Crystal ~~Crystal~~ Heating in a Third Generation Synchrotron X-ray Beam, a Significant Factor or Not?

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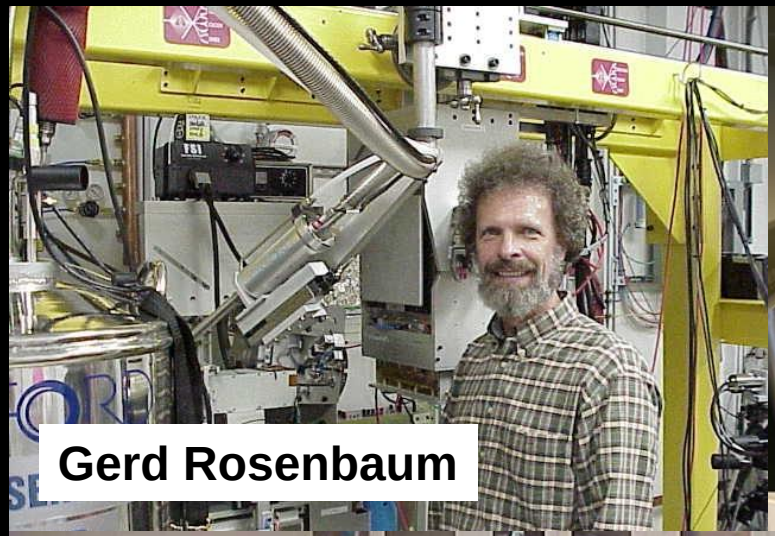
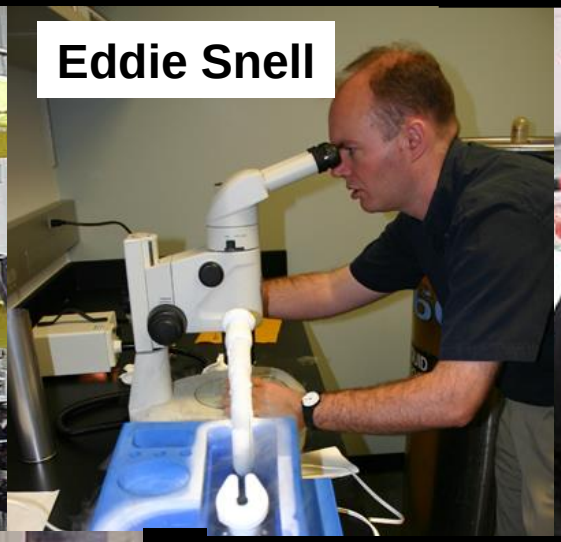
Who, what, where, why and how?

Then answer the question ….

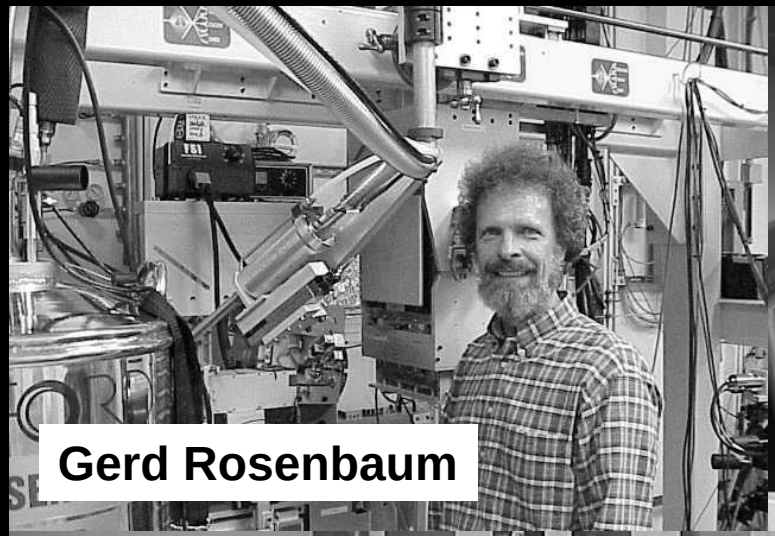

Gerd Rosenbaum


Eddie Snell


Henry
Bellamy


Mark van der Woerd

Who?



Converted to Grey

Where?

Preliminary studies at the South East Regional (SER)–CAT:

Beamlines 22-BM (developing the method) and 22-ID for the first tests.

The data presented here was collected on the Structural Biology Center (SBC) –CAT Beamline 19-ID

- **Energy 6.5 Kev (1.9 Å)**
- **Ring current ranging between 101 and 103 mA**
- **Intensity of 3.24 x 10^{12} Ph/s.**

Why?

Cryocooling is used to mitigate radiation damage due to propagation of free radicals formed as a result of X-ray irradiation.

Crystalline ice formation is a problem in cryocooling; the amorphous crystalline ice transition occurs ~140K.

To be successful the sample must be kept below 140K.

Hold on, free radicals are shown to be mobile at ~120-130K.

The crystal must be kept below 120K.

However the X-ray photons deposit energy. This is dissipated as heat. How significant is this heat and is it a problem?

How ?

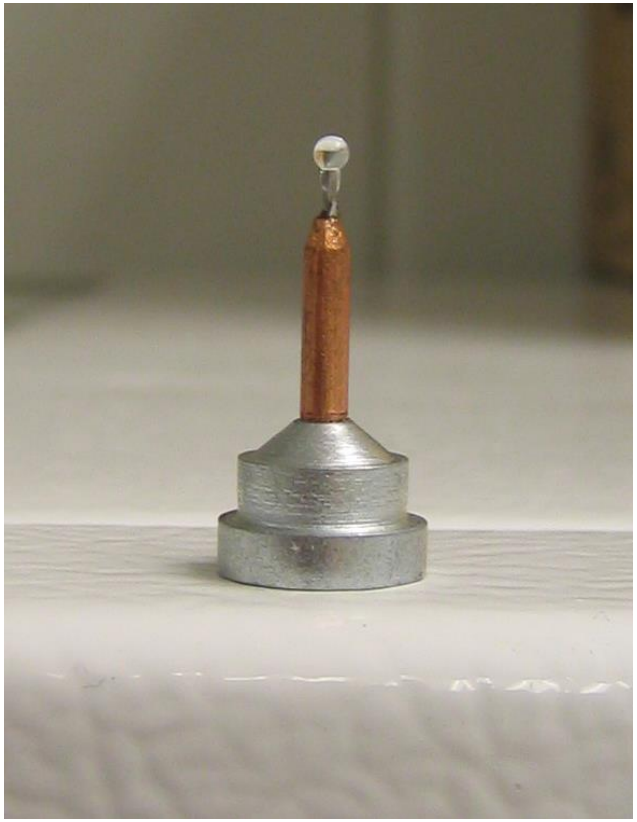
- Measure sample heating non-invasively
- Compare the result with current models to test and validate those models.
- If the models accurately predict the temperature rise then make use of them to extrapolate to other cases.

Sample

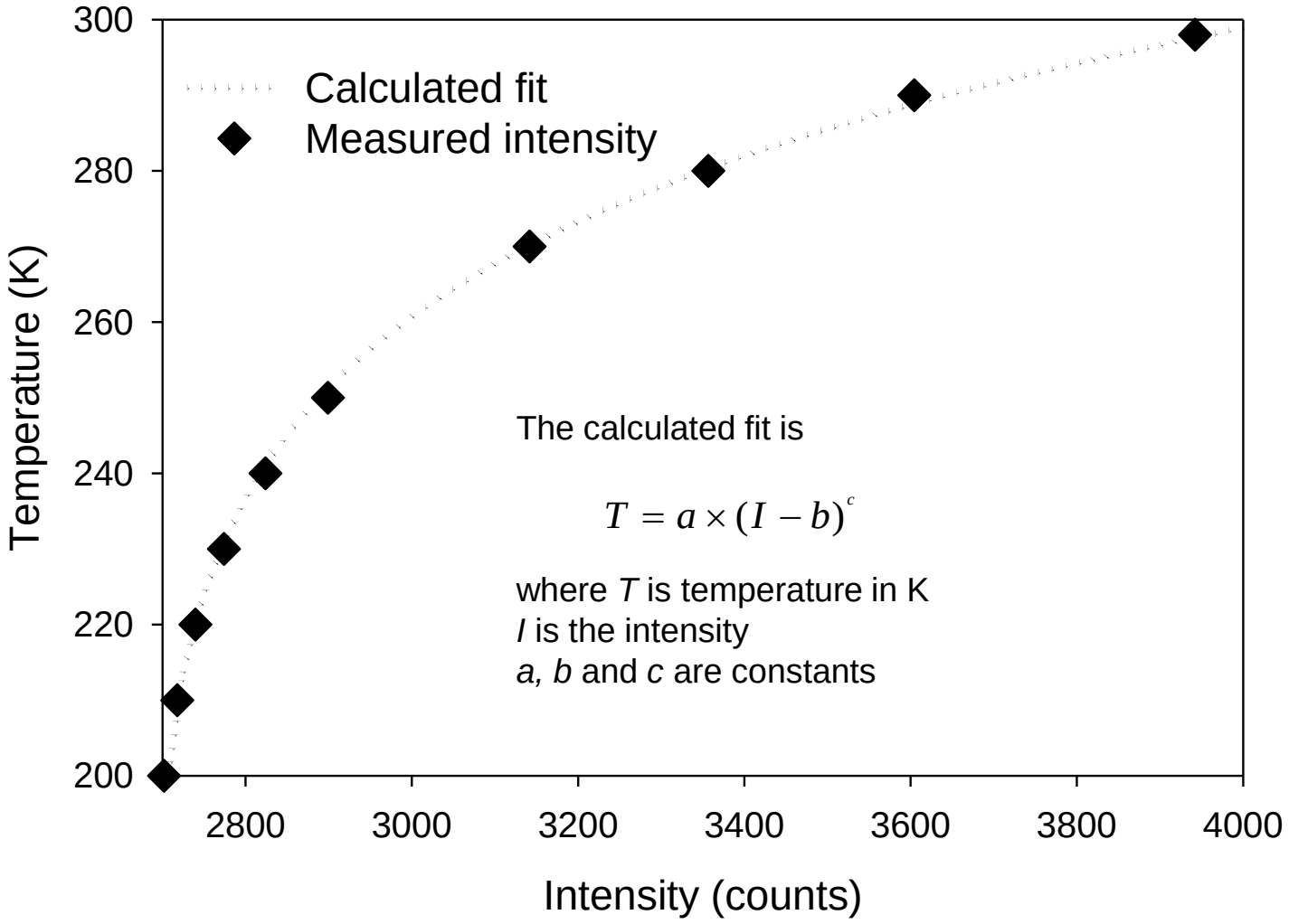
- **Surrogate crystals:**

- Sample 1: 2mm diameter glass bead
  - imaged at 100K with no beam (steady state calibration point)
  - Imaged with shutter opening (time resolved)
  - Imaged after shutter had been open for 1 minute (steady state).
  - Measurements repeated in 10K steps up to 290K
  - Final measurement with the cryostream off.

- **Sample 2: 1 mm diameter glass bead**
  - Imaged from 290K down to 100K in reverse of 2mm case.



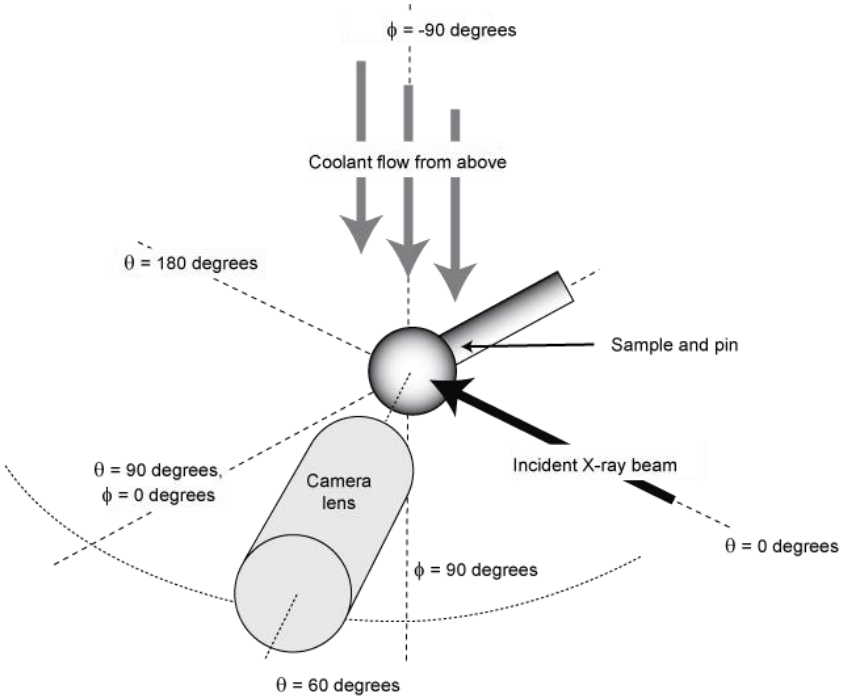
Calibration

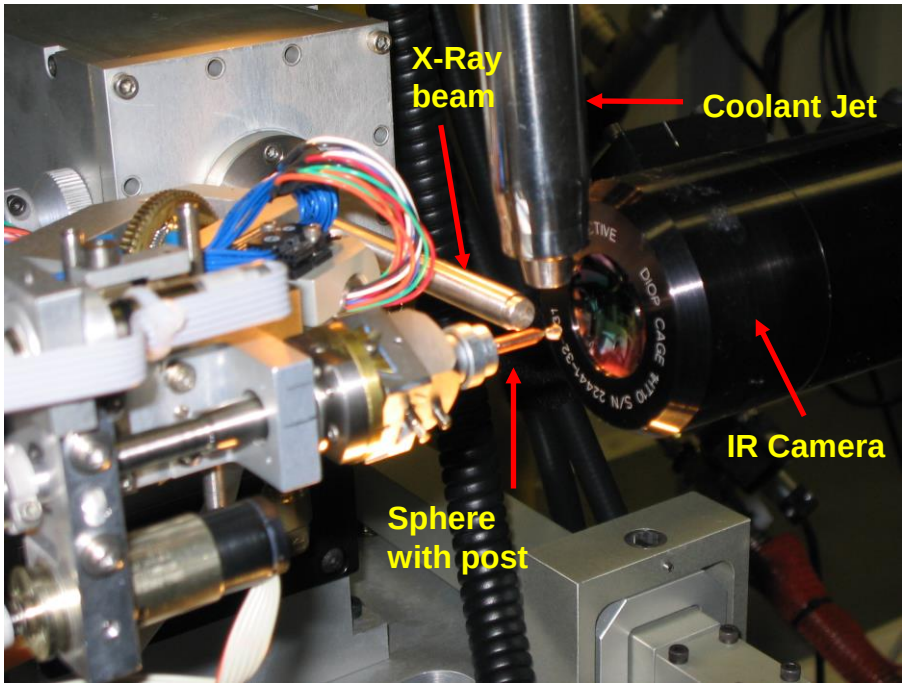


Why a glass bead, not a real crystal?

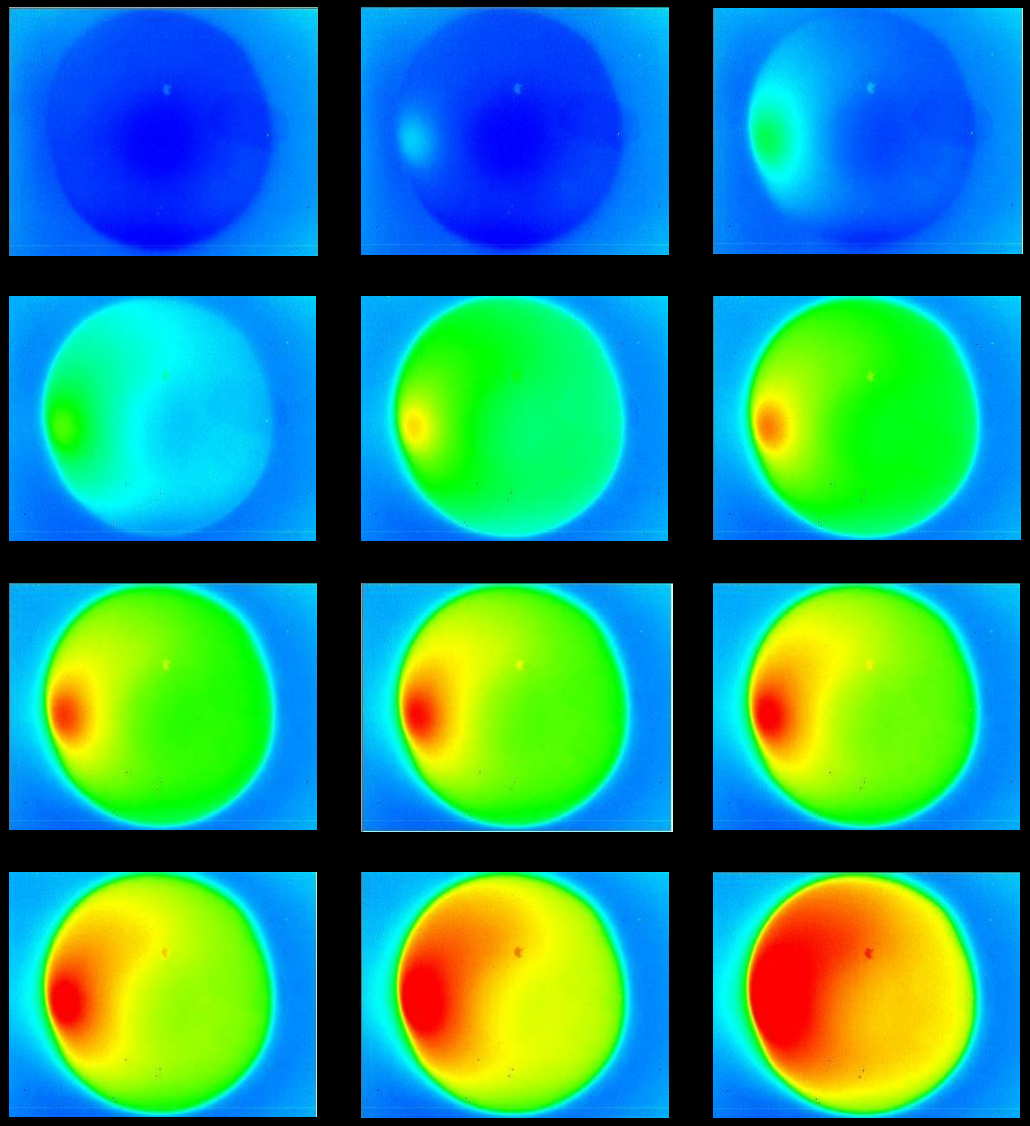
- Radiation damage causes significant changes in the infrared properties of macromolecular crystals.
- These are also seen in the visible spectrum as color changes (the crystal goes from clear to black).
- Because of this we have been unable to calibrate the temperature of a macromolecular crystal. We can image it as we warm the cryostream up but the infrared properties of the sample have already been changed by irradiation.
- We cannot calibrate the temperature before irradiation due to the amorphous/crystalline ice transition.
- (The next step is to use an infrared laser to put a heat load onto a real crystal and measure the thermal properties or build a better crystal).

Experimental





Shutter Opening

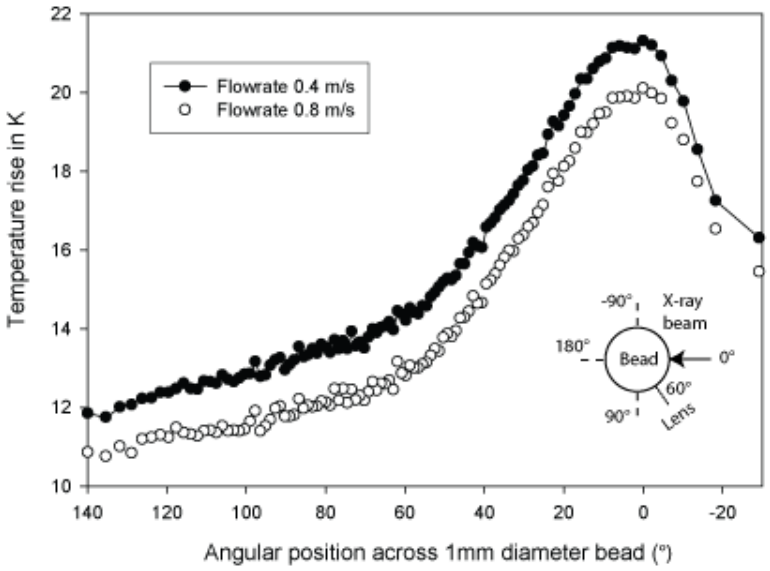


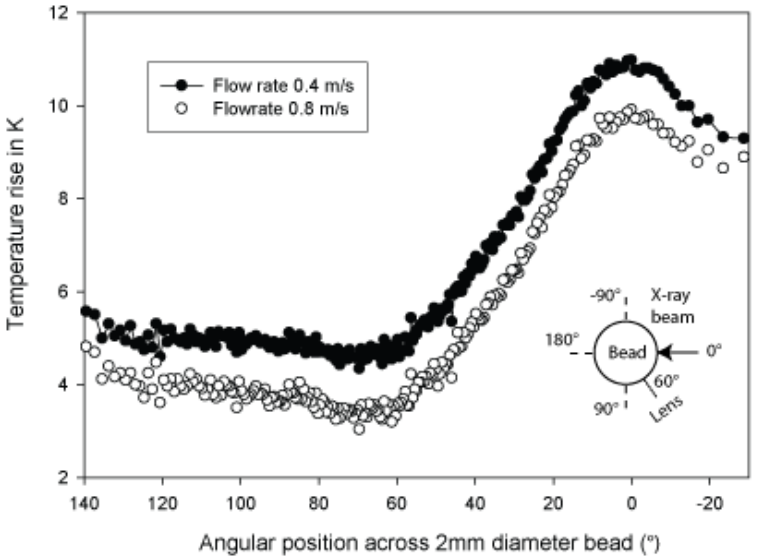
2mm glass bead at room temperature with no cryostream flow.

Beam hits bead from left, blue is cold, red is hot.

Temperature rise is 50K over 24 seconds.

Extreme case!



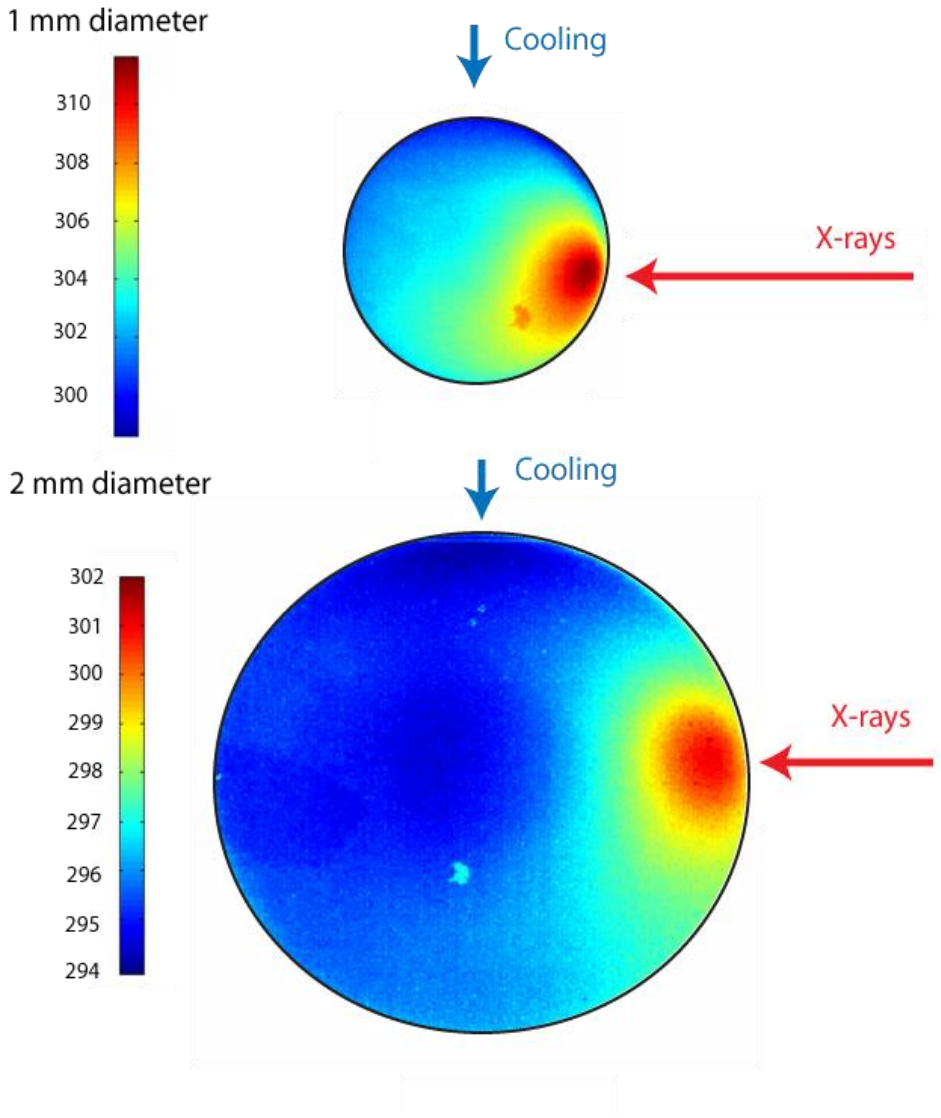


Heat across bead

For a 1 mm bead the peak heating where the beam strikes is 21K. Doubling the flow rate of the cryostream reduces this to 20K, a minimal effect.

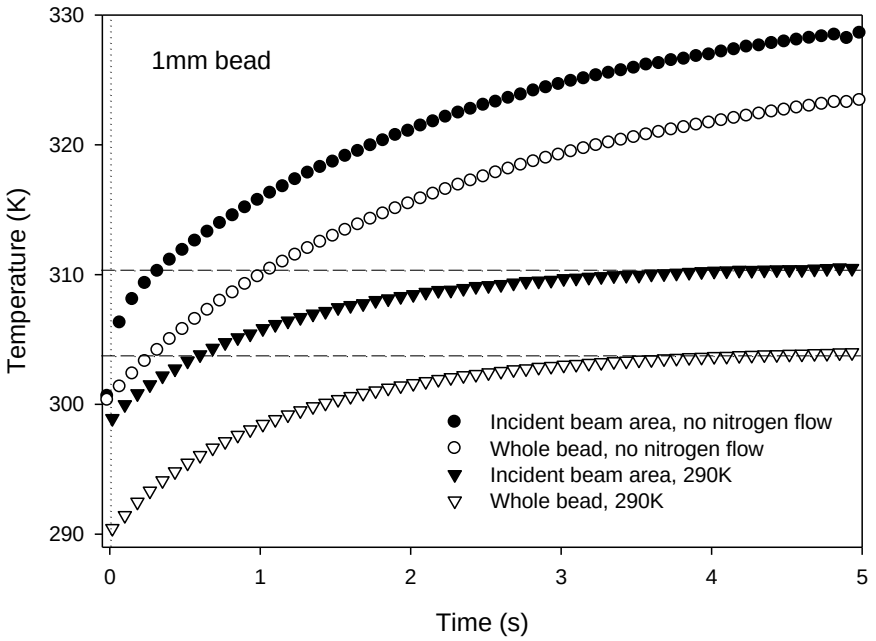
For the 2 mm case the heating is 11K. Again, increasing the flow rate has little effect on the temperature rise.

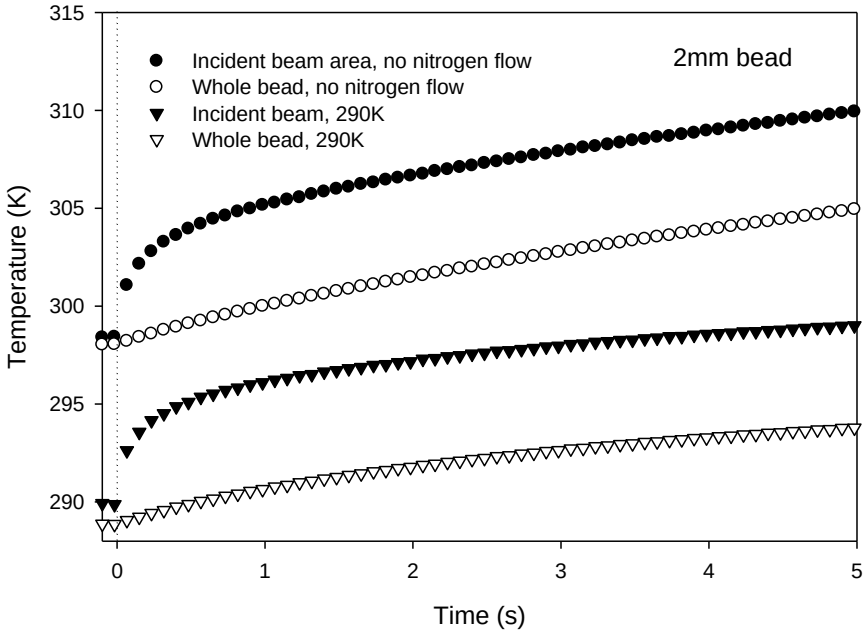
Spatial heat on the bead



Images color coded according to heat. Red is warmer than blue.

The incident beam is seen nicely.





Time resolved effect

Each graph shows solid points representing the incident beam position and open points representing the whole bead average. In the upper plots in each graph there is no cryostream flow, in the lower the cryostream is providing temperature control.

For the 1 mm bead with the cryostream flowing the steady state is reached in 4-5 seconds after the beam is incident.

For the 2 mm case after 5 seconds the temperature is still slowly rising.

Steady state is reached rapidly for small samples.

Compare results with current models

- Two related models: Kriminski et al. (2003) Acta Cryst. D59, 697-708, Mhaisekar et al.,(2005) J. Synchrotron Radiation 12, 318-328.
- For the external temperature rise between the sample surface and gas, ΔT_{ext}, the model samples are first scaled to the 1 mm diameter glass bead.
- Mhaisekar's more recent model predicts ΔT_{ext} = 12.7 K for the 1 mm bead. The measured average temperature rise for the 1 mm bead is ~14 K.
- Similar agreement seen for the 2 mm bead.
- The agreement between model and measurement appears to be good enough to justify extrapolating from Mhaisekar's 0.2 mm sphere model down to the "typical" 0.1 mm size biocrystal sample.
- What are typical values for a macromolecular experiment?

Typical data collection at the APS

- Average crystal size 0.02-0.10 mm, typical 0.05 mm.
- Average loop size 0.02-0.20mm, typical 0.10 mm.
- Average beam size 0.02-0.15 mm square, typically 0.08.
- Photon 12.4 keV (1 Å) or 12.658 keV.
- Exposure time per frame 0.5-3 s, typically 3 s.
- Fluxes 1.0-2.5 x 10^{12} photons s^{-1}, typically 1.5 x 10^{12} photons $s^{-1.}$

- For these parameters and the physical properties of nitrogen, amorphous ice and protein crystals the power absorbed by a typical sample (crystal and amorphous ice in a loop) is ~ 0.09 mW or about 1/40 of the power absorbed by the glass beads.

Applying the model – what does it tell us?

- For full details of the arguments see Snell, Bellamy, Rosenbaum and van der Woerd, Journal of Synchrotron radiation, 14, 109-115 (2007).
- We scale the experimental results for size and absorbed power then make use of Mhaisekar's model.
- For a "typical" 0.1 mm sample absorbing 0.087 mW of power the temperature rise can be extrapolated to ΔT_{ext} = 4.5 K
- Adding an internal temperature rise of less than 0.25 K, the maximum realistic temperature increase inside the crystal sample is then, ~5 K.
- Because of the wide variability of data collection parameters stated above, the temperature rise in any particular case may range from 0.2 to 2 times the rise extrapolated for the typical case, i.e. 1 to 10K.
- Beam heating is not significant with regard to free radical mobility (120K) if your sample is at 100K or below.

Implications for data collection

- The model calculations by Mhaisekar *et al.* (2005) show that for the **same** flux on the sample the temperature rise increases very little with decreasing sample diameter (about 20% for a four-fold decrease in diameter).
- However, if we have a four-fold increase in flux the temperature rise scales proportionally, i.e. if we go from 1.5 x 10^{12} photons s^{-1} to 6.0 x 10^{12} photons s^{-1} then our predicted temperature rise goes to 20K.
- Restating a previous slide, beam heating is not significant with regard to free radical mobility (120K) if your sample is at 100K or below.
- However, for microfocus cases if you try and maximize your flux instead of increasing exposure time you may get to a point where sample heating could cause problems (intensity of the spot is proportional to the product of the flux intercepted by the crystal, the crystal thickness and exposure time).
- Similarly, for radiation damage studies, care should be taken not to use a flux that could cause heating.

Where to go from here

- Our surrogate samples are far from real crystals.
  - Therefore, use improved surrogate samples.
- Our camera sensitivity is low at low temperatures.
  - Improve camera sensitivity.
- The current models are steady state.
  - Improve the models to provide dynamic heating and cooling information.

- Basically, get data closer to the actual conditions for macromolecular cryocooling i.e. lower temperatures, more realastic samples and higher sensitivity.

Acknowledgements

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Thanks to great referees!

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