Practical crystal mounting method for the longer wavelength SAD phasing

Nobuhisa Watanabe
Synchrotron Radiation Research Center,
Nagoya University, Japan
nobuhisa@nagoya-u.jp

These are the slides to display a method of preparing crystal mounting device for the longer wavelength S-SAD phasing. Some modifications have been made, but these slides were originally used at the workshop of ACA Chicago 2010.

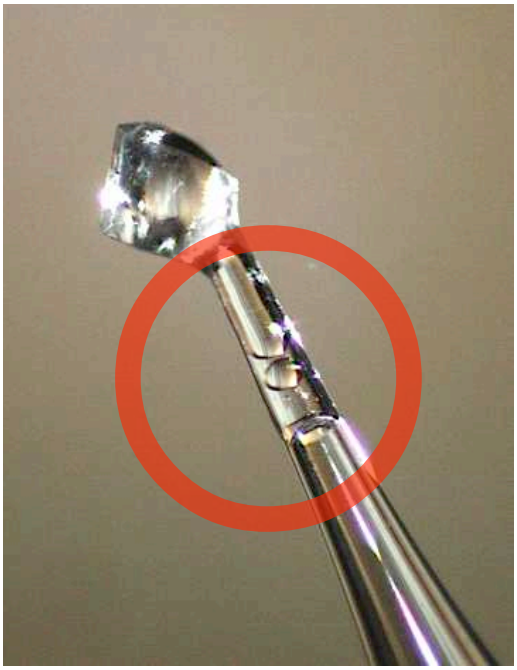
I hope you will be interested in this method, and you will try to use it at your home lab or at your synchrotron trip, and you will be able to get better SAD dataset.

Which is better for S-SAD?

First of all, do you think which crystal mounting method is better
for the longer wavelength SAD phasing?

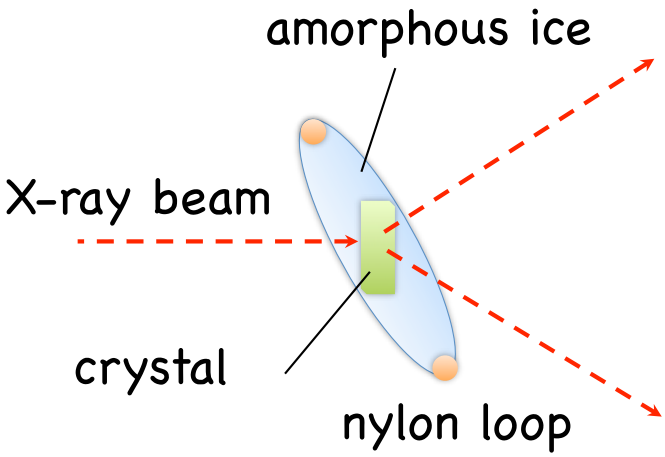
The answer is...

Which is better for S-SAD?



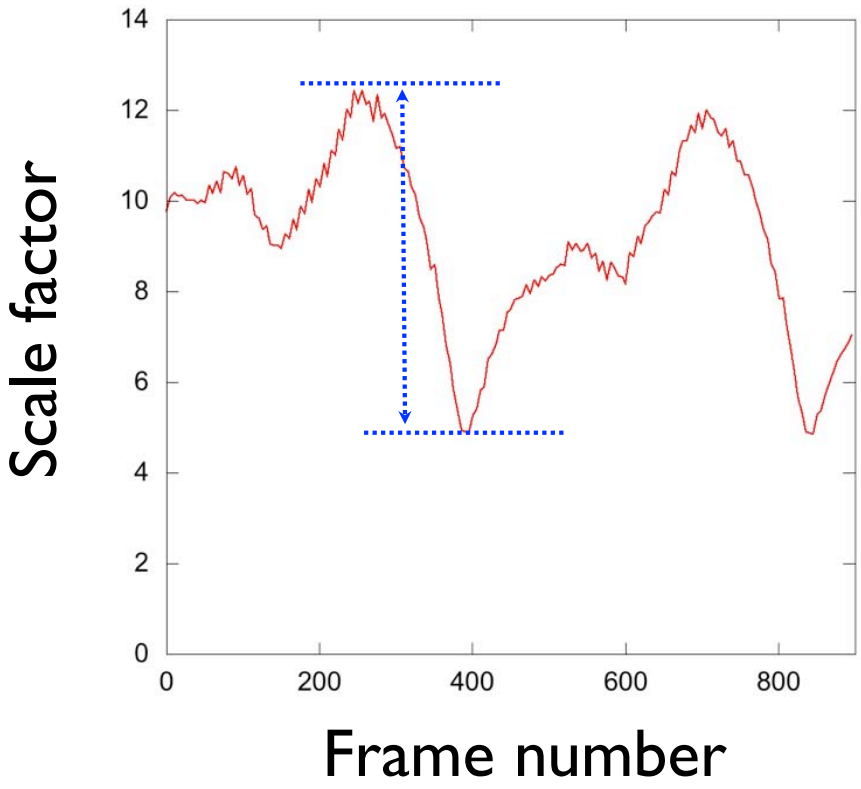
The mounting method of right side might be better. Because,

larger absorption effect of amorphous ice in the loop



If we use the standard loop mounting method, frozen buffer solution exists around the crystal. And the larger X-ray absorption by the amorphous ice reduces the quality of the SAD dataset.

That makes scaling difficult

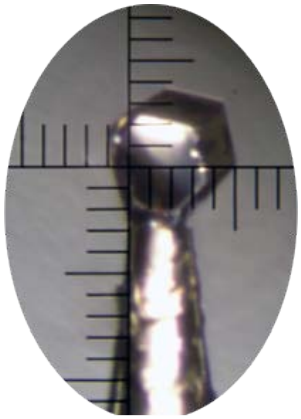


This is an example of the plot of the scaling factors of the dataset using the standard loop mounting method. Scale factor depends on the shape of the large amorphous ice around the protein crystal, and the distribution of the scale factor is very large. Difference between the smaller and larger scale factor sometimes becomes severalfold or more.

Even if we can use sophisticated software for data reduction, extracting only about 1% anomalous difference from the dataset scaled with such scale factors might be a problem.

In order to clear the obstacles,

Our solution is a "loopless" crystal mount



Kitago, Y., Watanabe, N. and Tanaka, I.
Acta Cryst., D61(8), 1013-1021 (2005).

We thought that the best way is removing the amorphous ice around the protein crystal.

If we can mount crystal without ice, it is possible to reduce the scaling error, and the absorption correction might work well because it depends only on the crystal shape.

glass micro-capillary with nylon loop

nylon loop

glass micro pipette

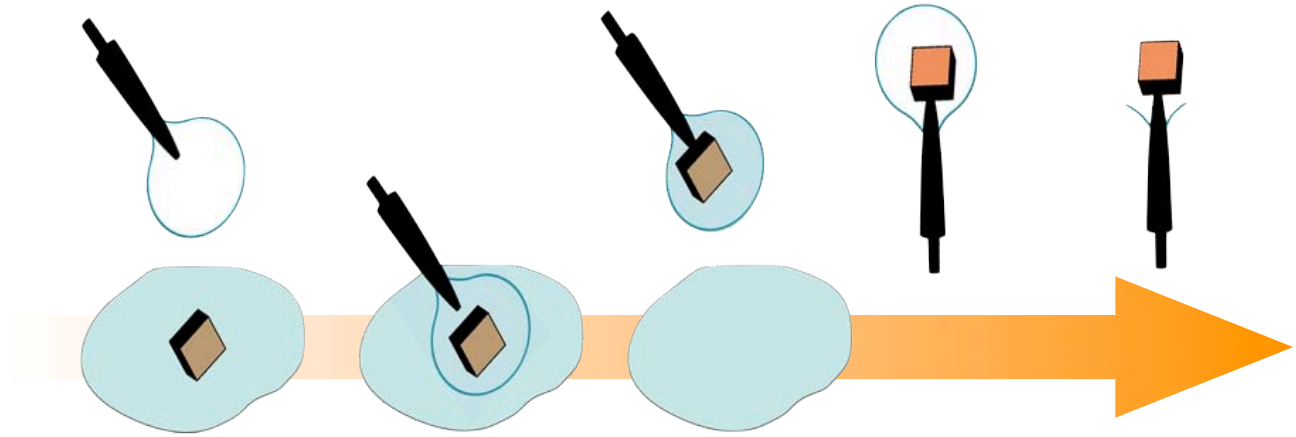
For this mounting method, we made a tool that has a loop at the tip of a tapered micro-capillary.

Both a loop and capillary are important.

As you might expect, the loop is necessary in the harvesting process.

because a loop is necessary

to pick up a crystal across the surface of the drop



Since this tool has a loop, it is possible to harvest a crystal from the crystallization drop beyond the surface tension of the mother liquid, in the same way as the standard cryoloop.

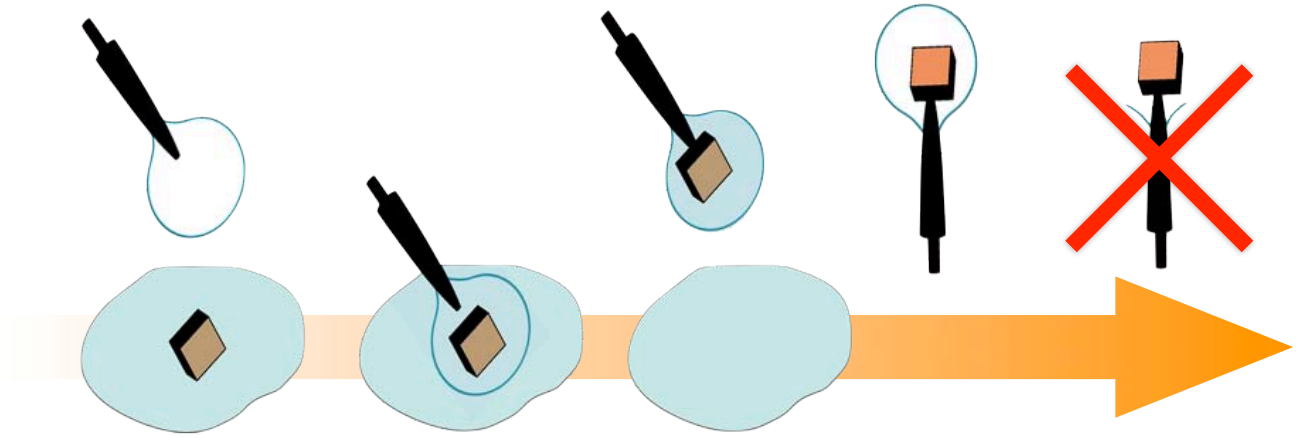
Then, on the goniometer head, the solution around the crystal can be quickly removed
by aspirating through the capillary.

In other words, we can keep crystal in the mother liquid until just before freezing the crystal. This is important to prevent unwanted dehydration of the crystal.

And, if we want, the nylon loop can be removed with a small hook or forceps.

and

removing loop is not so important



But, if the crystal is not so small, the absorption effect of the loop is not serious as frozen solution.

So, we usually keep the loop, like...

Our current approach

remove the frozen solution, but leave the loop

this photograph.

Leaving the loop has another merit. If we can leave the loop, we can reuse the same capillary several times.

How to make it by yourself

ingredients for the tool

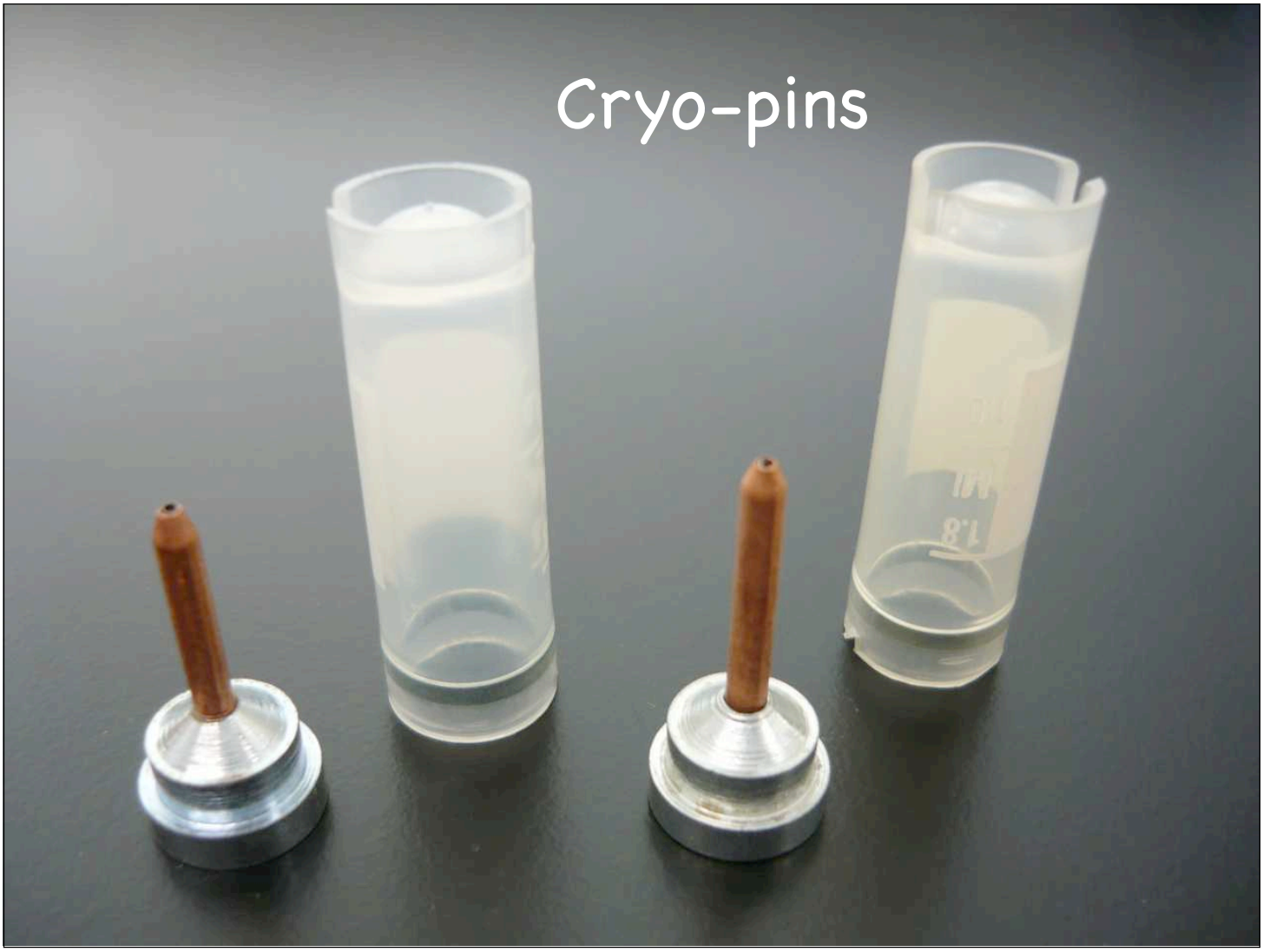
- glass capillary
- nylon loop
- cryo-cap copper
- tweezers & glue
- soldering iron & bees wax

To make the capillary, materials you should prepare are listed here.

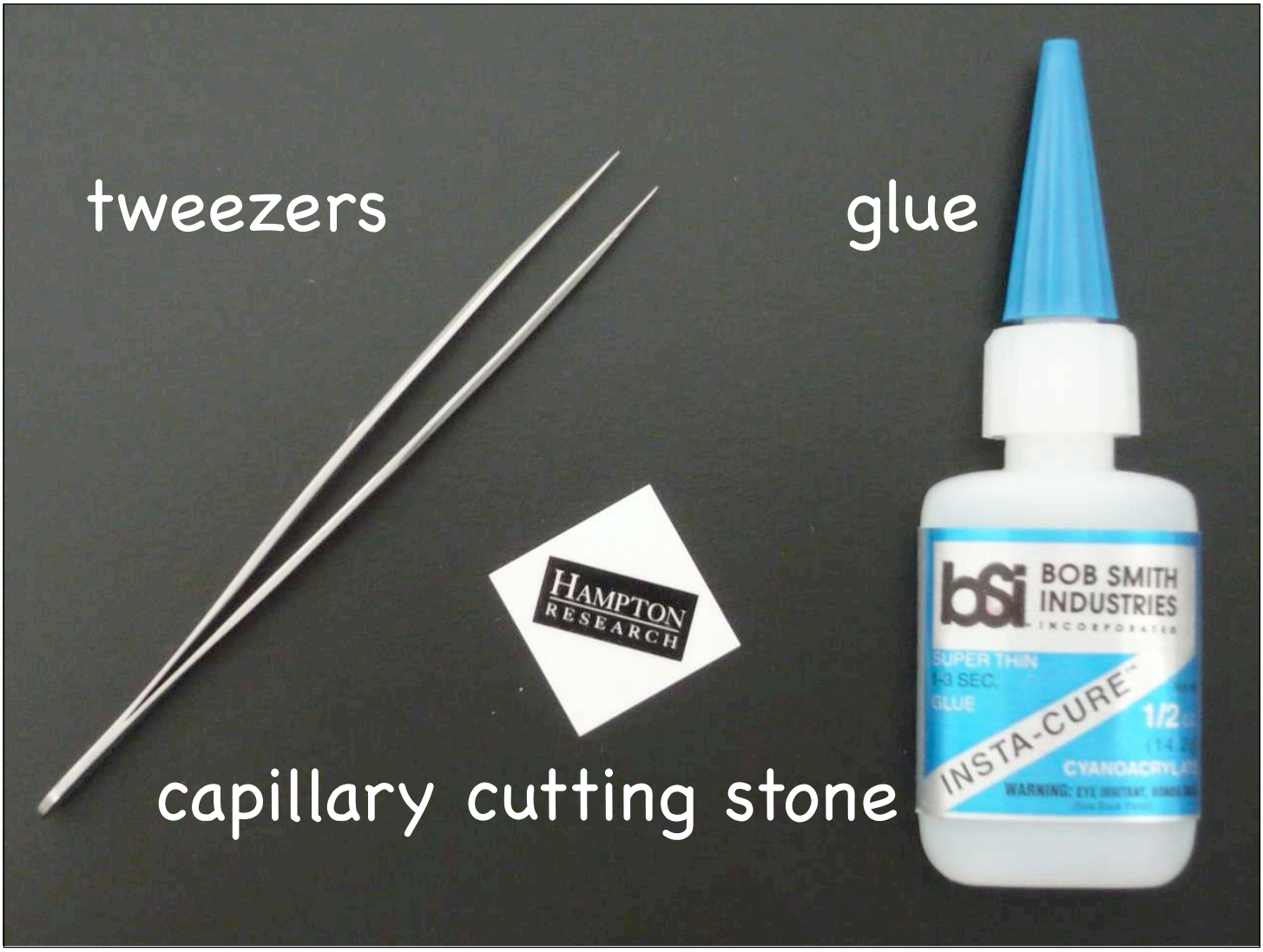
First of all, glass capillaries are necessary. We use 1mm capillary with an inner diameter of 0.6.

You can get ready-made nylon loops from Hampton Research.

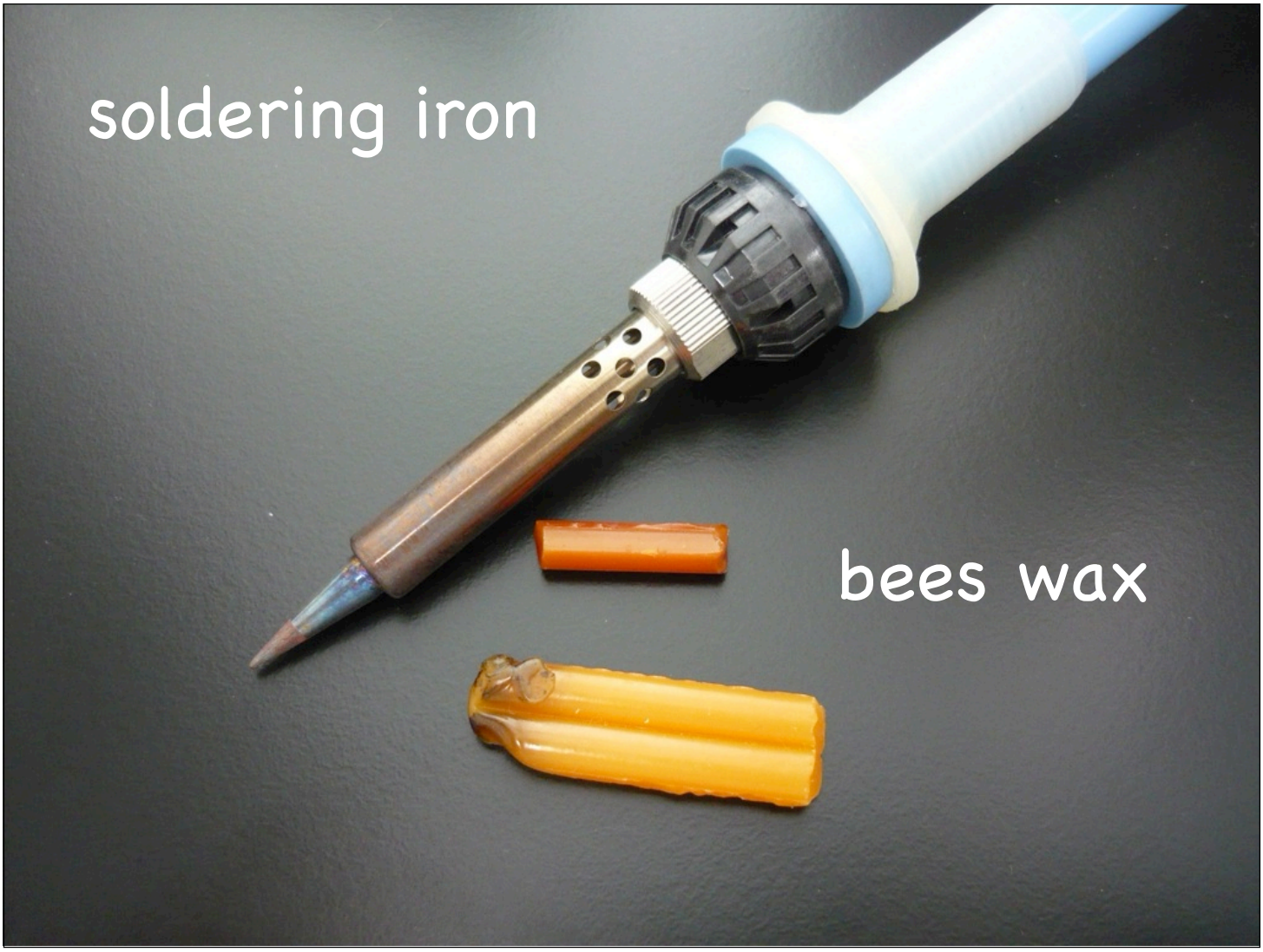
They have loops with several range of the diameter, but using one size larger loop than the standard loop mounting method is better for this method.



And we use the CrystalCap Copper as the base material of the modified cryo-pins.



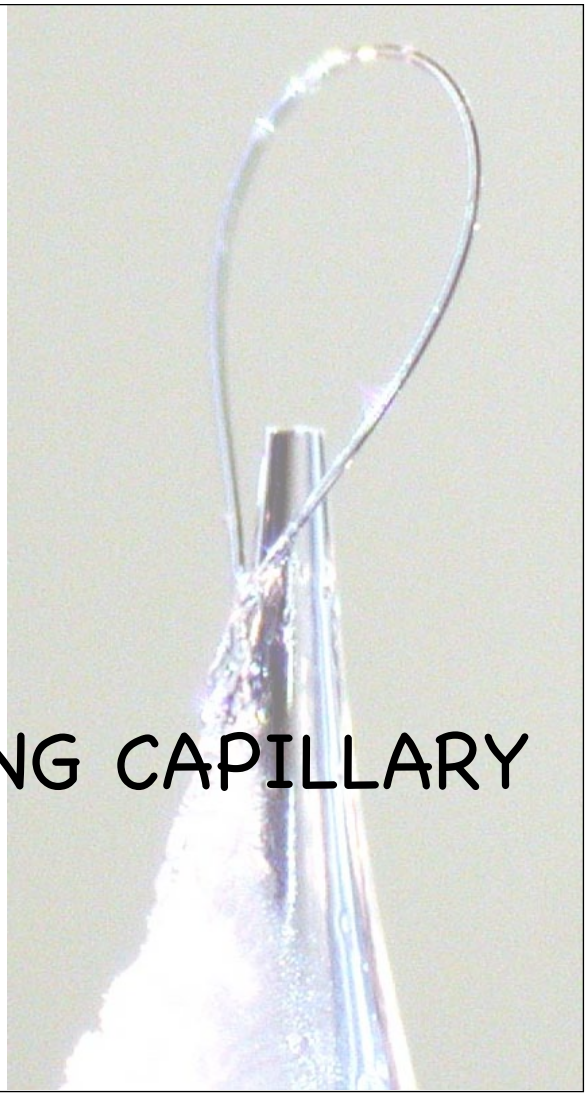
Tweezers, glue, and the capillary cutting stone are necessary, too.
You can get the cutting stone from Hampton Research.



And the last ones are a soldering iron and bees wax.
It is necessary to fix the glass capillary to the modified crystal-cap.
And I use bees wax there.

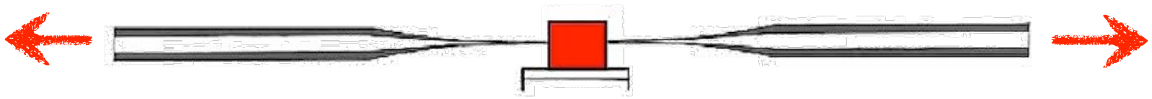
be good with your hands

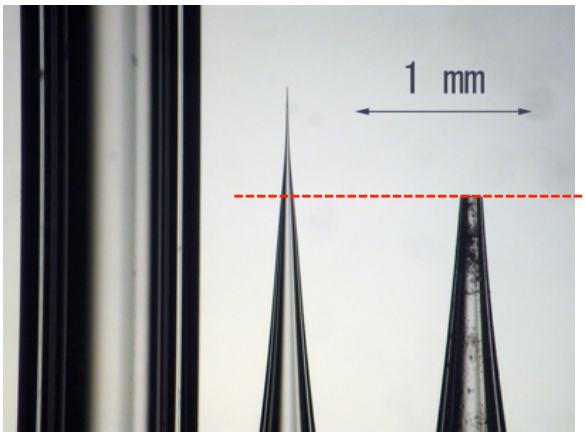
MAKING THE MOUNTING CAPILLARY



Now you have all the ingredients in your hands, and let’s start with making the capillary.

pull & cut a glass capillary





The first thing you should make is the tapered micro capillary.

To do this, pull the glass capillary, and cut it at the suitable diameter for your protein crystal.

If your crystal has a dimension of 100um, the inner diameter of the capillary should be smaller than that. Otherwise, your crystal will be drawn into the capillary.

tools for making micropipette for Cell Biology

pull

pipette puller
(Narishige PC-10)

cut

microforge
(Narishige MF-900)

polish

micropipette grinder
(Narishige EG-400)

We use these tools for making the micro capillary.

This is a pipette puller, and this is a micro-forge to cut the capillary at proper position. We also have a micro-grinder, but it is not essential.

These are the equipments for cell biology. They use these tools to make a micropipette for their experiment.

I think you can try to make the tapered capillary like this photograph.

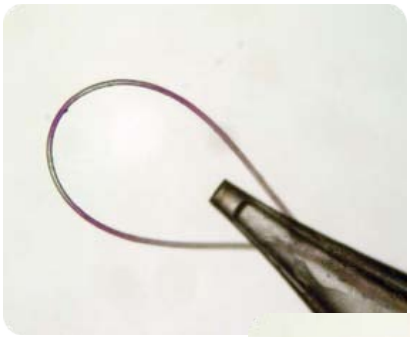
better way might be

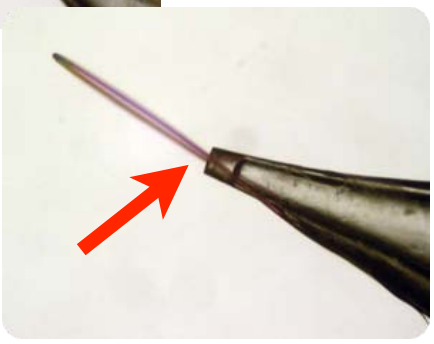
asking your friends in Cell Biology

But I guess an easier way is ask your friends in the cell biology lab to make tapered micro-capillary for you.

glue the loop

under any microscope





Once you can have tapered capillary which has a suitable diameter, then you should glue the nylon loop at the tip of the capillary under the microscope.

The important point is making the loop plane on the center of the tip of the capillary,
as indicated by the red arrow.

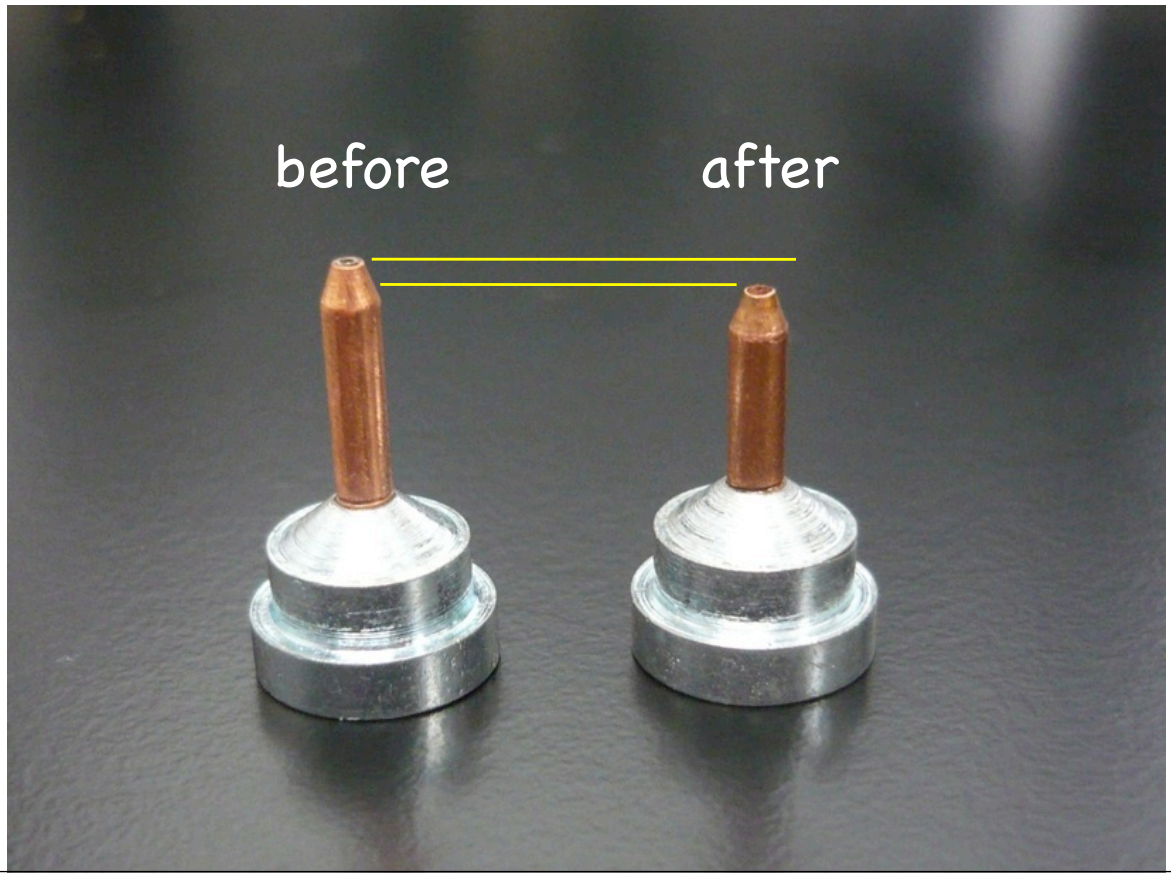
This is the most painful work of this cooking.
But if you can go through it, now you have the mounting capillary.

in no hurry to drill the pin

MAKING THE CRYO-PIN

The next step is modifying the cryo-pin for holding the mounting capillary.

cut & drill the crystal cap



Since we use an 1 mm capillary, we should modify the pin to accept it.

To do that, cut the end of the copper jacket and...

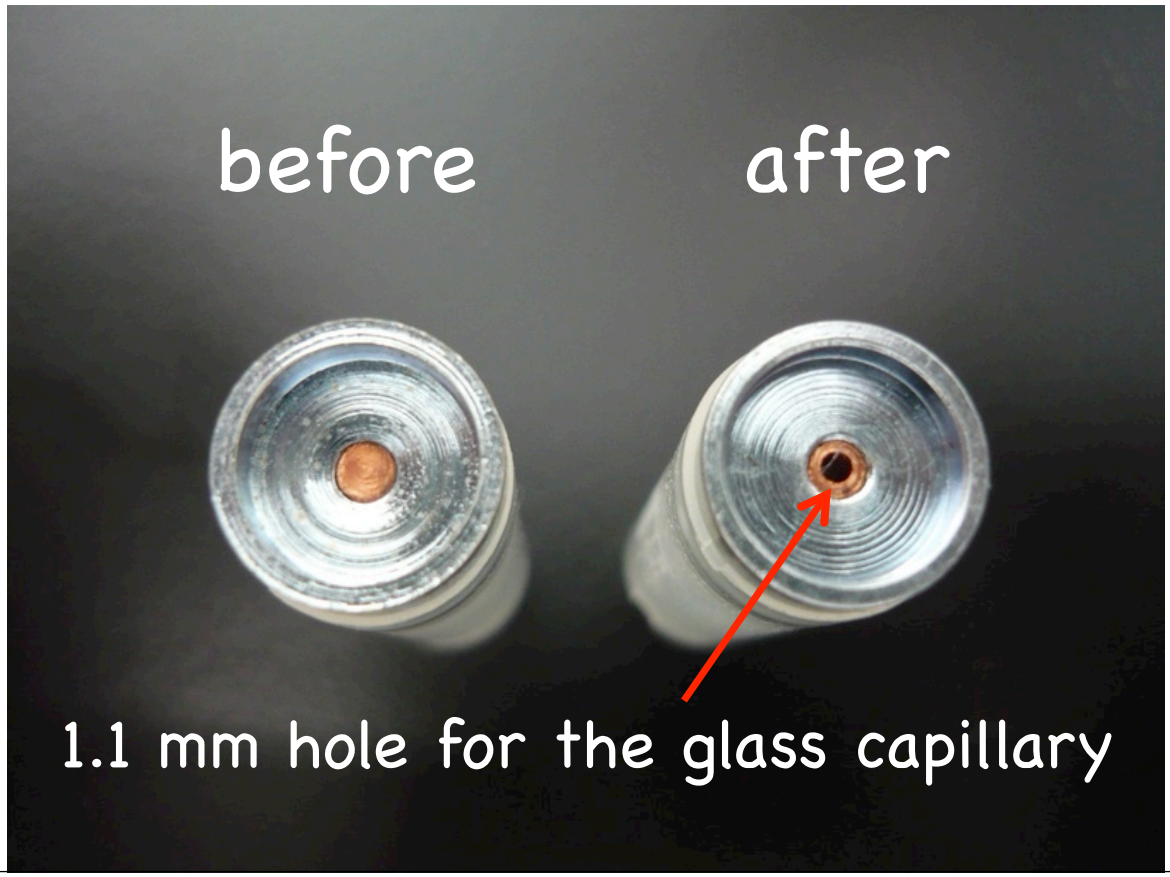
remove a steel pipe before drilling

if you use a Hampton pin

If you use a Hampton pin as the ingredient, remove the steel pipe in the copper jacket,
as you bone the fish.

Otherwise, the drill will be broken by this hard bone.

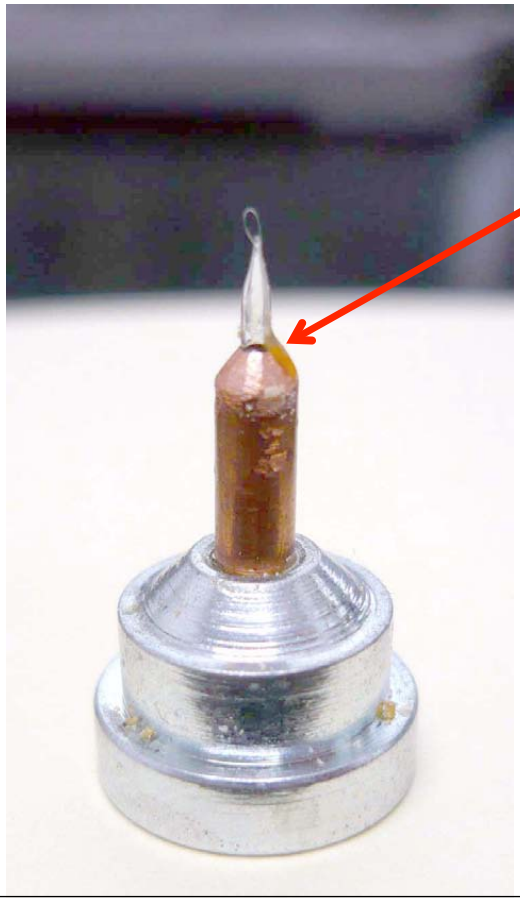
then drill the pin



Now you can drill the center of the copper jacket with for example 1.1mm drill.

In fact, it is not so easy to drill copper.
Usually, I break some drills when I do this by myself.

assemble the capillary and the pin

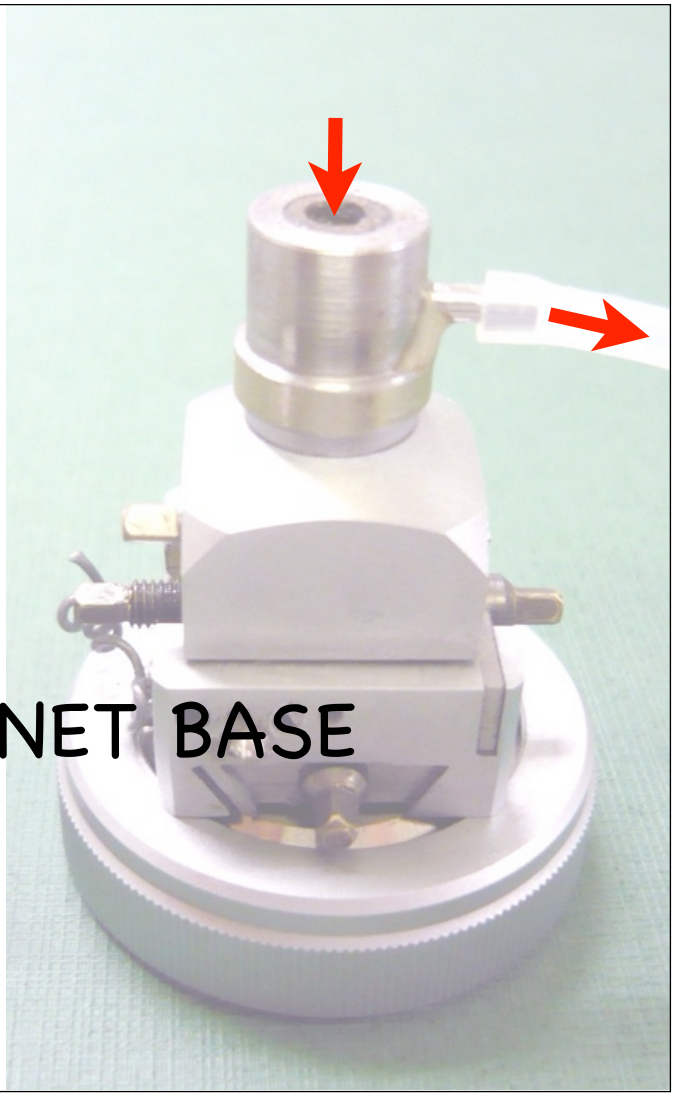


seal here with bees wax

The next step is putting the pieces together. Using tweezers, put the capillary to the modified cryo-pin like this photo. We use bees wax to fix the capillary.

Of course you can use any glue here, but I use bees wax, because it is easy to remove, and we can reuse the drilled copper base again.

to remove the mother liquid in the loop

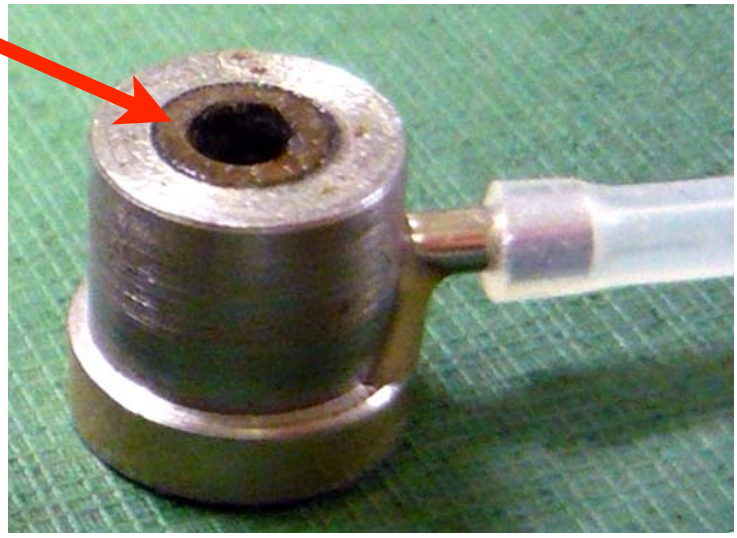


MAKING THE MAGNET BASE

The last step is making the magnetic adapter for the cryo-pin.

magnetic adapter for the cryo-pin

ring shaped
ferrite magnet

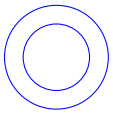


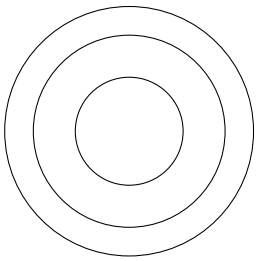
This is the adapter I made and we are using.

A ring shaped magnet is used, and the suction line is connected.

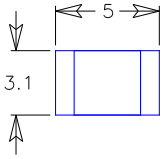
magnetic adapter for the cryo-pin

(example)

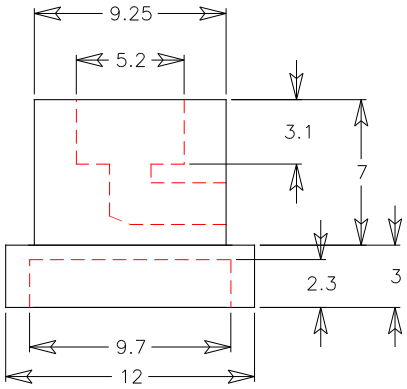


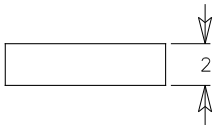


adapter
material: steel



ring shaped
ferrite magnet





short pipe of
2ϕ

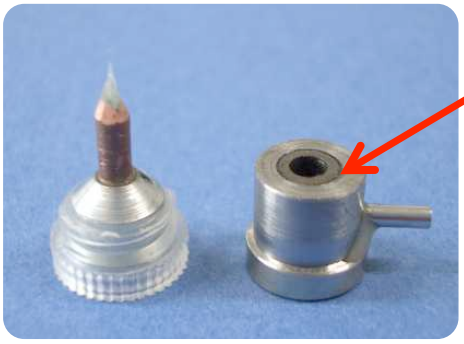
This is the drawing of our adapter.

This is just an example, and you can modify dimensions to fit the ring shaped magnet, which you can find.

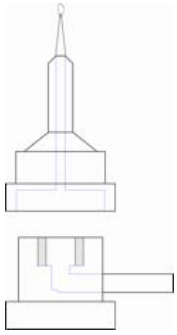
Since I like this kind of work, and I have my own lathe in my office room. Actually this is mine.

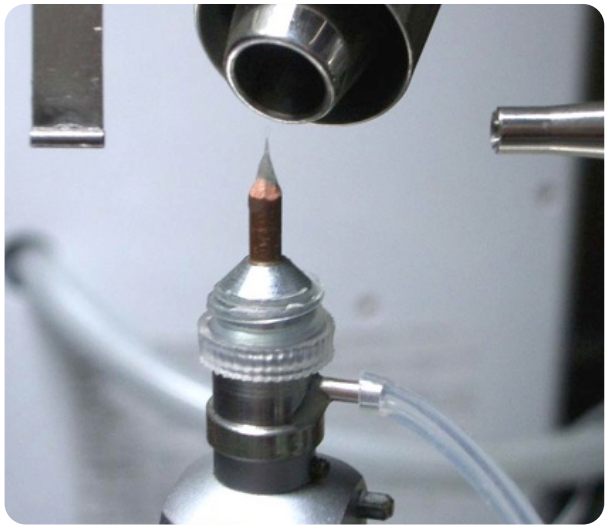
But I guess you do not have your own lathe. So, ask the machine shop of your faculty to make the adapter for you.

completion !



put a little grease here



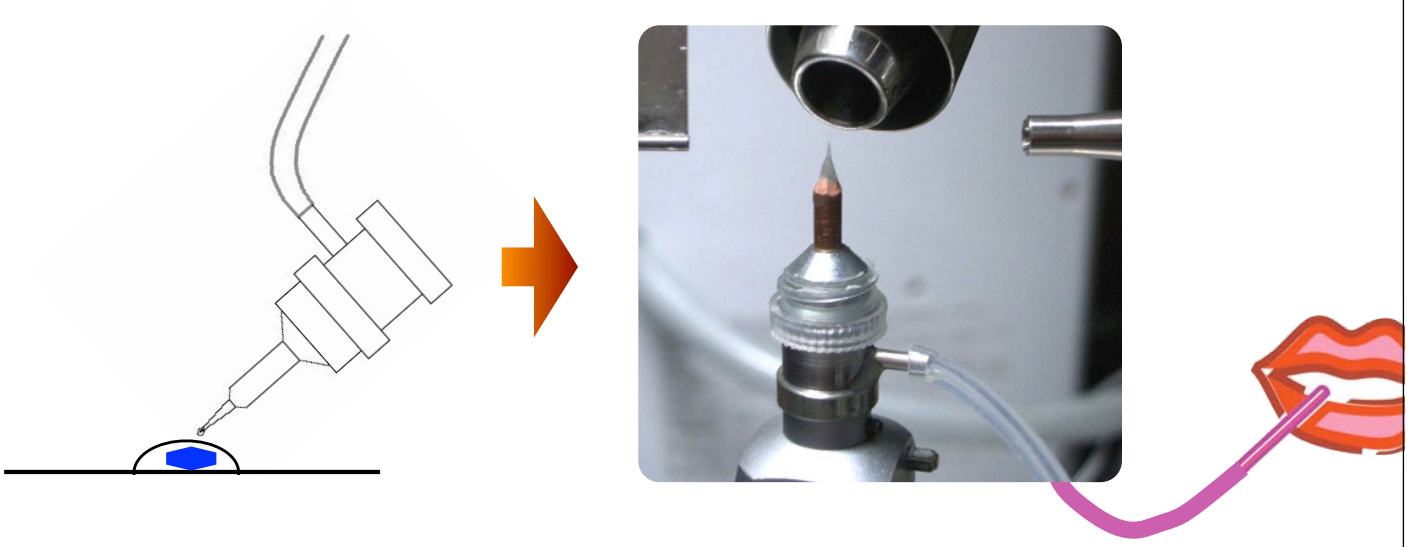


Anyway, if you can make the adapter, the cooking is finished. Now you have all tools in your hand.

You can use them on your standard goniometer like this photograph.

There is a little trick. That is a grease. Put a small amount of grease here to prevent vacuum leaks between this adapter and the cryo-pin .

usage of the tool is ... easy



Usage of this tool is not so difficult. At least, I think so.

Using this tool, you can harvest a crystal from the drop, and remove the solution around the crystal through the capillary, then flash freeze it immediately.

Once the crystal will be frozen, then you can recover and store the modified cryo-cap using a standard cryo-tong.

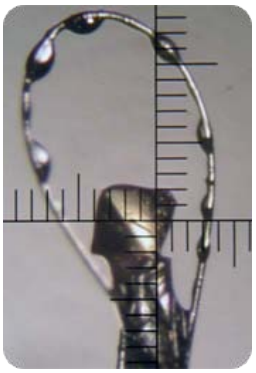
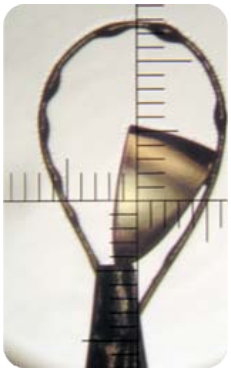
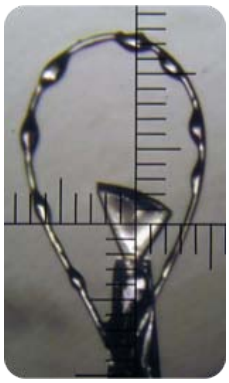
We can use this tool at synchrotron, too.

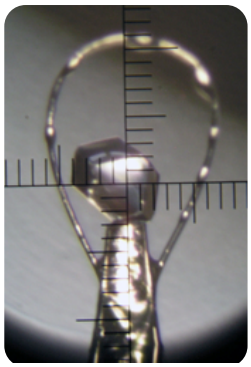
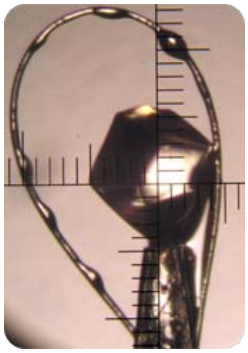
BL17A at the Photon Factory, Japan

You can also use this mounting tool on the vertical goniometer system at synchrotron beamlines.

This photo was taken at the BL17A at the Photon Factory, Japan.

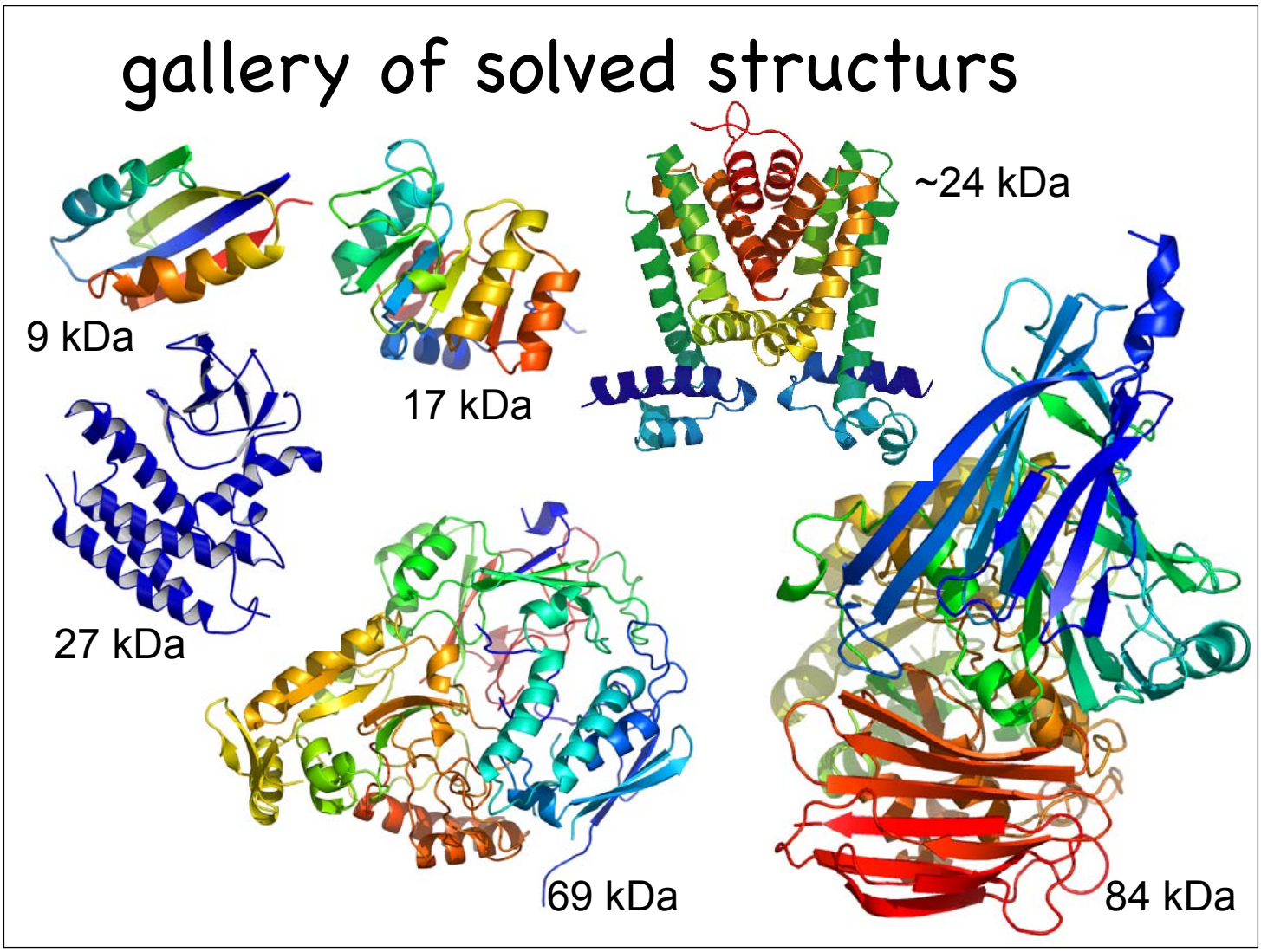
gallery of mounted crystals


50µm/div

This is a photo gallery of an examples of mounted crystals using this method.
As you can see, crystals with any shape can be mounted.

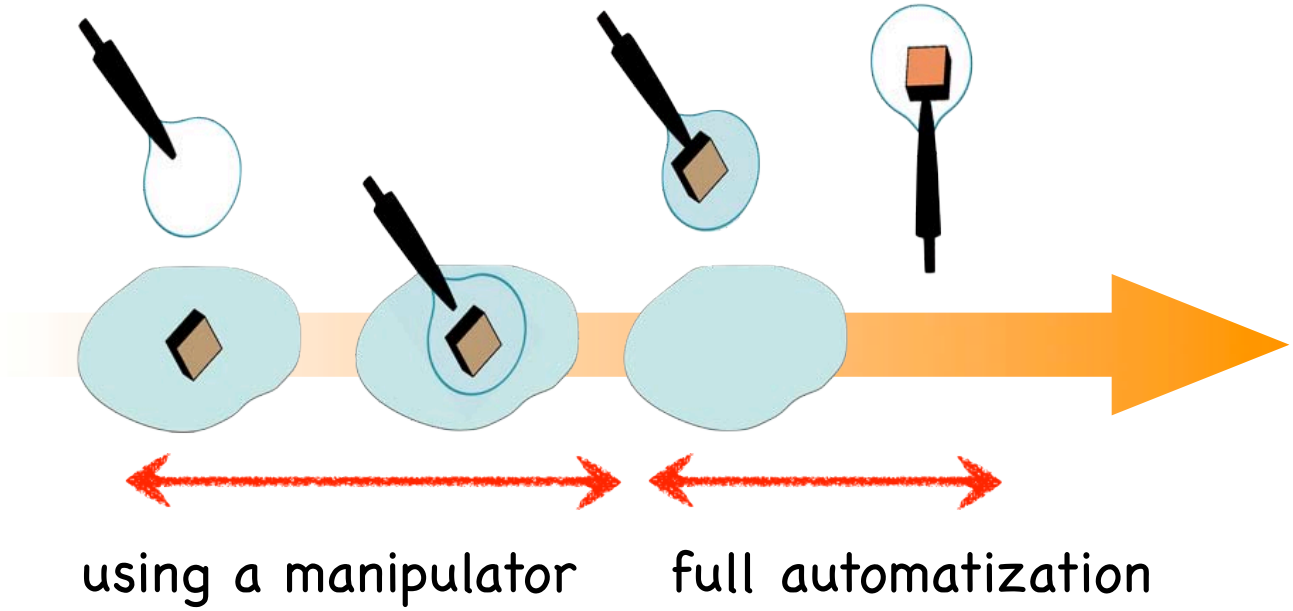


This is a photo gallery of the proteins solved by the Cr S-SAD using this crystal mounting method.

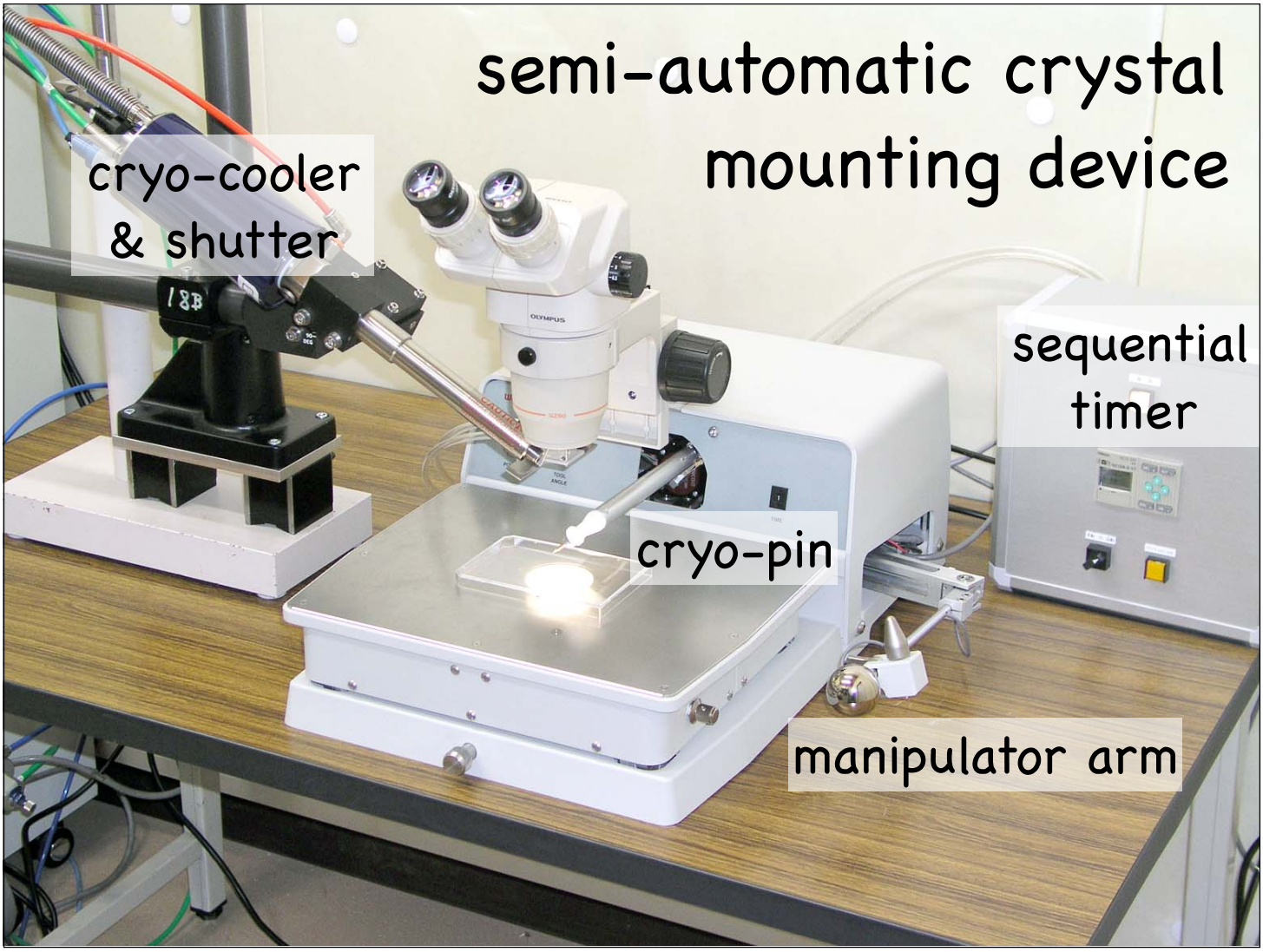
Please come to my poster & exhibition at the ACA meeting

OUR FURTHER DEVELOPMENT

semi-automatization of the mounting process



Our current development is automatization of the mounting process using micro-manipulator.
We have made an equipment, by which we can harvest the protein crystal from the crystallization drop. And further procedures, such as withdrawal of the solution around the crystal and subsequent flash freezing of the protein crystal, are carried out automatically.



This is the photograph of the device. We have modified the equipment for semiconductor chip production.

We can operate the cryo-loop by the mechanically linked manipulator. And once we have successfully harvest the crystal, it is possible to remove the solution and freeze the crystal automatically.

For the details, please check Acta Cryst., D61(8), 1013-1021 (2005).

references

Kitago, Y., Watanabe, N. and Tanaka, I.:
Structure determination of a novel protein by sulphur SAD using chromium radiation in combination with a new crystal mounting method,
Acta Cryst., D61(8), 1013-1021 (2005).

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From phasing to structure refinement in-house: Cr/Cu dual wavelength system and an loopless free crystal mounting method,
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Kitago, Y., Watanabe, N., and Tanaka, I.:
Semi-automated protein crystal mounting device for the sulphur SAD method,
J. Appl. Cryst., 43(2), 341-346 (2010).