

Digital Slide Scanning Sample Guidelines

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What are digital whole slide images (“digital slides”) and why would I want to digitize my slides?

Digital whole slide images (WSIs) or “digital slides” consist of a pyramidal series of Tiff images of a standard glass histological slide taken up to a specified level of magnification. Because of the pyramidal structure of the file, the resulting file can be viewed on a computer, using an appropriate (freely available) viewer, from any location within the scanned image and at any level up to the scanned magnification. This is analogous to how a traditional histological slide is viewed on a microscope.

The use of digital slides presents several advantages:

- Review of histological slides does not require a microscope or shipping of the glass slides, thus digitized slides are useful for remote work or collaboration.
- Images for publication can be taken from the digital file at any magnification or from any location on the slide. This is helpful for future publication or presentation since generation of figures will not require finding the original glass slide or obtaining access to a microscope and camera.
- Quantitative morphometry or pathology evaluation can be performed on the whole slide image rather than a pre-selected field-of-view (FOV) image. This reduces bias due to field selection.

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How do I request digital slide services?

Digital slide requests may be made as stand-alone services (submitting previously prepared slides for scanning) or as part of a histology or pathology request (submitting samples for preparation and/or samples for pathology evaluation). Samples may be submitted through MiCORES (https://umich.corefacilities.org/service_center/show_external/5711). Use Anatomic Pathology- Digital slide scanning submenu to make your request.

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What type of samples may be scanned?

Brightfield slides containing animal or human tissues for research use only and stained with HE, special histochemistry stains, or chromogenic immunohistochemistry stains may be scanned. Fluorescent stains cannot be accommodated at this time. Human tissues must be approved for research use and de-identified. No human clinical samples or samples with clinically identifying information will be accepted. For scanning of clinical slides that are not de-identified, please see the Digital Pathology Core of the UM Department of Pathology.

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What type of digital slide scanner will be used and what kind of files will be generated?

Our scanner is a [Leica Aperio AT2®](#) with the following specifications:

- Magnification options: 20x (standard) and 40x objective equivalents
- Scanning resolution: 50,000 pixels/inch (0.5 μm/pixel) for 20x, 100,000 pixels/inch (0.25 μm/pixel) for 40x
- Illumination: Brightfield
- Capacity: up to 400 slides per run
- Digital slide file format: sv5

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How will my digital slide files be provided?

Image files will be uploaded to Dropbox or to a flash drive. The delivery method may be specified in the MiCORES request.

You may supply a flash drive/ SSD, or the core can provide one for a fee. The standard flash-drive size is 64GB and limited-availability flash-drives are 128GB. Remember that svx files average ~0.5-1.2 GB each and plan accordingly.

Core folders on Dropbox are intended only as a means of file transfer to clients—clients are responsible for downloading and long-term storage of their own data. Older svx files (>3 years) on client-shared core Dropbox folders are subject to removal or archival. The core also maintains image files for approximately 3 months on its own server, however this is not accessible to clients. Files on the server are subject to deletion after 3 months and the client will be responsible for long-term storage of the data.

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What kind of digital file format will be generated? How big are the resulting files?

Digital slides will be generated as svx files, which are a proprietary slide format consisting of pyramidal tiff files that can be viewed at any magnification up to the scanned magnification. Svx image files are large files that can range from ~0.1-3.0 GB. Note that the file size (and the width/height of the files in pixels) varies depending on the size of the tissue section, not the glass slide itself.

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What information will be provided with my slides?

File names will consist only of sample ID, as printed on the slide, and the scanning magnification. Our core does not provide metadata curation services for digitized slide files. Unless requested otherwise, the sample label will be scanned with the slide – this provides an added internal check against file naming error. We strongly recommend that you provide a master spreadsheet with your accurate sample IDs at the time of sample submission. This will facilitate data entry and will allow for a second level of accuracy check regarding sample labeling.

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What magnification should I request?

We offer 20x or 40x scans, corresponding to typical 20x or 40x microscope objectives. Pricing is based on magnification and the number of slides submitted (price break points are at <20 slides, 20-50 slides, >50 slides). See MiCORES for specific pricing.

We highly recommend choosing 20x rather than 40x for most applications. Most pathology diagnoses are made at 4x, 10x, or 20x so the 20x scanning magnification is usually quite sufficient to see the features of interest. Digital analysis is usually also performed at this magnification. 40x scans are larger which requires greater storage space, more expense, and can complicate downstream analysis due to larger file sizes. When in doubt, we suggest you scan one test slide at 20x to determine if this will be sufficient for your needs.

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How will I view my digital slides?

Digital slide files may be viewed using a program called ImageScope that is freely downloadable from [Leica Aperio](#). Imagescope is usable on a PC but not a Mac. Imagescope is useful for viewing slides and taking images. For Mac users, we recommend using [QuPath](#), which is an open-source software available on GitHub. QuPath also works on PCs. Digital slides on Dropbox must be downloaded before viewing (Dropbox does not support ImageScope or QuPath). Remember that long-term storage of digital slide files is your responsibility, and our Dropbox digital slide folders will be purged at 3-year intervals.

Svs files can be converted to OME-TIFF format using [Bioformats](#), which is available as an ImageJ plugin, however many svs files are too large to open within ImageJ as full-size images (must be tiled).

Basic tutorials in using ImageScope to view slides from Dropbox and in opening digital slides in QuPath are available from the core as separate documents.

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Are there free software packages I can use to take images or perform simple quantitative analysis?

[Imagescope](#) is freely downloadable software provided by the scanner manufacturer and is useful for viewing slides and taking images. It has a very simple positive pixel assessment tool and can measure the area of manually drawn annotations, but no additional analysis is available in the free version of this software. [QuPath](#) is freeware available on Github and has a variety of analysis tools. It also permits export of annotations for analysis in other programs like Matlab. Imagescope only works on PCs and QuPath works on Macs or PCS. Our core has simple tutorials for taking images in Imagescope and performing simple analysis within QuPath. Other QuPath tutorials are available on [Youtube](#).

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Are there other software options for more advanced analysis?

Our core pathologists offer digital pathology analysis through the opensource program [QuPath](#) or a through a commercially available program called [Aiforia](#). QuPath is useful for generating size and color deconvolution-based pixel classifiers to count cells or objects or to quantify pixel area. Aiforia is useful for training deep learning models for segmentation and quantitation of cell, stain, or morphology-based features. With Aiforia, training, analysis, and validation can be performed on whole slide images entirely within the platform without a requirement for scripting or coding. For simple IHC or stain-based quantitation, QuPath is fairly straightforward but for more complex models it requires coding skills. For deep learning-based models, QuPath requires tiling and export of annotations to Matlab or other external platform, then re-importing tiles for assembly back within QuPath.

Please request a consultation or submit a Pathology request describing your pathology analysis needs in MiCORES.

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Can someone take representative images from my digital slides for me?

Yes. Our pathologists or technicians can generate representative images as individual Tiff files or as fully formatted figures or composites for publication. Please submit a Pathology request within MiCORES explaining what you would like represented in the image and giving any journal-specific information on figure formatting. Please remember to include us in your acknowledgements if you utilize figures or other data generated by our core in your publications.

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How should I prepare my slides for submission to the core for scanning?

Ensure that your slides meet specifications for the scanner:

- Standard 1 x 3 in² (~25 x 75 mm²) glass slides
- Max thickness (including coverslip and label) of entire slide: 1.48 mm (standard)
- Coverslip thickness: 0.17 mm (coverslip not required but recommended for best results)

Check coverslip, label, tissue placement, slide damage, slide cleanliness:

- Slide should be fully cured with no residual/excessive mounting media
- Coverslip should not protrude beyond the slide edge
- Tissue should not extend beyond the coverslip and be placed away from the edges: standard scanning region of the slide will be 22.9 mm (top to bottom) x 54.9 mm (from the right edge)
 - If you have multiple replicate tissue sections on the slide, consider scanning only one of them to decrease file size, time, and expense (additional tech time).
 - If you would like to scan only one of multiple replicate sections, indicate whether you need a specific one scanned or whether we can just pick the best quality one.
- Label should be fully adhered to the slide and not curling back on itself. Do NOT wrap tape or other labels all the way around the slide – this will make the bottom of the slide uneven, and your scans will be out of focus.
- Check for damage, cracks, or scratches – these will preclude scanning in most cases.
- Avoid markings or writing on the slide – these present a different focal plane. If you are planning to perform deep learning-based digital pathology analysis, these will be confounders for your model.
- **CLEAN YOUR SLIDES:** dust or fingerprints will create artifacts. Dirty slides will be returned or an additional fee for cleaning will be applied.

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