

Integrated dual-grating interferometric detection with polymer microbeams for bio-chemical sensing in liquid environment

Jin-yang Feng, Xiong-ying Ye, Yuan-fang Shang, Kang Wu, Feng Chen

State Key Laboratory of Precision Measurement Technology and Instruments, Department of Precision Instrument, Tsinghua University, Beijing 100084, People's Republic of China
E-mail: xyue@mail.tsinghua.edu.cn

Published in Micro & Nano Letters; Received on 27th April 2013; Revised on 25th June 2013; Accepted on 9th July 2013

An interferometric displacement detection method of polymer microbeams based on integrated dual-grating was developed for bio-chemical sensing in a liquid environment. Sensing microstructures made of polyimide (PI) with four symmetrically arranged L-shaped beams suspending a central plate were designed and fabricated on a glass substrate using a surface micromachining process. With these structures, deflection changes of the microbeams were transduced to out-of-plane movements of the central plate, which were then measured from the interference of light spots diffracted from the integrated gratings using a charge coupled device image measurement setup. To extend the displacement detection range, two adjacent metal gratings corresponding to a microbeam were formed inside and outside a groove with a depth of 55 nm, respectively, to achieve a phase shift of about $\pi/2$ in liquid. The detected noise of aluminium (Al) microcantilevers in ethanol slightly increased compared with that in air, and the noise of PI microbeams in phosphate-buffered saline was about three times larger than that of Al cantilevers in ethanol. The preliminary experimental results in ethanol show that the detection method based on polymer microbeams integrated with dual-gratings is feasible in a liquid environment. Antibody–antigen binding detection was also conducted with a rabbit immunoglobulin G (IgG) immobilised chip in 100 $\mu\text{g/ml}$ goat anti-rabbit IgG solution.

1. Introduction: In recent years, microcantilever sensors have been intensively studied as a promising platform for label-free and array bio-chemical sensing [1–3]. The adsorption of molecules on the cantilever surface may lead to change in the cantilever bending [4, 5] or shift in its resonance frequency [6, 7]. The detection method based on deflection is suitable in a liquid environment [8].

The measurement methods of microcantilever bending are based on a piezoresistor [9–11], a field-effect transistor (FET) [12], an optical lever [13, 14], interferometry [15] and so on. Detection sensitivity of the piezoresistive and FET methods is relatively low, whereas that of optical detection is higher. However, the optical lever detection method is not readily amenable for massively parallel detection because of difficulty in light beam alignment.

The interferometry read out is one of the most sensitive methods for displacement detection. An interferometric detection method based on an integrated diffraction grating has been proposed to detect displacement in an array with high accuracy [16, 17], which benefits from ease of integration with micromachining process and implementation for large array detection. This method meets the demands of high-sensitive and high-throughput detection of bio-chemical sensing. Furthermore, in comparison with the interferometric detection method based on an integrated single-grating, the one based on integrated dual gratings with a phase shift of $\pi/2$ reveals an obviously expanded displacement detection range, and the detection range is extended from less than the monotony range of the periodic output light intensity signal ($\lambda/4n$, λ is the wavelength of incident light, and n is the refractive index of the medium between the beam and the gratings) to multi periods without dramatically decaying the sensitivity [18, 19]. This method is mainly used for detection in air, and may suffer from the influence of the transparency and refractive index of the medium in liquid. In our previous work, the deflections of an aluminium (Al) microcantilevers array integrated with dual-gratings in the air were simultaneously detected with a charge coupled device (CCD) measurement setup [20].

Besides, polymer materials, such as SU-8 [21], parylene [22] and polyimide (PI) [23], have been recently investigated for bio-chemical sensing because of their low Young's modulus,

processing compatibility, chemical resistance, biocompatibility and low cost.

In this Letter, we demonstrate an interferometric measurement using PI microbeams with integrated dual-grating in a transparent liquid environment. PI microstructures with four L-shaped beams suspending a centred plate are designed and fabricated. The detection noises in liquid are characterised. The displacement of the polymer microbeams with multi periods is measured in ethanol and an antibody–antigen binding detection is conducted too.

2. Design and fabrication: Fig. 1 illustrates an integrated dual-grating microbeam on a glass substrate. A groove is etched on the glass substrate and two adjacent metal gratings corresponding to a microbeam are patterned inside and outside the groove. Each grating and the beam above form a phase sensitive diffraction grating [16, 17]. When a laser beam illuminates from the back side of the chip, diffracted light whose intensity is modulated by the distance between the microbeam and the grating as a regular Michelson interferometer, is generated. The phase shift of the two phase sensitive gratings is designed to $\pi/2$ through the groove with a specified depth $h = \lambda/8n$, depending on λ and n . The beam is designed as four L-shaped beams suspending a centred plate to realise out-plane translational motion for optical detection. The centred plate, acting as a reflector, is suspended above two adjacent gratings patterned inside and outside the groove. The L-shaped beams work as sensing beams which will deflect under external stimulates.

The chips were fabricated by using the surface micromachining process, as shown in Fig. 2. PI was used as the beam structure material. First, grooves with a depth of 55 nm were etched on a glass substrate (Fig. 2a). Next, 10 nm/50 nm/10 nm Ti/Au/Ti gratings were patterned inside and outside the grooves by lift-off (Fig. 2b). Then, a 5 μm -thick Al sacrificial layer was deposited and patterned (Fig. 2c). Afterwards, a 2 μm PI layer was spin-coated, patterned and then cured at 250°C to form the beams (Fig. 2d). Then, the microbeams were released in phosphoric acid solution (Fig. 2e). Finally, a 5 nm/10 nm thin Cr/Au layer was deposited on the top surface of the polymer microbeams as a

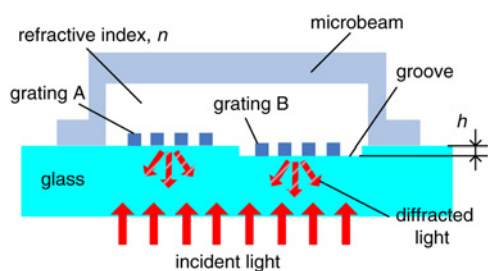


Figure 1 Schematic diagram of an integrated dual-grating microbeam

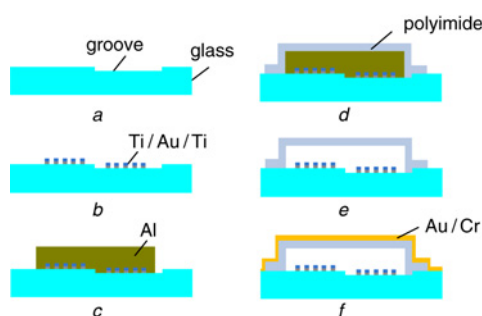


Figure 2 Fabrication process flow

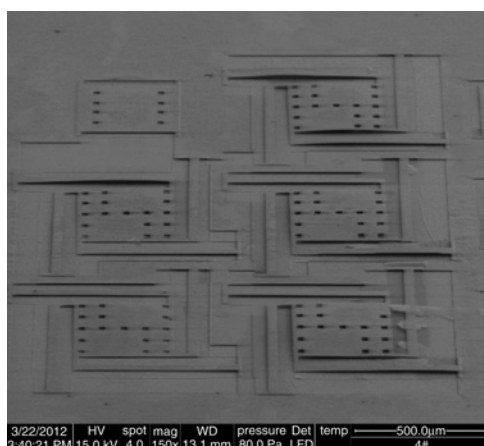


Figure 3 SEM picture of fabricated microbeams

substrate to anchor successive layers for immobilisation of probe molecules (Fig. 2f). Fig. 3 shows a scanning electron microscopy (SEM) picture of fabricated microbeams. The size of a sensing beam and the centred plate were designed to be $500 \times 40 \mu\text{m}$ and $370 \times 370 \mu\text{m}$, respectively. The dimensions of the plate are dependent on the size of the gratings which are designed to be $150 \times 150 \mu\text{m}$.

3. Experiments: Fig. 4a shows the schematic diagram of the optical measurement setup with a CCD. To carry out experiments in liquid, the chip was placed in a fluid cavity with an inlet connected to a syringe and an outlet. A collimated and expanded laser beam with a wavelength of 633 nm illuminated from the backside of the chip, and an image of light spots of the first diffraction light from the integrated gratings was recorded with a 12-bit CCD, as shown in Fig. 4b. The intensity changes of a pair of gratings corresponding to a microbeam were obtained from the recorded images. The optical intensity signals corresponding to a beam were divided into sequential sections where interferometric intensities of the two gratings were chosen alternately to avoid

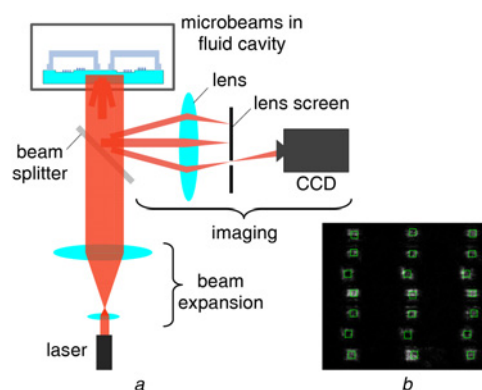


Figure 4 Schematic diagram of optical measurement setup with a CCD, and CCD image of light spot array
a Schematic diagram
b CCD image

the insensitive ranges, so that the range of displacement measurement was extended [20].

To compare the measurement noises with polymer and metal beams in liquid and in air, optical intensities of diffraction light spots were detected in static condition in air and liquid with metal beams and polymer beams, respectively. For measurement in liquid, a chip fixed in the fluid cavity was immersed in solution and then used for further experiments after stabilisation. First, integrated grating Al microcantilever array, which were fabricated by using a photoresist sacrificial layer [20], were used for noise detection. Fig. 5 shows the optical intensities of the diffracted light spots corresponding to an integrated grating Al cantilever array in air and in ethanol. The averaged relative standard deviations of the grey values in air and in ethanol were 0.43 and 0.74%, respectively. The maximum relative standard deviations were 0.59 and 1.00%, respectively. The measured noises with Al microcantilevers in ethanol were slightly larger than those in air, and this may be induced by the movement of a cantilever under the influence of Brownian motion in liquid and refractive index change of the liquid caused by laser-induced temperature change.

Afterwards, the noises of the integrated grating PI microbeam array were detected in phosphate-buffered saline (PBS) (pH = 7.4). Fig. 6 shows the optical intensities of the diffracted light spots with the integrated grating PI microbeams in PBS. Excluding the spot with significant drift shown in Fig. 6, which may be because of fine interference fringes in the edge area of the expanded laser beam, the averaged relative standard deviation of the grey values was 3.68% and the maximum relative standard deviation was 5.14%. The results indicated that the noise with the PI microbeams in PBS was three times larger than that with Al cantilevers in ethanol. This may be induced by the bad optical quality of the reflecting surface on PI microbeams and the interference fringes in each spot caused by the bending of the moving plates

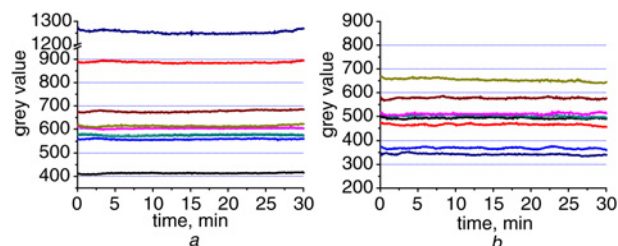


Figure 5 Optical intensities of diffracted light spots corresponding to integrated grating Al cantilevers
a In air
b In ethanol

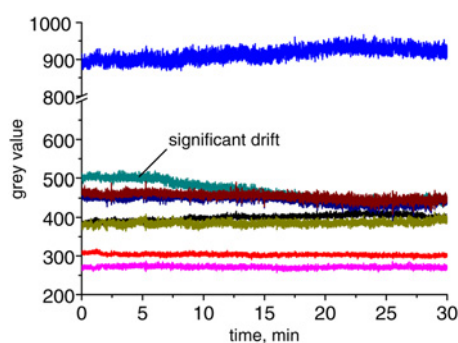


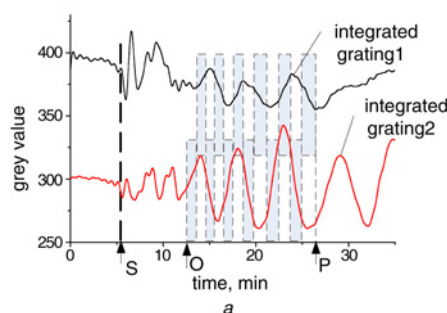
Figure 6 Optical intensities of diffracted light spots corresponding to integrated grating PI microbeams in PBS

with internal stress as shown in Fig. 3. It should be improved by reducing the roughness of the metallic sacrificial layer, placing the Au reflective film under the polymer layer and reducing the internal stress of the beams.

4. Results and discussion

4.1. Displacement detection in ethanol: PI swells in water and contracts when ethanol molecules expel water molecules [24]. To test the measurement method in a liquid environment, we detected the displacement changes of Au-coated PI microbeams caused by replacing water with ethanol. The centred plates translationally move in the out-plane direction because of the bending of the sensing beams when the polymer contracts by ethanol molecules expelling water molecules, but the Au layer does not change. In the experiment, the chip was fixed in the fluid cavity and immersed in the phosphate buffer solution first. After about 5 min when the beams stabilised, ethanol was injected and the intensity response of diffracted light spots was recorded.

To eliminate the high-frequency optical intensity noise from the mechanical motion signals with slow changes, the measured data were processed with a smoothing filter of fast Fourier transform (FFT). Fig. 7a shows the FFT filtered optical intensity changes of the two light spots corresponding to the two gratings under a moving plate when ethanol is injected and replaces the phosphate buffer solution. The microbeam responds immediately to the injection at point S, caused by the fluidic disturbance, and then deflects slowly because of absorption of ethanol. The intensity signals from point O to point P were divided into 12 marked sections, in which the signals were chosen from grating 1 or 2. The sectional calculated results of displacement of the plate and the accumulated results are shown in Fig. 7b. A displacement of about 850 nm was recorded. The preliminary experimental results show that the extended range displacement measurement method with the



integrated dual-grating PI microbeams is available in the liquid environment. According to the measured phase difference of the signals from the two gratings inside and outside the groove, we conclude that the distance between the plate and the substrate has a variance with the design. The measured phase difference of two signals was about 1.65 rad, and the calculated depth of grooves was about $1.65/(\pi/2) \times (\lambda/n_{\text{eth}})/8 = 62 \text{ nm}$ (n_{eth} is the refractive index of ethanol, $n_{\text{eth}} = 1.34$). The contrast of the output optical signals was low, which resulted in low sensitivity. This may be because of the mismatch of reflectivity of the grating reflector and the moving reflector, which should be improved by placing the Au reflective film under the polymer layer and reducing the roughness of the metallic sacrificial layer.

4.2. Detection of antibody–antigen binding: For the antibody–antigen binding detection experiment, a PI microbeam array chip was cleaned by oxygen plasma for 2 min and then immersed in 11-Mercap to undecanoic acid (11-MUA, 1 mM, prepared with ethanol) solution for 24 h as soon as possible, so that thiol molecules, as a successive layer, were bound well to the Au surface. Afterwards, rabbit immunoglobulin G (IgG) molecules were immobilised as probe molecules by incubating for 30 min at 35°C in a rabbit IgG solution (1 mg/ml) prepared with PBS (pH = 7.4). After that, all the non-specific binding sites left were blocked by incubating for 30 min at 35°C in bovine serum albumin (BSA, 10 mg/ml). For the measurement experiment, the functionalised chip was fixed in the fluid cavity and immersed in PBS. After about 2 min when the beams became stable, 100 µg/ml target goat-anti-rabbit IgG solution (in PBS) was injected and the intensity changes of diffracted light spots corresponding to the beam deflection were recorded.

Fig. 8 shows the optical output responses from the two gratings corresponding to an IgG immobilised microbeam when 100 µg/ml

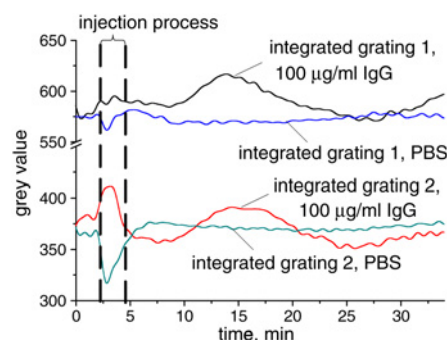


Figure 8 Optical output responses from the two gratings corresponding to a microbeam immobilised with rabbit IgG when injecting 100 µg/ml goat anti-rabbit IgG solution and phosphate buffer solution

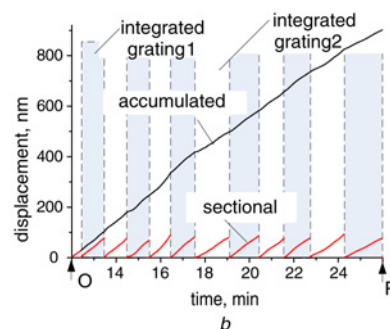


Figure 7 Optical output responses of two light spots corresponding to two gratings under a moving plate when phosphate buffer solution replaced by ethanol (after FFT filtering), and calculated results of displacement of plate from point O

a Optical output responses
b Calculated results

goat anti-rabbit IgG solution and PBS are injected, respectively. There were obvious disturbances during the injection process, and then the optical intensities changed periodically in the 100 µg/ml goat anti-rabbit IgG solution while they came back to equilibrium soon in PBS. The antibody–antigen binding process was still progressing after 30 min. As the phase difference of the two signals was very small, the measurement with extended range could not be used. During the measurement, the intensity change of each grating was about 1.5 periods, corresponding to about 350 nm displacement in 100 µg/ml goat anti-rabbit IgG solution. That the phase difference of the measured signals of the dual gratings departed far from $\pi/2$ may result from the inclination of the centred plate because of the asymmetry of internal stress or stress caused by antibody–antigen binding on the four sensing beams.

4. Conclusion: A polymeric microbeam array integrated with dual gratings was designed and fabricated for bio-chemical sensing in a liquid environment. The displacement of microbeams was measured by alternately using two quadrature phase-shifted interferometric signals obtained by a CCD image measurement setup. Noise analysis results show that the noise of Al microcantilevers in ethanol is slightly larger than that in air, and that of PI microbeams in PBS is three times larger than that of Al cantilevers in ethanol. The displacement measurement method with extended detection range based on integrated dual-grating proved to be feasible in a liquid environment by preliminary experiments with Au coated PI microbeams in ethanol. The measured displacement was 850 nm. The detection experiment of antibody–antigen binding was conducted and the measured displacement was 350 nm. In future work, the fabrication process should be modified to improve the optical quality of the reflector and reduce internal stress within the microbeams.

5. Acknowledgment: This work was supported by the National Natural Science Foundation of China (91023027).

6 References

- [1] Boisen A., Dohn S., Keller S.: ‘Cantilever-like micromechanical sensors’, *Rep. Prog. Phys.*, 2011, **74**, pp. 036101–036130
- [2] Lavrik N.V., Sepaniak M.J., Datskos P.G.: ‘Cantilever transducers as a platform for chemical and biological sensors’, *Rev. Sci. Instrum.*, 2004, **75**, pp. 2229–2253
- [3] Goeders K.M., Colton J.S., Bottomley L.A.: ‘Microcantilevers: sensing chemical interactions via mechanical motion’, *Chem. Rev.*, 2008, **108**, pp. 522–542
- [4] Fritz J., Baller M.K., Lang H.P., *ET AL.*: ‘Translating biomolecular recognition into nanomechanics’, *Science*, 2000, **288**, pp. 316–318
- [5] Berger R., Delamarche W., Lang H.P.: ‘Surface stress in the self-assembly of alkanethiols on gold’, *Science*, 1997, **276**, pp. 2021–2024
- [6] Li S.Q., Fu L.L., Barbaree J.M., *ET AL.*: ‘Resonance behavior of magnetostrictive micro/milli-cantilever and its application as a biosensor’, *Sens. Actuators B, Chem.*, 2009, **137**, pp. 692–699
- [7] Ilic B., Yang Y., Craighead H.G.: ‘Virus detection using nanoelectromechanical devices’, *Appl. Phys. Lett.*, 2004, **85**, pp. 2604–2606
- [8] Alvarez M., Lechuga L.M.: ‘Microcantilever-based platforms as bio-sensing tools’, *Analyst*, 2010, **135**, pp. 827–836
- [9] Boisen A., Thaysen J., Jensenius H., *ET AL.*: ‘Environmental sensors based on micromachined cantilevers with integrated read-out’, *Ultramicroscopy*, 2000, **82**, pp. 11–16
- [10] Wee K.W., Kang G.Y., Park J., *ET AL.*: ‘Novel electrical detection of label-free disease marker proteins using piezoresistive self-sensing micro-cantilevers’, *Biosens. Bioelectron.*, 2005, **20**, pp. 1932–1938
- [11] Yang S.M., Chang C.: ‘A piezoresistive bridge-microcantilever biosensor by CMOS process for surface stress measurement’, *Sens. Actuators B, Chem.*, 2010, **145**, pp. 405–410
- [12] Shekhawat G., Tark S.H., Dravid V.P.: ‘MOSFET-embedded micro-cantilevers for measuring deflection in biomolecular sensors’, *Science*, 2006, **311**, pp. 1592–1595
- [13] Arntz Y., Seelig J.D., Lang H.P., *ET AL.*: ‘Label-free protein assay based on a nanomechanical cantilever array’, *Nanotechnology*, 2003, **14**, pp. 86–90
- [14] Yue M., Stachowiak J.C., Lin H., *ET AL.*: ‘Label-free protein recognition two-dimensional array using nanomechanical sensors’, *Nano Lett.*, 2008, **8**, pp. 520–524
- [15] Koev S.T., Bentley W.E., Ghodssi R.: ‘Interferometric readout of multiple cantilever sensors in liquid samples’, *Sens. Actuators B, Chem.*, 2010, **146**, pp. 245–252
- [16] Lee W., Hall N.A., Zhou Z.P., *ET AL.*: ‘Fabrication and characterization of a micromachined acoustic sensor with integrated optical readout’, *IEEE J. Sel. Top. Quantum Electron.*, 2004, **10**, pp. 643–651
- [17] Karhade O.G., Degertekin F.L., Kurfess T.R., Micromech J.: ‘SOI-based micro scanning grating interferometers: device characterization, control and demonstration of parallel operation’, *Microengineering*, 2008, **18**, p. 045007
- [18] Shin D., Kin B., Micromech J.: ‘A laser interferometer encoder with two micromachined gratings generating phase-shifted quadrature’, *Microengineering*, 2011, **21**, pp. 085036–1–9
- [19] Van Gorp B., Onaran A.G., Degertekin F.L.: ‘Integrated dual grating method for extended range interferometric displacement detection in probe microscopy’, *Appl. Phys. Lett.*, 2007, **91**, p. 083101
- [20] Feng J., Ye X., Chen F., *ET AL.*: ‘Interferometric displacement measurement of microcantilevers based on integrated dual gratings’, *Opt. Precision Eng.*, 2012, **20**, pp. 1747–1753 (in Chinese)
- [21] Johansson A., Calleja M., Rasmussen P.A., Boisen A.: ‘SU-8 cantilever sensor system with integrated readout’, *Sens. Actuators A*, 2005, **123–124**, pp. 111–115
- [22] Katragadda R., Wang Z., Khalid W., *ET AL.*: ‘Parylene cantilevers integrated with polycrystalline silicon piezoresistors for surface stress sensing’, *Appl. Phys. Lett.*, 2007, **91**, p. 083505
- [23] Ibbotson R.H., Dunn R.J., Djakov V., *ET AL.*: ‘Polyimide microcantilever surface stress sensor using low-cost, rapidly-interchangeable, spring-loaded microprobe connections’, *Microelectron. Eng.*, 2008, **85**, pp. 1314–1317
- [24] Boisen A., Thaysen J., Jensenius H., Hansen O.: ‘Environmental sensors based on micromachined cantilevers with integrated read-out’, *Ultramicroscopy*, 2000, **82**, pp. 11–16