DOI 10.5162/sensor2015/C4.4

Ultrasonic Dispersion of Particles in Lab-on-Chip Systems for Enzyme-Linked-Immunoassays

Lukas Brandhoff[1], Helene Zirath[2], Johannes Peham[2], Herbert Wiesinger-Mayr[2], Heinz Redl[3], Andreas Spittler[4], Anna Haller[5], Guntram Schnetz[6], Michael J. Vellekoop[1]
[1] Inst. for Microsensors, -Actuators, & -Systems (IMSAS/MCB), University of Bremen, Germany
[2] Health and Environment Department, Austrian Institute of Technology, Vienna, Austria
[3]Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria
[4]Surgical Research Lab. and Core Facility Flow Cytometry, Medical University of Vienna, Austria
[5]Vienna University of Technology, Austria
[6]Biegler GmbH, Mauerbach, Austria

lbrandhoff@imsas.uni-bremen.de

Abstract:
We present a system for dispersing colloids on-chip via ultrasound. Superparamagnetic beads used in Enzyme-Linked-Immunosorbent-Assays (ELISAs) can be efficiently dispersed in the on-chip reaction chamber by this method. By keeping the ultrasonic transducer off-chip and focusing the ultrasound through a horn towards the reaction chamber, a low-cost disposable chip design can be used instead of highly complicated and expensive integrated systems. This is advantageous especially in biological and medical applications where single-use devices are needed to prevent cross contamination between samples.

Key words: Lab-On-Chip, ELISA, Immunoassay, Ultrasound, Actuator

Introduction

Enzyme-Linked-Immunosorbent-Assays (ELISAs) are one de-facto standard in detecting and quantifying antibodies or antigens [1]. They provide a specific detection method for immune system responses, from on-setting or fully active diseases to drugs and allergens in physiologically relevant quantities.

In its simplest form, an immobilized capture antibody is used to specifically collect antigens from a sample. Because of the way antibodies connect to their antigen counterparts this step is highly selective towards the marker. The remainder of the sample with all non-specific antigens is then washed away. Detection antibodies coupled to a catalyst, in many cases horseradish peroxidase (HRP), are introduced. They again bind to the collected and immobilized antigens, and produce a visible and measureable reaction together with a luminescent or colorimetric substrate.

Lab-on-chip integrated ELISAs are able to work with much smaller sample volumes than traditional well-plate based systems, opening possibilities for minimally invasive and neonatal diagnosis. They also provide quicker assay times and lower costs, taking minutes instead of hours while needing no or less supervision.

While in laboratory scale experiments immobilization to chamber or well-plate walls is often used, immobilization to superparamagnetic beads has favorable properties. Because of the vastly larger surface area, created by the small (<5µm) size of those beads, more antibodies can be attached to them. For washing steps and exchanging liquids, the beads are collected by a strong magnet. This keeps them unaffected inside the chamber while the liquid is removed or replaced.

Integrating a complete ELISA onto a chip creates new challenges for science and engineering to produce functional, effective and cheap systems.

One of the hurdles faced when designing a magnetic beads based ELISA is, that when the beads are collected by magnetic actuation, clusters are formed since the beads are attracted to each other. These clusters do not break up after the magnetic field is removed, since hydrophobic bonds hold them together. This drastically reduces the surface area of the

beads in contact with the liquid. Dispersion of the paramagnetic beads is necessary to ensure that all of the surface area of the particles is in contact with the sample liquid, especially during incubation phases. This increases sensitivity and decreases the time necessary for incubation. During washing steps, a good dispersion of the beads is also needed, since all of the non-specifically bound protein and reagent have to be washed away.

Our system uses the off-chip ultrasonic system further described in this paper for this purpose.

ELISA-Chip

Our superparamagnetic beads based ELISA chip for detection of inflammatory diseases [2], is made from a poly-methyl-methacrylate acrylonitrile-butadiene-styrene (PMMA-ABS) blend. It is shown in Figure 1 and contains only the liquid channels and the reaction-chamber, while pumps, valves, and optical components necessary for detection are kept on system level. The reason is to create a low cost, disposable chip that can be fabricated by injection molding without the need for expensive cleanroom technology. Shifting the total cost from the disposables to the system level, makes it much more attractive for high throughput testing such as in hospital or research laboratories.

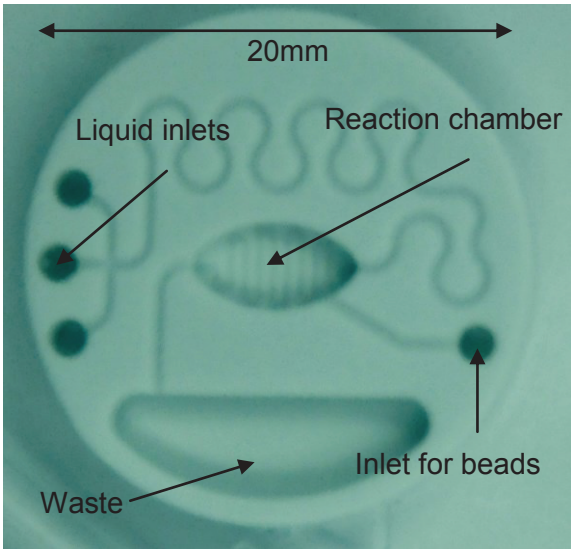

Fig. 1. PMMA-ABS based ELISA-Chip

The beads are stored on-chip and the reagents for the ELISA protocol are introduced one after the other, with washing steps in between. During the liquid exchanges the magnetic beads are attracted with a permanent magnet fixed over the reaction chamber.

The system also contains an ultrasonic system coupled to the chip for dispersion of the magnetic beads in the reaction chamber in addition to the already discussed [3] removal of non-specifically bound proteins from the chips surface. The ultrasound can be activated during washing steps to clean the chips surface and provide less background signal during the measurement.

Ultrasonic Driver

In standard ultrasonic cleaning applications the drivers are usually built as oscillators, with the piezoelectric transducer working as frequency defining element and actuator at once. This has the advantage of providing highly efficient on-resonance drive to the piezo.

In our case, such a freely oscillating driver is not sufficient, since we need to deviate from the main resonance frequency of the piezoelectric crystal to prevent standing wave formation in the microfluidic chamber.

Frequency definition and actuation have thus to be separated. A sine-wave oscillator, combined with a high power analog output stage would be the most precise way to control the piezo. Because of the efficiency problem of such a driver, resulting in a larger cost, and since we are aiming for a low cost approach, an H-Bridge based square wave oscillator is sufficient. A frequency sweep from 98 to 102 kHz is generated, and supplied to the circuit shown in Figure 2. The sweeping is used to prevent the formation of standing waves in the chamber which would otherwise concentrate the beads at the anti-nodes of the pressure waves in the chamber. The created high and low side drive signals are passed on to two half-bridge MOSFET drivers including charge pumps for driving the high-side MOSFETs. Both sides of the bridge are supplied inversely, generating a square wave output with twice the amplitude than a normal driving system. The supply voltage for the piezoelectric driver is 42 V.

In our ultrasonic dispersion system, a standard low-cost piezo (Pro-Wave) with 2 cm diameter is connected to the microfluidic chip and its chamber with an ultrasonic horn that focuses the sound into the microfluidic chamber. This has an advantage compared to on-chip ultrasound. Generated heat in the piezoelectric crystal is kept outside of the microfluidic device, eliminating its influence on the sensitive biological samples. Additionally, the larger piezoelectric crystal enables lower resonant frequencies and thus easier drive.

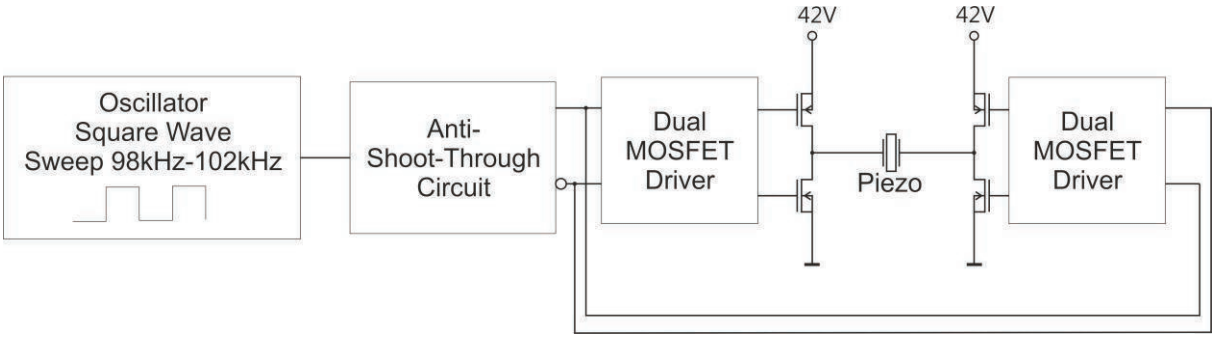

Fig. 2.: Driver Block Diagram.

Ultrasonic Horn

A lambda sized stepped horn is used to concentrate and couple the ultrasound into the microfluidic chamber. Such horns are usually used in high-power ultrasonic welding or chemistry applications, but work very well for microfluidic applications. The design of the aluminum horn is shown in Figure 3.

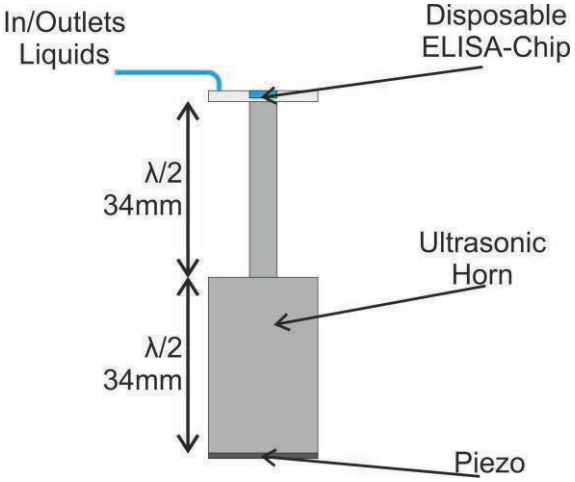

Fig. 3. Ultrasonic Horn in System (not to scale)

A Photograph of the Horn with dimensions is shown in Figure 4.

Since the speed of sound in aluminum is approximately 6800m/s, an excitation frequency of 100 kHz results in a total horn size of 68mm. Each part of the stepped horn will thus be 34mm long.

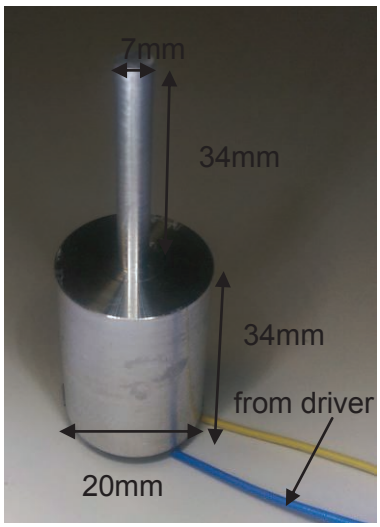

Fig. 4. Ultrasonic Horn with Annotations

Ultrasonic Dispersion

Ultrasonic dispersion is a technique used in standard laboratory practice [4], where colloidal suspensions are placed in an ultrasonic bath in which they are agitated by acoustically induced motion. This provides one of the most effective means for creating a uniform mixture, even for hard to disperse colloids.

This system miniaturizes the bench-top laboratory setup down to the microfluidic level. The horn connected to the microfluidic chip couples the ultrasonic energy into the chamber and disperses the beads through induced liquid motion as well as attraction of the beads into the changing standing wave anti-nodes of the chamber.

The progress of the dispersion is shown in Figures 5 and 6

Figure 5 shows the aggregated beads after they were magnetically attracted and the magnet was removed. Aggregation of the particles is clearly visible.

Figure 6 depicts the same chamber after 15 seconds of ultrasonic dispersion. The beads have been uniformly distributed in the chamber.

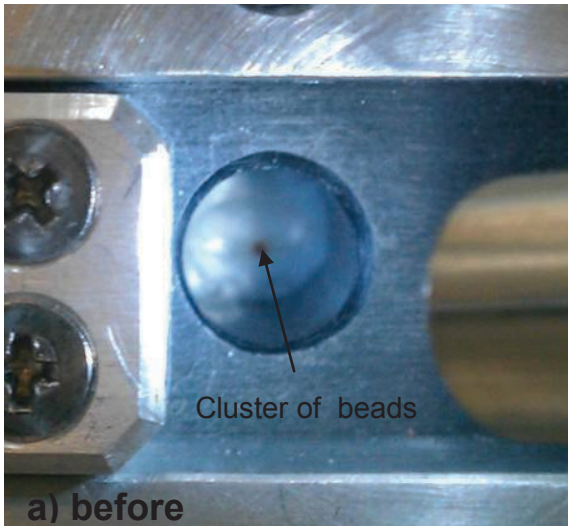

Fig. 5. Magnetic beads cluster before dispersion

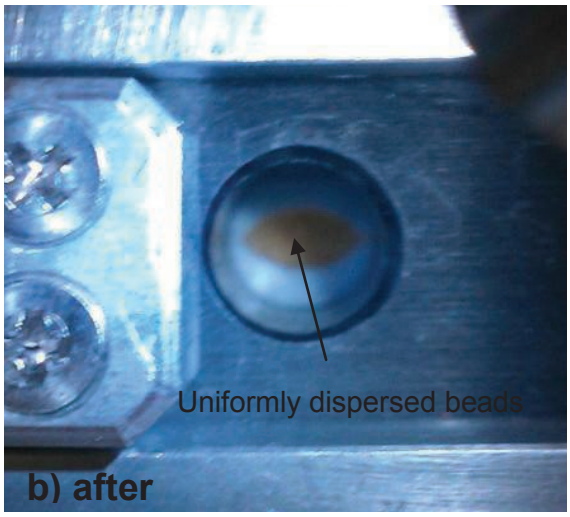

Fig. 6. Superparamagnetic beads after dispersion

Conclusion

Our ultrasonic system solves the problem of the aggregation of superparamagnetic beads in an ELISA after they have been attracted by a magnet via low power ultrasound. The ultrasound system also serves the purpose of mixing the liquids and aids in washing of the chamber. This provides an additional feature at no additional cost and is beneficial over hand dispersion. The whole system is designed to be simple and low-cost.

Acknowledgment

This work was funded by FFG-Bridge Programme and is a part of the project “Entwicklung eines innovativen Diagnosesystems für das Bed-Side Immunomonitoring - BSI” (829651).

References

[1] R. M. Lequin, „Enzyme Immunoassay (EIA)/Enzyme-Linked Immunosorbent Assay (ELISA)“, *Clinical Chemistry*, Bd. 51, Nr. 12, S. 2415–2418, Jan. 2005.

[2] H. Zirath, J. Peham, G. Schnetz, L. Brandhoff, A. Spittler, H. Wiesinger-Mayr, M. J. Vellekoop and H.Redl, A disposable microfluidic chip for rapid and sensitive detection of plasma biomarkers, Procedia Engineering, Volume 87, 2014, pp. 496-499, doi:10.1016/j.proeng.2014.11.410

[3] L. Brandhoff, M. J. Vellekoop, H. Redl, A. Haller, H. Zirath, J. Peham, H. Wiesinger-Mayr, A. Spittler and G. Schnetz, IEEE Sensors 2014, pp. 1862-1865, doi: 10.1109/ICSENS.2014.6985391

[4] J. S. Taurozzi, V. A. Hackley, und M. R. Wiesner, „Preparation of Nanoparticle Dispersions from Powdered Material Using Ultrasonic Disruption - Version 1.1“, National Institute of Standards and Technology, June 2012.