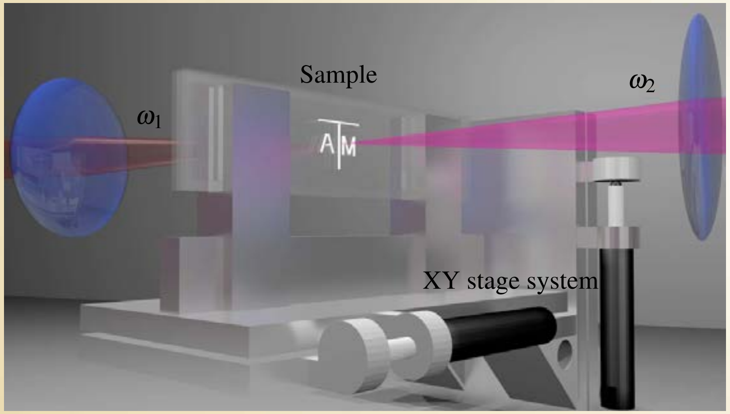
Solution:

We can employ **coherent** Brillouin spectroscopy.

By coherently exciting acoustic wave and scattering light off this wave, signal can be increased and data acquisition rate improved.

Stimulated Brillouin Imaging





f_1 and f_2 are provided by a pair of tunable, narrow-band, cw diode lasers

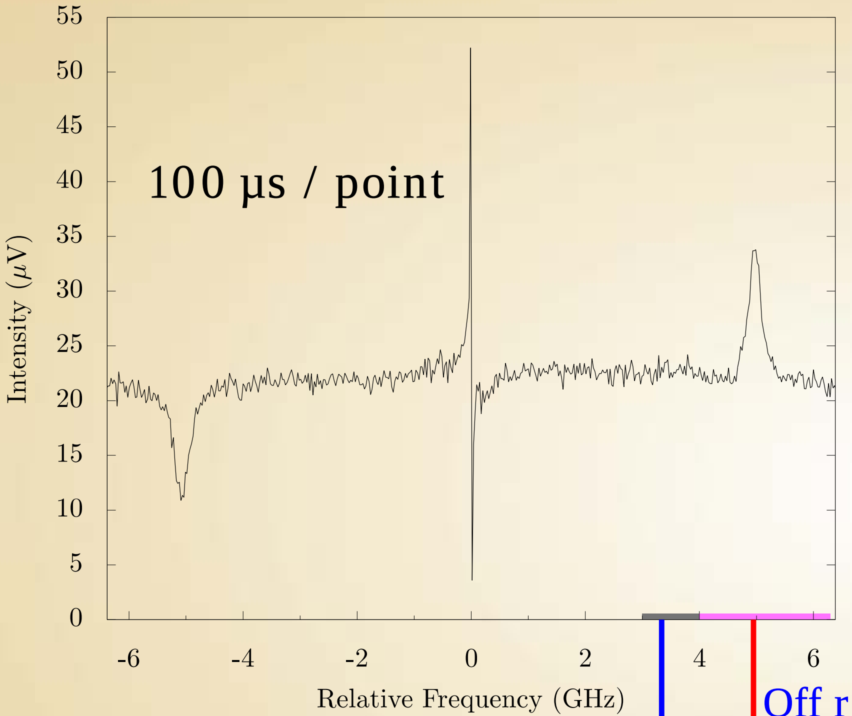
One beam is modulated and frequency tuned with respect to the other beam. Lock-in detection allows detecting small (~10^{-6}) gain/loss due to Brillouin interaction.

Structure is laser imprinted in glass and is immerged into water for imaging.

C. Ballmann, et al, *Scientific Reports* 5: 18139 (2015)

Stimulated Brillouin Imaging

SBS spectrum of water



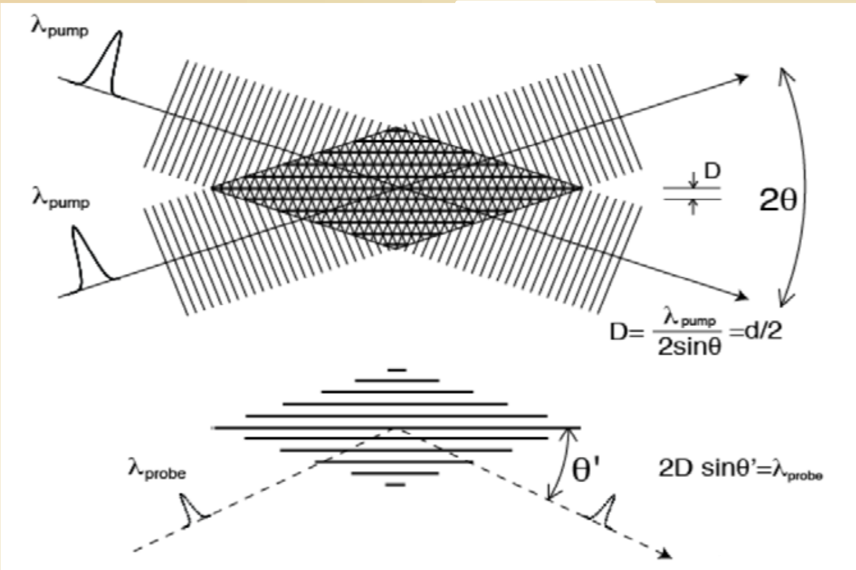
Off resonance

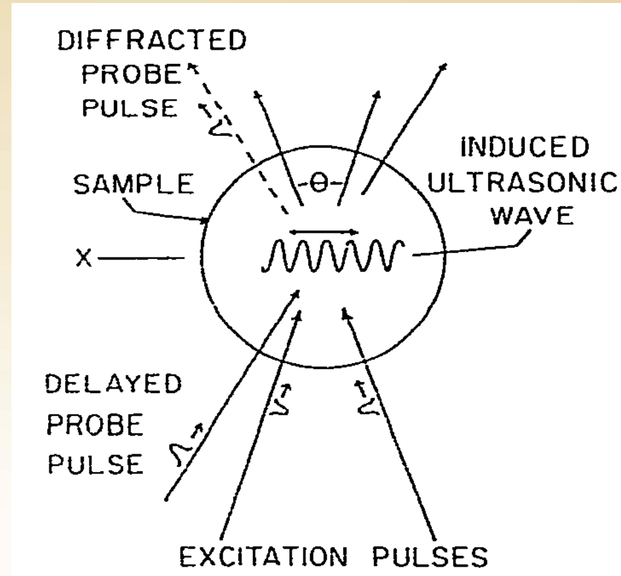
On resonance

To record the whole spectrum (~100 points), **10 ms** is needed

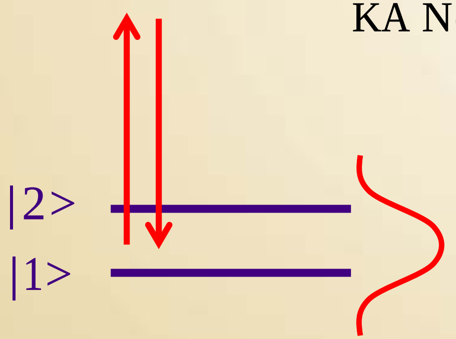
CW Ballmann, et al, *Scientific Reports* 5: 18139 (2015)

Impulsive Stimulated Scattering





KA Nelson, RJD Miller, MD Fayer, *J. Appl. Phys.* **53**, 1144 (1982).



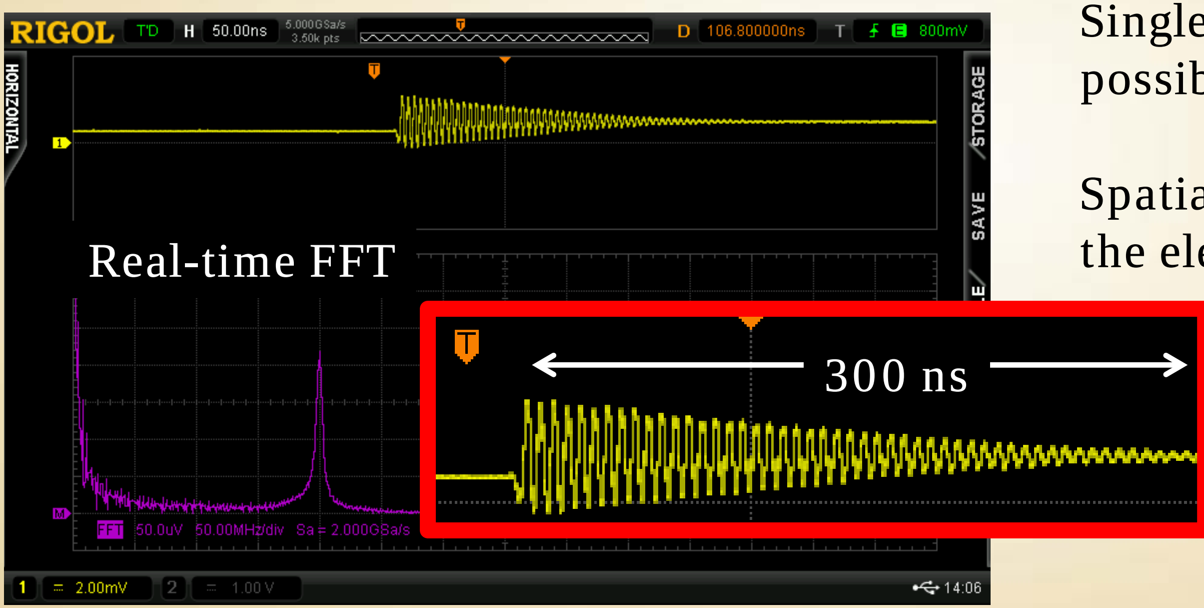
Two short pulses interfere in the sample and create two counter-propagating acoustic waves either through thermal effects or through electrostriction. Third (probe) pulse scatters off the acoustic grating recording temporal dynamics of this grating.

BISTRO measurements

Replace probe pulse with a cw beam and record temporal evolution of the diffracted beam using fast (1-10 GHz) photodiode and electronics.

Z. Meng, G. I. Petrov, and V. V. Yakovlev, *Analyst* **140**, 7160-7164 (2015)

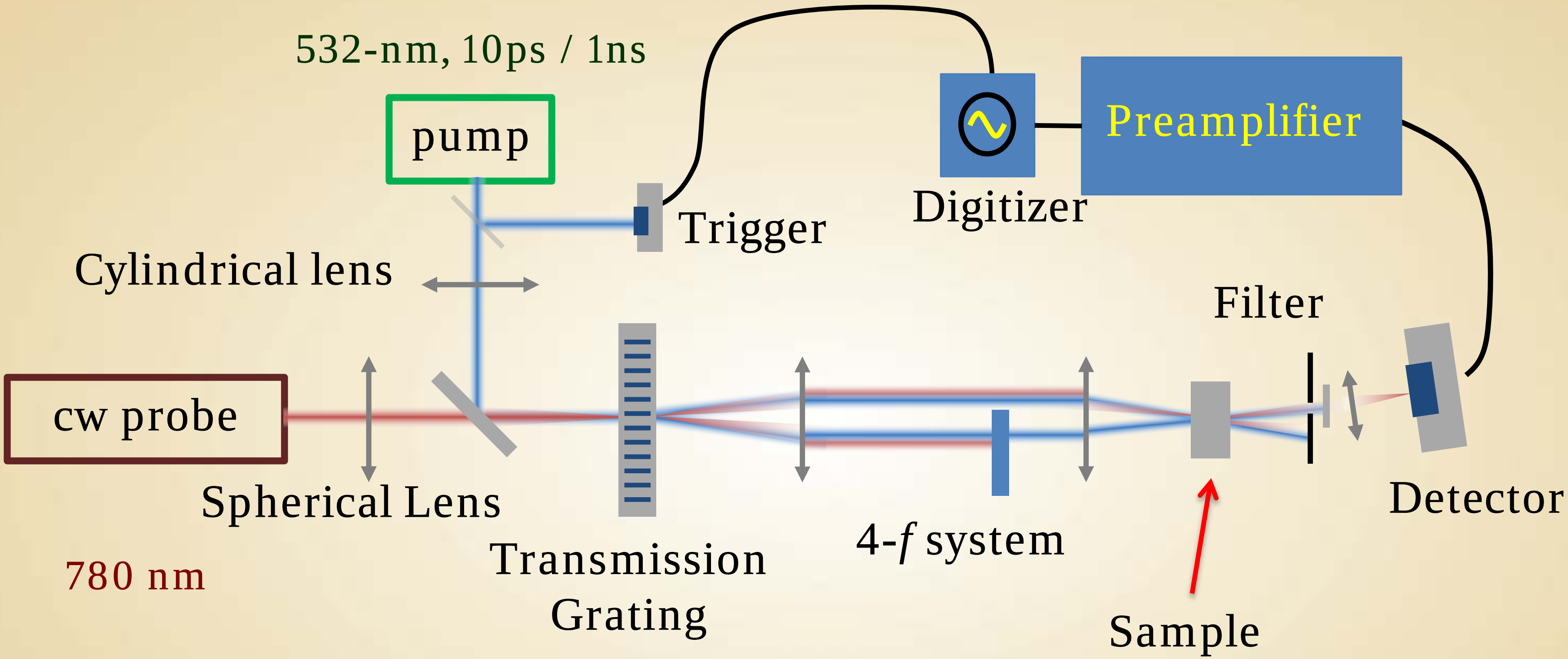
Brillouin Imaging/Sensing via Time-Resolved Optical (BISTRO) Measurements



Single-shot measurements are possible in a shot-noise limit.

Spatial resolution is limited by the electronic bandwidth

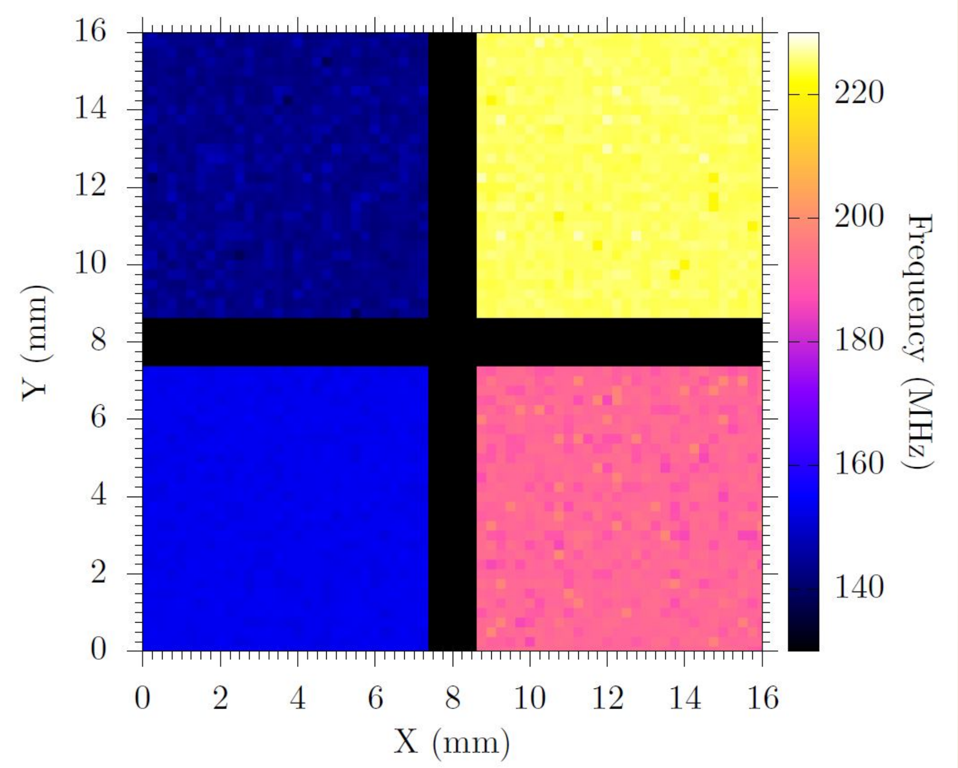
Experimental setup



Z Meng, et al, *Analyst* **140**, 7160-7164 (2015); CW Ballmann, et al *Optica* **4**(1), 124-128 (2017).

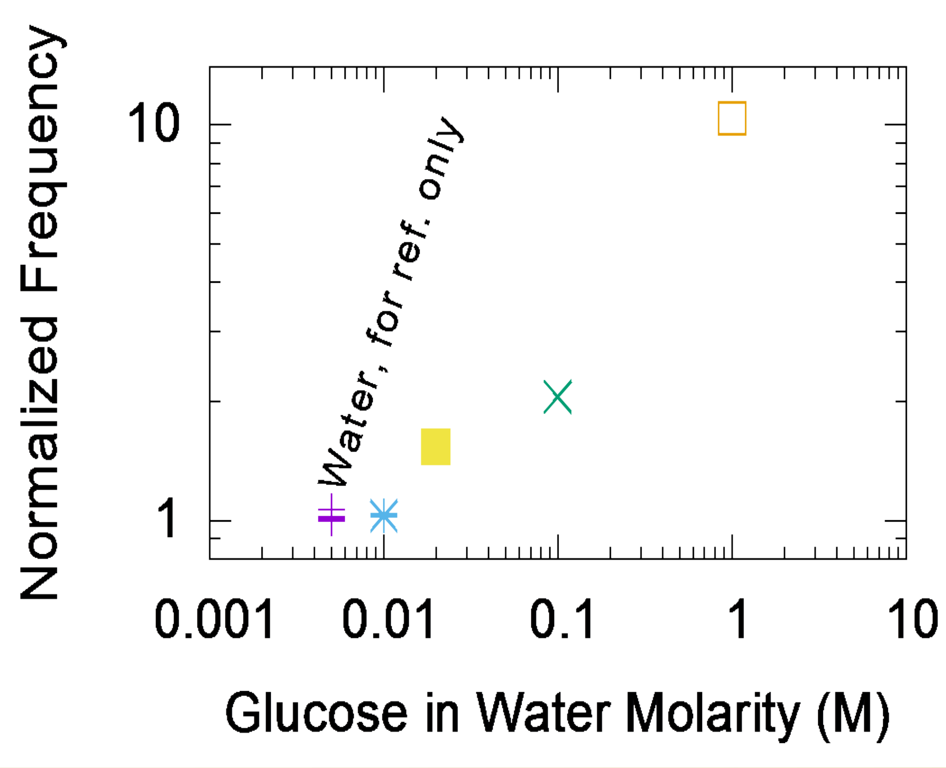
BISTRO measurements

Imaging



CW Ballmann, et al *Optica* 4 (1), 124-128 (2017)

Sensing



CW Ballmann, et al, *To Be Published*

Filling the technology gap

Summary

Brillouin spectroscopy is a sleeping giant of biomedical imaging

There is a clear need to assess viscoelastic properties of cells and tissues on microscopic level, and Brillouin spectroscopy / microscopy offers a plausible solution

Technology is lagging Raman spectroscopy / microscopy by ~20 years, which is clearly shown in the number of publications: for each publication on biological aspects of Brillouin spectroscopy there are more than 100 of publications which utilize Raman spectroscopy

Raman instruments (>50 vendors)

VS

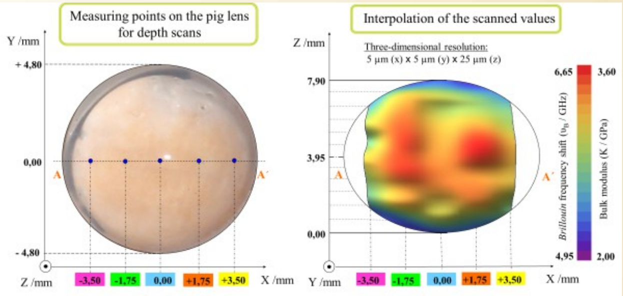
Brillouin instruments (2 vendors)

Summary

Fully automated instruments are not commercially available: there is a room for improvement. However, all existing research instruments are compatible with other optical microscopy techniques.

Future directions: Brillouin-Induced Kerr-Effect Scattering (BIKES) and Coherent anti-Stokes Brillouin Scattering (CABS); better spatial resolution (100 nm) through structured illumination.

Cornea imaging will likely be the first clinical application (MGH, U of Maryland, U Rostock (Germany))



Other applications involve eye imaging (retina detachment, glaucoma), developmental biology, cancer research, neuroscience, membrane biology, skin/bone diseases, biomaterials, dentistry, infectious diseases, cosmetics, gas and oil industry etc.

Acknowledgements

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Jessica Hanson
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Omar Yusufzai
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Marianna Peraza
Zachary Steelman
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