Label-free monitoring and manipulation of microfluidic water-in-oil droplets

Christoph Frey\textsuperscript{1,2,\#}, Jonas Pfeil\textsuperscript{1,\#}, Tobias Neckernuss\textsuperscript{3}, Daniel Geiger\textsuperscript{3}, Klaus Weishaupt\textsuperscript{1,2}, Ilia Platzman\textsuperscript{1,2}, Othmar Marti\textsuperscript{3}, Joachim P. Spatz\textsuperscript{1,2,4}

\textsuperscript{1} Department of Cellular Biophysics, Max Planck Institute for Medical Research, Heidelberg, Germany
\textsuperscript{2} Institute for Molecular Systems Engineering, University of Heidelberg, Heidelberg, Germany
\textsuperscript{3} Institute of Experimental Physics, University of Ulm, Ulm, Germany
\textsuperscript{4} Max Planck School Matter to Life, Heidelberg, Germany

Correspondence
Tobias Neckernuss, Institute of Experimental Physics, University of Ulm, Albert-Einstein-Allee II, 89081 Ulm, Germany.
Email: tobias.neckernuss@uni-ulm.de
Ilia Platzman, Joachim P. Spatz, Department of Cellular Biophysics, Max Planck Institute for Medical Research, Jahnstraße 29, 69120 Heidelberg, Germany.
Email: ilia.platzman@mr.mpg.de; joachim.spatz@mr.mpg.de

\#These authors contributed equally to this work.

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Abstract
Droplet-based microfluidic technology offers several benefits: the integration of multiple laboratory functions into a single microfabricated chip, manual intervention possibilities, minimal sample consumption, and increased analysis speed and data precision. These advantages have boosted the widespread application of this technology in biological and biomedical research. Despite recent progress, considerable challenges remain to be addressed for end-user applications. This especially concerns the difficulty of creating powerful and easy to implement methods for real-time analysis and active manipulation of passing droplets. Toward this end, we developed a very sensitive optical device equipped with smart algorithms for real-time label-free monitoring and active manipulation of passing droplets. We demonstrate the advanced properties of the developed optical device by measuring different droplet production parameters as well as the label-free detection of cells in droplets. Moreover, the newly developed technology was connected with a function generator system to allow for subsequent manipulation of the monitored droplets based on the measured parameters. As an example, we performed electric field-mediated, label-free sorting of cell-containing droplets from empty ones. Furthermore, we achieved an efficient size-based separation of droplets. We envision that the developed optical device will be a useful tool for the online monitoring of passing droplets and will be implemented for the integration and automation of various droplet-based microfluidic functional units.

KEYWORDS
active droplet manipulation, droplet-based microfluidics, high-throughput, label-free, real-time observation
INTRODUCTION

High-throughput droplet-based microfluidics have become a powerful technology for diverse biological and biomedical applications, including, but not limited to, single-cell analysis,[1,2] drug screening,[3] and synthetic biology.[4] During droplet production, different components, from single molecules to micrometer-sized objects like cells, can be encapsulated. Thereby, each microfluidic water-in-oil droplet represents a self-contained microreactor that is suitable for the high-throughput observation of its internal chemical and biological interactions among large populations.

To date, several real-time monitoring techniques have been developed to obtain precise information about droplet content and production parameters. Most of these monitoring techniques rely on fluorescence labeling of the droplets or their content.[5] Fluorescent staining became a powerful tool for analyzing cellular processes and dynamics on the single-cell level and facilitates fast recognition at the high-throughput droplet-based microfluidic application. At the same time, the fluorescence labeling processes can affect cell behavior or even be toxic to them, thereby compromising their potential clinical use in biomedical applications.[6]

To overcome the drawbacks related to fluorescence-based detection, several label-free monitoring methods have been developed to get a precise information about droplet production parameters such as size, production rate, and droplets content. These methods include software-based offline analysis of recorded videos,[7] real-time intensity measurements by using a photodiode as a camera trigger,[8] and real-time impedimetric measurements.[9] Another method such as differential detection photothermal interferometry (DDPI) relies on the measurement of light absorbance and has been adapted as a detection scheme for in-droplet colorimetric assays.[10] In the context of label-free cell monitoring, it is also important to mention the method that is based on opto-acousto-fluidic microscopy. By the sensing of acoustic waves induced by the intrinsic light-absorbance of matter this method allowed for three-dimensional detection of droplet shape and its content.[11] However, despite the remarkable progress these technologies are either slow in the detection rate or highly advanced and therefore complex to set up for end user. Moreover, due to their complexity most of these techniques would require substantial efforts for their integration toward droplet manipulation.

Here, we present a newly designed optical device for easy-to-implement label-free observation, characterization, and active manipulation of passing water-in-oil droplets. The device consists of a fast and sensitive CMOS camera sensor, an integrated data processing unit, and custom algorithms and can be simply installed into the optical path of a microscope. The brightfield images of the passing objects are evaluated in real-time manner on a field-programmable gate array (FPGA) using the developed algorithms. To achieve real-time monitoring, only the necessary information gets extracted from the taken images. The data extraction allows image analysis with ultra-short and fixed latency of only a few microseconds at frame rates of >10 000 fps over unlimited time ranges. We determined the sensitivity and error rate of the device by analyzing the droplet production rate, droplet size, and distance between droplets and then comparing the values to manually analyzed high-speed camera videos.

Moreover, we employed the developed device for label-free droplet content analysis, including the distinction between empty and cell-loaded droplets. Furthermore, we also demonstrate the ability of the developed optical device to generate a trigger signal each time a droplet with previous selected physical parameters passes the region of interest (ROI). This signal can be used to control a function generator that triggers electrodes in the microfluidic device for the manipulation of the passing water-in-oil droplets. As an example, for the active droplet manipulation we used the optical device to trigger droplet sorting based on droplet parameters such as the droplet size and the droplet content (i.e., cell-laden droplet vs empty droplet). The developed optical device requires the implementation of a single sensor-computing unit. Therefore, in comparison to other real-time intensity measurement systems,[6] the system complexity is reduced leading to faster droplet manipulation capabilities due to deterministic latency of <200 μs and a sustainable rate of 10 000 Hz. We envision that the developed optical device will expand the droplet-based microfluidic toolbox for biomedical, cell biology, and synthetic biology applications.

MATERIALS AND METHODS

2.1 Working principle of the optical device

Our optical device consists of an image sensor (PYTHON1300, ON-Semiconductor, USA) in which different freely configurable regions of interest (ROI) can be selected. In order to reduce the amount of data and to analyze the reduced dataset in real-time, the image data are processed directly on an FPGA (XCY7Z020CLG400-1, Xilinx, USA). Figure 1A illustrates the workflow. After the sensor captures an image, the background image is subtracted prior to image analysis. Dynamic background subtraction is done automatically based on the last 128 pictures. This reduces the susceptibility of the
FIGURE 1  Schematic representation of the data analysis flow: (A) The overview illustrates the entire workflow of the device. Images are captured by the optical device at a rate of 288 MB/s. An orthogonal projection of the intensity, either with or without prior binarization, reduces the data rate to 4 MB/s. After signal analysis and parameter calculation, the rate is reduced to 0.5 MB/s and enables further real-time processing. (B) An example of the binarization, projection, and height signal analysis for height detection of a droplet with an encapsulated cell is shown. The threshold needs to be set by the user.

image to slow changes, particularly in the occurrence of background illumination changes or slow vibrations and drift. Subsequently, two different data processing branches are computed in parallel to reduce the data rate. Both have in common that the intensity (i.e., the gray value) is projected to an orthonormal basis, either with or without prior binarization of the image. In the former case, an individually determined gray-value threshold is applied and for all further computations all pixels above the threshold become white, all below black (Figure 1B). The projection of the intensity is performed by integrating the pixel values of the preprocessed images, row by row horizontally and column by column vertically, leading to two orthogonal intensity vectors. This reduces the dimensionality of the data stream from: \( \text{data rate} \propto \text{image height} \times \text{image length} \times \text{frame rate} \) to \( \text{data rate} \propto (\text{image height} + \text{image length}) \times \text{frame rate} \). The definition of the height and length are explained in the supporting information (Figure S1).

The set of four intensity vectors is analyzed in the signal analysis step. For example, the area or size of the object can be determined by calculating the sum of the elements in either one of the vectors obtained from the binarized image. Parameter extraction from the image is shown exemplarily in Figure 1B where the height of an object is calculated by application of a threshold to the signals and intersection calculations based on these. As an additional parameter and excellent marker of a particle within the field of view, the summed squared difference (SSD) between the image and the background is determined. To achieve this, the squared intensity difference between the image and the background is calculated pixelwise and summed over the whole field of view. High values indicate that an object moves through the ROI. Furthermore, the total value of this measure is directly related to the optical contrast present in an image such that for a given object size the SSD is a sensitive marker for the content of an object. In total, the device is able to calculate more than 30 values (e.g., size, shape, position, morphology, velocity, orientation, brightness, absorption, and granularity) for each object simultaneously. Of these, we used a subset of parameters required for sufficient classification of the relevant subpopulation of empty or cell-laden droplets. However, it is important to mention that for more complex sorting
tasks additional parameters can be added without affecting sorting speed or response time. The described data reduction steps and the converting of complex image data to single parameters directly on the device is the reason why the presented technique is superior to all conventional label-free analysis. The entire measurement is documented and the analysis results for each passing object and each parameter is stored in a permanent memory. The sensor also contains a bypass of the analysis pipeline, thus enabling the recording of a short movie of the raw image sensor data. The high data rates limit the length of such movies to several seconds. The movies can later be used to validate the correct functioning of the sensor, as the result of the image pipeline are saved simultaneously.

For droplet manipulations like label-free sorting, the resulting scalar parameters are transmitted to a real-time softcore processor (Figure 1A). This enables deterministic runtimes, since it runs entirely on SRAM memory and, therefore, has no uncontrollable latencies. Any parameter range and combination that differentiates the objects can be chosen for the manipulation. For example, droplets with cells and without cells are clearly separated by their size and SSD value (see the description in the analysis of the content of water-in-oil-droplets part). The device automatically creates, based on chosen parameters, a program that runs on the real-time softcore processor, which then provides a gating signal (3.3V TTL) to an external output.

2.2 Production of polymer-stabilized water-in-oil droplets

Stable water-in-oil droplets were produced in the flow-focusing junction of the droplet production device (Figure S2a). A continuous HFE 7500 oil-phase (3 M, USA) containing 3 wt% perfluoropolyether-polyethylene glycol (PFPE-PEG) block-copolymer fluorosurfactants (Ran Biotechnologies, Inc., USA) and an aqueous-phase were injected into the device using syringe pumps (11 PicoPlus Elite, Harvard Apparatus, USA) and an aqueous-phase was injected into the device using syringe pumps (11 PicoPlus Elite, Harvard Apparatus, USA). One milliliter syringes (Omnilix®, F. Braun, Germany) connected by a cannula (Sterican®, 0.4 × 20 mm², BL/LB, F. Braun, Germany), and PTFE-tubing (0.4 × 0.9 mm, Bola, Germany) for liquid transfer. Different droplet production rates, droplet sizes, and distances between droplets were generated by adjusting the flow rates of the continuous aqueous and oil phases between 100 and 250 μL/h and 200 and 500 μL/h, respectively. To validate the performance of the optical device and to obtain visualized statistics on the droplet production parameters, a high-speed camera (Phantom 7.2, Vision Research, USA) was utilized to record videos of each experimental condition. The recorded high-speed camera videos were manually analyzed and compared with the obtained data from the developed optical device. Toward this end, we manually analyzed three videos of each flow condition including 50 droplets per video. With the sensor we measured for the respective flow rates (μL/h) a certain number of droplets. (100aq 200oil: 2033 droplets, 150aq 300oil: 2980 droplets, 200aq 400oil: 8798 droplets, 250aq 500oil: 13307 droplets).

2.3 Cell encapsulation into polymer-stabilized water-in-oil droplets

Chinese hamster ovary (CHO) suspension cells (Public Health England) were cultured in protein free medium (EX-CELL® ACF DHO Medium, Sigma-Aldrich, Germany) supplemented with 4 mM L-glutamine (Gibco® L-Glutamine, ThermoFisher, USA). Approximately 2 × 10⁶ cells in 100 μL medium were encapsulated into water-in-oil droplets at 600 μL/h aqueous phase flow rate. The associated flow rate for the oil phase was set to 500 μL/h. The droplet size with those flow rates was around 42 μm. The size of CHO suspension cells is between 14 and 15 μm.[13]

2.4 Label-free sorting based on droplet content and droplet size

For label-free droplet sorting based on their content, previously generated droplets with encapsulated CHO cells were injected into the microfluidic device (Pneumatic flow controller MFCS™-EZ, Fluigent, Germany) at 145 mbar (Figure S2b,c). To avoid electric field-mediated droplet coalescence and to achieve high sorting efficiency, a sufficient distance between the droplets is required. Therefore, pure HFE 7500 oil was injected into the separation oil inlet channel of the microfluidic device at 180 mbar (Figure S2b). Two populations of water-in-oil droplets were generated for sorting according to droplet size. Droplets of 35 μm diameter were generated using flow rates of 400 and 500 μL/h for the aqueous and oil phase, respectively. The bigger droplets with a diameter of 42 μm were generated with flow rates of 600 and 500 μL/h for aqueous and oil phase, respectively. The two populations were collected and mixed prior to injection into the microfluidic device at 90 mbar. The pressure for the separation oil was set to 125 mbar. For real-time, label-free sorting by the optical device, the ROI for detecting passing droplets was set in proximity to the electrode array and the Y-shaped channel (Figure 2). The electric field was set to 900 V at 4 kHz. The field was generated using a function generator (HM8150, Rohde & Schwarz, Germany) gated by the trigger signal of the
optical device, which had a sine output of 4.5 V amplitude and 4 kHz at 4.5 V DC offset amplified by a 100x piezo amplifier (Model 2210, Trek, USA). Figure 2 shows a schematic drawing of the setup for label-free droplet sorting using our novel optical sensor.

3 | RESULTS AND DISCUSSION

3.1 | Method validation

To validate the functionality and sensitivity of the developed method, we analyzed droplet production parameters under different flow rates and compared the online measured results of the device with manually analyzed high-speed camera videos of the same experiment. Figure 3A shows the ROI of passing droplets differing in size and spacing. As can be observed, the increased flow rates lead to smaller droplets with shorter spacing and therefore higher droplet production rates (Figure S3). To determine droplet production rates in real time, the distance between droplets and changes in droplets size were determined from bright-field signals at each flow rate using the optical device. These measurements were then compared to the results obtained by manual high-speed camera observations. Figure 3B shows droplet production rates obtained by the optical device and manual analysis in comparison. For the highest flow rates, the optical device measured a droplet production rate of 1740 Hz, similar to the manually determined rate of 1756 Hz. In the cases of lower flow rates, the obtained results were 694 versus 694, 285 versus 277, and 193 versus 194 Hz for the sensor versus manual measurements, respectively. The analysis of the droplet length (Figure 3C) revealed similar results for both methods. For example, at the highest flow rate the average droplet length measured with the device was 46.9 ± 0.8 μm compared to 46.8 ± 0.8 μm measured manually. The highest deviation in droplet length was obtained at the lowest flow rate. In this case, the average droplet lengths obtained from the optical device and manual measurements were 142.4 ± 2.7 and 139.0 ± 1.0 μm, respectively. In contrast to droplet length, droplet population densities (in other words, the space between droplets) showed the biggest difference at the highest flow rate. As shown in Figure 3D, interdroplet distances measured by the optical device and manual measurements were 40.2 ± 0.8 and 51.3 ± 2.0 μm, respectively. For detailed information see Table S1 in the supporting information, which summarizes all
FIGURE 3  Quantification of different droplet production parameters. (A) Representative bright-field images obtained with the optical device show droplets produced at different oil and aqueous flow rates. The corresponding oil and aqueous flow rates can be found above the image and the plot markers for the scatter plots are shown to the left of each image. Images (B) (C) and (D) show scatter plots of the quantification of droplet production rates, droplet size, and the distance between consecutive droplets obtained by optical device measurements as well as manual droplet counting from high-speed camera videos at the different flow rates. Error bars indicate the standard deviation of 3 independent experiments. The spectrogram (E) shows the variation of the droplet production rate over a measurement time of 5 s due to instabilities in the microfluidic system.

The differences between the hand counted and automatic determined values as computed by the sensor can be attributed to drifts in the experiment. In Figure 3E, a spectrogram of the droplet rate during the measurement with the highest flow rate is shown. The main peak (attributed to droplet production rate) varies by about 50 droplets per second over the measurement time. With a smaller ripple in the timescale of 0.1 s. These variations are caused by combined effects of all elastic elements in the microfluidic system, friction in mechanical components, and instabilities of the syringe pumps. This explains the relatively large error bars for the automatic frequency counting since it averaged over the whole measurement time including 9233 droplets while...
for manual counting only 150 droplets were considered. This continuous quality monitoring of microfluidic systems is not possible with conventional techniques within reasonable time and effort and opens a wide range of applications for the here presented system. Overall, the similarity in the obtained results proves the reliability and high sensitivity of the optical device for real-time analysis of droplet-based microfluidic processes.

3.2 Analysis of the content of water-in-oil droplets

The produced water-in-oil droplets form self-contained, stable microcompartments suitable for single-cell assays for various biomedical applications. In most of these studies, staining of cells was used for their monitoring. Labeling of cells helps in their recognition but, nevertheless, can affect cell behavior and thereby compromise potential clinical use. Therefore, we set out to assess the abilities of the developed optical system for label-free detection of the droplet content in real time. In particular, we aimed to determine the percentage of cell-laden droplets the exact number of encapsulated cells, and, if possible, their condition. The simplest method to determine the content of a droplet is shown in Figure 4A where the SSD between the intensity values of an image to the background image is plotted over the measurement time. Each droplet passing the ROI leads to a significant signal increase. The height of the peak indicates the amount of brightness moving through the image. Because cells appear as bright spots, droplets containing cells have higher peaks. However, the signal strength not only depends on the content but also on variations in the droplet size and on the optical properties of the encapsulated object, as can be seen in Figure 4A,B. In combination with the binarized size, which is mostly insensitive to the optical contrast, the parameter range for droplets containing a cell can be clearly determined. Figure 4B shows the scatter plot of the droplets’ SSD versus their binarized size of a subset of N = 1057 droplets. Empty droplets and droplets containing a cell are marked as blue spots and green crosses, respectively. The insets show the corresponding images, demonstrating that a very large SSD corresponds either to multiple cells in a single droplet or to a cell with comparably large contrast. In comparison, a very low SSD and a large binarized size correspond to a big cell and/or an image with low optical contrast, which could, for example, correspond to a cell that lost membrane integrity due to apoptosis.

Analysis of the data in the scatterplot allows the definition of the physical parameter space (thresholds) of a specific droplet population. These can be selected as the trigger parameter for further droplet manipulation. For example, to achieve label-free, cell-laden droplet sorting, the dielectrophoretic forces will be activated by a trigger signal each time a droplet with the specific preselected parameters (yellow polygon in Figure 4B) is detected. It is important to mention here that there is complete flexibility in choosing the polygon area (ie, parameter space of SSD and binarized size thresholds) and the chosen values of the sorting parameters can be optimized during the sorting process. For further experiments, the polygon can be adjusted such that only droplets with exactly one cell are sorted or especially cells with apoptotic appearance are neglected. Toward this end, one would have a larger dataset with more of the different subpopulations and carefully choose the sorting area around them. With the given optical resolution and contrast settings, we are able to detect bacteria having a similar optical contrast as the used cells with sizes of 2 μm. Polystyrene beads can be analyzed down to 1 μm diameter since they show a higher optical contrast.

3.3 Label-free sorting of water-in-oil droplets

Droplet sorting enables the possibility to identify and select rare events among large droplet populations and finds application in cell and microorganism screening. These sorting techniques are normally based on fluorescence readout that helps to identify the cells within the droplets. At the same time, in the case of several biomedical applications, the fluorescence staining of cells can affect their behavior and thereby compromising their potential clinical implementation, therefore, it can be favorable if the cells get sorted in their native state. Based on the microfluidic design principles developed for fluorescence-activated droplet sorting we set out to use the physical parameters obtained by the developed optical device for real-time, label-free droplet sorting. Toward this end, a combination of parameters including the SSD and the binarized size (yellow polygon in Figure 4B) was chosen to trigger the separation of cell-laden droplets from empty droplets (Figure 5A).

To demonstrate the capabilities of the optical device for label-free, cell-laden droplet sorting, we prepared a droplet mixture consisting of 82% empty and 18% CHO cell-laden droplets. Figure 5A shows the representative bright-field image of the sorting device and the summary of the sorting results. Note that with the applied flow rates the measured sorting rate was 131 droplets/s. An auxiliary high-speed camera was used as a control to monitor both of the outlet channels and the number of sorted droplets were manually counted. A total of 370 droplets were evaluated.
Among these were 70 cell-laden droplets (18.92%), 58 of which has been sorted correctly (deflected) into the “cell-laden outlet” channel and all of them contained a cell (0% false positives). A total of 312 droplets passed the channel without being deflected and were directed into the “empty droplets outlet” channel. Detailed analysis of these non-deflected droplets revealed that 300 (96.15%) of these were empty, which is equivalent to a false negative rate of 3.85%. Hence, the purity of the droplet population exiting the cell-laden outlet channel is 100%. Video S1 shows a high-speed camera recorded video of the label-free droplets sorting of cell containing droplets.

By the implementation of the developed label-free monitoring method, we can additionally distinguish between the droplet sizes by the difference in the emitted bright-field intensity of the droplets. Therefore, to demonstrate the flexibility of the developed optical device for active droplet manipulation based on different physical parameters of the droplets, we show label-free sorting of droplets based on their size. Toward this end, a droplet mixture
FIGURE 5 Content-based (A) and size-based (B) label-free sorting of water-in-oil droplets. The respective channels for sorting and the ROI for the measurement by the optical device is given in the images. The images are composed of several camera shots to illustrate sorting. If a droplet matching the defined parameter range enters this ROI, the optical device triggers an activation of the dielectrophoretic force that deflects the passing droplet into the narrow outlet channel. A, Droplets with (18.16%) and without (81.84%) CHO cells are analyzed by our device and 100% of cell-laden droplets are directed toward the “cell-laden outlet” channel whereas 97.68% of empty droplets are directed into the “empty droplet outlet” channel. B, For size discrimination, a mixture of 45.35% small (35 μm in diameter) and 54.65% large (42 μm in diameter) droplets was separated into two streams, one containing 99.25% small droplets and the other containing 94.42% big droplets.

containing 45.35% small (35 μm in diameter) and 54.65% large droplets (42 μm in diameter) was injected into the sorting device and triggered utilizing the developed optical device. The area in the parameter space for selecting small droplets was chosen as shown in Figure S4. The SSD shows only little contrast in this experiment. Higher peaks are related to multiple droplets within the ROI and both smaller and larger droplets show very similar peak heights (see Figure S4a). Therefore, sorting was achieved using the length and the size of the binarized image of the objects. The relative abundance of small and large droplets before and after sorting is shown in Figure 5B. To assess the quality of sorting a total of 624 droplets were traced within the ROI and both smaller and larger droplets were sorted. Among those droplets only two large ones were detected, equivalent to a droplet population purity of 99.25%. Among the droplets that were not deflected 339 large and 20 small droplets were detected, equivalent to a droplet population purity of 94.42%. The sorting rate was 118 droplets/s. Video S2 shows a high-speed camera video of label-free sorting based on droplet size. Important to mention here that the geometry of the sorting area can be optimized in order to reduce the incidence of false deflected droplets with the increase in a flow rate.\[17\]

4 SUMMARY AND OUTLOOK

In this study, we present a developed optical device for the label-free monitoring and active manipulation of passing water-in-oil droplets in real-time manner. In a first step, we validated the functionality and sensitivity of the device by measuring different droplet production parameters and comparing them with the manually measured results obtained from high-speed camera videos of the same experiment. Droplet production rate, droplet size, and distance between the droplets measured with the optical device matched well with the results obtained from the high-speed camera observations. Due to easy-to-implement integration and operation of the developed optical device, it can be used for the automated quality assurance of passing objects. Moreover, we showed the ability of the developed optical device for label-free droplet content monitoring. By measuring the change of the summed squared difference between the binarized size
of the droplets and the intensity of the background subtracted picture, droplets containing CHO suspension cells can be clearly and reliably identified. The analysis of the obtained data allowed us to define a physical parameter space consisting of the SSD and the binarized size parameter, which can be selected as a trigger for further manipulation of specific droplet populations. We selected the physical parameter space such as to achieve highly efficient label-free, cell-laden droplet sorting from a mixture of empty and cell-laden droplets in real time. We demonstrated that 96.15% of the cell-laden droplets were successfully sorted at a frequency of 131 droplets/s. To additionally assess the flexibility of the developed optical device for droplet sorting based on different droplet parameters, we employed this device to trigger size-dependent droplet sorting. Toward this end, droplets with a diameter of 35 µm were separated from those 42 µm in size at a frequency of 118 droplets/s. The obtained separated populations had 99.25% and 94.42% purity for small and big droplets, respectively. The basic applications and functionalities shown in this article set the path for various new experimental setups and measurements. The utilization of artificial intelligence for discrimination is currently in the testing phase. Using a machine learning approach, the manual selection of the parameter space will be further facilitated and automated. With a potent and optimized microfluidic setup,[17,18] the maximum achievable analysis rate of 10 000 Hz roughly leads to a theoretical sorting capacity of >3000 droplets/s. In addition to identification of cell-loaded droplets, a proper selection of analyzed parameters will enable classification of the cells inside the droplet, for example by their size, morphology, or stage. Moreover, the optical device gets currently optimized to measure several regions of interest simultaneously. This can increase the maximum sorting rate further by parallelization or can be used to do multistep manipulations on a single chip. This is the last step toward a real multipurpose lab-on-a-chip device that is able to contain an entire process requiring active surveillance. For example, single microfluidic droplet manipulation units for pico-injection,[19] droplet sorting, and release of the droplet content[20] could be implemented in a synergetic application and controlled with the developed optical device. This kind of microfluidic factory utilizing our developed optical device has the potential to be a game changer in basic biological research as well as in pharmaceutical and medical research and applications.

**CONFLICT OF INTEREST**

J.P., T.N., D.G., and K.W. are shareholders of the company Sensific GmbH producing and selling the described optical device under the name ODIN.

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**ORCID**

Christoph Frey [https://orcid.org/0000-0002-4545-4407]
Ilia Platzman [https://orcid.org/0000-0003-1239-7458]
Othmar Marti [https://orcid.org/0000-0003-2254-6656]

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