Perfect Optical Integration with JPK´s DirectOverlay™

Atomic force microscopy (AFM) is a powerful tool to investigate a huge variety of different samples with nanometre scale resolution under physiological conditions. As well as providing topographic measurements, information about interaction forces and mechanical properties like adhesion and elasticity can also be obtained. Perfect integration of AFM with an optical setup can increase the range of applications and opens up many possibilities for correlating structural information with optical information such as functionalized labelling of certain components.

DirectOverlay™ principle

To achieve the perfect combination of optics and AFM at the molecular scale, distortions must be prevented. This will result in two images, such as optical and AFM images, that do not perfectly overlay. Reasons for distortions include aberrations arising from the lenses and mirrors of the optics system. This nonlinear stretching, rotating and offsetting of optical images are present in nearly all types of optical setups.

To generate anyhow an ideal overlay of both techniques, JPK developed in 2005 a cutting-edge calibration method, called DirectOverlay™, which is using the accuracy of the AFM closed loop scanning system that enables a true display of absolute angles and length coordinates. The calibration procedure is done automatically and uses the known positions and offsets of the cantilever to calibrate the optical image into the AFM coordinates. To generate a perfect match of the optical and AFM image, 25 or ever more points are used in the calibration algorithm. At each point an optical image is acquired and the position of the cantilever tip is automatically recognized in each optical image, without needing input on cantilever angle, shape or magnification. This performs a nonlinear conversion, so the optical image is corrected for any lens imperfections and converted into the linearized AFM length coordinates. These provide a perfect integration of optical and AFM data with sub-diffraction limit precision.

Finally, the calibrated optical image is transferred into the JPK SPM software, so that scan regions can be selected within the optical image. Direct “in optical image” selection of AFM measurements (imaging, mapping and force spectroscopy) leads to more efficient experiments and reduces dramatically overview image scanning in AFM.

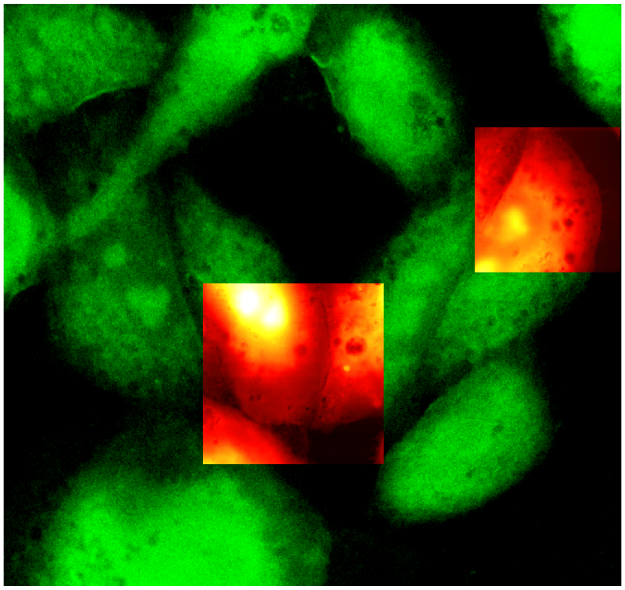


Fig. 1 *DirectOverlay™* of AFM topography insets in red and fluorescence in green of CHO cells with confocal microscopy

Features and benefits

DirectOverlay™ is giving faster results with lower tip contamination. This also protects functionalized tips for molecular recognition and avoids tip passivation from image scanning before force measurements are carried out. Next to optical contrast enhancement techniques including phase contrast and DIC all standard and advanced epi-illumination techniques like laser scanning confocal, TIRF, FRET, FLIM, etc. and fluorescence microscopy data can be perfectly overlaid by unique DirectOverlay™ calibration in the same window. Optical images can be taken with any optical device and can be imported directly using the integrated camera software.
To generate an optimal flexibility, DirectOverlay™ can be used with the JPK NanoWizard® AFM tip scanning system

as well as the Tip Assisted Optics (TAO™) module sample scanning system. Due to the high precision of the calibration, a co-localization of the optical and AFM sample features down to molecular scale is possible.

- **Unique DirectOverlay™ feature for optical and AFM image overlay**
- **DIC, phase contrast or fluorescence data perfect overlaid by unique calibration in the same software window**
- **AFM scan selection in the optical image**
- **More efficient experiments, e.g., for force spectroscopy or mapping – click directly in the optical image**
- **Corrects not only for scaling and rotation, but also image distortions**
- **Based on 25 or more points, not just 3**
- **Automatic procedure with selected cameras**

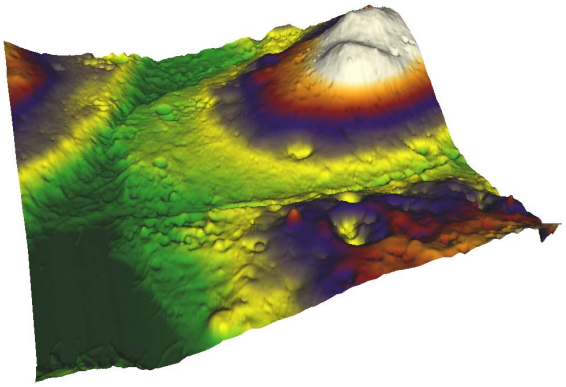


Fig. 2 3D AFM topography of the AFM middle inset in Fig. 1

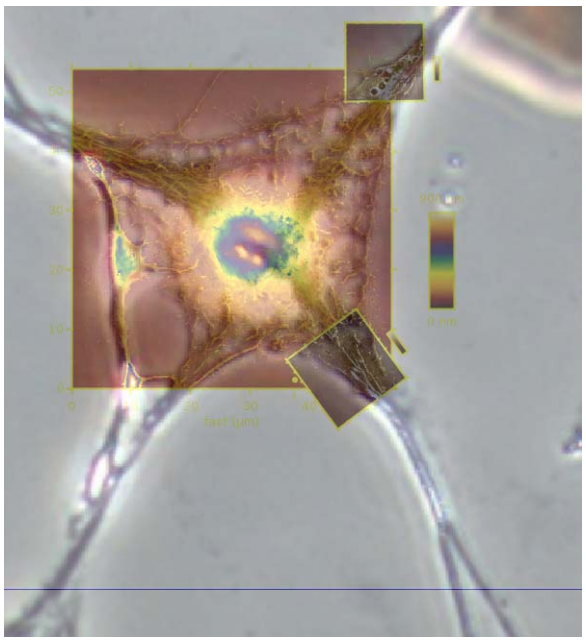


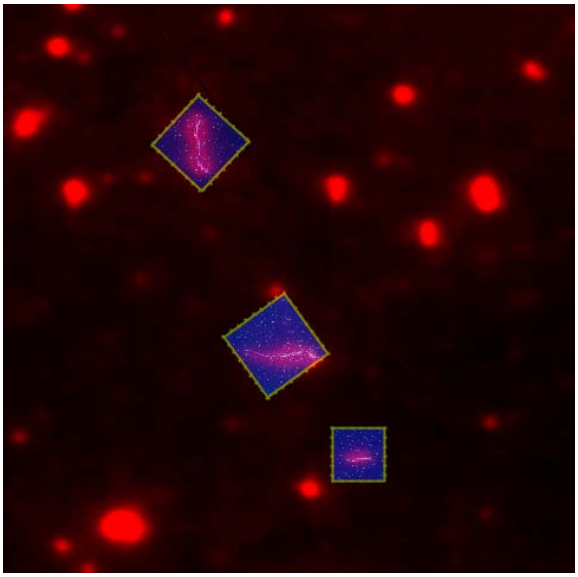
Fig. 3 *DirectOverlay™* of AFM (colored) with optical phase contrast of an astrocyte cell in a semi-transparent view

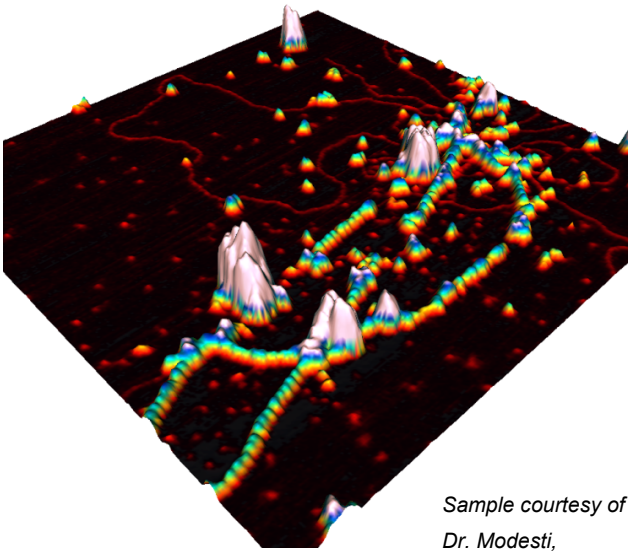
Application fields

The technique can be used for any combination of optics and AFM techniques. It can be operated on opaque and transparent samples in any environment.

Application overview:

- AFM and Optics with transmission illumination like brightfield, DIC, phase contrast, modulation contrast or others
- Fluorescence microscopy
- Confocal microscopy
- TIRF, FRET, FCS, FLIM
- Superresolution techniques such as PALM, STORM
- Spinning disc experiments combined with AFM
- Multiphoton applications
- Raman and TERS experiments
- Single photon counting applications with AFM





Sample courtesy of Dr. Modesti, CNRS Marseille; France

Fig. 4 and 5:
AFM and fluorescence images of Alexa555-labeled Rad51 proteins bound to DNA. Rad51 assembles into filaments along double-stranded DNA, which can be seen in the 3-D plot of the 700 nm topography image. In the fluorescence overview image, the Rad51 filaments are fluorescent in red and the AFM images are shown in blue showing individual DNA molecules, partially coated with Rad51. The fluorescence image was captured with an Andor™ iXon+ 897 EMCCD camera, fully integrated with the AFM software.

Figures 4 and 5 are displaying the combination of single molecule fluorescence microscopy and AFM measurements. Double-stranded lambda phage DNA is partly coated with fluorescent Rad51 proteins that form filaments along the DNA. The region of DNA molecules can be clearly identified in the fluorescence image recorded with an EMCCD. The single molecules can easily be resolved by AFM imaging.

Specifications

- Perfect overlay of optical and AFM data with sub-diffraction limit precision
- Can be operated on opaque and transparent samples
- No limitation regarding objective lenses
- Can used for all optical transmission illumination techniques, as well as advanced epi-illumination techniques
- Optical images by all standard CCD cameras also for high-end CCD's
- Easiest navigation for direct correlation of AFM and optical data
- Compatible with NanoWizard® 2 and 3 AFM´s and Tip Assisted Optics (TAO™) or CellHesion® module
- Minimum 25 points are used in patented calibration algorithm
- Direct imported of optical images using the integrated camera software
- Easy to use software interface
- Color and the overlay options are adjustable by the user
- Transparency of the overlay variable for maximum contrast