



## Webinar Transcript

### McCrone Associates: The Particle Approach

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#### Introduction

Hello and welcome. My name is Charles Zona and I would like to thank everyone for attending today's webinar. Our presenters today are Dr. Kent Rhodes and Dr. Craig Schwandt of McCrone Associates. Kent and Craig are going to talk to us about the capabilities at McCrone Associates and employing what they call "the particle approach." Kent is the Senior Vice President and Technical Director of McCrone Associates, and he also co-teaches our scanning electron microscopy course. In addition to his management of technical services at McCrone Associates, he performs surface analysis using x-ray photoelectron spectroscopy, secondary ion mass spectrometry, and electron microprobe analysis. Craig, a senior research scientist, is the Director of Industrial Services with McCrone Associates. He is also co-instructor for our basic and advanced scanning electron microscopy courses. Craig specializes in x-ray microanalysis of particles using energy and wavelength dispersive spectrometry methods with the scanning electron microscope and electron microprobe. Kent and Craig will field questions from the audience immediately following today's presentation. This webinar is being recorded and will be available on the

#### PRESENTERS:

##### Kent Rhodes, Ph.D.

Kent is the senior vice president and technical director of McCrone Associates (MA). He performs surface analysis using x-ray photoelectron spectroscopy, secondary ion mass spectrometry, and electron microprobe analysis. He co-teaches the SEM course at Hooke College of Applied Sciences.



##### Craig Schwandt, Ph.D.

Craig is a senior research scientist and the director of industrial services at MA. Craig specializes in x-ray microanalysis of particles using energy and wavelength dispersive spectrometry methods with the scanning electron microscope and electron microprobe. He is co-instructor for basic and advanced scanning electron microscopy courses at Hooke College of Applied Sciences.



McCrone group website under the Webinars tab. And now I will hand the program over to Kent and Craig.

##### Kent Rhodes (KR):

Thank you, Chuck, and thank you all for attending our webinar today. To begin with, let's just talk about particles. Well, I think all of us have a pretty good idea of what we mean when we talk about particles, and those are just small discrete pieces of a material. They are surrounding us constantly—the air we breathe, the food we eat—all have particles in it. I

like to divide them into different classes of materials; a lot of materials are, by their very nature, particulate: the dust in the air, the salt and sugar you use when you eat, those are fundamentally particulate in nature. Other materials go through a phase where they are particulate during the production of a final product; for example, coffee is ground into particulate extracted to form a final liquid material. The final type of particles that we deal with a lot here at McCrone Associates are contamination, which is basically foreign particulate matter usually where



A microscopist filters a sample in one of McCrone Associates' ISO Class 5 cleanrooms.

you don't want it to be. Those turn out to be the most interesting projects because they're forensic in nature—you have to try to determine what the material is and perhaps where it originated from—with the ultimate goal, of course, being to reduce or eliminate that foreign particulate matter.

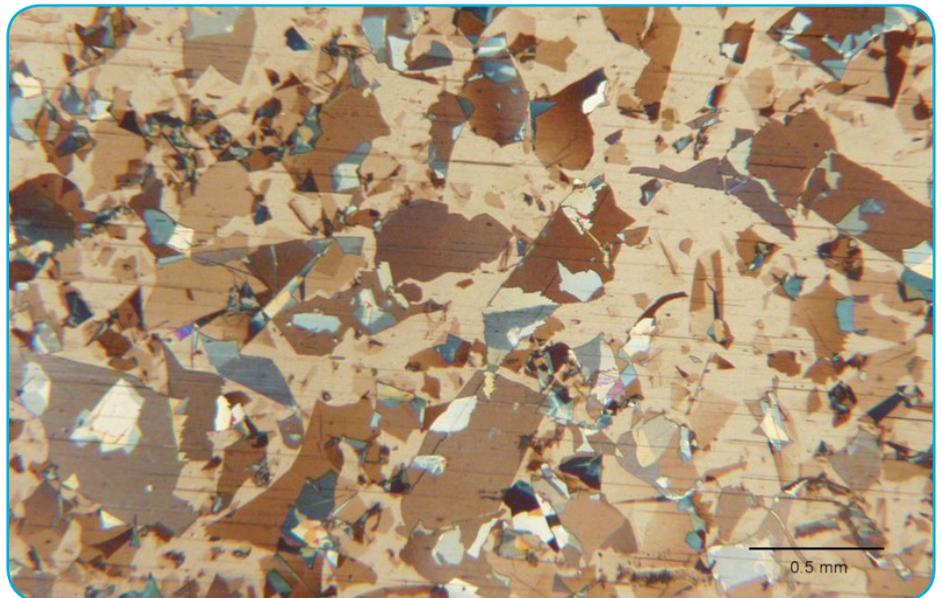
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### **Craig Schwandt (CS):**

As a tie in to the previous webinar—which was about glass delamination in injectable drug products—this image is an example of some glass delamination flakes on a filter after it was filtered from the product. In general, our clients are really interested in knowing “what is that foreign particle and how did it get there?”

Before we get to that question, or those two questions, we want to consider some concentration first. If we use an example of, say, a particle in a volume of material, you might think that you have a little piece of iron in it; it might be one speck in a large cubic meter of volume. So, you could say that was relatively a one part per million. If we had a cubic centimeter out of a cubic meter, that's effectively a one part per million concentration. So, analyzing materials at trace levels requires one method, but when we look at particles we often remove those particles from the matrix that they are in, so we actually start to look at the entire volume. If we have an alloy that is made up of different components, say we were looking at this collection of M&M's®, and each color M&M represents a different alloy element and the steel—well, iron actually—is the major component. If we focus our



Glass delamination flakes on a polycarbonate filter.

analysis just on the small volume at hand, then those constituent elements make up major percentages of our foreign particle. So, “concentration” in that sense is really a relative term. If you're looking at a big volume, then you might be considering trace elements, but if you're looking at just the particle of interest, those elements are in major element proportions.

**KR:** Just to convince you how daunting this analysis challenge can be, if you take the example of say a hundred mLs of aqueous solution and put in five stainless steel particles, this is a typical analysis request. We did contamination samples with very small numbers of particles. If you try to do this by bulk analysis, as Craig just described, your analytical challenge here would be to measure this at the pico gram per gram levels. This is a very challenging analysis for ICP, atomic absorption, or post-bulk analysis techniques, but happily, we can take advantage of the fact that these particles are not homogeneously distributed through this material. If we can concentrate the particles for analysis, we have a much better chance of doing this forensic identification of the particulate matter.

**CS:** So, before we go on, just a moment. Kent, one pico-gram per gram; that's maybe a foreign way of looking at it for some people. I think that's something like one part per

trillion. Wow, that is pretty amazing. That is trace, ultra-trace detection.

**KR:** Yes, if you don't take advantage of the fact that these particles are heterogeneously distributed, and in many cases, can be concentrated, you are going to have a very difficult time measuring small amounts of particulate in large volumes of material. Because remember, there's also the sampling issue. You can't take just a mL aliquot of that and have any assurance that you'll have a representative sampling of the particulate matter.

**CS:** That's right. So, our next question is, really, is the particle approach considered testing? And in that sense, it's not really testing. The particle approach is investigative or forensic analysis as opposed to compendial or specified testing, where you're testing a product to a specification—either an ASTM standard, a USP standard—some standard where you know what the concentration of the elements should be and you want to know, in a testing situation, is your product compatible? Or, also, at the same time, if you are doing a regular testing method and then you, perhaps, do discover that there's an elevated concentration of a particular analyte—that's when you would really need to look at investigative or forensic analysis.

So, we want to ask, “What exactly is it?” That's our main question then—what

we want to determine for the problem, and then we can ask “what is it” versus “how much is there.” Most of the time, we really want to know what it is rather than how much is there.

So, this comes into play—we have the quality elements involved, really—with testing, you’re really comparing the results against some specification, whether it’s for standard or for your product specification. And you could do a replicate analysis to improve your confidence in the results that you’re measuring. This is in a different contrast to investigational analysis where we’re going to perform periodic tests on our instrumentation, we’ll also do periodic testing of our analysts to demonstrate that both the instrumentation and our analysts know how to give the right answer when testing known materials. But, really, in an investigational analysis we don’t know what the answers are going to be beforehand. So, we’re doing a quality assurance task, whereas with testing we’re really looking at a quality control task; there is a big difference there. These are things that you want to ask when you’re considering what type of laboratory you need to address your particular analysis concerns.

The pros and cons with the bulk analysis testing method: It can alert you to the presence of contamination and it’s relatively fast and cheap; but it really doesn’t help you find the source of your contamination, whereas the particle approach is able to explicitly identify contaminants using a wide variety of methods. It greatly assists in identifying the source (of contamination) at the production plant. But, the downside of it is it can be a bit slow, and obviously it’s a little bit more expensive because it may require multiple methods in order to come to a complete conclusion.

**KR:** With that background information about what we mean by the particle approach, let’s talk about how we actually perform these kinds of tests. We mentioned previously that really the first step, and one of the most important, is to concentrate the material, or the particulate matter, for further investigation. There are a variety of methods we use at McCrone

Associates to do that. Probably one of the most common ones, and one that you might be familiar with, is filtration. We do a lot of filtration. One of the key points to realize here is that we really need to protect our samples from further contamination, from the environment, or from ourselves; we do most of our filtration in very controlled environments at McCrone Associates. Inside our clean rooms are laminar flow hoods to reduce the background, and to make sure we don’t further introduce additional contaminants that would confuse us or provide information that’s of no value on these particular samples.

Once we’ve concentrated these particles, we have to have a way of looking at them. And, of course, they come in all kinds of sizes from millimeter down to the nanometer range. To visualize these particles, we use a variety of different microscopies. So, this slide just shows you some of the size scales that we’re dealing with here. Everything from naked eye visible particulate down to, maybe, a tenth of a millimeter, through the range that is accessible by light microscopy to electron microscopy—both scanning electron microscopy and transmission electron microscopy. But, again, the idea here is once you have concentrated these small particles, can you get your analytical probe down to the same size so you can really take advantage of the analysis volume to get a better analysis of these small bits of material.

Once you have your particulate isolated in a form you can observe with a microscope, you can then isolate it for further analytical work by using microtools. We’ve developed a variety of microtools at McCrone Associates and they allow us to manipulate these small pieces of material, or small particles. This is just an example of a few flakes of material being observed on a filter, and in this case, it could be isolated for techniques like vibrational spectroscopy using Raman, or for compositional analysis using transmission electron microscopy. It is a fundamental tenet of particle analysis: isolate the particles, observe the particles, and then analyze the particles.

One of the most common methods we use to analyze particles is scanning electron microscopy (SEM) with energy dispersive spectroscopy, or EDS. The advantage of this technique is that it provides two pieces of information. Using the electron beam to scan across the sample, you produce two different types of signals. You produce electrons, which you can use to image the materials—so the image of the left shows us fly ash sample. It has been collected on a filter and then imaged using scanning electron microscopy. As that electron beam you’re hitting the sample with interacts with the atoms present in the material, it generates x-rays. You use a spectrometer to measure the energy of these x-rays and you can present them as a plot, as is shown on the right. The peaks correspond to the different elements present. So, again, going back to the left, you can see right in the center of the screen a bright particle with the red dot on it; that’s where the spectrum on the right was collected from. You can see it’s typical of a fly ash material with aluminosilicate with calcium. The interesting feature on this particular particle is the amount of barium in it, which is actually the cause of why it appears brighter in the left-hand image; barium is a more efficient scatterer of the electron beam, so it appears brighter. This is one of the key techniques we’ll talk about in the rest of our presentation to determine both the size and shape of the particles, and also the chemical composition.

Now we’re going to present two examples of how we might use the particle approach. The first example was a tablet that arrived with a brown stain. So, you can see on the left, here is the tablet mounted just as we received it. It’s mounted on an aluminum disk with a conductive carbon adhesive. And, again, this is for scanning electron microscopy. The image on the right shows the electron micrograph of this material. So, you can see the stain doesn’t show up too well against the background of the tablet, but if you look closely, you can the stain is composed of particulate matter.

Here’s what the stain looks like at a higher magnification. You can see on

the left in the electron image, it consists of particulate of a variety of different forms in terms of size, shape, and in brightness. Brightness, again, is a key to the composition of the material. If you do an overall analysis of the field of view shown on the left with x-ray spectroscopy, you obtain the spectrum shown on the right. And, you can see that immediately what stands out is there is a large iron peak. That's probably an indication of why the stain is discolored; it's brown, most iron materials/iron compounds are brown. But really, this isn't the particle approach. We're analyzing this whole stain and so the other components, we don't know the relationship. Are some of these elements associated with other elements? Which elements are coming from the tablet itself? Because we haven't done anything to isolate it, really, this is more of a bulk-type analysis.

We go to the next slide we can see the particle approach to this problem. Here we've removed the particulate, put it on a low atomic number substrate so we don't have a contribution from the tablet itself, and then we have gone in and analyzed individual particles of this stain. And, now we can get a much better breakdown of the materials causing this. Again, we see that many of the particles, if we look at the data plot on the right, we see that many of the particles are elevated in iron content, as we learn from the overall scan, but we also start to see that there are particles that contain iron

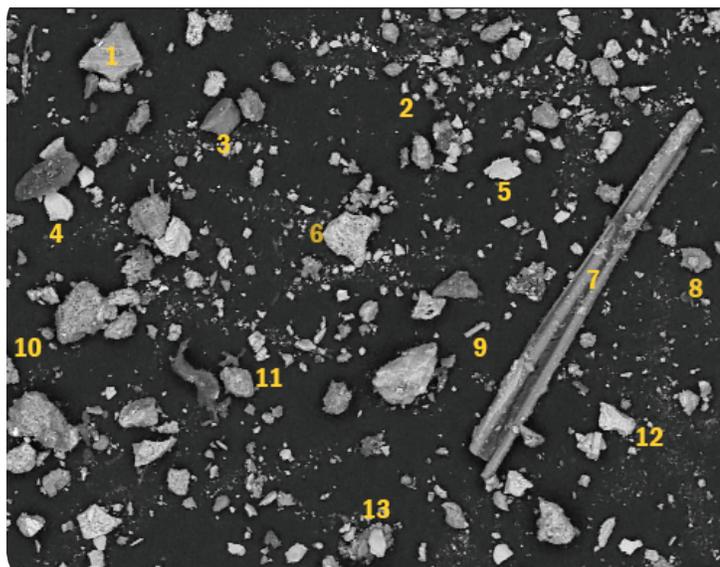
associated with sulfur and chlorine, which are typical elements in corrosive environments. And, then there are other ones that are particles that have iron associated with silicate materials. We can start to break down the individual components of the stain and feed this information back to our client to help him or her determine possible sources of this material. So, again, by going to a particle approach we get a lot more information about the nature of the material of interest, and hopefully then about the possible sources of this material.

The second example that we're going to present—a client who is producing a drug product using a raw material. The raw material is typically dissolved in solution, and when that step occurred during the production, the client observed some large brownish flakes of material in the solution. They asked us to investigate the foreign particulate matter present in the powder sample, so the powder sample was dissolved and filtered, and the particulate was collected on a filter.

In this case, we chose an automated analysis technique. The advantage of automated analysis is that you don't have to select a small subset of the particulate matter to investigate. You can analyze thousands—to millions—of particles. This animation shows how an automated analysis is performed. The electron image in the center is generated in the SEM, and the electron

beam is scanned across this image, and particulate is recognized as being brighter than the background. When a particle is found, the electron beam is then rastered over that particle to determine its size and shape. So, in the data being presented on the left, you can see the size and shape of the particles. The electron beam is then placed on the particle for just half of a second and an x-ray spectrum is collected. The elements are determined, and then it's classified based on a series of rules we develop. You can see a variety of fields of view that are done on one particular stub; you get a listing of all the particulate you've analyzed, and you can then start to classify them into various materials. In this example you can see silicates, spherical particles, calcium rich materials—you get a complete breakdown of materials.

So, here's the results from this particular analysis. Using automated analysis, we analyze for approximately 32,000 particles that were collected on the filter from dissolving the raw material. What we found is a very small number of these particles—about three in 10,000—were relatively large, greater than 10 μm, copper particles. So, these are the particles that the client was able to visually see in the solution once the material was dissolved. But, it turns out they are a very tiny fraction of the overall particulate matter in this material. You can see that there are large amounts of typical processing



Particle	Na	Mg	Al	Si	S	Cl	Ca	Fe
1	<1	6	6	24	<1	<1	32	3
2			<1	<1	15	<1	2	38
3		<1	4	49	<1	<1	1	4
4			<1	<1	<1	4	<1	70
5	<1		<1	<1	<1	7	<1	46
6			<1	<1	1	4	<1	37
7	9	2	2	24	<1	<1	3	8
8		15	<1	<1	<1	<1	28	6
9	2	3	5	18	2	2	22	21
10	1		<1	<1	23		19	3
11			<1	<1	15	2	13	17
12		<1	17	<1	2	2	2	20
13		<1	<1	<1	<1	2	<1	48

Individual particles of the stain are analyzed; components of the stain are broken down in the data plot.

residues from industrial production equipment: stainless steels, tungsten, silicon, and teflon-like fluorocarbons. So really, the large visible particulate was a very minor component of the material in this raw material. We gave this information back to the client, and they were able then to go back to their supplier and work on reducing not only the visible particulate matter, but also some of the other materials present in the raw material.

So, I hope we've given you a feel for how you would use the particle approach, and some of the advantages versus bulk analysis. From the two examples we gave you, I think you get a feel for some of the applications—looking at raw materials, or looking at contamination. There are also all kinds of applications in environmental studies, wear debris, and any other areas where you want to analyze either a small number of particles that you can isolate from the solution, or a whole population of particles.

Craig, did you want to add anything else?

**CS:** If they have additional questions, our email addresses are up on the screen. I would be happy to entertain any questions that they have that are of a more direct nature, but otherwise we are going to open it to a general question session now.

**CZ:** Yes, as Kent and Craig mentioned, thanks again for attending today's webinar. You can go ahead and type your questions into the question a box there, and we'll entertain some of them as they come in; we have a few already. "If I have an unknown particle contamination, a particulate contamination, what techniques other than FTIR can I use to identify the anion?"

**KR:** Well, again, if we had a totally unknown sample of particulate, we isolate it and then we would look at both the elemental composition using SEM EDS as we described here, and then if it turned out that it did appear that there was organic material present, you can use either IR or Raman spectroscopy. But, really

it depends. I think of it as a stepwise approach. It depends on what you find in your first analysis on what the additional techniques might be. So, for anions, it depends on if they are things like chloride or fluoride anions; techniques like SEM EDS will pick those up very readily. If they are carbonate or other organic-type materials, or inorganic carbon materials, it may require Raman spectroscopy, for example.

**CS:** That's right. We really do use SEM EDS, pretty much, in conjunction with the IR. You mentioned what anions, well actually, you wouldn't be sure; if it's a total unknown, you don't know that they are behaving in an anion capacity versus the cation capacity, so we want to understand what the components of the unknowns are. We'll use elemental techniques to determine what the elements are, we'll use spectroscopy methods to understand organic molecules that are present, and then once we understand those two portions of the problem, then we'll apply other methods like x-ray diffraction, electron diffraction, or Raman. Or maybe we want to take it in a different direction and look at a more surficial thing, and use ESCA to look at the valence state of material.

That is sort of a loaded question in the sense that you thought that there was something was an anion, but we don't know anything until we actually start to do the tests. So, we pair the elemental and the organic analysis together first to find out how to get down to the next path.

**CZ:** James is asking, "What equipment was used for the automated particle study showing elemental data and images?"

**KR:** So, that's SEM EDS and the particular piece of equipment is specialized microscope for doing automated analysis sold by FEI. It was formerly known as ASPEX, and now it's an FEI product, and it's optimized for doing automated particle analysis. Most software vendors of EDS systems also have automation packages you can buy as add-ons, or supplemental packages. I know Oxford, EDAX, and

Thermo all have automated analysis software packages.

**CZ:** That kind of answers our next question from Lynn, "Is automatic SEM EDS a software function?"

**KR:** Yes and no. So, as I said, most hardware vendors sell it as a software option. However, the example I showed in the presentation today is a system that's actually designed from the ground up for optimized particle analysis. So, we can run typically three to five times faster than most of the software add-on packages that are available. So, the answer is yes, it's an add-on package. If you really have high volume of samples or need a very large throughput of particulate analysis you may want to look at a more dedicated system.

**CS:** In addition to FEI, Tescan and Zeiss also manufacture dedicated systems for those uses.

**CZ:** We have a question from Sean: "What method do we use to ignore bad EDS results? Essentially, how do you ID what's the true data? How do you know what's there?"

**CS:** Bad data. Well, one thing that we do to make sure we produce as little bad data as possible is we'll mount our particles on substrates for which the background isn't going to be a problem. If we're looking at metals, we can mount them simply on carbon planchets and we can discount the inclusion of, or the incorporation of, the carbon. If we were interested in looking at organic materials, then we'll have those mounted on a beryllium planchets so that there is no carbon background from the substrate. We isolate the particle from its substrate and then analysis elements can be sorted.

**CZ:** "Can you speak to how you might use Raman versus FTIR spectroscopy in evaluating organic particles that may or may not have inorganic groups, too?"

**KR:** Well, in general, I believe Raman provides you more information about

the inorganic groups than IR, but the other consideration is the stability of your material under the lasers typically used for Raman versus IR. The wavelength difference produces a whole different resolution between the two techniques of Raman—you can go down to micrometer sizes whereas IR you're maybe at 20  $\mu\text{m}$ . So, it's more than just what type of analytical information it's providing. Is it even possible to analyze some of these particles, one versus the other?

**CZ:** James is asking a question here that relates to our upcoming webinar next month—I'll put the slide up there right now. He's asking, "How do the benchtop SEM-type systems like the NeoScope compare to the large systems for particle investigation?" Without giving away your entire talk, Craig, can you comment briefly on that?

**CS:** Yes. I think that they actually compare very well. There are a couple of key issues that you want to consider to be the most important for your selection of which system meets your needs best—all in all, it's a close call.

**CZ:** Okay, we'll learn more about that on December 17 when you and Alan address it directly in the next webinar. Let's see here, "Can you explain the filtration step a little bit more? I don't understand how you can concentrate your particles if you don't know what they are and what solvents they will respond to."

**KR:** That's an excellent point. A lot of times your filtration, the sample that's coming in, is already in a liquid, and the client is interested in insoluble material that liquid could begin with. Other samples will come in as solids, and the question is: Can you dissolve the matrix material in a liquid and keep the foreign particulate without dissolving it? You may have to try a couple different liquids to determine whether the foreign material is soluble in one versus another. It really depends on the nature of the samples and the client's request, but it can take some investigation. I think this is a point Craig was making, these aren't standard methods, a lot of times. It's something that you have to develop for each individual request.

**CS:** And, then depending on the type of contamination, in some cases it wouldn't be done by filtration. In fact, we have a number of staff clean room microscopists that are very adept at manually isolating the particles from the matrix using custom tungsten tools. In some cases, we might have to resort to that as opposed to a dissolution and filtration.

**CZ:** Roy is asking, "Do you know of an adhesive that is not carbon base to secure the particles for EDS?"

**KR:** You can use indium metal. You can press particles into indium metal, or maybe some other soft metals depending on how hard your particles are, but of course indium is going to have a very high atomic number versus carbon, so it's going to be pretty challenging to do EDS directly on it. I don't really know of any non-carbon-based adhesives, besides that.

**CS:** I don't either.

**CZ:** From Fiona, "Do you perform your SEM EDS for particle analysis under low vacuum conditions?" and then she has a second part, "Do you calibrate to compensate for the carbon contribution from the conductive carbon tape?"

**KR:** Alright, the first question is about low vacuum conditions. Most of the time these small particles won't have significant charging. If you're talking about micrometer sized particles there's not enough charging to worry about that. If they are larger than that or they're particularly nonconductive, you may have to either run them in low vacuum mode or carbon coat them, especially if you want really high-resolution imaging. The second part of the question was...

**CZ:** "Do you calibrate to compensate for carbon contribution from the conductive carbon tape?"

**KR:** That's a really, really difficult measurement, especially on particles, how you get an accurate contribution of carbon from the tape. As particles get smaller and smaller, it's more and more difficult to combine the interaction volume to the particles themselves. Overall, the answer is no. There's not really a robust method for doing that.



A microscopist isolates particles in one of McCrone Associates' ISO Class 5 cleanrooms.

I think the better solution is what Craig mentioned: put them on a substrate that doesn't have carbon in it to begin with; either boron or beryllium.

**CZ:** Looks like our last question here is, "What kind of filter is recommended for particle analysis?"

**KR:** The filters we use are membrane filters. They have a flat smooth surface. These are ideal for doing imaging directly on the filter, as we showed in the SEM, and also for manipulating particles with micro tools directly on them. Membrane filters can be purchased in a variety of different materials: polyester, polycarbonate, and also fluorocarbon materials, but those are ones we recommend.

**CZ:** Great. Well, that rounds out our questions, and I would like to thank Kent and Craig today for their information and everybody else out there who attended. Be sure to join us for our next webinar on December 17 when our presenters, again, Dr. Craig Schwandt will be with us and Alan Vitous of McCrone Microscopes & Accessories will compare the JEOL NeoScope to a full-sized scanning electron microscope. Thank you.