# Subcellular fractionation in a fluidic microsystem

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**Motivation:**
The availability of an easy-to-use, reproducible method for subcellular fractionation would significantly promote qualitative and quantitative analysis of subcellular protein composition (1, 2). Procedures currently employed are tedious, hampered by low yield or will not provide sufficiently pure organelle preparations.

**Objective:**
The goal of this study (*microPrep*) therefore consists in the fabrication of a micro device capable of dielectrophoretic separation of different types of organelles from a complex cell homogenate.

**Results:**
Design and fabrication methods as well as finished microsystems for the microPrep chip will be discussed. Microchannels contain deflector electrode arrays generating inhomogeneous electric fields (3). Particles such as organelles moving through the channels are polarized and experience dielectrophoretic forces whose magnitude depends on their size and dielectric properties. The small size of organelle of typically less than 1 µm requires both high field strength and a large degree of inhomogeneity and consequently small electrode separation in the order of 10 – 20 µm.

Cells were stained using MitoTracker Green or JC 1 dye and subsequently lysed by douncing resulting in a suspension with intact mitochondria. After removal of larger debris by low g centrifugation, the sample containing protein and mitochondria was pumped through the microsystem at rates of 1 – 200 µl/h. By variation of flow velocity, voltage amplitude and frequency, optimum parameters for manipulation of mitochondria were determined. Separation at frequencies ranging from 100 - 1000 kHz using a voltage amplitude of 10V at a channel height of 10µm was successfully demonstrated. Mitochondria move along the edge of a deflector array and are collected in the sample outlet.

References:

1. H. Lu, S. Gaudet, M. A. Schmidt, K. F. Jensen, Anal.Chem. 76, 5705-5712 (2004).

2. S. Brunet et al., Trends in Biotechnology 13, 829-637 (2003).

3. J. Kentsch et al., IEE Proc.-Nanobiotechnol. 150, 82-89 (2003).

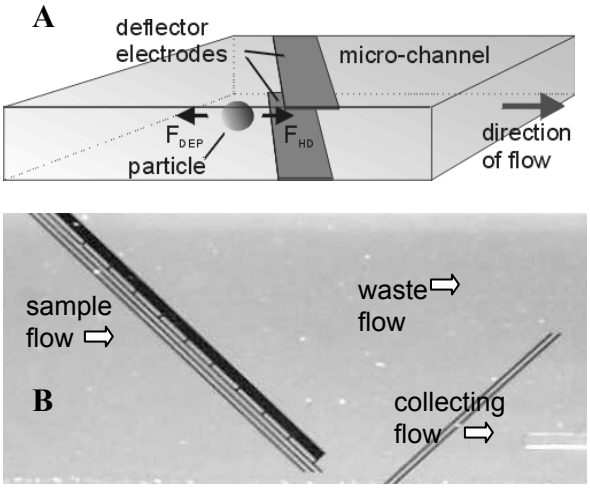


**Fig 1 A)** dielectrophoretic deflector array. Separation is achieved by a competition between hydrodynamic friction forces and dielectrophoretic forces. **B)** dielectrophoretic bandpass structure (shown through channel top face): the particle suspension flows from left to right in between the electrodes which are deposited on top and bottom face of the channel. Larger particles are deflected along the edge of the electrode whereas small particles penetrate the dielectrophoretic barrier. Two sequentially arranged deflectors allow for retrieval of any particle fraction from a mixture.

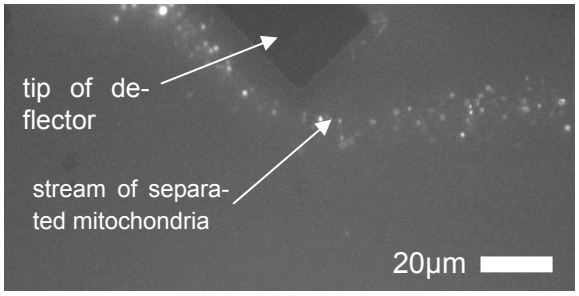


**Fig 2:** Mitochondria flow along the electrode and may be collected in the sample outlet of the microdevice.

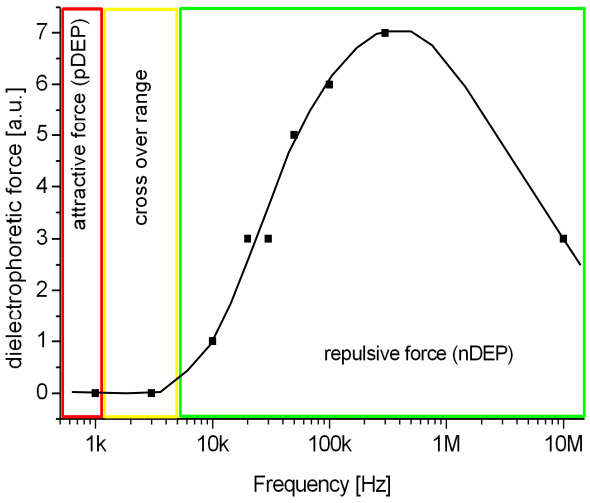


**Fig 3:** dielectrophoretic force acting on mitochondria in a saccharose/ HEPES buffer. At a frequency of approx. 300 kHz, the force is maximum repellent for this particle / buffer combination.


NSTI-Nanotech 2008, www.nsti.org, ISBN 978-1-4200-8504-4 Vol. 2