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Direct hemoglobin measurement on a centrifugal microfluidic platform for point-of-care diagnostics

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Abstract

We present a novel concept to determine relevant biochemical markers for applications in the field of emergency diagnostics. Our modular setup comprises a disposable polymer disk which hosts microfluidic structures and optical beam-guidance elements. The beam of a standard laser is guided by total internal reflection (TIR) at V-grooved retro reflectors which are monolithically integrated on a "lab-on-a-disk". This way, the optical path length through the commonly flat detection cell and thus the sensitivity of colorimetric assays is massively enhanced compared to direct (perpendicular) beam incidence. The reusable analyzer compactly incorporates a standard drive to spin the disk, a dispensing unit, a low-cost laser, and a spectrophotometer serving as flexible detector for different assay formats. The competitive performance of the setup was proven by the rapid and accurate determination of the concentration of hemoglobin (Hb) in human whole blood. Outstanding features are a high degree of linearity ($R^2 = 0.993$) between the optical signal and the Hb, a good reproducibility of CV = 2.9%, and a time-to-result of 100 s, only.

Keywords: Hemoglobin; Colorimetric assay; Centrifugal microfluidics; Point-of-care; Human whole blood

1. Introduction

The transfer of clinical diagnostics from centralized laboratories to point-of-care applications is subject of various efforts concerning scientific aspects [1–6] as well as commercialization [7,8]. To meet the requirements of clinical diagnostics, these "lab-on-a-chip" technologies seek to enable a full process integration, reduced consumption of sample and reagents as well as short time-to-result and ease of handling are the most prominent candidates. Great market opportunities are particularly expected for cost-efficient applications delivering "actionable diagnostic information", i.e. data which can immediately be used by the end user or health care professional therapy.

Among these "lab-on-a-chip" systems, we here consider centrifugal "lab-on-a-disk" technologies [8–18] which exploit the interplay of centrifugal and capillary forces for a full integration of analytical protocols, comprising entire process chains, e.g. sample preparation, reacting/separation and detection. Numerous "lab-on-a-disk" products have already been launched to the market [19–24].

In this work, we focus on the implementation of a disk-based, colorimetric hemoglobin assay. The Hb is defined as the amount of the protein hemoglobin in the erythrocytes (RBCs) with respect to the total blood volume. It is one of the most relevant markers in clinical diagnostics and routine blood screening, indicating certain diseases (polycythemia vera, chronic hypoxia), genetic defects (thalessemia) as well as severe physiological conditions (blood loss, dehydration). Furthermore, we developed an analyzer which features all reusable components for centrifugation, fluid metering and dispensing, and optical components.

2. Colorimetric assay on-disk

Colorimetric assays are based on the measurement of the intensity I of a probe beam of an incident intensity I_0 after

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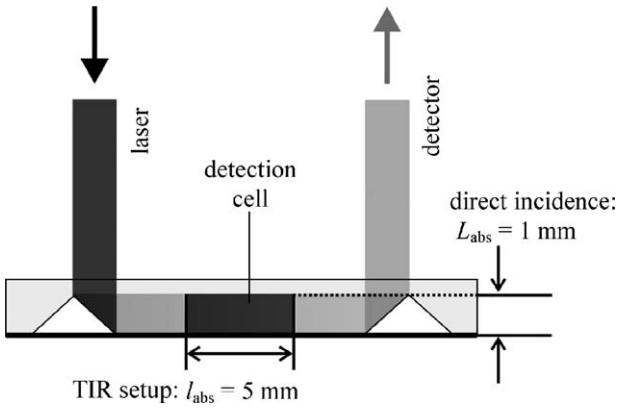

Fig. 1. Concept for an integrated optical beam guidance by total internal reflection (TIR). Next to the detection cell, integrated V-grooved retro reflectors deflect the beam by 90° into the disk-plane. After passing the detection cell, the attenuated probe beam is again reflected and then measured by a detector positioned above the disk. To ensure TIR, the incident angle α must be larger than the critical angle $\alpha_c \approx 41°$.

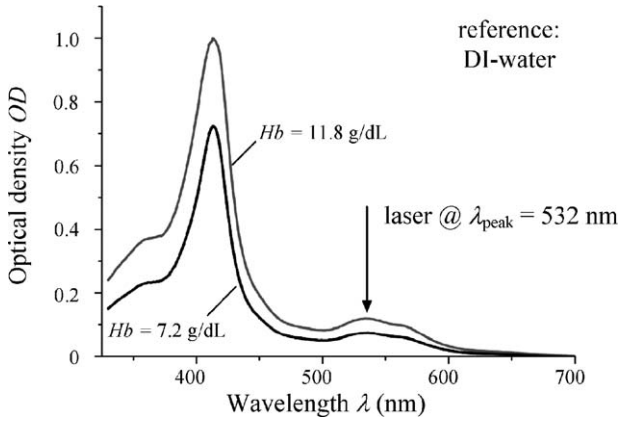

Fig. 2. Characteristic absorption spectra of processed blood with Hb at two different concentrations. A distinguishable side maximum is located near the wavelength of the probe laser (λ_{peak} = 532 nm) which features the highest difference in signal amplitude for different Hb.

passing the detection cell containing the analyte solution. According to the law of Beer–Lambert, the optical density (or absorbance)

$$\mathrm{OD} = \log \frac{I_0}{I} = \ln(10)\,\varepsilon(\lambda)\mathrm{Hb}\,l_{\mathrm{abs}} \quad (1)$$

linearly depends on the molar extinction coefficient $\varepsilon(\lambda)$ of the solution (which is governed by the products of the colorimetric reaction), the initial Hb, and the optical path length l_{abs} through the detection cell.

In our approach [25], the optical beam of a standard laser diode (λ_{peak} = 532 nm) [26] impinges perpendicular to the flat upper side of the disk. Via total internal reflection (TIR) at the side facet of the triangular V-groove, which is monolithically embedded into the reverse side of the chip (Fig. 1), the beam is then deflected by 90° into the plane of the flat detection cell. To ensure TIR, the angle of incidence α has to exceed the critical angle

$$\alpha_c = \sin^{-1}\left(\frac{n_{\mathrm{air}}}{n_{\mathrm{COC}}}\right) \quad (2)$$

which is governed by the refractive indices of the polymer substrate $n_{COC} \approx 1.5$ and the surrounding air n_{air}. To ensure TIR, the incident angle $\alpha = 45°$ is larger than the calculated critical angle $\alpha_c \approx 41°$. After passing the detection cell, the attenuated beam is reflected at another V-groove towards a spectrophotometer [27]. With this setup, the optical path length is appreciably extended to l_{abs} = 5 mm compared to the maximum path length of about 1 mm which can be realized for direct perpendicular incidence on typically flat microfluidic chips.

3. Biochemical concept

In our colorimetric reaction scheme, Hb is directly quantified in an untreated sample of human whole blood. The hemoglobin here reacts with a solution of sodium lauryl sulfate (SLS), a detergent, and buffer. Compared to the reference method for Hb-determination [28] using cyanide, the non-toxic SLS-method [29] is advantageous for point-of-care applications.

The reagents (V_r = 98 μL) first lyse the erythrocytes of the blood sample (V_{sample} = 2 μL) and then oxidize the hemegroups (Fe^{2+}) of the released hemoglobin to methemoglobin (Fe^{3+}):

$$\text{hemoglobin}\,(Fe^{2+}) \xrightarrow[\text{lysing and oxidation}]{\text{SLS}} \text{methemoglobin}\,(Fe^{3+})$$

In contrast to hemoglobin located in the erythrocytes, the released methemoglobin exhibits a distinct absorption band matching the emission of the laser (Fig. 2).

4. Assay protocol

In our lab-on-a-disk concept, the hydrodynamic actuation is delivered by the reusable and robust macroscopic centrifuge drive. On the other hand, the microstructured disk, which is made of cyclic olefin copolymer (COC) in the format of a conventional data CD, features passive, low-aspect-ratio microstructures in a basically planar architecture without moving parts, only. It can hence be fabricated in a very economic fashion. In our prototyping technology, microfluidic and optical elements are micromachined into the disk. Next, the surface is hydrophilized and dip-coated with PEtOx (poly(2-ethyl-2-oxazoline)) dissolved in methanol to prevent biofouling.

The frequency protocol $\nu(t)$ (Fig. 3) implements the backend liquid processing for the assay. An initial ramp-up to a maximum frequency ν_{max} = 20 Hz transports the sample and the reagent mixture into the detection cell. To enhance the diffusion-limited speed of the reaction, we then apply our previously developed "shake-mode", i.e. a frequent change of the sense of rotation between the maxima at ν_{max} = 8 Hz at a steep acceleration ramp $d\nu/dt$ = 32 Hz/s [30,31]. Within one second, the disk thus goes through a complete clockwise and counter-clockwise revolution. After an 80-s period of such inertially induced mixing and reacting, the optical density OD is measured at constant spinning (ν_{det} = 8 Hz) to eventually quantify the Hb (Eq. (1)).

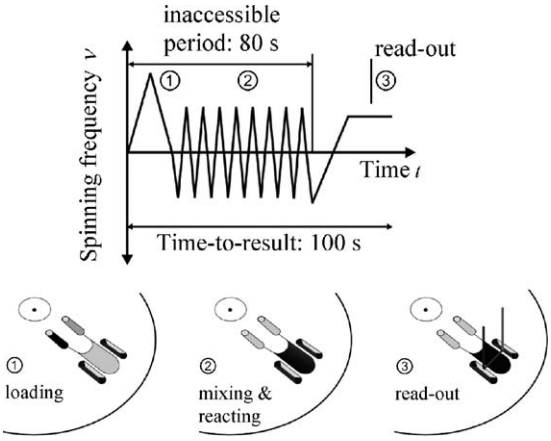

Fig. 3. Frequency protocol $\nu(t)$ to perform an on-disk hemoglobin assay within a time-to-result of 100 s. (1) After the sample and the reagents are loaded into the inlet reservoirs, the disk is spun to transport the liquids into the detection cell. (2) While applying the "shake-mode", convective currents are induced which accelerate mixing to enhance the chemical lysis of the erythrocytes and the subsequent oxidation of the hemoglobin. (3) The assay result is quantified during constant spinning.

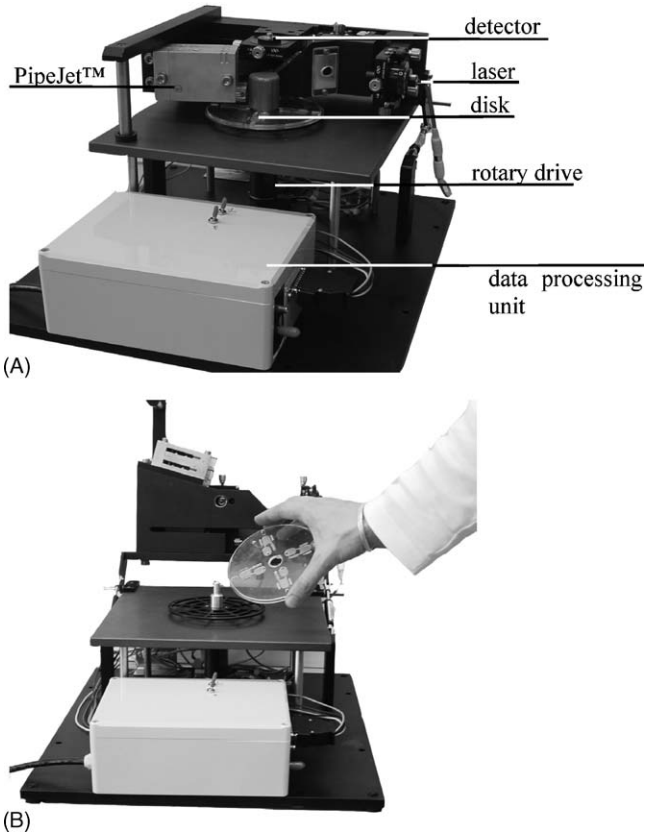

Fig. 4. Photograph of the reusable processing and read-out device. (A) A standard rotary drive underneath the table spins the disk. The laser beam I_0 is guided to the disk where it is attenuated to an intensity I according to the initial hemoglobin concentration Hb (Eq. (1)). Subsequently, the beam reaches the entrance aperture of the spectrophotometer. During rotation $\nu(t)$, the contact free dispensing unit (PipeJet^{TM}) supplies a defined volume of the reagents. (B) The disk is mounted by opening the system.

5. Integrated processing and read-out

Besides the microstructured disposable polymer disk, we have developed a reusable device for the fully integrated processing and the final read-out of the hemoglobin assay (Fig. 4). This bench-top device comprises a PC-controlled actuation unit, a synchronized free-droplet dispenser unit (PipeJet^{TM}) [32] to load the reagents, together with a low-cost laser [26] and a spectrophotometer [27] as components for the optical detection. The use of a spectrophotometer allows a flexible read-out of additional colorimetric assays or even fluorescence immunoassays (FIA) [33]. Thus, different types of assays may be run or different samples may be tested on the same disk, either simultaneously (in parallel), or one-after-another (sequentially) without the having to transfer of the disk between different devices.

The disk is first mounted on a disk holder and is thus defined in radial and azimuthal position and the sample (i.e., untreated blood) is loaded into the designated inlet. A standard rotary drive conducts the assay specific frequency protocol $\nu(t)$ which comprises the loading of the reagents (i.e., SLS) by the free-droplet dispenser. This conceptually simple PipeJet^{TM} [32] dispenser is constituted by an elastic polymer tube with circular cross section which is actuated by a piezostack driven piston. The piston squeezes the tube at a defined position near the open end by a significant fraction of its cross section (Fig. 4). The PipeJet^{TM} (Fig. 5) is able to dispense volumes in the nano- and microliter range and in a viscosity range between 1 and 200 mPa s at a CV below 1%.

After loading the reagents, the "shake-mode" accelerates mixing and reacting. During the final read-out step, a probe

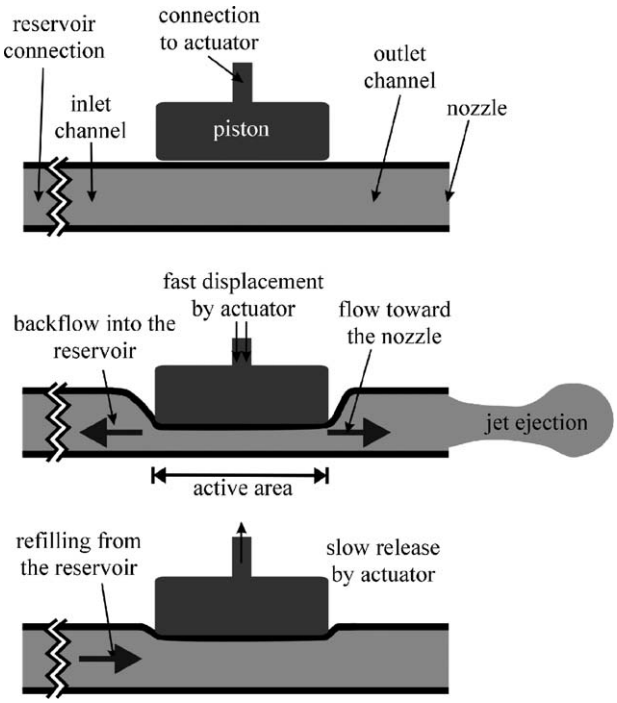

Fig. 5. Working principle of the PipeJet^{TM} dispenser. By a fast displacement of a piston connected to a piezostack, the tube is squeezed to drive the enclosed liquid towards both ends of the tube. Due to the proximity of the actuation region to the nozzle, most of the displaced volume is ejected as a free liquid jet.

beam I_0 emitted from the laser [26] is guided to perpendicularly impinge the disk surface. The probe beam then attenuates according to the initial hemoglobin concentration Hb and reaches the entrance aperture of the spectrophotometer where the optical signal is recorded. Direct data processing [34] based on a measured calibration curve yields to the requested result.

6. Results

The spectrophotometer transduces the incident signal intensity I into a count rate dN/dt accumulated during an adjustable interval τ to a total count $I = (dN/dt)\ \tau$ with a maximum number N_{max} limited by the 16-bit capacity of the incorporated counter. Following the Poisson statistics, the CV is proportional to $(\sqrt{N})^{-1}$, thus favoring to keep N close to N_{max}. To this end, we dynamically adjust time interval τ according to the signal intensity I, i.e. to increase τ with the OD.

The disk-based experiments are calibrated by a series of optical density measurements with a standard sample at known Hb [35]. With increasing Hb, the integration time τ is doubled twice to elevate the output signal and thus reduce the Poisson-noise

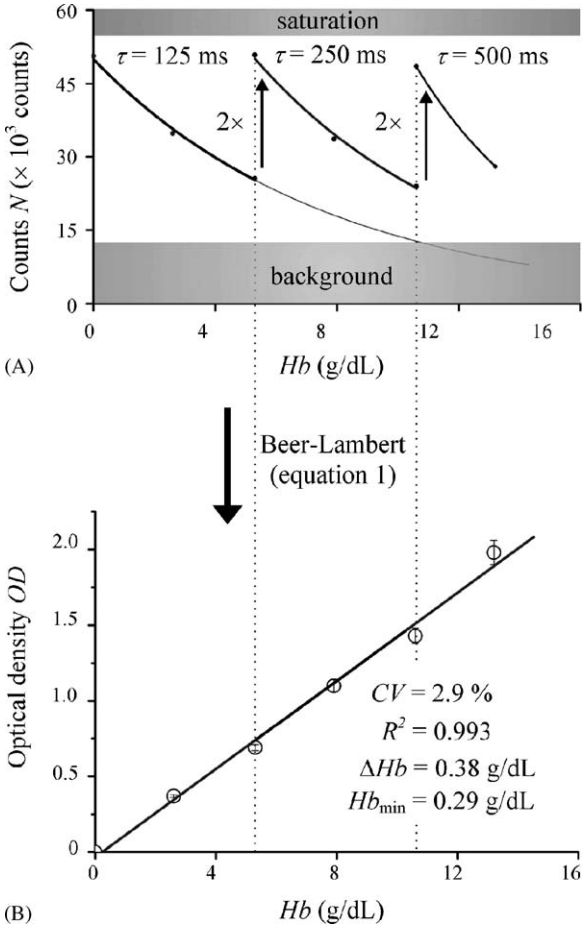

Fig. 6. Results of the Hb-determination: (A) the integration time τ of the spectrophotometer is increased in discrete steps (τ, 2τ, 4τ) to keep the output signal N of the detector in the upper working range. (B) The composite calibration curve is obtained by successive dilution of a calibrated standard sample.

(Fig. 6A). The displayed absorption characteristics clearly comply with the law of Beer–Lambert (Eq. (1)). We obtain a CV of 2.9%, a lower limit of detection of $Hb_{min} = 0.29$ g/dL together with a high resolution ($\Delta Hb = 0.38$ g/dL) and a good linearity between the hemoglobin concentration and the optical signal ($R^2 = 0.993$) (Fig. 6B). Additionally, by adapting τ, the CV was improved from 5% at constant τ to less than 3% at high Hb values.

7. Conclusion and outlook

We introduced a novel modular centrifugal platform for a rapid and automated processing of an emergency relevant hemoglobin assay. The assay is run on a cost-efficient modular platform comprising a passive polymer disk with monolithically embedded optical and fluidic structures as well as a reusable analyzer which features the spinning drive, the optical component, a detector and the dispensing unit. We successfully demonstrated the direct quantification of hemoglobin in whole blood at high accuracy.

In the future, the hemoglobin assay will be combined with other colorimetric assays at different probe wavelengths or fluorescence immunoassays (FIA) in a single read-out device for disk-based point-of-care applications.

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Biographies

J. Steigert obtained his graduate in civil engineering in microsystem technology in 2004 at the University of Freiburg, Germany. The topic of his diploma thesis was implementation and characterization of colorimetric assays on a centrifugal microfluidic platform. Since 2004 he is working as a PhD candidate at the laboratory for MEMS Applications at the Institute of Microsystem Technology (IMTEK) and being responsible for the further evaluation and optimization of technologies and assays within the "Bio-Disk" project.

M. Grumann was born in 1967 in Karlsruhe, Germany. He studied physics at the University of Freiburg and Ulm from 1989 to 1995. After that, he was working at Carl Zeiss AG in Goettingen as a service trainer for laser scanning microscopes. At 2001, he accepted a job as the director of the Muellheim production facility of Hoya-Lens GmbH. Since 2002 he is a PhD candidate in the department of MEMS applications at the Institute of Microsystem Technology (IMTEK) in Freiburg. Currently he is working in the Bio-Disk project which aims at the development of a microfluidic platform for blood analysis.

W. Streule obtained his graduate in civil engineering in microsystem technology in 2003 at the University of Freiburg, Germany. The topic of his diploma thesis was the theoretical and practical evaluation of sensor concepts to be integrated into nanoliter dispensers as well as first studies on the PipeJet™ dispensing system. Since 2004 he is working as a PhD student at the laboratory for MEMS Applications of the Institute of Microsystem Technology (IMTEK) being responsible for the further evaluation and optimization of the PipeJet™ technology.

L. Riegger was born 1978 in Freiburg, Germany. He studied Microsystem Technology at the University of Freiburg till 2004. Currently, he is a PhD candidate in the department of MEMS applications at the Institute of Microsystem Technology (IMTEK) in Freiburg. His research interests are the development of lab-on-a-disk systems for medical point-of-care diagnostics.

T. Brenner was born 1977 in Dortmund, Germany. He studied Microsystem Technology at the University of Freiburg till 2002. Currently, he is working towards his PhD in the department of MEMS applications at the Institute of Microsystem Technology (IMTEK) at the University of Freiburg. The focus of his work is the development of a centrifugal microfluidic platform for medical diagnostics.

P. Koltay studied physics at the Universities of Freiburg (Germany) and Budapest (Hungary) and obtained his PhD from the University of Freiburg 1999 for his work on solar cells and photovoltaic modules. End of 1999 he joined the chair of Prof. Zengerle at the Institute for Microsystem Technology (IMTEK) of the University of Freiburg. There he is heading the pL & nL dispensers group and the group fluidic simulation. His research interests are especially related to the development of microfluidic liquid-handing devices for various life-science applications as for example microdispensers, modeling of free surface flows and simulation of microfluidic devices by system simulation and computational fluid dynamic simulation.

R. Zengerle was born in 1965 and studied physics at the Technical University of Munich, Germany. From 1990 till 1995 he was a research engineer in the microactuator group at the Fraunhofer-Institute of Solid State Technology in Munich (today: FhG-IZM). Dr. Zengerle received his PhD degree

from the "Universität der Bundeswehr" in Munich with the development of an electrostatically driven micropump. From 1995 till 1999 he was the head of the Microfluidics Department at Hahn-Schickard-Society (HSG-IMIT) in Villingen-Schwenningen, Germany. Since 1999 Dr. Zengerle is professor at the University of Freiburg, Germany. He is heading the IMTEK laboratory for MEMS-Applications. This laboratory is a foundation of industry in order to stimulate the cooperation between industry and university. The laboratory of MEMS applications currently employs 30 research engineers and 10 master students. Since May 2005 Dr. Zengerle additionally is one of the new directors of HSG-IMIT in Villingen-Schwenningen. This institute currently employs 70 research engineers.

The research focus of Dr. Zengerle is in the field of microfluidics and incorporates highly parallel sub-microliter dispensing techniques, miniaturized autonomous liquid handling systems, miniaturized and implantable drug delivery systems, lab-on-a-chip systems, and micro- and nanofluidic simulation.

Dr. Zengerle is a member of the International Steering Committee of the IEEE-MEMS Conference and will be the general Co-Chair of the MEMS 2006 conference taking place in Istanbul. He also serves on the programme committees of various international conferences like the IEEE-Transducers conference, the International Conference on the Commercialization of Micro and Nano Systems (COMS), or the Actuator Conference. Dr. Zengerle is also the European Editor of the new Springer journal "Microfluidics and Nanofluidics".

J. Ducrée was born in the late 1960s in Essen, Germany. After that he studied physics at the University of Heidelberg and Münster. With two intermezzos in Uwe Thumm's group at the Kansas State University he finished his PhD in 1999 in Professor Andrae's group. Currently he works as a scientific assistant to the chair of MEMS applications at the Institute for Microsystem Technology (IMTEK) at the University of Freiburg in the field of microfluidics.