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# Testing commercial sample-mounting loops for movement in the cold-stream

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*This work performed in collaboration with F. J. Rotella and N. E. C. Duke*

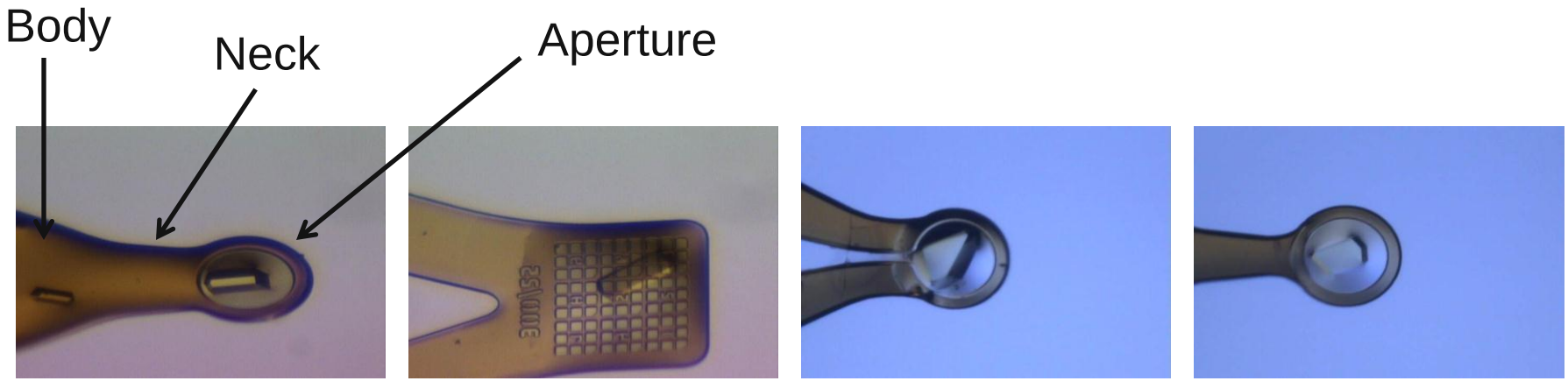
# Goals

- The object is to quantitatively measure the stiffness of commercial sample mounting loops and determine if they are moving under the influence of the cold-stream

- When completed, measure real protein samples and see if loop movement is present during normal data collection

- The loops under study are:
  - Mitegen
    - *Microloop*
    - *MicroMesh*
    - *Microloop HT*
    - *Dual Thickness Microloop*
    - *Microloop LD*
  - Hampton Cryoloop
  - Litholoop and Litholoop Mesh

# *Loops under study*

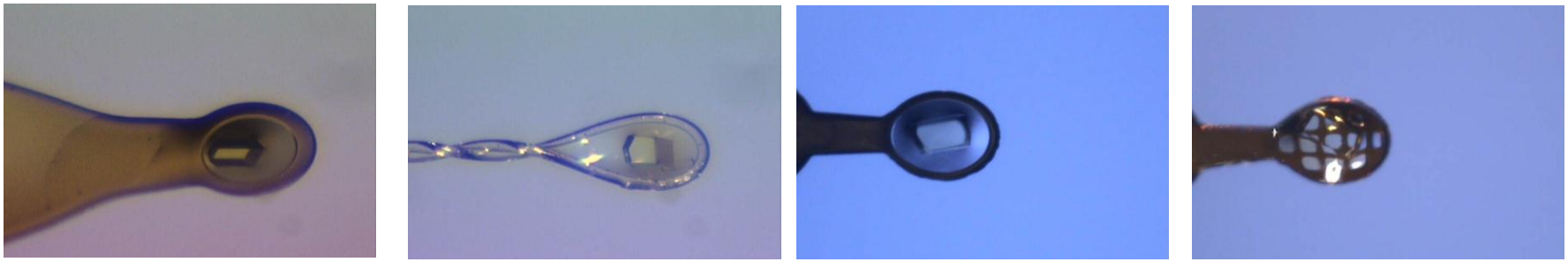



Mitegen
Microloop HT

Mitegen MicroMount
MicroMesh

Mitegen Dual
Thickness MicroMount

Mitegen
Microloop LD



Mitegen Microloop

Hampton Cryoloop

Litholoop

Litholoop Mesh

# Loop characteristics

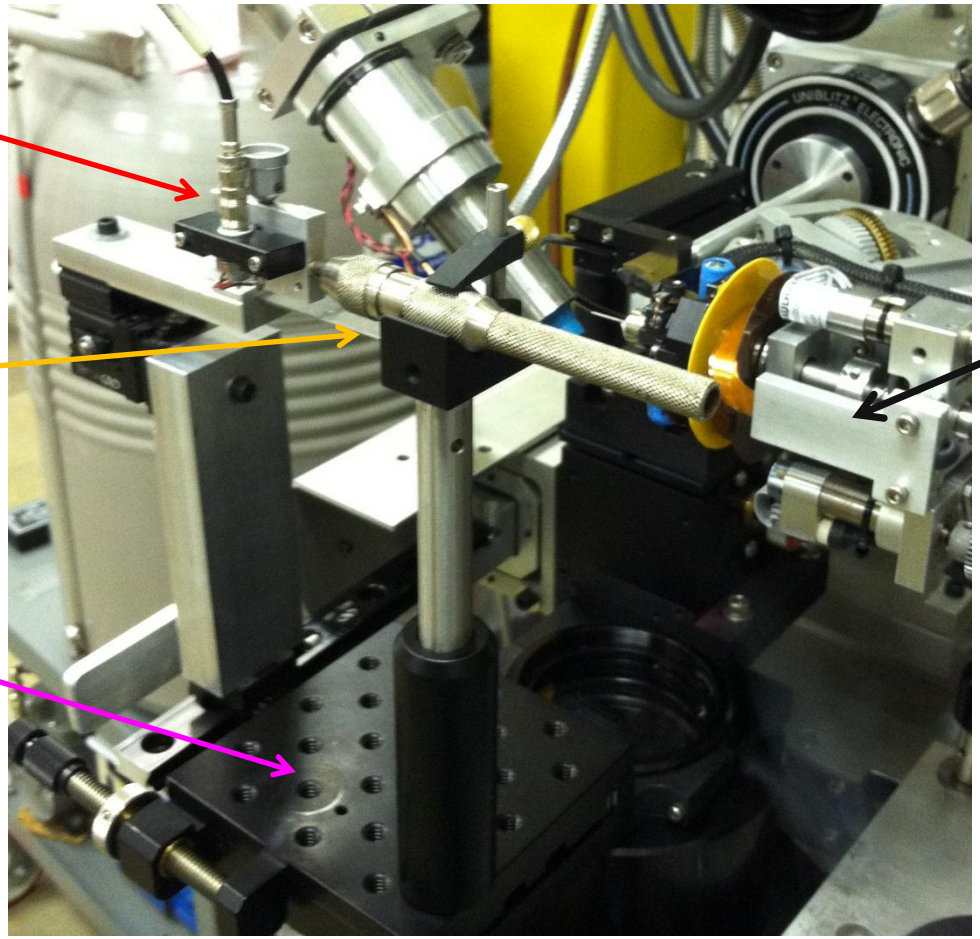
| Manufacturer | Aperture Thickness (µm) | Body Thickness (µm) | Neck/Body Width (µm) | Aperture Opening (µm) |
|---|---|---|---|---|
| Mitegen Microloop | 10 | 10 | 200/730 | 200 |
| Mitegen MicroMesh | 10 | 10 | 375/730 | 300 |
| Mitegen Microloop HT | 18 | 18 | 200/800 | 200 |
| Mitegen Dual Thickness (DT) MicroMount | 10 | 25 | 200/730 | 200 |
| Mitegen Microloop LD | 10 | 25 | 100/730 | 200 |
| Hampton Cryoloop | 20 | 20 | 40/40 | oval 100x200 |
| Mol. Dimensions Litholoop | 25 | 25 | 80/350 | 200 |
| Mol. Dimensions Litholoop Mesh | 25 | 25 | 80/350 | 200 |

# *Methods*

- Position cold-stream: Coplanar, 42° angle, facing the sample; offset 1.5mm vertically to center gas temperature profile on sample
- Sample loops all have ~0.2mm diameter apertures
- Attach Si crystal cubes, 0.2mm on edge, to loops using Apiezon-T grease
- Align loop with desired orientation facing the cold-stream

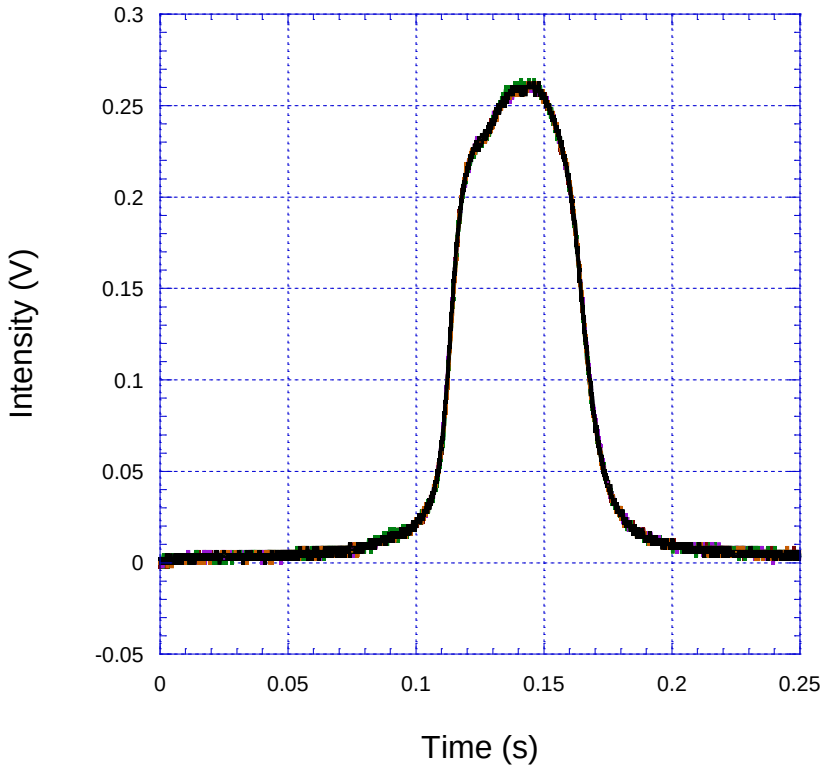
- Measure the Si(220) reflection
  - Find reflection in rotation angle and direction using CCD detector
  - Measure using unbiased photodiode with output sent directly to Tektronix oscilloscope
  - Timing trigger established by goniometer Heidenhain encoder: 180,000ct/deg
  - Perform repeat scans and determine standard deviations for angular and intensity measurement sets; all scans performed at 1sec/deg rate
  - Measure each loop-set a minimum of 12 times

# *Photodiode setup*



# *Instrumental baseline*

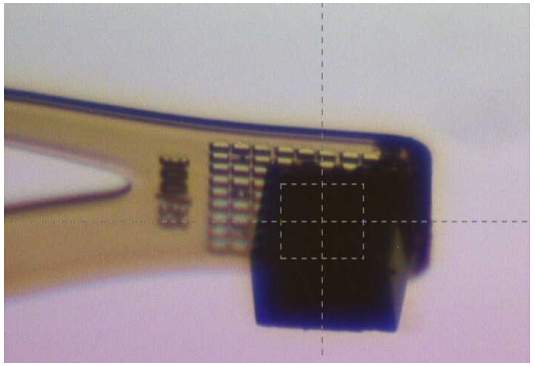
- To determine instrumental (no loop) baseline
  - Use Si cube mounted via epoxy to SS pin

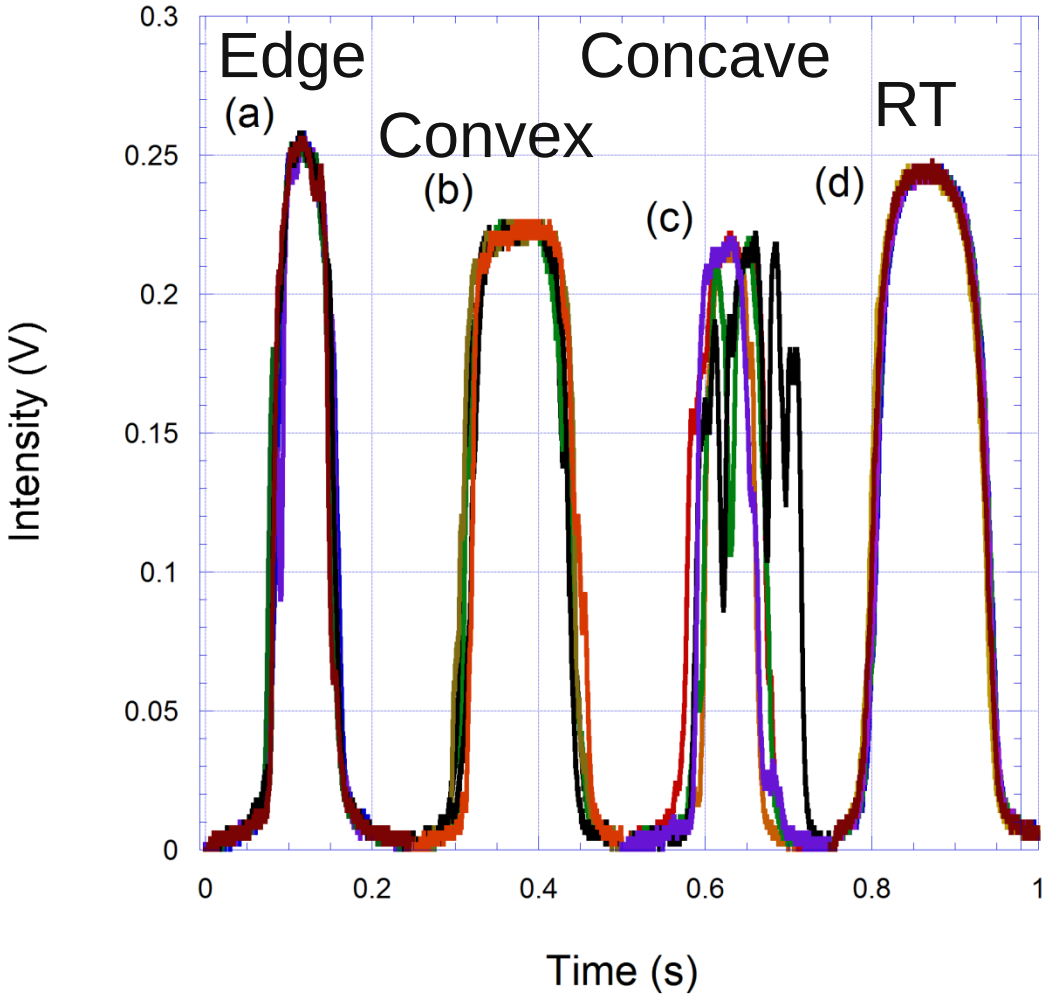



Six scans
RMS (pos) = 0.09mdeg
σ(I)/(I)% = 0.16

# *Mitegen MicroMesh, grease mount*

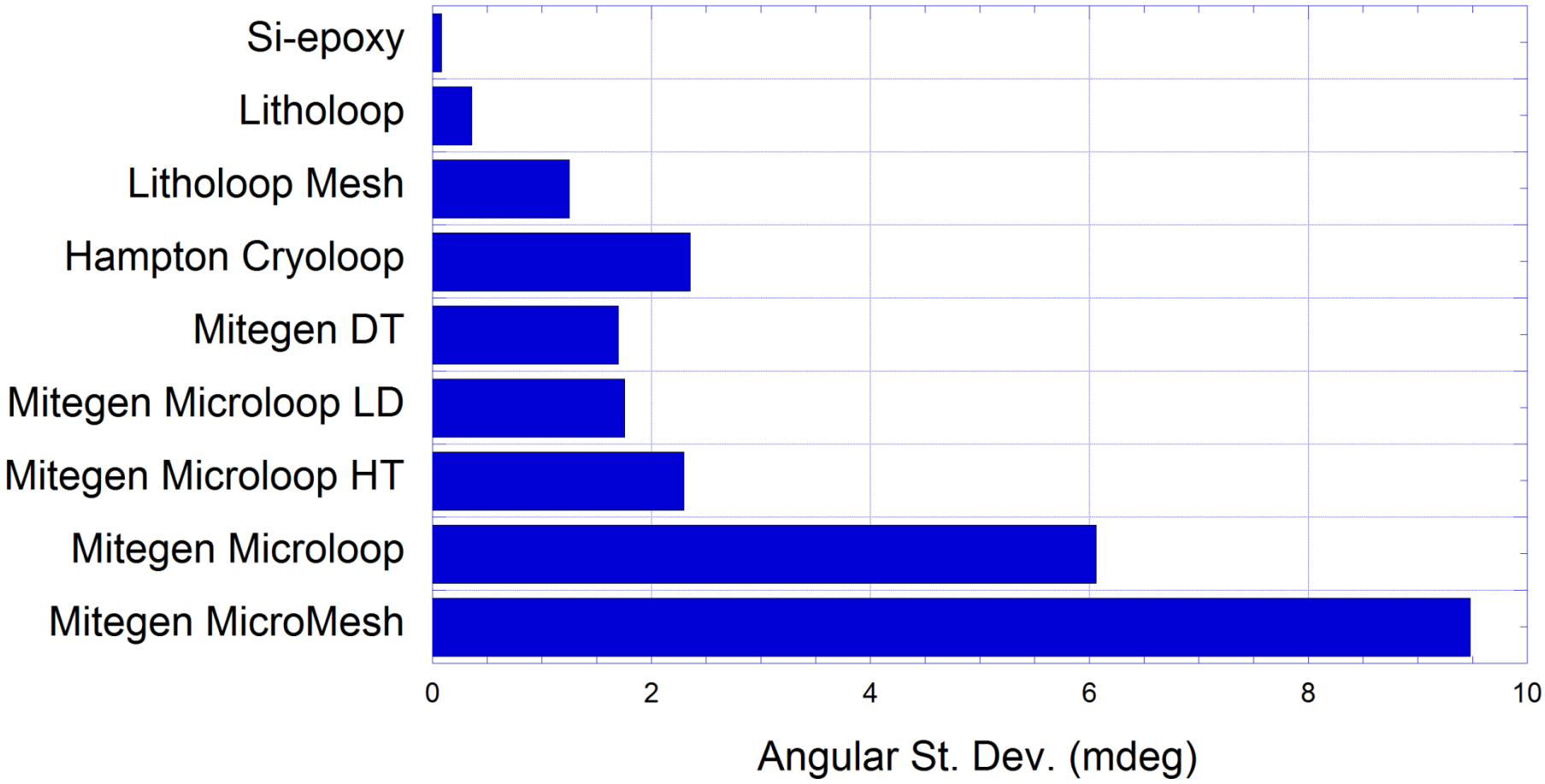
Think of Mitegen design like a fountain pen



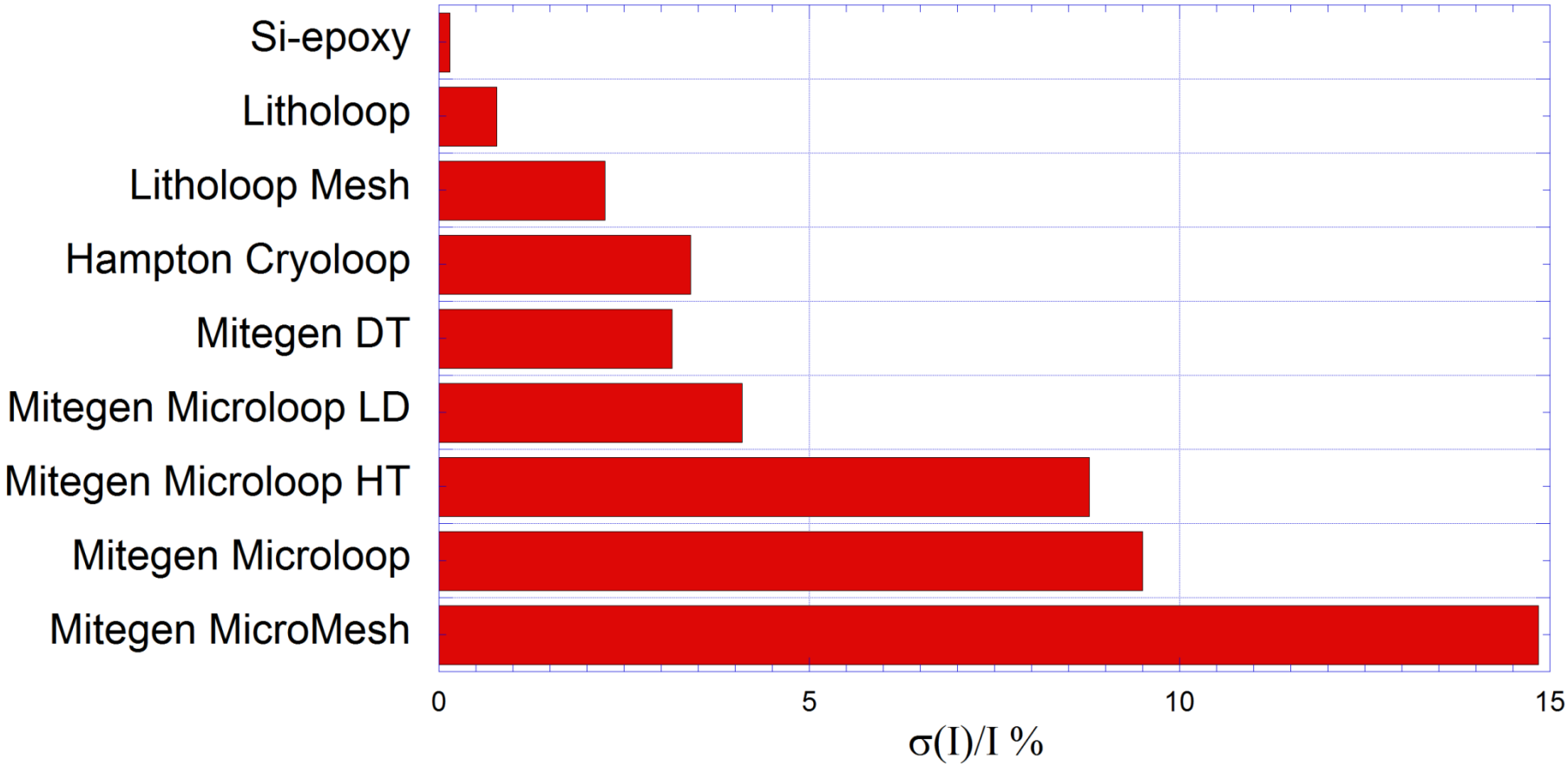



| | | RMS (mdeg), | σI/I% |
|---|---|---|---|
| | a) | 2.3 | 2.9 |
| | b) | 3.5 | 5.1 |
| | c) | 10.1 | 21.6 |
| RT | d) | 1.43 | 0.4 |

# Loop Angular Deviations (mdeg)



# Loop Integrated Intensity Deviations (%)



# *Silicon loop testing results*

- All loops showed motion relative to the “no loop” baseline

- Stiffness increases with loop thickness
- Loop motion depends upon loop orientation
- Loop motion can vary from loop to loop

- Grease mounting does not account for added support provided by frozen solvent -- this means the stress test only addresses relative loop strengths, not necessarily how it will perform under actual experimental conditions

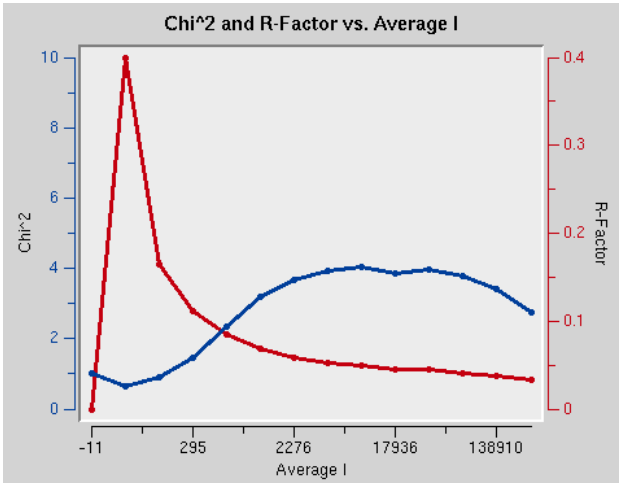
# *Lysozyme testing for loop motion – experimental setup*

- Differential measurement on the same crystal
  - Use two datasets taken at Kappa=0° and Kappa=-45° to avoid crystal-to-crystal comparisons
  - At midpoint of the 95° data collection range, widest part of loop faces cold-stream at Kappa=0°
  - At Kappa=-45°, loop is aligned parallel with cold-stream axis at scan midpoint

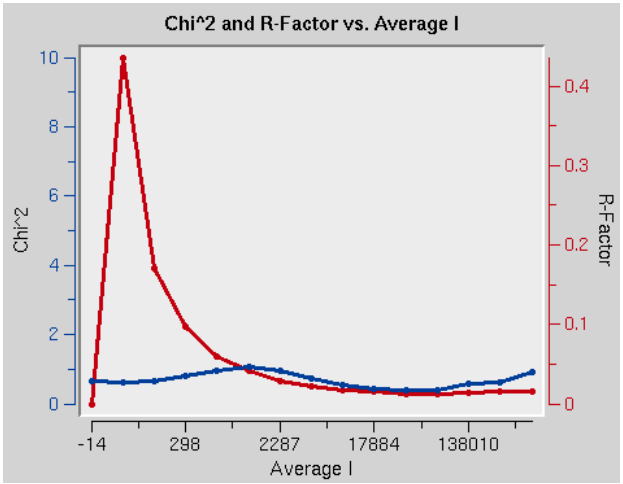
- All data collected at a scan rate of 1deg/sec, 12.66keV, 95° scan range with average redundancy 5.5 or better
- All data collected using Moore Tool table, single 1deg frames
  - 180,000ct/deg Heidenhain encoder

# *Motion detecting criteria – four separate trials on each loop*

- Rmerge must be significantly different between Kappa=0°and Kappa=-45° in the first two low resolution shells
- HKL3000 Scaling Chi Squared versus Average Intensity must show elevated profile at Kappa=0°
- The number of rejected reflections should be lower at Kappa=-45°
- If any one data set showed motion, the loop is labeled as showing motion




Motion present




Motion absent

# Lysozyme results

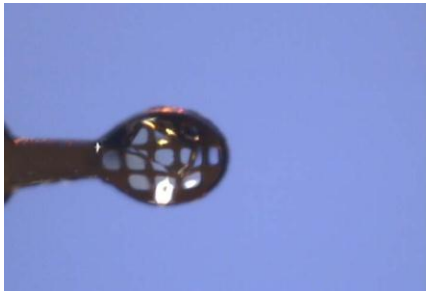
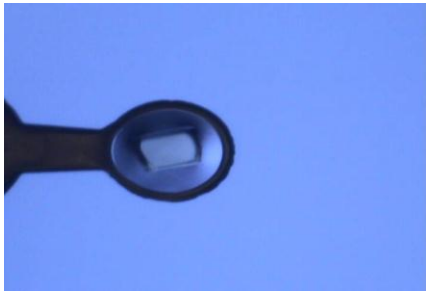
| | Mitegen HT | | Mitegen MicroMesh | | Mitegen DT | | Mitegen LD | |
|---|---|---|---|---|---|---|---|---|
| | Kappa0 | -45 | Kappa0 | -45 | Kappa0 | -45 | Kappa0 | -45 |
| Overall Linear Rmerge | 0.033 | 0.034 | 0.064 | 0.073 | 0.022 | 0.023 | 0.038 | 0.023 |
| Lin Rmerge <50-3.64> | 0.023 | 0.021 | 0.045 | 0.034 | 0.017 | 0.017 | 0.033 | 0.018 |
| Lin Rmerge <3.64-2.89> | 0.022 | 0.018 | 0.046 | 0.039 | 0.015 | 0.015 | 0.032 | 0.015 |
| # Rej Scale.log | 16 | 25 | 1988 | 45 | 137 | 76 | 612 | 39 |
| Iavg <50-3.64> /Avg Error | 816/11 | 573/8 | 331/5 | 256/5 | 1600/21 | 1505/20 | 1142/15 | 945/12 |
| Mosaicity Range | .19-.24 | .17-.19 | .19-.22 | .14-.17 | .26-.30 | .29-.35 | .22-.23 | .20-.25 |
| Total Reflections | 135888 | 135044 | 134153 | 116675 | 133658 | 135472 | 135421 | 137249 |
| Unique Reflections | 26695 | 26686 | 26700 | 26734 | 26840 | 26804 | 26758 | 26755 |
| % Complete <1.36-1.34> | 20.7 | 27.2 | 35.9 | 6.9 | 36.4 | 44.7 | 39.5 | 42.7 |
| Mean I/sigma | 46.7 | 35.7 | 27.5 | 18.5 | 60.2 | 51.1 | 52.8 | 50.6 |
| I/sigma <1.36-1.34> | 2.9 | 2.3 | 1.1 | 1.4 | 6.7 | 4.0 | 4.5 | 4.4 |
| Overall Redundancy | 6.7 | 5.9 | 5.6 | 5.5 | 6.3 | 6.1 | 5.8 | 5.6 |
| Loop thickness body/aperture | 18/18 | | 10/10 | | 25/10 | | 25/10 | |




# Lysozyme results

| | Mitegen Microloop | | Hampton Cryoloop | | Litholoop Mesh | | Litholoop | |
|---|---|---|---|---|---|---|---|---|
| | Kappa0 | -45 | Kappa0 | -45 | Kappa0 | -45 | Kappa0 | -45 |
| Overall Linear Rmerge | 0.051 | 0.038 | 0.040 | 0.035 | 0.023 | 0.024 | 0.021 | 0.021 |
| Lin Rmerge <50-3.64> | 0.037 | 0.022 | 0.033 | 0.026 | 0.018 | 0.018 | 0.015 | 0.017 |
| Lin Rmerge <3.64-2.89> | 0.038 | 0.020 | 0.031 | 0.024 | 0.015 | 0.015 | 0.014 | 0.014 |
| # Rej Scale.log | 351 | 35 | 187 | 167 | 57 | 41 | 42 | 29 |
| Iavg <50-3.64> /Avg Error | 491/7 | 395/6 | 683/9 | 787/11 | 1069/14 | 1158/15 | 1267/16 | 1047/14 |
| Mosaicity Range | .19-.26 | .20-.23 | .18-.22 | .21-.23 | .22-.27 | .22-.24 | .23-.24 | .22-.25 |
| Total Reflections | 135843 | 133315 | 138845 | 138165 | 135134 | 137357 | 135267 | 134629 |
| Unique Reflections | 26696 | 26675 | 26740 | 26768 | 26683 | 26741 | 26679 | 26702 |
| % Complete <1.36-1.34> | 30.2 | 23.1 | 32.1 | 45.4 | 40.3 | 47.6 | 28 | 36.8 |
| Mean I/sigma | 35.8 | 31.8 | 48.0 | 41.3 | 52.6 | 51.2 | 59.5 | 54 |
| I/sigma <1.36-1.34> | 1.8 | 1.7 | 3.4 | 2.6 | 4.6 | 4.4 | 5.2 | 4.6 |
| Overall Redundancy | 6.2 | 5.8 | 6.3 | 5.8 | 5.7 | 5.6 | 6.3 | 5.6 |
| Loop thickness body/aperture | 10/10 | | 20/20 | | 25/25 | | 25/25 | |



# *What can you do to minimize motion for already mounted samples?*

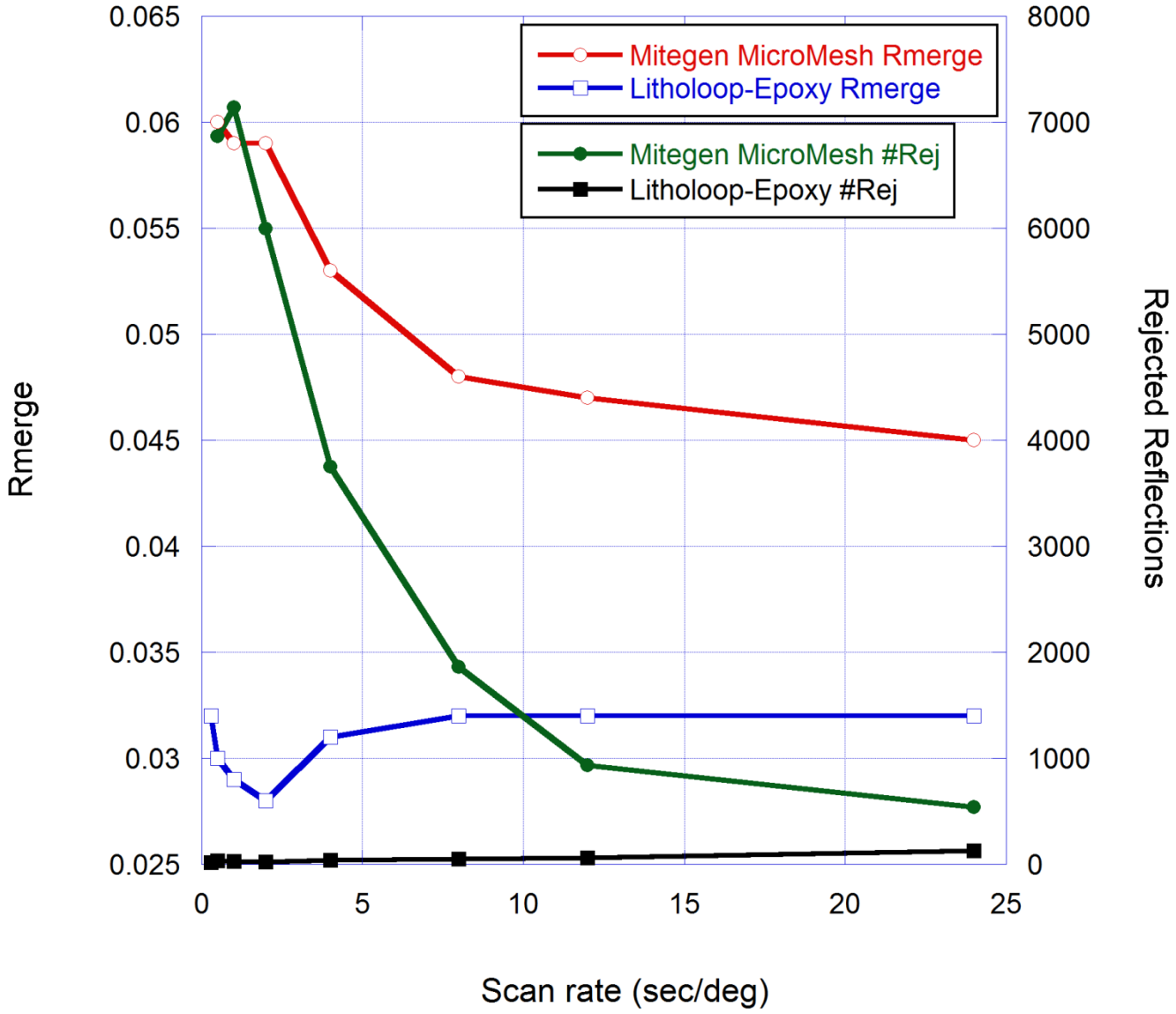
- Align loop along edge and/or convex side to minimize movement
- Time-average – lengthen scan rate to average out motion

- To confirm time-averaging
  - Lysozyme experiment as a function of scan rate
    - *Mitegen MicroMesh loop*
    - *Use attenuation to maintain similar counting statistics*
    - *Litholoop coated with epoxy for instrument baseline*

- Only one crystal for each series
  - Radiation damage not a significant contributor to results

- Report Rmerge and #Rejected reflections vs scan rate

# *Rmerge, #Rej vs scan rate*



# *Time-averaging results*

- Increasing time 1→24s/deg reduces overall Rmerge (.059 vs .045)
- Total number of reflections rejected decreased by factor of 13
- Data were still improving at a rate of 24sec/deg

- Time-averaging helps but not as much as a stiffener or thicker loop would have

# *Does any of this matter to real structure solution?*

- At present, the answer is “inconclusive”

- Lysozyme structure was solved via molecular replacement using the Mitegen MicroMesh data with 7100 rejected reflections
  - There were only small differences in the maps between motion and no motion data

- CCD detectors have a 1-2% Rmerge baseline due to calibration errors
  - Calibration errors increase as the spot size decreases
    - *1% or larger at 75um;*
    - *0.3% at 300um (best case)*
  - If loop motion error contributions are in this range, they may simply be absorbed

- Attempts are ongoing to collect and analyze anomalous data under loop motion conditions

# *Summary*

- Silicon testing results indicate that large variations in integrated intensities are possible at specific loop orientations
- Lysozyme results indicate that loop motion can occur under “normal” data collection conditions

- Thicker loops (25µm) perform better than thinner loops
- Both the protein crystal and frozen solvent can aid in stiffening loops

- Loop stiffness can be minimized by adding grease or epoxy for reinforcement
- Data degradation can be lessened by preferential loop orientation or time-averaging

# *Co-authors from the Structural Biology Center*

- Frank J. Rotella
- Norma Duke