ESRF	**Experiment title:** **Time-Resolved Study of Crystallization in Microfluidic Devices**	**Experiment number**: CH 4555
Beamline: ID13	**Date of experiment**: from: 25/11/2015 to: 29/11/2015	**Date of report**: 04/03/2016
Shifts: 12	**Local contact(s)**: Dr. Britta Weinhausen	*Received at ESRF:*

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Report:

The aim of experiment CH4555 was to observe the crystallization of calcium carbonate ($CaCO_3$) within water-in-oil (w/o) droplets with time-resolved micro-focused X-ray diffraction (μXRD) – and by doing so, develop droplet microfluidics coupled with synchrotron radition as a technique for studying crystallization *in situ*. Droplet microfluidics offers many advantages over single-phase flow. The two most important of these, in relation to crystallization, are the ability to mitigate device fouling by preventing the aqueous phase from wetting channel walls and the prevention of Taylor Dispersion, whereby diffusion and a laminar parabolic flow profile decrease time resolution as crystalites mix traveling downstream.[1] There have been some previous synchrotron-based studies of crystallization using microfluidics, but these have been mainly limited to single-phase flow, small-angle X-ray scattering (SAXS), and large protein crystals.[2, 3] To the best of our knowledge, this beamtime represents the first time anyone has successfully obtained powder XRD patterns from flowing microfluidic droplets.

In order to achieve this, we fabricated a new microfluidic device with X-ray permeable Kapton windows that could be mounted within the experimental hutch of beamline ID13. Each device was assembled from a central insert comprising the laser-etched channel design, two window inserts, two transparent silicone gaskets, and top and bottom poly(methyl methacrylate) (PMMA) base plates (Fig. 1). The top base plate contains ports for standard ¼ - 28 UNF chromatography fittings which serve as the fluid inlets and common outlet. The bottom plate contains self-tapping anchors for M5 bolts which are used to provide device sealing and structure. Additionally, it comprises a window that expands outwards at a 45° angle to allow diffracted X-rays to exit without encountering the device walls. The central insert is etched with a T-junction droplet generator possessing two aqueous inlets and one oil inlet. Downstream of the T-junction lies a serpentine flow channel that directs the droplets back and forth past the window that extends down the length of the base plates (Fig. 2a). There are 37 points where the channel is observable in the window (denoted as Positions 1-36 and the T-junction). The main channel is ~ 85 cm long and has a cross sectional area of 300 x 300 μm^2 which yields a residence time of 8.5 min at our minimum total flow rate of 9 μL/min. FC-40 oil with 2.5% w/w EA surfactant was used as the carrier phase while 25 mM $CaCl_2$ and 100 mM Na_2CO_3 were used for the precipitation of $CaCO_3$, which was chosen due to its polymorphism and multi-step crystallization pathway.[4] Calcium phosphate (CaP) and calcium sulfate ($CaSO_4$) were also attemped, but to lesser success. More beamtime will be required to conduct microfluidic X-ray analysis with these crystal systems.

Before the flow is initiated, the location of each viewing position within the window is mapped using the inline optical microscope – which also enables us to ensure the flow is regular before beginning X-ray analysis. Then using the Eiger X 4M detector at a sample-to-detector distance of ~ 95 mm, time-resolved total X-ray exposures of 10 s (with individual frames of 20 ms) are taken at each viewing position (at 13 keV beam energy). As droplets pass through the device window, the $CaCO_3$ crystals they contain are struck by the incident X-ray beam of 15 μm spot size. In Figure 2b one such diffraction event is observed at Position 15

where a $CaCO_3$ crystal of the calcite polymorph flows through the beam path. Diffraction patterns obtained 20 ms before and after this pattern ***do not*** contain this feature – confirming that the crystal is indeed ***trapped*** in the flow rather than fouling the device wall. We are also able to observe the multi-step crystallization pathway of $CaCO_3$. First, amorphous calcium carbonate (ACC) could be detected in early channel positions, then vaterite, and then finally calcite in positions far downstream. In similar experiments without the carrier oil flow, crystals foul channel surfaces close to the T-junction. Growth of these crystals depletes the aqueous phase of Ca^{2+} and CO_3^{2-} ions, preventing crystallization from taking place in the flow downstream and precluding the observation of single steps in the crystallization process. Ultimately, the channel continues to foul until the flow is blocked completely, further highlighting the advantages of utilising multiphase flow for microfluidic crystallization analysis. This beamtime served as an important ***proof-of-concept*** experiment for time-resolved XRD using droplet microfluidics. Further beamtime will be needed to continue to develop this method by extending it to more crystal systems and including additional crystallization processing ability (e.g. heating, additive insertion, droplet storage) on-chip.

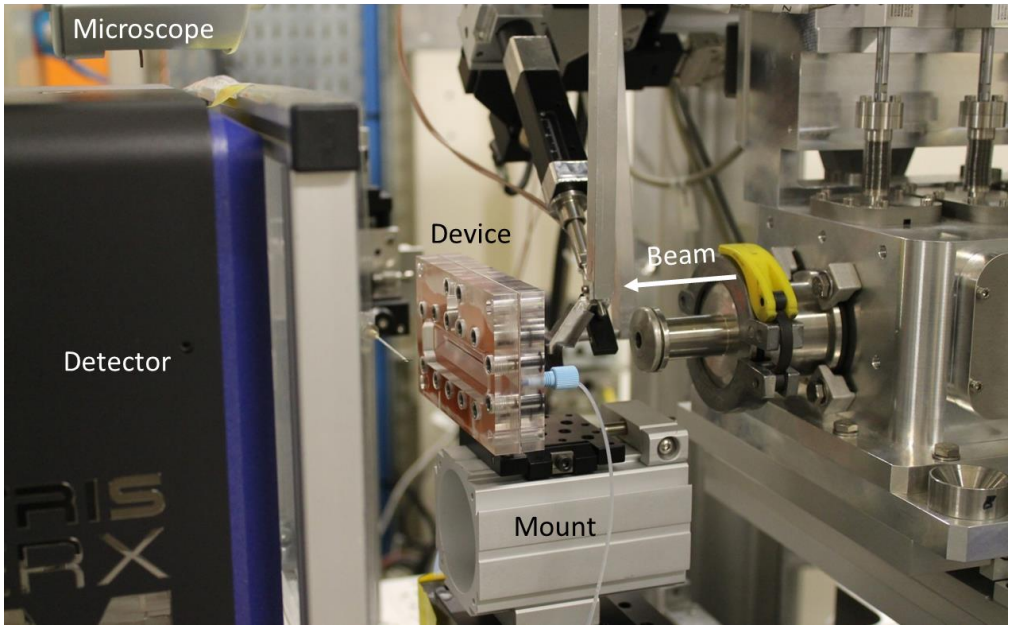

Figure 1: The microfluidic device mounted within the experimental hutch of ID13.

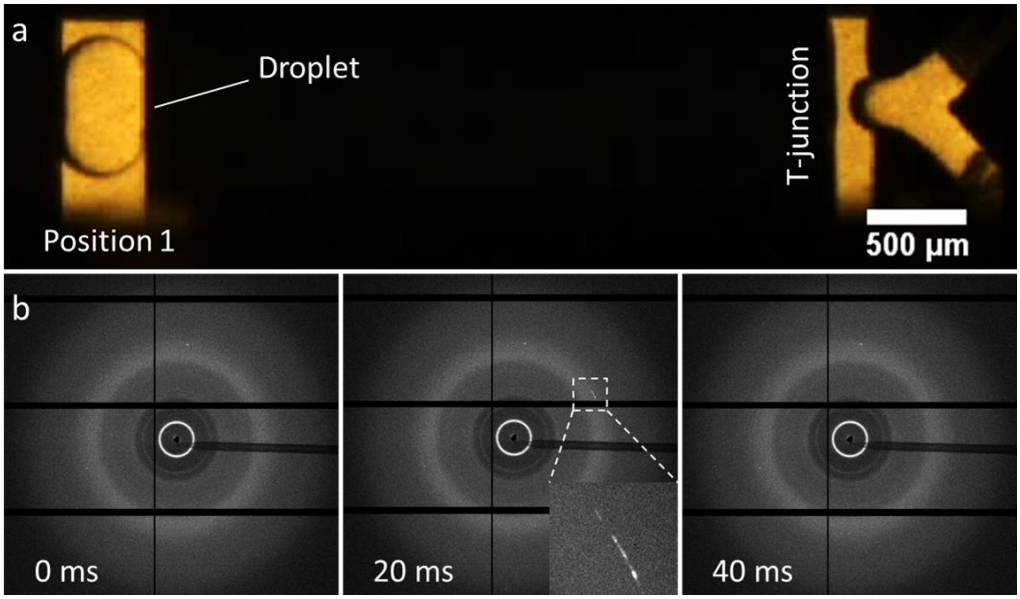

Figure 2: (a) Image of the device window showing the T-junction and Position 1 during flow. (b) Diffraction patterns from 3 sequential 20 ms exposures of the water-in-oil droplet flow within the microfluidic device (*left to right*). In the second exposure (middle image), a {104} reflection from a $CaCO_3$ crystal of the calcite polymorph can be seen (enlarged in inset).

References: [1] Lignos, Protesescu, Stavrakis, Piveteau, Speirs, Loi, Kovalenko and deMello, *Chemistry of Materials*, 2014, **26**, 2975. [2] Beuvier, Panduro, Kwasniewski, Marre, Lecoutre, Garrabos, Aymonier, Calvignac and Gibaud, *Lab on a Chip*, 2015, **15**, 2002. [3] Perry, Guha, Pawate, Bhaskarla, Agarwal, Nair and Kenis, *Lab on a Chip*, 2013, **13**, 3183. [4] Ihli, Wong, Noel, Kim, Kulak, Christenson, Duer and Meldrum, *Nature Communications*, 2014, **5**.