



Review Article

Basic concepts in digitizing slides: An overview

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Abstract

Background Digital pathology involves the conversion of physical slides into digital formats for advanced image analysis. Digitization begins with placing the physical slide on a scanner. Various file formats like MRXS, NDPI, iSyntax, DICOM, and SVS are used to store high-resolution images of the whole slides.

Objective This overview highlights the importance of each stage in digitizing slides for generating accurate and reliable results.

Method This overview compares famous software viewers; namely, 3DHistech's PANNORAMIC, Roche's navify®, Clinical Viewer and InstantViewer.

Results Digitizing and analyzing physical slides in digital pathology is a multi-step process involving sample preparation, scanning, image processing, and image analysis. Advances in digital pathology software, cloud storage, and image analysis algorithms are enhancing the field's capabilities, although challenges in image quality and integration persist.

Advances in knowledge While digital pathology offers significant advantages in terms of advanced analysis, data accessibility, and automation, it also presents challenges related to image quality, storage, integration, and the expertise required to utilize specialized tools.

Keywords: Slide Digitization, Image Analysis, Sample Preparation, Image Segmentation, Feature Extraction, Cloud Storage, Digital Pathology Software, Image Analysis Algorithm

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Introduction

It is important to digitize a physical slide and subsequently analyze it to ensure the accurate conversion of tissue or specimen slides into a digital format. This will make the extraction of valuable information possible. The key steps involved in this process are sample preparation, slide scanning, digital image storage, image preprocessing, region of interest selection, image segmentation, feature extraction, image analysis, and data interpretation and sharing[1].

The significance of proper sample preparation cannot be overstated, as the quality of the sample directly influences the accuracy and reliability of the subsequent analysis. This process's essential tasks include staining or labeling the sample to enhance specific features of interest. Once the sample is adequately prepared, slide scanners or digital microscopes are used to capture high-resolution images of the entire slide, so that an advanced image analysis can be conducted. Parameters such as the resolution, magnification, and focal plane must be adjusted carefully before the slide is scanned. The digitized images are then systematically stored in digital image repositories or databases. Effective organization and structured storage of the slides are essential to ensure the images can be easily accessed and retrieved as needed. This step is also necessary to maintain proper data management and archival practices [2].

Image preprocessing enables the application of noise reduction, color correction, and background subtraction measures to enhance the quality and clarity of the images. This step contributes to the accuracy of the analysis by minimizing unwanted variations in the images. Because not all parts of a digitized slide may be relevant to the analysis, so specific regions of interest (ROIs) within each digital image must be defined. These ROIs must then be further refined through image segmentation. Image processing techniques can be used to separate identified ROIs from the background of an image or adjacent tissues. This segmentation is vital for isolating the features of interest that require a detailed analysis. Feature extraction entails that relevant characteristics and information are derived from the segmented ROIs. These features may encompass texture, shape, intensity, and other distinctive attributes. These extracted features serve as the basis for the subsequent analysis.

Once the features have been extracted, the core of the analysis can begin. Depending on the research or diagnostic objectives, researchers or software algorithms can use analysis techniques, such as object detection, classification, quantification, or pattern recognition. This step provides quantitative insights into the samples, aids in identifying abnormalities, and supports classification tasks. The analysis results can then be interpreted within the context of the research or diagnostic goals. Quantitative measurements can be made, anomalies can be identified, and samples can be classified based on predetermined criteria. The analysis results, including measurements, interpretations, and visualizations, should be stored in a structured and well-documented format. Proper documentation is essential for traceability, reproducibility, and future reference. Well-documented records are vital for maintaining scientific rigor

and high quality standards. Sharing of the results with relevant stakeholders, researchers, or healthcare professionals is facilitated to support clinical decision-making, further research, and interdisciplinary collaboration[3,4].

Method

Specific software viewers included in this review are 3DHistech's PANNORAMIC, Roche's navify®, Clinical Viewer, and InstantViewer. These software viewers were chosen due to their prominence and widespread use in digital pathology. Peer-reviewed articles, technical reports, and comparative studies published within the past five years (from 2018 to 2023) were considered to ensure the relevance of these viewers to contemporary advancements and features. PubMed, IEEE Xplore, Google Scholar, and other academic databases were systematically searched using combinations of keywords such as "3DHistech's PANORAMIC," "Roche's navify®," "Clinical Viewer," "InstantViewer," "digital pathology software," "slide digitization," and "image analysis," among other related terms.

Emphasis was placed on software capabilities related to slide digitization, image processing, storage, image analysis algorithms, the user interface, integration with other tools, and any unique features contributing to the digitization process in pathology analyses. The review delved into features encompassing noise reduction, color correction, and background subtraction, all of which are measures that are critical for optimizing image clarity and reducing unwanted variations.

The review assessed the user interfaces of the software viewers, acknowledging the importance of intuition and navigability. Evaluating the ease of use, feature accessibility, and user interaction dynamics provided insights into how user-friendly the software viewers would be for pathologists and researchers. Official web pages and technical documentation from the manufacturers of the specified software viewers were accessed to gather detailed information, updates, features, and any relevant case studies or available comparisons.

Results

Frequently used digital slide formats

Digital slide scanning is conducted on a glass microscope slide containing a tissue sample or specimen. This slide is placed on the scanner for digitization. The scanner is the hardware device responsible for capturing high-resolution digital images of the microscope slide. It typically consists of a precision stage for moving the slide, a high-quality objective lens, a light source, and a digital camera for capturing images. The scanner moves the objective lens across the microscope slide in a grid pattern, capturing multiple overlapping images that are later stitched together to create a seamless whole-slide image. Whole-slide images are stored in specific file formats, such as SVS, NDPI, or MRXS, which support high-resolution imaging and annotations. Resolution refers to the level of detail in the digital image. In digital slide scanning, resolution is measured in

terms of dots per inch (DPI) or micrometers per pixel ($\mu\text{m}/\text{px}$). Higher resolution results in more detailed images but also larger file sizes.

Exporting digital slides comes in different formats. Famous examples follow:

- MRXS is a proprietary file format developed by Carl Zeiss MicroImaging for storing whole slide images. These files are used with the Mirax scanner and viewer. MRXS files are known for their high image quality and support for multiple image layers and annotations. Typically all 3DHistech scanners export in this format but Elsevier microscope does not support it.
- NanoZoomer Digital Pathology Image: NDPI is a file format developed by Hamamatsu Photonics for whole slide images. It is commonly used with Hamamatsu's NanoZoomer digital pathology scanners. NDPI files are known for their high-resolution images and efficient compression. All 3DHistech scanners can export images in this format, which is supported by Elsevier microscope.
- iSyntax is a proprietary file format associated with the Leica Biosystems digital pathology scanners. It is designed to store high-quality whole slide images and often includes features for annotations and image analysis.
- Digital Imaging and Communications in Medicine is a widely used medical image storage and exchange standard, including radiography and pathology images. DICOM files can contain a range of information beyond the image data, including patient and study information, making them suitable for healthcare information systems.
- Aperio ScanScope Virtual Slide: The SVS file format is used by Aperio ScanScope scanners for whole slide images. SVS files are known for their compatibility with various digital pathology software and platforms, making them widely adopted in the field.

Digital slides software viewers

Instead of storing these large image files locally on physical servers or computers, the images should be securely stored in the cloud. Cloud storage provides several advantages, including scalability, accessibility from anywhere with an internet connection, and robust data backup and recovery. This remote accessibility allows for telepathology, collaboration, and second opinions on the content of the scanned slide, which can be invaluable for medical consultations and research[5]. Cloud-based pathology workflows can be integrated with artificial intelligence (AI) algorithms and image analysis tools. These technologies can aid in the automated detection of anomalies, faster diagnoses, and the identification of potential biomarkers. The speed at which digital pathology images can be uploaded, retrieved, and analyzed in the cloud can be affected by latency issues. For real-time consultations and diagnoses, image retrieval or analysis delays can be a drawback[6,7].

Examples for digital slides software viewers are 3dhistech's PANNORAMIC DESK II DW, PANNORAMIC Midi II, PANNORAMIC SCAN II, PANNORAMIC Confocal, PANNORAMIC 250 Flash III, and PANNORAMIC 1000[8]. To view these slides, SlideViewer is a digital microscopy application specifically designed to support

microscope examination processes in bioscience. This application has been available since 2013, with the current release being SlideViewer 2.6. It is offered free of charge and caters to research purposes as a Windows application, compatible with file formats such as *mrxs*, *sys*, *dicom*, *ndpi*, *iSyntax*, and *tiff*. SlideViewer offers instant access and storage options through SlideCenter, be it local or cloud/server-based. It allows integration with AI tools through a Python interface, with optional image analysis tools like QuantCenter. The application also includes features like Marker Counter, 3DView for z-Stack slides, TMAtool (optional), comprehensive annotations, snapshot functionality, slide overview, tracking of inspected FOVs, parallel viewing of up to nine slides, color adjustment, and autofluorescence correction at an extra cost. It supports various input devices and is a valuable resource for research purposes. Other digital slide viewers include navify®, Clinical Viewer, and InstantViewer[9].

Roche's navify® Digital Pathology revolutionizes pathology laboratory workflow by enhancing efficiency through connectivity and automation. This cloud-based pathology workflow software seamlessly connects technicians and pathologists. Navify® is an iteration of Roche's uPath enterprise software, initially established in 2019, with continuous improvements and updates through 2022. Navify® Digital Pathology supports external algorithms for pathology decision support, and allows for comments and integration with external applications. Its unique characteristics include (a) one-click image analysis with compatibility for uPath whole slide image analysis digital pathology algorithms, (b) scalability through the deployment of additional cloud resources, (c) multi-slide canvas viewing for faster annotation and sign-out and (d) user-centric design with an innovative interface to enhance the overall user experience.

Clinical Viewer is a digital microscopy application crafted to support the histopathological diagnostic workflow and microscope examination processes. This application has been integral to the Panoramic Pathology Management System since 2020. The application operates on a case-based structure and provides comprehensive annotation features, a snapshot function, slide overview, and preview images. It allows tracking of inspected fields of view (FOVs), parallel viewing of up to nine slides, color adjustment, and autofluorescence correction. Various input devices are supported, making it a valuable tool for clinical and research purposes[10].

InstantViewer is the default application for opening slides within SlideCenter. It is a versatile, multi-platform slide viewer application compatible with Windows, Mac OSX, iOS, LINUX, and Android. InstantViewer is accessible free of charge and primarily intended for research purposes. It operates as a web browser-based tool and is also available as an iPad Viewer app. It is compatible with the *mrxs* file format and allows instant access to slides hosted on the server or in the cloud. InstantViewer provides annotations features, a snapshot function, slide overview, supporting brightfield (BF) and fluorescence (FL) slides. It ensures that slides are available anytime and on various devices, enhancing accessibility and convenience[11].

Table 1 presents a comparative overview of key digital slide viewers used in the field of pathology and research. This comprehensive breakdown encompasses several critical features that impact their utility in digitizing slides and subsequent analysis. Each viewer's distinct attributes are highlighted, heightening their functionalities, certifications, platform compatibility, and integration capabilities.

Table 1. Digital slides viewers

Feature	Navify®	Clinical Viewer	SlideViewer	InstantViewer
Primary Purpose	Pathology, Research	Clinical, Research	Research	Research
Year of Introduction	2022	2020	2013	2000
CE-IVD (IVDR) Certification	Yes	Yes	No	No
Operating System	Windows	Windows	Windows	Windows, Mac OS, iOS, LINUX, Android
Supported File Formats	Various	mrxs, sys, dicom,	mrxs, sys, dicom, ndpi,	mrxs, ndpi, iSyntax, tiff
Integration with AI Tools	Yes	Yes	Yes	No
Case-Based Structure	Yes	Yes	Yes	No
Instant Access and Storage	Yes, via PPMS	Yes	Yes,	Yes
Annotation Features	Yes	Yes	Yes	Yes
Snapshot Function	Yes	Yes	Yes	Yes
Parallel Viewing of Slides	Yes, with limits	Yes, with limits	Yes	Yes
Color Adjustment and Autofluorescence	Yes	Yes	Yes	Yes
Cloud-Based Storage Option	Yes	No	Yes	Yes
Integration with LIS	Yes	No	No	No
Multi-Platform Compatibility	No	No	No	Yes (Windows, Mac OS, iOS, LINUX, Android)

Image analysis algorithms and open-source libraries

Image analysis algorithms are used to process and extract information from digital images. These algorithms find applications in various fields, including healthcare, computer vision, remote sensing, and more. Many more specialized algorithms are designed for specific tasks and industries such as object detection algorithms, image

segmentation algorithms, feature extraction algorithms, texture analysis algorithms, image registration algorithms, and deep learning algorithms.

Object detection algorithms examples include:

- Haar Cascade Classifier: Used for face and object detection, this algorithm identifies features within an image.
- YOLO (You Only Look Once): A real-time object detection system that can identify multiple objects in an image in a single pass.
- Faster R-CNN: Combines region proposal networks with convolutional neural networks for object detection.
- Image Segmentation algorithms examples include:
- Watershed Algorithm is used for separating objects from the background in medical imaging and geospatial analysis.
- K-Means Clustering: Clusters pixels in an image based on color or intensity to segment objects.
- Graph-Cut Segmentation: Graph-based algorithm that separates images into regions using pixel connectivity.

Feature Extraction algorithms examples include:

- Scale-Invariant Feature Transform extracts distinctive features from an image that are invariant to scaling and rotation.
- Speeded-Up Robust Features is used for feature extraction and matching.
- Histogram of Oriented Gradients: Used for object detection by describing the gradient direction of local image regions.

Texture Analysis algorithms examples include:

- Gabor filters are used to analyze image textures by capturing spatial frequency and orientation characteristics.
- Grey-level co-occurrence matrix measures texture properties by calculating the occurrence of pixel pairs at various distances and angles.

Image Registration algorithms examples include:

- Affine transformation is used to align two images by applying translation, rotation, scaling, and shearing.
- Deformable image registration allows the registration of images with more complex, non-linear deformations.

Deep Learning algorithms examples include:

- Convolutional Neural Networks (CNN) are used for various tasks like image classification, object detection, and segmentation.
- Recurrent Neural Networks (RNN) are used for sequential data analysis, including image captioning.

- Generative Adversarial Networks (GAN) are used to generate images, modify images, and perform style transfer.

Edge Detection algorithms examples include:

- Canny Edge Detector identifies edges in an image by finding areas of rapid intensity change.
- Sobel Operator calculates the gradient magnitude to detect edges.

Thresholding algorithms examples include:

- Otsu's Method: Automatically calculates the optimal threshold to binarize an image.
- Adaptive thresholding adjusts the threshold value for different regions of an image to handle varying lighting conditions, as used by ImageJ. ImageJ is known for its extensibility and user-friendly interface, making it a versatile and widely adopted tool in image analysis and processing, especially in scientific and biomedical research. Its extensive plugin ecosystem allows users to customize and extend its capabilities for specific research needs.

Not all these algorithms can be operated with a user-friendly interface. Some of them require basic or advanced computational knowledge [12]. However, effort is made to render the required codes available on open-source libraries[13–15]. Examples for these libraries are:

- OpenCV is an open-source computer vision and image processing library. It provides a wide range of tools and functions for tasks like image and video analysis, object detection, feature extraction, and more. OpenCV is widely used in computer vision applications.
- TensorFlow is an open-source machine learning framework developed by Google. It is designed for building and training machine learning and deep learning models. TensorFlow supports a wide range of tasks, including image recognition, natural language processing, and more.
- PyTorch is an open-source deep learning framework developed by Facebook's AI Research lab. It is known for its dynamic computation graph and is widely used in research and applications related to deep learning, including image analysis and computer vision.
- scikit-image is an open-source image processing library based on scikit-learn. It provides a collection of algorithms and tools for image processing, manipulation, and analysis. scikit-image is used for various image-related tasks.
- SimpleITK is a simplified interface to the ITK library used for image segmentation and registration. It is designed to make medical image analysis tasks more accessible and easier to perform. SimpleITK is often used in the medics.
- Keras is an open-source high-level neural networks API that can run on top of TensorFlow, Theano, or Microsoft Cognitive Toolkit (CNTK). It simplifies the process of building and training neural networks and deep learning models.

- Matplotlib is a popular Python library for creating static, animated, and interactive visualizations in 2D and 3D. It is commonly used for creating plots, charts, and graphs to visualize data, including image data.
- Seaborn is a data visualization library built on top of Matplotlib. It provides a high-level interface for creating informative and attractive statistical graphics. Seaborn is used for data exploration and visualization.
- PyQt is a set of Python bindings for the Qt application framework. It allows developers to create cross-platform graphical user interfaces (GUIs) for desktop applications. PyQt is used for building interactive applications with GUIs.
- Tkinter is the standard GUI library for Python. It provides tools for creating simple and basic graphical user interfaces in Python applications. Tkinter is used for quick and straightforward GUI development.

It is worth noting that GUIs are not as suitable for fully automated or batch-processing tasks. Writing scripts or using command-line tools is more efficient in such cases. GUI-based applications can be resource-intensive, consuming more memory and processing power than command-line tools. GUIs for image processing and analysis tools are valuable for their ease of use and accessibility, particularly for inexperienced coding users. However, they may have automation, scalability, and flexibility limitations, making them more suitable for specific use cases. The choice between GUI-based and script-based tools depends on the specific requirements of the task and the user's expertise [14].

The best example of the intersection between research and practice in digital pathology is the algorithm quantification of Programmed Death-Ligand 1 (PD-L1) expression[16,17]. This involves processing immunohistochemistry stained slides using automated image analysis techniques[18,19]. OpenCV is usually used to perform PD-L1 analysis on a tested image. The following Python code suffices to load an image, convert it to grayscale, apply thresholding to segment the PD-L1-positive regions and find contour[20].

```
import cv2
import numpy as np
# Load the image
image = cv2.imread('pd-l1_image.png')
# Convert the image to grayscale
gray = cv2.cvtColor(image, cv2.COLOR_BGR2GRAY)

# Perform thresholding to segment the PD-L1-positive regions
# You may need to adjust the threshold value based on your specific images
_, thresholded = cv2.threshold(gray, 128, 255, cv2.THRESH_BINARY)
# Find contours in the thresholded image
contours, _ = cv2.findContours(thresholded, cv2.RETR_EXTERNAL,
cv2.CHAIN_APPROX_SIMPLE)
# Iterate through the contours and calculate area (a measure of PD-L1 expression)
pd_l1_areas = []
```

```

for contour in contours:
    area = cv2.contourArea(contour)
    pd_l1_areas.append(area)
# Calculate total PD-L1 expression area
total_pd_l1_area = sum(pd_l1_areas)
# Display the total PD-L1 expression area
print(f"Total PD-L1 expression area: {total_pd_l1_area} square pixels")

```

The specific steps and libraries used depend on the nature and goals of analysis. The code given below works for other libraries.

```

import pandas as pd
import seaborn as sns
import matplotlib.pyplot as plt
# Load your gene expression data as a DataFrame
# This is a simplified example; you should replace this with your actual data.
data = pd.read_csv('gene_expression_data.csv')
# Assuming you have a column for PDL1 expression
pdl1_expression = data['PDL1']
# Perform basic statistical analysis
mean_expression = pdl1_expression.mean()
median_expression = pdl1_expression.median()
max_expression = pdl1_expression.max()
min_expression = pdl1_expression.min()
print(f"Mean PDL1 expression: {mean_expression}")
print(f"Median PDL1 expression: {median_expression}")
print(f"Max PDL1 expression: {max_expression}")
print(f"Min PDL1 expression: {min_expression}")
# Visualize the PDL1 expression distribution
sns.histplot(pdl1_expression, kde=True)
plt.xlabel('PDL1 Expression')
plt.ylabel('Frequency')
plt.title('PDL1 Expression Distribution')
plt.show()

```

Conclusion

Digital slides are large files, which can be challenging to store and transmit. While digital slides can offer high-resolution images, the quality may not always match that of traditional slides. Factors such as scanner quality, slide preparation, and compression can affect the image quality. Managing and backing up large WSIs can be costly and require substantial storage space. Transmitting these files over networks can be slow and may require significant bandwidth. Integrating digital slides into the pathology workflow can be complex. Existing laboratory information systems (LIS) and picture archiving and communication systems (PACS) may need to be updated or replaced to support digital pathology.

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