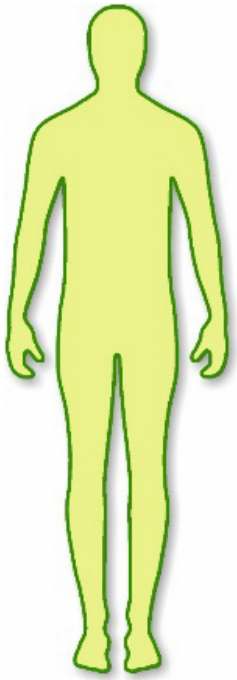
VyCAP

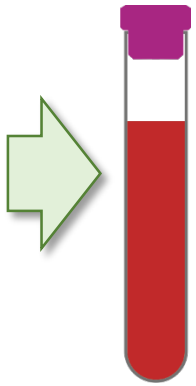
The Puncher platform

Antibody discovery and Cell line generation

NOV 2020

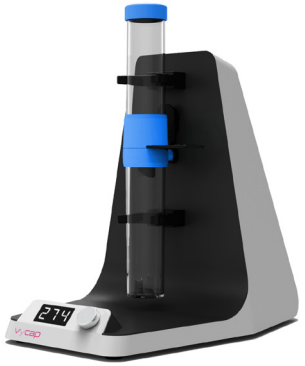
The Puncher Platform for isolation of Circulating Tumor Cells from liquid biopsies



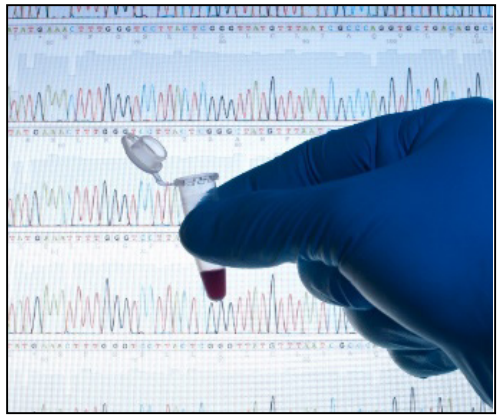


Liquid biopsy
Blood sample

Puncher Platform



Detection and isolation of
Circulating Tumor Cells (CTC)



Genetic fingerprint of the
Tumor Cells

Personalized Precision Therapy

Improved patient care

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The Puncher platform for antibody screening and cell line development

The Puncher platform for antibody screening and cell line development resulted from our expertise on the isolation of Circulating Tumor Cells in combination with new innovative technologies

We provide this Puncher platform to our customers as a **product** and as a **service**.

This gives our customers **large advantages** over existing technologies :

- It allows to screen thousands of single cells on their production of antibodies in days
- It measures the production of antibody at the single cell level
- Images of the cells provide 100% certainty on clonality
- The single cell that produces the right antibody can be isolated for sequencing and culturing

The Puncher platform proved to be a very powerful platform for
- **screening of antibody producing cells**
- **generating cell lines**

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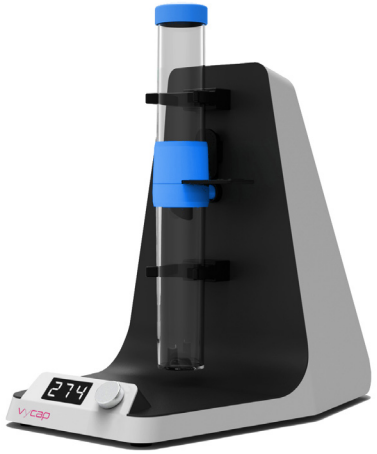
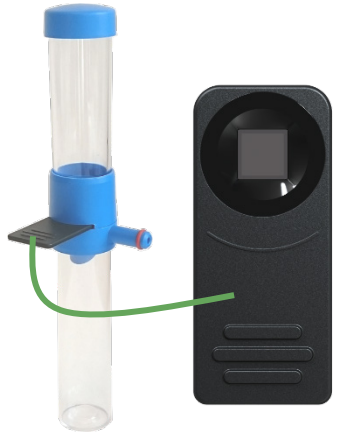
The Puncher platform for antibody screening and cell line development



Pump unit



Disposable Containing our Microwell chip

Clamp unit Collect the antibodies secreted by single cells

Puncher system Isolation of single cells

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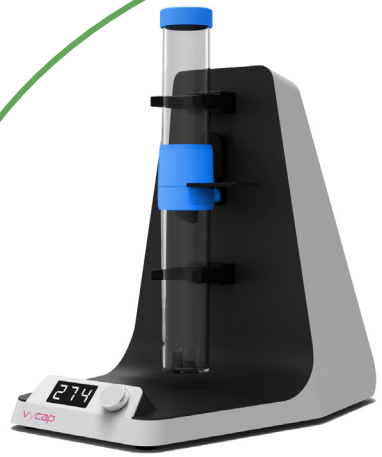
The Puncher platform for antibody screening and cell line development



Pump unit

Disposable
Containing our
Microwell chip

Clamp unit
Collect the antibodies
secreted by single cells

Puncher system
Isolation of single cells

Filling of the microwell chip by
forcing single cells in individual wells

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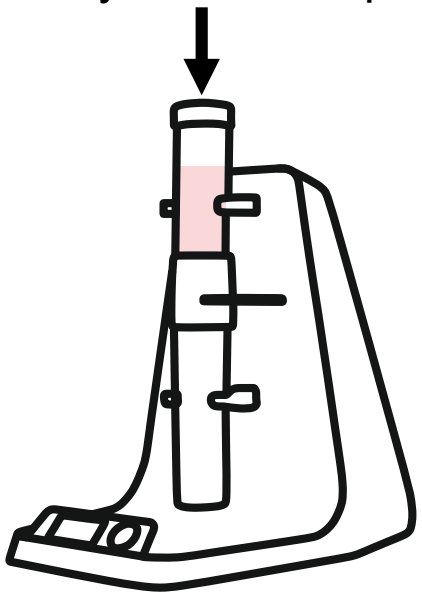
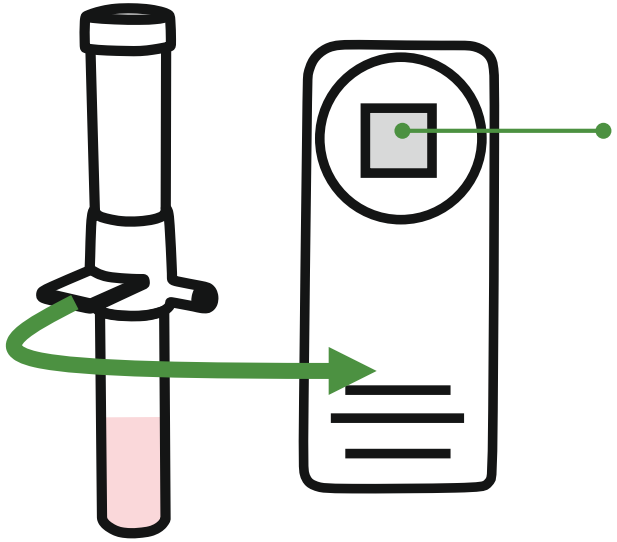
Capture single cells in individual microwells

Add your cell suspension



Pump unit with disposable

Remove the slide with microwells



Disposable with slide and microwell chip

Microwell chip

- **6400 individual wells** in an area of **8 x 8 mm**
- These wells are filled with with 6400 single cells

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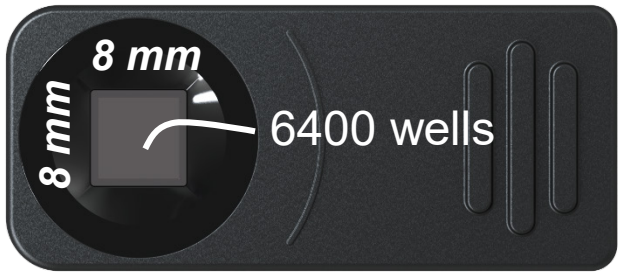
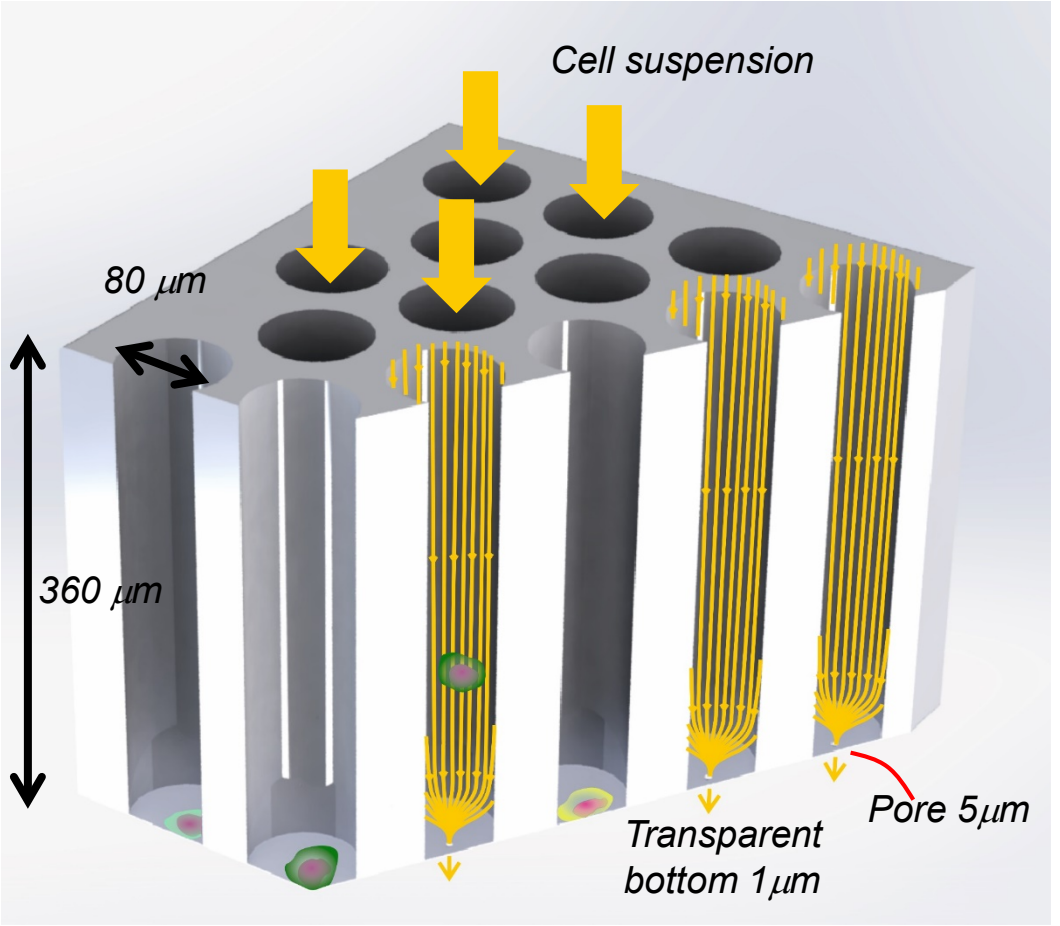
A closer look at the microwell chip

- ***6400** microwells in 8 x 8 mm^2 area*
- *Volume of a single well is **1.8nl***
- *Each well is closed with a 1μm thick **transparent** bottom*
- *Each well contains a **single pore** in the bottom (pore acts as a size selector)*
- *The microwell chip is made of Silicon*





Microwell chip single cell filling is not limiting dilution; No Poisson statistics

Microwell chip:

- A microwell contains 6400 individual wells
- Cells are forced into the microwells (no Poisson)
- Only one single cell per well

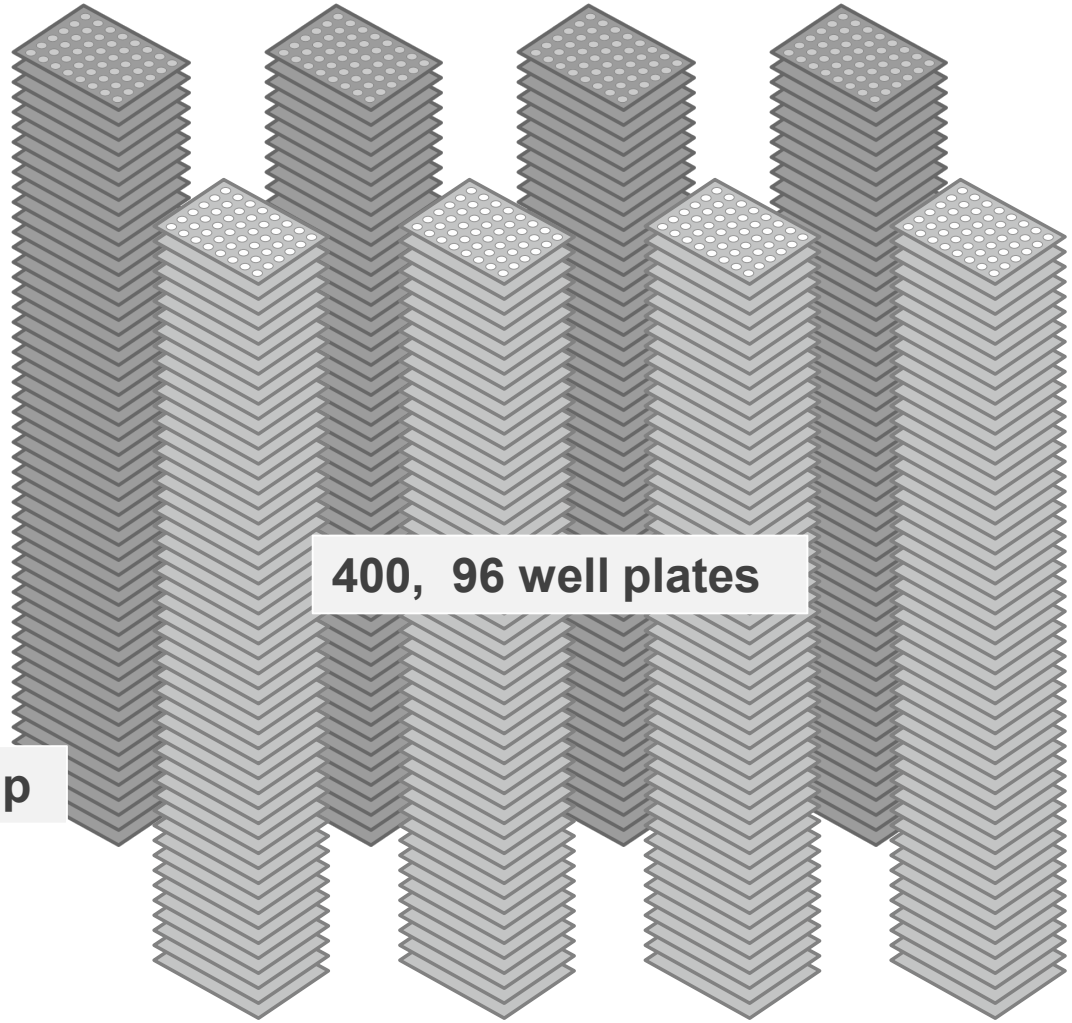
- Limited use of plastics
- Limited use of reagents
- Small footprint

1 microwell chip

equals

400, 96 well plates

One microwell chip



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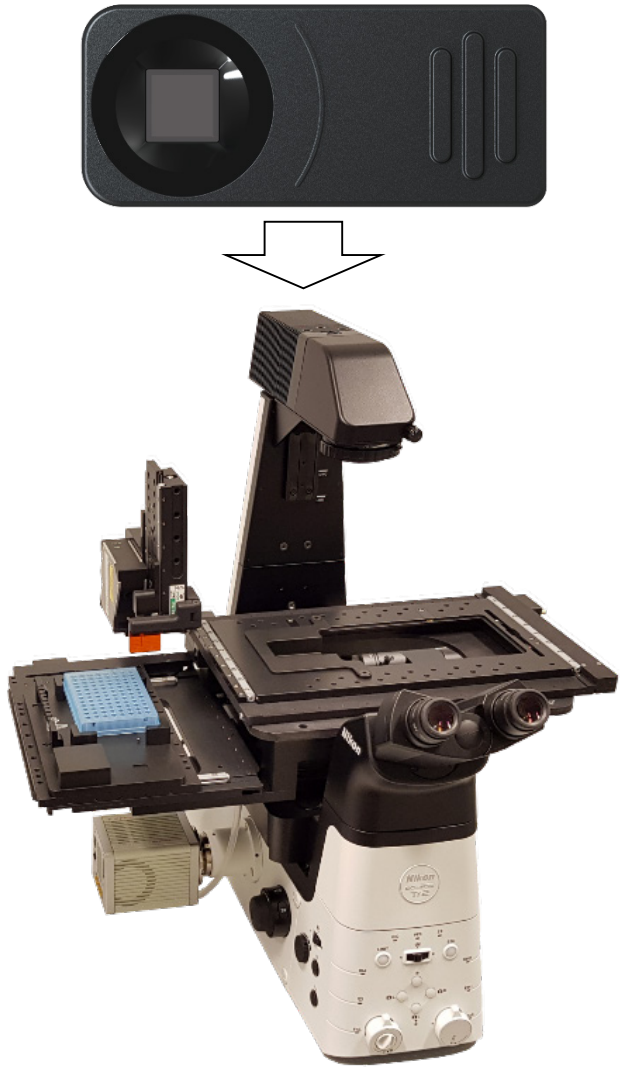
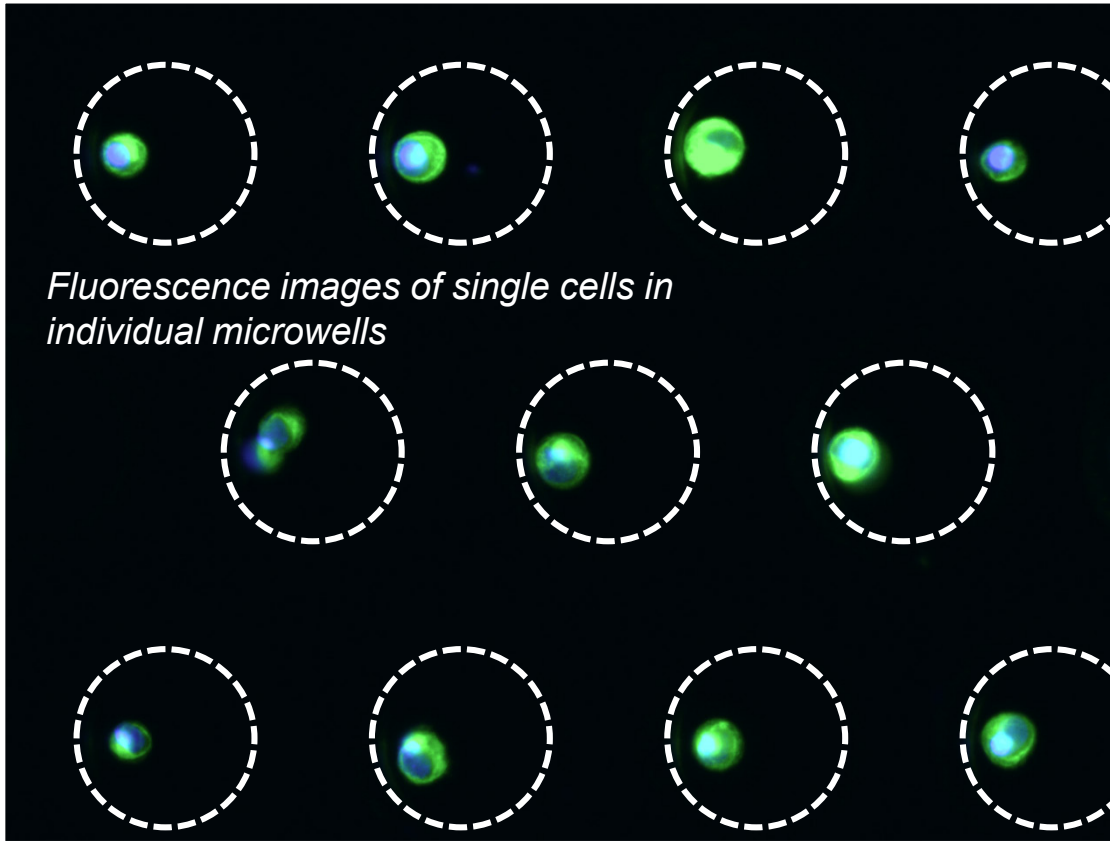
High resolution imaging assures clonality



VyCAP's Puncher system



After filling of the microwells high resolution images are acquired of each individual well.
The image of the cells assures clonality

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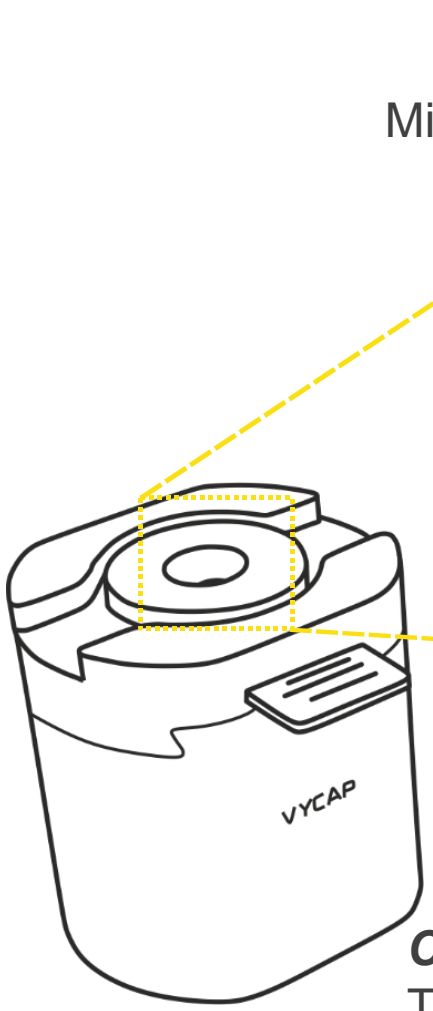
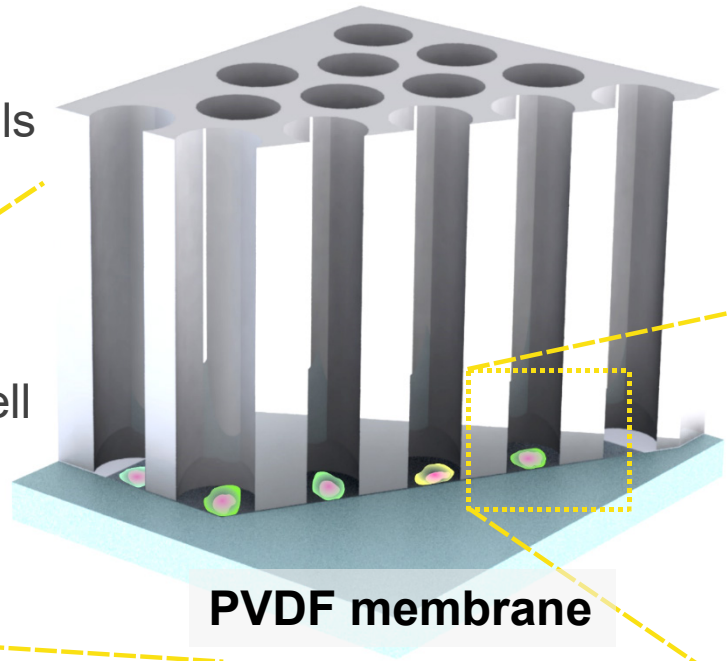
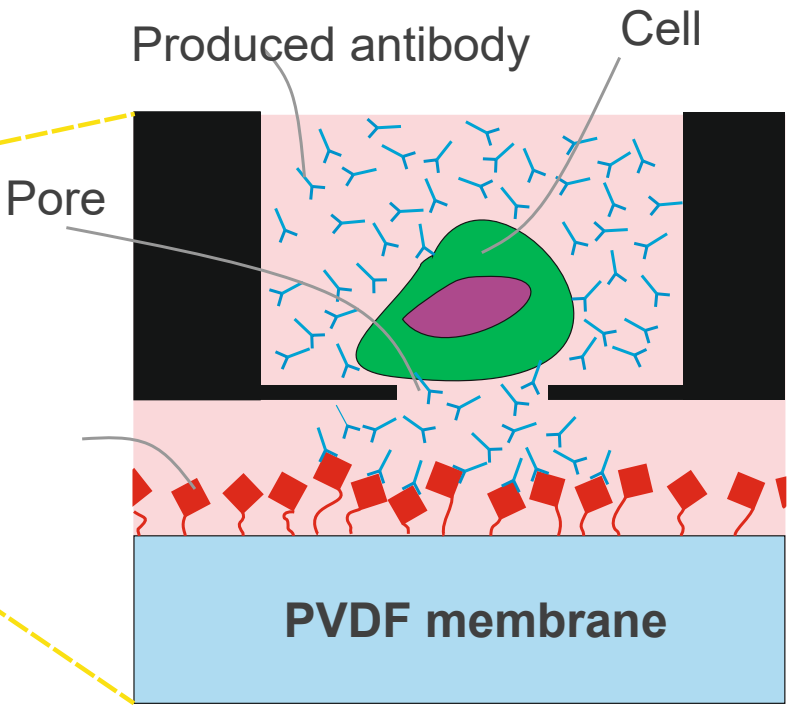
Next, capture the secreted antibody of each individual cell



Clamp unit
The membrane is pushed against the microwell bottoms





The membrane is labelled with anti-IgG, Prot A, etc…

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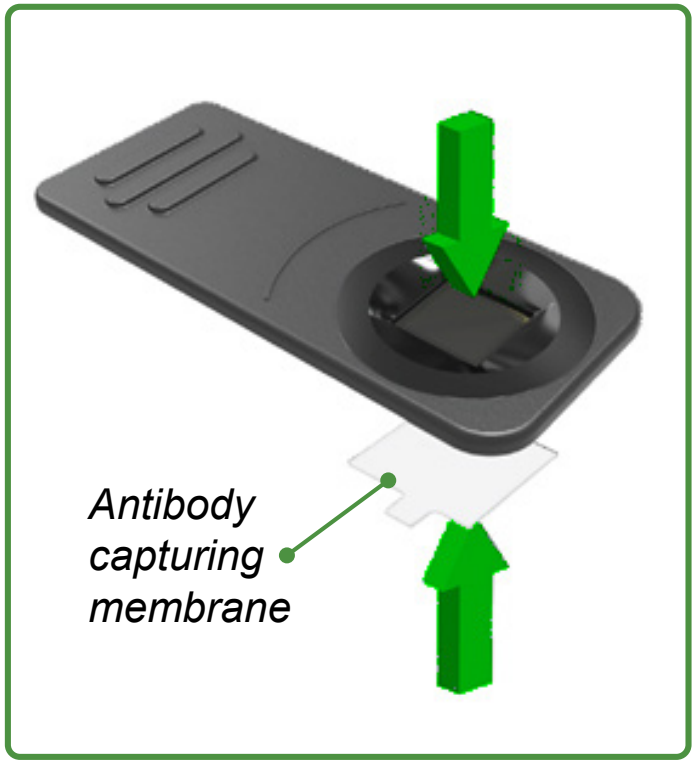
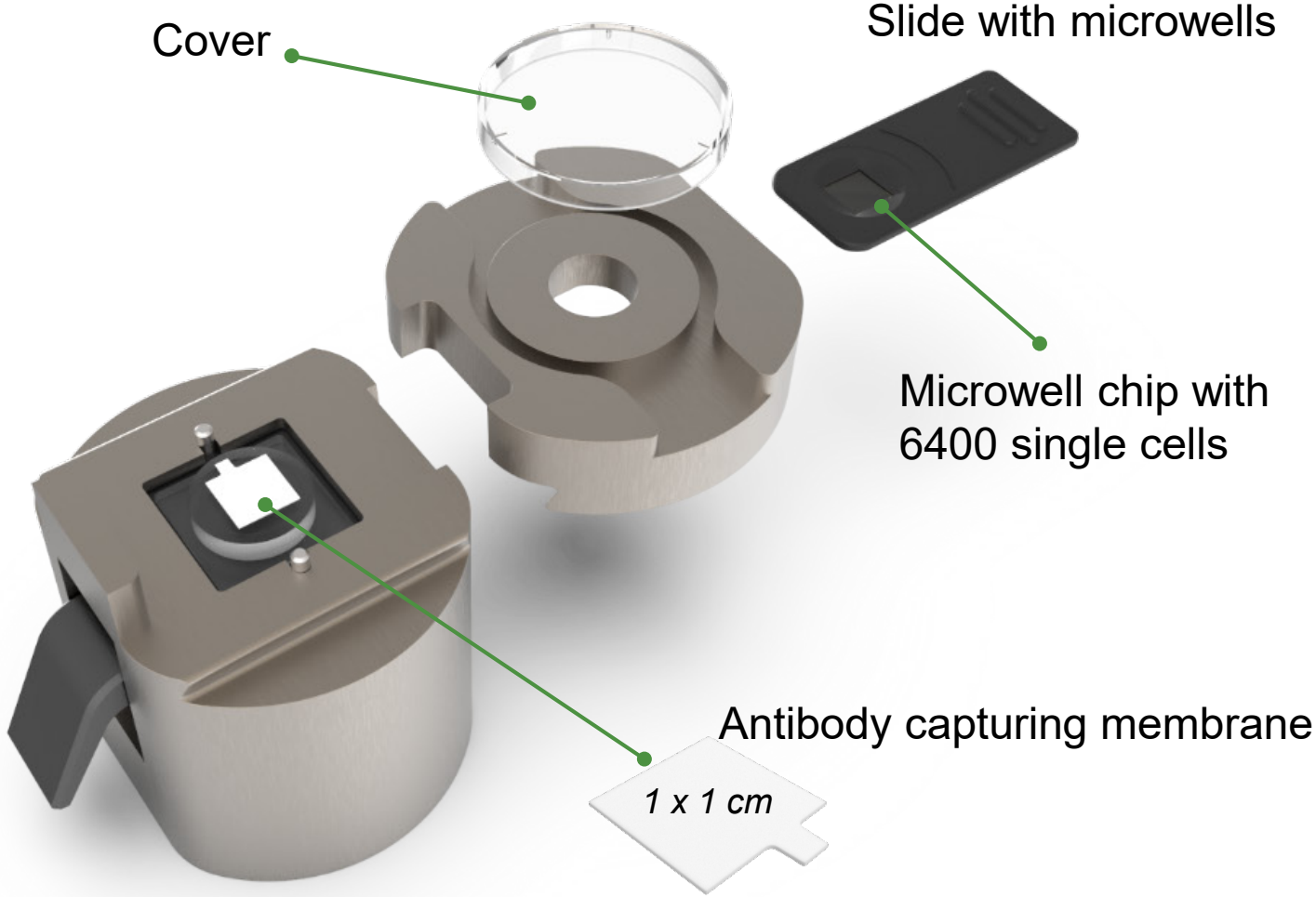
A closer look at the clamp unit





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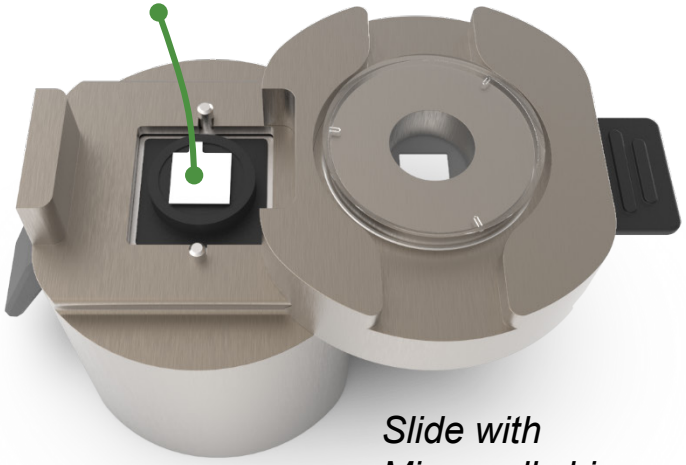
Prepare and incubate

Prepare separate parts

Capturing Membrane



Slide with Microwell chip Filled with cells

Loaded device

Incubator @ 37°C

The capturing membrane is mounted against the microwells
The unit with cells and membrane is placed in the incubator
The small footprint allows to place many units in a single incubator

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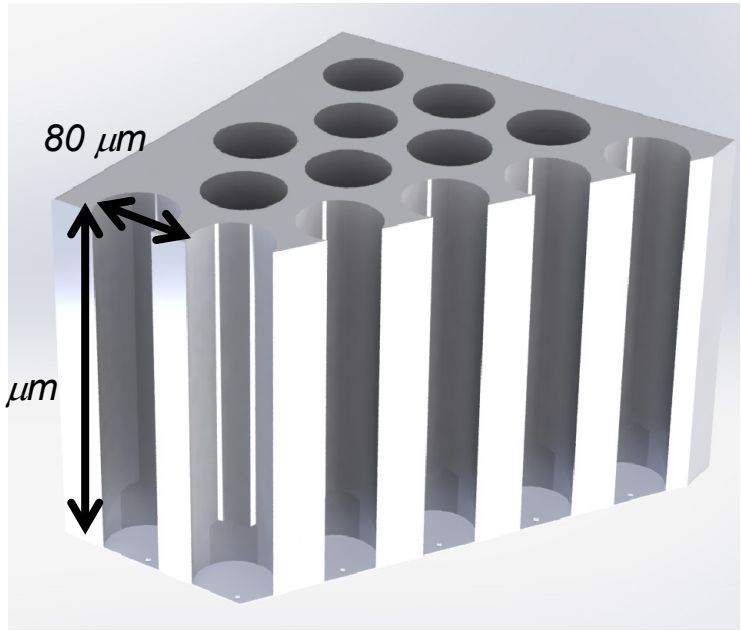
Short incubation times

For antibody producing CHO cells
4 hrs is already enough



Volume of a microwells is only 1,8 nl

↓

High local concentration

↓

Short incubation times

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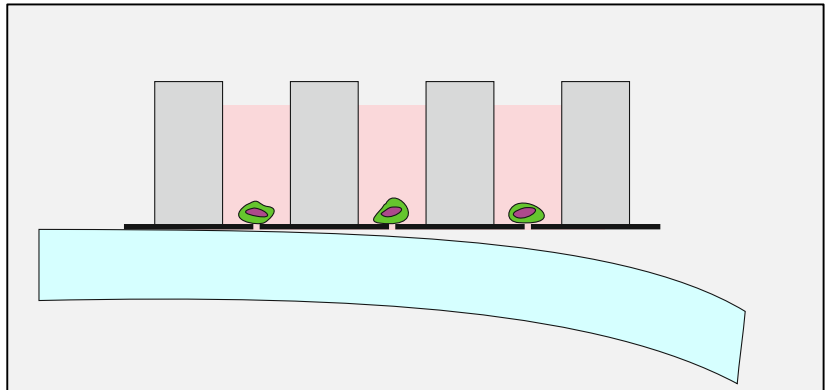
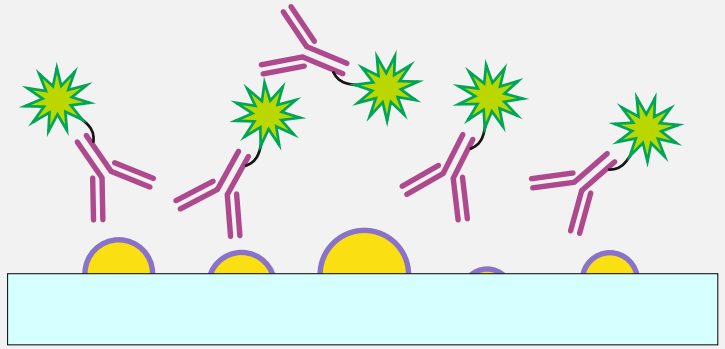
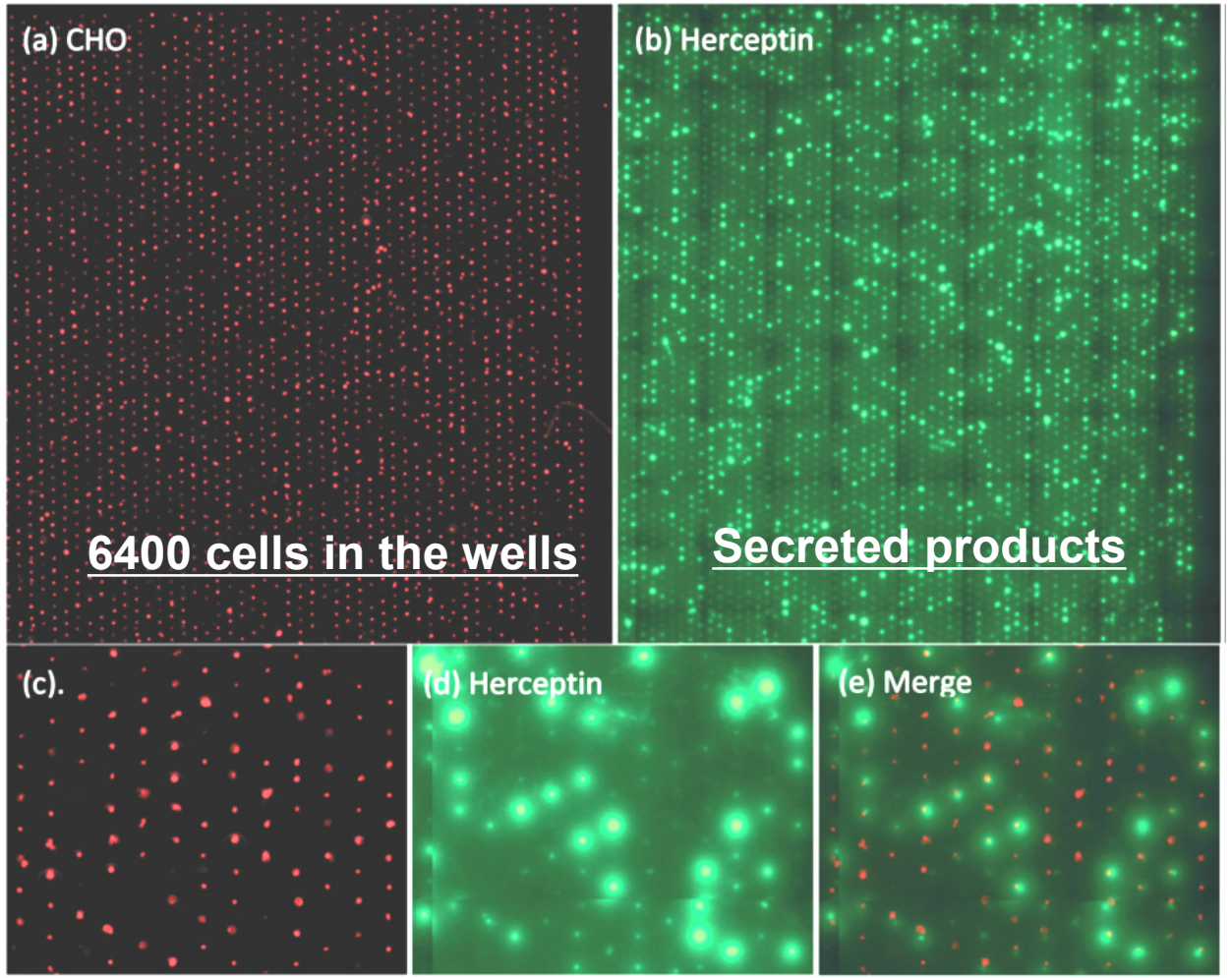
Example 1: After incubation remove and analyze the membrane



After incubation => remove the membrane



Label the membrane with common ELISA labeling strategies



Abali et al. Lab Chip, 2019, 19, 1850

Measure the secretion of 6400 single cells in parallel

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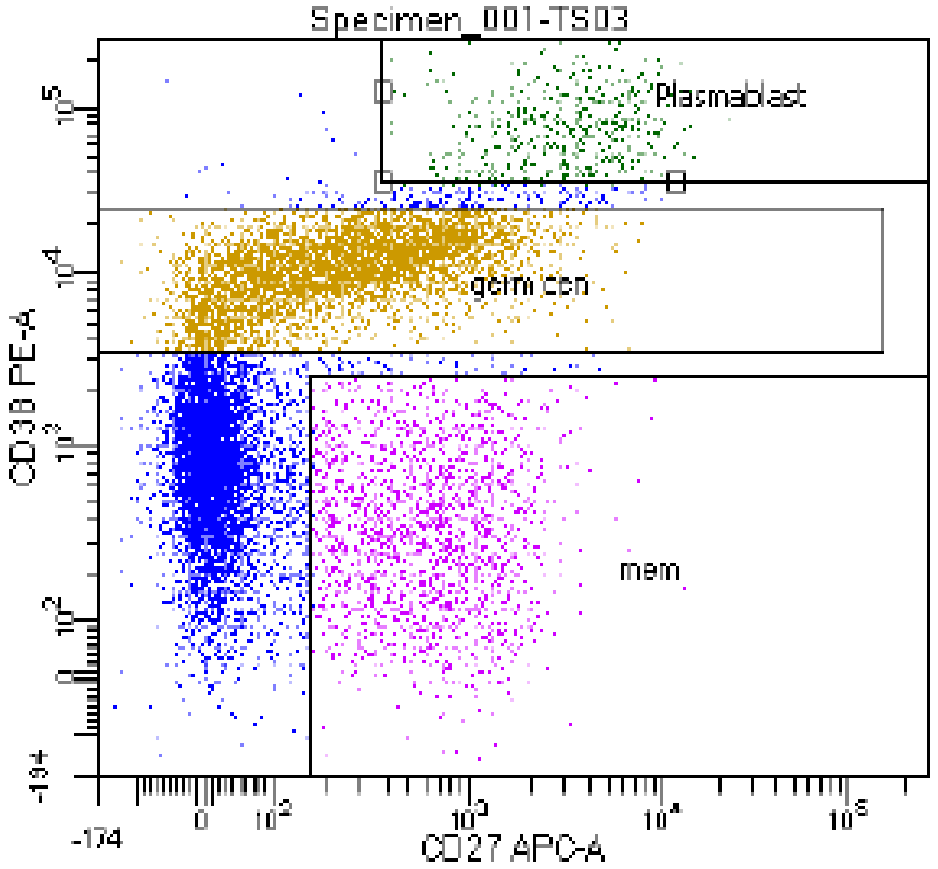
Example 2: Screen B cells on IgX production

1. Pre-sort different B-cell populations using FACS
   - *Naïve B cells: CD38- CD27-*
   - *Plasmablasts: CD38+ CD27+*
   - *Germinal centre B cells: CD38+ CD27 – or +*
   - *Memory B cells: CD38- CD27+*

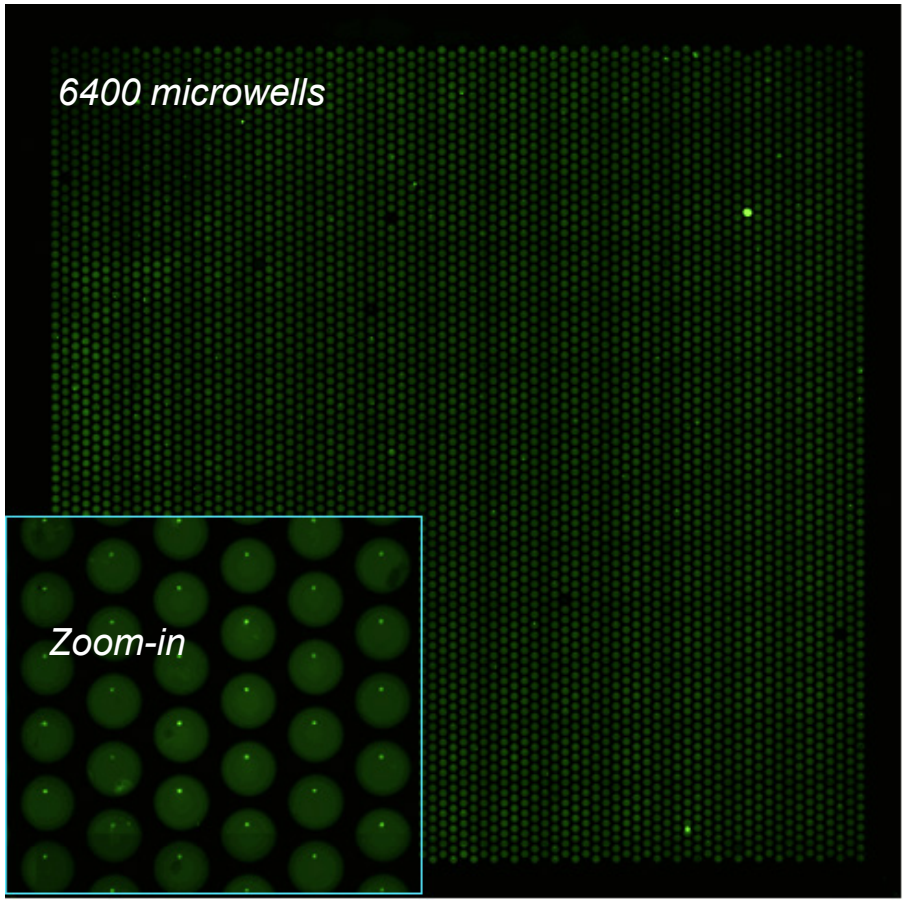
2. Transfer the different populations to the microwells
   - *Memory B cells require stimulation for antibody production. This can be done with various methods. Feeder cells can be used as well as culture media with different stimulants*
   - *Plasmablasts are antibody producing cells and are transferred to the microwells immediately after sorting*



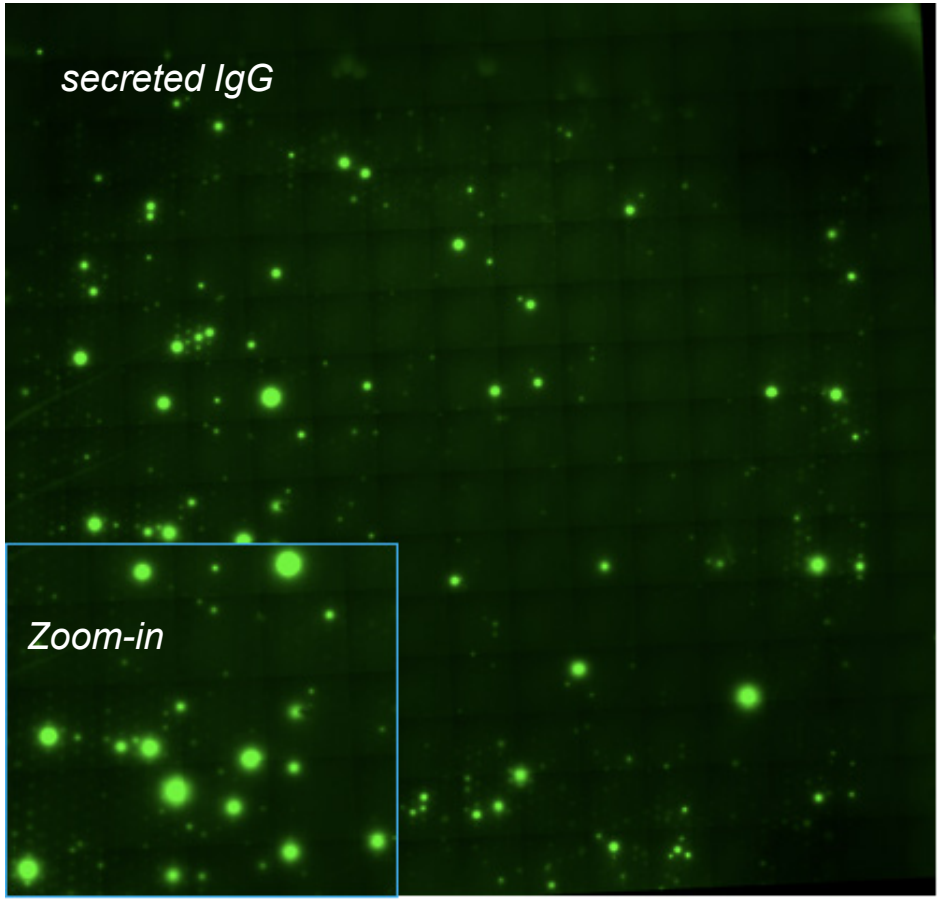
Example 2: Screen B cells on IgX production

Secretion of IgG by plasmablasts overnight

Overnight incubation yields more than sufficient antibody to measure the IgG production



Plasmablast cells in the microwell chip



IgG production of corresponding cells

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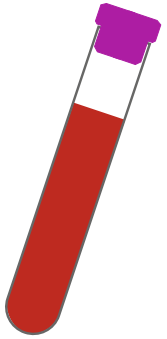
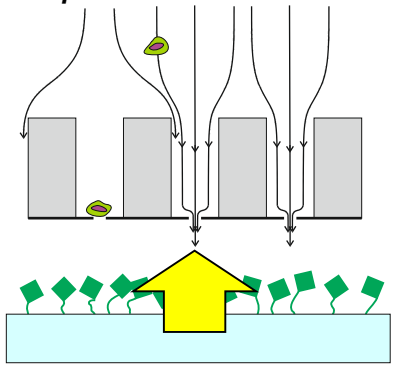
Example 3: B-cell screening for therapeutic antibody generation

Enrich B-cells



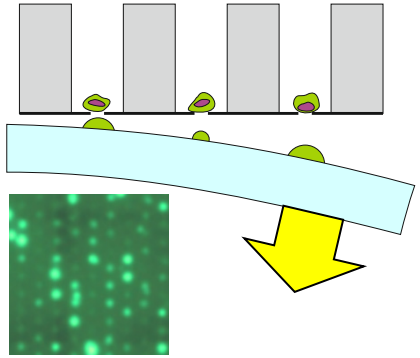
1

Single B-cell seeding
Clamp the membrane



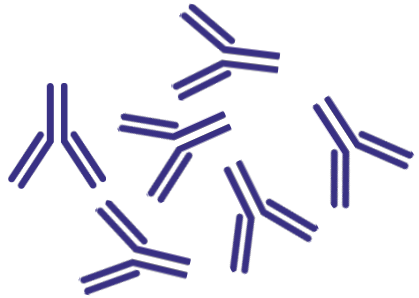
2

Remove the membrane
Identify the B-cell

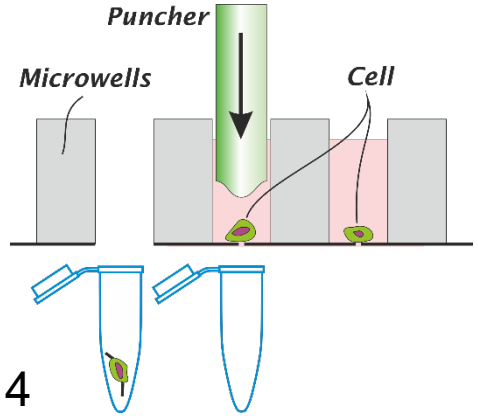


3

Antibody generating cell line

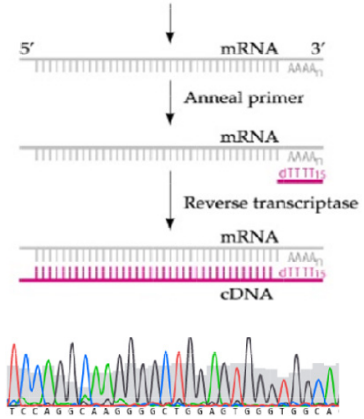


Isolation of the B-cell



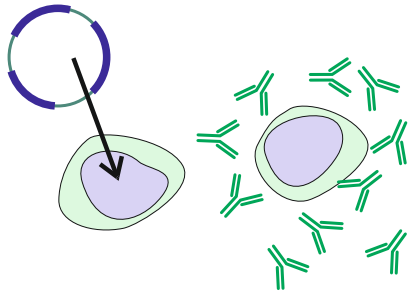
4

IgG RNA sequences



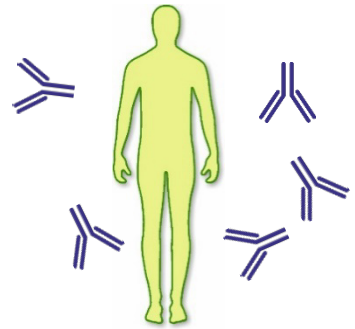
5

Transfection



6

Immuno therapy



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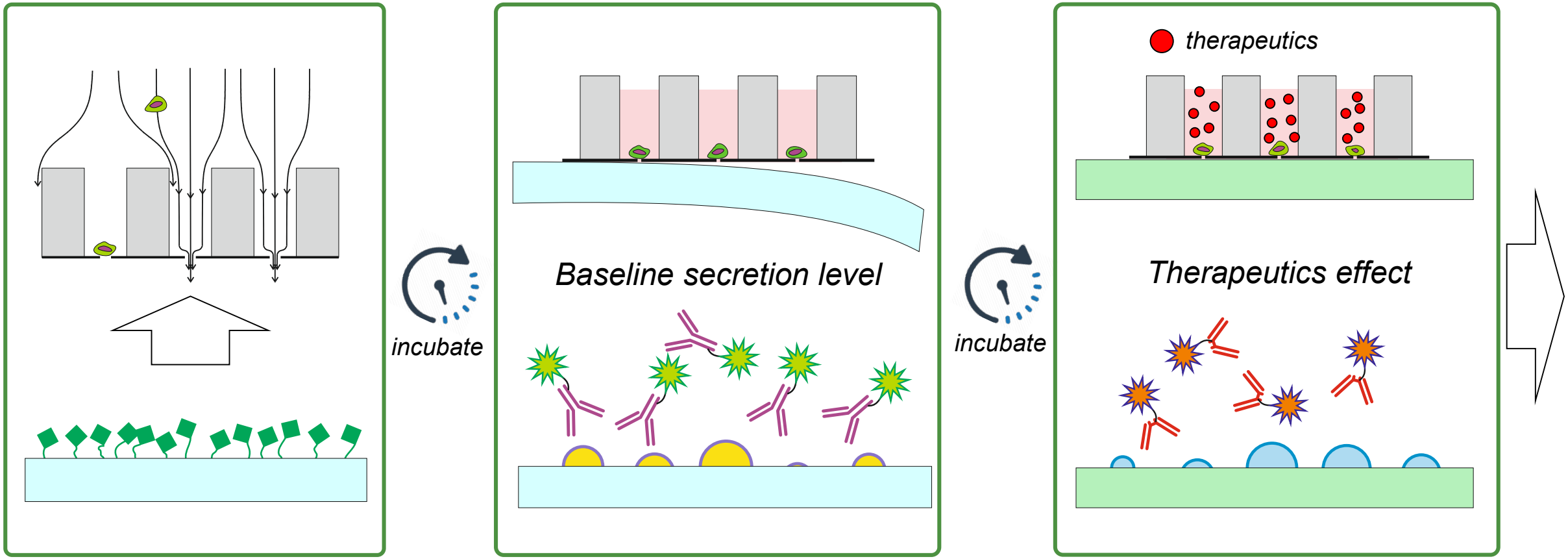
Example 4: Drug efficacy testing

Distribute cells and connect the membrane

Remove the membrane and label the membrane

Place a new membrane and add therapeutics



A total of up to 10 membranes can be placed and removed sequentially

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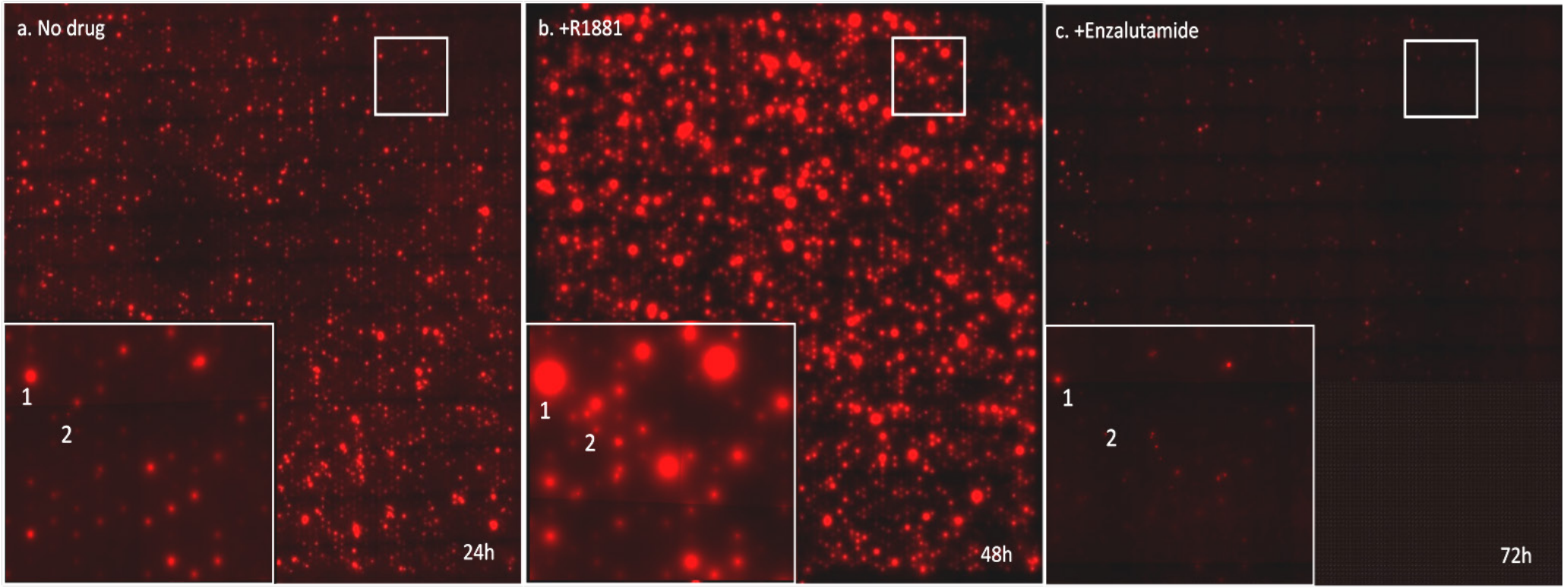
Example 4: Drug efficacy testing

PSA secretion and androgen inhibitors

Prostate cancer cells (LnCAPS) + R1881 + Enzalutamide



Membrane 1
Culture media

Membrane 2
+ R1881 PSA stimulator

Membrane 3
+ Enzalutamide

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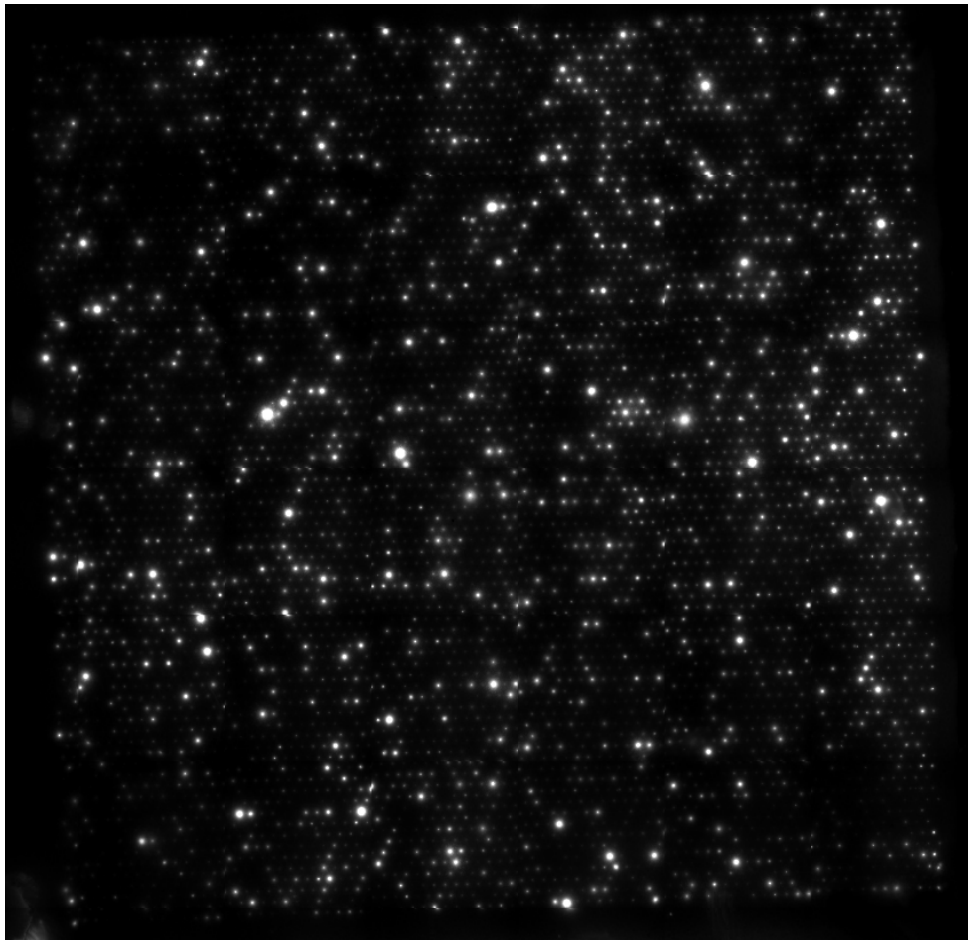
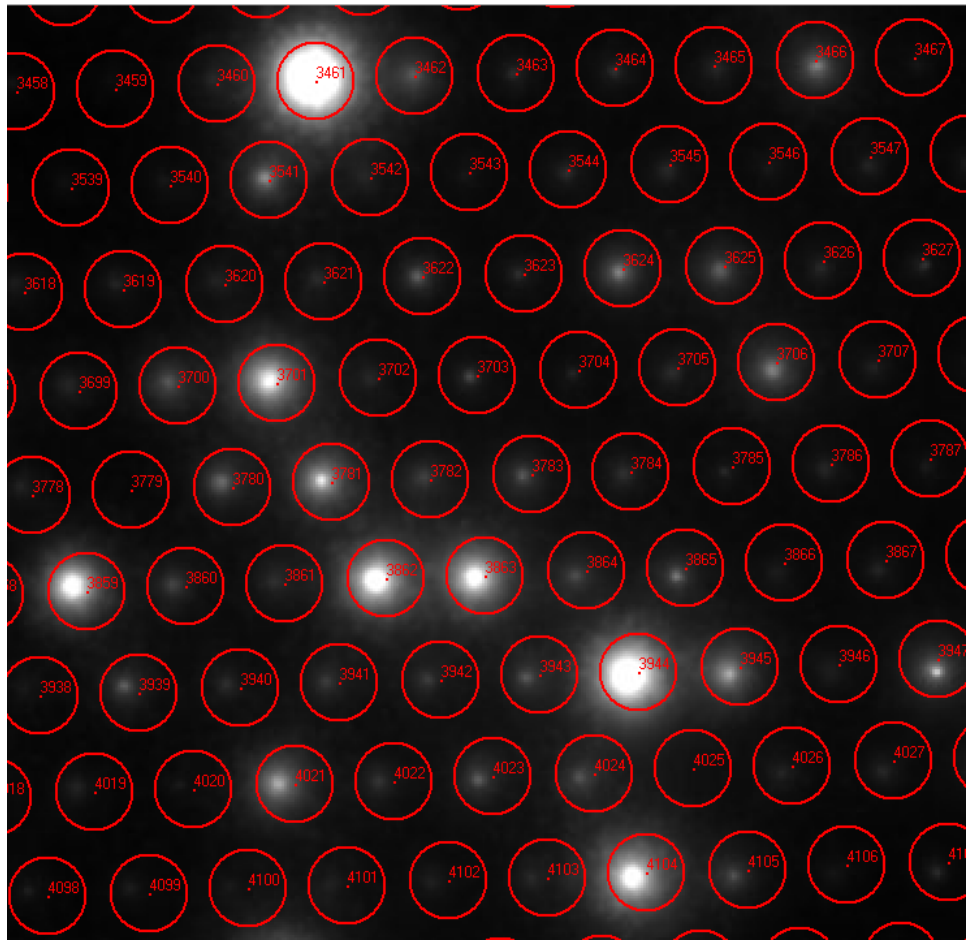
Determine the well ID of the high producers

Whole membrane



Correlate the location with the well number



Analyze the secretion of 6400 single cells in parallel

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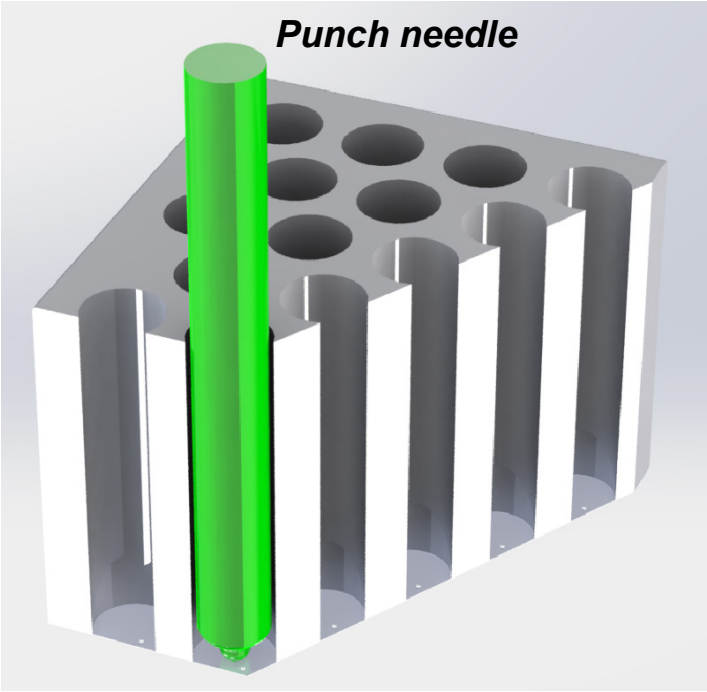
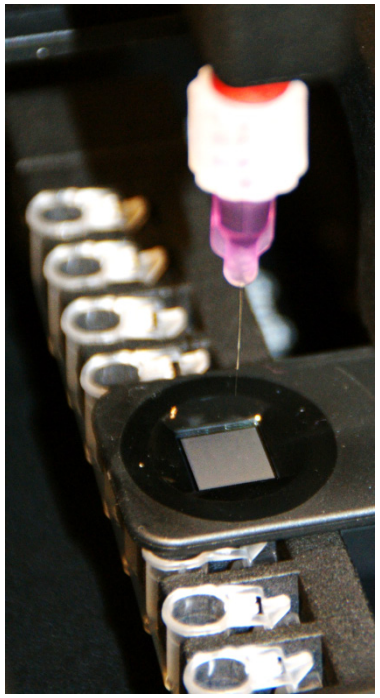
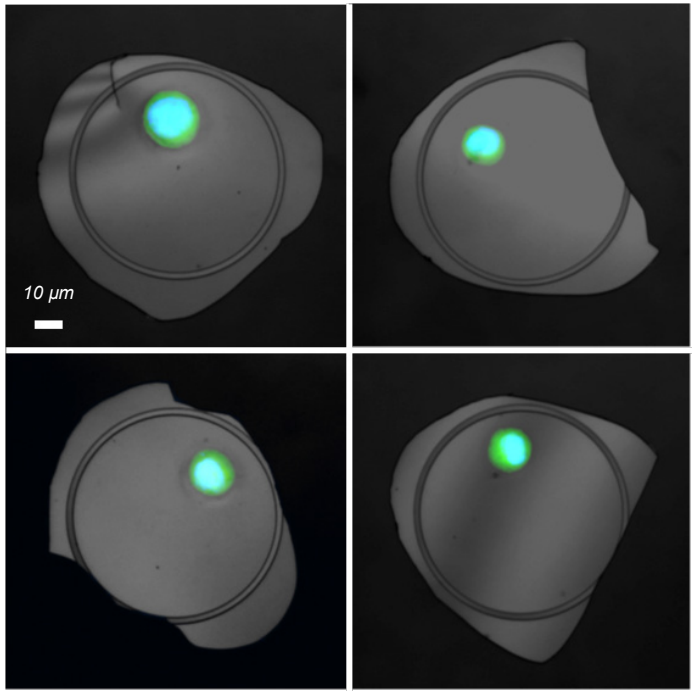
Isolation of the secreting single cells by Punching







- Isolation by punching the bottom + cell towards the preferred reaction tube
- Successful isolation > 95%
- 1 cell / second
- The design of the needle is such that it doesn't touch the cell during Punching

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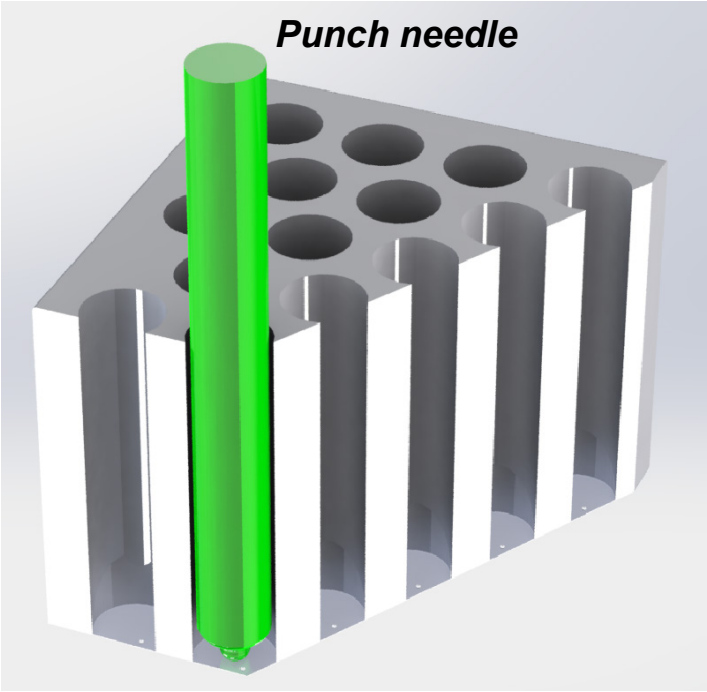
Isolation of the secreting single cells by Punching



- Isolation by punching the bottom + cell towards the preferred reaction tube
- The isolation efficiency > 95%
- 1 cell / second
- The design of the needle is such that it doesn’t touch the cell during Punching

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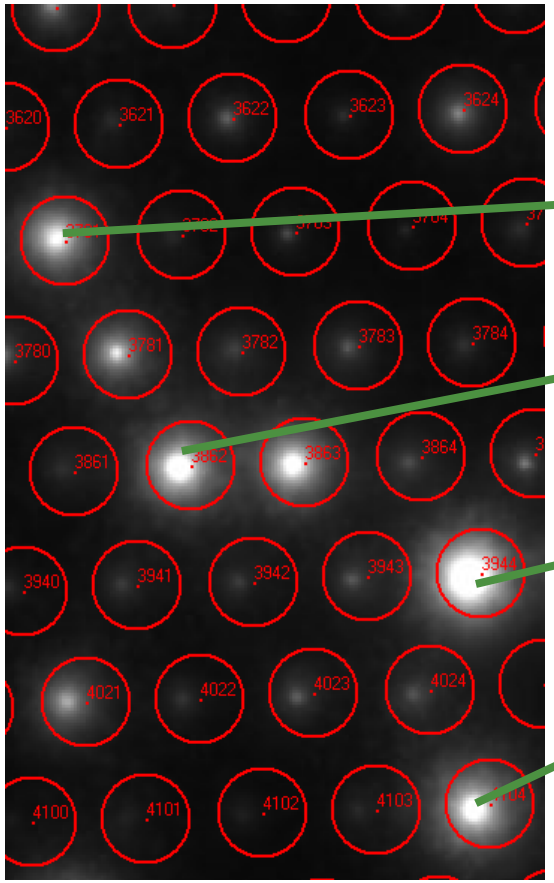
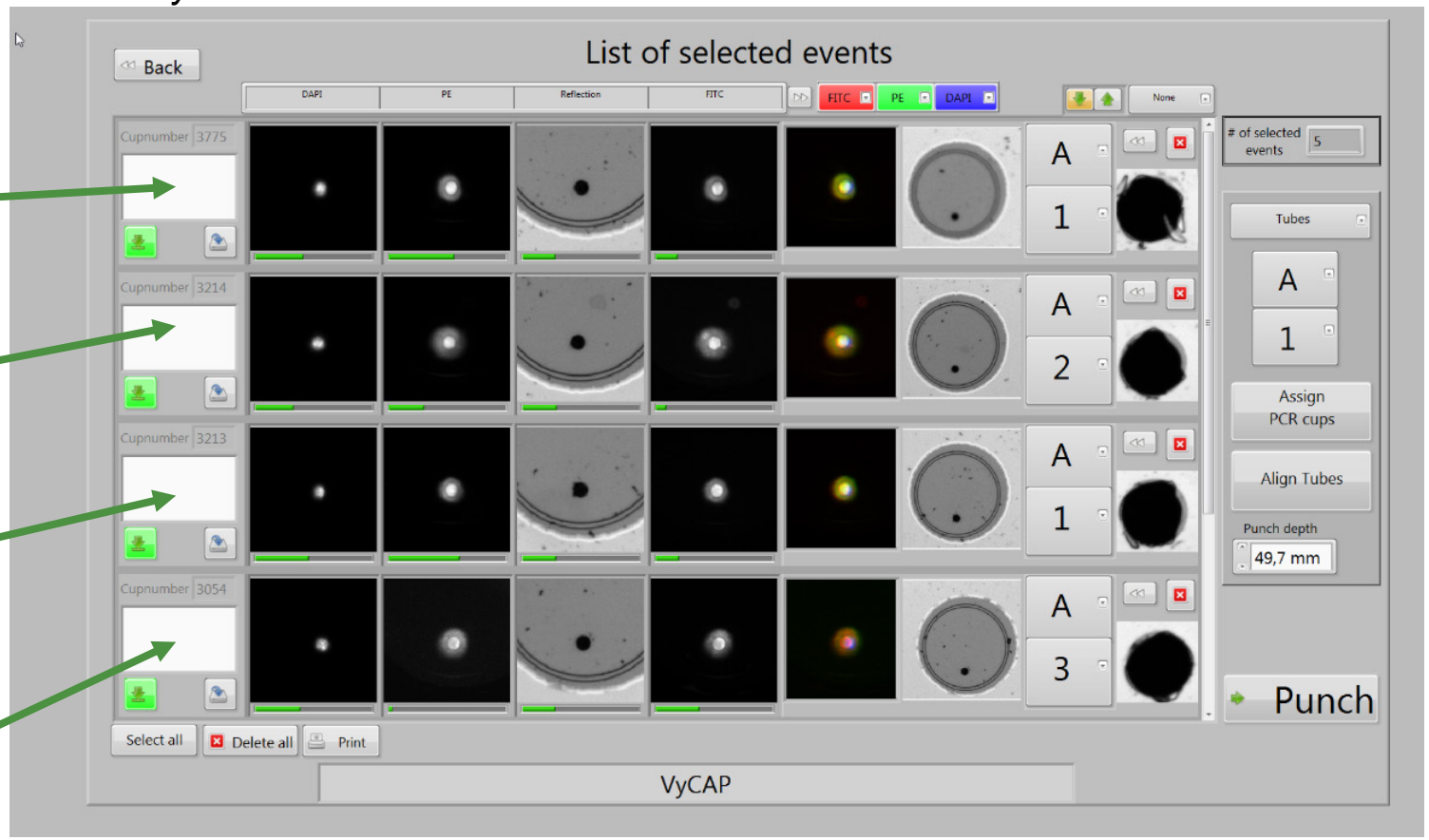
Automated system

- The Punching of the cell from the microwells chip is fully automated
- Isolation is verified by removal of the well bottom

Activated membrane



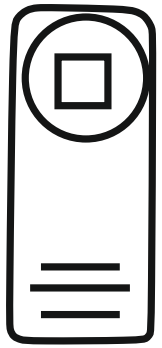
Puncher system software



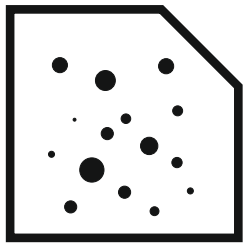
Overview of the whole procedure

Clamp unit to measure the antibody production of B-cells, hybridoma, CHO cells, …

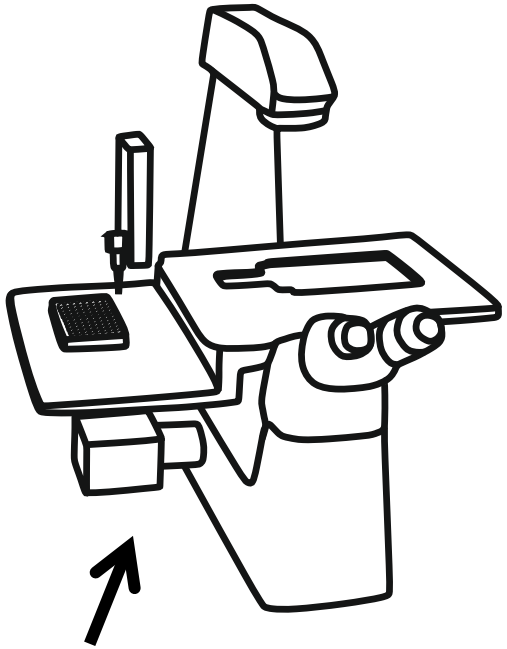
Slide with microwells



Antibody capturing membrane

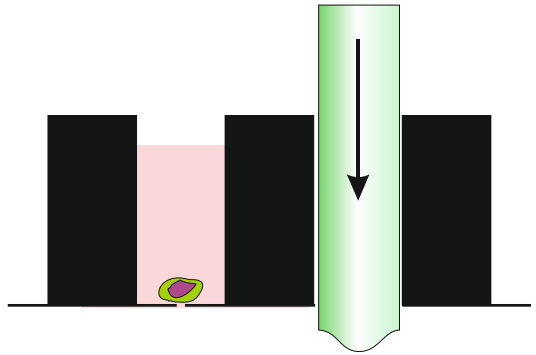


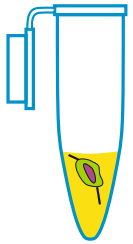
Puncher system



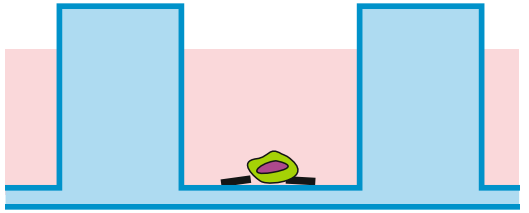
Feed the well ID numbers to the system

Isolate the single cell





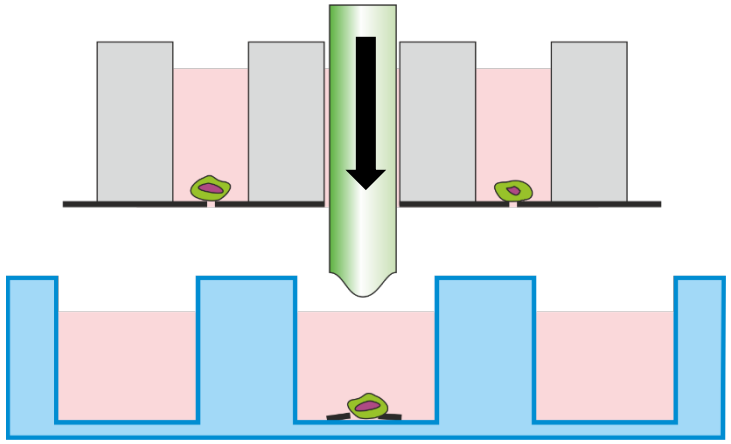
Or



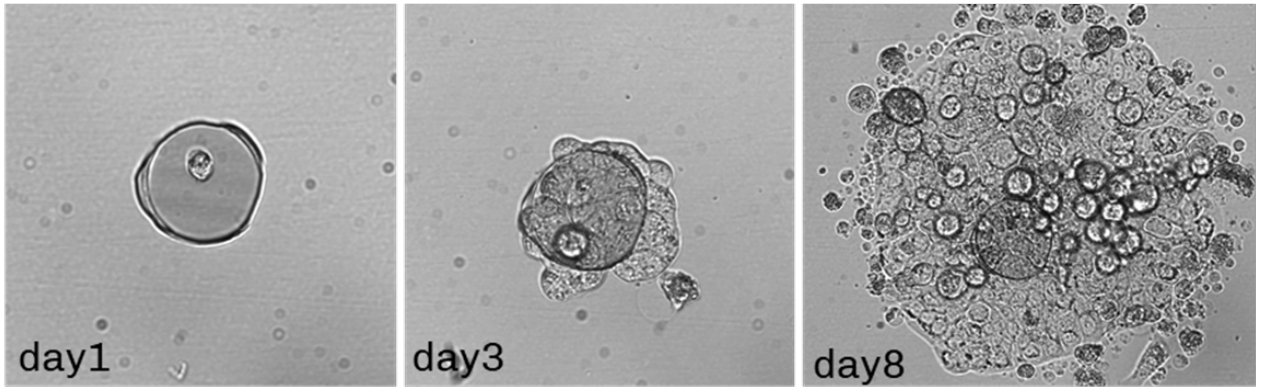
Isolate the cell from the microwell chip towards a DNA / RNA tube, well plate or culture plate

VyCAP

Isolation for clonal expansion



- Cells stay alive during isolation
- Cell line generation
- Monitoring the production of antibody producing cell cultures
- Improve production capacity



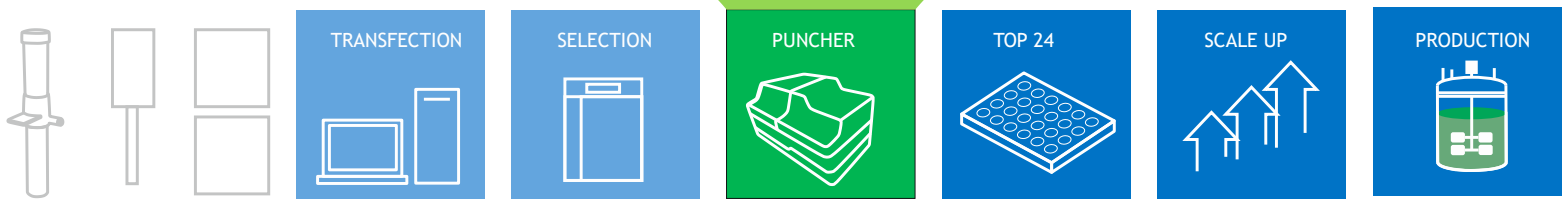
VyCAP

VyCAP's technology shortens the process from weeks to days

Common workflows for cell line generation

8 - 12 WEEKS

Cell Puncher workflow



3 DAYS

- The Puncher platform shortens process time to days
- Allows to screen thousands of cells in parallel
- Small footprint, saves incubator area
- Reduces the amount of culture media
- Reduces the large amount of cell culture plastics to a minimum
- Assures clonality

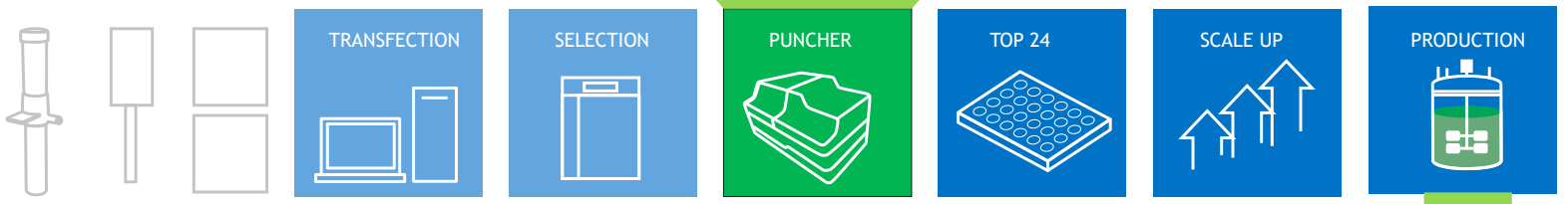
VyCAP

VyCAP's technology shortens the process from weeks to days

Common workflows for cell line generation

8 - 12 WEEKS

Cell Puncher workflow



3 DAYS

Simple and efficient methodology to check the quality of the production batches at the single cell level

VyCAP

In summary

The Puncher platform technology allows :

- Screening, selection and isolation of **antibody producing** single cells
- Fast and efficient **screening of B-cells**
- Analysis of all kind of **cellular secretions**
- Isolates **rare single cells** for DNA / RNA sequencing with a very high recovery percentage **> 95%**
- **Compatible** with DNA / RNA **sequencing** and protein analysis methods (**ELISA, Fluorospot**, ..)
- **Drug efficacy testing** on **single cells**
- **Fast, efficient, simple**
- **Affordable** (200 – 250 k€)

VyCAP

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Thank you for your attention

VyCAP