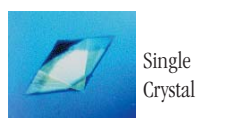
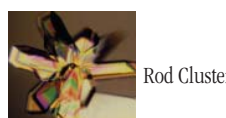
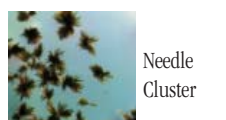
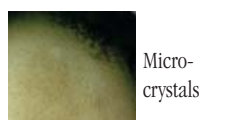
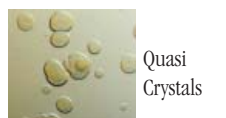
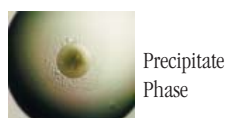
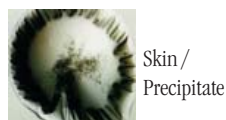


# Viewing Crystallization Experiments

## Crystal Growth 101

**Figure 1**  
Typical observations in a crystallization experiment



### Observing the Experiment

Gently set the plate onto the observation platform. 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 at 20 to 40x magnification. When something suspicious appears increase the magnification to 80 or 100x for a better view. Scan the entire depth of the drop using the fine focus control of 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 it will fall to a lower portion of the drop. Scrutinize everything until you are familiar with the differences between crystals, microcrystals, precipitate, and sweater fuzz. True 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 20 microns to 1 or more millimeters but most seem to fall in the range of 0.05 to 0.5 mm. Figure 1 shows typical examples of what one might observe in a crystallization experiment.

### Diffractable Crystals

Crystals useful for X-ray diffraction analysis are typically single, 0.05 mm or larger, and free of cracks and defects.

### Differentiating Between Microcrystals & Precipitate

Microcrystals (less than 0.02 mm) 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 (light colored shiny spots under a polarizer in dark field mode = crystals). Other tests to differentiate crystals from precipitate include streak seeding, or the use of a small amount (1 microliter) of colored, low molecular weight water soluble dye (crystal violet and methylene blue will often penetrate the solvent channels of macromolecular crystals and color them, where as precipitate will not be colored).

Precipitate can appear as clumps, fine wispy clouds, or anything in between and can range in color from white to yellow, brown or rust. During screening and very preliminary optimization one may wish to observe the drops immediately after set up, one day later and each day thereafter for the first week. Observations may be performed once a week thereafter until the drops turn into a crust of deceased sample and re-

agent. Never throw plates away until the drop is dead. Why? Most crystallization plates are made from polystyrene which allows for some evaporation over time. Evaporation leads to increased relative supersaturation and maybe crystals over time. Time also can lead to changes in the protein (denaturation of less stable forms, proteolytic cleavage, and other changes) which might promote crystallization. Take careful notes during observations and be especially conscious of changes that occur between observations. Most crystallization observations are done at room temperature since this is where one will find most microscopes and it is most comfortable.

### Cold Experiments

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 very destructive to a microscope so check with your maintenance group to keep the cold room as dry as possible. If a cold room is unavailable one is forced simply to work fast, moving plates from an incubator to the microscope carefully and making rapid yet thorough observations. Move only one plate at a time and gently close the incubator door between transfers since slamming the door will cause vibrations which can influence crystallization. Cold experiments tend to fog up rapidly especially if the light source is hot (if no infrared filters or light pipes are used). This is difficult to avoid and is one reason researchers prefer working in cold rooms.

### Technical Support

Inquiries regarding the viewing of crystallization experiments, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 5:00 p.m. USA Pacific Standard Time.

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