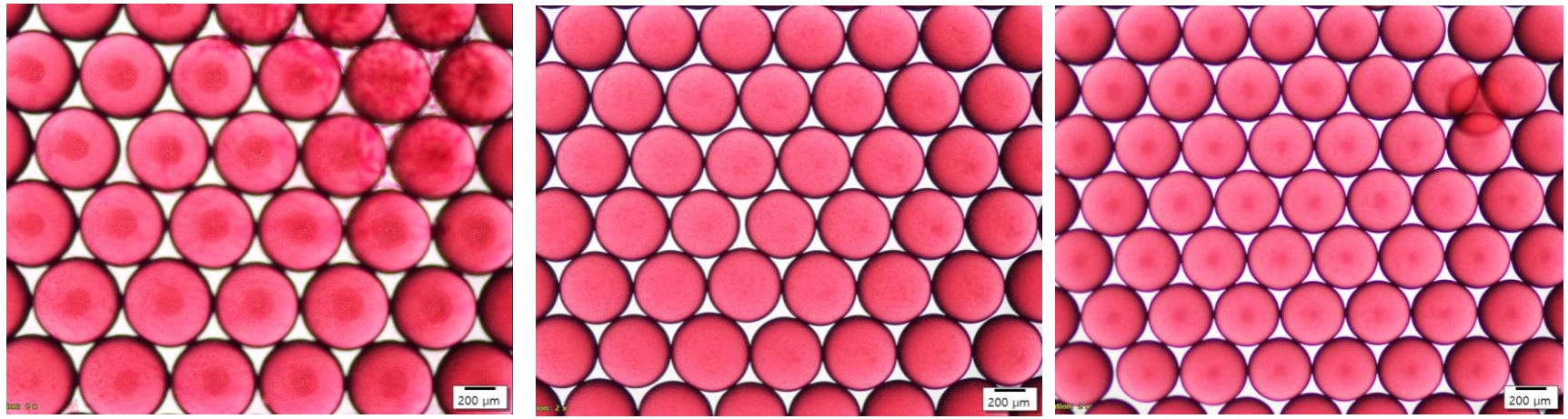
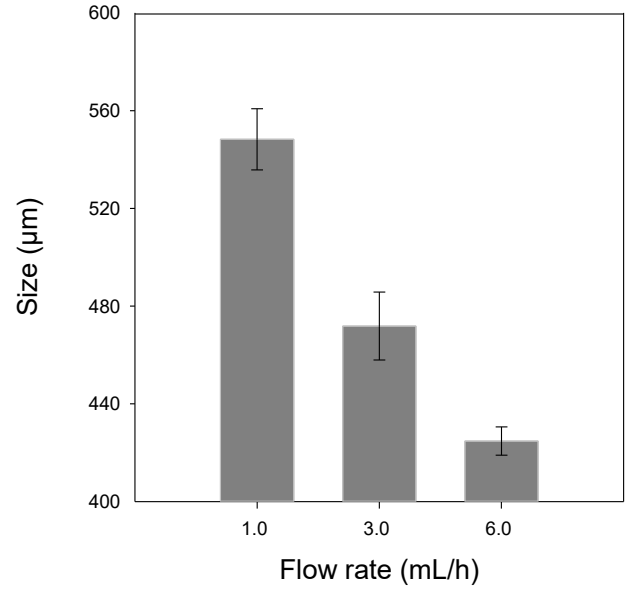
Emulsion fabrication

- DP : DCM + Oil Red, 0.6 mL/h
- CP : 2% PVA in DW, 1, 3, 6 mL/h

CP flow rate : 1 mL/h 3 mL/h 6 mL/h

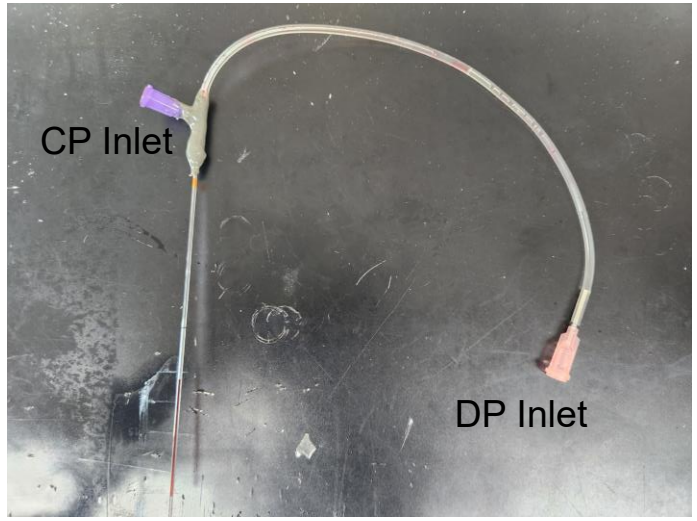




Size : 548.29±12.52 μm 471.82±13.88 μm 424.78±5.79 μm

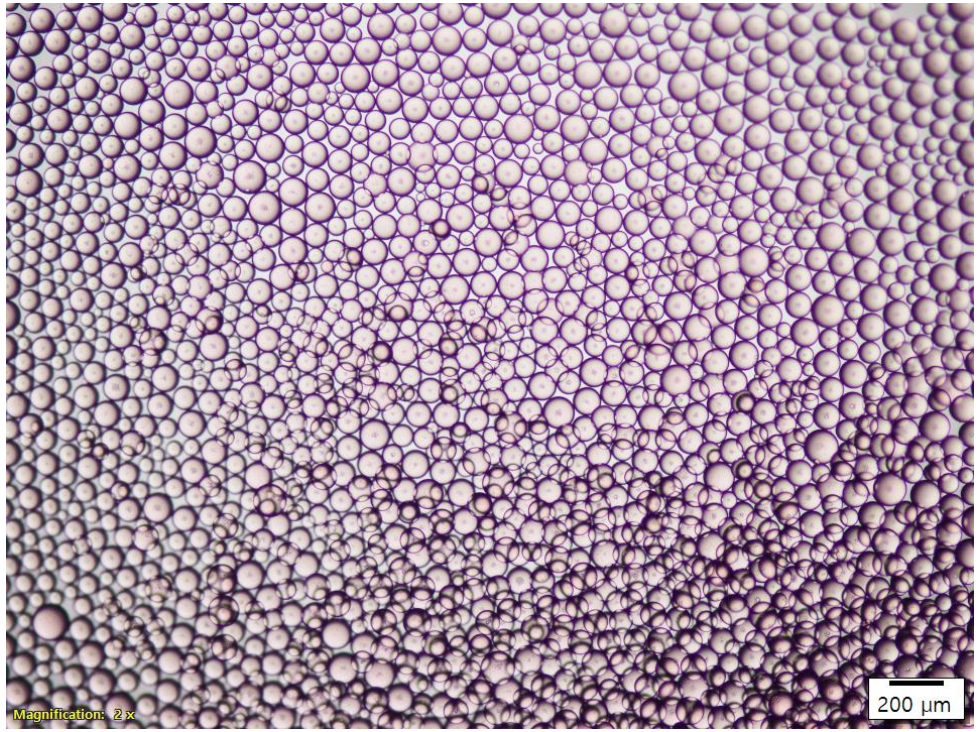
- **Results**

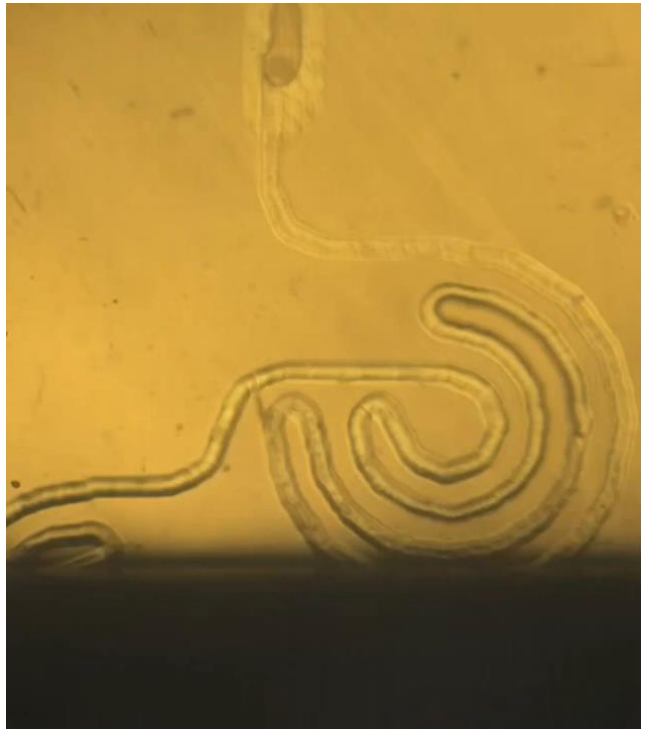
1) Emulsion size decreases as CP flow rate increases
2) CV values: 2.28%, 2.94%, 1.36% (<10%) → Emulsions are uniform

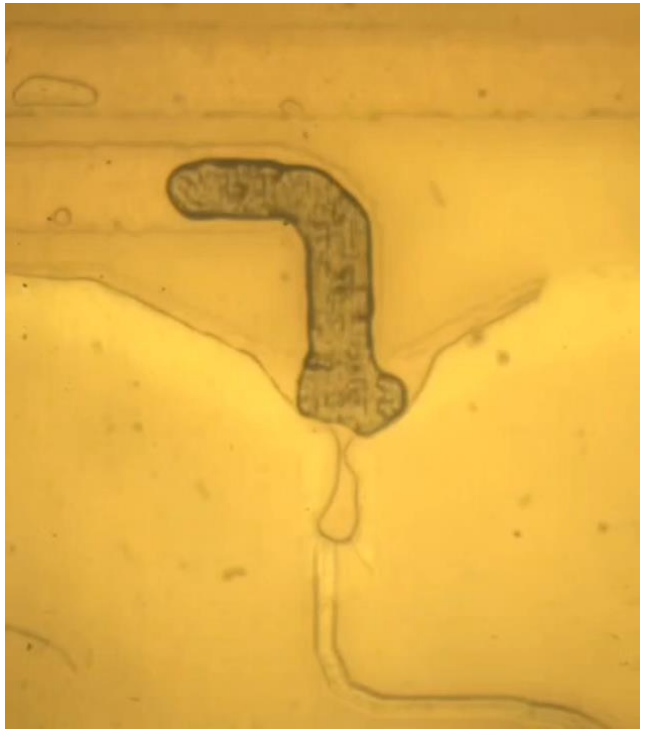


Emulsion fabrication

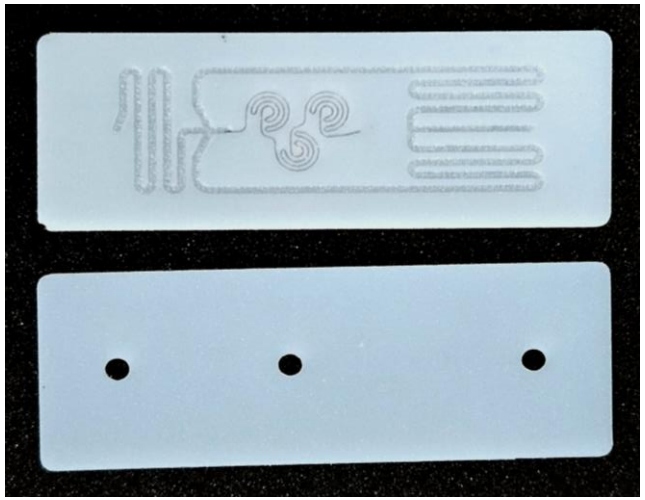
- DP : DCM + Oil Red, 0.5 mL/h
- CP : 2% PVA in DW, 5.0 mL/h







Size : 73.67±20.05 µm



- High volatility of DCM causes continuous bubble formation

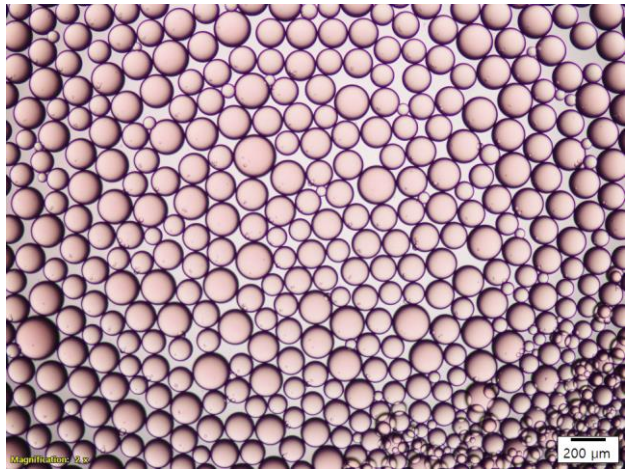
→ CV = 27.22% (>10%)

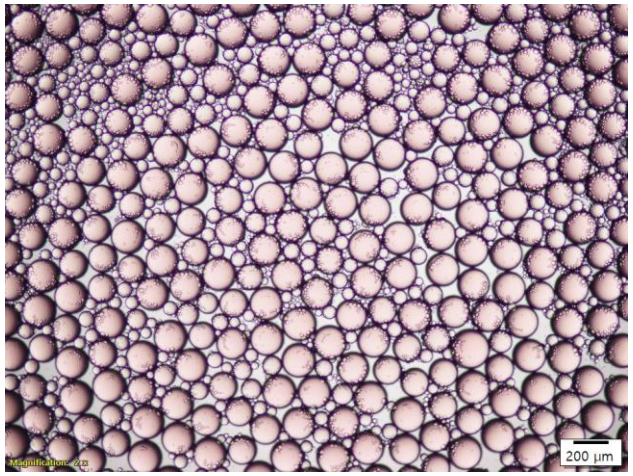
→ Emulsions are non-uniform

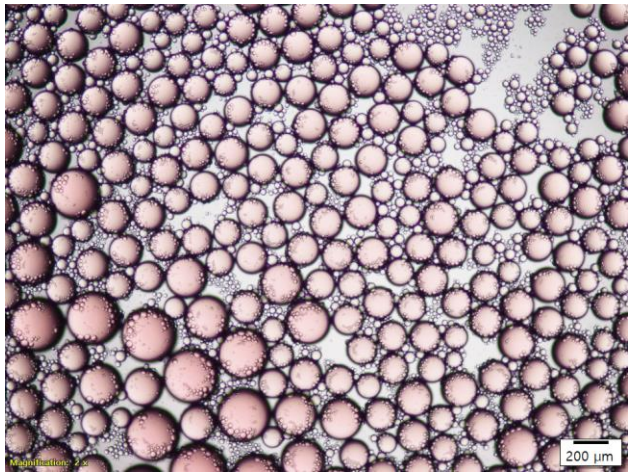
O/W Emulsion Fabrication

- DP : Isododecane + Oil Red, 1 mL/h
- CP : 2% Poloxamer in DW

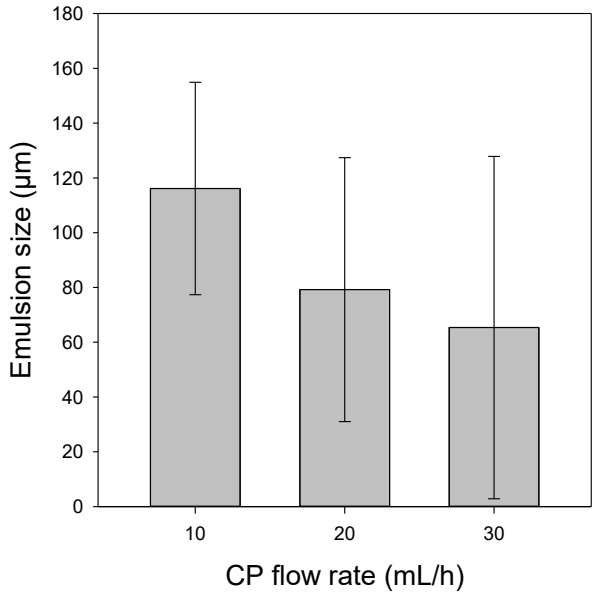
CP flow rate : 10 mL/h | 20 mL/h | 30 mL/h



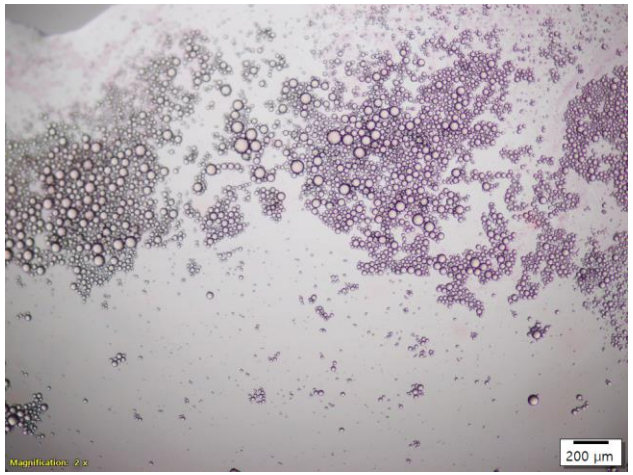


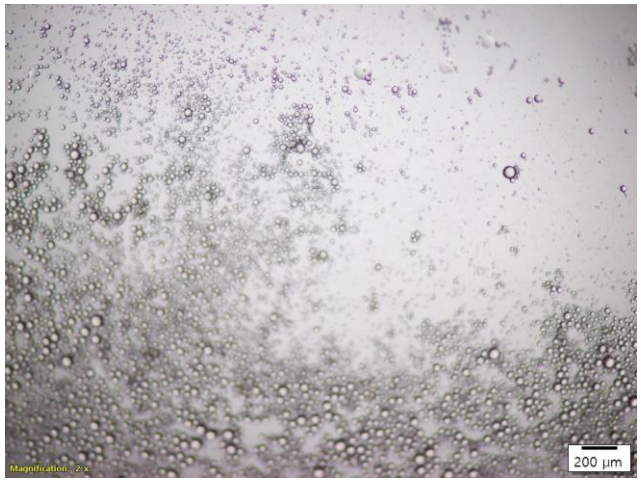


Size : 116.13±38.79 µm | 79.21±48.20 µm | 65.35±62.50 µm

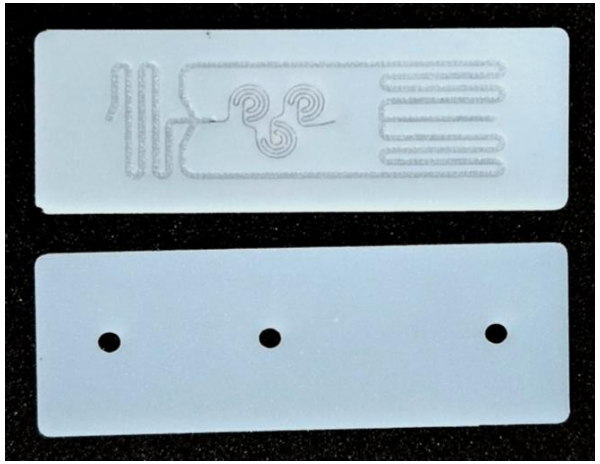


✓ CV values:
33.40%, 60.86%, 95.63%
(>10%)
→ Emulsions are uniform



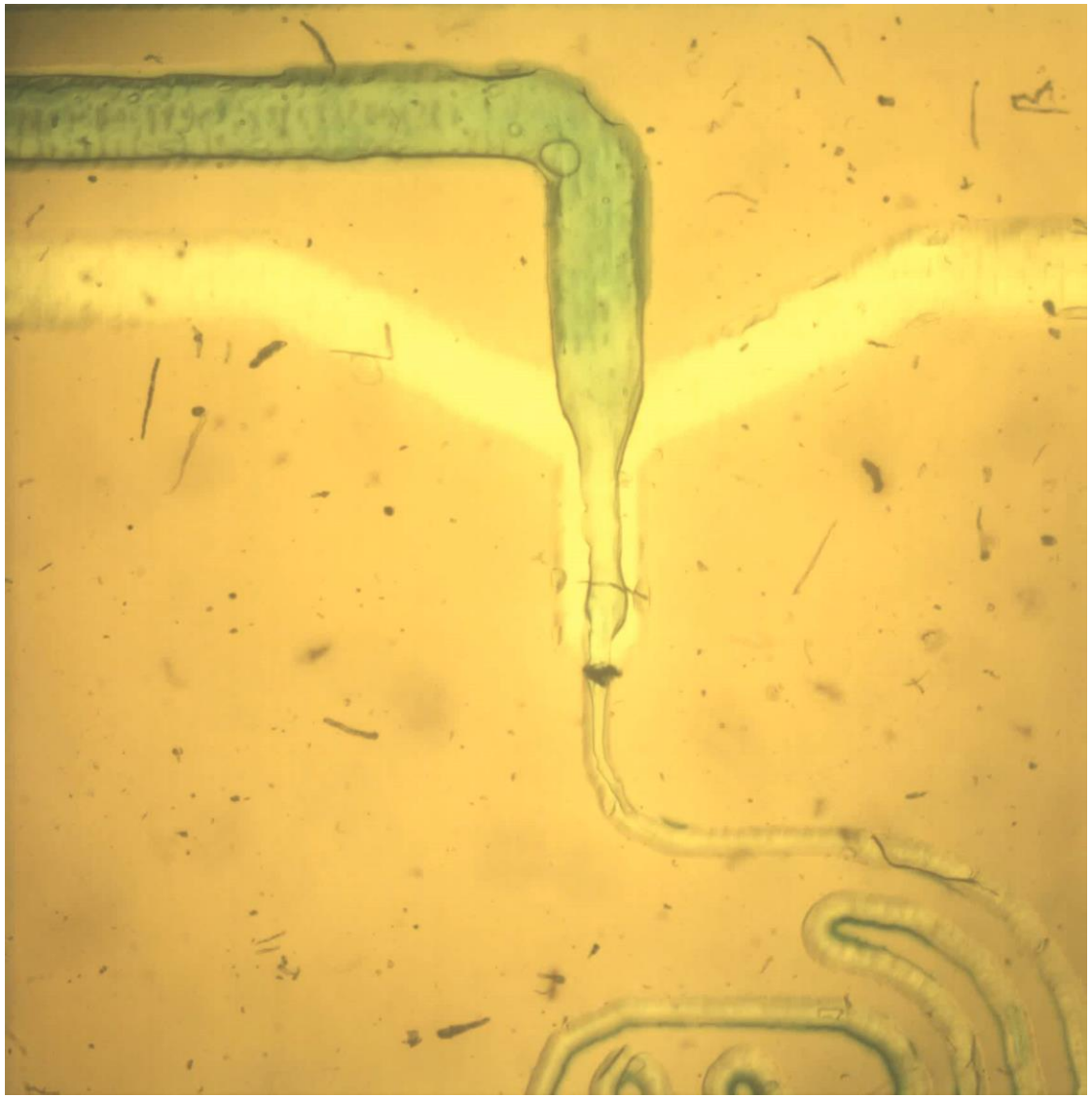


18.58±7.12 µm | 15.95±6.92 µm



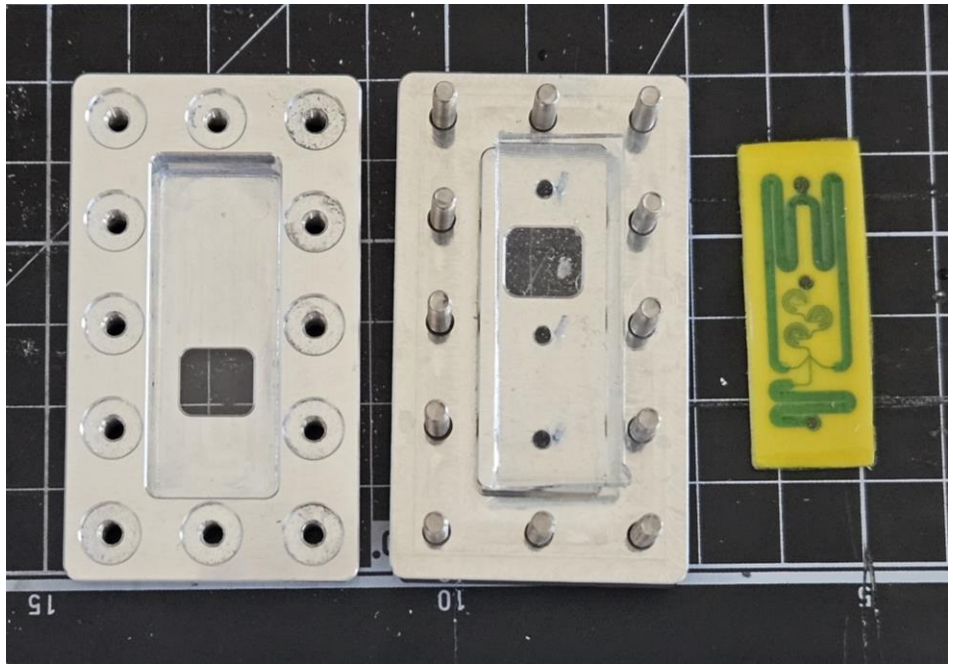
W/O Emulsion Fabrication

- DP : DW + metlyne blue, 1 mL/h
- CP : Isododecane + span80, 10 mL/h



Emulsions not formed at the junction
→ Formed as the channel narrows

Leak test

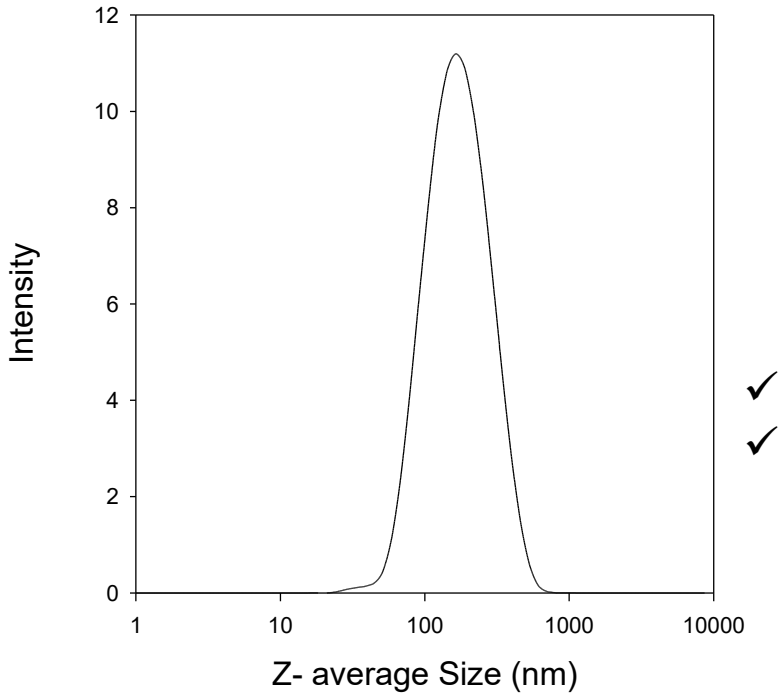


- Stable operation at TFR 5 mL/h for 1 hour
  → No leaks observed

- Upon disassembling the chip
  → Fluid only flowed through channels

LNP fabrication

- TFR : 5mL/h, FFR :3
- DP : 0.25% Lecithin in Ethanol , 1.25mL/h (0.02mL/min)
- CP : DW, 3.75mL/h (0.06mL/min)

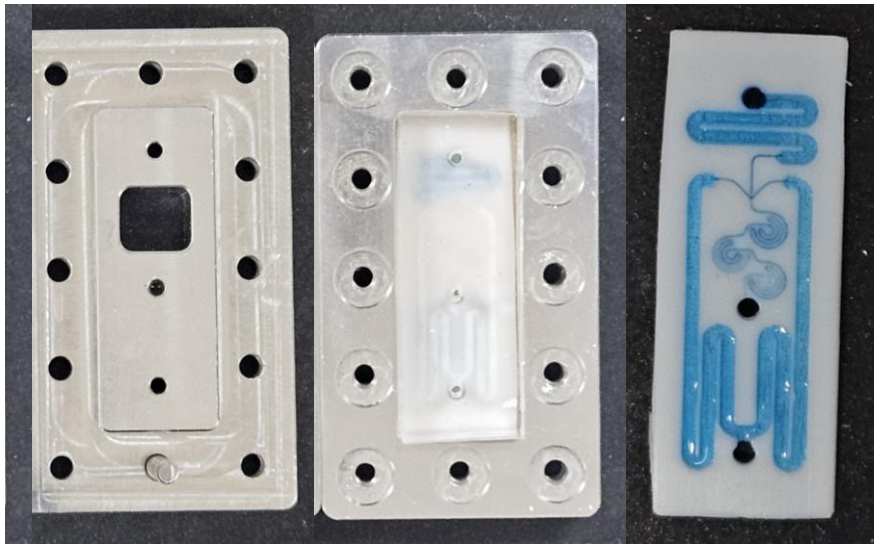


✓ Size :146.90±67.58 nm
✓ PDI : 0.21±0.005

- Using 8T PDMS,
  TFR above 5 mL/h prevented fluid entry into CP and DP
- PDMS thickness reduced to 6T

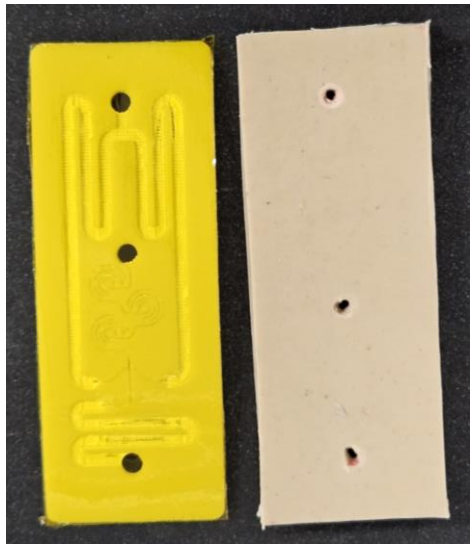
→ less compression, smaller LNPs expected

Teflon + Teflon Tape Leak Test



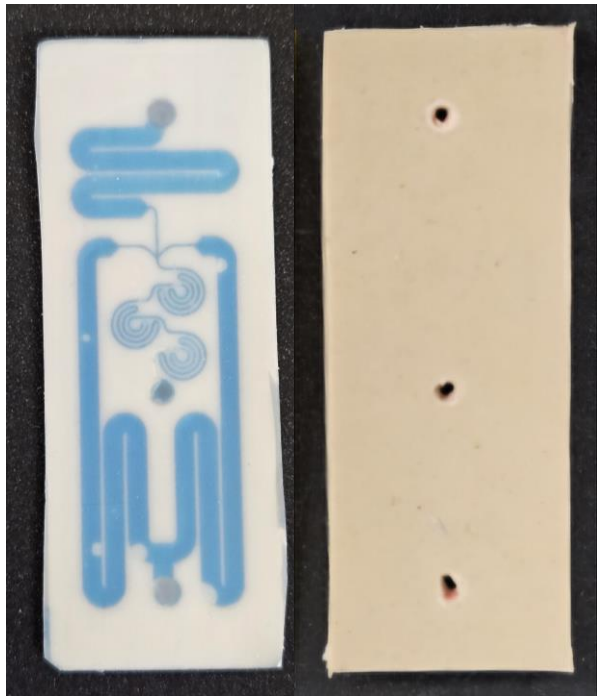
- Leakage was observed when using 6T PDMS
- With 7T PDMS, stable and leak-free operation was maintained for 1 hour at a TFR of up to 5 mL/h
- However, when the TFR was increased beyond 5 mL/h, leakage occurred at the CP inlet

Teflon + Keptone Tape Leak Test



- Further increases in TFR were limited by back pressure from excessive compression
- Therefore, the chip size was enlarged to 1.5× (60 mm × 22.5 mm)

Teflon + Teflon Tape Leak Test



60mm x 22.5 mm

- Using 3T isoprene,
  leak-free operation was confirmed up to a TFR of 30 mL/h

- When the TFR was increased to 40 mL/h,
  leakage occurred after 1 hour of operation.

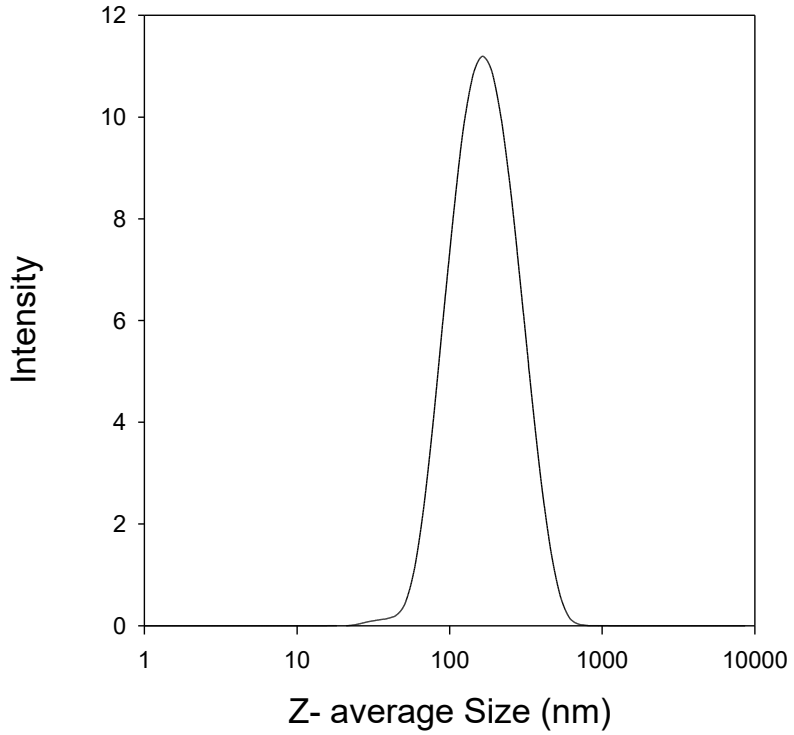
⇒ By increasing the chip dimensions by 1.5×,
a sixfold increase in the maximum achievable TFR was obtained

⇒ To further increase the TFR and fabricate smaller LNPs,
the channel layer will be fabricated
using thicker Teflon (1.5T) instead of 1T Teflon

⇒ After identifying the maximum stable TFR between 30 and 40 mL/h,
LNP formulation will be performed

LNP formulation

- TFR : 5 mL/h, FFR :3
- DP : 0.25% Lecithin in Ethanol , 1.25 mL/h (0.02 mL/min)
- CP : DW, 3.75 mL/h (0.06 mL/min)



✓ Size :146.90±67.58 nm
✓ PDI : 0.21±0.005

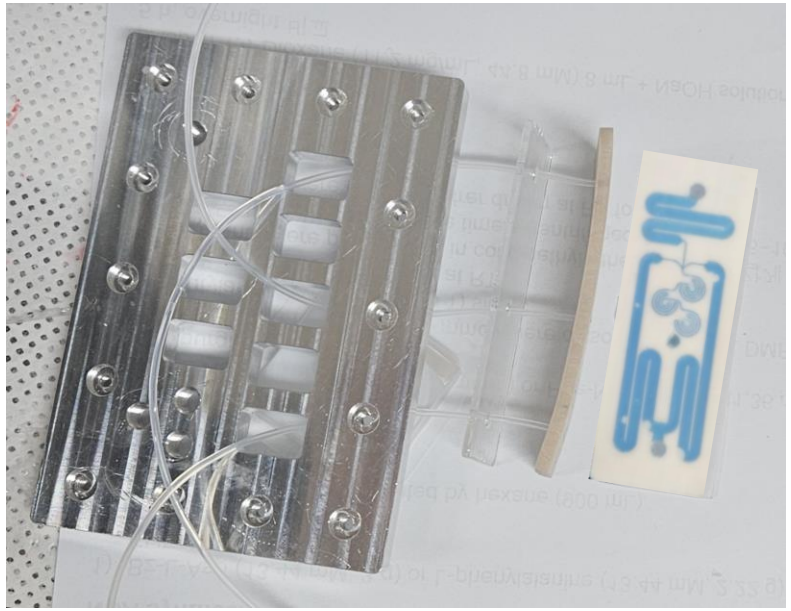
Teflon + Teflon Tape Leak Test

- Channel dimensions increased by 1.5×

→ Maximum TFR increased from 5 mL/h to 30 mL/h (6× increase)

- The chip size was increased by 2× to fit a wider jig,

→ resulting in an increase in the maximum TFR from 5 to 45 mL/h (9× increase)..



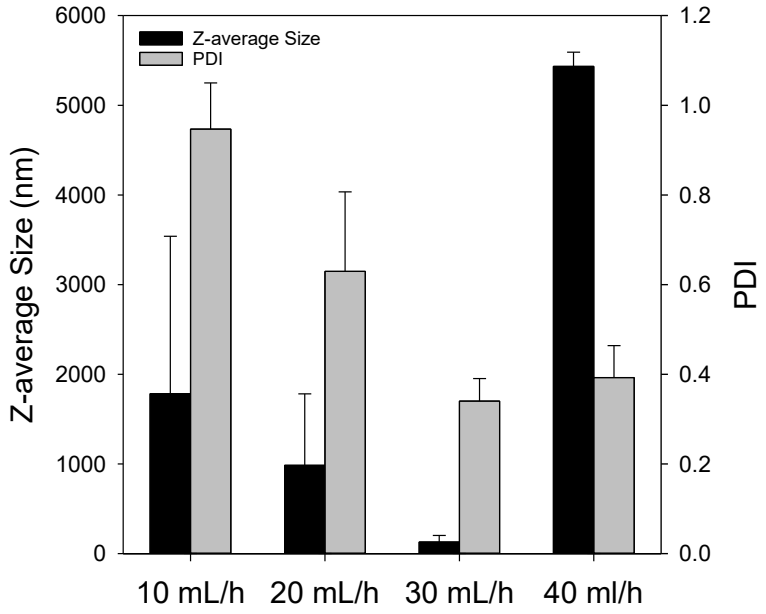
Assembly order:
acryl plate
Isoprene
channel

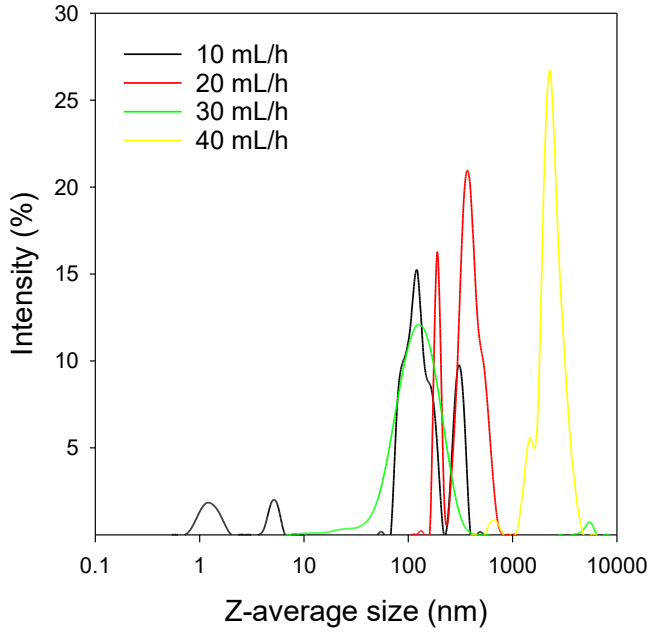
Chip size:
80 × 30 mm

➔ LNP fabrication planned at the maximum flow rate (45 mL/h).

LNP formulation

- TFR : 10 mL/h, 20 mL/h, 30 mL/h, 40 mL/h, FRR : 1:3
- DP : 0.25% Lecithin in Ethanol
- CP : DW





➔ Non-uniform particle formation is likely due to a decrease in flow rate over time. Previous flow tests were conducted using water only, flow rate tests using ethanol and water will be performed.

➔ The surface roughness of the laser-engraved Teflon channels is expected to hinder uniform dispersion, leading to particle aggregation in localized regions and resulting in larger particle sizes or broader size distributions..

	10 mL/h	20 mL/h	30 mL/h	40 mL/h
Z-average Size (nm)	1783.8±1755.06	984.58±798.46	128.32±74.26	5433.40±159.12
PDI	0.95±0.10	0.63±0.18	0.34±0.05	0.39±0.007