AUROX LASER FREE CONFOCAL

Correlative laser free confocal and atomic force microscopy

Application Note

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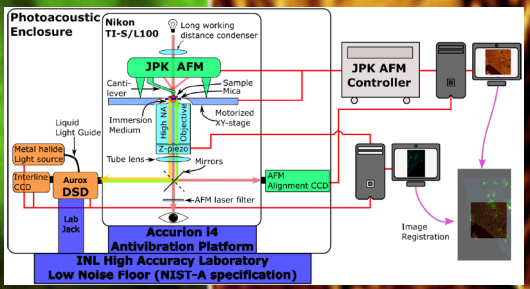

Figure 1. Experimental set-up

Introduction

Combined microscopy techniques offer a powerful tool to investigate complex biological systems and their interactions. Here, we present a new combined microscopy platform based on fluorescence optical sectioning microscopy using an Aurox Laser Free Spinning Disk Confocal, also known as Differential Spinning Disk (DSD) and an Atomic Force Microscopy (AFM) operated in an advanced force-spectroscopy based mode, referred to as Quantitative Imaging (QI). The illumination scheme of the Aurox unit, contrary to standard laser-based single or multi-point confocal microscopes (CLSM), provides a time-independent illumination of the AFM cantilever. This enables a distortion-free simultaneous operation of fluorescence optical sectioning microscopy and atomic force microscopy with standard probes.

Experimental Set-up

The anti-vibration platform is loaded with an inverted Nikon Ti-S optical microscope equipped with a JPK NanoWizard 3 from Bruker Nano GmbH. A metal halide light source provides excitation light for the Aurox unit (Revolution DSD, sold by Andor Technology) and the DSD image capture was performed by an Andor Clara CCD camera. A standard monochrome CCD camera is coupled to a microscope port for AFM cantilever laser spot alignment. Figure 1 depicts the experimental set-up for combined integration of an Aurox laser free confocal (DSD) imaging platform with a bioscience AFM system capable of advanced quantitative imaging.

One can estimate that the total light power incident on the AFM cantilever will be around an order of magnitude lower than for the DSD-AFM assembly as compared to the CLSM-AFM system integration for maximal illumination power available. However, for common operation, only a fraction of the available illumination power is used to avoid sample photobleaching. Simultaneous operation of AFM and a single point CLSM will intermittently deliver all fluorescence excitation energies to the AFM cantilever on the order of 1 Hz, while an AFM-DSD system integration will illuminate the AFM cantilever with a constant power level. A schematic optical layout and representation of the AFM cantilever illumination for a single point CLSM and an Aurox laser free confocal system (DSD) are shown in Figure 2.

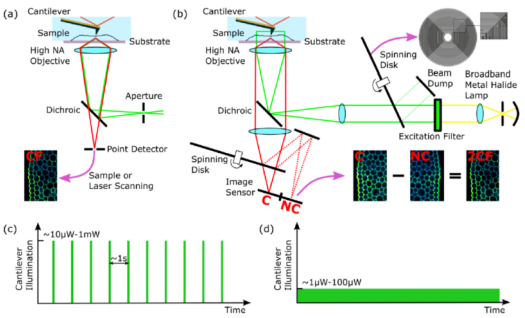

Figure 2. Schematic optical layout for a single point CLSM (a) and an Aurox laser free confocal (DSD) (b), a representation of the AFM cantilever illumination for CLSM (c) and a representation of the AFM cantilever illumination for DSD (d).

One challenge of combined optical and AFM microscopy is posed by the need for accurate image registration. Shear and stretch are commonly encountered distortions in the optical image. This has to be compared to the real-space image created with highly linear piezo-elements of an AFM. Correct overlay of the fluorescence data onto the AFM images is subsequently achieved by acquisition of fluorescence data, transfer to the AFM data acquisition PC, and application of a coordinate transform to the fluorescence image.

Example correlative images

Simultaneous measurements of DSD fluorescence optical sectioning microscopy and QI nanomechanical mapping AFM were used to study a 4:1 lipid mixture of DOPC and DOPS, respectively. Liposomes were prepared in a calcium buffer through lipid extrusion and labelled with the hydrophobic cyanine dye DiI. Subsequent incubation of the DOPC/DOPS (4:1) liposome solution with freshly cleaved mica for limited duration results in a partly formed lipid domain. Figure 3 shows a fluorescence optical sectioned image, a AFM QI extend adhesion image of a lipid structure and an image overlay produced using a system image registration algorithm.

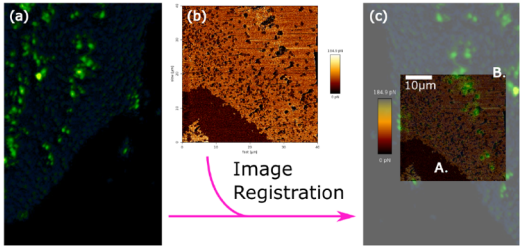

Figure 3. (a) DSD fluorescence image, (b) QI AFM adhesion retract map and (c) corresponding image overlay of a DOPC/DOPS (4:1) lipid domain labelled with DiI.

The simultaneous detection of fluorescence and nano-mechanical mapping provided an approach to distinguish and analyse the bio-physical properties of single cell populations in a mixed culture. The GFPþ MG-63 cell population could be distinguished by their green fluorescence. AFM provided information regarding cell topography and elastic properties.

Figure 4 shows representative correlative DSD-AFM imaging of Saos-2 and GFP+ MG-63 live cells. The AFM channel shows the topography of living bone cells (co-culture of Saos-2 and GFP+ MG-63 cells); the fluorescence channel shows the GFP+ signal from the MG-63 cell population. Using DSD, the fluorescence signal of interest can be detected at the same time as the AFM acquisition allowing the analysis of the bio-physical properties of single cell populations (in this case GFP+ MG-63 cells) in a mixed sample. The Young's modulus of each cell population was discriminated using the combined imaging platform, and the elasticity values of the two cell populations were statistically different (P = 0.0054).

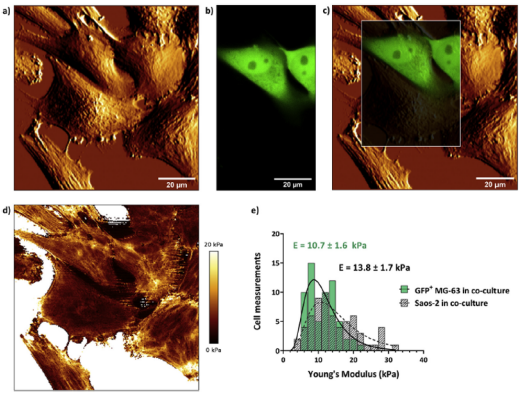

Figure 4. Correlative DSD-AFM imaging of Saos-2 and GFP+ MG-63 live cells. (a) AFM channel showing the topography of living bone cells (b) Fluorescence channel showing the GFP+ signal (c) Overlay image; (d) corresponding map of Young's modulus values (colour bar from 0 to 20 kPa); (e) the Young's modulus of each cell population.

Conclusions

Correlative laser free confocal and AFM can be used as a platform to distinguish different cell populations and to accurately determine cell mechanical properties. There is also scope in the future to study the mechanical phenotype of individual cell populations in heterogeneous samples that cannot be classified and discriminated using morphology alone. Such approaches to facilitate cell discrimination, are increasingly important in complex samples.

The compact size and laser free operation of the Aurox confocal system make it an ideal sectioning tool for use in complex experimental set-ups such as this one.

Here, the key technical advantage of integrating an Aurox laser free spinning disk confocal over a CLSM is that it provides a time-independent illumination of the AFM cantilever, allowing the simultaneous acquisition of data from fluorescence and nanomechanical mapping microscopy without time-dependent cantilever disruption and heating. This capability is of particular advantage in the observation of signalling processes in live-cell investigations in cellular biology.

References

A. Miranda et al, Review of Scientific Instruments 86, 093705 (2015).

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