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Guest editorial

Curious about stains? Need help with staining? Asking for a friend? Try the Biological Stain Commission's online *Stain Glossary*

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Trustees of the Biological Stain Commission have produced an online *Stain Glossary* of staining methods, stains and other reagents, plus some information on relevant historical figures and organizations in this field. Glossary items address dye-based ("tinctorial") routines and special stains, histochemistry and enzyme histochemistry, and immunostaining. To illustrate the breadth of topics covered by the glossary, all the words and phrases printed in red in this editorial are terms found in the *Stain Glossary.* Prior to giving some account of this new information tool, however, I first must present the back-story to the project.

Starting in the 19th century, the laboratory synthesis and industrial manufacture of synthetic dyes resulted in a colorful transformation of the world's clothing. There was, however, an unexpected consequence. The industrial production of such dyes coincided with the commercial availability of microscopes and with new medical institutions to create a new way to investigate biological problems, and a new medical diagnostic strategy using color coding of the machinery of life, i.e., cells, tissues and organs. In particular, pathological changes could now be visualized on the micro scale, and sometimes newly discovered microbial pathogens became directly observable. As a result, medical diagnosis took a giant leap forward.

Two hundred years later such methods remain widely used in biology and medicine. Many dye-based coloration mechanisms have been developed and new ways of generating and targeting the colored or color generating reagents, "stains," also have been devised. Thus we use histochemistry to identify chemical entities in tissues and cells, from iron deposits to lipid droplets, and enzyme histochemistry to detect individual enzymes. Then there are various "molecular" assays: immunostaining to detect, mostly, a variety of proteins; and a range of in

situ hybridization techniques to visualize particular nucleotide sequences using a labeled polynucleotide as a stain.

In all these methods, however, the strategy is the same: to color code an entity, ranging from a molecule to a cell organelle to a cell to a tissue, and hence to obtain simultaneously information concerning whether the entity is present and, if it is, where it is found. The staining mechanisms of the different approaches vary, however, not least in the way a color is retained by the target cell or tissue. Dyes generally are reversibly bound to biopolymers, as are some of the final reaction products of enzyme histochemistry. Other enzyme histochemical methods, some of which are used to label antibodies for use in immunostaining, generate insoluble pigments in the specimens under investigation. Another type of label widely used with antibodies and polynucleotides is to attach a fluorescent label to the biopolymer by a covalent bond.

Thus for nearly two centuries there has been one investigative strategy, i.e., color coding of entities of biomedical interest, spatially localized at a micro level, and many tactics. Yet even within the tactical variations, there are certain technical commonalities. For example, to generate consistent and intelligible preparations suitable for staining and viewing with a microscope, the biological specimens must be stabilized against mechanical, microbiological and endogenous damage. After all, what is not retained, whether of substance or shape, cannot be stained. The two most common processes used for stabilization are freezing and fixation. The latter is widely used and involves the transformation of native tissue substances into insoluble or non-extractable forms by protein denaturation, formation of crosslinks, complex formation and trapping. Another general requirement, except when using sophisticated

optical/computational systems, is the need to prepare thin preparations to facilitate microscopic observation. The latter feature is achieved in different ways depending on the physical form of the original specimen. For solid specimens sectioning is perhaps the most common strategy, using either a cryostat, which generates frozen sections from frozen specimens, or a microtome to cut sections from specimens embedded in some matrix such as paraffin wax or a plastic embedding medium. Cell imprints sometimes are obtained from soft solid tissues by dabbing, and monolayers of cells can be obtained from fluid tissues by preparing a smear or by using a cytocentrifuge. A final general requirement is that preparations intended to be viewed with a microscope must have minimal light scattering properties. Consequently the preparation viewed microscopically has typically been infiltrated by a mounting medium with a refractive index similar to that of fixed protein, the major constituent of most fixed preparations. Such a mountant is, at least initially, a viscous liquid, so it also acts to attach a coverslip to the surface of the specimen. Mountant and coverslip then serve to protect the thin specimen from mechanical damage, from being eaten by marauding invertebrates and microorganisms and, in the case of frozen sections, from autolysis by cellular hydrolases.

Many specimen manipulations and staining procedures were developed largely by trial and error. Nevertheless, there have been investigators, from Ehrlich and Giemsa, through Baker and Lillie, to current Biological Stain Commission members, who not only have devised useful and practical methods but also have pondered how the methods worked, and have therefore investigated the mechanisms of staining. These investigations have been undertaken because an understanding of staining mechanisms can lead to increased ability to troubleshoot existing

staining methods and to design new staining procedures. Indeed, the Biological Stain Commission, through its laboratory at the University of Rochester Medical Center and the publications of its members, continues to contribute in this way. Consider John Kiernan's classic textbook, *Histological & Histochemical Methods*, now in its 5th edition, or inspect *Trouble Shooting Histology Stains* by Richard Horobin and John Bancroft. These are printed books, however, and we are in the age of the internet. Consequently, in the 21st century we have a problem. There is too much relevant information for most bench workers to be acquainted with it all, let alone to remember it and too much to search for, even with, or perhaps especially with, electronic data sources. And when we do go online, whom do we trust? It is in this context that the Biological Stain Commission has sought to provide easier access to information about stains, staining and related topics in a way that the beginner and experienced practitioner can both find valuable.

What has emerged is an online *Stain Glossary*, devised and curated by members of the Biological Stain Commission, in particular by Richard Dapson, John Kiernan and me. This glossary is a new tool for the histotechnologist, the grad student and for their mentors and managers. This resource also may be useful even for the more expert practitioner as indicated by the fact that those of us who constructed this tool have learned a great deal from the process! The *Stain Glossary* also is a joy for the curious and intellectually playful. The *Glossary* is keyed to words, names and short phrases related to staining and microtechnique including fixation, tissue processing and, of course, staining using both histochemical and immunohistochemical methods. Notable features include thumbnail accounts of individual stains and dyes; short explanations of physical, chemical and immunological terms used; and

explanations of what the different kinds of fixation, processing and staining do to cells and tissues. There are abundant links between entries to assist speedy navigation within the *Glossary.*

But don't take my word for it. Put the "Biological Stain Commission *Stains Glossary*" into your browser and explore. For example, type H & E into the search box. This takes you to a concise definition, plus brief descriptions of the two colorants involved with links to more extended entries on hematoxylin and eosin. Find also the comments that these two colorants, or at least hemalum and eosin, constitute a basic dye and an acid dye, respectively, and there are links for more information about these classes of dyes, with additional examples of these classes noted. Of course, all entries have links to yet more information. The hematoxylin entry, for exam[le, has links to the widely used Delafield's, Gill and Harris' hematoxylins. As a result, we have created an information network with currently more than 500 entries, often with many separate terms within each entry, through which you can wander and become better informed, or just have fun by finding new and tasty facts. As example of this, if you happened to land at Heidenhain's iron hematoxylin, you would discover that there were two "stainers" bearing the Heidenhain name, Rudolf and Martin, who were father and son, both of whom on at least one occasion contributed to the development of the same stain.

A final couple of thoughts: should you have suggestions for additional topics, stains and people that might usefully be added, let us know. Also, if you notice errors, which are likely in a resource of this size, please let us know about them as well. We have only reached *Stain Glossary* version 1.1; there is plenty more to come!