

O is for Oil Red O

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 our A-Z staining series where we continue with Oil Red O. This dye is a hydrophobic, fat-soluble, red diazo compound and one of the oldest and simplest dyes for demonstrating lipids in tissue sections. Lipids are generally classified as simple (fats, oils and waxes), compound (phospholipids and glycolipids), and derived lipids (such as cholesterol). They have the common characteristic of being insoluble in water but soluble in organic solutions such as ethanol and chloroform. Consequently, frozen sections of formalin-fixed tissues are generally used as routine paraffin sections are not suitable for the demonstration of lipids as many of them are removed by the reagents used during tissue processing. Oil Red O has the chemical formula ($C_{26}H_{24}N_4O$) and is used for the staining of simple lipids such as neutral triglycerides (Figure 1a). Also known as Solvent Red 27 and Sudan Red 5B, the dye has

largely replaced the Sudan dyes III and IV as it provides a deeper red colour. The dye has many applications in cellular pathology such demonstrating fat emboli (Figure 1b) and diagnosing diseases such as steatosis (excess fat deposits in the liver) and liposarcoma (malignant tumour arising from fat cells).

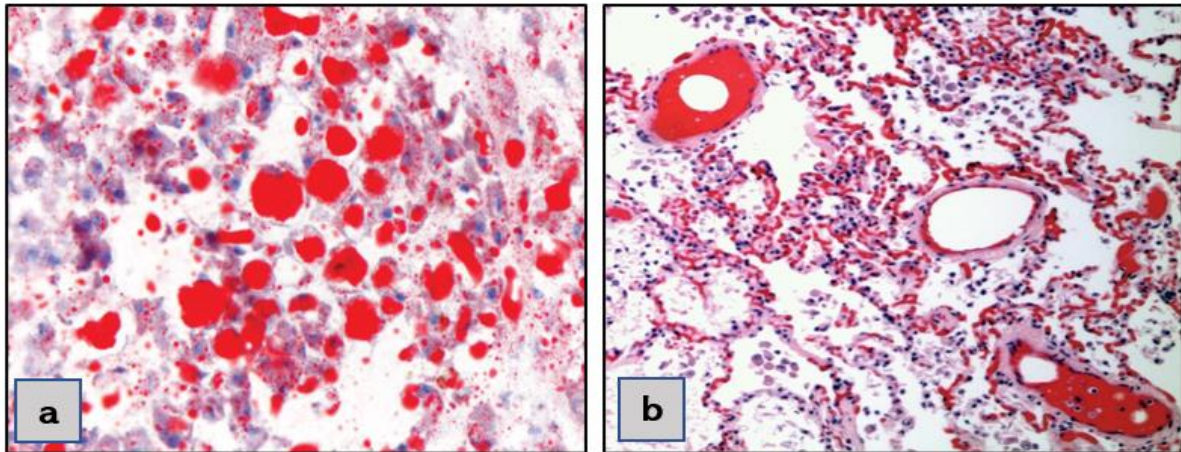


Figure 1. Oil Red O staining of (a) simple lipids and (b) fat emboli in lung

Oil Red O is also the stain of choice when assessing lipid distribution in the muscle of patients with lipid storage diseases. Excess accumulation of lipid in peripheral tissues is a key feature of many of these diseases and methods for quantifying it are important in understanding them. By staining with Oil Red O, the amount and distribution of lipid droplets and neutral lipids can be easily assessed in the muscle of patients with enzyme-deficient lipid myopathies such as multiple acyl-CoA dehydrogenase deficiency (MADD) and carnitine palmitoyl transferase (CPT) deficiency (Figure 2).

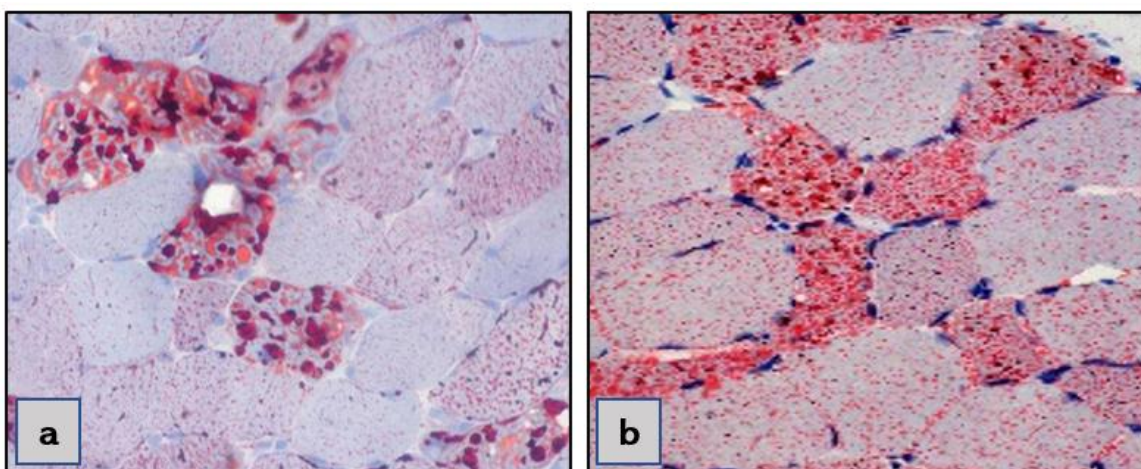


Figure 2. Oil Red O staining of muscle confirming the presence of excess lipid in the metabolic deficiencies of (a) MADD and (b) CPT

In addition, the labelling and tracking of exosomes in benign and malignant diseases also benefit from the staining properties of Oil Red O. Generated by all cells, exosomes are extracellular vesicles containing nucleic acids, lipids and proteins. The potential of them as diagnostic and therapeutic tools have enhanced their role as biomarkers in both the development and treatment of cancer. The method of Oil Red O is both sensitive and effective for monitoring the uptake of exosomes in live cells by exhibiting a distinct red fluorescence under confocal microscopy (Figure 3).

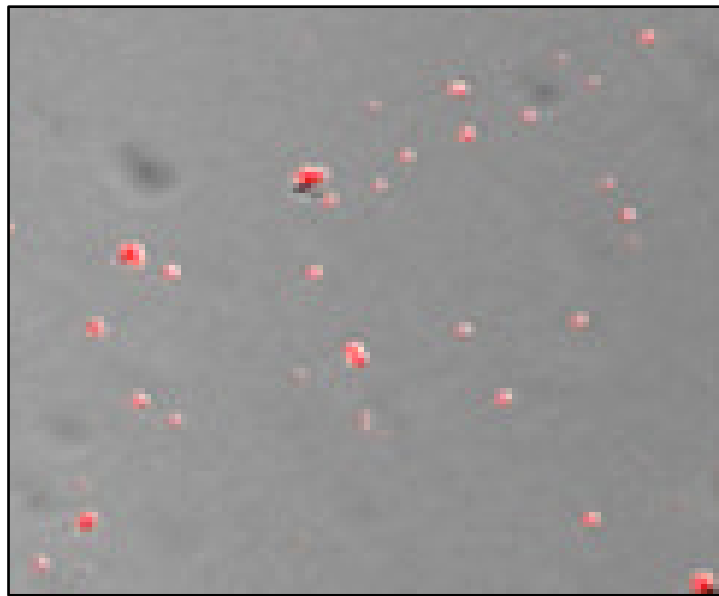


Figure 3. Exosomes showing red fluorescence with Oil Red O



Figure 4. Development of fingerprints on paper using Oil Red O

In forensic pathology, the dye has been used to develop latent fingerprints by interacting with fats and grease present on surfaces of wet or dry materials such as paper and cardboard (Figure 4). Although the effectiveness of the dye is known to decrease noticeably for prints older than four weeks, the technique is non-destructive and does not destroy or prevent the use of other techniques being used on the material. In addition, Oil Red O has been used to detect damage caused by oil and grease in the textile industry and is also an active component of red-coloured smoke in pyrotechnics.

Further reading

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<https://doi.org/10.1016/j.forsciint.2022.111417>

Mehlem A, Hagberg C et al. Imaging of neutral lipids by oil red O for analyzing the metabolic status in health and disease. *Nature Protocols* 2013;8:1149-1154.
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Thangaiah JJ, Booth CN et al. Oil red O staining of pulmonary macrophages in broncho-alveolar lavage specimens is not specific for vaping-associated lung injury. *American Journal of Clinical Pathology* 2022;158(6):723–729.
<https://doi.org/10.1093/ajcp/aqac118>

Dr Phil Bryant
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