Clinical Validation Protocol for Feature Detection Precision by WSI using Hamamatsu NanoZoomer S360 MD Digital Slide Scanner System

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PROTOCOL SIGNATURE PAGE

Sponsor's (Hamamatsu Photonics K.K.) Approval

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Name: Susumu Suzuki
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Protocol Number: HCT-P002

Study Title: Clinical Validation Protocol for Feature Detection Precision by WSI using Hamamatsu NanoZoomer S360 MD Digital Slide Scanner System

I have read this protocol.

By signing this protocol, I agree to conduct the clinical study according to this protocol, the principles of the Declaration of Helsinki (2008), the standards of Good Clinical Practice (as defined by the International Conference on Harmonization E6), ISO 14155 and applicable regulatory requirements. I will ensure that study staff fully understand and follow the protocol.

I understand that failure to comply with the requirements of the protocol may lead to termination of my participation as an investigator for this study.

Changes to the protocol will only be implemented after written approval is received from Hamamatsu Photonics K.K. and the Institutional Review Board or Independent Ethics Committee (as appropriate), with the exception of medical emergencies.

Investigator Name, Affiliation and Address_____

_____

Signature _____ **Date** _____

Table of Contents

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND TERMS

Abbreviation	Term
AE	Adverse Event
ANA	Average Negative Agreement
APA	Average Positive Agreement
CAP	College of American Pathologists
CI	Confidence Interval
CFR	Code of Federal Regulations
CMOS	Complementary Metal-Oxide Semiconductor
CRF	Case Report Form
CRO	Contract Research Organization
eCRF	Electronic case report form
EDC	Electronic Data Capture
EP	Enrolling Pathologist
FDA	Food and Drug Administration
FFPE	Formalin-Fixed Paraffin Embedded
FOV	Field of View
GCP	Good Clinical Practice
H&E	Hematoxylin and Eosin
ICH	International Conference on Harmonisation
ID	Identifier
IRB	Institutional Review Board
IT	Information Technology
LED	Light Emitting Diode
NA	Numerical Aperture
NPA	Negative Percent Agreement
PHI	Protected Health Information
PI	Principal Investigator
PIPS	Philips IntelliSite Pathology Solution
PPA	Positive Percent Agreement
R1	Reading 1
R2	Reading 2
R3	Reading 3
RP	Reader Pathologist

Abbreviation	Term
SC	Study Coordinator
TIFF	Tag Image File Format
US	United States
USB	Universal Serial Bus
VEP	Verifying Enrolling Pathologist
WSI	Whole Slide Imaging

1. PROTOCOL SUMMARY

1.1 Synopsis

TITLE OF TRIAL

NanoZoomer S360MD Digital Slide Scanner System Clinical Validation Protocol for Feature Detection Precision

TRIAL SITE(S)

Three (3) sites in the United States (US) and possibly Canada.

1. Ohio State University Wexner Medical Center, PI (Dr. Anil Parwani)
2. Cleveland Clinic Foundation PI (Dr. Bin Yang, Dr. Scott Fitzpatrick)
3. Washington University Medical Center, PI (Dr. Jon Ritter)

Detection of histologic feature by 3 reader pathologist for each of 3 substudies

Case screening and enrollment to use 1 pathologist to screen and enroll cases and 1 verifying pathologist to confirm case eligibility for enrollment

OBJECTIVES

Primary Objective:

To evaluate the repeatability and reproducibility of detection of histological features using WSI under following 3 sub-studies:

1. Scans within scanner (Intra-scanner precision substudy),
2. Scanners within a site (Inter-scanner precision substudy), and
3. Scanners between sites (Inter-site precision substudy).

ENDPOINTS

The endpoints are specific to each sub-study:

- Overall percent agreement between scans (e.g. between scan1 and scan2, or scan1 and scan3, or scan2 and scan3) within a given scanner (Intra-scanner precision)
- Overall percent agreement between scanners (Inter-scanner precision)
- Overall percent agreement between sites (Inter-Site precision)

METHODOLOGY

Pathologists will be asked to identify a particular histological feature present in a field of view (FOV) image from the WSI image of a single slide. The slides will be selected and will contain one particular screened and enrolled feature from an organ at a particular magnification as listed in Table 1. From these images, a TIFF image demarking the feature(s) of interest in a particular FOV will be created representing only a small discrete region of the entire slide consistent with field viewed at 10, 20 or 40x. The TIFF images will then be presented to the pathologist who will be asked to identify the feature(s) in the FOV from a list of possible multiple-choice features.

Additionally, “sham” images of known features will be inserted into the reading deck to reduce recall bias, but will not be included in the analysis.

Then, depending on the sub-study, the slide will be scanned three times on 1 scanner, or once on 3 different scanners.

For the intra-scanner substudy, three scanners will be used, the slides will be divided equally among the scanners and scanned 3 times per assigned scanner for a given reading pathologist, and each of 3 pathologists will identify the features across 3 reading sessions separated by a 2-week wash out period between readings.

For the inter-scanner substudy, 3 scanners will be used, the slides will be scanned once per scanner and 3 separate

pathologists will identify the features.

For the inter-site substudy, 3 sites, each with one scanner, will be used. The set of enrolled slides will be sent to site 2 and 3. These slides will be scanned once per scanner and 1 separate pathologist per site will identify the features in this substudy.

NUMBER OF SLIDES

A total of 21 features, 7 features at each of 3 viewing magnifications (10x, 20x, 40x), 3 organs within each feature, and 6 slides within each feature-organ combination resulting in 378 FOVs. There will be a total of 9 sham features, 3 at each of 3 viewing magnifications (10x, 20x, 40x), and 18 slides for each sham feature, within each sham features-magnification combination resulting in 162 FOVs sham slides.. The slides will be selected in consecutive order starting with slides at least 1 year old. An enrolling pathologist will screen and enroll the slides per the inclusion and exclusion criteria, which will be verified by a separate validation enrolling pathologist.

MAIN CRITERIA FOR INCLUSION / EXCLUSION

- Inclusion criteria:
  - Slides are selected from cases in a consecutive manner starting with cases at least one (1) year old since accessioning
  - Slide is a glass cover-slipped surgical pathology slide of human tissue
  - Slide stained with H&E or other stains
  - Slide has one of the designated primary feature in the FOV, which is readily observable in its natural environment although the slide may also have one or more secondary features from the same magnification group in the same FOV
  - Slide is available in the archives for use, or purchased commercially, is not damaged, has tissue on the slide which is still in good condition, has a stain that is not faded and otherwise passes all quality checks
- Exclusion criteria:
  - Slide is unable to be scanned, contains damaged tissue or has indelible markings
  - Slide comes from an active (less than 1 year old) case
  - Slide is from a patient who already has a slide enrolled in the study, only 1 slide per patient to be enrolled

DEVICE DESCRIPTION

Hamamatsu S360MD NanoZoomer Slide Scanner Digital Pathology System (NanoZoomer) is an automated digital slide creation, viewing, and management system. The Hamamatsu NanoZoomer S360MD Digital Slide Scanner creates diagnostic quality WSI digital images of glass slides containing formalin-fixed paraffin embedded (FFPE) tissue. Each image typically contains billions of image pixels, creating a digital image of the tissue on the original glass slide. The NanoZoomer captures digital images of entire slides for duplication, annotation, storage, retrieval, image sharing and viewing to permit the pathologist to make a primary diagnosis without needing to view those glass slides through a light microscope.

STATISTICAL METHODS

1. Intra Scanner Substudy

Each of 3 scanners will scan one-third of the slides 3 times for each reader (in such a way that at the end of this sub-study, all 378 study slides will be scanned by each scanner three times and all 378 slides will be evaluated by each reader, details are given in section 8.1). Three reading pathologist will evaluate all scanned slides across 3 reading sessions. In addition to 378 study FOVs selected, 162 “sham” FOVs of the different features will be selected per the same inclusion and exclusion selection criteria and inserted into the reading of slides battery to minimize or avoid bias by the reading pathologist, but the results of sham readings will not be included in any analysis.

The number of comparison pairs is calculated based on 3 readers where for each reader there are 3 pairwise comparisons, namely scan1 vs. scan2, scan1 vs. scan3, and scan2 vs. scan3, which results in 3 readers x 126 pairs per comparison x 3 pairwise comparisons =1134.

To evaluate Intra-Scanner precision for scanner A, three single 2x2 tables can be constructed for pairwise comparisons within scanner A and Reader1. The result of reading for each slide is "Yes" or "No" if the feature was detected correctly or not. Each feature shall be considered as of equal importance as the next feature; thus, the results of all features will be pooled.

Nine (9) 2x2 tables will be pooled within scanner A and across 3 readers to create a table for $scan_{ki}$ verses $scan_{kj}$ for Readers k (k=1,2,3) and i≠j (for i=1,2,3 and j>i). Then the Agreement Rate for each Scanner=$(a^1+d^1)/1134$ and 95% confidence interval (CI) will be estimated.

The overall Intra-Scanner Agreement Rate will be calculated by averaging all pairwise comparisons results over all 378 slides with selected features enrolled and all 3 pathologists. The overall number is 378 slides with selected features x 3 scanners x 3 readers = 3402. To preserve the correlation structure of multiple readings of the same feature and possibly multiple features on an FOV, the bootstrap method will be used to derive a two-sided 95% CI for the overall agreement rate. An FOV will be the bootstrap re-sampling unit.

The study acceptance criterion is 85% on the lower limit of the 95% CI for the overall agreement rate.

2. Inter Scanner Substudy

All same set of 378 study slides already enrolled with selected features and FOVs will be scanned once on each of 3 scanners all at the same site. In addition, the same 162 "sham" slides will be used in the inter-scanner study.

Three separate reading sessions will be performed by each of 3 new pathologists (different from pathologists used in Intra-Scanner Sub-study 1) with a washout period of at least two weeks between sessions. The 378 study slides that will be read during a reading session will be randomly selected without replacement from the slides scanned from 3 different scanners (details given in section 8.2). Per reading session, 54 different sham slides, 18 sham cards from each of 3 magnification FOVs will be added such that all 162 sham slides will be read by each reading pathologist. The sham slides are not used in determining precision.

To evaluate the inter-scanner precision, we need to estimate precision for 3 pairwise comparisons, namely scanner A vs. scanner B, scanner A vs. scanner C, and scanner B vs. scanner C. For pairwise comparison (scanner A vs. scanner B), three (3) 2x2 tables can be constructed for each of 3 readers. A 2x2 table can be created by pooling above three 2x2 tables for estimating agreement rate between scanner A, scanner B and scanner 3. The overall Inter-Scanner Agreement Rate will be calculated similar to one in Intra-Scanner study.

The study acceptance criterion is 85% on the lower limit of the 95% CI for the overall agreement rate.

3. Inter Site Substudy

In this study, there will be 3 sites and a different reading pathologist at each site, using 3 pathologists different from substudy 1 and 2. For inter-site study, the same set of 378 study slides will be scanned once at each site and each of the 3 reading pathologists will have only one reading session in which all slides scanned at their site will be read. The order in which the slides will be read at each site will be randomly ordered. No "sham" slides will be used.

The overall Inter-site Agreement Rate will be calculated similar to one in Intra-Scanner study.

The study acceptance criterion is 85% on the lower limit of the 95% CI for the overall agreement rate.

1.2 Schema

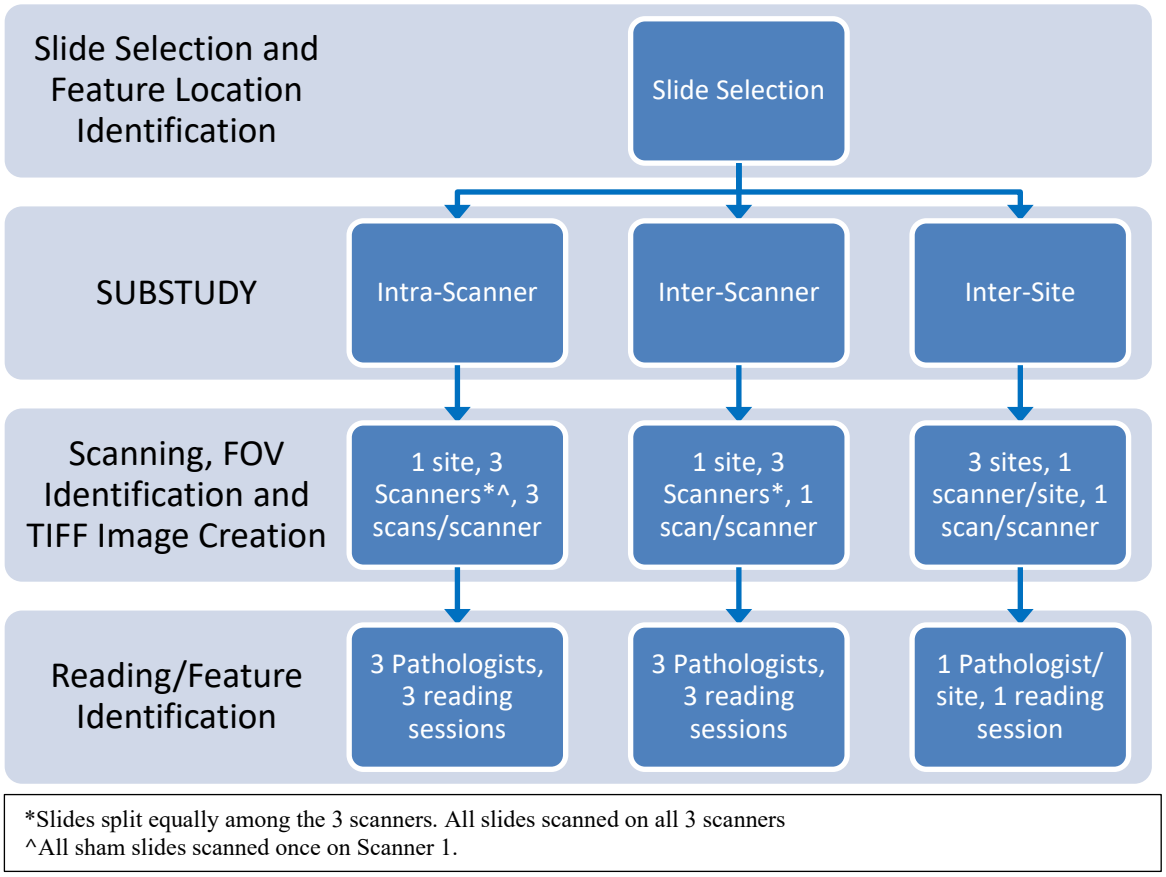


Figure 1. Study Schema

2. INTRODUCTION

2.1 Background

Whole Slide Imaging (WSI) has emerged as an alternative way to view pathology slides instead of the conventional method of viewing slides of tissue under a traditional light microscope used for primary diagnosis. There is growing adoption of the use of WSI for primary diagnosis in many countries (1-4). WSI is particularly advantageous for remote consultation where pathologists are not available over vast geographical regions, and for easier archiving of images of slide material that may have longer shelf life in digital form. In the United States (US), WSI is increasingly used for teaching, archiving, consultation, and research. Furthermore, the College of American Pathologists (CAP) has published recommendations to pathologists who wish to validate WSI in their clinical practices (5).

However, quite recently, in a clearance letter (6) and device summary (7), the Food and Drug Administration (FDA) has announced the approval of the Philips IntelliSite Pathology Solution (PIPS) for use of WSI for primary diagnosis, specifically permitting WSI for in-vitro diagnostic use as an aid to the pathologist to review and interpret digital images of surgical pathology slides prepared from formalin-

fixed paraffin embedded (FFPE) tissue, but not intended for use with frozen section, cytology, or non-FFPE hematopathology specimens.

Thus, with the clearance of the PIPS device for primary diagnosis, this allows the PIPS system to properly serve as a predicate device for any future 510(k) submissions for WSI systems seeking clearance for intended use of WSI, and, further, the FDA has indicated that a clinical study to prove repeatability and reproducibility of histological feature detection by viewing WSI images is a "special control" required to acquire that clearance.

Presently, Hamamatsu is developing a digital slide scanner system, the NanoZoomer S360MD Digital Slide Scanner System (NanoZoomer), for the same intended use as the PIPS system. Thus, Hamamatsu will make the submission in the form of a 510(k) premarket notification with understanding that the PIPS device is the predicate device, will accordingly adhere to the special controls that are established, and, in the spirit of "substantial equivalence," will follow similar study designs to test the Hamamatsu system. Further, Hamamatsu intends to use the data from this non-significant risk study for the 510(k) submission to clear its NanoZoomer system for the same intended use.

2.2 Rationale

In order for pathologists to make a primary diagnosis using a WSI image they need to be able to both detect and interpret various microscopic morphological features in the pathology digital image. For example, pathologists frequently rely on finding mitotic figures and/or necrosis in tumors to help them make a diagnosis of malignancy. This ability may vary among pathologists (e.g., skill increases with experience), between cases (e.g., simple versus complicated biological entities), and with diagnostic image quality (e.g., may be easier with higher resolution). In general, the reproducibility of feature recognition and related accuracy of diagnosis in the published literature is poor (15-23).

This study is being conducted in order to meet the regulatory requirements for feature detection of the Hamamatsu NanoZoomer S360 MD system. In particular, the study will determine how well a pathologist can repeatedly identify key histologic features present in a field of view" (FOV) of a region of the histological slide at 3 different magnification levels. The reader pathologist will read multiple TIFF images of these FOVs from multiple scans across the study.

There are three sub-studies evaluating the within and between scanner precision as well as the between site precision, namely:

1. Intra-Scanner Precision Substudy
2. Inter-Scanner Precision Substudy
3. Inter-Site Precision Substudy

Recently, the FDA has announced the approval of the PIPS for use of WSI for primary diagnosis in a clearance letter (6) and device summary (7), which included more information on the design, conduct, and acceptance criteria for the reproducibility and repeatability study of histologic features used to acquire clearance.

In light of the clearance of the Philips system, Hamamatsu recognizes that the PIPS device is the predicate device for clearance of its WSI system for that intended use, that the feature detection precision study is required as a special control, and, in the spirit of "substantial equivalence," is using this mentioned

information as guidance in how to design, execute and assess repeatability and reproducibility of the NanoZoomer 360MD system.

In two PowerPoint presentations (8, 9) the FDA has defined that manufacturers seeking clearance of their WSI devices for primary diagnosis in surgical pathology must demonstrate that a pathologist using WSI technology can achieve reproducible detection of histologic features. Those presentations provide only some general guidelines regarding the number of features, organ systems, magnification at which feature is detected, and number of scanners and readers. The additional information from the clearance letter and device summary provides deeper insight into the approval process for which substantial equivalence must be demonstrated

In the FDA information, three sub-studies for intra-scanner, inter-scanner, and inter-site repeatability and reproducibility are described (7) and further information is also provided related to the number of sites, scanners, pathologists, features, organs, magnifications, and examination of a limited FOV for detection of features. In addition, the precision of feature detection is mentioned as a special control for WSI clearance (6).

Outside of the FDA released information, further information is available from ClinicalTrials.gov study registration website (10) as well as presentations made by Philips (11). In May 2017, at the Pathology Informatics Summit in Pittsburgh, PA (12), Philips presented their features study with the features selected, the different magnifications, multiple choice selection of the expected feature, inclusion and exclusion criteria for slides with the desired feature, the limited FOV used for feature detection, and the acceptance criterion. The acceptance criterion was established as the lower limit of the 95% confidence interval greater than 85% for overall agreement.

2.3 Risk/Benefit

Since this is a retrospective examination of features from cases already available and archived at the site and that will be de-identified, and since there is no determination of diagnosis in this study, there is no apparent risk or benefit to the patient whose case is being used for the study.

3. OBJECTIVES AND ENDPOINTS

3.1 Primary Objective

To evaluate the repeatability and reproducibility of detection of histological features when using WSI under following sub-studies:

1. Scans within scanner (Intra-scanner Precision),
2. Scanners within site (Inter-scanner Precision), and
3. Scans between Sites (Inter-site Precision).

3.2 Primary Endpoint

The endpoints are specific to each sub-study:

1. Overall percent agreement between scans within a given scanner (Intra-scanner precision)
2. Overall percent agreement between scanners (Inter-scanner precision)
3. Overall percent agreement for scans between sites (Inter-Site precision)

4. STUDY DESIGN

The features study is designed to assess the precision of repeatability and reproducibility of the NanoZoomer image system. Readings of glass slides will not be included in this study.

Precision of the NanoZoomer will be assessed in three sub-studies, namely:

1. Intra-Scanner Precision Study
2. Inter-Scanner Precision Study
3. Inter-Site Precision Study

In the all three sub-studies, pathologists will review several slides consisting of different features (one per slide) from multiple organs and from different magnifications. These slides will be chosen to meet pre-specified inclusion and exclusion criteria. The pathologist will not be presented with the entire WSI image but with a very limited FOV in the form of a tag image file format (TIFF) image that contains a known particular feature. The TIFF images with the FOVs will be created from repeated 40x WSI scans from different scans, scanners, and sites, depending on the requirements of the sub-study. Figure 2 diagrams the slide selection process showing the magnifications, organs and number of slides. Figure 3, Figure 4, and Figure 5 diagram the scanning and reading process for the intra-scanner, inter-scanner and inter-site substudies, respectively.

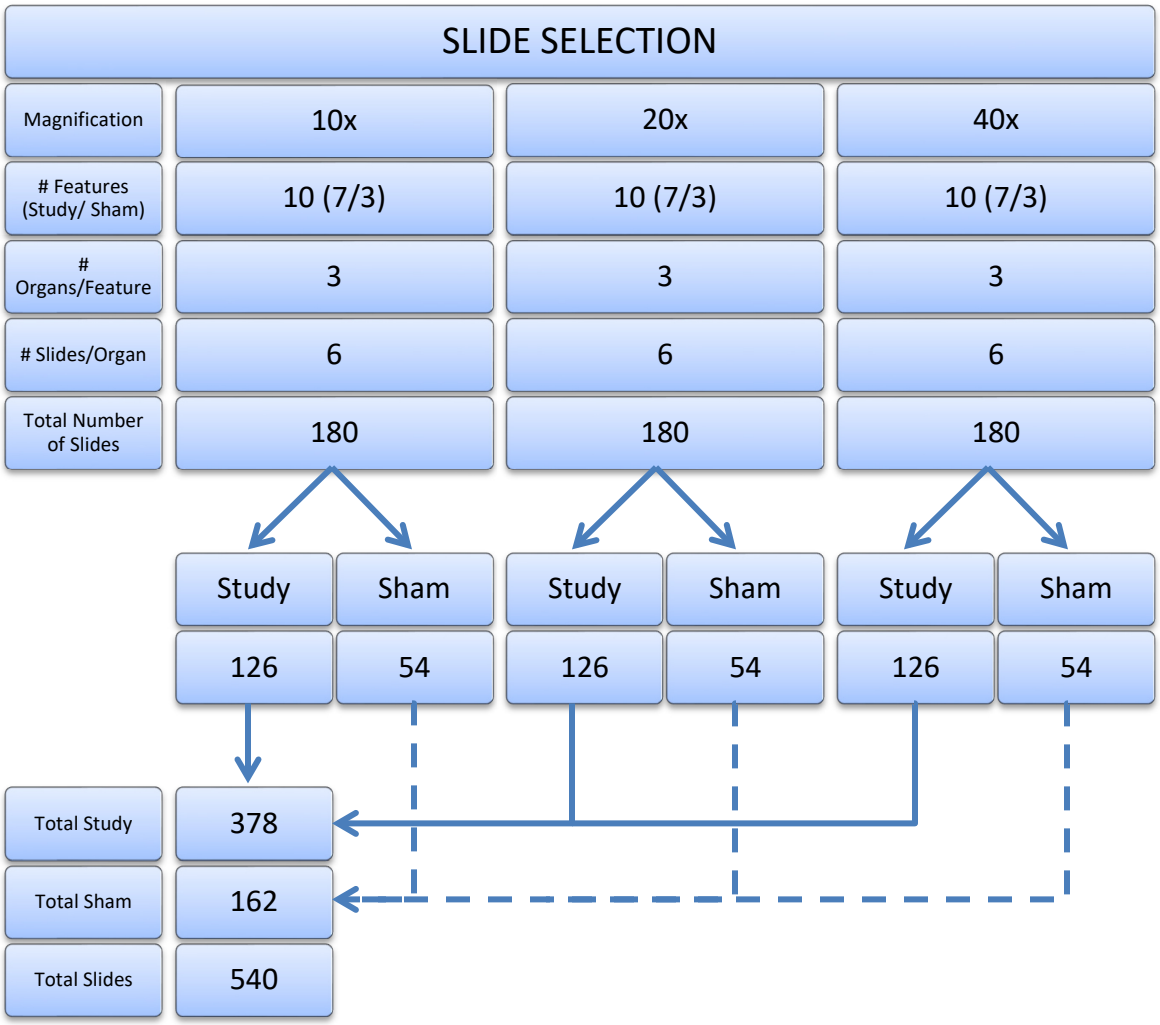


Figure 2. Slide Selection

Intra-Scanner Substudy

Site	**1**									
Scanner	**Scanner A**			**Scanner B**			**Scanner C**			**Scanner A**
Slide Allocation to Scanners	**Set-i* (set of 126 slides)**			**Set-j** (j ≠ i)**			**Set-k* (remaining slides)**			**162^{+} Sham Slides**
Scan	**Scan1**	**Scan2**	**Scan3**	**Scan1**	**Scan2**	**Scan3**	**Scan1**	**Scan2**	**Scan3**	**Scan**
RP 1		**Set 2**			**Set 3**			**Set 1**		
RS1	X					X		X		54^{++} (Set 2)
RS2			X		X		X			54 (Set 3)
RS3		X		X					X	54 (Set 1)
RP 2		**Set 1**			**Set 2**			**Set 3**		
RS1	X					X		X		54 (Set 1)
RS2			X		X		X			54 (Set 2)
RS3		X		X					X	54 (Set 3)
RP 3		**Set 3**			**Set 1**			**Set 2**		
RS1	X					X		X		54 (Set 3)
RS2			X		X		X			54 (Set 1)
RS3		X		X					X	54 (Set 2)

RP = Reading pathologist, RS = Reading session.
*** Randomly chosen 126 slides out of 378 slides such that the 126 includes 42 slides from each magnification.**
**** Randomly chosen 126 slides from remaining 252 slides such that the 126 includes 42 slides from each magnification.**
$^{+}$The 162 sham slides include 54 slides from each magnification.
$^{++}$ 54 sham slides read during each reading session and include 18 slides from each magnification.

Figure 3. Intra-scanner Substudy

Inter-Scanner Substudy

Site	1									
Scanner	**Scanner A**			**Scanner B**			**Scanner C**			**Scanner A**
Sets* of Slides	**Set 1**	**Set 2**	**Set 3**	**Set 1**	**Set 2**	**Set 3**	**Set 1**	**Set 2**	**Set 3**	**162 Sham Slides**
Scan**	**ScanA.1**	**ScanA.2**	**ScanA 3**	**ScanB 1**	**ScanB 2**	**ScanB 3**	**ScanC 1**	**ScanC 2**	**ScanC 3**	**Scan**
RP 1 (option 3)[#]										
RS1 (213)		X		X					X	54[+]
RS2 (132)	X					X		X		54
RS3 (321)			X		X		X			54
RP 2 (option 1)										
RS1 (132)	X					X		X		54
RS2 (213)		X		X					X	54
RS3 (321)			X		X		X			54
RP 3 (option 5)										
RS1 (312)			X	X				X	-	54
RS2 (231)		X	-		-	X	X			54
RS3 (123)	X				X	-		-	X	54

RP = Reading pathologist, RS = Reading session.
*** Three (3) sets of 126 slides out of 378 slides such that each set of 126 include 42 slides from each magnification.**
**** Three (3) sets of 126 slides will be scanned once on each scanner.**
[+]162 sham slides, 54 slides from each magnification, 54 read during each RE and include 18 slides from each magnification.
[#] For each RP, 1 of 6 Latin Squares selected. For each combination of sets, the first/second/third set comes from the first, second and third scanner.

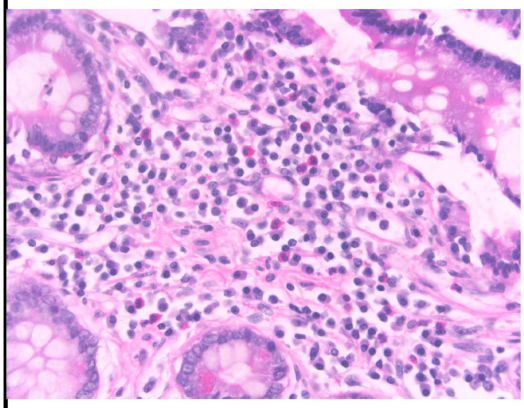
Figure 4. Inter-scanner Substudy

Inter-Site Substudy			
Site	1	2	3
Scanner	**Scanner A**	**Scanner B**	**Scanner C**
Sets of Slides*	Scan of 378 Slides	Scan of 378 Slides	Scan of 378 Slides
RP 1			
RS1	X		
RP 2			
RS1		X	
RP 3			
RS1			X

RP = Reading pathologist, RS = Reading session.
*** Slides to be transferred between sites via a Master Transfer Agreement (MTA). The same set of slides will be scanned once at each site, with a different scanner at each site and read one time by the RP at that site.**

Figure 5. Inter-site Substudy

The size of the TIFF image shall be standardized and comparable to the amount of surface area seen at that magnification. The pathologist will be asked to choose all the features detected in the FOV from the multiple-choice list. The multiple-choice list shall include 10 choices, the 7 study features and the 3 sham features for that magnification, such as in the following example (see Figure 6).



List of Features		
Feature #	Choice	40 x Magnification Group
1	Y or N	Cilia
2	Y or N	Eosinophil granules
3	Y or N	Mitotic Figure
4	Y or N	Nuclear Membrane
5	Y or N	Nucleolus
6	Y or N	Prickles Desmosomes
7	Y or N	Hemosiderin
8	Y or N	Intranuclear inclusion
9	Y or N	Melanin pigment
10	Y or N	Crystals

Legend: Eosinophil granules is the enrolled feature for this FOV. The magnification is 40x. The Reader Pathologist, however, may select any and all of the 10 multiple choices features listed, if detected in the FOV. The primary feature is present in the FOV, but other secondary feaures may also be in the same FOV.

Figure 6. Example CRF for Feature Detection at 40x Magnification

For the within scanner precision substudy, the same images will be acquired by scanning three times on the same scanner, allowing the scanner to cool down at least six hours between each iteration. For the between scanner precision substudy, the same images will be acquired by scanning on three different scanners. For the between site precision substudy, the same slides will be sent to the other sites. Fresh images will be acquired by scanning on different scanners at each of three different sites. Fresh TIFF FOV images will be created at the other sites. The pathologist at each of the sites shall read their own site's FOVs one time. The precision of these repeated readings from three different pathologists will be calculated and measured against a pre-established target of 85%. The lower limit of the two-sided, 95% confidence interval of the overall precision will be compared to 85%. For the between site precision, one pathologist from each site will be included (three pathologists overall). Different pathologists will be used for each of the 3 substudies.

The features to be tested are given in Table 1 and the organs in which the features may be found are given in Table 2. Overall there will be 21 features evaluated, 7 features for each of 3 viewing magnifications (10x, 20x and 40x). In addition a total of 9 sham features in the study, using 3 separate sham features for each of the 3 viewing magnifications.

Table 1: Features and Magnifications for Viewing			
Feature # / Magnification	**10x**	**20x**	**40x**
Rationale	**Tissue level**	**Cellular level**	**Subcellular level**
1	Small artery	Reed Sternberg cell	Cilia
2	Psammoma body	Polymorph neutrophil	Eosinophil with granules
3	Keratin pearl	Plasma cell	Mitotic Figure
4	Granuloma	Goblet cell	Infiltrating or metastatic lobular carcinoma
5	Adipocyte	Macrophage	Osteoid Matrix
6	Gland	Foreign body giant cell	Intercellular Bridges
7	Necrosis	Clear cell (of Renal Cell Carcinoma)	Hemosiderin
8	Cartilage	Myxoid stroma	Intranuclear inclusion
9	Duct	Muscle cell	Melanin pigment
10	Nerve	Calcification	Crystals

Table 2: Features Within Organs		
Mag	**Feature**	**Organ likely to be found in**
10x	Small artery	Any organ (including soft tissue including placenta)
	Psammoma body	Thyroid (PTC), Node (metastatic PTC), Ovary/Endometrium/ Peritoneum (serous carcinoma), Brain (meningioma, prolactinoma), Lymph Node
	Keratin pearl	Skin (SCC), Lung (SCC), Oral cavity (SCC), Larynx, Nodes (metastatic SCC), GE junction (SCC), Cervix
	Granuloma	Nodes, Lung, Colorectal (GI tract), Possibly any organ, Colon (Chron's disease)
	Adipose cell	Possibly any organ (especially breast, soft tissue, subcutaneous skin, GI tract mesentery, peritoneum, peri-nodal fat)
	Gland	Prostate, GI tract (includes stomach, pancreas, bile duct, colon), Uterus (includes cervix), Endocrine organs (thyroid, parathyroid, adrenal), Salivary
	Necrosis	Possibly any organ (with tumor necrosis)
	Cartilage	Lung, Larynx, Nose, Miscellaneous (bone/joint), Trachea
	Duct	Any organ
	Nerve	Possibly any organ (including soft tissue)
20x	Reed Sternberg cell	Possibly any organ with Hodgkin (Node, Mediastinum, Liver, Spleen, GI tract, Soft tissue)

Table 2: Features Within Organs		
Mag	**Feature**	**Organ likely to be found in**
	Polymorph neutrophil	Possibly any organ (with abscess, acute inflammation)
	Plasma cell	Possibly any organ (with chronic inflammation, plasma cell neoplasm)
	Goblet cell	Lung, GI tract, GE junction (Barrett's), Miscellaneous (conjunctiva), Bladder
	Macrophage	Possibly any organ (with chronic inflammation)
	Foreign body giant cell	Possibly any organ (with past surgery/sutures including skin, breast, soft tissue, GI tract), Miscellaneous (joints, gout)
	Clear cell (of Renal cell carcinoma)	Kidney, any organ with metastatic RCC
	Myxoid stroma	Soft tissue, Salivary gland tumors (pleomorphic adenoma), Uterus (leiomyoma with myxoid degeneration), Miscellaneous (joint myxoid degeneration, heart valve), Kidney
	Muscle cell	Possibly any organ (especially skeletal muscle, heart, bladder, uterus, GI tract/lung airway smooth muscle)
	Calcification	Possibly any organ (includes dystrophic & metastatic conditions such as blood vessels, tumoral calcinosis, degenerated heart valves, fat necrosis, pilomatrixoma, calciphylaxis, chondroblastoma)
40x	Cilia	Lung, Nose, Fallopian tube, Miscellaneous (middle ear, epididymis)
	Eosinophil with granules	Possibly any organ (especially nasal sinuses, eosinophilic esophagitis, dermatitis, colitis, nodes with Hodgkin, lung, component of chronic inflammation)
	Mitotic figure	Possibly any organ with proliferating tumor
	Infiltrating or metastatic lobular carcinoma	Breast, any organ with metastatic lobular carcinoma
	Osteoid Matrix	Any bone, any organ with metastatic osteosarcoma, any organ with ossification
	Intercellular Bridges	Skin, Lung (SCC), Oral cavity (SCC), Larynx, Nodes (metastatic SCC), GE junction (SCC), Cervix
	Hemosiderin	Possibly any organ (with past hemorrhage)
	Intranuclear inclusion	Thyroid (PTC), Node (metastatic PTC), Lung (adenocarcinoma), Cervix/Esophagus (herpes), GI tract (CMV), Brain (meningioma)
	Melanin pigment	Skin (benign, melanocytic lesion), Possibly any organ (with metastatic melanoma)
	Crystals	Salivary gland (amylase/tyrosine crystals), Cholesterol crystals (possibly any tissue include atheromatous plaque), Prostate, Nasal sinuses (Charcot-Leyden crystals), Testis (Reinke crystals of Leydig cell tumor)

4.1 End of Study Definition

It is anticipated that sites will have 3-5 months to screen and enroll slide, about 2-3 months to scan slides, and at least five to ten months to complete all reads. Each case will be considered complete when all study evaluations and study required assessments have been performed in accordance with the protocol.

5. INCLUSION AND EXCLUSION CRITERIA

5.1 Selection of Pathology Practice and Pathologists

Three sites shall be selected such that:

- The laboratory is accredited by the CAP
- The practice has sufficient numbers of cases to be able to provide the required number of features from the specified organ group(s)
- The site can send and receive slides between sites with material transfer agreements (for the inter-site study.)
- Site can and will conduct the study as approved by the institutional review board (IRB) and in accordance with Good Clinical Practice (GCP) and Human Subject Protection requirements.

The first site (Ohio State University Wexner Medical Center) conducting the 1st and 2nd substudy must have a minimum of nine board-certified pathologists, seven to serve as reader pathologists and two to serve as enrolling pathologists (EPs) and verifying enrolling pathologists (VEPs).

The other two sites (Cleveland Clinic Foundation, Washington University Medical center) doing only substudy 3 shall have one reader pathologist (RP) per site. The other 2 sites will not need an EP and VEP because slides are already enrolled at the first site and shared with the 2nd & 3rd site. The coordinates for feature and FOV location captured during enrollment process at the first site will be used to guide how to create the FOV, and will also be shared with site 2 & 3 so they can do the same.

Each pathologist shall be board certified, and have acceptable proficiency testing and site performance metrics (e.g., case review rate by tumor board, amended reports) not outside the practice's norm.

5.2 Selection of Cases

5.2.1 Inclusion Criteria

Glass slides are screened for the known features and will be considered eligible for the study only if all of the following criteria apply:

- Slides are selected from cases in a consecutive manner starting with cases at least 1 year old since accessioning
- Slide is a glass cover-slipped surgical pathology slide of human tissue
- Slide is stained with hematoxylin and eosin (H&E) or other stains
- Slide has the designated primary feature in the FOV, which is readily observable in its natural environment although the slide may also have one or more secondary features from the same magnification group in the FOV.

- Slide is available in the archives for use, or purchased commercially, is not damaged, has tissue on the slide which is still in good condition, has a stain that is not faded and otherwise passes all quality checks

5.2.2 Exclusion Criteria

Slides are to be excluded from the study if any of the following criteria apply:

- Slide is unable to be scanned, contains damaged tissue or has indelible markings
- Slide comes from an active (less than 1 year old) case
- Slide is from a patient who already has a slide enrolled in the study, only 1 slide per patient to be enrolled

5.3 Withdrawal or Discontinuation

5.3.1 Slide Withdrawal

It is unlikely that slides will be withdrawn from the study after they satisfy inclusion and exclusion criteria. However, if during the trial a slide somehow gets damaged or misplaced and cannot be used, or an inadvertent mix-up is discovered, this slide may be withdrawn and a protocol deviation noted. Such occurrence is expected to be very low. If necessary, a replacement slide from a new case may need to be screened, enrolled and added into the study in order to continue completing required imaging to satisfy the overall number of features examined for the trial.

5.3.2 Pathologist Withdrawal

If a reading pathologist is unable to complete study reads according to study schedules, a replacement pathologist may be selected. It is expected to be very low, but there is some possibility of staff turnover at any location.

For the intra-scanner and intra-site substudies, because of how the slides are split across readings sessions, the replacement pathologist will need to re-read any slides read by the original pathologist such that the three readings of the same slide are from the same pathologist.

For the inter-site substudy, because slides are only read once, the replacement pathologist will need to re-read any slides read by the original pathologist.

The primary analysis will not use any readings from the original/previous pathologist.

6. DEVICE UNDER STUDY

6.1 System Description

The NanoZoomer S360 MD Digital Slide Scanner System shall include:

1. The NanoZoomer unit to make whole slide digitized image scans of conventional histological glass slides of surgical tissue samples;
2. The software (C13220-01MD, Software version 1.0.0) to operate the scanner to create and store WSI images, as well as to view, manipulate, and annotate the digital WSI image on the monitor; and
3. The monitor for viewing the images.

The subsystems of the NanoZoomer system are connected over an information technology (IT) network. The IT hardware/software that supports the application software are not part of the system, but may be located in a central server room separate from the workstation with the viewing software and display. The communication of data between scanner and display may be linked by a customer-provided wired network or a direct, connected cable between the systems. The display will be validated as part of the clinical study.

Key Specifications are:

Glass slide size	26mm x 76mm
Objective lens	20x (NA 0.75)
Scanning mode	20x mode / 40x mode
Scanning resolution / pixel	20x mode (0.46micron) / 40x mode (0.23micron)
Image acquisition	Color CMOS sensor
Light source	LED
Slide capacity	360 slides

NA = numerical aperture, CMOS = complementary metal-oxide semiconductor, LED = light emitting diode.

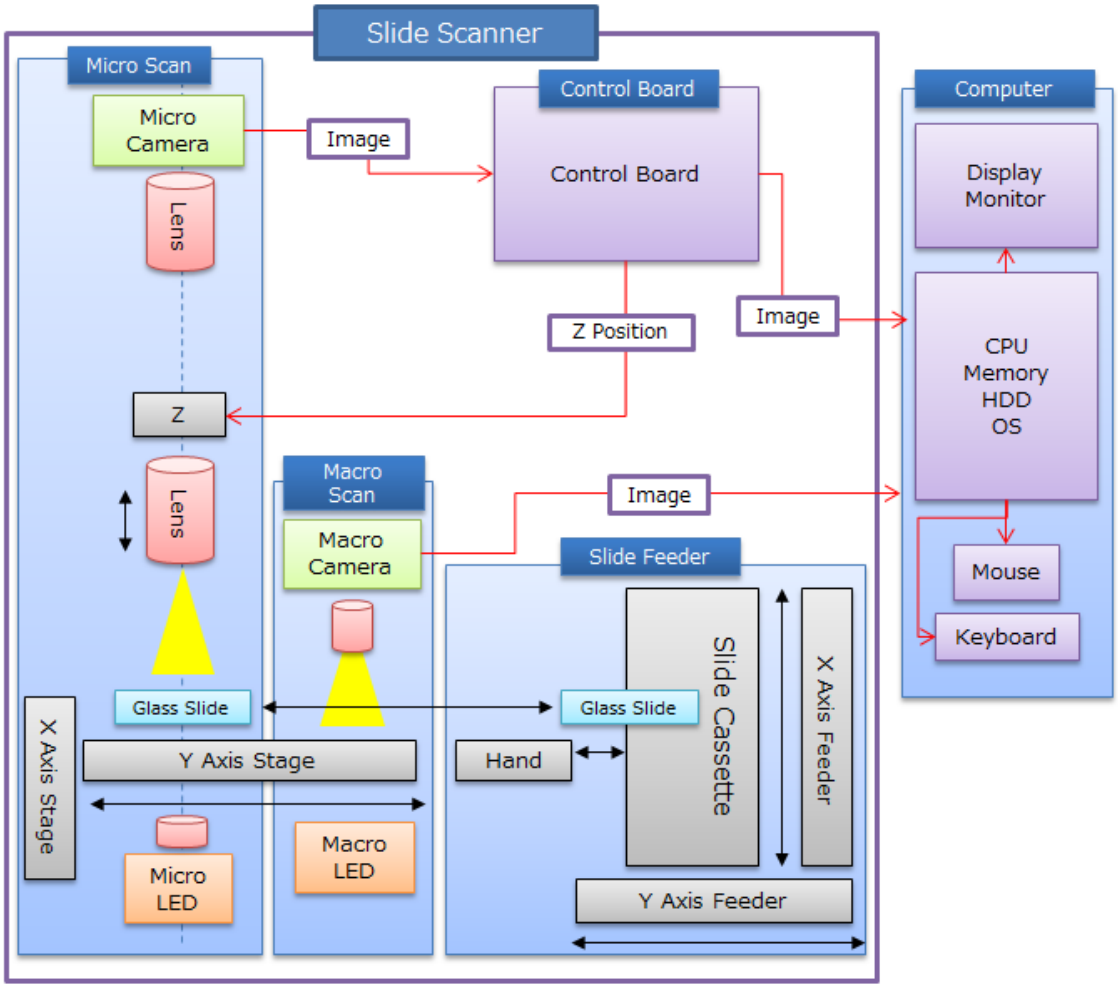


Figure 7. Communication Between Scanner and Display

The Slide Scanner consists of the following blocks as shown above in Figure 7.

- Slide Feeder: The slide feeder is manually loaded with glass slides and then transfers glass slides from the slide cassette to the macro scanning position using the mechanical hand.
- Macro Scan: When the glass slide has moved to the macro image scanning position, the macro LED illuminates the slide and acquires the macro image of both specimen and barcode label. The scanning area is automatically determined based on the macro image.
- Micro Scan: After acquiring the macro image, the glass slide is moved to the micro image scanning position. Then the micro LED illuminates the slide, and focusing points are automatically detected, and the scanning of micro images starts. After the scanning has finished, the glass slide is moved back to the slide cassette.
- Control Board: The control board controls both the micro and macro cameras together with the focusing lenses.

6.2 General Principles of Operation

The Hamamatsu NanoZoomer S360 MD Digital Slide Scanner (Figure 8) creates diagnostic quality digital images of glass slides containing FFPE tissue. Each image typically contains billions of image pixels, creating a digital image of the tissue on the original glass slide. The NanoZoomer captures digital images of entire slides for duplication, annotation, storage, retrieval, image sharing, and viewing to permit the pathologist to make a primary diagnosis without needing to view those glass slides through a light microscope.

The NanoZoomer can be loaded with as many as 360 glass slides with the mechanics of a slide feeder. The glass slide is transferred to the stage where the whole glass slide image is captured together with the barcode information present on each slide. Then the glass slide is moved to the scanning position. With the motorized stage, the 20x objective lens, the CMOS camera and the LED, the NanoZoomer detects focusing points and scans the slide to acquire the digital slide image. During the scanning, the image is automatically stored on a hard disc. After scanning, the fully digitalized image can be observed with the viewing software.

The computer controls the scanner via camera link and universal serial bus (USB) interface. It also stores the images on a hard disk. After the scanning has finished, the images can be displayed on the computer's monitor.

The system software does not have any software application that does automated image analysis to aid in computer-aided detection of diagnosis.

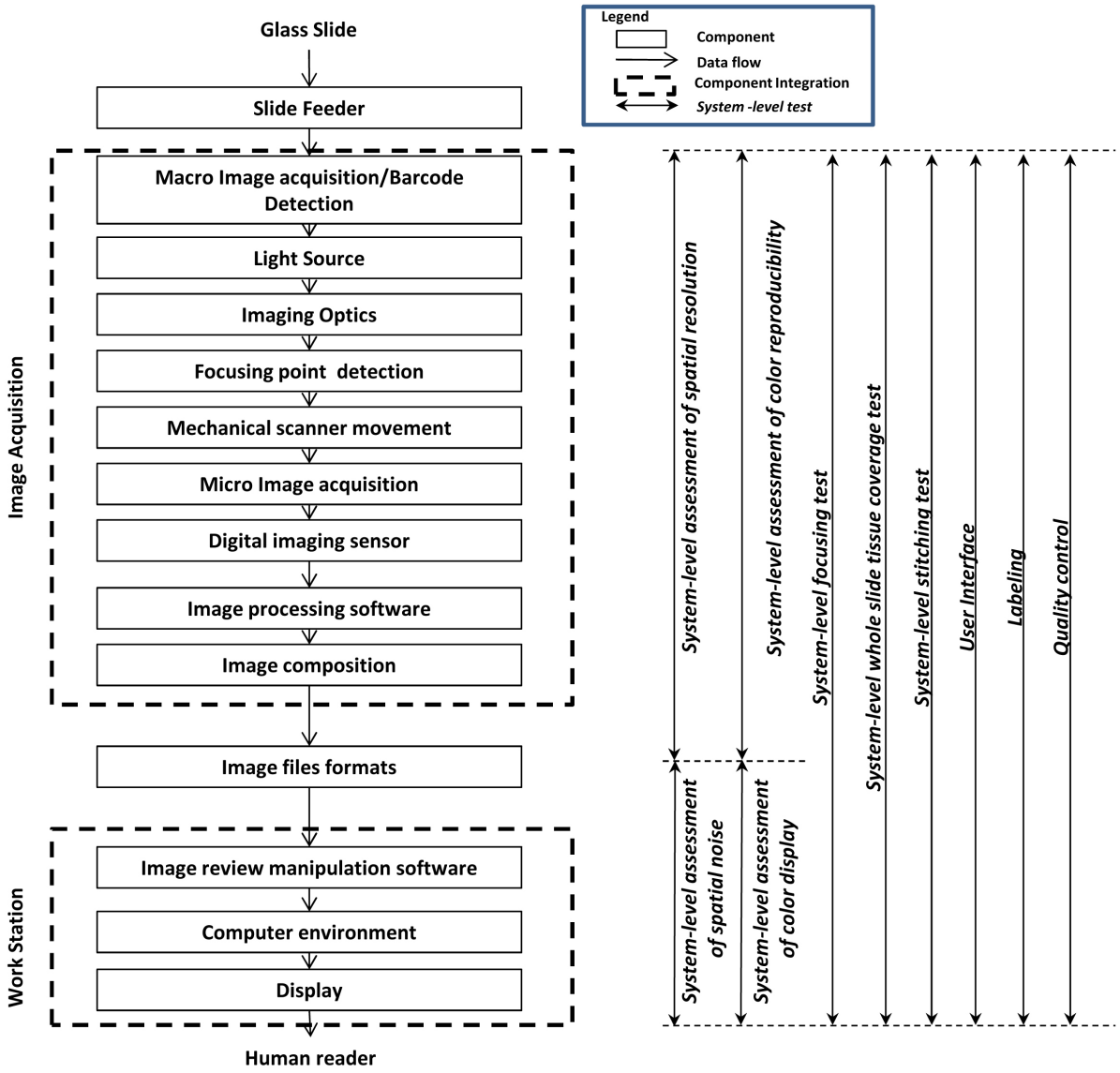


Figure 8. General Schematic of Scanner Operations

7. STUDY ASSESSMENTS AND PROCEDURES

7.1 Preparation of Facility

The Hamamatsu NanoZoomer S360 MD Digital Slide Scanner System to be installed at each of three sites and will include what constitutes the Hamamatsu system:

1. The Hamamatsu NanoZoomer S360 MD Digital Slide Scanner System to scan the slides into digital images
2. A monitor to view WSI images
3. The NanoZoomer viewing software
4. The NanoZoomer Operator's Manual for labeling information on operation of equipment

Additionally, there will be equipment that is not part of the product system, but will be used with the system, and which must meet general minimal requirements spelled out in the operator's manual, including:

1. A computer for scanner operation, data storage system for proper storage and retrieval of images, and operation of monitoring viewing and input device software to view images
2. An input device for manipulation of the image on the monitor, and possibly
3. The site's intranet for storage of WSI images

The Hamamatsu systems shall be labeled as "Investigational Device: For Clinical Research only."

The sponsor shall install a complete Hamamatsu NanoZoomer S360 MD Scanning Systems at all sites, with three scanners at one site, and one scanner apiece at the two other sites.

The sponsor product specialists shall ensure the devices are operational and that all sites have all proper records and any relevant certificates of manufacture. Likewise, the sponsor shall ensure any required maintenance is planned and conducted for the instruments prior to the start and during the course of study, as required; and that maintenance will be documented. Sponsor will track the serial number of each NanoZoomer installed at each site. The Sponsor will retain at the site any shipping records for each device. Device accountability logs will be used to account for equipment at site. The Sponsor will maintain accountability of each device. These records will be kept in a secure place at the site by the study coordinator.

The site IT department shall ensure there is proper and secure IT services for data storage of scanned images, recording of medical information, and retrieval of images for viewing, and for proper and secure use of EDC systems for data collection.

The WSI monitor viewing station cockpit and input device shall be standardized across all sites' viewing stations will be the only product monitor of the NanoZoomer system.

7.2 Training on Study Procedures and WSI Operation

The sponsor, or its designated contract research organizations (CROs) or clinical professionals, shall conduct training according to pre-established and documented training modules, which shall include procedures for:

- Screening and enrolling of features chosen, training slides, and self-familiarization slides

- Coding procedures for features and organ for the enrolled case
- Coding procedures for the reader pathologist chosen to participate in the study
- Storing, securing, and de-identifying Protected Health Information (PHI) for cases selected for study
- Use of WSI equipment for scanning and image storage, coordination with IT systems, and viewing workstation setup and operation
- Conduct of self-familiarization process
- Installation and maintenance of an CRO electronic data capture (EDC) system at the site
- Entering, storing, and securing data in CRO electronic Case Report Form (CRF)
- CRO Data management plans
- CRO Randomization procedures for scanning and reading slides
- Document and report protocol deviations and device deficiencies
- Document and reporting of other reportable events
- CRO Management of data integrity (verify, validate, lock database)
- CRO GCP requirements
- CRO GCP monitoring procedures

Each training event shall be recorded in a training log as to what training was done, who was trained, who did the training, where the training was performed, and the date of training. The training records shall be stored in a secure place by the site coordinator.

Training shall include training of:

- Entire study staff (Principal Investigator [PI], sub-PIs, study coordinator [SC], EP, VEP, RPs, WSI technicians, and IT personnel) on study protocol and processes relevant to their part of the study, GCP and human subjects training
- Coordinator on proper storage of equipment, enrolled slides and essential study GCP documents and records, eCRF and data records management
- Technicians on operation of NanoZoomer scanner equipment, how to scan, review, check, annotate, and store scanned image
- EP and Scanning Technicians how to locate feature on WSI from coordinates captured on glass, create TIFF image of FOV and processes how to randomize the location of the feature in different regions of the FOV, and randomized rotations of the image, and training on what and how to fill out the electronic CRF (eCRF) for scanning operation
- IT staff responsible for proper storage and retrieval of scanned images
- EP and VEP (site 1 only, Ohio State University Wexner Medical Center) on selection and validation of cases and glass slides for feature study, recording of known feature from glass slide and system to record coordinates where feature was present on the glass slide for later location when making the TIFF image from WSI image of that glass slide
- Pathologist “readers” on the protocol, the study processes, on instructions how to operate and view WSI slides for feature detection, and how to fill out the eCRF for each case, and self-familiarization process

The EP and VEP shall follow the same process to select 15 slides for self-familiarization, as well as establishing the designation of the known feature and feature coordinates.

7.3 Selection of Slides for Feature Detection

Glass slides shall be chosen per the procedure below and consistent with the inclusion and exclusion criteria for the study provided it has the specific feature required for that search. The exact slides, features, organs, and magnifications needed for the study are described in Table 1 and are the same for each of the three sub-studies all enrolled from the first site (Ohio State University Wexner Medical Center).

The coordinator and/or EP shall search the first site's database to locate cases that may contain glass slides with the specific feature from pre-specified organs as required by the protocol. Once the slides are obtained, the cases will be further screened by the EP to determine if they meet inclusion and exclusion criteria in order to be enrolled in the study.

Then, the EP will locate the known feature on the glass slide and the coordinates of the selected feature will be recorded. Also, the EP shall record if any other secondary features from same magnification group listed in Table 1 are within the FOV under the microscope view.

The slide will be scanned immediately to create a WSI image, which shall be a "locator WSI" used by the scanning technician to locate and document the WSI coordinates of the selected feature. The coordinates recorded from this locator WSI will be used to construct the study FOVs for each of the respective study scans for that specific enrolled feature.

Because of the wide variety of cases and features, the EP may seek the consult of other pathologists in the site's practice, as long as these other pathologists are not reader pathologists or VEPs. The Sponsor may choose to purchase known cases. In such a situation, the EP will follow the same procedure for purchased slides.

7.4 Validation of Slides for Feature Detection

The trained VEP will review and confirm the choice of slide, feature coordinates, the designated feature recorded and the presence of any coincidental features in the FOV. If there is a discrepancy of the primary feature determination between the EP and VEP, then both the EP and VEP will convene a consensus meeting to come to a decision. If the EP and VEP cannot come to a consensus decision, then that feature/slide choice shall be disqualified from the study.

The process shall be repeated to select an additional set of slides, labeled as the sham features, which follow the same feature selection criteria, but are not used in the analysis.

For each sham feature 6 slides will be selected from 3 different organs.

7.5 Storage of Slides for Feature Detection

The study and self-familiarization slides, the feature's known description, and organ, and feature coordinate information shall be delivered to the SC for safe and secure storage.

The SC shall:

a. Collect and store the cases, slides, organ and known feature description
b. Collect and store log of feature as well as FOV location and coordinates

c. Place proper de-identification labels (e.g., hide names, numbers, barcodes) on enrolled glass slides with a code known only to the SC, and confirm those labels will not interfere with the scanning process nor affect the integrity of the tissue on the slide
d. Remove all patient identifiers and assign coded-names to protect patient privacy
e. Ensure traceability is kept only at the site, stored in a secure location, not shared outside the practice, and kept blinded to reading pathologists

7.6 Preparation of WSI images and Creation of TIFF Files

The SC will provide the slides to the scanning technician.

The slide will be scanned at 40x magnification to create the WSI image into the standard Hamamatsu NDPI file format. For additional iterations, the NanoZoomer will be powered down for at least six hours for a complete cool down before the next scanning session. After each scan, the image quality will be checked. If image quality is not acceptable, scanning may be repeated up to 5 times.

If a slide is broken during the study, a decision will be made to record it as a protocol deviation, or to enroll a replacement slide for an incomplete substudy. The replacement slide will be scanned like the others in its set, but not for substudies already completed.

To make the TIFF FOV images for any enrolled slide, the scanning technician(s) shall utilize the locator WSI (made at screening & enrollment) and the sponsor's programs to create TIFF FOV images of the study scans using the originally recorded coordinates. These locator WSI files will be made at site 1 and shared with site 2 and 3 (See Figure 9)

Figure 9. Steps to Create the TIFF FOV Image

First, the scan technician shall locate the feature in the study scan WSI image, using the coordinate identified from the locator WSI for that feature. The scanning technician will create a series of TIFF FOV images at 10x, 20x, or 40x as specified in Table 1. The pixel density of the TIFF FOV image shall be identical to the designated software, display and driving computer to the end user. The size of the TIFF image shall be consistent with the size normally seen at those magnifications (10, 20 or 40X) under the microscope.

The location of the known feature in that TIFF shall be randomized to different locations in the image, and not always in the center of field.

The TIFF image of the feature FOV will be assigned a de-identified coded name kept confidential by the coordinator in a secure location. The TIFF images will be stored in a password protected secure drive of the NanoZoomer computer for later viewing by the reader pathologists.

The SC shall ensure this information is stored in a secure computer hard disk drive, and/or on a secure folder on the Intranet server with secure access known only to the SC. The reading pathologists shall be blinded to the feature information in the study.

The trained RPs shall be allowed to conduct a self-guided mock feature detection on a small set of training cases in order to become familiar with equipment operation for this study. The PI and sponsor's technical staff will be available for discussion of any questions.

7.7 Reading of TIFF Images

The SC shall provide instructions to the RP as to which TIFF images are to be viewed, and in what order, and which eCRFs are to be filled out.

Upon completion of those activities, the SC shall collect, return and store the materials once again to a secure place.

The SC shall provide the RP with the TIFF images to be read on the Hamamatsu workstation monitor in a pre-established randomized order in a password protected study hard drive or server.

Trained RPs in the selected sites will view the set of TIFF images on the monitor workstation of the NanoZoomer and will be asked to identify all the features of interest against a list of 10 possible choices, i.e., the 7 study feature and 3 sham features for that magnification. The RP will fill out the eCRF for the features identified from that FOV.

The TIFF FOV will be randomly rotated, when repeat readings of the same FOV by the same pathologist occurs. The rotations may be some multiple of 90 degrees only (i.e., 0° [no rotation], 90°, 180° or 270°).

When given the set of TIFF images for that reading, we anticipate that the time to read all images may lead to fatigue. Therefore, the RP shall be permitted to read images for a period of no more than two hours, at which time the RP must take a minimum one-hour break before continuing with the rest of the assigned readings.

The SC shall confirm all entries are filled in. The SC, scanning technician or RP shall record if any protocol deviation or device deficiency is observed. The SC shall report to sponsor if any such events have occurred and ensure the event is resolved per study and institutional procedures.

Different RPs will be used for each of the three substudies.

For the first two substudies: On the first day, each of 3 participating RPs will be required to conduct the first reading (R1) of their TIFF images in a random order with respect to the scanner (1, 2 or 3) and scan event (1 or 2) established by the statistician. After a minimum two-week washout period, the same RP will read the same set of slides again for the 2nd reading (R2) and another two-week washout period will occur before the 3rd reading (R3).

For the third substudy: Each participating RP, one at each of three sites, will be required to conduct the first and only reading (R1) of their TIFF images in a random order established by the statistician with the images produced only at their site.

7.8 Pilot Studies

Before the study starts, the sponsor may choose to conduct one or more optional pre-study mock pilot trials of certain key procedures, specifically the screening and enrollment, creation of FOVs, and reader pathologist detection of feature procedure. This pilot is an extension of the user acceptance testing and is to allow the sponsor to observe and optimize the combined operation and workflow of training, procedures and eCRF recordings to insure processes are operational as expected, and consistent amongst participants. In order to insure pilots are being done correctly, the sponsor and consultants may review pilot data and compare against de-identified pathology reports and scans of de-identified histochemical slides for that pilot case.

The pilot will include participation of the actual pathologists and scanning technicians who are assigned in the study. Each pilot may be a full or abbreviated test of the actual study procedure by those participants. Each pilot may use upwards of 10-20 de-identified cases from the archive. And, so to not interfere with the cases selection process, these pilot cases are to be selected with accession dates less than 1 year from non-active cases.

8. STATISTICAL CONSIDERATIONS

To minimize bias due to recall of FOVs from previous reads, different reader pathologists will be used in the 3 different substudies.

8.1 Intra-Scanner Precision Substudy

This substudy includes one site with three scanners (called scanner A, scanner B, and scanner C). The random ordering of slides for the study and the sham slides is described below. The 378 study slides will be divided equally into three sets of 126 slides, 42 slides from each of 3 viewing magnifications (called set1, set2, and set3) using stratified randomization and the three sets randomly ordered into six possible combinations (123, 231, 312; 132, 213, 321). These six combinations create twelve 3x3 Latin Squares. One combination of the sets (1 of the 12 Latin Squares) will be randomly selected (say Latin Square 1 [123, 231,312] as shown in the table below). Each number appears once on each row and on each column and each number represents a set of 126 slides, where each row and each column adds up to 378 slides.

Latin Square 1:			
	Column1	Column2	Column3
Row1	1	2	3
Row2	***2***	***3***	***1***
Row3	3	1	2
Each number appears once in each row and column. The number refers to a set.			

Assigning 378 Slides to Each Reader:

Step 1 - For a selected Latin Square there are 6 possible choices for assigning the rows to the 3 readers. These choices are:

	Choices for Assigning Each Combination to a Reader					
Pathologist	**1**	**2**	**3**	**4**	**5**	**6**
Reader 1	123	123	231	312	312	231
Reader 2	231	312	123	123	231	312
Reader 3	312	231	312	231	123	123

One option will be randomly chosen and assigned to readers. Suppose the selected option is choice 3. Then combination 231 will be assigned to Reader 1, combination 123 will be assigned to Reader 2 and Reader 3 will be assigned combination 312.

Step 2 - Then the combination will be assigned to three scanners in such a way that the first number of the combination is assigned to Scanner A, the second number is assigned to scanner B and the third number is assigned to Scanner C. For Reader 1 the combination is 231 and the 126 slides in each set is assigned to each of three scanners as shown below:

Scanner	**Set**
Scanner A	2
Scanner B	3
Scanner C	1

Step 3 - On each scanner the 126 slides will be scanned three times (called iteration1, iteration2, iteration3) with at least six hours downtime (ensuring full cool down) of the scanner between scanning iterations as shown below:

	Iteration		
Scanner	Iteration 1	Iteration 2	Iteration 3
Scanner A	2.1 [Set 2, Iteration 1]	2.2	2.3
Scanner B	3.1	3.2	3.3
Scanner C	1.1	1.2	1.3

Step 4 – From above scanner by iteration table one of six random 3x3 Latin Square can be created for the three scanners and three reading sessions for a given reader such that for a given session each set comes from a different scanner and a different iteration. One selected 3x3 Latin Square is given in Table 3 for three reading sessions for reader 1.

Table 3: Procedure to Randomly Select 378 Slides from Combination of Scanners and Iterations for Reader 1				
		Reading Session		
		1	2	3
Set	1	**Scanner C, iteration 2** 1.2 (n=126)	**Scanner C, iteration 1** 1.1 (n=126)	**Scanner C, iteration 3** 1.3 (n=126)
	2	**Scanner A, iteration 1** 2.1 (n=126)	**Scanner A, iteration 3** 2.3 (n=126)	**Scanner A, iteration 2** 2.2 (n=126)
	3	**Scanner B, iteration 3** 3.3 (n=126)	**Scanner B, iteration 2** 3.2 (n=126)	**Scanner B, iteration 1** 3.1 (n=126)
Total		378	378	378
Set1, set2, and set3 do not need to be equal, but they need to add up to 378 slides and each set should have equal number of slides from each of 3 viewing magnifications				

Three separate reading sessions will be performed by each of three reading pathologists, with a washout period of at least two weeks between reading sessions. All 162 sham slides will be scanned once on scanner A. Per reading session, stratified randomization will be used to select 54 different sham slides (18 for each sham feature of a specific viewing magnifications) to add to each reading session such that all 162 sham slides will be read by each reading pathologist. The rotation of the FOV will be randomly determined in 90-degree intervals (0° [no rotation], 90°, 180°, 270°).

Then the reading sessions for reader 1 can be displayed as follows using Table 3:

Session 1 reading for Reader 1 is combination of (1.2, 3.3, 2.1 + 54 sham slides)

Session 2 reading for Reader 1 is combination of (1.1, 2.3, 3.2 + 54 sham slides)

Session 3 reading for Reader 1 is combination of (2.2, 1.3, 3.1 + 54 sham slides)

As can be seen, each element of a given reading session comes from different rows and columns of the scanner by iteration table.

The process described above for Reader 1 will be repeated for readers 2 and 3. At the end, all 378 slides are scanned by each scanner and read by each reader.

8.1.1 Required Number of Slides

The precision will be based on three reading pathologists' assessments and identification of specific histologic features that are observed in TIFF images of FOV of FFPE H&E slides containing a primary feature. The twenty one (21) study features and 9 sham features selected for this study are provided in Table 1. Each selected feature will be selected from at least three organs. There will be 6 FOVs per organ within each combination of feature-magnification. The 21 study features will be evaluated at their relevant magnification. The levels of viewing magnification will be 10x, 20x, and 40x. Each level of magnification will include seven study features.

For each study feature, three organs will be selected. For each organ, six FOVs will be selected, and it is assumed that each FOV contains one primary feature. Thus, the required number of study slides is

21 study features x 3 organs/study feature x 6 FOV/organ = 378 slides, which include selected study features and selected FOVs (see Table 4).

Table 4: Required Number of Slides with Selected Features and Selected FOVs

Feature	Organ	Slides with Selected Feature &FOVs
1	1	1-6
1	2	7-12
1	3	13-18
2	1	19-24
2	2	25-30
2	3	31-36
.	.	.
.	.	.
.	.	.
.	.	.
21	1	361-366
21	2	367-372
21	3	373-378

In addition to the 378 FOVs selected for this study, 9 sham features, 3 for each viewing magnifications will be used. The number of sham slides is (3 viewing magnifications) x (3 features per magnification) x (3 organs per feature) x (6 FOVs per organ) = 3 x 9 x 6 = 3 x 54 = 162 sham slides, which include selected features and selected FOVs. The 162 sham FOVs will be selected from other glass slides. Sham FOVs will be used to minimize or avoid bias by the RP, but will not be included in any analysis. Thus, the total FOV set for the feature study is 540 FOVs.

8.1.2 Statistical Analysis of Intra-Scanner Precision

Each of three scanners will scan one-third of the slides three times; three RPs evaluate all scanned slides across three reading sessions. Table 5 shows number of comparison pairs for each scanner.

Table 5: Intra-Scanner Pairwise Comparison

Scanner	Number of Comparison Pairs
Scanner A	1134
Scanner B	1134
Scanner C	1134
Overall	3404

The number of comparison pairs, that is 1134, is calculated based on three RPs where for each RP there are three pairwise comparisons, namely scan1 vs. scan2, scan1 vs. scan3, and scan2 vs. scan3, which results in 3 reader x 126 pairs per comparison x 3 pairwise comparisons =1134 (see Table 6) comparisons per scanner.

Table 6: Number of Comparison Pair Per-Scanner

		Number of Pairwise Comparisons			Total # of Pairwise Comparisons
Scanner 1	**Reader**	**Scan1 vs. Scan2**	**Scan1 vs. Scan3**	**Scan2 vs. Scan3**	
	1	126	126	126	378
	2	126	126	126	378
	3	126	126	126	378
					1134

To evaluate Intra-Scanner precision for scanner A, three (3) single 2x2 tables can be constructed for pairwise comparison within scanner A and Reader1. Table 7 demonstrates a 2x2 table for the pairwise comparison of scan1 versus scan2 for Reader1.

The result of reading for each slide is “Yes” or “No” if the primary feature was detected correctly. Each primary feature shall be considered as of equal importance as the next primary feature; thus, the results of all primary features will be pooled.

Table 7: Intra Scanner/Inter Scan Precision for Scanner A

		Feature is correctly identified using scan2		
		Yes	**No**	
Feature is correctly identified using scan1	Yes	a	b	a+b
	No	c	d	c+d
		a+c	b+d	126

Nine (9) 2x2 Tables will be pooled within scanner A and across 3 readers to create Table 8 for $scan_{ki}$ versus $scan_{kj}$ for Readers k (k=1,2,3) and i≠j (for i=1, 2, 3 and j>i).

Table 8: Intra Scanner/Inter Scan Precision for Scanner A and all 3 Readers

		Feature is correctly identified using $scan_j$		
		Yes	**No**	
Feature is correctly identified using $scan_i$	Yes	a^1	b^1	a^1+b^1
	No	c^1	d^1	c^1+d^1
		a^1+c^1	b^1+d^1	1134

Then the Agreement Rate for each Scanner=$(a^1+d^1)/1134$ and 95% confidence interval (CI) will be estimated as shown in Table 9.

Table 9: Intra-Scanner Results

	Number of Pairwise Agreement	Number of Comparison Pairs	Agreement Rate	
			%	95% CI
Scanner A	a^1+d^1	1134	$(a^1+d^1)/1134$	
Scanner B	a^2+d^2	1134	$(a^2+d^2)/1134$	
Scanner C	a^3+d^3	1134	$(a^3+d^3)/1134$	
Overall	a^*+d^*	3402	$(a^*+d^*)/3402$	

The overall Intra-Scanner agreement rate will be calculated by averaging all pairwise comparisons results over all 378 slides with selected study features enrolled and all three pathologists. The overall number is 378 slides with selected features x 3 scanners x 3 readers = 3402. To preserve the correlation structure of multiple readings of the same feature and possibly multiple features on an FOV, the bootstrap method will be used to derive a two-sided 95% CI for the overall agreement rate. An FOV will be the bootstrap re-sampling unit.

Above analyses will be performed for primary feature.

The study acceptance criterion is 85%. The lower limit of the 95% CI for the overall agreement rate will be compared to 85% and if the lower limit is greater than 85%, then the study will have demonstrated intra-scanner precision.

In addition to the overall agreement given in Table 9, overall average positive agreement (APA) and overall average negative agreement (ANA) will be provided with their corresponding two-sided 95% CIs.

In Table 8, the percent agreement of scan i with scan j for a positive result is called positive percent agreement (PPA) and is defined as

$PPA = a^1/(a^1+c^1)$

The percent agreement of scan i with scan j for a negative result is called negative percent agreement (NPA) and is defined as

$NPA = d^1/(b^1+d^1)$

When obtaining PPA or NPA, one cannot assume either scan i or scan j is the reference. Thus, it is suggested that a reasonable summary of positive agreement between two scans is an average of the two PPAs, and a reasonable summary of negative agreement is an average of the two NPAs. These averages should be weighted to reflect the different marginal totals that are used when scan i or scan j is used as the reference. Clinical and Laboratory Standards Institute (28) defines APA and ANA as follows:

$APA = 2\ a^1/(2a^1+b^1+ c^1)$

$ANA = 2\ d^1/(c^1+ b^1+2d^1)$

Table 10 provides the overall APA and overall ANA rates with corresponding 2-sided, 95% CIs by pooling all pairwise comparisons over the three scanners.

Table 10: Intra-Scanner Overall APA and Overall ANA Rates					
	Number of Pairwise Agreement	**APA Agreement Rate**		**ANA Agreement Rate**	
		%	**95% CI**	**%**	**95% CI**
Overall	a^*+d^*	$2a^*/(2a^*+b^*+c^*)$		$2d^*/(c^*+b^*+2d^*)$	

8.1.3 Statistical Analysis of Secondary Features

The presence of secondary features in the FOVs is expected to be sparse because the FOV is selected for a specific primary feature.

The objective of the analysis is to investigate the frequency distribution of the absence or presence of the secondary features. Each feature will be considered primary for 18 FOVs (3 organs x 6 FOVs per organ) and will be considered secondary for the remaining 360 FOVs (this means n_1 of the 18 FOVs in set 1, n_2 of 18 FOVs in set 2, and n_3 of 18 FOVs in set 3, where $n_1+n_2+n_3=18$). It is possible that a given feature may not be present in the majority of the 360 FOVs.

The secondary analysis will be based on the repeatability/reproducibility regarding each secondary feature separately. To perform the secondary analysis for a specific feature, say feature X, 3 scans (scan1, scan2, and scan3) per scanner will be used. In the 3 scans from scanner A, there are n_1 FOVs primary for feature X among 126 FOVs and $126–n_1$ FOVs secondary for feature X. Thus, $126–n_1$ FOVs will be used to construct the following 2x2 contingency table.

Table 11: Intra Scanner/Inter Scan Secondary Analysis for Scanner A				
		Secondary Feature X is Observed Using Scan2		
		Yes	**No**	
Secondary Feature X is Observed Using Scan1	**Yes**	a	b	a+b
	No	c	d	c+d
		a+c	b+d	*126-n_1*

Nine (9) 2x2 tables will be pooled within scanner A and across 3 readers to create Table 12 for $scan_i$ versus $scan_j$ for reader *k* (k=1,2,3) and i≠j (for i=1,2,3 and j>i).

Table 12: Intra-Scanner/Inter-Scan Secondary Analysis for Scanner A Across all 3 Readers				
		Secondary Feature is Observed Using $Scan_j$		
		Yes	No	
Secondary Feature is Observed Using $Scan_i$	Yes	a^1	b^1	a^1+b^1
	No	c^1	d^1	c^1+d^1
		a^1+c^1	b^1+d^1	$1134 – 3(n_1+n_2+n_3)$

Repeatability and reproducibility of the reading results (i.e., overall APA and overall ANA) will be provided as shown in Table 13 for each of the 21 features that appear as secondary features. In total, up to 21 contingency tables, one for each secondary feature, will be provided, based on the 3240 (3402 – 9 x 18) pairwise comparisons across all 3 readers and all 3 scanners.

Table 13: Overall APA and Overall ANA Rates for Secondary Feature Analysis			
	Number of Pairwise Agreement	APA Agreement Rate	ANA Agreement Rate
		%	%
Overall	a^*+d^*	$2a^*/(2a^*+b^*+c^*)$	$2d^*/(c^*+b^*+2d^*)$

8.2 Inter-Scanner Precision Substudy

The same set of all 378 slides with selected features and FOVs will be scanned in this substudy, once on each of the 3 scanners at one site and the same 162 sham slides will be used in this substudy from the intra-scanner study.

Three (3) separate reading sessions will be performed by each pathologist with a washout period of at least two weeks between sessions. Per reading session, 54 different sham slides will be added such that all 162 sham slides will be read by each reading pathologist. The rotation of the FOV will be randomly determined in 90-degree intervals (0° [no rotation], 90°, 180°, 270°).

Sets 1, 2, and 3 created in the Intra-Scanner study will be used in the Inter-Scanner study. Each set will then be randomly assigned to be scanned in a random order on Scanner A, Scanner B and Scanner C using a Latin Square. As discussed in intra-scanner study, once all sets have been scanned, one 3x3 Latin Square out of 12 possible choices will be selected to assign sets to reading sessions, say

Latin Square 1:			
	Column1	Column2	Column3
Row1	2	3	1
Row2	1	2	3
Row3	3	1	2
Each number appears once in each row and column. The number refers to a set.			

Then combination 231 (row 1) will be assigned to reading session 1 (RS1), combination 123 will be assigned to RS2 and combination 312 will be assigned to RS3.

Then the combination will be assigned to three scanners in such a way that the first number of the combination is assigned to Scanner A, the second number is assigned to scanner B and the third number is assigned to Scanner C. For RS1 each set of the combination 231 (126 slides in each set) will be assigned to each of three scanners as shown below:

Scanner	Set
Scanner A	2
Scanner B	3
Scanner C	1

Thus, for Reader 1 a 3x3 Latin Square for combination of Reading Session-Scanner is as follows;

Slide Reading Schedule				
Reader	**Reading Session**	**Scanner A**	**Scanner B**	**Scanner C**
1	1	Set 2	3	1
1	2	1	2	3
1	3	3	1	2

RS1= read set 2 from Scanner A + read set 3 from Scanner B +read set 1 from Scanner C + read 54 sham

RS2=read set 1 from Scanner A + read set 2 from Scanner B +read set 3 from Scanner C +read 54 sham

RS3=read set 3 from Scanner A+ read set 1 from Scanner B +read set 2 from Scanner C +read 54 sham

Choosing 2 other 3x3 Latin Square out of 12 possible Latin Squares by random without replacement for reader 2 and reader 3 will provide Table 14.

Table 14: Slide Reading Schedule

Reader	Reading Session	Scanner A	Scanner B	Scanner C
1	1	2	3	1
1	2	1	2	3
1	3	3	1	2
2	1	1	2	3
2	2	2	3	1
2	3	3	1	2
3	1	3	1	2
3	2	2	3	1
3	3	1	2	3
Total scans read		1134	1134	1134

Table 15 provides the number of scans pooled over reading sessions.

Table 15: Slides Pooled over Reading Sessions			
Reader	**Scanner A**	**Scanner B**	**Scanner C**
1	378	378	378
2	378	378	378
3	378	378	378
Total	1134	1134	1134

To evaluate the Inter-Scanner precision, we need to estimate the precision for three pairwise comparisons, namely scanner A versus scanner B, scanner A vs. scanner C, and scanner B vs. scanner C. For the pairwise comparison, three (3) 2x2 tables similar to Table 8 can be constructed for each of 3 readers and each reading session. Inter-Scanner precision for each reader can be created by pooling the nine (9) 2x2 tables for each reader. In addition, the overall Inter-Scanner precision will be estimated by pooling three 2x2 contingency tables across 3 readers as shown in Table 16.

Table 16: Inter-Scanner Results				
	Number of Pairwise Agreement	**Number of Comparison Pairs**	**Agreement Rate**	
			%	**95% CI**
Reader 1	a^1+d^1	1134	$(a^1+d^1)/1134$	
Reader 2	a^2+d^2	1134	$(a^2+d^2)/1134$	
Reader 3	a^3+d^3	1134	$(a^3+d^3)/1134$	
Overall	a^*+d^*	3402	$(a^*+d^*)/3402$	

The two-sided 95% CI for overall Inter-Scanner agreement rate will be calculated in a manner similar to the one provided for the Intra-Scanner substudy.

The above analyses will be performed for the primary feature.

The study acceptance criterion is 85%. The lower limit of the 95% CI for the overall agreement rate will be compared to 85% and if the lower limit is greater than 85%, then the study will have demonstrated inter-scanner precision.

In addition to the overall agreement, overall APA and overall ANA rates for inter-scanner precision will be provided with their corresponding two-sided 95% CIs.

Analysis of secondary features will be similar to the analysis described for the intra-scanner substudy (see section 8.1.3).

8.3 Inter-Site Reproducibility Substudy

In this study, there will be three sites and different RPs at each site. For the inter-site study, the same sets will be scanned once at each site and each of the three RPs will have only one reading session in which all sets scanned at their site will be read randomly.

The overall inter-site agreement rate will be calculated in the same manner as for the intra-scanner study. The inter-site study results can be summarized in Table 17.

Table 17: Inter-Site Precision Results

	Number of Pairwise Agreement	Number of Comparison Pairs	Agreement Rate	
			%	95% CI
Site1 vs. Site2	a^1+d^1	378	$(a^1+d^1)/ 378$	
Site1 vs. Site3	a^2+d^2	378	$(a^2+d^2)/ 378$	
Site2 vs. Site3	a^3+d^3	378	$(a^3+d^3)/378$	
Overall	a^*+d^*	1134	$(a^*+d^*)/1134$	

The above analyses will be performed for the primary feature.

The study acceptance criterion is 85%. The lower limit of the 95% CI for the overall agreement rate will be compared to 85% and if the lower limit is greater than 85%, then the study will have demonstrated inter-scanner precision.

In addition to the overall agreement, overall APA and overall ANA rates for inter-site precision will be provided with their corresponding two-sided 95% CIs.

Analysis of secondary features will be similar to the analysis described for the intra-scanner substudy (see section 8.1.3).

9. DATA MANAGEMENT AND DATA QUALITY

9.1 Data Management

The study shall have a data management plan to define how the data is handled, managed, stored, secured, and how the processes for that are validated, and how the access is controlled.

CRFs and/or study logs will be used to collect the data over the course of the study.

For the CRF, that data will be entered into an EDC system

Data recordings shall include:

- During screening and enrollment:
  - Site name
  - Start date and time
  - Slide # and sub-number
  - Name of technician scanning slide
  - Name of enrolling pathologist
  - Name of primary feature of interest.
  - Organ of slide for primary feature
  - Coordinates for primary feature of interest on glass & WSI
  - Randomized sector where primary feature is to be placed.
  - Presence of any secondary features in FOV seen under microscope

  - Name of validation enrolling pathologist
  - Confirmation if VEP agrees or disagrees with EP
  - If disagreement, consensus meeting identifier (ID) between EP & VEP, if consensus reached, or if slide/feature disqualified
  - Record of protocol deviations
  - End date and time

- During scanning:
  - Site name
  - Start date and time
  - Slide #
  - Name and ID of technician scanning slide
  - Record of technical issues with slides, scanning and viewing images
  - Record of slides with insufficient image quality
  - Record of number of rescans
  - Record of protocol deviations
  - End date and time

- During creation of TIFF FOV:
  - Site name
  - Start date and time
  - WSI Slide #
  - Name and ID of technician scanning slide
  - Name of primary feature of interest
  - Record of technical issues with slides, scanning and viewing images
  - Record of protocol deviations
  - End date and time

- During reading of feature:
  - Site name
  - Name of reader pathologist conducting reading
  - Start date and time
  - Slide # and sub-number List of features in multiple choice format
  - Pathologist record of which feature(s) was identified when viewing TIFF image
  - Record of rotation for reading event
  - Record of protocol deviations
  - End date and time

The CRF instructions should clearly instruct how to complete the form.

A data manager shall create a pre-established validated database containing all the fields from the eCRF, which will be stored on a secure file server at the site or on a secure server of the CRO managing the EDC. The database will be locked and stored in a secure place and transferred to the CRO study statistician for analysis. All analyses will be conducted using data from that stored database.

9.2 Data Quality

For each and every case reading, the CRO data manager will promptly and thoroughly review each eCRF to ensure all fields are complete, and query the coordinator to request a pathologist to correct and complete as quickly as possible any incomplete or illegible entries.

9.3 Data Retention Procedures and Period

The Sponsor and site should retain all data and study records in a controlled location for a minimum of 2 years after completion of study or completion of FDA submission process.

9.4 Publication Policy

The results of this study may result in publication. Individual sites will not publish until the multicenter study is published.

The study will be registered on www.ClinicalTrials.gov.

10. DEVIATIONS FROM THE CLINICAL INVESTIGATION PLAN

The site personnel shall document all protocol deviations and device deficiencies in logs to document the event and date, as well as clear explanations of the event. Protocol deviations and device deficiencies shall be recorded in these logs by the scanning technician, coordinator or enrolling or reader pathologists. Any protocol deviations noted by CRO during monitoring and data management will be directed to site study coordinator to enter into protocol deviation log.

10.1 Protocol Deviations

A protocol deviation is defined as any change or alteration from the procedures stated in the clinical investigation plan, consent document, recruitment process, or study materials (e.g. questionnaires) that were originally approved by the IRB where the change or alteration itself is not IRB approved.

All protocol deviations must be reported to Hamamatsu or their authorized representatives (CRO study monitors) through the protocol deviation form. In addition, the Investigator is required to adhere to IRB of records' procedures for reporting protocol deviations. Deviations shall be documented in writing and maintained in the Investigator and clinical study management files.

Per 21 CFR §812.140 (a) (4), Investigators are required to maintain accurate, complete and current records, including documentation showing the dates of, and reasons for, each deviation from the CIP. Failure to comply with the CIP may result in Investigator termination of participation [21 CFR §812.46 (a)] in the current study.

Repeated protocol deviations will lead to site retraining. Continued deviation from the CIP may result in site or investigator suspension or disqualification and is at the discretion of the Sponsor. Suspension and/or study processes, including washout, modality read order, and matching of slides with correct case information will be closely managed. A deviation will be filed for any case part that has missing, damaged, or broken slides. These cases will be reviewed for consistency with the protocol across all Reading Pathologists.

10.2 Device Deficiencies

A device deficiency is when a device not working as designed. The site personnel shall document any device deficiencies by recording the event narrative and date. All device deficiencies must be reported to Hamamatsu or their authorized representatives or CRO (study monitors.). Any incident with the operation

of the WSI device will be recorded and assessed for impact on scanning, file storage and file viewing operations.

Sponsor may terminate study conduct due to poor product performance.

11. SAFETY AND SUBJECT CONFIDENTIALITY

11.1 Safety

For the purposes of this study, the Hamamatsu NanoZoomer S360MD Digital Slide Scanner System is not considered a significant risk IVD device (21 CFR §812.3(m) and poses a low risk to the physical safety of study participants. Findings from the current study will not be used to diagnose or impact treatment of subjects. No invasive sampling techniques will be utilized as cases/slides are selected from patient archives.

11.1.1 Adverse Events

The study does not anticipate any adverse events (AEs) as this is a retrospective study without possibility to effect patient care.

11.2 Patient Confidentiality

All reports and communications relating to Subjects in the study will identify Subjects by their Subject ID number only. The site coordinators will de-identify all cases and slides of patient identifiers, and replace the labels with a code for patient identification for cases and slides being used in the study. All other personnel of the study, with the exception of the sponsor or CRO study monitor, will be blinded to the actual Subject ID. The locked database that goes to the statistician CRO and to the sponsor shall be free of any patient information before transfer to sponsor and statistician CRO. If there is a privacy breach, the sponsor will take reasonable steps to expunge such private information from its systems.

11.3 IRB and Protocol Approval

The protocol must be submitted and approved by the site IRB of record before the start of the study. The study will ask for a waiver of informed consent consistent with other reported studies since active slides will not be included. The IRB will be informed that study results may lead to publication and presentation at scientific meetings. There is no anticipated risk to patients with this study. The site will not start the study until the site IRB approval is available.

Amendments to the CIP will be reviewed by and agreed upon by the Sponsor and the Principal Investigators prior to implementation. Amendments will be approved by the sites' IRBs as required by applicable IRB procedures. All amendments will be tracked in the CIP revision history.

Any additional requirements imposed by the IRB or FDA shall be followed.

12. COMPLIANCE AND GOOD CLINICAL PRACTICES (GCP)

The trial will be carried out in accordance with Good Clinical Practice (GCP) as required by US Code of Federal Regulations (CFR) applicable to clinical studies (45 C.F.R. Part 46, 21 C.F.R. Part 54, 21 C.F.R. Part 56, and/or 21 C.F.R. Part 812) and ICH E6.

All key personnel (all individuals responsible for the design and conduct of this trial) will have completed Human Subjects Protection Training.

12.1 Financial Disclosures

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested in accordance with 21 C.F.R. Part 54 to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

12.2 Monitoring

The sponsor, or sponsor-representative and/or CRO monitor, shall conduct site monitoring, at site qualification, initiation, interim, and close out periods, as needed, to ensure the integrity of the data collection and that the study is performed per GCP.

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REVISION HISTORY

Rev	Description of Change	Page Number	Date
1.0	First Release	n/a	January 10, 2019
2.0	Clarification of certain features, Added organ choices. Allowance of up to 5 scans for image quality. Statement of non-significant risk study. Rules how to manage a broken or missing slide. Clarification of statistical methods and devices labeling		June 19, 2019
3.0	Identification of sites participation in study. Clarification of data sharing between sites. Explanation of the image quality check of scanned image, and number of rescans. Clarification of Adverse Events. Clarification of study personnel who may access data. Clarification of Monitoring activity for check of data integrity as well as for GCP monitoring. Clarification of sponsor CRO who may partake in monitoring. Clarification of sponsor or CRO role to monitor GCP and protect patient confidentiality. Clarification of reporting of protocol deviations to Clinical Plan.		July 28, 2019
4.0	Change of 3 features in feature list and organ selection.		September 18, 2019
5.0	Change of 1 feature in feature list and organ selections.		May 11, 2020