Affordable Manufacturing of Droplet-based Microfluidics Devices

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Abstract

This article investigates the integration of droplet-based microfluidics into high school curricula to enhance scientific education. Traditional chemical reactions are usually performed in batch reactors, but continuous flow microreactors offer significant advantages such as improved efficiency, safety, and precise control over reaction conditions. Our main goal was to develop a cost-effective approach to production of such devices that enables high-schoolers to use school's equipment to research chemical, medical and biological applications of the technology. We developed a cost-effective and accessible lab-on-a-chip device using photopolymer 3D printing technology and a peristaltic pump system. The device, featuring microchannels with dimensions of 300 micrometers, allows students to observe and analyze fluid behavior under a microscope, making advanced scientific techniques accessible in a classroom setting. The fabrication process utilized photopolymer 3D printing for creating masterforms for polydimethylsiloxane (PDMS) chips, demonstrating a practical and scalable method for high school laboratories. The self-developed peristaltic pump system, controlled by Arduino, provided stable droplet formation and high flow rates, proving to be an effective alternative to conventional syringe pumps. Additionally, digital microscopy and computer vision analysis enabled detailed monitoring of droplet dynamics. This project highlights the feasibility and educational benefits of incorporating microfluidics technology into high school science programs, fostering a deeper understanding of modern scientific methods among students.

Introduction / Droplet-based microfluidics overview

Usually, chemical reactions are performed in batch reactors, such as flasks for synthesis in chemistry laboratories[1]. However, continuous flow microreactors exhibit numerous advantages that make them a revolutionary technology in chemical synthesis: small size, high surface-area-to-volume ratio of microstructures, residence time control[2], dealing with potentially hazardous substances, handling thermal runaways, and meeting efficient

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[1] Yoshida, Jun-ichi, Yusuke Takahashi, and Aiichiro Nagaki. 2013. "Flash Chemistry: Flow Chemistry That Cannot Be Done in Batch." *Chemical Communications* 49 (85): 9896–9904. https://doi.org/10.1039/C3CC44709J.

[2] Yoshida, Jun-ichi, Aiichiro Nagaki, and Takeshi Yamada. 2008. "Flash Chemistry: Fast Chemical Synthesis by Using Microreactors." *Chemistry - a European Journal* 14 (25): 7450–59. https://doi.org/10.1002/chem.200800582.

mixing requirements[3]. Innovative microfluidic devices have found widespread applications in chemical and pharmaceutical industries[4], leading to high efficiency of technological processes and yields of useful products. The potential of incorporating microfluidics technology into a curriculum has been acknowledged and explored previously, as it is not only a useful model to cover topics within science and engineering[5], but it also provides an insight into a cutting-edge field of science and exhibits numerous practical advantages: low reagent uptake, rapid analysis time and ability to observe unique liquid behavior in microchannels[6]. We are inspired by the utility and prospects of this technology and we hope to make it accessible in high school. Nevertheless, there are significant challenges in implementing microfluidics technology at the school level, because classrooms are usually not suited with high-level laboratory equipment[7]. Here, we utilize the Liquid Crystal Display (LCD) 3D printing technology and a programmed peristaltic pump system to create a lab-on-a-chip with all channel dimensions >= 300 micrometers, which can be observed under a microscope and analyzed via a computer-vision-based program. Given the fact that LCD 3D printing is an increasingly adopted technology in high schools, this device can be fabricated and modified further by educators to teach microfluidics in class.

The peristaltic pumps (x2) were purchased via an online marketplace. They provide flows from 40 ml/min up to 160 ml/min. The pumps were disassembled and connected to an Arduino, which enabled us to remotely control the pump flow. The lab-on-a-chip was

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[3] Toma Glasnov. 2016. *Continuous-Flow Chemistry in the Research Laboratory*. *Springer EBooks*. Springer Nature. https://doi.org/10.1007/978-3-319-32196-7.

[4] Bojang, Adama A., and Ho-Shing Wu. 2020. “Design, Fundamental Principles of Fabrication and Applications of Microreactors.” *Processes* 8 (8): 891. https://doi.org/10.3390/pr8080891.

[5] Wietsma, Jan Jaap, Jan T. van der Veen, Wilfred Buesink, Albert van den Berg, and Mathieu Odijk. 2017. “Lab-On-a-Chip: Frontier Science in the Classroom.” *Journal of Chemical Education* 95 (2): 267–75. https://doi.org/10.1021/acs.jchemed.7b00506.

[6] Vangunten, Matthew T, Uriah J Walker, Han G Do, and Kyle N Knust. 2019. “3D-Printed Microfluidics for Hands-on Undergraduate Laboratory Experiments.” *Journal of Chemical Education* 97 (1): 178–83. https://doi.org/10.1021/acs.jchemed.9b00620.

[7] Hemling, Melissa, John A. Crooks, Piercen M. Oliver, Katie Brenner, Jennifer Gilbertson, George C. Lisensky, and Douglas B. Weibel. 2013. “Microfluidics for High School Chemistry Students.” *Journal of Chemical Education* 91 (1): 112–15. https://doi.org/10.1021/ed4003018.

fabricated using the PDMS casting technique, for which a two-component silicone compound “Silagerm 8040” was purchased from LLC PA “Technology – Plast”.

Materials and Methods: Resin-based 3d-printing for masters fabrication

Manufacturing of lab-on-a-chip (LOC) usually involves soft lithography of PDMS on rigid masters. While the soft lithography process remained unchanged throughout our research, we had to implement a universal, budget-friendly, and straightforward way of creating rigid masters for LOCs. The chosen solution was resin-based LCD 3D-printing on Anycubic Photon 2 printer. This method provided an available, cost-effective and user-friendly approach that also ensured accuracy up to 50 micrometers in all 3 dimensions.

Alternatively, Direct Ink Writing (DIW)[8], photolithography or Fused Filament Fabrication (FFF) could be used. However, DIW printing and photolithography are not available to most scholars and are specialized methods that are challenging to use for beginners. While FFF printing is more available and cost-effective, it has proven to have significant difficulties when fabricating channels smaller than 500µm[9]. It can also be noted that resin-based 3D printing such as Sterelitography (SLA), Digital Light Processing (DLP), and LCD is getting more attention in industrial microfluidics due to its accuracy. One example being RapidBio - leading medical microfluidics company in Russia - which conducts research involving LCD.

Overall, 3D modeling is a very flexible manufacturing method since implementing changes to the design is undemanding due to the high-end 3D modeling software such as Fusion 360. The uncostly and time-efficient production process of masterforms allows high-schoolers to go through multiple iterations of the design cycle without significant labor or financial outlays.

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[8] Ching, Terry, et al. “Fabrication of Integrated Microfluidic Devices by Direct Ink Writing (DIW) 3D Printing.” *Sensors and Actuators B: Chemical*, vol. 297, Oct. 2019, p. 126609, www.sciencedirect.com/science/article/pii/S0925400519307981, https://doi.org/10.1016/j.snb.2019.05.086.

[9] Karayannis, Panagiotis, et al. “3D-Printed Lab-On-a-Chip Diagnostic Systems-Developing a Safe-By-Design Manufacturing Approach.” *Micromachines*, vol. 10, no. 12, 28 Nov. 2019, p. 825, https://doi.org/10.3390/mi10120825.

Materials and Methods: Design and creation of droplet-based masters

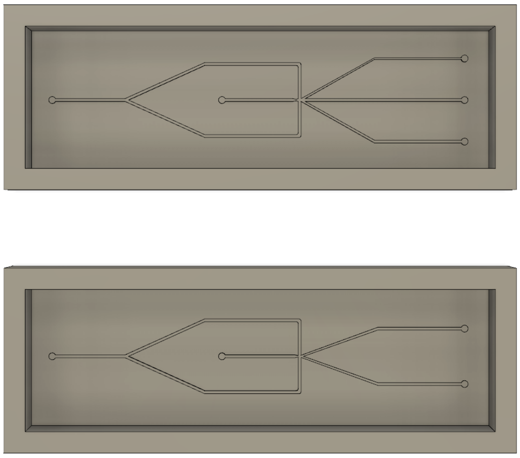

Figure 1: 3D models of droplet-based LOCs in Fusion360. Outlet is placed in the central part of the chip, while inlets are located on the sides. 3-input and 2-input chips are shown.

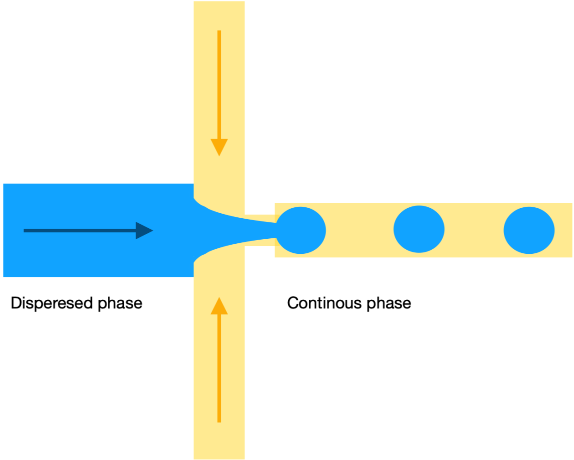

Figure 2: Planar flow focusing water-in-oil-out droplet formation. Mechanism of droplet formation. Inspired by [10]

The masters were modeled in Fusion360. Width of the channels varies from 200 to 300µm; height of the master is 20mm; therefore the resulting PDMS chip is thick, which secures inlets and outlets placement. The chosen way of droplet formation is planar flow

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[10] Nan, Lang, et al. “Development and Future of Droplet Microfluidics.” *Lab on a Chip*, vol. 24, no. 5, 1 Jan. 2024, pp. 1135–1153, https://doi.org/10.1039/d3lc00729d.

focusing (Figure 2) with water-in-oil-out droplets. The incoming liquids channels could vary in quantity as shown on Figure 1: two incoming liquids for reactions that involve two reactants (such as an indicator-base reaction) and three incoming liquids for reactions that involve three reactants (such as synthesis of magnetic iron oxide nanoparticles, or the Briggs-Rauscher oscillating reaction).

The full protocol of manufacturing a master form is composed of printing, washing in isopropanol, curing, washing it again, and finally letting it rest for approximately 24 hours. The thorough washing and resting stages ensure that no liquid photopolymer is still present as it will negatively affect the purity of the PDMS lab-on-chip.[11]

Materials and Methods: Molding the polydimethylsiloxane (PDMS) lab-on-a-chip

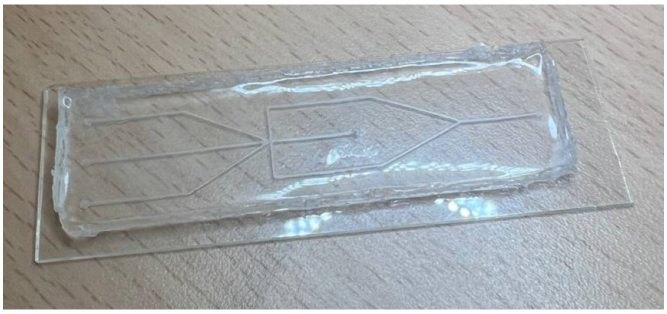

Figure 3: Lab-on-a-chip

The process of manufacturing a lab-on-a-chip (LOC) is straightforward and rarely implies any deviations from the standard procedure used by the majority of microfluidics companies across the world. First, the PDMS agents are mixed and placed in the master, degassed, and cured at approximately 70˚C; however, our masters were 3D printed which lowered the curing temperature to 50˚C, preventing masters from melting and increasing curing time. After that the chip is glued to either another PDMS slab or a glass slide, using air plasma surface activation method[12]. However, the majority of schools do not have access to such specialized equipment, and the most available way to sew together the chip and a glass slide is by silicone-based hermetic glue. Glue spread by a thin layer did not clog microchannels but still managed to keep the chip hermetic. After inlets and outlets were installed the chip was completely ready for the experiments.

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[11] Štaffová, Martina, et al. "3D Printing and Post-Curing Optimization of Photopolymerized Structures: Basic Concepts and Effective Tools for Improved Thermomechanical Properties." *Polymer Testing*, vol. 108, Apr. 2022, p. 107499, https://doi.org/10.1016/j.polymertesting.2022.107499.

[12] M.R. Howlader, et al. *Sequential Plasma Activation Process for Microfluidics Packaging at Room Temperature*. 28 July 2005, https://doi.org/10.1109/ectc.2005.1441332.

Materials and Methods: Design and creation of peristaltic pumps system

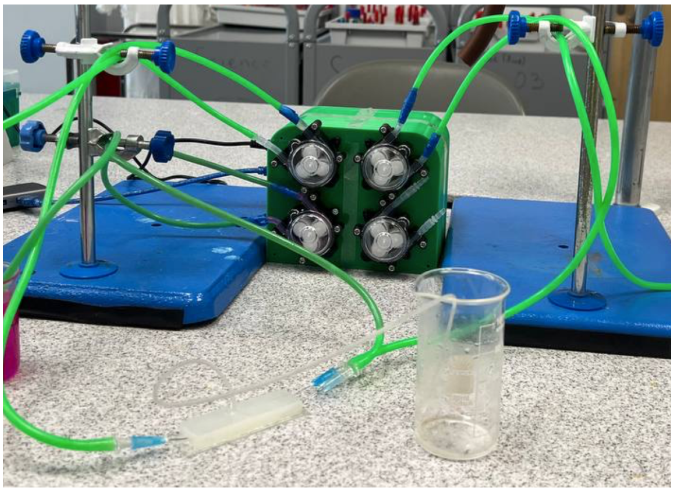

Figure 4: Peristaltic pumps system with the LOC. The following setup was at testing stage for the protoype of 2-input chip.

Fluid delivery method is a crucial element of the experimental setup. Active pumping, which is the most suitable option for droplet generation, comes in different forms such as pressure controllers, centrifugal pumps, and syringe pumps. Although providing high precision, extensive flow range, and low pulsatility, all the listed methods are not budget-friendly and do not ensure ease for beginners. In contrast, peristaltic pumps are cheap, undemanding in maintenance, and reliable due to their simple stepper mechanism.[13] In rare cases, a single perilstaltic pump can be sufficient to achieve droplet formation (Figure 5), but that proved to be a non-reliable method that lack demonstrative function that is important in a classroom setting.

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[13] Marquet, Colin. “Peristaltic Pumps: A Comprehensive Guide.” *Darwin Microfluidics*, 23 Feb. 2023, blog.darwin-microfluidics.com/peristaltic-pumps-a-comprehensive-guide/.

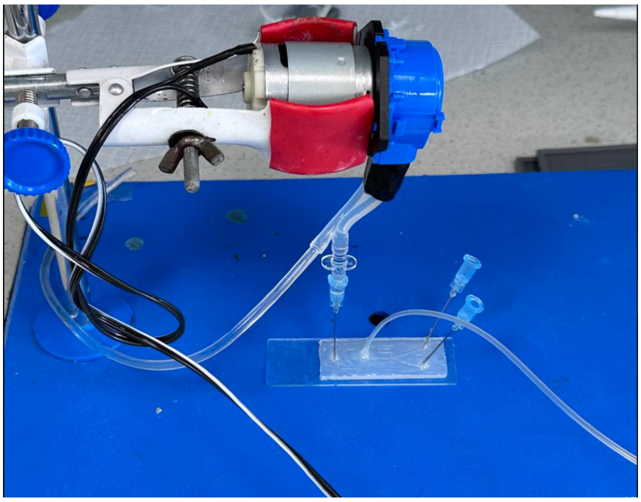

Figure 5: Early prototype of the pump system. Single oil input is connected to a perilstaltic pump - other two inputs were controlled manually.

Originally, 4 pumps were ordered via an online marketplace for a cost of approximately $30 each. It was decided to place all 4 pumps in one 3d-printed enclosure (Figure 4), saving space and enabling multitasking of the pumps: 3 reactant fluid pumps and 1 oil pump. The system was controlled via two Arduino motor shields using pulse-width modulation. Fluid delivery rate for each pump can be updated during the experiment which opens opportunities for dynamic regulation of the droplet generation rate which affects droplet size as well[14]. The program for the Arduino microcontroller initialized the motors rotation and updated them accordingly to the console inputs. Taking in consideration every component of the system, the cost for each pump summed up to approximately $48. Compared to the cost of an average syringe pump,[15] the cost of a peristaltic pump is less than half of that, which can be of drastic difference for some high-school labaratories.

Further tests showed that droplet formation is not that troubled by pulsatility on higher flow rates as it was expected. The peristaltic pumps have proved to be a suitable option for droplet-based microfluidics in our case — general research and introduction to microfluidics.

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[14] Hong, Yiping, and Fujun Wang. “Flow Rate Effect on Droplet Control in a Co-Flowing Microfluidic Device.” *Microfluidics and Nanofluidics*, vol. 3, no. 3, 30 Nov. 2006, pp. 341–346, https://doi.org/10.1007/s10404-006-0134-3.

[15] Iakovlev, Aleksei P., et al. “Novel Pumping Methods for Microfluidic Devices: A Comprehensive Review.” *Biosensors*, vol. 12, no. 11, 1 Nov. 2022, p. 956, https://doi.org/10.3390/bios12110956.

Materials and Methods: Digital microscopy

To verify the formation of liposomes and observe the fluid dynamics, our team has proposed an integration of a digital microscope to capture high-quality images and videos of the liposome droplets. Digital microscopy gained its prevalence during Covid-19[16] and since then has been implemented in many educational institutions. This method offers superior convenience for capturing and storing images without sacrificing any of the benefits of traditional microscopy and is used more often when dealing with microfluidics[17]. Beyond high-resolution imaging and efficient storage capabilities, the digital microscope facilitates the implementation of a computer vision program that might be used as a way to perform quantitative analysis inside the microfluidic channel.

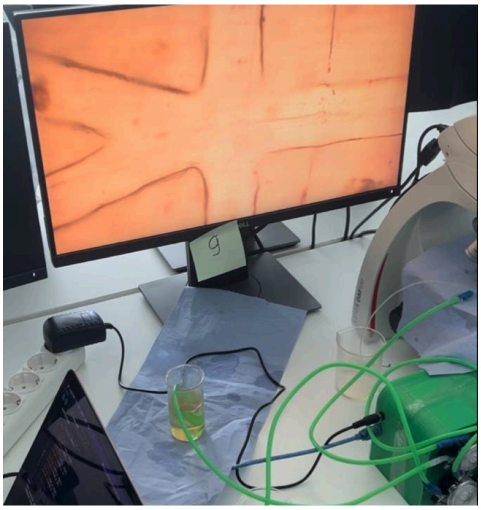

Figure 6: Channels of LOC displayed on a monitor.

The [model] digital microscope was precisely calibrated to document the formation and flow of liposomes, with real-time display on a nearby monitor (Figure 6). The video feed from the monitor was transmitted to a computer, enabling further processing and analysis with ease.

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[16] Sreeshyla, Huchanahalli Sheshanna, et al. “Digital Microscopy: A Routine Mandate in Future? A Leaf out of Covid-19 Pandemic Laboratory Experience.” *Journal of Oral and Maxillofacial Pathology*, vol. 27, no. 1, 1 Jan. 2023, p. 162, journals.lww.com/jpat/Fulltext/2023/27010/Digital_microscopy__A_routine_mandate_in_future__A.28.aspx, https://doi.org/10.4103/jomfp.jomfp_111_22.

[17] Funano, Shun-ichi, et al. “A Simple and Reversible Glass–Glass Bonding Method to Construct a Microfluidic Device and Its Application for Cell Recovery.” Lab on a Chip, vol. 21, no. 11, 2021, pp. 2244–2254, https://doi.org/10.1039/d1lc00058f.

Materials and Methods: Computer Vision Analysis of the Droplet Flow

To accurately monitor and analyze droplet formations in our microfluidic system, a computer vision system was integrated into our digital microscope setup. Using Python and OpenCV we developed a program to track droplet size and speed. High-resolution video feed from the digital microscope is converted into frames post-processed to enhance visibility and reduce noise.[18] While the computer vision model was mostly tested and trained on video feed of microfluidic droplets on open-source platforms like YouTube [19], we were able to run it on the actual captured feed. The program identifies circle-like shapes, calculates the average diameter, and with certain calibration, depending on the microscope's magnification, it was able to approximate flow's speed.

Results

As a model experiment, we decided to perform a base-indicator reaction inside the LOC, which requires accessible reagents: sodium hydroxide (NaOH) and phenolphthalein, which makes the mixture of two colorless solutions turn violet. The reactants were delivered into the double-channeled lab-on-a-chip device (Figure 1), reacted upon contact, and their purple mixture pinched off as water-in-oil droplets, also referred to as liposomes. This simple experiment enabled us to demonstrate the success of our device in stable and continuous droplet formation, as the resulting liposomes are visible in the sink tube (Figure 7). We managed to analyze image input of our setup, and got meaningful insights about droplet flow. We observed that droplet diameter ranged between 100 and 150 micrometers.

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[18] Durve, Mihir, Adriano Tiribocchi, Fabio Bonaccorso, Andrea Montessori, Marco Lauricella, Michał Bogdan, Jan Guzowski, and Sauro Succi. 2022. "DropTrack—Automatic Droplet Tracking with YOLOv5 and DeepSORT for Microfluidic Applications." *Physics of Fluids* 34 (8): 082003. https://doi.org/10.1063/5.0097597.

[19] Fluigent. "Microfluidic High Speed Droplet Generation." *YouTube*, 16 Nov. 2012, www.youtube.com/watch?v=9f1f1BRGnuo.

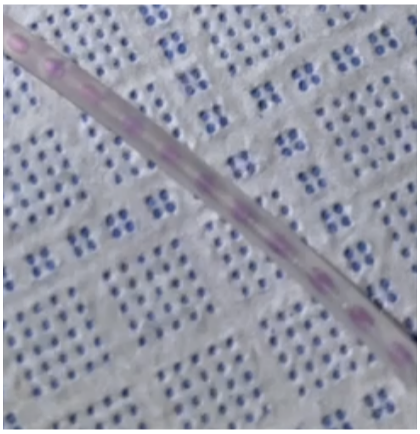

Figure 7. Base and indicator microdroplets in the sink tube.

On the financial side, the cost of this specific setup totalled $250, including additional materials like microtubes, sodium hydroxide, indicator and 3D-printing polymer. Taking in consideration that the setup is reusable, some high-school labarotories could be interested in conducting such experiments with relatively low impact on their budget.

Discussion

In this investigation, we have demonstrated how microfluidics technology can be cost-effectively implemented to perform chemical reactions and investigate them in the high school laboratory. The microreactor may be used for chemical synthesis, investigation of droplet formation, which can be used as a hands-on experience for chemistry students involving cutting-edge science. In the future, we plan to integrate computer vision technology into our Arduino-controlled pump system, allowing for dynamic adjustments to flow rates, thereby ensuring consistent and reproducible droplet formation.[20] By implementing advanced image processing combined with real-time pump control, high-school labaratories and sciences departments will be able to make a high-level microfluidic technology intuitive for hands-on access for even high school students in a field where it was previously unavailable.[21] The described procedure is accessible, does not require specialized equipment, and can be used or modified further for various

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[20] Mihir Durve, Sibilla Orsini, Adriano Tiribocchi, Andrea Montessori, Jean-Michel Tucny, Marco Lauricella, Andrea Camposeo, Dario Pisignano, and Sauro Succi. 2023. “Benchmarking YOLOv5 and YOLOv7 Models with DeepSORT for Droplet Tracking Applications.” *European Physical Journal E* 46 (5). https://doi.org/10.1140/epje/s10189-023-00290-x.

[21] Konry, Tania, Alexander Golberg, and Martin Yarmush. 2013. “Live Single Cell Functional Phenotyping in Droplet Nano-Liter Reactors.” *Scientific Reports* 3 (1). https://doi.org/10.1038/srep03179.

purposes. Besides, the microchannels may be successfully visualized and magnified using digital microscopy, and the quantitative parameters of droplets inside the device may be monitored and analyzed via a computer vision program, which simplifies the quantitative investigation of droplets. Having described the project that we have created, we believe that our insights can facilitate development of microfluidics in the high school settings.

Acknowledgements

Our team is grateful for Mikhailov V.N. and Zhukov A.S. for creating and sharing «Modern synthetic methods in chemistry: the path from flask to chip», which navigated us through the common methodology, manufacturing process and model experiments in microfluidics. We are as well greatful for the Letovo School resource center that provided required materials, placed orders on our behalf, and funded purchase of PDMS and perilstaltic pumps.

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