Clinical Validation Protocol for Test of Non-Inferiority of Primary Diagnosis by WSI using Hamamatsu NanoZoomer S360MD Digital Slide Scanner System Compared to Conventional Determination by Light Microscopy

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PROTOCOL SIGNATURE PAGE

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Protocol Number: HCT-P001

Study Title: Clinical Validation Protocol for Test of Non-Inferiority of Primary Diagnosis by WSI using Hamamatsu NanoZoomer S360MD Digital Slide Scanner System Compared to Conventional Determination by Light Microscope.

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** _____

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ABBREVIATIONS AND TERMS

Abbreviation	Term
AAP	All Available Population
AE	Adverse Event
AP	Adjudicator pathologist
CAP	College of American Pathologists
CFR	Code of Federal Regulations
CI	Confidence Interval
CMOS	Complementary Metal-Oxide Semiconductor
CRF	Case Report Form
CRO	Contract Research Organization
DPA	Digital Pathology Association
eCRF	Electronic case report form
EDC	Electronic Data Capture
EP	Enrolling Pathologist
FDA	Food and Drug Administration
FFPE	Formalin-Fixed Paraffin Embedded
GCP	Good Clinical Practice
GT	Ground Truth
H&E	Hematoxylin and Eosin
HER2	Human Epidermal Growth Factor Receptor 2
ID	Identifier
IRB	Institutional Review Board
IT	Information Technology
LED	Light Emitting Diode
LM	Light Microscopy
MMRM	Mixed Model Repeated Measures
NA	Numerical Aperture
PDF	Portable Document Format
PHI	Protected Health Information
PI	Principal Investigator
PIPS	Philips IntelliSite Pathology Solution
RP	Reader Pathologist

Abbreviation	Term
SAP	Statistical Analysis Plan
SC	Study Coordinator
ST	Study Technician
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

TRIAL SITE(S)

Four (4) 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. Tricore Reference Labs, PI (Dr. Richard Feddersen)
4. Washington University Medical Center, PI (Dr. Jon Ritter)

Primary diagnosis of glass slides and whole slide imaging (WSI) images done by four (4) reading pathologists

Case screening and enrollment to use 1 pathologist to enroll cases and 1 pathologist to confirm case enrollment per site.

OBJECTIVES

Primary Objective:

1. To evaluate the safety and accuracy of the Hamamatsu WSI test method compared to that of the reference method (conventional light microscope [Glass]) under clinical use conditions.

Secondary Objectives:

1. Evaluate the accuracy of the Hamamatsu WSI test method compared to that of the reference method by site, and by reader within sites.
2. Evaluate the accuracy of the Hamamatsu WSI test method compared to that of the reference method by site, and overall for diagnostic subtypes separately.
3. Evaluate the accuracy of the Hamamatsu WSI test method compared to that of the reference method by organ within site and overall.
4. Evaluate the accuracy of the Hamamatsu WSI test method compared to that of the reference method by procedure type, within site and overall.
5. Evaluate the agreement of the Hamamatsu WSI relative to glass slides for each diagnostic category and overall for each pathologist (intra-site/intra-reader/inter-modality precision).
6. Evaluate the pathologist-to-pathologist precision overall and within diagnostic category within each site and for each modality (inter-reader/intra-site/intra-modality precision).

PRIMARY ENDPOINT

- The primary endpoint is the indicator of major discordance in primary diagnosis between ground truth case diagnosis and case diagnosis by each modality, WSI and Glass.

METHODOLOGY

Cases will be selected in consecutive order by the enrolling pathologist starting with cases at least one year old and verified to match inclusion and exclusion criteria, and the case distribution for that site. Upon confirmation of enrollment, the slides necessary to make the diagnosis will be selected and included in the set of slides available to the reading pathologists. Additional slides for the case will be excluded. The original primary diagnosis reported in the clinical file for each case will be the Ground Truth (GT) diagnosis.

Each pathologist will read each case enrolled at their site by both conventional light microscope (Glass) and by WSI and record their primary diagnosis only, including the correct subtype of the lesion/neoplasm, and related

parameters (e.g., tumor subtype, tumor grade, margin status, lymph node involvement), if appropriate. The order of reading by Glass or WSI will be randomly determined; and, readings by the two modalities will include a minimum of 4 weeks washout period.

Upon completion of a reading at a site, two independent adjudicating pathologists will compare the primary diagnosis obtained using WSIs and using Glass to the GT diagnosis to determine if there is any discrepancy. Any mismatch will be further judged as major or minor based on pre-specified case rules cited in the literature to categorize a "discordance" into pre-designated categories and to decide if the discordance would have led to a change in patient treatment (i.e., major) or none at all (i.e., minor). If the two adjudicating pathologists do not agree on a "major" discordance, a 3rd independent pathologist shall review the case to determine majority agreement or if a consensus meeting of all three adjudicators is required.

NUMBER OF CASES

A total of 2000 cases will provide at least 90% power to demonstrate non-inferiority of WSI to Glass with a non-inferiority margin of $\delta = 4\%$ and type I error of 5%, if in fact WSI is non-inferior to Glass.

MAIN CRITERIA FOR INCLUSION/EXCLUSION

Inclusion criteria:

Cases are eligible to be included in the study only if all of the following criteria apply:

- Cases originating from and that were diagnosed at the local sites
- Cases are available in the site's archive
- Cases are at least 1 year old since accessioning
- Cases are selected because their primary diagnosis is consistent with the assigned target categories listed in **Table 2**
- Cases have a set of slides representative of the primary diagnosis for which it has been selected

Slide Selection for a given case must meet the following criteria:

- Slide obtained by surgical pathology and prepared from formalin-fixed paraffin embedded (FFPE) human tissue
- Slides must be stained with hematoxylin and eosin (H&E) and accompanying special stains (histochemical and/or immunohistochemical).
- All special stain slides (histochemical and/or immunochemical) where the slide and stain is used for diagnosis, not prognosis
- All chosen slides must demonstrate and be representative of the primary diagnosis; 1 H&E slide selection may suffice for biopsy cases
- For resection cases, a minimum of 5 H&E slides must be selected, which represent the primary diagnosis. If represented with less than 5 H&E slides, additional H&E slides (primary, secondary, or benign slides) from same case may be used to fulfill minimum number
- Slide is intact, has correct size/thickness, good edges, undamaged coverslip, without pen markings that can't be removed, no air bubbles, tidy labels, and fulfills the quality checks per the general clinical practice

Exclusion criteria:

Cases are excluded from the study if any of the following criteria apply:

- Case does not have relevant slides or if case information necessary for the study is missing
- Case is still active (less than 1 year old) at the local site
- Cases for which the control slides for immunohistochemistry and special stains are not available

- Two cases from same individual
- Gross only cases that have no slides
- Cases that are frozen section, cytology or hematology or immunofluorescence specimens only
- Cases where the only available set of slides have evidence only of secondary or no diagnoses and not the primary diagnosis for which the case is being screened.

Slides for a given case will be excluded if they meet the following exclusion criteria

- Glass slide that is broken, has abnormal size/thickness, beveled edges, poor coverslip (cracks, waviness, scratches), is sticky, has many pen markings or dirt, contains air bubbles and overhanging labels that can't be corrected, and if stain is severely faded.

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 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.

STATISTICAL METHODS

All Available Population (AAP) is defined as all cases for which at least one out of four readers provides an evaluable outcome for either the WSI or Glass modality and will be used for the primary analysis.

For the primary objective of demonstrating the WSI major discordance rate to be non-inferior to the Glass major discordance rate, a Mixed Model Repeated Measures (MMRM) logistic regression will be conducted. The dependent variable will be the major discordance status (yes/no) and the independent variables will include modality (WSI or Glass) as a fixed effect and site, reader, and case as random effects. From this model a two-sided 95% confidence interval (CI) for the difference in major discordance rate ($\pi_{WSI} - \pi_{Glass}$) will be constructed. If the upper bound of the 95% CI is less than the non-inferiority margin of 4%, then WSI would be considered non-inferior to Glass.

1.2 Schema

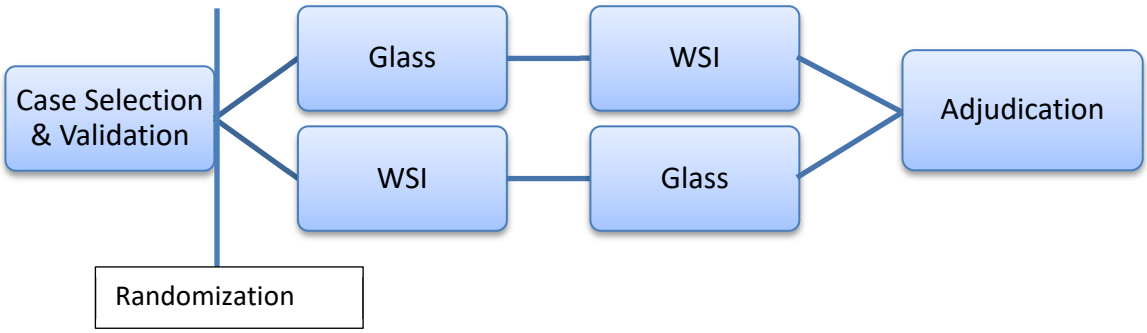


Figure 1. Study Schema

Table 1: Schedule of Assessments

	Screening				Reading				Adjudication		
	EP	**VEP**	**SC**	**ST**	**RP-1**	**RP-2**	**RP-3**	**RP-4**	**Adj-1**	**Adj-2**	**Adj-3[1]**
Enroll slide	X										
Confirm eligibility		X									
Enter GT Information	X		X								
Create WSI Images				X							
Read slides[2]											
WSI					X	X	X	X			
Glass					X	X	X	X			
Discordance Status Adjudication[3]											
WSI									X	X	
Glass									X	X	

EP = Enrolling pathologist, VEP = verifying enrolling pathologist, SC = study coordinator, ST = study technician, RP = reader pathologist, Adj = adjudicator.
1. When a major discordance differs between the first two adjudicators, a third adjudicator will review to break the tie. When all three results differ, a meeting will be held to reach consensus.
2. Slides are read in a random order, specified separately for each pathologist at a site.
3. Reader diagnosis concordance/discordance with the Ground Truth diagnosis will be adjudicated upon completion of a read.

2. INTRODUCTION

2.1 Background

Whole Slide Imaging (WSI) has emerged as an alternative way to view pathology slides for primary diagnosis instead of the conventional method of viewing slides of tissue under a traditional light microscope. There is growing adoption of the use of WSI for primary diagnosis in many countries (1-4). WSI is particularly advantageous for remote consultation over vast geographical regions where pathologists are not available, and for easier archiving of images of slide material that may have longer shelf life in digital form. In the United States, 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 de novo authorization letter (6) and device summary (7), the Food and Drug Administration (FDA) has announced the authorization 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, the authorization of the Philips device for primary diagnosis, PIPS system, serves as the 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 non-inferiority of WSI device is a "special control" required to acquire that clearance.

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 the PIPS device as the predicate device and will accordingly adhere to the special controls that are established. Hamamatsu will follow similar study designs to test the NanoZoomer system, and 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. Additional study design input became available from FDA presubmission review of protocol (20-25)

2.2 Rationale

In recent years, there has been an abundance of literature testing the concordance of primary diagnosis by WSI versus determination by standard review of light microscopy (LM) of glass slides (8-10). Many variations of experimental design are discussed encompassing topics of comparison to ground truth, concordance comparison, time necessary for washout period, targets for assessment of non-inferiority, sample size, choice of organ systems, effects of pre-training, adjudication of major and minor discrepancy, etc.

The CAP has published guidelines for pathology practices interested in using such technology for primary diagnosis (5). This society tried to standardize the methodologies for pathologists to consider for validation of WSI for primary diagnosis, further touching on the same study factors for consideration.

The FDA has been in close communication with leading pathologists in the field of digital pathology on the design of studies to support WSI for primary diagnosis, as well as the major vendors interested in pursuing that application, mainly through the Digital Pathology Association (DPA). The FDA has published guidance for technical specification (11). Yet, for clinical validation, the FDA has presented the Agency thinking on clinical validation protocol considerations in two PowerPoint presentations (12-13), touching on the kinds of cases to be tested, as well as the number of sites, pathologists and cases to be tested.

Quite recently, the FDA has announced the de novo authorization of the PIPS for the intended 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 non-inferiority study used to acquire clearance.

Hamamatsu is closely following the scientific study design and performance targets that were used by Philips.

2.3 Risk/Benefit:

Since this is a retrospective examination of cases, there is no apparent risk or benefit to the patient whose case is being used for the study.

Because this study design does not call for the re-assessment of the primary diagnosis of the retrospective case, we do not envision a situation in which the original diagnosis will be questioned and reassigned.

For that reason, and because the cases are retrospective and at least 1 year old, we do not see any need to notify the patient as protocol does not call for any change in diagnosis.

In the future, the device itself offers significant potential benefits, particularly for remote viewing of case material in geographies where pathologists are sparsely present.

3. OBJECTIVES AND ENDPOINTS

3.1 Primary Objective:

1. To evaluate the safety and accuracy of the Hamamatsu WSI test method compared to that of the reference method (conventional light microscope [Glass]) under clinical use conditions.

3.2 Secondary Objectives:

1. Evaluate the accuracy of the Hamamatsu WSI test method compared to that of the reference method by site, and by reader within sites.
2. Evaluate the accuracy of the Hamamatsu WSI test method compared to that of the reference method by diagnostic category within site and overall.
3. Evaluate the accuracy of the Hamamatsu WSI test method compared to that of the reference method by organ within site and overall.
4. Evaluate the accuracy of the Hamamatsu WSI test method compared to that of the reference method by procedure type within site and overall.
5. Evaluate the agreement of the Hamamatsu WSI relative to glass for each diagnostic category and overall for each pathologist (intra-site/intra-reader/inter-modality precision).
6. Evaluate the pathologist-to-pathologist precision overall and within diagnostic category within each site and for each modality (inter-reader/intra-site/intra-modality precision).

3.3 Endpoints

The primary endpoint is the indicator of major discordance between ground truth case primary diagnosis and case diagnosis by each modality, WSI and Glass, separately.

The secondary endpoints are:

- The difference in major discordance error rates of both WSI and optical LM (Glass) modalities compared to the original sign-out primary diagnosis rendered at the site, by site and by reader within each site
- The difference in major discordance error rates of both WSI and optical LM (Glass) modalities compared to the original sign-out primary diagnosis rendered at the site, by site and overall for each diagnostic category
- The difference in major discordance error rates of both whole slide imaging (WSI) and optical LM (Glass) modalities compared to the original sign-out primary diagnosis rendered at the site, by site and overall for each procedure type
- The difference in major discordance error rates of both WSI and optical LM (Glass) modalities compared to the original sign-out primary diagnosis rendered site, by organ within site and overall

- The percentage agreement of WSI relative to glass for each diagnostic category and overall for each pathologist
- The percentage agreement for each diagnostic category and overall between each pair of readers within a site for a given modality (WSI or Glass).

4. STUDY DESIGN

4.1 Overall Design

The study is a multi-site, randomized-read order, retrospective, paired-design evaluation of the Hamamatsu NanoZoomer S360MD Slide Scanner system consisting of a review of archived, de-identified and previously signed-out slides representing main organ systems within surgical pathology. Cases will include retrospective hematoxylin and eosin (H&E) stained formalin fixed tissue, and special stains and/or immunohistochemical stains (most commonly used is the brown chromophore) from the pathology practice, but will not include frozen sections, or cytological and hematological cases.

A total of 2000 cases consisting of multiple organ and tissue types will be enrolled. Cases will be divided over four (4) sites. At each site, four pathologists will read all the cases assigned to their site using both WSI and Glass modalities in a randomized order and with a washout period of at least four weeks between readings, resulting in a total of 8000 planned WSI reads and 8000 planned Glass reads. After any completed reader diagnosis case report form (CRF) at a site has been collected and cleaned and the CRF is completed, two adjudicators will review the reader's diagnosis against the original diagnosis (ground truth) to determine whether the new and original diagnoses were concordant, minor discordant, or major discordant. A third adjudicator will be used, if disagreement occurs between the first two adjudicators on the classification of a "major" discrepancy. If all three adjudication results differ, a meeting will be held to reach consensus among the three adjudicators. Figure 2 and Figure 3 display the process for the selection and reading of slides and the adjudication process, respectively.

4.2 Scientific Rationale for Study Design

The use of multiple organs and tissue types obtained from various procedures provides a variety of cases upon which to base generalizability of the results. Furthermore, a spectrum of diagnoses is included in these cases providing confidence in the generalizability of the results.

The randomized order of the two modalities attempts to remove the bias associated with reading the same slide twice. And, requiring that the two modalities be read with at least a four-week washout further decreases the chance of recall bias.

Additional source information on the study design comes from the Philips scientific abstract (14), Digital Pathology Association presentation webinar (15), and a Philips presentation at the May 2017 Pathology Informatics Summit in Pittsburgh, PA (16).

4.3 End of Study Definition

Each case will be considered complete when all study evaluations and study required assessments have been performed in accordance with the protocol.

It is anticipated that sites will have three to five months to screen and enroll slides, about two to three months to scan slides, and at least five to ten months to complete all reads. Adjudication will occur as soon as any one reading diagnosis is completed. Thus, adjudication will be occurring concurrently with reading of cases, and will end soon after all readings are completed.

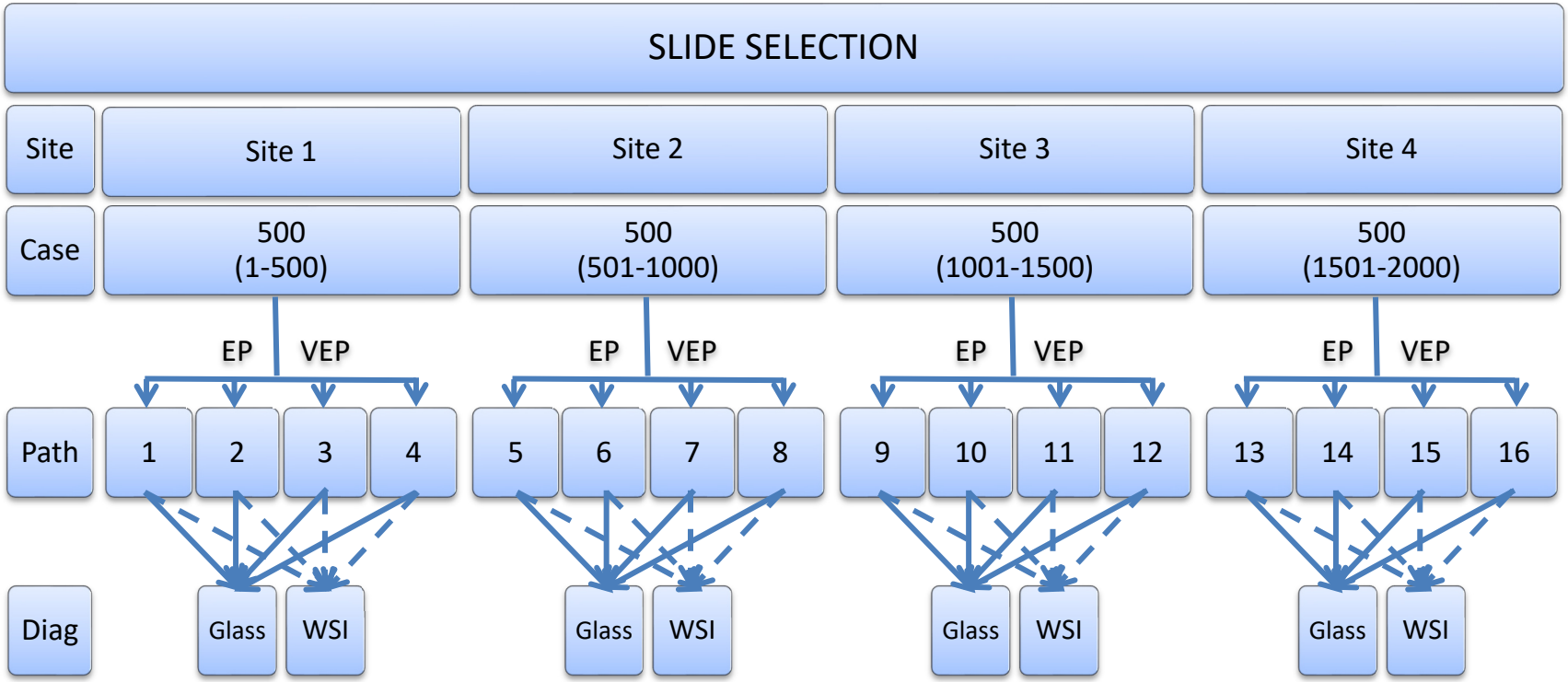


Figure 2. Study Process: Slide Selection to Diagnosis by Reading Pathologists

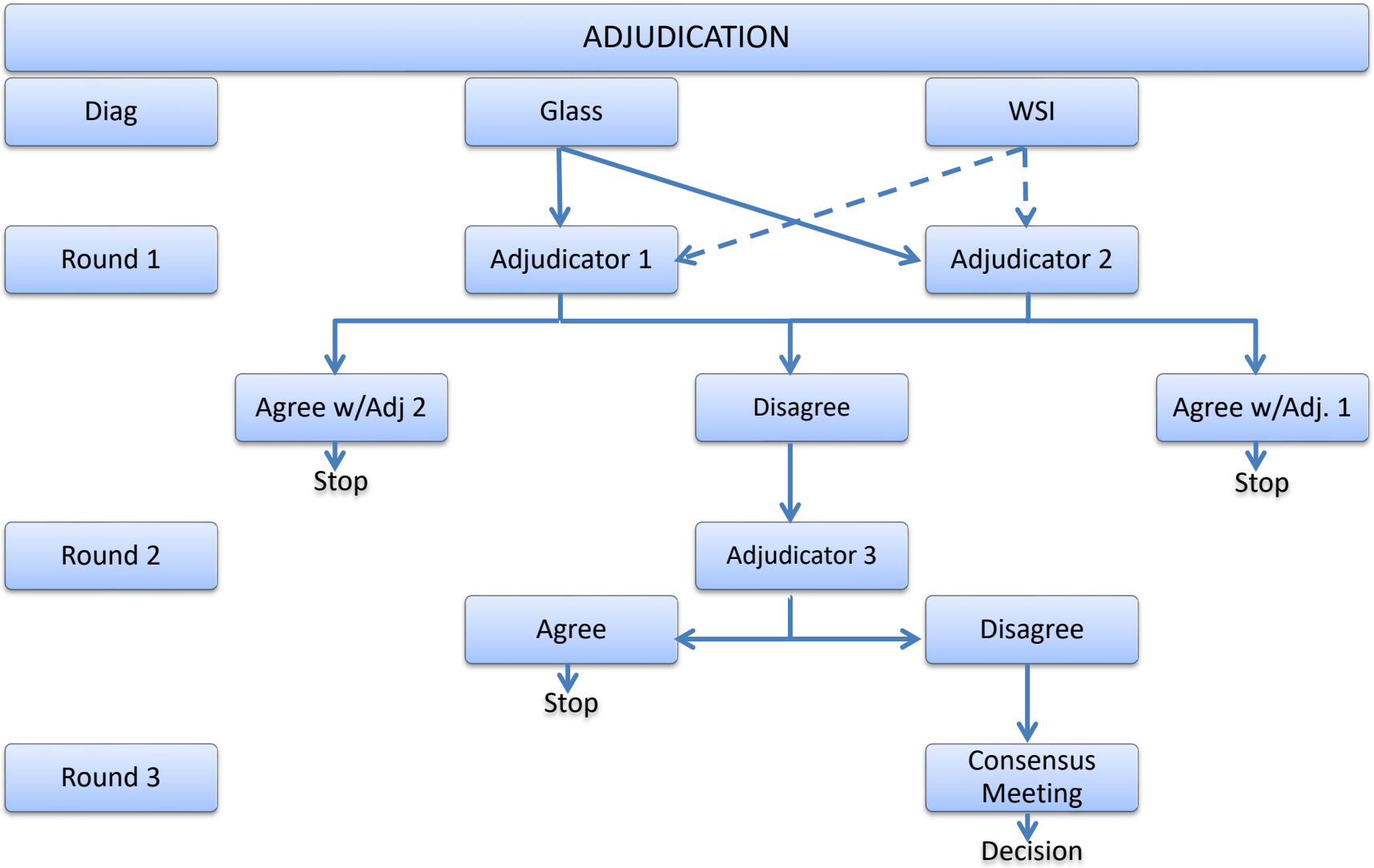


Figure 3. Study Process: Adjudication of Diagnoses

The completion of the study at the site is defined as the date of the last reading of the last pathologist from the last participating site in the study.

The end of the study is after the adjudicators have completed their review of the pathologists' diagnoses and classified each reading as concordant or discordant; and, if discordant, as major or minor and the adjudication data has been entered into the clinical database, and the locked database has been transferred to the statisticians.

5. ADJUDICATION COMMITTEE

A panel of expert pathologists will convene to establish a charter with clear pre-established rules of adjudication for each type of case listed in Table 2. Each case category from FDA list will have an adequate description of what constitutes a match, a minor discordance and a major discordance according to the definitions of major and minor discordance provided in Table 3. These pre-adjudication rules shall also specify that all deferrals shall be considered as minor or major discordances per application of adjudication rules for deferrals. A panel of adjudicators independent of the site shall be selected and trained on these rules.

The committee to establish the pre-adjudication rules shall classify discordant results according to the 4 harm categories (18) as shown in Table 3 and judge if the discordance in question would have led to a change in patient care or treatment plan. Any discordance not leading to a change in treatment will be considered as a "minor" discordance and considered a match. Any discordancy leading to a change in treatment plan for the patient would be judged as a "major" discordance, and therefore considered to not be a match.

6. INCLUSION AND EXCLUSION CRITERIA

6.1 Selection of pathology practice and pathologists

Four sites shall be selected such that:

- There shall be as many as 3 academic and at least 1 community and/or private practice pathology sites,
- The laboratory is accredited by the CAP
- Each site will be assigned cases consistent with the types of cases read at that site and from the cases listed in Table 2.The four sites together shall cover the entire range of cases required in the protocol and are:
  - Ohio State University Wexner Medical Center, Columbus, OH
  - Cleveland Clinic Foundation, Cleveland, OH
  - Washington University Medical Center, St Louis, MO
  - TriCore Reference Laboratories, Albuquerque, NM
- The practice has 4 board certified pathologists to serve as "reader" pathologist to conduct the study for primary diagnosis. The pathologists shall be both specialists and generalists, with acceptable proficiency testing and site performance metrics (e.g. case review rate by tumor board, amended reports) not outside the practice's norm.

- The practices shall dedicate at least 1 enrolling pathologist (EP) to conduct screening and enrolling cases per inclusion and exclusion criteria, and at least 1 validation enrolling pathologist (VEP) to confirm case enrollment of EP
- Each site shall have at least one dedicated scanning technician properly trained by the sponsor in WSI scanning operations per the sponsor's operator's manual.
- 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 Subjects Protection requirements

6.2 Selection of pathology practice and pathologists cases

The FDA has provided a table of specific diagnoses, which are to be considered when selecting cases for inclusion in the primary diagnosis study, including organ system, and sample size for particular subtypes and procedures. These are listed in Table 2 below.

Table 2: FDA List of Cases to be tested for Primary Diagnosis Study

(TOTAL 2000 CASES FOR THIS EXAMPLE)

CNB = Core Needle Biopsy; TUR = Transurethral Resection; LEEP = Loop Electrosurgical Excision Procedure; Dysp = Dysplasia; ECC = Endocervical Curettage; R/O ≡ Rule Out;

ORGAN	# OF CASES		SUBTYPES (procedures)
BREAST	300	50	Benign/Atypical CNB
		50	Benign/Atypical Lumpectomy
		50	In-Situ Carcinoma CNB
		50	In-Situ Carcinoma Lumpectomy
		50	Invasive Carcinoma CNB
		50	Invasive Carcinoma Lumpectomy
PROSTATE	300	120	Benign Core Bx
		30	Benign Resection
		120	Adenocarcinoma Bx
		30	Adenocarcinoma Resection
LUNG/BRONCHUS/Larynx/oral cavity/Nasopharynx	100	25	Benign/Inflammatory Bx Only
		25	Dysplasia Bx Only
		30	Carcinoma Bx
		20	Carcinoma Resection
COLORECTAL	150	50	Benign/Inflammatory Bx
		50	Adenomas Including Severe Dysp Bx
		40	Adenocarcinoma Endoscopic Bx
		10	Adenocarcinoma Resection
GE Junction	100	50	R/O Barrett's/Dysplasia Bx
		50	Non-Neoplastic/Inflammatory Bx

Table 2: FDA List of Cases to be tested for Primary Diagnosis Study

(TOTAL 2000 CASES FOR THIS EXAMPLE)

CNB = Core Needle Biopsy; TUR = Transurethral Resection; LEEP = Loop Electrosurgical Excision Procedure; Dysp = Dysplasia; ECC = Endocervical Curettage; R/O ≡ Rule Out;

ORGAN	# OF CASES			SUBTYPES (procedures)
Stomach	100		50	Inflammatory Including R/O H. Pylori Bx
			40	Polyps/ Neoplastic Bx
			10	Polyps/ Neoplastic Resection
SKIN	175		50	Non-Neoplastic/Inflammatory Bx
			50	Squamous/Basal Cell Neoplasms Bx
			75	Melanocytic Lesions Bx
LYMPH NODE	100		75	For Presence/Absence Of Metastasis
(no micrometastases smaller than 0.5 mm)			25	Non-Neoplastic
BLADDER	100		25	Benign/Inflammatory/Non-Neo Bx
			25	Dysplasia Bx
			25	Noninvasive Carcinoma (TUR Or Bx)
			15	Carcinoma TUR/Bx
			10	Carcinoma Resection
Gyn	150		40	Endometrial Bx/Curetting
			10	Hysterectomy for endometrial or cervical cancer
			25	Cervix Bx/Curetting (Bx, ECC)
			25	Cervix Bx/Curetting (Cone/LEEP)
			20	Ovary Benign/Non-Neoplastic
			30	Ovary Neoplastic
LIVER/BD, NEO	50		40	Core Bx
			10	Wedge Bx or Resection
Endocrine	100	ALL COMERS	50	Pancreas
			30	Thyroid
			10	Parathyroid
			10	Adrenal
BRAIN/NEURO	60		10	Non-Neoplastic
			25	Neoplastic Bx
			25	Neoplastic Resection
KIDNEY, NEOPLASTIC	50		50	All Comers (Consecutive Cases)
Salivary gland	50		50	
Hernial/Peritoneal	10		10	

Table 2: FDA List of Cases to be tested for Primary Diagnosis Study

(TOTAL 2000 CASES FOR THIS EXAMPLE)

CNB = Core Needle Biopsy; TUR = Transurethral Resection; LEEP = Loop Electrosurgical Excision Procedure; Dysp = Dysplasia; ECC = Endocervical Curettage; R/O ≡ Rule Out;

ORGAN	# OF CASES	SUBTYPES (procedures)	
Gallbladder	10	10	
Appendix	10	10	
Soft Tissue Tumors	20	20	
Anus/Perianal	50	50	Bx
Miscellaneous to reach 2000	15	15	

Sponsor should include a sufficient number of difficult and challenging diagnoses in the larger (>100) groups

The total number of cases is to be 2000. The total cases are to be subdivided among the four (4) independent clinical sites (academic, community hospital, and private laboratories.) Each site will be assigned cases consistent with the types of cases read at that site and from the cases listed in Table 2. The cases will be selected from actual cases diagnosed at that practice and taken from that site's own database.

However, it may be impractical for some sites to have sufficient cases of all types required under the protocol. In such eventuality, the PIs will review any gaps in cases enrolled across the study and propose adjustments of allotted cases at certain sites where some unbalance with respect to all numbers of cases for certain sites may occur. However, each site will enroll at least 400 cases and no more than 600 cases.

Ten extra cases at each site will be selected for self-familiarization testing after pathologist training. The selection of these ten cases will follow after the cases for the study have been collected.

6.2.1 Inclusion criteria:

Cases are eligible to be included in the study only if all of the following criteria apply:

- Cases originating from and that were diagnosed at that local site
- Cases are available in the site's archive
- Cases are at least 1 year old since accessioning
- Cases are selected because their primary diagnosis is consistent with the assigned target categories listed in Table 2
- Cases have a set of slides representative of the primary diagnosis for which it has been selected (see below for slide selection criteria).

Slide selection for a given case must meet the following criteria:

- Slide is obtained by surgical pathology and prepared from FFPE human tissue
- Slides must be stained with H&E and accompanying special stains (histochemical and/or immunohistochemical)
- All special stains slides (histochemical and/or immunohistochemical) where the slide and stain is used for diagnosis, not prognosis.

- A chosen slide must demonstrate and be representative of the primary diagnosis; 1 H&E slide selection may suffice for biopsy cases,
- For resection cases, a minimum of 5 H&E slides must be selected, which represent the primary diagnosis. If represented with less than 5 H&E slides, additional H&E slides (primary, secondary, or benign slides) from same case may be used to fulfill minimum number
- Slide is intact, has correct size/thickness, good edges, undamaged coverslip, without pen markings that can't be removed, no air bubbles, tidy labels, and fulfills the quality checks per the general clinical practice

6.2.2 Exclusion criteria:

Cases are excluded from the study if any of the following criteria apply:

- Case does not have relevant slides or if case information necessary for the study is missing
- Case is still active (less than 1 year old) at the local site
- Cases for which the control slides for immunohistochemistry and special stains are not available
- Two cases from same individual
- Gross-only cases that have no slides
- Cases that are frozen section, cytology or hematology or immunofluorescence specimens only
- Case where the only available set of slides have evidence only of secondary or no diagnoses and not the primary diagnosis for which the case is being screened.

Slides for a given case will be excluded if they meet the following criterion:

- Glass slide that is broken, has abnormal size/thickness, beveled edges, poor coverslip (cracks, waviness, scratches), is sticky, has many pen markings or dirt that cannot be removed, contains air bubbles and overhanging labels that can't be corrected, and if stain is severely faded.

6.3 Withdrawal or Discontinuation

6.3.1 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 (or case) may be withdrawn and a protocol deviation noted. Such occurrence is expected to be very low. If necessary, another slide from the same case may need to be added or a new case may need to be enrolled in order to satisfy the overall number of cases examined for the trial. As long as the new slide does not change the recorded ground truth primary diagnosis and synoptic information of subtype, grade, margin and lymph node involvement, then the RP will not be asked to re-read cases already read. These events will be documented.

6.3.2 Case Withdrawal

It is unlikely that cases will be withdrawn from the study after they satisfy inclusion and exclusion criteria. However, if during the trial a case can no longer be used, or an inadvertent mix-up is discovered, this case may be withdrawn and a protocol deviation noted. If necessary, a new case may need to be added to satisfy the overall number of cases examined for the trial. The substituted case shall be read by

all RPs for both modalities. These events will be documented. Such occurrence is expected to be very low.

6.3.3 Pathologist Withdrawal

If a reading pathologist is unable to complete the study reads according to study schedules, a replacement pathologist may be selected by Hamamatsu. In that situation there will be no re-reads of cases by the replacement pathologist (to be selected as similar as possible to the original pathologist), who will begin where the other pathologist left off, as long as the prior pathologist had completed reads for both modalities. The primary analysis will use all readings by the original/previous pathologist, with the readings by the replacement pathologist only being used for cases that the original/previous reading pathologist did not read with both modalities. Such occurrence is expected to be very low, but there is some possibility of staff turnover at any location.

7. DEVICE UNDER STUDY

7.1 System Description

The NanoZoomer S360MD 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, 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.

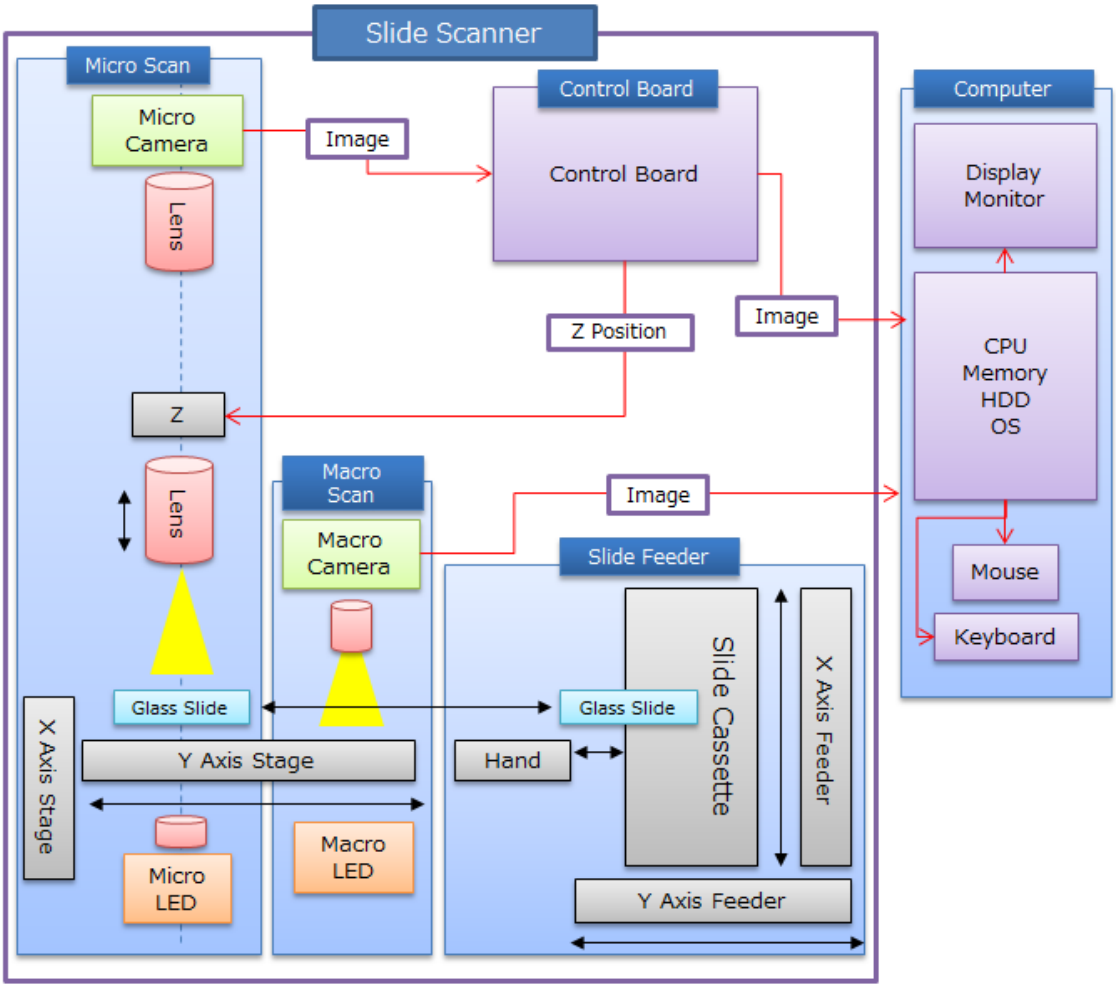


Figure 4. Communication between Scanner and Display

The Slide Scanner consists of the following blocks as shown above in Figure 4.

- 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.

7.2 General Principles of Operation

The Hamamatsu NanoZoomer S360MD Digital Slide Scanner (Figure 5) creates diagnostic quality digital images of glass slides containing FFPE tissue (11). 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.

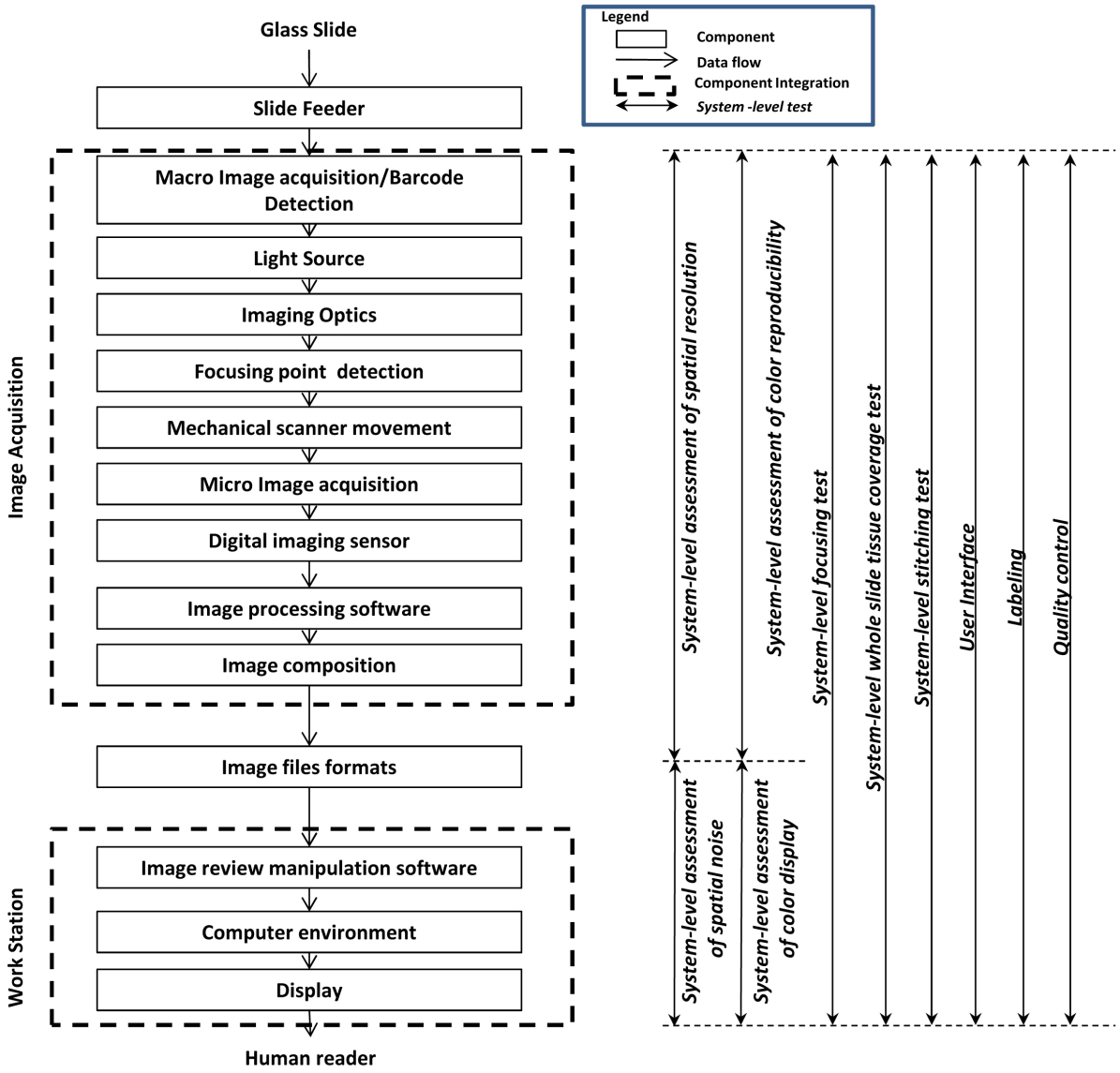


Figure 5. Example block diagram of typical components found in current WSI systems

8. STUDY ASSESSMENTS AND PROCEDURES

8.1 Preparation of Facility

The sponsor shall install a complete Hamamatsu NanoZoomer System at each of the 4 sites. The system shall include the NanoZoomer Slide Scanner System, monitor, product software, and Operator's Manual. In addition to the components of the Hamamatsu NanoZoomer system, additional off-the-shelf components shall be used with the system, such as a designated computer, input device, and perhaps a sponsor's server or the site intranet for data storage.

The sponsor's 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. 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. All study records will be kept in a secure place at the site by the site 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 electronic data capture (EDC) systems for data collection.

The WSI monitor viewing station cockpit and input device shall be standardized across all sites. Viewing images must be done at this defined monitor viewing station as the monitor is a required part of the NanoZoomer system as specified for the product and study.

The pathologist shall use the light microscope conditions normally used for reading of glass slides at the site.

8.2 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 may include:

- Selection of sites and pathologists to participate in study
- Shipment, installation, maintenance, and service of equipment by Hamamatsu personnel
- Screening and enrolling of cases, slides, and establishment of ground truth for non-inferiority study, recording of case notes to be made available to reading pathologist, training slides, and selection of self-familiarization slides
- Coding procedures for cases, slides, ground truth, and medical information selected 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
- Method to present case information available to pathologist
- Method to conduct of self-familiarization process
- CRO Installation, operation, and maintenance of EDC systems at site, validation and password protected access
- CRO Entering, storing, and securing data in electronic case report form (eCRF)
- CRO Randomization procedures for reading slides
- CRO Adjudication charter and process to adjudicate reader pathologists' diagnoses
- Documentation and reporting on device deficiencies and protocol deviations

- CRO Procedures for data management to manage the data integrity (data rejection, audit trail, verification, validation, and locking database)
- CRO GCP requirements training
- CRO GCP monitoring procedures

Training on Adjudication Process:

- Rules for Adjudication of "Major" and "Minor" discrepancies,
- CRO Operation of Adjudication Software

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 study coordinator (SC).

Training shall include training of the:

- Entire site study staff (PI, sub-PIs, study coordinators, enrollment and validation enrollment pathologists, reader pathologists, WSI technicians, IT personnel) on study protocol, study processes, essentials of GCP, and human subjects training or applicable to those individuals.
- Study coordinators on proper storage of equipment, 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, and training on what and how to fill out eCRF for scanning operation.
- Information Technology (IT) staff, if relevant, responsible for proper storage and retrieval of scanned images.
- EP on selection and validation of cases and slides for study, recording of ground truth, proper selection of slides from cases that demonstrate primary diagnosis, and collection of medical notes available to reader pathologist.
- VEP for review and confirmation of the cases, slides, supplemental information, and ground truth diagnosis selected by the EP.
- Reader pathologist (RP) on the protocol, study processes, instructions how to operate and view WSI slides for primary diagnosis, and how to fill out the eCRF for each case.
- Adjudication pathologists on procedures for adjudicating a) differences between EP and VEP enrollment case selection, and b) diagnosis of WSI and LM readings if identical to ground truth or discrepant and if any discrepancy is major or minor in respect to patient treatment.

Once trained, the reader pathologists shall be allowed to self-familiarize themselves in a mock primary diagnosis by WSI on a small set of training cases in order to become familiar with equipment operation. The pathologist will be allowed to conduct this trial diagnosis on 10 case slides chosen and selected per the same enrollment procedures for the cases for the study. Once the pathologist feels comfortable with the self-familiarization process, he or she may begin the study.

8.3 Selection of Case Slides

The site coordinator and/or EP shall search through the site's database to locate and withdraw from the archive the assigned number of consecutive cases starting with archival cases at least 1 year old since accessioning and moving consecutively back in time. They will choose cases categorized from FDA in

Table 2. The EP will screen and enroll cases per the inclusion and exclusion criteria, to record the ground truth primary diagnosis, to record the FDA category information (i.e., organ, procedure, subtype, and category), record the basic patient case note(s) that the reader pathologist will be presented with, to select the slides indicative of primary diagnosis and to collect the supplemental diagnostic information and synoptic checklist information, where available in site pathology report, and relevant to classification, subtype, grade, margin, status, etc. The EP may consult with other pathologists within the site practice who may be subject matter experts in certain case categories, as long as those pathologists are not reader pathologists or VEPs. This supplemental information will be in accordance with truncated CAP cancer protocols (17) developed to highlight the key synoptic information to take into account later when reader pathologist makes study diagnosis.

GT diagnosis is the primary diagnosis based on the original sign-out (reported) primary diagnosis of cases that are enrolled in the study. The ground truth will not be reestablished, and should not include secondary diagnoses. Only a review of the final sign-out report (and not a review of the glass slides) will be used to record this information. The original sign-out primary diagnosis (reported clinically), as well as any supplemental diagnostic information, will be captured verbatim. If site uses synoptic checklists in their pathology report, that information, too, will be recorded.

From those cases, the EP will choose the H&E slide or set of H&E slides in the case that are consistent with the primary diagnosis. The following rules will be followed when selecting slides:

- For biopsy cases, the EP will select at least the H&E slides representative of the primary diagnosis. In most biopsy cases, this can be done with just 1 H&E slide, but the EP may choose more if needed to define the diagnosis. To minimize selection bias, the EP shall select the first H&E slide that encompasses that primary diagnosis.
- For resection cases, the EP shall select the set of slides that are necessary to indicate the primary diagnosis, inclusive of the key CAP checklist abridged metrics of cancer type, grade, margins and if metastasized to lymph nodes.
    - If there are multiple levels, the EP may select only the level of the same block slide that is germane to the primary diagnosis.
    - To minimize selection bias, the EP shall choose the first available H&E slide that describes that condition(s), rather than the best slide.
    - A minimum of 5 H&E slides will be selected. If the set of slides selected to define the primary diagnosis is < 5, then the EP shall continue to pick H&E slides until a minimum of 5 is achieved, and thereby the EP may pick further primary diagnosis slides, secondary diagnoses slides or slides irrelevant to any recorded diagnosis, whichever comes first.

In addition, for any case where there are extra slides with special and/or immunochemical stains, the EP shall enroll all those, as long as these extra stains are used for making/confirming the diagnosis and not for prognosis or to guide treatment (e.g. human epidermal growth factor receptor 2 [HER2] stain in breast cancer).

For enrolled slides, the EP shall remove all markings from the coverslip. If not possible, the slide shall be excluded, and another slide must be chosen.

A priori defined slide and case data rejection criteria have been developed per the defined inclusion and exclusion criteria in section 6. Some case data may be spurious or artifactual and may need to be disregarded, or slides may be damaged, aged, or lost, or there may be corrupted digital files, etc.

For every case enrolled, the EP shall make a determination if this case can be considered to be a "difficult and challenging" case. The EP will select "difficult and challenging" when one or more of the following apply:

- It's professional opinion of EP that case is "difficult and challenging"
- Case used special stains or immunohistochemical stains to aid in diagnosis
- Case used molecular tests to aid in diagnosis
- Case used tumor boards to review case to aid in diagnosis
- Other reason, and brief narrative to elaborate.

The EP shall capture this information for every one of the 500 enrolled cases for that site.

8.4 Validation of case and slide selection

Upon completion of the screening and enrollment by the EP, then one validation enrollment pathologist (VEP) shall review and confirm the screening and enrollment selection of the EP. If the VEP disagrees with the case or slide selection of the EP, then both the EP and VEP shall meet for consensus decision. If agreement cannot be reached, then that case shall be disqualified.

All enrolled material and information shall be provided to the site coordinator for secure storage and to be kept blinded from reading pathologists.

8.5 Storage of case slides

The site coordinator shall:

a. Collect and store the cases, slides and medical notes, clinical history of the case, and final pathology report in a secure location,
b. Place proper de-identification labels (e.g., hide names, numbers, barcodes) on slides with a code known only to the site coordinator, and confirm those labels will not interfere with the scanning process nor affect the integrity of the tissue on the slide. The printer and label operations shall be validated before the study to prove the study labels do not interfere with the scanning process and are robust for the duration of the study, when the study labels can finally be removed and the material returned to the archive.
c. Remove all patient identifiers and assign coded subject ID to protect patient privacy, and
d. Ensure the traceability code-defining patient ID with study assigned code shall be kept only at the site, stored in a secure location, not shared outside the practice, and kept blinded to reading pathologists.

8.6 Whole slide scanning operations

The SC shall deliver the slides to be scanned to the scanning technician.

The scanning technician shall receive the batch of de-identified case slides to be scanned from the site coordinator. Then the technician shall:

a. Scan the slide at 40x magnification according to the standard Hamamatsu default scanning conditions and save the image in its proprietary native file format.
b. Conduct an initial quality check of the scanned slide to confirm that all material on the slide was properly scanned.
c. Assess if the image looks to be of sufficient quality to advance to the pathologist.
d. Transfer the WSI image to the assigned secure drive with proper file nomenclature consistent with the patient de-identified code.

If the technician deems that the scan quality is unsatisfactory, the technician may scan again, shall note that in the eCRF, review the slide quality of the re-scanned slide, and then store these images in the secure drive location. If re-scanning does not result in a suitable WSI, the technician shall note that in the eCRF, and then bring this to the attention of the EP and VEP to decide if that case or slide should be replaced. Data or re-scans will be recorded. Up to 5 scans will be allowed.

Once complete, all case and slide material shall be promptly returned to the coordinator for proper and secure storage. The WSI files shall be bundled into folders, each specific for each case.

8.7 Reading a Case

At each site and after appropriate training, four pathologists participating at that site will read the same set of case slides, unique to each site. Each pathologist shall read each case on a conventional light microscope (Reference Method; Glass) and on the Hamamatsu system (Test Method; WSI) in an order randomly determined.

RPs will receive from the site coordinator the slides and/or WSI images to be read. The case study identification, patient age and gender, relevant supplemental clinical history, part and procedure information, and specimen gross description in the EDC system in addition to corresponding slides (either glass or WSI) will be available for RP viewing, but RP will never have access to the final sign-out diagnosis or GT information. Pathologists may not request re-scans of any slide. No additional slides from the case can be requested. Reading Pathologists will not be permitted to order additional re-cuts or stains outside of what was cut/stained during the original clinical review process (i.e., no additional slides will be created for the purpose of this study) and selected by the EP and VEP.

All RPs will perform randomized (read-order) glass and WSI reads with a minimum 4-week washout period between modalities. The order of reading will be randomly assigned such that each case will either be read first using glass slides and LM and then (following a 4-week wash-out period) via WSI or vice versa. All digital reads will be executed using the Hamamatsu Pathology workstation. Pathologists will access the Hamamatsu system via secure login/password. For viewing by glass, the pathologist shall use the light microscope that they normally use at that site.

When the RP reads the cases, all WSI and glass diagnoses, as well as supplemental information based on truncated CAP checklists developed for this study, will be entered into an eCRF using an EDC system.

The RP will write a primary diagnosis in a narrative text in their words.

Next, the reader pathologist shall fill in an abridged CAP checklist to capture supplemental diagnostic information specific to each of the organ malignancy types in the study, if applicable. The abridged checklist will include only those items most relevant to the patient's management, i.e., type, grade, margin and whether infiltration into lymph nodes. The pathologist may use information from an abridged

CAP checklist to clarify and complete the diagnosis description that satisfies the primary diagnosis. The RP may use textbook reference materials to assist in determination of diagnosis.

The reading pathologist will be trained and given instructions how to use the CAP checklist information when writing the text that shall describe the reading pathologist's diagnosis.

Possibility of Deferral: Reader must do her or his best effort to determine a primary diagnosis. However if the reading pathologist feels strongly that he or she is not able to make a diagnosis, then the reader pathologist may record that in the case report form. The recording can be "deferral to Glass" in the case of WSI, or "deferral to specialist" in either case of Glass or WSI.

RPs will not be able to review eCRFs for which a diagnosis is finalized (case signed-out).

8.8 Adjudication of Discordant Readings

Once the RP eCRF is reviewed, queried, cleaned and completed, that reading will be sent to the adjudicators to adjudicate the endpoint.

The adjudicator pathologist (AP) is part of an independent board of subject matter expert, board certified pathologists, who shall review and compare diagnoses on WSIs and on Glass to the GT, the original diagnosis that was rendered in the clinical practice. The AP(s) shall not be presented with the slides or WSI images. The adjudicators shall be presented only with the ground truth primary diagnosis and synoptic information, the patient case notes available to the reading pathologist, and the reading pathologist primary diagnosis and supplemental CAP checklist records.

When the adjudicator is presented with a case to adjudicate, the 1st and 2nd adjudicator will independently review the case. If a disagreement involving a major discordance is present between the 2 adjudicators, such a case will go to the 3rd member to adjudicate. Based on the determination of the 3rd member, the majority choice will be selected. If there is no majority from the rulings of the 3 adjudicators, then the adjudication will proceed to a consensus meeting for final decision.

Table 3: Definition of Discordance

Severity	Definition
Minor	No Harm: a. Will not result in harm b. No change in prognosis or a change in prognosis that is unlikely to result in a change in treatment according to standard care.
	Minimal Harm [Grade 1] a. Further unnecessary noninvasive diagnosis test(s) performed [e.g., blood tests or non-invasive radiological examination]. b. Delay in diagnosis or therapy of < 6 months. **c.** Minor morbidity due to [otherwise] unnecessary further diagnostic effort(s) or therapy predicated on the presence of [unjustified] diagnosis.
Major	Moderate Harm [Grade 2]

Table 3: Definition of Discordance	
Severity	**Definition**
	a. Further unnecessary invasive diagnostic test(s) [e.g., tissue biopsy, re-excision, angiogram, radionuclide study, or colonoscopy]. b. Delay in diagnosis or therapy of > 6 months. **c.** Major morbidity lasting < 6 months due to [otherwise] unnecessary further diagnostic effort(s) or therapy predicated on the presence of [unjustified] diagnosis.
	Severe Harm [Grade 3] a. Loss of life or limb, or other body part, or long-lasting morbidity [lasting > 6 months.].

8.9 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, reader pathologist diagnosis and adjudication 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.

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 5-10 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. 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.

9. STATISTICAL CONSIDERATIONS

The statistical analysis plan (SAP) will be developed and finalized before database lock and will describe the set of data to be included in the analyses, and procedures for accounting for missing, unused, and spurious data. The SAP may also include additional exploratory analyses not explicitly mentioned in the following sections.

9.1 Statistical Hypotheses

The primary statistical objective of this study is to demonstrate that the safety and accuracy of the Hamamatsu system is non-inferior to the Diagnostic Reference Standard glass diagnosis in routine surgical pathology cases.

Assuming a non-inferiority margin of 4%, consistent with the literature (8-10) and with the target to which the PIPS' clearance was held, then the hypothesis to be tested can be written as:

$H_0: \pi_{WSI} - \pi_{Glass} > 0.04$ Versus $H_1: \pi_{WSI} - \pi_{Glass} \leq 0.04$

where π_{WSI} and π_{Glass} are the major discordance rates for WSI and Glass respectively.

9.2 Sample Size Calculations

Assuming $E\{d_{li}\} = \Delta$ and variance $Var\{d_{li}\} = \sigma^2$ where d_{li} is equal to average over differences of error rates between WSI and Glass compared to GT diagnosis for case i in site l, the following expression is applicable: $n = \left(Z_\alpha + Z_\beta\right)^2(\sigma^2) \Big/ (\delta + \Delta)^2$

$$= \frac{\left(Z_\alpha + Z_\beta\right)^2 \pi'_{WSI}(1-\pi'_{WSI})\pi'_{Glass}(1-\pi'_{Glass})\{1-\lambda+(r-1)(\xi-\eta)\}}{r(\delta+\Delta)^2}$$

For which π'_{WSI} and π'_{Glass} are the probabilities that WSI and Glass methods agree (or minor discordance) with the GT diagnosis classification retrospectively; $\lambda = Corr(a_{lij1}, a_{lij2})$, the correlation between the classifications for the two modalities by the same reader; $\xi = Corr(a_{lijk}, a_{lij'k})$, the correlation between two readers for a given modality; and, $\eta = Corr(a_{lij1}, a_{lij'2})$, the correlation between two modalities and between two readers.

Assuming power = $(1-\beta) = 0.90$, α = 5% two-sided, a non-inferiority margin of δ, a true difference between proportions of WSI and Glass equal to Δ in favor of Glass**,** number of readers r = 4 and number of sites = 4, the required sample size is provided in Table 4 for different ranges of π'_{WSI}, Δ, λ, and (ξ–η).

Table 4: Sample Size for 4 Readers and 4 Sites

π'_{WSI}	λ	(ξ–η)	Max σ^2 ($\sigma^2$for $\Delta = 0$)	$\Delta = \pi'_{WSI} - \pi'_{Glass}$				
				0.0	-0.5%	-1.0%	-1.5%	-2.0%
0.6	0.5	0.150	0.114000	749	976	1326	1904	2968
		0.125	0.105000	690	899	1221	1754	2733
		0.100	0.096000	631	822	1116	1604	2499
	0.6	0.150	0.102000	670	874	1186	1704	2655
		0.125	0.093000	611	796	1082	1553	2421
		0.100	0.084000	552	719	977	1403	2187
0.7	0.5	0.150	0.099750	665	852	1154	1653	2568
		0.125	0.091875	604	785	1063	1522	2366
		0.100	0.084000	552	718	972	1392	2163
	0.6	0.150	0.089250	587	762	1032	1479	2298
		0.125	0.081375	535	695	941	1348	2095
		0.100	0.073500	483	628	850	1218	1893
0.8	0.5	0.150	0.076000	500	646	871	1241	1920
		0.125	0.070000	460	595	802	1143	1768
		0.100	0.064000	421	544	733	1045	1616

Table 4: Sample Size for 4 Readers and 4 Sites								
π'_{WSI}	λ	(ξ–η)	Max σ^2 ($\sigma^2$for $\Delta = 0$)	$\Delta = \pi'_{WSI} - \pi'_{Glass}$				
				0.0	-0.5%	-1.0%	-1.5%	-2.0%
	0.6	0.150	0.068000	447	578	779	1111	1718
		0.125	0.062000	408	527	710	1013	1566
		0.100	0.056000	368	476	642	915	1414

This table shows, a total of 2000 cases will provide more than 90% power to demonstrate non-inferiority of WSI to Glass with a non-inferiority margin of 4% as long as the proportion of agreements with Ground Truth is at least 60%, λ is at least 0.5, (ξ-η) is at most 0.15, and $\Delta \geq$ -1.5%.

9.3 Populations for Analyses

All Available Population (AAP): AAP includes all cases for which at least one reader provides an evaluable outcome for either the WSI or Glass modality where an outcome is considered evaluable if the adjudication committee is able to classify the diagnosis as concordant, minor discordant, or major discordant with the Ground Truth diagnosis. If a reading pathologist is unable to complete study reads, a replacement pathologist may be selected by Hamamatsu, the re-reads by the replacement pathologist are supplementary, and the primary analysis will use all readings by the original/previous pathologist, with the readings by the replacement pathologist only being used for cases that the original/previous Reading Pathologist did not read by both modalities.

9.4 Statistical Analyses

This section is a summary of the planned statistical analyses of the primary and secondary endpoints described in detail in the study SAP.

9.4.1 Case Characteristics and Disposition, and Reader Pathologists

The characteristics of the cases selected at each site and overall will be descriptively summarized using numbers and percentages. The characteristics of the cases will include, the case breakdown as specified in Table 2, including organ system, procedure type, overall classification.

The number and percentage of deferrals will be summarized by reader, site, and overall for each modality. The reason for the deferral will also be provided.

The characteristics of the reader pathologists at each site and overall will be descriptively summarized in regard to years in practice and specialty.

9.4.2 Primary Analysis of Non-inferiority of WSI Relative to Glass

The AAP will be used for the primary analysis. To evaluate the major discordance rates between WSI and Glass, a mixed model repeated measures (MMRM) logistic regression will be performed. The indicator of major discordance (yes/no) will be the dependent variable with modality (WSI or glass), as a fixed effect; and site, reader and case entered into the model as random effects; glass will be the reference

group. From this model, a two-sided 95% confidence interval (CI) for the difference in major discordance rate (π_{WSI} - π_{Glass}) will be constructed. If the upper bound of the 95% CI is less than the non-inferiority margin of 4%, then WSI will be considered non-inferior to Glass.

A summary table will provide the number of deferrals and reasons for deferral (e.g., deferral to specialist, deferral to glass, etc.). Cases with diagnosis deferred for at least one modality (excluding missing observations) will be excluded from the primary analyses.

The point estimates and the corresponding two-sided 95% confidence intervals of the major discordance rates will be provided separately for WSI and glass. To support the clinical performance of NanoZoomer, the major discordance rate for WSI should not exceed 7%.

9.4.2.1 Sensitivity Analysis

Sensitivity analyses will be performed to evaluate the robustness of the results. All deferred plus missing data cases will be assumed as having major discordance with GT and included in the same MMRM logistic regression analysis as that of the primary analysis.

9.4.2.2 Generalizability for Readers and Sites

The WSI vs. glass difference in major discordance rate and associated 95% CI by reader will be constructed using the primary MMRM logistic regression model. The 16 such confidence intervals for the 4 readers at the 4 sites will be descriptively displayed in a Forest Plot. The extent to which the respective confidence intervals in the Forest Plot are overlapping sheds light on their homogeneity, and reasonably homogeneous confidence intervals can support generalizability with respect to readers in the sense of comparability of estimated differences between WSI and Glass for the 4 readers at the 4 sites.

Generalizability for sites will be addressed in a similar way to that previously discussed for readers within sites. The 4 confidence intervals for the 4 sites can be descriptively displayed in a Forest Plot. The extent to which the respective confidence intervals in the Forest Plot are overlapping sheds light on their homogeneity, and reasonably homogeneous confidence intervals can support generalizability with respect to sites in the sense of comparability of estimated differences between WSI and glass for the 4 sites.

9.4.3 Secondary Endpoints Analyses

The AAP will be used for all secondary endpoints analyses. Same as the primary analysis, missing observations and deferred outcomes will be excluded from these analyses.

For the first 4 secondary endpoints, summary statistics and corresponding two-sided 95% confidence intervals for the major discordance rates and differences between the major discordance rates within each subgroup of interest (site, organ, diagnostic category, and procedure type) will be provided.

To evaluate the Intra-reader/Inter-modality (within reader, between modality) precision, with glass as the comparator method, the percentage of overall agreement and 95% confidence intervals between the WSI and the Glass diagnostic categories will be computed for each reader at each site separately.

To evaluate the Inter-reader/Intra-modality (between reader, within modality) precision, with glass as the comparator method, the percentage of agreement and 95% confidence intervals will be computed for each pair of readers at each site separately (6 pairwise comparisons per site).

9.4.4 Other Analyses

The occurrence and rate of all technical issues during scanning and viewing of WSI images shall be tabulated.

10. DATA MANAGEMENT AND DATA QUALITY

10.1 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.

Case report forms (CRFs) and logs will be used to collect the data recorded in the course of the study, and shall be presented in an electronic data capture (EDC) system.

An eCRF for selection and enrollment of cases is to be completed for each case by the EP and confirmed by the VEP.

This information includes:

- Site ID, organ, procedure and subtype,
- Clinical history, age, gender, gross description notes
- Inclusion & exclusion criteria,
- Ground truth primary diagnosis, and diagnosis notes, and, where applicable, synoptic information of subtype, grade, margin and lymph node involvement
- Number of slides for the entire case, number of slides selected for the study, and organ of the slide, and stain for that slide
- Patient case notes.

An eCRF or log for WSI scanning operation for creation of WSI images to be filled out for each case by the technician scanning the glass slides which shall include:

- Clear instructions on what to do at what point along the way.
- Field entries for:
    - Date and Time
    - Name of technician
    - Site Name/Number
    - Case #
    - Slide # if there is more than one slide for the case
    - Condition of scanned image, if not acceptable, and why
        - If there is any gross out-of-focus, image aberration, color distortion, etc.
        - If all material was scanned.
        - If a rescan was required and why
        - If final scan acceptable
        - # of rescans

An eCRF for Primary Diagnosis recording to be filled out by the pathologist reader, which shall include:

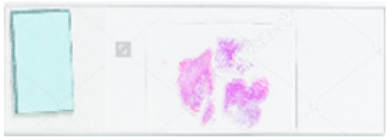
- An eCRF for case and modality, with WSI-specific fields shown only for WSI cases

- Clear instructions on what to do at what point along the way
- Field entries for:
  - Date and Time
  - Site name/number
  - Case #
  - Slide # if there is more than one slide for the case
  - Tissue and organ and stain used
  - Clinical history, age, gender, gross description notes
  - ID of pathologist conducting reading
  - Start time and date of reader's review
  - Checkbox if reading by light microscope or WSI
  - Record of "Deferral" if to Glass, to specialist, or "Other" with free text field to describe.
  - Record of Primary Diagnosis:
  - Record all Abridged CAP checklist information as appropriate for resection cases, including primary diagnosis text narrative, as well as subtype, grade, margin, and if lymph node involved, etc.
  - End time and date of reader's review

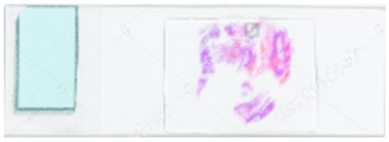
An eCRF for the Adjudication Panel to be filled out by each Adjudication Pathologist, which shall include

- Adjudication pathologist ID
- Record of match, major or minor mismatch, and reason for decision, if needed
- Record if adjudicator felt case should go to consensus meeting
- Date of consensus meeting, consensus ID, final determination of discordance and rationale for the decision if needed.

- **Note:** The example shown is a case of a breast lumpectomy specimen with sentinel node excision, from which 3 slides were selected for the trial.



Slide 1. Contains breast tumor section to determine primary diagnosis of carcinoma with subtype and grade



Slide 2. Contains section of specimen margin to determine if carcinoma is present / absent (orientation of margin not specified)

Slide 3. Contains lymph node fragments to determine if there is metastatic carcinoma present or not

- **Reading Pathologist's Name:** Dr. X entered
- **Case Number:** Case #1 entered
- **Primary diagnosis:** Invasive breast carcinoma
- **Tumor histologic type:** Invasive ductal carcinoma (or just ductal)
- **Histologic grade:** Overall grade (1, 2 or 3) entered
- **Margin status:** Involved or uninvolved by invasive carcinoma entered
- **Lymph node involvement:** Positive or Negative for metastatic carcinoma entered

Figure 6. Example of CRF (using truncated CAP checklist)

A CRO 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.

10.2 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.

Once the reader pathologist diagnosis is complete (whether WSI or glass), the data manager shall confirm completion of the case and advance the relevant data to the adjudicators.

10.3 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.

10.4 Publication Policy

The results of this study may result in publication.

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

11. 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, study coordinator or enrolling and/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.

11.1 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 log 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 disqualification actions, including the rationale, will be documented.

For protocol deviations, 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.

11.2 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) through an appropriate log. 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.

12. SAFETY AND SUBJECT CONFIDENTIALITY

12.1 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.

12.1.1 Adverse Events

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

12.2 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 Subject ID for cases and slides being used in the study. All other personnel of the study, with the exception of the study monitor of the sponsor, sponsor's agent or CRO, will be blinded to the actual patient information. The locked database that goes to the CRO statistician and to the sponsor shall be free of any patient information before transfer to sponsor and CRO statistician. If there is a privacy breach, the sponsor will take reasonable steps to expunge such private information from its systems.

12.3 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.

13. 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.

The sponsor, or sponsor-designated CROs or Clinical Professionals, shall conduct site monitoring, including site qualification, site initiation, site monitoring, and site closure, per ICH EG Good Clinical Practice (19) to ensure the study is undertaken according to GCP.

13.1 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.

13.2 Monitoring

The sponsor, or sponsor representatives 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 done per GCP.

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REVISION HISTORY

Rev	Description of Change	Page Number	Date
1.0	First Release	n/a	September 10, 2018
2.0	Clarification of study as non-significant risk study. Clarification of minimum numbers of slides as H&E slides. Clarification that any replacement case must be read by all 4 RPs for both modalities. Clarification of use of both logs and CRFs for data collection. Clarification that any protocol deviations noted by CRA or Data Manager staff will be directed to site study coordinator to be entered into on-site protocol deviation log. Clarification that protocol deviations and device deficiencies to be recorded in logs. Clarification of adverse events for retrospective study. Clarification of slide or case replacement. Identification of 4 sites. Clarification of participants in Monitoring and function to confirm the integrity of the data. Clarification of adjudicator board. Clarification of data review for pilots.		July 28, 2019
3.0	Modifications to the statistical analysis sections based on discussions with the Food and Drug Administration.		October 16, 2019
4.0	Modifications to be in accord with FDA instructions re: adjudication of deferrals, removal of period & sequence term for analysis, and removal of interaction terms for analysis.		July 9, 2020