

UV/Visible Light Imaging and BioSAXS

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Automated Drop Imaging

- Resolution: What is the smallest size crystal I can detect?
- Detection: Can I see crystals and any other important events even under difficult conditions (precipitate, membrane screens, etc)?
- Image analysis: How much time will I have to spend to score all my images?



New approach to combined UV/visible imaging

Single optical train – Directly compare images

Monochromatic camera – Highest resolution

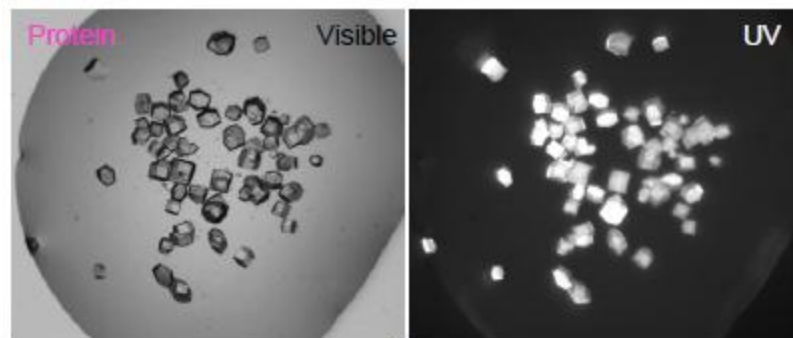
Integrated polarisation

Integrated UV source

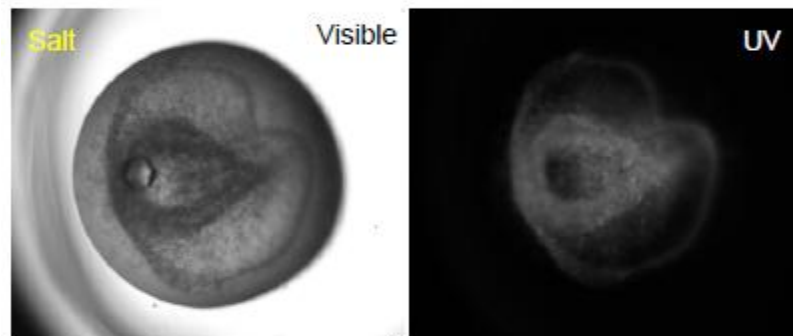
Microscope objectives

Our Solution: High Resolution and Automated Imaging

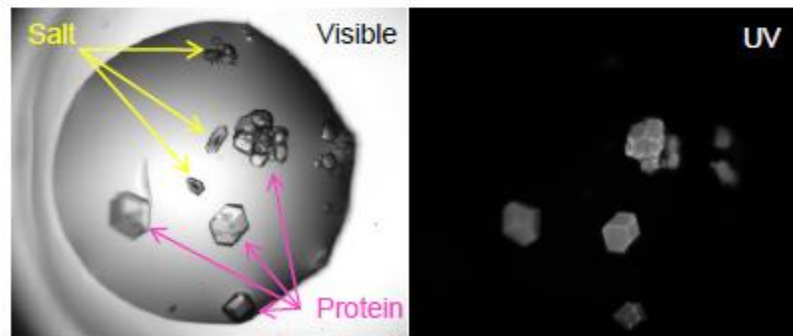
- We implemented 3 types of objectives in our new imager:



(a)



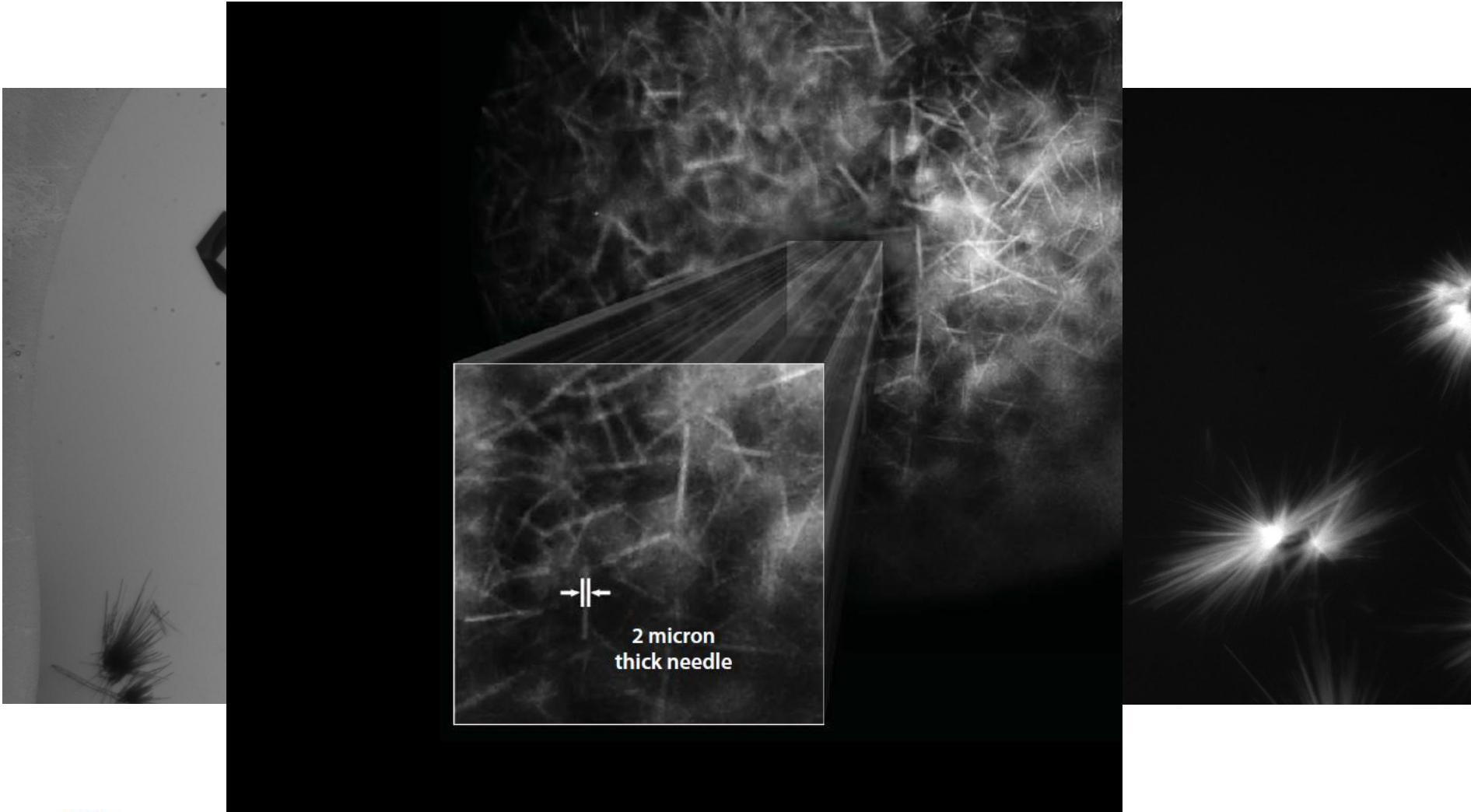
(b)



(c)

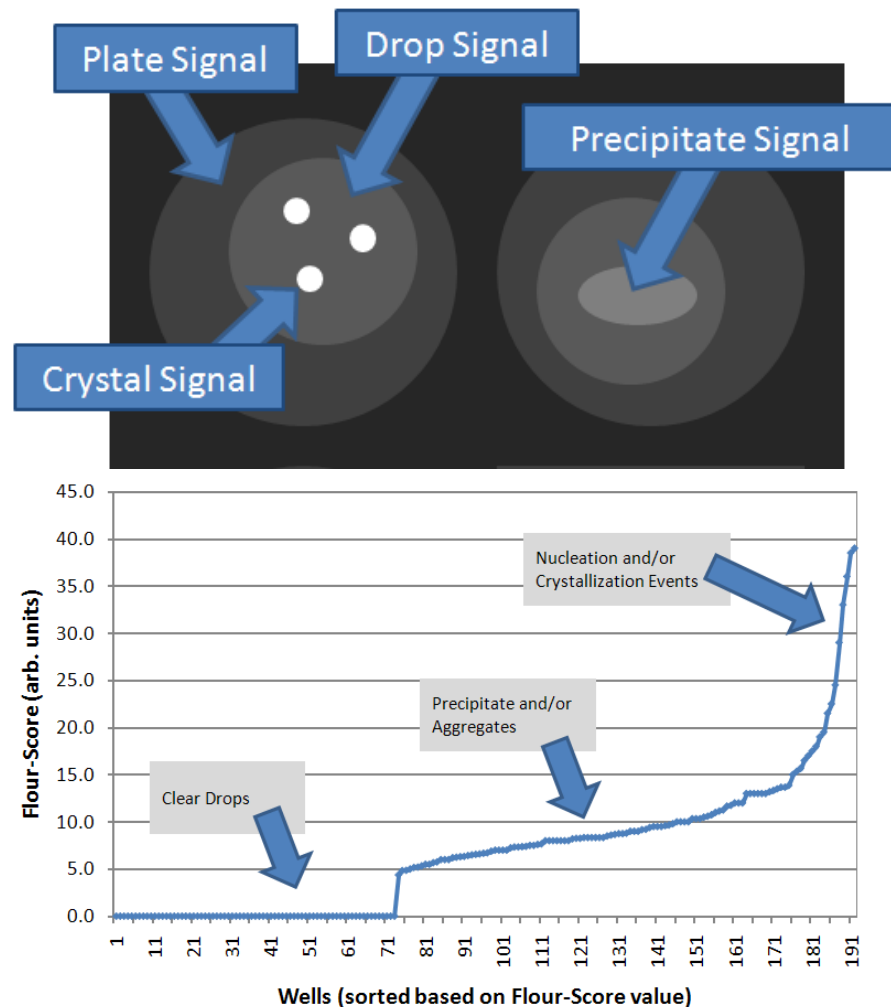


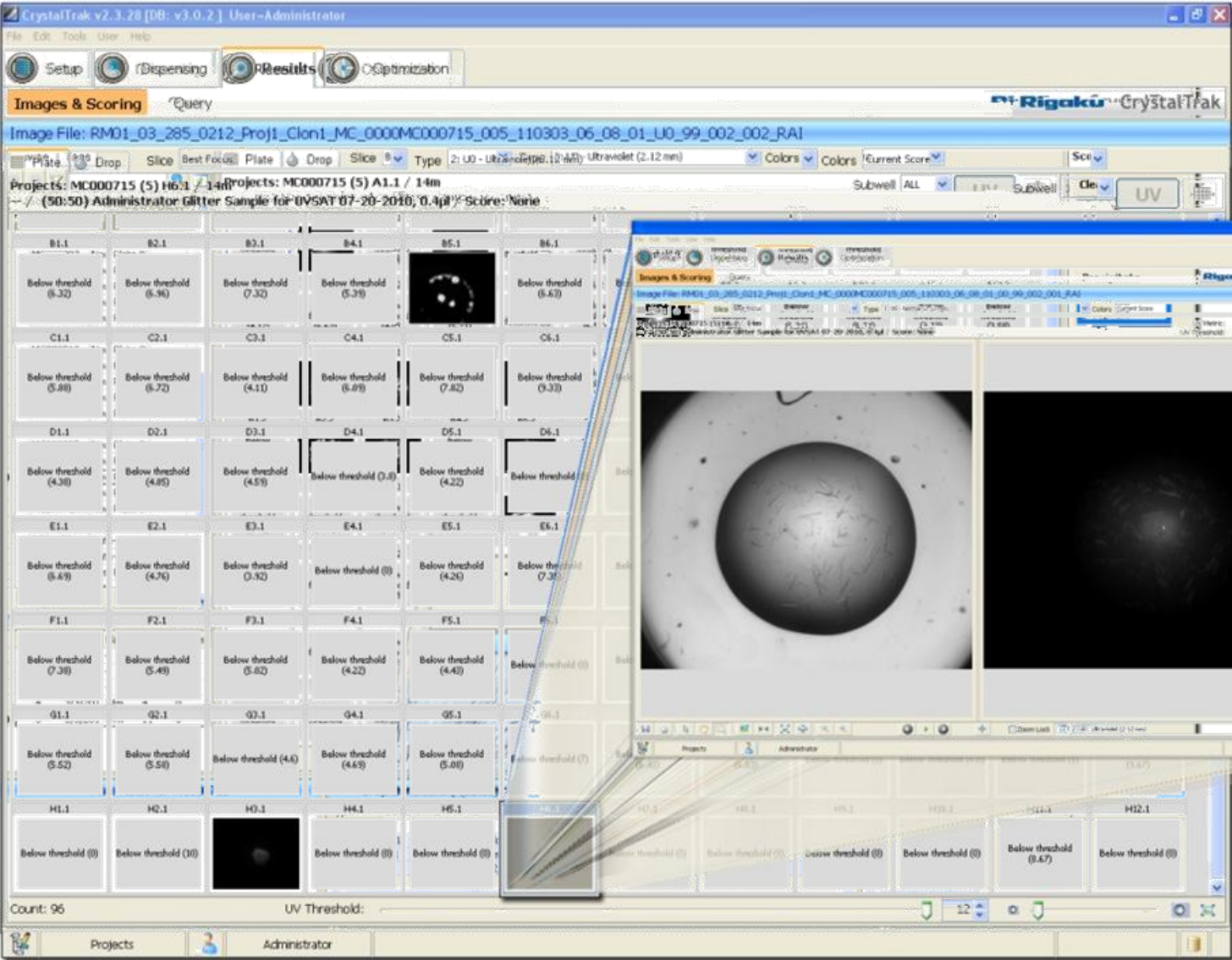
The Result



Introducing Fluor-Score: UV based auto-scoring

- Issue with conventional image analysis approaches:
False negatives (missed crystals)
- Fluor-Score focuses on detecting *clear* drops
- Score = Regional variations of fluorescence signal

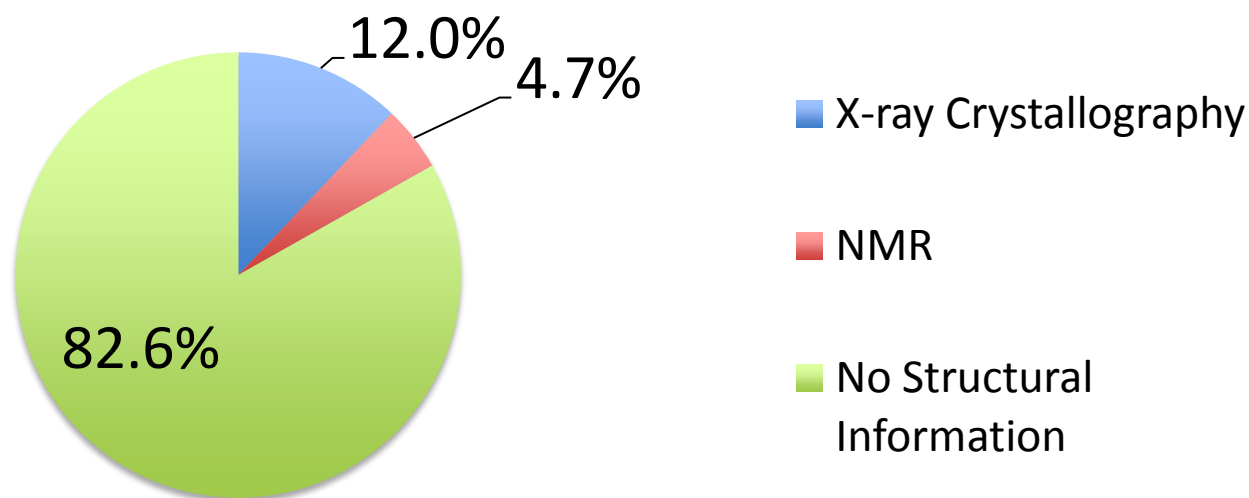




BioSAXS

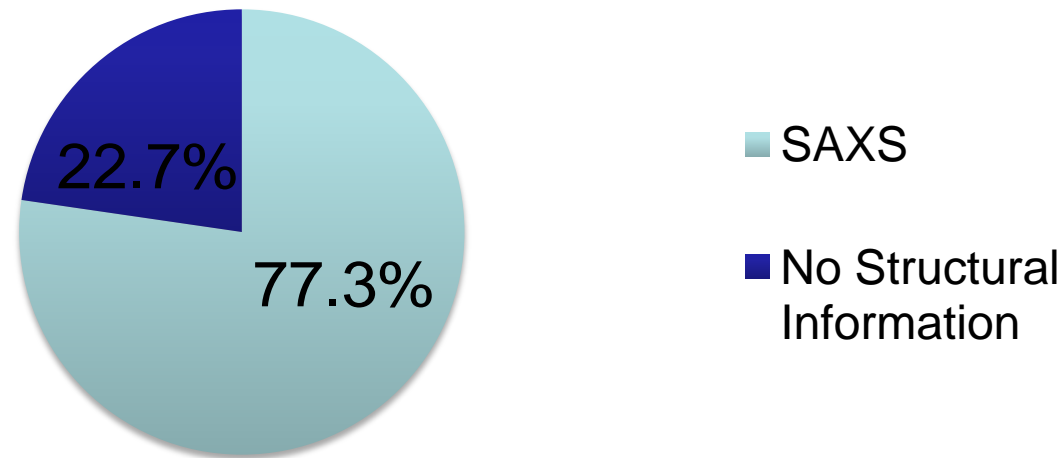
A Complementary Technique

High Throughput Structure Success



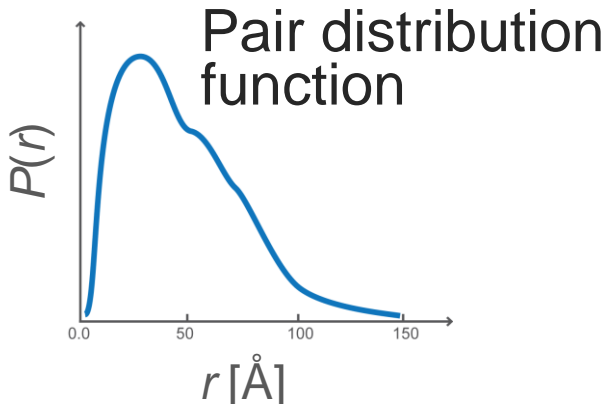
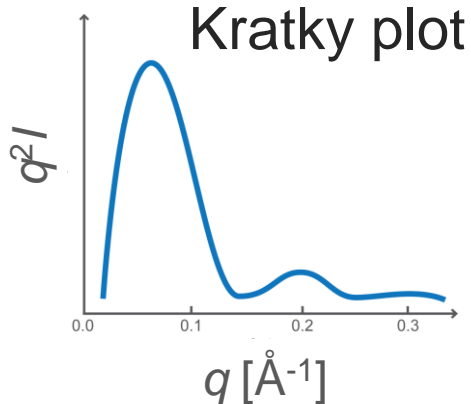
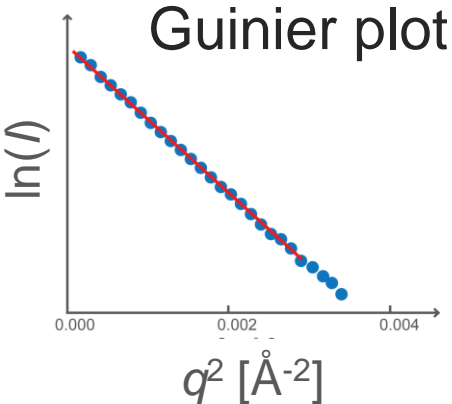
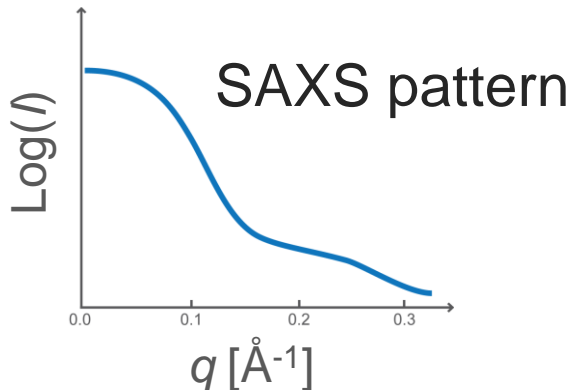
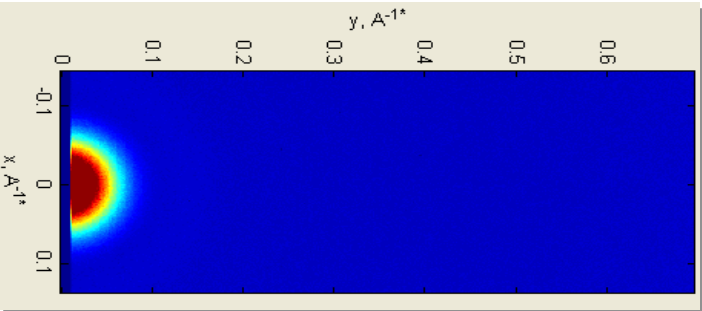
Northeast Structural Genomics Consortium

High Throughput SAXS Success



Northeast Structural Genomics Consortium

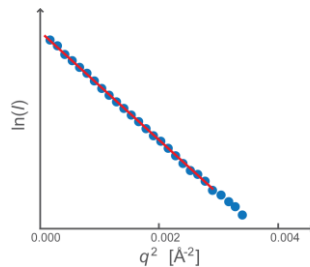
Profile conversion



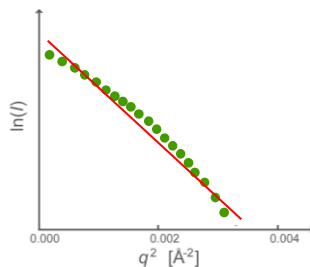
Biological applications of SAXS

- Predictor for crystallizability
- *Ab initio* shape determination of native protein state
- Monitoring conformational changes
- Ligand binding studies
- Characterization of oligomeric states and missing loops/residues
- Differentiation of monodisperse and aggregated proteins in solution
- Differentiation of folded and unfolded protein in solution
- Long distance constraint in NMR protein structure refinement

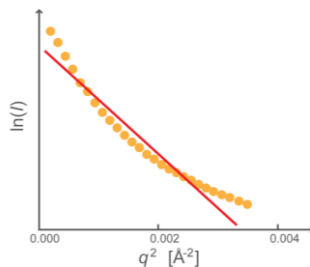
Predictor of Protein Crystallizability



Too Soluble

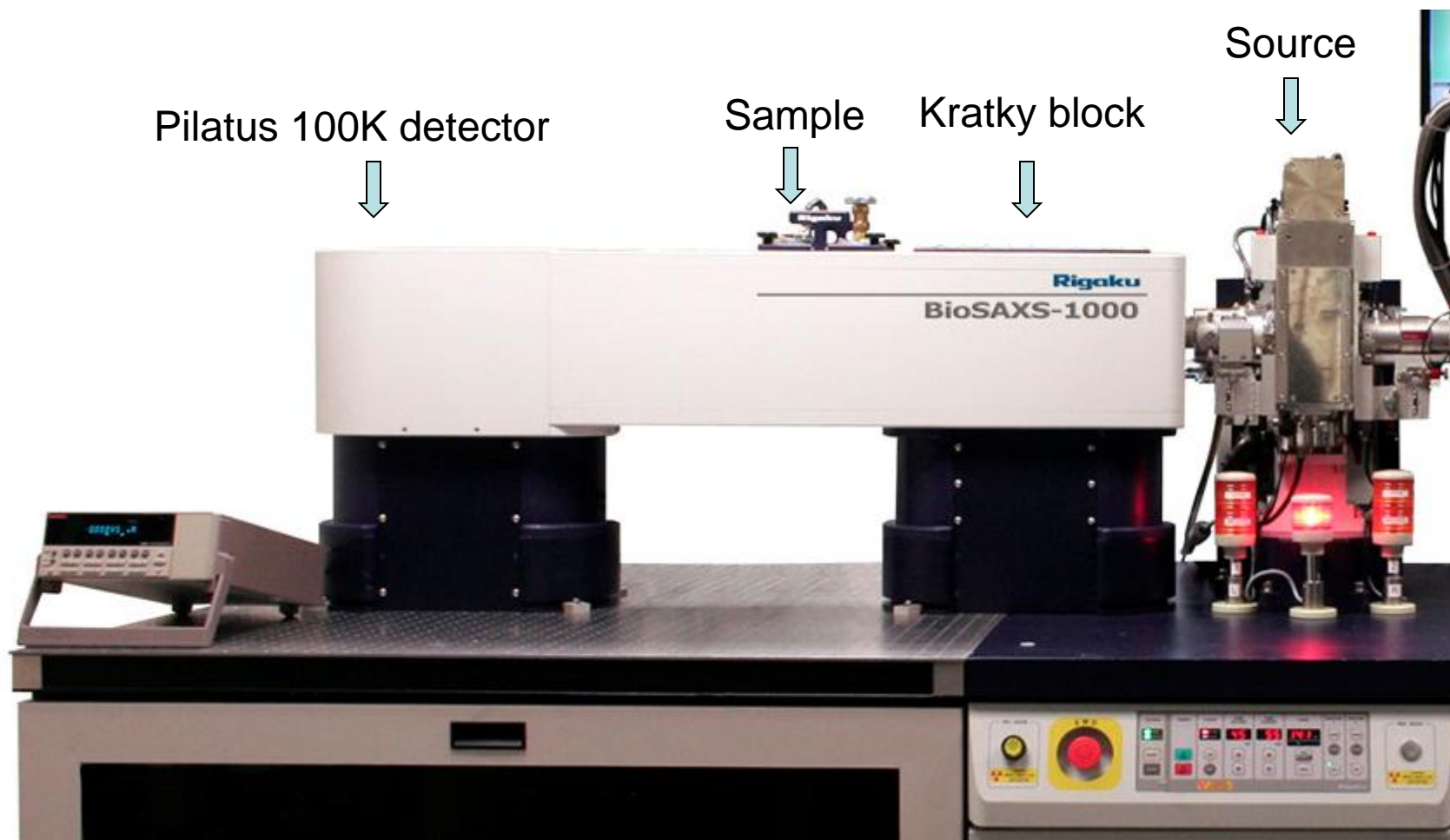


“Crystallization Slot”

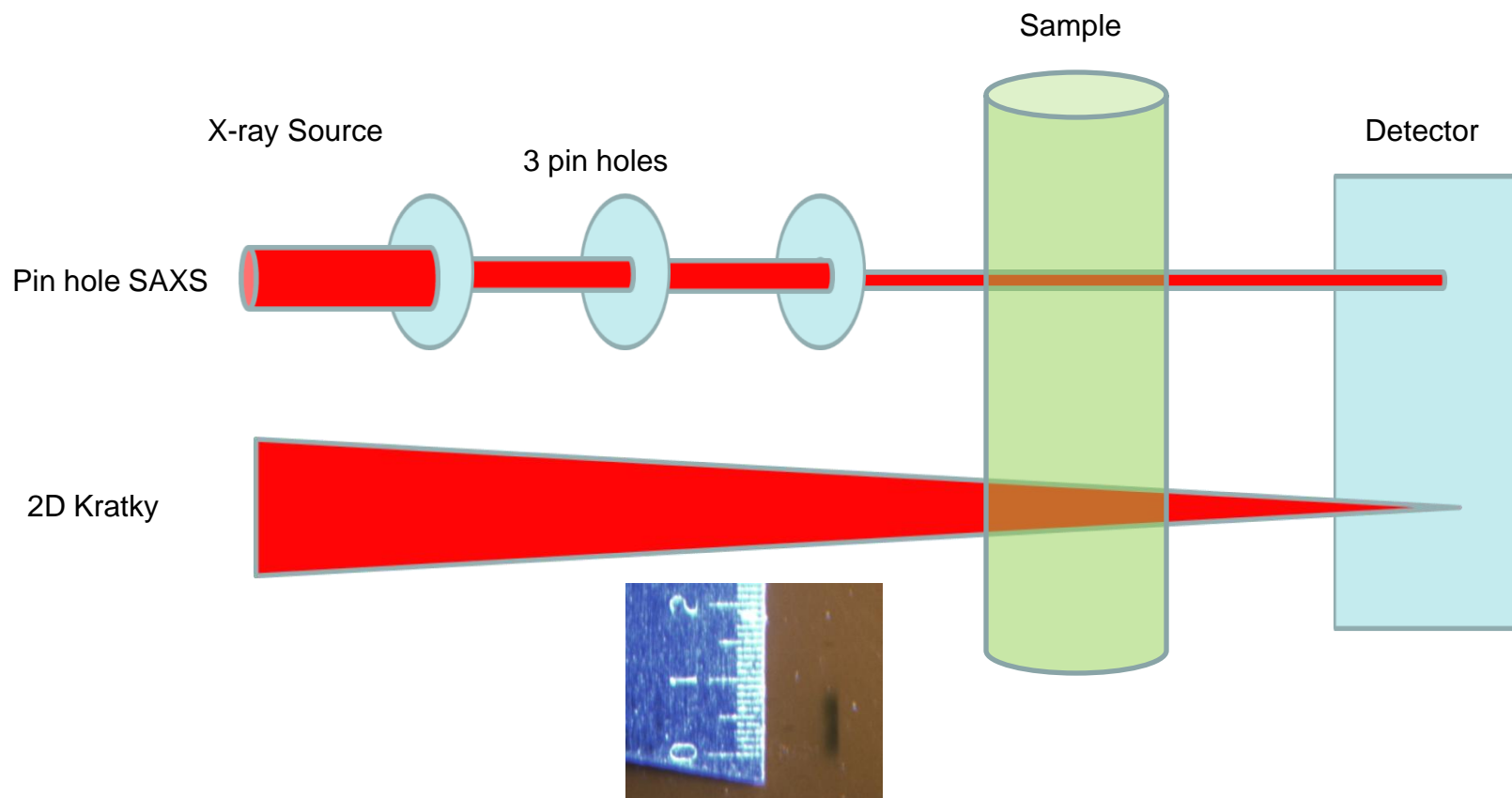


Amorphous Precipitate

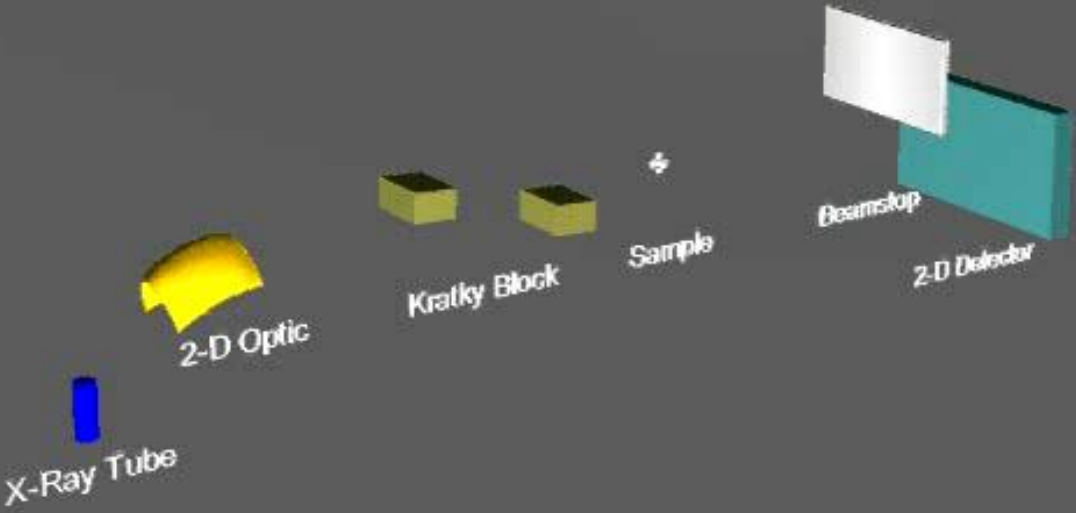
BioSAXS-1000



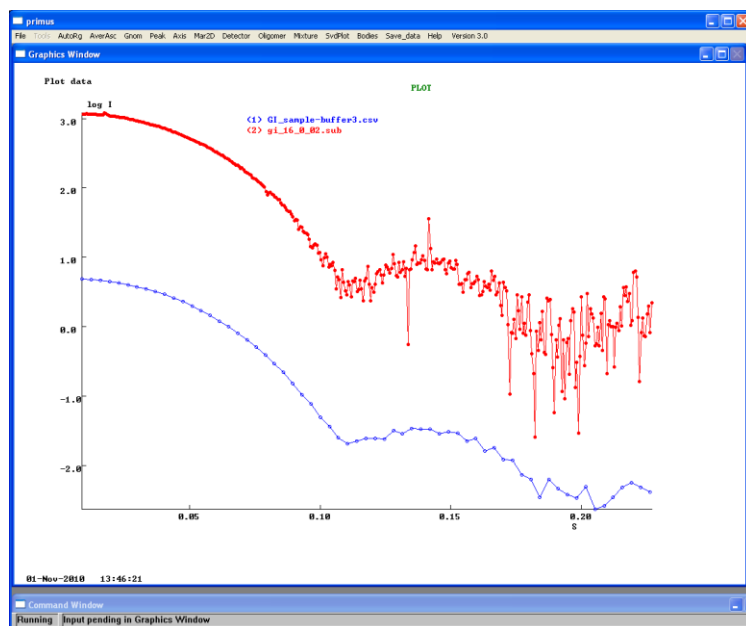
BioSAXS-1000 optic design



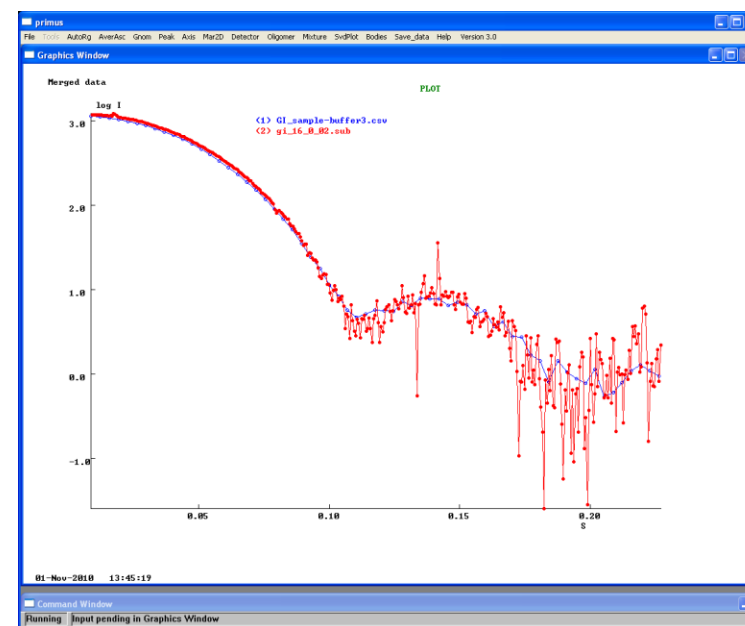
Rigaku 2-D SAXS Geometry



BioSAXS-1000 and Synchrotron Data Quality Comparison



Before scaling

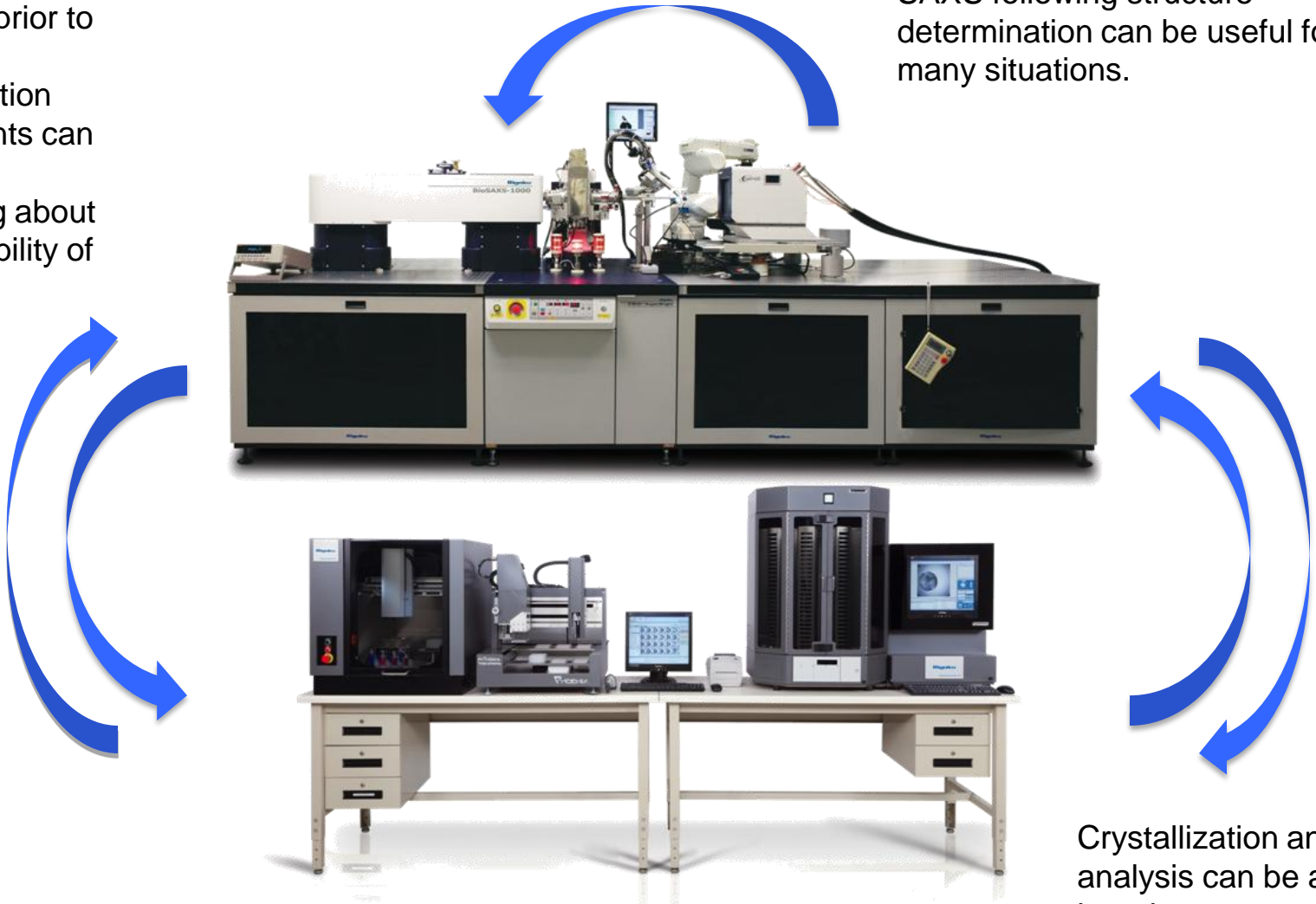


After scaling

Comparison data: BL4-2 at **SSRL (red)** and **BioSAXS-1000 (blue)**
SSRL data courtesy of T. Grant, J. Luft and E. Snell (HWI)

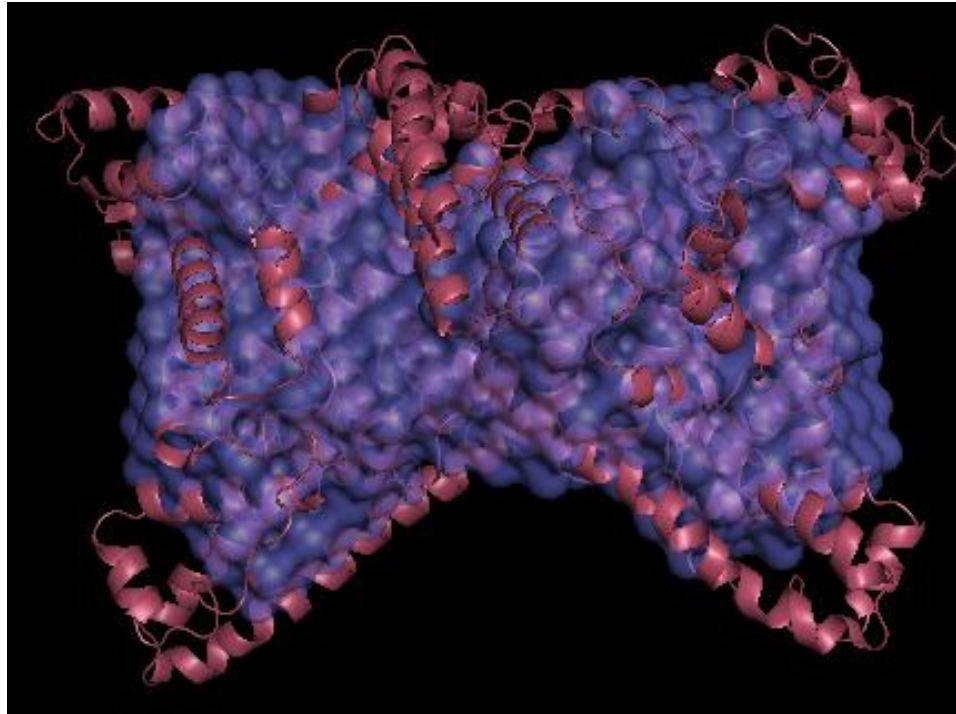
SAXS data collected prior to setting up crystallization experiments can tell you something about the probability of success.

SAXS following structure determination can be useful for many situations.



Crystallization and X-ray analysis can be an iterative process.

Thank You



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