On the effects of hydrodynamic interactions among cells for microfluidic holographic cyto-tomography

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bottega della **materia soffice**

Introduction to myself

- Massimiliano M. Villone
  - Naples, 07 Jan ‘87
- Chemical Engineer
  - MSc from the University of Naples Federico II in 2010
  - PhD in 2014
- Associate Professor at the University of Naples Federico II
- International collaborations:
  - TU/Eindhoven, The Netherlands
  - KTH Royal Institute of Technology, Stockholm, Sweden
  - Princeton University, US
- Research interests:
  - modelling and numerical simulation of the mechanical and rheological behaviour of multiphase systems
  - applications of machine/deep learning
- Teaching:
  - statistics
  - numerical simulation
  - soft matter

Motivation

- In the last decade, **imaging flow cytometry** (**IFC**) has established as a powerful measurement method in clinical diagnostics.
  - High resolution optical and/or spectroscopic information is added to conventional flow cytometry.
  - 👍 high throughput (~10^4 cells per second)
  - 👎 provides a 2D projection of a 3D object
  - 👎 based on fluorescent analysis

- **Quantitative phase imaging** (**QPI**) has been proposed as a low-cost solution to achieve rapid cell detection.
  - **Digital holography** (**DH**) is a **3D**, **label-free**, **non-destructive** microscopy technique allowing for QPI of cells in biomedical applications.

A Adan et al, Crit Rev Biotechnol, 2017; RJ Yang et al, Sens Actuators B, 2018; Y Han et al, Lab Chip, 2016; A Kleiber et al, Lab Chip, 2021; BK Goud et al, Opt Lasers Eng, 2019; C Trujillo et al, Opt Lasers Eng, 2019; AB Ayoub et al, Sci Rep, 2021; F Merola et al in: Advancement of optical methods & digital image correlation in experimental mechanics, Vol 3, Springer, 2019

Motivation

- In conventional DH imaging, cells are placed in petri dishes to complete the necessary recordings.
  - 👎🏻 discontinuous process
  - 👎🏻 low throughput

- This weakness is overcome by combining DH with **microfluidics** → **holographic flow cytometry** (**HFC**).
  - 👍🏻 continuous
  - 👍🏻 high throughput
  - 👍🏻 no need for cell focusing thanks to multi-refocusing ability of QPI.

W Bishara et al, Opt Express, 2010; P Paiè et al, Cytometry Part A, 2018

Motivation

- HFC demonstrated fruitful for many important **biomedical applications**:
  - detection of erythrocytes infected by *plasmodium falciparum*,
  - identification of cancer cells → **liquid biopsy**,
  - classification of human leukemic cells,
  - cell phenotyping.

- As large datasets are involved in HFC biomedical applications, **deep learning** can be effectively applied to them.

- Holographic microscopy can be employed to perform **phase contrast tomography** (**PCT**), allowing precise **3D refractive index** (**RI**) **mapping** at the subcellular level.

M Ugele et al, Lab Chip, 2018; L Miccio et al, View, 2020; M Ugele et al, Adv Sci, 2018; KCM Lee et al, Cytometry Part A, 2019; Z Wang et al, Light Sci Appl, 2021

Motivation

- In “traditional” PCT, the sample is fixed and a source of light rotates around it.
  - 👎 discontinuous
  - 👎 low throughput

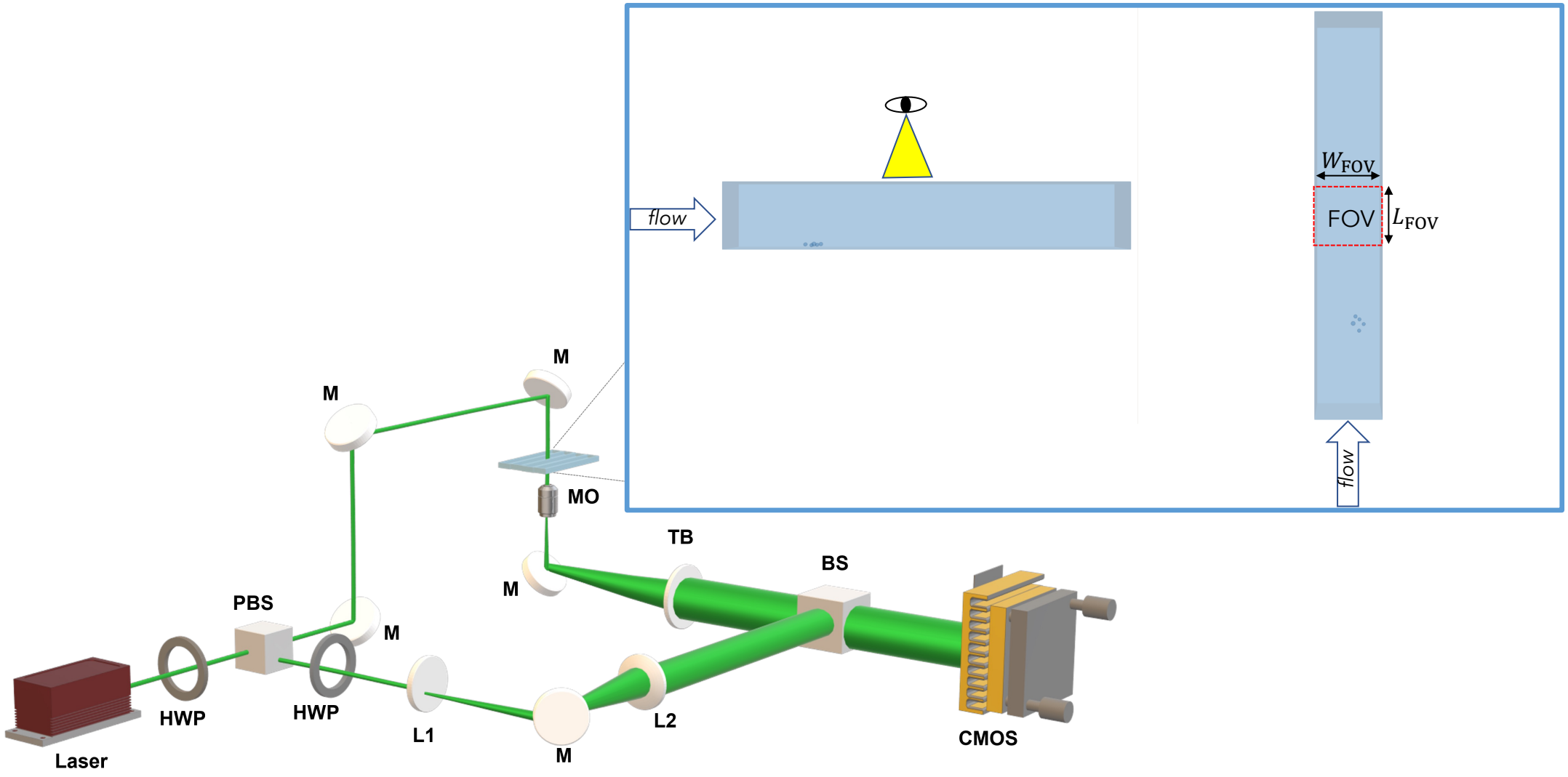
- By exploiting **hydrodynamic-induced rolling** of cells in microfluidic channels, PCT can be achieved by conventional DH systems (fixed in space).
  - 👍 continuous
  - 👍 high throughput

- The paradigm of **in-flow PCT** has been demonstrated by reconstructing the 3D RI distribution of
  - red blood cells (RBCs),
  - diatoms,
  - circulating tumor cells (CTCs),
  - …

F Merola et al, Light Sci Appl, 2017; MM Villone et al, Lab Chip, 2018

Motivation

- To achieve the PCT of cells flowing in a microfluidic channel, their **full rotation** within the field of view (FOV) of the holographic microscope needs to be assured.



Particle manipulation in microfluidics

- When a suspension flows through a microfluidic channel, a "transverse" motion of the suspended particles can be promoted to **focus** them onto specific streamlines.

- Active techniques: lateral migration achieved by the application of external fields, e.g.
  - electrical,
  - magnetic
  - acoustic,
  - …

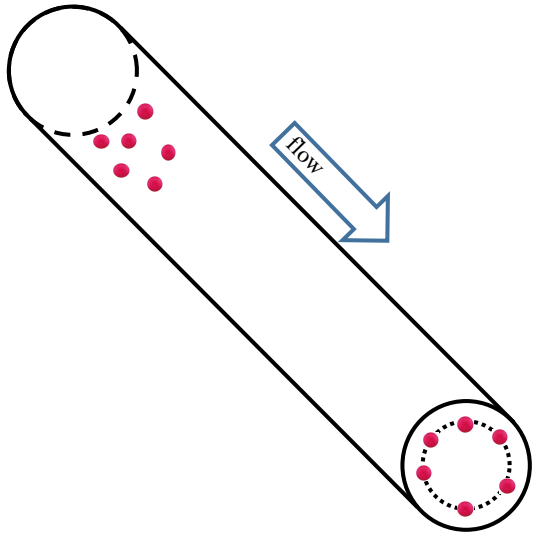
- **Passive techniques**: lateral migration achieved by tuning the forces connected to
  - **fluid dynamics/rheology** of the suspending medium,
  - **deformability** of the particles,
  - **hydrodynamic interactions** with the walls of the microfluidic device.

G D'Avino et al, Annu Rev Fluid Mech, 2017; MM Villone and PL Maffettone, Rheol Acta, 2019

Inertial particle migration in cylindrical channels

- Lateral forces act on **rigid particles** suspended in **Newtonian liquids** under pressure-driven flow in a tube:
  - wall effects push them particles toward the tube axis,
  - shear gradient forces push them toward the wall.

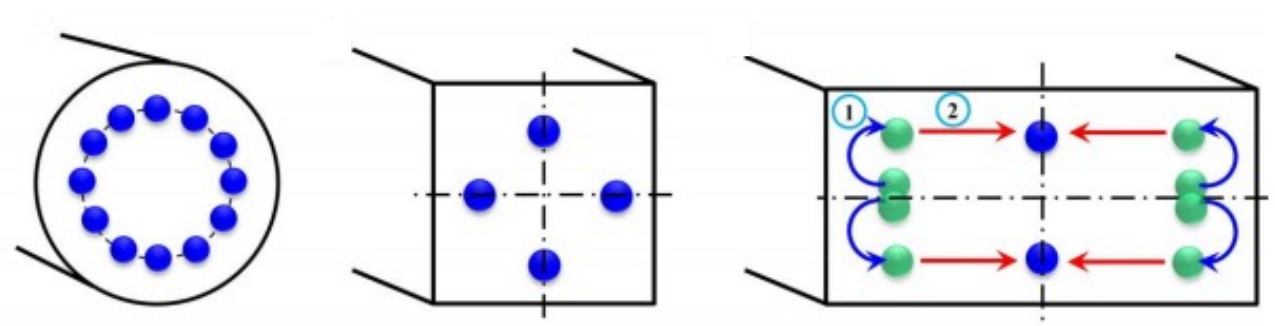
- **Segré-Silberberg effect**: spherical particles tend to an **annular region** at $\frac{r}{R}(\mathrm{Re})$.
  - In the Stokes regime $(\mathrm{Re} = 0)$, no lateral migration occurs.
  - Lateral migration is also influenced by particle confinement, i.e., particle to channel transversal size ratio.



G Segré and A Silberberg, Nature, 1961

Inertial particle migration in rectangular channels

- In cylindrical geometries, rigid spherical particles arrange on an annulus due to axial symmetry.

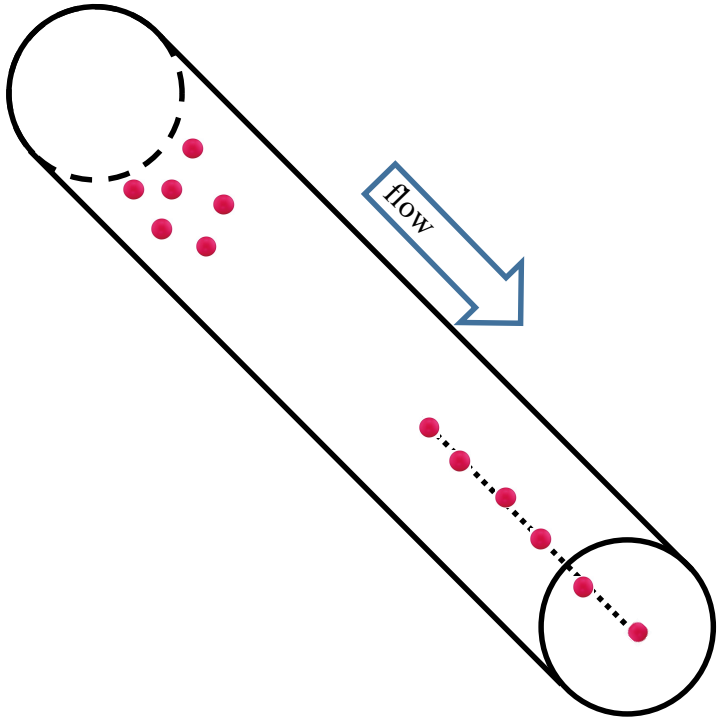


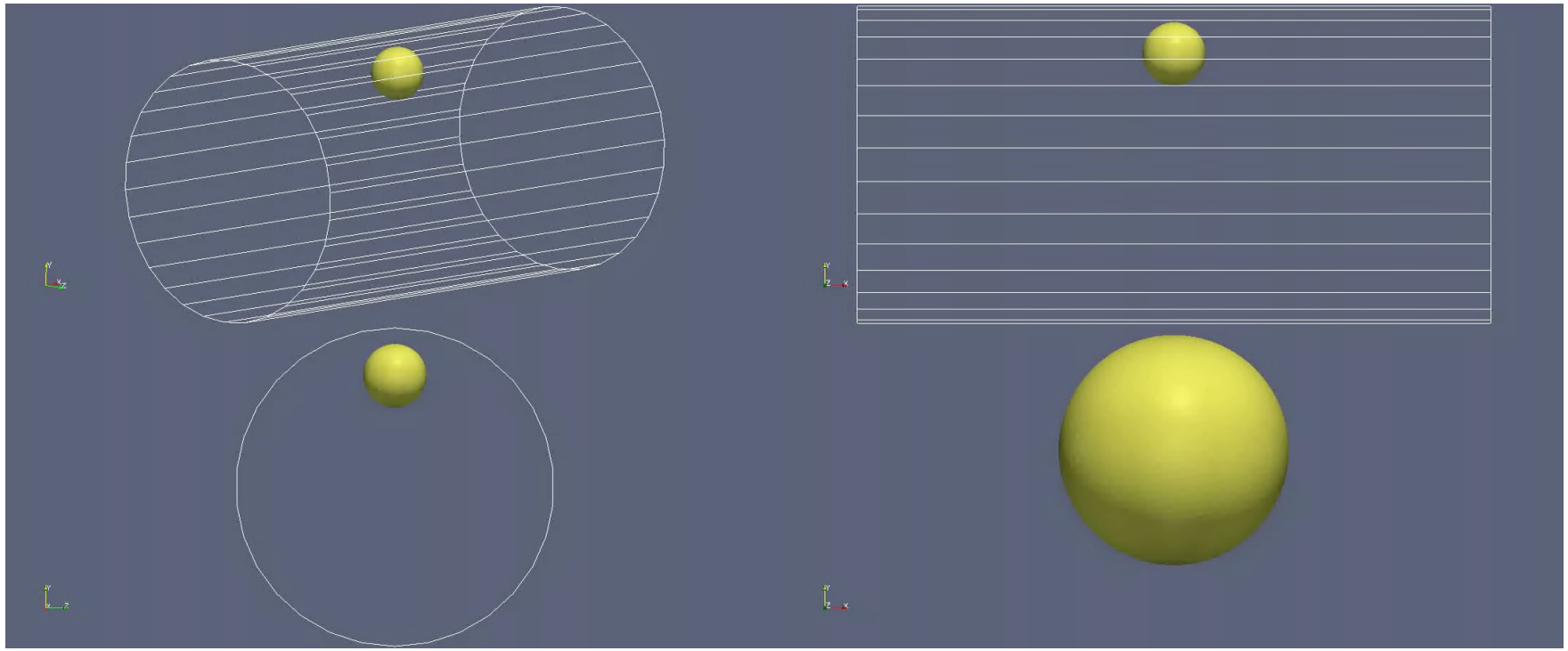
- In geometries with a rectangular cross-section, the axial symmetry is lost.
  - Particles migrate towards a **finite number of equilibrium positions** within the channel cross-section.

J Zhang et al, Lab Chip, 2016

Elastic particle migration in straight channels

- Lateral forces act on **soft particles** suspended in **Newtonian liquids** under pressure-driven flows in straight channels:
  - wall effects push them toward the channel axis,
  - elastic forces also push them toward the axis.
- Soft particles undergo lateral migration even in the Stokes regime ($\mathrm{Re} = 0$).





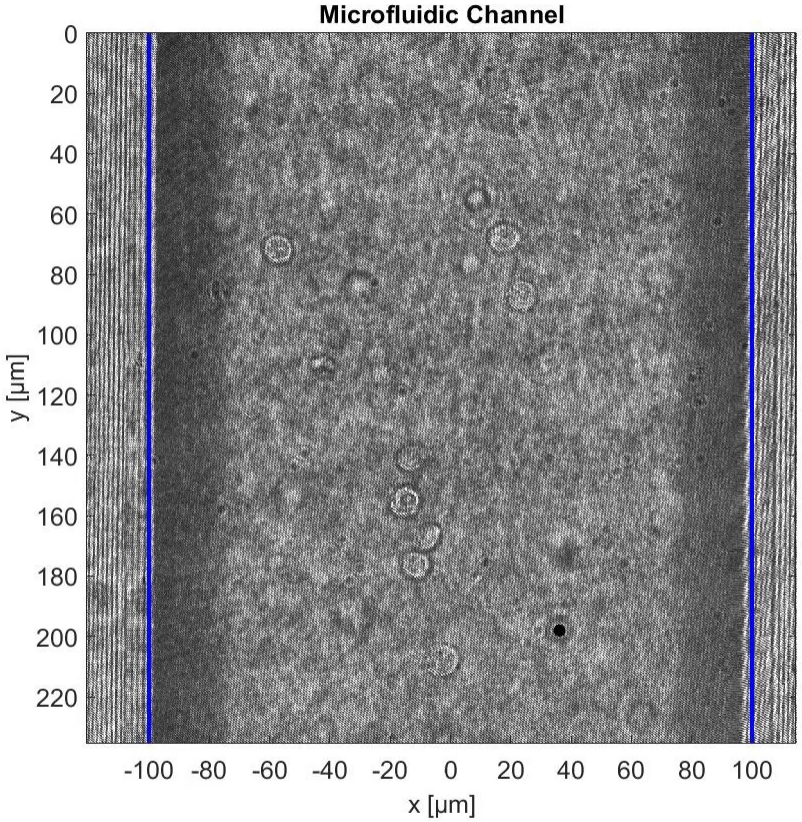
MM Villone and PL Maffettone, Rheol Acta, 2019

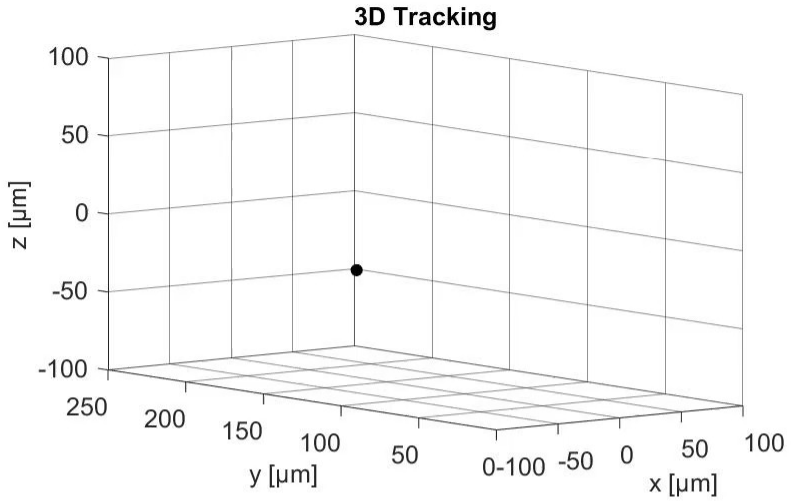
Aim of the work

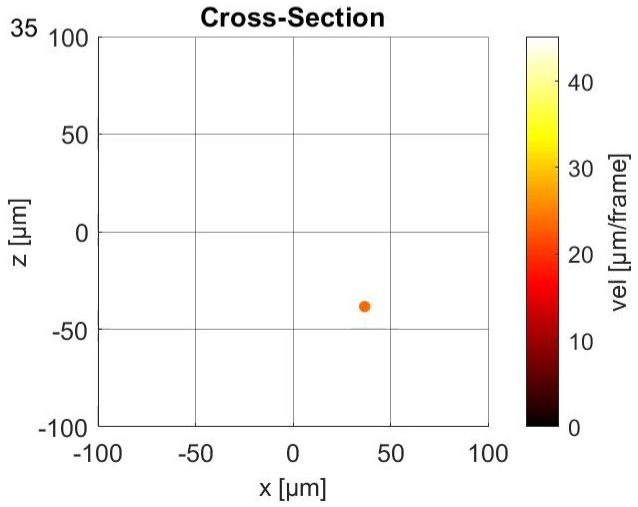
- To make the tomography of flowing cells, **three requirements** must be fulfilled:
  - the cells have to perform **at least one complete rotation** within the FOV of the imaging apparatus,
  - the cells have to travel along the FOV as fast as possible to increase the throughput, but **not too fast** to allow the camera to acquire a sufficient number of frames for the tomography,
  - the flow must **not deform** the cells above about ~5%.

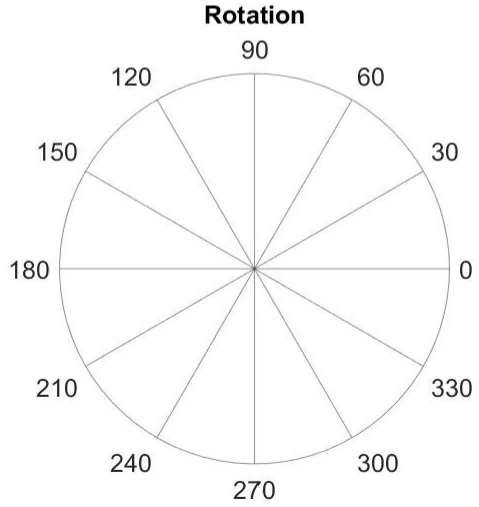
- We investigate by **direct numerical simulations** the effects of the **hydrodynamic interactions** among several cells on their **rotational behaviour** and mechanical deformation during the flow along a microfluidic channel.
  - These are two essential aspects connected to the possibility of recovering the tomograms.

Experimental data



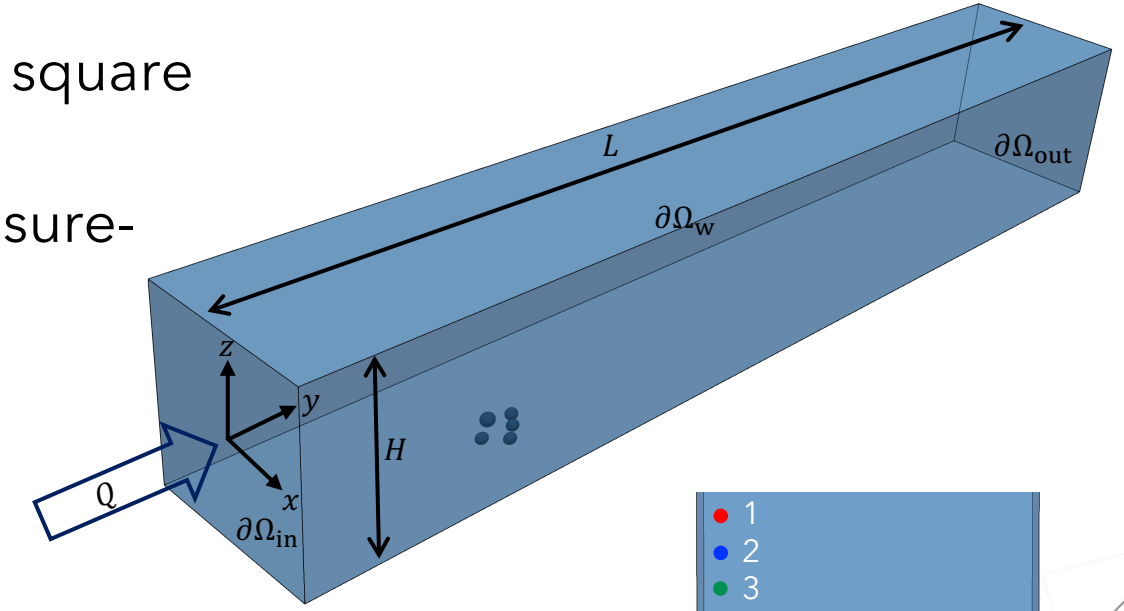


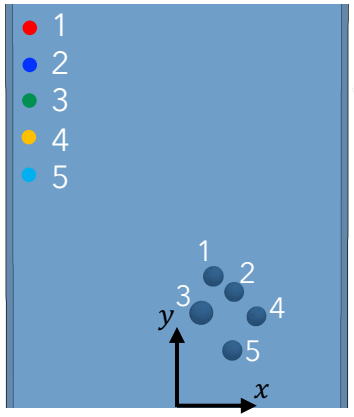


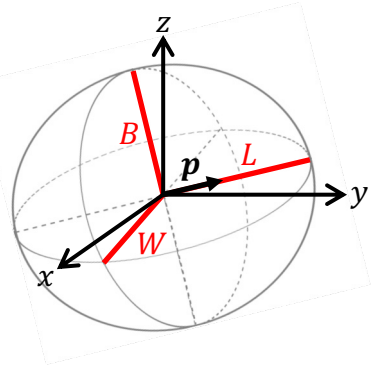


Mathematical model (1/2)

- We consider
  - a portion of a straight channel with square cross section,
  - a **Newtonian liquid** undergoing pressure-driven flow carrying **5 ISK cells**.







- **Mathematical model**:
  - continuity equation: $\nabla \cdot \boldsymbol{u} = 0$
  - momentum balance equation:
    $\rho\left(\frac{\partial \boldsymbol{u}}{\partial t} + \boldsymbol{u} \cdot \boldsymbol{\nabla u}\right) = -\boldsymbol{\nabla} p + \boldsymbol{\nabla} \cdot \boldsymbol{\sigma}$
  - Newtonian constitutive equation (liquid):
    $\boldsymbol{\sigma} = 2\eta \boldsymbol{D}$
  - neo-Hookean constitutive equation (cells):
    $\overset{\nabla}{\boldsymbol{\sigma}} = 2G\boldsymbol{D}$

ISK is a well-differentiated endometrial cancer cell line preserved in the Obstetrics and Gynecology Laboratory of Peking University People's Hospital.
T Ohashi et al, Biomed Mater Eng, 2002

Mathematical model (2/2)

- **Boundary conditions**:
  - no-slip/no-penetration on channel's walls:

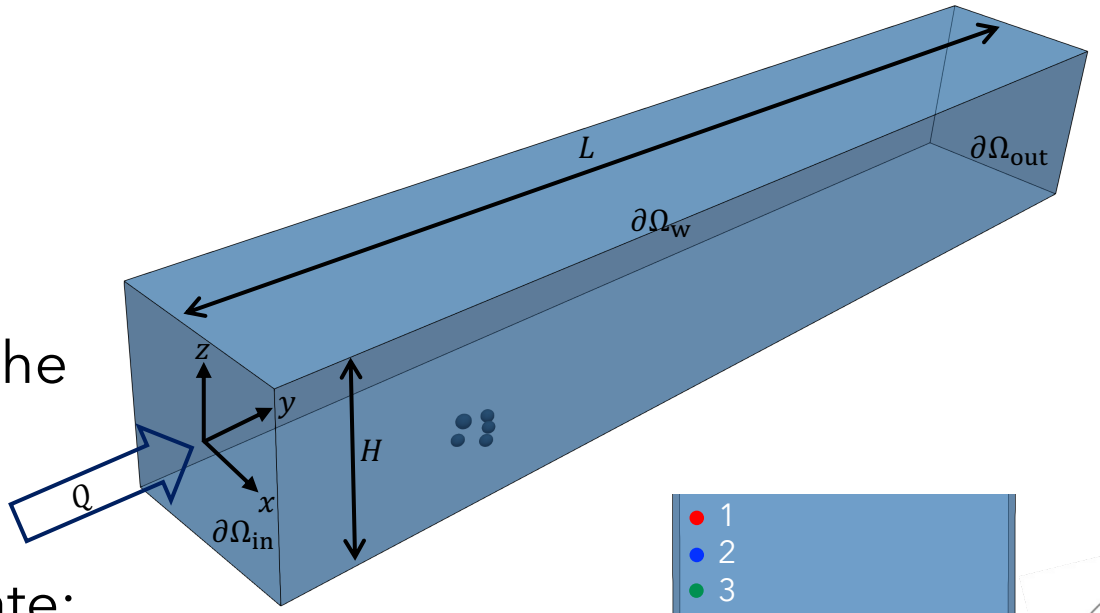
    $\boldsymbol{u} = \mathbf{0}$ on $\partial\Omega_{\mathrm{w}}$
  - periodicity of liquid velocity across the channel inlet/outlet sections:

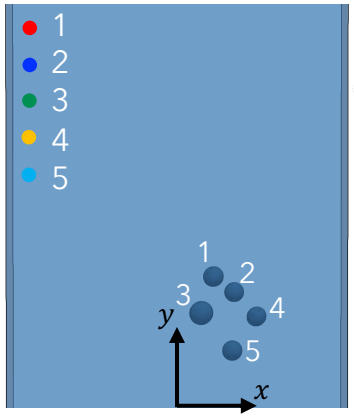
    $\boldsymbol{u}|_{\partial\Omega_{\mathrm{in}}} = \boldsymbol{u}|_{\partial\Omega_{\mathrm{out}}}$
  - imposed suspending liquid's flow rate:

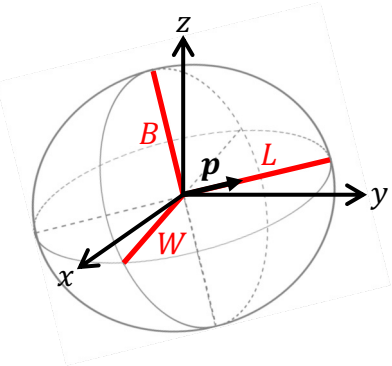
    $-\int_{\partial\Omega_{\mathrm{in}}} \boldsymbol{u}\cdot\boldsymbol{n}\, dS = Q$
  - continuity of velocity across cell-liquid interfaces:

    $\boldsymbol{u}_{\mathrm{c}} = \boldsymbol{u}_{\mathrm{l}}$
  - continuity of traction across cell-liquid interfaces :

    $\boldsymbol{\sigma}_{\mathrm{c}}\cdot\boldsymbol{n} = \boldsymbol{\sigma}_{\mathrm{l}}\cdot\boldsymbol{n}$

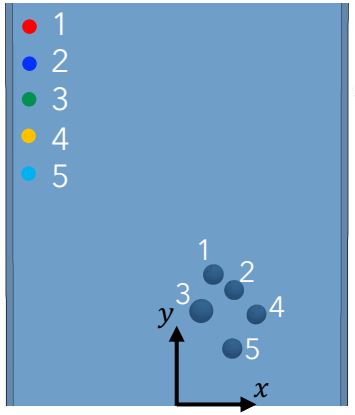


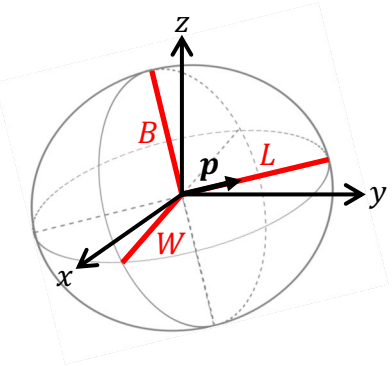




Dimensionless parameters

- Geometrical, operating, and physical parameters:
  - $H = 200\ \mu\text{m}$,
  - $\text{Q} = 1\ \mu\text{l/min}$,
  - $\bar{u} = Q/H^2 = 3.33\times10^{-4}\ \text{m/s}$,
  - $\rho = 1000\ \text{kg/m}^3$,
  - $\eta = 10^{-3}\ \text{Pa.s}$,
  - $G = 900\ \text{Pa}$,
  - $\boldsymbol{p}_0 = \left(\sqrt{3}/3, \sqrt{3}/3, \sqrt{3}/3\right)$.





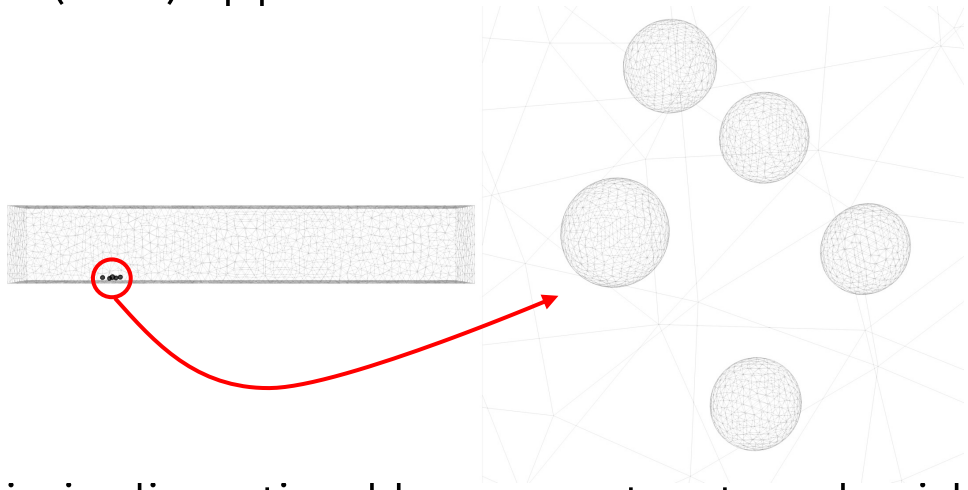
Cell number	Volume [μm^3]	L/B	L/W
1	1000	1.05	1.07
2	900	1.05	1.13
3	1600	1.07	1.10
4	900	1.12	1.18
5	950	1.07	1.17

- **Dimensionless parameters**:
  - Reynolds number $\text{Re} = \frac{\rho\bar{u}H}{\eta} \sim 10^{-2}$ → **negligible inertial effects**,
  - elastic capillary number $\text{Ca}_\text{e} = \frac{\eta\bar{u}}{HG} \simeq 2\times10^{-6}$ → **no cells' deformation**.

T Ohashi et al, Biomed Mater Eng, 2002

Some numerical details

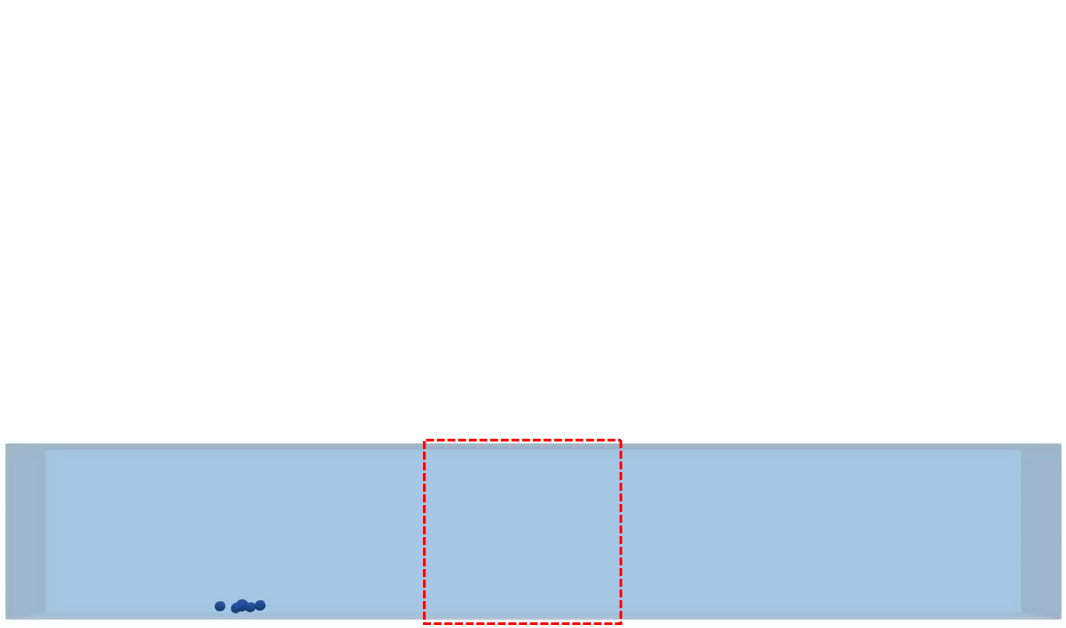
- Direct numerical simulations are made through the **finite element method** (FEM) with the **arbitrary Lagrangian-Eulerian** (ALE) approach[6].

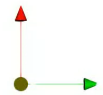


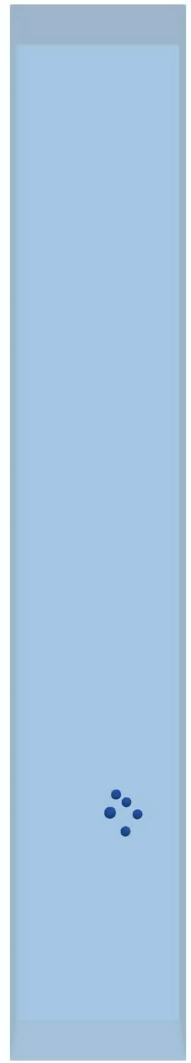
- The computational domain is discretized by an unstructured grid made of **tetrahedral elements**.
  - Any time the 'quality' of the mesh elements in the computational domain becomes unacceptable in terms of a threshold, a **remeshing** is performed and the solution is projected from the old mesh to the new one.
  - Space- and time-discretization **convergence** are verified.
- **Computational time:** about 2 days on 2 cores of DELL Power Edge M710HD blades with two Intel Xeon E5649 hexacore processors @ 2.53 GHz and 48 GB RAM.

MM Villone et al, Comput Fluids, 2014

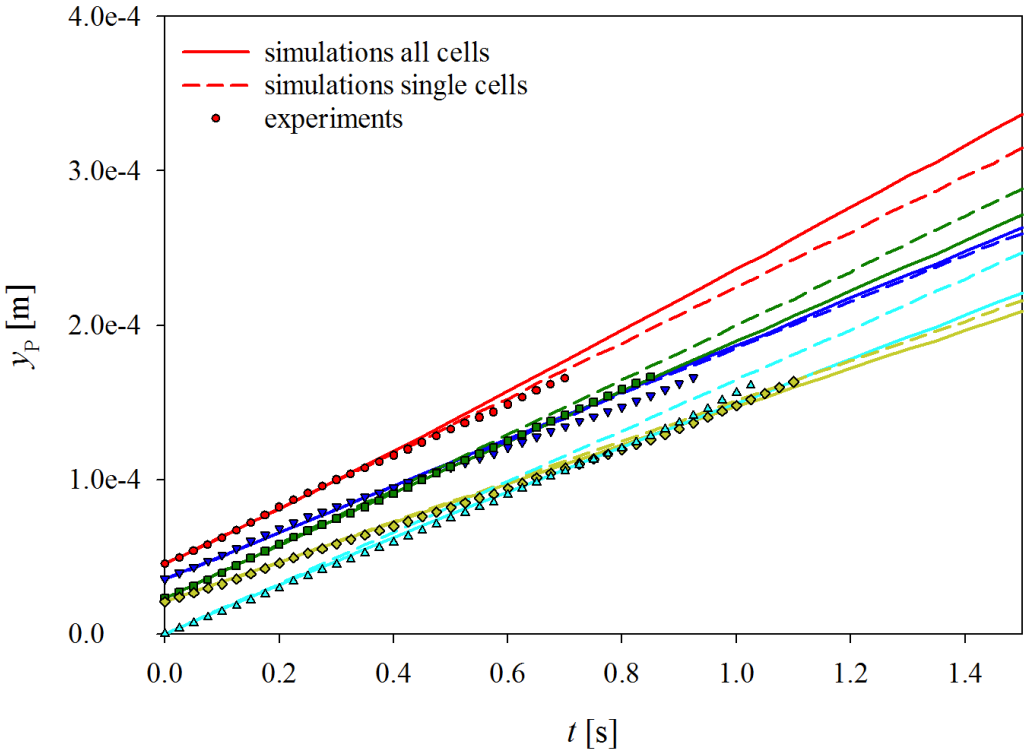
Cell dynamics from numerical simulations





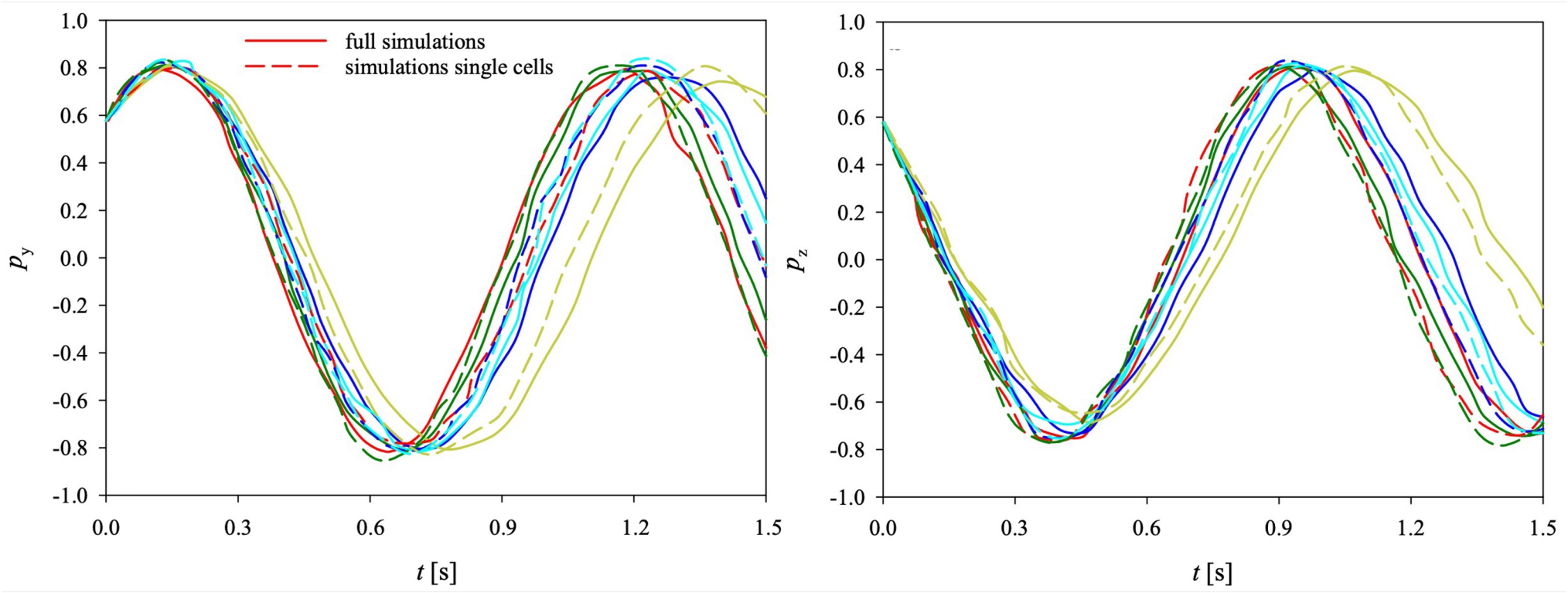


Cell translational dynamics



- **Experimental validation** of the simulations is achieved.
- **Little quantitative differences** between the trajectories of the cells with and without the others:
  - cell 1 slightly slower when alone (hydrodynamic interactions "push it forward" along the flow direction),
  - cells 2 and 5 slightly faster when alone (hydrodynamic interactions slow them down along the flow direction),
  - cells 3 and 4 show almost no difference between the two cases.
- **Differences are moderate** → hydrodynamic interactions do not alter cell translational dynamics drastically.

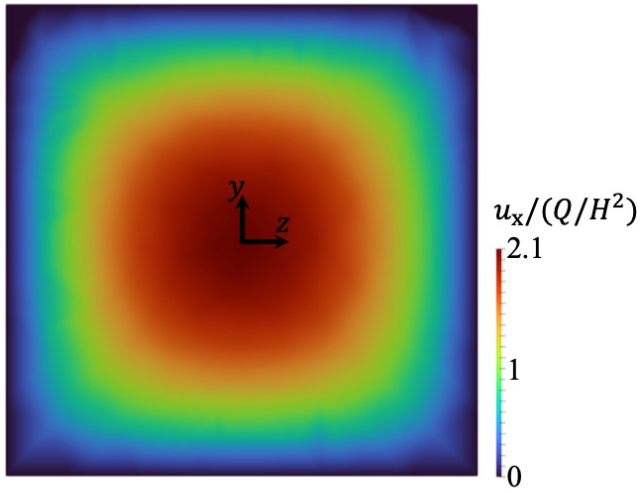
Cell rotational dynamics

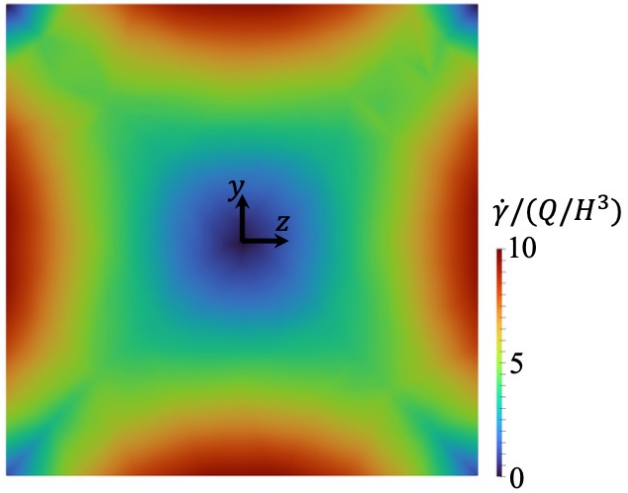


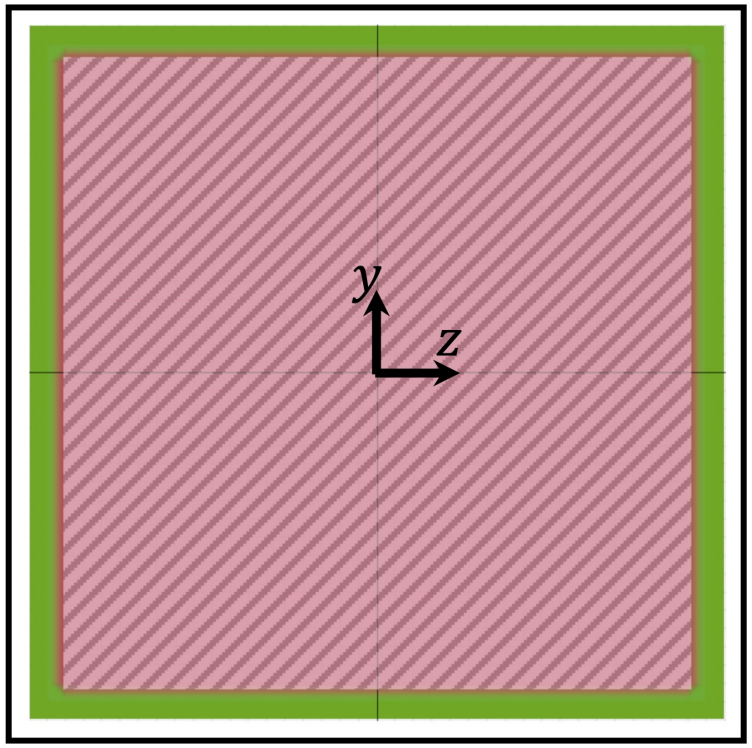
- Hydrodynamic interactions slightly **slow down** cell rotation.

Effect of the transversal position

- Let us assume $L_{\mathrm{FOV}} = H$







Conclusions

- Hydrodynamic interactions **slow down** cell rotation.
  - They need a longer space in the flow direction to complete a rotation compared to the case where they are isolated.

- Since the hydrodynamic torque that makes the cells rotate is connected to the gradient of the velocity of the carrier liquid in the cross section of the channel, the **best conditions for cell rotation** are found **close to the channel walls**.

- Also from point of view of translation, the best location is close to the walls, since the translational velocity is the lowest.
  - This could be disadvantageous in terms of throughput.

- Cell **deformation is negligible** in the characteristic given the operating parameters considered here.

If you want to know more…

- Vitolo, A., Villone, M.M., Maffettone, P.L., Numerical study of the effects of hydrodynamic interactions among cells for microfluidic holographic cyto-tomography, Frontiers in Physics, in press
- Pirone, D., Montella, A., Sirico, D.G., Mugnano, M., Villone, M.M., Bianco, V., Miccio, L., Porcelli, A.M., Kurelac, I., Capasso, M., Iolascon, A., Maffettone, P.L., Memmolo, P., Ferraro, P., Label-free liquid biopsy through the identification of tumor cells by machine learning-powered tomographic phase imaging flow cytometry, Scientific Reports 2023; 13:6042
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- Villone, M.M., Memmolo, P., Merola, F., Mugnano, M., Miccio, L., Maffettone, P.L., Ferraro, P., Full Angle Tomographic Phase Microscopy of Flowing Quasi-Spherical Cells, Lab on a Chip 2018;1:126-131

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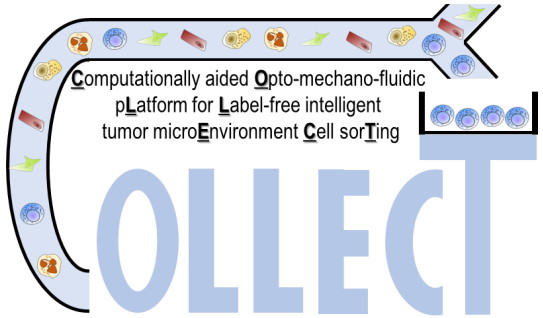

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