

The new JPK Side-view Cantilever Holder – Cell adhesion from a different angle

JPK Instruments recently designed a new Cantilever Holder which enables a side view of the cantilever tip-sample interaction. The special design fully integrates into transmission light techniques and there are no limitations for the parallel use with the NanoWizard® 3 AFM. Any applications that would benefit from a side view observation are possible: living cells, gels, micron sized objects or experiments with micro structured substrates or electrodes. This note describes the application of the Side-view Cantilever Holder in AFM based single cell force spectroscopy which provides an additional view on cell-surface/cell-cell interaction.

Flexible design

The Side-view Cantilever Holder is based on a mirror fixed with a 45° angle to the cross section plane. This unit deflects the side light down to the objective lens. Different light sources can be used to illuminate the sample for side view. Cold light fiber optics can be adjusted to illuminate the cantilever directly from the side. Mirror cubes provide reflection light and epifluorescence impression of the tip sample interaction.

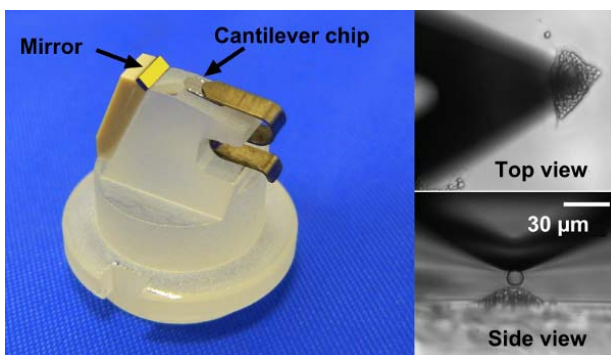


Fig. 1: A mirror is fixed to the Side-view Cantilever Holder in a 45° angle. This allows for analysis of the tip sample interaction in top and side view. The top and side view images show a colloidal probe (bead glued to a cantilever) in contact with a fixed neural crest cell of *Xenopus laevis*. Data courtesy of C. Gonnermann, Dr. D. Stamo and Dr. C. Franz, KIT Germany

There are neither restrictions in experimental design compared to standard AFM applications. Once the

cantilever and sample position as well as the optical light path have been adjusted for side-view experiments, the probe-sample interface can be examined.

Interactions of living cells

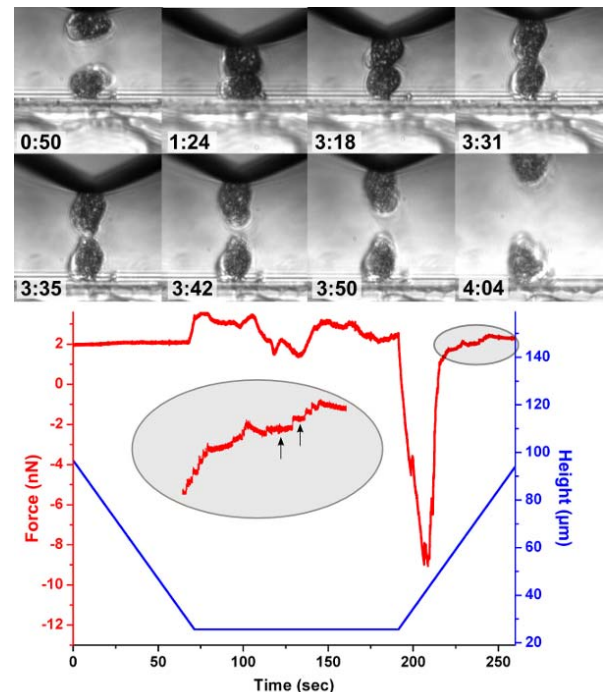


Fig. 2: Interaction of living *Xenopus laevis* cells during a single cell force spectroscopy experiment. The side view image series shows the approach (0:50 min.), contact (1:24 min.) and retract phase (3:18 - 4:04 min.) of the force spectroscopy cycle (cell width around 25 microns). See text for description of the diagram Data courtesy of C. Gonnermann, Dr. D. Stamo and Dr. C. Franz, KIT Germany

The Side-view Cantilever Holder is especially designed for the optical analysis of experiments like Single Cell Force Spectroscopy. A living cell is firmly attached to a soft cantilever and approached to a surface or another living cell. During the retract cycle the cell-cell/surface-cell interaction can pull out membrane tethers which are not visible using transmission light techniques. The special design with the 45° mirror makes such membrane tethers

visible and opens doors to new possibilities in the research of cell interactions.

The diagram of figure 2 displays the piezo height (blue) and the force acting between the two cells (red). The cells were allowed to interact for 120 seconds at constant height (plateau in the height signal). The cells changed their height during the contact period (red line during the plateau region). As the cells are retracted (increase in height curve) they deform and tethers are pulled out of the cell membrane. The highlighted magnification indicates the membrane tethers, which are visible as force plateaus (arrows).

Conclusion

The benefit of the JPK Side-view Cantilever Holder lies in the possibility to observe vertical interactions of any relevant materials optically during AFM based measurements. The mirror gives optical access to the vertical axis during force spectroscopy experiments and provides complementary information. Here the advantage for the combination with single cell force spectroscopy was demonstrated. The cell-cell interaction could not only be measured in terms of force, but also be visualized optically.

Specifications

- Side-view Cantilever Holder with 2x3 mm gold coated mirror plate
- Cleaning with mild detergents

- Sterilization with 70% ethanol
- For use in liquid and air

Compatibilities

- **Sample holders:**
 - o Standard SampleHolder, PetriDishHeater™, BioCell™, CoverSlipHolder™,
- **Stages:**
 - o Manual Precision Stage™, TAO™, Motorized Precision Stage, Precision Mapping Stage
- **Optical configuration:**
 - o Nikon (e.g. TE2000, Ti), Zeiss (e.g. Axio Observer, AxioVert 200), Olympus (e.g. IX line), Leica (e.g. DMI line)
- **Objective lenses:**
 - o Objective lenses with working distance (wd) of at least 4 mm, e.g.:
 - Olympus 20x "LC Plan" FI 20x/0.4 Ph1; wd 6.9mm
 - Zeiss LD "Plan-Neofluar" 20x/0.4 corr.; wd 7.9mm
 - Zeiss LD "Epiplan" 50x/0.50; wd 12mm