Microscopy in Meat Research

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As an introduction to this statement about microscopy, we offer the following quotation from *My Life and Hard Times* by James Thurber (Harper & Row, 1933).

> "We'll try it," the professor said to me, grimly, "with every adjustment of the microscope known to man. As God is my witness, I'll arrange this glass so that you see cells through it or I'll give up teaching. In twenty-two years of botany I . . ." He cut off abruptly for he was beginning to quiver all over, like Lionel Barrymore, and he genuinely wished to hold onto his temper; his scenes with me had taken a great deal out of him.
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> So we tried it with every adjustment of the microscope known to man. With only one of them did I see anything but blackness or the familiar lacteal opacity, and that time I saw, to my pleasure and amazement, a variegated constellation of flecks, specks and dots. These I hastily drew. The instructor, noting my activity, came back from an adjoining desk, a smile on his lips and his eyebrows high in hope. He looked at my cell drawing. "What's that?" he demanded, with a hint of a squeal in his voice. "That's what I saw," I said. "You didn't, you didn't, you didn't!" he screamed, losing control of his temper instantly, and he bent over and squinted into the microscope. His head snapped up. "That's your eye!" he shouted. "You've fixed the lens so that it reflects! You've drawn your eye!"

With that bit of humor, the first point is made – the effective use of the microscope is limited by the level of training and understanding of the microscopist. The ordinary light microscope has the distinction of being one of the most common pieces of laboratory equipment, yet is probably the one least effectively used.

So, our purpose in this reciprocation session is to encourage a better understanding of the use of the microscope as a tool for study of meat.

Why would the meat scientist want to know about microscopy? For many of us, it is an instrument which can be employed usefully in our own research and teaching. Therefore, we should not only know how to use it, but also appreciate the limitations of use. Many of us serve at some supervisory level. Therefore, if we supervise people who use microscopes, it is common sense to be at minimum conversant with the equipment our employees are using. And finally, as scientists, a knowledge of microscopy adds to our general interpretative ability.

What are the prerequisites for effective use of the microscope? The general design of the instrument should be appreciated, and proper adjustment must be mastered – this is very important. Since it is easy to look and see, the user must be careful in interpretation and try to invoke as many control situations as possible. Finally – and the most critical point to be made – nothing can be substituted for the trained eye. This means that the person wishing to do microscopy should make every effort to serve an apprenticeship with an individual experienced in both the science and art of microscopy. The trained eye sees things the untrained eye overlooks and has a feeling for identifying artifacts.

What are some typical uses for the microscope? We enumerate the following with the realization there may well be more: to identify something, to locate something, to make a quantitative measurement of something, to visualize a change, to relate function to structure, to check for normal versus diseased state, as a component of quality control, to see if bugs are present, to check for impurities.

The ordinary light microscope has been modified for special application. Some of these provide extremely powerful tools as, for example: phase-contrast, polarizing, darkground, Nomarski, interference and fluorescence.

Electron microscopy presents a special case, and has now been refined to consist not only of transmission but also scanning and high-voltage instrumentation. The techniques are extremely powerful but also costly and require special training. Our advice is to obtain the services of a competent electron microscopy unit if electron microscopy is required.

Preparation of biological specimens is an enormous topic in itself. Suffice it to say that in addition to fixed and sectioned or frozen-sectioned preparations, useful information can also often be obtained from nonsectioned preparations. A number of textbooks give simple, reliable step-by-step procedures. However, we reiterate that an apprenticeship is the easiest way to learn.

With recent combinations of micro-computers and

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digitizing pads, a vast improvement in speed of obtaining quantitative results has been made.

The use of microscopy in advancement of meat science is well documented. We note, for example, the classical work relating muscle contraction state to tenderness and the characterization of muscle growth and meat properties by microscopic methods.

What can be accomplished in the future? That depends upon the ideas of the investigator coupled with an appreciation of how microscopy can be best utilized to answer questions.

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*A selected selection of resource documents to aid both the beginner and expert.

Discussion

T. Silva: In my pictures, I have myofibers and what I think are Z-lines. But on the surface of my sample picture, I have open spaces. I have three treatments and a control and all exhibit the same behavior. My professor has asked what these spaces might mean and I told him I thought that water had evaporated. Do you think these are artifacts? Why do I have open spaces between the fibers?

R. Cassens: I'll at least give you my ideas. Open spaces do not occur in living biological tissues. You immediately have to be suspicious if you see cracks and open spaces. As far as preparation for scanning electron microscopy, I really have had no experience. Is there anyone who would like to comment on this further?

J. Sebranek: I'm not commenting from the standpoint of having a lot of experience with electron microscopy, but we might look at a couple of these slides. Scanning electron microscopy was used to study freezing rates and open spaces were related to supposed crystal size. In the slide, the four different freezing rates created the apparent size difference. We concluded that the ice crystals formed those kinds of textural differences. We are assuming that was the result of lost water.

Cassens: Artifactual formation of ice crystals is very much dependent on the condition of the muscle. Because with the exactly same freezing techniques, you can take several samples from different animals and some will look terrible in terms of ice crystal formation. Ice crystal formation is dependent on the state of the water in the muscle as well as the freezing technique. Obviously, you must have a good freezing technique but sometimes that is not enough.

M. Solomon: Some of the artifacts that you see from a section that is frozen improperly for histochemical staining can be created by just tampering with the hematoxylin. Depending on what enzyme you are going for, you can create a lot of artifacts that one would see if one was looking for ice crystal formation or membrane disruption. From the histochemical technique, it would be very difficult to ascertain using some of the criteria that are normally used when histochemists or pathologists look at sections to determine if a problem exists or whether it is an artifact. I have been interested in putting together a pamphlet on artifacts and various techniques for histochemistry. When you look at the literature, you see a lot of artifact and yet a lot of the artifact may be due to the sampling techniques. Also, people do not know that some of the solutions used can cause the artifacts.

A. Sosnicki: Right now, a lot of artifacts are very well