



Spot Analyzer TiVi80 User Manual

User Manual 3.2 Version 3.2 October 2013

PIONEERS IN TISSUE VABILITY IMAGING

Dear Valued Customer!

Welcome to the WheelsBridge Spot Analyzer TiVi80 system designed for automatic analysis of spatial and temporal variations in spot appearance. The Spot Analyzer TiVi80 was designed especially for observer-independent analysis of changes spot erythema in association with evaluation of skin care products and follow up of treatment regimes.

The Spot Analyzer TiVi80 utilizes a highly sensitive digital camera equipped with polarization filters making it possible to suppress direct surface reflections from the skin surface. 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 Spot Analyzer TiVi80 software the following are particularly helpful in the evaluation of spatial or temporal variations in spot erythema:

- ? Automatic capturing of photos.
- ? Zoom-in features to display details of image by clicking on the actual spot.
- ? Automatic batch calculation of TiVi values from photos.
- ? Automatic generation of spot intensity and extension.
- ? Project window displays the overall results of a test panel trial.
- ? All data can be exported to ASCII-format spread sheets.

We are convinced that the Spot Analyzer TiVi80 will be a productive tool in the assessment and follow-up of spot appearance following treatment with skin care products.

Thank you for choosing the WheelsBridge Spot Analyzer TiVi80.

WheelsBridge AB

Information in this document is subject to change without notice and does not represent a commitment on the part of WheelsBridge AB. No part of this manual may be reproduced or transmitted in any form or by any means, electronically or mechanically, including photocopying and recording, for any purpose without the written permission of WheelsBridge AB.

Any use of application of WheelsBridge products must be performed by properly trained and qualified personnel. WheelsBridge AB takes no responsibility for action taken on the basis of information derived from the use of the product described in this manual or from consequential damages in connection with furnishing, performance or use of this manual.

Licensee's limited rights are governed by the license agreement between WheelsBridge AB and MathWorks. Licensee may not modify or remove any license agreement file (MarthWorks or third party) that is included with the MCR Libraries ("MCR Library License"). Licensee of this Application accepts the terms of the MCR Library License.

The Spot Analyzer is patented and/or patents are pending in several countries.

WheelsBridge is a registered trade mark.

1. INTRODUCTION

Facial spots including acne appear most frequently during puberty with a varying degree of severity. About 90% of the adolescent population is believed to be affected by this skin disease. Numerous cosmetic products are commercially available for treatment of spots and new and more effective products are continuously released on the market.

To evaluate the performance of these skin care products, the product candidates are generally tested using a panel of volunteers with spots of varying severity. The result is assessed by naked eye observation at which both the intensity and the extension of the spot erythema are evaluated. These evaluations are generally performed by a skilful dermatologist and a severity score from one to five may be used to quantify the findings. Because of the unavoidable user-dependent element of these naked eye inspection procedures, results may vary among observers and inter-laboratory comparison of data may be difficult to perform.

The *Spot Analyzer TiVi80* - based on photos captured with a polarization spectroscopy camera that generates colour-coded images of the red blood cell concentration in the skin – streamlines the analysis process and delivers user-independent results regarding both spot intensity and extension.

The intended use of the *Spot Analyzer TiVi80* is to map erythema in spots in research and laboratory applications. It is not yet approved for the diagnosis and treatment of disease.

2. OPEARATING PRINCIPLE

The Spot Analyzer TiVi80 operates in the following way:

- 1. A sequence of photos of the same skin site is captured at different points in time.
- 2. This sequence of photos is uploaded to the Spot Analyzer TiVi80 main window.
- 3. Specific spot are brought into focus by clicking on them using the mouse.
- 4. The corresponding TiVi-images (RBC concentration maps) are displayed.
- 5. The intensity and extension of the erythema around the spots in focus are automatically calculated and displayed in the Chart window.
- 6. Up to 20 separate spot sequences from the Chart windows can be exported to and displayed in the Project window which displays the complete results of a test panel trial.

In the **GETTING STARTED** section these features are further explained by way of an example

3. GETTING STARTED

The basic features of the *Spot Analyzer TiVi80* are probably best explained by way of an example. In the following example it is assumed that the photos *Day-0001.jpg* to *Day-0004.jpg* have been captured and stored in the *TiVi80demonstration* folder. These photos demonstrate the initial four days healing process of experimental wounds made by a lancet on the volar side of the forearm. The task is to analyze the intensity and extension of the erythema associated with these wounds in the initial wound healing phase.

1. Open the Spot Analyzer TiVi80 main window.

Photo 4 Photo 5 Photo 8 Load Photos Set ROT Size View Angle Catculate & Diplay Thi Image Parameters 20 Note Spen 1004 Reset All	heels <mark>Bridge</mark> Photos - Photo 1	Photo 2		TMt
Set ROLSIDe View Angle Calculate & Diplay Tivi Image Parameters 20	-Photo 4	Photo 5	Photo 6	Load Protos @ Sequence () Kientical
Show TM Show TM 20				Load
20 View Angle Calculate & Digitary Tivi Image Parameters 20 View Angle Resort At Resor				Show TM
	20 X-Boals	View Angle Calcs	Manual Dn Color Spen 1000	Reset All

2. Click the **Load**-button, navigate to the *TiVi80demonstration* folder. Double-click the *Day-0001.jpg* icon to load a sequence of photos. The *Spot Analyzer TiVi80* main window should now look like:

J TiVi80 Spot Analyzer 3.1			× III -
File Settings Manual About De	no Assistant		
Wheels Bridge			TM:
Photo 1	Photo 2	Photo 3	
De 001(s) 4843 5.04		De-2003bg 407412 204	
- Photo 4	Photo 5	Photo 6	
			Lead Photos Sequence Identical
			Load
100-0004 (99) 120 412 2-004 18-909-0008 20 22 28			Show TM
- Set ROI Size	- View Angle Calculate	& Diplay TM Image Parameters	
20	Tep Vew	ual On Color Span 1000 *	Reset All

- 3. The four photos in the sequence are now uploaded demonstrating the initial healing process of an experimental wound.
- 4. Adjust the **X-Scale** and **Y-Scale** sliders until the values in the associated edit-boxes read 10. When the mouse is moved over a photo a rectangular white box is displayed. Move the mouse pointer to point at the center of the left wound spot in *Photo 1*.

TVISO Spot Analyzer 3.1	-	COMPANY AND A	100		10-10-10 (me
e Settings Menuel About Demo <i>l</i>	asistent				
Acole Oridge					THE
- Photo I-	Field 2-		holo 3		
Pything 1242 2.04	Pr. 100 pr	2540 704 Ng	Ming and		
10-Nov 2008204142 C Malatime	10-hov-2006203730	Model that	en 2010 10 10 11	entes	
					- Lead Photos ® Sequence O Uninkal
Dev-004.bg 42.412 2.04					Losd
15-Nov 2005 20 22 25 🕐 Hold The					Show TIVI
Set ROI Size	Yew Asge	Calculate & Dipay	TV inage Parameter	•	
40 V.Graie	Tep View	Manuel Or	Talua Tara 10		Rossi AI
Y-State			Caler Span	1001	

5. Click the left mouse button. *Photo 1* will now zoom-in and display the wound at the center of the zoomed-in photo.



6. Place the mouse pointer over the same spot in *Photo 2* and repeat the procedure. Repeat this procedure for all four photos. The *Spot Analyzer TiVi80* main window should now look like:



7. To display the TiVi-images corresponding to the zoomed-in photos click the **Show TiVi** button. The sequence of TiVi-images demonstrate how the erythema intensity and extension both are reduced as the wound heals (from day one to day four).

Settings Manual A	About Demo Assi	stant				
keels Bridge Photos						TiVe
- Photo 1		Photo 2		Phote 3		
De-300 pg 43+ 19-Me-2008 00+140 - Photo 4	r 2e Hattin	De-Willyg Griev-200223740 — Photo S	ASAN SAN Kasat Tat	Dw-4000 (pg 14-4er-2000 2013 (p Phote 6	AH Ze Saattu	
						Losd Photos Sequence Sequence Identical
54-504.90 414 5-Nex-5005.0020.05	2.41 Nation 1751					Load Shew Photo
iet ROISize	Scale	View Asgle	Calculate & Di	ley Th'inoge	Parameters	Reset All
10	-Scale	C Side View	Autometi	Celor Sp	2 *	

8. Click the **Automatic**-button to calculate the intensity and area of the four individual TiVi-images. The **TiVi80 Chart** window is now displayed showing the **Mean Intensity** of the **Erythema**.



9. Select **Both** and check the **Standard Deviation** check-box to display the **Erythema** stacked on the **Background** TiVi-values together with the associated standard deviations.



10. Click the Area button to display the area bar sequence.



- 11. Select **Save Chart** from the **File** pull-down menu. Navigate to the *TiVidemonstration* folder and print *MyFirst* in the **Filename** edit box. Click the **Save** button. The **TiVi80 Chart** data will now be saved under the name *MyFirst_SA.mat* (SA stands for Spot Analyzer TiVi80). Click the **Close**-button to close the **TiVi80 Chart** window. The **TiVi80 Chart** window can be opened again by clicking the **Open Chart** item in the **File** pull-down menu in the *Spot Analyzer TiVi80* main window. The data in the **TiVi80 Chart** window can also be loaded into the **Project window** (together with the results from other **Charts**).
- 12. Click the **New Proj** item in the **File** pull-down menu in the *Spot Analyzer TiVi80* main window to open a new **Project window**.



13. Click Load Object to load a new object into Project. Navigate to the *TiVi80demonstration* folder and double click the *W1_SA* file icon to load the first object into Project. The TiVi80 Project window should now look like:



14. To load five more objects into **Project**, again click the **Load Object** button. When the **Pick a file** window opens press the key-board Ctr-button while at the same time clicking the *MyFirst_SA*, *W3_SA*, *W4_SA*, *WA5_SA* and *WA6_SA* file icons. The **Pick a file** window should now look like:

🛃 TiViðð Project			-	-	-			
A Pick a file						22	1	
Lefai Tridgare plateer Skilvhood Bibliotek Dator Natverk	MyfintSA WJSA WJSA WSSA WSSA WWSSA	dination	← [No Number	Vode Erythems Background Daplay Veson kitelisity Vesian kitelisity Area X-axes File Number Vessurement
	Filomat	"W6_SAniat" "MyRnt, ("niat)	_SA.mat" "W3_SA.n	iat" "W4_SA.ina	**W6_SA.s.+	Oppne Avbryt		20 30
								Clear All
	1	2 3	4 errent Number	5		1		Load Object
-								Close



15. Click the **Open** button to load the selected files.

16. Check the Standard Deviation check-box to display the Standard Deviations.



17. Click the **Measurement** radio-button to display the results with the number of measurements in each TiVi-image sequence along the independent axis.



18. Click the brown panel in front of the *W6.mat* text to permanently remove this file from the **Project**. The **TiVi80 Project** window should now look like:





19. Check the **3D radio-button** to display the results in 3D mode.

- 20. Place the mouse pointer in the center of the diagram. The shape of the pointer now changes to circular. Press the left mouse button and move the mouse. The view angle of the 3D display now changes.
- 21. Click the Area radio-button to display areas in 3D.



22. Select **Export Data** in the **File** pull-down menu to save data in an **Excel** file. Print the file name in the **File Name** text edit box and press the **Save button**.

This completes the **GETTING STARTED** section.

4. DETIALED DESCRIPTION

4.1 Spot Analyzer TiVi80 main window

When the *SpotAnalyzer TiVi80* is started in tool-box mode from inside the *TiVi700 Analyzer*, no photos are loaded.

In the *Spot Analyzer TiVi80* window below the photo sequence A-0001.jpg – A-00026.jpg has been uploaded by clicking the **Load** button and then double-clicking the file icon A-0001.jpg in the *MNA* folder.



Each photo is displayed in a separate sub-photo panel. The name and capturing date as well as the size of the actual photo/TiVi image and whether in zoom-in (Z-In) or zoom-out (Z-Out) mode are displayed below each photo.

- 1. Load used for loading a new sequence of photos into the system. The first six photos in a sequence are automatically uploaded.
- 2. Show TiVi used for displaying the corresponding TiVi images instead of the photos after **ROI**s have been drawn..



- 3. Click the same button (now labeled Show Photos) to again display the photos.
- 4. Click the **Reset All** button to again display the original photos.
- 5. Place the mouse pointer over a specific photo. A white box, the dimensions of which are determined by the **Set ROI Size** panel edit boxes and sliders, now appears over the photo and follows the mouse pointer as the mouse is moved over the photos (without pressing the mouse buttons).
- 6. Move the **X-Scale** and **Y-Scale sliders** until the associated edit boxes read 25 and 15 respectively. These settings determine the size of the white ROI-box (as percentage of full scale). The settings can also be altered by printing the numbers directly in the edit boxes. Moving the mouse over the photo in photo-panel *Photo 4* should now display like:



7. When the mouse is positioned over the center of the bottom left erythema patch in *Photo 4*, click the left mouse button. A zoomed-in photo is displayed with the center corresponding to the mouse pointer position and the size corresponding to the white box settings. At the same time the size indicators change to the size of the photo displayed while the zoom indicator displays Z-In.

Photos Photo 1	Photo Z	Photo 3	
A001(6) 18 21 240	t A-100 (0) 2010 2010	A VETT (20) 500 2 - 004 21 - 000 102 1 - 40 - 500 2 - 004	
Phete 4	Photo 5	Photo 6	Load Photos © Sequence © Identical
Adonting Srist 2-is	A 200 gg 200 200	a Additing 20122 2014	Load
C RIGHTM		ALLER THE ALLER AL	Show (n/)
	A standard and standard an	ata 8 Distant Thilling and Decemptant	

- 8. To return to the original photo place the mouse pointer somewhere inside the actual photo and click the left mouse button.
- 9. Successively point to the center of the lower left erythema patch in all photos and click the left mouse button to place all photos in zoom-in mode. The *Spot Analyzer TiVi80* main window should now look like:

1. D.						
koola Oridge						TIVE
Photo 1	1	Photo 2		Phote 3		
4001 pg	5157 Zen	A-022 pp	115° 24	Andre pg	227 24	
Photo 4	0	Photo 5		Phote 6		1
Reg.	-	P	1	C	b .,	Ecod Photos Sequence C Identical
	A187 7.0	ANTIN		4.77.00	A147 7.40	Load
21-749-2008-03-22-10	Reserve	21-740-0006 09:02:40	Pieces Thy	21-740-2005-08-02-10	O NORTH	Show TiVi
Set ROI Size		View Angle	Calculate & I	iplay TVi Imag	e Parametera	4
251	x-Date	Top Vew	Manual	On Color 5	man 400 •	Reset All
and the second	Y-8000					

- 10. Click the **Show TiVi** button to display the TiVi images corresponding to the selected regions of interest in the photos.
- 11. **Color Span** use these settings to change the color scale of the TiVi-images displayed. In this particular case set the **Color Span** to 400. The *Spot Analyzer TiVi80* main window should now look like:



12. Side View - click this radio-button to display the TiVi-images in 45 degree side view.



13. Top View – click this radio-button to bring the system back to top view display mode.

14. If high TiVi-value areas not belonging to the reaction of interest appear in the TiViimage, these can be eliminated by placing the mouse pointer at the point in the TiViimage to be erased. Press the left mouse button and move the mouse. The erased areas are displayed in gray colour and in the further analysis treated as background by the system (i.e. not accounted for in the intensity and area calculations). Position the mouse pointer on the red spot in *Photo 4*, press the left mouse button and move the mouse to start erasing a selected area in the TiVi-image. The *Spot Analyzer TiVi80* main window should now look like:



- 15. To remove the erase section, click the **Reset TiVi** radio-button under the actual TiViimage.
- 16. **Erase Size** use this pull-down menu setting to determine the size of the erase area (1 = small area, 5 = large area). The actual size of the erase area is influenced also by the size of the TiVi-image.
- 17. Reset All to reset the system and again display the originally loaded photo sequence.
- 18. Automatic clicking this button automatically calculates the intensity and area of the displayed TiVi80 images with threshold values as set in the TiVi Settings window and generates the TiVi80 Chart to display the results.
- 19. **Manual On** clicking this button opens the **Manual Steps** panel with buttons that takes the user to the different steps towards calculating the intensity and area and to displaying the results in the **TiVi80 Chart**.

1 2				
heels Oxidge				TW1 127
- Photo 1	Phote 2	Photo	3	Manual Steps
				Calculate BG Calculate Hax
			State 1	Calculate Threahold
			State of the second	Internatly & Area
edio pg Sist S-No-200 (900) 🕜 And T	240 pg (00144 1-1240 00154 - 140	DIST 24 A00 tigs	a 1957 Den DE DE D	Generate Chart
- Photo 4	Photo 5	- Photo	16	
			<u> ()</u>	- Lond Photon © Sequence © Identical
				Losd
	S-m A-0001(pg M 01-Pask-2006-00.00-40	0157 24 A-0051pg 0 reset TWI 21-Pai-20	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Show Phote
Set ROI Size	View Angle	Calculate & Diplay	TiVi Image Parameters	

20. Click **Calculate Bg** to display the areas in each TiVi-image used as background (normal skin TiVi-value). Threshold settings from the **TiVi80 Settings** window.



21. Click **Calculate Max** to display the areas in each TiVi-image representing maximal values. Threshold settings from the **TiVi80 Settings** window.



22. Click **Calculate Threshold** to display the areas in each TiVi-image representing values above threshold. Threshold settings from the **TiVi80 Settings** window.

Notify Notify <th>D</th> <th></th> <th></th> <th></th>	D			
Photo 1 Photo 2 Photo 3 Photo 3 Calculate B33 Calculate Calculate B33 Ca	hoels Oxidge notos			TM: 109
A SCR UP STORE STO	Photo 1	Photo 2	Photo 3	Calculate B(3
Worksy 515 See Addings 515 See	1961 (8) 1157 2-11 1-16-335 (1923) (1966 1971) Photo 6	A-000(0) 51 87 2-9 21-96-205 81 21 10 10 10 10 10 10 10 10 10 10 10 10 10	A45011,000 01167 249 21496-3020 002145 (Masker trail Photo 6	Calculate Threshold
Additing 5157 Den Additing 5157 Den Pres-cost at 2240 State This Set ROI Size Rest Table Rest Ta	1	0		Load Photos © Sequence © Identical
Interview Description Description Description Description Shown Photo	-24 m 115 Je	Addring S17 Date	Additing Stiff Dec	Load
Set RDI Size View Angle Calculate & Diplay TN/ Image Parameters Reset All	1-940-3008 08 23 1 S	21-#60-0008 08 22 40 C Pased TVA	21-Me6-300k 08-28-16 C Ravet The	Show Photo
	et ROI Size	View Angle Calculate &	Diplay TM image Parameters	ReastAll

- 23. Click **Intensity & Area** to calculate the intensity and area with associated standard deviations for each TiVi80-image.
- 24. Click Generate Chart to display the TiVi80 Chart with information about intensity and area for each TiVi-image.
- 25. When the system displays TiVi-images the actual TiVi-value at the mouse pointer is displayed in the upper right corner of the *TiVi80 Spot Analyzer TiVi80 main* window.

4.1.1 TiVi-80 Spot Analyzer TiVi80 main window pull-down menus

- 1. File Open Chart to open an existing TiVi80 Chart window.
- 2. File Open Project to open an existing TiVi80 Project window.
- 3. File New Project to open a new TiVi80 Project window.
- 4. File Close to close the *Spot Analyzer TiVi80* main window.
- 5. Settings displays the TiVi80 Settings window (see below).
- 6. **Manual** the *Spot Analyzer TiVi80* on-line manual.
- 7. About –*Spot Analyzer TiVi80* About window.

The **TiVi80 Settings** window allows the User to set several TiVi80 parameters. These parameters are stored and saved in between sessions.

🛃 TiVi80_Settings 🛛 📃 💟
25 Y-Scale 15 Y-Scale
Background fraction 0.2
Maximum fraction
Threshold fraction
0.5 Default
Intensity fraction
0.9 Close

- 1. **X-Scale slider** and **edit box** to set the width of the ROI box (percentage of photo width). Also updates the corresponding controls in the *Spot Analyzer TiVi80* main page.
- 2. **Y-Scale slider** and **edit box** to set the height of the ROI box (percentage of photo height). Also updates the corresponding controls in the *Spot Analyzer TiVi80* main page.
- 3. **Background fraction** sets the lowest fraction of the TiVi80 image as background (normal skin TiVi-values).
- 4. **Maximum fraction** sets the fraction over above which the TiVi-values are used to calculate the maximum value.
- 5. Treshold fraction fraction value used as threshold for calculation of area extension.
- 6. **Intensity fraction** fraction above which values are included for calculation of the erythema intensity.

TiVi80 Spot Analyzer



4.2 TiVi80 Chart window

The **TiVi80 Chart** window displays the mean or median intensity and the area of up to six TiVi-images.



- 1. Mean Intensity to display the mean intensity calculated.
- 2. **Median Intensity** to display the median intensity calculated. To be used instead of mean intensity if single outlier values are present.

- 3. Area to display the extension of the erythema area.
- 4. **Erythema** to display the erythema superimposed on the background (normal skin TiVi-value) alone.
- 5. Background to display the background (normal skin TiVi-value) alone.
- 6. Both to display the erythema value stacked on top of background (see Fig. above).
- 7. Standard Deviation check to display the standard deviation bars.
- 8. Close to close the TiVi80 Chart window.

4.1.2 TiVi-80 Chart window pull-down menus

- 1. Save Chart to save the Chart.
- 2. Close to close the TiVi80 Chart window.
- 3. **Export Data** to export the calculated data as ASCII code to an *Excel* spread sheet. The following data sets are saved: *Mean Intensity, Median Intensity, Mean Background, Median Background, Standard Deviation for Intensity, Standard Deviation for Background* and *Area.*
- 4.

4.2 TiVi80 Project window

In the **TiVi80 Project** window the results from up to twenty different **Chart** calculations are uploaded and displayed. The **TiVi80 Project** window typically displays the overall result of a full test panel session.



- 1. Mean Intensity to display the mean intensities calculated.
- 2. **Median Intensity** to display the median intensities calculated. To be used instead of mean intensity if single outlier values are present.
- 3. Area to display the extension of the erythema areas.
- 4. **Erythema** to display the erythema superimposed on the background (normal skin TiVi-value) alone.
- 5. Background to display the background (normal skin TiVi-values) alone.
- 6. File Number sorts the results after file number (session) along the x-axes.
- 7. Measurement sorts the result after measurement number along the x-axes.
- 8. **2D** Displays the result as two-dimensional bar graphs.
- 9. **3D** Displays the result as three-dimensional bar graphs.
- 10. Standard Deviation check to display standard deviation bars.
- 11. Clear all click to clear the display.
- 12. Load Object to load and display a new object.

13. File Number – Displays the names of the files uploaded and displayed. Clicking the brown panel in front of the name permanently removes the associated file from the **Project**.

4.3.1 TiVi80 Project window pull-down menus

- 1. Save Project to save the TiVi80 Project.
- 2. Close to close the **TiVi80 Project** window.
- 3. **Export Data** to export the calculated data as ASCII code to an Excel spread sheet. Two-dimensional data for files and measurement number are saved in the following sheets: *Mean Intensity, Median Intensity, Mean Background, Median Background, Standard Deviation for Intensity, Standard Deviation for Background* and *Area*.