equiComet User Manual

version 1.0b
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1. Introduction

equiComet is a Macintosh program for making comet assay measurements using images created by most laboratory and consumer cameras. *equiComet* uses algorithms developed specifically to obtain accurate, objective results on large numbers of comet images. It overcomes measurement problems specific to comet analysis (thresholding, background subtraction, image rotation, etc.), while achieving high throughput via a simple user interface.

equiComet is as a macro program running under the application *Equipoise-Image*, a descendant of *NIH Image* and *Object-Image. equiComet* executes on all versions of Mac OS X in Classic mode, on Mac OS 8.6, and on all versions of Mac OS 9. All of the image manipulation tools available in *Equipoise-Image*, *NIH Image,* and *Object-Image*, are available to the *equiComet* macros user.

The unregistered version of *equiComet* implements a full set of quantitative measurement capabilities and a limited set of data export capabilities. It may be freely used and distributed.

Registered users have added data reporting capabilities, chiefly the export of measurements, results, and statistics from the analysis of multiple comets in spreadsheet format. Registration is accomplished by purchasing a license from Equipoise Imaging, LLC. License prices are:

individual user:	$100.00
single lab site license (2 - 10 users):	$250.00
unlimited departmental/divisional license:	$500.00
unlimited institutional license:	$1000.00

Registered users will receive further documentation and examples, and are entitled to minor upgrades. Visit the registration page at:

`http://www.eqpi.net/equiComet/registration.html`

for instructions on registering your copy and receiving a full license.

2. Installation

Installation entails the following steps:

1. Obtain the archive containing *equiComet* and *Equipoise-Image* from:

        http://www.eqpi.net/eqpi/equiCometDL.html

2. Expand this archive onto your hard drive. This will create a "Equipoise-Image *f*" folder, containing the following files and folders:

    Equipoise-Image 1.0 (application)
    Macros folder (example macros based on the core features from NIH Image)
    Plug-ins folder (containing a single example plugin)
    Kernels folder (containing filters)
    equiPoise macros folder (folder containing equiComet 1.0b and equiComet1.0b-Loader.obj)
    equiCometUserManual.pdf (this manual in Adobe Acrobat format)

3. Using *equiComet*: a brief tutorial

The following tutorial exercise will help familiarize you with *equiComet*.

1. Double-click the "equiComet1.0b-Loader.obj" file icon. This will launch Equipoise-Image and a dialog will open requesting you to navigate to and open the file "equiComet 1.0b". This two-step process is necessary because "equiComet1.0b-Loader.obj" initializes many *equiComet* settings before it opens and runs the actual "*equiComet* 1.0b" program. This step will also open a "LUT" window for manipulating the grayscale or color map that controls the on-screen appearance of pixels (the tall, thin window on the left side of the screen), a "Tools" palette containing a variety of image manipulation tools (to the right of the LUT window), an "Info" window (near the bottom left of the screen), and a "Map" window for controlling the brightness and contrast of images.

2. Use the "File>Open" menu item to navigate to and open a TIFF image containing comets that are to be analyzed. Many other image formats may be accessed using the "File>Import" menu item - see the documentation for NIH Image at the URL in section 4 below for details.

3. Make sure that the pixel values in this image are such that low values correspond to the image background while high values correspond to pixels in the comets. If this is not the case, the image data may be inverted by using the "Edit>Invert" menu item. The color or grayscale

scheme (know as a "lookup table", or LUT) that controls how an image is displayed may be inverted using the "Options>LUT Options..." menu item.

4. Measurement of individual comets may now begin. Use the mouse and mouse button to select a comet in the image, by pressing and holding the mouse button at a position above and to the left of the head of the comet to be measured and then dragging down and to the right of the comet before releasing the mouse button. This will select a rectangular "region-of-interest" containing the comet. Be sure that the entire comet is contained in this region-of-interest, and that it does not contain parts of nearby comets.

5. Press the [F5] function key. This will initiate the analysis, after which a new image window is created just below the comet region-of-interest. Note that this image contains a copy of the comet that has been rotated automatically to the horizontal. This ensures that comet measurements are along the comet main axis even when comets are not oriented horizontally in the main image. This window also contains a plot showing the vertically-integrated intensity of the comet as a function of left-to-right position. This calculation uses a locally adaptive background subtraction that ensures that the background signal does not influence the measurement of the comet. Note that a "dotted" vertical line selection is also created in the single comet window.

6. Using the <left-arrow> or <right-arrow> keys, adjust the position of the vertical line selection so that it lies over the comet head peak, based on the peak of the plotted curve and the appearance of the comet in the image beneath the plot. Once you are satisfied with the peak position, press the "F6" function key. This will initiate the remaining calculations, resulting in a new text window containing data and results, and a new image window containing a plot of the overall comet profile, the head profile, and the tail profile. This window also contains an image of the comet, with colored boxes indicating the extent of the head and tail regions (in red and blue, respectively). Note that the main image now has a numbered, dashed box identifying the measured comet. These markings exist on a separate "display layer" do not alter the data of the image.

7. Repeat steps 5 - 6 for each comet that you would like to measure. Notice that numbered data and profile plot windows are created for each comet that is measured.

8. Select the "Objects>Show Obj. Results" menu item. This will open the "Object Results" datasheet, which contains all of the measured and calculated results. Click on one of the entries in the "RoiA" column and look back at the comet image. Notice that the numeral identifying the comet associated with this row of data is now emphasized in the image, and that the rectangular marker for it is now filled. Do this for other entries in the "RoiA' column. This operation allows the user to browse particular entries in the datasheet and easily identify the associated comet in the image. Now go to the "Tools" palette and select the "finger" tool near the bottom right. Go

back to the main image window and click on one of the rectangular markers on a comet that has been measured. This will select that particular comet (evident by its emphasized number and marker) so that its data may be identified in the Object Results datasheet.

9. At the left of the "Object Results" datasheet, click on the double-headed "Statistics" arrow to select which statistics are calculated and displayed for each numerical measurement column.

10. With one of the comets selected (via either method of step 8), press the [F10] function key. This will remove the selected comet from the list of measured comets, delete all measurements made from it, and dispose (without saving) the individual plot image and data windows associated with it. The remaining comets are then renumbered and their summary statistics recalculated.

11. When you are finished, save the data windows (as TEXT files) or single comet images (as TIFF files) that you wish to retain. In the registered version of *equiComet*, the datasheet containing results from an entire set of measured comets may be exported as a tab-delimited text file for use by a spreadsheet, database, or word processing application.

12. To perform additional analyses using a different gel image, close the completed gel image, save or dispose of all open data and plot windows, and use the "Special>Reset" menu item to reset *equiComet* to its initial state. Open a new gel image, and repeat steps 3 - 11.

4. Resources and related information

NIH Image homepage: `http://rsb.info.nih.gov/nih-image/`
          - the home of the whole *Image* family, laden with information and related software

Object-Image homepage: `http://simon.bio.uva.nl/object-image.html`
          - especially useful for familiarization with *Object-Image*

Equipoise-Imaging homepage: `http://www.eqpi.net/eqpi/`
          - examples, macros, and related software

Please contact us at:

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with questions, comments, or suggestions. Your feedback is vital to our continuing work to improve *equiComet's* usability and performance and to add features requested by users.