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Promises and pitfalls of In-situ diffraction

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The ultimate test of any crystallization experiment is how well the crystals (if any) diffract x-rays. In particular, we would like this diffraction data to be as free from caveats as possible, so that we know one condition really does produce better crystals than another, and not that we had a great crystal somewhere, but it was messed up in the harvesting and cryo-preservation process. So why doesn't everyone do in-situ diffraction? The reason is because of background. The principle impediment to observing weak high-resolution spots is that they get lost in the background scattering, so every effort must be made to minimize it. Unfortunately, plastic, water, oil, amorphous protein and protein crystal all generate a similar number of background x-rays per unit thickness. This is because they are all made of similarly light elements (oxygen, carbon, nitrogen) and have similar densities (0.9 to 1.2 g/cm^3). There is no such thing as a "low background" material, and everything the main beam touches on its way in, through and out of whatever is holding the crystal generates background. This is why loop mounts are so popular: the total path of the x-rays through non-crystalline stuff in a typical loop mount is generally not much thicker than the crystal, and reducing this thickness further has diminishing returns because the background is now dominated by that from disorder in the crystal lattice itself. So, why not make ultra-thin in-situ trays? Not only are thin walls difficult to manufacture cheaply, but they also dry out a lot faster, which is problematic for growing the crystals in the first place. The future success of in-situ diffraction requires trays that are not only thin-walled and low-permeability, but cheap.

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