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PASCA – PLATFORM FOR ADVANCED SINGLE CELL HANDLING AND ANALYSIS

Content

- Why single cells?
- The SCM technology
- Experimental results
- Technical summary
- How did we get there
- Dissemination & Commercialization

SCM prototype instrument

Why Single Cells?

Typically cells are handled in batches of thousands or millions

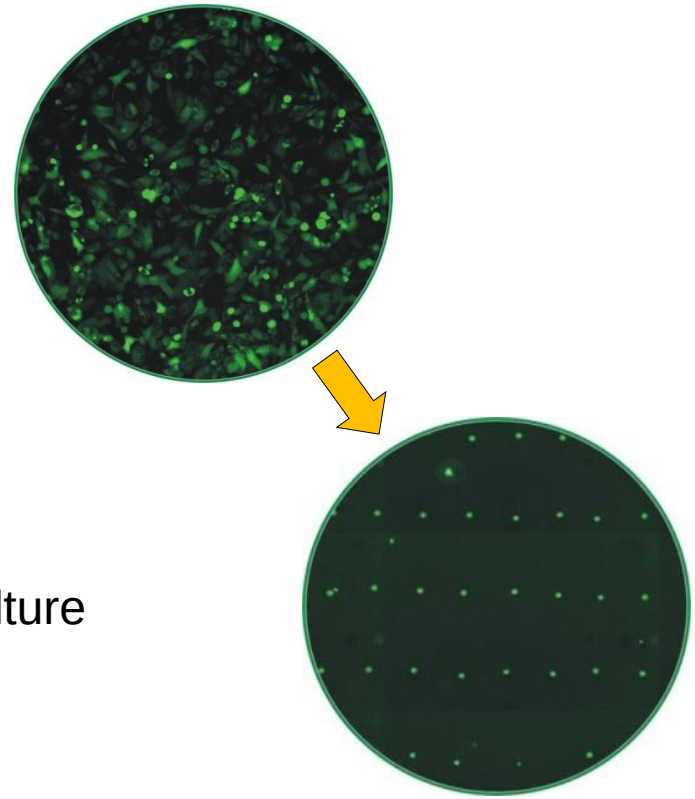
- Dispersed in solutions / co-cultures
- Tissue samples
- Adherent on substrates (Petri-dish, etc.)

Limits of large cell populations

- Cell heterogeneity only allows averaged results
- Cell-cell interactions influence experiments
- Technically difficult to track individual cells in a culture

“Improved single cell methods are helping to unravel biological complexity”

(nature methods | VOL.9 NO.1 | JANUARY 2012 | **35)**

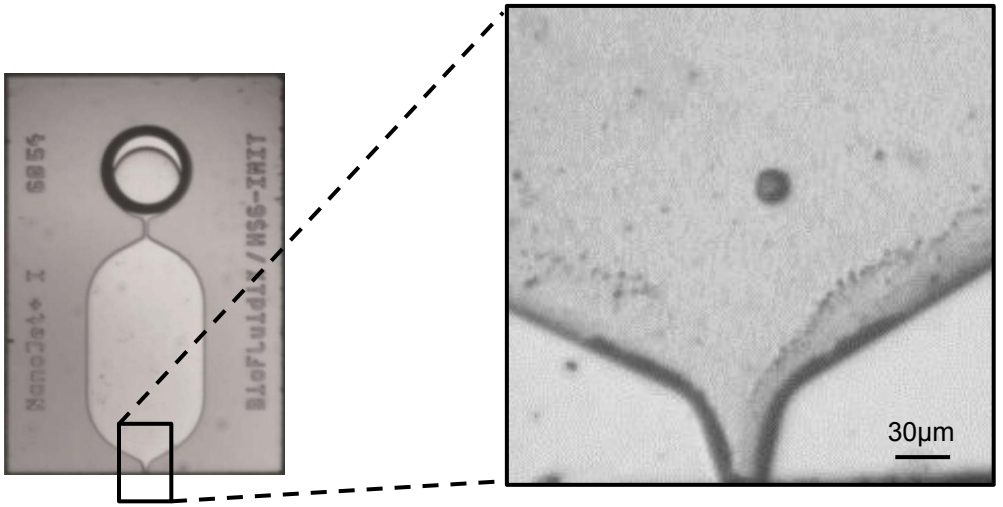


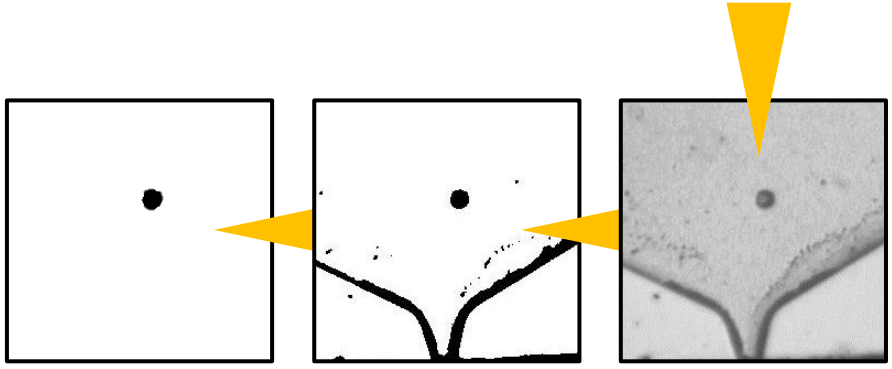
Fluorescent micrograph of a cell culture and an array of single cells

SCM Technology

Single cell printing based on optical imaging

- Transparent micro-chip
  - Direct displacement dosage
- High magnification camera
- Automatic image processing
  - Fast object detection
  - Image storage & retrieval
- ‘Stop n Go’ printing
  - Printing can be stopped and continued any time
  - No loss of cells during stop

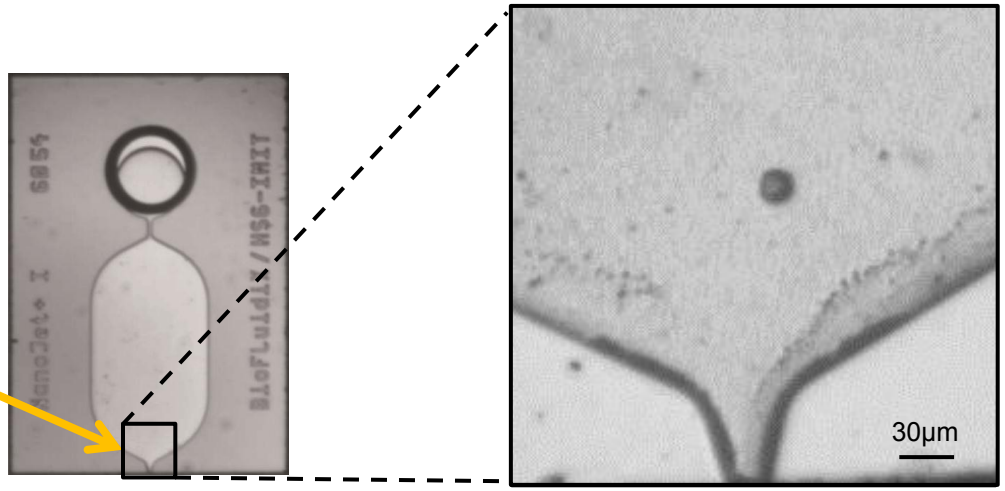


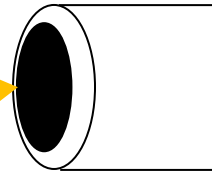


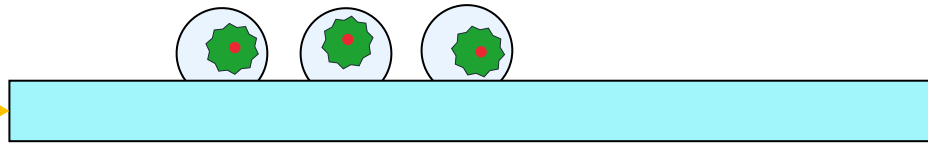
SCM Technology

Working principle

- Camera
  - Images nozzle
  - One image each droplet
- Object detection algorithm
  - Detects the cells
  - Cell number & properties
- Shutter system
  - Deflects unwanted droplets
- Target substrate



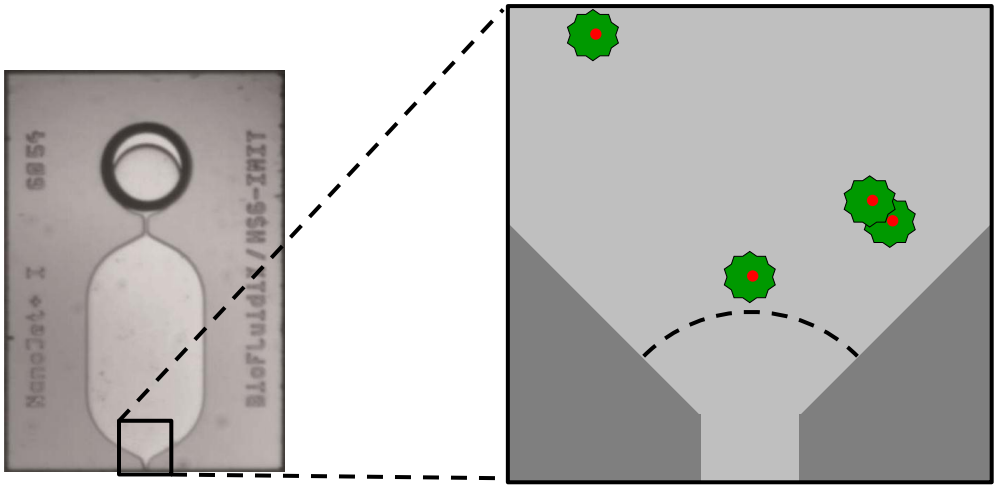


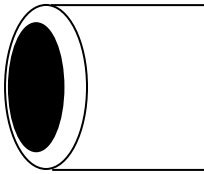


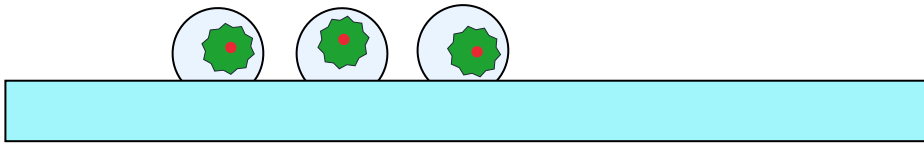
SCM Technology

Working principle

- Cells in nozzle: **0**
- Shutter: **ON**



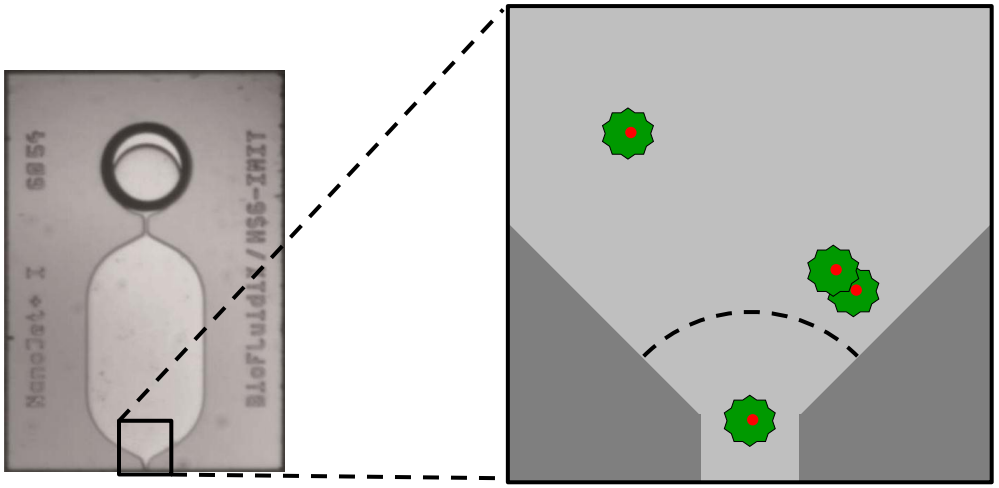


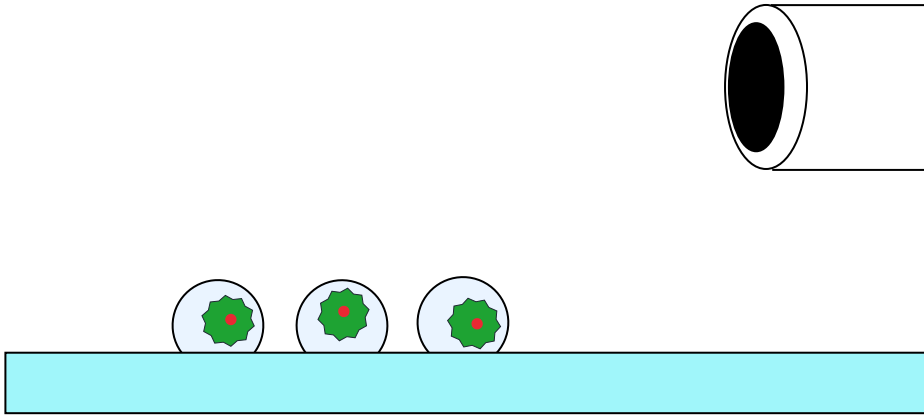


SCM Technology

Working principle

- Cells in nozzle: **1**
- Shutter: **OFF**

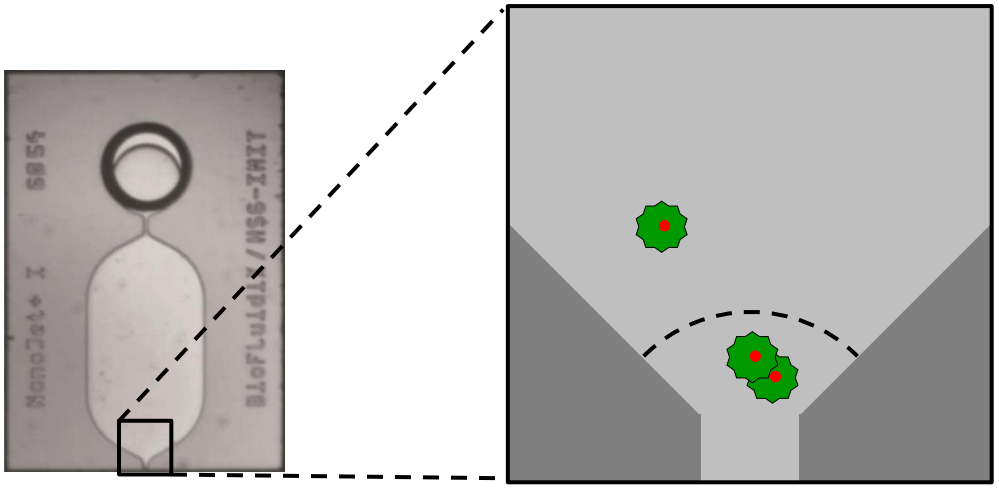


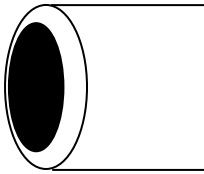


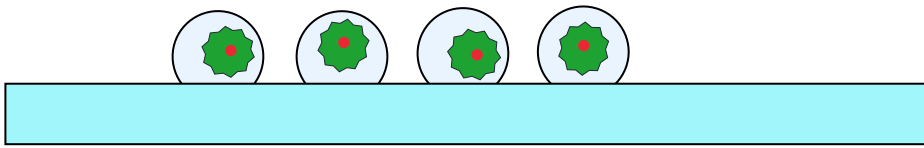
SCM Technology

Working principle

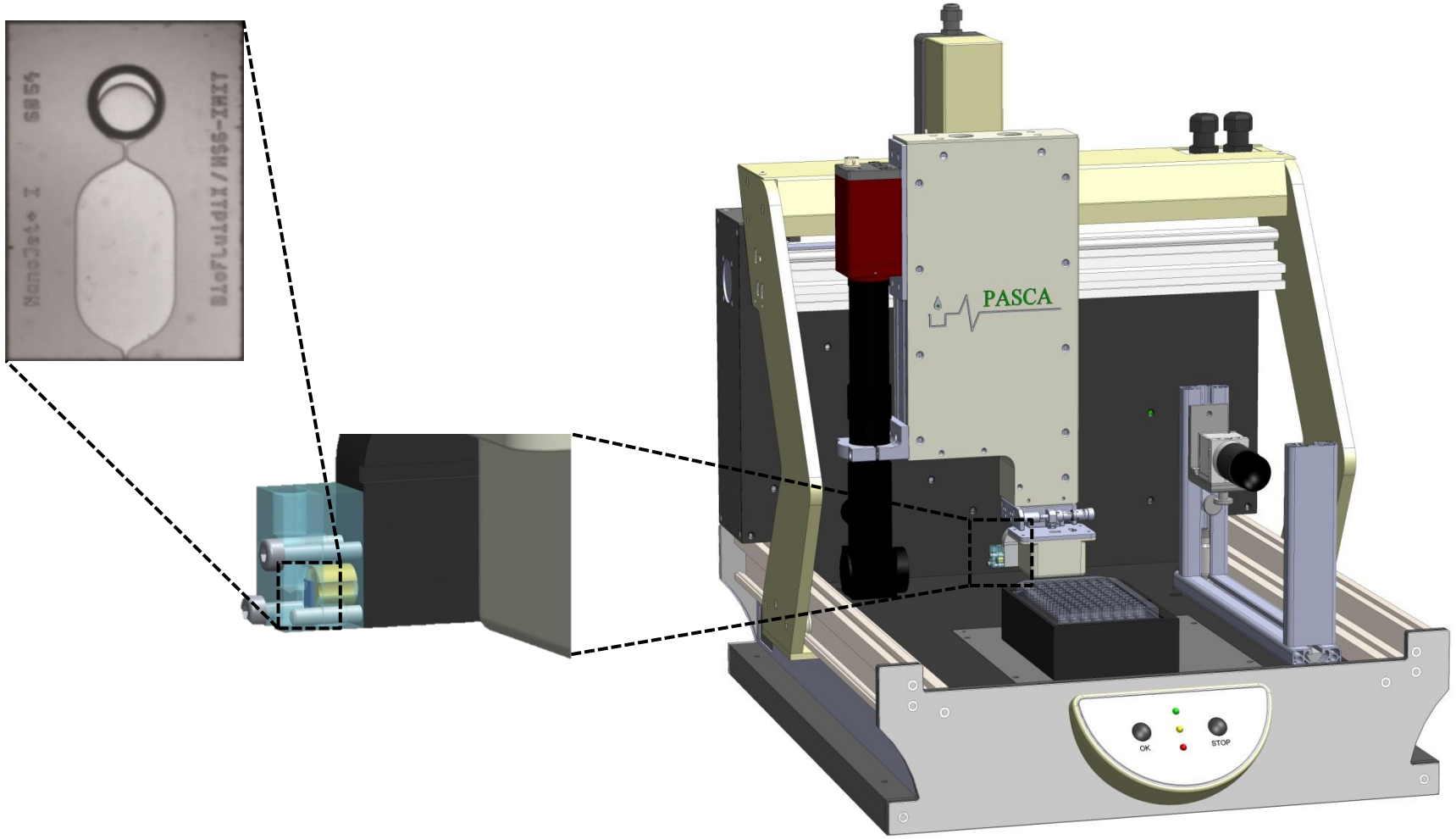
- Cells in nozzle: **2**
- Shutter: **ON**





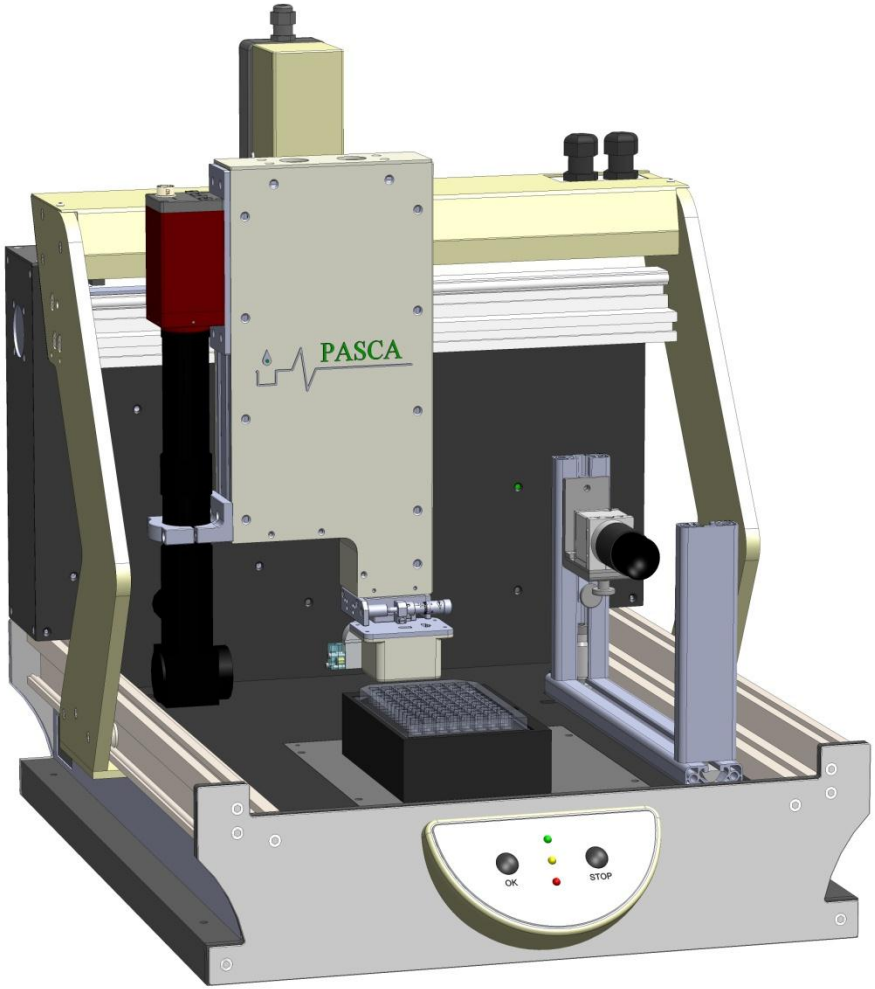


SCM Prototype Overview

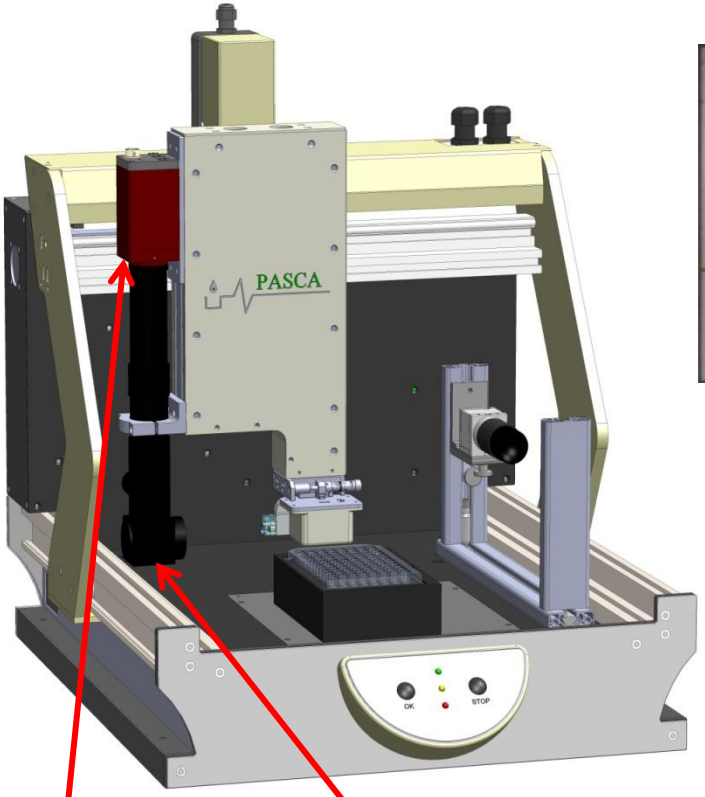


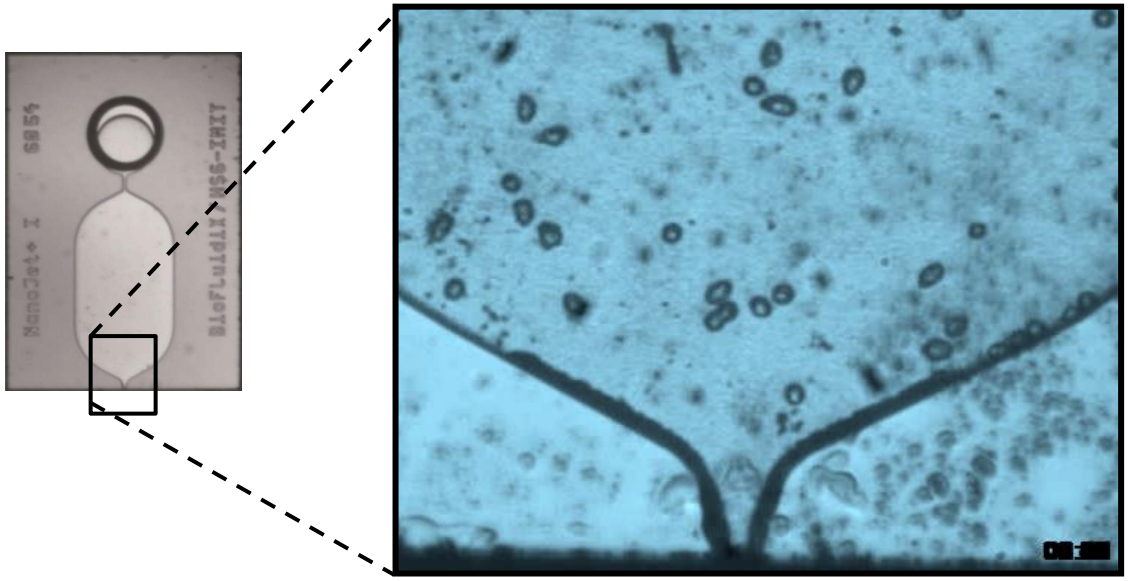
SCM Prototype Overview

- Drop-on-demand printing unit
- Chip camera
- Stroboscopic camera
- Substrate camera
- 3-axes lab robot
- Embedded PC



Cell Detection

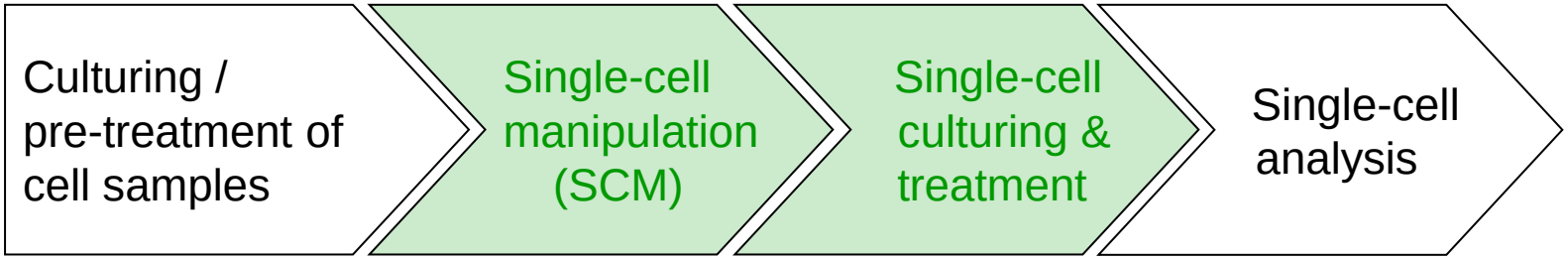




Live printing video of cells inside dispenser chip

- Zoom-optics & camera
- Image cells inside chip
- Live image of each cell
- Automatic image processing

The PASCA Platform



In the PASCA project technologies and workflows are developed that allow detection, separation and individual analysis of single cells

The PASCA platform…

- … is based on the presented SCM technology / instrument
- … is developed and optimized for certain chosen pilot applications
- … integrates into typical workflow of laboratories
- … is modular and extendable

PASCA Applications

- Cell-line optimization
- Pharmaceutical research
- Stem-cell research
- Cancer research
- Tissue engineering / organ models

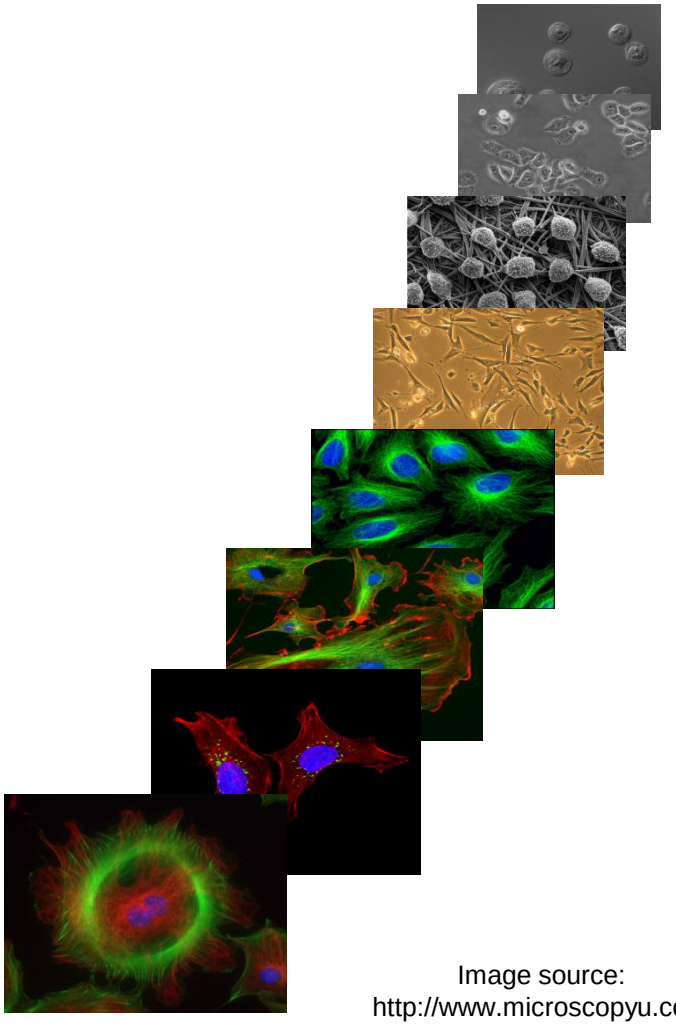
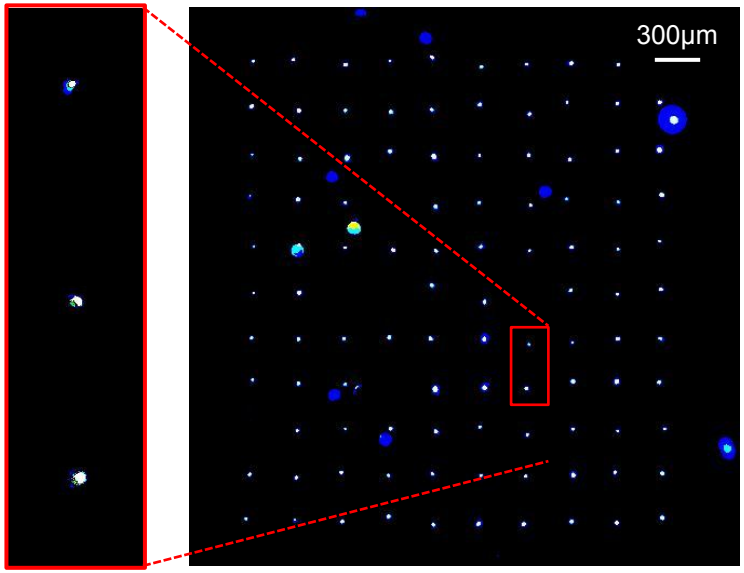


Image source:
http://www.microscopyu.com

Experimental Results

Single micro-beads

- Arrays on microscope slides
- Cell-sized beads (10 µm - 20 µm)



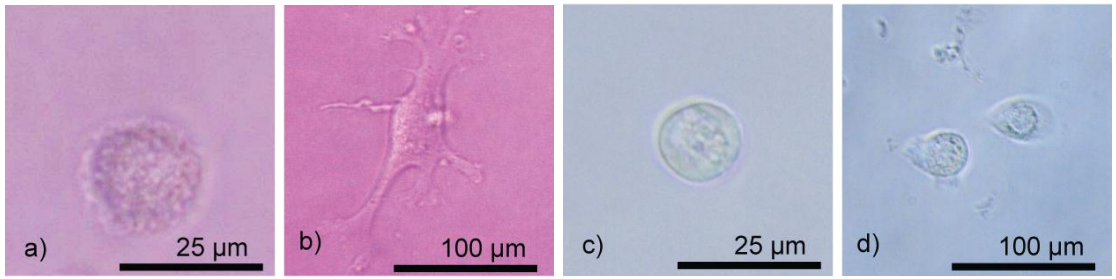
10x10 array of fluorescent Polystyrene beads (10 µm)

Viability of printed cells proofed for

- Yeast, HeLa, CHO, RBL, HEK, …
- Fibroblasts, Keratinocytes

Printed & cultured

- Micro-well plates (96, 384)
- Agar-plates

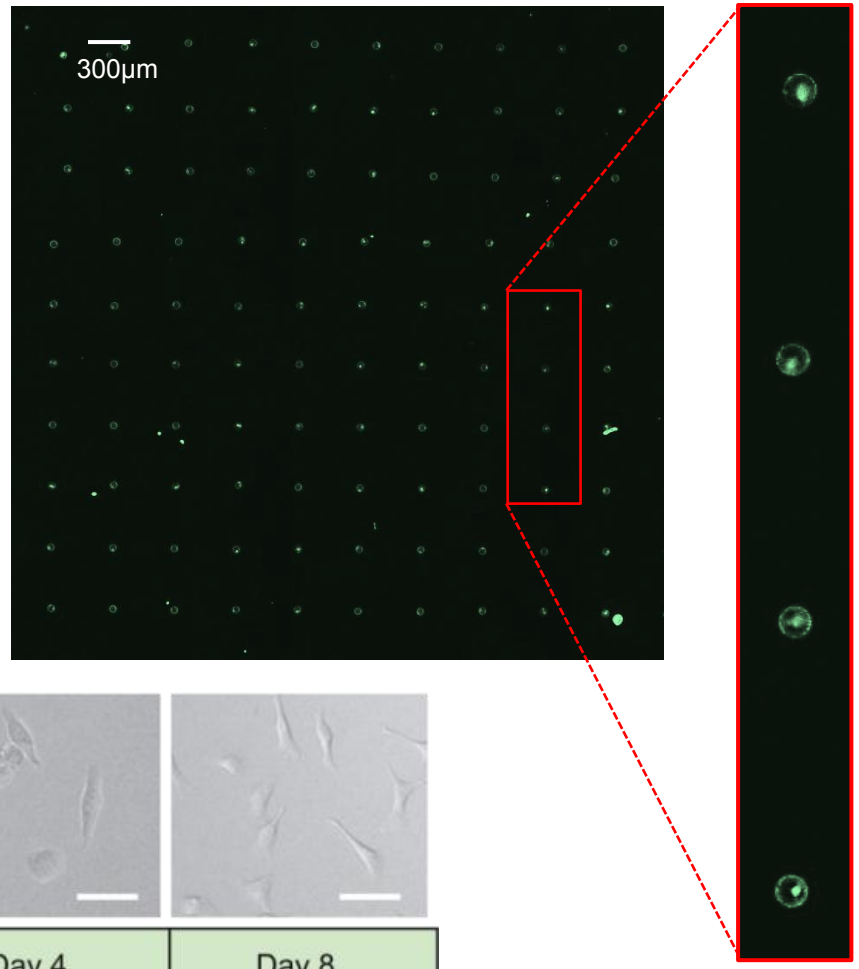


Same single printed, living Fibroblast & Keratinocyte directly after printing (a,c) and after 24 h incubation (b,d) respectively

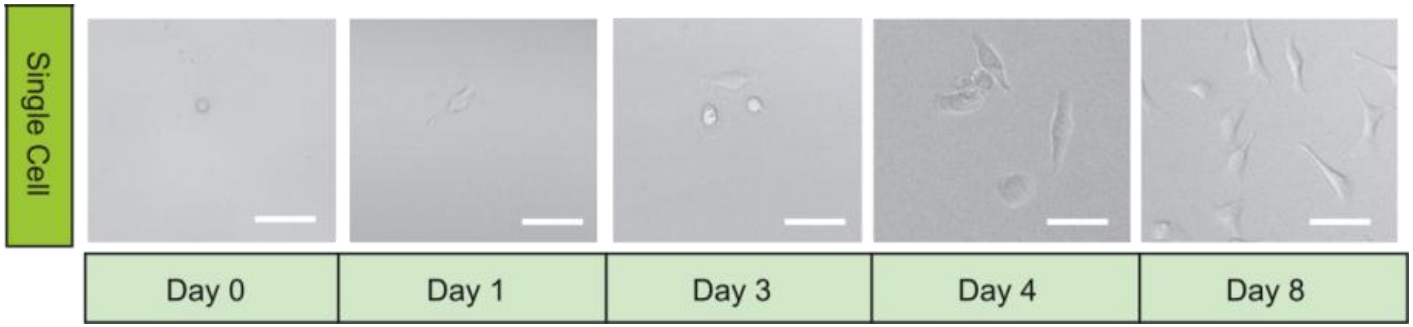
Experimental Results

Single cells

- Fluorescent GFP labeled HeLa cells on microscope slide (printing yield > 80 %)

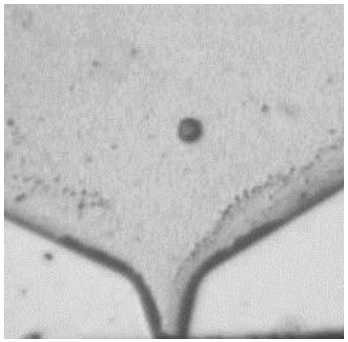


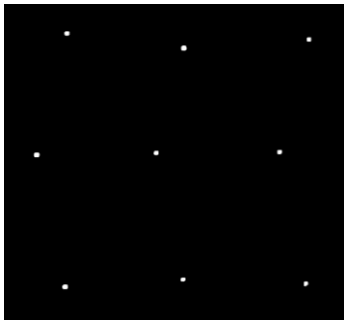
- Untreated HeLa cells directly after printing and 8 days after incubation respectively (printing yield 84 %)



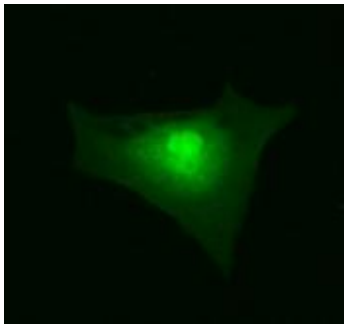
Benefits of SCM Technology

- Drop-on-demand (stop & go) printing
- Small sample volume
- Small dead volume
- Positioning in micro-scale resolution
- Various substrates





- Droplets with single cells only
- Untreated, label-free cells
- Pre- & post-printing images of each single cell
- Each cell can be delivered at any position



Technical Summary

The SCM Technology

- Core of single cell printing platform
- Enabled by label-free optical detection
- Drop-on-demand non-contact printing
- Delivered one year after project launch

Future technical perspectives

- Optical fluorescence detection
- Impedance spectroscopy
- Combine optical, fluorescent and impedance sensor signals for sorting of cells

How did we get there

Delivered five 1st generation prototypes one year after project launch

Only possible due to good project planning and good team spirit

- Formation of sub-teams
- Stringent specifications system
- Prototype design
  - Bases on proven technologies
  - Uses commercially available parts wherever possible

Project Team Structure

Two sub-teams within project

- SCM device team:

- Application team:

- Exchange of specifications / prototypes
- Team managers (BioFluidix/Sophion) control progress & ensure regular communication
- More members can join the applications team during the project

Specification System

Why specs system, why multilevel?

- Biologists “think” and “name” different than engineers

Specification system bases on three levels:

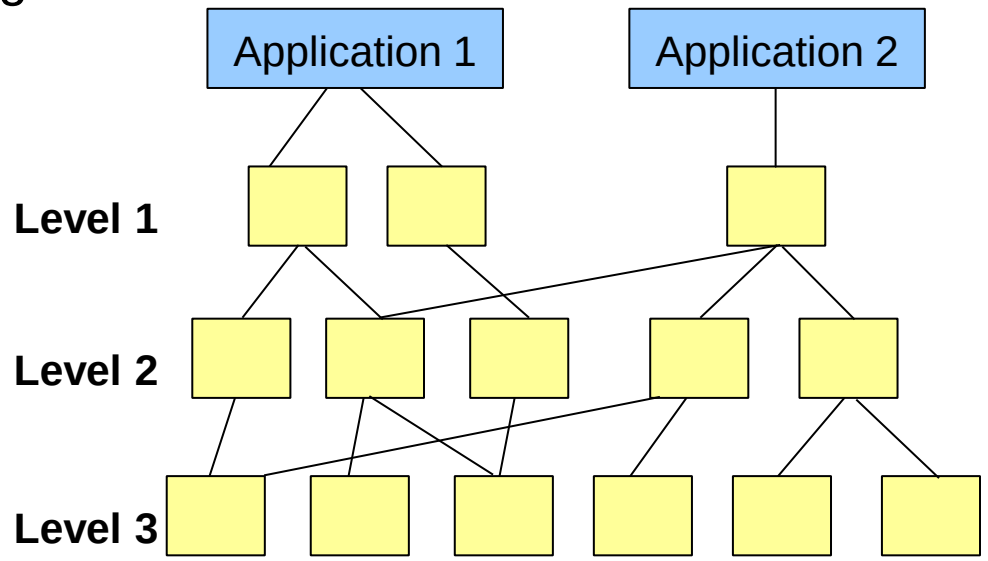
- Level 0: General description of the intended application
- Level 1: Biological characteristics (e.g. 98% of all dispensed cells should survive)
- Level 2: Non-biological measurable characteristics (e.g. droplet size 30±5µm)
- Level 3: Design characteristics (e.g. nozzle diameter 30 µm)

Centralized data storage/parallel editing (Google docs)

Specification System

Specifications tree

- All specifications are linked up to a higher level specification or application
- Fulfillment of all specs can be checked locally or by looking one level up
- All specs are classified according to MuSCow:
  - Must have
  - Should have
  - Could have
  - **Won't have**
- → Easy development process (Engineers don't need to understand all the bio-things)



Dissemination

Making the project visible as early as possible

- Project website www.pasca.eu
- Showcase running device at fairs (SLAS 2012 / Analytica 2012) & conferences

Expand the applications team by pilot-partner program

- Early adopters interested in the technology
- Send us a description of the experiments and intended results
- Can get a SCM prototype or make experiments in our labs
- Large interest, 5 potential partners so far, Yale running experiments

→ Optimize SCM design based on pilot partner input, potential customers

Commercialization Support Actions

COWIN (EU level)

- Helps to develop relationship to partners & investors to establish business case and financing

MicroTEC südwest / Promitis (local level)

- Helps to develop commercialization strategy, business case & partnerships in south-western germany

Market research (HTS-Tec)

- Determine specifications, applications and market size for potential products

Project Summary

All technical project goals could be reached so far

- Team-structure and specifications system helped a lot to realize that

Five first prototypes are available for evaluation inside the project but also for external pilot partners

First dissemination/commercialization approaches on-going