MICROFLUIDICS & LAB ON A CHIP

location: INBIT, Kamenice 34, ground floor, rooms 0.22 and 0.23

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I. WORKFLOW

- on-demand droplet generation
- droplet microfluidics and microscopy
- screening of reaction conditions on the chip
- capillary microfluidic platform

II. MOTIVATION

Microfluidics can be defined as the science and technology manipulating and analysing fluid flow in sub-millimeter dimensions. It is becoming important technology for many emerging applications and disciplines, especially in the fields of chemistry, biology and medicine. Concrete application examples are biosensor devices for molecular diagnostics, polymerase chain reaction chips, high-throughput screening, controlled drug delivery systems, drug discovery methods, forensic analysis instruments, and so on (1).

III. THEORETICAL BACKGROUND

III-A. On-demand droplet generation

A nice technique for droplet generation, when multiple distinct samples are necessary, is the on-demand generation using the commercial microfluidic device, Mitos Dropix from Dolomite Microfluidics, UK. The scheme of the droplet generation principle is shown in Figure 1.

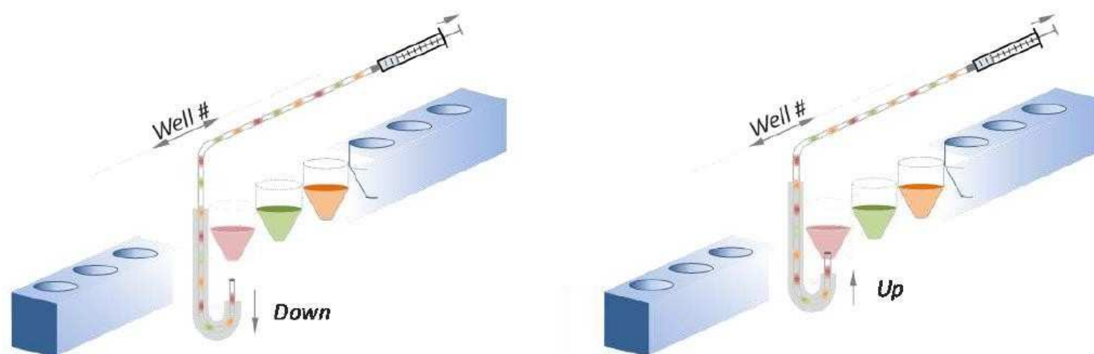

Figure 1 *Droplet on demand – a constant suction driven flow results in the creation of a segmented flow. The timing of the 'up' or 'down' position of the sampling hook dictates the droplet volume and spacing volume respectively. The transverse position of the hook dictates the selection of the sampling well. (Source: https://www.dolomite-microfluidics.com/wp-content/uploads/mitos-dropix-droplet-splitting-application-note-1.pdf)*

III-B. On-chip droplet formation

Formation of water in oil droplets in microfluidic chip has several benefits when compared to standard technology. Amongst such benefits belong low volume of reagents consumed, chip modularity, low cost and simple fabrication. When all pros combined properly one may encounter drop costs of screening million fold [3,4].

In this practice students will put hands on microfluidic chip technology. Prepared chips are to be used for water in oil droplet generation [5]. An example will follow with encapsulation of single *E.coli* BL21 DE3 cells to droplets. Finally, there will be observation of single cells in emulsions generated.

III-C. Screening of reaction conditions suitable for luciferase enzyme on the chip

Microfluidic devices are suitable for time-efficient and strictly precise mixing of solutions. Therefore, microfluidic chip allows to test big number of possible combinations in short time and it can be used for semi-automated screening of reaction conditions such as optimal pH, concentration of effectors or additional salts. Concentration of reaction components inside droplets are managed by setting proper flowrates of individual solutions. Gradient of pH can be established in droplets by mixing buffer acid (tricine) and buffer base (bis-tris propane = BTP) in proper ratios.

Renilla reniformis luciferase (RLuc) is oxidoreductase which emits blue light during conversion of its substrate coelenterazine (CTZ). The aim of this practical part is to test the activity of RLuc on a glass chip in different pH and approximately find out the pH optimum for this enzyme. Ultra-sensitive camera will be used for detection of enzymatic bioluminescence.

III-D. Capillary microfluidic platform

Technical setup (Figure 2) can be described separately as an optical, mechanical and microfluidic part. A precise calibration is one of the primary processes. First, the concentration of HCl must be determined by acid-base titration with potentiometric detection. Once we know the concentration of the acid, the titration of the working buffer must be performed to know the dependency between the proton concentration (pH respectively) and the fluorescence intensity of the probe. Working buffers with three different pH values are used for the "on-platform" calibration of the proton-sensitive assay – the buffer with initial pH ≈8.00 and several mixtures adjusted by titration with HCl to desired pH, respectively. The fluorescence intensity of the pH-sensitive probe must be measured at all check-points (loops) and for all ratios of reaction mixture and acid. Please note, that the fluorescent dye is temperature sensitive and therefore the calibration must be measured for all the combinations.

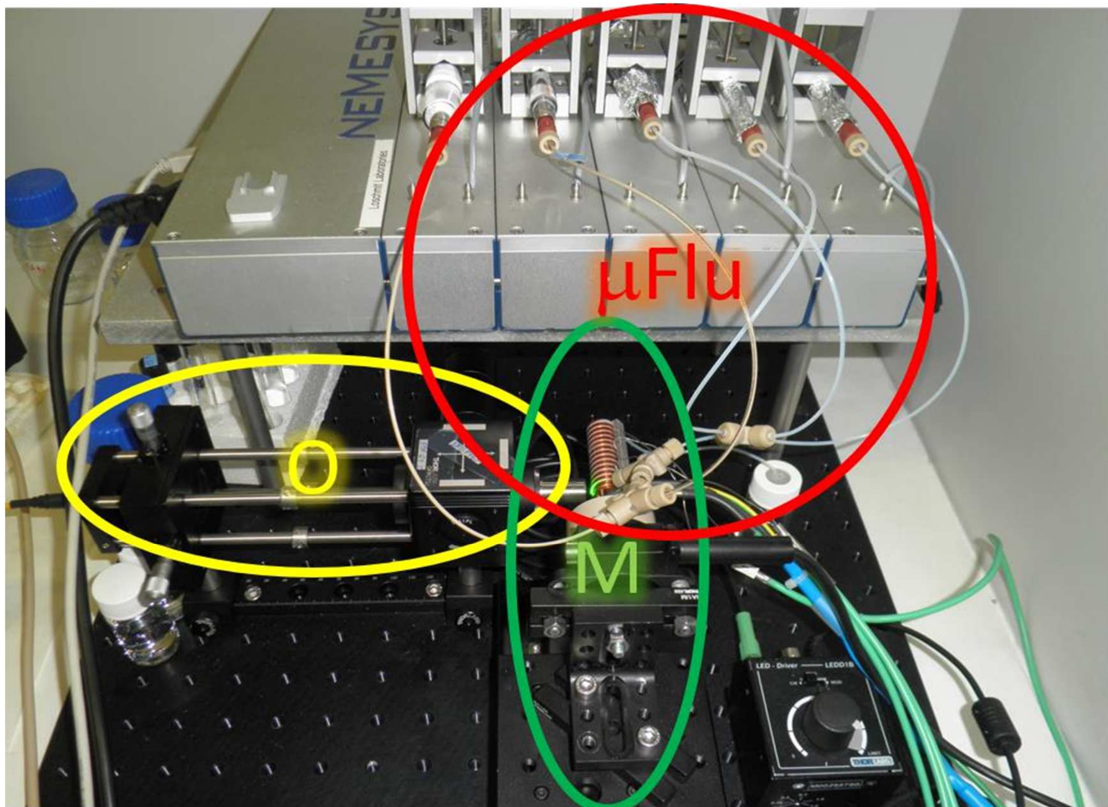

Figure 2 *A photography of the capillary-based microfluidic platform with highlighted optical (O), mechanical (M) and microfluidic (μFlu) parts.*

IV-A. PROTOCOL (On-demand droplet generation and fusion)

1. Prepare 1mL of 50 µM HPTS solution out of 1 mM HPTS stock solution. Using the 6 mM HCl stock solution, prepare five HCl calibration solutions of concentrations equally distributed between 1.5mM and 0.3mM. Use the just prepared 50 µM HPTS solution for dilution. Plan the pipetting so that you finally have at least 40 µL of each solution.
2. Insert 20 µL of the 50 µM HPTS solution and of each calibration solution into separate wells of the rack. Insert also 20 µL of the test-enzyme sample into another well. Markdown the numbers of the wells.
3. Start the microfluidic pump at 10 µL/min in a withdraw mode.
4. Write the sequence of droplets to be generated into the Dropix software. The order of samples is up to you, but it should have some logic. Just follow the rule that each aqueous phase droplet should be 150 nL and the oil phase in between the droplets should be 300 nL. Also, 10 droplets should be generated per each of the sample (per each well).
5. Start the droplet generation on Dropix.
6. When finished wait a while, until you visually see that the generated droplets in the tubing.
7. Start the detection on the LabView detection window.
8. Observe the droplets being detected – both with the naked eye and on the detector.

IV-B. PROTOCOL (On-chip droplet formation)

Solutions and reagents:

- HFE-7500 or FC-40 oil
- PicoSurf-2 surfactant
- Trichloro(1H,1H,2H,2H-perfluorooctyl)silane
- 1.5M NaCl
- deionized H_2O
- *E.coli* BL21 DE3 (calculate the concentration!)
- Percoll ®
- Isopropanol

Equipment:

- Chemyx Fusion 200 syringe pumps or precise neMesys syringe pumps
- gas-tight syringes (various volumes)
- PTFE tubing
- Microfluidic chips – various designs
- glass slide
- Inverted microscope

1. load syringes with HFE-7500 oil and 150 mM NaCl, 25 % (v/v) Percoll and properly diluted cells

2. attach PTFE tubing to the syringe and remove any bubbles
3. put syringes into the syringe pumps, lock tight and set proper syringe diameter
4. connect syringe via tubing into the chip
5. set liquid flow – 300 µL.h^{-1} for oil phase and 30 µL.h^{-1} for the aqueous phase
6. observe droplet formation under microscope at various magnification
7. verify cell occupation in emulsions on inverted microsope

IV-C. PROTOCOL (Screening of reaction conditions suitable for luciferase enzyme on the chip)

Solutions and reagents:

- HFE-7500 or FC-40 oil
- PicoSurf-2 surfactant
- 50mM bis-tris propane = BTP (pK_A = 9.0) + 150mM NaCl solution
- 50mM tricine (pK_A = 8.1) + 150mM NaCl solution
- *Renilla reniformis* luciferase (RLuc8) solution (diluted in mixture of BTP and tricine 1:9)
- coelenterazine (CTZ) solution (diluted in mixture of BTP and tricine 1:9)
- deionized H_2O
- isopropanol or ethanol

Equipment:

- glass microfluidic chip
- ultra-sensitive camera
- neMesys syringe pumps
- gas-tight syringes (various volumes)
- FEP tubing
- pH meter

1. load syringes with HFE-7500 oil, RLuc8, CTZ, 50mM BTP and 50mM tricine
2. remove any bubbles from the syringes, attach FEP tubing to the syringe and fill tubing with solutions
3. put syringes into the syringe pumps, lock tight and set proper syringe diameter
4. connect syringe via tubing into the chip
5. set liquid flow – 4 µL.min^{-1} for oil phase and 1 µL.min^{-1} for the aqueous phases
6. observe droplet formation under microscope
7. change flowrates of BTP and tricine to establish different pH in droplets
8. take images of the chip by FluorCam software for every flowrate settings
9. measure pH of bis-tris propane and tricine mixtures (in tested ratios) by pH meter

IV-D. PROTOCOL (Enzyme kinetic on the microfluidic platform - calibration)

Solutions and reagents:

- FC-40 oil
- 1.0M NaCl
- 50 mM Tricine
- 50 mM BisTrisPropane
- deionized H_2O

Equipment:

- neMesys precise pump
- gastight syringes (various volumes)
- PTFE tubing
- Microfluidic platform
- CCD spectrophotometer

1. Prepare 5 mL of 50 μM HPTS solution (use the 10 mM HPTS stock and UB1 buffer) and 0.5 mL of 2 mM HCl solution (use 100 mM stock solution and DW)
2. Fill the gastight syringes (HPTS, HCl, FC-40) and mount them into pump
3. Start the pumps at flowrates (Oil 20 $\mu L.min^{-1}$ / HPTS 8 $\mu L.min^{-1}$) – generating plugs
4. Increase the flowrate of HCl for increment of 0.05 $\mu L.min^{-1}$ till 0.5 $\mu L.min^{-1}$
4. Collect data and plot the calibration curves

V. HOMEWORK

1. Calculate the volumes to be pipetted to prepare all reaction solutions according to the protocol IV-A.

2. Estimate volume in pico-/femto- litres for monodisperse droplet formed at channel having dimensions 5, 10 and 20 µm, respectively (assume square cross-section forms spherical droplets of the same diameter).

3. For calculated droplet volumes estimate approximate cell density in $x.10^y$ per mL, to put single cell per droplet. There are approximately 1.10^8 cells in medium with OD_{600} 0.5. Cultivated cells have OD_{600} 4.8. In case that grown culture has insufficient density, calculate the factor for thickening cell media to sufficient level. Account for 10 % pipetting error.

VI. LITERATURE

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