Journée Thématique
Couplage Microscopies Optiques - Microscopie à Force Atomique

Organisée au Laboratoire Adhésion Inflammation
(LAI, U1067 Inserm, CNRS, Aix-Marseille Unviersité)
avec le soutien des 2 réseaux technologiques RéMiSoL et RTMFM

Vendredi 7 decembre 2018 de 9H30 à 17H30
163, Av. de Luminy, Bât TPR2 bloc 5, RDC
13288 Marseille Cedex 9, France

Inscription gratuite et obligatoire avant le 30/11/2018
https://indico.mathrice.fr/event/36/
PH Puech et F. Rico pour le Groupe de travail couplage AFM/Optique

PROGRAMME

9H30-10H00: Reception

Session #1: Present: coupled systems AFM/Optique in practice

10H00: L Costa (IBS, Montpellier): Correlative atomic force and photonic microscopies and spectroscopies operational schemes to study the structure and dynamics of membrane assemblies

10H30: S Janel (CIIL Lille): Coupling AFM to photonic microscopy in cell biology : applications, advantages and disadvantages

11H00: Cristina Flors (IMDEA, Madrid): Combining AFM with super-resolution fluorescence microscopy: some results and many challenges

11H30: PH Puech (LAI, Marseille): Simultaneous AFM force mode and florescence detection for mechanotrasduction studies

12h00: F Rico (LAI, Marseille): Combination of HS-AFM with spinning disk microscopy

12H45 - 14H00: Lunch (cantine)

14H00: Visit of the coupled systems at LAI

Session #2: Future: Other microscopies, improvements, perspectives and dreams
14H30: Round table with contributions to be proposed by the participants

17:15 – 17:30 Closing remarks

DESCRIPTION

Le Groupe de Travail (GT) s'intéresse aux problèmes liés au Couplage champ proche – optique au sens large. Le couplage s'entend comme un dispositif permettant de faire des acquisitions dans les deux modes soit simultanément soit de manière séquentielle.

Les problématiques de repositionnement sont discutées dans un autre GT (GT Repositionnement). Il est question ici de l'apport de chaque mode en fonction de l'échantillon et de la question posée, de la préparation des échantillons, des dispositifs pour réaliser les acquisitions de données et leur traitement.

Différentes modalités de couplages sont concernées: microscopie photonique et AFM (dans toutes ses composantes ; imagerie, spectroscopie de force, AFM rapide, AM-AFM...), nanoIR, spectroscopie Raman, SNOM,…

De plus en plus de systèmes couplant les deux modes de microscopies sont installés dans les laboratoires en plus des développements mixant les deux domaines. Une grande diversité d'appareillage émerge sans que les solutions techniques et les savoir-faire soient disponibles au sein de la communauté. Des solutions de couplage originaux sont développées voire au sein de deux laboratoires en même temps. Il est donc question de partager toutes ces expertises. Ainsi, des moyens techniques hardware et software peuvent-ils être multipliés et des verrous conceptuels levés par la mise en commun des réflexions sur le sujet.

Dans un premier temps nous proposons de faire un état des lieux des recherches et développements techniques en cours afin de pouvoir mieux appréhender la diversité du domaine et dégager des lignes d'approfondissements pour le GT en fonction des enjeux.

Cette thématique est soutenu par les deux réseaux de microscopie RéMiSoL et RTmFm de la Mission pour l’Interdisciplinarité.

Abstracts

Correlative atomic force and photonic microscopies and spectroscopies operational schemes to study the structure and dynamics of membrane assemblies

Costa Luca, Doucet Christine, Benistant Christine, Vial Anthony, Saavedra Oscar, Milhiet Pierre-Emmanuel.

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We will present the instruments and the protocols developed at the Center for Structural Biochemistry of Montpellier to investigate biological membranes both *in-vitro* and *in-cellulo* using correlative optical microscopy/spectroscopy and AFM.

Our developments involve a large variety of home-made fluorescence setups, including:

- wide-field conventional epifluorescence and TIRF excitation schemes, as well as
- PALM and STORM super-resolution
- confocal approaches for FRET, fluorescence spectroscopy FCS and FLIM
- sub-Kg AFM that can be mounted on synchrotron beamline hexapods permitting AFM and structural characterization by means of high energy photon scattering and diffraction (X-Rays).

We shall discuss the advantages and drawbacks of all instruments in terms of temporal-spatial resolutions, their mechanical stability and the relevant information that can be extracted and coupled to AFM imaging/mechanics.

Coupling AFM to photonic microscopy in cell biology : applications, advantages and disadvantages

Sébastien Janel, Elisabeth Werkmeister, Frank Lafont

CIIL CNRS UMR8204, Institut pasteur de Lille, France

We'll be presenting several biological applications using the coupling of bio-AFM with optical microscopy for cell studies, from bright field to super-resolution. We'll also discuss the advantage and disadvantages of each system in terms of combining the two techniques (crosstalk, software integration, etc…)

Presented systems will be :

- JPK BioMat

- JPK NW3/Zeiss Z1

- JPK NW3Ultra/Aberrior STED

- Bruker Catalyst/Zeiss 200m

- Bruker Resolve/Zeiss PALM P1

Simultaneous AFM force mode and florescence detection for mechanotrasduction studies

Pierre-Henri Puech
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Mechanotransduction explores how a mechano-biological signal is integrated by a given cell to explore her environment and react to it accordingly. To study this at single cell scale, AFM is a tool of choice to exert and record forces. Coupling it, in real time, with the capability to follow a fluorescent signal, reporting for example for calcium fluxes, opens the way to dissect finely some of the cellular signal pathways and integration methods that cells are using. Examples will be shown using T lymphocytes and macrophages, and the use of the technique for following optegenetic stimulation of the cell's cytoskeleton will be presented.

Combining AFM with super-resolution fluorescence microscopy: some results and many challenges

Cristina Flors
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In this talk, I will discuss the benefits and challenges of combining super-resolution fluorescence microscopy based on single-molecule localization with atomic force microscopy (AFM) *in situ*. The correlation between these techniques provides a high resolution topography image as well as specific chemical information, the latter with a spatial resolution approaching that of AFM. The technical aspects of the correlative microscope, including image alignment and sample preparation requirements will be discussed [1]. This tool can be used to validate novel super-resolution imaging methods [2], as well as to obtain complementary information about the structure and properties of (bio)materials [3]. The latter will be exemplified on a hybrid nanomaterial that consists of protein fibrils functionalized with organic fluorophores and quantum dots [3].

References

[1] Bondia, P.; Casado, S.; Flors, C., *Meth. Mol. Biol.* **2017**, 1663, 105.
[2] Monserrate, A.; Casado, S.; Flors, C., *ChemPhysChem* **2014**, *15*, 647.
[3] Bondia, P.; Jurado, R.; Casado, S.; Domínguez-Vera, J. M.; Gálvez, N.; Flors, C, *Small* **2017**, *13*, 1603784.

Combination of HS-AFM with spinning disk microscopy

Leda Lacaria and Felix Rico

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We have coupled a spinning disk head (CSU-X1, Yokogawa) to a tip scanning HS-AFM (PS-NEX, RIBM) mounted on a Nikon inverted optical microscope frame. Two laser sources are included for common fluorophores (GFP, mCherry...). An sCMOS camera (Photometrics Prime 95B) with large field of view, high frame rate (41 fps at 1200x1200) and single photon sensitivity is used for image detection. An IR cut filter will be included to prevent damage of the camera from the HS-AFM laser. The system will be exploited to correlate the mechanics at high rates with structural information of the cytoskeleton.

Preliminary measurements suggest that direct coupling of the spinning disk head through the lateral port using a C-mount introduces important mechanical noise. To avoid this, we will mechanically uncouple the two instruments, at the expense of losing some photons. The highly focalized excitation coming from the confocal head may induce sudden deflection of the cantilever. For that reason, at a first stage, confocal images are acquired with the cantilever in the retracted position and synchronization mechanisms for simultaneous or quasi-simultaneous optical and HS-AFM measurements will be explored. For fast focusing at different z positions to obtain z-stacks, common piezo nanofocusing rings for the microscope objective seem incompatible due to the large dimensions. Thus, we plan to adapt the sample stage to be moved at high velocity in z using three small piezoelectric elements. This will allow, in turn, the acquisition of long-range force curves, suitable for leukocyte studies.