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# Multi-parameter microcantilever sensor for comprehensive characterization of Newtonian fluids

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# 1. Introduction

Nanomechanical microcantilevers are widely used in sensing applications to detect changes in mass, stress or temperature to mention a few [1,2]. Another use of these sensors are rheological measurements in liquids [3–8]. As an example, the information about rheology of the blood can be used as a preventive diagnostics for heart disease, stroke, hemostatic disturbance and inflammation [9–12].

The vibrational motion of cantilevers in an incompressible liquid by external excitation is affected in two ways [13]: first, the vibrating cantilever must displace the surrounding liquid, which results in an inertial loading of the beam, also called virtual mass. This shifts the resonance frequencies (peak- and eigenfrequency, see below) to lower values. Second, liquids have a large external damping effect on the cantilevers, which results in reduction of

# ABSTRACT

We have developed a microcantilever-based sensor for the characterization of Newtonian fluids. The vibrating cantilever's peakfrequency, eigenfrequency and damping due to the surrounding liquid were simultaneously recorded. Various aqueous solutions of glycerol and ethylene glycol with defined densities and viscosities were injected and the cantilever response was tracked in real time. Our experiments demonstrate that only a sensor measuring several parameters simultaneously can discriminate between viscosity and density effects. The sensor is found to be sensitive to liquid properties with a resolution of 1.5% in viscosity and 0.06% in density. The results indicate that the effect of viscosity on the eigenfrequency cannot be neglected even at higher vibrational modes.

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the peak amplitude and broadening of the resonance curve (low quality factors Q). It also affects the phase response by reducing its steepness without shifting the eigenfrequency. The eigenfrequency is the frequency where the cantilever response is shifted by  $90^{\circ}$  in phase relative to the driving force. It is equal to the frequency at the turning point of the phase (see red dots in Fig. 1). Note that the damping shifts the peakfrequency to lower values relative to the eigenfrequency is a pure function of effective mass of the cantilever while the peakfrequency represents a combined effect of mass and damping. An overview about the effects of density and damping on a cantilever response is given in the supporting material, Section 1.

Here we demonstrate the application of a multi-parameter and real-time cantilever sensor for the characterization of Newtonian liquid rheology. The independent effect of viscosity and density of the liquid on the vibrating cantilever was studied and the experimental results were compared to theoretical calculations [14,15]. Furthermore, we studied the sensor sensitivity at various modes of resonance.

# 2. Materials and methods

Arrays of eight silicon cantilevers, each  $500 \,\mu\text{m}$  long,  $100 \,\mu\text{m}$  wide and nominally  $1 \,\mu\text{m}$  thick, were used for the measurements.

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**Fig. 1.** Measurement set-up. A frequency generator excites a piezo element mounted beneath the cantilever array (1). The response of the cantilever vibrating in liquid is evaluated using a laser beam deflection technique and a position sensitive detector (PSD, 2). The frequency analyzer (3) monitors the PSD output signal, which is compared to the excitation signal (4), resulting in amplitude and phase spectra (5). These raw data are post-processed to determine the desired parameters (eigen- and peakfrequency and quality factors) using the NOSEtool software. The peakfrequency (from amplitude spectra) and eigenfrequency (from phase spectra) are indicated by red dots. The NOSEtools post-processing software was used to analyze the raw data (6) and extract the eigen-, peakfrequency as well as the quality factors (7). The liquid handling system is also shown (8 and 9). The storage flasks are labeled with the corresponding liquids. Roman numbers according to the sections in Fig. 2 indicate the injection sequence. The corresponding liquid can be selected using a multiway valve (9). The liquid was continuously pumped by an air-pressure driven system through the 6 µl liquid chamber (10). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The laser beam deflection detection technique was used to monitor individual resonance frequencies of the eight cantilevers placed in a chamber volume of  $6 \mu l$  (flow-rate during liquid injections:  $10 \mu l/min$ ). The cantilever array was mounted on a piezo element. A sinusoidal excitation signal generated from a network analyzer swept the desired frequency range vibrating the cantilevers at resonance, thus yielding amplitude and phase responses as output. An overview on the measurement principle is given in Fig. 1. Amplitude and phase spectra were continuously recorded throughout the experiment. NOSETools software [16] was used to analyze the spectra and extract peakfrequencies and quality factors from the amplitude responses and eigenfrequencies from phase [16]. A compendium on the data processing is published elsewhere [17].

All chemicals were purchased from Fluka AG, Switzerland, including purified water (HPLC grade). At 20 °C, different concentrations of glycerol (density  $\rho$  = 1250 kg/m<sup>3</sup>, kinematic viscosity  $\eta$  = 1.42 kg/(m s)) and ethylene glycol ( $\rho$  = 1113 kg/m<sup>3</sup>,  $\eta$  = 18 × 10<sup>-3</sup> kg/(m s)) in water ( $\rho$  = 997 kg/m<sup>3</sup>,  $\eta$  = 0.913 × 10<sup>-3</sup> kg/(m s)) were investigated.  $\eta$  is the density independent kinematic viscosity. A detailed description of the solution preparation is presented in supplemental material (see Section 4). Glycerol at 5% (v/v) and ethylene glycol at 12% (v/v) concentration have the same density but different viscosity. The viscosity of ethylene glycol solution was larger by 19% (v/v) than the glycerol solution. Similarly, glycerol at 5% (v/v) and ethylene glycol at 5% (v/v) have different densities but same viscosity. The density of the glycerol solution is 0.8% larger than the ethylene glycol solution. The corresponding  $\rho$  and  $\eta$  values are shown in Table 1.

During all experiments liquids were injected in the following order: water, 5% glycerol, 12% ethylene glycol, 5% glycerol, 5% ethylene glycol and water. Liquid was continuously flushed at a rate of 10  $\mu$ l/min until clear saturation of the signal was observed.

To compare our results with theory, we used the models proposed by Van Eysden and Sader [15]. Note that we used approximations to estimate the eigenfrequencies compiled in Table 1. This model assumes that the fluid is incompressible in nature. Fur-

#### Table 1

Density, dynamic viscosity values and eigenfrequency shifts relative to water

Liquid (v/v)	Density (kg/m <sup>-3</sup> )	Dynamic viscosity (×10 <sup>-3</sup> kg/(m s))	$\Delta f_{\text{Exp}}$ (Hz)	$\Delta f_{\text{Theor}}$ (Hz)
Water	997	0.913	$0 \pm 3.7$	0
5% Glycerol	1010	1.055	$3513 \pm 3.5$	3378
12% Ethylene glycol	1011	1.257	$4824 \pm 6.1$	4487
5% Ethylene glycol	1003	1.058	$2122 \pm 3.8$	1963

The theoretical shifts ( $\Delta f_{\text{Theor}}$ ) were calculated according to [15]. The eigenfrequency in pure water was used to calibrate the cantilever thickness (experimental cantilever thickness: 0.923 µm).  $\Delta f_{\text{Exp}}$ : experimental eigenfrequency shifts. The errors are standard errors.

thermore we used the experimental eigenfrequencies in water to calibrate the cantilever thickness. We did not compare theoretical quality factors with the experiment because our experimental situation are out of the condition-range presented by Van Eysden and Sader [15].

# 3. Results

We tested the multi-parameter cantilever sensor to detect density and viscosity changes in liquids in real-time by exchanging the surrounding media, varying viscosity, density or both parameters. This allowed us to study these effects independently. The parameters monitored were peakfrequency, eigenfrequency and quality factor.

Fig. 2 shows the resonance frequency changes (peak- and eigenfrequency) and the quality factors throughout the experiment for mode 3 (at 14.6 kHz, Fig. 2A) and mode 12 (at 458 kHz, Fig. 2B) out of 16 modes (mode 1 is the fundamental mode) of resonance frequencies in a span of 1 MHz [14]. Peakfrequencies and quality factors were obtained from the amplitude response, whereas the eigenfrequencies (red) correspond to the turning-point positions in the phase response. All solutions were continuously pumped through the measurement chamber (volume 6  $\mu$ l, flow-rate 10  $\mu$ l/min) sur-



**Fig. 2.** Frequency and quality factor behavior for different densities and viscosities of the liquid. (A) Mode 3 and (B) mode 12. Various injections are separated by dotted lines and the corresponding solutions were constantly flown through the measurement chamber. I. Water, II. 5% glycerol, III. 12% ethylene glycol, IV. 5% glycerol, V. 5% ethylene glycol and VI. water. Peak frequencies and quality factors are obtained from the amplitude response while eigenfrequencies are extracted from the phase response. A 19% change in viscosity has significant effect both at low and higher modes, whereas a 0.8% change in density was within the noise floor at low modes. However, it was rather significant at mode 12. Correspondingly, quality factors decreased during viscosity increase and remained constant during density decrease.

rounding the cantilever sensor. Initially, the liquid chamber was filled with water (section I). Note that the difference between the peakfrequency and the eigenfrequency increases with the mode number as well as the quality of the resonance. After reaching a stable signal the water was exchanged against a 5% glycerol solution, changing density and viscosity of the liquid surrounding the cantilever (section II). The cantilever response reflects this change in all parameters, i.e. the peak- and eigenfrequencies as well as the quality factors decreased. In the next step, keeping the density constant but changing the viscosity, 12% ethylene glycol solution was injected (III). The resonance frequencies and the quality factors decreased. After injecting again the 5% glycerol solution (IV) all parameters exhibit almost the same values as in section II. Keeping the viscosity constant but changing the density, 5% ethylene glycol was injected (V). As response, the resonance frequencies increased but the quality factors remained constant. Finally, the water injection sets the resonance frequencies and quality factors to initial values (VI).

Two typical amplitude and phase response spectra from the saturation region in sections II (5% glycerol) and III (12% ethylene



**Fig. 3.** Amplitude and phase response for (A) viscosity increase. (B) Density decrease. Viscosity increase broadens the amplitude response and reduces steepness of the phase signal due to damping. Additionally, the increased viscosity leads to the rise of the shear forces between the water layers and therefore to an increased virtual mass (see Section 4). Both effects lead to a crossover in the phase spectra (arrow). For decreasing density of the liquid (panel B), the amplitude response shifts towards higher frequency keeping quality factors constant whereas the corresponding phase response shifts to higher frequency. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

glycol) of Fig. 2B are plotted in Fig. 3A. The surrounding solutions have different viscosities but almost identical densities (see Table 1). It can be observed that the amplitude response (red in color) in the more viscous medium has shifted to lower frequency and flattened indicating reduction in its quality factor. The corresponding phase response has shifted to slightly lower frequency and became less steep resulting in the crossover (marked in the figure) of the low viscosity phase response (black in color, compare to section 1 of supplemental material). Analogous spectra are shown in Fig. 3B. The cantilever response from the saturation regions of sections IV (5% glycerol) and V (5% ethylene glycol) implies a decrease of the density but not of the viscosity. The amplitude response (red in color) in the less dense medium shifted towards higher frequency without changing the quality factor. The corresponding phase response also shifted to higher frequency without anv crossover.

The effects of the liquid density and viscosity on the cantilever response at the first and the last few resonance modes below 1 MHz are shown in Fig. 4. The shift in frequency and quality factor are the differences obtained between two equilibrium regions during the flow of a particular liquid. In Fig. 4, Panel A demonstrates effect of the viscosity increase, while Panel B shows the influence of the density decrease. For viscosity increase at constant density, the frequency shift was negative and increased at higher mode num-



Fig. 4. Mode dependency for eigenfrequency shifts and quality factors. (A) 19% viscosity increase at constant density (compare to transitions II–III in Fig. 2). (B) Density decrease maintaining constant viscosity (corresponds to transitions IV–V of Fig. 2).

ber. This was also reflected in the quality factor behavior. Density decrease resulted in positive frequency shift due to lower virtual mass around the cantilever. Again these shifts were more pronounced at higher modes. We observed nearly constant quality factor at all modes. The quality factors were determined by analyzing the peak-shape that was not always possible, especially when the peak shifted to lower frequencies. In the latter case estimated values were calculated by the software from the peak width determined by a Lorentzian fit (e.g. mode 15 panel A, modes 16 and 17 panel B).

# 4. Discussion

The main observations in this study are

- Density change contributes to frequency shift, which increases with mode number; the quality factor is not affected.
- 2. Higher viscosity increases the shear forces between liquid layers and, therefore increases the virtual mass. Due to the mesoscopic size of the cantilever sensor, the viscosity changes cannot be neglected even at high modes.
- 3. Higher modes are more sensitive.

Fig. 2B shows that the eigenfrequency (in red, from the phase turning point) is always 0.2% higher than the peakfrequency for mode 12. For mode 3 (Fig. 2, panel A) this effect is hidden in the noise of the amplitude response as discussed below. This frequency difference is due to the damping of the system, which shifts the amplitude peaks but not the eigenfrequencies (see supplemental material). Furthermore, the amplitude frequencies at mode 3 are significantly noisier than those at mode 12 (Fig. 2, panel B) and the corresponding eigenfrequencies. This can be explained by the laser based deflection read-out technique being used, which is angle sensitive: the amplitude response at lower modes has a smaller magnitude than at higher modes. This low magnitude is the result of smaller cantilever beam bending [18] (see also discussion in supplemental material section 2). Also note that the quality factors are five times lower at mode 3 compared to mode 12 as discussed previously [14], which is caused by the hydrodynamic effects of the liquid environment. This effect broadens the amplitude and phase responses and results in additional source of noise at lower modes.

The response of the measurement set-up shows much higher sensitivities for environmental changes at higher modes (compare panel A and B of Fig. 2, see also Fig. 4). The kinetics of the liquid exchange highly depends on the viscosity of the liquid flown through the instrument due to the small diameters of the tubings connecting the storage flasks to the measurement chamber. Two liquid exchanges are of particular interest: first, the viscosity increases with constant density from sections II to III, and second, from sections IV to V the liquid density changes but not the viscosity. Changes in liquid density have a clear impact on the peak- and eigenfrequencies resulting in shifts towards lower values. Thereby the damping (quality factors) is not affected. The amplitude and phase spectra are merely translated to lower frequencies (see Fig. 2B). These results are in good agreement on current theoretical models (see Table 1). The increase of the liquid viscosity (sections II-III) shows a more complex pattern: the quality factors decrease as expected. However, changes of the peak- and eigenfrequencies are also observed-most distinct for mode 12 (Fig. 2, panel B). This is surprising at first glance since the older theoretical models that described best the resonance frequencies at high modes (>8) neglected the influence of the viscosity [19]. On the other hand, viscous models [20] failed to explain the resonance frequencies at higher modes (for comparisons see Ghatkesar et al. [14]). However, a close comparison of the theoretical calculations using the extended viscous model of Van Eysden and Sader [15] reveals that the observed frequency shifts can be clearly attributed to viscosity change and a slight decrease of the Reynolds number leading to a decrease of the resonance frequency (see the comparison of the theoretical and experimental values in Table 1). The relative change (0.26%) of the eigen- and peakfrequency is clearly detected at mode 12. This is due to the increase of the virtual mass. More precisely, the volume of the inertial liquid load of the cantilever beam increases with constant density. This directly correlates with the increased shear forces between the liquid layers at higher viscosity so that more water layers contribute to the inertial load of the cantilever beam. The slight increase of the virtual mass is only measurable due to the extremely small volume of the cantilever sensors: an example of how a mesoscopic structure translates events into the nanoscopic world. For low frequencies, experiments comparing the resonance frequencies with theoretical models taking the viscosity into account were already presented before [20,21], but these

models failed predicting the resonance behavior at higher modes (>8) [14].

The frequency shifts caused by viscosity changes lead to a crossover of the two phase spectra in different viscosity regimes (see Fig. 3A). This finding can be explained by the lower slopes of the phase transition and, additionally, by the shift of the eigen-frequency due to the increased virtual mass. The gap between the peak- and eigenfrequency increases during the injection of high viscosity solution since the damping of the system increases. The sensor used provides three different physical properties peak-and eigenfrequency as well as Q-factor. The study of this multiparameter information permits a comprehensive analysis of liquid characteristics. However, our data also strongly suggest using reference cantilevers as negative control in order to exclude artifacts in the signal interpretation of the positively functionalized cantilevers in dynamic mode mass adsorption experiments.

The viscosity effect is mode-dependent and approaches asymptotically a constant virtual mass. This is reflected in the frequency shifts which do not change significantly at modes higher than 14 (Fig. 4A). This saturation behavior depends on the geometry of the cantilever, so that a slower convergence is expected for narrower cantilevers [20]. In contrast to this finding, the shifts of the frequency increase with mode number upon density changes (Fig. 4B). This is due to the increased mass sensitivity at higher modes [16,18,22]. The quality factor shifts boost from modes 1 to 17 but the relative changes are constant around 7%. The absolute quality factors rise from 3.6 (mode 2) to 31 (mode 17, data not shown). For the density shift the changes of the Q-factor are within the measurement error and do not vary significantly (average relative change of 0.96%).

Determination of the sensitivity and resolution of our instrument is complicated by two facts: first, density and viscosity are intensive quantities. Therefore we compare only relative changes. Second, the dependency of the output parameters (eigenfrequency, *Q*-factors) are monotonic, but not a linear function of density and viscosity changes [15]. The sensitivity of the method toward 0.8% density changes at mode 17 is found to be  $2.5 \pm 0.1$  kHz. We estimated the resolution for density fluctuations up to 0.06% of the density change. The viscosity fluctuations are best detected by the change of the *Q*-factor (damping) at high modes where the frequency depended marginally on viscosity (Fig. 4A). At mode 17 we measured a sensitivity of  $2.5 \pm 0.2$  kHz in case of 19% viscosity change. The viscosity resolution is expected to be around 1.5%.

## 5. Summary, conclusion and outlook

Liquid characteristics (density, viscosity) were measured in realtime in small volumes within a few minutes using microcantilever sensors. A comprehensive analysis of the liquid properties was achieved using a multi-parameter cantilever sensor that allowed us to perform simultaneous measurements of the damping and the eigen- and peakfrequencies, respectively. The sensor detected a density change of 0.06% and a viscosity change of 1.5% in water. We experimentally demonstrated that the viscosity contributes to changes of the virtual mass even at high frequencies (high modes), in contrast to previous reports [13,19,23]. The sensitivity toward changes in density increases with mode number. This method can be extended to characterize the rheology of complex fluidics such as polymer solutions at high concentrations and blood taking also the liquid elasticity into account. We envisage the use of cantilever based real-time sensors to monitor chemical reactions, protein aggregation and blood-plasma rheology for medical applications.

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# Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.snb.2008.08.012.

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**Murali Krishna Chatkesar** received his M.Sc. degree in Electronics from University of Hyderabad, India, and the M.Sc. (Engg.) degree in 2001 from Indian Institute of Science, India for his work on tactile sensors. In 2001, he joined industry and implemented speech codecs on digital signal processors. He went back to Indian Institute of Science in 2003 to work on the development of thin film heat transfer gauges on high enthalpy aerodynamic structures in hypersonic shock tunnel. In 2007 he received his PhD on "resonating nanomechanical microcantilevers in liquid as biosensors" at Institute of Physics, University of Basel, Switzerland and now joined the California Institute for Technology (Caltech) in the USA. His research interest belongs to the areas of nanobiotechnology, microelectromechanical systems and biophysics.

**Ekaterina Rakhmatullina** studied chemistry at St. Petersburg State University from which she graduated in 2001 with M.Sc. degree in organic and macromolecular chemistry. Before starting her PhD she was working on the interdisciplinary project in collaboration with BIA Separations d. o. o., Ljubljana, Slovenia, Institute of Macro-molecular Compounds, Russian Academy of Sciences, and Institute of Experimental Medicine, Russian Academy of Medicine, St. Petersburg. In 2004 she started her PhD in the Department of Chemistry, University of Basel. Her current interests are synthesis and characterization of the amphiphilic block copolymers; copolymer self-assembly in aqueous media, polymer nanocontainers. Another direction of copolymer biomimetic membranes on gold and silicon surfaces.

Hans Peter Lang received his PhD in physics from the University of Basel in 1994 with a thesis on scanning tunneling microscopy on high temperature superconduc-

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**Christoph Gerber** is the Director for Scientific Communication of the NCCR (National Center of Competence for Nanoscale Science) at the Institute of Physics, University of Basel, Switzerland and was formerly a Research Staff Member in Nanoscale Science at the IBM Research Laboratory in Rüschlikon, Switzerland. For the past 25 years, his research has been focused on Nanoscale Science His current interests are biochemical sensors based on AFM technology, chemical surface identification on the nanometer scale with AFM, nanomechanics, nanorobotics, AFM research on insulators, single Spin Magnetic Resonance Force Microscopy (MRFM) and self-organization and self-assembly at the nanometer scale.

**Martin Hegner** received his M.Sc. in Life Science 1989, Swiss Federal Institute of Technology, Biochemistry and his PhD in Life Science 1994, Swiss Federal Institute of Technology. In 2006 he was awarded Endress professor for sensors in biotechnology at the University of Basel. His primary interests are related to the field of Nanobiology, investigating molecular interactions by optical tweezers and the development of biosensors based on nanomechanical cantilevers working at the institute of the Trinity College, Dublin, Ireland.

**Thomas Braun** received his M.Sc. in biophysical chemistry in 1998, University of Basel (Switzerland) where he continued his scientific work and acquired PhD degree in biophysics in 2002. His thesis was in the field of membrane protein biochemistry, high-resolution electron microscopy and digital image processing. As post-doc he was working on the development of kinetic microarray techniques with focus on nanomechanical sensors for membrane protein research and multimode measurements at the Institute of Physics, University Basel. He proceeds these projects as senior research fellow in the CRANN institute of the Trinity College, Dublin, Ireland.