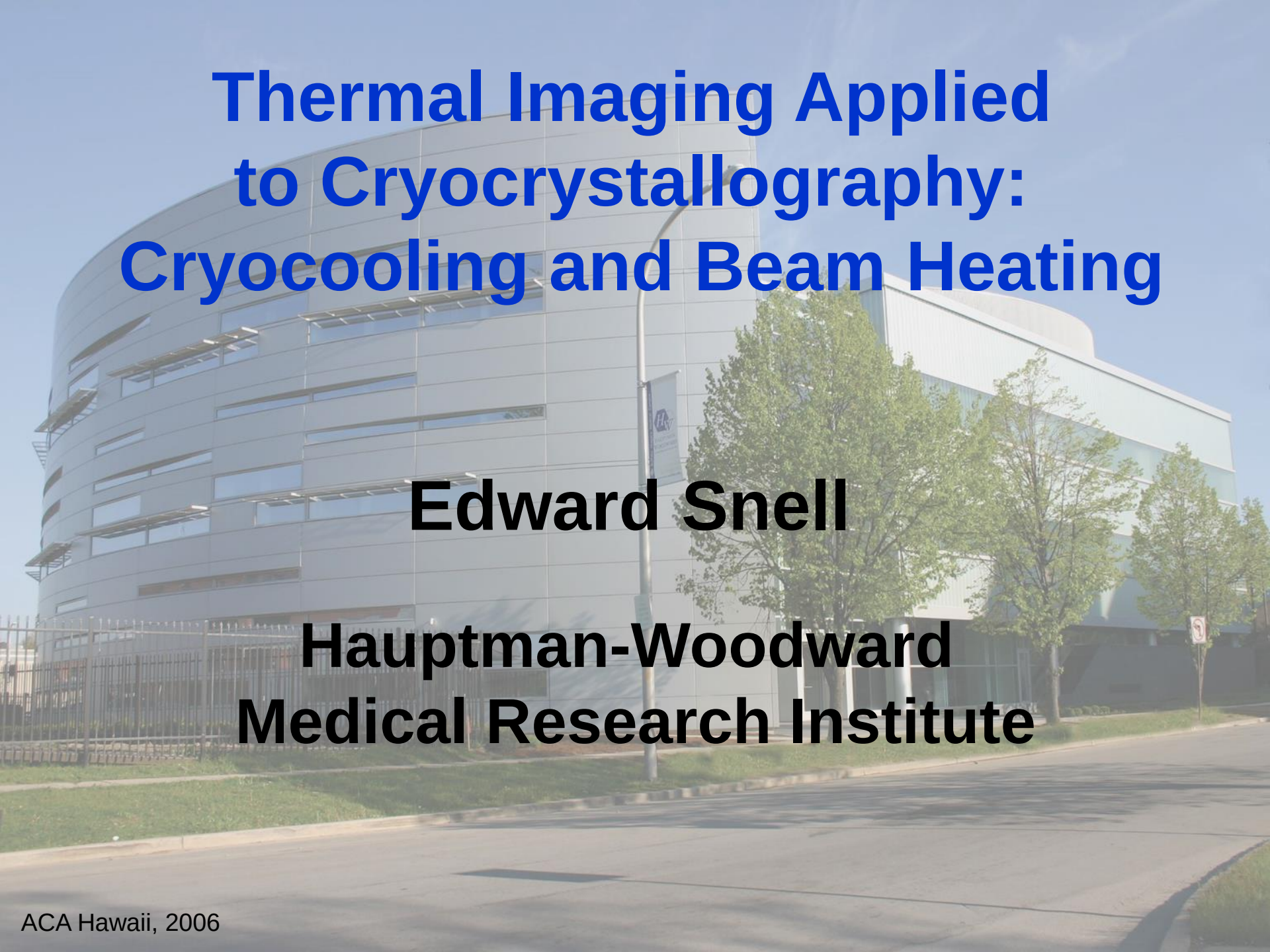
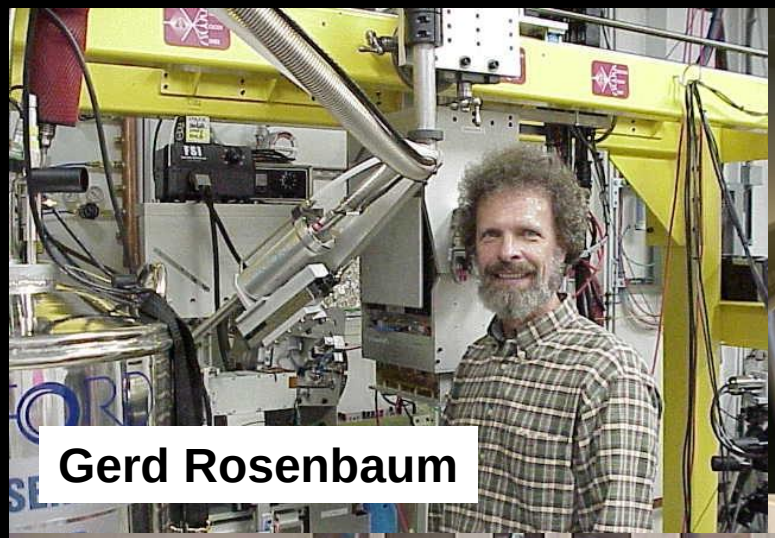
Thermal Imaging Applied to Cryocrystallography: Cryocooling and Beam Heating



Edward Snell

Hauptman-Woodward Medical Research Institute

ACA Hawaii, 2006



The research team

Outline of the talk

Infrared definitions and properties

Imaging Cryocooling

Finding a crystal

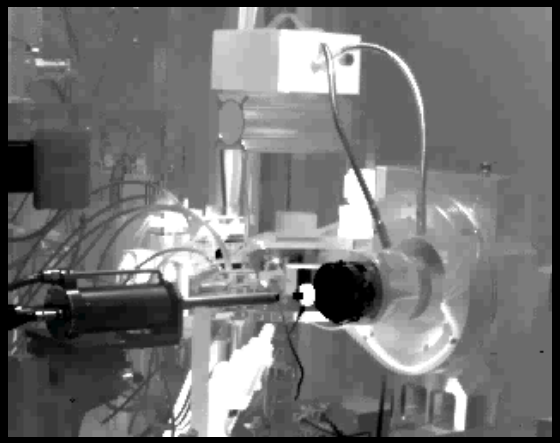
Quantifying the data: The speed of cool

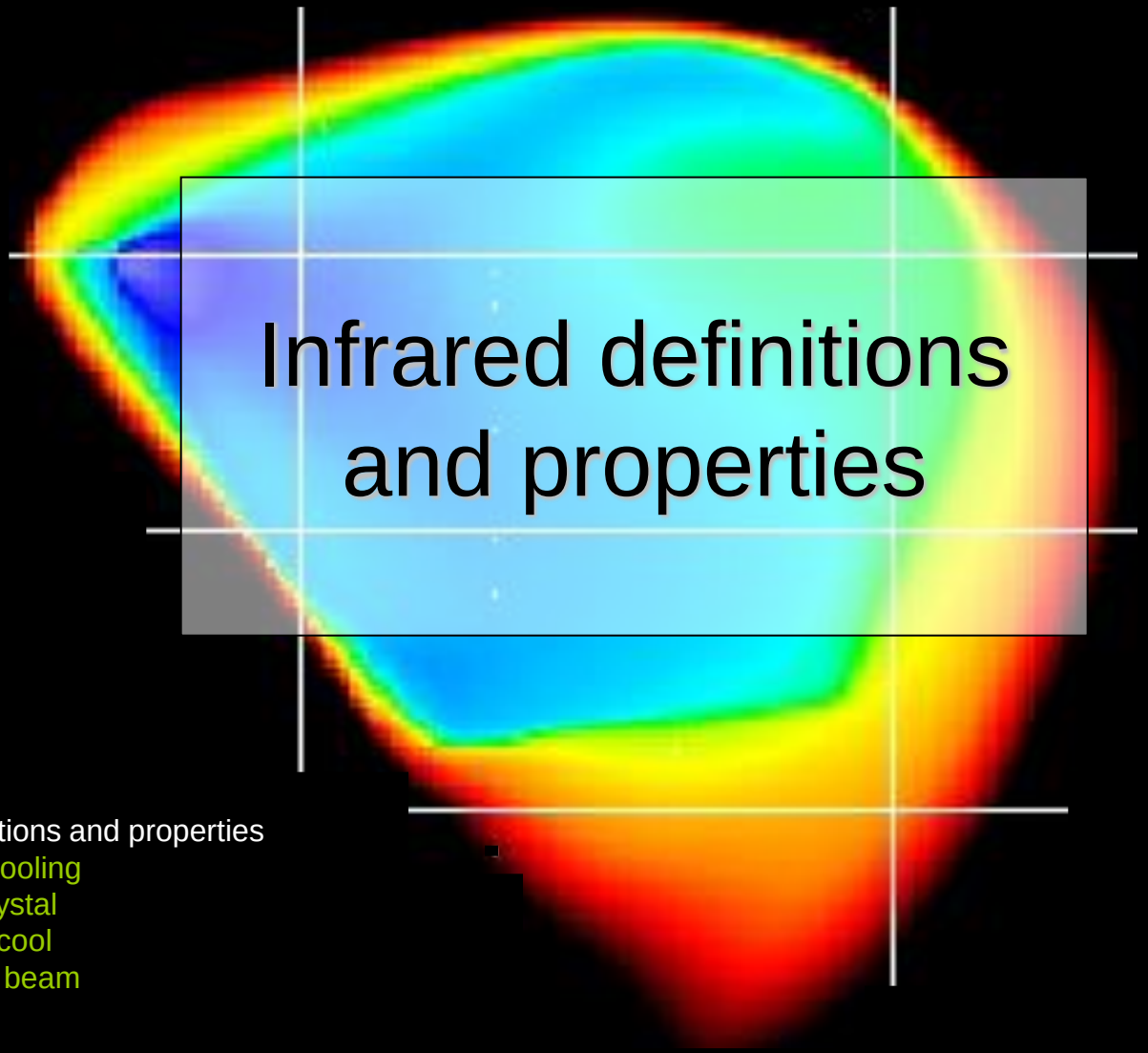
Heat from the beam

Modeling of the data

Summary

Future directions





Infrared definitions and properties

- Infrared definitions and properties
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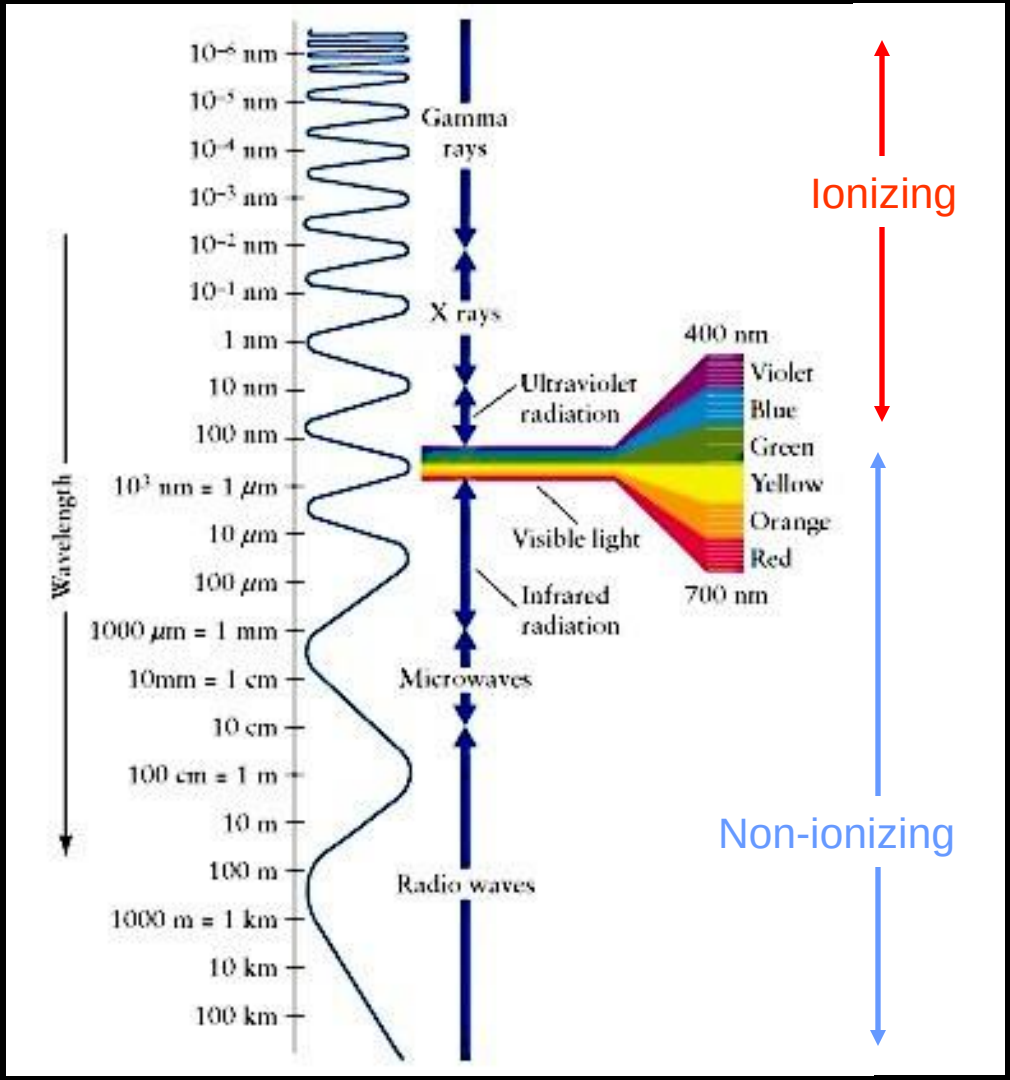
The Electromagnetic Spectrum

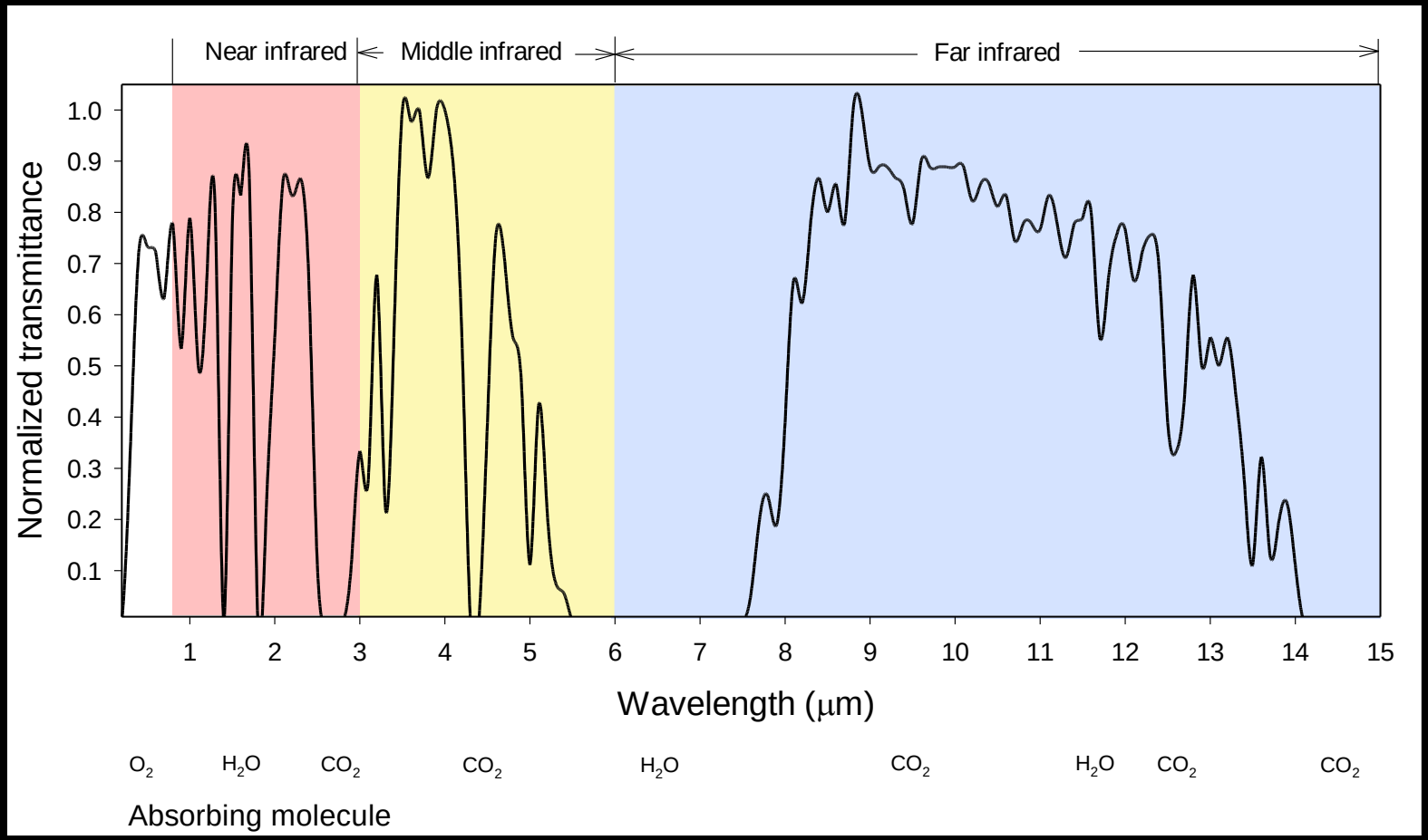
The electromagnetic spectrum can be divided into ionizing and non-ionizing radiation.

Ionizing, e.g. X-rays, high energy ultra violet *etc.* have enough energy to break chemical bonds – they are damaging to the molecules.

Non-ionizing radiation, *e.g.* visible and infrared does not have the energy required to break bonds. Observation with this type of radiation is non-invasive.



Infrared radiation is absorbed in the atmosphere.



There are three defined regions where absorption is minimized termed the far, mid and near infra-red. These “windows” in the atmosphere can be used for observation.

Black body radiation

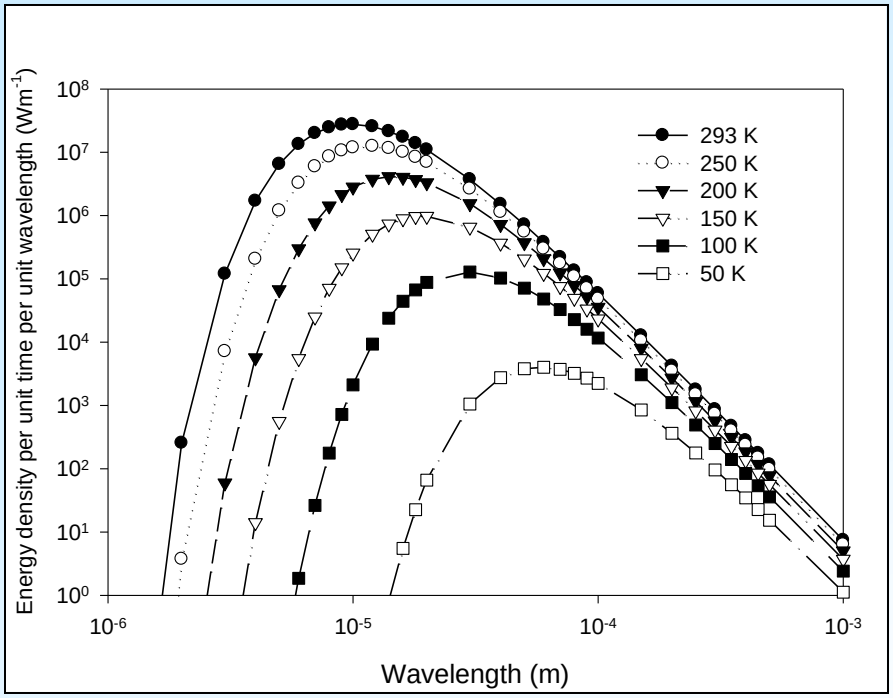
- All objects above 0K emit infrared energy as a function of their temperature.
- A black body perfectly emits and absorbs this thermal radiation.
- The energy spectrum for a black body is exactly given by Planck's radiation law;

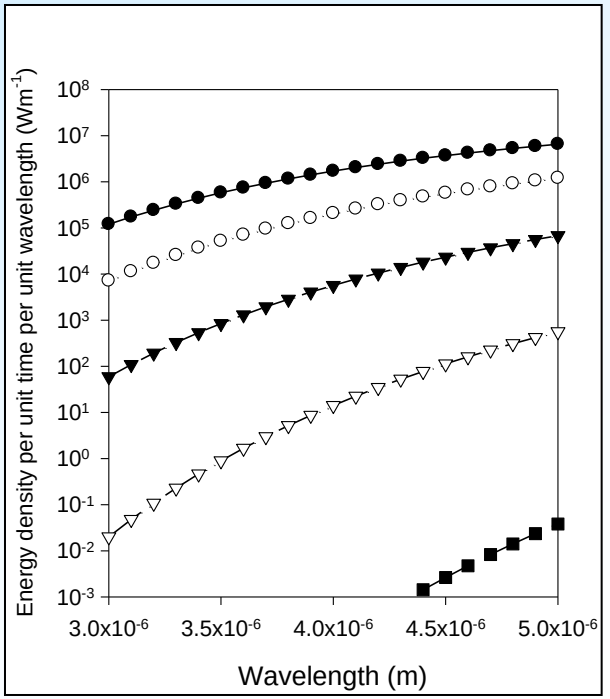
$$E(\lambda(T)) = \frac{2\pi hc^2}{\lambda^5 (e^{hc/\lambda kT} - 1)}$$

- Where λ is the wavelength, *c* is the speed of light, *k* is the Boltzmann constant, *h* is Plank's constant and *T* is the temperature in Kelvin.

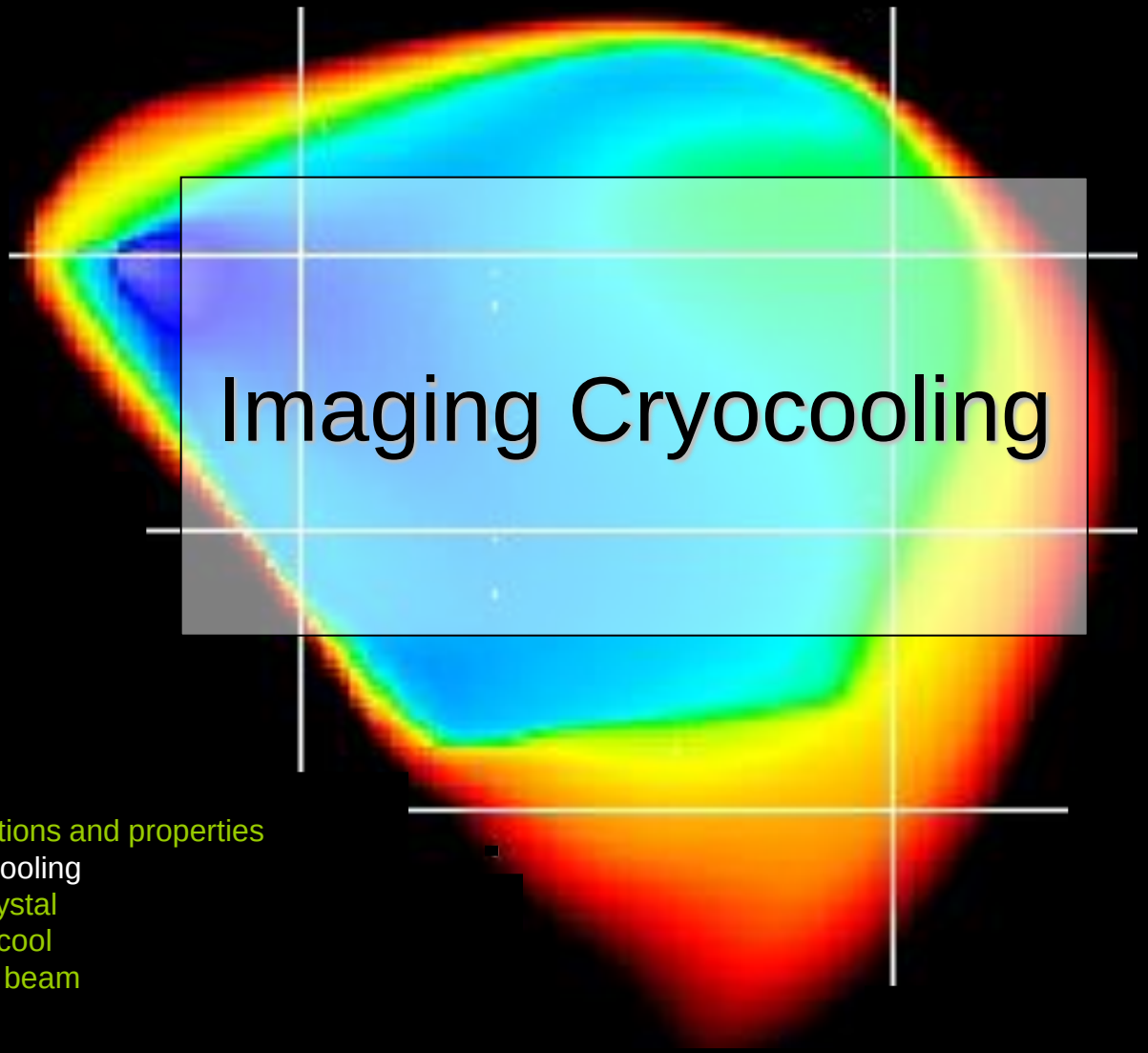
The energy spectrum for objects cooled below ambient conditions

The energy spectrum for the mid-range sensitivity of the infrared camera used





Imaging in the mid-range was chosen. This has a greater energy density and accuracy over the near-range and and greater response to temperature change over the far-range



Imaging Cryocooling

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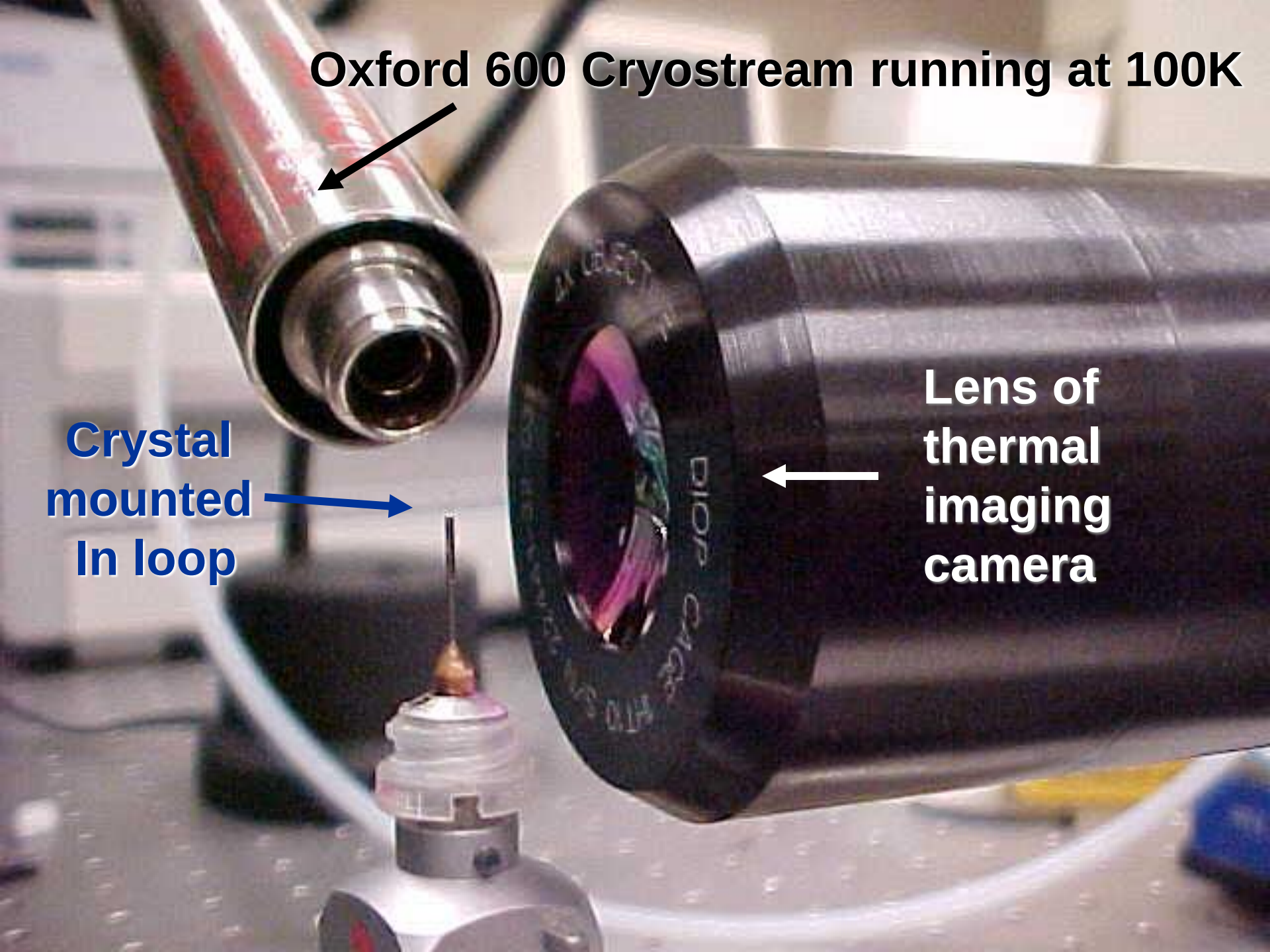
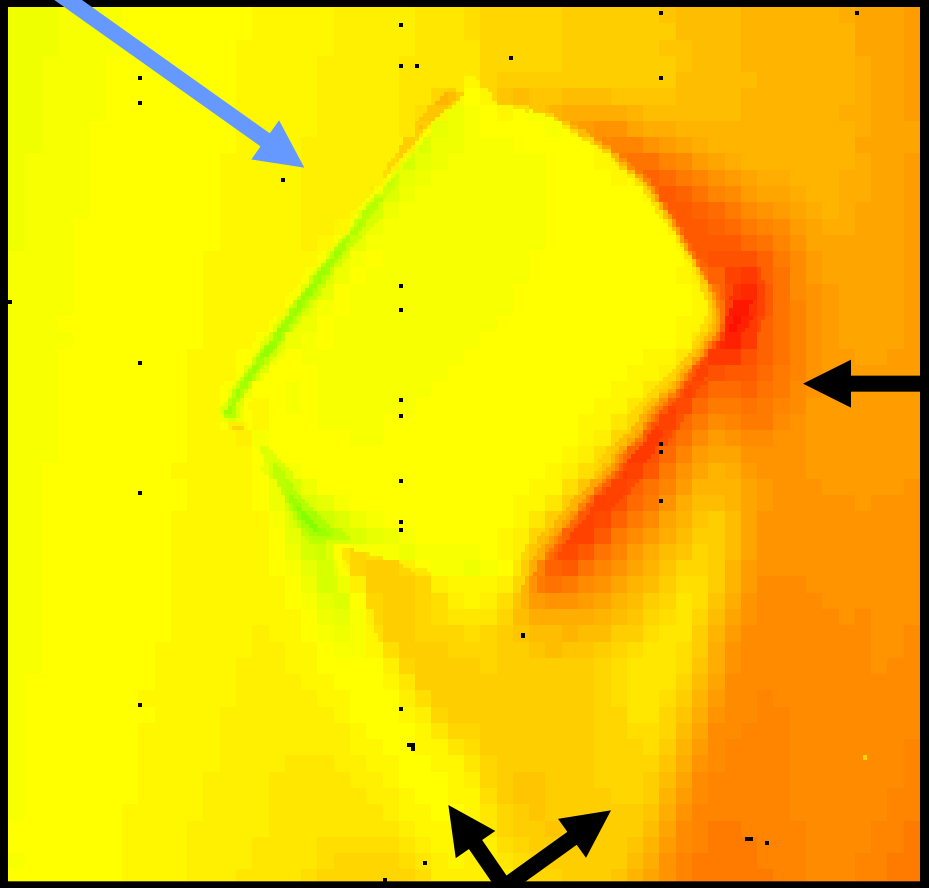
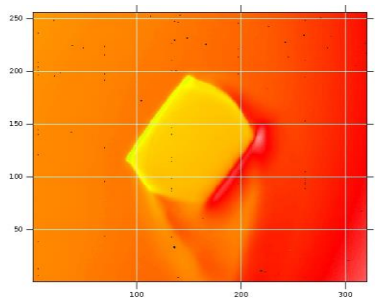

Oxford 600 Cryostream running at 100K
Crystal mounted In loop
Lens of thermal imaging camera

Cryostream at 100 K



Crystal

Nylon cryoloop



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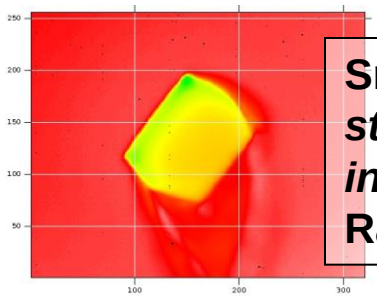

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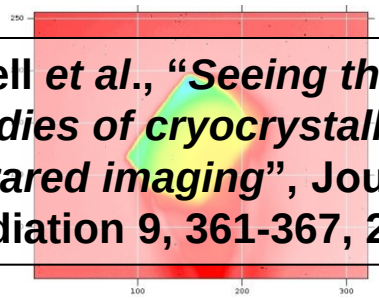

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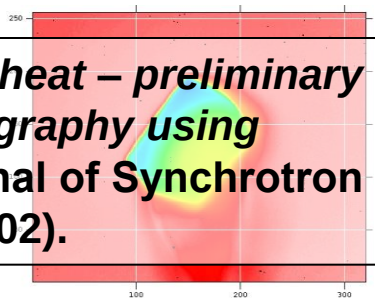

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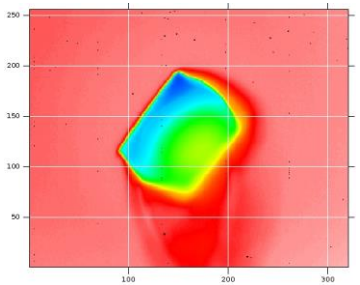
Snell *et al*., "*Seeing the heat – preliminary studies of cryocrystallography using infrared imaging*", Journal of Synchrotron Radiation 9, 361-367, 2002).



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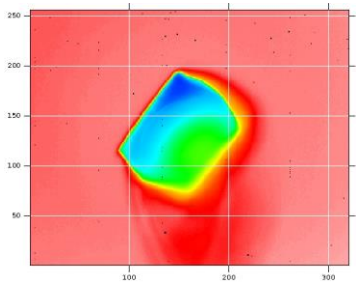

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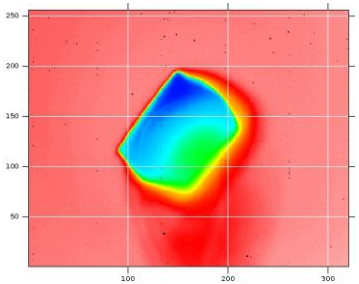

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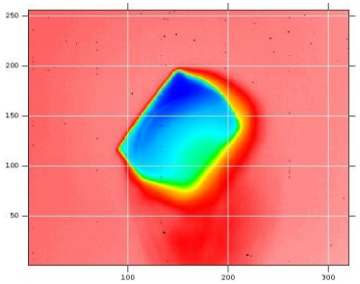

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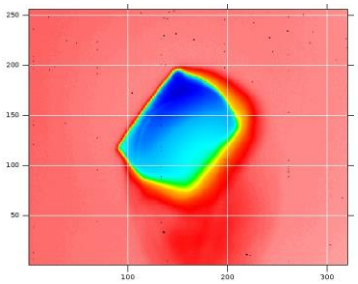

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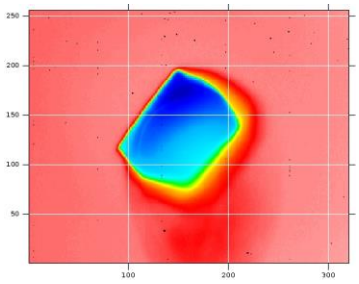

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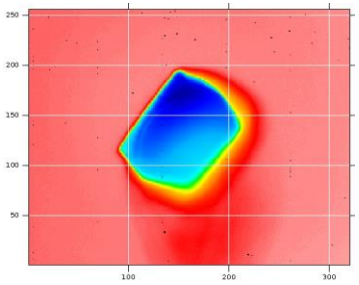

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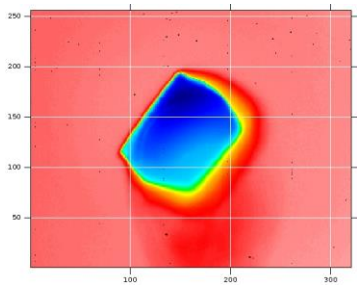

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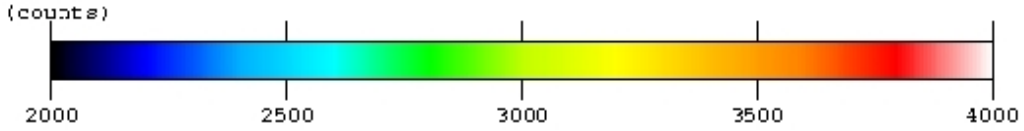


Properties of Infrared Radiation from a crystal (Why were the results qualitative?)

- Macromolecular crystals do not tend to be perfect black bodies:
  - They do not perfectly emit or absorb radiation
  - The spectral radiance is less than that predicted by Planck's law.

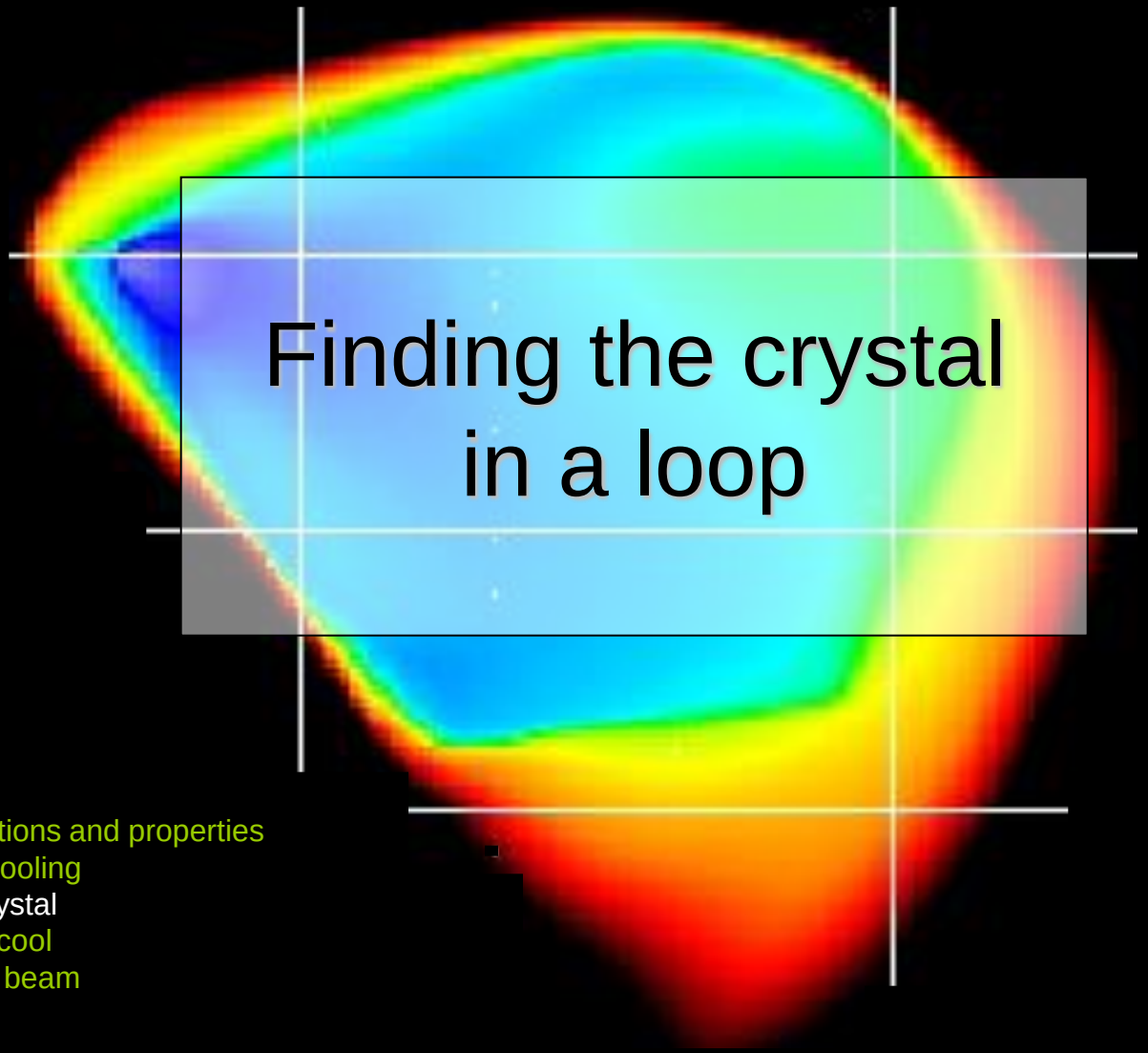
- Crystals tend to be illuminated by a number of infrared sources:
  - The ambient heat in the room
  - The experimenter
  - The illumination
  - The coldstream

- Crystals transmit and reflect heat
  - Heat is seen behind the object
  - Heat is seen reflected off the object

- Infrared properties of the crystal vary with wavelength and viewing angle

- Problem, it is not trivial to do quantitative studies

Solution – qualitative studies



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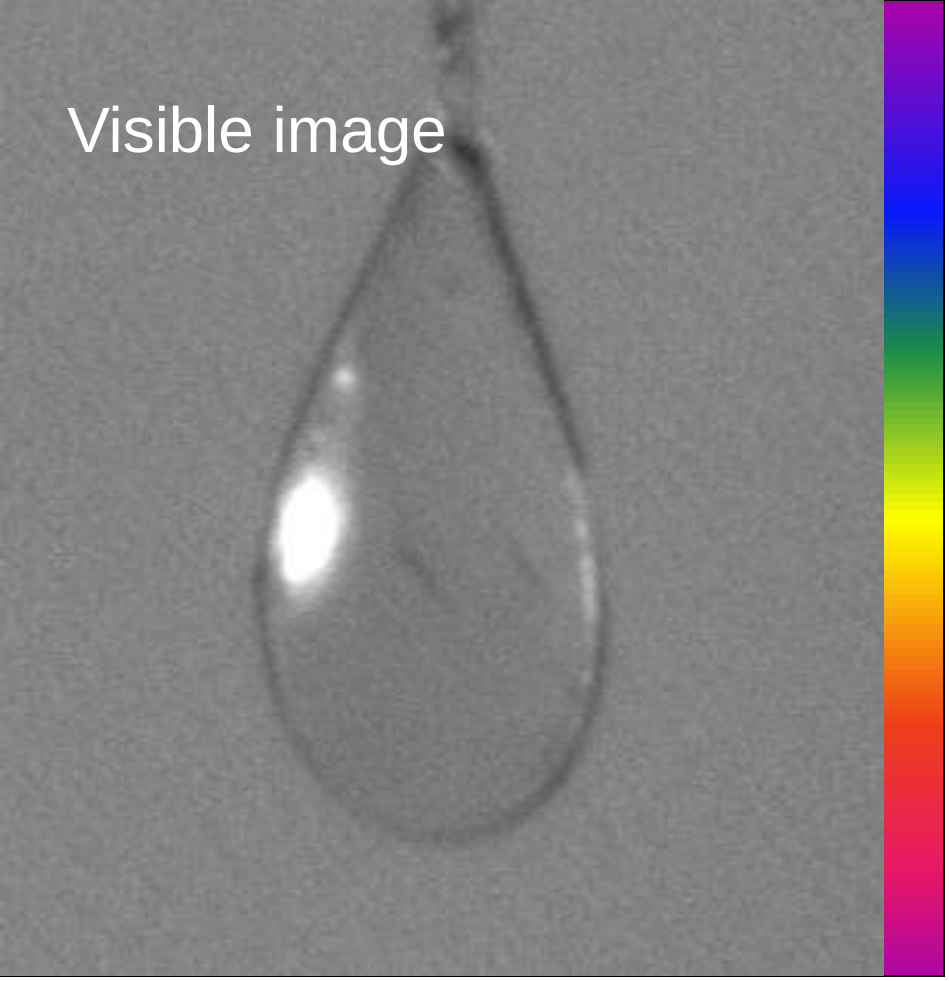
In the following images:

- The crystals are imaged after several minutes of cooling in the 100K nitrogen stream to ensure they have reached equilibrium.
- The loop used is 0.5 mm wide, all images are inverted.
- For visible light the best image of the crystal (illumination position and background are variables) is shown. No back illumination was available.
- For the infrared images the contrast and limits have been optimized to show the crystal. The dynamic range recorded is not visible without doing this.
- The infrared images are false colored (gray scale) according to heat. In this case white is hot, black cold.



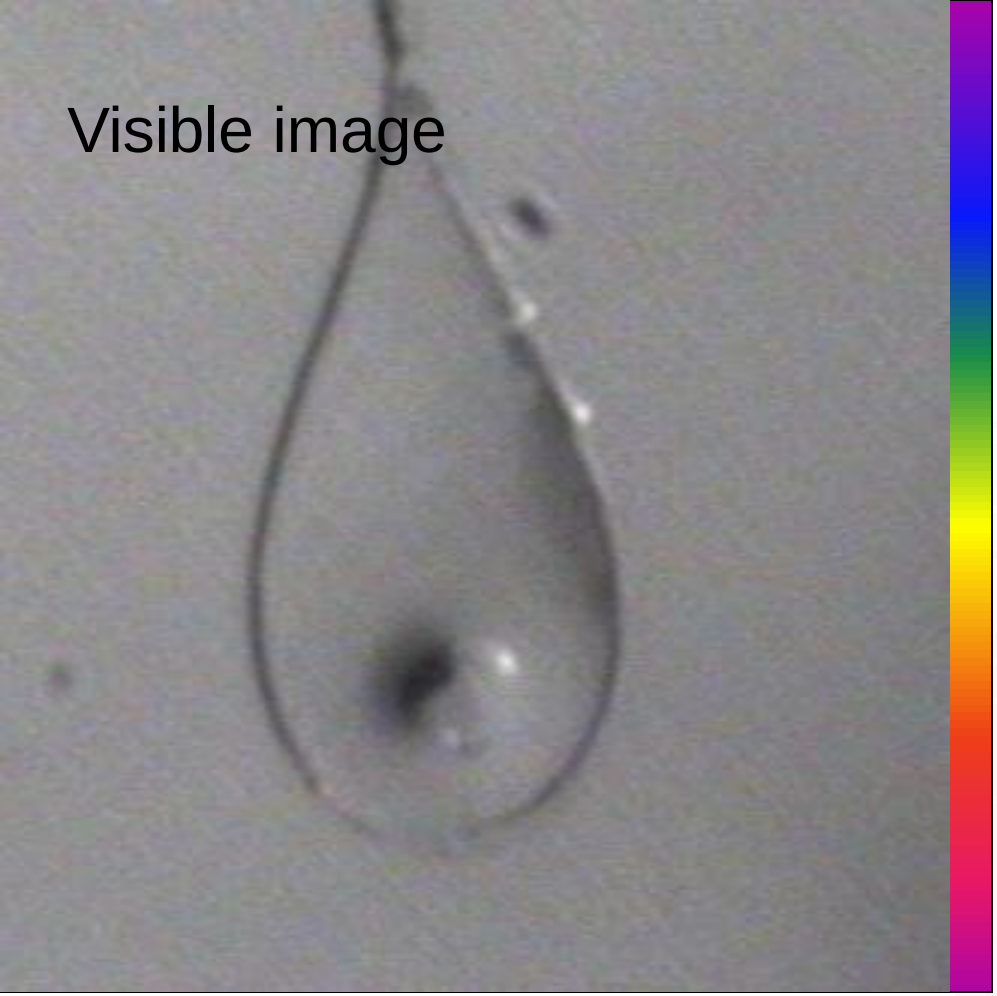
Lysozyme crystal
(0.14 x 0.11 x 0.06 mm^3)

Infrared image (lamp illumination at 45°)

Cell parameters: $P4_32_12$ 78.5, 78.5, 37.8 Å

Solvent content: 40%

Cryoprotectant: Ethylene Glycol



Glucose Isomerase crystal

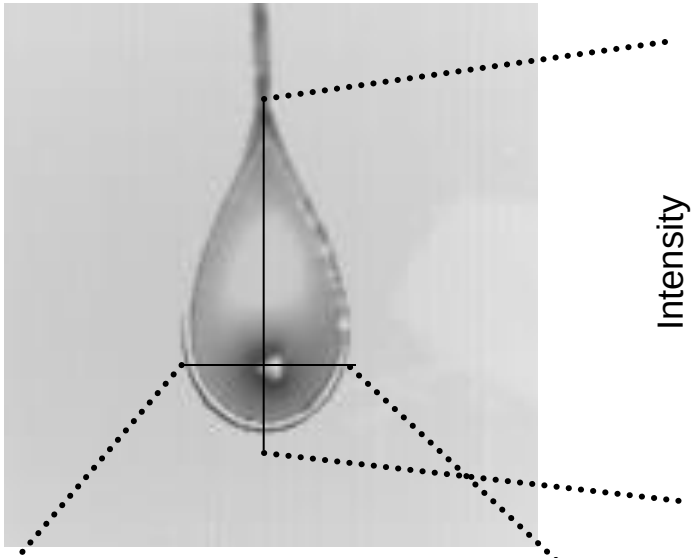
(0.08 x 0.08 x 0.04 mm^3)

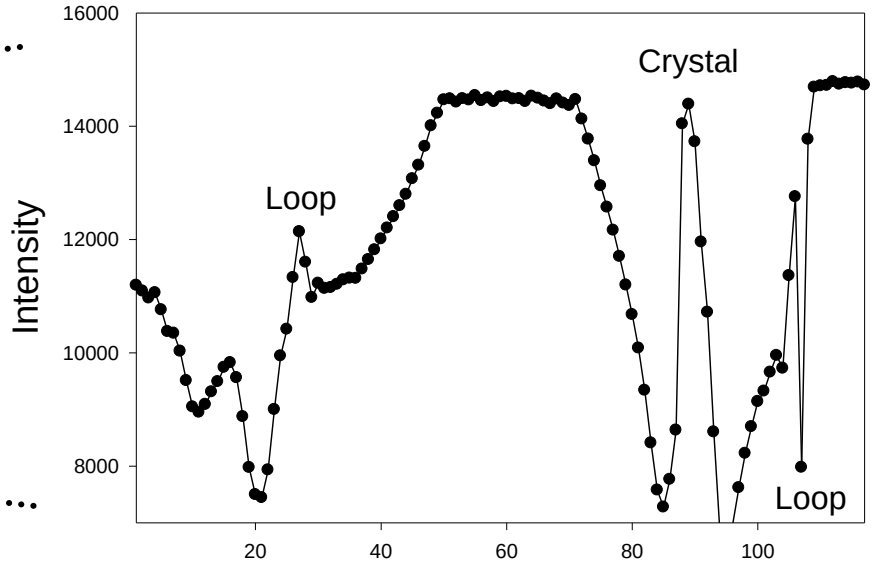
Infrared image (lamp illumination at 45°)

Cell parameters: I222, 92.5, 98.2, 102.2 Å

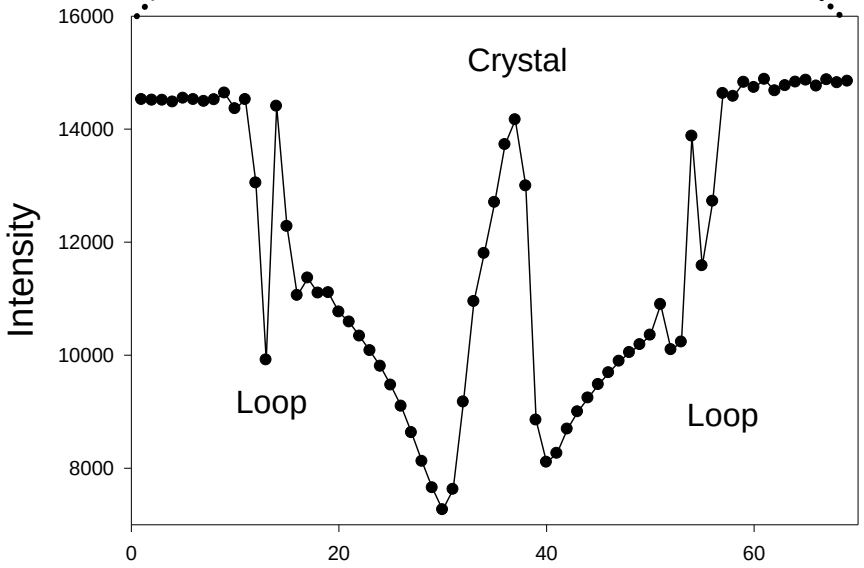
Solvent content: 50%

Cryoprotectant: Isopropanol, glycerol





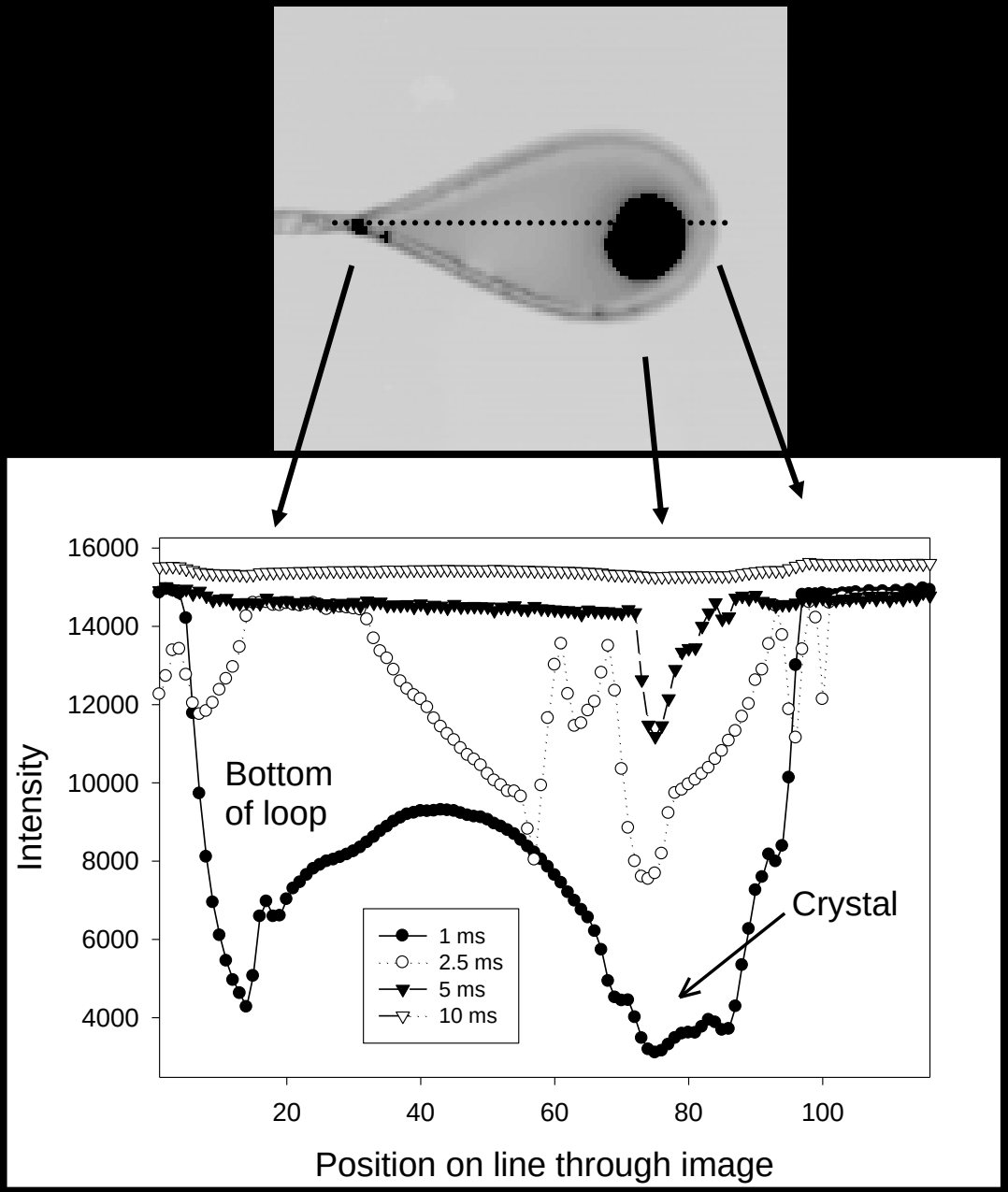
Vertical position across loop (top down in this inverted image)



Horizontal position across loop

In this case the crystal is a “warm” peak in a cold well – it is reflecting infrared radiation.

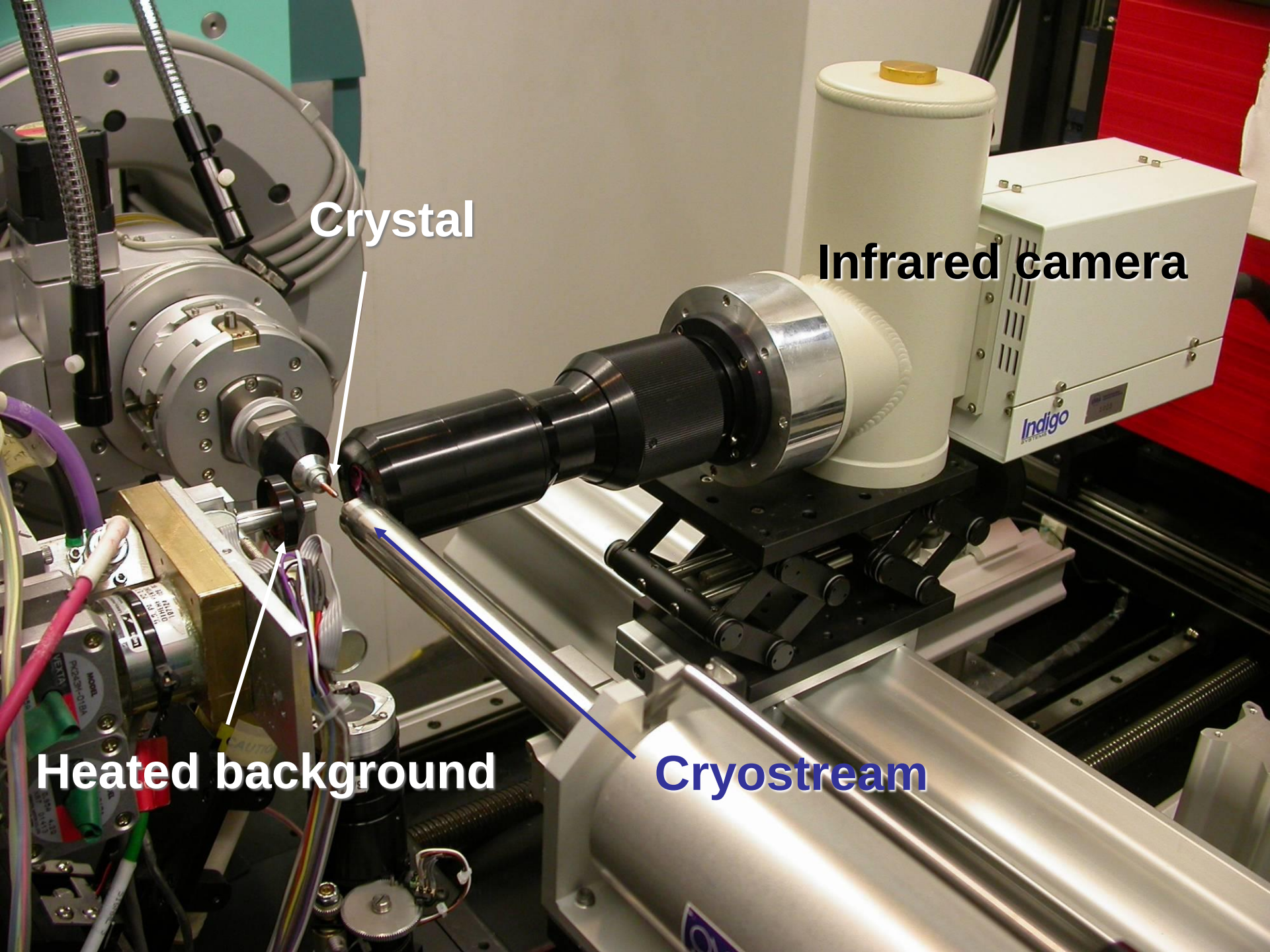
The bottom of the loop appears cold.



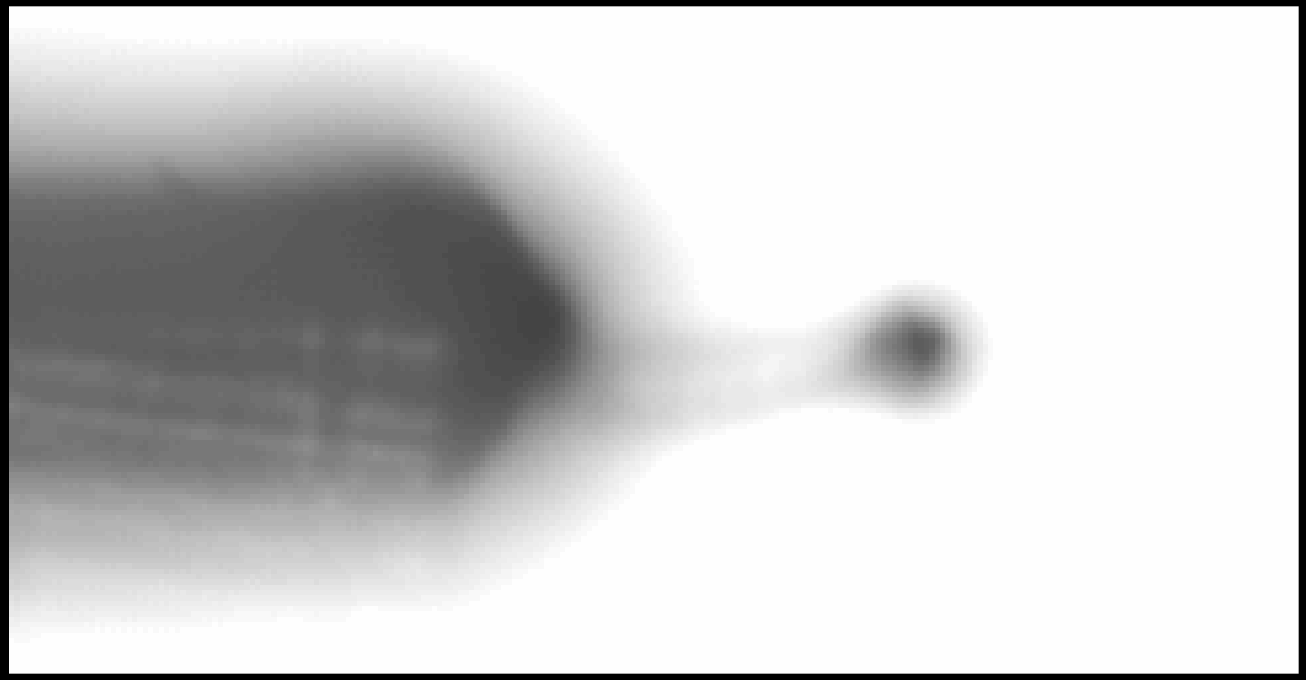
In this case the crystal (also glucose isomerase) is “cold” in comparison to the rest of the image – it is acts as an insulator to the infrared radiation from a lamp source behind.

This lamp is barely warm to the touch but acts as an infrared illumination source without perceptibly heating the crystal.

The bottom of the loop also acts as an insulator and appears cold.

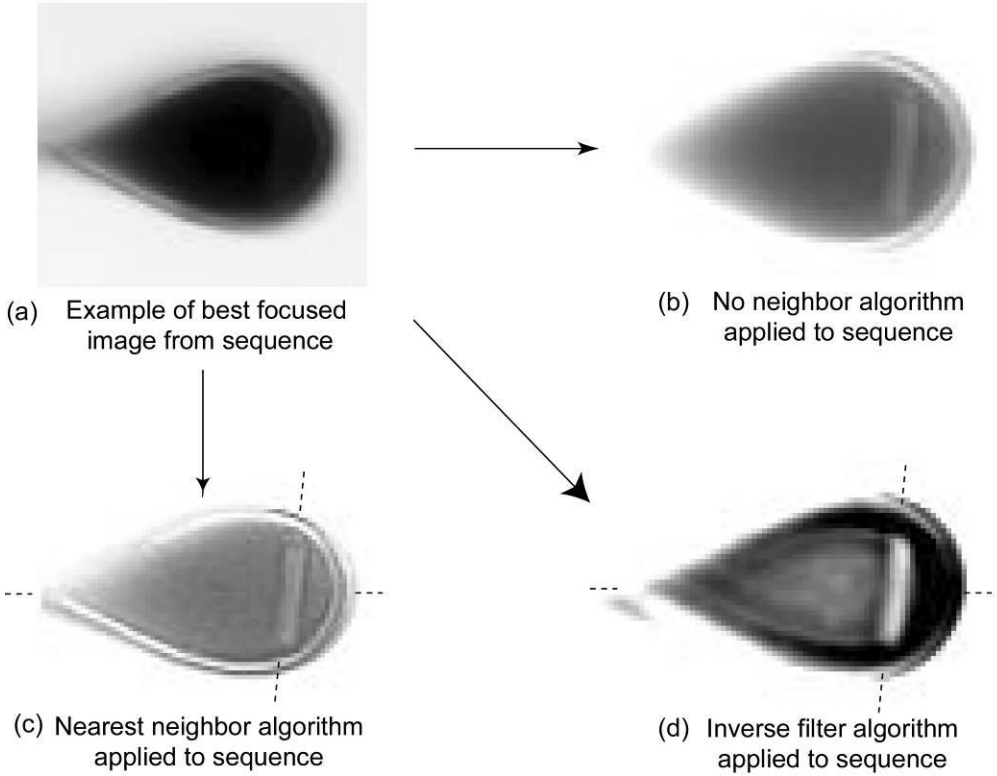

Crystal
Infrared camera
Heated background
Cryostream
Indigo

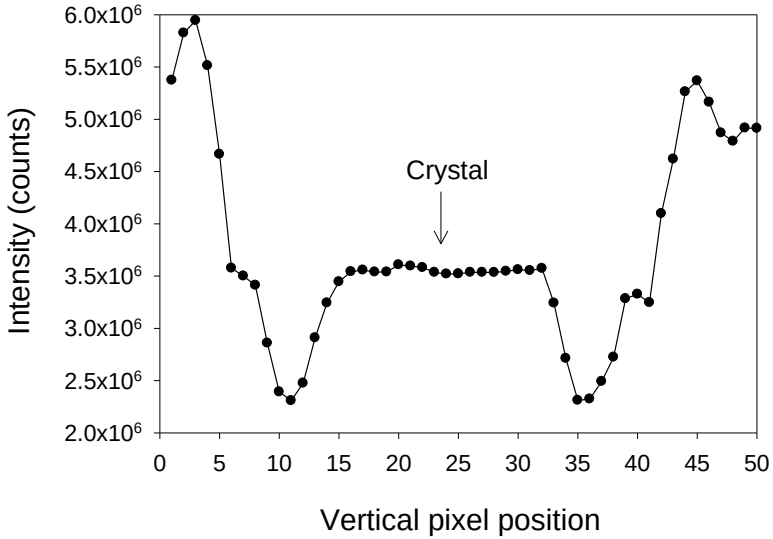
Infrared Image with crystal being moved in 10 μm step size

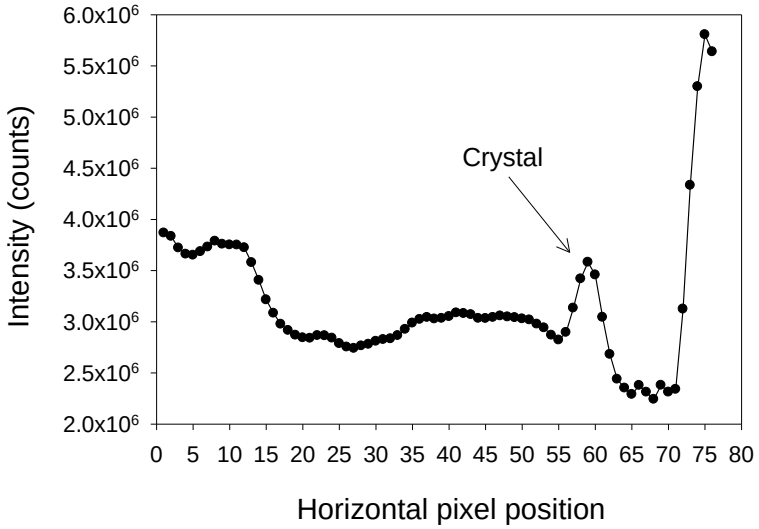


30% glycerol, real sample from structural genomics program.
Loop is 0.1 mm diameter.

Depth of field of is small, less than 10μm.

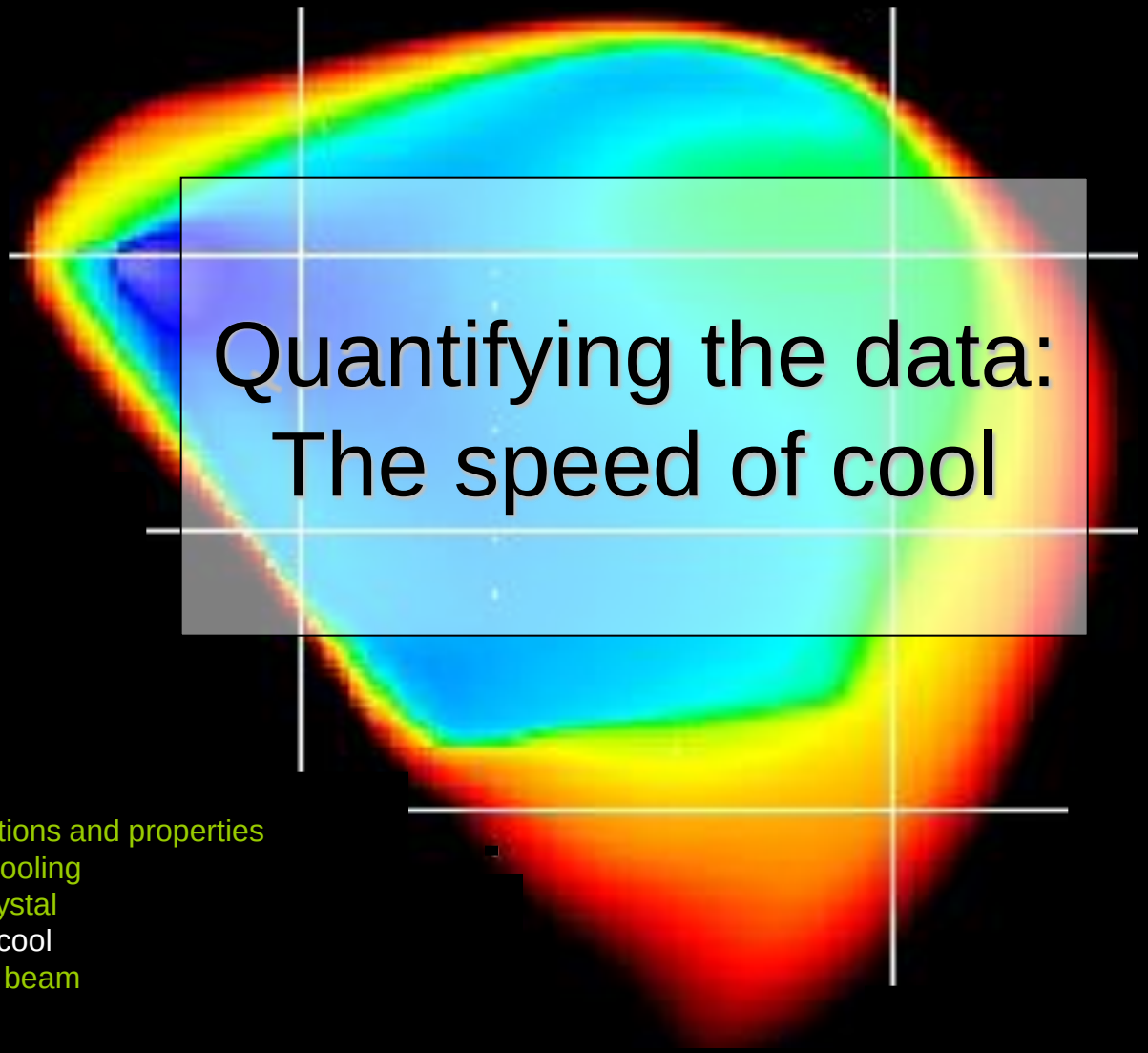

(a) Example of best focused image from sequence
(b) No neighbor algorithm applied to sequence
(c) Nearest neighbor algorithm applied to sequence
(d) Inverse filter algorithm applied to sequence


Intensity (counts)
6.0x10^6
5.5x10^6
5.0x10^6
4.5x10^6
4.0x10^6
3.5x10^6
3.0x10^6
2.5x10^6
2.0x10^6
Crystal
0 5 10 15 20 25 30 35 40 45 50
Vertical pixel position


Intensity (counts)
6.0x10^6
5.5x10^6
5.0x10^6
4.5x10^6
4.0x10^6
3.5x10^6
3.0x10^6
2.5x10^6
2.0x10^6
Crystal
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
Horizontal pixel position

Observations

- The complete crystal was seen as the camera was translated so different parts of the loop were in focus – the optics have a small depth of field.
- The whole crystal was never completely in focus due to the small depth of field.
- A single focused image can be generated using image processing techniques from the sequence of images at 10μm focal points.
- The background illumination and shielding was not optimized in this case.
- The crystals were approximately the same size as the loop used.
- There is still a way to go to make the system of practical use at the synchrotron.



Quantifying the data: The speed of cool

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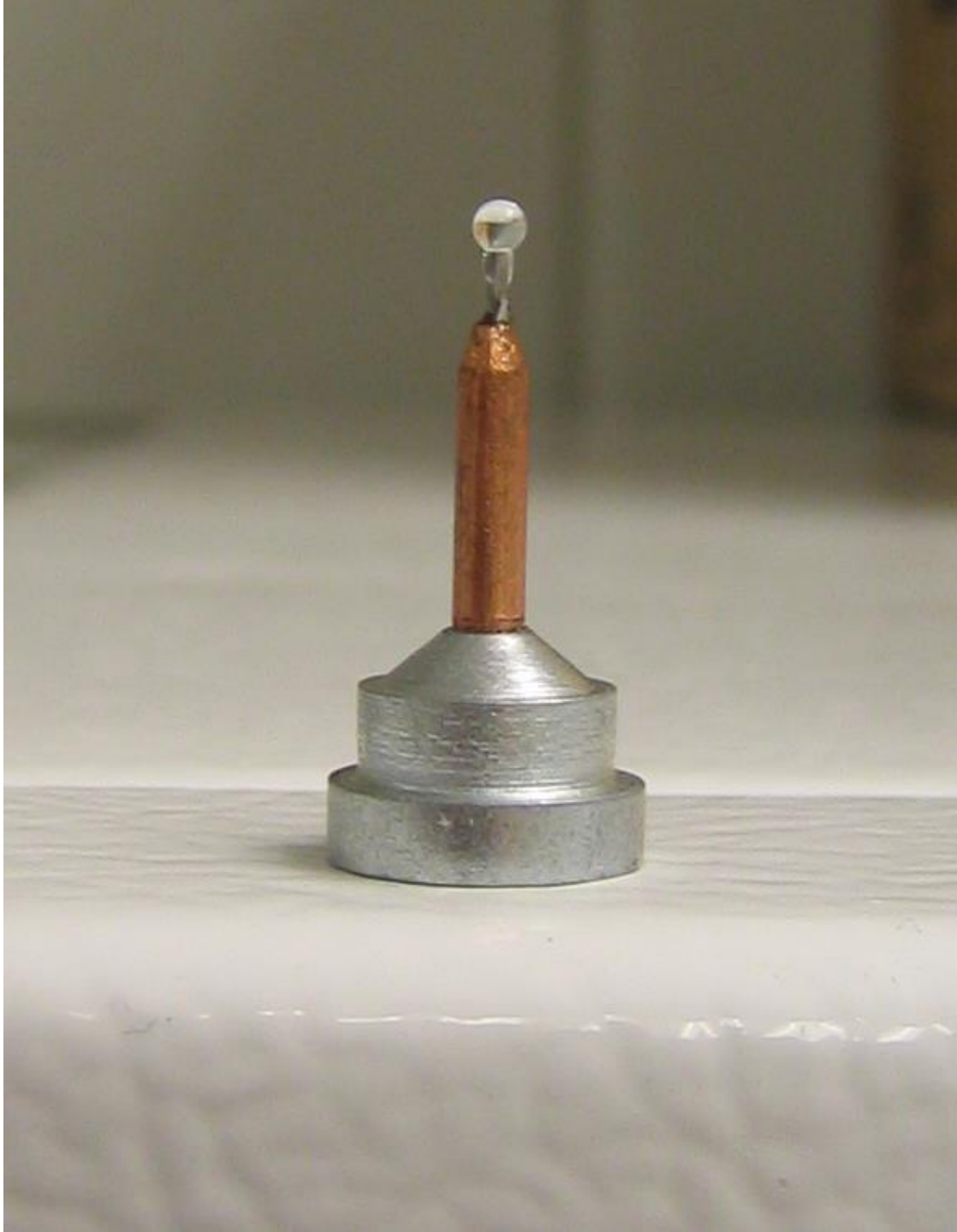
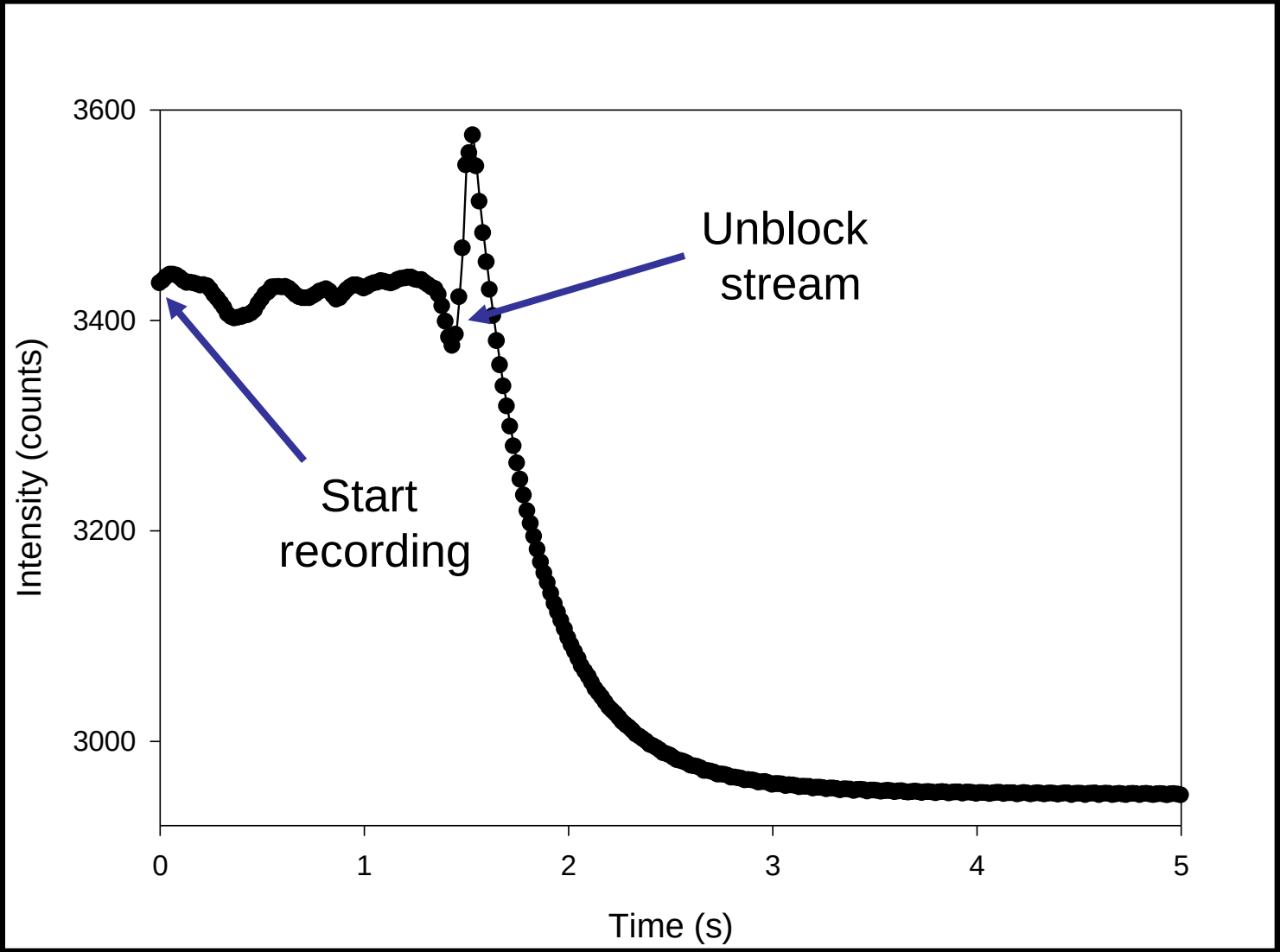
Test samples for model development

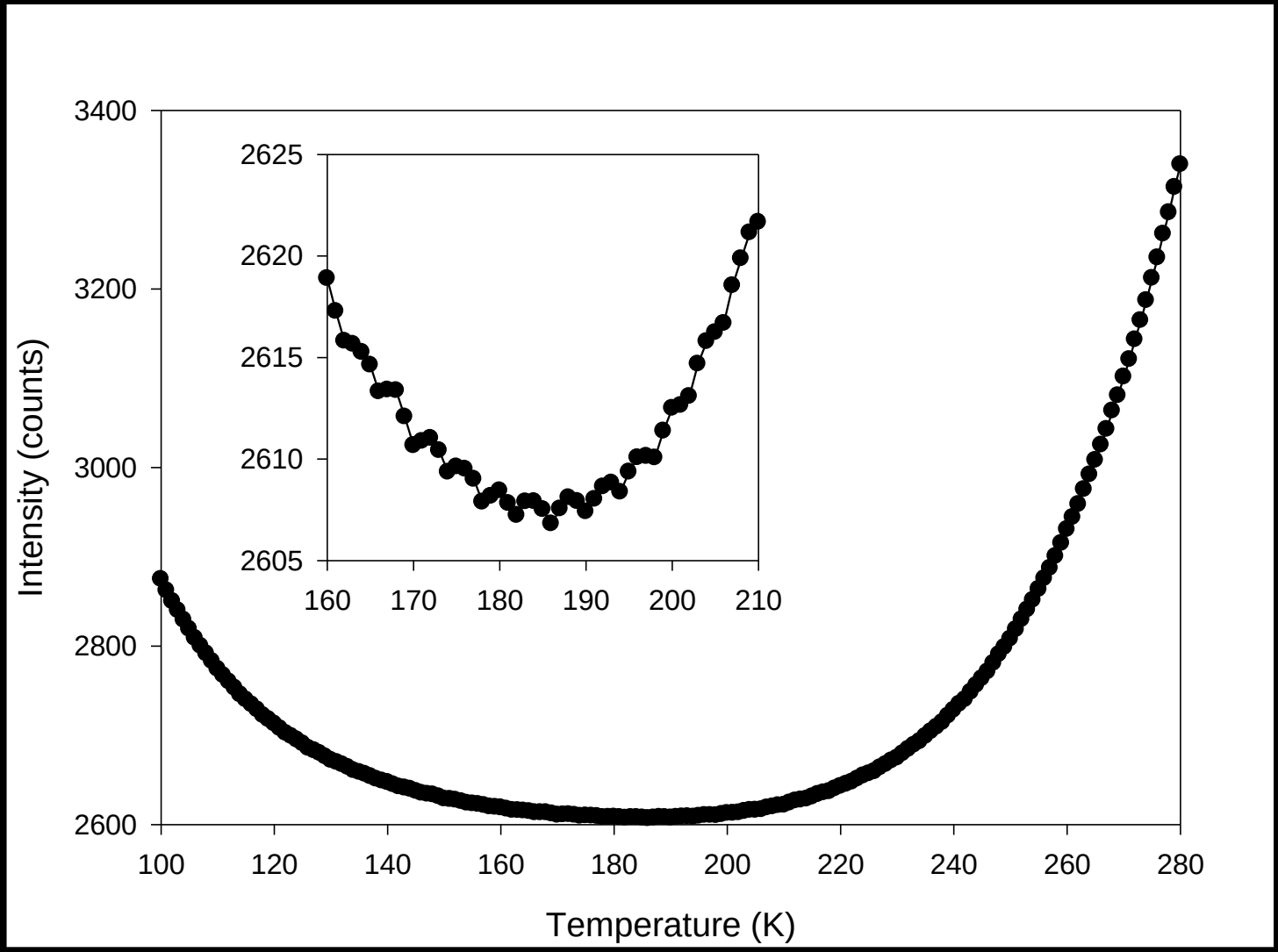
- Glass beads of 0.5, 1.0 and 2.0 mm diameter.
- Mounted on standard Hampton Research Cryomounts.
- Directly glued to the cryopin using Epoxy resin.
- Chosen as spherical samples allow for easy modeling.
- No special care needed to maintain glass at 100K – can study the system as a function of temperature.



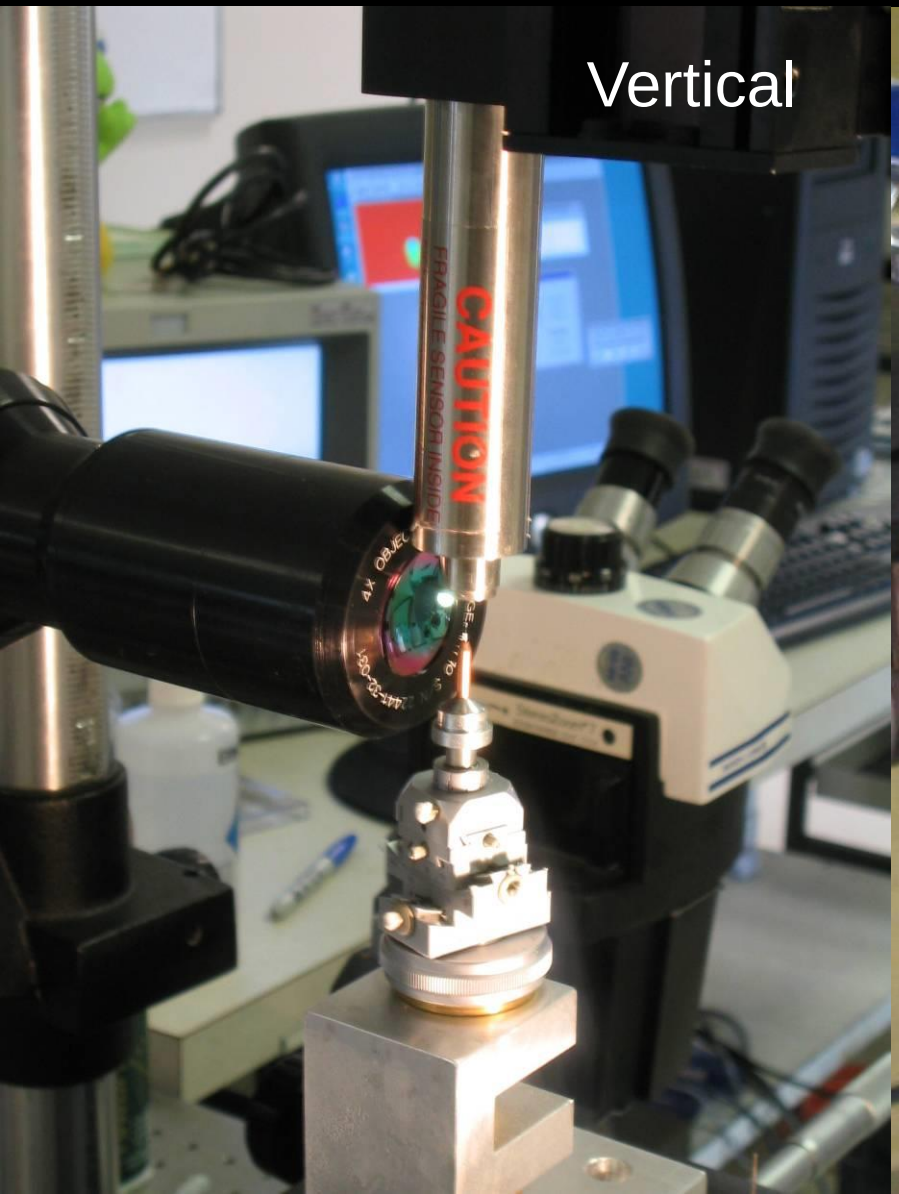
Data profile for 0.5 mm diameter glass bead flash cooled



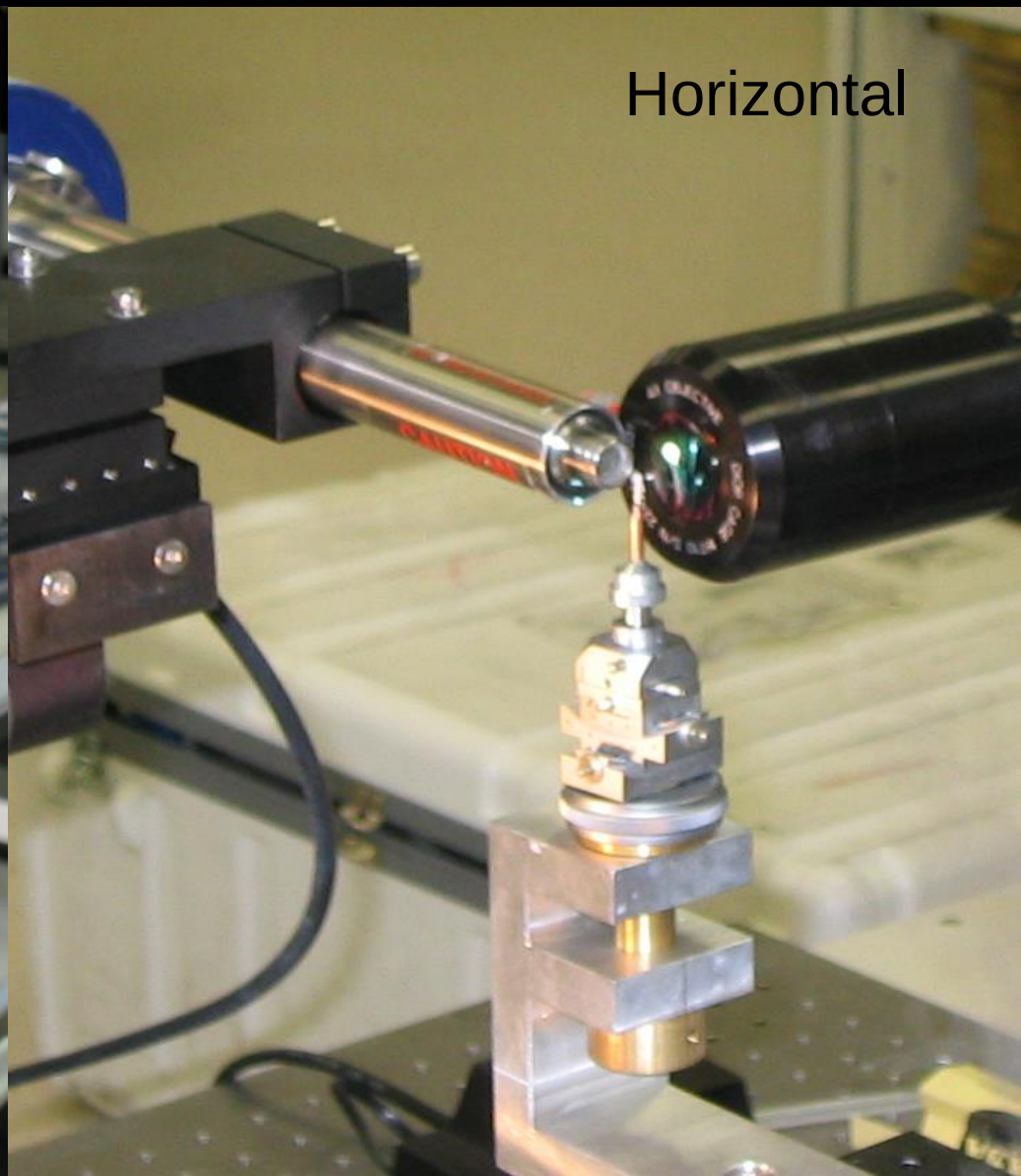
The bead is then warmed up at a known rate and the data collected for intensity to temperature calibration



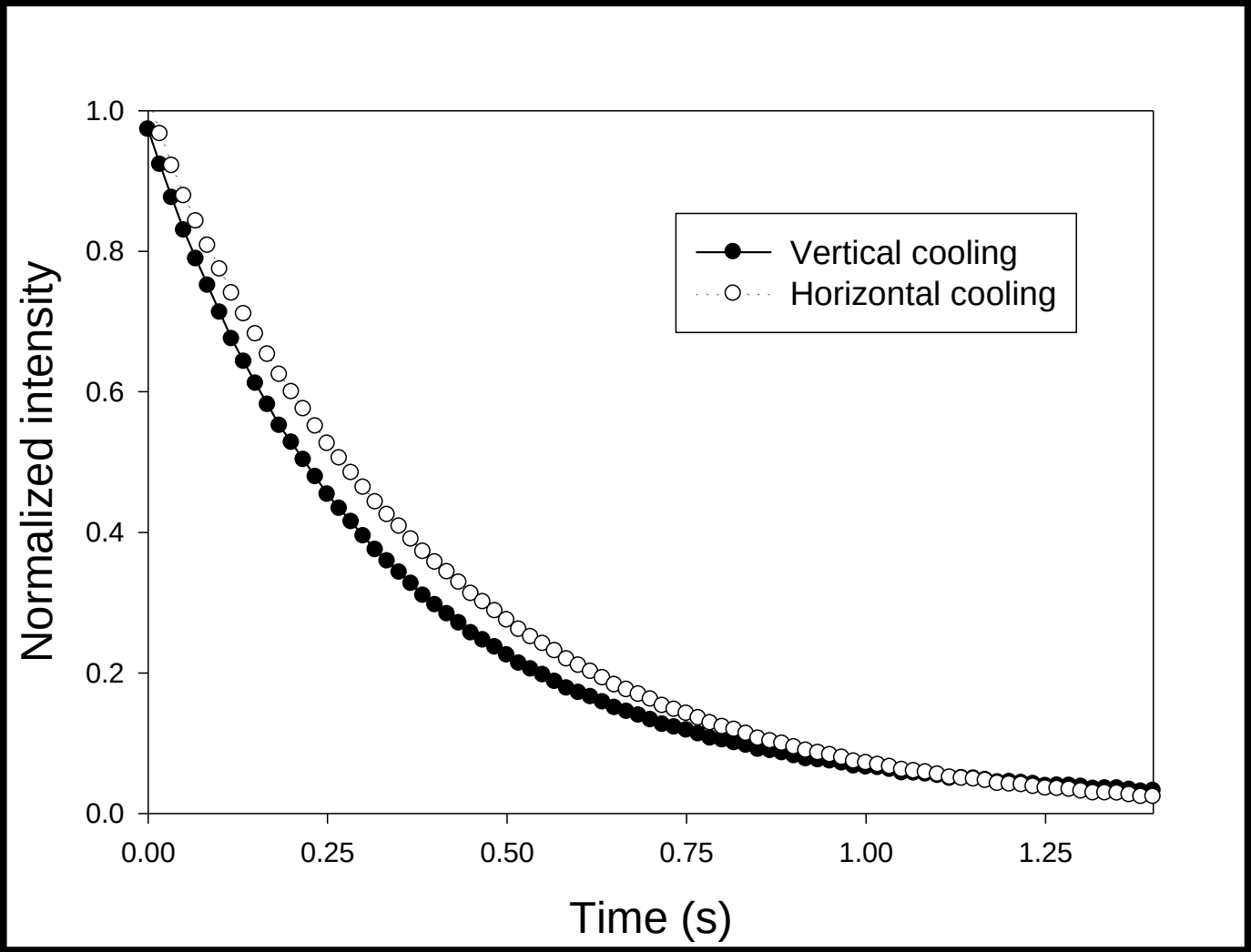
Before we look at rate which direction of cooling is best?



Vertical

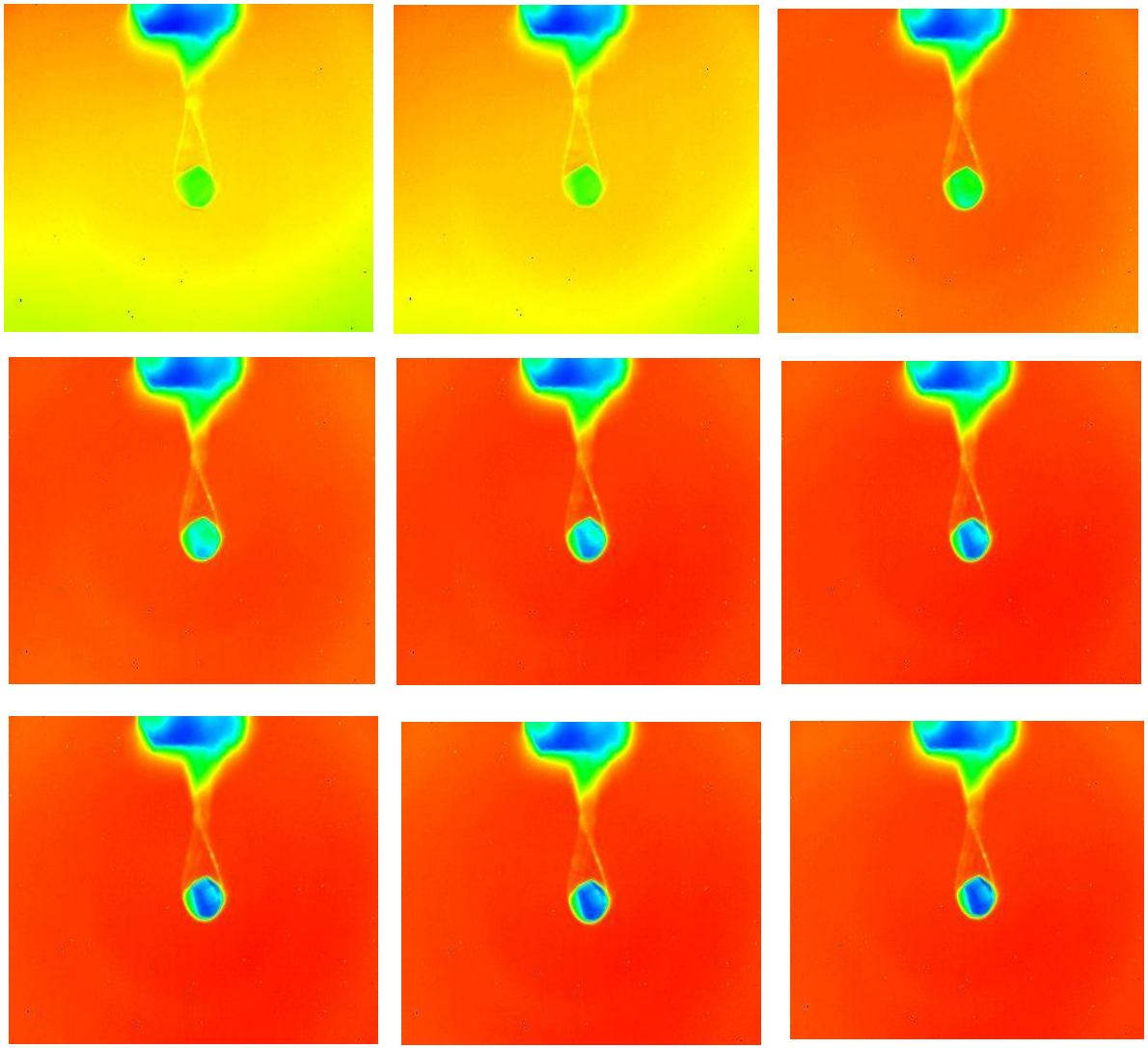


Horizontal



Little difference between vertical and horizontal cooling – direction is unimportant.

Example of a cryocooled lysozyme crystal



The crystal is 0.40 x 0.32 x 0.19 mm mounted in a 0.5 mm Hampton Research cryoloop.

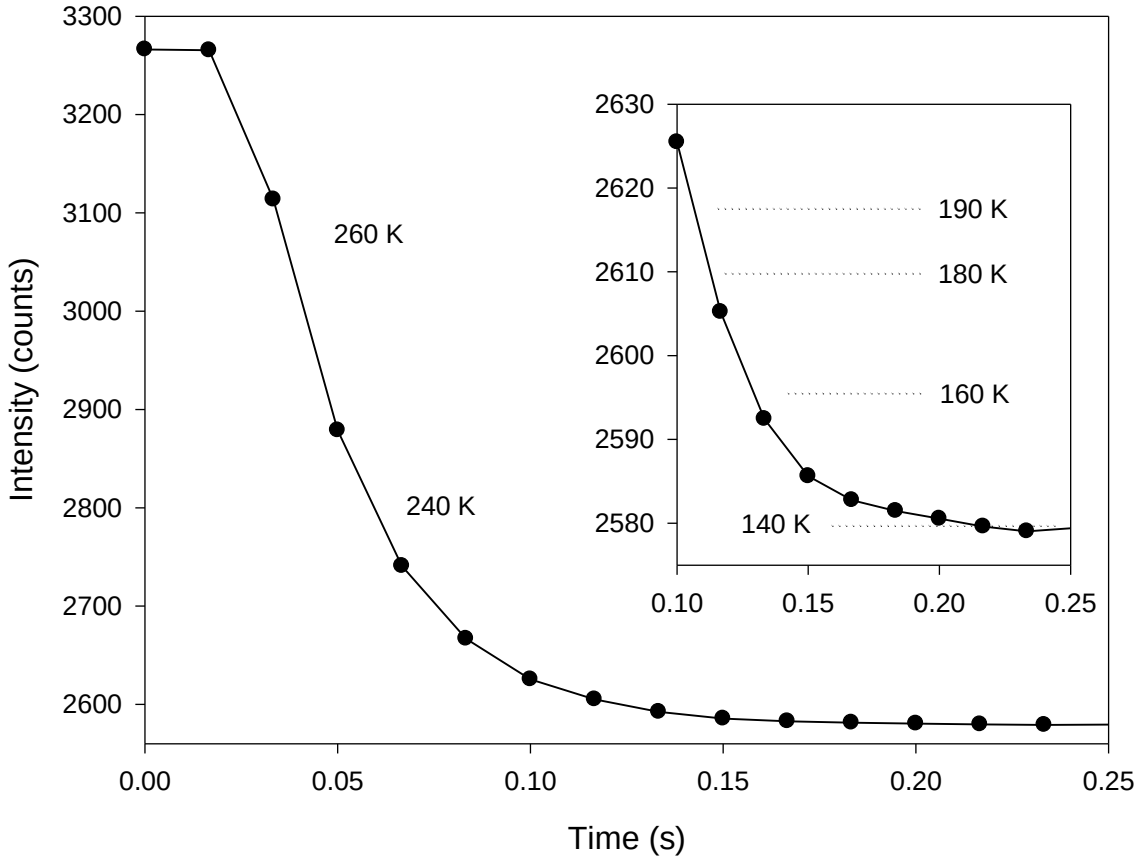
The images are shown inverted due to the microscope objective.

The images are false colored with blue (low intensity) being cold and red (high intensity) being hot.

Each image is taken 1/60 of a second apart.

The cryostream, at 100K, cools the crystal vertically from above (below in the image)

Lysozyme crystal cooling

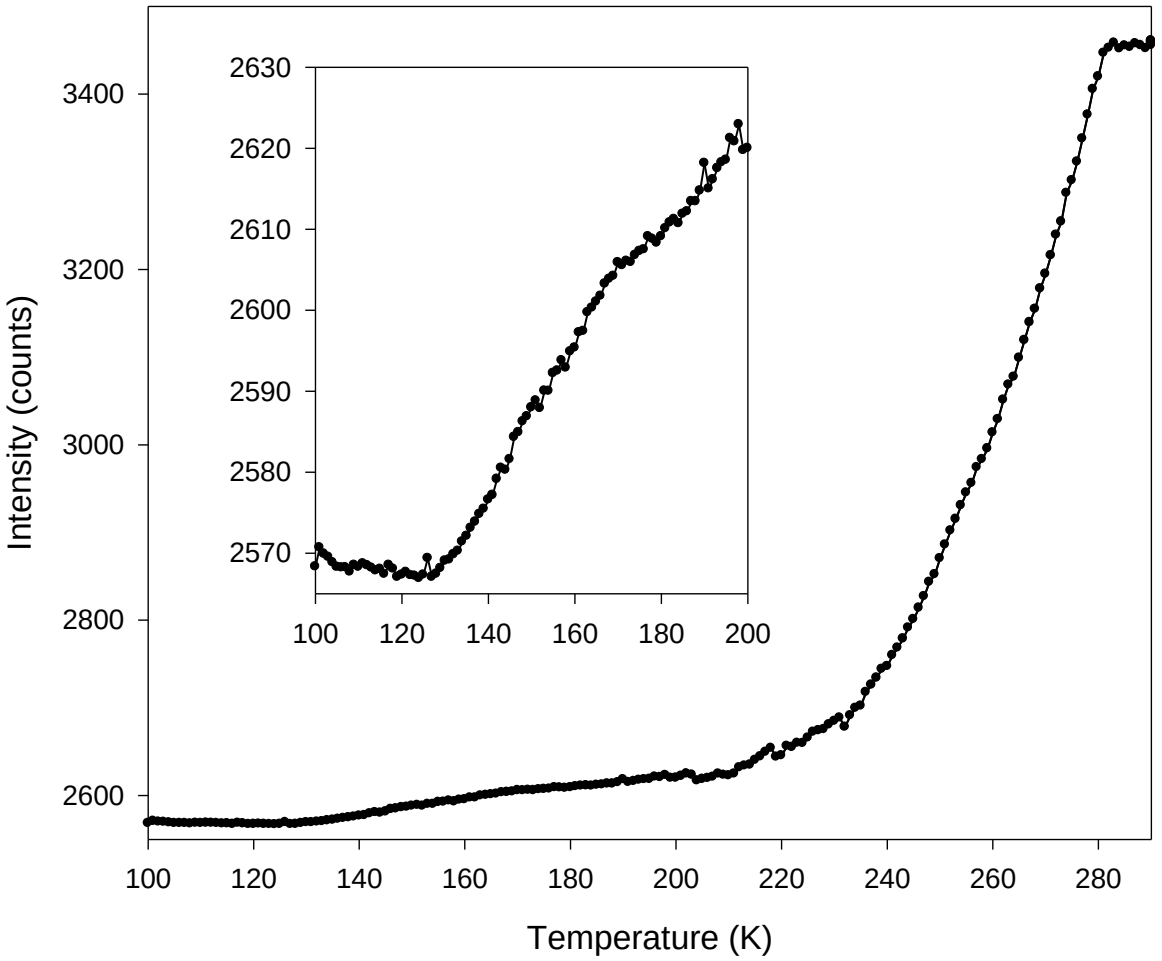


Experimental protocol similar to the glass bead:

1. Set stream to 100 K.
2. Block stream
3. Mount crystal
4. Focus
5. Start data collection (1000 images at 60 Hz)
6. Program stream to warm up at 2 K per minute once cooling data collection is complete.
7. Collect an image every 30s (1 K) from 100 K to 290 K

The cooling data is then calibrated from the warm up data. Due to background thermal radiation and crystal position each crystal has a unique value of intensity at a given temperature.

Lysozyme crystal calibration

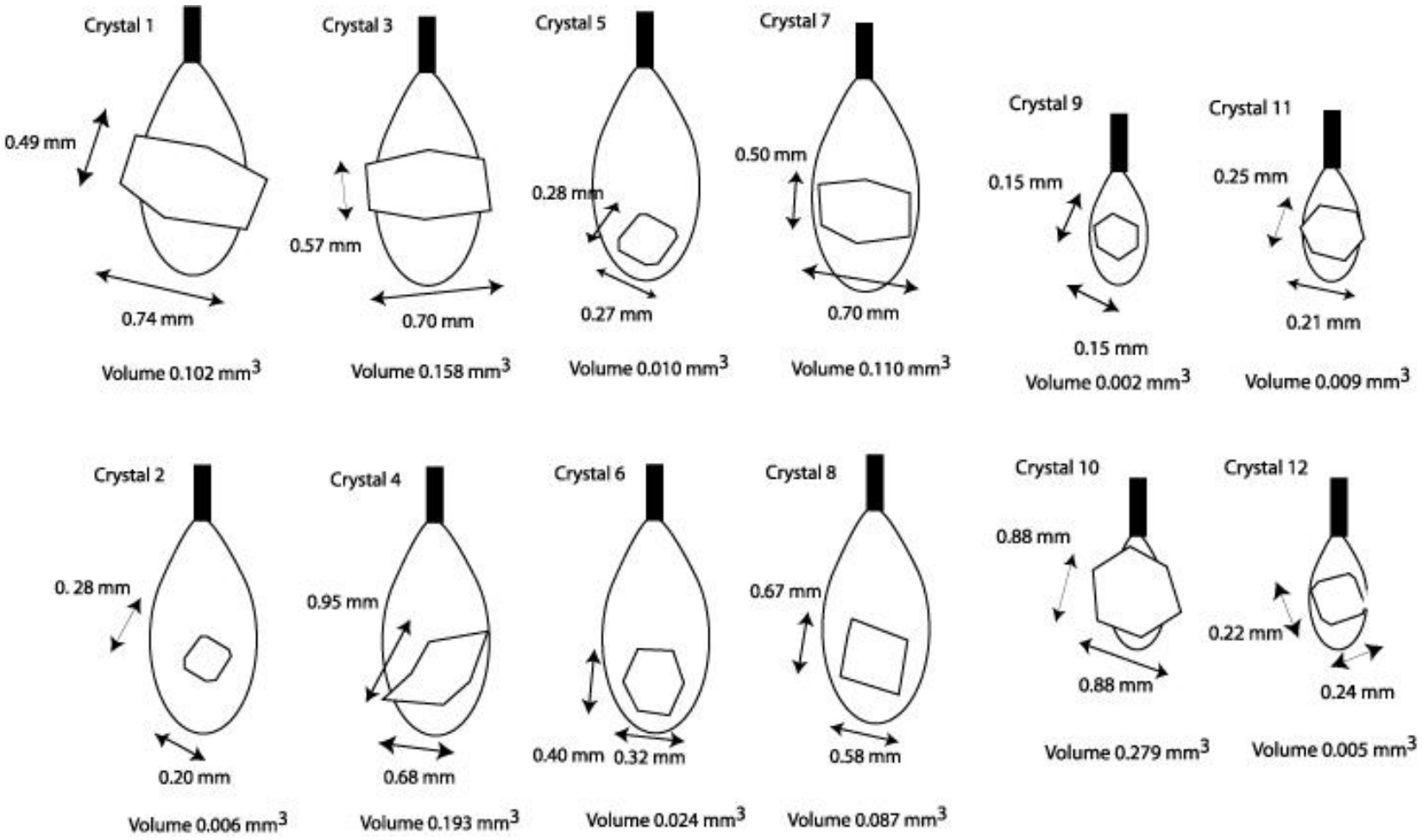


The calibration reveals that for lysozyme crystals the sensitivity of the camera is approximately 135 K.

This sensitivity is a property dependent on the non-ideality of the sample as a black body, i.e. emissivity. This is the ratio of the radiation emitted to that predicted by Plank's law.

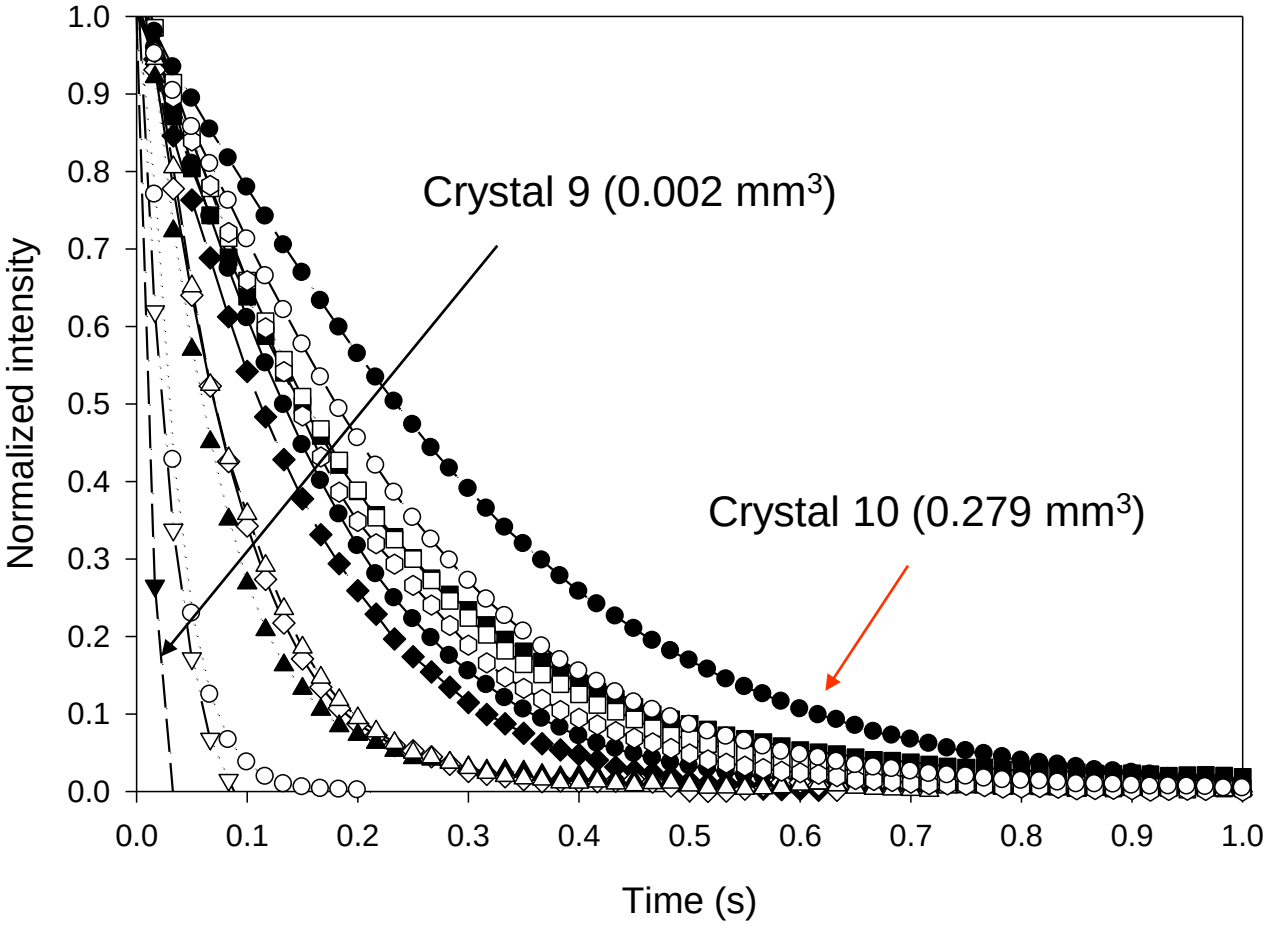
This emissivity and therefore camera sensitivity is sample dependent

Diagram of lysozyme crystal samples studied

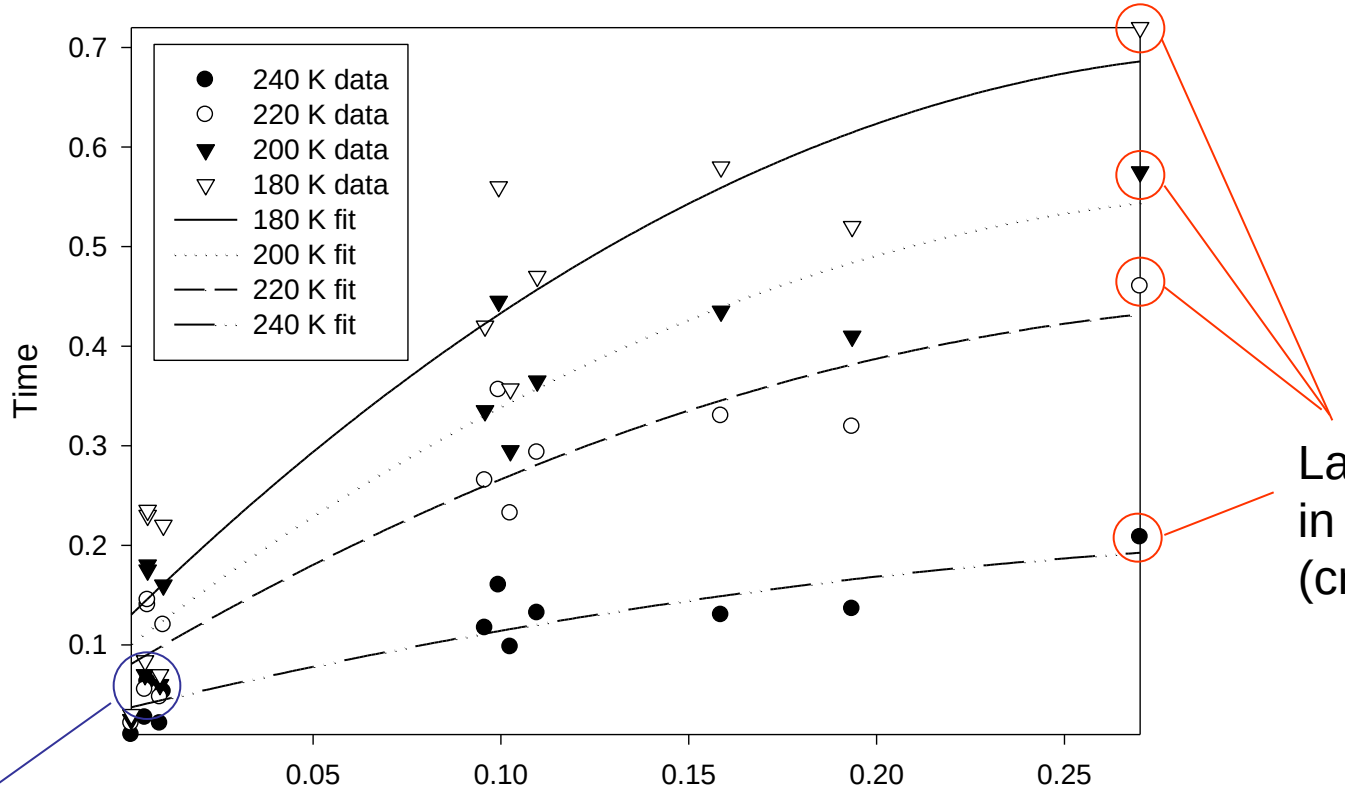


Two different loop sizes were used, 0.5 mm and 0.2 mm diameter.
Typically the smallest dimension is into the picture.

Normalized intensity profile of crystal samples during cooling.



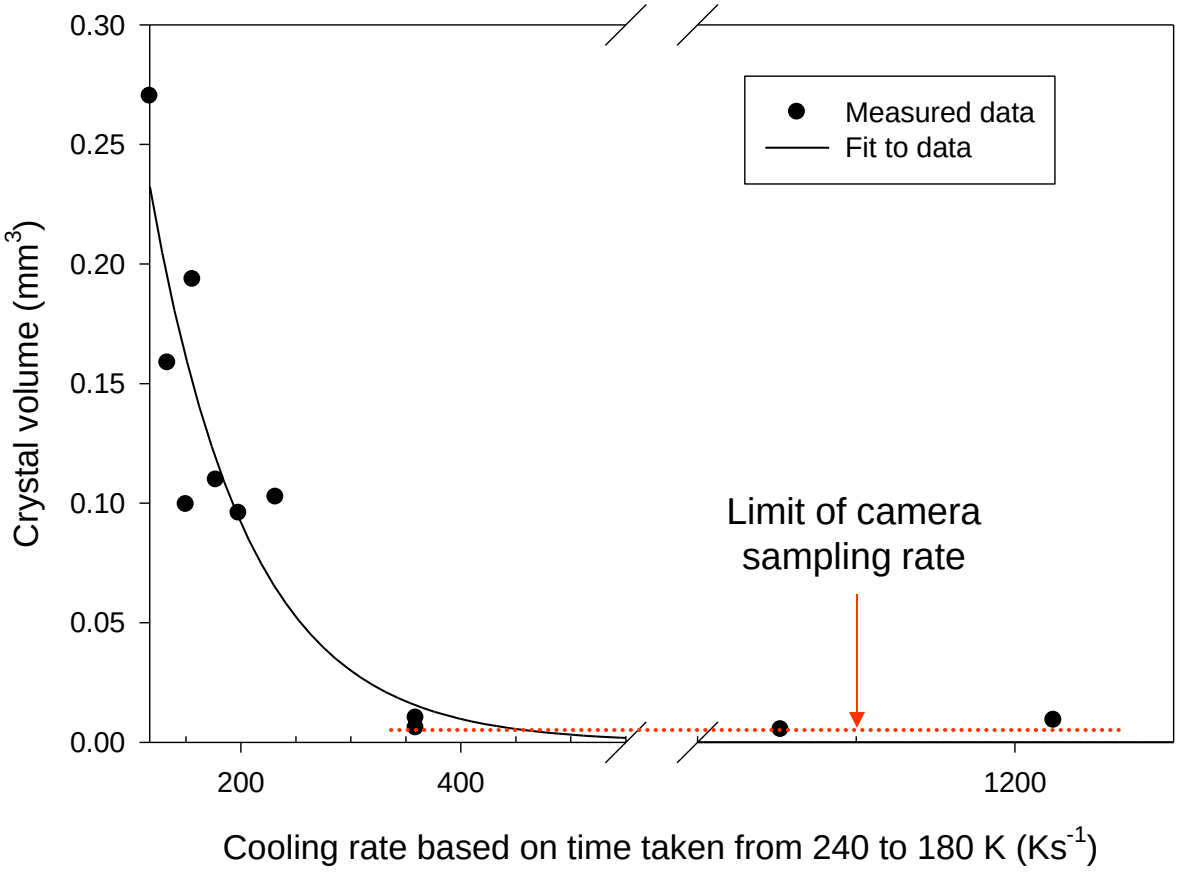
Cooling as a function of crystal volume



Large crystal in a small loop (crystal 10)

Small crystal in large drop (crystal 2)

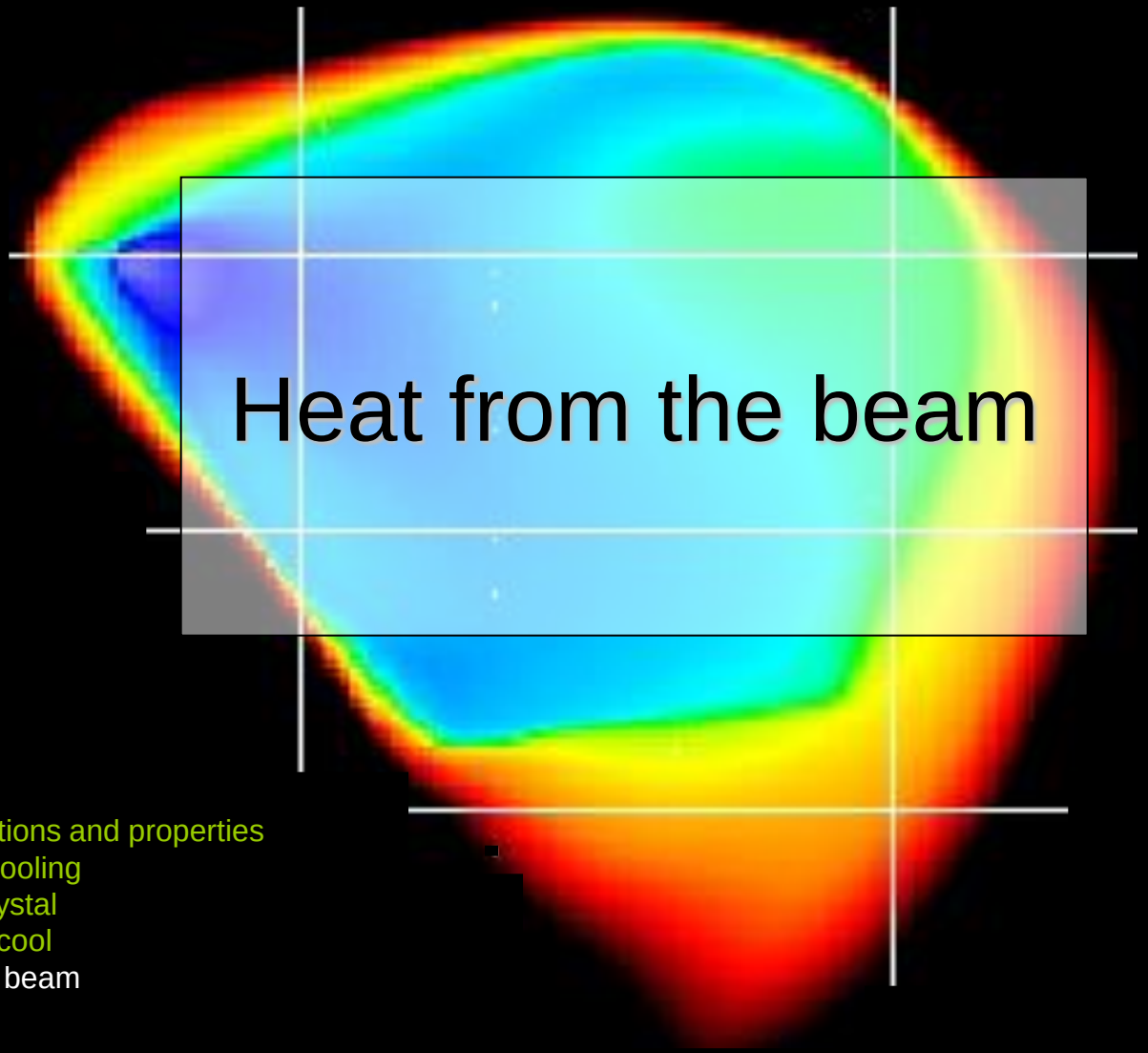
The speed of cool….



Note that for the fastest cooled crystals the data is at the limit of the camera in the setting used.

The camera can image digitally at faster rates, full frame 150 Hz, half size frame 300 Hz (images every 0.0067 and 0.0033 seconds respectively) but the video output is unavailable. It is not possible to focus the crystal easily.

The smallest crystals await optics with improved depth of field.



Heat from the beam

- Infrared definitions and properties
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Beam Heating Experiments

Initial studies

The South East Regional (SER) -CAT

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

For the data presented here

The Structural Biology Center (SBC) -CAT

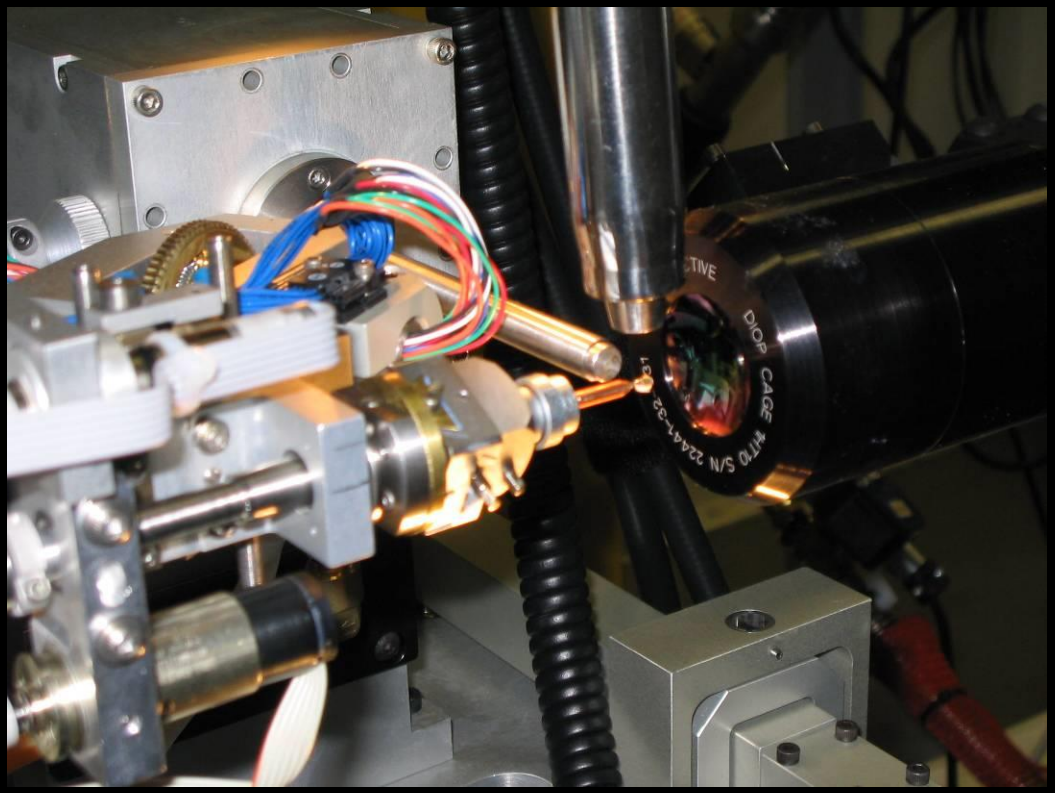
Beamline 19-ID

Energy 6.5 Kev (1.9 Å)

Ring current ranging between 101 and 103 mA

Intensity of 3.24 x 10^{12} Ph/s.

Experimental

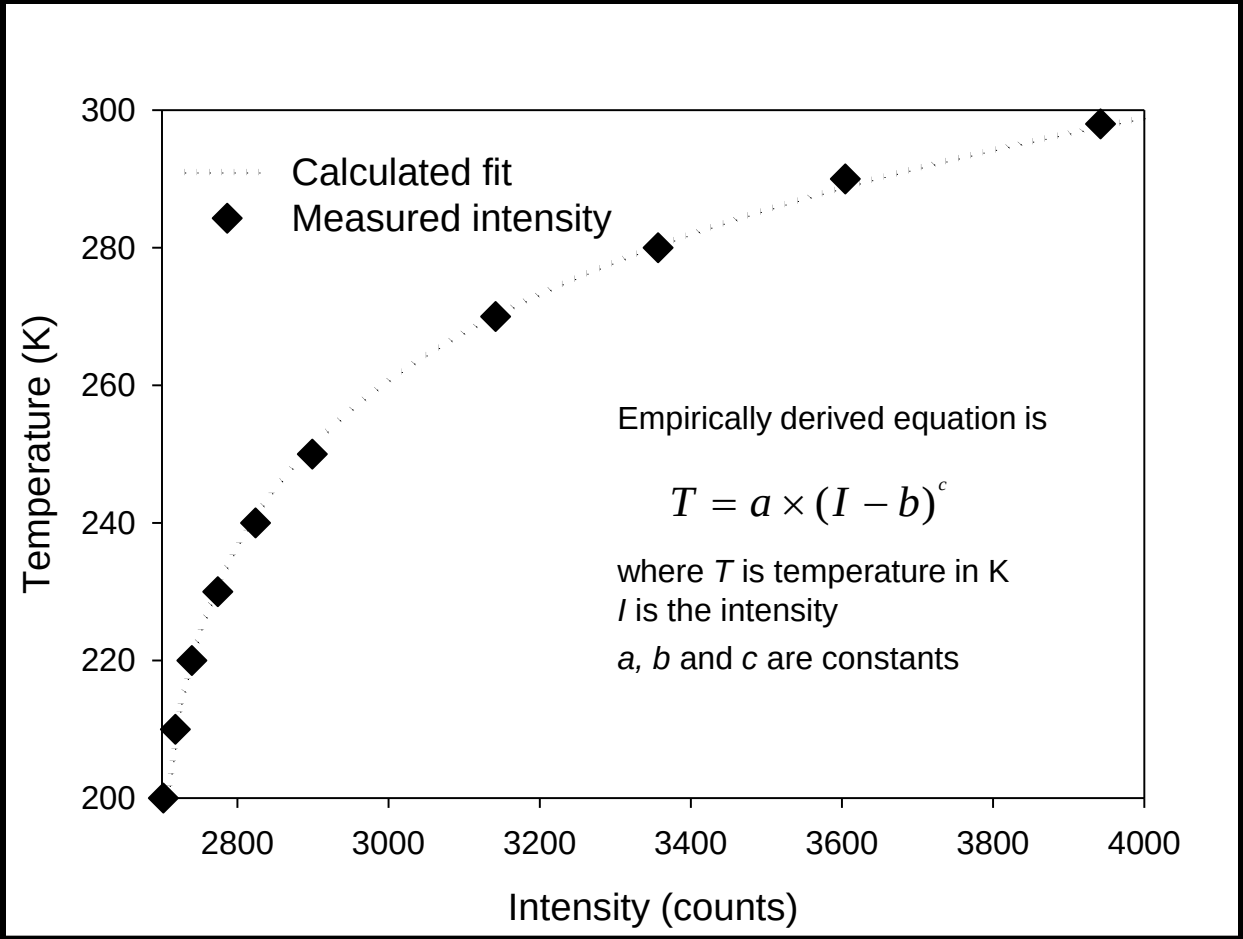


- Advanced Photon Source beamline 19-ID Structural Biology Cat
- Oxford 700 cryostream used to cool sample
- Samples imaged with thermal imaging camera

Glass Bead Samples and Protocol

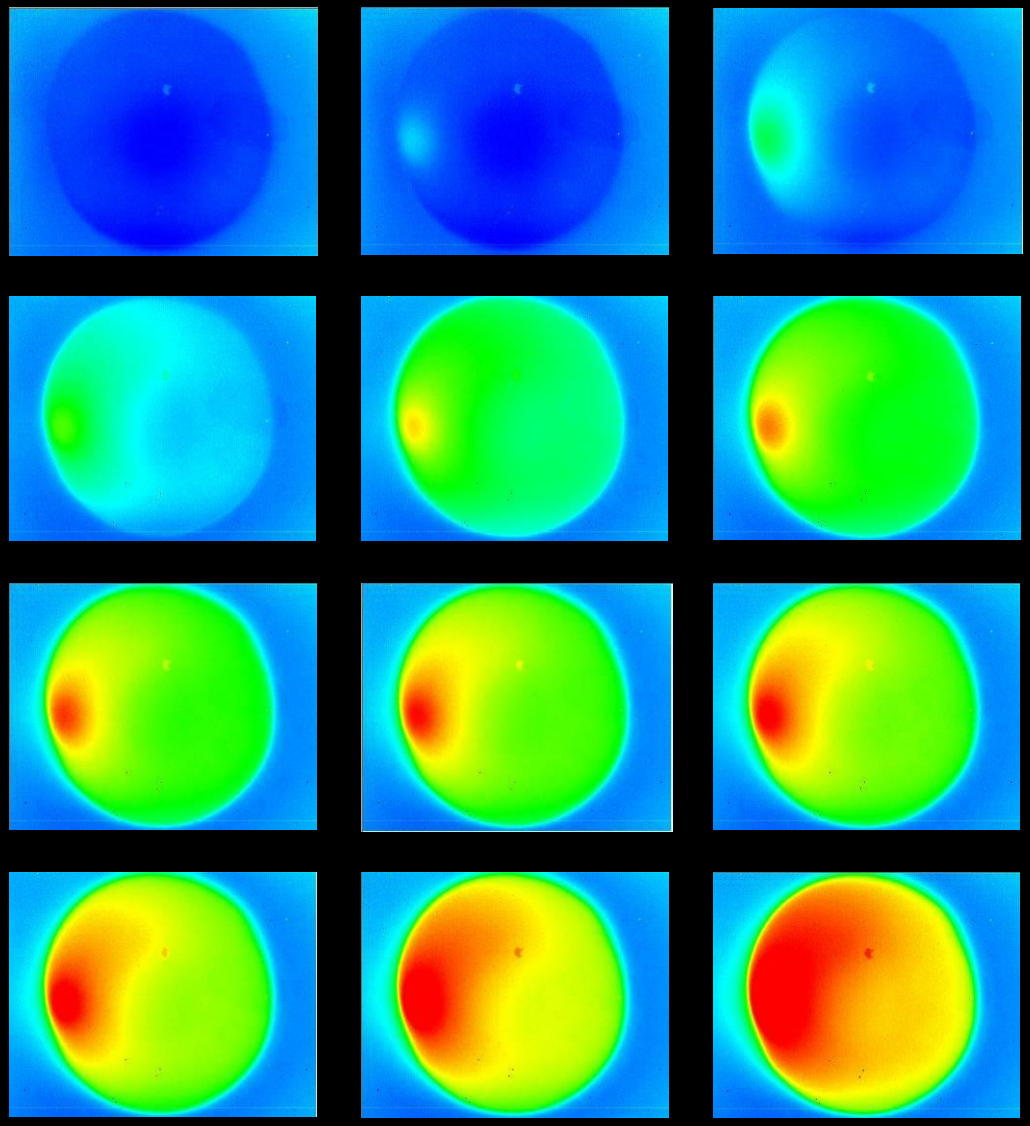
- Sample 1: 2mm diameter glass bead
  - imaged at 100K with no beam (steady state calibration point)
  - Imaged with shutter opening (time resolved)
  - Imaged after shutter had been open for 1 minute (steady state).
  - Measurements repeated in 10K steps up to 290K
  - Final measurement with the cryostream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

Temperature calibration



Bead at known temperature when shutter is closed. The intensity determined at this temperature and a calibration curve of intensity versus temperature calculated.

Shutter Opening



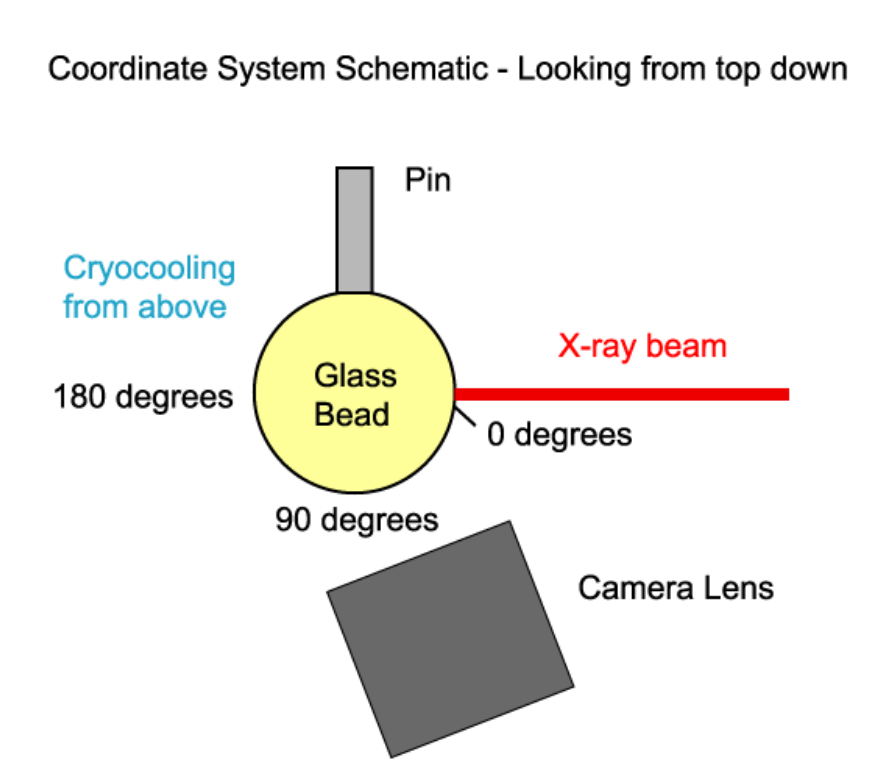
2mm glass bead at room temperature with no cryostream flow.

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

Temperature rise is 50K over 24 seconds.

Extreme case!

Experimental



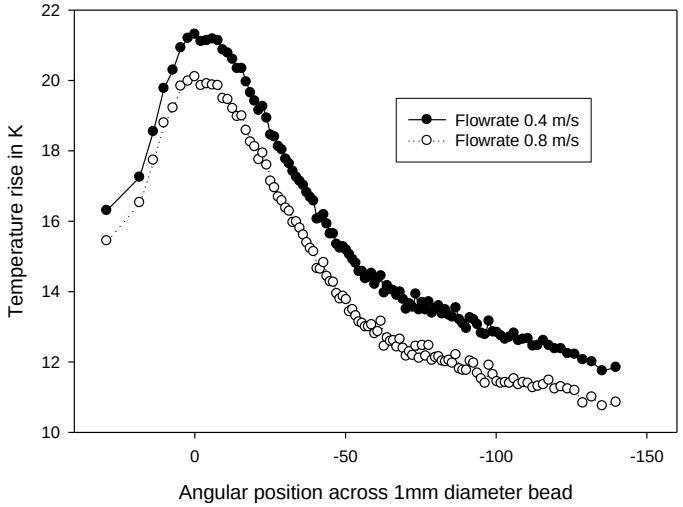
•For the 2mm glass bead the cryostream was set to 100K and a still image taken of the bead before the shutter was opened. A set of sequential images was then recorded as the shutter was opened. Finally a single image was taken with the shutter open when the bead had reached a steady state.

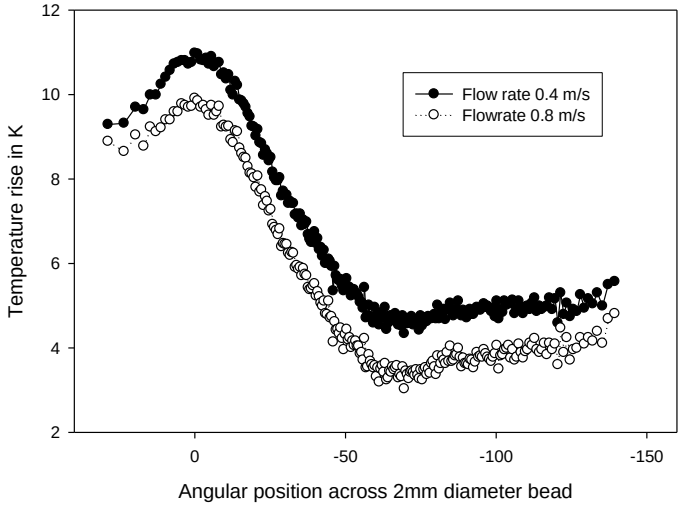
•The same protocol was repeated with cryostream settings in 20K increments until 200K and then 10K increments until 290K.

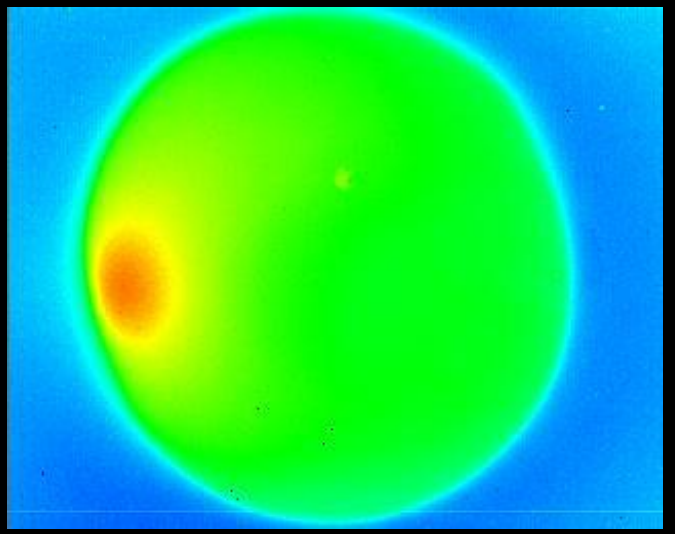
•At 290K an additional experiment was made with the cryostream at double flow. The cryostream was then switched off and the bead allowed to warm to ambient temperature, 298K, and the experimental measurement repeated.

•Following this the 2mm bead was replaced with a 1mm bead. Similar experiments were repeated.

Steady State Results







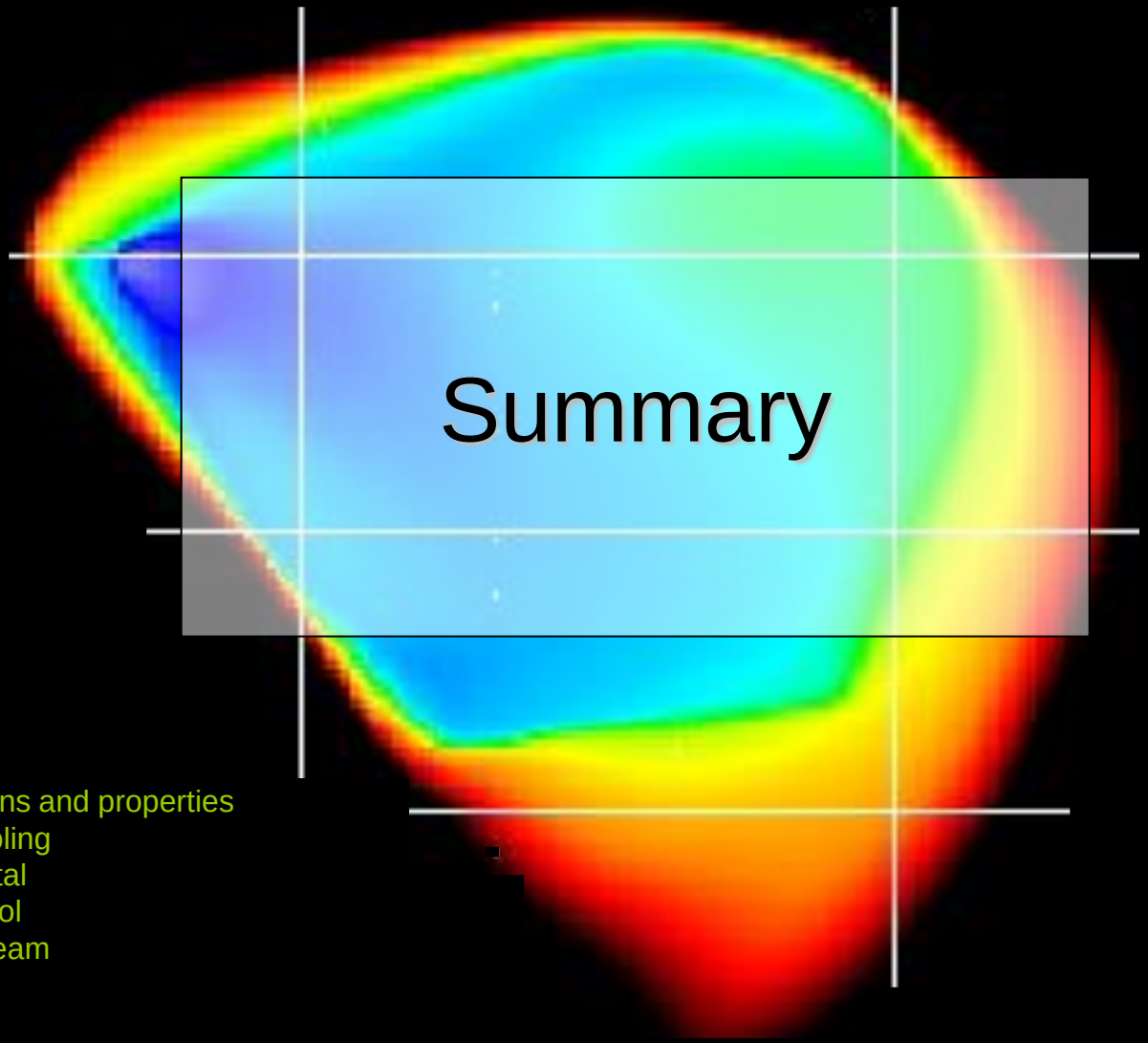
For 1mm glass bead, beam heating peaked at ~22K. For the 2mm case the heating peaked at ~11K. Doubling the gas flow rate decreased the heating but not by a significant amount.

With real crystals?

- Radiation damage causes significant changes in the infrared properties of the crystal. Also seen in the visible spectrum as color changes.
- We have been unable to calibrate the temperature of the crystal to the intensity recorded by the camera.
- The next step is to use and infrared laser to put a heat load onto a real crystal and measure the thermal properties for modeling protein samples.

Dynamic modeling?

- We have presented steady state data. We also have dynamic data with a movie over time of the heating due to shutter opening.



Summary

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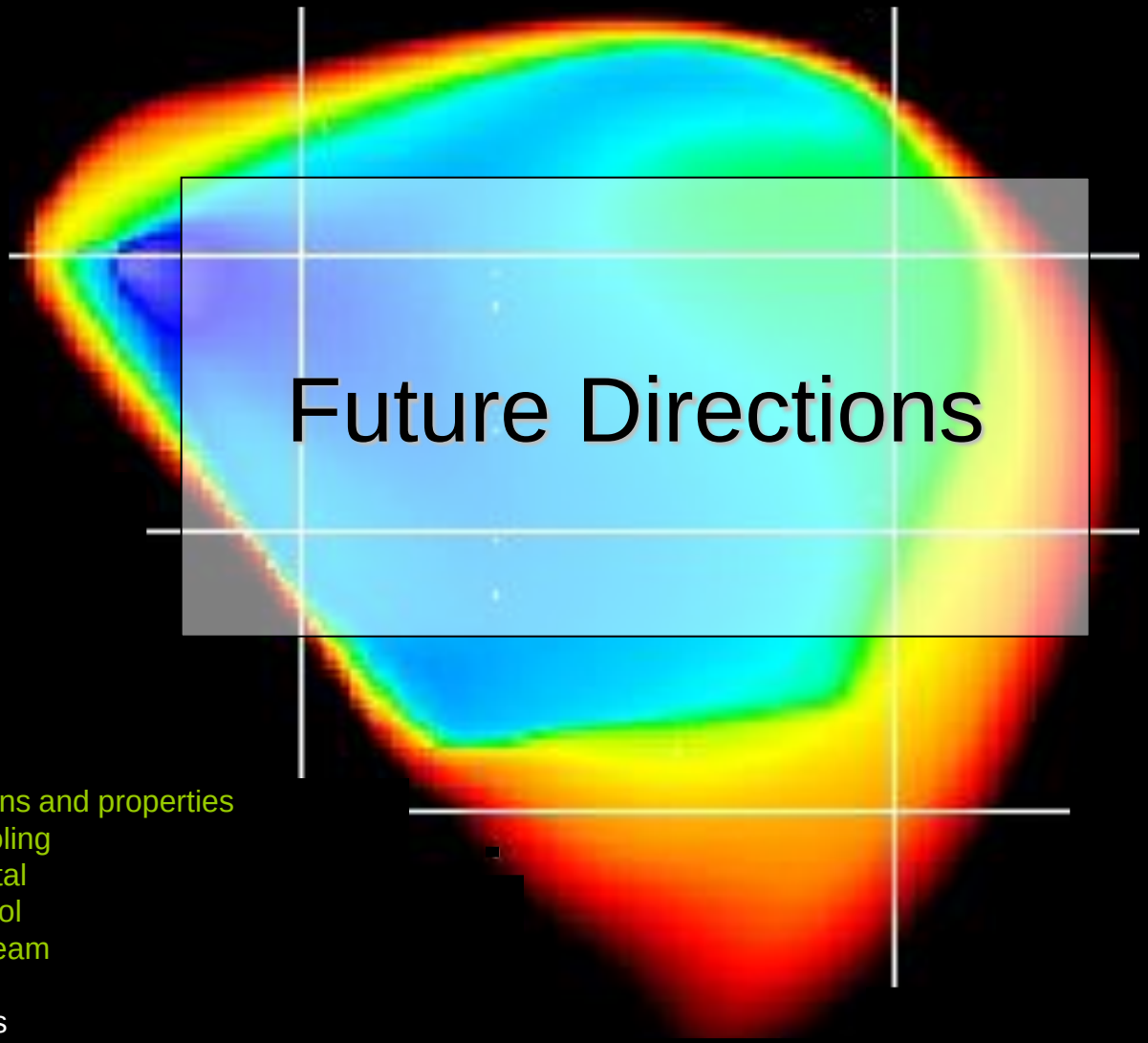
Summary

- Infrared imaging has been used to successfully to:
  - Show the process of cryocooling as a wave across the crystal.
  - Identify crystals in loops.
  - Determine that cooling direction is not an important variable.
  - Show that small crystal cool much more rapidly than larger crystals.
  - Determine the spatial heat load on a model sample when the shutter is opened.
- It is difficult to do quantitative studies
- There are problematic areas with the technique, i.e. very small depth of field.
- There are many more studies to which the technique can be applied.



Future Directions

- Infrared definitions and properties
- Imaging cryocooling
- Finding the crystal
- The speed of cool
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Future Directions

- Extend the crystal volume versus cooling rate to more macromolecular samples (Summer student 1)
- Gather data to help models of heat load for macromolecular samples by measuring thermal properties of crystals illuminated by infrared radiation.
- Improve the statistics on the current data.
- Use the change in infrared properties with radiation to rapidly assess the performance of free radical scavengers (everything to a hammer is a nail).
- Assess the performance of a wide range of cryoprotectants in different crystallization solutions (Summer student 2).
- Use the experimental data to confirm models of the beam heating process (Done – see next talk).
- Provide experimental data to develop and test models of the dynamic cooling and heating processes.

Acknowledgements

We would like to thank the Advanced Photon Source for beamtime. The staff at the SERCAT and SBC-CAT beamlines are thanked for help and patience throughout the development and use of the experimental methods. Similarly the Stanford Synchrotron Radiation Laboratory is thanked for beamtime and the staff for all the help and support provided.

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