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Streamline crossing: An essential mechanism for aerosol dispersion in the pulmonary acinus

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Abstract

The dispersion of inhaled microparticles in the pulmonary acinus of the lungs is often attributed to the complex interplay between convective mixing, due to irreversible flows, and intrinsic particle motion (i.e. gravity and diffusion). However, the role of each mechanism, the exact nature of such interplay between them and their relative importance still remain unclear. To gain insight into these dispersive mechanisms, we track liquid-suspended microparticles and extract their effective diffusivities inside an anatomically-inspired microfluidic acinar model. Such results are then compared to experiments and numerical simulations in a straight channel. While alveoli of the proximal acinar generations exhibit convective mixing characteristics that lead to irreversible particle trajectories, this local effect is over-shadowed by a more dominant dispersion mechanism across the ductal branching network that arises from small but significant streamline crossing due to intrinsic diffusional motion in the presence of high velocity gradients. We anticipate that for true airborne particles, which exhibit much higher intrinsic motion, streamline crossing would be even more significant.

Keywords

Lungs; Pulmonary Acinus; Inhaled aerosol; Particle dispersion; Microfluidics; Tracking velocimetry

1 Introduction

The past decades have witnessed significant efforts to uncover the complex flow phenomena thought to influence inhaled aerosol transport in the pulmonary acinar regions (Sznitman, 2013; Tsuda et al., 2013). In the 1980s, Heyder et al. set a milestone in the investigation of acinar particle transport with a series of *in vivo* experiments in human volunteers (Heyder et al., 1988). By letting subjects inhale a small volume (i.e. bolus) of monodisperse 1 μm aerosols and measuring the widening of such bolus peak upon exhalation, the authors observed increased dispersion for deep breaths reaching into the acinus compared to shallower ones. Such acinar dispersion phenomenon was previously thought to be weak, if

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Conflict of interest

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not absent, due to slow intrinsic motion of such particles (i.e. Brownian motion and sedimentation), while the low Reynolds numbers characteristic of oscillatory flows in the acinus were believed to yield reversible flows with no convective mixing (Davies, 1972; Pedley, 1977).

In the footsteps of Heyder et al., efforts have followed to unveil particle transport phenomena in the lung depths. One strategy has concentrated on characterizing acinar flow patterns and how these may give rise to irreversible mixing. Notably, methods have included numerical Lagrangian tracking in single alveoli and alveolated airway models (Tsuda et al., 1995; Haber et al., 2000; Henry et al., 2002; Tsuda et al., 2008; Hofemeier et al., 2014; Hofemeier and Sznitman, 2014), scaled-up *in vitro* experiments using velocimetry techniques (Tippe and Tsuda, 2000; Tsuda et al., 1999), *ex vivo* flow visualizations in excised lungs (Tsuda et al., 2002) and theoretical considerations (Butler and Tsuda, 2005; Tsuda et al., 2011).

In parallel, an alternative approach has tackled the problem through numerical modeling of particle dynamics, beginning with classic 1D models of gas transport extended to modeling particle transport (Darquenne and Paiva, 1994; Darquenne et al., 1997; Hofmann et al., 2008). Computational fluid dynamics (CFD) simulations have followed in which detailed acinar flow patterns and the intrinsic forces governing particle transport were solved in anatomically-inspired acinar geometries (Darquenne and Prisk, 2003; Sznitman et al., 2009; Ma and Darquenne, 2012; Hofemeier and Sznitman, 2015). These concluded that the intrinsic motion of sub-micron ($\sim 0.5\text{--}1.0\text{ }\mu\text{m}$) particles, though small, is nevertheless sufficient to yield significant dispersion in acinar environments characterized by sub-millimeter length scales. Notably, several studies (Sznitman et al., 2009; Ma and Darquenne, 2012) have underlined the effects of gravity in the acinus, where a small deviation from a given streamline (i.e. streamline crossing) in regions of high velocity gradients may cause a particle to reach a slower streamline, thus leading to net longitudinal translocations after a full breathing cycle.

In the absence of gravitational forces, streamline crossing remains nevertheless potent, as witnessed in *in vivo* experiments under microgravity conditions. For example, Darquenne et al. (1999) found that under microgravity inhaled $2\text{ }\mu\text{m}$ particles disperse less in the acinus compared to 1.0 or $0.5\text{ }\mu\text{m}$ particles. The authors assigned this phenomenon to the smaller diffusivity of such particles, indicating that Brownian diffusion of 0.5 and $1.0\text{ }\mu\text{m}$ particles, although allegedly weak compared to the convective flows, has nevertheless a non-negligible effect on overall dispersion. Yet, given the lack of suitable imaging modalities such intrinsic transport mechanisms have never been directly observed *in situ*.

Recently, our group has visualized time-resolved motions of airborne particles in the range of $0.1\text{--}1.0\text{ }\mu\text{m}$ (Fishler et al., 2015) using a breathing microfluidic *in vitro* network of alveolated airways (Fishler et al., 2013). Such efforts have offered for the first time a direct window to image at true scale the small, yet sub-stantive, effects of Brownian motion on particle transport, whereby such diffusion can ultimately determine whether a particle deposits within an alveolus or escapes. Despite such progress, there remains a lack of experimental quantifications tracing the origins of inhaled aerosol dispersion in the acinus as

in vivo experiments cannot typically resolve, in both time and space, the dynamics of inhaled aerosols at the scale of single particles. In turn, quantitative insight has mainly come from numerical simulations with few experimental data to support direct validations.

With the advent of microfluidics, we revisit the question of acinar particle dispersion at true scale. Our experiments are conducted in a controlled *in vitro* environment using a variant of the *acinus-on-chip* model (Fishler et al., 2013). Following dynamic similarity, liquid-suspended micron particles are tracked over cumulative breathing cycles within single alveoli and across the acinar tree. The rationale for using liquid-suspended rather than airborne particles lies in isolating the interplay between diffusional streamline crossing and convective mixing under conditions where gravity is negligible. By comparing results to experiments and numerical simulations in straight channels, our findings highlight the omnipresent role of such streamline crossing for particle dispersion in the acinus despite very low diffusion coefficients.

2 Methods

Fig. 1 shows a top view of the anatomically-inspired acinar tree, where airway generations are numbered. The microfluidic device is very similar to our recent *acinus-on-chip* design (Fishler et al., 2015) and comprises 5 generations of bifurcating ducts (110 μm wide and 100 μm tall) lined with cylindrical alveolar cavities (110 μm in diameter). Unlike the previous model (Fishler et al., 2015), a straight long channel (100 $\mu\text{m} \times 100 \mu\text{m} \times 1.2 \text{ cm}$) feeds directly into generation 1 where no auxiliary channels are used. Imaging is performed using an epi-fluorescence microscope (Eclipse Ti, Nikon) equipped with an sCMOS camera (Neo 5.5, Andor) and a 4X or 20X objective lens for resolving particle trajectories alveolar cavities or across the tree, respectively. Details on device microfabrication, anatomical limitations of the model, actuation, filling, and particle tracking techniques are available elsewhere (Fishler et al., 2015).

In a first step, we visualize particle trajectories within single alveoli spanning different acinar generations (Fig. 2). To ensure spatially-resolved visualizations at the local alveolar scales, the channels are filled with a 64/36 (v/v) glycerol/water mixture seeded with 1 μm fluorescent polystyrene particles (1.05 gr/cm^3). The terminal velocity for these particles is 0.0036 $\mu\text{m}/\text{s}$, such that they may be regarded as massless within the time scale of the experimental measurements. Flow inside the channels is induced following (Fishler et al., 2015). Briefly, the pressure of water inside the chambers surrounding the channels from the sides and the top is controlled via a syringe pump; this results in deformations of the thin PDMS walls leading to fluid flow inside the channels with a breathing period (T) of 4 s and a volume change of $\sim 30\%$ relative to the minimum airway volume (equivalent to functional residual capacity). The relevant experimental parameters, including non-dimensional flow numbers (i.e. Reynolds and Womersley number), are summarized in Table 1 (see middle column).

Next, we implement a modified protocol for visualizing particle trajectories spanning multiple acinar generations (Fig. 3). Here, larger 2 μm particles (1.05 gr/cm^3) are used for increased visibility of individual particles across the larger field of view (FOV). Unlike the

previous set of experiments in individual alveoli, the working fluid consists of a 16.5/83.5 (v/v) glycerol/water mixture to match the particle density and reduce the effects of gravity in the direction orthogonal to the bifurcation plane such that the resulting terminal velocity was $0.013 \mu\text{m/s}$. Note that using the glycerol/water ratio of 64/36 as earlier would have resulted in a positive buoyant terminal velocity of $\sim 0.3 \mu\text{m/s}$ which could lead to significant translocation within minutes. Flow inside the tree is induced by connecting the inlet of the leading channel to a syringe pump using a $10 \mu\text{l}$ glass syringe and thin Polyethylene tubing ($0.61 \mu\text{m OD} \times 0.28 \mu\text{m ID}$). The identical protocol is implemented for tracking particles in the long straight channel leading into generation 1 (see Results and discussion).

Given limitations in the imaging resolution to resolve the trajectories of particles across the acinar domain, the syringe pump is programmed to continuously repeat a flow cycle comprising a 5 s injection at a constant flow rate of $1.6 \mu\text{l/min}$ followed by a 1 s pause, then a 5 s withdrawal at $1.6 \mu\text{l/min}$ followed by another 1 s pause. Note that the inclusion of a short pause is adopted to ensure that convective mixing does not originate from possible unsteady effects that are known to occur near flow reversal between the inhalation and exhalation phases (Sznitman, 2013). Due to the intrinsic flexibility of the surrounding materials (e.g. tubing), resulting flows vary significantly from those set by the pump. Based on particle imaging in the ducts the flow cycle is measured to be ~ 4.33 s injection (i.e. inhalation) at a constant flow rate of $0.34 \mu\text{l/min}$ followed by a 1.67 s pause, then a 4.33 s withdrawal (i.e. exhalation) at $0.34 \mu\text{l/min}$ followed by another 1.67 s pause. To calculate the corresponding flow rate, an analytical solution for laminar flow in rectangular ducts was used (Bruus, 2008) where the velocity of the fastest particles observed ($v_{\text{max}} = 1.36 \times 10^{-3} \text{ m/s}$) was assumed to be equal to the peak velocity along the ductal centerline. The final prescribed flow conditions account for a volume change of $\sim 7\%$ of the minimum airway volume, where the tidal front spans the entrance of the acinar tree to the third generation (see Fig. 1). The relevant parameters for these experiments are summarized in the right column of Table 1.

To simulate the motion of particles in the straight channel, an in-house discrete element method (DEM) solver is used (Ostrovski et al., 2016). We implement a Lagrangian framework built on linear momentum conservation of single particles, where viscous Stokes drag and Brownian diffusional forces are considered and a time step of $5 \times 10^{-8} \text{ s}$ is selected following convergence tests. To allow a direct comparison between the simulations and the experiments in the long channel we match the channel dimensions, oscillatory flow conditions and particle parameters as defined in the experiments. An analytical channel flow profile is implemented (Bruus, 2008), where the pressure drop is selected to fit the experimentally measured peak velocity (v_{max}). A total of 1024 particles of $2 \mu\text{m}$ diameter are initialized across a regular rectangular grid located on a plane orthogonal to the channel's streamwise axis. To recreate a particle bolus in the simulations that matches the initial experimental conditions (see Fig. 4), a random distance in the streamwise direction (ranging between zero and $161.5 \mu\text{m}$) was added upon completion of the simulation to each individual simulated particle. Note that this procedure is exactly equivalent to adding a random number to the initial location of the particles due to the translational symmetry of the straight channel.

3 Results and discussion

3.1 Irreversible particle tracks inside single alveoli

Particle trajectories inside the alveolar cavities are tracked at generations 1, 3 and 5 (Fig. 2), respectively. In assessing the importance of intrinsic particle motion (Fishler et al., 2015), we first note that the (terminal) buoyant velocity of the $1\text{ }\mu\text{m}$ particles suspended in a 64/36 glycerol/water mixture (see Methods) is $0.0036\text{ }\mu\text{m/s}$ while the characteristic particle displacement in 1 s due to Brownian motion is $0.31\text{ }\mu\text{m}$ (See Table 1). The left panel of Fig. 2 shows a representative particle trajectory inside an alveolus at generation 1 over three breathing cycles. We recall that in proximal acinar airway generations, alveolar flow patterns are characterized by a large recirculation zone that evolves throughout the breathing cycle with intrinsically irreversible flow properties (Fishler et al., 2013; Sznitman, 2013). As observed here, the particle trajectory shows a considerable degree of irreversibility, where the net translocation may amount to more than a quarter of the alveolar diameter after completing a mere single flow cycle. Additionally, particles may escape (or enter) an alveolus, as seen for example in the trajectory that exits the alveolus near the proximal side (Fig. 2, left panel).

With increasing acinar depth, the extent of alveolar mixing is however mitigated. This is shown in generation 3 (Fig. 2, center panel), where the size and strength of the recirculation is reported to be much smaller and confined (Fishler et al., 2013; Sznitman, 2013). While particle trajectories in the close vicinity of the recirculation zone exhibit irreversible paths, trajectories further away from the vortex center are gradually more reversible. In the last acinar generations (Fig. 2, right panel), where flows are known to feature radial-like streamlines with the absence of recirculation patterns, particles exhibit quasi-reversible trajectories where slight deviations from the flow streamline are on the same scale as motions due to diffusion.

Our experimental results are in strong qualitative agreement with numerical simulations that track massless tracers inside alveoli (Hofemeier and Sznitman, 2014; Tsuda et al., 1995). Namely, in the presence of intrinsic motion due to diffusion (on the order of one particle diameter per flow cycle) convective mixing is significant at the local scale of alveolar cavities, though this phenomenon is likely to be absent in the most distal acinar generations. However, for airborne micron particles exhibiting much faster intrinsic motion, the coupling between convective and intrinsic motions would have to be simultaneously considered (Fishler et al., 2015; Ma and Darquenne, 2012).

4 Particle dispersion across acinar trees

While several numerical studies have characterized the irreversibility of flow pathlines and ensuing particle trajectories in single alveoli or alveolated ducts (Henry et al., 2002; Hofemeier et al., 2014; Tsuda et al., 1995), few efforts have addressed altogether particle dispersion across acinar tree structures (Darquenne and Prisk, 2003; Ma and Darquenne, 2012). To showcase the mechanisms leading to dispersion across the acinar network we track individual particles (see SM Video 1) as exemplified in the trajectories of Fig. 3. Here, we recall that the modified setup features $2\text{ }\mu\text{m}$ particles to guarantee particle visibility under

a lower magnification (4X), while the breathing period was increased to 12 s and the tidal volume reduced to ~7% (see Methods). Such conditions correspond to an average and peak Reynolds number of $Re = 0.08$ and 0.09 , respectively, in generation 1 (see Table 1).

The results of Fig. 3 highlight the irreversible nature of trajectories along the acinar ducts. We note that the irreversible pathlines seen in alveoli (Fig. 2) appear to have little influence on the overall ductal dispersion across the tree since few particles pass close enough to the alveolar openings where they may be affected by local alveolar mixing. While particles appear to trace similar trajectories over consecutive cycles, their exact location between exhalation and inhalation (marked by filled circles and numbered according to flow cycle) varies considerably from one cycle to the next. The net size of such cycle-to-cycle translocations ("steps") is larger in proximal generations compared to distal ones; this follows from mass conservation (Fishler et al., 2013) that gives rise to slower flows and thus shorter particle trajectories in the distal generations. At first, irreversibility could be erroneously attributed to an asymmetry in the inhaled and exhaled volumes leading to a shift in particle location from cycle to cycle. However, we have observed (not shown here) that in the course of one flow cycle, two initially close-by particles may often translocate in opposing directions.

To help uncover whether irreversibility is attributed to convective flow mixing or relies instead on diffusional streamline crossing, we compare the net dispersion of particles originating in the first generation of the model to that occurring in a straight channel. Here, we select the long entrance channel (1.2 cm) leading to the alveolated tree structure as a model (see SM Video 2). This strategy ensures that the volume of fluid passing through the straight channel is identical to that filling the model and correspondingly, that the flow rates are identical. For such experiments, we ensure that the tracked particles remain far from the tree entrance throughout time. We note that wall deformations in the leading channel are expected to be very small since the thin walls in the tree structure, which are only ~50 μm in width, are much more flexible compared to those of the straight channel measuring several millimeters in width.

Fig. 4 shows the locations of 171 individually-tracked particles inside the alveolated tree (left column) where experiments are limited to 4 consecutive flow cycles to maintain all particles within the FOV. Here, we compare particle dispersion to experiments in a straight channel using 171 particles (Fig. 4, middle column) and simulations in a straight channel using 1024 particles (Fig. 4, right column), where all flow and particle parameters are closely matched (see Methods). It immediately transpires that dispersion is not solely confined to the acinar tree. Qualitatively, the extent of dispersion in the straight channel is on the same order of magnitude as that seen in the tree network, underlining the likely importance of diffusional streamline crossing as the leading source of irreversibility in both geometries. These results are further supported by the DEM simulations, where any dispersion in the duct arises solely from particle Brownian motion.

5 Effective diffusivities

To assess more quantitatively dispersion phenomena between the alveolated tree and the straight channel, we extract the mean squared displacement (MSD) for the experiments of Fig. 4; these are further compared to freely diffusing particles in the absence of flow. In Fig. 5a, MSD curves are plotted as a function of normalized time (t/T) where we extract the effective diffusion coefficient using $\text{MSD}(t) = 4D_{\text{eff}}t$, since visualizations are limited to 2D projections of particle trajectories in the FOV (Fishler et al., 2015). While the experimentally determined diffusion coefficient is found to be $D_{\text{mol}} = 1.5 \times 10^{-13} \text{ m}^2/\text{s}$ (Fig. 4a, inset), corresponding to an 8% deviation from the Stokes–Einstein prediction, D_{eff} in the alveolated tree and straight channel (experiment) is $1.2 \times 10^{-10} \text{ m}^2/\text{s}$ and $2.5 \times 10^{-10} \text{ m}^2/\text{s}$, respectively. Such values are 3 orders of magnitude higher compared to D_{mol} where dispersion in the straight channel is observed to be larger (approx. twice) compared to that in the tree. We note that the DEM simulations in the straight channel yield an even higher D_{eff} of $4.9 \times 10^{-10} \text{ m}^2/\text{s}$; a discrepancy that could be attributed to the larger amount of particles required for sufficient ensemble statistics and to small inaccuracies in measuring velocity profiles in the channel.

We measure next for each experimentally tracked particle the characteristic “step size” defined as the average distance travelled by each particle over cumulative cycles; this is shown in the histograms and box plots of Fig. 5b. A Mann–Whitney U test indicates that the larger step sizes in the straight channel are statistically significant compared to the alveolated tree ($p < 0.001$). One important reason for such differences lies in the fixed inhaled volume in both the acinar structure and the straight channel. As such, particles in the channel experience higher velocities and longer trajectories compared to the tree, where Re drops by a factor of 2 with each bifurcation. Hence, net dispersion in the tree is comparatively *reduced* due to lower convective velocities.

In both experiments and simulations we observe significant dispersion from streamline crossing despite low particle diffusivity. Here, the average and peak particle Peclet numbers (Pe) in the leading straight channel (and generation 1) are 4.0×10^5 and 9.1×10^5 , respectively; a result that would imply negligible diffusion in classic steady uniaxial flows. Yet, the ballistic approximation is unfit, as highlighted in experiments in shallow microfluidic channels (Ajdari et al., 2006). Alternatively, for augmented Taylor–Aris dispersion (Aris, 1956; Taylor, 1953), where the timescale for radial diffusion is shorter than axial convection (but longer than radial convection), the condition $1 \ll Pe \ll L/a$ arises, where L is the length of the channel and a its width. Such dispersion regime would only be met for $L \gg O(10^{-1} \text{ m})$ which is not the present case. Theoretical and experimental works have extended such works to quantify axial diffusivity under oscillatory laminar flows both in straight pipes (Joshi et al., 1983; Watson, 1983) and branching tube networks (Kamm et al., 1984). These models predict that for a low dimensionless frequency parameter (i.e. Womersley number, $Wo = a(\omega/\nu)^{1/2}$ with ω the angular frequency and ν the fluid’s kinematic viscosity) the ratio $D_{\text{eff}}/D_{\text{mol}}$ will return to unity (Elad et al., 1992; Gaver et al., 1992); a trend different from our observations for $Wo = O(10^{-2})$ where the ratio $D_{\text{eff}}/D_{\text{mol}}$ yields $\sim 10^3$ and Pe values are intrinsically much larger (Kamm et al., 1984). While these results call for further investigation, we note as a final remark that net particle displacements

per cycle scale approximately according to a modified Fick's law, i.e. $\left(D_{eff} \frac{dv}{dr}\right)^{1/2} \cdot T$ ~50–100 μm , where v is the streamwise velocity and r is the spanwise direction. Such values fall within the range of experimentally measured step sizes shown in Fig. 5b.

6 Conclusion

It is often brought forward that the acinar airway environment is prone to substantial convective mixing during breathing; a mechanism that weighs heavily on the fate of inhaled aerosols in the acinus since they behave to a first order as passive tracers. By utilizing liquid-suspended micron particles with negligible sedimentation, our microfluidic experiments suggest that particle dispersion relies significantly on diffusional streamline crossing despite very low diffusivity. Extrapolating such results to the lung acinus, virtually all inhaled aerosols (~0.005–5 μm) acknowledged to reach these depths are prone to some degree of streamline crossing, and hence dispersion, as a result of intrinsic motion. While our experiments cannot dissociate entirely the coupling between convective mixing and intrinsic motion, there is likely to be no single particle size that can be truly regarded as a passive tracer. Namely, the dispersion mechanisms witnessed here are anticipated to be considerably augmented for sub-micron airborne particles due to their higher diffusivity in air (i.e. Stokes–Einstein equation), whereas gravity will become more important for streamline crossing with larger particles. This view is in line with previous analyses predicting that for 1 μm particles gravitational sedimentation can account for most particle dispersion in the acinus without requiring substantive heterogeneous wall motion or convective mixing (Darquenne and Prisk, 2003). Our experiments allude to a possible hypothesis for the low contribution of convective mixing to overall particle dispersion: the particles most affected by convective mixing may be those passing near alveolar openings, where streamlines are slow and particles are rarely exhaled but rather prone to deposit within the acinus. On the other hand, exhaled particles usually originate from more central and fast streamlines where streamline crossing is the main source of dispersion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Top view of the acinar tree model consisting of five acinar generations (numbered G1 to G5) that are lined with alveolar cavities. Flow within the device is controlled either by cyclically pressurizing the water chamber to deform the thin PDMS walls, or alternatively by leaving the chamber dry and connecting a syringe pump directly to the inlet. The inlet channel extends far beyond the shown field of view (FOV) to a distance of 2.2 cm and is used as a straight channel for comparative experiments (see Methods and Results and discussion).

Fig. 2.

Trajectories of 1 μm particles suspended in 64% (v/v) glycerol within alveolar cavities located at generations 1 (left), 3 (middle) and 5 (right) of the microfluidic acinar model. Alveolar walls are marked with a white line for clarity. Note that trajectories are overlaid on the first image in the sequence used for particle tracking. Color coding represents normalized time where dark blue indicates the starting time and red encodes the end time (see left panel). Note that the overall tracking time varies between the shown trajectories, spanning 3 to 14 cycles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Fig. 3.

Trajectories of 2 μm particles suspended in 16.5% (v/v) glycerol within alveolated ducts. Particle locations at the reversal between inhalation and exhalation are marked with circles and chronologically numbered according to cycle. Note that to maintain all particle trajectories within the FOV, experiments are limited to 4 cycles.

Fig. 4.

Experimental time lapse images of bolus dispersion showing particle locations at $t = 0, T, 2T, 3T$ and $4T$ in the first generation of the alveolated tree model (left column) and in the straight channel (middle column); a total of $n = 171$ are shown in both cases where the particle ensembles correspond to the superposition of multiple independent experiments (as exemplified in SM Video 1 and 2). Numerical simulations for the straight channel are shown (right column) with $n = 1024$. Note that particles are colored according to particle index.

Fig. 5.

(a) Mean square displacement (MSD) as a function of cumulative cycles for experiments in the acinar tree and in the straight channel, respectively. Statistics are extracted for a total of $n = 171$ tracked particles in each case. Inset: Corresponding experimental MSD curve for freely diffusing particles in the absence of flow ($n = 500$); also shown is the theoretical prediction following the Stokes–Einstein equation. (b) Histogram and box plot (inset) for the characteristic average step size in the acinar tree model (“tree”) and in the straight channel

(“duct”); see text for details. The bar plot depicts average and SD for step sizes in the duct and tree. A Mann–Whitney U test was used to test for statistical significance ($*p < 0.001$).

Table 1

Experimental conditions and relevant parameters.

	Field of view (FOV)	
	Individual alveoli	Tree and straight channel
Particle properties		
Particle diameter (μm), d_p	1	2
Particle density (gr/cm^3), ρ_p	1.05	1.05
Terminal velocity ($\mu\text{m}/\text{s}$)	-0.0036	0.013
Brownian displacement in 1 s (μm)	0.31	0.75
Fluid properties		
Glycerol/water volume ratio	64/36	16.5/83.5
Dynamic viscosity at 24 °C (Ns/m^2), μ	0.0178	0.00156
Fluid density (gr/cm^3), ρ	1.17	1.04
Flow properties		
Cycle time (s), T	4	12
Re_{max} (i.e. gen. 1 and straight channel)	0.08	0.09
Wo_{max} (i.e. gen. 1 and straight channel)	0.06	0.02