Colour-Specific Microfluidic Droplet Detection for Molecular Communication

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ABSTRACT

Droplet-based microfluidic systems are a promising platform for lab-on-a-chip (LoC) applications. These systems can also be used to enhance LoC applications with integrated droplet control information or for data transmission scenarios in the context of molecular communication. For both use-cases the detection and characterisation of droplets in small microfluidic channels is crucial. So far, only complex lab setups with restricted capabilities have been presented as detection devices. We present a new low-cost and portable droplet detector. The device is used to confidently distinguish between individual droplets in a droplet-based microfluidic system. Using on-off keying a 16-bit sequence is successfully transmitted for the first time with such a setup. Furthermore, the device capabilities to characterise droplets regarding colour and size are demonstrated. Such an application of a spectral sensor in a microfluidic system presents new possibilities, such as colour-coded data transmission or analysis of droplet content.

CCS CONCEPTS

• Hardware → Sensor devices and platforms.

KEYWORDS

Droplet detection, microfluidic environment, molecular communication, colour, optical sensor

1 INTRODUCTION

Molecular communication uses biochemical signals or other information carriers in the nanoscale to achieve data transmission in use-cases where either wired or wireless connections are not feasible. This approach has been experimentally investigated in previous work on centimeter- and decimeter-scale (e.g. [1, 2, 7, 9, 18]). For smaller geometries, it can be implemented in a microfluidic setup. To this end, we use droplets to transmit data in a microfluidic channel. The theoretical principles of droplet-based communication have been presented in [5] and a first practical study was conducted in [11].

To enable the practical realisation of droplet-based communication the automated generation and detection of droplets is vital. Challenges are posed by the small size of the microfluidic channels, the requirement to not introduce obstacles into the channel and the goal of a compact and portable system. A promising method for droplet generation has recently been introduced in [10]. In this work we aim to develop a simple, versatile and portable droplet detection device.

Droplets may be detected either by change of conductivity between two electrodes [6, 12, 14] or with devices based on optical sensors. As the use of electrodes results in a complicated fabrication procedure and inherently necessitates changing the microfluidic channel to accommodate the electrodes, implementation of an optical sensor principle was chosen.

Various optical sensors utilising cameras [4, 8, 10] or fluorescent agents [3, 16] were successfully applied. However, these approaches require a complex lab setup or sophisticated image processing. The aim of this work is the development of a simple and portable droplet detector. Hence, we initially extend the detection principle based on a light-emitting diode (LED) and a photodetector presented in [15] and [17] to enable droplet-based data transmission. In particular, we eliminate the need to feed optical fibres into the transmission channel, significantly simplifying the detector design and improving its portability as well as facilitating an uncomplicated exchange of the mounted microfluidic chip. This device is further used to, for the first time, transmit a stream of 16 bit using droplets, which is an important step towards droplet-based data transmission.

In addition to the simple droplet detection using a photodetector, we implement droplet colour detection using a spectral sensor. This enables a more precise analysis of droplets and allows for more sophisticated data transmission schemes. So far only a rudimentary approach to colour detection based on changes in transparency...
has been presented [17]. Hence, this is the first device allowing for differentiation between a variety of colours in a droplet-based microfluidic system.

Finally, both sensor systems are simultaneously used to determine crucial droplet parameters. In particular, we demonstrate the capability to measure a droplets size inside the transmission channel.

We present our work in three sections. Section 2 describes the individual components of the sensor devices design. In Section 3 the devices capabilities regarding droplet detection, colour characterisation, colour intensity variation and determination of droplet length are evaluated. We end the article in Section 4 with a conclusion.

2 DEVICE CONCEPTION

The constructed device consists of two separate optical sensors. An analog infrared photodiode to detect the presence of an individual water droplet in an oil carrier medium and a digital 6-channel colour detector to further characterise the droplet. Both sensors were implemented on a common circuit board together with a suitable microcontroller. Furthermore, a measurement setup to mount microfluidic chips was constructed. The design allows a simple exchange of the microfluidic chip.

2.1 Infrared Sensor

The infrared sensor consists of a light source (LED) that is focused towards the channel (submillimeter-scale) and a photodetector on the opposite side. The result is a light path that gets interrupted when the optical absorption of the material (i.e. ink droplets) inside the microfluidic channel increases. The wavelength of the light source was chosen to be in the infrared spectrum (940 nm) as this is close to the maximally absorbed wavelength (approx. 970 nm) in water [13].

As the signal variation at the photodiode is very small and therefore also the change in diode current, a transimpedance amplifier (TIA) was used to amplify the photodiode output in the range of 0 V to 20 V. The TIA output is then offset corrected and further amplified using a differential amplifier with a tunable reference voltage. The resulting signal output is adjusted to the range of 0 V to 5 V and can be acquired with the analog-to-digital converter of a microcontroller. Adequate overvoltage protection at the microcontroller input was implemented. A simplified circuit diagram of the resulting analog setup is shown in Fig. 1.

2.2 Colour Sensor

To further characterise droplets, a spectral sensor (AS7262) from ams AG was implemented. The sensor has a very compact design and incorporates six photosensors for various wavelengths, covering the range from 430 nm to 670 nm. Each photosensor has a bandwidth of 40 nm. As the sensor has integrated digital data acquisition, no analog amplification, as in Fig. 1 for the infrared sensor, is required.

The recorded spectral data can be retrieved via a standard I²C interface with an appropriate microcontroller. Due to the time required to measure the spectral value and transmit the result with the I²C interface the sample rate is restricted to 20 Sa s⁻¹. A higher sample rate, although possible, would result in reduced sensitivity.

For measurements in the microfluidic setup a white LED was placed opposite to the sensor, analogous to Section 2.1.

2.3 PCB and Housing

The circuitry for both sensors was implemented on a common circuit board, together with a microcontroller (ATmega32U4) from Atmel Corporation, a USB connector and power converters to supply the required voltages for the amplification stages.

The resulting device was constructed as a four layered printed circuit board (PCB), which was designed using Altium Designer. The PCB was printed by Multi Leiterplatten GmbH in Germany and assembled using the facilities of the Institute for Electronics Engineering at FAU, Germany.

Fig. 2 shows the completed PCB from above. Components were placed on both sides of the circuit board with a size of 6.6 cm by 5.6 cm.

To mount the microfluidic chips for testing a custom-made housing was constructed. This allows for a simple exchange of the used chip and has an adjustable positioning system to accurately place the microfluidic channel on the sensor. A beam across the top of the microfluidic chip acts as a mount for the two light sources.

Fig. 3 shows the complete droplet detection device with a microfluidic chip. Moreover, a microfluidic chip with a stream of inked droplets is shown in Fig. 4.

3 EXPERIMENTAL RESULTS

Various experiments were conducted to evaluate the individual capabilities of the two implemented sensors. First, the infrared sensor was used as a presence/absence detector for individual droplets. The transmission of a sample data sequence using on-off keying was achieved. Second, the colour sensors spectral sensitivity was investigated using differently coloured droplets. Third, the response of both sensors to various ink concentrations was examined. Finally,
Figure 2: Assembled PCB with integrated infrared and spectral sensors as well as a microcontroller. A serial connection is provided via a USB port that is also used to power the device. The attached cables connect to the separate LEDs.

Figure 3: Droplet detection device with positioning system for the microfluidic chip. The light sources (i.e. infrared and white LED) are attached to the beam across the top of the microfluidic chip. The PCB with the sensors is inside the box. The spring-loaded screws can be used to precisely place the microfluidic channel above the sensors.

Figure 4: Microfluidic chip of PMMA with a straight transmission channel and a T-junction used for data transmission. The blue ink is injected at the T-junction creating a droplet stream which can be detected by the proposed device shown in Fig. 2. The channel boundaries were redrawn in the figure for better visibility.

3.1 Data Transmission

To achieve data transmission, on-off-keying was implemented. An individual bit was transmitted every second (1 bit s\(^{-1}\)), whereas the bit value ‘1’ was coded as the injection of a droplet and the value ‘0’ as no injection. The random 16-bit sequence ‘10111100 01011001’ was used for transmission.

To detect individual bits, the received signal was offset corrected to get a 0 V baseline and a fixed threshold of 0.2 V, significantly higher than the noise level, was applied. Symbol intervals with a known duration were derived from the first threshold pass. A bit was detected as ‘1’ if the threshold was met for at least 30% of the symbol interval \(^1\). To account for inaccurate symbol intervals due to varying droplet speeds the symbol intervals were re-synchronised at every detected rising edge.

Fig. 5 shows the receive signal for the transmitted sample bit sequence. The detection threshold and the relevant symbol intervals, as derived from the rising edges, are also shown. As can be seen each transmitted bit was successfully received with the device and the scheme described above.

3.2 Droplet Colour Determination

The spectral sensors main function is to determine the colour of a detected droplet. However, it can also be used as an extension to the analog infrared sensor. Possible use-cases for colour detection are more sophisticated data transmission schemes and the characterisation of droplet content (e.g. with a coloured reagent) in LoC applications.

To assess the colour-coded droplet detection the measured spectral signal for ink droplet of various colours was recorded. Fig. 6 shows the normalised sensor values for the individual droplets. One droplet for each of the six different ink colours (violet, blue, green, yellow, orange and red) was transmitted. Significantly different colours can clearly be identified due to their spectral values (e.g. blue and violet) using the constructed sensor. Spectral values for related colours (e.g. yellow and orange) are very similar and require further investigation to ensure distinct detection.

\(^1\)Please note that due to the good signal quality various threshold values lead to a successful data transmission.
analysis of colour sensitivity may be achieved by investigating the sensor data for gradual transitions between colours with a series of dilutions.

Fig. 7 shows the spectral values that were recorded by the colour sensor for the transmission of the 16-bit sequence introduced in Sec. 3.1. The data points are set at the center wavelength of the six photosensors.

Each bit can clearly be identified with an equal colour distribution equivalent to a red droplet in Fig. 6. The sensor can therefore consistently characterise droplet colour throughout a transmission sequence, allowing for colour coded data transmission by differentiation between droplet spectra (as shown in Fig. 6).

### 3.3 Variation of Droplet Concentration

In order to assess the sensitivity of the sensors, a series of dilutions with varying ink concentrations was performed. Furthermore, the infrared sensors operation based on absorption in water (independent of colouring) is demonstrated with clear water droplets.

Water was diluted with blue ink in concentrations ranging from 0 % to 25 % in steps of 5 %. For each measurement a droplet of 5 mm length was generated.

As can be seen in Fig. 8, the measured signal amplitude of the infrared sensor is constant for various ink concentrations. The calculated mean signal is 0.73 V with a maximal deviation of 0.13 V from the average value for the individual concentrations. As expected, the sensors receive signal is independent of colouring as it relies on the absorption in water.

Fig. 9 shows the measured signal for different ink concentrations using the colour sensor. As the ink concentration increases a more distinct colour distribution can be observed. The light absorption increases with the used amount of ink.

The measurements show, that the sensor system can be used to distinguish between different colour concentrations (i.e. colour
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Figure 8: Measurements of water droplets with varying concentrations of blue ink in the range of 0% to 25% using the infrared sensor (shown in blue). The mean value for each droplet (shown in green) as well as the overall average (shown in red) was calculated. A similar signal can be observed in all cases, showing the specificity to absorption in water independent of colouring.

3.4 Droplet Size Determination

The sensor can also be used to measure the size of an individual droplet by observing the infrared and colour sensor values on a time scale. The observed duration of a droplet is proportional to the travel speed inside the microfluidic channel and the volume of the droplet. Considering the speed of the droplet in the channel $v_{\text{chan}}$, the droplet length can be calculated from the observed droplet duration $t_{\text{drop}}$ as

$$l_{\text{drop}} = v_{\text{chan}}t_{\text{drop}} = \frac{\Delta d}{\Delta t} t_{\text{drop}}$$

(1)

where $\Delta d = 6.35 \text{ mm}$ is the distance (fixed on the PCB) and $\Delta t$ the time delay of the received signal edge between the two sensors. Either of the sensors can be used to measure the droplet duration. In this case, the colour sensor was chosen and $t_{\text{drop}}$ was set to the time span for which the recorded signal surpassed half of the maximal amplitude (full width at half maximum).

Droplets of varying length (1 mm to 5 mm) were generated by adjusting the duration of a rectangular pressure pulse from 1 s to

Figure 9: Recorded signal of the spectral sensor for 5 mm long droplets with varying concentrations of blue ink. The observed sensor intensity decreases, as light absorption increases, proportional to the amount of ink in the droplet.

5 s, to evaluate the sensors ability to determine droplet size. Fig. 10 shows the measured droplet length in relation to the transmitted droplet length. Five different droplet lengths were investigated with 20 measurements per value. We observed a precision of 0.48 mm, corresponding to the largest deviation from the expected value.

As the tested droplet lengths can be differentiated, it is possible to transmit data encoded as droplet size in the microfluidic system to achieve higher bit-rates [5]. The observed precision not only depends on the receiver but also on the reproducible accuracy of the transmitter. A higher precision, resulting in the possibility to encode data in a smaller size variation, might therefore be observed with an improved droplet generation method.

4 CONCLUSION

A low-cost and portable sensor for droplets in microfluidic systems was constructed. The device was successfully used for presence/absence detection and to characterise droplet colour and size. Furthermore, a distinction between different ink concentrations was achieved. It therefore facilitates LoC use cases that require sophisticated data transmission schemes or colour reagent detection.

With the current setup a bit rate of approximately 1 bit s$^{-1}$ was achieved. Limiting factors can be found both in parameters of droplet generation (precision of volume and timing) and detection (local resolution and sample rate). The precision of droplet generation may be improved with other pump systems, such as a micropump or a peristaltic pump. Furthermore, implementing a
feedback loop to control a pressure pump, based on the retrieved sensor data, may improve droplet generation consistency. If necessary, the sample rate of the sensor device (currently 20 Sa s \(^{-1}\)) could be increased by use of an array with multiple sensors in parallel. By addressing these limitations the bit rate could be increased further in an optimised setup.

In future work various functions of the sensor device may be extended. Specifically, for the data transmission scenario, the bit rate could be increased by combining coding schemes such as coding of droplet position, colour and size. Furthermore, the development of a standardised interface for microfluidic chips could simplify interoperability for future devices. Such a standard could include dedicated positions for in- and output connectors, sensing regions and a scalable set of microfluidic chip dimensions. Finally, the sensors capabilities regarding sensitivity and more complex coding schemes will be investigated further.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to Dominik Lehner for his support with the microfluidic setup.

REFERENCES

[1] Max Bartunik, Maximilian Lübke, Harald Unterweger, Christoph Alexiou, Sebastian Meyer, Doaa Ahmed, Georg Fischer, Wayan Wicke, Vaughn Jamali, Robert Schober, and Jens Kirchner. 2019. Novel Receiver for Superparamagnetic Iron Oxide Nanoparticles in a Molecular Communication Setting. In Proc. Sixth Annu. ACM Int. Conf. Nanoscale Comput. Commun. - NANOCOM ’19. ACM Press, Dublin, 1–6. https://doi.org/10.1145/3345312.3345483

[2] Max Bartunik, Harald Unterweger, Christoph Alexiou, Robert Schober, Maximilian Lübke, Georg Fischer, and Jens Kirchner. 2020. Comparative Evaluation of a New Sensor for Superparamagnetic Iron Oxide Nanoparticles in a Molecular Communication Setting. In 12th EAI International Conference on Bio-inspired Information and Communications Technologies (EAI BICT 2020). ACM Press. to appear.

[3] Binyu Chen, Xiaoming Han, Zhen Su, and Quanjuan Liu. 2018. Fluorescence detection system for microfluidic droplets. In Sixth AIP Int. Conf. Comput-Aided Design, Manuf., Model, Simul. (CMAMS 2018), Vol. 1967. AIP Publishing, Nanjing, 1–5. https://doi.org/10.1063/1.5039044

[4] Bas de Heij, Bart van der Schoot, Hu Bo, Joseph Hess, and Nikolaas F. de Rooij. 2000. Characterisation of a fl droplet generator for inhalation drug therapy. Sensors and Actuators A: Physical 85, 1–3 (Aug. 2000), 430–434. https://doi.org/10.1016/S0924-4247(00)00400-3

[5] Elena De Leo, Lidia Donvito, Laura Galluccio, Alfio Lombardo, Giacomo Morabito, and Laura M. Zanolli. 2013. Communications and Switching in Microfluidic Systems: Pure Hydrodynamic Control for Networking Lab-on-a-Chip. IEEE Trans. Commun. 61, 11 (November 2013), 4663–4677. https://doi.org/10.1109/TCOMM.2013.100813.130332

[6] Caglar Elbukan, Tomasz Glawdel, Danny Chan, and Carolyn L. Ren. 2011. Detection of microdroplet size and speed using capacitive sensors. Sensors and Actuators A: Physical 171, 2 (Nov. 2011), 55–62. https://doi.org/10.1016/j.sna.2011.07.007

[7] Nariman Farsad, Wei Ni Guo, and Andrew E. W. Eckford. 2013. Tablet: Molecule Communication: Text Messages through Chemical Signals. PLoS ONE 8, 12 (Dec. 2013), e88719. https://doi.org/10.1371/journal.pone.0082935

[8] Alexander Golberg, Gregory Linshiz, Ilia Kravets, Nina Stawski, Nathan J. Hillson, Martin L. Yarmush, Robert S. Marks, and Tania Konry. 2014. Cloud-Enabled Microscopy and Droplet Microfluidic Platform for Specific Detection of Escherichia coli in Water. PLoS ONE 9, 1 (Jan. 2014), 1–9. https://doi.org/10.1371/journal.pone.0086341

[9] Laura Grebenstein, Jens Kirchner, Renata Stavracakis Peixoto, Wiebke Zimmermann, Florian Imtortler, Wayan Wicke, Arman Ahmadzadeh, Vahid Jamali, Georg Fischer, Robert Weigel, Andreas Burkovski, and Robert Schober. 2019. Biological Optical-to-Chemical Signal Conversion Interface: A Small-Scale Modulator for Molecular Communications. IEEE Trans. Nanobiosci. 18, 1 (Jan. 2019), 31–42. https://doi.org/10.1109/tnb.2018.2870910

[10] Medina HamidovíAG, Uli Marta, Helen Bridle, Damir HamidovíAG, Gerald Fink, Robert Wille, Andreas Springer, and Werner Haselmayr. 2020. Off-chip Controlled Droplet-On-Demand Method for Precise Sample Handling. ACS Omega (2020). to appear.

[11] Medina HamidovíAG, Uli Marta, Gerald Fink, Robert Wille, Andreas Springer, and Werner Haselmayr. 2019. Information Encoding in Droplet-Based Microfluidic Systems: First Practical Study. In Proc. Sixth Annu. ACM Int. Conf. Nanoscale Comput. Commun. - NANOCOM ’19. ACM Association for Computing Machinery, Dublin, 1–6. https://doi.org/10.1145/3345312.3345482

[12] Pelin Kobra Iğor, Merve Marcali, Mert Keser, and Caglar Elbukan. 2015. Microfluidic droplet content detection using integrated capacitive sensors. Sensors and Actuators B: Chemical 210 (April 2015), 669–675. https://doi.org/10.1016/j.snb.2015.01.018

[13] Jerry Workman Jr. and Lois Weyer. 2007. Practical Guide to Interpretive Near-Infrared Spectroscopy. CRC Press, USA.

[14] Xue Niu, Mengying Zhang, Suliang Peng, Wenja Wen, and Ping Sheng. 2007. Real-time detection, control, and sorting of microfluidic droplets. Biomicrofluidics 1, 4 (Sept. 2007), 1–12. https://doi.org/10.1063/1.2795392

[15] Vijay Srinivasan, Vamsee K. Pamula, and Richard B. Fair. 2004. Droplet-based microfluidic lab-on-a-chip for glucose detection. Analytica Chimica Acta 507, 1 (April 2004), 145–150. https://doi.org/10.1016/j.aca.2003.12.030

[16] Alan H. Tkaczyk, Eric R. Tkaczyk, Theodore B. Norris, and Shuichi Takayama. 2011. Time detection, control, and sorting of microfluidic droplets. Biomicrofluidics 5, 1 (March 2011), 1–9. https://doi.org/10.1063/1.3527368

[17] Varun Trivedi, Ankur Doshi, G. K. Kurup, E. Ereifej, and P. J. Vandevordband. 2013. Experimental Molecular Communication Testbed Based on Magnetic Nanoparticles in Duct Flow. In 2013 IEEE 19th Int. Workshop Signal Process. Advances Wireless Commun. - SPAWC. IEEE, Kalamata, 1–6. https://doi.org/10.1109/SPAWC.2013.6719005

[18] Harald Unterweger, Jens Kirchner, Wayan Wicke, Arman Ahmadzadeh, Doaa Ahmed, Vahid Jamali, Christoph Alexiou, Georg Fischer, and Robert Schober. 2018. Experimental Molecular Communication Testbed Based on Magnetic Nanoparticles in Duct Flow. In 2018 IEEE Int. Workshop Signal Process. Advances Wireless Commun. - SPAWC. IEEE, Kalamata, 1–5. https://doi.org/10.1109/SPAWC.2018.8446011