

CH-413 Nanobiotechnology

Single cell manipulation and study

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May 22, 2025

Housekeeping notes

- Uploaded evaluator's check list ("How_we_grade")
- If you need help, contact TAs to schedule a meeting

Exercise 4: some take-aways

- Thank you for completing this exercise!
- It was great to see you validate references. Most (all?) of you concluded that referencing is unreliable. **LLMs require fact-checking.**
- Some of you really went for it and strategically interrogated the AI on multiple dimensions. You asked precise follow-ups.
- Some of you expressed frustration with the way the AI interacted with you (too positive, too much text, not combative): you are the one asking the questions and setting the terms of the conversation. **You are the prompt engineers. You can state how you want the questions answered.**

The AI's perspective on your feedback

I got feedback from my students that your references cannot always be trusted, what do you say to that. summarize in a couple of sentences

That's valid feedback — my references aren't always reliable, especially if I'm generating them from scratch. I can sometimes provide inaccurate or fabricated citations, so it's important to fact-check anything I cite, particularly in academic work.

what would you say as the AI, if someone doesn't like the way you respond (length of text, positivity, etc), they could just tell oyu to react differently and you would do that, right?

Exactly — if someone doesn't like how I respond (too long, too formal, too enthusiastic, not critical enough, etc.), they can absolutely just tell me. I'll adjust immediately.

You can say things like:

- "Be more concise."
- "Don't sugarcoat it — I want blunt feedback."
- "Answer more like a skeptical reviewer."
- "Give me short bullet points."
- "Don't be so positive — critique it harder."
- "Act like a collaborator who challenges my assumptions."

And I'll shift my tone, style, or depth accordingly. My default aim is to be helpful and constructive, but **you're always in control of how we work together.**

It's like tuning a tool — you get to decide what settings suit your needs.

Some of you expressed frustration with the way the AI interacted with you (too positive, too much text, not combative): you are the one asking the questions and setting the terms of the conversation.

this reminds me of a term i heard, prompt engineer?

Yes, exactly — what you're describing is very much in line with the role of a **prompt engineer**.

A *prompt engineer* is someone who learns to shape an AI's behavior by crafting the right kinds of inputs — or *prompts*. In this context, your students are essentially prompt engineers: **they set the tone, depth, and direction of the interaction**.

If the AI is too positive, too verbose, or not challenging enough, it's because it's responding to the way it's being asked. Students can steer the AI by saying things like:

- "Critique this like a skeptical reviewer."
- "Be concise — max 3 bullet points."
- "Challenge my assumptions — don't just agree."
- "Play devil's advocate here."

The point is: **the AI will adapt — but only if it's told how**. Frustration often comes from expecting the AI to behave like a human peer without realizing that *you're in charge of setting the ground rules*. That's the skill — and the power — of good prompt design.

Links and references for 'prompt engineering'

- “Leveraging prompt engineering in large language models for accelerating chemical research” *ACS Cent. Sci.* **2025**, *11*, 511-519. DOI: 10.1021/acscentsci.4c01935
- https://guides.library.ucdavis.edu/genai/prompt?utm_source=chatgpt.com
- <https://libguides.nyit.edu/promptengineering/principlesofpromptengineering>
- https://www.aiforeducation.io/prompt-engineering-for-educators-webinar?utm_source=chatgpt.com
- https://educationaltechnologyjournal.springeropen.com/articles/10.186/s41239-025-00503-7?utm_source=chatgpt.com

Today's in-class activity

- I will present a paper.
- As a group, we will brainstorm: what is next? What are the potential next steps in this field? We will gather general ideas.
- Then, we will look at the data: who has cited this paper? What are researchers doing now? How has research progressed in this field?
- Collection of papers on Miro board
- Final discussion to summarize

Learning objectives

- **Understanding** of microfabrication applications and cell manipulation, cell pairing and fusion
- Identification of **research boundaries**: limitations and challenges of the existing research. **This is a core skill to train in research!**
- **Creative application and problem solving**: to propose new applications and optimizations for microfabrication techniques, to engage in creative problem-solving
- **Critical thinking and analysis**: Today's in-class activity will enhance your ability to critically analyze scientific research, assessing both its strengths and weaknesses.
- **Collaborative learning and communication**

Cell fusion for hybridoma production



ARTICLES

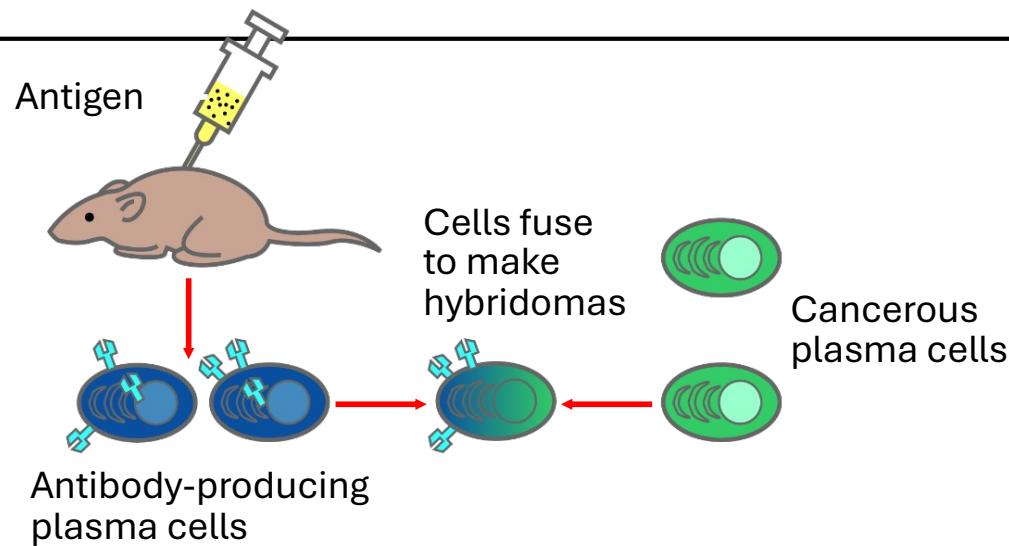
Microfluidic control of cell pairing and fusion

Alison M Skelley^{1,2,6}, Oktay Kirak^{3,6}, Heikyung Suh³, Rudolf Jaenisch^{3,4} & Joel Voldman^{1,2,5}

RECEIVED 18 AUGUST 2008; ACCEPTED 18 NOVEMBER 2008; PUBLISHED ONLINE 4 JANUARY 2009; DOI:10.1038/NMETH.1290

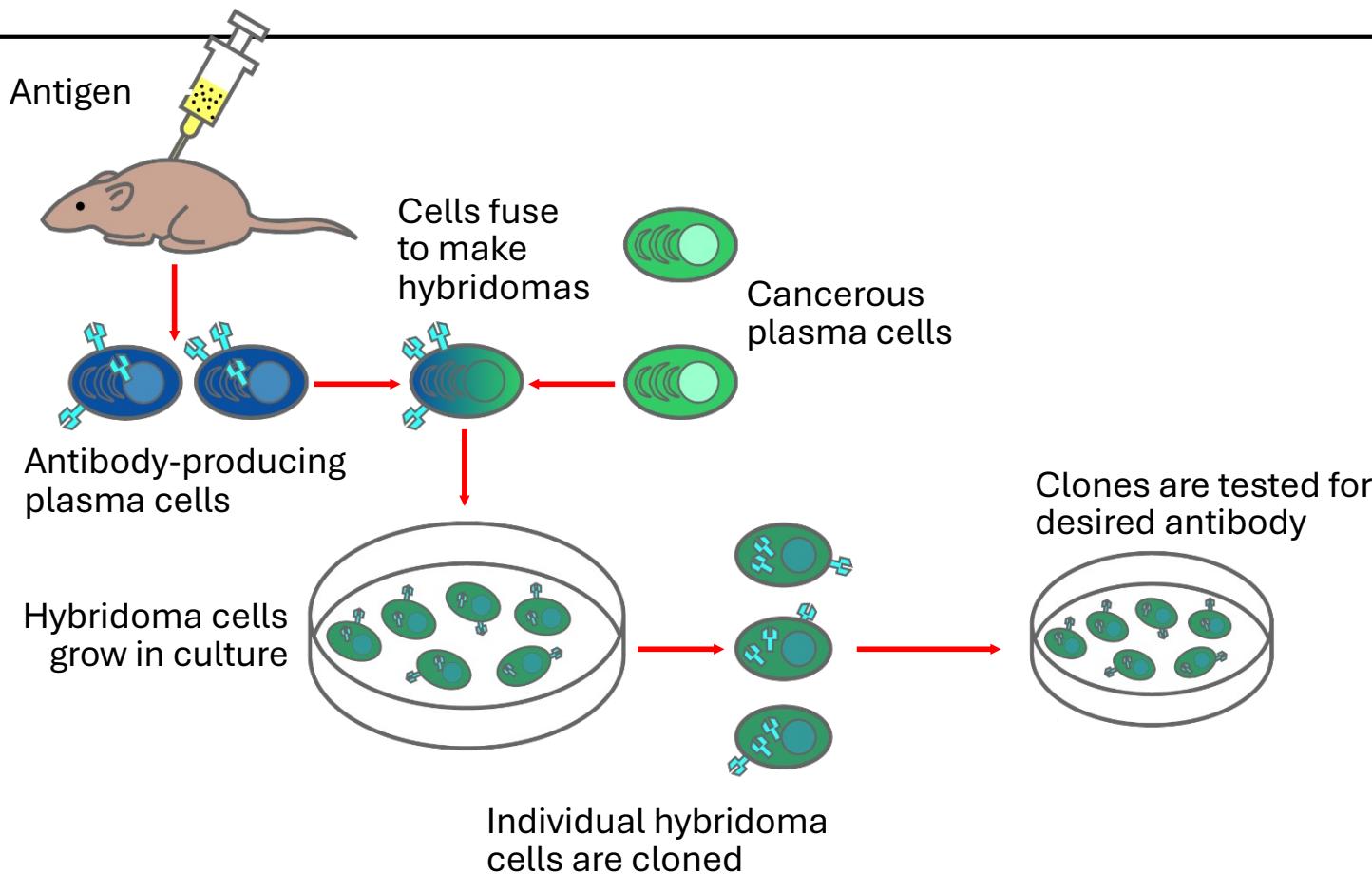
NATURE METHODS | VOL.6 NO.2 | FEBRUARY 2009 | 147

Cell pairing: Hybridoma formation



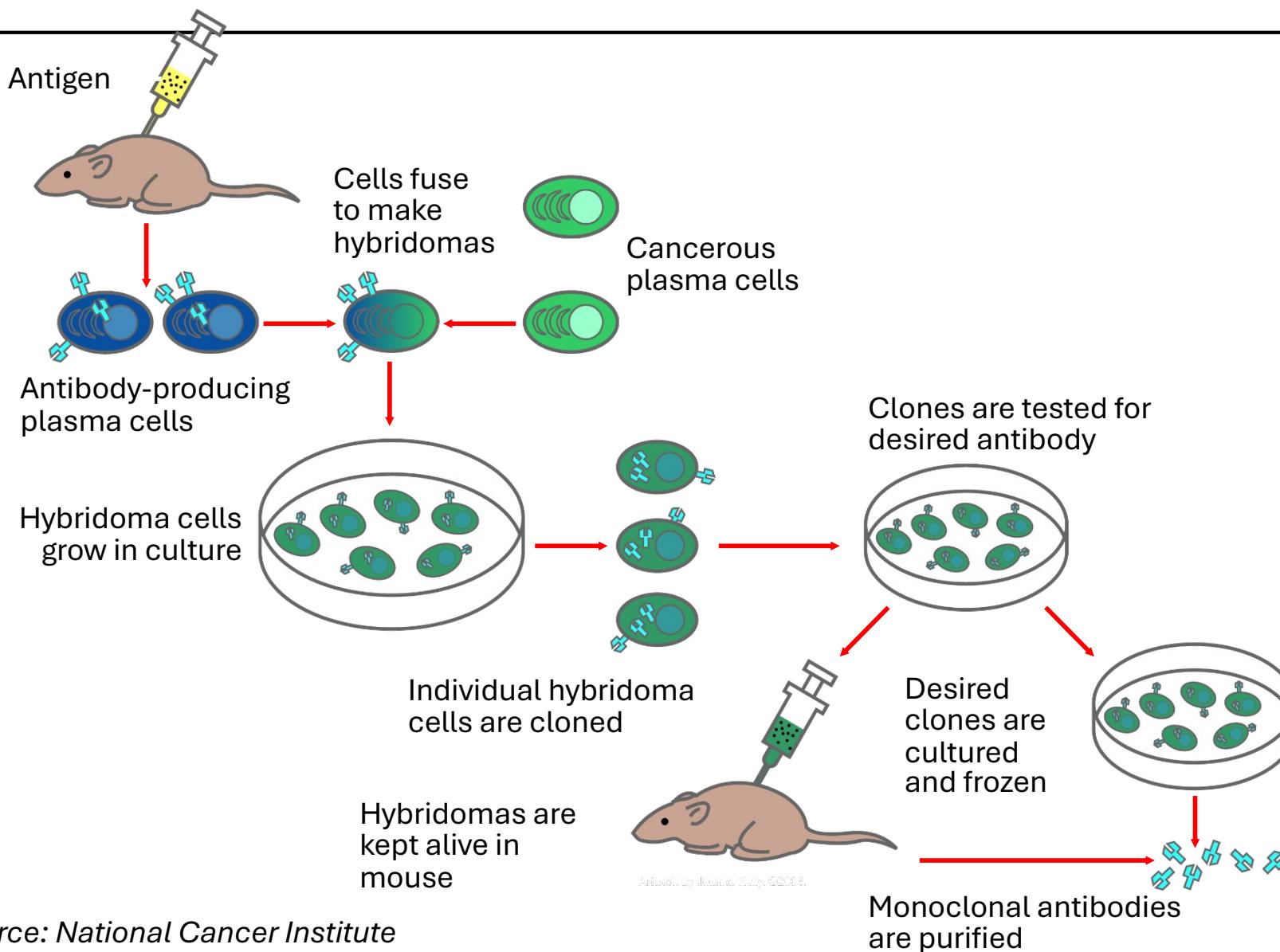
Source: National Cancer Institute

Cell pairing: Hybridoma formation



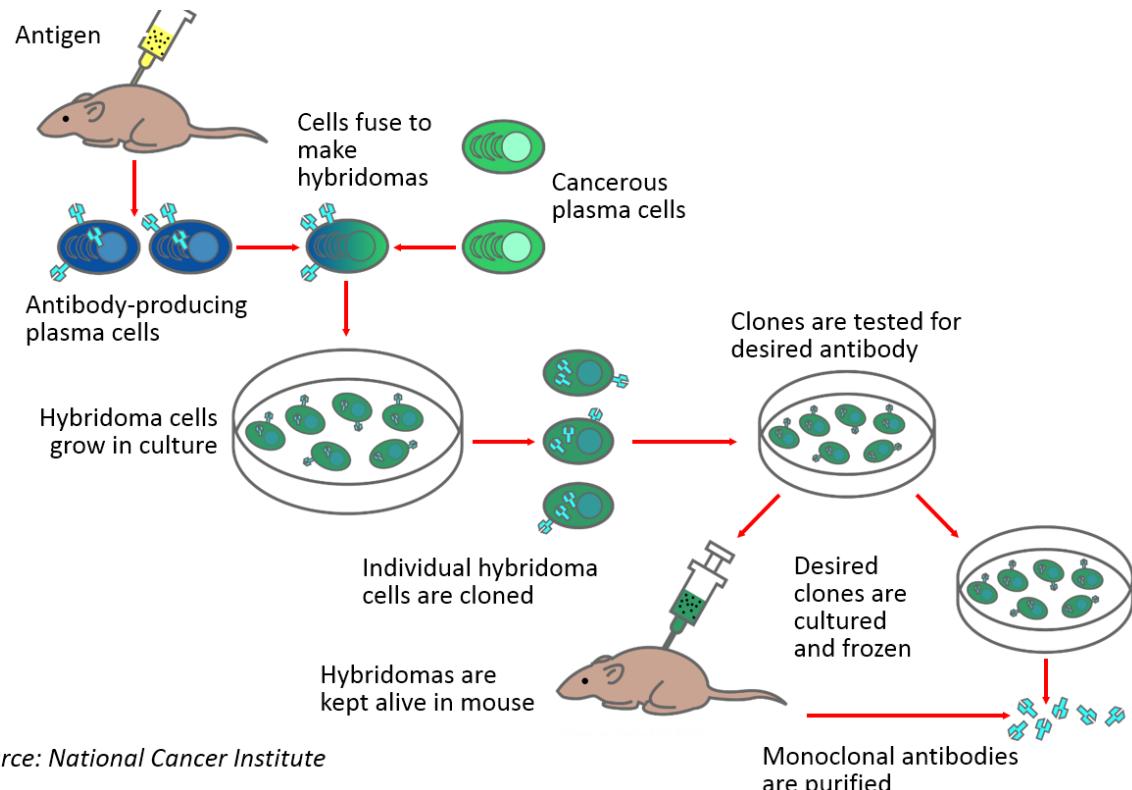
Source: National Cancer Institute

Cell pairing: Hybridoma formation



Source: National Cancer Institute

Cell pairing: Hybridoma formation



Source: National Cancer Institute

Source: National Cancer Institute

Cell fusion: Production of hybridomas, reprogramming

Electrical, chemical (PEG) →
Relies on random cell pairing

Biological (viruses, receptors)

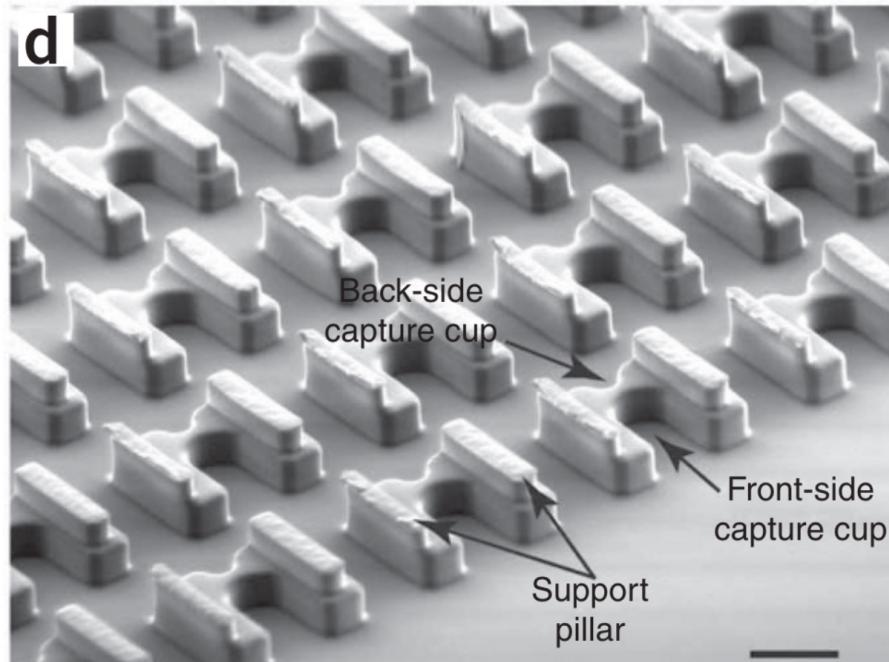
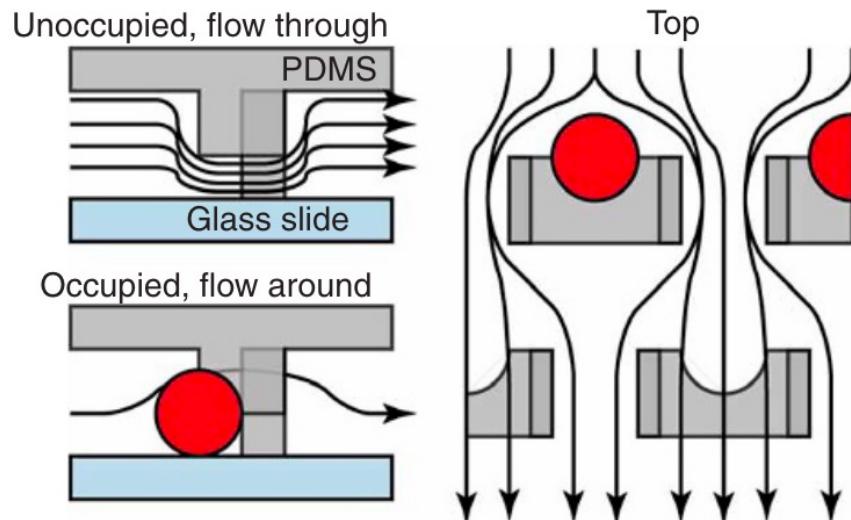
Problems:
Poor cell contacts
Random cell contacts

→ Low yield of hybridoma formation, antibiotic selection, lengthy subculturing

Microfluidic control of cell pairing and fusion

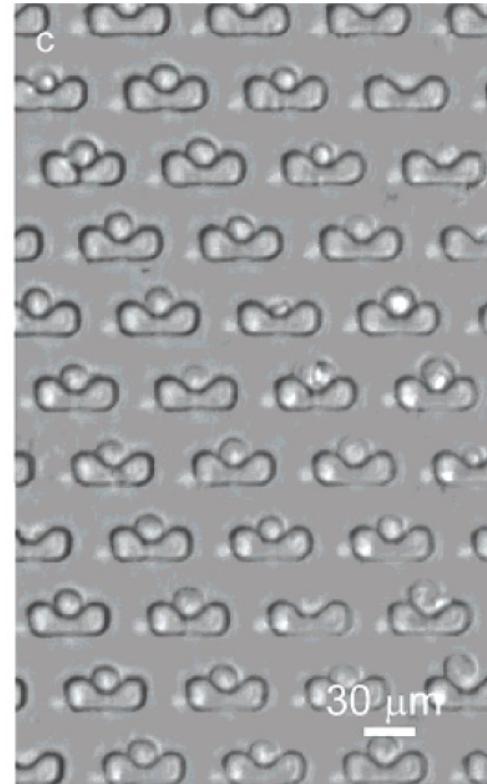
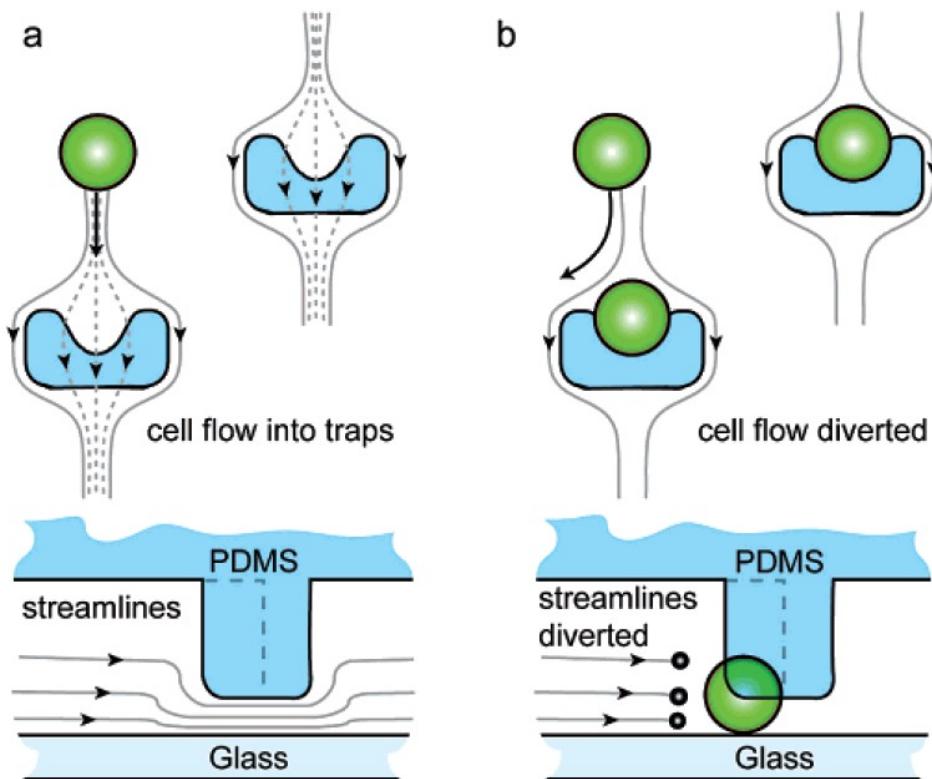
Here:

Microfluidic device containing dense array of **weir-type** passive hydrodynamic cell traps



Voldman & Jaenisch labs:
Skelley et al., *Nature Methods*, 2009

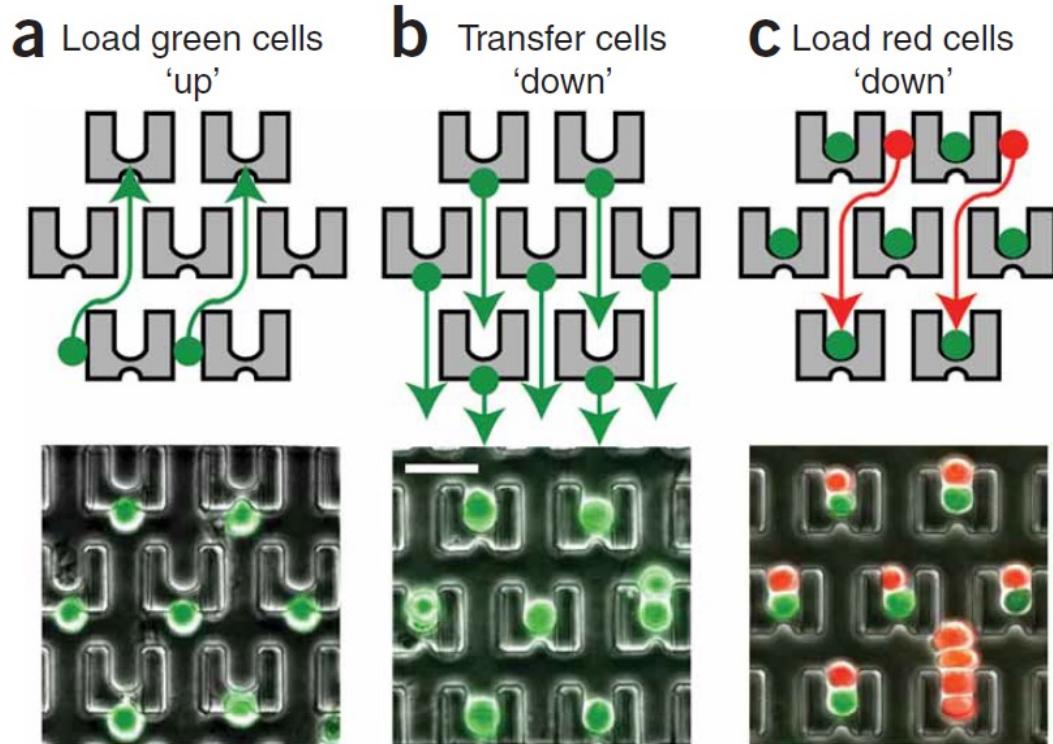
Arrays of hydrodynamic traps: Single cell trapping – uniform cell distribution



Di Carlo lab: Di Carlo et al., Anal. Chem 2006

- Traps with size of one cell
- Small channel under trap allows flow through obstacle
- Single cell fits into trap, closes channel
- Further cells are diverted into empty traps
- High efficiency of single cell trapping
- **Automated observation of regularly spaced single cells**

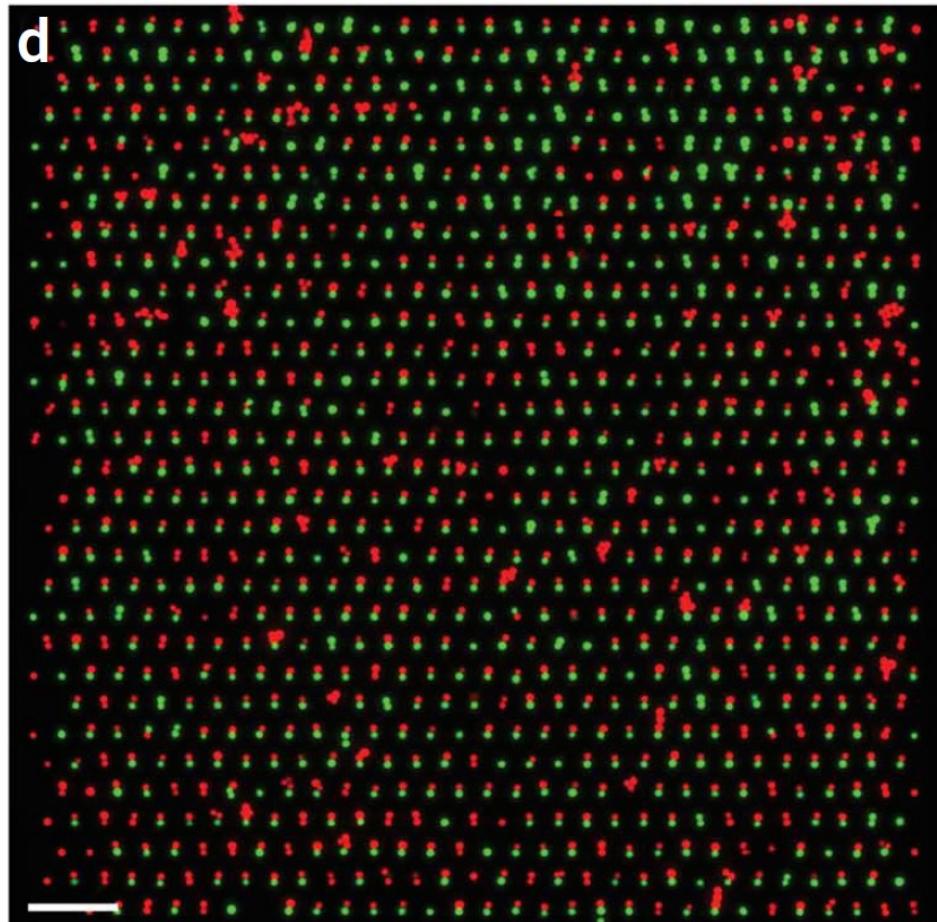
Pairing & fusion procedure



- Control of pairing using two flow directions in chip:
- First cells are loaded up, and transferred down
- Second cell batch are directly loaded down
- Microwell size optimized that mostly two cells are captured in the same trap
- Design allows fast solvent exchange, electric operations and microscopic observation

Voldman & Jaenisch labs:
Skelley et al., *Nature Methods*, 2009

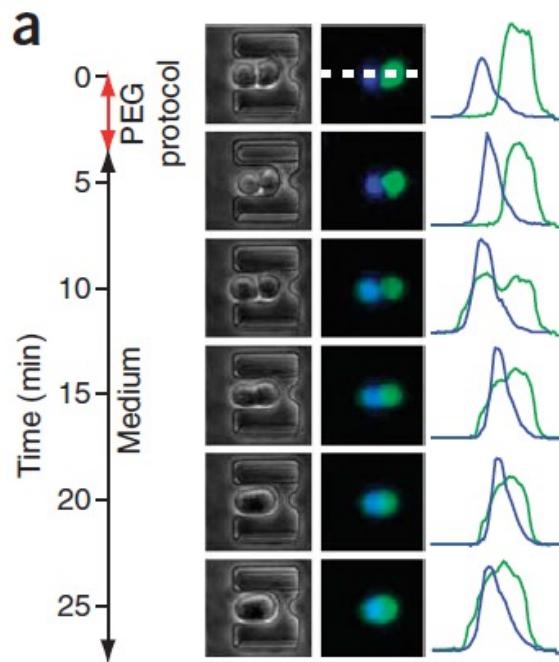
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Pairing & fusion procedure



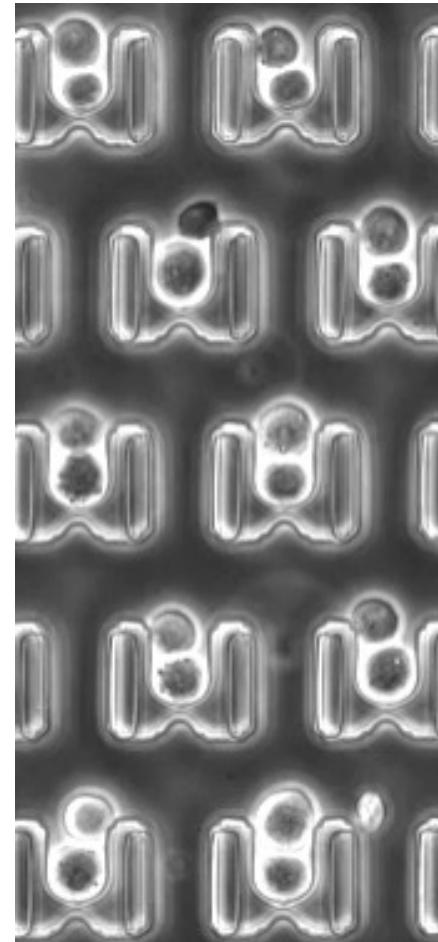
PEG based fusion

(osmotic shock)

GFP-mESC (mouse embryonic
stem cells)

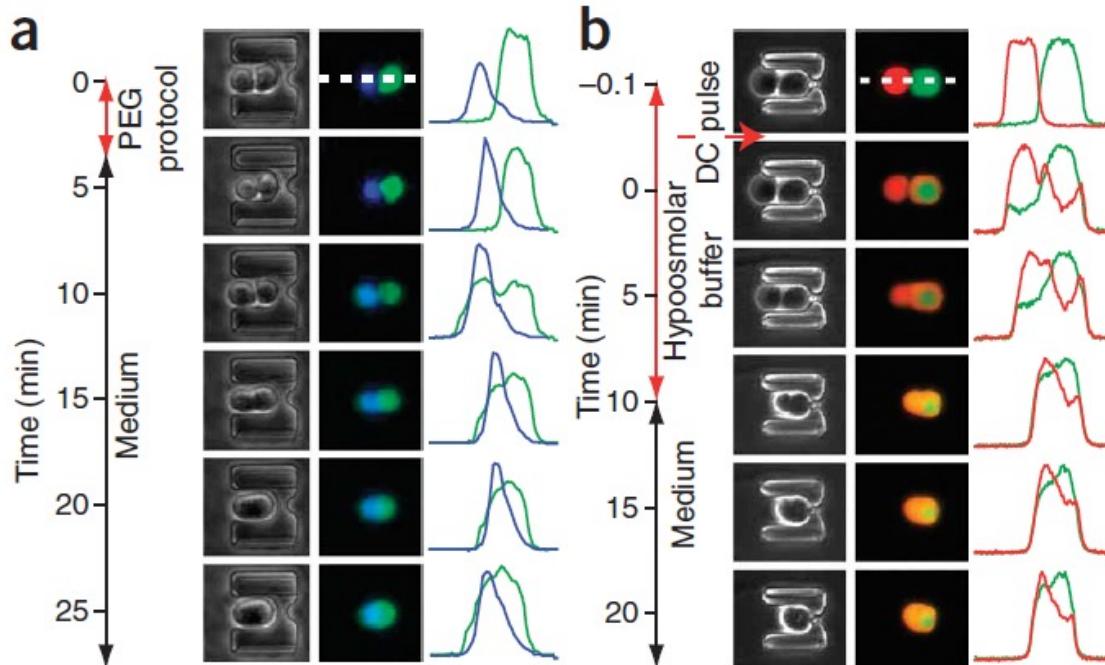
Hoechst-mEFs (fibroblasts)

*Voldman & Jaenisch labs:
Skelley et al., Nature Methods, 2009*



PEG flowing past immobilized 3T3 cells. Images were taken every 100 ms, and the video is playing at 4 frames/s.

Pairing & fusion procedure



PEG-based fusion

(osmotic shock)

GFP-mESC (mouse
embryonic stem cells)

Hoechst-mEFs (fibroblasts)

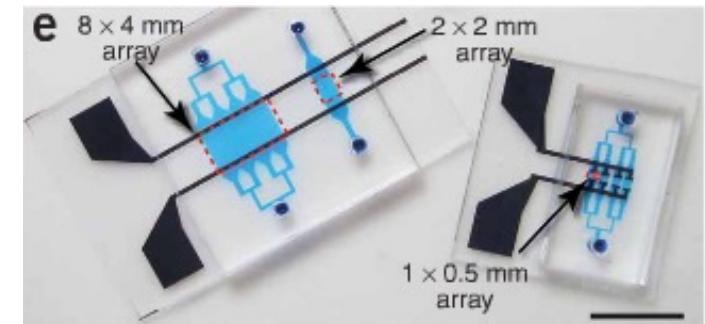
*Voldman & Jaenisch labs:
Skelley et al., Nature Methods, 2009*

Electrofusion

DsRED-3T3

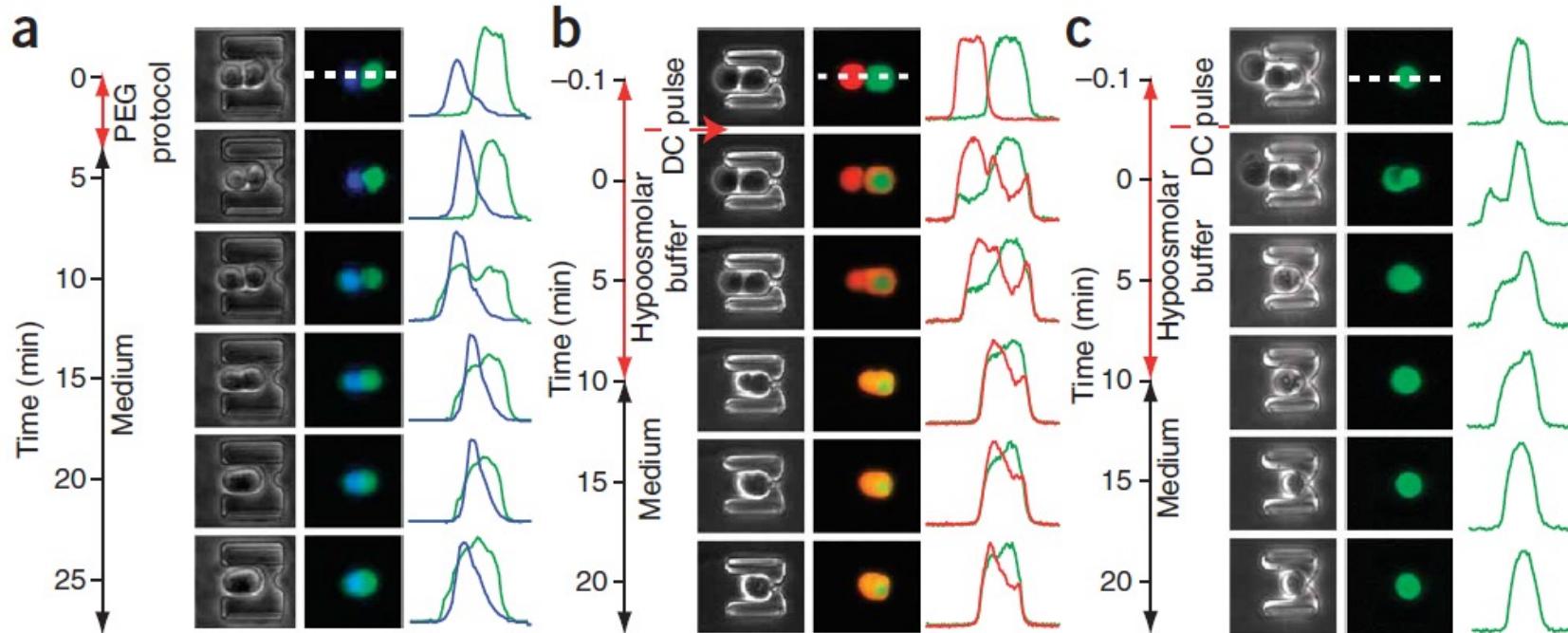
eGFP-3T3

- Induction of fusion: DC pulse
- Fluorescence change can be monitored in real time



Array, with electrodes

Pairing & fusion procedure



PEG based fusion

(osmotic shock)
GFP-mESC (mouse
embryonic stem cells)
Hoechst-mEFs (fibroblasts)

*Voldman & Jaenisch labs:
Skelley et al., Nature Methods, 2009*

Electrofusion

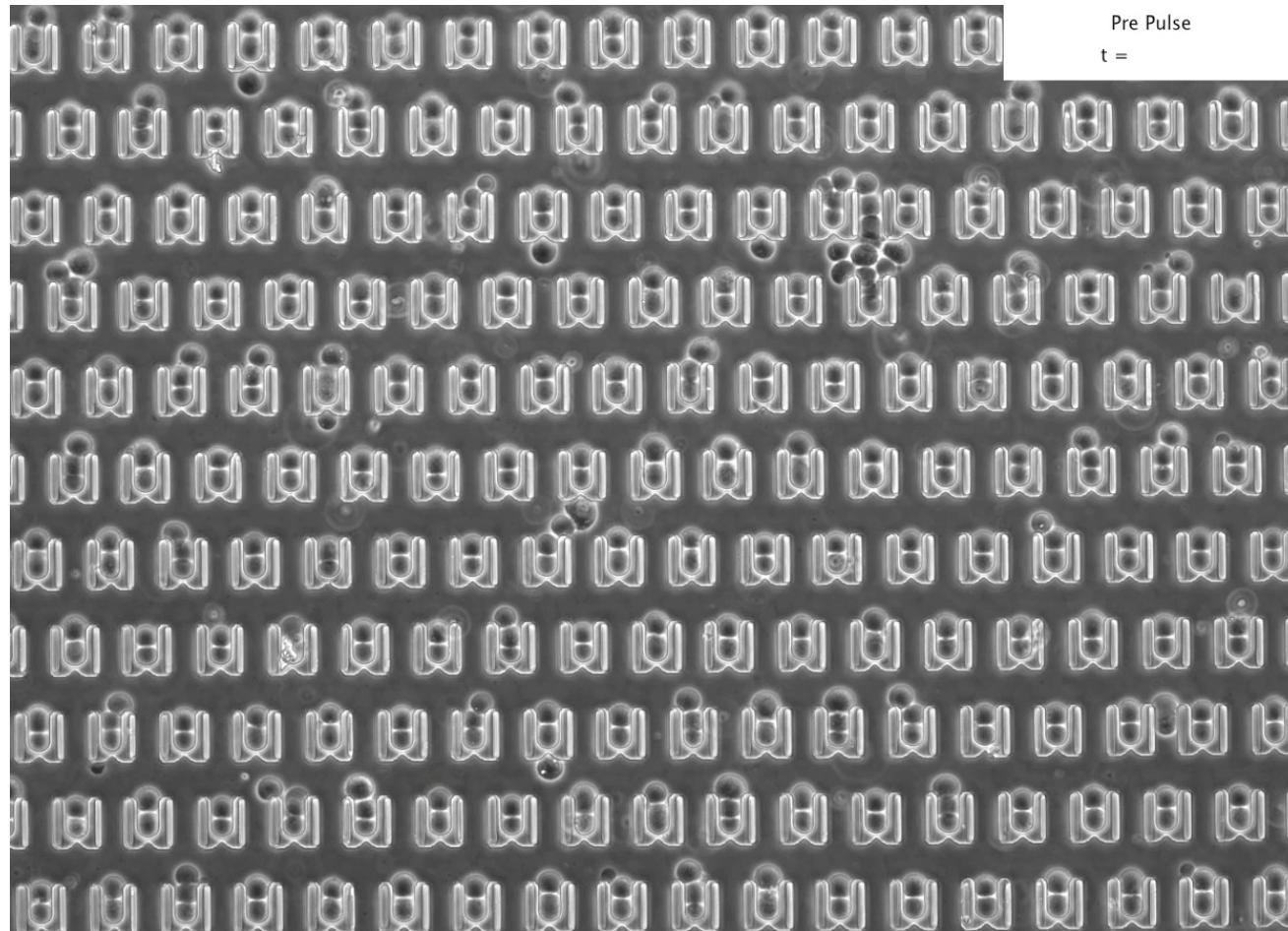
DsRED-3T3
eGFP-3T3

- Induction of fusion: DC pulse
- Fluorescence change can be monitored in real time

GFP-B cells (immune cells)

Myeloma (unstained)

Video 5: electrofusion



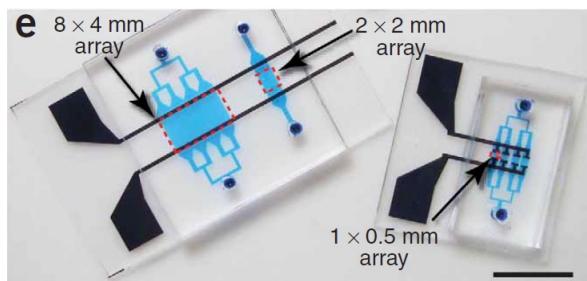
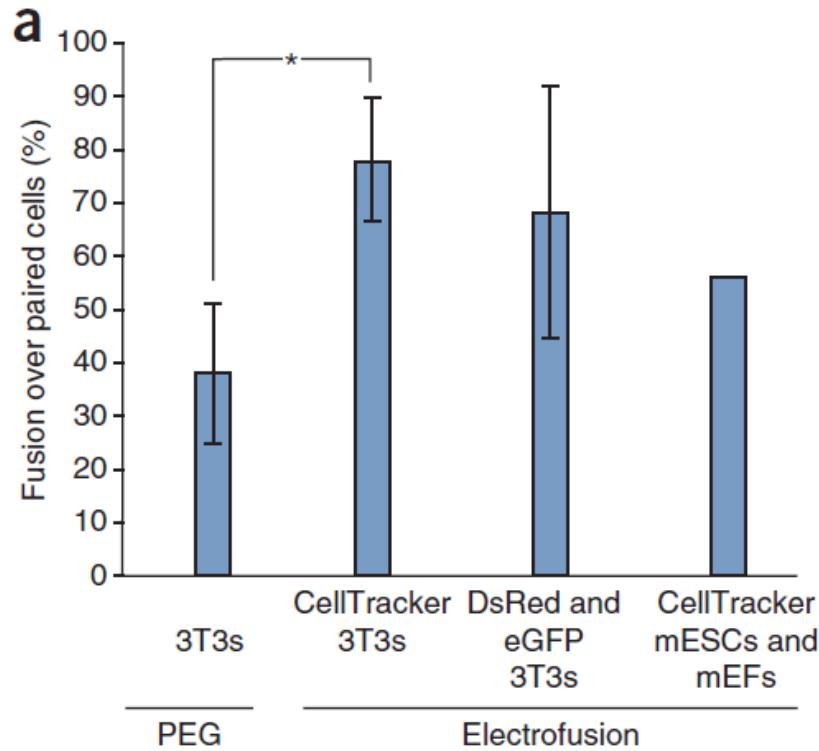
Both PEG-induced and electro-fusion cause **transient destabilization of membranes**

PEG-based fusion:
dehydration to rapid rehydration (shrinking then swelling)

Electrofusion:
Electroporation under hypoosmolar conditions (swelling/stretching)

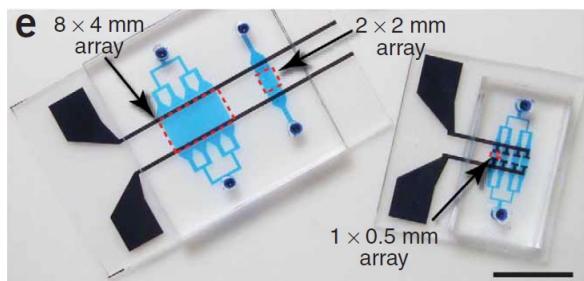
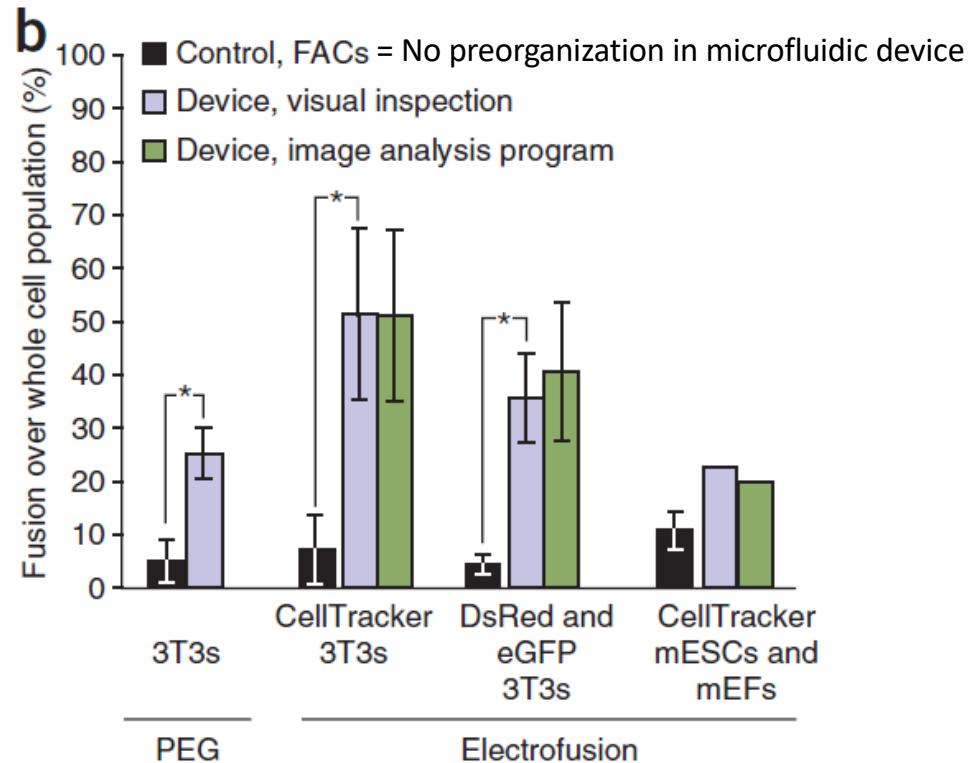
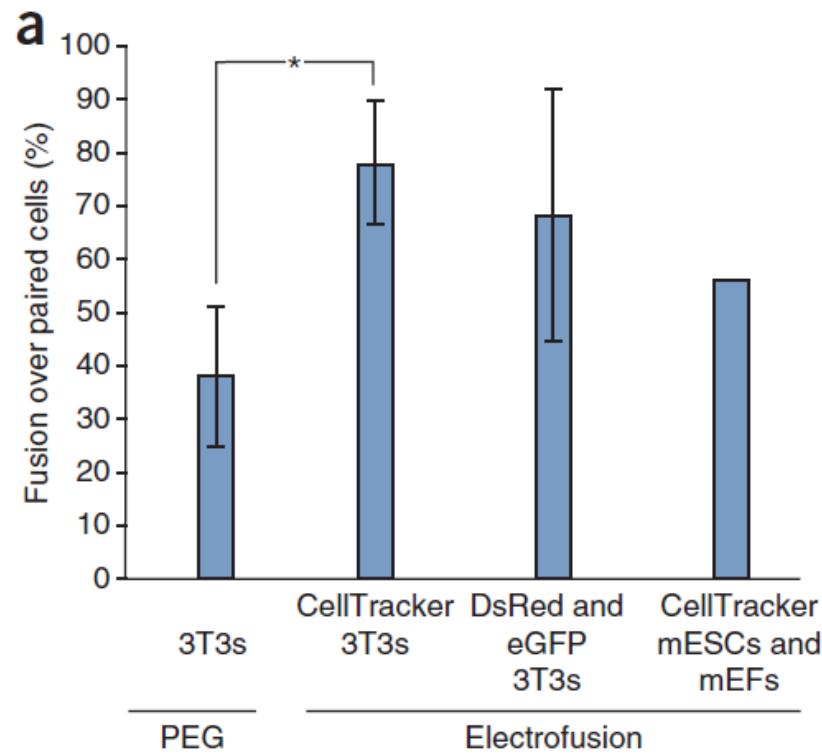
Video shows fusion of 3T3s with a single electrofusion pulse over entire field of view at 10× magnification. Images were taken every 5 minutes, and the video is playing at 2 frames/s.

Quantification of fusion efficiency



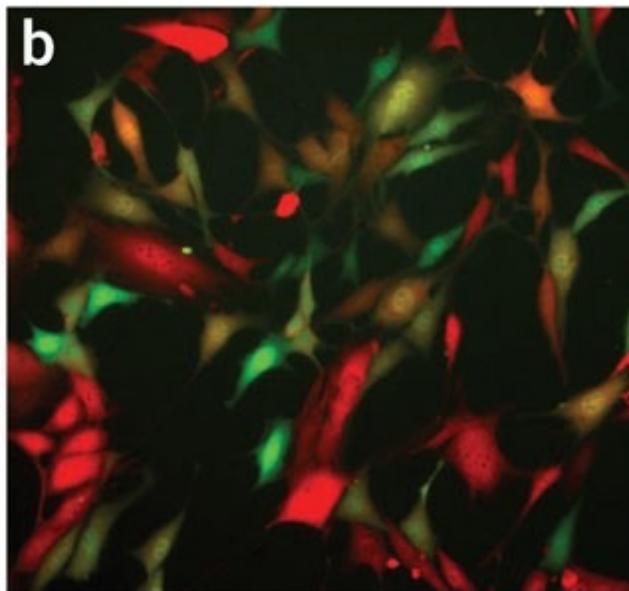
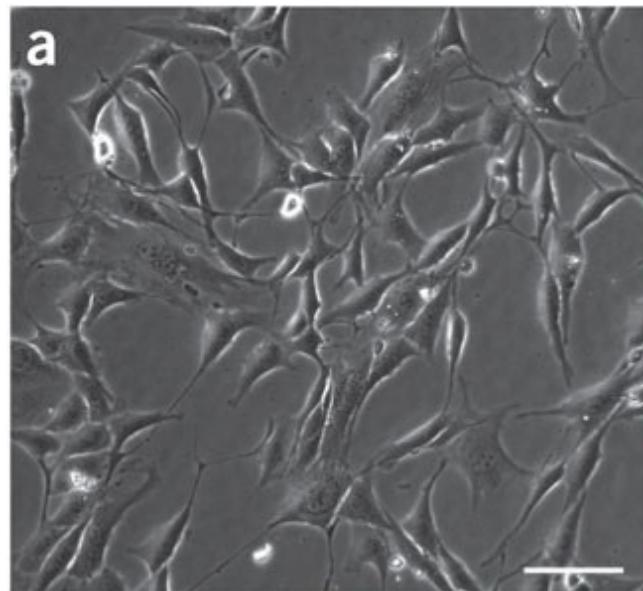
- Better yield compared to classical methods (up to 50%)
- Automated analysis of fusion yield
- Electrofusion yields better results than PEG based.

Quantification of fusion efficiency



- Better yield compared to classical methods (up to 50%)
- Automated analysis of fusion yield
- Electrofusion yields better results than PEG based.

Fused cells are viable



- a. Phase-contrast and
- b. Fluorescence image of DsRed-eGFP 3T3 cells 4 days after fusion

Conclusions

- Automated **device** was presented that can **fuse thousands of cells** with very high efficiency
- Different fusion protocols were evaluated on the single cell level
- Cells can be retrieved and cultured
- Fused cells are viable:
 - 3T3s were removed from device and cultured
 - Viable double positive red-green cells were observed
- Embryonic stem cells were fused with somatic cells (mESCs & mEFs)
- The resulting fusion cells remained stem cells in culture, demonstrating epigenetic reprogramming

Activity: What's the next step? PART A

1. Form pairs or groups of three.
2. Discuss potential future research directions or applications of the technology presented. These do not have to be very specific, they can be broad next logical steps.

Think about: Who would cite this paper? What type of research could they be doing? What research question could they be trying to answer?

After 5-10 minutes, we will discuss together and record 3-4 areas for further research (Ideas 1-4) on the Miro board.

https://miro.com/app/board/uXjVlxgiQ4s=/?share_link_id=707774651553

Activity: What's the next step? PART B

3. Use an online scientific database (Web of Science, PubMed, or similar) to do a forward search for papers citing the original study.
4. Use keywords discussed in groups to find a related research study.
5. Share a link/DOI of the study on the Miro quadrant category that fits best.
6. **On a linked note on the Miro board: Summarize in one sentence how this study is linked to the original paper.**

Another fun resource to make connections

- <https://researchrabbitapp.com/>

That's it!

- Thank you for your openness to learn,
for all the work you've put in.