



Isolating Adherent Single Cells – The Taming Of The Shrew

Dr. Chris Wetzel

MMI microscopic Single Cell Isolation
Eching/Munich



www.molecular-machines.com

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Introduction
who we are

MMI - the Company

Microscopic Single Cell Isolation

- Established: 1998 (1992 S+L GmbH)
- Headquarters: MMI GmbH, Germany
- Branches: MMI Inc., USA
- MMI Hong Kong
- MMI AG, Switzerland
- Represented in more than 65 countries
- More then 500 customer installations





MMI Product Range

mmi CellCut

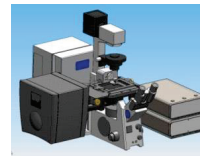
mmi CellManipulator



mmi CellEctor



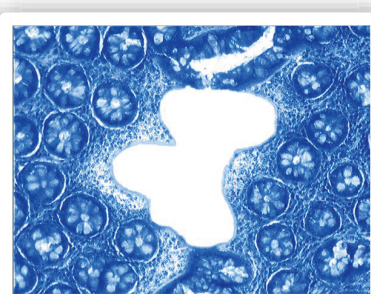
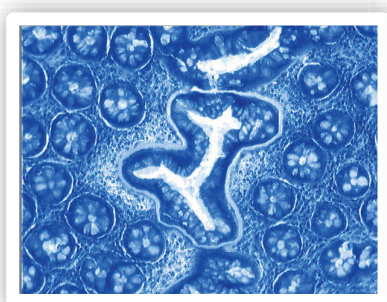
mmi CustomSolution



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MMI CellCut Laser Microdissection

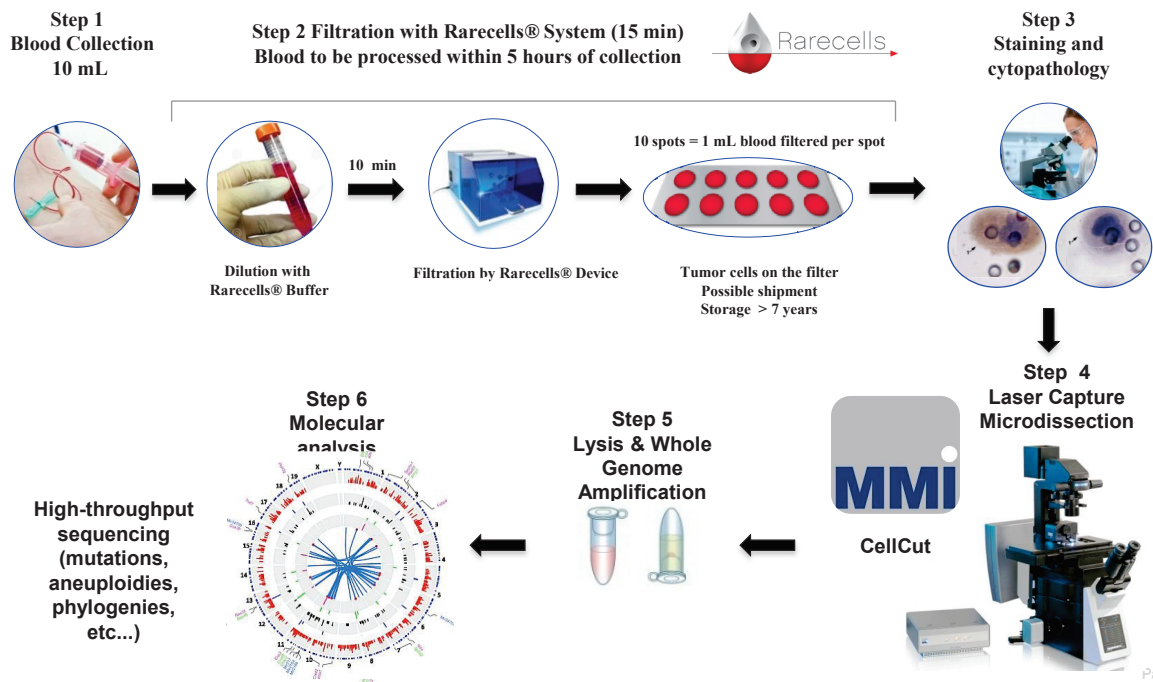


Clear-cut Laser Microdissection

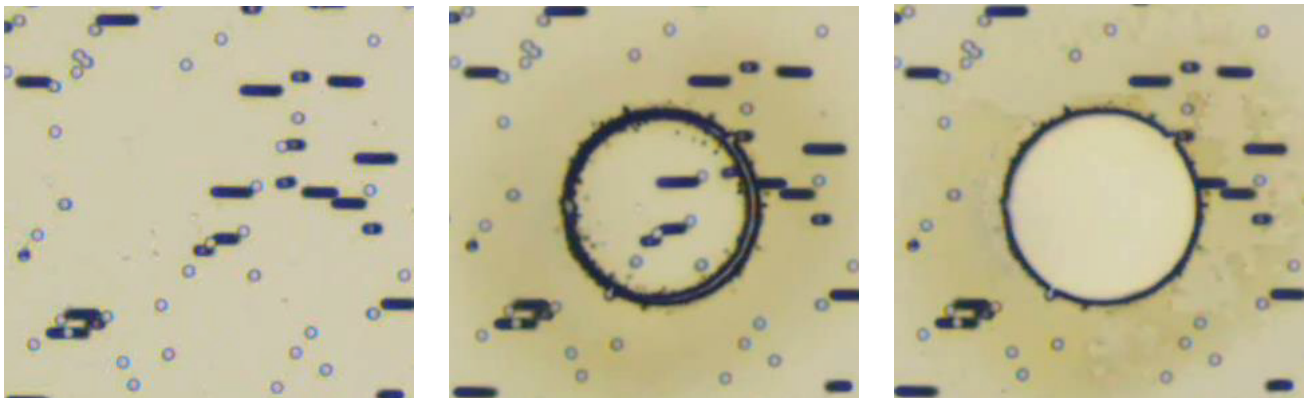
- excise cell clusters, single cells & subcellular compartments with sub-micron precision
- dissect various types of tissues
- inspect all steps during contamination-free & gentle sample capture

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Characterization of the genetic profile of individual Circulating Rare Cells



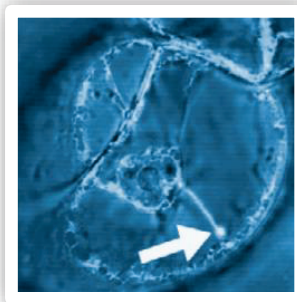
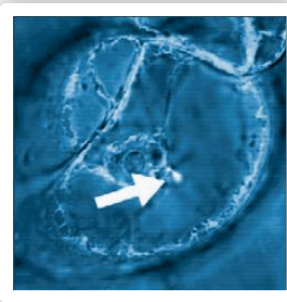
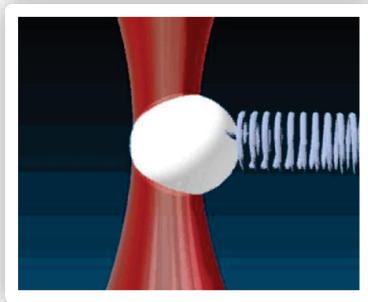
Laser microdissection of cell on ISET filter



→ See presentation of Prof. Paterlini-Bréchet



MMI CellManipulator (optical tweezer)



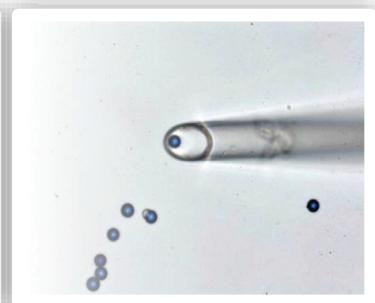
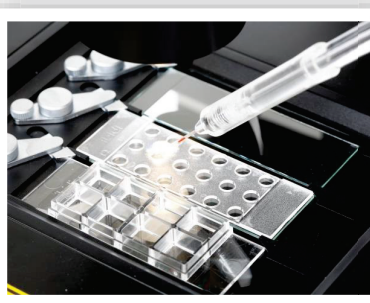
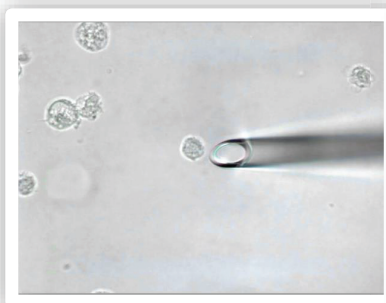
Precise & Contact-free Micromanipulation

- manipulate up to 20 microparticles & living cells
- create strongest inter- & intra-cellular forces
- force spectroscopy with automatic calibration

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MMI CellEctor



Selective Isolation of Single Cells

- specifically isolate single cells in nanoliter volumes
- collect cells with intuitive point & click tool
- pick & deposit cells with full visual control

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Workflow for Capillary Cell Sorting



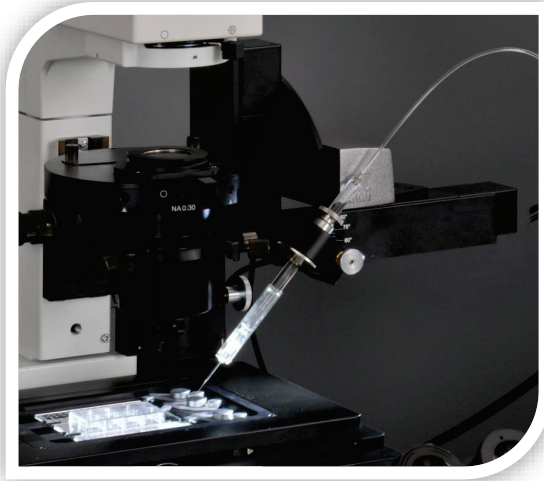
Product Features

MMI CellEctor

- **microscopes:**
 - **motorized**
 - Olympus IX83
 - Nikon Ti-E
 - **manual**
 - Olympus IX53 / IX73
 - Nikon TI-S / U
 - **upright**
 - Nikon NI
 - **Flexibility for other microscope brands**
- **3D - Micromanipulator**
 - with a motorized capillary holder
- **Motorized piston pump**



mmi 3D CellRobot



3D high precision software controlled xyz capillary mover

- Easy swiveling to grant full microscope stage access
- Capillary positioner to adjust and switch between hoover and scratching position
- Fully automatic software driven or manual controlled by joystick

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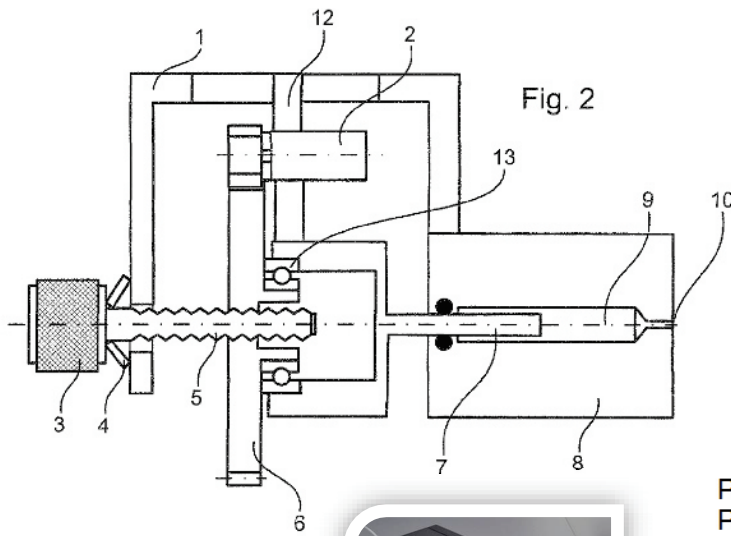
mmi CellPump

High precision, software controlled, piston and transparent pump head

- +/- 2 nanoliter resolution
- Easy controll of oil flow, air bubbles, plus adaptation for quick flush or adding second pump
- Additional manual pump control for quick refill or flush
- Modular design stackable with mmi controler



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Patented
Precision 2 nl
Manual and motorized control
Up to 60 nl/ s

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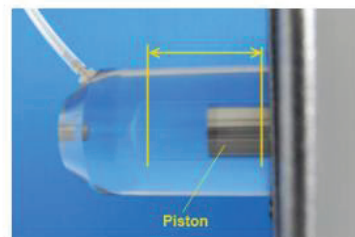


Figure 6.13: The mmi CellPump allows to pump the shown range.

Figure 6.12: The mmi CellPump allows to pump in and out by manually turning the rotation knob.

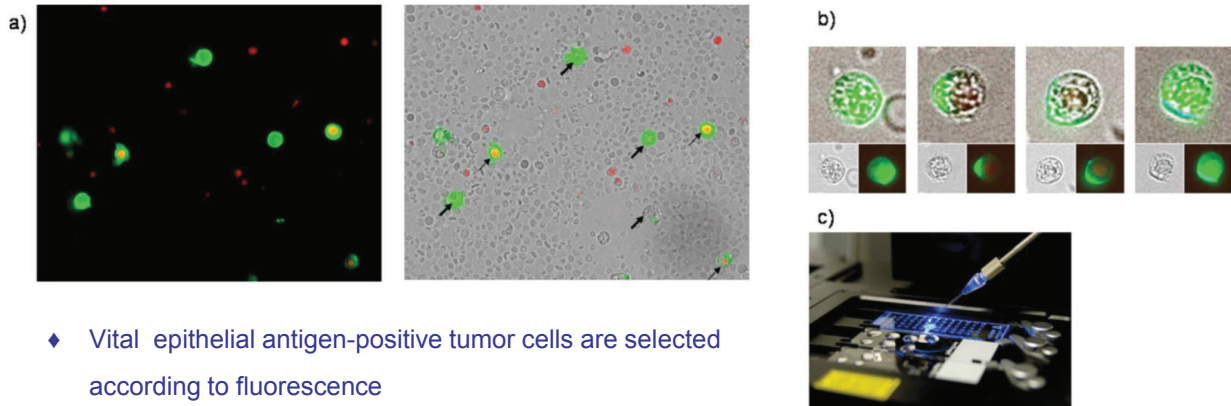
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www.nature.com/scientificreports

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CellEctor – Isolating Circulating Tumor Cells



- ♦ Vital epithelial antigen-positive tumor cells are selected according to fluorescence
 - green: epithelial antigen-positive
 - red: avital
- ♦ semi-automated aspiration of individual cells from cell suspensions and deposition into individual wells

Pizon *et al.*, *ecancermedicalsecience*, 2013

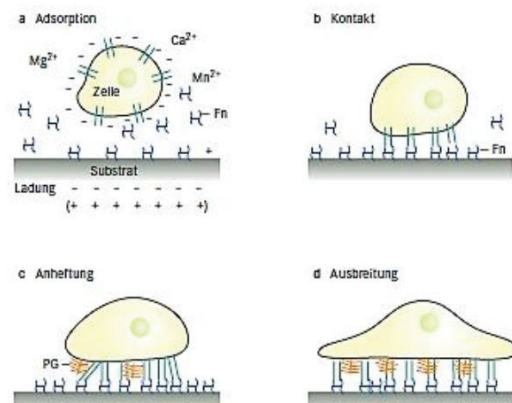
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What to do with adherent cells?

R&D strategy:

- Beads
 - Pumping parameters
 - Pick-up angle
 - Volumes
 - Dwell times
- HeLa cells
 - Pumping parameters
 - Dwell times
 - Vitality rates

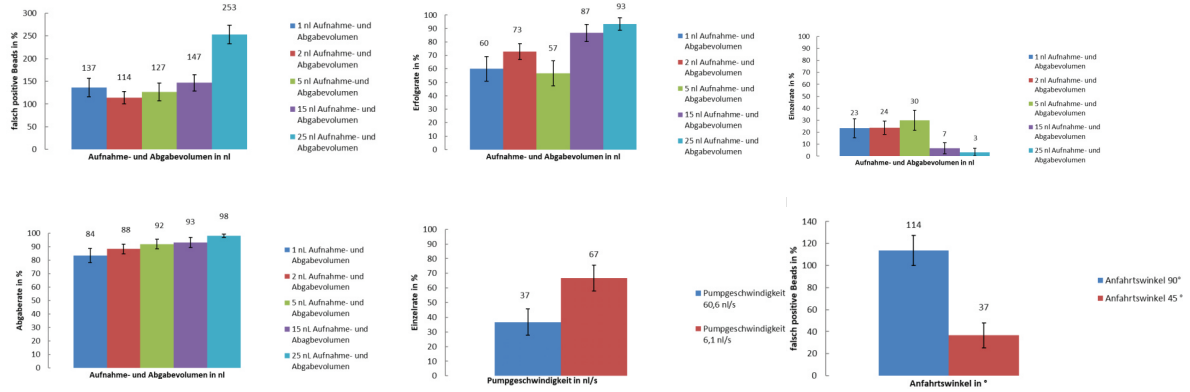


Gstraunthaler, G., & Lindl, T. (2013). *Zell-und Gewebekultur*. S.53-5

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Adherent Beads



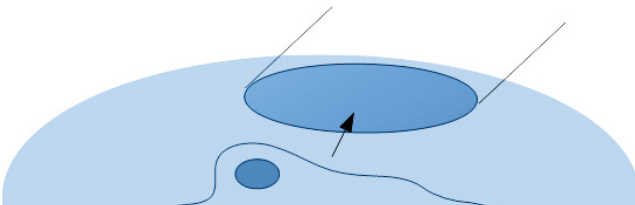
- Advantage of low pump volume, low angle, low dwell time
- BUT MORE IS NEEDED THAN JUST OPTIMIZING PUMP PARAMETERS**

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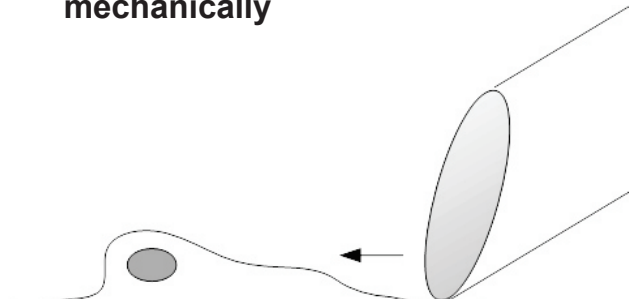


2 ways to detach cells

chemically



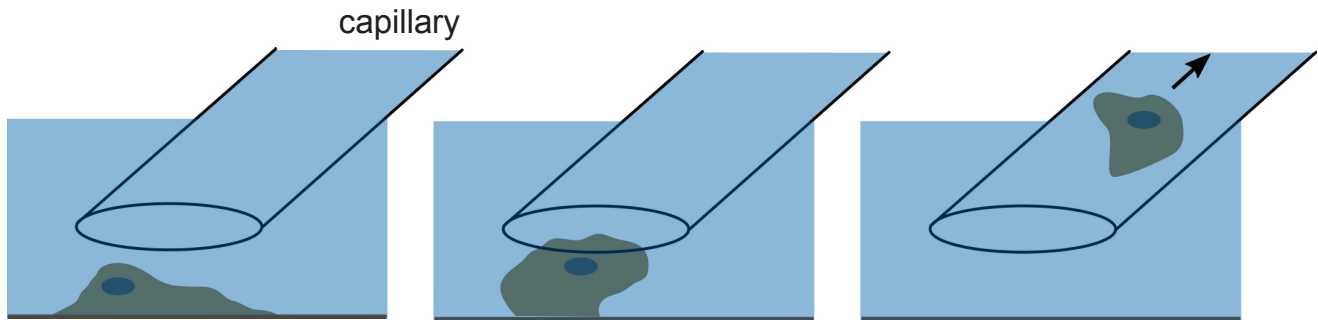
mechanically



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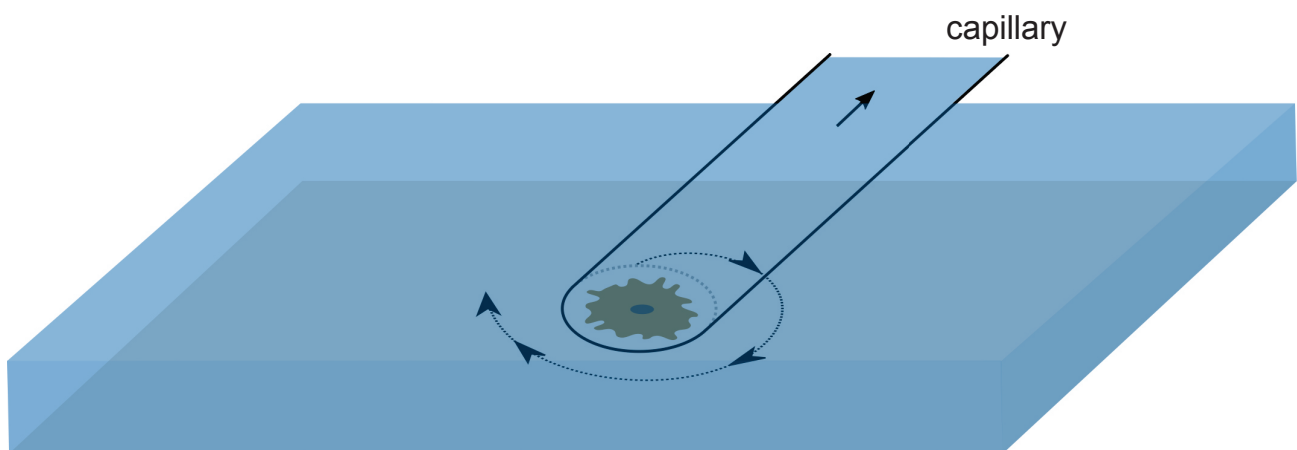
Using lysis buffer



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MMI developed 'shaking mode'



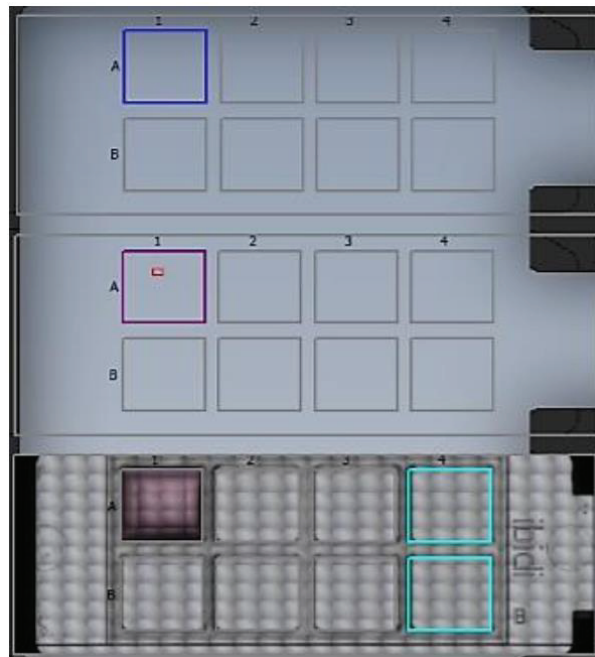
Microscope stage

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Transfer of HeLa cells



Aspiration well

Deposit well

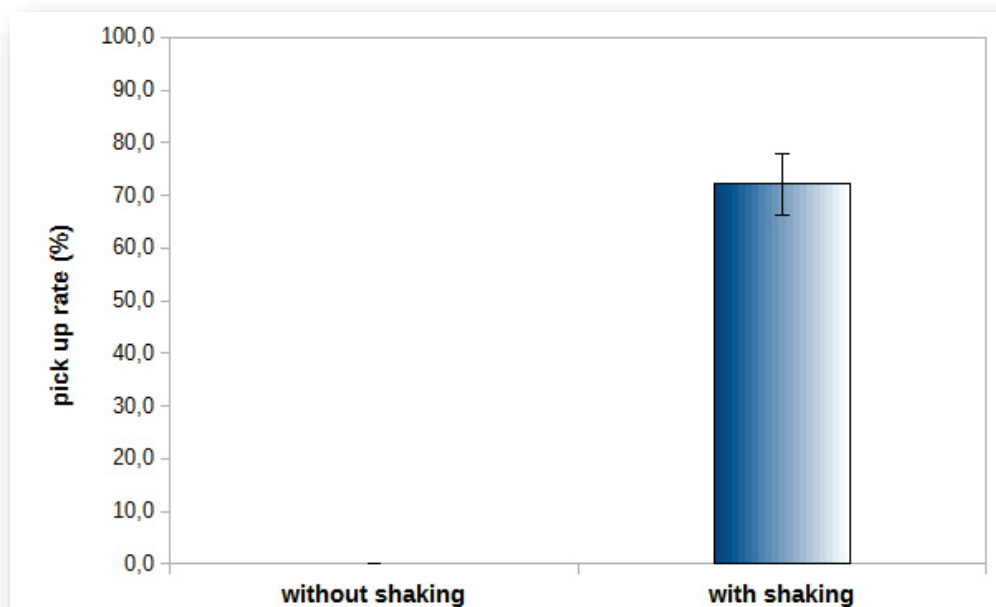
Rinsing well

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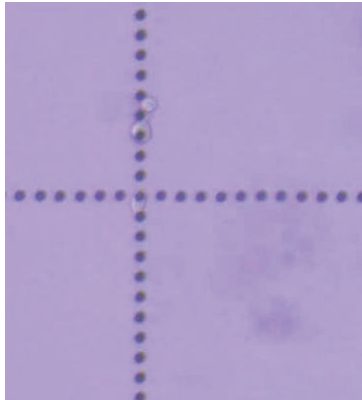
MMI

Pick up rate

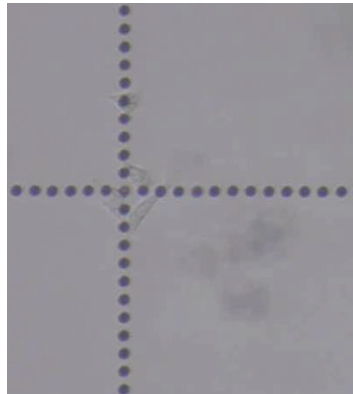




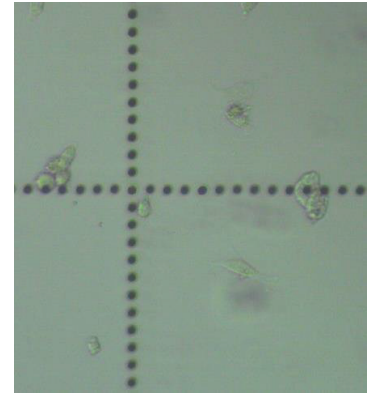
Vitality of transferred cells: proliferation



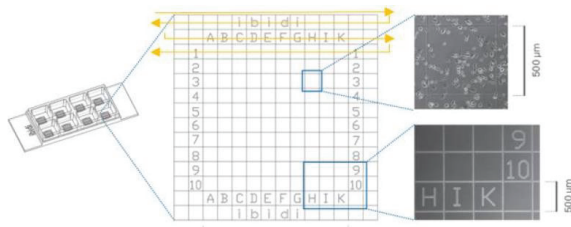
day 1



day 2



day 5



Sandra Lubos, B.S. Thesis

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Thank you for your attention!

Dr. Chris Wetzel

- Sandra Lubos
- Dr. Nils Körber

MMI
Breslauer Strasse 2,
D - 85386 Eching, Germany
Phone: +49 89 319 048 40
Email: wetzel@molecular-machines.com
www.molecular-machines.com

