Morpho-mechanical study of 3D cellular assemblies with confocal Brillouin light scattering

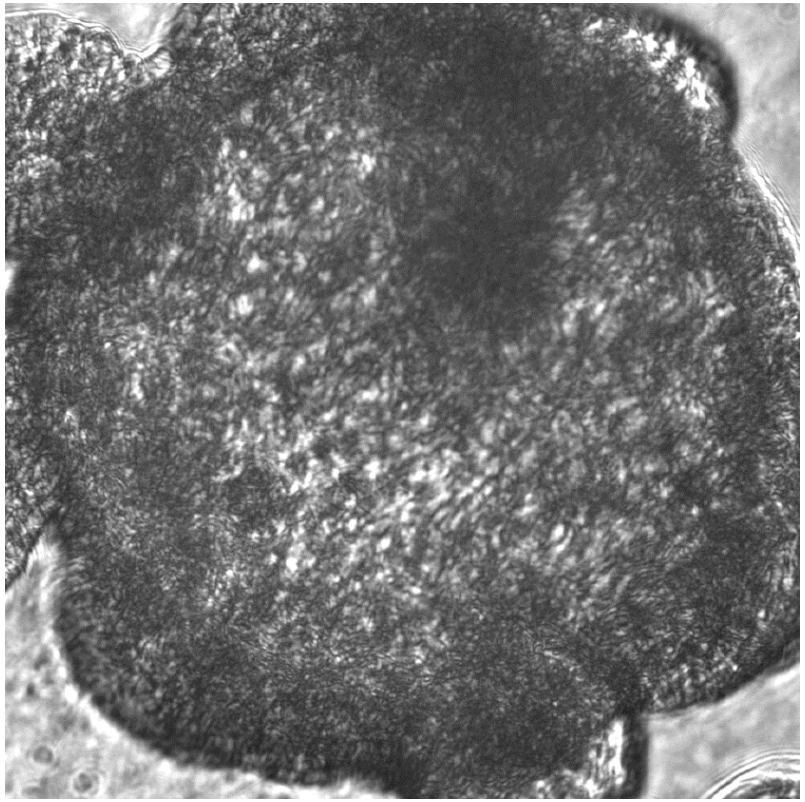
Pierre Bouvet

Congrès des 150 ans de la Société Française de Physique

07/07/2023

How can we analyze 3D cellular assemblies?

Brightfield Microscopy



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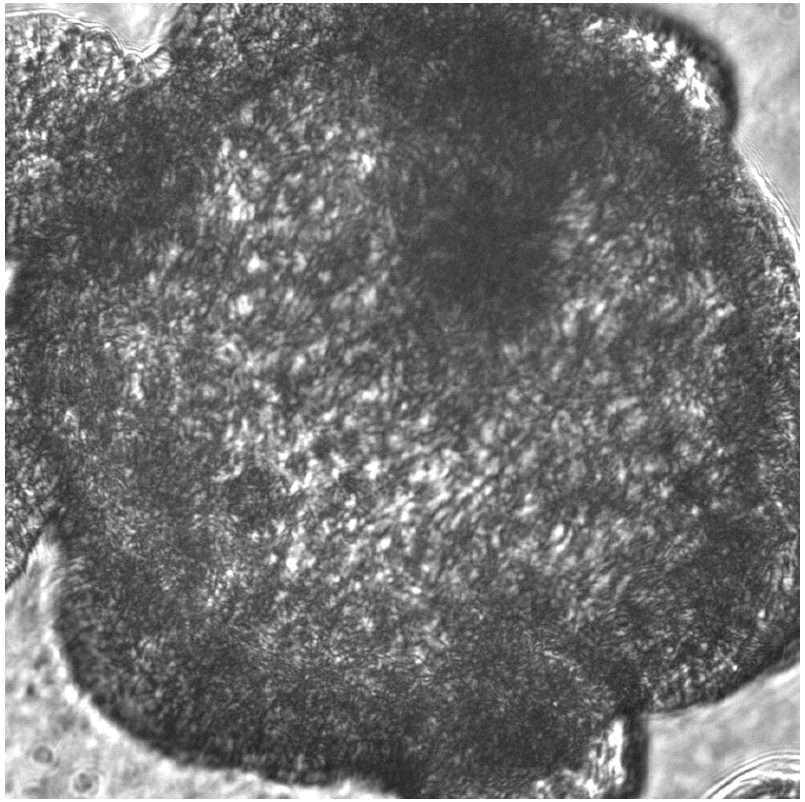
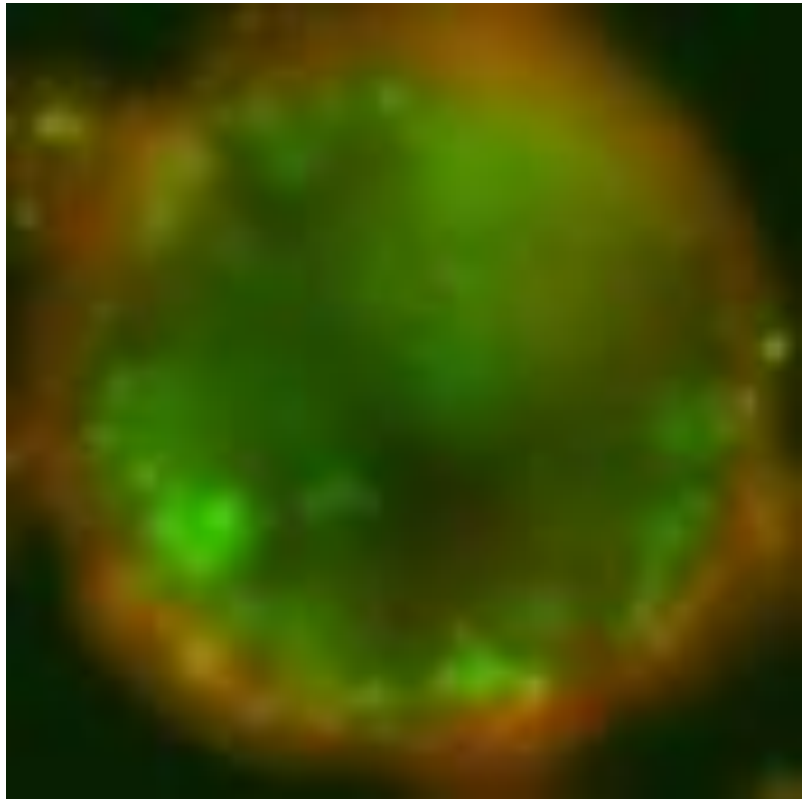


Fluorescence



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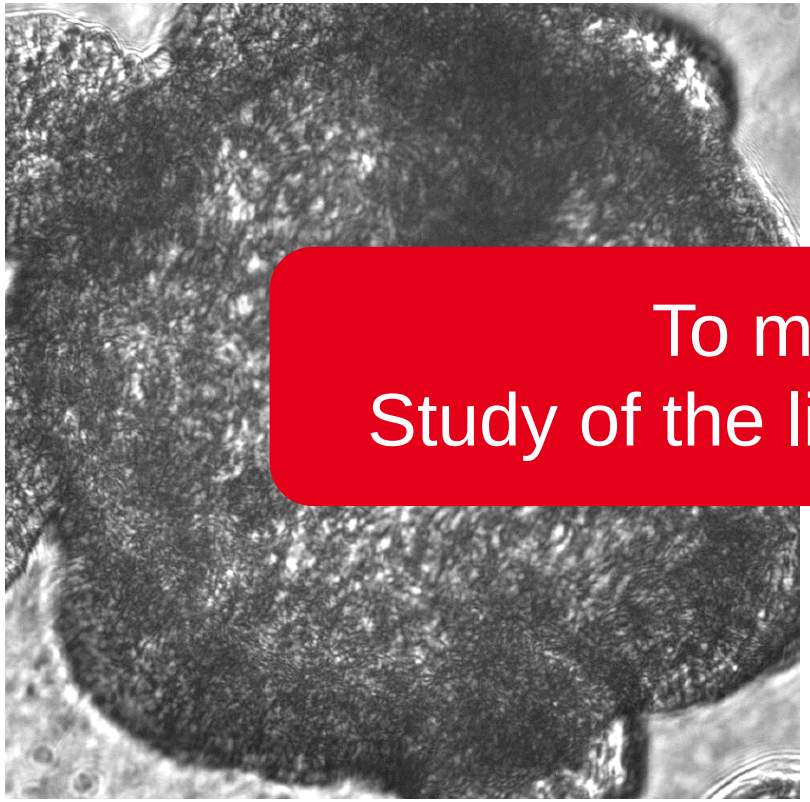

Fluorescence

To minimize invasiveness:
Study of the light incoming from the sample

How to measure 3D cellular assemblies without damaging them?

- Study the electromagnetic wave coming from the sample

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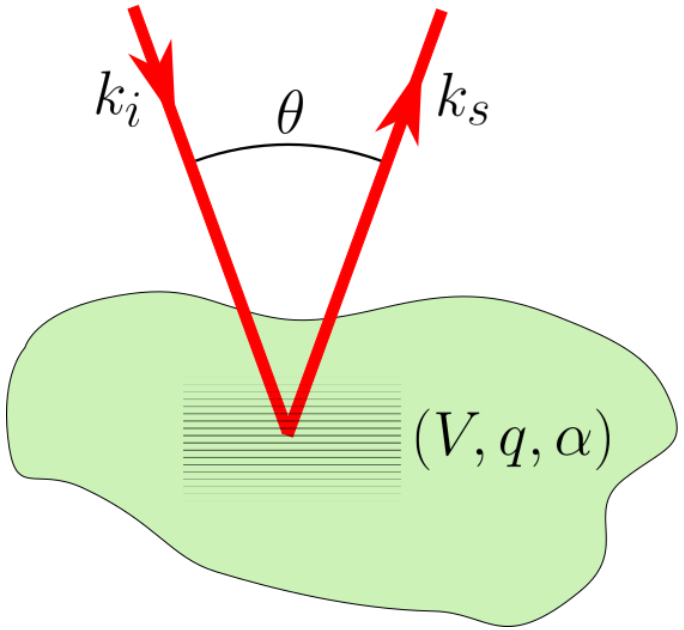

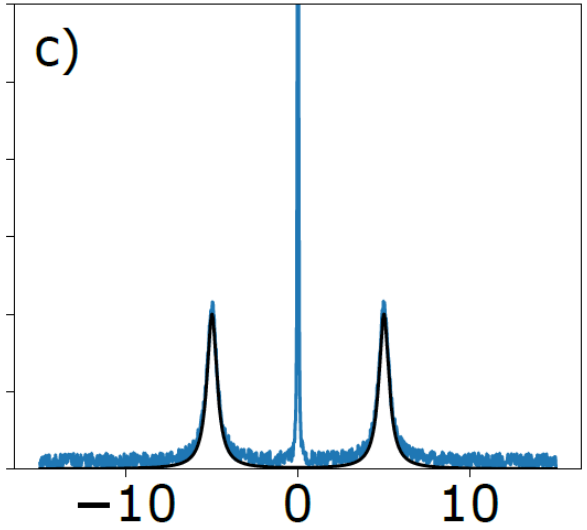

Frequency shift with respect to laser (GHz)

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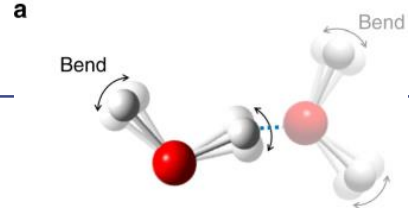
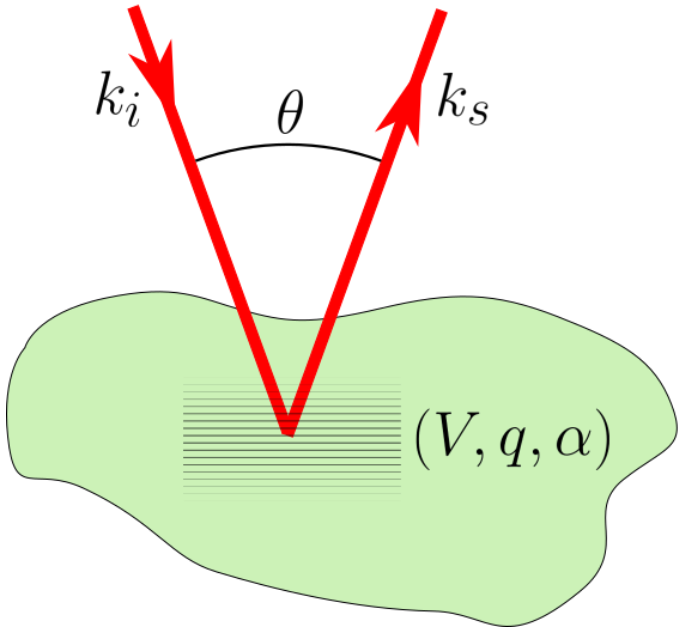
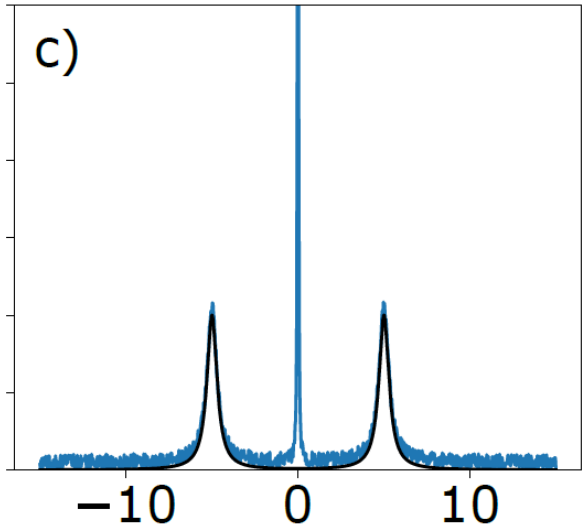
Raman scattering
High energy vibrations
⇒ Large shifts

Intra-molecular movements







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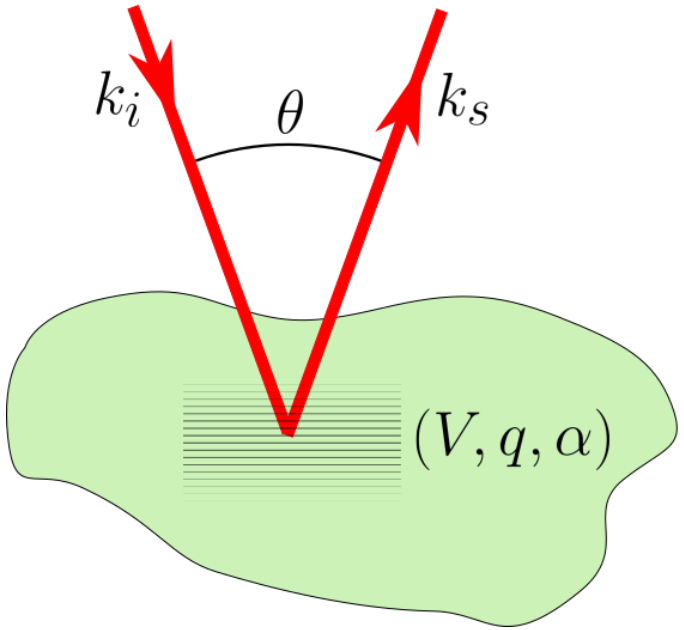

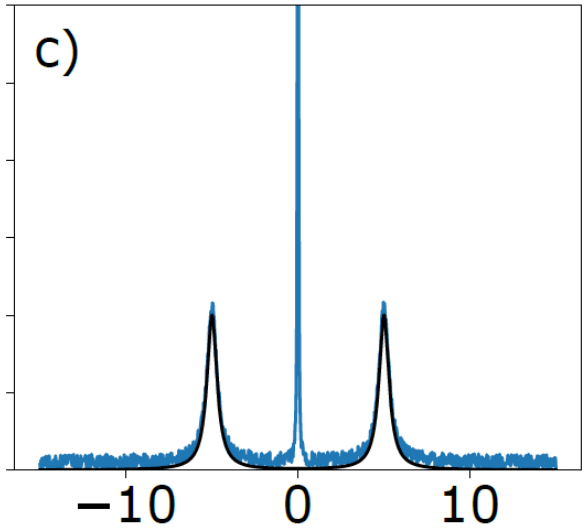

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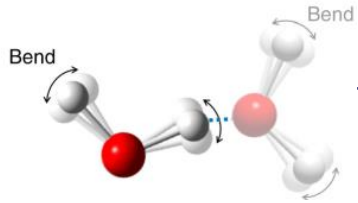
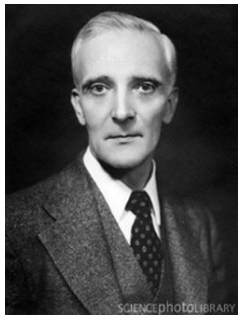
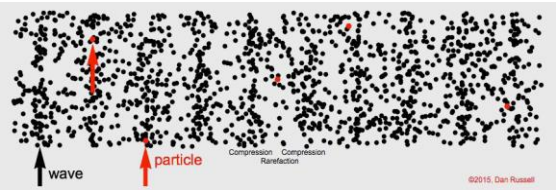




Brillouin scattering
Low energy vibrations
⇒ Small shifts

Inter-molecular movements



How to achieve the limit of diffraction in microscopy?

Confocal microscopy

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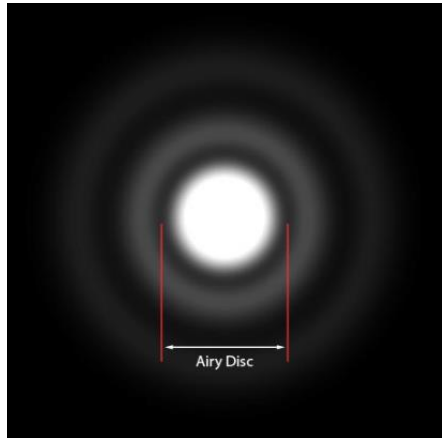

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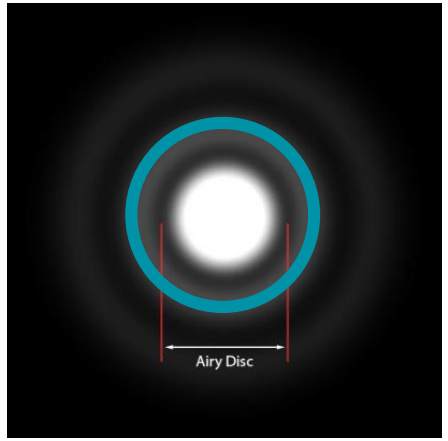

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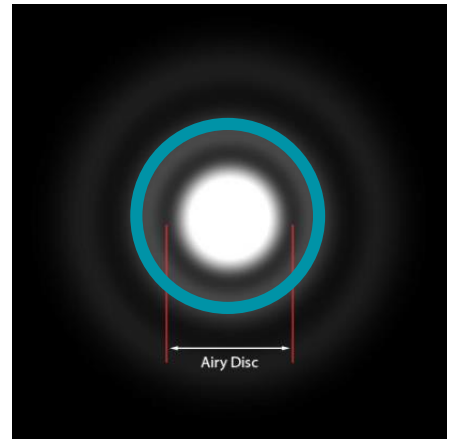

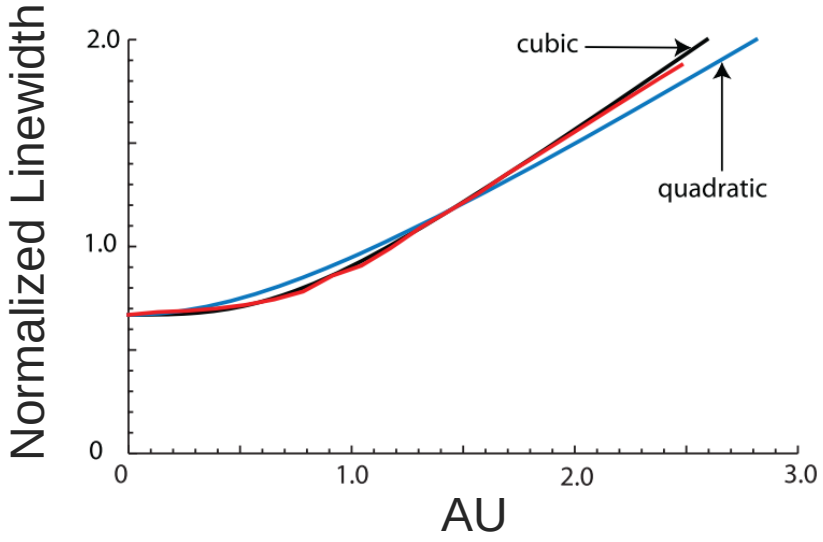

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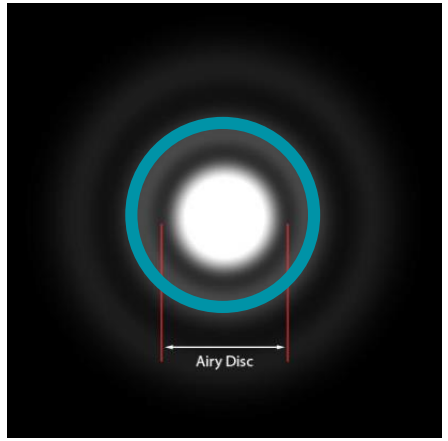

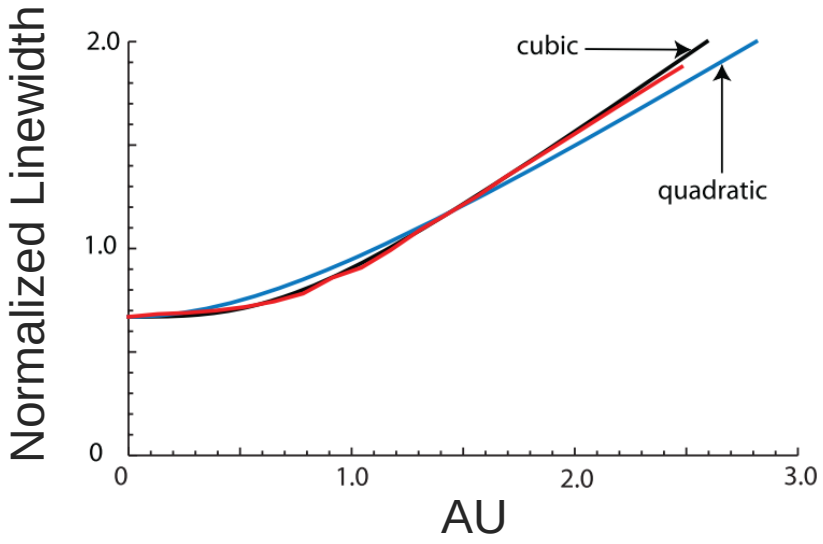

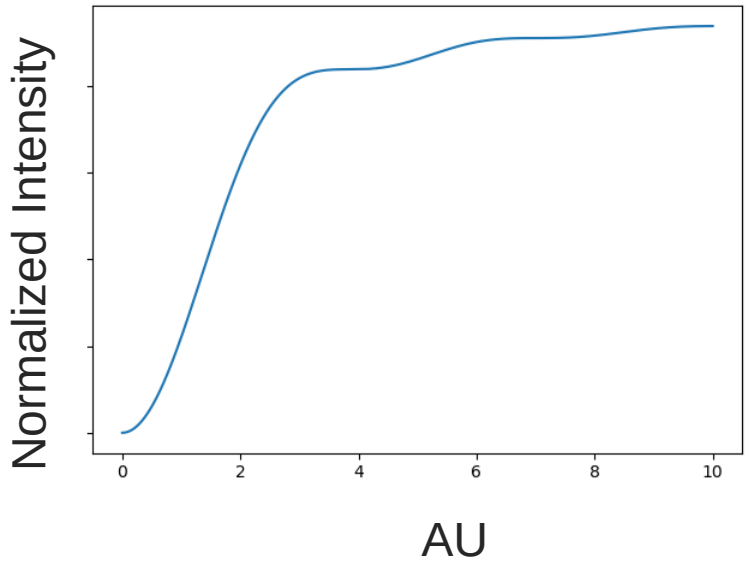

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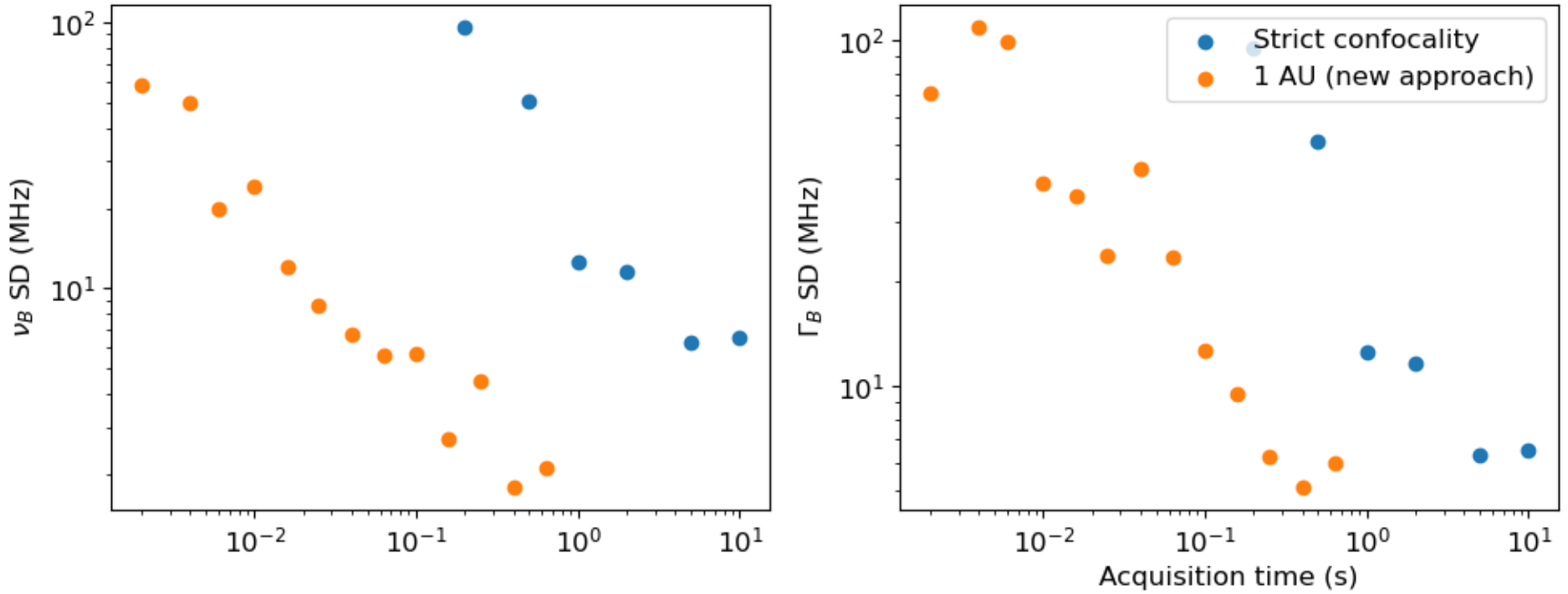
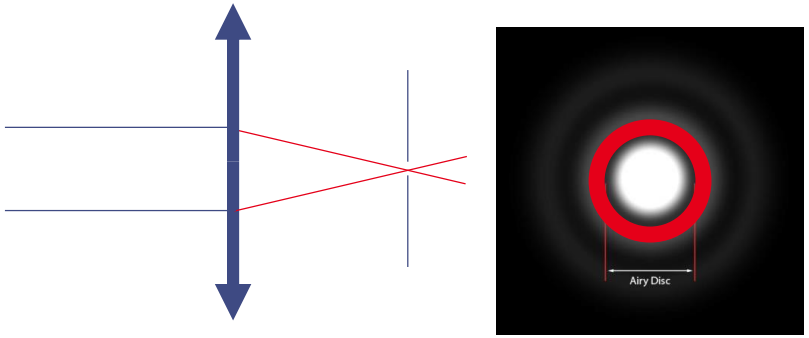
Comparison of approaches



Blue dots from: Yan, G., Bazir, A., Margueritat, J. & Dehoux, T. Evaluation of commercial virtually imaged phase array and Fabry-Pérot based Brillouin spectrometers for applications to biology. *Biomed. Opt. Express* **11**, (2020).

Confocality in fibered optical devices



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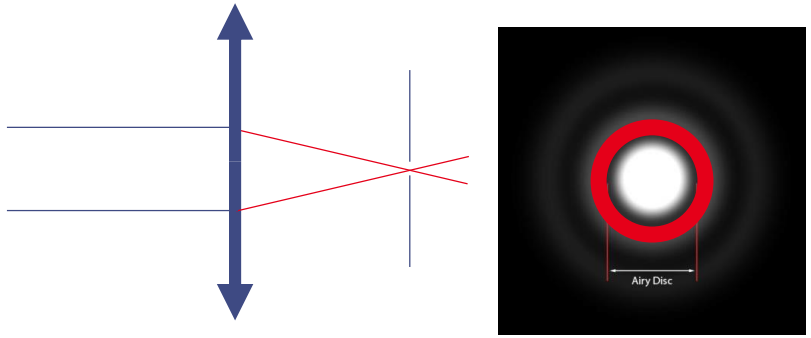

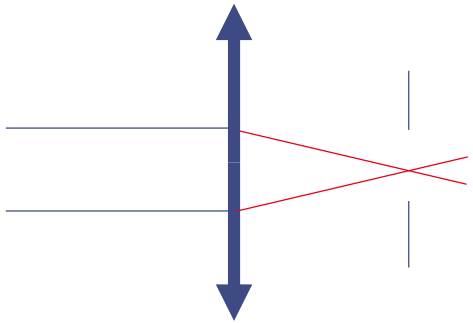

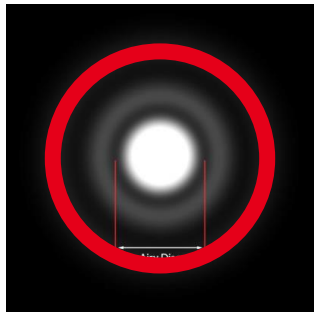

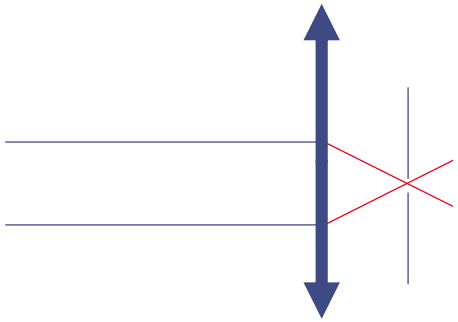

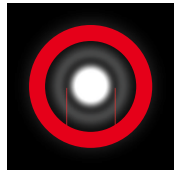

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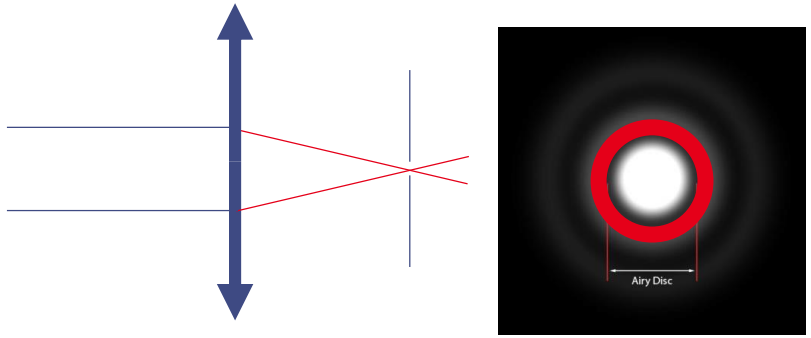
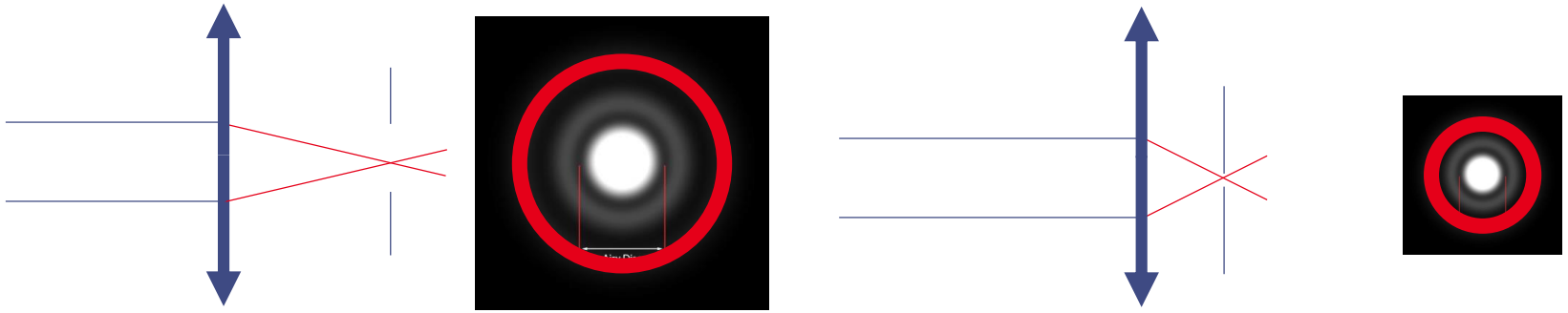
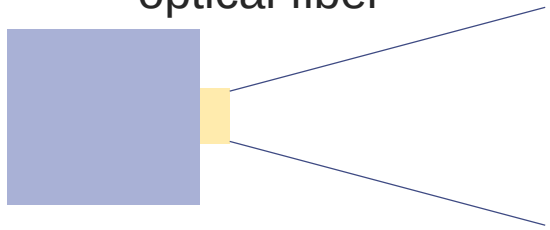




A simple approach of an optical fiber



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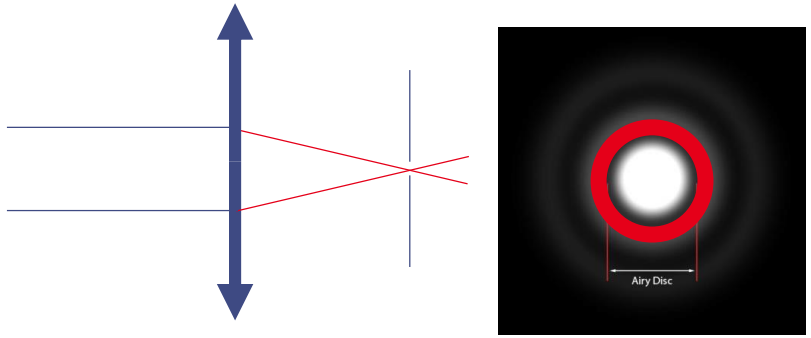

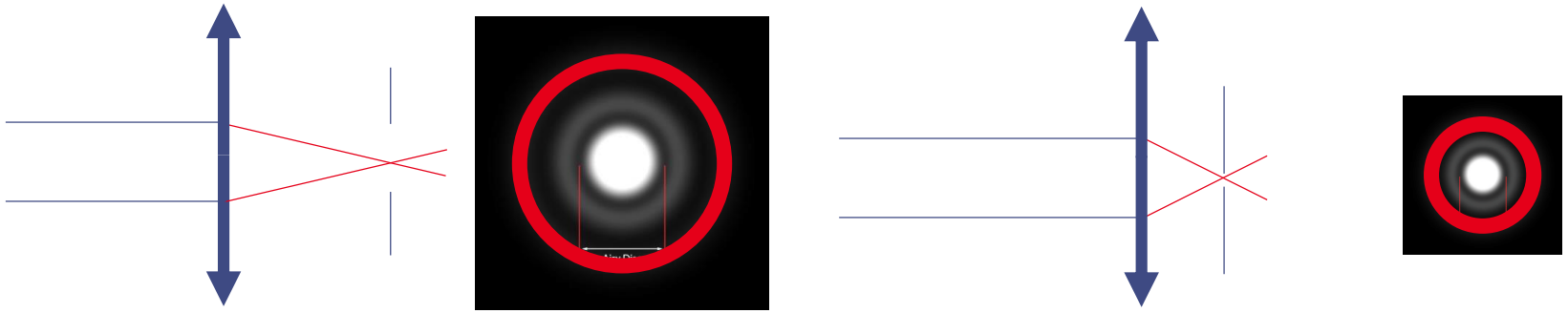

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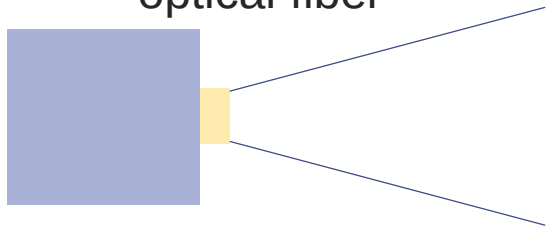
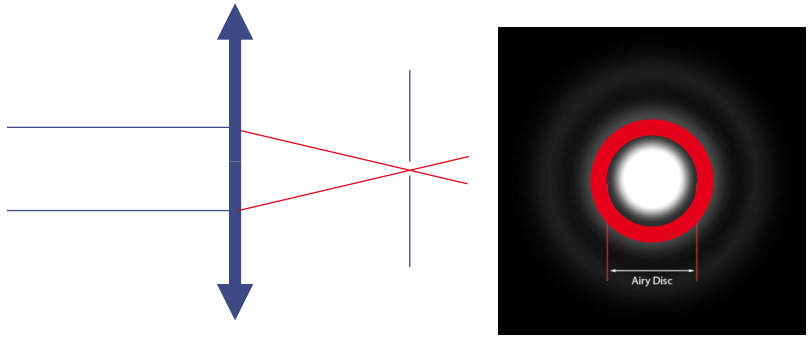
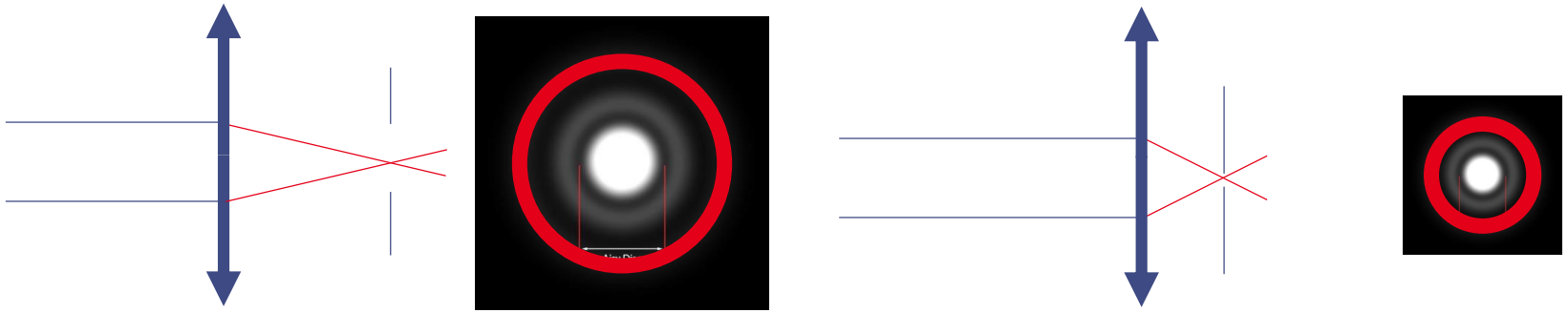


Problem: Optical fibers have finite NA

Confocality in fibered optical devices





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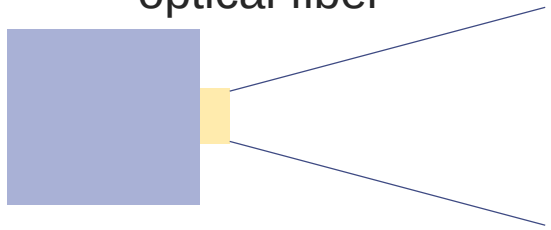

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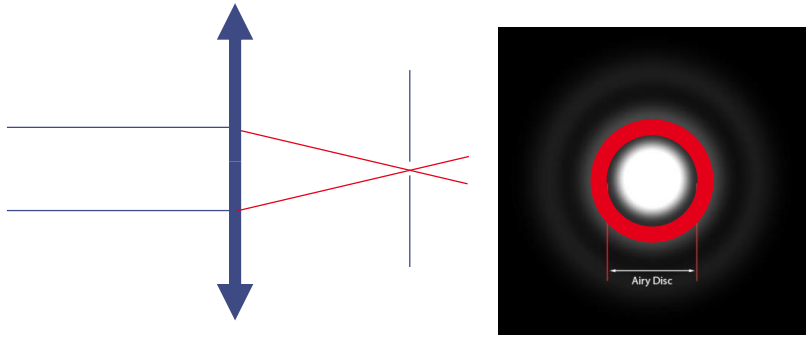
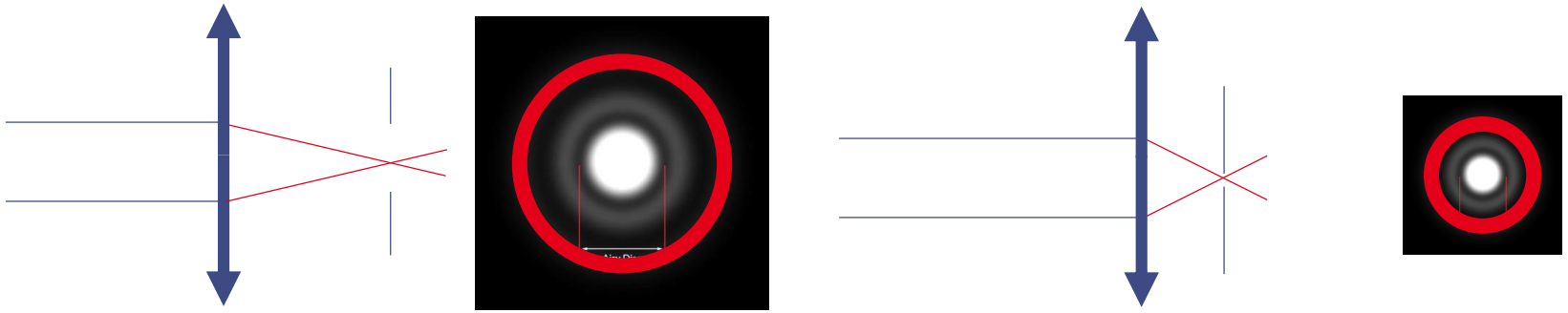
Confocality in fibered optical devices





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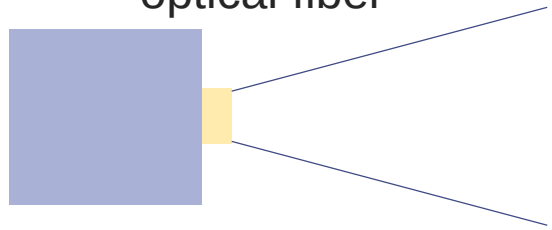
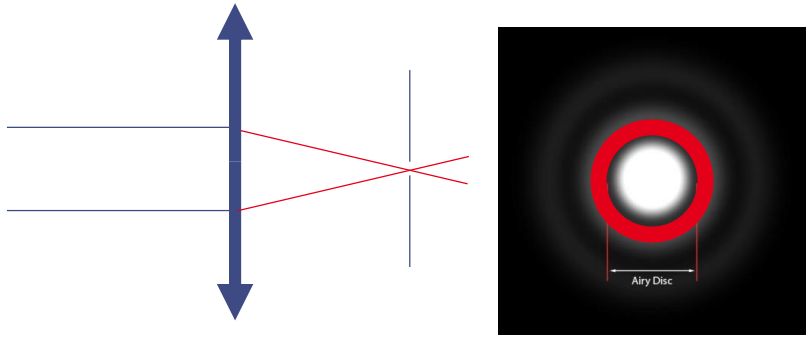


Problem: Optical fibers have finite NA

⇒ If we want to increase the pinhole size in a single-mode fiber, we will lose light to the NA

⇒ We need to use a fiber with a « larger core » = a fiber that carries more than 1 mode = a multimode fiber

Confocality in fibered optical devices



A simple approach of an optical fiber

Can we use a multimode fiber in a Brilouin spectrometer?

Problem: Optical fibers have finite NA

⇒ If we want to increase the pinhole size in a single-mode fiber, we will lose light to the NA

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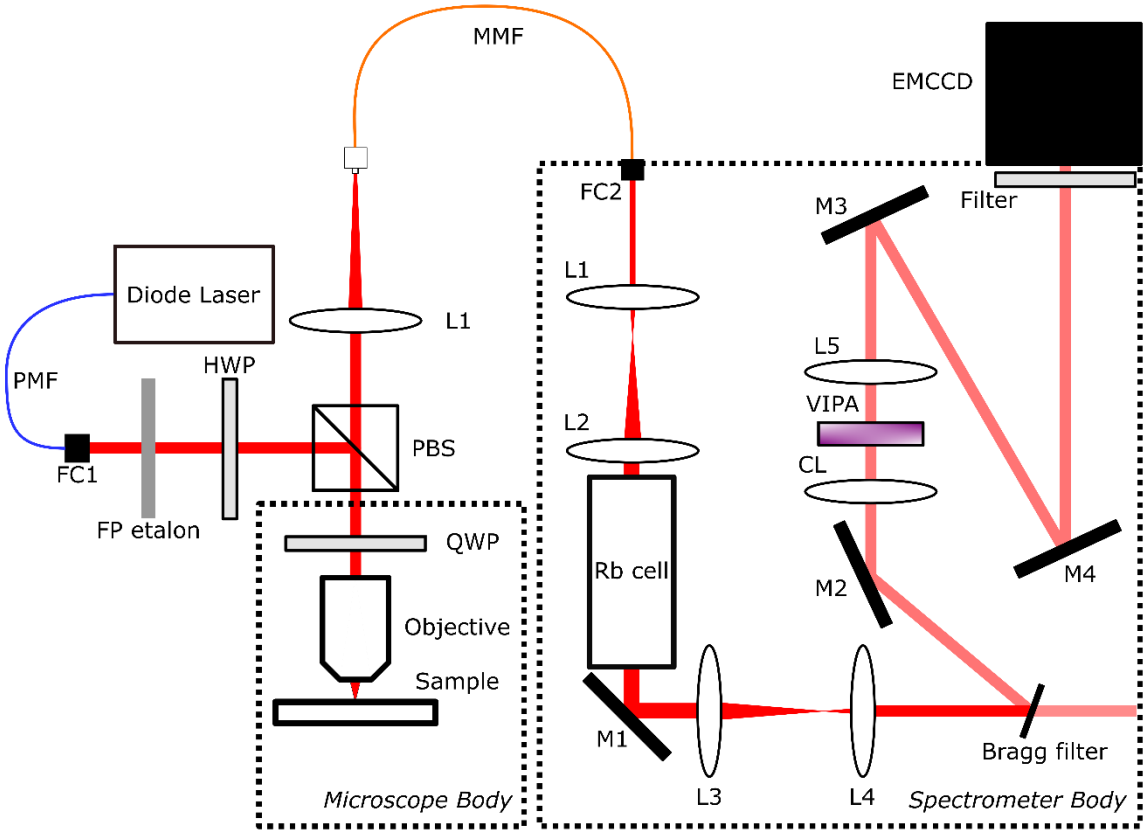

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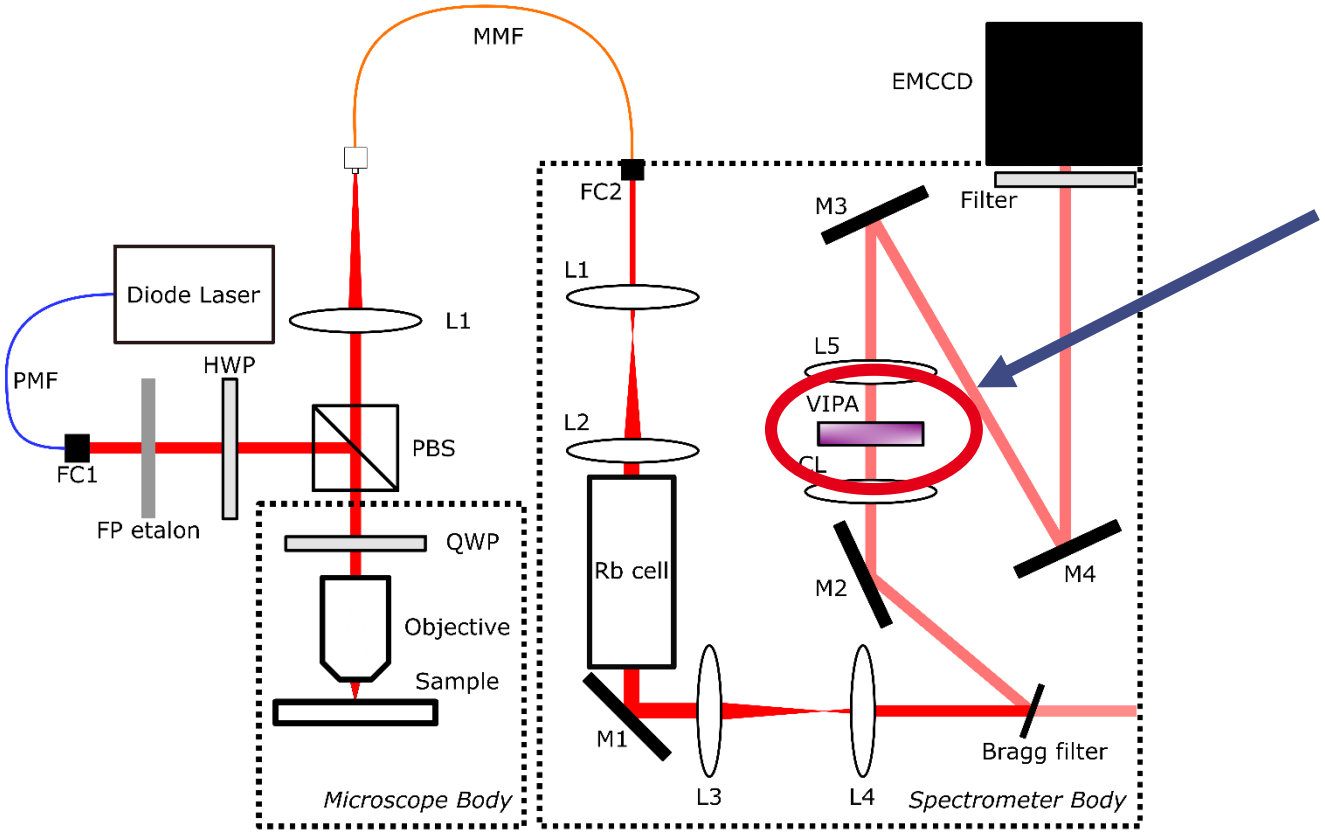

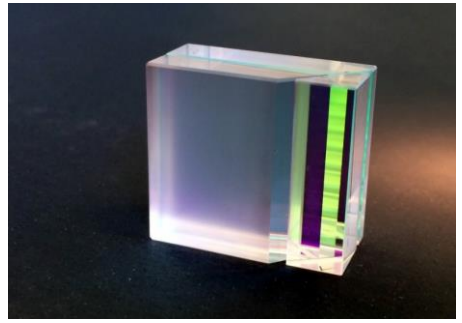

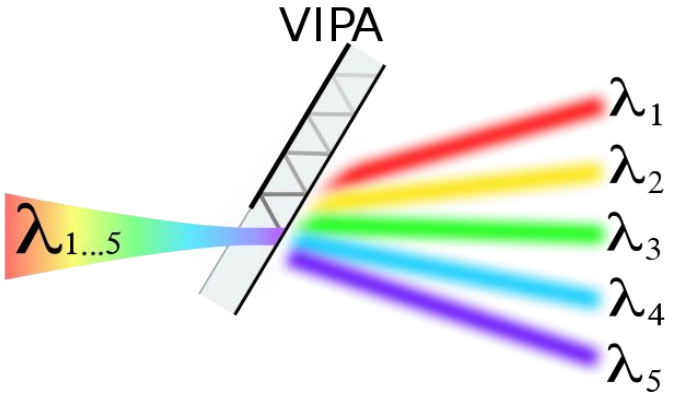

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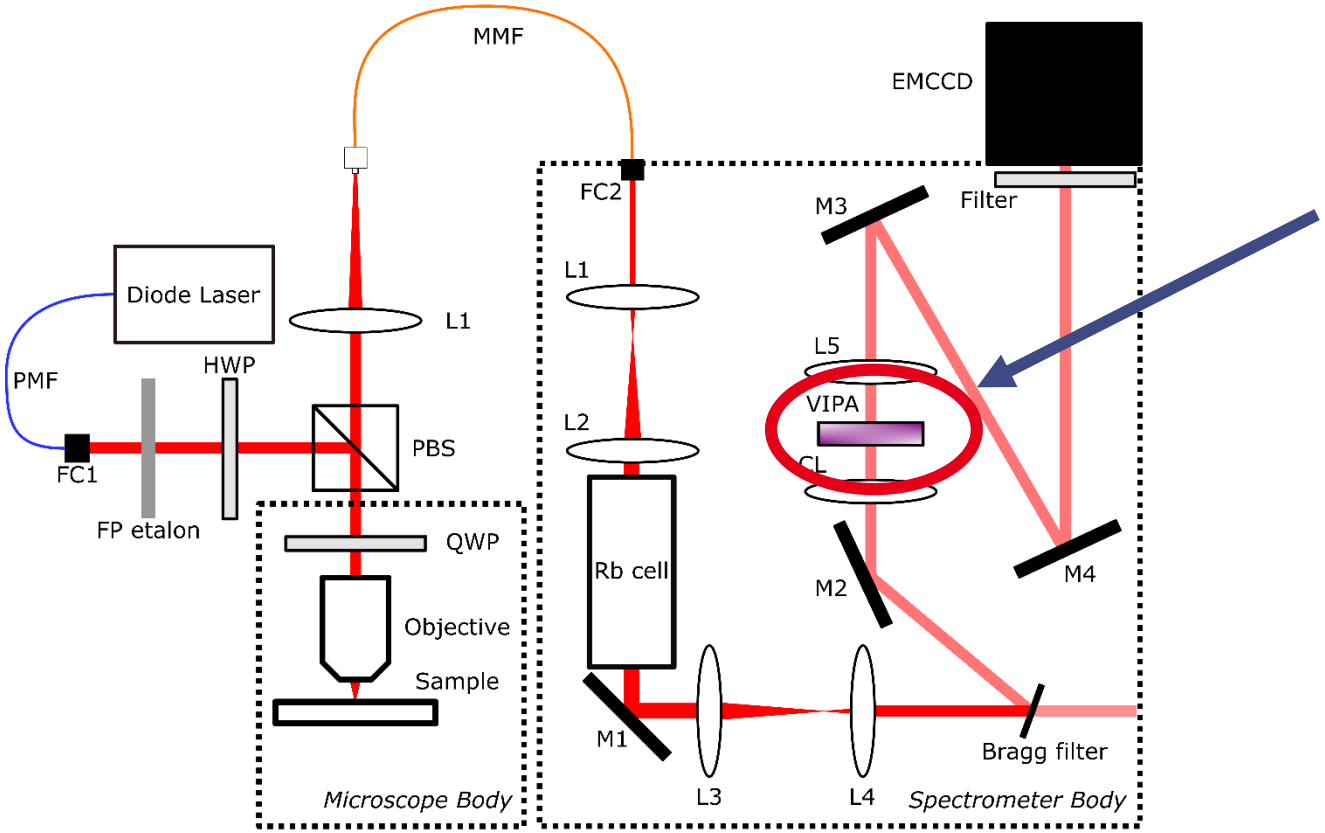

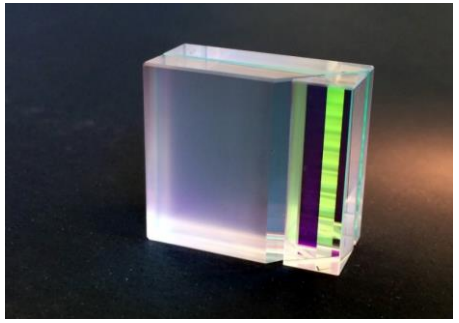

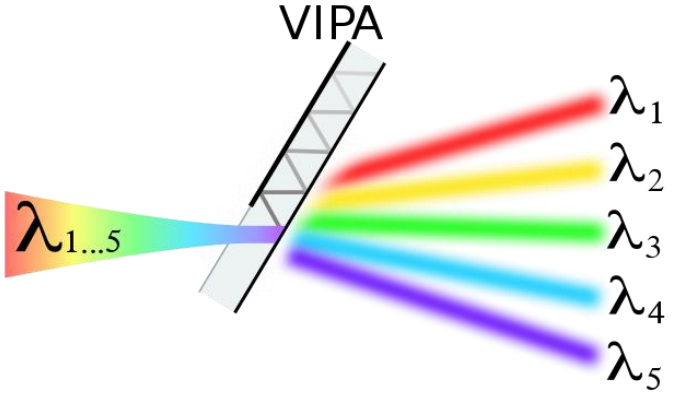

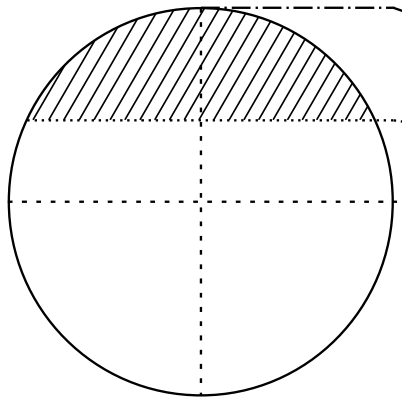

Clipping of the signal

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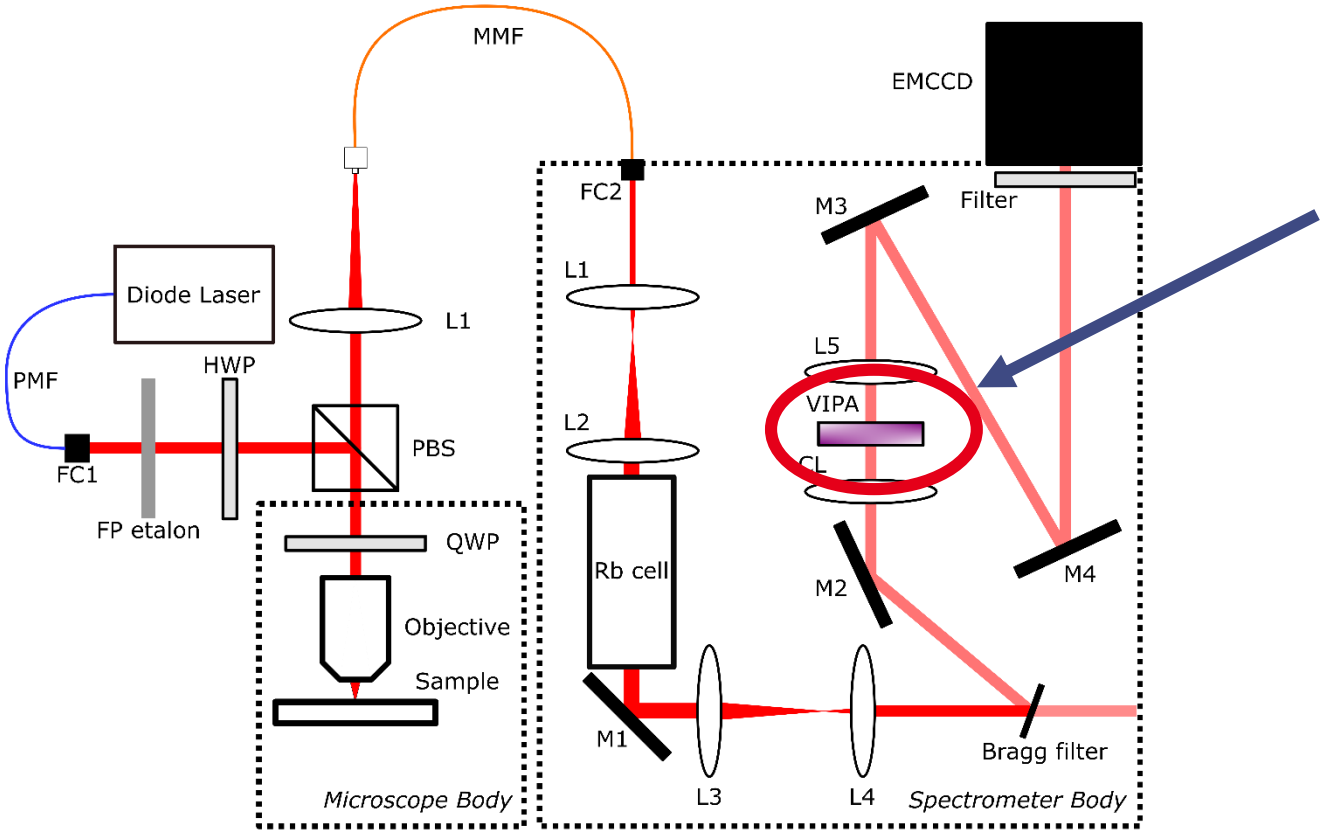
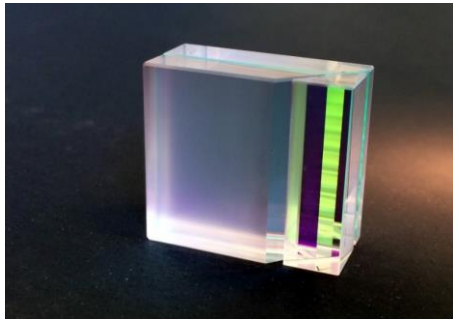
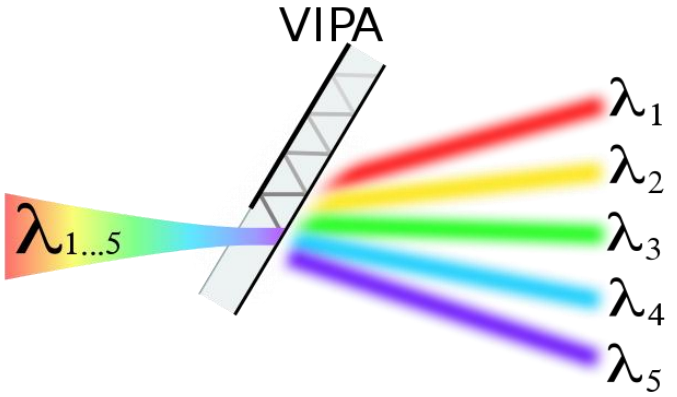
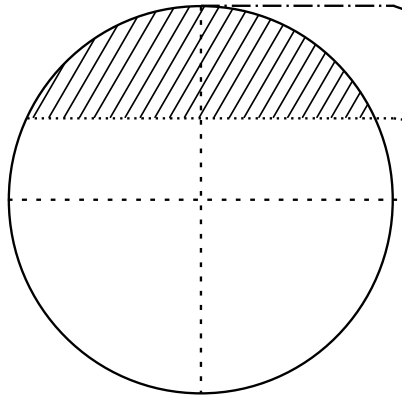
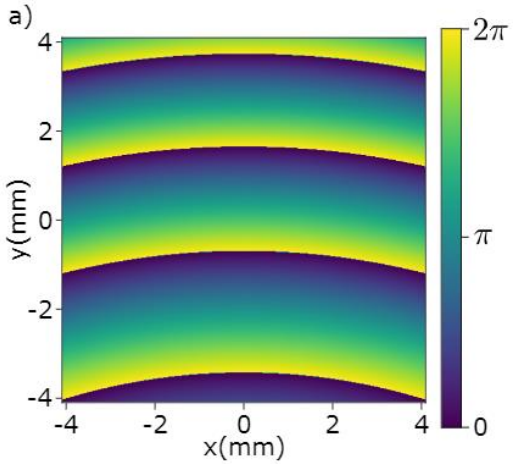






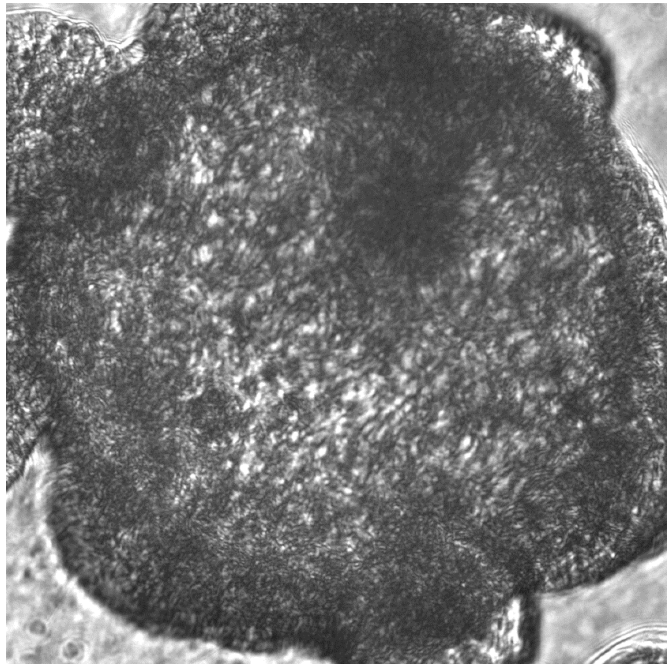




Clipping of the signal

Appearance of curves

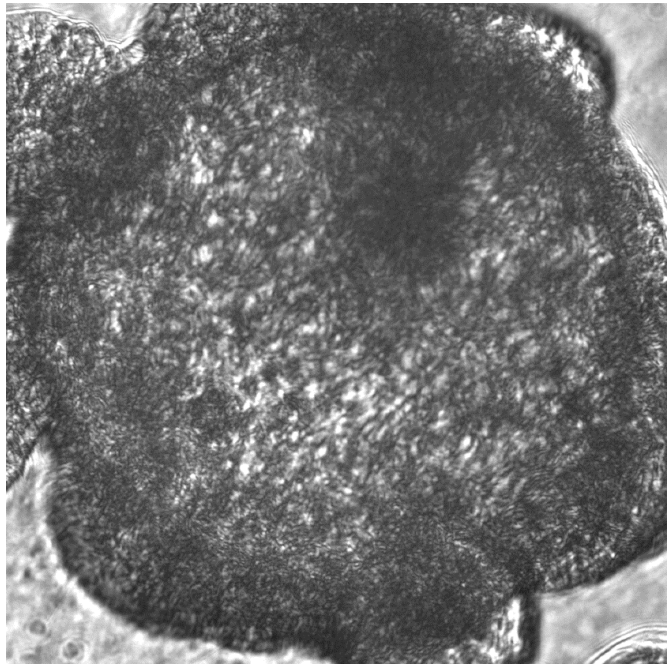
Brillouin image of a 3D assembly vs fluorescence



$\approx 1s$

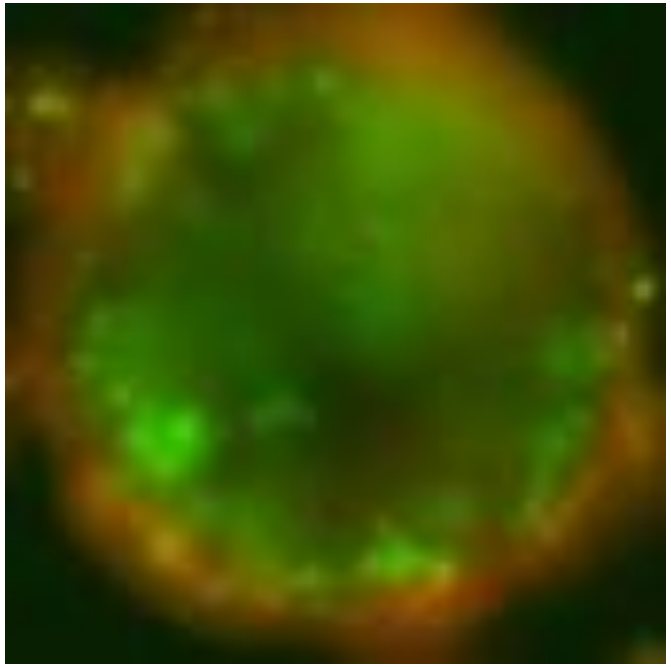
I don’t see anything
But I don’t damage the sample

Brillouin image of a 3D assembly vs fluorescence



$\approx 1s$

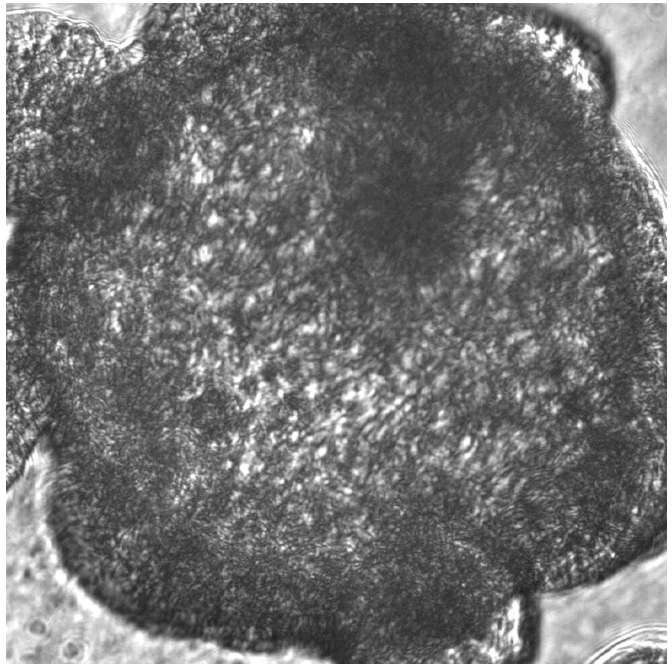
I don’t see anything
But I don’t damage the sample



$\approx 10s$

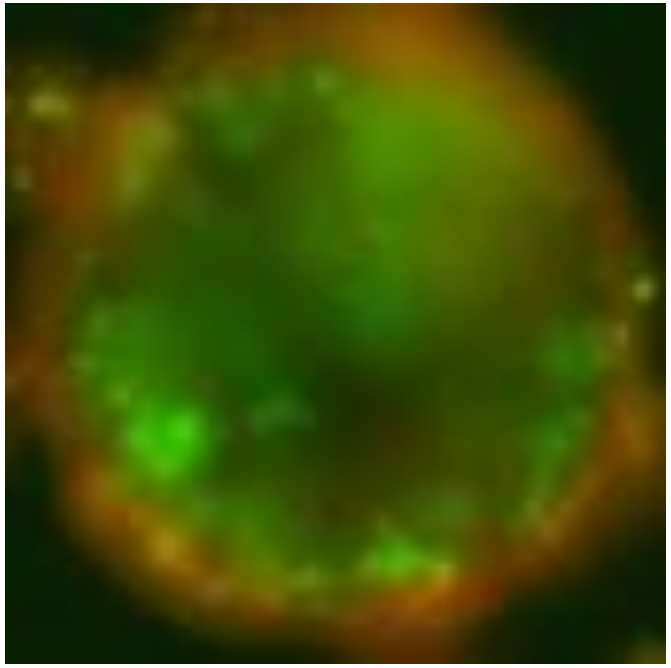
I see the information
But I’ve damaged the sample

Brillouin image of a 3D assembly vs fluorescence



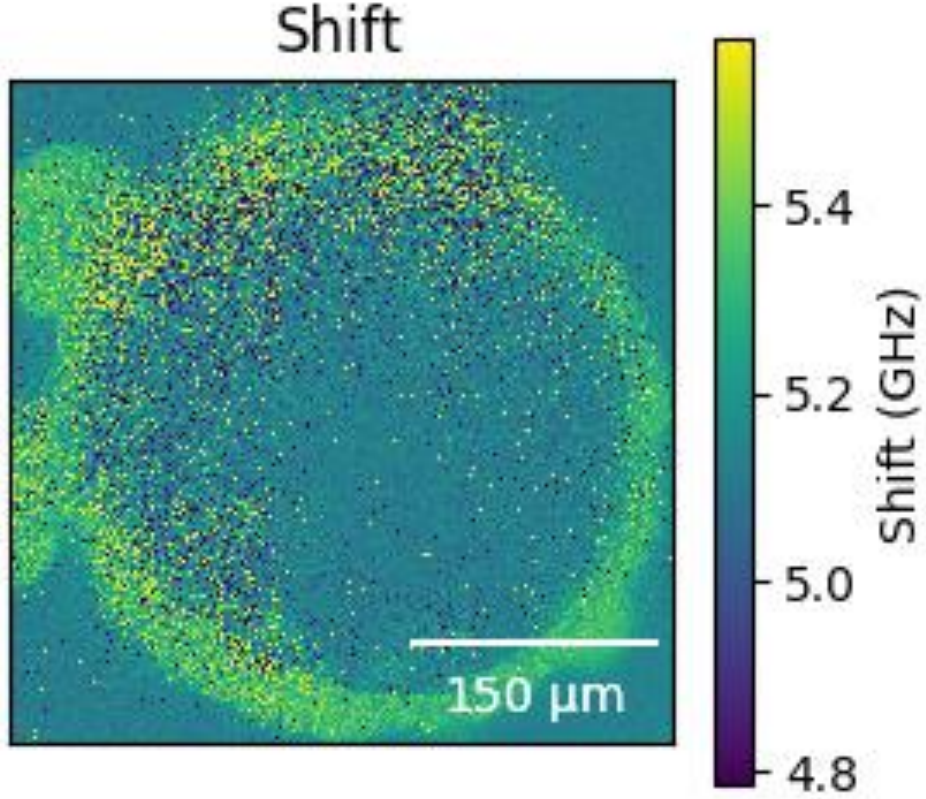
$\approx 1s$

I don't see anything
But I don't damage the sample



$\approx 10s$

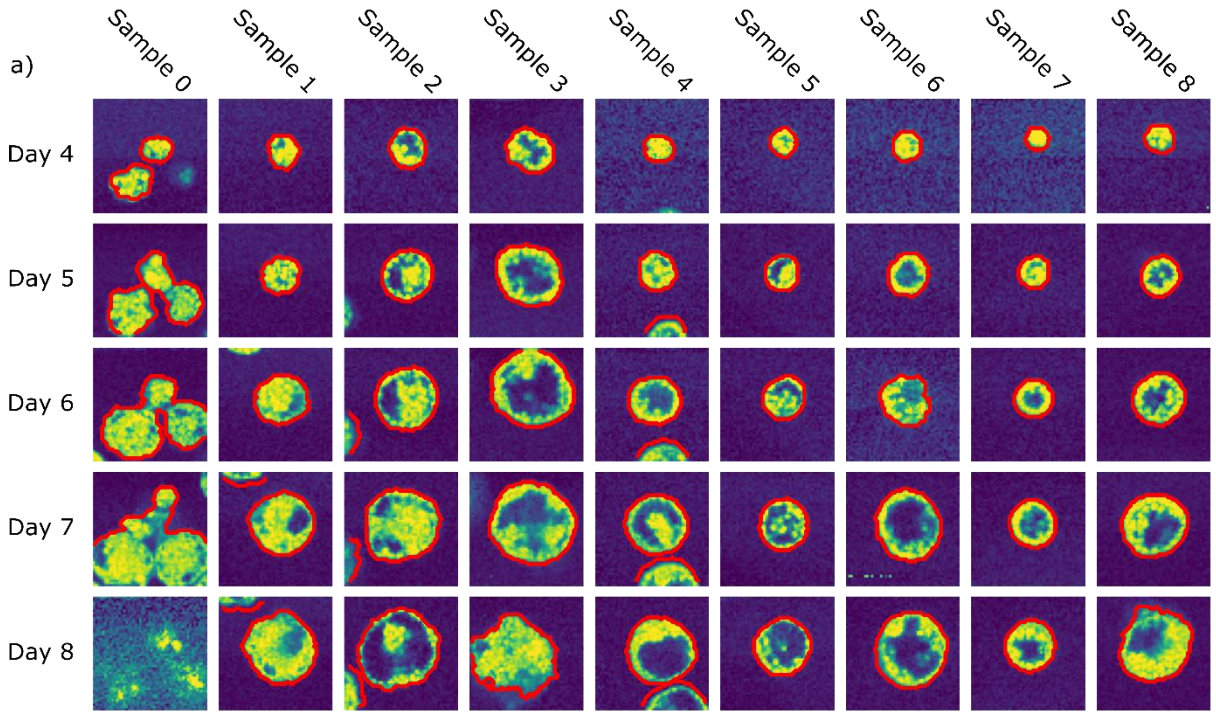
I see the information
But I've damaged the sample

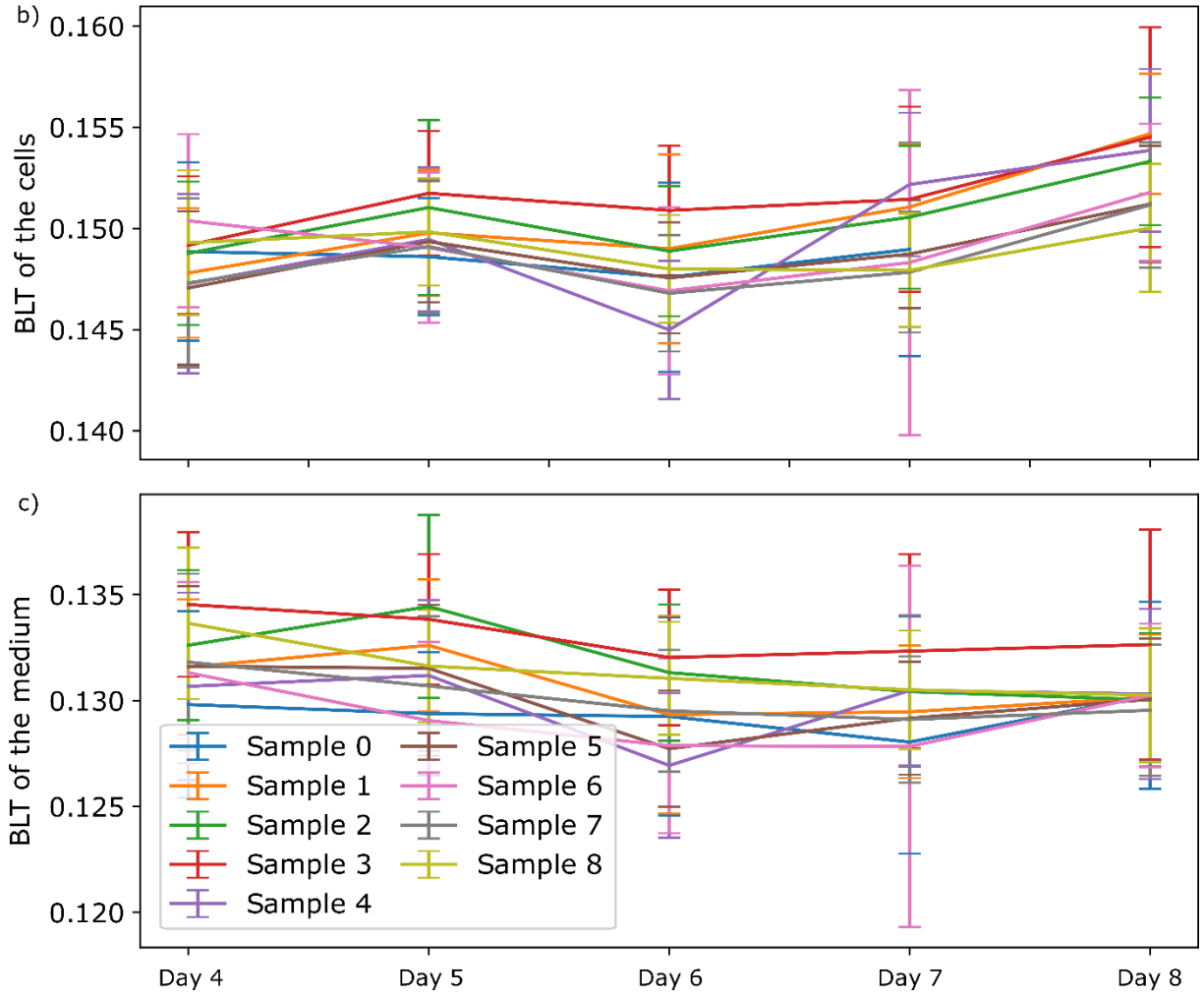


$\approx 15\text{min}$

I see the information
And I don't damage the sample

Mechanical studies of the formation of structures





Morpho-mechanical study of 3D cellular assemblies with confocal Brillouin light scattering

Thank you for your attention

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