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ON THE COVER

A curved, wispy rosette crystal resulting from a microcrystal test for the drug alprazolam, using a reagent of gold chloride with concentrated hydrochloric acid. See *New Microcrystal Tests for Controlled Drugs, Diverted Pharmaceuticals, and Bath Salts (Synthetic Cathinones)*, page 17. (Photomicrograph courtesy of McCrone Research Institute)

COVID-19 and *The Microscope Journal*

As you are aware, the COVID-19 pandemic and its socioeconomic impact have caused disruptions in many business operations, especially those of not-for-profits, independent research and higher-learning institutions, and publishers like McCrone Research Institute.

Many people, including the staff of Microscope Publications, have been working remotely under government stay-at-home orders. This situation has drastically affected the publishing workflow of *The Microscope* journal, and distribution of Vol. 68, No. 1 (2020) has been severely delayed. We apologize for this disruption but are pleased to present this issue to you as an online edition. This issue's informative content includes:

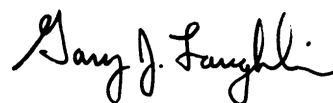
- Thermally modified phytoliths used for identifying debris from fires, by Russ Crutcher
- Brian J. Ford's Critical Focus column on unscrupulous "nonscience" research
- New microcrystal tests for illicit drugs, diverted pharmaceuticals, and psychoactive bath salts, by McCrone Research Institute
- A striking photomicrograph of dehydroacetic acid in Rheinberg illumination, by Mel Pollinger of the New York Microscopical Society

You will also find a large selection of microscopy resources and training on www.mccroneinstitute.org:

- *The Microscope* past issues in the Publications section; full articles from 2008–2012 can be viewed for free. Selected microscopy books, charts and graphs, and other useful resources are available to view or download for free.
- Microscopy courses (basic and advanced)
- Instructional videos on light microscopy and microanalysis
- McCrone applied research projects and publications

In these trying times, and always, it is our mission to keep you engaged in the fascinating world of microscopy and microanalytical research. A big "thank you!" to all of our subscribers and readers, authors and contributors, for your patience and understanding as we work to resolve the current difficulties as soon as possible.

We wish you good health and a relaxing summer and look forward to reaching out to you again in the near future.



Gary J. Laughlin, Editor

Thermally Modified Calcium Oxalate Phytoliths as Markers for Biomass Fire Sources

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ABSTRACT

Calcium oxalate phytoliths are present in more than 217 different families of plants. They concentrate in the bark and leaves, which are also the parts of plants that are consumed in wildfires and contain the highest ash content. Phytoliths have a variety of shapes, forms, or crystal habits based on the plant part where they occur and the genetics of the plant. The shape of the phytoliths are retained even after exposure to high temperature that changes their chemical composition and alters the optical properties of the phytolith.

Thermally modified calcium oxalate phytoliths indicate the types of plants (and plant parts) that have burned, as well as the type of temperature transition and intensity of the combustion, making the presence or absence of thermally modified phytoliths useful for identifying debris from the smoke of specific wildfires and combustion sources. Below are some examples of combustion sources:

- Bark and leaves, because of their high ash production, are not used in wood-burning stoves, pellet stoves, and fireplaces. However, bark and leaves contain phytoliths and are the major fuels for wildfires.
- Logs with their bark not removed and burned in a fireplace, for example, release phytoliths from the bark of that one species. The species burned will vary by geographic region but never includes the variety of species burned in a wildfire.
- Domestic open burning of bark and leaves tends to be a relatively low-temperature fire, and the phytoliths show low-temperature transitions.

- Prescribed fires are designed to burn primarily the understory of a forest. Wildfires tend to be intense and involve all of the plants and plant parts typical of that biome.

Keywords: calcium oxalate phytoliths, whewellite, weddellite, biomass ash, fireplace ash, wildfire ash, pellet stove ash, leaf ash, bark ash, wood ash, biomass fire markers, circular polarized light (CPL), reflected darkfield illumination, ring light, crystal habit, thermal transitions, smoke plume chemistry, stomata, trichomes, silica phytoliths

INTRODUCTION

Particles of combustion are common in all environmental samples. Major sources include trucks, automobiles, industrial boilers and power plants, fireplaces, pellet stoves, backyard fire pits, slash burns, and all forms of open burning (1, 2). However, the particles in the smoke from wildfires are unique due to the variety of plants and the parts of the plants that burn, which characterize a fire.

When a building is suspected of having had a major exposure to wildfire smoke, it is not sufficient to identify the presence of soot, charred plant material, or ash; these are always present to some extent (1). Rather, there are ample, unique, collections of particles in wildfire smoke to identify its presence with a high degree of confidence. Some of those particles are presented in this article with special attention to the thermally modified and fire-related changes that

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Figure 1



Figure 2



Figure 3



Figure 4

Figure 1. Forest in New Mexico two years after the Las Conchas Fire of 2011. Tree needles and twigs were burned away, bark is charred and burned, understory shrubs and grasses were burned away but are regrowing. Pine wood was not a significant fuel for this fire.

Figure 2. Burned chaparral in Southern California. White ash on the ground indicates intensity of the fire, but much of the woody stocks of the chaparral plants remain. Shrub oak, *Adenostoma* and *Ceanothus* species were the biggest sources of fuel for this fire. Their woody stocks and larger branches remain, but the leaves and bark, rich in calcium oxalate phytoliths, have all been consumed.

Figure 3. Grassland savannah fire in Washington State. Grassland fires are quick and tend to burn only the lower leaves and branches of shrubs in this environment. Shrub leaves and twigs, forbs, and grasses were consumed by the fire. Phytoliths in the shrubs and forbs are dominated by calcium oxalate; grasses are high in silica phytoliths.

Figure 4. Southern California grassland treed savannah fire. Grassland fires are fast moving and tend to burn only the lower leaves and branches of the trees, depending on the species. Trees with thin bark may be destroyed by these fires, but thicker bark generally protects trees from permanent damage. Leaves and twigs of the trees are a source of calcium oxalate phytoliths.

occur in calcium oxalate phytoliths.

Calcium oxalate phytoliths are common in the plants of nearly every biome (3–5, 6). They often have distinctive shapes that indicate the plant source and even the part of the plant (7–10). These phytoliths are typically the monohydrate or dihydrate of calcium oxalate, i.e., whewellite or weddellite, respectively. Whewellite is the most common and is easily distinguished from weddellite by its much higher numerical difference between principal refractive indices or birefringence; $B = 0.160$ for whewellite as compared to 0.021 for weddellite (11). Calcium oxalate phytoliths have another attribute that is useful: they pass through three well-defined transitions as a function of temperature (12). These transitions result in very specific optical changes, as shown in Table 1 (see page 15). The combination of distinctive crystalline habits that persist through the characteristic thermal changes make these phytoliths interesting as one of the markers for biomass fires in general and specifically for wildfires.

The abundance of calcium oxalate phytoliths in biomass is evident in the elemental content of biomass ash. The elemental composition of ash from biomass fires is often dominated by calcium (13–17). The extent to which calcium dominates is dependent on the plant and the parts of the plant that are being burned. Leaves and bark contain the most calcium and are of-

ten the major source of ash in a biomass fire (18–22). Fuels for pellet stoves and manufactured logs for fireplaces are selected to minimize the ash content (23, 24). Bark and leaf material are minimized in these manufactured fuels. Lumber is a low ash material, though the amount of ash varies by species of tree (22). The fuels for prescribed fires, slash burns, and wildfires, are dominated by the high ash, calcium-rich parts of plants, i.e., the leaves, twigs, and bark.

Figures 1–4 show the aftermath of wildfires in four different biomes: forest, chaparral, grassland savannah, and treed grassland. The wooden parts of plants tend to survive a wildfire. Thermally modified calcium oxalate phytoliths from the plants in the biome that are burning are distinct from wood being burned in a more controlled fire. Even if the wood being burned in a fireplace or fire pit has high bark content, it will generally produce phytoliths from one species rather than the mixture of plants in a biome. Recognizing thermally modified calcium oxalate phytoliths becomes an important part of identifying a combustion source.

Calcium oxalate phytoliths go through chemical and optical changes on exposure to heat over time. Both temperature and time at temperature are important. In a fireplace, the moisture content of the wood affects the types of particles that become part of the

smoke. The ash under the fire has a long exposure to high temperature and is completely combusted. The phytoliths recovered from a fireplace tend to show high temperature transitions. The phytoliths that are released to the atmosphere by a fireplace fire tend to show more variability than those under the grate. These phytoliths have been released from the wood at an elevated temperature but have not resided long in close proximity to high temperatures like the ash below the grate; instead, they cool rapidly as they leave the chimney. Backdrafts through a fireplace tend to show debris from the ash bed and higher temperature phytolith transitions. A poor draft in the chimney can result in wind-induced backdrafts that introduce more charred wood and lower temperature phytolith transitions into the home. These particles still tend to be from the single species of tree used for firewood in that region.

The conditions in a wildfire are far less controlled than a fireplace, but there is some similarity. The white ash of a smoldering fire is like the white ash in the fireplace, that is, the phytoliths tend to show higher temperature transitions. The phytoliths in wildfire smoke often show more variability. Some of them show the characteristics of temperature transitions below 420° C. For instance, pine needles can ignite shortly after their surface temperature reaches 350° C (25).

ANALYTICAL METHODS

A Nikon Optophot-Pol microscope with a trinocular head and a phase contrast condenser was used for this analysis (Figure 5). A sheet polarizing filter was mounted on the base of the microscope at about 15° from the normal to the optical axis of the microscope. Having the sheet polarizer mounted on the base of the microscope facilitates easy rotation. The off-axis angle prevents reflection off the polarizer back through the condenser, which would interfere with the image when reflected darkfield illumination is used. Reflected darkfield illumination is achieved by attaching a ring light to the 10× or 20× objective. Higher power objectives cannot be used because there is too much light loss due to specular reflection off the coverslip at the angle of incidence required. Transmitted darkfield is achieved by using an appropriate phase stop to eliminate the direct beam through the microscope. Samples are routinely scanned with transmitted circular polarized light (CPL) by inserting the quarter-wave compensator plate below the analyzer and using a sheet quarter-wave compensator on the polarizer. The sheet quarter-wave compensator is rotated so that it



Figure 5. A Nikon Optophot-Pol microscope with a trinocular head, a ring light attached to the 20× objective (blue cord), and a phase contrast condenser used for wildfire particle analysis.

is at 90° to the quarter-wave compensator below the analyzer (the background field should be black). The analyzer can be rotated to allow a lighter background, a configuration referred to as “off-crossed CPL.” This configuration allows for the rapid examination of every particle in a field of view using any combination of crossed polarized light, crossed linear polarized light, transmitted oblique illumination, transmitted dark-field illumination, and reflected darkfield illumination. The importance of this configuration is indicated in Table 2 (see page 15).

SAMPLES

The authors have analyzed hundreds of thousands of samples from buildings exposed to wildfires, structural fires, agricultural fires, prescribed fires, slash fires, cooking fires, refuse fires, fireplaces, and other combustion sources. The photomicrographs in this article are from environmental tape lift samples (tape lifts) collected in or near homes exposed to smoke from wildfires. The samples were collected with Scotch® Magic™ tape, which is a frosted tape with an acrylic adhesive and a cellulose ester plastic backing. The tape lift retains the spatial relationships of the particles and the particle concentration per unit area, while the particles are kept in a single plane on the slide.

After sampling, the tape lifts are adhered to the inside of a clean plastic storage bag for transport to the laboratory where they are removed then applied to clean microscope slides and placed in acetone to dissolve the plastic backing. This leaves the particles fixed

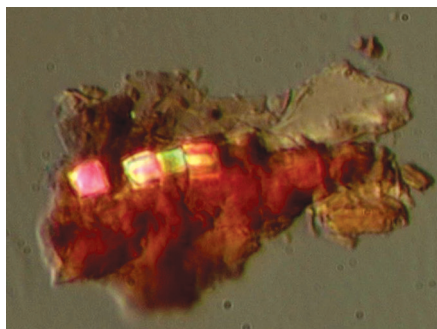


Figure 6

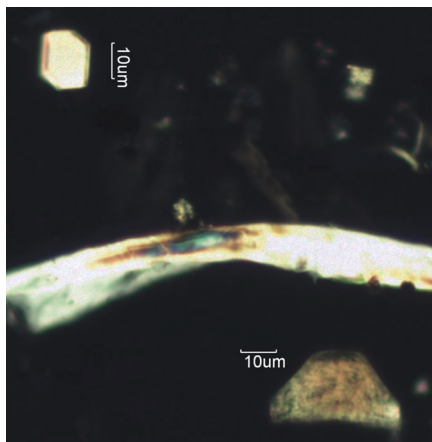


Figure 7

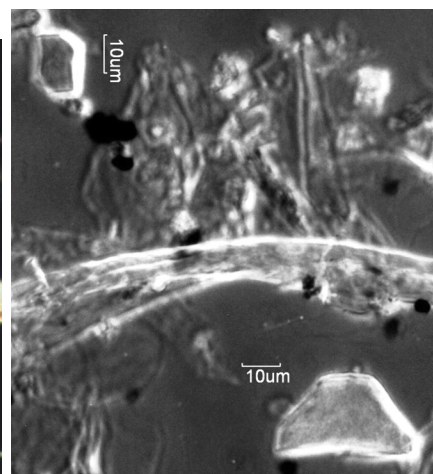


Figure 8

Figure 6. Composted leaf material, including calcium oxalate phytoliths from an environmental tape lift where no thermal modification has occurred; transmitted off-crossed CPL.

Figure 7. Phytoliths from an environmental tape lift; transmitted CPL. The phytolith in the upper left has not been exposed to temperatures higher than 120° C. The phytolith in the lower right was thermally modified by temperatures between 120° and 420° C and has lost most of its water of hydration. Lattice vacancies reduce the amount of light that can transmit through the crystal due to light scatter. A cellulose fiber is in the center.

Figure 8. Same as Figure 7; reflected darkfield illumination. The phytoliths in upper left scatters light only at its edges. The phytolith at lower right scatters light from the lattice defects that have resulted from the loss of water of hydration. The body appears lighter with reflected darkfield illumination due to light scatter from these sites. Many household dust particles are visible here, including the cellulose fiber (center), skin flakes, and other debris not related to the wildfire.

in the adhesive on the slides. A permanent mounting medium (SHUR/Mount™), with a refractive index of ≈ 1.490 , is then applied. Being a near match for the refractive index of the acrylic adhesive, the result is a high quality, permanent, isotropic optical mount that does not introduce any artifacts with any of the illumination techniques required to characterize the optical properties of these particles.

OPTICAL PROPERTIES AND THERMAL MODIFICATION

Calcium oxalate phytoliths are released into the environment when the plants that created them die. They are common in composted material which may appear dark but not due to combustion in a wildfire. The calcium oxalate phytoliths in the composted leaf material in Figure 6 show no thermal modification. In a wildfire, the first chemical and optical changes for calcium oxalate phytoliths occur early in the combustion process. Water of hydration is lost from calcium oxalate monohydrate beginning at 120° C and continuing to 235° C. The loss of water creates vacancies in the crystal structure, but the molecular framework stays basically intact (Figures 7 and 8). The particles retain the shape and size of the original crystal, and the optical properties, though changed, tend to be uniform

throughout the particle. The birefringence and the bulk density of the particle have decreased, but there is still an extinction position for the particle due to a single crystal lattice. The reflectivity of the crystal, when viewed with reflected darkfield illumination, has increased due to scattering centers at the crystal lattice vacancies created by the loss of water. Figure 7 shows an unaffected calcium oxalate phytolith in the upper left, above the cellulose fiber in the center of the image. A thermally modified calcium oxalate phytolith is in the lower right, below the cellulose fiber. The unaffected calcium oxalate phytolith in the upper left has not been exposed to the fire. The optical properties are uniform over the crystal, and there is no internal light scatter. The thermally modified phytolith in the lower right, below the cellulose fiber, began as a twinned calcium oxalate monohydrate crystal typical of oak leaves and bark. The interference colors of the crystal are consistent with its shape and thermal exposure, the edges and acute angles show more modification. When viewed with reflected darkfield illumination (Figure 8), the thermally modified phytolith shows more reflectivity through the crystal. The particle is brighter than the unmodified phytolith in the upper left. Figure 9 shows pine needle calcium oxalate phytoliths that have lost water of hydration but have not progressed into the next transition from γ -calcium oxalate to calcium car-

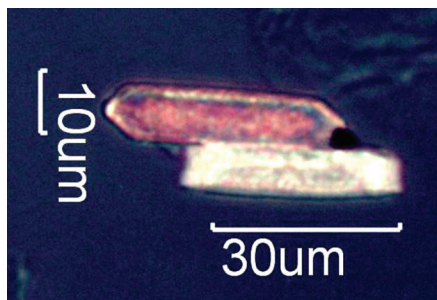


Figure 9

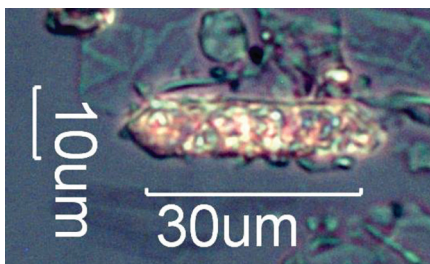


Figure 10

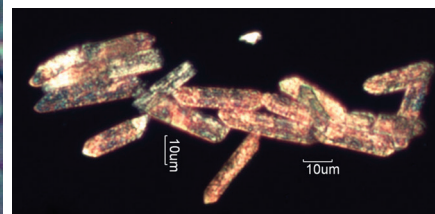


Figure 11

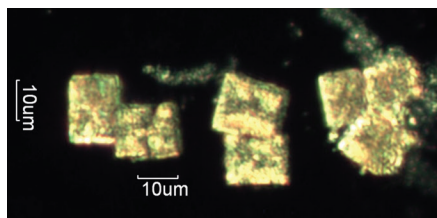


Figure 12

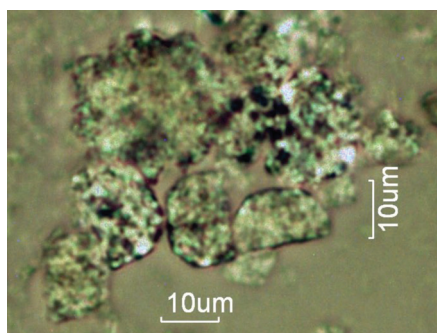


Figure 13

Figure 9. Thermally modified pine needle phytoliths from an environmental tape lift; transmitted CPL. These phytoliths are from pine needles and have been modified by exposure to heat from a wildfire. They have lost all of their water of hydration but have not converted to calcium carbonate.

Figure 10. Thermally modified pine needle phytolith; transmitted off-crossed CPL. This pine needle phytolith has been exposed to a temperature above 420° C long enough to have converted to calcium carbonate. That conversion occurs with the formation of multiple individual domains with orientations independent of one another. The phytolith becomes polycrystalline, retaining its original shape, but is now an aggregate of independent single crystals.

Figure 11. Thermally modified pine needle phytoliths from an environmental tapelift; transmitted off-crossed CPL. These phytoliths have been thermally modified to calcium carbonate by exposure to the high temperature from a wildfire. The crystals are pseudomorphs, where the general shape of the original phytolith habit remains. The atomic structure within each particle is one of multiple crystalline lattices at different orientation within the morphology of the original phytolith.

Figure 12. Thermally modified Douglas fir needle phytoliths; transmitted brightfield illumination. This is an example of the calcium carbonate domains in another phytolith habit.

Figure 13. Thermally modified shrub oak phytoliths; transmitted off-crossed CPL. These particles have begun to lose carbon dioxide and form calcium oxide. They are still quite mottled in appearance, but the interference colors are all low to moderate first order. Some of the crystals are in their extinction positions and appear dark.

bonate. This level of modification is seen in some of the phytoliths that are released early in the combustion process of their associated organic material and are carried by a cooler part of the smoke plume.

At 420° C, carbon monoxide begins to be released, and the chemical composition changes from calcium oxalate to calcium carbonate. The shape of the crystal persists, but it is now made up of numerous small independent crystals (polycrystals) with random orientations (Figure 10). Calcium carbonate has a high birefringence. The boundaries between the calcium carbonate individual crystal lattices tend to be locations of significant refractive index mismatches. The particle has no extinction position because there is no consistent orientation of the carbonate crystals. The interference colors are lower order, which is also a result of the random orientation of the small calcium carbonate crystals (Figures 11–13). The reflectivity of the particles is high due to the heterogeneity of the internal structure and the refractive index mismatches at the boundaries of the individual crystals (Figures 14–17).

Most of the phytoliths from wildfires show this level of modification. With transmitted light, these phytoliths appear dark because most of the light is reflected, instead of passing through the particle (Figures 18 and 19). At these temperatures, the organic host material has burned or at least charred sufficiently to crack and spall from adjacent material. The phytoliths become part of the particulate matter carried by the thermal plume and tend to cool to the extent that they are not further modified.

At a temperature beginning at 620° C and continuing to 860° C, carbon dioxide is lost and the material becomes calcium oxide. As the calcium carbonate converts to calcium oxide, the crystals lose their birefringence and interference colors. The last few calcium carbonate crystals appear bright against an isotropic background (Figure 13). Calcium oxide is cubic and therefore isotropic, so the particles are no longer birefringent. By this time, all of the calcium carbonate has been converted. The crystals have now lost 62% of their original mass, yet they retain most of their

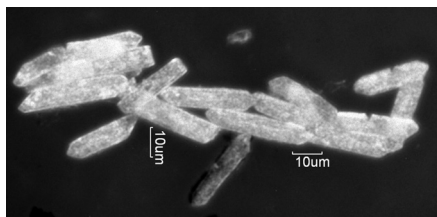


Figure 14

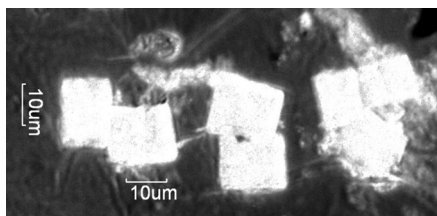


Figure 15

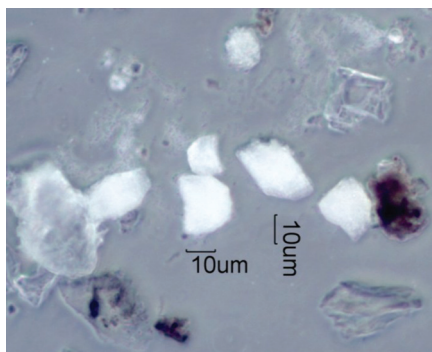


Figure 16

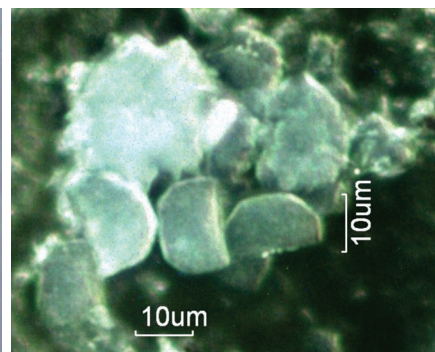


Figure 17

Figure 14. Same as Figure 11; reflected darkfield illumination. The phytoliths that have been converted to calcium carbonate scatter light and are highly reflective because of the irregular internal structure.

Figure 15. Same as Figure 12; reflected darkfield illumination. Light scatter makes these particles bright when viewed with reflected darkfield. Being able to switch rapidly between reflected darkfield and transmitted crossed CPL makes for rapid characterization. Scanning a sample using reflected darkfield can make the recognition of these particles quicker because they are so much brighter than most environmental particles.

Figure 16. Thermally modified shrub oak phytoliths; reflected darkfield illumination. Light scatter makes these particles stand out brightly against the background. The intensity of the background brightness can be controlled by changing the position of the substage condenser by raising or lowering it and can increase contrast by decreasing the background brightness.

Figure 17. Same as Figure 13; reflected darkfield illumination. The shape of the phytoliths is easier to see. The light scatter has decreased with the formation of calcium oxide. These particles resemble sponges and still retain their original shape (pseudomorphs), but they will ultimately retain only about 37% of their original mass.

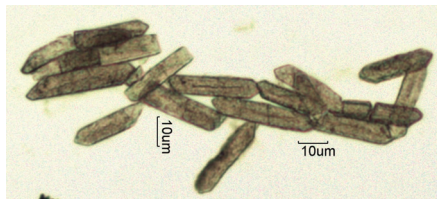


Figure 18

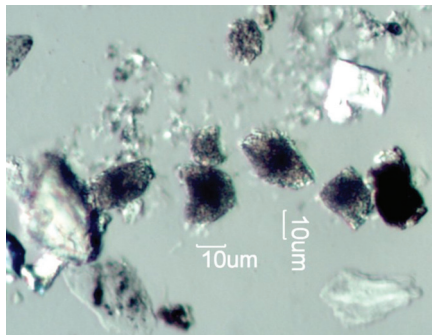


Figure 19

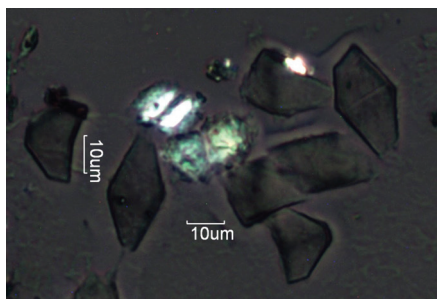


Figure 20



Figure 21

Figure 18. Same as Figures 11 and 14; transmitted brightfield illumination. Light scatter in these particles results in a significant reduction in the light that can pass through the particles. Due to light scatter, the particles appear darker than the background. Notice that the areas that appear darkest in this image are the lightest areas in Figure 14. The phytolith at lower center is an exception and may contain residual carbon from its original organic host.

Figure 19. Same as Figure 16; transmitted crossed CPL. Light scatter makes these particles quite dark when viewed with transmitted light. Switching rapidly between reflected darkfield, transmitted crossed CPL, and off-crossed polarized light allows quick characterization. The particle at far right center is dark, as it is in Figure 16.

Figure 20. Thermally modified shrub oak phytoliths; slightly off-crossed CPL. Most of the phytoliths in this image have converted completely to isotropic calcium oxide. The shape is visible only with the analyzer slightly uncrossed or with reflected darkfield illumination as in Figure 21.

Figure 21. Same as Figure 20; reflected darkfield illumination. Most of these phytoliths have converted completely to isotropic calcium oxide. Their shape is clear, but there is practically no internal light scatter. They reached temperatures in excess of 860° C.

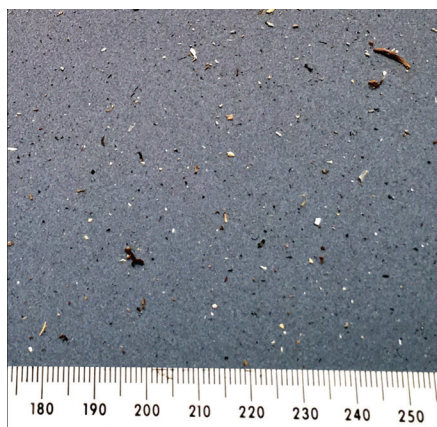


Figure 22

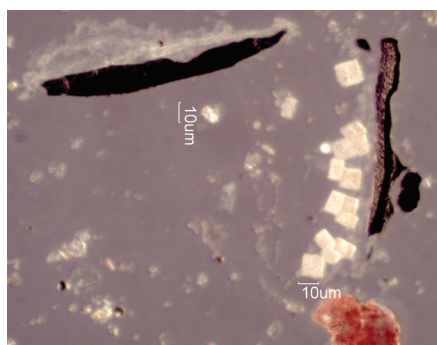


Figure 24

original shape (Figures 20 and 21) and the reflectivity drops. The boundaries between the calcium oxide crystals are not as effective at scattering light because they share the same refractive index, regardless of orientation. These particles are generated in the most intense part of the fire and dwell longer in the hottest part of the fire's thermal plume.

This discussion has described the changes that occur in calcium oxalate monohydrate phytoliths of whewellite. Weddellite, calcium oxalate dihydrate, begins to lose water of hydration on exposure to air. The orientation of the calcium oxalate in the crystal lattice is not the same as in whewellite, and it does not become whewellite as it transitions through a monohydrate form. The water of hydration is lost at lower temperatures as in the original transformation from calcium oxalate dihydrate to calcium carbonate. Once the transformation to calcium carbonate has occurred, the remaining transformations are the same.

TRANSPORT IN THE SMOKE PLUME

The thermally modified phytoliths would be of little value as markers if they were not carried in the



Figure 23

Figure 22. Ash fallout on the hood of a car in Redmond, WA (Sept. 5, 2017), with centimeter scale. The wildfire was more than 100 miles away. Combustion debris from numerous plants and forest floor duff are present.

Figure 23. Ash fallout on the hood of a car in Redmond, WA (Sept. 5, 2017). This is a photograph of the ash taken through a stereomicroscope. The large white particle in the upper right is a section of a Douglas fir needle. There are reddish brown fragments of charred forest floor duff, white particles of fully combusted wood, and black shiny particles of coked wood. All of the particles are from the ash fallout. Black is not a dominant color.

Figure 24. Environmental tape lift collected outdoors in Redmond, WA (Aug. 29, 2017); reflected darkfield illumination. This is debris from burned Douglas fir needles and burned clay that originated in a wildfire more than 100 miles away.

smoke plume from a fire. The thermal air currents created by a fire are quite powerful and can carry relatively large particles to considerable distances. Large fragments of burning biomass, called firebrands, can carry a fire hundreds of meters ahead of the fire front. Ash particles up to a centimeter in diameter can travel hundreds of miles in the smoke plume (Figures 22–24). Wildfires can cause air quality alerts in cities many hundreds of miles away (26). Even at these distances, particles from the wildfire can enter buildings.

Buildings function as a filter with perforations. Windows, doors, ventilations systems, people, and pets provide unfiltered access to the interior of a building. Even with windows and doors closed and the ventilation system off, the air exchange rate between the interior of a building and the exterior can be significant. The penetration of outdoor particles to the interior of buildings is important in this case. Thatcher and Layton review some of the studies done before 1994 and add their own research to the existing studies (27). They describe all the variables, designing models and generating data to calculate particle intrusion, but they omit people as a particle generator in the environment. As a result, they conclude that particles of all sizes entered the residence with no effective filtration (their data relies on raw particle counts and not identification of the particles in the two different environments). People are major particle generators in an environment for particles 10 µm and larger. A number of studies suggest values from 70% to 100% of the particles smaller than 2.5 µm in aerodynamic diameter enter a residence during periods

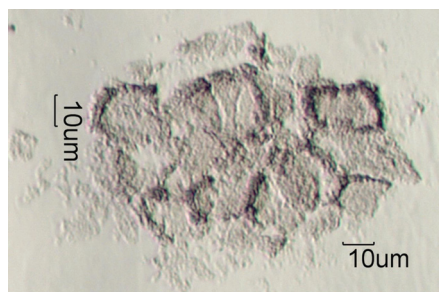


Figure 25

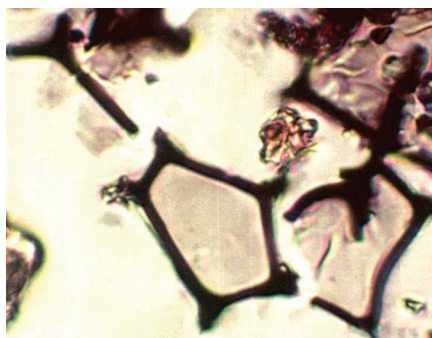


Figure 26

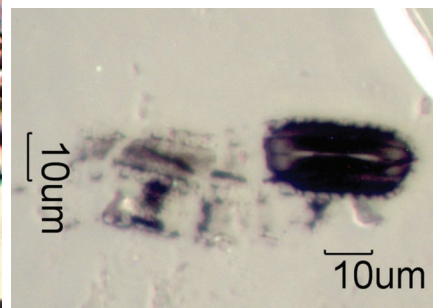


Figure 27

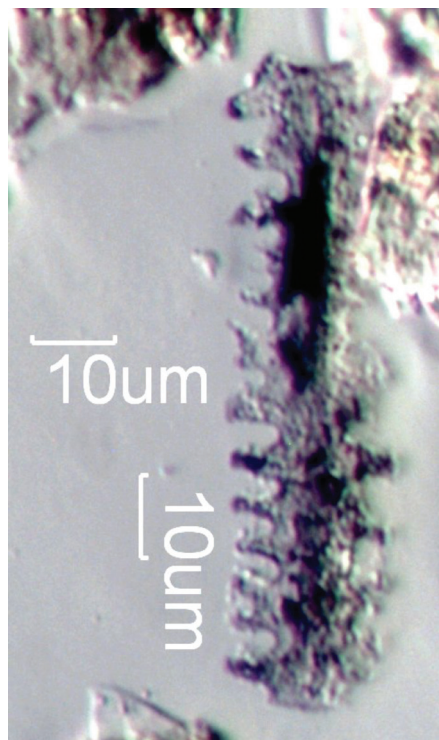


Figure 28

Figure 25. Ash particle showing leaf cell dimensions; transmitted brightfield illumination. This sample was fragmented after being flattened onto the tape lift. The cell walls are clearly visible and are easier to see with transmitted brightfield illumination. This is another example when switching rapidly between lighting conditions allows quick recognition of the particle.

Figure 26. Charred leaf fragments; transmitted brightfield illumination. This fragment of charred leaf shows the cell walls and the thin charred epidermis of the leaf. As biomass chars, it shrinks about 20%. This shrinkage results in elastic stress that “pops” char from the surface of a burning plant.

Figure 27. Charred leaf stomata; transmitted off-crossed circular polarized light and reflected dark-field. Leaf stomata are thicker, more fortified cells and often survive the fire as charred structures. Different plants have different forms of stomata.

Figure 28. Charred silica phytolith; transmitted off-crossed CPL and reflected darkfield illumination. Most plants have silica phytoliths on the upper, lower, or both sides of their leaves. Some have distinctive shapes characteristic of specific types of plants. These are common in most environmental samples, but when associated with a biomass fire, they may be coated with carbon.

found in samples collected indoors. A distinction between outdoor and indoor environments is that particles accumulate on interior surfaces. Outdoors, they are incorporated into the soil or carried away by wind and precipitation rather rapidly. At least some horizontal surfaces of a building with exposure to smoke from a wildfire have a few thermally modified calcium oxalate phytoliths in the 10 μm or less size range per square centimeter from the same wildfire many months later. In some cases, there are many thousands per square centimeter.

The ash in the smoke plume is not the same as the ash left at the site of the fire. The plume ash tends to have a lower mass-to-surface-area index, allowing rather large ash particles to be carried hundreds of miles. Another difference is the chemical exposure in the plume. There are a number of acidic gases released in the early stages of combustion that mix in the plume with the smoke created in the more intense part of the flame (36). Ash left on the ground is not exposed to these compounds. Two very significant gases in the plume are water vapor and carbon dioxide. These two gases create a weakly acidic environment in the plume that can react with strongly alkaline particles. The result is a reduction in the

of moderate outdoor temperatures. These particles see little to no filtration by the shell of the building. The results are also based on particle counts or mass per unit volume of air without identification of the particle types.

In the winter or with the windows and doors closed, some studies suggest that the value drops to 12–76% for these particles, depending on the age and design of the home (28–35). For particles up to 25 μm, the influx is lower but still significant. This is consistent with outdoor particles that have been routinely identified in indoor air samples by light microscopy. Tire wear, pollens, spores, wild bird feather barbules, plant parts, and other outdoor particles are typically

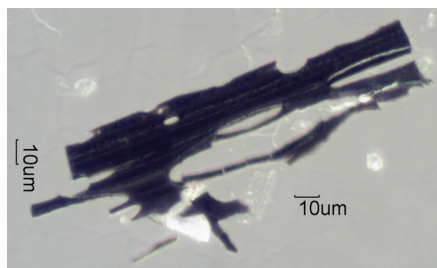


Figure 29



Figure 30

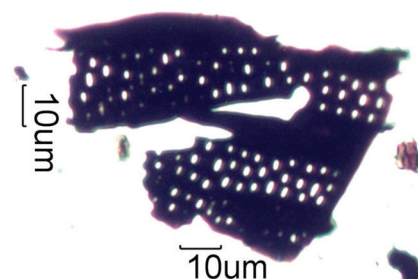


Figure 31

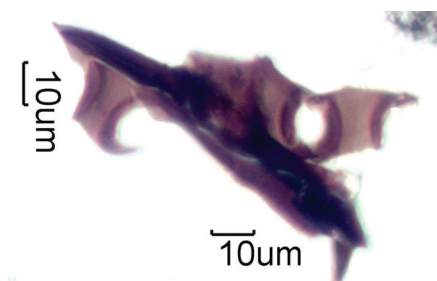


Figure 32

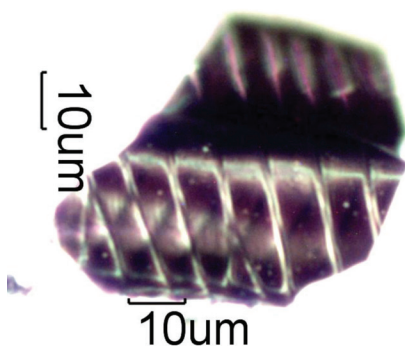


Figure 33

Figure 29. Charred hardwood (angiosperm) fragment; reflected darkfield illumination. This fragment of charred wood shows the narrow tracheid dimensions of a hardwood in the upper part of the fragment and the much larger diameter of the vessel cells in the lower part of the fragment. This structure is visible with reflected darkfield illumination. The background illumination must be reduced to the point that the eye or camera can adjust to the low light levels of the reflected light coming from fine details.

Figure 30. Charred softwood (gymnosperm) fragment; transmitted brightfield illumination. This fragment of charred wood shows the wide tracheid dimensions of a softwood cell. Softwoods do not have the vessel structure characteristic of hardwoods.

Figure 31. Charred hardwood (angiosperm) vessel fragment; transmitted brightfield illumination. This fragment of a hardwood vessel cell shows the characteristic sieve-like pore structure. There are no similar vessel structures in softwoods.

Figure 32. Charred spruce fragment; transmitted brightfield and reflected darkfield illumination. This fragment of two tracheids from a softwood shows the pore structure typical of spruce. The shape and orientation of the pore and its annulus help identify the genus of the fragment.

Figure 33. Charred douglas fir showing spiral thickenings; reflected darkfield and transmitted brightfield illumination. This fragment of charred Douglas fir shows its characteristic spiral thickenings, which are common in hardwood vessels and are also seen in yews, but not with the spacing or regularity seen in Douglas fir.

MARKERS FOR BIOMASS FIRE SOURCES

Thermally modified calcium oxalate phytoliths are only one of the markers used to identify the source of debris from a biomass fire (38). Ash particles often carry identifiable cell shapes characteristic of plant tissues (Figures 25–36). Burning leaves result in ash (Figure 25) and char showing cell structure (Figure 26–28). Charred wood fragments can indicate cell dimensions characteristic of the general types of wood burning (Figures 29–33). Pore structures on char fragments can result in identifying plants to the genus and even the species level (Figures 30–32). Other cell wall structures can add to the characterization of the plants burning, like spiral helical thickenings (Figure 33). Leaf structures, like stomata (Figure 27), trichomes (Figures 34 and 35), and charred silica phytoliths (Figure 28) aid in the identification of different plant fuels. Soil particles become airborne by the strong convective winds and are oxidized by the flame. They turn brick red in color as a result of biogenic iron typically associated with surface soils (Figure 24, lower right). Fire retardant spheres can be an additional marker (Figure 36).

alkalinity of the ash through the formation of carbonates in place of oxides or hydroxides. The pH of the ash in the smoke is significantly less than ash at the site of the fire as a result (13, 15, and 37). Ash at the site or from a fireplace is typically around pH 12, while airborne ash and ash settled at a distance is typically about pH 8. The pH is largely determined by the amount of potassium-containing compounds in the ash. These compounds are concentrated in the fragments of ash that outline cell structure, as seen in Figure 25. Carbonates of potassium and calcium are alkaline with a pH typically between 8 and 9. Oxides of calcium have a very low water solubility so the reaction with water vapor and carbon dioxide tends to be the formation of a thin film of what is probably aragonite (calcium carbonate) over their surface. Even here the shape of the original calcium oxalate phytolith is still present.

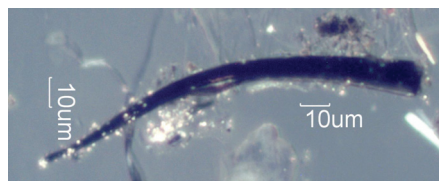


Figure 34

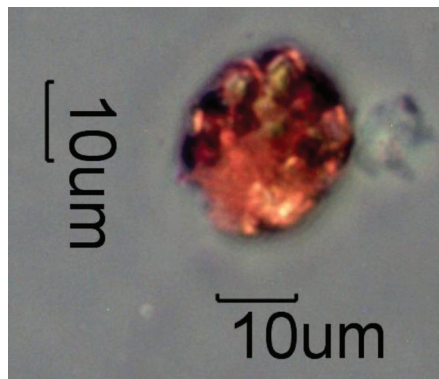


Figure 36

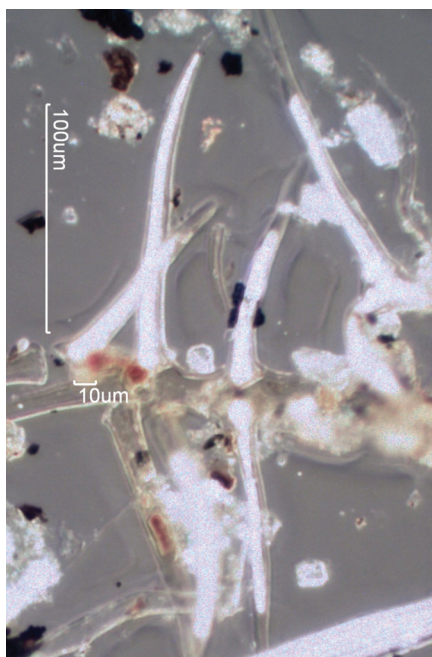


Figure 35

Figure 34. Charred trichome; transmitted off-crossed CPL. Most plants have trichomes on the upper, lower, or both sides of their leaves. Some have distinctive shapes, and others are rich in silica and may appear as charred trichomes or as thermally modified silica in deposits from a wildfire.

Figure 35. Fire exposed silica trichomes; transmitted off-crossed CPL and reflected darkfield illumination. Silica-coated trichomes and silica phytoliths often contain excess water in their structure. When heated, they can become “frothy,” as seen by the light scatter inside these trichomes from the Woolsey Fire in Southern California (2018). The interior appears black in only transmitted light.

Figure 36. Charred fire retardant sphere; transmitted off-crossed CPL and reflected darkfield illumination. Fire retardant spheres normally do not show any charring because they tend to be dropped ahead of the fire front. The faint pink cloud (right) that trails the main drop are fine spheres that are usually less than 10 μm in diameter. This particle was charred in the fire and was carried in the smoke plume; it then landed in a home exposed to the wildfire smoke.

The smoke plume of a wildfire is filled with markers for the plants and parts of plants being consumed. The markers for a specific wildfire often change as the fire burns into a different biome by moving up a slope or by entering a residential area. Markers persist that are far more specific than “char, soot, and ash.” When it is suggested that a building has been significantly impacted by exposure to a specific fire, this hypothesis can be tested. The first step is to identify the presence or absence of markers for that fire. Thermally modified calcium oxalate phytoliths are part of that analysis. The phytoliths found on surfaces in the building must be consistent with the plants burned by the suspect fire and show thermal modification.

CONCLUSION

Environmental particles can be used to uniquely identify their source. The source is not identified by a single particle but rather an assemblage of particles created by that source. Thermally modified calcium oxalate phytoliths help in the case of biomass fires. Leaves and bark are the primary fuels of wildfires, and these are the parts of plants that are rich in calcium oxalate phytoliths. Variations in the types of thermally modified calcium oxalate phytoliths indicate the variety of plants being consumed by a wildfire as well as the in-

tensity of the fire when the phytoliths were released.

It is necessary to use multiple types of illumination to identify these particles under the microscope. Table 3 (see page 16) illustrates the important type of illumination and how each is used. The characterization of thermally modified calcium oxalate phytoliths takes advantage of all the illumination techniques in the table.

The emissions from burning wood fuels for heat or enjoyment are marked by the combustion products of that single fuel. Charred wood fragments and wood ash dominate. Wood and lumber are low in phytoliths. Even if the wood includes bark, the major fuel for the fire is the wood.

The phytoliths that are carried by the smoke plume tend to be small, less than 10 μm in diameter. Particles of this size are most likely to penetrate into a building. If a building has had a significant exposure to smoke from a biomass fire, then thermally modified calcium oxalate phytoliths from the fuel should be present. Ash and char from those same fuels must also be present. Particles that are not from the biomass fire are of secondary importance. Recognizing the presence or absence of thermally modified calcium oxalate phytoliths and their likely plant source is an important part of identifying the source of smoke intrusion into a building.

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REFERENCES CITED

1. Thatcher, Tracy L.; Kirchstetter, Thomas; Malejan, Christopher J.; and Ward, Courtney E. "Infiltration of Black Carbon Particles from Residential Woodsmoke into Nearby Homes," *Open Journal of Air Pollution*, 3, pp 111–120, 2014.
2. Barn, Prabjit; Larson, Timothy; Noullett, Melanie; Kennedy, Susan; Copes, Ray; and Brauer, Michael. "Infiltration of Forest Fire and Residential Wood Smoke: An Evaluation of Air Cleaner Effectiveness," *Journal of Exposure Science and Environmental Epidemiology*, 18, pp 503–511, 2008.
3. Piperno, Dolores R. *Phytoliths*, AltaMira Press, 2006.
4. Persall, Deborah M. *Paleoethnobotany: A Handbook of Procedures*, 2nd ed., Academic Press, pp 355–496, 2000.
5. Franceschi, Vincent R. and Horner Jr., Harry T. "Calcium Oxalate Crystals in Plants," *The Botanical Review*, 46:4, pp 361–427, October–December 1980.
6. Arnott, H.J. and Pautard, F.G.E. "Calcification in Plants" in: *Biological Calcification: Cellular and Molecular Aspects*, Schraer, H. (Ed.), Appleton-Century-Crofts: New York, pp 375–446, 1970.
7. Kausch, Albert and Horner, Harry T. "Comparison of Calcium Oxalate Crystals Isolated from Callus Cultures and Their Explant Sources," *Scanning Electron Microscopy*, 1, pp 199–211, 1982.
8. Webb, Mary Alice. "Cell-Mediated Crystallization of Calcium Oxalate in Plants," *The Plant Cell*, 11, pp 751–761, April 1999.
9. Crutcher, Russ and Heidie Crutcher, "Calcium Oxalate Phytoliths in environmental Samples," *The Microscope*, 67:1, pp 3–11, 2019.
10. Crowther, Alison. "Re-viewing Raphides: Issues with the Identification and Interpretation of Calcium Oxalate Crystals in Microfossil Assemblages," *New Directions In Archaeological Science*, Chapter 8, pp 106–118, 2009.
11. Winchell, Alexander N. *The Optical Properties of Organic Compounds*, University of Wisconsin Press, p 26, 1943.
12. Frost, Ray L. and Weier, Matt L. "Thermal Treatment of Whewellite — A Thermal Analysis and Raman Spectroscopic Study," *Thermochimica Acta*, 2004.
13. Vassilev, S.V.; Baxter, D.; Andersen, L.K.; and Vassileva, C.G. "An Overview of the Composition and Application of Biomass Ash, Part 1: Phase-mineral and Chemical Composition and Classification," *Fuel*, 105, pp 40–76, 2013.
14. Bodí, M.B.; Sheridan, G.; Noske, P.; Cawson, J.; Balfour, V.; Doerr, S.H.; Mataix-Solera, J.; and Cerdà, A. "Types of Ash Resultant from Burning Different Vegetation and from Varied Combustion Processes," *Wood and Charcoal Anatomy. Problems and Solutions*, 11, pp 53–54, (no date).
15. Plumlee, G.S.; Martin, D.A.; Hoefen, T.; Korkaly, R.; Hageman, P.; Eckberg, A.; and Lamothe, P.J. *Preliminary Analytical Results for Ash and Burned Soils from the October 2007 Southern California Wildfires*, Open-File Report 2007-1407, USGS: Science for a Changing World, U.S. Department of the Interior, U.S. Geological Survey, 2007.
16. Balfour, V. "Variations in Wildfire Ash Properties and Implications for Post-Fire Hydrological Response Within Western North American Ecosystems," *Graduate Student Theses, Dissertations, & Professional Papers*, 10742, 2013.
17. Pereira, P.; Ubeda, X.; Martin, D.; Mataix-Solera, J.; Cerdà, A.; and Burguet, M. "Wildfire Effects on Extractable Elements in Ash from a Pinus Pinaster Forest in Portugal," *Hydrological Processes*, 28, pp 3681–3690, 2014.
18. Nosek, R.; Holubcik, M.; and Jandacka, J. "The Impact of Bark Content of Wood Biomass on Biofuel Properties," *BioResources*, 11:1, pp 44–53, 2016.
19. Jin, W.; Singh, K.; and Zondlo, J. "Pyrolysis Kinetics of Physical Components of Wood and Wood-Polymers Using Isoconversion Method," *Agriculture*, 3:1, pp 12–32, 2013.
20. Hytönen, J. and Nurmi, J. "Heating Value and Ash Content of Intensively Managed Stands," *Wood Research*, 60:1, pp 71–82, 2015.
21. Wang, L. and Dibdiakova, J. "Characterization of Ashes from Different Wood Parts of Norway Spruce Tree," *Chemical Engineering Transactions*, 37, pp 37–42, 2014.
22. Babayemi, J.; Dauda, K.; Nwude, D.; and Kayode, A. "Evaluation of the Composition and Chemistry of Ash and Potash from Various Plant Materials — A Review," *Journal of Applied Sciences*, pp 1820–2824, 2010.
23. Pellet Fuels Institute (PFI). "Standard Specifications for Residential/Commercial Densified Fuel," Nov. 9, 2018.
24. New York State Energy Research and Development Authority, "Elemental Analysis of Wood Fuels:

Final Report," June 2013.

25. Babrauskas, Vytenis. *Ignition Handbook*, Fire Science Publishers, Chapter 14, p 837, 2014.

26. Sapkota, Amir J.; Symons, Morel; Kleissl, Jan; Wang, Lu; Parlange, Marc B.; Ondov, John; Breyse, Patrick N.; Diette, Gregory B.; Eggleston, Peyton A.; and Buckley, Timothy J. "Impact of the 2002 Canadian Forest Fires on Particulate Matter Air Quality in Baltimore City," *Environmental Science & Technology*, 39:1, pp 24–32, 2005.

27. Thatcher, Tracy L. and Layton, David W. "Deposition, Resuspension, and Penetration of Particles Within a Residence," *Atmospheric Environment*, 29:13, pp 1487–1497, 1995.

28. Kenward, Alyson; Adams-Smith, Dennis; and Raja, Urooj. "Wildfires and Air Pollution: The Hidden Health Hazards of Climate Change," Climate Central, 2013; <http://assets.climatecentral.org/pdfs/WildfiresAndAirPollution.pdf> (accessed June 10, 2020).

29. Bytnerowicz, Andrzej; Arbaugh, Michael J.; Riebau, Allen R.; and Anderson, Christian (Eds.), *Wildland Fires and Air Pollution*, Elsevier, 2009.

30. Thatcher, Tracy L.; Kirchstetter, Thomas W.; Tan, Stella H.; Malejan, Christopher J.; and Ward, Courtney E. "Near-Field Variability of Residential Woodsmoke Concentrations," *Atmospheric and Climate Sciences*, 4, pp 622–635, 2014.

31. Siponen, Taina; Yli-Tuomi, Tarja; Tiittanen, Pekka; Taimisto, Pekka; Pekkanen, Juha; Salonen, Raimo O.; and Lanki, Timo. "Wood Stove Use and Other Determinants of Personal and Indoor Exposures to Particulate Air Pollution and Ozone Among Elderly Persons in a Northern Suburb," *Indoor Air*, pp 1–10, 2019.

32. Wheeler, Amanda J.; Gibson, Mark D.; MacNeill, Morgan; Ward, Tony J.; Wallace, Lance A.; Kuch-

ta, James; Seavoyer, Matt; Dabek-Zlotorzynska, Ewa; Guernsey, Judith Read; and Stieb, David M. "Impacts of Air Cleaners on Indoor Air Quality in Residences Impacted by Wood Smoke," *Environmental Science & Technology*, 48, pp 12157–12163, 2014.

33. Riley, William J.; McKone, Thomas E.; Lai, Alvin C.K.; and Nazaroff, William W. "Indoor Particulate Matter of Outdoor Origin: Importance of Size-Dependent Removal Mechanisms," *Environmental Science & Technology*, 36:2, pp 200–207, 2002.

34. Abt, Eileen; Suh, Helen H.; Catalano, Paul; and Koutrakis, Petros. "Relative Concentration of Outdoor and Indoor Particle Sources to Indoor Concentrations," *Environmental Science & Technology*, 34:17, pp 3579–3587, 2000.

35. Reisen, Fabienne; Powell, Jennifer C.; Dennekamp, Martine; Johnston, Fay; and Wheeler, Amanda J. "Is Remaining Indoors an Effective Way of Reducing Exposure to PM_{2.5} During Biomass Burning Events?" *Journal of the Air & Waste Management Association*, 1995.

36. Urbanski, Shawn; Hao, Wei Min; and Baker, Steve. "Chemical Composition of Wildland Fire Emissions" in *Wildland Fires and Air Pollution*, Bytnerowicz, Andrzej; Arbaugh, Michael J.; Riebau, Allen R.; and Anderson, Christian (Eds.), Chapter 4, Elsevier, 2009.

37. Pereira, P.; Úbeda, X.; Martin, D.; Mataix-Solera, J.; and Guerrero, C. "Effects of a Low Severity Prescribed Fire on Water-Soluble Elements in Ash from a Cork Oak (*Quercus suber*) Forest Located in the Northeast of the Iberian Peninsula," *Environmental Research*, 111:2, pp 237–247, February 2011.

38. Crutcher, Ernest R. III and Crutcher, Heidie K. "Assessing Wildfire Exposure in Homes Near Wildfires," Health Buildings America Conference, Boulder CO, 2015. ■

Table 1. Optical Properties of Calcium Oxalate Phytoliths with Temperature

Temperature	Ambient	120°–235° C	420°–520° C	620°–860° C
Whewellite Chemistry	$\text{Ca}(\text{C}_2\text{O}_4) \cdot (\text{H}_2\text{O})$	$\text{Ca}(\text{C}_2\text{O}_4)$ (anhydrous calcium oxalate)	CaCO_3 (calcite)	CaO (lime)
Whewellite Optical Properties	$\alpha = 1.490$ $\beta = 1.553$ $\gamma = 1.650$ $B = 0.160$	Diffuse, $B = 0.140$ Low light scatter	Polycrystalline 1st order colors High light scatter $B = 0.172$	Isotropic Low light scatter $B = 0$
Weddillite Chemistry	$\text{Ca}(\text{C}_2\text{O}_4) \cdot 2(\text{H}_2\text{O})$	$\text{Ca}(\text{C}_2\text{O}_4)$ (anhydrous calcium oxalate)	CaCO_3 (calcite)	CaO (lime)
Weddillite Optical Properties	$\omega = 1.523$ $\varepsilon = 1.544$ $B = 0.021$ $\varepsilon = 1.544$	Diffuse, $B = 0.030$ Low light scatter	Polycrystalline 1st order colors High light scatter	Isotropic Low light scatter $B = 0$

Table 2. Illumination Systems Required for Investigating Wildfire Particles¹

Illuminations	Reflected Darkfield	Transmitted Oblique	Circular Polarized	Linear Polarized	Transmitted Brightfield	Transmitted Darkfield
Morphology	X	X	X	X	X	X
Reflectivity	X					
Light Scatter	X				X	X
Birefringence			X	X		
Isotropic			X			
Refractive Index		X				
Optical Density					X	X

¹The configuration of the microscope, as described in the Analytical Methods section, permits rapid transitions between these illuminations without moving the particle. These transitions may involve pressing a button or using a filter and are made in a second or less. The change in the image of the particle is often sufficient to identify the particle and its source and history.

Table 3. Important Properties by Particle Type¹

Properties	Morphology	Reflectivity	Light Scatter	Birefringence	Isotropic	RI	Optical Density
Ash	X		X	X		+	X
Charred Bark	X	X					X
Charred Wood	X	X					X
Calcium Oxalate	X	X	X	X	X	+	X
Silica	X		X		X	–	
Fire Retardant	X	X		X			X
Burned Clay		X		X		+	X

¹This table lists the illumination techniques used to identify different particle types generated by wildfire.

Morphology is a catchall term and includes the outline shape, internal structure, texture, heterogeneity, shapes of surface features, shapes of internal features, size, aspect ratios, and the relative sizes of different features.

Reflectivity includes the pattern of the reflection, the relative intensity of the reflection, and the color of the reflection.

Light scatter has a relative intensity and a pattern that is related to the number of scattering elements per unit volume or area.

Birefringence is a property of directional differences in the bond polarity within a material. For a single crystal the three-dimensional bond polarity is fixed.

Isotropic: A material is isotropic if bond strengths are the same in all directions.

Refractive index (RI) is relative to the mounting medium and is either higher (+) or lower (–).

Optical density refers to how much light transmits through the particle; it ranges from opaque to transparent and includes transmission color.

New Microcrystal Tests for Controlled Drugs, Diverted Pharmaceuticals, and Bath Salts (Synthetic Cathinones)

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McCrone Research Institute¹

Beginning with this issue, *The Microscope* is publishing selected monographs from McCrone Research Institute's recently completed research, *New Microcrystal Tests for Controlled Drugs, Diverted Pharmaceuticals, and Bath Salts (Synthetic Cathinones)*, which contains newly developed microcrystal tests and reagents with 9 additional drugs: alprazolam, butylone, MDPV, 4-MEC, mephedrone, methylone, alpha-PVP, tramadol, and zolpidem. This installment includes an updated introduction from McCrone Research Institute's first drug compendium research, *A Modern Compendium of Microcrystal Tests for Illicit Drugs and Diverted Pharmaceuticals*, followed by monographs for these drugs and reagents:

- alprazolam: gold bromide with hydrochloric acid
- alprazolam: gold bromide with sulfuric acid and acetic acid
- butylone: palladium chloride with hydrochloric acid and phosphoric acid
- butylone: platinum bromide with sulfuric acid

Additional monographs will be published in future issues of *The Microscope*.

BACKGROUND

Traditional light microscopy and microcrystal tests have been used together for more than 100 years. They have proven useful when automated instrumental analysis is unavailable or impractical; for example,

if mixtures of one or more drugs, excipients, diluents or adulterants are present, or when the drug is held in alternative delivery devices such as gels or transdermal patches. Furthermore, while some crime laboratories may lack certain automated instrumental capabilities, most have light microscopes and properly trained microscopists.

Microcrystal tests using polarized light microscopy (PLM) can identify most illicit drugs, diverted pharmaceuticals, and synthetic cathinones (psychoactive bath salts) specifically and quickly (usually within a few minutes) and are inexpensive compared to other methods. In addition, proper use of the light microscope and microcrystal tests can check and confirm the results obtained by alternative methods. It should be explicitly noted, that good scientific practice requires the use of a positive and negative control that should be implemented with the use of microcrystal tests. This ensures that the reagents are functioning properly and that the analyst can recognize the crystal morphologies and optical properties that indicate a positive result, as well as the ability to recognize crystal morphologies and optical properties that do not indicate a positive result. The original compendium of microcrystal tests, together with the addition of these recently discovered microcrystal tests for 9 new drugs, will continue to fulfill a critical need for reliable analytical methods and assist forensic scientists and other researchers in their work.

This project was supported by Award No. 2016-IJ-CX-K010, awarded by the National Institute of Justice, Office of Justice Programs, U.S. Department of Justice. The opinions, findings, and conclusions or recommendations expressed are those of McCrone Research Institute and do not necessarily reflect those of the Department of Justice.

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MICROCRYSTAL TESTS

A Modern Compendium of Microcrystal Tests for Illicit Drugs and Diverted Pharmaceuticals includes 19 drugs for which microcrystal tests using various reagents have been previously developed. It is used today by forensic scientists in the crime laboratory and by researchers in the analytical chemistry laboratory. *New Microcrystal Tests for Controlled Drugs, Diverted Pharmaceuticals, and Bath Salts (Synthetic Cathinones)*, contains research on 9 additional drugs, including some psychoactive bath salts, for which microcrystal tests had not previously been discovered or developed. Together, the compendiums describe in detail the microcrystals formed for 28 drugs with various reagents for each test and include photomicrographs, morphology illustrations, optical properties, notes, and infrared (IR) spectra of the resultant microcrystals.

Most drugs in the original compendium include two or three reagents that may be used for their identification; in a few cases, only one reagent is provided. Reagents for the new research were first determined as candidates based on their availability, use, and success in microcrystal tests for related chemical compounds. The methods were derived from the technical literature with known compounds of interest and were subjected to rigorous testing. Once a reagent was found to form characteristic microcrystals, the reagent and test method were thoroughly evaluated. As a result, each reference listed in the new monographs for the 9 additional drugs is directly related to the reagent formula and a known documented alternative use for the reagent. For each of the 9 additional drugs, the authors include two reagents, which were found to be reliable, accessible, and practical.

Techniques have also been developed for the additional drugs with common adulterants that may inhibit or distort crystal formation, including butylone, BZP, caffeine, ethylone, lidocaine HCl, MDPV, 4-MEC, mephedrone, methylone, alpha-PVP, and TFMPP. All procedures were vetted and evaluated by McCrone Research Institute research microscopists, together with practicing forensic scientists in other collaborative laboratories. The compendium and monographs include recommended protocols, reagents, morphology of crystals (with numerous photomicrographs), IR spectra of microcrystals, and potential interferences. In addition, they include optical and crystallographic properties of the microcrystals.

Each monograph includes the following topics for each drug: reagents; test methods; sensitivity of the test and limit of detection; time required for crystal

formation; crystal morphology; evaluation of the tests in the presence of common excipients, diluents, and adulterants (for street drug samples) or combination drugs (for pharmaceutical preparations); and evaluation of the tests for drugs from selected pharmaceutical delivery devices, e.g. tablets, capsules, gels, transdermal patches, and oral solutions when applicable.

Limit of Detection

The limit of detection (LOD), or minimum amount of sample required to obtain a positive result, i.e. typical crystal formation, was determined for each drug and reagent in the compendium. Some previous researchers referred to using samples "the size of a period on a printed page." The amount suggests a minimum required sample quantity and provides a means to compare the sensitivity of all the microcrystal tests. An analogous unit of measurement was established for this compendium wherein sample size was measured in units of "PPP," a quantity with an approximate diameter the size of a single period on a printed page. This unit represents a quantity of sample that fills the area of a period printed or displayed at 100% in Times New Roman 10-point font. The weight of 1 PPP is approximately 0.1 mg. All microcrystal tests in the compendium specify a LOD (usually 1 PPP) for each drug and reagent; however, the LOD is a lower limit, and more material can be tested with similar results. Pharmaceutical products included in the compendium were tested at various dosages or concentrations, and in most cases, the lowest dosage pharmaceutical and the lowest quantity of material required for a successful test was specified.

Crystal Morphology

Descriptions of typical crystal morphology were often used in reference to Clarke (Figure 1). There are some cases where Clarke's general descriptions are used with additional terms for microcrystals that resemble easily recognizable objects: parallelograms, nails, bow ties, coffins, dahlia flowers, pants, wrapped candy, etc.

Pharmaceuticals, Adulterants, Other Drug Interactions, and Alternative Delivery Devices

The compendium and monographs include commonly encountered adulterants and excipients that were tested in several ratios with the drugs (5:1, 1:1, and 1:5) to determine the success of each microcrystal test and reagent. In most cases, the microcrystal test was successful and the drug was detected in these ratios. However, in a few cases, the drug produced no

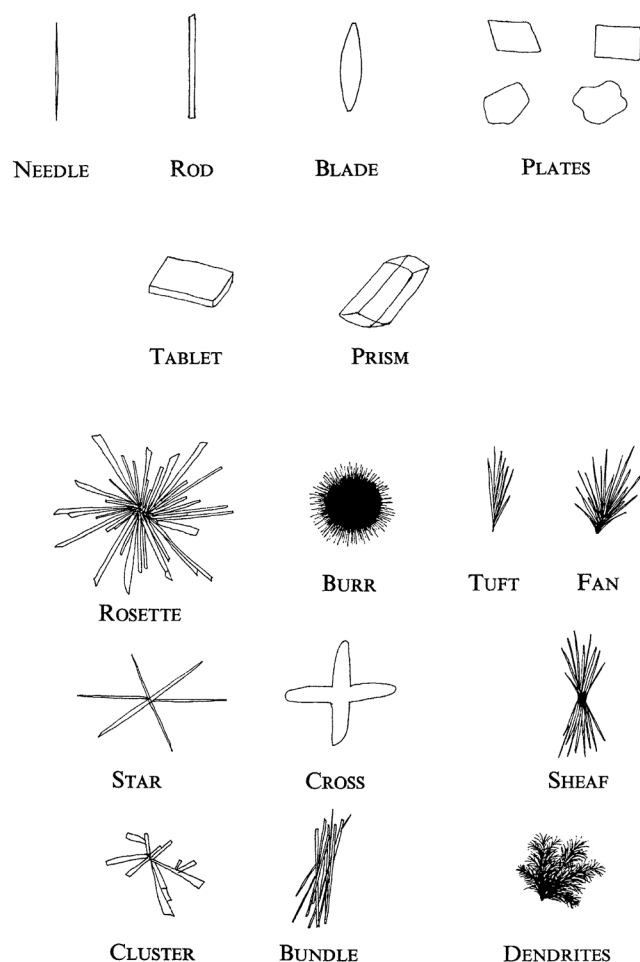


Figure 1. Microcrystal morphology (from Clarke; used with permission).

crystals, was not reproducible, or did not produce typical crystals in the presence of the adulterant or excipient. Some pharmaceuticals included several different drugs or ingredients, and the additional drugs interfered with the microcrystal test, or the drug was present in such low concentration that typical crystals were distorted or did not form. In these instances, micro-scale extractions were performed in order to extract, isolate, or concentrate the drug. The extractions carried out using microcentrifuge tubes take only a few minutes and are described in the compendium.

In addition to tablets and capsules, several pharmaceuticals employing alternative delivery devices (e.g. oral solutions, extended-release formulations, gels, and transdermal patches) were tested in order to determine the success or failure of the microcrystal tests. In some cases, the microcrystal test produced no positive results directly, and micro-scale extractions were required. After some modifications to the test

methods, many microcrystal tests were successful on alternative delivery devices and are described in the compendium.

Fourier Transform Infrared Microspectroscopy

Infrared spectra of the microcrystals obtained by Fourier transform IR microspectroscopy were not available in any of the references and are now included in this compendium. It was observed that the spectra obtained from the drugs are different than the spectra obtained from typical microcrystals of the same drug. There are sometimes differences in peaks and small shifts in peak positions, and there are differences that may occur in the spectra of microcrystals for the same drug when using different reagents.

IR spectra files (.spc and .jpg formats) for microcrystal tests are available for download on the McCrone Research Institute website, www.mccroneinstitute.org.

PLM OPTICAL PROPERTIES

Refractive Indices

The refractive indices of some microcrystals were difficult to determine because they must be dried, not obscured by recrystallized reagent, and excess liquid must be wicked away before applying the refractive index liquids. Crystals in ordinary aqueous reagents were most easily dried at room temperature, while those in acidic reagents needed to be washed with a solvent such as ethanol or chloroform and then dried. The following is the procedure used for washing the crystals: Excess reagent was wicked with a lab tissue or filter paper. A drop of solvent was placed on a slide near the typical crystals that formed, and then a tungsten needle was used to wick up some of the solvent to draw it over the crystals and wash free the reagent from the crystals. This may require multiple attempts to sufficiently remove the reagent. Some of the microcrystals have refractive indices greater than 1.700, which are considered very high and are above the limit at which many laboratories are capable of determining with readily available refractive index liquids. When microcrystals exhibited a refractive index greater than 1.700, this result was recorded, and exact values were not pursued further.

Estimated Birefringence

Birefringence (B) was determined by measuring the thickness of the crystal using a calibrated ocular scale and estimating the interference colors observed in crossed polars with PLM. The birefringence was then calculated using a Michel-Lévy interference

color chart or the classic birefringence equation: $B = R / (1000 \times T)$, where R is retardation (interference color value, in nanometers) and T is crystal thickness (in micrometers). Birefringence for the typical microcrystals was estimated to be low when the values were less than 0.010, moderate when they were between 0.010 and 0.050, or high when they were greater than 0.050.

Sign of Elongation

The sign of elongation was determined for microcrystals that are elongated. If the refractive index parallel to the long axis (length) is greater than the refractive index perpendicular to the long axis (width), then the crystal has a positive sign of elongation. If the opposite is true, it has a negative sign of elongation.

Interference Figures

Interference figures were difficult to obtain on the microcrystals. Many of the microcrystals were not a suitable shape or size or were not properly oriented to observe an interference figure. However, there are a few crystals in the compendium that did show good interference figures. When an interference figure was observed, its uniaxial or biaxial character was recorded together with the optic sign.

METHODS AND TECHNICAL NOTES

The procedures used throughout the research and presented in the compendium and monographs are standard procedures employed by most microscopy laboratories and will be familiar to any microscopist. Techniques that may be less common are explained in the appropriate section for each drug. However, there are technical details about the tests throughout the compendium that should be noted: most microcrystal tests are performed in an exposed reagent drop without using a coverslip. Unless specified, a coverslip was not used in performing these tests. Occasionally, a coverslip was placed on the reaction drop after crystal growth occurred in order to obtain better quality photomicrographs.

Most of the microcrystal tests required less drug material and, therefore, less liquid reagent than traditional laboratory dropper bottles provide. A micropipette was used to obtain smaller quantities of liquid. If a micropipette is not available, tiny drops of solvent or reagent can be made by using a tapered glass rod. A tapered glass rod is made from a length of cylindrical glass approximately 10 cm in length and 2–3 mm in diameter that has been drawn out in a flame to about 1 mm diameter at the tip, then polished to a flat, blunt

end. The glass rod can be used to obtain small drops of solvent or reagent by simply teasing a drop from a bottle dropper. The bottle dropper is squeezed slightly, allowing a small amount of liquid to exit the tip as the glass rod is drawn across the opening. This creates a micro-drop, approximately 5 μ L, on the tip of the glass rod. The drop can then be placed on a glass slide or coverslip in preparation for the microcrystal test. A 5 μ L drop, after being placed on the glass slide or coverslip, will be about 5 mm in diameter.

Glass rings used during the volatility tests have the following specifications: 17 mm outer diameter, 14 mm inner diameter, 1 mm wall thickness, and 5 mm height. Different diameter rings and glass concavity slides should give similar results, however, the microscope may have difficulty focusing with glass rings that are more than 5 mm in height, especially when using high-magnification objectives. Glass is the preferred material for the rings because it is inert, however, other materials may be substituted if they will not interfere with the microcrystal tests.

Reagent formulations are written using the quantities given in the original sources but can be halved, quartered, or otherwise adjusted as needed. Unless otherwise noted, the reagents are stable for years, if stored properly. If the age or condition of a reagent is uncertain, the test should be performed on a known drug sample to ensure the reagent is working properly.

Data, including photomicrographs, were obtained using research-grade drug standards in order to acquire the highest quality results. Pharmaceuticals and street drug samples tested with the reagents typically yielded the same microcrystals. However, in some rare cases, certain combination drugs or adulterants may have caused the test to be unsuccessful. These instances are noted in the appropriate drug and reagent sections, together with any alternative test methods.

Pharmaceutical tablets are often coated or encapsulated with inert ingredients that do not contain any drug material. Therefore, when sampling from a pharmaceutical tablet, the tablet was first broken in order to expose the inner portion. A needle or sharp instrument was then used to break off small pieces from the center, without the coating. The drug material is sometimes present as colorless particles, which can be distinguished from other fillers and binders (e.g. microcrystalline cellulose, starch, etc.), when viewed with a stereomicroscope. The drug particles may be euhedral (well-formed), causing them to appear shiny in reflected light. When these crystals are present, they should be selected and removed individually for the microcrystal tests.

CONCLUSION

A Modern Compendium of Microcrystal Tests for Illicit Drugs and Diverted Pharmaceuticals is presented in a PDF file and comprises 19 drugs. It includes reagents, microcrystal test methods, optical properties, and IR spectra. *New Microcrystal Tests for Controlled Drugs, Diverted Pharmaceuticals, and Bath Salts (Synthetic Cathinones)*, contains 9 additional drugs, including psychoactive bath salts, for which microcrystal tests had not previously been discovered or developed. Both publications will be updated with additional drugs, reagents, and microcrystal tests when such data become available.

AUTHOR CONTRIBUTIONS

Sebastian Sparenga and Meggan King performed and evaluated the microcrystal tests, documented the optical properties, and assisted in the format, layout, and design of the monographs. Sparenga performed the IR microspectroscopy. Dean Golemis designed the layout, created the pages, and edited the content. Gary Laughlin provided the editorial and technical review and overall project management. All authors read and approved the final documents.

ACKNOWLEDGMENTS

The authors would like to thank Kelly Brinsko Beckert (Microtrace LLC) for her contribution to the research and development of *A Modern Compendium of Microcrystal Tests for Illicit Drugs and Diverted Pharmaceuticals* (2015) and early contributions leading to the successful discovery and development of new microcrystal tests for drugs in this research. The authors would also like to thank James Dunlop (Kalamazoo County Sheriff's Office), Hiram Evans (San Bernardino County Sheriff's Department, Retired), and Skip Palenik (Microtrace LLC) for their advice and assistance throughout this project.

REFERENCES

- Clarke, E.G.C. *Isolation and Identification of Drugs, Vol. 1*, The Pharmaceutical Press: London, 1969.
- Fulton, C. *Modern Microcrystal Tests for Drugs*, Wiley-Interscience: New York, 1969.
- McCrone Research Institute. *A Modern Compendium of Microcrystal Tests for Illicit Drugs and Diverted Pharmaceuticals*: http://www.mccroneinstitute.org/uploads/A_Modern_Compendium_of_Microcrystal_Tests.pdf; 2015–2018. ■

See alprazolam and butylone monographs on pages 22–32.

Alprazolam: Gold Bromide with Hydrochloric Acid

REAGENT 1A: Gold Bromide (HAuBr₄) with Concentrated Hydrochloric Acid (HCl)

There are two ways to make this reagent: 1.3 g HAuBr₄ in concentrated HCl, make up to 30 mL. Alternatively, to convert gold chloride to gold bromide: 1 g HAuCl₄·3H₂O and 1.5 mL HBr (40%) in concentrated HCl, make up to 30 mL. Reagent does not keep and should be fresh when used.

Test Method

Direct test: Dissolve sample in 2 µL of 10% HCl. Place a 5 µL drop of reagent on a coverslip. Invert the coverslip and place it directly onto the dissolved sample. Or, dissolve sample in 5 µL of 10% HCl, then add 5 µL of reagent and gently mix drop with pipette tip, glass rod, or toothpick to induce crystallization.

References

1. Fulton, C. *Modern Microcrystal Tests for Drugs*, Wiley-Interscience: New York, 1969.

Limit of Detection

1 PPP

Time Required for Crystal Formation

≈9 minutes (pharmaceuticals up to ≈20 minutes).

Crystal Morphology and Test Notes

Curved, wispy rosettes. Crystals also form clusters of straight needles and blades.

Photomicrograph of Typical Crystals



Figure 1. 1 PPP of alprazolam in 2 µL of 10% HCl and 5 µL of HAuBr₄ with HCl reagent. Crystals form curved, wispy rosettes.

Pharmaceuticals, Adulterants, or Other Drug Interactions

These tests were successful on four different alprazolam pharmaceutical tablets and an alprazolam oral solution. Different procedures were necessary for successful results.

Acid-base extraction procedure, tablet. Tests of pharmaceutical tablets from two different manufacturers were successful after an acid-base extraction procedure: crush ½ a tablet (or smaller portion) and place it in a microcentrifuge tube. Add 200 µL of 10% H₂SO₄ and mix by aspirating with a transfer pipette. Slowly add 200 µL of saturated Na₂CO₃ solution and mix, then add 50 µL of chloroform and mix. (Note: If using more than ½ a tablet, add 500 µL of 10% H₂SO₄ and 500 µL of Na₂CO₃.) Use a micropipette to decant 10 µL from the chloroform layer (bottom) onto a glass slide. Allow to evaporate, then proceed

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PLM Optical Properties

Approximate Size Range	25–100 µm
Color/Pleochroism	Yellow-orange. Pleochroic: yellow to dark yellow-orange.
Refractive Indices (RI)	n-parallel ≈ 1.700 n-perpendicular > 1.700

Morphology Illustration



not to scale

How Crystals Were Dried for RI Measurement	Excess liquid was wicked away with lab tissue and dried at room temperature.
Estimated Birefringence	High
Extinction	Oblique (≈40°)
Sign of Elongation	Negative (–)
Crystal Optics and Optic Sign (Interference Figure)	Indeterminable

Alprazolam: Gold Bromide with Hydrochloric Acid (continued)

Pharmaceuticals, Adulterants or Other Drug Interactions

Continued from preceding page

with microcrystal test. These tests were successful with the tablets in the following required quantities:

- ½ tablet Sandoz® 0.25 mg alprazolam
- 1 tablet Actavis® 0.25 mg alprazolam
- ⅙ tablet Sandoz® 2.0 mg alprazolam

Note: Typical crystals form within 15–20 minutes.

Ethanol extraction procedure, extended release tablet. A test of a Greenstone® 0.5 mg extended release tablet was successful after an ethanol extraction procedure: crush ½ tablet and place into a microcentrifuge tube with 1 mL of ethanol and allow to sit for 30 minutes, then centrifuge. Use a micropipette to

decant 20 µL onto a glass slide, then proceed with test method. Note: Typical crystals form within 15–20 minutes.

Chloroform extraction procedure, oral solution. A test of an Intensol® oral solution containing 1 mg/mL of alprazolam was successful after a chloroform extraction: Transfer 100 µL of the oral solution into a microcentrifuge tube, add dilute NH₄OH to pH 9, then shake. Add 100 µL of chloroform and shake vigorously. Allow to separate, then use a micropipette to remove and transfer the chloroform/alprazolam (bottom) layer into a clean microcentrifuge tube. Adjust to pH 6 with 10% HCl and shake. Use a micropipette to decant 10 µL onto a glass slide, then proceed with test method. Note: Typical crystals form within 15–20 minutes.

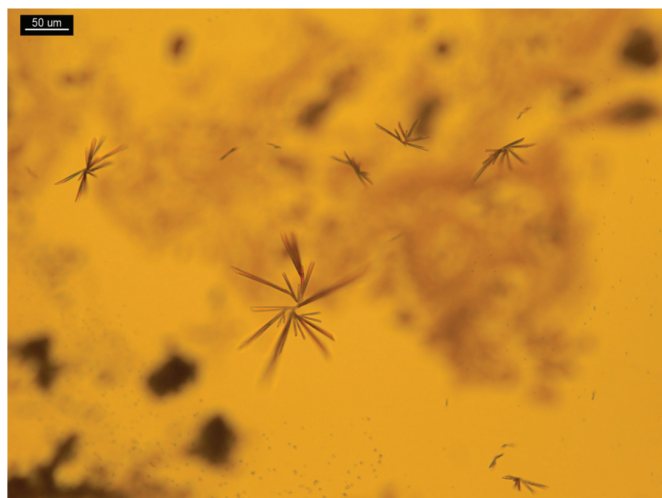


Figure 2. 1 PPP of alprazolam in 2 µL of 10% HCl and 5 µL of reagent: H₂AuBr₄ in HCl. Crystals form curved, wispy rosettes and clusters of straight needles and blades.



Figure 3. 1 PPP of alprazolam in 2 µL of 10% HCl and 5 µL of reagent: H₂AuBr₄ in HCl. Crystals form curved, wispy rosettes and clusters of straight needles.



Figure 4. 1 PPP of alprazolam in 2 µL of 10% HCl and 5 µL of reagent: H₂AuBr₄ in HCl. Crystals form curved, wispy rosettes and clusters of straight needles and blades.



Figure 5. ⅙ of a Sandoz® 0.25 mg tablet, after acid-base extraction procedure. Crystals form curved, wispy rosettes and clusters of straight needles and blades in 20 minutes.

Alprazolam: Gold Bromide with Hydrochloric Acid (continued)



Figure 6. 1/8 of a Sandoz® 2.0 mg tablet after acid-base extraction procedure. Crystals form curved, wispy rosettes in 16 minutes.

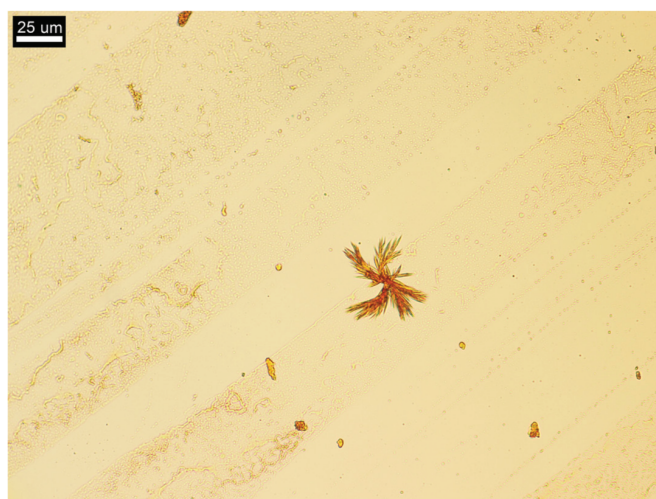


Figure 7. 1/2 of a Greenstone® 0.5 mg extended release tablet after ethanol extraction procedure. Crystals form curved, wispy rosettes in 20 minutes.



Figure 8. 1 Actavis® 0.25 mg tablet after acid-base extraction procedure. Crystals form curved, wispy rosettes in 15 minutes.



Figure 9. 100 μL from a 1 mg/mL Intenol® alprazolam oral solution after chloroform extraction procedure. Crystals form curved, wispy rosettes in 15 minutes.

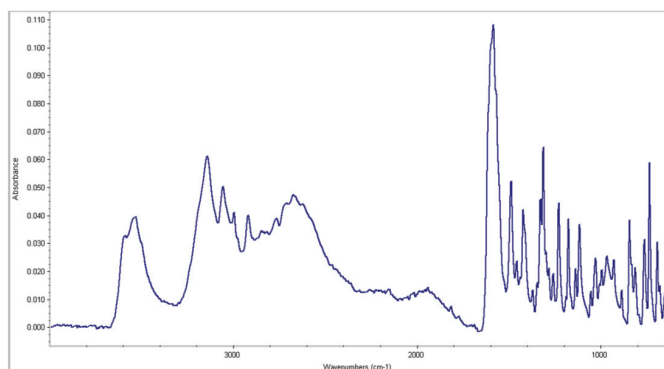


Figure 10. Infrared spectrum of alprazolam H₂AuBr₄ with HCl precipitate.

Alprazolam: Gold Bromide with Sulfuric Acid and Acetic Acid

REAGENT 1B: Gold Bromide (HAuBr₄) with Sulfuric Acid (H₂SO₄) and Acetic Acid (HOAc)

There are two ways to make this reagent: 1.3 g HAuBr₄ in (2+3) H₂SO₄, make up to 30 mL. (2+3) H₂SO₄ is dilute sulfuric acid made by combining two parts (e.g. 12 mL) of concentrated sulfuric acid with three parts (e.g. 18 mL) of water. Then add 10 mL of glacial HOAc. Alternatively, to convert gold chloride to gold bromide: 1 g HAuCl₄·3H₂O and 1.5 mL HBr (40%) in (2+3) H₂SO₄, make up to 30 mL. Then add 10 mL of glacial HOAc.

Test Method

Direct test: Place sample on glass slide. Add a 5 µL drop of reagent on coverslip, invert the coverslip, and place it directly onto the sample. Alternatively, add 5 µL of the reagent directly to the sample on a glass slide; no coverslip.

References

1. Fulton, C. *Modern Microcrystal Tests for Drugs*, Wiley-Interscience: New York, 1969.

Limit of Detection

1 PPP

Time Required for Crystal Formation

≈8 minutes (pharmaceuticals up to ≈15 minutes).

Crystal Morphology and Test Notes

Wispy rosettes of needles and blades. Crystals also form burrs, fans, and sheaves.

Photomicrograph of Typical Crystals

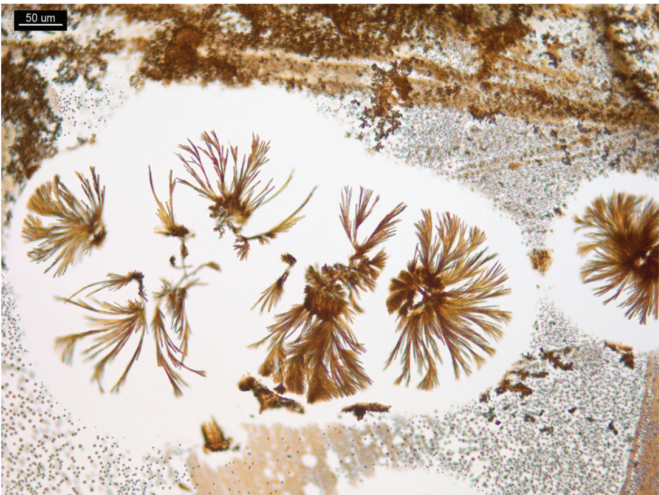


Figure 1. 1 PPP of alprazolam in 5 µL of HAuBr₄ with H₂SO₄ and HOAc reagent. Crystals form rosettes of needles and blades, burrs, fans, and sheaves.

Pharmaceuticals, Adulterants, or Other Drug Interactions

These tests were successful on four different alprazolam pharmaceutical tablets and an alprazolam oral solution. Different procedures were necessary for successful results.

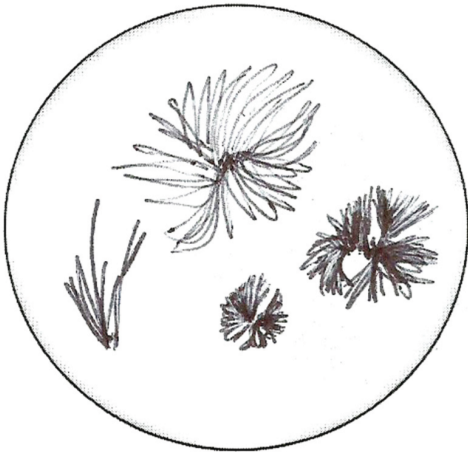
Acid-base extraction procedure, tablet. Tests of pharmaceutical tablets from two different manufacturers were successful after an acid-base extraction procedure: crush a small portion of tablet material and place it in a microcentrifuge tube. Add 200 µL of 10% H₂SO₄ and mix by aspirating with a transfer pipette. Slowly add 200 µL of saturated Na₂CO₃ solution and mix, then add 50 µL of chloroform and mix. Use a micropipette to draw off 10 µL from the chloroform layer (bottom) and place a small drop onto a glass slide (it does not need to evaporate)

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PLM Optical Properties

Approximate Size Range	20–100 µm
Color/Pleochroism	Yellow-orange. Pleochroic: yellow to dark yellow-orange.
Refractive Indices (RI)	n > 1.700

Morphology Illustration



not to scale

How Crystals Were Dried for RI Measurement	Excess liquid was wicked away with lab tissue and dried at room temperature.
Estimated Birefringence	High
Extinction	Oblique (≈40°)
Sign of Elongation	Positive (+) and negative (–)
Crystal Optics and Optic Sign (Interference Figure)	Indeterminable

Alprazolam: Gold Bromide with Sulfuric Acid and Acetic Acid (continued)

Pharmaceuticals, Adulterants or Other Drug Interactions

Continued from preceding page

and proceed with the microcrystal test. These tests were successful with the tablets in the following required quantities:

- ½ tablet Sandoz® 0.25 mg alprazolam
- ½ tablet Actavis® 0.25 mg alprazolam
- ¼ tablet Sandoz® 2.0 mg alprazolam

Note: Typical crystals form within 10–15 minutes.

Ethanol extraction procedure, extended release tablet. A test of a Greenstone® 0.5 mg extended release tablet was successful after an ethanol extraction procedure: crush ½ tablet and place into a microcentrifuge tube with 1 mL of ethanol and allow to sit for 30 minutes, then centrifuge. Use a micropipette to decant 20 µL onto a glass slide, then proceed with test method. Note: Typical crystals form within 10–15 minutes.

Chloroform extraction procedure, oral solution. A test of an Intensol® oral solution containing 1 mg/mL of alprazolam was successful after a chloroform extraction: Transfer 100 µL of the oral solution into a microcentrifuge tube, add dilute NH_4OH to pH 9, then shake. Add 100 µL of chloroform and shake vigorously. Allow to separate, then use a micropipette to remove and transfer the chloroform/alprazolam (bottom) layer into a clean microcentrifuge tube. Adjust to pH 6 with 10% HCl and shake. Use a micropipette to decant 10 µL onto a glass slide, then proceed with test method. Note: Typical crystals form within 10–15 minutes.

IR Spectrum

See Figure 9.

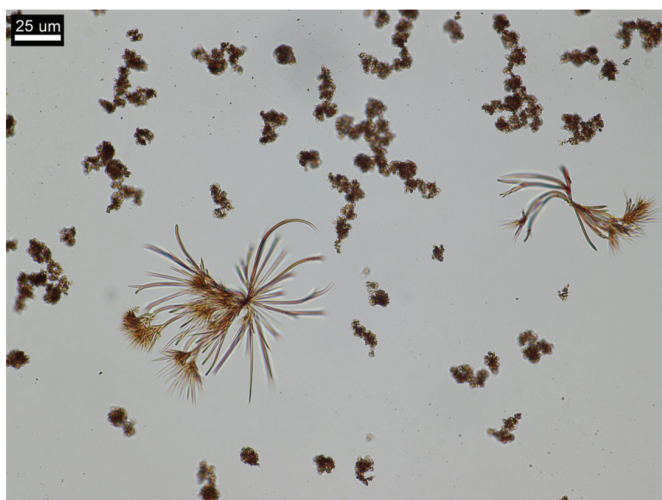


Figure 2. 1 PPP of alprazolam in 5 µL of HAuBr_4 with H_2SO_4 and HOAc reagent. Crystals form wispy rosettes of needles and blades.



Figure 3. 1 PPP of alprazolam in 5 µL of HAuBr_4 with H_2SO_4 and HOAc reagent. Crystals form wispy rosettes of needles and blades.



Figure 4. ½ of a Sandoz® 0.25 mg tablet after acid-base extraction procedure. Crystals form wispy rosettes of needles and blades in 10 minutes.



Figure 5. ¼ of a Sandoz® 2.0 mg tablet after acid-base extraction procedure. Crystals form wispy rosettes of needles in 11 minutes.

Alprazolam: Gold Bromide with Sulfuric Acid and Acetic Acid (continued)

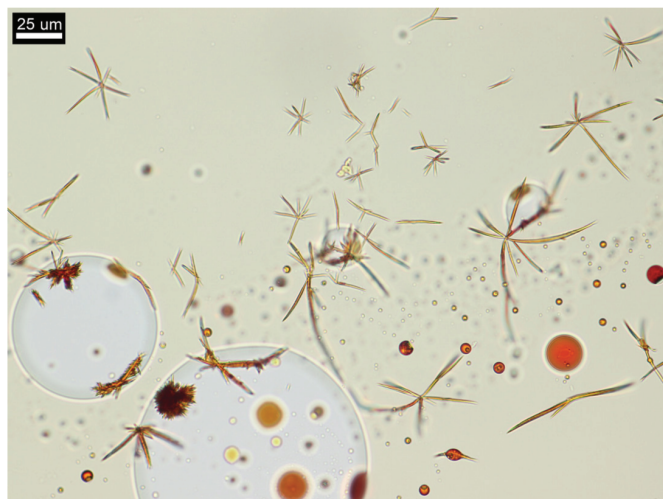


Figure 6. ½ of 0.25 mg Actavis® tablet after acid-base extraction procedure. Crystals form wispy rosettes of needles and blades in 15 minutes.

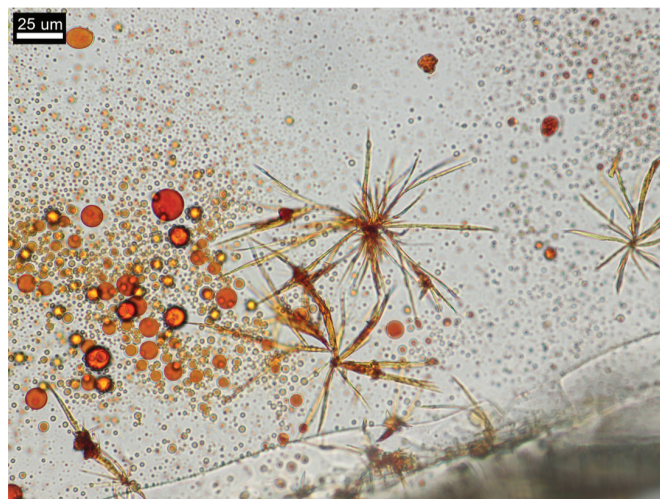


Figure 7. ½ of a Greenstone® 0.5 mg extended release tablet after ethanol extraction procedure. Crystals form wispy rosettes of needles and blades in 15 minutes.



Figure 8. 100 μL from a 1 mg/mL Intensol® alprazolam oral solution after chloroform extraction procedure. Crystals form curved, wispy rosettes in 15 minutes.

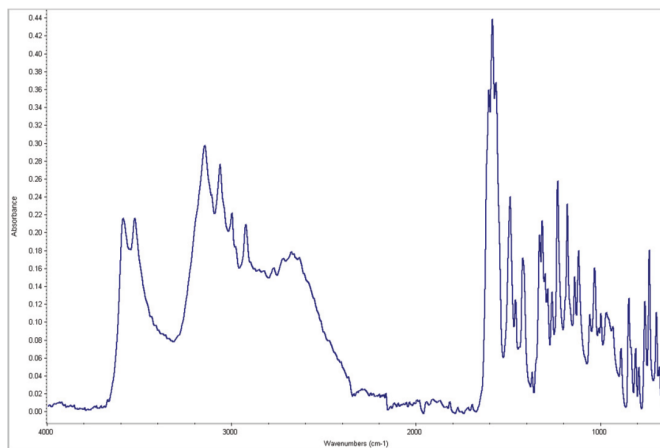


Figure 9. Infrared spectrum of alprazolam H₂SO₄ and HOAc precipitate.

Butylone: Palladium Chloride with HCl and H₃PO₄

REAGENT 1: Palladium Chloride (H₂PdCl₄) with Concentrated Hydrochloric Acid (HCl) and Concentrated Phosphoric Acid (H₃PO₄)

H₂PdCl₄ in concentrated H₃PO₄ is made by combining 1 g of PdCl₂ with 0.9 mL of concentrated HCl, then make up to 20 mL with concentrated H₃PO₄.

Test Method

Direct test: Place a 10 µL drop of reagent on a coverslip. Invert coverslip and place it directly onto the sample.

References

1. Fulton, C. *Modern Microcrystal Tests for Drugs*, Wiley-Interscience: New York, 1969.

Limit of Detection

1 PPP (A larger amount of sample results in faster and larger crystal growth.)

Time Required for Crystal Formation

≈3 minutes

Crystal Morphology and Test Notes

Rosettes of tablets.

Photomicrograph of Typical Crystals

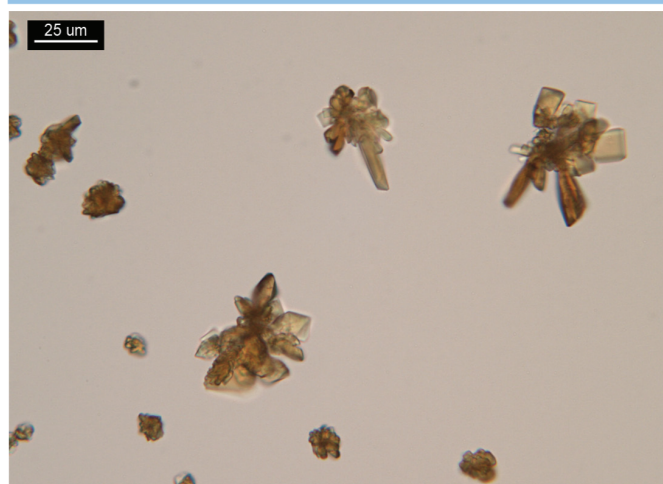


Figure 1. 1 PPP of butylone in 10 µL of H₂PdCl₄ with HCl and H₃PO₄ reagent. Crystals form rosettes of tablets.

Pharmaceuticals, Adulterants or Other Drug Interactions

Common adulterants that may inhibit or distort crystal formation include BZP, caffeine, ethylone, lidocaine HCl, MDPV, 4-MEC, mephedrone, methylone, alpha-PVP, and TFMPP. The detectability of butylone with selected adulterants is listed below:

- BZP:butylone — detectable at 1:5, 1:1, and 5:1
- Caffeine:butylone — detectable at 1:5, 1:1, and 5:1
- Ethylone:butylone — detectable at 1:5, 1:1, and 5:1
- Lidocaine HCl:butylone — detectable at 1:5, 1:1, and 5:1
- MDPV:butylone — detectable at 1:5, 1:1, and 5:1
- 4-MEC:butylone — detectable at 1:5, 1:1, and 5:1
- Mephedrone:butylone — detectable at 1:5, 1:1, and 5:1
- Methylone:butylone — detectable at 1:5, 1:1, and 5:1
- Alpha-PVP:butylone — detectable at 1:5, 1:1, and 5:1
- TFMPP:butylone — detectable at 1:5, 1:1, and 5:1

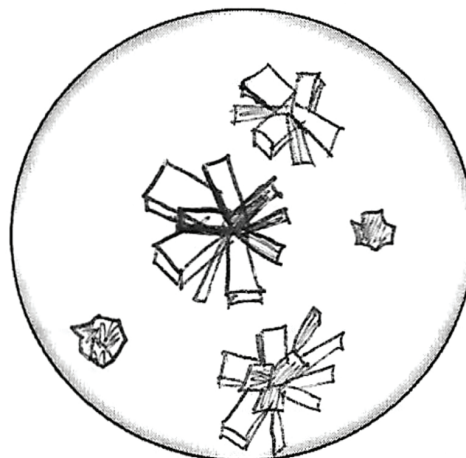
IR Spectrum

See Figure 6.

PLM Optical Properties

Approximate Size Range	10–115 µm
Color/Pleochroism	Yellow-brown. Pleochroic: dark brown to light yellow
Refractive Indices (RI)	$n_1 \approx 1.500$ $n_2 > 1.700$

Morphology Illustration



not to scale

How Crystals Were Dried for RI Measurement	Excess liquid was wicked away with lab tissue then washed with absolute ethanol and dried at room temperature.
Estimated Birefringence	High
Extinction	Oblique (individual tablets) and parallel
Sign of Elongation	Not applicable
Crystal Optics and Optic Sign (Interference Figure)	Indeterminable

Butylone: Palladium Chloride with HCl and H₃PO₄ (continued)

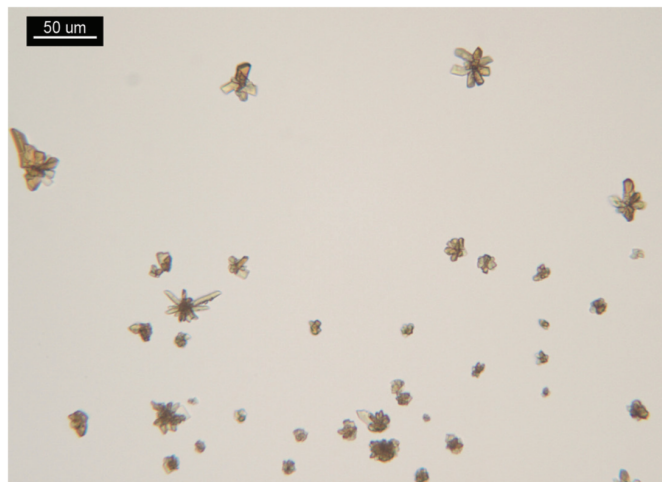


Figure 2. 1 PPP of butylone in 10 μL of H₂PdCl₄ with HCl and H₃PO₄ reagent. Crystals form rosettes of tablets.

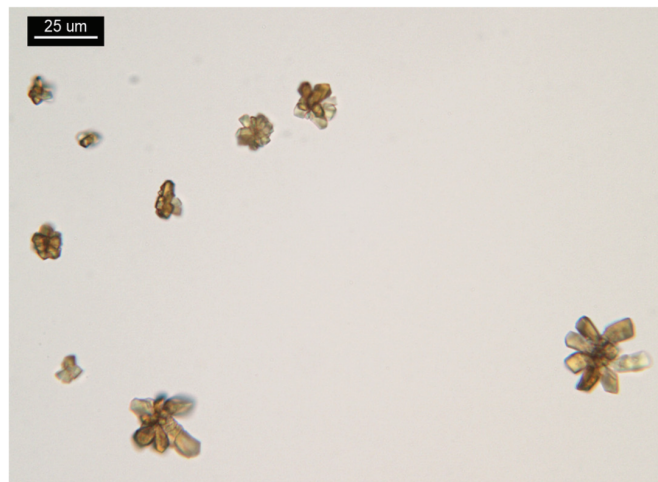


Figure 3. 1 PPP of butylone in 10 μL of H₂PdCl₄ with HCl and H₃PO₄ reagent. Crystals form rosettes of tablets.

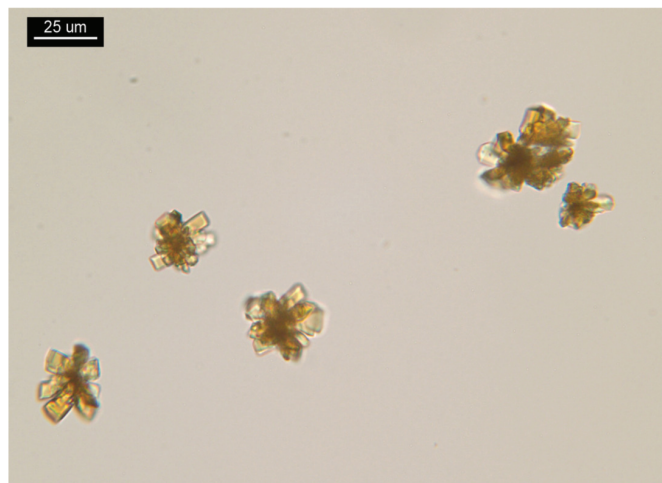


Figure 4. 4-MEC:butylone mixture (1:1) and 10 μL of H₂PdCl₄ with HCl and H₃PO₄ reagent. Crystals form rosettes of tablets.

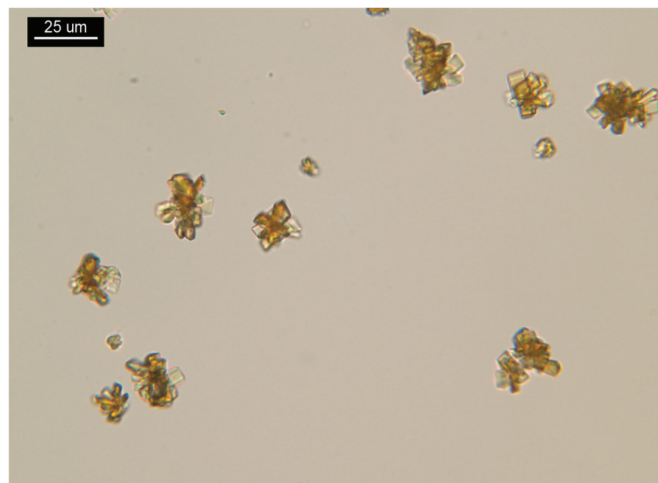


Figure 5. Methylone:butylone mixture (5:1) and 10 μL of H₂PdCl₄ with HCl and H₃PO₄ reagent. Crystals form rosettes of tablets.

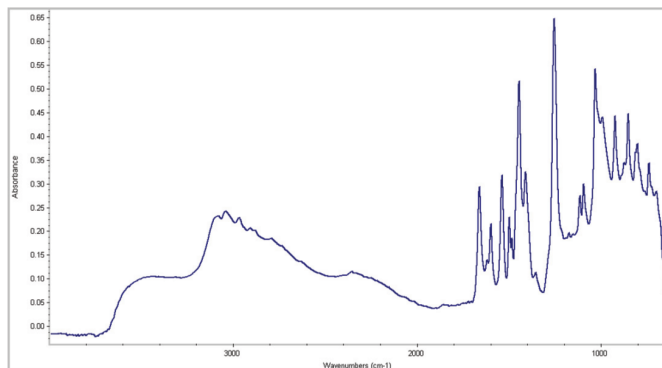


Figure 6. Infrared spectrum of butylone H₂PdCl₄ with HCl and H₃PO₄ precipitate.

Butylone: Platinum Bromide with Sulfuric Acid

REAGENT 2: Platinum Bromide (H₂PtBr₆) with Sulfuric Acid ((2+3) H₂SO₄)

There are two ways to make this reagent: 1.3 g H₂PtBr₆ in (2+3) H₂SO₄, make up to 20 mL. (2+3) H₂SO₄ is dilute sulfuric acid made by combining two parts (e.g. 8 mL) of concentrated sulfuric acid with three parts (e.g. 12 mL) of water. Alternatively, to convert platinum chloride to platinum bromide: 1 g H₂PtCl₆·6H₂O and 1.7 mL HBr (40%) in (2+3) H₂SO₄, make up to 20 mL.

Test Method

Direct test: Add 5 µL of reagent directly to sample; with or without coverslip.

References

1. Fulton, C. *Modern Microcrystal Tests for Drugs*, Wiley-Interscience: New York, 1969.

Limit of Detection

1 PPP (A larger amount of sample results in faster and larger crystal growth.)

Time Required for Crystal Formation

≈2–5 minutes

Crystal Morphology and Test Notes

Coffin lid-shaped tablets and rosettes of tablets with coverslip and rectangular tablets and rosettes of tablets without coverslip.

Photomicrograph of Typical Crystals

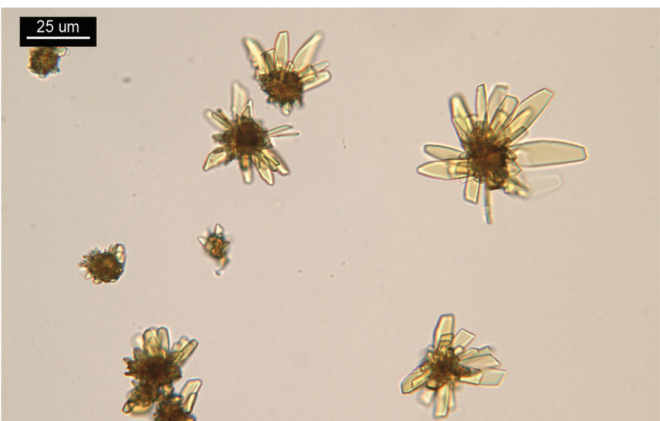


Figure 1. 1 PPP of butylone in 5 µL of H₂PtBr₆ with (2+3) H₂SO₄ reagent. Crystals form coffin lid-shaped tablets and rosettes of tablets.

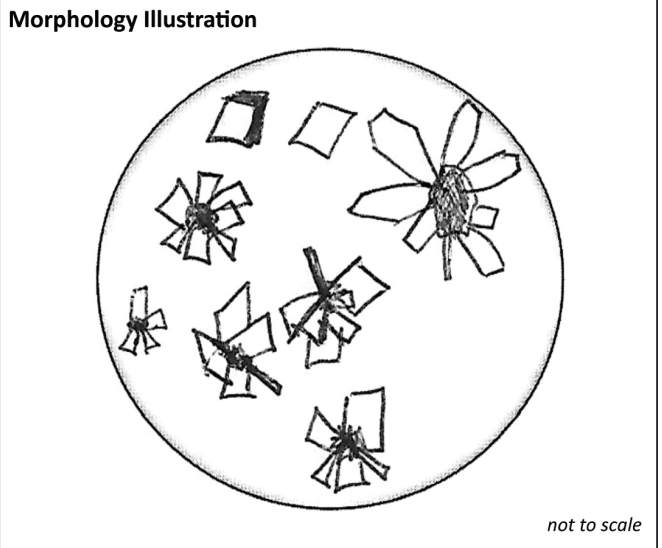
Pharmaceuticals, Adulterants or Other Drug Interactions

Common adulterants that may inhibit or distort crystal formation include BZP, caffeine, ethylone, lidocaine HCl, MDPV, 4-MEC, mephedrone, methylone, alpha-PVP, and TFMPP. The detectability of butylone with selected adulterants is listed below:

- BZP:butylone — detectable at 1:5, 1:1, and 5:1
- Caffeine:butylone — detectable at 1:5, 1:1, and 5:1
- Ethylone:butylone — detectable at 1:5, 1:1, and 5:1
- Lidocaine HCl:butylone — detectable 1:5; undetectable at 1:1 and 5:1
- MDPV:butylone — detectable at 1:5 and 1:1; undetectable at 5:1
- 4-MEC:butylone — detectable at 1:5, 1:1, and 5:1
- Mephedrone:butylone — detectable at 1:5; undetectable at 1:1 and 5:1.
- Methylone:butylone — detectable at 1:5, 1:1, and 5:1
- Alpha-PVP:butylone — detectable at 1:5, 1:1, and 5:1
- TFMPP:butylone — detectable at 1:5, 1:1, and 5:1

PLM Optical Properties

Approximate Size Range	10–60 µm
Color/Pleochroism	Yellow; not pleochroic
Refractive Indices (RI)	n ₁ = 1.700 n ₂ > 1.700



How Crystals Were Dried for RI Measurement	Excess liquid was wicked away with lab tissue then washed with water and dried with gentle heat.
Estimated Birefringence	High
Extinction	Oblique (individual tablets) and parallel
Sign of Elongation	Positive (+) and negative (–)
Crystal Optics and Optic Sign (Interference Figure)	Indeterminable

Butylone: Platinum Bromide with Sulfuric Acid (continued)

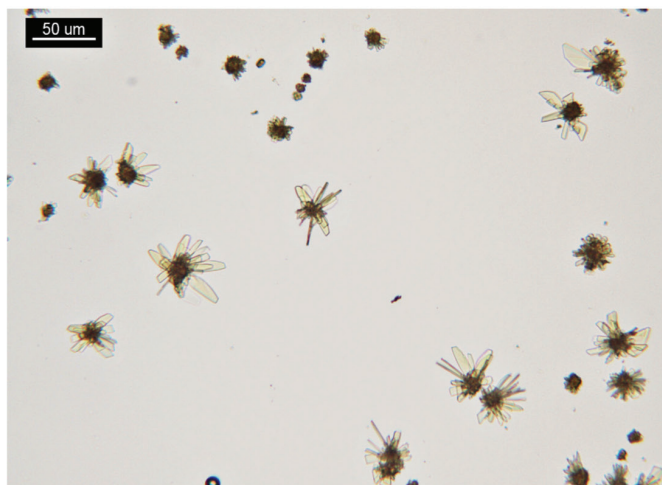


Figure 2. 1 PPP of butylone in 5 μL of H_2PtBr_6 with (2+3) H_2SO_4 reagent. Crystals form coffin lid-shaped tablets and rosettes of tablets.

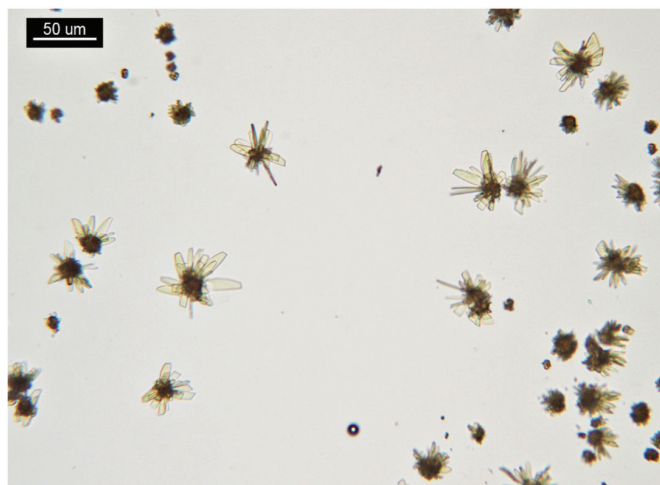


Figure 3. 1 PPP of butylone in 5 μL of H_2PtBr_6 with (2+3) H_2SO_4 reagent. Crystals form coffin lid-shaped tablets and rosettes of tablets.

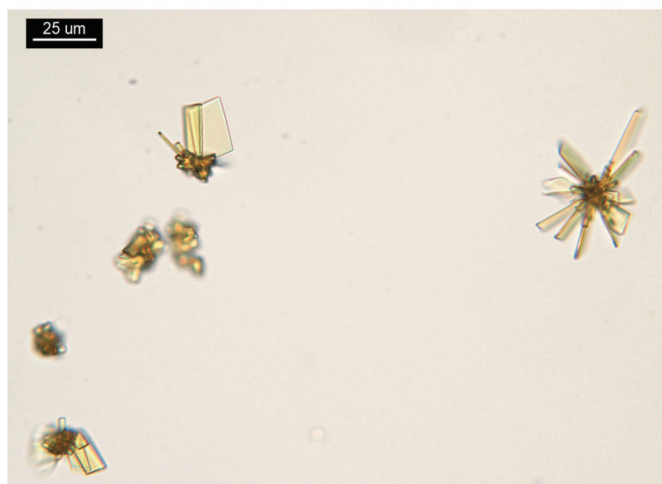


Figure 4. 1 PPP of butylone in 5 μL of H_2PtBr_6 with (2+3) H_2SO_4 reagent. Crystals form coffin lid-shaped and rectangular tablets and rosettes of tablets.

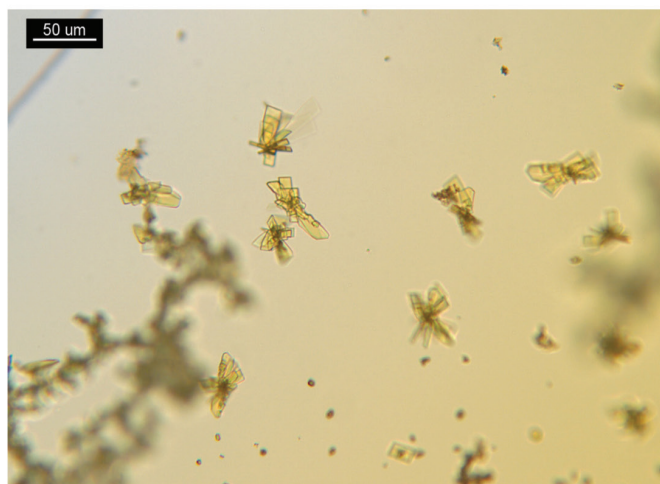


Figure 5. BZP:butylone mixture (1:1) and 5 μL of (2+3) H_2SO_4 reagent. Crystals form coffin lid-shaped and rectangular tablets and rosettes of tablets.

Butylone: Platinum Bromide with Sulfuric Acid (continued)

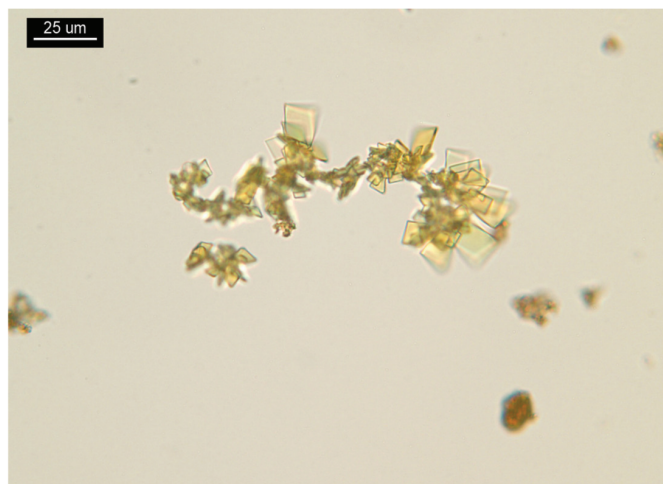


Figure 6. Caffeine:butylone mixture (5:1) and 5 μL of (2+3) H₂SO₄ reagent. Crystals form coffin lid-shaped and rectangular tablets and rosettes of tablets.

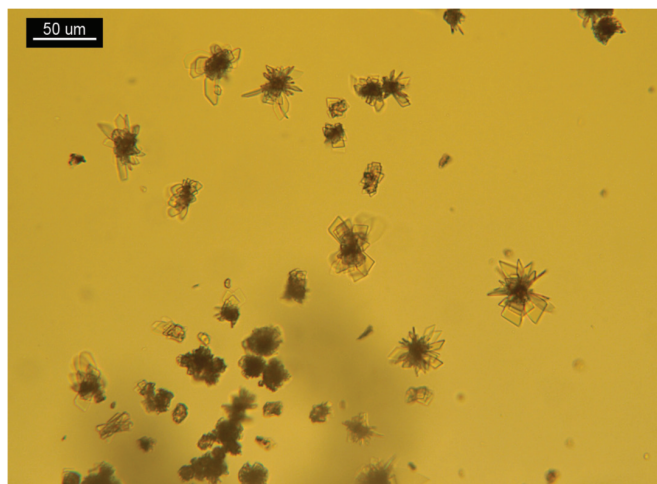


Figure 7. Lidocaine HCl:butylone mixture (1:5) and 5 μL of (2+3) H₂SO₄ reagent. Crystals form coffin lid-shaped and rectangular tablets and rosettes of tablets with distortions.

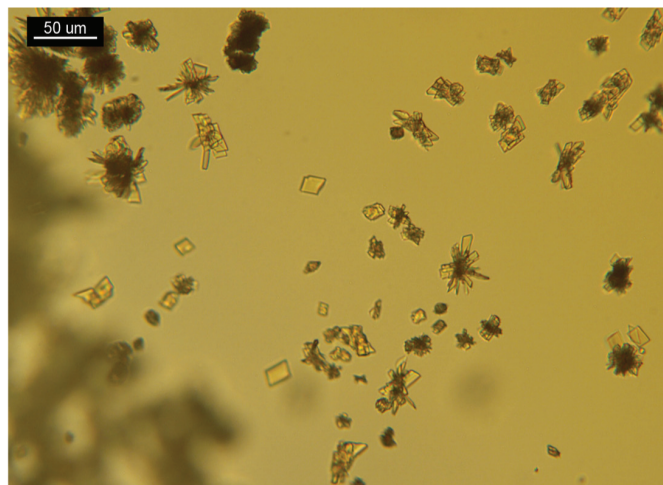


Figure 8. Alpha-PVP:butylone mixture (5:1) and 5 μL of (2+3) H₂SO₄ reagent. Crystals form coffin lid-shaped and rectangular tablets and rosettes of tablets.

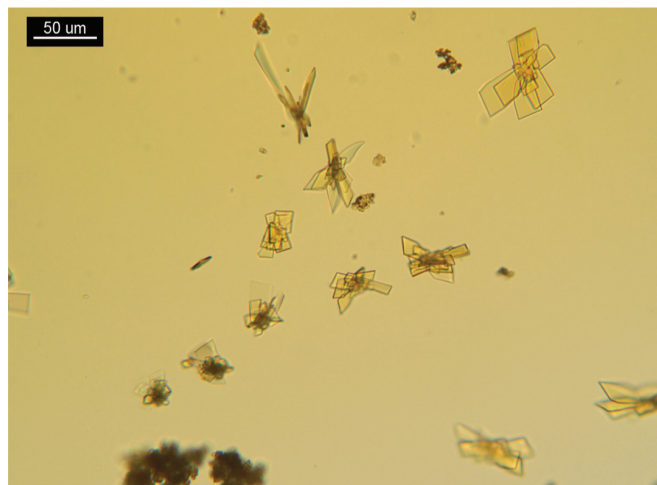


Figure 9. TFMPP:butylone mixture (5:1) and 5 μL of (2+3) H₂SO₄ reagent. Crystals form coffin lid-shaped and rectangular tablets and rosettes of tablets.

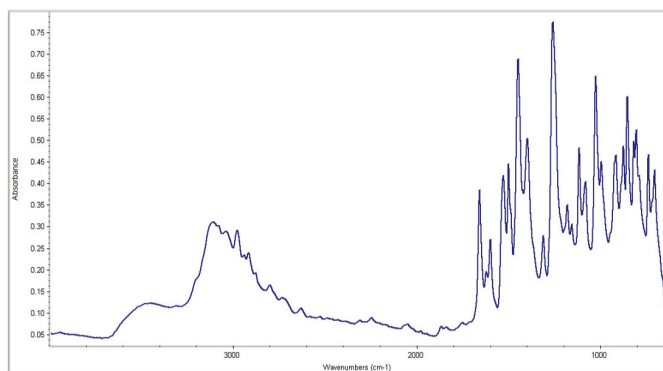


Figure 10. Infrared spectrum of H₂PtBr₆ with (2+3) H₂SO₄ precipitate.



CRITICAL FOCUS

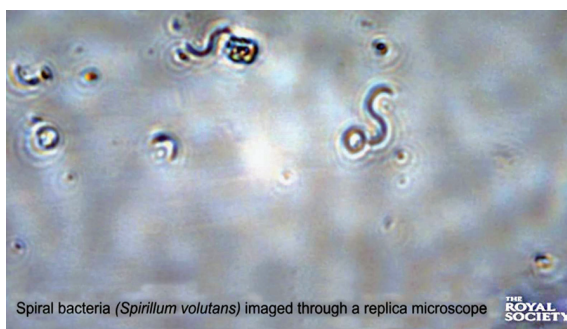
Brian J. Ford

Science? What Science?

Unscrupulous individuals and institutions are exaggerating their scientific research or are plagiarizing someone else's authentic work in the quest for inflated grants and recognition.

There was a time when science was everywhere, probing for truth, exposing hidden facts, and clarifying reality. That is (roughly) what I do in my day job, and it is the guiding principle behind this column. But it opened the door to exploitation, and people would sometimes appropriate the principle to suit themselves. They saw it as a way to patronize and confuse people with complex terms, as a means to claim large grants for small projects, and — above all — as a way of keeping the public firmly in their place. This is what I call “Nonscience.” It is a racket. And it is bigger now than ever before.

“Fake news” may be a coinage popularized by the current American president, but it's old news if you follow the machinations of Nonscience. People are sold myths all the time. You may like to see whether you can pick out the genuine subjects from this list: the Amazon as the lungs of the world, the legendary unicorn, single-use plastics poisoning our planet, the



Spiral bacteria (*Spirillum volutans*) imaged through a replica microscope

When I imaged these living bacteria for the first time through a Leeuwenhoek-type lens in 1989, it was widely acknowledged as groundbreaking. It now appears on the Royal Society website without permission and with the Society claiming the credit.

Large Hadron Collider opening our eyes to new physics, Crick and Watson discovering DNA, Darwin's unprecedented theory, and mermaids. Of course. Two stand out like beacons — mermaids and unicorns are the odd ones out.

Both of those are based on fact, whereas all the others are false.

In Critical Focus, we have discovered why the rainforests are not the lungs of the world (64:1, 2016) and also

gave the lie to the plastic pollution hysteria (67:1, 2019). The Large Hadron Collider is a ridiculously costly experiment that has made no significant change to our understanding of physics, though it has certainly proved how it is possible to draw down vast sums of public money to fund personal enthusiasms (64:3, 2016). DNA was actually discovered by the Swiss biochemist Fritz Miescher in 1869, while Crick and Watson's breakthrough was working out its structural formula, which was discussed here in 2015 (63:3), and Darwin was late on the scene with evolution, as

we discovered in 2011 (59:3). Yes, a large number of myths have been busted by this column.

It is surprising to reflect that the mermaid had factual origins (being a mistaken interpretation of manatees suckling their young) as did the unicorn, an early description from European explorers encountering the Indian rhinoceros for the first time (64:3, 2016). So you see? Topics which everybody believes to be sound and solid are fictitious; and the very things you would imagine to be fairy tales are the only ones rooted in reality.

Fifty years ago, I realized that unscrupulous individuals were exaggerating their work, bluffing their way to secure large grants, while authentic scientists were working away in the background and achieving real results. That is when the idea of Nonscience arose in my mind. At the time, a London publisher was producing a series of amusing paperback books called *The Bluffer's Guides*, and I realized that my new concept of people bluffing their way through science might work well on his list. That publisher was Peter Wolfe, so he arranged for us to meet over lunch in central London. He brought some examples of the guides for me to see and asked me to explain what I had in mind. As I expounded on my proposal, he became increasingly thoughtful. Eventually, he spoke.

"Hmm," he said. "This doesn't fit with the *Bluffer's Guide* idea." I was crestfallen — it had seemed to me the perfect fit. Peter smiled. "It doesn't fit only because it is far too important," he went on. "We have built our company on humorous paperback booklets, but this one is serious satire and it needs to be much more substantial. This will be our first hardback production. And it will be bigger — 200 pages." I was thrilled at the prospect. By the time dessert was finished we had agreed to a deal and shaken hands, and we didn't leave the restaurant until late afternoon. By then it was all mapped out; all we needed was a title. And so, as a joke, I later typed out something long and obscure, as a satirical comment on the way complicated words were used to baffle the outsider: *Nonscience and the Pseudotransmogrificationalific Egocentrified Reorientational Proclivities Inherently Intracorporated In Expertistical Cerebrointellectualised Redeploymentation with Special Reference to Quasi-Notional Fashionistic Normativity, The Indoctrinationalistic Methodological Modalities and Scalar Socio-Economic Promulgationary Improvementalisationalism Predelineated Positotaxically Toward Individualistified Mass-Acceptance Gratificationalistic Securipermanentalisationary Professionism, or How To Rule The World*. It was meant to amuse the office, but they all loved it. "This," said Peter, next time we met, puffing a cigar

that was even larger than usual, "this is going to be a classic. I can't tell you how happy we are to be publishing the book."

FAKE EXPECTATIONS

He was forgetting one thing — no such book existed. It now had to be written. There was plenty to satirize at that time, and the book was fun to write. Needless to say, the opening pages mentioned microscopes and then set the scene with mention of "magnificent, minute microorganisms with shells like spun silk." And for my critique there were so many examples of wanton exaggeration from which to choose. For one thing, Britain at the time wanted to boast of having more university students than anyone else, and was busily renaming the colleges as universities, so the number of so-called universities had gone up from 22 to 44 in the decade before the book was published. (Now there are more than 130.) Meanwhile, the value of the degree has steadily diminished, and academics were complaining of students who were illiterate and unable to function as graduates.

Microscopy was harder to satirize, because microscopists are hell-bent on finding out the truth, though I did tease the publicity surrounding Albert Crewe's research at the University of Chicago. Crewe, a physics professor, had constructed a field emission electron microscope with which he imaged thorium atoms. All the publicity suggested that this was the first time that atoms had been observed. This was false. The field emission instrument had been designed in 1951 by Erwin W. Müller at Pennsylvania State University, and he had famously photographed individual atoms on the tip of a tungsten needle in 1955. In 1970, Crewe had resolved individual thorium atoms — a considerable achievement for microscopy, but not what was being reported. He was claiming to have carried out an unprecedented experiment which actually belonged to someone else. However, that wasn't the reason for my teasing. The statement released to the press added the words: "He expects the tool to be useful in research on a cure for cancer." One of the keys to Nonscience is when any new discovery is related to an eye-catching cure for something unrelated to the research, and this was a wonderful example for my 1971 book. Imaging thorium atoms had nothing to do with curing cancer. The same picture was still circulating in 2013, this time sent out by documentary producers investigating thorium as a source of green energy to combat global warming, equally unrelated to Crewe's research. It is only a matter of time before the photo is repurposed as



Is thorium the answer to the world's energy woes?

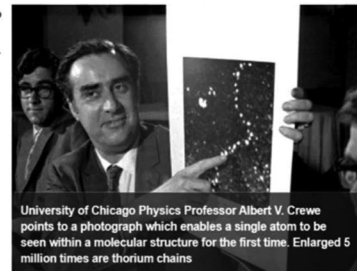
An Irish duo have set up an ambitious Kickstarter campaign for a documentary on the issue, they told TheJournal.ie.

May 26th 2013, 8:05 AM 11,975 Views 30 Comments

Share 105 Tweet 42 Email 45

TWO IRISH FILMMAKERS want to know if thorium could be the answer to the world's energy woes – and they have launched a Kickstarter campaign to raise enough money to finish a documentary on the topic.

Frankie Fenton and Des Kelleher want to raise £40,000 so that they can finish post-production on *The Good Reactor*, their documentary looking into the alternative nuclear fuel and whether it can generate clean, safe energy.



Left: Albert Crewe used a field emission electron microscope to image atoms at the University of Chicago in 1970, proclaiming that he was the first to do this, though tungsten atoms had been imaged by Erwin W. Müller at Pennsylvania State University 15 years earlier. The publicity exaggerated his research, claiming it could help cure cancer. Right: Decades later, in 2013, two Irish documentary producers used the same image to promote thorium as a source of green energy.

an answer to plastic pollution.

My finished book ran to 63,000 words and covered 206 pages. When it was published, *Nonscience* attracted positive reviews. It appeared on national television, and *The Sunday Times* of London wrote excitedly about this book by “a sharp young microscopist.” The youth magazine *Time Out* said it was “a very funny parody,” and even the lengthy review in *Nature* concluded by saying: “It made me laugh and Mr. Ford’s point is worth taking.” It sold well, and was soon published in Spanish, with each tortuous word in the extravagant title faithfully translated. Wolfe had never published a hardback book before, and this was to open up a new enterprise for them. He and I discussed other topics, and I mentioned how many microscopists had 35 mm slide collections that would be popular as reference and teaching resources, and from that small idea the Wolfe Medical Atlas series arose. These published extensive collections of anatomy and histology preparations, including an excellent *Color Atlas of Histological Staining Techniques*. It was this pioneering series that presented microscopical views in book form — in full color — for the first time in publishing history.

My satirical approach remained in popular demand, and a decade later *Nonscience* was extended and reissued as *The Cult of the Expert* (1982). In its German translation it became *Der Experten Kult*. A review on Amazon Deutschland, translated into English, summed it up: “A very socially critical book by a good author with entertaining and informative content!” To my great surprise, the original *Nonscience* became a collector’s item. Copies were soon selling

Nonscience: How to Rule the World
 Brian J. Ford
 Published by Mosby-Wolfe (1971)
 ISBN 10: 0723404496 / ISBN 13: 9780723404491
 (Used | Hardcover)
 Quantity Available: 1
 From: [GlassFrogBooks](#) (Hawthorne, CA, U.S.A.)
 Seller Rating: ★★★★★

US\$ 1,500.00
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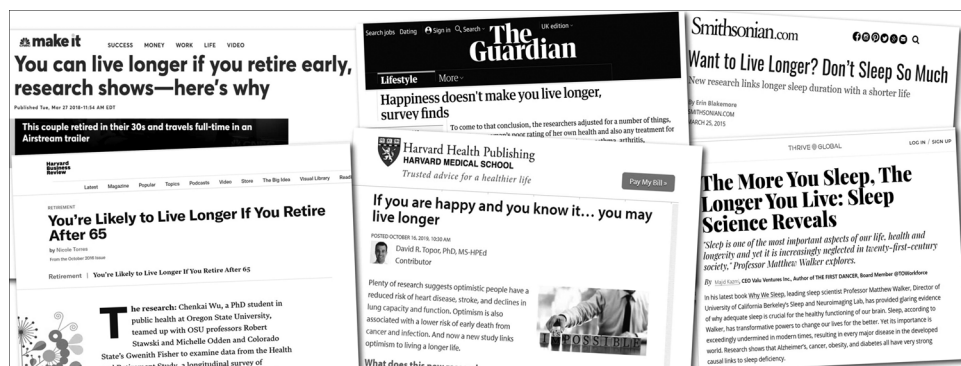
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Sort By:

Reviewers of *Nonscience* found the book amusing and timely. Its excessively complicated title was translated verbatim for the Spanish edition, which was published as a series. The new hardcover cost \$3, and collectors' copies now go for hundreds of dollars. One bookstore was asking \$1,500 (hope you didn't buy it for that price).

for hundreds of dollars and the highest price charged for a good copy was \$1,500 at Glass Frog Books in Hawthorne, CA.

The book continued to attract attention, and on Jan. 21, 2010, *The Daily Telegraph* in London ran a report by Gary Dexter on “How the Book Got Its Name,” explaining that this is “possibly the longest book title in British publishing history.” He added: “Brian J Ford was a scientist working chiefly in microscopy, who was incensed by the obscurantist language of his colleagues: the title was cunningly chosen to furnish an example of the book’s subject.” I wrote to Dexter to say: “Less of the ‘was’, if you please.” He apologized,



Contradictory clinical findings underpin the nature of Nonscience. University of Amsterdam researchers showed early retirement helps you live longer, though Oregon research showed the opposite. Harvard scientists insist happiness matters, but the *The Lancet* ran an article saying it doesn't. The University of California, Berkeley claims that more sleep adds to your years, but Warwick University research found that less is more.

but said that the book had been published so long ago that its author seemed to belong in the past tense. I forgave him, but did pour myself a large bourbon.

Much has happened in the intervening half century since *Nonscience* was written, and the predictions of huge sums of money being extorted by incomprehensible specialists to pursue their own idle aims has all come true. The U.S., being more centered on enterprise and accountability, hasn't suffered as much — but in Europe, where subservience to specialists has long been a cultural tradition, it had boomed. The book had become a memory — until December 2018, when a message arrived from Curtis Press, an enterprising new company, asking about a possible publishing project. My dinosaur book *Too Big to Walk* (2018) had dominated my writing schedule, and I wasn't keen to tackle a new book, but the 50th anniversary of *Nonscience* was approaching. Neil Shuttlewood, the publisher's CEO, was intrigued at the idea of reprinting it and agreed that it would have to be updated. I had kept files of ludicrous examples, collected from disparate sources over the decades, so adding those would be sensible. I had so many examples to cite: one team at Amsterdam University were funded in 2018 to prove that early retirement ensured a longer life, while others from Oregon State University had demonstrated in their 2016 research that retiring late gave the greatest benefit. Other clues to a long life were choice of religion (Protestants live longer than Catholics), being overweight, avoiding vitamin supplements and, best of all (from Exeter University) smelling someone's farts. These reports originated from a real paper, "The synthesis and functional evaluation of a mitochondria-targeted hydrogen sulfide donor" published in *Medicinal Chemistry Communications* in 2014.¹

Yes, there were endless examples to include in an

updated edition. Negotiations were soon completed, and it was agreed that the book would be reborn. *Nonscience Returns* was the title Shuttlewood proposed (I am hopeless at titles), so that was settled. The new book is due out in September 2020. One aspect that I discuss is the lack of public familiarity with the microscopic realm. Understanding living cells and how they behave underpins our existence and dictates how we shall die. They are the single most important aspect of science that people need to understand, yet they don't know anything about them (an observation I published for *Nature* on Dec. 11, 1975, "Microscopic Blind Spots"; doi:10.1038/258469a0). The discovery of the living cell and the microbial universe should be familiar to everyone in the world, but remains a fragment of forgotten information. Yet when microbes were first discovered it was one of the greatest single steps ever taken in the history of science.

MICROBE REVELATION

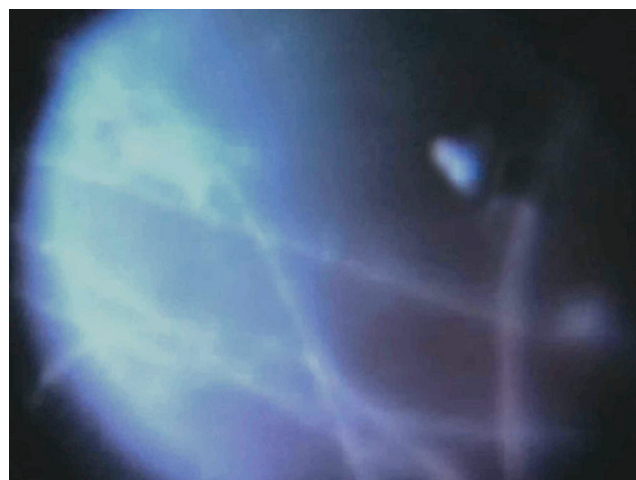
The door to the microbe world was opened on Oct. 9, 1676, by Antony van Leeuwenhoek, when he took a sample of lake water and examined it with a single-lens microscope designed by Robert Hooke in London and made by hand, at home, by Leeuwenhoek. He used descriptive powers that are so precise we can recognize the organisms he observed to this day. He wrote of "green tendrils," like the coils of a distiller's condenser, which are just like the spiral chloroplasts we see in cells of the chlorophyte alga *Spirogyra*. It has recently been suggested that he might have been observing the cyanobacterium *Dolichospermum* (which you will probably know as *Anabaena*), though those spirals are smaller, and he probably wouldn't have resolved them. In my view — after decades of opting for the conventional *Spirogyra* interpretation — I am now inclined to think he was observing *Spirulina magnifica*, a magnificent cyanobacterium with tight spirals very

¹This report is available for free from Exeter University online: www.exeter.ac.uk/news/research/title_393168_en.html.

like those of a distiller's condenser. Leeuwenhoek described rotifers meticulously "with two little legs near the head, and two little fins at the rear end of the body" and writes lyrically about *Euglena*, with its chloroplasts described as "green and glittering little scales." He saw "ashen gray" organisms, a good description of the cytoplasm of protozoa like *Paramecium*. And there were colonial chlorophytes everywhere, "very many small green globules together," he wrote, perhaps recording his first view of *Volvox*.

To this day, that sight of aquatic microorganisms remains one of the most astonishing spectacles in the realm of scientific inquiry. By chance, I am currently engaged in a project that involves taking videomicrographs of tropical zooplankton and the excitement of the sight never pales. The first images that I ever obtained through a simple microscope were published in 1971 (*British Journal of Photography*, 118:5793, pp 682–685, 1971), and by 1998, I had a vast and growing collection of color images that revealed what could be seen through a 17th century lens. They were featured in *Scientific American* ("The Earliest Views," 278:4, pp 50–54, April 1998) and created much international interest.

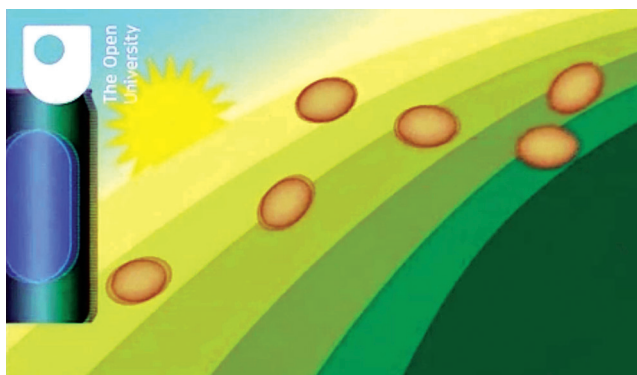
Nobody else had managed this, and producers were increasingly interested in my research. It came up one day when I was with Mark Thompson, the CEO of Channel Four television in London, though life was too busy at the time to take it further. Shortly afterwards, Thompson was appointed to head the BBC and he asked about a proposal for a short series on cells. Although my idea was rejected by their commissioning editor, we soon heard that a BBC science producer was planning a film about Leeuwenhoek. I watched the transmission with eagerness. In spite of their technical excellence and a substantial budget, the BBC were unable to match my results. Instead of beautifully resolved protozoa of the kind Leeuwenhoek had observed and written about so vividly, viewers had to be content with a faint blur in the corner of the screen that the presenter, Adam Rutherford, insisted was a "tiny, tiny microbe" that he could see "scooting about" — we reviewed this kind of patronizing approach in this column 10 years ago (58:3, 2010) and this led to my determination to build a library of videomicrographs of what could truly be seen with these little microscopes, to sit alongside the many still images that I had already obtained. Leeuwenhoek was a great inspiration. Reports of my investigations continued; in 2011, the journal *Nature* published a review generously insisting that I was "the world's leading expert on the topic" ("Early Microscopes Offered a



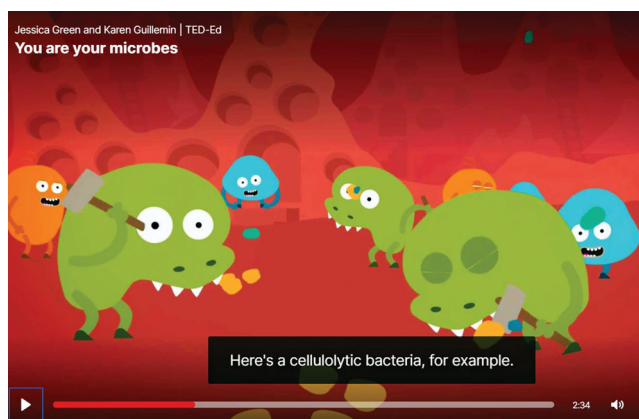
Top: A BBC program on cells had their presenter Adam Rutherford looking excitedly through a replica Leeuwenhoek microscope made by Hans Loenker. "You're looking at the wrong side," he advised Rutherford, who was ignorant of how the instrument was used. "I can see a tiny tiny bug scooting about!" said Rutherford, though only a blurred shape could actually be seen. Bottom: Using a replica Leeuwenhoek microscope lens ground by my colleague Esmond Reid of Cambridge, I recreated the view that Leeuwenhoek could expect to see. Here is *Stylonychia*, 150 μm long, actively swimming near a chlorophyte filament in pondwater. When properly adjusted, these diminutive lenses can create remarkably high-resolution images.

Sharp Vision," *Nature*, March 4, 2011; doi:10.1038/news.2011.116), and on May 20, 2015, *New Scientist* again celebrated my success in capturing images through Leeuwenhoek's own microscope, and many other accounts have since appeared online.

We should remember that Leeuwenhoek's lifetime of work had been triggered by the investigations of Robert Hooke, whose book *Micrographia* he had read when on a visit to London in 1666 and whose life we examined in a previous column five years



Videos of living microbes are rarely used in education. CGI cartoons take their place; they are less demanding than carrying out critical microscopy. This image of living yeast cells is from an Open University series on "Wonders of the Microbe World," which claims to introduce the microscopic realm. These blank oval shapes are a crude distortion of the visceral, enticing *Saccharomyces* cells that we can observe.



Jessica Green and Karen Guillemin created "You Are Your Microbes," a video with plenty of technical talk but no real microbes. Instead, we are treated to grotesque cartoons and a script that lacks literary merit (e.g., "a bacteria"). Would you use *SpongeBob SquarePants* to exemplify human interactions? If you were Green and Guillemin, you probably would.

ago (63:1, 2015). I showed in 1982 that the specimens which Leeuwenhoek had first sent to the Royal Society were a direct response to the published observations of Hooke (*British Medical Journal*, 285, pp 1822–1824). Hooke may not have known it, but he was the pioneer from whom Leeuwenhoek's lifetime of investigation had flowed.

LIFELESS CELLS

Although living cells captivated Leeuwenhoek back in the 17th century, they are rarely glimpsed on television in the 21st, and when they do, they are widely misrepresented (58:3, 2010). These days, when

BBC documentaries mention "living cells" you simply fade to tiny, shapeless blobs that float past like dust particles in the projector beam of a movie theater. As a rule, whenever television features microbial life it appears as computer generated imagery (CGI). The Open University has released a series of videos called "Seven Wonders of the Microbe World," and they start with the yeast *Saccharomyces*. Their editors included a host of diligently researched wall paintings from ancient Egypt, showing how beer was produced in that era. But, when we get to yeast itself, all we see are oval shapes drifting across the screen in a childlike illustration. Of real, visceral, living, juicy yeast there is no sign.

Search for "living cell" on Google Images and almost everything you find are CGI renderings, not real cells. Hit the Videos tab and it is just as bad: endless examples of crazy cartoons, and not a cell in sight. High on the list is the TED talk "Visualizing the Wonder of a Living Cell" by a medical illustrator David Bolinsky, who sets out to reveal the "truth and beauty" within the "living cell" but confuses viewers instead with mechanistic models that look nothing like cells and give no impression of the majesty of life. There is a presentation called "Meet your Microbes" in which Jonathan Eisen ensures that any glimpse of a microbe is absent from the entire talk. Instead, an assistant throws plastic toys into the audience that are as close to real microbes as Mickey Mouse might be to Michelangelo. Finally, let me cite a lecture compiled by Jessica Green and Karen Guillemin called "You Are Your Microbes." The script is fine, as far as it goes: "Here is a cellulocytic bacteria," it says. "Their one job is to break down cellulose ..." Have you noticed the creeping illiteracy that has people using the term "bacteria" as a singular noun? You must have; there are millions of websites using this grammatical collision. However, of the organisms themselves there was no sign. Instead, as you might by now have guessed, the images are cartoons of weird-looking dwarves wielding sledgehammers.

Appearances by real, genuine, voluptuously visceral living microbes are vanishingly rare. I wanted to commemorate this distortion of microscopical reality in my new *Nonscience Returns* book and soon found the perfect example. The last time the BBC included real microbes was in 2013 for a documentary series called "Wonders of Life" hosted by Brian Cox, a physicist who naively believes that you can account for all living phenomena through the Newtonian principles we studied in school. They featured low-magnification movies of *Paramecium*, trying to show

that it worked like a primitive robot that moved by backing off simplistically when it encountered an obstacle. Ciliates are infinitely more sophisticated than this, a fact I had presented at Inter/Micro 75 in Cambridge, England ("Towards a New Microscopy," *The Microscope*, 24:4, pp 295–302, 1976), though our parlous education about the cell meant that Cox didn't know anything about that. And where did he demonstrate these movie clips? In a laboratory, equipped for aquatic microscopy? Perhaps by the pool beneath a wooded glade where *Paramecium* abound? You won't believe where they chose. The production team drove 20 minutes west of Miami into the Everglades, and stopped at the tiny unincorporated settlement of Coopertown, FL, where there is a small restaurant whose specialties include frog's legs, catfish, and gator tail. They set up the presenter near a window, and projected images of *Paramecium* on the café wall, and also on his cheek, the napkin dispenser, and a bottle of Tabasco sauce.

You might have speculated that the microbes projected on the presenter's head would link to an explanation of the cell communities that comprise his cranium, or that the Tabasco was included because of the microscopic lactobacilli and oxidative yeasts whose fermentation create the sauce — but no. Cox insisted that *Paramecium* works like a simple diode, the potential across its cell membrane controlling it like the electricity in a lightning flash. This wanton misrepresentation was included to remind the viewer how simple are microbial cells and how close are those ingenious physicists to their pretense of a "theory of everything" that would allow them to account for life as a few formulae scattered across the page. There has never been a more utterly irrelevant, misleading, or bizarre screening of any microbe anywhere in the history of television. The BBC does not merely avoid microbes but will feature them only when they can be downgraded to childlike interpretations that promulgate the view that they are uncomplicated and easily understood.

It is remarkable to reflect on how hard it has been for people to capture video through simple microscopes. Present-day instruments adjust and focus themselves and rely on push-button simplicity (it cannot be long before they also dispense coffee). When I was researching this field and building up my library of movie sequences showing what Leeuwenhoek saw, Google reminded us that nobody else in the world had ever done so. Capturing images through a single lens is not complex, but it does demand experience, diligence and patience. The remarkable thing about the single-

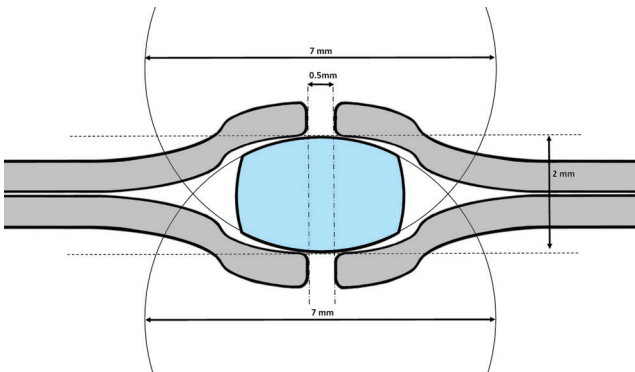
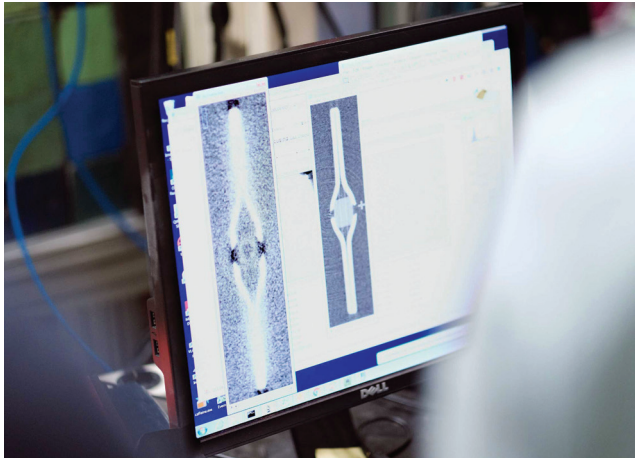


In 2013, the BBC featured microbes in a program presented by Brian Cox, a physicist distinguished by a baffling ignorance of life. They traveled to a small-town café located in Florida's Everglades to project videos of *Paramecium* on a wall, napkin dispenser, and bottle of hot sauce. No documentary in the history of television, anywhere, has ever produced anything as bizarre and misconceived as this.

lensed, simple microscopes that Hooke had designed — and which Leeuwenhoek used throughout his 50 years of microscopical investigation — is the clarity of the image they can generate. So many authorities say that Leeuwenhoek's lenses were spherical beads of glass (search for *leeuwenhoek biconvex lens* and you will find about 10,000 citations; but search instead for *leeuwenhoek bead lens* or *leeuwenhoek spherical lens*, and you find four times as many). Indeed, when the Rijksmuseum Boerhaave in Leiden produced their booklet on Leeuwenhoek (which contained their account of the specimens I had discovered, without acknowledgment) they misleadingly titled it *Beads of Glass*. It was written by Brian Bracegirdle, who never understood the way Leeuwenhoek created his magnificent lenses.

The history of microscopy is one area where academia fails the scholar. Everywhere you look in the field you find error, confusion, misstatement, and frank plagiarism. Almost all the accounts of Brownian Motion say that it was observed in the movement of pollen grains (Google lists over 8,000 websites saying so) whereas, as I have pointed out, it was actually observed in the microscopic particles *within* the grains of pollen (just 116 sites in the world get it right). Virtually every attempt to capture the appearance of microscopical specimens using pre-20th century microscopes results in failure.

Courses on the history of science (like the latest syllabus for the Honor School of Natural Science on the History and Philosophy of Science, which I have just been sent from Oxford University) feature Galileo



Top: The Technical University of Delft, Netherlands used neutron tomography to obtain a cross-sectional view of a Leeuwenhoek microscope from the Boerhaave museum in Leiden. All they did was show the metal plates and the barrel-shaped lens, but there was a cost: the microscope remained radioactive after the scan. Bottom: I decided to capture their low-resolution image from the previous picture, correct its perspective distortion, and refine it to produce a diagram that revealed the dimensions of its curiously shaped lens with its prominent mounting. The lens shows a focal length of 3.67 mm, providing a magnification of 69 \times , close to what the museum was claiming.

and Newton, before moving on to Lyell and Darwin. There is no mention of Hooke, Spallanzani, Leeuwenhoek, or Trembley, the people who created our modern world of bioscientific study. Nothing is said about microscopes, either; your only chance to glimpse one might be during the brief tour planned for the Museum of the History of Science. Throughout this Oxford University course, you won't ever encounter a living cell or marvel at the microscopical magnificence of a microbe. Drop into the Whipple Museum at Cambridge, one of the finest of its kind, and you will find the crudest and most uneducated presentation of images taken through their early microscopes. They are an amiable bunch at the Whipple; their director, Liba Taub, has been a friend for years, and I have no idea

why they don't modernize their approach. I have often worked there, and in 2017, I presented a workshop for them, "Complex Constructs from the Simple Microscope." They liked the subject so much they later invited the Boerhaave museum to mount a presentation with their replica Leeuwenhoek microscopes — yet, when you look into the quality of microscopy at the Whipple, the standards are crushingly low.

RESOLVING LEEUWENHOEK

Turn to the Royal Society, that temple of early modern science, and you will find repeated examples of curious misstatement. Their Leeuwenhoek specialist is the librarian, Keith Moore, who always claims that Leeuwenhoek's lenses were spherical, in spite of all the evidence which proves that they weren't. You may recall the enjoyment we shared when I reported that the Royal Society had signed off the text for their commemorative book *Seeing Further* (2010) by describing Leeuwenhoek's diminutive instruments as "tiny wooden paddles with a little bubble of glass embedded in them." As I pointed out in this column (62:2, 2014), they acted upon my good-humored intervention by removing the word "paddles" — but they substituted it with "dowels," which is even worse.

Some teams claim part of the action by exaggerating their findings. At the Technical University of Delft, neutron tomography has recently been used to obtain a crude sectional view of a Leeuwenhoek microscope from the Boerhaave museum. They issued a press release claiming that this "solved the mystery" of a Leeuwenhoek microscope, by showing the lens had been ground. This claim was something of an overstatement. The technique was known since the 1660s, everyone had always been familiar with the method, and the images were too indistinct to prove the matter one way or the other. We discovered nothing new about Leeuwenhoek's methods from this research, though I used their images to recreate a sectional diagram of the microscope and its lens, which hadn't been done before.

My discovery in 1981 that Leeuwenhoek's original specimens had actually survived for three centuries astonished microscopists and science historians everywhere. It was reported in the international press and was so prominent that it was even featured on the BBC's weekly humorous "News Quiz" program. I had found his specimen packets neatly labeled and hidden amongst his letters (59:1, 2011). I examined small portions of each surviving sample by light and scanning electron microscopy. Some of his dried specimens



The Boerhaave museum, which curiously spells Antony van Leeuwenhoek's first name with an "i," presents slides illustrating his achievements. The illustration of the optic nerve samples they published is taken from one of our original photographs, published without acknowledgment. Historians of science are a refuge for those who seek reality — this kind of misrepresentation is becoming widespread.



Sir Andrew Huxley (right) and I had some far-reaching discussions about Leeuwenhoek. Huxley, a Nobel Prize recipient and pioneering microscopist of great integrity, first proposed that I should work through Leeuwenhoek's letters at the Royal Society.

were reconstituted and restored to life-like appearance — just as they would have been when Leeuwenhoek examined them — and for the first time since his death in 1723, Leeuwenhoek's specimens were reunited with his original hand-made microscope, a unique event in the history of science. Hundreds of published papers and two books, *Single Lens, the Story of the Simple Microscope* (1985) and *The Leeuwenhoek Legacy* (1991) resulted from that research.

Meanwhile, the two previously unknown Leeuwenhoek microscopes that I had authenticated continued to attract widespread interest. Bert Degenaar wanted to keep details of his silver microscope, which I had identified in London in May 2014, close to his chest. I therefore proposed a detailed investigation,

with analysis of its composition, construction, and optical performance, which Degenaar did not want to pursue, though he did allow the Boerhaave museum to take some close-up photographs. Seven months later the other unknown Leeuwenhoek microscope came to light, when it was privately purchased by a Spanish microscope collector, Tomás Camacho, who terminated its sale on eBay by approaching the seller personally. Fortunately for scholarship, Camacho was much more interested in finding out as much as he could and sent it over by courier, with no end date for its return. At Cambridge university, I was able to utilize the scanning electron microscopes at the Cavendish laboratory to reveal that the instrument was of crude 17th century construction, with nothing in common with the present-day methods used to manufacture replicas. By the time I had examined the many details of this extraordinary little artifact, I had published about 30 more presentations and papers. All of this had stemmed from my original work on the Leeuwenhoek letters at the Royal Society, to which Sir Andrew Huxley had introduced me in 1981, when he was the Society's president. Since then, whenever there had been an exhibition, the Society had requested copies of my original photographs or sought advice on how the material might best be explained, and due acknowledgment was always given.

That was in the old days. Now, in the modern world, integrity counts for little. In a publication of the Boerhaave museum appears a plagiarized photographic study of Leeuwenhoek's slices of bovine optic nerve, which I had taken in 1981.

Getty Images is selling a still from the news report featuring my investigations of the Camacho microscope, without any explanation. Fernando Colonna



Brady Haran (right) interviews Keith Moore about the Leeuwenhoek research that I had conducted at the Royal Society. Moore, who is the Society librarian, is an amiable and obliging character, with gray locks and professorial eyeglasses. But Haran's questions led him to reveal his lamentable lack of knowledge of Leeuwenhoek, and Haran confessed he found it all "way more boring."

Rosman shows my historic micrograph of my own blood cells, imaged through a Leeuwenhoek microscope, and credits it to the University of Utrecht. The Linda Hall Library in Kansas City features one of the photographs I painstakingly captured, though saying nothing of its origins, and one of my most historic images, of living bacteria, which I succeeded in photographing through a single-lensed, Leeuwenhoek-type of microscope for the first time in history, is available from the Kelly Carrier website, credited to Ms. Carrier. Don't think too badly of her — the best people do it. If you look on the Royal Society website for Leeuwenhoek material you will see this same historic image, with the credit this time claimed by the Royal Society (see image on page 33).

In November 2018, that Society decided to promote Leeuwenhoek's rediscovered specimens in a video, with Moore (their librarian) showing the specimen packets to an interviewer named Brady Haran. When I had discovered the specimens in 1981, I had selected small portions of each one for microscopical examination, leaving the bulk of the material uncontaminated for future scientists to analyze with methods yet unknown. To my surprise, in the video the closed packets were being opened and the specimens within turned over casually, fully exposed to every contaminant. The Society had always said that they would keep them safe and sealed, and that they would never be inspected without my being consulted. That was now disregarded, and Moore remarked:

"This is so much more interesting, surely," only to be told: "No it's not, it's way, way more boring." Haran, the interviewer, turned to the annotation I had typed at the time and read it, saying: "This looks like some stuff science was done on in the 1980s." Moore agreed, adding: "That's right; the specimens have been looked at, in modern times of course, partly to see what it was possible for Leeuwenhoek to see using contemporary technology." The interviewer nodded. "Ah, so it was like, you know, 300 years later, what can we do. Wow." He thought for a moment, and said that the idea of seeing some of Leeuwenhoek's own specimens had originally made him "a little bit excited." Moore asked him: "And now?"

"Less so," was the reply. "Still liked it — but not a show-stopper." This was such a misrepresentation of Leeuwenhoek's work, and the sensational revelations his meticulously prepared specimens had offered. This film may be found on YouTube in a series that the Royal Society, with no hint of irony, calls "Objectivity" (search for "Lost Microscopes – Objectivity #188").

With his graying locks and abstracted manner, Moore — a man I like and with whom I have cooperated for decades — provided the image of a knowledgeable academic, yet he knows little about the subject and was similarly dismissive of the Leeuwenhoek microscope that had been found in landfill mud from the bottom of a Delft canal. On national television, its announcement in *Nature* had been greeted as a sensational discovery; but by the time it featured in the Royal Society's "Objective" video it was very different. "Every time they dredge a canal in Holland," mused Moore, "they tend to find one of these things. Leeuwenhoek seems to have dropped an awful lot into canals." That is an extraordinary way to misrepresent reality, and provides a noteworthy case study for scholars.

HIJACKED HISTORY

The president of the Royal Society is himself interested in microscopy, and we had been in contact by email about my Leeuwenhoek investigations. Although I have known all the Royal Society's presidents since the 1970s, and had met one or two before that, I had yet to meet the current one, Venkatraman Ramakrishnan. He was born in Tamil Nadu, India, and began studying ribosomes at Yale. In 2009, along with Thomas A. Steitz and Ada Yonath, he was awarded a joint Nobel Prize in Chemistry for elucidating the atomic structure of the 30S ribosomal subunit employed in organizing chromatin. We had hoped to

meet at the Society's 2020 Soirée, but both of us were called away and it hadn't come to pass. Ramakrishnan wrote to say he hoped we might meet, and a date was soon arranged. Leeuwenhoek was the topic in which he was most interested. I had been casting around for a tactful way to mention the repeated infelicities that had been heaped upon Leeuwenhoek's reputation, but to my surprise, Moore joined the two of us, and I did not want to be personally critical about his performance. Instead, the president (who had received my Leeuwenhoek bibliography several weeks earlier) was keen to know which of my papers I would recommend him to consult, because he said he was so interested in my work. We then discussed the possibility of my giving a brief presentation about Leeuwenhoek's discoveries to mark the 300th anniversary of his death in 1723. The president thought this might make a neat introduction to a longer lecture given by a Fellow. Moore asked whether I was still carrying out research on the microscopes, and I told him that all our findings had been published in full. He added: "I shall be going over to Leiden shortly to see the microscopes at the Boerhaave museum." Cordially, as you do, I asked him to give the Boerhaave staff my best wishes.

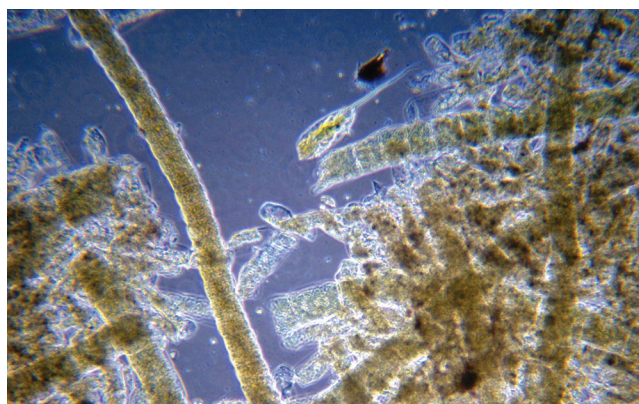
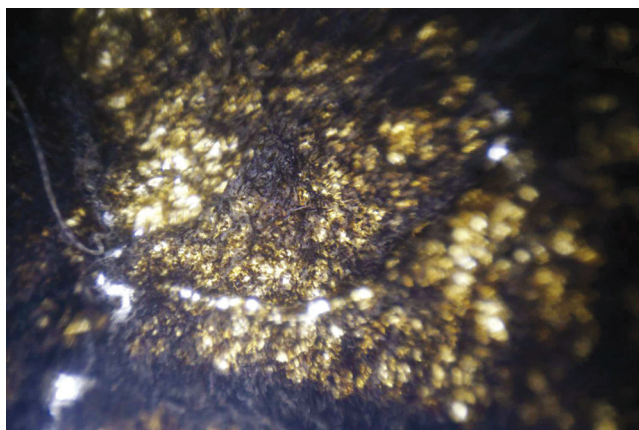
The president wrote to me next day saying how much he had enjoyed our meeting, and added that he had raised the forthcoming anniversary with the officers. By that time, he would have finished his term in office. On reflection, I was not keen on a short introductory lecture about Leeuwenhoek. I had given a presentation on my early research at the Royal Society in October 2010 and had to work hard to fit the subject into an hour, so I responded by saying: "I would not want the Society to be thought of as consigning the subject to an abbreviated time slot." It was agreed that the idea would be discussed when the new president had been installed — and there the matter rested. I was left wondering how I could give a positive presentation to a crowded meeting of the Royal Society when it would inevitably highlight how they were misrepresenting Leeuwenhoek. However, matters were shortly to deteriorate even further — the insidious specter of Nonscience was about to rear its head.

On Oct. 16, 2019, I heard that a press release was being sent out to the scientific media. The signatories were a distinguished group of academic institutes: the Rijksmuseum Boerhaave, the University of Cambridge, and the Max Planck Institute — spearheaded by the Royal Society. Its opening words were unambiguous. "What may be the earliest surviving objects seen by a microscope — specimens prepared and viewed by the early Dutch naturalist Antoni van Leeu-

wenhoek — have been reunited with one of his original microscopes," it began. This event gave science historians "the opportunity for taking photographs through the original microscope," it continued, promising to recapture the "look" of 17th century science with "stunning high-resolution color photographs for the first time."

Microscopists everywhere were stunned. The journal editors to whom it was sent declined to publish it as it stood. The comments I received were unanimous: this was an amateurish attempt to hijack a major landmark in the history of science. Everybody was aware that those earliest microscope specimens had been reunited with an original Leeuwenhoek microscope almost 40 years earlier, and there was nobody in the field who didn't know the story. The idea that these were the first high-resolution color photographs was widely known to be absurd: my pioneering micrographs had been on film, which has a resolution far higher than that of modern digital media. In 2001, our pictures had been digitized, allowing the images to be optimized so that they looked as they had appeared originally. Recreating the "look" of the specimens Leeuwenhoek had studied had been achieved in 1981, and had not been repeated by anyone else since. Even so, Amito Haarhuis, director of the Boerhaave said: "We are finally able to see in full detail what van Leeuwenhoek might have seen 350 years ago. We couldn't be more excited!" Added Moore: "Our first color views of the sections cut by Leeuwenhoek's razor, with the lens made by the same hand, was a heart-stopping moment." No wonder he and the Royal Society's president had been so intent on learning about my current interests on Leeuwenhoek. The calumny of these curious claims was strange enough; and this fiction was being touted around the media by some of the greatest institutions in science.

The research was published online by Sietske Fransen, who was based at the university of Cambridge Centre for Research in the Arts, Social Sciences and Humanities, under Professor Sachiko Kusukawa. Her department is known by the curiously applicable acronym of CRASSH (as one colleague said to me in Cambridge: "CAR CRASSH would have been even better") which was established in 2001 to build bridges between art and science. That was a field I had pioneered in 1967 at the Newport College of Art, now part of Cardiff University, and led later to my book *Images of Science, a History of Scientific Illustration* (1992). Similar wording was used when the new press release was published on the websites of the Max Planck Institute, the Royal Society, and the Boerhaave museum.



Top: The Boerhaave museum released this image of a dried algal film that Leeuwenhoek had sent to London in 1687. They stated that they had obtained this view through a Leeuwenhoek lens, but little microscopical detail can be discerned. In the 1981 research, these films had been imaged with a scanning electron microscope, and the specimens had then been reconstituted to reveal hidden details. Bottom: After reconstitution, the specimens were restored to the condition in which Leeuwenhoek would have examined them. Among the chlorophyte filaments, several rotifers were observed (these were among the first aquatic microbes that Leeuwenhoek described, and they are mentioned in his account from 1674). I could still identify the rotifer *Trichocerca cristata* after almost three centuries.

What compounded the affair was that, though none of the other sites gave an acknowledgment, Fransen had conceded my precedent by citing: "Brian J. Ford, 'The Van Leeuwenhoek Specimens,' *Notes and Records of the Royal Society*, 36, pp 37–59, 1981." Fransen's reference set it out in full.

DISTORTED VIEWS

Perhaps because a digital camera has been used, the micrographs that appear online look strange. They do not have the typical appearance of the view through a single-lensed microscope. Whenever an im-

age is taken with an instrument of that sort, the periphery of the picture always reveals the image of the aperture holding the lens. Even with the blurry ghost of an organism seen through a replica microscope, which the BBC managed to capture (see top image on page 37), you can see the curved outline. It is always visible. Yet in the pictures taken by Wim van Egmond for the Boerhaave museum there is simple vignetting, which is what you usually observe if a macro lens is improperly adjusted. No doubt there is an explanation, but nothing has been published on the methods involved. When I took the first historic photographs back in 1981, I designed an optical stand, which meant there was no ancillary camera lens in use. The only thing between the specimen and the film emulsion was the Leeuwenhoek lens itself. This captured a pure and direct image at full resolution; with a camera lens in between, the photograph might have been subtly compromised.

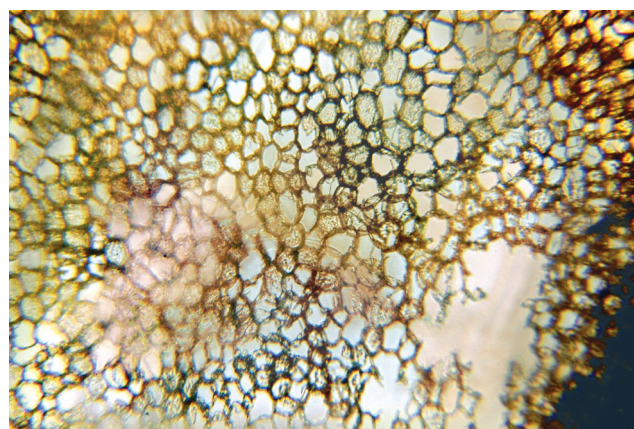
Colleagues reminded me that the first line of intervention is to contact those responsible, so I sent emails to the heads of each department. Not one replied. I emailed the president at the Royal Society, but he suddenly became uncommunicative. Having known Keith Moore since the 1980s, I contacted him personally and he explained that this was the culmination of a project called "Making Visible," which had been set up with a \$1,000,000 grant from the Arts and Humanities Research Council (AHRC). The project had been set up to catalogue the images in the collection of the Royal Society, though none of us could see why such a huge sum of money was necessary. Microscopy was a part of this, and the project embraced the re-creation of Leeuwenhoek's view of nature. Research into the early days of microscopy is always welcome (there is far too little of it around) though I have to say that it is probably better to undertake something original, rather than pretend that somebody else's well-known ideas were now yours. Moore insisted: "The press release was very carefully written and agreed with all parties." He added: "There was nothing untruthful about it." This was a clear breach of the conventions of scholarship, but was it plagiarism? Wikipedia offers this definition: "paraphrasing someone else's original idea without citation." I consulted Cambridge University, to find that their statement is unequivocal: plagiarism is defined as "using someone else's ideas, words, data, or other material produced by them without acknowledgement," while the University of Oxford sets it out in a modern context: "Plagiarism is presenting someone else's work or ideas as your own ... by incorporating it into your work without full

acknowledgement. All published and unpublished material, whether in manuscript, printed or electronic form, is covered under this definition.” Plenty of ticked boxes there.

When this happens, it provides yet more memorable material for articles (like this) and offers much new content for my lectures. Without people generously providing me with this kind of first-rate material, I might be hard-pressed to select a suitable subject. The new publication of *Nonscience Returns* includes this episode, of course, as it’s a classic. That book may be satire (well, I know it made the publisher’s editor laugh aloud), but there is a serious message here: we are facing an era where we are being bamboozled by authority, and integrity is regarded as an outdated handicap, rather than a crucial prerequisite.

As I stated in an article for this journal, plagiarism is a compliment (58:1, 2010). It shows that people who plagiarize regard your work far higher than their own. It also shows that, when they cannot think of anything to do, it was your insight they misappropriated. This latest episode is an exemplar of our new age when the integrity of original inquiry is banished, and opportunistic connivance takes its place. That is the hallmark of *Nonscience*, along with acquisition of huge sums of unaccountable money. I know that the CRASSH project catalogued the 400-odd pictures published in the first 50 years of *Philosophical Transactions*, and I see that Fransen has published a catalogue of the 200-odd drawings from Leeuwenhoek’s writings. Her paper is not scholarly — there is no attempt to identify the details in the images or to account for how they were obtained or whether they are accurate — it is little more than a list. There is also a handful of still images and a few minutes of video from the Leeuwenhoek samples taken at the Boerhaave museum, a good afternoon’s work, though I am not sure that this can explain where all of the million dollars went. My own modest research resulted in some 3,000 black and white negatives and 1,000 color slides, with approximately 100 videos taken with simple microscopes running to about 10 hours playing time, and the revelation that there are two unknown Leeuwenhoek microscopes, an extensive program of research published in a bibliography of over 400 items. In all, it cost about \$30,000 — say, 300 times as many results for one-thirtieth of the money.

How can we summarize? Studying the history of science, at university level, is dead. There is no attempt by its practitioners to keep up to date, and recycling a 1930s curriculum satisfies everyone because



Top: This frame is said to show the appearance of a Leeuwenhoek cork section under an original microscope at the Boerhaave museum. It is curiously dissimilar to conventional micrographs taken with these instruments, because neither spherical nor chromatic aberration are seen, as one might expect. There is no sign of the lens periphery, which should always be seen. Bottom: Leeuwenhoek’s ability to cut fine section material is evident from this micrograph of his cork specimen. To capture this image, I used the Leeuwenhoek microscope at Utrecht University, with its higher magnification and superior resolution. The lens periphery is clear (lower right corner) and the effect of aberration, which is clearly evident, does little to detract from the overall image.

students know no better. There is little need for people to understand their instrumentation, to know about the most basic facts, to conduct honest inquiry or even to act with integrity. Research projects are easy to find — just appropriate someone else’s and claim it as your own. Then you can acquire seven-figure sums from authorities too ill-informed to know what you’re up to and too busy to check up on what you did. I know what you’re thinking — this is how the new *Nonscience Returns* is being written. Brian is being satirical.

But is he? ■

F. Donald Bloss

1920 – 2020

Dean Golemis

Senior Managing Editor, *The Microscope*

F Donald Bloss, widely regarded as the father of modern optical mineralogy, died on April 22, 2020, just over a month before his 100th birthday, in Blacksburg, VA. A prolific author, esteemed instructor, and innovator of the detent spindle stage for the polarized light microscope, Dr. Bloss inspired generations of mineralogists, geologists, and microscopists.

Dr. Bloss was a longtime professor in the department of geosciences at Virginia Tech University and is renowned for his extensive research in optical mineralogy and crystallography. His three textbooks — *An Introduction to the Methods of Optical Crystallography* (1961), *Crystallography and Crystal Chemistry* (1971), and *Optical Crystallography Simplified, The Spindle Stage: Principles and Practice* (1981) — are regarded as classics in their field and are still used by researchers and students today. (The books are available from the Mineralogical Society of America, minsocam.org.)

He pioneered analytical techniques that employ the detent spindle stage, a single-axis rotation device used on the polarized light microscope for identifying and characterizing single crystals and for determining a crystal's indicatrix. In 1973, Bloss teamed with Virginia Tech mathematics professor Dean Riess to create EXCALIBUR, a computer program that solves optical extinction data with the spindle stage and



Montage image from the cover of *The Microscope* special issue honoring the work of F. Donald Bloss (Vol. 40:1, 1992).

determines the optic axial angle $2V$ and the orientation of a crystal's optical indicatrix. EXCALIBUR has since been revised and updated several times, including the Microsoft Windows-compatible EXCALIBRW, and most recently, the spreadsheet-based EXCELIBR.

Dr. Bloss championed polarized light microscopy in geology and other microanalytical fields, which drew the praise of Dr. Walter C. McCrone in a special issue of *The Microscope* honoring Dr. Bloss's research (Vol. 40:1, 1992): "No one, this century, has done more than Don Bloss for optical crystallography and mineralogy. Most mineralogists today are abandoning the polarized light microscope for SEM/EDS and other microprobe instruments. Don continues to

demonstrate how much more direct, more precise and more complete, mineral characterization and identification is with an instrument that costs a tenth as much..."

Fred Donald Bloss was born in Chicago on May 30, 1920. As a youngster, he developed an affinity for writing and had his poetry published in the *Chicago Daily News*. He entered the University of Chicago as an English major but soon switched to geology after hearing a lecture by noted geologist J Harlen Bretz.

He served three years in World War II and then returned to the U. of C. to earn his B.S. (1947), M.S. (1949), and Ph.D. (1951). Dr. Bloss then launched his

distinguished teaching career, serving as a professor of geology at the University of Tennessee (1951–57), Southern Illinois University (1957–67), and Virginia Tech (1967–1991). In 1972, he was appointed Virginia Tech's first Alumni Distinguished Professor, a position he held for 20 years.

Dr. Bloss also played prominent roles in the Mineralogical Society of America. From 1972 to 1975 he was the executive editor of MSA's *American Mineralogist* journal and also served as the society's vice president (1976) and president (1977).

He won numerous accolades for his contributions to microscopy. Among them were the Award of Merit and Honor by the State Microscopical Society of Illinois (1979) and the Ernst Abbe Memorial Award from the New York Microscopical Society (1988). In 1981–82, the University of New Mexico selected him to occupy the State of New Mexico's first endowed chair, the Caswell Silver Distinguished Visiting Professor of Geology. From 1988 to 1990, he served as Chairman of the Department of Geological Sciences at Virginia Tech. Blossite, a mineral of copper vanadium oxide, was named in his honor in 1987.

Dr. Bloss came out of retirement in 2003 to teach a three-day course in spindle stage techniques at McCrone Research Institute in Chicago, along with his protégés Mickey Gunter, Bryan Bandli, Shu-Chun Su, and Robert Weaver, who hosted 14 students. The course also covered EXCALIBUR/EXCALIBURW and how to make your own spindle stage.

Beyond the lab and classroom, Dr. Bloss excelled in chess and also published a biography of Mark Twain. He is survived by his wife of 74 years, Louise, and their three daughters: Terry Kensler, Janet Shuff, and Jill Bloss.

Mickey Gunter, Dr. Bloss's last Ph.D. student, an Emeritus University Distinguished Professor at the University of Idaho, reflected: "While Don is no longer physically with us, his legacy will live on with his books, his research publications, and those of us fortunate enough to have had him as a mentor, colleague, and friend. But what's overlooked by those who never interacted personally with him is his wit — e.g., he once wanted to title a paper on Biotite, '2V or not 2V.' Don also was very compassionate, once explaining to me how he 'herded' a bunch of newborn turtles from the beach into the ocean. Thus, he showed there's lots more to being a famous scientist than just science!"

As Don Halterman, a mineralogist, said in an on-line message board after Dr. Bloss's death: "He called his students his children, and I have tried for the last 20 years to make him proud." ■

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Dehydroacetic Acid

Mel Pollinger, New York Microscopical Society

Dehydroacetic acid, quickly melted under a coverslip and slow cooled for 2 hours under a 3 oz. brass weight; Rheinberg illumination and polarized light. (Courtesy of the *NYMS Newsletter*, October 2019)

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