Physics and Instrumentation of Brillouin microscopy

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Mechanical properties in Biomedicine

Organs

Muscoloskeletal system, Circulatory system, Respiratory system

optitrack.com

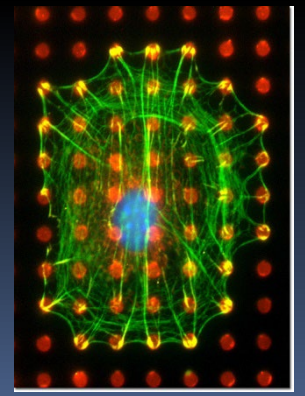
Tissue Biomechanics

Disease diagnosis, Morphogenesis, Tissue engineering

study.com

Cell Biomechanics

Lineage specification, Tumor progression



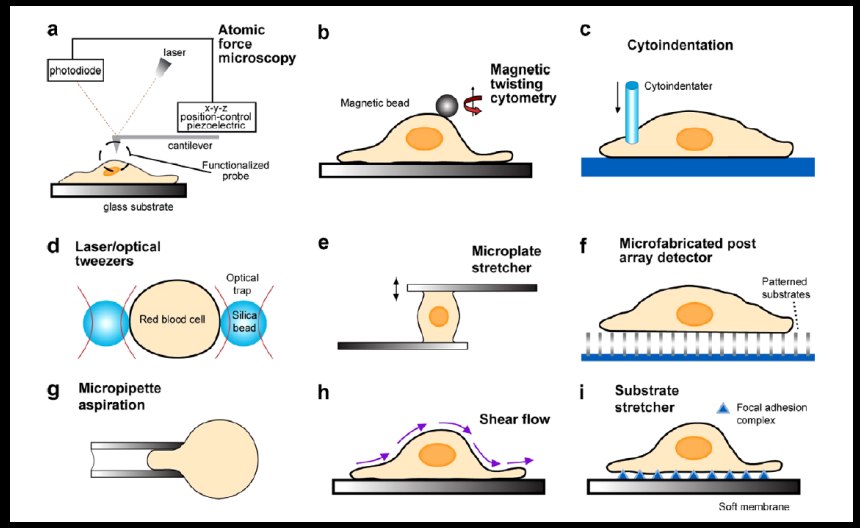
faculty.washington.edu

How do we measure mechanical properties?

Instron Machine

tainstruments

Rheometer



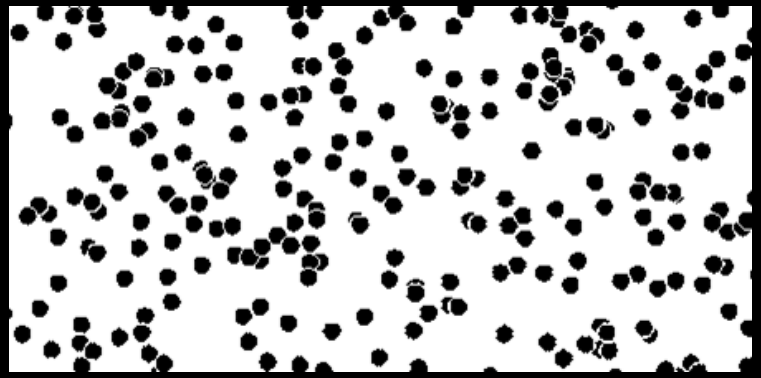
Bao Suresh, Nature materials (2007)

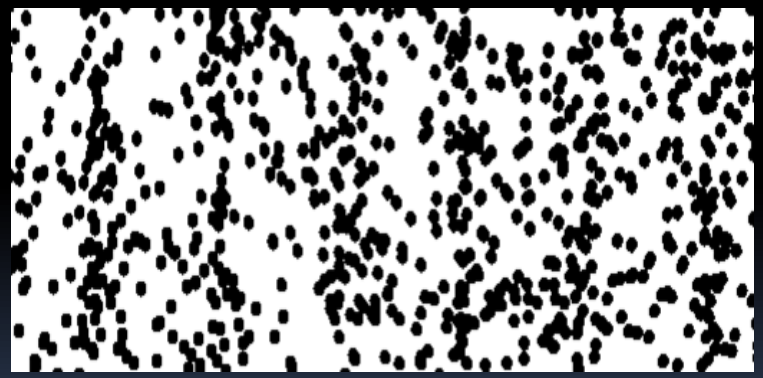
Our goal:

Measuring mechanical properties with an optical microscope

Phonons

Molecules everywhere in the universe move due to their non-zero temperature





$$\mathbf{u}(\mathbf{r},t) = \mathbf{u}_0\, e^{-i(\mathbf{q}\cdot\mathbf{r}-\Omega t)-\Gamma q^2 t}$$

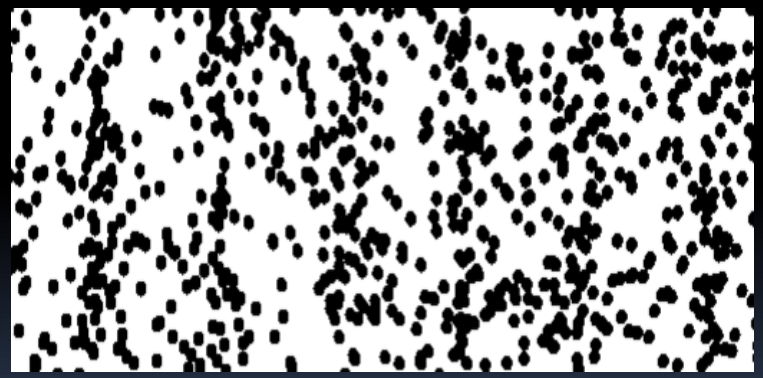
This motion can be decomposed as superposition of oscillations

- Propagating along 3 axis: X-Y-Z
- On 3 modes: 1 longitudinal and 2 transverse
- Of an infinite number of frequencies

Every one of these traveling waves is a thermal acoustic phonon

Phonons

The phonon dispersion relationship links the phonon propagation and attenuation to the medium properties: $\Omega = qv - i\Gamma q^2$



$$\mathbf{u}(\mathbf{r},t) = \mathbf{u}_0\, e^{-i(\mathbf{q}\cdot\mathbf{r}-\Omega t)-\Gamma q^2 t}$$

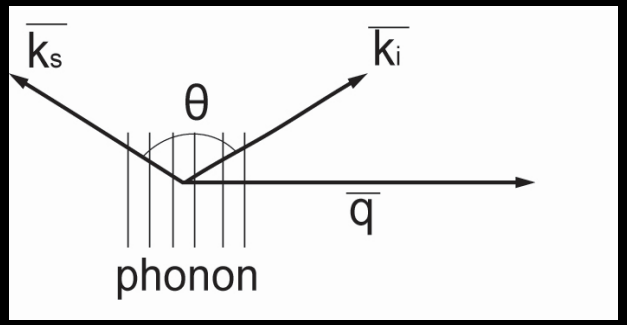
$$v^2 = \frac{c_{ij}}{\rho} = \frac{M'}{\rho} \qquad \Gamma = \frac{\eta}{\rho} = \frac{M''}{\rho}$$

Elastic

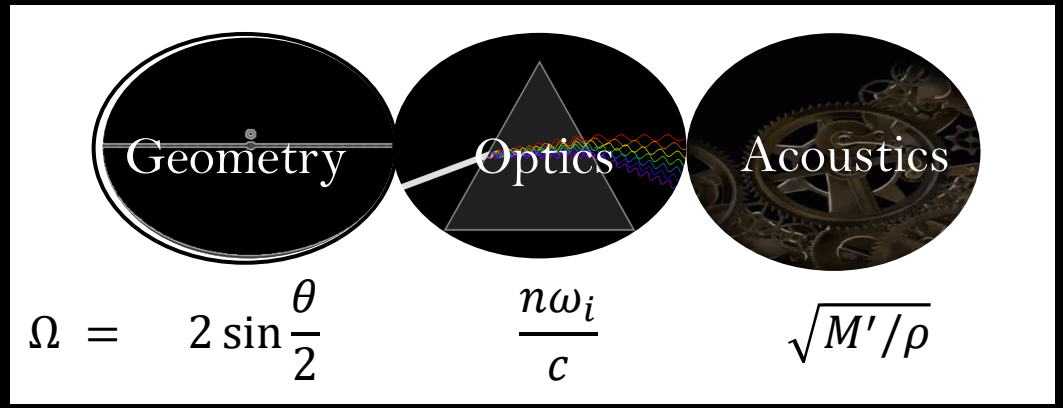
Viscous

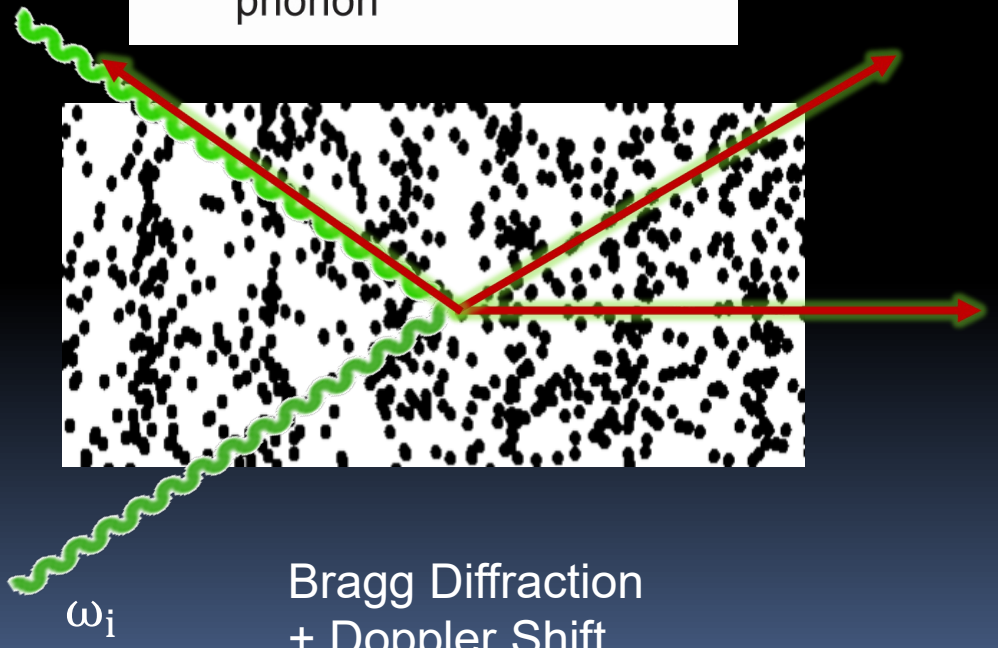
Longitudinal Modulus

Brillouin scattering

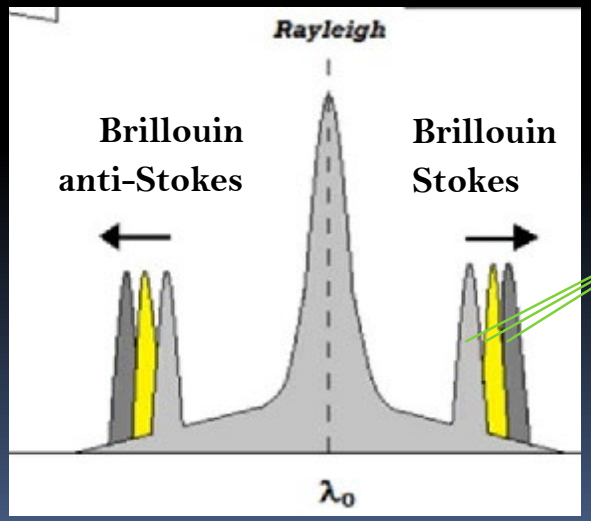


$$\mathbf{k}_s = \mathbf{k}_i \pm \mathbf{q}$$





Bragg Diffraction
+ Doppler Shift

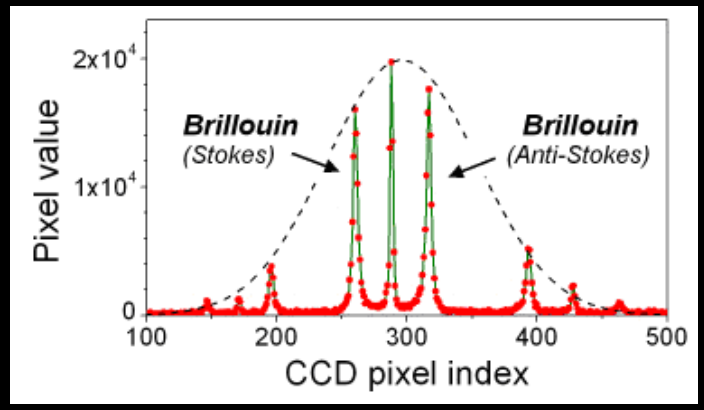


M^*, n, ρ

- Frequency
- Temperature
- Pressure
- Concentration
- Chemical composition
- Water content
- …

Campanella et al., IEEE ICIT, 2016

Brillouin scattering spectroscopy



Brillouin shift ~2-8 GHz (3 – 10 pm)

frequency shift ➡ longitudinal modulus

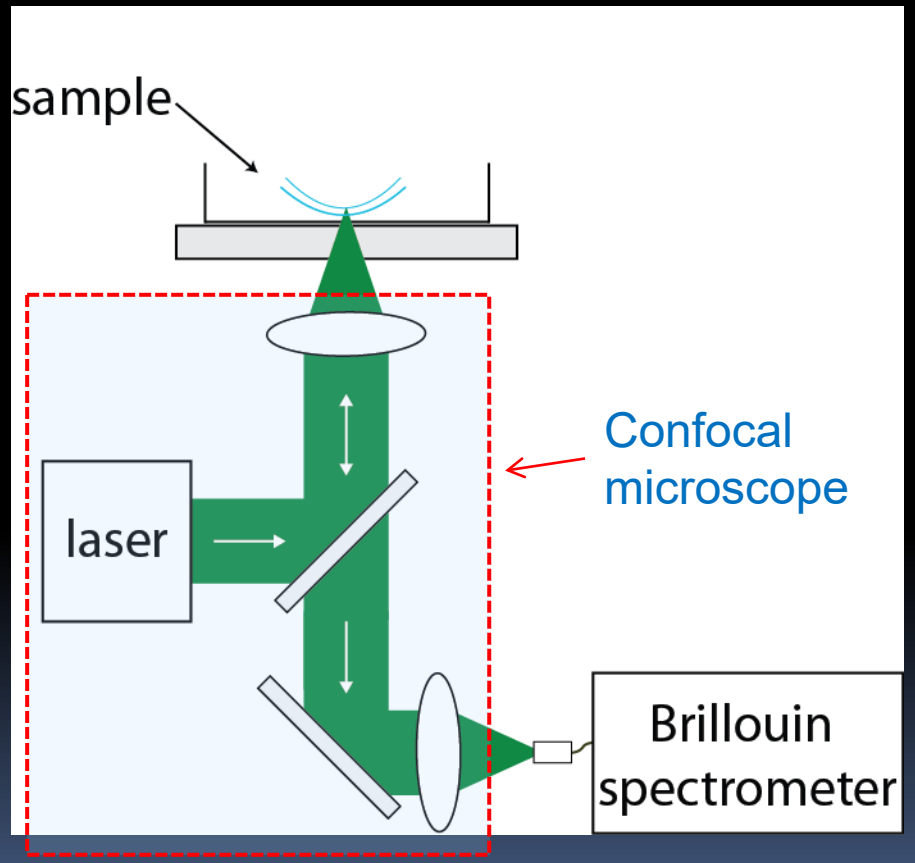
$$\nu = \frac{2n}{\lambda}\sqrt{\frac{M'}{\rho}}\cos\left(\frac{\theta}{2}\right)$$

linewidth ➡ viscosity

$$\Delta\nu = \frac{\alpha}{\pi}\sqrt{\frac{M}{\rho}}$$

Our plan....

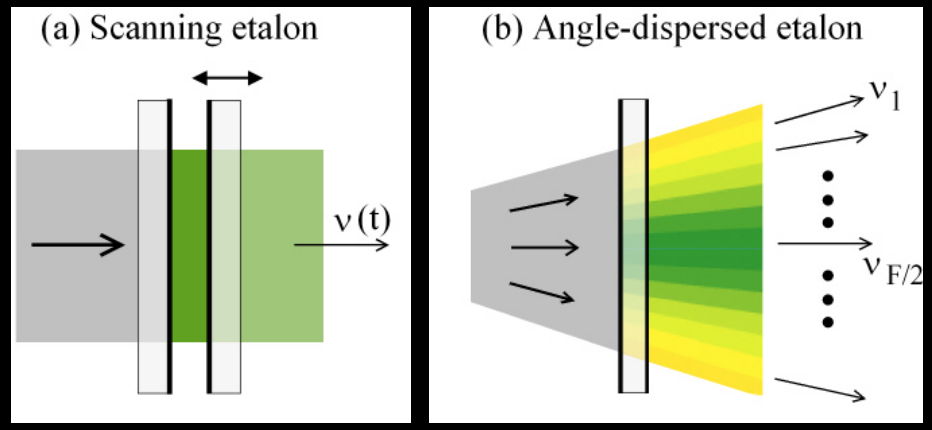
Brillouin Spectroscopy + Optical Microscopy



Challenges

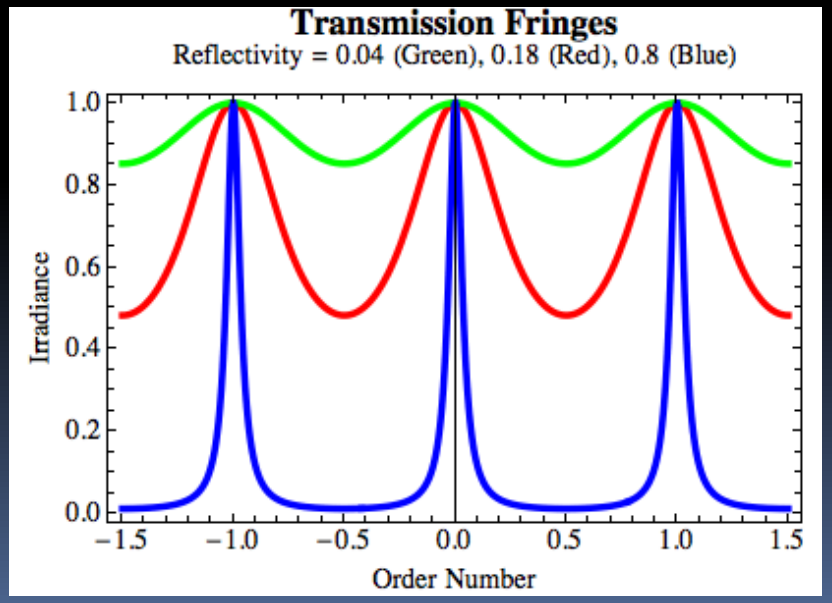
- ***spectral resolution (< 0.001 nm)***
- ***weak (<10^{-9})***
- ***spectral extinction (>> 60 dB)***

Speed challenge



Sandercock Itoh + Koski-Yarger

Single shot
Same losses



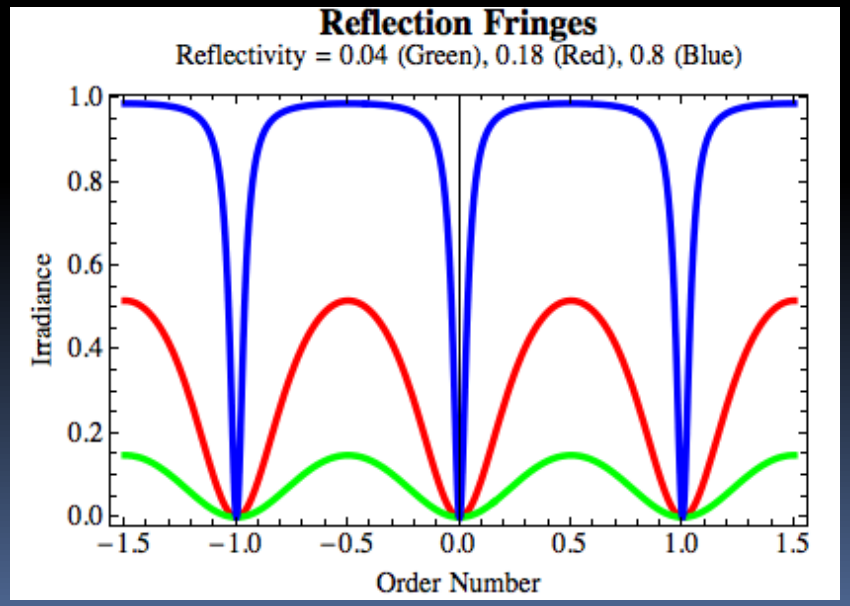
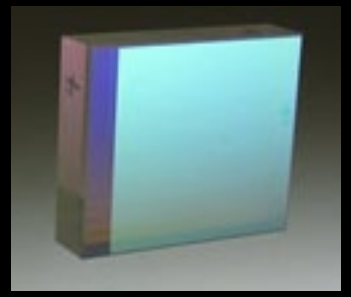


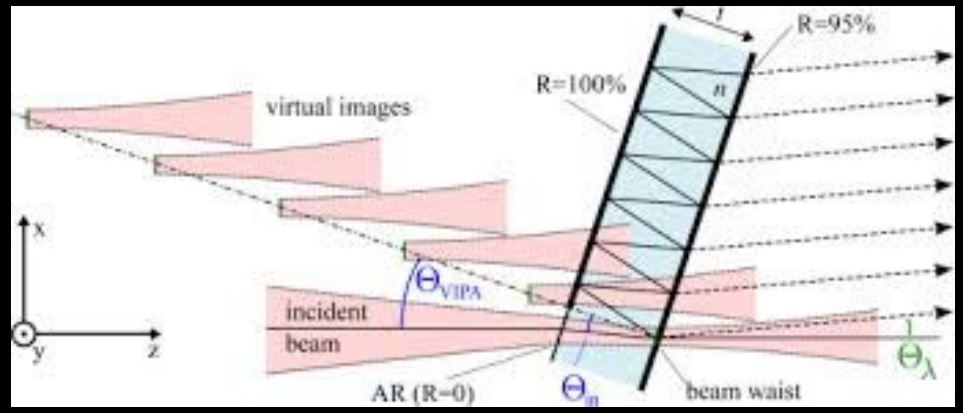
image credit: optics.arizona.edu

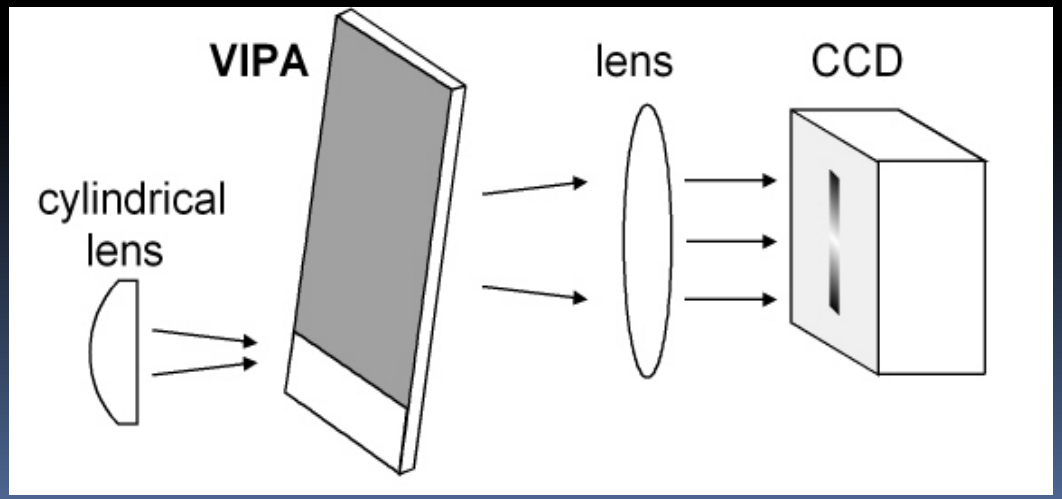
Our throughput solution: VIPA

Virtual Image Phased Array



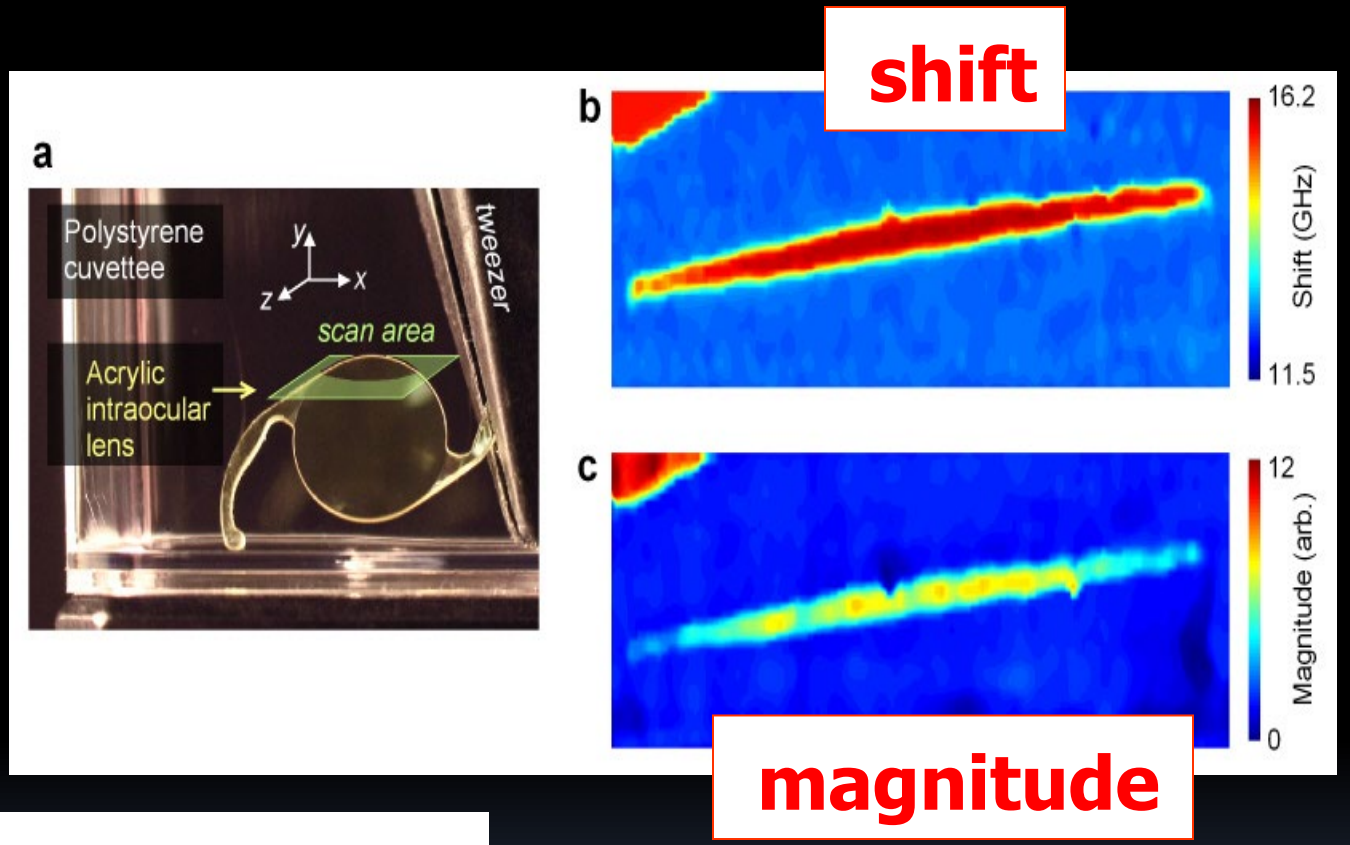
Shirasaki, Weiner





- **"equivalent" to FP**
- **Higher throughput ∝ $\mathcal{F}inesse$**

Brillouin imaging



LETTERS

Confocal Brillouin microscopy for three-dimensional mechanical imaging

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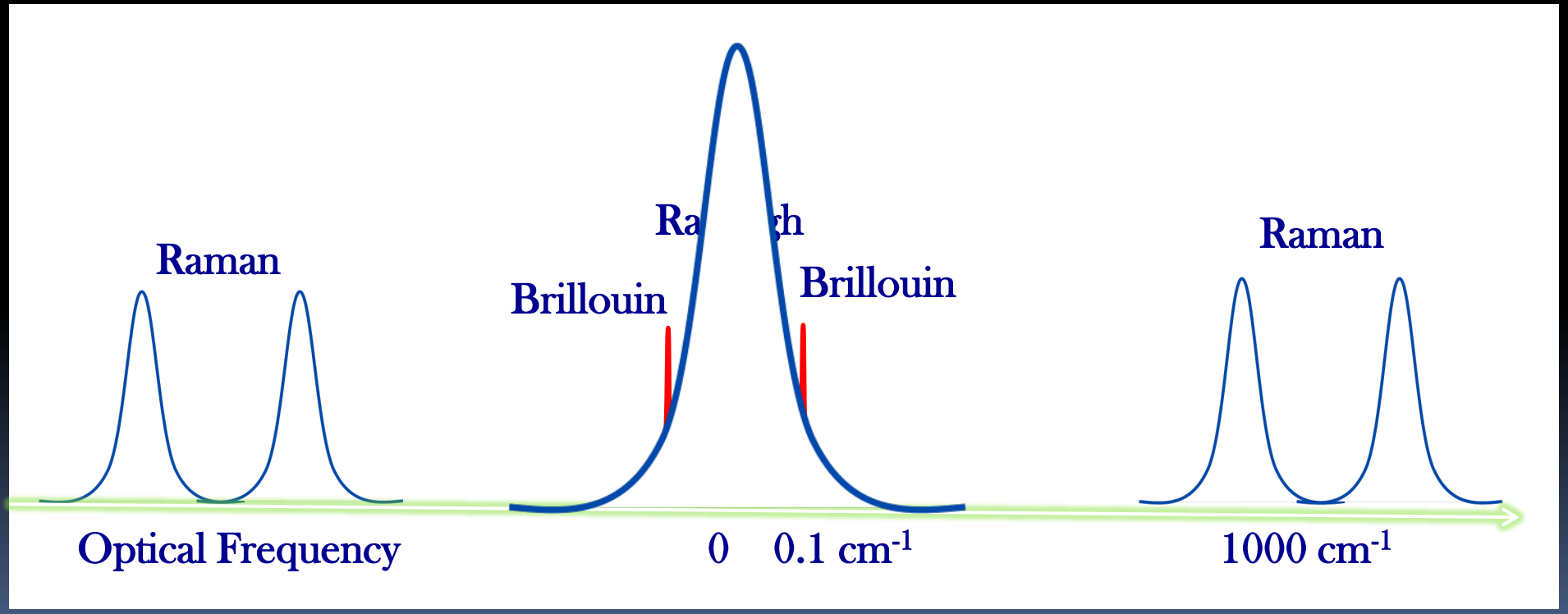
Scarcelli & Yun, Nature Photonics 2, 39 (2008)

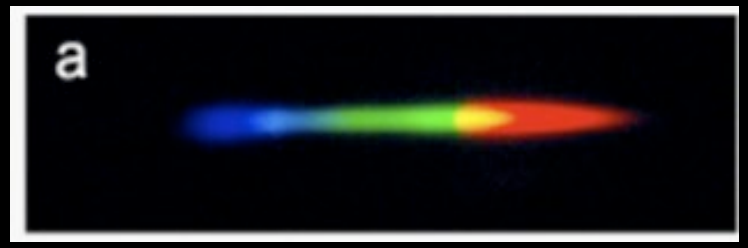
Extinction/contrast challenge…

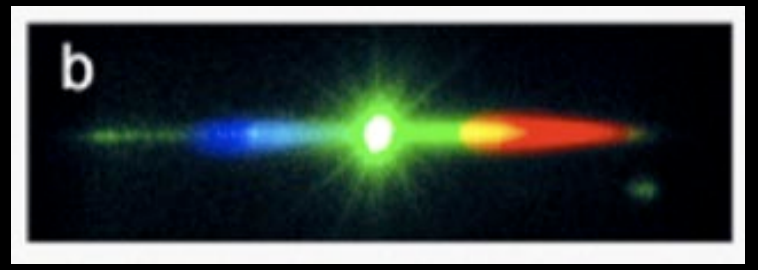
Rejecting elastic scattering

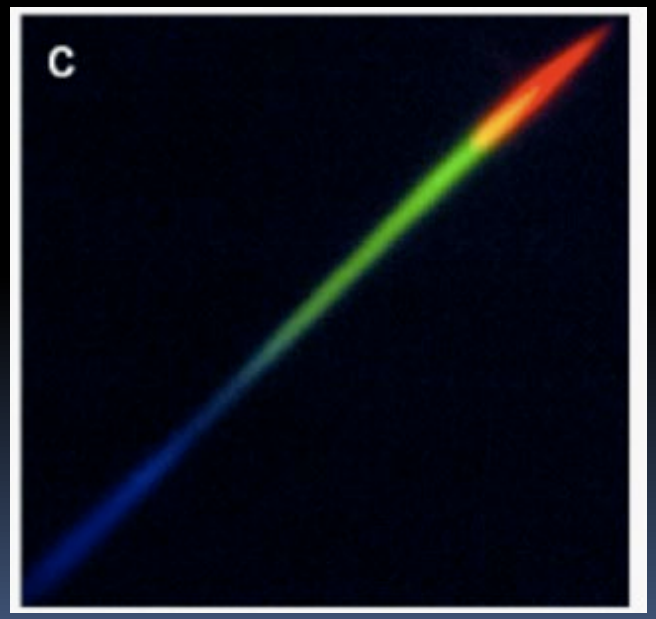
1- Cross-Axis Cascade
2- Apodization
3- Coronagraphy

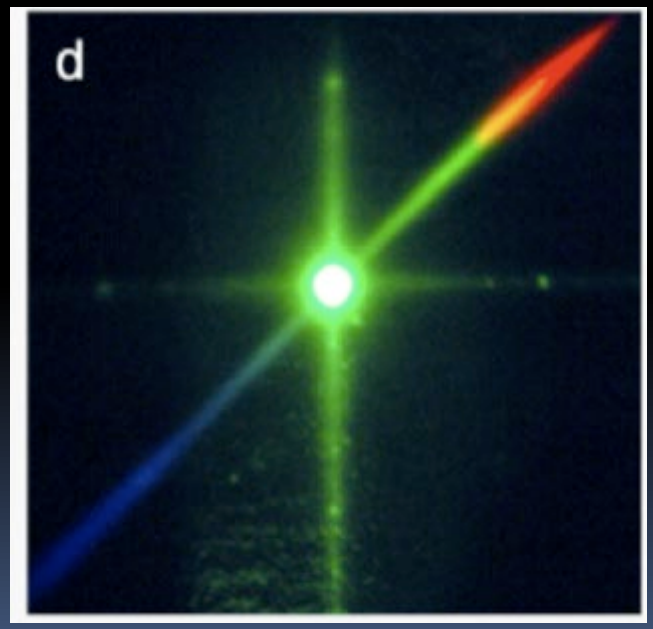


Cross-Axis Configuration



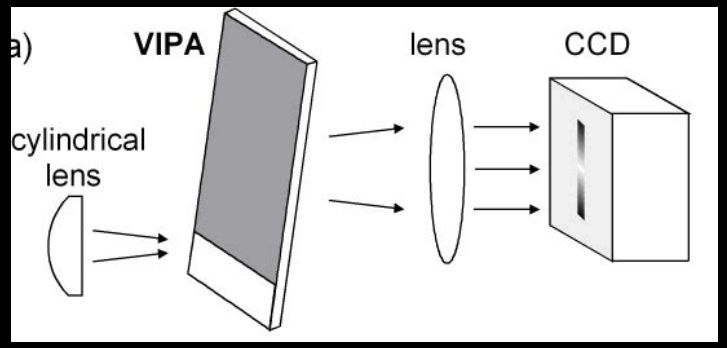




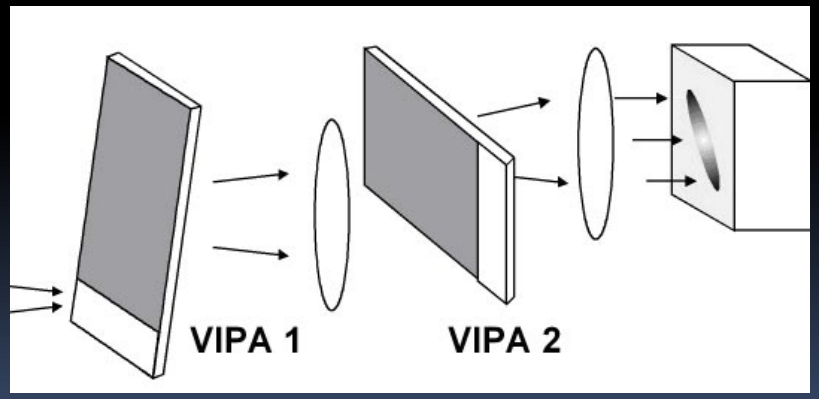


Scarcelli & Yun, Optics Letters (2008)

Cross-axis spectroscopy



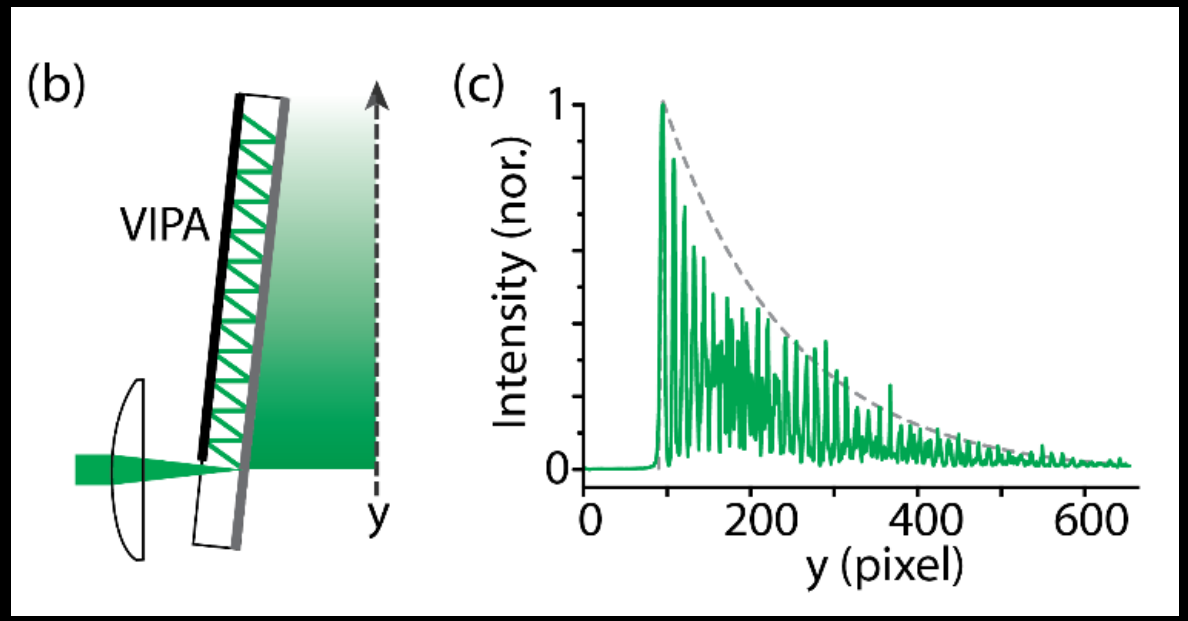
~30 dB

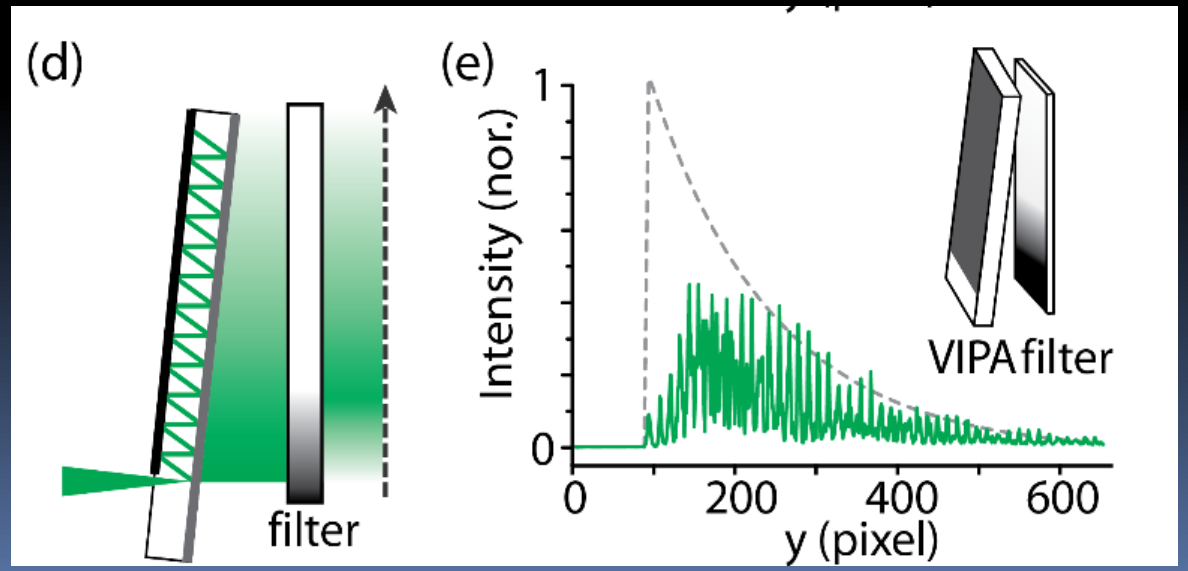


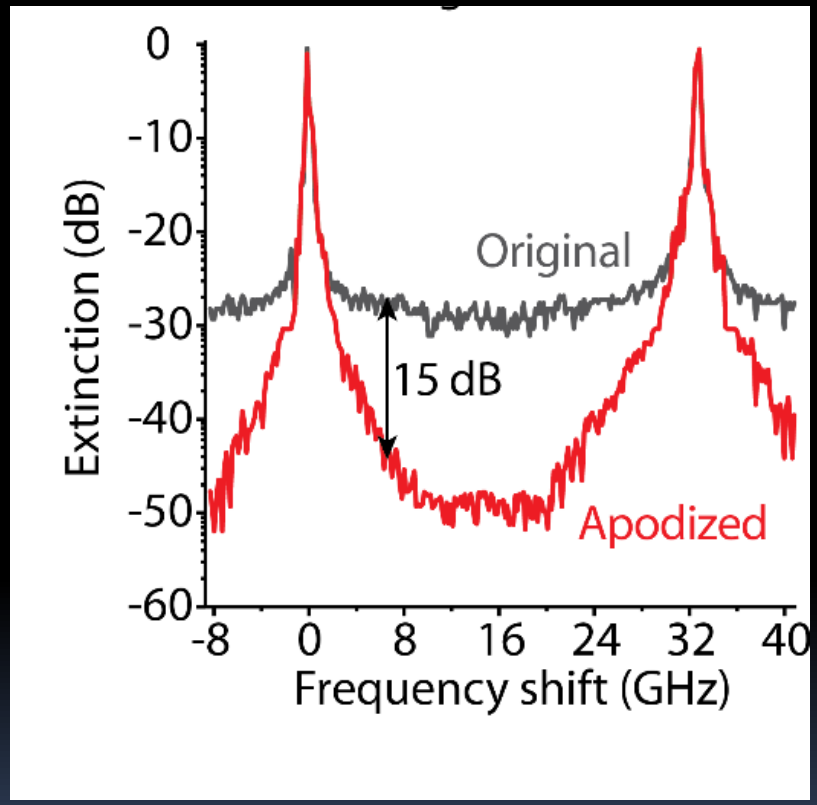
~60 dB

Scarcelli & Yun, Optics Express (2011)

Apodization

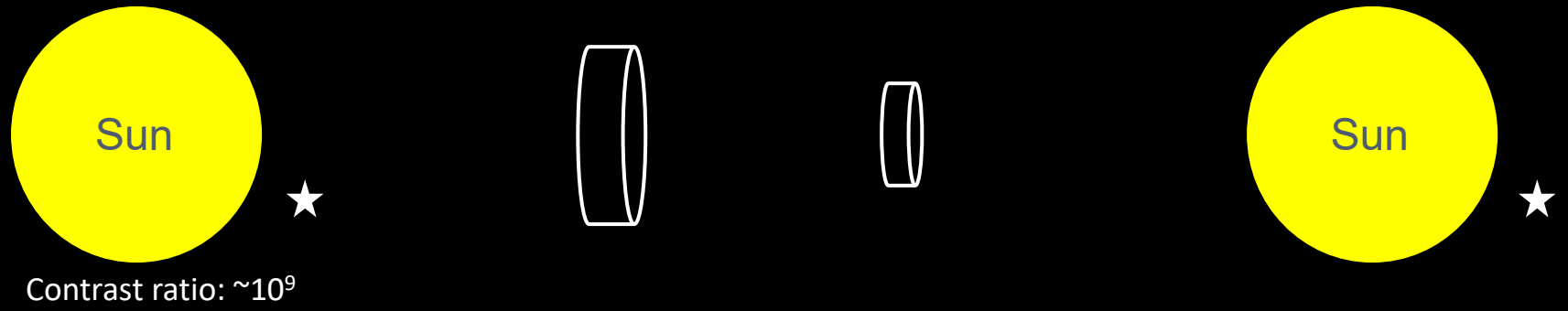






Coronagraphy

Direct imaging of planets



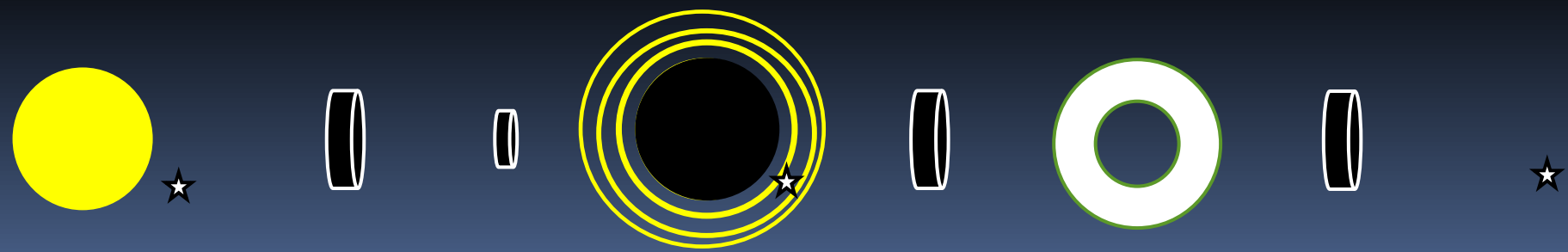
Contrast ratio: ~10^9

Object plane

Telescope

Imaging plane

Lyot coronagraph (Bernard Lyot, 1939)



Object plane

Telescope

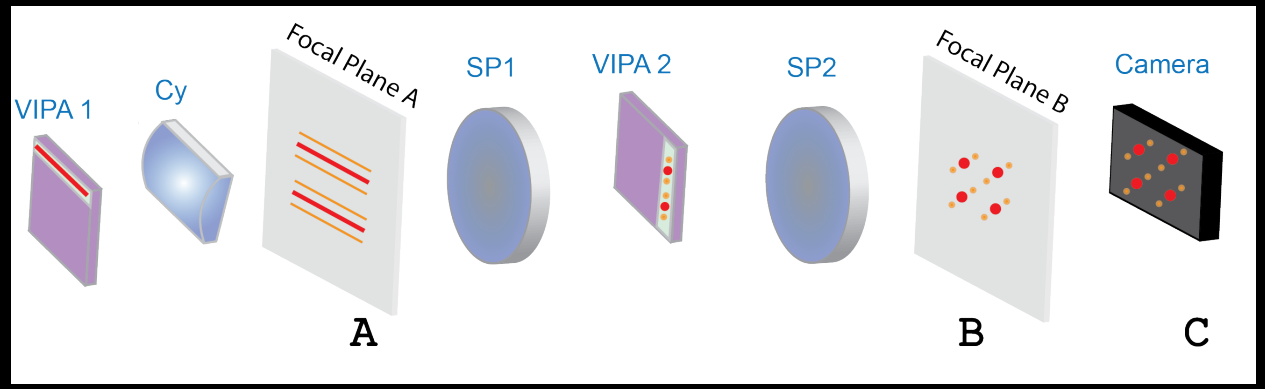
First imaging plane, masked center

Fourier plane with "Lyot stop"

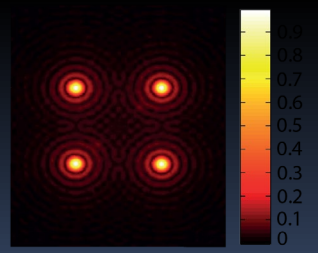
Final imaging plane

The spectral analog of planet imaging

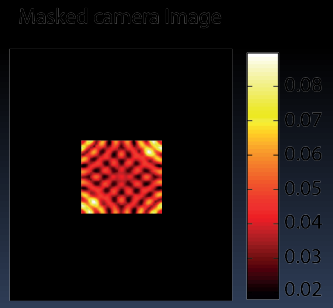
Double stage VIPA spectrometer



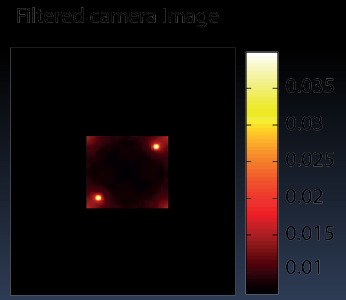
The output of the spectrometer (illustration):



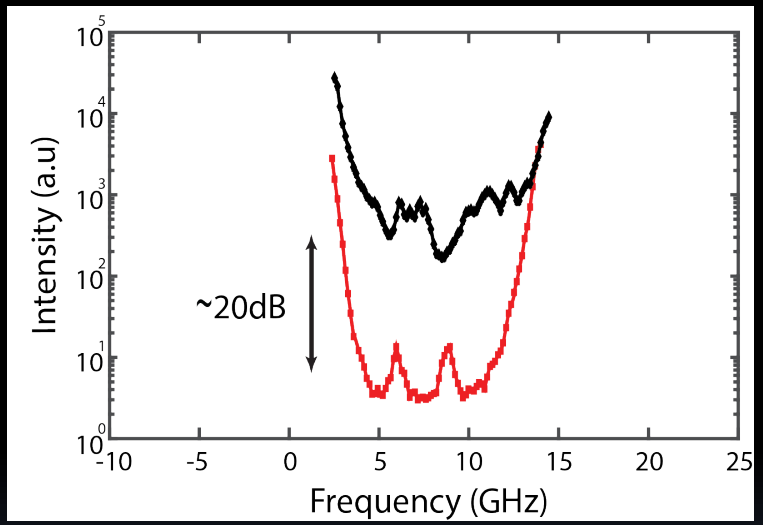
Airy patterns surrounding the high intense peaks



Covering the peaks doesn't remove the Airy patterns

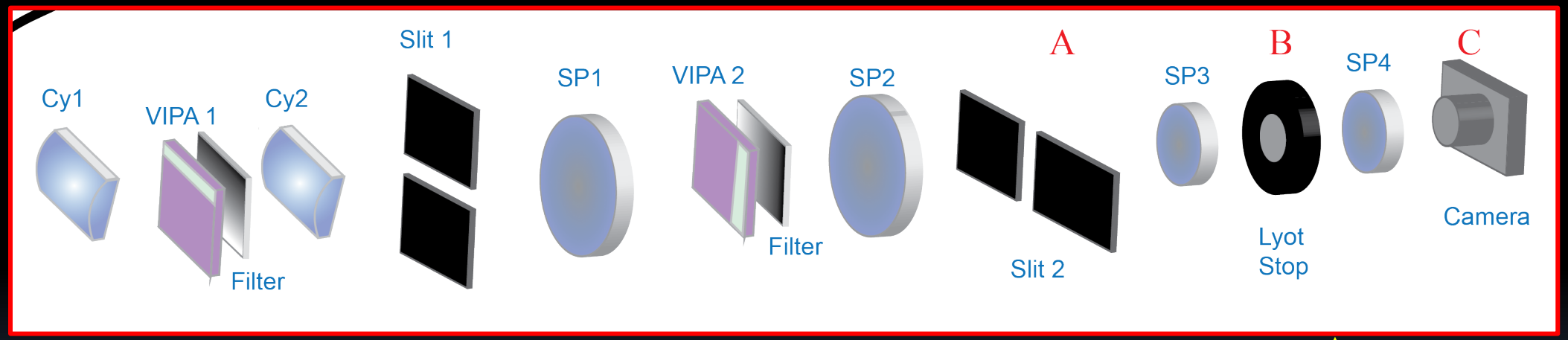


High frequency removal by the Lyot coronagraph



Full spectrometer

Two orthogonal VIPA-stages



Two Apodizations

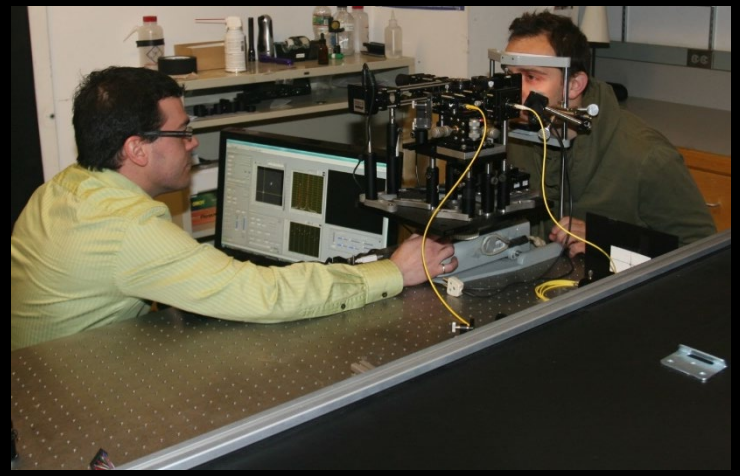
Coronography

Zhang and Scarcelli, ***Nature Protocols***, 16, 1251 (2021)

Brillouin Instruments (+ disclosures)

Brillouin ophthalmoscope

- *Start-up: Intelon*

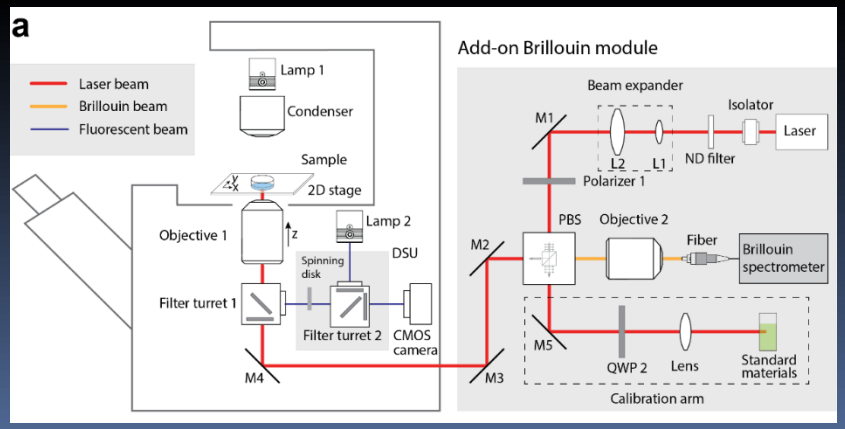


Scarcelli, Besner, Pineda, Yun JAMA Opth. 133, 480 (2015)

Brillouin flow cytometer

Zhang et al., ***Lab on Chip***, 17, 571 (2017)

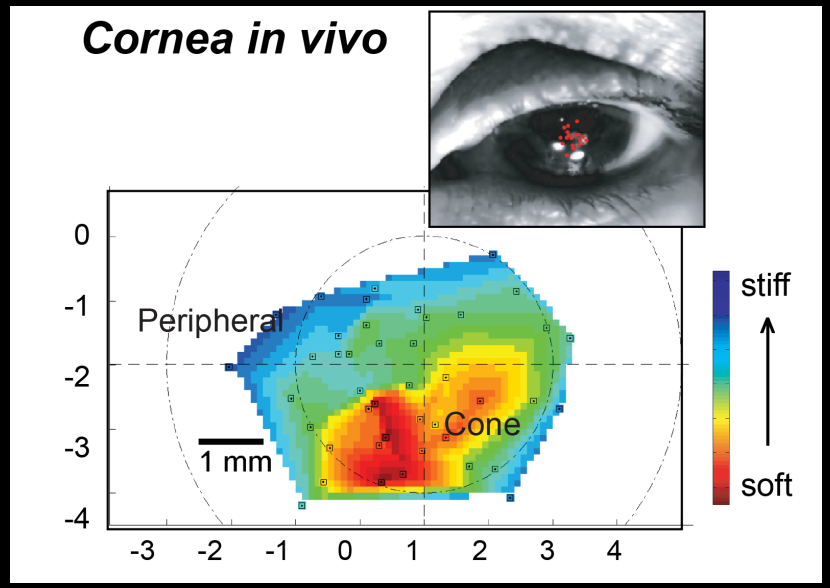
Add-on Brillouin module for confocal microscopes

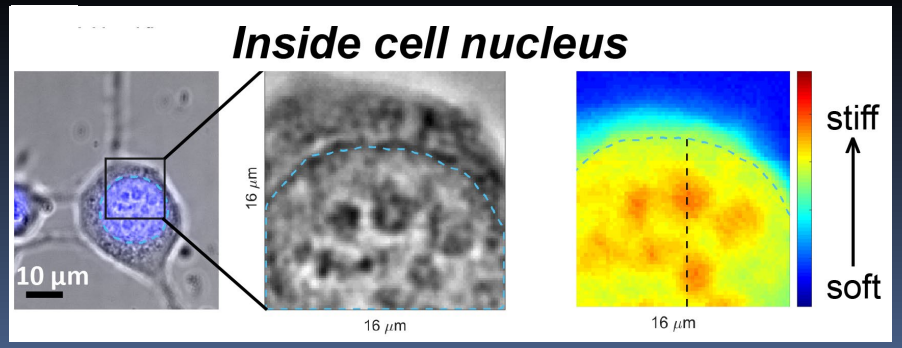


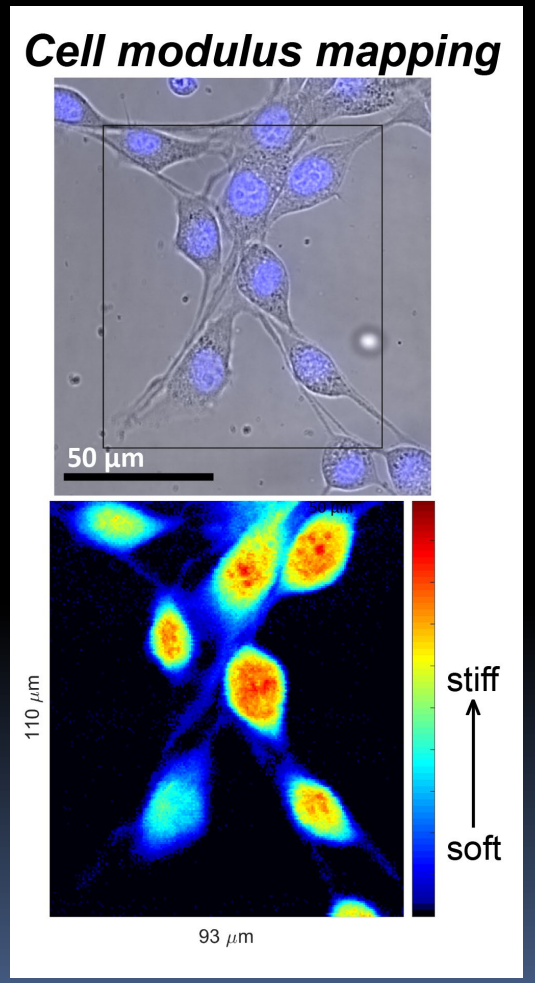
Zhang and Scarcelli, ***Nature Protocols***, 16, 1251 (2021)

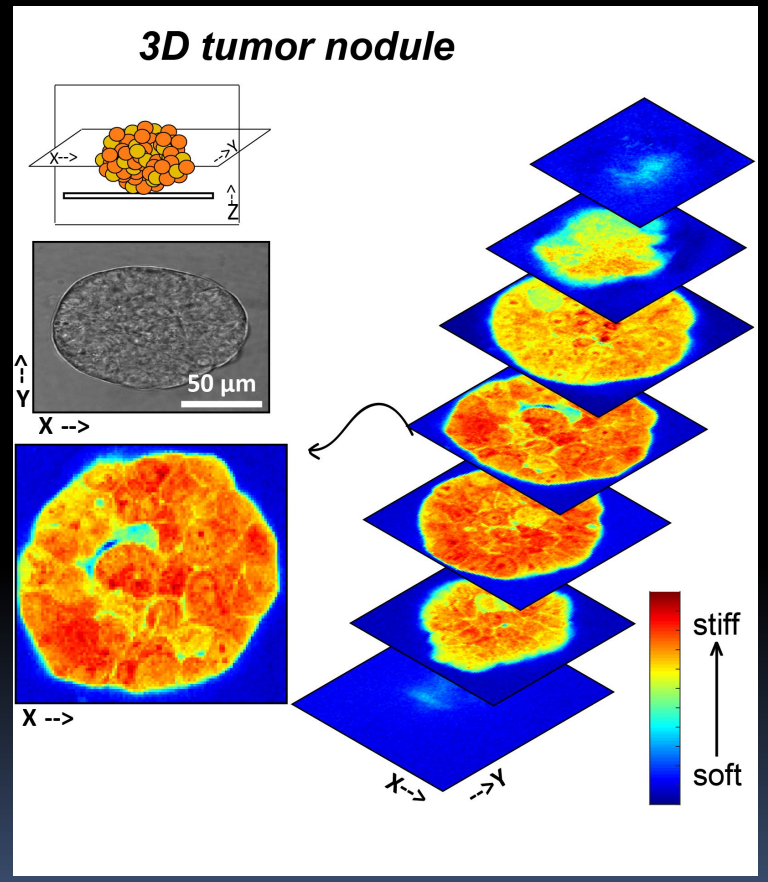
Confocal Brillouin microscopy.....

10 mW….20ms….10 MHz

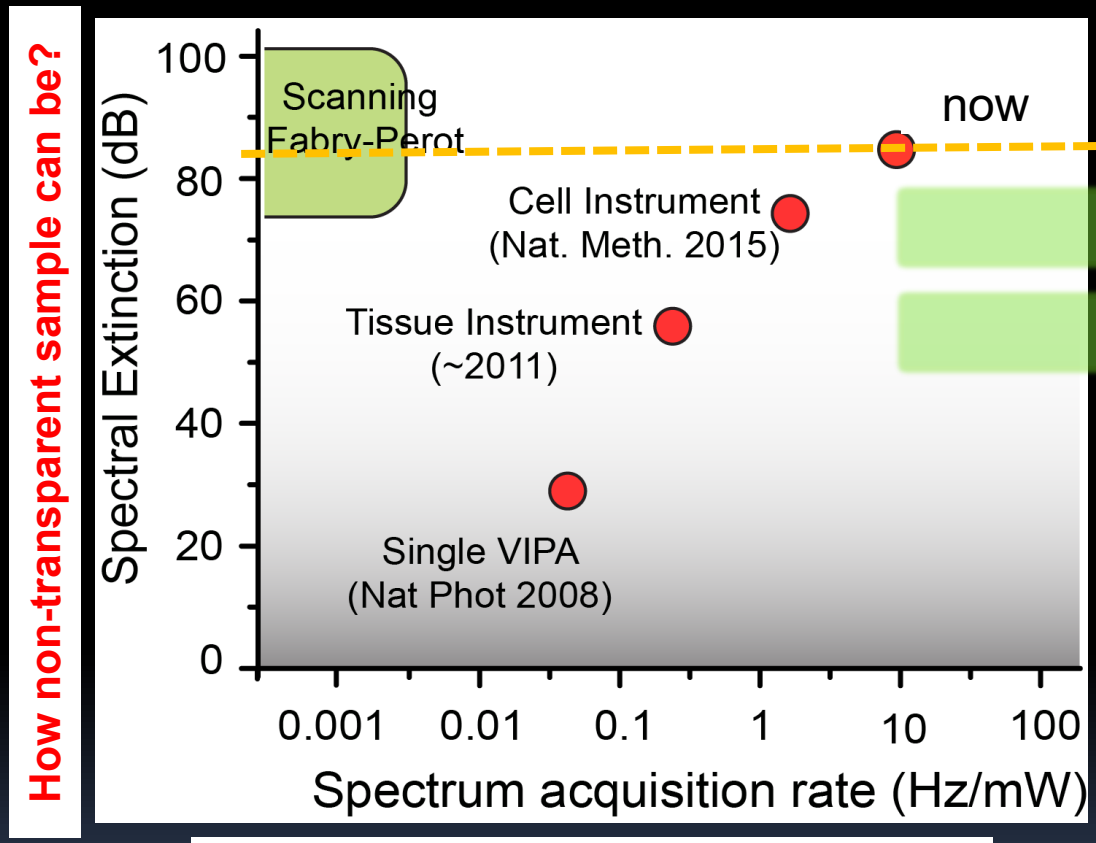








Summary



Shot-noise limit in tissue

Fiore et al. APL(2016)

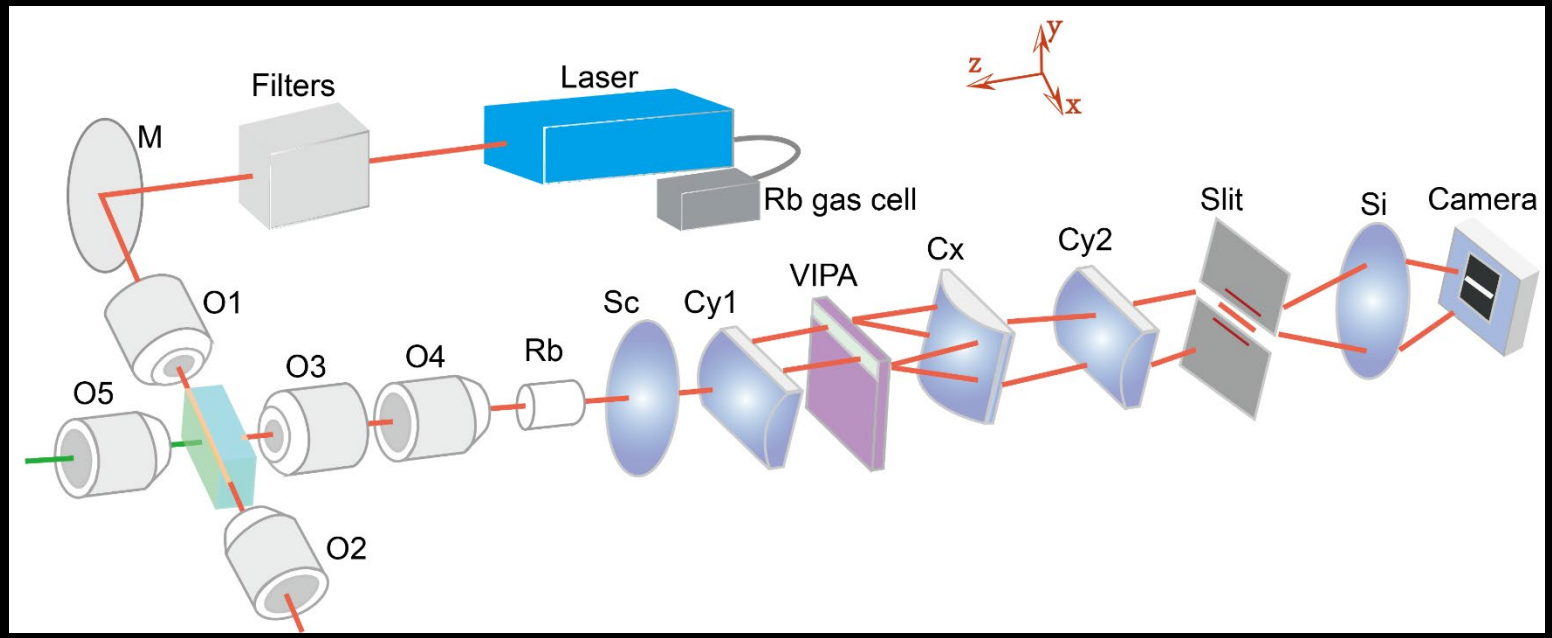
live-cell imaging 2014 -

Safe in vivo human ocular tissue 2011 -

Edrei and Scarcelli, Applied Physics Letters, 119, 243602, (2018)
Edrei, Gather and Scarcelli, Optics Express 25 6895 (2017)
Zhang, Fiore, Yun, Kim and Scarcelli, Scientific Reports 6, 35398 (2016)
Shao, Besner, Zhang, Scarcelli and Yun, Optics Express 24, 22232 (2016)
Berghaus, Zhang, Yun, Scarcelli, Optics Letters, 40, 4436 (2015).
Scarcelli and Yun, Optics Express 20, 9197-9202. (2012).
Scarcelli and Yun, Optics Express 19, 10913; (2011).
Scarcelli , Kim and Yun, Optics Letters 33, 2979; (2008).

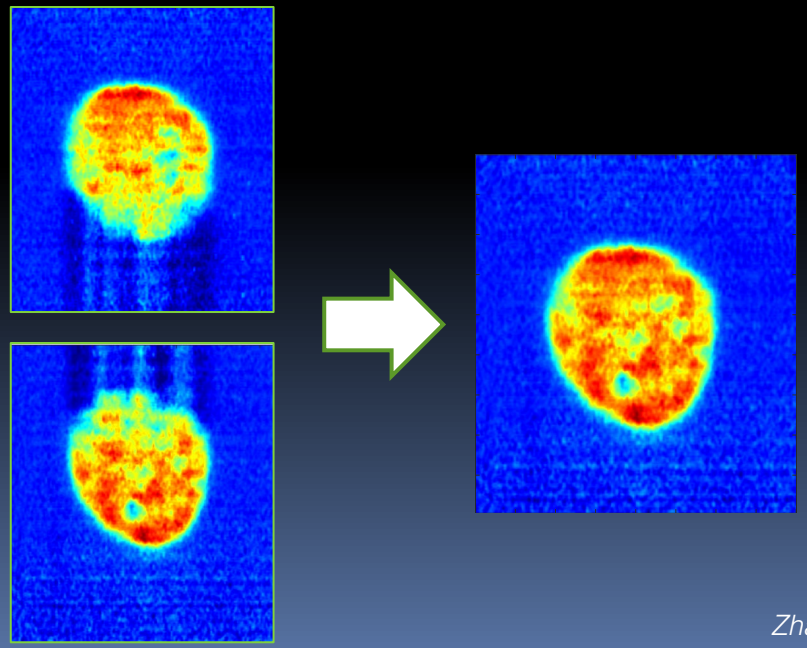
Towards high-throughput

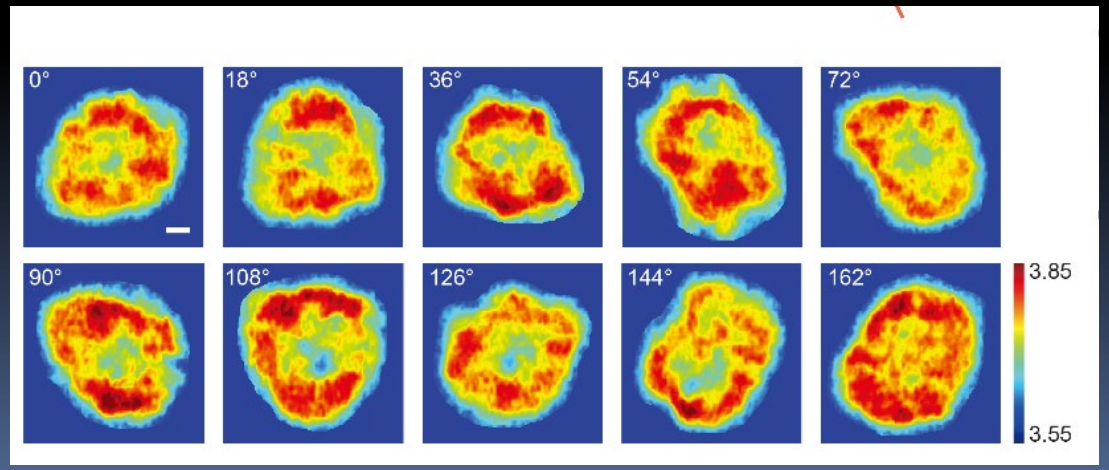
Dual Linescan…



1 ms/pixel

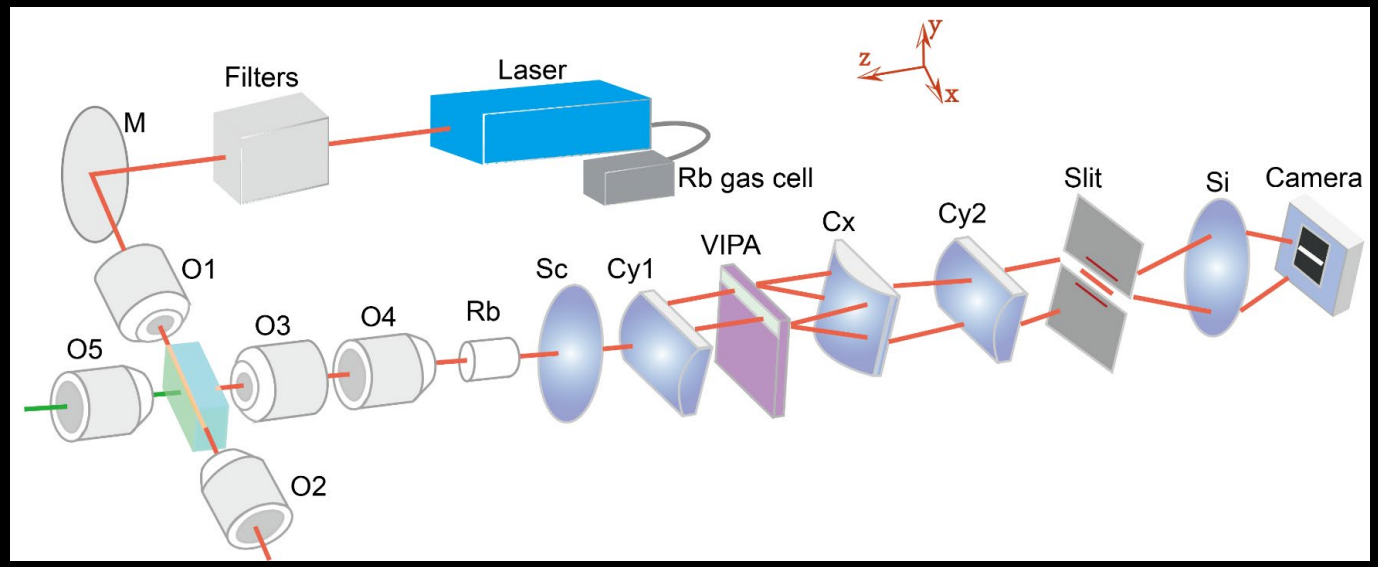
10x less light-dose



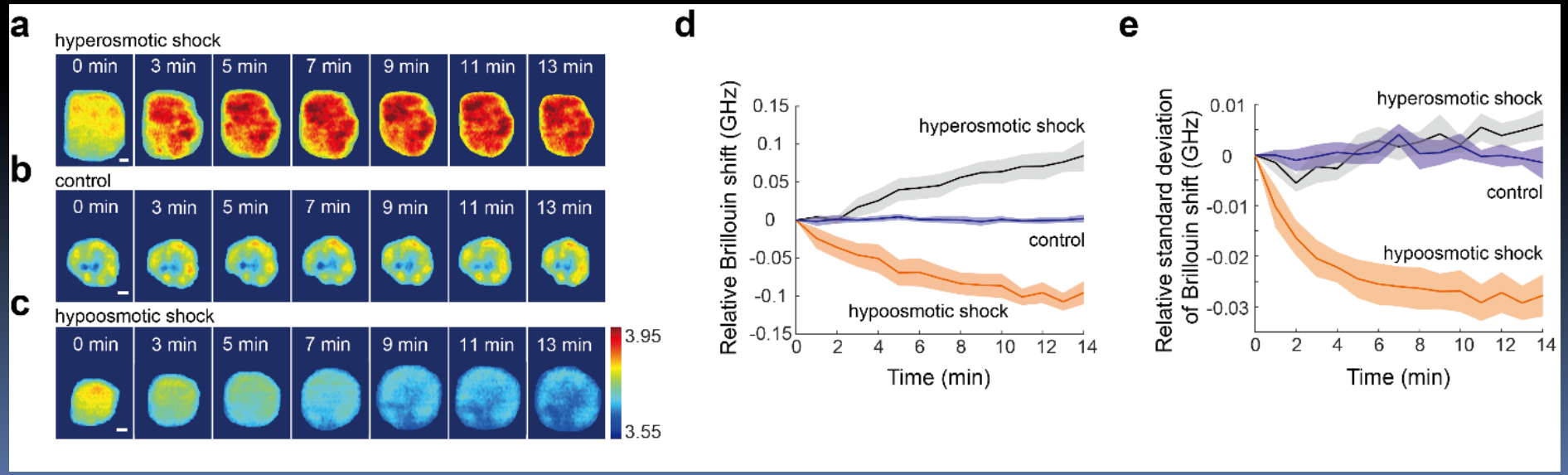


Zhang, Nikolic, Tanner & Scarcelli, Nature Methods (in revision)

Dual Linescan…

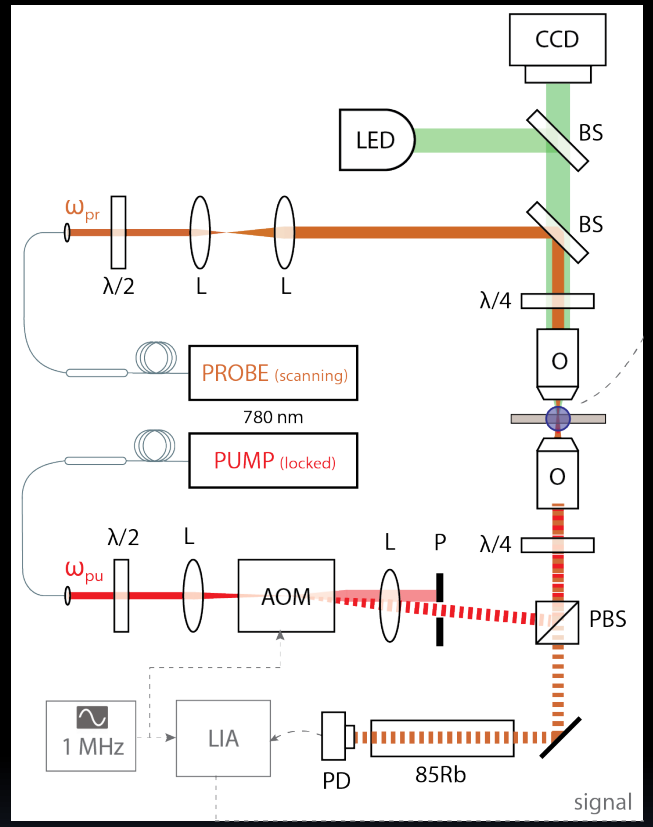


Osmotic shock



Zhang et al., Nature Methods (in revision)

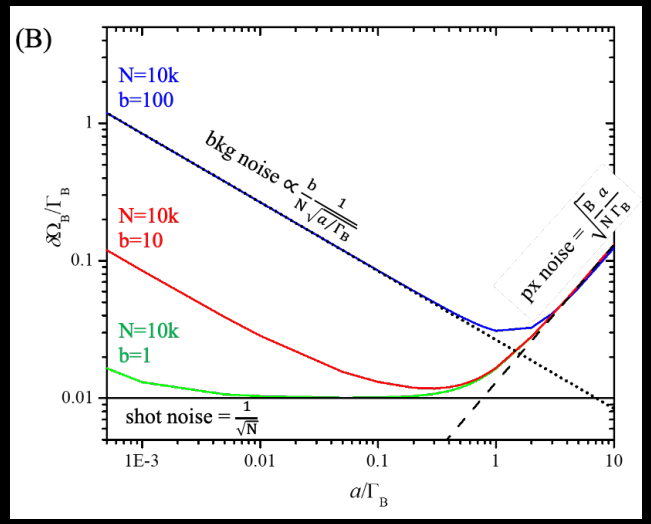
Stimulated…

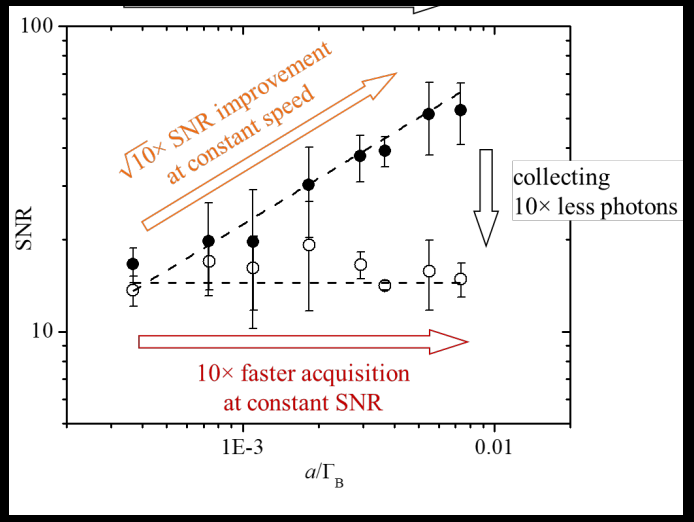


Remer & Bilenca. Nature Methods (2020)

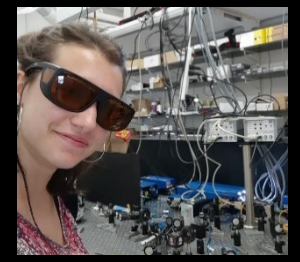
CW….

Localization-based detection





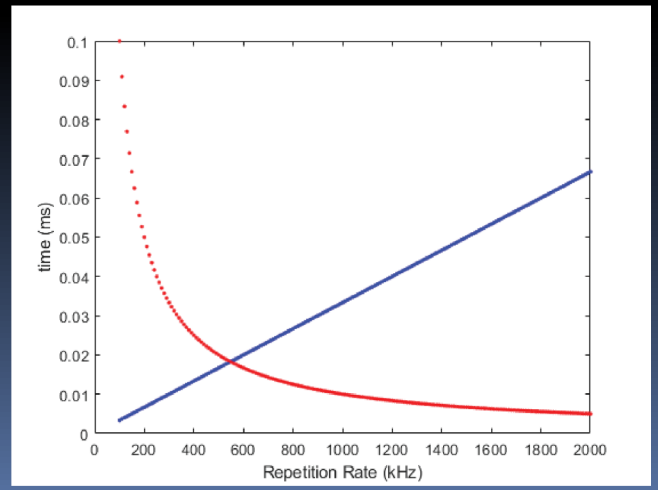
Zanini & Scarcelli, APL Photonics 7, 056101 (2022)



Dr. Giulia Zanini

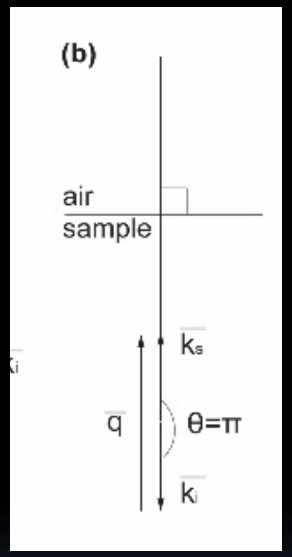
10x speed-up

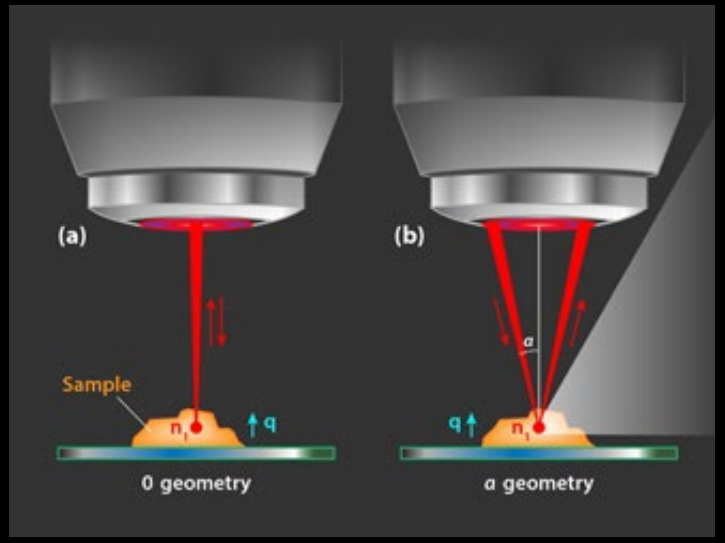
Pulsed SBS (with B. Redding, NRL)

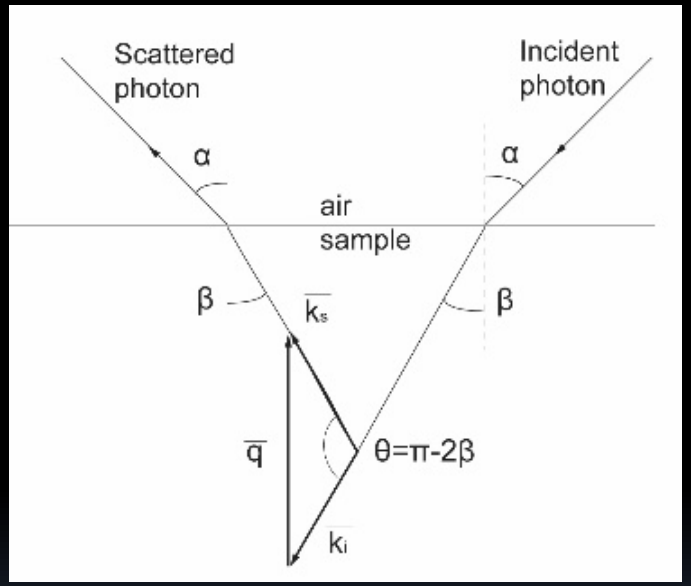


Photon-phonon label-free contrast mechanisms

Dual-geometry Brillouin measurements







PHYSICAL REVIEW LETTERS **122,** 103901 (2019)

Editors' Suggestion Featured in Physics

Potential of dual-geometry Brillouin interaction

Antonio Fiore

OPTICAL DISPERSION

$$\mathbf{k} = \frac{\omega}{c}\mathbf{n} = \frac{\omega}{c}[\mathbf{n}_1(\omega) + i\mathbf{n}_2(\omega)]$$

ACOUSTIC DISPERSION

$$\mathbf{q} = \frac{\Omega}{\mathbf{v}(\hat{\mathbf{q}}, \Omega)} + i\frac{\Gamma(\hat{\mathbf{q}}, \Omega)\mathbf{q}^2}{2\mathbf{v}(\hat{\mathbf{q}}, \Omega)}$$

shift

$$\rightarrow \quad n_1(\omega_i) = \left(\frac{\sin^2\alpha}{1-R^2}\right)^{\frac{1}{2}}$$

Index (real part)

$$v(\hat{q}) = \frac{\Omega_0 c}{2\omega_i}\left(\frac{\sin^2\alpha}{1-R^2}\right)^{-\frac{1}{2}}$$

Acoustic speed

linewidth

$$\rightarrow \quad n_2(\omega_i) = \frac{1}{2}\left(\frac{sin^2\alpha}{1-R^2}\right)^{\frac{1}{2}}\frac{\Delta\Omega_\alpha - \Delta\Omega_0}{\Omega_\alpha - \Omega_0}$$

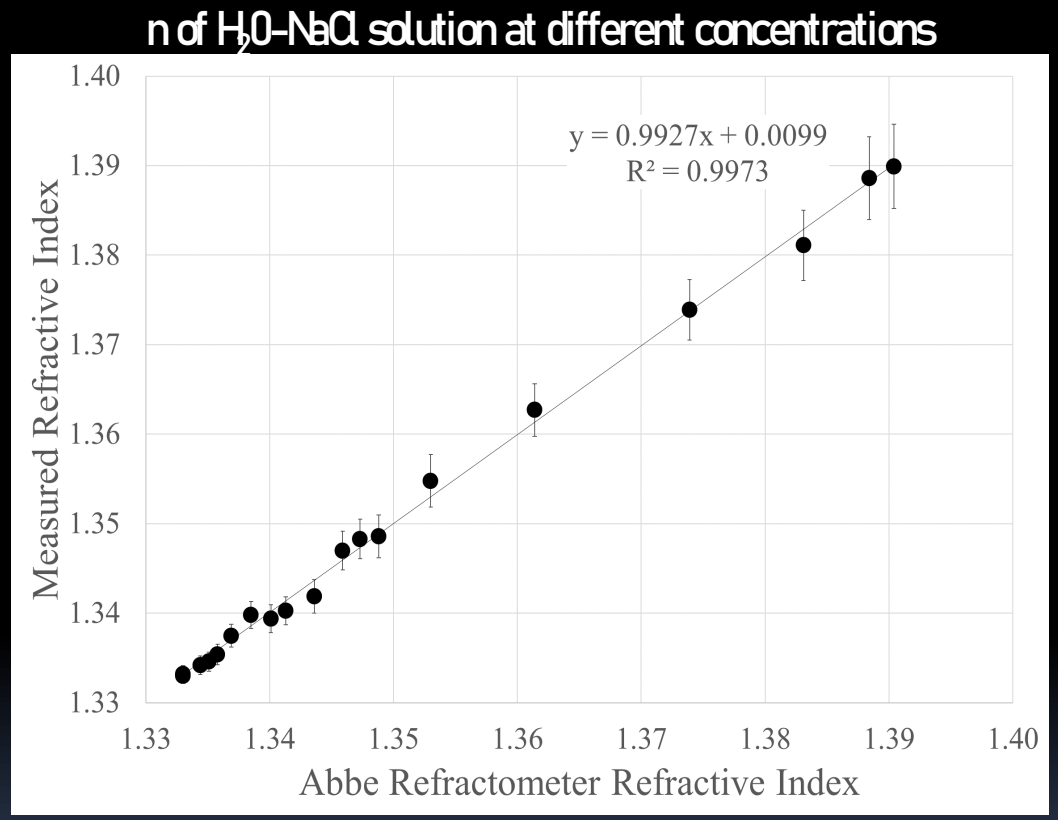
Index (imaginary part)

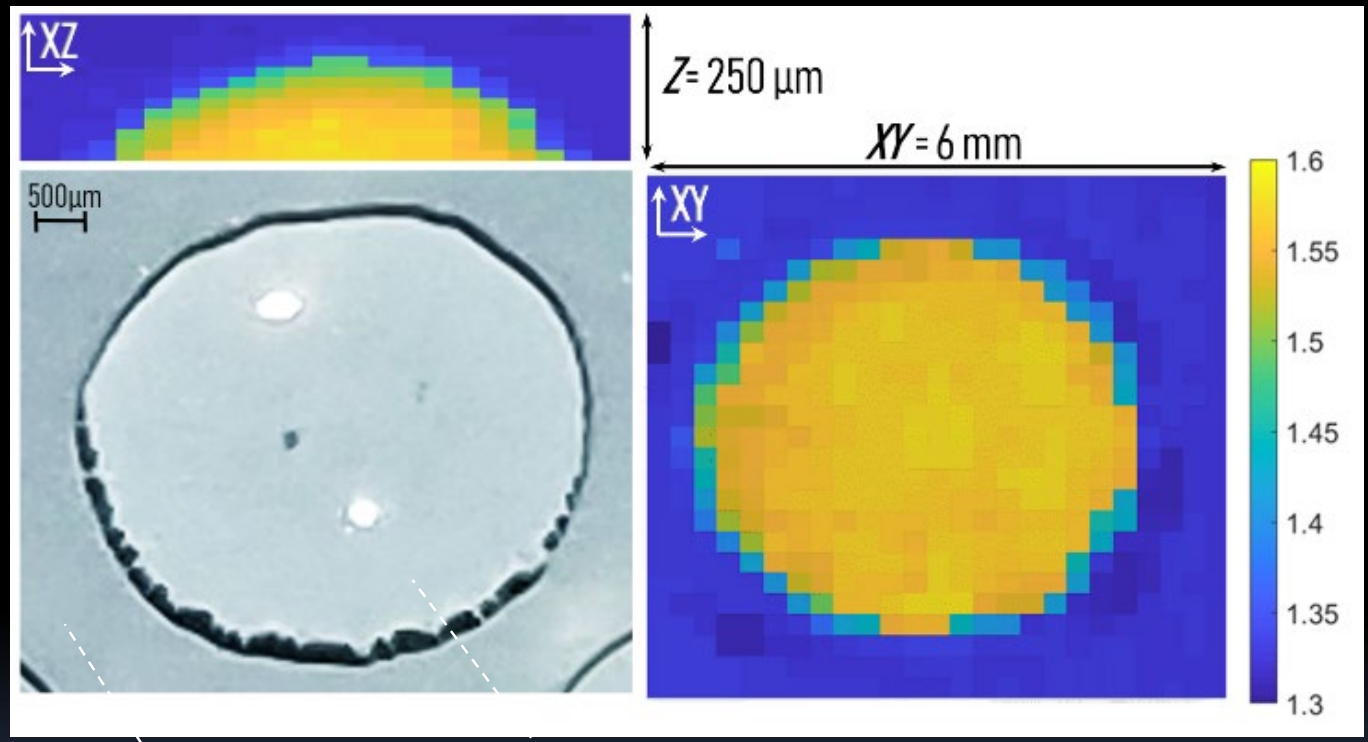
$$\Gamma(\hat{q}) = \frac{c^2}{4\omega_i^2}\left(\frac{sin^2\alpha}{1-R^2}\right)^{-1}\left(\frac{\Omega_\alpha\Delta\Omega_0 - \Omega_0\Delta\Omega_\alpha}{\Omega_\alpha - \Omega_0}\right)$$

Viscosity

Fiore & Scarcelli, Physical Review Letters (2019)

3D Refractive index mapping in epi-detection





CH-BOH (n=1.329)

NOA61 (n=1.56)

Fiore & Scarcelli, Physical Review Letters (2019)