510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K190332

B. Purpose for Submission:

New Whole Slide Imaging (WSI) system

C. Measurand:

Not applicable

D. Type of Test:

Digital pathology whole slide imaging

E. Applicant:

Leica Biosystems Imaging, Inc.

F. Proprietary and Established Names:

Aperio AT2 DX System

G. Regulatory Information:

1. Regulation section:

   21 CFR 864.3700

2. Classification:

   Class II (special controls)

3. Product code:

   PSY

4. Panel:

   88 - Pathology

H. Intended Use:

1. Intended use(s):

   The Aperio AT2 DX System is an automated digital slide creation and viewing system. The Aperio AT2 DX System is intended 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. The Aperio AT2 DX System is not intended for use with frozen section, cytology, or non-FFPE hematopathology specimens.

   The Aperio AT2 DX System is composed of the Aperio AT2 DX scanner, the ImageScope DX review application and Display. The Aperio AT2 DX System is for creation and viewing of digital images of scanned glass slides that would otherwise be appropriate for manual visualization by conventional light microscopy. It is the responsibility of a qualified pathologist to employ appropriate procedures and safeguards to assure the validity of the interpretation of images obtained using the Aperio AT2 DX System.

2. Indication(s) for use:

   Same as Intended Use.

3. Special conditions for use statement(s):

   For *in vitro* diagnostic (IVD) use only

   For prescription use only

4. Special instrument requirements:

   Aperio AT2 DX digital slide scanner (for software Aperio Console DX 102.0.4.46 and Aperio Controller DX 102.0.4.85_x64)

   Viewing Workstation executing ImageScope DX viewer software (version 1.0.0.5018)

   Display (MR2416)

I. Device Description:

The Aperio AT2 DX System is an automated digital slide creation and viewing system. The system is composed of the following components:

- Aperio AT2 DX digital slide scanner
- Viewing Workstation executing ImageScope DX viewer software

- Display (MR2416)

The Aperio AT2 DX digital slide scanner digitizes microscope slides at diagnostic resolutions: 0.5 µm/pixel (20x objective equivalent) and 0.25 µm/pixel (40x objective equivalent), to create digital WSI images. The Aperio AT2 DX scanner has a capacity of 400 1x3 inch glass slides. After the slides are loaded the scanning process begins when the operator starts the Aperio AT2 DX scanner and finishes when the scanner has completed scanning of all loaded slides. The image acquisition components include a white LED light source, Olympus Plan Apo 20x lens with a Numeric Aperture (NA) of 0.75, a 10/90 beam splitter, a 2D area-scan camera and a 1D line-scan camera which is the primary image capture device. The 10/90 bean splitter sends 10% of the photons toward the 2D area-scan camera and remaining 90% of the photons toward the 1D line-scan camera. Two motorized stages are used with one stage for moving the slide to be scanned and the other stage for moving the objective lens for focusing purposes. During scanning with a 20x objective lens, the motion of the slide stage combined with the speed of the 1D line-scan camera, the field of view of the objective lens and the number of pixels of the camera result in a resolution of 0.5 µm/pixel for 20x magnification. 40x magnification is achieved with a magnification doubler, with 0.25 µm/pixel resolution. Focus during the scan is maintained using a triangulated focus map built from individual focus points determined in a separate step before scanning is started. Proprietary software is used for image processing during acquisition. Leica's proprietary format, SVS, is used to store and transmit the images between the Aperio AT2 DX digital slide scanner and the ImageScope DX viewer on viewing station. Software Aperio Controller DX runs on the AT2 DX workstation as a system service and controls the operations of the scanner. Software Aperio Console DX is a desktop program that runs on the AT2 DX workstation with a display monitor (Dell MR2416) and provides user interface for an operator to control operations of AT2 DX scanner.

The ImageScope DX application is a software only subsystem to be used with the display monitor (Dell MR2416) and runs on a separate viewing workstation PC. The ImageScope DX software opens the WSI images acquired with Aperio AT2 DX scanner from the image storage attached to local network and uses the color profile to render the image data to the calibrated display monitor to deliver the image view at the appropriate magnification and color. Functionalities of ImageScope DX include navigating the slide similar to using an optical microscope, panning across the slide to examine all tissue regions, zooming in and out to allow review at different magnification levels, and allowing pathologist annotations during review.

The different subsystems of the Aperio AT2 DX System are connected over an IT network at the user site. The IT hardware/software that supports the ImageScope DX Application Server & Storage software is not provided as part of the Aperio AT2 DX System, but may be located in a central server room separate from the workstation with the ImageScope DX viewing software and Display. The communication of data between Aperio AT2 DX scanner and ImageScope DX application is via a customer provided wired network or a direct connected cable between these subsystems. Aperio AT2 DX System includes a display that has been validated as part of the pivotal clinical study.

The Aperio AT2 DX System allows pathologists to view and evaluate digital images of formalin-fixed, paraffin-embedded (FFPE) tissue slides that would otherwise be appropriate for manual visualization by conventional brightfield (light) microscopy. The Aperio AT2 DX System does not include any automated image analysis applications that would constitute computer aided detection or diagnosis.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Philips IntelliSite Pathology Solution (PIPS)

2. Predicate 510(k) number(s):

   DEN160056

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The Aperio AT2 DX System is an automated digital slide creation and viewing system. The Aperio AT2 DX System is intended 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. The Aperio AT2 DX System is not intended for use with frozen section, cytology, or non-FFPE hematopathology specimens. The Aperio AT2 DX System is composed of the Aperio AT2 DX scanner, the ImageScope DX review application and Display. The Aperio AT2 DX System is for creation and viewing of digital images of scanned glass slides that would otherwise be appropriate for manual visualization by conventional light microscopy. It is the responsibility of a qualified	The Philips IntelliSite Pathology Solution (PIPS) is an automated digital slide creation, viewing, and management system. The PIPS is intended 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. The PIPS is not intended for use with frozen section, cytology, or non-FFPE hematopathology specimens. The PIPS comprises the Image Management System (IMS), the Ultra Fast Scanner (UFS) and Display. The PIPS is for creation and viewing of digital images of scanned glass slides that would otherwise be

Similarities		
Item	Device	Predicate
	pathologist to employ appropriate procedures and safeguards to assure the validity of the interpretation of images obtained using the Aperio AT2 DX System.	appropriate for manual visualization by conventional light microscopy. It is the responsibility of a qualified pathologist to employ appropriate procedures and safeguards to assure the validity of the interpretation of images obtained using PIPS.
Specimen type	Surgical pathology slides prepared from FFPE tissue	Same
Principle of operation	After conducting Quality Control (QC) on the glass slides per laboratory standards (e.g., staining, cover-slipping, barcode placement, etc.), the technician loads the slides into the Aperio AT2 DX scanner. The scanner scans the slides and generates WSI image for each slide. The technician performs QC on scanned WSI images by checking image data and image quality. When QC is failed, the slide will be re-scanned. The acquired WSI images are stored in an end user provided image storage attached to the local network. During review, the pathologist opens WSI images acquired with the Aperio AT2 DX scanner from the image storage, performs further QC to ensure image quality and reads WSI images of the slides to make a diagnosis.	Same
Device Components	WSI scanner (Aperio AT2 DX scanner), Image Management System (ImageScope DX application), and color monitor display	Same (PIPS Ultra Fast Scanner, Image Management System and color monitor display)

Differences		
Item	Device	Predicate
Whole Slide Imaging Scanner	Aperio AT2 DX scanner with loading capacity of 400 slides	Ultra Fast Scanner with loading capacity of 300 slides
Graphical User Interface	ImageScope DX	Image Management System
Monitor Display	Dell MR2416	PS27QHDCR

K. Standard/Guidance Document Referenced (if applicable):

Guidance for Industry "Technical Performance Assessment of Digital Pathology Whole Slide Imaging Devices", dated April 20, 2016.

Special controls under 21 CFR Sec. 864.3700 Whole slide imaging system.

L. Test Principle:

The Aperio AT2 DX System is an automated system designed for scanning and digitizing surgical pathology slides prepared from FFPE tissue. These digitized images can then be reviewed and interpreted by pathologists for clinical (patient care) purposes.

Prior to scanning the slide on the Aperio AT2 DX scanner, the technician conducts quality control of the slides per the laboratory's standards (e.g., staining, cover-slipping, barcode placement, etc.). The technician then places the slides into slide feeder (Autoloader) which is a component of the Aperio AT2 DX scanner as a batch. With AutoLoader, the scanner automatically mounts each slide on the scanning stage, generates a macro image that includes the slide label and a low magnification image of the entire slide. The system then locates specimen tissues within the slide scan area, which are subsequently scanned at high resolution (0.50 µm/pixel for 20x and 0.25 µm/pixel for 40x). After the slide is scanned, it is returned to the same slot of the same rack from which it was originally obtained.

The images scanned in the Aperio AT2 DX scanner are compressed and saved using proprietary SVS file format. The SVS image files are then transmitted to customer provided Medical Device Data System (MDDS). The SVS image files acquired on Aperio AT2 DX scanner can be reviewed through the supplied ImageScope DX review application only. ImageScope DX application allows users to open a case, review the WSI images of the slides (eSlides) by navigating around and viewing the WSI image at the desired magnifications. The pathologist is responsible for ensuring the validity of the interpretation of the digital images obtained from the Aperio AT2 DX System.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   The objective of this study was to evaluate intra-system, inter-system/site, within-pathologist and between-pathologist precision for the Aperio AT2 DX System.

   The precision of the device was based on five reading pathologists' assessments and

identification of specific histopathologic "features" that are observed in FFPE Hematoxylin and Eosin (H&E) stained slides. Twenty-three (23) primary features were selected for the analytical studies. The selected primary features were evaluated at their relevant magnifications with twelve (12) primary features evaluated at 20x magnification level and eleven (11) primary features evaluated at 40x magnification level.

The Precision Panel Slide Curator (curator) identified study slides by conducting a Lab Information System (LIS) search and reviewing the microscope slides from consecutive cases that potentially contain the study features (Table 1). A representative H&E stained slide or a re-cut slide containing the feature(s) of interest was obtained from each case. After the curator selected and marked the study features on the slides, the slides were scanned at magnification levels designated to the study features, either 20x or 40x magnification. The curator reviewed the glass slides through the microscope while reviewing the WSI images on the monitor to extract Field of Views (FOVs) containing the study features from the WSI images using a lossless compression type. For each subsequent WSI images of the same slide, the annotations were manually replicated by a technician using the image viewing software to extract and randomly rotate the FOVs by 90, 180 or 270 degrees to minimize recall bias. A trained study personnel verified the FOV filenames and also that the FOV extraction areas were correct and enrolled the FOVs in the study. During precision study, the extracted FOV images were restricted for both the area of tissue visible to the reading pathologists and for the viewing magnification. The reading pathologists were not allowed to navigate the WSI image or change magnification or get access to any clinical information associated with the study slides. Additional slides that may or may not include any of the study features were included as "wild card" slides to reduce recall bias. The "wild card" FOVs were extracted by following the same procedures as the study FOVs.
As shown in Table 1, each primary study feature was represented in three (3) FFPE sections of different organ types. From each slide, two (2) or three (3) FOVs containing either one primary study feature, or a primary study feature and secondary study feature(s) in each FOV were selected. A total of 202 FOVs were selected from 69 glass slides. Out of 202 FOVs, 46 FOVs (from 24 slides) contained multiple histologic features and 156 FOVs (from 62 slides) contained one primary histologic feature. In addition, 36 wild card FOVs were selected from 12 glass slides.

Table 1: Primary Histologic Study Features in Precision Study

Magnification Level	**Primary Feature**	**Organ Type**
20x	Chondrocytes	Toe
		Femoral Head
		Osteosarcoma of humerus
	Fat Cells (adipocytes)	Axillary lymph node
		Femoral head
		Prostate

	Foreign Body Giant Cells	Left knee synovium
		Shoulder
		Sigmoid colon
	Goblet Cells in Intestinal Mucosa or Intestinal Metaplasia	Gastroesophageal junction
		Sigmoid colon
		Tubular adenoma (intestine)
	Granulomas	Colon
		Iliac crest (bone)
		Cervical lymph node
	Infiltrating or metastatic lobular carcinoma	Iliac crest (bone)
		Jejunum
		Left breast
	Intraglandular Necrosis	Left lung
		Liver
		Right colon
	Osteoclasts	Sacrum
		Toe
		Paget's disease of spine
	Osteocytes	Foot
		Maxilla
		Osteosarcoma of femur
	Pleomorphic nucleus of malignant cell	Left 11th rib
		Sacrum
		Vertebra
	Serrated intestinal epithelium (for example sessile serrated polyp)	Appendix
		Ascending colon polyp
		Sigmoid colon
	Skeletal Muscle fibers	Right lower leg
		Shoulder
		Spine
40x	Asteroid Bodies	Axillary lymph node
		Liver
		Synovium
	Clear Cells (renal cell carcinoma)	Humerus
		Retroperitoneal lymph node
		Right kidney
	Foreign bodies (for example plant material or foreign debris)	Distal femur
		Foot
		Wrist
	Hemosiderin (pigment)	Knee synovium
		Liver
		Osteosarcoma of femur
	Megakaryocytes	Cervical Spine
		Femur (margin of sarcoma)
		Tibia

	Necrosis	Femoral head
		Left para-aortic lymph node
		Right leg
	Nerve Cell Bodies (for example ganglion cells)	Ganglioneuroma
		Small bowel
		Stomach
	Nuclear Grooves	Cervical lymph node (papillary thyroid carcinoma)
		Iliac crest (bone) (Langerhan's cell granuloma)
		Ovary (Brenner tumor)
	Osteoid Matrix	Femur
		Humerus
		Lung
	Psammoma bodies	Cervical lymph node (metastatic papillary carcinoma of thyroid)
		Fallopian tube (papillary ovarian carcinoma)
		Left ventral cranial region (meningioma)
	Reed-Sternberg Cell	Axillary lymph node
		Neck mass
		Spleen

A total of five reading pathologists reviewed the FOVs in precision study. Three pathologists reviewed for within- and between-pathologist precision, one pathologist reviewed for intra-system precision and one pathologist reviewed for inter-system/inter-site precision. For each magnification (20x or 40x), a separate checklist containing eleven (11) features (for 40x) or twelve (12) features (for 20x) was developed. For each FOV, the reading pathologist recorded the presence or absence of each of 11 features (for 40x) or 12 features (for 20x) on a checklist. Only the recorded results from study FOVs were used for statistical analysis. The results of wild card FOVs were not included in the statistical analysis.

Intra-System Precision

The panel of 69 study slides was split equally among the three (3) sites (i.e., each site scanned a separate subset of slides). Each site has a single scanning system. For each system, 23 slides were scanned once on each of three (3) days, producing three (3) sets of scans for each slide. FOVs were extracted from scanned WSI images. The FOVs from three scanning sessions were evaluated by one reading pathologist over three different reading sessions with at least a two-week washout period in between the reading sessions. In each reading session, the reading pathologist evaluated all 202 study FOVs from each of three scanning sessions (across three scanning systems), plus unique "wild card" FOVs to assist in preventing recall bias between reading sessions.

For each system, agreements between scan 1 versus scan 2, scan 1 versus scan 3 and scan 2 versus scan 3 were analyzed. The overall intra-system precision was based on the pooled data of all three systems. A bootstrap approach was used to calculate 95% Confidence Intervals (CIs) with the following exception. When the agreement estimate was 100%, the Arcsine (variance stabilizing transformation) approach that corrected for the continuity was used to calculate CIs.

Table 2: Intra-System Precision Study Results

System	Number of Pairwise Agreements	Number of Comparison Pairs	Agreement Rate and 95% CI	
			% Agreement	95% CI
System 1	193	201	96.0	(91.0, 100)
System 2	201	201	100	(98.2, 100)
System 3	199	204	97.5	(93.6, 100)
Overall	593	606	97.9	(95.9, 99.5)

Inter-System/Inter-Site Precision

At each of three study sites, a study technician scanned the entire set of 69 study slides once. FOVs from three scanning sessions (resulting from one session from each of the three sites) were extracted. From all the scanning systems, 606 FOVs were transferred to a single reading pathologist for evaluation in three separate reading sessions. In each reading session, the reading pathologist evaluated all 202 study FOVs from a single site, plus unique "wild card" FOVs to assist in preventing recall bias between reading sessions.
Agreements between systems at Site 1 versus Site 2, systems at Site 1 versus Site 3 and systems at Site 2 versus Site 3 were analyzed. The overall inter-system/inter-site precision is based on the pooled data from each system to system comparison. A bootstrap approach was used to calculate 95% CIs.

Table 3: Inter-System/Inter-Site Precision Study Results

System	Number of Pairwise Agreements	Number of Comparison Pairs	Agreement Rate and 95% CI	
			% Agreement	95% CI
Site 1 vs. Site 2	195	202	96.5	(94.1, 99.0)
Site 1 vs. Site 3	194	202	96.0	(93.1, 98.5)
Site 2 vs. Site 3	193	202	95.5	(92.6, 98.0)
Overall	582	606	96.0	(93.6, 98.2)

Within-Pathologist and Between-Pathologist Precision

The entire set of 69 slides were scanned once at one site. FOVs were extracted and saved in three different orientations. From all the orientations, 606 FOVs were presented to each of three reading pathologists for evaluation in three reading sessions. In each reading session, each reading pathologist evaluated all 202 study FOVs plus unique "wild card" FOVs to assist in preventing recall bias between reading sessions.
For within-pathologist precision, agreements between FOVs in orientation 1 versus orientation 2, orientation 1versus orientation 3, and orientation 2 versus orientation 3 were analyzed for each reading pathologist. The overall within-pathologist precision was based on the pooled data from each reading pathologist. A bootstrap approach was used to calculate 95% CIs.

Table 4: Within-Pathologist Precision Study Results

Pathologist	Number of Pairwise Agreements	Number of Comparison Pairs	Agreement Rate and 95% CI	
			% Agreement	95% CI
Pathologist 1	561	606	92.6	(89.6, 95.7)
Pathologist 2	595	606	98.2	(96.3, 99.7)
Pathologist 3	571	606	94.2	(91.4, 96.9)
Overall	1727	1818	95.0	(92.9, 96.8)

For between-pathologist precision, agreements between pathologist 1 versus pathologist 2, pathologist 1 versus pathologist 3, and pathologist 2 versus pathologist 3 were analyzed. The overall between-pathologist precision was based on the pooled data from each reading pathologist. A bootstrap approach was used to calculate 95% CIs.

Table 5: Between-Pathologist Precision Study Results

Pathologist Comparison	Number of Pairwise Agreements	Number of Comparison Pairs	Agreement Rate and 95% CI	
			% Agreement	95% CI
Pathologist 1 vs. Pathologist 2	572	606	94.4	(91.6, 96.9)
Pathologist 1 vs. Pathologist 3	562	606	92.7	(89.9, 95.4)
Pathologist 2 vs. Pathologist 3	579	606	95.5	(93.1, 97.7)
Overall	1713	1818	94.2	(91.7, 96.4)

b. Linearity/Assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Not applicable

d. Detection limit:

Not applicable

e. Analytical specificity:

Not applicable

f. Assay cut-off:

Not applicable

2. Technical studies:

Multiple studies were conducted to evaluate the performance assessment data associated with the technical evaluation of the Aperio AT2 DX System.

a. Slide feeder

Information was provided on the configuration of the slide feed mechanism, including a physical description of the slide, the number of slides in queue (carrier), and the class of automation. Information was provided on the user interaction with the slide feeder, including hardware, software, feedback mechanisms, and Failure Mode and Effects Analysis (FMEA).

b. Light source

Descriptive information associated with the lamp and the condenser was provided. Testing information was provided to verify the spectral distribution of the light source as part of the color reproduction capability of the Aperio AT2 DX scanner.

c. Imaging optics

An optical schematic with all optical elements identified from slide (object plane) to digital image sensor (image plane) was provided. Descriptive information regarding the microscope objective, the auxiliary lenses, and the magnification of imaging optics was provided. Testing information regarding the relative irradiance, optical distortions, and lateral chromatics aberrations was provided.

d. Mechanical scanner movement

Information and specifications on the configuration of the stage, method of movement, control of movement of the stage, and FMEA was provided. Test data to verify the repeatability of the stage movement and to verify the mechanism that the stage movement stays within limits during operations was provided.

e. Digital imaging sensor

Information and specifications on the sensor type, pixel information, responsivity specifications, noise specifications, readout rate, and digital output format. Test data to determine the correct functioning of the digital image sensor that converts optical signals of the slide to digital signals which consist of a set of numerical values corresponding to the brightness and color at each point in the optical image was provided.

f. Image processing software

Information and specifications on the exposure control, white balance, color correction, sub-sampling, pixel-offset correction, pixel-gain or flat-field correction, and pixel-defect correction were provided.

g. Image composition

Information and specifications on the scanning method, the scanning speed, and the number of planes at the Z-axis to be digitized was provided. Test data to analyze the image composition performance was provided.

h. Image files format

Information and specifications on the compression method, compression ratio, file format, and file organization were provided.

i. Image review manipulation software

Information and specifications on continuous panning and pre-fetching, continuous zooming, discrete Z-axis displacement, ability to compare multiple slide simultaneously on multiple windows, image enhancement and sharpening functions, color manipulation, annotation tools, and digital bookmarks were provided.

j. Computer environment

Information and specifications on the computer hardware, operating system, graphics card, graphics card driver, color management settings, color profile, and display interface were provided.

k. Display

Information and specifications on the technological characteristics of the display device, physical size of the viewable area and aspect ratio, backlight type and properties, frame rate and refresh rate, pixel array, pitch, pixel aperture ratio and subpixel matrix scheme, subpixel driving to improve grayscale resolution, supported color spaces, display interface, user controls of brightness, contrast, gamma, color space, power-saving options, etc., via the on-screen display menu, ambient light adaptation, touchscreen technology, color calibration tools, and frequency and nature of quality-control tests was provided. Test data to verify the performance of the display was provided.

l. Color reproducibility

Test data to evaluate the color reproducibility of the system was provided.

m. Spatial resolution
Test data to evaluate the composite optical performance of all components in the image acquisition phase was provided.

n. Focusing test

Test data to evaluate the technical focus quality of the system was provided.

o. Whole slide tissue coverage

Test data to demonstrate that the entire tissue specimen on the glass slide is detected by the tissue detection algorithms, and that all the tissue specimens are included in the digital image file was provided.

p. Stitching error

Test data to evaluate the stitching errors and artifacts in the reconstructed image was provided.

q. Turnaround time

Test data to evaluate the turnaround time of the system was provided.

3. <u>Human factors studies:</u>

Human factors studies designed around critical user tasks and use scenarios performed by representative users for both histopathology technicians and pathologists were conducted. Information included a list of all critical user tasks and a description of the process that was followed to identify them. A systematic evaluation involving simulated use by

representative users performing all tasks (including critical tasks) required for operation of the device, and subjective assessment of failure was provided. All participants were able to perform all tasks (including the critical tasks) and no critical task failures were observed. There were some occasional difficulties that are generally expected with any piece of new software and instrument but learnability and ease of use appeared to be considerably high. All difficulties observed were of little influence on the perception of the usability, and no difficulties or failures were observed on tasks that could lead to patient harm. In all instances both pathologists and histopathology technicians were able to easily identify cases and ensure that everything was complete.

4. Clinical studies:

A multi-center study was conducted to demonstrate that viewing, reviewing, and diagnosing digital images of surgical pathology FFPE tissue slides using the Aperio AT2 DX System is non-inferior to using optical (light) microscopy. The primary endpoint was the difference in major discordance rates between WSI review modality (WSIR) and microscope slide review modality (MSR) when compared to the reference (main) diagnosis, which is based on the original sign-out pathologic diagnosis rendered at the institutions, using an optical (light) microscope.

Five sites were used in the study. At each site, a case curation pathologist or lab technician searched the site's LIS to identify candidate cases with a sign-out pathologist diagnosis. Candidate cases were identified consecutively, starting with cases at least one (1) year old (sign-out diagnosis was made at least one year before) and continuing with sequentially older cases. The case curation pathologist then checked whether the candidate case met the study eligibility criteria. The case curation pathologist reviewed the sign-out diagnosis and supporting documentation available at the time of the diagnosis (i.e., pathology report). By reviewing the microscopic slides used to make the sign-out diagnosis, the slide(s) that were representative of the sign-out diagnosis for the case were identified. The selected slides included H&E, immunohistochemistry (IHC), special stains and any slides that were not critical to diagnosis but supported the final surgical pathology report (e.g., margins, lymph node status, vascular and neural invasion), and any slides representing required elements of the College of American Pathologists (CAP) applicable cancer protocols. In the case of IHCs and special stains, the inclusion of control slides was required to fulfill the quality checks according to general clinical practice. The site's curation verification pathologist verified that the selected slide(s) reflected the main diagnosis for the case as well as required ancillary information for cancer cases, met all inclusion criteria and none of the exclusion criteria, and then the case was enrolled.

Across five sites, a total of 2045 cases (5849 slides) consisting of multiple organ and tissue types were enrolled. At each site (with exception of Site 1 that had three pathologists), four pathologists read all the cases assigned to the site using both MSR and WSIR modalities in an alternating fashion and randomized order and with a washout period of 31 days or longer between the MSR and WSIR reads, resulting in a total of 7781 planned WSIR reads and 7781 planned MSR reads. The reading pathologists were

provided with all representative slide(s) of a case at once. An electronic case report form (eCRF) was completed to document the reading pathologist's diagnosis. Three adjudicators reviewed the reader diagnoses against the corresponding sign-out diagnoses and determined whether the reader diagnoses were concordant, minor discordant or major discordant. A major discordance was defined as a difference in diagnosis that would be associated with a clinically important difference in patient management. A minor discordance was defined as a difference in diagnosis that would not be associated with a clinically important difference in patient management. For each case, two adjudication pathologists independently reviewed the eCRFs to determine whether or not the diagnosis was consistent with the sign-out diagnosis. In the event that there was a disagreement between the two adjudicators, a third adjudication pathologist reviewed the case to achieve majority vote. In cases where all three adjudicators had a different opinion, consensus was arrived at in an adjudication panel meeting consisting of the same three adjudication pathologists.

Inclusion criteria:

- All glass slides with human tissue obtained via surgical pathology of the original case were available.
- The original sign-out diagnosis and ancillary supporting information were available.
- The selected slide(s) for the main diagnosis and the control slide(s) (e.g., controls for IHC stained slides) matched the study requested organ type and fulfilled the quality checks according to general clinical practice.
- For cases with multiple diagnoses in a single sign-out pathology report, only the main diagnosis and ancillary information were included.

Exclusion criteria:

- The H&E stained slides that were used for the original sign-out diagnosis were not available at the site and re-cuts were not available.
- If applicable, the IHC or special stain slides that were used for the original sign-out diagnosis were not available at the site or had unacceptable artifacts. Control slides for IHC or special stain were not available.
- Cases for which slides needed to support the original sign-out diagnosis required either a special light source (e.g., a mercury lamp for fluorescence microscopy) or special filters (e.g., for polarized light).
- Cases with slides containing unremovable markings or were damaged.
- Cases that contained frozen sections or gross specimens only.
- Cases that were missing significant clinical and ancillary information that was available at the time the case was originally interpreted (e.g., X-rays).
- Cases that were signed out less than one year prior to the date of curation.
- Only one case could be enrolled per patient.

For the primary objective of demonstrating the WSIR major discordance rate to be non-inferior to the MSR major discordance rate, a Generalized Linear Mixed Model

(GLIMMIX) logistic regression was conducted. For each reading result the dependent variable was the major discordance status and independent variables included modality as a fixed effect (WSIR vs. MSR) and site, reader, and case as random effects. A two-sided 95% CI for the modality effect, i.e., the overall major discordance rate difference (WSIR minus MSR), was constructed from this analysis. If the upper bound of the 95% CI was less than the non-inferiority margin of 4%, WSIR would be considered non-inferior to MSR.

A total of 2045 cases were included in the Full Analysis Set with 5849 slides and 7781 planned diagnoses for each modality. During the study, reading pathologists deferred cases by either modality per the site's standard procedure, mimicking the intended use. 172 cases were deferred in both modalities, 87 cases (1.1% of total planned reads) were deferred only in MSR modality and 100 cases (1.3% of total planned reads) were deferred only in WSIR modality. 7509 WSIR diagnoses and 7522 MSR diagnoses that were adjudicated by the adjudication panel and had consensus scores, were included in the statistical analyses. Deferred diagnoses were excluded from the data analysis as missing data.

The observed overall major discordance rate, i.e., over all sites, reading pathologists and organs, was 3.73% for WSIR modality and 3.28% for MSR modality. The WSIR-MSR difference in major discordance rate was 0.45%. The major discordance rates as estimated by the GLIMMIX logistic model ("modeled") resulted in similar, slightly lower, proportions, i.e., 3.64% for WSIR modality and 3.20% for MSR modality. The WSIR-MSR difference in major discordance rate was 0.44%, with derived two-sided 95% CI of [-0.15%, 1.03%]. The upper limit of this confidence interval was less than the pre-specified non-inferiority margin of 4%, and therefore, the WSIR modality using the Aperio AT2 DX System was demonstrated to be non-inferior to the MSR modality using light microscopy with respect to major discordance rate with comparing to the main diagnosis. Thus, the study met the primary objective as shown in Table 6.

Table 6: Clinical Study Results Based on Major Discordance Rates

	Whole Slide Imaging Review (WSIR)			Light Microscope Slide Review (MSR)			Difference (WSIR – MSR)	
	Total Reads	% discordant	95% CI	Total Reads	% discordant	95% CI	% discordant	95% CI
Observed	7509	3.73	-	7522	3.28	-	0.45	-
Model		3.64	(3.21, 4.12)		3.20	(2.80, 3.65)	0.44	(-0.15, 1.03)

The observed differences in major discordance rates by organ types between WSIR and MSR are shown in Table 7.

Table 7: Major Discordance Rates by Organ

Organ	Major Discordance Rate (%)		% Difference in Major Discordance Rates (WSIR – MSR)
	Whole Slide Imaging Review (WSIR)	Light Microscope Slide Review (MSR)	

Anus/perianal	3.95	2.79	1.16
Appendix	0.00	0.00	0.00
Bladder	10.40	9.47	0.93
Brain/neuro	3.09	2.54	0.55
Breast	4.29	3.53	0.76
Colorectal	2.46	2.46	0.00
Endocrine	4.04	4.57	-0.53
Gastroesophageal junction	3.69	3.16	0.54
Gallbladder	0.00	0.00	0.00
Gynecological	4.28	3.18	1.10
Hernia/peritoneal	0.00	0.00	0.00
Kidney	1.14	1.69	-0.56
Liver/bile duct	1.59	0.53	1.06
Lung	5.24	3.68	1.55
Lymph node	1.09	1.87	-0.78
Prostate	3.00	3.44	-0.44
Salivary gland	0.55	1.69	-1.14
Skin	4.74	2.72	2.03
Soft tissue	4.23	4.83	-0.60
Stomach	3.15	2.09	1.06

Three (3) organ types, appendix, gallbladder and hernial/peritoneal, had major discordance rates of 0.00% for both modalities. For all organ types, the absolute values of the observed differences in the major discordance rates between WSIR diagnosis and MSR diagnosis (relative to the reference diagnosis) were $\leq 2.03\%$. When examining the major discordance rates, 'Bladder' had the highest major discordance rate of 10.40% for WSIR and 9.47% for MSR. However, statistical model showed that the modality-by-organ interaction was not statistically significant.

Secondary analysis of agreement between the WSIR and MSR diagnosis showed that there was also strong agreement between the two modalities. There were 7423 pairs of readings with both adjudication outcomes for WSIR and MSR. Results showed 96.1% of the paired readings resulted in no major discordance for both modalities (94.7%) or major discordance for both modalities (1.47%).

The modality-by-organ interaction was not statistically significant. The clinical study was not powered to analyze the results by individual organ site or diagnosis. The number of unbalanced diagnoses in which the WSIR modality had major discordance and the MSR modality had concordance with the reference diagnosis in each tissue/organ type category ranged from 0 cases for kidney to 15 cases in breast, corresponding to 0.0% to 4.9% of the total cases in each organ type group. Similarly, the number of unbalanced diagnoses in which the MSR modality had major discordance and the WSIR modality had concordance with the reference diagnosis in each tissue/organ type category ranged from 0 cases in skin to 13 cases in breast, corresponding to 0.0% to 4.3% of the total cases in each organ type group. Thus, unbalanced diagnoses represented a small percentage of the

overall cases and occurred with similar frequency for both modalities. When the specific diagnoses were examined by tissue/organ type, there were no apparent trends in any tissue/organ type that suggested WSIR is more inherently prone to major discrepancies compared to MSR. When examining this data by reading pathologist, none of the reading pathologists showed a tendency to have more types of specific major discrepancies with WSIR as compared to MSR. In every case the observed rates were within the known and established rate of inter-pathologist variation in diagnosis as reported in literature.

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

   Not applicable

N. Instrument Name:

Aperio AT2 DX System

O. System Descriptions:

1. Modes of Operation:

   Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

   Yes ____X____ or No _____

   Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

   Yes _____ or No ____X____

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X____ or No _____

3. Specimen Identification:

   Glass slides are identified by barcodes on slide label.

4. Specimen Sampling and Handling:

   Glass slides are loaded manually into the slide feeder (Autoloader) which is a component of the Aperio AT2 DX scanner as a batch. With AutoLoader, the scanner automatically loads each slide in the batch to the stage and scans the glass slide to generate WSI image. After the scanning of all slides in the batch is completed, the slides are unloaded from the scanner and stored according to the lab procedure.

5. Calibration:

   The Aperio AT2 DX scanner is calibrated and verified at the factory before shipment. No further manual calibration or verification is required by the customer. Annual preventive maintenance performed by Leica Biosystems Technical Services is recommended to ensure the scanner is operating correctly. The scanner provides automatic calibration and verification every time a slide is scanned by performing calibration during the scan.

   The monitors provided with the Aperio AT2 DX System have been calibrated at the factory and may require periodic re-calibration in the field by authorized Leica Biosystems technicians. The user should not attempt to change the monitor settings as doing so may interfere with the monitor calibration.

   The ImageScope DX application does not require calibration.

6. Quality Control:

   It is the responsibility of the laboratory staff to conduct and maintain quality control of the slides per their laboratory standards (e.g., staining, cover-slipping, barcode placement) prior to loading the slides into the Aperio AT2 DX scanner. After completing a scan, the lab technician checks image data and image quality using the Aperio ConsoleDX application installed on the scanner workstation per the instructions for use. Before review, the pathologist performs quality control on the WSI images of the slide per instructions for use.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type under 21 CFR 864.3700.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.