WHITEPAPER

Sectioning: A Deeper Look

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Sectioning: A Deeper Look

There are numerous factors that must be considered when preparing tissue for IHC staining. Due to the diversity of tissue and media that may be involved, there is significant variability in sample preparation and no single answer for best practices. However, some key contributing factors can be identified. One oft-overlooked factor is sectioning thickness.

The amount of light permitted to pass through the tissue is critical for microscopic examination.[2] This is partially controlled by section thickness, which affects the contrast, sharpness, and morphological details of the tissue under the microscope, thus greatly influencing staining quality.[1]

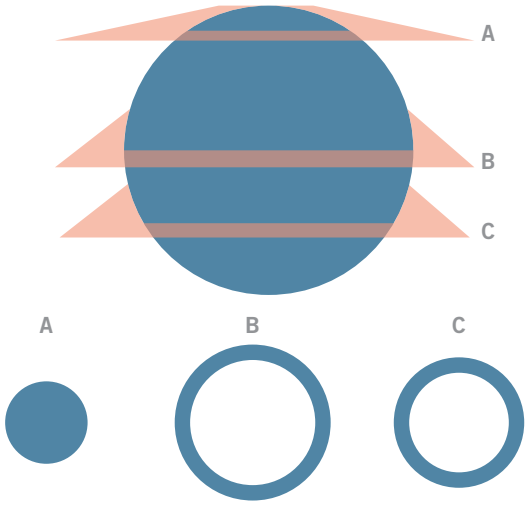
Generally, thicker sections demonstrate greater staining intensity due to more protein being present and labeled in a thicker three-dimensional structure. For example, a 7zwm section will have increased staining intensity compared to a 4µm section. In addition, what is visible in a 7µm-thick section may be lacking in a 4µm-thick section since less tissue is present.

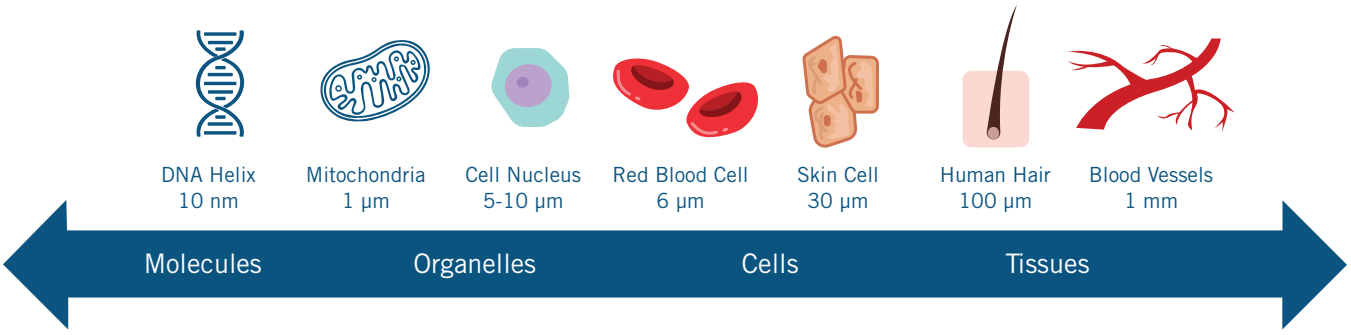
Another area of consideration in sectioning thickness is how it affects the contents and composition of the block. Cells can range in size from 1 to 100 µm. In mammals, organelles and cellular components similarly vary in size. A ribosome may be 0.2 µm in diameter, while a cell nucleus may have a diameter of 6 µm. As a result, different-sized sections will better visually represent different cellular components, such as the membrane, cytoplasm, or nucleus.[1,2] Tissue sections >5 µm can produce more variation in staining intensity and make the assessment of cytoplasmic and membrane staining more complex than for nuclear staining.[3]

Tissue preservation and embedding medium should also be considered when determining section thickness.[1] Tissue is made firmer by the fixation process to preserve its structure and then may be infiltrated with a medium, such as wax or plastics, to support it, or may be fresh frozen. Section thickness typically ranges from 8-15 µm for frozen sections, 4–10 µm for wax sections, and 0.5–3 µm for plastic histological sections. For IHC staining, sections are cut between 3-5 µm.

Sectioning thickness is often overlooked as a factor in IHC staining outcomes but should always be considered in terms of tissue preservation, embedding medium, and staining quality.[1] At a minimum, consistency in sectioning is critical for the quality of patient care to prevent variability and confounding results. Sectioning thickness has significant implications in medical care, both now and in the future as digital imaging pathology becomes more prominent.[3]

Sectioning a sphere with a wall of finite thickness





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3. Shinobu Masuda, MD, PhD. Ryohei Suzuki, ME et al. (2021) Tissue Thickness Interferes with the Estimation of the Immunohistochemical Intensity: Introduction of a Control System for Managing Tissue Thickness. Applied Immunohistochemistry and Molecular Morphology 2021;29: p118–126