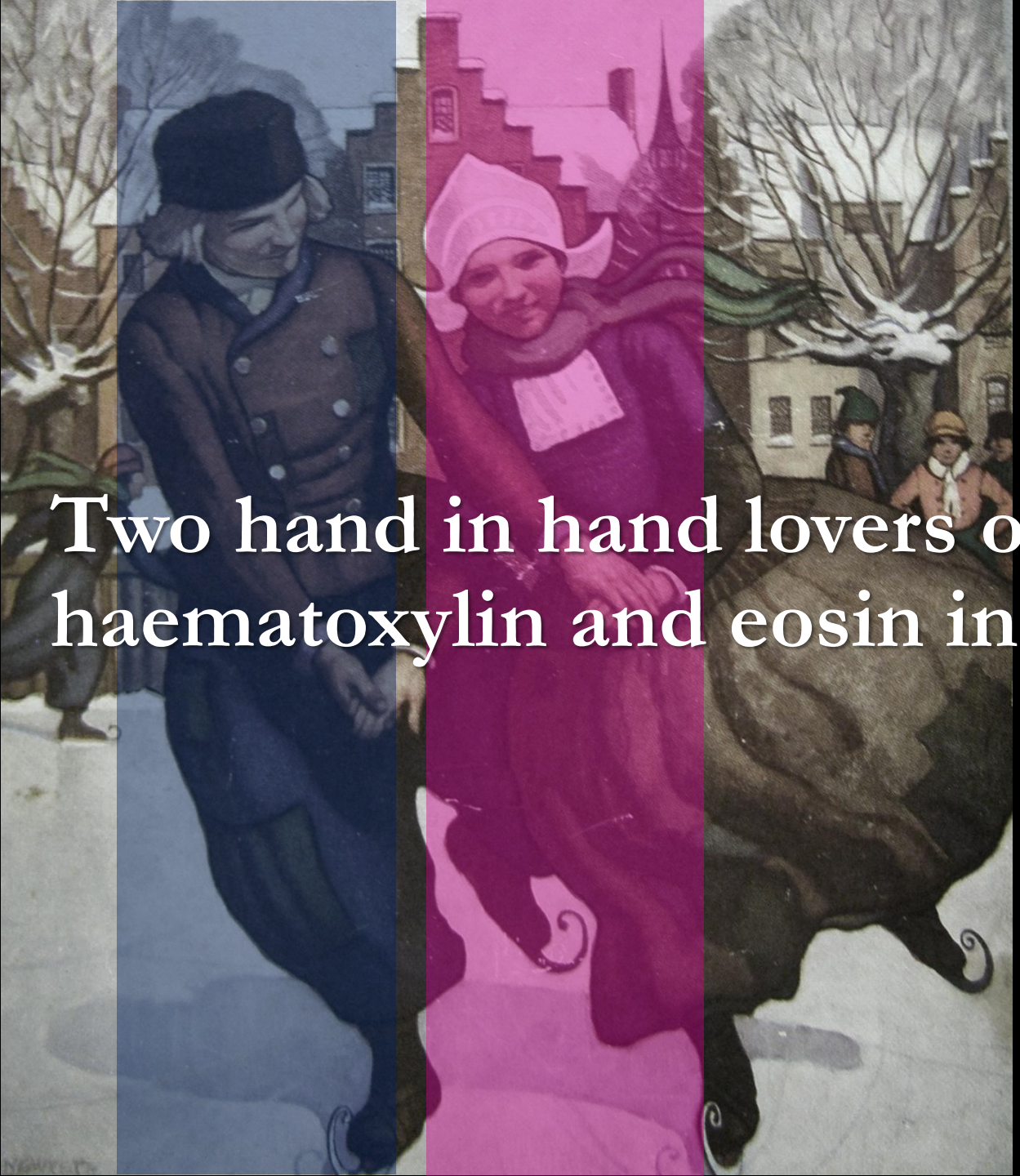


Two hand in hand lovers on a glassy path: haematoxylin and eosin in histology

Raffaella Santi, MD PhD

Careggi University Hospital, Florence, Italy

Paraffin-embedded tissue sections are routinely stained with H&E nowadays. Only when clinical history, gross examination or H&E study suggests it are other "special" stains employed to delineate better features poorly or not all brought out by H&E

Staining is such an integral part of modern histology that it is difficult to think of being without it. H&E have become the inimitable scaffold on which many diagnoses are made every day

HAEMATOXYLIN & EOSIN

HAEMATOXYLIN

Haematoxylum Campechianum
(Logwood)

Titford M, 2005

In the early 16th century when the Spanish explored the coast of the Yucatan Peninsula in search of gold and silver, they found logwood and they introduced it to European textile makers as a competitor to indigo. Logwood soon became a target of piracy, with English, French and Dutch forces all seeking to profit from its use. Its value was such that Spain initially claimed a monopoly on all logwood sales and later, the right to profit from logwood plantations, was part of the political statement that followed the Seven Years' War (1756-1763) between Britain, France and Spain

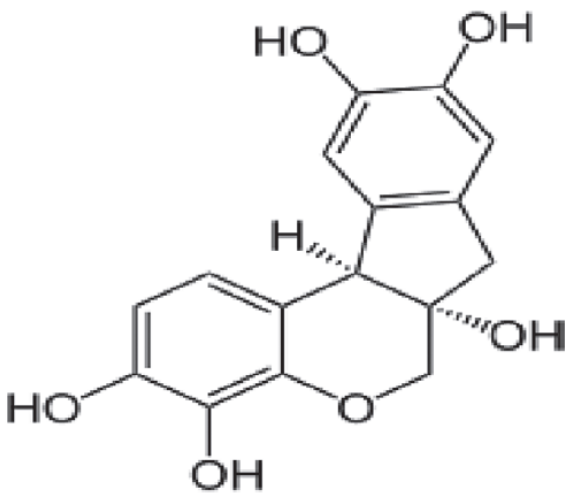
Treaty of Paris (1763)

The earliest recorded European settlers to Belize were shipwrecked British sailors, otherwise known as the "Baymen" who first arrived in 1638. The Baymen became loggers, sending tons of logwood back to England throughout 1700 and 1800s

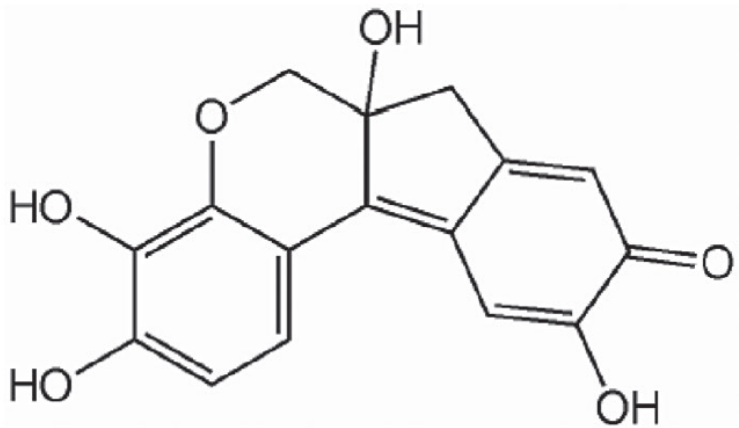
Such was their notoriety that the national flag and currency of Belize depict the Baymen still today

Orchard GE, 2018

Haematoxylin was mainly used as a fabric dye and stained the uniforms of soldiers in the American Civil War (1861-65) and subsequently in the First and the Second World Wars. Its use as a dye underwent a renaissance during the Second World War since allied forces sought sources of dye other than German-manufactured aniline dyes



The molecular structure of haematoxylin prior to 'ripening', via oxidization by natural air and sunlight with boiling water or chemically using either sodium iodate or mercuric oxide and potassium permanganate

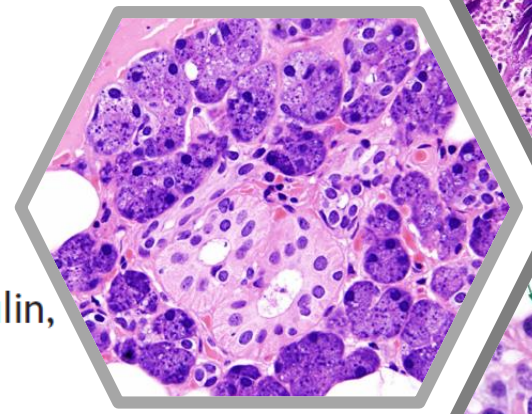


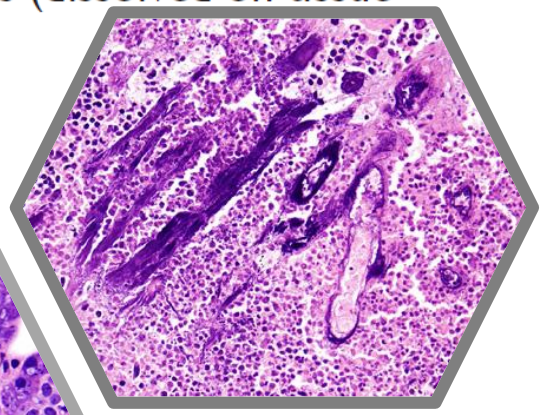
The molecular structure of hematein following the oxidization of the haematoxylin molecule.

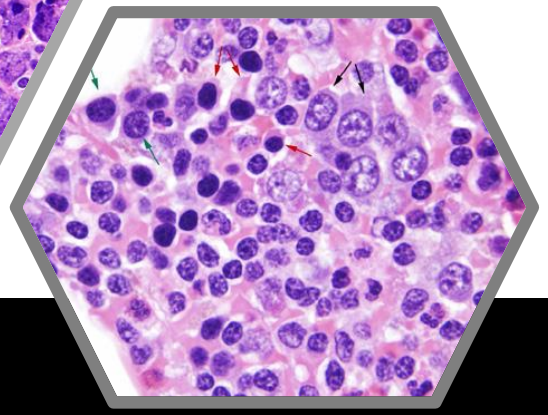
Paul Mayer (1891)

Table 1. Staining Properties of Cellular Constituents by Hematoxylin–Eosin.

Basophilic (Blue)	Eosinophilic (Pink)	No Staining (Empty)
• Nucleus (including nuclear membrane and heterochromatin) • Rough endoplasmic reticulum[a] • Ribosomes[a] • Zymogen granules of serous acinar cells in salivary gland • Catecholamine dense-core (neurosecretory) granules • Acidic mucin	• Cell membrane (including microvilli) • Cilia • Mitochondria • Lysosome • Most types of dense-core (neurosecretory) granules • Smooth endoplasmic reticulum • Intermediate filaments • Myofilaments • Microtubules • Proteins, for example, immunoglobulin, hemoglobin • Nucleolus • Neutral mucin	• Lipid vacuoles (dissolved on tissue processing)







Chan JK, 2014

Owing to its short-lived “fugitive” colorant effect, logwood was initially outlawed in England during the Elizabethan era (1558-1625). This paved the way for the introduction of the mordant, which imparted a long-lasting permanency of the dye

Mordants are derived from heavy metals such as aluminium, iron, lead, tungsten and molybdenum. They are di/trivalent salts or hydroxides of metals which combine with the dye by displacing a hydrogen atom from the dye

The remaining valences of the mordant serve to bind the dye-mordant complex to the tissue components such as phosphate groups to nucleic acids, resulting in a more permanent dye colour

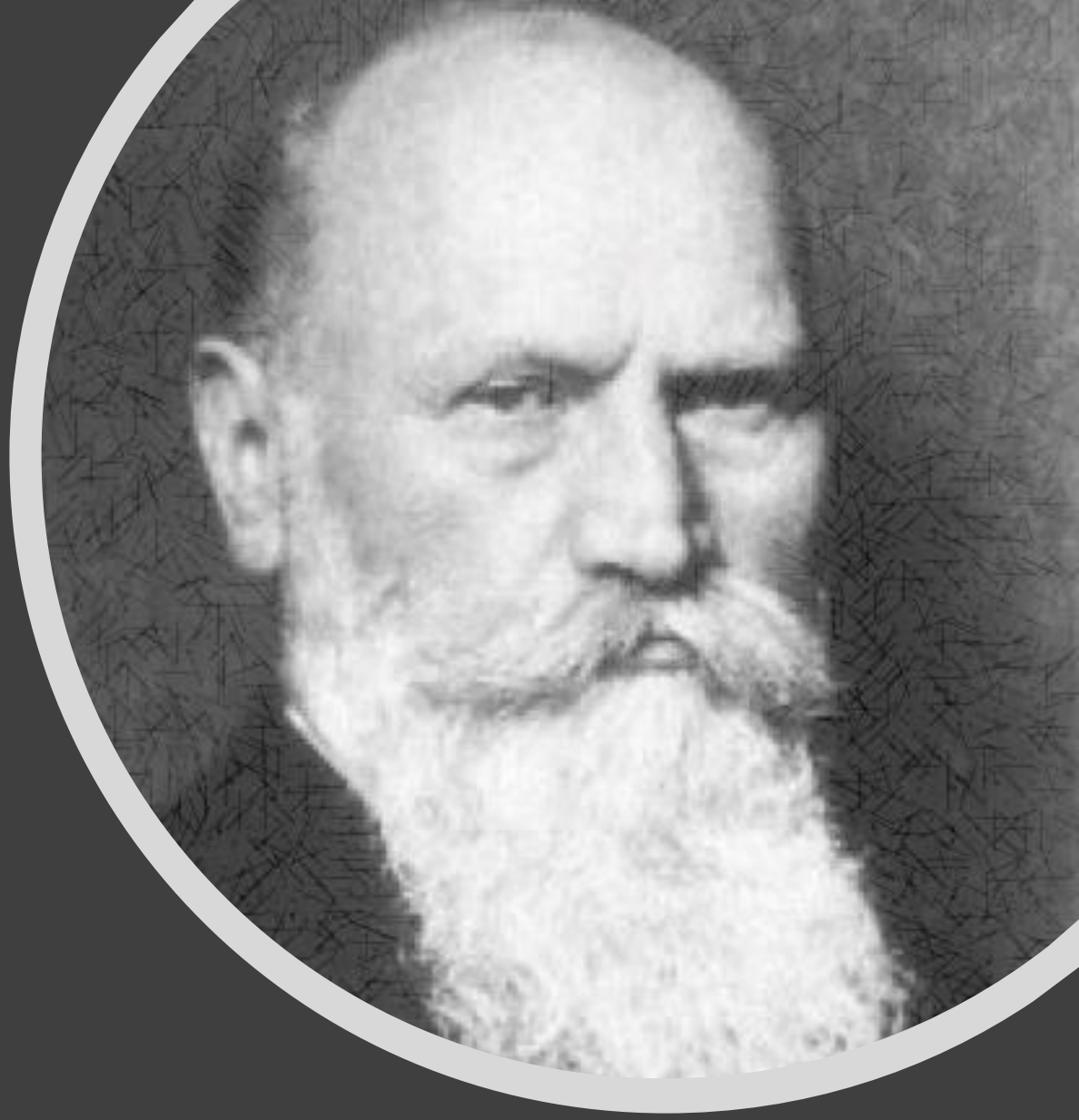
Aluminium haematoxylins	Delafield's haematoxylin (1880)
	Ehrlich's haematoxylin (1886)
	Harris' haematoxylin (1900)
	Mayer's haematoxylin (1903)
	Cole's and Carazzi's haematoxylin (1943)
Iron haematoxylins	Heidenhahn's haematoxylin (1896)
	Weigert's haematoxylin (1904)
	Verhoeff haematoxylin (1908)
Tungsten haematoxylins	Phosphotungsten acid haematoxylin (PTAH)
	Mallory haematoxylin (1897)
Molybdenum haematoxylins	Thomas's haematoxylin
	Mallory haematoxylin (1891)

Coleman R, 2006

Heinrich Wilhelm Gottfried von Waldeyer Hartz (1836-1921)

The first histologic application of haematoxylin is attributed to Waldeyer when in 1863 unsuccessfully applied a crude aqueous extract of logwood chips to tissue sections. However, debate continues with reference to Reichel's work much earlier (1758), with the use of logwood for microscopic staining to study plant material

In 1865, Franz Böhmer, adding alum as a mordant, did succeed in staining tissues with haematoxylin. Alum had already been widely used in the dye industry as a mordant, which Waldeyer had overlooked



King DF, 1986

Paul Ehrlich (1854-1915)

More selective nuclear staining was achieved when in 1886 Ehrlich added acetic acid to the solution

A Nobel Prize (1908), famous for his studies in the field of immunology and renowned for his original contribute in the fields of batteriology, hematology, experimental oncology and chemotherapy as well in histology

During the late 1870's Paul Ehrlich applied specific acid ad basic aniline dyes to blood smears and observed that different blood cells stained with basic dyes, but others with acidic dyes. This led to the terms "eosinophil, basophil and neutrophil". Ehrlich's stain formulation is the predecessor of the Romanovsky stain that is currently used today


EOSIN

Table 1. Staining Properties of Cellular Constituents by Hematoxylin–Eosin.

Basophilic (Blue)	Eosinophilic (Pink)	No Staining (Empty)
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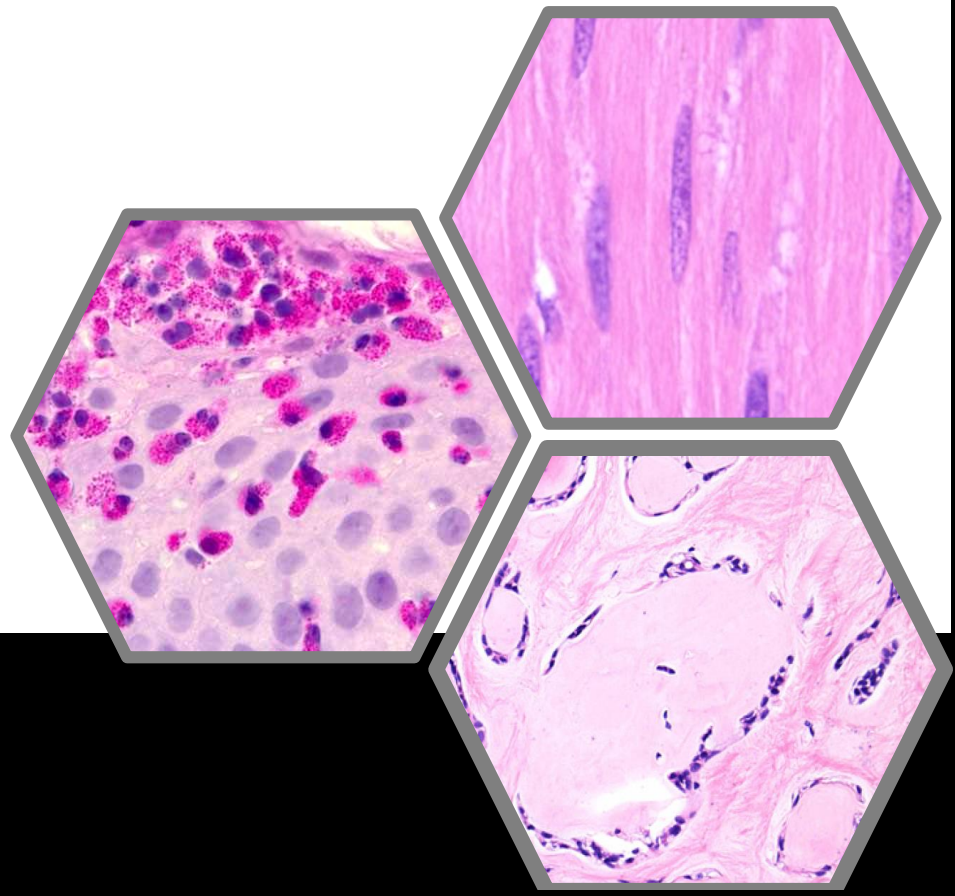
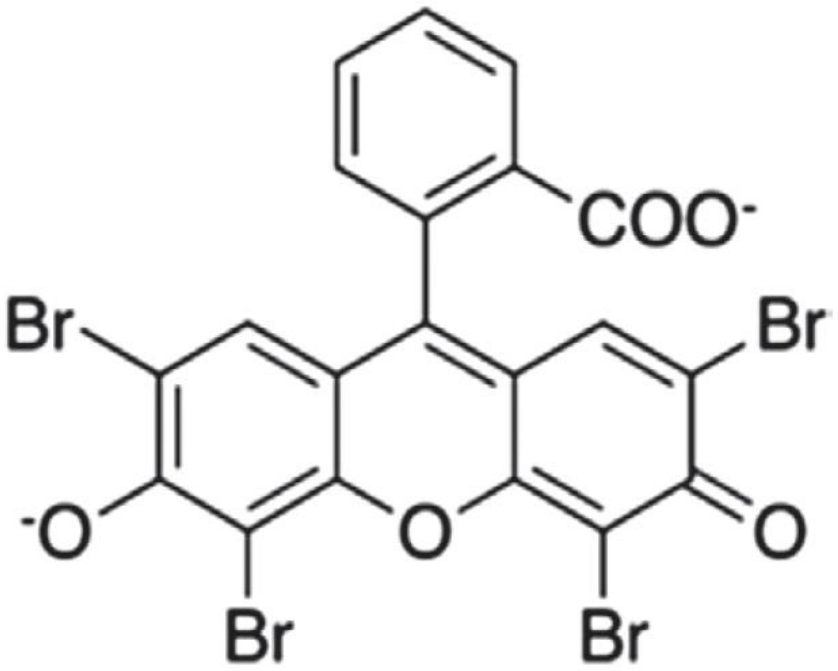
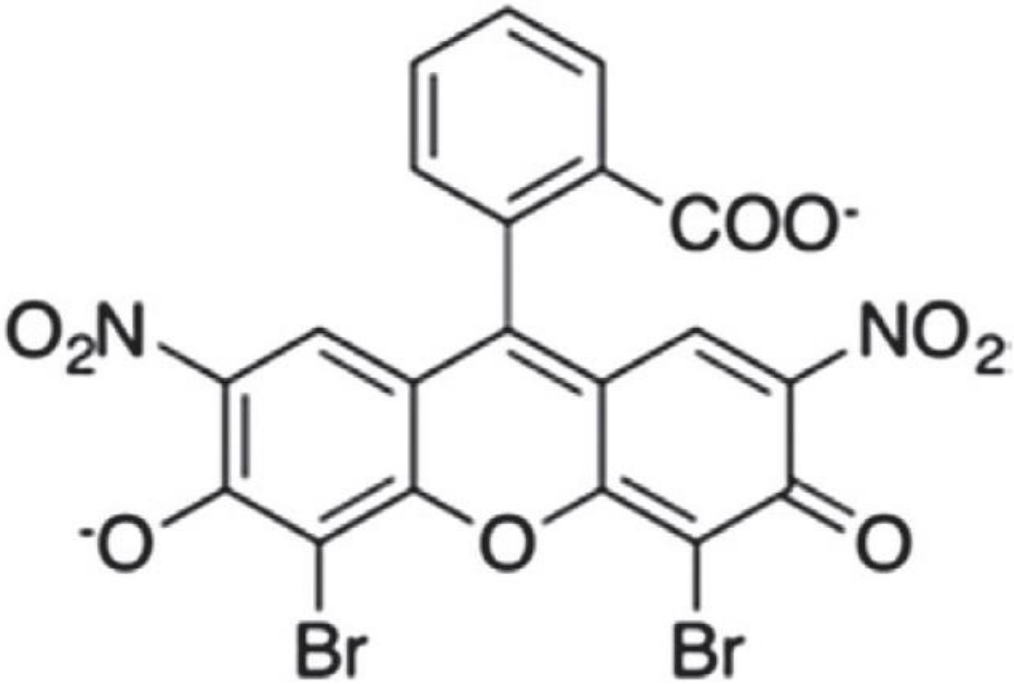


Chan JK, 2014

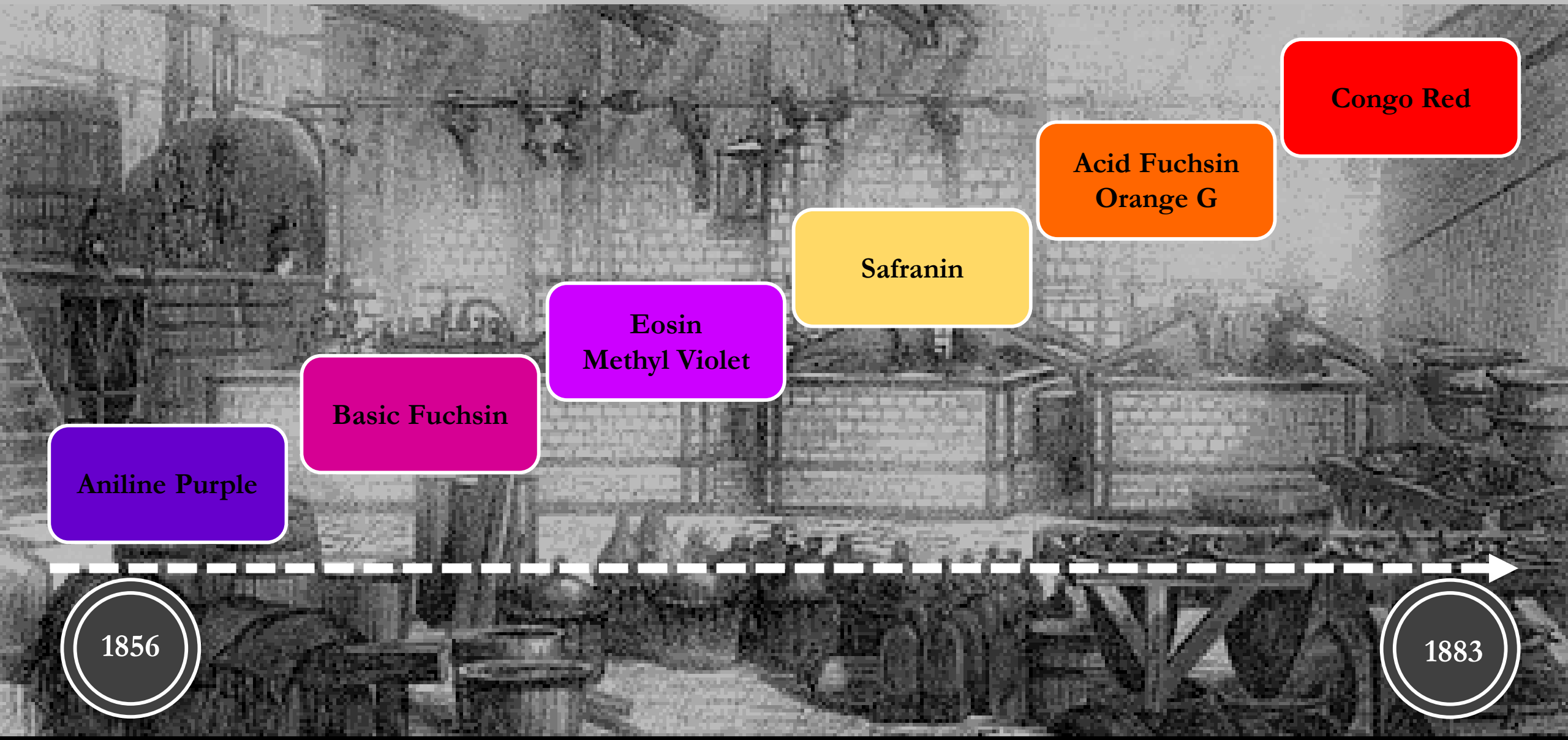


Eosin Y; a tetrabromo derivative of fluorescein

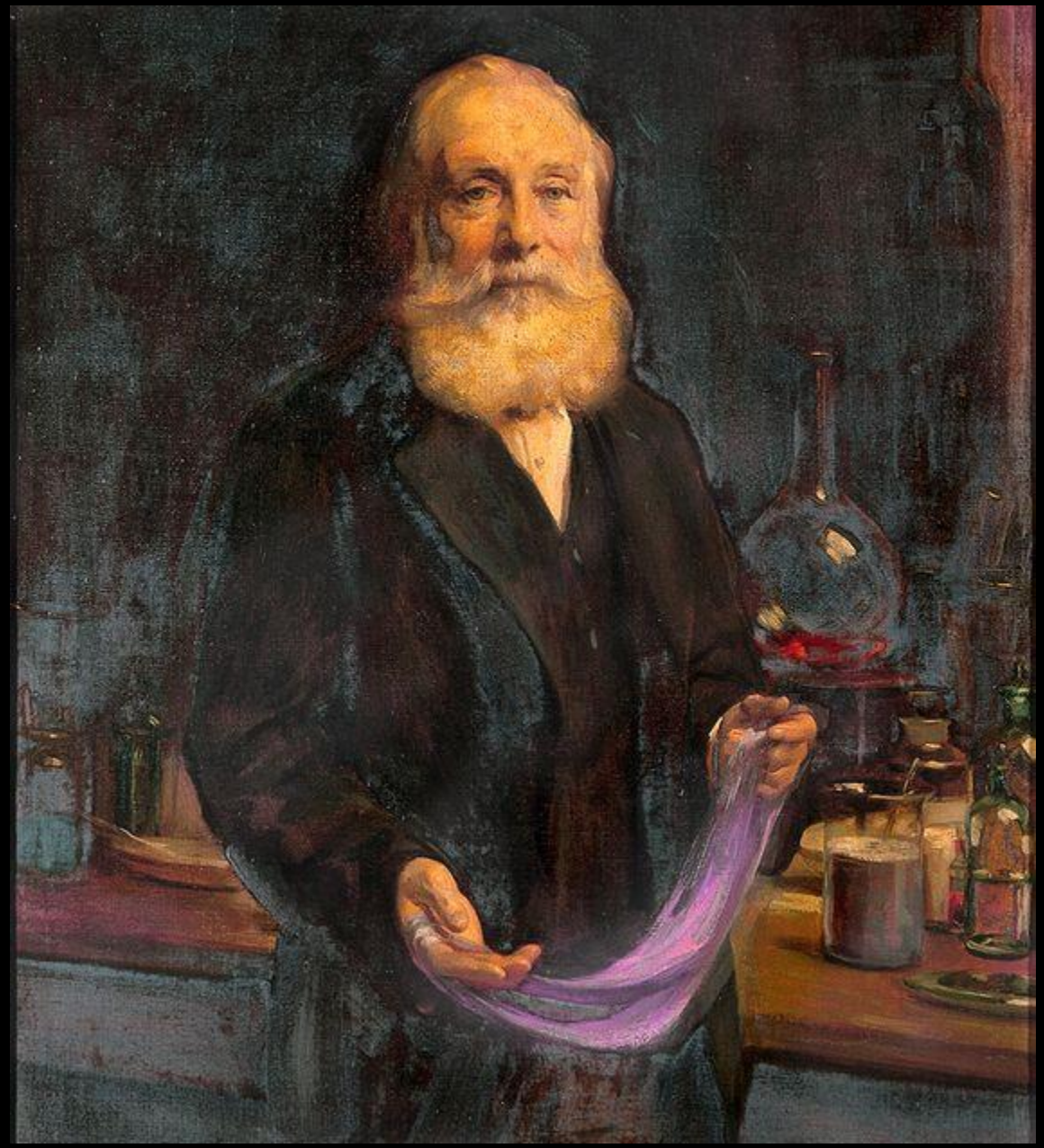


Eosin B; a dibromo-dinitro derivative of fluorescein

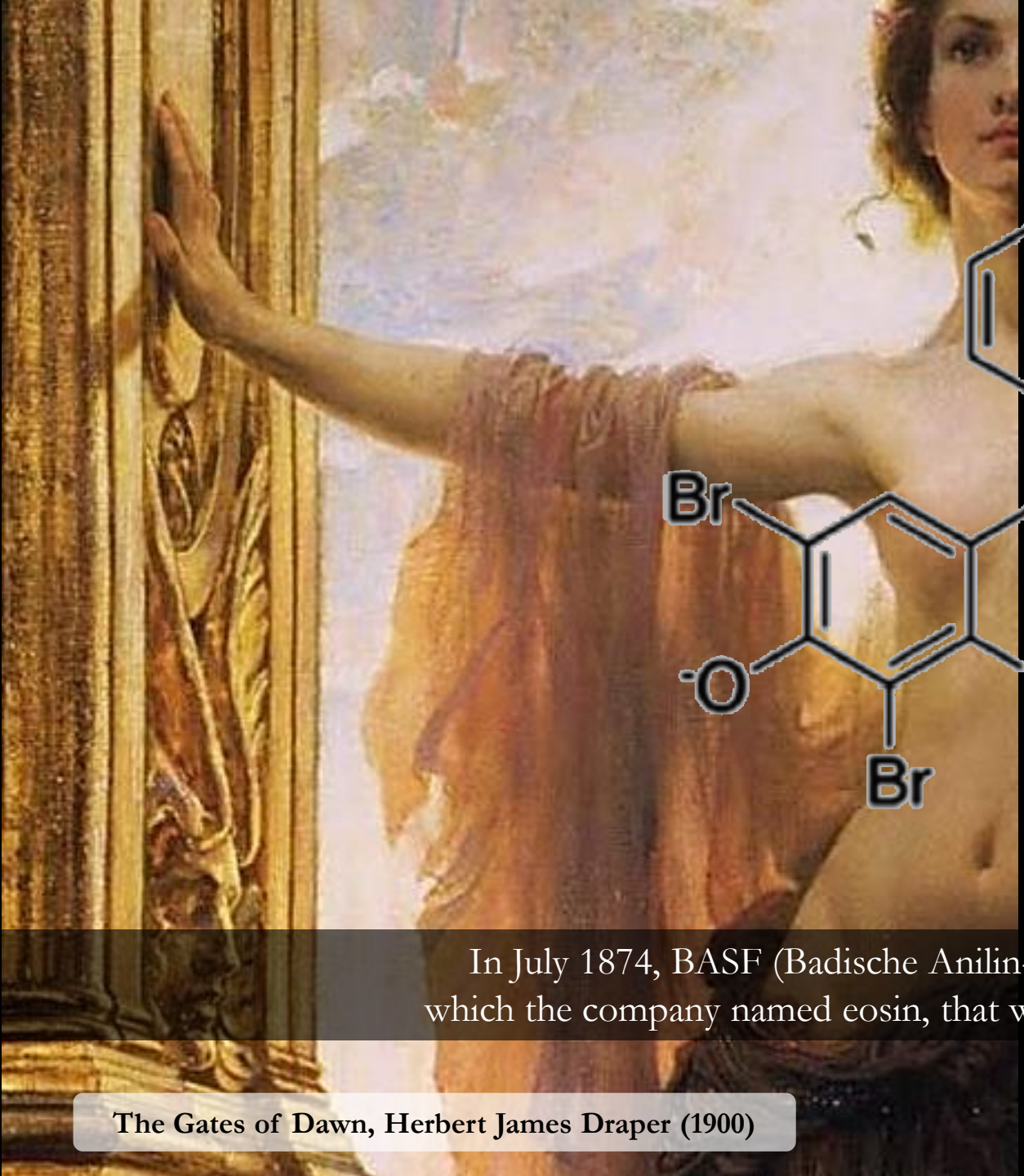
Aniline Dyes

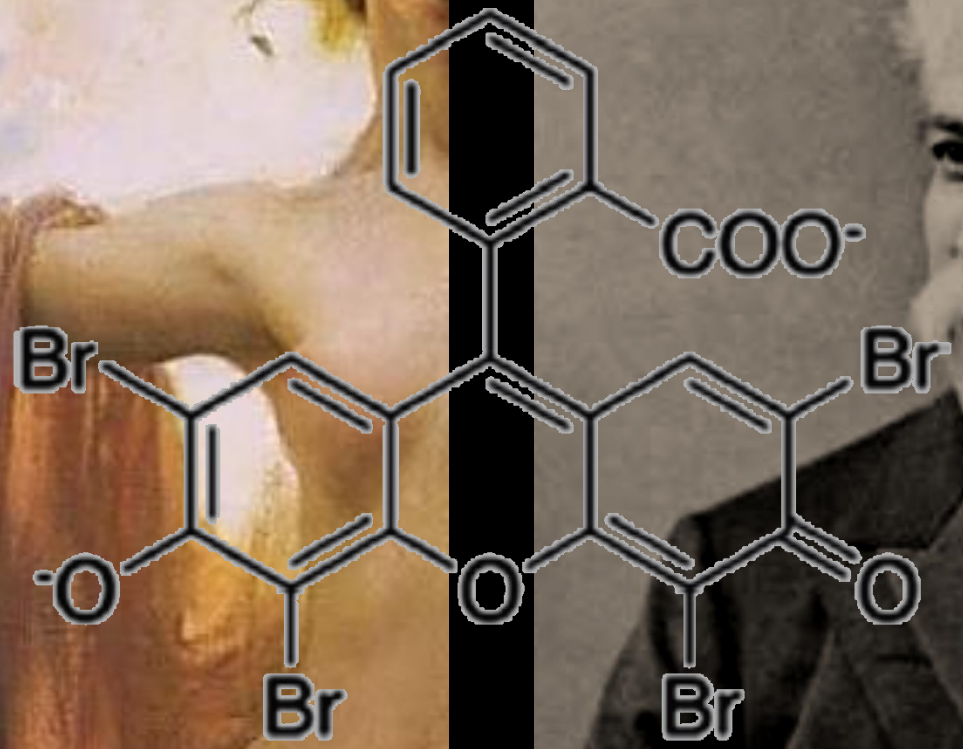


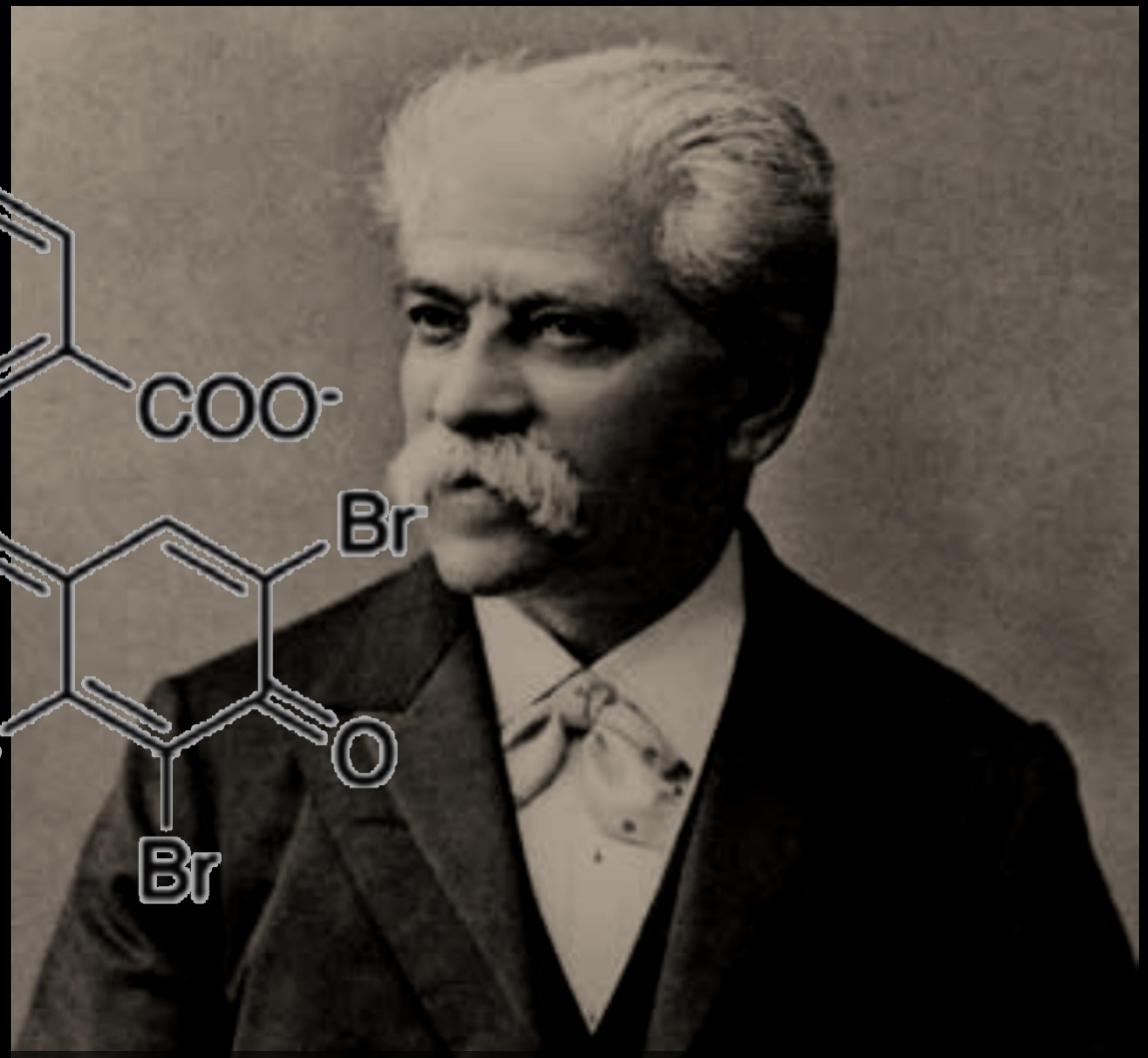
Mod. From Gal AA, 2001



William Henry Perkin (1838-1907)



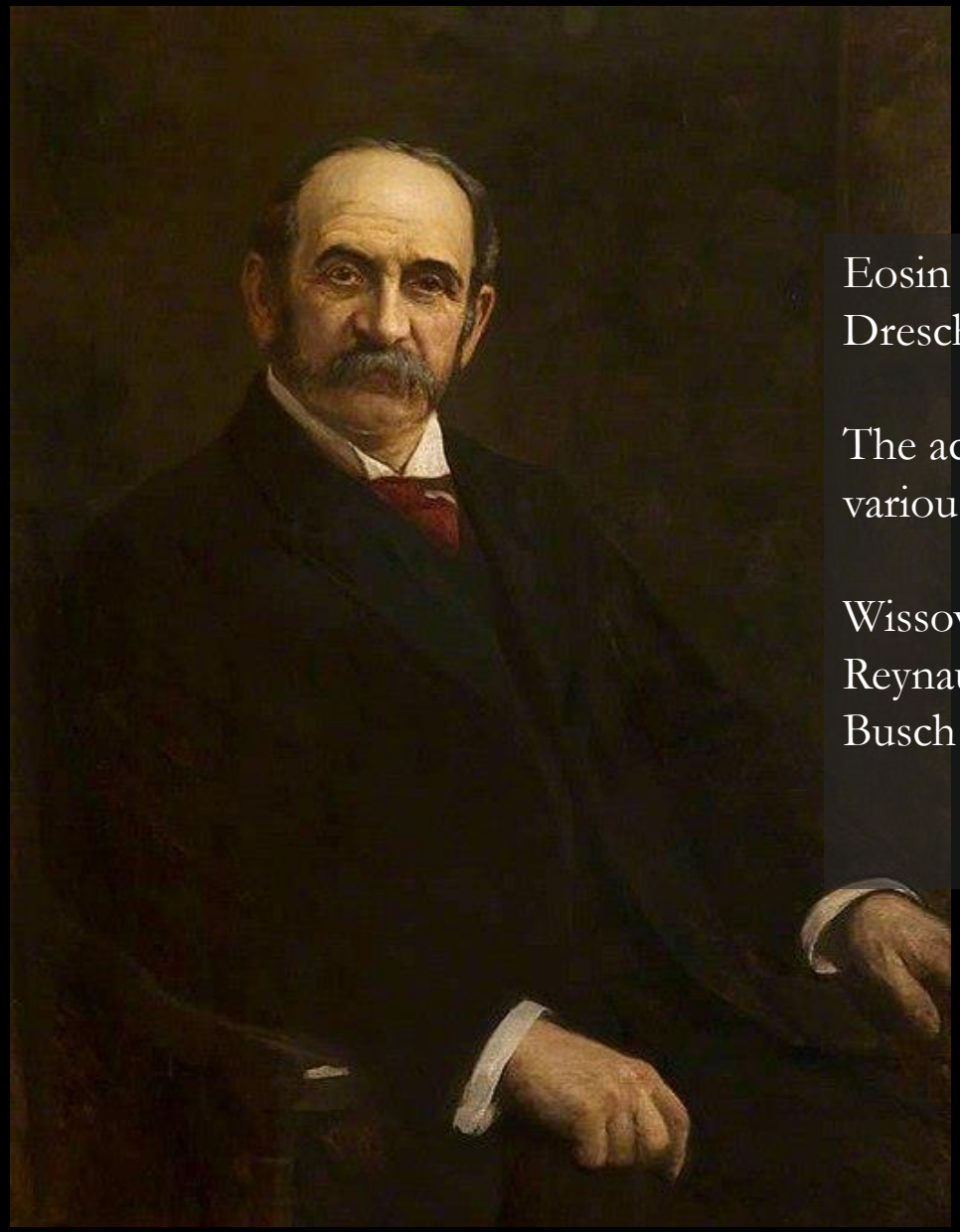




In July 1874, BASF (Badische Anilin-&Soda-Fabrik) announced a new dye, which the company named eosin, that was claimed to be especially useful for silk

The Gates of Dawn, Herbert James Draper (1900)

Heinrich Caro (1834-1910)



Eosin had been reported as a general stain for tissues by Dreschfeld and Fischer in the 1870s

The actual combination of H&E to form a single method has been variously attributed to:

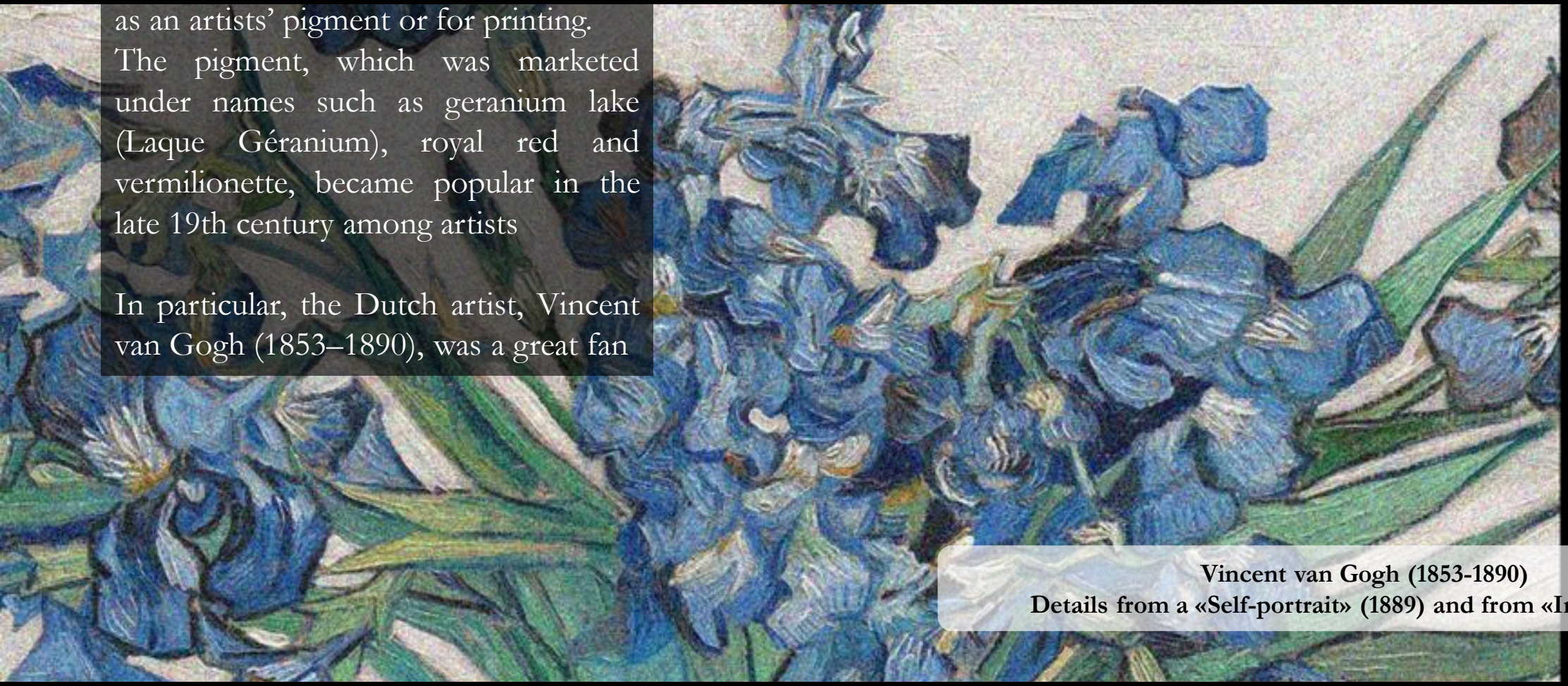
Wissowzky (1875)
Reynaud (1876)
Busch (1876-1878)

Julius Dreschfeld (1845-1907)

Cook HC, 1997

Soon after the introduction of eosin as a dye, it was found that eosin gave a bright red lake that could be employed as an artists' pigment or for printing.
The pigment, which was marketed under names such as geranium lake (Laque Géranium), royal red and vermilionette, became popular in the late 19th century among artists

In particular, the Dutch artist, Vincent van Gogh (1853–1890), was a great fan



Vincent van Gogh (1853-1890)
Details from a «Self-portrait» (1889) and from «Irises» (1890)

Cooksey CJ, 2018

…Then I have a canvas one meter long by only 50 centimeters high, of fields of wheat, and one that makes a pendant of undergrowth, lilac trunks of poplars, an underneath them some flower-dotted grass, pink, yellow, white and various greens…

Vincent van Gogh, Undergrowth with Two Figures (1890)

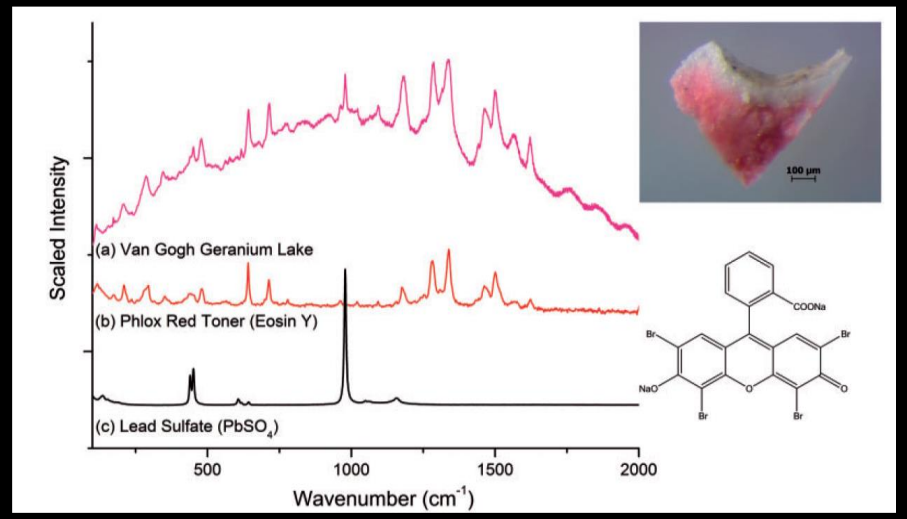
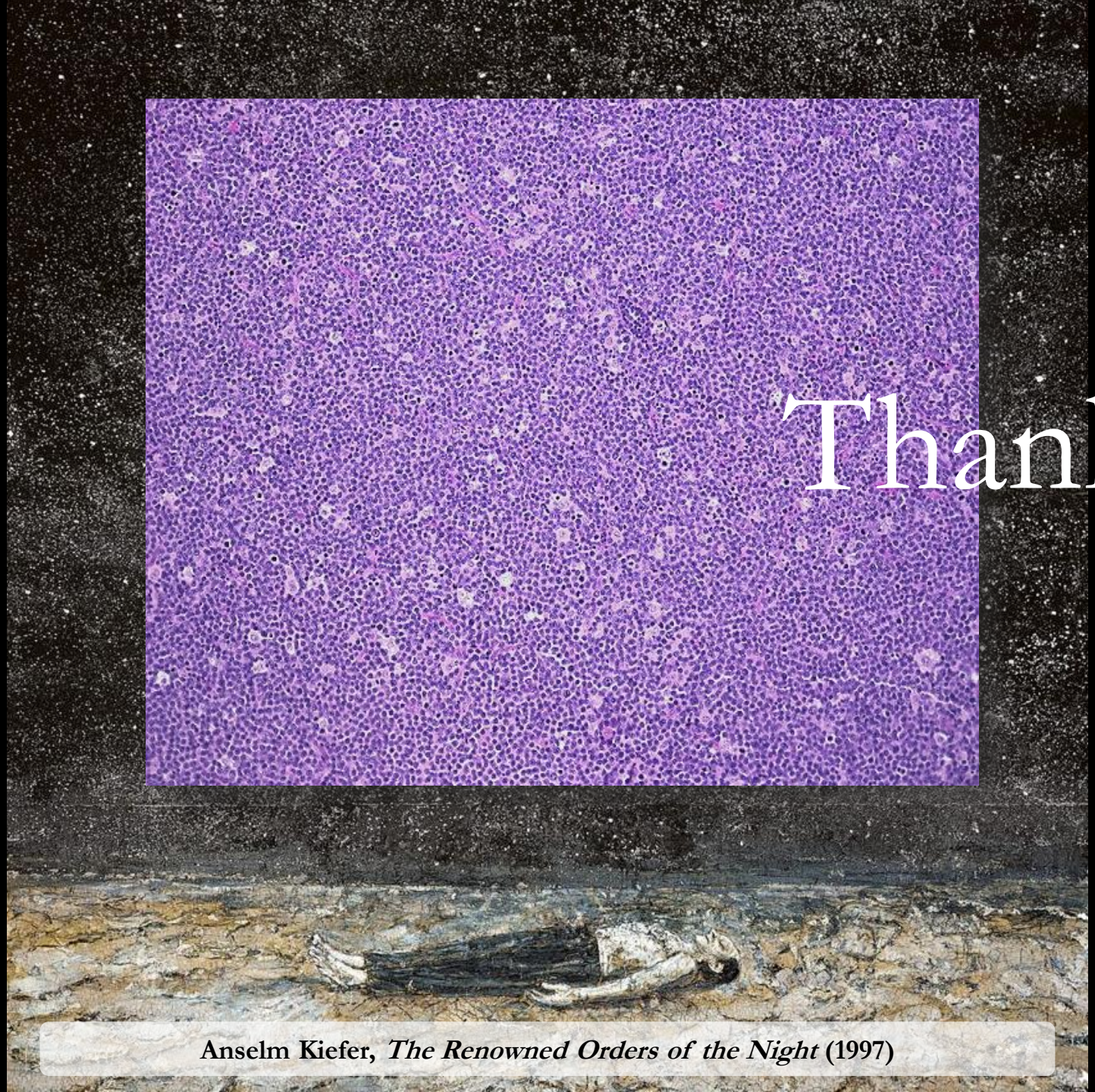


Table 1. Pigments found in *UwTF* and the relevant analytical techniques used to identify them.

Pigment	Chemical formula	Excised (E) or in situ (I)	Raman	SEM-EDS	XRF
Geranium lake	Eosin dye on $PbSO_4$	E, I	X	X	X
Lead white	$2PbCO_3.Pb(OH)_2$	E, I	X	X	X
Zinc white	ZnO	E, I	X	X	X
Chrome yellows[69]	$PbCrO_4$, $PbCrO_4 \cdot xPbSO_4$ ($x < 0.4$)	E, I	X	X	X
Vermilion	HgS	E, I	X	X	X
Ultramarine	$Na_{8-10}Al_6Si_6O_{24}S_{2-4}$	E, I	X		
Cobalt blue	$CoO{\cdot}Al_2O_3$	E, I		X	X
Prussian blue	$Fe_4[Fe(CN)_6]_3$	E, I	X		X
Viridian	$Cr_2O_3{\cdot}2H_2O$	E, I	X	X	X
Emerald green	$Cu(CH_3COO)_2{\cdot}3Cu(AsO_2)_2$	E, I	X		X
Carbon black	C	E	X		
Red lead	Pb_3O_4	E, I	X		X

Fieberg JE, 2017

..paintings fade like flowers…



Anselm Kiefer, *The Renowned Orders of the Night* (1997)

Thank You