

Enlightening mechanics: Brillouin microscopy for high-throughput automated spheroid stiffness mapping in lab-on-a-chip devices

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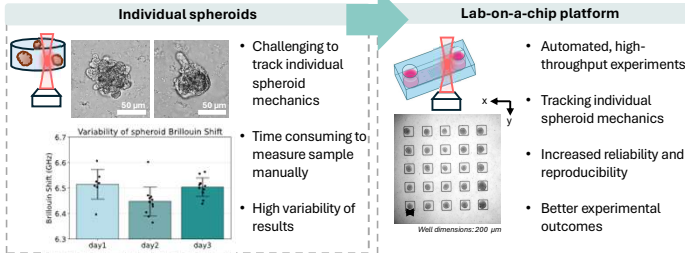
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1. Motivation: mechanobiology-ready platform

Mechanical properties of biological tissues provide critical insights into their functional state [1]. Viscoelastic properties of the microenvironment have an impact on the cell phenotype [2]. To model these systems in vitro, spheroids embedded in viscoelastic hydrogels are widely used as they mimic the native three-dimensional tissue microenvironment and preserve cell-to-cell interactions. Therefore, developing reliable, high-throughput methods to probe the mechanics of spheroids and their microenvironment could open new opportunities for understanding patho-physiology and phenotype changes in in vitro models.

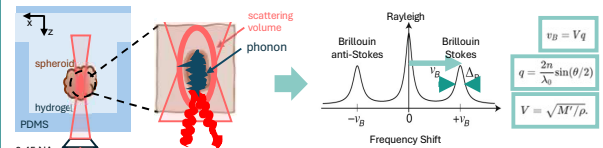


AIMS:

- Integrate Brillouin microscopy with a viscoelastic hydrogel lab-on-a-chip platform encapsulating spheroids.
- Automated, reliable and high-throughput quantification of both gel and spheroid mechanics over time.

2. Brillouin microscopy

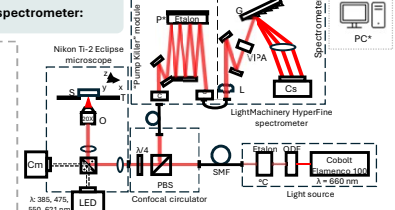
Brillouin microscopy is non-invasive, label-free and offers mechanical contrast based on the inelastic scattering of photons off spontaneous, thermally-induced acoustic vibrational modes (phonons). The resulting frequency shift depends on the local sound velocity within the material.



- Larger Brillouin shift: stiffer medium
- Larger Brillouin linewidth: more viscous medium

LightMachinery Brillouin spectrometer:

- Spectral resolution: 0.5 GHz or 0.7 pm.
- ~60 dB laser wavelength suppression.
- Up to ~30 mW incident power at the sample.

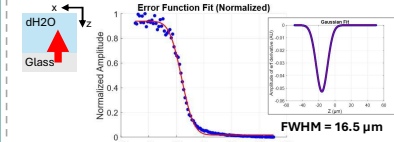


3. Development of robust, high-throughput Brillouin micro-spectroscopy pipeline for lab-on-a-chip devices

How do we achieve reliable and reproducible Brillouin data?

- Impact of environmental conditions
- Spatio-mechanical resolution
- Sample-specific measurement strategy and data analysis

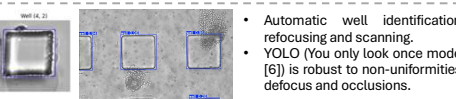
3.1 Axial spatio-mechanical resolution



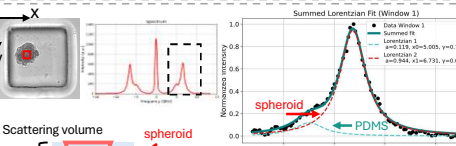
- Axial focal spot dimensions are compatible with bulk spheroid mechanics sampling with 0.45 NA objective lens.

3.3 Software development for automated experiments

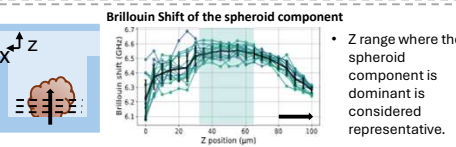
- Custom pymicro-plus based microscope control and a comprehensive multi-dimensional acquisition engine [5].



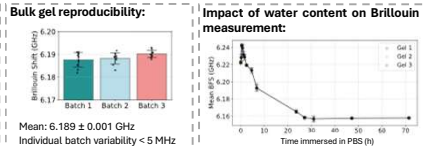
- OpenCV library allows extracting relevant spatial features [7].
- Spheroid localisation and automatic quality assurance.



- Sum of Lorentzians is fitted to exclude PDMS contribution (5 GHz) from gel or spheroid measurements.



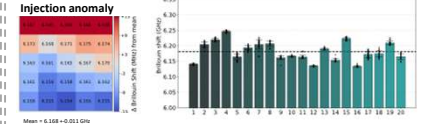
3.4 Robust viscoelastic PEGNorb hydrogel platform establishment



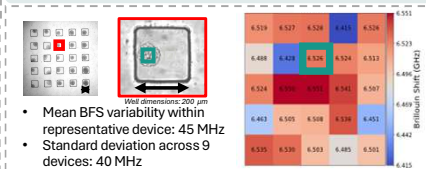
Reproducibility of gel BFS in devices:

- 80% of the channels exhibit uniform BFS (mechanical microenvironment), comparable to bulk gel.

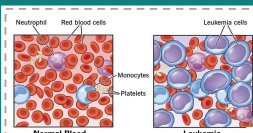
Injection anomaly



3.5 Preliminary spheroid data



4. Outlook: Mechanobiology-Based Medicine



- About 9,900 new cases of leukaemia are diagnosed each year in the UK.
 - Difficult to diagnose in early stages.
- People with CHIP mutations have a higher risk of developing blood cancers.

How does the underlying mutational landscape impact the mechanical properties of cells?

Bone marrow niche:

- Advanced Hydrogel
- MSC Spheroid
- MNCS
- Fine tuning of viscoelastic properties.

Miniaturisation for high throughput:

- Bone marrow niche is compatible with microfluidic devices.

Automated Brillouin microscopy on BM niche:

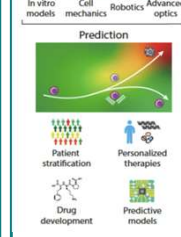
- Custom software + data analysis compatible with microfluidic devices.

Application of nano-vibration:

- Vibration can be used for in vitro phenotypic control [8].
- Nano-stimulation (e.g., 100 nm; 1 kHz) to observe a measurable mechano-transduction response with Brillouin microscopy.

A pipeline for distinguishing between healthy and diseased bone marrow models.

In vitro models



Learn more about our work!

The work presented in this poster is part of Mechanomedicine Phase 2 project.



References

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