Book of Abstracts

BioBrillouin for MechanoBiology: Materials, Methods and Perspectives

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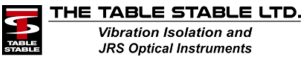

List of abstracts

Bringing GHz Phonons to the Clinic: Sub-Cellular Mechanical Imaging via Fibre-Based Brillouin Endoscopy

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What if we could detect cancer before it takes hold, just by listening to how cells respond to sound? Recent advances in optical physics suggest we can via Brillouin scattering. It leverages GHz-frequency phonons to probe nanoscale mechanical properties non-invasively, enabling investigation of critical mechanobiological processes like early carcinogenesis. As a first step toward clinical application, we have developed a fibre-based Brillouin microscopy and endoscopy platform that stimulates large-amplitude phonons and measures their time-of-flight (ToF) as they propagate through a specimen. This system delivers optical lateral resolution while breaking the diffraction limit axially via sub-optical wavelength phonons, yielding extremely high axial resolution without parasitic fibre scattering. This enables precise mechanical imaging from the tip of a glass optical fibre, offering a clinically compatible endoscopic solution. Using this technique, we performed mechanical property mapping with sub-micron voxel resolution in both single cells and complex multicellular organisms [1, 2]. We have also demonstrated for the first time that the ToF information can be used to train a neural network to identify the acoustic signature of individual cancer cells with >90% classification accuracy demonstrating its diagnostic potential [3]. Beyond biomedicine, the technique also shows promise in sustainable agriculture, where soil-induced changes in rice root cell elasticity have been linked to biochemical signalling that affects root development [4].

Throughout my PhD research, I aim to unlock the clinical potential of the phonon endoscopy technology. My work will focus on: (1) developing clinically relevant case studies and prototype applications, using fibre probes to interrogate stiffness changes during tumorigenesis and the development of precancerous symptoms; (2) optimising this technology's clinical performance by improving acquisition speed, penetration depth, cost, size, and biocompatibility; and (3) advancing AI-assisted diagnostics with emphasis on data interpretability. Within the first 4 months of my PhD, I have developed an AI model that classifies cancer in ToF signals, and applied gradient-weighted class activation maps to preliminary identify cancer-associated signal regions, a crucial step toward elucidating model behaviour and support future biologically meaningful interpretations.

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Mechanical properties and polymerization of the egg glue of the Southern green stink bug, *Nezara viridula* L. (Hemiptera: Pentatomidae)

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The Southern green stink bug, *Nezara viridula* L. (Hemiptera: Pentatomidae), is a globally significant pest affecting various plant families. Like many insect species, *N. viridula* females secrete an egg glue during oviposition that serves as a natural adhesive, securing eggs together and to the substrate to enhance protection and stability. This study aims to investigate the mechanical properties, polymerization dynamics, and adhesion strength during the polymerization period of the egg glue. Using the SA2 Nano indenter with the Berkovich diamond tip, we quantified the Young's modulus and hardness of the polymerized egg glue as 4.578 ± 0.916 (n=4) GPa and 0.227 ± 0.083 (n=4) GPa, respectively. The insect egg glue was found to be twice as elastic when compared to the chicken egg albumin. The hardening process of the glue was monitored over time using Brillouin micro-spectrometry. The analysis revealed rapid polymerization occurring within the first hour of air exposure, transforming the material from a viscous fluid into a stiffer and more rigid state. Once formed, the glue layer surrounding the eggs was analyzed through mechanical mapping. The glue exhibited mechanical heterogeneity at the micrometric scale, with variations in Brillouin frequency shift of approximately 5% between thicker and thinner regions. The adhesion strength of the egg glue was assessed using the BIOPAC force sensor system at various intervals after egg deposition, showing an increasing trend in the force required to detach the eggs from the substrate, which aligns with the polymerization behavior we observed through the Brillouin analysis. These findings provide a mechanistic understanding of this natural adhesive and offer important insights for developing pest management strategies. Also, the mechanical properties of *N. viridula* egg glue can be important for the potential applications for the development of biomimetic adhesives.

Keywords: Insect egg glue, bio-adhesives, adhesion force, polymerization

Towards early detection of retinal pigment epithelium dysfunction in an in vitro model of age-related macular degeneration with Brillouin micro-spectroscopy

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Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in the elderly[1]. AMD is associated with a dysfunction of the retinal pigment epithelium (RPE) that metabolically supports light-detecting photoreceptors[2]. The structural remodelling of ageing RPE is characterised by changes in cell size and arrangement, potentially leading to altered mechanical balance[3]. Therefore, obtaining the biomechanical signature of early AMD is instrumental for understanding the patho-physiology of retinal ageing.

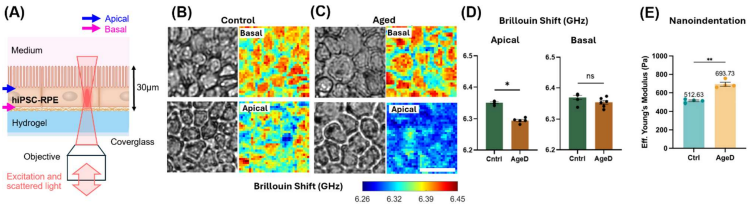

Figure 1: *A: Brillouin microscopy setup for characterising hiPSC-RPE mechanics. B, C: Representative brightfield images of cell structures and the corresponding Brillouin shift maps from the apical and basal regions (scale bar: 25 µm). D: Preliminary results: apical layers yield a smaller Brillouin shift in AgeD samples compared to the control, whereas the difference in basal layers is not significant. E: Effective Young's modulus of an apical surface is higher for AgeD samples, as shown by nanoindentation measurements.*

We integrated Brillouin microscopy[4] with a novel *in vitro* model (Fig.1, A), where human iPSC-derived RPE undergoes apoptosis, mimicking the cell density and functional decline seen in ageing RPE (Apoptosis-generated Density – AgeD). As epithelia show a balance between forces at their apical versus basal sides[3], our preliminary measurements with LightMachinery Hyperfine VIPA-based spectrometer (1 pm spectral resolution; 20X 0.45 NA air objective) at apical and basal layers of the monolayer (Fig.1, B, C) yield a position-dependent difference in mean Brillouin shift between AgeD and control samples (Fig.1, D). Furthermore, vertical line scans through the monolayer exhibit a peak in stiffness at basal layers and high heterogeneity. Nevertheless, challenges remain in optimising data acquisition and analysis strategies to accurately extract mechanical information across the 15-30 µm thick monolayer and ascertain the contributions from the medium and hydrogel to the Brillouin spectra. Furthermore, nanoindentation (Fig.1, E) indicated increased Young's modulus in AgeD samples compared to control. This discrepancy between trends in Brillouin and nanoindentation data may reflect variations in apical microvilli orientation and distribution[5], cell height heterogeneity and a difference in thickness in control and AgeD samples. These preliminary results highlight the complexity of interpreting mechanical properties in aging RPE and the need for careful, sample-specific data analysis methods for Brillouin microscopy in biological preparations.

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Hypoxia activates Piezo1/IK axis in human glioblastoma cells: role in volume regulation and cell survival

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Glioblastoma (GBM) is the deadliest form of human brain tumor, with a very poor prognosis. Its malignancy is strongly associated with the presence of hypoxic regions within the tumor, due to the ability of hypoxia to promote migration/invasion and death resistance through the overexpression of the hypoxia-inducible factor (HIF-1) and the subsequent modulation of gene transcription. To acquire the more aggressive phenotype, GBM cells must survive to the hypoxic insult, known to induce cell swelling and necrotic death in many cell types. **(1)** Here we show that GBM cells can revert the hypoxia-induced cell swelling and prevent necrotic cell death through the activation of the mechanosensitive Piezo1 channel, the rise of cytosolic Ca^{2+} and the resulting activation of the intermediate-conductance Ca^{2+}-activated potassium (IK) channels. The consequent IK mediated K^+ efflux, accompanied by Cl^- efflux through the volume-regulated anion channel, previously shown to be also modulated by the hypoxic insult and crucial for preventing hypoxia-induced cell necrosis, results in an osmotically driven water efflux and volume decrease, allowing the cell to survive. (2) Notably, the large-conductance Ca^{2+}-activated potassium (BK) channel, also largely expressed in these cells, cannot contribute to this process due to its fast inhibition by hypoxia. Taken together, these results suggest that the Piezo1/IK axis plays a key role in the survival of GBM cells in a hypoxic environment, potentially offering a new therapeutic target for this malignant tumor.

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Characterisation of hydrogel mechanics and macrophage-substrate response using Non-invasive Brillouin Microscopy

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The mechanical properties of cells, biomaterials and tissues is critical to their function and performance. However typical modalities, such as atomic force microscopy (AFM), optical tweezers, micropipette aspiration, particle-tracking micro-rheology, and optical coherence elastography, are invasive, do not permit longitudinal observation, and are limited to surface and/or bulk measurements. Brillouin microscopy is a groundbreaking optical modality, that is non-invasive, capable of mapping mechanical properties in real-time, with a working principle based on the inelastic scattering produce by the interaction between photons with acoustic waves (phonons).[1]

In this study, agarose and gelatin hydrogels at varying concentrations, as well as synthetic polyacrylamide gels, with a continuous percentage of bulk material, were prepared. The mechanical properties of these hydrogels were evaluated using compression tests, and compared with the results obtained with Brillouin microscopy using a Discoverer Brillouin microscope (Cellsense, Germany) with dual crossed VIPAs configuration and equiped with a 780 nm laser.

To investigate cell–material interactions, human blood-derived M0 macrophages were seeded onto polyacrylamide hydrogels and compared with M0 macrophages seeded on glass. For further comparison, additional macrophages cultured on glass were stimulated toward an M1 phenotype using IFN-γ or an M2 phenotype using IL-4. Brillouin imaging was performed 24 hours post-seeding, and the data were analyzed to determine the average Brillouin frequency shift (ABS) in the nucleus and cytoplasm across all conditions. ELISA assays were also conducted to evaluate cytokine and chemokine secretion profiles under each condition.

The study revealed distinct apparent Brillouin shifts (ABS) in hydrogels with varying bulk material concentrations and hydration levels, which correlated well with results from conventional mechanical measurements. Additionally, macrophage interactions with polyacrylamide substrates showed differences in ABS values in both the cytoplasm and nucleus compared to cells cultured on glass. Notably, the cytoplasm of M0 macrophages cultured on hydrogels exhibited significantly more solid-like viscoelastic properties than those seeded on glass. However, no significant differences were observed in the nuclear compartment between these two culture conditions. When comparing the nuclear ABS of M1 and M2 polarized macrophages seeded on glass with that of M0 macrophages cultured on hydrogels, a significant difference was found between M0 and M1 states, whereas no significant difference was observed between M0 and M2 states in this compartment.

Overall, this work highlights Brillouin microscopy as a powerful tool for non-invasive mechanical characterization of hydrogels and their interaction with immune cells, offering valuable insights into cell–substrate mechanics relevant to tissue engineering and immunomodulatory biomaterials.

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Piezo1 is an essential player in the regulation of cell volume in human glioblastoma cells

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ABSTRACT

Cell volume regulation is a complex homeostatic process employed by nearly every cell to respond to osmotic changes or to facilitate essential processes such as proliferation and migration. In this regard, ion channels and transporters play key roles by controlling intracellular water content through the mediation of osmolyte movement across the plasma membrane. We recently found in human glioblastoma (GBM) cells that hypotonic-induced Ca^{2+} influx through mechanosensitive channels is crucial for activating both Ca^{2+}-activated K^+ (K_{Ca}) channels of large (BK) and intermediate (IK) conductance, which in turn drive the subsequent regulatory volume decrease (RVD) (1). Although increasing evidence indicates that the mechanosensitive Piezo1 channel mediates hypotonic-induced Ca^{2+} influx, definitive proof of its involvement in cell volume regulation in GBM cells has been lacking due to the absence of selective inhibitors (2). To clarify this point, we generated a stable Piezo1 knock-out U87-MG cell line using the CRISPR-Cas9 technique. Our study demonstrates that Piezo1 is essential in regulating the volume of U87-MG cells during the hypotonic-induced RVD process. In the absence of Piezo1, the activation of the two predominant K_{Ca} channels (IK and BK) in response to hypotonic stress is abolished. On the other habd, Piezo1 deficiency did not affect the activity or functional expression of the volume-regulated anion channel (VRAC), also essential in the RVD process of these cells. Furthermore, activating Piezo1 with Yoda1 under isotonic conditions induces a biphasic volume response characterized by an initial swelling due to Na^+ influx, followed by shrinkage resulting from the Ca^{2+}-dependent activation of K_{Ca} channels. Since Piezo1 is expressed in nearly all animal cells, its contribution to volume changes is likely broadly applicable.

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Probing Nuclear Mechanics: Brillouin Microscopy Reveals Nucleoplasm Stiffening During Nucleus Shape Deformation

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The structural organization and mechanical properties of the cell nucleus are increasingly recognized as critical regulators of gene expression, cellular differentiation, and tissue development, both in physiological and pathological contexts [1,2]. However, how these mechanical properties contribute to the cellular mechanotransduction process remains largely unclear. In particular, the effect of nuclear deformation—resulting from cytoskeletal forces or external mechanical stimuli—on the mechanical properties of the nucleoplasm is not fully elucidated. In this study, we investigated whether deformation of the cell nucleus can modulate the mechanical properties of the nucleoplasm. To this end, we engineered micro-patterned culturing platforms designed to control the organization and tension of the actin cytoskeleton, enabling us to induce precise and reproducible nuclear deformations in human adipose-derived stem cells (hASCs). By printing adhesive islands with varying geometries and spatial constraints, we achieved controlled micron-scale confinement of the cell body, thereby exerting graded mechanical stresses on the nucleus [3]. Finally, we used Brillouin microscopy to quantitatively assess the mechanical response of the nucleoplasm under different levels of nuclear deformation. Our results demonstrate that nuclear shape deformation leads to measurable changes in nuclear stiffness. These findings offer new insights into the dynamic interplay between nuclear mechanics and cell function, with implications for understanding stem cell behavior and mechanobiology.

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Bending fluctuation spectroscopy of actin filaments in entangled suspensions

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The mechanical properties of soft materials can be probed on small length scales by micro-rheology. A common approach tracks fluctuations of micrometer-sized beads embedded in the medium to obtain information about the medium's rheological properties on the same length-scale of the bead's size.

Recently, filament micro-rheology, which involves the use of filaments instead of particles as probes, has been proposed as an alternative to traditional micro-rheology. These probes are much less invasive than particles and the study of their bending dynamics using a bending mode decomposition allows one to obtain information about the mechanical properties of the material simultaneously on different length-scales. However, this approach has been only tested on model viscoelastic samples that are not representative of biological systems.

In this work, we test the validity and feasibility of filament micro-rheology on a biological system. In particular, we use fluorescence-stained actin filaments as probes to investigate the rheological properties of a cytoskeletal-model actin suspension. In addition, the actin filaments in the suspension are labeled with a fluorescent dye of a different color, which allows us to observe the medium under study by video-microscopy. This, together with the fact that the probes and the medium are made of the same material, improves the reliability of the method. We analyzed the actin suspensions for four different monomer concentrations. We defined the experimental protocol to obtain homogeneous networks of actin polymers in a quasi-2D chamber. Using fluorescence microscopy and advanced image analysis techniques, we propose a complete characterization of the bending dynamics of the probes with high spatial resolution. Finally, we obtain preliminary results for the shear modulus $G(\omega)$ of the suspensions.

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Investigating mechanical properties of plant roots using Brillouin microscopy

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The plant cell wall is a dynamic and structurally complex matrix that maintains cell shape, enables growth, and plays a central role in development by mediating mechanical and biochemical signals essential for both normal growth and adaptive responses to environmental stimuli (1-2). While the importance of the cell wall in plant physiology is well established, our understanding of its mechanical properties remains limited. Existing data is mostly based on indirect, invasive techniques and/or obtained from non-living samples, which restrict insights into its in vivo biophysical behavior (3). In this context, Brillouin microscopy emerges as a powerful, non-invasive, label-free technique for in vivo characterization of viscoelastic properties with subcellular resolution. Using this approach, we mapped the viscoelastic properties of cell walls across root tissues in wildtype and mutant seedlings, in different developmental stages and in response to treatments. Intriguingly we observed that the properties can both differ between cell layers and rapidly respond to osmotic stress. Mutants for specific cell wall components show limited changes in mechanical properties compared to wild-type plants. In contrast, mutants deficient in cell wall integrity sensors exhibit marked differences compared to wild type, suggesting that manipulating CWI sensors has more profound effects than manipulation of individual cell wall components. Taken together, the results highlight the valuable insights generated by Brillouin microscopy enabling us to uncover the role of cell wall mechanical properties in plants (3-4).

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Deep Learning-Based Segmentation and Analysis of the Actin Cytoskeleton in Adipose Stem Cells with Novel Quality Metrics

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The actin cytoskeleton plays a critical role in regulating cell morphology, stiffness, adhesion, and motility. Stress fibers, contractile actomyosin bundles, are particularly vital for mechanotransduction processes [1], [2]. This study introduces a novel approach for analyzing the actin cytoskeleton in fluorescence microscopy images, with specific focus on stress fibers using deep learning techniques. Adipose stem cells (ASCs) were cultured, stained with Alexa 488 phalloidin, and imaged via confocal microscopy. Our dataset consisted of 170 paired images (fluorescence image + binary mask). Initial segmentation was performed using SFEX, a MATLAB-based open-source software package, which generated binary masks subsequently utilized to train U-Net [3] and DeepCell [4] neural networks (NNs). Due to the limited dataset size, we employed data augmentation techniques with a test split of 0.2 and validation split of 0.1. The networks were trained for 100 epochs on Google Colab using the Adam optimizer with a learning rate of 1e-4, achieving a loss below 30%. While standard segmentation metrics showed acceptable performance (Dice coefficient and IoU parameters >70%), these measures fail to capture critical morphological aspects of actin filaments. Specifically, the Dice coefficient cannot adequately penalize filament discontinuities, thickness variations, or false segmentations—all crucial for accurate cytoskeletal analysis. We propose a novel connectivity-preserving metric that evaluates both pixel-wise accuracy and structural integrity of actin filaments, ensuring proper representation of continuous structures, accurate filament thickness, and robust discrimination between true filaments and artifacts. This metric will enable more relevant evaluation of segmentation quality for mechanobiological studies where filament continuity and morphology directly impact functional interpretation.

Keywords: mechanobiology, cytoskeleton, stress fibers, deep learning, image segmentation, U-Net

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Brillouin frequency shift analysis reveals intrinsic differences between normal and keloid fibroblast cells and their subcellular responses to TGF-β treatment

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Brillouin Light Scattering (BLS) is an inelastic scattering of light by spontaneous thermal fluctuations in a material, enabling access to viscoelastic properties. Traditional biomechanical characterization methods-such as magnetic bead twisting, deformation microscopy, micro-rheology, and atomic force microscopy (AFM)- are typically contact-based, invasive, or lack sufficient resolution. In contrast, BLS imaging is non-contact, label-free, and non-destructive, making it particularly suitable for investigating biological samples at the GHz/micron scale, with strong potential for clinical diagnostics. This work aims to compare the Brillouin frequency shifts of normal and keloid fibroblast cells to better understand the pathogenesis of keloids and explore their potential therapeutic targets. Mattana et al.[1] demonstrated the feasibility of non-contact mechanical and chemical analysis of fibroblast cells using BLS, successfully distinguishing subcellular compartments and cellular states.

Keloids are fibrotic skin lesions characterized by excessive collagen deposition and growth beyond the initial wound, often following surgery or burns [2]. They are recognized as chronic inflammatory conditions with similarities to cancer [3]. Fibroblasts, which play a pivotal role in keloid formation, are highly responsive to mechanical and biochemical cues in their environment [2]. Interaction with immune cells drives fibroblast differentiation into myofibroblasts, promoting collagen overproduction [4] and reinforcing a feedback loop of stiffness-induced migration, collagen deposition, and activation [2]. Understanding the interplay between mechanical signaling and inflammation in keloid development is crucial [5]. In this project, BLS is employed to assess the Brillouin frequency shift of normal and keloid fibroblast cells. Statistical comparisons revealed no significant difference in Brillouin shift between the cytoplasm and nucleus of untreated normal fibroblast cells ($p=0.92$). However, upon treatment with TGF-β, a significant increase in nuclear Brillouin shift was observed compared to the cytoplasm ($p<0.001$). A similar analysis in keloid fibroblasts showed no significant difference between cytoplasmic and nuclear regions, both before ($p=0.65$) and after TGF-β treatment ($p=0.21$). When comparing untreated normal and keloid fibroblasts, both cytoplasmic and nuclear regions in keloid cells exhibited significantly higher Brillouin shift ($p<0.001$), what agrees with our findings that show elevated levels of actin filaments and increased expression of α-smooth muscle actin (α-SMA) in keloid fibroblasts compared to normal fibroblasts, indicating enhanced cytoskeletal remodeling and stress fiber formation. These observations further support the aggressive fibrotic phenotype seen in keloid cells. TGF-β treatment in normal fibroblasts led to a significant increase in Brillouin shift of the nucleus ($p<0.001$), while cytoplasmic changes were not statistically significant ($p=0.08$). In contrast, TGF-β treatment did not significantly affect Brillouin shift in either the nucleus ($p=0.06$) or cytoplasm ($p=0.71$) of keloid fibroblast cells.

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Brillouin microscopy to unravel the relationship of macrophage polarization with metabolic demands and mechanotransduction in response to biomaterial stiffness

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Introduction: Macrophages act in several biological processes adopting multiple phenotypes with specific functions (i.e. M1, M2a, M2c) in response to their surrounding environment (1). Evidence shows substrate mechanical properties can modulate macrophage metabolism and in turn influence polarization (2). We hypothesize a direct relationship between macrophage polarization, metabolism and viscoelasticity. In this study we assessed human monocyte-derived macrophage (MdM) polarization using the innovative Brillouin microscopy for non-invasive mapping of cell viscoelasticity joined with Fluorescence Lifetime Imaging Microscopy (FLIM) for cell metabolism delineation to unravel this complex crosstalk in a label-free, non-destructive, 3D manner.

Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats by LymphoprepTM density gradient and anti-CD14 magnetic beads. After 6 days M-CSF stimulation for macrophage differentiation, MdMs were reseeded in gelatin-coated glass-bottom dishes, then treated with IFN-γ, IL-4&IL-13 and IL-10. Real time metabolic and viscoelasticity property were assessed using Brillouin and 2P-FLIM analysis. RT-PCR and ELISA assays were used to assess polarization state.

Results: IFN-γ treatment showed increased glycolytic metabolism and lower stiffness with M1 polarization markers compared to IL-4&IL-13, where M2a markers were upregulated and a higher oxidative phosphorylation metabolism was detected and higher stiffness both in nuclei and cytoplasm. IL-10 treatment showed intermediate characteristic between the previous two and production of M2c markers.

Discussion: M1 polarisation shows an enhanced glycolytic metabolism and reduced cellular stiffness, consistent with its pro-inflammatory function. M2a and M2c macrophage subtypes display distinct metabolic and biomechanical profiles. M2a macrophages rely primarily on and show higher cellular stiffness compared to both M1 and M2c phenotypes, a higher degree of cytoskeletal organization consistent with their involvement in fibrotic matrix remodelling. In contrast, M2c macrophages display high glycolytic activity and decreased cytoskeletal engagement, suggesting a more metabolically active but mechanically quiescent state.

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Keywords: Brillouin, 2P-FLIM, stiffness, metabolism, polarization

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Brillouin Microscopy: A Tool for 3D Imaging in Mechanobiology

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In recent years, Brillouin imaging has rapidly advanced, finding new application areas thanks to its capability to measure mechanical parameters in three dimensions and on live samples. We present initial results obtained with our newly developed Brillouin microscope, which integrates this technology with existing optical imaging methods.

We showcase several examples, including single-cell measurements and tissue sample analyses. Its 3D capability makes this method particularly valuable for 3D cell culture applications, with initial results on complex organoids being presented. Additionally, there is a growing number of applications in plant physiology, and we will share results using the Arabidopsis root model system.

In summary, Brillouin microscopy is a versatile tool that can visualize differences in mechanical properties, quantify them, and observe dynamic events on time scales relevant to developmental biology.