Increment 57/58 Science Symposium

Light Microscopy Module Biophysics -2 (LMM-B2)

Presented by:

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LMM-B2

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Research Opportunities in Complex Fluids and Macromolecular Biophysics

Solution Convection and the Nucleation Precursors in Protein Crystallization

- Experiments to be performed on the LMM.
- Experiments explore the effects of an unstudied factor for the nucleation of protein crystals: solution shear flow.
- Benchmarking against data obtained at zero flow rates, only possible in space.
- Methods of detecting and monitoring protein aggregates leading to nucleation require experiments in solution layers significantly thicker than 100μm.
- Launch scheduled for June 28th 2018 on SpaceX-15.

Dr. Peter Vekilov [NASA Team: UH] PI / Provide flight samples, science requirements, and data analysis	Departments of Chemical and Biomedical Engineering University of Houston Houston, TX 77004-2610 Tel: 713-743-4315; vekilov@uh.edu
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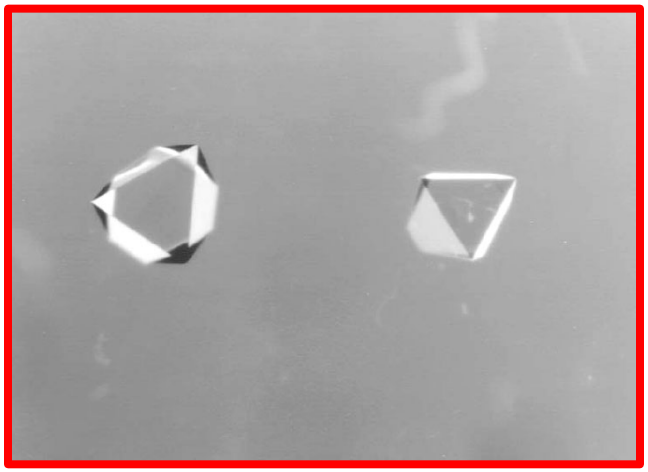
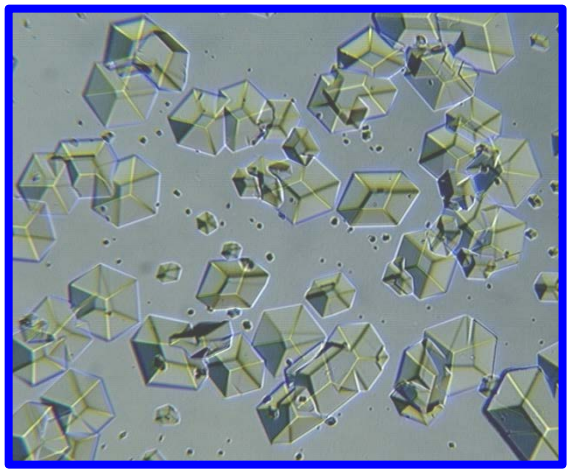
Light Microscopy Module Biophysics -2 (LMM-B2)

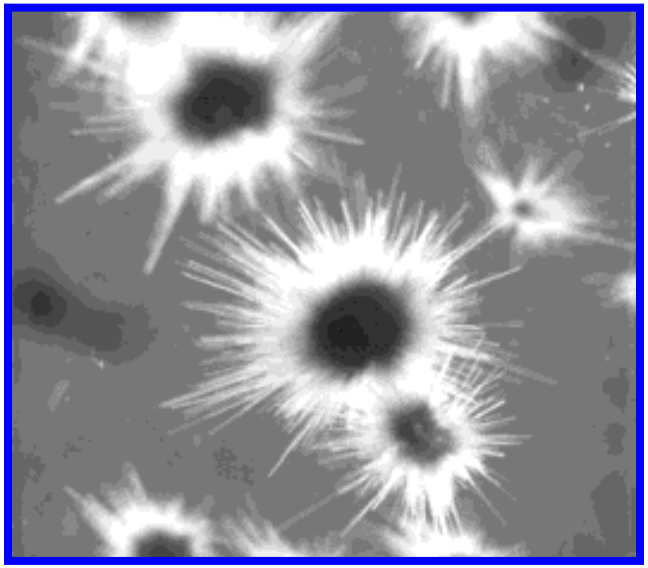
- Science Background and Hypothesis
- Investigation goals and objectives
- Measurement approach
- Importance and reason for ISS
- Expected results and how they will advance the field

Science Background and Hypothesis – 1/3

Nucleation Determines …







- ✓ Number
- ✓ Polymorph
- ✓ Morphology
- ✓ Habit
- ✓ Size
- ✓ Size distribution

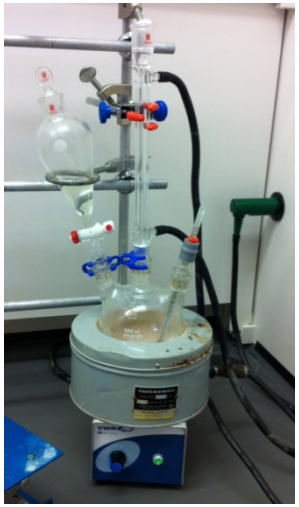
Requires data on:

- ✓ Solution PChem
  - Phase diagrams
  - Metastable states
- ✓ **Nucleation mechanisms**
- ✓ Growth mechanisms
- ✓ Agglomeration

Science Background and Hypothesis – 2/3

Problem: How Does Solution Convection Affect Nucleation?

- Industrial crystallization



Transfer from laboratory (volume = 1 L) to production reactors (1000 L)

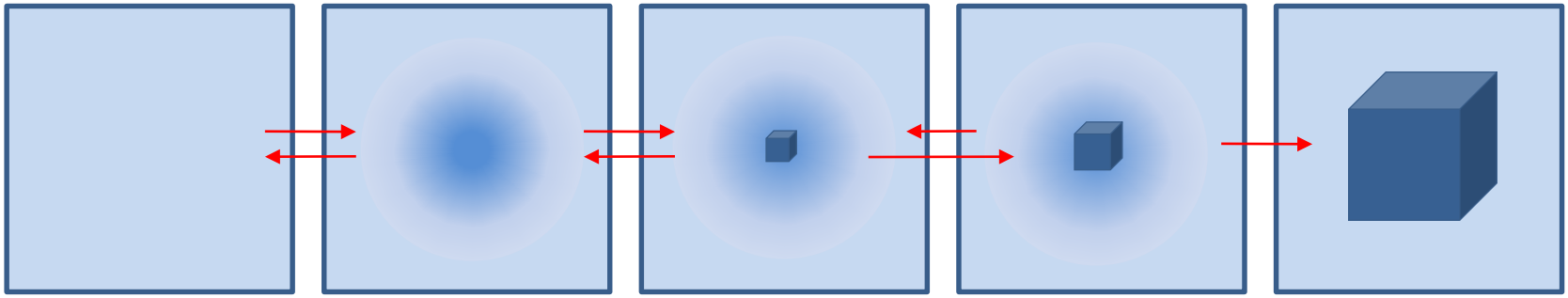
polymorph shift
number of crystals
time of nucleation
size distribution

nucleation proceeds differently

Solution flow patterns: flow rate O(cm s^{-1}), shear rate O(10 s^{-1})

Science Background and Hypothesis – 3/3

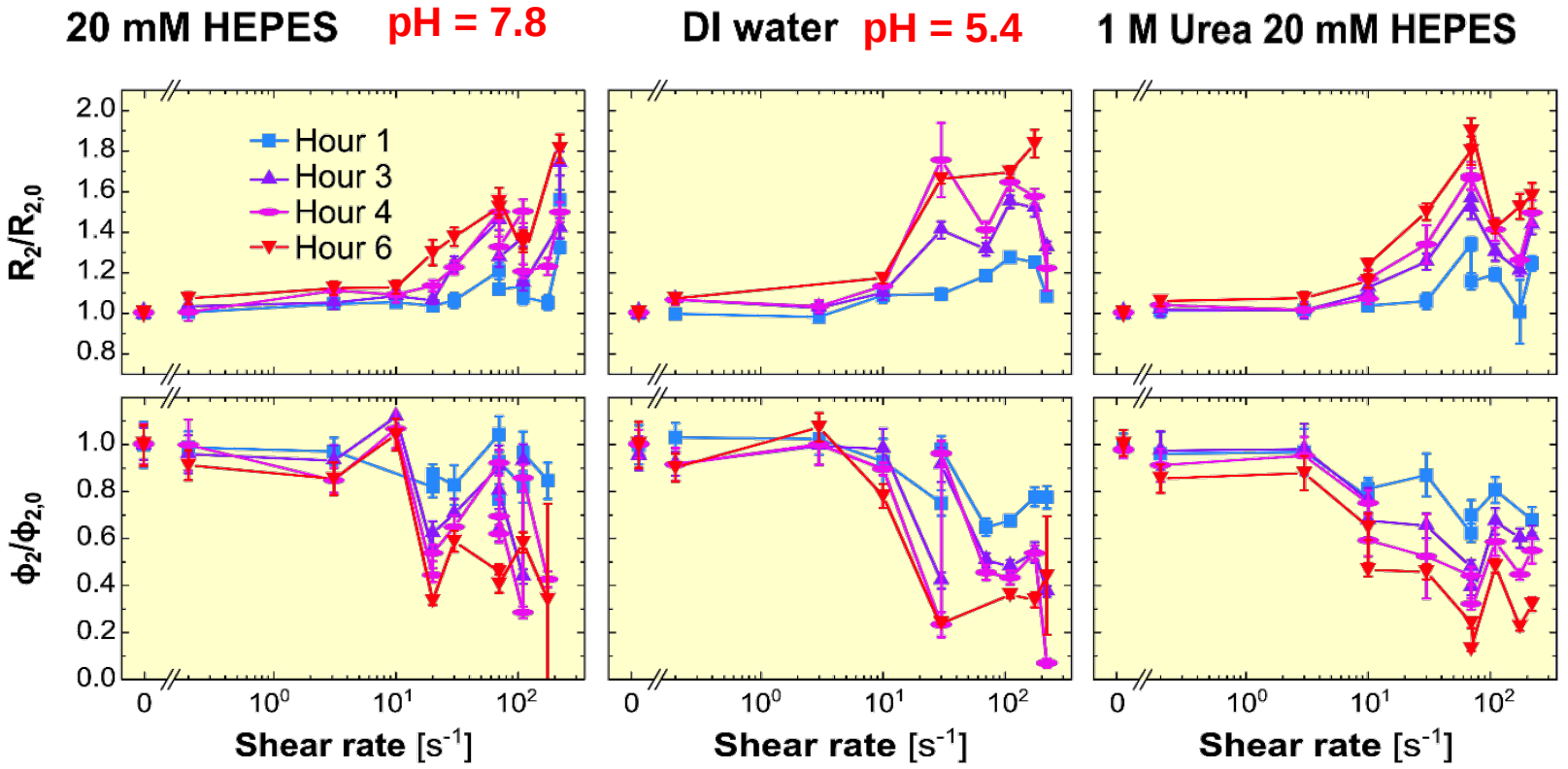
The Two-step Nucleation Mechanism



Galkin, O.; Vekilov, P. G.
Proc. Natl. Acad. Sci. USA **2000**, *97*, 6277-6281
Vekilov, P. G.
Crystal Growth & Design **2004**, *4*, 671
Vekilov, P. G.
Crystal Growth & Design **2010**, *10*, 5007

LMM-B2 Investigation Goals and Objectives

Effects of Shearing on the Cluster Population



- Enhanced intramolecular repulsion at pH = 5.4 (Z ↗ from 7 to 10)
- Destabilization of protein conformation by urea
  - lower threshold for shear effects
  - enhance shear effects

➢ **Destabilization of protein conformation is a part of the cluster mechanism**

LMM-B2 Measurement approach

We will be using a flight-hardened Commercial-Off-The-Shelf (COTS) microscope

[pictured on next page]

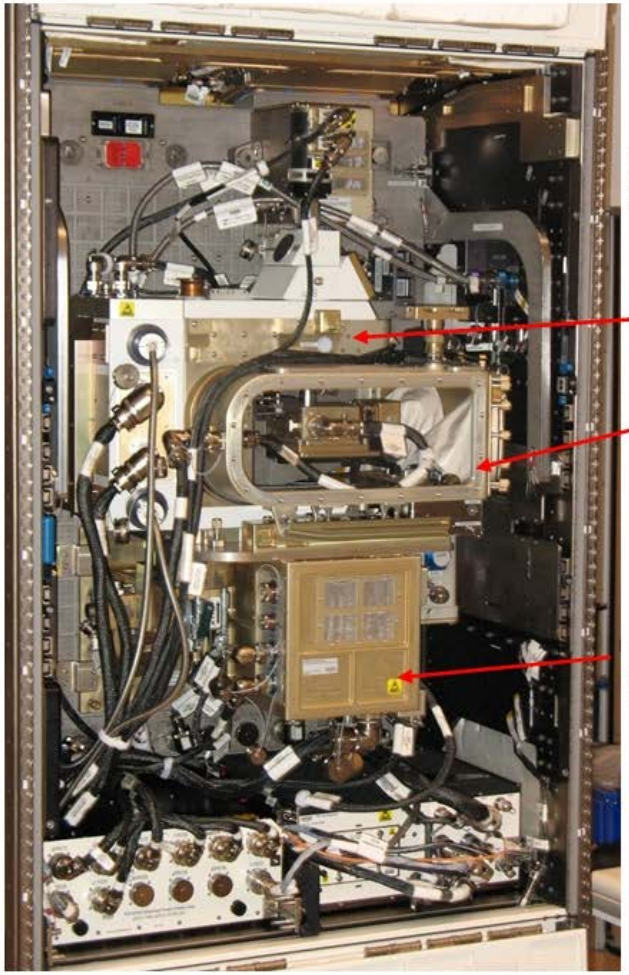
and a

Macromolecular Biophysics sample module

[pictured later]

Measurement approach – 1/5

Light Microscopy Module (LMM) in the Fluid Integrated Rack (FIR)



LMM in the Closed Position or Operating Configuration

LMM in the Open Position or Installation/Service Configuration

Measurement approach – 2/5

LMM Implementation Philosophy

Philosophy: Maximize the scientific results by utilizing the existing LMM capabilities. Develop small sample modules and image them within the LMM

Payload specific and multi-user hardware customizes the FIR in a unique laboratory configuration to perform research effectively.

Light Microscopy Module

Payload Specific Hardware

- Sample Cell with universal Sample Tray
- Specific Diagnostics
- Specific Imaging
- Fluid Containment

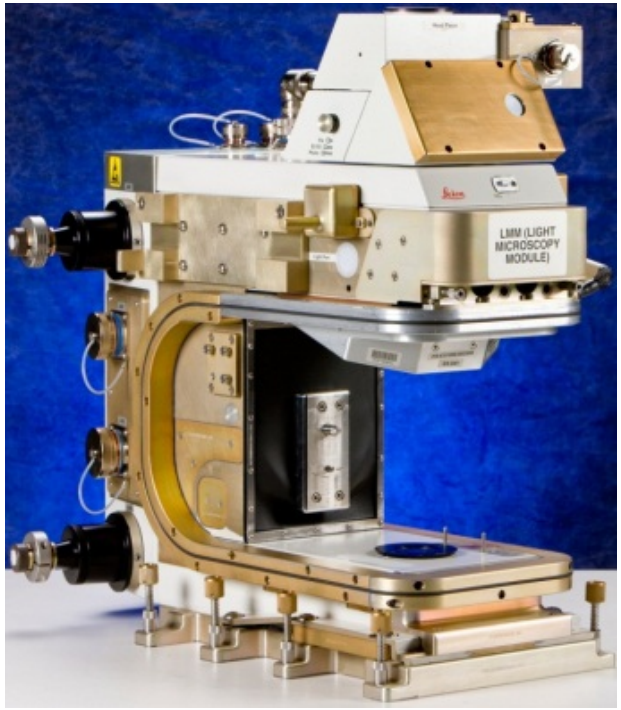
Multi-Use Payload Apparatus

- Test Specific Module
- Infrastructure that uniquely meets the needs of PI experiments
- Unique Diagnostics
- Specialized Imaging
- Fluid Containment

FCF Fluids Integrated Rack

- Power Supply
- Avionics/Control
- Common Illumination
- PI Integration Optics Bench
- Imaging and Frame Capture
- Diagnostics
- Environmental Control
- Data Processing/Storage
- Light Containment
- Active Rack Isolation System (ARIS)

Measurement approach – 3/5

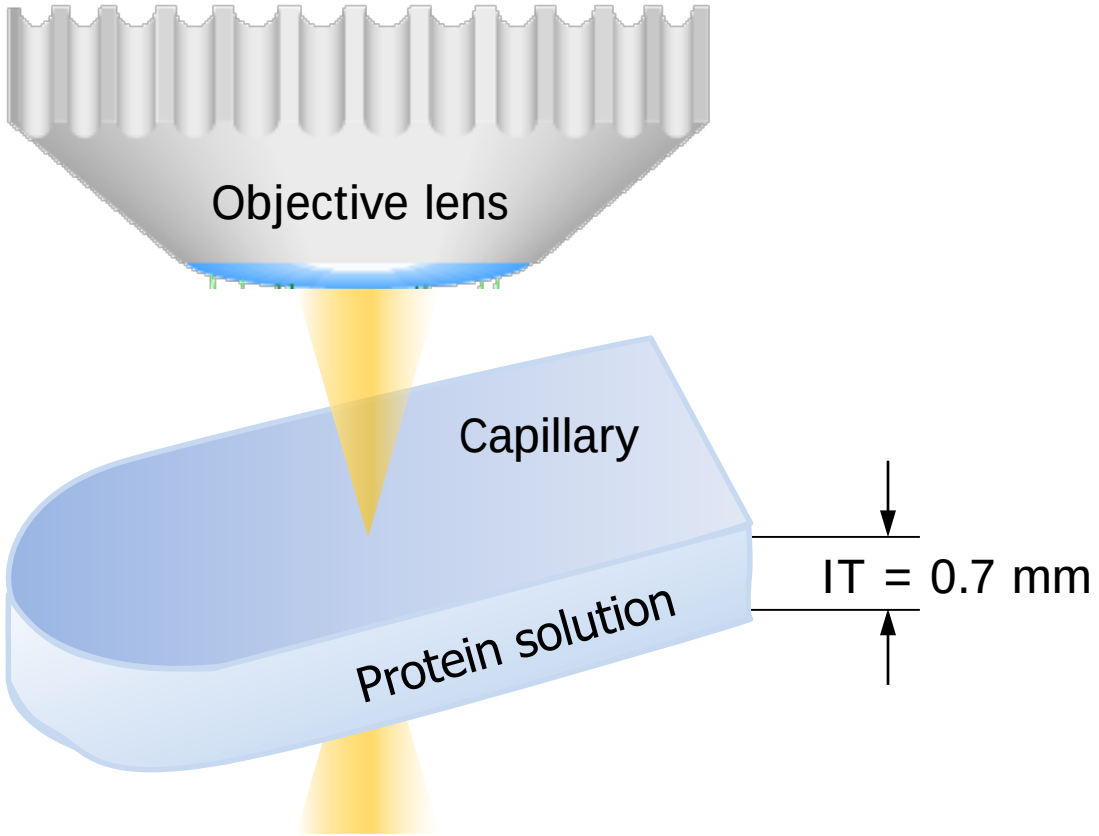


Light Microscopy Module (LMM)

LMM-B1 Sample Assembly Prototype that will contain up to 8 square 100mm capillaries

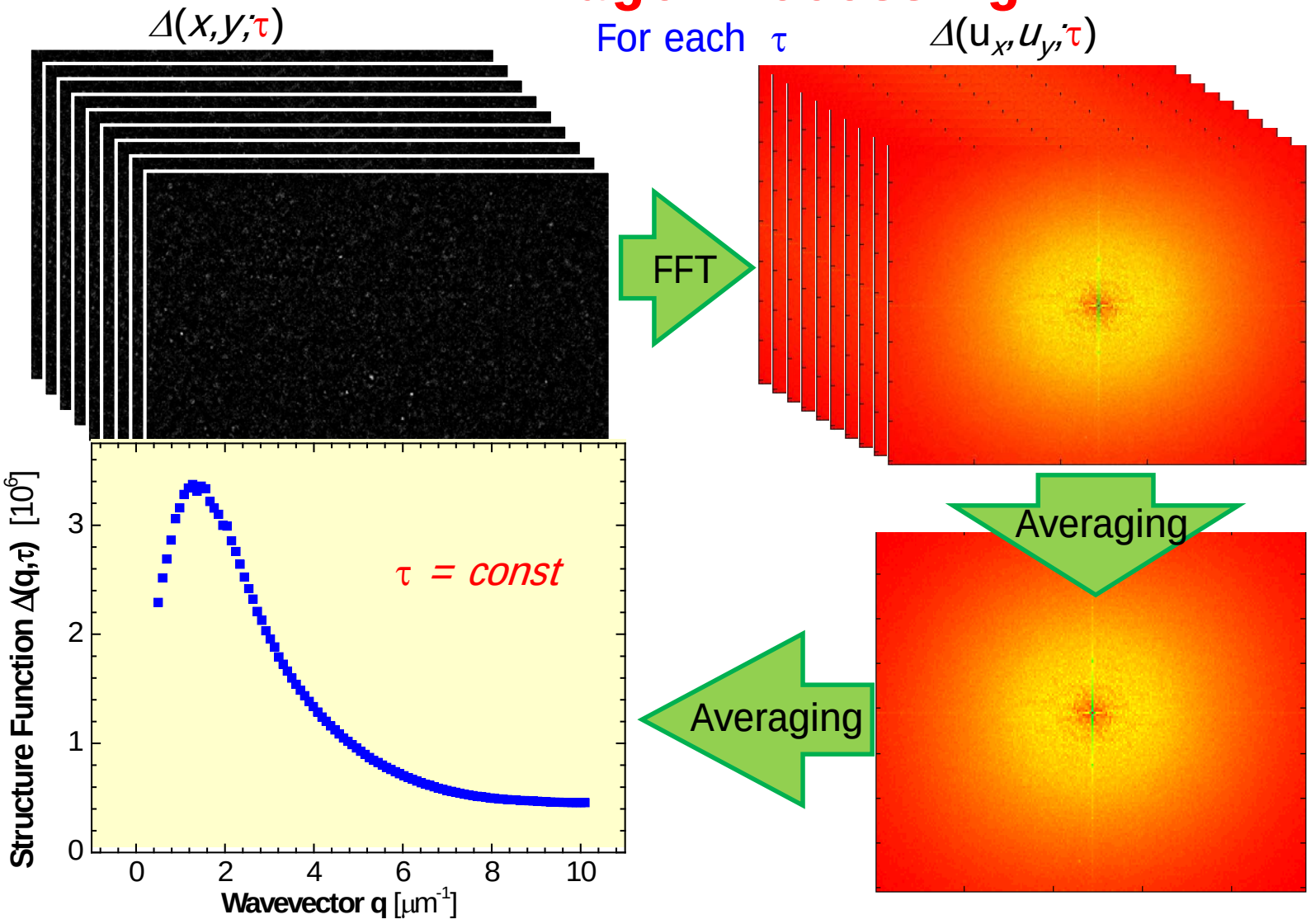
Measurement approach – 4/5

Schematic of DDM Implementation on LMM

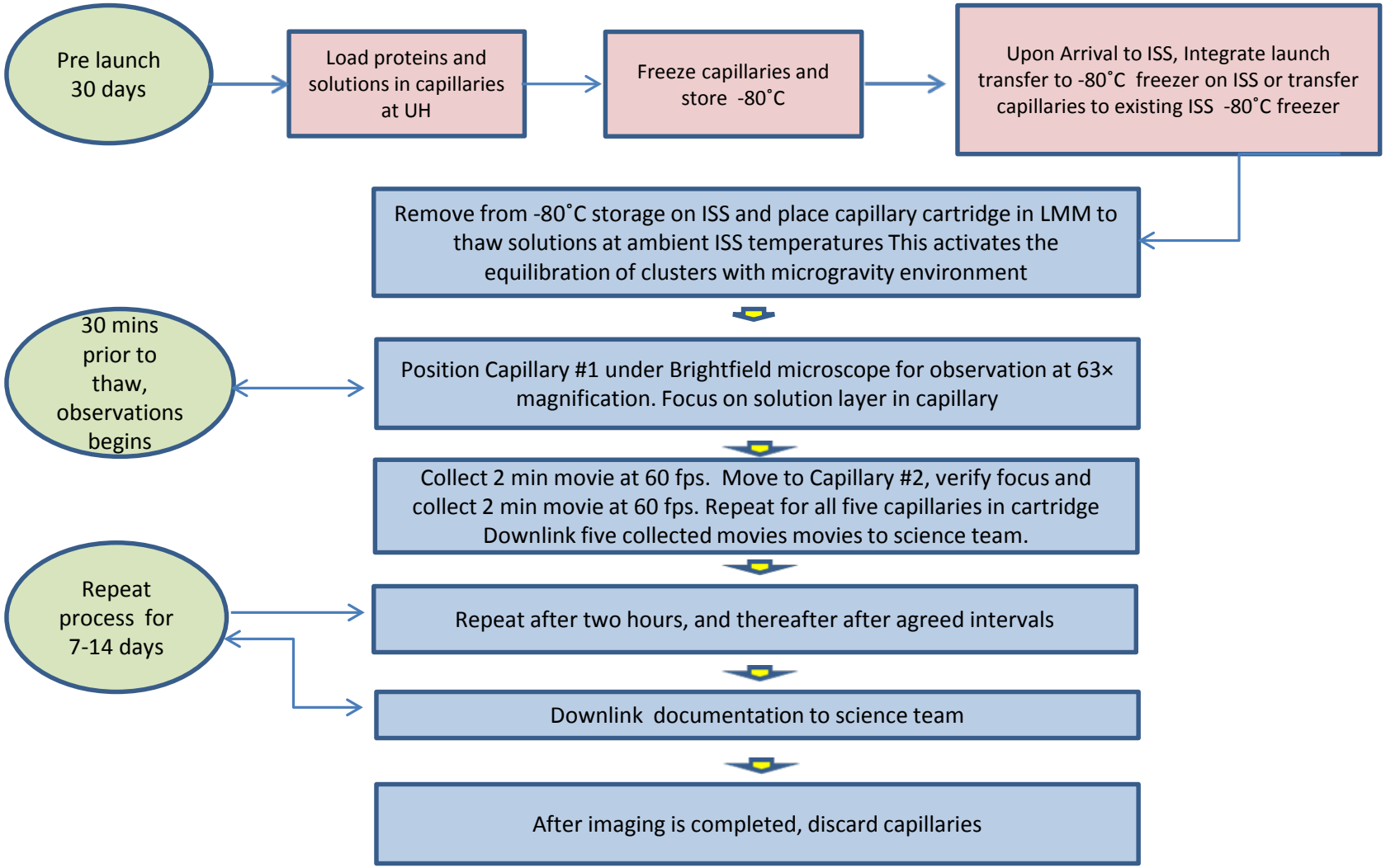


Measurement approach – 6/6

DDM Image Processing



- Experimental Procedure for Capillary Observation and Photo Documentation on ISS



The Need for Microgravity Experiments

- Nucleation determines many crucial properties of crystal populations
- Solution flows may strongly affect crystal nucleation rates
- Current theories do not provide understanding of solution flow effects
- **Fundamental understanding of flow effects on crystal nucleation needed**
- We need new data that will suggest novel mechanisms of flow effects
- Shear rate to be probed: from **0** to 30 s^{-1}
- Flow rate to be probed: from **0** to 300 $\mu m\ s^{-1}$
- Why do it with proteins?
  - Proteins provide a set of issues related to flow effects on nucleation
  - Proteins are an excellent model system for nucleation studies

Expected results and how they will advance the field

Gathering of knowledge eventually leading to an understanding of physical mechanisms of nucleation and subsequent growth is a long-standing endeavor. These sets of experiments will provided sufficient data to determine the role of shear in the nucleation process.

Proteins to be investigated are: Lysozyme (standard), Adult human hemoglobin and P53 (which is involved in fighting cancer in the body). Details of the role and mechanism are complicated and change with denaturation. In addition to protein crystal nucleation, denaturation may also result in fibril formation that can be dependent on shear formation.