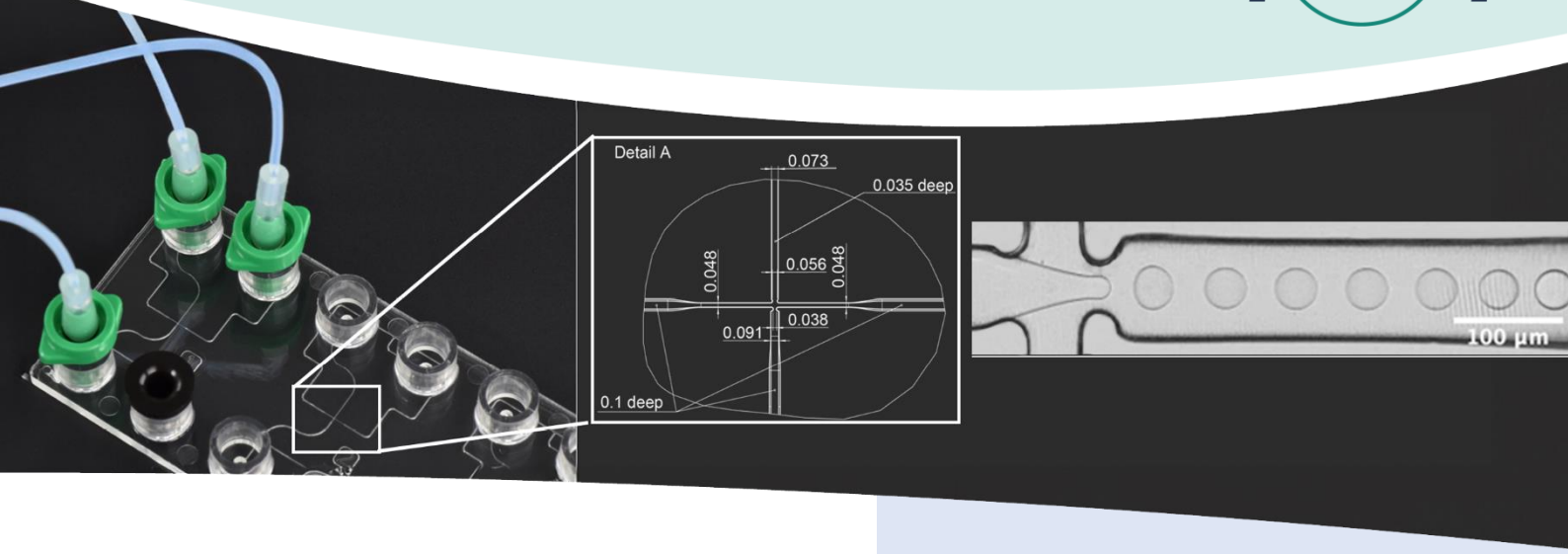
ZELLMECHANIK DRESDEN

microfluidic ChipShop



Application Note –

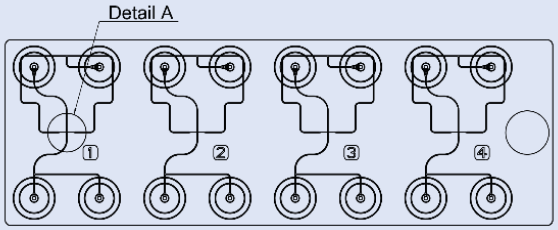
Real-time Analysis of Droplets

Enhancing Microfluidic Droplet Generation through Advanced Real-Time Analysis

In the realm of microfluidics, droplet generation stands as a pivotal technique, offering unique possibilities for various applications. Our collaborative effort with ZELLMECHANIK DRESDEN GmbH introduces a cutting-edge solution, combining *microfluidic ChipShop*'s Fluidic 537 microfluidic chips for efficient droplet generation with ZELLMECHANIK DRESDEN's advanced device DeCellerator and software designed to detect and analyze these microscale droplets on the fly.

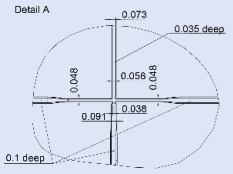
Droplet generation, often employed in applications ranging from pharmaceuticals to biochemical assays, demand precise control and ideally instantaneous feedback for optimal results. ZELLMECHANIK DRESDEN's innovative software RecoTeem enables real-time analysis of critical droplet parameters such as size, velocity, production rate, and total count. What sets this solution apart is its flexibility – not only can these real-time insights be obtained, but they can also be easily integrated into customized programs that implement a feedback loop (Example python scripts can be provided.) This functionality opens avenues for external component control, allowing, for instance, the regulation of pumps based on dynamic droplet characteristics.

The challenge of achieving real-time analysis in microfluidics is particularly pronounced in droplet generation. This application note explores the capabilities of our joint technology, shedding light on the complexities of droplet generation and the pivotal role of instantaneous analysis in fields where precision is essential.



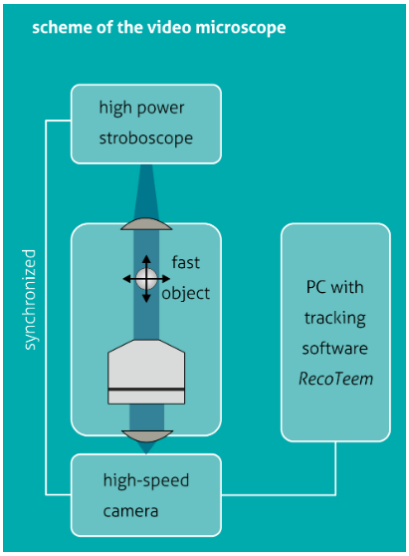
Chip Description – Fl. 537

The droplet generator fluidic 537 possesses a classic flow-focusing geometry, perfectly suited to generate simple droplets. With four identical droplet generator units on one microfluidic chip. This device is ideal for anyone who wants to try or compare multiple experimental setups, without the need of ordering a new chip for every experiment. Operation of one unit of Fluidic 537 requires a microfluidic pump with the ability to control two individual flows, one for the continuous and one for the disperse phase. Please be aware that this chip is one of the few droplet generators devices with Luer interfaces and appropriate Luer compatible accessories are required.



Parameter Fluidic 537	Description
Chip format	75.5 x 25.5 x 1.5 mm^3
Interface type	Luer
Droplet generator units	4 x 1
Nozzle type	Flow-focusing
Nozzle size	38 µm
Lid thickness	140 µm (Topas), 175 µm (PC)

High-Speed Video Microscope: DeCellerator



The DeCellerator is a high-speed brightfield video microscope equipped with a synchronized stroboscopic illumination. A modern high intensity monochromatic LED features microsecond short pulses to suppress motion blurring. This enables capturing of fast processes in video recordings. Full frame images (1 280 x 864 px) can be recorded at up to 1 000 frames per second and recording of up to 10 000 frames per second is possible for reduced regions of interest using the designated acquisition software: RecoTeem. The DeCellerator package contains an AxioObserver microscope body (ZEISS) with x-y-stage, adjustable sample holder and a 40x NA 0.65 objective to provide a solid base for your droplet experiments. Synchronized LED illumination enables bright field imaging with minimal motion blurring for objects moving as fast as 0.5 m/s while being imaged with micro meter resolution. The high speed imaging system consists of a Mikrotron 1.1CXP2 camera and a PC that is able to record and process the accumulated image data. RecoTeem offers a graphical user interface that enables user-friendly control of the imaging system. It helps the experimenter to record high-speed videos or still images, but also enables real-time analysis of droplet parameters such as: size, velocity, production rate and total count.

Figure 1: Standard video microscopy. Low frame rates don't allow high resolution motion tracking. Very strong motion blurring makes it impossible to determine particle shapes.

Figure 2: Fast video microscopy with standard illumination. Finer resolution in time, but motion blurring affects/hinders image analysis. Shorter exposure times can reduce motion blurring in exchange for worse signal to noise ratio.

Figure 3: DeCellerator. High resolution in time and optimal imaging without motion blurring through synchronized pulsed illumination.

Figure 4: Setup of the DeCellerator with Hardware and Software.

Nice to Know: The microfluidic setup with a pumping system and the DeCellerator can be operated either via a syringe pump system or we recommend the Fluigent Pressure Pump Setup with the LineUp™ Series.

Materials

Hard- and software

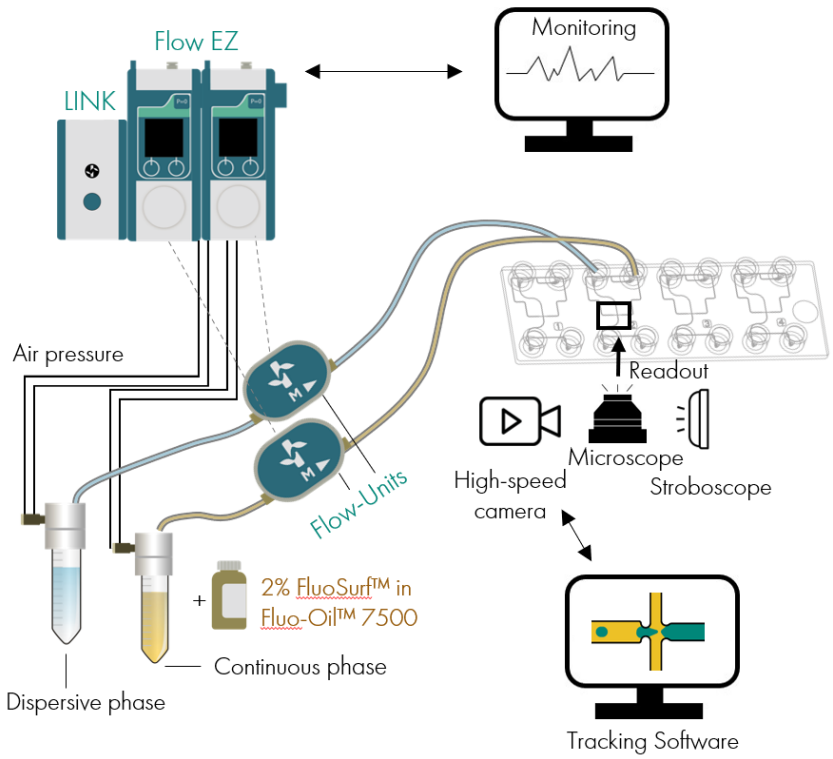
- Pump system with little pulsation e.g. pressure pump Fluigent system (LineUp™ – Flow EZ System, Flow-Units and P-Caps with reservoirs)
- Microfluidic chip: Fluidic 537 or 947 for droplet formation
- Tubing and adaptors for (Mini)Luer on chip and for syringe; Capillary PEEK tubing (ID: 0,02", OD: 1/32") from *microfluidic ChipShop* (Article 10002000), Male Luer tube tuck connectors from *microfluidic ChipShop* (Article 10002011)
- Handling frame (Article 10000043)
- DeCellerator setup (ZELLMECHANIK DRESDEN): High-speed imaging microscope with stroboscopic illumination
- RecoTeemST software (ZELLMECHANIK DRESDEN)

Reagents

- Continuous phase: 2% FluoSurf™ in Fluo-Oil™ 7500 (emulseo)
- Dispersed phase: Millipore dH_2O

Expert Tip: For silicone-based oils, Topas chips are preferred, whereas mineral oils necessitate PC chips. Fluorinated oils are best used with PC and Topas.

Experimental Setup (Using LineUp™ Series from Fluigent)



1. Connect Flow EZ™ with pneumatic tubing to an external pressure supply and the ouputs with the P-CAP pressure reservoirs.
2. Connect the Flow-Units with PEEK capillaries on both sides between the reservoir and the microfluidic setup and to the Flow EZ™ using USB cables.
3. Fill reservoirs of pressure pump for continuous phase (2% FluoSurf™ in Fluo-Oil™ 7500) and dispersed phase (Millipore dH_2O).
4. Connect the LINK module to a computer and launch the Fluigent software OxyGEN to control and monitor the flowratefor each Flow-Unit.
5. Launch the DeCellerator RecoTeemST software (ZELLMECHANIK).
6. Connect the chip using suitable fluid adaptors (Luer, Mini-Luer) and corresponding tubing.
7. The chip outlet can be connected with tubing to a collection container.
8. Put the chip on a microscope and adjust focus and stage to image the position on the chip where dispersed and continuous phase intersect.
9. Induce flow, **note „Expert tip" for detection for maximum velocity** of detected objects.

10. Position chip into detection region of interest (ROI). Depending on chip a position downstream where the channel gets wider might be ideal. Aim is to assess droplets one after another, while having to operate at not too high velocities.
11. Start detection in RecoTeemST.

Expert Tip: Guideline for ROI and framerate

- A ROI size of 50 x 50 pixels is ideal for high frame rates up to 10 kHz
- Position of ROI should be chosen in place where droplets are well separated
- Ideally an object is imaged in the ROI at least 3 times while move through the ROI
- Calculate displacement from channel geometry and flowrates to meet criteria above, it might help to choose lower magnifications

Results

Our investigation focused on real-time droplet generation using two distinct designs, Fluidic 537 and Fluidic 947, revealing key parameters and characteristics for each setup.

Fluidic 537, 38 µm nozzle:

- Imaging by DeCellerator (400 x 90 pixels, 10 kHz)
- Total flow rate: 2 µl/s
- Sheath flow rate (FluoSurf): 1.66 µl/s
- H_2O flow rate: 0.33 µl/s
- Droplet generation rate: 3000 droplets/s
- Droplet diameter: ~74.5 µm
- Droplet velocity: 160 mm/s

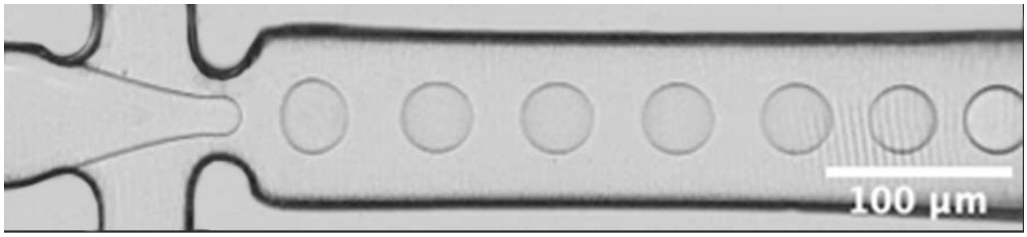


Figure 5: Imaging with DeCellerator (400 x 90 pixel, 10 kHz) of droplets generated with Fluidic 537.

Fluidic 947, 30 µm nozzle:

Total flow rate: 0.217 µl/s
Sheath flow rate (FluoSurf): 0.167 µl/s
H2O flow rate: 0.050 µl/s
Detection frame rate: 10 kHz
Droplet generation rate: 2800 particles/s
Droplet diameter: ~15 µm
Droplet velocity: 201 mm/s

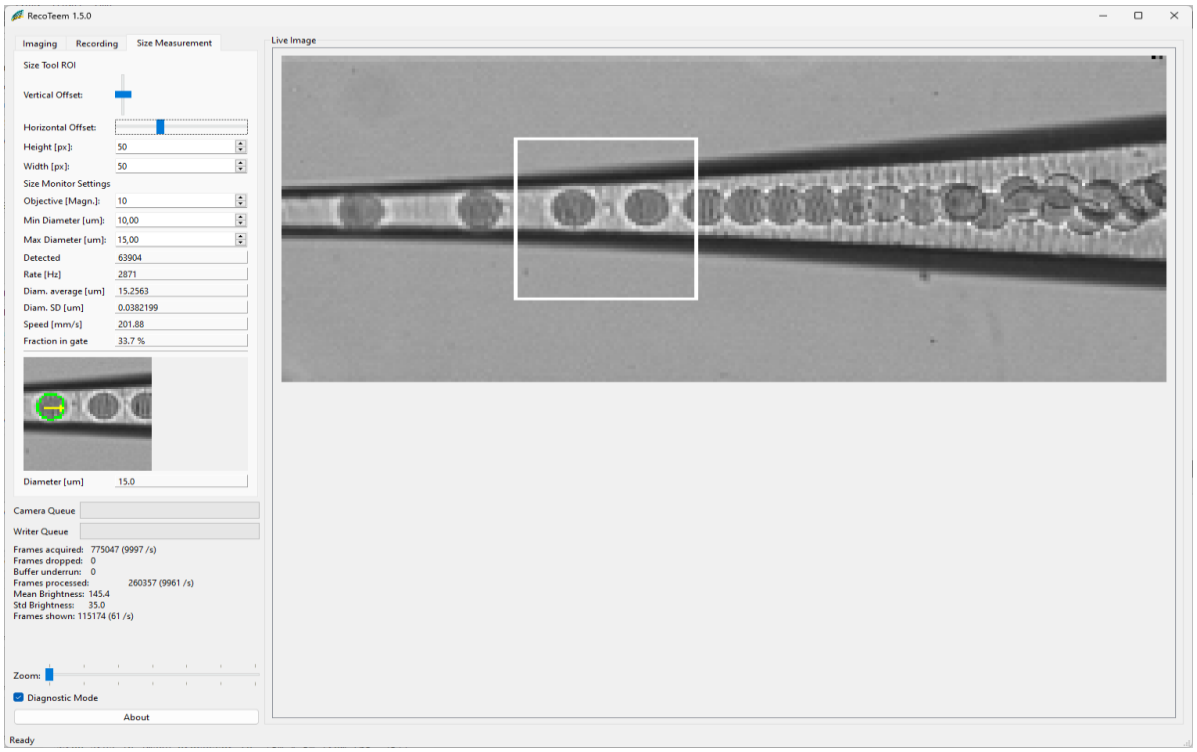


Figure 2: Main Window of RecoTeem software. On the right, live view image with ROI in white. On the left, controls to set up ROI and detection setting. Live view of processed image with detected contour (green) and displacement arrow (yellow).

Conclusion

In conclusion, the real-time measurements conducted on both Fluidic 537 and Fluidic 947 have demonstrated our ability to monitor droplet dynamics instantaneously.
Fluidic 537, with a larger droplet diameter of ~74.5 µm, showcased its potential for generating larger droplets with a higher flow rate, while Fluidic 947, featuring smaller droplets (~15 µm) and higher velocity, demonstrated a different range of applications.

This capability to measure droplets in real-time opens doors to enhanced control and understanding of microfluidic processes. The findings underscore the significance of real-time analysis in microfluidics, emphasizing the potential for further innovations and optimizations in droplet generation technologies.

As we continue to delve into the intricacies of microfluidic systems, we look forward to expanding our understanding and refining our methodologies for even more accurate and versatile real-time measurements.

Other Applications

In addition to monitoring droplet generation, the utilization of high-speed cameras in microfluidics opens up a plethora of further application possibilities.

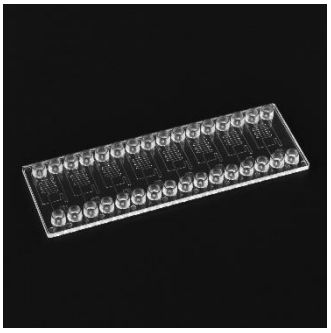
1. **Quality assurance:** Monitoring of droplet variations.
2. **Chemical Synthesis and Pharmaceutical Development:** Monitoring rapid mixing and reaction processes in microfluidic channels allows for real-time optimization of reaction conditions.
3. **Sorting and Analysis:** Utilization of high-speed imaging for rapid cell sorting and characterization based on morphological features or fluorescence signals. Sorting of droplets using techniques such as droplet FC or FACS.
4. **Bubble Flow Regulation and Oxygen Transfer:** Observation of bubble dynamics in microfluidic systems provides insights into bubble flow regulation and oxygen transfer processes.
5. **Lab Automation and Diagnostics:** Real-time visualization of flow patterns and pressure distributions in microchannels enables optimization of fluid transport, enhancing capabilities in lab automation and diagnostics.
6. **High-Throughput Screening**: Implementation of high-throughput screening applications in drug discovery.

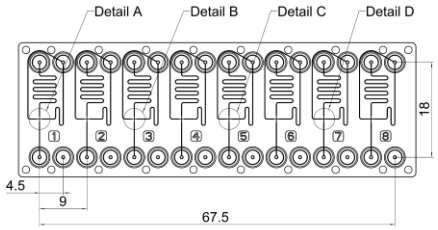
Related Products

To facilitate droplet generation, a range of products is essential, including polymer chips, fluid connectors, tubing, pumps and oil with surfactant. Additional products such as plugs for sealing or handling frames for enhanced experimentation can be utilized. For further technical inquiries, please reach out to us at inquiries@microfluidic-ChipShop.com.

Microfluidic chips

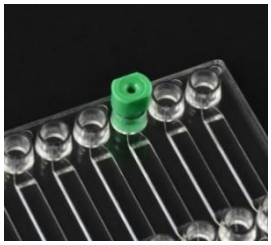
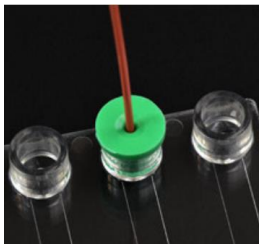
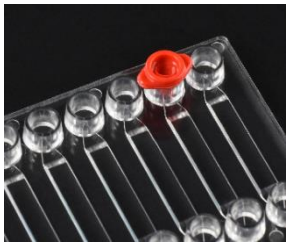
Product Code Fl. 537	Surface Treatment	Droplet Generation	Material	Interface	Lid Thickness [µm]	Nozzle Size [µm]
10000466	Untreated – hydrophobic surface	W/O	Topas	Luer	140	38
10000467	Untreated – hydrophobic surface	W/O	PC	Luer	175	38
10002124	Treated – hydrophiliic surface	O/W	Topas	Luer	140	38
10001535	Treated – hydrophilic surface	O/W	PC	Luer	175	38
Product Code Fl. 947	**Surface Treatment**	**Droplet Generation**	**Material**	**Interface**	**Lid Thickness [µm]**	
10001972	Untreated – hydrophobic surface	W/O	Topas	Mini Luer	140	10 - 30
10001337	Untreated – hydrophobic surface	W/O	PC	Mini Luer	175	10 - 30





Tubing and accessories

Product Code	Description	Material	Quantity
10002009	Capillary PEEK tubing, orange, ID: 0.508 mm (0.02“), OD: 0.794mm (1/32“)	PEEK	3.049 m


Product Code	Fluidic	Description	Type	Material	Color
10001764	997	Mini Luer tube tuck connector for 1/32“ tubing	Single	TPE	Green
10002011	1580	Luer tube tuck connector for 1/32’’ tubing	Single	TPE	Green
10000205	438	Male Mini Luer plugs – Low volume displacement	10 pc/pack	TPE	Opaque
10001595	1223	Male Luer plug - Low volume displacement	10 pc/pack	TPE	Opaque
10000042	352	Handling frame with high skirt	1 pc	PC	Orange

Droplet oil and surfactant

Explore our selection of oils and surfactants available in diverse formats. For expert guidance, feel free to reach out to us at inquiries@microfluidic-ChipShop.com

ZELLMECHANIK DRESDEN

Tatzberg 47-49 • 01307 Dresden • Phone: + 49 (0)35141884430
info@zellmechanik.com • www.zellmechanik.com

microfluidic ChipShop GmbH
Stockholmer Str. 20 • D - 07747 Jena • Germany

Phone: +49 (0) 36 41 – 347 05 0 • Fax: +49 (0) 36 41 – 347 05 90
inquiries@microfluidic-ChipShop.com • www.microfluidic-ChipShop.com