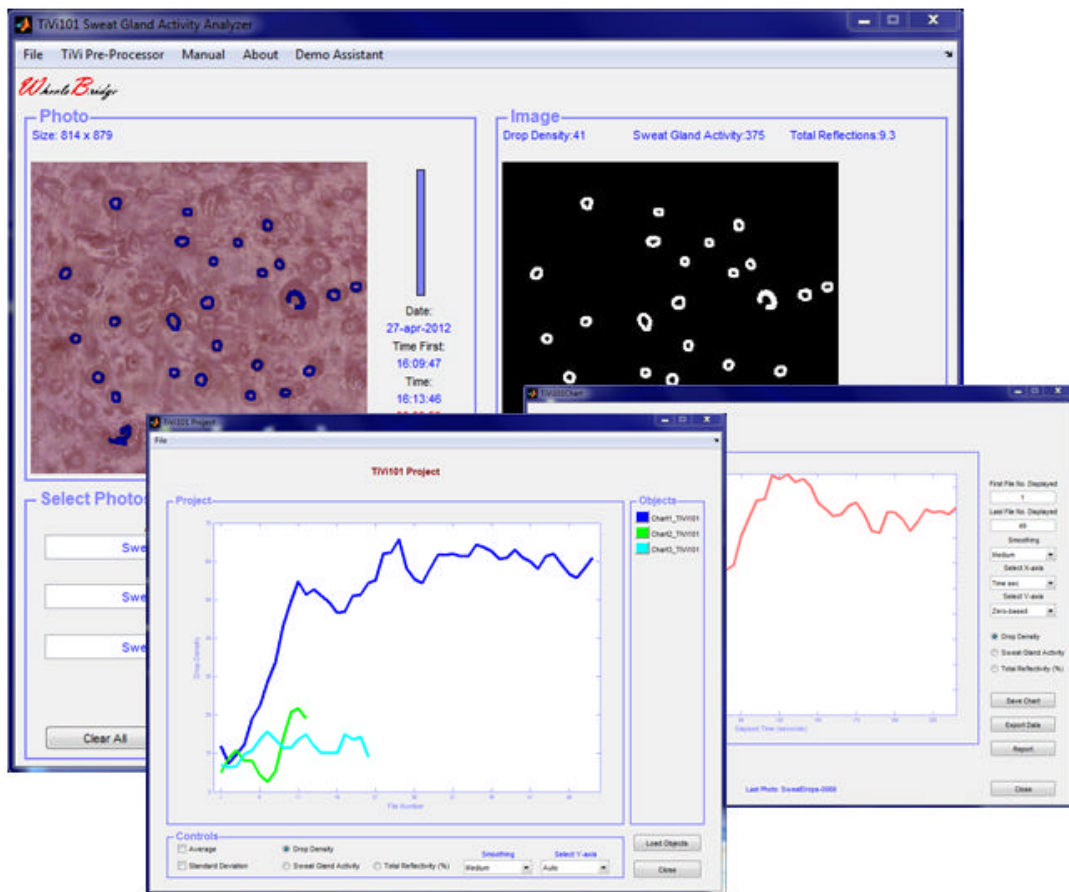




Wheels Bridge



Sweat Gland Activity Analyzer TiVi101 User Manual

User Manual 1.5
Version 1.5
October 2013

PIONEERS IN TISSUE VIABILITY IMAGING

TiVi101 Sweat Gland Activity Analyzer

Dear Valued Customer!

Welcome to the WheelsBridge TiVi101 Sweat Gland Activity Analyzer system intended for automatic analysis of human skin sweat gland activity. The TiVi101 Sweat Gland Activity Analyzer was designed for observer-independent analysis of sweat gland activity in association with evaluation of skin care products and for medical research applications.

The WheelsBridge TiVi101 Sweat Gland Activity Analyzer utilizes a highly sensitive digital camera equipped with magnifying lenses and polarization filters making it possible to detect surface reflections from individual sweat drops on the skin surface in co-polarized 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 TiVi101 Sweat Gland Activity Analyzer software the following are of particular interest:

- ? Automatic capturing of photos.*
- ? Automatic tracking of sweat drops.*
- ? Display of Sweat Gland Activity map.*
- ? Automatic generation of curves displaying the Sweat Gland Density.*
- ? Operates in co-polarization mode.*
- ? All data can be exported to ASCII-format spread sheets.*
- ? Project window displays up to 20 curves simultaneously.*
- ? Printable Report easy to generate.*

We are convinced that the TiVi101 Sweat Gland Activity Analyzer will be a productive tool in the assessment and follow-up of human skin sweat gland activity following treatment with skin care product and in medical research.

Thank you for choosing the WheelsBridge TiVi101 Sweat Gland Activity Analyzer.

WheelsBridge AB

TiVi101 Sweat Gland Activity Analyzer

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TiVi101 Sweat Gland Activity Analyzer

1. INTRODUCTION

Transepidermal Water Loss (TEWL) sometimes referred to as insensible perspiration is composed of two parts – the diffusion of water vapor through the epidermis and water evaporation of sweat drops discharged by active sweat glands. While there is a continuous diffusion of water vapor through the epidermis, transepidermal water loss is substantially increased when the sweat glands become active. The body utilizes an increased sweat gland activity to dissipate heat through evaporative heat loss by activating the sweat glands. An altered sweat gland activity may further be observed in diseases such as hyperhydrosis and in association with peripheral neuropathy frequently accompanying diseases such as diabetes.

Up to now it has been difficult to investigate the water evaporation caused by increased sweat gland activity separately because of lack of suitable technology. The *TiVi 101 Sweat Gland Activity Analyzer* makes such investigations possible.

The intended use of the *TiVi101 Sweat Gland Activity Analyzer* is to analyse the number of active sweat glands in experimental and research applications. It is not yet approved for the diagnosis and treatment of disease.

2. OPEARATING PRINCIPLE

The *TiVi101 Sweat Gland Activity Analyzer* operates in the following way:

In this application the TiVi system employs the *TiVi Camera Microscope* adapter to attain the sufficient magnification to visualize reflections of polarized light from the minute sweat drops as they emerge from the sweat glands. By this arrangement a lateral resolution of about 5 micrometer per pixel is attained. The skin site to be investigated is held against the aperture (diameter 15, 30 or 45 mm) at the frontal end of the *TiVi Camera Microscope* adapter (Fig 1).



Fig 1. The TiVi camera equipped with the *TiVi Camera Microscope* adapter in measurement position over the palm of the hand.

The internal Illuminator of the *TiVi Camera Microscope* adapter comprises a ring including 15 light emitting diodes (LEDs) emitting linearly polarized light (Fig 2).



Fig 2. Internal Illuminator ring of the *TiVi Camera Microscope* adapter comprising 15 light emitting diodes emitting linearly polarized light.

A second polarizing filter with a polarization direction in parallel with that of the filter in front of the LEDs effectively enhances surface structures while diffusely backscattered and depolarized light is suppressed. This configuration enhances surface reflections such as the reflection of polarized light from the LED ring backscattered from the surface of a sweat drop. Fig 3 shows the reflection pattern as seen by the camera after reflection in 5 – 6 sweat drops emerging from active sweat glands.

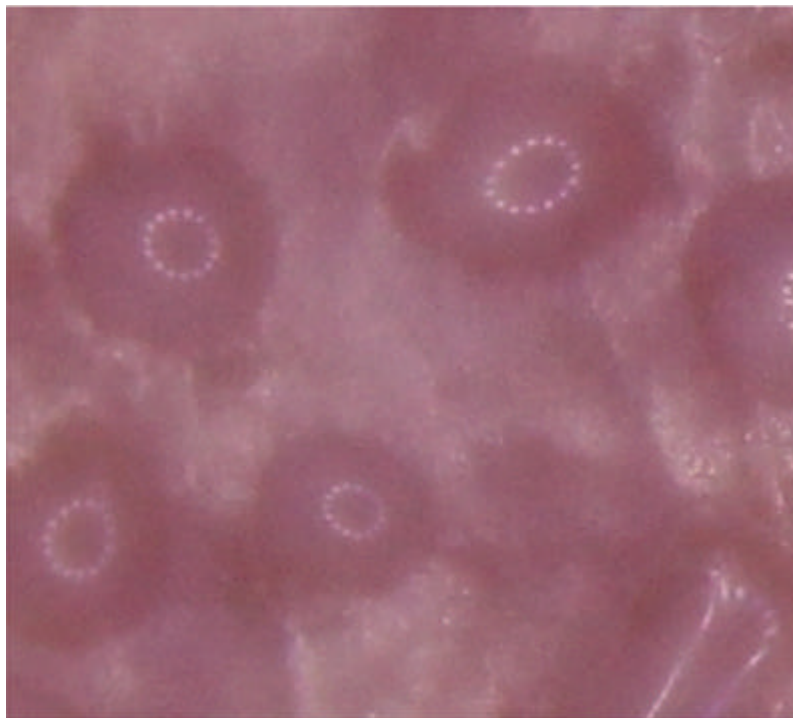


Fig 3. Reflection pattern from sweat drops emerging from active sweat glands, while illuminated by polarized light detected in co-polarization mode.

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In Fig 3, the extension of the sweat drop is defined by the darker uniform circular area from the centre of which the light from the LED ring is reflected. When a sweat drop evaporates the circular ring of LED reflections becomes distorted and eventually disappears. The size of the entire photo displayed in Fig 3 is 388 x 431 pixels or approximately 1.9 mm x 2.2 mm while the diameter of the sweat drops displayed is in the order of 100 pixels or 0.5 mm. The purpose of the algorithm discussed below is to automatically identify uniform rings of different sizes and to classify these rings as representing a sweat drop.

In most cases larger areas (up to typically 1000 x 1000 pixels corresponding to an area of about 5 mm x 5 mm) are analyzed. Within such an area up to several hundred active sweat glands can sometimes be observed, depending on what site of the body surface is investigated. In Fig 4 a skin area of a size of 5 mm x 5 mm with a multitude of active sweat glands is displayed. Using a larger skin site than about 1000 x 1000 pixels (5 mm x 5mm) makes it difficult to attain sufficient focus over the entire photo because of the shallow focal depth at high magnification. To attain a sufficiently large focal depth the shutter Av-value should be set to 10 or higher in the *TiVi701Camera* window (**Shutter** pull-down-menu) before capturing the photos.

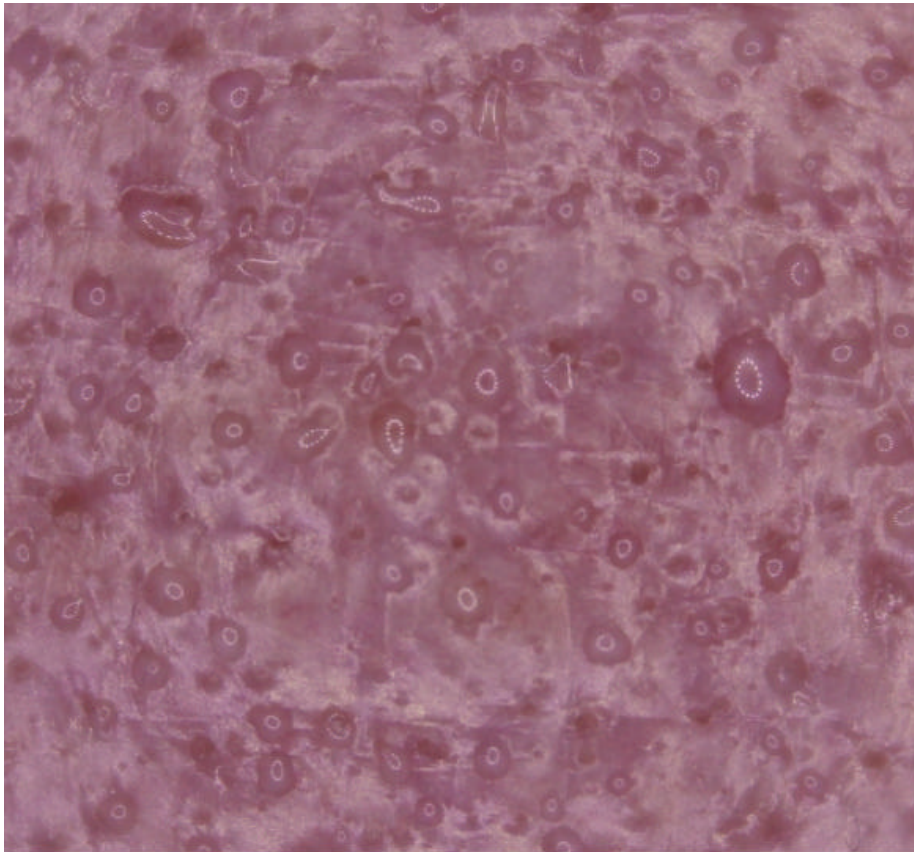


Fig 4. Forehead skin area (5 mm x 5 mm) with many active sweat glands in different states.

3. SETTING UP AN EXPERIMENT

When capturing a set of photos from which the local sweat gland activity is to be analyzed proceed as follows:

Capturing photos:

1. Attach the *TiVi Camera Microscope* adapter to the TiVi camera. Select a microscope front end cover with 15, 30 or 45 mm diameter aperture. Set the focus switch on the zoom-in lens to **AF** (*Auto Focus*) and rotate the distal part of the *TiVi Camera Microscope* adapter until the red dots are in close position (the system is now in co-polarized mode).
2. Click **Shutter** in the *TiVi101Camera* window pull-down menu and select '20'.
3. Set the camera **Select Photo Size** to *Large Fine*, **No of Photos** to *100* and **Delay** to *5 sec*. These settings capture a new photo every 5 seconds with a lateral resolution of about 5 micrometers per pixels, sufficient to display the reflections of co-polarized light rings in the emerging sweat drops. Click the **Save Photos** button to select a folder and name under which the photos are to be saved.
4. Wipe of the skin surface to be investigated before start of measurements to remove possible water drops or a liquid film from the skin surface. Place the aperture in contact with the skin and click the **Capture Photos** button to initiate the photo capturing process.
5. The photo capturing process can be halted at any time by clicking the **Pause** button. The photo capturing process can be continued again by clicking the same button, now labelled **Continue**. The capturing process can be terminated by clicking the **Reset** button.
6. Because of the high degree of magnification, the photo capturing process may be prone to movement artefacts. To capture a high resolution photo of good quality it is important to keep the *TiVi Camera Microscope* adapter aperture in firm contact with the skin surface investigated. Further, it is important to avoid body movements during the photo capturing process. With the focus switch set to **AF**, the camera automatically adjusts the focus before capturing a new photo. This automatic focusing process is based on the fact that there is sufficient contrast on the skin surface to focus on, otherwise the error message 'Can't Focus' is displayed and the photo capturing process is interrupted. If this happens, click the **Capture Photos** button to continue, possibly after moving the aperture a short distance sideways to an adjacent skin area with better contrast. Because of the high degree of magnification the focal depth is shallow and a sufficient focus may not be possible to attain over the entire surface of a large photo with 18 Mpixel resolution. In practice an area with adequate focus of 1000 x 1000 pixels or less is selected for the further analysis.

Analyzing photos:

1. Upload the sequence of photos to the *TiVi700 Analyzer* main window.
2. Click the **Automatic** button to scan through the photos. The skin site under investigation can now in some cases be seen to have shifted somewhat in position from photo to photo. At this point the *TiVi Pre-Processor* can be used to align the photos if necessary. Please refer to the *TiVi Pre-Processor* manual for further information.
3. The photos in the sequence may now be inspected for sufficient focus. Select an area in the photos of a size no larger than 1000 x 1000 pixels for further analysis. Draw a rectangular ROI around this area and click the **Show ROI** button.
4. Click the **Automatic** button again to scan through the zoomed in photo sequence. If there are active sweat glands in the selected skin area, small rings corresponding to reflections of polarized light in the emerging sweat drops can be observed. Profound sweating eventually causes substantial and irregular reflections in the liquid film appearing on the surface of the skin.
5. Click the **Crop & Save** button to save these selected areas for further analysis of sweat gland activity or alternatively click the **Undo Show ROI** button, move the ROI to another position and repeat the procedure.
6. The zoomed in photo sequence can now be automatically analyzed with respect to *Drop Density*, *Sweat Gland Activity* and *Surface Reflectivity*. *Drop Density* is by definition the number of identified sweat drops per unit skin area, while *Sweat Gland Activity* in addition accounts for the size of the rings of light reflection (proportional to the size of sweat drops). The algorithm used to automatically identify sweat drops on the skin surface is described in detail in the next section. *Surface Reflectivity* represents the overall relative area above the threshold value used in the algorithm (the white area in Fig 5 below). If the skin is dry the *Surface Reflectivity* value is generally high and is reduced as a liquid film from sweat drops spreads out over the skin surface. The *Surface Reflectivity* is normalized to 100% at its highest value in a sequence before displayed in the *TiVi101 Chart*. The relative reduction in *Surface Reflectivity* can be used as a measure of the extent of the liquid film formed on the skin surface following sweat gland discharge.

4. ALGORITHM EMPLOYED

The algorithm employed in the *TiVi101 Sweat Gland activity Analyzer* provides a way of identifying active sweat glands in the skin site investigated.

This algorithm works in several steps of which the first step includes converting the colour photo into a gray-scale and ultimately a black-and-white (BW) image with appropriate threshold settings. In the BW image objects too small or too large to be considered reflections of the illumination ring of LEDs in sweat drops are eliminated. The resulting reduced BW image calculated from an example photo may look like:

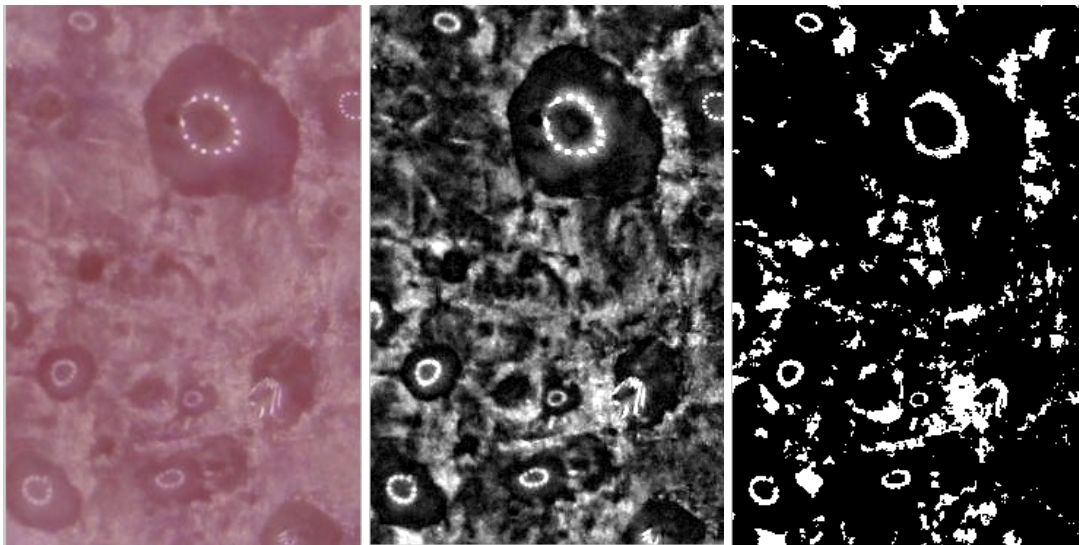


Fig 5. Generation of a gray-scale image (middle) from the original photo (left). After thresholding, the gray-scale image is converted into a black and white (BW) image in which different sweat drops reflection rings can be observed.

In the next step the BW image is split up in different objects. These objects are then individually investigated as candidates for representing reflections from individual sweat drops.

The pattern-recognition algorithm uses inclusion and exclusion criteria for classifying individual objects as reflection rings from sweat drops.

These criteria are:

1. The centre of an object is below a set threshold limit.
2. The ratio of the object area to perimeter is larger than a set threshold limit.
3. The object is reasonably circular.
4. The object is not too small.

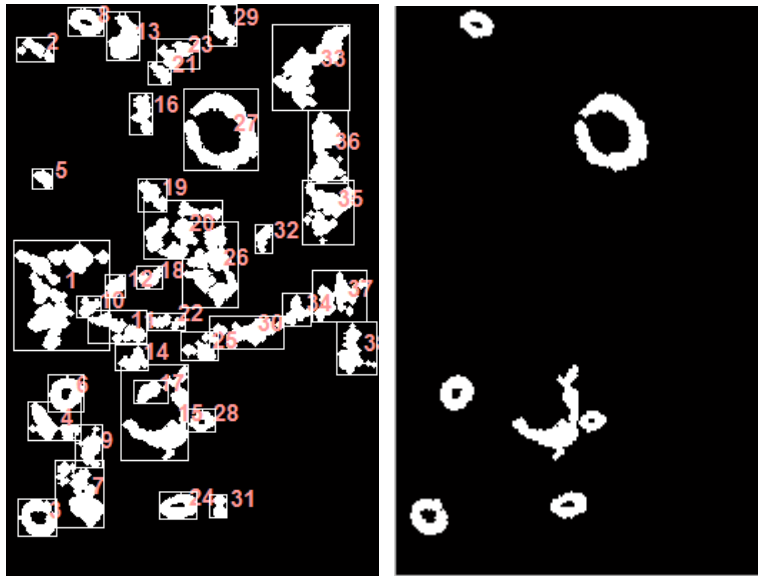


Fig 6. In the present example 38 objects are identified in the photo. Out of these 7 objects fulfil the criteria for being initially classified as polarised light reflection rings from sweat glands.

In the third step the objects are dilated to close rings not yet fully closed. These closed rings are then filled and the resulting image is calculated as the image with unfilled holes subtracted from the image with filled holes to generate an image identifying sweat drops (Fig 7).

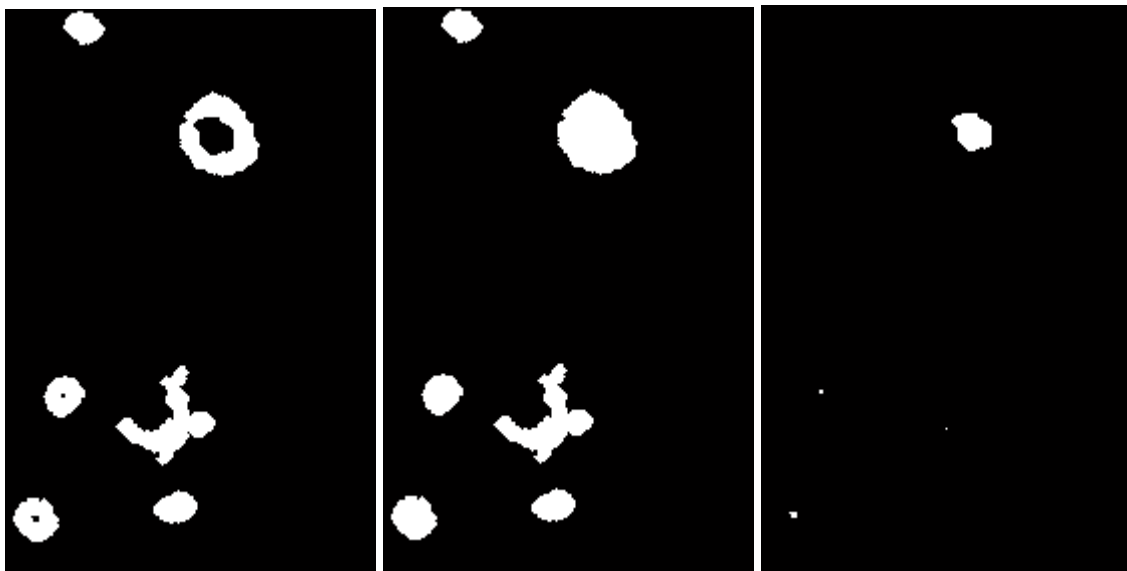


Fig. 7. Dilated objects to close the rings (left), filled ring (middle) and difference image (right) to identify the sweat drops.

If this procedure is applied iteratively, both small and large rings can be correctly identified.

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After removing too small objects to be classified as sweat gland related rings, the resulting *Swaet Gland Object Map* is displayed superimposed on the original photo to identify the sweat glands.

When a sweat drop emerges from the sweat gland duct the reflected ring of light becomes large enough to be classified as a marker for an active sweat gland. When the drop starts to evaporate or the liquid is spread over the skin surface, the ring disintegrates and the associated sweat gland is no longer classified as being active.

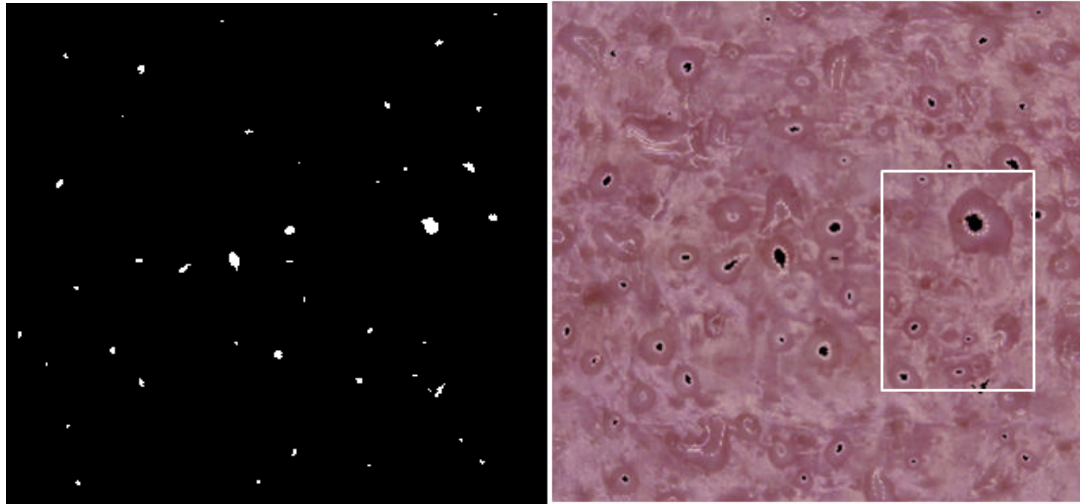


Fig 8. *Sweat Gland Object Map* indicating the positions of the individual active sweat glands and size of corresponding sweat drops (left) and *Sweat Gland Object Map* superimposed on the original photo (right). The white frame indicates the area used to explain the algorithm in the example above.

The *Drop Density* is calculated as the number of objects in the *Sweat Gland Object Map* divided by the size of the map.

The *Sweat Gland Activity* is calculated as the area occupied by all objects in the *Sweat Gland Object Map* divided by the size of the map.

The *Total Reflectivity* is calculated as the total area above threshold (the white area in Fig. 5) in relation to the the total area. The highest value in a sequence is set to 100%.

In the [GETTING STARTED](#) section the *Sweat Gland Object Map* is continuously calculated and updated based on a sequence of photos with increasingly active sweat glands.

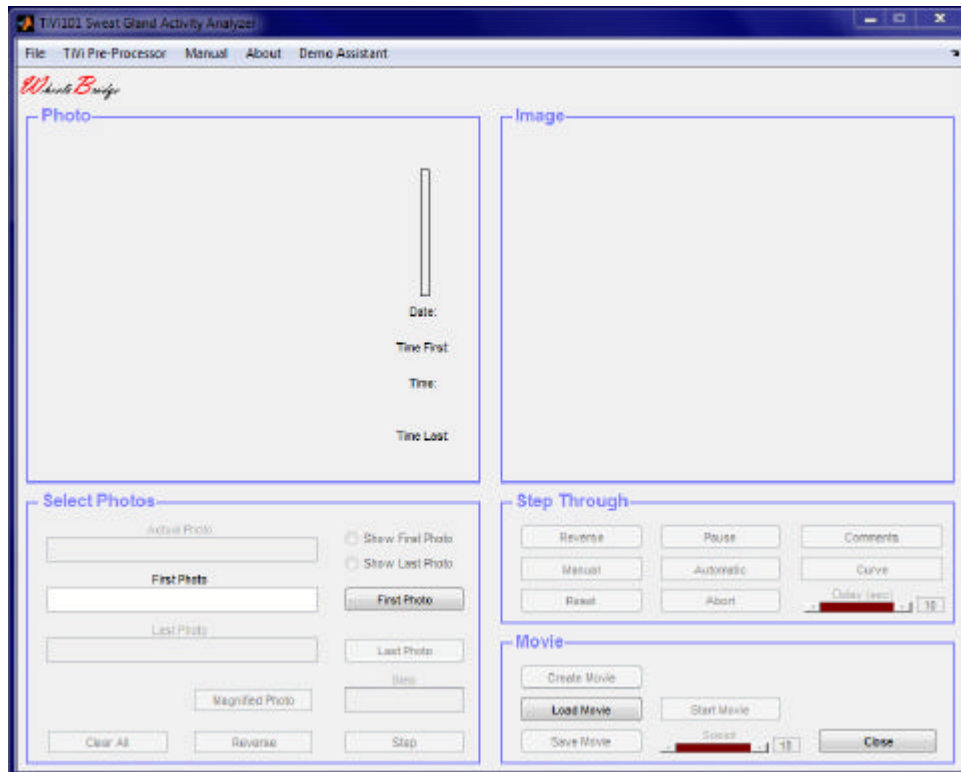
5. GETTING STARTED

The basic features of the *TiVi101 Sweat Gland Activity Analyzer* are probably best explained by way of an example. In the following example it is assumed that the photos *SweatDrops-0020.jpg* – *SweatDrops-0068.jpg* have been captured by a Canon EOS550D TiVi camera

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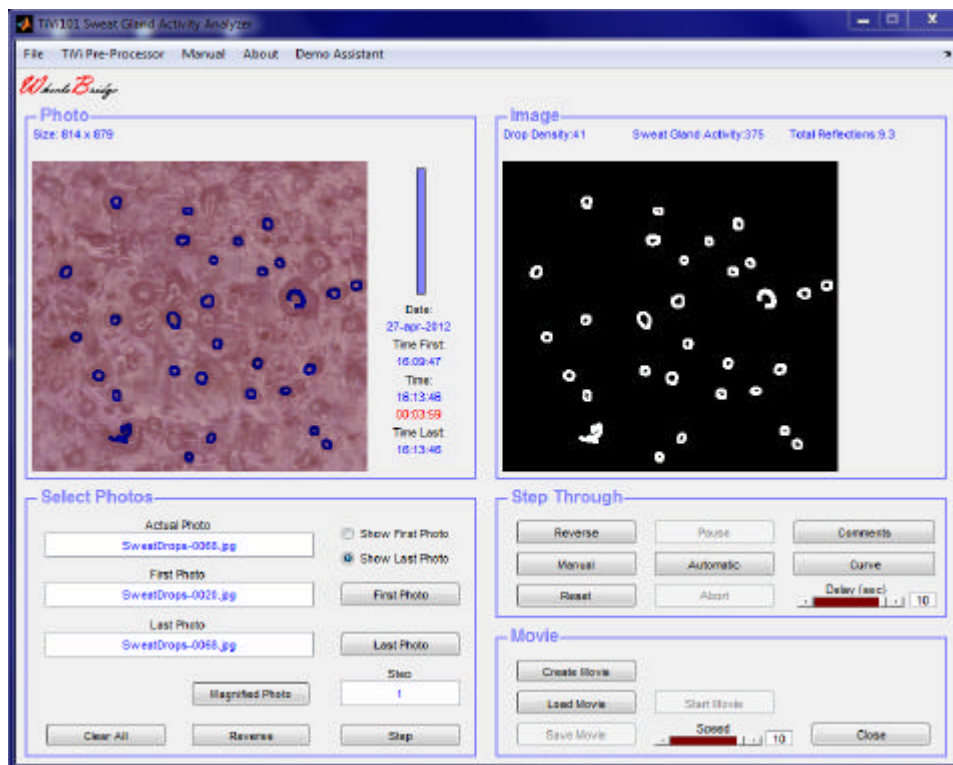
system and stored in the *TiVi101demonstration/SweatDrops* folder. These photos demonstrate the formation of active sweat glands captured from the forehead following physical exercise.

1. Open the *Sweat Gland Activity Analyzer* toolbox from inside the TiVi700 Analyzer main window.



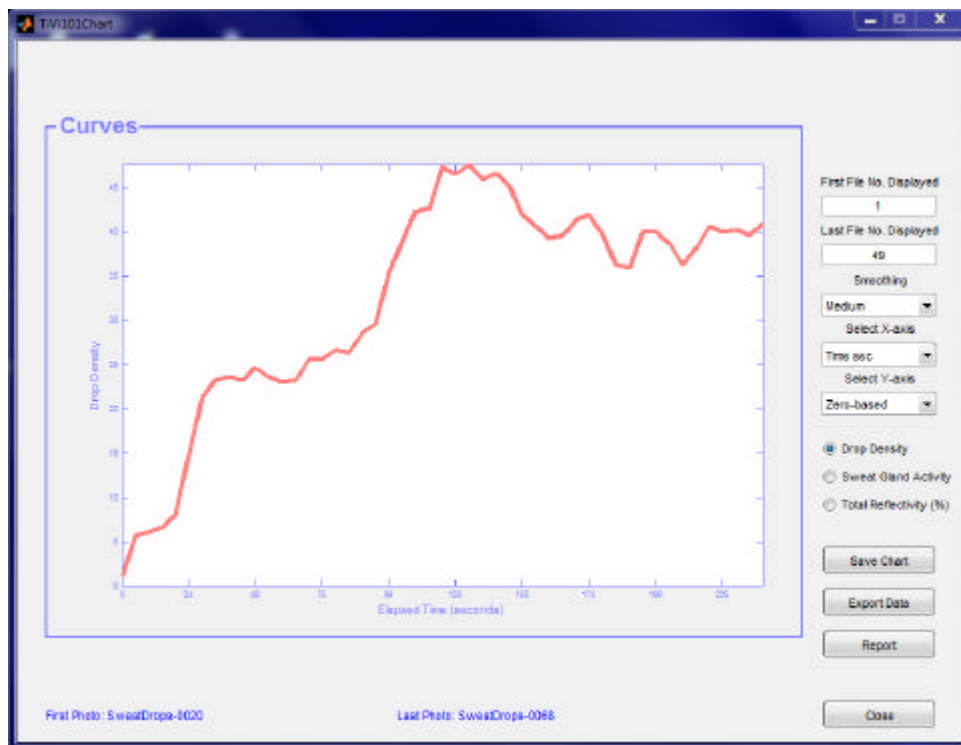
2. Click the *FirstPhoto* button and select the *SweatDrop-0020.jpg* photo in the folder *TiVi101 \ TiVidemonstration \ SweatDrops*.

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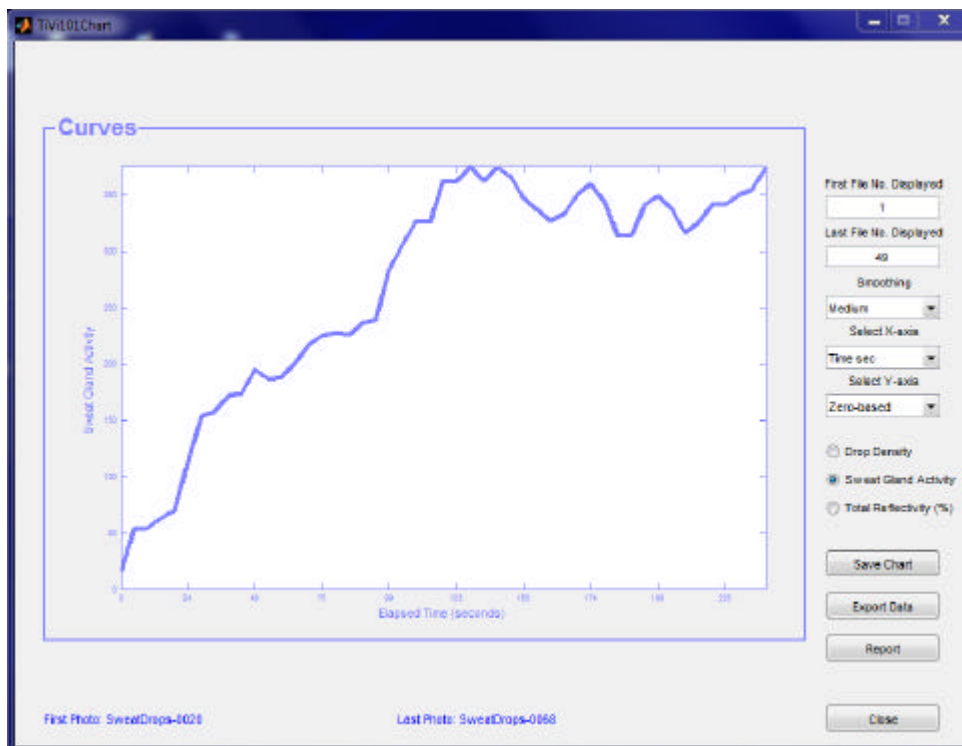


3. Click the *Curve* button to start the analysis process. As the photos are successively analyzed with respect to active sweat glands, the actual *Sweat Gland Activity Map* is displayed in the **Image** panel. This process generally takes several minutes. When all photos have been analyzed the *TiVi101 Chart* window will automatically open to display the sweat *Drop Density* as a function of elapsed time in the photo sequence analyzed. Observe that for LED rings reflections that are not closed or appear too weak the corresponding sweat gland is not classified as active.

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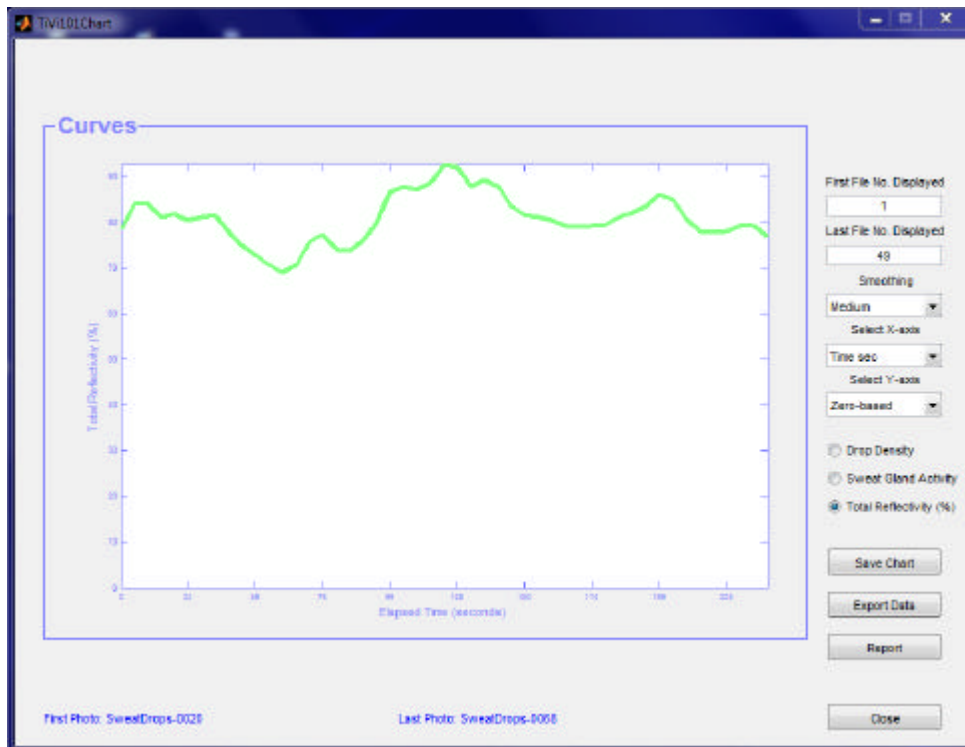


4. This curve displays the number of active sweat glands per unit area (*Drop Density*) for the sequence of photos analyzed.
5. Check the **Sweat Gland Activity** radio-button to display the *Sweat Gland Activity* curve.



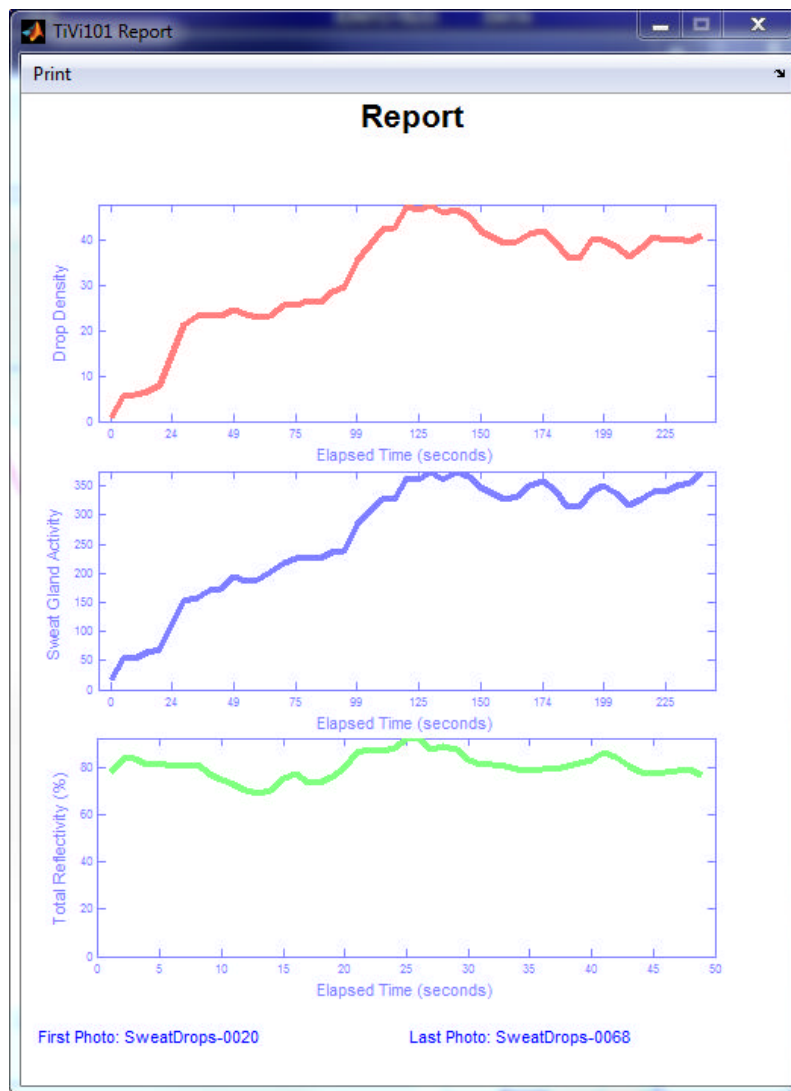
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- Click the **Total Reflectivity** radio-button to display the total reflections from the skin surface.



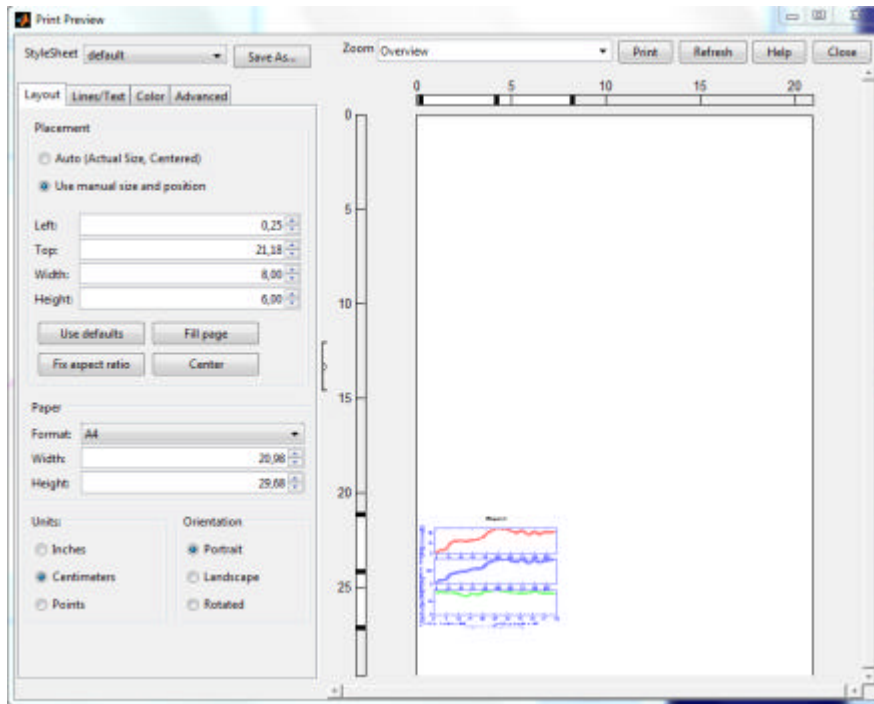
- Click the **Save Chart** button to save the actual *TiVi101 Chart*.
- Click the **Export Data** button to export the data to an *Excel* spread-sheet from which the analysis can be further continued.
- In the *TiVi101 Sweat Gland Analyzer* main window select **Open Chart** under the **File** pull-down menu navigate to the folder in which the *Chart* was saved and double click the *Chart* icon to open the *Chart* once again.
- Click the **Report** button to open the printable *Report* window.

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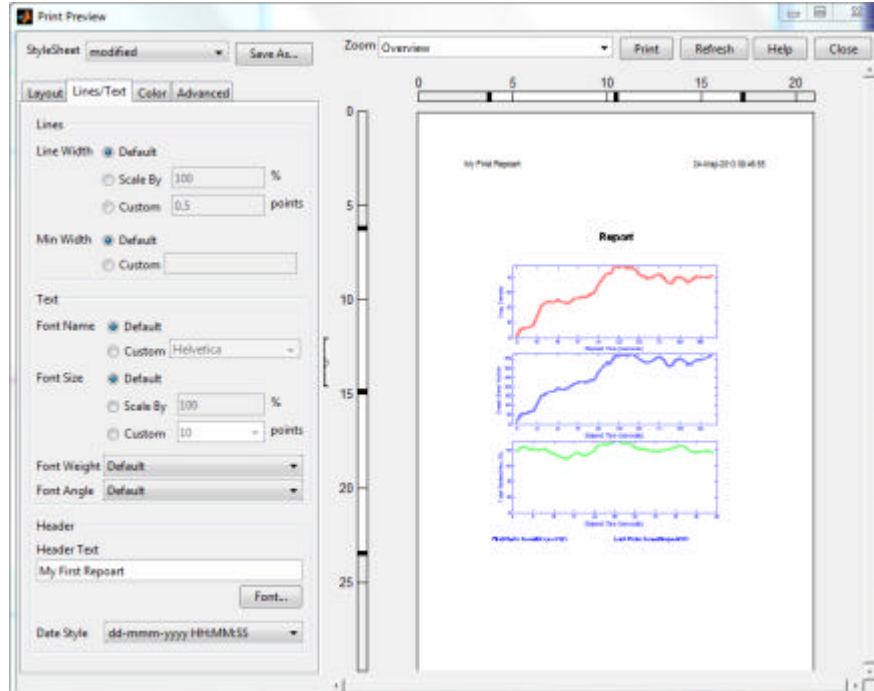


11. Click the **Print** button to open the *Print preview* window.

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- Click the **Auto** radio-button to place the content in the centre of the page. Click the **Line /Text** page and print “My First Report” in the **Header Text** edit box. Set the *Date Style* format. The Preview window should now look like:



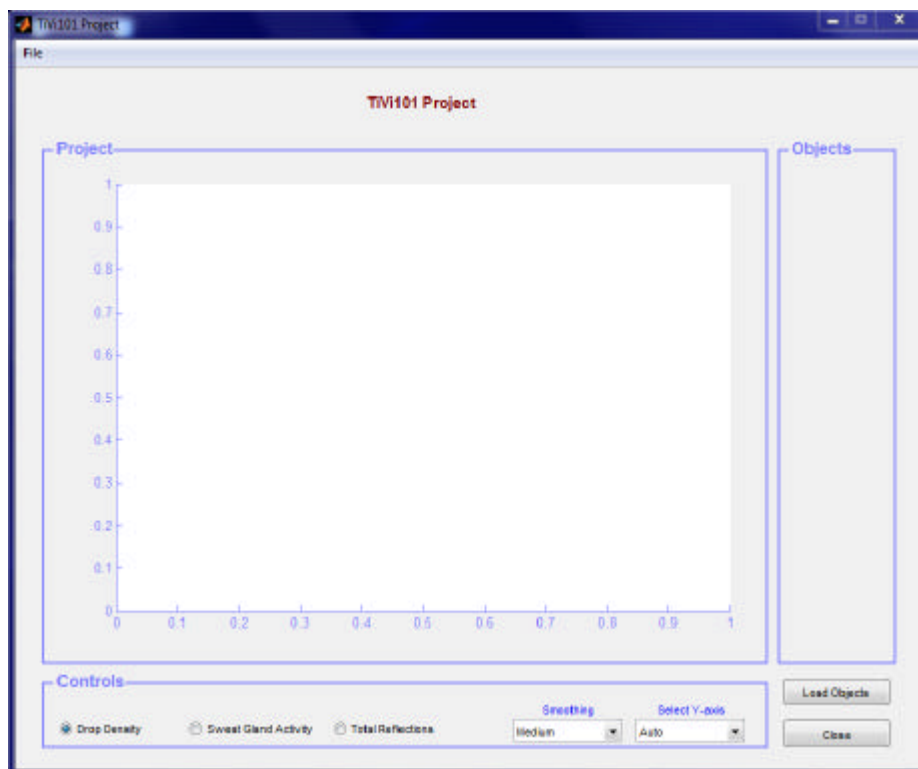
- Click the **Print** button to print out the *Report*.

This concludes the **GETTING STARTED** section.

6. WORKING WITH PROJECTS

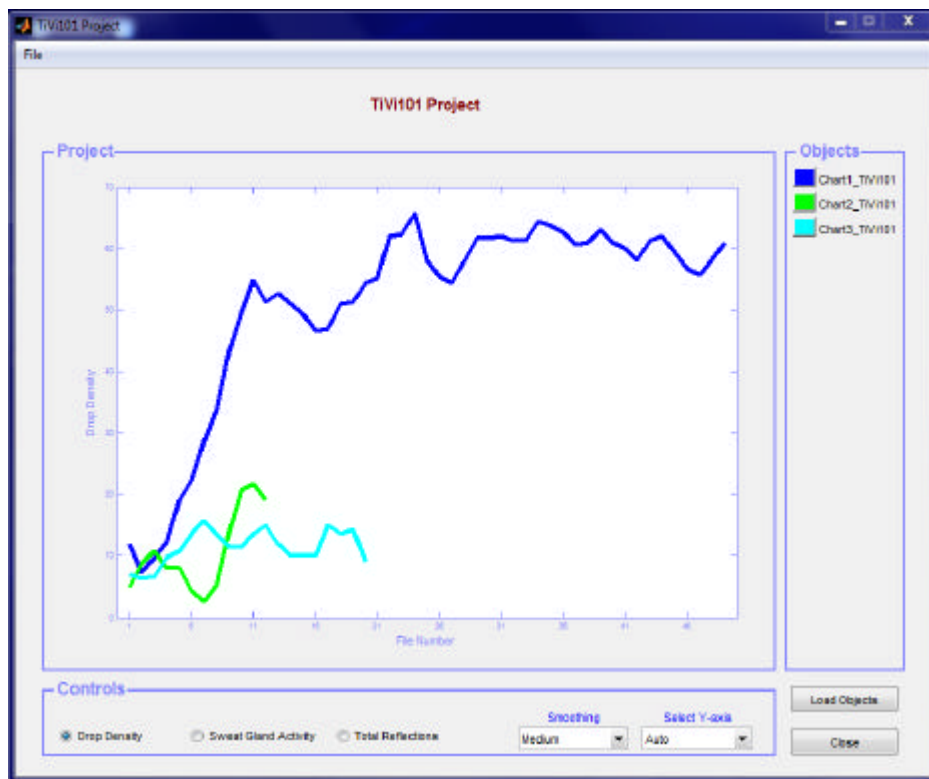
After individual *TiVi101 Charts* have been generated, the different curves can be uploaded and displayed in *TiVi101 Project* for comparison. The following example explains the essentials of *TiVi101 Project*. It is assumed that the three *TiVi101 Charts* have been generated and saved under the names *Chart1_TiVi101*, *Chart2_TiVi101* and *Chart3_TiVi101* respectively.

1. In the *TiVi101 Sweat Gland Activity Analyzer* main window, select *File* \rightarrow *New Project* to open a new project.

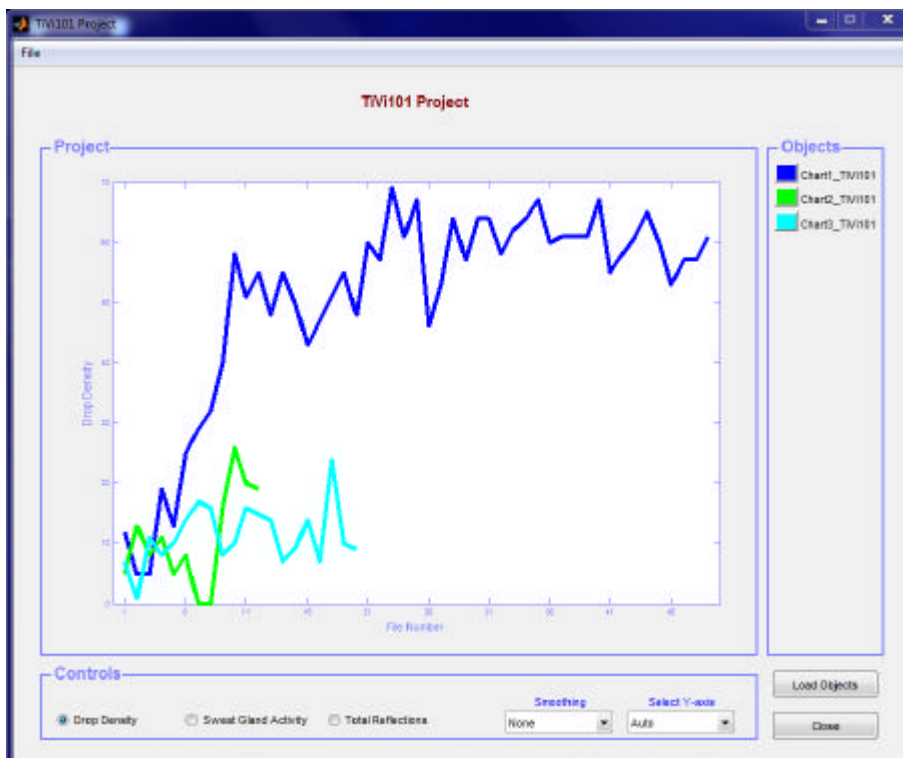


2. Click the **Load Objects** button and navigate to the folder in which the three *TiVi101 Charts* are stored.
3. Press the *Shift*-button on the keyboard and select the three files. Click the **Open** button to upload the three *TiVi 101 Chart* curves to the *TiVi101 Project* window.

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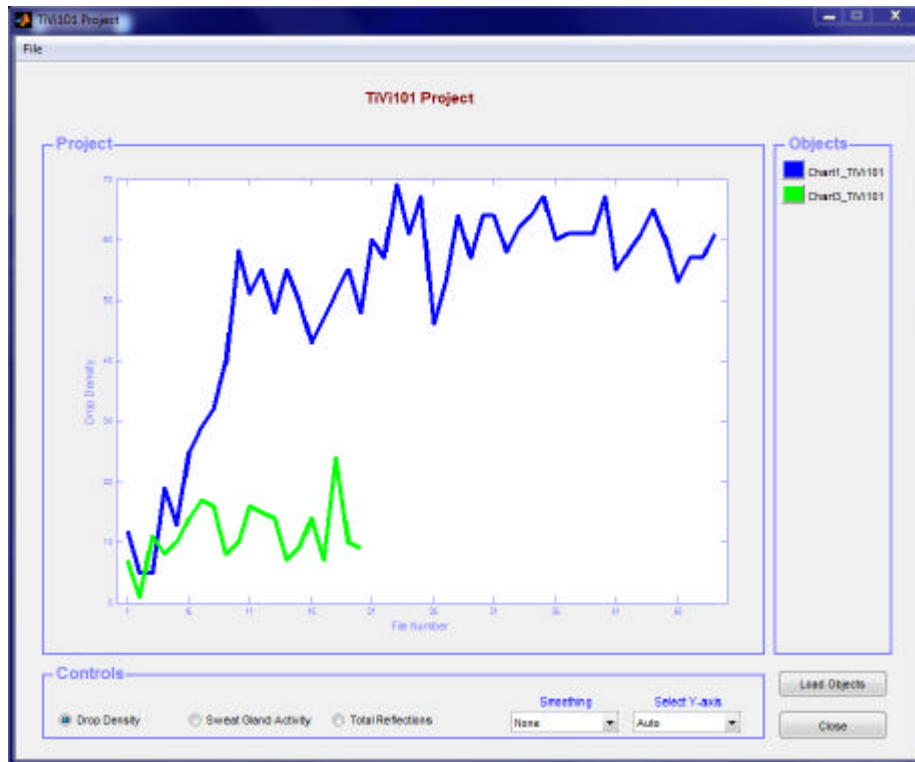


4. Select “None” in the **Smoothing** menu to change the smoothing of the curves displayed.



5. Click on the green panel in the *Objects* panel to off-load the *Chart2_TiVi101* curve from the *Project* window.

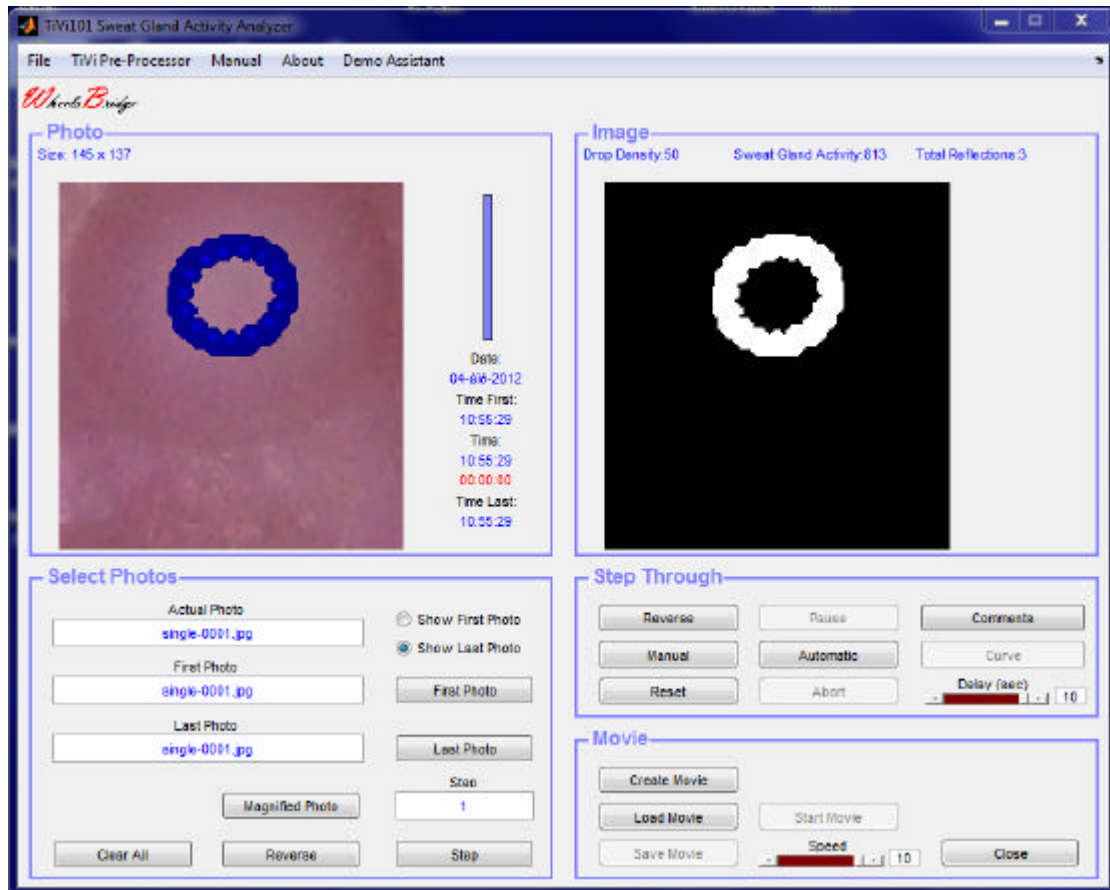
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6. Click the **Load Objects** button and select to *Chart2_TiVi101* file to upload this curve again to the *Project* window.
7. To save the *TiVi101 Project* window select *File* \gg *Save TiVi Project*. The Project can be re-opened by selecting *File* \gg *Open Project* in the *TiVi101 Sweat Gland Activity Analyzer* window.
8. Click the **Close** button to close the *TiVi101 Project* window.




7. DETAILED DESCRIPTION

7.1 The TiVi101 Sweat Gland Activity Analyzer window

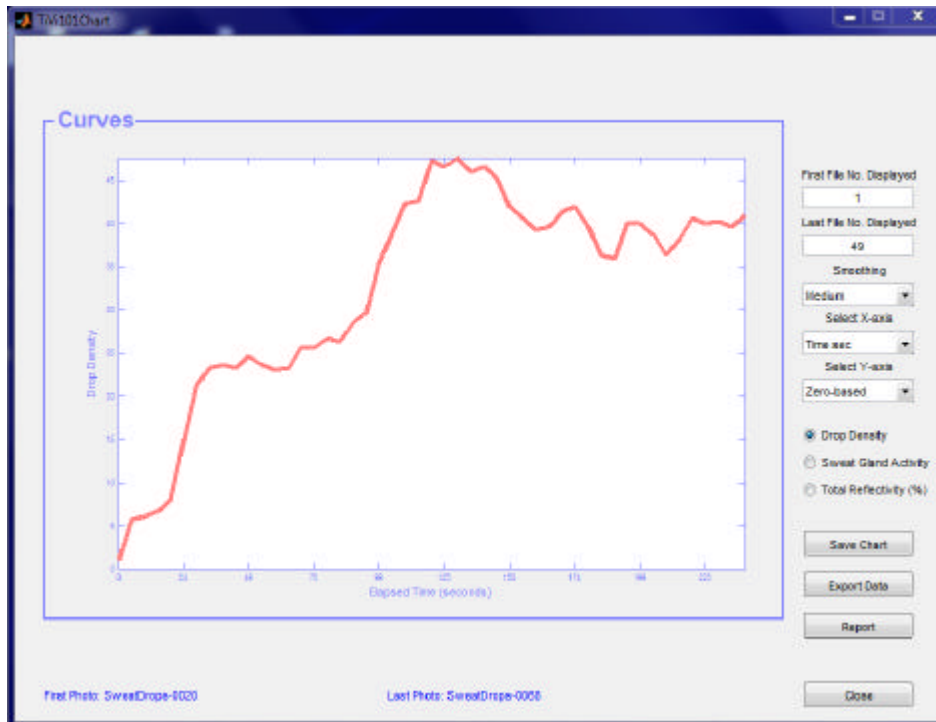


1. **First Photo** button – to upload the first photo in a sequence.
2. **Last Photo** button – to upload the last photo in a sequence.
3. **Show First** button – to display the first photo in the sequence.
4. **Show Last** button – to display the last photo in the sequence.
5. **Clear All** button– clears all displays and edit boxes and resets system to start position.
6. **Step** button – click to change the step in the photo sequence incrementally.
7. **Reverse** button – to reverse from incremental to decremental step selection.
8. **Step** edit box – print the step to be used.
9. **Magnified Photo** button – to display a magnified version of the photo or the photo with superimposed *Sweat Gland activity Map*.

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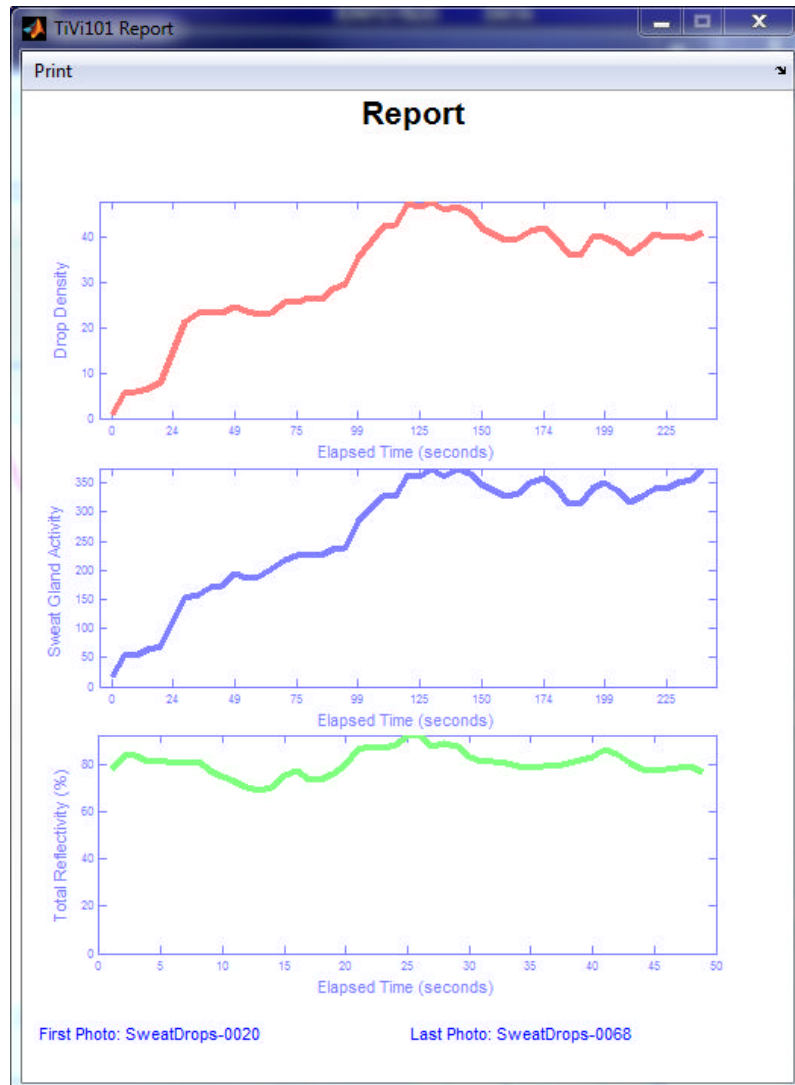
10. **Manual** button – to manually step through the photos in the sequence while at the same time displaying the actual sweat gland map.
11. **Reverse** button – click to step through the photo sequence in reverse direction.
12. **Reset** button – resets the step through process.
13. **Automatic** button – to rapidly step through the photo sequence without displaying the associated sweat gland activity maps.
14. **Pause** button – click to halt the curve generation process.
15. **Abort** button – to stop the curve generation process.
16. **Comment** button – to add comments to the photo *Wordpad*.
17. **Curve** button – to start the generation of the number of active sweat glands curve. While the curve is generated the associated photo and *Sweat Gland Map* is displayed.
18. **Delay** slider – to set the speed of the automatic display process.
19. **Create Movie** button – to create a video clip showing the generation of the active sweat gland activity maps.
20. **Load Movie** button – to upload an existing video-clip.
21. **Save Movie** button – to save a created video-clip.
22. **Start Movie** button – to start the display of a video-clip.
23. **Close** button – to close the *TiVi101 Sweat Gland Activity Toolbox* window.
24. **Speed** slider and edit box - to set the speed of the playback of a video-clip.
25. **File**  **New Project** pull-down menu – to generate a new *TiVi101 Project*.
26. **File**  **Open Project** pull-down menu – to open an existing *TiVi101 Project*.
27. **File**  **Open Chart** pull-down menu – to open an existing *TiVi101 Chart*.
28. **TiVi Pre-Processor** pull-down-menu – to start the *TiVi Pre-Processor*.
29. **Manual** pull-down menu – to display the online manual.
30. **About** pull-down menu – to display the *About* box.

7.2 The TiVi101 Chart window



1. **First File No. Displayed** edit box – to set the first file number to be displayed.
2. **Last File No. Displayed** edit box – to set the last file number to be displayed.
3. **Smoothing** pulldown-menu – to select the smoothing of the curve displayed.
4. **Select X-axis** pulldown-menu – to select units along the X-axis.
5. **Select Y-axis** – to select the scale of the Y-axis.
6. **Drop Density** radio-button – to display the *Drop Density* curve.
7. **Sweat Gland Activity** radio-button – to display the *Sweat Gland Activity* curve.
8. **Total Reflections** radio-button – to display the total reflections from skin surface.
9. **Save Chart** button – to save the actual *TiVi101 Chart* to file. This *Chart* can be re-opened from the **File** pull-down menu in the *TiVi101 Sweat Gland Analyzer* main window.
10. **Export Data** button – to export curve data to an *Excel* spread-sheet.
11. **Report** button – to generate a *Report* and display the *Report* window.
12. **Close** button – to close the *TiVi101 Chart* window.

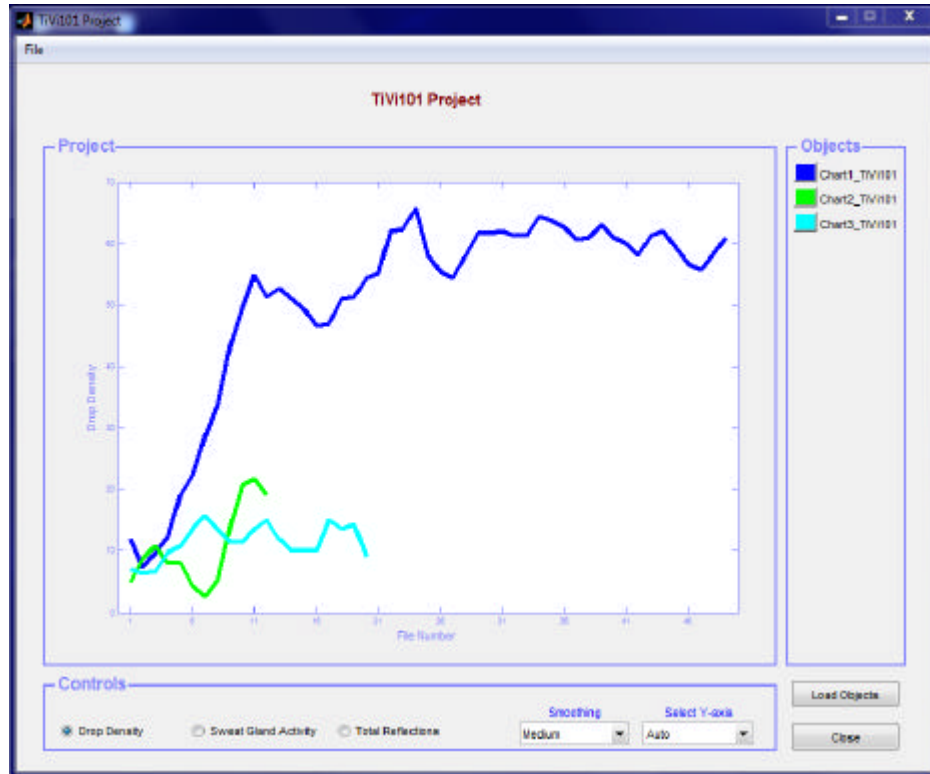
7.3 The Report window



1. **Print** pull-down menu– opens the **Print Preview** window in which further settings such as adding a title and date as well as placing the content centrally in the report. Clicking the **Print** button in the **Print Preview** window generate a hard copy report on a printer.

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7.4 The TiVi101 Project window



1. The curves represent the uploaded *Drop Density* or *Sweat Gland Activity* curves from different saved *TiVi101 Charts*.
2. **Load Objects** button– to upload one or more curves from *TiVi101 Charts*.
3. **Smoothing** menu – to select the degree of curve smoothing (None, Medium or More).
4. **Select Y-axis** menu– to select the scale of the y-axis.
5. **Drop Density** radio-button – to display the *Drop Density* curves.
6. **Sweat Gland Activity** radio-button – to display the *Sweat Gland Activity* curves.
7. **Total Reflections** radio-button – to display the *Total Reflections* of co-polarized light from the skin surface.
8. **Save TiVi Project** pull-down menu– to save the *TiVi Project* window (under **File** pull-down menu).
9. **Export Data** pull-down menu – to export data to Excel (under **File** pull-down menu).

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10. **Close** button– to close the *TiVi 101 Project* window (under **File** pull-down menu and as separate button).
11. **Objects** panel – lists the name of the *TiVi101 Charts* curves displayed in the diagram. To permanently off-load a curve click the coloured panel in front of the name.