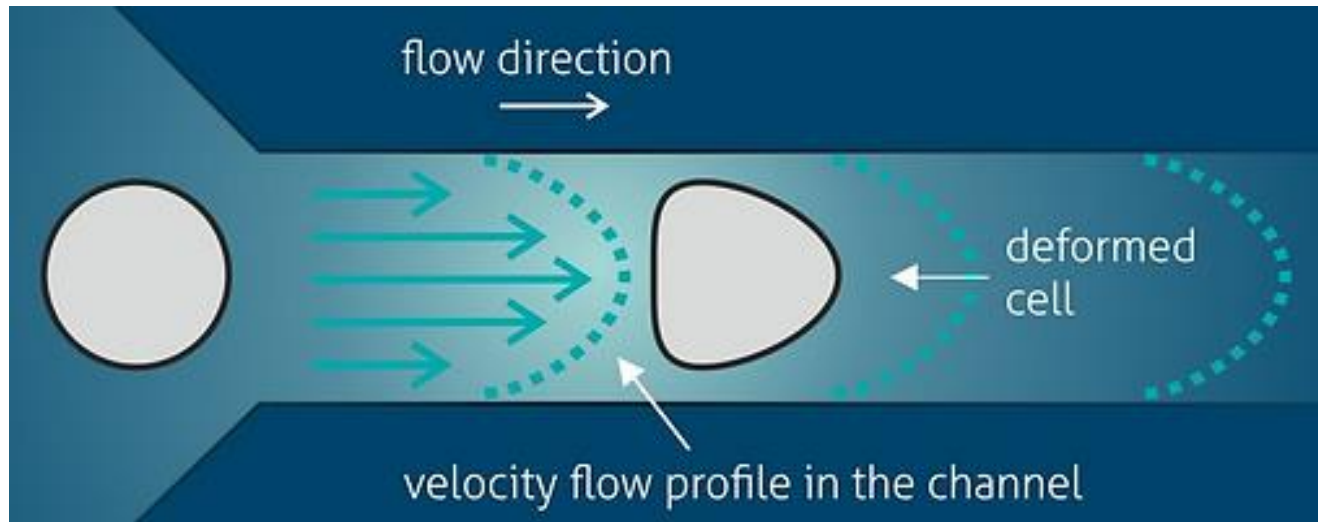


Real-Time Deformability Cytometry



<https://www.imaging-cytometry.com/media/thumbnails/00e/26b76ef75c23be860c3ad9d72ea1d00e/5d6182a0/6.jpg>
Accessed: 05/24/19

Seminar Talk on 28.05.2019

By Mariella Bosch

Outline

1. Introduction

- Stem cell isolation
- Detection methods for mechanical properties

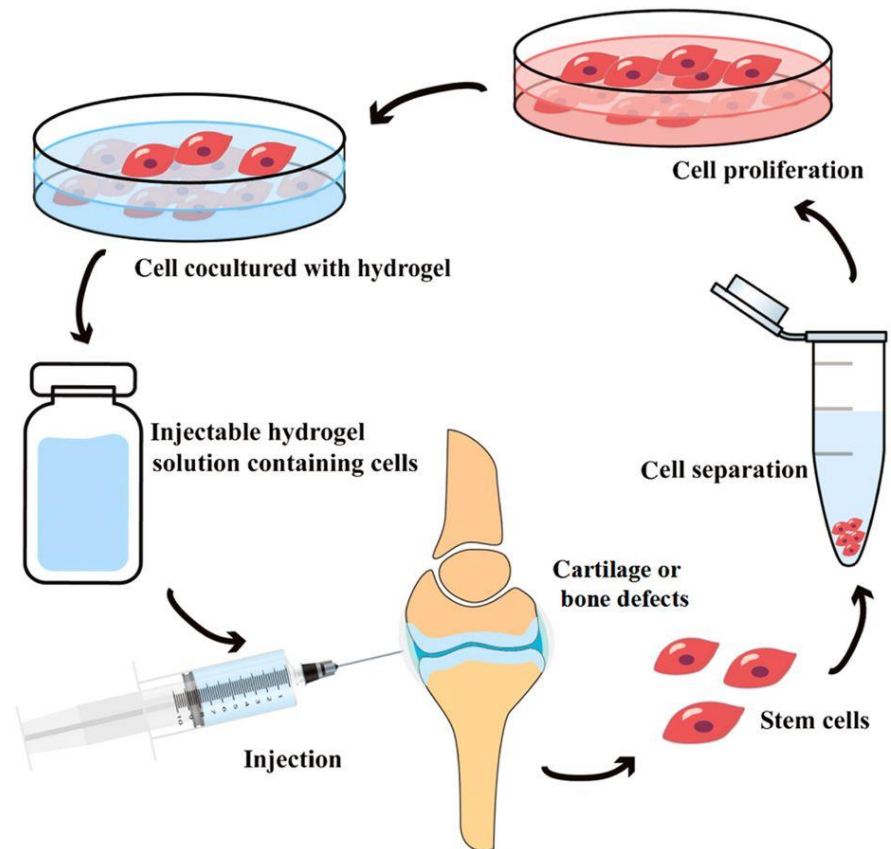
2. Real-time Deformability Cytometry (RT-DC)

- Setup
- Application

3. Summary & Conclusion

Introduction

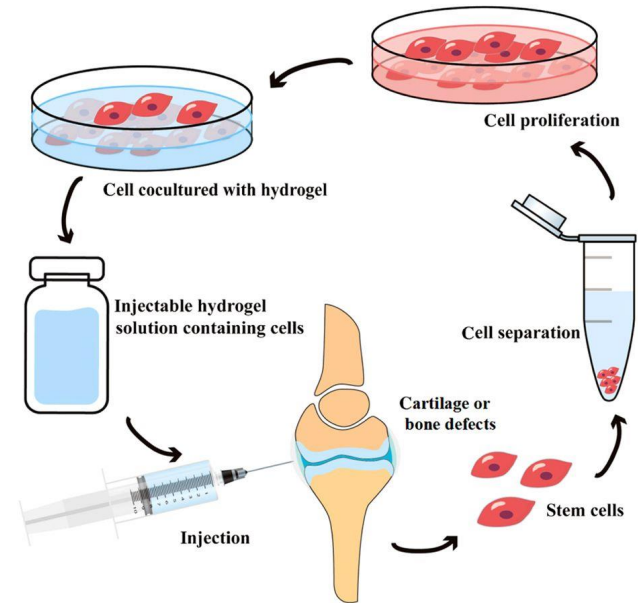
- Regenerative medicine/Tissue engineering
 - E.g. bone and cartilage repair with **skeletal stem cells (SSCs)**



https://media.nature.com/lw926/nature-assets/boneres/2017/boneres201714/images_hires/boneres201714-f1.jpg
Accessed: 05/25/19

Introduction

- Regenerative medicine/Tissue engineering
 - E.g. bone and cartilage repair with skeletal stem cells (SSCs)
- **Need:** homogeneous SSC population
- **Problem:** Mixture of different cells
 - E.g. SSCs very small fraction of bone marrow cells



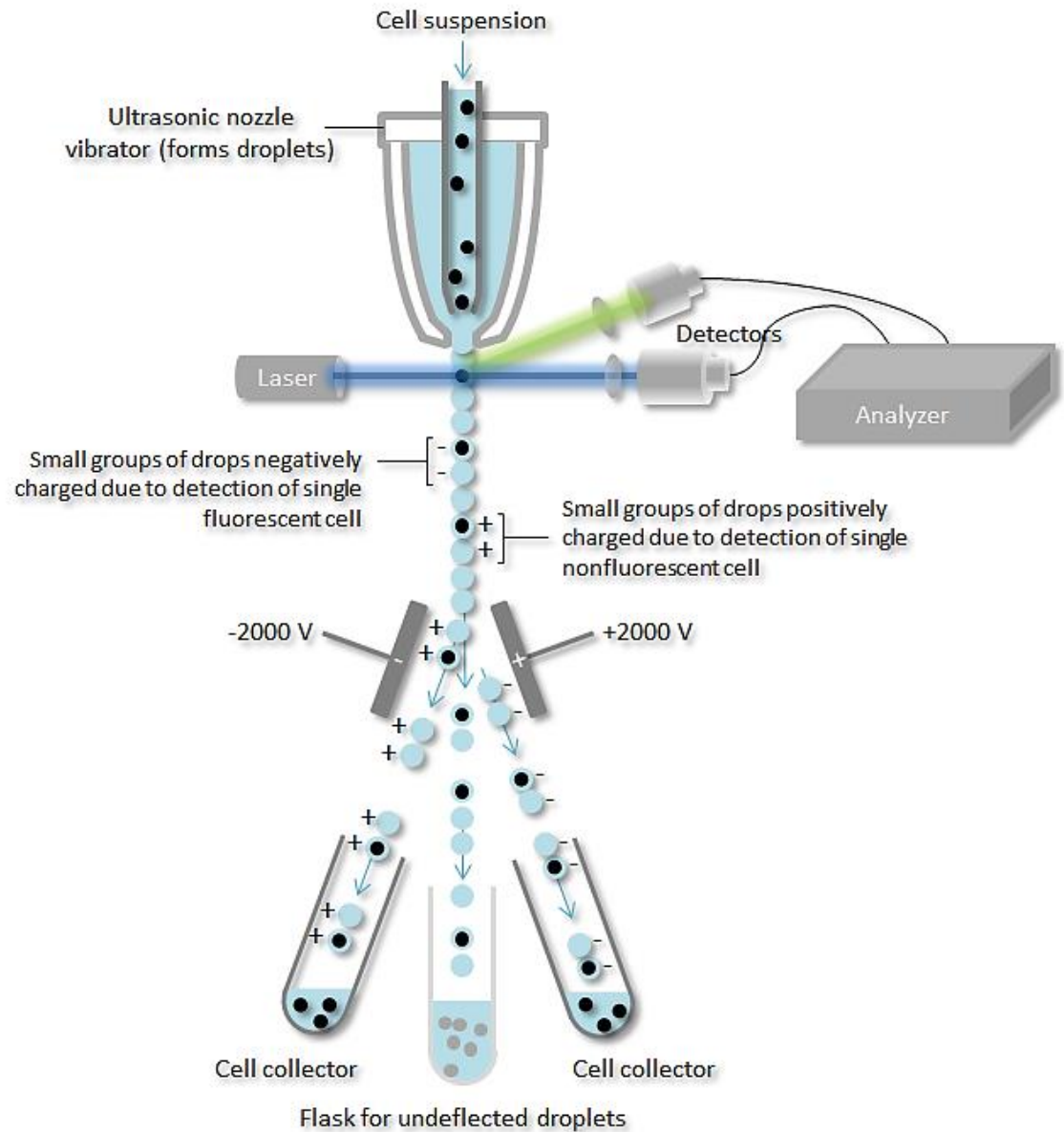
https://media.nature.com/lw926/nature-assets/boneres/2017/boneres201714/images_hires/boneres201714-f1.jpg
Accessed: 05/25/19

How can certain cells be isolated from others?

- Various isolation methods*:
 - Centrifugation
 - Selective culture media
 - Magnetic activated cell sorting (MACS)
 - Fluorescence activated cell sorting (FACS)
 - ...

* Gothard, David; Tare, Rahul (2011): In search of the skeletal stem cell: isolation and separation strategies at the macro/micro scale for skeletal regeneration. *Lab Chip* **11**, 1206–1220

Fluorescence activated cell sorting (FACS)



https://cdn1.sinobiological.com/styles/default/images/pdymg/FCM/flow_cytometry_fcm_fac fluoresce-activate/u14.png; Accessed: 05/19/19

How can certain cells be isolated from others?

- Various isolation methods*:

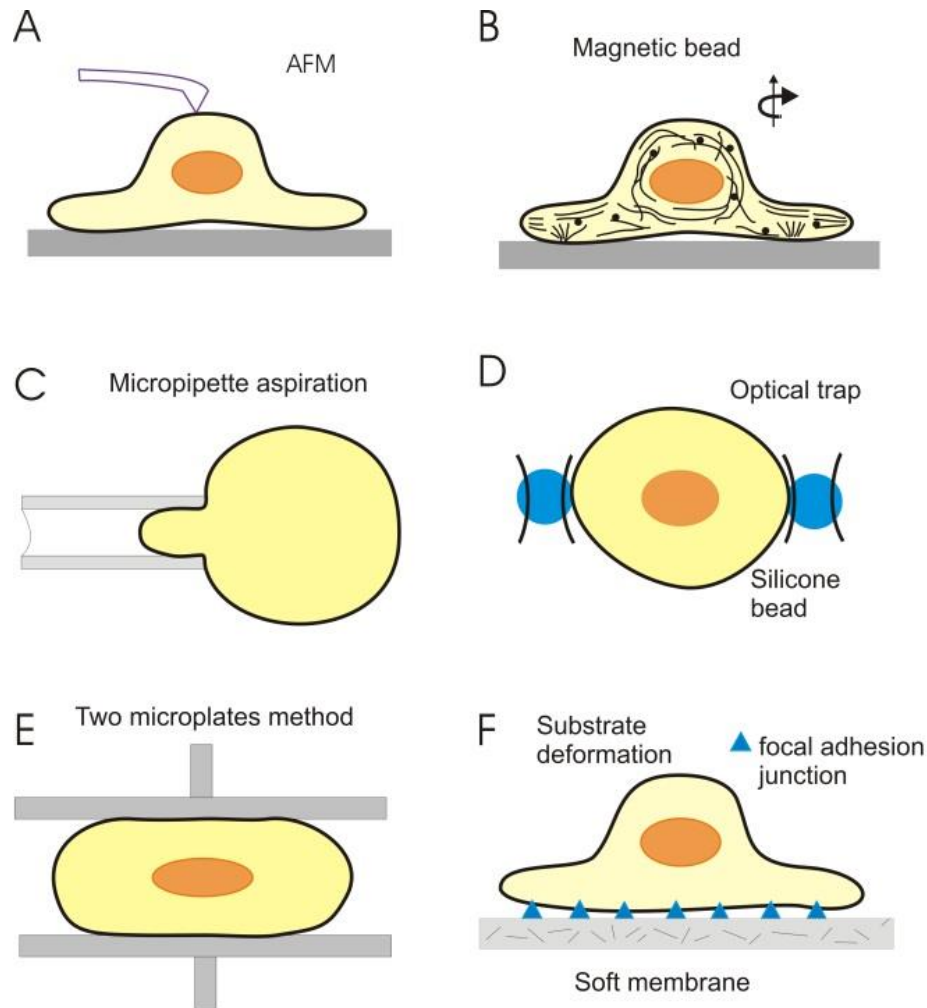
- Centrifugation
 - Selective culture media
 - Magnetic activated cell sorting (MACS)
 - Fluorescence activated cell sorting (FACS)
 - ...
- } • High number of cells
• Lack in purity
- } • High number of cells
• High(er) purity
• Cells are labelled

* Gothard, David; Tare, Rahul (2011): In search of the skeletal stem cell: isolation and separation strategies at the macro/micro scale for skeletal regeneration. *Lab Chip* 11, 1206–1220

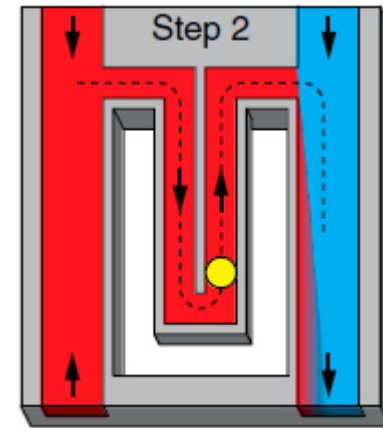
Need: Marker-free, non-destructive method with high purity

→ **Idea:** Measure mechanical properties

Mechanical Properties - Detection methods



Measuring weight and density



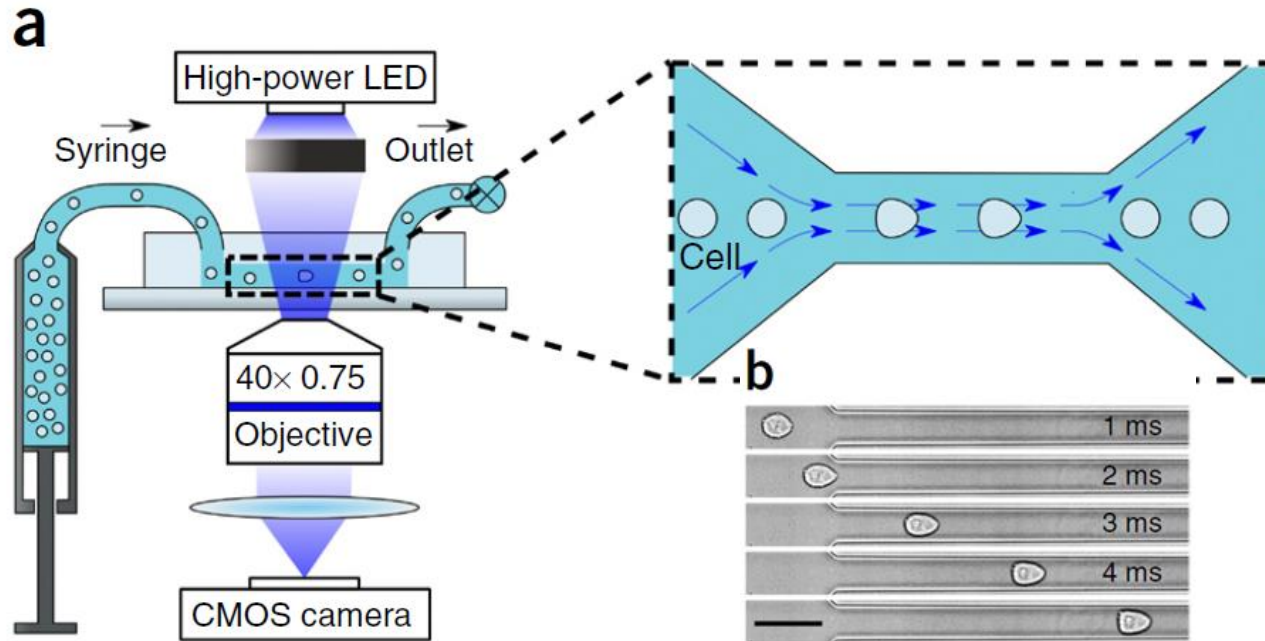
Grover, William; Bryan, Andrea et al. (2011):
Measuring single-cell density *Proc. Natl. Acad. Sci.*
108, 10992–10996. (Figure 2)

Problem:

- Low throughput
- Long analysis time

<https://api.intechopen.com/media/chapter/22184/media/image7.jpg> Accessed: 05/25/19

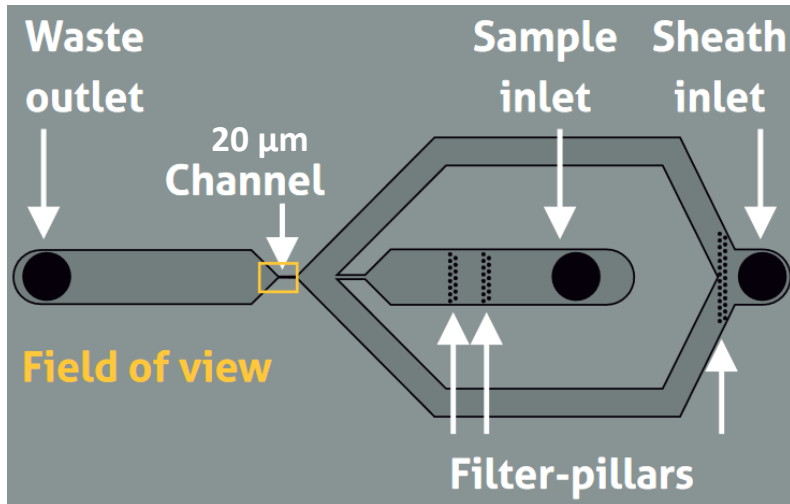
Real-time Deformability Cytometry (RT-DC)



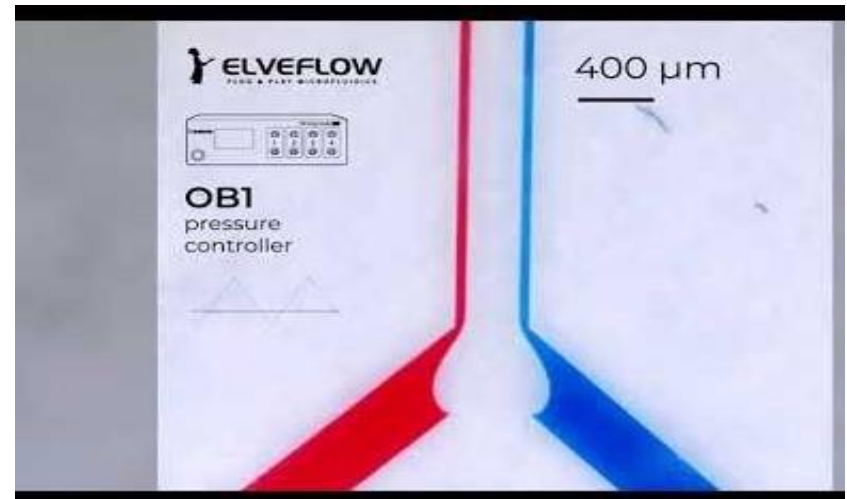
Otto, Oliver; Rosendahl, Philipp et al. (2015): Real-time deformability cytometry. On-the-fly cell mechanical phenotyping. *Nature methods* 12 (3), 199-202. DOI: 10.1038/nmeth.3281. (Figure 1a,b)

- Contactless, non-destructive, marker-free
- High-throughput (1000 cells/s)
- Real-time image analysis

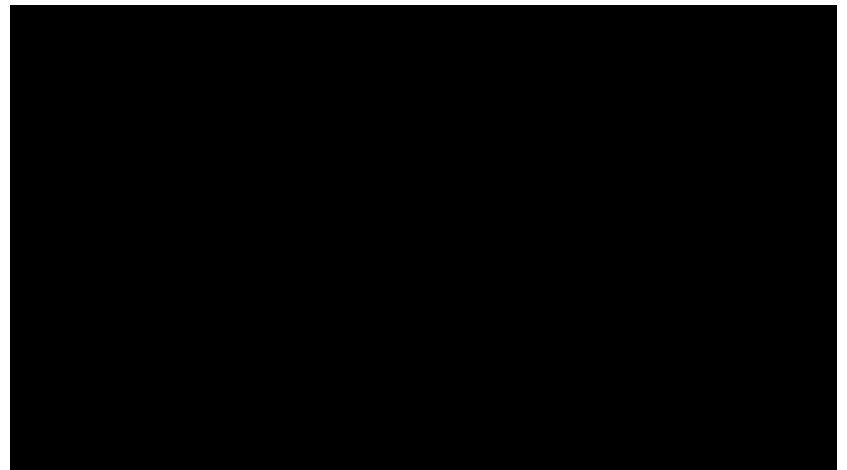
RT-DC Setup



https://www.zellmechanik.com/media/tyfo150-42f638c3f11dbdcedc9f1083766c6bc0/short_manual.pdf

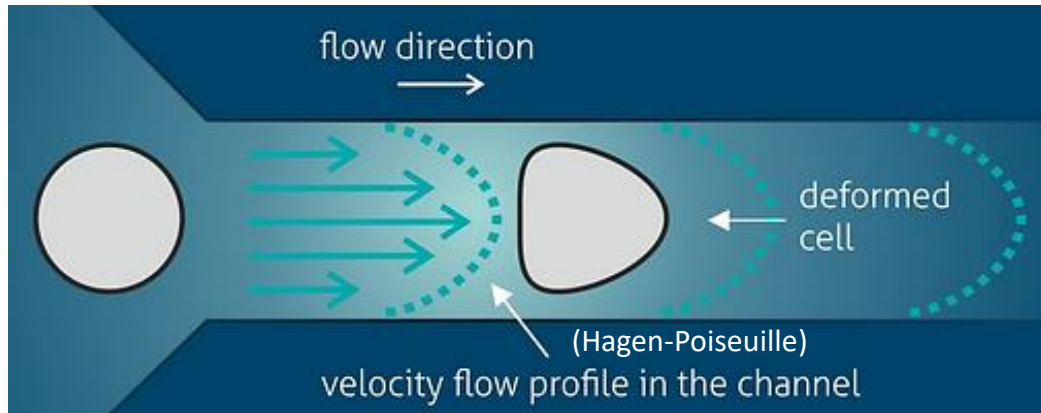


https://www.youtube.com/watch?v=hZT2K1znc_I



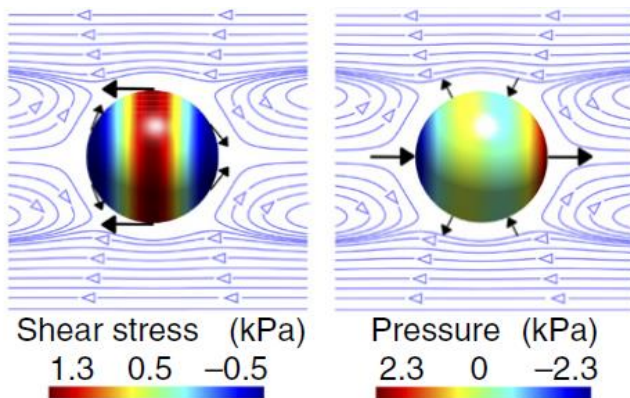
https://www.youtube.com/watch?time_continue=9&v=jAhdIcnWMBE

RT-DC: Deformation

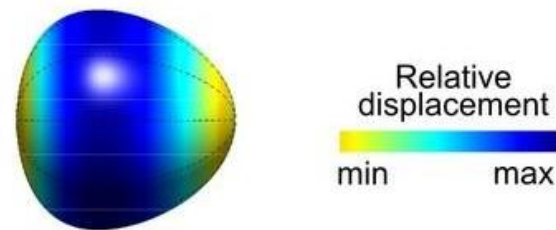


Forces can be controlled by flow velocity and viscosity of the medium

<https://www.imaging-cytometry.com/media/thumbnails/00e/26b76ef75c23be860c3ad9d72ea1d00e/5d6182a0/6.jpg>
Accessed: 05/24/19



Characteristic „bullet“ shape:

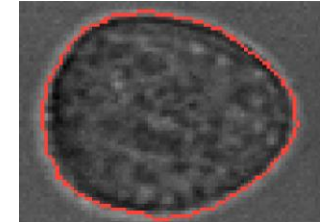


Otto, Oliver; Rosendahl, Philipp et al. (2015): Real-time deformability cytometry. On-the-fly cell mechanical phenotyping. *Nature methods* 12 (3), 199-202. DOI: 10.1038/nmeth.3281. (Figure 1d, suppl. Figure 1b)

RT-DC: Measurement of deformation

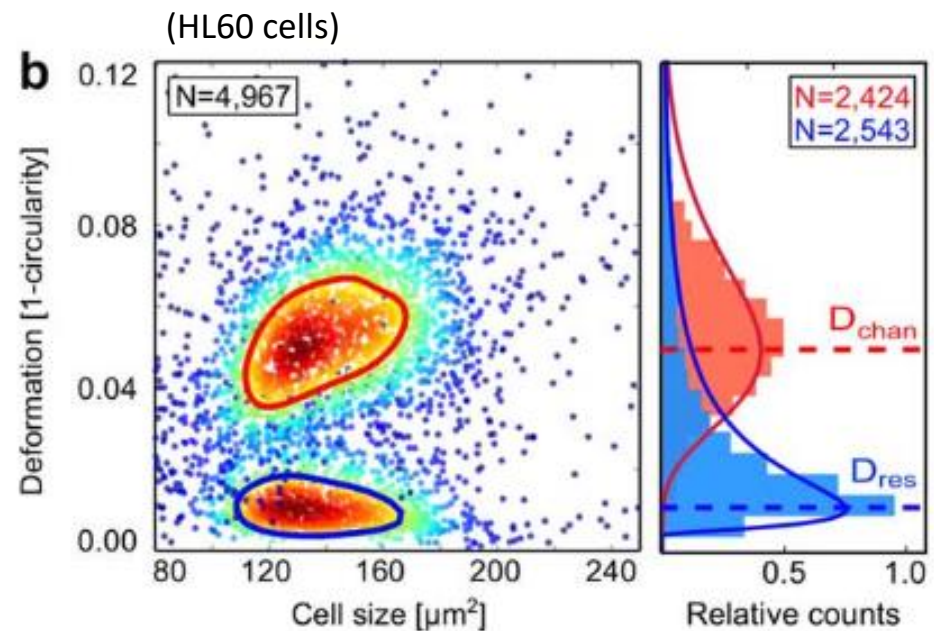
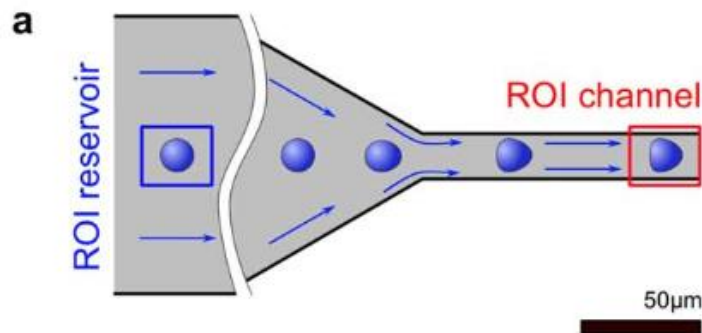
- Circularity: $c = \frac{2\sqrt{\pi A}}{P}$ (A : cross-sectional cell area, P : perimeter)

- circle: $c = 1$; any deformation: $c < 1$



- Deformation $D = 1 - c = 1 - \frac{2\sqrt{\pi A}}{P}$

- **Real-time image analysis**

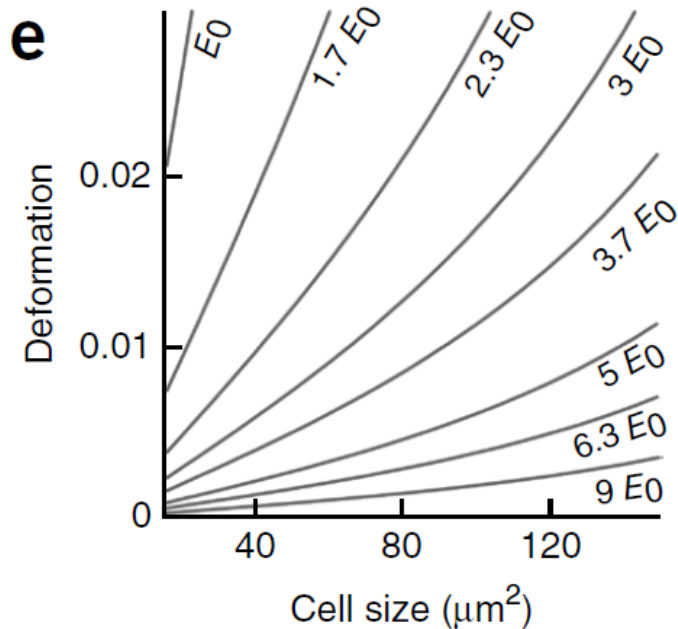


Golfier, Stefan; Rosendahl, Philipp et al. (2017) High-throughput cell mechanical phenotyping for label-free titration assays of cytoskeletal modifications. *Cytoskeleton* 74, 283–296. DOI: 10.1002/cm.21369. (Figure 1a,b)

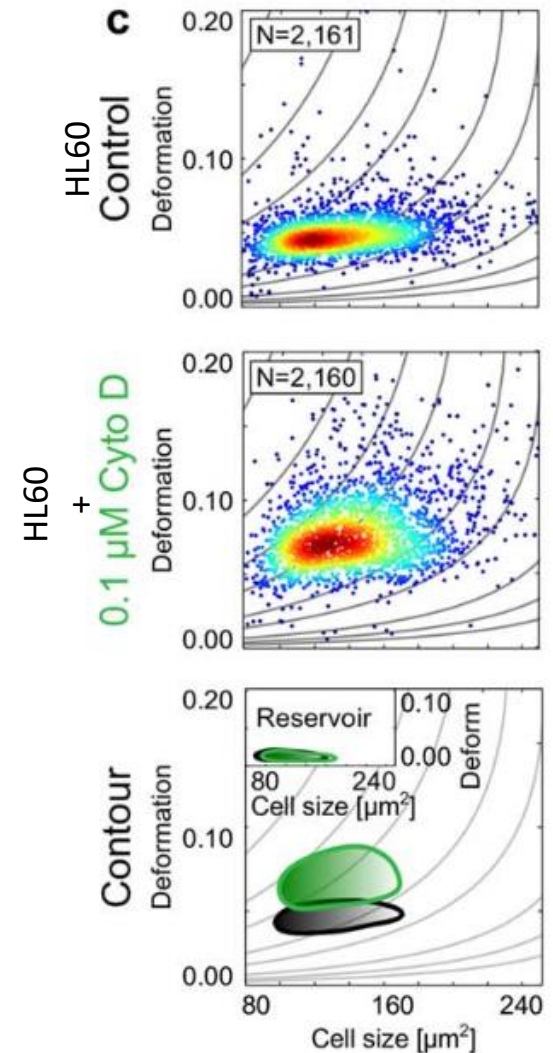
Linear elastic model

- “Problem”: Cell deformation depends on cell size
- larger cell $\rightarrow$ higher shear stresses

“linear elastic model which effectively decouples size and deformation by the introduction of isoelasticity lines”

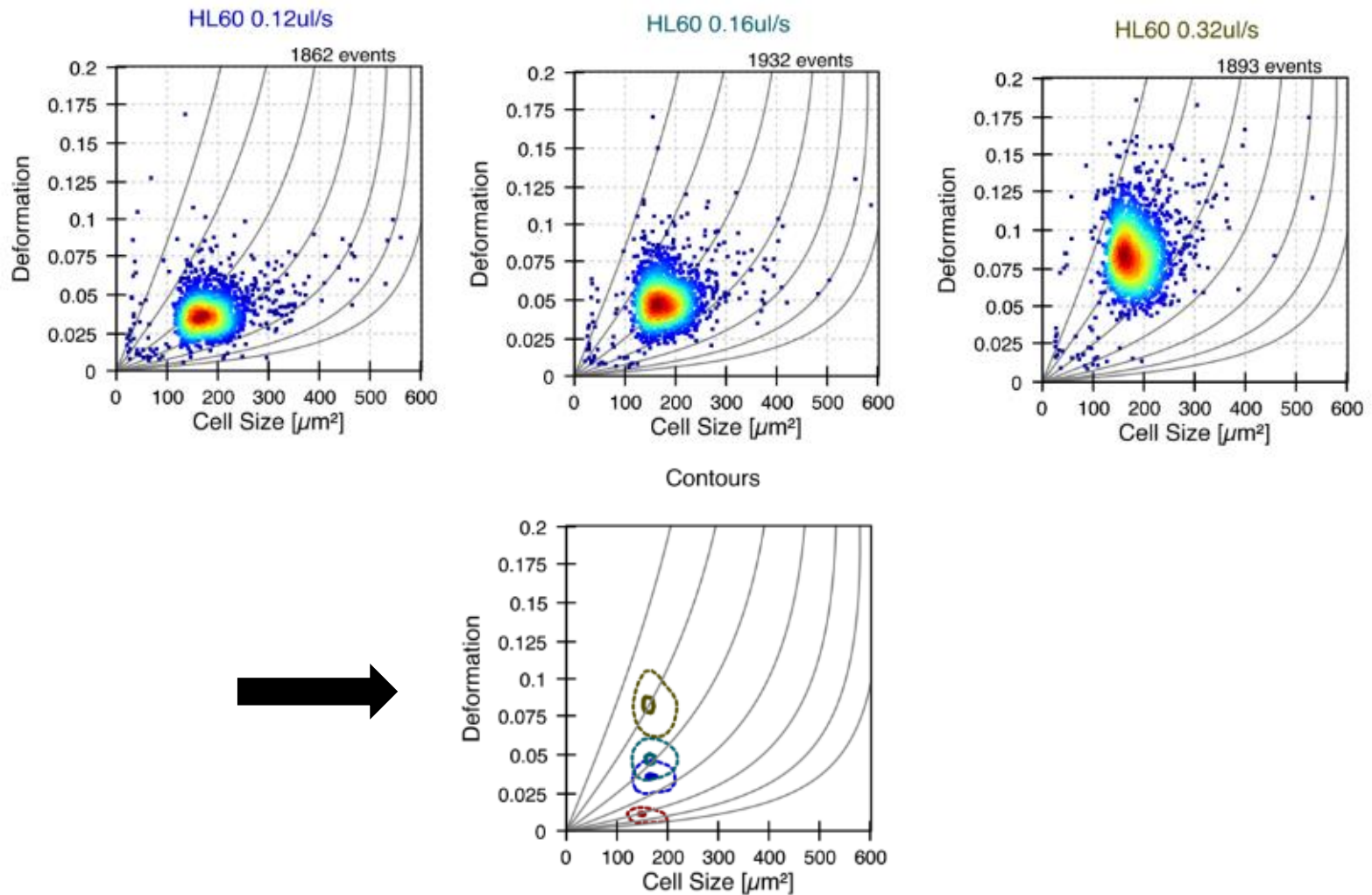


Otto, Oliver; Rosendahl, Philipp et al. (2015): Real-time deformability cytometry. On-the-fly cell mechanical phenotyping. *Nature methods* 12 (3), 199–202. DOI: 10.1038/nmeth.3281. (Figure 1e)



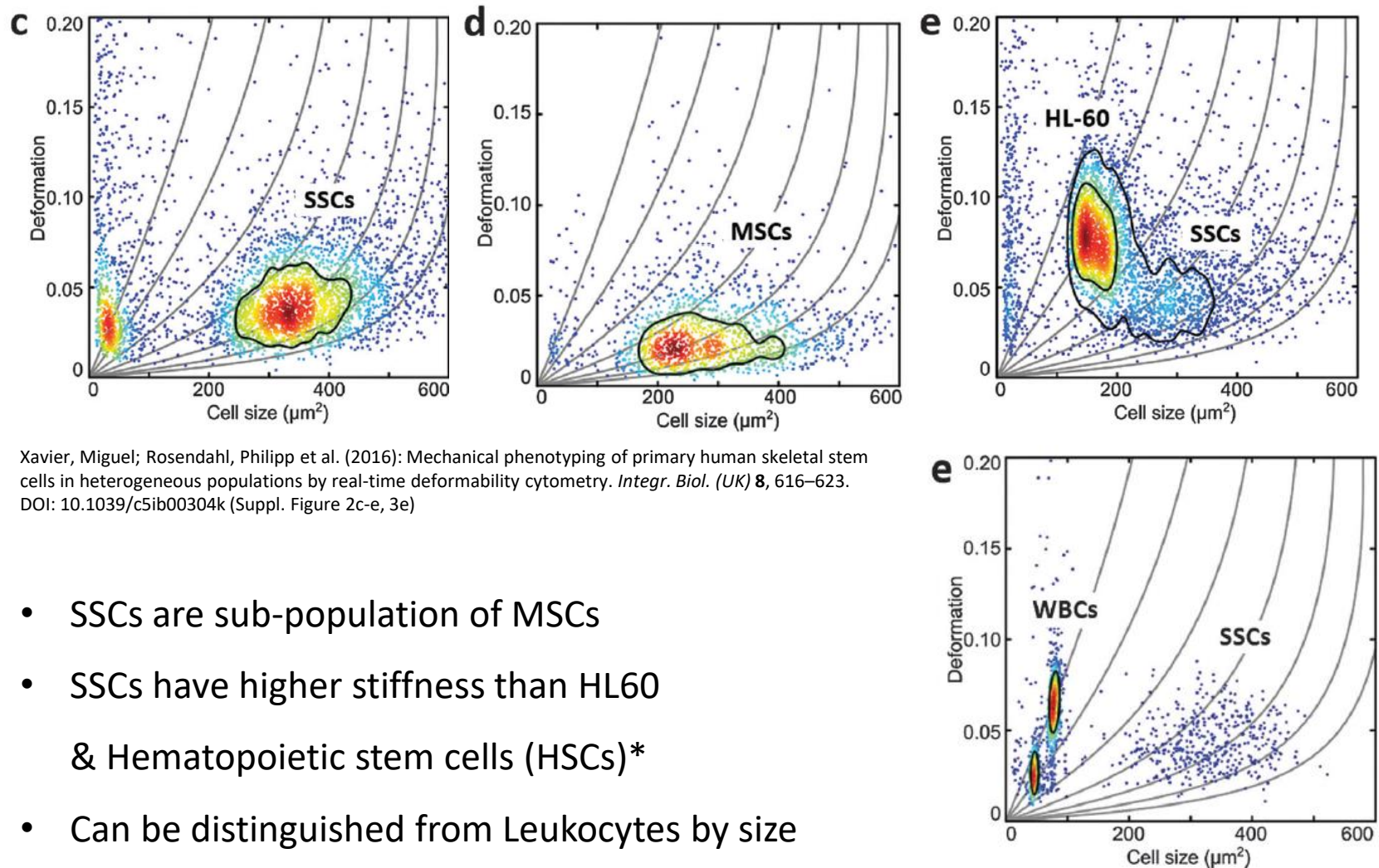
Golfier, Stefan; Rosendahl, Philipp et al. (2017): High-throughput cell mechanical phenotyping for label-free titration assays of cytoskeletal modifications. *Cytoskeleton* 74, 283–296. DOI: 10.1002/cm.21369. (Figure 1c)

Impact of flow rate on deformation



Xavier, Miguel; Rosendahl, Philipp et al. (2016): Mechanical phenotyping of primary human skeletal stem cells in heterogeneous populations by real-time deformability cytometry. *Integr. Biol. (UK)* 8, 616–623. DOI: 10.1039/c5ib00304k (Suppl. Figure 1)

RT-DC Application: SSC isolation

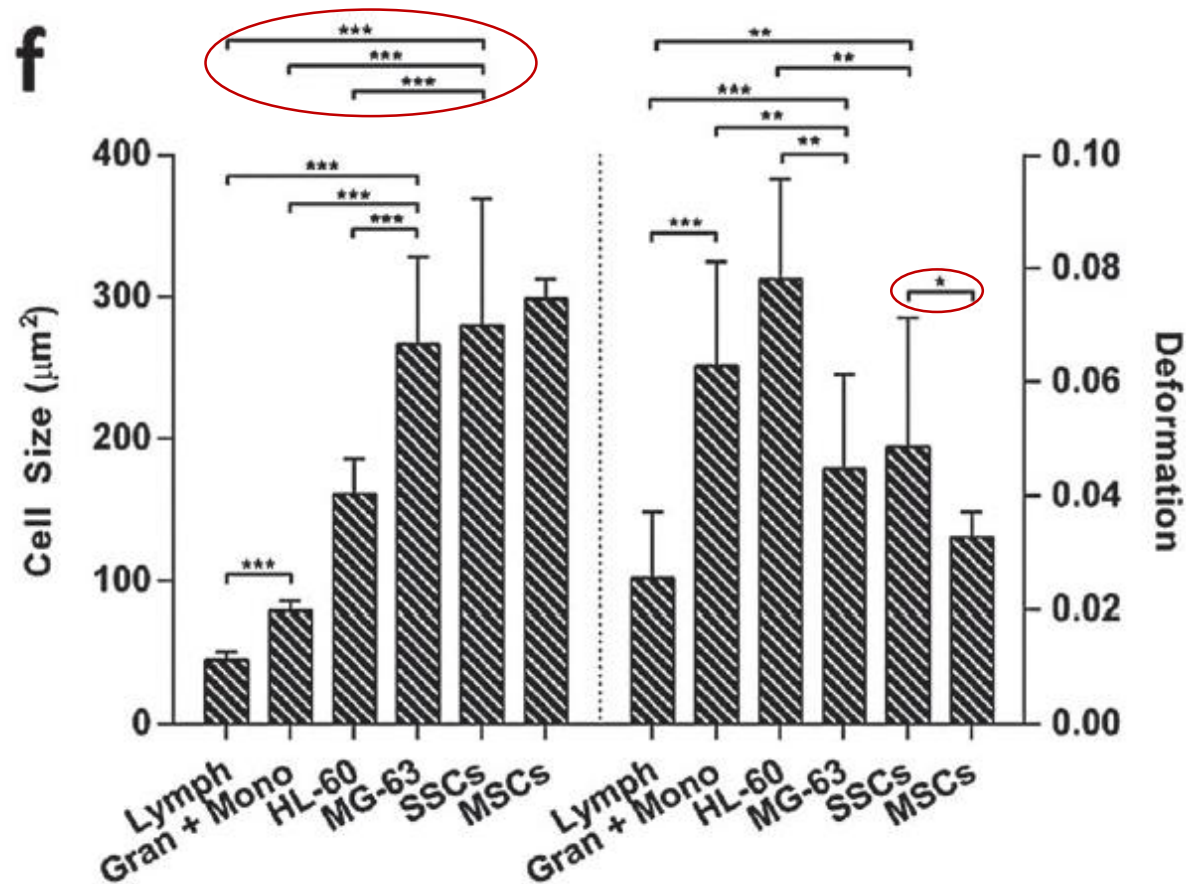


Xavier, Miguel; Rosendahl, Philipp et al. (2016): Mechanical phenotyping of primary human skeletal stem cells in heterogeneous populations by real-time deformability cytometry. *Integr. Biol. (UK)* 8, 616–623. DOI: 10.1039/c5ib00304k (Suppl. Figure 2c-e, 3e)

- SSCs are sub-population of MSCs
- SSCs have higher stiffness than HL60 & Hematopoietic stem cells (HSCs)*
- Can be distinguished from Leukocytes by size

* Otto, Oliver; Rosendahl, Philipp et al. (2015): Real-time deformability cytometry. On-the-fly cell mechanical phenotyping. *Nature methods* 12 (3), 199–202. DOI: 10.1038/nmeth.3281.

RT-DC example: Summary SSC isolation



Xavier, Miguel; Rosendahl, Philipp et al. (2016): Mechanical phenotyping of primary human skeletal stem cells in heterogeneous populations by real-time deformability cytometry. *Integr. Biol. (UK)* **8**, 616–623. DOI: 10.1039/c5ib00304k (Figure 3f)

RT-DC example: Summary SSC isolation

Could SSCs be isolated with this method?

- RT-DC could distinguish cells by their stiffness & size
- *No cell sorting yet*

→ RT-DC could improve SSC isolation in future

→ Possibility of combination with e.g electrostatic cell sorting or recently introduced “Intelligent Image-Activated Cell Sorting”

Further RT-DC Application

- Cell stiffness gives information about
 - Cell cycle
 - Differentiation of cells
 - Pathological changes
 - ...
- Application:
 - Detection of tumor cells
 - Investigation of blood and stem cells
 - Medical drug screenings
 - Combination with FACS for increased specificity

Summary RT-DC

- Mechanics as new biomarker
 - label-free, non-destructive forces
- High throughput
 - Ability to study large populations in a relatively short time
- Real-time image analysis
 - Opens possibility for active cell sorting

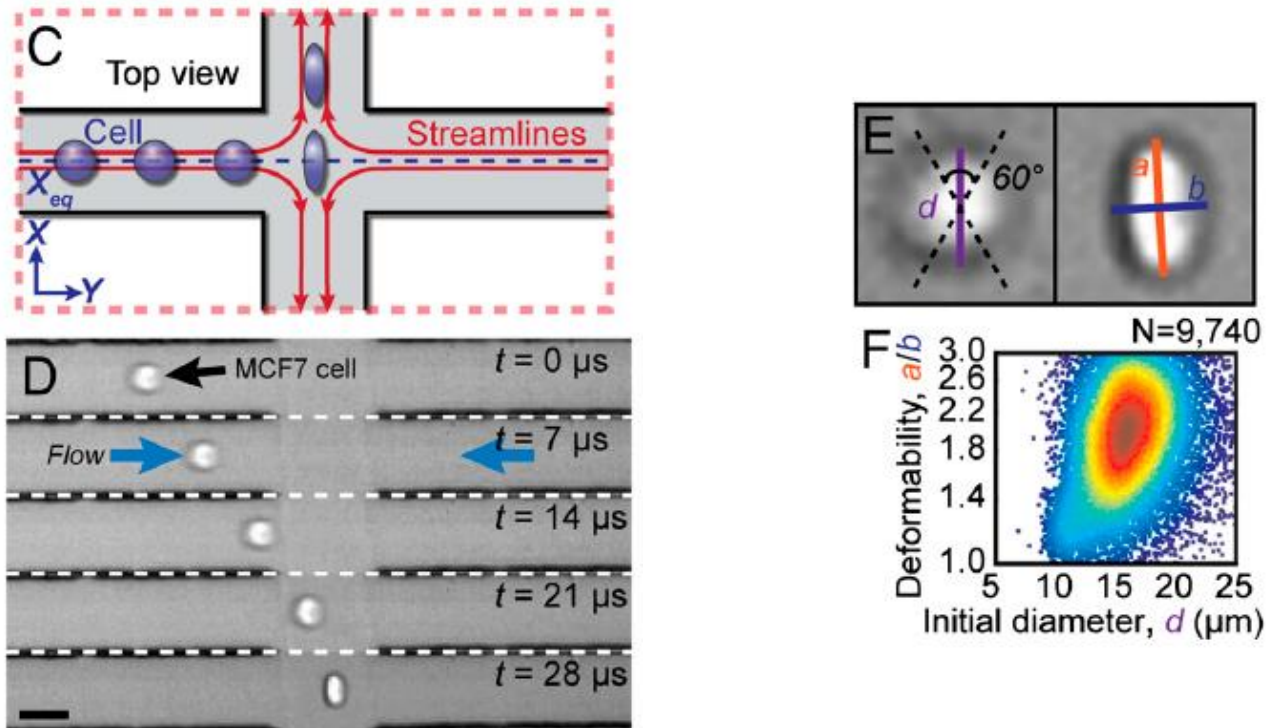
Thank you for your attention!

References

- Gothard, David; Tare, Rahul (2011): In search of the skeletal stem cell: isolation and separation strategies at the macro/micro scale for skeletal regeneration. *Lab Chip* **11**, 1206–1220
- Grover, William; Bryan, Andrea et al. (2011): Measuring single-cell density *Proc. Natl. Acad. Sci.* **108**, 10992–10996.
- Otto, Oliver; Rosendahl, Philipp et al. (2015): Real-time deformability cytometry. On-the-fly cell mechanical phenotyping. *Nature methods* **12** (3), 199–202. DOI: 10.1038/nmeth.3281.
- Golfier, Stefan; Rosendahl, Philipp et al. (2017) High-throughput cell mechanical phenotyping for label-free titration assays of cytoskeletal modifications. *Cytoskeleton* **74**, 283–296. DOI: 10.1002/cm.21369.
- Xavier, Miguel; Rosendahl, Philipp et al. (2016): Mechanical phenotyping of primary human skeletal stem cells in heterogeneous populations by real-time deformability cytometry. *Integr. Biol. (UK)* **8**, 616–623. DOI: 10.1039/c5ib00304k
- <https://www.zellmechanik.com>
- <https://www.imaging-cytometry.com/>

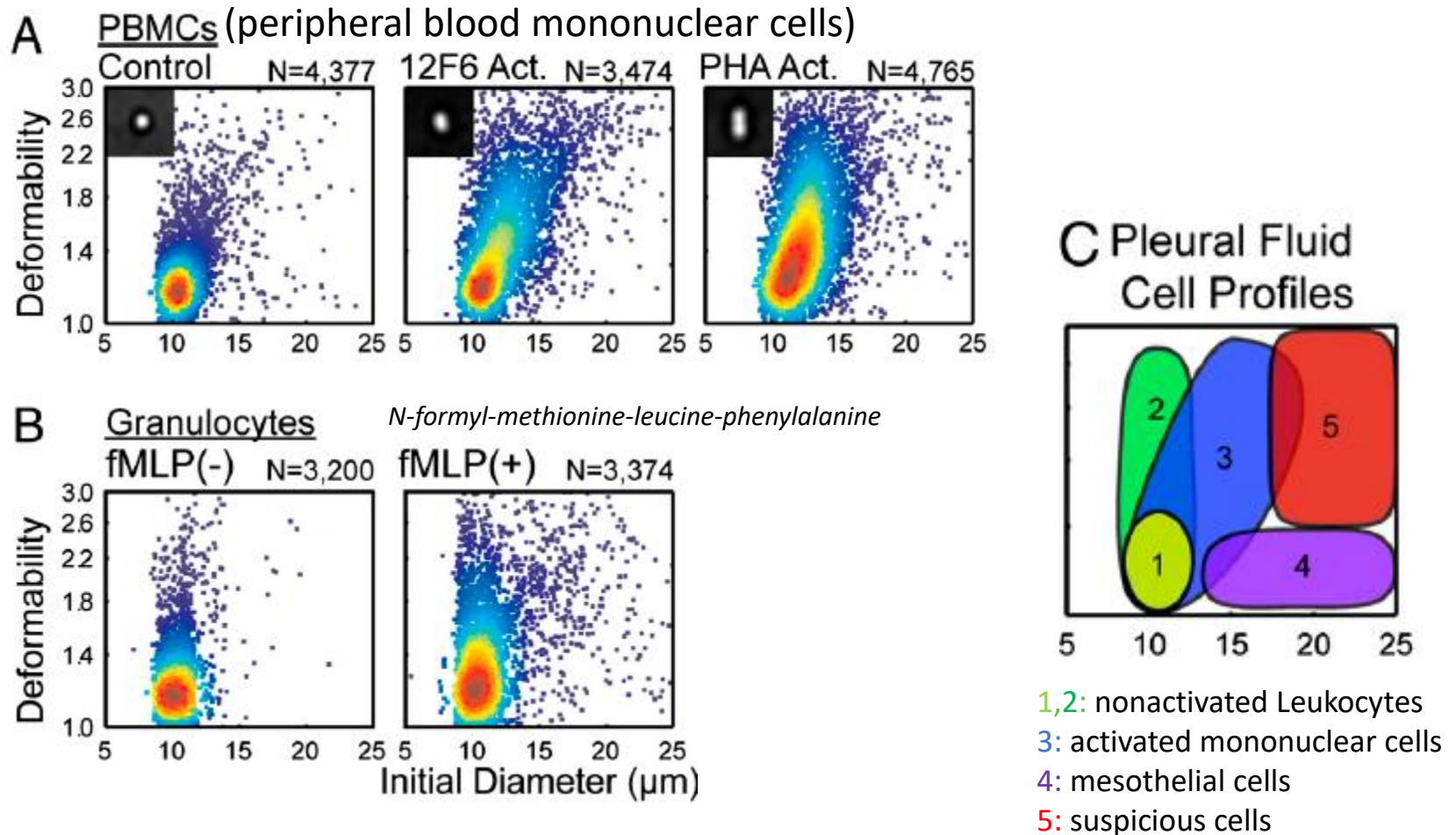
Supplementary Figures

Deformability Cytometry (DC)



Gossett, Daniel; Tse, Henry et al. (2012): Hydrodynamic stretching of single cells for large population mechanical phenotyping. *Proc. Natl. Acad. Sci.* 109, 7630–7635. DOI:10.1073/pnas.1200107109 (Figure 1)

Deformability Cytometry (DC)



Gossett, Daniel; Tse, Henry et al. (2012): Hydrodynamic stretching of single cells for large population mechanical phenotyping. *Proc. Natl. Acad. Sci.* 109, 7630–7635. DOI:10.1073/pnas.1200107109 (Figure 2A-C)

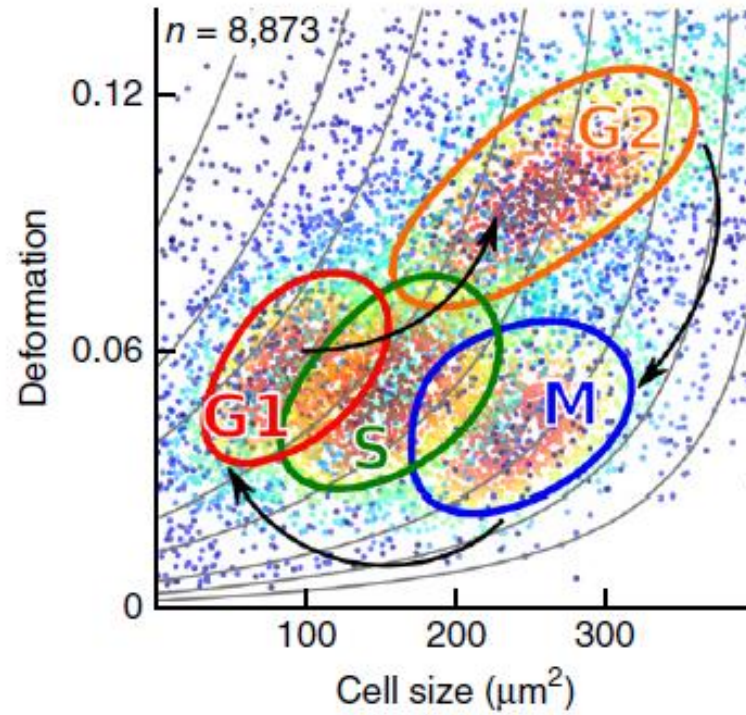
RT-DC



<https://www.youtube.com/watch?v=FoTI6gxqgVM&feature=youtu.be>

- Company “Zellmechanik”
- Price of an *AcCellerator* starts from 118 000 €
- Until now 6 devices sold

RT-DC

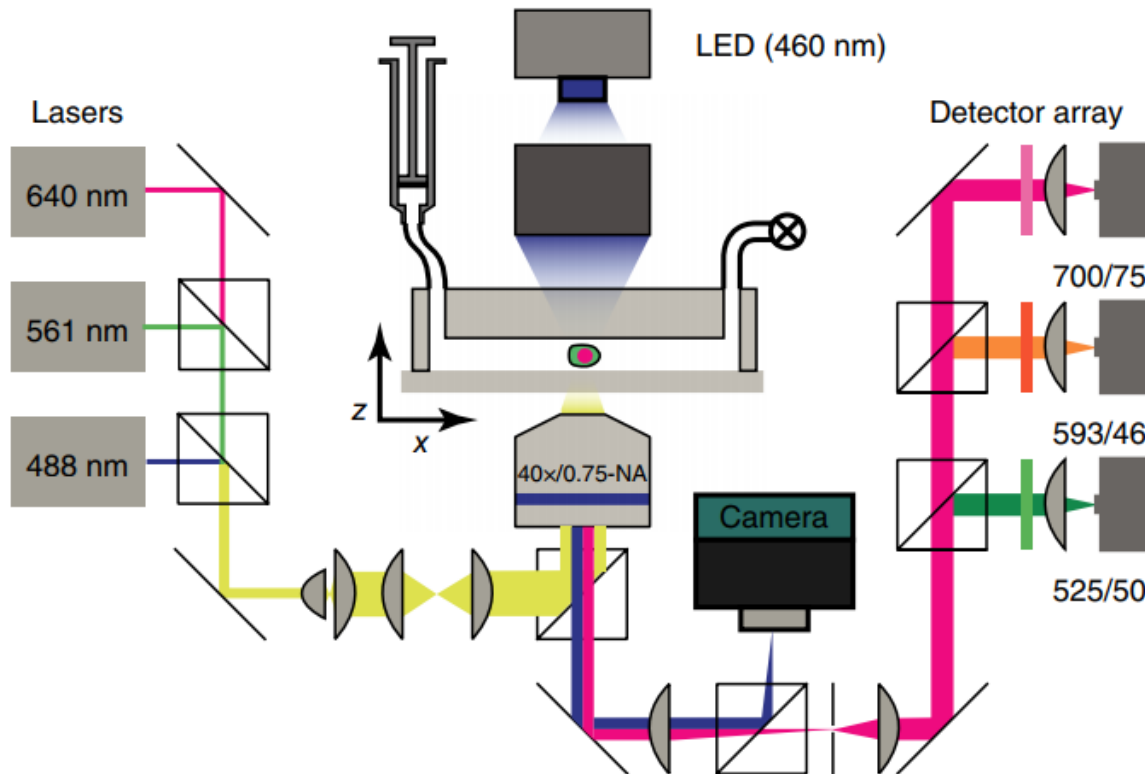


Otto, Oliver; Rosendahl, Philipp et al. (2015): Real-time deformability cytometry. On-the-fly cell mechanical phenotyping. *Nature methods* 12 (3), 199-202.
DOI: 10.1038/nmeth.3281. (Figure 2b)

RT-FDC

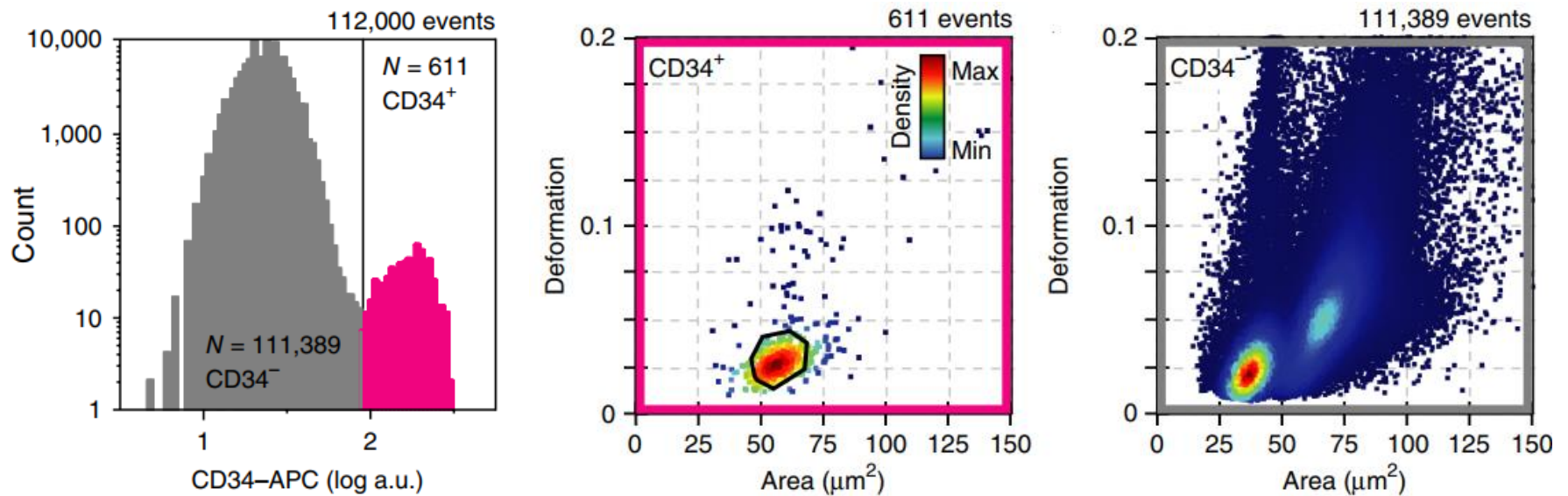
Problem with RT-DC: Lack of molecular specificity

→ Addition of florescent markers



Rosendahl, Philipp; Plak, Katarzyna et al. (2018): Real-time fluorescence and deformability cytometry. *Nature methods* 15 (5), S. 355–358. DOI: 10.1038/nmeth.4639. (Figure 1a)

RT-FDC



Rosendahl, Philipp; Plak, Katarzyna et al. (2018): Real-time fluorescence and deformability cytometry. *Nature methods* 15 (5), S. 355–358. DOI: 10.1038/nmeth.4639. (Figure 1c-e)