Proposition de sujet de thèse – 2017-2020

Titre : High Speed -bio-Scanning Microwave Microscopy (HS-bio-SMM)

Responsable : E. Lesniewska
Axe(s) scientifique(s) : 1
Equipe(s) de recherche : OSNC

Coresponsable(s): E. Bourillot – F. Canon (Inra Dijon)

Contexte scientifique général, caractère innovant et impact global du sujet :

In the European collaborative project **PHC AMADEUS "Bio-microwave"** and **ANR 2015 project "BioSound-IR"**, we explore and extend the use of scanning microwave microscopy (SMM) in biological research, and thereby, we improve and then establish this new exciting bio-SMM technology. Since 2008, the Institute of Biophysics (Linz university) and the Institute Carnot Bourgogne (Bourgogne university), collaborate through a consortium on High-Speed Atomic Force Microscopy Imaging.

In the AAP i-Site 2017, operated by the Federal University Burgundy Franche-Comté (UBFC), the **I-SITE 2017 "HS-bio-SMM"** project aims at creating a stimulating international environment in order to attract talented students on **a multidisciplinary approach combining applied physics, instrumental development, and biophysics on cell membrane.**

"Dynamics" is one of essential attributes of biological macromolecules and supramolecular assemblies. Their physiological functions are produced through their dynamic structural changes and dynamic interactions with other molecules. In addition, their functions are basically produced at the single molecule level. Therefore, the most straightforward approach toward understanding their functional mechanisms is to directly observe their dynamic behaviors at the single molecule level. Despite this obvious view, such observations were infeasible before the recent advent of HS-AFM developed by Ando's lab. Both institutes have the same equipments. Scanning Microwave Microscopy has been developed by the Keysight research lab in Linz in close relation with Linz University group (in the frame of a FP7 Marie-Curie ITN 'Nanomicrowave' from 2013-2016). To promote the wide-spreading of this new microscopy over biological research communities, the partners have decided to join forces to promote this technique in France and in Austria where bio-AFM experts have been actively studying on a wide range of biomolecular systems such as membrane proteins, lipids, DNA etc. This consortium is supported by the industrial research lab of Keysight Labs Austria including access to early SMM prototype development both for advanced hardware and software capabilities.

Prof. Hinterdorfer's team, Johannes Kepler University from Linz, focused their researches on atomic force microscopy related techniques and have used the force spectroscopy method with functionalized probes for years to address different biological problems. Scanning microwave microscopy is a brand new technique commercialized in 2008 and its applications are performed independently by our and Prof. Hinterdorfer's team from the early stage of the development of microscope. Our project aims to reveal the electric properties (conductivity and capacity) of oral epithelial cells and the variations of these properties after the formation of the salivary pellicle (i.e. the thin layer of salivary proteins anchored onto the cells of the oral mucosa). The very first analyzes were carried out on the dehydrated cells because our SMM setup is limited to analyze in air. Prof. Hinterdorfer's team has recently developed an extinction mode that enables to perform SMM analysis in liquid medium. The use of this extinction will be very interesting for our project. Indeed, this extinction will allow observing the impact of the mucosal pellicle on the epithelial cells in their morphological medium. The collaboration between these two teams provides to exchange expertise on force spectroscopy method and feedbacks on novel characterization technique, scanning microwave microscopy. These exchanges between our and Prof. Hinterdorfer's team would be a highly valuable source of information for advancement of our projects. In combination with the support of the Keysight research team to get access to internal developments, several synergies are expected for the University scientific work and publication activities.

One of our project concern the interactions between the salivary proteins anchored onto the oral mucosa and flavor compounds (i.e. aroma compounds and tannins) (MUFFIN, Grant ANR-14-CE20-0001-01) which are a center of interest for research groups at European and international level, as it could be involved in aroma persistence and astringency perception. Despite the importance of these sensations in food acceptability, there is a clear lack of knowledge on the molecular interactions occurring at the cell surface between the transmembrane proteins of the oral cells and salivary proteins and between the salivary proteins anchored at the surface of the oral cells and flavor compounds. Therefore, our project proposes to elucidate the molecular basis of the epithelial cells – mucosal pellicle and the flavor compounds – oral mucosa interactions using scanning microwave microscopy and related characterization techniques such as force spectroscopy. In order to achieve the objectives of the project, we propose a multidisciplinary approach associating expertise in cell culture, biochemistry of proteins and bio-suitable characterization techniques such as force spectroscopy and scanning microwave microscopy.

Atomic force microscopy (AFM) allows studying the topography and elastic properties of surfaces from nanometric to micrometric scale. The topographic features of the model of oral mucosa are investigated in liquid medium by AFM. In order to investigate the hydrophobicity of the oral mucosa and monitoring of the flavor compounds – epithelial cell surface and the flavor compounds – mucosal pellicle interactions, we have adopted the force spectroscopy method with functionalized probes. Different flavor compounds and alkanethiols with hydrophobic (CH_3) and hydrophilic (OH) ends are used for covering and modifying AFM probes. The working mechanism of an AFM is based on detection of the inter-atomic forces between a sample and a probe. Thereby, the adhesion forces between the various components and the

model of oral mucosa are measured. The hydrophobic tips are used to investigate the hydrophobicity of the model of oral mucosa. The recent results indicates that the presence of mucosal pellicle on epithelial cells decreases the hydrophobic character of cell surface, probably due to the physico-chemical properties of the anchored salivary proteins. The experiments concerning the flavor compounds – epithelial cell surface and the aroma – mucosal pellicle interaction are initiated.

Scanning microwave microscopy (SMM) provides the detection of local electric properties of a wide range of materials by combining AFM with microwave measurements in high frequency range (300 MHz to 16 GHz). SMM measures the complex reflection coefficient, Γ, (a complex number with magnitude and phase) of the tip – sample contact point. The measured phase and amplitude of SMM signals are directly related to capacitance and conductance, respectively. In biomaterials, the water and ionic content dominate the conductivity of sample. In addition, the density of sample could influence the dielectric constant and thereby the capacitance of sample. SMM analyses were performed to observe the electric properties and subsurface features of the in vitro model before and after the mucosal pellicle formation. Our preliminary results indicate that the presence of the transmembrane MUC1 proteins on the cell surface yield an increment at SMM amplitude. The differences at the SMM amplitude signal can be related to their different ionic and water content. This could indicate that the epithelial cells with the transmembrane MUC1 proteins have greater ability to retain water molecules and/or higher ionic content, which is also observed after saliva depositions. The mucosal pellicle forms the filamentous networks on the epithelial cells including some aggregates. A dephasing is observed at SMM phase for aggregated areas on the cell surface. The dephasing at SMM phase image indicates a variation at the dielectric constant. Therefore, we assume that these aggregates due to the saliva deposition have different dielectric constant than the rest of the cell surface and they can decrease the hydrophobicity of cell surfaces, as observed, and increase water retention. A more detailed multi-frequency SMM analysis is envisaged under physiological solution to support these findings. Other biological samples of interest such as bacteria and yeasts useful for biofuel production will be analyzed with these techniques. Carrying out these efforts, we expect develop the SMM methodology in biological research and gain insights into the biomolecular interactions, assembling and disassembling and the functional mechanisms of biomolecular systems. Without the collaboration between the complementary teams, these pioneering studies are impossible to carry out.

The student may have to travel in Linz. The student will be eligible to be accepted as a PhD student in France.

Proposed work:

1. Improvements of microwave microscopy enabling the study of individual cells
2. Implementation on a high-speed atomic force microscopy platform in a close collaboration with university of Kanazawa (Japan)
3. Implementation of force spectroscopy on a close collaboration with Linz university (Austria)
4. Application to the study of bacteria or buccal cells in collaboration with Inra Dijon (France).

Background:

- Instrumental physics
- Knowledge in biology and physics.
- Excellent knowledge of English essential

Contact :

Eric Lesniewska (lesniew@u-bourgogne.fr) – Eric Bourillot (eric.bourillot@u-bourgogne.fr) – Francis Canon (francis.canon@inra.fr)