Isolation of Exosomes Using Viscoelastic Fluids in a Microfluidic Device

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Overview

- Exosomes
- Exosome Isolation
- Viscoelasticity-based sorting
- Numerical Model
- Fabrication Process
- Conclusion

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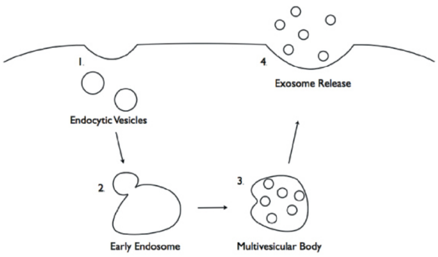
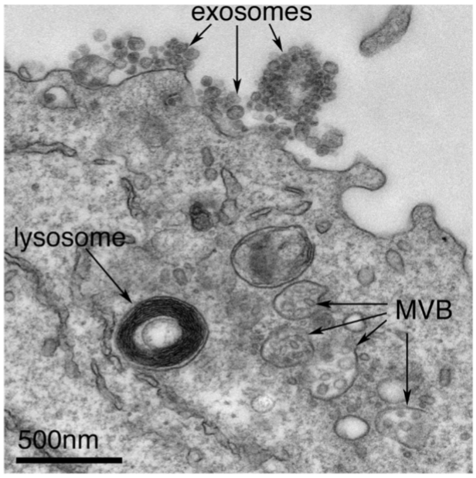
Exosomes

- Extracellular vesicles
- 30 – 150 nm in diameter
- Secreted by most cells
- Abundantly found in most biological fluids
- Carry proteins, DNA's and RNA's
- Reflect origin cells
- Intercellular communication
- Neurodegenerative disorders
- AIDS
- Cancer





BMC Biology 2016, 14:46
DOI: 10.1186/s12915-016-0268-z

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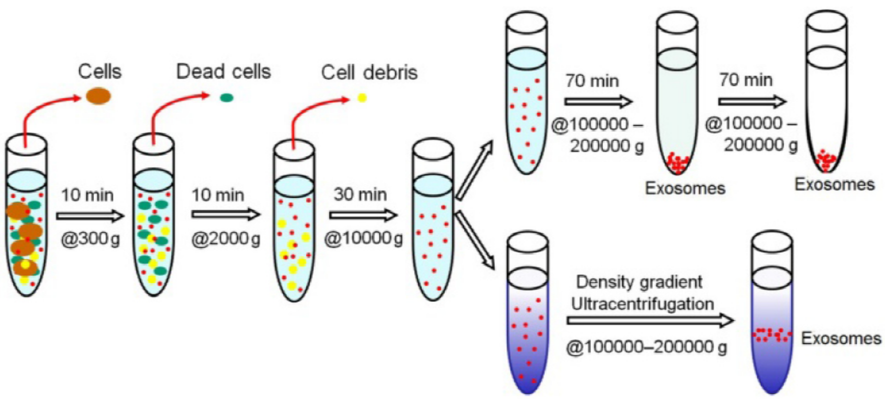
Exosome Isolation

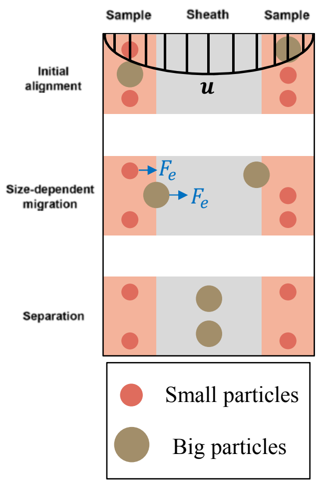
- Ultracentrifugation (current golden standard)
- €40,000 – 80,000 for the ultracentrifuge
- €2,500 per annum running cost
- Dedicated specialists
- 4 – 5 hours per sample
- 5 – 25% yield

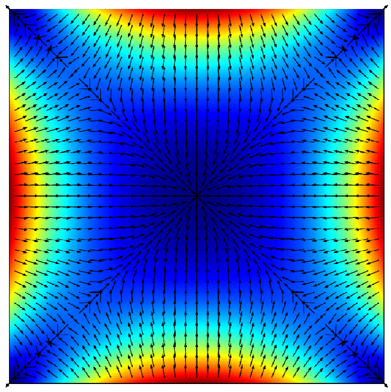


LSA2018 - LÜBECK
Theranostics 2017; 7(3): 789-804.
DOI: 10.7150/thno.18133

Viscoelasticity-Based Sorting

- Particle migration is always from the walls into the center of the microfluidic channel
- Continuous separation of particles
- No external devices
- No external fields
- No complex geometries





COMSOL simulation of the shear rate in a channel cross-section with elastic force vectors. Red colour represents regions of highest shear rate and dark blue colour represents regions of lowest shear rate.

Viscoelasticity-Based Sorting

The Convected Jeffrey (Oldroyd-B) Fluid Model

$$\rho\left(\frac{\partial \boldsymbol{u}}{\partial t} + \boldsymbol{u}\cdot\nabla\boldsymbol{u}\right) = \nabla\cdot\boldsymbol{\sigma}$$

$$\boldsymbol{\sigma} = -p\boldsymbol{I} + \boldsymbol{\tau}$$

$$\boldsymbol{\tau} = \eta_s\boldsymbol{\epsilon} + \boldsymbol{\tau}_p$$

$$\boldsymbol{\tau} + \lambda_1\hat{\boldsymbol{\tau}} = -\eta_0(\boldsymbol{\epsilon} + \lambda_2\hat{\boldsymbol{\epsilon}})$$

$$\boldsymbol{\epsilon} = (\nabla\boldsymbol{u}) + (\nabla\boldsymbol{u})^T$$

$$\hat{\boldsymbol{\epsilon}} = \frac{\partial}{\partial t}\boldsymbol{\epsilon} + \boldsymbol{u}\cdot\nabla\boldsymbol{\epsilon} - \{(\nabla\boldsymbol{u})^T\cdot\boldsymbol{\epsilon} + \boldsymbol{\epsilon}\cdot(\nabla\boldsymbol{u})\}$$

$$\hat{\boldsymbol{\tau}} = \frac{\partial}{\partial t}\boldsymbol{\tau} + \boldsymbol{u}\cdot\nabla\boldsymbol{\tau} - \{(\nabla\boldsymbol{u})^T\cdot\boldsymbol{\tau} + \boldsymbol{\tau}\cdot(\nabla\boldsymbol{u})\}$$

$$\eta_0 = \eta_s + \eta_p$$ (Water + Biocompatible polymer)

$$\eta_p = 0.072\eta_s c M_w^{0.65}$$

$$[\eta] = 0.72 M_w^{0.65}$$

$$\lambda_1 = 0.463\frac{[\eta]M_w\eta_s}{N_A k_B T}\left(\frac{c}{c^*}\right)^{0.65}$$

$$\lambda_2 = \frac{\eta_s}{\eta_0}\lambda_1 \qquad c^* = \frac{0.77}{[\eta]}$$

$\boldsymbol{\tau}$ – Total Deviatoric Stress Tensor
$\boldsymbol{\epsilon}$ – Strain-Rate Tensor
λ_1 – Relaxation Time
λ_2 – Retardation Time
η_0 – Total Fluid Viscosity
η_s – Solvent Viscosity
η_p – Polymer Contribution to the Viscosity
$[\eta]$ – Intrinsic Viscosity
M_w – Polymer Molecular Weight
T – Temperature
c – Concentration
c^* – Critical Overlap Concentration
N_A – Avogadro Number
k_B – Boltzmann Constant
$\langle \hat{\ } \rangle$ – Upper-Convected Time Derivative

Viscoelasticity-Based Sorting

Dominant Forces Acting on the Particles

$$F_p = F_D + F_V + F_e$$ (Particle Force Balance)

$$F_e = C_e d_p^3 \nabla N_1$$ (Elastic Lift Force) $$N_1 = 2\eta_p \lambda_1 \dot{\gamma}^2$$ $$\dot{\gamma} = \sqrt{2\boldsymbol{\epsilon} : \boldsymbol{\epsilon}}$$

$$F_V = \frac{1}{12}\pi d_p^3 \rho_f \frac{d(\boldsymbol{u} - \boldsymbol{V}_p)}{dt}$$ (Virtual Mass Force)

$$F_D = \frac{1}{\tau_p} m_p (\boldsymbol{u} - \boldsymbol{V}_p)$$ (Drag Force) $$\tau_p = \frac{4\rho_p d_p^2}{3\eta C_D Re_r}$$

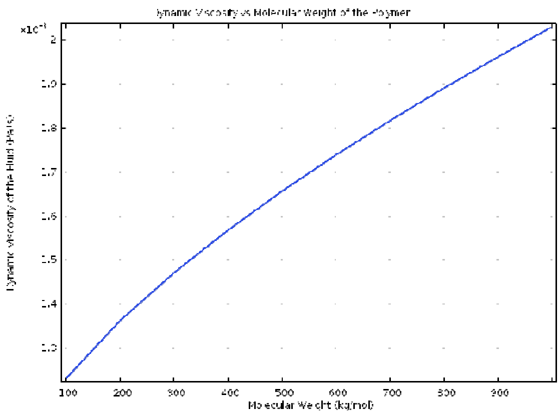
C_e – Elastic Lift Coefficient
d_p – Particle Diameter
N_1 – The First Normal Stress Difference
$\dot{\gamma}$ – Shear Rate
V_p – Particle Velocity
m_p – Particle Mass
ρ_f – Fluid Density
ρ_p – Particle Density
C_D – Drag Coefficient
Re_r – Reynold's Number
$\langle : \rangle$ – Contraction Operator

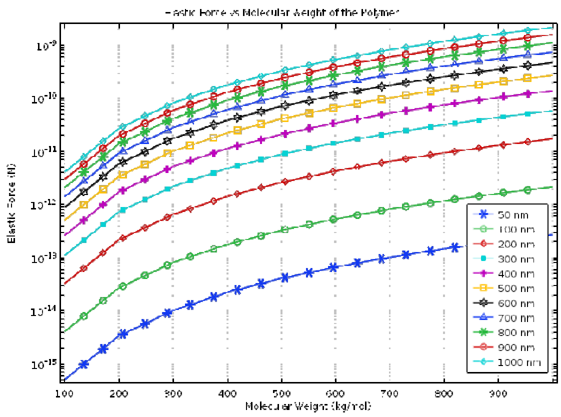
Numerical Model

$c = 0.18$ wt% In a 20x100 µm channel

$V_1 = 8$ µL/min ⟶ 0.07 m/s

$V_2 = 60$ µL/min ⟶ 0.49 m/s





Numerical Model

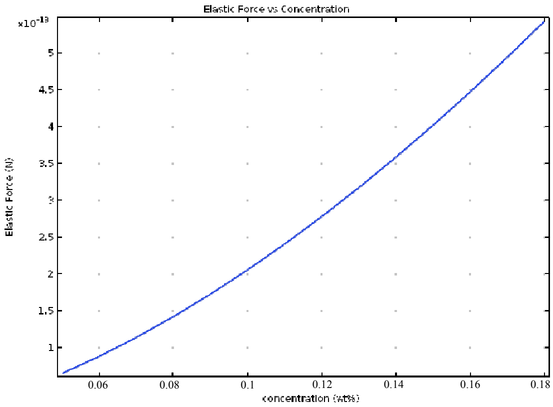
$c = 0.18$ wt%

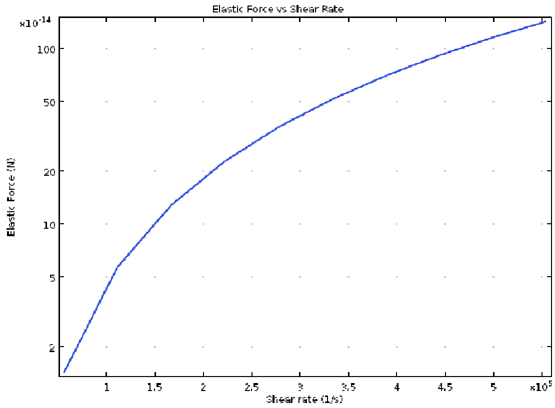
$V_1 = 8$ µL/min

$V_2 = 60$ µL/min

$M_w = 600$ kg/mol

$d_p = 100$ nm





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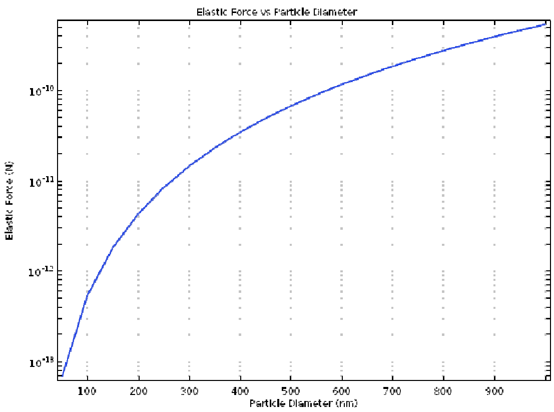
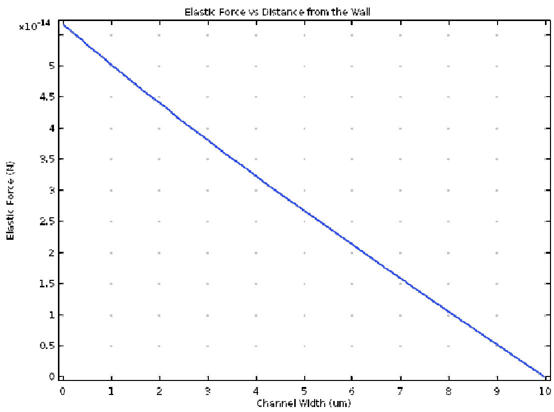


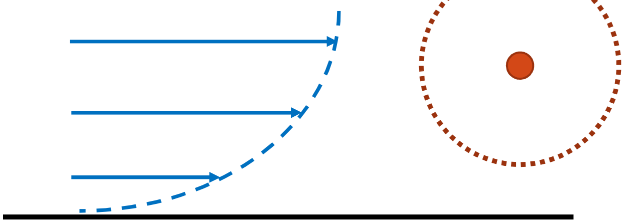


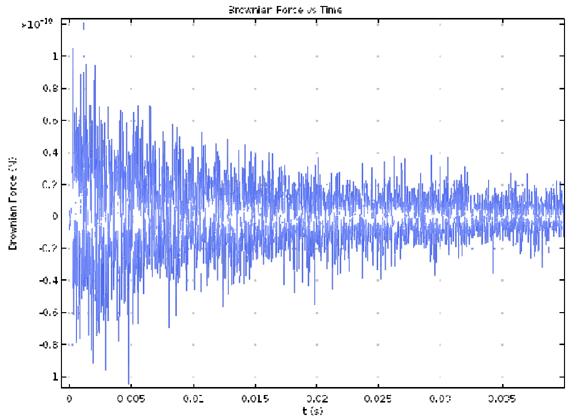
Numerical Model

Gaussian White Noise Process

$$F_B = \zeta \sqrt{\frac{6\pi k_B \eta_0 T d_p}{\delta t}}$$

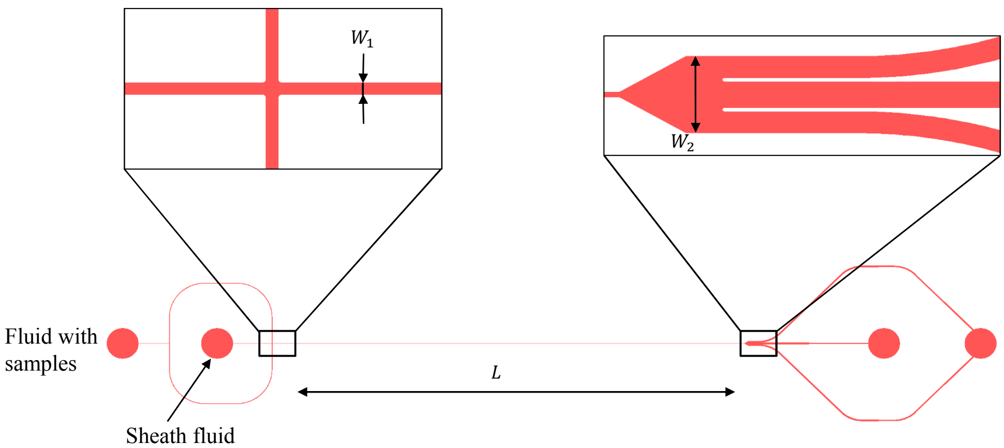
$$\Delta = \sqrt{2nD\delta t}$$





ζ – Normally Distributed Random Number
δt – Time Step
Δ – Root Mean Square of the Displacement

Numerical Model



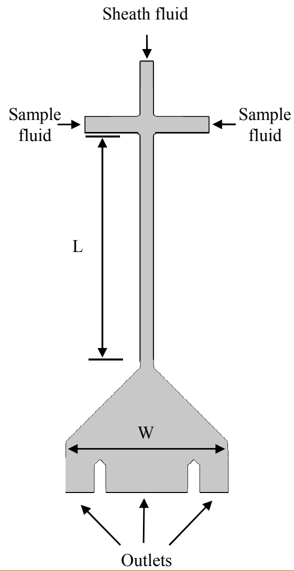
Microfluidic system geometry.

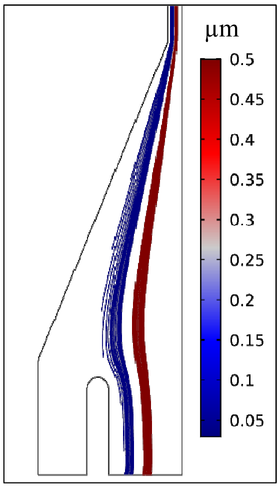
By varying

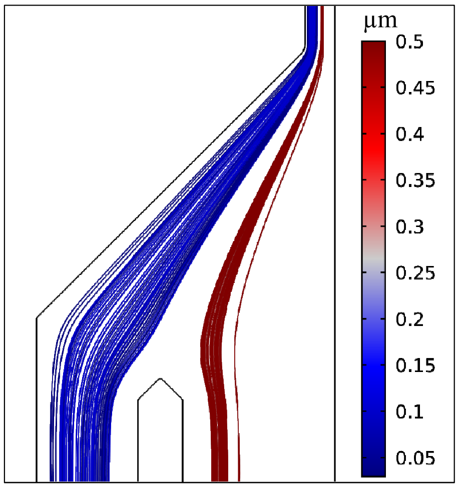
- The channel length L
- Widths, W_1 and W_2
- Fluid speeds
- Rheological properties of the fluid

One can control the separation

Numerical Model

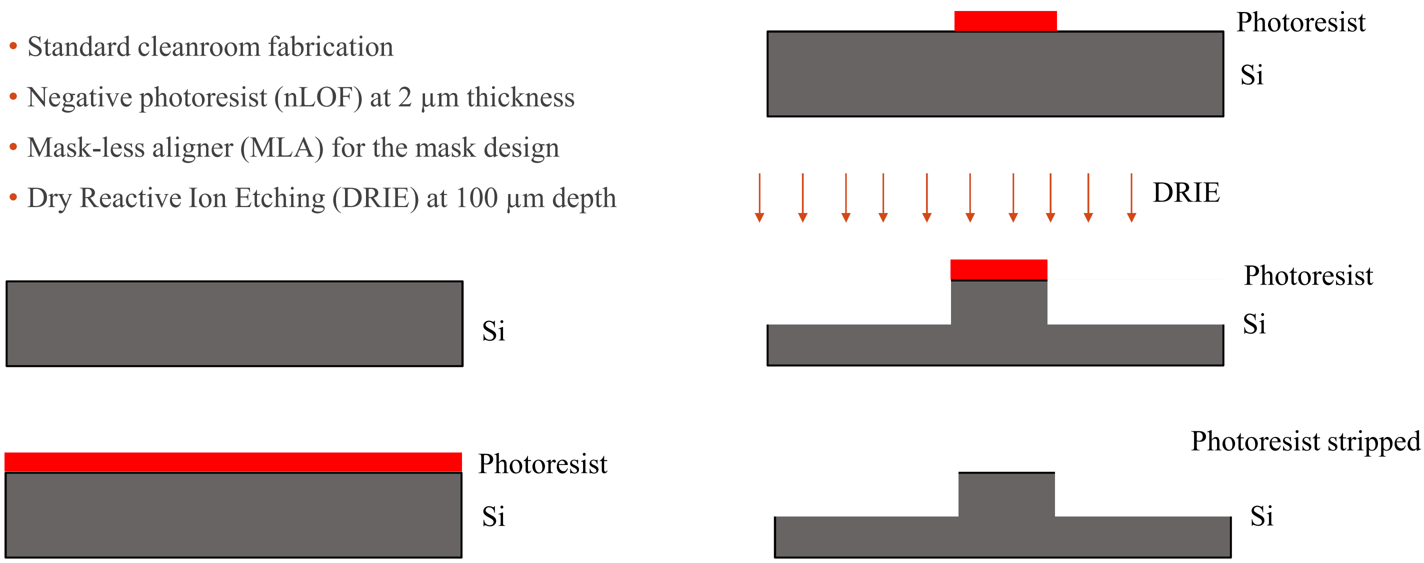




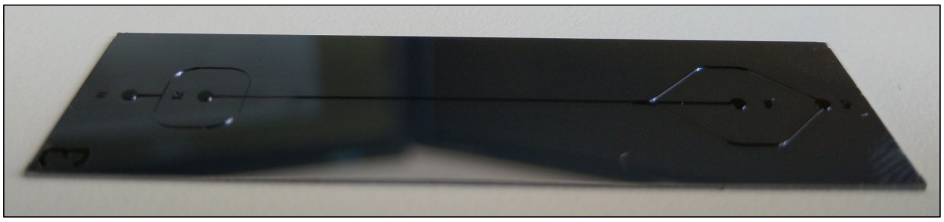


Fabrication Process

- Standard cleanroom fabrication
- Negative photoresist (nLOF) at 2 µm thickness
- Mask-less aligner (MLA) for the mask design
- Dry Reactive Ion Etching (DRIE) at 100 µm depth

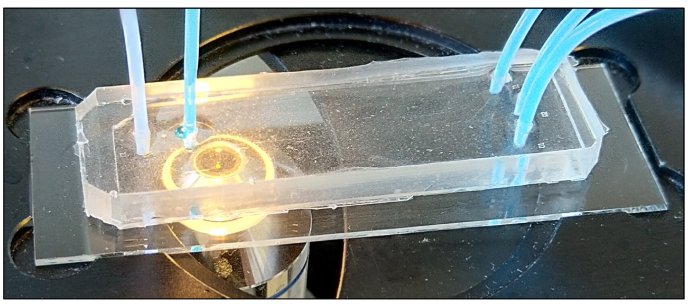


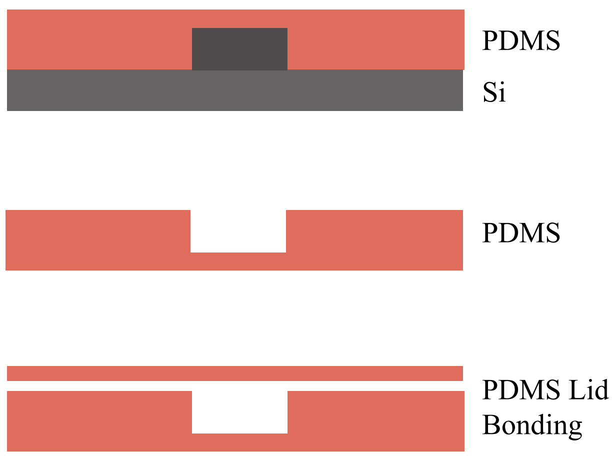
Fabrication Process



Fabrication Process

- PDMS (polydimethylsiloxane) + thermocurable polymer
- 3 hours in a 70°C oven





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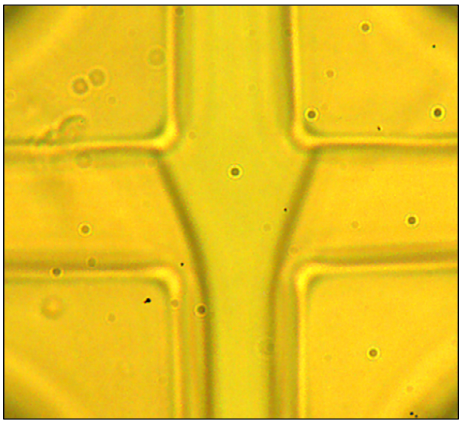
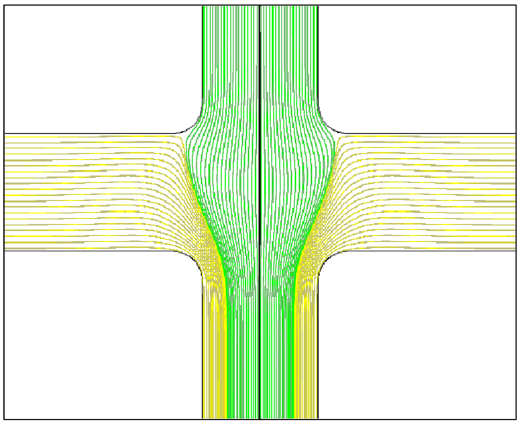
Fabrication Process





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Conclusion

- High yield (~90%)
- High purity (~90%)
- Low price
- Low volume handling (~100 μL)
- Short processing time (< 1 s)
- Simple design
- No material loss due to external fields
- Possibility to isolate other nanoparticles

Thank you for your attention