# Numerical investigation of microfluidic sorting of microtissues

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# Abstract

We characterize through simulation a microfluidic-based particle sorting approach instrumental in flow cytometry for quantifying microtissue features. The microtissues are represented herein as rigid spheres. The numerical solution employed draws on a Lagrangian-Lagrangian (LL), Smoothed Particle Hydrodynamics (SPH) approach for the simulation of the coupled fluid – rigid-body dynamics. The study sets out to first quantify the influence of the discretization resolution, numerical integration step size, and SPH marker spacing on the accuracy of the numerical solution. By considering the particle motion through the microfluidic device, we report particle surface stresses in the range of  $\sigma = [0.1, 1.0]$  Pa; i.e., significantly lower than the critical value of 100 Pa that would affect cell viability. Lift-off of non-neutrally buoyant particles in a rectangular channel flow at the target flow regime is investigated to gauge whether the particle shear stress is magnified as a result of dragging on the wall. Several channel designs are considered to assess the effect of channel shape on the performance of the particle sorting

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device. Moreover, it is shown that a deviation in flow rate does not influence the focusing of the particles at the channel outlet.

Keywords:

Microfluidic, particle sorting, direct numerical simulation, smoothed particle hydrodynamics

# 1. Introduction

Biomedical research and clinical applications rely on accurate devices for sorting and separating of cells. For decades, single cells have been successfully purified using fluidic sorting techniques. Those techniques were mostly limited to purification of small cells with diameters  $d < 10 \,\mu\text{m}$ . On the contrary, many of the conventional cell sorting techniques have been inefficient for the purification of cell aggregates or 3D microtissues; i.e., spheroids with diameter  $d > 100 \,\mu\text{m}$ . For instance, cell separation techniques such as Magnetic Activated Cell Sorting (MACS) and affinity chromatography rely on capture molecules that adhere to the cell surface. Their success rate decreases when handling large particles as they rely only on the surface properties of the aggregates. Alternatively, in charge based techniques, such as Fluorescence Activated Cell Sorting (FACS), the particle is deflected in an external electric field. Large inertia of cell aggregates decreases the deflection induced by the electric field thus requiring a larger travel distance for successful particles separation. The approach is highly sensitive since slight perturbations in particle trajectory can magnify over long distances and negatively impact the controlled particle motion.

Large particles have traditionally been sorted manually, i.e. under a mi-

croscope. This approach, however, is less desirable owing to its low throughput and issues related to repeatability, sensitivity, and quantification of experiments. A successful automated physical separation based on optical parameters; i.e., a FACS methodology, was implemented in the COPAS Bio-Sorter [11]. The sorting mechanism is pneumatic and consists of a microvalve actuated in response to particular optical features. Unwanted particles are diverted into a waste tank, while desired particles take the default flow path and pass through. The system, which provided significant efficiency gains for sorting of large particles ranging from  $10 \,\mu\text{m}$  to  $1500 \,\mu\text{m}$ , is limited in that it employs single photon optics, which cannot analyze the interior of aggregate cells deeper than 50 µm. Buschke and collaborators recently developed a multiphoton flow cytometry system capable of deep optical penetration of large aggregates [6], and designed a microchannel for particle sorting [5]. The electromechanical sorting apparatus is shown schematically in Figure 1. The device relies on an interrogation zone to identify particles displaying certain optical features, and two branches controlled by microvalves used to divert particles based on the identified optical features. Upon detection of a certain feature in the interrogation region, an open/close command signal is sent to the microvalves. The valves operation is delayed to account for the particle travel time. The device was shown to improve the efficiency of large particles sorting.

Herein, we consider the sorting solution in [5, 6] to demonstrate how numerical simulation can be used to analyze the particle sorting attributes for different device designs and flow regimes. The simulation framework can predict particle stresses and lift-off and thus ensure that the apparatus



Figure 1: Schematic illustration of the device used for sorting of large particles and 3D microtissues. At the normal condition, branches B and C are respectively open and closed, letting the desired particles move freely through branch B. Upon the detection of a desired property in the interrogation region, the solenoid valves switch the status of B and C to direct undesired particles to the waste container.

will not damage the cells and microtissues due to impact or large fluidinduced stress. Direct numerical simulation can also be used at different Reynolds (Re) numbers to predict the particles' location and velocity, thus predicting how certain design attributes control the aggregate dynamics in the interrogation region.

# 2. Simulation framework

The approach adopted here is based on a Lagrangian-Lagrangian formulation of the fluid and solid phases. The SPH method is used to represent the dynamics of fluid flow and maintain the two-way coupling with rigid body dynamics by regarding body geometries as moving boundaries. The 3D rigid body rotation is characterized by means of a set of three translational coordinates and four Euler parameters [12].

#### 2.1. The Smoothed Particle Hydrodynamics method

SPH [10, 18] is a meshless numerical discretization approach that has been used in problems involving celestial dynamics, fluid dynamics, elastic deformations, etc. [17, 19, 22]. At its core, SPH introduces a *smoothing* scheme for any space dependent field value as well as a discretization scheme using Lagrangian *particles*. For the mathematical identity given as

$$f(\mathbf{x}) = \int_{S} f(\mathbf{x}')\delta(\mathbf{x} - \mathbf{x}')d\mathbb{V},$$
(1)

the smoothing attribute is formulated as

$$f(\mathbf{x}) = \int_{S} f(\mathbf{x}') W(\mathbf{x} - \mathbf{x}', h) \mathrm{d}\mathbb{V} + O(h^2), \qquad (2)$$

where W is a smoothing kernel function whose smoothness is controlled by the characteristic length h. The kernel function is a symmetric,  $W(\mathbf{r}, h) = W(-\mathbf{r}, h)$ , and normalized,  $\int_S W(\mathbf{r}, h) d\mathbb{V} = 1$ , function of distance **r**. Additionally, it approaches the Dirac delta function as the size of the support domain tends to zero; i.e.,  $\lim_{h\to 0} W(\mathbf{r}, h) = \delta(\mathbf{r})$ . An example kernel function, and the choice used in this study, is the cubic spline function [23]

$$W(q,h) = \frac{1}{4\pi h^3} \times \begin{cases} (2-q)^3 - 4(1-q)^3, & 0 \le q < 1\\ (2-q)^3, & 1 \le q < 2\\ 0, & q \ge 2 \end{cases}$$
(3)

where  $q \equiv |\mathbf{r}| / h$ . This cubic spline has a support domain with radius 2h.

The spatial discretization of the equations of motion in SPH relies on moving Lagrangian particles:

$$\langle f(\mathbf{x}) \rangle = \sum_{b} \frac{m_b}{\rho_b} f(\mathbf{x}_b) W(\mathbf{x} - \mathbf{x}_b, h) , \qquad (4)$$

where  $\rho_b$  and  $m_b$  are the density and mass associated with particle b, respectively. To simplify notation, in the remainder of this document we use  $f(\mathbf{x})$ to represent  $\langle f(\mathbf{x}) \rangle$ . Moreover, the term *particle* is used henceforth for rigid spheres in 3D motion while the term *marker* is used to refer to what the SPH community uses for the spatial discretization. Figure 2 illustrates in 2D the kernel function W for a marker denoted as a.



Figure 2: Illustration of the kernel, W, and support domain, S – shown for marker a. For 2D problems the support domain is a circle, while for 3D problems it is a sphere. SPH markers are shown as black dots. Marker b represents a generic marker in the support domain of marker a.

Using the discretization scheme introduced in Eq. 4 as well as the SPH discretization of its gradient [22], the continuity and momentum equations; i.e.,

$$\frac{d\rho}{dt} = -\rho \nabla \cdot \mathbf{v}, 
\frac{d\mathbf{v}}{dt} = -\frac{1}{\rho} \nabla p + \frac{\mu}{\rho} \nabla^2 \mathbf{v} + \mathbf{f},$$
(5)

are discretized at an arbitrary location  $\mathbf{x} = \mathbf{x}_a$  within the fluid domain as [24]

$$\frac{d\rho_a}{dt} = \rho_a \sum_b \frac{m_b}{\rho_b} \mathbf{v}_{ab} \cdot \nabla_a W_{ab},$$

$$\frac{d\mathbf{v}_a}{dt} = -\sum_b m_b \left[ \left( \frac{p_a}{\rho_a^2} + \frac{p_b}{\rho_b^2} \right) \nabla_a W_{ab} - \frac{(\mu_a + \mu_b) \mathbf{x}_{ab} \cdot \nabla_a W_{ab}}{\bar{\rho}_{ab}^2 (x_{ab}^2 + \varepsilon \bar{h}_{ab}^2)} \mathbf{v}_{ab} \right] + \mathbf{f}_a.$$
(6)

Above,  $\mu$ ,  $\mathbf{v}$ , and p are dynamic viscosity, velocity, and pressure, respectively; quantities with subscripts a and b are associated with markers a and b (see Figure 2), respectively;  $\mathbf{x}_{ab} = \mathbf{x}_a - \mathbf{x}_b$ ,  $\mathbf{v}_{ab} = \mathbf{v}_a - \mathbf{v}_b$ ,  $W_{ab} = W(\mathbf{x}_{ab}, h)$ ,  $\nabla_a$  is the gradient with respect to  $\mathbf{x}_a$ ; i.e.,  $\partial/\partial \mathbf{x}_a$ ; and  $\varepsilon$  is a regularization coefficient. Quantities with an over-bar are averages of the corresponding quantities for markers a and b. A weakly compressible SPH model was considered herein, where the pressure p is evaluated using an equation of state [22]

$$p = \frac{c_s^2 \rho_0}{\gamma} \left\{ \left(\frac{\rho}{\rho_0}\right)^{\gamma} - 1 \right\} .$$
(7)

In this equation,  $\rho_0$  is the reference density of the fluid,  $\gamma$  is used to adjust the stiffness of the pressure-density relationship, and  $c_s$  is the speed of sound. The value  $c_s$  is adjusted depending on the maximum speed of the flow,  $V_{\text{max}}$ , to keep the flow compressibility below some arbitrary value. Herein,  $\gamma = 7$ and  $c_s = 50V_{\text{max}}$ .

The fluid flow equations (6) are solved together with

$$\frac{d\mathbf{x}_a}{dt} = \mathbf{v}_a \tag{8}$$

to update the position of the SPH markers.

# 2.1.1. Regularization of the velocity field

Since SPH does not require the velocity field to be single value, multiple markers with different velocities may occupy the same location. At high Mach number, this can cause irregular marker interpenetration. Relying on the fact that the markers transport velocity need not be equal to momentum velocity, Monaghan proposed an extended SPH approach, XSPH, which modifies the Lagrangian velocity based on the Eulerian velocity [20]. In other words, markers' velocity are modified by the velocity at neighboring markers' location according to

$$\langle \mathbf{v}_a \rangle = \mathbf{v}_a + \zeta \sum_b \frac{m_b}{\bar{\rho}_{ab}} \mathbf{v}_{ab} W_{ab} , \qquad (9)$$

where  $0 \leq \zeta \leq 1$  adjusts the contribution of the neighbors' velocities. The modified velocity calculated from Eq. (9) replaces the original velocity in the density and position update equations, but not in the momentum equation [7]. According to Eq. (9), the velocity of each marker gets closer to the Eulerian velocity, calculated as a collective property of markers overlapping at a specific location; therefore, nearby markers move with nearly identical velocities and velocity fluctuations and irregular interpenetrations are remedied.

Other regularization techniques are proposed to reduce the markers clustering through artificial viscosity [22] or pressure [21] approaches. By assimilating the artificial pressure into markers transport velocity, Adami et al. proposed a transport velocity that resolves markers clustering and irregular penetration [2]. All the information required therein can be obtained with a small computational cost increase while calculating the rate equations, i.e. Eqs. (6). This is a computational improvement over XSPH where the markers modified velocities need to be determined before evaluating the rate equations.

Relying on our previous validations [28], the XSPH methodology [7, 20]

along with a small base pressure [21] was used herein. Originally,  $\zeta$  was missing from Eq. (9), implying a value of one;  $\zeta \simeq 0.5$  is suggested in [22] and used herein. Nevertheless, a parametric study is carried out in Section 3.3.2 to show the dependency of the results on  $\zeta$ .

#### 2.2. Rigid body dynamics

The dynamics of the rigid bodies is fully characterized by the Newton-Euler equations of motion, see for instance [12],

$$\frac{d\mathbf{V}_{i}}{dt} = \frac{\mathbf{F}_{i}}{M_{i}},$$

$$\frac{d\mathbf{X}_{i}}{dt} = \mathbf{V}_{i},$$

$$\frac{d\boldsymbol{\omega}'_{i}}{dt} = \mathbf{J}'_{i}^{-1} \left(\mathbf{T}'_{i} - \widetilde{\boldsymbol{\omega}'}_{i}\mathbf{J}'_{i}\boldsymbol{\omega}'_{i}\right),$$

$$\frac{d\mathbf{q}_{i}}{dt} = \frac{1}{2}\mathbf{G}_{i}^{T}\boldsymbol{\omega}'_{i},$$

$$\mathbf{q}_{i}^{T}\mathbf{q}_{i} - 1 = 0,$$
(10)

where  $\mathbf{F}_i$ ,  $\mathbf{T}'_i$ ,  $\mathbf{X}_i$ ,  $\mathbf{V}_i$ ,  $\boldsymbol{\omega}'_i$ ,  $\in \mathbb{R}^3$ , denote the force, torque, position, velocity, and angular velocity associated to body *i*, respectively;  $\mathbf{q}_i \in \mathbb{R}^4$ ,  $\mathbf{J}'_i$ , and  $M_i$ , are the rotation quaternion, moment of inertia, and mass, respectively; and  $i \in \{1, 2, 3, ..., N_r\}$  is the rigid body index where  $N_r$  is the total number of rigid bodies in the system. Quantities with a prime symbol are represented in the rigid body local reference frame. Given  $\boldsymbol{\omega}'_i = [\omega_x, \omega_y, \omega_z]^T$  and  $\mathbf{q} = [q_x, q_y, q_z, q_w]^T$ , the auxiliary matrices  $\widetilde{\boldsymbol{\omega}'}_i$  and  $\mathbf{G}$  are defined as [12]

$$\widetilde{\boldsymbol{\omega}'_{i}} = \begin{bmatrix} 0 & -\omega_{z} & \omega_{y} \\ \omega_{z} & 0 & -\omega_{x} \\ -\omega_{y} & \omega_{x} & 0 \end{bmatrix}, \quad \mathbf{G} = \begin{bmatrix} -q_{y} & q_{x} & q_{w} & -q_{z} \\ -q_{z} & -q_{w} & q_{x} & q_{y} \\ -q_{w} & q_{z} & -q_{y} & q_{x} \end{bmatrix}. \quad (11)$$

#### 2.3. Moving boundary method using Lagrangian markers

Several methods are proposed to enforce a fixed or moving solid boundary [1, 4, 13, 24, 28]. In our previous work [28], we selected the so-called Boundary Condition Enforcing (BCE) markers, distributed on the rigid body, to investigate particle migration in channel flow by resolving immersed rigid bodies. The BCE markers, shown in Figure 3, can be interpreted as fluid markers attached to the solid object to enforce no-slip and impenetrability conditions. At the fluid-solid interface, each BCE marker captures an interaction force due to its inclusion in the proximity of the nearby fluid markers through Eqs. (6). The velocity of a BCE marker is replaced by the local velocity of the moving boundary, while the pressure relies on a projection from the fluid domain [15]. Wall boundary condition is enforced in a similar fashion by attaching BCE markers to the stationary wall.

To improve the fluid velocity at the boundary, Adami et al. [1] proposed a generalized velocity for the BCE markers. In this approach, the velocity of a BCE marker,  $\mathbf{v}_w$ , is calculated so that it complements the fluid contribution toward the wall velocity, i.e.

$$\mathbf{v}_w = 2\mathbf{v}_a - \tilde{\mathbf{v}}_a,\tag{12}$$

where  $\mathbf{v}_a$  is the prescribed wall velocity and  $\tilde{\mathbf{v}}_a$  is an extrapolation of the smoothed velocity field of the fluid phase to the BCE markers,

$$\tilde{\mathbf{v}}_a = \frac{\sum\limits_{b} \mathbf{v}_b W_{ab}}{\sum\limits_{b} W_{ab}}.$$
(13)

The pressure of a BCE marker draws on a force balance at the wall interface,

which is calculated as

$$p_w = \frac{\sum_{f} p_f W_{wf} + (\mathbf{g} - \mathbf{a}_w) \cdot \sum_{f} \rho_f \mathbf{r}_{wf} W_{wf}}{\sum_{f} W_{wf}}.$$
(14)

Although it might not be as accurate as the method proposed in [24], the generalized BCE method [1] given by Eqs. (12) and (14) can be easily applied to arbitrary geometries.

Our result, provided in [29], demonstrate that using Eqs. (12) and (14) improves wall boundary condition; particularly, there is no need for any artificial boundary force in gravitational flow fields. Relying on our previous validation efforts, however, we adopted a boundary model similar to [28] for this work.



Figure 3: Fluid-solid interaction using BCE markers attached to a rigid body. BCE and fluid markers are represented by black and white circles, respectively. The BCE markers positioned in the interior of the body (markers g and f in the figure) should be placed to a depth less than or equal to the size of the compact support associated with the kernel function W.

Once the fluid-solid interaction between BCE and fluid markers is ac-

counted for, it can be reduced to the total rigid body force and torque; for instance by summing the BCE forces and their induced torques over the entire rigid body. These fluid-solid interaction forces are subsequently added to the other forces, e.g., external and contact forces.

# 2.4. Rigid bodies impact

Classical lubrication theory predicts that when immersed in a fluid, impact between two *smooth* spheres does not occur due to an infinite amount of pressure buildup at the interface. In reality, however, solid-solid contact/impact occurs due to surface imperfections [9]. A normal viscoelastic force, expressed as

$$\mathbf{F}_{ve} = k_n \delta_{ij} \mathbf{n}_{ij} - \gamma_n \mathbf{v}_{n_{ij}},\tag{15}$$

is employed to model the direct impact of rigid surfaces, including particles and boundaries [8]. Herein,  $k_n$  and  $\gamma_n$  are material-dependent normal stiffness and damping coefficients, respectively;  $\delta_{ij}$  and  $\mathbf{v}_{n_{ij}}$  are relative interpenetration distance and velocity, respectively; and  $\mathbf{n}_{ij}$  is the unit normal vector at the contact location defined from surface j to surface i.

Depending on the cell properties and concentration, interaction of the rigid bodies can be expressed through different forms including lubrication [14, 28], hybrid viscoelastic-lubrication [16], cohesive, and Van der Waals. Denoting as  $\mathbf{F}_{rr}$  the force that models the rigid-rigid interaction at distance  $d_{rr}$ , where  $d_{rr} \geq \Delta$ , a hybrid, normal force model assumes the form

$$\mathbf{F}_{n_{ij}} = \begin{cases} \mathbf{F}_{rr}, & d_{rr} \ge \Delta \\ \alpha \mathbf{F}_{rr} + (1 - \alpha) \mathbf{F}_{ve}, & 0 \le d_{rr} < \Delta \\ \mathbf{F}_{ve}, & d_{rr} < 0 \end{cases}$$
(16)

where  $\alpha = d_{rr}/\Delta$ . We examined a hybrid viscoelastic-lubrication force model for cell-cell and cell-wall interactions. Due to a low concentration assumption, however, no significant difference was observed; yet a smaller time step was required due to the larger stiffness of the lubrication force compared to the viscoelastic force. Therefore, the viscoelastic model expressed in Eq. (15) was used.

Although usually negligible at the size range considered in this work, the cohesive force can sometimes be important. Depending on the cell and aggregate properties, cohesive forces can be included through Eq. (16). Nevertheless, the tests conducted were motivated by experiment on glass beads [6] where the volume fraction is small and the cohesive force is negligible.

#### 2.5. Time integration

A second order explicit Runge-Kutta method [3] is used to approximate the time evolution of both fluid and rigid markers. At the beginning of each time step, a neighbor list is assembled via a map data structure to indicate the set of markers that fall within the kernel support of each marker. The simulation algorithm was implemented to execute in parallel on Graphics Processing Unit (GPU) cards using the Compute Unified Device Architecture (CUDA) [25]. The main parallelization layers include: (i) force calculation on SPH markers; (ii) reduction of BCE markers' forces to rigid bodies' forces and torques; (iii) time stepping of the fluid markers; (iv) time stepping of the rigid bodies; (v) location update of BCE markers. Details are provided in [27].

#### 3. Simulation tool validation

The solution approach described above has been implemented into a simulation framework validated for particle suspension and migration dynamics in [28]. Further investigations were conducted herein to demonstrate the robustness of the simulation framework in relation to changes in the SPH discretization resolution (changing the value of h); numerical integration step size; markers spacing (number of SPH markers per unit volume), and the XSPH implementation.

#### 3.1. Resolution independence

The velocity profile obtained from the SPH simulation of a transient Poiseuille flow is compared with the exact solution [24] in Figure 4. The simulation setup consists of a 3D channel with dimensions  $(l_x, l_y, l_z) = (1, 0.2, 1)$  mm and confining walls in the z direction. Periodic boundary conditions were considered in the y direction to generate a 2D flow in the x - z plane.

Table 1 lists the exact values of the maximum velocity,  $V_{max}^{exact}$ , at four different time instances. These values were used to calculate the relative error of the numerical solutions,  $\epsilon_r$ , based on

$$\epsilon_r = 100 \times \frac{|V_{max}^{exact} - V_{max}|}{V_{max}^{exact}}.$$
(17)

A different number of SPH markers in the range of  $[28, 887] \times 10^3$  were used to discretize the physical domain. The relative error  $\epsilon_r$ , listed in Table 2 for different resolutions, indicate the mesh independence of the results.

## 3.2. Step size independence of the transient results

Two step size related sensitivity analyses are reported. In the first test, the accuracy of the velocity profile in transient Poiseuille flow was measured

Table 1: Exact value of the maximum velocity of the transient Poiseuille flow,  $V_{max}^{exact}$ , at four different time instances.

Time [s]	0.04	0.08	0.16	$\infty$
$V_{max}^{exact} (\rm mm/s)$	3.82	6.64	9.84	12.5

Table 2: Investigation of the mesh independence in transient Poiseuille flow in a fixed size channel. The table lists the value of  $\epsilon_r$  (%) for different resolutions and time instances.

<b>T</b> :	$N_m (\times 10^3)$						
rime (s)	28	60	122	212	450	887	
0.04	0.9	1.0	0.9	0.9	1.1	1.1	
0.08	0.9	0.3	0.2	0.1	0.2	0.0	
0.16	2.0	1.5	1.1	0.8	0.5	0.6	
$\infty$	2.2	1.6	0.7	0.7	0.9	1.4	



Figure 4: Velocity profile of transient Poiseuille flow obtained from simulation (dots) and series solution (continuous lines) at different times.

as a function of step size. For the flow condition depicted in Figure 4, the maximum relative deviation of the velocity profile,  $\epsilon_r$ , was obtained for different choices of step sizes in the range  $[1, 16] \times 10^{-6}$  s. The results, which were obtained for  $h = 7.0 \times 10^{-6}$ , were virtually the same for all choices, with a maximum relative error of 0.5% when compared to the exact solution.

The second test assessed the effect of step size on the trajectory of the rigid body. To this end, a spherical particle was released at a point close to the channel inlet of the microfluidic channel shown by the hatched area in Figure 5. The particle was tracked through the entire channel length until it arrived at the straight part; i.e., section A, which practically includes the interrogation region. This is a simplified model of the microfluidic channel used for the cell separation with the only difference being the number of the channel curves.



Figure 5: Microfluidic channel used for particle sorting. Only the hatched section was considered for step size analysis.

Figure 6 shows the rigid body displacement in y direction and rigid body velocity as functions of time. Several simulations were run with step sizes in the range  $[1.25, 10] \times 10^{-5}$ s. Therein, the y displacement is measured from the straight channel's lower wall. The velocity and vertical location of the particle at the end of its trajectory; i.e. at the time it is about to enter the interrogation region, are compared to the ones obtained at  $t = 1.25 \times 10^{-5}$ s in Table 3. This table indicates that over the range of step sizes considered, the numerical results are virtually independent of the choice of step size.

#### 3.3. Discussion

## 3.3.1. Markers spacing

For accurate simulations, several authors have suggested a choice of  $\beta = \Delta/h < 0.8$ , where  $\beta$  is the dimensionless inter-marker spacing and  $\Delta$  is the average minimum distance between two markers. For instance, in a Cartesian initialization of the markers,  $\Delta$  denotes the distance between two

Table 3: The errors in body's x displacement,  $d_x$ ; y displacement,  $d_y$ ; and velocity v at t = 10 s, obtained from simulations with different time steps,  $\Delta t$ . The relative errors are calculated with respect to the absolute values obtained from a simulation with  $\Delta t = 1.25 \times 10^{-5}$  s, where  $d_x = 16.4$  mm,  $d_y = 1.40$  mm, and v = 1.36 mm/s. The channel width at the measurement location is 3.04 mm and  $d_y$  is measured from the channel's lower wall.

$\Delta t$	$\epsilon_x\%$	$\epsilon_y\%$	$\epsilon_v\%$	$\epsilon_{ar{R}e}\%$
$1.77\times10^{-5}~{\rm s}$	1.25	1.0	0.1	0.7
$2.50\times 10^{-5}~{\rm s}$	1.24	4.6	2.1	0.5
$3.54\times10^{-5}~{\rm s}$	1.20	3.4	6.0	0.8
$5.00\times10^{-5}~{\rm s}$	2.97	0.8	1.9	0.2
$7.07\times10^{-5}~{\rm s}$	2.26	5.0	3.2	0.4
$1.00\times 10^{-4}~{\rm s}$	2.76	1.2	2.7	0.6



Figure 6: Rigid body's location and velocity as functions of real time, obtained from several simulations with different time steps: (a) location in the y direction; (b) velocity.

neighbor markers in each direction. With  $\beta = 0.8$ , each marker in a 3D setup includes approximately n > 75 markers within its support domain. A series of stability analyses using different kernels was performed in [24] by monitoring the response of the system to an initial applied wave. For an inviscid 3D problem, with SPH markers initially arranged on a cubic lattice and without XSPH refinement, the authors showed that the main stable ranges for a cubic spline kernel are  $0.67 < \beta < 0.8$  and  $1 \leq \beta < 1.11$ . Other stability regions exist outside the aforementioned ranges, but they are not of interest owing to heavy computational burden or accuracy issues discussed later in this section. Although the influence of viscosity and XSPH on the stability regions were not quantified in [24], they are expected to improve the stability by removing the high frequency error modes.

We choose a cubic spline kernel due to its computational benefits. Regardless of the stability criteria, accuracy can be improved through decreasing  $\beta$  which is equivalent to including more sampling points in SPH discretization scheme. However, larger values of  $\beta$  are computationally preferred since the computation load scales at the rate of  $\beta^{-3}$ .

Fortunately, the partition of unity, i.e. the numerical approximation of '1' is valid for a wide range of  $\beta$ . However, it should be pointed out that the backbone of SPH relies on Eq. (2), where the first order term is removed due the fact that the kernel function has a zero slope at the origin. It is possible, however, that a poor discretization invalidates the second order accuracy in Eq. (2). Specifically, by using a Taylor expansion of W at h,

$$\delta(\mathbf{r}) = W(\mathbf{r}, h) - hW'(\mathbf{r}, h) + O(h^2) , \qquad (18)$$

Eq. (4) is written as

$$\langle f(\mathbf{x}) \rangle = \sum_{b} \frac{m_b}{\rho_b} f(\mathbf{x}_b) W(\mathbf{x} - \mathbf{x}_b, h) - h \sum_{b} \frac{m_b}{\rho_b} f(\mathbf{x}_b) W'(\mathbf{x} - \mathbf{x}_b, h).$$
(19)

Figure 7 shows the error,  $\epsilon$ , associated with the first order term in Eq. (19); i.e., the second term on the right hand side, as a function of  $\beta$ . The error, measured for different values of  $\beta$  and several random initialization with homogeneous distribution, grows rapidly for  $\beta > 1.08$ . Therefore, using  $\beta = 1$  along with cubic spline maintains stability and accuracy. To further investigate the influence of  $\beta$ , the maximum error in the velocity profile of the transient Poiseuille flow was obtained for the flow condition shown in Figure 4 and values of  $\beta$  in the range [0.7, 1.2]. The results, shown in Table 4, confirm that the choice of  $\beta \leq 1$  maintains the accuracy.

#### 3.3.2. Transient behavior using XSPH

In the XSPH approach,  $\langle \mathbf{v}_a \rangle$  given in Eq. (9) replaces  $\mathbf{v}_a$  in Eq. (8) as well as in the discretized form of  $d\rho_a/dt$  in Eq. (6). Although such a low pass



Figure 7: First order error of marker-based approximation, plotted as a function of marker distance.

filtering is not expected to affect the transient behavior of the flow or induce any dissipation [20], we carried out a transient Poiseuille flow simulation for different values of  $\zeta \in [0.1, 0.9]$ . The results, shown in Table 5, confirm the independence of the velocity profile from the choice of  $\zeta$ .

To evaluate the efficiency of XSPH and determine the best value for  $\zeta$ , the Root Mean Square of the error of the SPH markers velocity  $\epsilon_{v,rms}$  was evaluated for steady state solution and different values of  $\zeta \in [0, 1]$ , see Table 6. Therein, the exact velocity [24] was used as the reference solution; for a typical marker *a*, the velocity error was obtained as  $\epsilon_{v,a} = v_a - V^{exact}(\mathbf{x}_a)$ . Table 6 lists the relative errors, defined as  $\epsilon_{v,rms}^r = \epsilon_{v,rms}/V_{max}^{exact}$ . Inferred from the results, the influence of the XSPH on the simulation accuracy is only marginal. The main reason is that the flow regimes considered herein are all at moderate to low Reynolds number where the irregular interpenetration is insignificant. As expected, larger values of  $\beta$ , e.g.  $\beta > 0.6$ , result in more

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Time (s)	0.7	0.8	0.9	1.0	1.1	1.2
0.04	0.48	0.01	0.45	0.62	0.25	1.40
0.08	0.04	0.67	0.13	0.08	0.97	3.35
0.16	0.27	1.46	0.76	0.46	1.28	7.13
$\infty$	0.88	2.53	2.53	0.33	1.95	4.54

Table 4: The relative error in maximum velocity,  $\epsilon_r$  (%), obtained for different values of marker spacing,  $\beta$ .

Table 5: The relative error in maximum velocity,  $\epsilon_r$  (%), obtained for different values of XSPH regularization coefficient,  $\zeta$ .

			~			
Time $(s)$		0.0	ς	0.0	0.0	1 0
	0.0	0.2	0.4	0.6	0.8	1.0
0.04	0.6	0.6	0.6	0.6	0.6	0.6
0.08	0.1	0.1	0.1	0.1	0.1	0.1
0.16	0.5	0.5	0.5	0.5	0.5	0.5
$\infty$	1.1	0.7	0.8	0.2	2.2	2.9

Table 6: The root mean square of the markers velocity deviation from the exact Eulerian solution,  $\epsilon_{v,rms}$ , as a function of regularization coefficient,  $\zeta$ .

ζ	0.0	0.2	0.4	0.6	0.8	1.0
$\epsilon^r_{v,rms}$ (%)	0.9	0.8	1.1	0.9	2.6	4.2

dispersion [20].

#### 3.3.3. Boundary displacement

A correct positioning of BCE markers on the surface of the immersed solid bodies is necessary since it directly affects the buoyancy and viscous forces. Improper BCE positioning will, for instance, result in an incorrect buoyancy force and therefore unrealistic particle deposition or flotation. The outermost layer of BCE markers must be positioned at a depth of  $\xi\Delta$  below the real surface. Although theoretically  $\xi = 0.5$ , numerical artifacts such as force magnification close to the curved boundaries and low discretization resolution may call for a different value of  $\xi$ . To ensure adequate BCE placement, a parametric study was performed where a neutrally buoyant sphere was positioned in a stationary fluid. By applying the gravity, a value  $\xi = 0.55$ proved sufficient to capture the particle buoyancy.

#### 4. Results

The geometry of the particle sorting microchannel is shown in Figure 5. The dilute suspension flows through the channel at the rate  $Q \in [0.4, 1.0]$  ml/min. The particles are spherical with diameter in the range [140, 500]  $\mu$ m and density  $\rho = 1050$  kg/m<sup>3</sup>.

#### 4.1. Particles shear stress

The fluid stress,  $\boldsymbol{\sigma}$ , is expressed as the sum of deviatoric,  $\boldsymbol{\sigma}'$ , and volumetric stresses,

$$\boldsymbol{\sigma} = \boldsymbol{\sigma}' - p\mathbf{I} \ . \tag{20}$$

In turn, the deviatoric stress at the location of a marker a can be approximated within the SPH framework as [30]

$$\boldsymbol{\sigma}_{a}^{\prime} = -\mu \sum_{b} \frac{m_{b}}{\rho_{b}} \left[ \mathbf{v}_{ab} \otimes \nabla_{a} W_{ab} + (\mathbf{v}_{ab} \otimes \nabla_{a} W_{ab})^{t} - \frac{2}{3} \left( \mathbf{v}_{ab} \cdot \nabla_{a} W_{ab} \right) \mathbf{I} \right].$$
(21)

Using Eq. 21, the maximum particles stresses was measured in the range [0.1, 1.0] pa. It is worth mentioning that the critical stress that affects cell vitality is around ~ 100 pa.

# 4.2. Particles lift-off

One design criteria for the sorting device is that of avoiding flow conditions in which the particles are dragging on the wall – a phenomena that can affect the cell viability and cause surface rupture. Preventing wall drag calls for the study of particle lift-off. In [26], the authors considered the lift-off of circular particles in a 2D Poiseuille flow. They based the lift-off criteria on the dimensionless shear Reynolds number defined as

$$R = \rho \dot{\gamma} d^2 / \mu , \qquad (22)$$

where  $\dot{\gamma}$  is the shear rate and d is particles diameter. For the considered flow condition, the maximum shear Reynolds number; i.e. the one obtained based on the maximum flow rate and the largest particle size, has a value of approximately 5.6. This value is very close to the critical lift-off shear Reynolds

number reported for w/d = 4, see [26]. Although it serves as a good starting point, the 2D assumption of a motion of spheres in duct flow is limiting. Therefore, the particle lift-off was considered herein at the target flow condition for different particle densities in the range  $\rho_p \in [1000, 1160] \text{ kg.m}^{-3}$ . The fluid flow properties are those of the *in vitro* cell sorting [5]:  $\rho = 1000 \text{ kg.m}^{-3}$ ,  $\mu = 0.001 \text{ kg.} m^{-1} \cdot s^{-1}, w \times e = 3 \text{ mm} \times 1 \text{ mm}, \text{ where } w \text{ and } e \text{ are the chan-}$ nel cross section dimensions in y and z directions, respectively. The gravity  $\mathbf{g} = -9.81 \text{ m.s}^{-2}$  is applied in the z direction. Two flow rates close to the working condition; i.e., with different mean velocities  $V_m \in \{2, 4\}$  mm.s<sup>-1</sup>, were considered herein. This choice of the flow conditions results in channel Reynolds numbers  $Re_e = \rho V_m e/\mu \in \{2, 4\}$ . The flow was initialized as fully developed and the particle linear and angular velocities were initially equal to the fully developed flow velocity at the particle location and a zero vector, respectively. The results, shown in Figure 8, demonstrate particle sedimentation for  $\rho_p \ge 1060 \text{ kg.m}^{-3}$  at  $Re_e = 2.0$ , and  $\rho_p \ge 1080 \text{ kg.m}^{-3}$  at  $Re_e = 4.0$ . Therefore, the target particle density  $\rho_p = 1050 \text{ kg.m}^{-3}$  [5] does not result in particle-wall impact.

## 4.3. Particles focusing

Focusing the particles toward the channel center line increases the performance of the flow cytometry since it improves the predictability of the particles location. For straight channels, Segre and Silberberg showed experimentally that in the pipe flow of a dilute suspension, particles stabilize radially on an annulus with a predictable radius [31]. While remarkable, this phenomenon is of limited relevance in particle sorting since the transition distance is large. If R is the pipe radius, the transition distance x > 150R,



Figure 8: Lift-off of non-neutrally buoyant particles at the working flow condition with  $\rho = 1000 \text{ kg.m}^{-3}$ ,  $\mu = 0.001 \text{ kg.m}^{-1}.s^{-1}$ , and different flow rates: (a)  $Q = 360 \mu \text{l/min}$ , equivalent to  $Re_e = 2.0$ ; (b)  $Q = 720 \mu \text{l/min}$ , equivalent to  $Re_e = 4.0$ . Gravity is applied in the z direction with the magnitude of  $-9.81 \text{ m.s}^{-2}$ . Curves titles show the value of particle density,  $Re_p$ , in kg.m<sup>-3</sup>.

which leads to a large fluidic device. It makes sense to consider instead a meandering shape for the channel, see Figure 5, a topology that intensifies the flow inertia effect thus reducing the length required for particle focusing.

To show the effectiveness of the microfluidic channel, particles with density  $\rho_p = 1050 \text{ kg.m}^{-3}$  were released at different locations at the pipe entry and their location was measured after passing through several periods of the channel. This test was repeated for various channel designs shown in Figure 9, with geometries different in terms of the values of  $(D_{i1}, R_{i2}), i \in \{1, 2, 3, 4\}$ (see Figure 5). Figure 9 shows only the geometry of one period of each design. The real test included seven periods of the curved section in a setup similar to the one in Figure 5.

Table 7 summarizes particle focusing test results, where w is the channel



Figure 9: Microfluidic channel designs used for the analysis of the particle focusing. Only one period of the curved section, attached to the straight channel, is shown. Each test included a channel with seven periods.

width; i.e., channel dimension in the y direction, at the entry and outlet. The initial and final distance between particles in the y direction is denoted by  $l_{start}$  and  $l_{end}$ , respectively. Two tests were performed for each channel design where particles were released at two different initial locations with  $l_{start} = 1.2$  mm. Same tests, with similar particles initial locations, were repeated for all channel designs and the value of  $l_{end}$  was obtained accordingly. The results demonstrate the best focusing efficiency for design e, which has the same dimensions as those in Figure 5 except  $R_{12} = 3.1$  mm and  $R_{32} =$ 4.3 mm. Unsurprisingly, particle focusing improves by increasing the number of channel periods, as reported in Table 8 for channel design 'e'.

Finally, a test was performed to show that the focusing of particles is independent of the flow rate. In this test, neutrally buoyant particles were released at the same location and subjected to different flow rates. The location of particles in the y direction, when they enter the section A of the channel shown in Figure 5, was measured and compared in Table 9. This

Table 7: Particle focusing results obtained for different channel designs shown in Figure 9. For all scenarios, channel width, w, is the same at entry and outlet. Two tests, i.e. with two different particle initial locations were performed for each design. The relative distance between initial and final locations are denoted as  $l_{start}$  and  $l_{end}$ , respectively.  $l_{start} = 1.2$  mm was assumed for all scenarios.

Channel design	a	b	С	d	e	f
$w \pmod{m}$	1.78	2.52	2.52	1.78	2.52	1.78
$l_{end} \pmod{mm}$	0.72	0.81	1.02	0.28	0.16	0.28

Table 8: Effect of the number of periods on the focusing efficiency.

period number	$0: l_{start}$	1	3	5	7
relative distance $l$ (mm)	1.2	0.41	0.33	0.32	0.16

table shows that the deviation in the flow rate does not affect the particles' final position. More importantly, it can be concluded that the final particle velocity scales linearly with the flow rate. This is a useful insight when accounting for the delay time required in the operation of the solenoid valves at branches B and C.

Table 9: Particle focusing at different flow rates in the range [103, 252] ( $\mu$ l/min). Similar at the inlet, the y position is compared at the outlet for different flow rates.

$Q \; (\mu l/min)$	103	154	202	252
$y \pmod{2}$	3.279	3.280	3.276	3.279

# 4.4. Particles separation

Without any underlying sorting mechanism, particles released into the channel shown in Figure 1 might clump very close to each other. A valve opening would then allow both desired and undesired particles to move into the same branch due to lack of separation. Increasing the separation between consecutive particles thus becomes a critical success metric of a design. Modeling and simulation can help in this regard by running "what if" scenarios. For instance, the curved microfluidic channel in Figure 9 was confirmed to increase particle separation by tracking the particles, initially delivered as an agglomerate with small inter-particle distances, throughout the microfluidic channel, see Figure 10.



Figure 10: Particles declustering using the curved channel.

# 5. Conclusions

A numerical simulation methodology is presented that can characterize the sorting of 3D microtissues using a microfluidic technique. The simulation methodology relies on a two-way coupled fluid-solid interaction approach as enabled by the SPH method. Resolution and step size analyses were carried out to demonstrate the robustness of the simulation framework. Robust and accurate simulation results can be achieved by using a cubic spline interpolation kernel along with any SPH discretization that satisfies  $\beta = \Delta/h \leq 1.0$ . The XSPH refinement improves the stability of the simulation without compromising the transient behavior in the numerical solution.

It was shown that the hydrodynamics stresses on the particles' surface stays below 1 Pa, which guarantees damage-free channel passage. For flow condition usually encountered in practice, i.e.  $\rho = 1000$  kg.m<sup>-3</sup>,  $\rho_p =$ 1050 kg.m<sup>-3</sup>,  $\mu = 0.001$  kg.m<sup>-1</sup>.s<sup>-1</sup>, and  $V_m = \{2,4\}$  mm.s<sup>-1</sup>, the liftoff forces prevented the particles from impacting the channel wall. Therefore, at a minimum flow rate  $Q = 360 \ \mu$ l/min, the particles experiences hydrodynamics stresses but not impact stresses. The lift-off analysis in a rectangular channel showed that sedimentation occurs at Stokes number  $St = (2/9) (\rho_p Va/\mu) = 0.71$  when Re = 2.0, and St = 1.44 when Re = 4.0.

The simulation setup was leveraged in channel geometry selection. Six channel designs were analyzed to assess their focusing attribute, which was shown to improve as the number of channel periods increased. Finally, it was shown that the particles position at the channel exit are independent of the flow rate.

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