## Artifacts and Their Sources\*

McCrone Research Institute

Artifacts in preparations are sample contaminants and may constitute a nuisance if they are not recognized, or an error if they are mistaken as part of the sample. Most artifacts have two characteristics: they are common substances and they are usually minor or trace components of the sample. Occasionally, artifacts are found as a major part of what is assumed to be the sample; for example, microscopical analysis of a plugged pneumatic valve showed cornstarch as the major component. It was soon discovered that the valve had been sent in a plastic bag dusted with cornstarch. Only one percent of the particles were actual sample.

Samples can be contaminated with artifacts during collection and during slide preparation. Contaminants that occur during sample collection are the most difficult to categorize as artifacts, especially if their sampling history or source is unknown. Fortunately, the composition of particles from clean rooms, stacks, process lines and products can be predicted, so the artifacts should be outstanding.

Artifacts that occur during sampling are nearly limitless. Some common ones are metal pieces and filings from instrument threads, valves, etc., and fragments from tools used to scrape, pry or abrade the sample. Particles collected on membrane filters are occasionally removed by dissolving the filter and "freeing" the particles by repeated centrifuging and solvent washing. Consequently, a small amount of membrane filter may be found in the dried sample residue. The dry sample substrate or container can also contribute artifacts. In rough decreasing order of the quantities of particles they contribute, facial tissue, filter paper, glass fiber filters, transparent tape, glass vials, plastic bags, membrane filters and plastic vials can all contaminate samples.

The microscopist and his environment contribute most artifacts. The microscopist's clothes, skin and hair generate hundreds of thousands of particles. Fragments from the cover glass, fibers from lens tissue or facial tissue used to clean the slide and cover glass, and general laboratory dust are also prime artifacts. The microscopist can, however, control or monitor these artifacts. The best control is a clean bench with a laminar flow of HEPA-filtered air (rated to remove 99.97% of 0.3 µm and larger particles), though this preventive measure is costly. It is cheaper to identify or at least recognize, particles in the sample work area. This can be done by sampling the lab dustfall by exposing a clean slide. This slide can then serve as a permanent standard of that area's artifacts. From time to time, similar standards should be prepared to account for seasonal artifacts such as pollen, insect parts, oil soot, etc.

An alternate procedure is to keep handy a photographic reference of common artifacts, such as the accompanying set of figures. As an additional aid, the captions contain a very brief description of the artifacts' prominent features. These and hundreds of other particles are fully described in The Particle Atlas<sup>2</sup>. An atlas of artifacts found in histological sections is also available<sup>3</sup>. All of the photomicrographs here were prepared in the Aroclor 5442 mounting media (refractive index = 1.66) and photographed with slightly uncrossed polars.

\*Adapted from an article originally published in The Particle Analyst in 1968, available from McCrone Research Institute (1).

(1) McCrone Research institute, 2820 South Michigan Avenue, Chicago, IL 60616-3292. VOICE (312) 842-7100; FAX (312) 842-1078; www.mcri.org.

(2) The Particle Atlas, Electronic Edition, is available on CD-ROM from McCrone Research Institute, from McCrone Accessories and Components, 850 Pasquinelli Drive, Westmont, IL 60559, or from McCrone Scientific Ltd, 73 Maygrove Road, London NW6 2BP.

(3) An Atlas of Artifacts Encountered in the Preparation of Microscopic Tissue Sections. Samuel Wesley Thompson, D.V.M., M.S. and Lee G. Luna, D.Lit., H.T. (ASCP); Charles C. Thomas Publisher, Springfield, IL (1978), 190 pgs., illus. (500 photomicrographs).





Figure 1 Cotton

250X Figure 2 Nylon



Figure 3 Human hair

Figure 4 Epithelial cells



250X

Figure 5 Orlon

Figure 6 Viscose rayon

100X

Figure 1. Cotton fibers are colorless or dyed, birefringent ribbons, generally twisted, resembling slightly wound rubber bands. Their refractive indices are less than the refractive index of the medium. Extinction is seen, if at all, as a traveling black band as the microscope stage is rotated. The most common sources of cotton fibers are personal clothing and lab coats.

Figure 2. Nylon fibers are smooth, colorless, transparent fibers with high birefringence for a fiber (0.060). These have a uniformly round crossection. The refractive indices are less than the refractive index of the medium. These fibers are primarily generated from fabrics. Figure 3. Human hair has a scale pattern not easily seen in this mounting medium. The central canal, or lumen, if seen, appears dark and may be continuous or fragmented Some fibers are so heavily pigmented that they are nearly opaque. The refractive indices are less than the refractive index of the medium. Dandruff flakes, paper fibers and other debris are often attached to the fibers.

Figure 4. Epithelial cells are colorless, transparent flakes. Aggregates are light tan sheets (dandruff). They are isotropic but occasionally appear weakly birefringent. The refractive index is below 1.66.

Figure 5. Orion fibers have a uniform diameter and are often dumbbell-shaped in cross section, giving the appearance of having a central canal or a bright line through the center. The fibers are usually delustered with titanium dioxide and appear pigmented. These textile fibers are weakly birefringent, having indices less than 1.66 and a negative sign of elongation.

Figure 6. Viscose rayon is uniform and transparent, with a convoluted cross section making the surface appear striated. The birefringent fiber has indices below 1.66, and usually shows first- and second-order polarization colors.

250X



Facial tissue fibe



Figure 11 Quartz



Glass

Figure 8 Air bubbles: with top lighting

250X

Figure 7. Glass fragments are transparent, colorless, with sharp edges and corners, equant or flattened but rarely elongated. The fractured surface is rippled or conchoidal. This artifact generally comes from the cover glass or a container and is isotropic, with an index below 1.66. (Some leaded glasses have indices above 1.66.)

250X

Figure 8. Air bubbles are perfectly circular in low-viscosity media. They can be distorted by contact with particles or by "trapping" them in a high-viscosity medium. Air bubbles are dark due to bordered total reflection, with a bright center. Shallow air bubbles show only a dark outline with a large bright center. The easiest way to identify them is by pressing on the cover slip, causing them to move or deform.

Figure 9. Facial tissue fibers in slide preparations are often from the tissue used to clean the slide and cover glass. Most facial tissues consist of bleached, chemical softwoods. These birefringent, ribbonlike fibers are translucent, colorless and occur individually rather than as bundles. One or two rows of pits, sometimes bordered, can be seen on the longer fibers. Hardwood fibers are usually a minor component of facial tissues. Their presence can be detected by vessel elements which are large, filmy, baggy cells with many rows of pits. All of these elements are birefringent with indices below 1.66.

Figure 10. Glass fibers are very smooth, uniform, transparent, colorless, isotropic and generally short. Ends are broken and jagged just like those of macroscopic glass rods. The refractive index is less than 1.66. Glass fiber filters are usual in this contaminant.

Figure 11. Quartz is a very common atmospheric contaminant. It resembles glass fragments in shape, surface texture and refractive index, but it is distinguished from glass by its birefringence. Quartz particles occasionally have gaseous and liquid inclusions.





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Figure 18 Graphite; with top lighting 250X

Figure 19 Insect parts

250X

Figure 14. Calcite crystals usually show high-order white polarization colors; even fivemicron particles show second-order polarization colors. Twinning, which is common, is evidenced by parallel extinction bands that bisect the acute rhomb angle. Because one of calcite's refractive indices is near 1.66, the particles seem to disappear and reappear as the stage is rotated in plane polarized light.

250X

Figure 15. Trichomes are colorless transparent hairs from grasses and leaves. Species differ greatly in shape - from single tubular fibers to shield-like plates with radiating fibers. Trichomes show first order (to yellow) polarization colors and have indices below 1.66.

Figure 16. Oil soot particles are black, translucent to opaque, hollow spheres or cages. They exhibit a moderate luster and can be broken by pushing on the cover glass. The surface of the oil soot varies from smooth to rough and pitted, due to oxidation and temperature exposure differences.

Figure 17. Pulverized coal flyash has three main components: 1) black, opaque, rough to uneven, partially coked coal; 2) partially fused coal minerals with white and red areas in a brown to black matrix; and 3) translucent to transparent and colored to colorless glass spheres.

Figure 18. Graphite resembles anthracite coal in its high reflectivity, opacity, and blackness. Graphite, however, trends to be platy, occasionally showing a six-sided particle.

Figure 19. Insect parts, when large enough, show an organized structure. Many parts are covered with fine hairs. Body and leg fragments are translucent to transparent and colorless to orange-brown. Almost all of the fragments are partly birefringent, with indices below 1.66.