

DIODE LASER GENERATED ULTRASOUND FOR HUMAN BLOOD CELL LYSIS

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ABSTRACT

The lysing of human blood leukocyte cells prior to performing e.g. DNA analysis is performed on a routine basis in most biochemical labs. Well-established techniques include using a lysis agent or using mechanical methods such as high-energy ultrasound – also known as cell sonication. When porting these trivial methods to microfluidic analysis systems (Lab-on-a-chip, microTAS - systems) challenges become substantial. Adding an extra reagent, such as a lysis agent, to a microsystem or integrating ultrasonic transducers, adds considerably to the complexity of a microsystem (note: when performing PCR, lysis buffer will inhibit the enzymatic process and must thus be removed/inactivated)

We have devised a method and build and tested a system whereby we use a focussed infrared laser diode (808nm, 500mW) to generate high-energy ultrasound in a microchannel, and thereby perform lysis cellular matter. We are able to lyse human blood in a microchannel in 5 seconds.

Keywords: cell lysis, cavitation, sonication, sample preparation, lab-on-a-chip

1 BACKGROUND

The basis of the FluimediX technology is the utilization of focused laserenergy in connection with microfluidics. Our primary invention was the laser driven micropump [1] with which we are able to manipulate enclosed liquids. The present work is closely related to the laser driven micropump, as the involved technical means and thus the driving principles involved are identical.

The driving principles are based on the following means: the focusing of laser energy and thus the generation of extremely high optical energy densities into a microfluidic channel or reservoir followed by the subsequent vaporization of enclosed liquid.

The involved physical mechanisms are to some extent similar with the mechanisms of the well know bubble-jet (ink-jet) printer technology. The ink-jet printhead comprise a microfluidic chamber, wherein a microheater will boil an insignificant part of the enclosed ink within a few microseconds; the expanding bubble will thus act as a

piston expelling part of the enclosed ink within 5-15 μ s. The vapour bubble will cool, condense and thus contract and provide a vacuum that will ultimately aid in refilling the chamber. The forces involved are measured in 10's of atmospheres. Theoretical pressures of 10-20 atmospheres upon bubble growth and up to 40 atmospheres at collapse has been mentioned in relation with inkjet printing technologies.

2 TECHNICAL DESCRIPTION

Referring to the schematic representation of our system (fig. 1) the laserdiode is pulsed for a period of 3 μ s whereby a small amount of the – in the microchannel enclosed – liquid is brought to a super heated state. The liquid will rapidly nucleate and expand into a vapour-bubble and thus exert a substantial pressure onto this, where after it will cool, condense and thus collapse. The cycle is repeated every 50 μ s as illustrated in figure 2, (laser pulse with the resulting bubble size superimposed) thus eventually producing 20kHz ultrasound that is the preferred frequency for cell sonication.

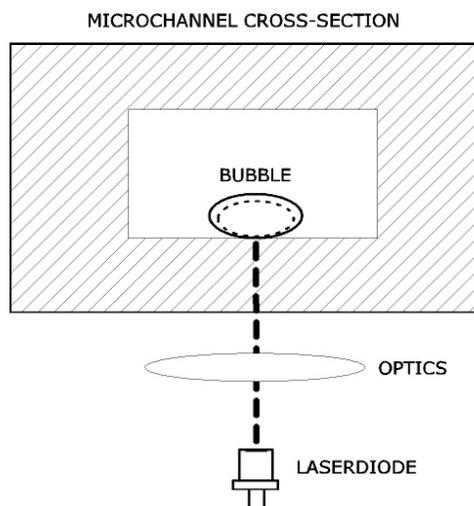


Fig. 1 – schematic representation

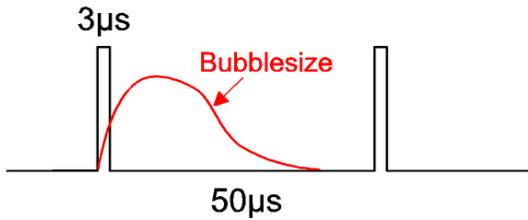


Fig. 2 – laserpulse and resulting bubble size.

3 POLYMER MICRO-FLUIDICS

The microfluidic circuit comprise a hot embossed polymer (PETG) microreservoir “titterplate”, that has been surface treated with infrared absorbing dye, so that laserlight is absorbed and converted to heat in the immediate surface of the channel after passing through the bulk of the polymer. The microfluidic “titterplate” contains a grid of elongated reservoirs of length 500μm, width 200μm and depth 100μm (fig. 3). We have previously performed the operation on smaller structures (100X25μm width/depth -channels) with success, but in order to get enough lysate for macro-world analysis, we devised the depicted structure.

The plate was primed with whole blood (EDTA blood) suspended in isotonic saline (40/60% - blood/saline) and a lid was placed onto the droplet and held in place by capillary forces (cover-slip fashion) The state of the enclosed liquid and the components herein, was recorded through a microscope with an attached digital camera.

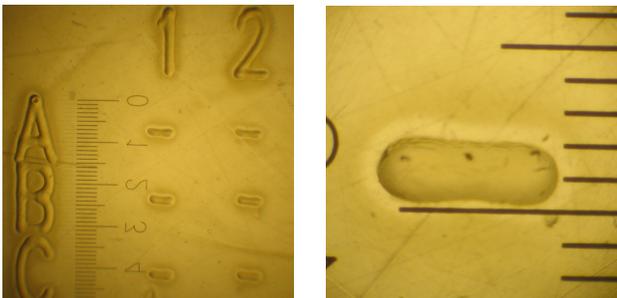


Fig. 3 – hot embossed reservoirs (200X500μm)

4 EXPERIMENTAL RESULTS

The primed microfluidic circuit was placed under a microscope with the laserbeam being focused from below in line with the schematic representation in fig. 1. The laser was initially run at a constant *low* power (25-40mW depending on focus) so that the focus of the beam would be clearly identifiable through the attached digital camera (note: 808nm infrared light will be visible through most digital cameras although invisible to the naked eye) It is of paramount importance to get the beam focussed at its minimum size in order to achieve the necessary energy

density. We are able to focus a beam of around 700μm² with the resulting energy of around 350mW (500mW optical output with optical loses etc.) and we will thus get an energy density of around 500MW/m². To put matters in perspective, the energy density of the surface of the sun is around 200MW/m². According Hewlett Packard inkjet researchers around 500MW/m² is applied to an inkjet reservoir in order to propel an ink droplet.

The following stills (fig. 4, 5 & 6) depicts the state of the enclosed blood/saline at time t=-0s, t=0s (laser run according to fig. 2) t=5s (end of lysing) and t=+5s (laser of

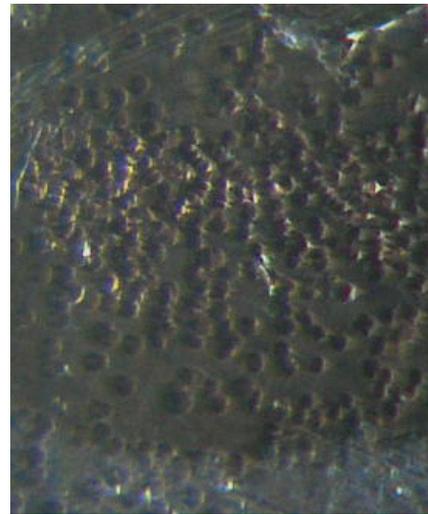


Fig. 4 – blood in saline before lysing (t<0s)

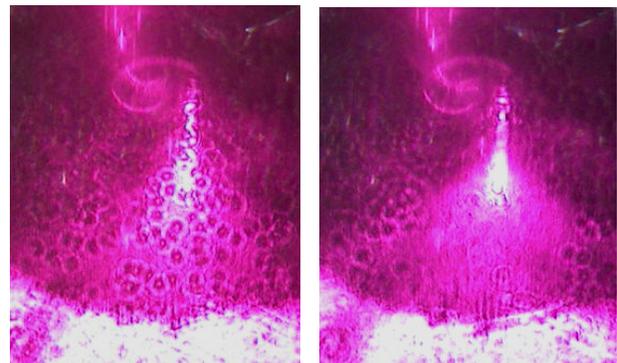


Fig. 5 – laser on, t=0s and t=5s respectively

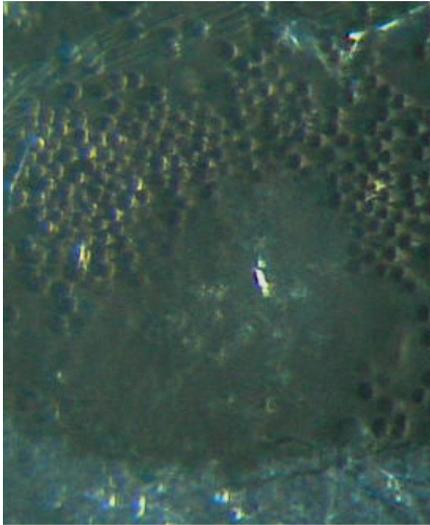


Fig. 6 – blood in saline after lysing ($t > 5s$)

As can be readily identified – even through our digital camera – the cellular matters appear to have vanished in an area around the laserbeam influence ($\sim 100\mu m$ radius) However - as the bulk of the cellular blood components are erythrocytes (red blood cells) sceptics might argue that what appears to be cell lysing by sonication, was merely heat induced *haemolysis* (haemoglobin escaping erythrocyte cell membranes) In order to gain confidence in the fact that we see cell sonication, the state of the cellular remains were analysed visually by means of high magnification microscopic inspection (fluorescence microscope, magnification 400X). The sub-micrometer cellular debris, that is characteristic of cell lysing by sonication, was clearly identifiable as they were influenced by “Brownian motion”. Also, we were not able to identify any haemolysed (empty) erythrocyte cell membranes, as would have been the case if the involved action was haemolysis. Finally we conducted a comparative experiment to check whether the applied heat would have any influence on the effect; as we are applying $3\mu s$ of $\sim 350mW$ every $50\mu s$, we should be able to induce the same amount of joule heat if we drove the laser at $3/50 \times 350mW$ at a 100% duty cycle (always on). We saw no action on the enclosed components whatsoever, even after applying double the amount of energy, we only registered some thermal movement (convection)

5 DISCUSSION

We see a clear industrial application to the described technology. There is an obvious need for alternatives to the cumbersome solutions to the lysing of cells in a microfluidic system, that we have seen over the years. As mentioned initially, the everyday practice of pipetting, spinning, rinsing, decanting etc. that works in the hands of a skilled laboratory technician, are just not applicable to the

low-cost, easy-to-use lab-on-a-chip solutions that has been sought for years.

We have not yet explored the boundaries of the technology, e.g. laser-power, surface influence, geometry dependencies etc. but we feel that we have a robust technology, which can be applied to a number of areas:

The efforts towards addressing bio-war terrorism using micro-TAS solutions, has raised the issue of “hard-to-lyse” biological matters such as Anthrax spores, and the violent forces of sonication, has been mentioned as *the* (only!) way to achieve fast and efficient lysis of Anthrax. In connection with nano-medicine therapy the effect of nanoparticles “clumping” may pose an issue, however applying short bursts of ultrasound may aid in *de-agglomerating* nanoparticles [2]. Finally recent studies has demonstrated that light sonication, may replace *cell-electroporation* used in connection with DNA-transfection of e.g. dendritic cell immunotherapy [3].

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