

T is for Toluidine Blue

A-Z of Staining - a series of articles where we share a little extra information about stains, staining techniques and some of the interesting chemicals associated.



Welcome to ABC of Staining series where we continue with the letter 'T' for the dye Toluidine Blue.

Toluidine blue (TB) is a cationic thiazine dye with a high affinity for acidic tissue components. Also known as tolonium chloride, it is a basic metachromatic dye that produces different colours by interacting with the acidic elements, causing the dye to change its light absorption properties. This phenomenon is known as metachromasia and leads to a colour shift from that of the original dye. When staining cartilage, the dye binds to complex carbohydrate molecules known as glycans, resulting in a reddish-purple colour to the chondrocytes against a blue background (Figure 1a). Similarly, TB stains mast cell granules purple (Figure 1b) as a result of their heparin-rich content, while Nissl bodies of nerve cells stain dark blue due to the high amount of RNA present (Figure 1c).

Another important use of TB is in the rapid diagnosis of frozen sections, where it provides effective

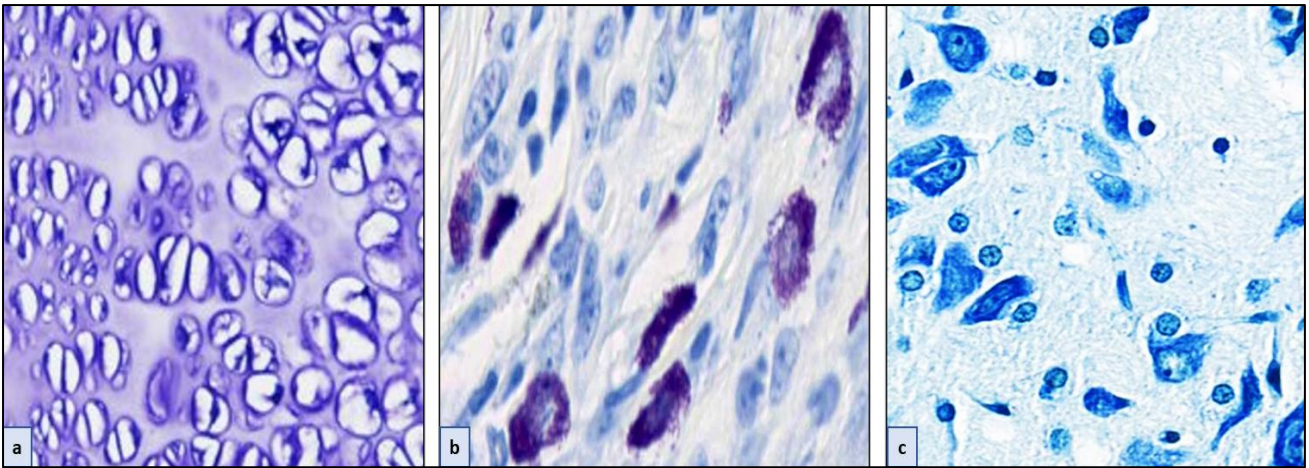


Figure 1. Toluidine blue staining of Chondrocytes (a), Mast cell granules (b), and Nissl bodies (c)

preparation and analysis of tissue samples taken during both general and Mohs micrographic surgery. In the Mohs technique, the dye imparts an identifiable staining pattern on skin samples by highlighting islands of cancerous cells in blue. With surrounding stromal tissue mucins metachromatically staining a magenta colour, the dye has become a valuable diagnostic tool in the Mohs workflow for assessment of surgical tumour margins (Figure 2).

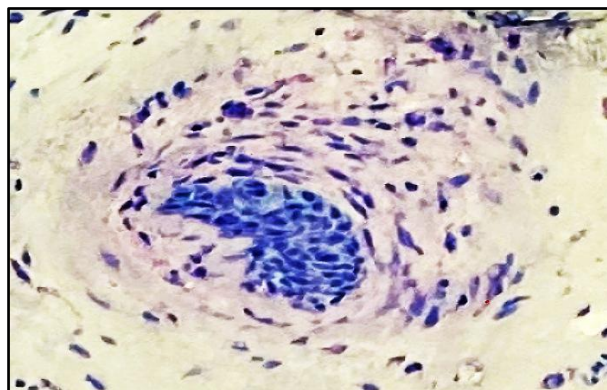


Figure 2. Toluidine blue staining of basal cell carcinoma and stromal mucin

For tissues that have been embedded in resin, TB is a useful dye to highlight tissue components in resin sections. For example, when examining undecalcified bone, the dye has been used to stain both mineralized (Haversian systems and cement lines) and unmineralized (osteoid) bone in resin sections (Figure 3). The method is an important tool for studying the histomorphometry of bone as it stains the mineralization front where new bone is being deposited. The stain is also ideal for use in electron microscopy where it is used especially for orientating semi-thin resin sections by providing high-resolution detail of samples prior to the cutting of ultra-thin sections for EM studies. Although all structures stain blue, cellular details are clearly visible because of the thinness of the sections.

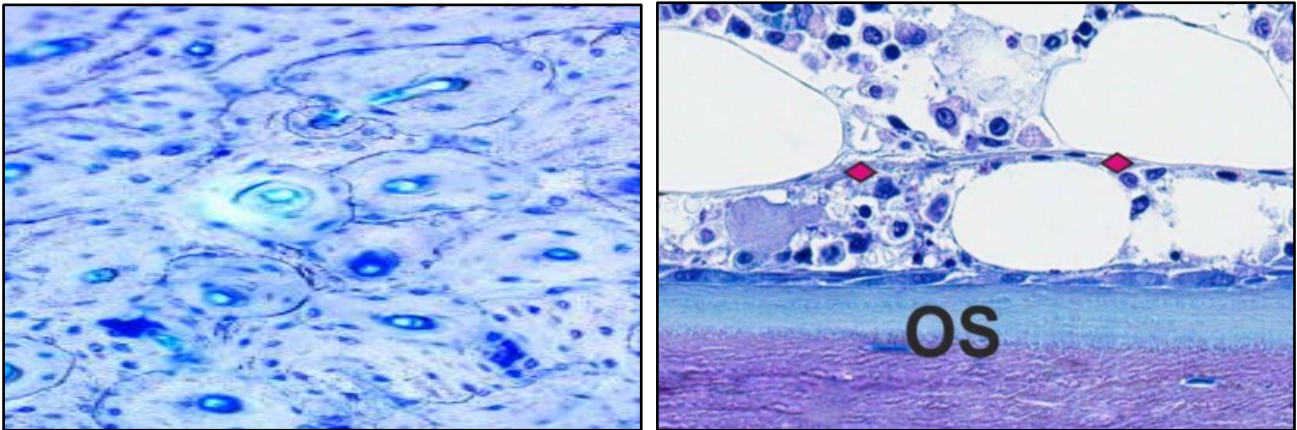


Figure 3. Toluidine blue staining of undecalcified bone showing osteons with central Haversian canals and cement lines (left image). On the right, the unmineralized osteoid seam (OS) is stained pale blue, whilst the mineralized bone matrix below, and the bone marrow cells above stain darker

As well as staining fixed tissue paraffin and resin sections, Toluidine blue can also be used as a vital stain, where it is used to stain cells and tissues in the living state. There are two techniques of vital staining, namely, intravital staining in the living body (*in vivo*) and supravital staining outside the body (*in vitro*). Although the stain has been widely used for detecting mucosal abnormalities of the cervix, another application has involved an oral rinse to identify suspicious oral lesions that are at a higher risk of progressing to cancer. Because abnormal cells have a higher nucleic acid content, they retain the dye more intensely than surrounding healthier tissue. *In vivo* staining of oral epithelium has been recommended as a means of surveillance in patients who are at a risk of developing oral cancer and for those who had confirmed neoplasms of the aerodigestive tract.

Further reading:

1. Sridharan G & Shankar AA (2012). Toluidine blue: A review of its chemistry and clinical utility. *Journal of Oral & Maxillofacial Pathology* 2012;16(2):251-255. doi: 10.4103/0973-029X.99081
2. Long T et al (2023). The role of toluidine blue in Mohs micrographic surgery: A systematic review. *Cutis* 2023;112(6):E6-E11. doi: 10.12788/cutis.0915
3. Peev S et al (2024). A modified protocol for staining of undecalcified bone samples using toluidine blue. *Applied Sciences* 2024;14(1):461. doi: 10.3390/app14010461

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