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INTRODUCTION TO MICROFLUIDICS

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Ultrasonic Particle and Cell Separation and Size Sorting in Micro-channels

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Ultrasonic particle and cell separation and size sorting in micro-channels

Lecture 5

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Introduction

Increasing needs in biotechnology, environmental science, and medical applications in continuous flow analysis require filtration of basic fluids from particles and cells that can interfere with the on-line analysis. Such continuous flow separators and size sorter are also needed in a fast developing field of microfluidics

Various techniques of filtration, particle and cell separation and size sorting

- **Large scale biotechnology processes** are used centrifuge and membrane filters that obstruct continuous flow process.

- **Small scale biotechnological processes** are often based on specific chemical bonding to extract certain constituents with high degree of resolution, purity, and effectiveness: density gradient centrifugation, fluorescent activated sorting (FACS), magnetic associated cell separation (MACS), and laser capture micro dissection (LCMD).

An alternative approach is to use bulk forces to conduct continuous flow separation and size sorting

- Obvious realization of this idea is to use high frequency ultrasonic standing waves to generate a radiation pressure that tends to move particles within a fluid toward nodal or antinodal planes.

- This phenomenon is well known though did not gain widespread technological applications since it is highly sensitive to perturbations and involves acoustic forces that are usually rather weak compared with, e.g. viscous forces in a flow.

- The well known Kundt figures are resulted from this phenomenon, namely dust particles subjected to an acoustic standing waves are collected in orthogonal lines separated by one half wave length of sound in the medium.

Forces acting on particles in acoustic waves

- Primary acoustic radiation forces acting in the direction of the wave vector depend on acoustic energy gradients both axially and transversally to the sound propagation direction.

- Secondary particle-particle interaction forces originating from scattering of the incident wave from surrounding particles, so called Bjerkness forces. The latter can be either attractive or repulsive but effect is negligible for particle distances of several particle radii.

- In a plane standing wave that is superposition of two contrpropagating traveling waves particles move under influence of the primary radiation forces towards velocity anti-nodal planes (or pressure nodal planes) of maximum velocity (minimum pressure) separated by a half wave length.

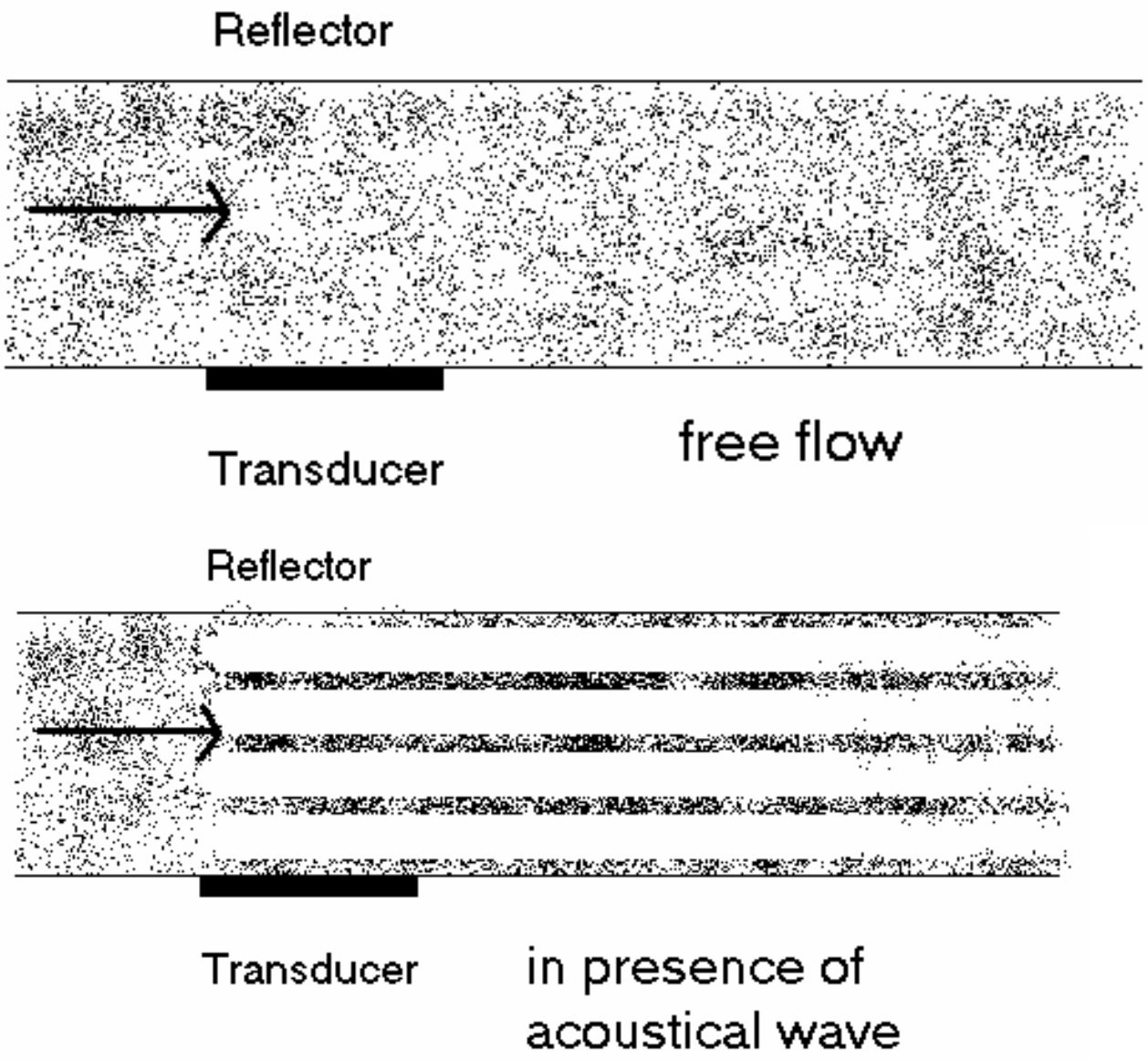
High frequency (MegaHertz range) is desirable to work with due to several reasons:

1. To avoid cavitation –nucleation of gas bubbles in a fluid due to local pressure drop below the vapor pressure of the fluid that maybe detrimental for handling biological material such as cells.

2. To make this technique suitable for implementation in microfluidic devices, where the dimensions of the fluidic channel can be on the order of the sound wave length. Indeed, acoustic wave length in water at 10 MHz is 150 μm and still much larger than the particle (cell) radii used for sorting.

3. Short acoustic path length is advantageous from sound attenuation point of view.

Thus, acoustic trapping in microfluidic channels requires to use integrated miniature ultrasonic transducers in microfluidic devices



Distance between the lines is $\lambda / 2$, where λ is wave-length.

Theoretical background

When a standing wave is set up in a fluid, the acoustic force acting on a particle, which radius, *R*, is much larger than the sound wavelength (*kR*<<1, where *k* is the sound wave number), is given in the approximation of zero viscosity by:

$$\overline{F}_{st} = \frac{2\pi (kR)^3 2\overline{E}_{st}}{k^2} \Phi(\Lambda, \sigma) \sin 2\vec{k}\vec{r}_0$$

Here $\overline{E}_{st}$ -is the energy density of the standing waves

$\sigma = c_p / c$ -is the ratio of the sound velocity of a particle c_p and a fluid c

$\Lambda = \rho_p / \rho$ -is the ratio of the particle ρ_p to fluid ρ density

R -is the particle radius; $\vec{r}_0$ -is the vector normal to the force node

$$\Phi(\Lambda, \sigma) = \frac{1}{(1+2\Lambda)^2} \left\{ \left(\Lambda - \frac{1+2\Lambda}{3\Lambda\sigma^2} \right)^2 + \frac{2}{9}(\Lambda - 1)^2 \right\}$$

Derivation of expression for radiation force

Estimates of fluid flow regime for efficient separation

According to the theory, particles are accumulated in the nodes of the acoustic force (pressure). For efficient particle trapping transverse mixing by flow should be eliminated, i.e. separation should occur in a laminar flow at $\mathrm{Re} \equiv \rho V d / \eta \leq 1$, where *V* is the flow velocity, and *d* is the channel diameter.

For our experiments we use a micro-channel of the width *a*=160 μm and the depth of *b*=150 μm, $\rho = 1.027 g/cm^3$, the fluid discharge Q=100 nl/s that results in $\mathrm{Re} \cong 0.7$

Role of particle diffusion in separation efficiency

The diffusion length passed by a particle during time *t* is defined as $h^2 = 2Dt$, where *D* is the particle diffusion coefficient defined as

$$D = \frac{k_B T}{6\pi\eta R}$$

In the experiment the characteristic distance between transducer and a channel fork is about 2 mm. Then at *Q*=100 nl/s it will be covered by a particle in *t*=0.48 s, for which particles of $R = 5\,\mu m$ have the diffusion length $h \cong 0.2\,\mu m$ that is negligible for interface smearing.

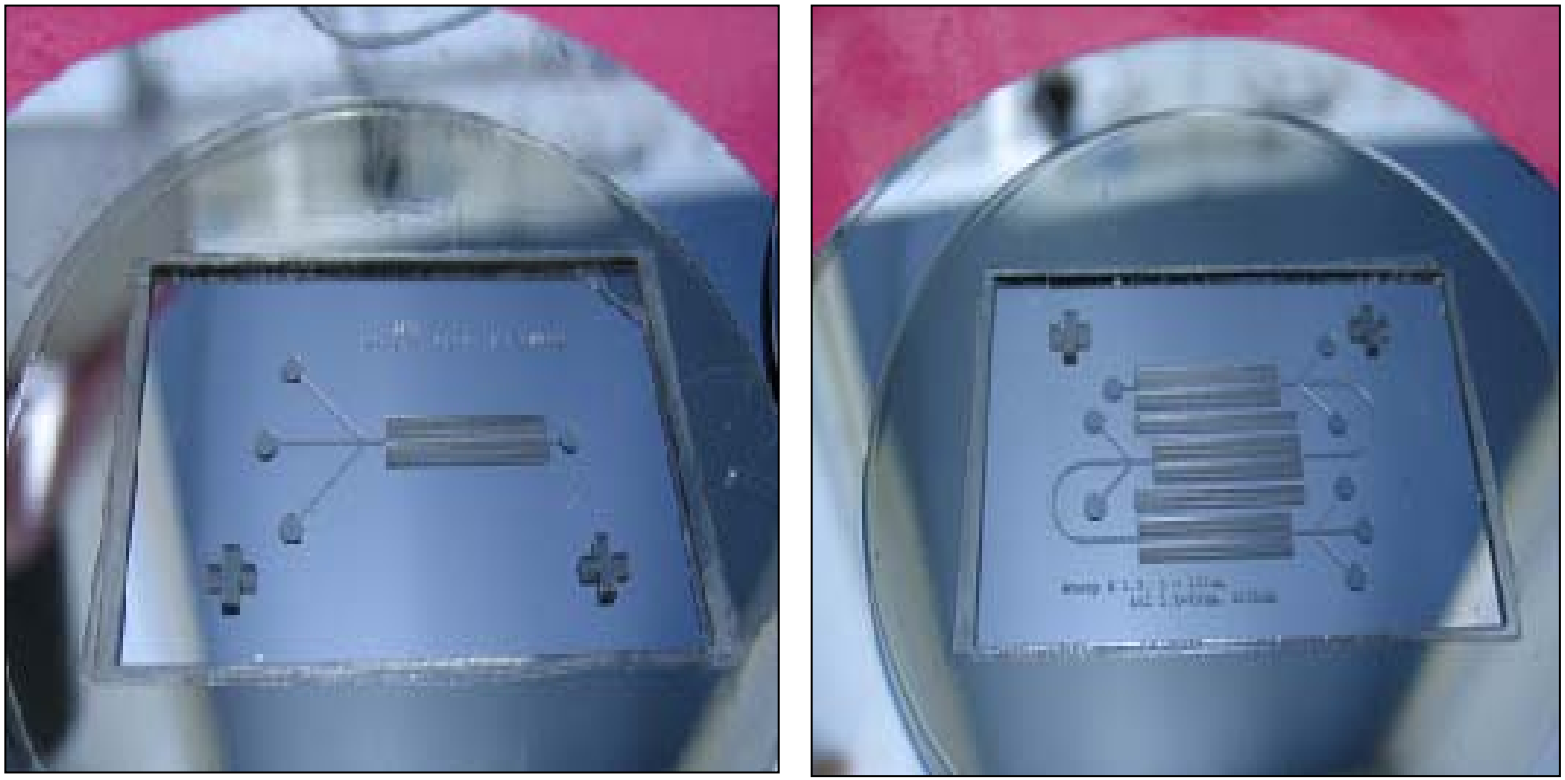
Solution preparation

- Commercially available particles (ORGASOL 2002 EXD NAT 1, ultrafine powder of polyamide 12 with narrow size distribution and nearly round particle shape were used.
- Particle density $\rho = 1.03 g/cm^3$, diameter $2R = 10 \pm 2 \mu m$

- To dissolve particles in water the following protocol is used:

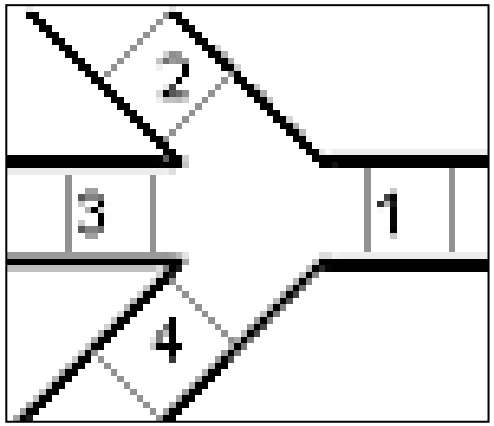
Surfactant (MAFO CAB- BASF) 6.8%
Polymeric dispersant (polyacrylate salt)(Darvan 7) 2.5%
Defoamer (Plurafac RA40-Basf) 1.4%
Water 89.3%

Molds for one-part (left) and two parts (right) micro-channels

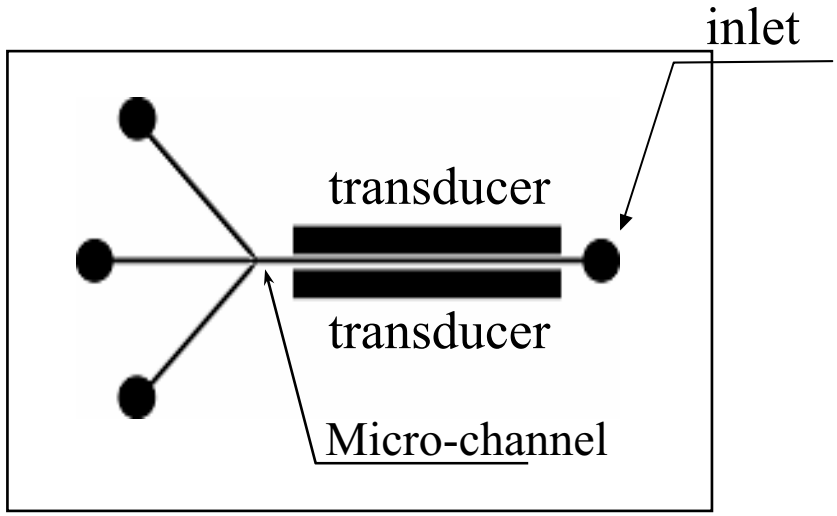


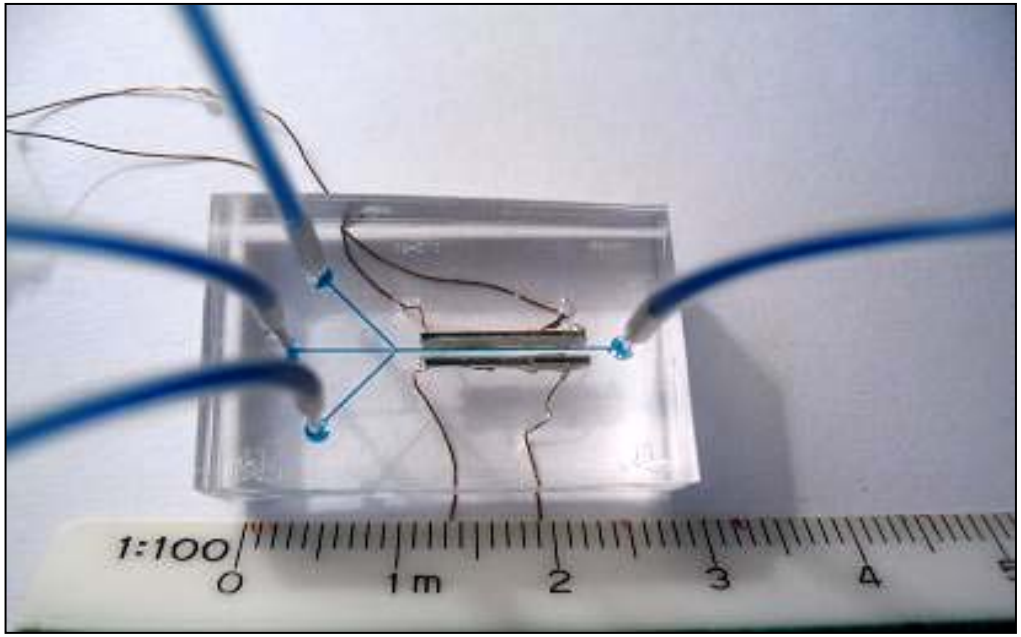
One-stage micro-channel separator (schematic view and photo)

outlet

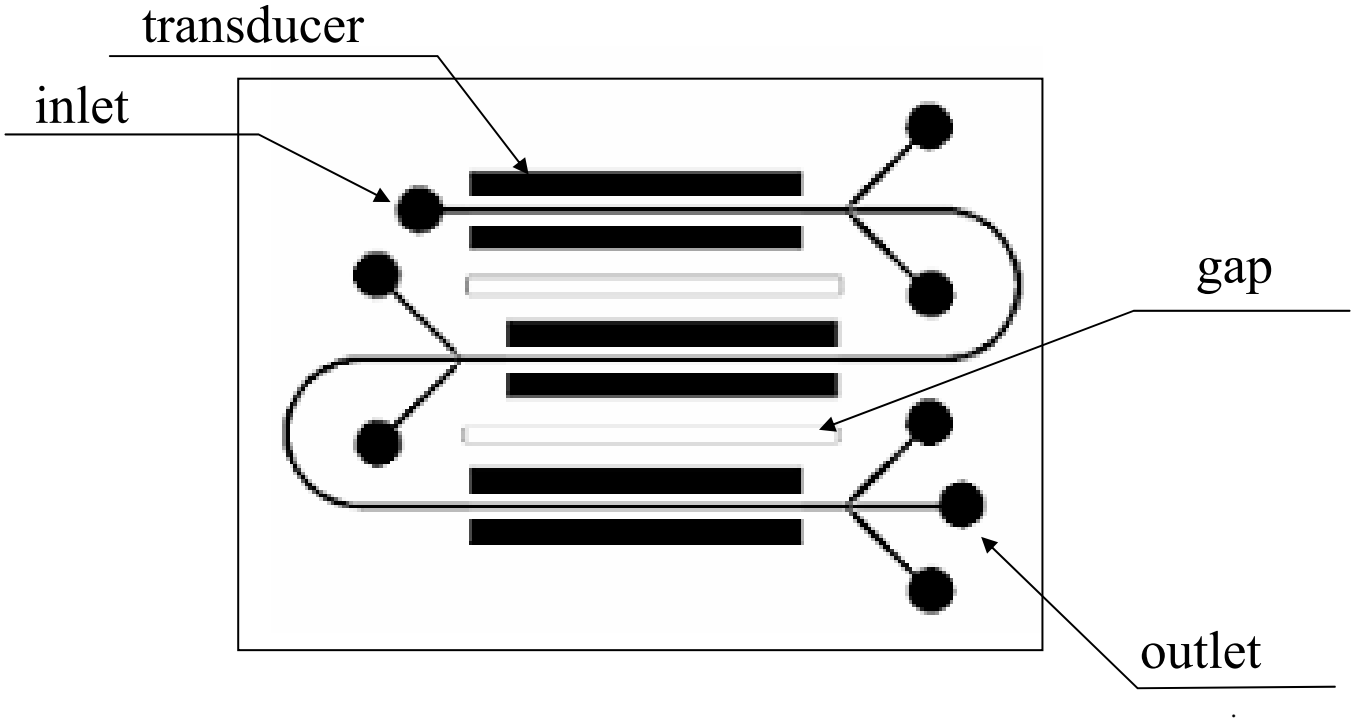


Microfluidic chip is made of a silicone elastomer Sylgard 184 with curing time 4 hours at 65C.
The width $a=160\ \mu m (\approx \lambda/2)$ and depth $b=150\ \mu m$
Transducers are mounted on both sides of the micro-channel at 800 μm from the channel center

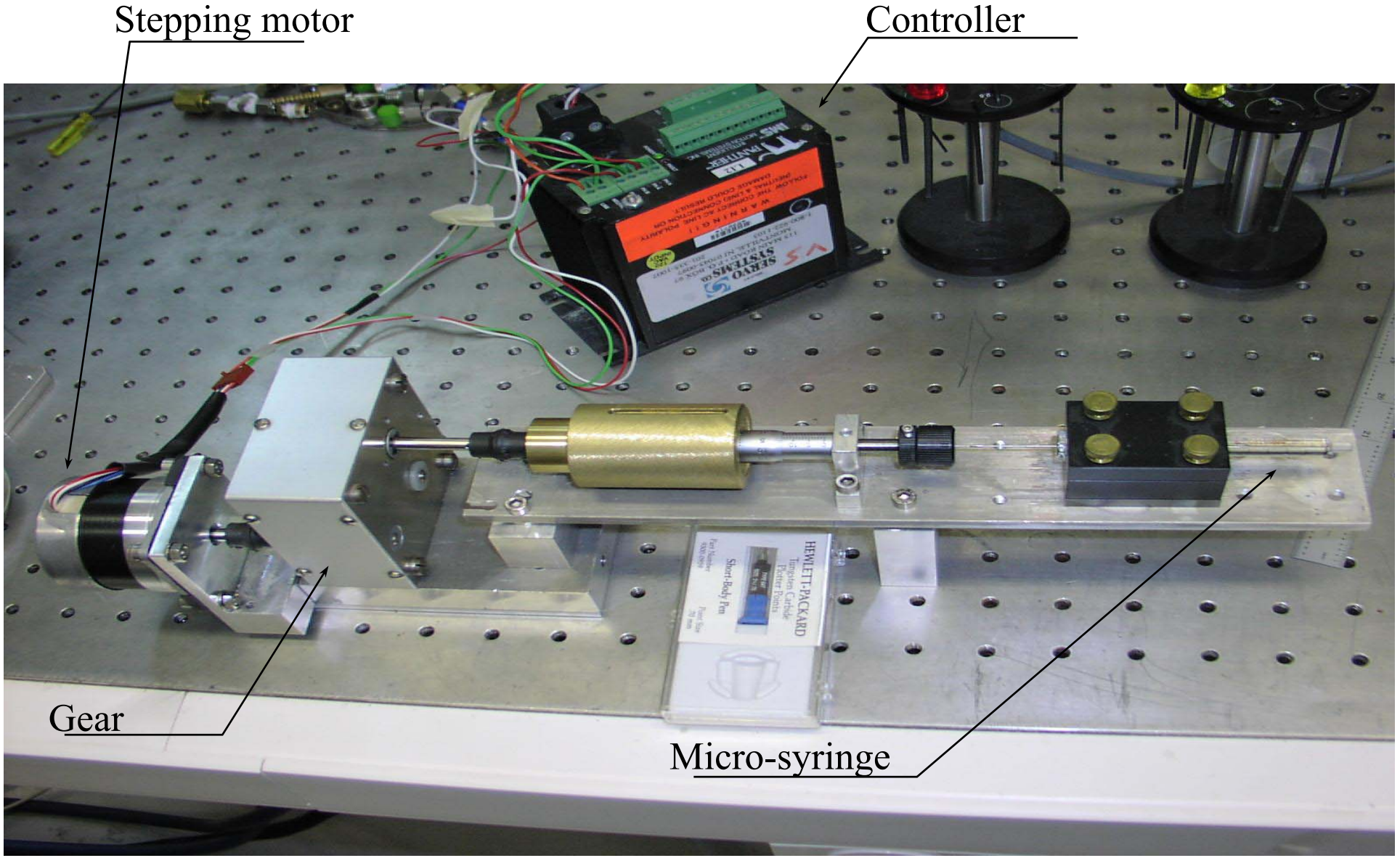




Three-stage micro-channel size sorter (schematic view)



Flow rate controller



Numerical simulations

To describe dynamics of a particle in a channel flow and particularly to estimate its characteristic time of reaching the force node, the following equation of particle motion was solved numerically :

$$m\ddot{Y} = F_{st}\sin(2kY) - C\dot{Y}$$

Here Y is the coordinate across the channel, $C = 6\pi\eta R$ is the Stokes coefficient, F_{st} is the ultrasonic force amplitude defined as

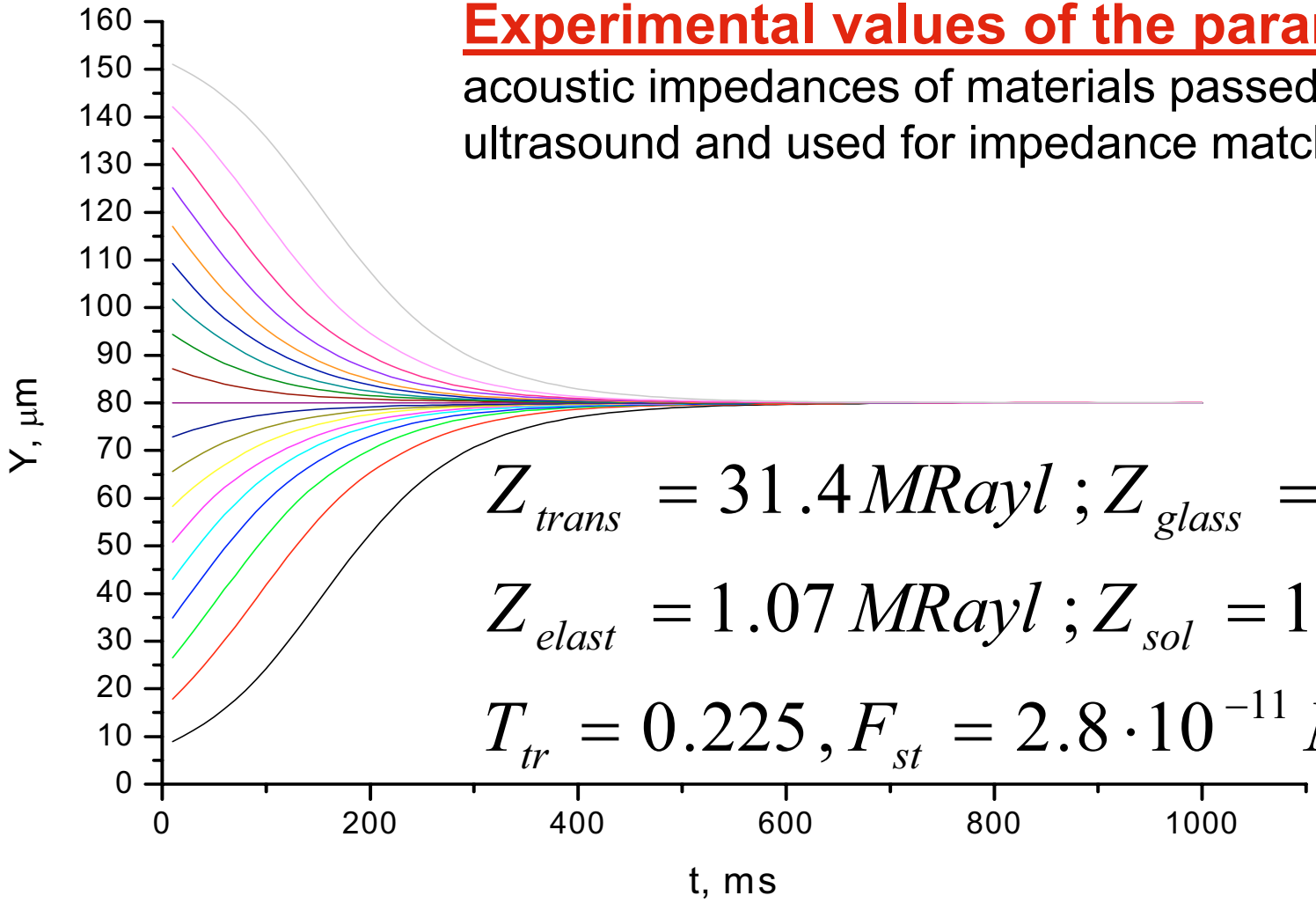
$$F_{st} = \frac{2\pi(kR)^3 2E_{st}}{k^2}\Phi(\Lambda,\sigma)$$

$$E_{st} = 8\pi^2 f^2 R^3 d_{33}^2 U^2 \rho T_{tr}$$

Here E_{st} is the energy density of the standing wave, f is the frequency, U is the applied voltage on the transducer, d_{33} is the longitudinal piezoelectric elasticity coefficient, and $T_{tr} = {}^{4Z_1Z_2}\!/_{(Z_1+Z_2)^2}$ is the transmission coefficient

Experimental values of the parameters:

acoustic impedances of materials passed by ultrasound and used for impedance matching and force

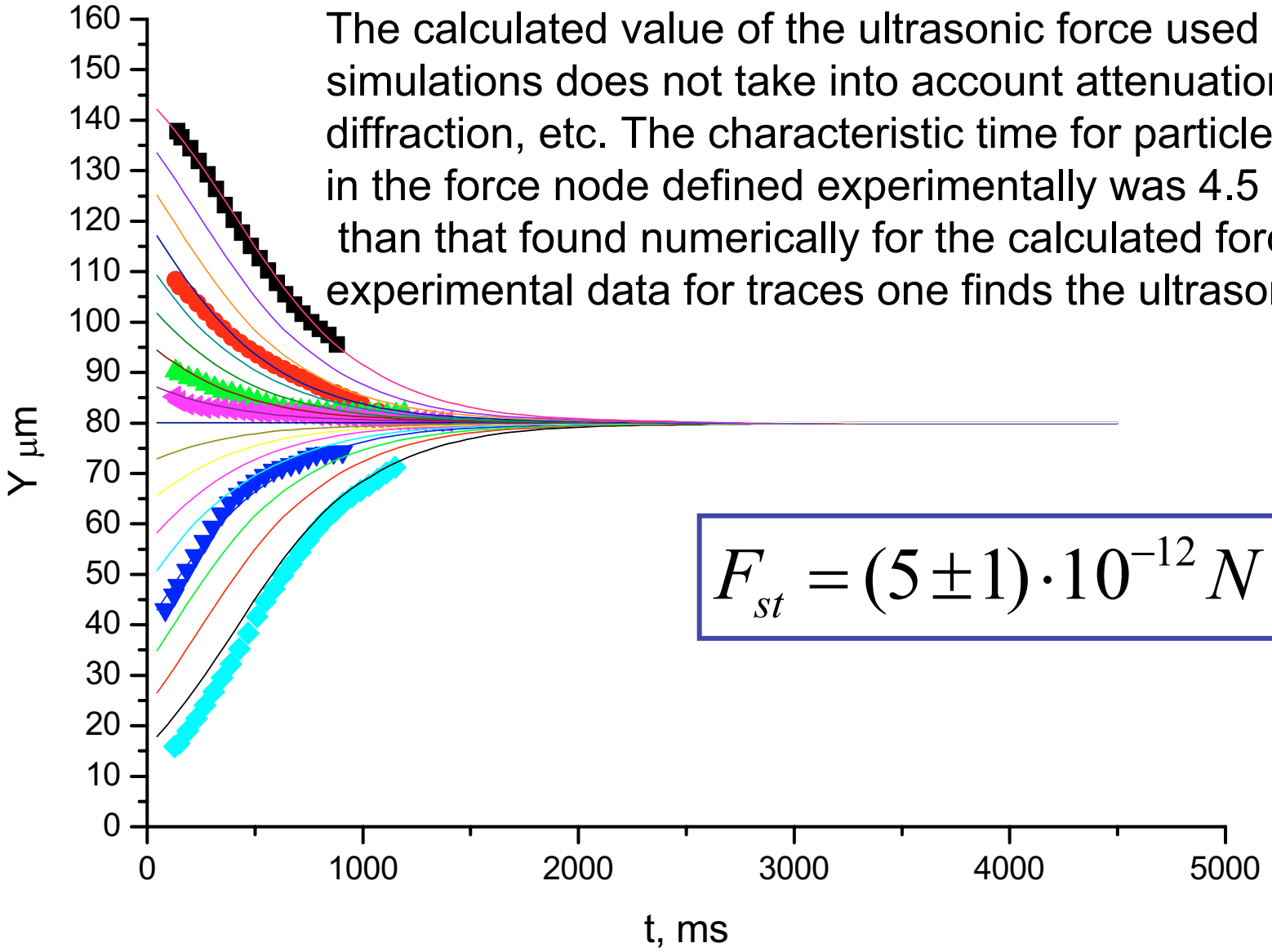


$$Z_{trans} = 31.4\,MRayl\,; Z_{glass} = 13\,MRayl\,;$$

$$Z_{elast} = 1.07\,MRayl\,; Z_{sol} = 1.5\,MRayl$$

$$T_{tr} = 0.225\,, F_{st} = 2.8 \cdot 10^{-11}\,N$$

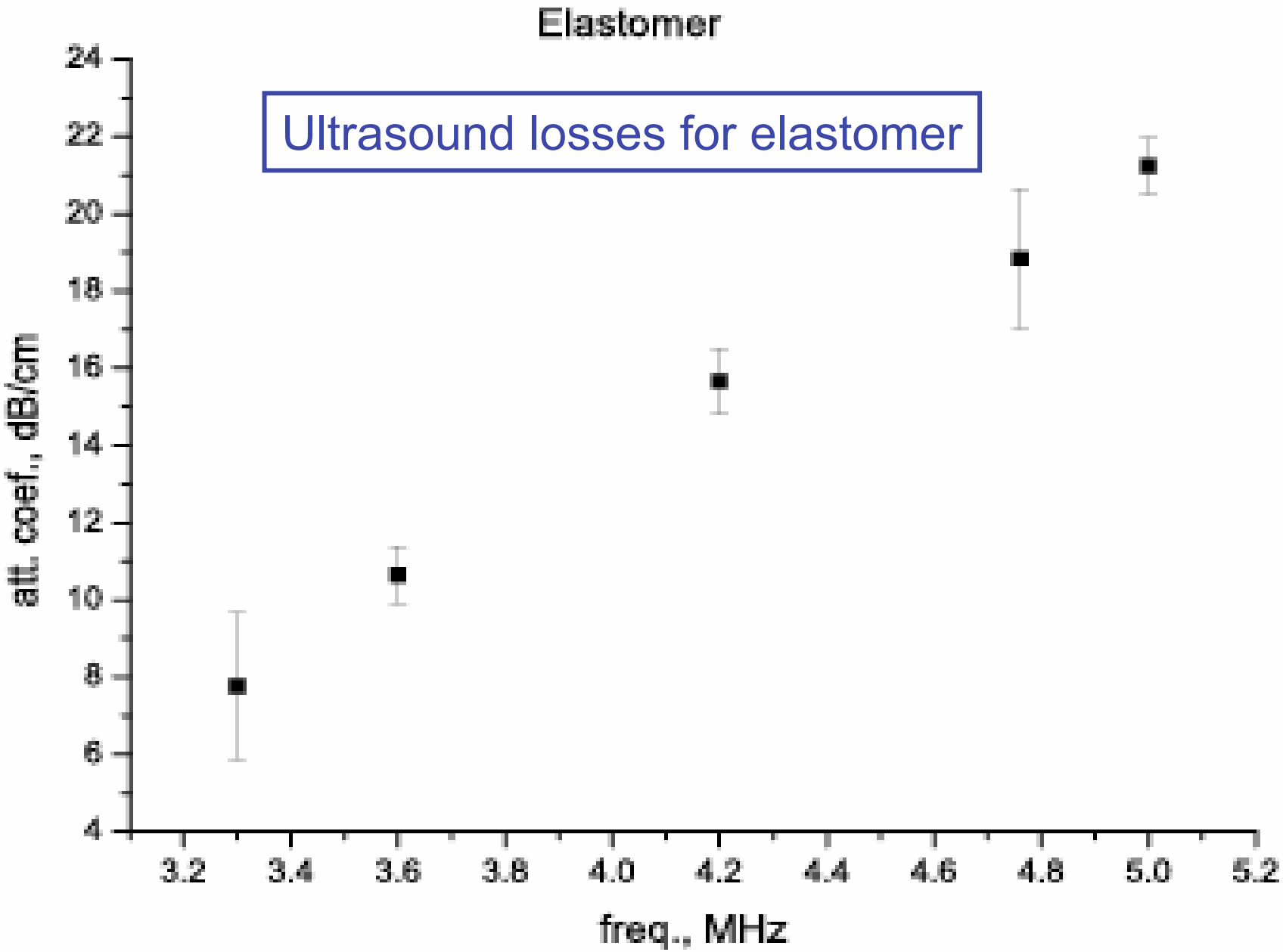
Position of 10 μm particles in the micro-channel as a function of time and initial position

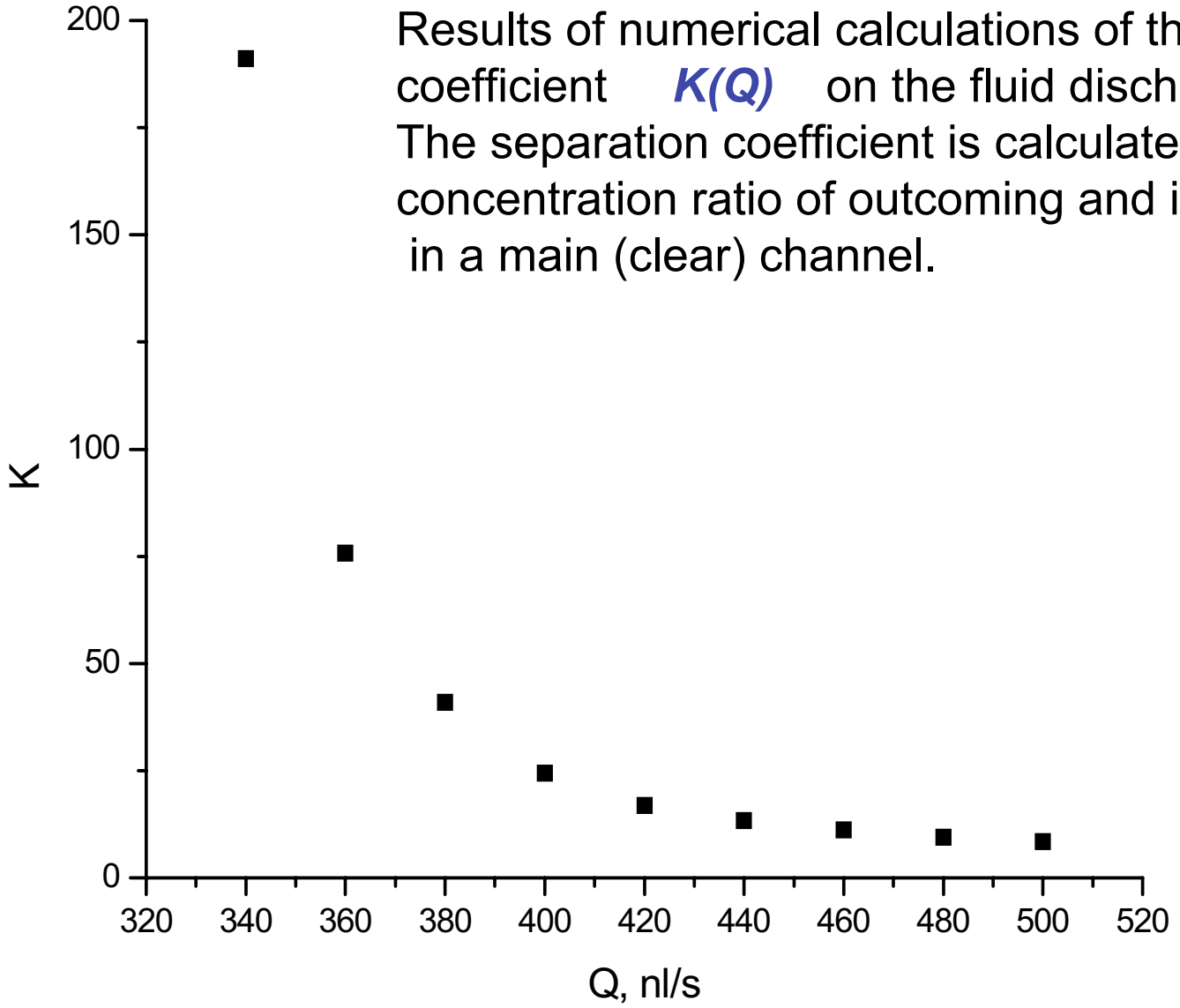


The calculated value of the ultrasonic force used in numerical simulations does not take into account attenuation, interference, diffraction, etc. The characteristic time for particle accumulation in the force node defined experimentally was 4.5 times longer than that found numerically for the calculated force. Fitting the experimental data for traces one finds the ultrasonic force:

$$F_{st} = (5 \pm 1) \cdot 10^{-12}\, N$$

Tracks of particles in the micro-channel as a function of time and initial position are shown: solid lines (numerical calculations) and dots (experimental data)


Elastomer
Ultrasound losses for elastomer
att. coef., dB/cm
freq., MHz
24
22
20
18
16
14
12
10
8
6
4
3.2
3.4
3.6
3.8
4.0
4.2
4.4
4.6
4.8
5.0
5.2



Results of numerical calculations of the separation coefficient ***K(Q)*** on the fluid discharge ***Q***.
The separation coefficient is calculated as the concentration ratio of outcoming and incoming particles in a main (clear) channel.

Flow velocity profile

The numerical estimates of the dependence of the separation coefficient in a rectangular cross-section micro-channel with the transducers of 1 cm long shown in the previous figure are made using the flow velocity profile in a rectangular cross-section channel: $-a \le y \le a$, $-b \le z \le b$ given by

$$u(y,z) = \frac{16a^2}{\eta\pi^3}\left(-\frac{dp}{dx}\right)\sum_{i=1,3,5,\ldots}^{\infty}(-1)^{(i-1)/2}\left[1-\frac{\cosh(i\pi z/2a)}{\cosh(i\pi b/2a)}\right]\frac{\cos(i\pi y/2a)}{i^3}$$

$$Q = \frac{4ba^3}{3\eta}\left(-\frac{dp}{dx}\right)\left[1-\frac{192a}{\pi^5 b}\sum_{i=1,3,5,\ldots}^{\infty}\frac{\tanh(i\pi b/2a)}{i^5}\right]$$

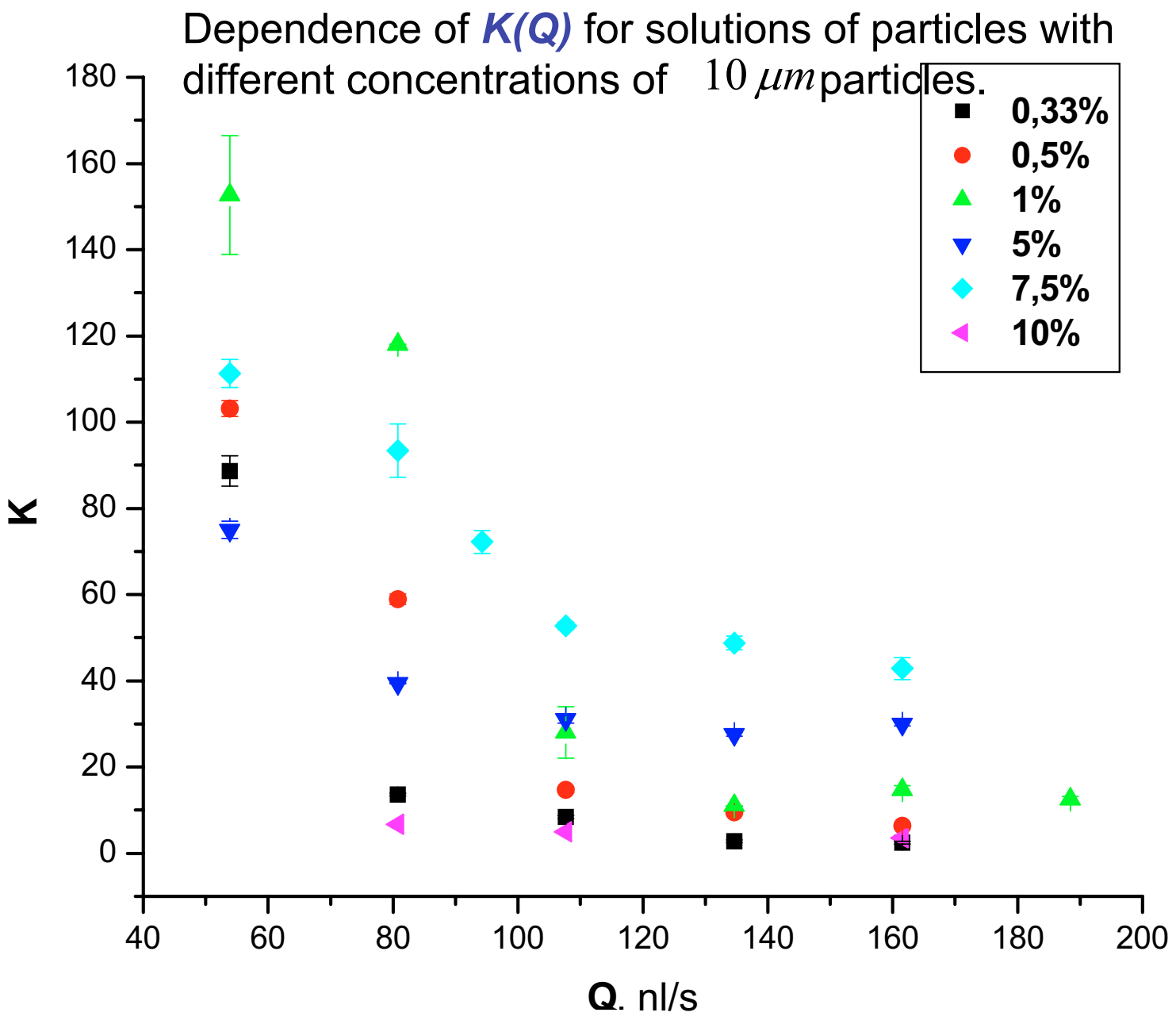
Measurements

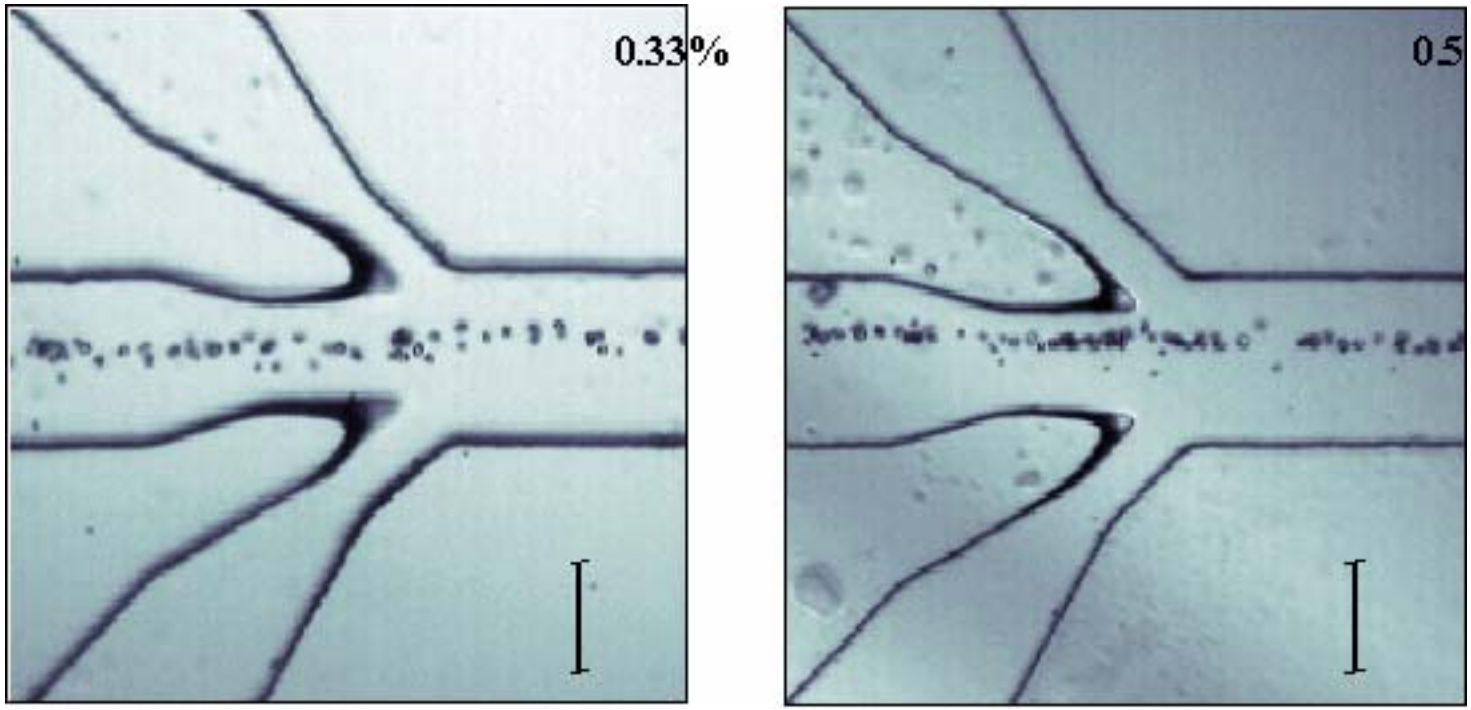
1. The goal of the first group of the experiments on particle separation was to determine the clearance coefficient dependence on the flow rate, ***Q***.

2. The goal of the second group of the experiments was to use the same setup for separation of blood cells from the serum in one-stage and three-stage channels.

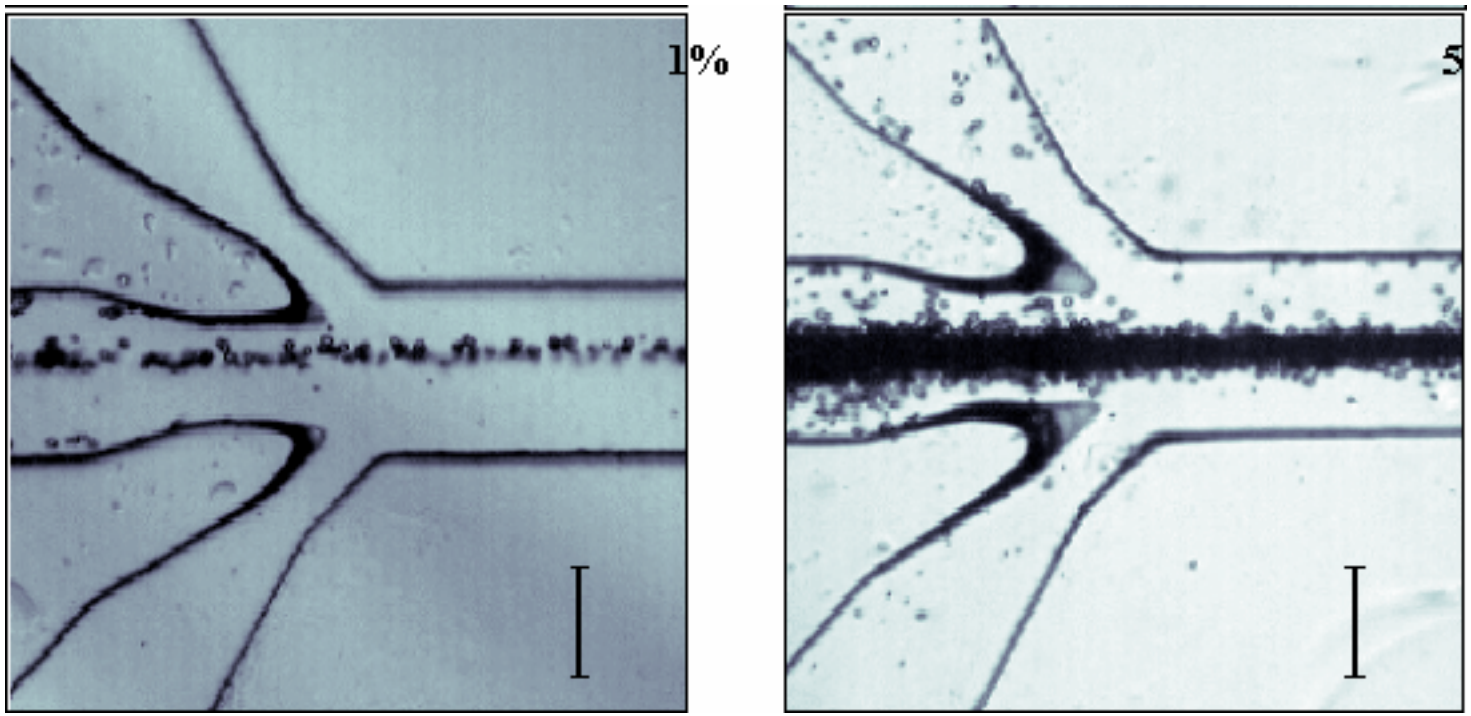
3. One can conclude from the data that ***K(Q)*** is affected by scattering of ultrasonic waves on particles particularly at higher concentrations that reduces the separation effectiveness.

Using three-stage channel ***K=3900*** at ***Q=162nl/s*** was achieved.

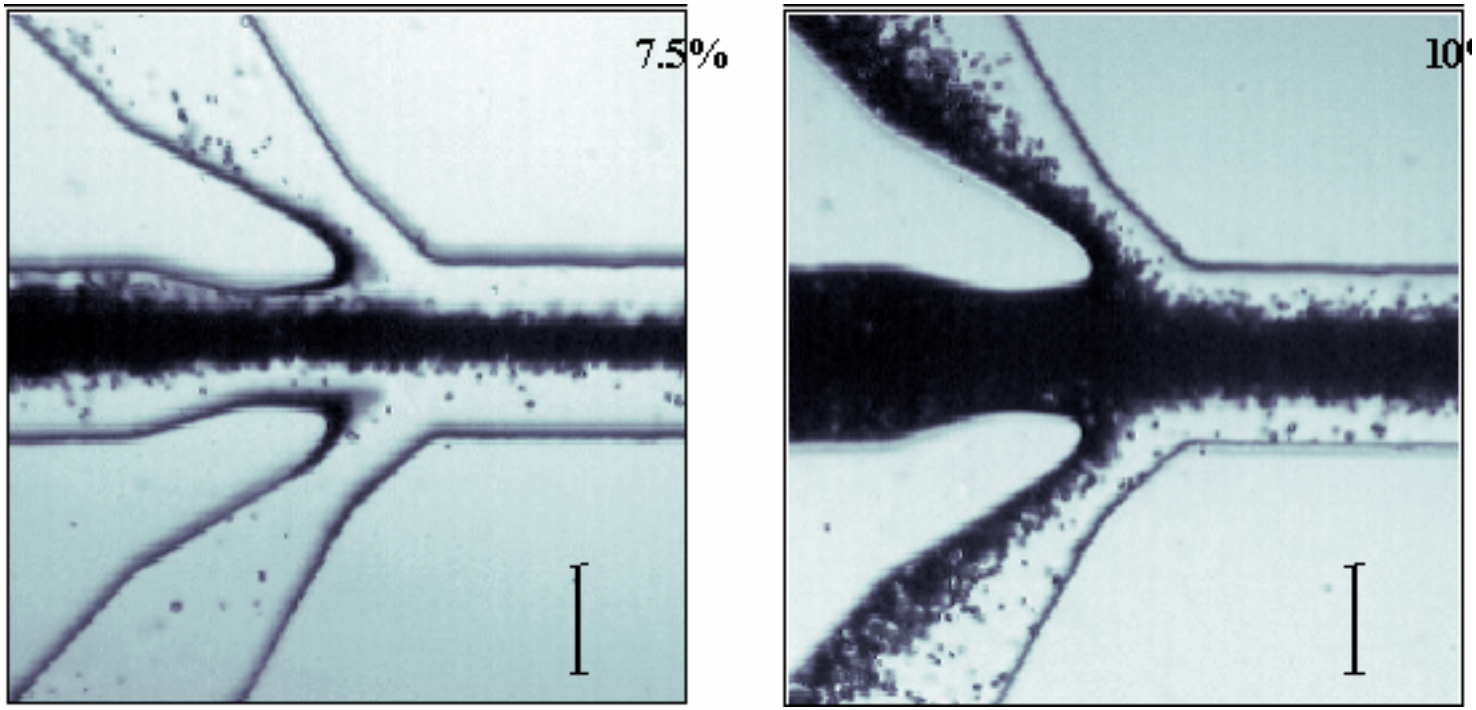

Dependence of K(Q) for solutions of particles with different concentrations of $10\,\mu m$ particles.
0,33%
0,5%
1%
5%
7,5%
10%
K
Q. nl/s
180
160
140
120
100
80
60
40
20
0
40
60
80
100
120
140
160
180
200



Images of particle separation in 0.33% and 0.5% solutions of 10 μm particles by volume. Black line is 100 μm

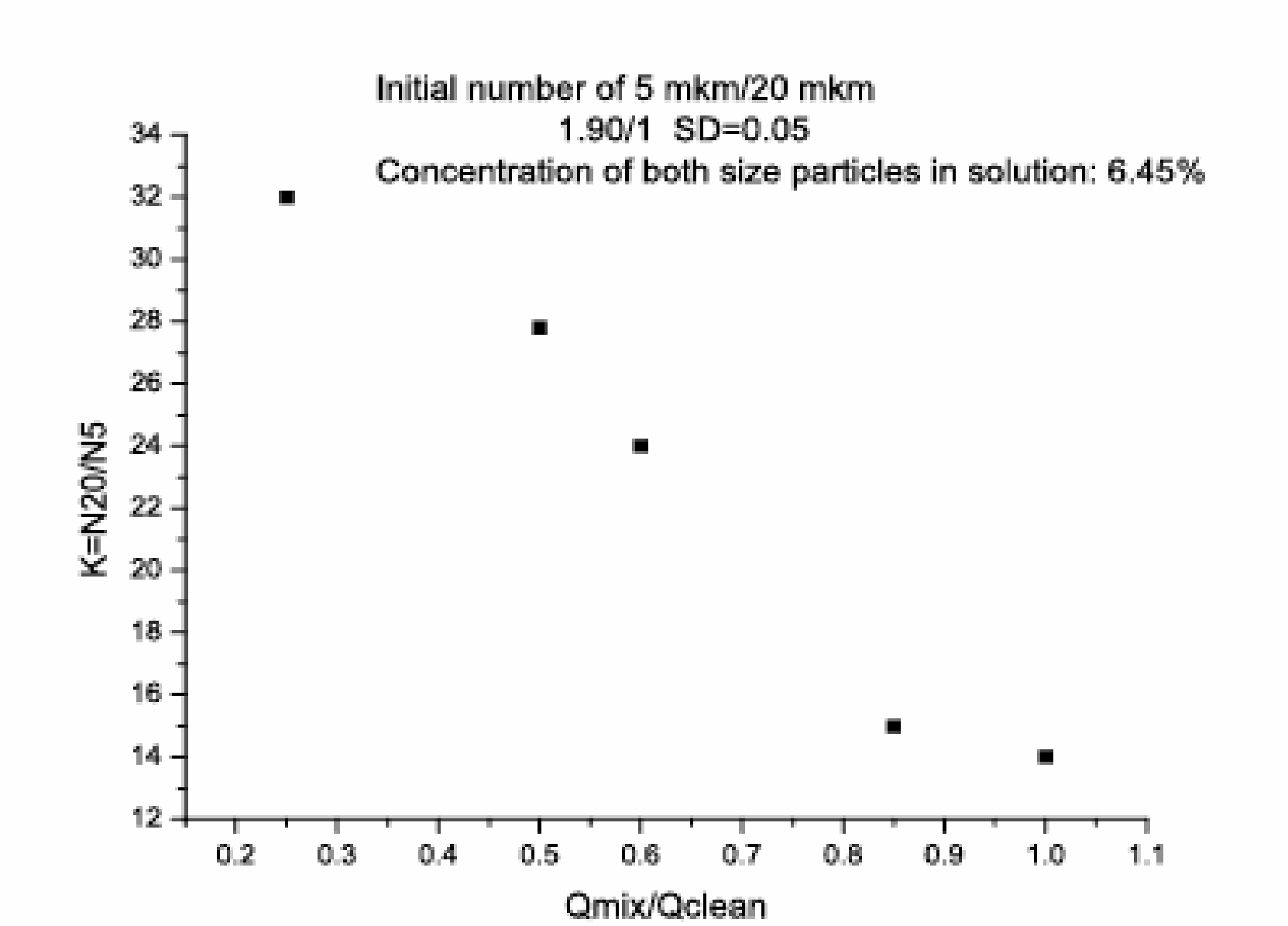


Images of particle separation in 1% and 5% solutions of 10 μm particles by volume. Black line is 100 μm



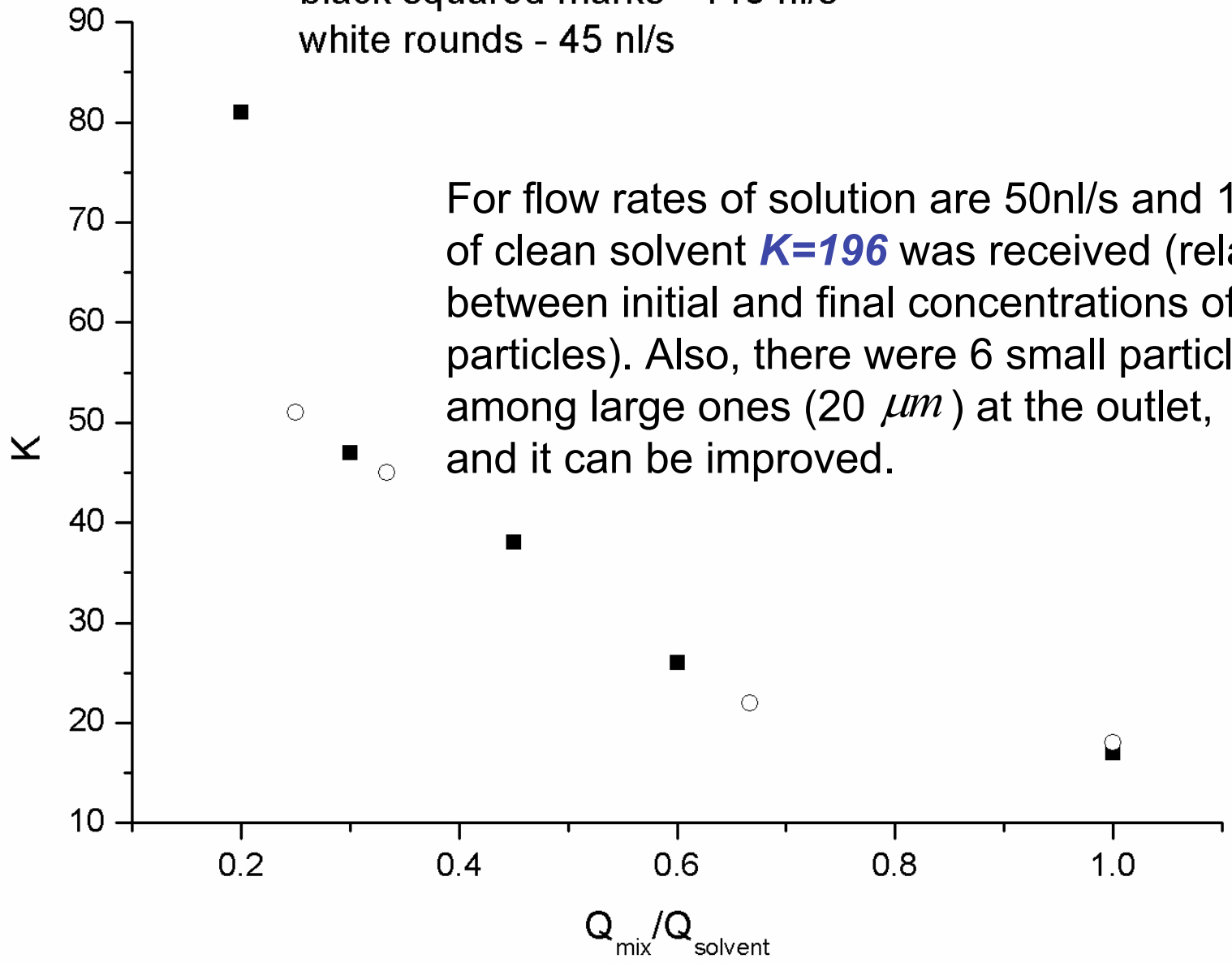
Images of particle separation in 1% and 5% solutions of 10 μm particles by volume. Black line is 100 μm

First results are obtained on size sorting.



Another experiment on size sorting

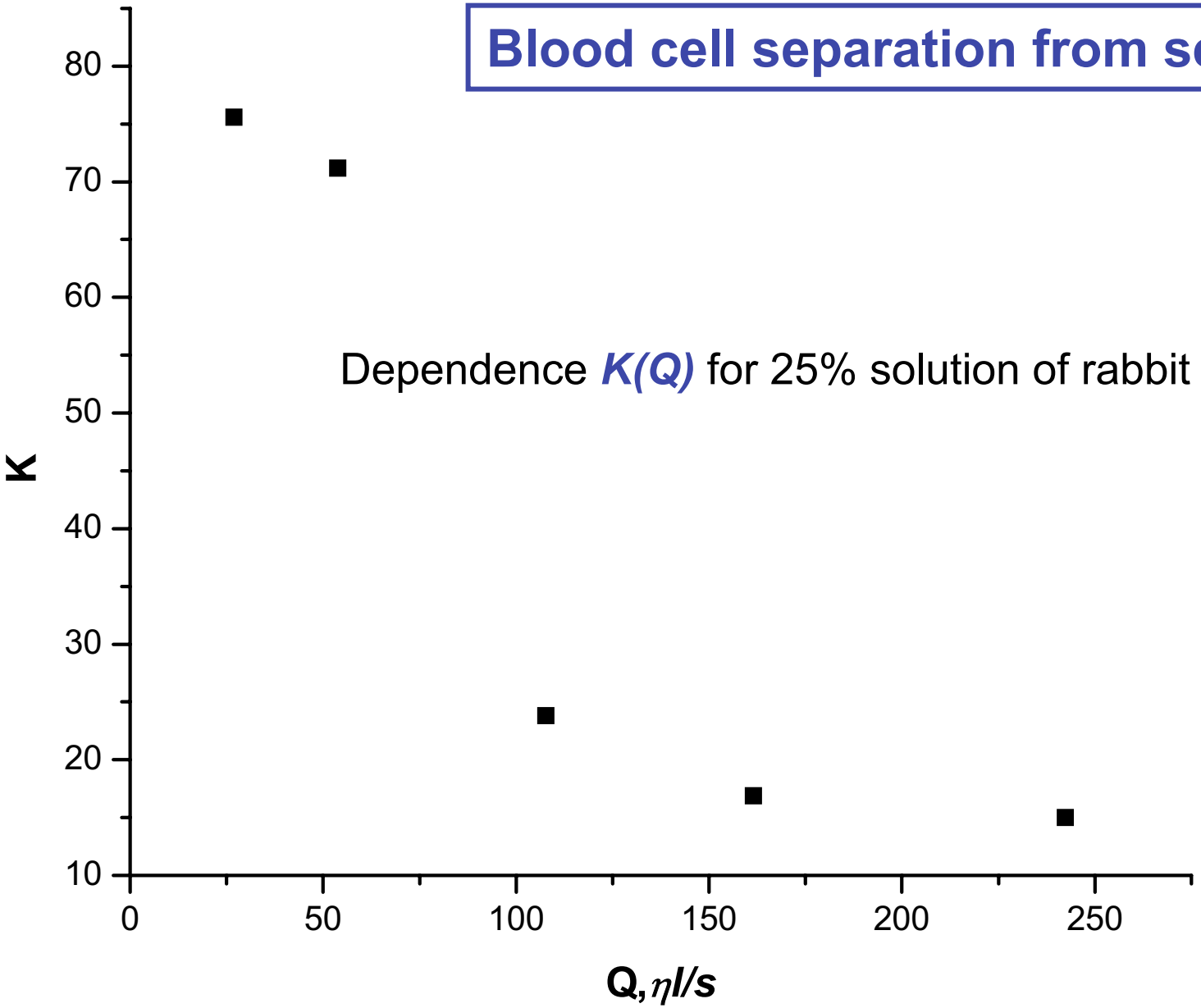
K for both flow rates of clean solution:
black squared marks - 145 nl/s
white rounds - 45 nl/s



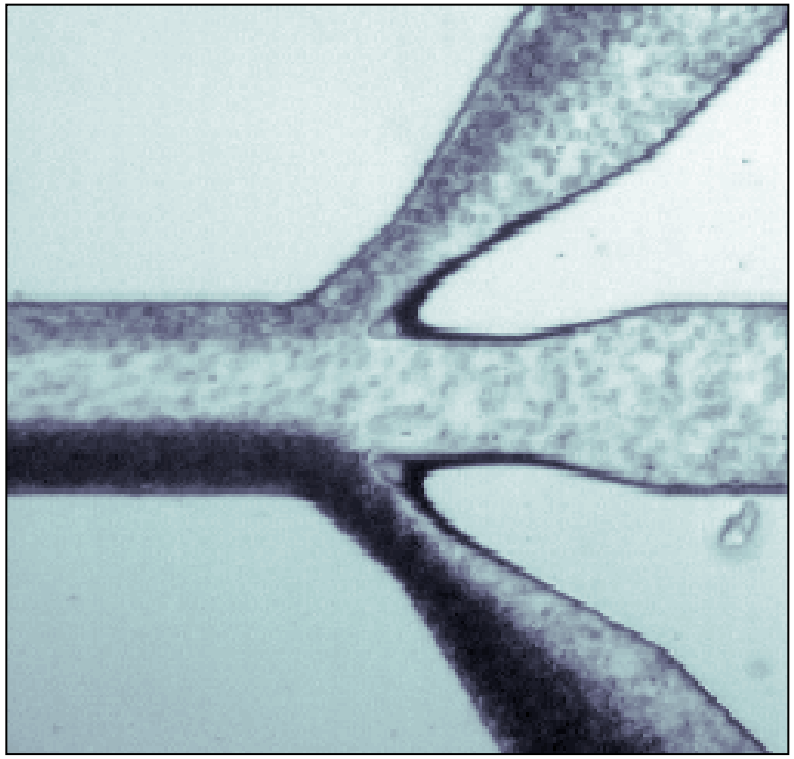
For flow rates of solution are 50nl/s and 150nl/s of clean solvent ***K=196*** was received (relation between initial and final concentrations of large particles). Also, there were 6 small particles (5 μm) among large ones (20 μm) at the outlet, and it can be improved.

Blood cell separation from serum

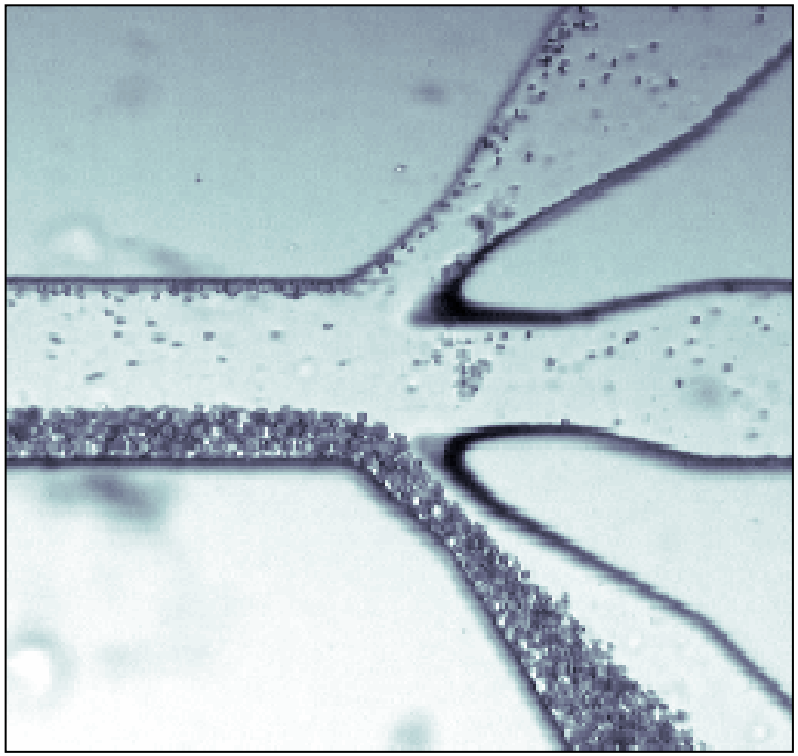
Dependence ***K(Q)*** for 25% solution of rabbit blood in PBS



Images of separation of blood cells from the serum in the three-stage channel



First stage of separation



Second stage of separation

Conclusions

- High frequency ultrasound standing waves can be used effectively for particle trapping, separation and size sorting as well
- Feasibility of the method in microfluidic channel flow was shown and rather high separation ratio was achieved
- It was also demonstrated the possibility to use the technique for cell blood separation from the serum