

MICRO-COSMIC SNAPSHOTS OF PROTEIN CRYSTAL GALAXIES

Developing User Sample Assessment Kits for remote viewing and characterization before beamtime



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BACKGROUND

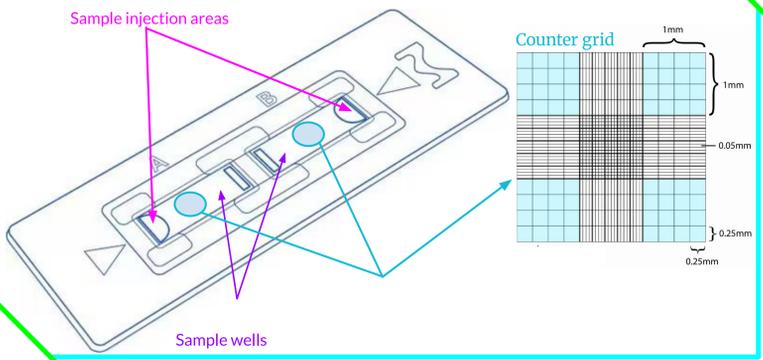
- Protein crystal concentrations, size distributions and shapes contribute to the success of sample delivery during current beamtimes, especially in future high repetition rate beamtimes.
- Characterizing crystal slurries beforehand could minimize excessive troubleshooting time and sample consumption during LCLS experiments by offering a *priori* information about jet performance without the need to consume sample *before* an experiment.

OBJECTIVES

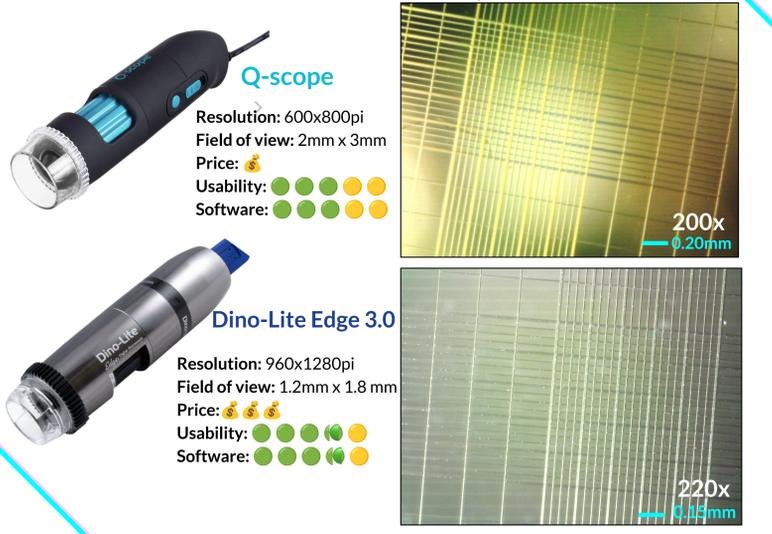
- Reduce delivery issues and sample consumption by encouraging early sample characterization from users
- Develop portable, practical tools to assess protein crystal size and concentration
- Provide reliable, repeatable information to SED with minimal staff burden
- Ensure methods are simple, resource-light, and do not require XFEL-experience or user equipment

TOOLS

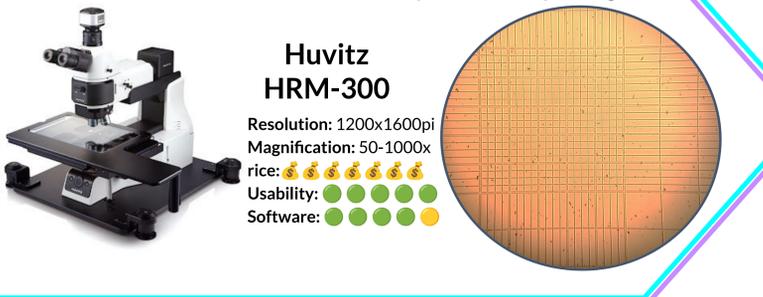
Disposable hemocytometer: cell counter repurposed to count crystals



Handheld Digital Scope Candidates: remotely snapshot protein crystals

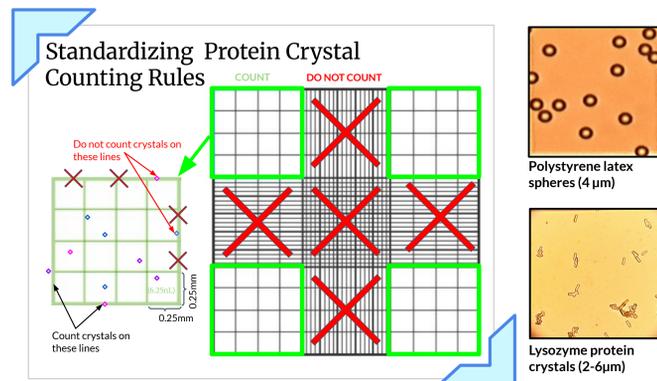


High-resolution reference: Used to obtain reference images to compare with scope images

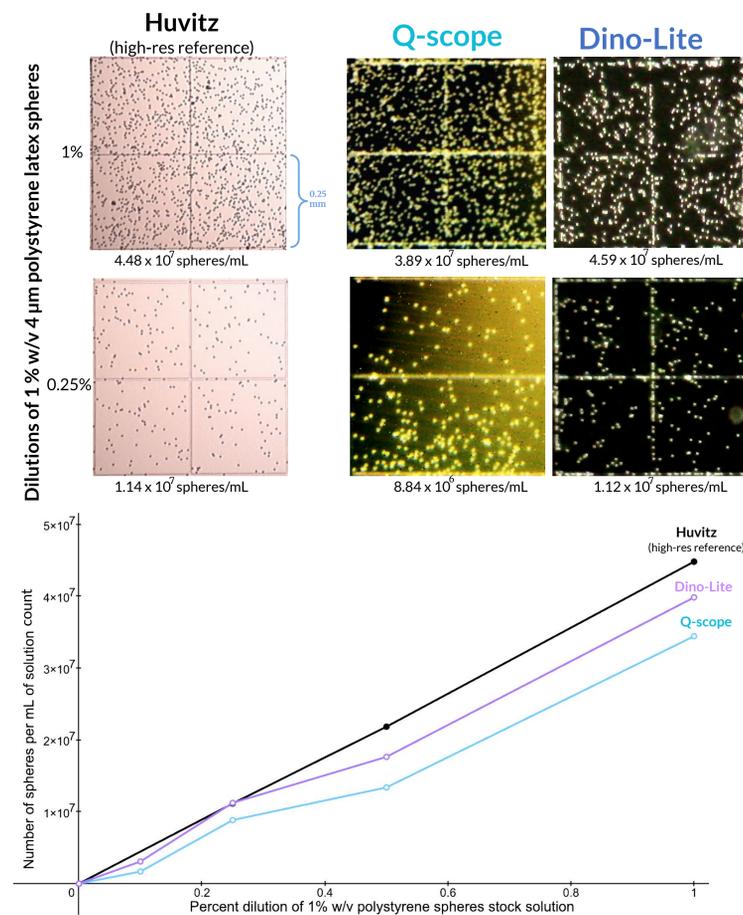


ASSESSING SCOPE CAPABILITIES

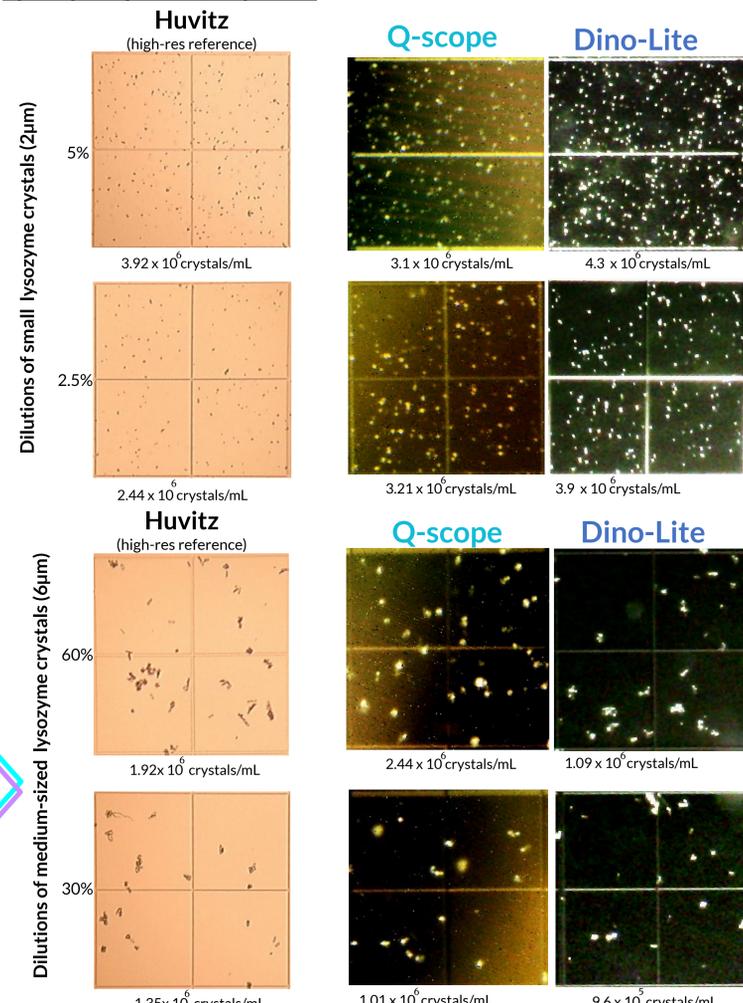
(for accurate counts regardless of users' lab equipment)



Polystyrene latex spheres:



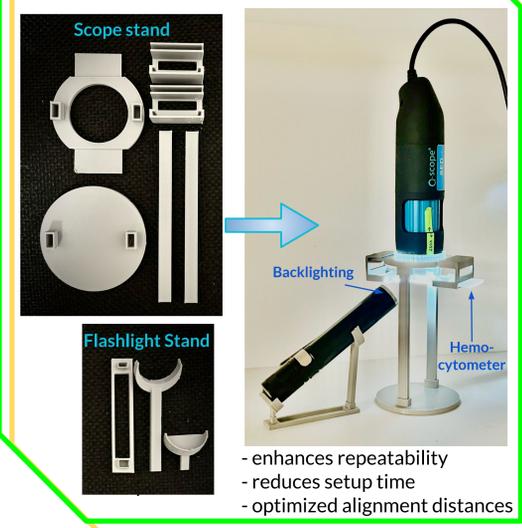
Lysozyme protein crystals:



RESULTS/CONCLUSIONS

- Feasible protein counting using the hemocytometer requires diluted sample slurries (typically at least 1-2 orders of magnitude), thus very little sample expenditure is needed before experiments.
- Despite the Q-scope's limited resolution, it could consistently resolve particles as small as 4 μm for a remote measurement of users' samples before experiments.
- Crystal clusters remain a challenge, as it relies on personal judgment to attribute each cluster to a certain number of crystals
- If sample is returned to the SED team, a more exact measurement could be made on a better microscope with image processing.
- Imaging crystals requires specialized scope stand setup and backlighting:

Designed and 3D-printed a self-assembly scope and backlighting setup



FUTURE DIRECTIONS

- Standardize protein counting rules for attributing crystal counts to clusters
- Verify that disposable hemocytometer is compatible for different crystal slurries
- Develop **USER SAMPLE ASSESSMENT** protocol
 - Standardize procedure to ship and return equipment to and from users
 - Design at-home follow-up tests to verify purity of imaged crystals
- Test higher magnification/resolution scopes
- Implement ImageJ counting and size distribution
- Improved 3D-printed pieces for setup
- Correlate appropriate concentrations/sizes with sample delivery method and repetition rates (requires beamtime)

ACKNOWLEDGEMENTS

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