Deterministic Lateral Displacement – Challenges and Perspectives

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Abstract

The advent of microfluidics in the 1990s promised a revolution in multiple industries, from healthcare to chemical processing. Deterministic Lateral Displacement (DLD) is a continuous-flow microfluidic particle separation method discovered in 2004 that has been applied successfully and widely to the separation of blood cells, yeast, spores, bacteria, viruses, DNA, droplets, and more. DLD is conceptually simple and can deliver consistent performance over a wide range of flow rates and particle concentrations. Despite wide use and in-depth study, DLD has not yet been fully understood or fully optimised, with different approaches to the same problem yielding varying results. We endeavour here to provide an up-to-date expert opinion on the state-of-art and current fundamental, practical, and commercial challenges as well as experimental and modelling opportunities. Since these challenges and opportunities arise from constraints on

hydrodynamics, fabrication and operation at the micro- and nano-scale, we expect this article to serve as a guide for the broader micro- and nanofluidic community to identify and address open questions in the field.

Keywords — microfluidics; nanofluidics; Deterministic Lateral Displacement; mathematical theory; experiment; modelling and simulation; particle sorting; particle separation

1 Introduction

Deterministic Lateral Displacement (DLD) is a method of separating particles by size. The phenomenon was discovered accidentally in 2004 while looking for ways to exploit asymmetric diffusion of DNA in obstacle arrays [1, 2]. DLD has been used to separate DNA fragments [1], exosomes [3], oil droplets [4], fungal spores [5], blood [6], blood parasites [7], circulating tumour cells [8] and many other bio-particles (see Refs. [9, 10] for DLD review papers).

A standard DLD array is made of a flat microfluidic channel filled with a regular array of micro-pillar obstacles as shown in Fig. 1. Each subsequent row of the array is shifted laterally by a certain distance $\Delta\lambda$, creating an array inclination α with respect to the average flow direction (along the x-axis) through the channel. This setup creates a periodic flow pattern, where the flow through

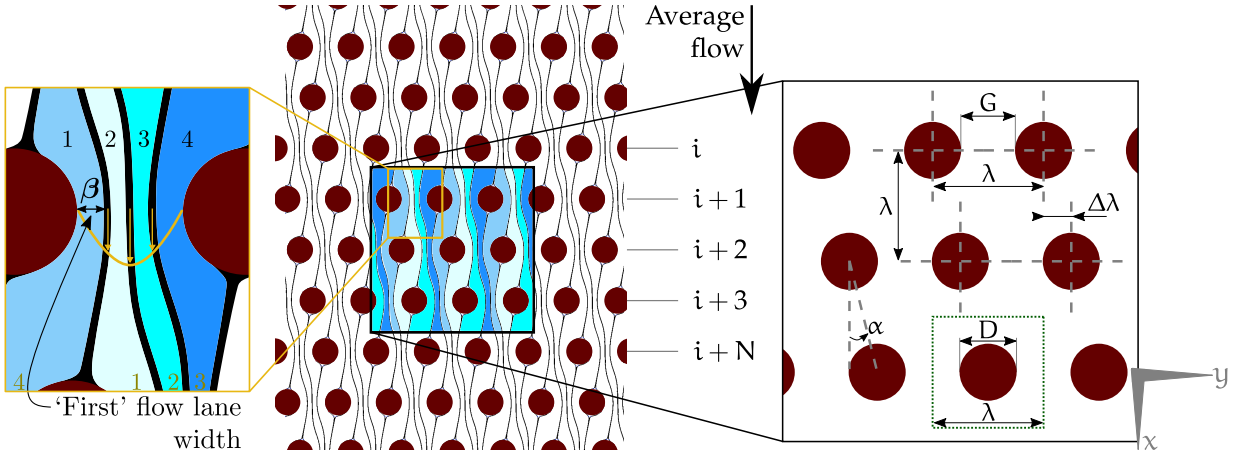

Figure 1: Top view of a typical DLD geometry. Rightmost panel shows geometric details. G is the gap size, λ is the pillar array pitch (here identical for x- and y-directions), D is the post diameter, and $\Delta\lambda$ is the row shift. The row shift fraction in this example is $\varepsilon = \Delta\lambda/\lambda = 1/4$, and the tilt angle is $\alpha = \tan^{-1}(\varepsilon)$. The shifted post arrangement separates the fluid flow into distinct flow lanes (shown in different shadings) separated by a periodic pattern of stagnation streamlines (centre panel). The leftmost panel shows these flow lanes ($N = 1/\varepsilon = 4$ here) through a single gap. The width of the 'first' (pillar adjacent) flow lane β through each pillar gap gives a first order approximation of the critical separation radius for the DLD.

each gap is divided by multiple stagnation streamlines, as shown in the left and central panels of Fig. 1. These flow bifurcations create distinct *flow lanes* through each gap of the array.

Particle separation is achieved when particles above a certain size experience repeated interactions with the posts that result in small and identical net lateral displacements (along the y-axis), leading to particles moving at the array inclination α. These large particles are therefore separated from smaller particles that experience no net displacement and follow the fluid flow direction. Particles following the array inclination angle are in the *displacement mode* (or *bump mode*) since they bump into a post and are laterally displaced at each row; particles tracing the average fluid flow direction are in the *zigzag mode* since their track lines appear to zigzag between posts.

A sharp transition in particle trajectory is usually seen at a critical particle diameter commonly denoted as D_c which is typically small and can be made much smaller than the gap G, making DLD ideal for clog-free, size-based particle separation. The DLD principle relies on deterministic, rather than stochastic or diffusive effects, which gives DLD a high size resolution. Thermal diffusion of particles still takes place; this effect is usually negligible for micron-sized particles, but becomes relevant on the nano-scale (see Sec. 2.4). DLD is robust and fault tolerant because separation relies on repetitive action. For example, a fabrication defect such as a broken or missing pillar will disrupt separation at that pillar, but effective separation can still be achieved with a longer device.

Shortly after the discovery of DLD, its potential for both cellular and nano-scale separation was identified: in 2005, BD (formerly Becton Dickinson) challenged Austin and Sturm to make a chip to separate plasma from blood [6]; and the bumping of 200 nm particles was first demonstrated in 2008 [11]. While applications for the sorting of biological cells flourished, more practical applications of nano-scale DLD took another decade [3]. DLD has been used as an integrated upstream sample processing method and has shown applications in single live-cell analysis [12], gene sequencing [13] and disease diagnostic [14]. DLD shows a robustness and applicability comparable with other microfluidic cell separation technologies like dielectrophoresis, optical tweezing or surface acoustic waves [15]. Within the realm of healthcare, DLD devices may be used to rapidly isolate, purify, enrich, and sub-fractionate circulating bio-markers from complex bio-fluids, augmenting or displacing existing preparation methods to streamline the diagnostic workflow. Further evidence for the impact that DLD has had to date is given by the summary of industrial use in Sec. 4.2.

Despite recent progress and wide application, many challenges and opportunities remain. These are multidisciplinary, usually arising from the interaction of device, fluid and particle. Fig. 2 outlines the significant factors influencing DLD design and performance, and references them back to relevant sections in the main text (see also Fig. 4 in the appendix for a causal loop diagram). Practical use adds complexities, such as design and fabrication approaches, and influences best operational practice and device cost. We focus on the most interesting and pressing concerns which we believe should shape future work and could increase commercial potential. We distinguish between fundamental challenges (Sec. 2), practical challenges (Sec. 3) and commercial challenges (Sec. 4).

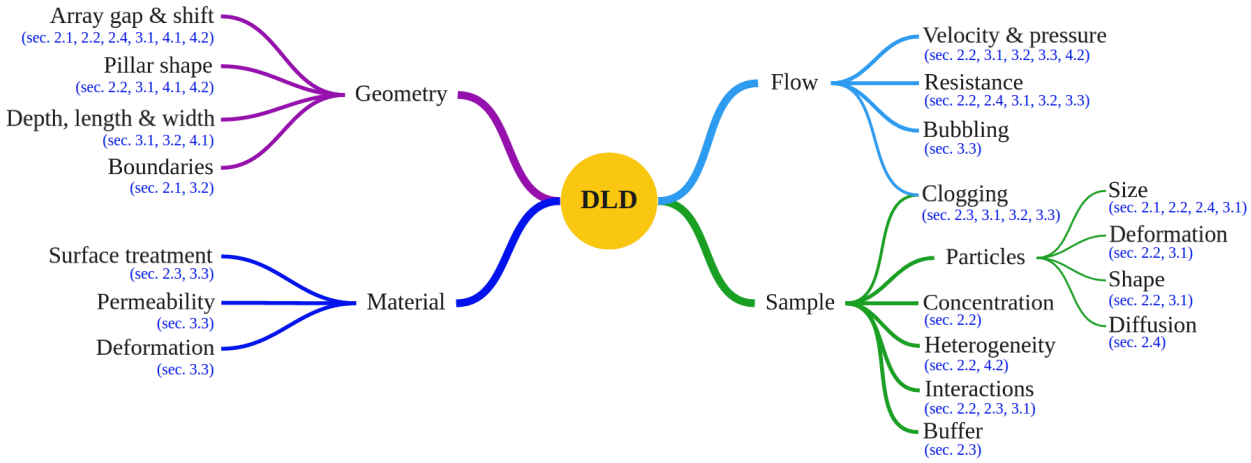

Figure 2: Tree-navigation diagram of parameters to be considered for DLD design, fabrication and operation. The figure back-references these parameters to relevant sections in the main text (Sec. 2 for fundamental challenges, Sec. 3 for practical challenges, and Sec. 4 for commercial challenges). The list is not exhaustive but picks out the most important and commonly considered factors. The causal dependencies between these factors are illustrated in a causal loop diagram in Fig. 4 in the appendix.

In Sec. 5, we summarise the main challenges and opportunities for future DLD research.

2 Fundamental challenges

Although DLD has been employed for 15 years, there are still important open questions about its fundamental principles. These challenges are related to fluid-only behaviour (Sec. 2.1), the interactions of particles with the fluid and the device (Sec. 2.2), surface chemistry (Sec. 2.3) and scaling to nano (Sec. 2.4).

2.1 Fluid-only behaviour

The first step toward mastering DLD is understanding the steady-state flow field. This flow field is primarily determined by the device, *i.e.* the shape, dimension and arrangement of the posts and other solid boundaries. The first conceptual model of DLD proposed that the critical diameter D_c is equal to twice the width of the first flow lane β (Fig. 1) [1]. If the flow profile in the gap is assumed approximately parabolic, the width of this flow lane can be calculated analytically for any array angle [16]. The approximation $D_c \approx 2\beta$ explains much of the experimental data on critical size, but remains incomplete (Fig. 3).

Due to the lack of detailed experimental flow field measurements, the large parameter space and the challenge of solving the (Navier-)Stokes equation, a variety of numerical flow solvers are used,

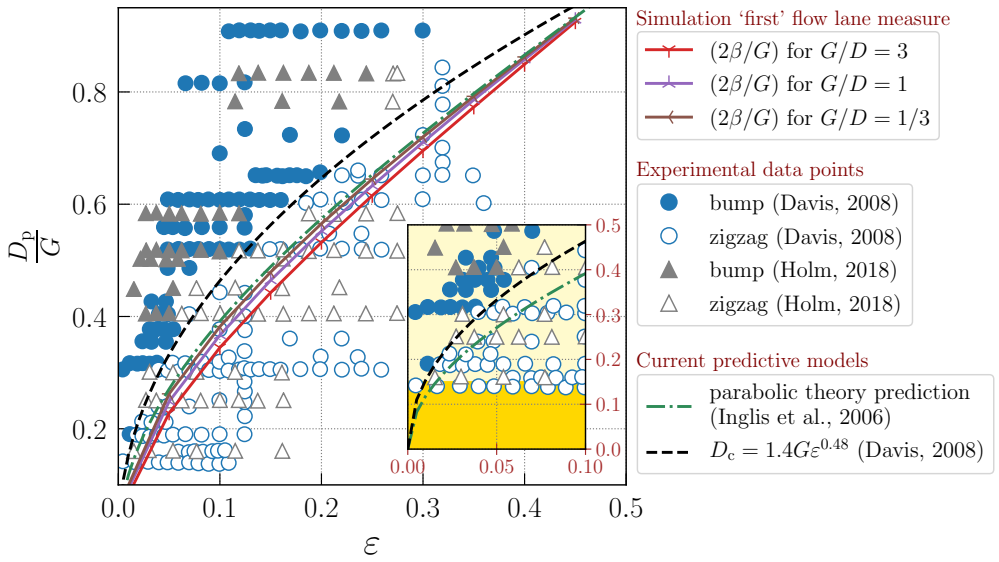

Figure 3: Selection of experimental data for spherical beads with diameter D_p in *bump* and *zigzag mode* as function of the DLD row shift fraction ε and gap G. Open symbols denote zigzagging particles, solid symbols bumping particles. Dashed (black) line gives an empirical best fit curve [17] for critical diameter D_c, which is seen to be always larger than the numerically obtained values (solid lines) of the first flow lane width 2β from fluid-only simulations. Though these simulation measures agree well with the analytical prediction [16] (dot-dashed line, green) of $2\beta = f(\varepsilon, G)$, from an assumed parabolic velocity profile, we see fluid-only predictions are insufficient to explain experimentally observed critical size behaviour. The inset shows zoomed-in data for the often used row shift range ($0 \leq \varepsilon \leq 0.1$), highlighting a finite size limit on the experimentally achievable particle separation for $D_p \lessapprox 0.15G$. A full understanding of particle mode behaviour remains elusive, even for simple rigid spherical particles in DLD with cylindrical pillars.

such as finite element (*e.g.* COMSOL), finite volume (*e.g.* ANSYS Fluent), lattice Boltzmann [18], dissipative particle dynamics [19], and boundary integrals [20]. For correctly imposed boundary conditions and sufficient grid/mesh resolution, fluid-only simulations are normally accurate and reliable.

Most numerical models cannot capture long-range flow patterns (such as anisotropic device permeability which leads to a deflection of the flow direction away from the pressure gradient direction [21]), nor are they routinely used for the design of complete devices. *Simulating entire DLD devices (centimetres) with the necessary resolution (micrometres) is normally not done as it requires computationally expensive simulations.*

A related problem is the design of the non-periodic lateral device boundaries. Strategies for improving the flow patterns and particle dynamics near boundaries exist [22–24]. *However, no*

approach has yet delivered perfect DLD boundaries in 3D.

To reduce computational cost, 2D rather than 3D simulations are often employed. Since the flow patterns vary through the depth of a device, 2D simulations are sufficient only when all fluid features are much smaller than the post height. *Until computational constraints are overcome and full 3D simulations of complete devices from inlet to outlet are routinely accessible, designers will need to continue to synthesise analytical models and approximations with 3D simulations of device sub-domains.*

2.2 Particle-flow interaction

Full knowledge of the flow field allows an estimation of the critical particle size in some circumstances, but even in the creeping flow limit the critical diameter D_c is not equal to twice the first flow lane width.

Fig. 3 reminds us that, for typical situations, D_c has always been found to be larger than twice the width of the first flow lane (2β). This first-order approximation ignores particle-flow interactions where the presence of the particle in the gap alters the velocity field and any notion of a stationary flow lane. It has also been shown that for non-circular posts, such as I-shape pillars, the velocity profile on the cross-section between two neighbouring pillars is symmetric and approximately parabolic, and yet the sorting performance is quite different [25]. Existing analytical treatments for this coupled problem are limited in their applicability. For example, the Faxén and Maxey-Riley equations [26] predict the particle trajectory, but they assume that the particle is far from any boundary or other particles, which is invalid in DLD. *Since the particle-induced flow perturbation, and with it the true particle trajectory, is a complicated (and unknown) function of particle properties and boundaries, an accurate representation of particle motion in fluid flow is overwhelmingly more challenging than the oversimplified idea that particles behave like tracers in a well-known flow field combined with steric repulsion from the wall.*

Even though the motion of particles in fluid flow is inherently 3D, the majority of microfluidic systems generally allow a 2D approximation in modelling and simulation, which is simpler and computationally less demanding and may still deliver semi-quantitative or at least qualitative results. For instance, modelling of spherical particles as circles in 2D can deliver quantitative predictions of their critical size [19], whereas capturing the motion of deformable RBCs in DLD [18, 27] generally requires 3D modelling. It is important to keep in mind that hydrodynamic interactions in 2D and 3D are not the same, which may result in a under-/overestimation of drag and lift on the particles. *It is generally difficult to predict when 2D approximations are suitable and when they fail, but 2D simulations can identify promising device configurations and flow conditions, which can guide computationally more expensive and more predictive simulations of 3D models.*

Particle-post contact — In the absence of direct contact, particle-particle and particle-post interactions are mediated by the fluid. In continuum hydrodynamics, lubrication refers to the forces that arise when the fluid layer between solid objects is small and flow is laminar. Analytical solutions to the Navier-Stokes equations for smooth rigid objects in this limit predict a lubrication force that is inversely proportional to the distance between the objects [28].

In the lubrication model and at negligible inertia, direct contact between a fixed cylinder and a moving sphere does not occur, and the trajectory of the sphere is time-reversible. In real-world DLD devices, additional effects come into play, such as multi-obstacle effects, finite inertia, surface roughness, short-range electrostatic repulsion (see Sec. 2.3), and a breakdown of the continuum approximation at small distances. The latter is certainly significant as the limits of nano-DLD are explored (see Sec. 2.4). Particle softness and non-spherical shape, as discussed below, also break the time reversibility symmetry. *Although simulations without special surface interaction effects show qualitative and quantitative agreement with experiments [19, 27], a rigorous mathematical model for the conditions leading to bumping and zigzagging does not exist. Such a model would likely explain some of the deviations highlighted in Fig. 3.*

Biological and deformable particles — Precise control of particle trajectories within DLD devices depends on a number of parameters, including device geometry, fluid properties, particle shape and deformability. Therefore, efficient separation or sorting of particles of interest requires fine-tuning of various conditions such that the corresponding device serves as an optimal sensor or separator of the targeted particles.

Biological particles constitute the group most widely studied in DLD devices. Compared to the polystyrene beads used to develop most microfluidic systems, biological particles present a range of complications including, but not limited to, non-spherical shapes, deformability, non-hydrodynamic particle-surface and particle-particle interactions. What is more, these properties are often extremely heterogeneous within a sample. For example, whole blood contains about 45% erythrocytes $< 1\%$ leukocytes, $< 1\%$ thrombocytes, and potentially circulating tumor cells, bacteria, parasites, DNA, and extra-cellular vesicles [29].

Deformable cells can have features (*e.g.* nucleus size, membrane viscoelasticity) with potentially large effect on their dynamics and therefore trajectories. For example, unlike rigid particles, deformed particles experience additional lift forces in shear flow [30]. Also, the frequency of the intrinsic dynamics of the cell in flow (*e.g.* tumbling or tank-treading) interferes with the encounter frequency with the posts, which strongly affects trajectories [31]. For all deformable particles, such as macromolecules [1, 32], droplets and cells, the degree of deformation is affected by viscous and inertial forces, so that separation performance changes with flow rate [33]. A related problem is the shape of the DLD posts [19, 34]. It has been shown experimentally [25] and numerically [35] that soft particles interact with different post shapes in non-trivial ways, which allows for the separation

of particles by deformability or other cellular properties.

The requirement of high efficiency and sensitivity of devices for a specific target suggests that a custom design is needed for every particular case of interest. *There is currently no comprehensive understanding of the interplay of DLD geometry, particle deformation, dynamics and trajectories. In particular, we do not understand how pillar shapes can be optimised to achieve desired separation outcomes for soft particle populations.*

DLD with finite inertia — Operating DLD devices with high pressure drops (and therefore high flow rates), or using larger-scale DLD devices (*e.g.* millimetre-range gap size) can give rise to flows with Reynolds numbers (Re) well above unity. In these cases, inertia is important and leads to at least two distinct effects. First, the streamlines deform, and the flow field between posts can no longer be considered parabolic [36]. Secondly, particles at $\mathrm{Re} > 1$ experience inertial lift forces that may affect trajectories and therefore separation outcomes [37, 38]. *Not much experimental or modelling work has been conducted to investigate particle dynamics in inertial DLD flows. It is unclear how this can be exploited for tunable or optimised separation performance, or how one can work around undesired inertial effects at high throughputs.*

Multi-particle effects — Practical applications usually involve high particle concentrations such that particles invariably interact with each other, either due to direct contact or hydrodynamically. This may cause zigzag movement of a particle that would otherwise bump [39].

Interactions of rigid particles have been reported to decrease separation effectiveness when particle volume fraction is increased to 10% [37]. DLD can be run at red blood cell concentrations greater than 10% [40, 41], but operation with rigid particles at this density has not been reported. DLD is based on 'deterministic' interactions between particles and the device, but particle-particle interactions tend to be chaotic and the key advantage of DLD – high size resolution – is therefore degraded at higher particle concentrations. *Although simulating dense particle flows in DLD is possible [39, 40], improved particle-particle interaction models, including lubrication and friction, are necessary to make simulations more predictive.*

2.3 Chemistry and surface effects

At or near contact, a range of non-hydrodynamic forces between particles and posts become relevant. Even in cases where these surface forces may be weak, particles interact with hundreds to thousands of obstacle during transit, and weak interactions can amplify deviations in particle trajectories. The role of particle-obstacle surface interactions, particularly localised forces and chemical interaction, is an open frontier in DLD development.

An electrostatic effect in DLD has been demonstrated and modelled for cases of low to moderate

salt concentrations of 0 to 150 mM, where the Debye layer can be on the order of 10 to 100 nm. This variation in ionic strength generated an effective gap that was smaller than the physical one [42]. Changing media ionic concentrations (from ultra-pure DI water to saline solution) can very precisely modulate the effective critical diameter (D_c) by ~ 1.1 μm, a significant shift in a gap size of 2 μm.

The Debye length provides the order of the range of electrostatic interactions, which alone is not sufficient to precisely predict DLD performance since electrostatic forces are persistent on length scales beyond the Debye length. *The role of electrostatics in DLD performance requires further exploration.*

Surface areas inside DLD devices are considerable, and measures are normally taken to decrease surface interactions; however, these interactions hold biological information that can be used as a handle for separation. Little work has been done to engineer intentional surface interactions in DLD to enable selective sorting [43–46]. Many of the current methods used in biology for preparing affinity matrices can be translated to DLD surface. It is a propitious time for this undertaking, as a growing realisation of phenotype heterogeneity in cells is pushing developments in microfluidics [47, 48]. Gleghorn *et al.* [46] showed how DLD geometries could be engineered to drive size-based capture of cells on antibody coated surfaces. These approaches allow for 'chromatographic DLD'. *It is unclear how electrostatic and chemical particle-post interactions can be designed and modified such that clogging is minimised and surface-chemistry-specific factors can be exploited as intrinsic sorting mechanisms. An elementary model incorporating hydrodynamics with particle-surface forces is needed to understand and identify design requirements.*

2.4 Scaling to nano

Lowering the minimum-size limit for separable particles towards nano-scale in DLD devices is a natural research direction. The first journal publications on experimental nano-scale DLD are from 2016 [3], showing that DLD can be used to separate nano-scale particles, including exosomes, down to 20 nm. With decreasing particle size, diffusion becomes important and may affect trajectories and separation efficiency. The ratio of a particle's advection rate v to its rate of diffusion in a fluid is quantified by the Péclet number

$$\mathrm{Pe} = \frac{v\ell}{D} = v\ell\frac{6\pi\eta r}{k_B T} \tag{1}$$

where D is the thermal diffusion coefficient (approximated with the Stokes-Einstein relation) of the particle in the fluid, η is the fluid viscosity, k_B is the Boltzmann constant, T is the fluid temperature, r is the particle radius, and ℓ is the typical particle advection distance. The particle advection distance for a bumping particle is the flow lane width; however, for particles well below the critical size the advection distance may be the total array length. Diffusion affects the microscopic transport of a bumping particle if diffusion enables a shift across a streamline. The likelihood of

such events increase with decreasing Pe. Diffusion also causes lateral dispersion of small particles, resulting in downstream broadening of their lateral position. The separation angle of the DLD array must be kept sufficiently large to overcome this broadening. Diffusion is unhelpful in DLD, but unavoidable in the low Pe environments of nano-DLD.

Heller & Bruus [49] investigated the effects of diffusion in DLD systems theoretically. Their model uses a simplified DLD flow consisting of straight streamlines over which they superimposed Brownian motion without considering the effects of pillar size. The model suggests that diffusion allows supercritical particles to escape lateral displacement, leading to an increase in critical diameter, and potentially, a fundamental limit on scaling DLD to nano. *However, little data exists on critical sizes in the nano regime.*

Diffusion has also been shown to enhance spatial dispersion of particles in the device [50, 51]. The geometry of the DLD device may lead to effective diffusion coefficients much larger than the intrinsic particle diffusivity [52]. Given that the entire system is dominated by boundaries, the Stokes-Einstein diffusion model is too simplistic to settle the debate. *A unified picture of the impact of diffusion on particle trajectories in DLD is not available. It is not known how the critical size changes at the nano-scale and how strongly this scaling is affected by diffusion.*

3 Practical challenges

DLD-based technologies must offer a manageable and preferably user-friendly work flow for wider use. Many challenges arise in device design (Sec. 3.1), fabrication (Sec. 3.2) and operation (Sec. 3.3) that so far have inhibited application of DLD by a larger community.

3.1 Design

DLD design usually starts with the array, where a critical size is chosen such that it lies midway between the two particle sizes to be separated. At least three modifications to Davis's equation [17] for critical size are useful. 1) When the lateral and forward unit-cell distances (λ) are unequal, Zeming *et al.* [53] introduced a modification to the row shift fraction ε

$$\varepsilon_{\text{effective}} = \frac{\lambda_{\text{forward}}}{\lambda_{\text{lateral}}} \tan \alpha, \tag{2}$$

where α is the angle that the column of obstacles make with the average flow direction, and λ_{forward} and λ_{lateral} are flow-wise and lateral-to-flow array pitch distances, respectively. 2) Loutherback showed that the critical size is modulated by the number of obstacle verticies when these are regular polygons [54]. 3) Dincau *et al.* [55] showed a significant reduction in critical size for a particular geometry under moderate Reynolds number. *However, more robust and general design rules are*

required, both for the low and intermediate Reynolds number regimes.

Once the array parameters are defined, the array length and width are worked out based on the array layout employed. There are a number of different array layouts, including mirrored arrays, chirped arrays and cascaded arrays [6, 9, 10]. The length of an array L is a geometrical calculation that involves the array angle and the incoming sample stream width W (for $W > \lambda$) with

$$L > \frac{W}{\tan(\alpha)}. \tag{3}$$

Keeping a DLD array as short as possible while maintaining its performance is a key aim since shorter devices have less flow resistance and require less space. Therefore, splitting the sample stream into identical parallel arrays allows each individual array to be be narrower and shorter. *If the lateral boundary problem described in Sec. 2.1 can be solved, then DLD arrays can be made just one obstacle wide.*

Although having only a weak impact on the critical diameter, the device depth plays an important role in the design process. Increasing the device depth decreases the array's flow resistance and can reduce clogging due to particles being less confined. Non-spherical particles show different dynamic behaviour in DLD when they can rotate freely, rather than being confined by the bottom and top walls [18, 20, 31]. In practice, the device depth cannot be arbitrarily large; the depth of field of microscopes is limited, and geometric aspect ratios are constrained by fabrication requirements. *Changing device depth and using multiple depths in a single array have shown potential in improving DLD performance, but a robust optimisation strategy is lacking.*

Even with known design rules and guidelines, it is challenging to design or simulate entire devices, including inlet channels, the array, and outlet channels (see Sec. 2.1). Laying out a complete wafer with multiple DLD devices and more than one million pillars is difficult for many CAD packages. Despite the lack of whole-device simulations, mesoscale simulation techniques (*e.g.*, lattice Boltzmann, dissipative particle dynamics and multi-particle collision dynamics) in combination with models of particles and deformable cells allow studies of cellular behavior in DLD. These approaches are gaining popularity, owing to the new insights they offer for DLD, and can be used in the device-design process [21, 35]. *Integration between physical layout software and whole-device simulation, ideally including realistic particle models, would dramatically improve the design workflow.*

The DLD design process must also consider properties of the sample. Biological samples can adhere and aggregate; the impact of surface fouling and non-specific adsorption can rapidly lead to clogs. Thus, DLD devices can usually process samples for a limited amount of time. To reduce these effects at the chip layout stage, designers have included on-chip, upstream filters that are intended to isolate clogs away from DLD arrays [3, 6]. Combined with surface modification techniques (see Sec. 3.3), chip lifetimes in nano-DLD have been dramatically extended [56]. *Maximising chip lifetime is critical and starts with the layout. DLD designers should consider shear stresses throughout the*

chip, not just in the DLD array, and eliminate high-shear regions to avoid cell rupture or shear-induced aggregation when biological samples are involved. Clear guidelines for minimising clogging are not yet available.

Other aspects that are often overlooked in the DLD design process but are important for usability are the fluid-to-plastic volume ratio and the chip-to-world interfaces. In particular for nano-DLD, dead volume must be aggressively reduced. *Innovations and standardisation in chip interfacing and packaging are necessary and need to be part of the design process.*

Once a DLD device has been designed, there is still the drawback that each DLD array is useful only for a narrow range of pre-determined particle sizes. Traditional DLD performance is independent of flow rate, as long as i) the Péclet number remains large; ii) soft particles are not strongly deformed; and iii) inertia is negligible. If one of these conditions is not met, the flow rate can be used for separation control, *e.g.* for nanoparticles [32], biological cells [18, 35, 57] or inertial effects [55]. Mechanical tuning has been achieved by squeezing/stretching the device [58] or by turning a central piece to alter the angle α between the array and the flow [59]. Additional forces, such as those generated by applied electric fields [60, 61], intrinsic electrostatics [42], gravity [62], and viscoelastic liquids [63] have also been employed for sorting. *There is strong need for methods that allow control of the critical diameter. This would open avenues to using the same array for several separations.*

3.2 Fabrication

DLD fabrication is demanding because of the desire for high volume throughput. This requires high aspect-ratio features (pillar diameter/distance versus device depth) and nearly vertical sidewalls. Deep silicon etching and thick resist photolithography are well suited and widely available in research settings. 3D printing approaches will continue to grow in capability and popularity and fit into a trend toward fabrication methods that do not need a clean room or specialised tools. For commercial applications, device cost is the key driver, and there are a number of significant challenges (see Sec. 4). In research and some foreseeable commercial settings, it is desirable to sterilise, clean and re-use devices as this reduces the cost per run. *Although sterilisation is a common and usually straightforward process, cleaning is more challenging. It is questionable whether cleaning will ever be possible or practicable for low-cost DLD devices, unless devices are open [64]. Alternatively, a modular approach appears promising in which only parts of the device (e.g. the array) are single-use and other parts (e.g. inlets, outlets, holder, collection vials) are re-usable.*

Despite the demonstrated potential of nano-DLD and the high-precision fabrication of nano-scale silicon obstacles *via* thin film techniques, the robustness and applicability of nano-DLD to a larger set of use cases remains limited at present. Due to the small scale, some challenges that have already been solved for micro-scale DLD are still present at the nano-scale. Several of these challenges have

been addressed in the form of an integrated nano-DLD device [56]; however, much work remains to propel the technology forward and better define use cases. Advancements in these areas could pave the way for a host of new, currently inaccessible applications, *e.g.* in the chemical and environmental sectors and other industries dealing with the separation of nano-scale colloids. *To unlock its full potential, nano-DLD must overcome limitations in flow rates, chip lifetime, functionality, and ease of operation.*

3.3 Operation

Surface treatment — Surface treatment, which may be considered the first step before introducing a sample or the last step in fabrication, is critical for DLD operation. Incompatibility between the DLD surfaces and sample/fluid can lead to poor wetting, incorporation of defects (*e.g.* bubbles), and particle adhesion. Fouling often limits the length of time a device can be operated for. Surface treatment can improve device longevity, reproducibility and capability to handle complex sample mixtures. There are four major strategies: i) surface energy modification, *e.g.* charged or polar moieties to increase surface wettability [65]; ii) competitive additives, such as serum albumin and dry milk added to running buffer [66]; iii) polymer brushes, such as a triblock co-polymer of hydrophobic propylene glycol flanked by two hydrophilic blocks of polyethylene glycol [67]; iv) tethered lipid bilayers which are frequently used in sensor applications to reduce non-specific binding [65, 68]

Fouling has been greatly inhibited in microfluidic environments using textured surfaces the bind a lubricating oil, but these have yet to be translated to DLD [69]. Metabolic inhibition [66] and tailoring the molecular hydrophobicity of the surface using mixed hydrophilic/hydrophobic moieties [70] may further limit biofouling in DLD. *Novel strategies for anti-fouling, such as new polymer brush formulations or nano-structured surfaces, need to be developed or translated from related fields to DLD.*

Running — A DLD run typically involves wetting the device, introducing the sample, flowing at a fixed operating point for some time while collecting the sample, then disposing of the device or preparing the chip for re-use. Strategies for loading the sample without introducing bubbles are critical and specific to the device material and chip connection strategy. Methods such as PDMS pre-degassing [71], wetting agents, or capillary actions of low surface tension fluids such as chloroform or ethanol [3] have been used to prime the device with fluids before replacing it with a suitable sorting medium. In most settings, tubing and connectors between chip and sample must be set up, which may introduce bubbles. Systems need to be implemented to ensure that untrained users can achieve reliable results. These systems might include bubble traps and in-line degassing, but there is no standard approach that works with all DLD systems. *DLD, along with many other*

microfluidic devices, requires better and material-dependent strategies to avoid bubbles entering the device. This is particularly challenging due to the large surface-to-volume ratio of DLD devices.

A high flow rate is desirable in most applications, but the maximum rate for a given application is limited and highly variable because hydraulic resistance grows strongly with reducing channel cross-section. Limiting factors include: i) high shear stress which can reduce cell viability and lead to sample aggregation even when surface fouling is marginal [72]; ii) devices have a maximum positive fluid pressure that they can withstand before rupture, leakage or significant deformation. This could be a fraction of an atmosphere for PDMS/glass devices [73], or up to 20 atm for bonded glass/silicon devices [3]; iii) at moderate Re, separation performance changes as inertial effects become significant (Sec. 2.2). Once a flow-rate limit for a given device has been reached, multiple identical devices can be run in parallel to increase overall flow rate, which is particularly important for nano-DLD. In-plane parallelisation has been demonstrated many times in nano- and micro-separations [8, 32, 56] while out-of-plane stacking is less common [74, 75]. Finally, for micro-scale separations, run time, flow rate, and tubing size need to be adjusted to avoid excessive settling in tubing and reservoirs. *For nano-DLD, scaling up flow rates is the biggest challenge. For cellular separations, scaling flow rates without inducing excessive shear stresses is a limiting factor to be overcome.*

4 Commercial challenges

This section first revisits DLD fabrication, now with a commercial view, which brings about its own set of challenges (Sec. 4.1). Sec. 4.2 summarises the commercial uptake of DLD and its main bottlenecks.

4.1 Manufacturing

For successful commercialisation of DLD-based systems, regardless of their specific application, manufacturing technologies have to be employed which allow a robust, reproducible high-volume production of the microstructures. In the case of DLD devices, this is particularly challenging due to the following characteristics: i) comparatively small feature size (pillar diameter typically of the order of 5–20 µm) with potentially complex shape and tight tolerance requirements; ii) high aspect ratios; iii) large number and high density of individual features (pillars); and iv) large structured areas (typically several cm^2) for devices which require a high flow throughput. *The size, geometry and number of features make the use of relatively expensive photo-lithographic processes practically unavoidable.*

There are two main fabrication strategies for DLD devices available. First, using photolithography directly to manufacture devices, either in a photoresist, such as SU-8 [61], or by using deep-

reactive ion etching (DRIE) in silicon following the initial lithography step [42]. *While this strategy provides high-performance devices in the academic world, direct DRIE is cost-prohibitive for commercial devices with typical costs of the order of \$5–10 per cm^2 for medium volume production (1000 wafers/year), decreasing to about \$1–2 per cm^2 for >10k wafers/year.*

The second approach is to employ photolithography for generating a master structure which can then be replicated into a polymeric material. Soft lithography methods used to replicate a silicon or SU-8 master structure into a soft elastomeric material (typically PDMS) have proven very successful in the academic world. *However, these methods are not well suited for commercial production due to the long cycle time of the polymerisation step, the high cost of the elastomeric material, and its properties, e.g. low mechanical stiffness.*

More promising are replication methods into thermoplastic materials, such as hot embossing [76] or injection molding. *While soft embossing has proven its suitability for prototyping and low-volume production with an excellent feature reproducibility, injection molding has the potential for high-volume production [77]. However, the size and aspect ratio requirements for most devices exceed the process envelope of conventional injection molding.*

In order to achieve a good replication and mold filling of high-aspect pillar structures, two specific modifications have been useful. 1) To prevent premature solidification of the polymer melt due to a higher cooling rate of the large surface-to-volume pillar structures, the tool temperature has to be increased, leading to the use of a so-called 'variotherm' molding process [78]. 2) For a high structural replication accuracy, after the initial polymer melt injection, a compression step is introduced, requiring an injection molding tool specifically designed for this process. *Both modifications increase the process cycle time for molding, and hence the cost; but it still remains significantly shorter than the cycle time of hot embossing.*

Other challenges arising from the basic geometries of DLD devices are related to the lifetime of the molding master structure. As the initial structure in current designs has to be lithographically defined, the only way to obtain a metal master structure is to electroplate this initial structure, yielding a molding master in nickel or nickel alloy. Nickel masters have a shorter lifetime compared to standard steel masters which can be generated using ultra-precision mechanical machining. Furthermore, during production, the individual pillars represent 'vulnerable' structures. If such a pillar breaks during the molding process (especially critical is the moment when the part is separated from the mold, the so-called 'demolding'), it will stick in the master structure. This will lead to a defect at that location in all following parts. Removal of the stuck material from the master is next to impossible. *Durability of the molding master remains a crucial challenge for commercial DLD device fabrication.*

A commercial DLD product needs to be manufactured in high volume and at an as-low-as-possible cost. Addressing the challenges identified in this section will help lower that cost. Finally, a direct translation of existing designs from academic publications will rarely yield a device that can

be successfully manufactured. Achieving manufacturability may require reducing the aspect ratio, introducing draft angles to the pillars, and maximising feature sizes. *The impact of design changes for manufacture need to be understood and compensated for where possible.*

4.2 Industrial uptake

The first DLD patent was issued in 2003 [79], with adjusted expiration at the end of 2023. There have been multiple entities taking up the commercial challenge. In 2008, Artemis Health (later acquired by Illumina) published some details of their efforts to isolate fetal nucleated red blood cells for non-invasive prenatal testing [80]. GPB Scientific is commercialising DLD technology for use in processing blood and blood products. They initially demonstrated a disposable, commercially produced plastic chip for small volumes that eliminated the need for any centrifugation or lysing/washing steps [76]. Recently, GPB Scientific demonstrated the use of a highly parallel DLD design in commercially produced plastic, targeted towards a closed-system processing of apheresis blood products, such as Car-T-cells [74].

In 2014, Cytovera Inc. (Massachusetts) [81] filed a patent on a DLD-inspired size filtration system. The system uses a line of pillars in a channel (rather than an array). It uses the same hydrodynamic separation principle as DLD but may be capable of a larger dynamic range (maximum particle size passed divided by smallest particle deflected). The patent also deals with obstacle shape variations, including egg and teardrop shapes, and a high-throughput version where a membrane replaces the row of pillars. This arrangement is similar to cross-flow filtration (*e.g.* [82]).

PACT Pharma (California) is interested in using DLD as a purification step in a T-cell therapy and filed a patent in 2018 that draws on DLD [13, 83]. The filing shows the use of I-shaped pillars for continuous flow separation and streamline engineering for cell trapping. The patent also shows some pillar shape optimisation with complex non-symmetric obstacles. Berkeley Lights Inc. (California) has also recently filed a patent for a DLD device to sort a population of activated lymphocytes for immunotherapy [84].

In 2013, the Massachusetts General Hospital partnered with Veridex and others to establish a research centre on circulating tumor cells (CTC) technology. Veridex made CellSearch, an immunomagnetic CTC counting systems that was the only commercially available device for capturing and enumerating circulating tumor cells. In 2014, the Scientist magazine [85] reported that Johnson & Johnson had licensed technology from CTC-ichip [86, 87] with plans to commercialise the device in 2015. The CTC ichip was developed at Massachusetts General Hospital's Centre for Engineering in Medicine. The commercial CTC landscape was altered by the emergence of Grail (founded in late 2015 to do early cancer detection using cell-free nucleic acid).

More recently, inertial lift forces have been combined successfully with DLD-style separation [38]. The patent has been licensed by Micromedicine (Massachusetts) who are developing a commercial

device for large cell purification. The chip comprises three stages: pre-filter, separating large cells using inertial DLD, increasing cell concentration using inertial focusing combined with branch flow fractionation [88].

These examples show that, in certain situations, DLD is replacing traditional methods of cell processing, such as lysis, centrifugation for de-bulking blood and fluorescence or magnetic activated cell sorting for finding rare cells. The examples also show mixed success, with none yet demonstrating sustained and substantial market uptake/penetration.

DLD requires a level of expertise that most companies do not possess. Therefore the cost for commercial use is twofold: companies must license the technology, and they must outsource, hire or develop some specialised skills in design and manufacture. Like most technologies it is one among many bits of intellectual property that are needed to make a successful product, so we do not expect that the patent expiry hinders industrial use. Industrial uptake of DLD will be most significantly impacted by new developments in volume fabrication. Perhaps this is unsurprising, given that most microfluidics researchers have little or no experience or expertise in this field.

5 Conclusion and outlook

Deterministic Lateral Displacement (DLD) is a promising passive microfluidic technique for particle separation and a candidate for diagnostic point-of-care devices. DLD devices can separate pathogens and rare cells, can be produced *en masse*, are resistant to changes in humidity and temperature, and can be operated by inexperienced personnel or through a simple control system. However, there are significant challenges to overcome before such a 'chip in a lab' can be deployed in the field and used as actual diagnostic device, a genuine 'lab on a chip'. Despite more than 15 years of research efforts, DLD has not yet reached full commercial maturity.

In this paper we have identified key fundamental, practical and commercial challenges of DLD that need to be tackled in order to make the technology more attractive for industrial uptake.

- **Fundamental challenges:** Simulations of entire devices in 3D (including upstream and downstream features) are currently not possible due to numerical limitations. More computationally efficient 3D models of the device geometry would make the design process more effective and efficient. It is difficult to predict when 2D approximations are sufficient to generate sufficient insight and effectively guide 3D design. We cannot reliably predict particle trajectories in DLD, neither in the small nor the intermediate Reynolds regime. The governing particle-device interactions are not well understood. Biological and other soft particles bring additional challenges due to their deformability, non-spherical shapes and bio-chemical interactions with other particles and the device. The behaviour of these particles is more difficult to predict, with reliable numerical models lacking. Also, the impact of pillar shape

on particle behaviour is not well understood. It is unclear how the lateral device boundaries should be optimally designed for the wide range of posts and geometries used. Electrostatic effects play an important role in certain situations, but have been generally neglected in the absence of reliable models. For nano-scale applications, particle diffusion becomes important, but the effect of diffusion on particle behaviour is poorly understood.

- **Practical challenges:** Despite the existence of some basic design rules, the community would benefit from clearer and more robust DLD design guidelines. DLD array length, width and depth can be optimised further to reduce flow resistance and array footprint while maintaining or even increasing separation performance. Better integration between device simulation and layout software would simplify the design process. Chip lifetime due to fouling and clogging is an issue. The effect of shear stress and the use of pre-filters needs to be considered to minimise clogging and maximise sample integrity. Although sterilisation of DLD devices is possible, cleaning is a problem that limits re-usability. Flexible control of the critical diameter D_c would bring the technology closer to multi-purpose applications. The interface between the actual DLD chip and everything else needed (*e.g.* tubing, sample recovery) is often neglected and requires standardisation across the field. This interface has implications for reliable operation, *e.g.* avoiding bubbles that interfere with device performance. The design and fabrication of nano-DLD devices is still challenging and expensive.

- **Commercial challenges:** Geometric requirements demand expensive photo-lithographic or deep-reactive ion etching; cheaper alternatives are desirable. Injection molding, for example, is not reliable at high aspect ratios. Improvements to the fidelity, longevity and cycle time in replication processes are critical to reducing device cost. Devices need to be specifically designed for mass manufacture. The changes to DLD design required for reducing cost are not always obvious, and optimisation strategies are lacking.

DLD faces many challenges, but three areas stand out as being particularly exciting. Developing our understanding of DLD at the extremes of high Reynolds number and the nano-scale may give rise to new separation mechanisms with lower critical sizes, higher resolution, less clogging, and higher throughput. Intentionally using and understanding the surface interactions that are forced to occur so thoroughly and predictably in DLD may open a new form of chromatographic-style separations. Continuing to examine and experiment with obstacle and array geometries may improve throughput and reduce clogging. By continuing to look critically and creatively at the various challenges for DLD, we expect to see improvement in all aspects of performance.

These improvements will enable wider use of DLD in fields where it is already known, such as cell separation and healthcare. They may also enable DLD to have an impact in other globally significant challenges such as i) energy, through the processing of hydrogen generating micro-algae;

ii) the environment, through the separation of micro-plastics; and iii) food security and clean water, through the detection of harmful micro-organisms.

We hope that this opinion article generates new ideas and outcomes, and leads to a fresh perspective in DLD research with a view toward the fundamental, practical and commercial challenges.

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References

1. Huang, L. R., Cox, E. C., Austin, R. H. & Sturm, J. C. Continuous Particle Separation through Deterministic Lateral Displacement. *Science* **304,** 987–990. ISSN: 0036-8075 (2004).

2. Sturm, J. C., Cox, E. C., Comella, B. & Austin, R. H. Ratchets in Hydrodynamic Flow: More than Waterwheels. *Interface Focus* **4.** ISSN: 2042-8898 (Dec. 2014).

3. Wunsch, B. H. *et al.* Nanoscale Lateral Displacement Arrays for the Separation of Exosomes and Colloids down to 20 Nm. *Nature Nanotechnology* **11,** 936–940. ISSN: 1748-3387 (Nov. 2016).

4. Joensson, H. N., Uhlén, M. & Svahn, H. A. Droplet Size Based Separation by Deterministic Lateral Displacement- Separating Droplets by Cell-Induced Shrinking. *Lab on a Chip* **11,** 1305–1310. ISSN: 14730189 (Apr. 2011).

5. Inglis, D. W., Herman, N. & Vesey, G. Highly Accurate Deterministic Lateral Displacement Device and Its Application to Purification of Fungal Spores. *Biomicrofluidics* **4.** ISSN: 19321058 (2010).

6. Davis, J. A. *et al.* Deterministic Hydrodynamics: Taking Blood Apart. *Proceedings of the National Academy of Sciences* **103,** 14779–14784. ISSN: 0027-8424 (Oct. 2006).

7. Holm, S. H., Beech, J. P., Barrett, M. P. & Tegenfeldt, J. O. Separation of Parasites from Human Blood Using Deterministic Lateral Displacement. *Lab on a Chip* **11,** 1326–1332. ISSN: 1473-0197 (2011).

8. Loutherback, K. *et al.* Deterministic Separation of Cancer Cells from Blood at 10 mL/Min. *AIP Advances* **2.** ISSN: 21583226 (2012).

9. McGrath, J., Jimenez, M. & Bridle, H. Deterministic Lateral Displacement for Particle Separation: A Review. *Lab on a Chip* **14,** 4139–4158. ISSN: 1473-0197 (2014).

10. Salafi, T., Zhang, Y. & Zhang, Y. A Review on Deterministic Lateral Displacement for Particle Separation and Detection. *Nano-Micro Letters* **11,** 77. ISSN: 2150-5551 (Sept. 2019).

11. Morton, K. J. *Micro and Nanofluidic Structures for Cell Sorting and Genomic Analysis* Ph.D. (Princeton University, United States – New Jersey, 2008). 134 pp.

12. Jing, T. *et al.* Single Cell Analysis of Leukocyte Protease Activity Using Integrated Continuous-Flow Microfluidics. *Analytical Chemistry* **88,** 11750–11757. ISSN: 0003-2700 (Dec. 6, 2016).

13. Ng, A. H. C. *et al.* MATE-Seq: Microfluidic Antigen-TCR Engagement Sequencing. *Lab on a Chip.* ISSN: 1473-0189 (Aug. 8, 2019).

14. Song, Y. *et al.* Bioinspired Engineering of a Multivalent Aptamer-Functionalized Nanointerface to Enhance the Capture and Release of Circulating Tumor Cells. *Angewandte Chemie International Edition* **58,** 2236–2240. ISSN: 1521-3773 (2019).

15. Barrett, M. P. *et al.* Microfluidics-Based Approaches to the Isolation of African Trypanosomes. *Pathogens* **6,** 47 (Dec. 2017).

16. Inglis, D. W., Davis, J. A., Austin, R. H. & Sturm, J. C. Critical Particle Size for Fractionation by Deterministic Lateral Displacement. *Lab on a Chip* **6,** 655–658. ISSN: 1473-0197 (2006).

17. Davis, J. A. *Microfluidic Separation of Blood Components through Deterministic Lateral Displacement* Dissertation Thesis (Princeton University, 2008).

18. Kruger, T., Holmes, D. & Coveney, P. V. Deformability-Based Red Blood Cell Separation in Deterministic Lateral Displacement Devices-A Simulation Study. *Biomicrofluidics* **8.** ISSN: 1932-1058 (2014).

19. Zhang, Z., Henry, E., Gompper, G. & Fedosov, D. A. Behavior of Rigid and Deformable Particles in Deterministic Lateral Displacement Devices with Different Post Shapes. *Journal of Chemical Physics* **143.** ISSN: 00219606 (Dec. 2015).

20. Kabacaoğlu, G. & Biros, G. Sorting Same-Size Red Blood Cells in Deep Deterministic Lateral Displacement Devices. *Journal of Fluid Mechanics* **859,** 433–475 (Jan. 2018).

21. Vernekar, R., Krüger, T., Loutherback, K., Morton, K. & Inglis, D. W. Anisotropic Permeability in Deterministic Lateral Displacement Arrays. *Lab on a Chip* **17,** 3318–3330. ISSN: 1473-0189 (Sept. 26, 2017).

22. Feng, S. L., Skelley, A. M., Anwer, A. G., Liu, G. Z. & Inglis, D. W. Maximizing Particle Concentration in Deterministic Lateral Displacement Arrays (Vol 11, 024121, 2017). *Biomicrofluidics* **11.** ISSN: 1932-1058 (2017).

23. Ebadi, A. *et al.* Efficient Paradigm to Enhance Particle Separation in Deterministic Lateral Displacement Arrays. *SN Applied Sciences* **1,** 1184. ISSN: 2523-3963, 2523-3971 (Oct. 2019).

24. Inglis, D., Vernekar, R., Krüger, T. & Feng, S. The Fluidic Resistance of an Array of Obstacles and a Method for Improving Boundaries in Deterministic Lateral Displacement Arrays. *Microfluidics and Nanofluidics* **24,** 18. ISSN: 1613-4990 (Feb. 17, 2020).

25. Zeming, K. K., Ranjan, S. & Zhang, Y. Rotational Separation of Non-Spherical Bioparticles Using I-Shaped Pillar Arrays in a Microfluidic Device. *Nature Communications* **4.** ISSN: 2041-1723 (Mar. 2013).

26. Maxey, M. R. & Riley, J. J. Equation of Motion for a Small Rigid Sphere in a Nonuniform Flow. *The Physics of Fluids* **26,** 883–889. ISSN: 0031-9171 (Apr. 1, 1983).

27. Henry, E. *et al.* Sorting Cells by Their Dynamical Properties. *Scientific Reports* **6,** 34375. ISSN: 2045-2322 (Dec. 2016).

28. Batchelor, G. K. *An Introduction to Fluid Dynamics* xviii, 615 p. ISBN: 0-521-09817-3 (Cambridge University Press, London [England], 1967).

29. Antfolk, M. & Laurell, T. Continuous Flow Microfluidic Separation and Processing of Rare Cells and Bioparticles Found in Blood – A Review. *Analytica Chimica Acta* **965,** 9–35. ISSN: 0003-2670 (May 1, 2017).

30. Geislinger, T. M. & Franke, T. Hydrodynamic Lift of Vesicles and Red Blood Cells in Flow — from Fåhræus & Lindqvist to Microfluidic Cell Sorting. *Advances in Colloid and Interface Science. Special Issue in Honour of Wolfgang Helfrich* **208,** 161–176. ISSN: 0001-8686 (June 1, 2014).

31. Chien, W., Zhang, Z., Gompper, G. & Fedosov, D. A. Deformation and Dynamics of Erythrocytes Govern Their Traversal through Microfluidic Devices with a Deterministic Lateral Displacement Architecture. *Biomicrofluidics* **13,** 044106 (2019).

32. Wunsch, B. H. *et al.* Gel-on-a-Chip: Continuous, Velocity-Dependent DNA Separation Using Nanoscale Lateral Displacement. *Lab on a Chip* **19,** 1567–1578 (2019).

33. Beech, J. P., Holm, S. H., Adolfsson, K. & Tegenfeldt, J. O. Sorting Cells by Size, Shape and Deformability. *Lab on a chip* **12,** 1048–51. ISSN: 1473-0189 (Mar. 2012).

34. Al-Fandi, M., Al-Rousan, M., Jaradat, M. A. K. & Al-Ebbini, L. New Design for the Separation of Microorganisms Using Microfluidic Deterministic Lateral Displacement. *Robotics and Computer-Integrated Manufacturing* **27,** 237–244. ISSN: 07365845 (Apr. 2011).

35. Zhang, Z., Chien, W., Henry, E., Fedosov, D. A. & Gompper, G. Sharp-Edged Geometric Obstacles in Microfluidics Promote Deformability-Based Sorting of Cells. *Physical Review Fluids* **4,** 1–18. ISSN: 2469990X (2019).

36. Lubbersen, Y. S., Dijkshoorn, J. P., Schutyser, M. A. I. & Boom, R. M. Visualization of Inertial Flow in Deterministic Ratchets. *Separation and Purification Technology* **109,** 33–39. ISSN: 1383-5866 (May 9, 2013).

37. Lubbersen, Y. S., Schutyser, M. A. & Boom, R. M. Suspension Separation with Deterministic Ratchets at Moderate Reynolds Numbers. *Chemical Engineering Science* **73,** 314–320. ISSN: 00092509 (May 2012).

38. Mutlu, B. R. *et al.* Non-Equilibrium Inertial Separation Array for High-Throughput, Large-Volume Blood Fractionation. *Scientific Reports* **7,** 1–9. ISSN: 2045-2322 (Aug. 30, 2017).

39. Vernekar, R. & Krüger, T. Breakdown of Deterministic Lateral Displacement Efficiency for Non-Dilute Suspensions: A Numerical Study. *Medical Engineering and Physics* **37,** 845–854. ISSN: 18734030 (2015).

40. Holm, S. H. *et al.* Microfluidic Particle Sorting in Concentrated Erythrocyte Suspensions. *Physical Review Applied* **12,** 014051. ISSN: 2331-7019 (July 2019).

41. Inglis, D. W., Lord, M. & Nordon, R. E. Scaling Deterministic Lateral Displacement Arrays for High Throughput and Dilution-Free Enrichment of Leukocytes. *Journal of Micromechanics and Microengineering* **21.** ISSN: 09601317 (May 2011).

42. Zeming, K. K., Thakor, N. V., Zhang, Y. & Chen, C.-H. Real-Time Modulated Nanoparticle Separation with an Ultra-Large Dynamic Range. *Lab on a Chip* **16,** 75–85 (2016).

43. Liu, Z. B. *et al.* Rapid Isolation of Cancer Cells Using Microfluidic Deterministic Lateral Displacement Structure. *Biomicrofluidics* **7.** ISSN: 1932-1058 (2013).

44. Liu, Z. *et al.* High Throughput Capture of Circulating Tumor Cells Using an Integrated Microfluidic System. *Biosensors and Bioelectronics* **47,** 113–119. ISSN: 09565663 (Sept. 2013).

45. Gleghorn, J. P. *et al.* Capture of Circulating Tumor Cells from Whole Blood of Prostate Cancer Patients Using Geometrically Enhanced Differential Immunocapture (GEDI) and a Prostate-Specific Antibody. *Lab on a Chip* **10,** 27–29. ISSN: 1473-0189 (Jan. 7, 2010).

46. Gleghorn, J. P., Smith, J. P. & Kirby, B. J. Transport and Collision Dynamics in Periodic Asymmetric Obstacle Arrays: Rational Design of Microfluidic Rare-Cell Immunocapture Devices. *Physical Review E* **88** (Sept. 2013).

47. Hur, S. C., Henderson-MacLennan, N. K., McCabe, E. R. B. & Di Carlo, D. Deformability-Based Cell Classification and Enrichment Using Inertial Microfluidics. *Lab on a chip* **11,** 912–920 (Mar. 2011).

48. Salek, M. M., Carrara, F., Fernandez, V., Guasto, J. S. & Stocker, R. Bacterial Chemotaxis in a Microfluidic T-Maze Reveals Strong Phenotypic Heterogeneity in Chemotactic Sensitivity. *Nature communications* **10,** 1877 (Apr. 2019).

49. Heller, M. & Bruus, H. A Theoretical Analysis of the Resolution Due to Diffusion and Size Dispersion of Particles in Deterministic Lateral Displacement Devices. *Journal of Micromechanics and Microengineering* **18.** ISSN: 09601317 (Aug. 2008).

50. Cerbelli, S., Giona, M. & Garofalo, F. Quantifying Dispersion of Finite-Sized Particles in Deterministic Lateral Displacement Microflow Separators through Brenner's Macrotransport Paradigm. *Microfluidics and Nanofluidics* **15,** 431–449. ISSN: 16134982 (Oct. 2013).

51. Dorfman, K. D. & Brenner, H. "Vector Chromatography": Modeling Micropatterned Separation Devices. *nan* **238,** 390–413. ISSN: nan (June 2001).

52. Cerbelli, S., Garofalo, F. & Giona, M. Effective Dispersion and Separation Resolution in Continuous Particle Fractionation. *Microfluidics and Nanofluidics* **19,** 1035–1046 (Aug. 2015).

53. Zeming, K. K., Salafi, T., Chen, C.-H. & Zhang, Y. Asymmetrical Deterministic Lateral Displacement Gaps for Dual Functions of Enhanced Separation and Throughput of Red Blood Cells. *Scientific Reports* **6,** 22934. ISSN: 2045-2322 (Mar. 10, 2016).

54. Loutherback, K. *et al.* Improved Performance of Deterministic Lateral Displacement Arrays with Triangular Posts. *Microfluidics and Nanofluidics* **9,** 1143–1149. ISSN: 16134982 (Dec. 2010).

55. Dincau, B. M., Aghilinejad, A., Hammersley, T., Chen, X. & Kim, J.-H. Deterministic Lateral Displacement (DLD) in the High Reynolds Number Regime: High-Throughput and Dynamic Separation Characteristics. *Microfluidics and Nanofluidics* **22,** 59. ISSN: 1613-4982 (June 2018).

56. Smith, J. T. *et al.* Integrated Nanoscale Deterministic Lateral Displacement Arrays for Separation of Extracellular Vesicles from Clinically-Relevant Volumes of Biological Samples. *Lab on a Chip* **18,** 3913–3925. ISSN: 14730189 (2018).

57. Holmes, D. *et al.* Separation of Blood Cells with Differing Deformability Using Deterministic Lateral Displacement. *Interface Focus* **4.** ISSN: 2042-8898 (2014).

58. Beech, J. P. & Tegenfeldt, J. O. Tuneable Separation in Elastomeric Microfluidics Devices. *Lab on a Chip* **8,** 657–659. ISSN: 14730189 (2008).

59. Du, S. & Drazer, G. Deterministic Separation of Suspended Particles in a Reconfigurable Obstacle Array. *Journal of Micromechanics and Microengineering* **25.** ISSN: 13616439 (Oct. 2015).

60. Beech, J. P., Jönsson, P. & Tegenfeldt, J. O. Tipping the Balance of Deterministic Lateral Displacement Devices Using dielectrophoresisY. *Lab on a Chip* **9,** 2698–2706. ISSN: 14730189 (2009).

61. Beech, J. P., Keim, K., Ho, B. D., Guiducci, C. & Tegenfeldt, J. O. Active Posts in Deterministic Lateral Displacement Devices. *Advanced Materials Technologies.* ISSN: 2365709X (2019).

62. Devendra, R. & Drazer, G. Gravity Driven Deterministic Lateral Displacement for Particle Separation in Microfluidic Devices. *Analytical Chemistry* **84,** 10621–10627. ISSN: 0003-2700 (Dec. 2012).

63. Li, Y. *et al.* Dynamic Control of Particle Separation in Deterministic Lateral Displacement Separator with Viscoelastic Fluids. *Scientific Reports* **8,** 1–9. ISSN: 20452322 (2018).

64. Tran, T. S. H., Ho, B. D., Beech, J. P. & Tegenfeldt, J. O. Open Channel Deterministic Lateral Displacement for Particle and Cell Sorting. *Lab on a Chip* **17,** 3592–3600 (2017).

65. Shirtcliffe, N. J., Toon, R. & Roach, P. in *Microfluidic Diagnostics: Methods and Protocols* (eds Jenkins, G. & Mansfield, C. D.) 241–268 (Humana Press, Totowa, NJ, 2013). ISBN: 978-1-62703-134-9.

66. Hochstetter, A. *et al.* Microfluidics-Based Single Cell Analysis Reveals Drug-Dependent Motility Changes in Trypanosomes. *Lab on a Chip* **15,** 1961–1968 (2015).

67. Menachery, A. *et al.* Counterflow Dielectrophoresis for Trypanosome Enrichment and Detection in Blood. *Scientific reports* **2,** 1–5. ISSN: 2045-2322 (Jan. 2012).

68. Xu, Y., Takai, M. & Ishihara, K. Phospholipid Polymer Biointerfaces for Lab-on-a-Chip Devices. *Annals of Biomedical Engineering* **38,** 1938–1953. ISSN: 1573-9686 (June 1, 2010).

69. Wong, T.-S. *et al.* Bioinspired Self-Repairing Slippery Surfaces with Pressure-Stable Omniphobicity. *Nature* **477,** 443–447 (Sept. 2011).

70. Ostuni, E., Chapman, R. G., Holmlin, R. E., Takayama, S. & Whitesides, G. M. A Survey of Structure-Property Relationships of Surfaces That Resist the Adsorption of Protein. *Langmuir* **17,** 5605–5620 (Sept. 2001).

71. Tottori, N. & Nisisako, T. Degas-Driven Deterministic Lateral Displacement in Poly (Dimethylsiloxane) Microfluidic Devices. *Analytical Chemistry* **91,** 3093–3100. ISSN: 0003-2700 (Feb. 2019).

72. Nesbitt, W. S. *et al.* A Shear Gradient–Dependent Platelet Aggregation Mechanism Drives Thrombus Formation. *Nature Medicine* **15,** 665–673. ISSN: 1546-170X (June 2009).

73. Inglis, D. W. A Method for Reducing Pressure-Induced Deformation in Silicone Microfluidics. *Biomicrofluidics* **4.** ISSN: 19321058 (2010).

74. Campos-González, R. *et al.* Deterministic Lateral Displacement: The Next-Generation CAR T-Cell Processing? *SLAS Technology: Translating Life Sciences Innovation* **23,** 338–351 (Jan. 2018).

75. Loutherback, K. *Microfluidic Devices for High Throughput Cell Sorting and Chemical Treatment* red. by Princeton University. 108 p. ISBN: 978-1-267-01032-2 (2011).

76. Civin, C. I. *et al.* Automated Leukocyte Processing by Microfluidic Deterministic Lateral Displacement. *Cytometry Part A* **89A,** 1073–1083. ISSN: 1552-4922 (2016).

77. Becker, H. & Gärtner, C. Polymer Microfabrication Technologies for Microfluidic Systems. *Analytical and Bioanalytical Chemistry* **390,** 89–111. ISSN: 1618-2650 (Jan. 1, 2008).

78. Chu, J.-S., Gilchrist, M. D. & Zhang, N. in *Encyclopedia of Microfluidics and Nanofluidics* (ed Li, D.) 2085–2101 (Springer, New York, NY, 2015). ISBN: 978-1-4614-5491-5.

79. Huang, L. R. & Sturm, J. C. *US Patent* 7150812B2 (2006).

80. Huang, R. *et al.* A Microfluidics Approach for the Isolation of Nucleated Red Blood Cells (NRBCs) from the Peripheral Blood of Pregnant Women. *Prenatal Diagnosis* **28,** 892–899. ISSN: 0197-3851 (2008).

81. Huang, L. *US Patent* 8679751B2 (2014).

82. Dijkshoorn, J. P., Schutyser, M. A., Sebris, M., Boom, R. M. & Wagterveld, R. M. Reducing the Critical Particle Diameter in (Highly) Asymmetric Sieve-Based Lateral Displacement Devices. *Scientific Reports* **7,** 1–10. ISSN: 20452322 (2017).

83. PENG, S. *et al. pat.* WO2018085453A1 (WO) (2018).

84. Loutherback, K. D., Bronevetsky, Y., Beemiller, P. J., Wang, X. & Chapman, K. T. *pat.* WO2018018017A1 (WO) (2018).

85. Zeliadt, N. *Capturing Cancer Cells on the Move* <https://www.the-scientist.com/lab-tools/capturing-cancer-cells-on-the-move-37721> (2014).

86. Ozkumur, E. *et al.* Inertial Focusing for Tumor Antigen–Dependent and –Independent Sorting of Rare Circulating Tumor Cells. *Science Translational Medicine* **5,** 179ra47–179ra47. ISSN: 1946-6234, 1946-6242 (Apr. 3, 2013).

87. Karabacak, N. M. *et al.* Microfluidic, Marker-Free Isolation of Circulating Tumor Cells from Blood Samples. *Nature Protocols* **9,** 694–710. ISSN: 1754-2189 (Mar. 2014).

88. Martel, J. M. *et al.* Continuous Flow Microfluidic Bioparticle Concentrator. *Scientific Reports* **5,** 11300. ISSN: 2045-2322 (June 10, 2015).

A DLD causal loop diagram

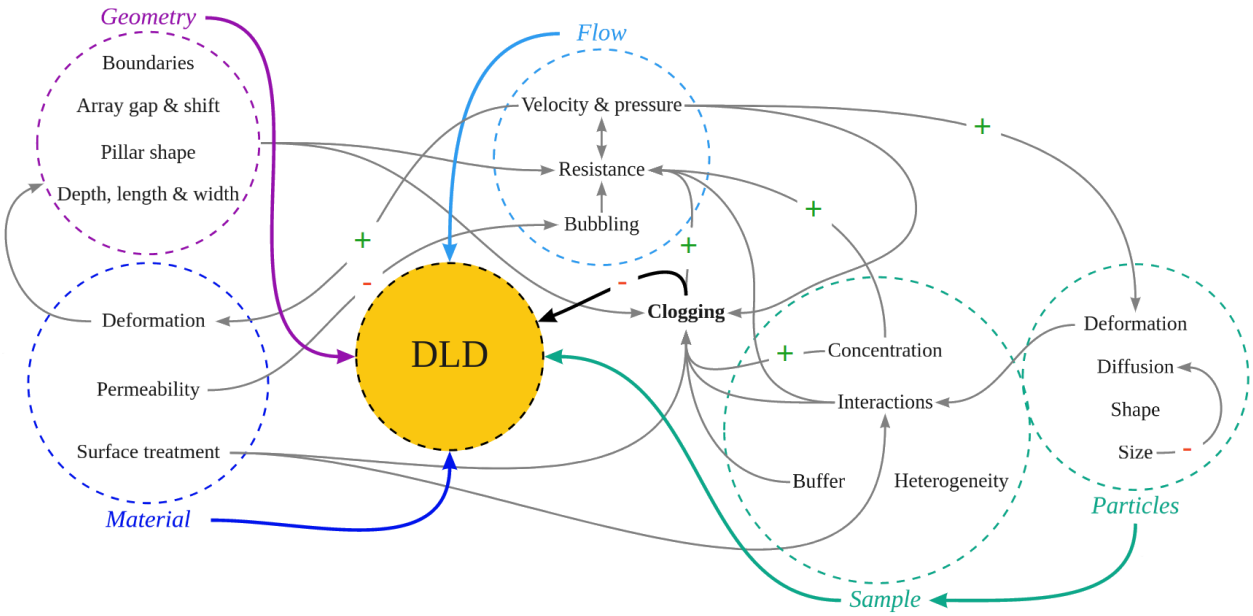

Figure 4: Causal loop diagram of factors impacting the performance of DLD. The illustrated factors are identical to those in Fig 2, and only the most common and important factors are included. For many applications, additional factors (such as sample bio-chemistry, sample viscosity and viscoelastic properties) also play a role. Interrelations are shown as arrows. Arrows with a green plus (+) sign indicate that an increase (or decrease) in the upstream factor causes an increase (or decrease) in the downstream one. Arrows with a red minus (−) sign indicate that increasing (or reducing) the upstream factor reduces (or increases) the downstream one. Arrows without signs indicate complex or unclear relation between factors. Factors are divided into four groups: i) device geometry, ii) device material, iii) sample/particle properties and iv) flow/operational properties (colours are used solely for classification). Clogging plays a special role since it is affected by various factors. The central circle labelled 'DLD' stands for all desired outcomes of the application (*e.g.* separation efficiency, critical size).