… I'm in the Lysozyme business.

User **S**ample **A**ssessment DIY Kit

Lysozyme Characterization

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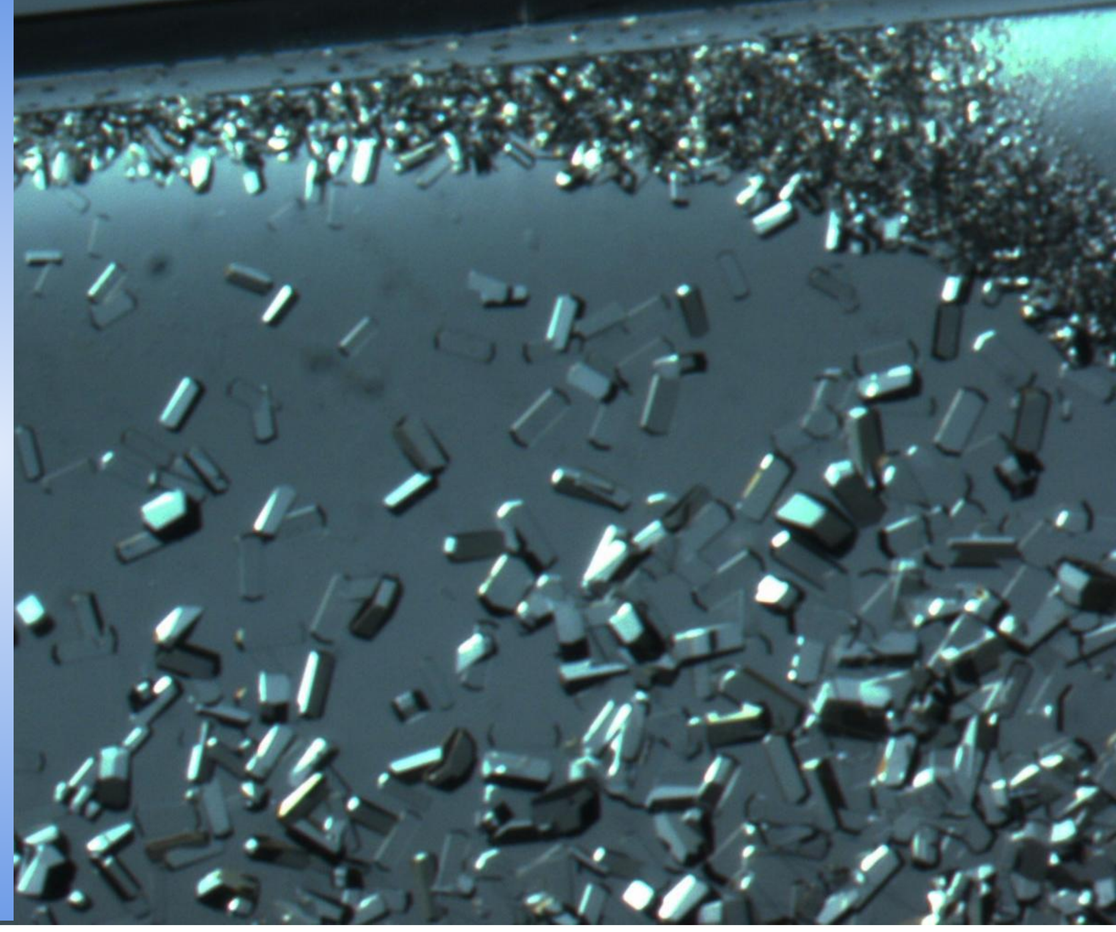
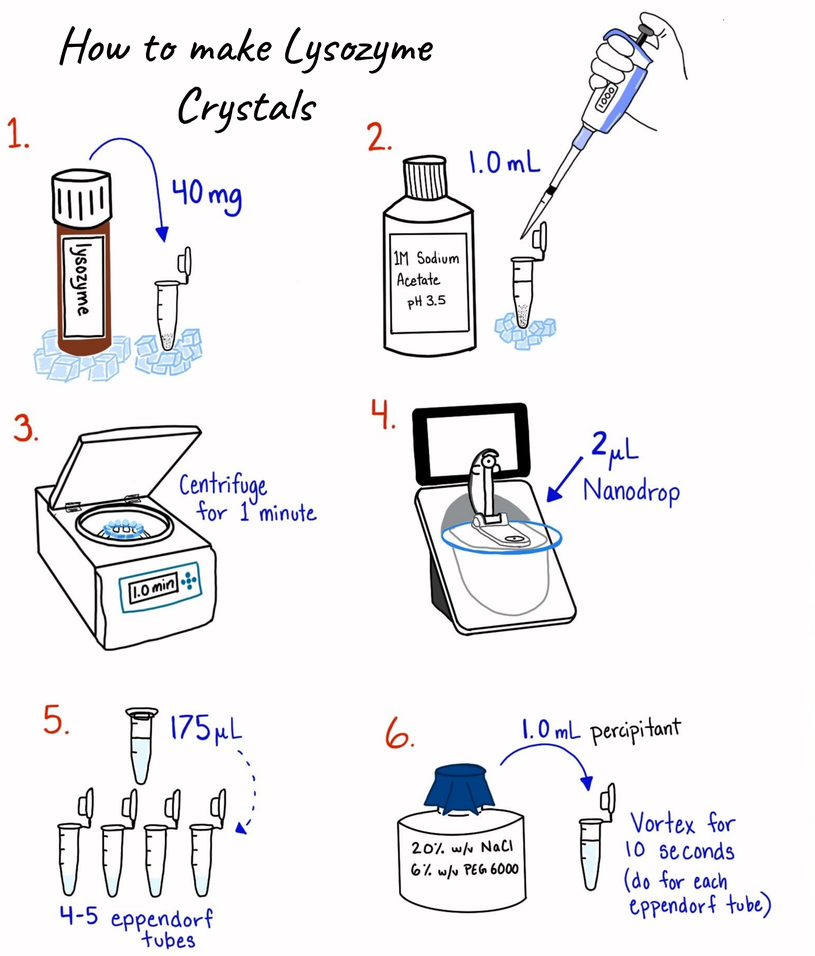


Background

To improve beamline efficiency and reduce injector issues, we seek to develop a low-cost, user-friendly kit that allows researchers to pre-screen their sample before arriving at LCLS. This effort supports the development of a standardized lysozyme protocol for the Crystal Library. The goal is to reduce injector clogs and beamtime delays. It will enable users to perform basic characterization independently, with no prior XFEL experience.

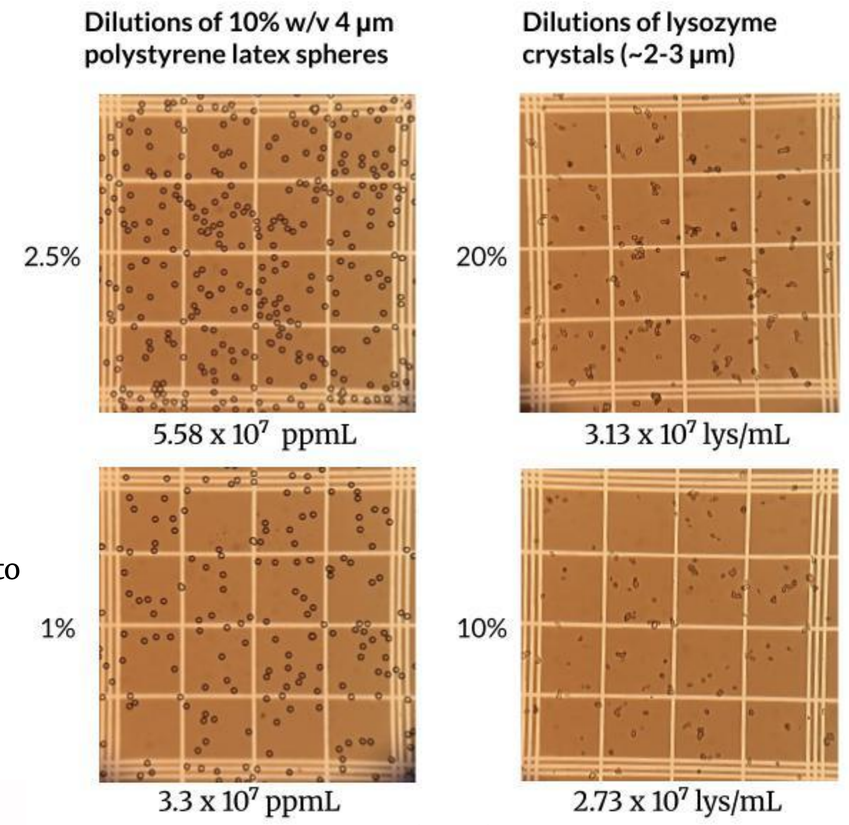
Lysozyme Crystallization Overview

- Calibrate detectors.
- Validate/troubleshoot SFX experiments
- Simplify the process of preparing lysozyme crystals to reduce staff burden
- Easy for non-biologists to make their own
- How to make different sized crystals and concentrations?

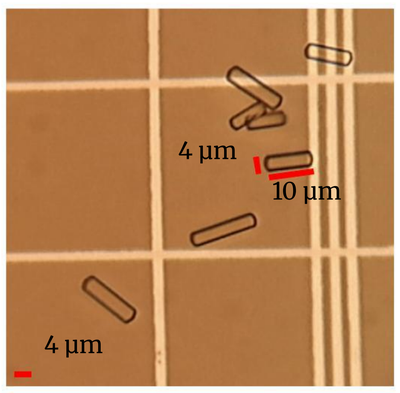


Results

10% w/v 4 µm Polystyrene Latex Spheres compared to concentrations of lysozyme crystals. (~5.58 x 10^9 ppmL)



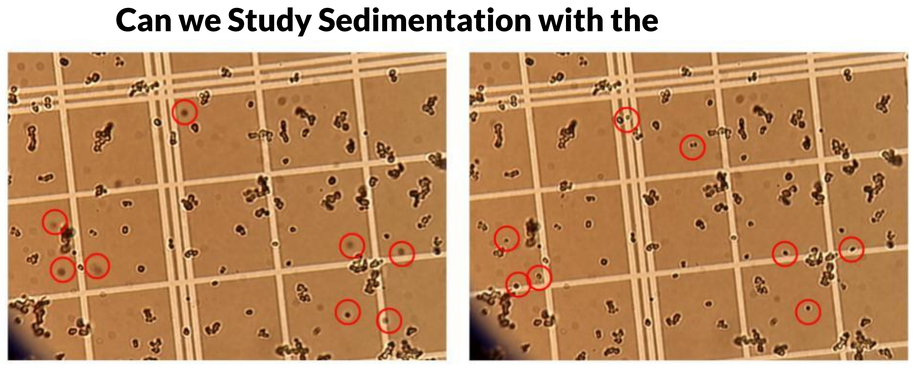
Huvitz Microscope



Large Lysozyme Crystals

- Diluted protein solution created larger lysozyme crystals.
- Number of larger crystals was less than normal concentration of small lysozyme crystals.

Can we Study Sedimentation with the

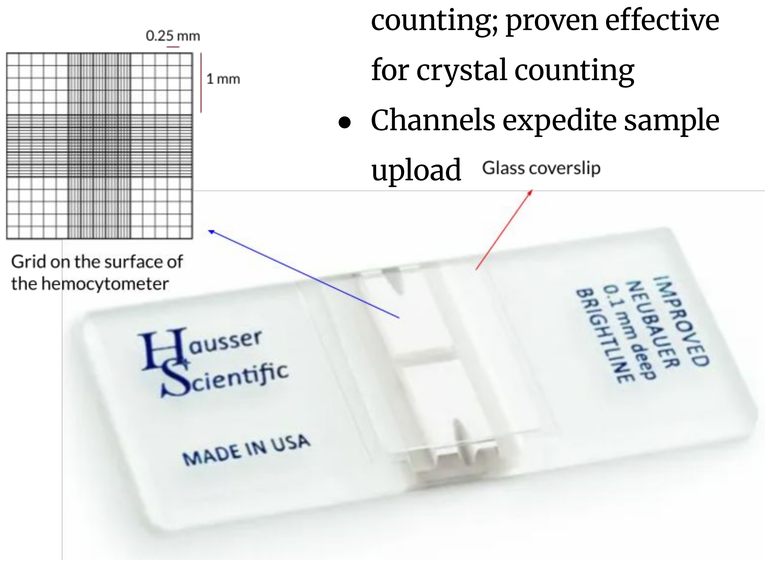


0 minutes

10 minutes

Hemocytometers

- Designed for cell counting; proven effective for crystal counting
- Channels expedite sample upload



Future Work

<u>Develop simple viscosity test for jet compatibility</u>: Characterize the viscosities of glycerol, cellulose, pluronic, LCP both alone and with lysozyme crystals. Essential for tuning flow properties in HVE injectors.

<u>Glycerol</u>

- Well understood viscosities to test in liquid jets (e.g. GDVN, DFFN, MESH)

<u>Pluronic F-127:</u>

- A non-ionic surfactant with thermo-reversible gelation: liquid at 4℃ and a gel at room temperature. Effective for delivering hydrophobic molecules.
- When lysozyme crystals are added, its ability to return to liquid at cold temperatures is lost.
- Potential to test novel HVE redevelopments

<u>Hydroxyethyl Cellulose and Lipidic Cubic Phase</u>:

- LCP is a matrix of monoolein and water that can crystallize biologically important systems, such as hydrophobic membrane proteins like GPCRs.
- A biocompatible, low-cost alternative to LCP. HEC can serve as a viscous medium for crystal suspension in HVE injection systems, especially soluble proteins.

<u>Crystal Library</u>:

- Finalize and validate a simple, reproducible lysozyme crystallization protocol.
- Expansion of the library will include new crystals and various growth methods for jet testing and beamtime needs.

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