

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

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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.

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Photo Settings First Photo Last Photo	First Photo	Coordinates x-coord = 2.5 y-coord = 2.5 Height = 5 Width = 5	Image Settings Eraser Size 1 Erase Background Dis	Reset	Image Type Red plane Colour Map copper Background Type auto

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.

TiVi98 Microstructure Analyzer Ver 3.1 Manual About Demo Assistant	_ <u> </u>
Wheels Builgo Photo Use mouse pointer to move the zoom-in area	Image Set Background Discrimination
Coordinates x-coord = 1728 y-coord = 2592 Height = 374 Width = 561 Cast Photo Last Photo	Image Settings Image Type Eraser Size Red plane 1 Colour Map Erase Reset Background Discrimination Background Type 4 0 auto
Name: ColoredHair-0001.jpgStep	Rotate Analyze Close

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.



TiVi98 Microstructure Analyzer Ver 3.1	
Manual About Demo Assistant	
Wheels Buildge Photo Use mouse pointer to move the zoom-in area	Image Erase points, mark the object and/or click the Analyze button
Photo Settings Coordinates x-coord = 1909 y-coord = 2885	Image Settings Eraser Size Image Type 1
First Photo First Photo First Photo	Erase Reset Copper 💌
C Last Photo Last Photo Save	Background Discrimination Background Type
Name: ColoredHair-0001.jpg Step	Rotate Analyze Close

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.

TiVi98 Microstructure Analyzer Ver 3.1	
Manual About Demo Assistant	
Wheels Bridge	
Use mouse pointer to move the zoom-in area	Set Background Discrimination
2	
Photo Settings Coordinates x-coord =	Image Settings Eraser Size 1
y-coord = Height = 403 Width = 605	3 Colour Map Erase Reset copper V
C Last Photo Save	Background Discrimination Background Type
Name: ColoredHair-0001.jpg Step	Rotate Analyze Close

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.

TiVi98 Microstructure Analyzer Ver 3.1	
Manual About Demo Assistant	
Wheels Bridge Photo Use mouse pointer to move the zoom-in area	Set Background Discrimination
-	
Photo Settings Coordinates x-coord = y-coord = y-coord = Height = 403 Width = 605 Vidth = 605	Image Settings Eraser Size Image Type 1 Red plane Colour Map Frase Reset Copper
Last Photo Save	Background Discrimination Background Type auto
Name: ColoredHair-0001.jpgStep	Rotate Analyze Close

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.

Analysis98 Export Data				_ D X
Colormap :copper Image Photo / Image Set points to create profile	Type :Red plane	Name :C	oloredHair-0001.jpg Display Spectrum patial Variations	
Height: 404 Width: 606 Min. Area: 23716		-0.05 -0.1 0 100 20	0 300 400 Pixels	500 600
Photo / Image Controls		- Curves / Spectrum Control:	S	
Display Photo O Display Image Lower Limit	Display Profile Ipper Limit	Display Curves	Ymin -0.1	Ymax 0.1
Small Object Analysis No. of Objects: Start Display Statistics	Eraser Size	Display Spectrum	Xmin 0	Xmax 600
Small Object Removal	Reset			Close

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.

🛃 Analysis98			– – ×
Export Data			
Colormap :copper	Image Type :Red plane	Name :ColoredHair-0001.jpg	
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	-	0.02	-
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Height: 404 Width: 606 Min. Area: 2371	6	0 10 20 30 Frequency	40 50
Photo / Image Controls		Curves / Spectrum Controls	
 Display Photo Display Imag Lower Limit 	e O Display Profile	Ymin	Ymax
		O Display Curves 0	0.035
No. of Objects:	Eraser Size	Xmin	Xmax
Start Display Statistics	1 -	Display Spectrum 0	50
Small Object Removal	Erase on		
	Reset		Close

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.

Export Data Colormap :copper Image Type :Red plane Name :ColoredHair-0001.jpg Photo / Image Select Display Curves or Display Spectrum Select Display Spectrum Select Display Curves or Display Spectrum 0.02 Periodogram 0.015 0.015 Power =0.085 1 0.005 0.005	alysis98	
Colormap:copper Image Type:Red plane Name:ColoredHair-0001.jpg Photo / Image Set points to create profile Setect Display Curves or Display Spectrum 0.02 0.015 0.015 0.015 0.015 0.015 0.015 0.015 0.015 0.015 0.015 0.015 0.015 0.015 0.015 0.015 0.015 0.015	t Data	
Photo / Image Curves / Spectrum Set points to create profile Select Display Curves or Display Spectrum 0.02 0.015 0 0.015 0 0.005	Colormap :copper Image Type :Red plane	Name :ColoredHair-0001.jpg
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neigin, 404 Wildin, 606 Mill, Area, 25716 Trequency	reigin, 404 Wildin, 606 Mill, Area, 23/16	
Photo / Image Controls Curves / Spectrum Controls	oto / Image Controls	Curves / Spectrum Controls
O Display Photo O Display Image O Display Profile Ymin Ymax Lower Limit Upper Limit O Display Curves O O.02	Display Photo Display Image Display Profile Lower Limit	Ymin Ymax Display Curves 0 0.02
Small Object Analysis Xmin Xmax	- Small Object Analysis	Xmin Xmax
No. of Objects: Claser Size	No. of Objects:	Display Spectrum 1 10
Small Object Removal	Small Object Removal	Close

- 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.

Analysis98 Export Data		
Colormap :copper	Image Type :Red plane	Name :ColoredHair-0001.jpg
Photo / Image		Curves / Spectrum
Set points to create profile		Select Display Curves or Display Spectrum
		Periodogram
Height: 404 Width: 606 Min. Area: 2371	8	$\begin{array}{c} 0.015 \\ \hline 0.015 \\ \hline 0.005 \\ 0 \\ \hline 0 \\ 2 \\ \hline 4 \\ \hline 6 \\ 8 \\ 10 \\ \hline \\ Frequency \\ \hline \end{array}$
Photo / Image Controls		Curves / Spectrum Controls
Oisplay Photo Oisplay Imag	e 💿 Display Profile	Ymin Ymax
Lower Limit	Upper Limit	O Display Curves 0 0.02
Small Object Analysis		Xmin Xmax
No. of Objects:	Eraser Size	Display Spectrum 1 10
Start Display Statistics Small Object Removal	Erase on Reset	Close

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.

Analysis98	
Export Data	
Colormap :copper Image Type :Red plane	Name :ColoredHair-0001.jpg
Photo / Image	Curves / Spectrum Select Display Curves or Display Spectrum
	Periodogram
Height: 404 Width: 606 Min. Area: 23716	0.015 0.015 0.005 0.
Photo / Image Controls	Curves / Spectrum Controls
Display Photo Display Image Display Profile Lower Limit Upper Limit	Ymin Ymax O Display Curves 0 0.02
- Small Object Analysis	Xmin Xmax
No. of Objects:	Display Spectrum 1 10
Start Display Statistics Erase on Small Object Removal 20 Reset	Close



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.

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Manual About Demo Assistant	
Wheels Builgs Photo Use vertical sider to zoom in	Image Set Background Discrimination
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Photo Settings Coordinates x-coord = y-coord = y-coord = Height = 403 Width = 605 Width = 605	Image Settings Eraser Size Image Type 1 Red plane Colour Map Erase Reset Copper V
Last Photo Last Photo Save	Background Discrimination Background Type
Name: multihair-0001.jpgStep	Rotate Analyze Close

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.

TiVi98 Microstructure Analyzer Ver 3.1	
Manual About Demo Assistant	
Wheels Builgs Photo Use mouse pointer to move the zoom-in area	Image Set Background Discrimination
Photo Settings	Image Settings
Coordinates	Eraser Size Image Type
y-coord = 2743	Colour Map
First Photo First Photo Width = 2648	Erase Reset Copper -
C Last Photo Last Photo Save	Background Discrimination Background Type
Name: multihair-0001.jpgStep	Rotate Analyze Close

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



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

Analysis98 Export Data					X
Colormap :copper Image	Type :Red plane	Name :r	multihair-0001.jpg		
Photo / Image Set points to create profile		Curves / Spectrum Select Display Curves o	r Display Spectrum		
	/	1	Spatial Variation	15	
	~	0.8- ep 0.6- ep 0.4- 0.2 -			
Height: 883 Width: 1325 Min. Area: 556		0 0.5	1 Pixels	1.5 2	
- Photo / Image Controls-		Curves / Spectrum Control	ls		
Display Photo Display Image Lower Limit	Oisplay Profile Upper Limit	Display Curves	Ymin O	Ymax 1	
Small Object Analysis No. of Objects: Start Display Statistics	Eraser Size	O Display Spectrum	Xmin 0	Xmax 2	
Small Object Removal	Erase on Reset			Close	

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

Analysis98	
Export Data Colormap :copper Image Type :Red plane Photo / Image Set points to create profile	Name :multihair-0001.jpg Curves / Spectrum Select Display Curves or Display Spectrum
Height: 883 Width: 1325 Min. Area: 556 Photo / Image Controls Display Photo Display Photo Display Profile Lower Limit Upper Limit Upper Limit Small Object Analysis Small Object Removal Small Object Removal 20 Reset	Curves / Spectrum Controls Ymin Ymax Image: Display Curves 0 1 Xmin Xmax Display Spectrum 0 2 Close Close

Analysis98	
Export Data	
Colormap :copper Image Type :Red plane	Name :multihair-0001.jpg
Photo / Image	Curves / Spectrum
Set points to create profile	Select Display Curves or Display Spectrum
Photo / Image Controls	Curves / Spectrum Controls
Display Photo	Vmin Vmav
Lower Limit Upper Limit	
	Display Curves
Small Object Analysis	Xmin Xmax
No. of Objects: 13 Eraser Size	Display Spectrum 0 2
Start Display Statistics Erase on Small Object Removal 20 Reset	Close

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.

Analysis98	×
Export Data	
Colormap :copper Image Type :Red plane Photo / Image Set points to create profile	Name :multihair-0001.jpg Curves / Spectrum Select Display Curves or Display Spectrum
Height: 883 Width: 1325 Min. Area: 556 Erase ON)//
Photo / Image Controls	Curves / Spectrum Controls
Display Photo Display Image Display Profile Lower Limit Upper Limit	Ymin Ymax
Small Object Analysis No. of Objects: 13	Display Spectrum 0 2
Start Display Statistics Erase Off Small Object Removal 20 Reset	Close

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

📣 Analysis98		_ _ ×
Export Data		
Colormap :copper Image Type :Red plane Photo / Image Set points to create profile	Name :multihair-0001.jpg - Curves / Spectrum Select Display Curves or Display Spectrum	
Height: 883 Width: 1325 Min. Area: 556 Erase ON) / /	
Photo / Image Controls	Curves / Spectrum Controls	
Display Photo Display Image Display Profile	Ymin	Ymax
	Display Curves 0	1
Small Object Analysis	Xmin	Xmax
No. of Objects: 13	O Display Spectrum 0	2
Small Object Removal		
20 Reset		Close

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.

TiVi98 Microstructure Analyzer Ver 3.1	
Manual About Demo Assistant	
Use mouse pointer to move the zoom-in area	Image Set Background Discrimination
Photo Settings	Eraser Size Image Type
x-coord = 1728	1 Red plane
y-coold = 2392 Height = 2139	Colour Map
First Photo First Photo Width = 3209	Erase Reset Copper V
C Last Photo Save Save	Background Discrimination Background Type
Name: Stubbles-0001.jpg Step	Rotate Analyze Close

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:

Analysis98 Export Data				
Colormap :copper	Image Type :Red plane	Name :C	ColoredHair-0001.jpg	
Photo / Image Set points to create profile	на: 34732	Curves / Spectrum Select Display Curves of 0.2 0.15 0.1 0.1 0.1 0.05 -0.05 -0.1 -0.15 0.2 0.2 0.2 0.1 0.1 0.1 0.1 0.1 0.2 0.2 0.2 0.1 0.1 0.2 0.2 0.1 0.2 0.2 0.2 0.2 0.1 0.2 0.2 0.2 0.2 0.1 0.2 0.2 0.2 0.1 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2 0.2	r Display Spectrum ipatial Variations	800 1000
Photo / Image Controls		Curves / Spectrum Control	s	
Display Photo Display Photo Disp Lower Limit	lay Image O Display Profile Upper Limit	Display Curves	Ymin -0.15	Ymax 0.2
Small Object Analysis No. of Objects: Start Display Sta Small Object Removal	Eraser Size 1 Erase on 20 Reset	Display Spectrum	Xmin 0	Xmax 1000 Close

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.

Analysisso		
Export Data		
Colormap :copper Image Type :Red plane	Name :ColoredHair-0001.jpg	
Photo / Image Set points to create profile	Curves / Spectrum Select Display Curves or Display Spectrum	
	0.2 Spatial Variations]
	0.15	-
	0.1 ≝ 0.05	
		-
	-0.05	-
	-0.1	-
Height: 599 Width: 898 Min. Area: 34732	-0.15 200 400 600 800 11 Pixels	000
- Photo / Image Controls	- Curves / Spectrum Controls-	
Display Photo Display Image Display Profile Lower Limit	Ymin Ymax	
	Display Curves -0.15 0.2	
Small Object Analysis	Xmin Xmax	
Start Dienlay Statistice	Display Spectrum 0 1000	
Small Object Removal Erase on		1
20 Reset	Close	

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.

 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.

Analysis98 Export Data	
Colormap :copper Image Type :Red plane	Name :multihair-0001.jpg
Photo / Image Set points to create profile	Curves / Spectrum Select Display Curves or Display Spectrum
Height: 692 Width: 1037 Min. Area: 35	
Photo / Image Controls	Curves / Spectrum Controls
Display Photo Display Image Display Profile	Ymin Ymax
	Display Curves 0 1
Small Object Analysis	Xmin Xmax
No. of Objects: 33 Eraser Size	O Display Spectrum 0 2
Start Display Statistics Erase on Small Object Removal	Close

- 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.

🛃 Statistics98	_ 0	X
Export Data		
BW-image	Data Actual Object Area: 318 Major Axis: 81.99 Minor Axis: 15.42 Eccentricity: 0.98 Orientation: -2.54 EquivDiameter: 20.12 Perimeter: 215.19 All Objects Average Min Area: 539.16 ± 198.15 318 Major Axis: 114.6 ± 37.71 55.3 Minor Axis: 8.27 ± 3.18 5 Eccentricity: 0.99 ± 0 0.98 Orientation: -0.53 ± 57.13 -89.2 BuivDiameter: 25.81 ± 4.65 20.1 Attage 34.8 Perimeter: 229.56 ± 69.45 Obj/MPixels: 16.72	
Controls Indicator Settings Select Property Indicator Settings Area 1 Show Histogram Step Through	e O Large Size No. of Bins t O Long Dist 10 Close	

- 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).

TiVi98 Microstructure Analyzer Ver 3.1	
Manual About Demo Assistant	
Wheels Bridge Photo	Image
Use mouse pointer to move the zoom-in area	Set Background Discrimination
-	<i>i</i>
- Photo Settings	Image Settings
x-coord =	1 Red plane
y-coord = Heinbt = 344	Colour Map
First Photo First Photo Width = 516	Erase Reset copper 💌
	Background Type
C Last Photo Save Save	auto
Name: Stubbles-0001.jpgStep	Rotate Analyze Close

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

Analysis98	
Export Data	
Colormap :copper Image Type :Red plane	Name :Stubbles-0001.jpg
Photo / Image Set points to create profile	Curves / Spectrum Select Display Curves or Display Spectrum
	Spatial Variations
113 13200	0.8
	ep 0.6-
I BE MI AN	0.2
Height: 345 Width: 517 Min. Area: 21	0 0.5 1 1.5 2 Pixels
	Curves / Spectrum Controls
Display Photo O Display Image O Display Profile Lower Limit Upper Limit	Ymin Ymax Image: Display Curves 0 1
Small Object Analysis No. of Objects: Eraser Size	Xmin Xmax
Start Display Statistics Erase on Small Object Removal	Display Spectrum U 2
, 20 Reset	Liose

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:

Analysis98 xport Data				
Colormap :copper	Image Type :Red plane	Name :S	stubbles-0001.jpg	
- Photo / Image Set points to create profile		Curves / Spectrum Select Display Curves or	r Display Spectrum	
			1	·]
	E BALLA	0.6		-
1192		0.4		
	9.9 A.S	0.2		
Height: 345 Width: 517 Min. Are	a: 21	0 0.5	1	1.5 2
Photo / Image Controls		Curves / Spectrum Control	s	
Display Photo Display Photo	lay Image O Display Profile		Ymin	Ymax
		Display Curves	0	1
Small Object Analysis			Xmin	Xmax
No. of Objects: Start Display Stat	istics Erase on	O Display Spectrum	0	2
	20 Reset			Close

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 (*TiV*i-value = 48) displayed in the TiVi main window below.

TiVi700 A	Analyzer	1.1.1	Wizard	Maintena	nce He		Tool Boxer	Demo Assis	tant			
		h Iol _				s congooge	. 100100xc3	Demo Assi				
V kanto B	ridys	ROIOFF		Subtract OF	F	Mask OFF				Show	v / Crop)
- Photo Rols:	Pol:CR	Dist(mm)	:0 :	Zoom:0		Photo Size: 17915904 Date: 31-0ct-2010 Time First: 10:55:20 00:00:00 Time Last: 10:55:20	Lower	ge Limit:0	Upper Limit:	100 T	TVE	Mean 300 48 Media Media 270 46 240 0 210 SD 210 SD 180 Min 150 1/1 150 55/85 90 5184 60 3456 30 Points 0 156561
- Select	t Photo	Actual Pho Stubble2-000 First Photo	to 1.jpg		⊙ sr ⊚ sr	now First Photo now Last Photo		Control Contro		Pause utomatic Abort	Color Si	Comments
	s	Stubble2-000	1.jpg			That Photo						
	5	Stubble2-000 Last Photo Stubble2-000	1.jpg) 1.jpg			Last Photo		p Images			Mask	x1: x2:
	5	Stubble2-000 Last Photo Stubble2-000 Subtracted	1.jpg) 1.jpg Photo			Last Photo Step 1		p Images Show ROI Overlay		tract F	Mask iFr V	xi: x2: yi: y2: Reset Mask

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.

TIVi98 Microstructure Analyzer Ver 3.1	_ _ ×
Manual About Demo Assistant	Image
Photo Settings Coordinates x-coord = 2.5	Image Settings Eraser Size 1 Red plane
First Photo First Photo First Photo	Colour Map Erase Reset Copper 💌
C Last Photo Last Photo Save	Background Discrimination Background Type auto
Name: ForeheadA-0001.jpg	Rotate Analyze Close

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.

TiVi98 Microstructure Analyzer Ver 3.1	
Manual About Demo Assistant	
What Bridge Photo Use mouse pointer to move the zoom-in area	Set Background Discrimination
Coordinates x-coord = y-coord = y-coord =	Eraser Size Image Type
First Photo First Photo Width = 269	Erase Reset copper Background Discription Background Type
C Last Photo Last Photo Save	83 auto
Name: ForeheadA-0001.jpg Step	Rotate Analyze Close

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.

📣 Analysis98					X
Export Data					
Colormap :copper	Image Type :Red plane	Name :Fo	oreheadA-0001.jpg		
Photo / Image Set points to create profile		Curves / Spectrum Select Display Curves or	Display Spectrum		
		0.4 SI	patial Variations		
		0.3			
		월 0.1	M.		
		Maga 0.	Man. M	Mat	
D		-0.1	I W WWW	μų į	
		-0.3 50	100 15	50 200	
Height: 180 Width: 270 Min.	Area: 5081		Pixels		
Photo / Image Controls		Curves / Spectrum Controls			
 Display Photo 	isplay Image 💿 Display Profile		Ymin	Ymax	
Lower Limit	Upper Limit	Oisplay Curves	-0.3	0.4	
- Small Object Analysis	Erapar Siza		Xmin	Xmax	
No. of Objects:	1	O Display Spectrum	0	200	
Start Display S Small Object Removal	Erase on				
	20 Reset			Close	
I					

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

Analysis98				
Export Data				
Colormap :copper	Image Type :Red plane	Name :Foreh	ieadA-0001.jpg	
- Photo / Image		Curves / Spectrum		
Set points to create profile		Select Display Curves or Disp	play Spectrum	
		Pe	eriodogram	
		0.2	1.1	
Heipht 180 Width: 270 Min.	Area: 5081		Power =1.51	8 10
– Photo / Image Controls		Curves / Spectrum Controls		
 Display Photo 	isplay Image 💿 Display Profile		Ymin	Ymax
Lower Limit	Upper Limit	C Display Curves	0	0.2
Small Object Analysis		C Display Curves		
- Smail Object Analysis	Fraser Size		Xmin	Xmax
NO. OT UDIECTS:	1 -	Display Spectrum	1	10
Start Display S	tatistics Erase on			
Small Object Removal	20 Reset			Close

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.

TiVi98 Microstructure Analyzer Ver 3.1	
Manual About Demo Assistant	
Wheels Bridge Photo- Use mouse pointer to move the zoom-in area	Erase points, mark the object and/or click the Analyze button
Photo Settings Coordinates x-coord = 1150 y-coord = 2406 Height = 347 Width = 521 347	Image Settings Eraser Size 1 Colour Map Erase Reset Copper V
C Last Photo Last Photo Save	Background Discrimination Background Type
Name: DamagedHair-0001.jpg Step	Rotate Analyze Close

Analyze98 window with Spatial Variations below

Analysis98	
Export Data	
Colormap :copper Image Type :Red plane	Name :DamagedHair-0001.jpg
Photo / Image	Curves / Spectrum Select Display Curves or Display Spectrum
	Spatial Variations
	on Mal Man My A
Company of the owner of the owner	₹0.2 \V \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\ \\
	≥ -0.4
	-0.6
	-0.8
	-1 0 100 200 300 400 500
Height: 346 Width: 522 Min. Area: 22375	Pixeis
Photo / Image Controls	Curves / Spectrum Controls
Display Photo Display Image Display Profile	Ymin Ymax
	Display Curves -1 0.6
Small Object Analysis	Xmin Xmax
Stat Display Statistics	Display Spectrum 0 500
Small Object Removal	
, 20 Reset	Close

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.

Analysis98	
Export Data	
Colormap :copper Image Type :Red plane	Name :DamagedHair-0001.jpg
Set points to create profile	Select Display Curves or Display Spectrum Periodogram 1
Height: 348 Width: 522 Min. Area: 22375	0.8 0.6 0.4 0.2 0.2 0.4 0.2 0.4 0.2 0.4 0.6 0.6 0.6 0.6 0.6 0.6 0.6 0.6
Photo / Image Controls	Curves / Spectrum Controls
Oisplay Photo Oisplay Image Oisplay Profile Lower Limit	Ymin Ymax O Display Curves 0 1
Small Object Analysis Eraser Size No. of Objects: 1 Start Display Statistics	Xmin Xmax © Display Spectrum 1 10
Small Object Removal Erase on Reset	Close

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

Export Data		
Colormap :copper Image Type :Red plane	Name :DamagedHair-0001.jpg	
Photo / Image Set points to create profile	Curves / Spectrum Select Display Curves or Display Spectrum	
	0.5	1
	0.4 - Power =1.939 -	
CT COLUMN		
	0.1-	
Height: 348 Width: 522 Min. Area: 22375	0 2 2.2 2.4 2.6 Frequency	J
Photo / Image Controls	Curves / Spectrum Controls	
Display Photo Display Image Display Profile	Ymin Ymax	
	O Display Curves 0 0.5	
Small Object Analysis	Xmin Xmax	_
	Display Spectrum 2 2.75	
Start Display Statistics Erase on Small Object Removal	Close	

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.

nual About					
Phote Bridge Photo Use vertical slider to z	oom in		Image		
			K		
Photo Settings——		Coordinates	Image Settings	Image I	Tune
		x-coord		Red place	100
		y-coord		Celeur	•
First Photo	First Photo	Height Width	Erase	copper	wap •
				Backgroun	nd Type
C Last Photo	Last Photo	Save	Background Discrimination	0 auto	•

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.

anual About				
Uheels Bridge Photo Use mouse pointer to n	nove the zoom-in area		Set Background Discrimination	
	-			
Photo Settings	First Photo	Coordinates x-coord = 3023 y-coord = 2064 Height = 188 Width = 282	Image Settings Eraser Size	Image Type Red plane v Colour Map copper v
	Last Photo	Save	Background Discrimination	Background Type auto
Last Photo				

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.

nuar About				
Photo Use mouse pointer to	move the zoom-in area	Rector Contraction	Erase points, mark the object and/or click th	ne Analyze button
				Erase On
*				
Photo Settings	First Photo	Coordinates x-coord = 3023 y-coord = 2064 Height = 188 Vvidth = 282	Image Settings Eraser Size 3 Stop Erase Reset	Image Type Red - Green pl v Colour Map pink v
Photo Settings First Photo Last Photo	First Photo Last Photo	Coordinates x-coord = 3023 y-coord = 2064 Height = 188 Vidth = 282 Save	Image Settings Eraser Size 3 Stop Erase Reset Background Discrimination	Image Type Red - Green pl • Colour Map pink • Background Type auto •

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

nual About				
Theels Bridge				
Photo	ve the zoom-in area	A Contraction of the second se	Erase points, mark the object and/or click the	9 Analyze button
-				
-				
Photo Settings First Photo	First Photo	Coordinates x-coord = 3023 y-coord = 2064 Height = 188 Width = 282	Image Settings Eraser Size 3 • Erase Reset	Image Type Red - Green pl V Colour Map pink V
Photo Settings First Photo Last Photo	First Photo Last Photo	Coordinates x-coord = 3023 y-coord = 2054 Height = 188 Width = 282 Save	Image Settings Eraser Size 3 Erase Reset Background Discrimination	Image Type Red - Green pl • Colour Map pink • Background Type 88 auto •

20. Click the **Analyze** button to get to the *Analyze*98 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:

7.2.1 SNR:1033502636 e Edit Images Analysis □ □ □ □ □	s Camera:EDSDK Wizards Maintenance Z 🗗 😽 📥	Help Tool Boxes Demo As	iistant ₹		
Thends Bridger ROI OFF Subtract OFF Mask OFF			Show Images		
ROIs: 0	Photo	Image Size 17915904	Image Mean 18 Median 400 17 360 SE.M. 0 320 S.d.		
		Date 05-Nov-2010 Time First 10:01:45 Time 10:01:45 00:00:00 Time Last 10:01:45	280 10 407 700 200 Min 120 5184 80 Height 3455 40 Points 0 43904		
Select Image List			Step Through		
Actual Image 1. Select First Image	Data-0001	 Show First Image Show Last Image 	Reverse Pause Image Size (MPix) Manual Automatic Speed Reset Abort Image Size (MPix)		
2. Select Last Image 3. Select Step	Data-0001	Last Image Step	Azimuth Color Span Plot Method		

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

7.2.1 SNR:1033502636 File Edit Images Analysis	s Camera:EDSDK Wizards Maintenance	Help Tool Boxes Demo As	sistant 1		
S 🖬 限 🖳 🔡 🧱 Z 🔁 🚧 🔺 🌆 🧱 👬 🜌 📰 🥕			Crop Images		
ROIS: 1	Photo	Image Size 17915904	Image Mean 18 400 17 360 S.E.M. 0 320 S.d.		
	1	Date 05-Nov-2010 Time First 10:01:45 Time 10:01:45 0:00:00 Time Last 10:01:45	280 10 Max 240 70/2 200 Min 2/2 160 2/2 160 2/2 160 4 120 5184 80 Height 3456 40 Points 90 43904		
Actual Image	Select Image List	Show First Image	T Crop Images Show ROI Subtract FFF = 1222 ± 4283 Upper Limit Upper Limit = 1109 ± 2784		
1. Select First Image	Calibration-0001	Show Last Image First Image	Crop and Save		
2. Select Last Image 3. Select Step Clear All	Data-0001 1 Reverse	Last Image Step OK	Azimuth 0 Rotate Azim 400 View Surface v Elevation 89 Rotate Elev 1		

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

🖬 🕏 🖶 🔡	🐹 Z 🔁 🔯 📥 📶	🖉 iii 🗖 📰 📝 🤉	7		
Ukurde Bridger ROI ON Subtract OFF Mask OFF			Crop Images		
ROIs: 1	Photo	Image Size 5016512	Image	Mean 2 Mediar	
		Date 05-Nov-2010		400 2 360 S.E.M. 0 320 S.d. 280 0 Max 240 5/4 200 Min	
		Time First 10:01:45 Time 10:14:42 00:00:00 Time Last 10:01:45		200 Min 2/2 160 Width 120 3044 80 Height 1648 40 Points 0 896	
;	Select Image List		Crop Images	a 1222 b 4283	
Actual Image	Calibration-0001	Show First Image Show Last Image	Undo Subtract F# y Accept As BO Upper Limit > 100 Crop and Save & 84	a 1139 b 2784 Accept Refresh	
n ooloot in ot in ago		Lastimage	View		

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.