Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary Data:

Materials and Methods:

Cantilever thiolated-Biotin functionalization and Streptavidin preparation

The gold coated cantilevers were cleaned in Piranha (7.5 ml 98 % H_2SO_4 added to 2.5 ml of 30% H_2O_2). The cantilever chips were immersed in the solution for 10 min. The chips were then transferred to Milli-Q-Water for washing for 1-2 min in each container, followed by a 4 min rinse in absolute ethanol and 4 min rinse in isopropanol. The chips were stored in a closed container at low humidity. The biochemical reaction was performed the same day as the cleaning process to minimize contamination of the gold surface.
Cantilevers were functionalized with EZ-link Biotin-HPDP (Thermo Scientific) as a linker molecule to immobilize streptavidin on the cantilevers surface [S1]. 50 µl of 10 mM EZ-link Biotin-HPDP stock solution was diluted with 950 µl absolute ethanol. The cantilever chips were placed in a 1.5 ml eppendorf tube. 1 ml of EZ-link Biotin-HPDP solution was added and placed on a shaking table for 24 hours at room temperature at low shaking rate. The chips were transferred to another vial with ethanol, washed for 10 min and then dried in air.
Streptavidin solution was prepared (~1.6 nM) by diluting 10 µl of 1 mg/ml stock streptavidin with 90 µl 10 mM PBS buffer.
The streptavidin was added to the reservoir, wetting all cantilevers on the chip. The chips in contact with streptavidin solution were incubated at room temperature for 10 min, then the sample was removed and the reservoirs were washed with PBS and Milli-Q-Water to remove salts.

BAM – anti-BAM assay preparation

The chips were microspotted with BAM haptens EQ0031 conjugated to ovalbumin using a CantSpot system from Nanonord A/S, DK.
We used a conjugation buffer composed of 1.68 g sodium hydrogen carbonate dissolved in 200 ml Milli-Q-Water. BAM haptens EQ0031 were first activated by taking 35.3 µmol EQ0031 and 35.3 µmol BOP (Benzotriazol-1-yloxy-tris (dimethylamino)phosphonium hexafluorophosphate) and dissolving it in 1.5 ml dry DMSO (Dimethyl sulfoxide) [S2]. Subsequently 18.5 µl DIEA (N-Ethyldiisopropylamine) was added. 5 µl activated EQ0031 was added to 1 ml (1 mg/ml) solution of freeze dried ovalbumin dissolved in conjugation buffer and then shaken for 2 hours in darkness.
The spotting quality was monitored via a web camera and recorded. The spotter head was placed in a vial containing the sample, and 4-5 µl of spotting solution was sucked up using 1 µl increments. A tip voltage of 100 V and pulse width of 20 V was used. Each cantilever was spotted with ~0.1 nl drops fully covering only the top surface of the cantilever with the coatings reported in Table S1.

After spotting the chips were kept in a solution containing µfreeze dried ovalbumin dissolved in Milli-Q-Water. Once the stock solution (1 mg/ml) was made, it was distributed in 100 µl PCR tubes and kept in the freezer until use. The anti-BAM used in this work was made from mouse serum at Statens Serum Institut DK, HYB 273-01, Batch nr.03102P01/071008. Antibodies were diluted prior to use to 0.1 mg/ml adding 40 µl stock AntiBAM to 210 µl PBS 0.05%.

DVD-based Detection platform

Detection of cantilever bending is carried out with an astigmatic detection system (ADS). Here the ADS is implemented with a commercial DVD pickup head (PUH) placed below the disc that holds the cantilever array. A schematic of this detection platform is illustrated in figure S1(a). The cantilever deflection results in a change in the defocus distance, which can be detected with a focus error signal, S_{FE}, in a linear region as shown in figure S1 (b). In this setup, the cantilever chips are clamped in the disc that can be spun by a spindle motor. We use the voice coil motor (VCM) of the DVD PUH to move the objective lens along the Z axis to focus the laser beam on the back plane of the cantilever array. The VCM can also move in the X axis, such that the laser spot can be moved along the long axis of the cantilevers. A cantilever 3D reconstruction can be obtained by moving the X axis actuator while spinning the disc.

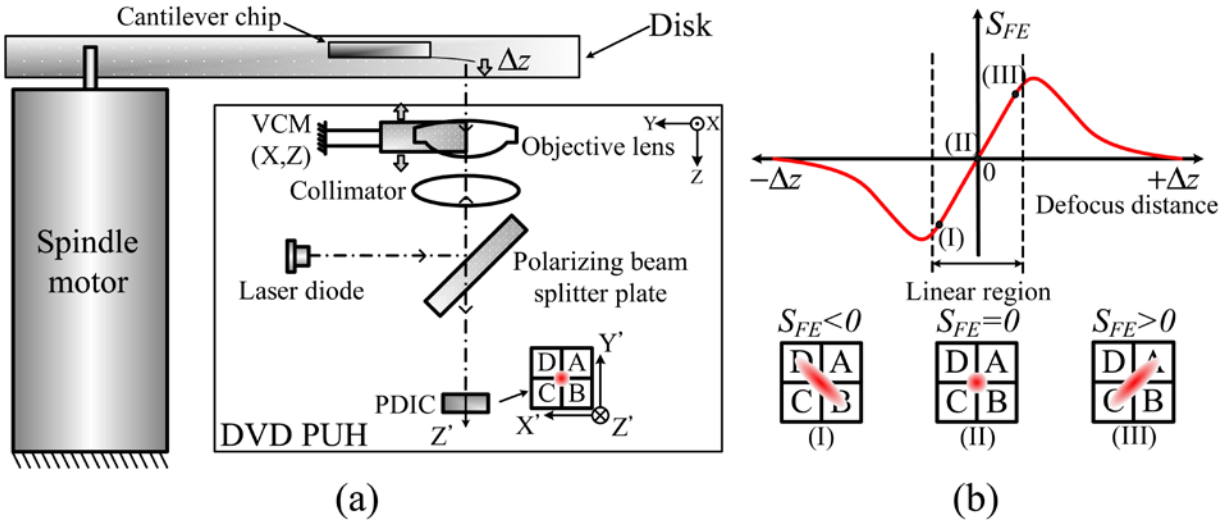

Figure S1. The detection scheme of the cantilever read out system. (a) Schematic diagram of the system. (b) S_{FE} vs. defocus distance

Inside the DVD PUH, a laser light emitted from a laser diode is collimated and focused onto the cantilever surface by collimator and objective lens. The laser reflected from the surface of the cantilever passes through a polarizing beam splitter plate then impinges onto a photodetector integrated chip (PDIC). The PDIC has four quadrant photo elements (*A*–*D*) with a current preamplifier for each of them. The polarizing beam splitter plate works as beam splitter and astigmatic shaping device.

The laser light spot on the PDIC surface is circular shaped, when the cantilever surface is right on focus (defocus distance Δz=0) of the objective lens. When the cantilever surface is higher or lower than the focus point, the laser spot becomes more elongated in A-C or B-D direction, as shown in Fig. S1(b). The four quadrant photo elements are used to detect the shape change of the laser spot. The detection signal which is called focus error signal (S_{FE}) is defined as $(S_A+S_C)-(S_B+S_D)$, where S_A~S_D are PDIC output voltages of the photo elements *A*–*D*, respectively. The PDIC has a very high working bandwidth that is capable of measuring S_{FE} with 80MHz (3dB). The resolution of S_{FE} is

better than 0.1 nm, which means that the height resolution of the 3D reconstruction is in sub nano scale.

The relation between S_{FE} and defocus distance shows a typical S curve. The linear region of S_{FE}, is used to measure the cantilever displacement. The linear region is defined by the numerical aperture (NA) objective lens. Typically, the NA and linear region of DVD PUH is 0.6 and 6~8 µm, respectively. The laser spot size is c.a. 560 nm (FWHM), which is beneficial for sub micron sized cantilever measurement. Compared with optical lever technique, the PUH has the same or even higher resolution. Furthermore, the PUH can tolerate the surface angular deviation θ of each cantilever from -8 to 8 degree during the measurement process, no angular tilt or PSD position readjustments are needed (see figure S2).

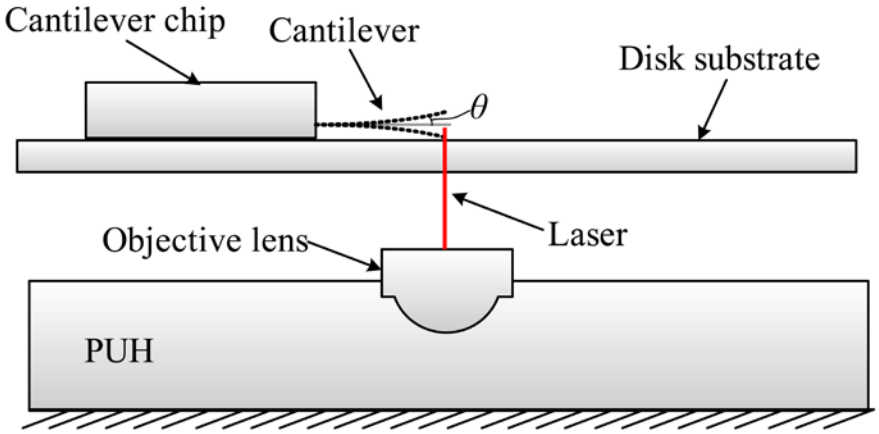

Figure S2. The angular tolerance of the DVD measurement platform.

Multiple PUH system configuration and data treatment

The use of multiple optical pick-up heads (PUH) in our system has several advantages. In the present configuration we employ 4 PUH, three of which are used to perform simultaneous monitoring of bending, surface roughness and resonance frequency, while the fourth is used for calibration.
The role of the calibration PUH is illustrated in figure S3. The distance between laser and cantilever apex can vary during disc revolution due to inhomogeneities in disc and assembly. Therefore, an automated protocol was required to keep the lasers continuously focused on the microcantilevers.
In our setup we used the feedback loop of the Focus Error Signal to readjust the Z-position of the laser spot through the vertical movement of the Voice Coil Motor (VCM) [S3].
PUH 1 was focused on a 100 nm Al coated ring patterned 500 µm away from the position of the apex of the cantilevers. The feedback loop measured the voltage cycle needed to keep the laser focus at constant distance from the disc surface and superimposed it phase-shifted to the voltages driving PUH 2, 3 and 4. The real-time approach in the calibration process ensured that any variation of the disc-PUH relative distance was instantaneously recorded and the VCM position of the sensing PUH corrected.

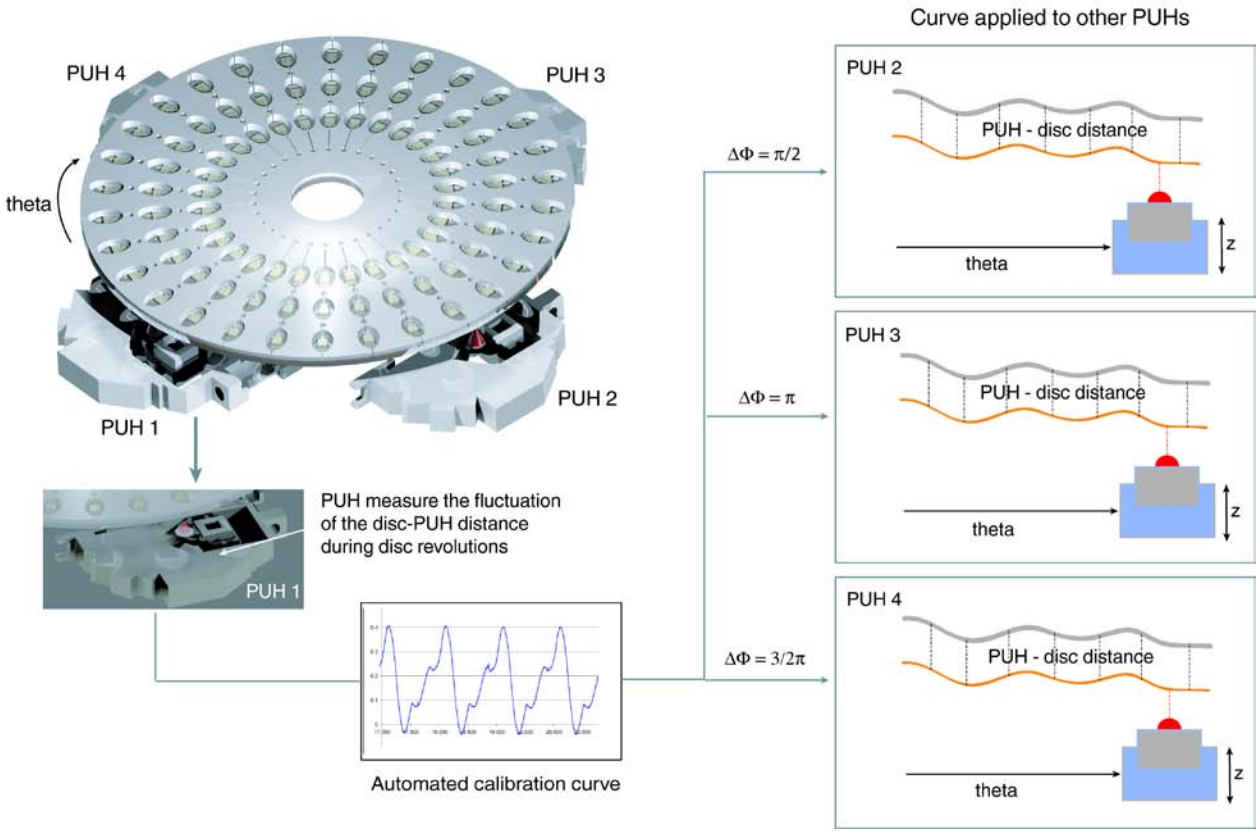

Figure S3. Scheme of the 4-PUH auto-calibration approach.

In figure S4 the data acquisition workflow is presented. Each sensing PUH simultaneously performed one of the three analysis techniques. The whole process was mainly composed by two steps: the characterization block and the sensing block. Our platform performed statistical characterization of *M* cantilevers over *N* measurements (revolutions), before the biological sample was dispensed to the sensing reservoir. During the characterization 3*M* measurements were thus acquired at each nth revolution of the disc. Considering the generic single measurement *V* acquired at the point *p* over cantilever *m* at revolution *n* as $V_m^n(p)$, the evaluation of average and variance during the characterization process was obtained from:

$$\overline{V}_m(p) = \frac{1}{N}\sum_{n=1}^{N} V_m^n(p) \qquad \sigma^2{}_m(p) = \frac{1}{N}\sum_{n=1}^{N}\left(V_m^n(p) - \overline{V}_m(p)\right)^2 \tag{1)-(2)}$$

Typically, 10-20 characterization revolutions were performed. Average values were stored to be used as reference for the sensing part, while the variance was used to evaluate the reliability of the measurements. In case of the bending profiles, the noise was higher at the outer 10-15 microns of the cantilever profile, and this region was generally removed before data processing (standard deviation greater than one part per thousand of the absolute value).

To evaluate the surface roughness the point average obtained with PUH 3 was subsequently averaged over the set of points *P* composing the profile (surface) *V* and the variance was used to estimate the roughness of the surface of the cantilever:

$$R_m = \frac{1}{P}\sum_{p=1}^{P}\left(\overline{V}_m(p) - \overline{V}_m\right)^2 \tag{3}$$

The peak frequencies and the Quality factors measured by PUH 4 were similarly averaged over the *N* revolutions for each of the *M* cantilevers.

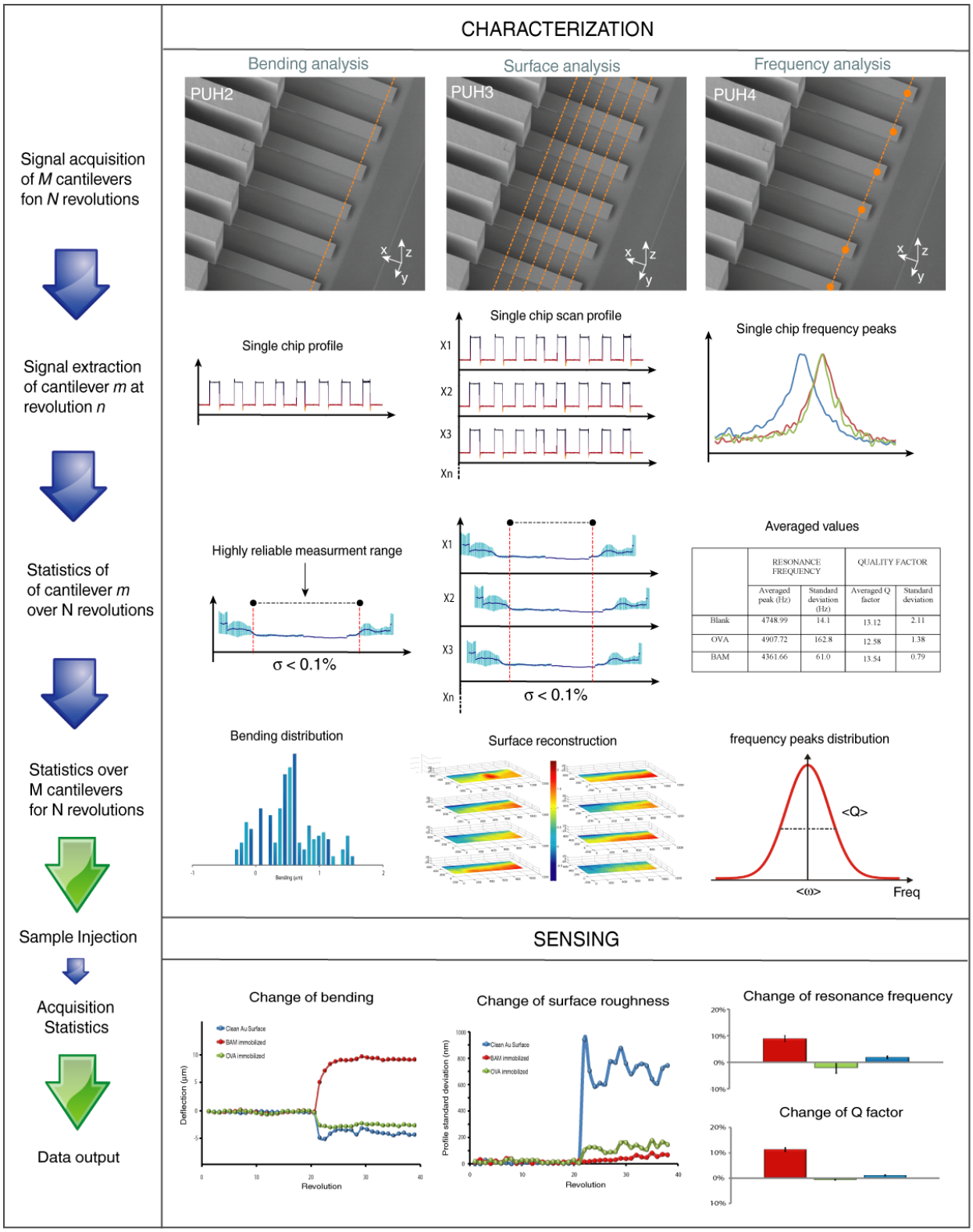

Figure S4. Workflow for statistical microcantilever sensing.

After the reliability of each characterization measurement was ensured, statistical analysis over the sample of cantilevers was made. The total averages and variances were calculated from:

$$\overline{V}(p) = \frac{1}{M}\sum_{m=1}^{M}\overline{V}_m(p) \qquad \sigma^2(p) = \frac{1}{M}\sum_{m=1}^{M}\left(\overline{V}_m(p) - \overline{V}(p)\right)^2 \qquad \text{(4)-(5)}$$

After data processing, it was possible to obtain a detailed statistical analysis of the initial conditions of the cantilevers in air. After the characterization process has been completed the biochemical sample was injected in the sensing chamber and the measurement protocol was repeated.
By substracting the statistical results obtained before and after the sample injection we got the differential signal.

Cantilever 1	1.0 mg/ml ovalbumine in 1x PBS
Cantilever 2	1.0 mg/ml ovalbumine in 1x PBS
Cantilever 3	0.75 mg/ml BAM-ovalbumine conjugate in 1x PBS
Cantilever 4	0.75 mg/ml BAM-ovalbumine conjugate in 1x PBS
Cantilever 5	0.75 mg/ml BAM-ovalbumine conjugate in 1x PBS
Cantilever 6	0.75 mg/ml BAM-ovalbumine conjugate in 1x PBS
Cantilever 7	Blank
Cantilever 8	Blank

Table S1. Functionalization configuration of 8 cantilevers on a single chip for the BAM-Anti-BAM assay.

References

[S1] W. Shua, E. D. Laue, A. A. Seshia, *Biosensors and Bioelectronics* **22**, 2003–2009 (2007).

[S2] L. Bruun, C. Koch, B. Pedersen, M. Havsteen Jakobsen, J. Aamand, *Journal of Immunological Methods* **240**, 133-142 (2000).

[S3] C. L. Chu, K.C. Fan *Measurement Science & Technology* **15,** 734-740 (2004).