<table>
<thead>
<tr>
<th>Terminology Table</th>
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<tbody>
<tr>
<td><strong>Image Analysis</strong></td>
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<tr>
<td>The extraction of meaningful information from digital images by means of digital image processing techniques.</td>
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<td><strong>Convolution</strong></td>
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<td>Image processing technique in which each pixel is altered by some function of the surrounding pixels.</td>
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<tr>
<td><strong>Deconvolution</strong></td>
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<td>Analyses that removes the effects of convolution from measured data.</td>
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<tr>
<td><strong>Colocalization</strong></td>
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<td>Analysis that determines to the contribution of each stain at every pixel location.</td>
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<td><strong>Intensity</strong></td>
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<td>The strength of the light emitted or passing through something. In image analysis an intensity value of 0 = black and 255 = pure white.</td>
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<td><strong>Color Vector</strong></td>
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<td>The Red, Green, Blue (RGB) component value that represents a specific color.</td>
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<td><strong>Immunohistochemistry (IHC)</strong></td>
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<td>A method for staining cells; antibodies to specific proteins are used to analyze specimens and identify specific types of cells, especially for certain types of cancer</td>
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<td><strong>Angiogenesis</strong></td>
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<td>The formation and differentiation of blood vessels.</td>
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Image Analysis Illustrated

Image Analysis extracts meaningful data:

- **Stains are used to highlight features.**
  - Tissue and cell components are revealed.
    - Nuclei, membranes and cytoplasm.
  - Specific protein expressions such as, ImmunoHistoChemistry panels.
    - HER2, ER and PR.
- **Image analysis can help answer questions.**
  - Where and how much staining is there?
  - Where and how many objects are there?
    - Tumor cells.
  - How much staining is there on different objects?
    - Cell nuclei.
    - Cell membranes.

Image Analysis tools can present a markup image to highlight analysis results.
How Image Analysis works:

- Analyzing a digital slide is done by applying an algorithm directly to the digital slide or selected region of the digital slide.
  - Stained slides result in a multicolored image. Image analysis acquires the RGB color of the pixels.

- Image Analysis tools organize the color (RGB pixels) of stains.
  - Classifies pixels by color (stain).
  - Measures pixel (stain) intensities.
  - Identifies objects by color, shape or size.
  - Measures object (stain) intensities.

Intensity value of 0 = black & 255 = white

Rare Event

Nucleus  Membrane
Image Analysis Tools

Image Analysis tools and their specific applications:

- **Positive Pixel Count** – quantifies the area and intensities of positive and negative staining.
- **Color Deconvolution** – accurately separates up to 3 stains and measures intensity.
- **Colocalization** – quantifies the area and intensity of “colocalized” markers.
- **Nuclear** – optimized for IHC ER & PR stained breast tissue.
- **Membrane** – optimized for IHC HER2 stained breast tissue.
- **Microvessel Analysis** – detects and quantifies microvessels.
- **Rare Event Detection** – detects micrometastasis of tumor cells.
- **Digital IHC Analysis** – streamlined workflow for IHC analysis.
- **Genie** – smart tissue classifier.

*Positive Pixel Count is licensed without fee with other Aperio Software.
**Color Deconvolution is our professional version of our Positive Pixel Count algorithm.
Aperio Image Analysis Capabilities

Cell Quantification
- Nuclear Membrane

Area Quantification
- Positive Pixel Count
- Color Deconvolution
- Colocalization

Event Detection & Quantification
- Microvessel Analysis
- Rare Event Detection

FDA Cleared: *Her2 510(k) & **ER/PR 510(k)

Feature Classification
Genie – Tissue Classifier
Image Analysis Applications

Blood Vessels

Stain Separation

3 colors

Nuclear Staining Quantification

Membrane Staining Quantification
Image Analysis Applications

Fatty Vacuoles

H&E Nuclear Counting

Cytoplasmic Quantification

Rare Event Detection
Performing Analysis

Run Analysis several ways.

- **ImageScope Analysis**
  - Use the Analysis command of ImageScope to analyze either a local digital slide image (that is, an image on your workstation or on the network where your workstation can see it via Microsoft file sharing).
  - Use ImageScope to connect an image on an Aperio ImageServer1.

- **Spectrum Plus Analysis**
  - Analyze a single digital slide image that resides on the ImageServer by using Spectrum’s **Analyze** command.

- **Batch Analysis**
  - Analyze a batch of digital slide images that reside on the ImageServer by selecting multiple images and using Spectrum’s **Analyze** command.

In most cases, it is expected that a digital slide will be opened in Spectrum. For the purpose of training all images will be opened in Spectrum.
Performing Analysis

Selecting Areas to Analyze

- Analyze entire digital slide or selected areas.
- Use ImageScope drawing tools to select or exclude areas to analyze.

- Pen - draw free-form area of interest.
- Negative Pen – draw free-form area to exclude from analysis.
- Rectangle – draw a rectangular area of interest.

View annotation information in ImageScope’s Annotations window
Performing Analysis

Analysis Window

• When performing analysis via Spectrum the Algorithm Server Job displays registered macros.

Analysis Buttons
Test – modify existing macro for selected algorithm and test before saving.
Create – creates new macro based on selected algorithm.
Analyze – run analysis.
Cancel – cancel current analysis job.