Komet™ imaging software

dna damage & repair analysis
Komet™ imaging software:

**Key Features**

- One-click capture and analysis of comets ensures rapid scoring.
- Fully automatic or interactive computation of Head/Tail %DNA, Olive Tail moment, etc.
- Database captures and stores cell image and data.
- Database Viewer enables data presentation, decoding and data summaries for statistical analysis.
- “Experiment” mode guides the user through analysis - slide by slide.
- Scoring can be suspended and resumed in multiple sessions.
- Pop-up Live/Open button speeds scoring and minimizes user fatigue.
- Powerful Microsoft Excel™ Macros speeds manipulation of data.

**Introduction**

Komet 5.5 has been designed to capture and analyze samples produced using the Comet Assay. This assay permits the quantification of DNA damage and repair in single cell preparations and is applicable to any eukaryotic cell. The assay can be used in both in-vitro and in-vivo testing and has been shown to be a powerful and sensitive predictor of genetic toxicity. Kinetic Imaging first implemented image analysis for the technique in a commercial imaging system in 1991 and since the company was purchased by Andor Technology in 2004, the product continues to be supported and developed.

Komet is quite simply the most advanced and powerful software solution for analysis, data management and presentation of comet assay samples. Over 400 Komet systems have been sold to the world’s leading academic, clinical and contract research laboratories. Komet 5 introduced the use of Databases which store an image of each cell along with its analysis data. The Komet Database Viewer (DBV) is supplied as a separate module and can run stand alone to allow scoring review, QA and training, decoding of blind-scored studies and data summary creation for submission to statistical analysis. DBV also allows presentation of results in Dose Response curves and other graphical forms.

Komet is now available in 3 configurations:

- **Komet GLP** FD 21 CFR Part 11 compliant version.
- **Komet 5.5** Research version with live image capture from a CCD camera and microscope.
- **Komet 5.5F** Research version with scoring from image files – many formats.

www.andor.com
Scoring Protocols used to organize settings & calibration:

- Scoring Protocols are handled through the Protocol Manager, which guides you through Wizards for Calibration, Camera Settings and Comet Options.
- Comet Processing Options allow you to customize the way Comet images are processed to allow for specimen preparation variations.
- Protocols store all settings to ensure repeatable and reliable analysis.

- Komet can create a Database (password protected) containing an image of every cell scored along with its data. This enhances scoring QA and validation.
- Data Storage path is user-defined (“Automatic Data Directories”) - can be remote/network storage.
- All Protocol settings are stored in the Database for QC purposes.
- Scoring can begin once a Protocol is defined and Experiment mode is entered.

Figure 1a and 1b. Komet Protocols contain all settings for scoring a study, including calibration, camera and analysis settings. Data paths and output options such as use of databases are also stored here.
toolbar provides instant access to key functions:

Rapid scoring, multi-session capability & automatic data organization

- Once a scoring session is in progress, speed is of the essence. The user acquires and scores cells quickly with a single right mouse click.
- Komet toolbar allows rapid and convenient program control.
- Scoring sessions can be suspended and resumed at a later time with the session manager.
- Background correction for every cell scored and automatic detection of head and tail extent. Instantaneous computation of all parameters.
- Immediate visual feedback of analysis results, on image, in data summary and in Analysis Graphics.
- Head DNA, Tail DNA, Tail Length, Olive and Extent Tail Moments and T/L (Tail Length to Head Diameter Ratio) are presented in the Key Results panel (figure 3).
- 24 parameters are computed from the comet image based on intensity and migration patterns.
- Comet integrated intensity, moment, skew and inertia are included.
- User can over-ride to interactive scoring on-demand - especially useful for “hedgehog” or heavily damaged comets.
- Cell saturation check prevents unsuitable analysis and warns you to adjust the camera or illumination.

Figure 2. The Komet Toolbar provides quick access to the most common functions. In addition Live and Open buttons are presented on the Image Window for convenient and rapid comet scoring.

Figure 3. ‘Key Results’ are tabulated below the Image Window as shown above. In the Comet Options setup phase they colored Red to make them stand out. In Experiment mode they are colored black. These examples show typical key parameter values for a heavily damaged comet and slightly damaged comet respectively. Heterogeneous comet morphology is common within a single sample, so statistical methods are applied to data from approximately 100 cells (randomly selected) per specimen. As with any scientific experiment, good experimental design plays an important part in the reliability of conclusions.
Live Image Scoring

- One right mouse click initiates capture and analysis.
- Automatic Pop-up Live button (figure 4) appears after each comet analysis, minimizing mouse movements and operator fatigue.
- As each cell is scored measurement calipers provide visual feedback on accuracy of analysis.
- For each comet analysis results and intensity profile data are instantly shown in the Analysis panel (figure 5).
- Software control of wide range of cameras from cooled CCD to video and Firewire (IEEE1394).
- Scoring is fast - 300-400 comets per hour.

Scoring from file

- Standard and proprietary 8, 12, 16 bit gray and 24 bit color files are handled seamlessly.
- Komet 5.5F automatically loads files from a user-defined directory on demand.
- “Flip or Rotate on Open” function allows images (with comet tails to left) to be automatically oriented for analysis.
- Apply Calibration on Open” calibrates images for real measurements.
- Automatic Pop-up Open button minimizes mouse movements and reduces operator fatigue.
- Analysis and presentation of results proceeds as in Live scoring.

Presentation of Results during Scoring

Figure 4. Scoring – A live image appears in the image Window (shown pseudo-colored here). A right mouse click initiates capture and analysis for DNA migration. Intact DNA remains in the comet ‘Head’, damaged DNA migrates to the ‘Tail’. Vertical calipers (in red) show the extent of the Head and Tail. Courtesy Marie Vasquez Helix3 Inc.

Figure 5. The Analysis window shows comet intensity profiles and 24 comet parameters are recorded in a data grid. The integrated intensity profiles of comet, background, head and tail and can be useful for identifying background debris and comet overlap.
data output formats & content:

Images, Intensity Profiles and Data Grid

- As comet scoring continues, the results are written to the Analysis panel (figure 5).
- Print images and intensity profiles direct from Komet.
- Copy images to Windows Clipboard for use in other programs or reports.
- Save images to TIFF, BMP and JPEG formats.
- Apply Histogram Mapping and color palettes for visual presentation of Comet Images (figure 6).

Excel compatible files and Macros

- As each slide or specimen is completed the data is written out to an ASCII data file (to a user-defined or system managed folder) for import into any preferred analysis program.
- Komet’s unique Excel macros to simplify and streamline data analysis.
- Komet’s Excel macros allow plotting of single dose Histograms, multi-dose Histograms (3D) and Dose response curves with error bars (Excel 2000).

Figure 6. Examples of comet images showing similar levels of damage, but using different visualization. The top image was acquired with a digital color CCD camera from a specimen labelled with SYBR-Green, showing how the cell would look in the microscope eyepiece. The bottom image has been pseudo-colored with a color scale in which blues represent low intensities ranging to green and red for higher values.

Figure 7. The Excel Macros developed by Andor prior to the development of Komet Database Viewer, which are still supplied with the Komet software.
Komet Databases and Database Viewer

- If the user selects to save the data to a Komet Database, the data and image of every comet scored is transmitted to a Database.
- Data output includes details of scoring session – user, time, date, protocol name and all settings.
- Database Viewer provides Decoding for blind-scored studies, essential for GLP purposes.
- Create and print a Gallery of comets for each specimen or treatment.
- Data can be organized by treatment group and statistical summaries produced for significance and dose response analysis.
- Locked Database provides security and validation of data.
- Create single specimen Histograms, multi-specimen Histograms (3D) and Response curves with error bars.
- Database Viewer can be distributed free of charge with the data for review at other sites.
- Integration and comparison of data from multiple databases.

Figure 8. Komet Database Viewer loads the scored datasets and displays the analysis data and image of each comet scored. This is the basic View/Review tool and you can scroll down the dataset and view the cells in a kind of “movie” by holding down the keyboard scroll down key. This dataset is from a GLP study and shows audit trail, decoding records and summary data performed by the study director. Database Viewer lets you create a Gallery of comet images from a Database. This Gallery can be printed or used for review and training.

Figure 9a. 3D Histogram presents study data where a Dose Response was detected. The progression from Negative control (in grey) through the dose groups to the Positive control (orange) is apparent in the data distributions for Tail DNA. Figure 9b. The data can also be presented in Response Curve format and Summary data table.
Komet 5.5 is available to suit your requirements

Komet 5.5 F – provides you with cost-effective tools to analyze your comet images from image files, to create databases and perform data presentation and manipulation prior to statistical testing. It is supplied complete with Excel macros and Komet Database Viewer. Database Viewer can be distributed free of charge with your data to colleagues and third parties.

Komet 5.5 – the busy researchers’ solution enables capture from a wide range of CCD cameras coupled directly to the fluorescence microscope. Live imaging delivers the fastest route from specimen slides to high quality data. Komet Database Viewer provides data organization, summarization and presentation. Summary tables can be imported directly into your statistics program.

Komet 5.5 GLP – The 21 CFR part 11 compliant version of Komet delivers all the same ease of use and power in a secure and traceable framework. It provides user management (admin, study director and scorer), audit trails, scoring security, database password protection. This product has been validated in a number of contract research labs in the US and Europe.

Komet Systems - Komet 5.5 preloaded on a powerful PC, with image capture interface and Camera such as the Andor Luca™.

Microscopes - We can supply a suitably configured fluorescence microscope for a complete single source imaging solution. Scopes from your preferred supplier can be accommodated.

Andor overview:

Andor Technology strives to bring innovative solutions to the demanding needs of researchers and manufacturers. We are a reliable source for the researcher in the lab as well as a trusted supplier to more than half of the top twenty analytical instrumentation companies in the world. We understand their requirements and share an in-depth knowledge of their applications. Whether you require a single or multiple components, off-the-shelf or a custom-designed solution, Andor is the logical choice, enabling greater efficiencies, lower costs and better results.

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