

# Natural and synthetic dyes in histology

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Counterstains can be of high relevance for the evaluation of immunostaining because cellular details and immunological reactivity are often easier correlated in counterstained tissue sections than in simply immunostained specimens. There are many kinds of dyes in histology. Some are useful for counterstainings while others are not; a careful selection is necessary. Even a given type of dye, f.e. haematoxylin, will not give under all circumstances comparable results. Hence, it is recommended for high reproducibility to choose a favorite supplier of a special dyeing product. A number of counterstains and counterstaining procedures are presented in the section *Laboratory methods*.

## Dyes for microscopy

In histological sections, cellular structures are not significantly different to one another. Hence, dyes are used whenever defined intra- or extracellular elements have to be displayed. The first use of a dye is credited to A LEEUWENHOEK (1673) who worked with "saffron", a natural dye extracted from saffron crocus to stain histological structures, but genuine work on histological dye staining started not before the second half of the 19<sup>th</sup> century when C WEIGERT, J GERLACH, P EHRLICH and H GIERKE systematically studied dyes for histology. At that time coloring materials were still of natural origin from which *carmine* or *cochineal* was the most used dye.

The human eye is able to perceive wavelengths of light between 400 and 700 nm. Dyes appear colored because they absorb a particular wavelength in the visible region, and the eye senses the reflected light as the complementary color. Absorption of light energy occurs when the compound has electrons which can be promoted to higher energy levels.. The energy difference between the ground state and the excited state will determine the wavelength of light absorbed. The staining procedure itself has to provide conditions which promote the binding of a dye to cellular components; the utility of a given staining lies in its ability to bind to selected structures. In classical histological staining, the interaction between a dye and cellular structures are mainly due to ionic, covalent or hydrophobic binding.

According to their sources, coloring agents are discerned as *natural* or as *synthetic* dyes. So-called general stains will dye the tissue uniformly (indifferently) while selective stains have affinity for special cell or tissue components. A new chapter in the history of staining began when WH PERKIN discovered the first aniline dye (from extracts of coal tar) in 1856 which should revolutionize the dyeing industry. From here, the majority of aniline dyes including those for microscopy have developed. One of the first manufacturers to make necessary dyes available for histologists were E MERCK & Company and G GRÜBLER & Company in

Germany with hundreds of dyes (natural and aniline dyes including indigo, haematoxylin etc.).\*

### **Citation from CERTISTAIN® Standards for microscopical staining**

Published for the Merck Group by BDH Diagnostics Division

(...) Many staining techniques in use today were first described in the late nineteenth and early twentieth centuries, being subsequently modified and republished by later workers. Joseph Gerlach (1820-1896) Professor of Anatomy and Physiology at Erlangen, was the first to introduce and record staining methods in histology and is often rightly referred as 'the father of modern histology'. Commercial scale dyestuff manufacture was pursued vigorously in Germany, and German dyes were acknowledged to be the best available prior to the First World War. (...)

Dyes can be roughly divided into *acidic dyes* and *basic dyes*. Basic dyes stain acidic components, and acidic dyes stain mainly basic components. The method used by P EHRlich (1877) for using mixtures of acidic and basic dyes was an important milestone in the development of staining techniques. The subsequent microscopical picture with its color intensity and contrast is essentially determined by the quality of the dye solution and the technical procedure employed.

The exact chemical description of dyes was very often not known, and, dye nomenclature was for a long time chaotic until the Society of Dyers and Colourists (England) published in 1922 the first edition of the *Colour Index*. This systematic approach to dye nomenclature provided a means of identification by the Colour Index Number for each individual dye which was assigned on the basis of chemical structure. Then, for reasons of histological staining consistency and to set standards of performance for microscopical stains, the formation of the Biological Stains Commission was prompted at the same time in the USA.

After the First and Second World wars the companies of GT GURR, E GURR, HARLECO and E MERCK became renowned names in the field of microscopy throughout the world. In the years between 1970 and 1980, E MERCK Industries acquired the HARLECO product line, BDH Chemicals Ltd. and HOPKIN & WILLIAMS (including the 'GURR' name and trademark). Then, in 1982, the Merck group started a project to establish worldwide a standard of excellence for microscopical stains under the Merck, Gurr and Harleco labels; this is named 'CERTISTAIN'. Certistain® dyes are analyzed products according to strict specifications including functional performance. They guarantee consistent batch to batch performance adapted to the criteria given by the Biological Stain Commission (CONN HJ, Biological Stains, 10<sup>th</sup> Ed. 2002, BIOS Scientific Publishers, Oxford UK).

For a comprehensive list of histological dyes with their respective Color Indices we refer to the homepages of BD LLEWELLYN (<http://www.stainsfile.info/StainsFile/bdl.htm>), A EISNER (<http://www.aeisner.de/daten/farbinh.html>) and CHROMA Gesellschaft (<http://www.chroma.de>) where one can also find a number of preparation and staining methods for microscopy.

## **Histological dye staining**

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\* Dyes and other chemicals in histological staining can be toxic and carcinogenic. They must be handled with great care

Histological staining is usually done by staining of cut sections inasmuch as a dye in solution is offered to bind to defined tissue structures. *Progressive* and *regressive* techniques can be differentiated including *direct* and *indirect* procedures.

One can operate with mixture of dyes *simultaneously* or *successively* in order to discriminate different tissue textures by the respective dyes used. So, double, triple and multiple stainings can be achieved. Multiple staining in its proper sense is obtained when f.e. cell nuclei are stained in red color by carmine with concurrent staining of elastic material in dark violet by resorcin fuchsin. Effects of multiple tissue staining can be also obtained by a diffusely staining dye which is superimposed by staining of certain areas by a second dye.

Many dyes have *a priori* only poor affinity to tissues, but this can be overcome by the use of metal salts. Those enhancing compounds are then called “mordants”. Their mechanism of action is not yet clear, but it seems that mordants have a role in coordination bonding between the metal and the dye as well as in further coordination between that dye complex and tissue structures. It can be expected that many hundreds of staining protocols exist, and many of them are up to hundred years old.

A uniform theory of histological dye staining does not exist. This is because the mechanisms of dye binding to the different tissue components are quite heterogenous. Hence, histological dye methods cannot be interpreted as an isolated dye reaction, but must be seen in connection with the chemical and morphological behaviour of the stained structures. The process of staining can rely on both *chemical* or *physical* grounds (for details see PISCHINGER A [1926], ROMEIS B [1968], LILLIE RD and FULLMER HM [1976], BURCK HC [1988], KIERNAN JA [1999], BANCROFT JD and GAMBLE M [2007]).

Dyes may be divided according to chemical or practical viewpoints. Yet, colors and dyes are often differently grouped in textbooks on color chemistry, and this is often dependent on personal views. Furthermore, dyes are not uniformly named, and often fancy names are used.

In a *chemical classification* one divides organic dyes into:

- Azo dyes (the greatest group), e.g. acid and basic azo dyes,
- Nitro and nitroso dyes,
- Chinone dyes, e.g. benzochinones, naphthachinones,
- Di- and triphenylmethan dyes,
- Xanthene dyes with the subdivisions of pyronines and phthaleines,
- Acridine dyes,
- Azine dyes,
- Oxazine dyes,
- Thiazine dyes,
- Vat dyes.

The *practical classification* of dyes relies on their coloring effects:

- Basic dyes, e.g. methyl green, safranin and fuchsin,
- Acidic dyes, e.g. eosin, acid fuchsin and some anthraquinone dyes,
- Substantive dyes, e.g. benzopurpurin and Congo red,
- Mordant dyes, e.g. haematoxylin and carmine,

- Developing dyes, e.g. aniline black, naphthol red, Echtsrot,
- Vat dyes, e.g. indigo and indanthrene dyes,
- Sulfur dyes, e.g. vidual black and pyrogene blue.

Other dyes are mainly of industrial interest and not for microscopy.

## References for further readings

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