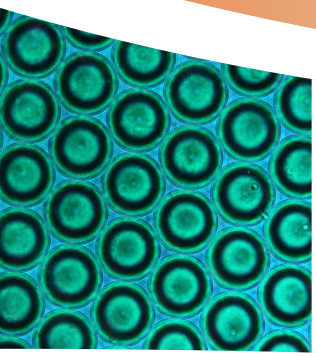
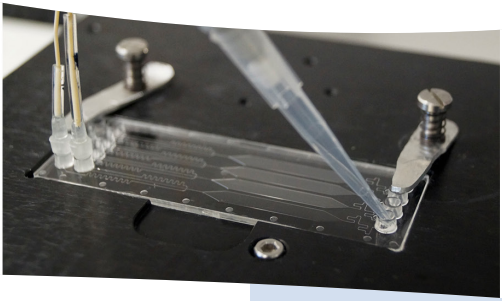
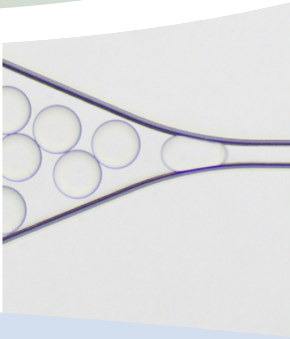
emulseo

microfluidic ChipShop







Application Note - Surfactant Application for Droplet Generation and ddLAMP

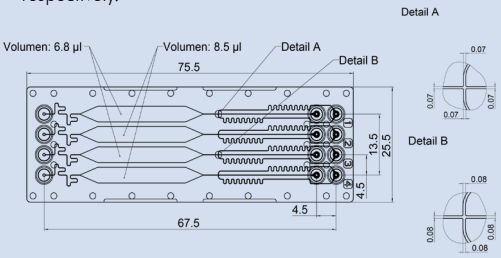
Loop-mediated isothermal amplification (LAMP)

microfluidic ChipShop offers different PCR-on-chip systems for the amplification and detection of bacterial nucleic acids, with relatively small reaction volumes ranging from 2.5 to 50 µL. Tremendously higher throughput can be achieved using droplet-based target gene amplification methods, with smaller reaction volumes. DNA samples are divided into thousands of small volume droplets in the range of approximately 0.2 – 2 nL. Amplification of target genes is performed in each droplet.
This technique requires fewer samples than other commercially available, digital PCR systems. It reduces costs and preserves valuable quantities of samples and reagents. Furthermore, digital droplet-based amplification methods enable cost-effective, absolute quantification of the DNA amount/sample, without including standard curves. Thus, these methods are perfectly suited for high throughput experiments, e.g., determination of microbial cell counts, viral loads or genetic differences.

Recently, isothermal amplification methods, such as loop-mediated isothermal amplification (LAMP), were developed. LAMP is highly specific, eleminated the need for complex thermocycling processes and is better suited for microfluidic applications. In this application note, we analyze the commercially available fluorinated surfactant FluoSurf™ for digital droplet LAMP (ddLAMP) on a microfluidic chip. Due to the standardized microscope slide format, the chip allows to perform the individual experiment steps with common laboratory equipment.
At first, flow-rate-controlled droplet generation is performed, which assures monodispersed droplet formation. Subsequently, ddLAMP is operated on the microfluidic device placed on a common heating plate and then visualized using a conventional fluorescence microscope.

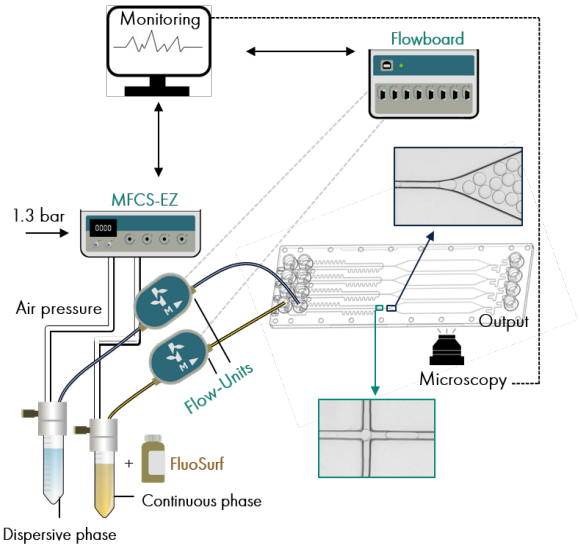
Chip description - Fluidic 1147

The droplet generator Fluidic 1147 was used for ddLAMP. It features four functional droplet generation units with a simple flow-focusing nozzle of 70x70 µm and 80x80 µm to generate droplets of different sizes. The Mini-Luer interfaces enable connection of continuous (oil) and a dispersive phase (water). The droplets can be captured, monitored and manipulated in the observation chambers downstream of the nozzle. The monitoring chambers come at a height of 80 µm and 100 µm, with volumes of 6.8 µl and 8.5 µl, respectively.



Parameter Fluidic 1147	Description
Chip format	Microscopic slide (75 mm x 25 mm)
Interface type	Mini Luer
Droplet generator units	Four
Nozzle type	Single cross, flow focusing
Nozzle size	70x70 µm / 80x80 µm
Droplet storage	Yes
Chamber depth	80 µm / 100 µm
Chamver volume	6.8 µL / 8.5 µL
Surface treatment	None
Lid thicknesss	175 µm

Experimental Setup and Materials



Reagents

Continuous phase

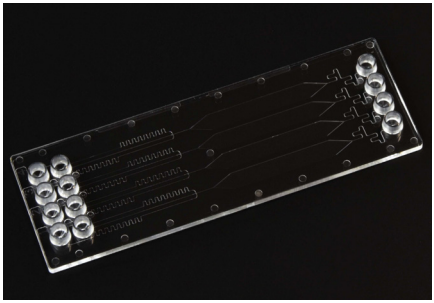
- FluoSurf™ Neat (Emulseo) 2% w/w- diluted in HFE 7500 (3M)

Dispersive phase / LAMP reaction

- Isothermal Amplification Buffer (1x)
- MgSo4 (6 mM)
- dNTP-Mix (1.4 mM)
- FIP & BIP primer (each 1.6 µM)
- F3 & B3 primer (each 0.2 µM)
- Loop F & B primer (each 0.4 µM)
- Eva Green dye (1x)
- Bst Warm Start Polymerase (320 U)
- Nuclease free water
- Template DNA (2 ng µL-1 isolated from *Bacillus thuringiensis*)

Hard- and Software

- Microfluidic chip: Fluidic 1147
- Fluigent system (MFCS-EZ, Flowboard, Flow-Units, Fluiwell with reservoirs)
- External light source and camera (Basler acA800) equipped with 4x objective
- PC-Monitoring with Pylon Viewer Software (Basler) and A-i-O 2019 Software (Fluigent) working with gelling ECM, like cooling pipette tips, etc.)
- Capillary PEEK tubing (ID: 0,005", OD: 1/32") with silicon tubing sleeves (ID: 0,76 mm, OD: 1,65 mm)
- Male Mini Luer fluid connectors
- Low-volume displacement Mini Luer plugs
- Pneumatic tubings
- Thermal cycler or heating block (with prevention of light)
- Fluorescence microscope; 10x magnification; FITC channel filter systems, imager (Zeiss Axio)



Quick start guide

Setup preparation

1. Connect the MFCS-EZ pressure controller input with pneumatic tubings to an external pressure supply that provides 1.3 bar and the outputs with the Fluiwell air pressure inputs for two reservoirs.
2. Connect the Flowboard to the Flow-Units and to a computer using USB cables.
3. Fill the two reservoirs with FluoSurf™ 2% in HFE 7500 (continuous phase) and with LAMP reaction Mix (dispersed phase) and place them in the Fluiwell.
4. Connect the Flow-Units with PEEK capillaries and silicon tubing sleeves on both sides, and with the Fluiwell liquid output of the two reservoirs.
5. One side of the tubing is located at the bottom of a liquid reservoir (volume 2 mL) whereas the other side interfaces with the inlet of the droplet generator.
6. Turn on all necessary electronics and devices such as light source, camera, computer and MFCS-EZ.
7. Launch the pylon viewer imaging software (Basler) and the A-i-O 2019 software (Fluigent). With the easy handling A-i-O software air pressure and/or the flowrate can be controlled and monitored for each Flow-Unit.
8. Connect the inputs on chip for to the dispersed and continuous phase with Male Mini Luer fluid connectors on silicone sleeves from step 4.
9. The chip outlet can be connected with tubing to a collection container or the flow-through can be removed during the process by pipetting.

Expert tip

In replacement of the described Fluigent flow system any other sufficient pressure, syringe or peristaltic pump system can be applied to generate monodispersed droplets via a continuous flow and determined flow rates to increase droplet generation performance.

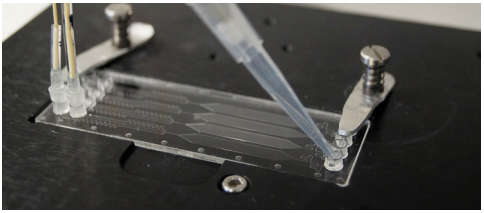
In this experiment, the droplet generation is performed with pressure-driven flow control using a Fluigent-MFCSTM-EZ pressure control system and A-i-O 2019 – Software.

Droplet generation

Expert tip

Prior, degassing of the reagents is recommended because to prevent the formation of bubbles inside the chip. Air can spread over the chamber surface and reduce the droplet stability.

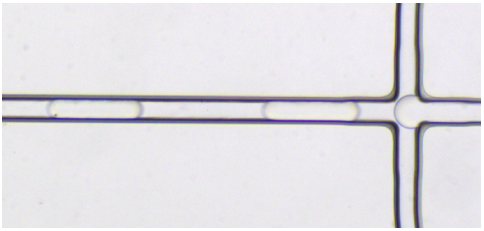
1. The pump pressurizes the liquids leading to liquid flow inside the tubing and drop formation into the droplet generator.
2. Fill the fluidic chip with FluoSurf™ (continuous phase) to remove air and avoid bubble formation in the observation chamber with a pressure of 200 mbar (≈ 2.4 µL min-1) and keep the pressure for the dispersed phase at 30 mbar (≈ 0.2 µL min-1) for 1-2 minutes.
3. Increase the dispersive phase (LAMP mix) to the inlet keeping the pressure rate at 180 mbar (≈ 1.4 µL min-1).
4. Waste like continuous phase, air bubbles or droplets varying in size can be removed at the Mini Luer outlet by simply pipetting or using a waste container.



Droplet generation step 4: liquid removal at the chip output.

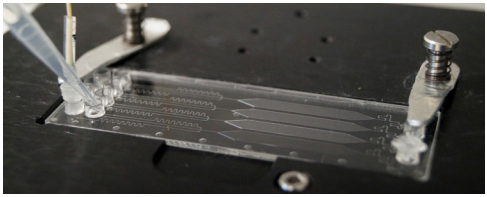
Expert tip

The flowrate is freely adjustable, but should be equally or lower than the continuous phase and at a minimum as given values to achieve desired droplet size and droplet generation frequency. By increasing the aqueous flow-rate and keeping the oil flow rate constant, the droplet diameter increases.



Droplet generation

5. Run the droplet generation for at least 5 min to guarantee complete air removal from the dispersed inlet and to ensure stable droplet formation.
6. Fill the collection chamber with monodispersed and densely arranged droplets.
7. Close the output port with a low-volume displacement plug, quickly remove the Mini Luer connection from the dispersive phase input and turn off the flow of the dispersive phase.
8. Displace the dispersive phase completely from the channel with FluoSurf™ (continuous phase) for approximately 1-2 min and remove it by pipetting.



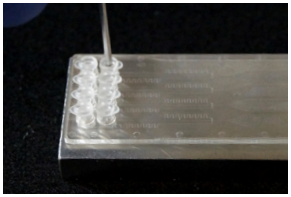
Droplet generation step 8: liquid removal at the chip input for the dispersal phase.

Expert tip

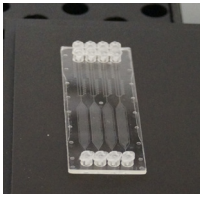
The chip chamber provides the advantage to observe droplets during the generation process (i.e. monodispersity, size, amount) with (exact values) or without (estimated values) using a microscope equipped with a camera. The droplets can also be collected in a PCR vial to perform target amplification like ddLAMP or conventional ddPCR off-chip.

Loop-mediated isothermal amplification (LAMP)

1. Close all chip ports tighly with low-volume displacement plugs, e.g., with a pen.
2. Place the chip on a heating plate or in a thermal cycler for 40 min at 66°C or respective LAMP conditions.
3. Analyze the chip chambers with a fluorescence microscope equipped with FITC channel filter systems (ex: 480nm / em: 535 nm) and visualize amplified products with Zeiss Axio imager, for example.



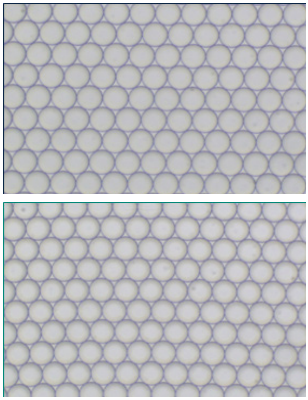
LAMP step 1: Closing chip ports



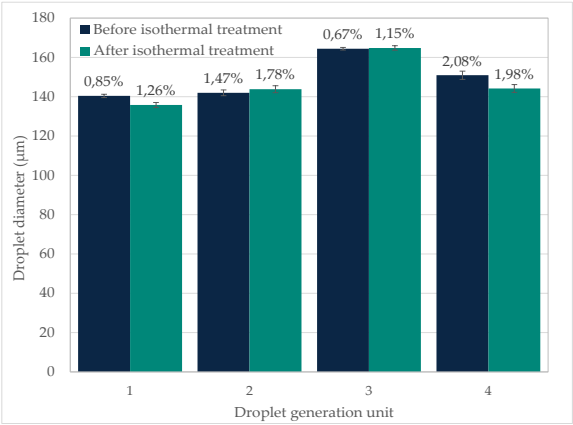
LAMP step 2: Placement on heating plate

Results

Stable droplet emulsion for digital LAMP has been performed with a high monodispersity rate below 2% (Fig 3 & 4). Depending on the different droplet generation units, with different nozzle and chamber sizes, average droplet sizes between 140 μm and 165 μm were achieved, with volumes between 1.24 nL and 1.79 nL, respectively. The droplets remain very stable during the heating process at 66°C for 40 min.



Monodispersed droplets in chip chamber before (top) and after (below) heating at 66°C for 40 min.

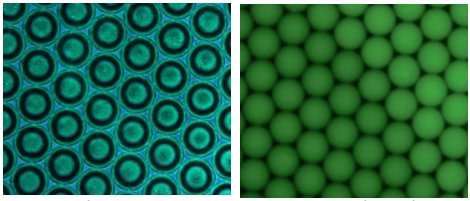


Droplet size and monodispersity (CV%) before and after chip heating at 66°C for 40 min.

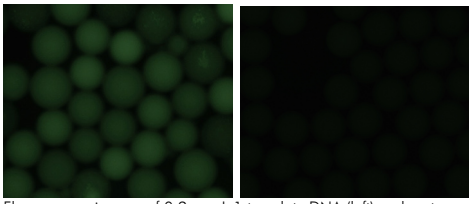
High fluorescence intensities of the droplets can be seen with the fluorescence microscope (ex: 480 nm/em: 535 nm) that indicate successful amplification of the target gene (Fig 5). When using a higher amount of DNA (2 ng µL-1), there is a positive fluorescence signal in all droplets. At a tenfold lower amount of DNA (0.2 ng µL-1), not every droplet contains a template that can be amplified and therefore only some fluorescing droplets can be detected.

Expert tip

To avoid fluorescence signal bleaching, pay more attention working under dark conditions and apply only short exposures to light.



Green light filtered (Ex: 480 nm/ Em: 535 nm) bright field (left) and fluorescence (right) images showing LAMP of target genes (2 ng µL-1 template DNA) within droplet reactors.



Fluorescence images of 0.2 ng µL-1 template DNA (left) and no template control (NTC, right) images showing LAMP products in droplets.

Conclusion

The main advantage of the system on a chip is that only one chip is needed for droplet generation, amplification of target genes and detection. The easy chip handling ensures droplet formation and storage with high monodispersity and stability, and successful target amplification and detection by ddLAMP.

Due to the lower temperature input, the droplets remain more stable on chip with the LAMP-reaction than has been tested at higher temperatures used in conventional digital droplet PCRs. Furthermore, droplet-based LAMP has the advantage that target amplification is more specific than ddPCR and a conventional heating plate is sufficient.

Further Applications

- **Single cell encapsulation**
  e.g., microbial cultivation
- **Emulsion stabilitystudies**
  e.g., long-term applicability of w/o- or o/w-emulsions
- **Antimicrobial or metabolical screening**
  e.g., AST
- **Crytallography in droplets**
  e.g., growth of protein crystals
- **Chemical-cell interaction testing**
  e.g., tumor cell treatment

Related Products

The droplet generation chip Fluidic 1147 is available in two different polymers, Topas (COC) and Polycarbonate (PC). The droplet generation chip Fluidic 1114 has a similar design, but different nozzle and chamber designs with lower volume capacity. This fluidic design is well applicable for short-time experiments (e.g., cell cultivation, monodispersity tests).

A family of droplet generator chips in various designs allows the generation of droplets in different sizes and frequencies or for droplet storage, enable a wide variety of experiments. Our Ready-to-Use Kit and First-User Kit offer a variety of different droplet generator fluidics, already integrated fluorinated oil with surfactant and accessories to start your droplet generation application.

Ready-to-Use-Kit

Product	Contents	Article	Material	Quantity	Price [€]
10002033	Fluidic 440 50 - 80 µm nozzles	10000174	1 x PC	1	785.25
		10000040	1 x Topas	1	
	Fluidic 947 10 - 30 µm nozzles	10001336	1 x Topas	1	
	Fluidic 1032 Double cross; 100 µm nozzles	10001335	1 x PC	1	
	Fluidic 162 Double cross; 70 µm nozzles	10000003	1 x PC	1	
	Fluidic 912 Single cross; 80 µm nozzle	10001333	1 x PC	1	
		10001985	1 x Topas	1	
	Fluidic 163 Double cross; 140 µm nozzle	10000004	1 x PC	1	
	Fluidic 488 Double cross; 74 µm, storage	10000511	1 x PC	1	
	Fluidic 1147 Single cross; 70 µm; 80 µm	10001777	1 x PC	1	
	Droplet oil: Fluo-Oil™ 7500	10002034	-	1 x 21 mL	
	Surfactant: FluoSurf™	10002035	-	1 x 0.5 g	
	Male Mini Luer fluid connectors	10000116	TPE	2 x 10 pcs	
	Male Mini Luer plugs	10000054	TPE	2 x 10 pcs	
	Silicone tube, ID: 0.76 mm, OD: 1.65 mm	10000031	Silicone	1 x 1 m	
	Micro tubes, PTFE, ID: 0.5 mm, OD:1.0 mm	10000032	PTFE	2 x 1 m	

First-User-Kit

Product	Contents	Article	Material	Quantity	Price [€]
10002037	Fluidic 440 50 - 80 µm nozzles	10001972	1 x PC	1	230.49
	Fluidic 947 10 - 30 µm nozzles	10001336	1 x Topas	1	
	Fluidic 488 74 µm nozzle	10000511	1 x PC	1	
	Droplet oil: 2% FluoSurf™ in Fluo-Oil™ 7500	10002036	-	4 mL	
	Male Mini Luer fluid connectors	10000116	TPE	1 x 10 pcs	
	Male Mini Luer plugs	10000054	TPE	1 x 10 pcs	
	Silicone tube, ID: 0.76 mm, OD: 1.65 mm	10000031	Silicone	1 x 1 m	
	Micro tubes, PTFE, ID: 0.5 mm, OD:1.0 mm	10000032	PTFE	1 x 1 m	

Droplet Generators for ddLAMP

Product	Fluidic	Nozzle [µm]	Material	Price [€/chip] 1+	10+	100+
10001745	1147	70; 80	Topas	36.20	24.30	16.10
10001777	1147	70; 80	PC	36.20	24.30	16.10
10001753	1114	50; 60	Topas	42.20	34.40	26.10
10001776	1114	50; 60	PC	42.20	34.40	26.10

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