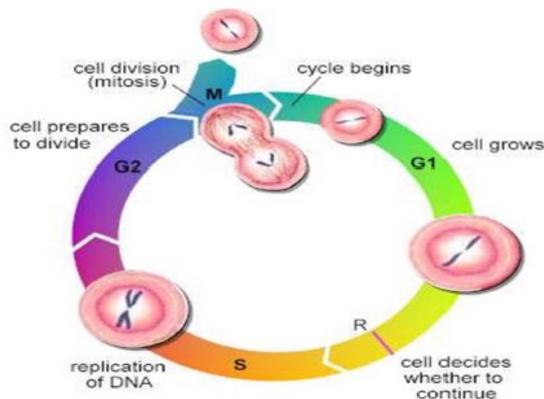


Ploidics™ Analysis System

FULLY AUTOMATIC DNA PLOIDY ANALYSIS
WITH NO USER INTERACTION
AND SUPPORT OF VIRTUAL SLIDES

Ploidy analysis is a test performed on cells using either an optical microscope hooked to a computer or a flow cytometer. It is based on the fact that tumors with a diploid pattern of DNA amounts tend to be benign. On the other hand, tumors with highly variable DNA patterns tend to be more malignant. It analyzes a cell cycle as the cell duplicates its DNA content. When a cell is ready to divide, its DNA content becomes diploid (2C) or tetraploid (4C). DNA ploidy measurements as computed in Ploidics™ can easily indicate cells which have elevated amounts of DNA and therefore engaged in proliferation.



Ploidics™ is a stand-alone, software product that performs image processing and image analysis on digital images captured with an optical microscope and stored on a computer. It also processes virtual slide images of any size and depth. Ploidics is an automatic tool to measure abnormal DNA content in individual cells in a mixed population of cells. It includes a fully automatic, powerful, and innovative segmentation process, an editing tool to manually annotate the segmentation result if desired, and an automatic computation of a histogram showing the DNA mass distribution of the cell population.

Ploidics™ Processing Architecture

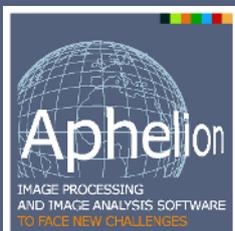
The Ploidics process begins with analyzing a set of images captured from a control slide that contains cells representative of normal cells. Images of these cells must be captured using an imaging system having characteristics identical to the imaging system used to capture the images of the patient slide (i.e., same setup of camera, microscope, magnification, and staining).

The results of this analysis serve as a reference for analyzing patient slides. Next, patient slides are automatically processed by Ploidics to generate a histogram of DNA Ploidy for the patient slide set, with the associated measurements saved on disk. Analyzing the number of peaks and their sizes and locations in the histogram characterizes the proliferation process for the subject slide set.

The basic element in the ploidy analysis is the optical density (OD) of a pixel. This is computed as the logarithm of the grey-level value of a pixel falling inside a selected cell. All of the OD values for pixels within a selected cell are summed to obtain the *integrated optical density* (IOD) for that cell. Finally, the histogram for all selected cells is computed based on the number of cells falling within user-specified IOD ranges (i.e., classes). By enabling the user to specify the range of the histogram classes the user can fine tune the histogram to show the number of significant peaks (diploid, tetraploid, etc.). The IOD of a patient slide is then compared to the IOD computed for the control slide.

Examples of cells used for a control slide include:

- Rat hepatocytes for epithelial cells
- Normal lymphocytes for lympho-proliferative disorders



Main benefits of Ploidics™ system:

- Fully automatic segmentation including nuclei separation
- Support of all localizations (i.e., breast, colon, prostate)
- Support of multiple stains
- User control of all analysis parameters
- Support of virtual slides and large images
- Based on the Aphelion Image Processing Software product

User Interaction

Compared to other ploidy analysis products, the Ploidics™ software product has the following capabilities:

- Automatic splitting of touching nuclei;
- User selection of nuclei size used in the analysis;
- User decision to keep or discard heterogeneous nuclei;
- User specification of color band to be processed based on the stain selected;
- User modification of segmentation results to select/deselect cells used in the ploidy analysis.

User Interface and Analysis Output

Figure 1 displays the segmentation result for one image of a patient image series being analyzed. An image gallery is provided composed of image thumbnails for all images in the series.

A typical ploidy histogram is displayed in Figure 2. It is a graph of DNA mass in picograms normalized by the reference IOD (X axis) and the numbers of cells having a given DNA mass (Y axis). The final analysis is based on the determination of the number of peaks in the histogram and the DNA amount of those peaks. Normal cells are represented by a histogram with a peak in 2C, one in 4C if cells are splitting, etc. Abnormal cells have an impact on the shape of the histogram, and the peak position.

All cell measurements are saved in Aphelion ObjectSet files. Data derived from the ploidy histogram are saved in an xml file, including the following measurements:

- Number of peaks
- For each peak: mass of the peak, DNA index, area, and number of cells
- Statistical analysis for each class (i.e. mean, Standard deviation, CV, percentage and number of cells) Total cell count
- DNA mass base

Ploidics™ software enables the user to effectively manage the control slide and patient slide data by saving this data and the associated ploidy analysis results in a patient-specific folder. Control slide and patient data histograms are saved on disk as Tiff or pdf files.

System Requirements

Ploidics™ is a stand-alone, software product that can be installed on any personal computer running Windows™ XP or higher. Images need to be accessible from a hard drive or from network storage, and organized in folders to be processed by the software. All Ploidics™ measurements are calibrated and output in real-world units. The calibration value is manually entered in the Ploidics Software as a single value.

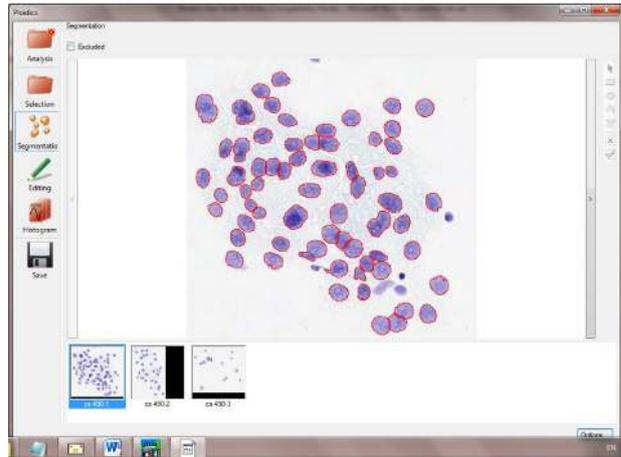


Figure 1 – Segmentation results for one image of an image sequence, with Image Gallery shown.

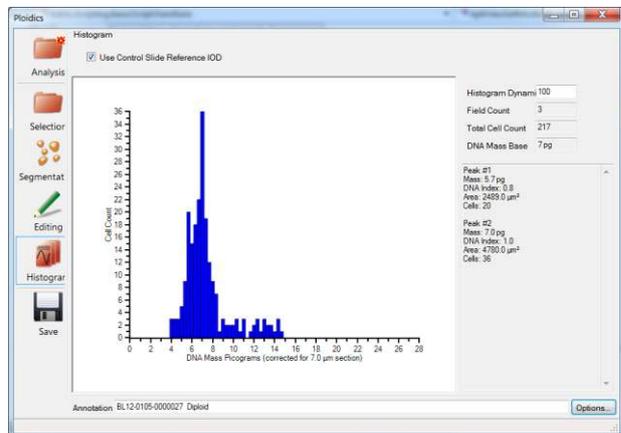


Figure 2 – Typical ploidy histogram