510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
 k043519

B. Purpose for Submission:
 Adaptation of the Applied Imaging Ariol™ system (k031715) to fluorescence in situ hybridization (FISH) in addition to IHC

C. Measurand:
 Her2/neu gene copy number in formalin-fixed paraffin-embedded human breast cancer tissue specimens

D. Type of Test:
 Computer-assisted image analyzer for fluorescence in situ hybridization (FISH)

E. Applicant:
 Applied Imaging Corporation

F. Proprietary and Established Names:
 Ariol™ HER-2/neu FISH

G. Regulatory Information:
 1. Regulation section:
    21 CFR 866.4700, Automated Fluorescent in situ Hybridization (FISH) Enumeration Systems
 2. Classification:
    II
 3. Product code:
    NTH, System, automated scanning microscope and image analysis, for fluorescence in situ hybridization (FISH) assays
 4. Panel:
    Immunology 82

H. Intended Use:
 1. Intended use(s):
    Ariol™ is an automated scanning microscope and image analysis system. It is intended for *in vitro* diagnostic use as an aid to the pathologist in the detection, classification, and counting of cells of interest based on particular color, intensity, size, pattern, and shape.

    This particular Ariol application is an accessory to the PathVysion® HER-2/*neu* DNA Probe kit (PathVysion, Vysis, Inc., Downers Grove, IL). PathVysion is designed to detect amplification of the HER-2/*neu* gene via fluorescence *in situ* hybridization (FISH) in formalin-fixed, paraffin-embedded human breast cancer tissue specimens. Results

from the PathVysion kit are intended for use as an adjunct to existing clinical and pathologic information used as prognostic factors in stage II, node-positive breast cancer patients. The PathVysion kit is further indicated as an aid to predict disease-free and overall survival in patients with stage II, node positive breast cancer, treated with adjuvant cyclophosphamide, doxorubicin, and 5-fluorouracil (CAF) chemotherapy. The PathVysion kit is also indicated as an aid in the assessment of patients for whom HERCEPTIN® (Trastuzumab) treatment is being considered. While the PathVysion kit provides the probes that offer direct visualization and manual enumeration of the HER2 and Chromosome 17 genes with a fluorescent microscope, the Ariol may be used as an accessory that provides automated enumeration.

2. Indication(s) for use:
   Same as intended use.
3. Special conditions for use statement(s):
   For Prescription use
4. Special instrument requirements:
   The Applied Imaging Ariol™ system

I. Device Description:

Ariol™ is an automated scanning microscope and image analysis system. The system is comprised of a computer, monitor, keyboard, mouse, printer, installed software, microscope, monochrome CCD camera, focus and filterwheels supplied with 3x25 mm filters optimized for bright field scanning and RGB capture. The automated microscope includes motorized stage, focus drive, brightfield and fluorescence filter wheels, brightfield and fluorescence lamps, eyepieces, condenser and camera adapter. The Monochrome CCD camera acquires the microscope images, which are then transferred to the PC through the image acquisition board for subsequent analysis by the software algorithm.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Ariol™ HER2 IHC
2. Predicate 510(k) number(s):
   k031715
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Tissue specimen used and application	To detect amplification of the HER-2/*neu* gene in formalin-fixed, paraffin-embedded human breast cancer tissue specimens. Indicated as an aid in the assessment of patients for whom HERCEPTIN® (Trastuzumab) treatment is being considered.	Same

Similarities		
Item	Device	Predicate
Method of cell detection	Colorimetric pattern recognition by microscopic examination of prepared cells by size, shape and intensity of counterstained nuclei as observed by an automated computer controlled microscope and/or by visual observation by a health care professional.	same
Device components	- Automated microscope - Image analysis system - Video camera - PC with windows-based operating system - Keyboard and control panel - Color monitor for display of information - Color printer for reports	Same

Differences		
Item	Device	Predicate
Intended use	Ariol™ HER2/neu FISH is an accessory to the PathVysion® HER-2/*neu* DNA Probe kit (PathVysion, Vysis, Inc., Downers Grove, IL). PathVysion is designed to detect amplification of the HER-2/*neu* gene via fluorescence *in situ* hybridization (**FISH**).	Ariol™ Hersight application is an accessory to the HercepTest™ (DAKO, USA, Carpinteria, CA). It is intended to provide semi-quantitative immunohistochemical (**IHC**) results to aid in the determination of Her-2/neu over-expression.
Indications for use	Results from the PathVysion kit are intended for use as an adjunct to existing clinical and pathologic information used as prognostic factors in stage II, node-positive breast cancer patients. The	

Differences		
Item	Device	Predicate
	PathVysion kit is further indicated as an aid to predict disease-free and overall survival in patients with stage II, node positive breast cancer, treated with adjuvant cyclophosphamide, doxorubicin, and 5-fluorouracil (CAF) chemotherapy.	
Device components	• Joystick controller for automated stage • Slide Loader or multi-bay stage	• Dual Control Stick
Microscope objectives	1.25x, 5x, 10x, 20x, 40x	10x, 20x, 40x
Light Source	Mercury Lamp	Halogen Lamp

K. Standard/Guidance Document Referenced (if applicable):
FDA guidance documents on software validation and off-the shelf software.

L. Test Principle:
Although the pathologist is responsible for making a classification or diagnostic decision, the Ariol™ system assists in this by enumerating the signals from the DNA probes in each nucleus and computing their ratio. The PathVysion® assay is performed by the Ariol™ system either by using a Two Pass scanning method or by Region Cam method. After the selected region has been scanned at 40x, the user selects the cells of interest for the system to count the Spectrum Orange and Spectrum Green signals. The total number of each probe as well as their ratio is presented to the user in the data grid, along with an image of the cell. Once an adequate number of cells have been chosen and scored, the pathologist then enters a score for the slide. This may or may not be based on the score computed by the Ariol system, at the discretion of the pathologist. The final score so entered is then printed out on a final report.

M. Performance Characteristics (if/when applicable):
1. <u>Analytical performance:</u>
   a. *Precision/Reproducibility:*
   The Ariol™ HER-2/*neu* FISH application was evaluated for precision in simulated clinical settings. Ariol precision was assessed via three precision studies, namely within-day, within-instrument (P1); between-day, within-instrument (P2), and between-instrument (P3).

   A total of five slides were evaluated in multiple "runs" for each precision study. Each slide set consisted of the Vysis PathVysion® Negative Control, (target ratio 1.0) n=2; the Vysis PathVysion Mid-Low Amplified Control (target ratio 1.8 to 2.2), n=2; and a

highly amplified control. (target ratio of >2.3), n=1. The lot numbers for the two commercial controls were: Negative- Lot 55301/55392, and Mid-Low Amplified- Lot 56287/56011. The highly amplified control slides were represented by slides prepared from breast cancer cell line SKBR3. All the slides were stained by one operator in the same staining batch, and the precision slides were forwarded to Applied Imaging within one day of their staining. Each precision evaluation is described below.

Within-day, within-instrument (P1)
Study P1 was performed in one day with one Ariol instrument (Ariol #1). The five slides were scanned and interpreted three times in succession by re-scanning the same regions, and then this experiment was done two more times with the same slides, but with different regions of the slides. It was necessary to scan different regions for the 2nd and 3rd set of scans, as it is well known that the FISH signals fade and eventually disappear with repeated exposure to the microscope light.

Between-day, within-instrument (P2)
Study P2 was performed over three days with a different Ariol instrument (Ariol #2) from the one used in Study P1. Each of the five slides were scanned and interpreted three times in one day, and different regions of the slides were used for each day of testing. The slides were stored at 2-8 degrees and in the dark between days in order to preserve the staining.

Between-instruments (P3)
Study P3 was performed over several days, and this study evaluated a new set of slides on a third Ariol system.

The data are summarized in the following table, and they show %CVs ranging between 5% and 16% across the various samples.

ID #	ID Ratio Her2/ Chr 17	Set #	P1				P2				P3			
			Mean	SD	%CV	Grand %CV	Mean	SD	%CV	Grand %CV	Mean	SD	%CV	Grand %CV
1	Control 1.0	1	1.28	0.04	2.74	7.76	1.32	0.11	8.6	10.89	1.25	0.17	13.3	8.35
		2	1.14	0.11	9.35		1.19	0.09	7.33		1.22	0.06	2.79	
		3	1.13	0.01	1.02		1.18	0.18	15.5		1.24	0.10	8.42	
2	Control 1.0	1	1.25	0.08	6.43	10.08	1.19	0.14	11.9	9.26	1.23	0.13	10.6	14.7
		2	1.38	0.04	2.90		1.24	0.06	5.11		1.22	0.18	15.1	
		3	1.12	0.08	6.91		1.11	0.10	8.68		1.26	0.28	22.5	
3	Control 1.8	1	2.09	0.04	1.91	4.75	2.00	0.10	4.9	4.77	2.07	0.05	2.48	5.69
		2	2.01	0.11	5.33		1.94	0.12	6.27		1.94	0.12	6.14	
		3	1.92	0.03	1.31		1.90	0.04	2.00		2.09	0.13	6.33	
4	Control 1.8	1	2.09	0.12	5.68	5.95	2.03	0.08	3.7	4.17	2.10	0.05	2.14	4.94
		2	1.92	0.07	3.61		2.07	0.09	4.12		1.98	0.11	5.58	
		3	2.03	0.13	6.4		1.94	0.05	2.68		2.02	0.12	6.00	
5	Control ~5.0	1	4.73	0.15	3.08	8.91	5.30	0.42	7.85	15.67	4.67	0.51	10.9	16.5
		2	5.00	0.09	1.80		4.91	0.07	1.39		5.01	0.08	1.60	
		3	4.22	0.44	10.38		3.79	0.36	9.51		3.53	0.28	7.80	

b. *Linearity/assay reportable range:*
Not applicable.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
The analytical traceability of the system depends on the Vysis PathVysion® HER-2/neu DNA Probe kit. Ariol PathVysion system uses ProbeChek control slides, supplied by Vysis, to assess the accuracy of signal enumeration and to monitor the assay. Control slides are required to run on each day of FISH testing and with each new kit lot and with each run of patient slides processed.

d. *Detection limit:*
Not applicable.

e. *Analytical specificity:*
The analytical specificity of the test result is dependent on the analytical performance of the Vysis PathVysion® HER-2/*neu* DNA Probe kit.

f. *Assay cut-off:*
The assay cut-off of the test result is dependent on the analytical performance of the Vysis PathVysion® HER-2/*neu* DNA Probe kit.

2. Comparison studies:

a. *Method comparison with predicate device:*
The substantial equivalence studies were based on comparison to conventional manual microscopy performed in accordance with Vysis PathVysion® HER-2/*neu* DNA Probe kit .

The concordance study was performed at Applied Imaging with one Ariol system. The clinical slides were obtained from four independent clinical sites. The study included a total of 82 cases, consisting of 32 cases of normal (ratios of approximately 1.0), 17 cases of low-moderate amplification (ratios between 1.8 and 2.2), and 33 cases of high amplification (ratios >2.2). For each case, the sites selected frozen blocks from their clinical inventories and prepared new slides. Slides were stained with both H&E reagents and HER2 FISH reagents, and the slide pairs were blinded as to their results and forwarded to Applied Imaging. At Applied Imaging, the H&E slides were used to mark the regions of tumor cells, and then these regions were then selected on the FISH slides. The ratio results from the original clinical interpretations, all performed by manual readings and by following the instructions in the reagent package insert, were considered the "true" results for the basis of comparison to Ariol interpretations.

For the manual method, the sites counted 20 cells and then calculated the average HER2/chromosome 17 ratios. If the result was between 1.8 and 2.2, another 20 cells were counted (as per the reagent package insert recommendation) and the final ratio was based on the average from counting 40 cells. The same protocol was followed for the Ariol (automated) method. If the Ariol reported a ratio between 1.8 and 2.2 with the first 20 cells, the operator was instructed to count another 20 cells, and then the final average calculation from the 40 cells was reported.

Matched pairs t-test (two tail) was performed to compare the mean values of the two methods. Pearson correlation coefficient and orthogonal regression was also performed. Bootstrapping was performed to generate a 95% CI for the y-intercept. Pure arithmetic concordance was calculated by determining the percent agreement within a category.

		Manual Method		
		≤1.74	1.75 to 2.25	>2.25
Ariol Method	≤1.74	31	1	0
	1.75 to 2.25	1	16	0
	>2.25	0	0	33

The overall concordance across all three clinical categories was 98% (80/82 cases).

     b. *Matrix comparison:*
     Not applicable.
3. Clinical studies:
     a. *Clinical Sensitivity:*
     The clinical sensitivity of the test result is dependent on the analytical performance of the Vysis PathVysion® HER-2/*neu* DNA Probe kit. The pathologist must follow the recommendations of the Vysis PathVysion® HER-2/*neu* DNA Probe kit.
     b. *Clinical specificity:*
     The clinical specificity of the test result is dependent on the analytical performance of the Vysis PathVysion® HER-2/*neu* DNA Probe kit. The pathologist must follow the recommendations of the Vysis PathVysion® HER-2/*neu* DNA Probe kit.
     c. *Other clinical supportive data (when a. and b. are not applicable):*
     Not applicable.
4. Clinical cut-off:
     The clinical cut-off of the test result is dependent on the analytical performance of the Vysis PathVysion® HER-2/*neu* DNA Probe kit. The pathologist must follow the recommendations of the Vysis PathVysion® HER-2/*neu* DNA Probe kit.
5. Expected values/Reference range:
     Not Applicable.

N. Instrument Name:
Applied Imaging Ariol™ system

O. System Descriptions:
1. Modes of Operation:
     Semi-automated computer assisted interpretation.
2. Software:
     FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:
     Yes ___√_____ or No _____
3. Specimen Identification:
     Slides are barcoded before they are loaded into the instrument.
4. Specimen Sampling and Handling:
     The Ariol system can be configured with three different kind of stages. One stage is used by the slide loader for automatic loading of the slide and the other two stages are for manual slide loading. SL50 stage is a single bay stage designed specifically for use with the slide loader. MB8 stage has 8 manual bays and MB4 stage has 4 manual bays. There

are two ways to load a slide manually on the SL50 system, one uses the slide loader and the other is a manual placement of a slide on the stage. A barcode reader is integrated into the slide loader. With the SL-50, clicking on the bar code button initializes the slide loader to read the barcodes on the active tray and produces a list of slides in the loader. The assay column shows the assay type and which pass is the next to be scanned and assigned priority for each slide. Barcode labels are not a requirement for systems with a multibay stage. A unique slide name can be used but it can be used for only one slide and should be assigned to the slide in the Entry application. Before a slide can be scanned it must first be entered in the Entry application. Each slide node must have an Assay field and Slide Type. Scan looks for the assay name to set up scanning parameters. Bar codes are read by the slide loader.

The PathVysion® assay is performed by the Ariol™ system either by using a Two Pass scanning method or by Region Cam method. In the Two Pass method, the tissue is scanned at a magnification of 5x to capture images of nuclei counterstained with DAP1. Based on these images, the user selects regions of interest for a second pass. In the second pass, a magnification of 40x is used to capture images of the DAP1 counterstained nuclei, as well as the Spectrum Orange and Spectrum Green fluorescent signals from the DNA probes. Region Cam method is a manual scan method where the user selects frames for image acquisition using the sample slide based on the last scan pass configured in the assay. Selection is done using a 40x objective. After the selected region has been scanned at 40x, the user selects the cells of interest for the system to count the Spectrum Orange and Spectrum Green signals. The total number of each probe as well as their ratio is presented to the user in the data grid, along with an image of the cell. Once an adequate number of cells have been chosen and scored, the pathologist then enters a score for the slide. This may or may not be based on the score computed by the Ariol system, at the discretion of the pathologist. The final score so entered is then printed out on a final report.

5. Assay Types:
   Computer-assisted image analysis of fluorescence in situ hybridization signals in interphase nuclei of cell in formalin-fixed, paraffin-embedded human breast cancer tissue specimens.

6. Reaction Types:
   Fluorescent microscopy

7. Calibration:
   The calibration module of the Ariol™ system provides user interface and functionality necessary for calibrating inter-dependent system hardware. System calibration includes the spatial calibration of stage steps per pixel and microns per pixel and calibration of the microscope lamp and capture card settings for each filter. One is at the factory level and the other one is performed automatically every day before use.

6. Quality Control:
   The accuracy of the system depends on the laboratory following the quality control instructions recommended in the labeling of the fluorescence in situ hybridization (FISH) assay kit associated with the Ariol™.

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

None

Q. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

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