

Single-step centrifugal hematocrit determination on a 10-\$ processing device

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Abstract We present a novel concept to process human blood on a spinning polymer disk for the determination of the hematocrit level by simple visual inspection. The microfluidic disk which is spun by a macroscopic drive unit features an upstream metering structure and a downstream blind channel where the centrifugally enforced sedimentation of the blood is performed. The bubble-free priming of the blind channel is governed by centrifugally assisted capillary filling along the sloped hydrophilic sidewall and the lid as well as the special shape of the dead end of the two-layer channel. The hematocrit is indicated at the sharp phase boundary between the plasma and the segregated cellular pellet on a disk-imprinted calibrated scale. This way, we conduct the hematocrit determination of human blood within 5 min at a high degree of linearity ($R^2=0.999$) and at a high accuracy ($CV=4.7\%$) spanning over the physiological to pathological working range. As

all processing steps including the priming, the metering to a defined volume as well as the centrifugation are executed automatically during rotation, the concept is successfully demonstrated in a conventional PC-CDROM drive while delivering the same performance ($R^2=0.999$, $CV=4.3\%$).

Keywords Bubble-free priming · Hematocrit · All-in-one · CDROM · Centrifugal microfluidics

1 Introduction

Apart from clinical and near patient environment applications, microfluidic point-of-care applications are attracting growing interests (van den Berg and Oosterbroek 2003). The often named benefits of these miniaturized systems are reduced times-to-result, less error-prone handling steps, increased reliability, and minimized costs. So-called “lab-on-a-chip” systems aim to integrate all required processing and readout steps. Among these labs-on-a-chip, rotating “lab-on-a-disk” platforms (Madou and Kellogg 1998) additionally exploit centrifugal forces for metering, sedimentation of suspended particles (e.g., the cellular constituents of whole blood), and mixing in order to run fully process integrated assays. The high attractiveness of such lab-on-disk technologies for clinical diagnostics and research applications is endorsed by numerous commercial products (e.g., *LabCD* 2007, Abaxis Piccolo).

In this paper, we implement a novel lab-on-a-disk concept for hematocrit determination based on centrifugal sedimentation. The hematocrit (*Hct*) is defined as the volume fraction of the red blood cells with respect to the total blood volume. It is one of the most relevant markers in

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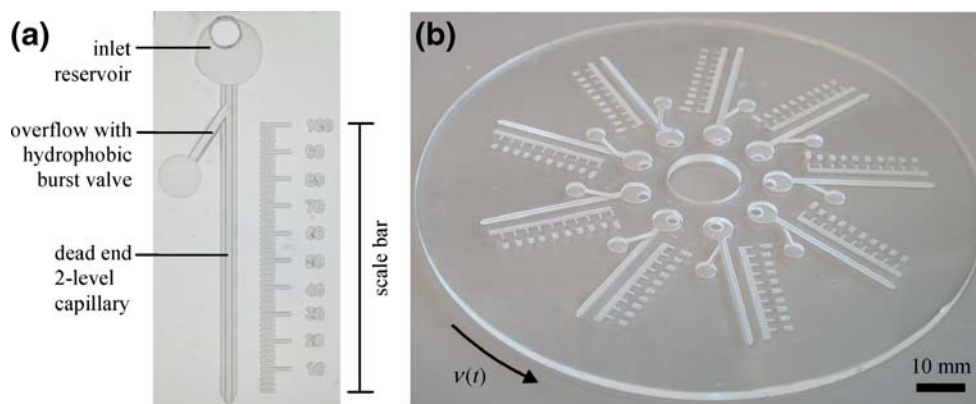


Fig. 1 (a) The hematocrit structure features an inlet reservoir, an overflow channel confined by a hydrophobic burst valve, and a two-level measurement capillary. Imprinted calibrated scale bars enable the direct visual readout of the hematocrit after sedimentation of the red

blood cells has terminated. (b) Microfluidic disk and channel structure. The disk which is spun at frequency μ follows the format of the common compact disc (CD) and presently permits up to a nine-fold degree of parallelization

medical diagnostics and routine blood screening, indicating certain diseases (anemia, polycythemia) as well as severe physiological conditions (blood loss, dehydration).

The *Hct* determination by sedimentation is a rather common method in medical diagnostics whenever the number of samples to be analyzed is small (Hermle 2007). This simple standard method additionally constitutes a reference for the *Hct* determination (Bull et al. 1994).

In our approach (Riegger et al. 2005), fast spinning of the disk by a standard centrifugal drive or even a conventional PC-CDROM drive appreciably accelerates the surface-tension driven self-priming of the dead end capillary, meters the blood volume, and enforces a total sedimentation of the red blood cells within 5 min, only. In contrast to the common benchtop-scale and centrifuge-assisted hematocrit determination using a glass capillary (Bull et al. 1994), error-prone handling steps like sealing of the capillary, mounting of the capillary into the centrifuge rotor, and the alignment of the calibration-template with respect to the processed capillary are significantly facilitated or even automated; simply apply a droplet of blood on the disk, start the process and read-out the hematocrit!

2 Microfluidic structure

The microfluidic structure (Fig. 1(a)) comprises an inlet reservoir for the uptake of the sample (i.e., untreated blood), an overflow featuring a hydrophobic burst valve for metering, and a straight two-level capillary (Fig. 2(a)) for sedimentation. The top and the bottom levels measure a depth of 400 and 800 μm , and their radial lengths are 25 and 25.4 mm, respectively. The calibrated scale bar facilitates the readout of the hematocrit by simple visual inspection. To demonstrate parallelization, up to nine structures have been integrated on a single disposable disk (Fig. 1(b)).

3 Bubble-free priming of a dead end sedimentation capillary

The concept of bubble-free priming of a dead end capillary is depicted in Fig. 2. Capillary forces—assisted by centrifugation—first transport the blood from the inlet into the blind capillary. A distinct depth profile (Steinert et al.

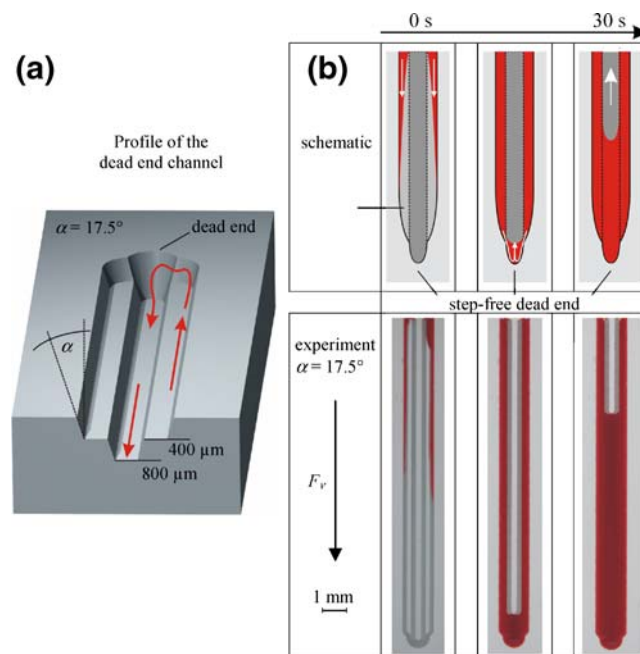


Fig. 2 Bubble-free priming of a dead end channel. (a) Channel profile of the dead end section of the channel. The channel features a depth of 800 μm in the center and 400 μm at the shoulders, respectively. The side walls are sloped by $\alpha=17.5^\circ$. The red arrows indicate the path of the meniscus. (b) Initially, the blood propagates along the boundary between the top level of the channel and the sealing lid. After reaching the step-free dead end of the channel, the transition of the fluid to the bottom central level occurs, thus initiating the reverse filling of the channel. The centrifugal force F_v shifts the whole process from the minutes to the seconds range

2004) combined with sloped side walls reliably suppresses trapping of gas pockets upon filling. The sealed and hydrophilized microfluidic capillary channel features a two-level structure where the top level is wider than the bottom central level (“T-shaped cross-section”).

As the edge acts as a capillary barrier, a fluidic separation of the two levels at the adjacent edge is established between the two levels. Additionally, capillary filling of the dead end channel is promoted at the top level (i.e., at the shoulders of the “T”) where the fluid is exposed to an elevated capillary pressure generated at the edge of the sloped side walls and the lid (Goldschmidtboeing and Woias 2005). As the fluid progresses in the downstream direction, air is hence removed through the lower central level.

As the fluid reaches the step-free dead end where the sharp, adjacent edge between the two levels vanishes (Fig. 2(a)), the capillary-driven fluid can penetrate the bottom central level to fill the capillary in the reverse direction without trapping of air bubbles.

Both priming phases can appreciably be accelerated from minutes to seconds by simultaneous centrifugation. The capillary priming can even further be improved by increasing the slope angle α of the side walls of the bottom and the top levels (shoulders), respectively. This way, the dependency of the capillary pressure on geometrical constraints is

exploited in two aspects. On the one hand, the increased slope angle α reduces the critical angle between the side wall of the shoulders and the lid, thus locally increasing the capillary pressure (Goldschmidtboeing and Woias 2005). On the other hand, the capillary pressure at the bottom level is reduced by the same effect. Overall, the pressure gradient is enhanced and priming at the top level is accelerated.

4 Disk fabrication

The hematocrit-disk, made from cyclic olefin copolymer (COC 2007) which was selected due to its good compatibility with our surface coating procedures, is structured by CNC-micromachining by a tool tapered at α . The geometrical dimensions of the microstructures without undercuts would simply allow the transfer to industrial scale production technologies (e.g., injection moulding).

The milled disk is then activated by an O₂-plasma and a hydrophilic polymer displaying photoreactive groups is deposited onto the disk by dip-coating. The polymer is based on a partially hydrolyzed poly(ethyloxazoline) to which photoreactive benzophenone units are coupled by means of a polymer analogous reaction (Chang et al. 2002). The coating is then UV cured at 365 nm, 3 mW cm⁻² for 1 h, yielding both crosslinking and chemical attachment to

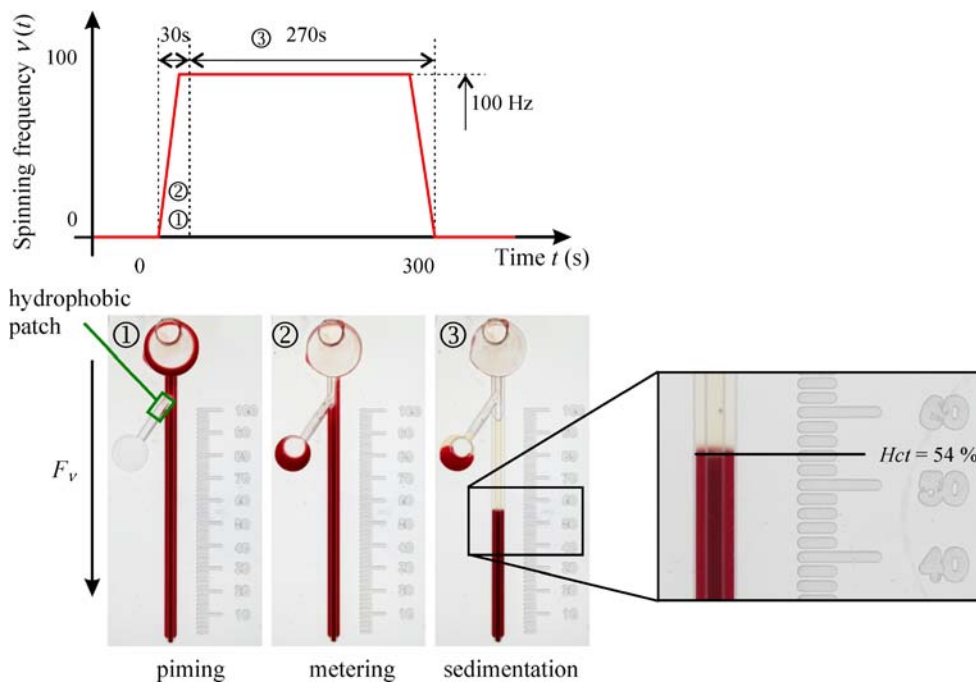
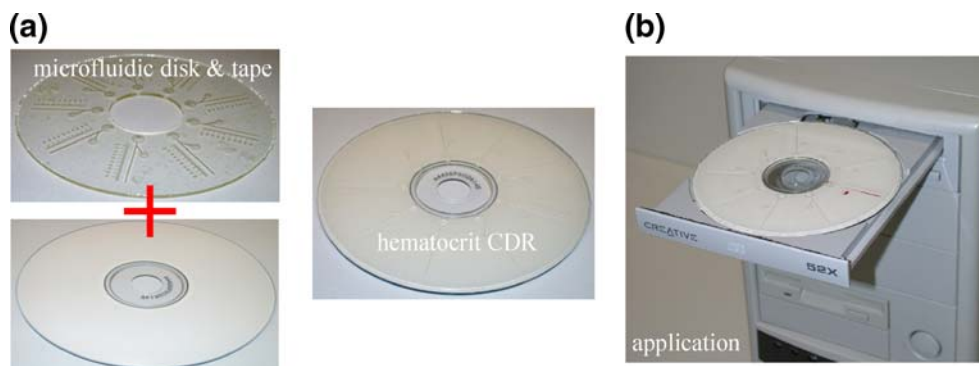


Fig. 3 Priming, metering, and sedimentation of a blood sample, induced by the centrifugal force F_v . ① After an unmeasured volume of whole blood is dispensed into the inlet reservoir, the capillary is primed within 1–3 s upon acceleration. ② Beyond 30 Hz, the

capillary barrier breaks and thus removes the excess liquid via the overflow channel. ③ After sedimentation, the hematocrit can be determined by inspecting the imprinted scale, e.g. $Hct=54\%$

Fig. 4 (a) The microfluidic disk is bonded to a conventional CDR by double-sided adhesive tape. (b) This test disk can readily be inserted into any conventional CDROM drive. For safe operation, a common CAV (constant angular velocity) drive is selected and the rotational speed has to be limited to 6,000 rpm (i.e., 30×) which can be done with the ASPI hardware protocol



the surface. This hydrophilic layer also blocks the surface against biofouling, i.e. protein or cell adsorption to the channels are efficiently suppressed.

Next, a modified felt pen is used to precisely place a droplet of dissolved Teflon (1 vol-%) at the inlet of the overflow channel to define a capillary burst valve (McNeely et al. 1999) for the metering of the blood. The disk is finally sealed by a rigid double-layer foil made of COC by temperature diffusion bonding.

5 Experimental protocol

After loading of the blood sample, the capillary priming of the microfluidic structure initiates. This priming process can appreciably be accelerated by simultaneous centrifugation to finish priming within seconds, compared to less than 1 min with the disk at rest (Fig. 3).

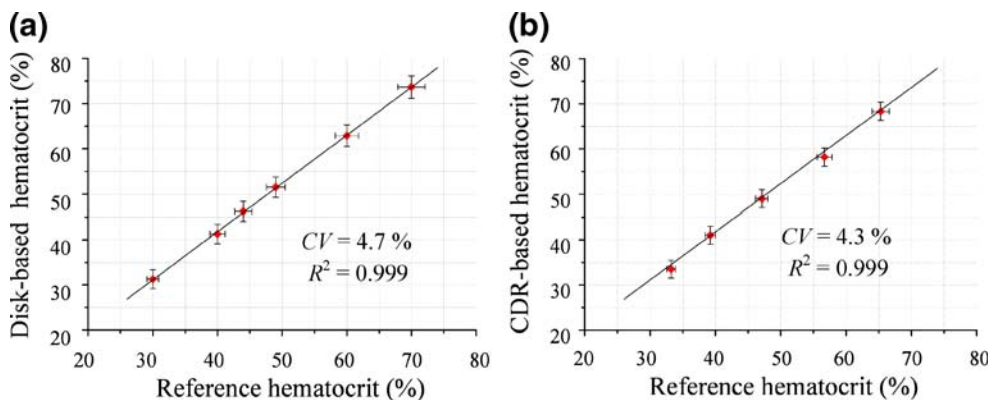
Once the whole capillary is filled, the investigated blood volume is metered to 20 μl by an upstream overflow channel. This channel opens when the burst frequency of the capillary valve at its entrance is surpassed at 30 Hz. Next, the metered volume is centrifuged for 5 min at 100 Hz (equaling approximately 16,000 g at a mean radial position of 4 cm). A shock interface between the plasma in

the supernatant and the concentrated cells evolves, moving rapidly towards the dead end after reaching 100 Hz. While the sedimentation proceeds, the shock interface decelerates and eventually reaches a stationary radial position after about 300 s. At this point the centrifugation is discontinued to allow the readout. The hematocrit is determined at rest by visual inspection of the plasma-pellet interface on the calibrated, disk-imprinted scale.

6 Processing in a PC-CDROM drive

For use in a conventional PC-CDROM drive, a double-sided adhesive tape is sandwiched between the disk and a standard, pre-written CDR (Fig. 4(a)). Unmetered samples of whole blood are introduced into the inlet reservoirs. Next, the disk is inserted into the CAV (constant angular velocity)-based CDROM (Fig. 4(b)) and the CD-ROM data track is completely read to provide a constant rotational frequency. For safety reasons, the data transfer rate is limited to 30× (corresponding to 6,000 rpm) which can be done by the ASPI (Advanced SCSI Programming Interface) hardware protocol. During the acceleration phase, the blood sample is precisely metered. Finally, the hematocrit can directly be read out on the disk-imprinted, calibrated scale bar.

Fig. 5 The experimental results of the hematocrit measurements are calibrated by the microhematocrit method (Bull et al. 1994). The low CV of 4.7% and 4.3% and the high linearity of $R^2=0.999$ allow high precision measurements with a standard centrifuge (a) as well as a conventional PC-CDROM drive (b), respectively. The small offset in contrast to the reference measurements accounts to a pre-sedimentation during the priming and metering phase



7 Experimental results

The disk-based experiments are benchmarked against the established microhematocrit method (Bull et al. 1994) with EDTA-treated whole blood (Fig. 5(a)). To this end, a set of samples were spiked (i.e., after sedimentation in a standard tube, supernatant plasma was removed or added to vary the *Hct*) as a preparative step. The so determined *Hct* was quantified via reference measurements. It shall be noted that the error of the measurements is dominated by the visual readout (i.e., constant error of one scale bar equaling 2% *Hct*).

We obtain a CV of 4.7%, a competitive turnaround time of 300 s, and a high linearity between the disk-based hematocrit determination and the reference measurement ($R^2=0.999$). Even by processing in a conventional PC-CDROM drive, the same performance is achieved (Fig. 5(b), $R^2=0.999$, CV of 4.3%). The offset in respect to the reference measurements can be explained by a pre-sedimentation during the priming and metering phase.

8 Conclusions

We introduced a novel hematocrit test on an unmetred amount of untreated whole blood drawn from the finger tip which is carried out automatically either in a standard centrifuge or by a conventional, PC-controlled CDROM drive. The test is implemented by a frequency protocol on a simple, 2-level priming structure. It requires a blood volume of only 5–20 μl which is comparable to standard turbidimetric tests (e.g., Diaglobal 2007, 10 μl) and which is lower than the volume required for the microhematocrit method (e.g., Hermle 2007, 60 μl). The precision of the test is slightly higher than that of the microhematocrit method (e.g., Hermle 2007, CV=3%) which can be explained by the smaller length of the capillary used for the readout. However, also considering the higher cost per test of 10 ct (if assuming mass production of the polymer disks) in comparison to the 2 ct for the microhematocrit capillaries (Hermle 2007), it should still be acceptable due to the practical elimination of all the critical manual handling steps and the lower cost of the processing device.

Further, the technology to run the test in a PC-CDROM drive can be easily applied to all tests based on readout by visual inspection (e.g., agglutination tests). The aspect of using an ubiquitously installed, 10-\$ device technology in combination with substrates that are very close to standard polymer mass fabrication processes for the disposable substrates opens attractive perspectives for a cost-efficient implementation of microfluidic technologies at the end-user level (e.g. patient home care and sports medicine).

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