Chemistry & Biology on the Small Scale: Continuous & Segmented Flow Microfluidics

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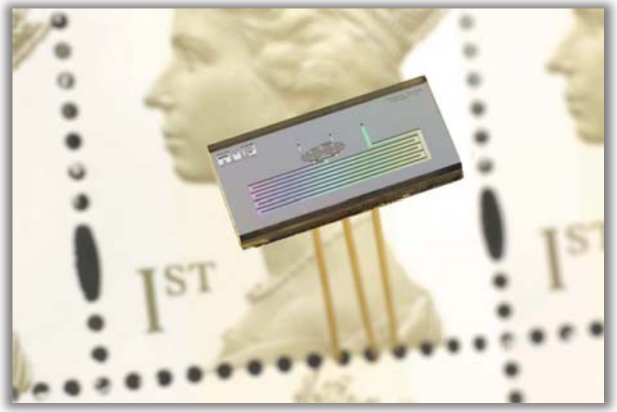
why miniaturize?

benefits of miniaturization

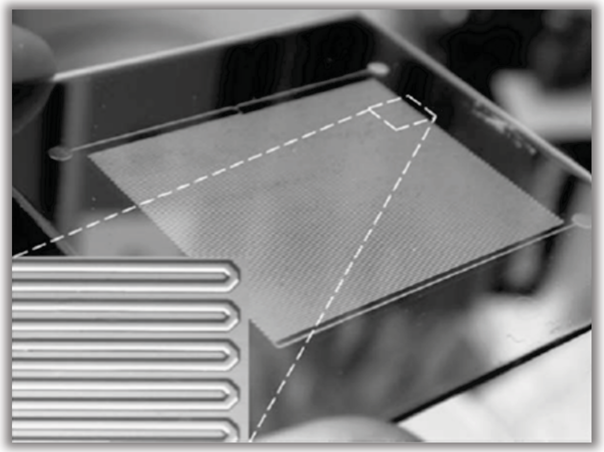
- cost economies through micromachining
- reduced sample/reagent and power consumption
- portability (e.g. point-of-care/in-the-field applications)
- superior analytical performance (*speed*, *efficiency* and *control*)
- facile process integration and automation (*c.f. microelectronics*)
- high analytical throughput
- functionality



Anal. Comm. 1999 36 213

why chose microfluidics?

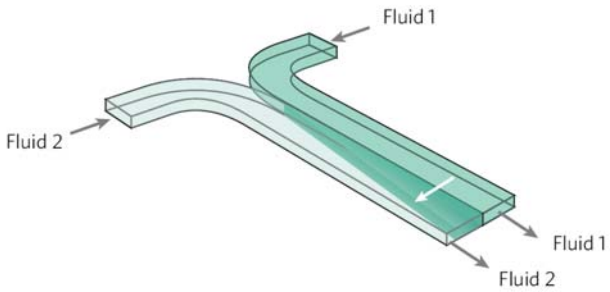
- scale dependence of heat and mass transfer
- improved performance (speed/efficiency/control/throughput)
- functional integration of components facile



Angewandte Chemie 2007 119 2933

superior quality and rate of generation of chemical / biological information

the importance of scale on fluid flow



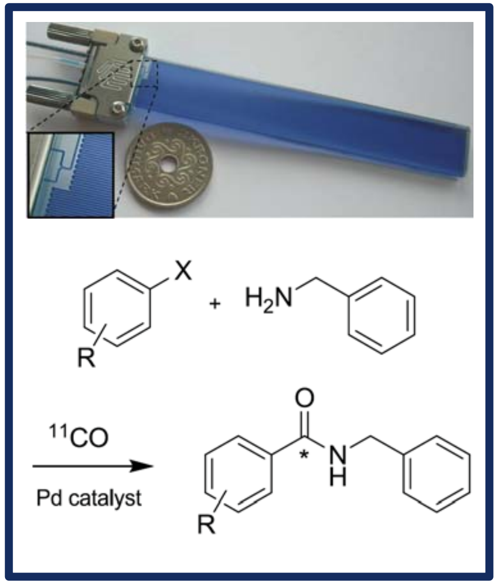
$$Re = \frac{\rho v \delta}{\mu}$$

$$Pe = \frac{v\delta}{D}$$

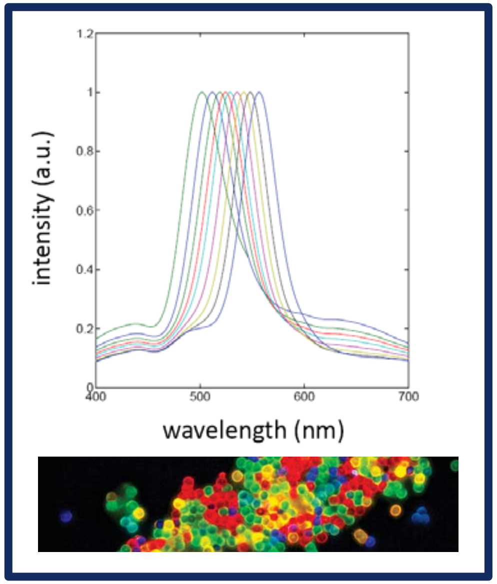
$$Ca = \frac{v\mu}{\gamma}$$

- large surface area-to-volume ratios allow for highly efficient mass and heat transfer in microsystems
- mass and energy are transferred more quickly when creating or homogenizing solute & temperature gradients
- as system dimensions are reduced fluids are increasingly influenced by viscosity rather than inertia, which results in laminar flow.
- the relative importance of diffusion and convective bulk flow for mass transport is given by the Péclet number and can be controlled by flow velocity and system dimensions.
- high surface area-to-volume typical on the microscale ensures that surface tension influences fluid behaviour.

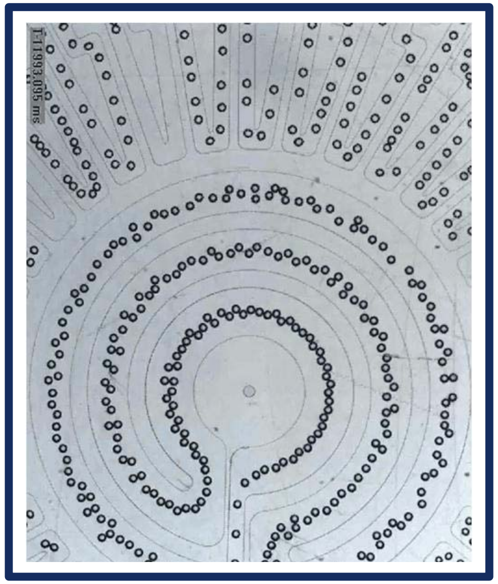
microengineered reaction systems



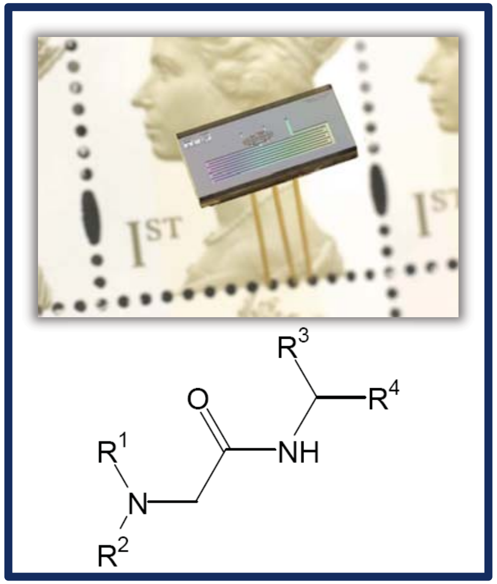
radiochemistry



nanoparticle synthesis

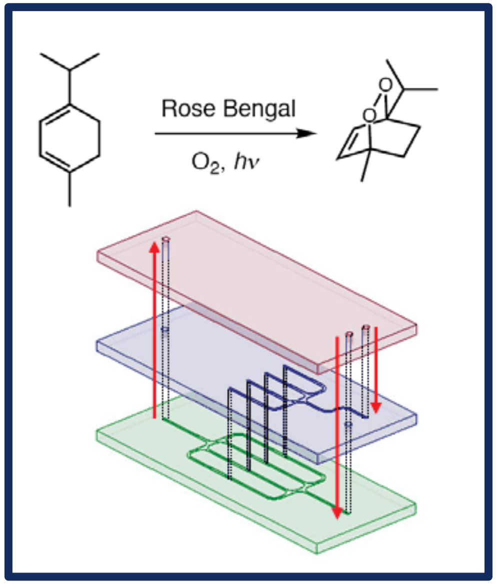


DNA amplification



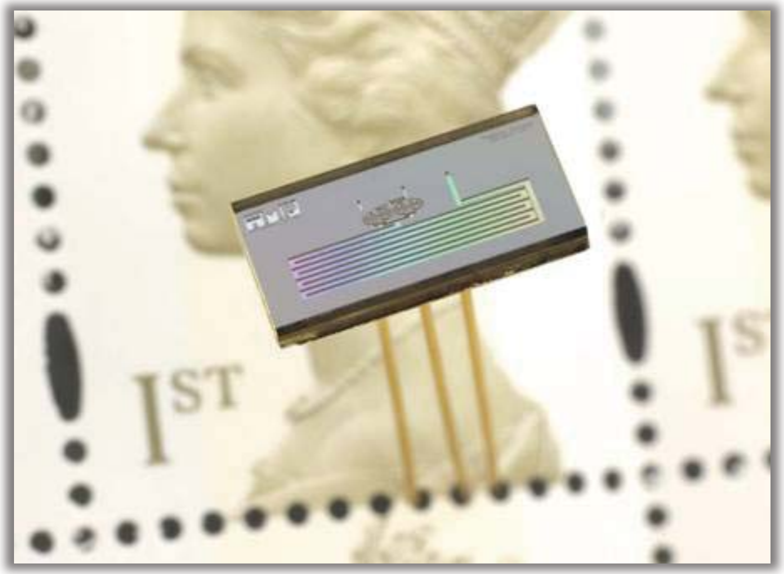
combinatorial chemistry

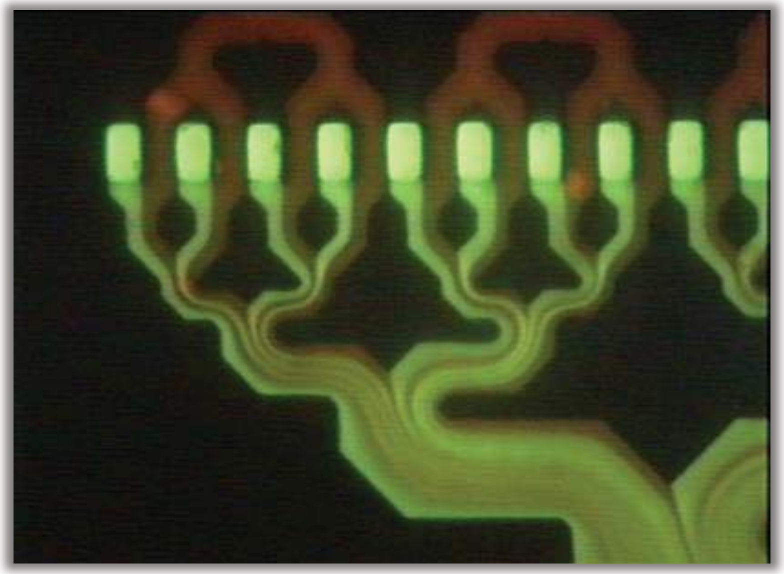
intelligent synthesis

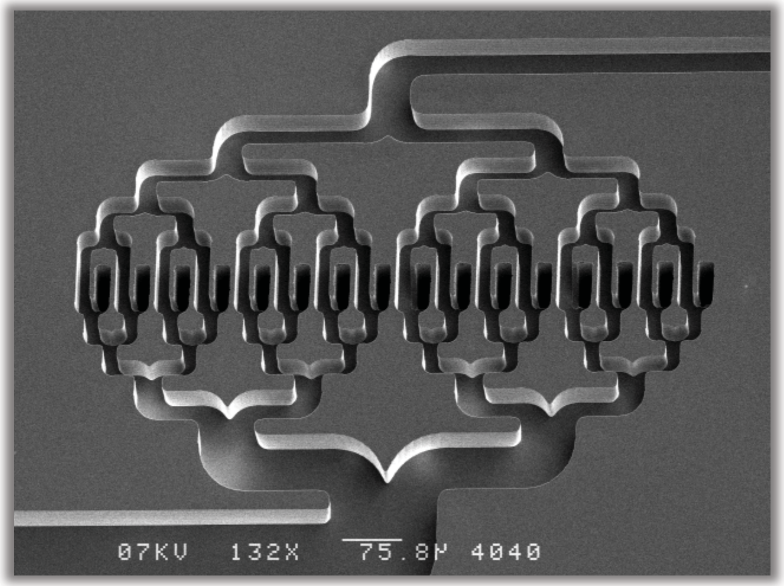


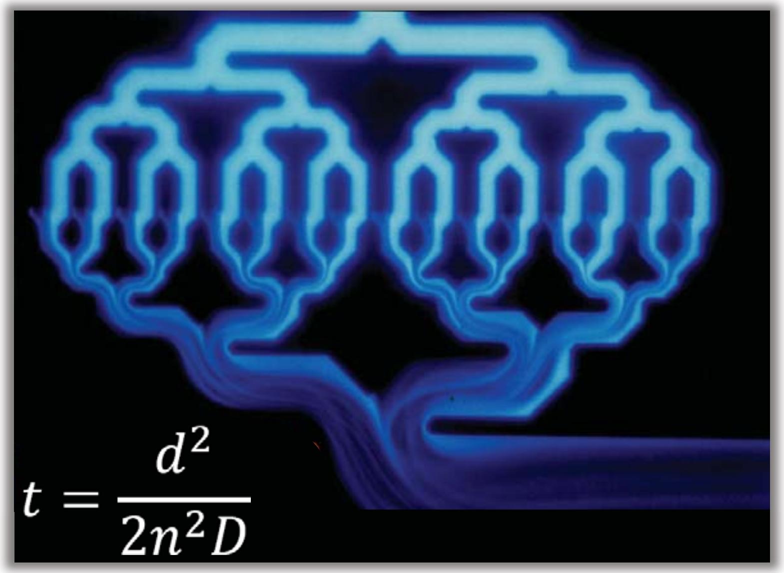
photochemistry

microengineered systems for efficient fluidic mixing





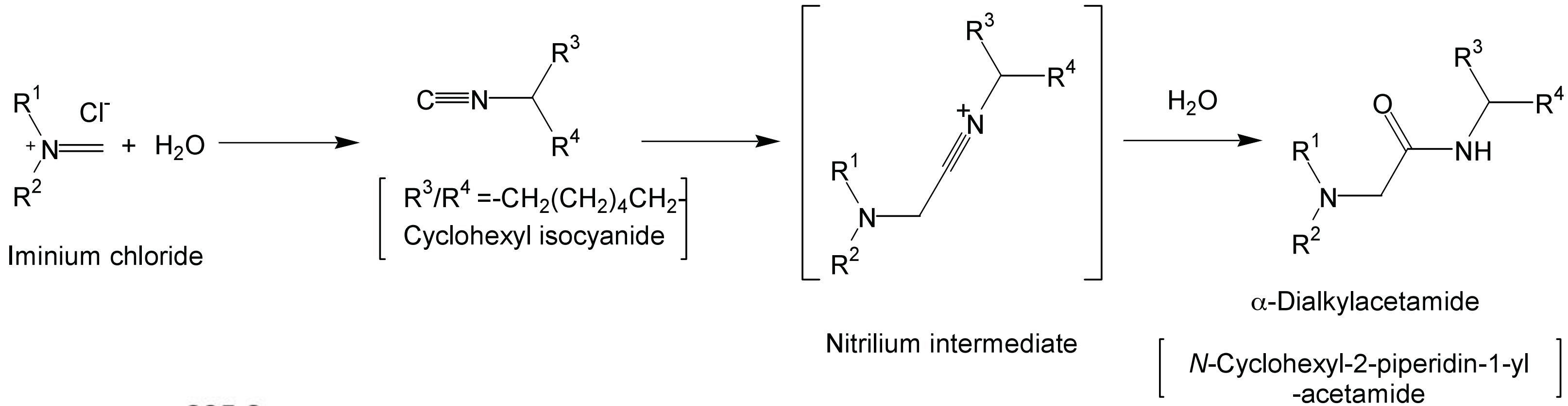


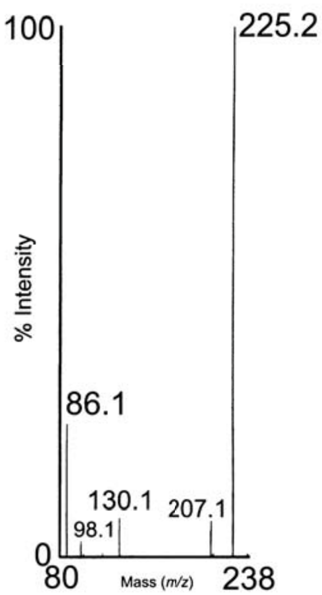


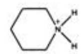
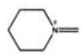
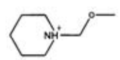
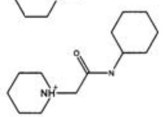
Anal. Comm., 36, 213, 1999

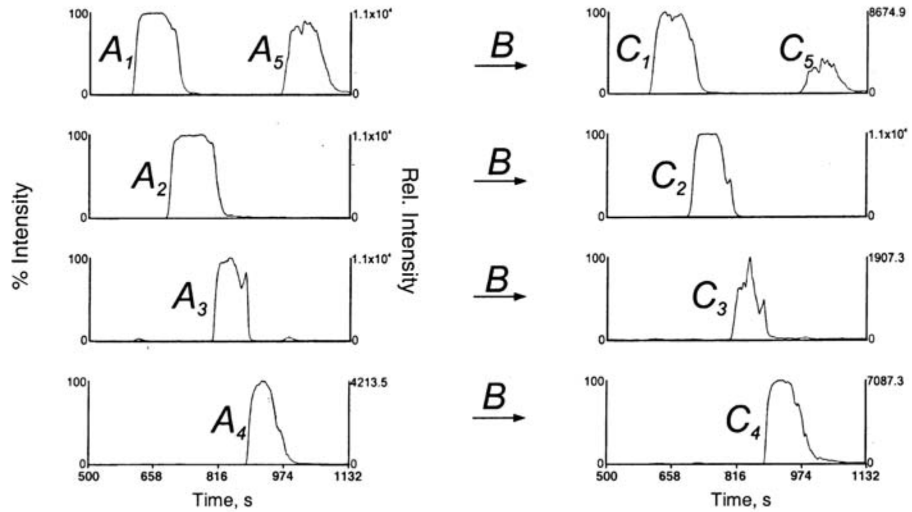
high-throughput solution phase chemistry

multicomponent reactions are one-pot reactions for library generation, provide rapid generation in molecular complexity / variability, are highly exothermic



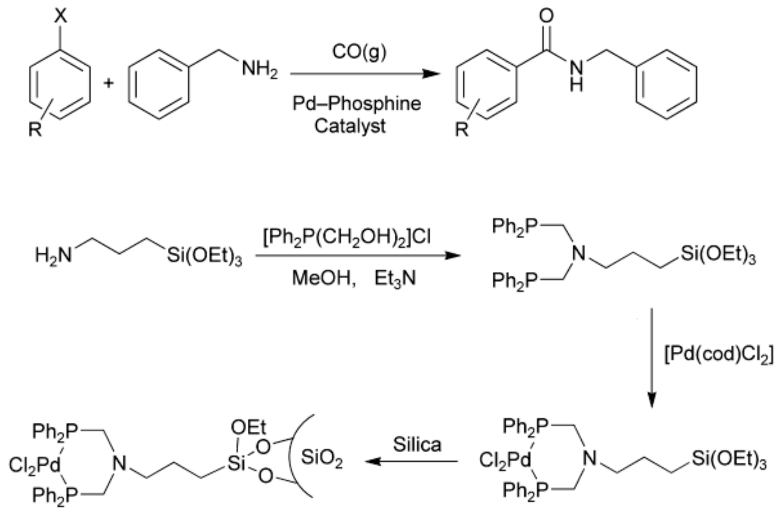


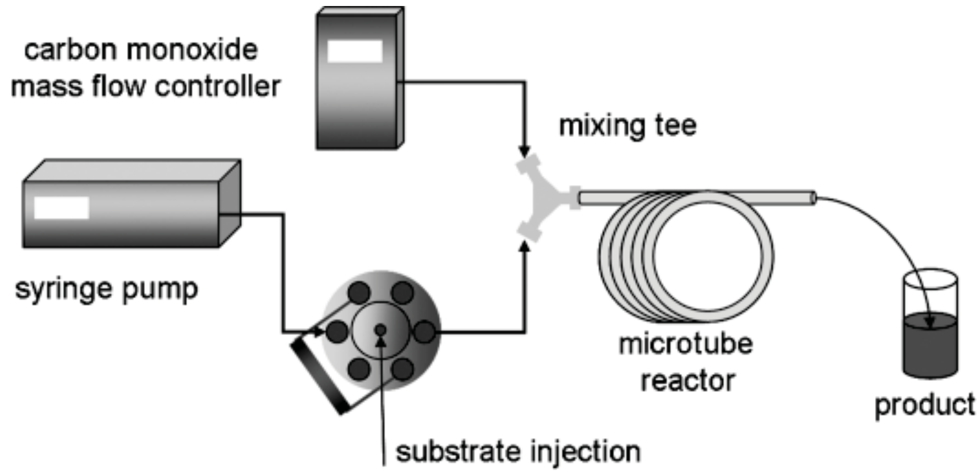
Mass (m/z)	Identity
86.1	
98.1	
130.1	
207.1	
225.2	



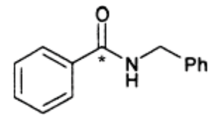
J. Chem. Soc. Perkin Trans, 1,514, 2002

multiphase reactions: application in PET ^{11}C-radiolabeling

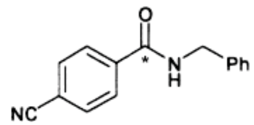




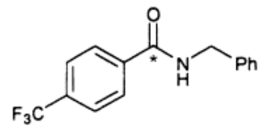
- synthesis of palladium–phosphine complex followed by attachment to the silica-support
- packed channel increases S/V by a factor of 2×10^4
- carbonylative cross-coupling reactions on microscale yield enhanced yields
- ^{11}CO carbonylative cross-coupling reactions to yield ^{11}C labeled amides successful
- ^{11}CO produced on-line by reduction of cyclotron generated $^{11}CO_2$ using a molybdenum catalyst.



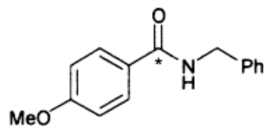
radiochemical yield – 79%
radiochemical purity – 96%



radiochemical yield – 70%
radiochemical purity – 95%



radiochemical yield – 68%
radiochemical purity – 90%



radiochemical yield – 45%
radiochemical purity – 80%

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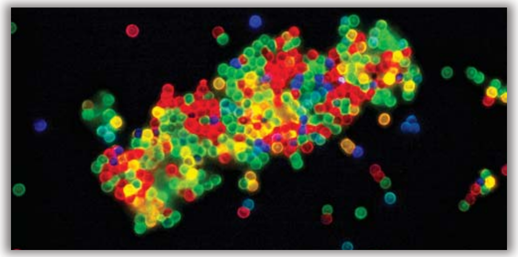
compound semiconductor nanoparticles

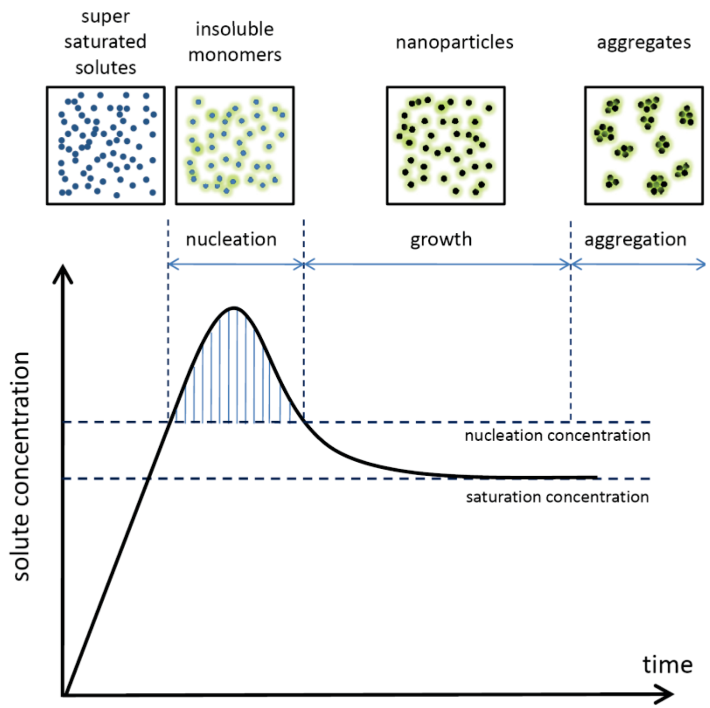
nanoparticles

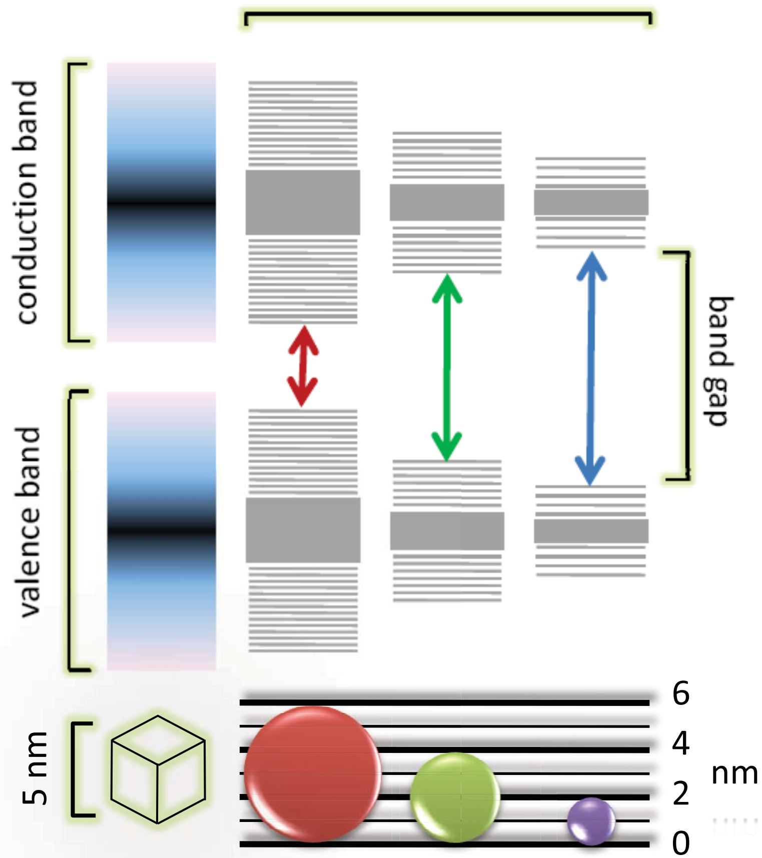
size range from 1 – 50 nm (between molecular & bulk scale)
small crystal size → quantum confinement effects
tuneable optical and electronic properties



nanoparticle synthesis in bulk

bottom up approaches involve the use of templates, post hoc size selection or control of reaction conditions





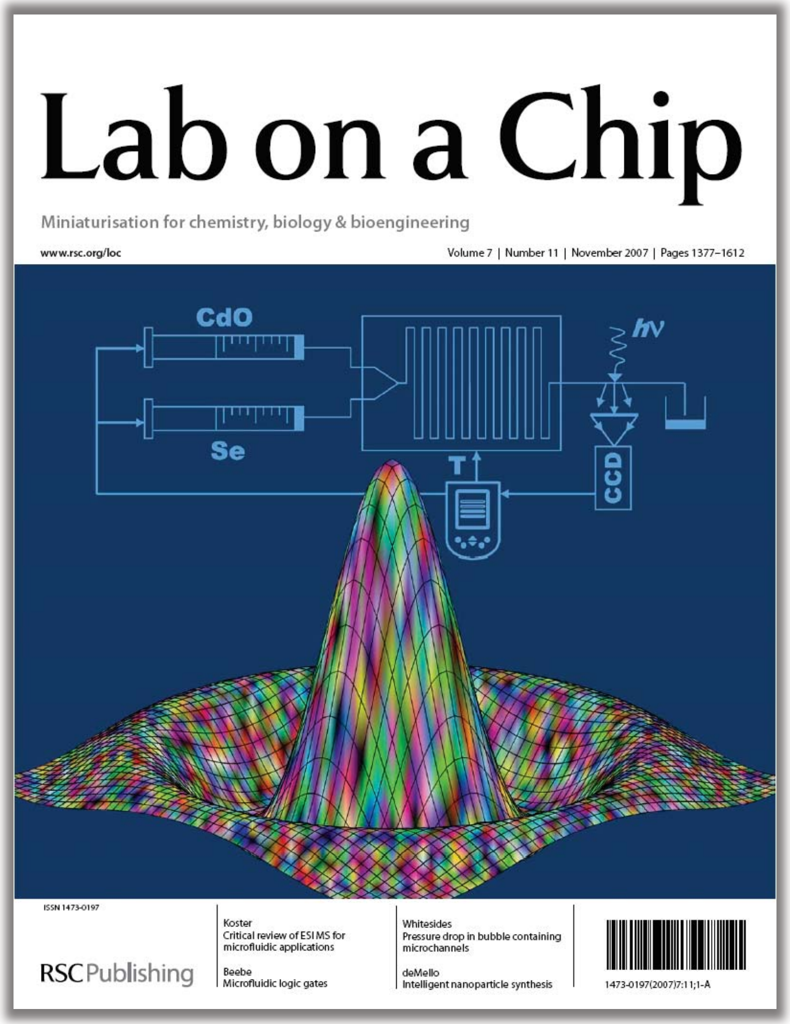
properties depend on size and shape of nanoparticles

high monodispersity needed for many applications
current synthetic routes are complex & yield poor quality particles

Lab Chip 2004, **4**, 11-15N

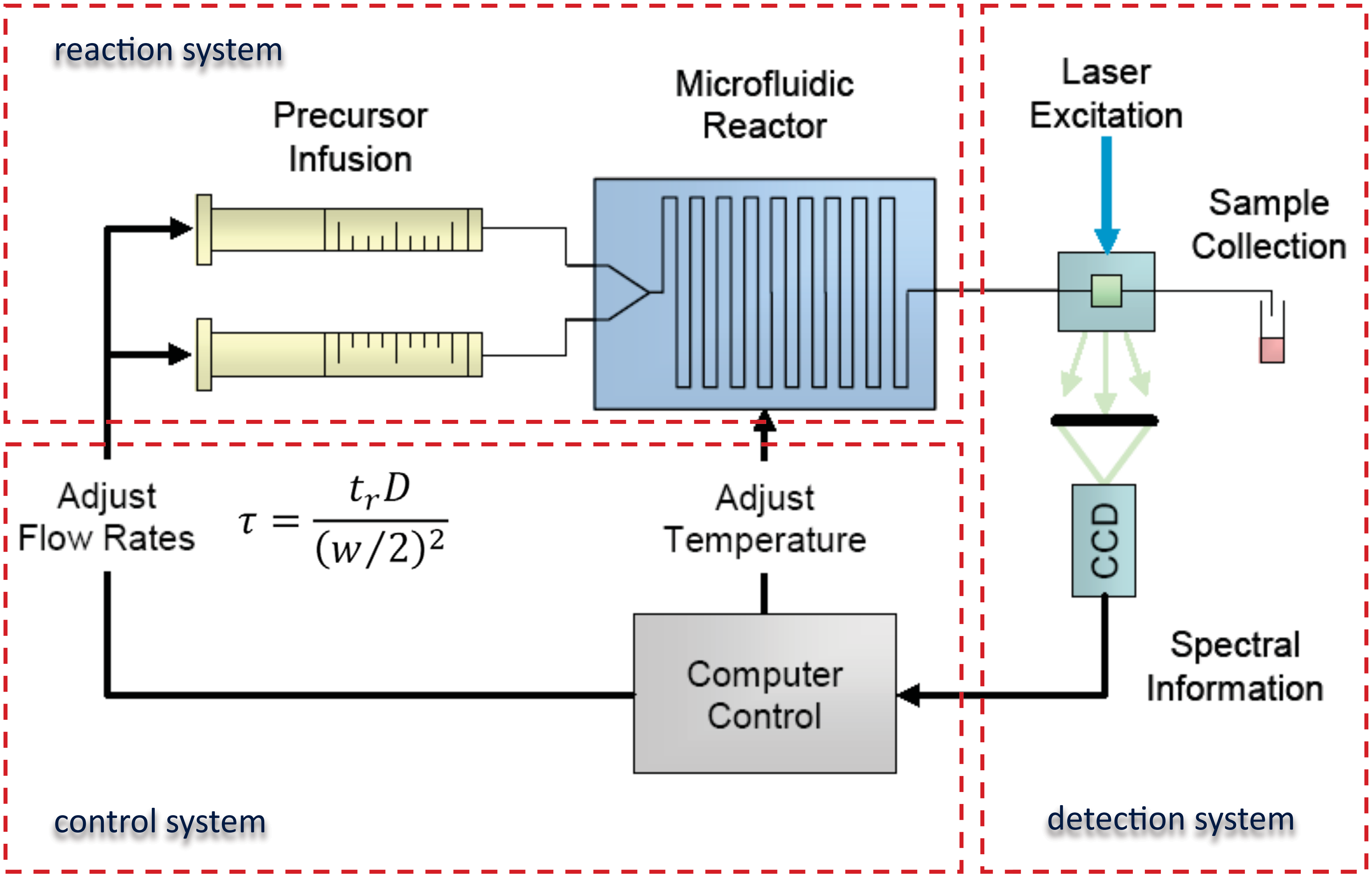
intelligent synthesis of nanoparticles

- aim is to create an autonomous 'black-box' system to controllably synthesize nanoparticles.
- system incorporates a reactor, detector, control algorithm and a means of changing system variables (such as temperature, reaction time and reagent concentration).
- approach leverages control of mass and thermal transport and the ability to measure reaction success.



Lab Chip, 2008, **7**, 1434

intelligent synthesis of nanoparticles: instrumentation



Lab Chip, 2008, **7**, 1434

challenges associated with reaction optimisation

problems

- the mechanisms of nanoparticle formation are poorly understood → no process models
- nanoparticle synthesis usually involves the optimisation of several properties at once, which creates multiple minima in the chemical parameter space
- ideally need to map all parameter space, but this requires more measurements than is practical

two-part approach

- a utility function defines a scalar dissatisfaction coefficient to characterise emergent particles
- find a balance between *local* searching in vicinity of identified optima and *global* searching of unexplored regions where superior optima may exist (SNOBFIT, Neumaier et al).

$$u_\gamma(\gamma_c) = \frac{|\gamma_c - \gamma_t|}{|\gamma_w - \gamma_t|}$$

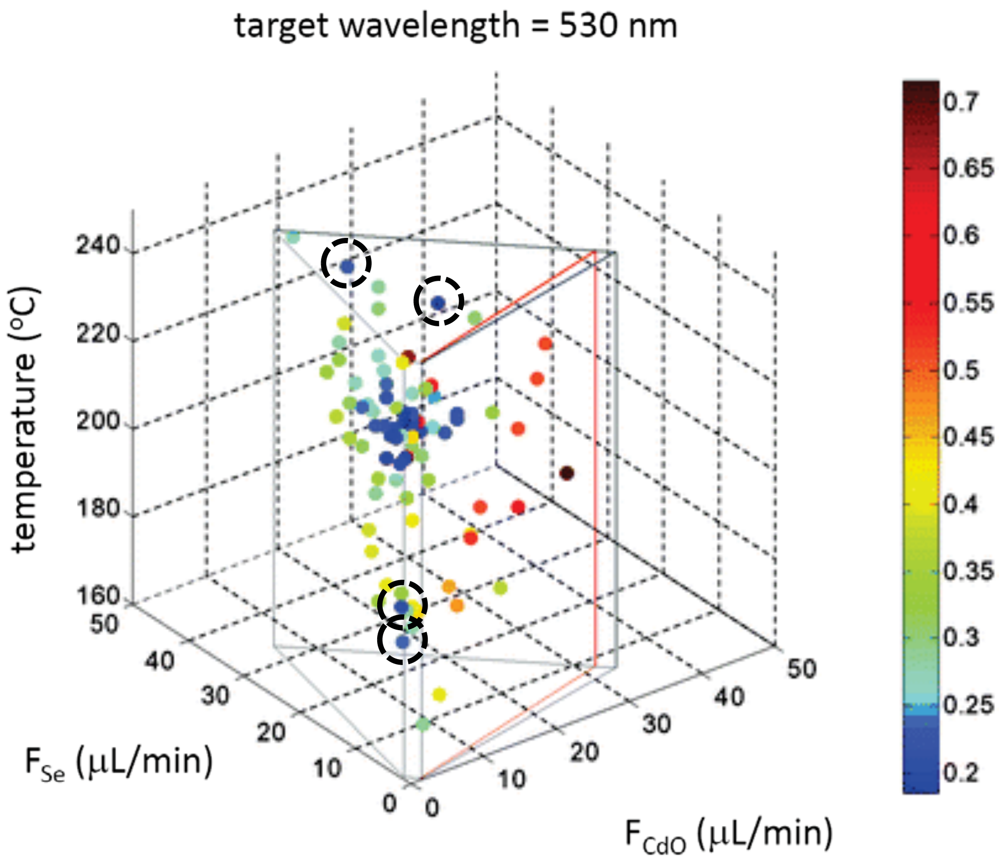
dissatisfaction coefficient

$$U(\lambda_c, I_c) = \frac{1}{k}\left(k\alpha\frac{|\lambda_c - \lambda_t|}{|\lambda_w - \lambda_t|} + 1\right)\left(k\beta\frac{|I_c - I_t|}{|I_w - I_t|} + 1\right) - \frac{1}{k}$$

multi-attribute utility function

three-dimensional chemical optimisation

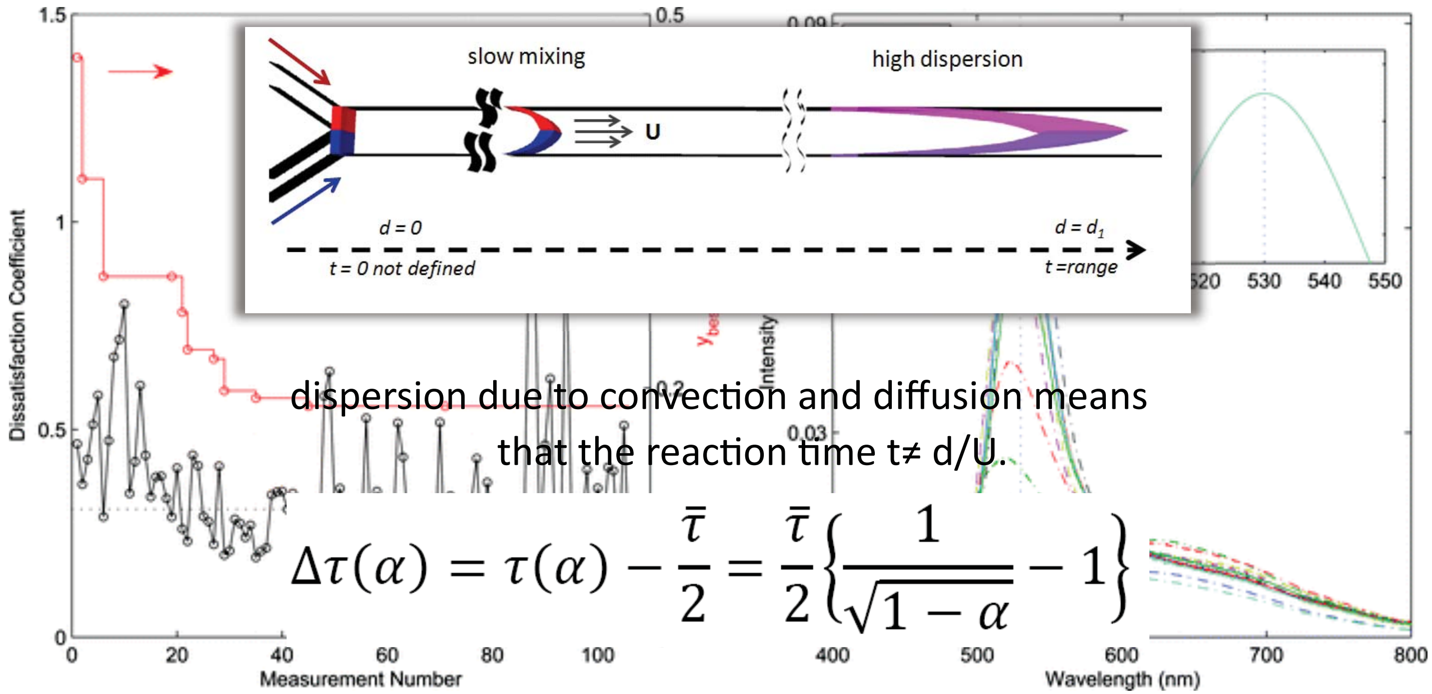
$$U(\lambda_c, I_c) = \frac{1}{k}\left(k\alpha\frac{|\lambda_c - \lambda_t|}{|\lambda_w - \lambda_t|} + 1\right)\left(k\beta\frac{|I_c - I_t|}{|I_w - I_t|} + 1\right) - \frac{1}{k}$$



- F_{Se} and F_{CdO} & temperature tuneable
- want to maximise emission intensity for a specified target wavelength (530 nm)
- wire frame cage indicates reaction space
- preferential sampling of low flow rate zone and high temperature zone
- dark blue points suggest multiple optima in accessible reaction space

Lab Chip, 2008, **7**, 1434

three-dimensional chemical optimisation



dispersion due to convection and diffusion means that the reaction time t≠ d/U.

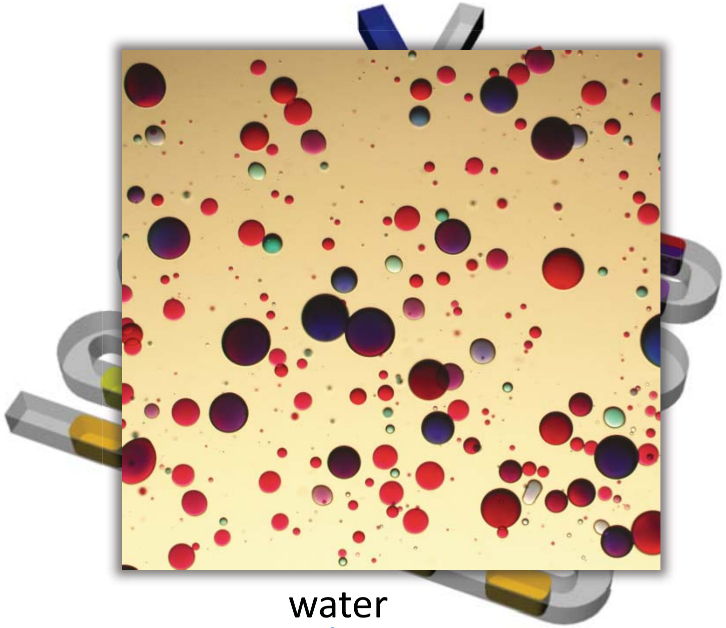
$$\Delta\tau(\alpha) = \tau(\alpha) - \frac{\bar{\tau}}{2} = \frac{\bar{\tau}}{2}\left\{\frac{1}{\sqrt{1-\alpha}} - 1\right\}$$

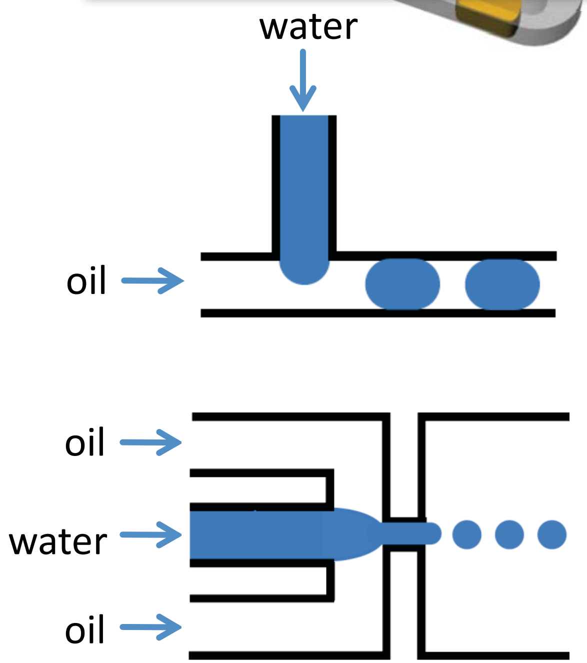
- DC values fluctuate since algorithm alternates between local (low DC) and global (high DC) searching
- staircase function describes temporal variation of $y_{best,}$
- control algorithm finds best case scenario in <u>71 iterations</u> without intervention
- bias between wavelength and intensity is crucial in defining the optimum
- current studies addressing the synthesis of core shell particles & control of defects

Lab Chip, 2008, **7**, 1434

droplet-based microfluidics

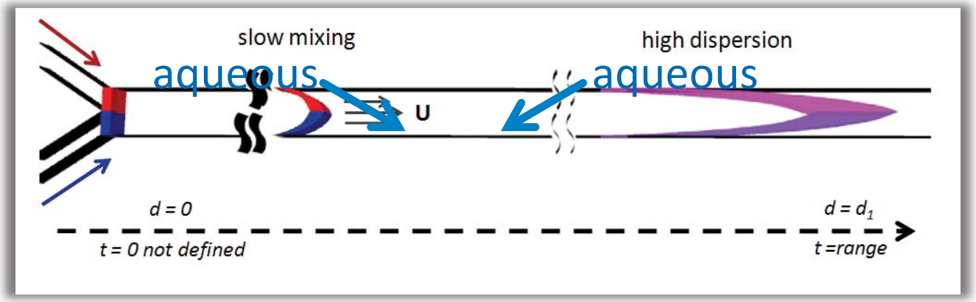
- discrete liquid droplets are encapsulated by a carrier fluid that wets the channel surface and forms the continuous phase
- droplets are isolated and form the dispersed phase in which reactions may occur
- droplet size is well-defined (monodisperse)
- mass transport occurs without dispersion
- droplets can be dosed with varying amounts of input reagents
- droplets can be generated at kHz frequencies
- can be achieved for both L/L and G/L systems
- droplets easily generated using flow-focusing and tee-junctions



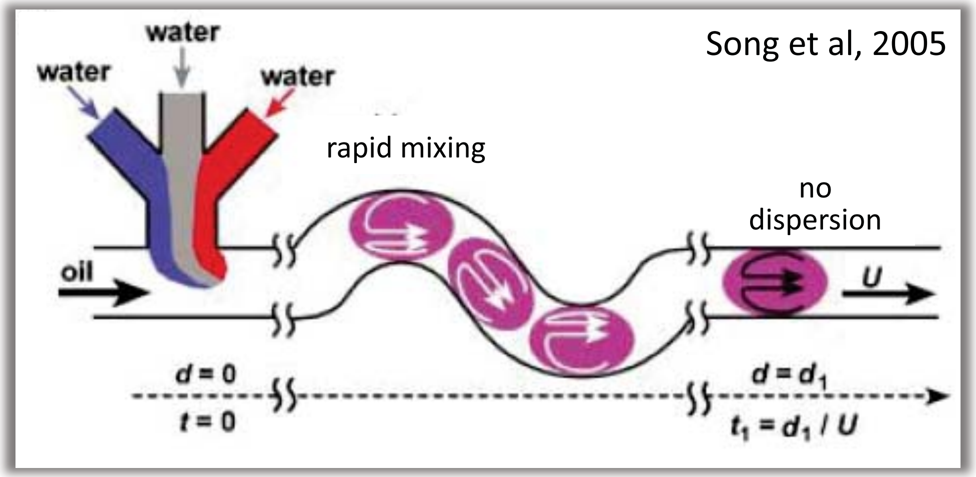


Lab Chip, 2008, **8**, 1244; Nature, 2006, **442** 394

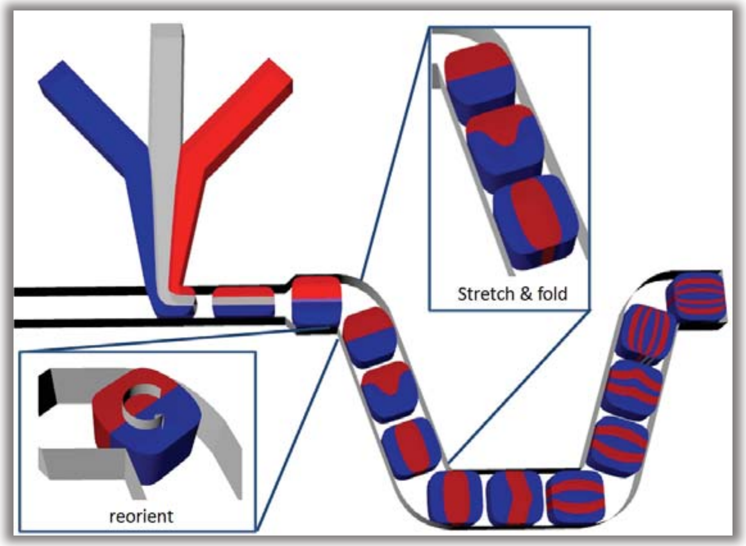
droplet-based systems: enhanced timing and mixing



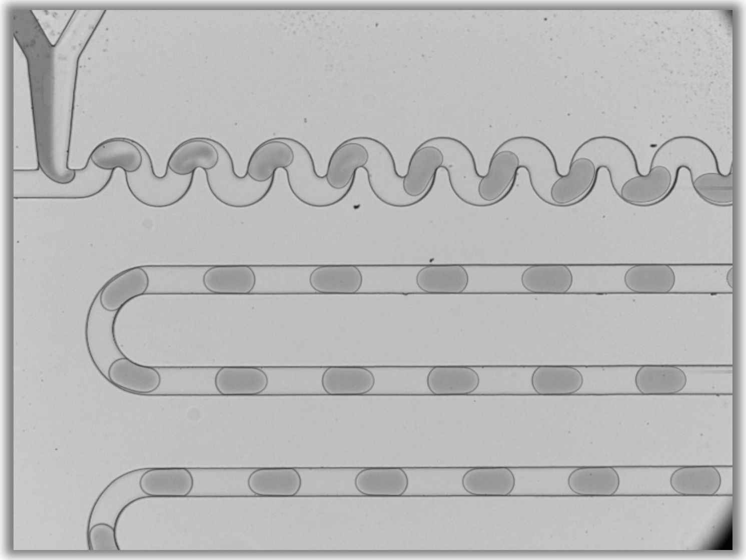
- dispersion due to convection and diffusion means that the reaction time t≠ d/U.



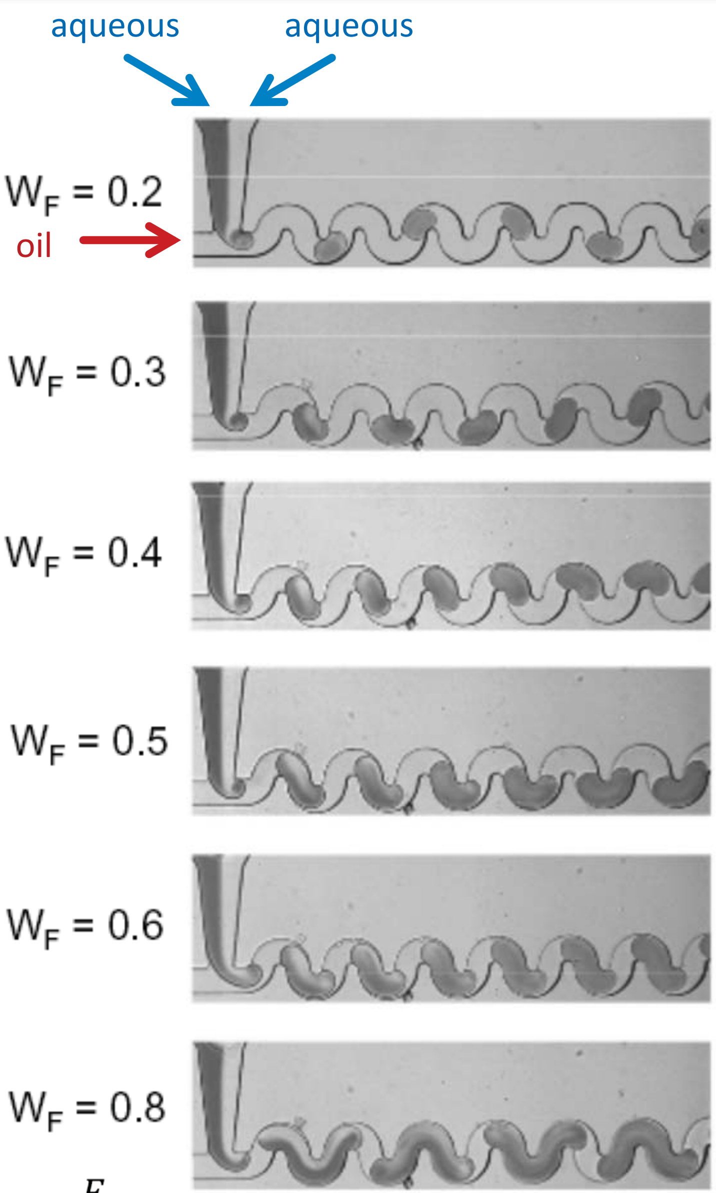
- dispersion due to convection and diffusion eliminated since reagents are encapsulated
- accurate control of reaction times is facile and residence time distributions are eliminated



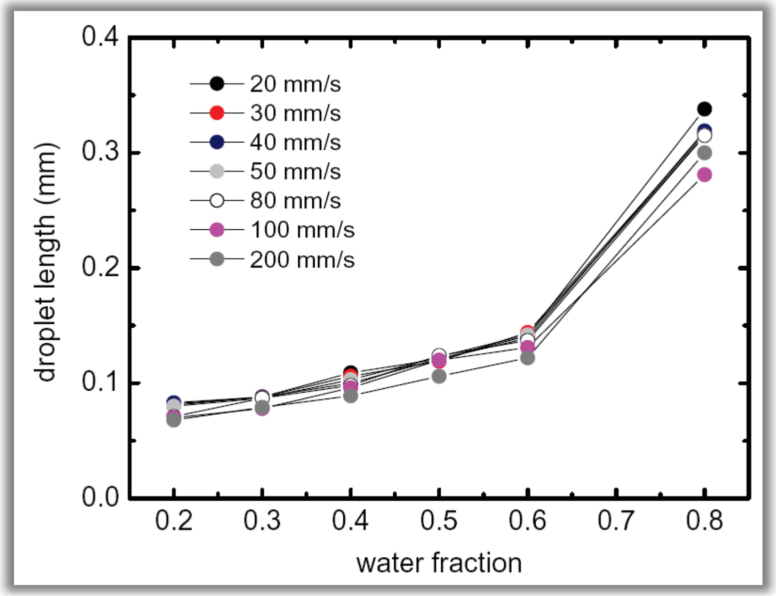
- interfaces are reoriented, stretched and folded as the droplet moves

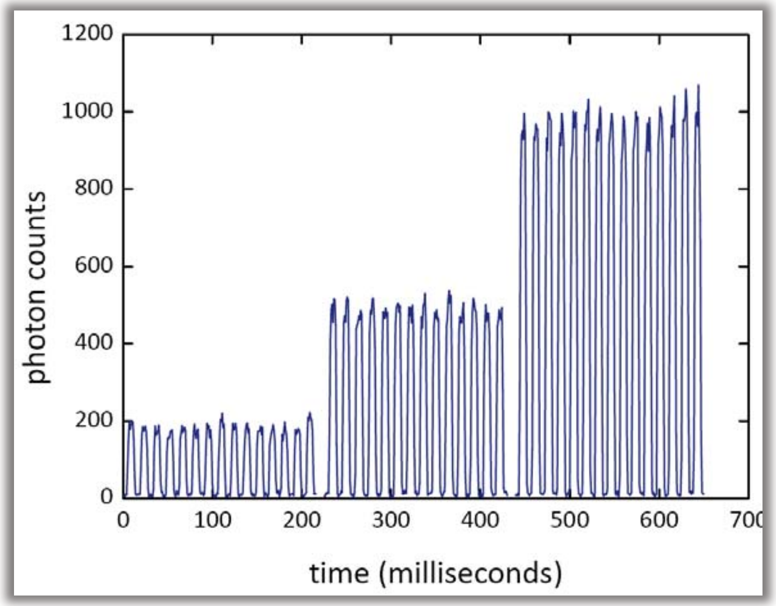


control of droplet characteristics



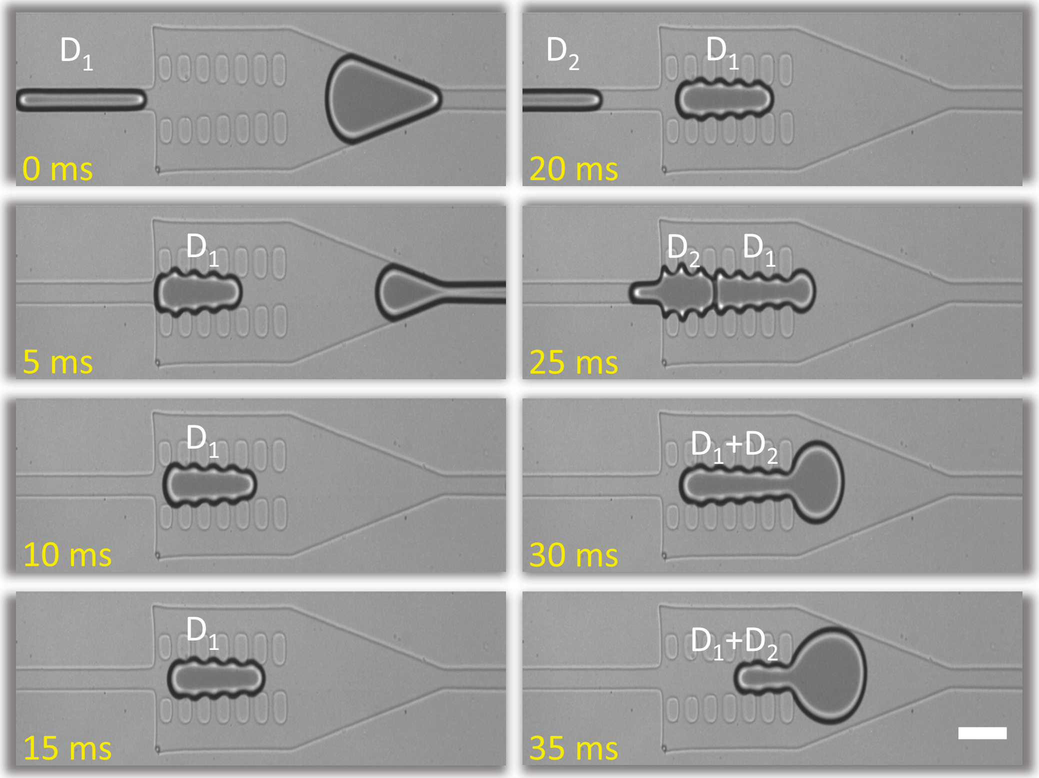
$$W_f = \frac{F_w}{F_w + F_o}$$

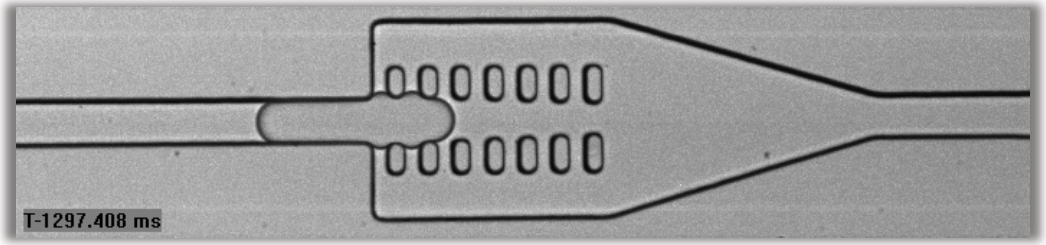


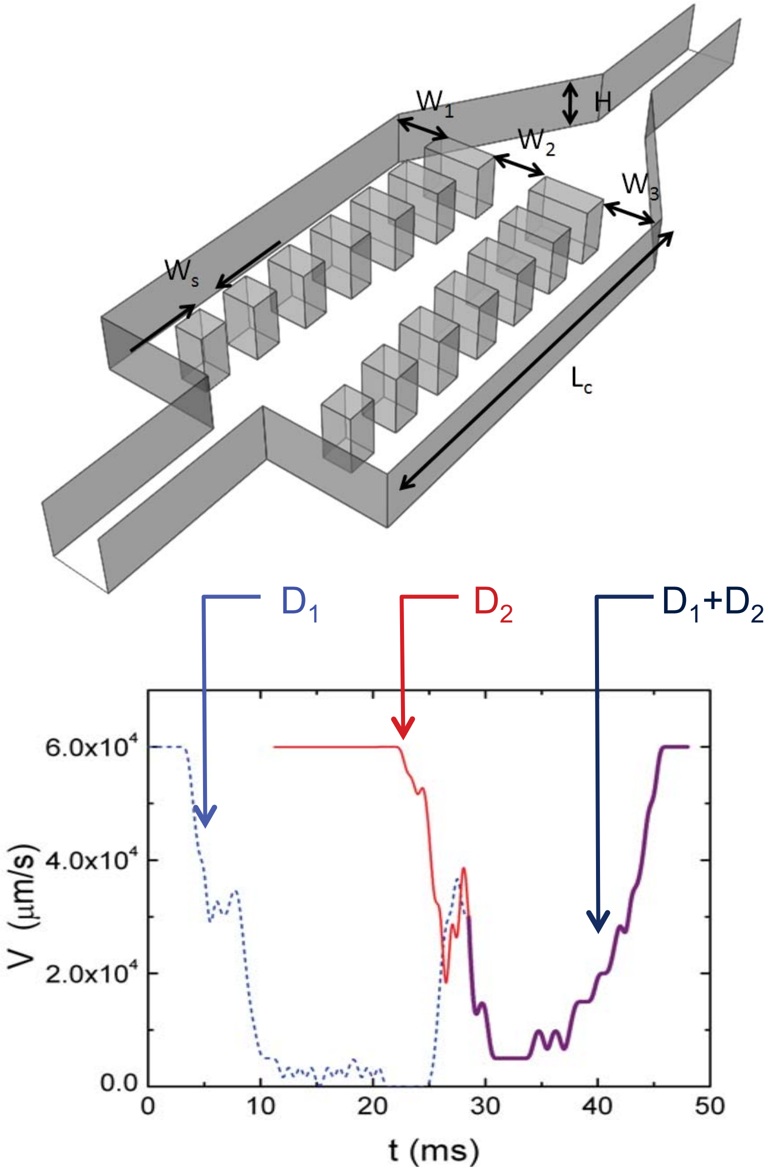


Analytical Chemistry, 2007, **79**, 6682

unit operations: controlled droplet fusion

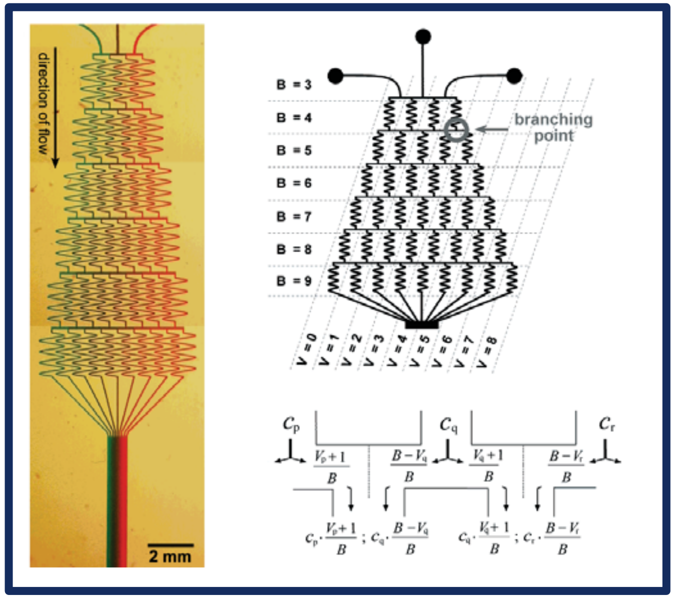




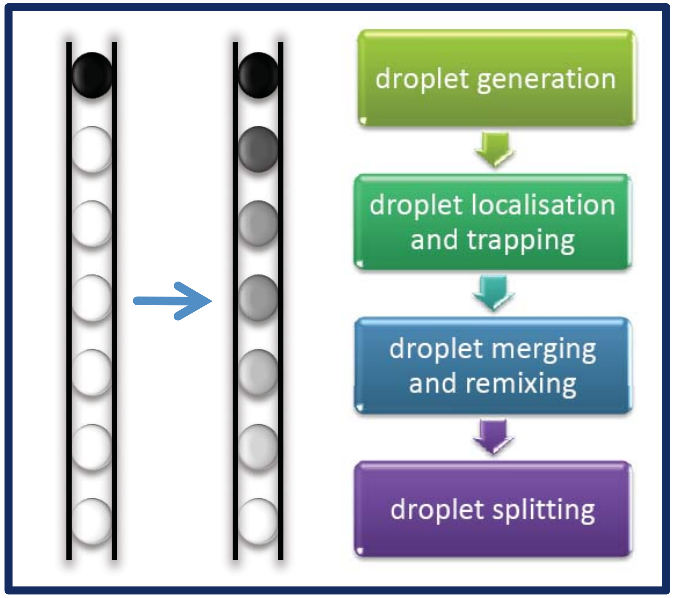


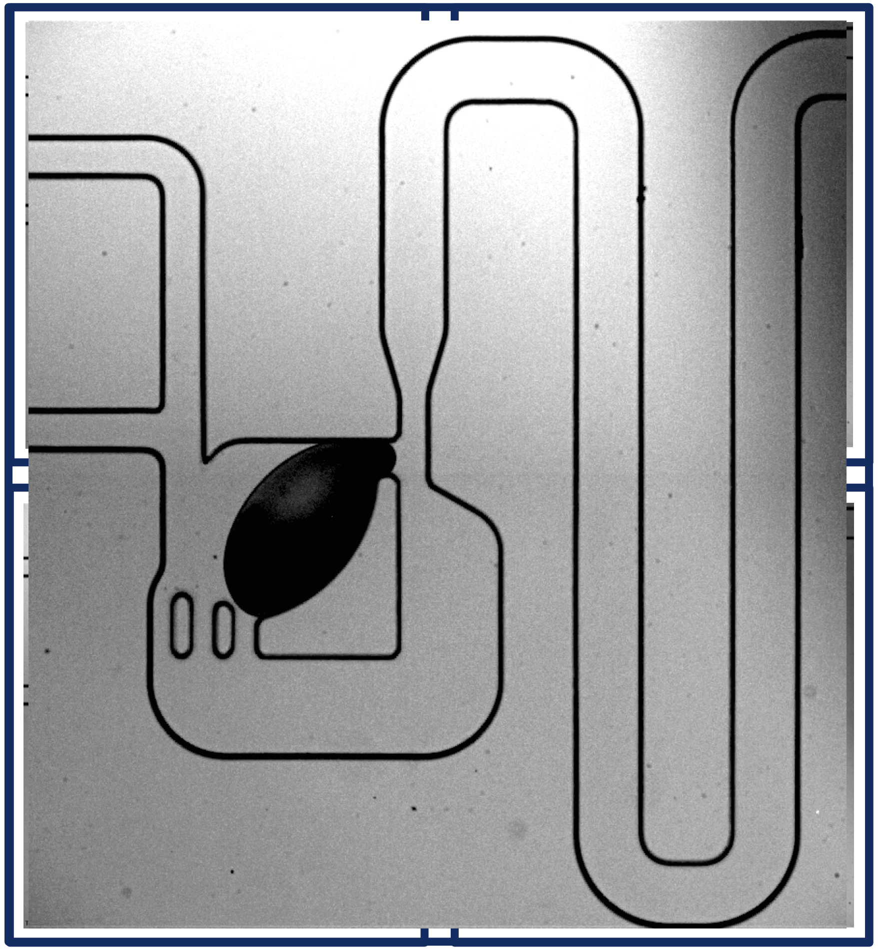
Anal. Chem. 2009, **81**, 7321; Lab Chip, 2008, **8**, 1837

droplet manipulations: dilution on the microscale



Whitesides (Analytical Chemistry, 2001)

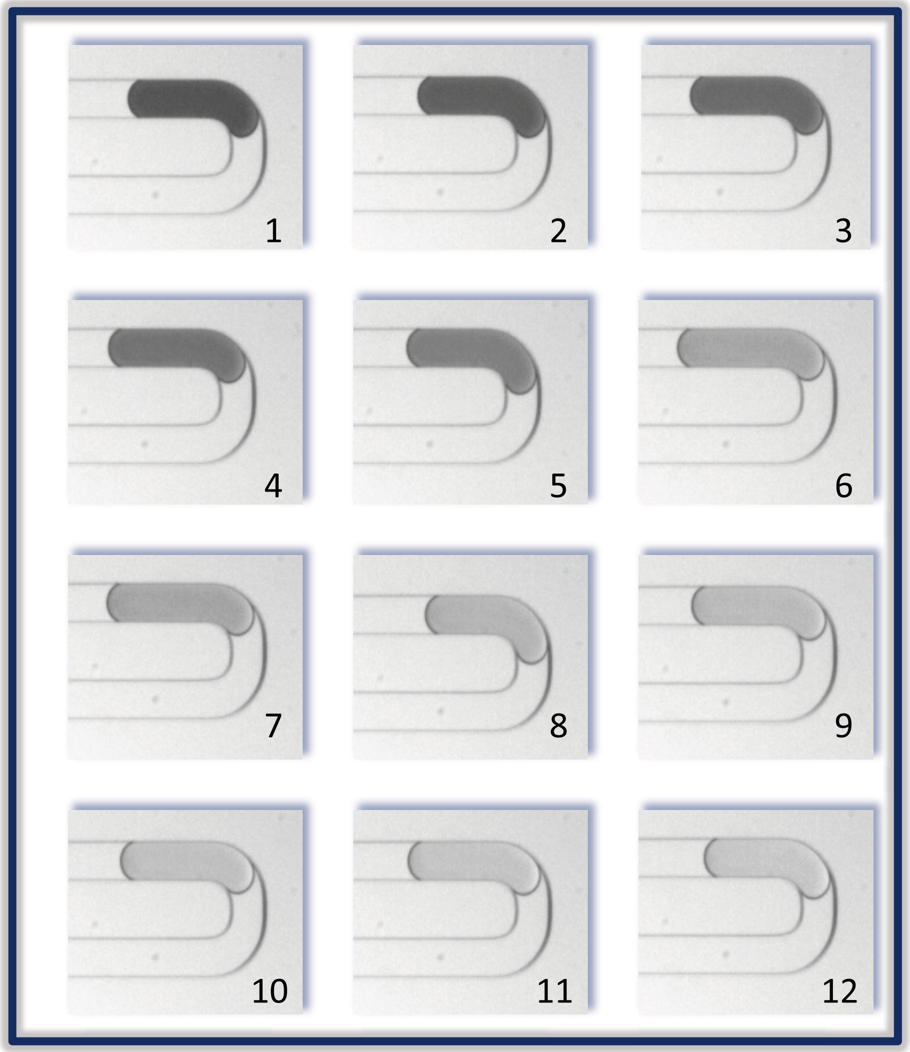


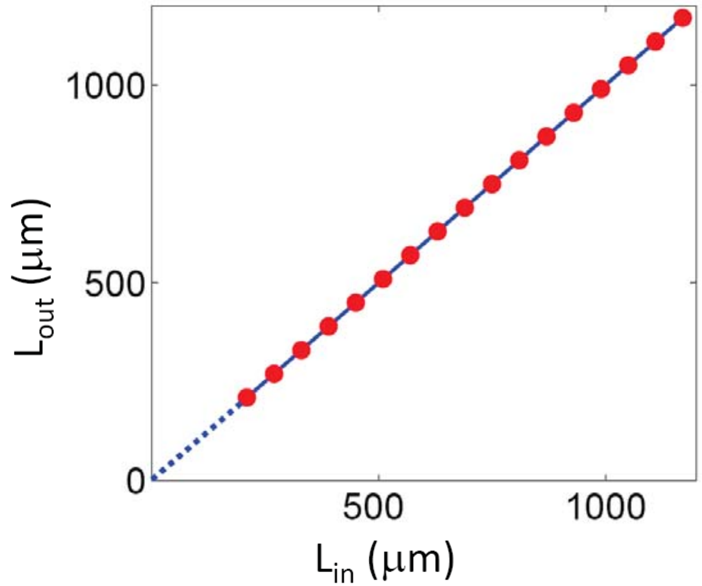


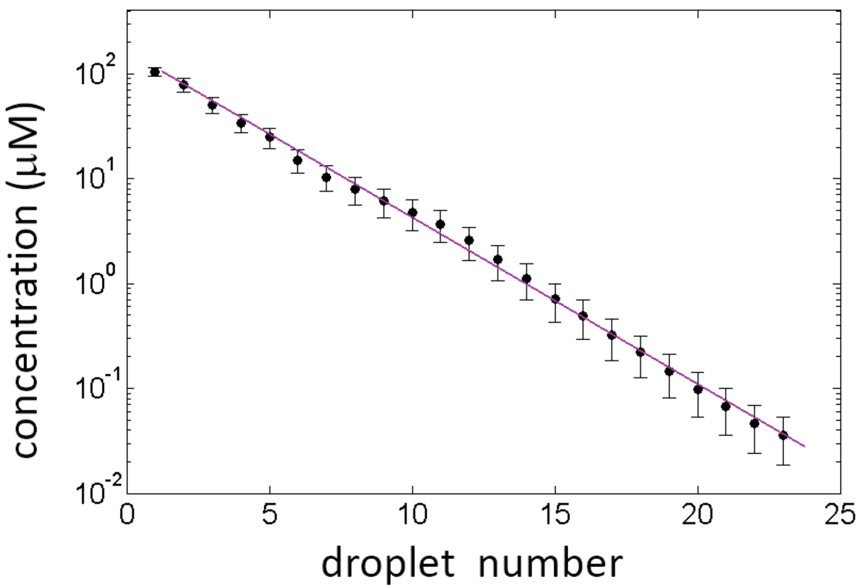
passive dilution structure is based on L/L and L/S structure hydrodynamic interactions

$$\Delta p_{surf} \propto \gamma cos(\theta + \alpha)\left({}^{1}/_{d_1} - {}^{1}/_{d_2}\right)$$

droplet manipulations: dilution on the microscale

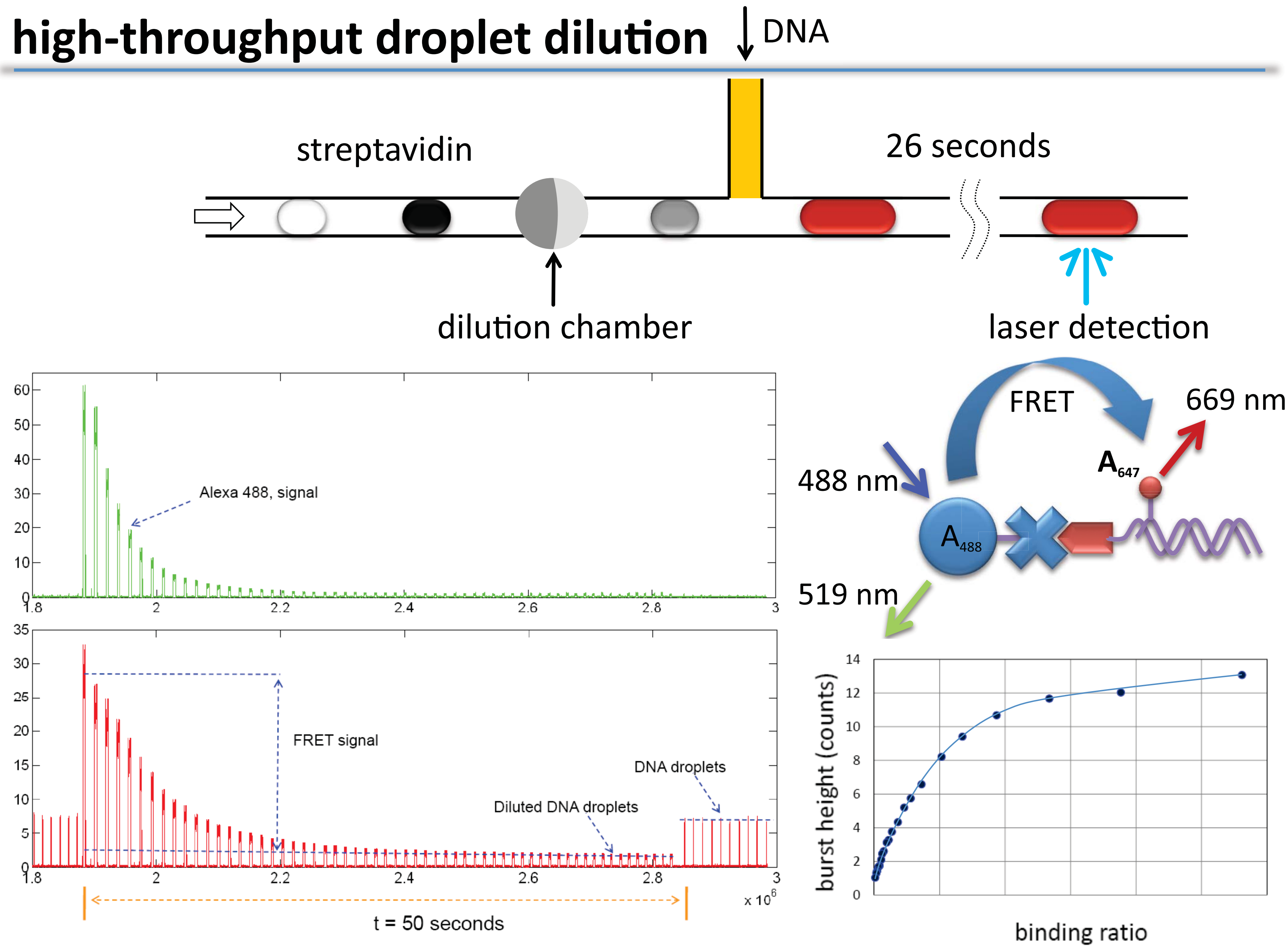




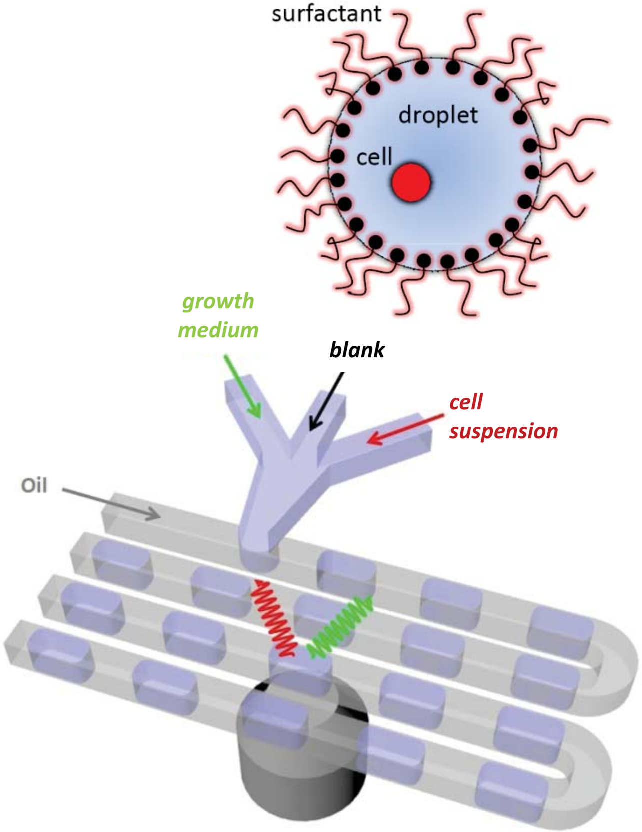


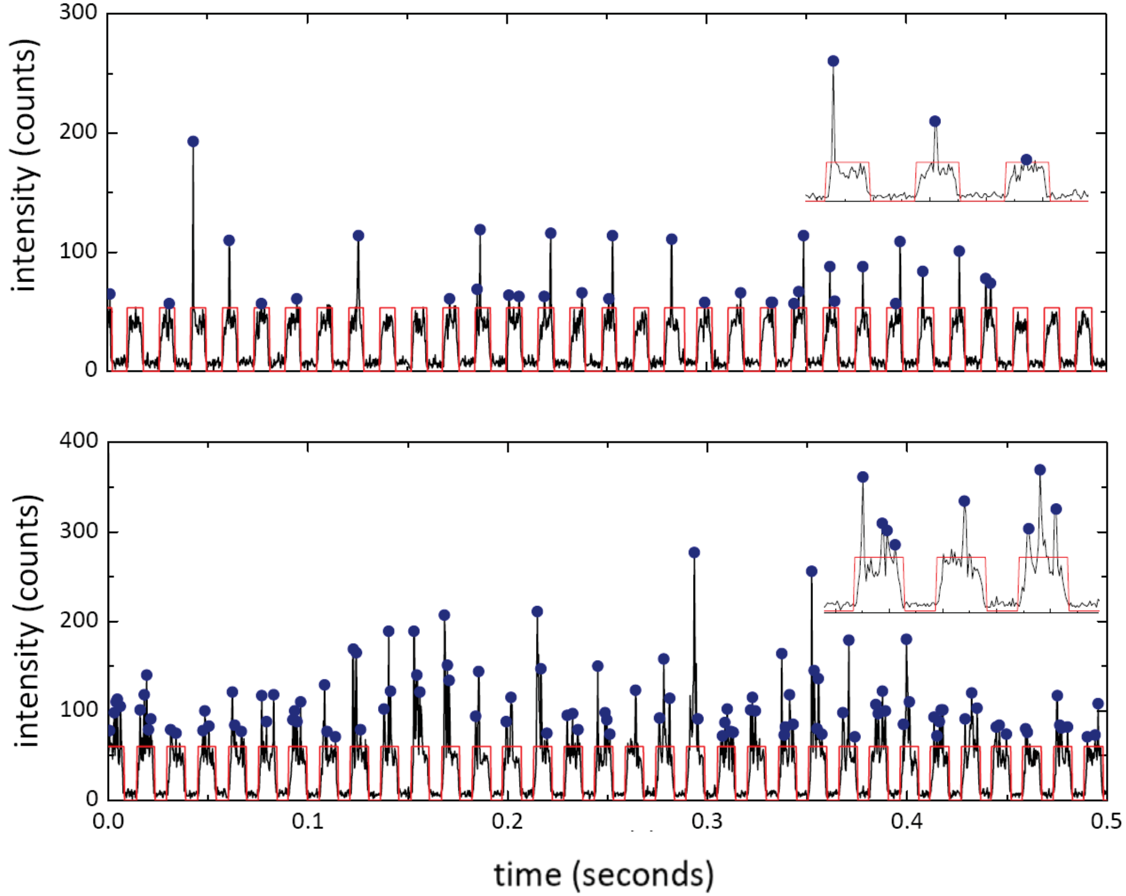
23 output droplets access 4 orders of magnitude of concentration (100 μM - 36 nM)

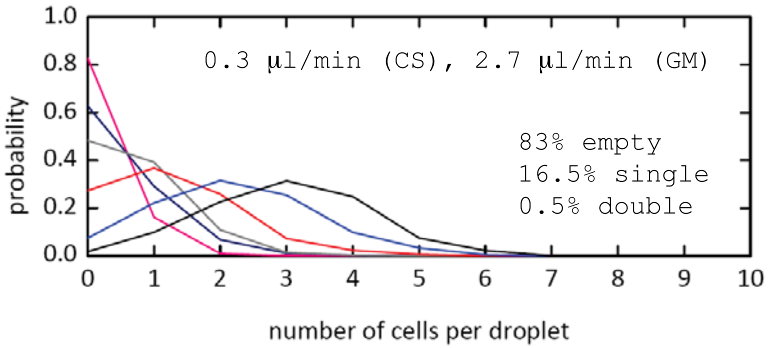
high-throughput droplet dilution

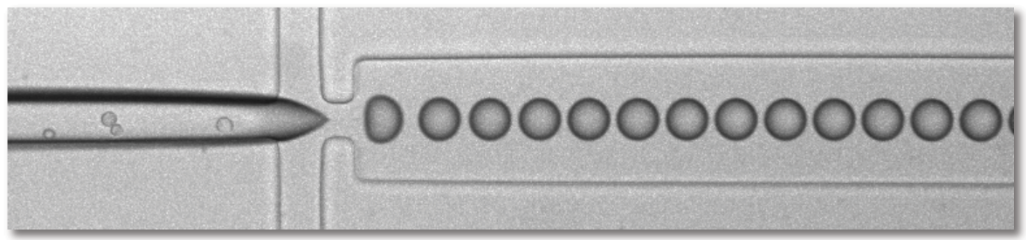


controllable compartmentalisation of single cells



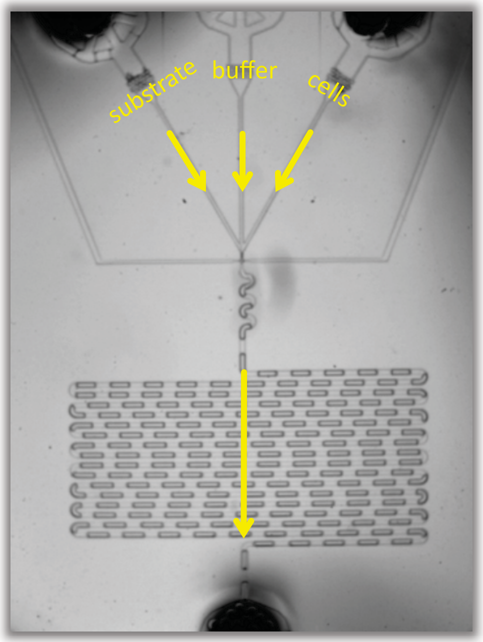


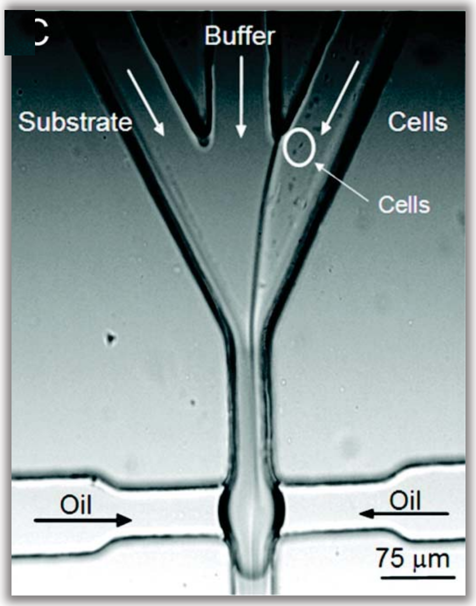


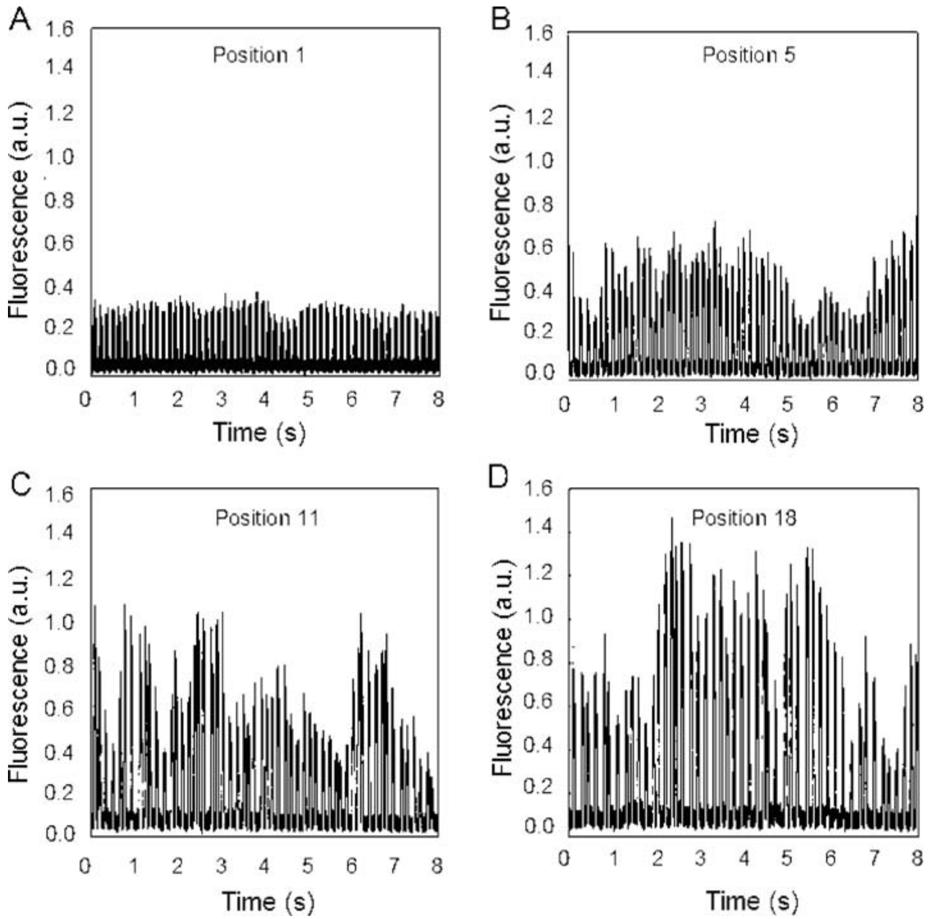


Chem Comm., 2007, 1218

extracting kinetics: cell-based enzyme assays



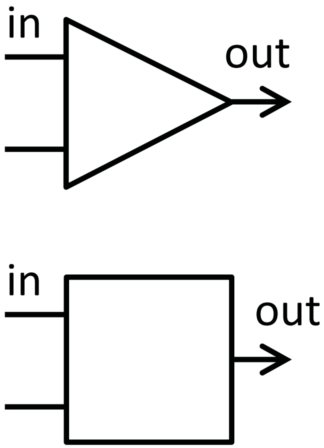


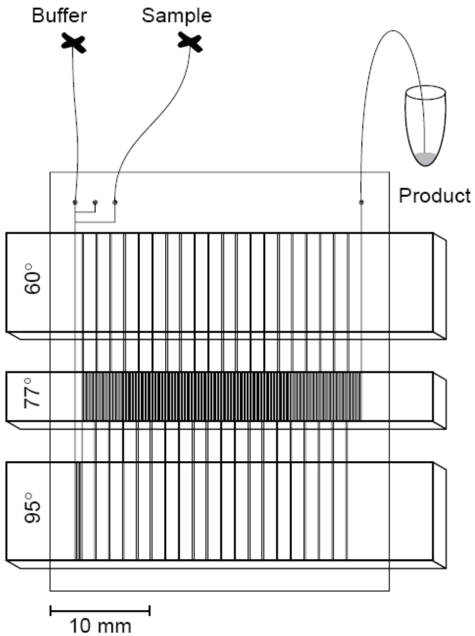


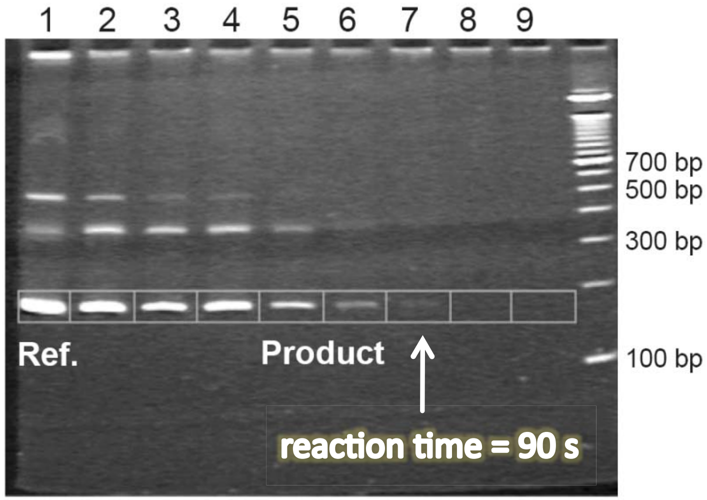
- substrate – o-methylfluorescein phosphate
- cells- e coli expressing alkaline phosphatase
- droplet volume - 800 pL at 6 Hz
- residence time - 46 seconds
- encapsulation and throughput are key

Analytical Chemistry 2008 **80** 3890

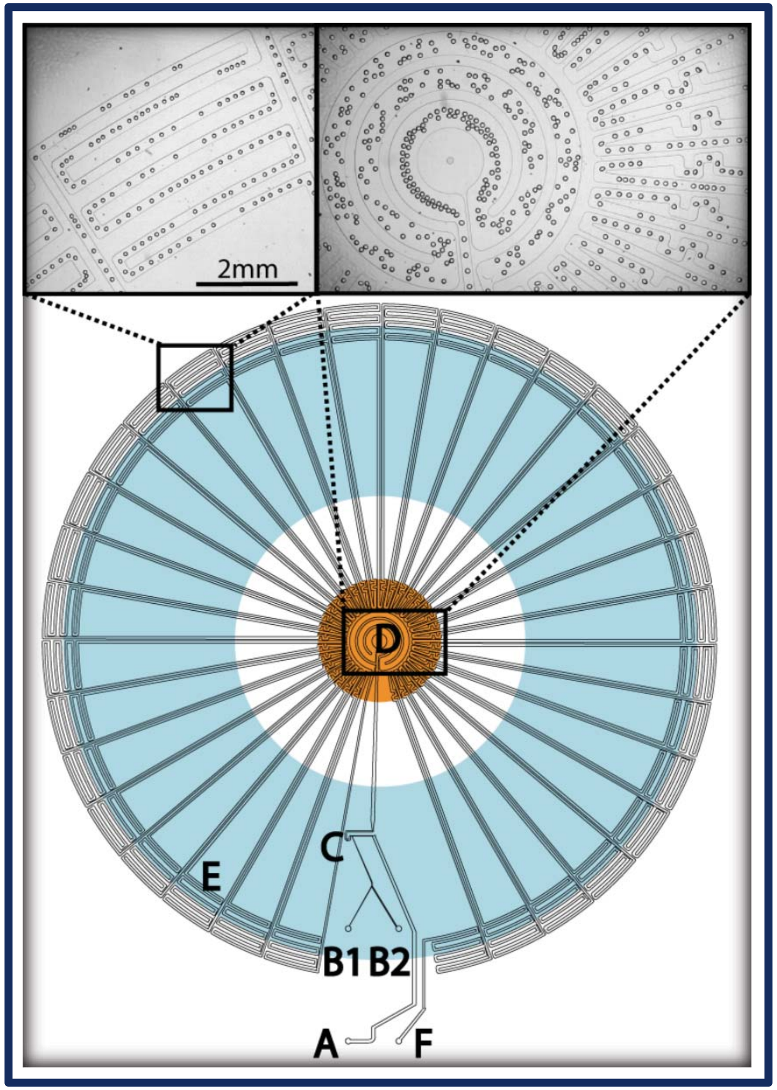
continuous flow PCR: the old and the new







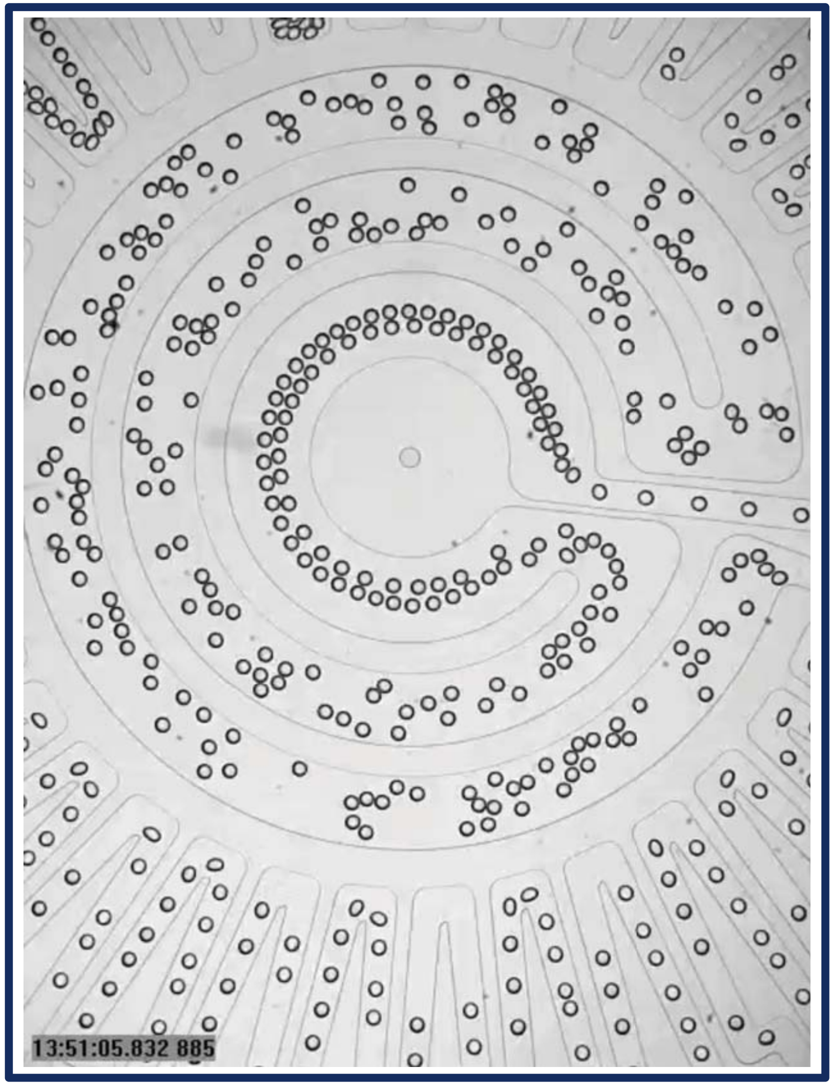
Science **280** 1046 (1998)

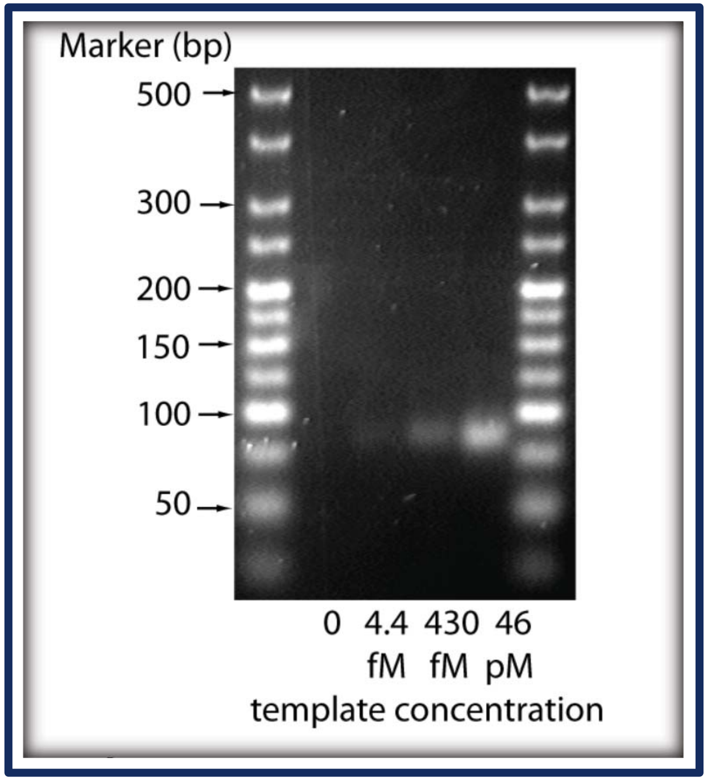


(A) oil in, (B) aqueous in, (C) droplet formation, (D) denaturation, (E) annealing/extension, (F) exit

Analytical Chemistry **81** 306 (2009)

dna amplification by the drop

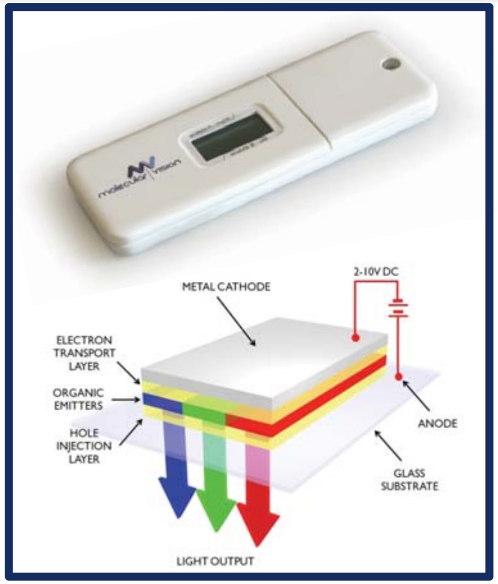




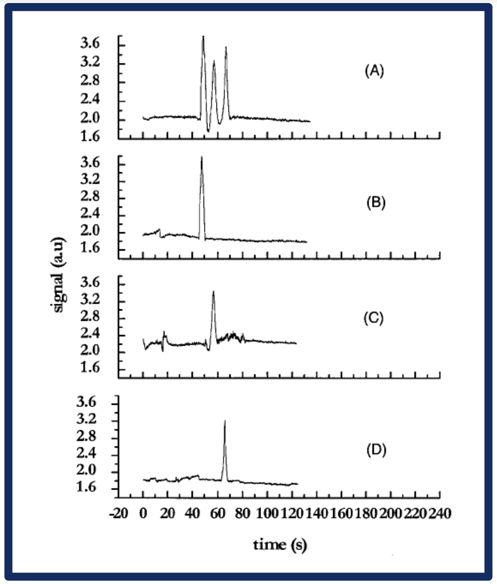
- 29 s per cycle and 17 min total residence time
- 160 µl/hr (droplet volume ~ 130pL)
- 4.4 fM → to 0.3 template/droplet
- high amplification factors (<10^6) observed
- move to online detection of droplets

Analytical Chemistry **81** 306 2009

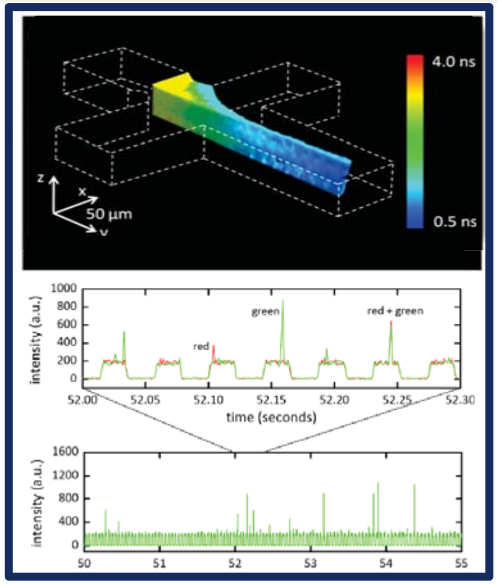
small volume detection



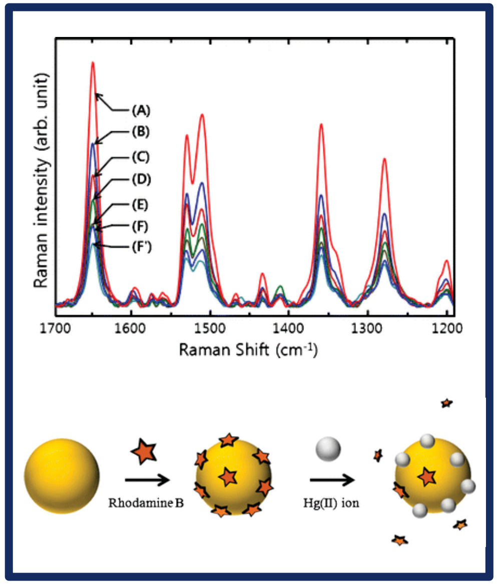
miniaturised optics



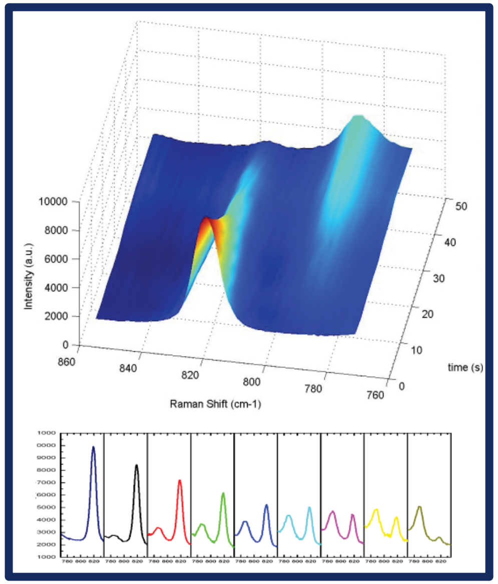
indirect fluorescence



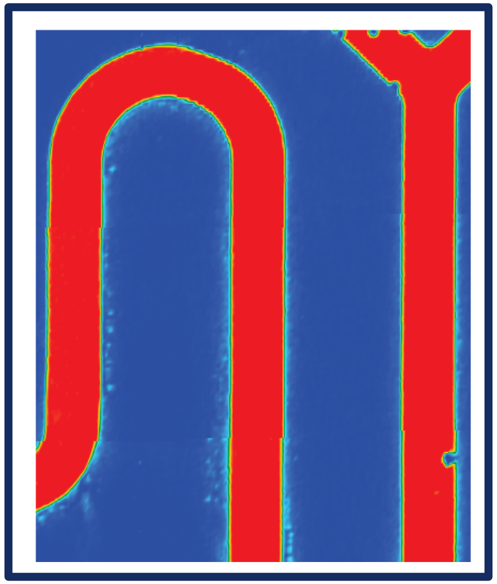
fluorescence spectroscopy



SERS



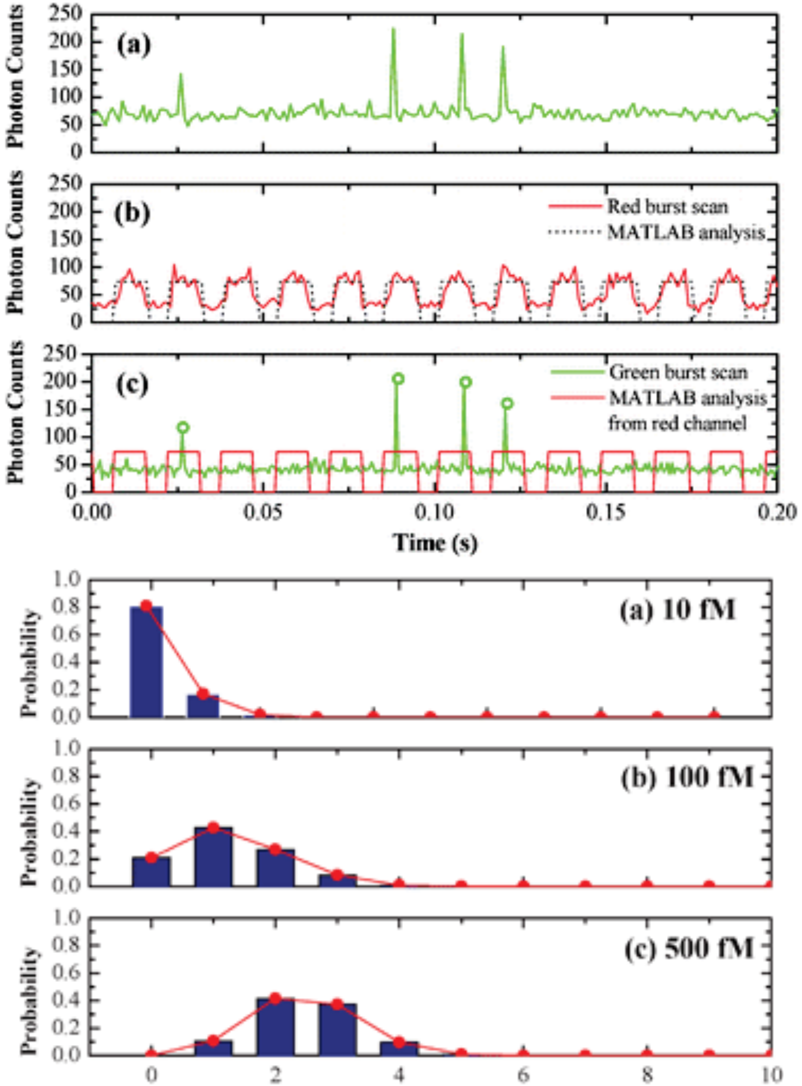
Raman spectroscopy



ATR-IR spectroscopy

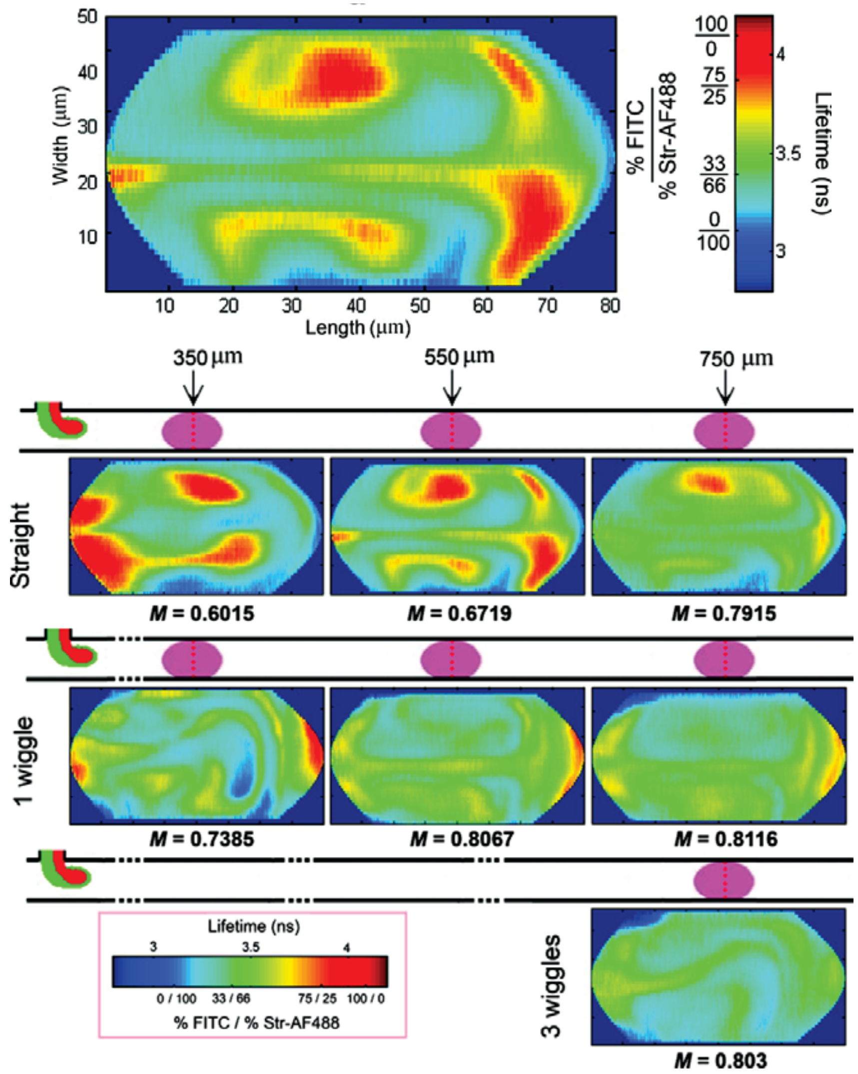
detection in picoliter-sized droplets

single molecule detection



Chem Comm, 2009, 6548 - 6550

fluorescence lifetime imaging

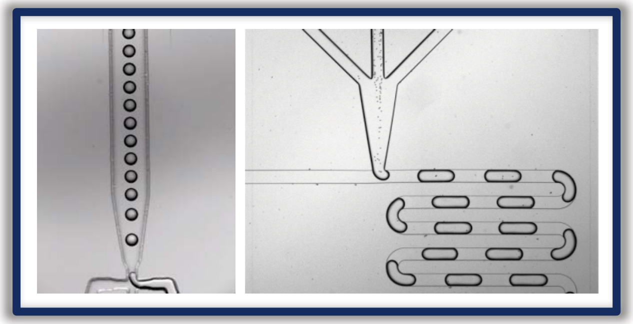


Analytical Chemistry, 2010, **82**, 3950

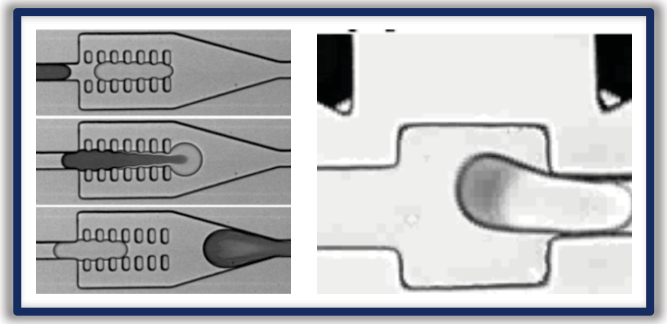
creating a droplet toolkit

TOOLS

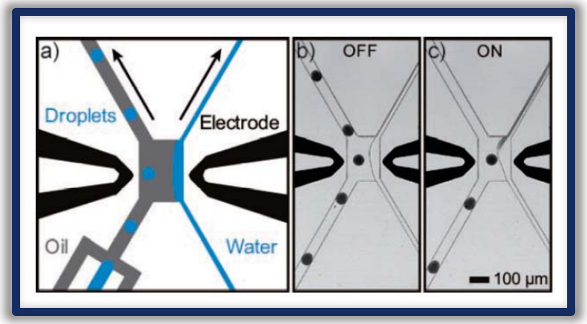
- generation
- fusion
- sorting
- splitting
- dilution
- PCR
- incubation
- separation



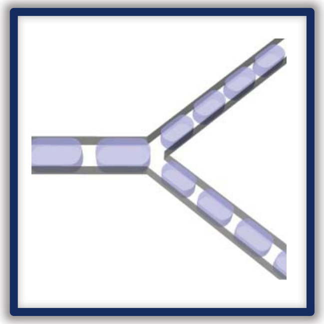
droplet generation



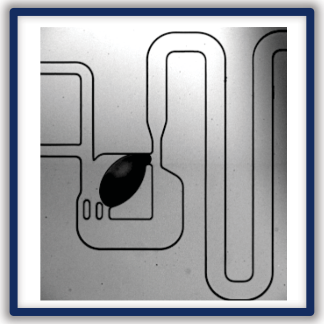
droplet fusion



droplet sorting

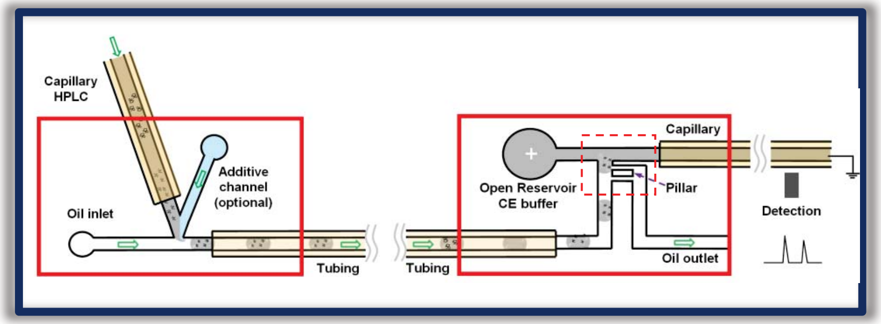


splitting



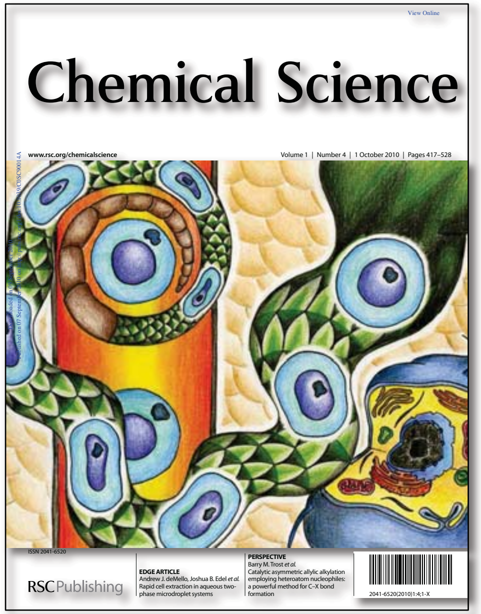
dilution

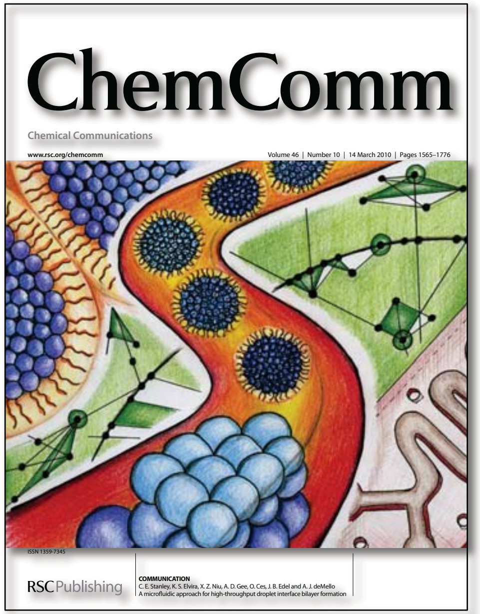
PCR

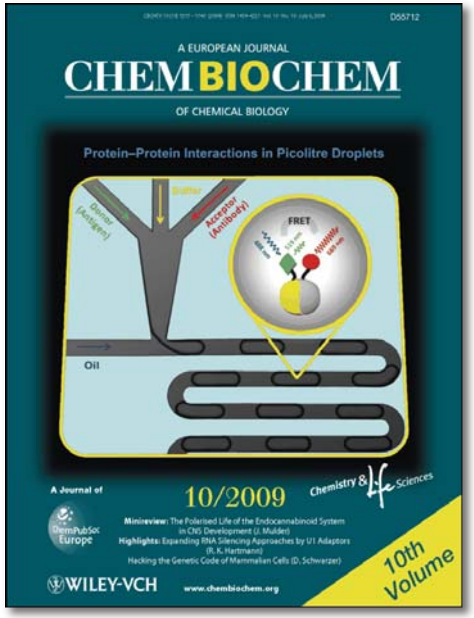


2D-separations

applications in biological analysis







rapid cell extraction in aqueous two-phase microdroplet systems

Chemical Science, 2010, 1, 447

microfluidic high-throughput droplet interface bilayer formation

ChemComm, 2010, 46, 1620

analysis of protein-protein interactions using droplet-based microfluidics

ChemBioChem, 2009, 10, 1605

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small molecule chemistry

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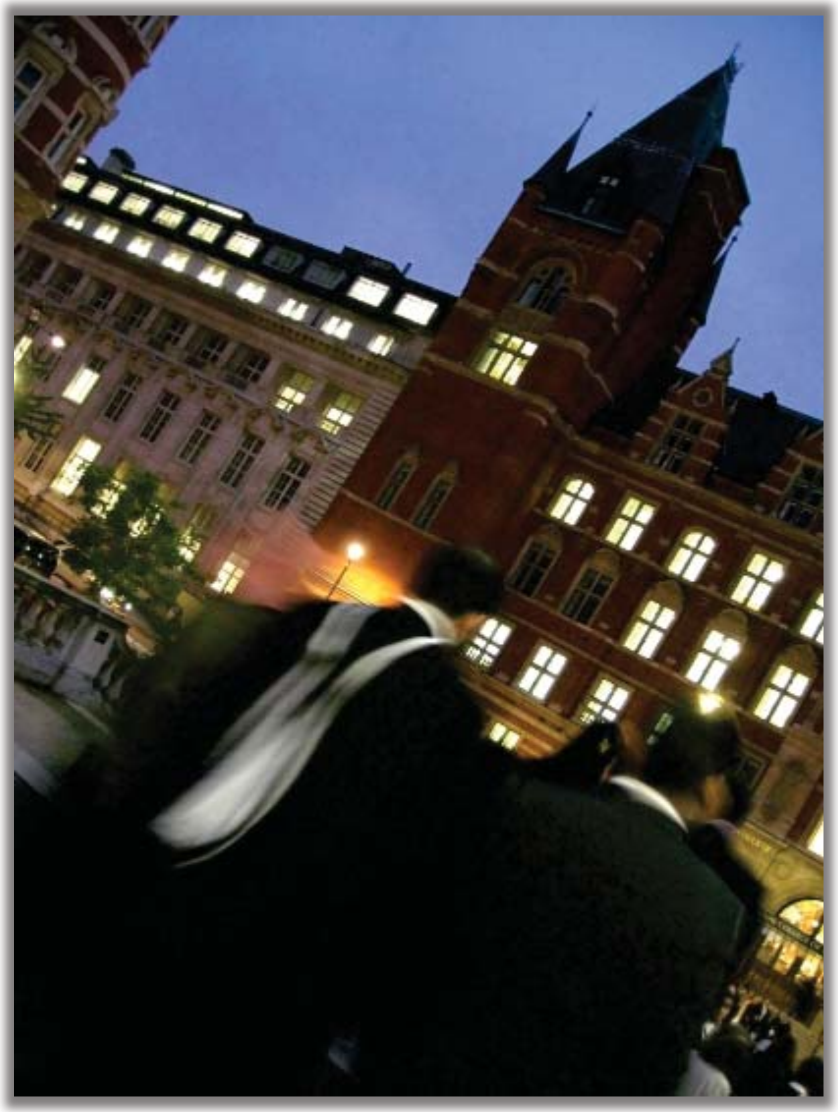
Chris Abell, Florian Hollfelder, Wilhelm Huck, Carol Robinson, Joshua Edel, Jongin Hong, Xize Niu, Shelly Gulati, Katherine Elvira, Monpichar Srisa-art, Fabrice Gielen, Graeme Whyte, Ansgar Huebner, Yolanda Schaerli, Rob Wootton, Bo Zhang, Suwan Jayasinghe, Claire Stanley, Oscar Ces



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