Near-IR micro-fluidic sensing by total internal reflective diffraction

Y Sarov¹,², V Sarova¹, I Capek³ and I Rangelow¹

¹ Department of Micro- and Nano-electronic Systems, IMNE, FEI, Ilmenau University of Technology, PF 100565, 98684 Ilmenau, Germany
² Institute of Optical Material and Technologies, Acad G. Bonchev Str., Bl. 109, 1113 Sofia, Bulgaria
³ Polymer Institute, Institute of Measurement Science, Slovak Academy of Sciences, Dubravska cesta 9, Bratislava, Slovakia

E-mail: ysarov@yahoo.com, yanko.sarov@tu-ilmenau.de

Abstract. This work studies how the sensitivity of a recently reported novel integrated micro-fluidic system [1] can be improved optimizing the conditions of measurements. The considered lab-on-a-chip system is based on a diffraction grating, operating under total internal reflection and illuminated by near-IR light (1.55 μm). The interaction between the evanescent field of the totally reflected light with dipoles of the analyte results in phase and amplitude changes in the reflected diffraction orders. In this work the dependence of the sensing signal on the measured diffraction order, polarization of the light and angle of incidence are investigated. The conditions giving highest sensitivity are determined. The feasibility of the integrated micro-fluidic system for analysis of nano-dispersions is demonstrated by samples of oil-based dispersion of composite CuFe₂O₄ nanoparticles. A resolution toward the CuFe₂O₄ concentration of 0.1 wt.% is obtained. The possibilities for further increase of the sensitivity are discussed.

1. Introduction
The fast development in the modern field of micro-chemical and microbiological instrumentation is motivated mainly by two reasons. The first stimulus is related with the broad applications of the micro-fluidic on-chip systems in the biotechnology and bio-analysis, process control, environmental and medical sciences [1]. Moreover, the micro-reactors and micro-sensors benefit of the times of magnitude quicker processing and lower consumption of samples [1-2]. Second, the micro-fluidic transporting part can be easily and cheap integrated into the microchip applying the standard, parallel microstructuring and microfabrication [2]. A complete fabrication of integrated lab-on-a chip microsystems gives a requirement that the sensor part to be also capable of being integrated on the chip and its fabrication to be compatible with the standard micro-fabrication technologies. An optical analysis of micro-fluidic samples is especially interesting in the near-infrared (NIR) range as a result of the absorption peculiarities of the bio-substances due to the organic absorption bands of the infrared (IR) region.

Very recently, a novel integrated micro-optical system for micro-fluidic analysis has been reported [3-4]. Its principle of operation is based on the diffraction from a grating under Total Internal Reflection.
Reflection (TIR) [5-7]. Later, the integrated sensor system has been modified and applied for near-IR micro-fluidic sensing and analysis [8].

This work presents the further optical investigation of the integrated IR micro-optical sensor and its application for micro- and nano-fluidic diagnostics and analysis. The sensor is calibrated with standard water solutions of sucrose, which absorption coefficient changes linear with the concentration at that spectral region. The dependence of the sensitivity of the detector on the angle of incidence and the polarization of the light is studied for the both 0th and the 1st diffraction orders. The possibilities for increasing of the sensitivity are discussed. The potential applications of integrated IR system for micro-fluidic investigation and concentration analysis are demonstrated in case of oil-based dispersions of composite CuFe$_2$O$_4$ nanoparticles with size below 10nm.

2. Principle of sensing and optical setup

Figure 1 presents the optical setup and explains the principle of operation of the integrated IR micro-fluidic sensor. The NIR light source is a diode laser with $\lambda=1550$nm (1). Its light is coupled with a visibly beam from another diode laser (2) by a beam-splitter (4), which allows easy optical adjustment. The direction of the light polarization is chosen by the rotator of the polarization (3). The incident IR beam is directed to the prism facet in such a way, that the beam inside the prism (5) fulfills the TIR condition for the interface between the micro-prism (5) and the fluid in the micro-channel (7). On this interface a metal diffraction grating (6) is deposited.

Figure 1. Near IR micro-fluidic sensor chip employing total internal reflection of light: 1) NIL diode laser, 2) visible diode laser, 3) rotator of the polarization, 4) beam-splitter, 5) silicon micro-prism, 6) diffraction grating, 7) microfluidic channel, 8) photodetectors.

The fluidic sensing is realized with the help of the evanescent electromagnetic field, which penetrates in the investigating medium from the spacing between the lines of the grid. The conversion of the harmonic waves into the evanescent TIR field at the two media interface, caused by the dielectric discontinuity of the media, is influenced by the optical constants of the micro-fluid. When the micro-channel is filled with absorbing fluid having a complex refractive $n_2 = n_2 (1 + i\kappa)$, an effect of frustrated TIR occurs, i.e. the light reflects particularly due to the absorption of the evanescent TIR field. The reflection amplitude of the TIR $r$ is given by:

$$r_{s, p}^{s, p} = 1 - 2n_2 \kappa d_{s, p}^{s, p} / \lambda$$

(1)

where $n_2$ and $\kappa$ are the indices of refraction and absorption, respectively, $\lambda$ is the wavelength and the relation $\kappa << 1$ is supposed. The superscripts $s$ and $p$ corresponds to s and to p polarization of the light, when transferal to the plane of incidence oscillates the electric or the magnetic vector, respectively. The quantity $d$ is the penetration depth of the evanescent filed [9], calculated by:

$$d_s = \frac{\lambda n \cos(\varphi)}{\pi n_1 \sqrt{\sin^2 \varphi - n_2^2 (1 - n_s^2)}}$$

$$d_p = d_s \frac{2\sin^2 \varphi - n_2^2}{(1 + n_s^2) \sin^2 \varphi - n_2^2}$$

(2)
where \( n = n_j/n_1 \) is the relative refractive index, \( n_j \) is the refractive index of the denser medium (silicon) and \( \varphi \) is the angle of incidence at the base of the prism. The phase of the totally reflected light is determined by:

\[
tg(\Phi^j) = -\frac{2\sqrt{\sin^2 \varphi - n^2 \cos \varphi}}{n^2 + 1 - 2\sin^2 \varphi}, \quad tg(\Phi^m) = \frac{2n^2\sqrt{\sin^2 \varphi - n^2 \cos \varphi}}{n^4 \cos^2 \varphi + n^2 - \sin^2 \varphi}
\]

(3)

The (1-3) show that the amplitude of the TIR light is influenced by the absorption of the fluid, while the TIR phase depends on its refractive index. At the same time the reflection from the lines of the grating (figure 1) creates a constant reference signal. The TIR light from the lines spacing interfere with the light, reflected from the metallic lines. As a result a diffraction pattern (8) is created. The Diffraction Efficiency (DE) of the 0th (\( \eta_0 \)) and the 1st (\( \eta_1 \)) diffraction orders is given by [7]:

\[
\eta_0 = \frac{1}{4} (r^2 + m^2 + 2mm \cos(\Phi - \Phi_m)) \quad \eta_1 = \frac{1}{\pi^2} (r^2 + m^2 - 2mm \cos(\Phi - \Phi_m))
\]

(4)

where the \( m \) and \( \Phi_m \) are the amplitude and the phase of the metallic reflection. Any change in the fluidic optical constants result in change in the values of \( \eta_0 \) and \( \eta_1 \). Thus, the fluidic sensing is realized by measuring of the energy diffracted in the 0th and the 1st order (figure 1). For that purpose fixed photodetectors (8) are used, since the angles of diffraction are constant [4-5].

3. Experimental

The micro-sensors are realized by fitting of two <100> silicon wafers, as reported in [3]. The microprisms are created in one of the wafers. First, it is oxidized, the SiO\(_2\) is etched by hydrofluoric acid (HF) from the reverse side of the wafer and Cr diffraction gratings are deposited there by lift-off technique. Then, the SiO\(_2\) layer front side of the wafer is patterned by photolithography and chemical oxide etching. The resist is stripped and the microprisms are micromachined by wet anisotropic potassium hydroxide (KOH) etching using the patterned SiO\(_2\) as a mask. This process reveals the (111) crystal planes of the silicon, which are etched 100 times slower than the <100> direction. The resulting prisms have an angle of 54.7° (the angle between the (100) and (111) planes in a cubic crystal cell). The microchannels are structured by realized in the second wafer. It is oxidized, and the SiO\(_2\) is patterned from the both sides of the wafer by photolithography and consequent SiO\(_2\) chemical etching. Then, again bulk micromachining by KOH etching is performed until meeting of the both etch fronts. Finally, the two wafers are adjusted, bonded together and the sensor chips are separated mechanically. A picture of the micro-fluidic chip placed in its fluidic-supply holder is shown in figure 2. A smaller top-left situated box shows a top- and back-side view of the chip itself.

Oil soluble CuFe\(_2\)O\(_4\) composite nanoparticles have been successfully synthesized. A suitable surface functionalization and choice of solvent are crucial to achieving sufficient repulsive interactions to prevent aggregation so as to obtain a stable colloidal solution of magnetic nanoparticles, a ferrofluid. In the magnetic nanoparticles, the carboxylate ends of long-chain carboxylic acids such as oleic acid bind well to the particles so that the aliphatic chains extending out from their surface of the particle make them effectively hydrophobic and dispersible in nonpolar solvents such as hexane. Fe(acac)\(_3\) (1 mmol), Cu(acac)\(_2\) (1 mmol), 1,2-hexadecanediol (10 mmol), oleic acid (6 mmol), oleylamine (6 mmol), and benzyl ether (20 mL) were mixed and magnetically stirred under a flow of argon for 30 min. The mixture was heated to 200 °C for 30 min and then, in argon atmosphere, heated to reflux (265 °C) for another 30 min. The black-brown mixture was cooled to room temperature in several hours by removing the heat source. Then, ethanol (40 mL) was added to the mixture, and a black material was precipitated and separated via centrifugation (ca. 5000 rpm, 10 min). The black product was dissolved in hexane in the presence of oleic acid (~ 0.05 mL) and oleylamine (~ 0.05 mL). Centrifugation (6000 rpm, 10 min) was applied to remove any undispersed residue. The product, CuFe\(_2\)O\(_4\) nanoparticles, was then precipitated with ethanol, centrifuged (6000 rpm, 10 min) to remove
the solvent, and redispersed into hexane. All precipitation and centrifugation runs were performed under an argon atmosphere.

### Figure 2.
Micro-fluidic sensor: single chips and the micro-sensor placed in a fluidic holder.

#### 4. Results and discussion

The dependence of $\eta_0$ and $\eta_1$ (the 0th and the 1st order diffraction efficiency) on the concentration $c$ of aqueous solutions of sucrose is shown in figure 3. Figures 3a) and 3b) correspond to s and p polarization of the IR laser beam. The signs ▲, ● and ▼ are related to the 0th order and note a deviation from normal incidence to the front prism's facet of $\alpha=-30^\circ$, $0^\circ$ and $30^\circ$, correspondingly. The hollow signs □, ○ and △ denote the 1st order, respectively. The lines represent a linear fits of the experimental data, as expected before [3]. In all cases the measurements agree with the linear fits within the experimental error.

#### Figure 3.
Diffraction efficiency of the 0th (filled signs) and 1st (hollow signs) diffraction orders with a variation of the analyte's concentration at different angles of incidence (square for $-30^\circ$, circle for $0^\circ$ and triangle for $30^\circ$) a) case of s-polarized light, b) p-polarized light.

It visible from figure 3, that the polarization of the light has a critical influence on the characteristics of the sensor. Figure 4 shows that the polarization behaviour of the diffraction orders is described by curves with a maximum and a minimum at ca $90^\circ$ polarization angle. At TIR the reflected
beams undergo simultaneously both amplitude and phase changes. The phase dependence is especially significant, as the difference between the phases for s and p polarization is close to $\pi$ [9]. Hence, the maximum DE for a given polarization corresponds to a minimum for the orthogonal one. Moreover, the DE change with the polarization is about 2 times higher for the 0th order, because both the +1st and -1st order contribute to the energy transfer. The polarization characteristic of the integrated micro-sensor is compared with a $\cos^2$ function, which is typical for the amplitude diffraction gratings. The good agreement between them confirms the validity of a theoretical description of such grating's system by an amplitude transmission model, as was made in the previous works [3-4, 8].

Based on the above investigations, it could be concluded, that the presented IR integrated system can be applied for concentration analysis of the micro-fluidic mixtures. The resolution of analyte concentration $\Delta c$ is given by $\Delta c = |d\eta/dc|^{-1}\Delta\eta$. In all cases the absolute experimental uncertainty in the diffraction efficiency is $\Delta\eta < 0.001$, which is determined by multiple measurements and statistic processing of the data. Also, the experimental data coincide with the theory within such error. Table 1 summarizes the DE sensitivity $d\eta/dc$ from the experiments with sucrose solutions for all combinations between light polarization, observed diffraction order and deviation angle $\alpha$. The concentration resolution is also calculated and presented. It is clearly visible, that the sensitivity of the micro-fluidic sensing system can be increased several times by properly choosing the conditions of the measurements. In such a case the concentration of sucrose can be determined with a precision, better than 1 wt.%. The measurements are better by p-polarized light in the 0th diffraction order and smaller possible value of the deviation angle $\alpha$. The latter corresponds to incidence at the prism base at angle, closer, but still exceeding the critical angle. Then the penetration depth of the evanescent filed is higher, i.e. effective absorption path is longer and thus the sensitivity is higher. The measurements range of concentration practically does not depend on the angle of incidence because the total internal reflection is fulfilled for any angle of incidence due to the higher Si refractive index. On the other hand, a value of $\alpha$, smaller then -45° to -60° is not desirable due to the increased reflection losses at sloped incidence (even at normal incidence the reflection from the silicon is about 50%).

Table 1. DE sensitivity and resolution for sucrose concentration.

| $s$ polarization | 0th order | 1st order | $p$ polarization | 0th order | 1st order |
|------------------|-----------|-----------|------------------|-----------|-----------|
| $\alpha=$30°     | $\alpha=$0° | $\alpha=$30° | $\alpha=$0°     | $\alpha=$30° | $\alpha=$0° |
| $d\eta/dc \times 10^{-4}$ | -8.5 | -7.3 | -6.4 | -2.2 | -1.7 | -1.4 | -11.2 | -9.0 | -7.9 | -4.8 | -3.7 | -2.5 |
| $\Delta c$, wt.% | 1.2 | 1.4 | 1.6 | 4.5 | 5.9 | 7.1 | 0.9 | 1.3 | 2.1 | 2.7 | 4 |

The next experiment with composite CuFe$_2$O$_4$ nanoparticles in hexane (figure 5) proves the feasibility of the micro-fluidic system for analysis of nano-dispersions. Such magnetic nanoparticles bear two attractive features, fluorescence and superparamagnetism. The size of the CuFe$_2$O$_4$ particles here is below 10 nm. The measurements are led in the most-beneficial configuration: 0th order, p polarization, $\alpha=$ -30°. The influence of the continuous medium could be excluded, because hexane has no absorption peculiarities at the considered wavelength [11]. A clear effect of decrease of the DE with the concentration of the nanoparticles can be reported. The data are compared with a linear function, as the angular coefficient defines the concentration sensitivity $|d\eta/dc|^{-1}\Delta\eta=0.02$. The experimental uncertainty of the considered results $\Delta\eta=2.10^{-3}$ is determined by the statistical processing of the data of multiple-repeated measurements and determination of the deviation between the data points and the fit line. The corresponding resolution toward the concentration of the CuFe$_2$O$_4$ nanoparticles is $\Delta c = |d\eta/dc|^{-1}\Delta\eta=0.1$ wt. %.
Figure 4. Diffraction efficiency of the 0th and the 1st orders at different polarization angle of the incident light.

Figure 5. Diffraction efficiency of the 0th order at p polarization and external angle \(\alpha = -30^\circ\) from the concentration of CuFe₂O₄ nanoparticles.

A slight saturation effect (deviation from the linear behavior) in the DE can be observed. It could be explained with the increased influence of the multiple scattering at higher particles concentration. Other reason could be the specific photo-properties of such nanoparticles. MnFe₂O₄ and Fe₃O₄ nanoparticles are known to absorb light and transform to photoexcited once [10]. The photoexcitation of ferromagnetic nanoparticles led to the demagnetization of ferromagnetic nanoparticles. The photoexcited CuFe₂O₄ nanoparticles may be deactivated by the nonradiative process. Thus, absorption of the incident light and light scattering by metal nanoparticles are expected to decrease additionally the reflected light intensity or DE. Furthermore the self-quenching of excited states of nanoparticles is known to increase with the higher concentration (or the number) of metal nanoparticles. We should exclude the participation of organic shell (composed of oleic acid and oleyl amine) on the excitation and deactivation processes (absorption of light, light-scattering and quenching of excited states). Anyway, a certain conclusion cannot be made due to the relative high error of the experiment.

Finally, we have proven that the analysis of the experiments leads to more precise measurements. On the other hand, the measurements can be made simultaneously in the both 0th and the 1st order and/or both with s and p polarization. Such investigation will additionally increase the precision of the measurements and will eliminate the influence of casual mistakes.

5. Conclusions

This work presents for a first time an investigation of the sensitivity of a novel integrated IR laser system for micro and nano-fluidic investigation on the measurement conditions. Increased sensitivity is found out for the most-beneficial configuration of p-polarized light, 0th diffraction order and minimal possible angle of incidence. Main advantages of the micro-fluidic detector are the simplified fabrication technique, the fixed positions of the optical elements (there are no moving parts) and the operation with a monochromatic NIR source (no spectral analyzer is required). Moreover, it is sensitive to transparent and absorbing fluids and is applicable for strongly absorbing, highly concentrated and nano-disperse media. The last application is proven for concentration analysis of oil-based dispersion of composite CuFe₂O₄ nanoparticles. In this case, a resolution below 0.1 wt. % toward the CuFe₂O₄ nanoparticles is demonstrated. Other applications of the integrated micro-system are chemical, medical and biological diagnostic of liquids and dispersions of drugs, serums, proteins, peptides, DNA, RNA, viruses, etc.
Acknowledgement
This work is supported by AvH Foundation, the VEGA Projects No. 2/0037/10 and 2/0160/10 and ASFEU project 26240220011 OPVaV-2008/4.2/01-SORO.

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