

Small Sample, Big Answers: Low-Volume Rheology and Automated Crystal Analysis for XFEL Sample Characterization

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Background

Experiments at X-ray Free-Electron Lasers (XFELs) like LCLS are often hindered by unpredictable sample delivery and injector clogging. This results in loss of valuable beamtime and sample. This project aims to continue the development of a kit that enables researchers to screen samples *before* (*a priori*) arriving at the beamline. To that end the work focuses on two components of the kit:

- Project 1: Viscosity Test – Use of shear thinning rheology to determine viscosity of user samples
- Project 2: A YOLO (You Only Look Once) deep learning model trained to rapidly and accurately quantify protein crystal count.

Underlying Principle: Thin Film Rheology

- The intended device uses a stepper motor to move a smearing blade at precise, programmable speeds over standard glass slides as seen in Figure 1.

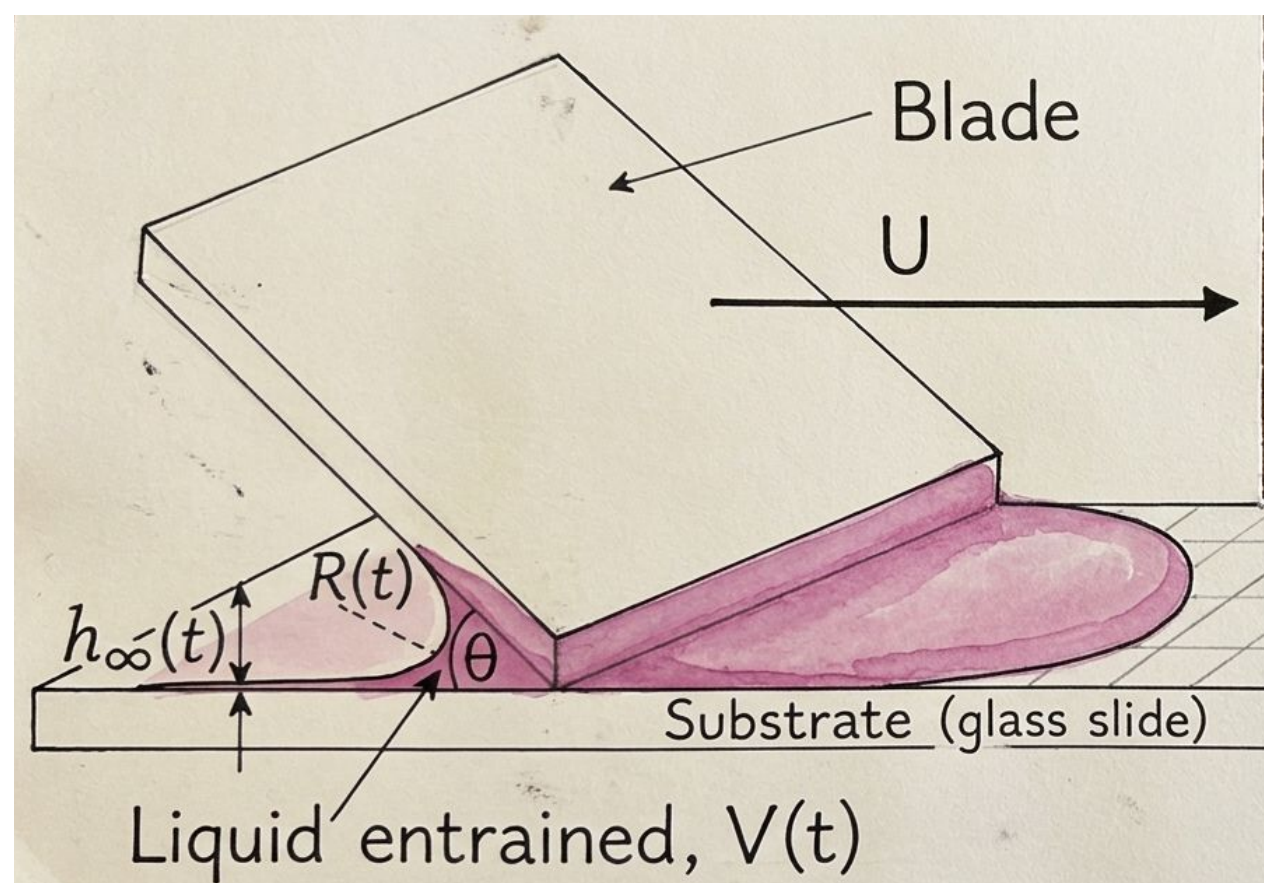


Figure 1. Drawing of the smearing technique. Adapted from "A viscosity measurement technique for ultra-low sample volumes," by M. A. Mir and M. S. Tirumkudulu, 2024, *Soft Matter*.

- With the velocity (U), the shear rate ($\dot{\gamma}$) and h_{∞} representing the thickness of the film, this platform obtains viscosity characterization info for low sample volumes (5–10 μ L) using the following formula.

$$\dot{\gamma} = \frac{U}{h_{\infty}}$$

- By attaching a force transducer in the form of an load cell into the smear device we can extract shear stress information which then lets us find the viscosity (η)

$$\eta = \frac{\tau}{\dot{\gamma}}$$

Current Iteration of Smear Machine

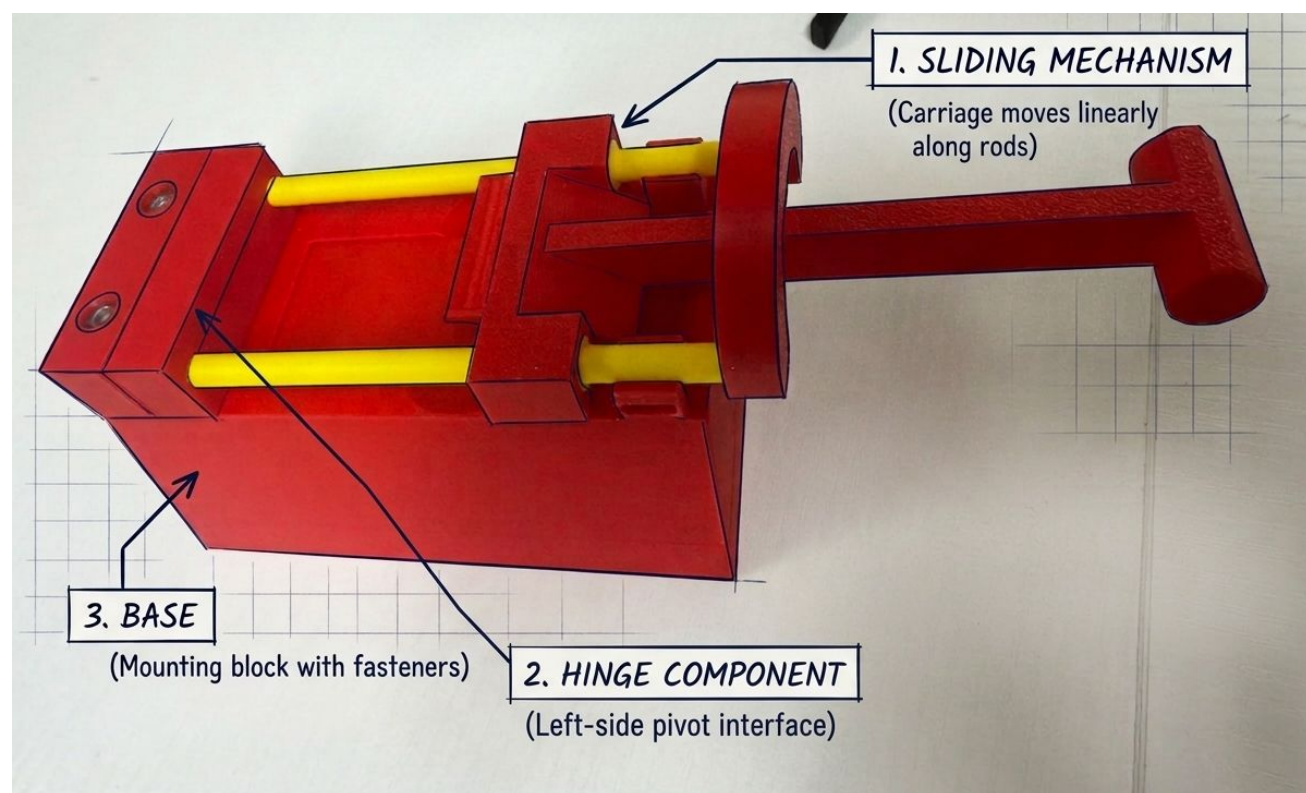
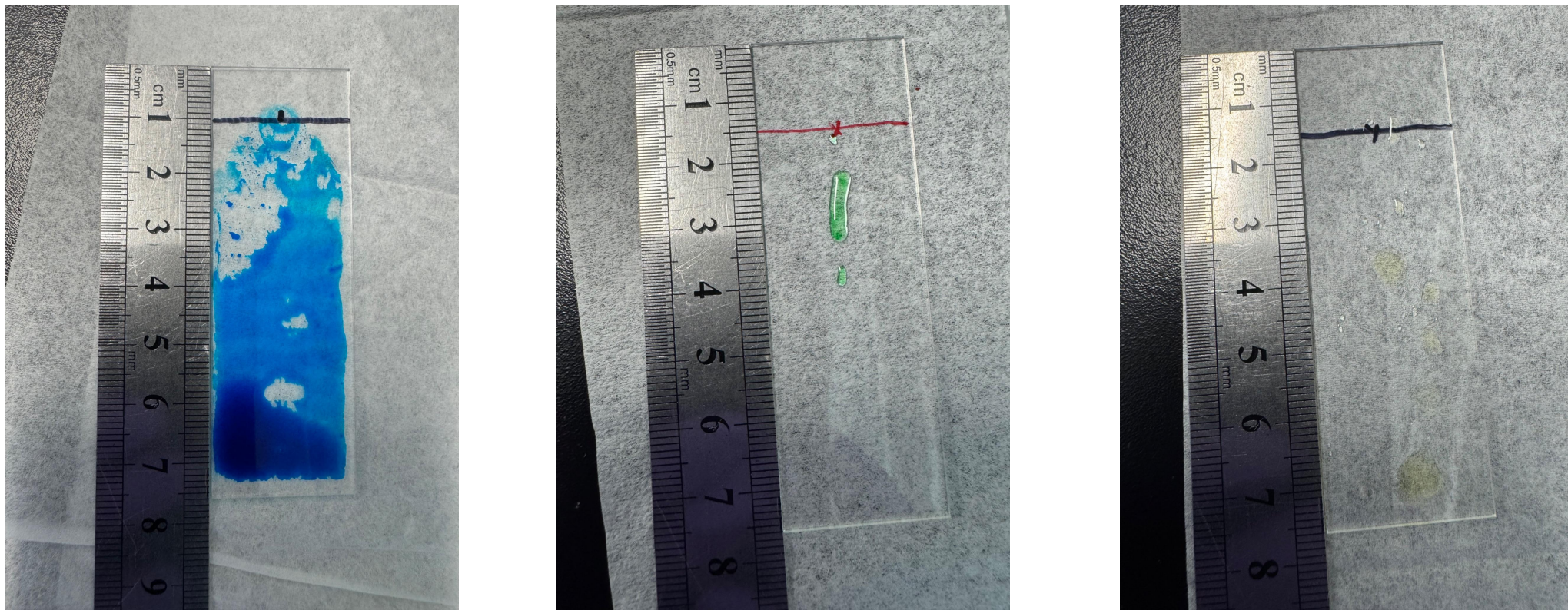


Figure 2. Current semi automatic smear device. Original design adapted from autohaem (McDermott et al., 2022)

Smear Length as a Proxy for Fluid Viscosity

- As an initial proof of concept, manual smear tests were conducted to validate the relationship between fluid mechanics and smear geometry prior to integrating hardware automation.
- Procedure starts with a drop of sample pipetted onto a slide at a fixed distance then a secondary slide at a 30 degree angle spreads the sample pausing briefly to allow capillary action to draw the fluid along the edge before pushing forward in one smooth, continuous sweep

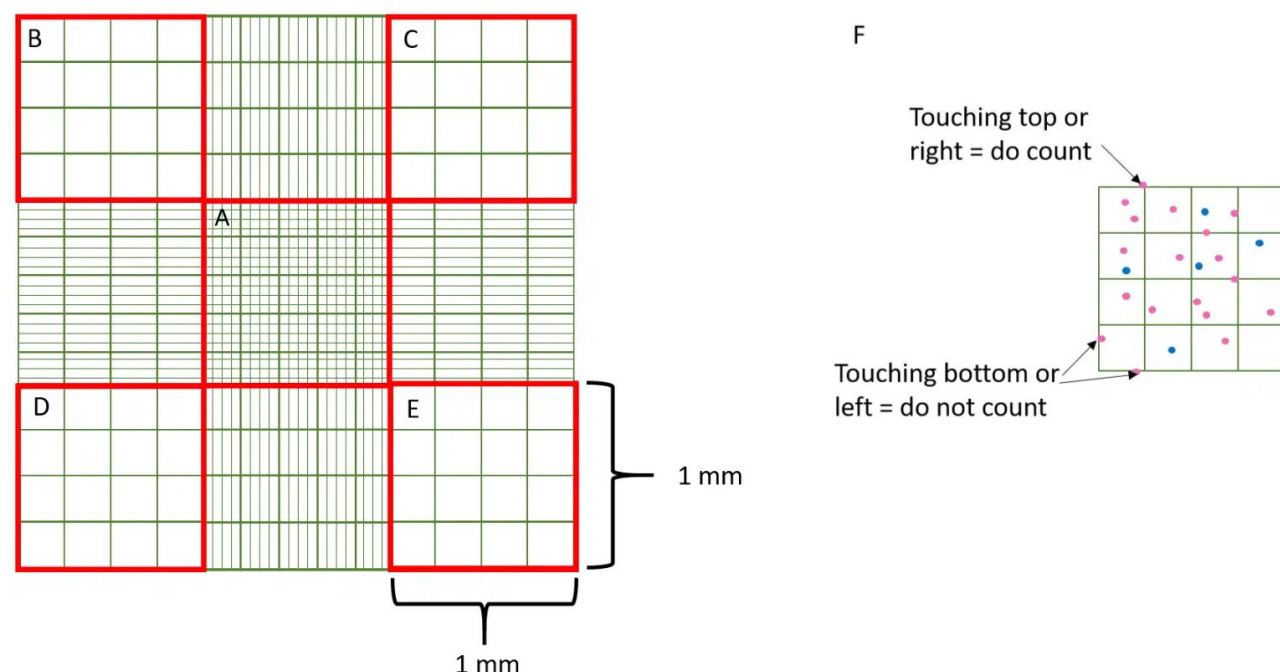


Note. The tested samples were from left to right, distilled water, 75% glycerol (aq), and PEG 6000

Measuring smear length using Smear Machine



Manual Hemocytometer Counting

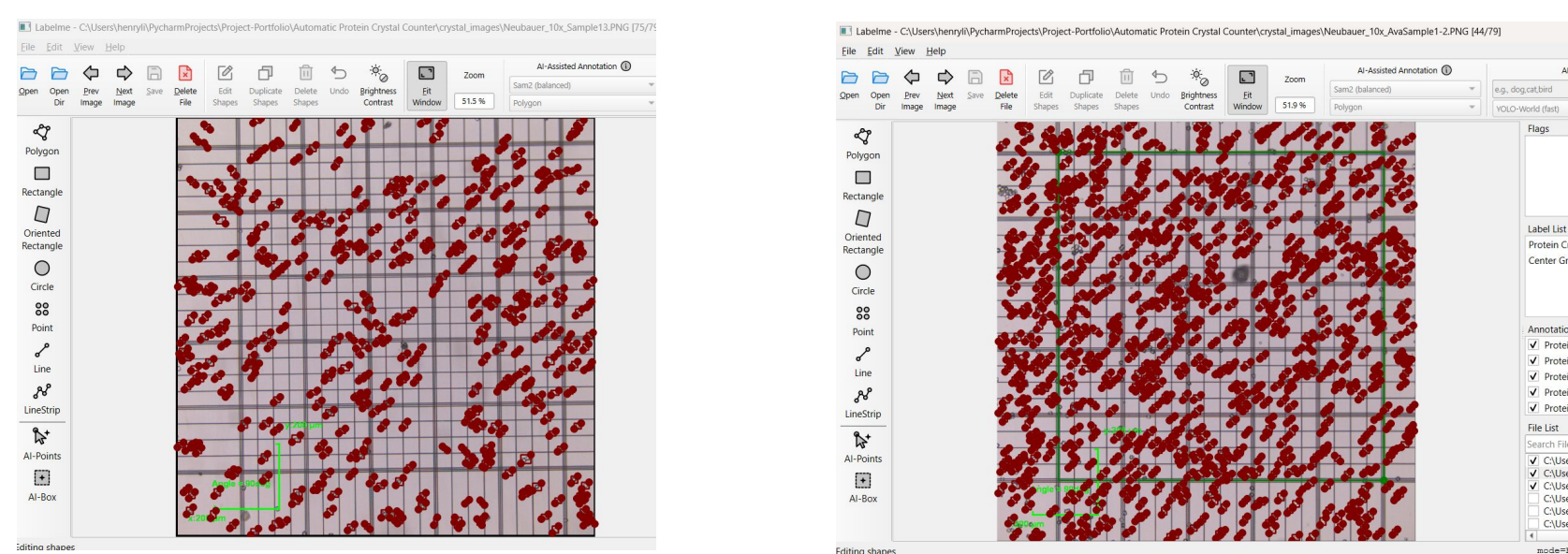


- A hemocytometer slide is used to view a measured grid under a microscope so we can count the number of tiny particles in a liquid sample.
- Useful for counting protein crystals because it tracks how many crystals grow in a specific volume and checks their size and shape.
- Protein crystal solutions can contain anywhere from 10 to 5000+ crystals in a single hemocytometer image!

Protein Crystal Counting Pipeline

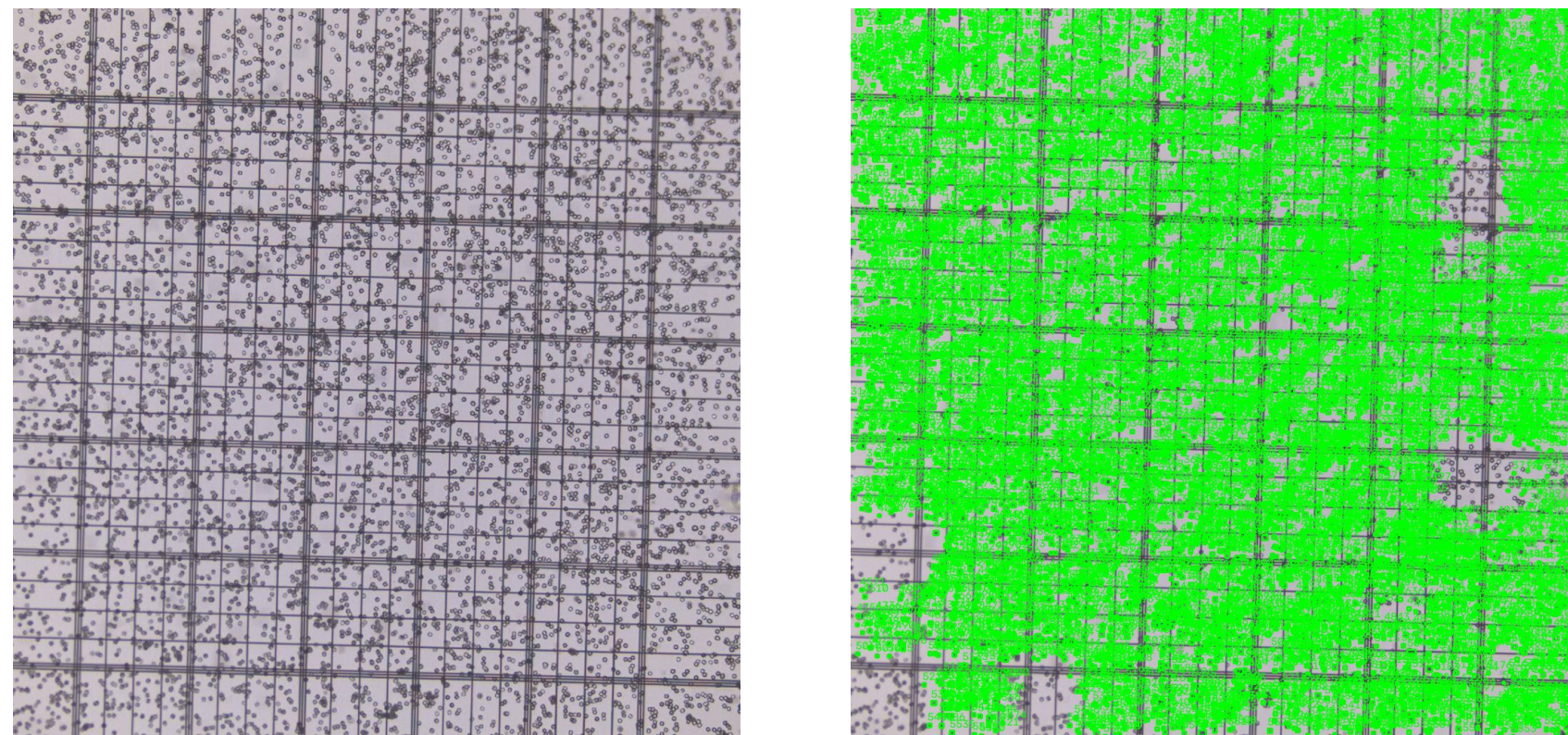


Bounded Box Manual Labeling Process



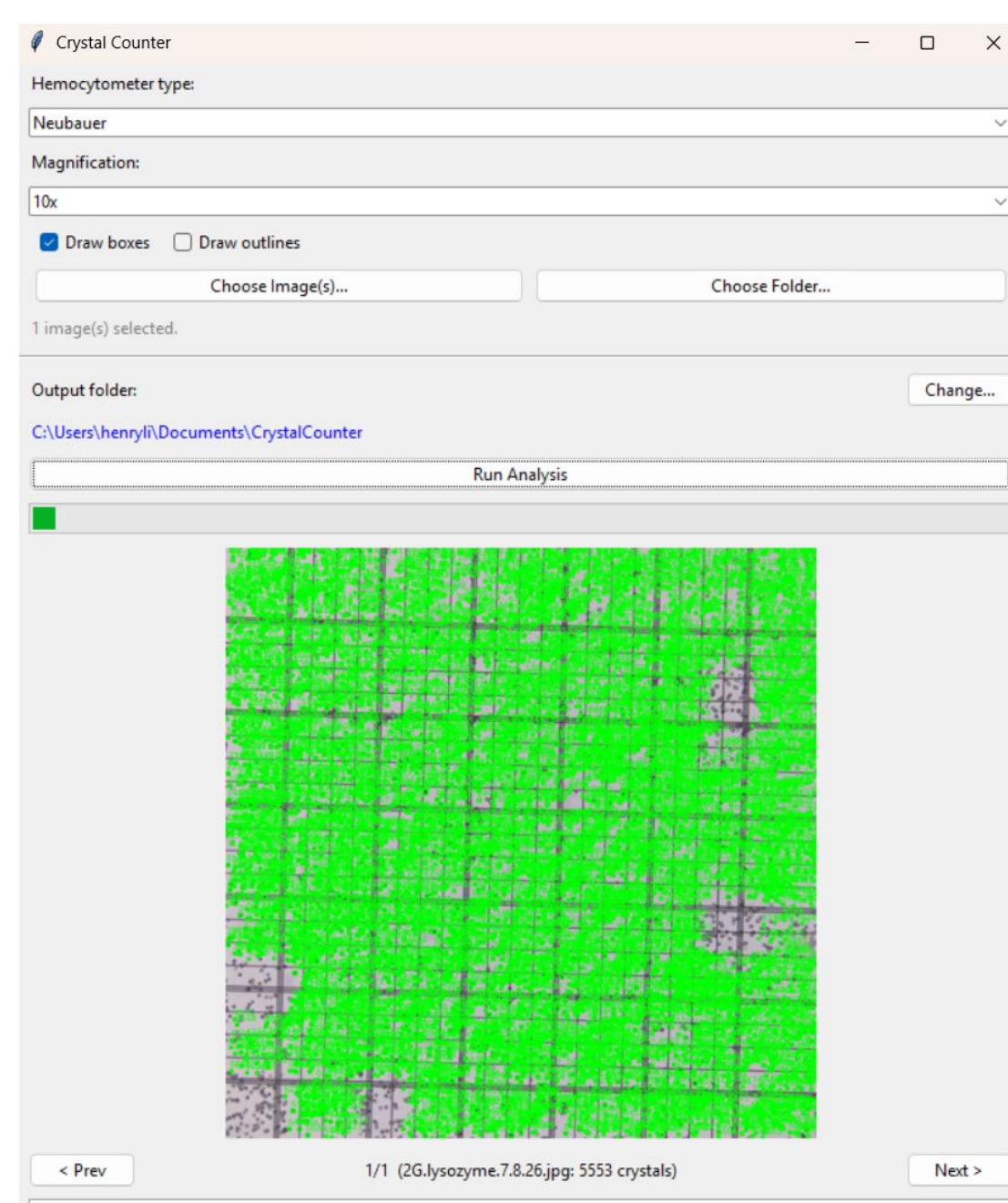
Performance of Model

- Overall performance of most up to date model is around ~84% accuracy. Trained with a total of 150 images, 100 in training, 25 in test, 25 in validation.



Example run. Original image on left, post model count on right. Manual counting regime count: ~6600, Model count: 5553, Percent accuracy: 83.29%

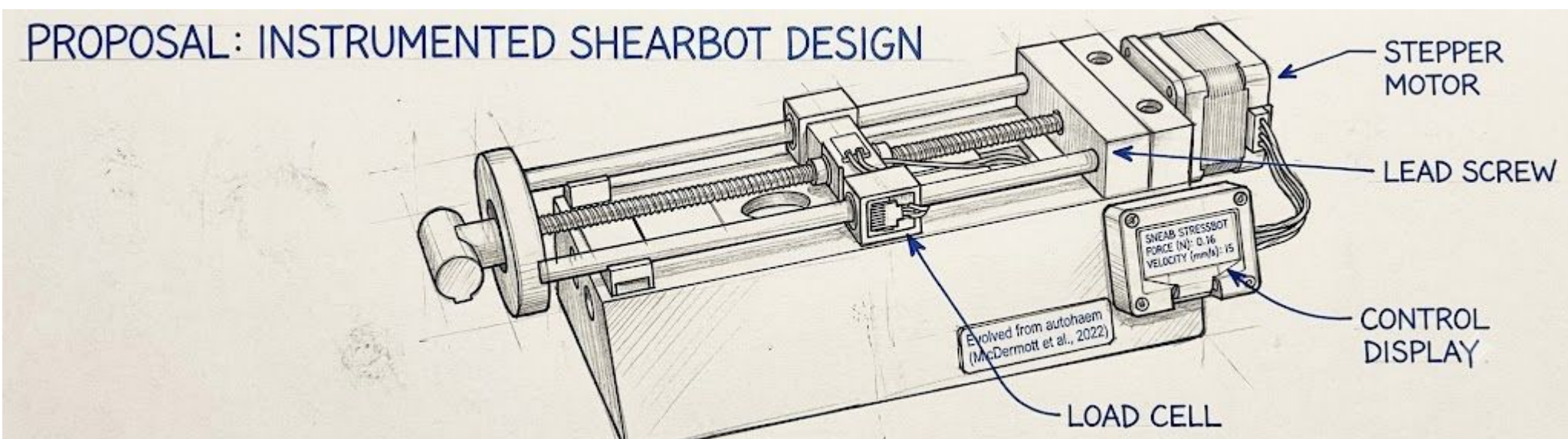
Protein Crystal Counter APP GUI



- Standalone .exe file works natively on windows and apple OS
- Runs locally with no need for internet connection

Future Work

- One possible route is to implement quantitative instruments such as an load cell and stepper motor to capture shear stress and velocity



- Continue manual smear tests and extend them to incorporate various non-newtonian fluids like oobleck, crystal slurries, viscous carrier media, and ketchup to see shear thinning and thickening effects.
- For crystal counter switch from object detection (YOLO) to segmentation (UNet) in order to get size of crystals and a resulting size distribution graph
- Incorporate other differently shaped protein crystals such as rods, spikes, cylinders to training sets.

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