Finding the cold needle in a warm haystack:

Infrared imaging applied to locating cryocooled crystals in loops

Edward Snell and Mark van der Woerd

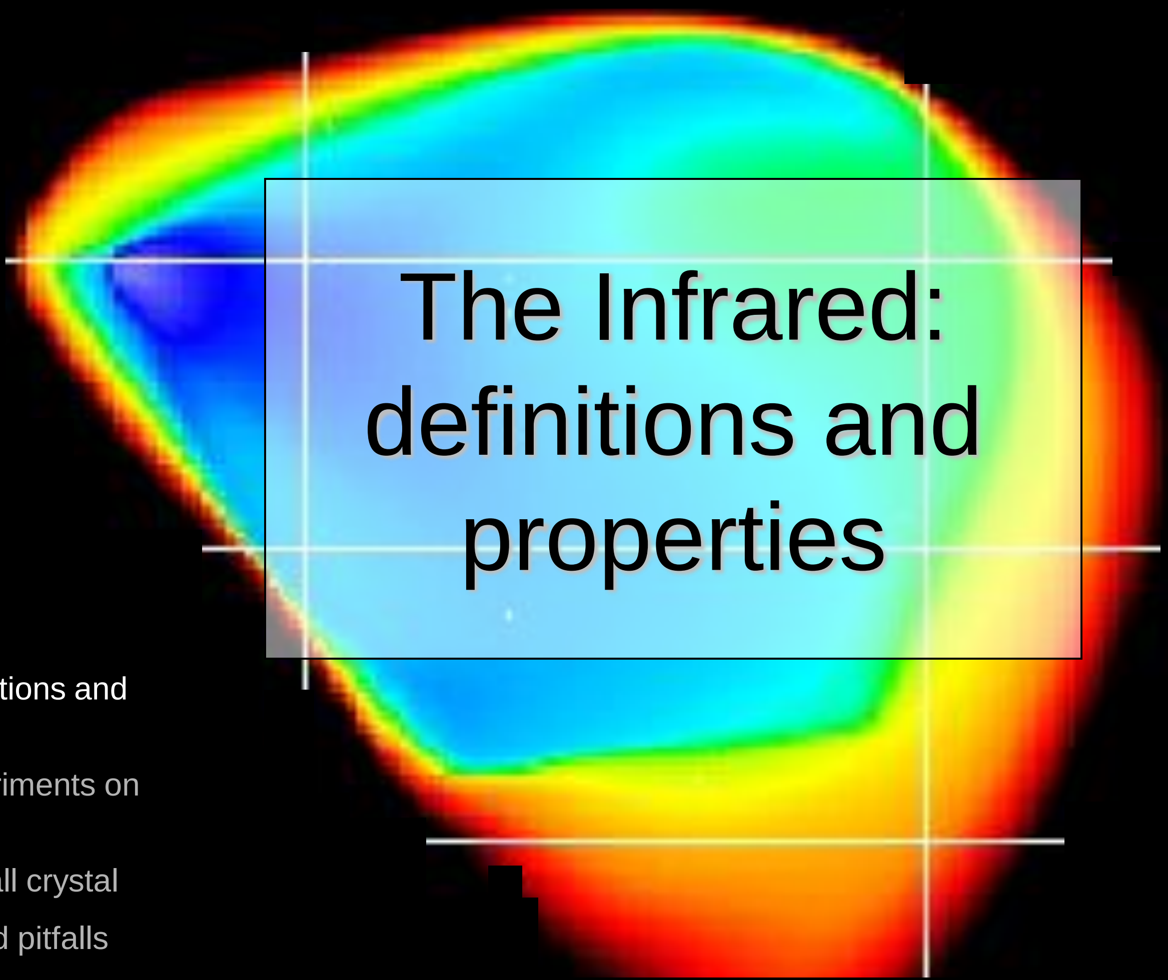
NASA Laboratory for Structural Biology, MSFC

Acknowledgements

Ashley Deacon, Russell Judge, the staff at Stanford Synchrotron Radiation Laboratory and funding support from NASA.

Stating the obvious

- A key step in automation of the data collection process is centering of the crystal in the X-ray beam.
- In a number of cases visual observation of the cryocooled crystal, the solution and the loop holding it does not reveal the crystal position.
- In this study we explore infrared imaging as an alternative or addition to visual imaging.



The Infrared: definitions and properties

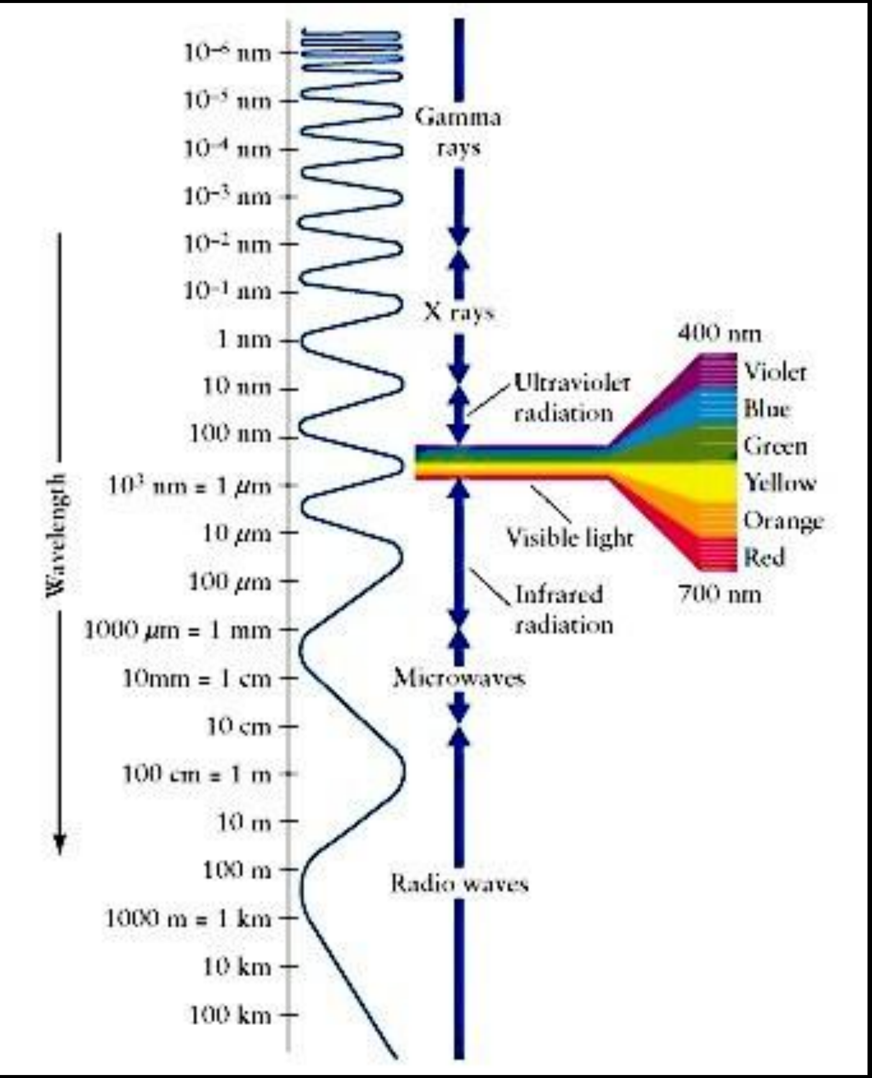
- Infrared definitions and properties
- Original experiments on cryocooling
- Finding a small crystal
- Problems, and pitfalls
- Future directions

The Electromagnetic Spectrum

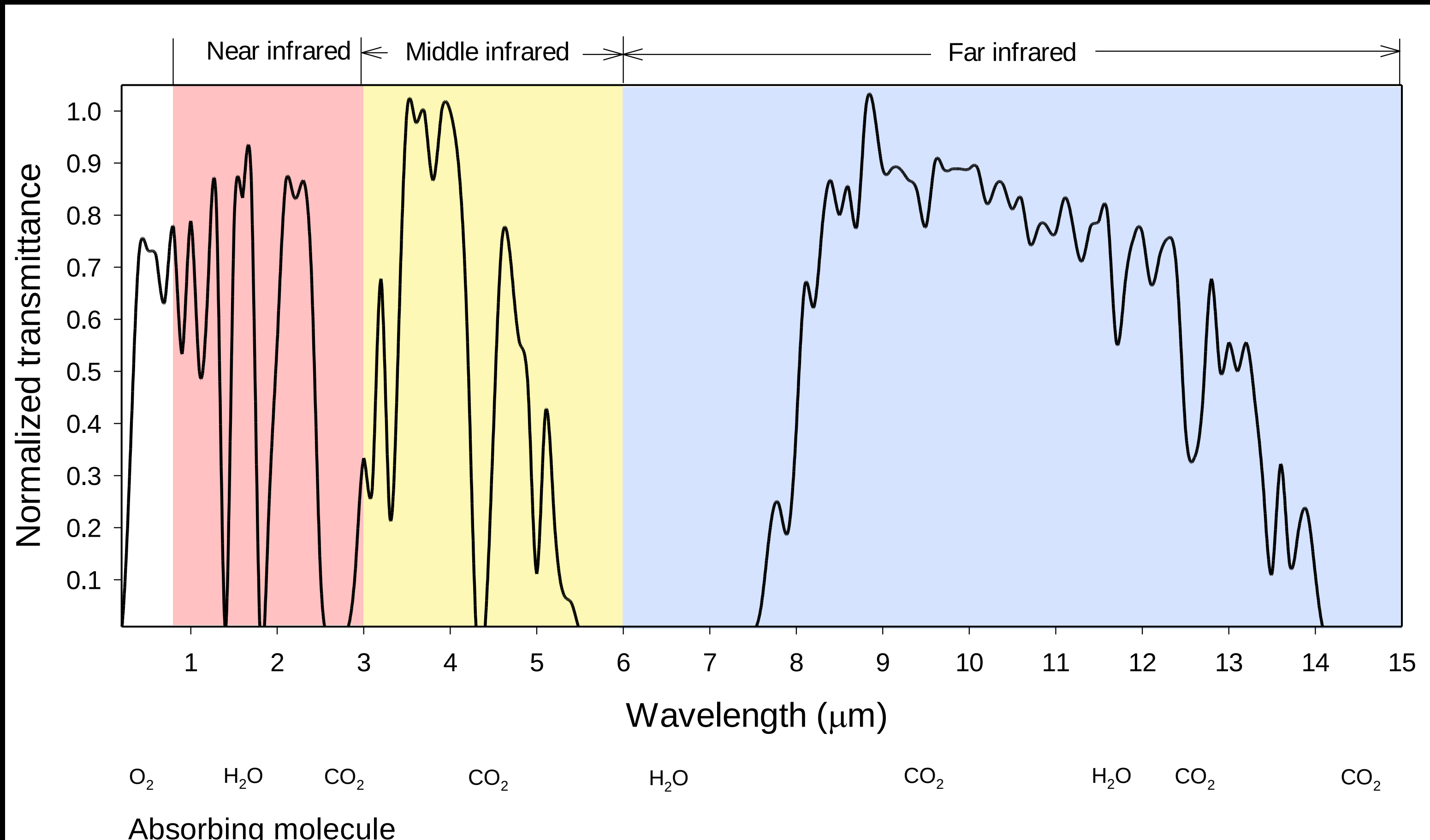
The electromagnetic spectrum can be divided into ionizing and non-ionizing radiation.

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 windows which can be used for observation, Far, mid and near.

Black body radiation

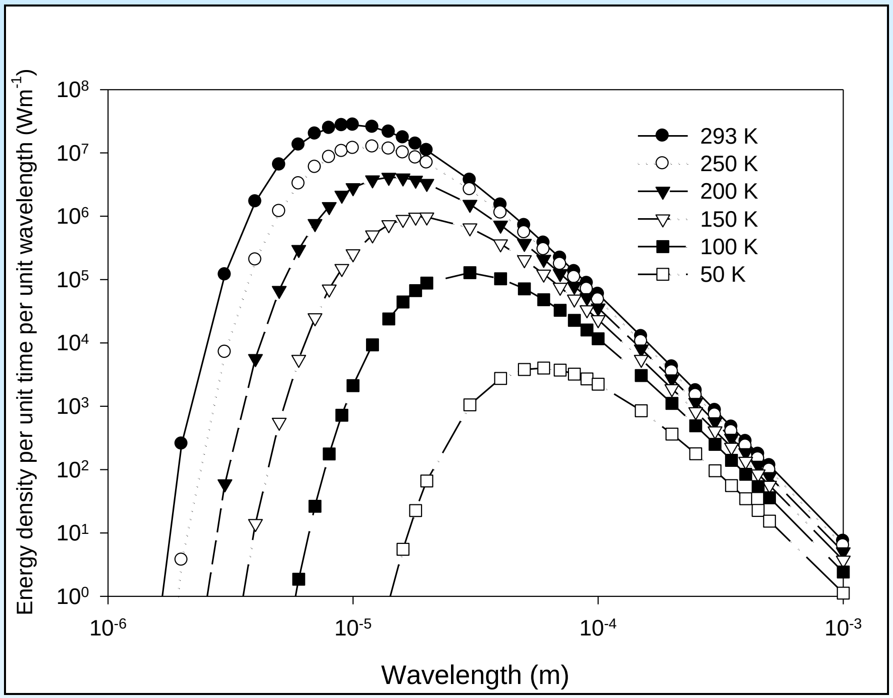
- All objects above 0K emit infrared energy as a function of their temperature.
- A black body perfectly emits and absorbs thermal radiation.
- The energy spectrum for a black body is given by Planck's radiation law;

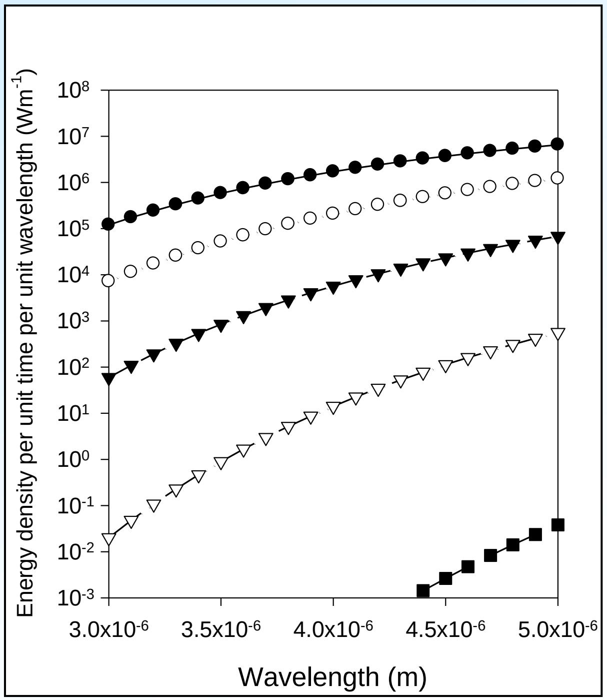
$$E(\lambda(T) = \frac{2\pi hc^2}{\lambda^5 (e^{hc/\lambda kT} - 1)}$$

- 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

Radiation from a real object

- Real objects 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.
- Real objects tend to be illuminated by a number of infrared sources:
  - The ambient heat in the room
  - The experimenter
  - The illumination
  - The coldstream
- Real objects transmit and reflect heat
  - Heat is seen behind the object
  - Heat is seen reflected off the object
- Infrared properties of the object vary with wavelength and viewing angle
- It is not trivial to do quantitative studies

Lights, Camera ….

Indium Antimonide detector.

3.0-5.0 μm bandpass.

320 x 240 30mm^2 pixels.

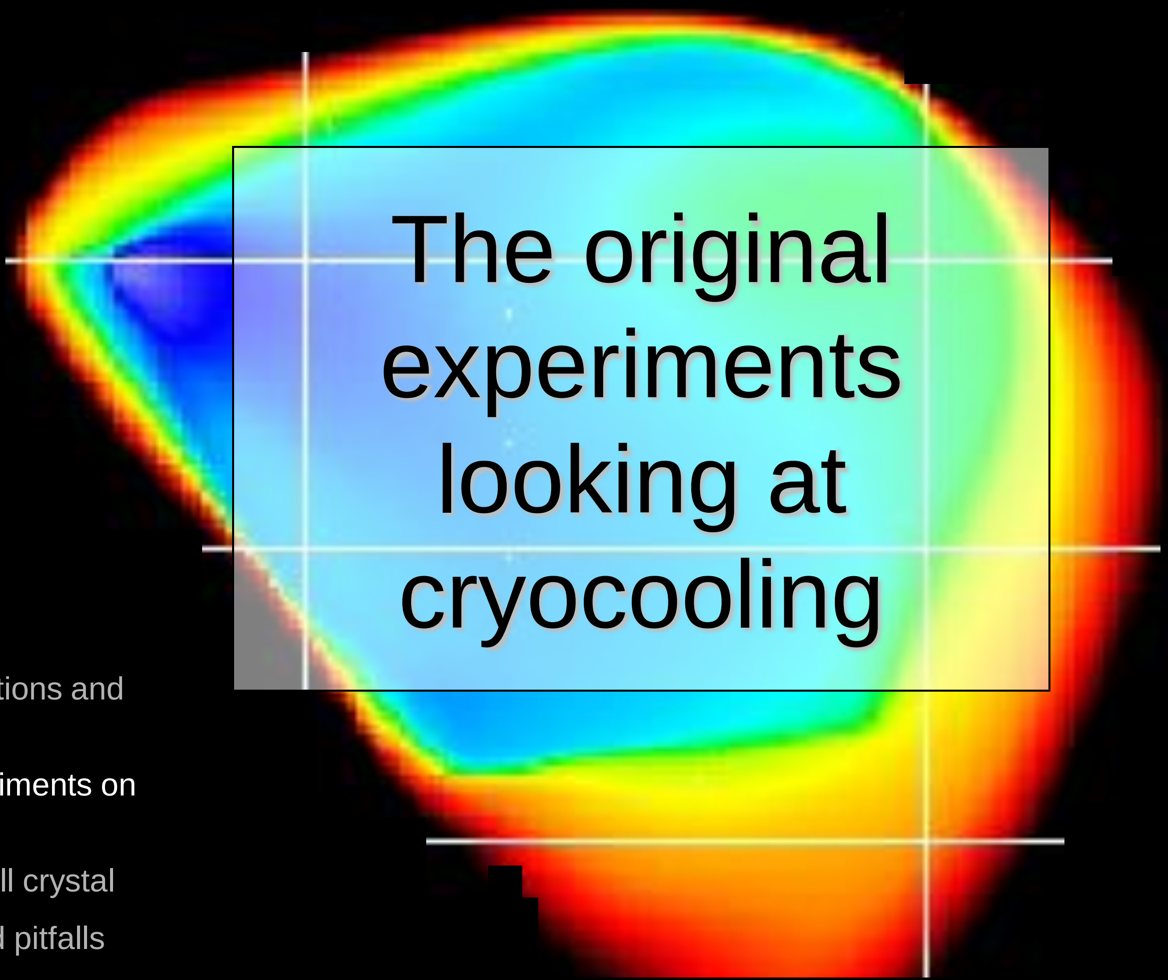
60 Hz frames.

Integration time 10 μs to 17 ms.

12 bit digital video output.

Cooling to 77K by integral Stirling system.

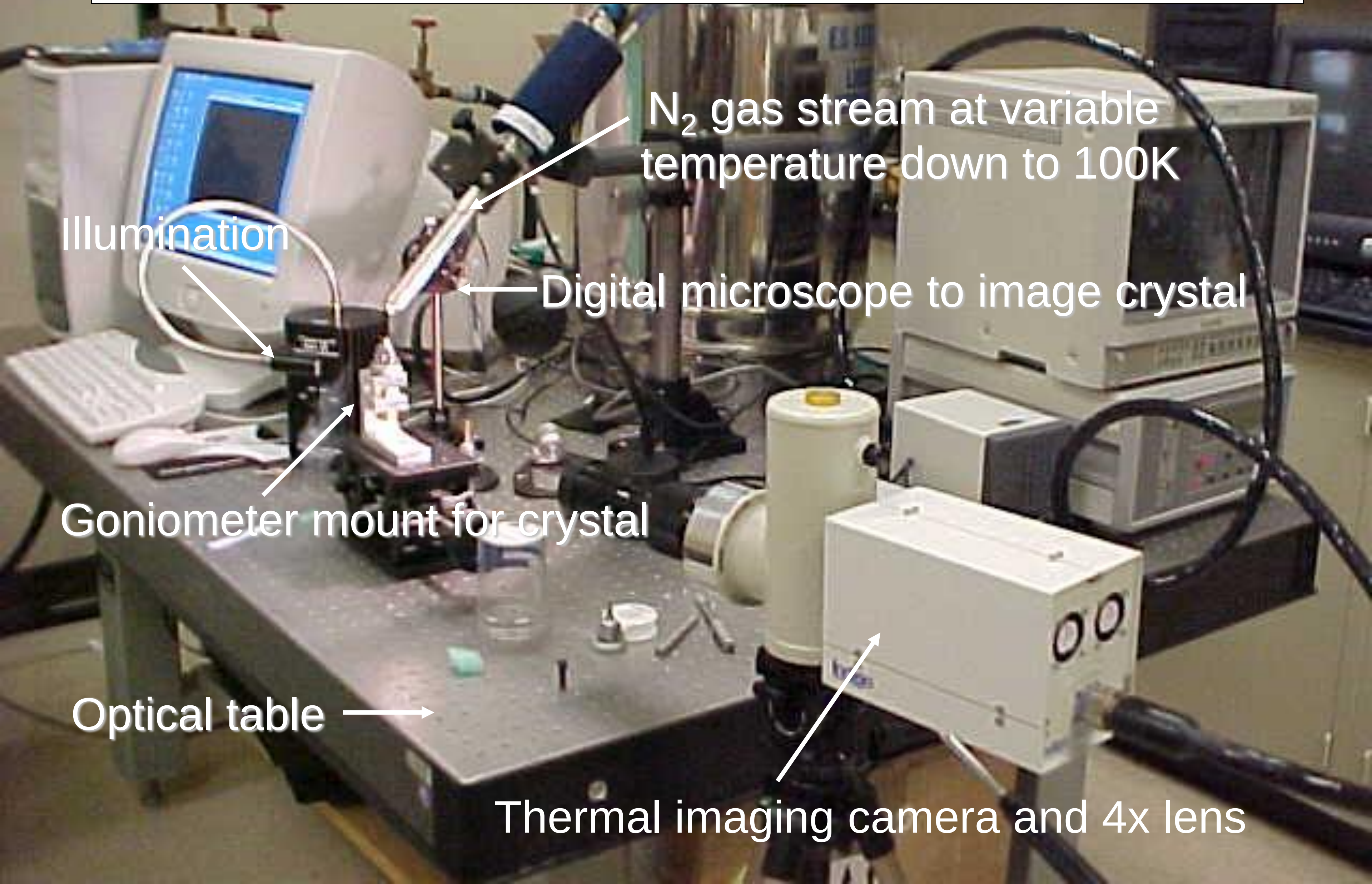
Size, 13 x 12 x 23 cm.

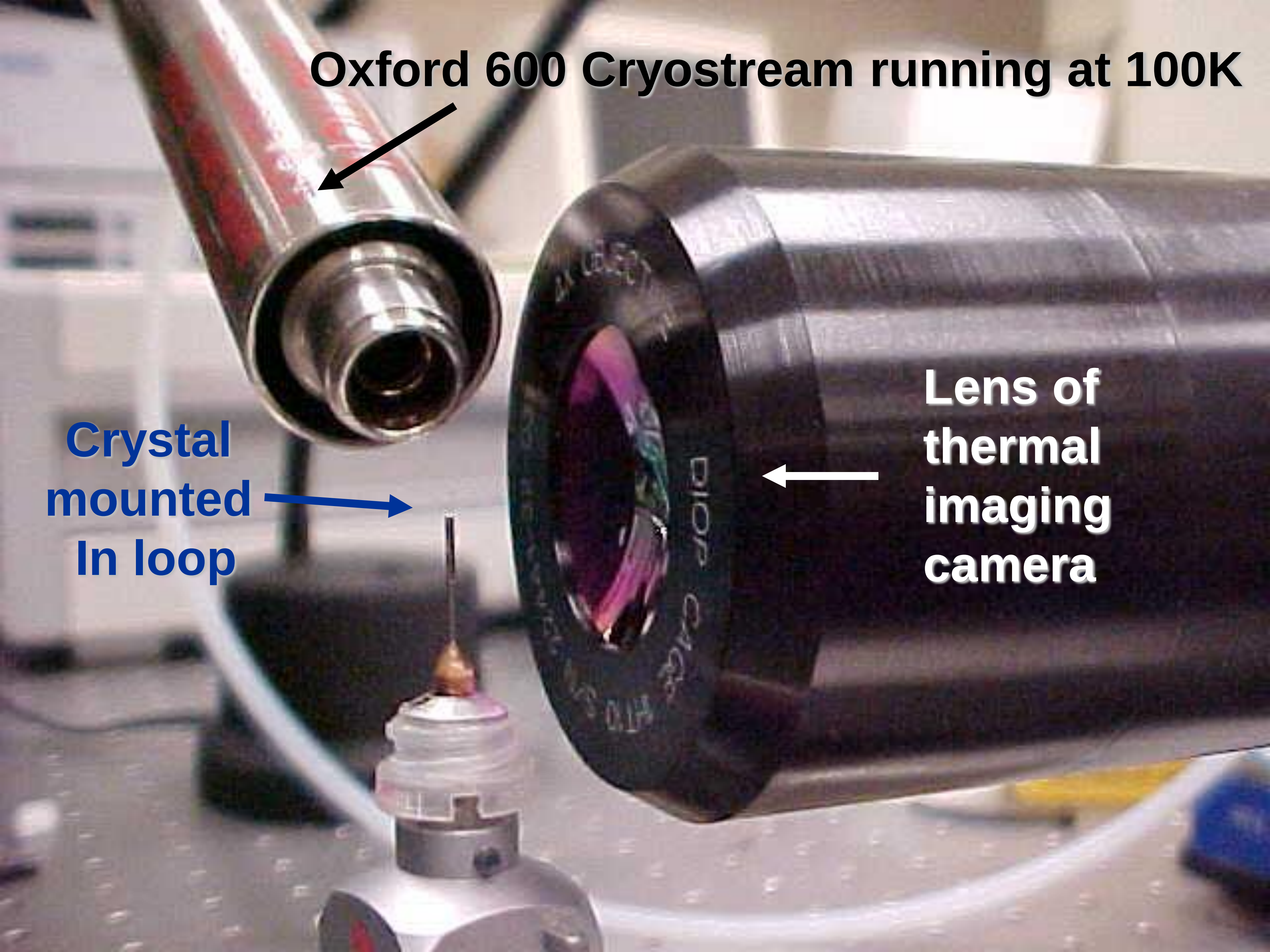


The original experiments looking at cryocooling

- Infrared definitions and properties
- Original experiments on cryocooling
- Finding a small crystal
- Problems, and pitfalls
- Future directions

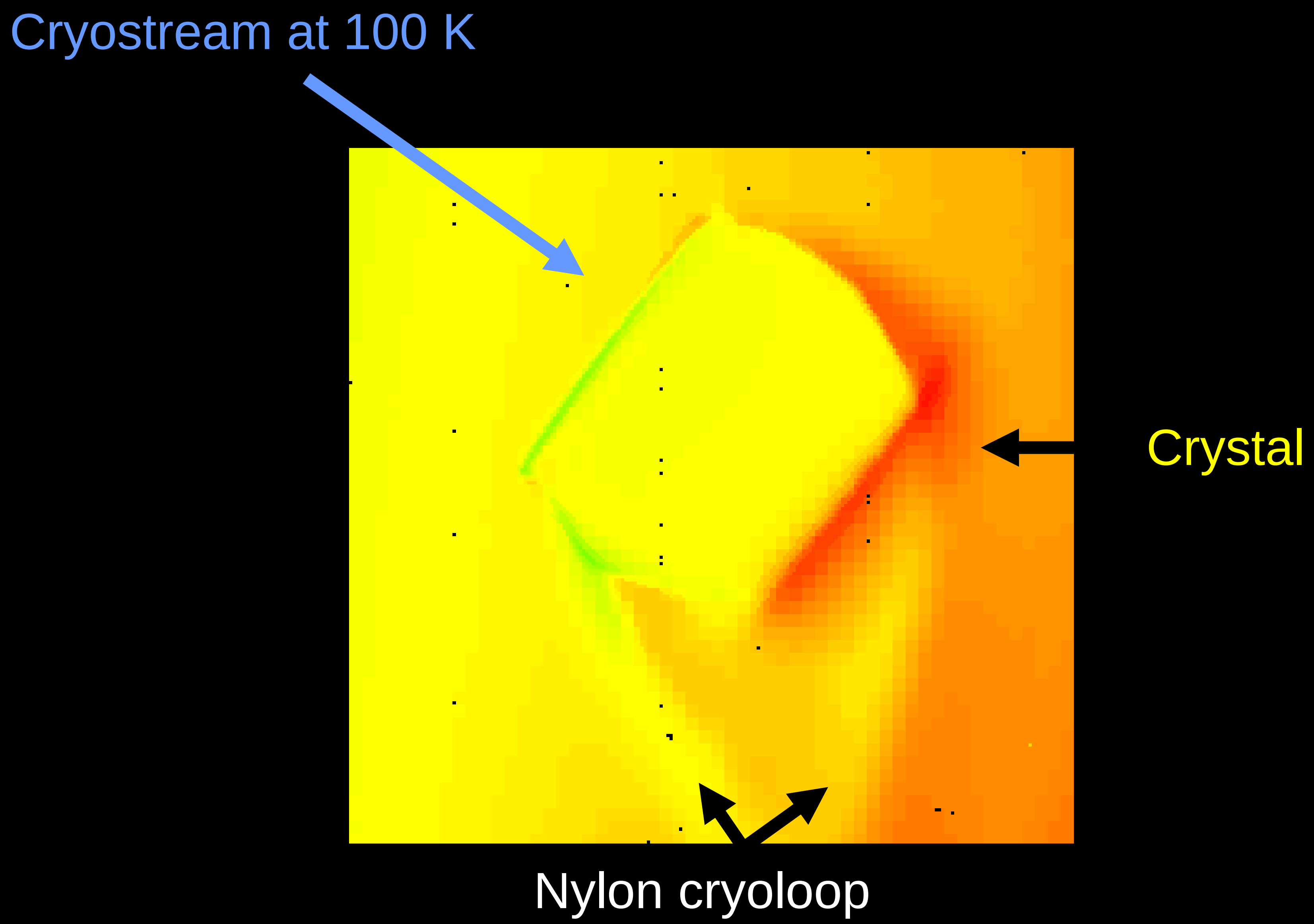
System setup in the Laboratory

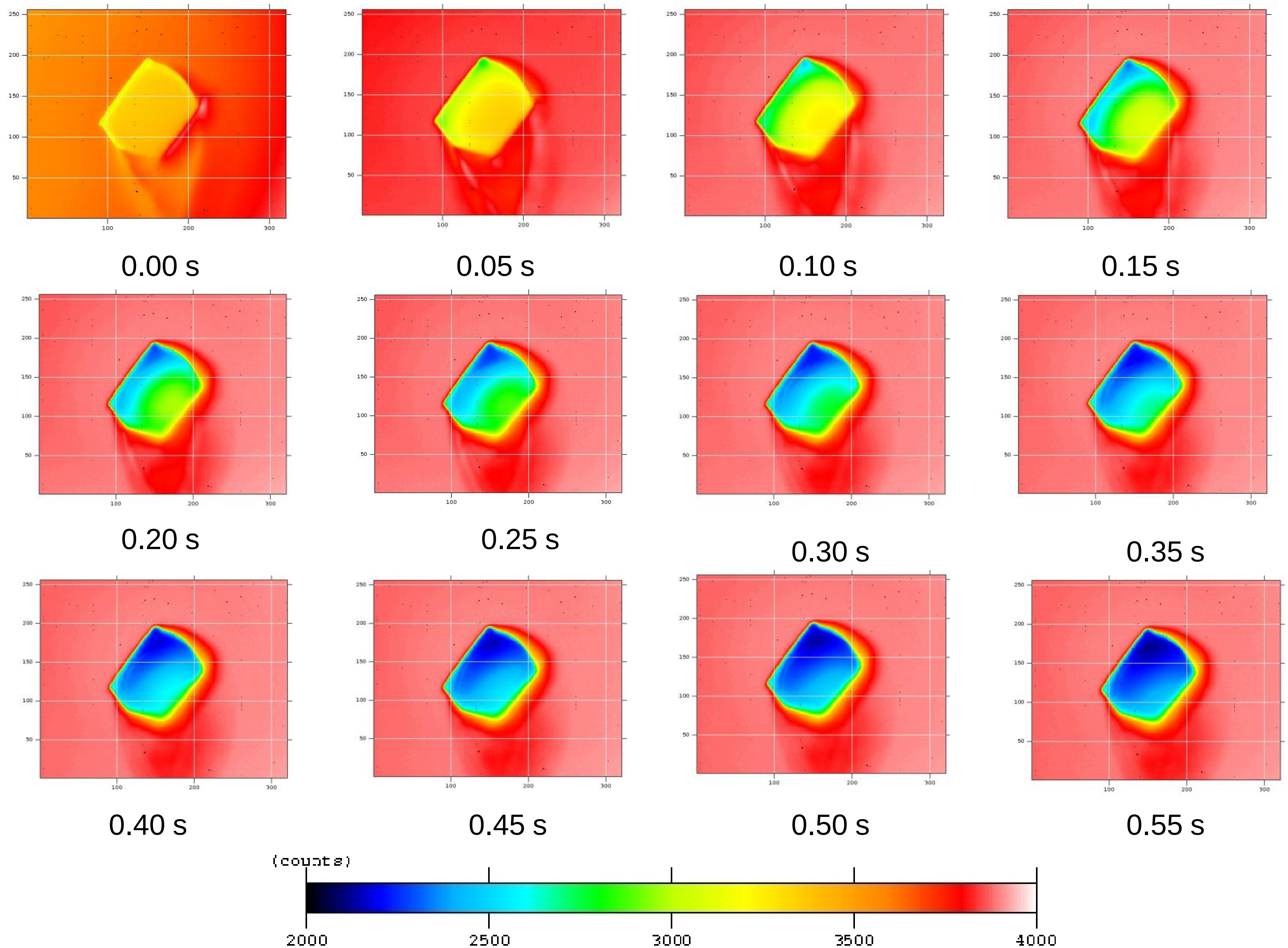


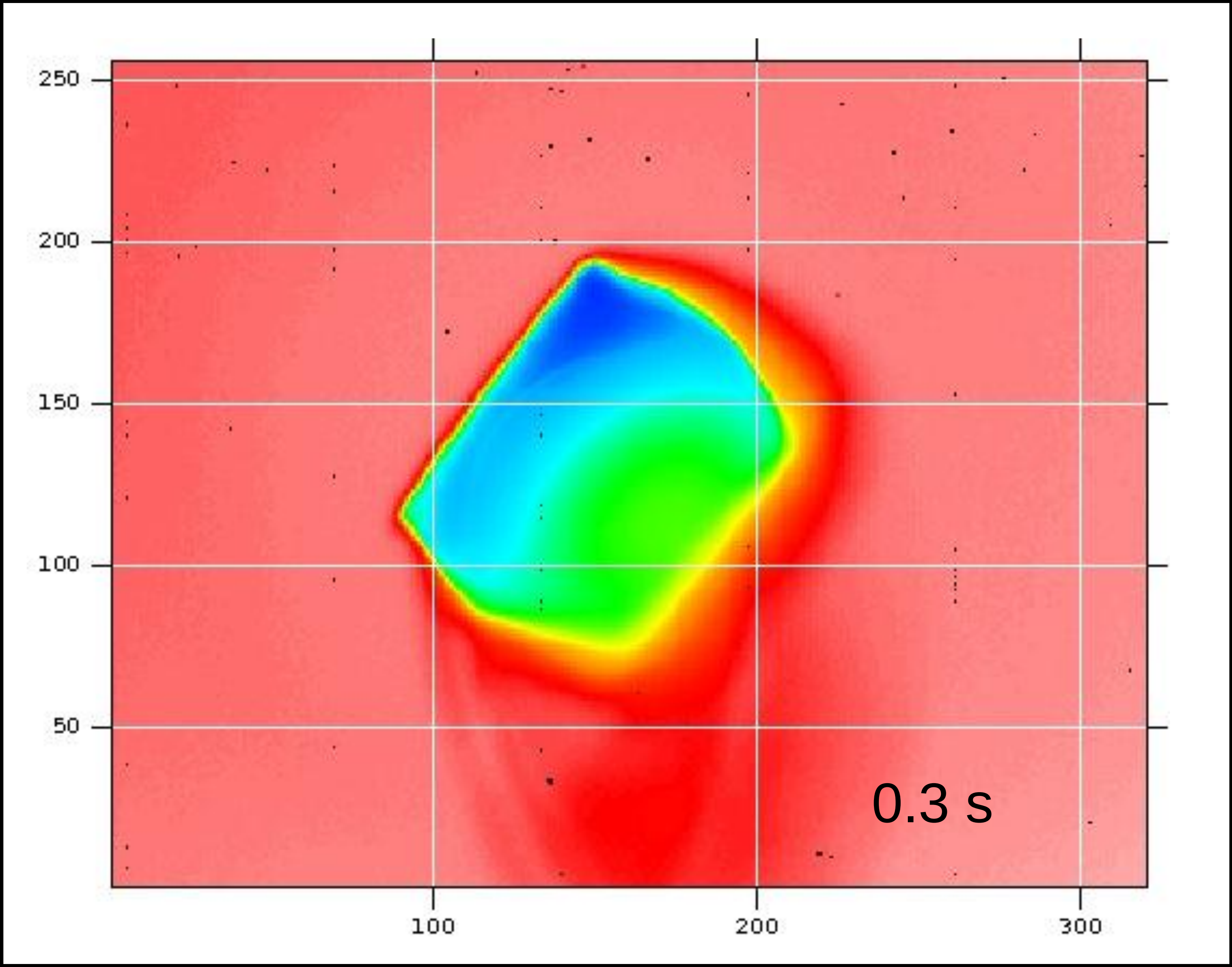

Oxford 600 Cryostream running at 100K
Lens of thermal imaging camera
Crystal mounted In loop

Example of one of the experiments

- Lysozyme
  - Lysozyme solution 75 mg/ml in 0.1 M, pH 4.5, Sodium Acetate
  - Reservoir solution 0.9 M Sodium Chloride with 25% Ethylene Glycol and 0.1 M, pH 4.5, Sodium Acetate
  - Drop solution, 6 μl of lysozyme and 4 μl of reservoir
  - Hanging drop crystallization
- Crystal size used for experiment
  - 1.00 x 0.72 x 0.24 mm
    (somewhat larger than typical)

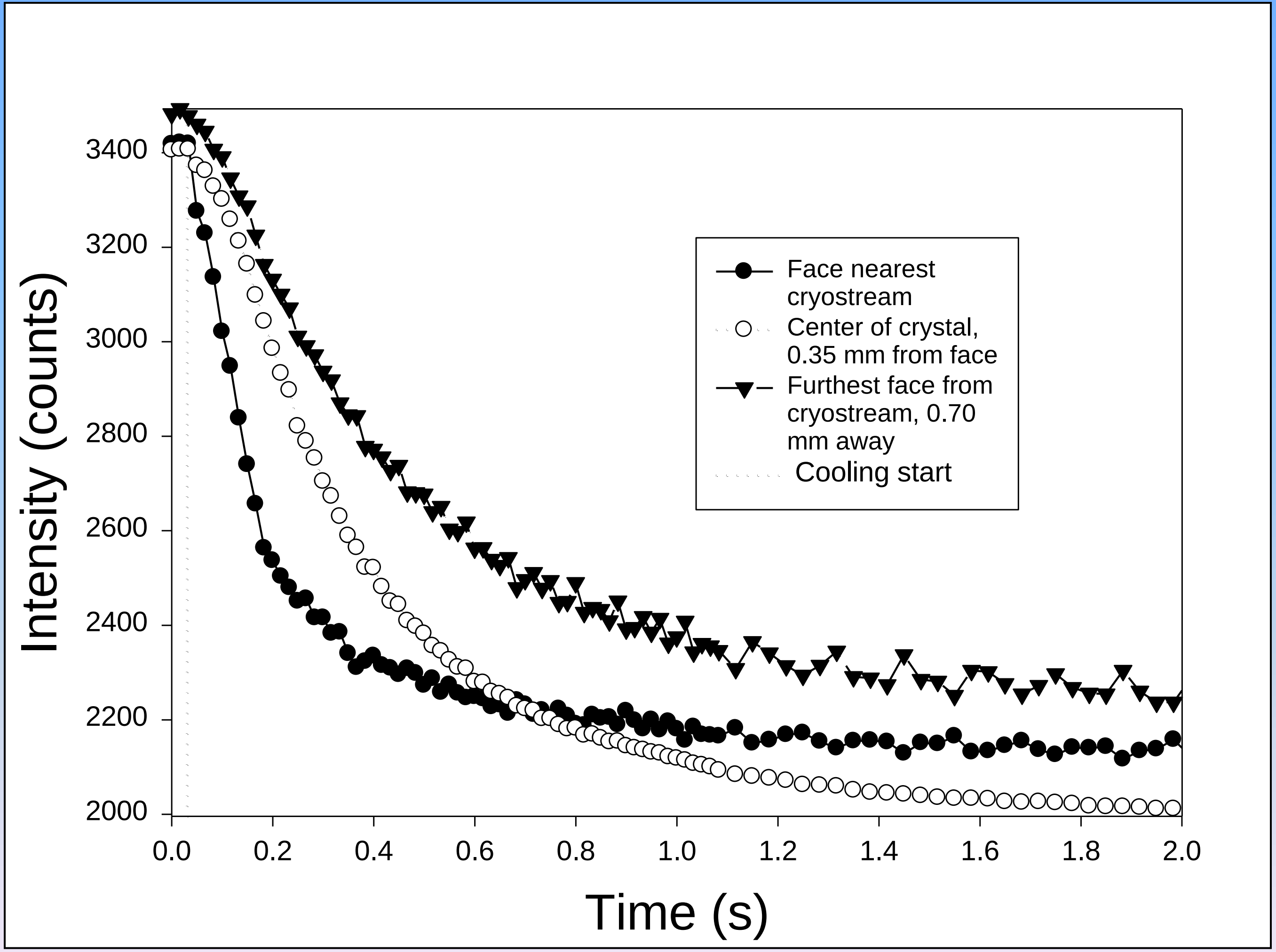

Cryostream at 100 K
Crystal
Nylon cryoloop


0.00 s
0.05 s
0.10 s
0.15 s
0.20 s
0.25 s
0.30 s
0.35 s
0.40 s
0.45 s
0.50 s
0.55 s
(counts)
2000
2500
3000
3500
4000



250
200
150
100
50
100
200
300
0.3 s

Intensity over time



Summary

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

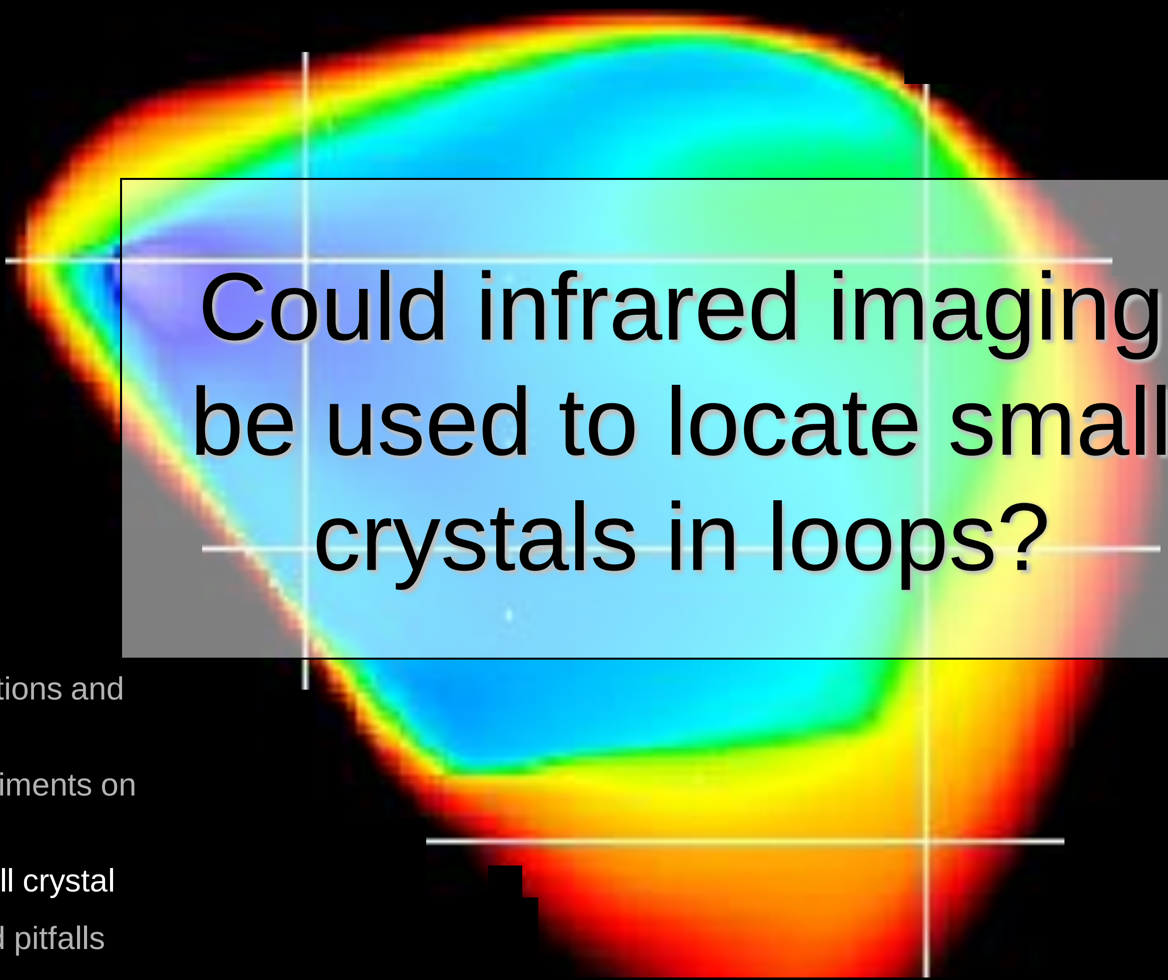
- Crystals cooled in a wave from the point nearest the cryostream to the point furthest away.

- The crystals studied were large to deliberately bring out the detail in the images.

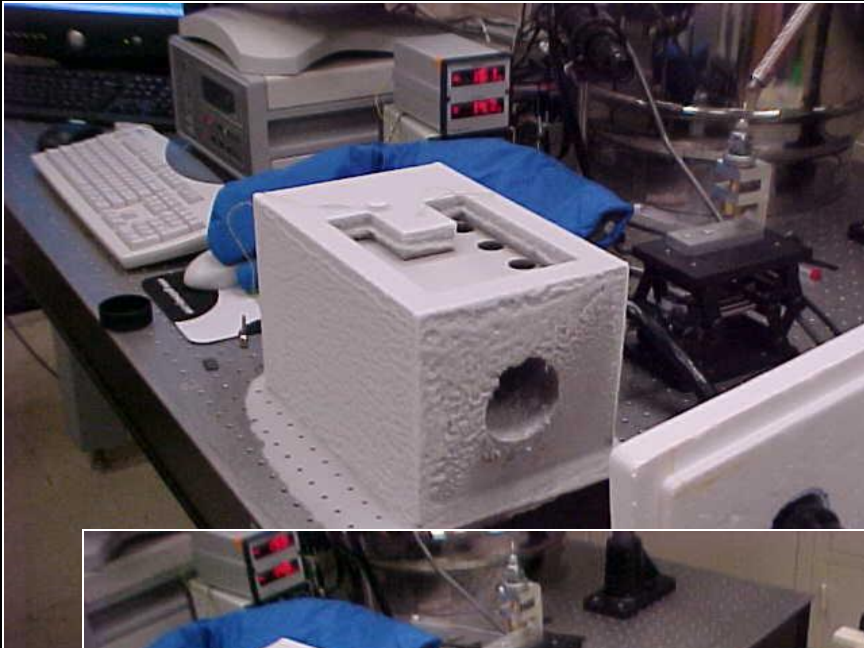
Of interest here:

- In each case the crystal was clearly distinguished from the loop.

Could infrared imaging be used to locate small crystals in loops?



- •Infrared definitions and properties
- •Original experiments on cryocooling
- •Finding a small crystal
- •Problems, and pitfalls
- •Future directions



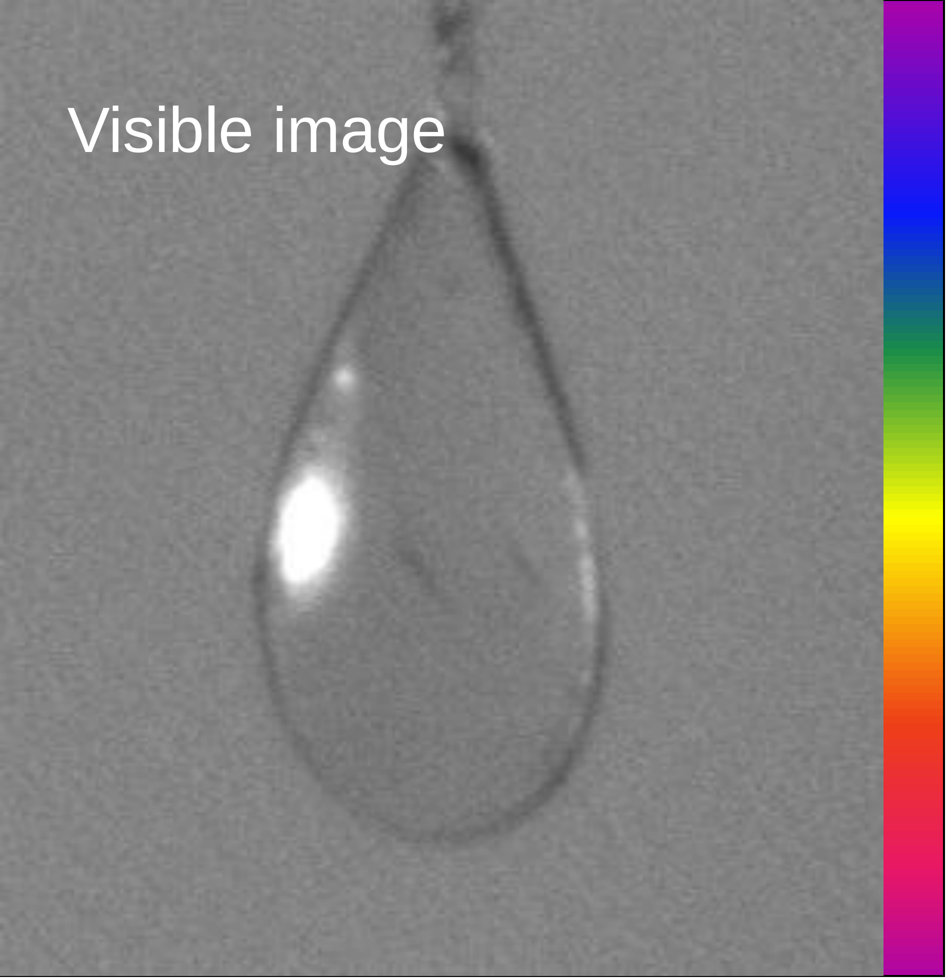
A blackbody source at 213 K is used for non-uniformity correction of the detector.

The infrared camera (cooled to 77 K) and digital microscope are then focused on the crystal position.

Measurements begin

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

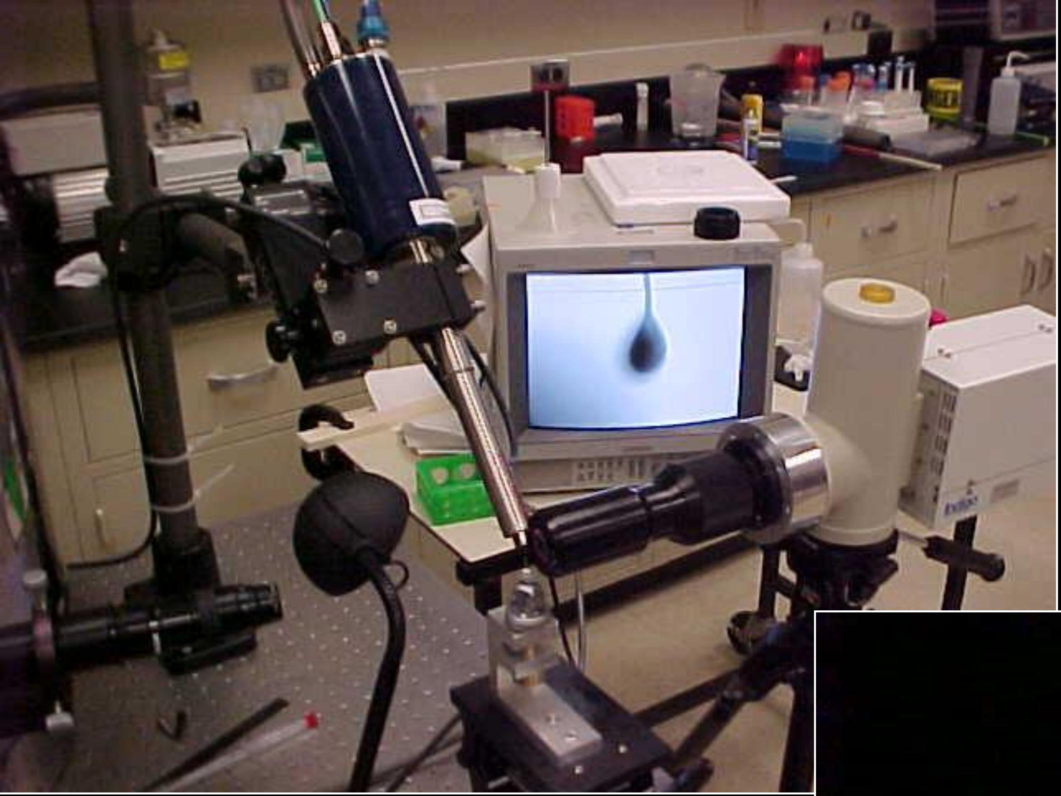
(0.14 x 0.11 x 0.06 mm^3)

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

Solvent content: 40%

Cryoprotectant: Ethylene Glycol

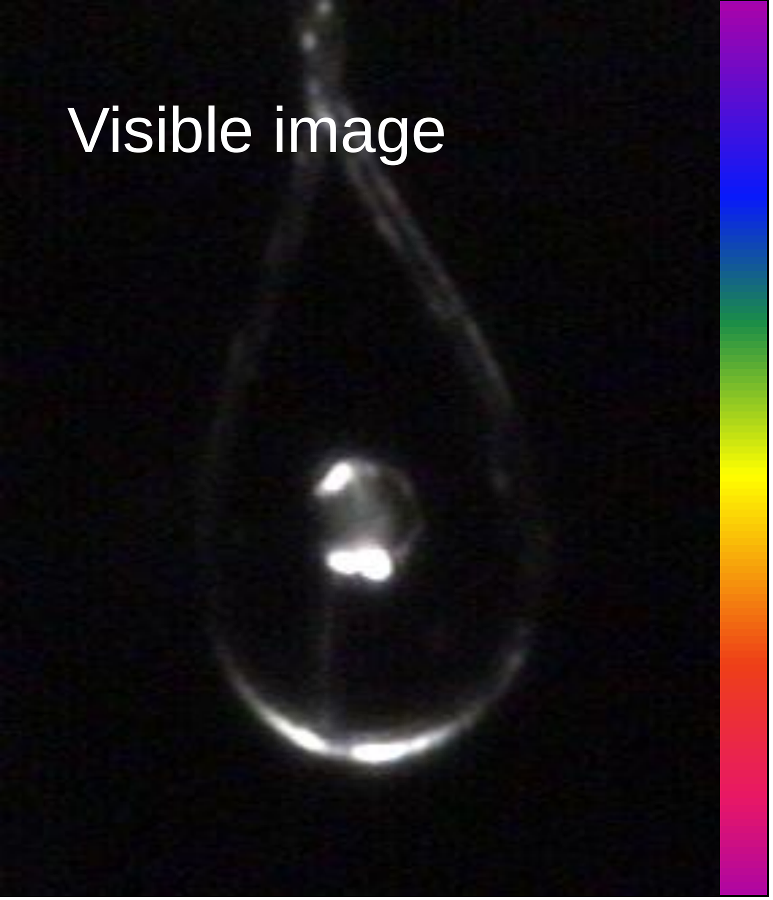
Infrared image (lamp illumination at 45°)



Lights on….

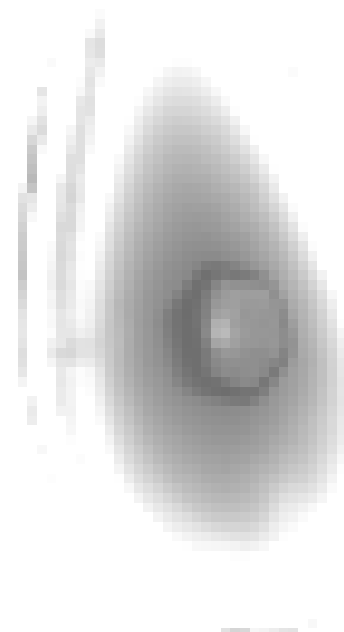
(note the lamp for visual illumination, off in this case)

Lights off….



Basic Fibroblastic Growth Factor/DNA complex crystal

($0.11 \times 0.17 \times 0.05$ mm^3)



Cell parameters: P622, 112.8, 112.8, 450.2 Å

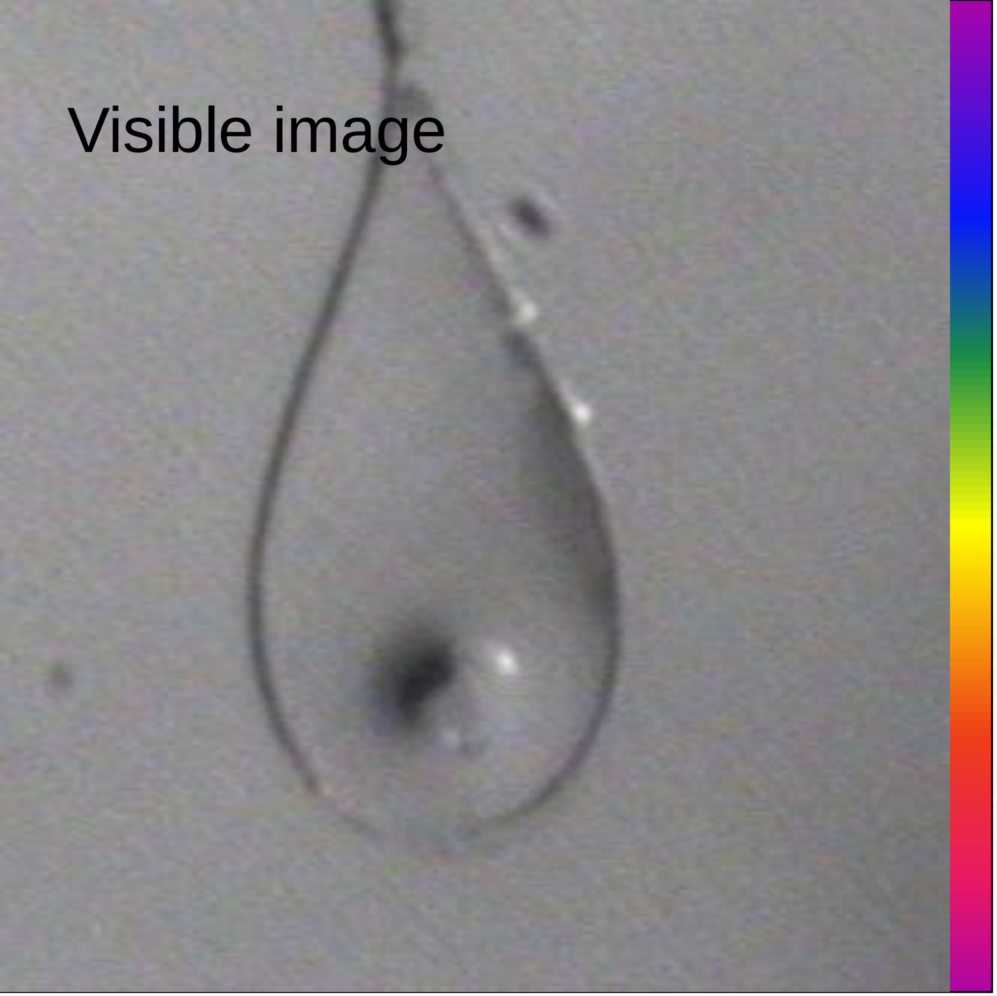
Solvent content: Unknown

Cryoprotectant: Butanediol

Infrared image (lamp illumination at 45°)

Glucose Isomerase crystal

(0.08 x 0.08 x 0.04 mm^3)

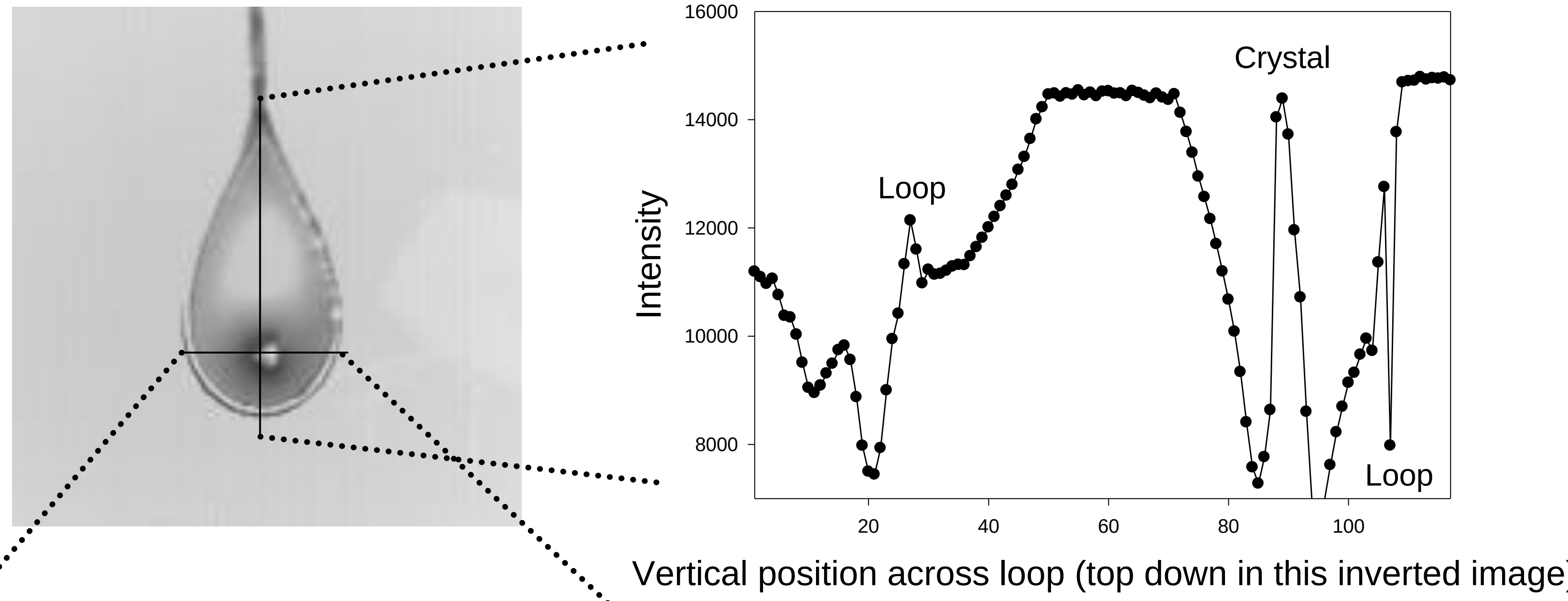


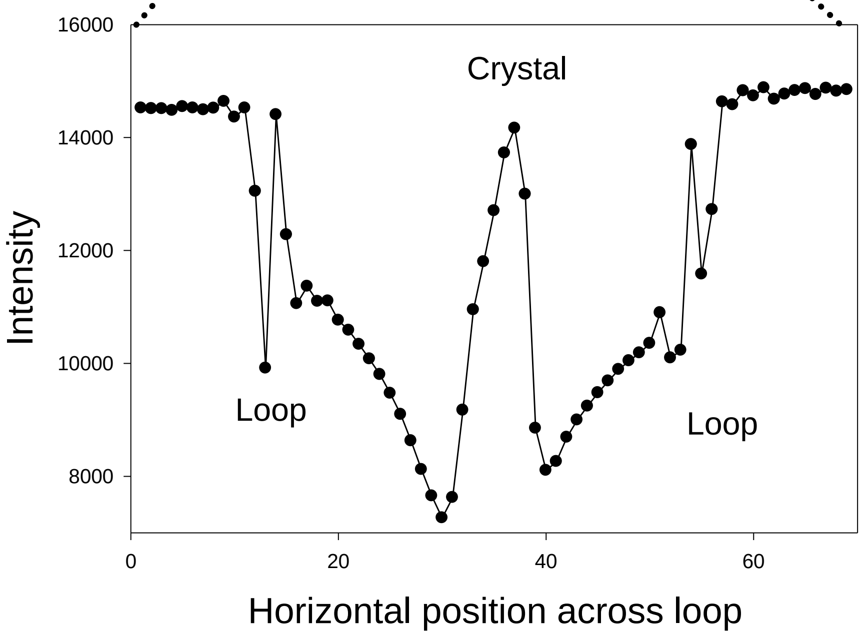
Infrared image (lamp illumination at 45°)

Cell parameters: I222, 92.5, 98.2, 102.2 Å

Solvent content: 50%

Cryoprotectant: Isopropanol, glycerol





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.

The effect of exposure time

Visible

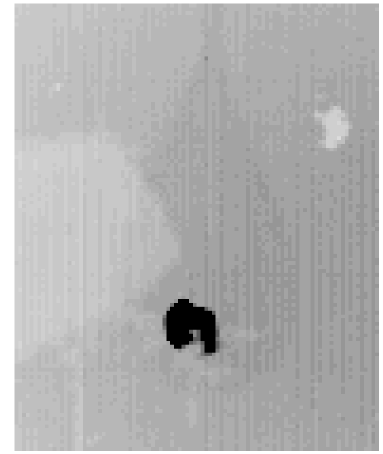
Glucose isomerase

White background

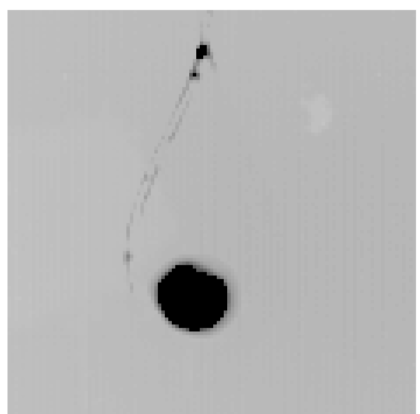
No background

Infrared (lamp illumination from behind)

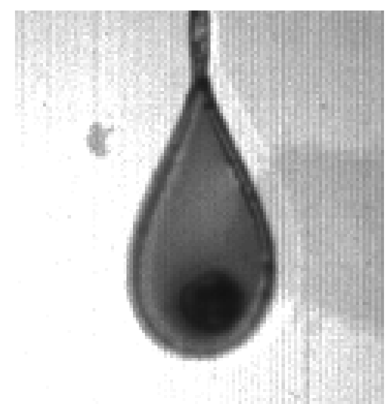
1.0 ms exposure



5.0 ms exposure

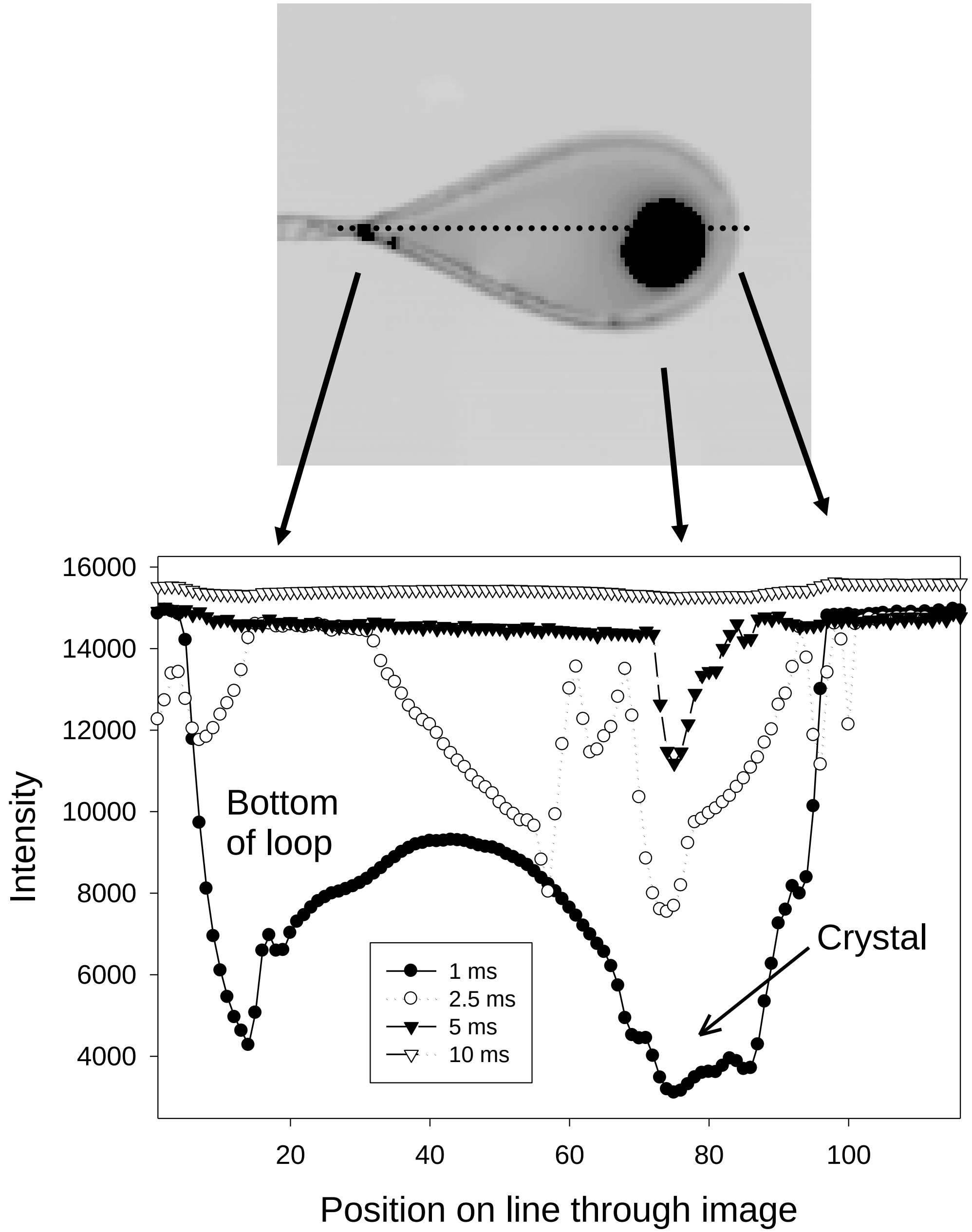


2.5 ms exposure



10.0 ms exposure

(0.22 x 0.15 x 0.06 mm)

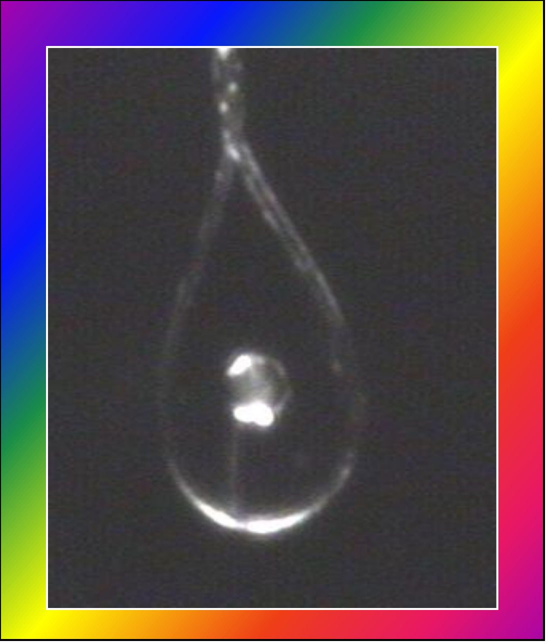


In this case the crystal (also glucose isomerase) is “cold” in comparison to the rest of the image – it is acts as an insulator to the infrared radiation from a lamp source behind.

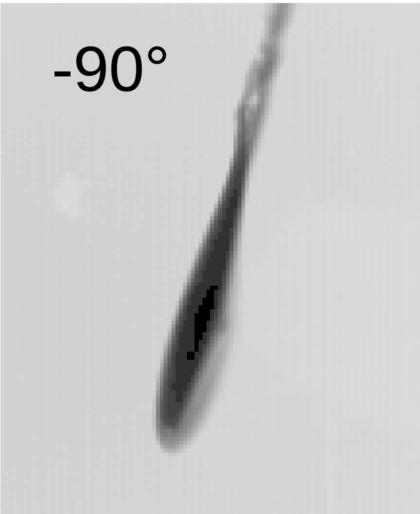
This lamp is barely warm to the touch but acts as an infrared illumination source without perceptibly heating the crystal.

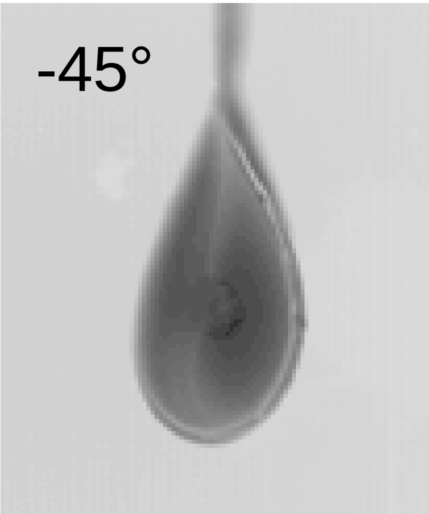
The bottom of the loop also acts as an insulator and appears cold.

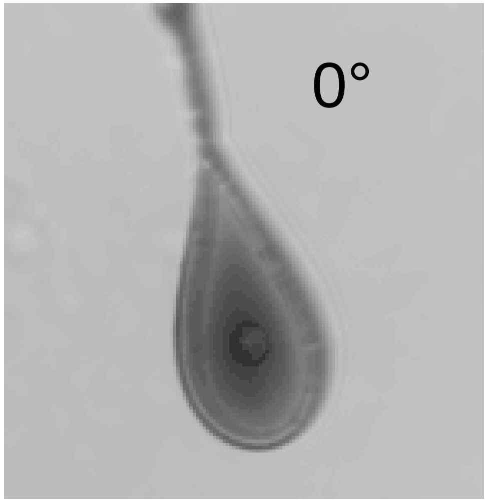
Angular sensitivity

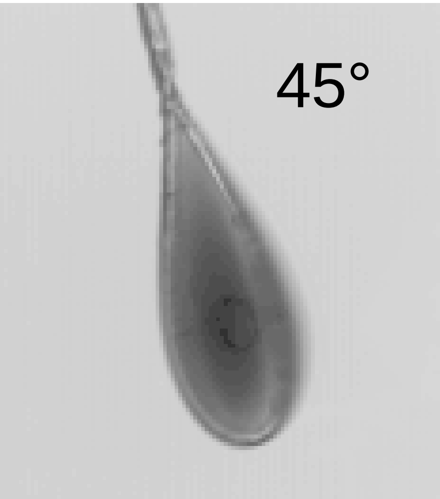


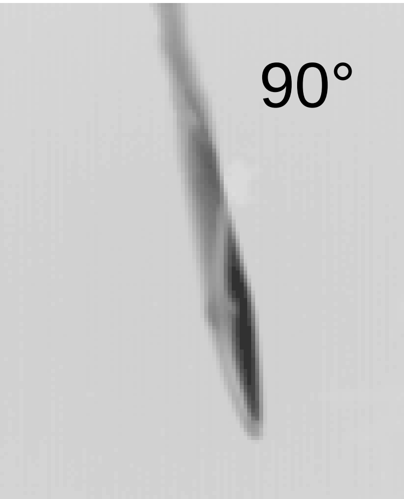
1.0 ms exposures









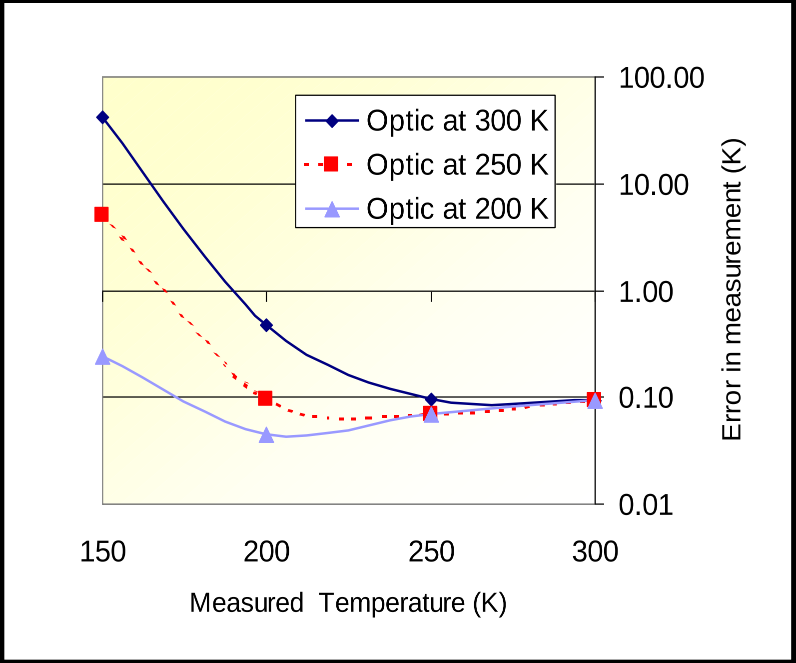


Observations

- Infrared imaging distinguished the crystal in typical cryo mounts.
- The crystals appeared at higher intensity (temperature) or lower intensity than the vitrified liquid in the loop depending on illumination.
- Visual observation also showed the crystals but the light illumination had to be optimized for each case.

Why can we see the crystals?

- The system is at equilibrium at 100K.
- We do not see the temperature difference due to deviations from 100K.
- We see the crystals primarily due to the transmission and reflection of infrared radiation

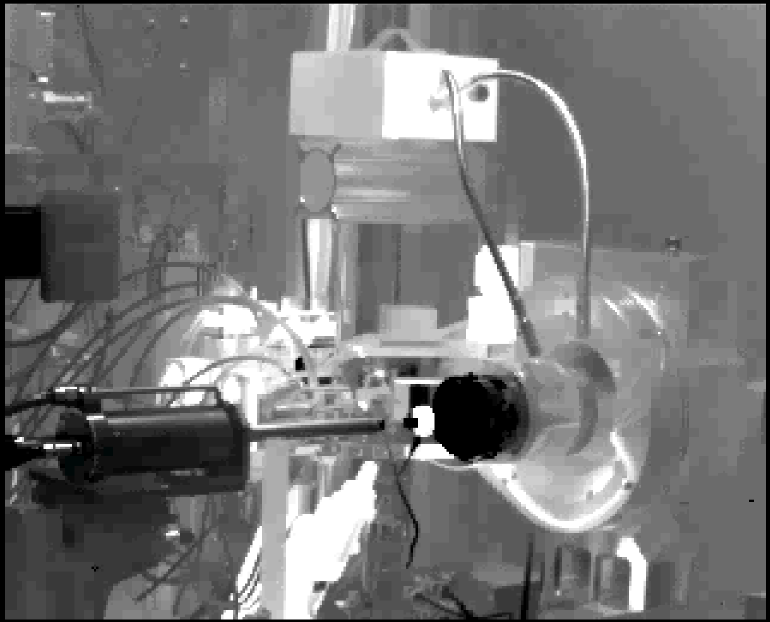


We know from our other studies that the camera in the configuration we are using is sensitive to 130 K (somewhat better than the calculated graph shown). By using it at maximum sensitivity we may also be able to measure the emissivity properties of the samples further enhancing the technique

Answer – yes, but…
Problems and pitfalls

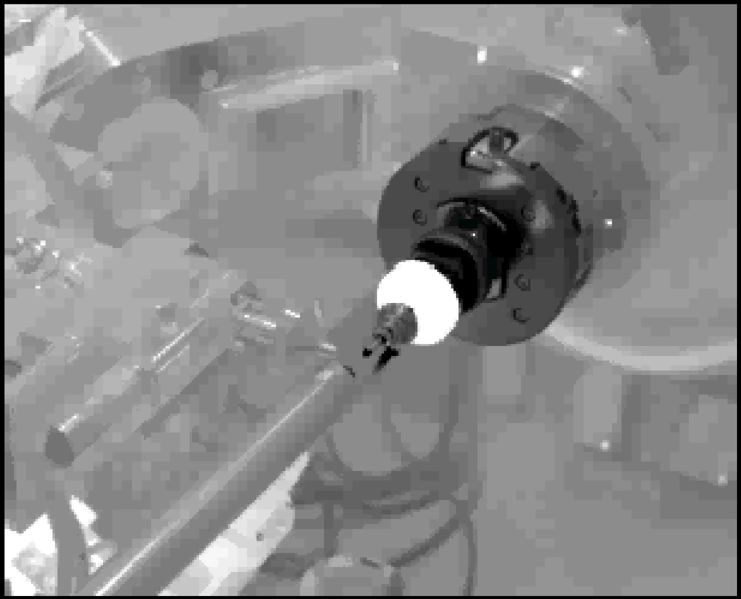
- Infrared definitions and properties
- Original experiments on cryocooling
- Finding a small crystal
- **Problems, and pitfalls**
- Future directions

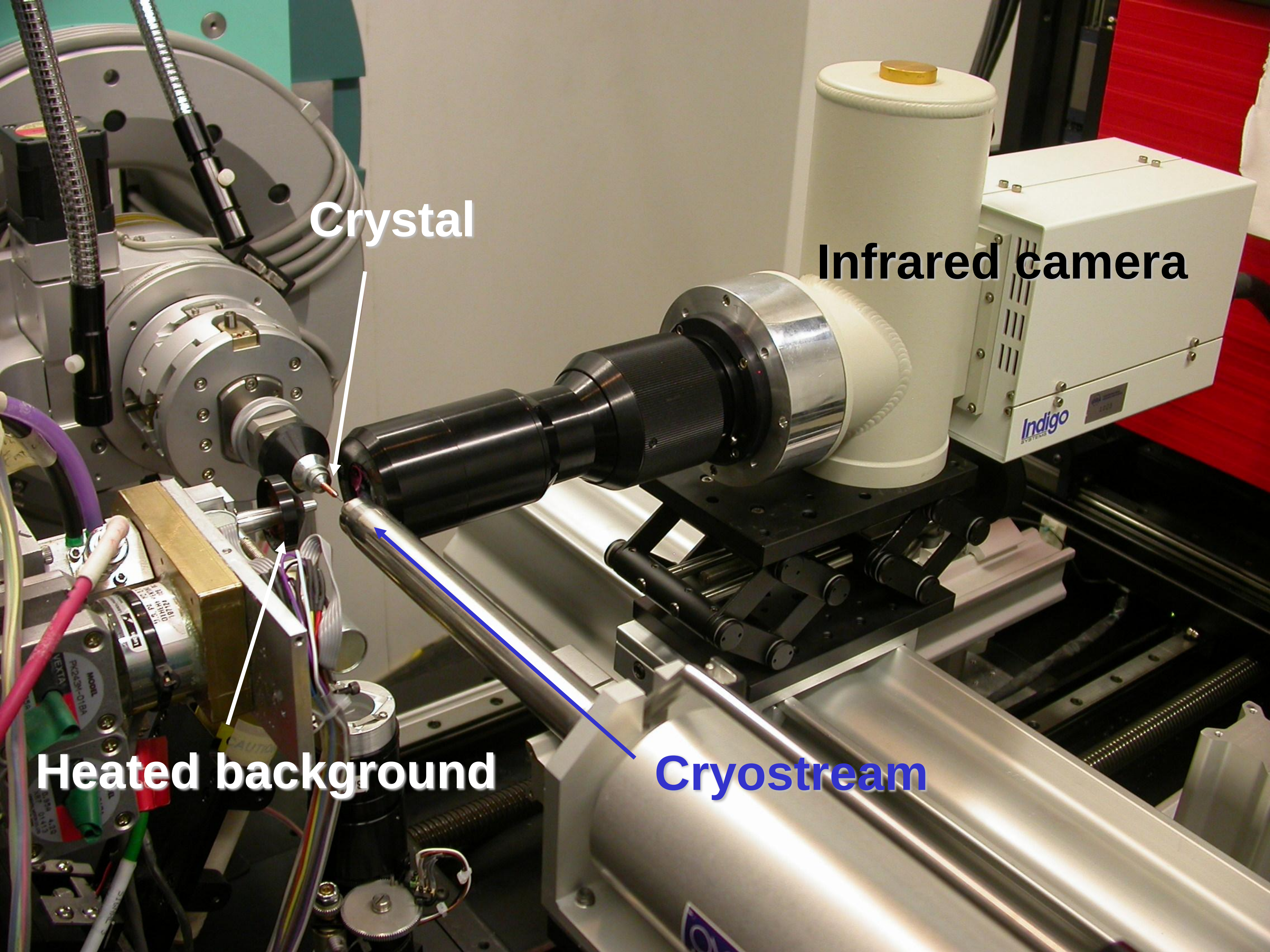
A real world case – SSRL Beamline 11-1



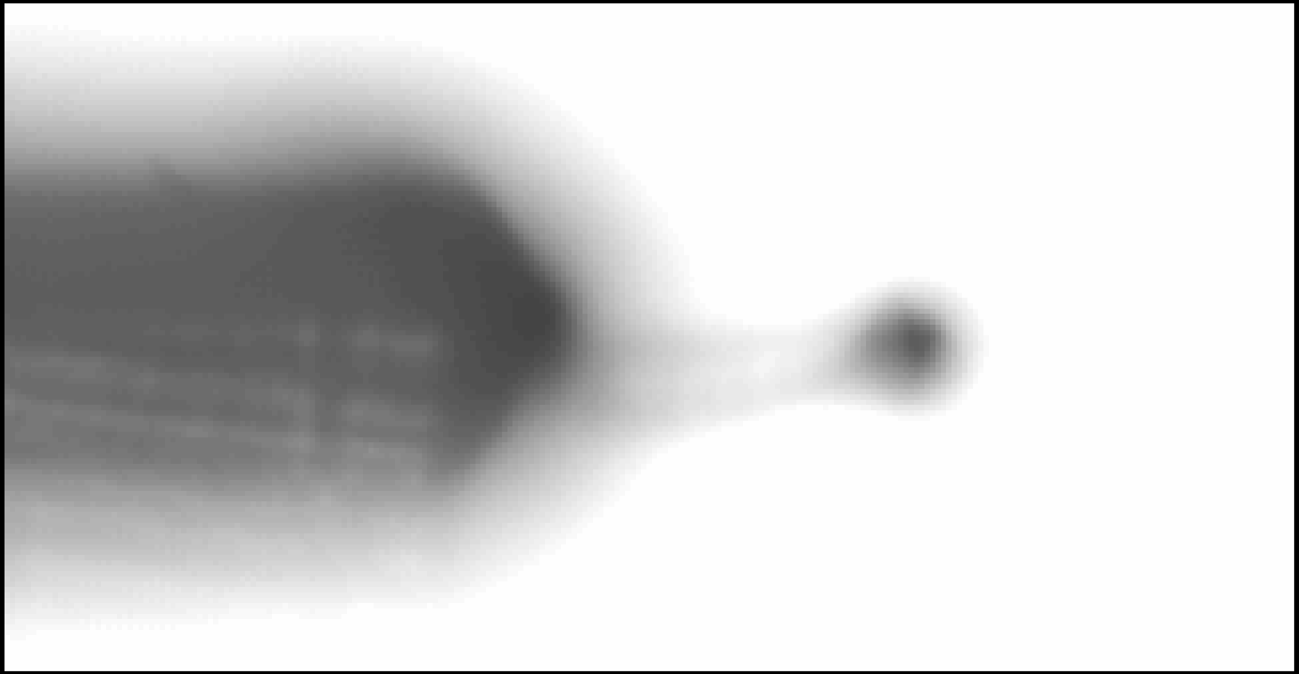
Mounting a crystal

Un-mounting a crystal




Crystal
Infrared camera
Heated background
Cryostream

Infrared Image with crystal being moved in 10 μm step size

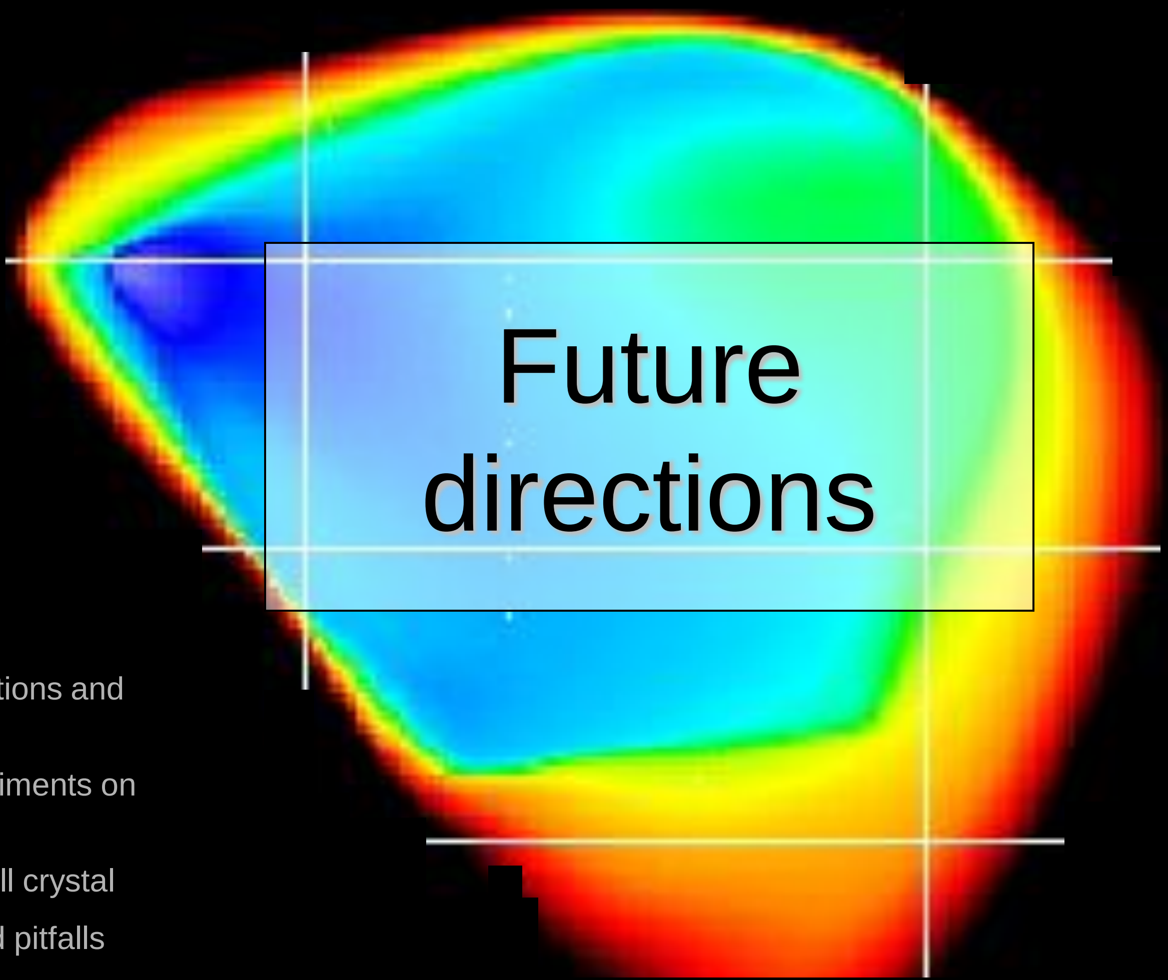


30% glycerol, real sample from structural genomics program.
Loop is 0.1 mm diameter.

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

Observations

- The complete crystal was seen as the loop was moved through the depth of field of the camera.
- The whole crystal was never completely in focus.
- Work is in progress to generate a single focused image 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.



Future directions

- •Infrared definitions and properties
- •Original experiments on cryocooling
- •Finding a small crystal
- •Problems, and pitfalls
- •Future directions

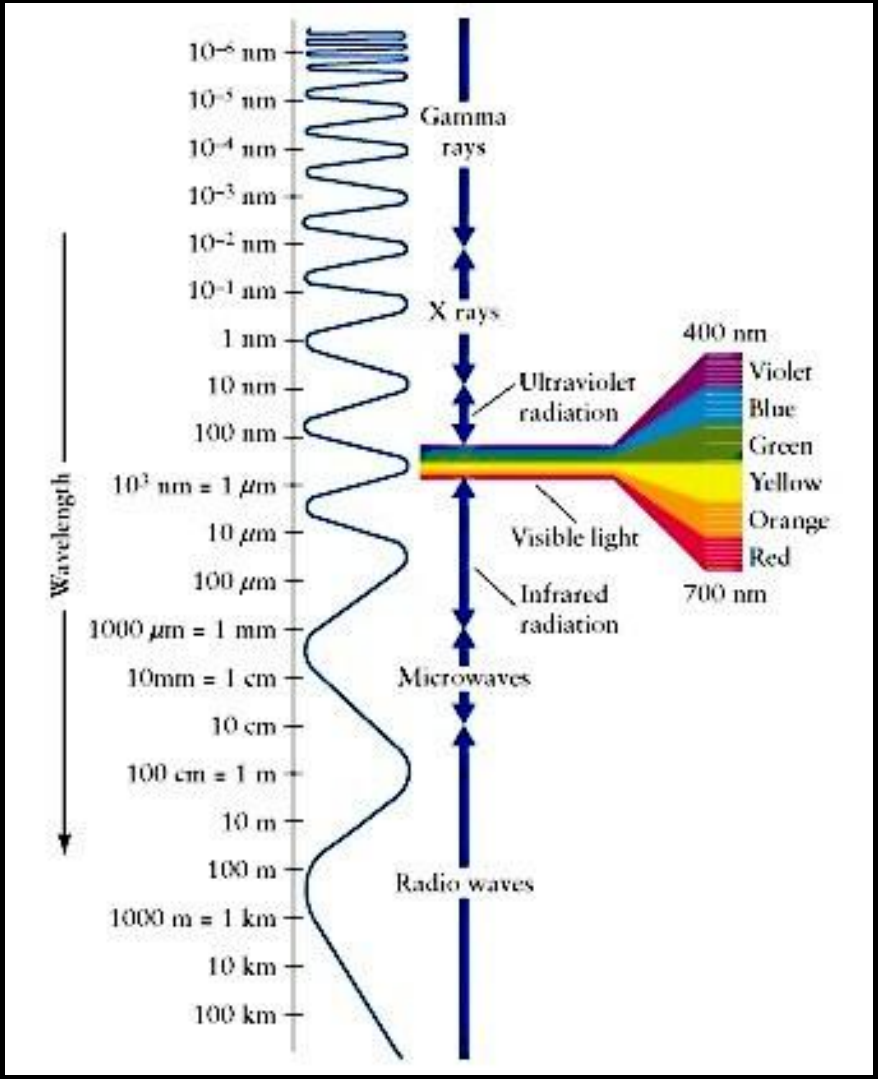
- Imaging samples where the crystal has the same refractive index as the cryocooled solution
  - Artificially done through cryoprotectant
  - Real example case
- Increasing the sensitivity and improving the optics of the camera
  - Determining camera limits, non-uniformity correction at those limits
  - Cooling the lens, increasing depth of field and working distance
  - Increasing exposure time
  - Back infrared illumination or heat shielding entire system
- Examining salt crystals
  - ‘Very’ preliminary experiments show promise in this area (but I am an optimist)

Infrared and visual light imaging makes use of only a small part of the electromagnetic spectrum.

Microwaves and radio waves are alternative methods.

Acoustic methods also offer sensing opportunities.

Another approach may be phase based imaging.



Summary

- Infrared imaging shows the crystal position in a cryoexperiment.

- Infrared imaging is non-invasive.

- It has yet to be proven that infrared imaging can reveal samples that visual imaging could not. There is however, reason to consider this a likely outcome.

- There are pitfalls that will need to be fully explored.

- Infrared imaging opens up another small part of the electromagnetic spectrum for crystallographers.

Acknowledgements

- Dr. Mark van der Woerd
- Dr Russell Judge
- Dr Ashley Deacon
- Dr. Mitch Miller
- Darren Ferree
- Richard Kephart
- Janet WIlks
- Dana Roeber
- Funding: NASA NRA Award
- SSRL