Laser Diodes in Optofluidics and Microfluidics

ABSTRACT

Microchips are extremely useful when studying sample sizes less than a milliliter in volume. Researchers from Massachusetts Institute of Technology and Georgia Institute of Technology both used microchips in conjunction with infrared diode lasers to conduct experiments on small-scale environments. The absorption spectrum of water dictated the wavelength choices made by the researchers. On one hand, negligible heating of the water (via absorption) was desired, so the wavelength was chosen away from an absorption peak of water. In the opposite scenario, the laser was utilized to heat up the liquid in the microchip. As such, the wavelength was aligned with a water absorption peak. In both cases, the lasers play a crucial role in the positive outcomes of the two experiments.

OVERVIEW

Microfluidics, as the name suggests, encompasses study of fluids on the micro-scale (less than mL volume). Optofluidics builds upon this idea, incorporating optics into the mix.

Both fields can utilize custom-built microchips made out of materials such as plexiglass. These microchips are fabricated to precisely control various experimental parameters, such as sample temperature, laser absorption, and reaction volume.

Performing experiments on the micro-scale is important, as described by [1]:

"The need to isolate small numbers of specific cells from background populations is ubiquitous, with applications in pathology, clinical diagnosis, cloning, and cell biology research."

This sentiment is echoed in [2]:

"There is therefore a need for technologies capable of high temporal frequency sampling...to measure gene expression kinetics in single cells or cell populations in real time."

Both [1] and [2] report results where lasers are used in a crucial portion of the experiment. The methods in which the lasers are used are quite different between the two experiments, and are further described in the next section. In both cases, however, precision and stability are important parameters.

ROLE OF LASERS

LASERS IN OPTOFLUIDICS

Researchers from Massachusetts Institute of Technology laid out a grid array of 10,000 particles in microwells, ~30 µm in diameter. The particle diameter under study was ~9 µm. Once the cells were distributed in the microwells, automated fluorescence imaging and location analyzation was performed. The techniques used in [1] describe the ability to selectively sort the cells, either based on whole cell fluorescence, or on chromophore-specific fluorescence (nuclear fluorescence). **Figure 1** shows the imaging and cell removal microscope configuration used.

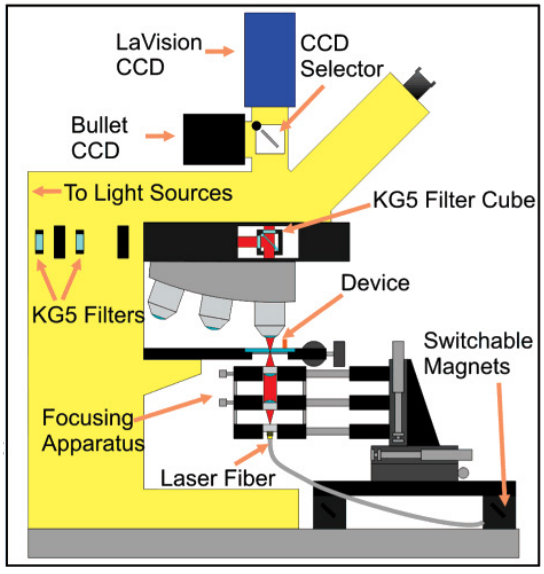

Figure 1. Experimental configuration used for both imaging and cell removal. Adapted with permission from [1]. Copyright 2007 American Chemical Society.

Once sorted, based on either fluorescence method, particles exhibiting the desired parameters were then removed from the microwells using an infrared diode laser (emitting at 980 nm, fiber-coupled). The location of the desired cells was stored during the fluorescence measurements, and the laser beam was then steered to these locations to remove the particles.

Removal of the particles was accomplished via a method similar to optical tweezing. **Figure 2** shows the removal sequence. The microchip structure was designed to allow transmission of infrared light. The optical scattering force, once focused on a particle, then levitated the particle vertically out of the well, allowing the fluid flow to remove it from the microgrid array for storage. Once the beam was aligned to a chosen spot, it took between 3-10 seconds to remove the particle from the well.

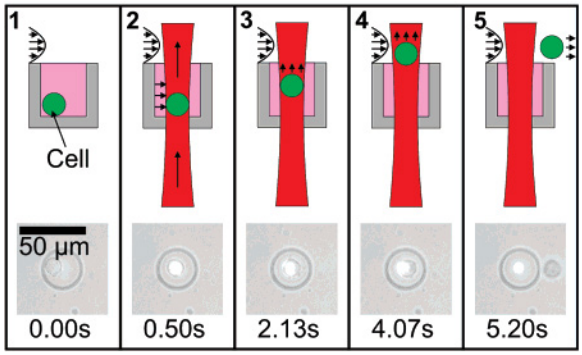

Figure 2. Cell levitation and removal sequence. Adapted with permission from [1]. Copyright 2007 American Chemical Society.

Infrared light was chosen for two main factors. First, infrared light has much higher damage thresholds for cells, allowing for enough optical power to reach the cell to levitate it out of the array without damaging it. Second, water is not strongly absorbing at the wavelength chosen, producing low cell temperature changes. This again allows for minimal cell damage during the process.

LASERS IN MICROFLUIDICS

Researchers from Georgia Institute of Technology utilized the laser for the opposite approach. Instead of choosing a wavelength where water absorption does not contribute meaningfully to the temperature of the medium, they utilized a water absorption peak to vary the temperature of the microchip [2].

In this way, the temperature range required for polymerase chain reaction (PCR) could be achieved. This PCR required the cell's medium to vary in temperature between 72°C and 93°C (shown in **Figure 3**) in 50 second cycles.

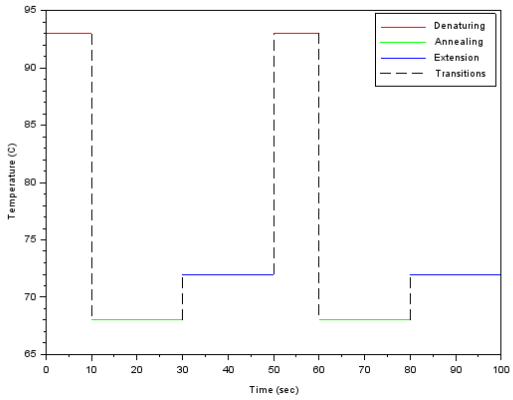

Figure 3. Representation of two thermal cycles used for the microchip PCR. For the 500 base-pair PCR reported, 30 cycles were used. Data from [2].

Again, the microchip was designed to allow transmission of light (here a 1450 nm infrared diode laser) through the base of the microchip, with negligible heating of the chip itself. 1450 nm was chosen due to its alignment with an absorption peak of water, enhancing the temperature change of the water (the cell's surrounding medium) upon radiation.

The heating and cooling cycles required for PCR are well known. In this application, a calibration between the laser setpoint voltage (i.e. output current) and the temperature of the water needed to be applied to ensure the cycling was easily and precisely repeatable. This was achieved empirically, by varying the driving voltage of the laser and recording the corresponding temperature of the water.

To collect the data for the calibration, the voltage of the laser was changed, and radiation allowed to occur for 2 minutes to reach a steady-state temperature of the fluid. To reach the desired temperatures, the entire operation range of the laser was used.

Once the calibration was finalized, it was found that the heating and cooling rates were on the order of 3°C/s, with cooling having a slightly higher rate than heating.

RESULTS

OPTOFLUIDIC RESULTS

The results of [1] suggest that utilizing a wider area beam to levitate the particles out of the well is possible. Pairing this with the selective sorting system available with multiple sorting parameters gives the method advantages over current systems employed for cell sorting. **Figure 4** shows the ability to remove selected cells (in this case, the red cells were removed).

In addition to the spatial sorting allowed via imaging, this system exposes the temporal characteristics of the particles being studied, allowing enhanced study.

The ability to sort based on either whole-cell or nuclear fluorescence is another advancement in cell-sorting. This allows for "complex phenotype sorting."

The researchers also describe the ability to scale up to allow the study of >10,000 particles easily. The main limitation of the experimental configuration is the image data analysis, due to the high number of sites.

It was found that the chosen optical characteristics of the laser (infrared wavelength and large spot size), along with the loading and unloading of particles had no impact on the viability of the particles under study.

MICROFLUIDIC RESULTS

Using a laser as the source for heating water, the experiments in [2] showed extremely repeatable results. 57% system efficiency was reached, which is slightly lower than conventional systems. It was reported that consistent amplification of DNA and RNA was possible with this system.

The temperature tuning rates were reported to be faster than conventional systems and other microfluidic PCR systems. In addition to the speed of cycling, this experimental configuration was capable of reverse transcription PCR as well.

Additional comparison of final results shows this microfluidic PCR to have comparable yield and improved specificity over conventional thermocyclers.

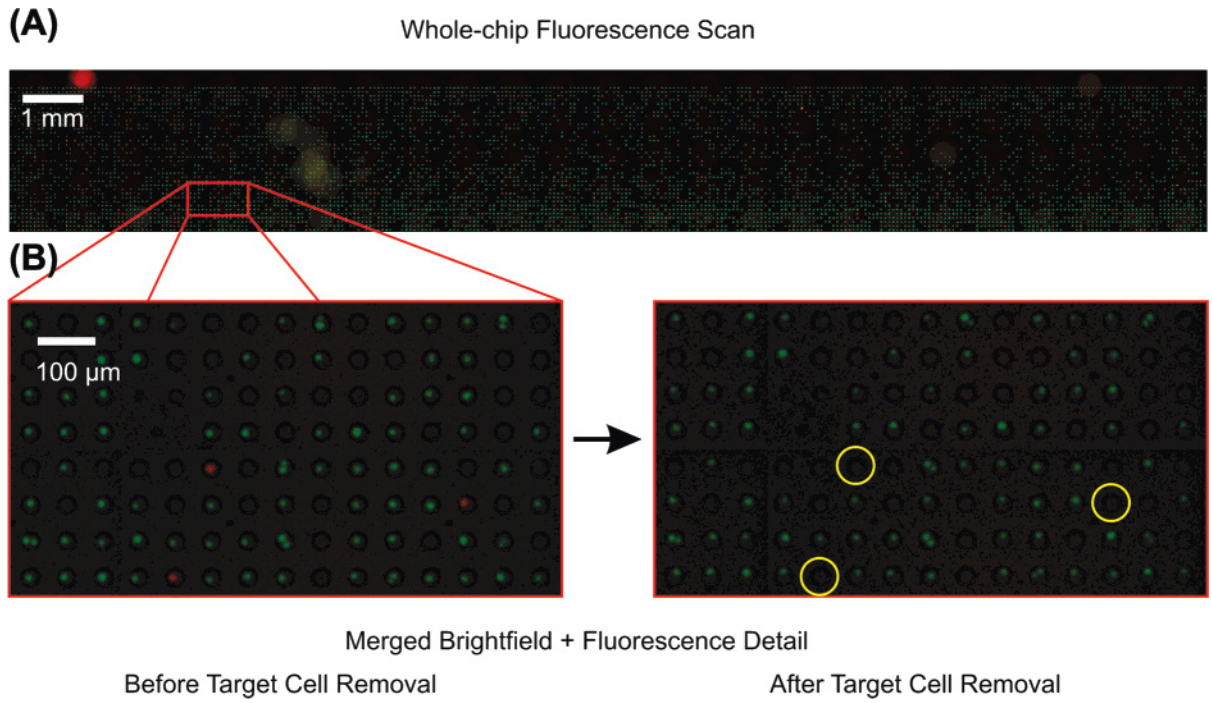

Figure 4. Using the imaging and cell removal methods described, the researchers were able to selectively remove the red colored particles from the microgrid array. Reprinted with permission from [1]. Copyright 2007 American Chemical Society.

LASER DIODE CONTROL SOLUTIONS

In both instances described here, infrared wavelength was chosen for the way it interacts with the media in the respective experiments. Additionally, the absorption spectra of water varies drastically in the infrared, and the researchers used that to their advantage.

In both cases, the laser power delivered to the microchip was a crucial experimental parameter. In [1], the power needed to be well-controlled to ensure the viability of the cells being removed. In [2], the delivered power directly impacts the results of the PCR, since PCR requires specific temperatures for each phase.

In each case, the laser driver plays a role in the quality of the output. Wavelength's LDTC2/2 utilizes the WLD3343 laser driver, which has 200 ppm stability, and was used to drive the cell-removal laser. The PLD5K-CH drove the infrared laser used to thermal cycle the PCR microchip. The PLD has <100 ppm stability, allowing precision power output of the infrared laser.

For the cell-removal experiment, the WTC3243 is used for temperature control. The WTC allows for temperature control on the order of 0.009°C. This stability minimized wavelength fluctuations.

This data is summarized below, in **Table 1**.

EXPERIMENT	CONTROLLER	STABILITY
Cell Removal	LDTC2/2	200 ppm (laser) 0.009°C (temperature)
PCR	PLD5K-CH	<100 ppm

Table 1. Stability specifications for the controllers used in the described experiments.

REFERENCES

1. J.R. Kovac and J. Voldman, "Intuitive, Image-Based Cell Sorting Using Optofluidic Cell Sorting," Anal. Chem. **79**(24), 9321-9330 (2007).

2. D.C. Saunders, G.L. Hols, C.R. Phaneuf, N. Pak, M. Marchese, N. Sondej, M. McKinnon, C.R. Forest, "Rapid, quantitative, reverse transcription PCR in a polymer microfluidic chip," Biosensors and Bioelectronics **44**, 222-228 (2013).

USEFUL LINKS

- PLD5K-CH Product Page
- LDTC2/2 Product Page

PRODUCTS USED
PLD5K-CH, LDTC2/2

KEYWORDS
optofluidic, microfluidic, cell sorting, PCR, laser control, temperature control, optical tweezers, water absorption

REVISION HISTORY

Document Number: CS-LDTC05

REVISION	DATE	NOTES
A	April 2019	Initial Release