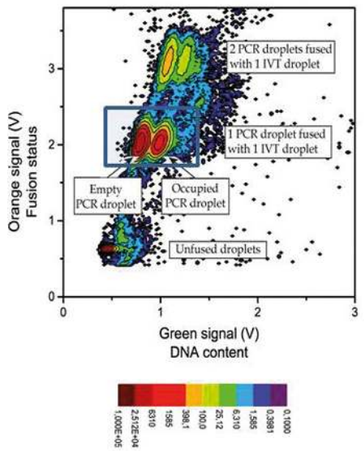
Droplet fusion and digital PCR analysis



This graph represents a count of droplets with given orange and green signals: the darker red a dot is signifies a large amount of droplets with these characteristics. Light blue represents low amount of droplets. The green signal shows the presence of DNA in the PCR droplet, and the orange one is relevant for the occurrence of the fusion.

Unfused droplets result in a low orange signal: less than 1 volt represent unfused droplets.

1/1 fusion has a twice the intensity in orange signal in the 2 volt region. The differentiation between those where the PCR droplet was empty or not is on the green signal: lower is empty, higher is occupied.

Successful fusion of 2 PCR droplets with 1 IVT droplet fusion is seen at the top of the graph, three times higher than unfused droplets.

Results:

- **Fusion 1/1** : 85% to 88% success rate (no fusion: 10%, double fusion: 5%)
- Very stable and synchronized flows
- **Automated set up** allows the experiment to be run without constant adjustment resulting in time saving, and offering the ability to perform systemic screening.
- **Process control**: by continuously measuring flow-rates and pressures, one can assess the performance of the fluidic system at a glance.

CONCLUSION

Flow-rate control solution based on pressure actuation provides:

- High droplet monodispersity (even at low flow-rates over long time-periods.)
- Straightforward set-up which can be easily automated
- Precise volume and flow control, even with complex, multi-channel chips. “Cross-talk between flow channels is minimized.
- Ability to detect and compensate for small disruptions such as air bubbles.

ACKNOWLEDGEMENTS & REFERENCES

- **University of Strasbourg, ISIS Team**
  Michael Ryckelynck et al.

FLUIGENT
SMART MICROFLUIDICS™

MULTI-STEP DROPLET MANIPULATION FOR DNA ANALYSIS

From high throughput generation to single fusion

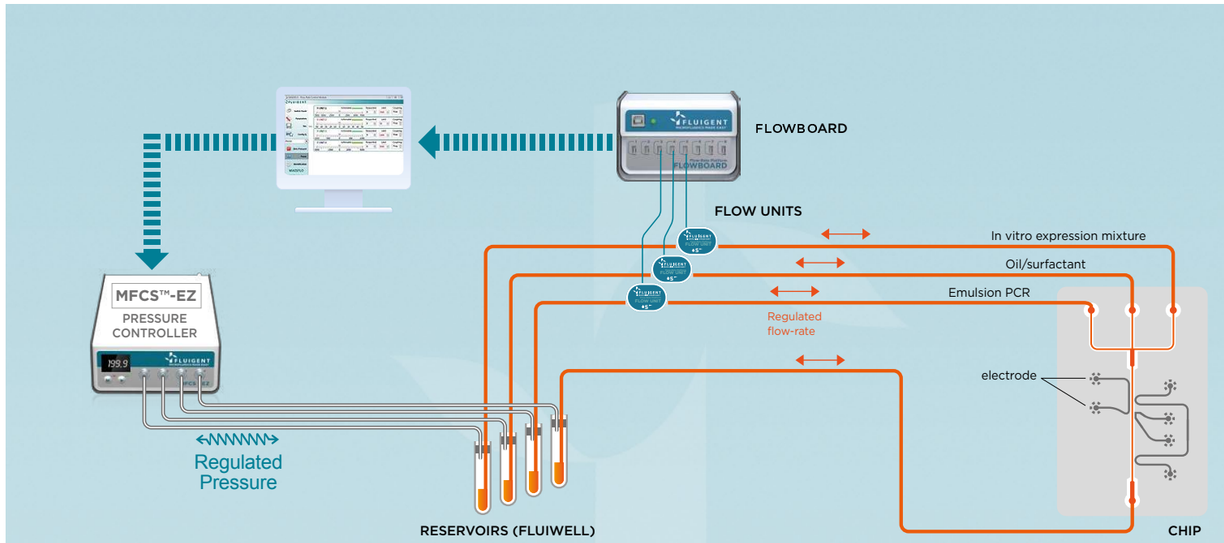


***Figure 1**: Diagram of the microfluidic path for the 1/1 fusion of IVT droplets and PCR emulsion*

INTRODUCTION: DROPLETS IN MICROFLUIDICS

The use of water in oil droplets in microfluidics in high-throughput screening is rapidly gaining acceptance. The main application areas currently involve screening cells as well as genetic material for various mutations or activity. Here the aim is to isolated single DNA molecules and analyse the enzymes resulting from their expression.

There were several steps to the study. First, two separate emulsions were generated. The first one encapsulated an aqueous-phase PCR and DNA mixture in an organic continuous phase on a dedicated chip.

The second emulsion was generated encapsulating an aqueous-phase IVT *(In Vitro Transmission)* mixture in the same organic phase.

Lastly, both emulsions were then fused one droplet at a time to control which DNA sequence is translated at the end of the process.

For these studies, it is very important that the droplets are generated at the correct frequency, and at uniform size.

A graphic representation of the droplet chip used to combine the PCR and IVT droplets is above.

FOR ANY FURTHER INQUIRY, PLEASE CONTACT US AT:
FLUIGENT SA || WWW.FLUIGENT.COM || CONTACT@FLUIGENT.COM || +33 1 77 01 82 68
SUBJECT TO MODIFICATIONS WITHOUT NOTICE || SEPT. 2016
: @FLUIGENT

MATERIAL USED TO CONTROL FLOWS

PRODUCTS	NAME & USE
	**MFCS™-EZ** Microfluidic pressure pump to generate fast and pulseless fluid movement (1 bar).
	**FRP: FLOW RATE PLATFORM** Flexible fluidic flow-rate measuring with the best precision.
	**FRCM : FLOW-RATE CONTROL MODULE** To control and monitor the flow-rate on up to 16 channels
	**FLUIWELL (1C & 4C)** Pressurized reservoirs (2ml and 15ml) for mixtures and continuous phase.

APPLICATION: HIGH-THROUGHPUT GENERATION AND SINGLE FUSION

A green fluorescent marker is added to the PCR primers to show at the end of the process which droplets contain DNA to be enhanced or which ones are empty. An orange dye is also added to measure the size of the droplet, the intensity of the light captured by a CCD camera being proportional to the size of the droplet.

APPLICATION NOTE
MULTI-STEP DROPLET MANIPULATION FOR DNA ANALYSIS
From high throughput generation to single fusion

PART ONE: GENERATION OF THE PCR/DNA DROPLETS

Results :

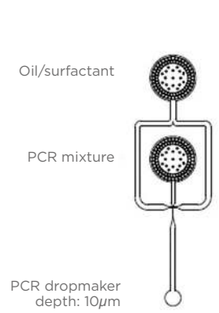
- 2pl droplets, highly monodispersed **CV 1.63%**
- Frequency: **10 to 12kHz**

Flow-rate and stability :

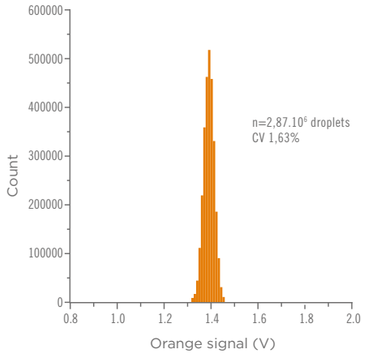
- Aqueous Phase 800nl/min, **CV 0.3%**
- Oil Phase 3400nl/min, **CV 0.5%**
- Fast start-up time.

Stable flow rates and droplet sizes were achieved in several seconds as opposed to minutes by other methods.

Rapid start/stop time due to the MFCS™-EZ, minimizes the use of expensive reagents.



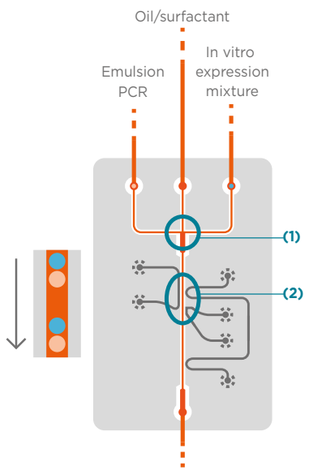
PCR dropmaker



Emulsion PCR 2pL-droplets production

PART TWO: FUSION OF ONE PCR DROPLET WITH ONE IVT DROPLET GENERATED ON CHIP.

After amplification, the PCR droplets are injected in a second chip. This chip generates IVT droplets, while also synchronizing them such that one PCR droplet bet is inserted between two IVT droplets. The IVT droplets contain an orange dye to differentiate the contents. Fusion is processed by the application of an electric field.



Objectives :

- Simultaneous injection of PCR emulsion and generation of IVT droplets **(1)**
- Fusion of PCR droplet and IVT droplet by electrical field **(2)**

Challenges :

- Only one oil phase: need to control the 3 phases (oil, PCR emulsion, IVT)
- Need for stable conditions to synchronize PCR droplet injection and IVT droplet formation
- Strong coupling between the channels. Very difficult to reach steady regime by using syringe-pumps

SUBJECT TO MODIFICATIONS WITHOUT NOTICE || SEPT. 2016