Shelley W. Judkins, MT(ASCP)
P. Joanne Cornbleet, MD, PhD

Synovial Fluid Crystal Analysis

ABSTRACT *Synovial fluids comprise less than 5% of the fluid analyses performed by the typical laboratory. The microscopic technique used in crystal analysis can be challenging for the inexperienced technologist. This article discusses the theory of crystal detection and the operation of a polarizing microscope with a first-order red compensator. It provides tips on procedures that will enhance the detection and identification of crystals in synovial fluids.*

This is the second article in a three-part series on body fluids. On completion of the series, participants should be able to prepare high-quality cytocentrifuge slides from cerebrospinal fluid and other body fluids, identify crystals in synovial fluid, and distinguish benign from malignant cells.

From the Clinical Laboratories and Department of Pathology (Dr Cornbleet) at Stanford (Calif) University Medical Center.

Reprint requests to Ms Judkins, Stanford Health Services, Clinical Laboratories, Room H-1524, Rt 6, 300 Pasteur Dr, Stanford, CA 94305-5272.

Crystal detection and identification play an important role in synovial fluid analysis. A polarizing microscope and first-order red compensator can reveal birefringent properties, facilitating crystal identification.

Synovial fluid, a filtrate of plasma plus hyaluronic acid, is produced within the fibrous capsule surrounding a joint space. The term *synovial* is from the Greek *syn* and *ovum* meaning "with egg," noting the fluid's egg-white appearance.[1] Crystals develop in synovial fluid for one of these reasons:

1. Crystallization of an elevated plasma constituent that becomes highly concentrated in the joint
2. Formation of crystals from a degenerative process involving cartilage or bone calcification
3. Introduction of a substance, such as corticosteroids, directly into the joint space

The presence of crystals leads to acute inflammation, producing increased WBC counts and a neutrophil-predominant infiltrate. Identifying these causative crystals in the fluid, especially if they are found intracellularly, in neutrophils or macrophages, is pathognomonic for a crystal-induced arthritis.[2–5]

Crystal-Induced Arthritides

Gout and pseudogout comprise the two major crystal-induced arthritides[1] (see "Terms for Analyzing Crystals in Synovial Fluid"). Gout affects 275 of 100,000 Americans and is the most common inflammatory joint disease of men older than 40 years of age. Affected men outnumber women at least 7 to 1. The frequency of pseudogout is half that of gout, and men have only a slight edge over women, 1.5 to 1.[3,4] The highest incidence of pseudogout is found among people who are 60 to 90 years old.[3]

Gout is associated with prolonged hyperuricemia. The increase in uric acid can result from faulty purine metabolism, decreased renal excretion, therapeutic drugs, or increased nucleic acid metabolism from high cell turnover, as in myeloproliferative diseases. Underexcretion of uric acid by the kidneys makes up the largest group of patients with gout. Women usually do not develop gout until after menopause because estrogen helps excrete uric acid. Drugs like cyclosporine, aspirin, and diuretics decrease the clearance of urates and can precipitate gout.[2,4] The presence of intracellular monosodium urate (MSU) crystals in neutrophils and macrophages is found in 90% of patients having an acute attack of gout. These crystals, however, also are seen in synovial fluid between attacks in 75% of patients, suggesting that multiple factors contribute to an acute episode.[1,2]

Pseudogout, or calcium pyrophosphate deposition disease, is characterized by the presence of calcium salts in cartilage and calcium pyrophosphate dihydrate (CPPD) crystals in synovial fluid. Unlike gout, no single serum metabolite is responsible for the disease. Hereditary metabolic problems or endocrine disorders, such as hypothyroidism and hyperparathyroidism, that elevate calcium levels in the blood can lead to pseudogout.[5] More commonly, pseudogout is associated with degenerative arthritis, demonstrated by

X-ray evidence of articular cartilage calcification. As many as 5% of adults may have CPPD deposits in their joints at the time of their deaths.[5]

Hydroxyapatite and other members of the basic calcium phosphate (BCP) family of crystals are minor contributors to crystal-deposition disease.[6] These crystals form as byproducts of a degenerating, calcifying joint and further irritate the joint space. They can be stained with alizarin red S, which stains all calcium compounds, including CPPD;[7] however, specific characterization requires X-ray diffraction or electron microscopy studies.[5]

Other crystals implicated in joint inflammation include calcium oxalate and corticosteroid preparations.[1,8–10] Corticosteroids, injected into the joint space to treat various arthritides, can produce a "post-ingestion flare." This transitory crystal-induced inflammation lasts several weeks or months after injection.[2] Calcium oxalate crystals can be found in patients on long-term renal dialysis.[6]

Techniques for crystal analysis focus on MSU and CPPD, the two most common crystals. MSU is needle-shaped and CPPD is often rhomboid, but considerable overlap exists in morphology, especially when the crystal is small and viewed intracellularly.

Role of Birefringence in Crystal Analysis

MSU and CPPD are birefringent materials. The basic property of birefringent materials is the ability to bend or refract light due to the presence of a "molecular grain." Produced by the nonrandom, linear internal structure of the crystals, the grain can run down the length of the crystal, similar to a wood log, or across the crystal. These properties cause incident light passing through the crystal to split into two rays:[10,11] a fast ray, which passes with the molecular grain, and a slow ray, which passes 90 degrees to the fast ray, or against the molecular grain.

A "sign" also is associated with birefringence. MSU crystals exhibit negative birefringence and CPPD exhibits positive birefringence. The sign of birefringence is determined by a formula:[12]

$$BR = (RI_{\parallel} - RI_{\perp})$$

where BR is the sign and magnitude of the birefringence, $RI_{\parallel}$ the refractive index parallel to the long axis of the crystal, and $RI_{\perp}$ the refractive index perpendicular to the long axis of the crystal.

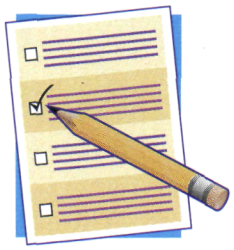
For MSU, the molecular grain runs parallel to the long axis (Fig 1). Incident light passes quickly because the refractive index is low. The slow or high refractive index path is perpendicular to the long axis. This means the value for $RI_{\parallel}$ will be smaller than $RI_{\perp}$, so the sign of the resulting birefringent value will be negative.

For CPPD, the reverse is true. The grain runs perpendicular to the long axis of the crystal, so $RI_{\parallel}$ is greater than $RI_{\perp}$. The sign of the resulting birefringent value is positive. This difference in birefringence allows for the differentiation of the two crystals using polarizing microscopy and the aid of a first-order red compensator.

Microscopic Procedure for Crystal Analysis

A polarizing microscope is a standard microscope equipped with two identical dicrotic or Polaroid filters. One filter, placed in a fixed position above the specimen and below the eyepieces, is called the analyzer. The second filter, placed in a rotating collar below the specimen and above the light source, is the polarizer. Each filter allows light of only one direction to pass. If the bottom filter is rotated until it is aligned 90 degrees in relation to the top filter (crossed), the light coming through the polarizer is blocked by the analyzer so a dark field is seen by the viewer. A specimen containing a birefringent crystal is introduced. Placed in the light path above the polarizer, the crystal refracts the light, allowing it to pass through the analyzer, and appears as a shiny object against the dark background (Fig 2).

MSU crystals usually are seen in the form of medium-size rods or needles, whereas CPPD crystals often are small and shaped like a diamond, rhomboid, or square. However, CPPD



Test Your Knowledge
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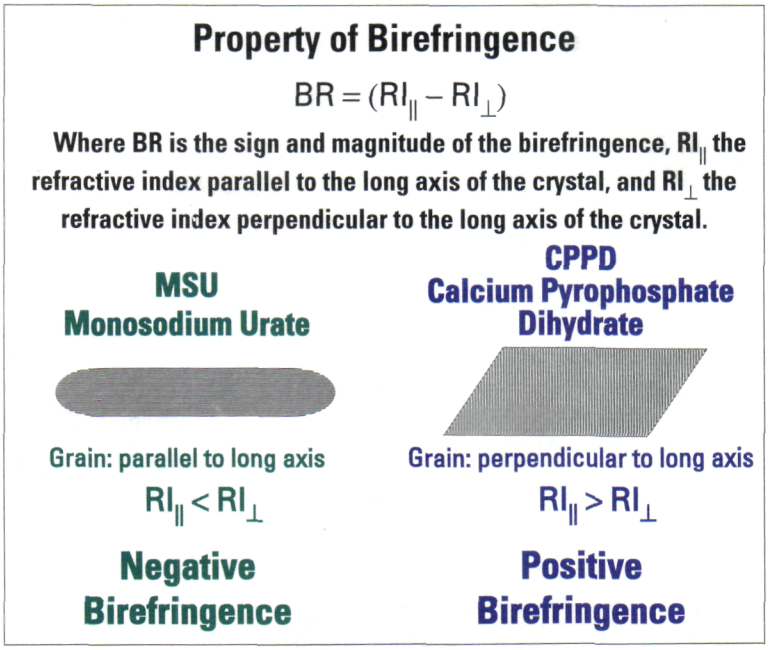
Fig 1. The direction of the molecular grain relative to the long axis of the crystal determines the sign of birefringence.



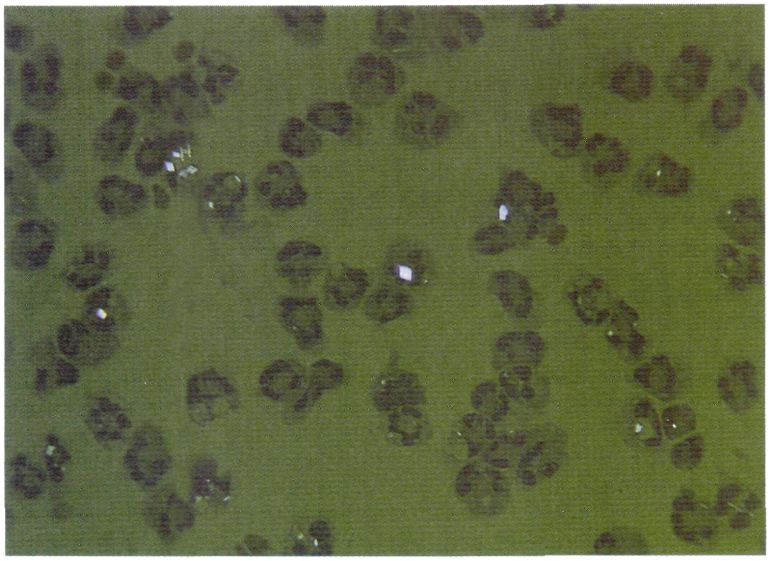


Fig 2. Calcium pyrophosphate dihydrate crystals (Wright-Giemsa stain on a cytocentrifuge preparation; ×400, polarized microscopy).

crystals also may be rod and needle-shaped, thus appearing similar to MSU. It is important that incident light be bright enough to highlight CPPD crystals because they are weakly birefringent. Only a few crystals may be present in the prepared specimen. It is important to scan the entire area under the coverslip.

Terms for Analyzing Crystals in Synovial Fluid

Arthritides—plural term for arthritis

Birefringence—ability of a substance to refract light and split the incident light into two rays: a fast ray and a slow ray

Calcium pyrophosphate dihydrate crystals (CPPD crystals)—causative agent of pseudogout

First-order red compensator—a birefringent crystal of specific properties that, when used with polarizing filters, provides a reference point for determining the birefringence of a superimposed crystal

Gout—crystal-induced arthropathy caused by prolonged hyperuricemia

Molecular grain—the nonrandom, linear structure of molecules in a birefringent material that causes incident light to be split into a fast ray, for light passing with the grain, and a slow ray, for light passing against the grain

Monosodium urate crystals—causative agent of gout

Polarizing filter—allows light wavelengths of only one direction to pass through

Pseudogout—historic name for a crystal-induced arthropathy caused most commonly by degenerative arthritis with articular calcification (also called calcium pyrophosphate deposition disease)

First-Order Red Compensator

A first-order red compensator (a platelike device) is used to determine the sign of birefringence of the crystal. When the plate is placed in a fixed position above the polarizer, the plate's thickness filters the light, and the field turns magenta. The compensator, a large birefringent crystal with a molecular grain,[11] will split the incident light into a fast ray and a slow ray. The direction of the slow ray is indicated by the manufacturer on the compensator. The color of a superimposed crystal and its orientation to the slow ray of the compensator is used to determine the sign of birefringence of that crystal.

The color is visual evidence of the direction of the molecular grain of the crystal. A negatively birefringent crystal, such as MSU, is aligned so that the slow axis of the compensator is parallel to the long axis of the crystal. After passage through the crystal, the difference between the velocity of each light ray from the compensator is smaller after leaving the crystal than it was before the light entered the crystal, and the crystal turns yellow against the magenta background (Fig 3A).

CPPD, a positively birefringent crystal, will appear blue. The light from the compensator is affected differently. The difference between the initial velocities increases after passage through the crystal and the color changes from magenta to blue (Fig 3B).

If the compensator is rotated 90 degrees, the crystal, now perpendicular to the slow ray, changes to the opposite color (yellow to blue, or blue to yellow).

Technique Tips

Consider these important points[13] when analyzing crystals:

1. Perform the analysis as quickly as possible after collection. White cells, particularly neutrophils, start disintegrating after 2 hours and significantly after 6 hours.[14] There is disagreement on how storage affects crystal preservation,[10,14,15] but performing a crystal analysis immediately after the cell count should prevent problems with crystal dissolution and allow for an accurate assessment of whether the crystals are present within phagocytic cells.
2. A fresh wet preparation is the specimen of choice. Pretreating the sample with hyaluronidase to reduce viscosity may make the specimen easier to handle but also may introduce debris.

3. Inspect the microscope slide and coverslip used in preparing the wet specimen. They should be free of scratches, talc, and dust. Such contaminants will refract light but usually have amorphous shapes or nonparallel sides and jagged edges.[1]
4. Avoid combining parts from different manufacturers to assemble a polarizing system. Purchase a crystal analysis attachment kit made for the microscope model. This will ensure the easiest setup and best-quality optics for the analysis.[10,16]
5. Introduce as much light to the specimen as possible before rotating the polarizer disk to darken the field. Focus the specimen under the 10× objective. Fully open the iris on both the light source and the condenser. Bring the condenser up closer to the slide, making sure the RBCs and WBCs are clearly visible.
6. Make sure the darkened field is not too dark. Turn the bottom polarizing filter until the field is at its darkest point, then "back off," or rotate the filter back a bit, letting in a small amount of light, so that the cells are still clearly seen. If the field is too dark, it will be difficult to keep the fields in proper focus as the slide is scanned.
7. Always scan the specimen for the presence of crystals under the 10× objective with only the polarizing filters. Do not swing the color compensator immediately into place. A blue or yellow object against magenta is much more difficult to see than a bright white object against a dark background.[1,16] Scan under the 10× objective for a bright form, then change to the 40× objective and refocus. Swing the compensator into place.
8. Develop a mental association for linking the color of the crystal when parallel to the slow axis of the compensator and the type of crystal. *Yellow* and *parallel* have two Ls. Associate the color yellow with *urine*, which sounds like *urate*, and the MSU crystal is identified. CPPD is the opposite.
9. In addition to the wet preparation, prepare an unstained, coverslipped slide using the cytocentrifuge. This method can maximize the number of crystals present, facilitating detection and identification, and preserve the white cells. The slide can be kept for later review. Warning: Centrifugation also may increase the amount of debris in the preparation.
10. Do not rely on Wright-stained preparations to detect and identify crystals. Crystals, particularly MSU, may be lost during the staining process.[1,17]
11. Occasionally, *both* CPPD and MSU crystals may be present.[10]
12. Note on the report whether the crystals were seen in or outside the cells. If the crystals are present

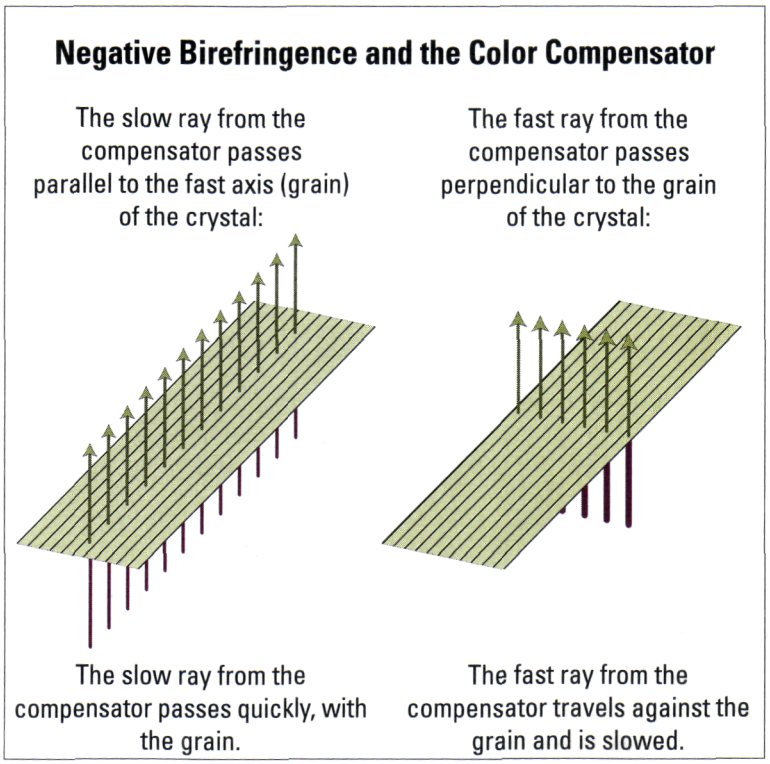


Fig 3A. Light passing from the compensator is changed after passage through a negatively birefringent crystal. The difference between the velocities of the fast ray and slow ray is smaller after leaving the crystal. The color changes from magenta to yellow.

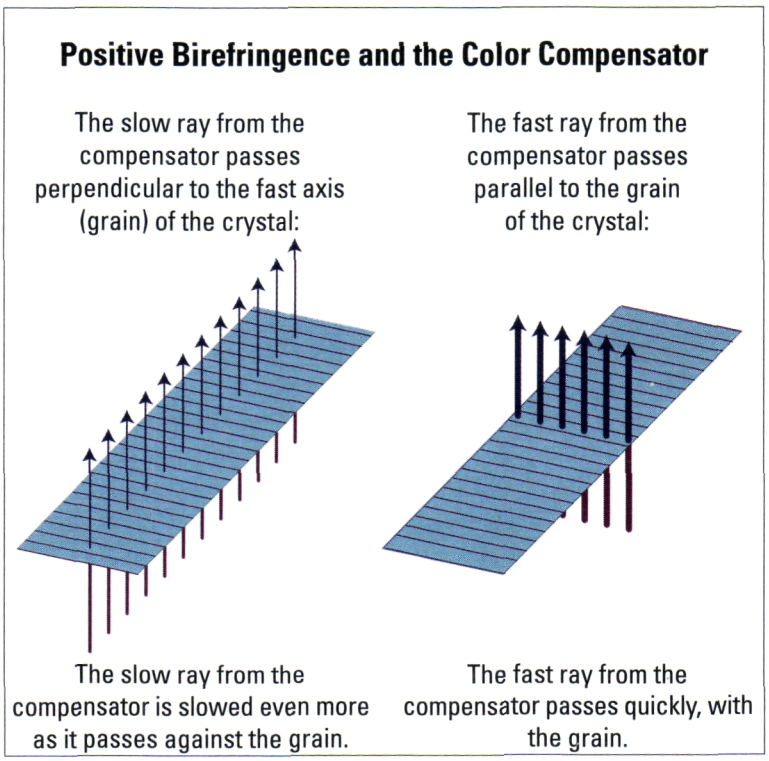


Fig 3B. Notice the change in the light velocities after passage through a positively birefringent crystal. The difference between the velocities of the fast ray and slow ray is larger after leaving the crystal. Instead of yellow, the color changes to blue.

BIREFRINGENT MATERIALS THAT MAY BE FOUND IN SYNOVIAL FLUID

Material	Shape	Birefringence
Crystals		
Calcium oxalate	Bipyramidal	Strong (no axis)
Calcium pyrophosphate dihydrate (CPPD)	Often rhomboid, may be rodlike, diamond or square; usually <l0 μm long	Weak (+)
Cartilage, collagen	Irregular shaped, rodlike	Strong (+)
Cholesterol	Flat, platelike, with notch in corner, occasionally needlelike; often >100 μm	Strong Plates (no axis) Needles (−)
Hydroxyapatite	Small (<1 μm), only aggregates seen	Weak (no axis)
Monosodium urate (MSU)	Needle, rodlike, with parallel straight edges; usually 8–10 μm long	Strong (−)
Steroids		
Betamethasone acetate (Celestone [Schering, Kenilworth, NJ])	Rods, 10–20 μm, blunt ends	Strong (−)
Cortisone acetate	Large rods	Strong (+)
Methyl prednisone acetate	Pleomorphic, small fragments, tending to clump	Strong (no axis)
Prednisone tebulate (Hydeltra TBA [Merck, West Point, Pa])	Small, pleomorphic with branched and irregular configuration	Strong (+)
Triamcinolone acetonide	Pleomorphic, small fragments, often clumped	Strong (no axis)
Triamcinolone hexacetonide (Aristospan [Fujisawa, Deerfield, Ill])	Large (15–60 μm) rods with blunt, squared, or tapered end	Strong (−)
Other Materials		
Debris	Small, irregular with jagged, rounded nonparallel edges	Variable
EDTA (dry, dipotassium)	Small, amorphous	Weak
Fat (cholesterol esters)	Globules	Strong (Maltese cross)
Lithium heparin (not sodium)	May resemble CPPD	Weak (+)
Starch granules	Varying size, round	Strong (Maltese cross)

+ indicates positive; −, negative.

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only outside the cells, it may point to another cause of the inflammation, such as infection.[10]

13. Have two technologists verify the presence or absence of crystals. This will increase the overall experience of the staff and increase the sensitivity of the procedure.

14. Provide readily available reference materials, with pictures, that reinforce quality techniques.[18]

Other Birefringent Materials

Many other birefringent materials may be present in a joint fluid (Table). Steroid crystals can be strongly birefringent and rodlike.[8] They sometimes are avidly phagocytosed. Generally, these rodlike forms are bigger than MSU or CPPD. Betamethasone, a steroid that appears as a strongly negative birefringent rod, can be used to prepare a reference slide for the polarizing microscope (Fig 4). Apply one to two drops of this substance to a slide, add the coverslip, and seal with nail polish.

Anticoagulants, such as sodium EDTA, ammonium oxalate, and lithium heparin, also can form crystals.[10] Synovial fluid samples should be collected in liquid EDTA, sodium heparin, or a plain tube.

Conclusion

Many hospital laboratories see only one specimen of synovial fluid a month. A technologist's experience in crystal analysis is, therefore, minimal. Initial training, detailed procedures, modern equipment, and readily available reference materials are important to ensure quality synovial crystal analysis technique.

Fig 4. Reference slide preparation of betamethasone crystals (×600, with a first-order red compensator). The direction of the compensator's slow ray is parallel to yellow crystals.

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