A Guide to Digital Slide Scanners and Associated Infrastructure, Frequently Asked Questions

On behalf of the Alzheimer's Disease Research Center Digital Pathology Working Group
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Digital Pathology is a field focused in generating data from digitized specimen scanned slides, sometimes referred to as whole slide imaging (WSI), a succession from traditional microscopy. In its full potential, it is anticipated the further use and development of digital pathology equipment and infrastructure will allow:

- Data/information to be transferred across large distances quickly
- Advancements in research and educational fields
- Advancements in computational analyses such as artificial intelligence/machine learning (scalable deeper phenotyping of specimens)

As many institutions/departments/laboratories may be considering purchasing a slide scanner, the Alzheimer's Disease Research Centers (ADRC) digital pathology working group has developed this Frequently Asked Questions (FAQ) Sheet to aid in common inquires investigators may have. In addition, a corresponding excel workbook with worksheets containing comparisons of common slide scanning systems, server/data storage options, and open source image viewing programs is available upon request. By no means are these resources exhaustive or are slighted to endorse certain products or methods.

Limitations and Disclaimer:

This document was developed with the intent to be a transparent, yet limited and non-exhaustive resource for persons interested in setting up a digital slide scanner system, geared towards those within Alzheimer's Disease Research Center community without certifying one vendor, brand, and/or institution above another. The commercial products on this page do not constitute an endorsement by the National Alzheimer's Coordinating Center (NACC), National Institute of Aging (NIA), and/or other persons/affiliated institutions with the ADRC digital pathology working group. Neither NACC, NIA, and/or other affiliated personnel/institutions with the ADRC digital pathology working group, assume any responsibility for errors or omissions found in this document. Similarly, this resource document is provided "as is" without warranty of any kind, either express or implied, including the implied warranties of merchantability, fitness for a purpose or non-infringement. The NIA, NACC, and other persons/affiliated institutions with the ADRC digital pathology working group further disclaim any liability for any information provided on this document or other related materials provided here.

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1. How can I afford a WSI digital slide scanner and what should I include in my budget?

There can be many means for amassing resources to purchase and set-up infrastructure for a digital slide scanner. A recent survey reported in ADRC neuropathology cores, half received institutional support (Table 1). Others reported the use of funding from specific grants (i.e. NIA, NINDS, NCI) or administrative supplements to existing grants, departmental funds for recruitment, and/or philanthropy. It may also be important to identify and reach out to other departments and/or centers at your institution (i.e. cancer, neurology, neuroscience, pathology, dermatology, GI, and/or telehealth) as they could benefit from the resources and could contribute to initial costs and/or service contracts. It can be very

Table 1. Types of funding used to cover the purchase and operational cost of digital slide scanners (results are not mutually exclusive).

ANSWER CHOICES	RESPONSES	
NIA funding (R01, U, P grants, administrative supplements, etc.)	11.54%	3
NINDS funding (R01, U, P grants, administrative supplements, etc.)	0.00%	0
NCI funding (R01, U, P grants, administrative supplements, etc.)	7.69%	2
Philanthropy	15.38%	4
Institutional support	50.00%	13
Unsure	26.92%	7
Other (please describe)	23.08%	6
Total Respondents: 26		

advantageous to amass multiple stakeholders within your institution as many small investments will allow purchase of a slide scanner and infrastructure/support needed.

In respect to finances, the purchase of the slide scanner may have a hefty initial price cost-- and there are additional costs to account for such as the purchase, set-up, and maintenance of a file-sharing/file storage system, personnel expenses (i.e. a 20% effort of a staff member to aid in slide scanning and management), an uninterruptable power-supply, and allocation of space to accommodate the equipment. All these are recommended to be worked into the budget/resource list.

Table 2. Common slide scanner brands used by ADRC neuropathology cores (responses are not mutually exclusive).

ANSWER CHOICES	RESPONSES	
Aperio/Leica	62.96%	17
Olympus	7.41%	2
Zeiss	14.81%	4
Perkin Elmer	14.81%	4
Philips	11.11%	3
Keyence	3.70%	1
Huron	0.00%	0
Don't know	14.81%	4
Other (please specify)	14.81%	4
Total Respondents: 27		

2. What personnel and other infrastructure should be considered?

An investment in personnel is important for the efficient use of the slide scanner and for data management. In most cases, the vendor will install the machinery and then train 1-2 personnel on staff for the hardware/software aspects of the slide scanner. These 1-2 personnel are then "primary users" who should commit their efforts into developing standard operating protocols, carrying out operations (i.e. slide loading, slide scanning, and general software set-up) and finally exporting/managing data. There is also the topic of data management, and without dedicated personnel this can cause disarray of what has been scanned in. Institutional personnel (such as those in IT) should also be involved in conversations regarding slide scanner purchases as they may be needed to advise on optimal network connectivity for data input/output from an onsite or approved offsite server.

3. What hardware features should I consider for a WSI digital slide scanner?

When choosing a WSI digital slide scanner, one should list all potential purposes for the machine and understand what features would be needed to support them. Table 2 represents common slide scanner brands used by ADRC neuropathology cores (responses are not mutually exclusive). Each brand may have different models and based on the distribution there is a clear representation that there is no defined choice for the "best" slide scanner. Below are some details on features to consider:

I. Load capacity (i.e. how many slides can be loaded and continuously run at a time). There are many affordable, smaller scale slide scanners (with a load capacity of 5 - 6 slides/run) and although these can be very efficient it can be time

consuming for personnel to operate when working with large quantities of slides. A higher capacity load slide scanner (with load capacities at 100 or more slides) may cost more, however, the ability to load slides less frequently and let the machine continuously run until complete may be more efficient and offset the cost associated with personnel in the long-term.

II. Brightfield/Immunofluorescent capabilities. Brightfield capabilities allow for scanning of H&E, histochemical, and immunohistochemical stains at a reported 1.0 – 4.0 mins/slide (standard size) based on tissue area and objective. Immunofluorescent (IF) capabilities are available, and increase costs considerably, and can be considered an optional feature available in some slide scanners. Furthermore, IF scan speed can be lengthy depended on the number of channels, exposure time, and typically takes much longer and files sizes much larger than traditional brightfield. If IF is important to your research, consider the number of channels your slides will have when choosing a WSI digital slide scanner.

III. Compatible Objectives. Microscope objectives for slide scanners typically range from 5x – 40x, with some commercial models advertising optional objectives up to 63x/100x.   Typically, slides scanned at higher magnifications take additional time to scan and can be much larger in their WSI file size. It is important to determine if the needs of your research require a true objective or could be met by a digital zoom (i.e. 20 x objective moved closer to appear at a 40x resolution, sometimes referred to as a pseudo doubler).

IV. Slide Size. The standard slide size supported by all slide scanners is 26 mm × 77 mm, with a glass/glass cover thickness 0.9 mm - 1.2 mm in depth. If your research/institution uses another slide size, ask if the slide scanner offers optional cassette sizes— this will depend on the manufacturer. Some slide scanner manufactures have reported support for the following slide sizes:

> 52 mm x 75 mm
> 75 mm x 100 mm
> 100 mm x 126 mm (thickness from 1.1 mm–1.4 mm)

V. Slide Format. Based on the slide scanners you choose, the slide format may vary (i.e. TIFF, JPEG- see Table 5 for examples). It may be beneficial to work with a slide scanner that exports files in format compatible with current software (comparison of open source image programs are contained within the companion xls file that is available upon request) or contain relevant metadata important to you study.

Table 3. Average slide scan file size across ADRC neuropathology cores.

ANSWER CHOICES	RESPONSES	
100MB or less	3.70%	1
101MB to 500MB	3.70%	1
501MB to 1GB	3.70%	1
Greater than 1GB but less than 4GB	29.63%	8
Greater than 4GB	3.70%	1
Unsure	37.04%	10
N/A: We do not currently use a slide scanner in our ADC	18.52%	5
TOTAL		27

4. **Where should I place my slide scanner?**

Slide scanners are costly investment, and it is recommended to place the slide scanner in a safe location with low chance of disruption (i.e. personnel traffic or workflow). Additionally, be mindful of room ventilation, airflow, and duct/pipe work as it would be tragic if there was a water leak that could cause physical damage to the machine and associated infrastructure. The lighting of the room may also be important. Some slide scanners advertise a "closed environment" (having all machinery encased on a sturdy covering), however, having a setting with multiple windows may still affect immunofluorescence capabilities or cause overheating due to increased room temperature. Lastly, as many slide scanners are often a shared resource one may consider placing their machine in a centrally located room with controlled access (as a side note, having a slide scanner as a shared resource can aid as leverage when asking permission for additional space from your institution).

With respect to file-sharing and storage, slide scanners create large data amounts of data per slide (see Table 3 and Table 4). If you will be transferring data, it is important to note that many slide scanners have a minimum requirement for connectivity (such as 10 – 100 MB/sec) to assure optimal transfers. Furthermore, it is always advantageous to check

with your institution on what file sharing options are approved, especially if you are within a healthcare setting and your slides may contain person health information (PHI).

5. What viewing/analysis software options (proprietary or open-source) are available-- and are we restricted to certain file formats?

Many slide scanners may have proprietary software (included, or optional during the purchase of the slide scanner) that work well with the scanner's native file format types (for examples see Table 5). Although the native file format allows the image to contain important metadata, it comes at the expense that it restricts which software options may open the file. Furthermore, many software packages may require substantial computational power for analysis, and this can be taxed further with large image files (i.e. software may easily analyze a specific anatomic region of a mouse brain but could stall when doing similar analysis on a larger human specimen). It is important to discuss with the vendor your specific needs to make sure you have a system to work optimally for your needs.

There are open-source software options (i.e. ImageJ) that can open and work with some images in their native file format types but may require some basic programming skills and trainings. In some circumstances where the slide scanners native file format needs to be changed to be viewed on another platform or for analysis, there are often options in the proprietary and open-source software to exported the file format into a more universally used file format (i.e. TIFF) but this can take some computer processing and graphical power depending on image/file size. Lastly, as many investigators may be within an institution or health care center, one should check with appropriate personnel to denote if the software they intend to use/install is approved by their institution/center. One should consider benefits/limitations of available software and file format as well as open-source software options.

Table 4. Current total storage space used across ADRC neuropathology cores.

ANSWER CHOICES	RESPONSES	
Less than 1TB	8.33%	2
Greater than 1TB but less than 10TB	8.33%	2
Greater than 10TB but less than 20TB	4.17%	1
Greater than 20TB but less than 30TB	4.17%	1
Greater than 30TB but less than 40TB	8.33%	2
Unsure	50.00%	12
If greater than 40TB, please state the estimated amount:	16.67%	4
TOTAL		24

Table 5. Types of digital pathology slide formats across ADRC neuropathology cores.

ANSWER CHOICES	RESPONSES	
SVS	52.00%	13
CZI	12.00%	3
TIFF	16.00%	4
QPTIFF	8.00%	2
JPEG	0.00%	0
VSI	4.00%	1
iSyntax Philips proprietary file	4.00%	1
Unsure	28.00%	7
Other (please specify)	16.00%	4
Total Respondents: 25		

6. What file storage or server/file-sharing options are available for digital images?

An average digital slide scan file within the ARDC can range from 1.0 to 4.0 GB in size (depending on percent compression- see Table 3), and if an institution is scanning multiple cases containing multiple slides, this can create TBs of data quickly. Although scanning onto a computer's internal hard drive (HDD) or onto an external HDD may seem appealing and easy, we recommend a long-term storage plan as well as dedicated method for data management (involving your institution's IT department and/or data core personnel are highly recommended). Having files directly scanned onto the internal HDD may cause the computer to crash (overburdening local memory) and may ultimately cause data loss. There are several options to explore for long-term storage, as well as additional options such as server/file-sharing integration which allows you to store data and access it from multiple locations (referred to as "endpoints"). Table 6 is an overview of how digital slide storage has been handled within ADRCs.

Long-term storage can be accomplished by several means. If most of your ADRCs work is internal, one can contact their IT department or other members of institution/center to set-up an approved on-site server (if the institute allows) which can guarantee a secure method to access or archive (back-up) data. The server can be set-up to connect with the slide scanner and an uninterruptable power-supply (UPS) so there is a dedicated method of transfer from the slide scanner to on-site server—this will prevent error or data loss in case of emergency power shut-downs. If your ADRC is expanding its collaborative efforts, then it can be reasonable to move toward a server/file-sharing platform. A cloud-based server (i.e. Globus, OMERO, etc.) and file-sharing platform (Amazon AWS S3, Google Drive, Box, Dropbox, OneDrive, etc.) differ in the method and structure that data is stored and shared. We recommend understanding if servers/platforms are HIPAA compliance, how much storage is allowed and costs, what speed of access is needed (immediate access, or glacial), and any associated fees with large file transfers- uploads may be free but downloading may come at a cost. The services provided by each option are scalable (will work pending on the site and/or reach of your institution), and an annual cost based on your needs. Always check/consult with your institution as certain storage means may not be approved, and a risk assessment may be warranted.

Furthermore, there may be initial set-up required, and the most important may include a reliable network connectivity (i.e. network speed and manageable firewall rules). There are means to test connectivity such as the following website: https://www.speedtest.net/ . There is no firm recommendation for one service or another, however, back-up and reliable archival in addition to data loss prevention features are invaluable and must be considered. Overall, one may want to engage in conversations with their institution IT team to discuss options.

Table 6. Types of digital slide storage (top) and sharing mechanisms (bottom) across ADRCs (categories are not mutually exclusive).

ANSWER CHOICES	RESPONSES	
Onsite storage directly controlled by the ADC (i.e., on-premises network attached storage)	34.78%	8
Onsite storage directly controlled by an entity other than the ADC	30.43%	7
Offsite storage directly controlled by the ADC	4.35%	1
Offsite storage directly controlled by a department (i.e., shared departmental server)	17.39%	4
Offsite centralized storage (i.e., shared server with other departments)	26.09%	6
Offsite Cloud storage provided by a third-party vendor	4.35%	1
Other (please specify)	17.39%	4
Total Respondents: 23		

ANSWER CHOICES	RESPONSES	
Web portal (e.g., eSlide Manager)	28.13%	9
File sharing (e.g., Google Drive, Box)	12.50%	4
External hard drives	12.50%	4
Unsure	3.13%	1
N/A: We have scanned slides but do not share	25.00%	8
N/A: We do not utilize a digital slide scanner	34.38%	11
Total Respondents: 32		

7. How should one approach file organization and transferring/sharing?

Organizing file names and indexing slides is essential once the slide scanner and infrastructure are in place. The following should be considered when creating an index or file naming scheme: Patient/Specimen Identifier, Case #, tissue region, and tissue stain. Additional details relevant to your slides can be added as necessary. With respect to transferring/sharing, as ADRCs are funded by the National Institutes of Health, they are required to report each year on resources they have shared, furthermore there may be specific acknowledgement wording for utilizing the WSIs as well as restrictions on sharing with third parties. Hence, if one is transferring/sharing WSIs, one may want to set up a request system, and establish data use agreement (DUAs) and/or collaborative agreements. For sharing files, we have stated above in section 5 some options, depending on what information is contained within the WSIs, one may want to consider an institutional approved HIPPA compliant means.

8. Should I opt-in for a slide scanner service contract and if so, how do I support the finances when my grant runs out?

There are often service contracts and warranties available at the time of purchase which cover maintenance and other specific instances when things break. Many vendors offer annual service contracts on their slide scanners, which

can be approximately 10 percent of the initial slide scanner cost (in some cases 13,000.00 USD annually) and one can even add additional years to the service at the initial time of purchase. In some cases, vendors have instead moved to a business model that supports repairs free of cost, on a per-case/per-repair bases or includes the costs of repairs with the initial costs. These plans should be discussed with the vendor, as well as if there are applicable discounts for the purchase if a service contract is purchased.

9. Are there any additional resources for digital pathology?
Yes, please follow the link to the following resources:

Digital Pathology Association: https://digitalpathologyassociation.org/
College of American Pathologists Digital Pathology Topic Center: https://www.cap.org/member-resources/councils-committees/digital-pathology-topic-center
National Alzheimer's Coordinating Center (NACC): https://www.alz.washington.edu/BiospecimenTaskForce.html

Contact information:
Please contact Dr. Brittany Dugger (bndugger@ucdavis.edu) with an email titled "A Guide to Digital Slide Scanners comments and contributions" to submit additional resources in a follow-up draft. We thank all our contributors in advance.