

Viewing Crystallization Experiments

Observing the Experiment

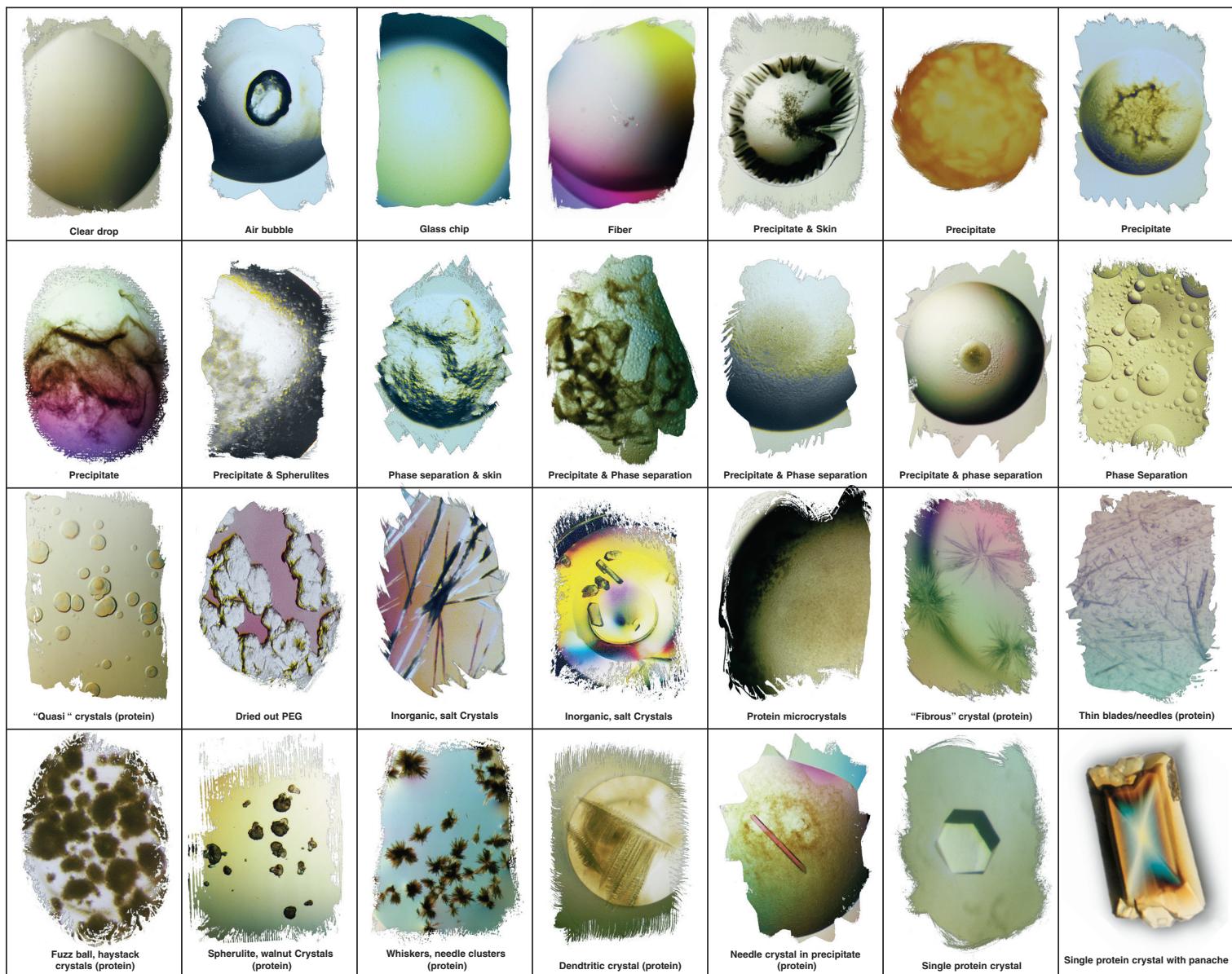
A stereomicroscope with zoom (8 to 12.5:1) and 10 to 100x magnification, LED illuminators to minimize temperature change during observation, polarizing optics to discern birefringence, camera attachment, and a viewing platform large enough to support a crystallization plate when viewed from any and all wells is a good starting point for observing the experiment. Automated imaging systems are also available, as well as imaging systems with ultraviolet and other capabilities.

Gently set the plate onto the observation platform of the microscope. If the platform is smooth and free of protrusions one may simply slide the plate in the X and Y directions on top of the viewing platform to view each of the

drops. Use low magnification to view and center the drop in the field of view. Scan the drops between 10 to 100x magnification. Drops can be viewed at 20 to 40x, and when something suspicious or interesting appears, increase the magnification up to 100x for a better view. Scan the entire depth of the drop from the top to the bottom using the zoom control on the microscope. Sometimes crystals will form at different depths of the drop because different areas of the drop can equilibrate at different rates. Also, crystals sometimes form at the top of a drop and as the crystal gains mass, fall to a lower portion of the drop. Look closely at the edge of the drop where relative supersaturation may be greatest, since in a vapor diffusion experiment this is the spot where water leaves the drop as it equilibrates with the reagent well.

Figure 1

Typical observations in a crystallization experiment



Viewing Crystallization Experiments

Scrutinize and document everything in the experiment, from clear drops to drops with amorphous material, various forms of precipitate, phase separation, and crystals. One is not only looking for crystals, but also different forms of changes in solubility over time, such as clear drops, phase separation or precipitation, since this information can be useful for further screening or optimization.

Be careful to not view the experiment in a single location for too long, or leave the plate unattended on the microscope with the light source on as this can elevate the temperature of the experiment, damaging or dissolving the crystal, or creating condensation on the seal, making viewing difficult.

When to Observe & Score

Observe and score the results immediately after setting the experiment, then each and every day thereafter for the first week, then once a week for 3 more weeks, and then once a month thereafter until the drops are no longer viable and either contain reagent crystals or have dried into a crust. "If no crystals form, dump the samples in the sink and curse the darkness". Alexander McPherson, 1982.

Typical Visible Results

Clear Drops are free of crystals, precipitate, phase separation, or other insoluble outcome. Clear drops indicate the relative supersaturation (protein and reagent concentration) did not reach the point of saturation that would nucleate and grow crystals.

Amorphous Material includes fibers, glass, dust, and debris that was present in the plate, sample, or reagent when the experiment was set and sealed. This should be observed and noted immediately after setting the experiment in order to assist differentiating this outcome from precipitate or microcrystals.

Precipitate appears in many forms and shades of color, but is always without an edge. Yellow or brown precipitate that is often heavy, often clumped is an indication of denatured protein which will not crystallize. White precipitate, which is often amorphous, wispy and cloud like, and more often light than heavy, can indicate a precipitated but not denatured or unfolded protein, which still has the possibility of crystallization.

Skin on the drop can be an indication of oxidized or denatured protein formed at the air/oil/drop interface and crystals often times do not appear in such drops. If desired, the skin can be removed with a probe and the experiment continued.

Phase Separation appears as few large or many small droplets. When this phase separation is protein based, crystals can sometimes form afterwards within, or at the edge of the drops. The droplets are often considered a protein rich phase that separates from the original drop solution. Sometimes the phase separation is not protein based, and can be a polymer, polyol, or non-volatile organic that is not soluble in the presence of the reagent's salt concentration. The presence or disappearance of the phase separation can be temperature dependent. Some phase separation appears or disappears below room temperature and sometimes above room temperature, so one can move the experiment to a different temperature to manipulate the presence of protein and reagent based phase separation.

Crystals can appear as microcrystals or microcrystalline precipitate, as near one dimensional needles, as two dimensional plate, or as three dimensional objects or clusters or needles or plates. Crystals will feature edges. Precipitate does not have edges. Crystals can appear as needles, blades, walnuts, spherulites, plates, and various geometric shapes. Crystals vary in size anywhere from a barely observable 5 microns to 1 millimeter or more. Larger drops can produce larger crystals than smaller drops.

Differentiating Microcrystals from Precipitate

Crystals smaller than 20 microns (microcrystals) can be difficult to differentiate from precipitate, especially under low power or with a low to medium quality microscope. Differentiate microcrystals from amorphous precipitate by looking for birefringence using polarizing optics. Birefringence appears as light colored shiny spots under a polarizer in dark field mode (crossed polarizers) and this birefringence is an indication of crystalline material.

Streak seeding from the drop into a new drop can be used to differentiate crystals from precipitate. Seeding can produce crystals from microcrystals, but not from true precipitate.

Introducing a small volume or colored dye to the drop can color protein crystals, where the precipitate will not take up the color of the dye.

Differentiating Salt from Protein Crystals

See "Crystal Growth 101 Salt or Protein Crystals?"

Viewing Experiments Below Room Temperature

To avoid temperature fluctuation while viewing the experiment, it is best to view the experiments at the same temperature as incubation. This is not always possible or practical. 4°C experiments may be observed in a cold room by moving the microscope into the cold room. Allow time for the microscope to equilibrate to 4°C to prevent fogging of the optics as well as unnecessary temperature transfer from the warm microscope to the cold experiment. Wear a warm jacket with gloves to stay as comfortable as possible in the cold room. Excessive moisture in a cold room can be destructive to a microscope so check with your maintenance group to keep the cold room as dry as possible and keep a keen eye on the microscope. If a cold room is unavailable and one is forced to work promptly at room temperature, so be it. Move plates from an incubator one plate at a time to the microscope, carefully, without door slamming and plate juggling and dropping and make rapid yet thorough observations and notes. Experiments incubated in the cold or warm tend to fog up rapidly during room temperature observation. This is difficult to avoid and is one reason one prefers working in cold rooms or using a combination imaging and incubator system. The experiment seal can be removed for viewing, and replaced with a new seal for sitting drop experiments, but this will allow evaporation from the drops, and be more significant in smaller than larger drops, and introduce a variable into the experiment.

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