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Webinar Transcript

What is Scientific Imaging, and Why is it Special?

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PRESENTER: Joseph G. Barabe

Joe is the Director and Senior Research Microscopist at Barabe & Associates LLC. He served McCrone Associates for 24 years as a Senior Research Microscopist and Director of Scientific Imaging where his analytical duties included analyzing art objects such as paintings and prints, ancient documents and historical and archeological artifacts using analytical tools including polarized light microscopy, SEM with X-ray spectrometry, infrared and Raman spectroscopy and other analytical methods.

Introduction

My name is Charles Zona and I would like to welcome everyone to today's McCrone Group webinar. Our presenter is Joe Barabe of Barabe and Associates. Joe is going to talk to us about What is Scientific Imaging and Why is it Special?

Before we get started, I would like to tell you a little bit about Joe's background and experience. Joe worked for more than 24 years at McCrone Associates as a Senior Research Microscopist and Director of Scientific Imaging. His analytical duties included the analysis of art objects, such as paintings and prints, ancient documents, and historical and archeological artifacts. He has used a variety of analytical tools to analyze these specimens, including polarized light microscopy, scanning electron microscopy with X-ray spectrometry, infrared and Raman spectroscopy, and other analytical methods as appropriate. He has performed forensic document examinations, including forged and altered documents; paper, ink and toner comparisons; writing sequence determinations, and printing process identification. His scientific imaging specialties include photomacrography and photomicrography and invisible radiation photography.

Joe also teaches courses at Hooke College of Applied Sciences, including Pigment Identification, Photomicrography and Scientific Imaging, and Printing Process Identification.

After the presentation, Joe will field questions from the audience.

This webinar is being recorded and will be available on The McCrone Group website. And now I will hand the program over to Joe.

Joseph Barabe (JB): Thank you, Chuck for your kind introduction. Good afternoon all, and welcome to the Hooke College of Applied Sciences webinar "What is Scientific Imaging, and Why is it Special?" I'm Joe Barabe, and it'll be my pleasure to share some thoughts on this subject with you today.

Just how this topic was chosen may be of some interest. Some months ago, the Hooke folks asked me to develop a workshop on photomicrography. After considerable discussions, we decided to enlarge the topic a bit and call it Photomicrography and Scientific Imaging. In this Hooke

Joe Barabe shows students how to set up axial transillumation for photomacrography.

College of Applied Sciences class the emphasis will be on photomicrography, which is photography through the microscope, but we will also include photomacrography, special subject illumination, and other scientific imaging techniques.

I should mention that before coming to McCrone, I was a medical photographer for 10 years, and previous to that I was a commercial photographer. At one point I had a bit of an epiphany: I came across this short publication by Kodak called "Photomacrography" by H. Lou Gibson, and after reading it, I felt called to make photomacrography and microscopy my career direction. I did that, and it was a good fit.

So, what do we mean by scientific imaging? How does it differ from conventional photography?

Conventional photography covers a lot of ground, from casual snapshots to professional wedding and portrait photography, commercial catalog and advertising, fine art, and the like. With the invention of smartphone cameras, photographs have become an integral part of our daily lives. Not only are we recording significant moments with our phones, we're also using our phones to gather information; and this leads us to what scientific imaging is all about: information.

Scientific images are data. Scientific images are produced within a scientific discipline for a scientific purpose. The subject matter of the image is, essentially, scientific. It may be a picture of an object, such as the ink well from an archaeological site, or a photomicrograph of a cross section taken from that inkwell.

In addition to scientific subject matter, the images may involve scientific instrumentation, such as microscopes, telescopes, or other instruments. Even if done for aesthetic reasons, such as photomicrographs, they still qualify as scientific images. The imaging energy can be photons, such as with the light microscope, or electrons, as in the scanning electron microscope, or in ultrasound waves, which have given expectant parents such joy to see their developing child!

Some of the fields utilizing this technique include medicine, biology, astronomy, archaeology, paleontology, mineralogy, and geology—the list goes on. My own experience is in medicine, industrial and forensic microscopy, and in art, documents, and historical objects conservation. We'll look at examples from a number of applications. Let's take another look at those inkwell images. The challenge was to determine whether this first century inkwell was bone or degraded ivory. The cross section image indicates that the Haversian canals are rounded, not flat, thus confirming bone.

Also, a scientific image is all about information or visual data. Nevertheless, scientific imaging does have its own aesthetic criteria of a sort, and within each scientific field certain criteria apply as to what an image should include and how it should be made.

The visual data represented in the photomicrograph was important in the analysis of this Benin bronze casting. The thick red cuprite corrosion layer revealed through a polished and etched cross section was key to determining the object's probable authenticity. Note also that imaging is only one of many analytical techniques employed in the analysis.

The aesthetic properties guiding scientific images differ somewhat from those we employ in creating conventional photographs. The entire purpose is to present the data with unambiguous clarity; no fancy compositions—just the subject matter centered within the field on a clear background. What is permissible, however, are any standards that might add information to the image—in this case, the scale, which was photographed with the subject.

This documentation of a ricocheting bullet exemplifies many of the characteristics of a well-made scientific image in the non-medical realm. It includes a scale for size—in this case, the scale was included in the original image. Also, the subject was placed on translucent plastic, which was trans-illuminated sufficiently to eliminate any distracting shadows. The subject was illuminated to show the morphology of the bullet and the specific striations resulting from the ricochet. The subject occupies almost the entire frame and all the lines are straight. This was photographed on 4" x 5" film with a high-quality macro lens; thus, it is of high informational value and is an attractive image.

Scientific images should be of high informational value. The photographic equipment should be of the highest quality affordable for the laboratory. A professional quality FX format camera and high-quality macro lenses might be one approach. Each specialty has its own criteria.

As mentioned above, scales are often used to communicate object size and magnification. Let's take a few minutes to look at a few that I use in my own work. Other disciplines will use others.

The choice of a scale will often be dictated by your application. These short, inexpensive rulers actually provide a lot of useful information, and are widely used in medical, forensic, and industrial applications. It provides rulers in both centimeters and inches, a neutral white or gray for color balance and density, and crossed circles for photogrammetric use.

In recent years, the X-Rite ColorChecker has found a home in my camera bag. While not as compact, it includes a huge amount of information. In addition to a scale in centimeters, it includes a six-swatch gray scale for tonal values, and CMY, RGB and other color references. The calibrated light-gray field is perfect for creating a custom white balancing in the field.

For photographing cultural artifacts, such as paintings, sculptures and historical objects, the American Institute for Conservation PhD targets are invaluable. They include scales for size in both centimeters and inches, a six-swatch gray scale, blue-green-red and yellow-magenta-cyan color references, and a shadow pin with photometric arc indicators. It is also well-suited for ultraviolet fluorescence photography, and includes a magnetic plate, unseen but off to the right in the illustration, for insertion of date and other artifact information.

The X-Rite ColorChecker.

In the case of microscopy, digital photography has a major advantage over film: it is easy to create scale bars for insertion into the image. For this, we must calibrate our microscopes to the software we are using or our photomicrographic method. For this, we need a size standard: a stage micrometer. The one I have is microphotographically etched; a 1 mm ruler with 100 subdivisions, each 10 µm in length (one thousand micrometers = one millimeter). Using this standard, we can calibrate our microscopes and create a scale bar for each objective, and even photo eyepieces if available. The scale bar included in this paint cross section was created by calibrating my microscope to the stage micrometer image and placed into the image using Adobe Photoshop.

Medical photography and imaging, including X-radiography and sonography, has a long history. Protocols have been established for poses and camera positions; even illumination. As an example, virtually all standard cranial photography is positioned by the Frankfort plane; this allows comparisons over time. The Frankfort plane is defined as a line from the top of the tragus of the ear to the top of the infraorbital ridge.

A good example of this is in this eye motility series. This can be repeated time after time, allowing the physician to see the progression of the condition or the effectiveness of the treatments. Sticking with such a protocol enables repeatability. Another important application of this method includes before and after cosmetic surgery documentation.

This image here looks like a conventional photomicrograph, but it's not. This is from a photomacrographic series of images of a pediatric heart defect taken at about 3X magnification, too low for use with the compound microscope. It was made with a simple microscope: a single objective lens on a bellows camera. A condenser transilluminator was used to provide maximum contrast and resolution. It was one of a series of slices; the resulting transparencies all had to be exactly the same magnification and exposure, and in roughly the same position. This was a difficult project, demanding precise alignment of the optics and exacting calibration before photographing the entire series.

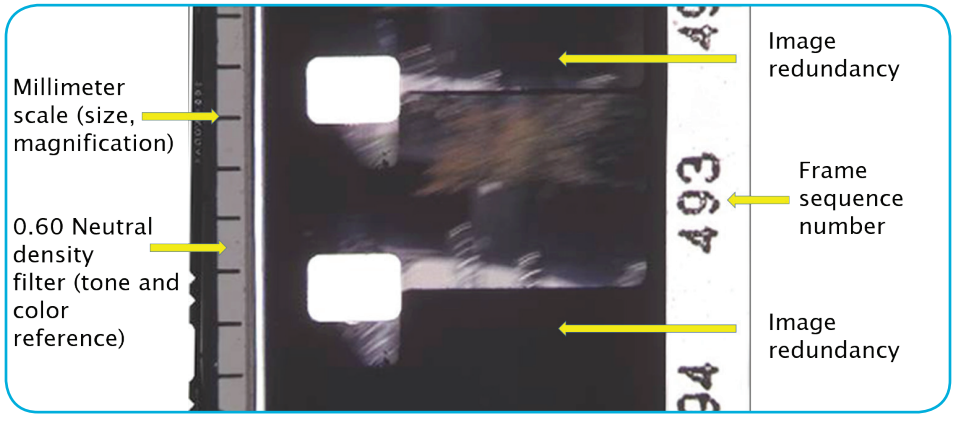
Another somewhat exotic application comes from the field of forensic document examination. This is not my project, but it illustrates the value of infrared luminescence to detect alterations in a document.

A less exotic example from the industrial quality control world is this use of coaxial reflected illumination to document a printing defect. This image confirms that the source of the defect was a droplet, probably water, that led to a series of events resulting in the swelling and bursting through the ink layer. Coaxial illumination—this is reflecting coaxial illumination—provides excellent information regarding surface irregularities.

Perhaps my most famous project was helping to create an image of every frame of Abraham Zapruder's 8 mm film of the assassination of President John F. Kennedy. These images were used to reconstruct a complete film of the assassination, including slow motion and enlarged portions that could be conveniently analyzed.

Here was the strategy:

- Photograph every frame in its entirety, from sprocket holes to the edges, including all of the information, including those around the sprocket holes, using 4 x 5 inch film designed for transparency duplication.
- Three sheets of film were made of each image.
- Include a millimeter scale in each image.
- Include a standard for color balance and density; in this case, a 0.60 Wratten neutral density filter was placed in every frame.
- Include a sequence number within each image so we knew exactly where we were on the film itself.



Frames of the Zapruder film.

- Also, include the visual information on both sides of the frame (image redundancy). In case a frame was missed, the information could be duplicated from these redundant images.

This strategy would be useful today, but a high-quality digital approach would more likely be employed. In 1996, though, sufficient quality was not available, and also, the ease with which digital can be altered was a significant issue in our planning.

The next images are from a recent project from my own laboratory. In addition to analyzing the paints in the painting, the painting was fully documented photographically, including invisible radiation imagery. The work was originally photographed with balanced illumination at a 1:1 contrast ratio for even lighting. The varnish on the painting was pooled in many areas and highly reflective, so the painting was also photographed with crossed polarized light.

Crossed polarized light does result in an overly-contrasty image, so it should only be used as an alternate to the standard image, but here it made the painting elements a little more visible. The effectiveness of crossed polarized light is most obviously effective in the signature, almost unreadable in ordinary light, but notice how minimizing the reflections with crossed polarized illumination makes this signature much more readable.

Photographing it with ultraviolet illumination, being careful to absorb the reflected UV by use of an ultraviolet absorbing filter (a Wratten 2H in this case) allows us to see and record only the fluorescence, which would show us recent restorations as dark spots. None in these particular images, but note the fluorescent portions from the AIC PhD Reference. This was all part of the scale's design. You can see in the scale the white lines and the orange lines are ultraviolet fluorescing, and that provides further information on this image.

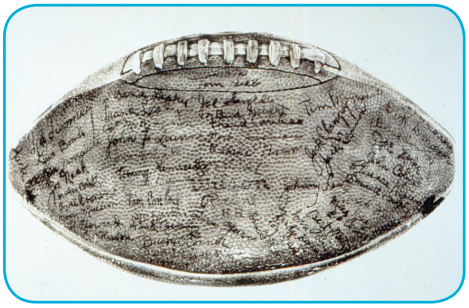
Photographing the painting with a camera modified for infrared photography allows us to see up to 950 nm. Infrared radiation penetrates many paint films, allowing us to see pentamenti and other ways the painting looked while being transformed into the painted surface we see today. Notice how different the figures appear in this view. Here's the regular view again.

X-rays are also high-energy, very short-wave electromagnetic radiation and provide information about the density of the paint pigments. We can see the great difference between the lead white seen in the very light areas of the painting, and the zinc white, with much lower densities. In this view, the horse, painted with zinc white, has disappeared. Note also a change in the position of the sun or moon.

Finally, a great deal can be learned about an artist's technique and materials by examining a cross section of the paint layers. In this painting, raking light illumination provides information about the texture of the painted surface, and in this case, shows that the painting was altered: the figure's right shoulder was originally draped, then painted over. This was confirmed in the X-ray of the painting.

Just for fun: why might we classify a photo of a signed football as a scientific image? Perhaps when that football is a historical object, and the project assignment included restoring the flattened football, identifying all of the signatures on the ball, and determining the year all of those players were on the team. Also, the object presented several photographic challenges. The signatures were made more readable by photographing the object with Tech Pan (Technical Pan, a high contrast film), with a Wratten 25 red filter to lighten the ball, and using crossed polarized light to manage reflections, which otherwise would have obscured the signatures. It turned out that the ball was signed by Knute Rockne on the other side of the lacing, and that the names coincided with his Notre Dame team, the 1929 National Champions.

Finally, we should note that scientific imaging practices can be used to create images in which the aesthetic value is as important as the information gathered, or at least of some importance. The cover illustration was



Notre Dame football.

produced as a fusion preparation of metol and hydroquinone, two photographic developing agents, with a polarizing light microscope, using crossed polarized light with a first order compensator.

We mentioned that camera phones are ubiquitous. When I purchased my own, my very first image was through the microscope, of one of my favorite pigments, emerald green. I use it on my business card.

Thank you so much for your attention, and I invite your questions.

CZ: Thanks, Joe. Thanks again to everyone for attending today's webinar. If you have any questions, please go ahead and type them into the questions field.

Let's start in on the questions here. We have one from Ken: "Do you ever use photo stacking for increasing depth of field?"

JB: Ken, that is a great question. Yes, I do. I probably should have had an example of it in this talk; I use it on a pretty regular basis. It is pretty easy to do, for those of you that have never tried it. My mind is a blank right now as to what the software I use—Helicon—that's it. You simply make a series of photographs, all at precisely the same magnification, but working your way through the subject matter—the 3D subject matter, making sure that you have within your depth of field good images throughout the whole thing. Usually, I use in my microscopy are roughly 1 µm difference between a group of images, and then they are pieced together in the software going by the highest contrast and the highest resolution planes. It works out really well, and I have used it quite a bit.

CZ: From Shane, we have: "What are the state of the science software programs that you are keeping your eye on?"

JB: I am not paying a huge amount of attention. I am primarily looking at imaging software and I have a subscription to Adobe Photoshop and Lightroom, and some other programs, that are always current. I have mentioned Helicon software; that is excellent. I am not watching a whole bunch of other things at this point.

CZ: From Ed: "What is your experience with compensating for defective pixels in the imaging chip, or defects in the lens system?"

JB: If I have defective pixel, if it is simply one pixel, I can simply clear that up in Photoshop. Generally speaking, I am not working at such a level of magnification that a single pixel would alter the state of the image, other than creating a tiny red spot in my final image. In really noisy images, which can happen, you can also do a subtraction. It is a little bit of trouble and I don't usually use that, but it can work.

CZ: Okay, from Jacquelyn: "Regarding line sequence determination, have you been successful in establishing which line of inked entries was written over or under the other line?"

JB: Thank you, Jacquelyn. That is a very difficult question. In line sequencing determination, it is a lot easier if you are dealing with something like ball point pen over laser printing or that sort of thing. You can sometimes, depending on the depth of the writing and of the two writings and how they cross over one another, sometimes you can tell the sequence, but it is an area where you want to be very conservative in your analysis. Thank you.

CZ: Question from Hugh: "How can I prepare a clean cross section for analysis of layer structures, such as film laminated structures?"

JB: That is a good question, too. It depends on the subject matter. I am not exactly sure. When you say film laminated structure, it reminds me of a project that I did with McCrone where we had to look at garbage bag layering—plastic garbage bag layering. That was a very difficult specimen preparation problem and it was handled by our cleanroom. They basically used liquid nitrogen to make a very stiff thing and then break it, and with that clean edge, we could do some very good imaging, but that can be difficult. With paint films, it is not a problem because you can embed a small paint film that is half a millimeter in diameter or so, and do a nice polish on that, and then usually those are photographed at about 200X magnification.

CZ: Another question from a different Hugh: "Have you performed scientific videography, and if so, do you have any tips on inclusion of scale?"

JB: I actually have not performed any scientific videography. In terms of inclusion of scale, certainly the safest way to do that would be to include a physical scale within the frame, so that whatever you're videographing, it will always be there. If that is not completely possible, you might also—if everything is at the same magnification—you could do a separate image, and through video editing you could include that scale in every frame, but you would have to have constant magnification for that to be valid.

CZ: Okay, one more from Orsalya: "Can you tell us about a case from your related work that resulted in surprisingly different results from what you had expected?"

JB: Every project I enter! I enter every project with the expectation of I don't know what I am going to get, but I am open to wherever the data leads me. Any kind of bias is really important, and can be difficult to see in yourself. I know that I am biased in various ways, and I always try to suppress that. Oftentimes in my reports I will try to be careful to express any opinions very carefully. Any conclusions have to be completely validated, and if there is any doubt, that doubt must be made very clear. That is a really good question, and I can't think of any particular case where I can give you a short synopsis, but certainly that has happened, that you never know what you are going to get and sometimes you are absolutely delighted with your results, and other times you scratch your head and wonder where you go from here.

Joe Barabe teaches students how to create balanced light for a scientific photograph.