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MIXING BY ON-CHIP GENERATED GAS BUBBLES FOR ASSAY AUTOMATION IN STANDARD LABORATORY CENTRIFUGES

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Summary. We present **bubble based mixing** as a **new unit operation for centrifugal platforms**. In contrast to existing centrifugal mixing concepts relying on fast acceleration and/or deceleration [1,2], bubble based mixing is **performed at constant rotation frequencies** and is thus **applicable to standard laboratory centrifuges**. Inspired by **bubble column reactors** for macroscopic process engineering [3], we developed an **on-chip gas source**, **scaled down volumes** and **scaled-up gravity through centrifugation** enabling similar fluid mechanical patterns as in the macroscopic set-up. An **excellent mixing performance** is demonstrated for DNA extractions from whole blood, which shows an **increased DNA yield by > 20 %** when mixing steps are performed by on-chip bubble based mixing compared to manual vortex mixing.

Introduction. Recently, assay automation on standard laboratory centrifuges has been proposed to lower market barriers for centrifugal microfluidic applications [4]. Since mixing is critical for many biochemical assays such as DNA extractions and existing centrifugal micromixers are not compatible to standard laboratory centrifuges, new mixers are required which enable mixing at constant frequency.

Functional principle. The new mixer uses the catalytic decomposition of hydrogen peroxide into water and oxygen as on-chip gas source. Fig.1 shows a possible chip design with separate chambers for the oxygen releasing reaction and the bubble based mixing process that allows for gas transfer while preventing contamination of the mixing chamber with hydrogen peroxide.

Experiments. DNA extraction from whole blood is chosen as an example of a biochemical application which strongly depends on mixing quality. Vortex mixing steps for lysis or binding are replaced by bubble based mixing on a centrifugal disk within the standard work flow of a manual DNA extraction (QIAamp DNA Mini, Qiagen). During on-disk lysis, 200 µl blood and 25 µl Proteinase K are mixed with 200 µl lysis buffer on a centrifugal disc at 30 Hz. Corresponding centrifugal discs (130 mm diameter) include milled fluidic structures as shown in Fig.1 and are sealed by an adhesive foil. The same design is also used for on-disc binding preparation which requires mixing of lysate and 200 µl ethanol. Resulting DNA yields of the extractions are analyzed via 18S-qPCR and compared to DNA extractions using vortex mixing.

Results and conclusion. High buoyancy in the centrifugal gravity field causes a fast rise of the gas bubbles in the mixing chamber. Thus, the bubbles become instable and separate into numerous small bubbles as shown in Fig.2. Drag forces and entrainment lead to strong convective flows that cause mixing.
Replacing manual vortex mixing of the lysis step with on disk bubble mixing increases the DNA yield by more than 30 % (Fig.3). When lysis and binding are performed on disk, the DNA yield increases more than 20 %. Furthermore, it was shown that with lysis on disk, a DNA yield of 70 % compared to the manual reference can be obtained with only 1 minute of bubble based mixing (Fig.4). With mixing times of > 7 minutes the DNA yield is beyond the standard procedure. This **excellent performance** in combination with requiring only **cheap and easy to prestore reagents** make bubble based mixing attractive for assay automation on standard laboratory centrifuges.

Word Count: 498

References:

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4. “Microfluidic Apps for off-the-shelf instruments”, D. Mark et al., Lab Chip, 12(14), 2464-8 (2012)

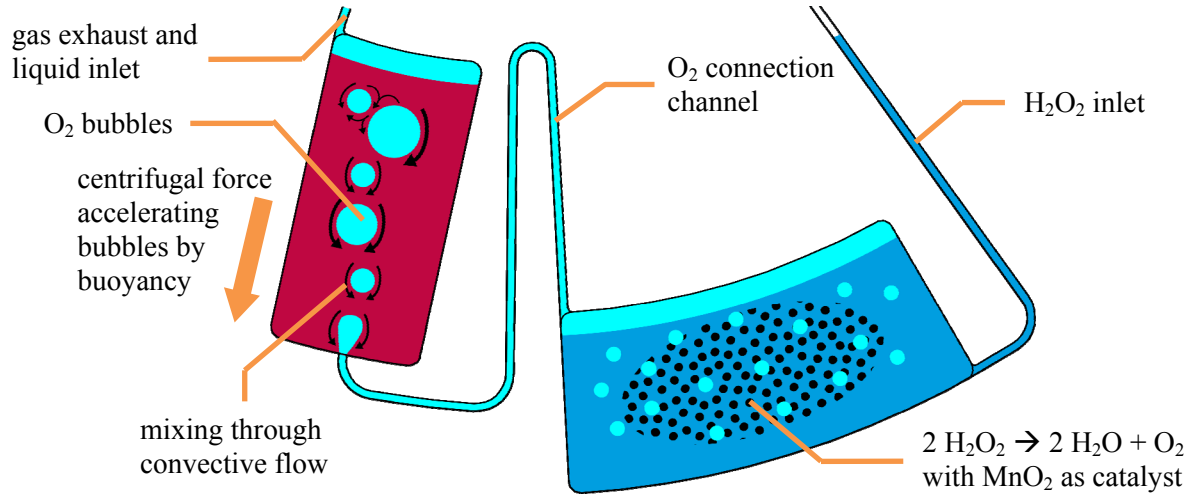

Figure 2: Oxygen bubble formation in a fluidic chamber filled with water. Each bubble is at a different state after ejection from the adjacent channel. The instability caused by centrifugal acceleration and subsequent separation in numerous small bubbles is clearly visible.

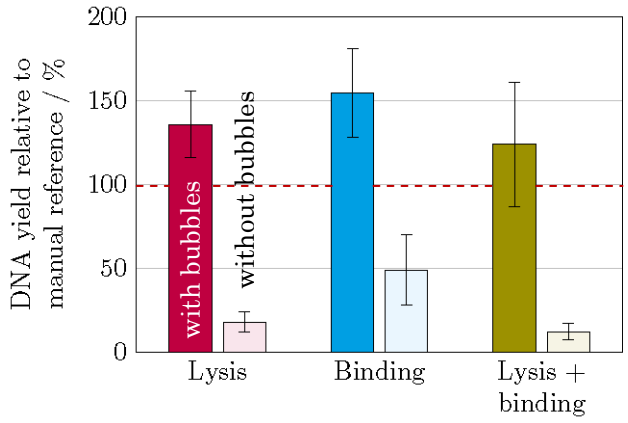

Figure 3: DNA yields with different on-chip performed mixing steps with and without gas development indicated by opaque and light bars, respectively. The experiments were executed on two different days with two different blood samples, 3 - 4 repetitions per blood sample and triplicates at the 18S- qPCR for a total number of 21 – 24 values per bar.

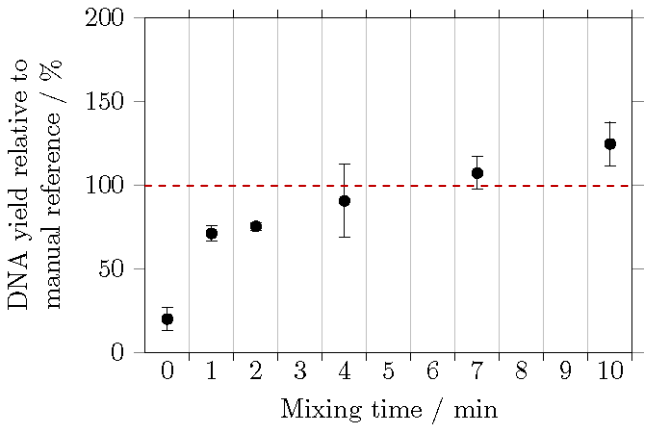

Figure 4: DNA yield for different mixing durations during lysis. The total lysis time (mixing and incubation) was always 10 min.