Anti-Gravity Protein Crystal for SFX

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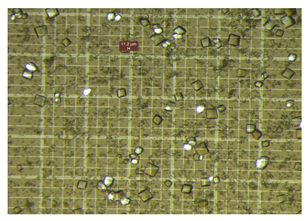
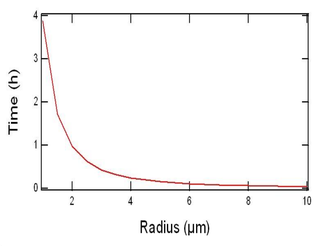
Introduction

Protein crystal settling creates costly wasted experimental time (LCLS costs ~1 burrito/second!)



Protein sample tends to be a mixture of different shape and size, thus creating variation in settling time. This project aims to characterize this process, as well as slowing it down to about 6 hours (0.5 shift), or ideally 12 hours (1 shift)



Theory

1. Reynold's number

$$\mathbf{Re} = \frac{\rho u L}{\mu} = \frac{u L}{\nu} < 1 \rightarrow \text{Stoke's flow}$$

2. Brownian Motion

Random motion of particles suspended in a fluid resulting from their collision with the fast-moving molecules in the fluid

3. Stoke-Einstein Equation

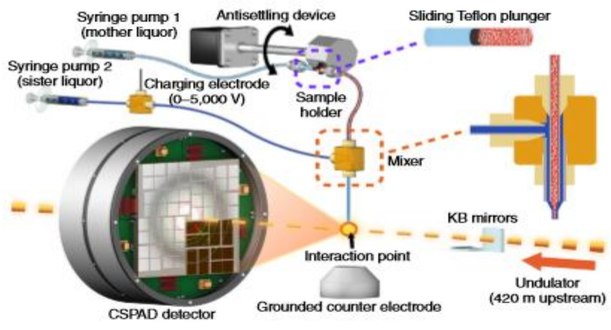
$$D = \frac{k_B T}{6\pi\eta r_h} \Rightarrow 6\pi\mu a\mathbf{U} = \tfrac{4}{3}\pi a^3(\rho - \rho_{fl})\mathbf{g}$$

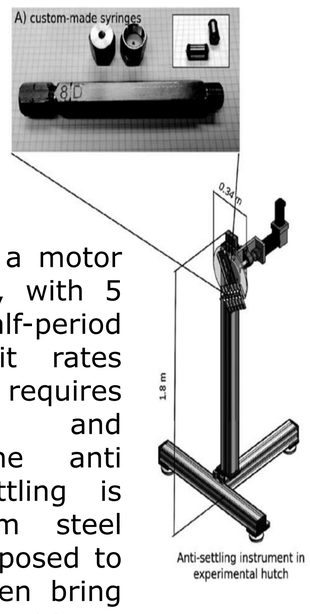
Drag force = Buoyancy

Terminal Velocity: $\mathbf{U} = \frac{2}{9}\frac{a^2}{\nu}\left(\frac{\rho}{\rho_{fl}} - 1\right)\mathbf{g}$

Background

Sierra et al, 2016



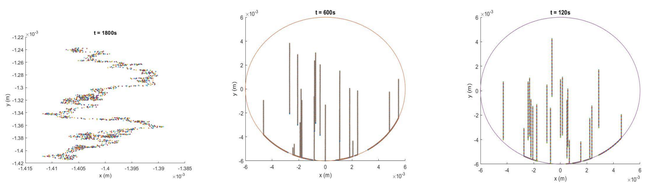


Anti-settling instrument in experimental hutch

Currently, LCLS is using a motor oscillating through 225º, with 5 seconds of rest every half-period (above). Diminished hit rates during an experiment, requires further understanding and characterization of the anti settlers. Visualizing settling is difficult in the custom steel reservoirs (center) as opposed to clear syringes. Users even bring custom devices to help mitigate settling (Lomb *et al*. 2012).

Method

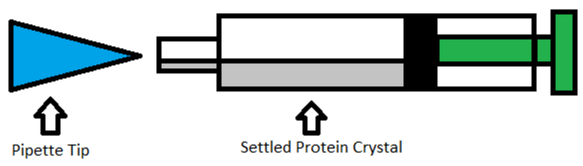
1. ***Simulation***:
- Brownian Motion is simulated by using Monte Carlo method
- Random number generation at each time step emulates *drunken walk*
- Sedimentation of protein crystal is simulated using terminal velocity equation (see Theory); terminal velocity achieved quickly
- Boundary condition is also simulated so that particles don't fall out of the simulated reservoir
- Mirror diffusion outside the wall, set to wall if fallen below
- Assumed centripetal force is negligible for these masses and rotation speeds
- Intra-particle interactions are ignored
- 2D diffusion only



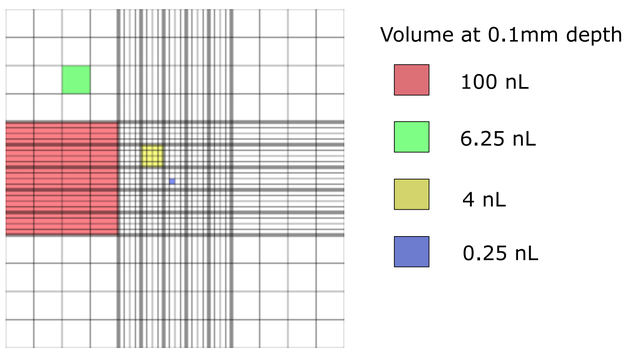
Zoom in

Simulated results of crystals settling
Radius: 1μm (left), 10μm (middle), 20μm (right)

2. Experiments:
- Concentration is taken using a pipette and measured using a hemocytometer



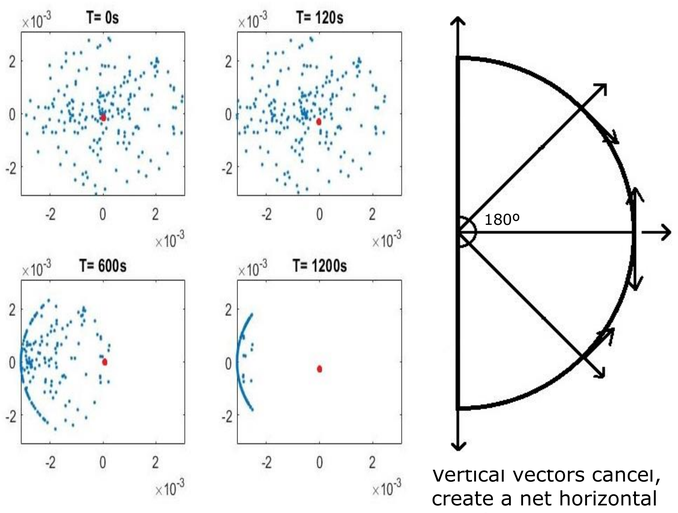
Schematic of sample withdrawal



Hemocytometer (Ref: Wikipedia)

Results

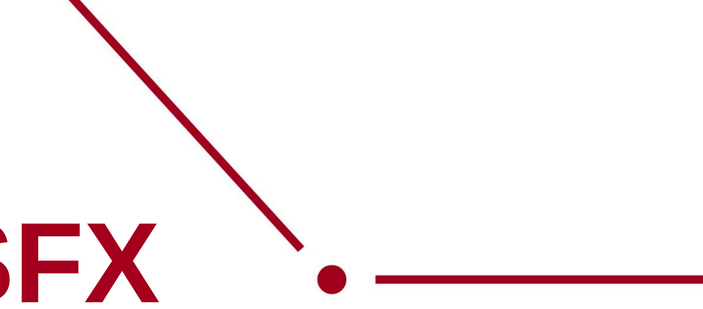
1. Rotation of 180º without rest time
- Simulation indicated a counter-intuitive results of settlement toward the side
- Experiment confirms simulation results



Simulation result of 180º rotation

vertical vectors cancel, create a net horizontal velocity

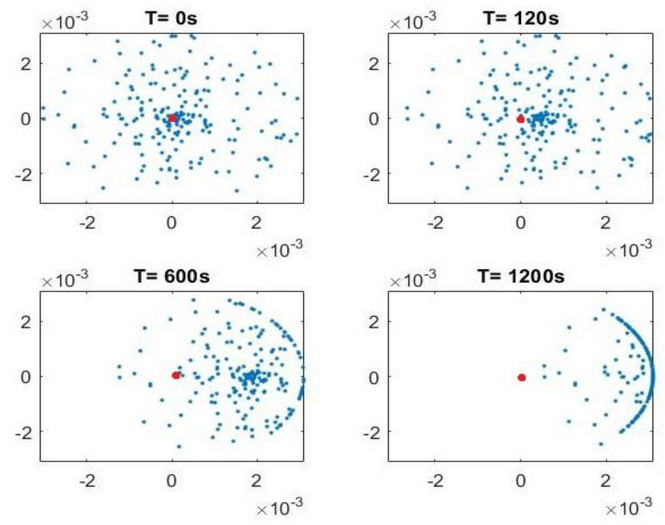
Results (Cont.)



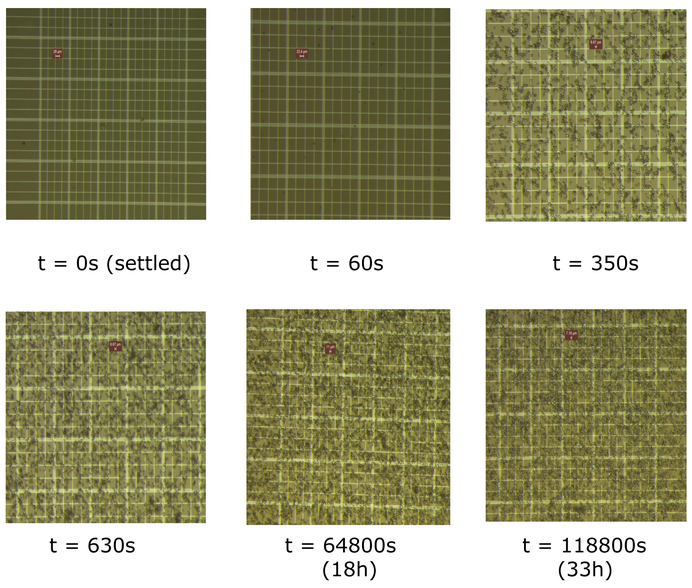
t = 0s t = 300s t = 600s

Experimental results of 180º rotation using lysozyme

2. Rotation of 360º without rest time:
- Simulation indicates sedimentation of crystals
- Experiments demonstrate that not only protein sample will not settle, this rotation can actually resuspend settled solution.



Simulation result of 360 rotation



t = 0s (settled) t = 60s t = 350s

t = 630s t = 64800s (18h) t = 118800s (33h)

Experimental results of 360º rotation using lysozyme

Conclusions

- Rotation of 360º is capable of resuspending sedimented sample; verify efficacy during actual beamtime is needed
- Future projects include simulation and experiments of rotation of 225º with resting time, particle interactions, 3D modeling and different samples and liquids, heterogenous mixtures
- Leverage other physics, such as centripetal forces or acoustic levitation

Acknowledgments

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