CometScore Tutorial

http://AutoComet.com

This tutorial will demonstrate how to acquire comet metrics from image data with CometScore. CometScore requires a Windows Bitmap image where all comets are oriented as head on the left and tail on the right.
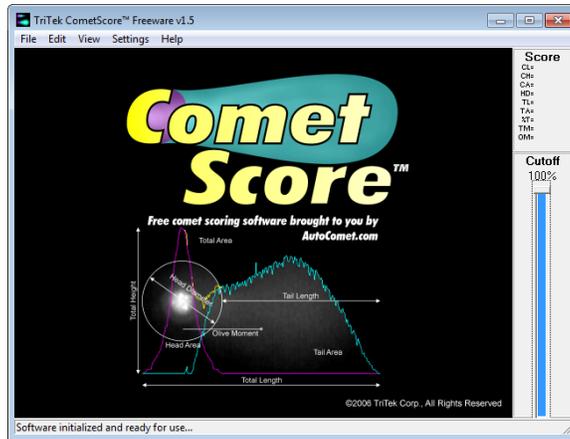

1. To begin, double-click on the CometScore.exe program icon to launch the CometScore analysis software.

2. Click File - Open to load a bitmap into the software.
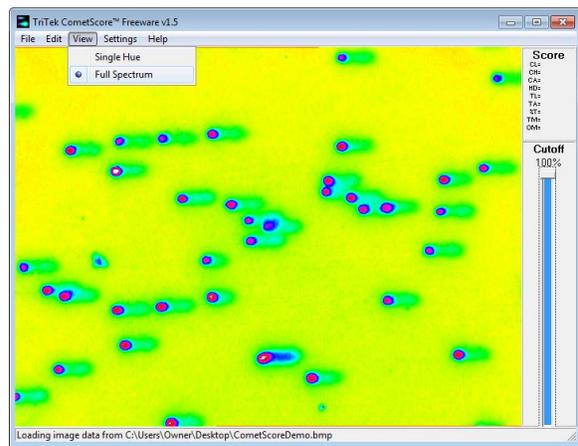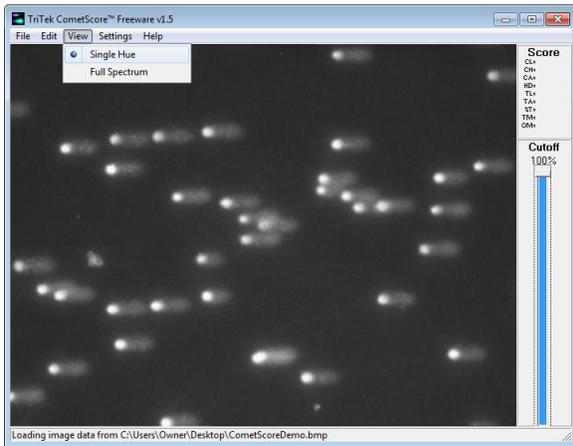
3. Use the cutoff bar to eliminate the background from the field of view. This will isolate the comets from background noise during analysis and produce more reliable metrics. (In "Full Spectrum" viewing mode, all black pixels will not factor into any metrics.)
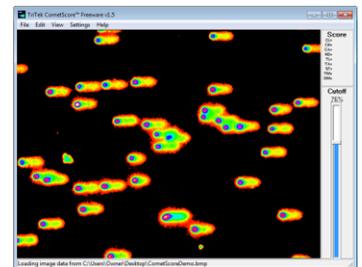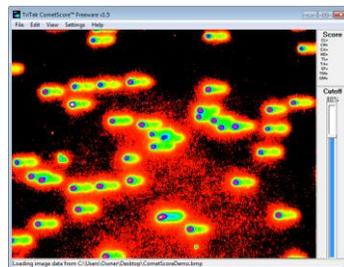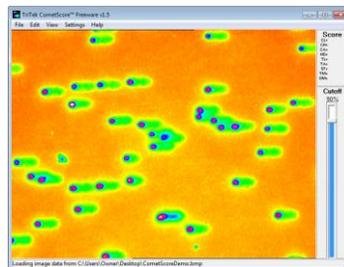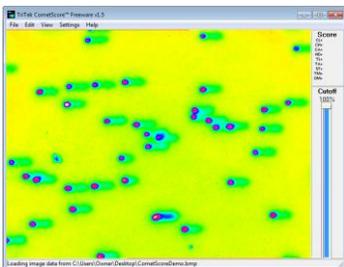
4. Click the left mouse button over the field of view and drag a rectangle around a single comet. Make sure that the rectangle does not overlap other comets in the field.
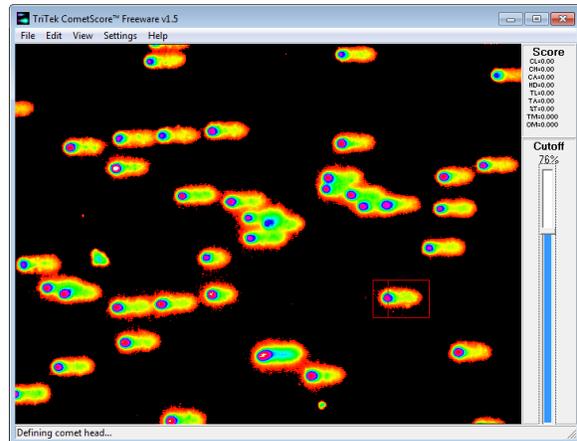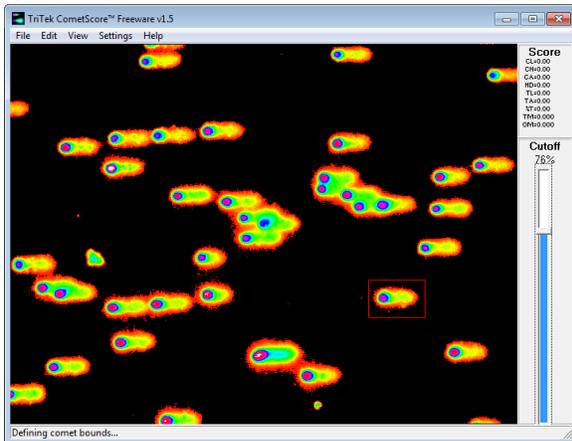
5. Move the mouse over the head of the currently selected comet and left click on the center of the head.

   At this point, the program will prompt you for an output filename. This occurs only one time during the program's lifecycle. To begin a new data file, you must close and reopen CometScore.

6. Repeat steps 4 and 5 until every isolated comet is scored in the field of view.
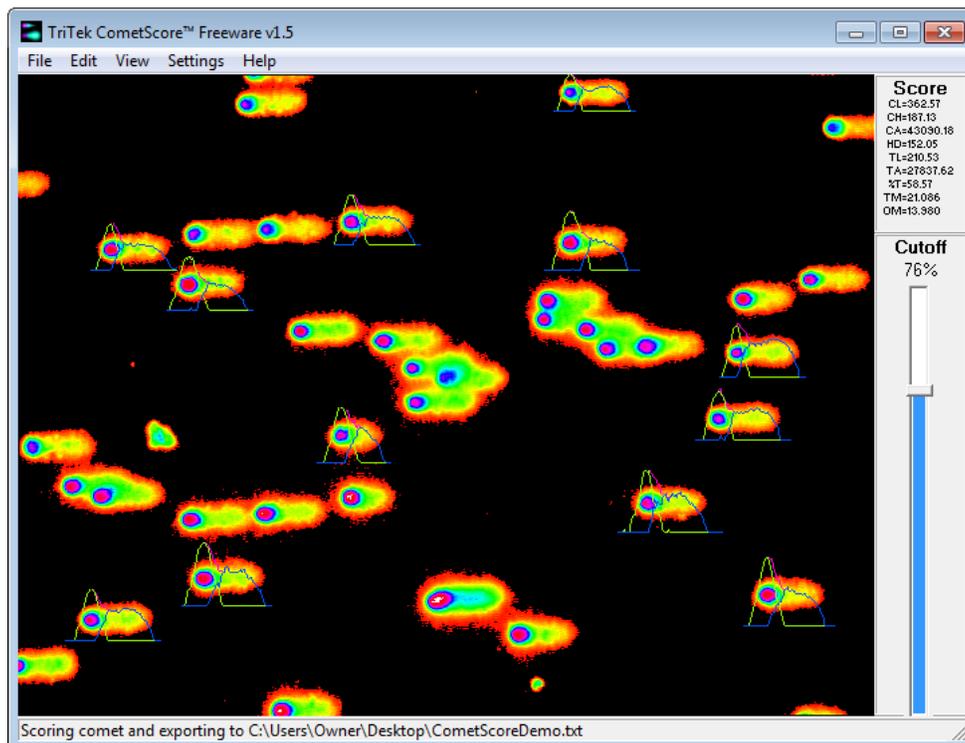
7. Repeat steps 2 and 3 to load another bitmap image into the software. All data collected from this and subsequent images will be appended to the same data file.

8. When all data has been collected, the file may be opened in any text viewer. The data is tab-delimited and is more easily viewed in a third party spreadsheet program, such as Microsoft Excel. (To open in Excel or OpenOffice, rename the file extension to XLS and then double-click on the file.)

For a description of each equation used in the CometScore software, see Appendix: Equations.
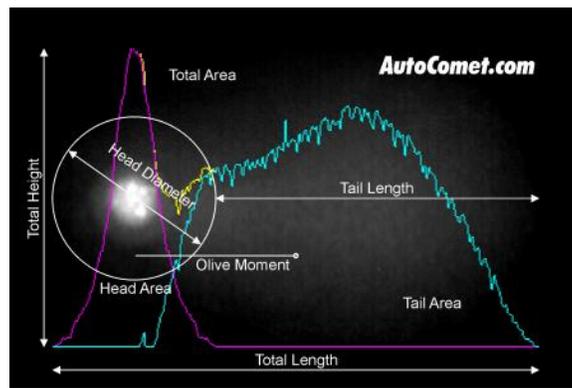
Appendix:
Equations

Scoring equations for the CometScore software are defined below. Algorithms are based on these equations.

Comet
A "comet" is defined as all adjoining pixel locations in a designated shape after segmentation processing where each pixel intensity corresponds to the amount of DNA at that location.

Comet Length
The overall length of a comet is the number of pixels in the horizontal direction of the comet.

$$L_c = N_x$$

Comet Height
The overall height of a comet is the number of pixels in the vertical direction of the comet.

$$H_c = N_y$$

Comet Intensity
The total comet intensity is the sum of all pixel intensity values in the comet.

$$I_c = \sum I_{(x,y)}$$

Comet Area
The total area of a comet is the number of pixels in the comet.

$$A_c = N_{(x,y)}$$

Mean Comet Intensity
The mean comet intensity is the average of all pixel intensity values in the comet.
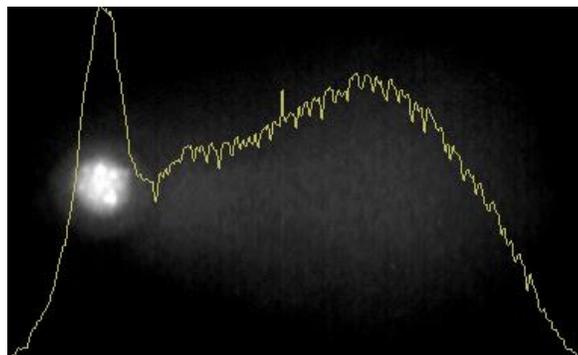
$$\mu_I = \frac{I_c}{A_c}$$

Intensity Integral
The comet intensity integral is the vertical summation of all pixel intensities in the comet, column by column. A graphic representation is superimposed on the digital image.

$$P_c = \int I_y d_y$$

Head Intensity Integral
The comet head intensity integral is the vertical summation of all pixels intensities in the comet head, calculated by mirroring the comet intensity integral about a designated center point. A graphic representation is superimposed on the digital image.

$$P_h = \int I_{h_y} d_y$$
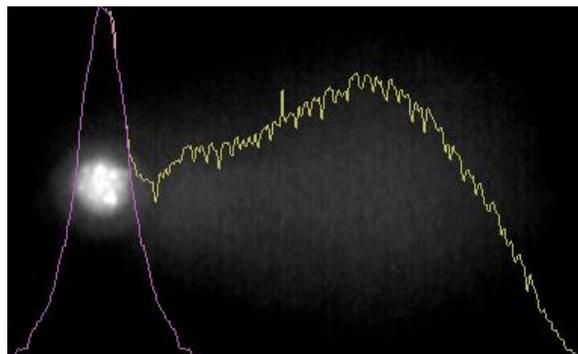
Head Diameter
The diameter of the head is the number of pixels in horizontal direction of the comet head.

$$L_h = N_{h_x}$$

Head Area
The total area of the comet head is the number of pixels in comet head.

$$A_h = N_{h_{(x,y)}}$$

Total Head Intensity
The total comet head intensity is the sum of all pixel intensity values in comet head.

$$I_h = \sum I_{h_{(x,y)}}$$

Mean Head Intensity
The mean comet head intensity is the average of all pixel intensity values in the comet head.

$$\mu_I = \frac{I_h}{A_h}$$

%DNA in Head
The percentage of DNA in the comet head is computed as the total comet head intensity divided by the total comet intensity, multiplied by 100.

$$\%DNA_h = 100 \times \frac{I_h}{I_c}$$

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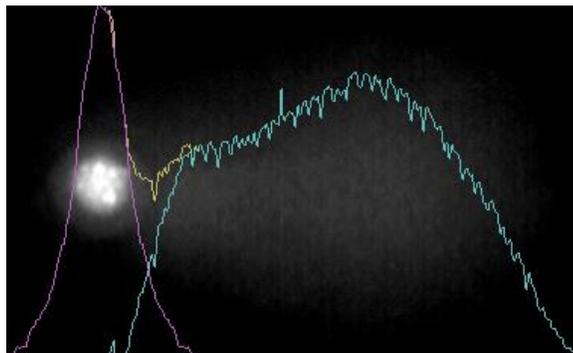## Tail Intensity Integral
The comet tail intensity integral is the vertical summation of all pixels intensities in the comet tail, calculated by subtracting the comet head intensity integral from the comet intensity integral. A graphic representation is superimposed on the digital image.

$$P_t = \int I_{t_y} d_y$$

Tail Length
The length of the comet tail is computed as the comet head diameter subtracted from the overall comet length.

$$L_t = L_c - L_h$$

Tail Area
The total area of the comet tail is the number of pixels in the comet tail.

$$A_t = N_{t_{(x,y)}}$$

Total Tail Intensity
The total comet tail intensity is the sum of all pixel intensity values in comet tail.

$$I_t = \sum I_{t_{(x,y)}}$$

Mean Tail Intensity
The mean comet tail intensity is the average of all pixel intensity values in the comet tail.

$$\mu_I = \frac{I_t}{A_t}$$

%DNA in Tail
The percentage of DNA in the comet tail is computed as the total comet tail intensity divided by the total comet intensity, multiplied by 100.

$$\%DNA_t = 100 \times \frac{I_t}{I_c}$$

Tail Moment
The tail moment is computed as the %DNA in the comet tail multiplied by the tail length.

$$M_t = \%DNA_t \times L_t$$

Olive Moment
The "Olive" tail moment is computed as the summation of each tail intensity integral value, multiplied by its relative distance from the center of the head (the point at which the head integral was mirrored), and divided by the total comet intensity.