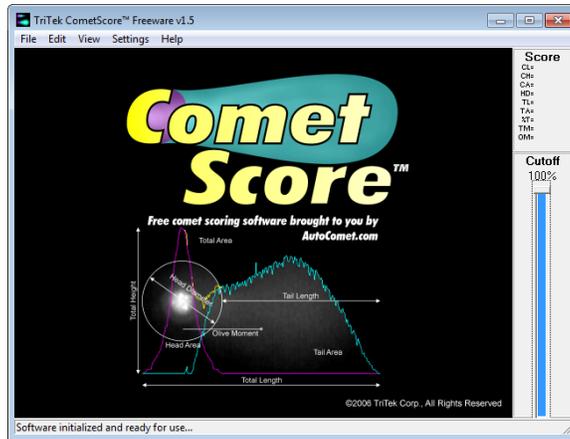


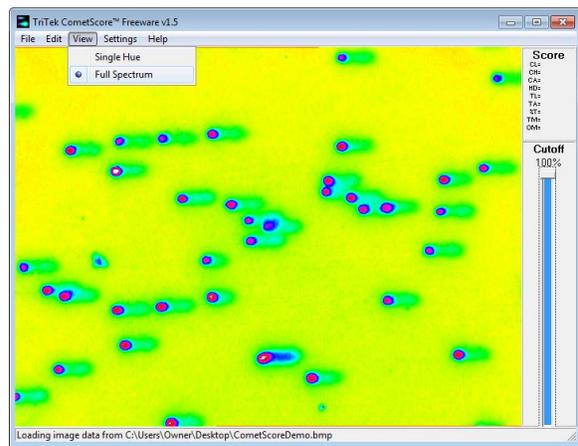
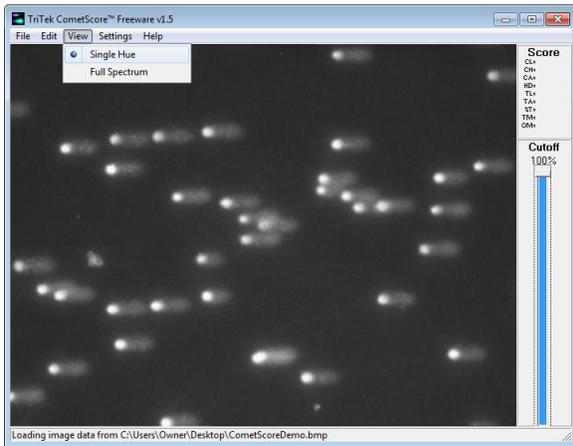
CometScore Tutorial

<http://AutoComet.com>

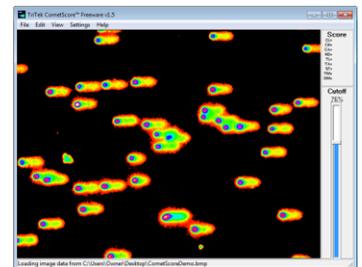
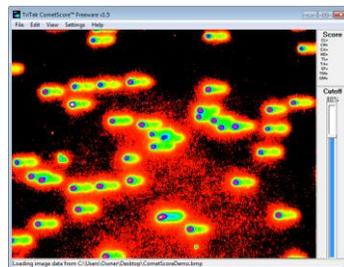
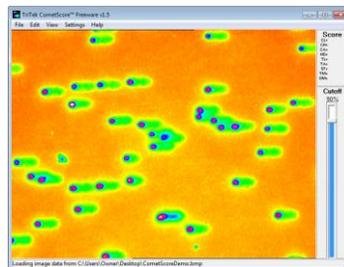
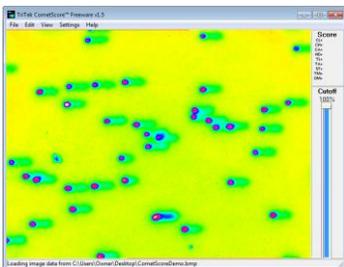
This tutorial will demonstrate how to acquire comet metrics from image data with CometScore. CometScore requires a Windows Bitmap image where all comets are oriented as head on the left and tail on the right.



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.



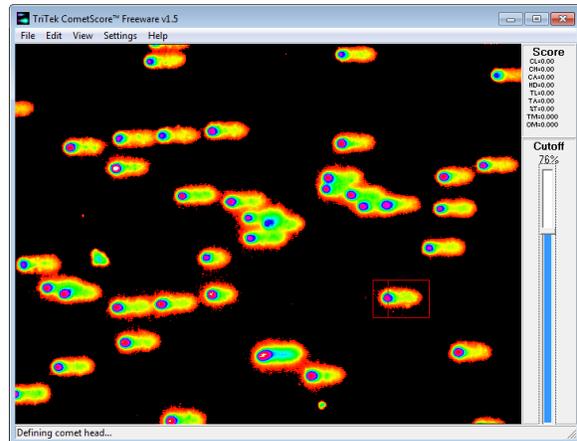
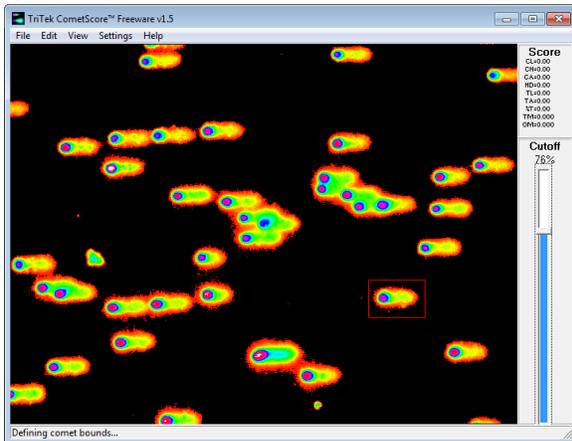
3. Use the cutoff bar to eliminate the background from the field of view. This will isolate the comets from background noise during analysis and produce more reliable metrics. (In "Full Spectrum" viewing mode, all black pixels will not factor into any metrics.)



CometScore Tutorial

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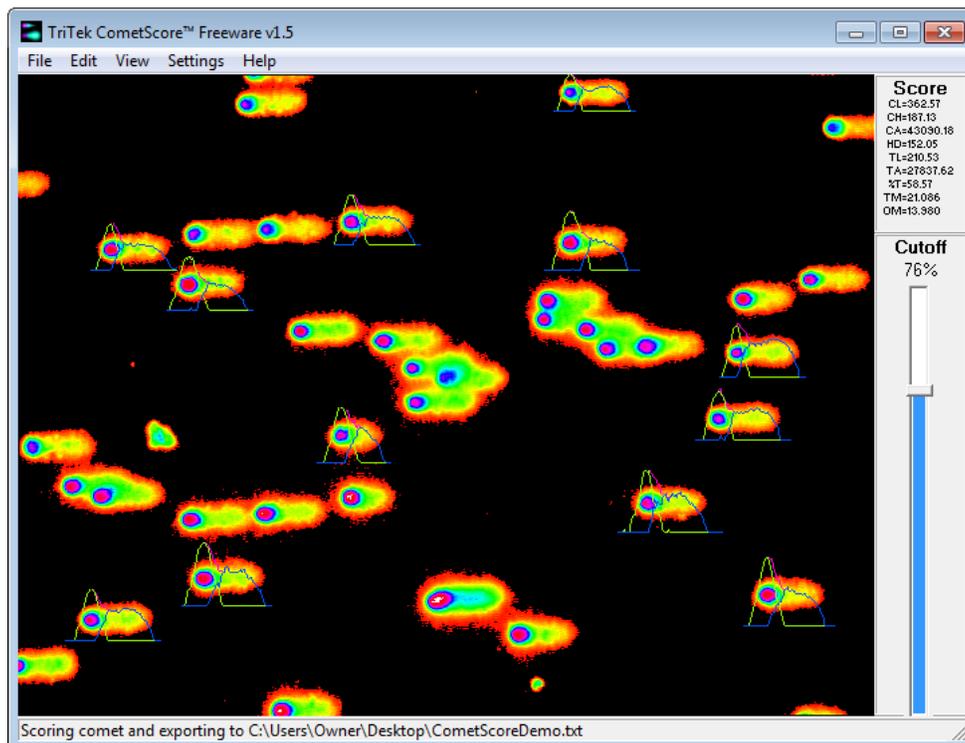
- Click the left mouse button over the field of view and drag a rectangle around a single comet. Make sure that the rectangle does not overlap other comets in the field.



- 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.

- Repeat steps 4 and 5 until every isolated comet is scored in the field of view.



CometScore Tutorial

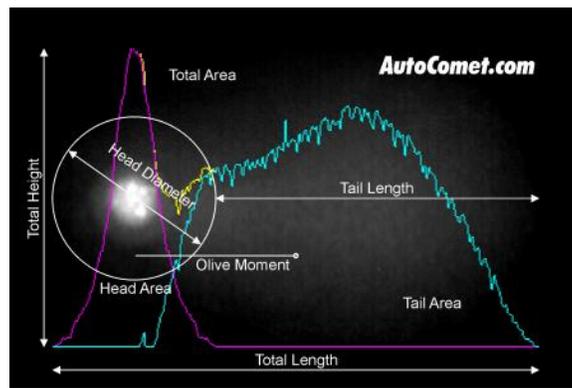
<http://AutoComet.com>

- 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.

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | | |
|----|------------|-------------|------------|----------|------------|-------------|-----------|---------|-----------|--------------|-----------|-------------|-----------|-----------|-----------|--------------|-----------|-------------|--------------|
| 1 | Comet Leng | Comet Heigh | Comet Area | Comet In | Comet Mean | Head Diamet | Head Area | Head In | Head Mean | Head %DNA in | Head | Tail Length | Tail Area | Tail Int | Tail Mean | Tail %DNA in | Tail | Tail Moment | Olive Moment |
| 2 | 333.33334 | 181.28655 | 42987.586 | 114079 | 90.754974 | 128.65497 | 14636.982 | 46402 | 108.41589 | 40.675321 | 204.67836 | 28350.604 | 67677 | 81.636909 | 59.324676 | 20.763636 | 14.75311 | | |
| 3 | 374.26901 | 216.37427 | 47159.809 | 127390 | 92.378532 | 210.52632 | 18262.029 | 52256 | 97.857681 | 41.020489 | 163.74269 | 28897.781 | 75134 | 88.915977 | 58.979511 | 16.514263 | 13.116893 | | |
| 4 | 339.18117 | 204.67836 | 44834.309 | 124029 | 94.606407 | 163.74269 | 18706.611 | 58460 | 106.87386 | 47.134137 | 175.4386 | 26127.697 | 65569 | 85.823296 | 52.865863 | 15.859758 | 11.551403 | | |
| 5 | 339.18117 | 210.52632 | 45107.895 | 123975 | 92.991661 | 175.4386 | 22468.451 | 70181 | 106.8204 | 56.608993 | 163.74269 | 22639.445 | 53794 | 81.259819 | 43.390077 | 12.149482 | 10.143844 | | |
| 6 | 345.02924 | 216.37427 | 46920.422 | 130526 | 95.135567 | 175.4386 | 22707.842 | 71613 | 107.85091 | 54.864931 | 169.59064 | 24212.578 | 58913 | 83.210449 | 45.135069 | 13.08917 | 10.417648 | | |
| 7 | 350.8772 | 169.59064 | 45176.293 | 124456 | 94.213478 | 152.04678 | 15628.74 | 51170 | 111.96937 | 41.114932 | 198.83041 | 29547.553 | 73286 | 84.821762 | 58.885068 | 20.020924 | 14.425114 | | |
| 8 | 350.8772 | 263.1579 | 53418.145 | 145920 | 93.418694 | 163.74269 | 22844.637 | 69514 | 104.06287 | 47.638431 | 187.13451 | 30573.51 | 76406 | 85.465324 | 52.361566 | 16.755701 | 12.454756 | | |
| 9 | 356.72516 | 175.4386 | 40251.703 | 112787 | 95.825829 | 128.65497 | 12037.892 | 41106 | 116.77841 | 36.445689 | 228.07018 | 28213.809 | 71681 | 86.886069 | 63.554311 | 24.78618 | 14.227828 | | |
| 10 | 345.02924 | 169.59064 | 42850.793 | 112736 | 89.972862 | 128.65497 | 10875.141 | 33790 | 106.25796 | 29.972678 | 216.37427 | 31975.65 | 78946 | 84.434227 | 70.027322 | 25.910109 | 16.692068 | | |
| 11 | 321.62742 | 134.50293 | 33890.77 | 88993 | 90.810295 | 116.95906 | 9575.5957 | 31287 | 111.73929 | 34.766036 | 204.67836 | 24315.174 | 58706 | 82.568214 | 65.239564 | 22.831888 | 15.052326 | | |
| 12 | 280.70175 | 245.61403 | 38199.789 | 99819 | 89.363472 | 163.74269 | 19014.398 | 54654 | 98.298561 | 54.753101 | 116.95906 | 19185.391 | 45165 | 80.508018 | 45.246896 | 9.049379 | 7.553823 | | |
| 13 | 362.57309 | 187.13451 | 43090.18 | 118035 | 93.678574 | 152.04678 | 15252.557 | 48899 | 109.63902 | 41.427544 | 210.52632 | 27837.625 | 69136 | 84.933662 | 58.572459 | 21.086086 | 13.980395 | | |
| 14 | | | | | | | | | | | | | | | | | | | |

- 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.



CometScore Tutorial

<http://AutoComet.com>

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 Area

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

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

Comet Intensity

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

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

Mean Comet Intensity

The mean comet intensity is the average of all pixel intensity values in the comet.

$$\mu_I = \frac{I_c}{A_c}$$

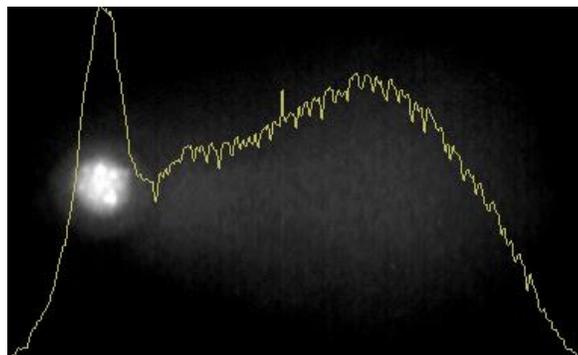
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Intensity Integral

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.

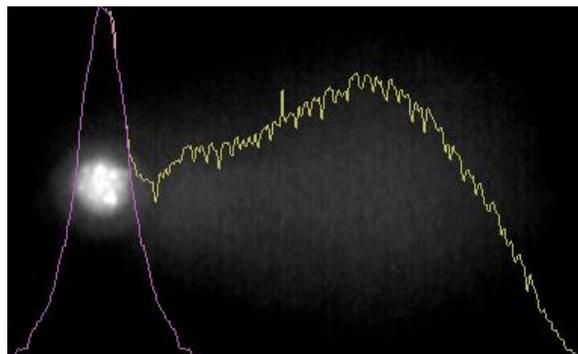
$$P_c = \int I_y d_y$$



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.

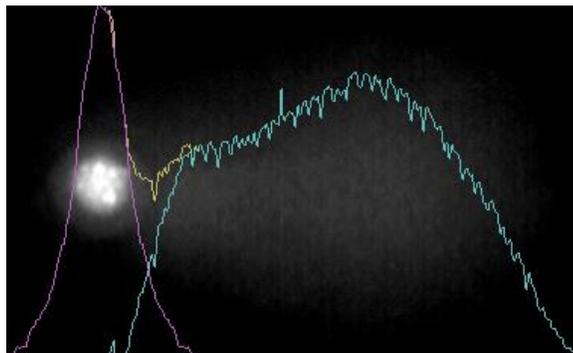
$$\%DNA_h = 100 \times \frac{I_h}{I_c}$$

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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.