

QI mode

In QI mode a complete force-distance curve is recorded at each pixel. Force curves are performed in a sinusoidal motion rather than triangular. Perfect direct force control by JPK's ForceWatch technology results in zero setpoint drift. The off-resonant QI mode with its linear motion with constant speed, compared to methods guarantees accurate values for stiffness and adhesion. A major advantage of QI is, that no lateral forces act on the sample during scanning. This preserves the sample and the tip and is in particular important when combining QI.

For soft samples such as cells, the Set Point needs to be fine adjusted to low forces (~ 100 pN), but the stickiness of biological samples such as cells often requires reasonable pull-off forces and often lengths >1 μm to completely withdraw. Extra height on withdrawal is advantageous for cells and similar structures to ensure removal of all adhesive forces.

One of the many parameters that can be determined is the contact point. The contact point is the height that the cantilever tip initially contacts the surface (starts deflecting). The contact point is the point of zero force at contact. Set as a reference point to deformation and nanomechanical calculations, an important point to determine. For cells this point can be difficult to determine. The cell membrane is not ideally smooth or consistent, especially due to the glycocalyx (a highly charged layer of membrane-bound biological macromolecules attached to a cell membrane acting as a barrier between a cell and its surrounding) with a low Young's modulus of 0.025 kPa. Negligible lateral forces are applied in QI mode which removes many anomalies, especially for soft biological samples.

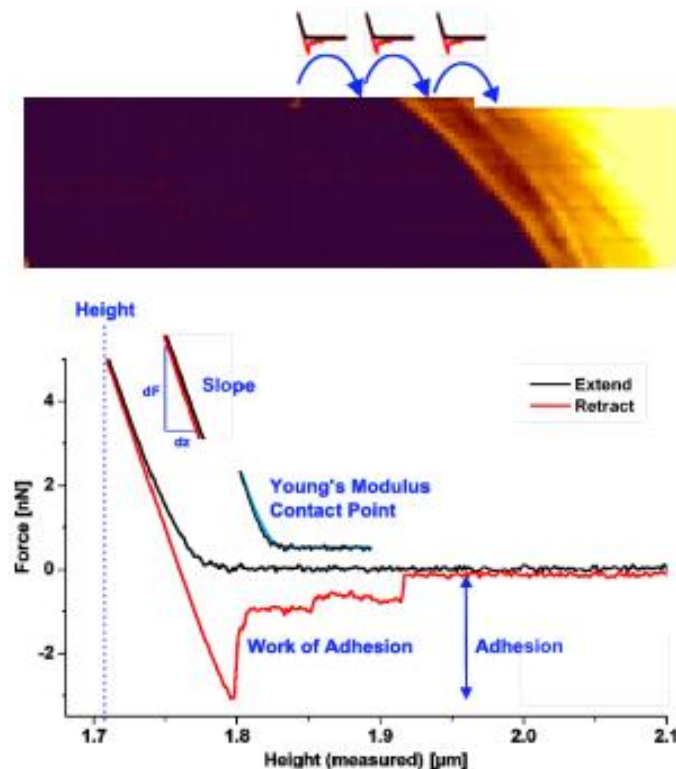


Figure 1: Principle of JPK QI mode.

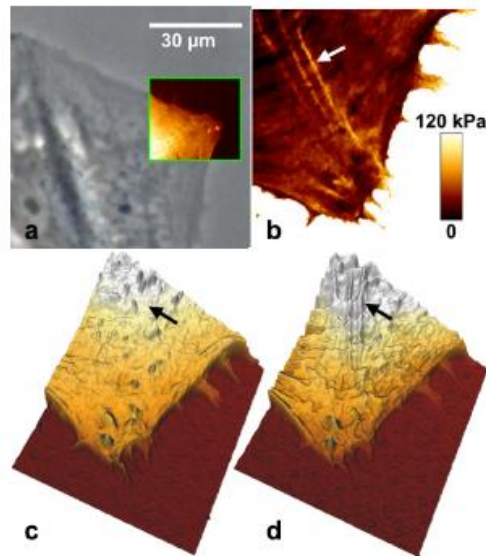


Figure 2: (a) Phase contrast image, (b) Young's modulus image, 3D reconstruction of the contact point at (c) height range 950 nm, and (d) height image at 650 nm height range, 1 nN Set Point of a living fibroblast cell.

Advantages of QI over standard triangular force curves and scanning include:

- improved analysis of steep edges, loosely attached particles, soft or sticky samples.
- Air or liquid
- No cantilever tuning
- Interaction force as low as 50 pN to minimise compression or deformation of soft materials

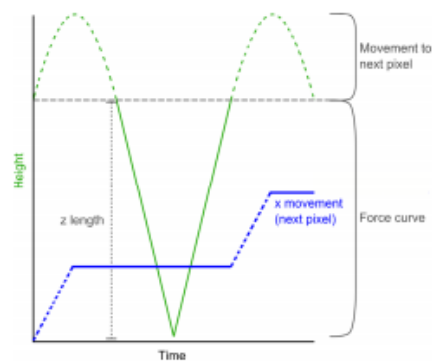


Figure 3: Tip movement in QI mode.

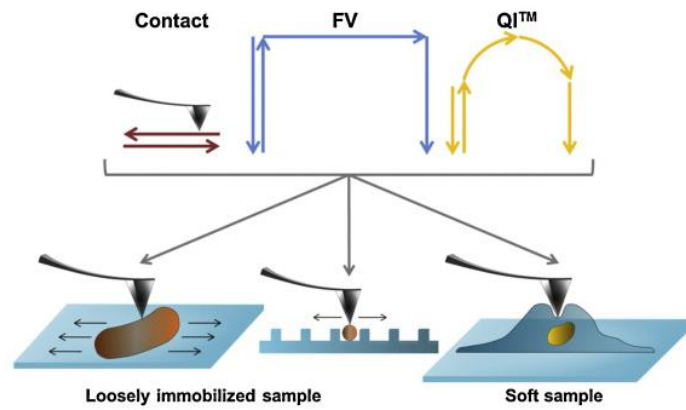


Figure 4: Schematic of contact, force volume, and QI mode on loosely bound and soft samples.

-Smolyakov, G., Formosa-Dague, C., Séverac, C., Duval, R.E. and Dague, E., 2016. High speed indentation measures by FV, QI and QNM introduce a new understanding of bionanomechanical experiments. *Micron*, 85, pp.8-14.

-<https://www.jpk.com/app-technotes-img/AFM/pdf/jpk-tech-quantitative-imaging.14-1.pdf>

-<https://doi.org/10.1016/j.bpj.2012.11.1412> (25/02/21)

-Delgadillo, L.F., Marsh, G.A. and Waugh, R.E., 2020. Endothelial glycocalyx layer properties and its ability to limit leukocyte adhesion. *Biophysical journal*, 118(7), pp.1564-1575.

-Extra channels, additional to height, slope, and adhesion are available such as Young's modulus.

-<https://www.jpk.com/products/atomic-force-microscopy/qi-mode>

-Chopinnet, L., Formosa, C., Rols, M.P., Duval, R.E. and Dague, E., 2013. Imaging living cells surface and quantifying its properties at high resolution using AFM in QI™ mode. *Micron*, 48, pp.26-33.

-<https://www.jpk.com/app-technotes-img/AFM/pdf/jpk-app-living-cells-qi.14-1.pdf>