

THE LAI AUTOMATED COMET ASSAY ANALYSIS SYSTEM (LACAAS)

The LAI Automated Comet Assay Analysis System (LACAAS) is a modular system of hardware and software that offers all the features and components needed for efficient collection and rapid automated analysis of large sets of fluorescent single cell comet images, as required for effective and accurate Comet Assay evaluation. The Comet Assay, also known as the Single Cell Gel Electrophoresis Assay, permits quantitative assessment of the effects of DNA damaging or apoptosis inducing agents and mechanisms in a wide variety of important target cell types. The assay also has great utility in studies of DNA repair.

The Comet Assay is based on the electrophoresis and quantitative analysis of populations of target cells, suspended in an agarose gel layered onto a microscope slide (or other appropriate support substrate). Electrophoresis causes the migration of unwound or fragmented DNA out of the nucleus of the cells, resulting in a characteristic, comet like, appearance that can be visualized microscopically, with fluorescent staining of the DNA. Appropriate measurement and quantitative analysis, of fluorescent intensities exhibited by the cells, yields measures of cellular DNA content, distribution, and damage.

The LACAAS reflects LAI's two decades of experience in the design, production, sale and support of advanced image analysis tools. All LACAAS software is designed to operate under the Windows operating system on Pentium Class computers, and provides the LACAAS with the highest degree of analytic precision, automated functionality, and technical sophistication, available anywhere today for comet analysis. With LACAAS, comet images are automatically and efficiently background corrected, analyzed, and data-based, with the highest regard for data accuracy and integrity, immediately as they are collected and assigned to user defined study groups.

Accurate Quantitative Image Collection

The LACAAS offers a unique and proprietary image capture system that cost effectively provides extended dynamic range imaging (EDRI) capabilities, to provide the most stable foundation for accurate quantitative analysis of comet images. LAI's EDRI system overcomes critical limitations, exhibited by conventional image capture systems that can lead to serious errors in the analysis of comet images. Unlike conventional image capture systems, the EDRI system enables detection and digital recording of the full range of fluorescent intensities important to accurate analysis of comet images. The EDRI system is comprised of a thermoelectrically cooled, computer controlled, instrument grade, solid state, digital CCD camera, and LAI's exclusive image capture control and processing software.

To accommodate users who choose to use other means of image collection, the LACAAS software can also import comet images collected by a variety of third party image capture systems that record images in compatible TIFF or BMP formats.

Adaptive Automated Background Correction

Comet images are corrected to remove any superimposed background noise signals, before quantitative analysis for DNA damage proceeds.

Nonspecifically bound fluorescent dye, auto-fluorescence and light scattering are all factors that contribute to unwanted background signals. These factors typically combine to create a non-uniform, spatially varying background signal. Consequently, subtraction of a single average background value from each comet image, as most competitive analysis systems do for background correction, is wholly inappropriate.

The LACAAS uses a more physically realistic and robust approach to background correction. LACAAS automatically samples the background signal, in the cell free areas, above and below the comet being analyzed. It uses this local information to compute a best estimate of the actual shape and magnitude of the background field superimposed on the comet, which it then subtracts to obtain an appropriately corrected comet image. This approach benefits from the facts that it makes direct use of the actual background information contained in the image, is locally adaptive, and doesn't require the system user to make improper generalized assumptions as to the average magnitude and spatial stability of the background signal.

Objective Automated Delineation of Comet Features

Intelligent image processing algorithms, exclusive to the LACAAS, eliminate the need for subjective user directed comet assessments by providing completely objective, fully automated delineation and analysis of head and tail regions within comitized cells. These sophisticated algorithms even perform well with highly damaged cells, where little of the DNA remains in the nucleus or head of the comet. Such cells present serious problems for most competitive comet analysis systems, causing analytic failures requiring manual interventions, which greatly slow the analysis process and throughput rate. The fully automated analysis enabled by the LACAAS greatly speeds the processing of comet images and makes practical the rapid analysis of large numbers of cells needed for a high degree of statistical confidence.

Multiple Quantitative Measure Generation and Databasing

The LACAAS automatically generates multiple quantitative measures of each cell analyzed. These include standardized measures of comet tail length, area, moment, moment arm and moment of inertia, as well as measures of cellular DNA content, percent of total cellular DNA in the tail and a measure of total cellular DNA fragmentation. All measures are automatically data based, along with their corresponding comet images, for subsequent review and statistical analysis.

Data Review, Visualization and Reporting

Special visualization capabilities permit the coordinated review of measured data and comet source images and comparison of multiple study groups, with respect to the distributional properties of selected measure types. The system can generate and output study reports detailing all individual cellular measures and a variety of useful summary group statistics. Data can also be exported to other software packages such as Microsoft Excel for further statistical analysis.

GLP Compliance Support

The LACAAS also incorporates a number of important features designed to facilitate GLP compliance. LACAAS provides program and data security features with password protected multilevel access. The system also provides a data audit feature that tracks the time, date and responsible system user relating to all significant study events, along with any relevant data and user comments or notes. All audit trail information is recorded in a study linked and data based log file that can be printed out for regulatory or supervisory review.

Ease of Operation

The high degree of analytic precision, automated features and technical sophistication offered by the LACAAS does not come at the expense of a high degree of operational complexity. The LACAAS is simple to operate. It has a simple Windows based user interface that has been intelligently designed and streamlined to maximize user productivity and data throughput, without compromise of functionality. This all combines to make LACAAS the best and most intelligent system choice for comet assay analysis.