



Mechanical phenotyping of cells flowing through microfluidic constrictions

Magalie Faivre

Equipe Dispositifs pour la Santé et l'Environnement

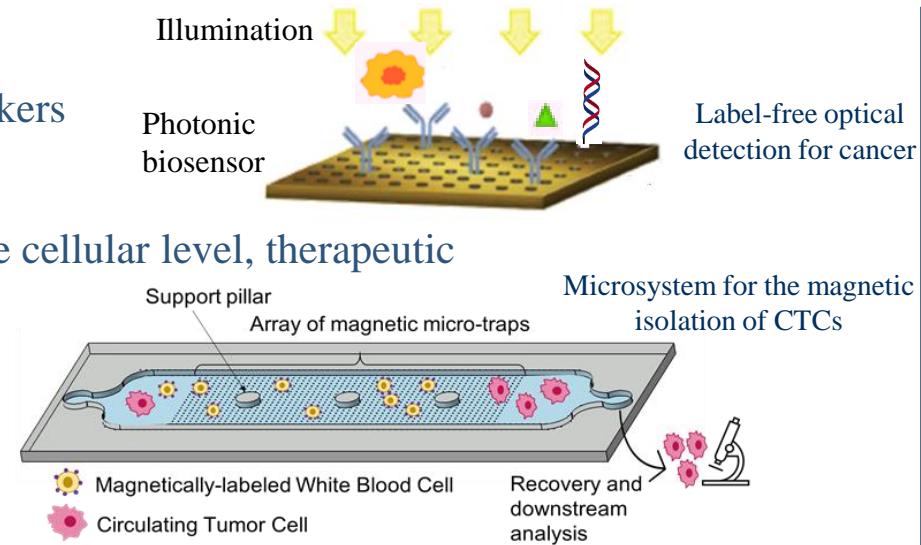
Univ Lyon, CNRS, INSA Lyon, ECL, UCBL, CPE Lyon, INL,
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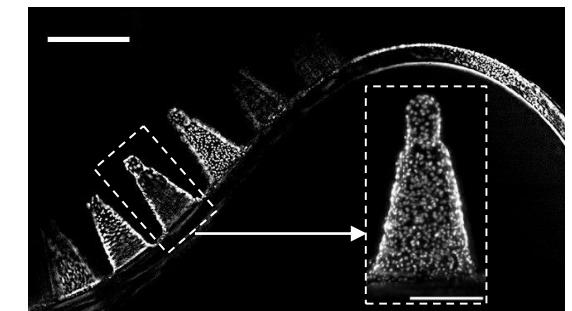
Team “Dispositifs pour la Santé et l’Environnement”

Diagnosis, personalized medicine, home care and patient follow-up

- **Biosensors**
Assay and monitoring of molecular biomarkers
- **Lab-On-a-Chip / Lab-On-a-Disc**
Information on biophysical properties at the cellular level, therapeutic resistance...
- **Synthesis of nanoparticles**
For diagnosis, imaging and therapy
- **4D biomimetic systems**
Artificial biomimetic systems towards a substitution of the animal model
- **On-board sensors for patient monitoring**
Measurement of physical parameters (T° , bio-impedance, ECG, activity...)



Impedance monitoring
for dialysis patients

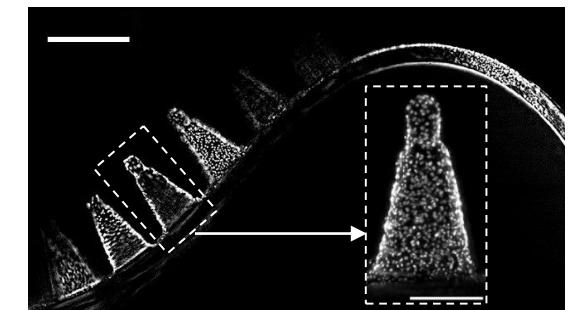
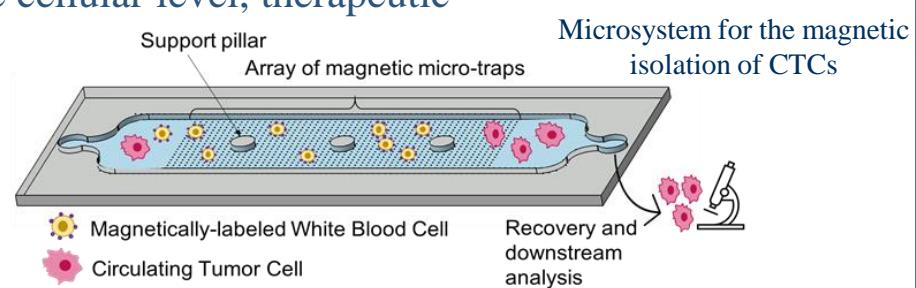
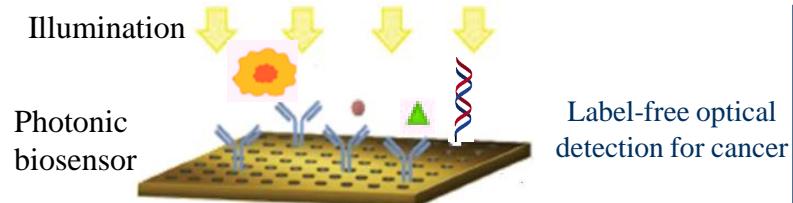


Reproduction of vilosities for the development of a gut-on-a-chip

Team “Dispositifs pour la Santé et l’Environnement”

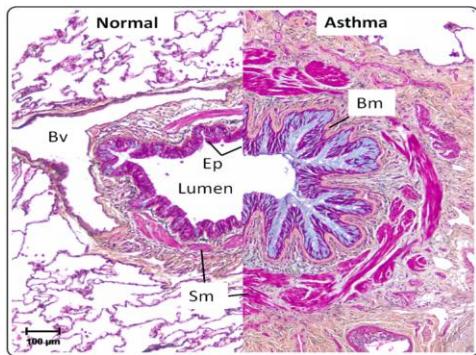
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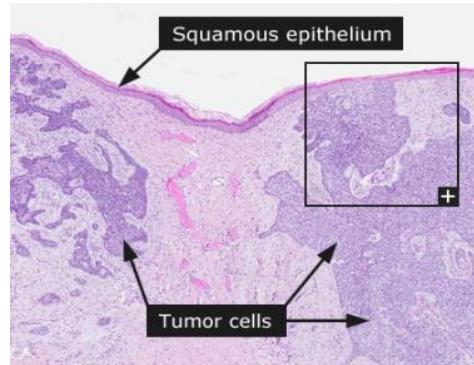
Mechanical phenotype for diagnostic applications

Asthma



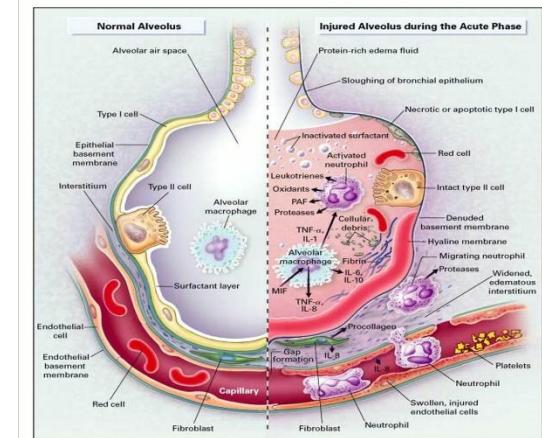
Wadsworth et al. *Asthma and Glycosylation in Airway Epithelial Repair*, (2012)

Cancer



www.proteinatlas.org

ARDS



www.clinicaladvisor.com

Malaria



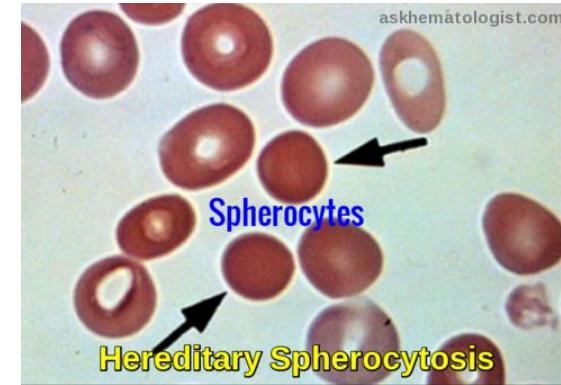
www.blog.rottenecards.com

Sickle Cell Disease



www.lifl.fr

Hereditary Spherocytosis

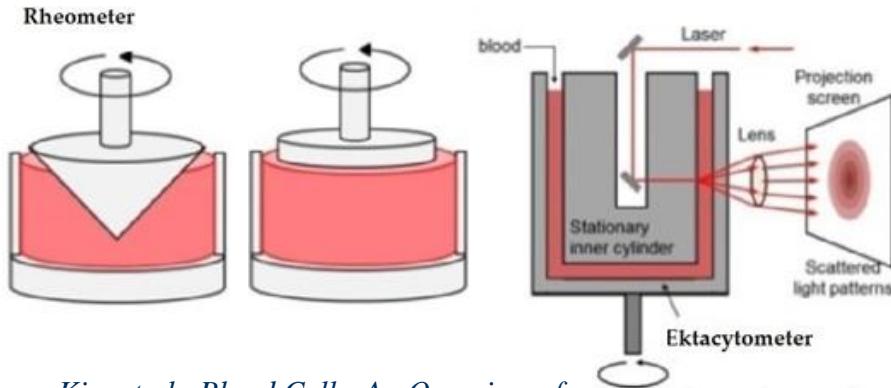


www.askhematologist.com

Mechanical signature of cells appears to be a relevant physical biomarker

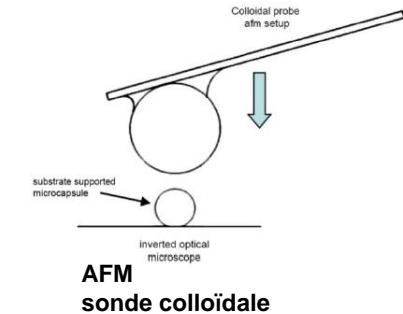
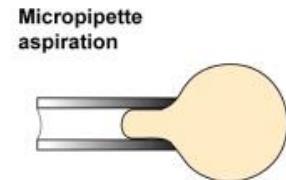
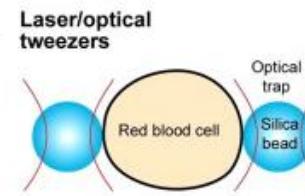
Mechanical phenotyping techniques

Collective techniques



Kim et al., Blood Cell - An Overview of Studies in Hematology - Chap10 (2012)

Single cell techniques



Dulinska et al., J. Biochem. Biophys. Meth. (2006)

Suresh, Acta Biomaterial (2007)

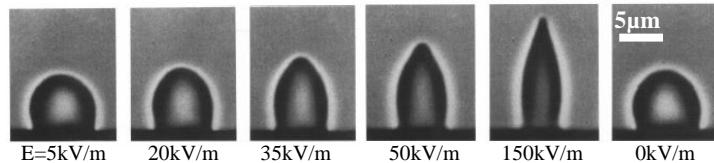
Not adapted for low number of pathological cells

High throughput

High sensitivity
(single cell detection)

Low throughput

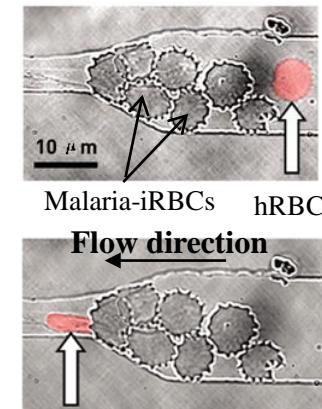
Microfluidic techniques



Engelhardt et al., Biophys. J. (1988)

actives

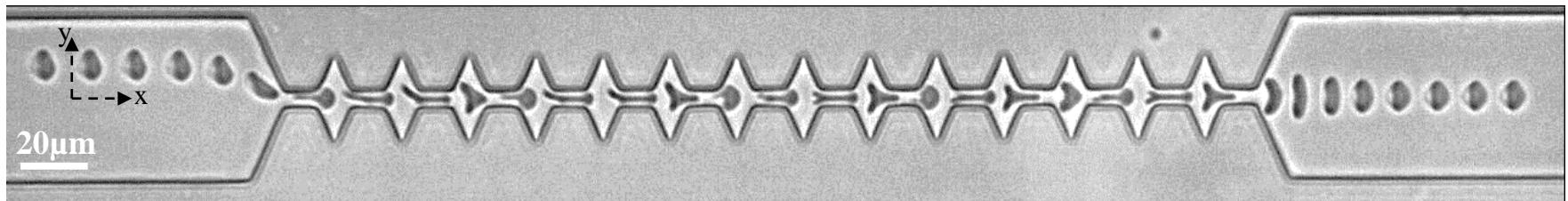
passives



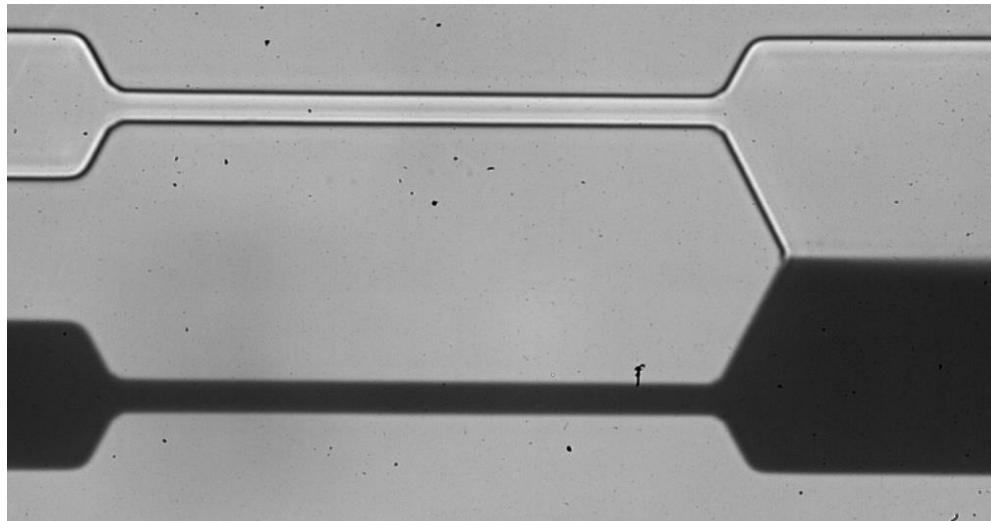
Shelby, PNAS (2003)

Outline of my presentation

Cell dynamical behaviour at the exit of a restriction

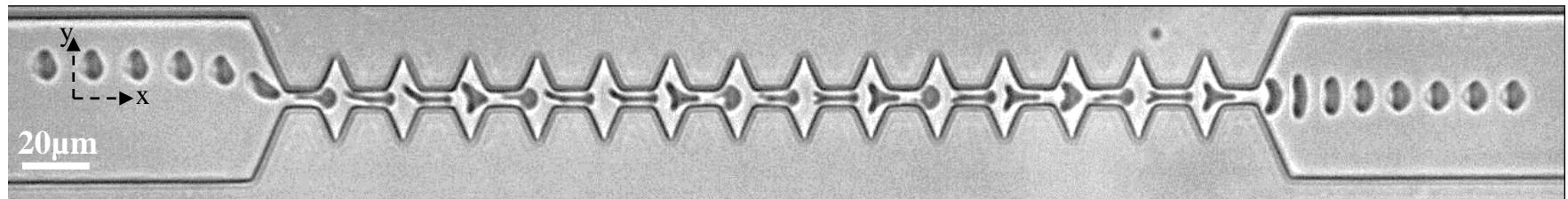


Pressure Drop measurement



Outline of my presentation

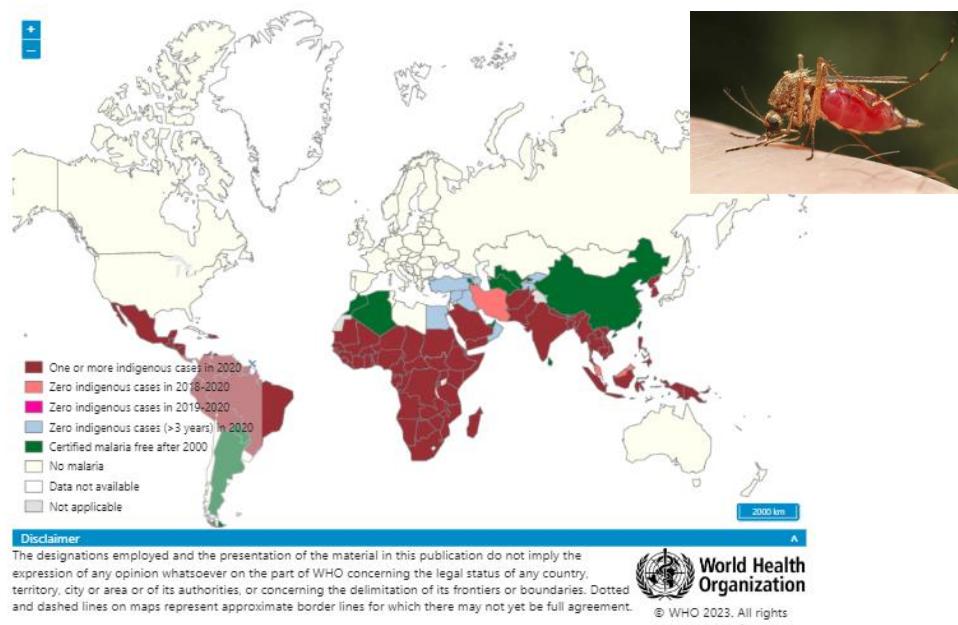
Cell dynamical behaviour at the exit of a restriction



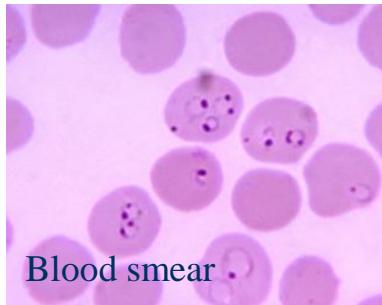
Pressure Drop measurement



Detection of malaria



Current detection methods:

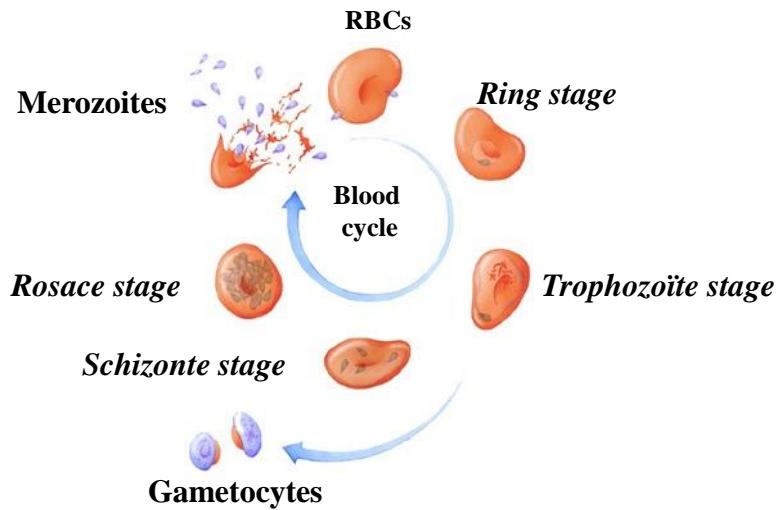


Not suitable for the field

False-positive leading to unnecessary drug use which contributes to the emergence of treatment-resistant strains

“247 million cases of malaria in 2021, and the estimated number of malaria deaths stood at 619 000. The WHO African Region carries a disproportionately high share of the global malaria burden.”

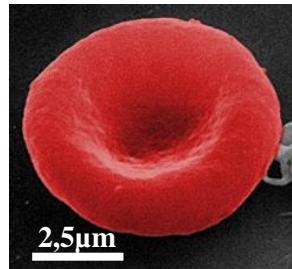
www.who.int/



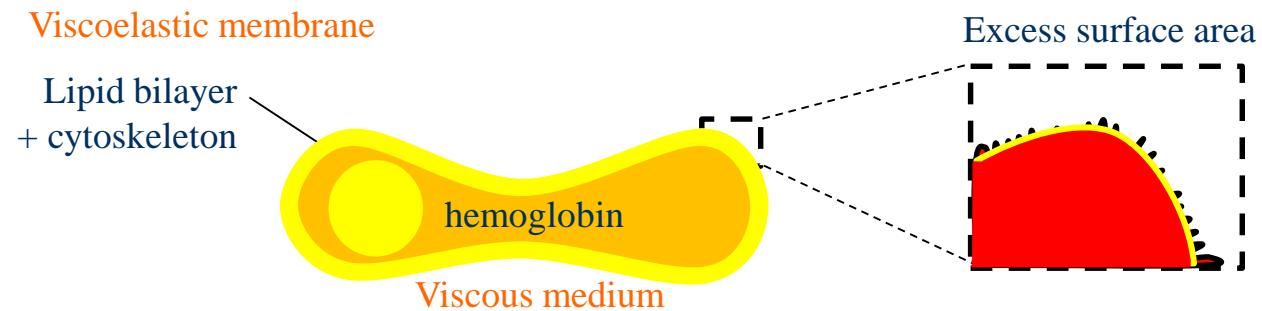
www.pasteur.fr

Direct detection of the presence of parasites within the host cell → mechanical phenotyping

The RBC : from structure to mechanical properties



www.altitude.org



Malaria



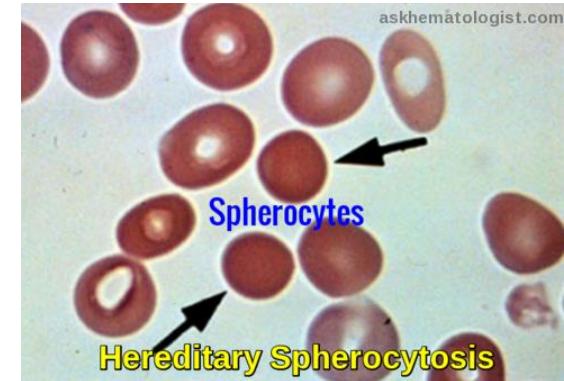
www.blog.rottenecards.com

Sickle Cell Disease



www.lifl.fr

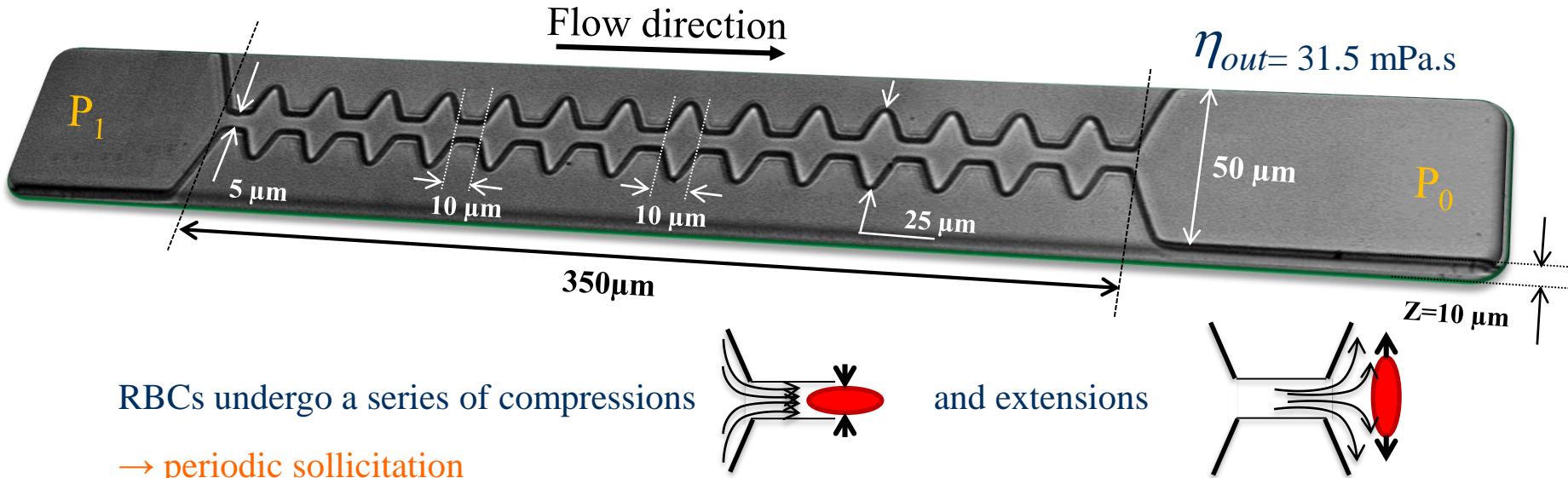
Hereditary Spherocytosis



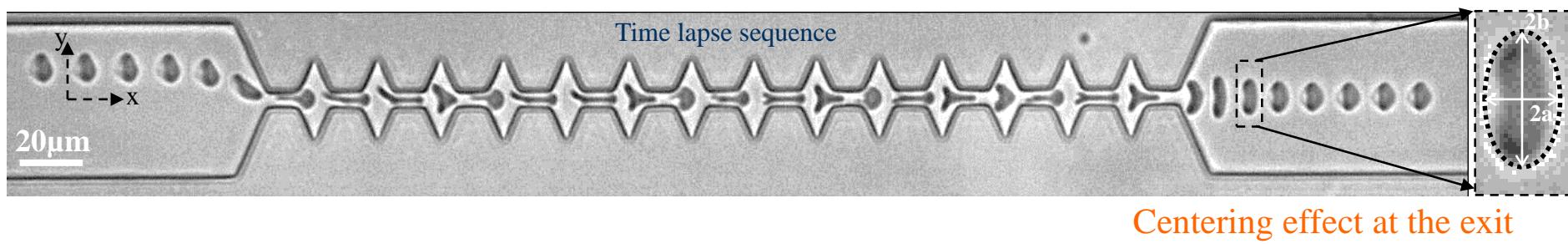
www.askhematologist.com

Cell dynamical behaviour at the exit of a restriction

Our approach multi-constricted channels:



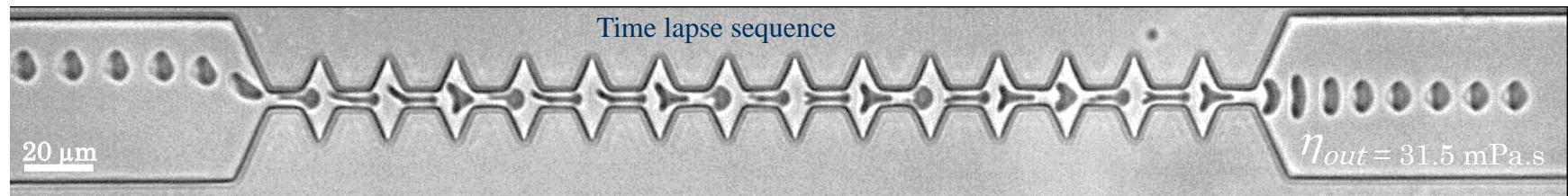
Typical flow behavior for a healthy RBC:



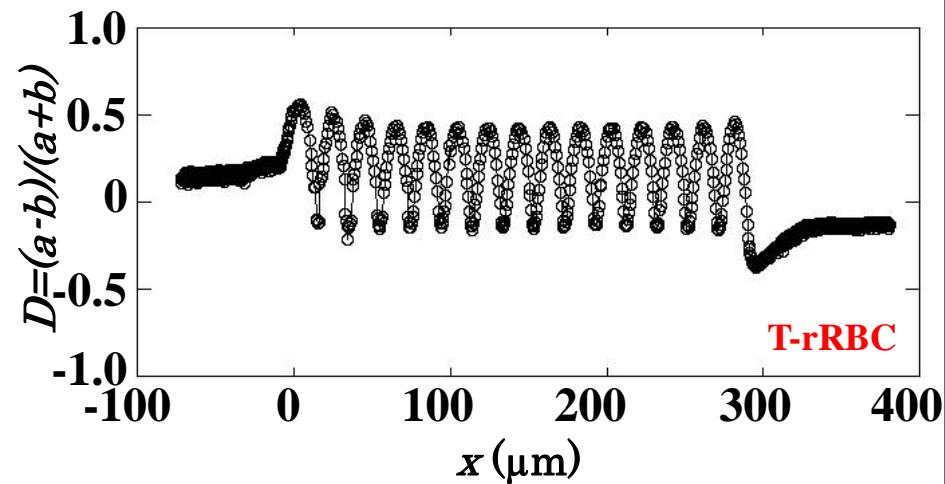
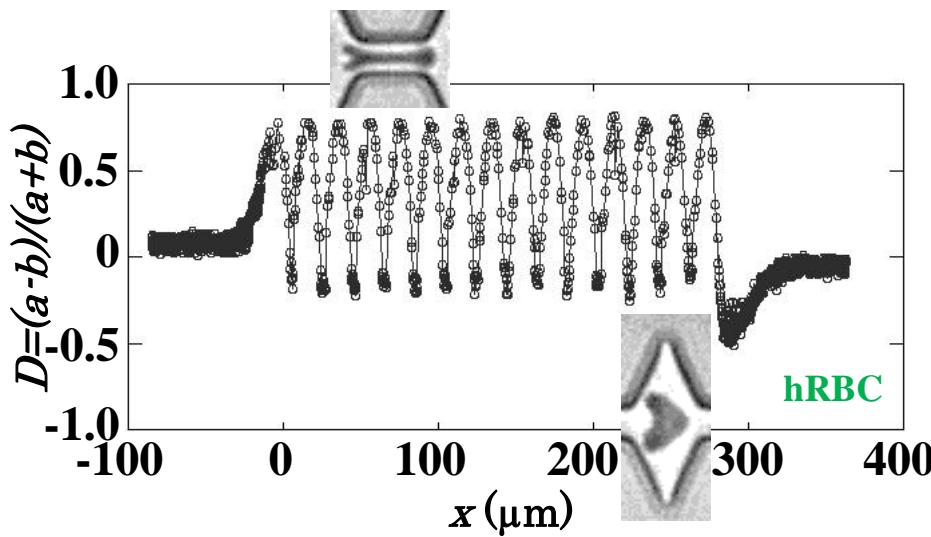
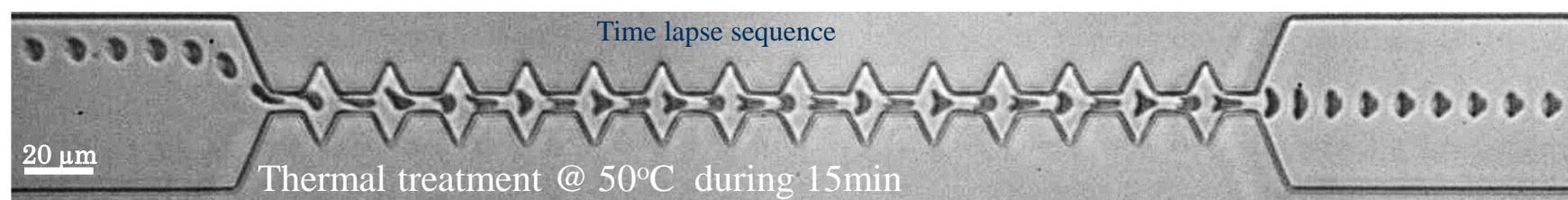
Identification of mechanically impaired RBCs

Experiments performed with temperature rigidified RBCs:

hRBC

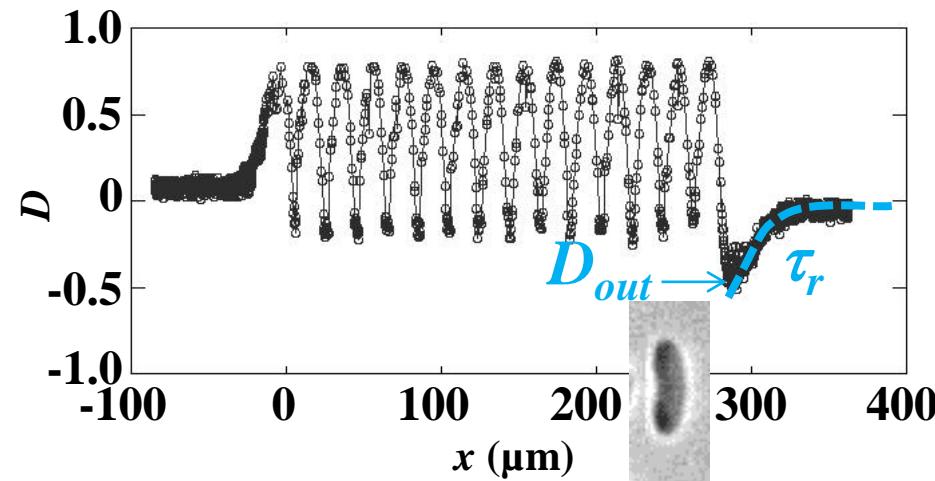
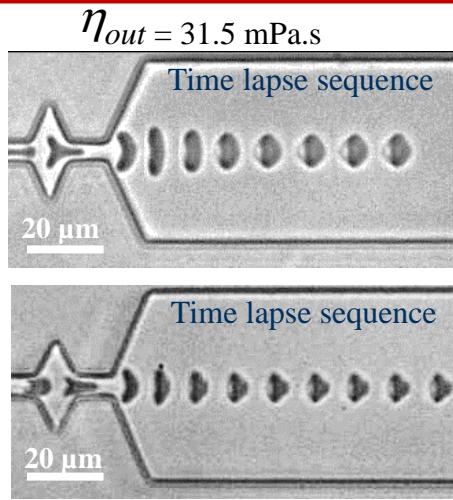


T-rRBC



Discrimination at the exit

hRBC TrRBC



Discrimination using D_{out} and τ_r

What about specificity ?



Are you ~~rigid~~ ?

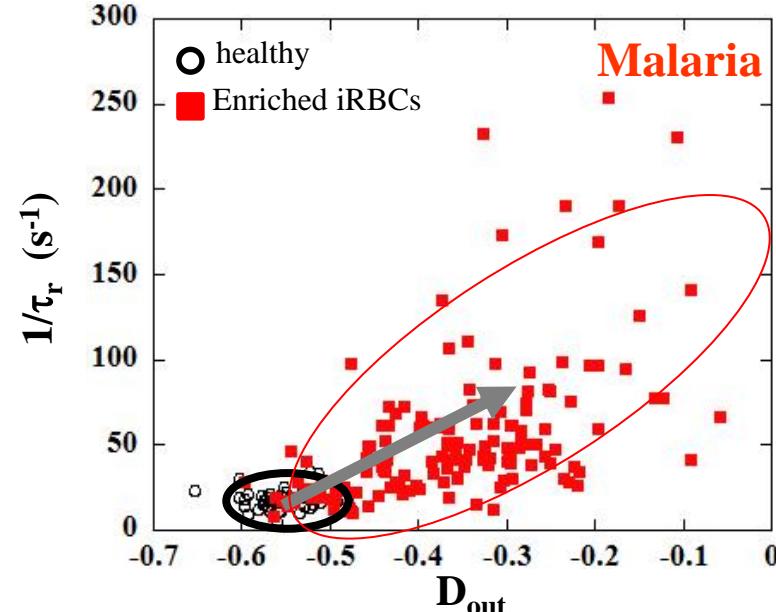
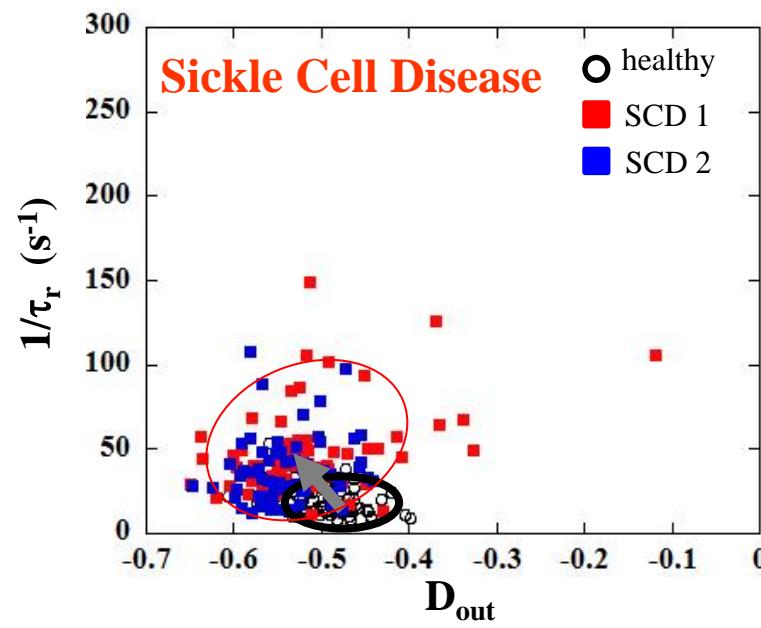
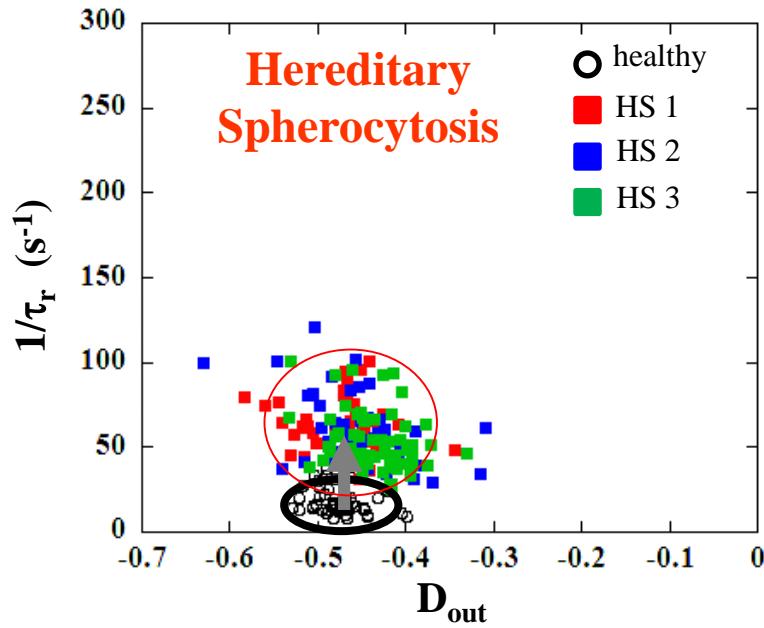
Do you have malaria ?

Do you have SCD ?

Do you have HS ?

Differentiation between several diseases which all impact overall cell deformability ?

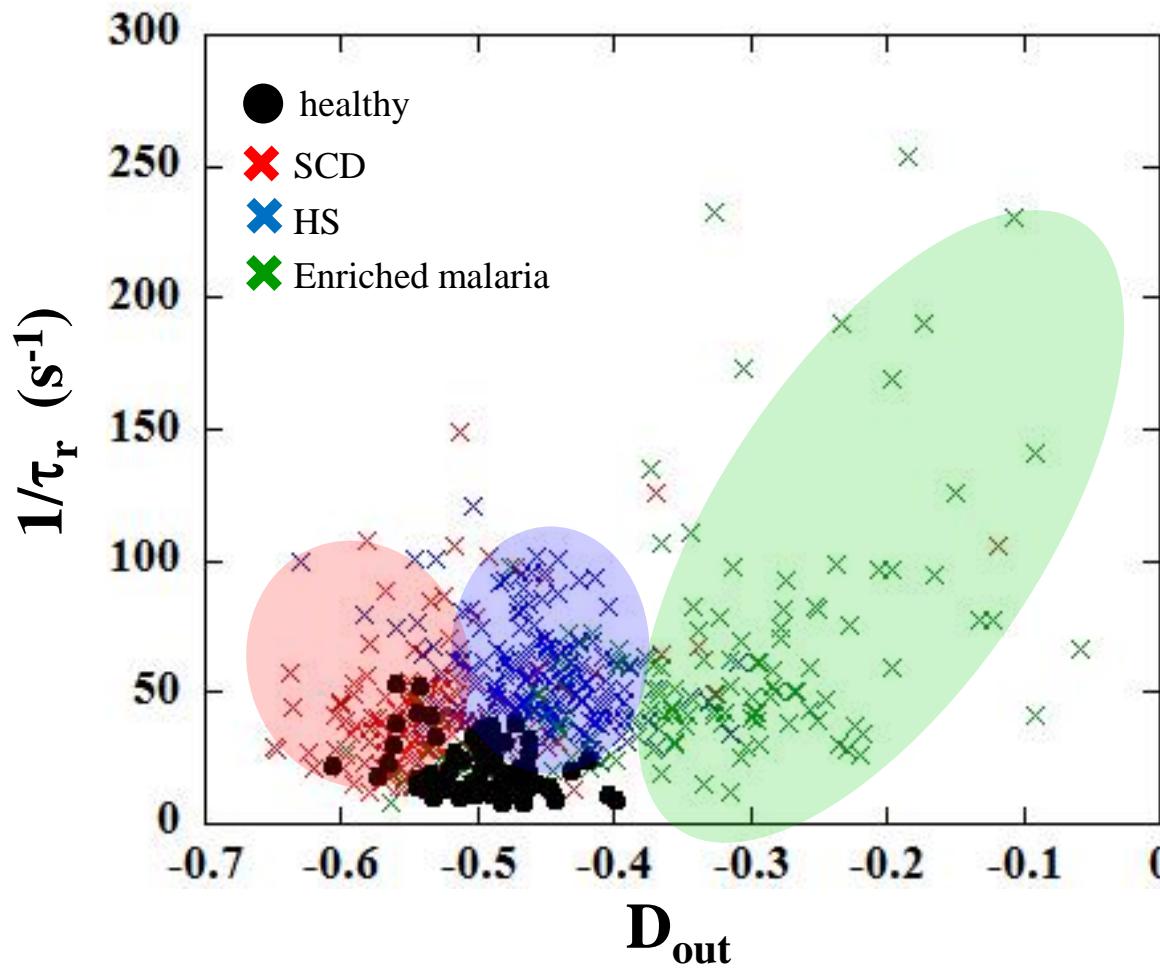
Discrimination between several pathologies ?



Faivre *et al.*, *Frontiers in Physiology* (2020)
Amirouche *et al.*, *Biomicrofluidics* (2020)

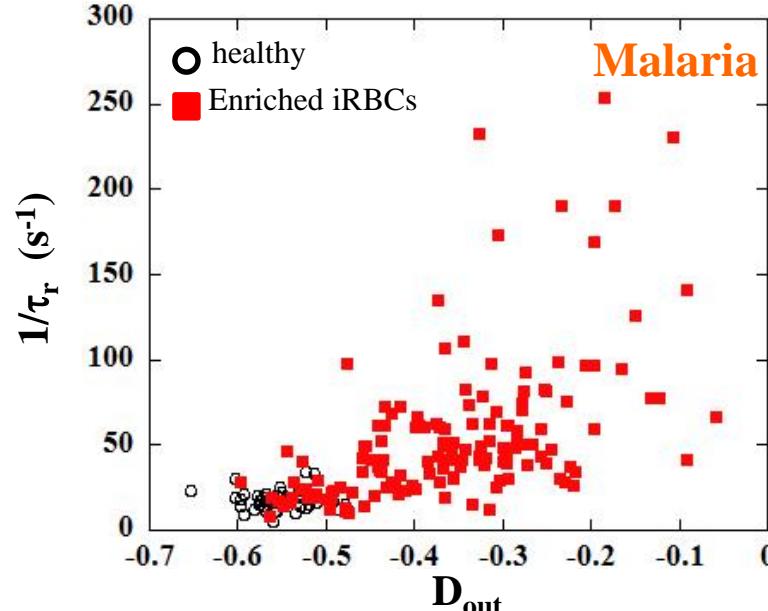
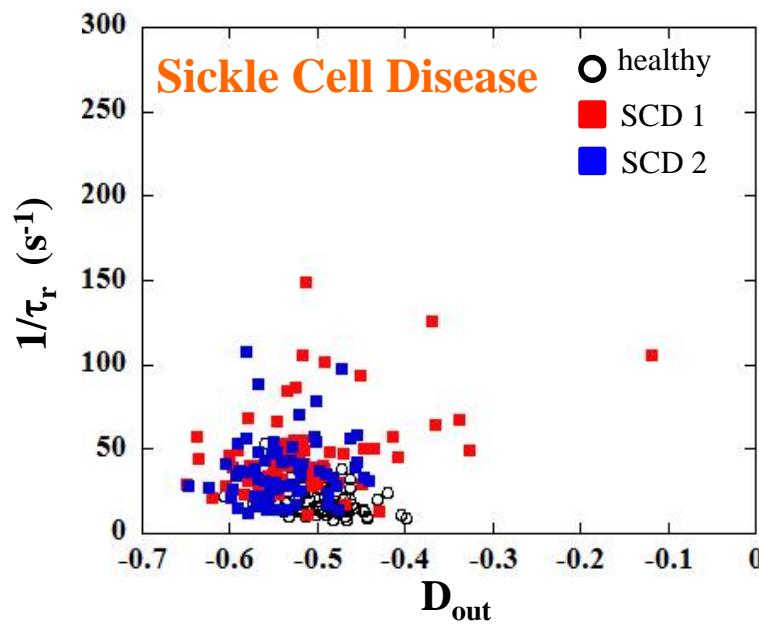
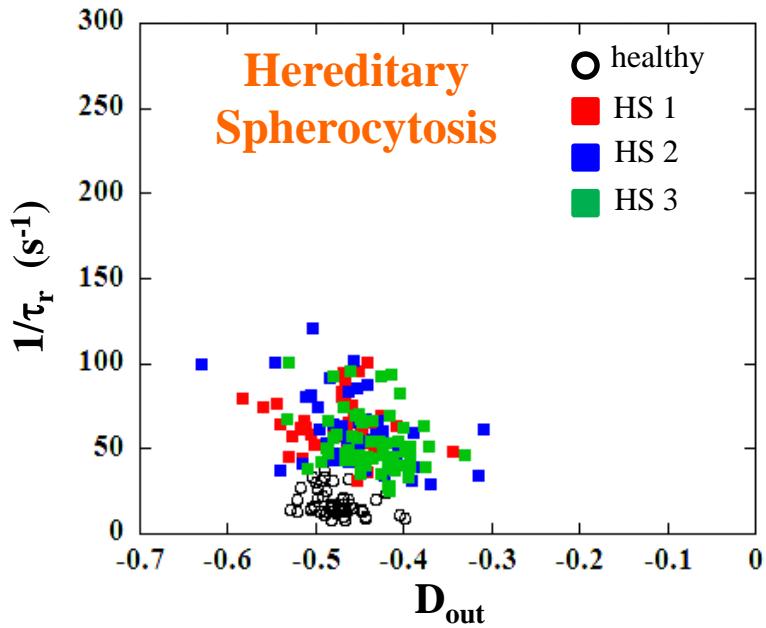
Discrimination between several pathologies ?

12



Discrimination possible between SCD, HS, malaria using D_{out} and τ_r

Is there more information ?



Quantitative analysis in progress
(T. Grenier) using machine learning

CREATIS

Faivre *et al.*, *Frontiers in Physiology* (2020)
Amirouche *et al.*, *Biomicrofluidics* (2020)

Conclusions and outlooks

○ Conclusions:

- ✓ Possibility to identify healthy and mechanically impaired RBCs using dynamical behaviour at the exit of a constriction (D_{out} and τ_r).
- ✓ Allows differentiating 3 different diseases which impact different structural component of the RBCs while decreasing the overall cell deformability.

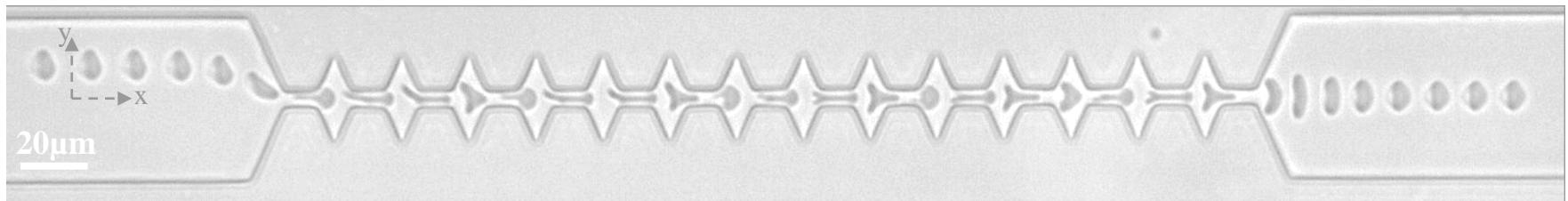
→ Great potential as a new diagnostic tool !

○ Outlooks:

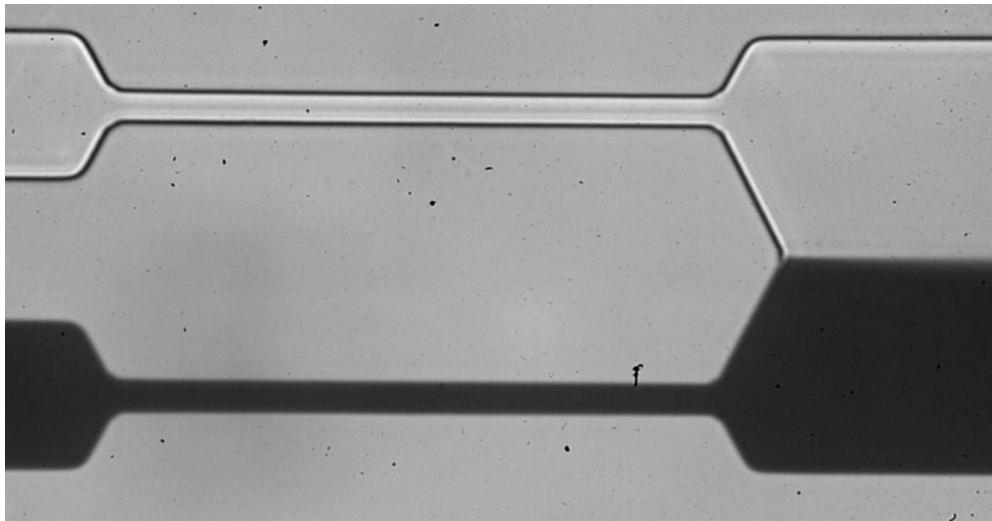
- Evaluate the possibility to quantify sub-populations of RBCs
 - Evaluate variability inter patients (on healthy, then on pathological samples)
- Could bring new insights in the diagnostic, prognostic and therapeutic follow-up

Outline of my presentation

Cell dynamical behaviour at the exit of a restriction



Pressure Drop measurement

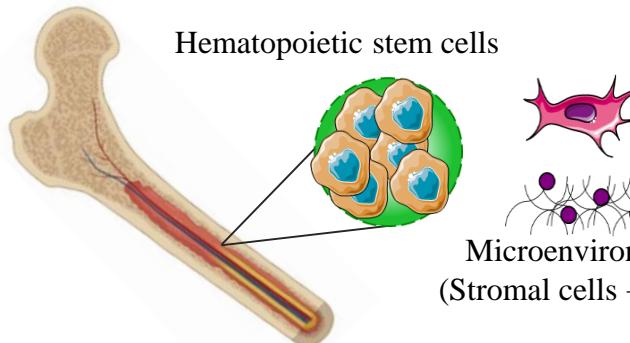


From mechanical properties to treatment outcome

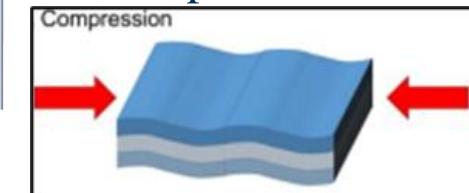
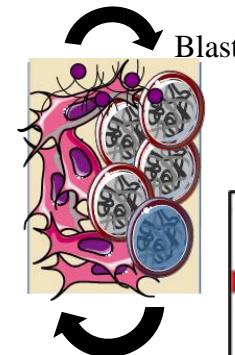
Acute Mieloid Leukemia (AML)

5-year survival rates of 30–35%

Kantarjian et al., Blaood Cancer J. (2021)



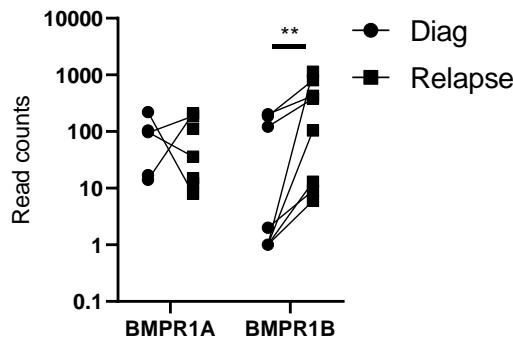
→ AML



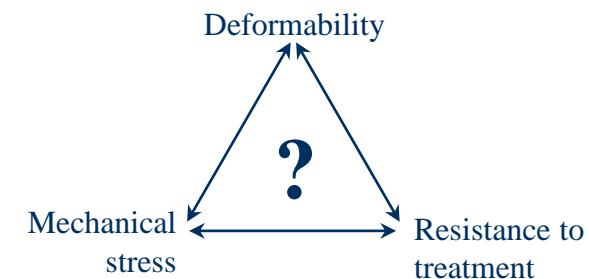
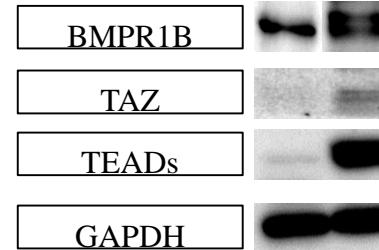
→ Cell deformability can be influenced by environment

CRCL

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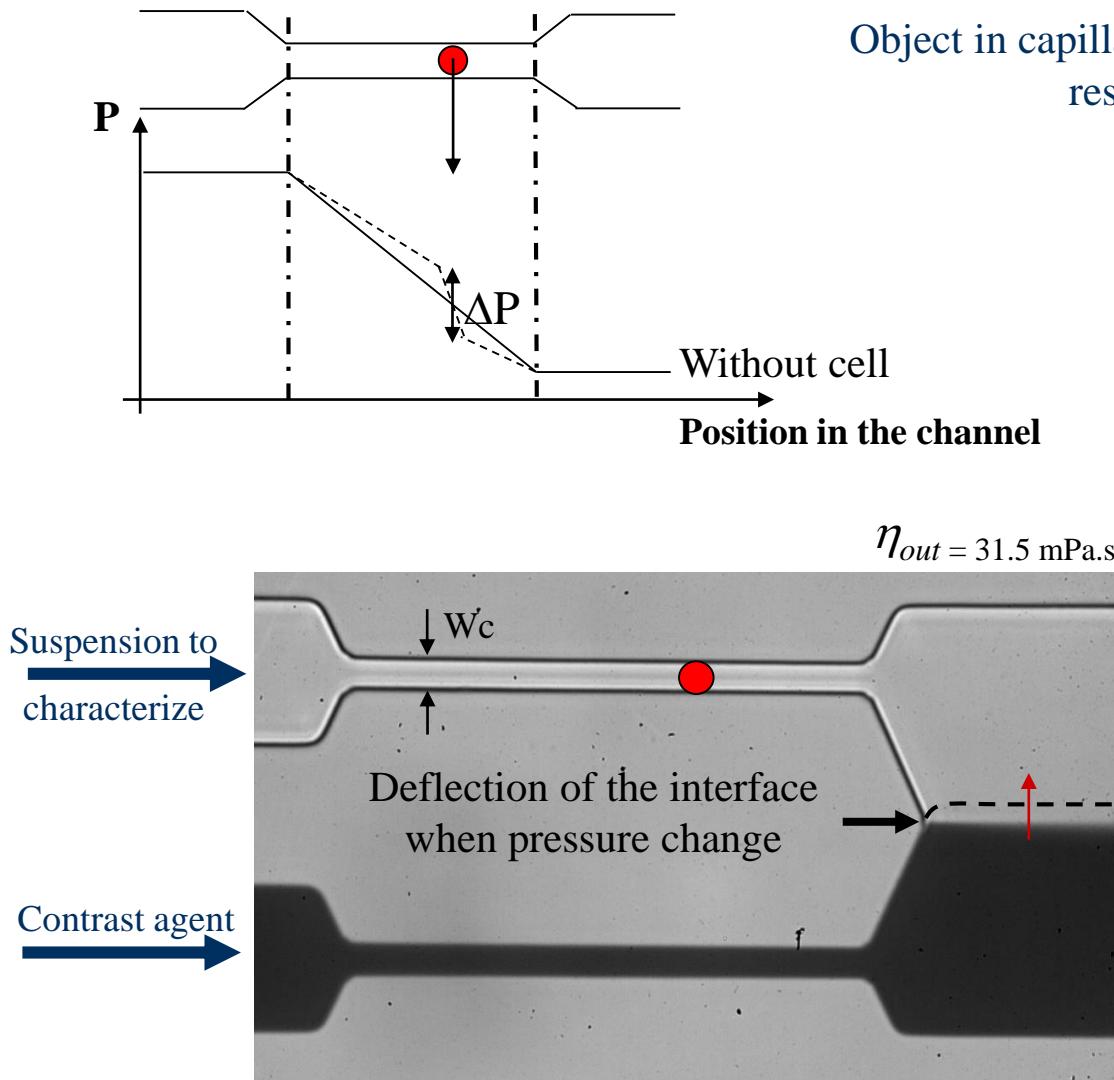
ML2-S
ML2-R



- ↗ Mechanosensitive pathways in cells from AML patients with relapse and commercial cell lines resistant to chemotherapy.

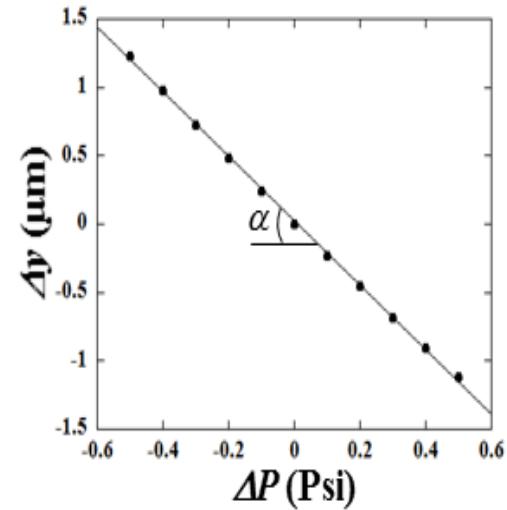
Lefort., Unpublished results (2022)

Pressure Drop Measurement



Object in capillary induces change in hydrodynamic resistance into the channel

Calibration



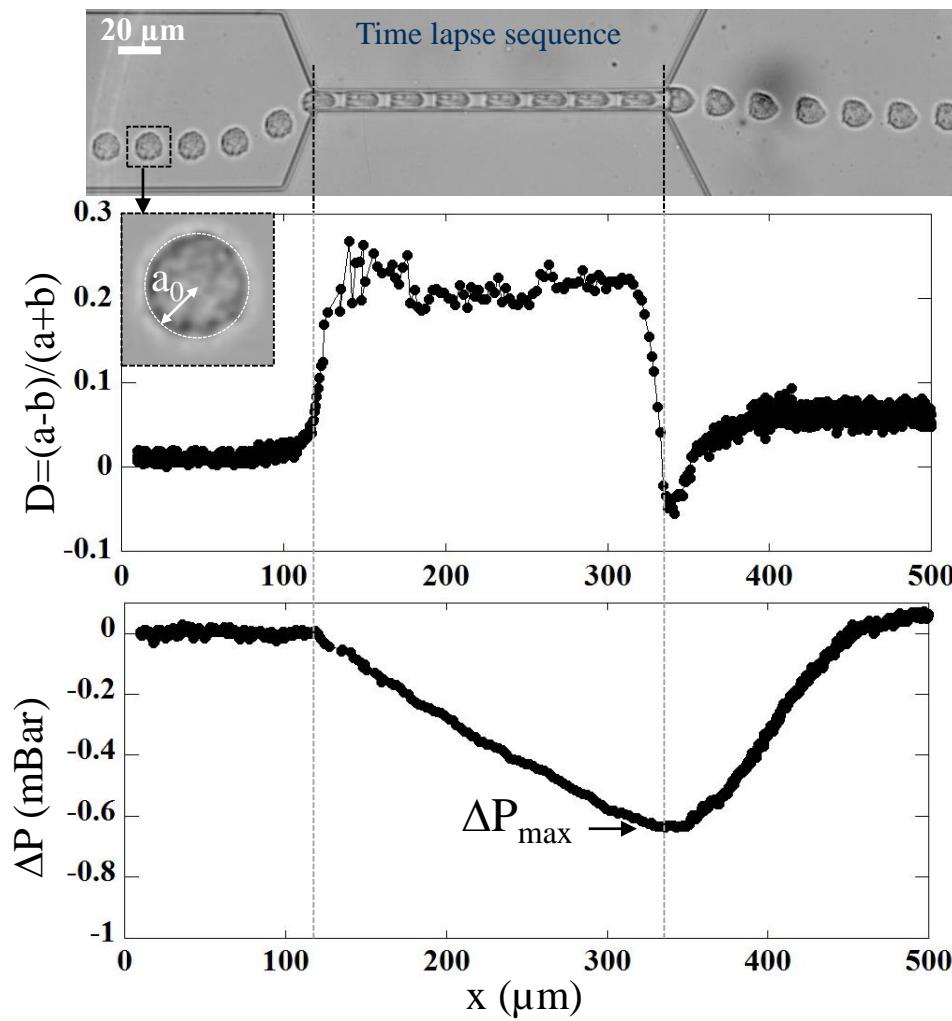
Object in capillary induces a change in flow rate downstream which can be translated into a change in pressure.

Abkarian et al., PNAS (2006)

Institut des Nanotechnologies de Lyon UMR CNRS 5270

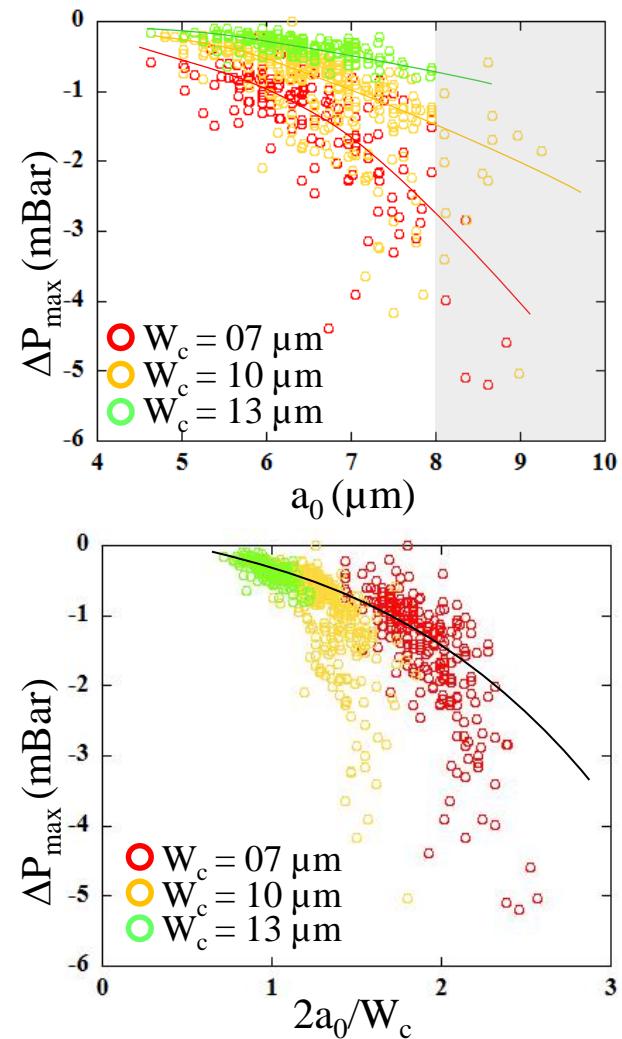
<http://inl.cnrs.fr>

Typical behaviour of ALM cells



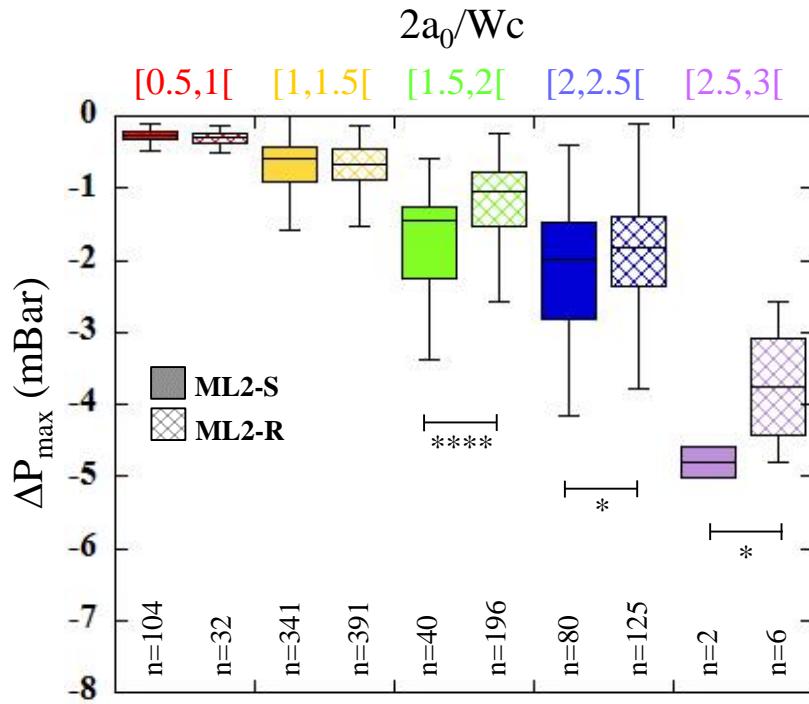
ΔP_{\max} varies with both **cell volume** and **deformability**

ΔP_{\max} traduces the occupation rate $2a_0/W_c$ of the cells in the constriction



Mechanical signature of the resistance to treatment

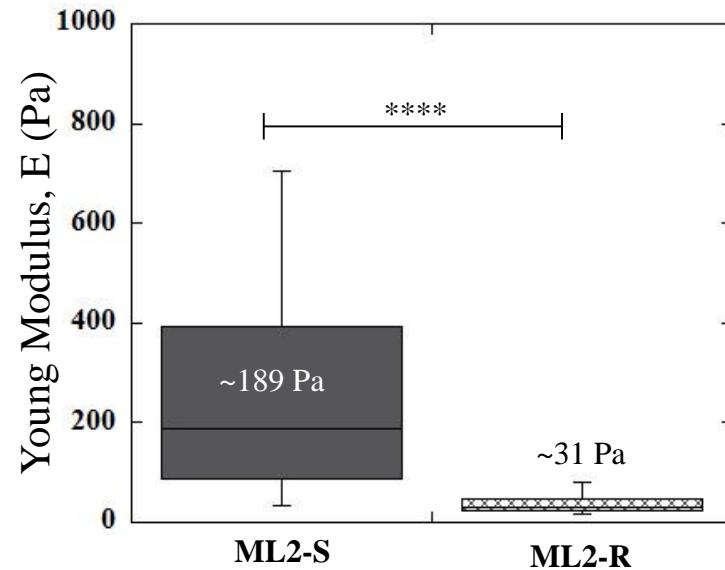
Comparaison of sensitive and resistant cell lines:



At a given occupation rate, ML2-S are less deformable than ML2-R.

Discrimination possible between sensitive and resistant cells to chemotherapy

Results confirmed by AFM measurements



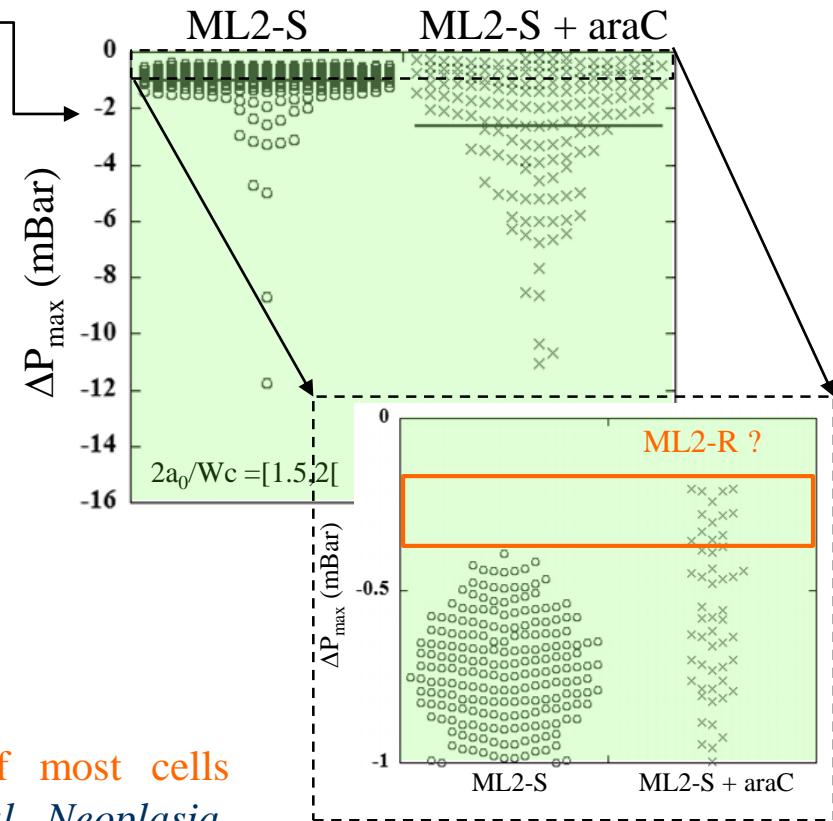
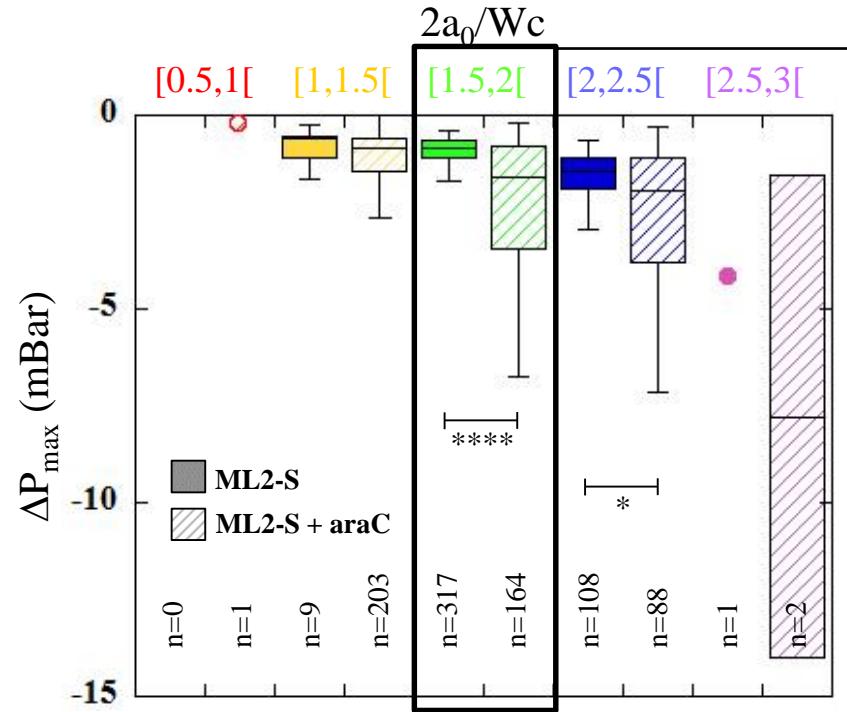
Literature on HL-60 (AML) :

< 100 Pa W.A. Lam et al., *Neoplasia*, 109(8):3505-3508, 2007

200-1400 Pa M.J. Rosenbluth et al. *Biophys. J.*, 90(8):2994-3003, 2006

Effect of treatment ?

Mechanical signature upon chemotherapy exposse for 24H00:



Upon chemotherapy exposure, stiffness of most cells increases (as stated in literature W.A. Lam *et al.*, *Neoplasia*, 109(8):3505-3508, 2007)

Conclusions and outlooks

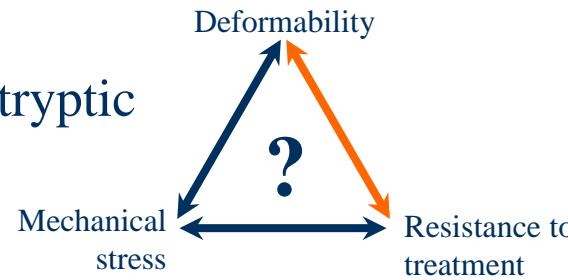
○ Conclusions:

- ✓ Pressure drop measurement is sensitive to both volume and deformability
- ✓ Possibility to discriminate between sensitive and resistant AML commercial cell lines.

→ Great potential as a new prognostic tool !

○ Outlooks:

- Test on primary cells issued from patients whose treatment outcome is known (relapse or not).
- Evaluate the other sides of the tryptic



Acknowledgements

At INL...



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(LIBM)



Lydie Da Costa
(hôpital Robert Debré,
Paris)



**Philippe
Connes**
(LIBM)



Sylvain Lefort
(CRCL)



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IEEE FRANCE sensors chapter

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