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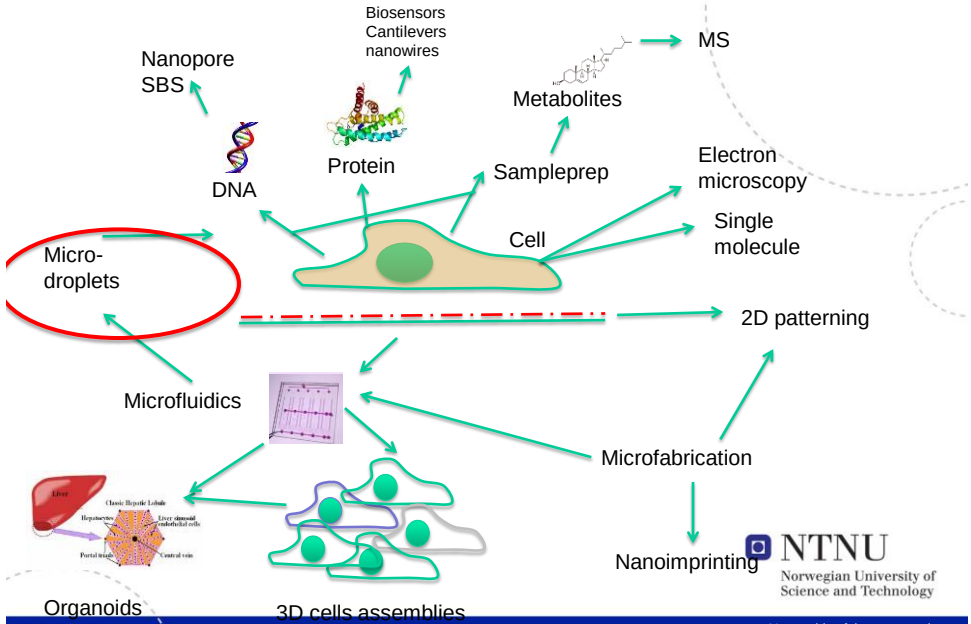
Digital (droplet) microfluidics

Øyvind Halaas, IKM
Mol3014 Nanomedicine I bioanalysis

Course outline : nanotechnology for biomedicine : analysis



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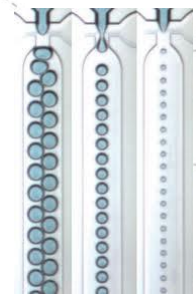
Digital, discrete droplets -one at the time

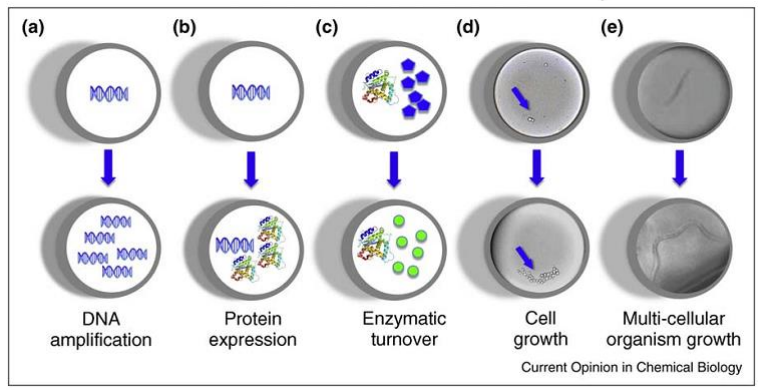


Standard microfluidics needs continous fluid flow

- Waste of reagents
- No flowing spatial resolution (only stationary)
- For discrete events, additional steps needed

Solution: Biphasic microfluidics

Biological assays performed in microdroplets



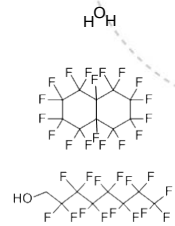
Pensum
DOI10.1016/j.cbpa.2010.08.013

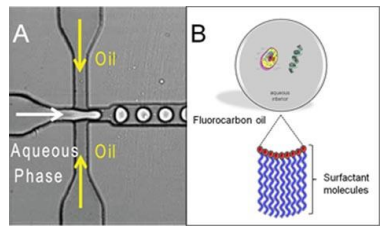
Microfluidic droplets: A two-phase system

1. Water-based reaction chamber

2. Superhydrophobic carrier (continous fluid)
   FC40, FC70 (DuPont)
   perfluorodecalin

3. Surfactant (interphase between phases)
   pentadecaflouoro-1-octanol
   Krytox (perfluorinated polyether, DuPont)





http://www.youtube.com/watch?v=O7rqq4DHckM

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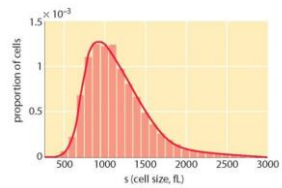
Microfluidic droplets – a volume consideration

- 10-200 μm diameter
- 0.5pL - 20nL
- =1/100.000 volume of standard assay volume
- Speed x1.000
- Cost 1/1.000.000 (2/3 of cost in HTS screen from pipette tips)

10^{-18}	1 attolitre	Volume of a typical virus (5 attolitres, a million million times a hydrogen atom)	
10^{-15}	1 femtolitre	Volume of a human red blood cell (90 femtolitres, 9×10^{-17} m3)	
10^{-12}	1 picolitre	A very fine grain of sand (0.063 mm diameter, 3 micrograms, 130 picolitres, almost a million times a virus	
10^{-9}	1 nanolitre	A medium grain of sand (0.5 mm diameter, 1.5 milligrams, 62 nanolitres, almost 500 very fine sand grains)	
10^{-6}	1 microlitre	A large grain (granule) of sand (2.0 mm diameter, 95 milligrams, 4 microlitres, 64 medium sand grains)	
10^{-3}	1 millilitre	(1 cubic centimetre)	1 teaspoon = 3.55 ml to 5 ml (about 1000 large sand grains) 1 tablespoon = 14.2 ml to 20 ml
10^{0}	1 litre	(1 cubic decimetre)	200 5ml teaspoons

Volume distribution of a L1210 mouse lymphoma cell



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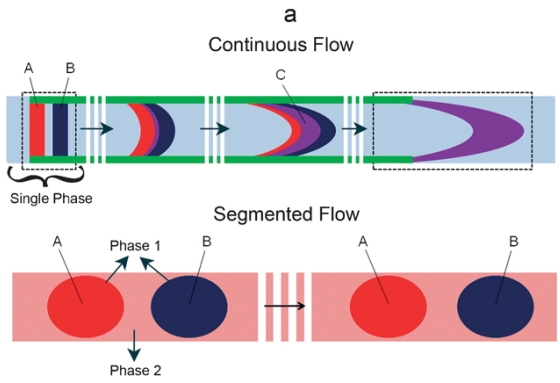
Advantages of segmented flow



Comparison between continuous and segmented flow microfluidics: microfluidic droplets elegantly address several issues of continuous flow, such as Taylor dispersion of reagents due to parabolic flow: the enlargement of the dotted area illustrates this spreading effect; cross-contamination: the single continuous phase allows diffusion between different fluid portions—in this case A and B eventually combine and become C (radial diffusion is omitted for the sake of simplicity); and reagent adsorption on channel walls (illustrated as green channel edges) leading to reagent loss and cross-contamination

doi: 10.1039/c0cc02474k

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Chips for making droplets (previous lectures)

- PDMS (most common)
- Glass
- Thiol-ene
- Silicon
- PMMA

Surfaces must be treated with «teflon» = fluorinated substrate

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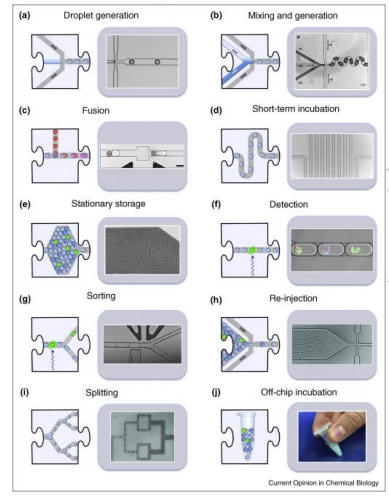
Handling droplets

- Forming (easy)
- Mixing (easy)
- Merging
- Splitting
- Addressing/sorting
- Analyzing



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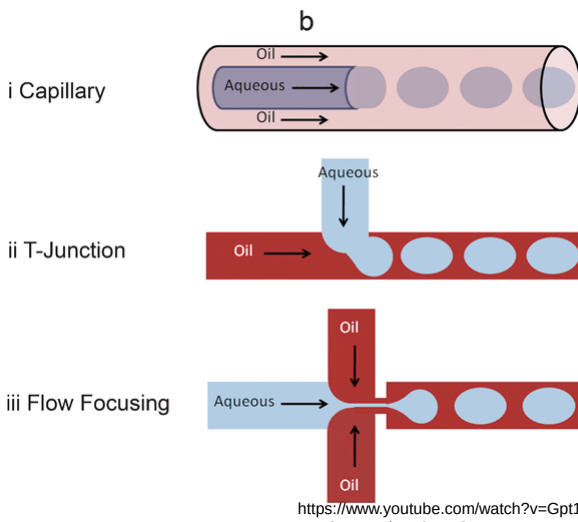
Formation of droplets



kHz, x000/sec

https://www.youtube.com/watch?v=Gpt1vkVAZJo&index=4&list=PLLvC_Sfeh477POXgpTHleTn0uoKQ-ZXoN

doi: 10.1039/c0cc02474k https://www.youtube.com/watch?v=YUw5808pohY

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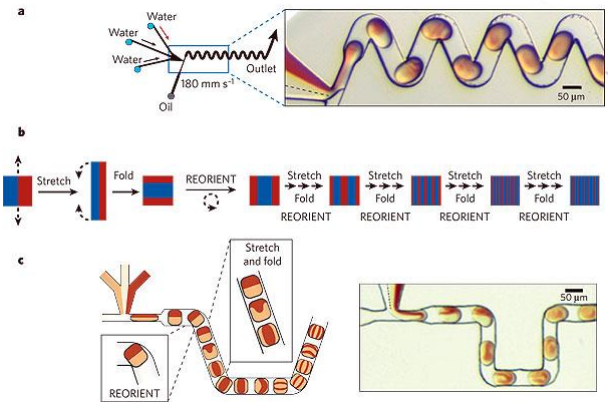
Mixing droplet content



https://www.youtube.com/watch?v=X3_eIm0nB0o&list=PLkexjjHnqVfZcGduZwQXFKu7kekgLP9rM&index=6

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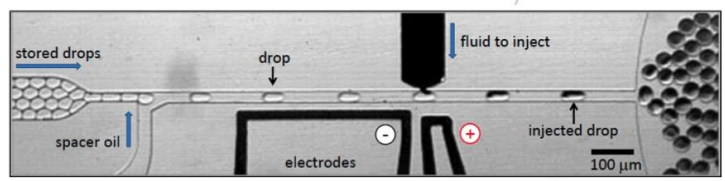
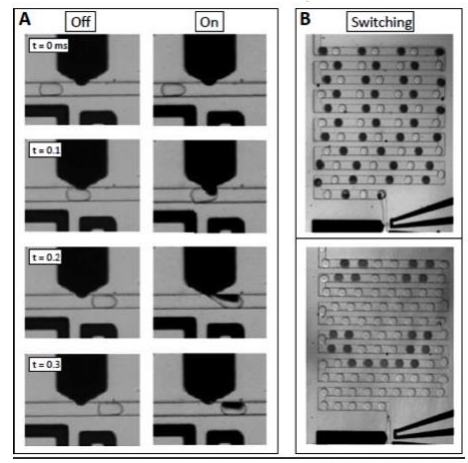


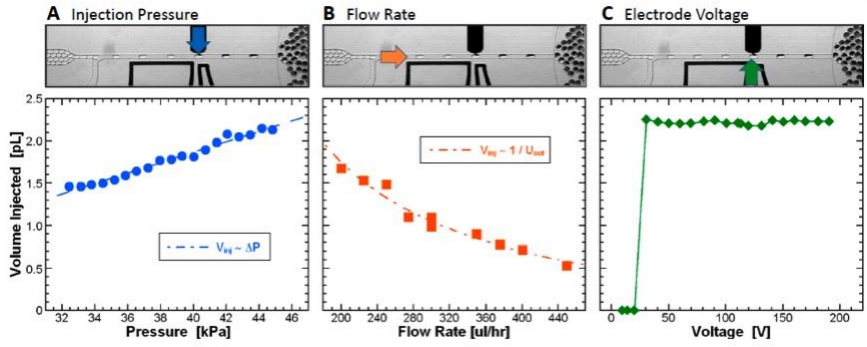
Injecting reagents



https://www.youtube.com/watch?v=vRue2Hk46D8

Amount of reagent injected dependent on

Injection pressure
Flow rate of droplet
Voltage



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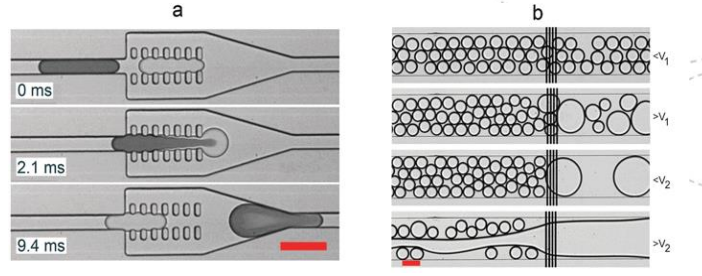
Merging droplets

Employs the principles of surface effects and $V_{in}=V_{out}$



https://www.youtube.com/watch?v=LlOiKdtgy3k

Chem Commun (Camb). 2011 Feb 21;47(7):1936-42. doi: 10.1039/c0cc02474k. Epub 2010 Oct 22.
Droplet microfluidics: recent developments and future applications.
Casadevall i Solvas X, deMello A.

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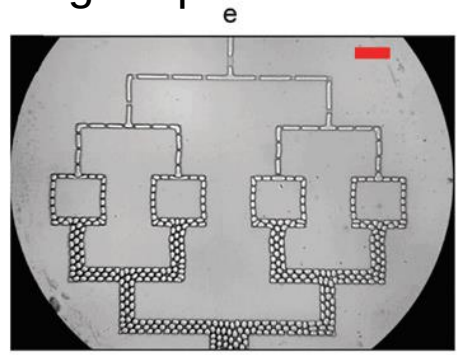
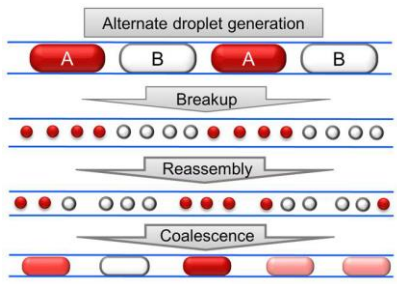
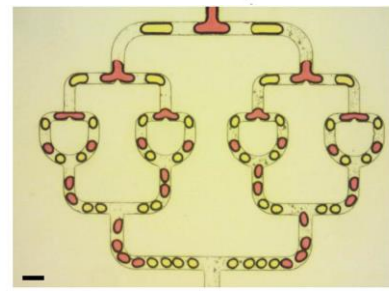
Splitting droplets



By simply applying splitters, droplets will tendend to split

Serial manipulation of microdroplets





Serial dilution of droplets

A general principle in biology is dose-response and titration

http://www.youtube.com/watch?v=7Kk14AKkZeY

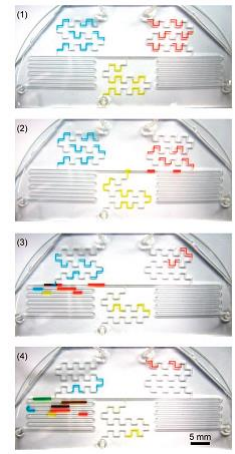
Nat Chem. 2011 Jun;3(6):437-42. doi: 10.1038/nchem.1046.
A microdroplet dilutor for high-throughput screening.
Niu X, Gielen F, Edel JB, deMello AJ.

Handling of droplets

https://www.youtube.com/watch?v=2KhIjcxgnkk

Microprocessing of Liquid Plugs for Bio/chemical Analyses

http://www.youtube.com/watch?v=AENnU0vdjPo



Anal. Chem., 2008, 80 (16), pp 6206–6213
DOI: 10.1021/ac800492v

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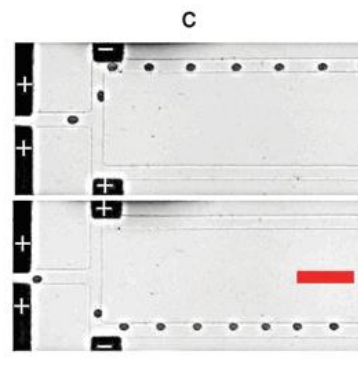
Dielectrophoretic sorting of droplets

Represents same challenges as in cell sorting



https://www.youtube.com/watch?v=RNpwgHCB5hQ

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Analyzing droplets

1. Imaging-based droplet analysis
    a. Bright-field microscopy
    b. Fluorescence microscopy

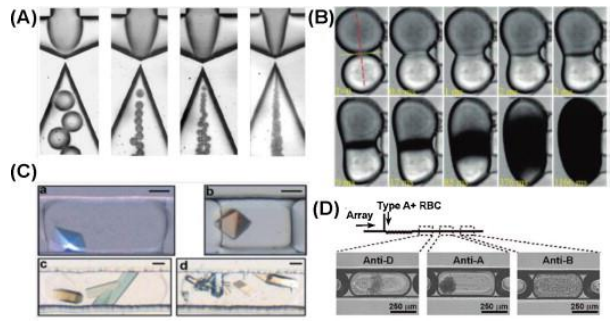
2. Laser-based molecular spectroscopy
    a. Laser-induced fluorescence (LIF) detection
    b. Raman spectroscopy

Electrochemical detection

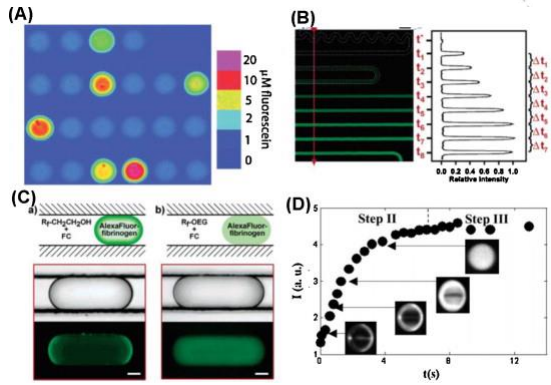
3. Capillary electrophoresis (CE)
4. Mass spectrometry
5. Nuclear magnetic resonance spectroscopy
6. Absorption and chemiluminescence detection

http://dx.doi.org/10.1016/j.aca.2013.04.064

Analyzing droplets: Brightfield microscopy



Analyzing droplets: Fluorescence microscopy



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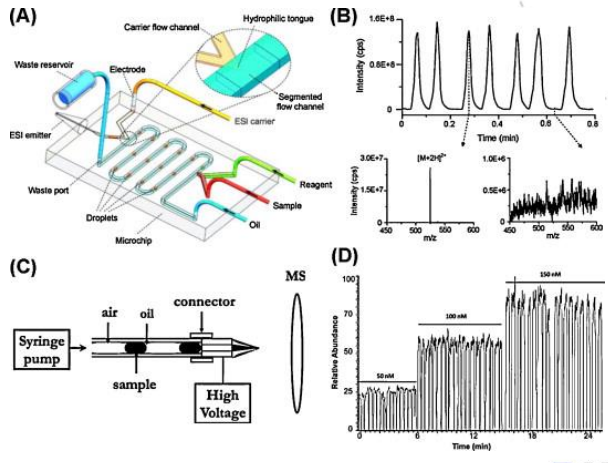
Analyzing droplets: MassSpec



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Single-cell applications in microfluidics chips includ

- Polymerase chain reaction (PCR)
- Culturing of cells
- Cytotoxicity
- Sorting
- Separation
- clone formation
- Lysis
- gene expression
- protein expression
- antibody secretion

Analyzing biomarkers in droplets

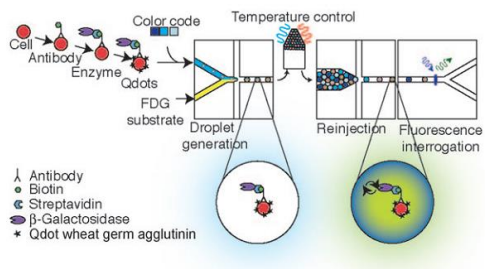

Figure 1. Schematic depiction of the complete assay. Cells were labeled for a specific cell-surface protein with an antibody-coupled enzyme and a quantum dot wheat germ agglutinin (WGA) stain for visualization. Cells were then encapsulated in droplets on the microfluidic chip with a sample-specific color code and a fluorogenic enzyme substrate. After incubation to let the signal develop, each droplet's fluorescence was analyzed. FDG = fluorescein-di-β-D-galactopyranoside.

http://dx.doi.org/10.1002/anie.200804326

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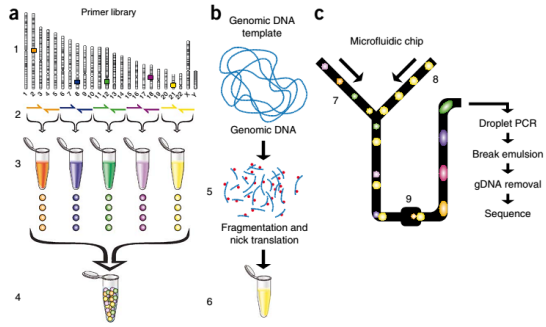
Microdroplet-based PCR enrichment for large-scale targeted sequencing



1.5mill discrete droplets formed

doi:10.1038/nbt.1583

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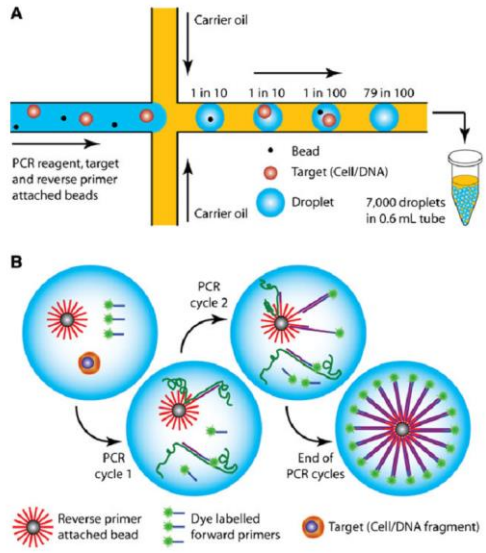
Single-molecule genomic amplification



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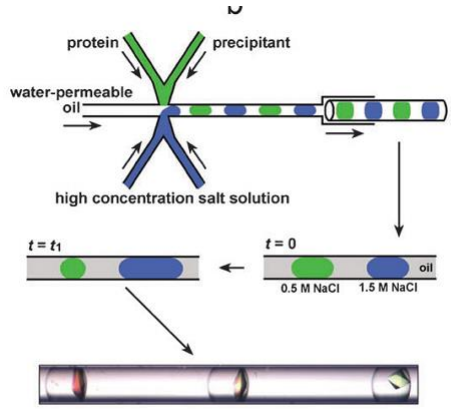
Crystallization of proteins for structure determination



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Encapulating cells

Primary human cells
Cell lines
Bacteria
Nematodes
Zebrafish embryos

High viability but low proliferation inside droplets

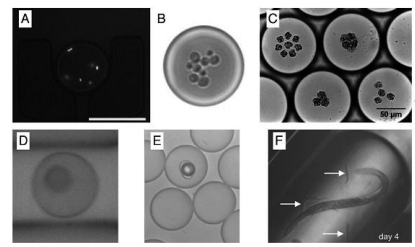

Figure 3. Many different cell types have been encapsulated and demonstrated to retain their viability in droplets. A) *E. coli* cells expressing RFP.[97d] B) Droplet-cultured *S. cerevisiae* clones proliferating in droplets.[30b] C) The green microalgae *C. reinhardtii*.[70] D) Human monocytic cell line U937.[115] E) Adherent insect cells *B. mori* growing on a bead surface.[65] F) Two generations of *C. elegans* cells cultured in droplets. The arrows indicate the second generation of droplet-cultured worms.[52] (Reprinted with permission from: (A), (B), and (C), The Royal Society of Chemistry 2009, 2008, and 2011, (D) Wiley-WCH Verlag GmbH & Co KGaA 2009, (E) and (F), Elsevier 2010 and Chemistry & Biology 2008.)

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Design and operation determines cell/capsule distribution

Size of droplet

Concentration of cells

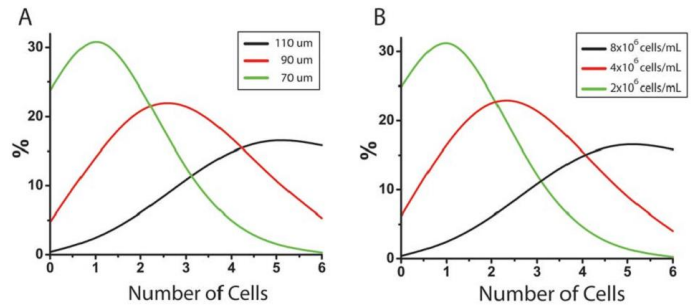

Figure S2. Theoretical Poisson Distribution of Cells in Cell-laden Microgels

Microencapsulation of cells in microfluidic devices

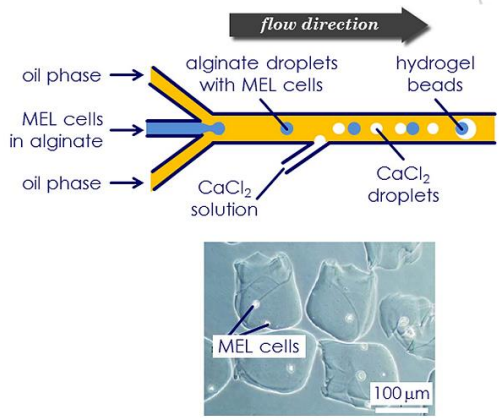

Fig. 7. Cross junction microfluidic chip for the fabrication of alginate microparticles encapsulating mouse erythroleukemia (MEL) cells. The calcium chloride solution was pumped into the main channel by a lateral T-junction.
Adapted and reproduced with permission from [99], ©Springer.

http://dx.doi.org/10.1016/j.addr.2013.07.021

Thermal gellation in agarose for microencapsulation

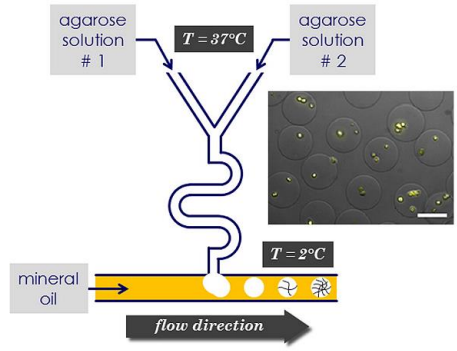

Fig. 12. Production of agarose microparticles with tunable mechanical characteristics by a T-junction chip geometry, showing the serpentine channel used to mix the two solutions of agarose at different concentrations. A representative optical microphotograph of the obtained microparticles entrapping murine embryonic stem (mES) is reported. Adapted and reproduced with permission from [116], @2010 Elsevier.

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http://dx.doi.org/10.1016/j.addr.2013.07.021

A variant is cell-laden fibre formation

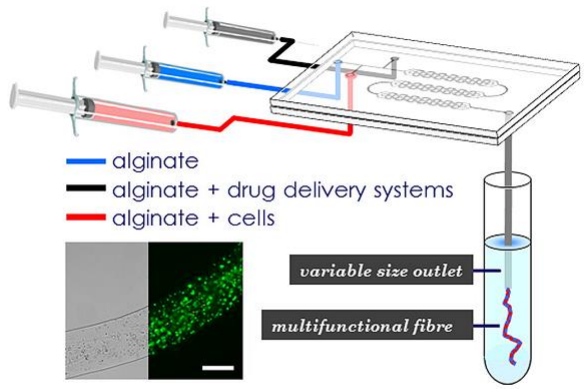

Fig. 16. Microfluidic set-up for producing multifunctional alginate microfibers by a glass microfluidic chip and micrograph of the produced microfibers entrapping Wharton's jelly mesenchymal stem cells (WJMSCs), bar corresponds to 250 μm. Adapted and reprinted with permission from [140], The Royal Society of Chemistry.

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Hepatic structures as coaxial fibres

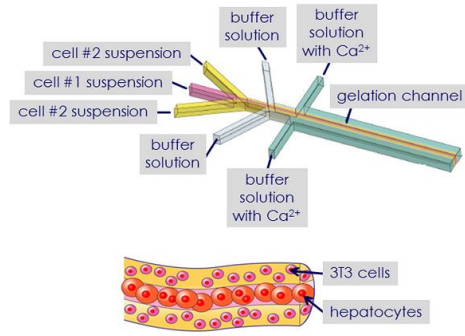

Fig. 17. Microfluidic chip for the fabrication of anisotropic alginate hydrogel microfibers containing hepatocytes (cell #1) and mouse embryonic fibroblast cell line (3T3) (cell #2) and schematic illustration of the obtained microfiber.
Adapted and reproduced with permission from [141], @2012 Elsevier.

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Forming cellular barriers inside microchannels

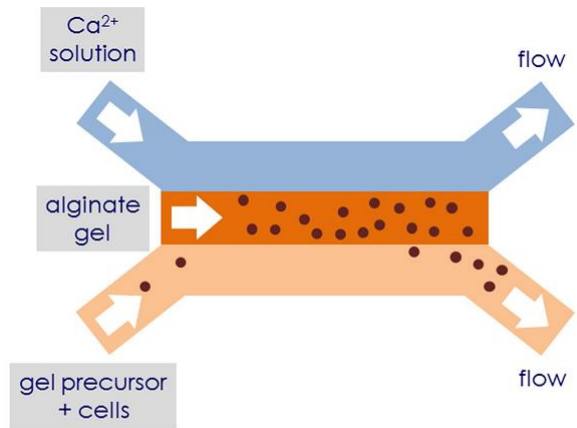

Fig. 20. Top view of microfluidic device during the alginate gel formation and cell entrapment.
Adapted and reproduced with permission from [148], ©Springer.

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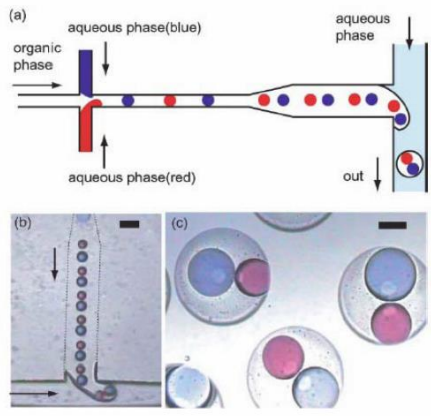
Two droplets in a droplet



Nisisako et al. (2005)

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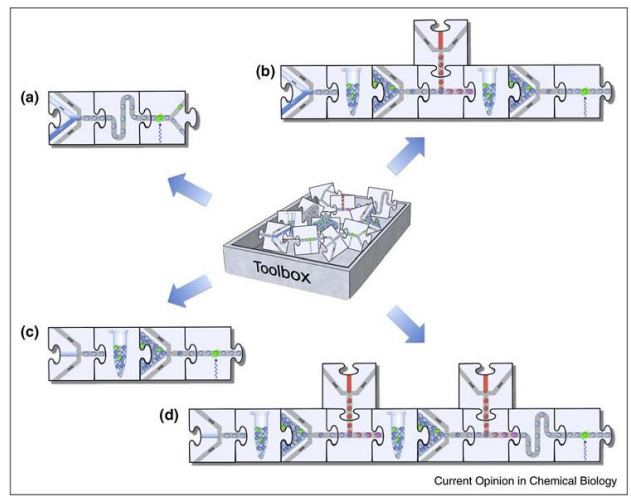
Module-based design for integration



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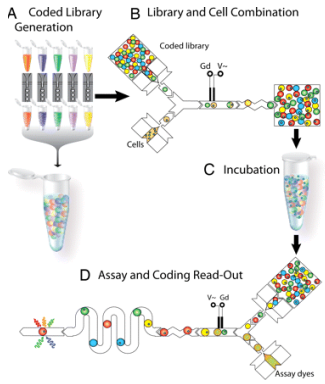
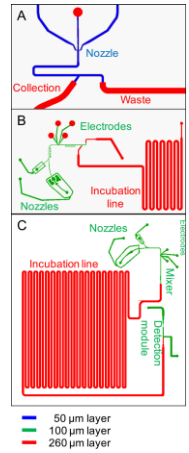
Single cell viability studies in droplets in a multimodular microfluidic device (700pL droplets/second): Putting it all together

Work flow

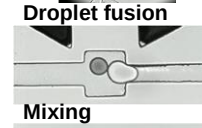


Device design

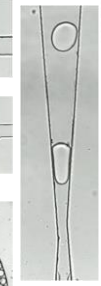


Alternation

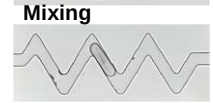
Droplet fusion



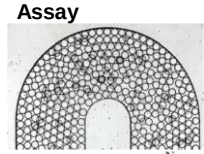
Detection



Mixing



Assay



Droplet microfluidic technology for single-cell high-throughput screening.
Proc Natl Acad Sci U S A. 2009 Aug 25;106(34):14195-200. Epub 2009 Jul 15.
Brouzes E, et al

Movies http://www.pnas.org/content/suppl/2009/07/15/0903542106.DCSupplemental/

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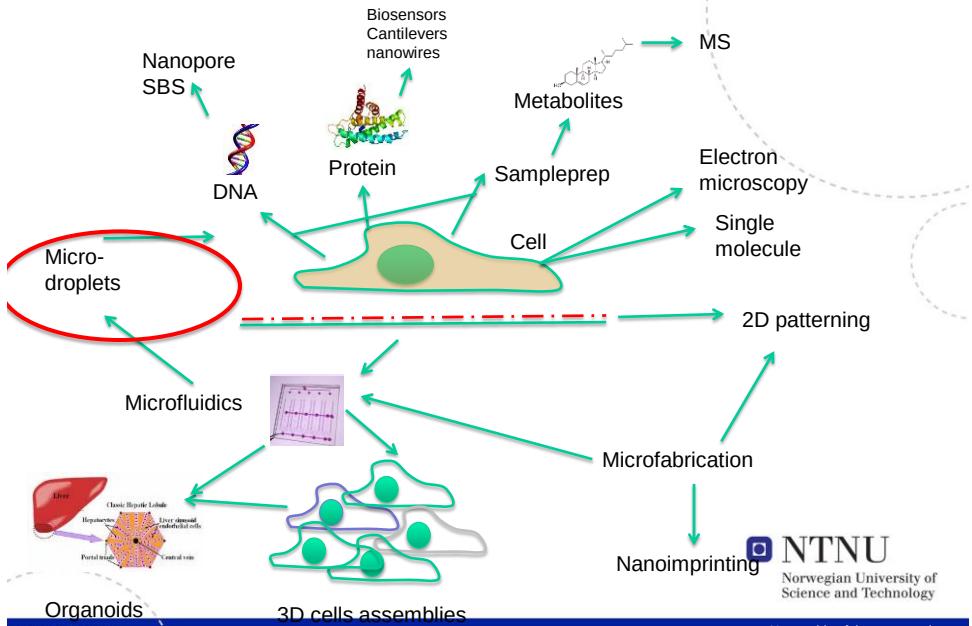
Course outline : nanotechnology for biomedicine : analysis



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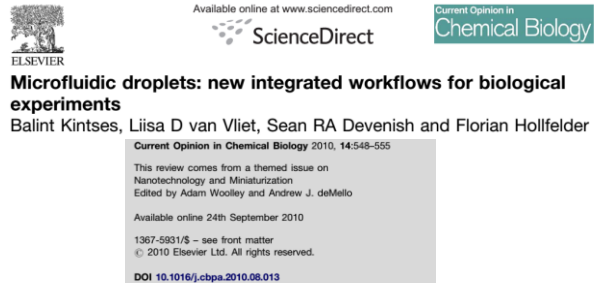

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Microfluidic droplets: new integrated workflows for biological experiments

Balint Kintses, Liisa D van Vliet, Sean RA Devenish and Florian Hollfelder

Current Opinion in Chemical Biology 2010, **14**:548–555

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1367-5931/$ – see front matter

DOI 10.1016/j.cbpa.2010.08.013

Additional reading

Angewandte Reviews

H. N. Joensson and H. Andersson Svahn

Life in a Bubble

DOI: 10.1002/anie.201200460

Droplet Microfluidics—A Tool for Single-Cell Analysis

Haakan N. Joensson and Helene Andersson Svahn*

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