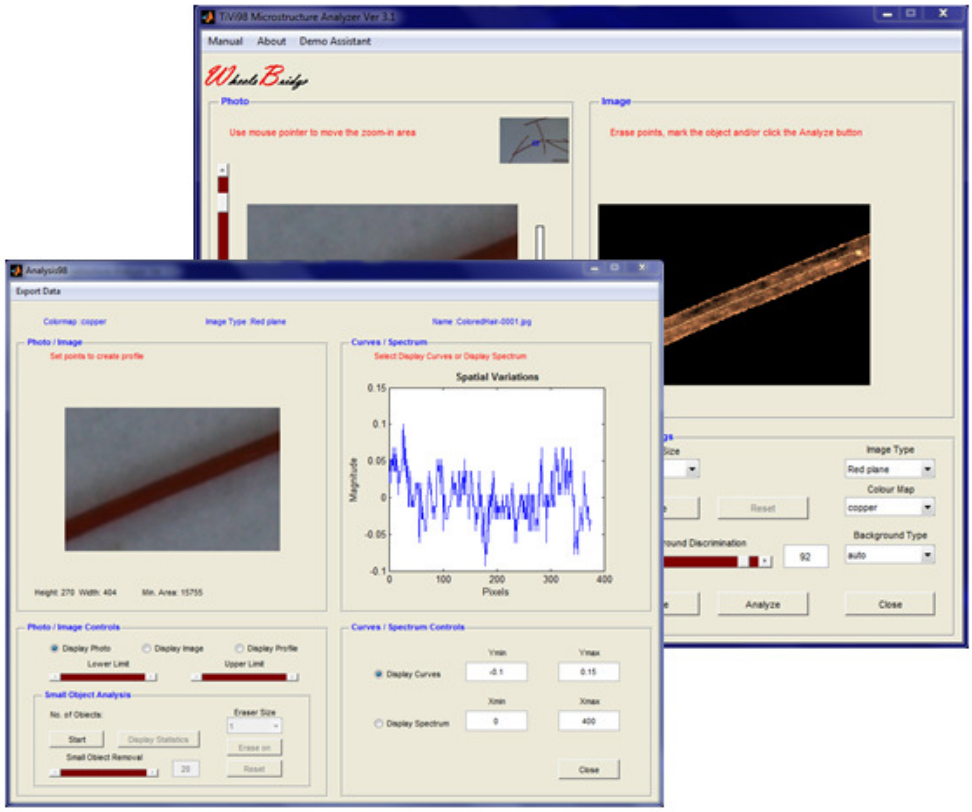
WheelsBridge



Microstucture Analyzer TiVi98
User Manual

User Manual 3.1
Version 3.1
January 2012

PIONEERS IN TISSUE VIABILITY IMAGING

Dear Valued Customer!

Welcome to the WheelsBridge TiVi98 Microstructure Analyzer system intended for automatic analysis of small object characteristics in in-vitro and in-vivo applications such as objective assessment of hair surface smoothness. The TiVi Microscope TiVi98 comprises a 60 mm tube including polarizing filters, magnifying lenses, illumination devises and sample holders all integrated in a 60 mm diameter tube of length 90 mm that is attached directly to the TiVi camera. The TiVi Microscope TiVi98 has a resolution of about 2.7 micrometers per pixel.

The TiVi98 Microstructure Analyzer utilizes a highly sensitive digital camera equipped with a macro zoom-in lens system and polarization filters making it possible to suppress direct surface reflections (cross-polarization mode) or alternatively enhance surface structures (co-polarization mode). The versatile system software – based on the MATLAB® high performance language for technical computing – allows for rapid and easy capturing and analysis of images. Among the many useful features of the TiVi98 Microsctructure Analyzer software the following are of particular interest:

- *Automatic capturing of 18 MPixel photos in batch mode.*
- *Operates both in in-vivo and in-vitro applications.*
- *Utilized a Macro lens with zoom-in function.*
- *Digital zoom-in and moving window analysis of a subset of the photo pixels.*
- *Automatic background elimination.*
- *Automatic profile generation to determine length and diameter of small objects.*
- *Surface irregularity analysis and frequency analysis.*
- *Automatic ensemble analysis of a multiplicity of many small objects simultaneously.*
- *Results can be transferred to spreadsheets for further analysis.*

We are convinced that the TiVi98 Microstructure Analyzer will be a productive tool in the objective and cost-effective day-to-day assessment of hair surface structures and other miniature objects.

Thank you for choosing the WheelsBridge TiVi98 Microsctructure Analyzer.

WheelsBridge AB

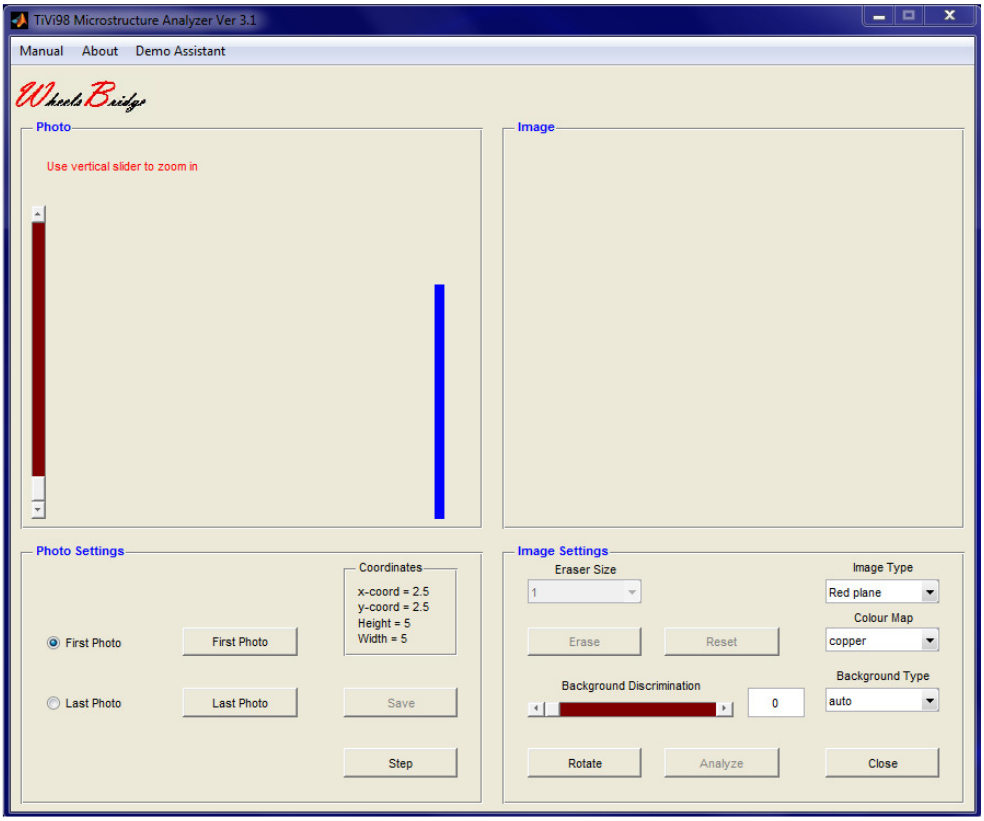
1. INTRODUCTION

The *TiVi98 Microstructure Analyzer* is intended for the study of small objects such as hair and stubble, both *in-vitro* and *in-vivo* with a best resolution of 2 - 3 micrometers per pixel. The *TiVi98 Microscope* comprises a tube (diameter 60 mm, length 90 mm) that is attached to the TiVi camera. This tube integrates magnifying lenses, a ring of light emitting diodes for object illumination and a sample holder (for *in-vitro* studies of small objects) or alternatively an aperture suitable for *in-vivo* applications. The standard TiVi camera zoom-in lens system is replaced by a macro zoom-in lens system. The *TiVi98 Microsctructure Analyzer* software includes features for digital zoom-in and moving a region of interest window over the photo, distance measurement (diameter and length of object), surface irregularity assessment and ensemble analysis with statistical measures of a multiplicity of small objects.

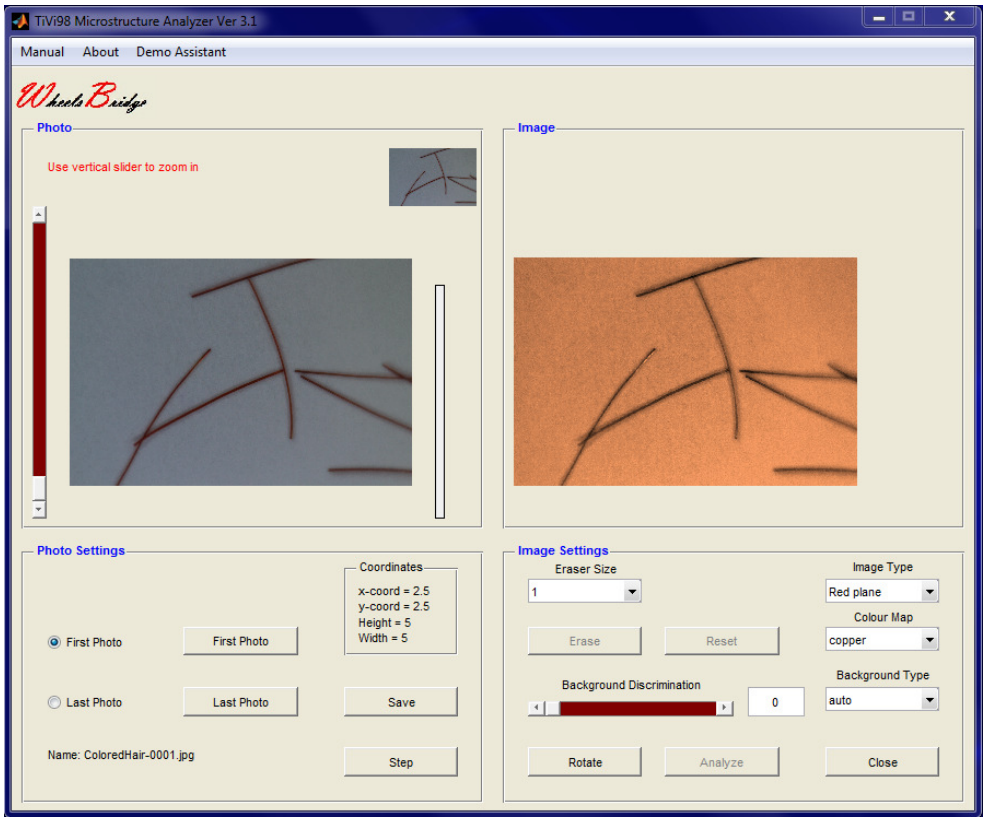
2. GETTING STARTED

The basic features of the *TiVi98 Microstructure Analyzer* are probably best explained by way of an example. In the following example it is assumed that the high resolution photo *ColoredHair-0001* has been captured by the *TiVi700 Analyzer* system and stored in the folder *TiVi98demonstration*. This photo displays coloured hair samples. The task is to measure the hair diameter and analyze surface irregularities along the hair.

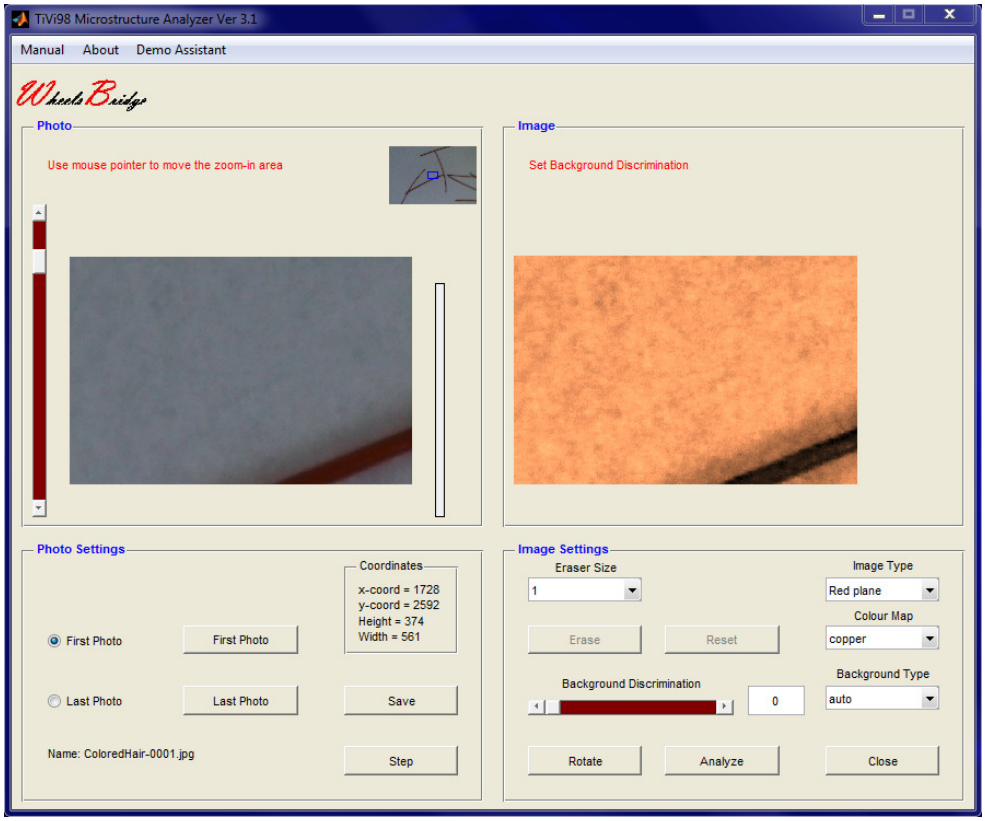
1. Select *TiVi98 Microstructure Analyzer* in the *Tool Boxes* pull-down menu in the *TiVi700* Analyzer window to open the *TiVi98 Microstructure Analyzer* window.



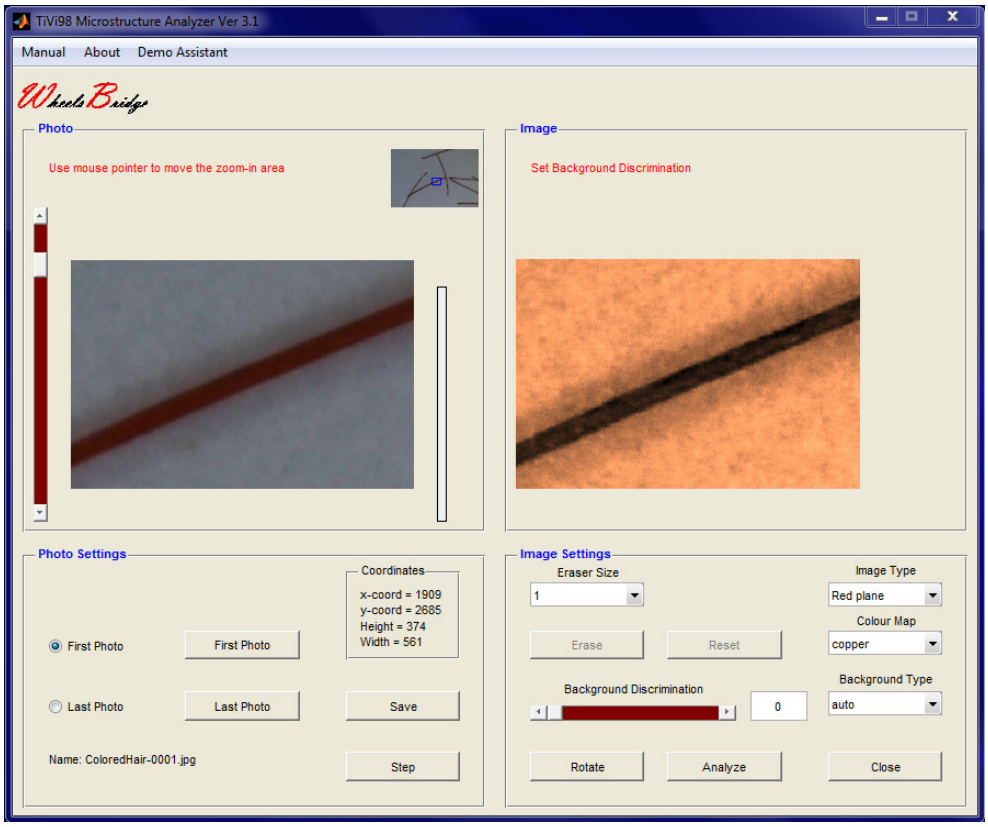
2. Click the **First Photo** button and open the *ColoredHair-0001* photo located in the *TiVi98demonstration* folder.



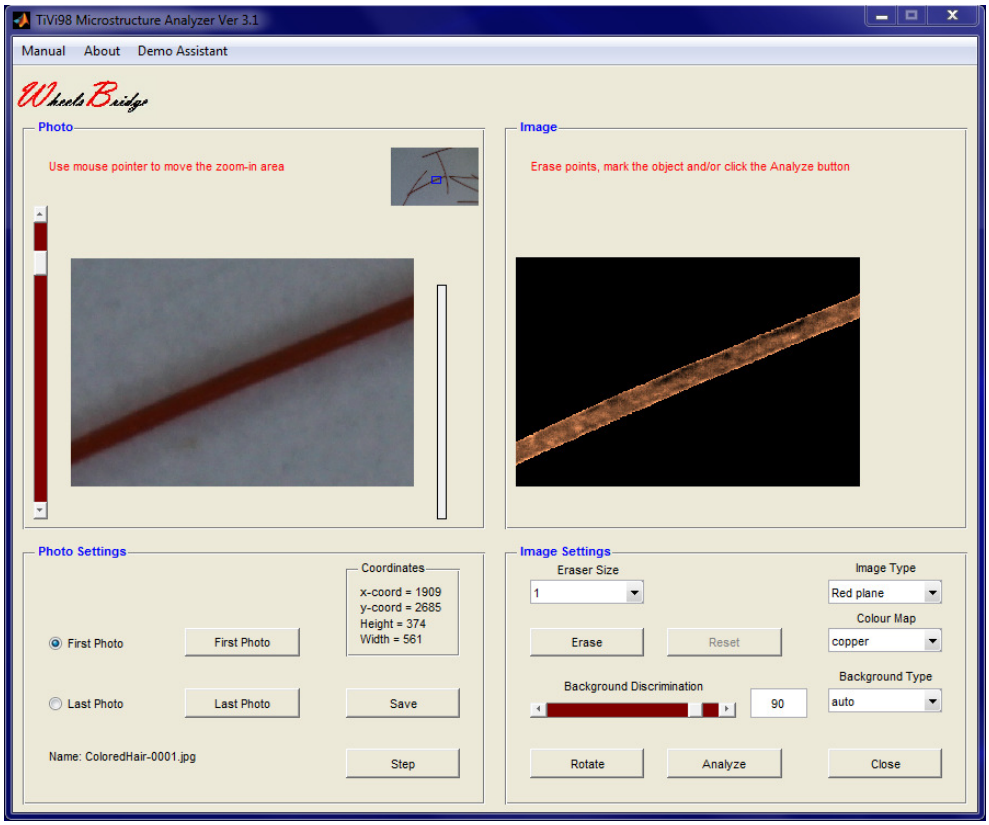
3. Move the vertical slider next to the photo to about 80% of its maximal position to zoom-in on the centre area of the photo. The position of the zoomed-in area in the original photo is displayed in the upper right corner of the **Photo** panel.



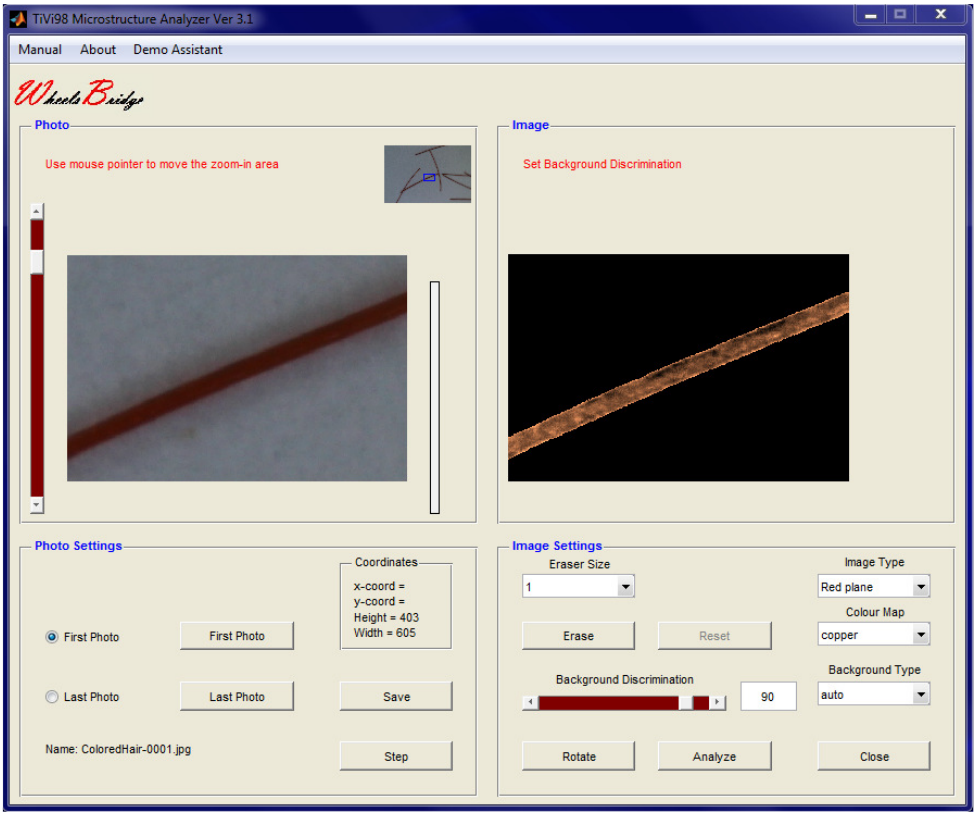
4. Place the mouse pointer in the centre of the photo and drag the mouse with the left mouse button pressed. The zoomed-in area will now move over the photo. Drag the mouse until the hair is in the centre of the zoomed-in photo.



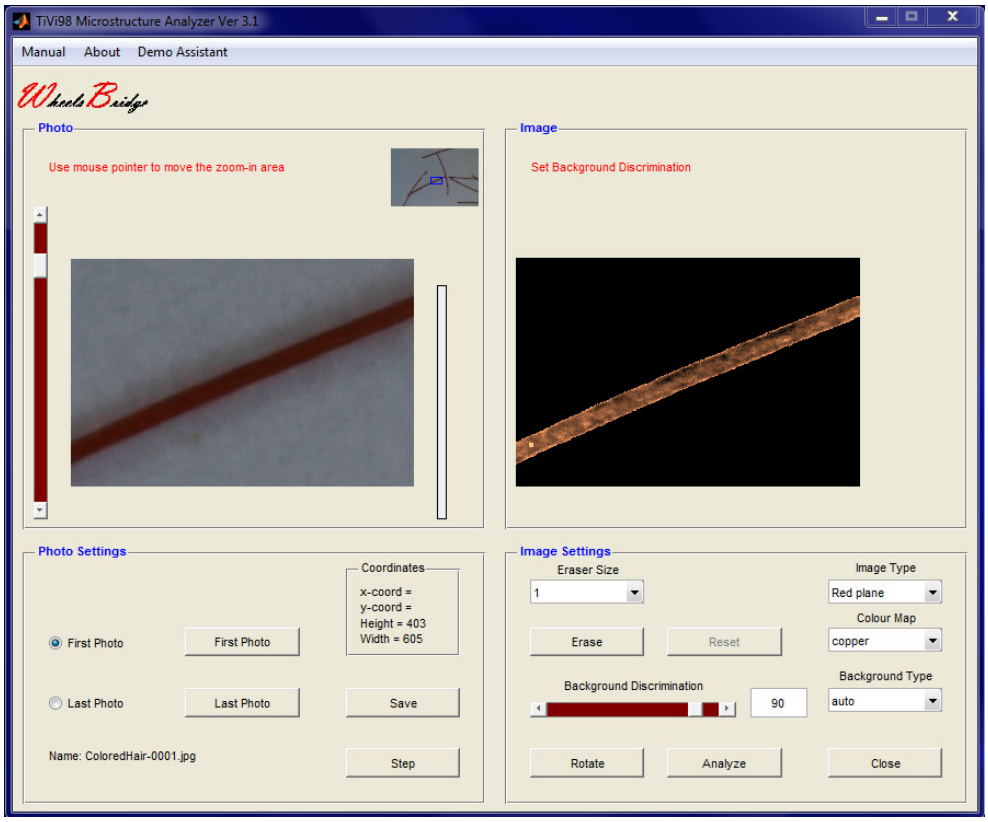
5. Move the **Background Discriminator** slider to about 90% of its maximal value.



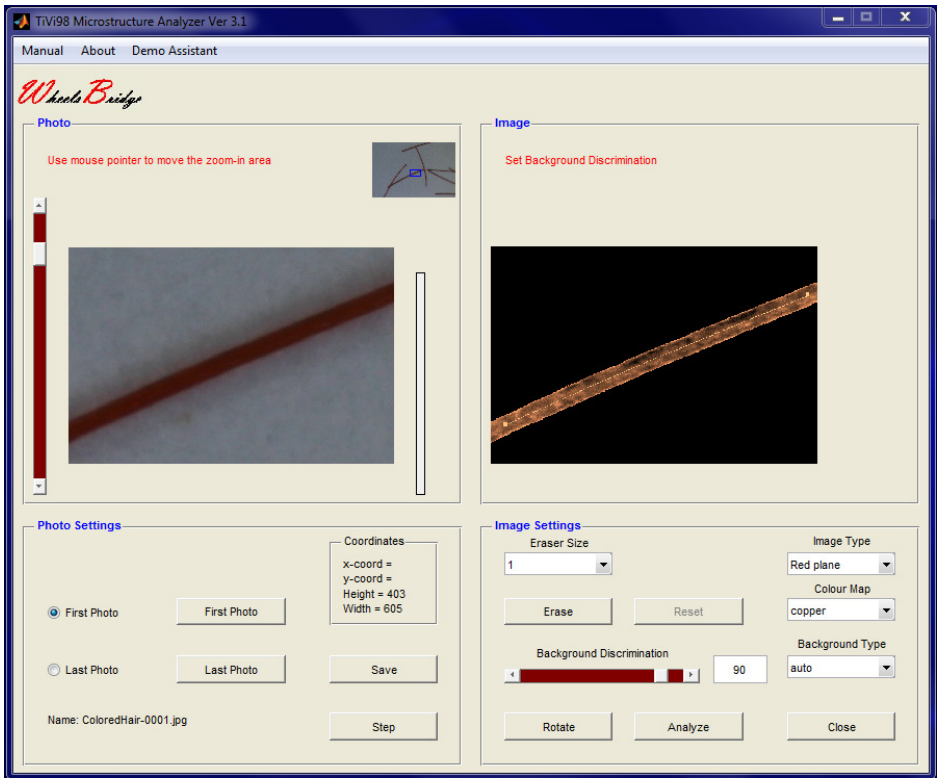
6. The surface structure of the hair is now displayed against a black background in the **Image** panel. In this particular case a **copper colorscale** is used to display irregularities in the **Red plane** of the photo. To further zoom in and display the surface irregularities, move the vertical slider next to the photo somewhat further upwards.



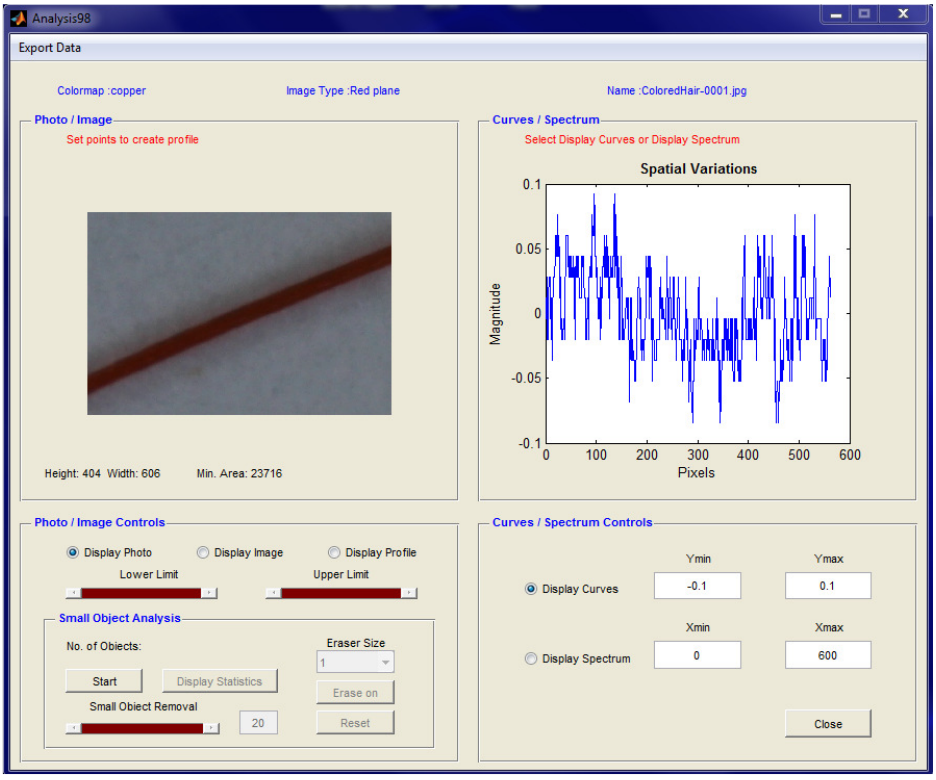
7. Position the mouse pointer inside the hair object in the **Image panel** close to its left end. Click the left mouse button to place the first reference point.



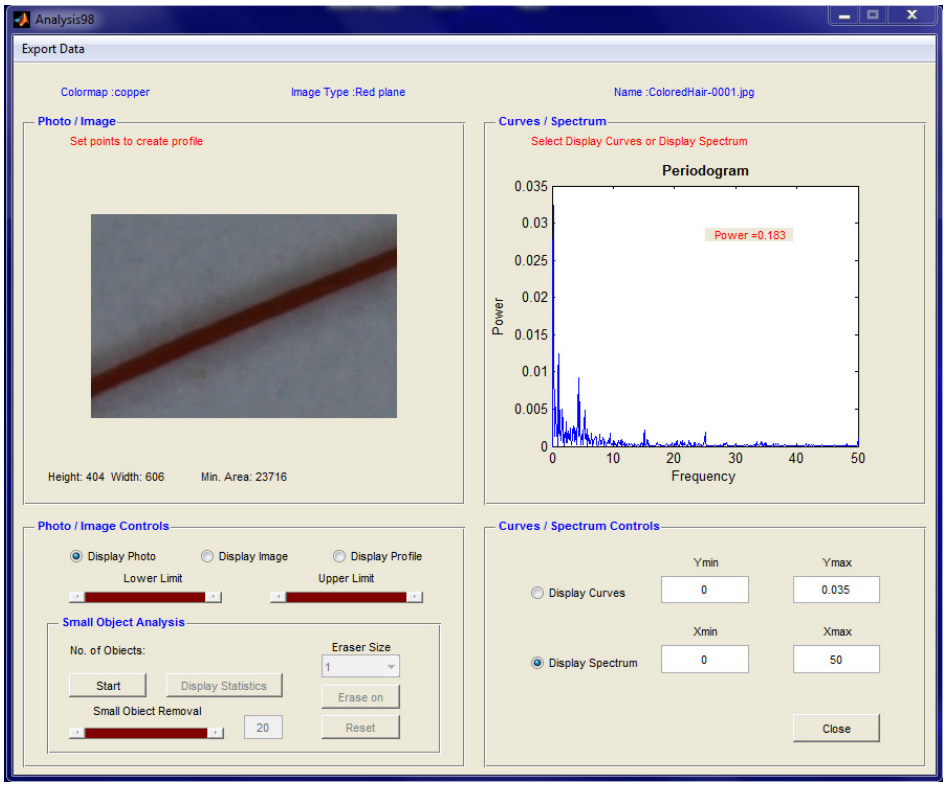
8. Position the mouse pointer inside the hair object in the **Image panel** close to its right end. Click the left mouse button to mark the second reference point. A line following the centre trajectory of the object is now drawn between the two reference points.



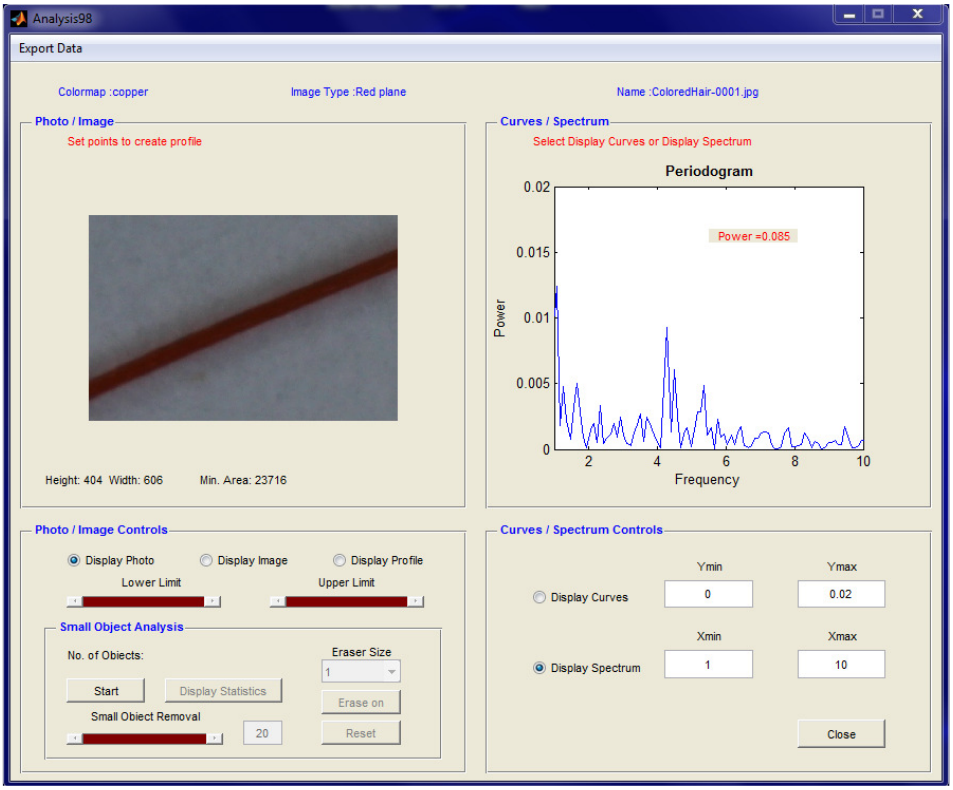
9. Click the **Analyze** button to open the **Analysis98** window.



10. In the **Photo/Image** panel the photo is displayed while the **Curves / Spectrum** panel displays the irregularities in the hair along the line between the two reference points. Click the **Display Spectrum** radio-button to display the power spectrum of the hair surface irregularities along the line between the two reference points.

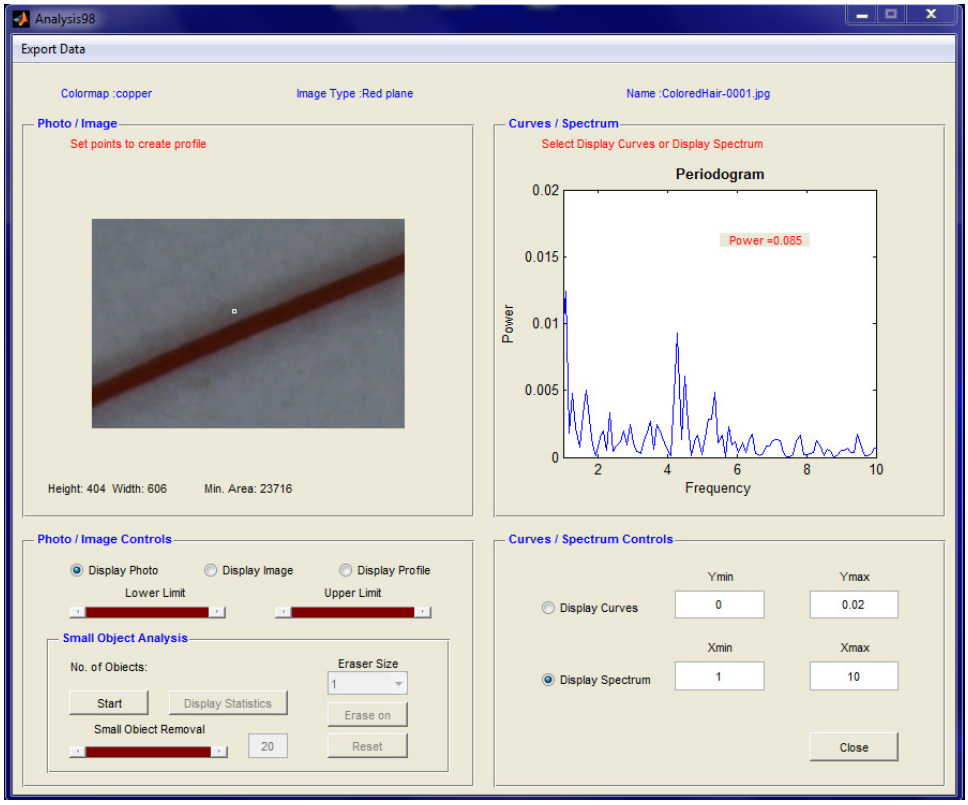


11. The total power (the surface area under the power spectral density curve displayed) is displayed in text line (red on gray background) in the power spectral density diagram. The total power represents an index that quantifies the overall irregularities of the hair within the frequency range selected while the power spectral density indicates how the irregularities are distributed over different frequency intervals. Print 10 in the **Xmax** edit box and 0.02 in the **Ymax** edit box and press **Enter** on the computer keyboard.

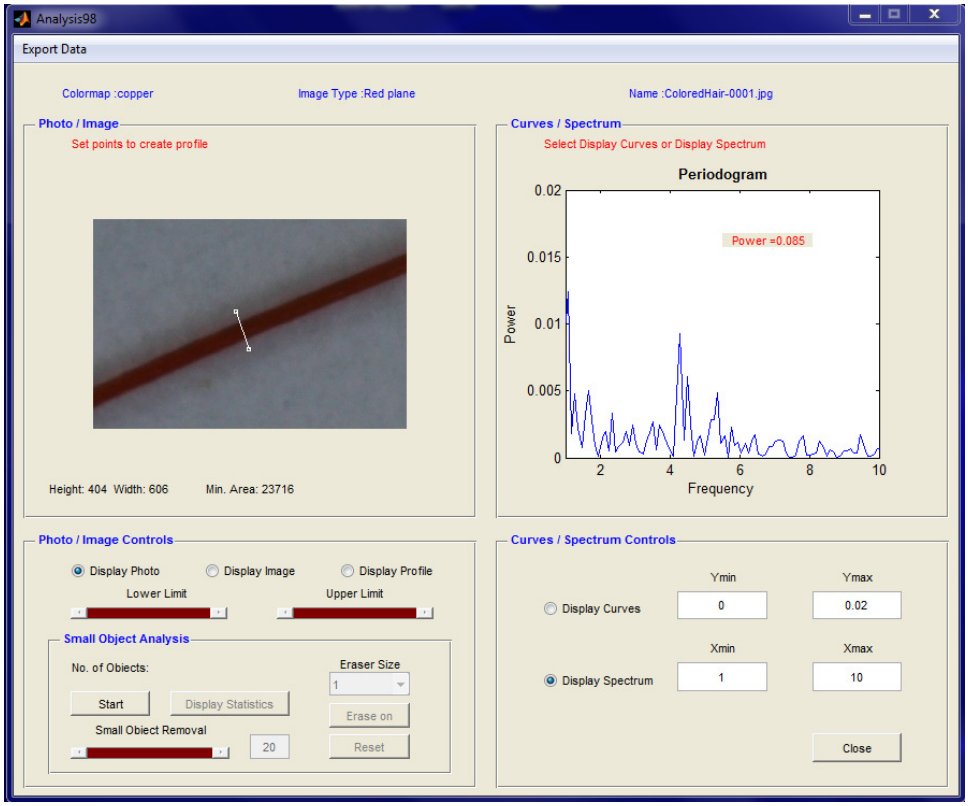


12. The power spectrum now displays the distribution of irregularities while the power index value shows the total magnitude of irregularities within the selected bandwidth (1 to 10).

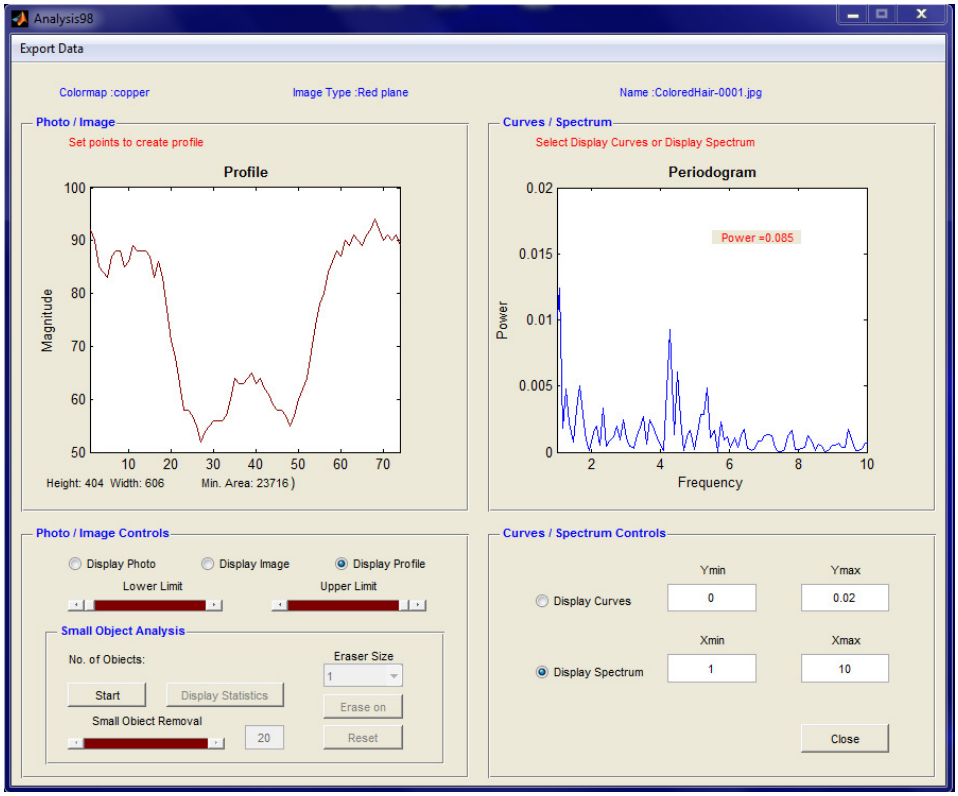
13. To calculate the diameter of the hair displayed place the mouse pointer next to the hair in the photo in the **Photo/Image** panel and click the left mouse button to generate the first profile reference point.



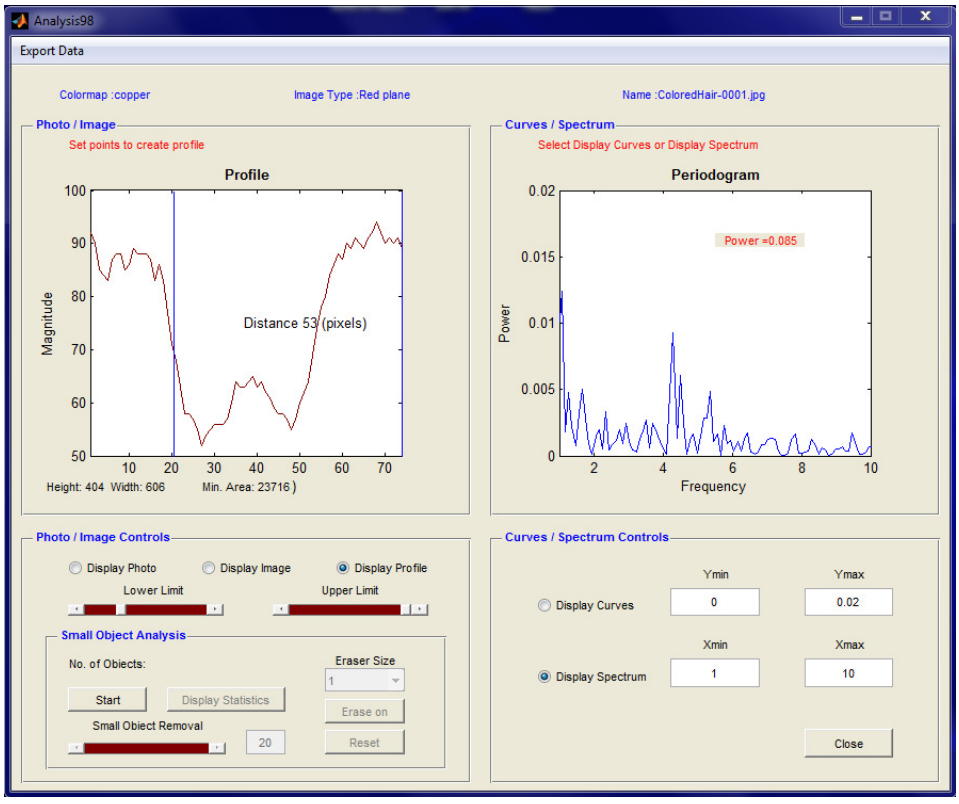
14. Position the mouse pointer on the other side of the hair and click the left mouse button again to generate the second profile reference point. A line is generated between the two profile reference points along which the profile is calculated.



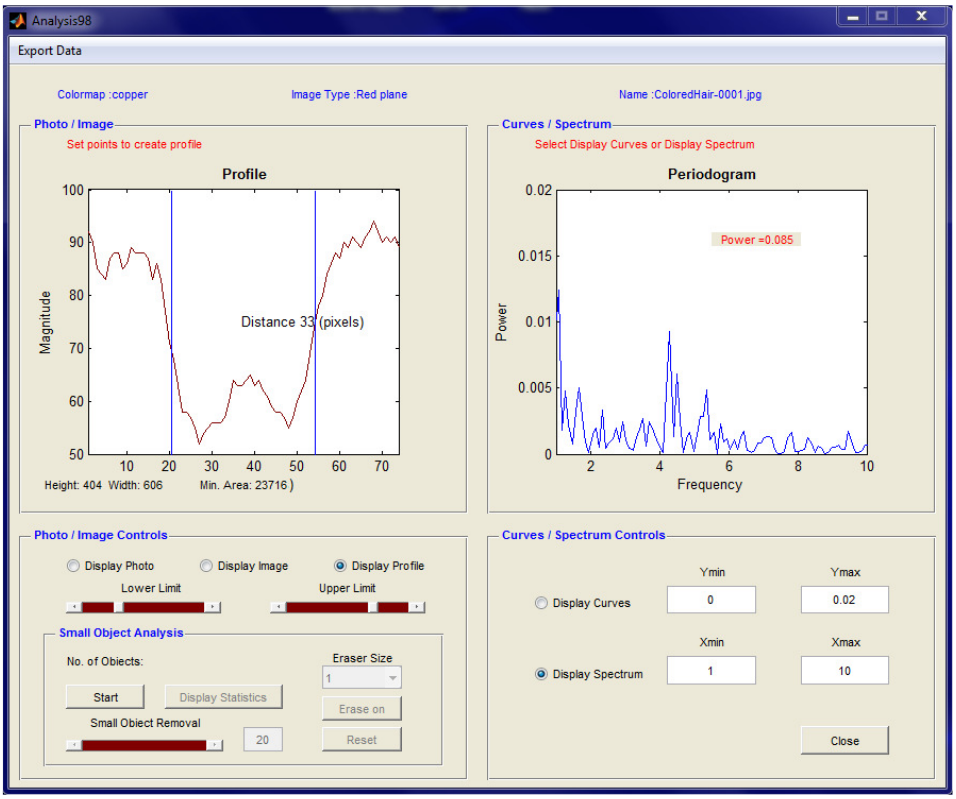
15. Click the **Display Profile** radio-button to display the profile along the line generated.



16. Move the **Lower Limit** slider until the vertical line coincides with the left side boundary of the hair profile.



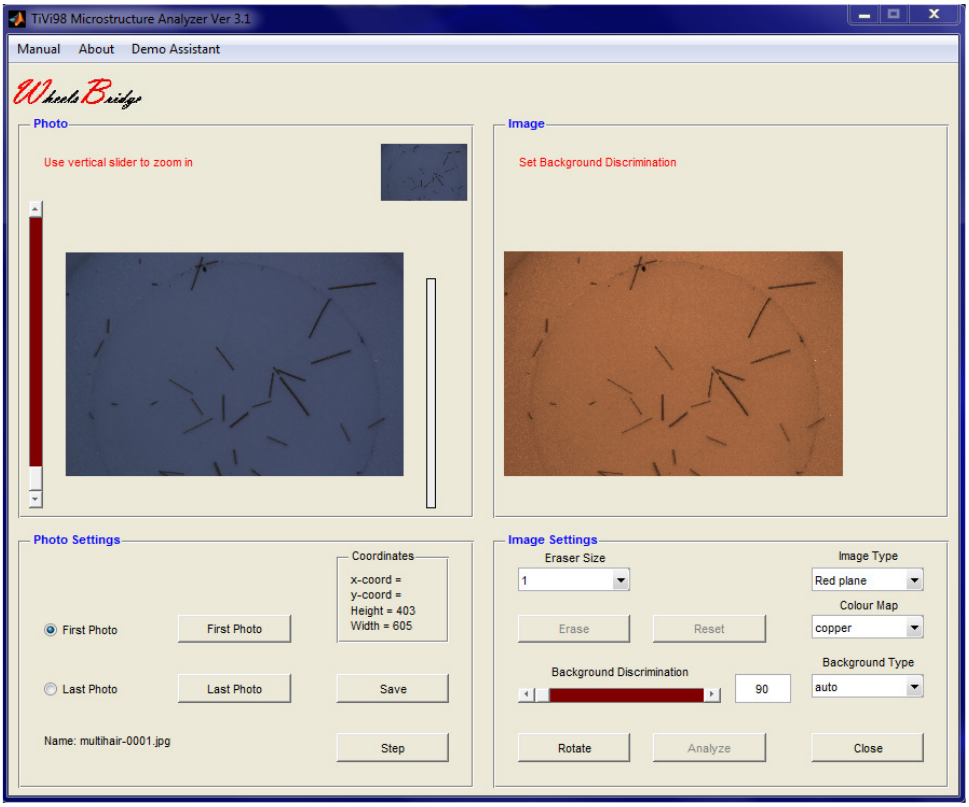
17. Move the **Upper Limit** slider until the vertical line coincides with the right side boundary of the hair profile.



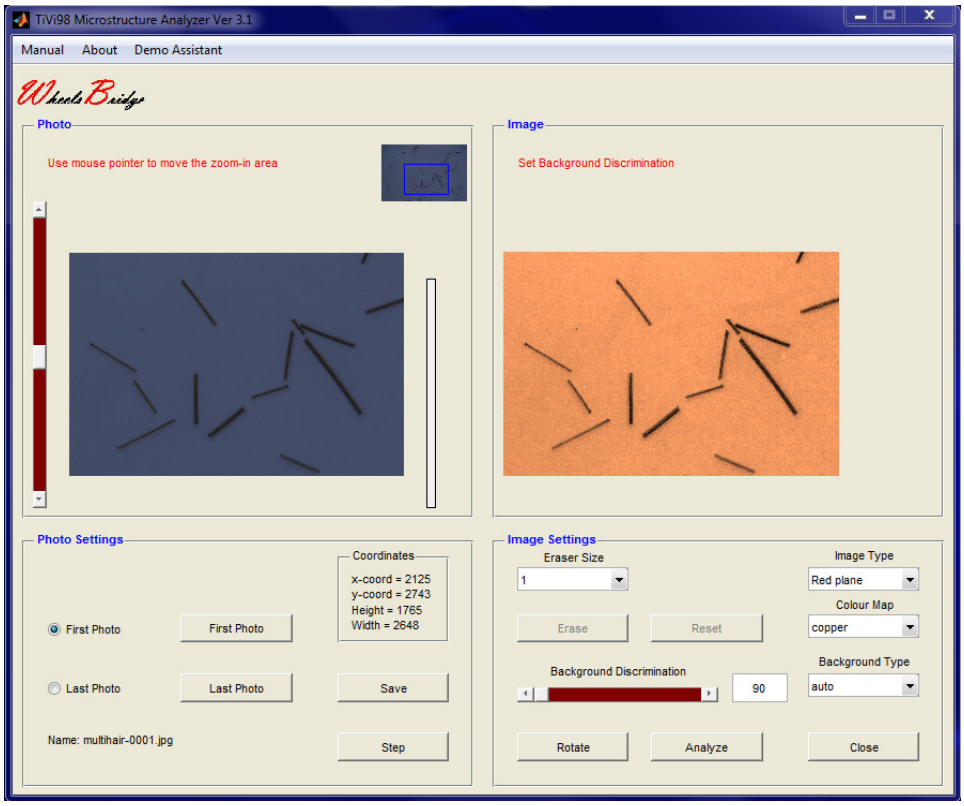
18. The diameter of the hair sample can now be read to be 33 pixels corresponding to 99 micrometers provided the TiVi microscope has been calibrated to 3 micrometers per pixel.

19. The example below describes how to estimate average property values for an ensemble of objects (short hair samples).

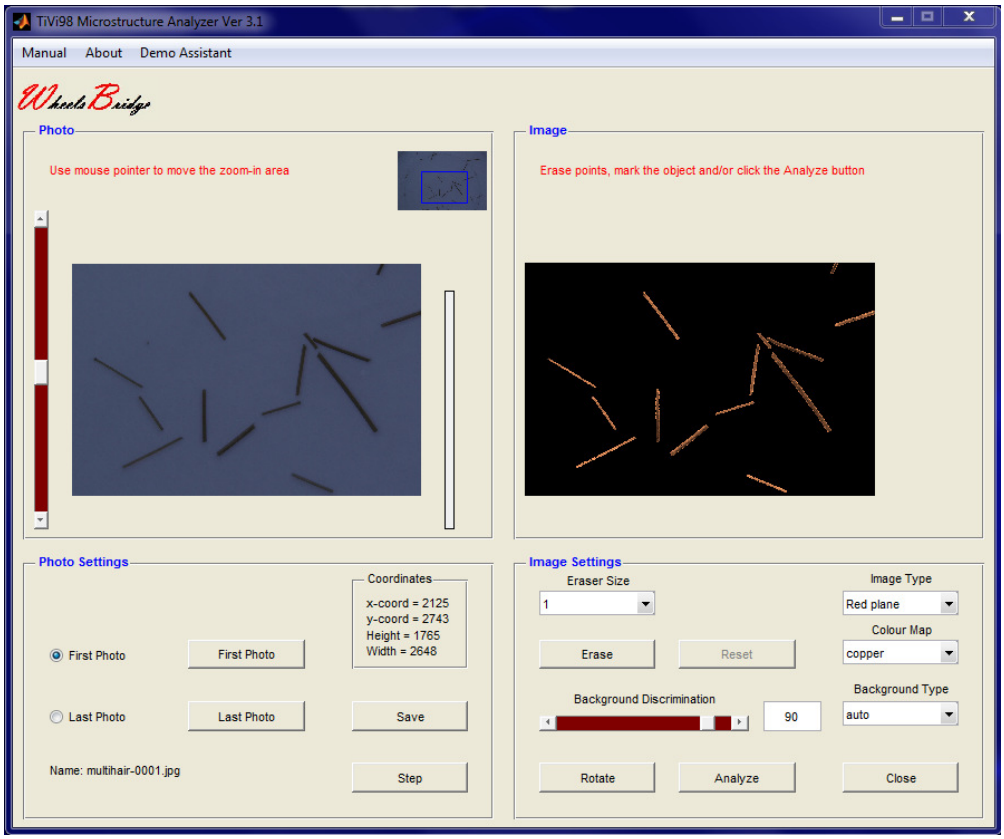
20. In the *TiVi Camera Microscope* window click the **First Photo** and upload the file *multihair-0001.jpg*. The task is to determine average properties of the objects such as the diameter.



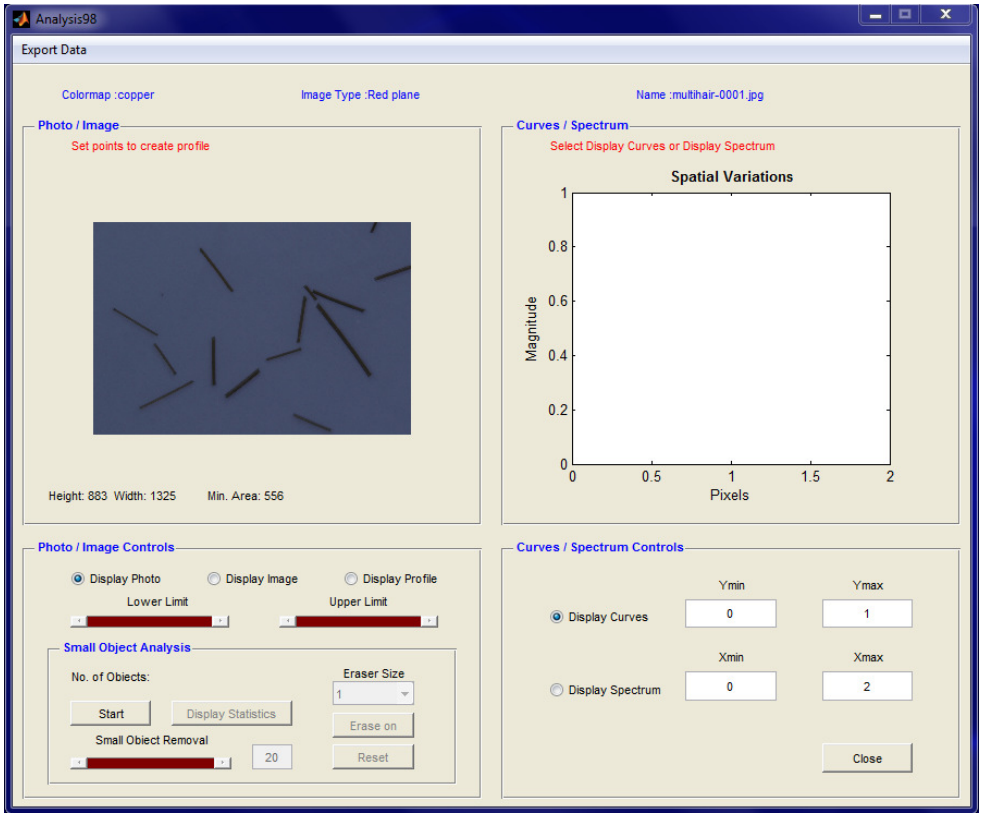
21. Zoom –in the photo by moving the vertical slider upwards and the move the centre of the zoom-in window by use of the mouse.



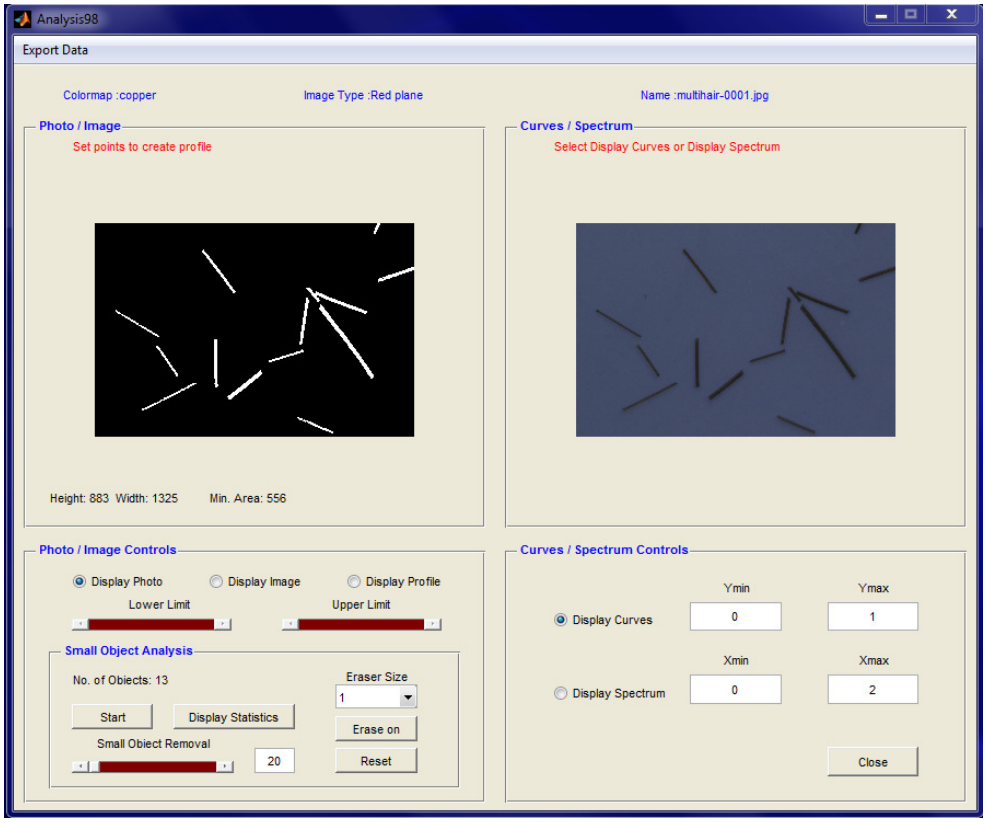
22. Drag the **Background Discrimination** slider to the right to create an image background.



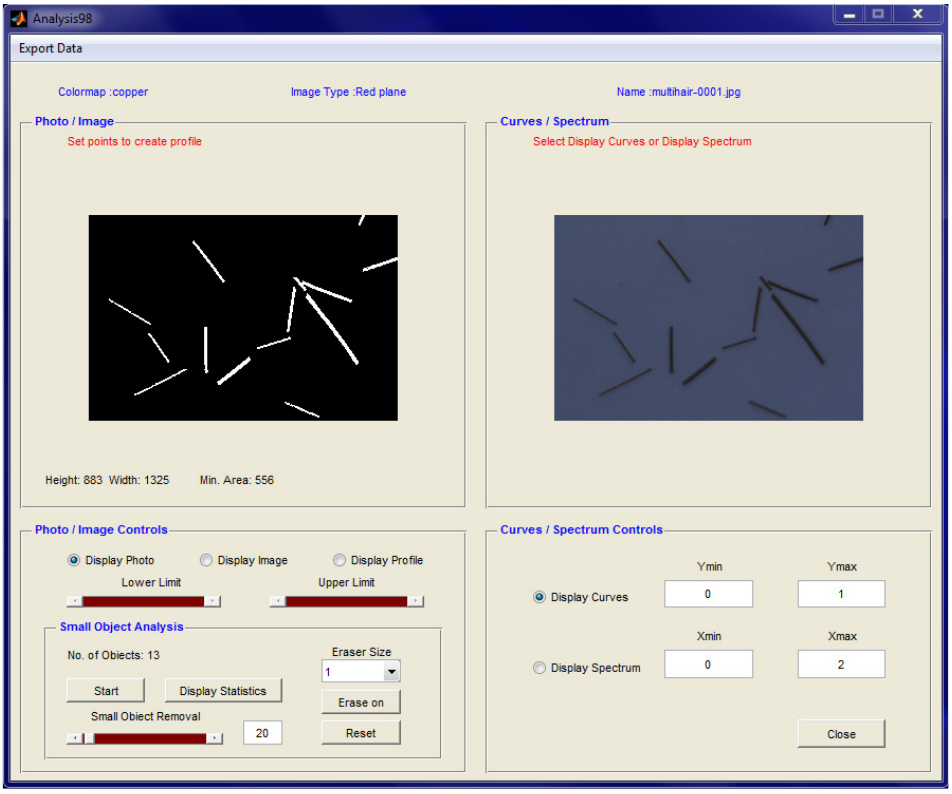
23. Click the **Analyze** button to open the *Analyze98* window.



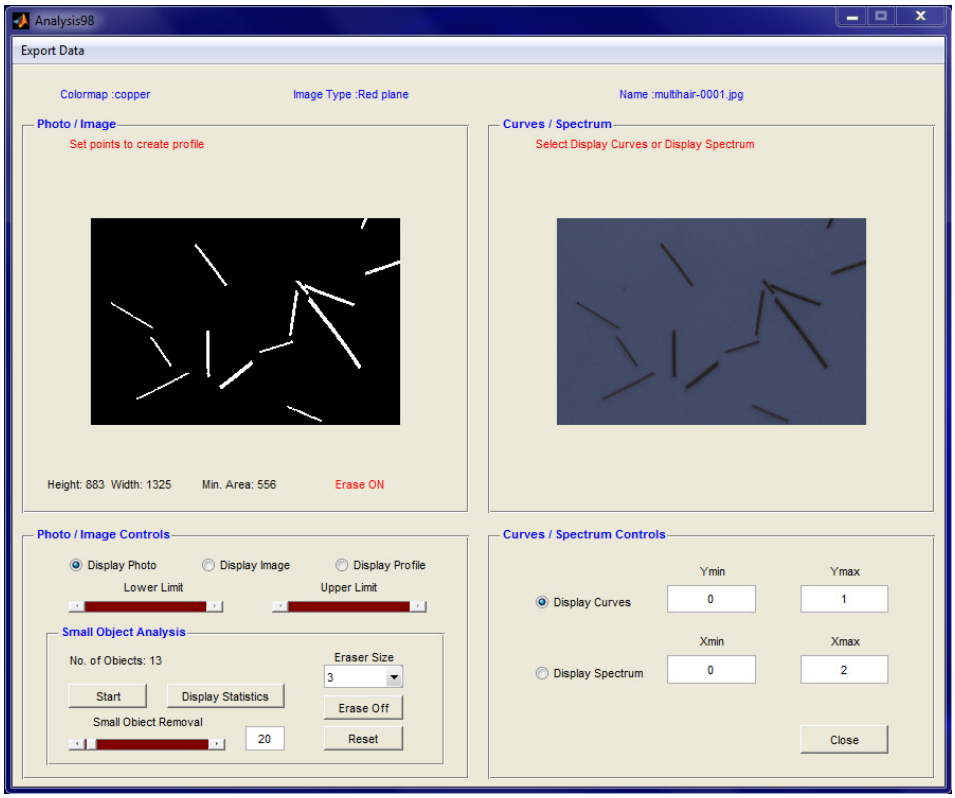
24. Click the **Start** button in the **Small Object Analysis** panel to create a black and white image from the photo.



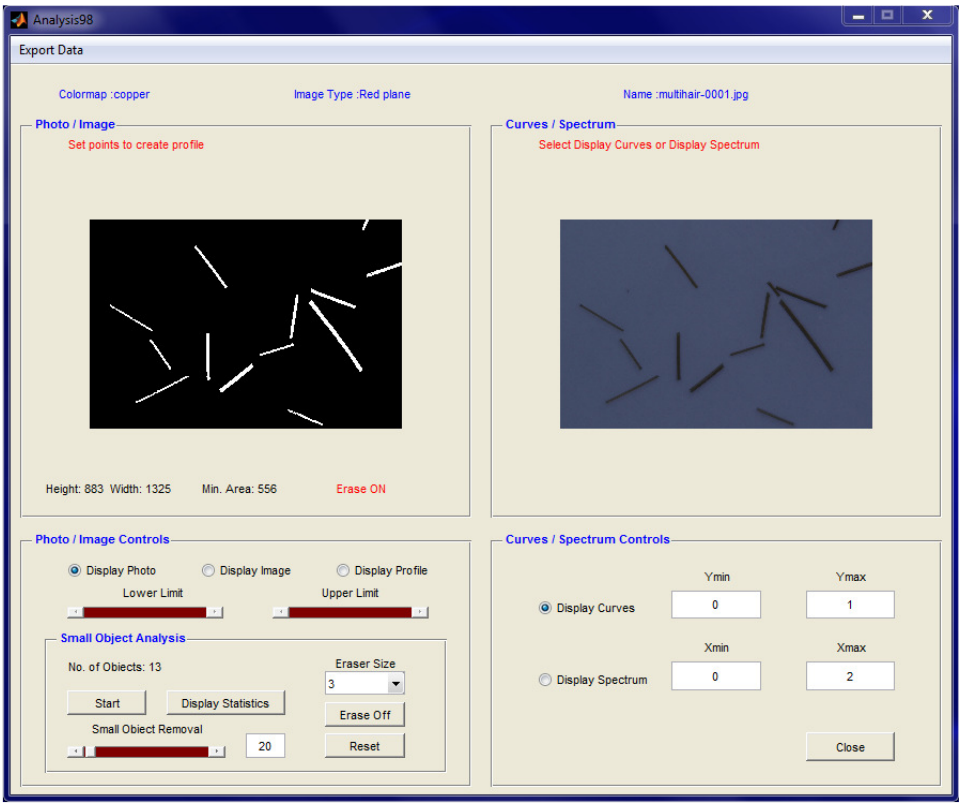
25. Drag the **Small Object Removal** slider until the **No.of Objects** reads 13.



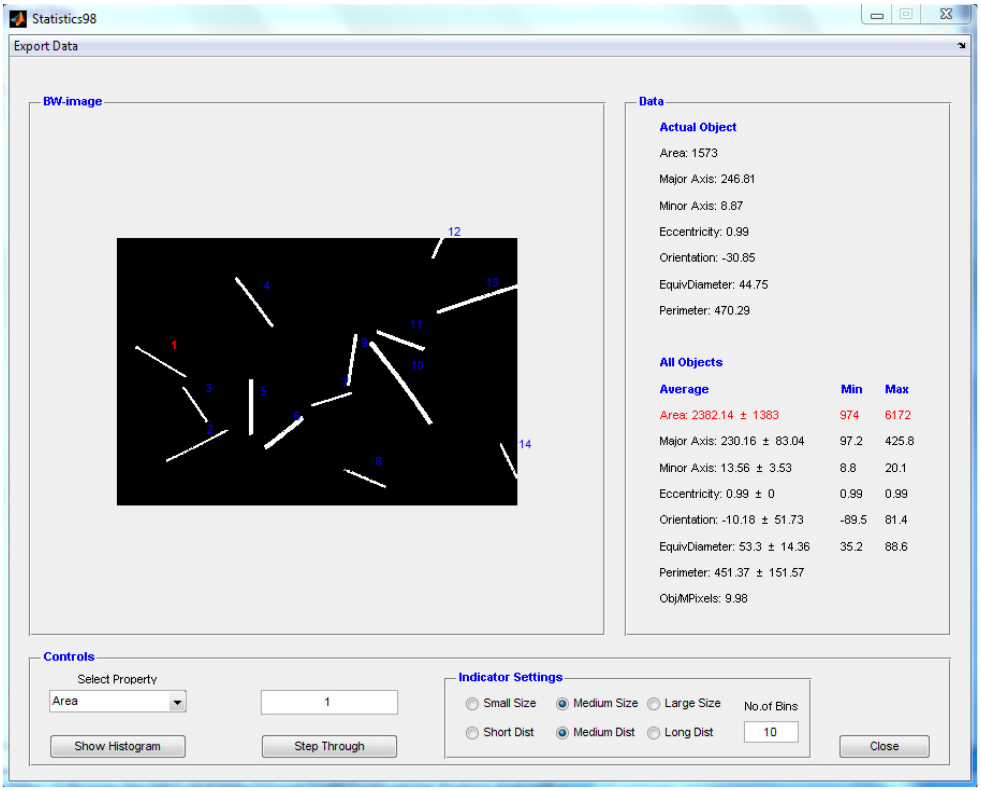
26. Click the **Erase On** button and set the **Eraser Size** to 3 in the **Eraser Size** pull-down menu.



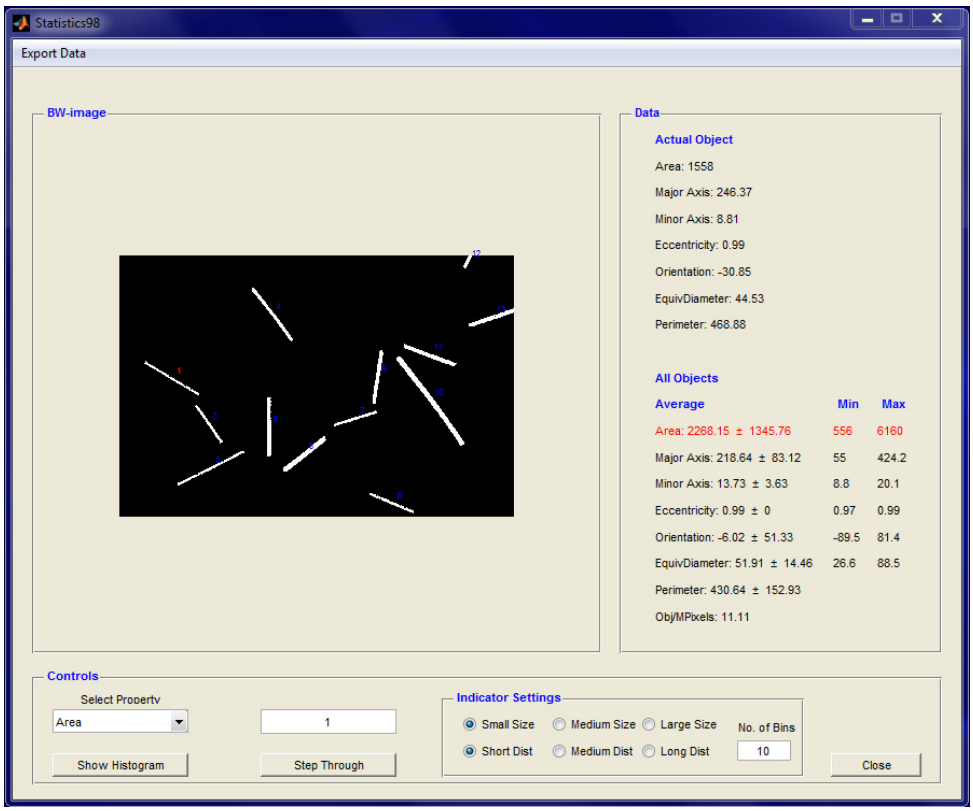
27. Position the mouse pointer above the smallest object in the black and white photo. Press the left mouse button and erase this object.



28. Click **Display Statistics** to open the **Statistics98** window.

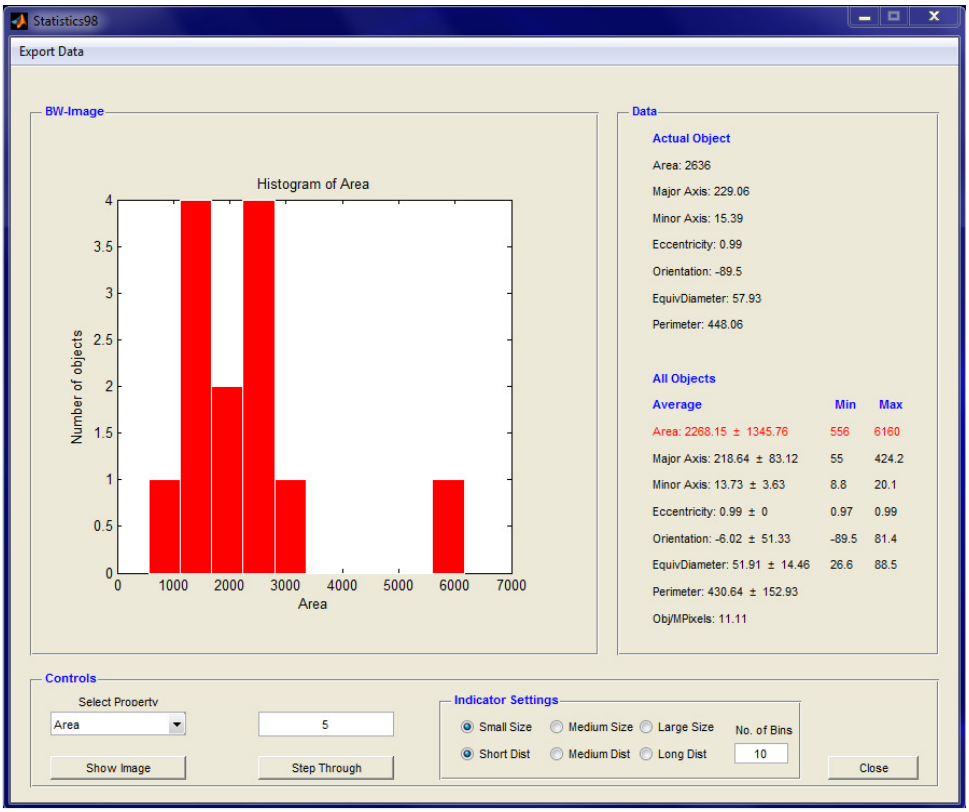


29. Check the **Small Size** and the **Short Distance** radio-buttons to reduce the size of the object number indicators and move them closer to their respective object.

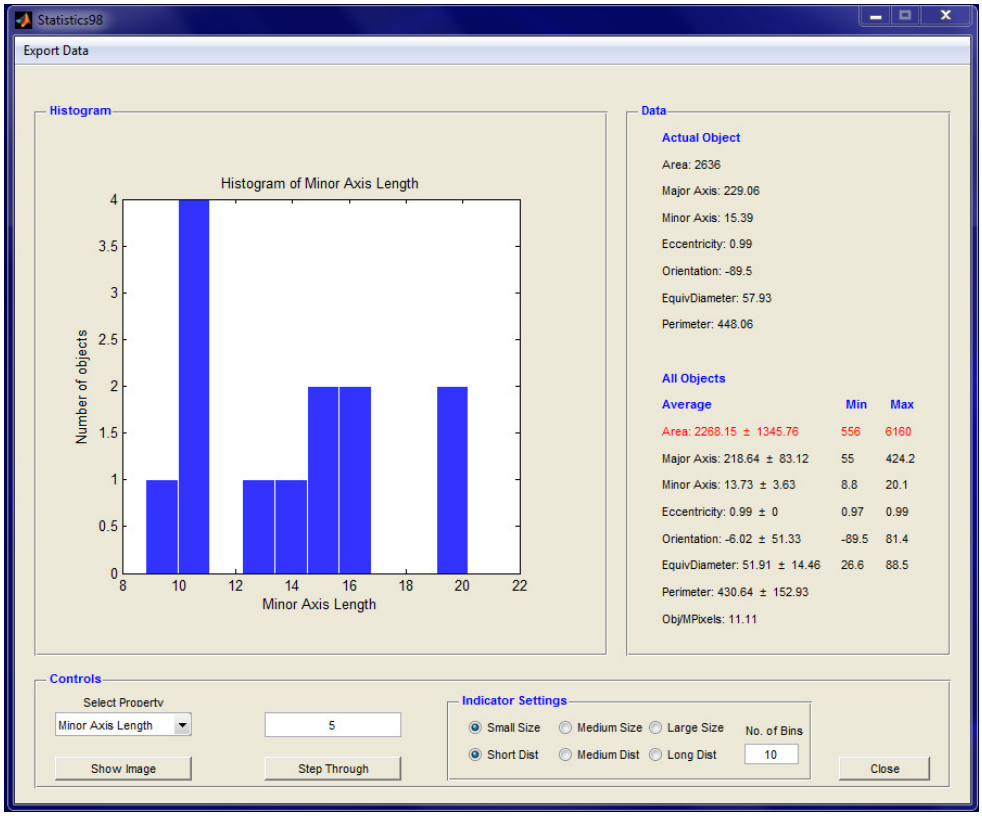


30. Click the **Step Through** button several times to shift the focus between the objects. The **Property Data** for the object in focus is displayed in the **Data** panel.

31. Click the **Show Histogram** button to display the distribution of object areas.



32. Select **Minor Axis Length** from the **Select Property** pull-down menu to display the distribution of the object minor axis (which is also the diameter of the hairs).



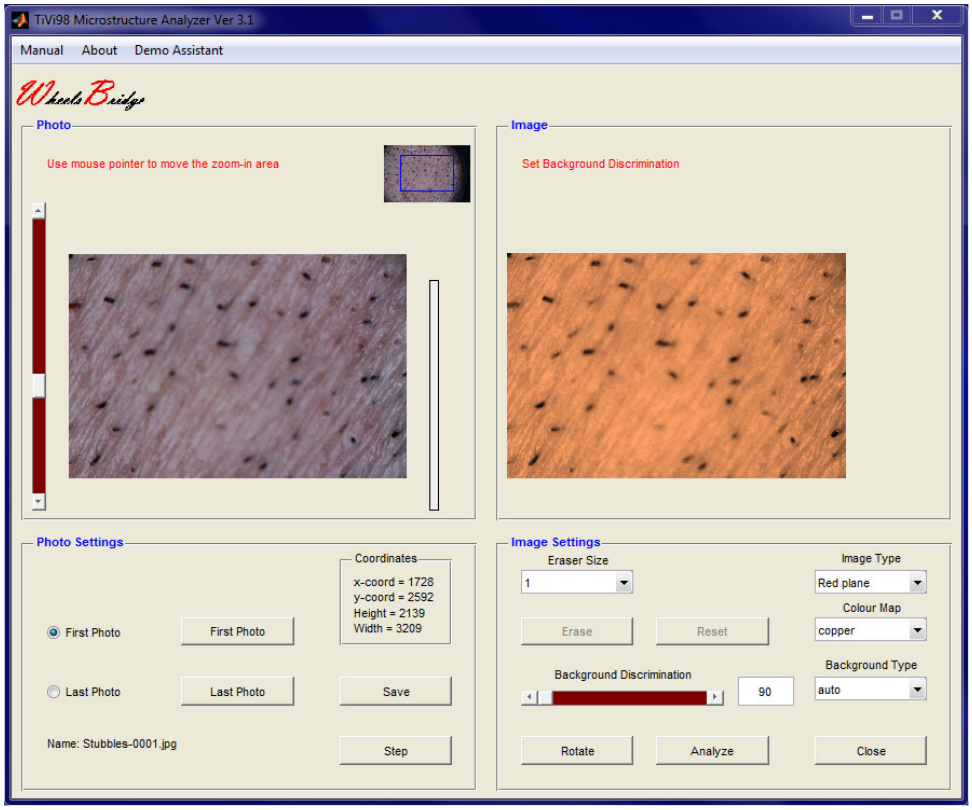
33. The average value of the **Minor Axis** is displayed in the **Data** panel (13.73 pixels).

34. Print "5" in the **No. Of Bins** edit box to reduce the number of histogram bins to 5.

35. This completes the **GETTING STARTED** session.

3. DETAILED DESCRIPTION

When the *TiVi Microscope TiVi98* is started from the *TiVi600 Tissue Viability Imager*, the actual photo is exported and the *TiVi Microscope TiVi98* main window is displayed.



The **Photo** panel displays the zoomed-in photo and the original photo (upper right) in which the position of the zoomed-in photo is outlined.

1. The vertical **zoom-in** slider – to set the degree of photo magnification (zoom-in).

2. With the mouse pointer pointing to a position inside the photo and the left mouse button pressed, dragging the mouse will cause the zoomed-in window to move over the original photo as indicated in the original photo displayed in the upper right corner of the **Photo** panel.

The **Image** panel displays the zoomed-in **Image** of which the structure and colour scale is determined by the selected **Image Type** and the **Color Map** settings.

1. After the **Background Discrimination** is set, placing the mouse pointer on the object and clicking the left mouse button, places the first reference point on the object (bright colour).

2. Placing a second reference point on the object, draws a line between the two reference points, along which the surface irregularity is recorded and further analyzed in the **Analyzer98** window.

The **Photo Settings** panel includes controls for photo manipulation.

1. **First Photo** radio-button – to select the first photo in the uploaded sequence.
2. **Last Photo** radio-button – to select the last photo in the uploaded sequence.
3. **First Photo** button **–** to upload the **First Photo.**
4. **Last Photo** button **–** to upload the **Last Photo.**
5. **Save** button – to save the zoomed-in photo.
6. **Step** –sets the step in an uploaded sequence of photos to be displayed.
7. The **Coordinates** panel displays the **centre coordinates** and the **Width** and **Height** of the zoomed-in photo.

The **Image Settings** panel includes controls for image manipulation.

1. The **Eraser Size** menu – to select the size of the Eraser brush (1, 2, 3, 4, 5 or 10 pixels).
2. **Erase** – to switch on or switch off the **Eraser**. With the **Eraser** switched on moving the mouse pointer with the left mouse button pressed over the **Image** will erase object pixels and turn them into background (black) pixels. This feature is useful for isolating an object and delete non-relevant pixels to be able to generate a reference line for irregularity analysis.
3. **Reset** – to reset the **Image** to its original version.
4. **Background Discrimination** slider – drag to set the background (black pixels) and prepare the **Image** for further analysis.
5. **Background Discrimination** edit box – same function as the **Background Discrimination** slider**.**
6. **Image Type** menu **–** to select what colour plane or combination of colour planes in the photo that forms the basis for generation of the **Image**. Features of the object appear in different discernment using different **Image** types. The available **Image** types are: *Red plane (*default*), Green plane, Blue plane*, the *difference between the Green and the Blue plane* and the *difference between the Red and Green plane*.
7. **Colour Map –** to set the colour map employed to display the **Image** (does not influence the calculated irregularity profile). The available maps are: *copper* (default), *gray, pink* and *hot*.

8. **Background Type –** select to match the colour of the physical background used. The available background types are: *auto* (default), *white, black, green* and *blue. Auto* automatically adapts to a physical background of white or black colour and should be tested as the first alternative.

9. **Rotate** – to rotate the **Image** successively by 90 degrees. The hair should preferably be as much in a horizontal position as possible to facilitate the drawing of the line between the two reference points along which the irregularity is calculated.

10. **Analyze** – to open the *Analyse98* window for further numerical analysis of object features such as surface irregularity and diameter.

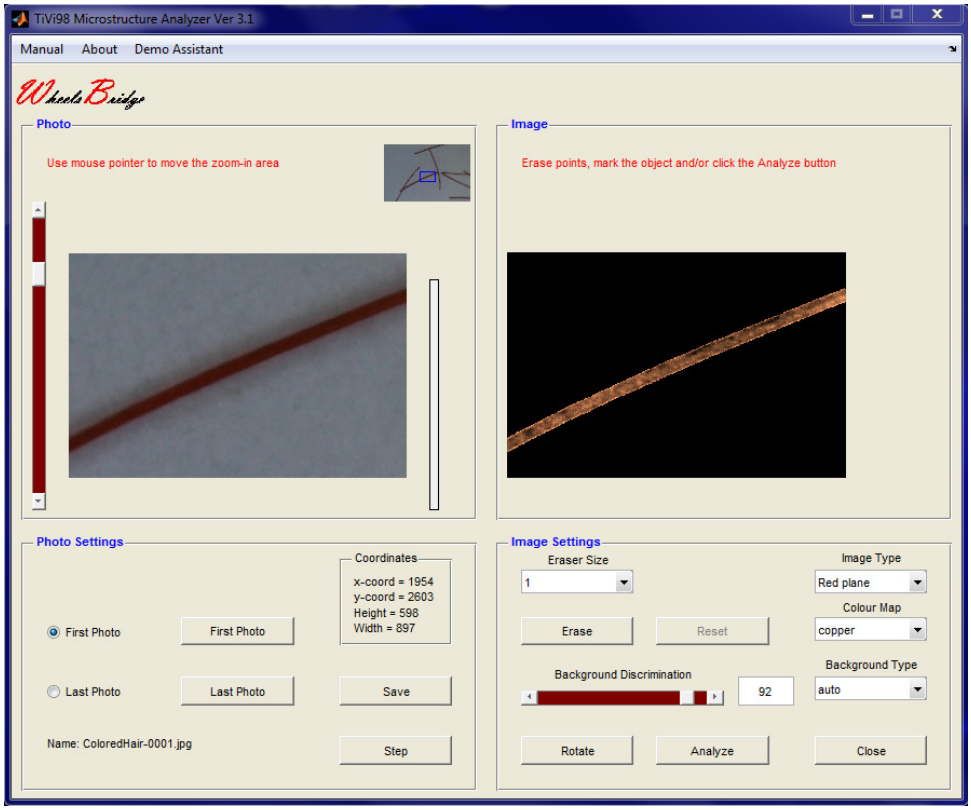
11. **Close** – to close the *TiVi Microscope* window.

12. **Manual** pull-down menu **–** open the on-line version of this manual.

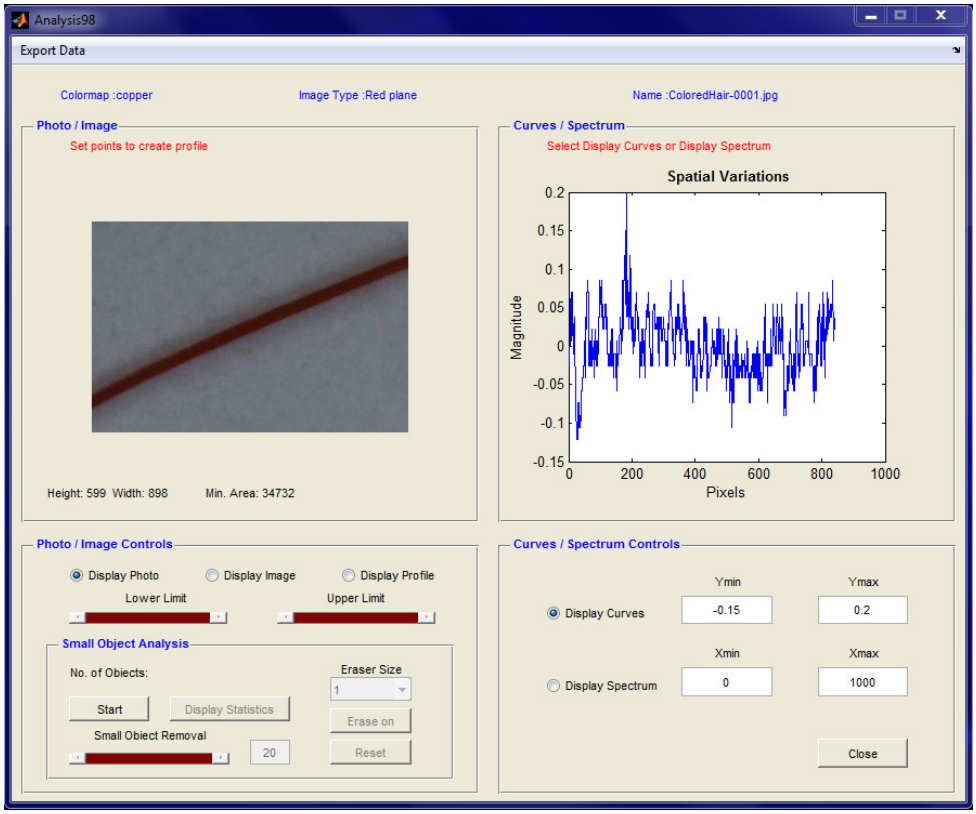
13. **About –** about the *TiVi Microscope Tivi98*.

When the **Analyze** button is clicked the *Analyz98* window is opened. In the example below a hair has been zoomed in and the two reference points have been set in the **Image.**

Preparation of the object in the *TiVi Microscope TiVi98* window:

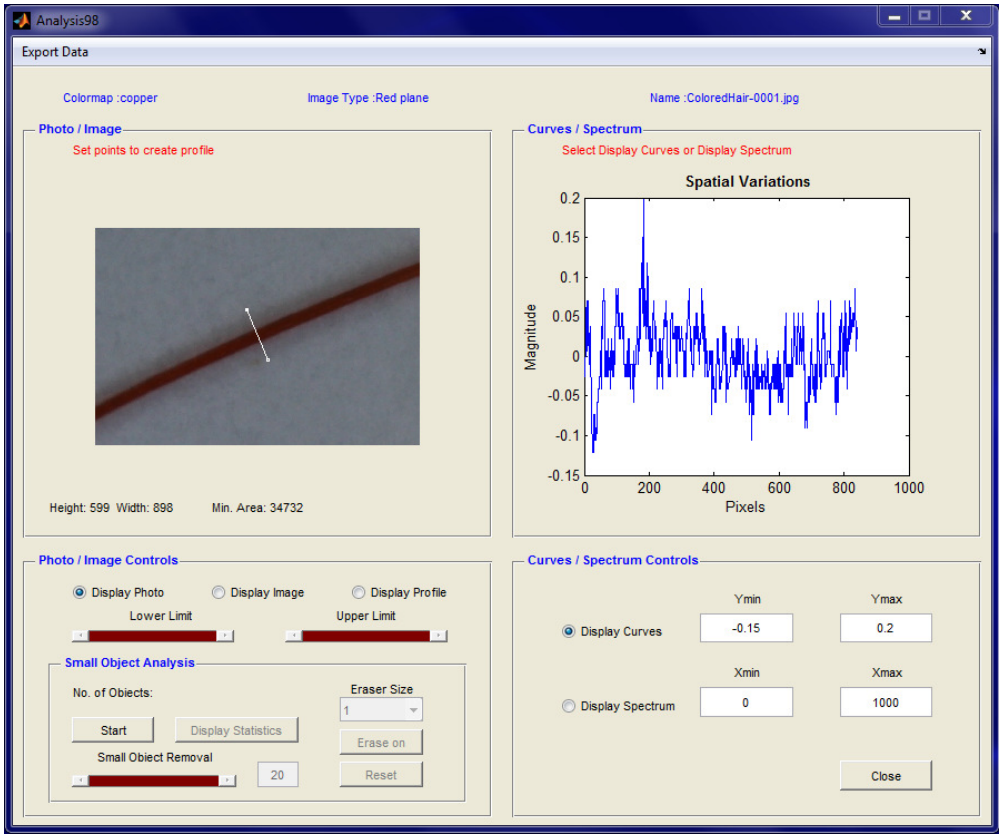


After having clicked the **Analyze** button to open the *Analyze98* window:



The **Photo / Image** panel displays either

1. The **Photo**, or
2. The **Image** with superimposed reference points and the line along which the irregularities are calculated, or
3. The **Profile** along the cross profile trajectory.
4. Two reference points can be positioned in the **Photo** by use of the mouse to generate a line along which the cross profile is generated.



The **Curves / Spectrum** panel displays either

1. The **Spatial Variations** in surface irregularity along the line trajectory between the two reference points, or

2. The **Periodogram** with the power spectral density corresponding to these surface irregularities. The red text on black background shows the total power of the segment displayed.

The **Photo / Image Controls** panel

1. **Display Photo** radio-button – to display the photo of the object with drawn cross profile trajectory if this exists.

2. **Display Image** radio-button – to display the Image with reference points and reference line.

3. **Display Profile** radio-button – to display the cross profile if the cross profile line is generated.

4. **Lower Limit slider –** to set the lower limit vertical line in the **Display Profile**.

5. **Upper Limit slider –** to set the upper limit vertical line in the **Display Profile**.

The **Curves / Spectrum Controls** panel

1. **Display Curves** radio-button – to display the **Spatial Variations** diagram in the **Curves / Spectrum** panel.

2. **Display Spectrum –** to display the **Periodogram** in the **Curves / Spectrum** panel**.**

3. **Ymin –** to set the minimum value of the y-axes in the diagram displayed in the **Curves / Spectrum** panel.

4. **Ymax –** to set the maximum value of the y-axes in the diagram displayed in the **Curves / Spectrum** panel.

5. **Xmin –** to set the minimum value of the x-axes in the diagram displayed in the **Curves / Spectrum** panel.

6. **Xmax –** to set the maximum value of the x-axes in the diagram displayed in the **Curves / Spectrum** panel.

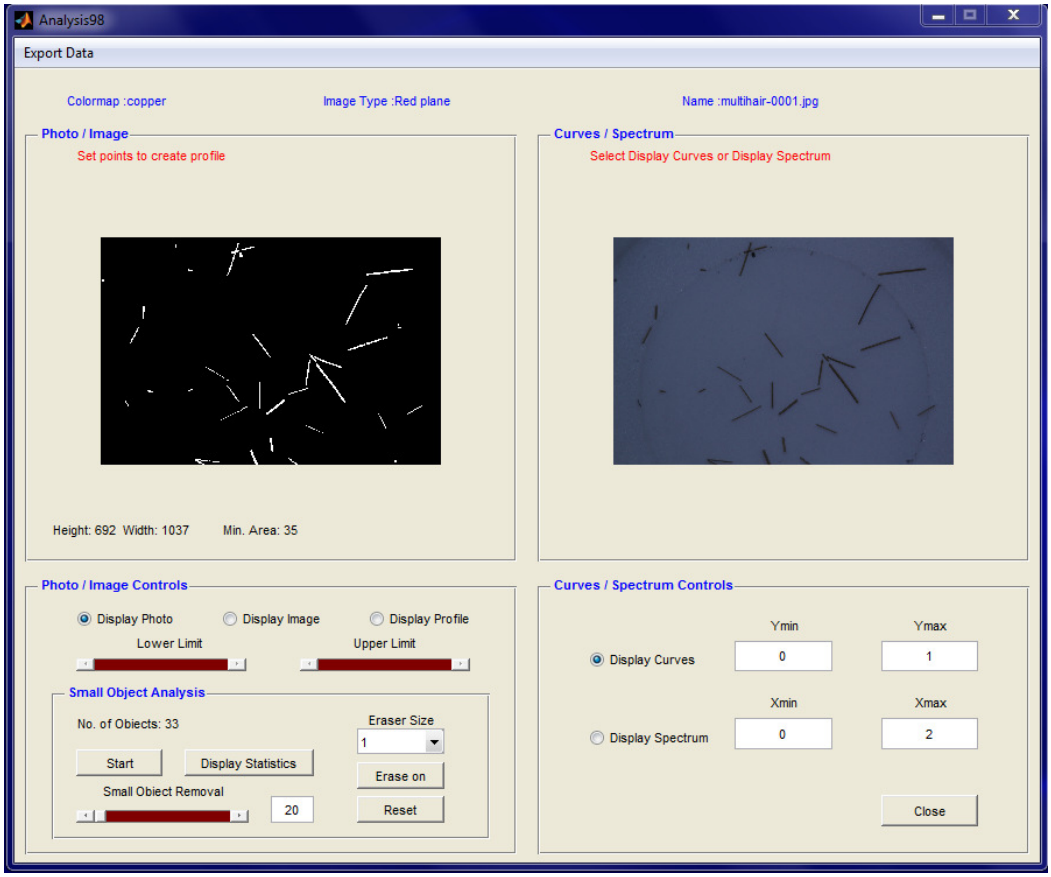
7. **Close –** to close the *Analysis98* window.

8. **Export Data** pull**-**down menu – to export the results generated to an Excel® spreadsheet.

The **Small Object Analysis** panel

The controls in this panel prepare for analyzing various properties of small similar objects in the photo.

1. **Start Button** – to covert the photo to a black and white image outlining the contours of the objects. The associate colour photo is temporarily displayed in the **Curves / Spectrum** panel.



2. **No. of Objects** – displays the number of objects identified and displayed.

3. **Erase On** – to activate the erase tool. With the eraser activated, positioning the mouse at a point in the black and white photo and pressing the left mouse button will erase pixels when the mouse is moved. This feature is useful when one wants to split up overlapping objects into separate objects.

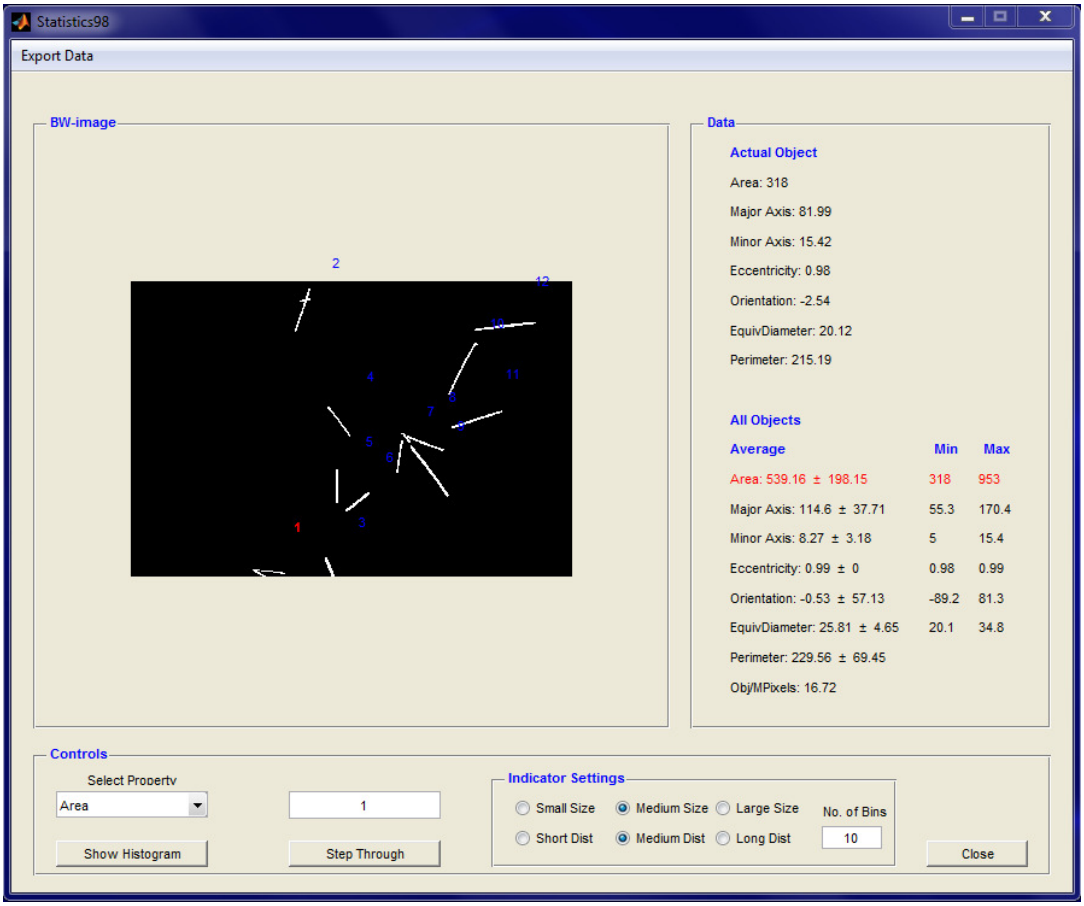
4. **Eraser Size** – to set the size of the eraser brush.

5. **Reset** – resets the black and white photo.

6. **Small Object Removal slider** – to remove objects with a size below the actual threshold. When using this feature the actual number of remaining objects is updated in the **No.of Objects** text-string.

7. **Small Object Removal edit box** – displays the setting of the **Small Object Removal slider** as the threshold area limit (pixels) for an object to be deleted.

8. **Display Statistics** – to export the black and white photo and open the **Statistics98** window.



1. **BW-image** – displays the black and white image of each object numbered by an unique index label. The object in focus is indicated by a red label.

2. **Data panel** – Display the **Actual Object** data (indicated by the red index in the black and white photo) as well as the average, minimum and maximum values for all properties of the black and white photo.

    a. **Area** – area of object in pixels.

    b. **Major Axis** – length of the major axis of an ellipse that encapsulates the object (generally the length of the object).

    c. **Minor axis** – the length of the minor axis of an ellipse that encapsulates the object (generally the width or diameter of the object for a straight object).

    d. **Eccentricity** – the eccentricity of an ellipse that encapsulates the object.

    e. **Orientation** – the angle (in degrees) between a horizontal line and the major axis of an ellipse encapsulating the object.

    f. **EquivDiameter** – the diameter of a circle that has the same area as the object (pixels).

    g. **Perimeter** – the distance around the boundary of an object.

h. **Obj/MPixels** – number of objects per one million pixels (object density).

3. **Control panel** - holding controls for manipulation of the displayed image and histogram.

4. **Select Property** pull-down menu – to select the black and white image property that is to be displayed in the **Histogram.**

5. **Show Histogram / Show Image** – alternatively displays the **Histogram** and black and white image.

6. **Step Through button** – to successively display the various objects in focus. The index of the actual object in focus is displayed in red colour. The actual index is also displayed in the **Step Through** index edit box.

7. **Step Through edit box** – displays the index of the object in focus. To jump directly to a certain object print the actual index in the edit box.

8. **Indicator Settings** – to set indicator parameters and the number of bins in the **Histogram**.

9. **Small Size** – click to use a small size indicator number.

10. **Medium Size** – click to use a medium size indicator number.

11. **Large Size** – click to use a large size indicator number.

12. **Small Dist** – click to use a small object-indicator distance.

13. **Medium Size** – click to use a medium object-indicator distance.

14. **Large Size** – click to use a large object-indicator distance.

15. **No. of Bins** edit box – to set the number of bins in the **Histograms**.

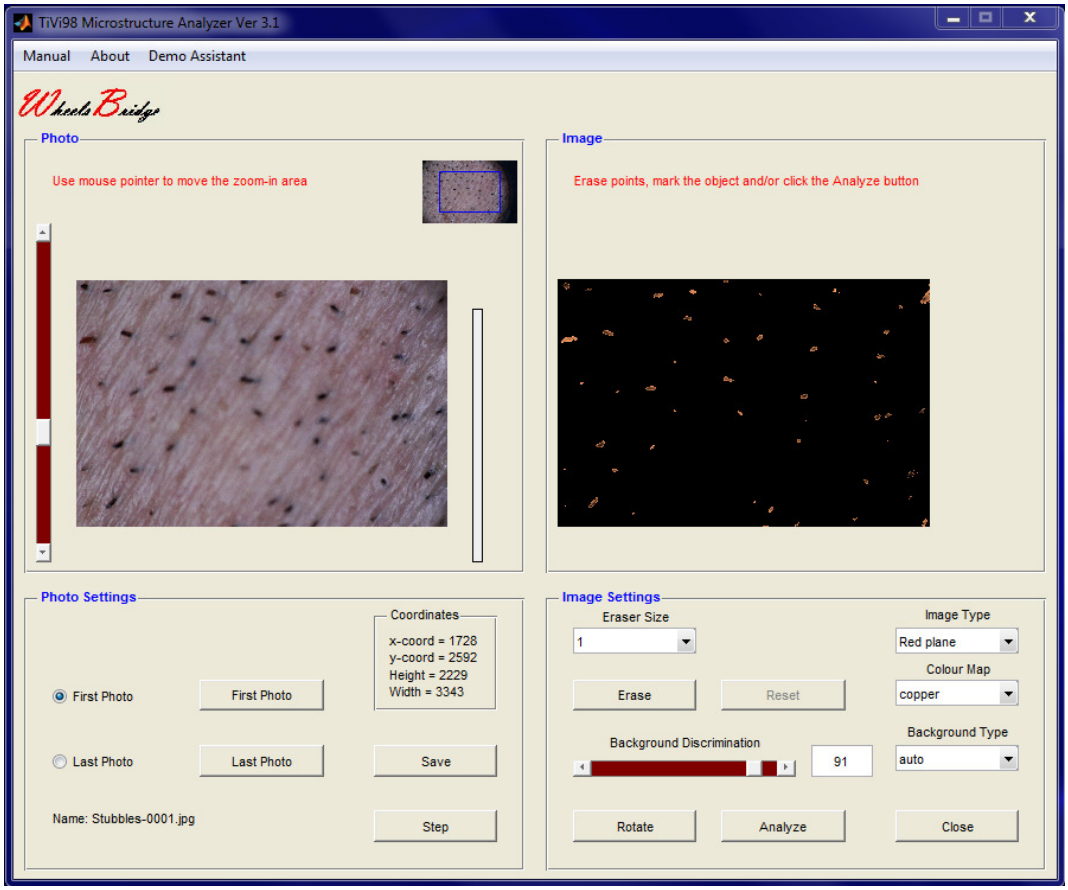
16. **Export Data** – to export data to external spread-sheet.

17. **Close –** to close the window.

4. EXAMPLES

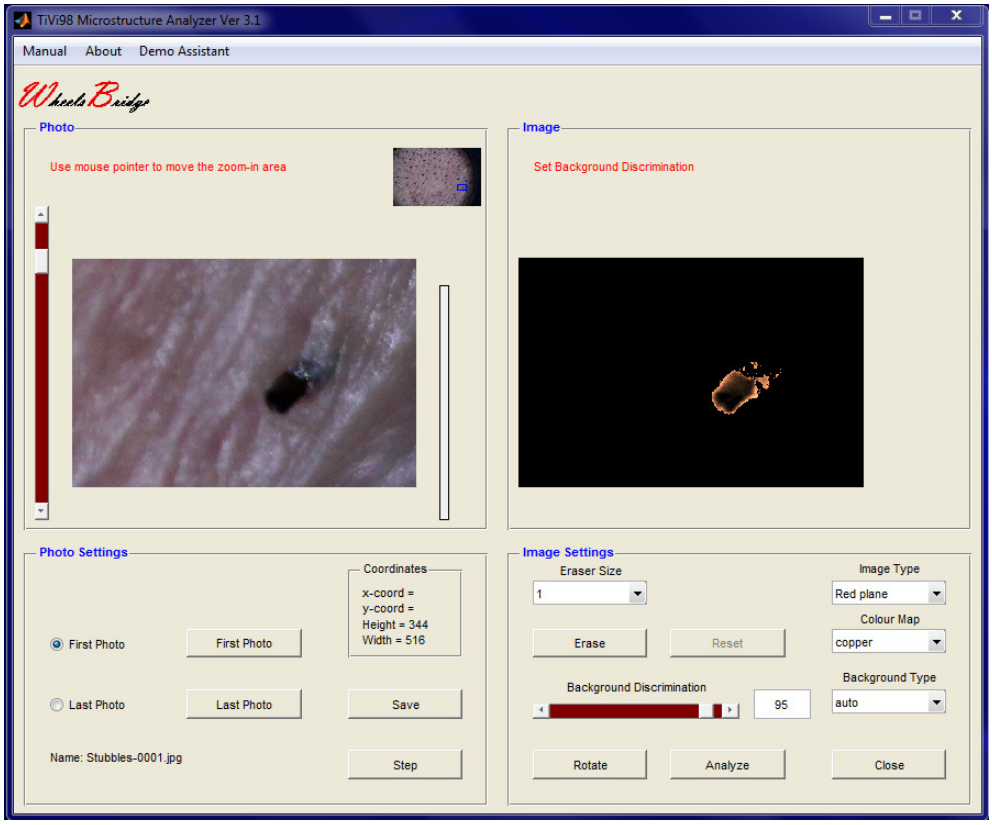
IN_VIVO APPLICATIONS

Assessment of stubble on cheek (24 hours after last shaving)

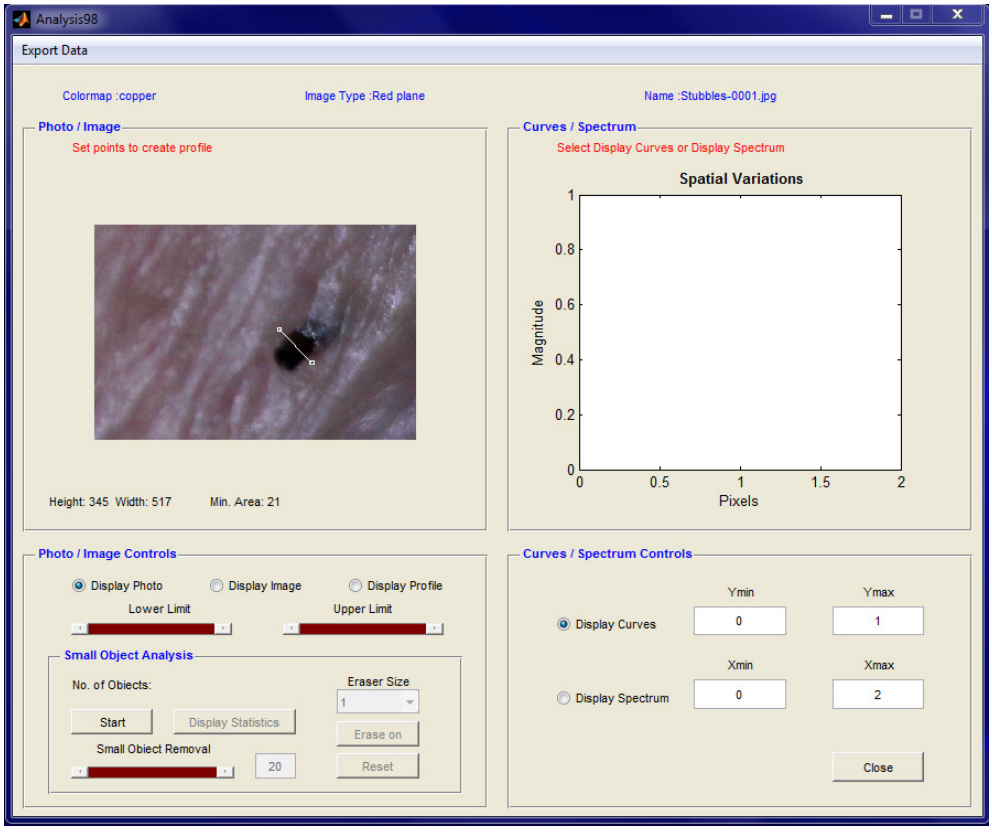


The field of view is 2136 x 3204 pixels or about 6mm x 9 mm. After having set the background threshold density estimates can be performed in the **Image**. In this section there are about 0.8 objects per square mm.

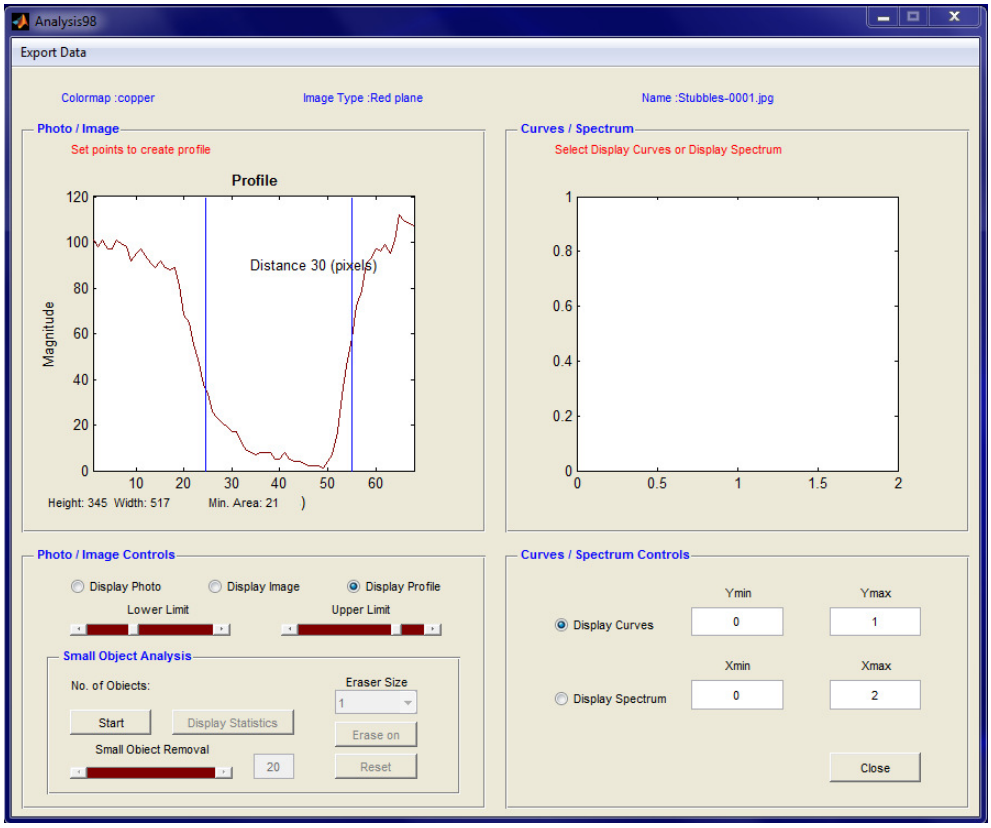
Zooming-in on individual objects makes it possible to estimate individual object diameter and length (and thereby the growth-rate).



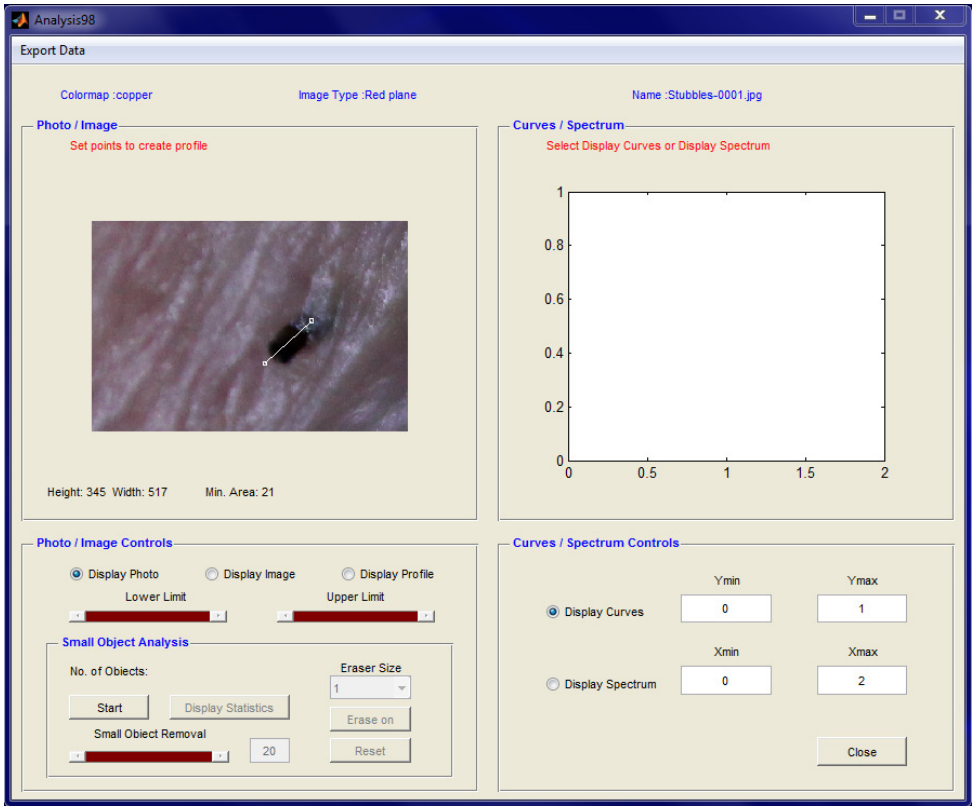
After opening the *Analyze98* window, a cross profile line is generated.



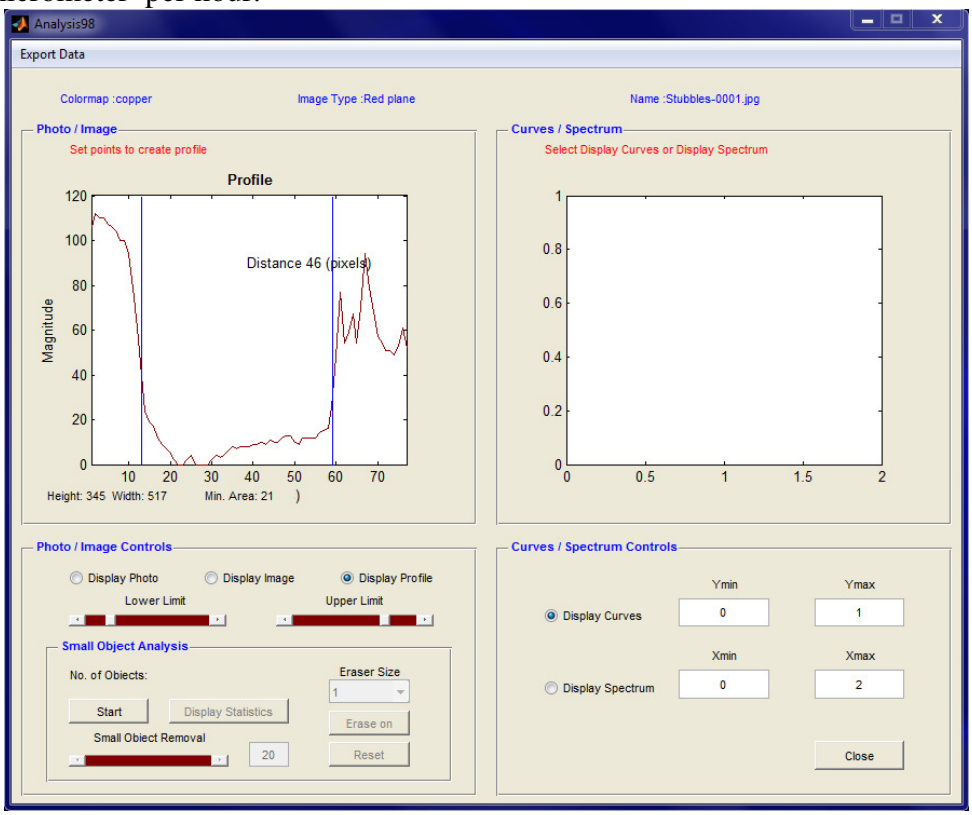
After clicking the **Display Profile** button and adjusting the **Upper Limit** and **Lower Limit** sliders to coincide with the boundaries of the hair, the diameter can be estimated to be 30 pixels or equivalently for a calibrated system 90 micrometers.



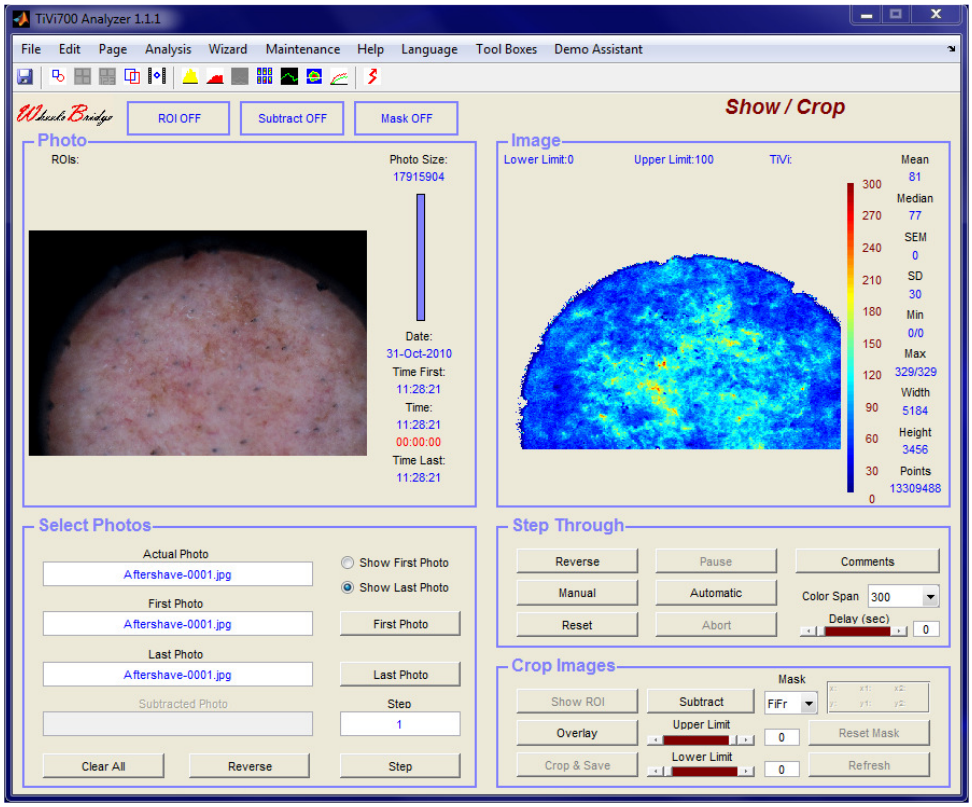
Going back to the **Display Photo** view and generating a new cross profile line:



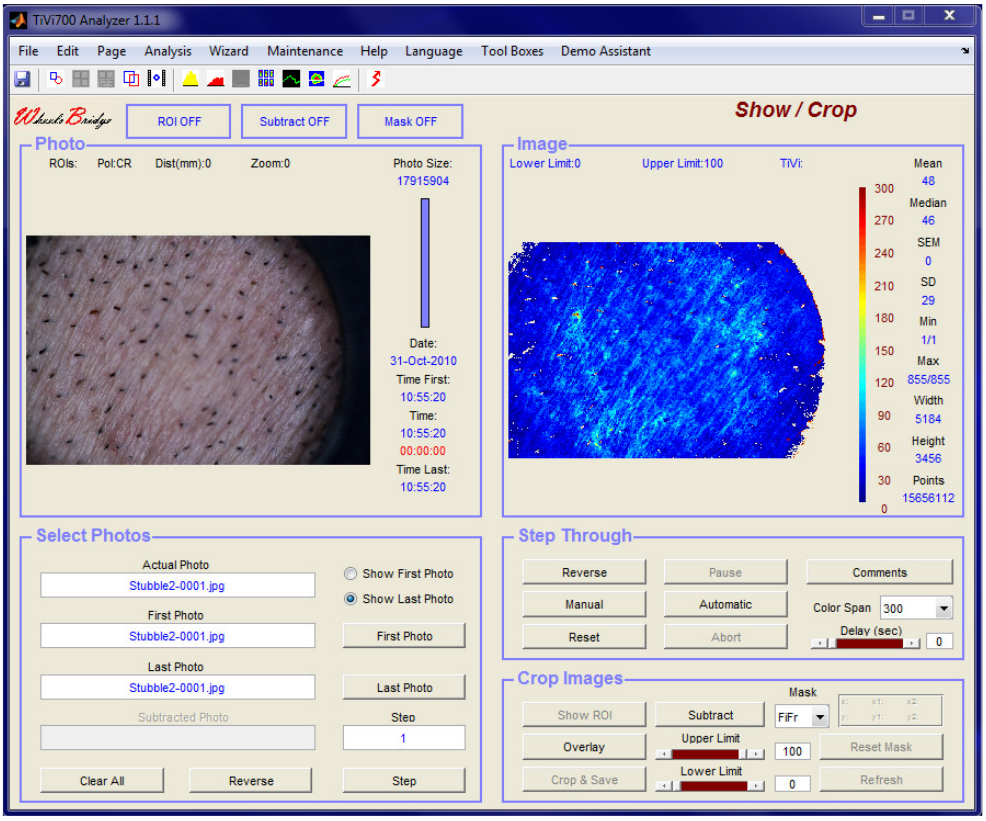
After clicking the **Display Profile** button and adjusting the **Upper Limit** and **Lower Limit** sliders to coincide with the boundaries of the hair, the length can be estimated to be 46 pixels or equivalently for a calibrated system 138 micrometers, corresponding to a growth rate of about 5.6 micrometer per hour.



Assessment of stubble and skin erythema immediately after shaving



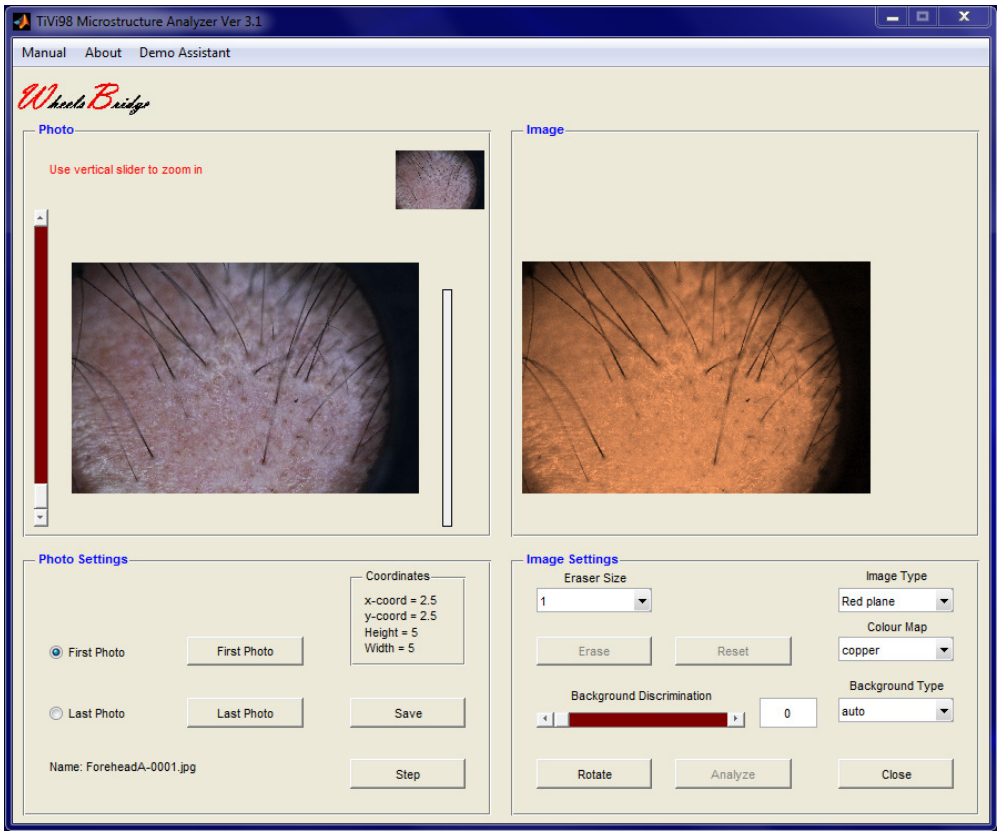
The **Photo** and **Image** in the *TiVi700 Analyzer* window above illustrates the situation immediately after shaving. Local areas of erythema due to skin irritation caused by the razor are mixed with less influenced areas. The average *TiVi* value is 81 *TiVi*-units, considerably higher than in the corresponding area 24 hours after shaving (*TiVi*-value = 48) displayed in the TiVi main window below.



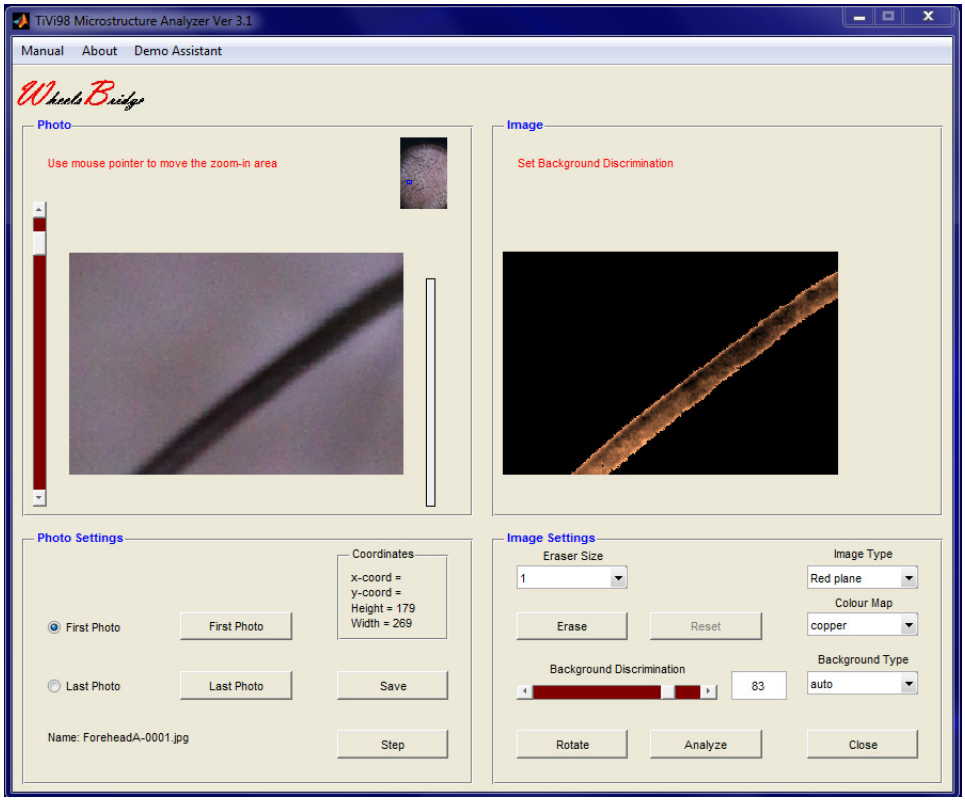
After exporting the photo captured immediately after shaving to the *TiVi Microscope* software and zooming-in on an individual stubble, the length and the diameter of the actual stubble using the same method as above could be estimated to 10 and 27 pixels respectively (corresponding to 30 and 81 micrometers).

Assessment of forehead hair

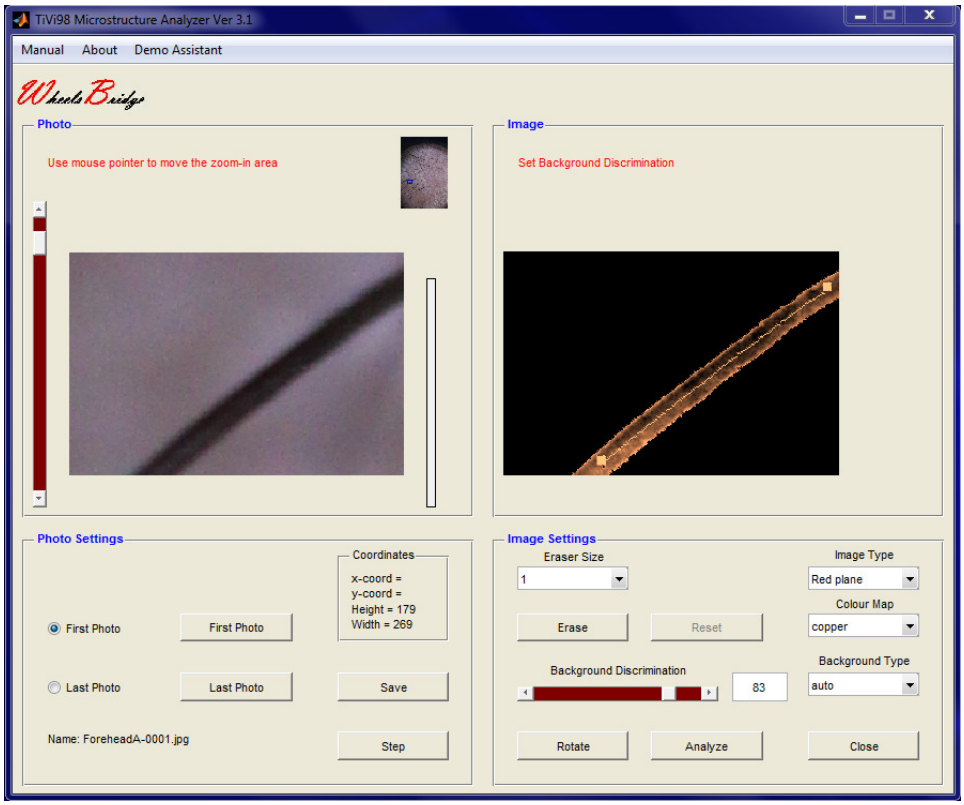
The **Photo** in the *TiVi Microscope* window below covers a field of view of 3456 x 5184 pixels corresponding to an area of about 10mm x 15 mm.



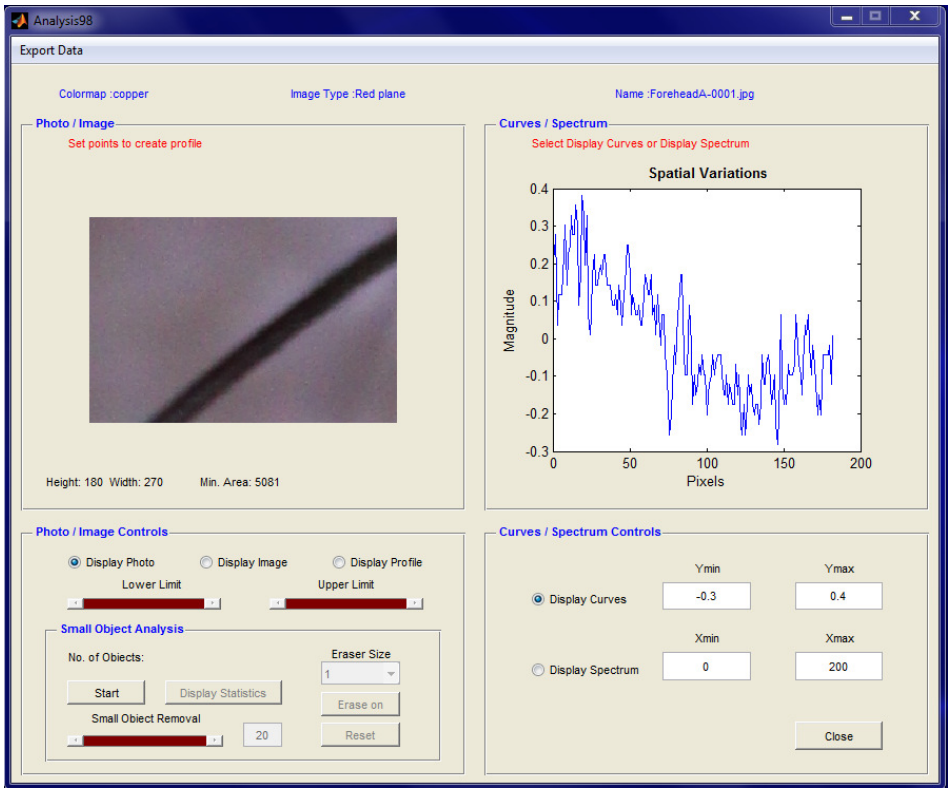
After having zoomed-in on an individual hair in focus, rotated the **Photo** and **Image** and adjusting the **Background Discrimination** the following window is displayed.



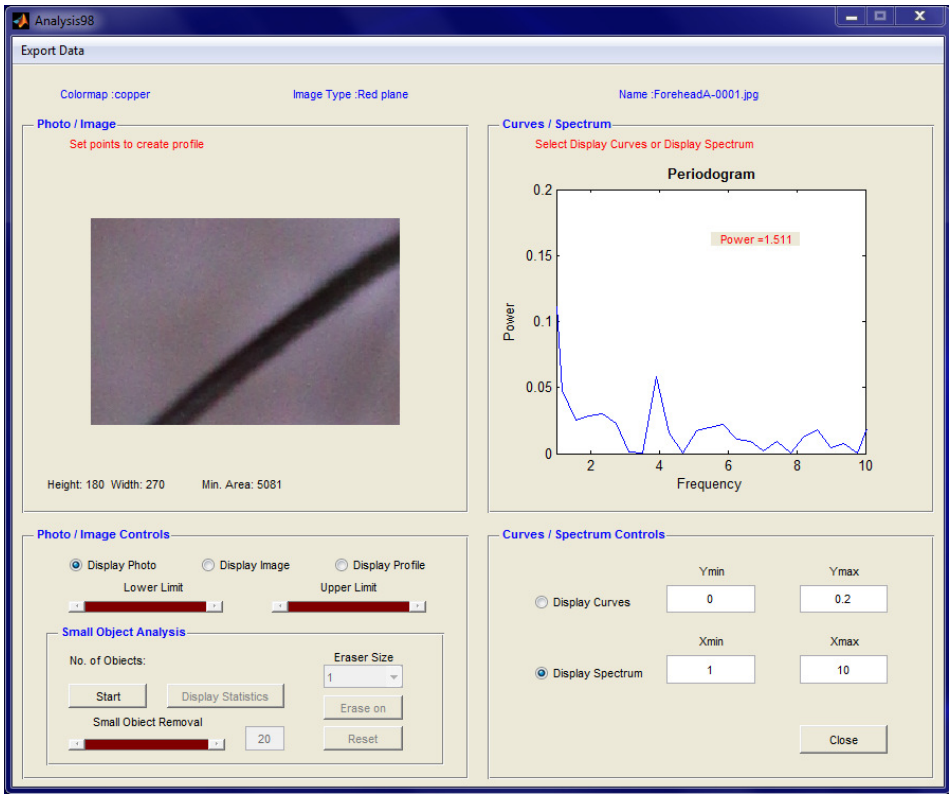
Two reference points are placed on the object to generate a line along which the irregularities cab be analyzed.



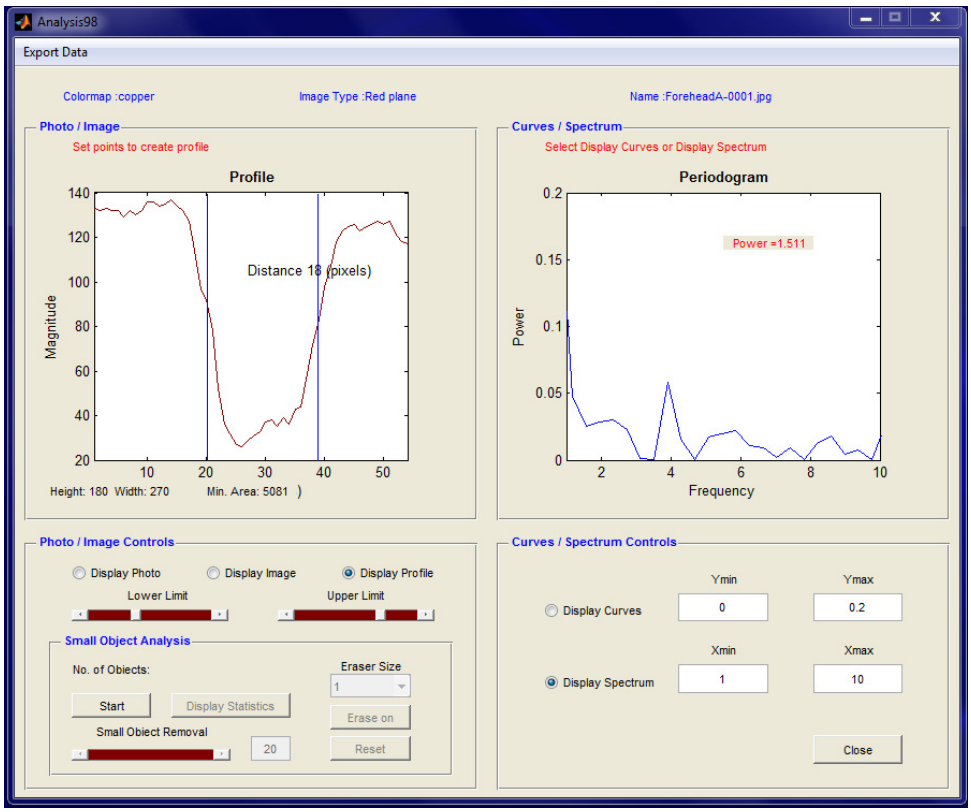
After clicking the **Analyze** button to open the *Analyze98* window the irregularities in the surface structure are displayed in the **Spatial Variations** diagram.



After clicking the **Display Spectrum** radio-button, the **Periodogram** is displayed.



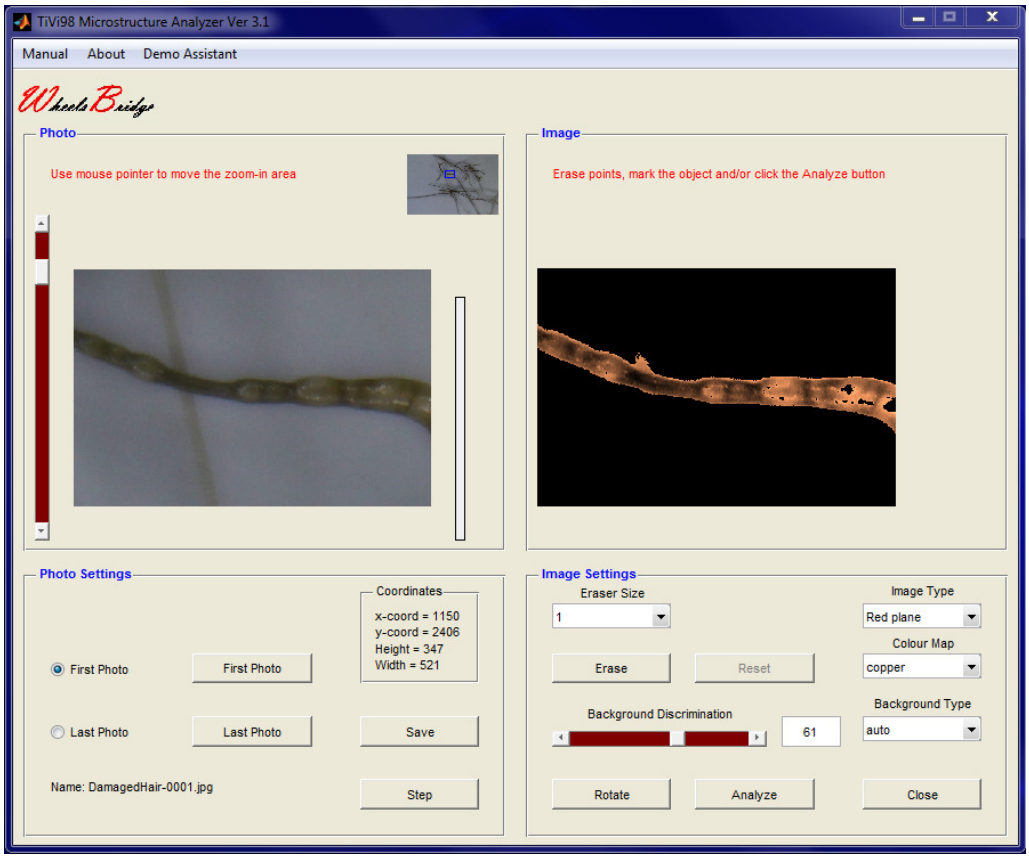
After having set reference point in the photo for generating the reference cross line along which the cross profile is generated, the diameter of the hair can be calculated to 18 pixels (54 micrometers).



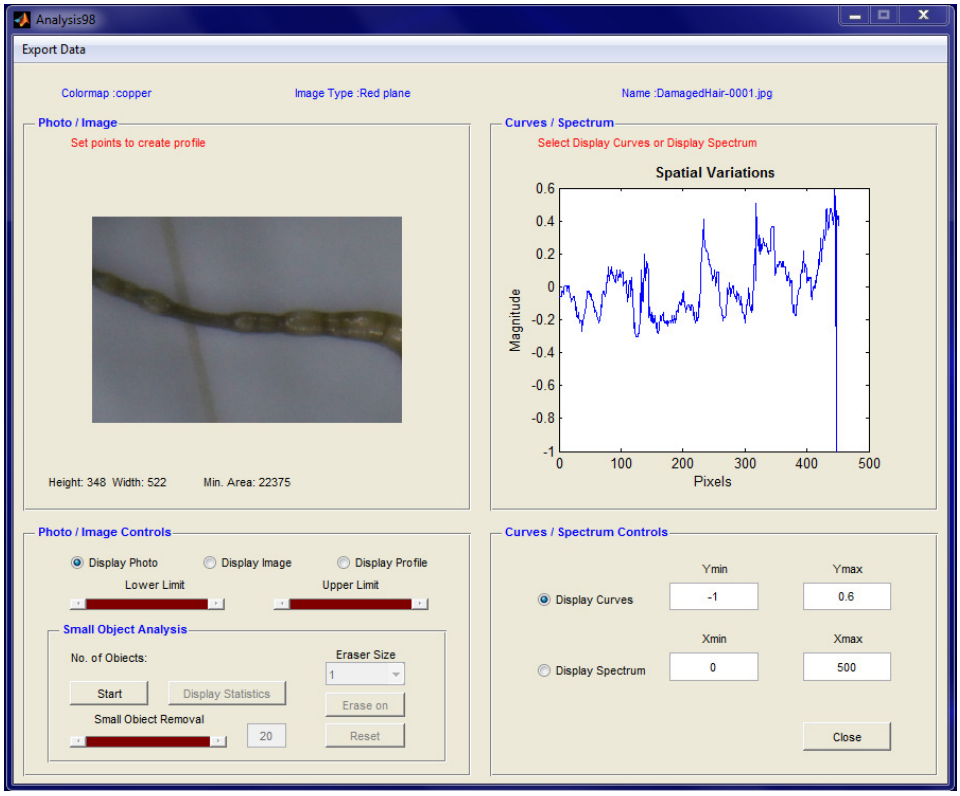
IN-VITRO APPLICATIONS

Assessment of damaged hair

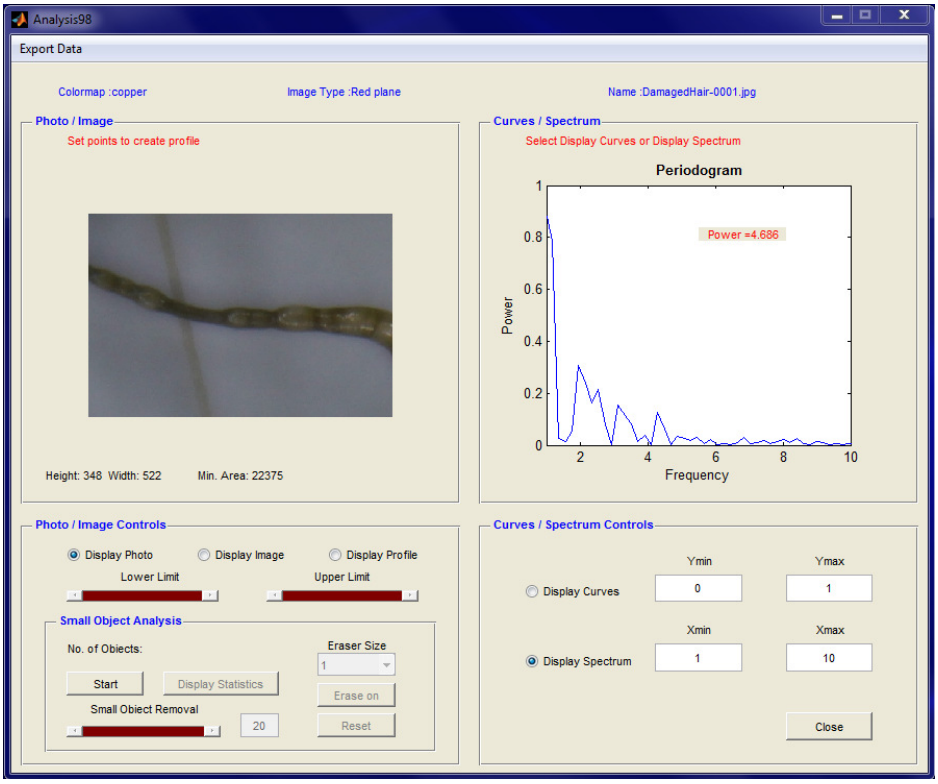
The *TiVi Microscope* window below displays a hair sample damaged by heat. Note the irregularities in the surface structure of the hair in the **Image** which are reflected in the **Spatial Variations** diagram and the **Periodgram** in the *Analyze98* window.



Analyze98 window with **Spatial Variations** below



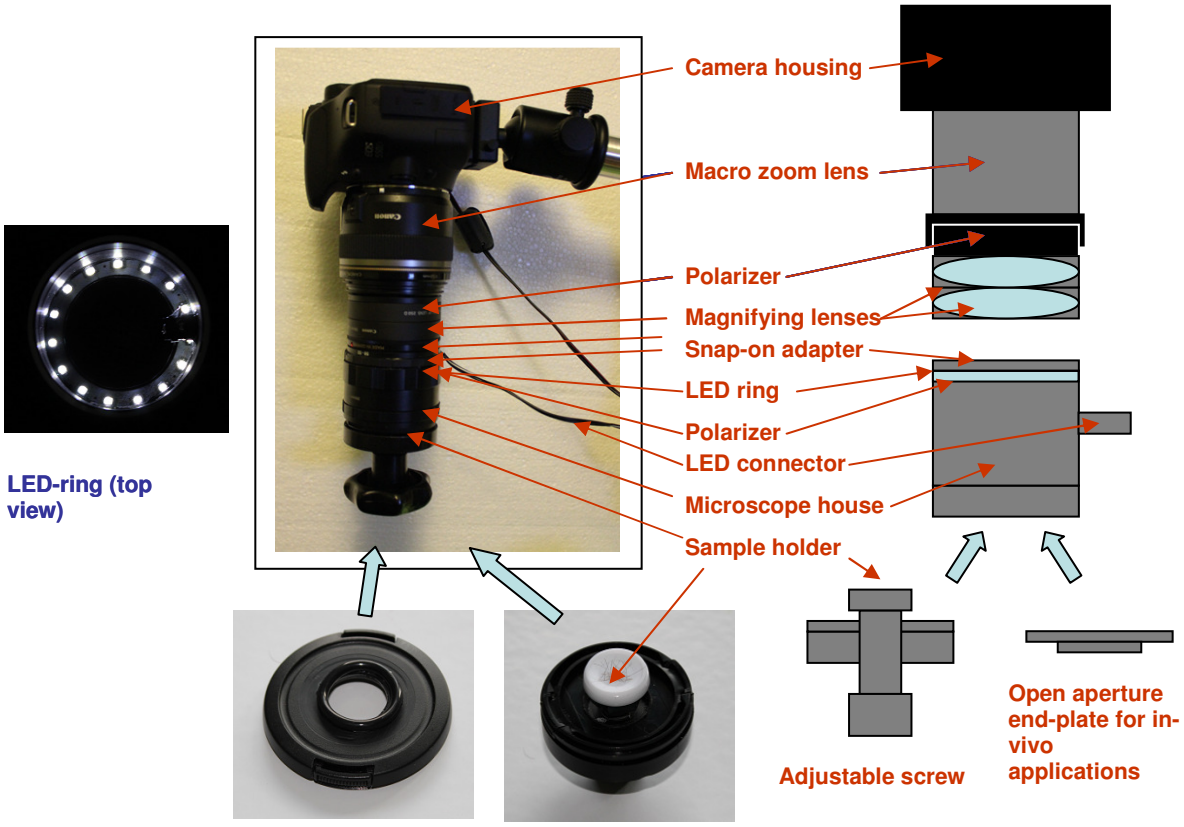
Note that periodicity in the **Spatial Variations** are reflected as a peak in the **Periodogram** at a frequency of about 2, characterizing the damage in terms of an index. The power of irregularity within the frequency range 2 to 2.75 can be calculated after setting the **Xmin** and **Xmax** to include this bandwidth only.



The **Periodogram** with **Xmin** and **Xmax** set to 2 and 2.75 respectively results in a power index of 1.939.

5. SETTING UP THE TiVi MICROSCOPE

This section describes how to set up the *TiVi microscope* and how the set it to operate in cross- and co-polarization mode respectively. The Fig. below shows a photograph and a cross-section diagram of the *TiVi Microscope*.



Assembling the TiVi Microscope

1. Start with replacing the standard *TiVi700* Canon EFS 18-55 mm zoom-in objective with the Canon Macro Lens EF-S 600 mm objective.

2. Combine the two magnifying lenses into a single package (screw thread).

3. Screw this package onto the polarizing filter in Canon Macro Lens EF-S 600 lens.

4. Snap on the microscope house to the lower magnifying lens.

5. Select the sample holder to be used and screw it onto the adjustable screw.

6. Connect the LED ring power supply to the LED connector.

7. Place the sample to be analyzed in the sample holder and attach this to the microscope house.

8. The microscope is now ready for use.

Adjusting the TiVi Microscope

1. To prepare the *TiVi Microscope* for either co- or cross-polarization mode, first rotate the adjustable screw with the sample holder clockwise to its end-point position.

2. Then turn the Macro lens focusing ring anti-clockwise to its end-position.

3. The LEDs are now visible in the camera view window.

4. Turn the Microscope house in relation to the magnifying lenses and note how the LEDs appear brighter (co-polarized mode) or darker (cross-polarized mode).

5. Adjust the Microscope house until the LEDs appear brightest (co-polarized mode).

6. Turn the Macro lens clock-wise until it reaches its end-point position. This corresponds to the highest magnification.

7. Turn the adjustable screw with the sample holder anti-clockwise until the object is in focus as viewed through the camera view window.

8. The TiVi Microscope is now ready for capturing photos.

6. CAPTURING A PICTURE

This section explains how to capture a *TiVi Microscope* photo of hair samples.

1. If the hair sample is dark, select a white sample holder background to attain optimal contrast.

2. Place the sample hairs on the sample holder.

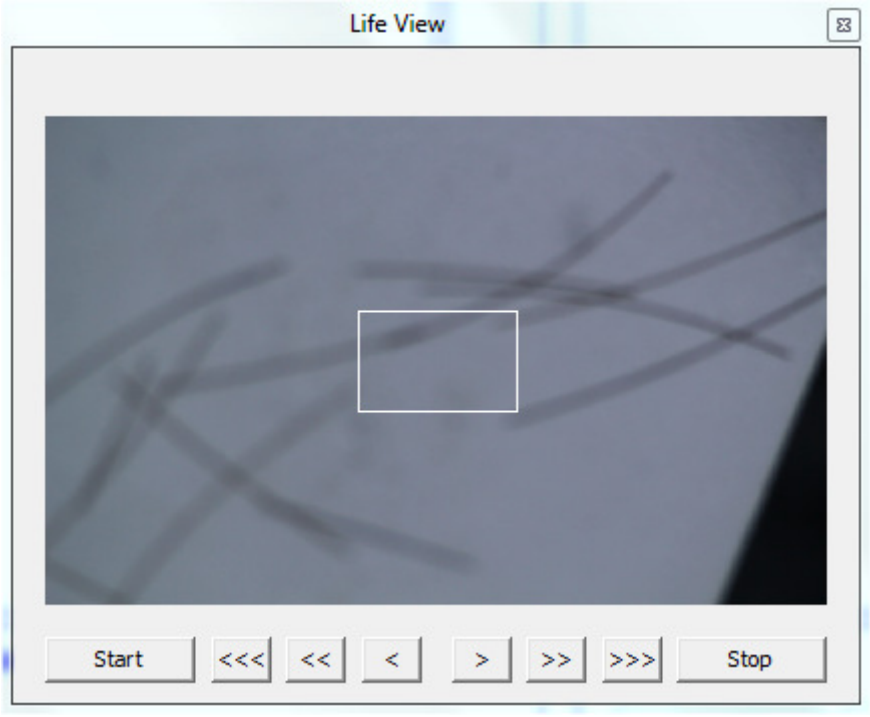
3. Snap on the sample holder plate to the lower part of the *TiVi Microscope*.

4. Turn the *TiVi Microscope* tube to capture a photo in cross-polarized or a co-polarized mode (see section above).

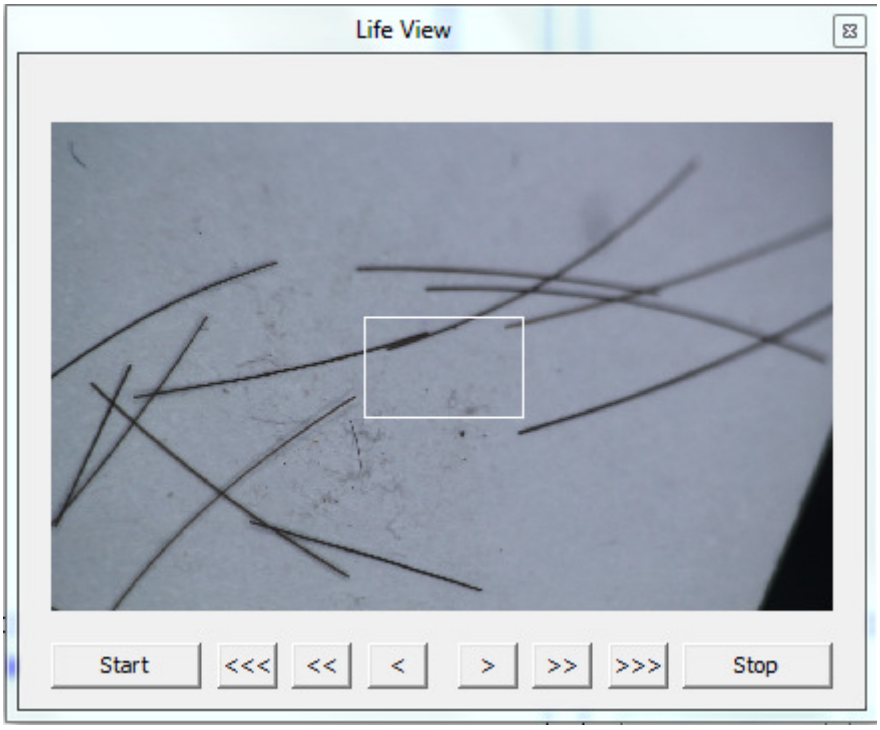
5. Select **Start Camera** from the *TiVi600* main window pull-down menu to open the *EDSDK Camera Settings* window.

6. Click the **Life View** button to start *Life View*.

7. Click the **Start** button. The **Life View** should now display the object



8. Adjust the focus with the **Arrow** buttons or with the Zoom ring on the Macro lens.



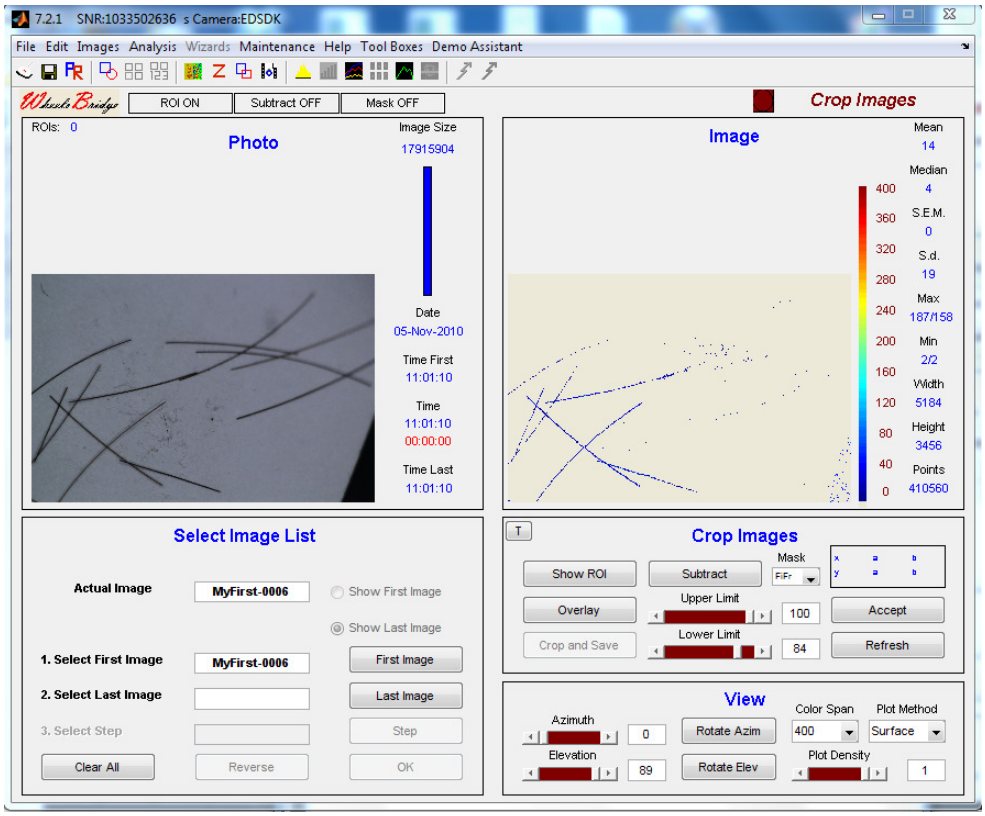
9. Rotate the sample and re-focus as necessary.

10. Click the **Stop** button to stop video recording and then click the delete (cross) button in the upper right corner of the **Life View** window to close the window.

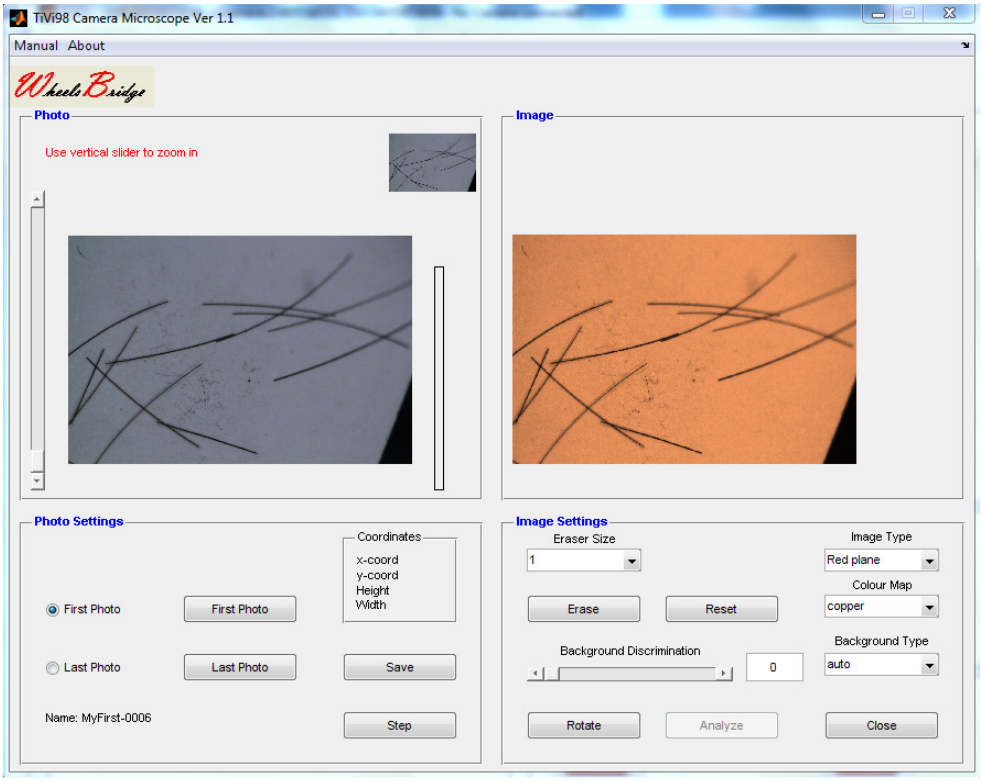
11. Click the **Save Photos** in the *EDSK Camera Settings* window and write "MyFirst" in the FileName edit box. Click **Save**. The photo to be captured will now be saved under the name *MyFirst-0001* in the folder selected.

12. Set **Select Photo Size** to **Large Fine** and **No of Photos** to 1.

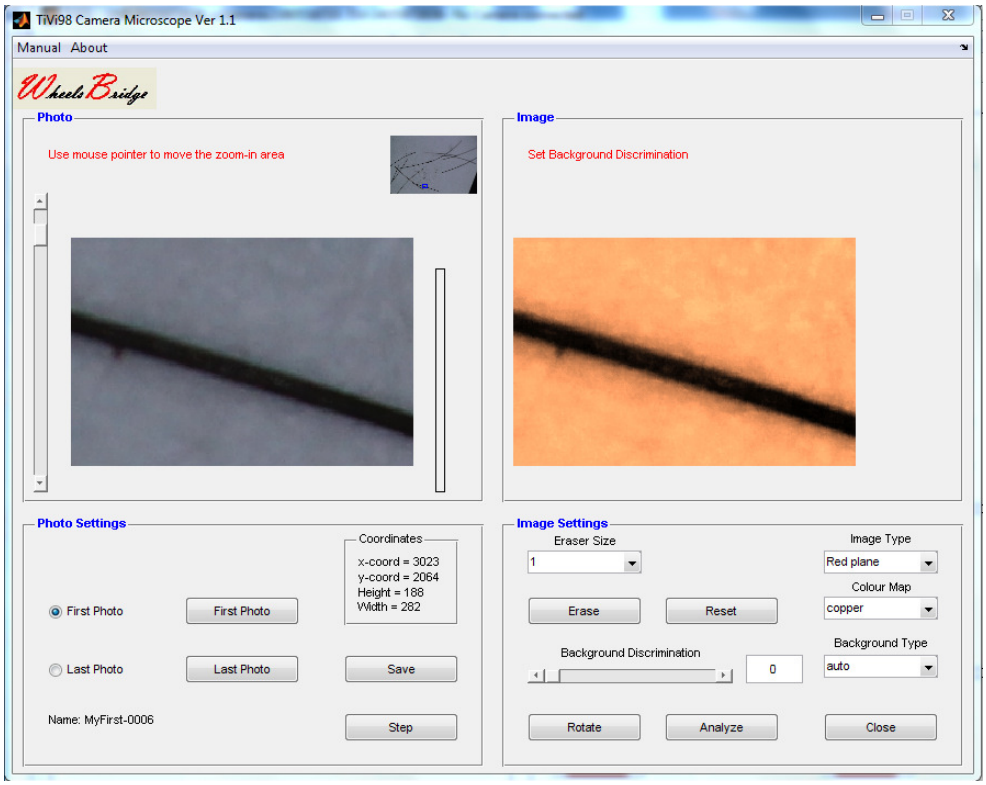
13. Click the **Capture Photos** button to capture the photo. After a few seconds the *TiVi600 main* window should look like:



14. Select *TiVi Microscope* in the *Tool Boxes* pull-down menu to export the photo to the *TiVi Camera Microscope* window.

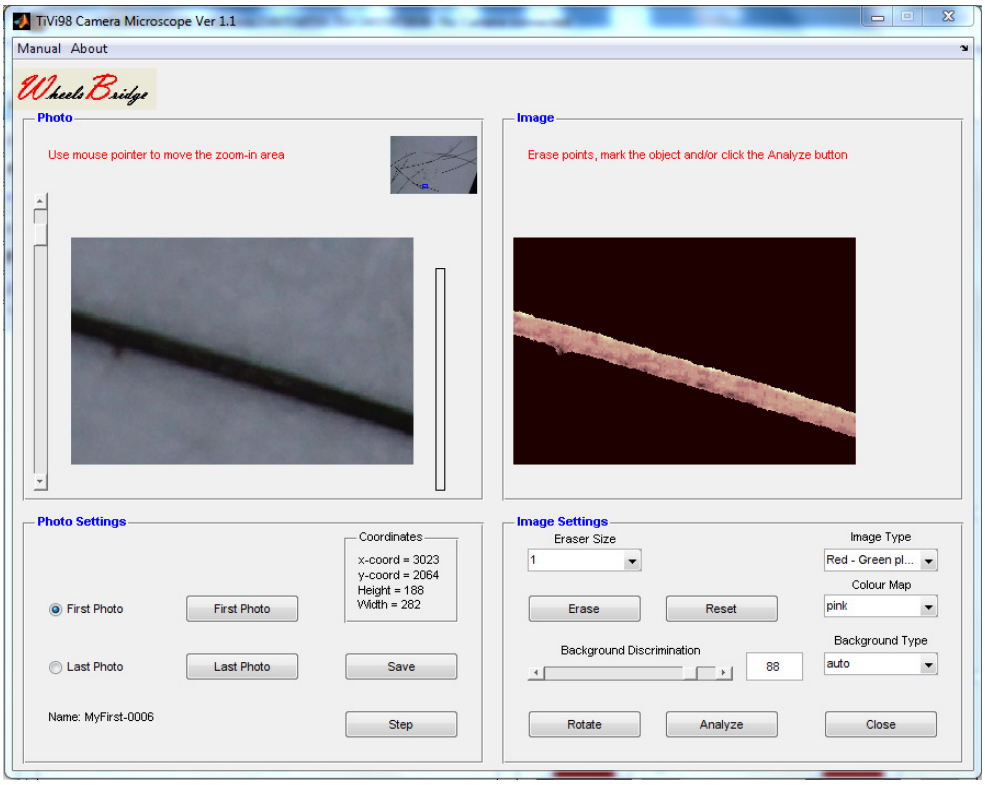


15. Move the vertical slider upwards to about 80% of its maximum value. Move the zoomed-in window until a single hair at good focus is displayed.

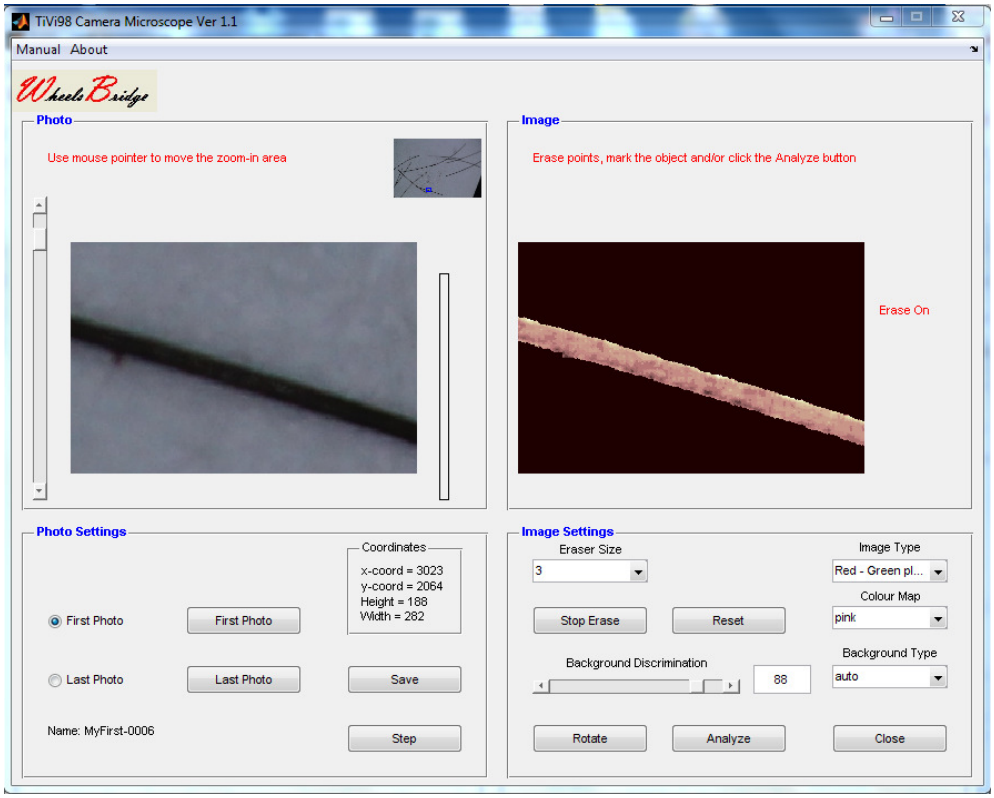


16. Drag the **Background Discrimination** slider to the right to generate the background.

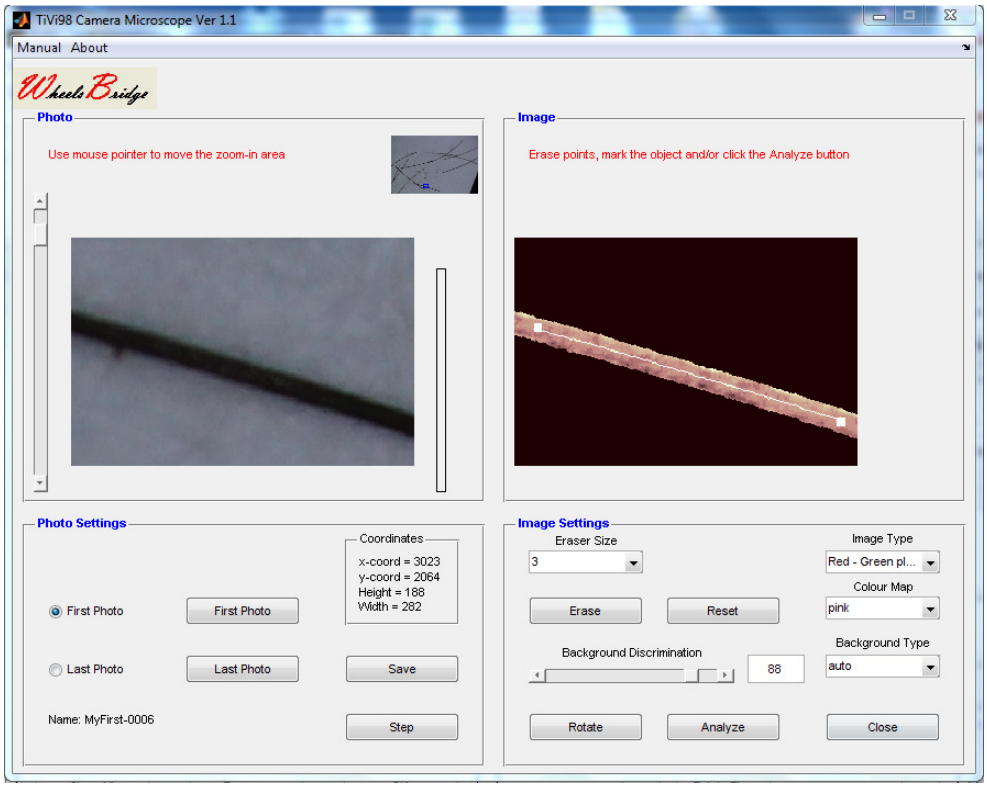
17. Examine the effects of choosing different **Image Types** and **Colour Maps** on the **Image**. After selecting *Red – Green Plane* as **Image Type** and *pink* as **Colour Map** the **Image** should look like:



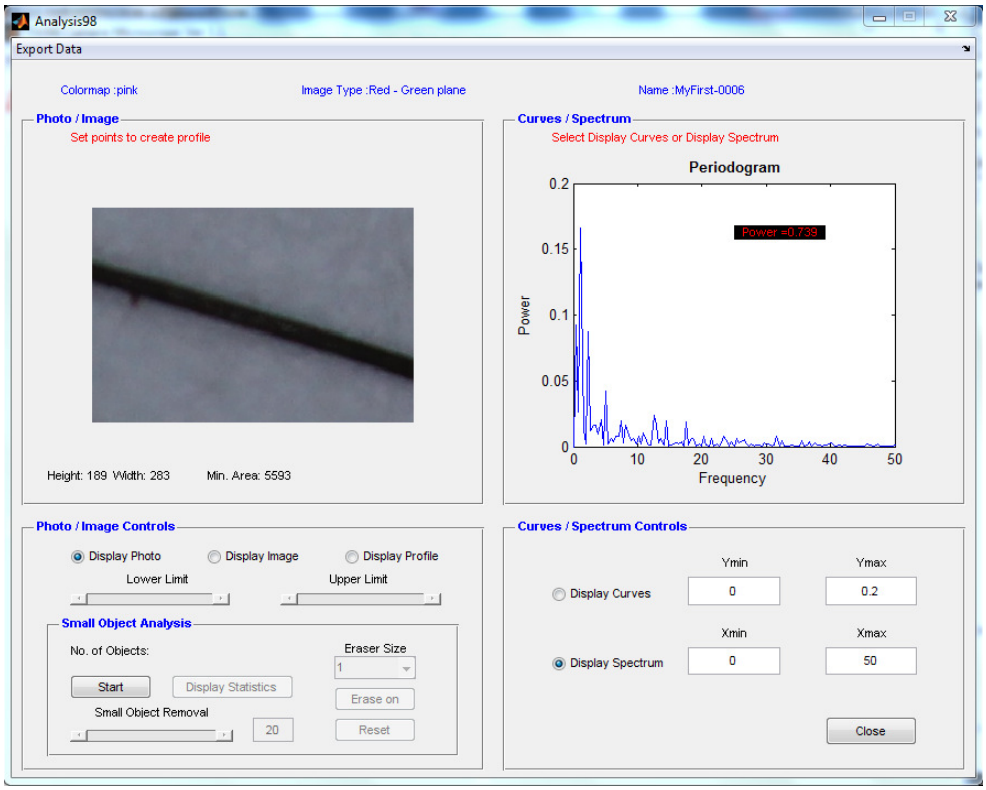
18. Click the **Erase** button to activate the **Erase** tool. Select **Erase Size** 3 and carefully move the mouse pointer with the left mouse button pressed to remove irregularities at the edge of the hair.



19. Click the **Stop Erase** button and place two reference points towards the ends of the visible parts of the object.



20. Click the **Analyze** button to get to the *Analyze98* window and continue with the analysis of the hair sample.



This completes the **CAPTURE A PICTURE** session.

7. CALIBRATION

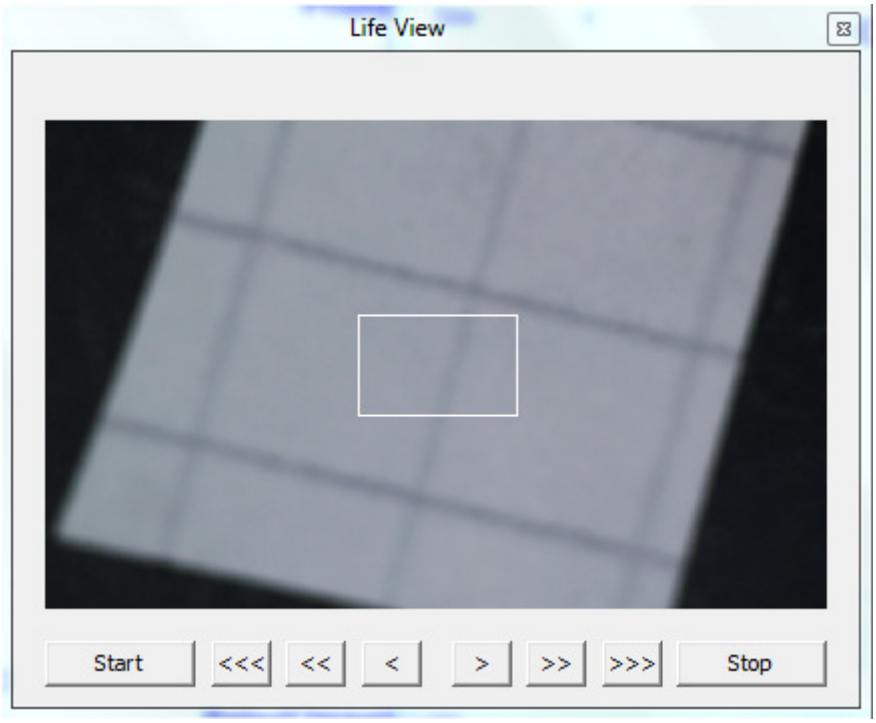
This section explains how to calibrate the *TiVi Microscope* magnification factor.

1. Place a paper with a known metric pattern such as squares of 5 x 5 mm on the sample holder.

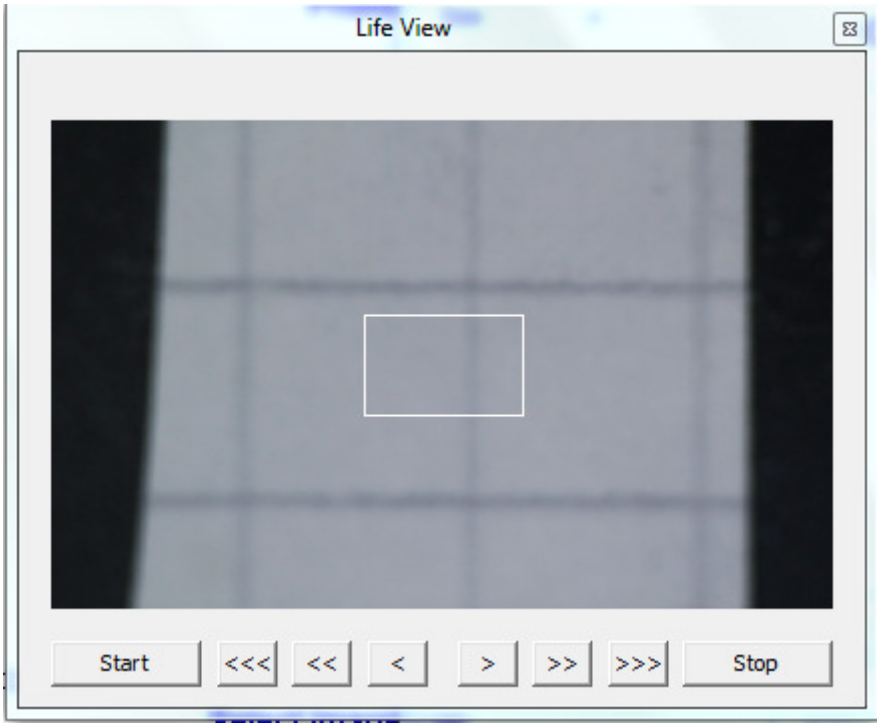
2. Select **Start Camera** from the *TiVi600* main window pull-down menu to open the *EDSDK Camera Settings* window.

3. Click the **Life View** button to start *Life View*.

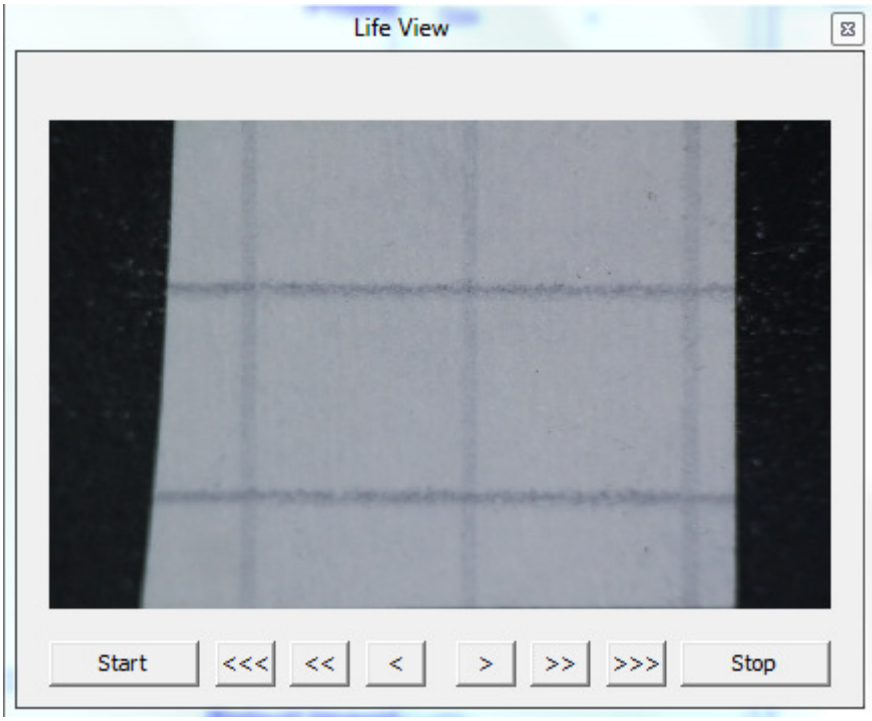
4. Click the **Start** button. The **Life View** should now display the object.



5. Rotate the object by turning the sample holder until the lines are parallel with the sides of the display window.



6. Adjust the focus with the **Arrow** buttons in the **Life View** window.

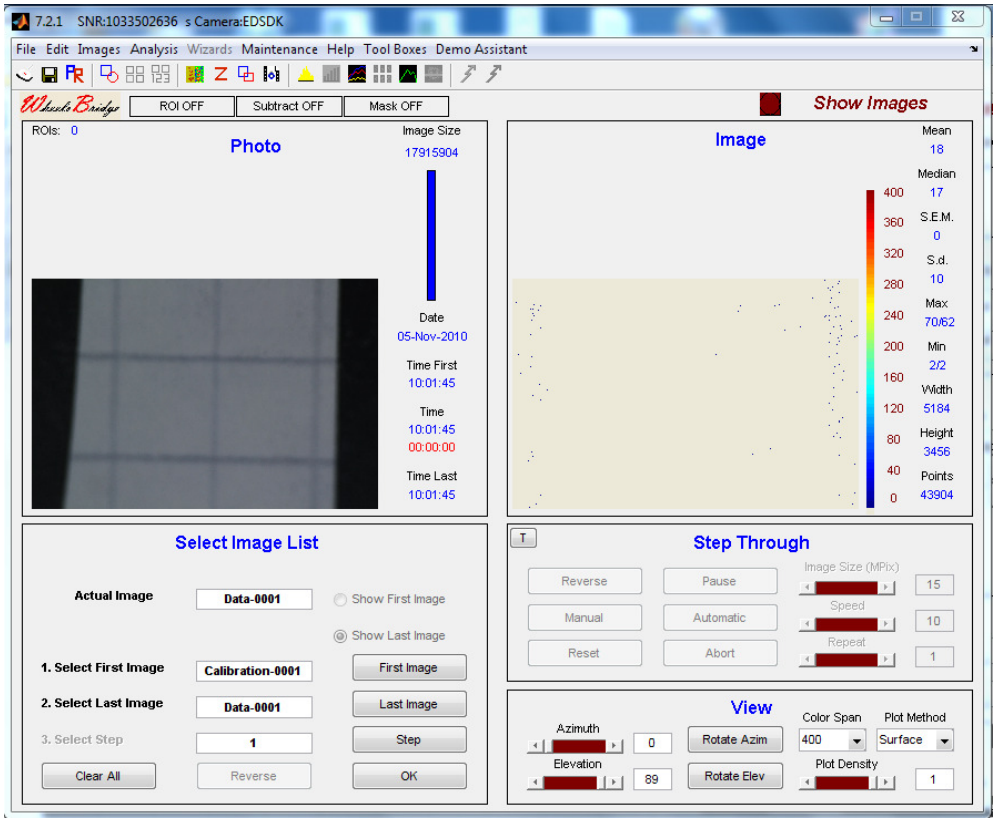


7. Click the **Stop** button to stop video recording and then click the delete (cross) button in the upper right corner of the **Life View** window to close the window.

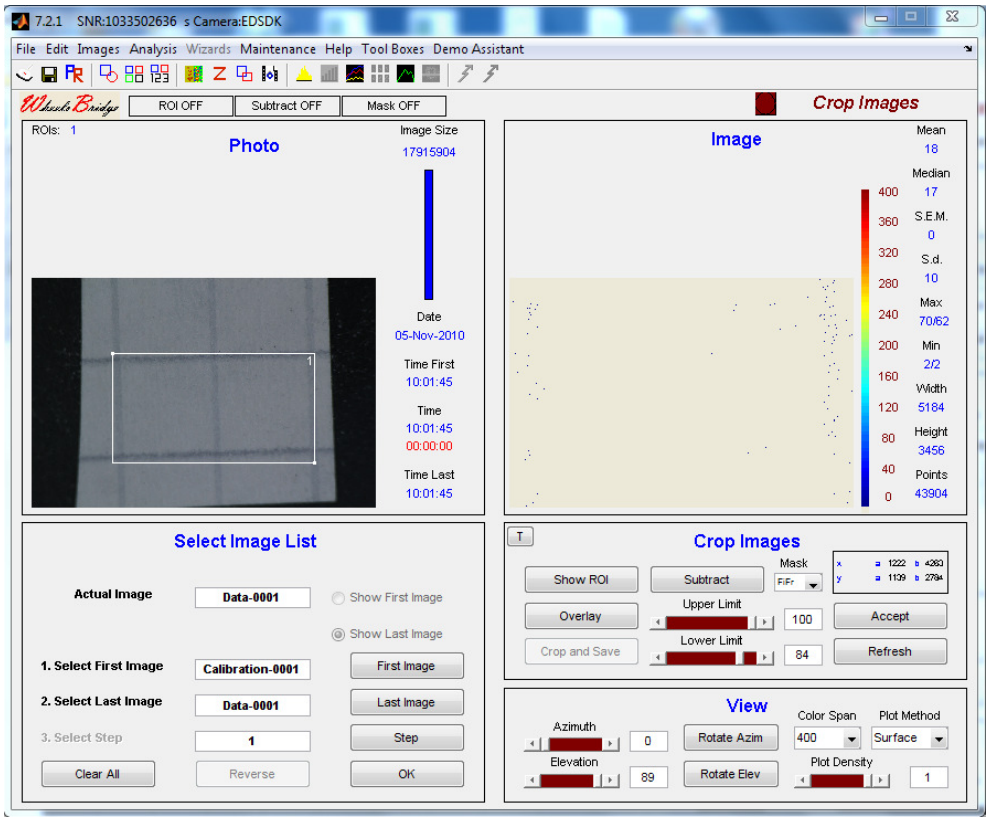
8. Click the **Save Photos** in the *EDSK Camera Settings* window and write "Calibration" in the FileName edit box. Click Save. The photo to be captured will now be saved under the name *Calibration-0001* in the folder selected.

9. Set **Select Photo Size** to **Large File** and **No of Photos** to 1.

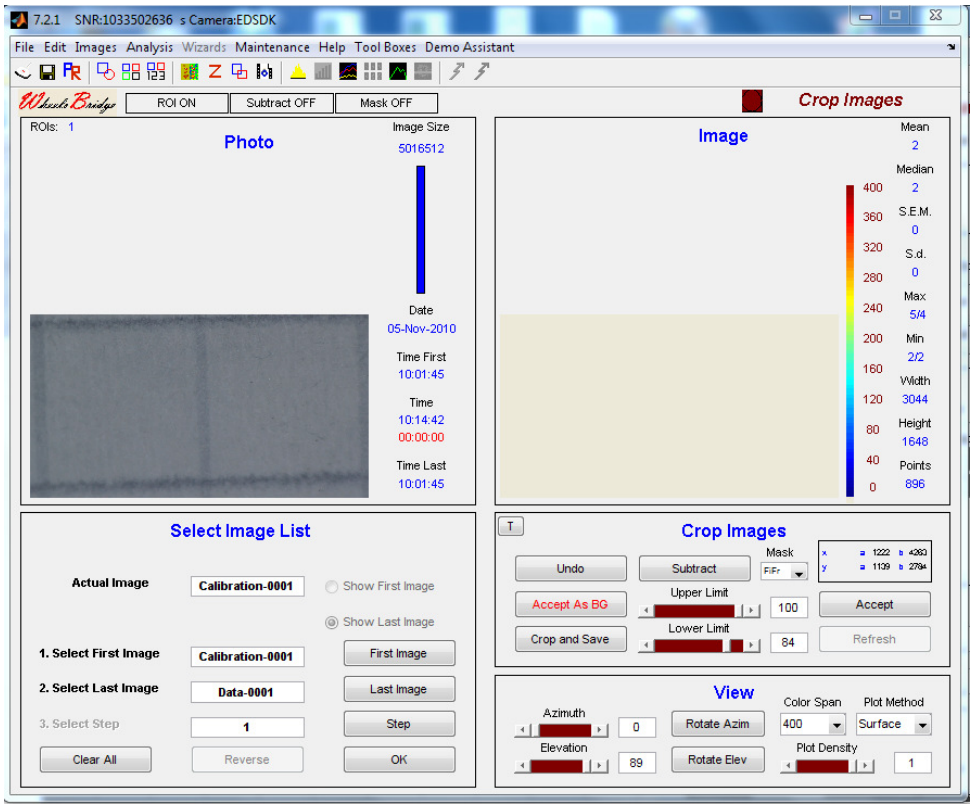
10. Click the **Capture Photos** button to capture and display the photo. The *TiVi600* main window should now look like:



11. Draw a **ROI** to cover the two centre squares of the object.



12. Click the **Show ROI** button.



13. The width of the object displayed is now 10 mm which corresponds to a **Width** of 3044 pixels (displayed in lower right corner of the **Image** panel). The calibration factor for this particular *TiVi Microscope* setup is therefore 10.000 micrometers per 3044 pixels or 3.29 micrometers per pixel.

A somewhat larger degree of magnification can be attained by inserting yet another magnifying lens in the TiVi Microscope, albeit at the expense of a reduced focal depth. The degree of magnification selected can also be adjusted by manually changing the Zoom-in of the Macro lens and by modifying the object-camera distance by turning the sample holder.

This completes the **CALIBRATION** section.