

CREATING DIGITAL READY SLIDES

A PRACTICAL GUIDE



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DIGITAL READY SLIDES

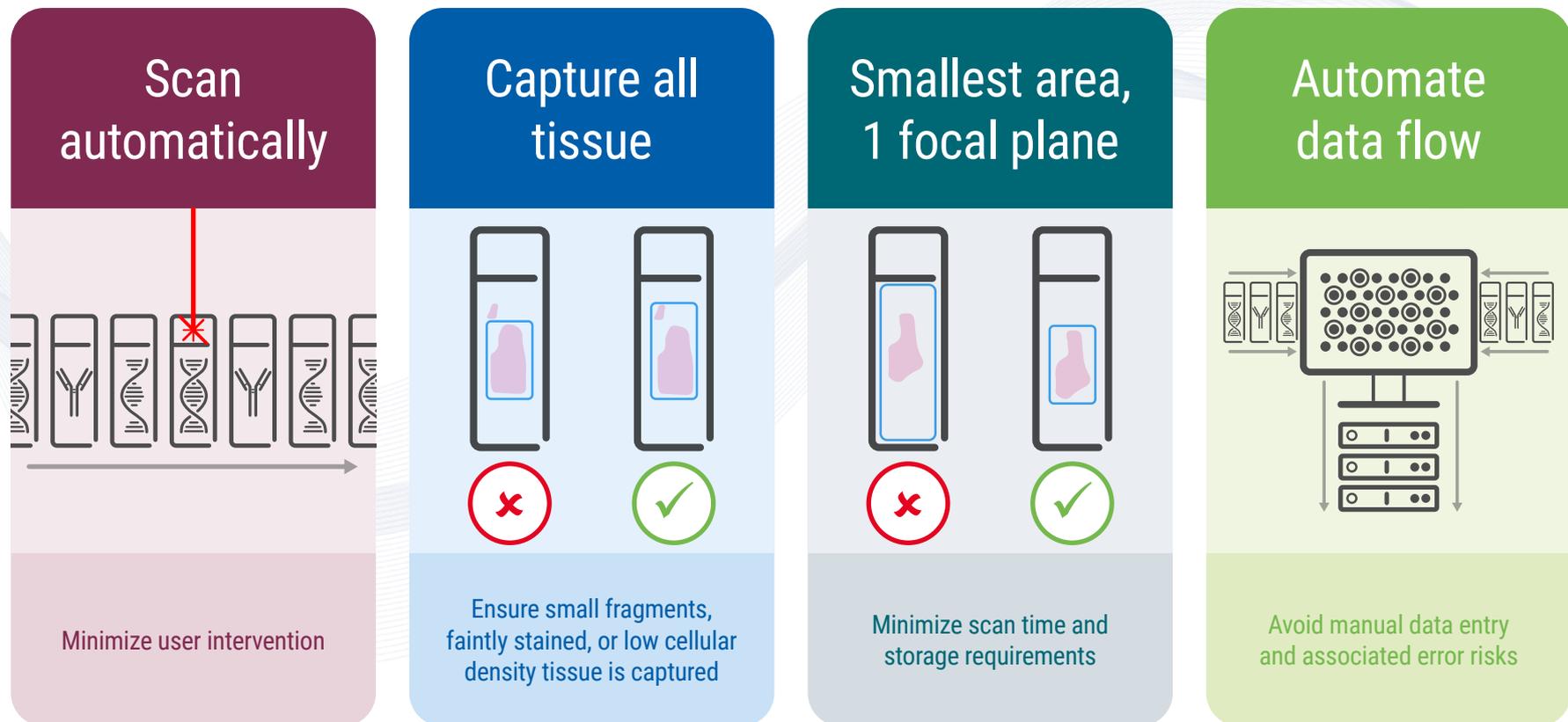


The last two decades have seen significant advances in digital pathology to the point where the acquisition speed, image quality and infrastructure to support whole slide images (WSIs) is at a level of maturity to facilitate routine, on-screen diagnostics¹⁻³. Moreover, there is growing acceptance for the use of digital pathology in the clinical setting in normal and emergency times⁴⁻⁶.

In the 1950's John McCarthy et al coined the term "Artificial Intelligence" (AI)⁷, referring to a branch of computer science whereby machine-based approaches emulate what a human might do in the same situation. Development of AI for pathology applications is growing rapidly, and typically requires large datasets of WSIs for training and validation⁸⁻⁹.

High-quality WSIs are the foundation for clinical utilization of digital pathology and the building blocks for development of histopathology AI tools. Collation of WSI datasets is facilitated by the creation of **Digital Ready Slides**, which are optimized for whole slide imaging.

KEY FEATURES OF DIGITAL READY SLIDES



Creation of Digital Ready Slides affects multiple process steps within the histopathology laboratory, which should be taken into consideration when implementing digital pathology either for routine use or AI development.

EMBEDDING

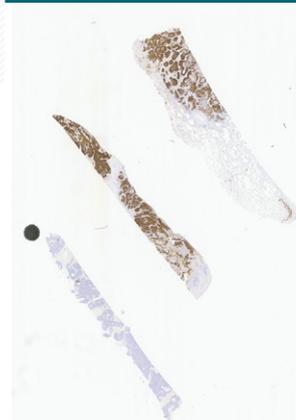
When embedding more than one tissue sample in a single block, such as multiple needle core biopsies, the following points should be considered.

Cluster samples



The samples should be placed close together without overlap in the center of the cassette. This will ultimately enable a smaller scan region for the final slide and ensure all parts of the sample are within the scan region on the slide.

Align samples



Place multiple samples in the same orientation where possible as this will enable optimal positioning at microtomy to match the tissue detection and focusing methodology of the scanner.

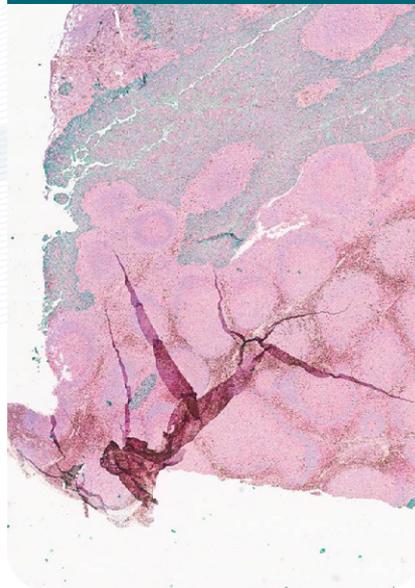
MICROTOMY

In addition to the normal best practices in microtomy to avoid tears, folds, contamination, or chatter in tissue, there are some additional points to consider when creating Digital Ready Slides.

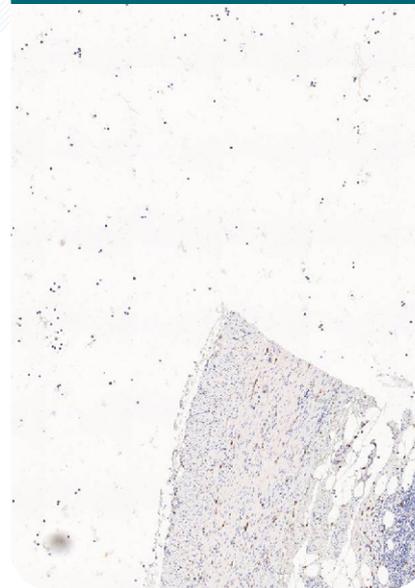
Tears



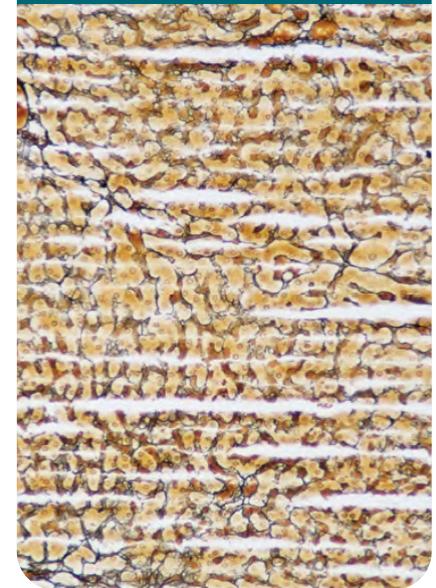
Folds



Contamination



Chatter



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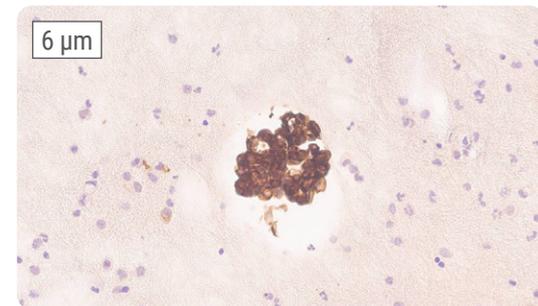
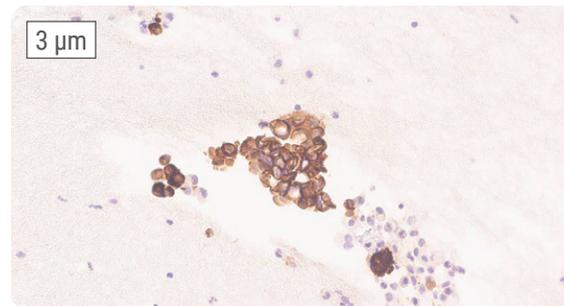
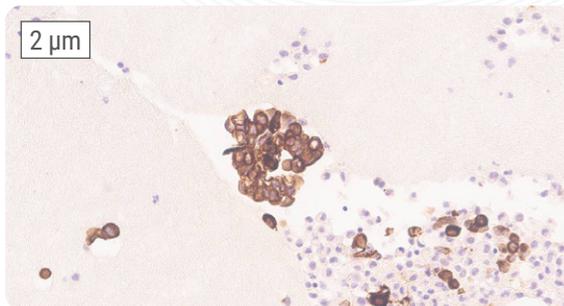
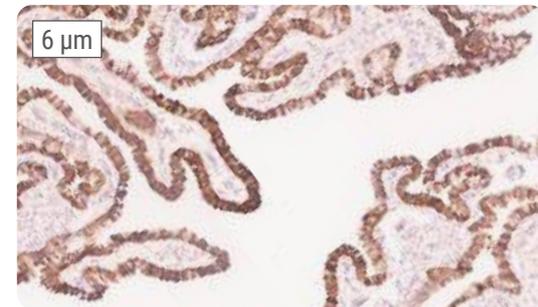
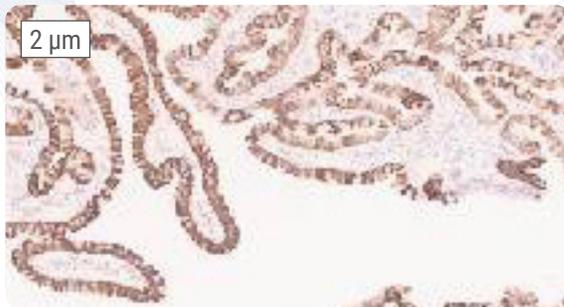
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SECTION THICKNESS

A section thickness of 3-4 μm , which may be thinner than routine sectioning, is optimal for scanning as it allows for capture of the tissue in a single focal plane without the need for z-stack scanning. In addition, section thickness has been shown to impact the staining intensity of immunohistochemical (IHC) markers when interpreted manually or digitally¹⁰⁻¹¹. Therefore, care should be taken to ensure uniform section thickness when considering development of AI tools for IHC stained slides.



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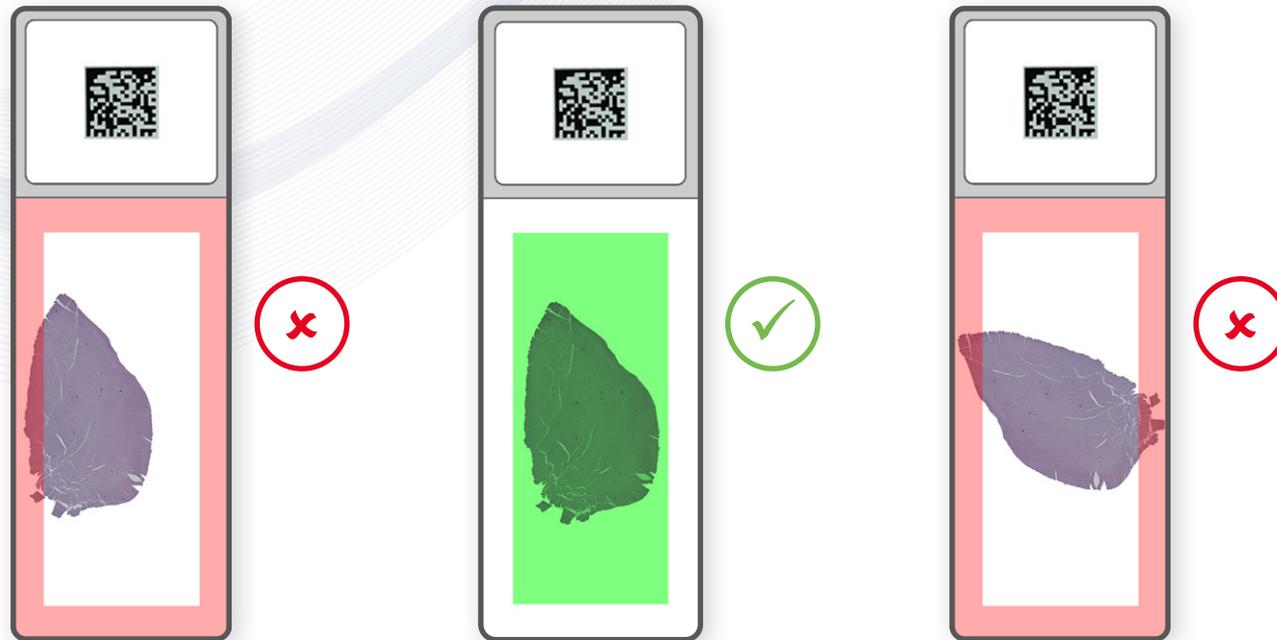
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TISSUE PLACEMENT

Tissue should be placed centrally on the slide, with care taken to avoid tissue being too close to the edge of the slide or coverslip which can impact tissue detection and focusing. Moreover, whole slide scanners will often have a small area close to the edge of the slide, which is outside the scanning region, meaning any tissue in this region will not be captured in the WSI. Therefore, to ensure capture of all tissue, care should be taken to locate the sample away from the edge of the slide.



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Pathology departments adopting digital pathology should consider using barcoded slide labels, if not already in place. While handwritten or human-readable slide labels can be scanned, this can quickly become a rate-limiting factor when scanning larger slide volumes for routine use or amassing slide sets for AI development. As a guiding principle, laboratories scanning more than 100 slides per day should consider barcoding when adopting digital pathology.



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MINIMIZE THE RISK OF DATA ERROR

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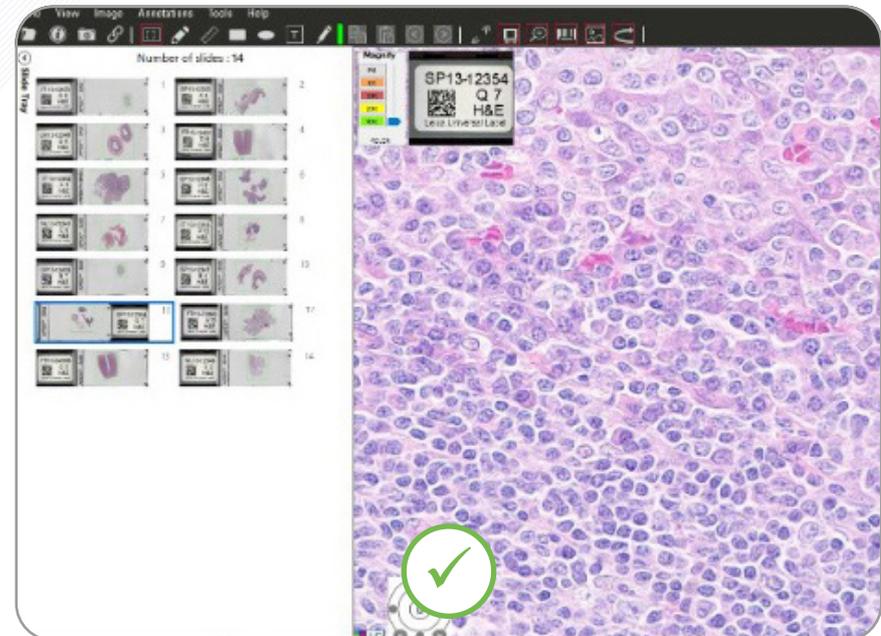
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AUTOMATIC SLIDE IDENTIFICATION

Due to their file size and format WSIs require specialized image management or picture archiving and communication system (PACS) software. Integration of the image management system with the laboratory information (management) system (LIS/LIMS) enables automatic association of slide identifiers and metadata with the WSI. This integration facilitates automated slide aggregation into slide sets, eliminating the need for manual slide sorting.



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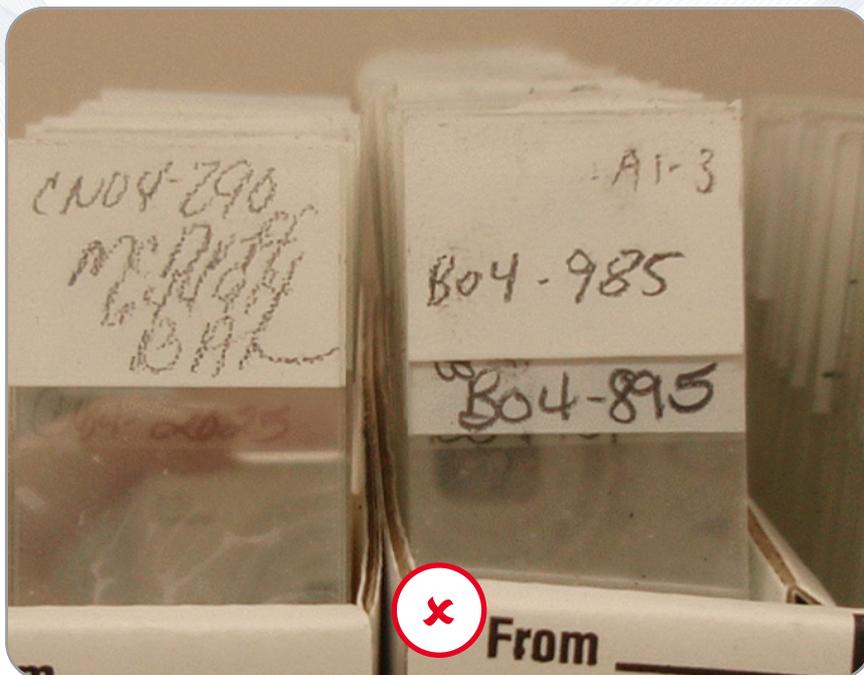
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SLIDE LABELING

MINIMIZE THE RISK OF DATA ERROR

Manual transcription of data is inherently prone to typographical and key stroke errors¹² such as when transcribing from slide labels or requisition forms into digital systems. Automating this step removes this risk, while also freeing up time for laboratory personnel to spend on other tasks rather than data entry.



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SINGLE SLIDE LABEL

When using sticky slide labels, as opposed to printing directly onto the slide, care should be taken to ensure the label does not overhang the edge of the slide. As best practice, only one sticky label should be placed on a slide to ensure the slide-thickness tolerance for the scanner is not exceeded. Overhanging or thick overlapping labels can cause the slide to get stuck in the rack/carrier or on the scanning stage.



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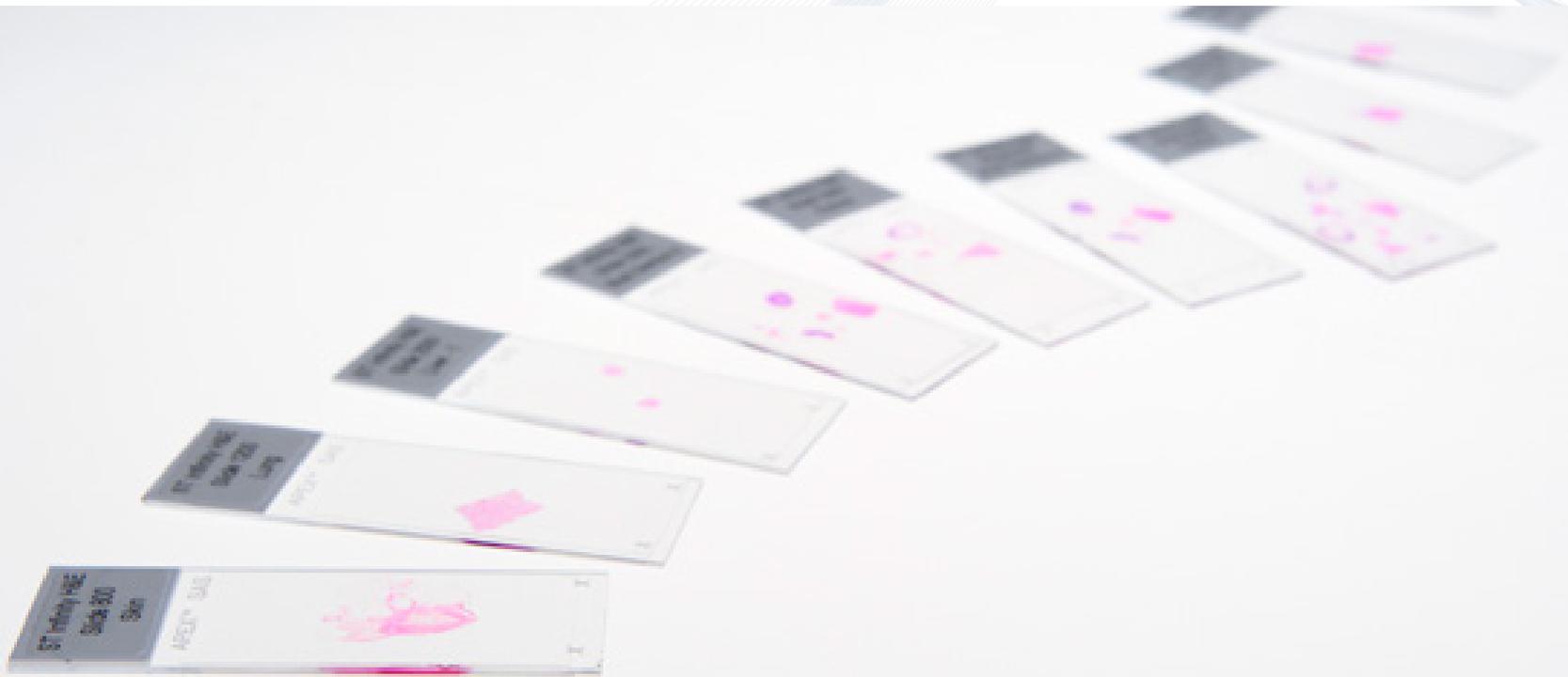
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Digital Ready Slides should exhibit consistent, reproducible staining, which can provide benefits for both on-screen and automated interpretation. Laboratories may want to consider the use of ready-to-use reagents and automated processing to reduce staining variability commonly seen with reagent dilution and manual staining protocols.



STANDARDIZATION OF STAINING

FREE OF DEBRIS AND ARTIFACTS

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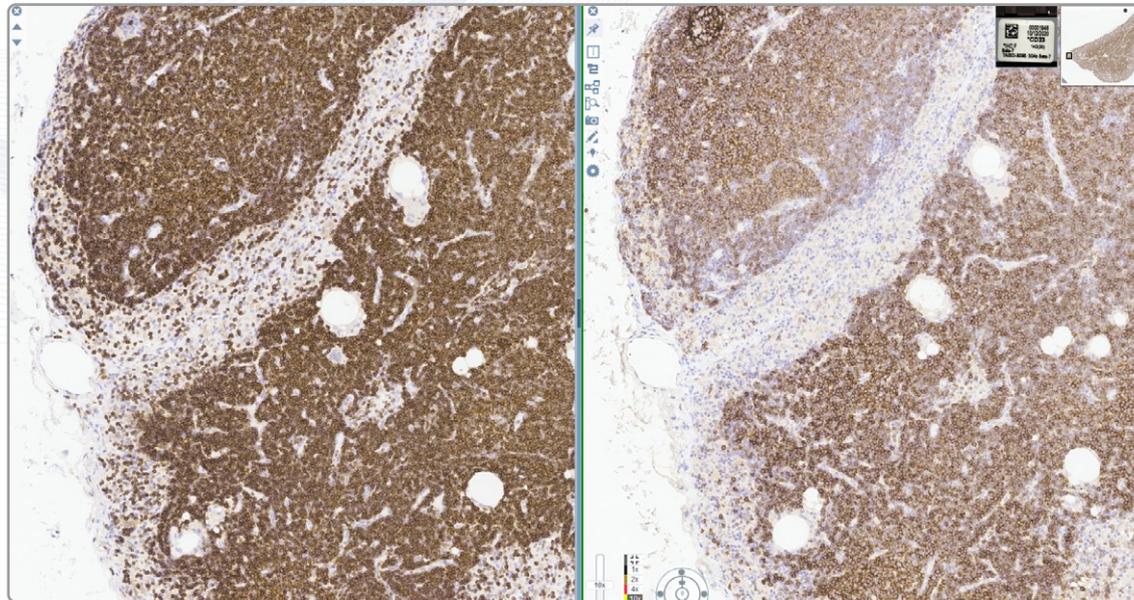
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STANDARDIZATION OF STAINING COLOR AND INTENSITY

Moving from microscopic to on-screen, review of slides is a paradigm shift and requires time for pathologists to familiarize themselves with the slight differences in tissue appearance between the two modalities. Uniform and consistent staining removes a variable from review, helping to increase confidence that differences seen are in the tissue and not from staining inconsistency. Moreover, some digital pathology viewers can present multiple WSIs on one screen, which can highlight variability in staining that the human eye automatically adjusts for when conducting single slide microscopic review.



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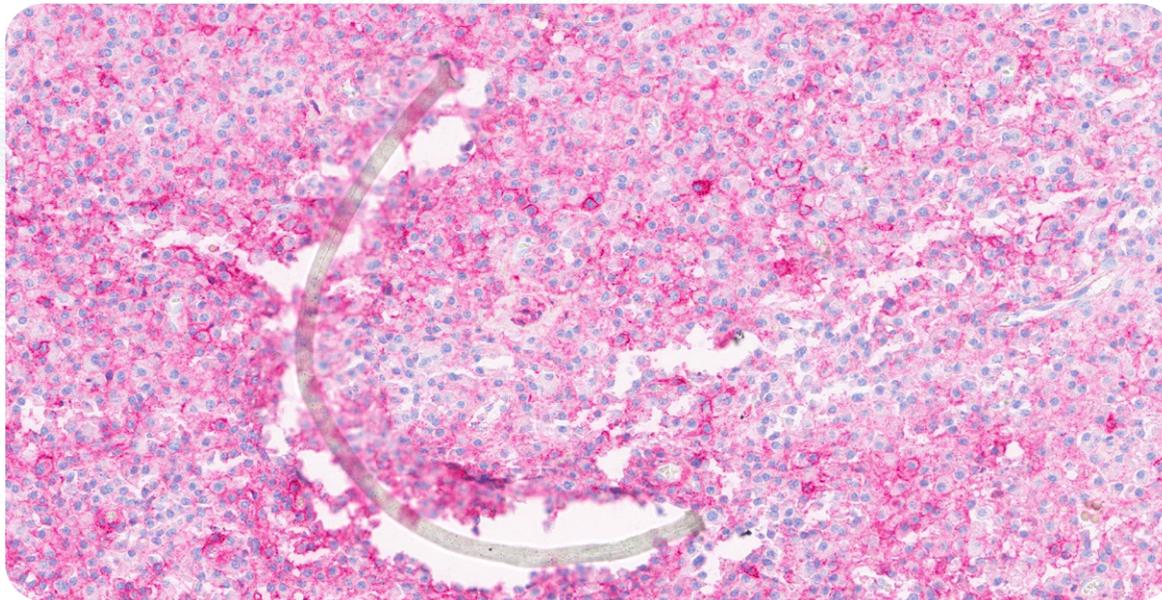
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FREE OF DEBRIS AND ARTIFACTS

Care should be taken to include appropriate rinse steps post-staining to minimize the presence of debris or staining artifacts on the slide. While different slide scanners employ a range of methodologies for tissue detection to define the scan area and focus points, this typically utilizes variance in contrast between the glass slide and tissue. Artifacts or debris on the slide located away from the tissue can lead to larger scan regions, and thus slower scan throughput plus larger file sizes. However, if the debris or artifact is co-located with the tissue, the scanner may focus on this rather than the tissue, leading to regions of tissue which are out of focus.



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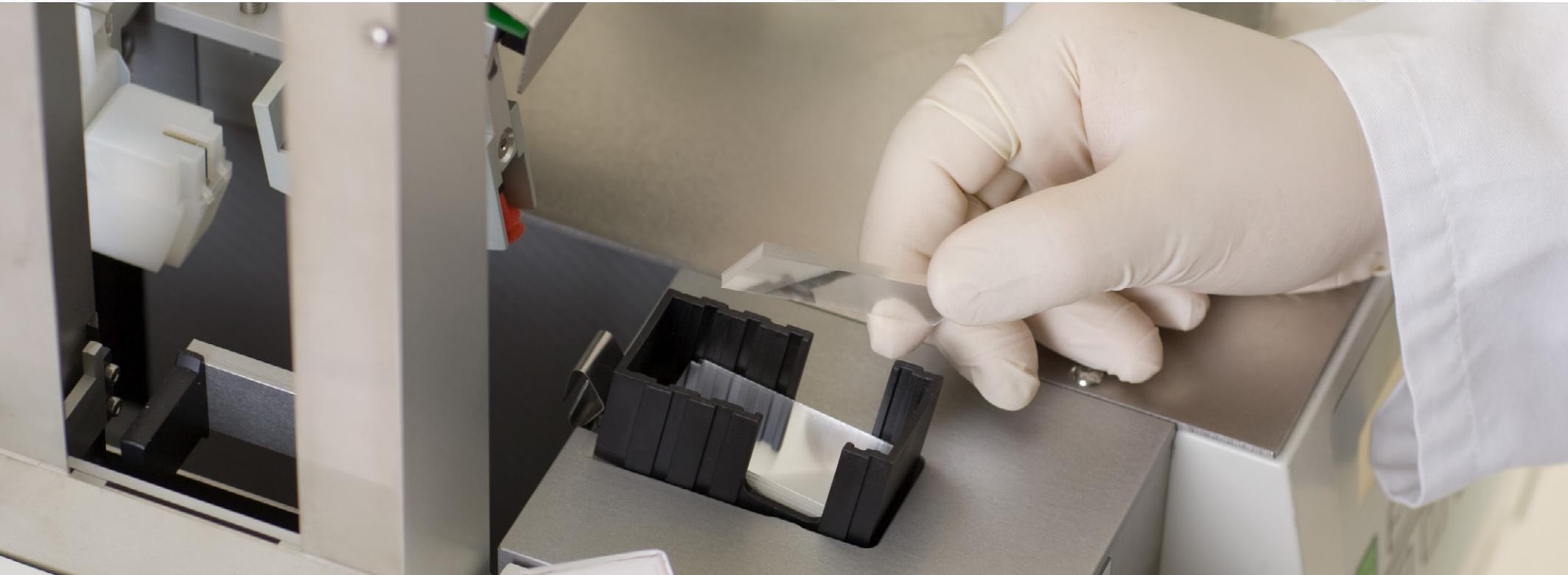
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Coverslipping is an essential step in creating slides for routine review and is of paramount importance when digital pathology is used due to the added sensitivities introduced with whole slide scanning. Careful consideration should be given to leveraging automated coverslipping in laboratories where manual coverslipping is the routine methodology employed.



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NO EXCESS MOUNTING MEDIA

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Bubbles in mounting media are troublesome for microscopic review of slides and even more so for digital pathology. Some scanners will misidentify bubbles as tissue and include them within a scan area. Furthermore, if the bubble is on top of the sample, the scanner may focus on the bubble rather than the tissue. In this case, the underlying tissue may not be captured in focus.



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Digital slide scanners employ a variety of methods to move the slide under the objective for high resolution capture. Hardened surplus mounting media, which extends beyond the coverslip can cause slides to get jammed in the slide moving mechanisms, which may lead to a pause in scanning, or in some situations, breakage of the slide.



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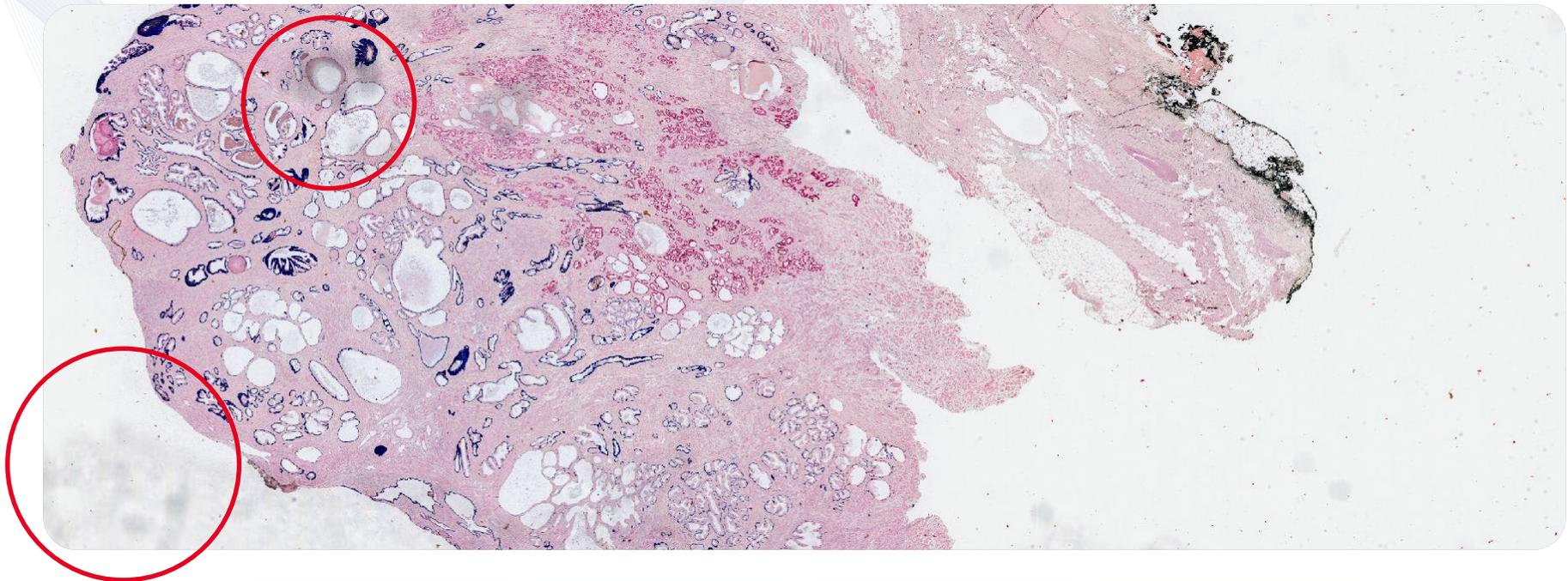
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CLEAN, DRY SLIDES

Coverslips should be clean and free from dust, fingerprints, and debris, which can interfere with tissue detection and focusing, in particular for slides with small tissue fragments or faintly stained samples. Slides which are wet, or have semi-dried tacky mounting media run the risk of sticking to the slide carriers or scanning mechanisms. In addition, if the scanner does not hold the slide in a fully horizontal position, there is a risk that the coverslip can slip off the slide or become skewed, possibly dragging the sample across the slide.



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Slides should have uniform coverslipping which sits inside the boundaries of the slide without any overhang. Misaligned coverslips are at risk of snagging in the scanner's slide carriers or racks, which could lead to breakage of the slide and damage to the scanner.



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Adoption of digital pathology is a multifaceted project involving many stakeholders across the pathology department. The impact on the laboratory is not isolated to simply installing a scanner, but rather affects the whole workflow to generate optimized **Digital Ready Slides**. Standardization of histological slide preparation requires focus on both optimization of individual workflow steps as well as a holistic overview of the complete process from sample acquisition right through to diagnosis. Knowing this in advance and taking appropriate steps to effectively support change management can promote engagement and pave a path to success.

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