IMISX Crystallization

Place: Crystallisation Facility X06DA-PXIII and WSLA/026
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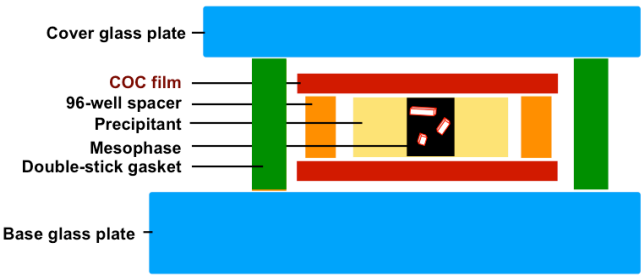
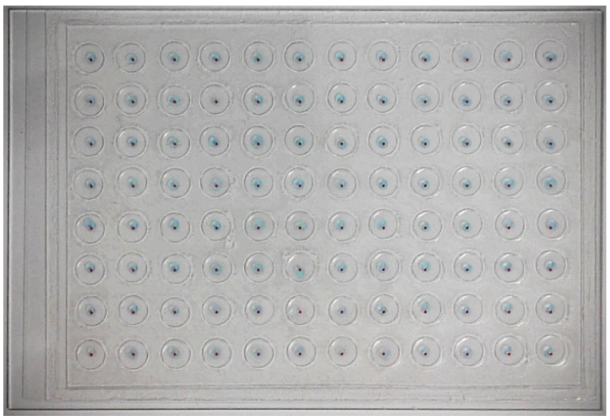
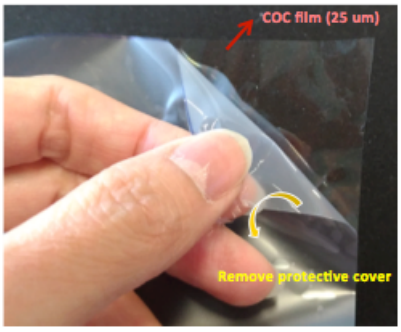
Describtions of the exercise:
Setup of LCP, assembly IMISX plate and sample mounting.

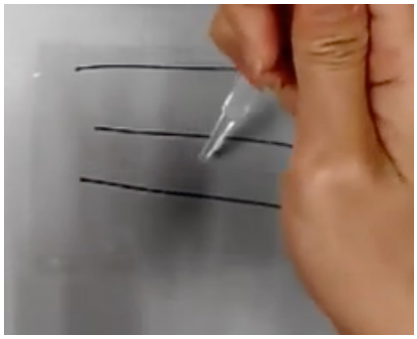


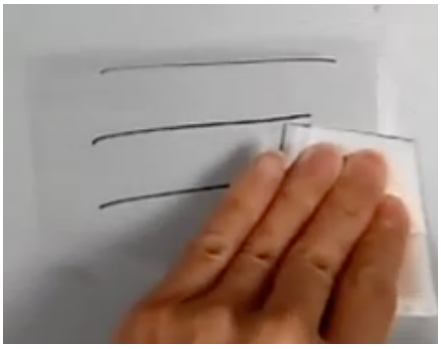


Setting up IMISX plates

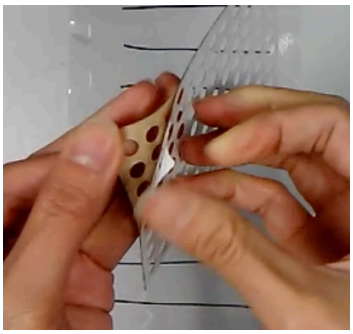
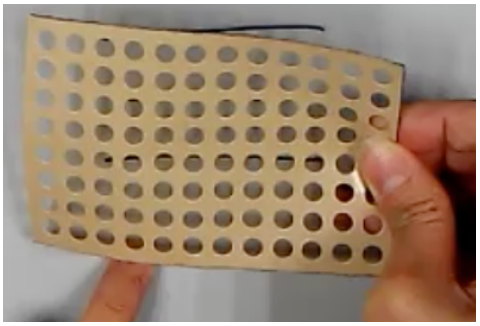
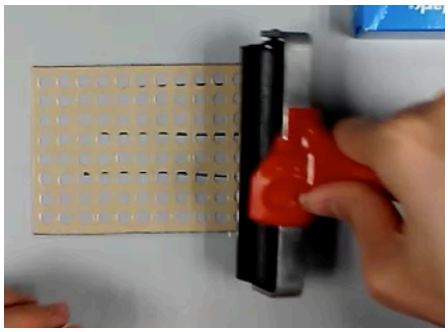
Step 1. With the aid of a piece of adhesive tape, remove one of the protective covers from both COC films, treat the exposed surface with silanizing agent, cleanse with water and blot dry with a tissue. This creates the base and cover of the COC sandwich plate.



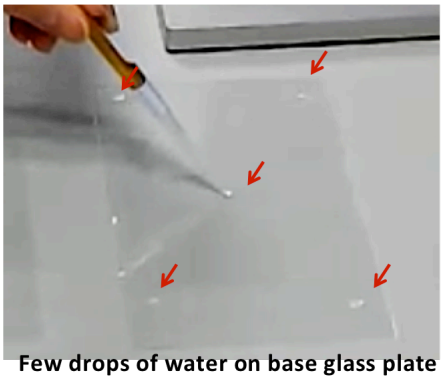


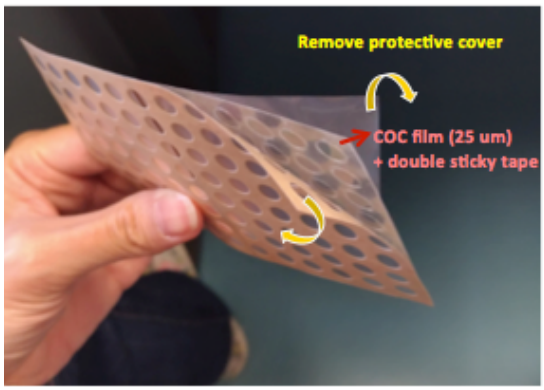


Step 2. Remove the protective cover from one side of the perforated double-stick spacer tape and apply it, sticky side down, to the silanized surface of one of the COC films from Step 1. Use the brayer to produce a tight seal. This step provides the COC base plate plus wells in which crystallization will take place.

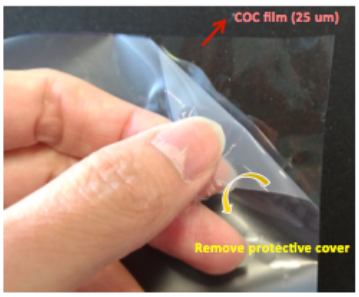




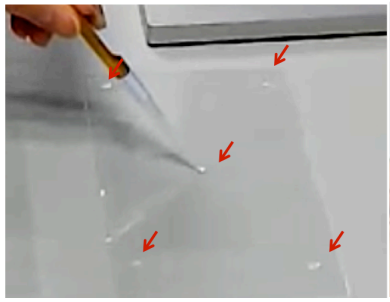
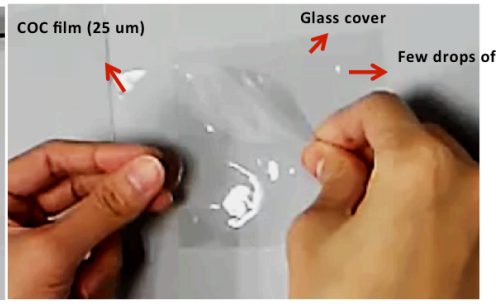
Step 3. Remove the protective cover from the upper surface of the double-stick gasket. Place a drop of water on the surface of the glass base plate. With the aid of a piece of adhesive tape, remove the two remaining protective covers from the perforated double stick tape/COC film prepared in **Step 2** and place it, tape side up, firmly onto the glass plate to be held in place by capillarity. This step generates the bottom section of the double sandwich in which sits the base of the COC plate ready for loading with mesophase and precipitant.





Step 4. Take the second COC film and remove the remaining protective cover from the film, place it with its non-silanized surface down and center it 2 mm from each edge on the cover glass plate to which has been applied a drop of water to bond the two by capillarity. With a clean tissue, force out excess water. This creates the cover plate with which to seal both the COC plate and the glass sandwich plate.



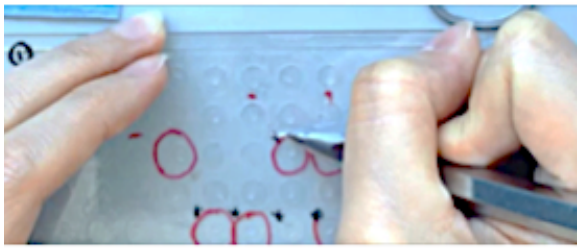



Step 5. Place the base plate from **Step 3** on a desk or on the deck of the *in meso* robot and load into each of the 96 wells protein-laden mesophase and precipitant solution manually or robotically. Seal the filled plate with the cover plate from **Step 4**, COC film facing down. Place on top of the cover plate a thick standard glass plate and brayer to provide a tight, uniform seal with the gasket. Note that without the standard glass plate, the COC film can deform by the brayering action and smear the mesophase bolus and the precipitant.

Opening the IMISX plate. Mounting, snap-cooling and storing IMISX wells

Step 1. With a glass cutting tool score lines in the cover glass of the double sandwich plate around the well/s of interest and remove the cover glass. Use a sharp blade or scalpel to free a square- or rectangular-shaped section of the COC sandwich plate about the exposed well/s and retrieve it with a forceps. Brush away all glass shards. Reseal the opened plate with crystal-clear tape and store it in an incubator until required.

A



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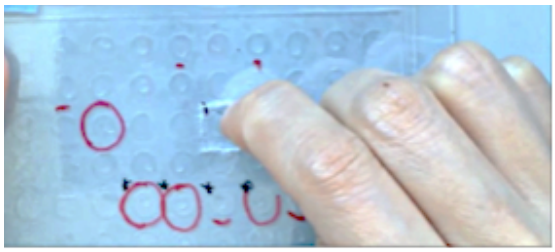

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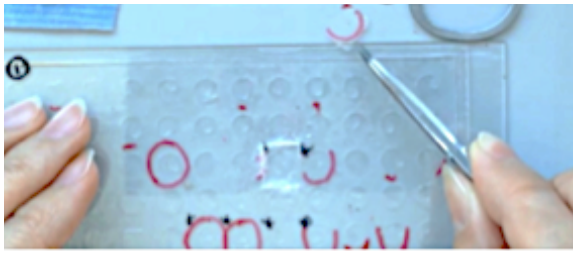

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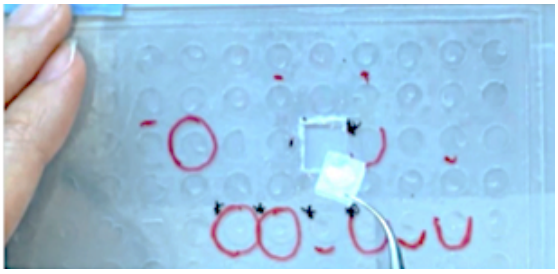
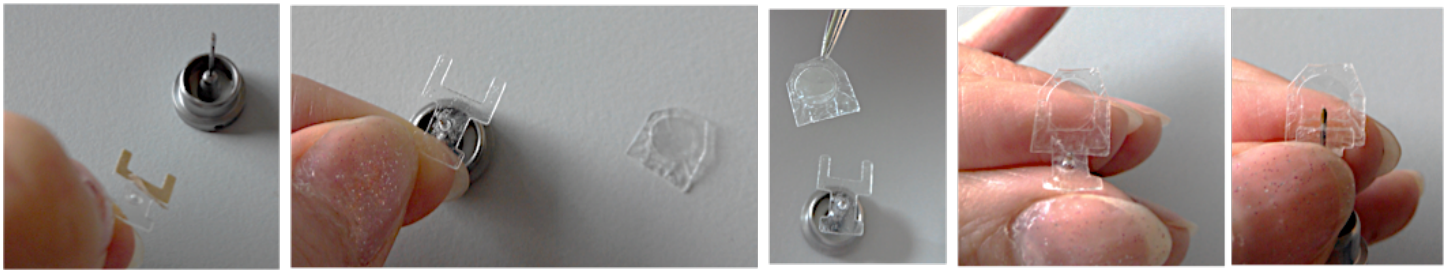
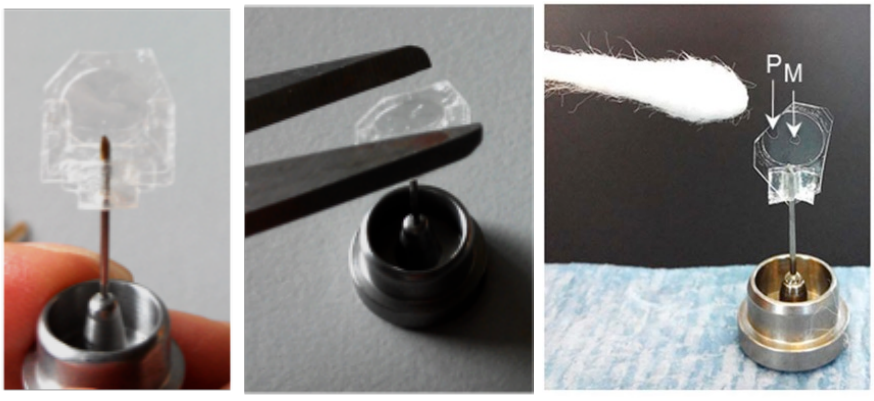


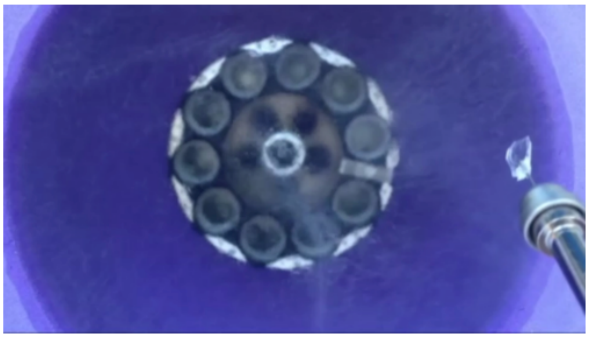
Step 2.1 Preparing the well for IMISXcryo: Remove the protective cover from the double stick tape on the Y support on the goniometer base. Affix the IMISX well to the support on the Y-shape. Fold the base of Y to clip IMISX between Y support. Use a forceps to press the well and the support firmly together to ensure a strong bond. The orientation of the 'flat' face of the well should be recorded on the bottom of the goniometer base to facilitate proper robotic and manual positioning of the sample in the X-ray beam and in the cryo-stream.

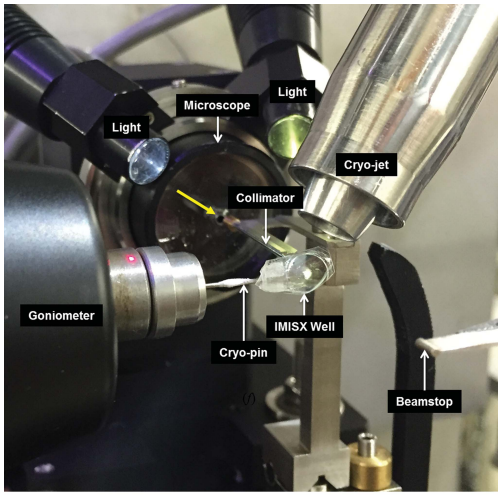


Step 2.2. Trim the corners of the well with a sharp scissors leaving a continuous strip of double stick tape around the edge of the well for structural stability. The trimmed well must be small enough to fit comfortably into a cryo-vial. Trim a minimum off one side of the well to expose the precipitant solution. By touching the precipitant with a cotton bud, gently wick away most of the precipitant from around the mesophase bolus.



Step 3. Immediately plunge the well into a loading Dewar filled to the brim with liquid nitrogen. When the sample has equilibrated thermally transfer it into a precooled storage puck in the same loading Dewar. Using the mark on the bottom of the goniometer base orient the base in the puck to ensure the well will be aligned correctly in the beam and in the cryo-stream upon manual or robotic mounting. Place the puck in a shipping Dewar and ship to the synchrotron.





References:

1. Huang, C.Y. *et al.* (2016) In meso in situ serial X-ray crystallography of soluble and membrane proteins at cryogenic temperatures. *ActaD* **72**, 93–112.

2. Huang, C. Y. et al (2015) *In meso in situ* serial X-ray crystallography of soluble and membrane proteins. *ActaD* **71**, 1238–1256.

3. Li D. et al., Crystallizing membrane proteins in the lipidic mesophase. Experience with human prostaglandin E2 synthase 1 and an Evolving Strategy. Cryst. Growth Des. 2014, **14**, 2034–2047.

4. Li D. et al., Host lipid and temperature as important screening variables for crystallizing integral membrane proteins in lipidic mesophases. Trials with diacylglycerol kinase. Cryst. Growth Des. 2013, **13**, 2846–2857.