MILESTONE 2

Stains and fluorescent dyes

Fluorescein powder dropped into a solution of tap water under ultraviolet light, after approximately 15 seconds have elapsed. Image is reproduced with permission from Bricksnite and licensed under the Creative Commons Attribution 3.0 Unported License (http://creativecommons.org/licences/by/3.0/)

Robert Hooke may have been the first to describe the light microscopic appearance of a stained object, in the form of dyed wool and hair (see MILESTONE 1). And although others subsequently reported the use of staining solutions, any differential staining that was observed is thought to have been mostly accidental. Hartig and Osborne, who independently observed the colouring of the cell contents in plants, are sometimes cited as the discoverers of staining in microscopy, but neither contributed significantly to the development of the technique. Hartig even casually mentioned the use of carmine, a dye derived from female scale insects, in an 1854 paper.

It was Joseph von Gerlach who, in 1858, while experimenting with solutions of carmine and leaving a section of brain tissue in a dilute carmine solution overnight, reported good differential staining of the nucleus and nuclear granules compared with little or no staining of the cytoplasm and intercellular substance. He concluded that previous staining solutions had been too concentrated, and also noted that the dye was absorbed by specific cellular elements and could not be washed out. Von Gerlach therefore deserves credit for recognizing the importance of staining and for carefully describing his staining method.

> “… it was not long before Adolf von Bayer synthesized the first fluorescent dye …”

The late 1800s and early 1900s marked the discovery of many cytological phenomena and the development of new stains and synthetic dyes. The use of silver staining in cytology was pioneered by Camillo Golgi in 1873 and made famous by Santiago Ramón y Cajal's detailed neuroanatomical observations. The notion that basic and acidic dyes are histologically distinct was important for the development of the haematoxylin and eosin stain (Paul Mayer, 1896), which became a key diagnostic stain. An acidic dye (eosin) and a basic dye (methylene blue) also form the basis of the Giemsa stain (Gustav Giemsa, 1904), which is still used to diagnose malaria and other parasites. Robert Feulgen's discovery in 1924 that chromosomal material can be stained by a chemical reaction based on acid hydrolysis of DNA became a cornerstone of cytochemistry. The cytochemical staining of peroxidase activity, as reported by Graham *et al.*, was subsequently developed as an immunohistochemical approach by Sternberger *et al.* in 1970.

Whereas stains provide finite contrast by changing the light absorption properties of different cellular structures, fluorescence provides infinite contrast with the right equipment — although such equipment obviously did not exist when fluorescence was discovered, and is still being improved today. The earliest description of fluorescence is thought to date from the sixteenth century, when Nicolás Monardes reported the fluorescent properties of wood extract from *Lignum nephriticum* (Athanasius Kircher described similar observations nearly a century later). John Herschel's description of the fluorescent properties of quinine sulphate in 1845 is considered the 'modern' milestone for observing fluorescence and realizing what it was, together with George Stokes's 100-page monograph published in 1852 that describes a vast collection of fluorescent substances, from quinine sulphate to Oporto wine. Although David Brewster first used the term 'internal dispersion' to describe fluorescence phenomena in 1838, it was Stokes who coined the term 'fluorescence' to describe light emission induced during excitation.

With the development of the synthetic dye industry, it was not long before Adolf von Bayer synthesized the first fluorescent dye, fluorescein, in 1871. Paul Ehrlich used the fluorescent dye uranin (a sodium salt of fluorescein) in 1882 to determine the pathway of secretion of aqueous humour in the eye — representing the first use of a fluorescent dye in animal physiology. In 1914, not long after the first fluorescence microscope was developed (see MILESTONE 4), Stanislav von Provazek used fluorescent dyes at a microscopic level as a means to enhance the autofluorescence of cells and tissues — representing the first use of a fluorescent dye as a stain in cell biology.

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Nature Reviews Molecular Cell Biology

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