Chip-Scale Universal Detection Based on Backscatter Interferometry

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An on-chip detector based on backscatter interferometry has been developed to perform subnanoliter-volume refractive index measurements. The detection system consists of a simple, folded optical train based on the interaction of a laser beam and an etched channel, consisting of two radii joined by a flat portion, thus defining a curved surface in the shape of a hemisphere in a silica (glass) plate. The backscattered light from the channel takes on the form of a high-contrast interference pattern that contains information related to the bulk properties of the fluid contained within the probe volume. Positional changes of the interference pattern (fringes) allow for the determination of standard absorbance determinations. With the advent of lasers and their unique properties, laser-induced fluorescence (LIF) can provide extremely low detection limits, with most laboratories able to detect as few as 10\(^3\) molecules and with recent developments allowing single-molecule detection to be performed “on-chip”. However, not all molecules fluoresce at available laser lines and therefore chemical derivatization steps are often necessary.

Other approaches to on-chip detection have included thermal conductivity, electroluminescence, electrochemical methods, and even mass spectrometry. While strides have been made toward developing broadly applicable detection methodologies for chip-scale analyses, a need still exists for a simple, inexpensive, sensitive, universal, chip-based detector. Refractive index (RI) detection, a common technique used in chemical and biochemical analysis, could potentially fill this void and has been successfully applied to several small-volume analysis schemes including universal detection in \(\mu\)HPLC, CE, and nanoliter-volume temperature measurements. For various reasons, RI detection represents an attractive alternative to fluorescence and absorbance, even with chip-scale systems. These include simplicity, compatibility with a wide range of buffer systems, universality (theoretically allowing detection of any solute), making it particularly applicable to solutes with poor absorption or fluorescence properties, and analytically useful sensitivity.

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The most promising RI schemes for on-chip detection involve the use of either waveguiding or interferometry and are in great part a result of technical advances in lasers and micro-optics. Among the RI techniques that have been applicable to chip-scale detection are the Mach–Zender approach, the porous silicon-based optical interferometer, the surface plasmon resonance (and related) techniques, the "on-chip" spiral-shaped waveguide refractometer, and the holographic forward scatter interferometer. While each of the aforementioned RI measurement techniques can produce impressive results, they all have limitations when applied to on-chip separations. In general, the path length dependency of surface-base techniques, such as SPR or the Mach–Zender interferometer, demand detection volumes incompatible with separation techniques such as CE. Thus far, the most promising approach for on-chip universal or RI detection with flowing stream analysis, such as CE, uses a holographic grating and a forward scattering optical configuration. In this work, it was recently shown that microchannel CE and RI detection could be used to rapidly separate and detect 33 mM sucrose, N-acetylglucosamine, and raffinose with reported detection limits in the 10 mM range for carbohydrates. While this work is impressive, showing the potential for doing on-chip RI sensing, the performance of the forward scatter technique employed is inherently limited because the probe beam passes only once through the detection channel. In previous nonchip investigations, it has been shown that backscatter interferometry can circumvent some of the drawbacks of "standard" interferometric or forward scatter methods. In the microinterferometric backscatter detector (MIBD), a "capillary" (cylindrical object) is illuminated with parallel coherent rays producing an interference pattern in the backscatter direction, and due to a multipass optical configuration, high-sensitivity RI measurements have been possible in picoliter detection volumes. Here we show that similar on-chip RI detection can be performed using a simple optical train consisting of an etched microchannel (hemisphere), a He/Ne laser or a diode laser, and a photodetector and that the on-chip interferometric backscatter detection (OCIBD) can be used to detect solutes at the micro- to the sub-mM level (139 fmol/12.8 pg) in just 188 pl. The response of the detector to changes in RI is linear (>2 decades in concentration) and highly reproducible. Likewise, a functional model of OCIBD will be put forth, allowing a detailed comparison of theoretical and experimental data for the optical system. In this paper, it will be demonstrated that backscattering interferometry can be used for on-chip refractive index detection.

**EXPERIMENTAL SECTION**

The block diagram for the OCIBD is shown in Figure 1. A low-power He/Ne laser (Melles Griot) or an intensity-stabilized diode laser (633 nm, µLaser Systems), acting as the illumination source, was mounted on Al support brackets that were bolted to an optical bench. The laser beam was directed toward a mirror positioned 55 cm from the laser's aperture. The mirror was angled at 45° with respect to the laser beam in order to direct the beam toward the etched substrate mounted on the front surface of an aluminum support affixed to an X–Y translation stage. Upon striking the channel, the laser beam produced an interference pattern (or an array of scattered light) that was monitored using a solid-state detector. The voltage output from the photosensor was digitized using an analog-to-digital converter (PPIO-AIO8, CyberResearch, Branford, CT) that was interfaced to a PC computer.

Detection of fringe movement was accomplished using either a slit/photodetector assembly or a silicon avalanche photodiode. The slit/photodetector assembly consisted of a pin photodiode integrated with a 632.8-nm interference filter (Coherent-Ealing) wired with a simple current to voltage circuit. A 50-µm precision air slit (Melles Griot) was mounted vertically in the center of the active surface area of the photodetector. The slit/photodetector assembly was housed in a 6 cm × 2.5 cm × 2 cm aluminum dye cast box (ITT Pomona Electronics, Pomona, CA) and securely mounted to a translation stage. The voltage output from the photodiode was then amplified (gain, 100) by a low-noise preamplifier (Stanford Research Systems) using a 30-Hz low-pass filter (12 dB/ octave). The diameter of the avalanche photodiode (APD) manufactured by Advanced Photonix, Inc. (Camarillo, CA) was 0.3 mm. The responsivity (R) of the APD was 30 A/W at 632.8 nm. The APD was wired in the reversed bias configuration in series with a 15-MΩ resistor and powered by a 213-V power supply, which was 2 V below breakdown. Due to the small diameter of the active surface area of the detector, no precision air slit was used to mask the detector. The APD was securely housed in an aluminum die cast box (Pomona Electronics) that was mounted on a high-precision translation stage. The analog signals from both detectors were digitized with an external DAQ board (PPIO-AIO8, CyberResearch) and displayed on a PC computer running a digital stripchart recorder (Labtech For Windows).

All chemicals were reagent grade or better. Distilled/deionized water was used as the blank solution.
RESULTS AND DISCUSSION

Experimental Investigations. Figure 2A shows a top view of the silica chip that contains a serpentine etched channel with a radius of 50 \( \mu \)m. At each end of the channel, holes are laser drilled to allow for introduction and removal of sample from the system. The unaltered laser beam is allowed to impinge the etched channel of the chip at a distance that is \( 1 \) mm from the exit port and is graphically shown in Figure 2A. A cross-sectional side view of the etched channel is shown in Figure 2B. The chip consists of two substrate pieces that are fused together, with the channel itself taking on the form of a hemisphere (half of a capillary) having two 50-\( \mu \)m radii connected by a 10-\( \mu \)m flat section. A cartoon of this feature is shown in an end-on view in Figure 2C.

Surprisingly, a hemispherical surface etched in a chip produces a unique multipass optical configuration when an unfocused laser beam interacts with the curved structure, even when the surface has a flat section (due to the etching process) between the radii at the apex of the hemisphere. A typical interference pattern produced by an unmodified chip filled with distilled/deionized water is shown in the false color intensity profile (black is no photons and white is detector saturation) shown in Figure 3. Even though the channel has the general shape of a hemisphere or half-circle containing a flat section, relatively high contrast interference fringes, somewhat similar to those seen with MIBD in full capillaries, are observed. Drawing on our previous research with the MIBD,\(^{19,21-24,36}\) it was postulated that positional changes in the backscatter fringe pattern would be observed upon changing the refractive index of the fluid contained within the probe volume. Figure 3 illustrates this response. As shown in the figure, the position of the backscatter fringes shift significantly (191 \( \mu \)m/mRIU) as the refractive index for the fluid in the channel is changed (water to 100 mM glycerol). This is the case even for a truly noncylindrical structure that consists of two radii separated by a flat region.

These observations are important because (1) the features on the chip that produce the interference fringes are quite common and easy to manufacture, (2) no additional optics are needed, and (3) the fringes are very high contrast, implying interferometry with high finesse\(^{24}\) which should lead to sensitive detection of optical path length changes in the channel.

The simplest way to quantitatively evaluate the refractive index sensitivity of on-chip interferometric backscatter is with a slit/photodetector assembly that measures shifts in interference fringe position. In this configuration, the slit/photodetector assembly was located on the order of 28 cm from the front surface of the chip and was aligned on the edge of a fringe so that fringe movement produces an intensity change. As the fringe shifts, a small voltage output from the photodetector is observed, which is linearly proportional to a change in refractive index (\( \Delta n \)). Locating the detector assembly on the edge of the sloping intensity gradient of the working fringe, at \( I = 1/e^2 \) of the essentially Gaussian intensity distribution, facilitates the maximization of sensitivity and dynamic range.

The evaluation of the refractive index sensitivity of OCIBD began with the generation of a calibration curve using a He/Ne laser and the slit/photodetector assembly under static conditions. To generate this calibration curve, a series of glycerol solutions ranging in concentration from 0 to 100 mM were prepared in distilled/deionized water, filtered with a 0.4-\( \mu \)m filter, and introduced sequentially from lowest to highest concentrations. The calibration curve resulting from this experiment is linear over 2 decades (\( r^2 = 0.999 \)) and is highly reproducible (STD = \( \pm 0.04 \) V). These initial experiments using a slit/photodetector for OCIBD yield a 3\( \sigma \) detection limit of 18.33 mM (57.6 pmol/ 5.3 ng) in a
probe volume of 3.14 nL, which corresponds to a \( \Delta n \) of 2.39 \times 10^{-4} \text{ RIU}.

These preliminary results suggest that OCIBD is an analytically useful technique; however, the need to improve the S/N of the measurement is clear. In comparison to MIBD, in on-chip interferometric backscatter, less light is contained within each fringe (probably due to the substrate shape not being a full cylinder and scattering of light by the cover plate). Therefore, a more sensitive photosensing method, such as an avalanche photodiode, should improve performance. Thus, the slit/photodetector assembly was replaced with a small-area (0.3 mm diameter) avalanche photodiode. The APD was aligned on the edge of the interrogated fringe (third from the centroid) and in a procedure similar to that used with the slit/photodetector assembly, a calibration curve of intensity versus glycerol concentration was generated. Positional changes in the backscatter fringe pattern were again quantifiable as intensity changes and linear with variations in solute concentration (refractive index). The pattern were again quantifiable as intensity changes and linear on the detector assembly was replaced with a small-area (0.3 mm diameter) avalanche photodiode. The APD was aligned on the backscatter fringe, less light is contained within each measurement is clear. In comparison to MIBD, in on-chip interference (with variations in RI), the detection limit was determined to be 742 \text{ \mu M}, which corresponds to a mass detection limit of 139 \times 10^{-15} \text{ mol} (139 fmol) or 12.8 \times 10^{-12} (12.8 pg). So at the detection limit, a \( \Delta n \) of 9.67 \times 10^{-6} \text{ RIU} is detectable at the 99.9% confidence level in only 188 pL. In other words, by using the diode laser and the new chip, the detection limit of the system was improved by a factor of 5.5 (4.1 \text{ mM} to 742 \text{ \mu M}) while the probe volume was reduced 16.7-fold (3.14 nL to 188 pL).

A few important points about these improved detection limits should be made at this juncture. First, lower detection limits were achievable in large part due to the intensity-stabilizing correction circuit of the diode laser, which allows a reduction in the predominant noise in the system, intensity instabilities of the probe source. No such intensity correction feedback loop was employed for the He/Ne laser, which are known to have relatively large intensity fluctuations. Second, the chip used for the last measurements was prepared with greater care (had fewer scratches in the substrate surface and nonuniformities in the glass plate), producing a higher definition fringe pattern (higher finess) and less unwanted scattered light. Finally, while there is a desire to improve on the detection limits of 742 \text{ \mu M} (139 fmol/12.8 pg), this concentration range does provide analytically useful sensitivity (68.3 ppm).

**Ray Tracing and Performance Modeling.** One of the intriguing aspects of applying backscattering interferometry backscatter for detection in chip-scale analyses is that, unlike capillaries, etched channels have two curved surfaces and a flat region between them. A capillary may be viewed as a geometrical figure consisting of four bent surfaces: two forming an inner cylinder and two forming the outer cylinder. Each of these surfaces contributes to ray bending, reflecting, refracting, and a general deviation from a straight-line propagation of light across a RI interface (Figure 5). The combined result of these unique interactions is a multiple-pass optical configuration whereby the light interacts multiple times for a fixed volume defined by the beam and the capillary. Yet the cross section of a typical etched channel contains four optical interfaces (surfaces), of which only one is curved. This curved surface forces the incoming rays to deviate from their initial direction of propagation while the flat

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**Figure 4.** Calibration curve of the refractive index response detected by the avalanche photodiode for a series of glycerol solutions using a 75-\mu m-diameter probe beam.

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surfaces produce a lateral displacement of the rays with the "direction" of propagation maintained.

It has been shown above that a chip with only one refracting surface is capable of producing a set of high-contrast fringes (Figure 3) that move with optical path length changes. But does this optical system give a multipass advantage and what parameters influence system performance? To begin to answer these questions, an optical model for OCIBD was constructed using the sophisticated optical software program, ASAP 6.6 (Breault Research Organization, Inc.). An etched channel in fused silica (RI = 1.45702) was created and covered with a flat slide having the same refractive index. The local coordinate system was oriented in such a way that the x axis coincides with the channel's central axis and the chip itself is perpendicular to the z axis. A coherent light source emitting light in the -Z direction was centered on the z axis at some +Z distance away from the chip. Since the emitted light consists of parallel rays and the source itself does not possess any physical properties other than wavelength, the backscattered light traveling in the +Z direction passes "through" the source unperturbed (e.g., the actual position of the source is irrelevant). The detector plane was placed 20 cm away from the chip and perpendicular to the z axis. A convenient aspect of the model is that all parameters of the system such as channel radius, chip thickness, detector plane location, wavelength, number of emitted rays, etc., can be changed to accommodate particular experimental settings.

Using the model, a series of preliminary theoretical investigations were performed. To mimic the experimental setup, a 100-μm channel was created and illuminated, from the curved surface side, with a 632.8-nm light source consisting of nine initial base rays (Figure 6). Illumination from this side was chosen as a result of chip manufacturing constraints. Each base ray was accompanied by eight parabasal rays and allowed no more than three splits at each optical interface. The middle plane of the chip simulates a boundary where the cover slide and the channel containing substrate are fused together. These surfaces are assumed to be of the same refractive index as fused silica. Since the index of refraction is unchanged at the boundary interface, its presence does not affect the rays passing through. The rays that exit the chip on the side opposite of illumination do not contribute to the formation of the signal in the backscatter direction and are dropped completely from the simulation. A typical theoretical fringe pattern is shown in Figure 7. It can be seen that the shape, position, and relative intensity of the fringes are qualitatively similar to those observed experimentally (Figure 3). For comparison to the experimentally observed response, a calibration curve of fringe movement versus glycerol concentration was created using the calculated refractive index of the glycerol solutions employed. The results from these calculations are shown in Figure 8. The model produces a linear response of the fringe

<table>
<thead>
<tr>
<th>laser</th>
<th>beam diameter</th>
<th>channel radius (μm)</th>
<th>detection volume (L)</th>
<th>detector</th>
<th>concn DL (M)</th>
<th>mass DL (mol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>He/Ne</td>
<td>0.8 mm</td>
<td>50</td>
<td>3.14 × 10^{-9}</td>
<td>slit/photodetector</td>
<td>18.3 × 10^{-3}</td>
<td>57.6 × 10^{-12}</td>
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<tr>
<td>He/Ne</td>
<td>0.8 mm</td>
<td>50</td>
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<td>APD</td>
<td>4.10 × 10^{-3}</td>
<td>12.9 × 10^{-12}</td>
</tr>
<tr>
<td>diode laser</td>
<td>75 μm</td>
<td>40</td>
<td>188 × 10^{-12}</td>
<td>APD</td>
<td>742 × 10^{-6}</td>
<td>139 × 10^{-12}</td>
</tr>
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Figure 5. Cross-sectional view of the optical ray trace model for MIBD using a diode laser with a 250-μm inner diameter, 350-μm outer diameter capillary, and a 20-μm thick polyimide coating. Only three selected rays with three splits are shown.

Figure 6. Cross-sectional view of the optical ray trace model for the OCIBD. He/Ne laser impinges directly onto the 100-μm etched channel. Only a few significant rays are shown for illustration purposes.

Figure 7. Theoretically generated profile of typical backscattered fringe pattern observed at 20 cm away from the chip.
position with respect to changes in refractive index and is similar to that observed experimentally.

Using the theoretical model, further investigations on the fundamental limits such as optimization of the optical train and evaluation of various physical configuration parameters for OCIBD were undertaken. For example, Figure 9 shows the theoretical model can be used to predict how the ratio of the cover plate to channel substrate thickness influences overall fringe spacing and fringe shift (sensitivity). Qualitatively, these simulations predict that the width of individual fringes is dependent upon the thickness of the cover slide and as the cover plate to channel substrate thickness ratio increases the spatial frequency of the fringe pattern increases. Furthermore, the fringe pattern almost completely extinguishes when the cover slide thickness becomes small or the ratio of the cover slide to the substrate thickness is <0.01. A more quantitative and extremely valuable investigation of OCIBD can be performed using these theoretical chip structures. Here calibration curves of refractive index sensitivity were generated for each chip (thickness ratios of 0.2, 0.5, 1.0, and 2.0). Then the slope for each of these response functions was plotted at the particular thickness ratio (Figure 10). The solid curve represents the log-normal fit to the data and shows that the best performance with respect to refractive index sensitivity is predicted when the cover plate is approximately half the thickness of the substrate containing the channel. Chips with these physical parameters are currently being manufactured to verify these theoretical predictions, and expanded theoretical investigations are currently underway. These studies should lend insights regarding how OCIBD performance is influenced by substrate material (for example, plastics), channel radius, substrate thickness, and excitation wavelength.

CONCLUSION

It has been demonstrated theoretically and experimentally that refractive index measurements can be performed directly on-chip using the simple optical configuration based on backscatter interferometry. A theoretical model of OCIBD was constructed and compares well with experimental observations. As shown in Table 1, detection limits range from 57.6 pmol to 139 fmol within low nanoliter (3.14 nL) to picoliter (188 pL) probe volumes. Since the technique is based on measurements of optical path length changes, the response should be universal, making OCIBD applicable to many nonabsorbing or nonfluorescing solutes. Even so, it is certain that efforts underway will lead to improved sensitivity for on-chip RI detection. Finally, considering the simplicity of this detection scheme, applicability to separation techniques such as capillary electrophoresis appears quite tractable and encouraging since initial results to this end have been demonstrated.38

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