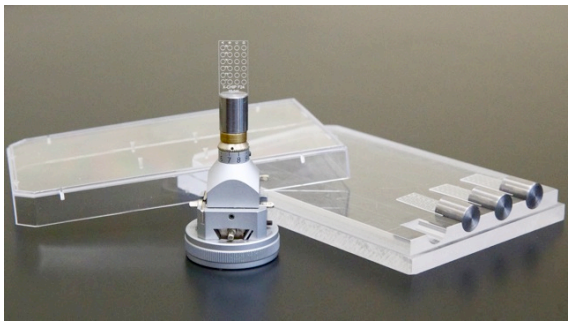
X-CHIP Manual

Crystallization Setup & Data Acquisition Procedures

http://www.x-chip.ca



Version: X-CHIP Official Protocol 4.3

1.0 Crystallization Setup

Prepare the protein at the same concentration that would be used for vapor diffusion crystallization. Pipette 0.10-0.15 uL of the precipitant solution onto the hydrophilic circle[1] (Fig.1) then subsequently add an equal volume of the protein solution and mix by pipetting (*e.g*. Snarpette M1, *Starstedt*, or P2 pipette, *Gilson* etc.).

Once this is done, immediately add 0.7-1.0 uL of oil[2] on top of the droplet to prevent drop evaporation. Add the oil slowly, starting at the top of the droplet and 'guiding' the oil around the droplet evenly. A coat of oil covering all sides of the aqueous drop should be formed. This is the most difficult step, as it is very easy to get an oil coat that exposes some part of the drop to air, leading to rapid evaporation.

Inspect the coating to make sure it is “complete”. It is evident when a part of the drop is exposed to air, as you can see a break in the coating. In this case, try adding an additional small amount of oil, but avoid a great excess of oil because the oil may then spill over[3] (Fig.2). For some projects, place a moist sponge or tissue on the Plexiglas platform next to the X-CHIPs under the lid to minimize or prevent undesirable drop evaporation.

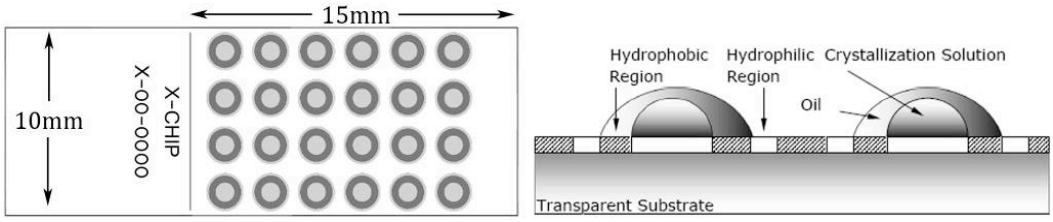


Fig. 1. Schematic of the X-CHIP

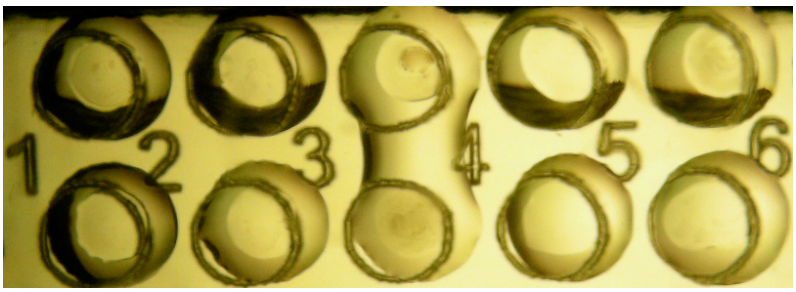


Fig. 2. Examples of drops on the X-CHIP

[1] The actual hydrophilic and hydrophobic circles may be slightly shifted from the etched boundaries. It is easy to identify their exact position by breathing on the chip and holding it against a black surface.

[2] Paraffin oil has been used quite successfully to cover the drops. Other oils that have been used with equal or greater success are 50:50 mixtures of Paraffin or Mineral oils with NVH oil.

[3] When oil spills over it will cause neighboring drops to merge, thinning out both of their oil coatings. Although these drops can still form crystals, they usually evaporate very fast and are hard to reproduce. One way to fix a “spilled drop” is to carefully take up as much oil as possible with a pipette, and then attempt to coat it one more time.

2.0 Diffraction Testing and Data Collection

Crystals grown in the droplets on the chip can be tested with most standard setups. The chips are designed to sit on a goniometric head just like a pin would. The metallic base of the X-CHIP will be securely held by the goniometer magnetic base[1] (Fig.3.) Once on the goniometer, align the chip perpendicular to the beam and the droplets facing the beam.

To screen for diffraction quality, collecting an image from a centered crystal on a perpendicularly set chip is enough. To collect full data sets using a finely focused beam, it is important to align the individual crystal visually at first, and then align by diffraction[2]. It is important to attenuate the beam in order to prevent extensive heat damage to the crystal.

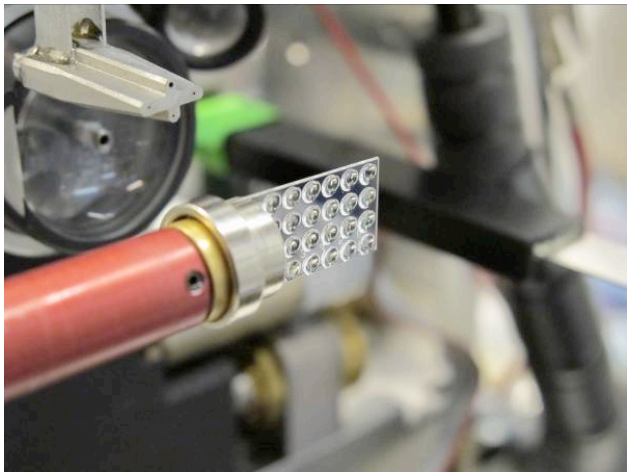


Fig. 3. X-CHIP on the goniometer

[1] Special care must be taken when placing the chip on the goniometer. If the chip is allowed to simply "snap" onto the strong goniometer magnet, it may lead to drop merging as well as disturb crystals in multiple drops on the X-CHIP.

[2] Even though the chip is very thin, it has a moderately high refraction index. Therefore, when the chip is not perpendicular to the camera axis, the visible crystal is just above or below (left or right) of where it appears visually. Shifting the C-XHIP towards or away from the camera by 50nm will address the issue. This parallax effect produced by the X-CHIP thickness is the reason it is necessary to align the crystal using diffraction prior to data collection.