

QImaging® Camera Application Notes H & E Stain Tissue Documentation

Haematoxylin and Eosin (H & E) is one of the most widely used histological staining methods of all and is a primary contrast method in medical diagnosis of biopsy specimens. Properly administered, the H & E stain can yield a surprising amount of useful information. Though H & E stains were first implemented at least a century ago, the generalized method is still considered essential for recognition of tissue types and for recognizing morphological indicators diagnostic of cancer pathology. H & E staining has been in use relatively unchanged for many years, the approach has evolved to include a variety of specialized, though related, protocol chemistries for different tissues and contrast emphasis. H & E staining is remarkably robust and works well with a variety of fixatives and is used to discriminate between a broad range of cytoplasmic, nuclear and extracellular matrix features.

The H & E staining method involves application of haematoxylin, a basic dye, to yield a blue-purple contrast on basophilic structures. An acidic eosin counterstains eosinophilic structures bright pink. Various hues can also be present in the sample, including yellow and brown due to intrinsic pigments such as melanin. Hydrophobic structures remain clear; such structures include adipocytes, myelin around neuronal axons, and Golgi membranes.

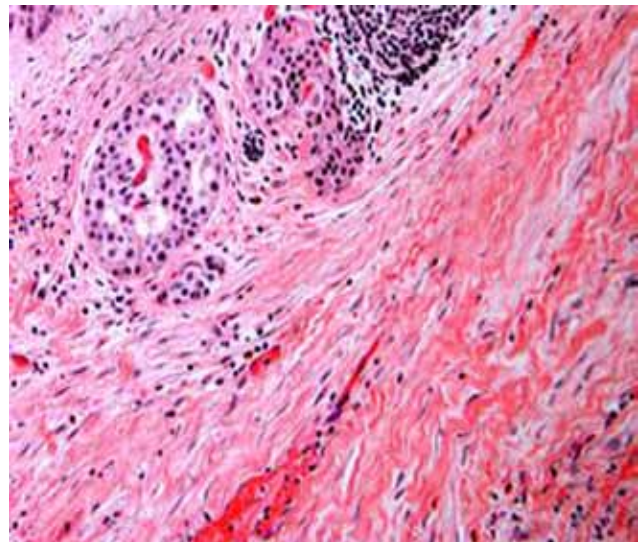


Figure 1. Haematoxylin and eosin stained section of pancreas tissue

General Concept

The H & E staining first involves application of the basic dye haematoxylin. Haematoxylin stains basophilic structures, primarily those containing nucleic acid moieties such as chromatin, ribosomes and cytoplasmic regions rich in RNA. Nuclei show varying cell and cancer specific patterns of heterochromatin condensation that are of noteworthy importance in pathological diagnosis.

The original haematoxylin is the oxidized product of the logwood tree, known as haematein. Presently most haematein is synthesized and oxidized or 'ripened', under controlled and standardized conditions.

Haematoxylin requires a 'mordant' or binding agent to facilitate binding to the tissues, in practice this is generally a metal cation such as aluminum or tungsten or iron. The mordant can influence the intensity and hue of staining. Aluminum mordanted solutions (haemalum) are most common.

The staining strategy for application of haematoxylin can be grouped into two major paradigms: progressive and regressive. A progressive staining approach refers to a process of bathing the sample for an amount of time in the stain, the length of this step determines the staining characteristics and fewer steps are required overall. In a regressive staining strategy, the specimen is effectively overstained then put through a series of acid ethanol washing steps to remove unwanted staining to the desired depth of color and staining characteristics. This washing step is referred to as 'differentiation'.

Haematin exhibits pH indicator-like properties; haematin is red and more soluble under alcoholic acidic conditions and becomes blue and less soluble under aqueous-alkaline conditions. When applied, Haematin is administered from an acid medium and the initial staining is brick red. In this phase the haematin is soluble and must be converted to the blue insoluble form or it will leach into the mounting medium under the coverslip. The conversion is done through altering the pH of solutions bathing the slide. It is customary to place the sample in an alkaline solution to perform the 'blueing' step then to wash with water to neutralize the pH of the tissue section.

Eosinophilic structures are generally composed of protein. Most of the cytoplasm is eosinophilic. Nucleoli stain with eosin.

There are a variety of synthetic eosins, varying in hue. There are three commonly used forms of eosin: eosin yellowish (eosin Y), eosin bluish (eosin B), and eosin alcohol soluble (ethyl eosin). These dyes are all similar in staining characteristics and can be used both progressively and regressively, eosin Y is the most popular and also displays a yellow-green fluorescence.

Eosin staining can be used effectively to distinct intensities of pink hue; eosinophils show the most staining, followed by bright pink erythrocytes. Muscle tissue is somewhat paler but still pink, and collagen generally exhibits a pale pink color.

Documenting H & E stained tissue

H & E stained tissue carries a remarkable amount of information to the trained eye, and accurate and reliable reproduction of the many hues at high resolution in a manner consistent with the image as perceived through the eyepieces of a microscope is a challenging application of camera technology. Furthermore, the

modern constraints of a clinical setting, where emphasis is on rapid diagnosis and high volume, require technologies that produce such images quickly and permit real-time adjustment of acquisition parameters and focus.

The QImaging® Go series of cameras provides a variety of affordable, single-exposure color research-grade cameras ideal for documentation of H & E stained specimens for pathology. QImaging®'s Go-21 camera permits an ideal blend of flexibility through combining fast readout color CMOS technology with an innovative pixel-shifting strategy to permit variable resolution up to a staggering 21-million pixels in 24 or 30 bit color. The Go series' USB 2.0 interface provides high-speed, convenience, and simple plug-and-play installation via a single cable.

What constitutes an ideal H & E stained slide differs from person to person; the requirements of an anatomist may differ from those of a pathologist depending on the structures that need to be discriminated and evaluated. For this reason, user-friendly flexibility in camera acquisition parameters is in important consideration.

QImaging® QCapture Suite software is a part of the Go camera package and provides user friendly and comprehensive control of camera parameters such as white balance, hue, gamma and color saturation for accurate and reliable documentation of staining contrast.

To learn more about the high-performance Go cameras from QImaging, please visit:

<http://www.qimaging.com/products/cameras/publication/index.php>