1st Conference on Brillouin light Scattering for life science and biomedical applications

NOVEMBER 14-17TH, 2022

LYON - FRANCE

Our sponsors

OXXIUS
Simply Light

Optique Peter
OPTICAL & MECHANICAL ENGINEERING

LightMachinery
Excellence in Lasers and Optics

HAMAMATSU
PHOTON IS OUR BUSINESS

Platinum sponsor

TABLE STABLE

Monday 14th

14h00	**Thorsten Hamann (invited)** *Using Brillouin microscopy to investigate changes in stiffness in Arabidopsis thaliana seedling roots*
14h40	**Caterina Czibula** *Exploring Brillouin light scattering of lignocellulosic fibers*
15h00	**Michelle Bailey** *Spectroscopic measurement of the viscoelastic properties and water structure of gelatin hydrogels*
15h20	**Marketa Samalova** *Expansins control cell wall stiffness and root growth via cell type-specific expression in Arabidopsis*
15h40	**Coffee break**
16h10	**Andreas Karampatzakis** *Probing the internal micromechanical properties of bacterial biofilms by Brillouin imaging*
16h30	**Mengting Yao** *GHz optoacoustic lenses for sub-optical resolution imaging*
16h50	**Fehima Ugarak** *Brillouin Light Scattering Characterization of Gray Tone 3D Printed Isotropic Materials*
17h10	**Flash talks** Luis Alonso Baez Julia Garcia Baucells Franziska Busse Jennifer Illibauer
17h30	**Posters and cocktail**
19h30	**Diner at Valpré**

Tuesday 15th

9h00	**Jitao Zhang (invited)** *Brillouin microscopy for tumour spheroid and embryo*
9h40	**Yogeshwari S. Ambekar** *Combining Optical Coherence Tomography and Brillouin Microscopy to Study Murine Neural Tube Development*
10h00	**Marketa Schmidt Cernohorska** *Material properties of centrosome*
10h20	**Coffee break**
10h50	**Francesca Palombo (invited)** *Brillouin microscopy to probe viscoelastic properties of tissues in health and disease*
11h30	**Alessandra Anna Passeri** *Mechanical Imaging Of Single Cells Investigated By correlative Brillouin and Raman Microscopy*
11h40	**Theresa Schlamp** *Brillouin Imaging of the Plant Cell Wall*
12h10	**Lunch**
14h00	**Lyon visit & free time**
19h30	**Gala diner at Valpré**

Wednesday 16th	
9h00	**Chris Lorenz (invited)** *Molecular dynamics simulations of biological systems*
9h40	**Giuseppe Antonacci** *Ultracompact on-chip notch filter on a silicon nitride ring resonator*
10h00	**Marjolaine Gonon-Caux** *Brillouin spectroscopy for lubricated bearing contacts*
10h20	**Coffee break**
10h50	**Antoine Coulon (invited)** *Magnetic manipulation of chromosomes in living cells*
11h30	**Salvatore La Cavera III** *3D Brillouin endo-microscopy of biological matter*
11h40	**Fan Yang** *Pulse-enhanced stimulated Brillouin scattering microscopy*
12h10	**Lunch**
14h00	**Wouter Roos (invited)** *Single-molecule approaches to study bionanodynamics in real-time*
14h40	**Hamid Keshmiri** *The dynamic role of anisotropy in the GHz regime*
15h00	**Laurent Belliard** *Correlative Imaging of Motor neuron Cell Elasticity by Pump and Probe Spectroscopy*
15h20	**Rafael Fuentes-Dominguez** *Parallel phonon microscopy for cell elasticity imaging*
15h40	**Coffee break**
16h10	**Silvia Caponi** *Brillouin imaging of micro-structured samples: size and environment effect*
16h50	**Pierre Bouvet** *GHz optoacoustic lenses for sub-optical resolution imaging*
17h10	**William Hardiman** *Phonon microscopy of live cells*
17h30	**Giulia Guerriero** *Predicting nanocarriers efficacy in 3D models with Brillouin Light Scattering spectroscopy*
19h30	**Diner at Valpré**

Thursday 17th	
9h00	**Claude Verdier (invited)** *Cancer cell and tissue rheology using AFM*
9h40	**Timon Beck** *Quantifying optomechanical properties of phase separated protein condensates*
10h00	**Alexis Viel** *Probing cell crowding in cells and tissues with Brillouin light scattering*
10h20	**Coffee break**
10h50	**Martina Alunni Cardinali** *Brillouin and Raman Spectroscopy application to mark the "boundaries" of bacterial colonies infecting bone tissue*
11h10	**Virgile Larrauri** *Mechanical and chemical characterization of dental tissues affected by molar and incisor hypomineralization*
11h30	**Evgenije Novta** *Dental composite's photo-activation using optical fibers – a holographic, thermographic, and Raman study*
11h50	**Closing remarks**
12h10	**Lunchbox**

Monday 14th

Using Brillouin microscopy to investigate changes in stiffness in *Arabidopsis thaliana* seedling roots

Laura Bacete.[1]; Yan Guqi[2]; Jeremie Margueritat J.[2]; Thomas Dehoux T.[2] and Thorsten Hamann[1*]

[1]Institut Lumière Matière, UMR5306, Université Lyon, 1-CNRS, 69622 Villeurbanne, France.

[2]Institute for Biology, Faculty of Natural Sciences, Norwegian University of Science and Technology, 7491 Trondheim, Norway.

**Corresponding author:* Thorsten.hamann@ntnu.no

Plant cell walls surround all plant cells, provide support during growth and development and form the interface with the environment. In this context they frequently also form the first line of defense against biotic and abiotic stress originating in the environment. The walls consist mainly of different types of polysaccharides such as cellulose, hemicellulose, pectins, proteins and dependent on the specific wall type also lignin. In order to meet the different functional requirements wall composition and structure change dynamically, which also affects wall stiffness. In the past atom force microscopy has been used frequently to investigate changes in wall stiffness of epidermal cell layers. More recently Brillouin microscopy has been used to investigate changes in wall stiffness in subepidermal cell layers in *Arabidopsis thaliana*.

We are interested in understanding the mode of action of the cell wall integrity (CWI) maintenance mechanism, which is monitoring the functional integrity of the wall and is initiating adaptive changes in composition and structure to maintain integrity in case cell wall damage occurs. We have initially used Brillouin microscopy to investigate if and how manipulation of turgor pressure and CWI affects stiffness in Arabidopsis seedling roots. We proceeded then to determine how different signaling components involved in response to CWD and osmotic stress responses modulate stiffness. Here we will present our latest findings on this topic.

Exploring Brillouin light scattering of lignocellulosic fibers

Caterina Czibula[1, *], Ulrich Hirn[1], Kristie J. Koski[2]

[1] *Institute of Bioproducts and Paper Technology, Graz University of Technology, Austria*

[2] *Department of Chemistry, UC Davis, USA*

**Corresponding author: caterina.czibula@tugraz.at*

Plants are built up by fibers which contribute to the structure and function of, f.e., the tree. In the case of lignocellulosic fibers as they exist in wood, the main components are cellulose, hemicellulose, and lignin. Cellulose is the most abundant biopolymer and its products such as paper, tissue, and paper board are a necessity for our daily life. With the biobased industrial sector gaining importance, the demand is going to increase further.

However, a lot of the influencing parameters on the final product's properties are unknown. Especially figuring out the interplay between the single fiber and the fiber network level is challenging. One reason is that single fibers are complicated to investigate. They exhibit a complex hierarchical structure and have anisotropic properties which depend on moisture content. Furthermore, the geometry of such fibers is making them difficult to handle, with a diameter of about 20-50 µm and a limited length of 2-5 mm. Tensile testing is the main technique to investigate single fiber behavior. Besides high scattering and the need for fixation on a sample holder, it yields only properties in one fiber direction. With atomic force microscopy, the testing of more fiber directions is possible, however, very tedious and error-prone [1].

Here, Brillouin spectroscopy comes into play as a technique which enables the measurement of the whole elastic stiffness tensor in a non-contact manner. Using laser light to probe phonons in different directions and assuming a crystal geometry like hexagonal for fibrous materials [2] enables the determination of the elastic constants. In this talk, first results of such investigations will be presented for different types of lignocellulosic fibers and comparisons to mechanical testing will be drawn.

References:

[1] C. Czibula, A. Brandberg et al, Sci. Rep., **11**, 22411 (2021)

[2] K. Koski et al, Nat. Mat., **12**, p262-267 (2013)

Brillouin Imaging of the Plant Cell Wall

Theresa Schlamp[1], Yoselin Benitez-Alfonso[2], Thomas Greb[1, *]

[1] *Centre for Organismal Studies (COS), Heidelberg University, Heidelberg, Germany*

[3] *Centre for Plant Science, University of Leeds, Leeds, UK*

*Corresponding author: **thomas.greb@cos.uni-heidelberg.de***

Unlike in animals, the extracellular matrix of plants forms a rigid wall that fixes the position of cells relative to each other. Remodeling the plant cell wall is therefore an essential aspect of plant growth and development. Plant cell walls are very ambivalent in their provided properties, on the one hand providing a rigid structure that supports and protects the plant and define the growth vector for cells by restricting turgor driven growth to the desired directions only. On the other hand, it has to be flexible to allow turgor-driven cell growth which can only take place if the cell wall is flexible enough to allow extension. This contradicting behavior of the plant cell wall is made possible by changes in chemical composition, the crosslinking of polymers and the variability of their orientation.

Expecting that monitoring these properties will help us to decipher the importance of cellular mechanics, we have initiated a project to use Brillouin imaging for studying the elongation zone of the root in *Arabidopsis thaliana*. As a proof of concept, we revealed that the composition of a cell wall-like hydrogel influences the Brillouin shift. Moreover, cell walls of cells of different developmental states and orientation show differences in their Brillouin shift, demonstrating the potential of the technique for investigating the mechanical aspect of plant morphogenesis.

Expansins control cell wall stiffness and root growth via cell type-specific expression in *Arabidopsis*

Marketa Samalova[1*], Kareem Elsayad[2], Alexis Peaucelle[3], Jaromir Gumulec[4], Alesia Melnikava[1], Evelina Gahurova[1] and Jan Hejatko[1]

[1]*CEITEC MU, Brno, Czech Republic*

[2]*Medical University of Vienna, Vienna, Austria*

[3]*INRAE, Versailles-Grignon, France*

[4]*Masaryk University, Brno, Czech Republic*

[*]*Corresponding author: marketa.samalova@sci.muni.cz*

Changes in the cell wall (CW) mechanics are a driving force of plant growth and development as predicted by a number of biomechanical models. Expansins facilitate cell expansion via mediating pH-dependent cell wall loosening [1]. However, the role of endogenous expansins in the control of biomechanical CW properties in the tissue and organ context remains elusive. We determined hormonal responsiveness and specificity of expression and localization of expansins predicted to be direct targets of cytokinin signaling in *Arabidopsis thaliana*. Using both transcriptional as well as translational fusions we found EXPA1 localized dominantly in the epidermis of lateral root cap. The response to exogenously applied plant hormone cytokinins was moderate unlike the robust response to auxins. Further, we found EXPA10 and EXPA14 localized predominantly at the three-cell boundaries of epidermis/cortex in various root zones. Cell type-specific localization of EXPA15 overlapped with higher CW stiffness measured via Brillouin light scattering (BLS) microscopy [2], implying possible role of expansins in the control of biomechanical CW properties.

The ability of EXPA1 to increase the CW stiffness was confirmed in stable transgenic lines following dexamethasone-induced [3] *EXPA1* overexpression and indicated by higher Brillouin frequency shift (BFS), as well as the apparent Young modulus [4] measured via atomic force microscopy (AFM). *In situ* measurement of the refraction index in living cells using a holotomographic microscope excluded the possibility that the observed EXPA1-induced increase of BFS is due to changes in the CW mass density. The *EXPA1* overexpression resulted in the root growth arrest phenotype, severity of which was enhanced by lower pH.

We propose that proper growth of roots in *Arabidopsis* requires delicate orchestration of biomechanical CW properties via tightly controlled expansins' activity and localization to specific cell types. Disturbing the balance has severe consequences for the plant growth.

References:

[1] S. McQueen-Mason et al, Plant Cell, **4**, p1425-1433 (1992)

[2] K. Elsayad et al, Trends in Cell Biol., **29**, p608-611 (2019)

[3] J. Craft et al, Plant J., **41**, p899-918 (2005)

[4] A, Peaucelle, J. Vis. Exp., **89**, p51317 (2014)

Probing the internal micromechanical properties of bacterial biofilms by Brillouin imaging

Andreas Karampatzakis[1,2], Radek Macháň[3], Peter Török[2,3,*]

[1] *Centre for BioImaging Sciences, National University of Singapore*

[2] *Blackett Laboratory, Department of Physics, Imperial College London, United Kingdom*

[3] *Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University Singapore*

**Corresponding author: peter.torok@ntu.edu.sg*

Bacterial biofilms consist of adherent bacterial cells embedded in a matrix of extracellular polymeric substances (EPS). The EPS matrix accounts for the majority of the biofilms dry mass and its functions include facilitating adhesion to surfaces and protecting the bacterial cells. In this way the matrix contributes to increased antibiotic resistance of biofilms and makes it harder to remove them from contaminated surfaces. Different aspects of biofilm EPC matrices have been, therefore, extensively studied, including their mechanical properties. Confocal Brillouin imaging is well suited for this task as it can probe the local mechanical properties in the interior of living biofilm colonies at micrometre scale in non-invasive and label-free manner. We present results obtained with a custom designed confocal Brillouin microscope equipped with a VIPA based spectrometer and a common path interferometer used to supress elastically scattered light. Our results show that mechanical properties of bacterial biofilm colonies vary with depth (distance from the top of the colony towards the substrate) as well as with the distance from the edge towards the centre of the colony. Smaller colonies tend to be stiffer in the centre, while older, lager colonies exhibit a softer core [1].

References:

[1] A. Karampatzakis et al, npj Biofilms and Microbiomes, **3**, p1-7 (2017)

GHz optoacoustic lenses for sub-optical resolution imaging

Mengting Yao[1], Rafael Fuentes-Dominguez[1], Fernando Perez-Cota[1], Salvatore La Cavera III[1], Richard J. Smith[1], Matt Clark[1*]

[1]*Optics and Photonics Group, University of Nottingham, University Park, Nottingham, NG7 2RD, UK*

**Corresponding author: eeymy@nottingham.ac.uk*

Phonon microscopy has demonstrated 3D elasticity imaging of living biological cells by measuring the Brillouin frequency using coherent phonon fields (i.e., time-resolved Brillouin scattering). Since the generated phonons are in the GHz frequency regime, this opens a route to sub-optical axial resolution images ($\lambda_{acoustic} = 280nm$) [1]. However, the lateral resolution is limited by the optical system.

To achieve the 'real' acoustic resolution in both axial and lateral, we propose a method to focus coherent phonon fields using novel GHz optoacoustic lenses. For instance, Fresnel zone-plate and concave lenses can be used for this purpose. The first one is a flat lens – easy for nanoscale fabrication, and the second one is a common acoustic focusing transducer design. Furthermore, these lenses can also be fabricated at the tip of hair-thin optical fibres and be compatible with ultrasonic endoscopic imaging system [2].

In this talk, we will present the design of Fresnel zone-plate as GHz optoacoustic lenses and demonstrate its capabilities to focus coherent phonon fields for sub-optical resolution imaging.

References:

[1] Smith, R.J., Pérez-Cota, F., Marques, L. *et al.* 3D phonon microscopy with sub-micron axial-resolution. *Sci Rep* **11,** 3301 (2021).

[2] La Cavera, S., Pérez-Cota, F., Smith, R.J. *et al.* Phonon imaging in 3D with a fibre probe. *Light Sci Appl* **10,** 91 (2021).

Brillouin Light Scattering Characterization of Gray Tone 3D Printed Isotropic Materials

Fehima Ugarak , Gwenn Ulliac , Julio Andres Iglesias Martinez , Johnny Moughames , Vincent Laude , Muamer Kadic & Alexis Mosset*,

Institute FEMTO-ST, CNRS, University Bourgogne Franche-Comte, 25000 Besancon, France

**Correspondence: alexis.mosset@femto-st.fr*

Three-dimensional direct laser writing technology enables one to print polymer microstructures whose size varies from a few hundred nanometers to a few millimeters. It has been shown that by tuning the laser power during writing, one can adjust continuously the optical and elastic properties with the same base material. This process is referred to as gray-tone lithography. In this paper, we characterize by Brillouin light scattering the complex elastic constant c11 of different reticulated isotropic polymers, at longitudinal phonon frequencies of the order of 16 GHz. We estimate the real part of the c11 constant to vary from 7 to 11 GPa as a function of laser power, whereas its imaginary part varies between 0.25 and 0.6 GPa. The linear elastic properties are further measured at a fixed laser power as a function of temperature, from 20◦C to 80◦C. Overall, we show that our 3D printed samples have a good elastic quality with high Q factors only ten times smaller than fused silica at hypersonic frequencies.

Keywords: 3D printed materials, Brillouin light scattering, Elastic properties of polymers

References:

[1] F. Ugarak et al. Brillouin Light Scattering Characterization of Gray Tone 3D Printed Isotropic Materials. Materials, **15**(12), 4070, (2022)

Studying the mechanical characteristics of plant roots using Brillouin microscopy

Luis Alonso Baez[1], Laura Bacete[1], Thomas Dehoux[2], Thorsten Hamann[1, *]

[1]*Institute for Biology, Faculty of Natural Sciences, Norwegian University of Science and Technology, 7491 Trondheim, Norway*

[2]*Institut Lumière Matière, Université Lyon 1, Villeurbanne, France*

**Corresponding author:* thorsten.hamann@ntnu.no

Plant cell walls are dynamic and versatile entities providing mechanical support, protection against abiotic and biotic stresses, and contributing to plant morphogenesis and patterning. During growth, plant cell walls need to be adaptive to respond to developmental and environmental cues. Turgor pressure in plant cells, which generates tensile stresses comparable to those found in bike tires, generates mechanical forces which can drive growth if the composition and structure of the cell walls allow cell elongation and expansion. Mechanical properties of plant cell walls are usually studied employing invasive techniques that require physical contact and are often limited to probing surface characteristics. In addition, some methods require fixing the sample and therefore don't allow to investigate molecular processes *in vivo*. Brillouin microscopy, being a contactless non-disruptive technique that allows investigation of deeper tissue layers, provides an excellent alternative to study physical properties in plant samples with cellular resolution (1).

The relatively simple tissue architecture and transparency of *Arabidopsis thaliana* roots allows to visualize and track cellular processes like cell elongation and responses to environmental signals over longer time intervals. How changes in turgor pressure affect the mechanical properties of *Arabidopsis* root cell walls have been measured using Brillouin microscopy (2). In addition, it was discovered that hormones play a role in adjusting osmotic levels when cell wall composition and/or structure are altered, providing feedback into cell wall mechanics. By combining genetics and Brillouin microscopy we found molecular components in the Abscisic acid (ABA) signalling pathway that regulate mechanical characteristics of cell walls, measured by a Brillouin frequency shift in the respective tissue. ABA is involved in responses to drought by decreasing the turgor pressure in plant cells (hyperosmotic stress), therefore, acting as a link between cell wall mechanics and plant adaptation. However, the molecular mechanisms involved during cell wall damage sensing and responses are just beginning to be discovered.

References:

[1] Elsayad et al, Cellulose, 27:4209-4220 (2020)

[2] Bacete et al., PNAS Vol. 119, No.1, e2119258119 (2022)

Centrosome mechanical properties in *C. elegans* embryos

Júlia Garcia Baucells[1,2], Manuel Rufin[3], Carlo Bevilacqua[4], Orestis Andriotis[3], Philipp Thurner[3], Robert Prevedel[4], Marketa Schmidt Cernohorska[1*], Alexander Dammermann[1*]

[1]*Max Perutz Labs, University of Vienna, Vienna, Austria*

[2]*Vienna BioCenter PhD Program, Doctoral School of the University of Vienna and Medical University of Vienna, Vienna, Austria*

[3]*Institute of Lightweight Design & Structural Biomechanics, Vienna University of Technology, Vienna, Austria*

[4]*Cell Biology and Biophysics Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany*

**Corresponding authors: marketa.schmidt.cernohorska@univie.ac.at alex.dammermann@univie.ac.at*

Centrosomes are non-membrane-bound organelles whose main function is to organize the microtubule cytoskeleton. They are composed of a pair of centrioles surrounded by an accumulation of proteins called the pericentriolar material or PCM. The main function of the PCM is to nucleate and anchor microtubules. During mitosis the PCM increases in size and microtubule nucleating capacity, which is critical for spindle assembly. In turn, the mitotic spindle controls the accurate segregation of chromosomes to the two daughter cells. At the end of mitosis, the PCM disassembles in a manner facilitated by pulling forces exerted on microtubules anchored at the centrosome. Centrosome abnormalities, both numerical and structural, have been linked to chromosome missegregation and aneuploidy, a hallmark of cancer.

Despite decades of research, a key question remains: How do centrosomes react to the pulling forces coming from the microtubules they nucleate? The answer to this question lies in the **mechanical properties of centrosomes** and how these change over time, particularly during the course of mitotic cell division.

In order to study the mechanical properties of centrosomes, we are taking advantage of the *C. elegans* early embryo, a system in which centrosome assembly is arguably best understood and centrosomes are significantly larger than in other experimental models. Using quantitative live cell imaging in one and two-cell stage embryos we found that the PCM is deformable and exhibits an elastic response to microtubule forces during early stages of mitosis. To further characterize these material properties *in vivo*, we imaged metaphase one-cell embryos using **Brillouin light scattering microscopy** and found that centrosomes have mechanical properties that are clearly distinct from condensed chromosomes and the surrounding cytoplasm. In a complementary approach, we further probed isolated centrosomes *in vitro* using Atomic Force Microscopy (AFM), to better put our measurements in context of those established for other cellular structures (see abstract by Marketa Schmidt Cernohorska).

The work briefly outlined above seeks for the first time to describe the mechanical properties of centrosomes, which are of key relevance for mitotic cell division. In the future, we want to

investigate how centrosome mechanical properties change during the cell cycle and study the effects on spindle function when these are perturbed.

Multi-modal microscope for simultaneous mapping of cellular forces and Brillouin scattering with high resolution

Franziska Busse[1], Andrew Meek[1], Nils M. Kronenberg[1], Giuliano Scarcelli[2], Malte C. Gather[1, *]

[1]*Humboldt Centre for Nano- and Biophotonics, Department of Chemistry, University of Cologne, Germany*

[2]*Fischell Department of Bioengineering, University of Maryland, USA*

[*]*Corresponding author: malte.gather@uni-koeln.de*

The field of mechanobiology studies the mechanical properties of cells and their critical role in development and health. Important factors are cell forces and viscoelastic properties, for which several measurement techniques have been established. To gain a better understanding of cell behavior, it is crucial to probe the interplay of all the different physical properties at play. However, various technical challenges have so far precluded the simultaneous acquisition of all relevant mechanical properties in cells. Here, we present a fully integrated microscope that contributes to closing this gap by combining elastic resonator interference stress microscopy (ERISM) and Brillouin microscopy. Both techniques are non-invasive, which is a major advantage in probing cell and tissue behaviour. Brillouin microscopy has been established as an optical technique to investigate the stiffness and viscosity of biological samples with very high resolution.[1] ERISM is a recently developed technique that utilizes interference within an elastic optical microcavity to detect cell forces with piconewton precision.[2] To combine both measurement techniques, we developed an elastic microcavity with minimal optical absorption. This cavity allows high-quality ERISM measurements, but unlike the conventional cavities, which are partially absorbing, avoids damage from the intense laser used for probing the Brillouin scattering. We also introduce an unequal armed Michelson interferometer to supress back reflection of the Brillouin laser at the microcavity surface. To demonstrate the utility of our combined microscope, we record high resolution images of cellular force, stiffness and viscosity of fibroblast cells.

References:

[1] G. Scarcelli et al, Nat. Photonics, **2**, p39-43 (2008)

[2] N. Kronenberg et al, Nat. Cell Biol., **19**, p864-872 (2017)

Correlative Brillouin Light Scattering study of blood plasma viscosity in relation to disease: a COVID-19 patient study

Jennifer Illibauer[1], Tamara Seitz[2], Alexander Zoufaly[2], Judith Aberle[1], Manuela Foedinger[2], Kareem Elsayad[1]

[1]*Medical University of Vienna, Vienna, Austria*

[2]*Klinik Favoriten, Vienna, Austria*

**Corresponding author:* kareem.elsayad@meduniwien.ac.at

The bulk viscosity (η_B) of fluids is a somewhat illusive quantity, the practical usefulness of which is primarily associated with the propagation of shock/acoustic waves. This is usually not of much relevance for typical rheological problems and can generally be neglected in fluid flow. Measuring η_B is also less trivial, typically done using Acoustic Spectroscopy (AS) or Brillouin Light Scattering (BLS). Here we present BLS as well as shear dynamic viscosity measurements on COVID-19 patient plasma as a function of disease severity and other factors. Our overarching goal is two-fold: (1) Can BLS serve as a proxy for assessing the dynamic viscosity and identify e.g. high-risk patients? We find that even if there is a similar trend it cannot be used as a quantitative equivalent. It nevertheless may serve as a qualitative indicator, and together with dynamic shear viscosity measurements provide additional insight into patient health. (2) Can η_B tell us something unique about disease severity, and what is the biophysics behind this? We find that the bulk and shear viscosity are separately perturbed in many COVID ICU patients but follow unique temperature scaling, that can be attributed in part to fibrinogen content. We propose this also results from the formation of fibrin micro-clots that are consistent with the observed anomalies in the scaling. This may have relevance for Long-COVID diagnosis/prognosis as data suggests fibrin micro-clots can persist post infection. Finally, we show η_B can become relevant for blood flow subject to external physical perturbations and pressure fluctuations which may lead to reported medical complications. In summary, we show BLS allows for assessment of often-neglected viscous plasma properties with potential prognostic and diagnostic relevance.

Tuesday 15th

Brillouin microscopy for tumour spheroid and embryo

Jitao Zhang *

Biomedical Engineering Department, Wayne State University, Detroit, MI, 48202

**Corresponding author: zhang4@wayne.edu*

Since first reported in 2008, confocal Brillouin microscopy has been opening up new possibilities for investigating the mechanical properties of biological specimens on both cell and tissue level. As a unique complementary tool to the conventional methods such as AFM, Brillouin microscopy provides access to the interior of biological sample and allows *in-situ* measurement. The new data acquired by Brillouin technique has been helping elucidate the role of biomechanics in physiological and pathological conditions. On the other hand, the specific requirement of the biological samples also drives the innovation of the Brillouin technology to achieve faster acquisition speed and lower phototoxicity. In this talk, we will first present our recent work on developing dual-line scanning Brillouin microscopy for rapid mechanical imaging at low irradiation level. We will then demonstrate the promising applications of Brillouin technique in biomedical research, where we focus on tumour spheroid and embryonic development.

Combining Optical Coherence Tomography and Brillouin Microscopy to Study Murine Neural Tube Development

Yogeshwari S. Ambekar[a], Manmohan Singh[a], Alexander W. Schill[a], Jitao Zhang[b], Christian Zevallos[a], Behzad Khajavi[a], Salavat R. Aglyamov[c], Richard H. Finnell[d], Giuliano Scarcelli[e], and Kirill V. Larin[a,f,*]

[a]Department of Biomedical Engineering, University of Houston; [b]Department of Biomedical Engineering, Wayne State University; [c]Department of Mechanical Engineering, University of Houston; [d]Department of Molecular and Cell Biology, Molecular and Human Genetics, and Medicine, Baylor College of Medicine; [e]Fischell Department of Bioengineering, University of Maryland; [f]Department of Molecular Physiology and Biophysics, Baylor College of Medicine

**Corresponding author: klarin@uh.edu*

During embryogenesis, there are many complex processes that are required for healthy development. However, these processes can be disturbed leading to development defects. One such process is the formation of the neural tube, called neurulation. It is postulated that mechanical forces govern neurulation. Thus, it is important to understand the interplay between forces and tissue stiffness during development. Due to the sub-optimal measurement techniques, it remains poorly understood. Brillouin microscopy is a non-invasive high-resolution all-optical imaging modality capable of mapping tissue stiffness. However, it lacks the ability to provide structural images and thus, is limited for imaging dynamic processes such as neurulation. To overcome this limitation, we have combined Brillouin microscopy with optical coherence tomography (OCT) in one synchronized and co-aligned instrument. The Brillouin-OCT system could map the layer-by-layer distribution of biomechanical properties of the neural tube in mouse embryos with OCT guidance at various developmental stages. Our data indicate that the biomechanical properties of mouse embryos were different at different embryonic days. At E 9.5 and 10.5, the neuroepithelial layer was stiffer compared to the mesoderm and ectoderm layers. The stiffness of the neural plate and neuroepithelium increased with the development stage.

Material properties of centrosome

Marketa Schmidt Cernohorska[1,*], Julia Garcia Baucells[1,2], Manuel Rufin[3], Carlo Bevilacqua[4], Orestis Andriotis[3], Philipp Thurner[3], Robert Prevedel[4], Alexander Dammermann[1*]

1Max Perutz Labs, University of Vienna, Vienna, Austria

2Vienna BioCenter PhD Program, Doctoral School of the University of Vienna and Medical University of Vienna, Vienna, Austria

3Institute of Lightweight Design & Structural Biomechanics, Vienna University of Technology, Vienna, Austria

4Cell Biology and Biophysics Unit, European Molecular Biology Laboratory (EMBL), Heidelberg, Germany

**Corresponding authors: marketa.schmidt.cernohorska@univie.ac.at*
alex.dammermann@univie.ac.at

Centrosome are cellular organelles that play a critical role in cell division as they nucleate and organize a bouquet of microtubules (the mitotic spindle) to which chromosomes attach and re segregated to the two daughter cells. Precise spatio-temporal organization of spindle assembly and chromosome segregation is required to ensure that no sister chromatid is lost, broken or amplified, and cells complete division without resulting in cell death or, worse, aneuploidy and tumorigenesis. Interestingly, although centrosomes are small (~2um) and lack a clearly defined boundary, they are capable of withstanding considerable forces being applied on them via the microtubules they nucleate without being pulled apart. The recently reported overall decrease in stiffness of tumor cells suggests that material properties of cellular structures, centrosome included, may be substantially altered in disease states. Consistent with this, centrosome structural abnormalities have been observed in a majority of human cancers and, through affecting the fidelity of chromosome segregation and cell division, may be a driving force in tumorigenesis.

While centrosomes have been well characterized in terms of their molecular composition, function and regulation, their material (biomechanical) properties remain essentially unknown. In order to address these material properties, we examined centrosomes in vitro isolated from C. elegans early embryos, which are well characterized in terms of their molecular composition and, critically, are substantially larger than those found in vertebrate somatic cells, facilitating microscopy-based approaches. Centrosomes purified on sucrose gradients continued to be able to nucleate and organize microtubules, indicating that they retain functionality. We next optimized measurements of their material properties by Brillouin light scattering microscopy and corroborated those results by atomic force microscopy (AFM) using both fine tip and colloidal probes to assess their surface topography and viscoelastic properties. Having successfully established methods to probe centrosome material properties in vitro, we are now applying fluorescence-correlated Brillouin microscopy in vivo in the C. elegans early embryo, to assess the stiffness of centrosomes as cells undergo mitosis (see also abstract by Julia Garcia Baucells).

In conclusion, centrosomes are measurable in Brillouin microscopy despite their small size, revealing mechanical properties in line with other intracellular components. The ability of Brillouin microscopy to examine subcellular structures in living cells opens up the possibility

to apply the same methods to assess centrosome function and dysfunction in human cancer cells.

Brillouin microscopy to probe viscoelastic properties of tissues in health and disease

Francesca Palombo[1, *]

[1] *School of Physics and Astronomy, University of Exeter, UK*

**Corresponding author: f.palombo@exeter.ac.uk*

Mechanical properties of live cells and tissues are often determinants of their biological function and impairments in these properties can lead to complications in diseases such as osteoarthritis and atherosclerosis. Mechanics in living systems have a spatio-temporal gradient in that biological matter is viscoelastic, hence measurements performed at different frequencies can lead to remarkably different scales of the properties investigated. In addition, there are different types of elastic moduli which quantify these properties, the most common ones being the Young's, bulk and shear moduli.

Imaging mechanical properties in a contactless, depth- and spatially resolved manner with micro-scale resolution is important to understand the inner working of biological systems. For instance, native tissues need to be preserved in their 3D environment for accurate mechanical testing. Brillouin micro-spectroscopy lends itself well for this type of investigations, since it probes viscoelastic properties on a microscale without the need for an external load, transducer or label applied to the specimen of interest. A sister technique to Raman spectroscopy, Brillouin spectroscopy is based on the interaction of light with thermally induced acoustic phonons which, by propagating inside a material, sense its viscous and elastic properties providing a complementary approach to chemical analysis.

This talk will cover the fundamentals of Brillouin microscopy and its application in biomedical sciences for tissue imaging in health and disease, a key priority of Exeter BioSpec team's research in last decade. Emphasis will be put on the strengths of the method in investigating the effects of hydration, phase transition and structural anisotropy in model and real tissues, and the importance of a correlative approach with complementary techniques such as Raman or infrared spectroscopy for quantitative analysis.

Mechanical Imaging Of Single Cells Investigated By correlative Brillouin and Raman Microscopy

Alessandra Anna Passeri,[1*] Chiara Argentati,[3] Ilaria Tortorella,[3] Francesco Morena,[3] Martina Alunni Cardinali,[1] Igor Neri,[1] Massimo Vassalli,[4] Daniele Fioretto,[1] Maurizio Mattarelli,[1] Sabata Martino,[3] Silvia Caponi[2]

[1]*Department of Physics e Geology, University of Perugia, I-06100 Perugia, Italy*

[2]*Istituto Officina dei Materiali, Italian National Research Council (IOM-CNR), Unit of Perugia*

[3]*Department of Chemistry, Biology, and Biotechnology, University of Perugia, 06123 Perugia, Italy*

[4]*James Watt School of Engineering, University of Glasgow, Glasgow G12 8LT, UK*

[*]*Corresponding author: alessandraanna.passeri@studenti.unipg.it*

Mechanical and chemical properties of biological systems are directly linked to their correct functionality. Therefore, the identification of these parameters is crucial to understand their influence on the system behaviour. The combined micro-Brillouin and micro-Raman setup [1], recently upgraded with an inverted microscope, extracts simultaneously these fundamental properties with subcellular spatial resolution, allowing the analysis of the mechanical and chemical modulation in different cellular compartments. The combined experimental setup was used to characterize in-vitro systems consisting of HEK cells with different levels of expression of the mechanosensitive ion channel Piezo1 [2], and primary fibroblasts (rFFFs) at different stages of Amyotrophic Lateral Sclerosis type-1 (ALS1) [3].

Brillouin spectra were analysed taking into account the sample heterogeneity in the scattering volume and the peak broadening induced by the use of high numerical aperture objective [4].

Our investigation highlights i) the mechanical properties of the distinct subcellular compartments identified by Raman spectroscopic markers, ii) the variations of the mechanical properties on the different cell types and iii) the existence of a positive correlation between the frequency shift and the width of the Brillouin peak, as usually found in liquid-like systems.

References:

[1] F. Scarponi et al, Physical Review X **7**, 031015 (2017)

[2] L. Sforna et al, J Cell Physiol. **237**, 1857– 1870 (2022)

[3] I. Bicchi et al, Biomedicines **9**(9), 1080 (2021)

[4] S. Mattana et al, Science & Applications **7**, 17139 (2018)

Spectroscopic measurement of the viscoelastic properties and water structure of gelatin hydrogels

Michelle Bailey[1], Daniele Fioretto[2], Francesca Palombo[1,*]

[1] *School of Physics and Astronomy, University of Exeter, UK*

[2] *Department of Physics and Geology, University of Perugia, Italy*

**Corresponding author: f.palombo@exeter.ac.uk*

The mechanical properties within the biological environment are crucial to the health and vitality of living systems, and alterations in mechanics can thereby indicate disease. Brillouin spectroscopy provides an all-optical means of discerning the micromechanics of biological samples through the scattering of light from acoustic waves or phonons. Previous work within our group has used Brillouin microscopy to measure the viscoelastic properties of elastin and collagen fibres [1], cross-sections of human hair [2], and tissue mimicking hydrogels [3], amongst other tissues. These results have shown Brillouin spectroscopy to give a unique description of the viscoelastic properties across a wide range of physical states, from the highly hydrated to the solid-like phase, and the transition between the two.

This contribution will focus on the evolution of mechanical properties and water structure of gelatin hydrogels as the water content is tuned. Brillouin spectroscopy has revealed the onset of a glass-like transition as the concentration of water in the hydrogels was reduced, observed through a sigmoidal evolution of the Brillouin frequency shift and a corresponding maximum in the linewidth. In addition to this, the use of Raman and FTIR spectroscopy as correlative techniques will also be discussed, which reveal further information about the structure of water during the dehydration process. Raman spectroscopy, enables the chemical composition to be determined simultaneously to the micromechanical properties probed by Brillouin spectroscopy, thus providing a comprehensive assessment of the sample. This combined approach demonstrates the potential of Brillouin-Raman spectroscopy for the study of physiology and disease, and here, provides a comprehensive understanding of biopolymer hydrogels, opening the way for expansion to more complex biological systems.

References:

[1] F. Palombo et al., J. R. Soc. Interface, 11(101), 20140739, (2014).

[2] N. Correa et al., J. Biophotonics, 14(6), e202000483, (2021).

[3] M. Bailey et al., Sci. Adv. 6(44), eabc1937, (2020).

Wednesday 15th

Molecular dynamics simulations of biological systems

Chris Lorenz

Biophysical & Soft Matter Research Group

Department of Physics, King's College London

Molecular dynamics simulations provide a molecular scale perspective of the interactions between molecules that lead to larger scale phenomena. By integrating Newton's second law, molecular dynamics simulations produce trajectories of the positions of the atoms/molecules within a given simulation and from those trajectories, structural, dynamic, and energetic properties of the system of interest can be determined. In this talk, I will give an overview of how molecular dynamics simulations work. Also, I will provide some examples of biological systems we have investigated which are (hopefully) of interest to the Brillouin light scattering community. Finally, I will provide some examples of how we have applied graph theoretical and machine learning approaches to assist in the analysis of these systems.

Ultracompact on-chip notch filter on a silicon nitride ring resonator

Giuseppe Antonacci[1,*], Kareem Elsayad[2], Dario Polli[1,3]

[1]*Specto Photonics, Via Caradosso 12, 20123 Milan, Italy*

[2]*Division of Anatomy, Center for Anatomy and Cell Biology, Medical University of Vienna, Vienna A- 1090, Austria*

[3]*Dipartimento di Fisica, Politecnico di Milano, 20133 Milan, Italy*

**Corresponding author: giuseppe@spectophotonics.com*

Collection of the elastic background light represents a major obstacle in the detection of the Brillouin spectrum. While several filtering methods have been successfully demonstrated and provide high extinction ratio [1-4], instrumental complexity given by the employment of a large number of optical components still represent a barrier in the adoption of Brillouin spectroscopy. Here, we demonstrate a fully integrated and ultracompact notch filter based on an optical ring resonator fabricated using photonics integrated circuits (PICs) on a silicon nitride platform [5]. Our on-chip ring resonator filter was measured to have an extinction ratio ~ 10 dB and a linewidth of 3 GHz at 532 nm central wavelength. Results herald the opportunity to further develop ultracompact and miniaturized modules based on silicon photonics for Brillouin spectroscopy and microscopy.

References:

[1] Meng, Z., et. al., Background clean-up in Brillouin microspectroscopy of scattering medium. Optics express, **22**, 5410-5415 (2014).

[2] G. Antonacci et al, Elastic suppression in Brillouin imaging by destructive interference, Appl. Physics Letters, **107**, 061102 (2015)

[3] Fiore, A., et. al., High-extinction virtually imaged phased array-based Brillouin spectroscopy of turbid biological media. Appl. Physics Letters, **108**, 203701, (2016).

[4] Shao, P., et. al. Etalon filters for Brillouin microscopy of highly scattering tissues. Optics express, **24**, 22232-22238 (2016).

[5] Antonacci, G., Elsayad, K., Polli, D., On-Chip Notch Filter on a Silicon Nitride Ring Resonator for Brillouin Spectroscopy. ACS Photonics, **9**, 772-777, (2022).

Brillouin spectroscopy for lubricated bearing contacts

Marjolaine Gonon-Caux[1], David Philippon[1], Jérémie Margueritat[2], Philippe Vergne[1], Laetitia Martinie[1, *]

[1]*Univ Lyon, INSA Lyon, CNRS, LaMCoS, UMR5259, 69621 Villeurbanne, France*

[2]*ILM, UMR5306, Université Lyon 1-CNRS, 69622 Villeurbanne, France*

**Corresponding author: laetitia.martinie@insa-lyon.fr*

Extreme conditions experienced by a lubricant in the multiple contacts of a rolling element bearing (high confinement, high pressures and high shear) may strongly influence the resulting macroscopic friction. Unfortunately, experimental tools to characterize the behavior of lubricants under such conditions are very limited.

An experimental set up is developed to perform Brillouin spectroscopy on lubricants in order to better understand and then to better predict friction in highly loaded lubricated contacts. Indeed, Brillouin spectroscopy turns out to be a very convenient tool for such application as it should enable to characterize the fluid inside the contact itself, and record data independently of the friction measurement.

We consider a classical ball on plane contact. This converging geometry at the contact inlet leads to very dynamic flow conditions. Indeed, the lubricant experiences a very strong (several GPa) and very rapid (on a few tenths of millimetres) pressure rise, as well as a strong increase of shear rate (up to $10^7 s^{-1}$). In addition, the heating generated by viscous dissipation can lead to temperature gradients of several tens of degrees. In order to understand the influence of each parameter, the project is divided into three steps.

The first step focused on the influence of pressure and temperature on the behavior of the lubricants. This is the subject of this presentation. Measurements have been performed with both a tandem Fabry-Perot interferometer and a VIPA interferometer, revealing a good agreement with both devices. Brillouin spectroscopy makes it possible to derive information on the longitudinal properties of lubricants under pressure, in particular their modulus and their relaxation time, and raises the question of the influence of glass transition on the macroscopic friction.

The next step will consist in performing Brillouin measurements in a rheometer, at atmospheric pressure and variable temperature, to highlight the influence of an external mechanical stress on the properties of the probed fluid.

Finally, the VIPA spectrometer will be installed onto the tribometer in order to simultaneously record Brillouin spectra and friction inside the contact. This step is challenging due to the very small thickness of the fluid flowing in the contact (few hundreds of nanometres).

Magnetic manipulation of chromosomes in living cells

Veer I. P. Keizer[1,2,3,4], Simon Grosse-Holz[1,2,3,5], Maxime Woringer[1,2,3], Laura Zambon[1,2,3,4], Koceila Aizel[1,3], Maud Bongaerts[1,3], Fanny Delille[1,6], Lorena Kolar-Znika[1,2,3], Vittore F. Scolari[1,2,3], Sebastian Hoffmann[1,4], Edward J. Banigan[5], Leonid A. Mirny[1,2,5], Maxime Dahan[1,3,#], Daniele Fachinetti[1,4,*], Antoine Coulon[1,2,3,*]

[1]*Institut Curie, PSL Research University, Sorbonne Université*

[2]*CNRS UMR3664, Laboratoire Dynamique du Noyau, 75005 Paris, France.*

[3]*CNRS UMR168, Laboratoire Physico Chimie Curie, 75005 Paris, France.*

[4]*CNRS UMR144, Laboratoire Biologie Cellulaire et Cancer, 75005 Paris, France.*

[5]*Department of Physics and Institute for Medical Engineering and Science, Massachusetts Institute of Technology, Cambridge, 02139 MA, USA.*

[6]*ESPCI Paris, CNRS UMR8213, LPEM, 75005 Paris, France.*

[#]*Deceased. *Corresponding authors. Lead contact: antoine.coulon@curie.fr*

Over the past decades, our understanding of the 3D organization and dynamics of the mammalian genome in the nucleus has improved tremendously. However, the physical principles underlying this organization remain partially understood due to the lack of tools to directly exert and measure forces on interphase chromosomes *in vivo* and probe their material nature. To address this gap, we have developed a novel approach to mechanically manipulate chromosomes in the nucleus of a living cell using magnetic forces [1]. It consists in targeting iron-containing nanoparticles to a genomic locus of interest and applying a controlled magnetic field. With this approach, we made the first measurements of how an interphase chromosome, in a living cell, responds to a point force and recoils after force release. We observed viscoelastic displacements over microns within minutes in response to near-picoNewton forces. The trajectories we measured are surprisingly consistent with a Rouse polymer model. Our results highlight the fluidity of chromatin, with a moderate contribution of the surrounding material, revealing minor roles for crosslinks and topological effects, and challenging the view that interphase chromatin is a gel-like material. We also characterized, for the first time, physical parameters and properties of interphase chromosomes (force-displacement relationship, fluctuation-dissipation...). Our new approach opens avenues for future research to probe how the physical properties of the genome relate to genome functions, including transcription, chromosome segregation, DNA damage repair and replication.

References:

[1] Keizer V. et al, (2022) Science, 377:6605, p489-495

3D Brillouin endo-microscopy of biological matter

Salvatore La Cavera III[1, *], Fernando Perez-Cota[1], Veeren Chauhan[2], William Hardiman1, Mengting Yao[1], Rafael Fuentes-Dominguez[1], Kerry Setchfield[1], Richard J. Smith[1], Matt Clark[1]

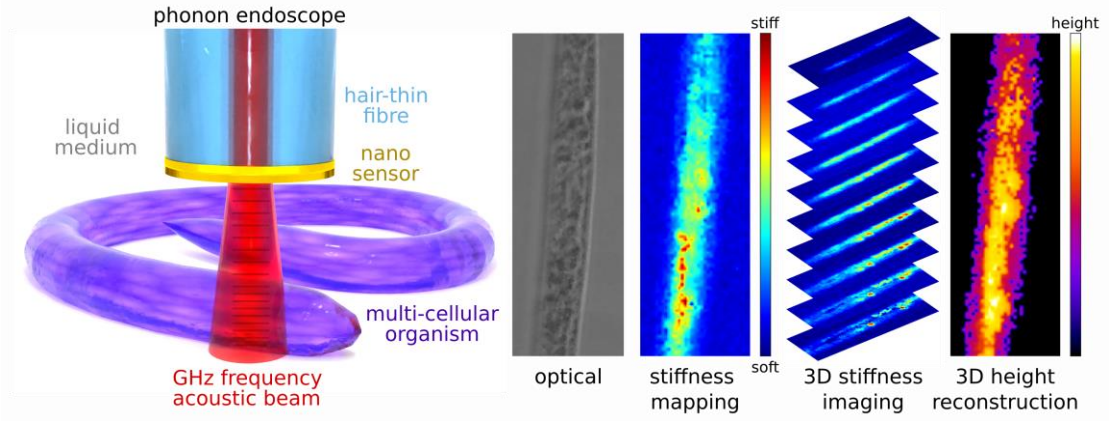
[1]*Optics and Photonics Group, Faculty of Engineering, University of Nottingham, University Park, Nottingham, NG7 2RD, UK*

[2]*Advanced Materials & Healthcare Technologies, School of Pharmacy, University of Nottingham, University Park, Nottingham, NG7 2RD, UK*

**Corresponding author: salvatore.lacaveraiii@nottingham.ac.uk*

The phenomenon of Brillouin scattering empowers powerful new technologies [1-3] to visualise *elastic* properties of biological specimens from single cells [3] to multi-cellular organisms [4]. Exciting work in the field of mechanobiology is beginning to reveal a close relationship between the mechanical properties of biological tissue environments and the progression of myriad diseases [2,5]. Non-destructive, label-free, and high resolution elasticity imaging techniques such as Brillouin and phonon microscopies are undergoing rapid development to meet the challenges of characterising tissue elasticity with the aim of using it as a disease biomarker for future clinical diagnostics.

In order to realise the clinical potential of Brillouin scattering-based techniques, it is critical to develop an endoscopic probe for measuring elasticity in future in-vivo environments. Engineering this technology is particularly challenging for Brillouin scattering, since the glass optical fibre that underpins endoscopy is highly photoelastic which ultimately shrouds light scattered from the microscopic region of interest. We have developed a *phonon probe* which actively injects high amplitude GHz strain pulses into specimens allowing us to utilise industry-standard optical fibre products and have demonstrated proof of concept this technique can be used for high resolution 3D imaging [6]. In this talk we show that this new technology is highly applicable to the 3D elasticity imaging of biological tissue from the single-cell scale to multi-cellular organisms and provides a future pathway for the clinical application of in-vivo Brillouin spectroscopy of tissue.



References:

[1] G. Scarcelli et al, Nat. Photonics, **2**, p39-43 (2008)

[2] R. Prevedel et al, Nat. Methods, **16**, 969-977 (2019)

[3] F. Perez-Cota et al, J. Appl. Phys., **128,** 160902 (2020)

[4] I. Remer et al, Nat. Methods, **17,** 913-916 (2020)

[5] C. Alibert et al, Biol. Cell, **109,** 167-189 (2017)

[6] S. La Cavera et al, Light Sci. Appl., **10,** 91 (2021).

Pulse-enhanced stimulated Brillouin scattering microscopy

Fan Yang[1], Carlo Bevilacqua[1,7], Sebastian Hambura[1], Ana Neves, Anusha Gapolan[1], K. Watanabe[1], Magdalena Schindler[2], Ling Wang[1], Nicoletta Petridou[2], Jan Ellenberg[1], Alba Diz-Muñoz[1], Simone Köhler[1], Georgia Rapti[2], Martin Jechlinger[3] and Robert Prevedel[1,4-7]

[1] *Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, Heidelberg, Germany.*
[2] *Developmental Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany.*
[3] *Molit gGmbH, Heilbronn, Germany.*
[4] *Epigenetics and Neurobiology Unit, European Molecular Biology Laboratory, Monterotondo, Italy.*
[5] *Molecular Medicine Partnership Unit (MMPU), European Molecular Biology Laboratory, Heidelberg, Germany*
[6] *German Center for Lung Research (DZL), Heidelberg, Germany*
[7] *Interdisciplinary Center of Neurosciences, Heidelberg University, Heidelberg, Germany*
Corresponding authors: prevedel@embl.de and fan.yang@embl.de

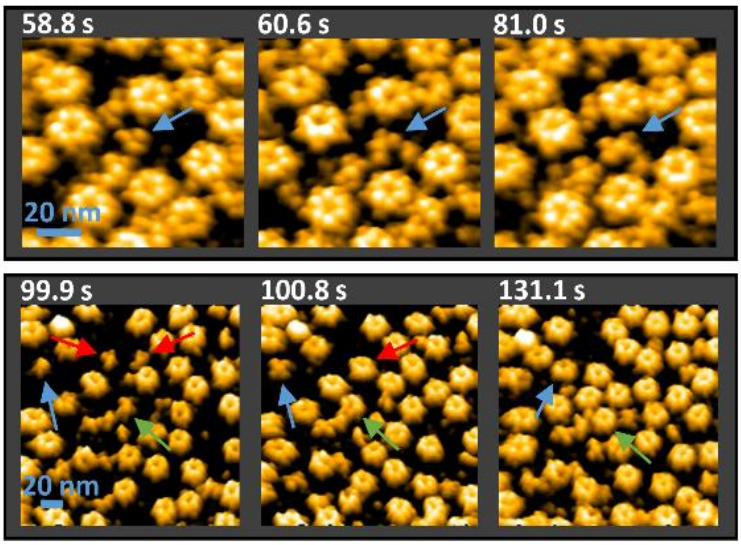
Stimulated Brillouin scattering microscopy (SBSM) can be used for label-free, non-contact mechanical imaging in biology with high sensitivity and high spectral resolution. However, typically the optical power required in SBSM is >250 mW which severely limits the applicability in biology. Here we propose a new pulse-enhanced SBSM approach that can substantially decrease the optical power demands and thus increase the usability of SBSM for live biological samples.

Single-molecule approaches to study bionanodynamics in real-time

Wouter H. Roos

Moleculaire Biofysica, Zernike Instituut, Rijksuniversiteit Groningen, Groningen, the Netherlands.

Cellular life harbours a fascinating variety of complex processes and we are still at the beginning of our understanding of these processes. Using (High Speed) Atomic Force Microscopy (HS-AFM) and fluorescent Optical Tweezers we are now able to scrutinize the dynamics of these processes at the nano scale, in real time, in liquid. I will show how we are using these techniques to study the fascinating physics of sub-cellular dynamics and biomimetic assembly processes. This will be illustrated by discussing assembly and disassembly of ESCRT-III protein complexes and High speed AFM visualization of the dynamics of self-replicators. Furthermore, dual-trap optical tweezers studies of the self-assembly of virus-like-particles (VLPs) are shown that reveal real time binding of capsid proteins to dsDNA and the formation of stable VLP structures around the genome. Furthermore the mode of action of antibiotics will be discussed. Finally the formation dynamics of 2D capsid protein assemblies will be analysed, particularly revealing how complex the kinetics of viral self-assembly can be, with multiple assembly pathways and continuously occurring assembly and disassembly events (see figure).



References:

[1] Maity et al., PNAS, 119, e2113927119 (2022)

[2] Shukla et al., Nature, 608, 390 (2022)

[3] Buzón et al., Science Advances, 7, eabg0811 (2021)

[4] Bruinsma et al., Nature Reviews Physics, 3, 76 (2021)

The dynamic role of anisotropy in the GHz regime

Hamid Keshmiri[1]†, Domagoj Cikes[2]‡, Marketa Samalova[3], Lukas Schindler[4], Lisa-Marie Appel[5], Michal Urbanek[6], Ivan Yudushkin[4], Dea Slade[5], Wolfgang J. Weninger[7], Alexis Peaucelle[8], Josef Penninger,[2,9] Kareem Elsayad[1,7]*

[1]*Advanced Microscopy Facility, VBCF, Vienna Biocenter; Vienna, Austria.*

[2]*Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Austria.*

[3]*Department of Experimental Biology, Masaryk University; Brno, Czech Republic.*

[4]*Center for Molecular Biology, University of Vienna; Vienna, Austria.*

[5]*Max Perutz Labs, Medical University of Vienna; Vienna, Austria.*

[6]*CEITEC Nano, Brno University of Technology; Brno, Czech Republic.*

[7]*Division of Anatomy, MIC, Medical University of Vienna; Vienna, Austria.*

[8]*Institut Jean-Pierre Bourgin, INRAE, Université Paris-Saclay, Versailles, France.*

[9]*Life Science Institute, University of British Columbia, British Columbia, Canada*

†Current address: Federal Institute for Materials Research & Testing; Berlin, Germany

‡ Current address: Center for Pathobiochemistry and Genetics, Medical University of Vienna; Vienna, Austria

*Corresponding author: kareem.elsayad@meduniwien.ac.at

We will discuss the measurement and interpretation of viscoelastic anisotropy in the GHz regime in relation to various biological processes. Using a custom angle-resolved BLS microscopy setup we show that these can be dynamically mapped in living cells, revealing an often surprisingly heterogeneous and dynamic landscape that is “invisible” to quasi-static elasticity measurements. We will focus on studies of plant cell walls and the cell nuclei, and their manifestations in, as well as relevance for, “macroscopic” phenomena. We will discuss mechanisms for transduction of information on these time-scales between chemical and material properties, and show how in certain cases nature manages to hover in the vicinity of an order-disorder critical state that allows for the energy efficient modulation of mechanical parameters relevant for morphological/structural changes and organization on the sub-micron and micron level.

Correlative Imaging of Motor neuron Cell Elasticity by Pump and Probe Spectroscopy

Laurent Belliard[1*], Océane Sénépart[2], Emmanuel Péronne[1], Claire Legay[3], Fannie Semprez[3], Maxime Schneider[4], Ahmed Hamraoui[2]

1 Sorbonne Université, CNRS UMR7588, Institut des Nanosciences de Paris, 4 place Jussieu, 75005 Paris, France

2 Sorbonne Université, CNRS UMR7574, Laboratoire de Chimie de la Matière Condensée de Paris, 4 place Jussieu, 75005 Paris, France

3 Saints-Pères Paris Institute for the Neurosciences, CNRS UMR8003, Université de Paris, Paris Descartes, Faculté des Sciences Fondamentales et Biomédicales, 45 rue des Saints-Pères, 75006 Paris, France

4 Centre de recherche de l'ECE Paris-Lyon, Immeuble Pollux-37 quai de Grenelle-CS 71520-75015 Paris, France 5 Université

Corresponding author:* **Laurent Belliard laurent.belliard@sorbonne-universite.fr

Because of their role of information transmitter between the spinal cord and the muscle fibers, motor neurons are subject to physical stimulation and mechanical property modifications. We report on motoneuron elasticity investigated by time resolved pump and probe spectroscopy [1-3]. A dual picosecond geometry simultaneously probing the acoustic impedance mismatch at the cell-titanium transducer interface and acoustic wave propagation inside the motoneuron is presented. Such noncontact and nondestructive microscopy, correlated to standard atomic force microscopy or a fluorescent labels approach, has been carried out on a single cell to address some physical properties such as bulk modulus of elasticity, dynamical longitudinal viscosity, and adhesion. The characterisation of a growth medium such as collagen will also be discussed.

References:

[1] A. Viel, et al., Picosecond ultrasounds as elasticity probes in neuron-like cells models, Appl. Phys. Lett. 115, 213701 (2019); https://doi.org/10.1063/1.5129783

[2] A. Hamraoui et al., Correlative Imaging of Motoneuronal Cell Elasticity by Pump and Probe Spectroscopy, Biophysical Journal (2021), https://doi.org/10.1016/j.bpj.2020.12.021

[3] E. Péronne et al., Data-Clustering Analysis of Scanning Ultrafast Acoustic Experiments: Revealing Acoustic and Structural Properties of a Motoneuron, Phys. Rev. Applied **18**, 034051

Parallel phonon microscopy for cell elasticity imaging

Rafael Fuentes-Dominguez[1,*], Mengting Yao[1], Will Hardiman[1], Salvatore La Cavera III[1], Fernando Perez-Cota[1], Richard J. Smith[1], and Matt Clark[1]

[1]*Optics and Photonics Group, University of Nottingham, University Park, Nottingham, NG7 2RD, UK*

**Corresponding author: ezzrf1@nottingham.ac.uk*

Phonon microscopy has accessed to new elasticity information within biological cells with sub-optical axial resolution [1]. This technology generates and detects coherent phonon field using an asynchronous optical sampling (ASOPs) pump-probe system that enables measurements of time-resolved Brillouin scattering signals along the depth of the specimen with sub-optical resolution ($\lambda_{acoustic}$=280 nm) [2].

Although phonon microscopy is much faster than an optical system with a mechanical delay line, each pixel takes between 1 and 2 seconds to acquire which will depend on the required signal-to-noise ratio (SNR). With the current state-of-art system is very challenging to track elasticity changes in living cells (while keeping same SNR) due to the cell movement changes over time. Therefore, it is critical to speed up phonon microscopy measurements.

In this talk, we will present a ~6-fold increase in acquisition speed by using a fibre bundle to detect multiple phonon microscopy signals in parallel while keeping the axial and lateral resolution.

References:

[1] Pérez-Cota, Fernando, et al. "Picosecond ultrasonics for elasticity-based imaging and characterization of biological cells." Journal of Applied Physics 128.16 (2020): 160902.

[2] Smith, R.J., et al. 3D phonon microscopy with sub-micron axial-resolution. Sci Rep 11, 3301 (2021).

Brillouin imaging of micro-structured samples: size and environment effect

Silvia Caponi[1] *

[1]*Istituto Officina dei Materiali, Italian National Research Council (IOM-CNR), Unit of Perugia, c/o Department of Physics and Geology, University of Perugia, Via A. Pascoli, I-06123 Perugia, Italy*

**Corresponding author: silvia.caponi@cnr.it*

Brillouin microscopy is emerging as an innovative optical method able to provide the mechanical images of a large variety of samples without any physical contact, but exploiting the light-matter interaction. It holds great promise: to allow mechanical analysis inside soft and heterogeneous materials, addressing the fundamental role played by viscoelastic properties in physiological and pathological processes occurring in living cells and tissues. Nevertheless, extending the Brillouin imaging in the context of life science is especially challenging when turbid media are under consideration [1], and when the analysis focuses on structures mechanically heterogeneous at the micro and nano scale. It poses a critical question about the actual spatial resolution reachable in the mechanical maps [2-3]. In this talk, I analyse the key quantities that define the spatial resolution in the Brillouin scattering process highlighting that not only the light focusing, but also the acoustic excitations present in the material influence the information collected by Brillouin imaging [4].

References:

[1] M. Mattarelli et al. ACS Photonics 9, 2087-2091 (2022)

[2] M. Mattarelli et al. ACS Photonics 7, 2319–2328 (2020)

[3] S.Caponi, D. Fioretto, M. Mattarelli Optics Letters 45, 1063 (2020)

[4] A.A. Passeri et al. unpublished

Probing differentiation of 3D cellular assemblies with Brillouin light scattering

Pierre Bouvet[1*], Flora Clément[2], Anastasia Papoz[2], Thomas Dehoux[3], Jean-Charles Baritaux[1],
[1]*Univ. Grenoble Alpes, CEA, LETI, F-38000 Grenoble, France*

[2]*Univ. Grenoble Alpes, CEA, IRIG, BIOMICS, F-38000 Grenoble, France*

[3]*Institut Lumière Matière, UMR5306, Université Lyon 1-CNRS, Université de Lyon, 69622 Villeurbanne,France*

**Corresponding author: Pierre.Bouvet@cea.fr*

Morphological traits of 3D multicellular complexes can reliably discriminate features and gene functions [1]. With the development of 3D models, new ways of extracting these characteristics and eventually predict them are needed.

One of these singular morphologies is the acinus: a multicellular complex that has differentiated to form a hollow core. These structures happen in a range of biological phenomena, from embryogenesis [2] to carcinogenesis [3].

Acini derive from single cells, which divide to form cellular assemblies where cells will collectively create the structure. Today, we identify acini either by fluorescent markings or by hand-selection through a bright field microscope image after differentiation. However, little is known about the evolution of a cellular complex to an acinus and there are no methods to non-destructively predict the outcome of a cellular assembly that might create an acinus.

Here, we study this evolution through the mechanical properties of the complex. Using Brillouin imaging, we show that we can identify acini, and possibly predict their apparition before their full differentiation.

References:

[1] A. V. Taubenberger et al, Acta Biomaterialia, vol. 36, p. 73, 5 (2016)

[2] M. Kowalska et al, Journal of Morphology, vol. 281, n°11, p. 110, 1 (2020)

[3] B. Laperrousaz et al, Nucleic Acids Research, vol. 46, n°112, p. e70, 7 (2018)

Phonon microscopy of live cells

William Hardiman[1*], Fernando Pérez-Cota[1], Matt Clark[1], Claire Friel[2], Alan Huett[2], Kerry Setchfield[1], Amanda Wright[1,]

[1]*Optics and Photonics Group, University Park, University of Nottingham, Nottingham, NG7 2RD, UK*

[2]*School of Life Sciences, Queen's Medical Centre, University of Nottingham, Nottingham, NG7 2UH, UK*

**Corresponding author: William.Hardiman@nottingham.ac.uk*

Phonon microscopy has demonstrated elasticity-derived contrast and enhanced resolution by using coherent phonon fields [1]. The coherent field is generated and detected with picosecond laser ultrasonics in an ASynchronous OPtical Sampling (ASOPS) configuration, allowing time-resolved Brillouin scattering measurements with sub-optical resolution [2].

Biocompatibility is a challenge for any live cell study, and this is especially true for raster scanning techniques where an image of a cell may take several minutes. This is compounded by the high laser powers typically required for Brillouin imaging techniques. Recent advances have demonstrated enhanced biocompatibility by moving towards longer wavelengths [3] and improved signal generation in phonon microscopy [1].

In this talk, we will demonstrate the non-damaging nature of phonon microscopy for long-term study of live cells by combining near-infrared wavelengths with improved opto-acoustic transducer designs.

References:

[1] Pérez-Cota, F., et al. "Picosecond ultrasonics for elasticity-based imaging and characterization of biological cells." *Journal of Applied Physics* 128.16 (2020): 160902.

[2] Smith, R.J., et al. 3D phonon microscopy with sub-micron axial-resolution. *Sci Rep* 11, 3301 (2021).

[3] Nikolić, M., et al. "Long-term Brillouin imaging of live cells with reduced absorption-mediated damage at 660nm wavelength." *Biomedical optics express* 10.4 (2019): 1567-1580.

Predicting nanocarriers efficacy in 3D models with Brillouin Light Scattering spectroscopy

Giulia Guerriero[1], Veronica Feltri[1], Alexis Viel[2], Guqi Yan[2], Thomas Dehoux[2], Giovanna Lollo[1,*]

[1]*Laboratoire d'Automatique, de Génie des Procédés et de Génie Pharmaceutique, Université Claude Bernard Lyon 1, CNRS UMR 5007, 43 bd 11 Novembre 1918, 69622, Villeurbanne, France*

[2]*Institut Lumière Matière, UMR5306, Université Lyon 1-CNRS, Universitè de Lyon, 69622 Villeurbanne, France*

**Corresponding author: giovanna.lollo@univ-lyon1.fr*

The three-dimensional (3D) multicellular tumor spheroids (MCTs) model are interesting tools that recapitulate in vitro the complexity of solid tumors and can be used to screen therapeutic candidates. So far, the efficacy and penetration of drug-loaded nanosystems into MCTS is mainly investigated by means of microscopy techniques that are highly invasive and require fluorescent labels [1]. To overcome these drawbacks Brillouin Light Scattering (BLS) spectroscopy has recently gained a great interest in the pharmaceutical field as a promising technique for quantitative high-throughput screening of nanocarriers penetration in 3D tumor models. The Brillouin allows to quantify in depth drug efficacy in a non-contact, non-destructive and label-free manner [2].

In this study, MCTS were engineered from the spontaneous aggregation of a colorectal carcinoma cell line (HCT116) in agarose micro-patterned array and treated with polymeric nanoparticles loaded with a platinum derivative drug (DACHPt) and oxaliplatin water solution, used as control [3,4]. The anticancer treatment efficacy was evaluated quantitively by BLS. Results exhibited a decrease in the frequency shift in a dose-dependent manner, suggesting the progressive destruction of the spheroids under treatment. These results confirm the possible use of the frequency shift as a quantitative indicator of nanocarrier efficacy.

Globally, BLS analysis on HCT116 spheroids grown in agarose microwells has turned out a promising tool allowing high-throughput screening of nanocarriers. In future we will extend our measurements to other cells line to confirm the reproducibility of the screening procedure on various types of tumors.

References:

[1] E.C. Costa et al, Biotechnology Advances, 34(8), 1427-1441 (2016)

[2] J. Margueritat et al, Physical Review Letters, 122, 018101 (2019)
[3] S. Goodarzi et al, The Royal Society of Chemistry, 21, 2459-2510 (2021)
[4] K. Matha et al, European Journal of Pharmaceutics and Biopharmaceutics (2020)

Thursday 16th

Cancer cell and tissue rheology using AFM

C Verdier

Laboratoire Interdisciplinaire de Physique - LIPhy, CNRS - Université Grenoble Alpes

B.P. 87, Saint-Martin d'Hères, 38402 cedex, France

We present data from microrheology measurements using AFM on various sytems. Polyacrylamide gels are studied first to validate the method [1]. Then results are obtained using different types of cancer cells, leading to interesting data on the influence of invasiveness on their rheological properties. Furthermore, the role of the substrate is studied and it is shown that cancer cells adapt their response in a mechanosensitive way [2]. Then we study spheroids made of the same cells and analyse their rheology in the presence of collagen [3].

All this information is discussed in terms of rheological models, and possible comparisons with Brillouin scattering data are made.

References:

[1] Y. Abidine, V.M. Laurent, R. Michel, A. Duperray, L.I. Palade, C. Verdier, Physical properties of polyacrylamide gels probed by AFM and rheology, Europhys. Letters, 109, 38003 (2015)

[2] Y. Abidine, A. Constantinescu, V.M. Laurent, V. Sundar Rajan, R. Michel, V. Laplaud, A. Duperray, C. Verdier, Mechanosensitivity of cancer cells in contact with soft substrates using AFM, Biophys. J., 114, 1165-1175 (2018)

[3] D. Tsvirkun, J. Revilloud, A. Giannetti, C. Verdier, The intriguing role of collagen on the rheology of cancer cell spheroids, J. Biomechanics, 141, 111229 (2022)

Quantifying optomechanical properties of phase separated protein condensates

Timon Beck[1, *], Raimund Schlüßler[2], Lize van der Linden[2], Kyoohyun Kim[1], Mark Leaver[3], Paul Müller[1], Simon Alberti[2], Jochen Guck[1]

[1]*Max Planck Institute for the Science of Light, Erlangen, Germany*

[2]*Biotec, TU Dresden, Dresden, Germany*

[3]*Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany*

**Corresponding author: timon.beck@mpl.mpg.de.*

The organization of intracellular material is a complex task and cells have different strategies for compartmentalization. One way is to enclose certain components with a membrane and thereby separate them from the rest of the intracellular fluid. But there are also membraneless organelles that are involved, for example, in metabolic control and DNA repair. The underlying process of phase separation and percolation is tightly controlled by many parameters as temperature, ion and protein concentration, as well as crowding conditions. Changes in these parameters have an impact on the intermolecular interactions and accordingly tune optical and viscoelastic characteristics of the condensates. Despite the dynamic development of the research field in the last years, there is a lack of tools to measure such physical properties in a quantitative way.

A combination of Brillouin microscopy with quantitative phase imaging, providing information about refractive index and density, gives access to a set of various optomechanical quantities and in particular the longitudinal modulus. Importantly, this approach does not require physical contact to the specimen and can also be applied in vivo [1]. By varying temperature and ion conditions, we were able to tune intermolecular interactions within phase separated protein droplets and found that the introduced variations are reflected in the opto-mechanical properties of the condensates. Accordingly, we could show that conditions that where associated with strong interactions also showed an increased longitudinal modulus and refractive index. All in all, we provide a new set of non-invasive tools introducing additional parameters for the characterization of biomolecular condensates and demonstrate their application in a controlled in vitro system.

References:

[1] R. Schlüßler & K. Kim et al, eLife, **11:e68490** (2022)

Probing cell crowding in cells and tissues with Brillouin light scattering

Alexis Viel[1], Guqi Yan[1], Gaëtan Jardiné[1], Malèke Mouelhi[1], Sylvain Monnier[1*] and Thomas Dehoux[1*]

1 - Institut Lumière Matière, UMR5306, Université Lyon 1-CNRS, Université de Lyon, 69622 Villeurbanne, France

*Corresponding author: sylvain.monnier@univ-lyon1.fr, thomas.dehoux@univ-lyon1.fr

Volume regulation is key in maintaining important tissue functions, such as growth or healing [1]. This is achieved by modulation of active contractility, as well as water efflux that change molecular crowding within individual cells. Local sensors have been developed to monitor stresses or forces in model tissues, but these approaches do not capture the contribution of liquid flows to volume regulation. Here we use a new tool based on Brillouin light scattering (BLS) that uses the interaction of a laser light with inherent picosecond timescale density fluctuations in the sample [2]. To investigate volume and density variations, we induced osmotic perturbations to compress single cells and multicellular spheroids (MCS). During osmotic compressions we observe an increase in the BLS frequency shift that reflects local variations in the refractive index and compressibility. We also measure a non-linear increase of the linewidth of the BLS frequency for large compressions suggesting a non-Newtonian behaviour. To elucidate these data, we propose a model based on constitutive equation that describes the increase of molecular crowding upon reduction of the intracellular fluids. Comparison with the data suggests a non-linear increase of the loss modulus due to the dense crowding that induces hydrodynamic interactions between the cellular polymers.

References:

[1] Cadart, et al. Nat. Phys. 15, 993–1004 (2019)

[2] Scarcelli, et al. Nat. Methods 12, 1132–1134 (2015).

Brillouin and Raman Spectroscopy application to mark the "boundaries" of bacterial colonies infecting bone tissue.

Martina Alunni Cardinali[1*], Sara Stefani[2], Marco Govoni[3], Dante Dallari[3], Alessandra Maso[4], Elisa Storni[4], Francesca Valenti[4,5], Melania Maglio[5], Maurizio Mattarelli[1], Alessandra Anna Passeri[1], Silvia Caponi[6], Assunta Morresi[2], Paola Sassi[2], Daniele Fioretto[1]

[1]*Department of Physics and Geology, University of Perugia*

[2]*Department of Chemistry, Biology and Biotechnology, University of Perugia*

[3]*Reconstructive Orthopaedic Surgery and Innovative Techniques - Musculoskeletal Tissue Bank, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy*

[4]*Laboratory of Microbiology and GMP Quality Control, IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy*

[5]*Complex Structure of Surgical Sciences and Technologies, IRCCS istituto Ortopedico Rizzoli, Bologna, Italy*

[6] *Istituto Officina dei Materiali, Italian National Research Council (IOM-CNR), Unit of Perugia*

**Corresponding author: martina.alunnicardinali@unipg.it*

Prosthetic-joint infections are a growing problem in the public health management system, due to both the increasing need to resort to arthroplasty and the progressively high resistance to drug treatment of pathogenic and/or opportunistic bacteria. Furthermore, the difficulty in detecting and recognizing these pathogens with traditional methods of synovial fluid analysis, because pathogens remain strongly adhered to the bio-device via resistant biofilms, necessitates the development of new in vivo diagnostic methods that allow an analysis of infected tissue at the surgical site, enabling the surgeon both to remove the contaminated tissue and to maintain as much healthy tissue as possible to ensure the new prosthesis is successfully sealed. Here, we present some preliminary results obtained through a combined Raman and Brillouin spectroscopy approach on infected portions of human femoral diaphysis with strains of Staphylococcus aureus, a species responsible for most implant bone infections, showing how the technique can be effectively used to identify the pathogen and delineate the biofilm it creates on the inner surface of cortical bone and the outer portion of the periosteum, paving the way to the application in the biomedical field.

References:

[1] G. Scarcelli et al, Nat. Photonics, **2**, p39-43 (2008)

[2] G. Scarcelli et al, Nat. Methods, **12**, p1132 (2015)

Mechanical and chemical characterization of dental tissues affected by molar and incisor hypomineralization

Virgile Larrauri[1], Philippe Djémia[2], Daniel Neuville[3], Elsa Vennat[4], Nicolas Roubier[4], Elisabeth Dursun[1], Aurélie Benoit[1,*]

[1]*Université Paris Cité, UFR Odontologie, URB2i*

[2]*Université Sorbonne Paris Nord, LSPM*

[3]*Université Paris Cité, IPGP*

[4]*CentraleSupélec, LMPS*

**Corresponding author: aurelie.benoit@u-paris.fr*

Molar incisor hypomineralisation (MIH) is a dental illness causing unsightly opacities that can worsen into post-eruptive breakdowns and dental caries [1]. Its global prevalence is 13.1%, its aetiology is not fully elucidated yet and dental restorations on affected teeth undergo many failures. It is therefore a growing concern for young patients, their parents and dentists.

Understanding the characteristics of affected tissues is essential to adapt dental cares and improve the success of restorations. Microstructure, chemical composition, mineral density and mechanical properties were investigated in the literature using various experimental techniques [2]. To our knowledge, no study has employed Brillouin spectroscopy, that could however be an interesting clinical tool to better delineate the lesion extent.

The objective of this study was to develop a multimodal characterization protocol of MIH lesions using nanoindentation, Raman and Brillouin spectroscopies on two affected teeth.

Compared to sound enamel, MIH enamel showed a higher proteins content under Raman spectroscopy and a reduced longitudinal elastic modulus under Brillouin spectroscopy. Nanoindentation revealed a 77% reduced Young modulus and an 85% reduced hardness for white MIH enamel. Furthermore, mechanical and chemical properties followed a gradient between sound enamel, white MIH enamel, yellow MIH enamel and dentin.

These results demonstrated the feasibility of this multimodal characterization and supported a relationship between chemical and mechanical properties of MIH enamel.

References:

[1] KL. Weerheijm et al, Caries Res., **35**(5), p390-1 (2001)

[2] K. Elhennawy et al, Arch. Oral Biol, **83**, p272-81(2017)

Dental composite's photo-activation using optical fibers – a holographic, thermographic, and Raman study

Evgenije Novta[1,*], Tijana Lainović[1], Dušan Grujić[2], Svetlana Savić-Šević[2],

Dejan Pantelić[2], Elvira Toth[3], Željka Cvejić[3], Larisa Blažić[1,4]

[1]*University of Novi Sad, Faculty of Medicine, School of Dental medicine, Serbia*

[2]*University of Belgrade, Institute of Physics, Belgrade, Serbia*

[3]*University of Novi Sad, Faculty of Sciences, Department of Physics, Novi Sad, Serbia*

[4]*Dental Clinic of Vojvodina, Novi Sad, Serbia*

**Corresponding author: evgenije.novta@mf.uns.ac.rs*

Lately, a new category of dental resin-based composites (RBC) has been introduced – bulk-fill RBCs, permitting deep cavity restoration (4-5 mm) in one single increment. However, in such bulk layers, problems such as increased polymerization shrinkage stress (PSS) and inadequate degree of monomer conversion (DC), may arise. This study aimed to examine the influence of a novel photo-activation protocol using optical fibers on PSS, RBC temperature change, and DC.

Models with standardized tooth cavities were filled with the RBC, and the proposed two-step curing was designed as follows: in the first step, three optical fibers (ϕ 1mm) connected to a commercial dental LED curing unit were inserted into the dental filling to cure the RBC from within; in the second step, fibers were extracted, remaining voids were filled with the RBC, and final conventional curing was performed. In the control group of samples, conventional curing was applied. Tooth model deformation, as a secondary manifestation of PSS, was measured in real-time using digital holographic interferometry (DHI), while simultaneously monitoring temperature change with an infrared thermal camera. DC was measured at various depths, immediately after curing and after 24 h of dark storage, using Raman spectroscopy.

Statistical analysis of the DHI results revealed a significant deformation decrease in the optical fiber approach, compared to the control (on average 36%, p=0). Meanwhile, the temperature measurements showed gradual and lower temperature increase compared to the conventional curing, postponing reaching the maximum temperature (estimated vitrification point during polymerization). No significant influence of the photo-activation protocol on DC was revealed for all three measured locations (p=0.214).

Additional examination with Brillouin spectroscopy could contribute to the presented research by real-time monitoring of viscoelastic changes during cross-linking of polymers.

Supported by Mikodental Dental depo (Shofu® (Japan) dental products) Šabac, Serbia.