Practical tips and experiences from remote data collection

Caveat Emptor

The tips, experiences and opinions presented are those from the user side after many remote data sessions involving many investigators. They do not necessarily represent the views of the user support at the synchrotron, are frequently being revised, and may change with time, more practice and better sleep. They are in addition to and not replacing those tips available on the user guides at SSRL. The tips and experience may be obvious or new, hopefully they will be useful!

If you have any tips to add they would be most welcome.

Asimov's Laws of Robots

- Zero'th law – A robot must not merely act in the interests of individual humans, but of all humanity.
- First law – A robot may not harm a human being, or through inaction, allow a human being to come to harm.
- Second law – A robot must obey orders given by human beings except where such orders would conflict with preceding laws.
- Third law – A robot must protect its own existence as long as such protection does not conflict with the preceding laws.

…applied to remote data collection

- Zero'th law – A robot must not merely act in the interests of individual crystals, but in the best interests of the crystallographer.
- First law – A robot may not harm a crystal, or through inaction, allow a crystal to come to harm.
- Second law – A robot must obey orders given by crystallographers but may first question them if it senses evidence of sleep deprivation and lack of common sense.
- Third law – A robot must protect its own existence and reproduce at other beamlines as long as such protection does not conflict with the preceding laws….

First get your beamtime

A successful proposal

- A successful proposal has three components:
    1. Important structural target/s, and/or a novel and useful experiment
    2. Sound justification of the need for synchrotron time
    3. Experience and previous results (can you carry out the experiment)
- Many times the first and third component are well described but the second is weak as users may not have had too much synchrotron experience or do not take the time to think about that aspect.
- Make your proposal strong by balancing all three components.
- Contact the support staff beforehand for advice if you have any questions to ask. Most, with rare exceptions, do not bite.
- Make a reasonable estimate of time and a determination the appropriate beamlines that could be used. How much time does it take to screen, how much time does it take to collect data, how many samples do you have then ask for one shift extra?
- Look at other successful proposals if you can.

Justifications for Synchrotron Time

Examples include:

- A spectrum of energy (wavelength) unavailable in the laboratory. Required for anomalous diffraction experiments.
- An increased intensity to push the resolution limit further. What resolution do you have, why do you need to extend it further? What question cannot be answered at the current resolution?
- The ability to resolve longer unit cells. What can the system at home resolve, what can you expect to resolve at the synchrotron? Look into the beamline instrumentation, do a quick calculation.
- In the robotic case, study many samples much faster than at home. What percentage of crystals provide good diffraction, how many do you need to screen efficiently? What time would that take at home.
- Does the experiment requires many measurements in a rapid as possible time?
- Why remote time? Standard experiments, limited travel budget, save the funding agency money, enable you to do more?

Now you have your beamtime think about:

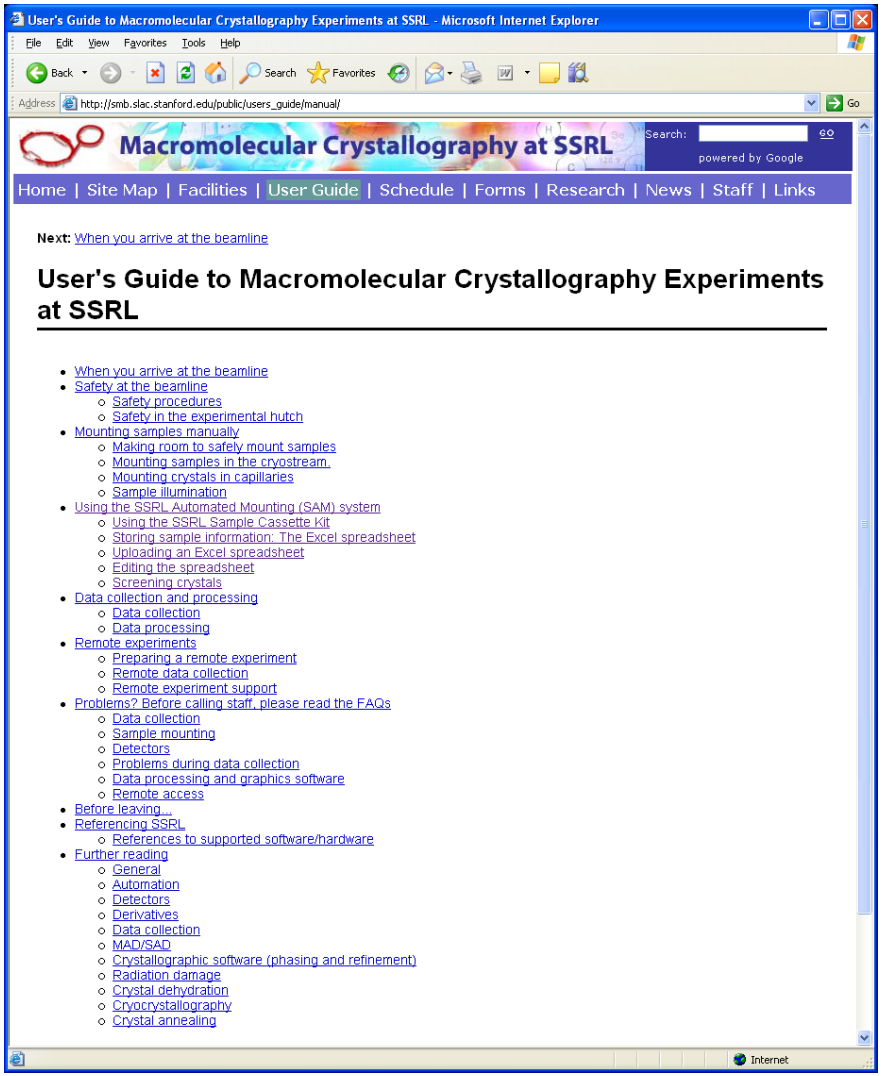
- Preparation – Things to do beforehand
- Filling – Putting samples in the cassette
- Shipping – Options and tips
- Screening – Quick look data collection
- Planning – Going from screening results to data collection
- Data collection – Tips and tricks
- Data backup – Getting it home
- Finishing – Acknowledgements, lessons and feedback

Preparation

Robotic beamtime is no substitute for the real thing. To use robotic beamtime to its fullest potential you should try and ensure that at least one member of the team is competent in data collection at the synchrotron and understands the differences from home source collection. Personal contact with the beamline staff is also very useful for transferring the latest ideas, advice etc. Stanford also has very nice restaurants….

General Preparation

- Know where to find, read and understand the user guides.
  - These contain very useful practical tips and are updated regularly from comments received by users.
- Get details on the software available on the beamline and make sure you know how to use it. Print out the manuals and have them to hand.
- Make sue your remote access software works in advance and that you have an alternate plan if you should have network problems
  - Try and find a 24 hour Starbucks and kill internet access and caffeine needs at the same time ☺ Most users at HWI now collect data from home. DSL or cable internet access are more than sufficient.
- Know how to develop a good data collection strategy, how to integrate and scale the data. Be prepared to do this rapidly during screening and data collection.
- Know your crystal, screen it or others in the laboratory beforehand. If possible know the space groups and expected unit cells.
- Think about the sequence of experiments assuming that the experiment could end unexpectedly at any time (one advantage of robotic beamtime is that your sample can usually be saved for later if necessary).


User's Guide to Macromolecular Crystallography Experiments at SSRL - Microsoft Internet Explorer
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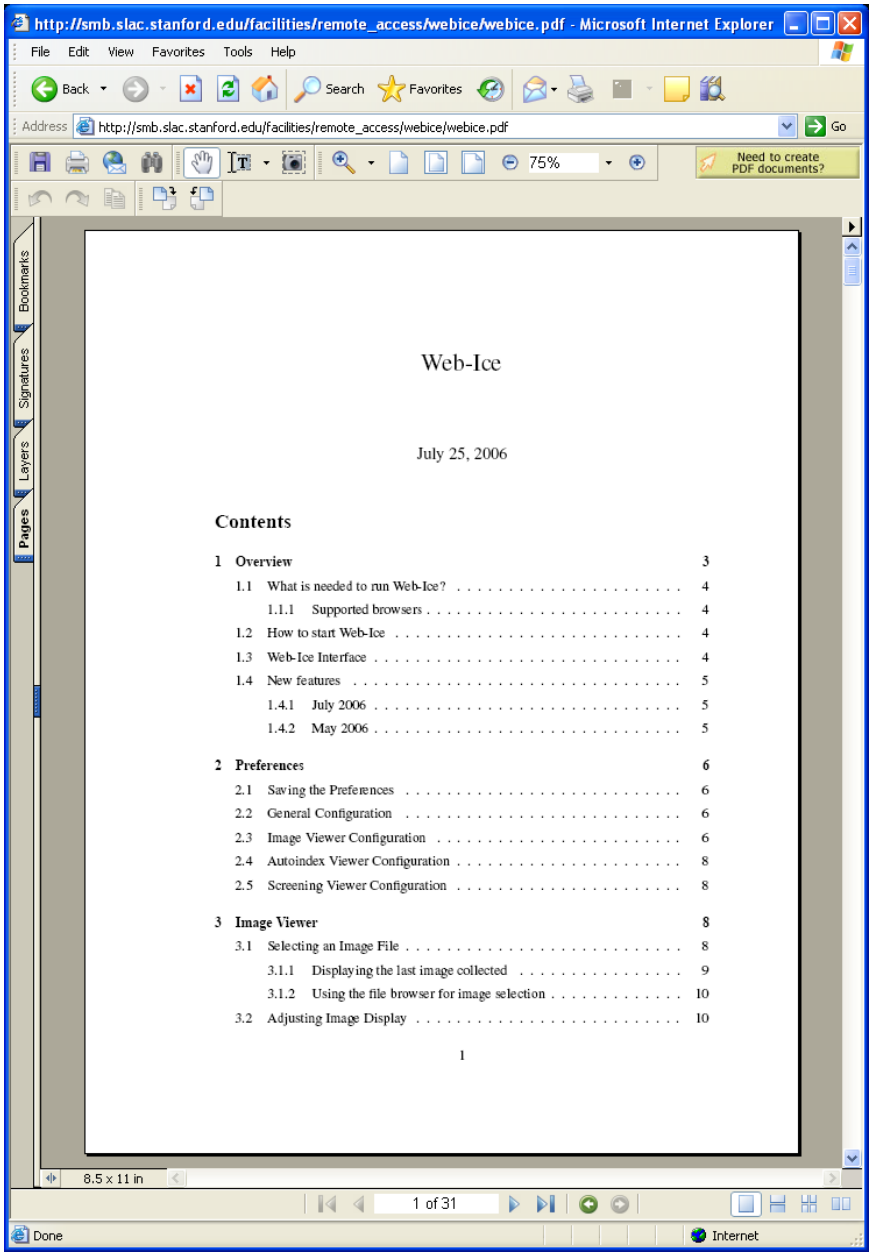
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Need to create PDF documents?
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July 25, 2006
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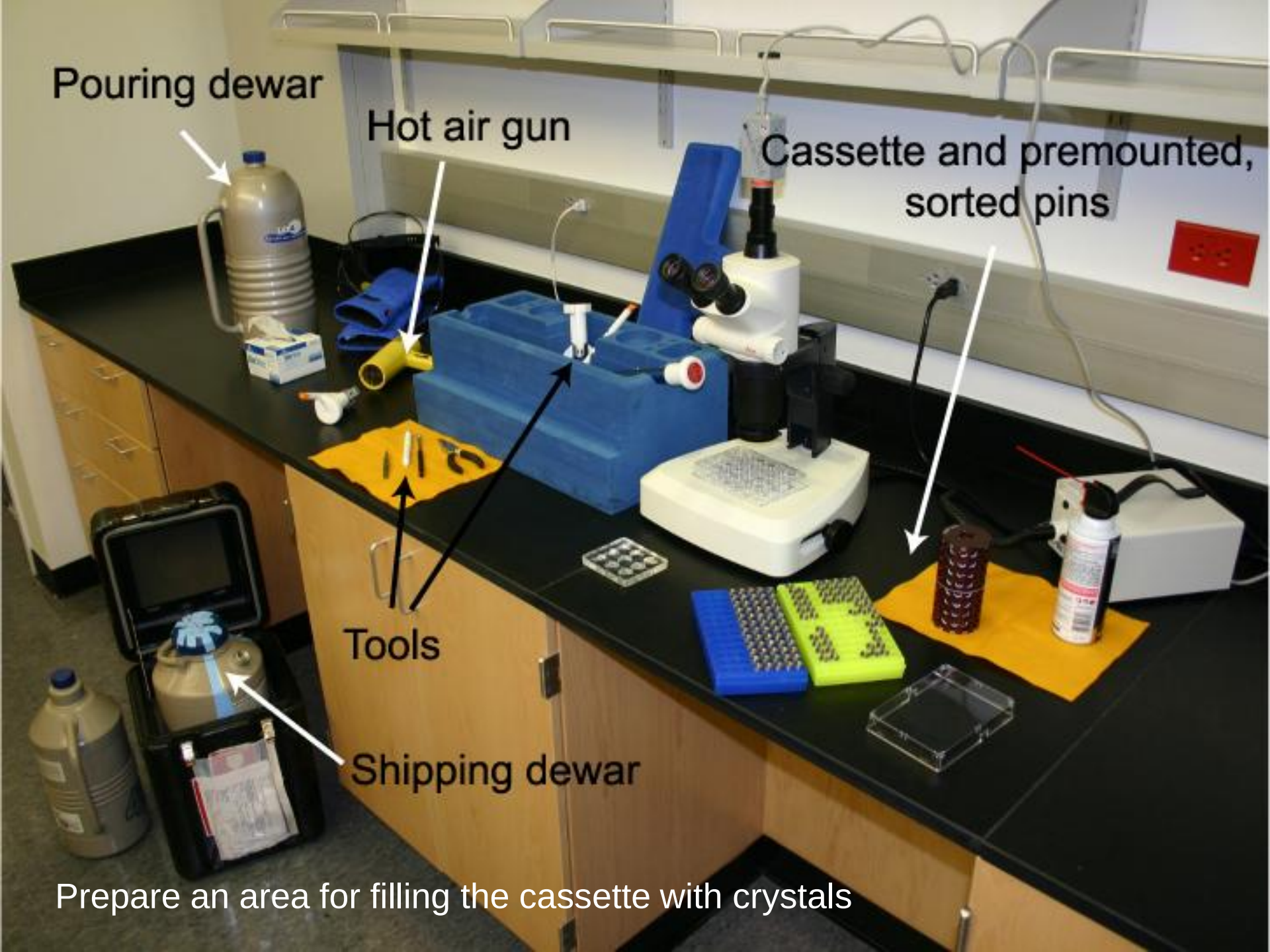
Preparation for Filling

Preparing pins

- Prepare a selection of pins and loops to suit crystal size.
- Prepare several at once.
- Select an appropriate loop to put in the pin.
- Glue the loop in the pin, 5 minute epoxy on the bottom of the loop holder to be inserted into the pin works well. Avoid getting epoxy on the outside of the copper mount.
- Check the loop under a calibrated microscope, make sure it is intact then write the size on the magnetic base.

Preparing for filling

- Make sure you have an excess of different size loops in standard height pins. Loops and pins can be cleaned and reused.
- Have loops and pins organized by size ready for mounting (see the 96 well organizers).
- Make sure the loop size can be read.
- If feasible, switch your entire laboratory over to SSRL compatible height pins. This will save problems later. Loops that are too short confuse the automatic centering and stop the process.
- Make sure the loops and pins are clean and the loop is solidly held in the pin (test and verify). A pair of pliers is a useful tool to have around.



Prepare an area for filling the cassette with crystals

Testing Loops

- Use forceps to test that the loop posts are firmly seated in the magnetic base
- If not, extract the loop with a pair of pliers and if the post is clean either re-glue the original loop or put a new one in its place.
- Test all loops before each data collection if reusing them (see last section).

Preparing for filling

- Make sure liquid nitrogen is available for the filling.
- Use a phase separator on the liquid nitrogen delivery tube. These are available for approximately $50 and reduce the amount of gas released when transferring liquid nitrogen. They are good for both safety and economy reasons as the amount of nitrogen used seems lower with these.
- With practice and the use of a phase separator, a single 100 liter liquid nitrogen Dewar is sufficient for the filling of two shipping Dewars and four cassettes with nitrogen left over (commercially the cost of nitrogen for this is about $100).
- Have safe handling equipment available; gloves, face shield and safety glasses as appropriate to your institutions liquid nitrogen handling policy. .
- Have two or more pouring Dewars ready and a suitable funnel to help fill them.
- Have a cassette (cryocane) Dewar ready. This is useful for washing the cassettes and brief storage when emptying the shipping dewar of liquid nitrogen.

A phase separator is a useful piece of equipment to fit on the end of a transfer hose. It cuts down significantly on the gas rather than liquid that is transferred, only costs about $50 and seems to make our large Dewar last longer between refills.

Liquid should flow fairly rapidly. If not ice has formed inside the separator and it needs to be warmed and dried.

Preparing for filling (continued)

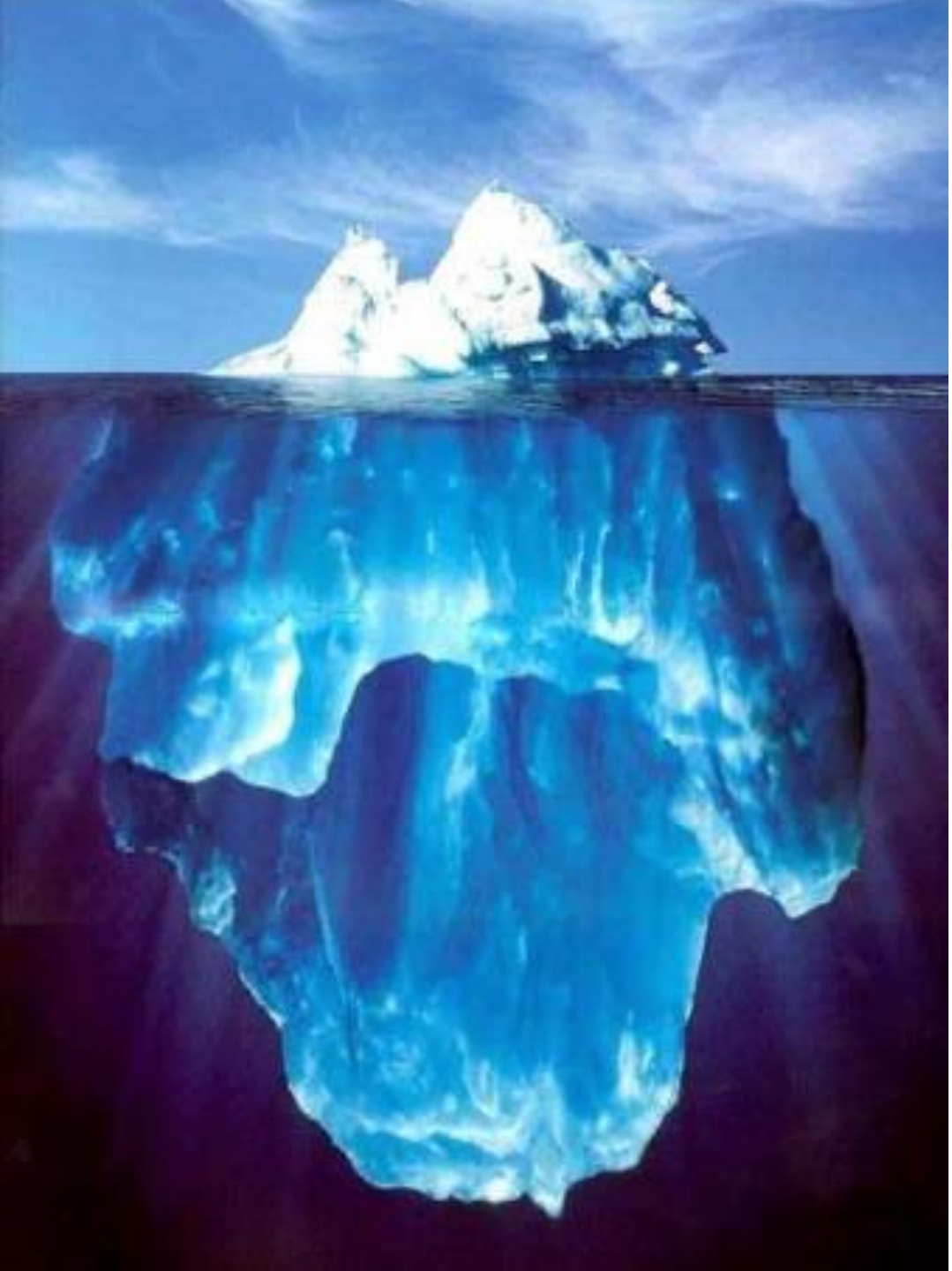
- If the shipping Dewars show evidence of ice allow them to warm and dry before use. Sometimes ice can build up if the Dewars are left dry but cold for a prolonged period.
- Before filling make sure the shipping Dewars have been fully charged and have not lost vacuum. Follow the manufacturers instructions for filling.
- Keep them filled with liquid until ready for shipping.
- Make sure the handle on the Dewar insert is firmly attached and the Dewar insert can be easily removed and replaced in the Dewar. The handle can come loose, this is not a disaster but it is useful to know before ‘discovering it’ during filling.
- Keep a spare microscope bulb and know where to find it.
- Keep a check list and follow it and amend it as necessary.
- Have a small sieve for removing ice from the liquid nitrogen in the blue reservoir.

Preparing for filling

- Have control crystals available, *e.g.* cryoprotected lysozyme, for each cassette. These can be used to tell if something untoward happened to the Dewar during shipping, e.g. if it were opened or tipped and warmed a little.
- A dry Dewar will keep below 100K for over two weeks if unopened (but don't let it).
- If available fill in a fume hood to create an updraft of boiling nitrogen and reduce ice formation.
- Have a log book ready to note down crystal details and position.
- Decide on the order of filling and cassettes to use.
- If shipping an odd number of cassettes remember that the styrofoam insert is not trash! If you loose this ship an empty cassette on top of the full one.
- Practice dry and if possible under liquid nitrogen.
- Have the microscope next to the filling reservoir.
- Make sure the cassette or cassettes are empty and clean. Sometimes the loop holder can come out of the pin and is still lodged in the cassette. Filling this position will cause severe problems.

Filling the cassette

(The first point where things can go wrong)



The number one practical tip:

Ice is the Enemy

Filling should be done as rapidly as possible.

Filling – Ice is the Enemy

- Note the cassette number and order to be used.
- Make sure the shipping Dewar is still full of liquid nitrogen, top it off, keep it closed as much as possible.
- At all times assume the cassette could fall during transfer. Use cryogloves beneath it, not to hold it, but to catch it just in case.
- Each cassette contains 96 experiments. If several people are filling the result could only be as good as the worst skilled
- Make sure the nitrogen level is maintained.
- The blue lid covers the filling reservoir, it does not prevent boil off of the liquid nitrogen. If loading in a fume hood it is better not to use it.
- If floating ice appears put the cassette in the full shipping Dewar, empty and dry the reservoir and then replace the nitrogen.
- If in doubt, use fresh nitrogen.
- If frost appears on the cassette wash it by pouring liquid nitrogen over it.
- Do not leave for a prolonged period, nitrogen boils off fast.

Filling (continued)

- Keep the pouring Dewar filled.
- Use good illumination.
- Make notes during the filling and transfer them to the spreadsheet as soon as possible. Even better, enter them as filling.
- Cover the strong magnet on the wand with tape to remind you not to use it.
- Use two tool sets if you have them. Dry one set as you use the other set. Swap over every 4 crystals or sooner if needed.
  - Ice on the end of the tool can cause the pin to stick to the tool.
  - Ice in the guide tool can cause the crystal to hit the cassette edge.
- If the pin will not dismount push it back in then angle both the wand and guide tool. The pin should remain in the cassette (see next slide).

The magnet head can sticks to the wand due to ice between the two. To overcome this push the magnet in with the wand following the guide tool, then tip both the wand and guide tool together. The magnet will remain in the cassette. New tools with a push release can also develop the same problem (with a similar solution).

Filling (continued)

- Mount several samples of each crystal.
- If possible use a little bit more cryoprotectant than needed.
- Empty the shipping Dewar at the last moment before have it picked up or take it to FedEx.
- Make sure all the liquid is emptied (follow the manufacturers instructions, e.g. Invert, stand upright for some time, invert again etc.) A little liquid left can cause an amazing paperwork mountain if discovered. Future shipments would be jeopardized not to mention possible civil and criminal penalties.
- Wash the cassettes in liquid nitrogen if necessary to remove ice and then put then in the shipping Dewar.
- Tape the Dewar lid and write “Contains sample” on the tape (advice from the FedEx guy).

Shipping the cassette

(Another major point where things can go wrong)

Shipping

There are several ways to ship,

- Ground shipping in a dry Dewar (several days)
- Ground shipping in a Dewar or dry shipper with liquid nitrogen (several days)
- Overnight in a dry shipper (the usual method)
- Overnight in a Dewar or dry shipper with liquid nitrogen.

- Shipping liquid nitrogen, *i.e.* in a Dewar or with the dry shipper filled with liquid requires lots of paperwork and a training course. For further details see me later or check out:

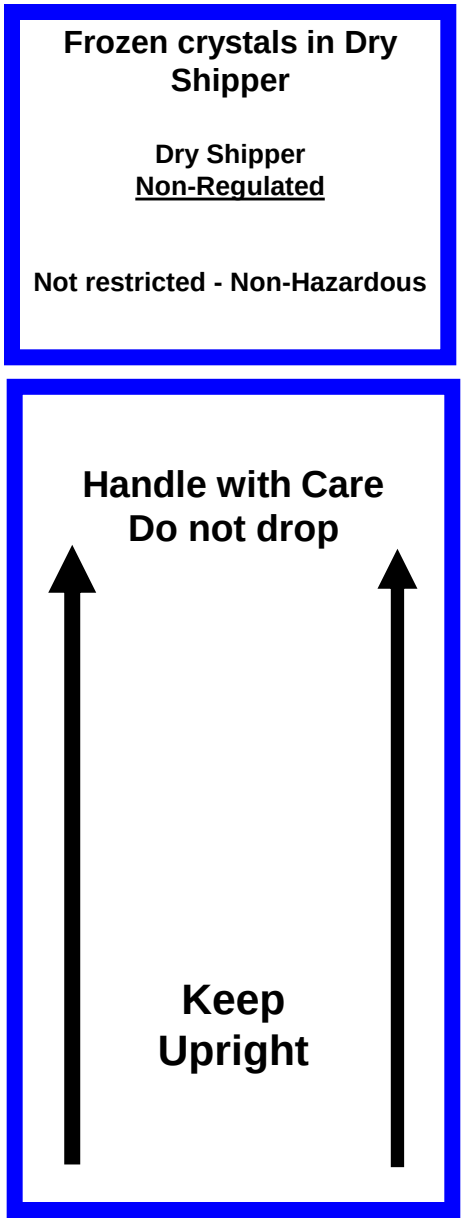
  https://prosperitylms.com/req/fedex_student/

  and

  http://www.fedex.com/us/services/options/express/dangerousgoods/seminars.html

Shipping Dry (magic words)

- To avoid delays in shipping your Dewar (shipping dry) should have a label with the magic words “Non-regulated”, “Not-restricted” and “Non-Hazardous”.
- Any label signifying liquid nitrogen should be removed if shipping dry (your Dewar will be returned to you if this is not removed).
- For shipping in liquid nitrogen the rules are very different and beyond the scope of this workshop – please ask me later if you are interested in these details.
- Ship to arrive at least a day in advance of the beamtime and arrange to have the Dewar filled on arrival. Note that shipments are not accepted over the weekend so if your beamtime starts Monday aim to have the Dewar arrive on the Friday.



Shipping (continued)

- Track the shipment to ensure no surprises. First time Dewar shipments can be like a boomerang until the local FedEx office becomes used to the sight of those strange boxes.
- Label the Dewar with your name, similarly label the shipping box on the outside.
- Put the return shipping label inside the shipping box.
- Note: Our own experiences on icing during shipping have been mixed hence the use of a control crystal.

Planning

(This will help make your experiment efficient)

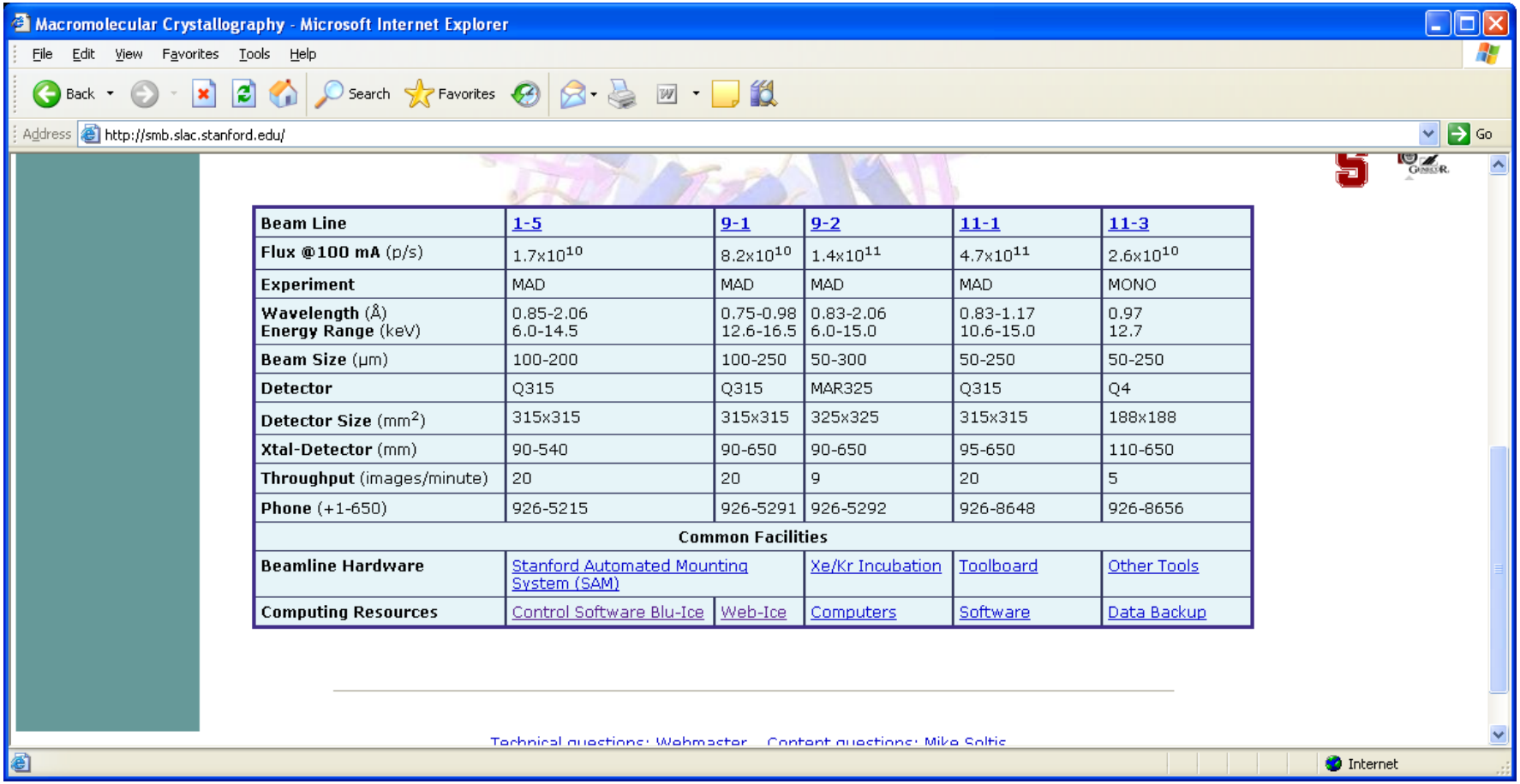
Planning – Communication

- Contact the support staff before the run, let them know how many cassettes are planned.
- Share all the contact details of the team with each other and the support staff. Try and have one or at most two numbers where the person collecting data can be contacted at all times.
- Get the cell phone number for the support staff (tip, look on the website at SSRL using the browser available in the remote software).
- During available hours, 9:00 am – 9:00 pm California, don't be afraid to contact the support staff. Check if they will be taking calls outside these hours and if not, do not call.
- If you fail to contact your staff support look on the schedule to see who else is on support and try them next … if it is urgent.
- Have a good communication plan within the team collecting data, especially if many different locations are being used. Instant messenger has worked quite well but a chat window available in Blu-Ice would be lovely ☺

At the synchrotron

(Setting up at home, screening and data collection)

Know your beamline



Beam Line	1-5	9-1	9-2	11-1	11-3
Flux @100 mA (p/s)	$1.7x10^{10}$	$8.2x10^{10}$	$1.4x10^{11}$	$4.7x10^{11}$	$2.6x10^{10}$
Experiment	MAD	MAD	MAD	MAD	MONO
Wavelength (Å) **Energy Range** (keV)	0.85-2.06 6.0-14.5	0.75-0.98 12.6-16.5	0.83-2.06 6.0-15.0	0.83-1.17 10.6-15.0	0.97 12.7
Beam Size (µm)	100-200	100-250	50-300	50-250	50-250
Detector	Q315	Q315	MAR325	Q315	Q4
Detector Size (mm^2)	315x315	315x315	325x325	315x315	188x188
Xtal-Detector (mm)	90-540	90-650	90-650	95-650	110-650
Throughput (images/minute)	20	20	9	20	5
Phone (+1-650)	926-5215	926-5291	926-5292	926-8648	926-8656
Common Facilities					
Beamline Hardware	Stanford Automated Mounting System (SAM)		Xe/Kr Incubation	Toolboard	Other Tools
Computing Resources	Control Software Blu-Ice	Web-Ice	Computers	Software	Data Backup

Setting up at home

- It is possible to efficiently run the screening and data collection from a single computer but it is much easier to use two or three.
- One computer should be dedicated to the Blu-Ice control software. The other can be used for data processing, structural solution, refinement, web surfing etc.
- If using a computer with a small screen or low resolution the effective screen size needs to be increased so that software such as HKL2000 will run.
- Different session names should be used. If you have multiple users it is good to incorporate their name into the session name. That way you do not accidentally terminate their session instead of your own.
- If you loose connectivity the current process will not stop. Log in again and hit passive to gain control of the software.
- You cannot log into Blu-Ice until the support staff enable you and your beamtime.
- However, you can log into the “blcpu” computers and create a directory for the data collection.


Blu-Ice for data collection with HKL2000 processing
Web-Ice and beamline video system
Structural data processing (CCP4)
Strong Coffee
Phone to call staff

Screening

- Make sure your spreadsheet is uploaded and the appropriate directory is set for screening.
- One person needs to monitor screening throughout – failure of auto centering can stop the screening, crystals may be shot out of the beam etc.
- Scoring is fairly accurate however it should be checked for choosing the top samples to study:
  - Bad crystals may not be too bad. Icing can produce bad statistics but washing or even returning the crystal and remounting it effectively removes small amounts of surface icing.
  - Good crystals can be bad. Ice crystals can mislead scoring.
- The screening images can be integrated and a strategy determined however if the crystal is returned to the Dewar and remounted the crystal position often changes. Strategy will have to be run again.
- If it is a critical experiment collect data as soon as the screening shows a good crystal (see tips in data collection).

Screening

- Use good names for data directories. Process in a separate directory with a related name. Write these down in a logbook.
- Use the video cameras to troubleshoot – check the ion chambers, that the phi axis is correct and matches the software setting. Make sure the beam is coming through, *i.e.* no attenuation etc. then call the support staff. If out of hours call a more experienced person in the lab to have a look. If this does not do the trick go home and get some sleep and come back when the support staff are on call again.
- Generate a plan for data collection priority as screening progresses
- Each sample takes about 4 minutes (for a few seconds exposure time and two images). The largest part of this is mounting and dismounting.
- *It is quicker to center manually if you can keep up the pace and are ready to do this immediately after mounting (no longer an option).*
- Make sure you know what is happening and keep stuff happening, It is easy to be whiling away the time while screening has stopped for any number of reasons. Make notes on diffraction that looks good, compare with the automatic scoring routine.

Data Collection

- Follow a plan and know how to process the data before you collect it – practice with other data sets.
- Check the directory before collecting into it. Check the directory again, check you have the correct crystal, check the crystal and the directory!
- Plan on having rested before data collection. On a long run the person screening should get some sleep before starting data collection.
- Don’t be greedy with resolution if collecting a MAD data set.
- Collect in dose mode but if you change beam size, dose needs renormalizing.
- Use a low (possibly medium) and high-resolution pass in that order if the data needs it. Low resolution can use a much shorter exposure time than high and can also use a wider oscillation range. Use about 20% resolution overlap depending on the number of reflections (a sufficient number must be common to scale the sets).
- Understand how to use distance, wavelength, oscillation and offset and the consequences in terms of data completeness where appropriate. Never think of the detector as square but use it as a round one (with no offset). Know the spectrum of the beamline and how to use it most effectively.

Data Collection (continued)

- For MAD data save the MAD scan data. Note down the wavelengths, and f’ and f’’. Know the sequence, molecular weight and number of sites expected.
- Know how to make a good data strategy and the differences between an anomalous and a completeness strategy.
- Use testgen in Mosflm or equivalent to determine the most appropriate oscillation angle/s. Check out the program Best.
- Collect complete data sets, if more time is available collect even more data.
- Process your data as soon as it is collected even if the processing is a quick and dirty job. Make full use of the scripts available (have 2 people working during data collection). If cannot be immediately processed resolve the problem with the help of the support staff.
- There is no advantage to having the detector any closer than the edge of the diffraction. However the edge is difficult to determine by eye.
- If several people are collecting have a good communication plan.
- Let the support staff know if you are going to finish early.

Being polite
(several projects during the same beamtime)

- If there are several projects collecting data remember that any waste of time by you eats into time that could be used productively by someone else.
- Don’t waste time and have a plan beforehand on the priority of samples.
- Let others know the approximate time you will finish.
- Keep an eye on the data collection and process continuously so that any problems can be identified early on.
- If this is your critical experiment only when you are happy should you remove that crystal (and take a lower priority next time).
- Those who send salt shall be shot ☺

Finishing up

(Data transfer, sample return, pin cleaning etc.)

Transferring data

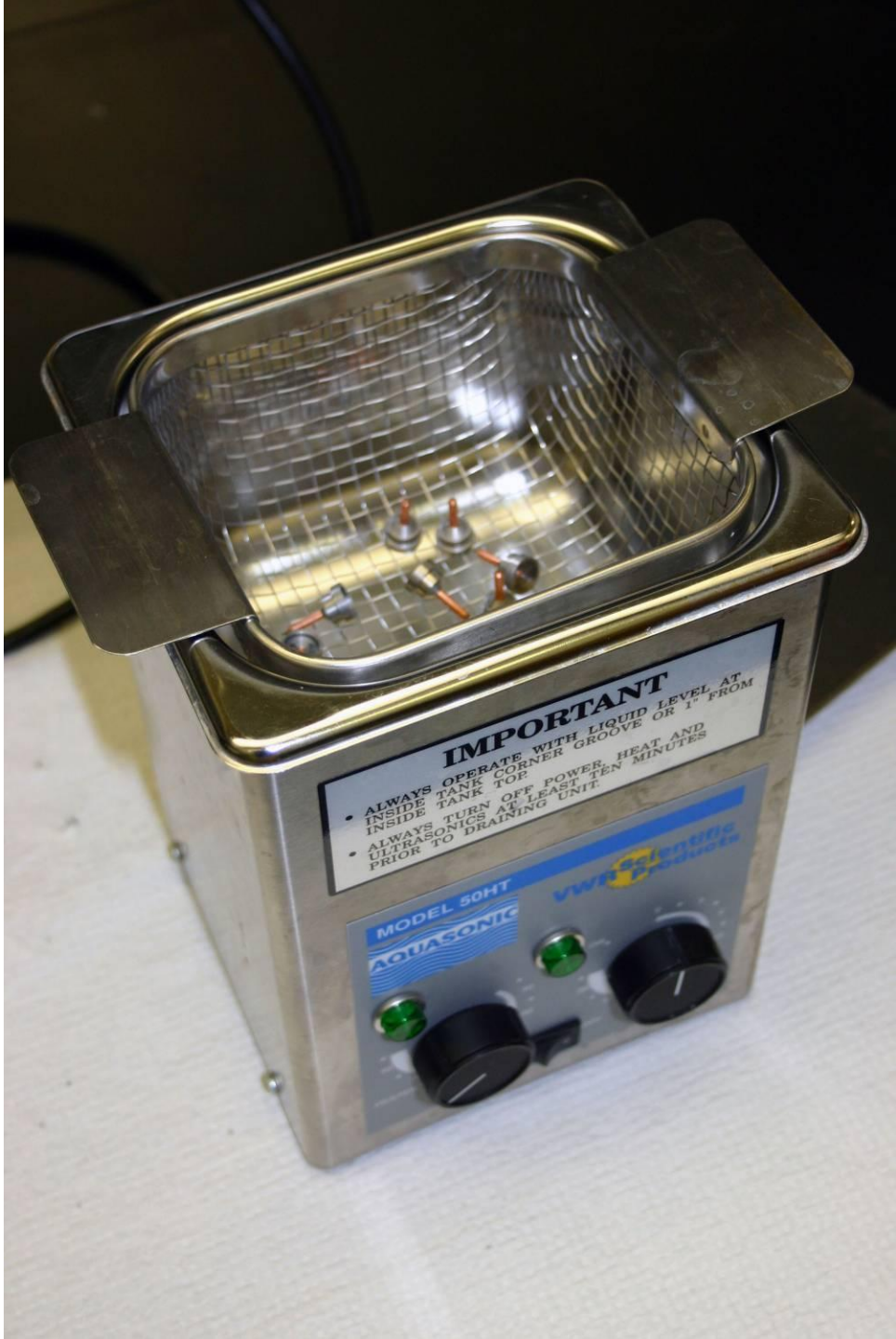
- It is best to process on the SSRL computers and ship the integrated intensities and log files back. The data will follow assuming you have requested it.
- It will take a few weeks for CD/DVD's to be received from the experimental run.
- Use descriptive names for the CD/DVD's. You, hopefully, will end up with a lot of them.

Choices to be made – sample return

- Cassettes can be left at SSRL. Remember this when collecting data. It may be better to spend more time collecting the best data set from one sample than several marginal datasets from another ….
- But, the more manipulation of the crystal that takes place the more chances to lose it!

Cleaning pins

- Pins and loops can be easily cleaned using a sonicator bath.
- We use a water wash followed by a 30% ethanol wash.
- The pins and loops are then dried and examined.
- Broken loops are pulled out of the pin and replaced with new loops.
- Loops still dirty are washed again.
- Best results are achieved using only a single layer of pins.
- About 80% of loops are reusable.



After the run

- Make notes of any problems and suggestions. Send them in the end of run report.
- Contact the support staff directly if you wish (email is best).
- A lot of effort goes into making the robotic system work well and ensuring the resulting data is the best possible -
  - acknowledge the support staff who helped out
  - reference appropriate publications about the robotic system and Blu-Ice
  - acknowledge SSRL
  - Get to know the habits of your support staff. When you see them encourage those habits, e.g. buy a beamline scientist a beer (or anything chocolate for particular individuals), say hello at meetings etc.
- Publish the paper, acknowledge the developments at SSRL that made it possible.
- Look forward to new developments.

Universal Laws of Remote Data Collection

- The most important experiment has the least amount of time available.
- Quick things never are.
- Unwatched nitrogen boils faster.
- Ice forms behind your back.
- When you think someone else is doing it, they are waiting for you.
- The computer will never tell you it is waiting.
- Planning to have something done early will get it done just in time. Planning for just in time will mean it will be late.
- The best crystal was the other one, not the one you collected data on.
- Whenever you set out to do something, something else must be done first.
- When all else fails, read the instructions.
- A difficult task will be halted near completion by one tiny, previously insignificant detail.
- Never trust modern technology. Trust it only when it is old technology.

The Universal Law of Remote Data Collection (from the synchrotron side)

The user will always want more.

Bottom Line

The system works well and is continuously evolving. Feedback to the synchrotron facility is a vital part of this evolution. Let them know what works well for you, what could work better, what doesn’t work and what you would to see.

With a big word of thanks to

The SSRL team that developed the remote data collection infrastructure and now support it for the users and the investigators at Hauptman-Woodward Medical Research Institute that sent crystals and contributed to these lessons