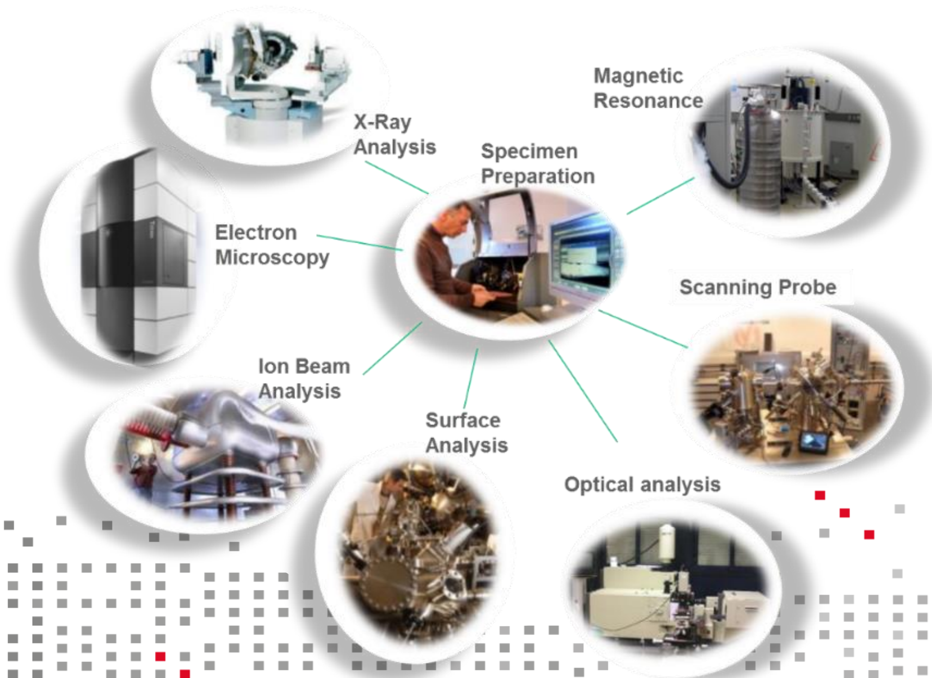
Platform For Nano Characterization (PFNC)



SNAP : STANDARDIZED NANO MECHANICAL ATOMIC FORCE MICROSCOPY PROCEDURE

COST ACTION TD 1002 (2010-2014)

http://afm4nanomedbio.eu/ https://afmbiomed.org/

" This COST Action aims to organize scientific and technological cooperation with experts of different backgrounds. **This transdisciplinary Action** relies on scientists from different fields such as physics, chemistry, mathematicians, biochemistry, biology and medicine, as well as those in more recently developed and developing fields such as genomics, proteomics, metabolomics and microscope design assembly of synthetic and biological molecules, which is critical for the understanding biological functions at the nanoscale. A Trans-Domain COST Action is believed to provide a key strategic platform and community to bundle these research activities."

"**COST (European Cooperation in Science and Technology) is a funding organization for research and innovation networks**. Our Actions help connect research initiatives across Europe and beyond and enable researchers and innovators to grow their ideas in any science and technology field by sharing them with their peers. COST Actions are bottom-up networks with a duration of four years that boost research, innovation and careers."

https://www.cost.eu/

THE PUBLICATION

SCIENTIFIC REPORTS

OPEN

Standardized Nanomechanical Atomic Force Microscopy Procedure (SNAP) for Measuring Soft and Biological Samples

Received: 10 January 2017
Accepted: 26 May 2017
Published online: 11 July 2017

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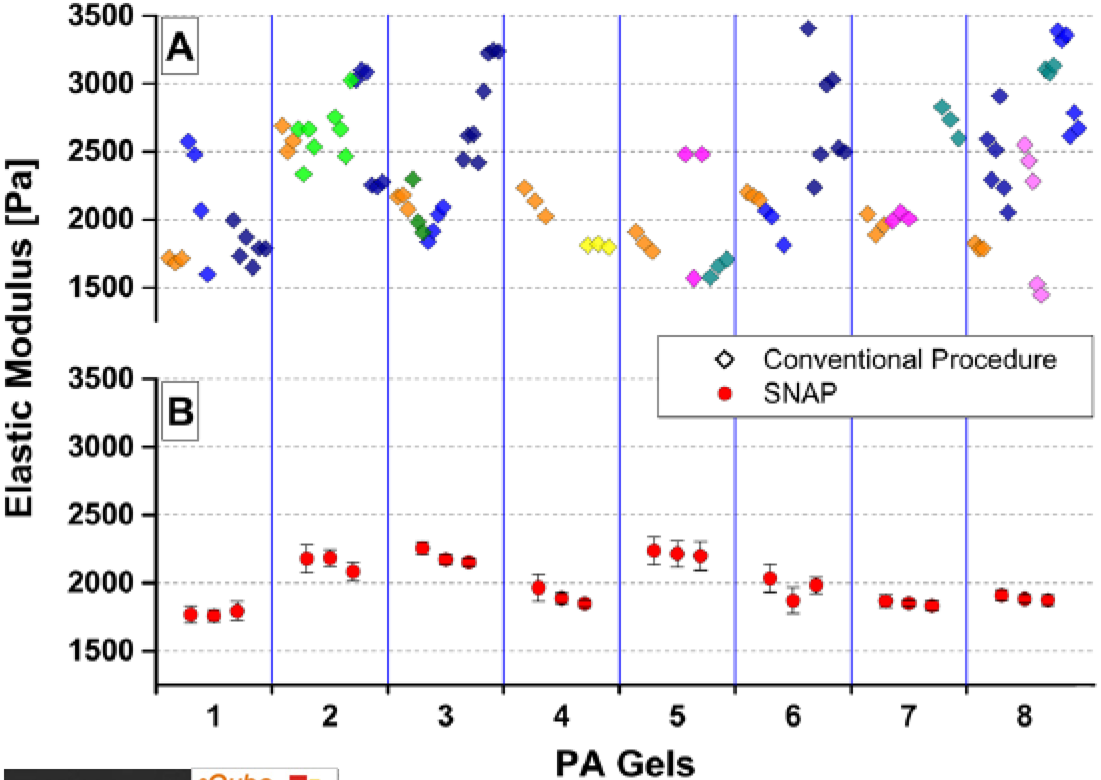
https://www.nature.com/articles/s41598-017-05383-0

Supporting information : https://static-content.springer.com/esm/art%3A10.1038%2Fs41598-017-05383-0/MediaObjects/41598_2017_5383_MOESM1_ESM.pdf

THE REPORT (ON POLYACRYLAMIDE GEL)



JPK (NanoWizard III)
Bruker (Catalyst and Multimode)
Park System (XE120)
Asylum (MFP3D)
[SNAP "OFF"]

MFP3D @ Bremen
[SNAP "ON"]

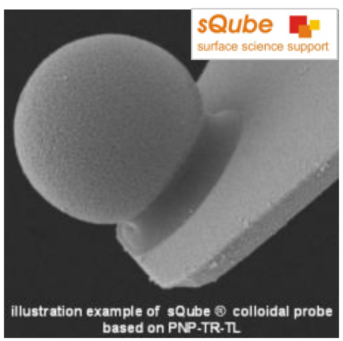


illustration example of sQube ® colloidal probe based on PNP-TR-TL

CP-PNP-SiO-C
SiO_2 D=6,62 µm
Triangular cantilever
K [nominal] = 0.08 N/m
K [LDV] ~ 0.05 N/m

Force-Volume (10 µm)² / (10 pixels)²
Z scan rate = 1 Hz (tip velocity of 8 µm/s)
Z range = 4 µm
Trigger deflection = 50 nm (~ trigger force of 2.5 nN)
in water at room temperature.
Center of the gel piece (75 × 25 mm²)
Modeling : HERTZ

WHY ? (SOURCE OF ERROR)

- Hypothesis 1. The force constants used in analysis (calculated from the AFM thermal spectra) were wrong
  => *No effect of the software/equipment whatever K is obtained by LDV or not.*

- Hypothesis 2. The different gels exhibited different elastic moduli (E) since their properties changed because of time or travel conditions
  => *No, there is no ageing of the gel (re-checked at Bremen before and after)*

- Hypothesis 3. The **deflection sensitivity** had been calibrated erroneously, which would affect the force and indentation values calculated.
  => YES !

"Measurements of soft hydrogel samples with a well-defined elastic modulus using different AFMs revealed that the uncertainties in the determination of the deflection sensitivity and subsequently cantilever's spring constant were the main sources of error. SNAP eliminates those errors by calculating the correct deflection sensitivity based on spring constants determined with a vibrometer."

[N.B. : other possible systematic (and hence constant) error sources like problems in calibrating AFM thermal spectra (SI § 2) or in analyzing thermals (SI §3) and the accuracy of the fitting procedure (SI §7)

GO BACK TO BASIC

E (what we want)

$F = K.\Delta Z$ (what we measure)

K [N/m]

i) by LDV (no need of S)

ii) By Thermal Tune (need S')

S' = amplitude sensitivity (cantilever is free)

$\Delta Z\,[m] = S[m/V].\text{Photodiode Output}\,[V]$

S = deflection sensitivity (cantilever is on contact)

THE SNAP “9 STEPS”

1. **MANDATORY : use a cantilever with well-characterized (known) force constant (e.g. using LDV)**

2. **Use a tip with well-defined geometry (e.g. spherical)**

3. **On your AFM software, calibrate the deflection signal [on very stiff surface] <u>in the same buffer that will be used later in the real experiment</u> => $S_{AFMSOFTWARE}$**

4. **Do not change laser adjustment or instrument settings, which will affect the deflection calibration.**

5. **Record a thermal tune (far away from the surface)**

6. **Analyze the thermal tune using the JAVA applet to obtain the apparent force constant in the AFM ($K_{TiltCorrected}$) and the correction factor λ, then recalculate the deflection sensitivity with $S_{CORRECTED} = \lambda . S_{AFMSOFTWARE}$**

8. Enter this two new values ($K_{TiltCorrected}$ & $S_{CORRECTED}$) on the AFM software

7. **Do you experiment of interest : record force curves or force maps**

8. **Analyze the force curves with the Hertz model or any other appropriate model to extract elastic moduli values (using the JAVA applet or others software, i.e. AtomicJ, Nanoscope Analysis, etc.)**

LASER DOPPLER VIBROMETRY (LDV)

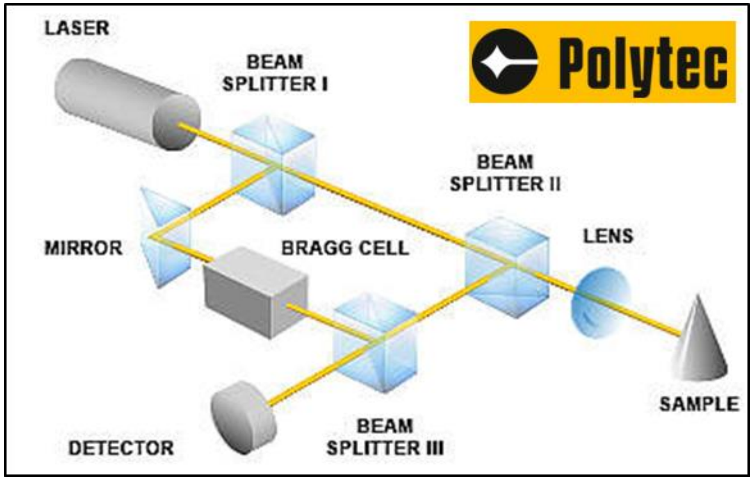
Nanotechnology

PAPER

Accurate and precise calibration of AFM cantilever spring constants using laser Doppler vibrometry

Richard S Gates[1] and Jon R Pratt[2]

Published 24 August 2012 • 2012 IOP Publishing Ltd
Nanotechnology, Volume 23, Number 37



https://iopscience.iop.org/article/10.1088/0957-4484/23/37/375702

- SAA-HPI-30 0.25N/m, k certified, controlled end radius, 5-PK
- RTESPA-150-30 5N/m, k certified, controlled end radius, 5-PK
- RTESPA-300-30 40N/m, k certified, controlled end radius, 5-PK
- RTESPA-525-30 200N/m, k certified, controlled end radius, 5-PK

THE DRAFT (SI §8) – STEP #1 : TILT CORRECTION

1. Calculate of the correction factor k

Cantilever name

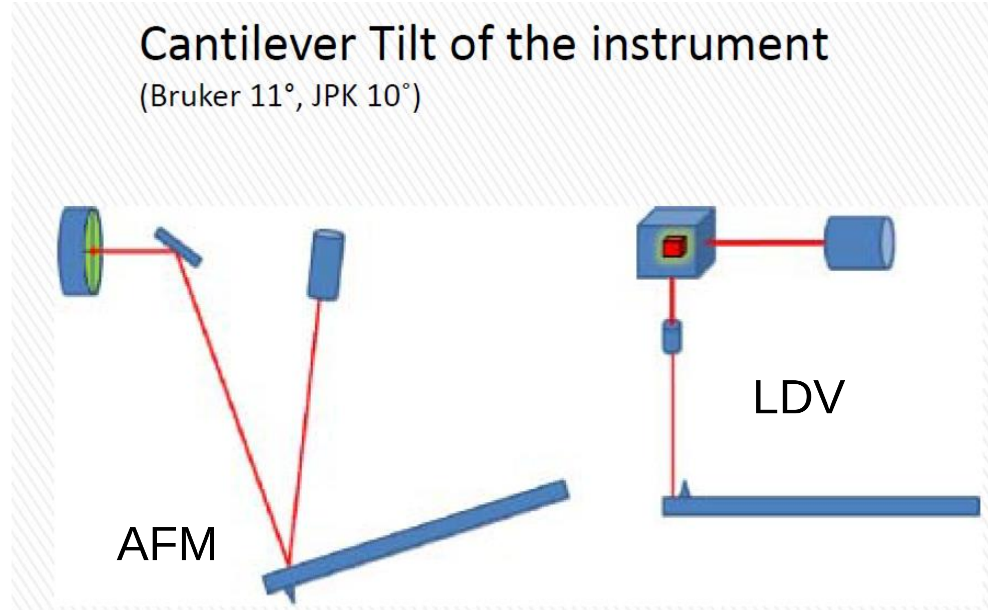
Spring constant determined with a vibrometer $k_{vibrometer}$:

Cantilever tilt of your instrument (α):

Calculate the effective (tilt corrected) force constant $k_{TiltCorrected}$ using the following equation:

$$k_{TiltCorrected} = k_{vibrometer} * \frac{1}{cos^2\alpha}$$

effective (tilt corrected) force constant $k_{TiltCorrected}$:



[adapted from H. Schillers slides “AFM in cancer research – some basics for biomechanical measurements”
Courtesy J.L. Pellequer CEA@IBS]

Question : is the tilt already taken into account in the thermal tune software of AFM ?

THE DRAFT (SI §8) – STEP #2 : THE Ƙ FACTOR

Set the κ factor in your software to a value of 1.1. This number may be called amplitude sensitivity correction or similar.

[N.B. : Ƙ = 1.1 for the soft triangular cantilevers !]

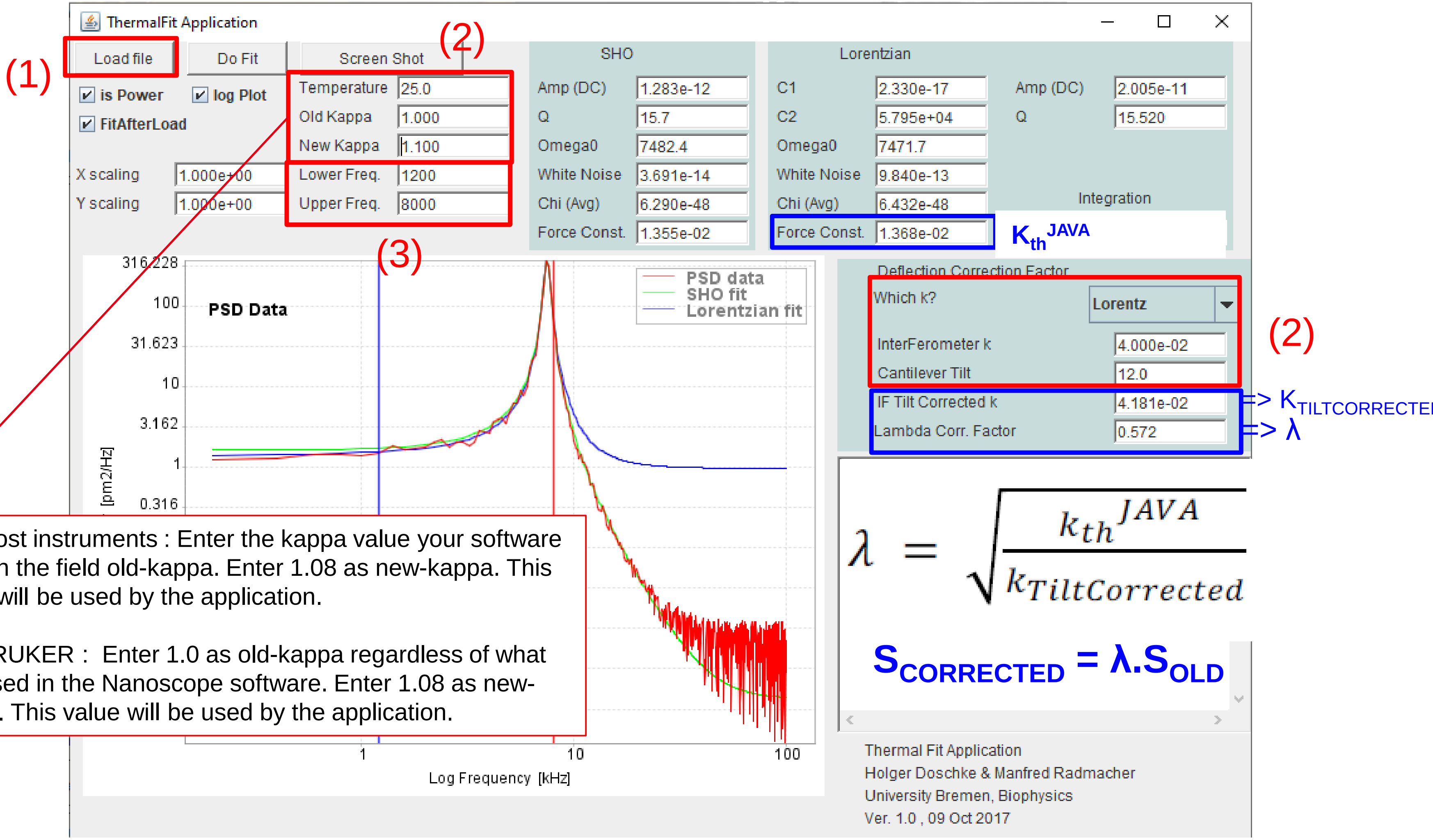
κ factor: ____________1.1__________ 2

“**The vibrational modes of a cantilever are different for a free cantilever (as used in a thermal) and a supported cantilever (as in contact with the sample), so the sensitivity factor used for converting photodiode signal (measured in volts) to cantilever deflection (in nanometers) is different for the two situations. Since the amplitude sensitivity of a free cantilever cannot be calibrated easily in AFM, usually a correction factor κ is used, to calculate the amplitude signal from the measured deflection signal.**”

$$\kappa = \frac{DeflectionSensitivity_{free}}{DeflectionSensitivity_{fixed}}$$

N.B. little bit confusing on the applet : “We believe that 1.08 is a good value for soft triangular cantilevers used in bio-AFM” !

THE JAVA APPLET : THERMALFIT APPLICATION



For most instruments : Enter the kappa value your software used in the field old-kappa. Enter 1.08 as new-kappa. This value will be used by the application.

For BRUKER : Enter 1.0 as old-kappa regardless of what you used in the Nanoscope software. Enter 1.08 as new-kappa. This value will be used by the application.

$$\lambda = \sqrt{\frac{k_{th}{}^{JAVA}}{k_{TiltCorrected}}}$$

$$S_{CORRECTED} = \lambda . S_{OLD}$$

http://www.biophysik.uni-bremen.de/start/radmacher-group/data-analysis/

http://www.biophysik.uni-bremen.de/start/radmacher-group/data-analysis/thermalfit/thermal-fit-instructions/

THE JAVA APPLET : THERMALFIT APPLICATION
- LOADING A FILE -

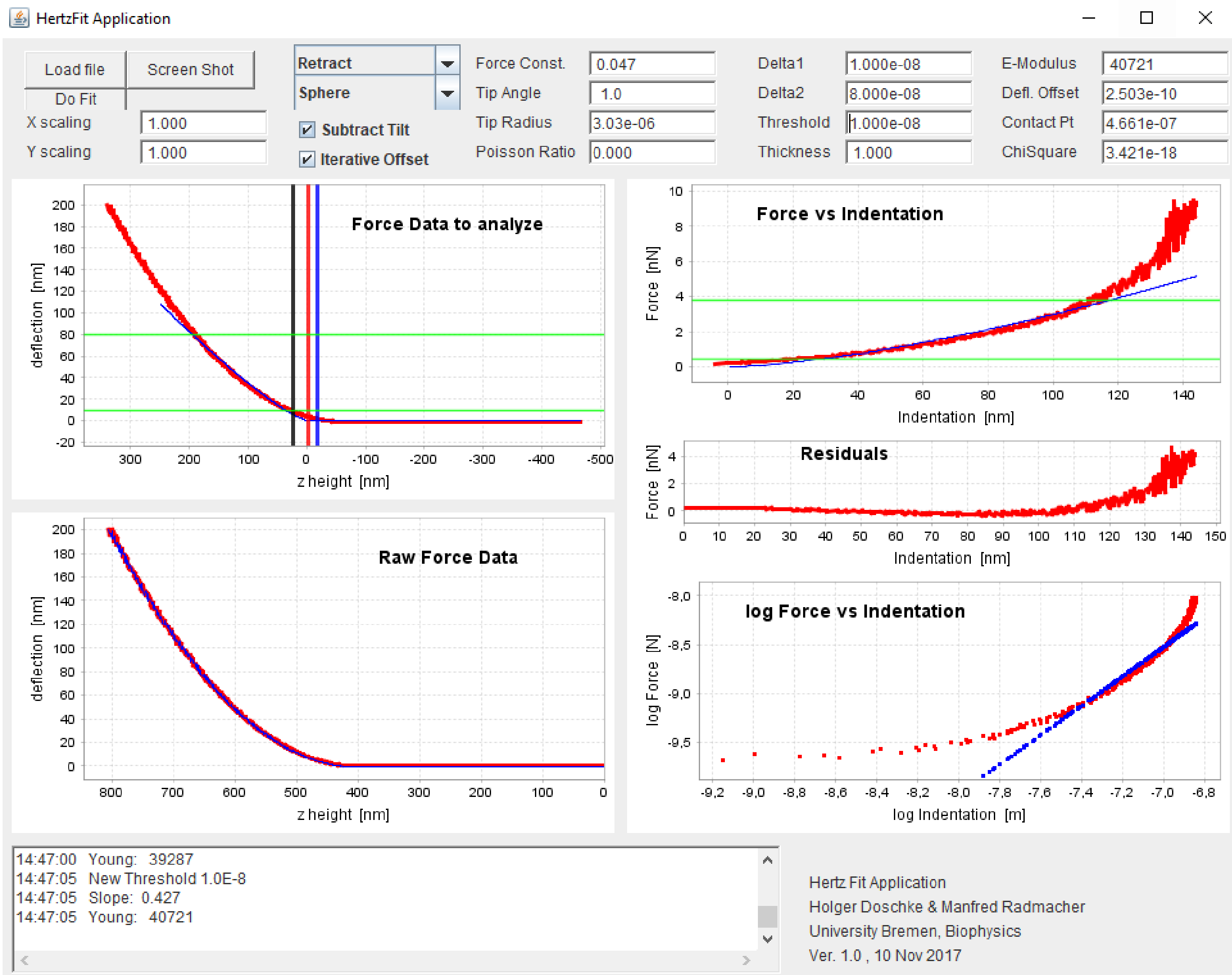
Data Import

This application imports ASCII files, which have two columns of data: column 1 is the frequency and column 2 is either the amplitude or the power, more technically it is the PSD (power spectral density) or the ASD (amplitude spectral density). You have to check, which quantity your instrument actually exports. ASDs will be of the order of pm (10e-12), PSDs will be on the order of 10e-24. You have to set the radio button "is Power" accordingly.
Units of the data are expected to be "Hz" or "m" or "m*m".
If your data, have different units, e.g. they are scaled in kHz, you can use the xscaling or yscaling for correcting this, e.g. by using a scaling factor of 1e3.
You may want to download a sample file here.

Frequency (kHz)	PSD Power (m^2/Hz)
0	0
38.146389	2.2287636e-24
76.292778	3.490037e-24
114.43917	3.8363391e-24
152.58556	5.1401944e-24
190.73195	5.1156671e-24
228.87834	5.1765005e-24
267.02472	7.3931468e-24
305.17111	5.7797204e-24
343.3175	4.4296185e-24
381.4639	5.018257e-24
419.61029	5.0821863e-24
457.75668	7.6143955e-24
495.90308	5.1421815e-24
534.04944	4.6139733e-24
572.19586	4.5056647e-24
610.34222	3.0822072e-24
648.48865	3.8954433e-24
686.63501	3.7392464e-24
724.78143	3.2833405e-24
762.9278	3.1650123e-24
801.07422	3.60852e-24
839.22058	3.2062593e-24
877.367	3.1796421e-24
915.51337	2.9459178e-24
953.65979	2.4333547e-24
991.80615	2.3772493e-24

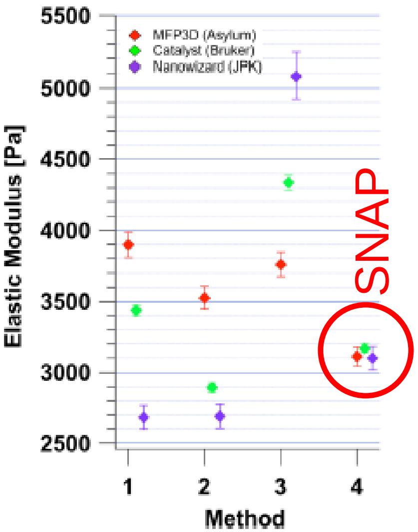
(downloadable file as a sample/example)

THE JAVA APPLET : HERTZFIT APPLICATION



http://www.biophysik.uni-bremen.de/start/radmacher-group/data-analysis/hertzfit/hertz-fit-downloads/

SNAP, USEFUL ?



Method	Method of force constant determination	Method of deflection sensitivity calibration
1	thermal analysis using the AFM software	Force curve
2	thermal analysis using the JAVA applet	Force curve
3	vibrometer by manufacturer	Force curve
4	vibrometer by manufacturer	SNAP

Figure 2. SNAP reduces the variability of mechanical measurements on the same gel in different locations. Elastic moduli of the same gel measured with three different instruments using the same kind of colloidal probes. The data were then analysed with different methods numbered from 1 to 4. In 1 to 3 ones, the deflection sensitivity was calibrated with a force curve on a stiff substrate and the force constant was determined from the analysis of the thermal with the AFM software (1); from the analysis of the thermal with the JAVA applet (2) and from the vibrometer measurements (3). In method 4, SNAP was applied: the force constant was measured using the vibrometer and deflection sensitivity was re-calibrated with the correction factor λ.

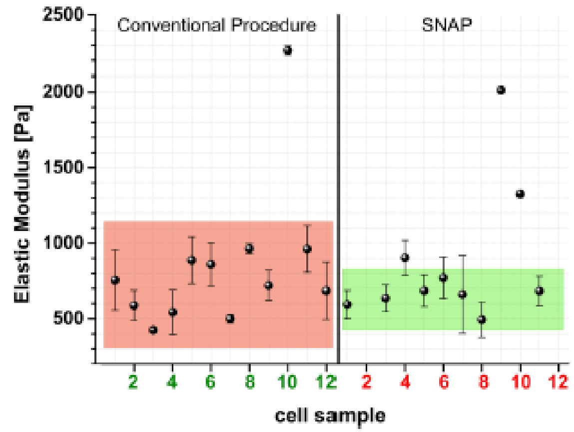


Figure 3. Mechanical properties of MDCK C11 cells. Elastic moduli were determined by participating labs with and without the application of SNAP (using the deflection sensitivity extracted from AFM thermal spectra with Java applet). Data represent peak value and the width of the histograms of Young's moduli determined by fitting a Gaussian function locally around the peak value (see *Methods* section for details). The average and standard deviation of all typical values for each lab (i.e. each cell sample) are depicted as colour bars showing the increased reproducibility of SNAP (green) compared to the conventional (red) one.

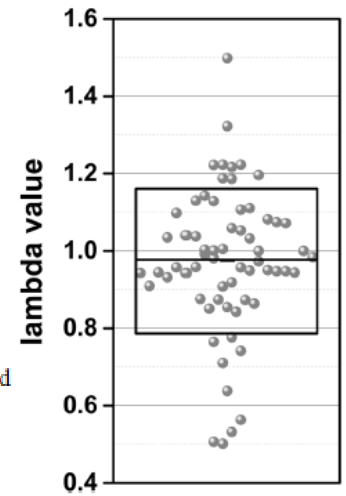


Figure 4. Values of the correction factor lambda (λ) identified for each applied SNAP. Lambda values presented as box-plot showing raw data (spheres), standard deviation (whiskers), 25 and 75 percentile (box) and the median (horizontal line).

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