

Fluorescence Microscopy

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My main objective this morning is to discuss the *understanding* of objects through the accurate application of Fluorescence Microscopy. To that end, I want to express both *caution and encouragement* in the use and application of fluorescence microscopy, discuss the *implications* of this technology to the changing role of the conservation profession and *how to* connect consumer and suppliers of this service.

I will not be covering very much of the technical side of microscopy and treatment case studies normally associated with this sort of talk. You will find more of that covered in Susan Buck's presentation. Please bear with me, but I believe it may be useful to review some of the basics of fluorescence microscopy.

Primary Fluorescence Microscopy Primer

Essentially we are taking advantage of how coating materials absorb or pass light:

shorter - higher energy - excitation

longer - lower energy - emission

Light for fluorescence microscopy is usually generated by a mercury or xenon lamp. This passes through a filter cube which *first* "narrows" the band of light to a desired excitation. *Second* it reflects this light down to the specimen via a di-chromic mirror causing emission of visible light with materials that fluoresce and *third*, the barrier filter portion of the cube passes the emitting light safely to the viewer eliminating residual exciting light. Understanding the nature of filter cubes and matching them to a particular need is essential to proper fluorescence microscopy.

My focus today is on primary fluorescence, for which ultraviolet or violet filter cubes were designed. I have been using a Nikon V2-B cube and a Leitz D cube having excitation and transmission values similar to those depicted here:

Leitz D	EX 355-425
	DM 455
	SUP 460

Nikon V2-B	EX 380-425
	OM 430
	SUP 460

Sampling

This is often tiresome and difficult. It pays to think carefully about where the most useful samples are likely to come from, and how necessary the sample is. Often more than one is necessary for a reliable reading of a surface. In addition, be aware of the casting media's possible impact on the sample. So ends my primer on the "nuts and bolts" of fluorescence microscopy, sampling and related issues.

Now some cautions on the subject. In 1785, the English microscopist Henry Baker published a book entitled *Miscroscopes and the Discoveries Made Thereby*. I will quote here from the XV chapter of that book:

“When you employ the microscope, shake off all prejudice nor harbour any favorite opinions: for if you do, it is not unlikely fancy will betray you into error, and make you think you see what you would wish to see. Beware of determining and declaring your opinion suddenly on any object, for imagination often gets the start of judgement, and makes people believe they see things which better observation will convince them could not possibly be seen. Pass no judgement upon things out of their natural state without making suitable allowances. Therefore, assert nothing till after repeated experiments and examinations in all lights and in all positions. Remember that truth alone is the matter that you are in search after, and if you have been mistaken, let no vanity seduce you to persist in your mistake.” (from a Leitz reprint)

Fluorochromes

It is possible to obtain more information from a sample when fluorochromes are applied. Fluorochromes are essentially histological or hematological dyes used to cause specific materials to fluoresce; a phenomenon known as secondary fluorescence. It is worth noting that the medical-biological field today uses secondary fluorescence more often than primary fluorescence which is the opposite of what I have found to be true when characterizing surface coatings on furniture. Be aware of this in your reading, studies, and interaction with medically focused microscopists.

Case Studies Notes

Cautions

- Use normal light microscopy in conjunction with ultraviolet light.
- Remember that an extracted sample is out of its original context.
- Most of the visual information for transparent coatings can come through primary fluorescence.
- There is an additional level of knowledge and skill needed when using and interpreting fluorochromes.
- Despite great expectations, fluorescence microscopy does not always give the answers that we hope for.
- Focusing solely on microscopy and letting other skills go limp is not a good idea.

Encouragement

- The complexity of a coating can be seen through this technology.
- The history of parts from an object may be discernable.
- The detection of surface problems and solutions may be possible.
- It may be possible to place the history of change in an object.

Connecting

- Connecting consumers and practitioners may best be achieved through the AIC Referral system.

Summary

What conservators are able to produce is more than a cared for object. Our role with historic/ artistic objects has been expanding into the area of helping to discern the nature and history of objects. The developing conservation profession is uniquely situated to help in a substantial way to add to the understanding of an object which should lead to a more accurate interpretation of it.

To these ends, it is critical that we embrace strict standards in our microscopy work including proper cubes, avoiding misconstrued samples and the like.