WheelsBridge
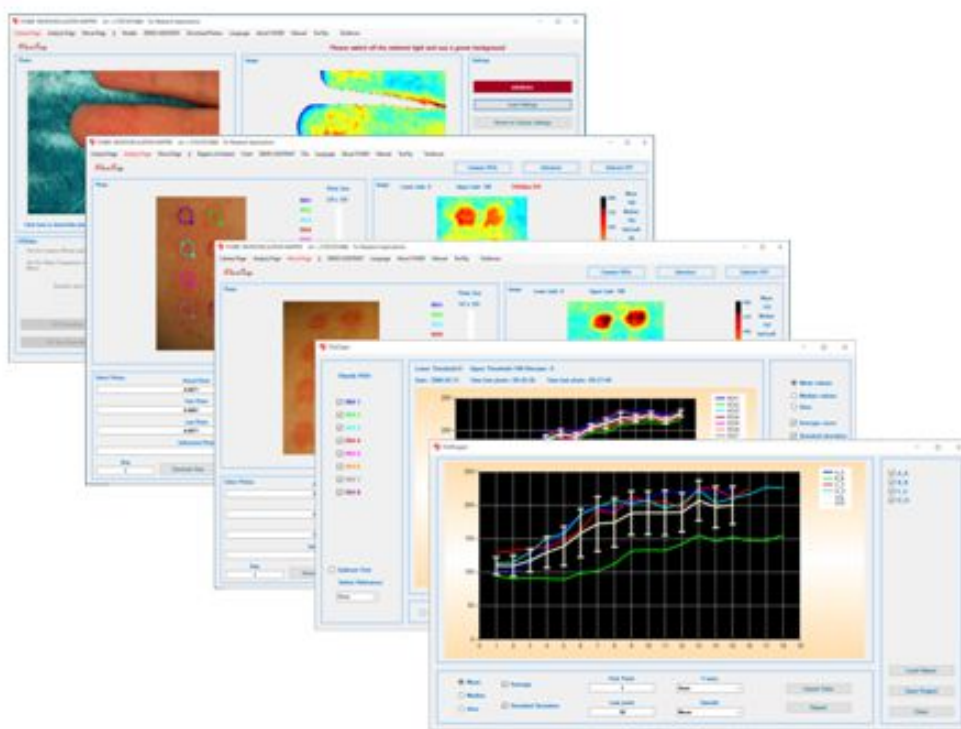

TiVi700 2.0 Tissue Viability Imager
Manual

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The *TiVi700 2.0 Tissue Viability Imager* is not registered as a Medical Device. It is intended for research applications only.

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> Warning! The TiVi cover includes super-magnets. A pacemaker could switch into test mode and cause illness. A heart defibrillator may stop working. If you wear these devices keep sufficient distance to magnets. Warn others who wear these devices from getting too close to magnets.

CONTENT

PART I – Basic System TiVi700 2.0

INTRODUCTION

The *TiVi700 2.0 Tissue Viability Imager* is a polarisation spectroscopy imaging system intended for investigation of skin and other tissue microcirculation in research applications. More precisely it can be used for quantification of *erythema* and *blanching* in irritation grading, assessment of microvascular effects of vaso-active substances topically applied to the skin as well as in the validation of skin care products and pharmaceuticals with respect to *safety* and *efficacy*. Its use is not restricted to investigation of the skin - it is useful in tissue microvascular research in general.

The *TiVi700 2.0 Tissue Viability Imager* is further suitable for the study of the microcirculation of objects in motion, because the recorded images are not affected by movement artefacts.

After recording videos or a sequence of photos, these are converted to Tissue Viability (TiVi) images depicting high-resolution colour coded maps of the microcirculation. By capturing the separate photos from which the TiVi-images are generated at a rapid rate, both a high temporal and spatial resolution is simultaneously attained. The TiVi camera can be attached to a table mounted stand or hold by hand, when capturing the photos or videos (Fig 1) and is controlled by the system software in which case the photos and videos are streamed to the hard drive or used as a stand-alone camera operating disconnected from the computer temporarily storing photos and videos in the camera SD-card.
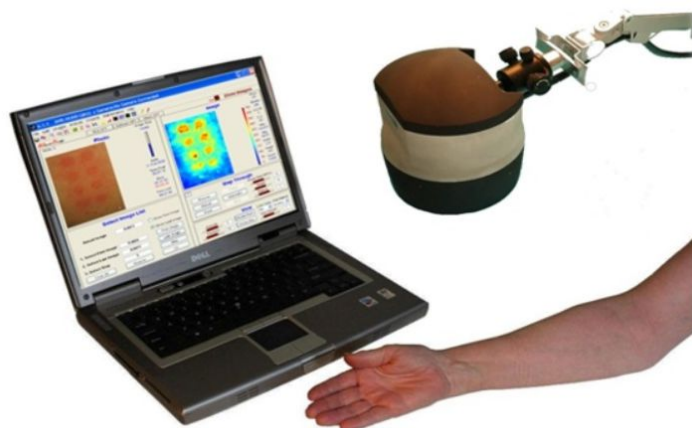
Fig 1. The *TiVi700 2.0 Tissue Viability Imager* camera device attached to a table mounted stand.

The *TiVi700 2.0 Tissue Viability Imager* software flow chart is shown in Fig.2. Parameters for capturing of photos and videos are set by the controls in the *Camera Page*. In the *Analysis Page*, sequences of photos are displayed, and the corresponding TiVi-Images are generated and analysed. Regions of Interest ('ROIs') are drawn for calculation of local average TiVi-values. In the *Movie Page*, individual TiVi-images can be compiled to videos, showing a time compressed version of microvascular events following stimulation of the tissue microvascular network.

Average TiVi-values from individual 'ROIs' are exported to the *TiVi Chart* window in which a diagram shows the time course of the average TiVi-values of individual 'ROIs'. In the *TiVi Project* window, the average curves from single *TiVi Charts* – each representing the results from a single test subject – are displayed.
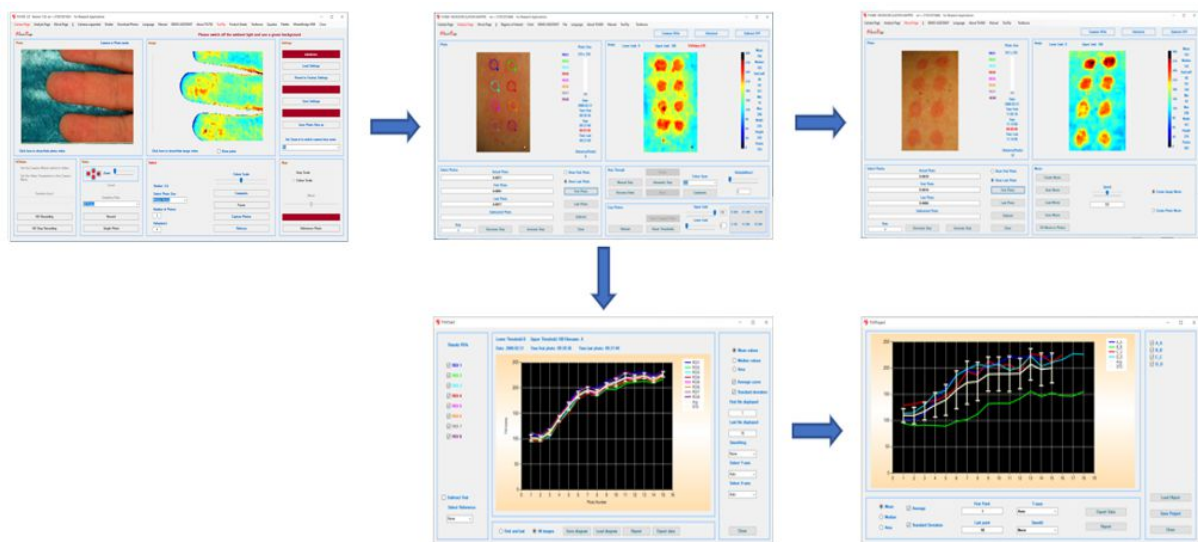
Fig 2. The *TiVi700 2.0 Tissue Viability Imager* software flowchart (Upper left: *Camera Page*; Upper middle: *Analysis Page*: Upper right: *Movie Page*; Lower left: *TiVi Chart* window; Lower Right: *TiVi Project* window)

An optional camera zoom-in lens (*TiVi Magnifier* lens) can be attached to the camera housing to produce photos and images of a resolution of about 5 micro-meters per pixel.

A variety of optional software toolboxes for analysis of skin parameters such as pigmentation, wrinkles, sweat gland activity, stubble elements and colour alterations can optionally be used for further analysis of photos captured by the *TiVi700 2.0 Tissue Viability Imager.*

GUIDANCE

This manual

This manual describes all features and functions of the *TiVi700 2.0 Tissue Viability Imager* camera and software. This manual can be used as an on-line manual on the computer screen or printed out as a hard copy (recommended) provided a pdf-reader is installed. When used as an on-line manual, different topics can be easily found by using the search tool in the ADOBE pdf-reader.

The DEMO ASSISTANT

The DEMO ASSISTANT can be used as an interactive tool that guides the user step-by-step in using many different functions and features of the *TiVi700 2.0 Tissue Viability Imager.* The DEMO ASSISTANT is opened by clicking DEMO ASSISTANT in the pull-down menu and selecting the topic of interest.

Tooltips

Each button is equipped with a tooltip that explains the function of the actual button. The tooltip function can be activated or deactivated by clicking *Tooltip* in the pull-down menu.

Demonstration videos

Many demonstration videos are available on the www.wheelsbridge.com WEB-site. For more information, please see the *Demo Videos* and *TiVi Tutorials* pages.

Click the link below to play the video: Tissue Viability Imaging Presentation.

http://media.wheelsbridge.se.loopiadns.com/2020/09/TiViPresentationOneShot-1.mp4

Click the link below to play the video: Introduction to Tissue Viability Imaging.

http://media.wheelsbridge.se.loopiadns.com/2020/08/IntroductionToTiVi-2.mp4

TiVi700 CAMERA

Operating principle

TiVi700 2.0 Tissue Viability Imager is a versatile technology intended for mapping the tissue microcirculation [*O´DOHERTY, J., HENRICSON, J., ANDERSON, C., LEAHY, M, NILSSON, G and SJOBERG, F. Sub-epidermal Imaging using Polarized Light Spectroscopy for Assessment of Skin Microcirculation. Skin Research and Technology 2007, 13; 472-484*].
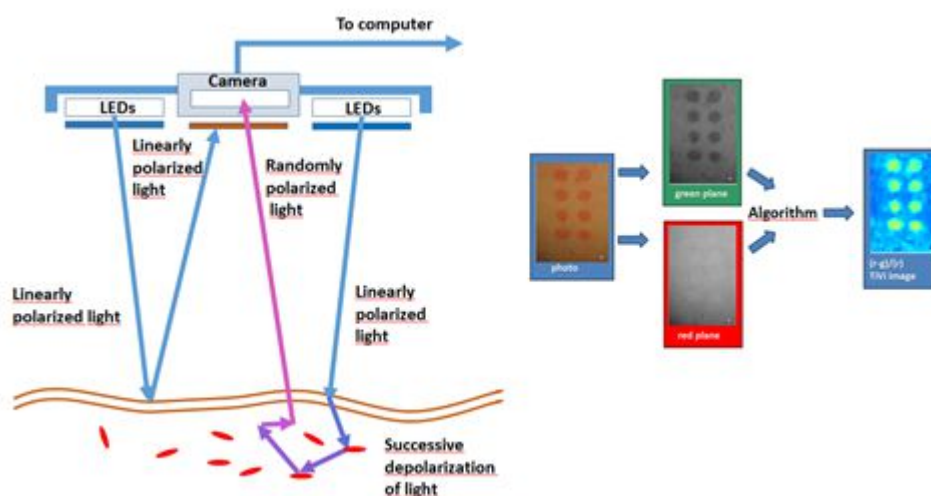
Fig 3.Operating Principle of the *TiVi700 2.0 Tissue Viability Imager*.

In the *TiVi700 2.0 Tissue Viability Imager*, linearly polarized white light from white light emitting diodes (LEDs) illuminates the tissue surface under investigation. A digital camera equipped with polarizing filters captures individual photos or video clips. The light illuminating the skin is partially reflected both in the skin surface and within the upper layer of the epidermis, as well as partially scattered in a diffuse manner into the deeper dermal layers where the microvascular network resides. The light directly reflected from both the surface and near-surface layers of the skin preserves its linear polarization, while the light diffusely scattered in the deeper dermal layers quickly becomes randomly polarized. The directly reflected light approaching the camera is effectively blocked by a polarizing filter with a polarization direction in a plane perpendicular to that of the linearly polarized light produced by the LEDs Illuminator. A portion of the diffuse, randomly polarized light is backscattered from within the deeper dermal layers, passes through the filter and finally reaches the detector. The resulting "sub-epidermal" image gives the impression that the camera can "see beneath" the top layer of the skin and probe the microvascular network. The polarizing filters on the camera lens and LED light source are set to operate in cross-polarized mode.

The green component of the backscattered randomly polarized light reaching the camera detector array is attenuated to an extent determined by the relative presence of RBCs in the

scattering volume, while the red component of the light is virtually unaffected due to its low absorption rate in RBCs. In contrast to what is the case with the RBCs in the skin microvascular network, the surrounding tissue absorbs both green and red light to approximately the same extent. After capturing a colour photo (or a frame in a video clip), the *TiVi700 2.0 Tissue Viability Imager* system software divides the image into discreet red, blue, and green matrices. A software algorithm subtracts the colour value of each picture element within the normalized green colour matrix from the colour value of the corresponding picture element within the red colour matrix. These calculations result in the generation of a pseudo-colour output matrix (TiVi-Image) which is displayed on a PC monitor or a tablet computer screen, effectively mapping the local RBC concentration. In contrast to what is the case with methods based on the detection of time-variable phase factors (*Laser Doppler Imaging* and the *Laser Speckle* devices), the *TiVi700 2.0 Tissue Viability Imager* is not affected by movement artefacts. It is therefore well suited also for assessment of the microcirculation in moving tissues.

Setting up the TiVi camera

1. Attach the stand to a table.

2. Attach the camera housing to the swivel and secure it with the front know.

Secure the camera housing with this knob.

Secure the camera housing position with this knob.
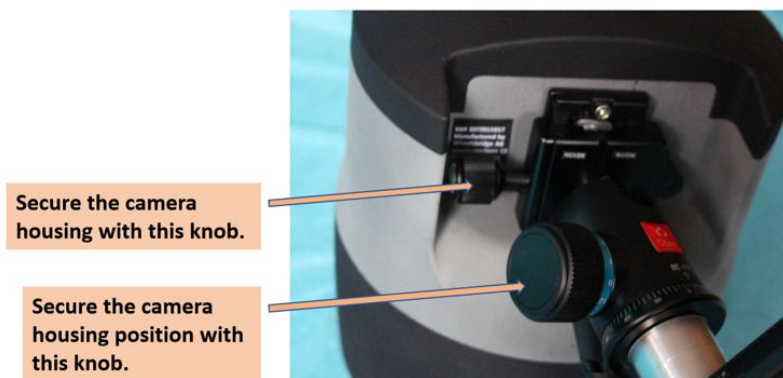
3. After tilting the camera housing secure the position of the camera housing using the large knob.

4. Connect the three cables to the respective power supplies and a computer USB port.

5. If the Illuminator is not in the ON state, flip the Illuminator switch.

ON/OFF Switch
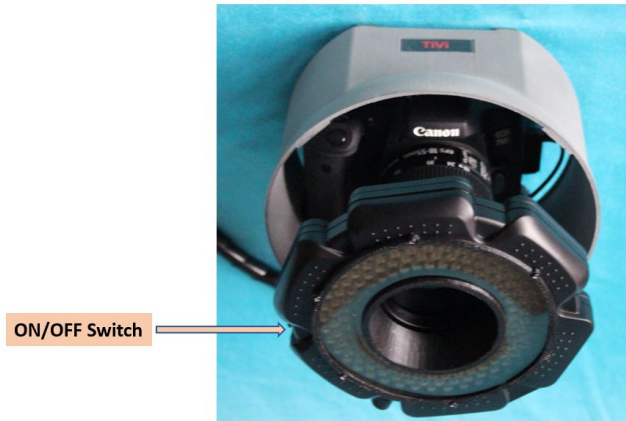
6. Adjust the arm so the bottom of the camera housing is positioned about 10 - 20 cm above the table. Place a green coloured background on the table below the camera housing.

7. Switch on the computer and start the TiVi700 program by clicking on its icon on the desktop.

8. Switch off or dim the ambient light in the room and place your hand on the green background. Move your hand and watch the photo video and the image video, the latter displaying the local concentration of RBCs in the skin.
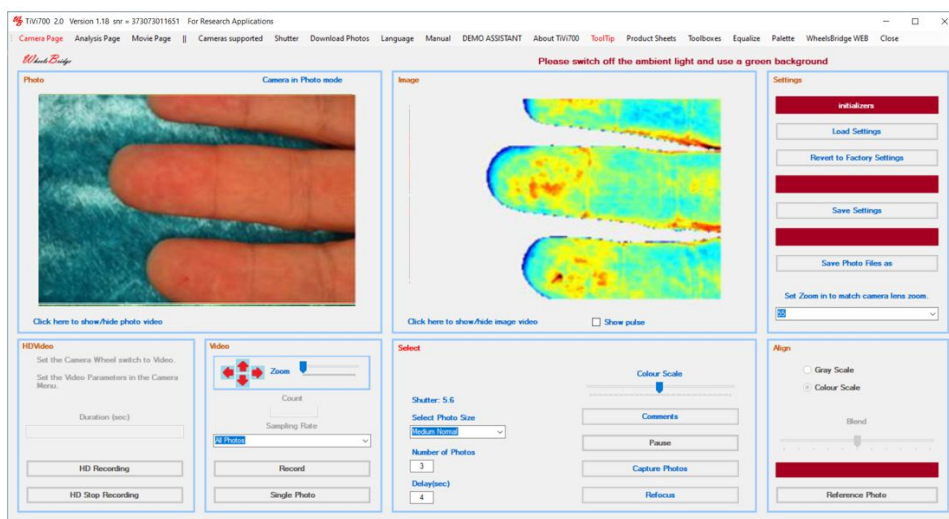
Click the link below to play the video: Setting up.

http://media.wheelsbridge.se.loopiadns.com/2020/09/1_B_SettingUp_OK-1.mp4

Click the link below to play the video: Camera positioning.

http://media.wheelsbridge.se.loopiadns.com/2020/09/2_B_CameraPositioning_OK-1.mp4

Camera Settings

1. The black top cover and the lower black protection shield are attached to the middle section of the camera housing by super-magnets. Remove these top and bottom covers of the camera housing to get access to the camera controls.

2. The camera switch can be set to ON or OFF (if the camera does not work check this setting). The camera switch is set to HD video when a HD video is to be captured and stored on the camera SD card.

3. The camera wheel should always be set to "Av".

4. The camera is normally set to "AF" (autofocus) to make it automatically focus on the object. If there is little contrast in the photo (such as in a uniformly coloured background or skin), autofocus does not work well – try to set this switch to MF (manual focus) to operate with a fixed focal distance.

5. Turn the lens to alter zoom in and out. This feature is not controllable from the software.
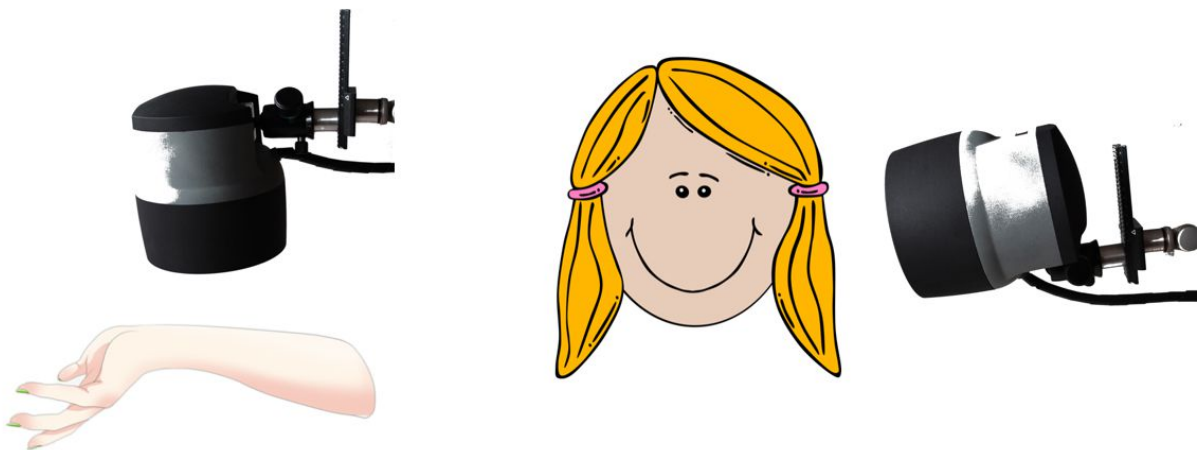
Positioning the camera for top view photos (left), side view photos (right) or bottom view photos (through a transparent glass plate) (below).

Transparent glass plate.
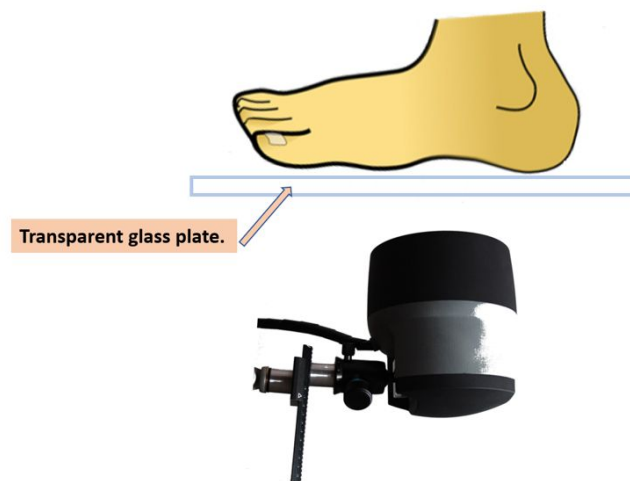
Click the link below to play the video: Camera Controls.

http://media.wheelsbridge.se.loopiadns.com/2020/09/3_B_CameraControls_OK-1.mp4

Operating the camera disconnected from the computer

In some applications it is convenient to operate the camera remotely and disconnected from the computer and power supplies. To operate the camera in stand-alone mode, proceed as follows:

1. Remove the black top and bottom parts of the camera housing.

2. Detach the camera from the stand.

3. Remove the swivel camera holder to release the camera.
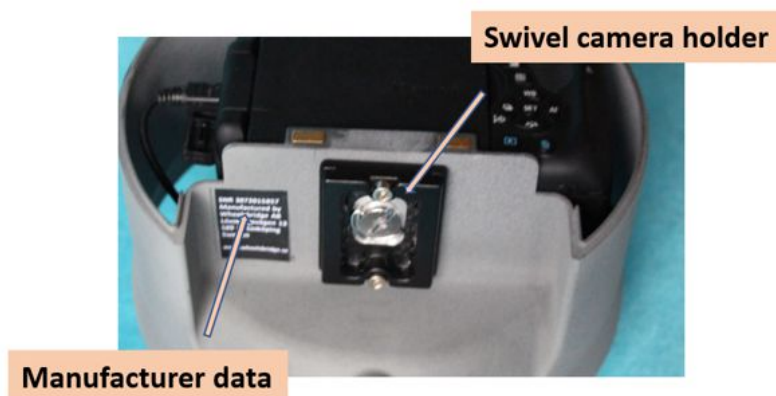
4. Disconnect the computer USB cable, the Illuminator cable and the Canon DC coupler located inside the camera housing after opening the DC coupler door.

5. Insert a charged camera battery in the camera housing Canon DC coupler cavity and close the DC coupler door.

6. Insert 6 x 1.5 V (rechargeable) batteries in the cavities on the rear side of the Illuminator.

7. Switch on the Illuminator using the switch on the side of the Illuminator and switch on the camera by setting the camera switch to ON.

8. The camera is now ready for photo capturing. Capture a new photo by pressing the shutter button on the front of the camera housing. New photos are saved on the camera SD card.

9. If these photos are to be analysed using the TiVi700 software, the photos must be downloaded to the computer by selecting *Download Photos* in the *Camera Page*.

Observe that photos saved on the camera SD-card must be downloaded before new photos are captured using the controls on the *Camera Page* in the TiVi700 software.

Adjusting zoom in

The zoom can be set using the knob attached to the lower black cover by a pair of super-magnets. Move the knob slowly along the scale (55 = max zoom in, 24 = max zoom out) to adjust the zoom lens. This knob can be removed after adjusting the zoom lens but can also be left in set position.

A removable handle attached to the cover by a pair of super-magnets.

Illuminator options

Left: Illuminator front side with 160 LEDs and polarising filter, Centre: Illuminator rear side with a second fixed polarising filter in the centre set to operate in cross-polarised mode (standard) and Right: Illuminator rear side with a second polarising filter that can be set to operate in either cross- or co-polarised mode - red clamp around the magnet = cross-polarised mode; black clamp around the magnet = co-polarised mode (optional).

The TiVi Magnifier

The TiVi Magnifier lens (optional) is to be used when photos of high spatial resolution (better than 5 micro-meters per pixels) are to be captured.

The TiVi Magnifier can be set to operate in cross- (subepidermal photos) or co-polarized (surface photos) mode by rotating the frontal part of the lens.

Connect the TiVi Magnifier lens to the camera housing by bayonet coupling .

The front end of the TiVi Magnifier lens is to be in contact with the object when capturing photos.

1. First remove the standard Illuminator from the camera housing by pressing the black button at the camera housing while turning the Illuminator.

2. Attach the TiVi Magnifier lens by pressing the black button at the camera housing while sliding the lens in place (bayonet coupling). The white dot on the lens should match the position of the white dot on the camera housing.

3. Connect the TiVi Magnifier lens to the 12V power supply. The LED ring inside the TiVi Magnifier lens should now emit white light.

4. Set the TiVi Magnifier lens to operate in cross- or co-polarised mode by turning the frontal end of the lens. When the red indicator pin is touching the metallic super magnet, the system is set to operate in cross-polarised mode.

5. When capturing photos or videos, the objects should be in contact with the front end of the lens.

6. If the photo cannot be focused automatically when operating it in autofocus mode, try focusing manually be setting the switch on the lens to MF (manual focus) and turn the lens manually.

TiVi700 SOFTWARE

TiVi700 File names convention

1. A TiVi700 file name is composed of a *Prefix* determined by the user and a minus sign followed by a number automatically set by the system.

2. A *Prefix* must start with a letter.

3. A *Prefix* may be a combination of letters and numbers.

4. A *Prefix* must not include a minus-sign.

5. If e.g. the files A-0001, A-0002 and A-0003 exist and the user selects a *Prefix* 'A' for the next photo to be captured, this will automatically be named A-0004.

6. When the user selects the file name of the first file to be uploaded, the system automatically calculates the minimal step and the name of the last photo file in the sequence.

TiVi700 window

The main *TiVi700 2.0 Tissue Viability Imager* window includes 3 pages (*Camera, Analysis* and *Movie Page*).

TiVi700 Main window: Camera Page
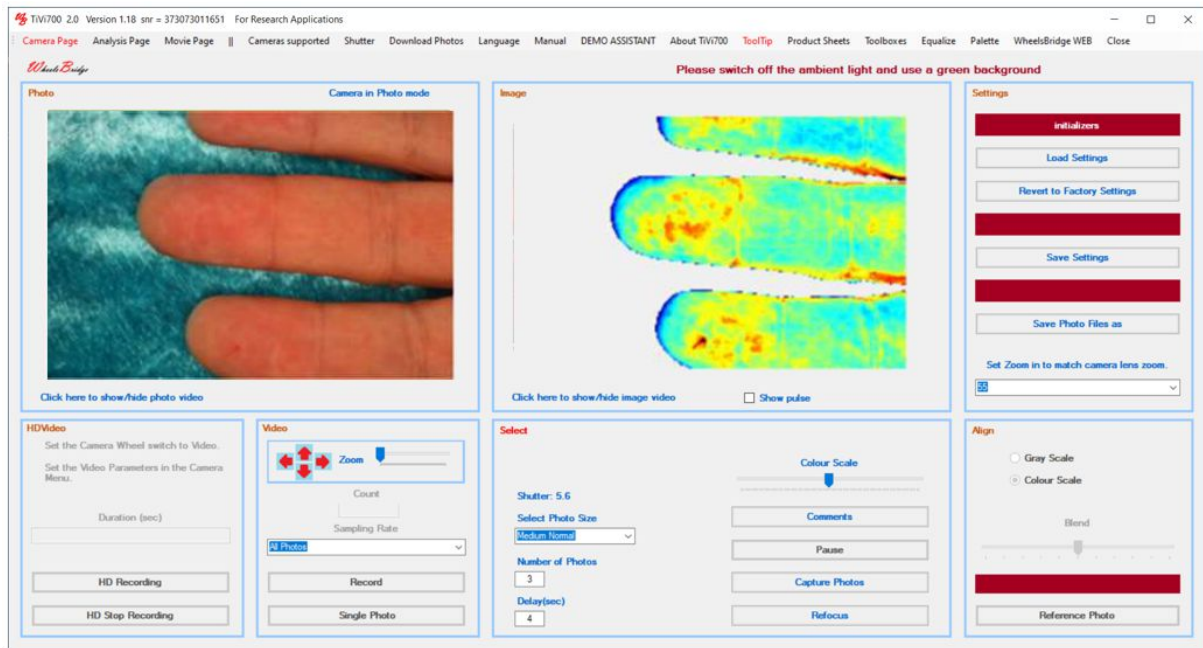
Camera Page – this page is used for capturing new photos and videos as well as for aligning new photos to a reference photo and setting various parameters.

TiVi700 Main window: Analysis Page

Analysis Page – this page is used for uploading a sequence of photos for analysis and for converting these photos to TiVi-images. 'ROIs' are drawn directly in the photo and the average TiVi-values within the 'ROIs' are used for further analysis.

TiVi700 Main window: Movie Page
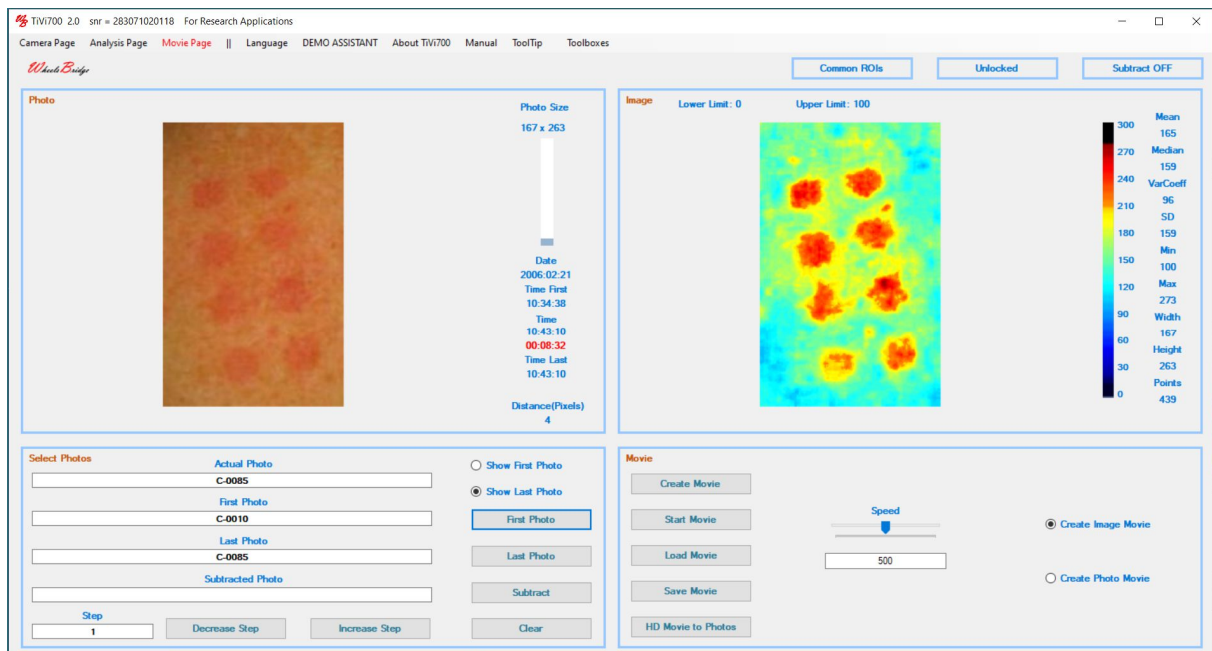
Movie Page – this page is used for creating and playing TiVi-Image video clips generated from a sequence of photos and for splitting up a HD video into separate photo files for further analysis.

TiVi Chart window
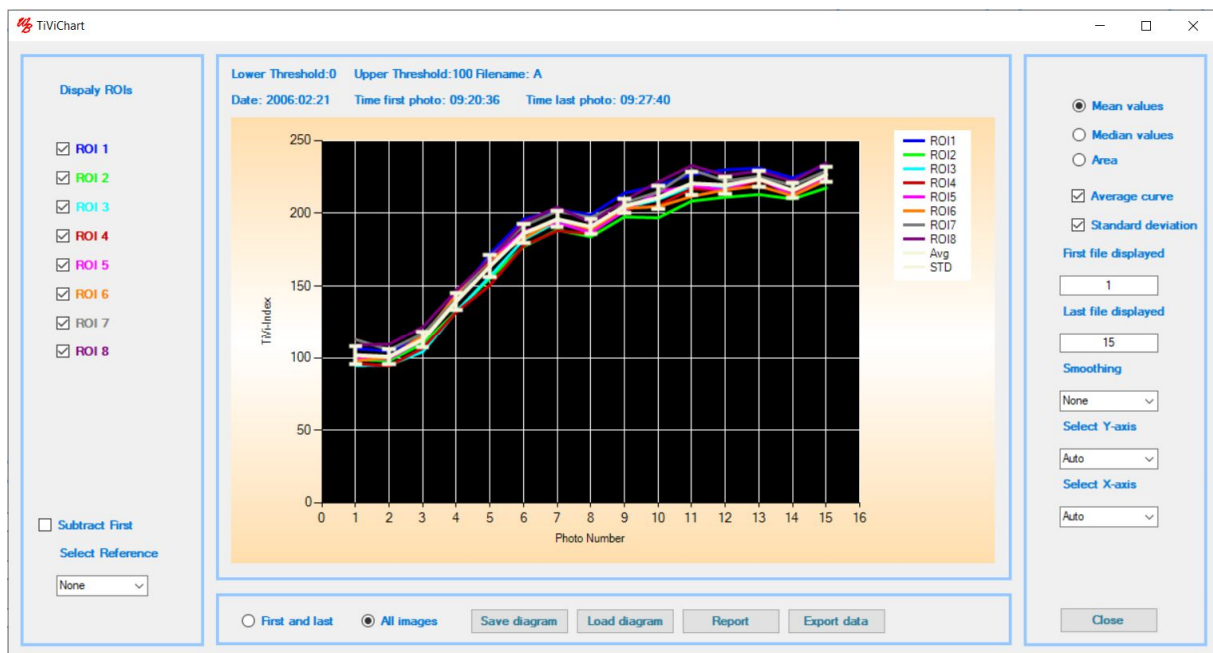
TiVi Chart - this window displays the average TiVi-values within selected 'ROIs' of the different photos in the sequence for a single test subject.

TiVi Project window
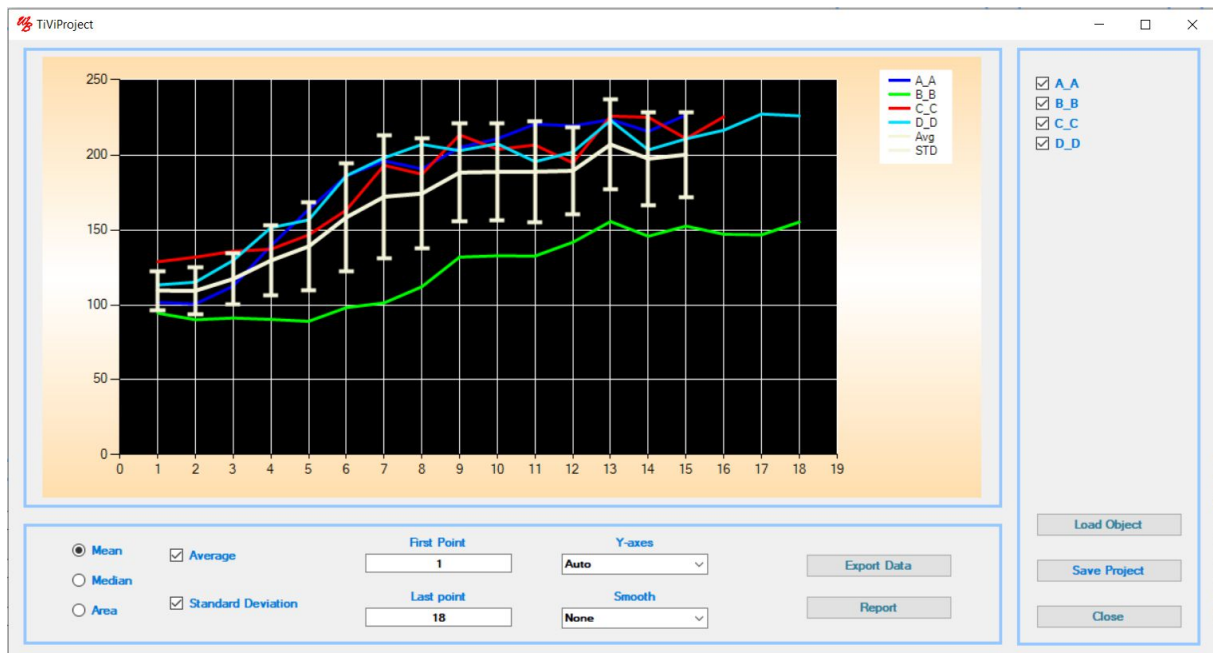
TiVi Project – this window displays - in a single diagram - the average TiVi-value curves generated in the different *TiVi Charts,* each curve representing a single test subject.

Toolboxes (optional)

The following (optional) toolboxes can be installed for specific applications:

TiVi60 Skin Damage Visualizer – for investigation of skin vascular damage caused by aggressive chemicals and vibrating tools.

Click the link below to play the video: TiVi60 Skin Damage Analyzer.

http://media.wheelsbridge.se.loopiadns.com/2020/10/100_H_TiVi60SkinDamageAnalyzerProject.mp4

TiVi70 Skin Colour Tracker – displays alterations in skin appearance by tracking colour changes over time.

Click the link below to play the video: TiVi70 Skin Colour Tracker.

http://media.wheelsbridge.se.loopiadns.com/2020/10/101_H_TiVi70SkinColourTracker.mp4

TiVi80 Spot Analyzer - observer-independent assessment of spot erythema intensity and extension.

Click the link below to play the video: TiVi80 Spot analyzer.

http://media.wheelsbridge.se.loopiadns.com/2020/10/103_H_TiVi80SpotAnalyzerProject.mp4

TiVi90 Wrinkle Analyzer – analysis of critical wrinkle parameters.

Click the link below to play the video: TiVi90 Wrinkle Analyzer.

http://media.wheelsbridge.se.loopiadns.com/2020/10/104_H_TiVi90WrinkleAnalyzerProject.mp4

TiVi95 Surface Analyzer – analysis of critical surface parameters.

Click the link below to play the video: TiVi95 Surface Analyzer.

http://media.wheelsbridge.se.loopiadns.com/2020/10/105_H_TiVi95SkinSurfaceAnalyzerProject.mp4

TiVi97 Pigment Analyzer – analysis of skin pigmentation.

Click the link below to play the video: TiVi97 Pigment Analyzer.

http://media.wheelsbridge.se.loopiadns.com/2020/10/106_H_TiVi97PigmentAnalyzerProject.mp4

TiVi98 Microstructure Analyzer – analysis of hair and other small objects.

> Click the link below to play the video: TiVi98 Microstructure Analyzer.
>
> http://media.wheelsbridge.se.loopiadns.com/2020/10/107_H_TiVi98MirostructureAnalyzerProject.mp4

TiVi101 Sweat Gland activity Analyzer – analysis of sweat gland discharge.

> Click the link below to play the video: TiVi101 Sweat Gland activity Analyzer.
>
> http://media.wheelsbridge.se.loopiadns.com/2020/10/108_H_TiVi101SweatGlandActivityAnalyzerProjec

TiVi102 Nevus Analyzer – analysis of nevus lesions core parameters.

> Click the link below to play the video: TiVi102 Nevus Analyzer.
>
> http://media.wheelsbridge.se.loopiadns.com/2020/10/109_H_TiVi102NevusAnalyzerProject.mp4

TiVi103 Wound Analyzer – analysis of wounds and the tissue repair processes.

> Click the link below to play the video: TiVi103 Wound Analyzer.
>
> http://media.wheelsbridge.se.loopiadns.com/2020/10/110_H_TiVi103WoundAnalyzerProject.mp4

TiVi104 Stubble Analyzer – analysis of stubble elements and microcirculation.

> Click the link below to play the video: TiVi104 Stubble analyzer.
>
> http://media.wheelsbridge.se.loopiadns.com/2020/10/111_H_TiVi104StubbleAnalyzerProject.mp4

TiVi106 Oxygen Mapper – analysis of trends in tissue oxy- and deoxy-hemoglobin.

> Click the link below to play the video: TiVi106 Oxygen Mapper.
>
> http://media.wheelsbridge.se.loopiadns.com/2020/10/112_HTiVi106OxygenMapperProject.mp4

Most toolboxes are equipped with library functions and trend monitors.

All calculated parameters and data can be exported to other software packages such as Excel® for further analysis.

Main window

Menus (general)

Camera Page – click to display the *Camera Page*.

In the *Camera Page* new photos and videos are captured and displayed.

Analysis Page – click to display the *Analysis Page*.

In the *Analysis Page* a sequence of existing photos can be uploaded and analysed. *Regions of Interest* ('ROIs') can be set and displayed.

Movie Page – click to display the *Movie Page*.

In the *Movie Page* video-clips can be created from a sequence of photos or alternatively recorded HD video-clips can be split into individual frames and stored as photos for further analysis.

DEMO ASSISTANT– displays the *DEMO ASSISTANT*.

The *DEMO ASSISTANT* is an interactive step-by-step guidance for using different features of the *TiVi 700 2.0 Tissue Viability Imager*.

Voice: Yes - if synthetic voice is to be used in the *DEMO ASSISTANT*.

Voice: No - if synthetic voice is to be muted.

DEMO ASSISTANT shows how to use the *DEMOASSISTANT*.

TiVi File Names – shows filename conventions.

Loading Photos – shows how to load photos.

Crop Save – shows how to crop and save photos.

Thresholding – shows how to use threshold in images.

Movies - shows how to create and show a video-clip.

Image Subtraction – shows how to subtract a selected image from the other images.

ROI Managements: **Draw ROIs** shows how to generate and show 'ROIs'.

ROI Managements: **Delete ROIs** shows how to delete a 'ROI'.

ROI Managements: **Move ROIs** shows how to move a 'ROI'.

ROI Managements: **Stretch ROIs** shows how to stretch a 'ROI'.

ROI Managements: **Set ROI Parameters** shows how to set ROI parameters manually.

ROI Managements: **Save Load Parameters** shows how to save and load 'ROIs' to file.

Chart – shows how to generate TiVi curves in a *TiVi Chart*.

Project – shows how to generate a *TiVi Project* diagram based on average TiVi curves.

Capture Photos: **Capture** shows how to capture a selected number of photos.

Capture Photos: **Video** shows how to capture and save frames from a video as photos.

Capture Photos: **HD Video** shows how to record a HD video.

Capture Photos: **Align Photos** shows how align new photos to a reference photo.

Language - selects working language.

TiVi700 2.0 Tissue Viability Imager supports the Chinese, English, French, German and Japanese language.

Voice Control – select if voice control of the photos and videos capturing process is to be employed (only operational if the English-US language engine is installed).

. **On** – click if voice control is to be used. *Voice Control* label is displayed in red colour.

Off – click if voice control is not to be used. *Voice Control* label is displayed in black colour.

Information: click to open the *Information* window which displays which voice commands to use. Click the *Play*-buttons to listen to the commands.

Voice control can be used for starting and stopping photo capturing and video recording without clicking any control buttons. Voice control works best in a noise-free environment to avoid unintentional start and stop of capturing and recording processes. When not in use click the **Voice Control: Off** button.

Click the link below to play the video: Voice Control.

http://media.wheelsbridge.se.loopiadns.com/2020/09/17_A_VoiceControl_OK.mp4

About TiVi700 – displays version and release date.

Manual: Main Manual – displays this online manual.

This manual is printed as a pdf-document and stored in the TiVi700→Manual folder. This manual can be printed out using a printer (recommended).

Manual: Equalizer – displays the online *Equalizer Appendix*.

Manual: PreProc Manual – displays the online *Pre-Processor Manual.*

Tooltips – selects if tooltips are to be displayed.

> **Show** – displays tooltips when hoovering the mouse over a button.

> **Hide** – click to hide tooltips from being displayed.

Toolboxes – click to open the toolbox selector. The toolboxes utilize code from the high-level language *MATLAB* and clicking this button first opens the *MATLAB* library (displaying a black command window) before the *Toolbox* selection window is opened.

Product Sheets – to display the TiVi700 2.0 Product Sheet.

Equalize – the equalizer is used to brighten the somewhat darker corners (as compared to the centre) of a photo. Equalizing a photo is particularly import when a large field of view is set by turning the camera zoom lens to a value of 24 or 35, as compared to a small field of view set by turning the camera zoom lens to a value of 55. If the Equalizer is activated, an equalized sequence of photos is created immediately after termination of a photo capturing process, a video process or the conversion of an HD video to separate photos. The names of the equalized photos are the same as the name of the basic photos with the additional prefix "_eq" added to the original prefix of the name. The equalizing process is running on a separate thread implying that other features of the TiVi700 software can be used simultaneously with running the equalizing process. The time consumed for equalizing a photo depends on its size. A large photo takes about 30 seconds to equalize while photos of medium and small sizes take about 20 and 10 seconds, respectively. For the equalizer to operate accurately, the "*Set Zoom in to match camera lens zoom*" combo-box <u>must</u> be set in accordance with the camera lens zoom value, manually set by turning the lens.

The best result is generally attained if the camera lens is set to 55 (small field of view). The actual area included in a photo can be extended or reduced by increasing or decreasing the distance between the camera and the object.

When the equalizing process is running, an indicator is displayed above the TiVi-image in the Image panel, showing the progress of the process. The "*Stop*" button to the right of the indicator can be used to prematurely terminate the equalizing process.

> **Yes** - Equalizer is activated.

> **No** - Equalizer is de-activated.

> Click the link below to play the video: Equalizing photos.
>
> http://media.wheelsbridge.se.loopiadns.com/2020/09/18_A_EqualizerProject_OK.mp4

Wheelsbridge WEB – access to the Wheelsbridge website.

Home Page - click to get to the Home Page in the Wheelsbridge website.

References Page - click to get to links to listed references.

Demo videos Page – click to view TiVi demo videos.

TiVi Tutorial Page – click to view tutorial videos on TiVi.

Close – to close the main window.

Menus (Camera Page only)

Cameras supported – lists the serial number of the cameras supported.

Shutter – selects camera shutter.

A low camera shutter value results in a short shutter opening time reducing the adverse effects of movement artefacts when capturing photos. A high camera shutter value results in a longer shutter opening time thereby increasing the focal length (the distances at which good focus can be attained).

Download Photos- to download photos directly captured from the off-line camera.

Photos can be captured with the camera disconnected from the computer and power supply (storing the photos in the camera SD-card and operating the camera on the re-chargeable battery). By also operating the Illuminator using six rechargeable batteries, the camera with Illuminator is fully disconnected from the power supplies and the computer. New photos are captured by use of the camera shutter button. Photos stored in the SD card to be analysed by the TiVi700 system, must be downloaded using the *Download Photos* button in the *Camera Page* pulldown-menu after connecting the camera to the computer via the USB-cable. This process will tag the photos as TiVi-photos recognized by the TiVi700 software. Observe that the camera needs to be connected to the computer before starting the TiVi700 software.

Click the link below to play the video: Download photos.

http://media.wheelsbridge.se.loopiadns.com/2020/09/15_A_DownloadPhotos_OK.mp4

Menus (Analysis Page only)

Regions of Interest: When a 'ROI' is drawn in the photo the name of the 'ROI' is displayed in the upper left corner of the 'ROI' and to the right of the photo. This name can be altered by using the **Name ROIs** feature (see below). The dot in the lower right corner of the 'ROI' is used for stretching the 'ROI'. A maximum of 12 'ROIs' can be drawn.

Regions of Interest: **Rectangular ROIs** - to select rectangular 'ROIs'.

Regions of Interest: **Elliptical ROIs** - to select elliptical 'ROIs'.

Regions of Interest: **Freehand ROIs** - to select freehand 'ROIs'.

Regions of Interest: **Common ROIs -** to make the 'ROIs' setup identical in all photos.

Regions of Interest: **Just this Photo -** to move this 'ROI' in just this photo.

> Upload a photo sequence and draw some 'ROIs'. Click the **Manual Step** button several times (or insert the file name in the *Actual Photo* textbox) to go to the selected photo in the sequence. Then click the *Just this photo* in the *Regions of Interest* pull-down menu. Finally move one of the 'ROIs' to a new location within the photo by 1) pointing at the centre of the 'ROI' (the hand pointer is displayed), 2) press the left mouse button and move the mouse pointer with the mouse button pressed and 3) release the mouse button. Click the *Automatic Step* button to watch the result.

Click the link below to play the video: Just this photo.

http://media.wheelsbridge.se.loopiadns.com/2020/09/37_D_JustThisPhotoProject_OK.mp4

Regions of Interest: **All upcoming photos -** to move this 'ROI' in all remaining photos.

> Upload a photo sequence and draw some 'ROIs'. Click the **Manual Step** button several times (or insert the file name in the *Actual Photo* textbox) to go to the selected photo in the sequence. Then click the *All upcoming Photos* in the *Regions of Interest* pull-down menu. Finally move the 'ROI' to a new location within the photo by 1) pointing at the centre of the 'ROI' (the hand pointer is displayed), 2) press the left mouse button and move the mouse pointer with the mouse button pressed and 3) release the mouse button. Click the *Automatic Step* button to watch the result.

Regions of Interest: **Lock on white spot** - to lock the 'ROI' setup to the white spot in all photos.

This feature works only on a sequence of photos with a white spot included somewhere in the photo. Place a small (3x 3 mm) white piece of paper on the object before photo capturing or video recording or insert a white spot manually in each photo using the window under **File: Add White Marker.** Verify that this marker is the only white spot in the photo. First click *Show First Photo* radio-button and draw some 'ROIs' in the vicinity of the white mark in the first photo. Then click **Regions of Interest**: **Lock on white spot.** The TiVi700 system will now scan through the photo sequence locking the 'ROIs' to the white spot. Verify that all 'ROIs' are inside the photo. When clicking the **Automatic Step** button, the 'ROIs' drawn in the first photo are displayed locked to the white spot. This feature is useful when analysing photos captured from objects in motion, locking the 'ROIs' to the moving object.

Regions of Interest: **Adjust ROIs** - to set the 'ROIs' to the same size as the selected 'ROI'.

Click the **Regions of Interest**: **Adjust ROIs** to open the *AdjustROIs* window. Select the 'ROI' to which the shape of the other 'ROIs' is to be adjusted. Click the OK button.

Regions of Interest: **Set ROI Coordinates** - to set the coordinates of the 'ROIs' manually.

Click the **Regions of Interest**: **Set ROI Coordinates** to open the *SetROIs* window. Select the 'ROI' which shape is to be set manually. Set the coordinates of the 'ROI' corners by inserting values in the four text boxes. Click the OK button.

Regions of Interest: **Name ROIs** - to give each 'ROI' a specific name.

Click the **Regions of Interest**: **Name ROIs** to open the *ROINaming* window. Insert names in the 'ROI' text box to be renamed. In this window the size of the 'ROI' text (*Small Text, Medium Text* or *Large Text)* as well as the line width (*Narrow Line*,

Medium Line or *Large Text*) can also be set. Click the OK button to accept the inserted values and close the *ROINaming* window.

Regions of Interest: **Remove Selected Areas -** to make the selected areas look like background in the images.

Draw some 'ROIs' in the photo displayed. Select **Regions of Interest**: **Remove Selected Areas.** The system will now scan through the photos in the sequence, insert a green area in the 'ROIs' and save these new photos in a folder named *xx-sub.* Navigate to this folder and double-click the first photo file. The new photos with green areas within the 'ROIs' are now displayed. In the corresponding TiVi-Image, the green areas in the photo corresponds to a grey (background) area. This feature is useful if any areas of the photo (such as reddening due to an injection trauma) are to be removed in the TiVi-Image.

File: Open a TiVi Chart - to open an existing *TiVi Chart*.

Click **File: Open a TiVi Chart.** In the *Open File Dialog* window select the *TiVi Chart* in which curves corresponding to the TiVi values within 'ROIs' are displayed.

File: Project: New Project - to open a new *TiVi Project*.

Click **File: Project: New Project** button to open a new *TiVi Project* window into which average TiVi curves in different *TiVi Charts* can be uploaded. Up to 20 different curves can be uploaded into a *TiVi Project.*

File: **Project: Open a TiVi Project -** to open an existing *TiVi Project*.

Click the **File: Project: Open a TiVi Project.** In the *Open File Dialog* window select an existing *TiVi Project* window into which average TiVi curves from different *TiVi Charts* can be uploaded. Up to 20 different curves can be uploaded into a *TiVi Project.*

File: Save As - to save the photos under a new name.

Click the **File: Save As** button to save the sequence of photos under a new name in a separate folder. This feature is useful if the filename (after multiple analysis processes) becomes long.

File: Load ROIs - to load a saved set of 'ROIs'.

Clicking the **File: Load ROIs** button opens an *Open File Dialog* window. Navigate to the folder in which the saved 'ROIs' file is stored and double click on the file to draw the saved 'ROIs' in the photo.

File: Save ROIs - to save the present set of 'ROIs'.

Clicking the **File: Save ROIs** button opens the *Save File Dialog* window. Insert a name and click the *Save* button to save the current set of 'ROIs' as drawn in the actual photo.

Click the link below to play the video: Save and load ROIs.

http://media.wheelsbridge.se.loopiadns.com/2020/09/39_D_saveAndLoadROIsProject_OK.mp4

File: Add White Marker – opens the *Add White Marker* window.

If no white marker is already added to the photos captured of objects in motion, the *White Marker* window can be used to add white markers manually so the photos can be analysed using adaptable 'ROIs'.

Click the **File: Add White Marker** button to open the *White Marker* window. Click the *First Photo* button to open the *Open File Dialog* window. Double-click a photo file to insert this file as the first photo. The system will automatically identify the first and last photo as well as the step in the photo sequence. Optionally, the last photo in the uploaded sequence can be selected by clicking the *Last Photo* button. Click the *Show First Photo* radio-button to display the first photo. Click at the spot in the photo where the white spot is to be inserted, verified by a click sound. The system automatically moves to the next photo in the sequence. Repeat the procedure until all photos in the sequence are marked, indicated by a window verifying that the procedure is done. The marked photo files are now saved in a subfolder named *xx_marked* and these marked files can now be used for analysis in the TiVi700 software.

File: Reduce Photo Size - to reduce the size (in pixels) of the photos in a sequence. This feature is helpful when working with many and large photos files. By reducing the size of the photos, the analysis time can be significantly reduced.

File: Export Image: All Images – exports basic TiVi values to a spreadsheet for all images.

> Clicking this button opens the *Save File Dialog* window. Navigate to the target folder and insert a name under which the individual TiVi values are to be saved as part of a spreadsheet. This spreadsheet can be opened by LIBRE office or MS EXCEL.

File: Export Image: Just This – exports basic TiVi values to a spreadsheet for just this image.

> Clicking this button opens the *Save File Dialog* window. Navigate to the target folder and insert a name under which the individual TiVi values are to be saved as part of a spreadsheet. This spreadsheet can be opened by LIBRE office or MS EXCEL.

File: Gallery – click to display a gallery of all photos or TiVi-images in the actual sequence.

File: Melanin Filter – click to open the Melanin filter window.

File: Reference Photo – click to open the Reference Photo window.

File: Pulse – click to open the Pulse window.

Camera Page

HDVideo panel

HDVideo panel – click somewhere in the panel to activate *HD video* and make it ready for *HD video* recording. <u>Set the wheel on the camera housing to video.</u>

Duration – insert the duration (in seconds) to determine the length of the video to be stored on the SD-card in the camera.

> Setting the duration automatically stops *HD Video* recording after the duration time expires. If the duration textbox is left empty the *HD Video* recording stops by clicking the *HD Stop Recording* button.

HD Recording – click to start the recording of the *HD video*. The number of frames per second is about 45 and the size of each frame is by defaults 1280 x 720.

> When *HD video* recording is started, the duration is continuously displayed in the *Duration* text box.

> When the recording is finished, a *Save File Dialog* window will open. Insert the name of the *HD video* file to be saved and klick OK. The *HD video* will now be saved to file under the name selected. Terminate the *HD video* session by setting the wheel on the camera housing to "ON".

HD Stop Recording – click to stop the recording of the *HD video*.

> When the recording is finished, a *Save File Dialog* window will open. Insert the name of the *HD video* file to be saved and klick OK. The *HD video* will now be saved to file under the name selected. Terminate the *HD video* session by setting the wheel on the camera housing to "ON".

Click the link below to play the video: High definition video.

http://media.wheelsbridge.se.loopiadns.com/2020/09/13_A_HDVideo_OK.mp4

Video panel

Video panel - click somewhere in the panel to activate the *Video* mode and make it ready for *video* recording saving the frames as individual photo files.

Zoom panel – to digitally zoom and move the photo and TiVi-image in *Video* mode.

Count – displays the number of files recorded.

Sampling Rate – select either All Photos (about 10 photos per second), every second, forth, eight or 16th photo.

> Setting the *sampling rate* to a lower value makes it possible to record a video as individual photo files over a longer time without filling up too much of the disk space with many photo files. Setting the *sampling rate* to a higher value makes it possible to record a shorter video clip with higher temporal resolution. Settings *sampling rate*

to All Photos generates about 600 photos per minute, while setting *sampling rate* to Every 16th photo generates about 35 photos per minute.

Record – when clicking this button, the frames are streamed to the hard-drive and saved as individual photo files, provided a file name has been inserted by clicking the *Save Photo File as* button. To stop the recording again click the same button (now named *Stop Recording*). The number of photos files saved is indicated in the *Count* textbox. The size (in Pixels) of the saved photos is determined by the digital zoom setting.

Single Photo button – to capture and save a single frame in the video as a photo file.

Click the link below to play the video: How to capture a standard video.

http://media.wheelsbridge.se.loopiadns.com/2020/08/Video-4.mp4

Click the link below to play the video: Standard video.

http://media.wheelsbridge.se.loopiadns.com/2020/09/11_A_StandardVideo_OK.mp4

Select panel

Select panel - click somewhere in the panel to activate the *Select Capture* mode.

Colour Scale trackbar- move the slider until the image colours reach a desired target value.

> The image colours displayed do not influence the recorded TiVi values. The image colours can be set to produce the best visual representation of the TiVi-Image.

Comment button – opens a window into which a text string can be inserted. This text string will be saved as meta-data with all photo files. The text string can be retrieved and edited when analysing the photos and images (see *Analysis Page*).

Select Photo Size – sets the size of the photo (in pixels) to either *Large Fine* (6000 x 4000 pixels, 9.4 MB), *Large Normal* (6000 x 4000 pixels, 8.1 MB), *Medium Fine* (3984 x 2656 pixels, 4.6 MB), *Medium Normal* (3984 x 2656 pixels, 4.0 MB), *Small Fine* (2976 x 1984 pixels, 2.9 MB), *Small Normal* (3984 x 2656 pixels, 2.4 MB).

> A large photo size makes it possible to zoom in at a specific area with still good spatial resolution, while a small photo size speeds up the capturing and analysis process.

Number of Photos – insert the number of photos to be captured in the session.

> The capturing process automatically stops when the set number of photos is reached. The capturing of new photos can be stop at any time by clicking the *Stop Capturing* button.

Delay(sec) – insert the delay time in seconds between two consecutive photos.

Large size photos can be captured at a minimum delay of 5 seconds, while medium size photos and small size photos can be captured at delays longer than 4 and 3 seconds respectively. In case photos need to be captured at a higher rate, instead record the photos as videos or HD videos.

Pause – double click this button to temporarily halt the capturing process.

Capture Photos – click to start the capturing process.

The capturing process can be terminated at any time by clicking this button again (now named *Stop Capturing).*

Click the link below to play the video: Capturing Photos.

http://media.wheelsbridge.se.loopiadns.com/2020/09/10_A_CapturingPhotos_OK.mp4

Click the link below to play the video: How to capture a sequence of photos.

http://media.wheelsbridge.se.loopiadns.com/2020/08/Capturing-2.mp4

Click the link below to play the video: Miscellaneous.

http://media.wheelsbridge.se.loopiadns.com/2020/09/195_A_MiscellaneousProject_OK.mp4

Refocus – click to refresh the focus.

Optionally if two cameras are connected:

Camera 1 radio-button – check to use camera 1.

Camera 2 radio-button – check to use camera 2.

Alternate radio-button – check to use camera 1 and camera 2 alternatively.

Click the link below to play the video: Using two cameras.

http://media.wheelsbridge.se.loopiadns.com/2020/09/19_A_UsingTwoCameras_OK.mp4

Align panel

Align panel - click somewhere in the panel to activate the *Align* mode. This feature is used to reach a good overlap between a reference photo and the photos to be captured.

Gray Scale radio-button – to display the reference photo in gray scale.

Colour Scale radio-button – to display the reference photo in colours.

Blend Trackbar – move this slider to change the mixture of the current photo displayed and reference photo. The mixed overlap photo is displayed in the photo panel.

Reference Photo button – click to open an *Open File Dialog* window from which the reference photo is selected.

> The reference photo is displayed in the Image panel while the overlap photo is displayed in the photo panel.

Click the link below to play the video: Aligning photos.

http://media.wheelsbridge.se.loopiadns.com/2020/09/14_A_AlignPhotos_OK.mp4

Settings panel

Load Settings button – click this button to load an existing initializer file including information about *Language, Image Size, Shutter Setting, Number of Photos* to capture, *Delay, Voice Synthesis, Tooltip Settings* and *Voice Control* for capturing photos. The file name is displayed in the text box above the button. These parameters are stored when the program is closed and automatically uploaded again when the program is re-started.

Revert to Factory Settings button – click this button to load the factory settings (of information parameters) from the *initializersFactory* file.

Save Settings button – click this button to save the present setting under a selected file name. To change the default settings (settings used at start up), double click the *initializers* file in the *Open File Dialog* window. The *initializersFactory* file cannot be overwritten.

Save Photo File as button – click this button to insert a file name (prefix) of the photo files to be saved to file. The system will automatically fill out the actual number of the photo file in the sequence.

Set Zoom in to match camera lens zoom combo-box – set to match the manual setting of the camera zoom lens. Matching this combo-box setting with the actual setting of the camera lens is of vital importance for the photo equalizing process to operate accurately.

Click the link below to play the video: Camera settings.

http://media.wheelsbridge.se.loopiadns.com/2020/09/16_A_CameraSettings_OK.mp4

Photo Panel: Click here to show / hide photo video – click this text string to show or hide the photo video.

Image Panel: Click here to show / hide image video – click this text string to show or hide the image video. Hiding the image video when recording a video tends to increase the number of frames per second saved to file as photo files.

Show pulse checkbox – check to display just the pulsatile component and uncheck to display the entire RBC concentration.

Update rate – change the update rate when in pulsatile mode.

Analysis Page

Common ROIs info – displays information if all 'ROIs' are common to all photos or 'ROIs' are different for different photos in the sequence.

Locked info – displays information about if the 'ROIs' are locked to an adaptable anchor point (white spot) or fixed in relation to the photos.

Subtract info – displays information about if the system is in subtract mode or default mode as set by the *Subtract* button.

Photo panel

Photo Size – shows the size of the displayed photo in pixels.

The bar below the *Photo Size* indicator displays the relative position of the present photo in the sequence.

Below the bar the capturing date and the time for the first, present and last photo file is displayed. This information is saved as meta-data when the photo is captured and cannot be altered. The elapsed time (from the first photo capturing time) is displayed in red colour.

When 'ROIs' are drawn in the photo, the distance (in pixels) from the upper left corner to the lower right corner of the 'ROI' is displayed. This information is useful for measuring the distance between two point (in pixels) in the photo.

All distances are displayed in terms of pixels. The TiVi system can, however, be calibrated by capturing a photo of a paper with squares (e.g. 5 mm x 5mm) to relate the mm-scale to pixels. Such a calibration procedure is valid only for the specific setting of the zoom lens and the camera – object distance.

When a 'ROI' is drawn, the name of the 'ROI' is displayed to the right of the photo. Clicking on this 'ROI' name displays the 'ROI' area in the Photo panel and the corresponding TiVi-image area in the Image panel. The name of the displayed 'ROI' area is now printed in *italic style* letters. The parameters column at the rightmost part of the Image panel now represents the Image in the 'ROI' area. Clicking the (*italic style*) name of the 'ROI', again reverts to displaying the full photo and image.

'ROIs' can also be drawn in the TiVi-image but the 'ROIs' are always displayed in the photo alone.

Image Panel

The *Upper* and *Lower Threshold* limits are displayed above the TiVi-Image.

When the mouse pointer is hovered over the TiVi-image, the TiVi-value at the mouse pointer location is displayed in red colour.

The *Colour scale* is displayed to the right of the image with the numbers changing as the *Colour scale* is set in the *Colour Span* combo-box. Changing the *Colour Scale* does not alter the TiVi-values.

The rightmost column within the Image panel displays information about the mean value, median value, variation coefficient, standard deviation, minimum value, maximum value, width (in pixels), height (in pixels) and the number of points within set thresholds of the image displayed. The number of points constitutes a measure of the area within thresholds values (in pixels).

Select Photos Panel

Actual Photo textbox – displays the name of the photo currently displayed. The photo currently displayed can be altered manually to jump directly to a desired photo file in the sequence uploaded by inserting the name of the photo file in the *Actual Photo* textbox.

First Photo textbox – displays the name of the first photo in the sequence as selected by using the *First Photo* button.

Last Photo textbox – displays the name of the last photo in the sequence as selected by using the *Last Photo* button.

Subtracted Photo textbox – displays the name of the photo of which the corresponding image is subtracted from all the other images in the sequence pixel by pixel as selected by the *Subtract* button.

Show First Photo radio-button – click to display the first photo in the sequence.

Show Last Photo radio-button – click to display the last photo in the sequence.

First Photo button – click to select the first photo in the sequence to be uploaded. The last photo in the sequence and the sequence step is automatically identified. After selecting the first and last photo in the sequence by clicking this button, the last photo in the sequence is displayed.

Last Photo button – click to select the last photo in the sequence to be uploaded.

Click the link below to play the video: Load and display images.

http://media.wheelsbridge.se.loopiadns.com/2020/09/30_D_LoadAndDisplayImages_OK.mp4

Subtract button – click to select the photo which corresponding image is to be subtracted from all the other images in the sequence pixel by pixel. This feature is useful when alteration from baseline is investigated. Positive differences in TiVi-values are displayed in colour, while negative differences are displayed in grey scale. The object must not move from one photo to another. To be used only for objects not in motion.

Click the link below to play the video: Image subtraction.

http://media.wheelsbridge.se.loopiadns.com/2020/09/43_D_ImageSubtractionProject_OK.mp4

Step textbox – displays the actual step for the photos and images to be displayed.

Decrease Step – decreases the step.

Increase step – increases the step.

Clear – clears the photo and image picture boxes and makes the system ready for uploading a new photo sequence.

Step Through Panel

Manual Step button – click to step through the photos and images sequentially.

Reverse Order button – changes the order in which the photos and images are stepped through.

Automatic Step button - click to step through the photos and images automatically. Click the same button to stop the step through process (button now labelled *Stop*)

Pause button – double-click to temporarily halt the step through process. Click the OK button in the continue window to proceed.

Colour Span – select a number to alter the colour span of the images. Changing the colour span does not affect the calculated TiVi-values.

Comments button – click to edit or insert comments to the photo files. The comments are saved as meta-data to the photo files.

> If comments were inserted at the capturing process, these comments are displayed in all photos in the sequence and can be edited using this *Comments* button in the Camera Page. If a comment is edit using the *Comments* button in Analysis Page, only the comment in the actual photo is altered.

Delay (milliseconds) – change the slider position or content of the associated text box to change the speed of the automatic step through procedure.

Crop Photos Panel

3D Plot – click to display the TiVi-image in 3D Plot. Place the mouse pointer on the image, press the left mouse button, and move the mouse to change the view angle.

Click the link below to play the video: 3D presentation of images.

http://media.wheelsbridge.se.loopiadns.com/2020/09/45_D_3D_OK.mp4

Refresh button – click to delete all 'ROIs' and display the full photo and image.

Save Cropped Photo – click to save a sequence of photos cropped to the selected 'ROI' size.

> When a 'ROI' area is displayed clicking this button opens a *Save File Dialog.* Navigate to the target folder and insert a file name. When the OK button is clicked the cropped areas of all photos and the sequence are saved to file. This feature is useful as the initial step in the image analysis process by cropping out only the section of the photos that are of interest in the analysis. By opening this sequence of photos using the *First Photo* button, a sequence of cropped photos of smaller size with information of interest can be used as the new original photos thereby speeding up the image analysis process.

Reset Thresholds – click to reset the thresholds and update the TiV Image.

Upper Limit slider or text-box – drag this slider or insert a value (between 0 and 100) in the textbox to alter the upper limit threshold. Pixels with TiVi-values above set *Upper Threshold* are replaced by the corresponding photo pixels.

Lower Limit slider or text-box – drag this slider or insert a value (between 0 and 100) in the textbox to alter the lower limit threshold. Pixels with TiVi-values below set *Lower Threshold* are replaced by the corresponding photo pixels.

Click the link below to play the video: Thresholding.

http://media.wheelsbridge.se.loopiadns.com/2020/09/44_D_ThresholdingProject_OK.mp4

Coordinate panel – displays the coordinates of the current 'ROI'.

ROI Management (in the Photo)

Draw a ROI – draw a new 'ROI' by first placing the mouse pointer at the upper left corner of the 'ROI' to be drawn. Hold down the left mouse button while moving the mouse pointer to a new position. Release the mouse button. A new 'ROI' is now drawn, and the name of this 'ROI' is displayed to the right of the photo. Clicking this 'ROI' text displays the' ROI' part of the photo in the photo panel, updates the image and recalculates the image data. Clicking the 'ROI' text again brings back the original photo with 'ROIs' drawn. 'ROIs' of the same size can be positioned in the original photo by clicking somewhere in the photo without moving the mouse pointer. Up to twelve 'ROIs' can be drawn. 'ROIs' can also be drawn in the TiVi-image but the 'ROIs' are always displayed in the photo.

Delete a ROI – to delete a 'ROI', move the mouse pointer to the centre of the 'ROI'. The arrow-shaped mouse pointer will now be replaced by a hand-shaped mouse pointer. Click the left mouse button without moving the mouse. A window is now displayed prompting the user to confirm the delete action. After deleting a 'ROI', the remaining 'ROIs' are re-named and re-coloured.

Move a ROI – to move a 'ROI', move the mouse pointer to the centre of the 'ROI'. Hold down the left mouse button while moving the mouse pointer to a new position. Release the mouse button. The 'ROI' is now moved to the new position.

Stretch a ROI – place the mouse pointer at the lower right corner of the 'ROI' (indicated by a dot). The mouse pointer turns into a cross-shape pointer. Hold down the left mouse button while moving the mouse pointer to a new position. Release the mouse button. The 'ROI' will now appear as a stretched 'ROI'. Apply both *Stretch a 'ROI'* and *Move a 'ROI'* to change the shape of the 'ROI' and move it to a new position.

If one or more 'ROIs' already are drawn in the photo, a new 'ROI' of the same size as the first 'ROI' can be placed in the photo simply by pointing at a pixel in the photo and clicking the left mouse button without moving the 'ROI', where the centre of the new 'ROI' is to be positioned.

Click the link below to play the video: Basic ROI manipulations.

http://media.wheelsbridge.se.loopiadns.com/2020/09/32_D_BasicROIManipulationsProject_OK.mp4

Click the link below to play the video: Applying ROIs.

http://media.wheelsbridge.se.loopiadns.com/2020/09/33_D_ApplyingROIsProjectOK.mp4

Click the link below to play the video: ROI and chart curves.

http://media.wheelsbridge.se.loopiadns.com/2020/09/34_D_ROIandChartCurvesProject_OK.mp4

Click the link below to play the video: ROI resizing.

http://media.wheelsbridge.se.loopiadns.com/2020/09/41_D_ROIResizingProject_OK.mp4

Movie Page

Movie Panel

Create Movie – click to create a videoclip from the uploaded sequence of photos or images.

> If the create image movie radio-button is checked, a TiVi-Image video clip will be generated, while if the create photo movie radio-button is checked, a photo video clip will be generated.

Click the link below to play the video: Create a TiVi movie.

http://media.wheelsbridge.se.loopiadns.com/2020/09/41_E_CreateTiViMovieProject.mp4

Start Movie – click to display a created or uploaded video clip.

> The video-clip is displayed in the Image panel (for Image Movie created) or in the Photo panel (for Photo Movie created). The playback velocity can be modified by setting the *Speed* at generation of the video clip.

Click the link below to play the video: Show movies.

http://media.wheelsbridge.se.loopiadns.com/2020/09/42_E_ShowMoviesProjec.mp4

Load Movie – click to load a video-clip from file.

Save Movie – click to save a created video clip.

> The saved video clip can be played back in the *Windows* video player or inserted in a *Power Point* presentation.

HD Movie to Photos – click to load a HD movie from file and split if up into individual frames to be saved as photo files.

> After splitting up a HD movie into separate photo files, these files can be analysed as ordinary photo files in the *Analysis Page* or *Movie Page*.

Speed trackbar – sets the speed by which the video clip is played back.

Create image movie radio-button – click this radio-button to create an image video clip.

Create photo movie radio-button – click this radio-button to create a photo video clip.

Chart window

The *TiVi Chart* window includes a diagram showing the average TiVi-values within the 'ROIs' of all images in the photo sequence, thus displaying the time course of the development of *erythema* and *blanching*.

Display ROIs - ROYx checkbox – check to include the curve corresponding to average TiVi-values within 'ROIx'.

Display ROIs - Subtract first checkbox – check to subtract the first TiVi-value of each curve from the remaining TiVi-values of the same curve (the resulting curve displays alterations from baseline).

Display ROIs - Select Reference combo box – select a curve to be subtracted from all the remaining curves (the selected curve generally represents a reference substance applied to a normal skin test site).

> By subtracting a reference curve (representing TiVi -values of normal skin throughout the different photos in the sequence) from all the other curves (representing TiVi-values of skin areas onto which a vaso-active substance is topically applied), overall changes in skin microcirculation throughout the entire experiment can be

compensated for. In addition, if the first TiVi-value is subtracted, the baseline variability at different skin sites may also be compensated for. The remaining curves thus uniquely represent alterations in skin microcirculation caused by the substance under test.

First and last radio-button – click to display just the first and last point of the curves.

Use this feature to display just the baseline and the end point TiVi-values.

All images radio-button – click to display the full curves.

Save diagram button – click to save the current diagram (*TiVi Chart)* under a user defined name.

A saved diagram (*TiVi Chart*) can be re-loaded for further analysis or loaded into a *TiVi Project* for analysis of average TiVi curves from different *TiVi Charts* representing different test subjects.

Load diagram button – click to load an existing diagram (*TiVi Chart*) and display it in the chart window.

Report button – click to generate a printable report (as pdf-document).

Export Data button – click to generate a report as a spreadsheet (readable by e.g. MS EXCEL® or LIBRE Office).

3D button – click to display data in a 3-dimensional diagram. Set rotation and inclination by using the trackbars in the movable panel. Click the same button again (now labelled 2D) to revert a 2-dimensional presentation of the diagram.

Mean values radio-button – click to display the mean TiVi-values.

Median values radio-button – click to display the median TiVi-values.

Area radio-button – click to display the areas of the patches as set by the lower and upper threshold trackbars.

Use this feature in combination with threshold settings to display the extent of erythema areas.

Average curve check-box – check to display the averages of the curves displayed.

This average curve can be exported to a *TiVi Project* to display how different test subjects react to a specific vaso-active substance systemically administrated or topically applied to the skin.

Standard deviation check-box – check to display the standard deviation of the curves displayed.

First file displayed textbox – insert a value to define the start point of the curves displayed.

Last file displayed textbox – insert a value to define the end point of the curves displayed.

Use the First and Last File displayed textboxes to zoom in and focus on a specific section of a curve.

Smoothing combo box – select *None, Minor* or *Major* to smooth the curves to different degrees.

Select Y-axis combo box – select the maximum value of the Y-axis.

This feature is useful if many *TiVi-charts* are displayed and compared.

Select X-axis combo box – select photo number or elapsed time.

By using photo number, the distance between two consecutive points along the x-axis are equidistant. If elapsed time is used the distance between two consecutive points along the x-aces is determined by the actual capturing time.

Close button – click to close the chart window.

Click the link below to play the video: How to analyze a sequence of photos and generate chart.http://media.wheelsbridge.se.loopiadns.com/2020/08/AnalyzeASequenceOfPhotos.mp4

Click the link below to play the video: TiVi Chart Basics 1.

http://media.wheelsbridge.se.loopiadns.com/2020/09/43_F_ChartBasics1Project_OK.mp4

Click the link below to play the video: TiVi Chart Basics 2.

http://media.wheelsbridge.se.loopiadns.com/2020/09/44_F_ChartBasics2Project_OK.mp4

Click the link below to play the video: TiVi Chart Basics 3.

http://media.wheelsbridge.se.loopiadns.com/2020/09/45_F_ChartBasics3Project_OK.mp4

Click the link below to play the video: TiVi Chart Basics 4.

http://media.wheelsbridge.se.loopiadns.com/2020/09/46_F_ChartBasics4Project_OK.mp4

Project window

An existing or a new *TiVi Project* is opened from the *File* pull-down menu in the TiVi700 main window.

Load object button – click to load an object (average TiVi-value curve from a *TiVi Chart*). Up to 20 objects can be loaded.

When a new object is loaded a corresponding checkbox is displayed. Unchecking the checkbox temporarily hides the corresponding curve. Checking the checkbox again displays the curve. To permanently remove a curve, point at its label, press the left mouse button and drag the mouse downwards. When the mouse button is released a window prompting the user to determine if the corresponding curve is to be permanently removed.

Save project button – click to save the current *TiVi Project* under a user selected name.

> This *TiVi Project* can be opened again for further editing and addition of new average TiVi-curves.

Close button – click to close the *TiVi Project* window.

Mean radio-button – click to display the mean values of the average TiVi-value curves from the different *TiVi Charts* (generally different test subjects).

Median radio-button – click to display the median values of the TiVi-value curves from the different charts (generally different test subjects).

Area radio-button – click to display the areas of the patches corresponding to the TiVi-value curves from the different charts (generally different test subjects). Works in combination with threshold settings.

Average check-box – check to display the average curve of the individual curves displayed.

Standard deviation check-box – check to display the standard deviations of the average curve.

First point textbox – insert a value to define the start point of the curves displayed.

Last point textbox – insert a value to define the end point of the curves displayed.

> Use *First and Last point* to zoom-in on a section of interest of the displayed curve.

Y-axes textbox – insert a value to define the highest value of the y-axes. Selecting *Auto* adapts the y-axes to the curves.

Smooth textbox – select *None, Minor* or *Major* to set the smoothing of the curves displayed.

Report button – click to generate a printable report (as pdf-document).

Export Data button – click to generate a report as a spreadsheet (readable by e.g. MS EXCEL or LIBRE Office).

3D button – click to display data in a 3-dimensional diagram. Set rotation and inclination by using the trackbars in the movable panel. Click the same button again (now labelled 2D) to revert a 2-dimensional presentation of the diagram.

Reduce Photo Size window

In this window the size (in pixels) of the photos can be reduced by a scale factor 2, 4 or 8. This feature is useful when working with many and large photo files. By reducing the photo size, the analysis time is significantly reduced.

Reduction Factor – select a reduction factor (2, 4 or 8) by which the size of the photo is to be reduced.

Reduce Photos – click this button to start the photo reduction process after selecting a name for the folder in which the reduced photos are to be saved.

First Photo button – click to select the first photo in the sequence to be uploaded. The last photo in the sequence and the sequence step is automatically detected. After selecting the first and last photo in the sequence by clicking this button, the first photo in the sequence is displayed.

Last Photo button – click to select the last photo in the sequence to be uploaded.

Show First Photo radio-button – click to display the first photo in the sequence.

Show Last Photo radio-button – click to display the last photo in the sequence.

Close button – click to close the window.

Actual Photo textbox – displays the name of the photo currently displayed.

First Photo textbox – displays the name of the first photo in the sequence as selected by using the *First Photo* button.

Last Photo textbox – displays the name of the last photo in the sequence as selected by using the *Last Photo* button.

Photo Size – the size of the displayed photo in pixels.

The bar below the *Photo Size* indicator displays the relative position of the present photo in the sequence.

Below the bar the capturing date and the time for the first, present and last photo file is displayed. This information is saved as meta-data when the photo is captured and cannot be altered.

Click the link below to play the video: Reduce photo size.

http://media.wheelsbridge.se.loopiadns.com/2020/09/40_D_ReducePhotoSizeProject_OK-1.mp4

Add white marker window

In this window a white marker can be added manually by clicking somewhere in the photos with the mouse pointer. When a marker is positioned in a photo, the next photo in the sequence is automatically uploaded and the process proceeds. This is used to identify an anchor point to which the 'ROIs' can be locked when photos have been captured from an object in motion.
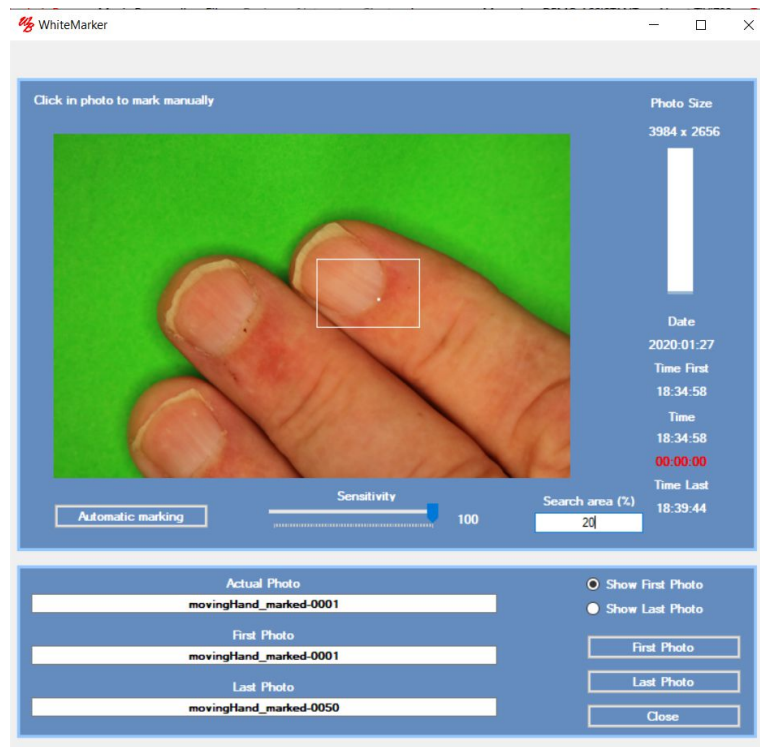
Automatic marking – if there is a white marker attach to the skin when the photos are captured, this feature can be used to automatically amplify the white colour of this maker and optimize its size. After clicking this button, insert a name of a folder into which the resulting photos are to be saved. When clicking the *Save* button the white marker generation process starts. If the white marker is placed outside the rectangle, stop the white marker generation process and repeat the procedure with other settings of the search area and /or sensitivity.

Sensitivity slider – set between 1 and 100%. A low sensitivity implies that markers of faint white colour can be detected. A high sensitivity implies that only points of bright white colour are detected. The sensitivity is automatically set to an optimal value when a photo with a marker is uploaded.

Search area (%) – set between 1 and 100. The area inside the rectangle is searched for a white marker in each new photo. A low value results in a small search area which is useful if movement of the object is small from photo to photo. If larger movements of the object from photo to photos is present, a larger search area is required.

First Photo button – click to select the first photo in the sequence to be uploaded. The last photo in the sequence and the sequence step is automatically detected. After selecting the first and last photo in the sequence by clicking this button, the first photo in the sequence is displayed.

Last Photo button – click to select the last photo in the sequence to be uploaded.

Show First Photo radio-button – click to display the first photo in the sequence.

Show Last Photo radio-button – click to display the last photo in the sequence.

Close button – click to close the window.

Actual Photo textbox – displays the name of the photo currently displayed.

First Photo textbox – displays the name of the first photo in the sequence as selected by using the *First Photo* button.

Last Photo textbox – displays the name of the last photo in the sequence as selected by using the *Last Photo* button.

Photo Size – the size of the displayed photo in pixels.

The bar below the *Photo Size* indicator displays the relative position of the present photo in the sequence.

Below the bar the capturing date and the time for the first, present and last photo file is displayed. This information is saved as meta-data when the photo is captured and cannot be altered.

Adjust ROIs window

This window is used for adjusting the size of all 'ROIs' to the selected 'ROI'.

Select ROI combo box – select the 'ROI' to which size all the other 'ROIs' are adjusted.

OK button – click to accept the selection.

Cancel button – click to cancel the selection.

Set ROI Coordinates window

This window is used to manually set the coordinates of a selected 'ROI'.

Select ROI combo box – select the 'ROI' the coordinates of which are to be altered.

X upper left textbox – insert the new x upper left coordinate of the 'ROI'.

Y upper left textbox – insert the new y upper left coordinate of the 'ROI'.

X lower right textbox – insert the new x lower right coordinate of the 'ROI'.

Y lower right textbox – insert the new y lower right coordinate of the 'ROI'.

OK button – click to accept the selection.

Cancel button – click to cancel the selection.

Name ROIs window

This window is used for renaming the individual 'ROIs', alter the text size and linewidth.

ROIx textbox – insert the name of the 'ROI'. This name will appear in upper left corner of each 'ROI', the ROI text to the right of the photo and in the *TiVi Chart*.

Small text radio-button – click to set the text size to small.

Medium text radio-button – click to set the text size to medium.

Large text radio-button – click to set the text size to large.

Narrow line radio-button – click to set 'ROI' border line width to narrow.

Medium line radio-button – click to set 'ROI' border line width to medium.

Wide line radio-button – click to set 'ROI' border line width to wide.

Save button – click to save the content of the Name ROIs window under a selected name.

Load button – click to load the saved content of the Name ROIs window.

OK button – click to accept the selection.

Cancel button – click to cancel the selection.

Click the link below to play the video: ROI renaming.

http://media.wheelsbridge.se.loopiadns.com/2020/09/38_D_ROINamingProject_OK.mp4

Gallery window

This window is used for displaying all photos or TiVi-images in a single panel.

Photo radio-button – click to display photos.

TiVi-Image radio-button – click to display TiVi-Images.

Close – click to close the window.
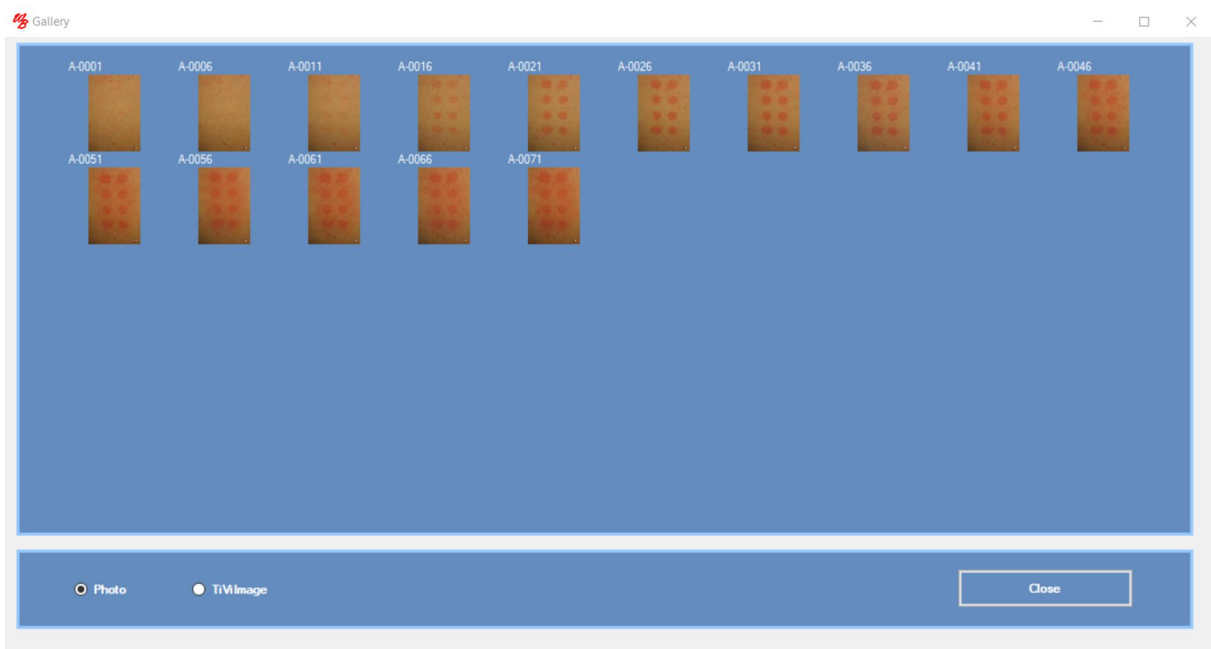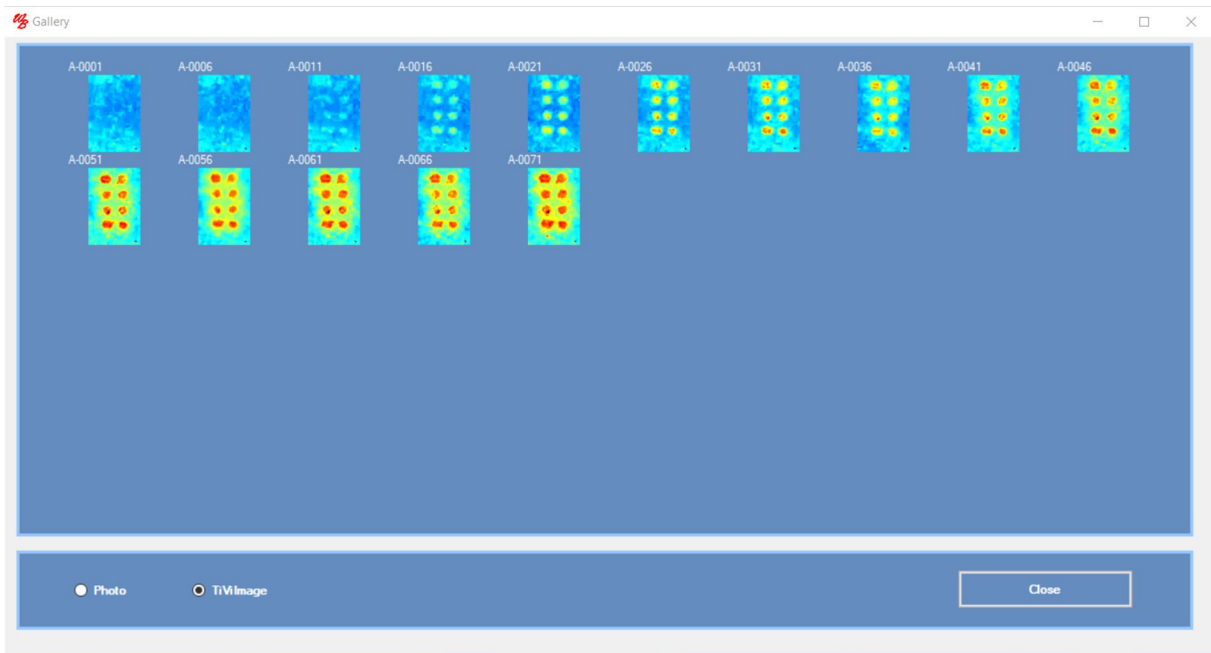
Click the link below to play the video: Gallery.

http://media.wheelsbridge.se.loopiadns.com/2020/09/46_D_Gallery_OK.mp4

Melanin Filter window

This window is used for creating sub-photos of recorded photos in which the coulor of pigments has been removed or altered. The target colour of non-erythema areas in the sub-photos can be set to coincide with the coulor of Caucasian (low-pigmented) skin or any other colour as selected by the user. By this arrangement changes in erythema areas in a sequence of photos are preserved while the baseline colour is changed to a different colour, making it easier to estimate the change in erythema related to baseline skin colour.

The functionality of the Melanin Filter is probably best explained by an example.

1. Upload a sequence of photos (in the case the A-0001.jpg – A-0071.jpg photo files) and check the show First Photo radio-button.
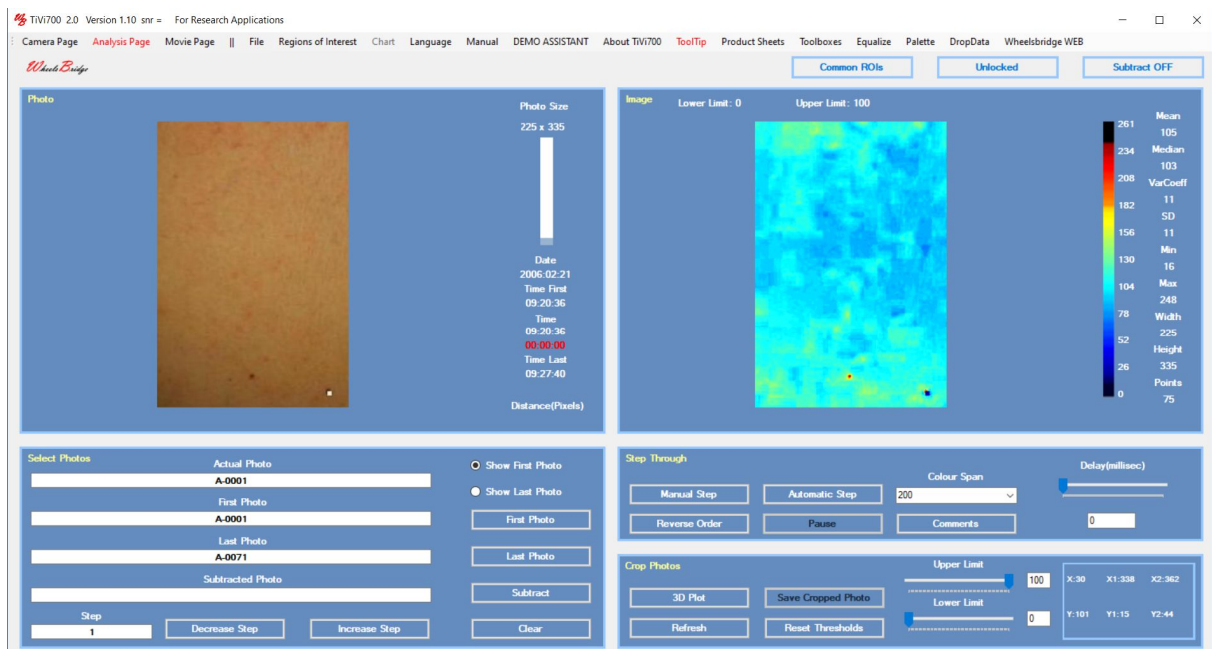
2. Draw a ROI on a non-erythema area.
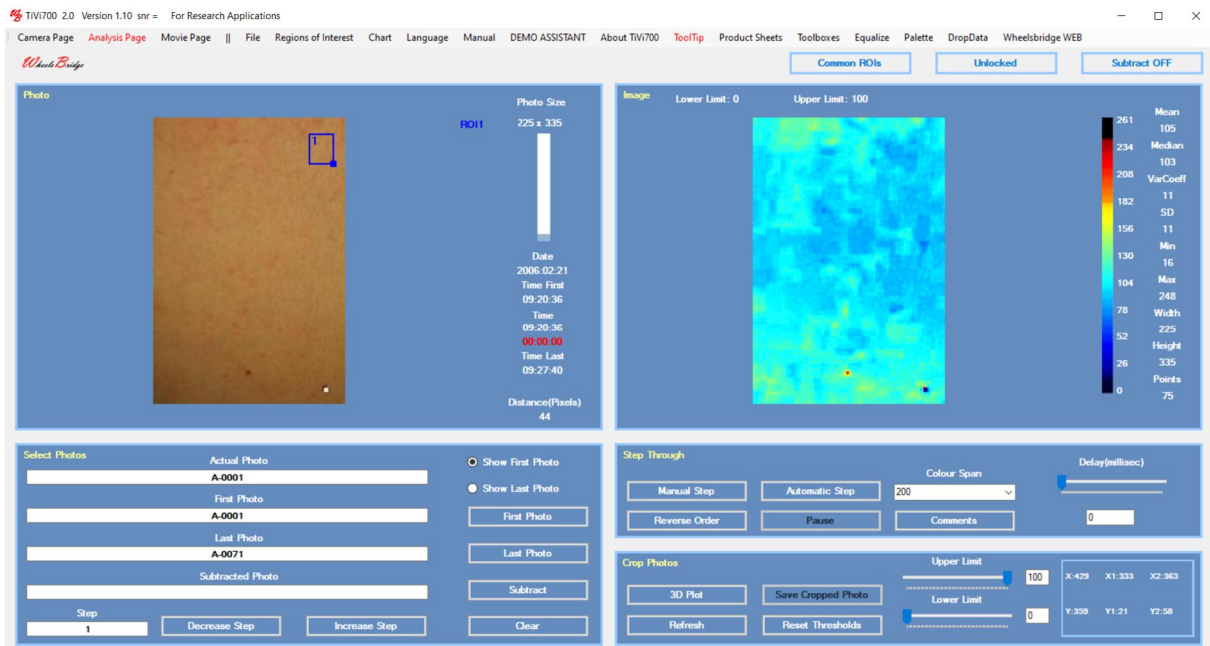
3. Select *Melanin Filter* from the *File* pull-down menu to open the Melanin Filter window.

4. Click the *ROI1* text in the main window to display the zoomed in ROI1 area.
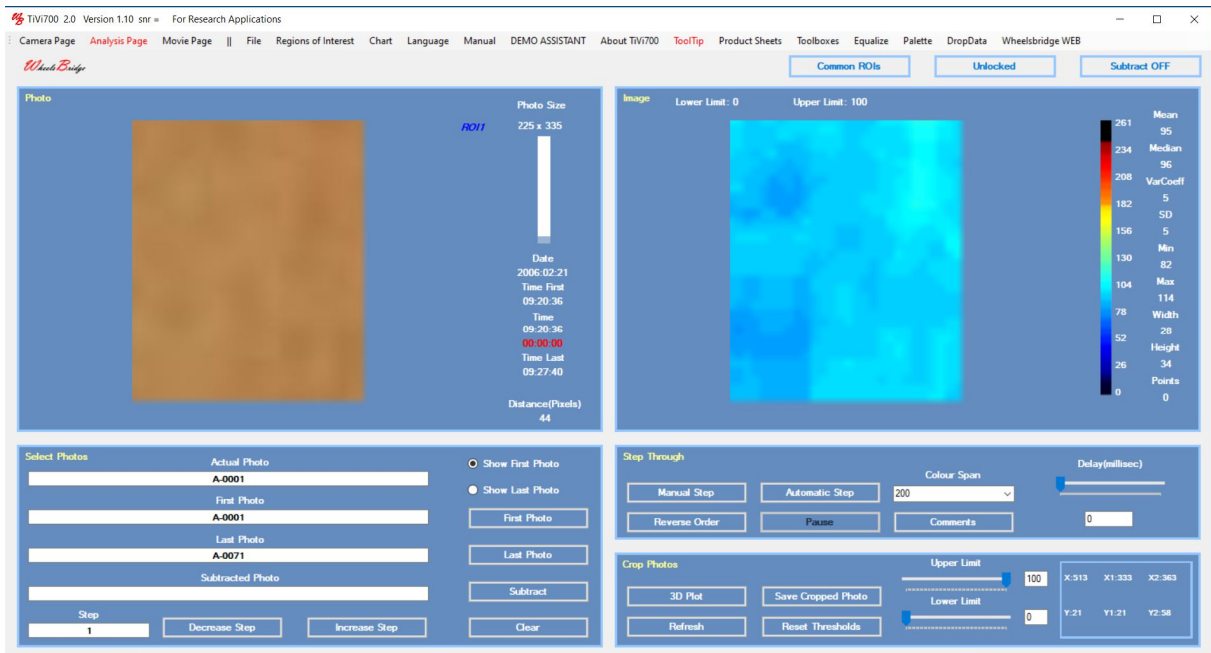
5. Click the *Import* button in the *Melanin Filter* window to import and calculate the average RGB colours of the selected non-erythema area in the ROI1 area.

6. Click the *Calculated Photo* radio-button to display the first sub-photo in the sequence, which is the filtered version of the first photo (*A-0071.jpg*).
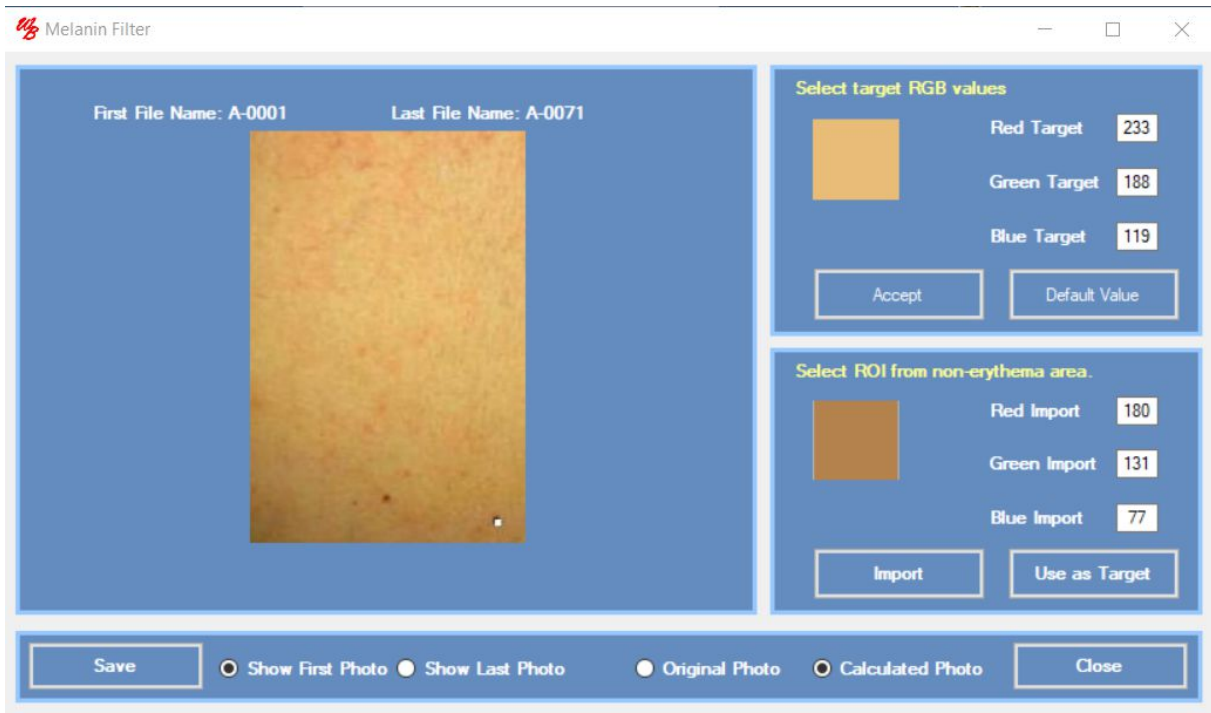
7. Click the Save button and select a name of the sub-folder into which the filtered sub-photos are to be saved. All sub-photos are now generated and save to the subfolder. Eventually the last sub-photo is displayed.

8. In the main window upload the original photo sequence, draw ROIs around the patches and select Chart in the pulldown-menu to generate the corresponding TiVi-chart.
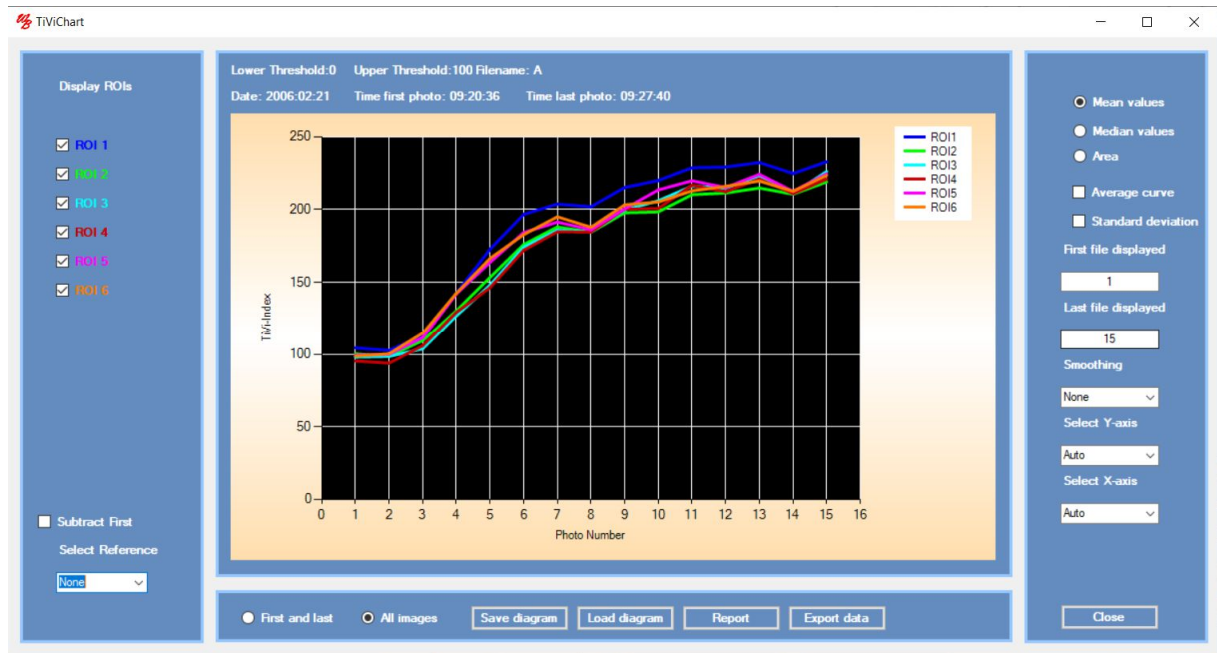
9. Repeat the procedure uploading the generated melanin filtered sub-photo sequence instead.
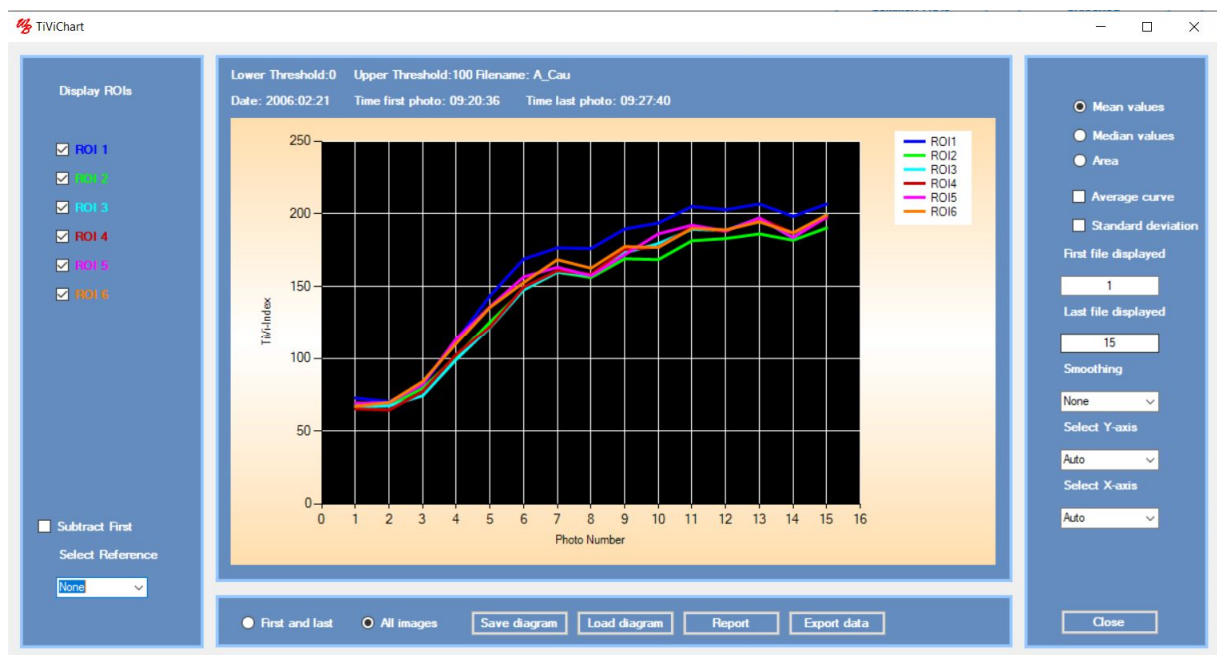
10. Observe that the baseline values have now been shifted towards 60 - 70 TiVi units from about 100 TiVi-units, while the erythema reaction is of about the same magnitude in both photo sequences.

Red Target textbox – displays the red target value (1 – 255). To change this value, insert a new value between 0 and 255 and click the *Accept* button.

Green Target textbox – displays the green target value (1 – 255). To change this value, insert a new value between 0 and 255 and click the *Accept* button.

Blue Target textbox – displays the blue target value (1 – 255). To change this value, insert a new value between 0 and 255 and click the *Accept* button.

Accept-button – click to accept new target RGB values (colour displayed in the associated picture-box).

Default-button – click to upload default skin target values representing Caucasian skin. Click the *Accept*-button to accept the default values as target values.

Import-button – click to import actual RGB values from the ROI zoomed in on in the photo displayed in the main window.

Use As Target – click to import actual RGB values from the ROI zoomed in on in the photo displayed in the main window and use these as target values.

Save button – to generate and save the filtered photos as sub-photos under a selected name in a selected folder.

Show First Photo radio-button – to display the first photo in the sequence.

Show Last Photo radio-button – to display the last photo in the sequence.

Original Photo radio-button – to display the original photos.

Calculated Photo radio-button – to display the calculated photos.

Close-button – click to close the Melanin Filter window.

Click the link below to play the video: Melanin filter.

http://media.wheelsbridge.se.loopiadns.com/2021/01/51_D_MelaninFilter_OK.mp4

Reference Photo window

This window is used for uploading a reference photo and its associated ROIs. When Photos are captured at different points in time it is sometimes difficult to place the object exactly in the same position in all photos. By using the reference photo with ROIs as a reference, a second photo of the same object is uploaded to the main window with its associated ROIs. If the object is somewhat displaced, the ROIs can be moved to overlap the same skin area on the object in the actual photo as in the reference photo.

Filename – displays the name of the photo file.

Rectangular ROIs radio-button – set the shape of the ROIs to rectangular (enabled only is non-freehand ROIs are employed).

Elliptical ROIs radio-button – set the shape of the ROIs to elliptical (enabled only is non-freehand ROIs are employed).

TiVi Image /Photo button – click to display the TiVi image or the photo.

Load Photo button – click to upload a selected photo.

Load ROIs button– click to upload a selected set of ROIs.

Refresh button – click to remove all ROIs from the photo.

Close button – click to close the window.

ROI1 – ROI12 labels – displays the labels for the ROIs in the selected ROI file. Click to display the selected ROI section only. Click again to display the entire photo.

Pulse window

This window displays pulsatile alterations in RBC concentration over a heart cycle (Video) or the maximum pulse amplitude during a heart cycle (Max Pulse).

Start with creating a three seconds long HD video from a non-moving object. Then split up this video into individual photos by clicking the *HD Movie to Photos* button in the *Movie*

Page window. Upload these photos in the *Analysis Page* window. Crop the photos as necessary and click the *Save Cropped Photos* button to save these cropped photos. Upload the cropped photos. Check if there is a pulsatile component in the photos by drawing a ROI and select *Chart* to generate a chart. In the chart generated check the *Subtract First* checkbox. If there is a pulsatile component 3 – 5 pulse cycles should be visible. Close the chart window and select *Pulse* from the *File* pull-down menu. The uploaded photos are now searched to extract a single pulse cycle. Eventually the maximum pulse amplitude is displayed in the *Max Pulse* image.

Moving the mouse pointer over the image displays the image coordinates and the pulsatile component as percentage of total RGB concentration.
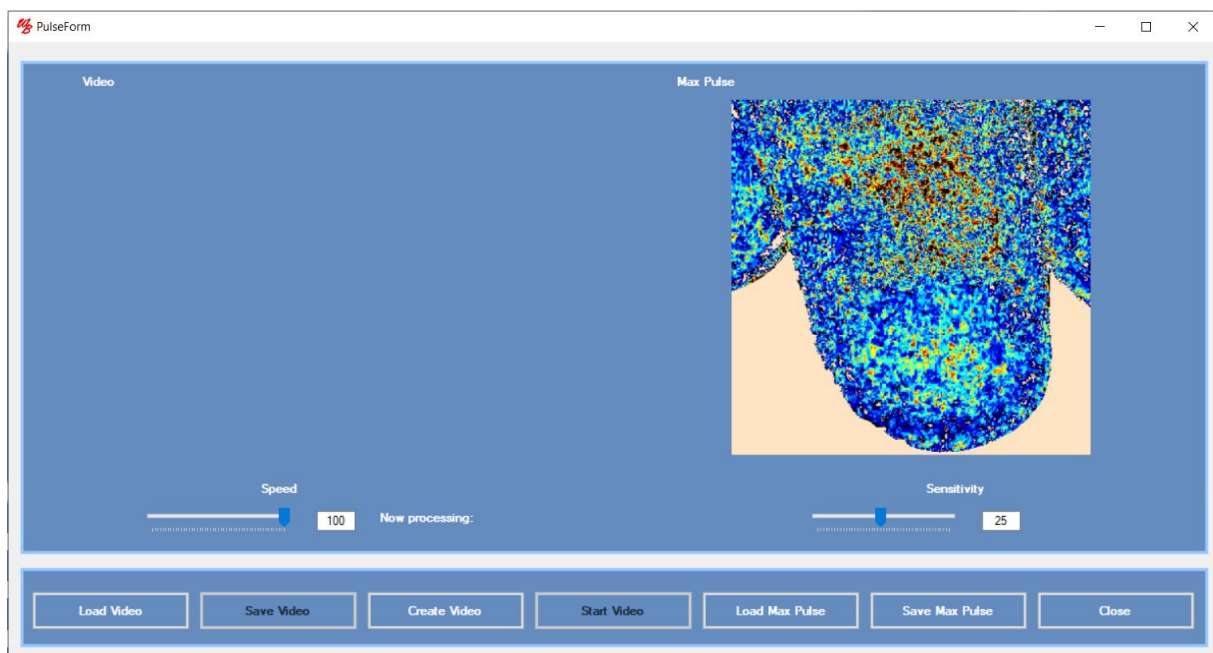
Create Video – creates a video clip of the pulse component for the actual heart cycle.

Start Video – plays the video created from uploaded photos repeatedly.

Speed – to change the speed of the video.

Sensitivity – to change the colour scale of the image displayed.

Load Video – to load an already saved video clip.

Save Video – to save the video displayed.

Load Max Pulse – to load an already saved *Max Pulse* image.

Save Max Pulse – to save the displayed *Max pulse* image.

Close – to close the *Pulse* window.

DropData window

This window is used for exporting image data such as *File name, ROI number, average TiVi-value, median TiVi-value, area above threshold, Min-value, Max-value and standard deviation*, into a table for later export to a spreadsheet. This is achieved by clicking the *Import* button in the *DropData* window exporting image data to the table. Selected ROI data from different images can be combined into a single table for further data analysis in a spreadsheet.

1. To display the *DropData* window, click *DropData* in the *Analysis Page* pull-down menu.
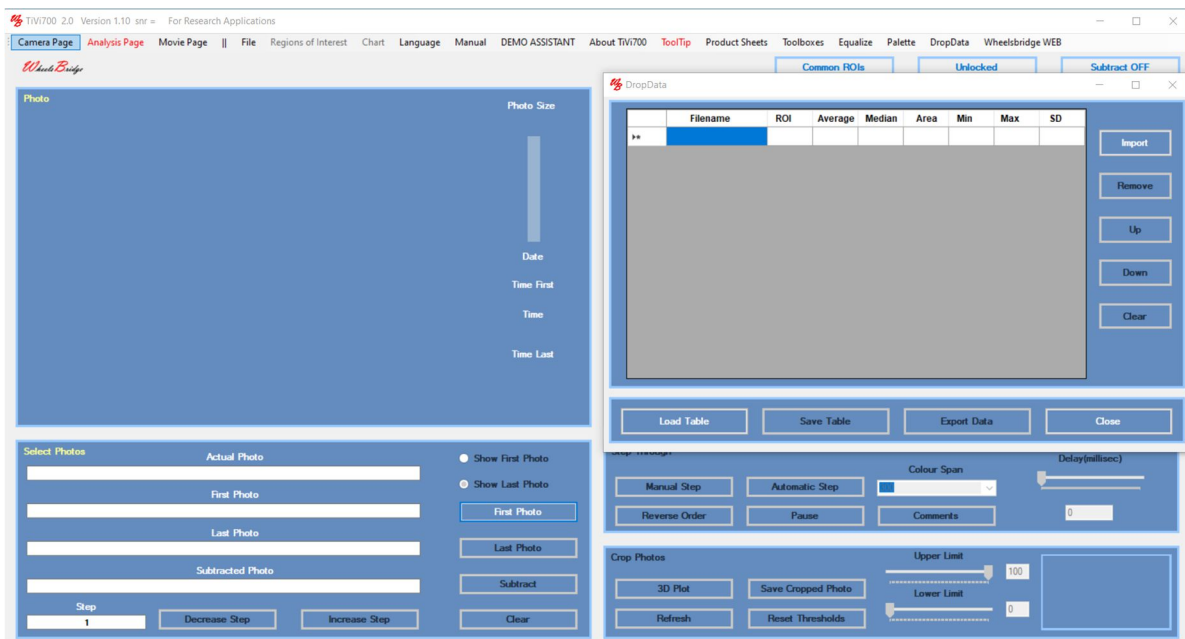
2. Open a photo-file and draw some ROIs. Display one of the ROIs by clicking on the name of the ROI to the right of the photo. Click the *Import* button in the *DropData* window.
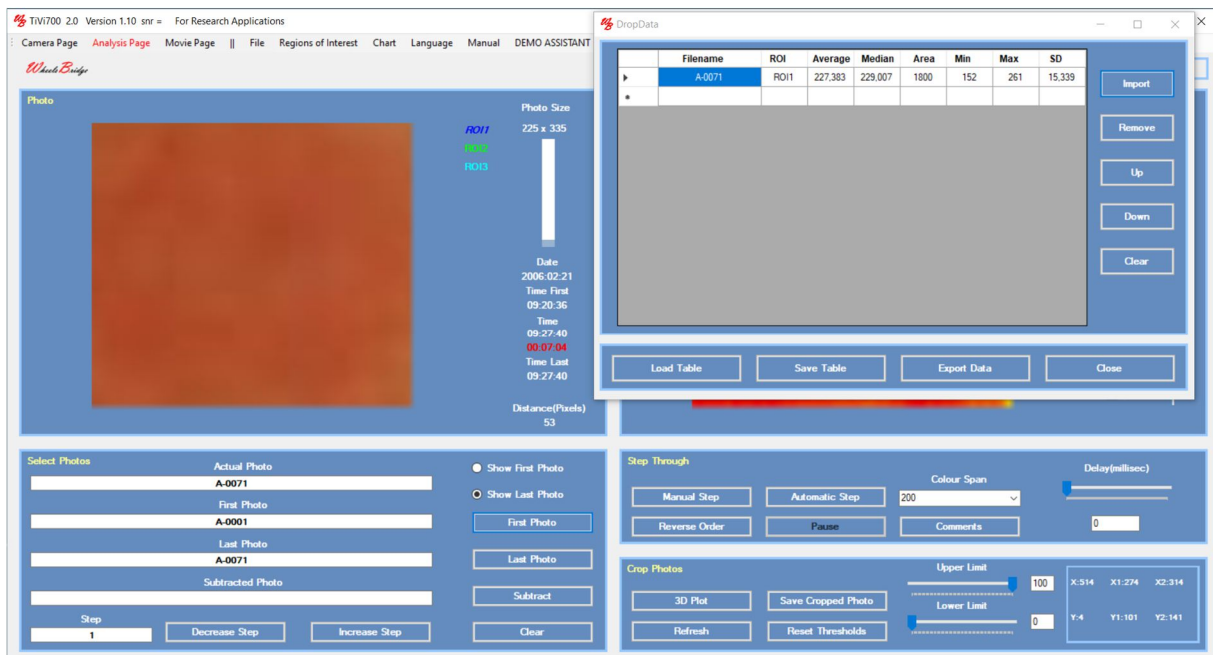
3. Select another ROI and click the *Import* button again.
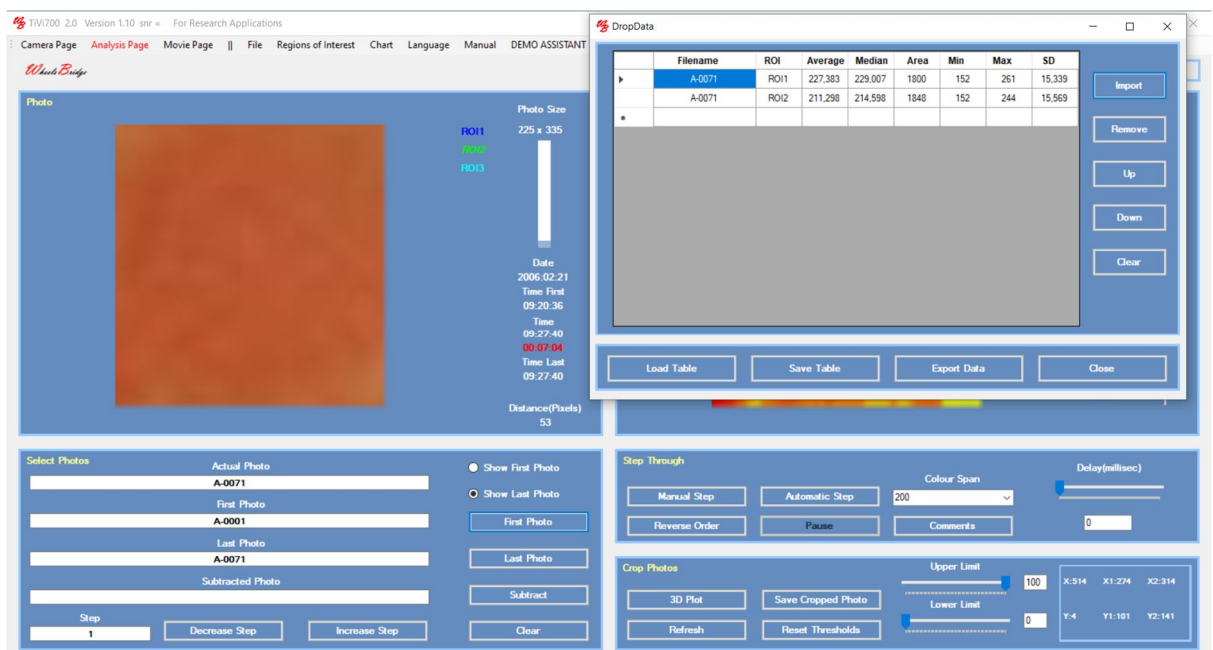
Data Grid View – displays data on Filename, ROI name, Average TiVi-value, Median TiVi-value, Area, Min value, Max value and Standard Deviation.

Import button – click to import new data from the *Analysis* page.

Remove button – point at the row to be removed and click the *Remove* button.

Up button – point at a row and click the *Up* button to move the row upwards.

Down button – point at a row and click the *Down* button to move the row downwards.

Clear button – click to clear the *Data Grid View*.

Load Table – click to upload a saved table.

Save Table – click to save the table to file.

Export Data – click to export the data in the table to a spreadsheet for further analysis.

Close – click to close the *DataDrop* window.

Click the link below to play the video: Drop Data.

http://media.wheelsbridge.se.loopiadns.com/2020/09/47_D_DropData_OK.mp4

HOW TO USE THE TiVi700 TISSUE VIABILITY IMAGER

In this section numerous full analysis scenarios are presented on how to analyse photos and videos recorded by TiVi700. Demonstration photos are stored in the folder named *'TiViDemonstration'* located in *'Program Files (x86): Wheelsbridge: TiVi700: TiVi700: TiVi700Demonstration'.*

Analysis of erythema caused by methyl nicotinate topically applied to the skin

Sequences of photo files captured from the skin of different test subjects to which methyl nicotinate has been topically applied using *Finn Chambers®* patch testing to produce erythema are used in this session. Typically, methyl nicotinate produces skin erythema that peeks after about ten minutes. After another ten minutes the erythema fades causing recorded TiVi values to return to baseline.

1. Start the TiVi700 program by clicking the TiVi700 2.0 icon on the desktop. Select the *Analysis Page*.

2. Click the *'First Photo'* button to open the *Open Dialog* window. Navigate to the *Program '(x86): Wheelsbridge: TiVi700 2.0: TiVi700: TiVi700demonstratio: MN DATA'*. Double click the *'A-0001'* photo file. This file is now uploaded. The last photo file as well as the step in the photo file sequence are automatically detected. The last photo file name is *'A-0071'*.

3. Select '*Freehand ROIs'* from the '*Region of Interest'* pull-down menu. Draw a 'ROI' around the upper left patch.
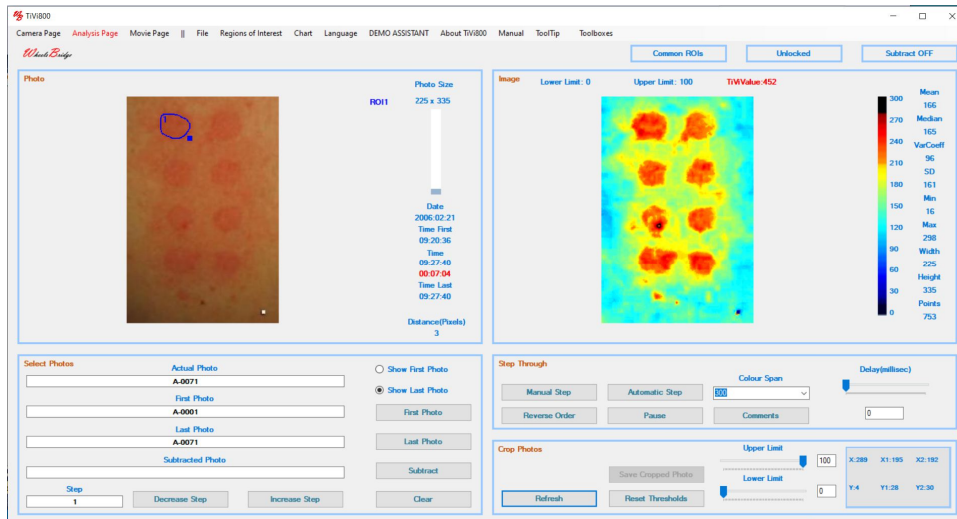
4. Click in the centre of the remaining patches while keeping the mouse still to generate seven more 'ROIs'.
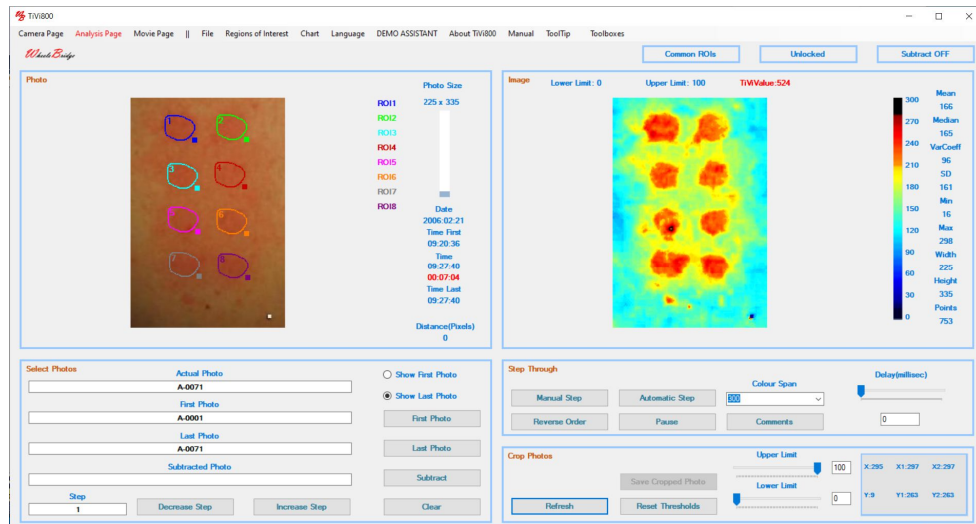
5. Select '*Name ROIs'* from the '*Regions of Interest'* pulldown menu. Insert '*one*', '*two*' and '*three*' in the '*ROI1'*, '*ROI2'* and '*ROI3'* textboxes. Check the '*Small Text'* and the '*Narrow Line'* radio-buttons.
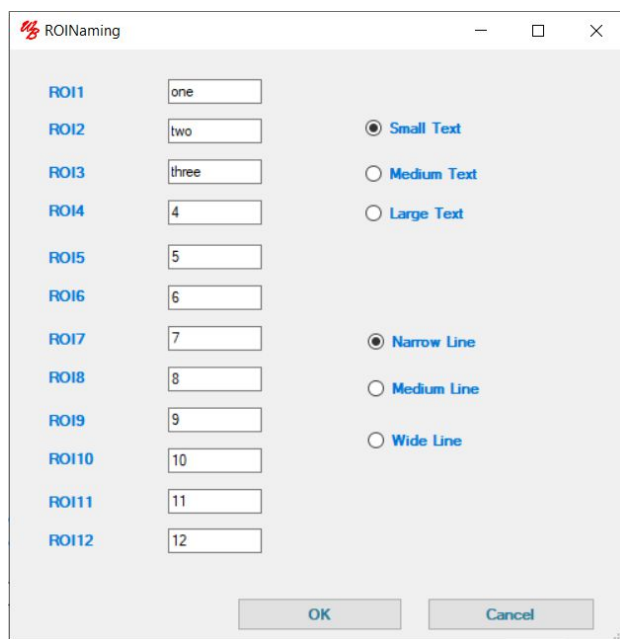
6. Click the '*OK'* button. The *Main* window should now look like:
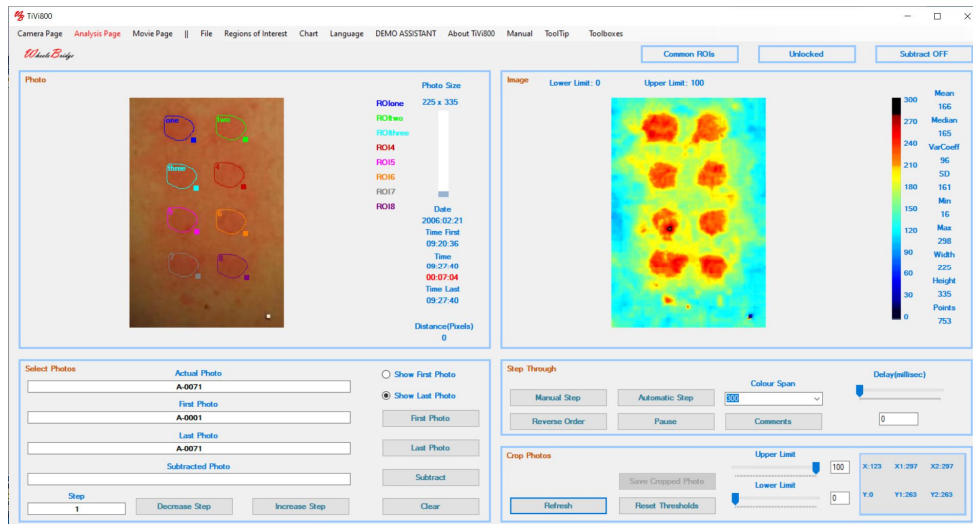
7. Click the *'Automatic Step'* button to scan through the photos and images in the sequence. Then click *'Chart'* I the pulldown menu to generate the chart showing the time course of the mean TiVi Values within the 'ROIs'.
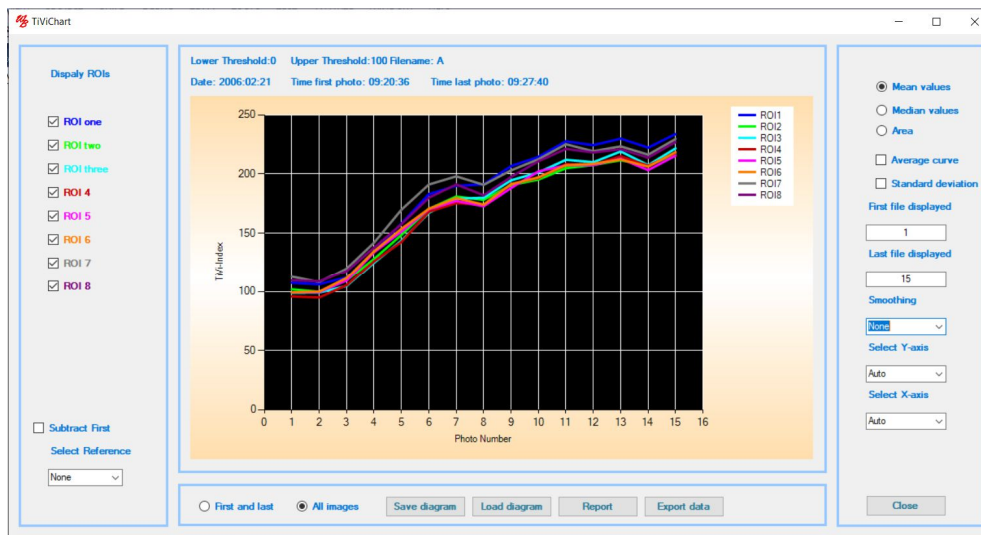
8. Check the 'Average curve' and the 'Standard deviation' checkboxes to display the mean values and the standard deviation of the curves displayed.
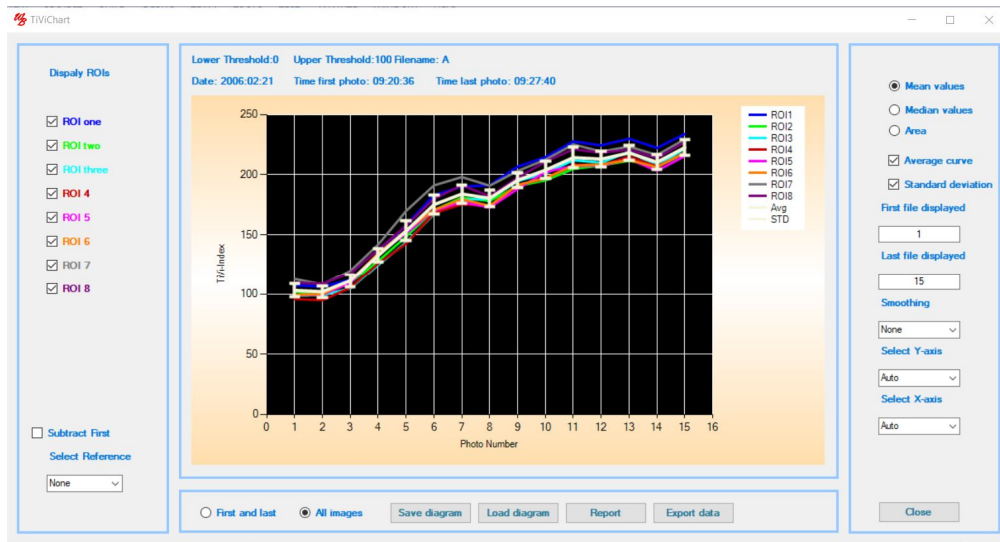
9. Set *'First file displayed'* to 5 and '*Last file displayed'* to 10 to zoom-in on the centre of the curve.
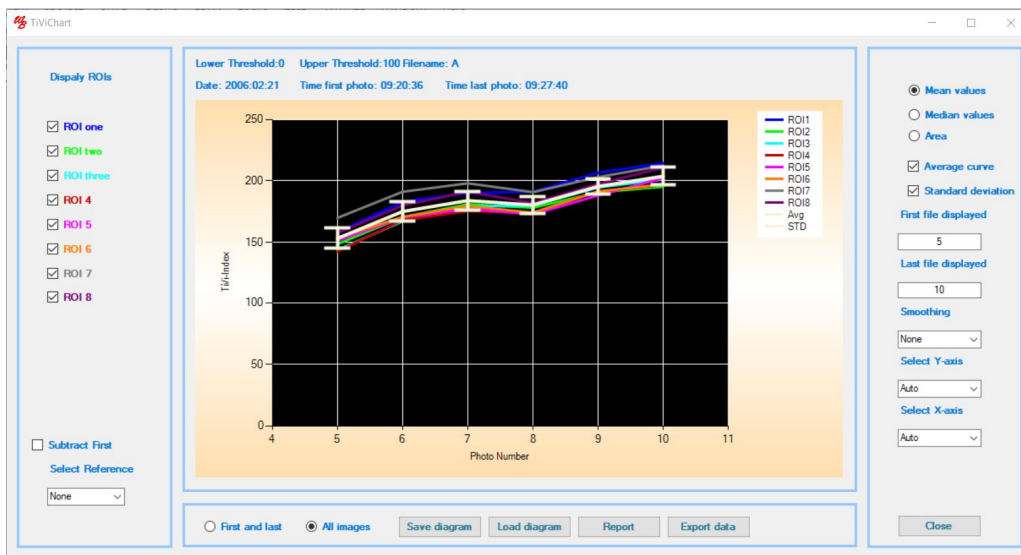
10. Set *'First file displayed'* to 1 and '*Last file displayed'* to 15 to display the entire curve again.

11. Uncheck and recheck the check-boxes in the '*Display ROIs'* panel to remove and reinsert specific curves.

12. Check all the 'ROI' checkboxes again and then check the *'Subtract First'* checkbox. All curves are now subtracted by the first point TiVi-value (baseline).
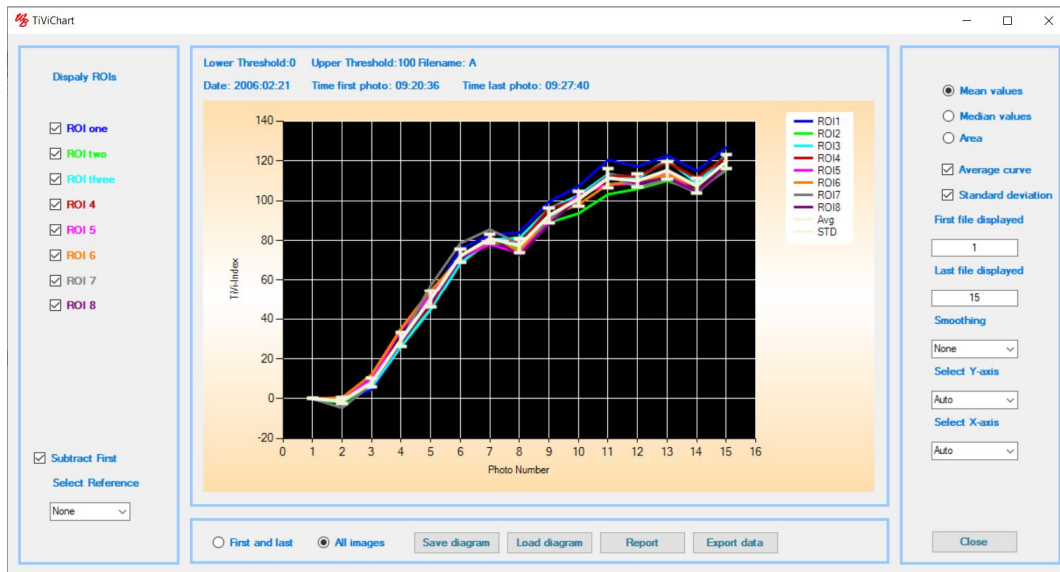
13. In the '*Select Reference'* combo-box, select '*ROI2'* (green curve). The '*ROI2'* curve is now subtracted from all the other curves.
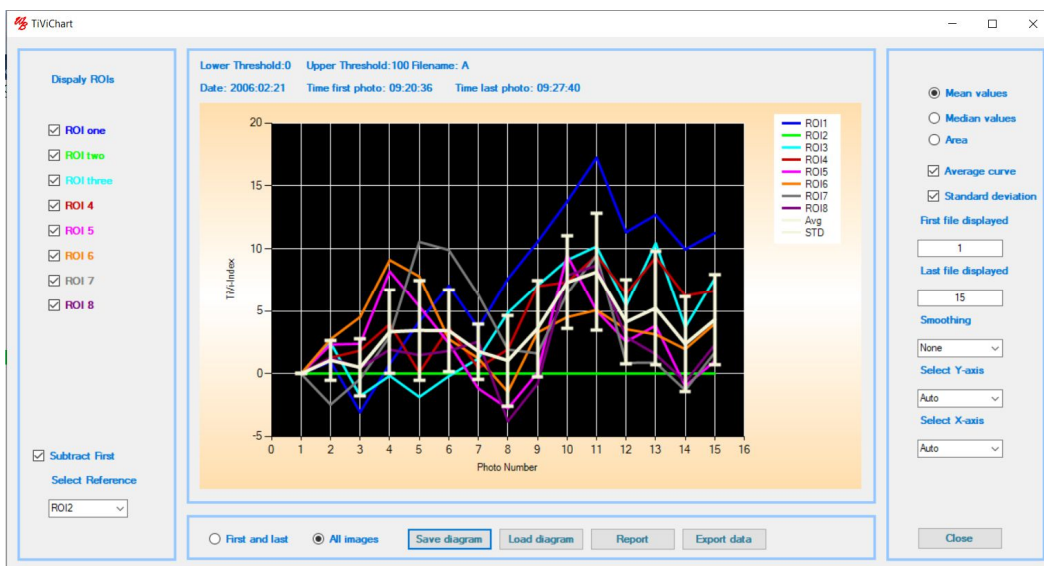
14. Uncheck the '*Subtract First'* checkbox and select '*None'* in the '*Select Reference'* combo-box.

15. Click the '*Save Diagram'* button to save the current chart. In the '*Save File Dialog'* window, insert '*AA'* and click the '*Save'* button. The parameters of the chart are now saved as a text file under then name '*AA.dia'*. This file can be uploaded into a '*Project'* or opened as a chart using the '*Load diagram'* button in the '*Project'* window or the '*Open a TiVi Chart'* in the '*Main'* window pull-down menu.

16. To generate '*Chart'* diagrams also for the files '*B-0013 - B-0098', 'C-0010 – C-0085', 'D-0010 – D-0095'* and '*E-0010 – E-0085*', repeat this procedure from point 2 to here (save

the chart files under the names *'BB', 'CC', 'DD'* and *'EE'*). This will generate the chart parameter dia-files *'BB', 'CC', 'DD'*, and *'EE'* each representing a single test subject.

17. Click the '*Close'* button to close the '*Chart*' window.

18. In the '*Main'* window, select *'File→Project→New Project'* to open a new project.
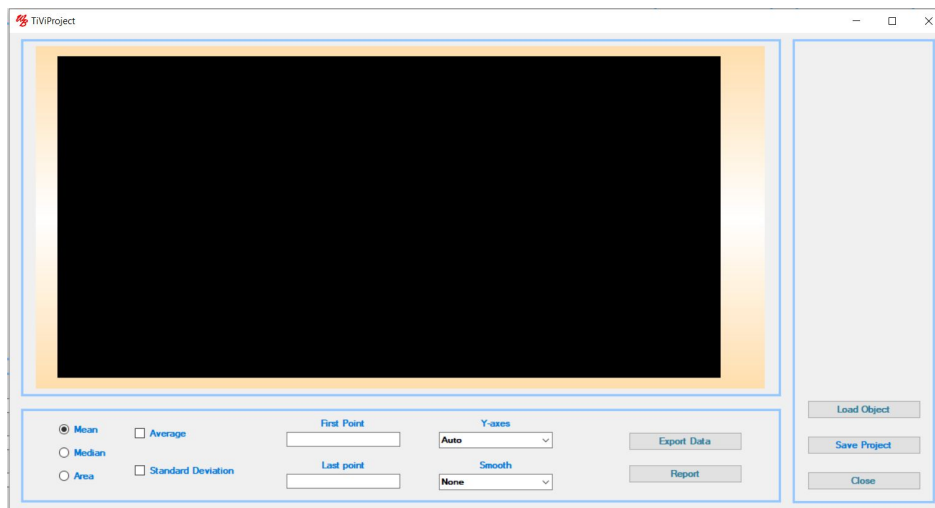
19. Click the *'Load Objects'* button to open the *'Open File Dialog'* window. Select the files '*AA', 'BB', 'CC', 'DD'* and *'EE',* while keeping the Ctrl-button on the computer keyboard pressed.
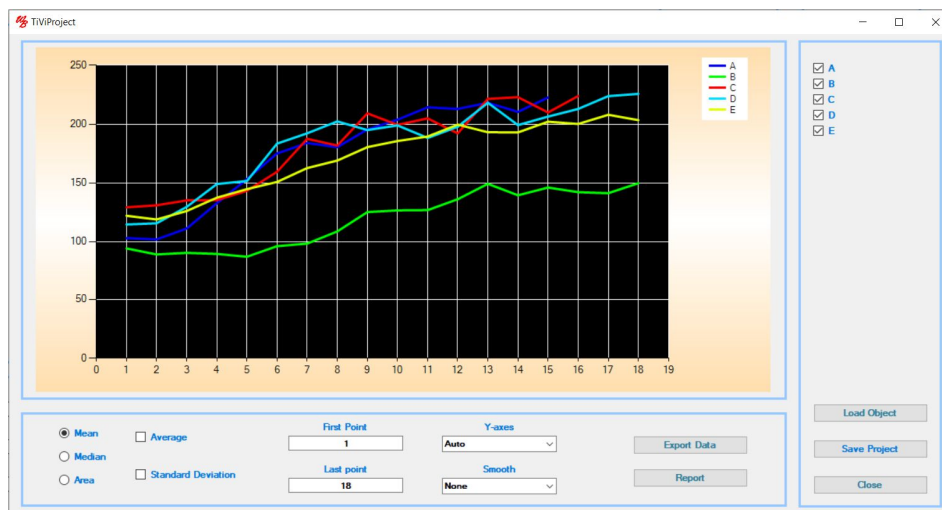
20. The curves displayed represent the average TiVi- value curves from five different test subjects. Check the *'Average'* and the '*Standard Deviation'* check-boxes to display the average curve with standard deviation.

21. Uncheck the checkbox 'BB' in the rightmost panel to remove this curve from the diagram.

22. Check the checkbox 'BB' again to bring this curve back to the diagram.
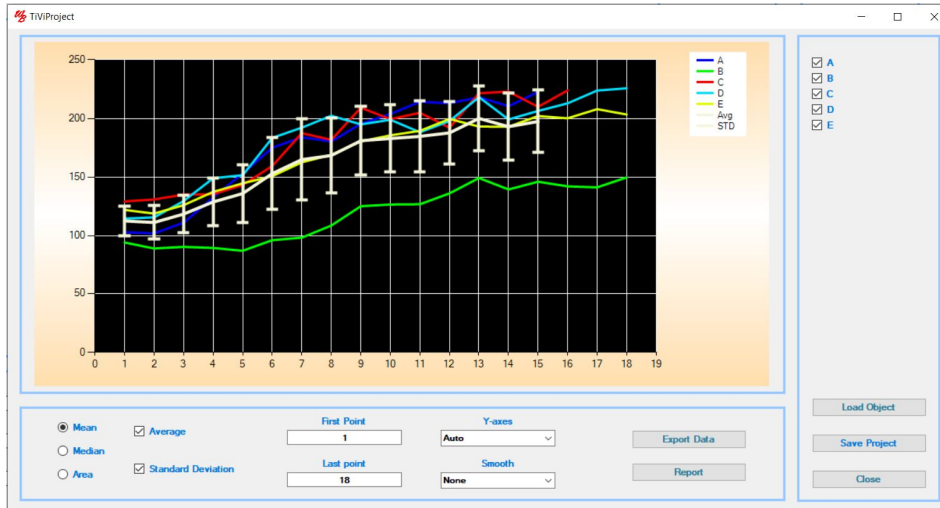
23. Click the *'Export Data'* button to generate a spreadsheet readable by e.g. *MS Excel.*

24. Click the *'Report'* button to generate a report (readable by a pdf-reader).

25. Click the '*Save Project'* button to open a *'Save File Dialog'* window and save the '*Project*' window parameters.

26. Click the *'Close'* button to close the *'Project'* window.

27. In the '*Main'* window - '*Analysis Page'*, select the '*Fil: Project: Open a TiVi Project',* to open the '*Open File Dialog'* window. Select the project file just saved to open it again.

28. This completes *the 'Analysis of erythema caused by methyl nicotinate topically applied to the skin' session*.

Analysis of capillary refilling recorded using HD Video.

This session demonstrates how to analyse photo files recorded during capillary refilling after local occlusion of the microvascular network. Since the refilling of the skin capillaries takes place over a short period of time (1- 2 seconds), an HD video is recorded from which the individual frames are extracted and saved as individual photo files. From these photo files an image video is generated which can be replayed inside the TiVi700 software or e.g. a *PowerPoint* presentation. The HD video file recorded is saved in the *'Capillary Refill'* folder.

1. Start the TiVi700 program by clicking on the TiVi700 icon on the desktop. Select the *Movie Page*.
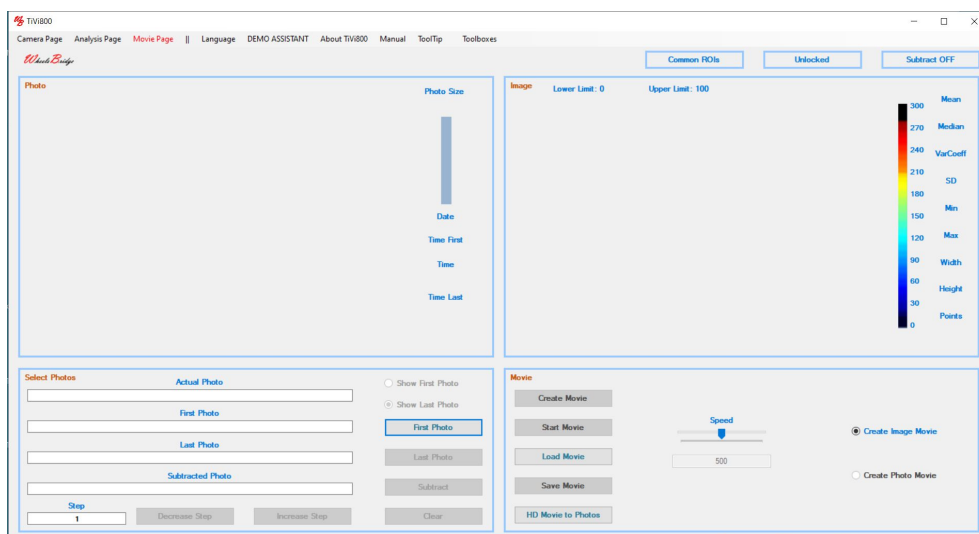
2. Click the '*HD Movie to Photos'* button to open the '*Open File Dialog'* window. Navigate to the *Program '(x86): Wheelsbridge: TiVi700 2.0: TiVi700: TiVi700demonstration: Capillary Refill'*. Double -click the '*capRefill'* movie file. The *'capRefill'* movie file is now opened and split up into individual photo files stored in the same directory under the names '*capRef-0001'* to '*capRef-0220'*.

3. Click the *'First Photo'* button and double-click the *'capRefil-0001'* file. The 'Main' window should no look like:
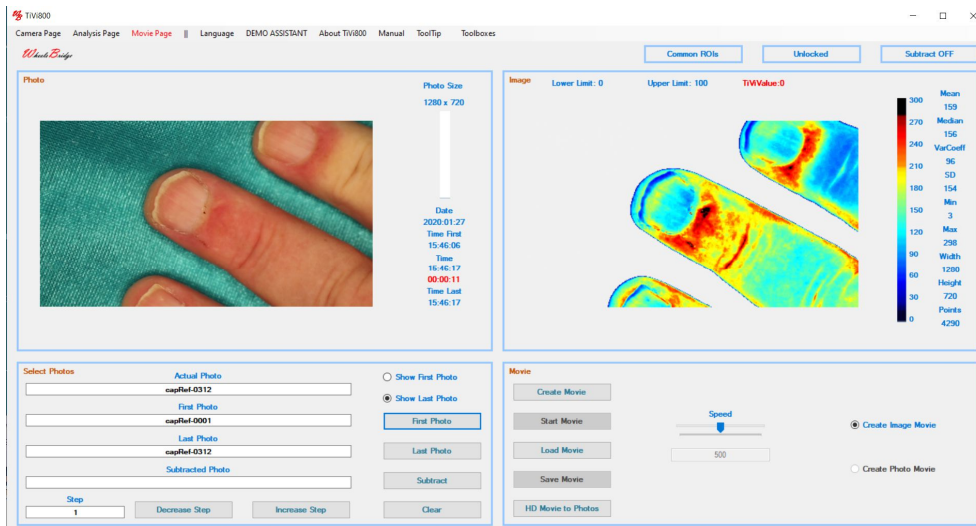
4. Click the '*Create Image Movie'* button to generate the 'Image Movie' file. When the movie is created, click the 'Start Movie' button to play the video clip.

5. Click the 'Save Movie' button to open the '*Save File Dialog'* window. Navigate to a folder into which the video clip is to be saved, insert a file name and click the 'Save' button to save the video clip. This video clip can be replayed in the Windows movie player or inserted into e.g. a *PowerPoint* presentation.

6. Select *'Analysis Page'* in the pull-down menu.

7. Click the 'First Photo' button and double-click the *'capRed-0075'* file.

8. Click the *'Show First Photo'* radio-button.
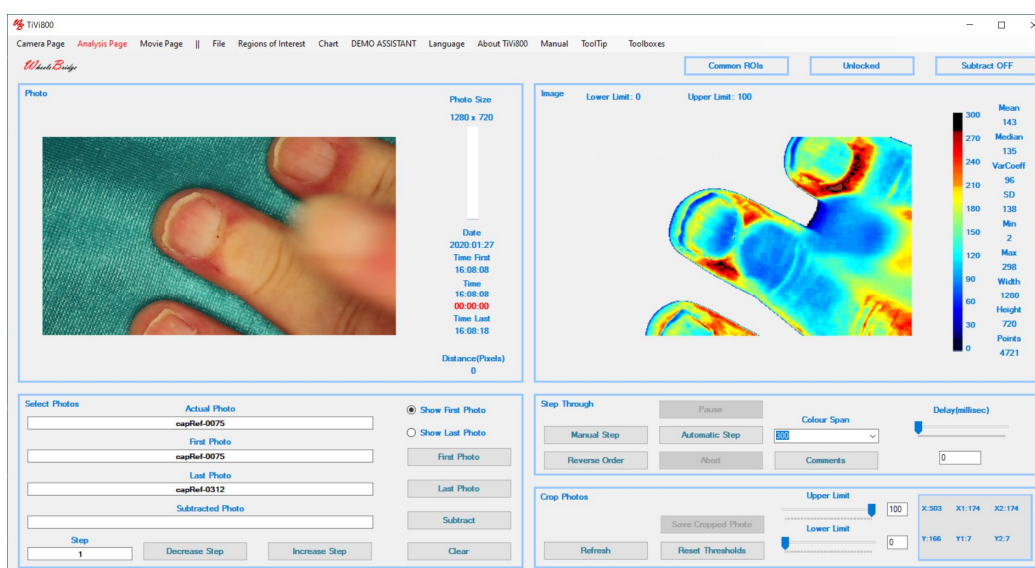
9. Select *'Elliptic ROIs'* from the *'Regions of Interest'* pull-down menu.

10. Draw two *'ROIs'* in the photo like this:
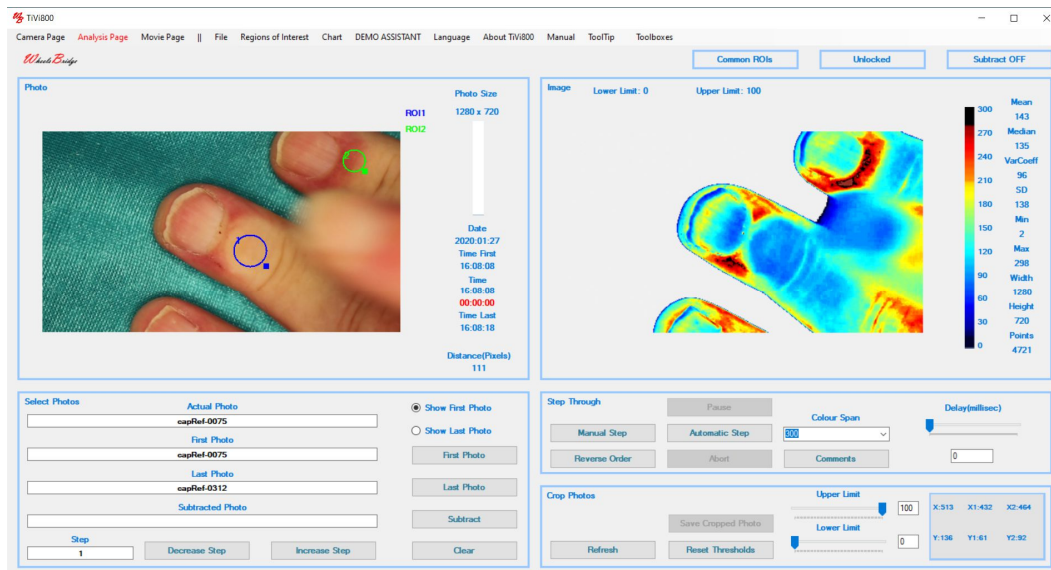
11. Select *'Chart'* from the pull-down menu to display the chart.
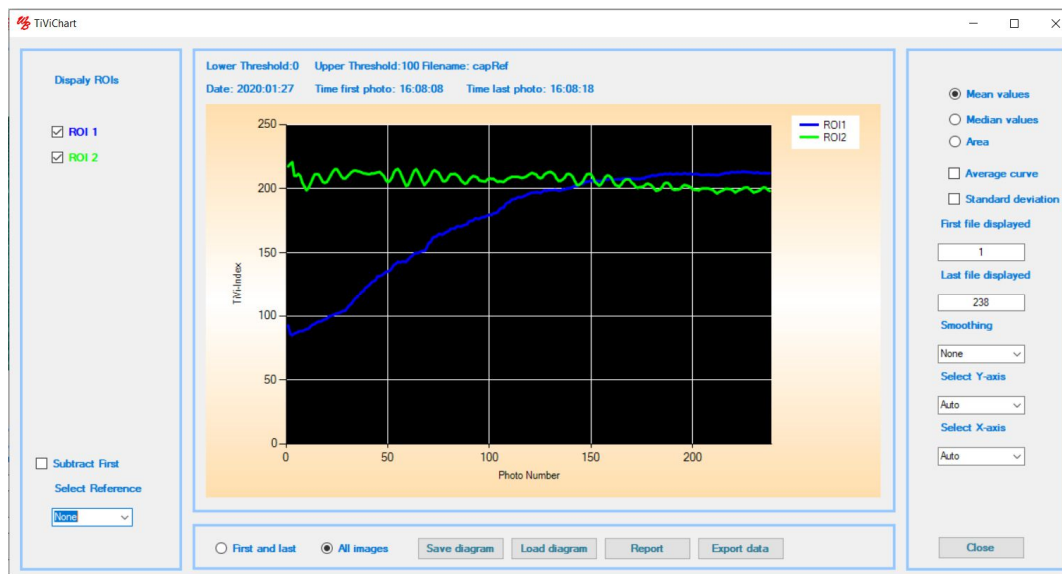
12. This completes the '*Analysis of capillary refilling recorded using HD Video'* demonstration

Click the link below to play the video: How to analyze a capillary refilling experiment using TiVi.

http://media.wheelsbridge.se.loopiadns.com/2020/08/CapillaryRefill-1.mp4

Analysing skin microcirculation in a moving object.

This session describes how to work with adaptable 'ROIs' to analyse the microcirculation in a moving object. A scenario in which a white marker has been attached to the skin before photo capturing is first addressed. Then it is demonstrated how 'ROIs' can be adapted by manually adding a white marker to the skin.

Recorded photo files named *'scratch-0001 – scratch-0050'* with white markers are saved in the folder *'(x86: Wheelsbridge: TiVi70: TiVi700 2.0: TiVi700demonstration: Scratch'*.

Recorded photo files named *movingHand-0001 – movingHand-0050'* with white markers are saved in the folder *'(x86): Wheelsbridge: TiVi700 2.0: TiVi700: TiVi700demonstration: Moving Objects'*.

1. Start the TiVi700 program by clicking on the TiVi700 icon on the desktop. Select the *Analysis Page*.
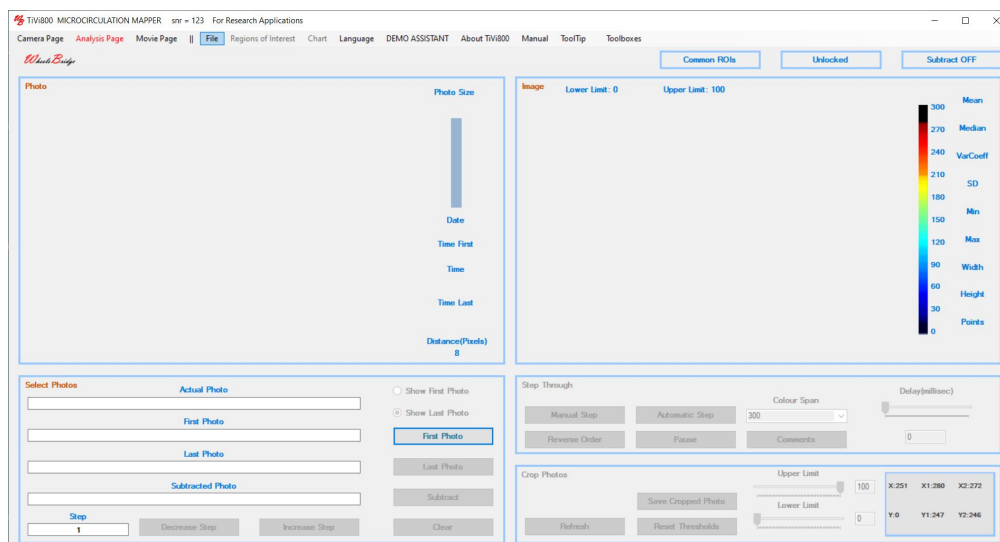
2. Select '*Add White Marker*` in the '*File*' pulldown menu.
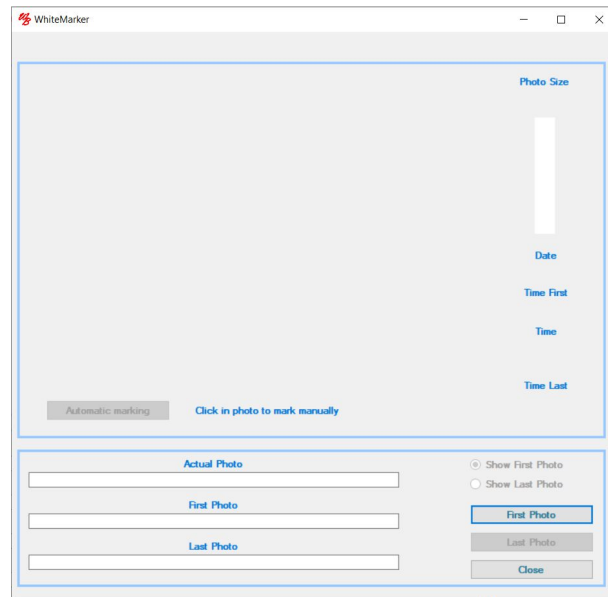
3. Click the 'First Photo' button to open the *'Open File Dialog'* window. Navigate to the *'Scratch'* folder and double-click the *'scratch-003'* photo file.
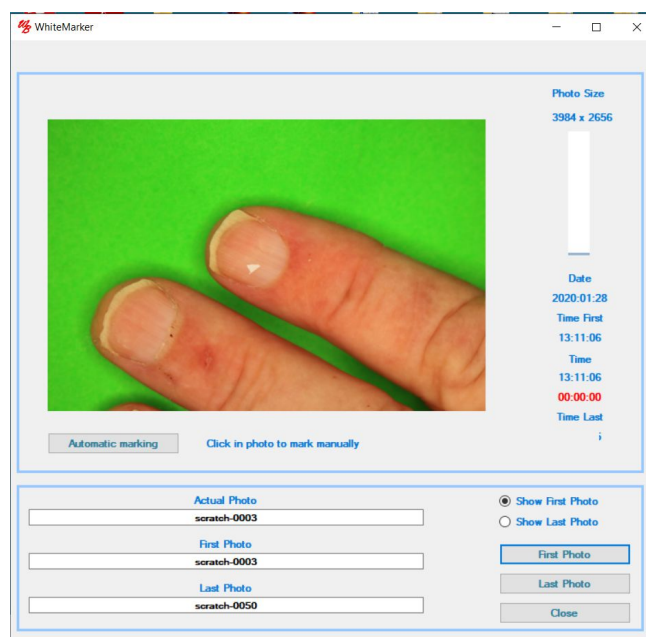
4. The white marker was placed on the nail of the point finger at photo capturing. Click the 'Automatic marking' button to open the *'Save File Dialog'* window. Insert the text *'Myfolder'* and click the *'Save'* button. The system will now scan through the photos, check for the brightest point, automatically insert a bright white marker point and save the marked photos in the folder named '*scratch_MyFolder_marked*' under the file names *'scratch_marked-003, scratch_marked-0004'* etc.

5. When all files are marked click the *'Close'* button to close the *'WhiteMarker'* window.

6. In the *'Analysis Page'* in the *'Main'* window, click the *'First Photo'* button to open the '*Open File Dialog'* window, navigate to the '*scratch_MyFolder_marked'* and double-click the '*scratch_marked-003'* photo file.
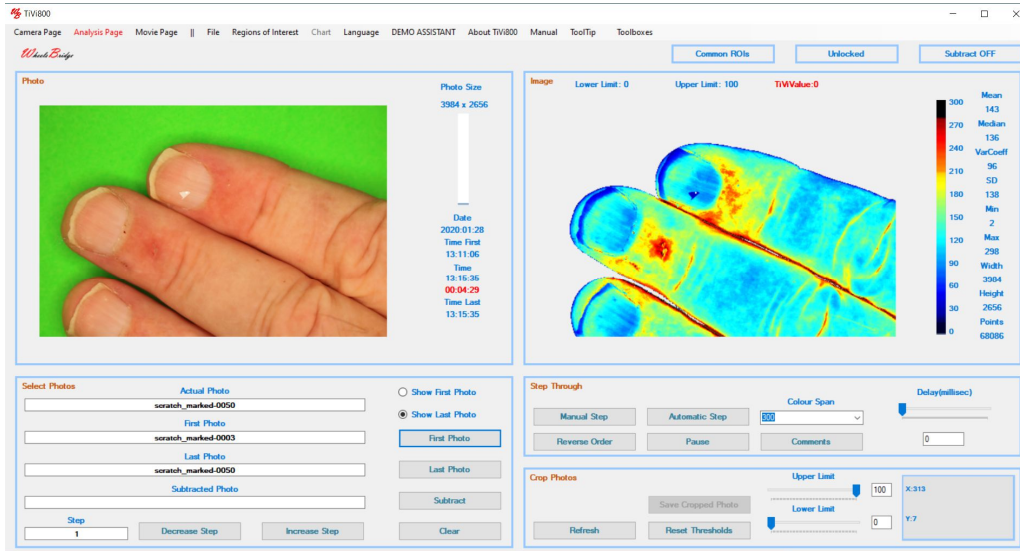
7. Observe the small bright white marker point added to the physical white paper marker. Check the '*Show First Photo'* radio-button. In the 'Regions of Interest' pulldown menu, select the 'Freehand ROIs' option. Draw a freehand 'ROI' just proximal to the nail in the point finger.
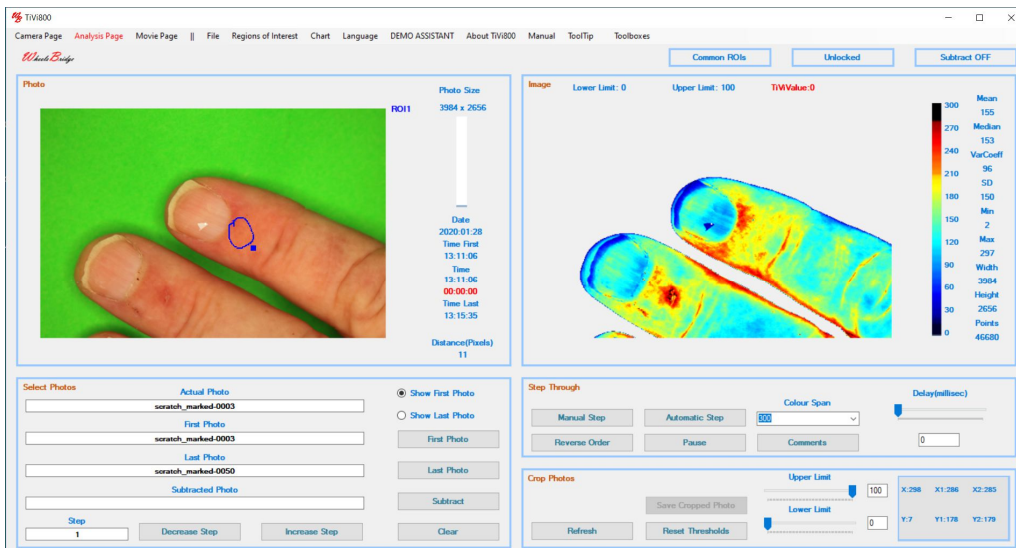
8. In the '*Region of Interest'* pulldown menu, select the '*Lock to white spot'* option and watch how the '*ROI'* drawn locks to the white spot while the system scans through the photos in the sequence.

9. Click the '*Manual Step*' button successively to inspect the positions of the *'ROI'* while scanning through the photo sequence. At *'Actual Photo'* named 'scratch_marked-0009', the 'ROI' is obviously misplaced due to an erroneous position of the white marker.
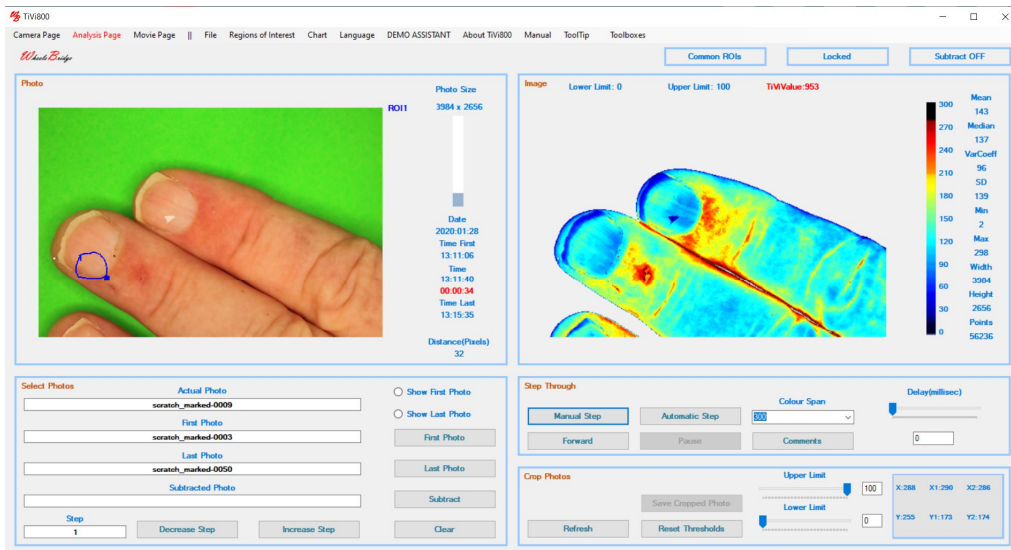
10. With the photo file *'scratch_marked-0009'* displayed, select *'Just this photo'* in the *'Regions of Interest'* pulldown menu. Point at the centre of the 'ROI', press the mouse button and drag the 'ROI' to the position shown below.
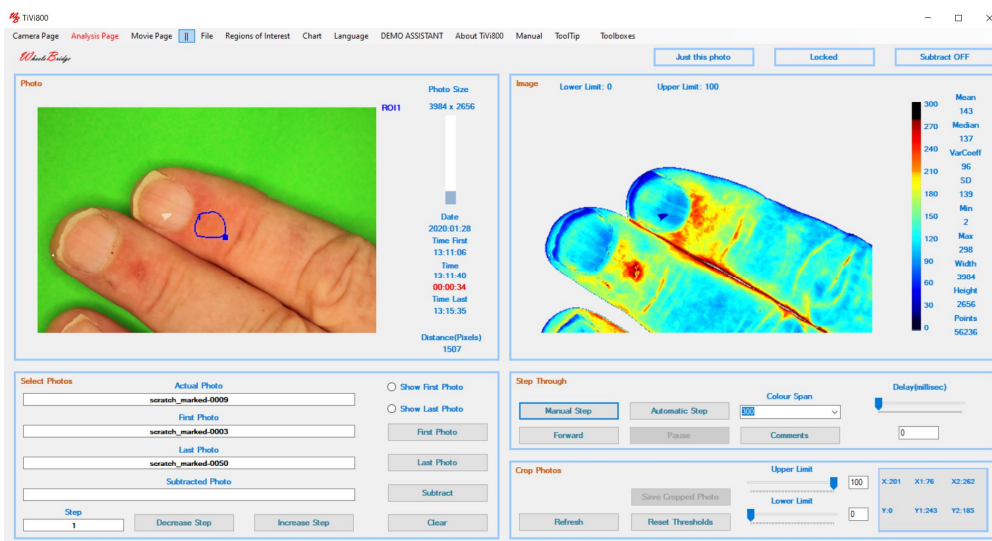
11. Click the '*Automatic Step*' button to scan through all the photos and verify that the 'ROI' is in the correct place in all photos.

12. Click *'Chart'* in the pull-down menu to display the chart.
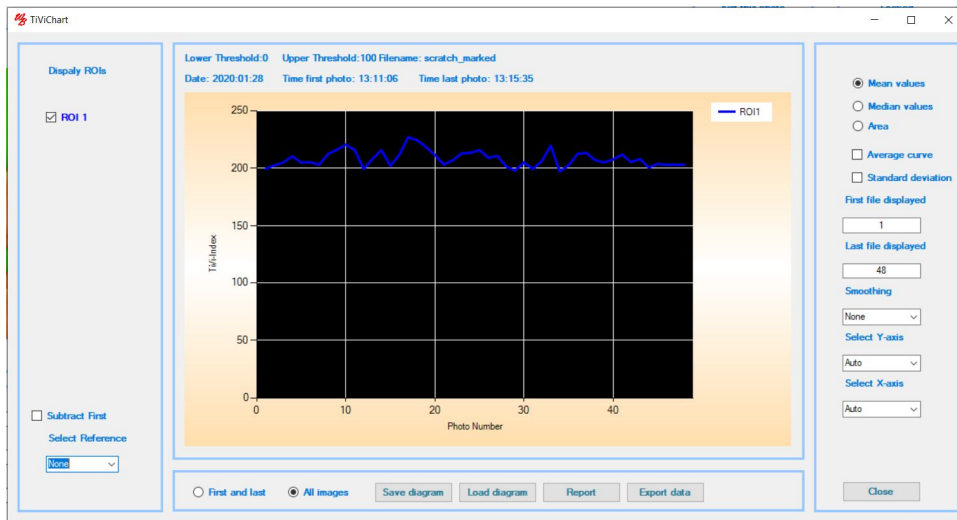
13. Close the *'Chart'* window and select *'Add White Marker'* from the *'Regions of Interest'* pulldown menu. Click the '*First Photo'* button to open the '*Open File Dialog'* window. Navigate to the *Moving Object* folder and double-click the *'movingHand-001'* photo file that does not include any white marker.
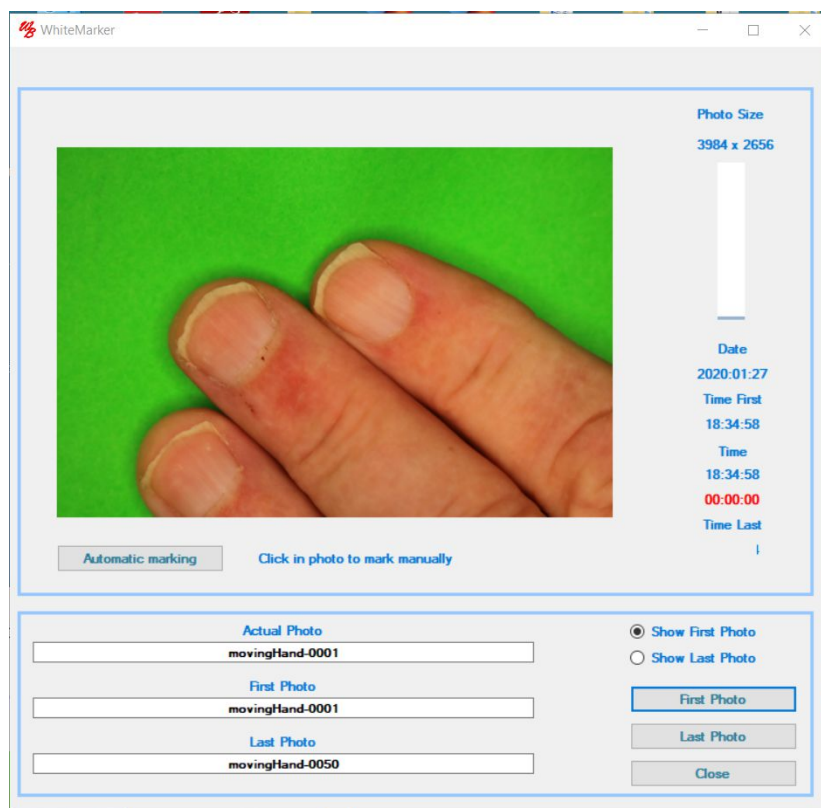
14. To place a white marker at the base of the nail of the finger, click at this point with the mouse pointer. At a click sound the bright white marker point is placed and displayed. The system then displays the next photo file in the sequence. Repeat the procedure with all photos until the 'Done' window is displayed. All photo files are now manually marked and displayed in the *'movingHand_marked'* folder.
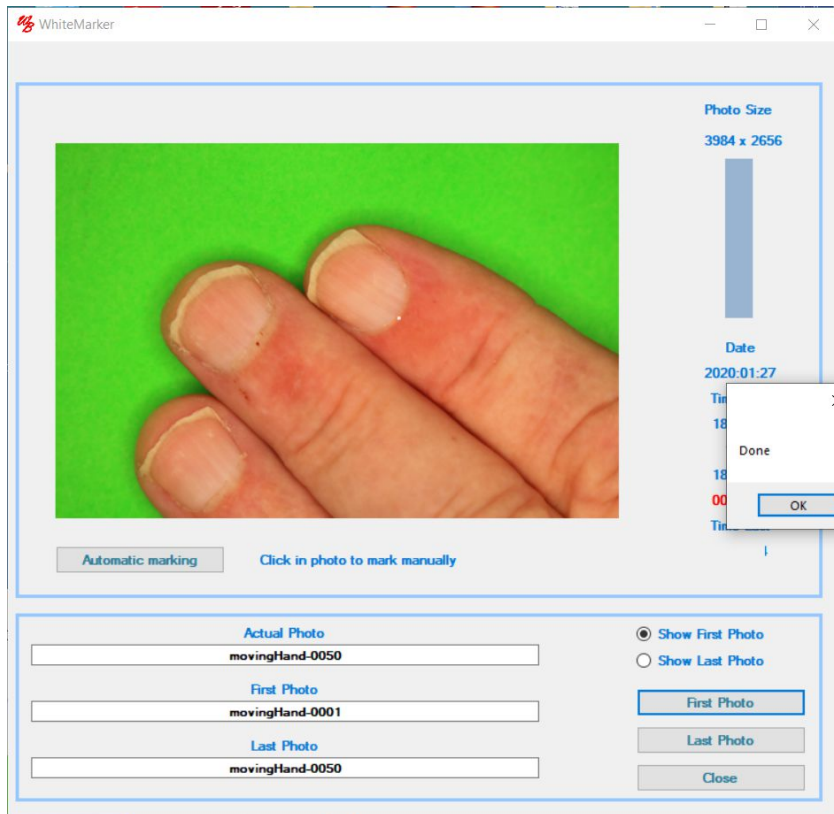
15. In the *'Analysis Page'* in the *'Main'* window, click the *'First Photo'* button, navigate to the *'movingHandMarked'* folder and double click the '*movingHand_marked-0001'* photo file. Click the '*Show First Photo'* radio-button to display the first photo in the sequence.
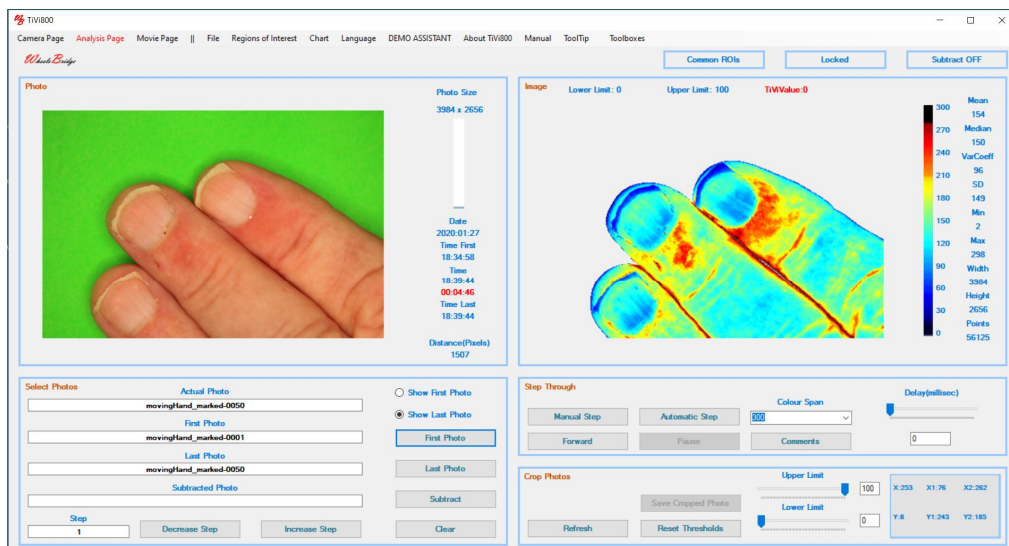
16. Select *'Elliptical ROIs'* from the *'Regions of Interest'* pulldown menu and draw two 'ROIs' in the photo.

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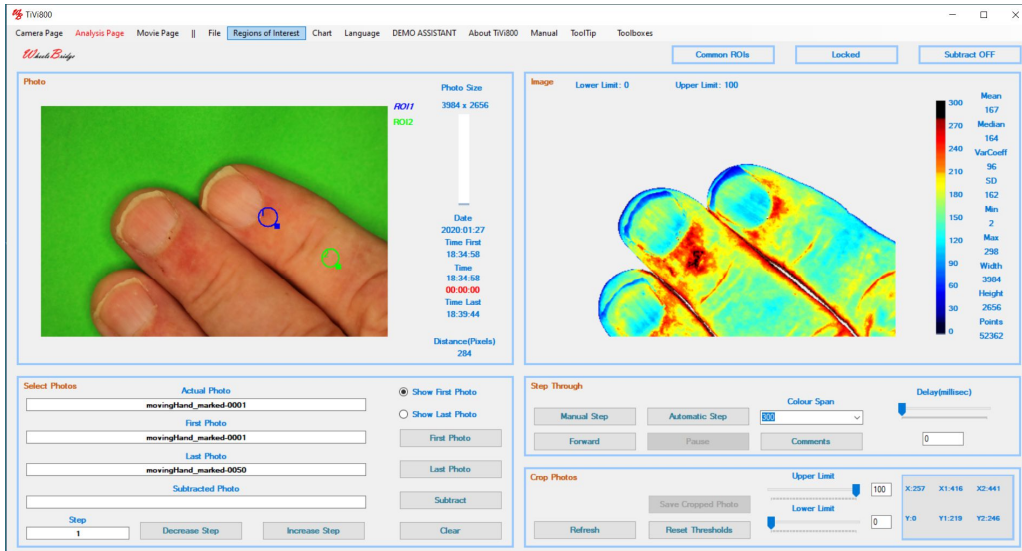
17. Select '*Lock to white spot'* in the '*Regions of Interest'* pulldown menu.

18. Click '*Chart'* in the pulldown menu.
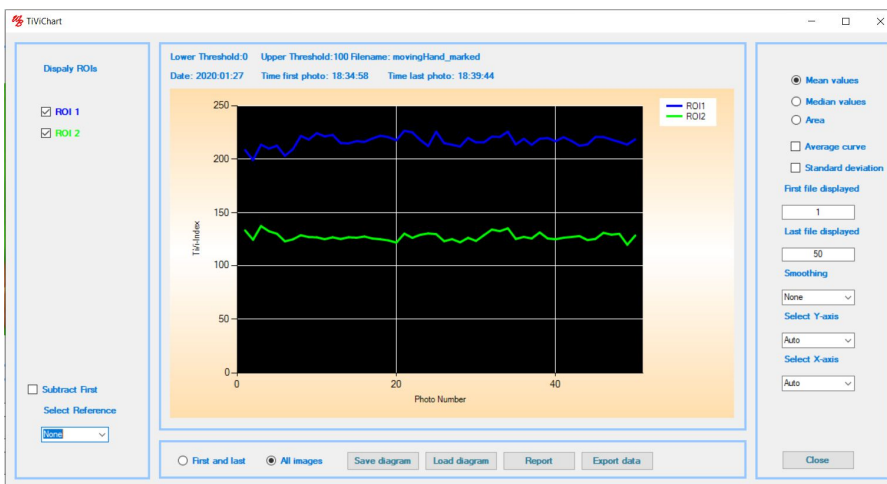
19. Click the '*Close'* button in the '*Chart'* window.

20. This completes the '*Analysing skin microcirculation in a moving object'* session.

Iontophoresis with an active and a reference electrode.

This session describes how to analyse a sequence of photos representing and iontophoresis experiment with one well with acetylcholine and one well with saline alone. 103 photos were captured with the delay set to 8 seconds and the photo size to 4128 x 2716 pixels, resulting in a total recording time of about 12 minutes.

1. Navigate to the folder *'Program Files(x86): Wheelsbridge: TiVi700 2.0: TiVi700: TiVi700demonstration: Iontophoresis'* and double-click the photo file named *'Ionto-0001'*.
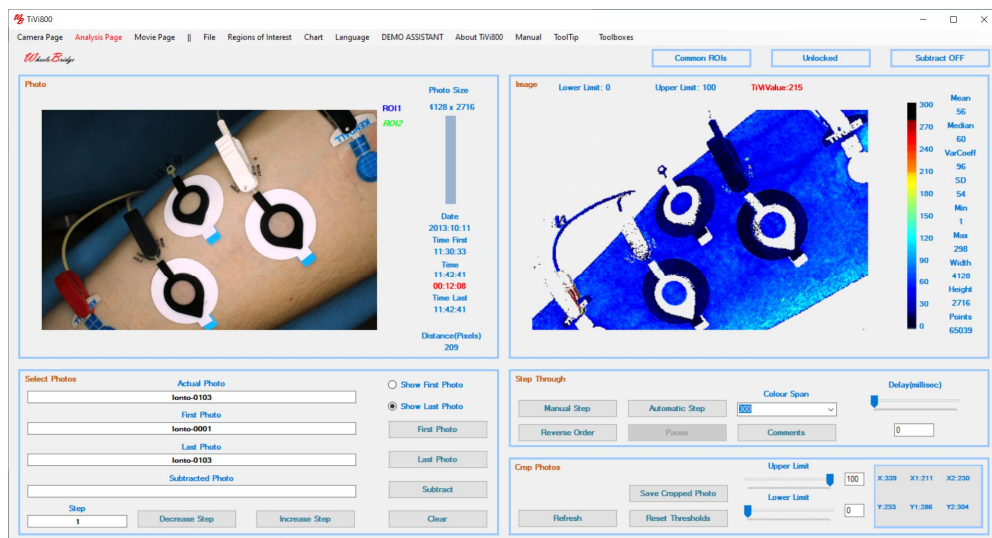
2. The lower (left) electrode is the active electrode with acetylcholine, while the upper (middle) well is the reference electrode.

3. Since the interesting part of the photo is the area of these two electrodes, start with drawing a rectangular 'ROI' including these two electrodes only.

4. Click the 'ROI1' text next to the photo to display the 'ROI' are alone.
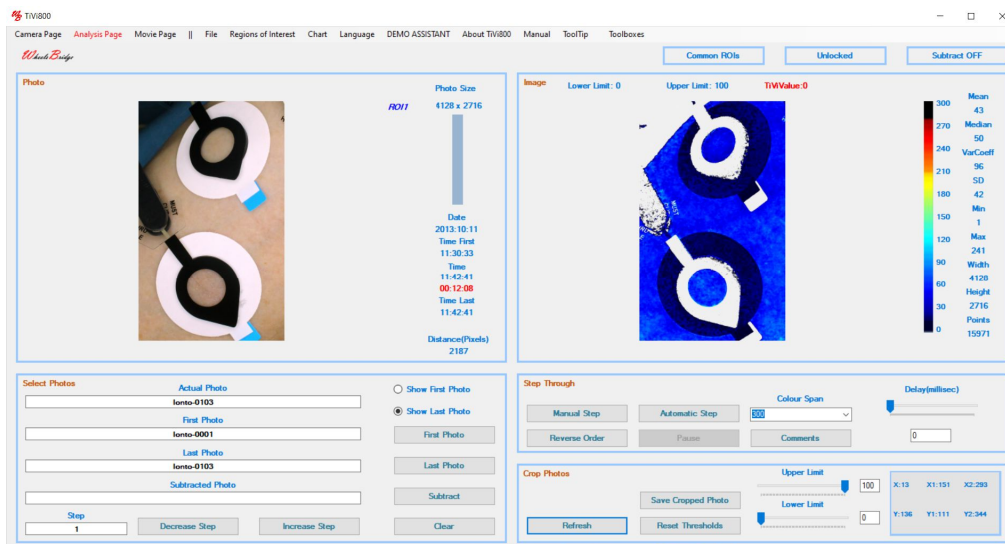
5. Click the '*Save Cropped Photo's* button to open the '*Open Save Dialog'* window.

6. Print *'cr_'* in the filename textbox and click the *'Save'* button.

7. Click the *'First Photo'* button, navigate to the *'cr_ionto'* folder and double click the cr_Ionto-0001 photo file.
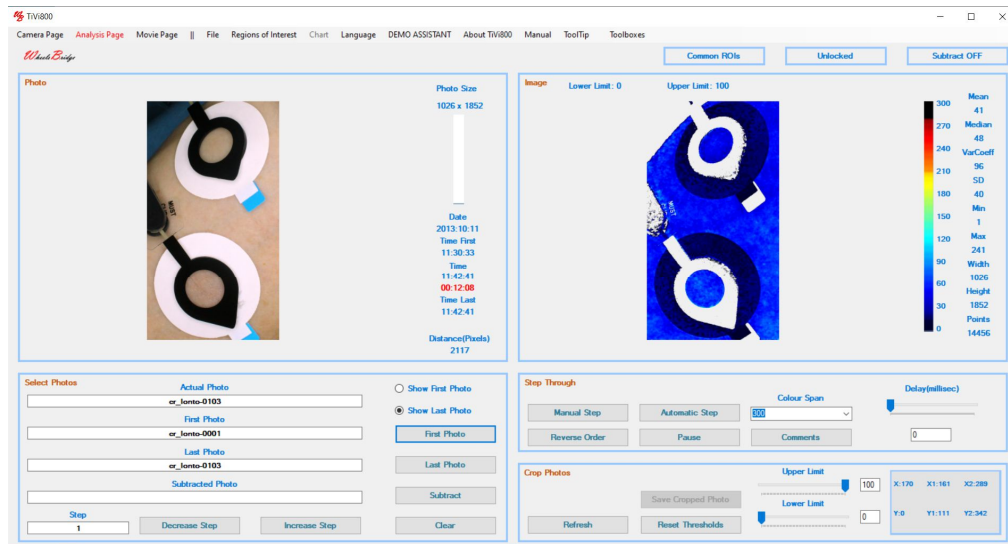
8. Observe that the cropped photos are now smaller and hence quicker to analyse. Set the '*Colour Span'* to 100 and click the *'Show First Photo'* radio-button.
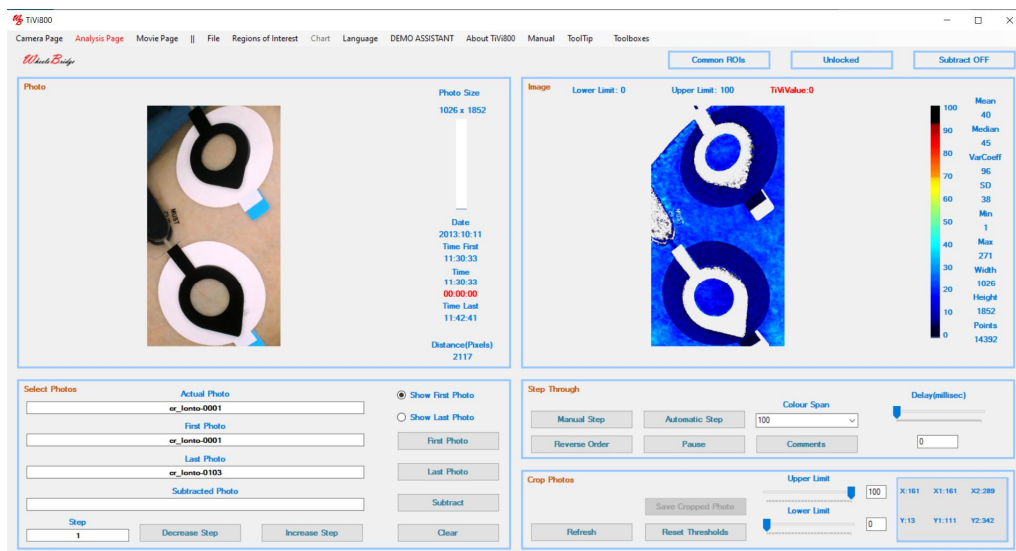
9. The colour of the image is now approximately the same in the two electrodes, indicating that the baseline TiVi-values are about the same inside the two electrodes. Click the *'Show Last Photo'* radio-button to jump to the last photo of the sequence.
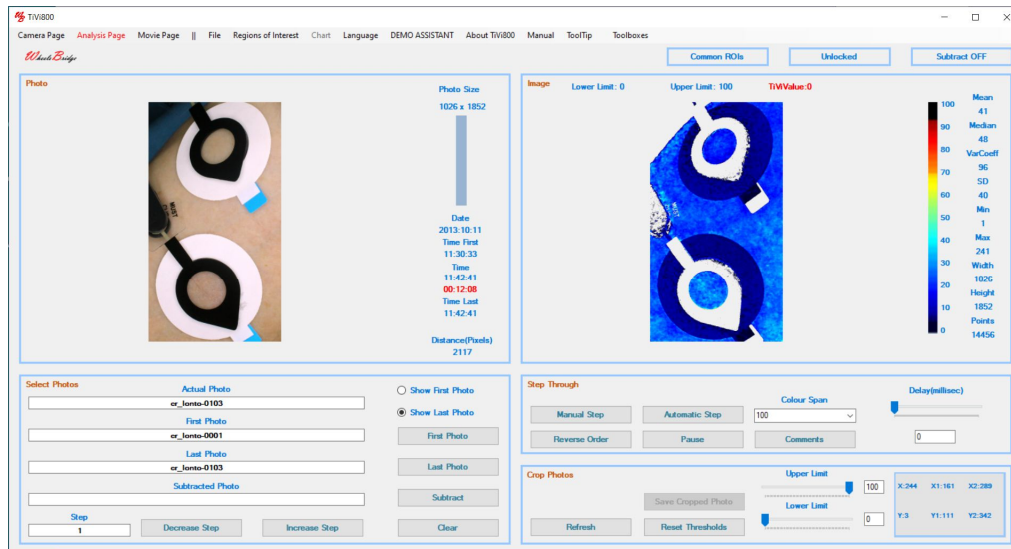
10. The colour of the image inside the lower electrode (with acetylcholine) is now indication a higher average TiVi-value as compared to the reference electrode (with saline alone).

11. Select *'Elliptical ROI'* under the 'Regions of Interest' pulldown menu. Place 'ROIs' inside the electrodes.
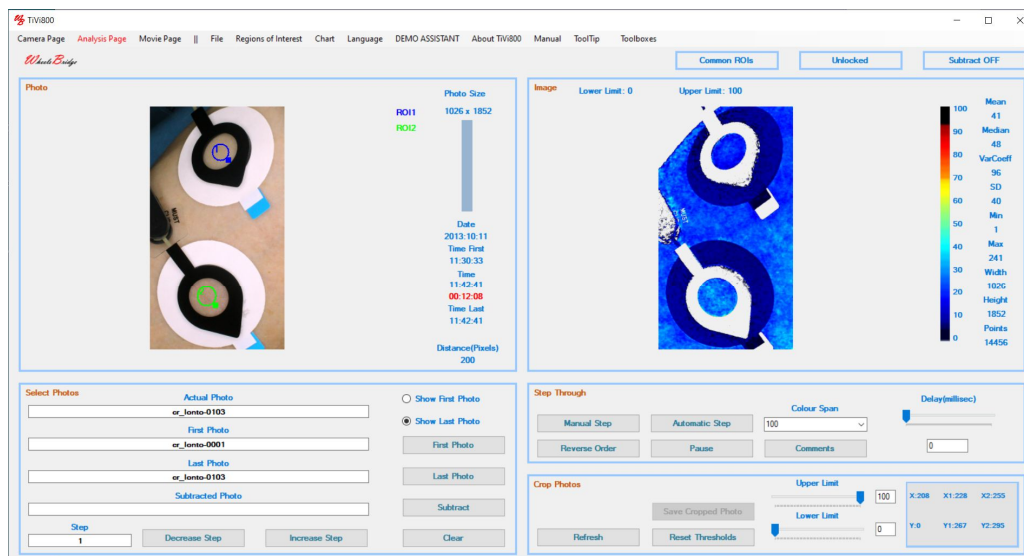
12. Click the 'Automatic Step' button to scan through the photos and images to verify that the 'ROIs' are positioned inside the electrodes in all photos. Click 'Chart' in the pulldown menu to generate the chart.
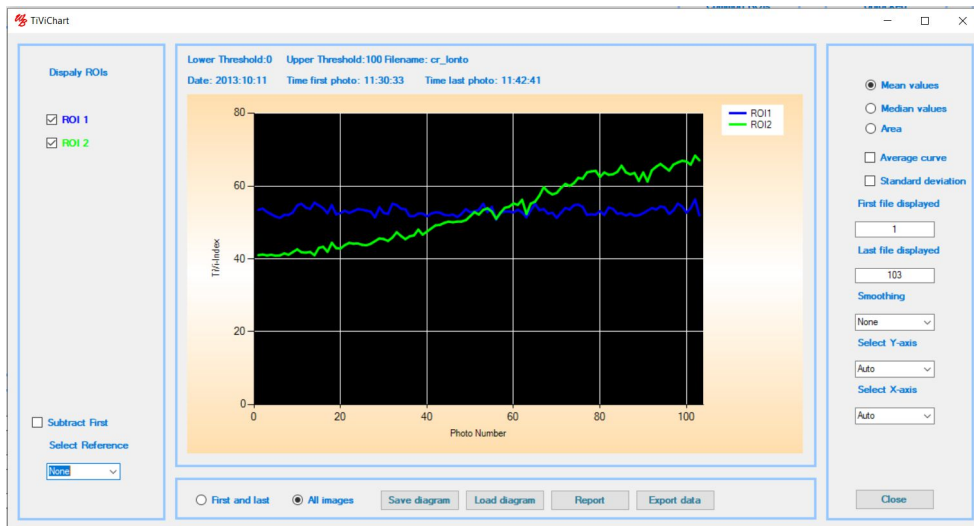
13. In the chart the blue curve represents the average TiVi-values inside the reference electrode, while the green curve represents the average TiVi-values inside the active electrode. Check the 'Subtract First' to subtract the baseline value from both curves.
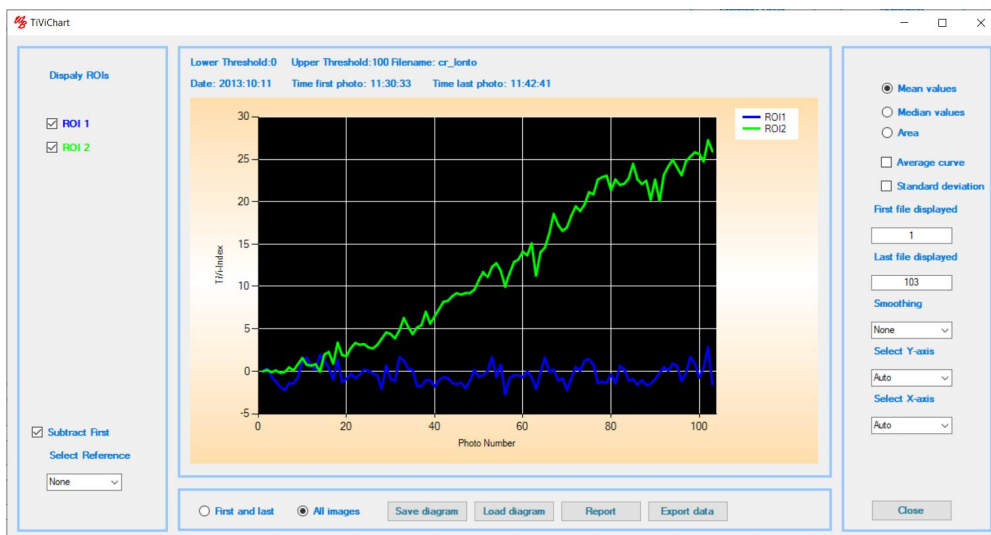
14. Set *'Select Reference'* to 'ROI1' to subtract the reference electrode TiVi-values from the TiVi-values of the active electrode curve. The resulting curve represents the increase in number of red blood cells caused by the iontophoresis of acetylcholine.
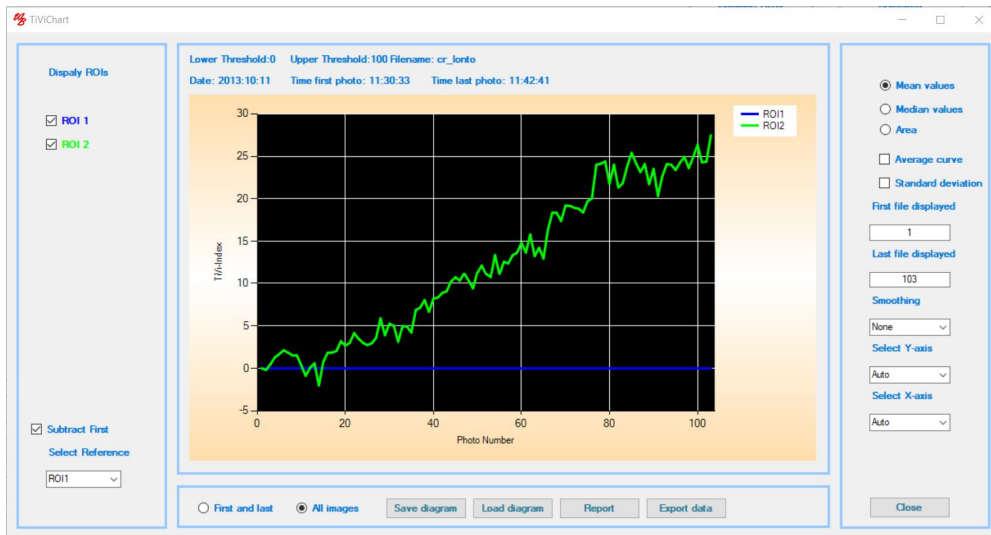
15. Set '*Smoothing'* to '*Major*' to smooth the curve.
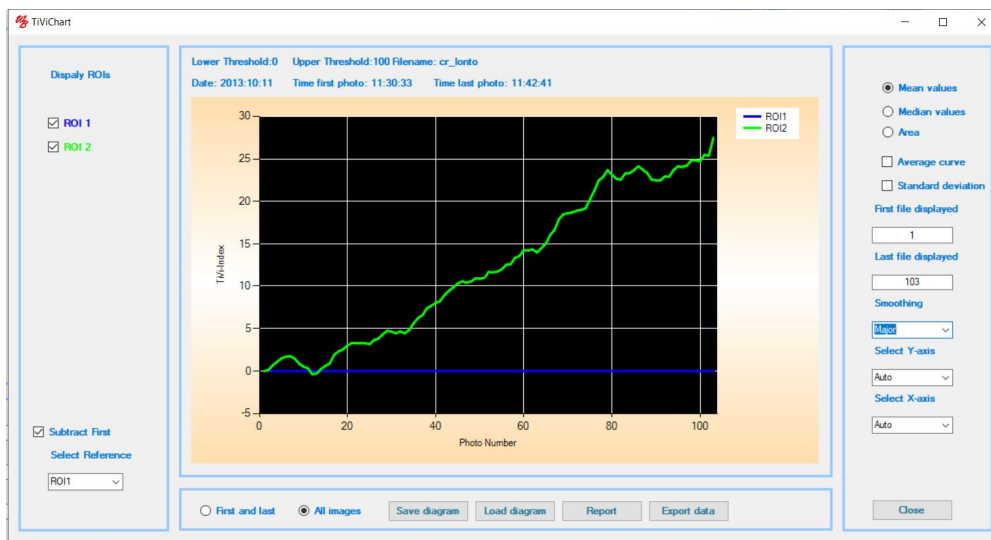
16. Click the 'Area' radio-button to display the areas of the TiVi-values above threshold inside the two *'ROIs'*.
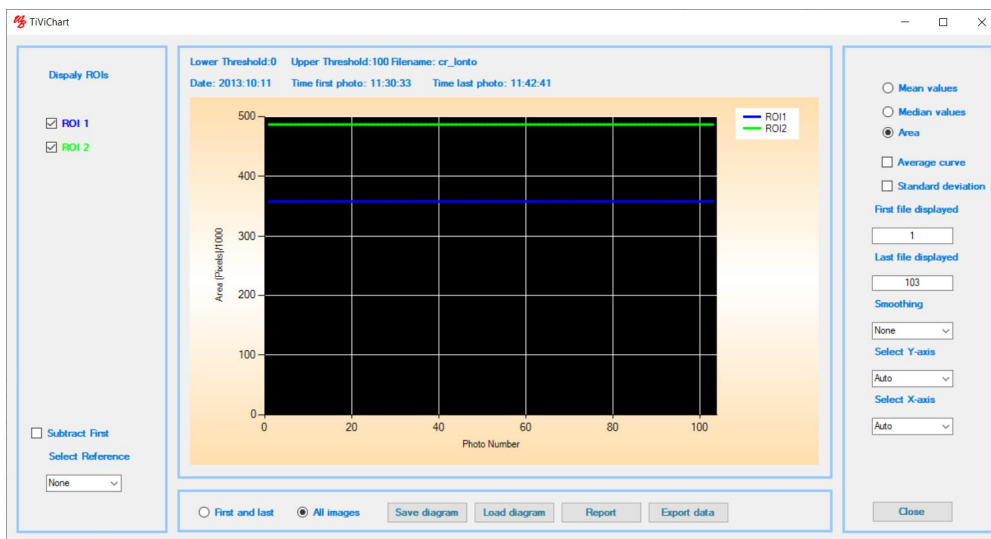
17. The two curves are flat because the areas are determined by the size of the 'ROIs' (in pixels). To investigate the extension of the erythema areas inside the electrode wells proceed as follows.

18. In the *'Main'* window, set the *'Lower Limit'* threshold to 25 and click the *'Show First Photo'* radio-button.
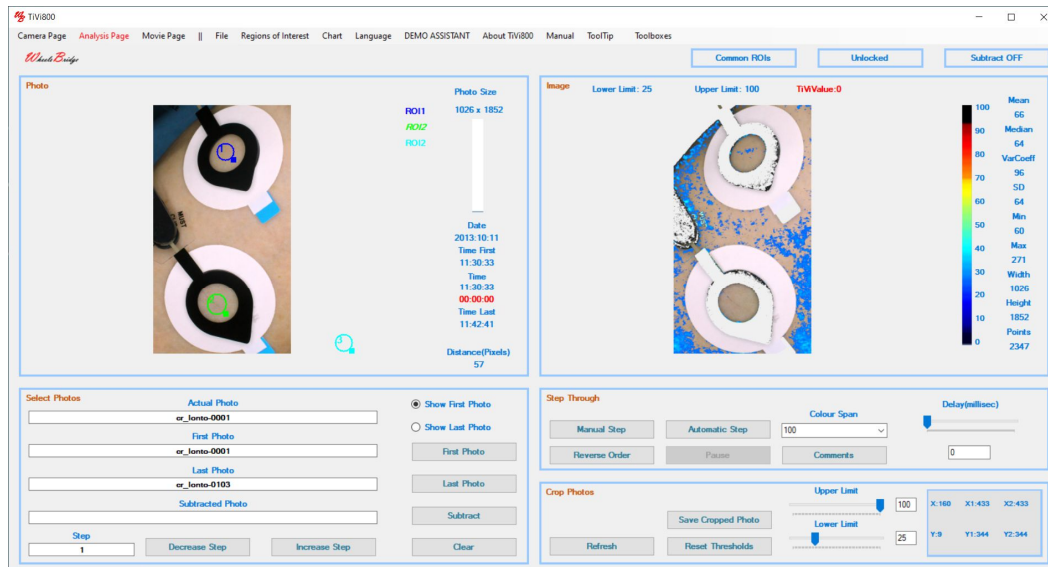
19. The TiVi image shows that there are only few pixels inside the electrode wells that have TiVi-values above the set *'Lower Limit'* threshold. Click the *'Show Last Photo'* radio-button.
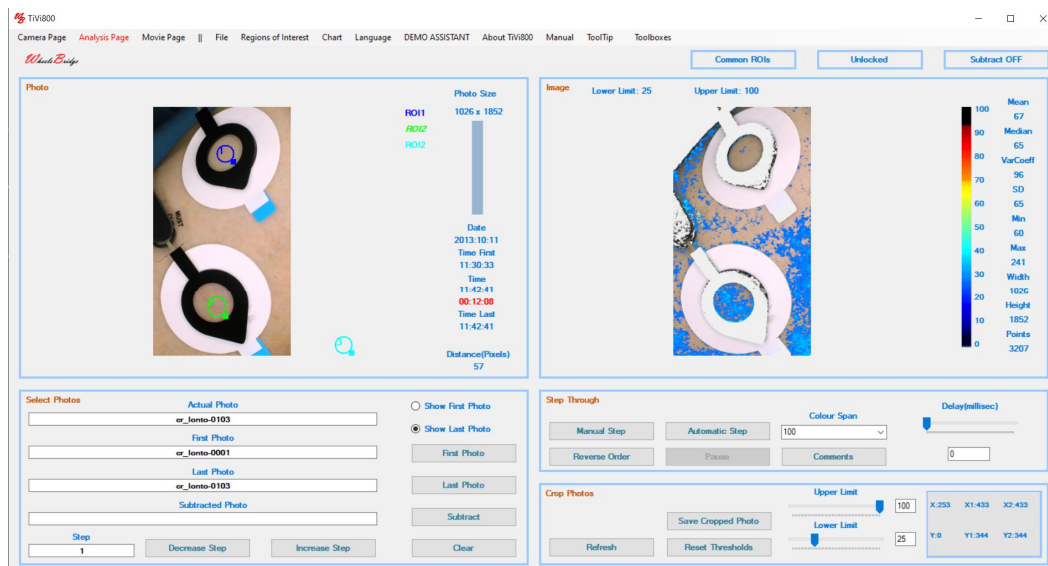
20. The last image in the sequence now displays a substantial number of pixels with TiVi-values above set threshold, while almost all TiVi-values within the reference electrode are still below threshold.

21. Click *'Chart'* to generate a new chart based on the new settings of threshold. In the chart click the 'Area' radio-button to display the number of pixels with TiVi-values above set 'Lower Limit' threshold.
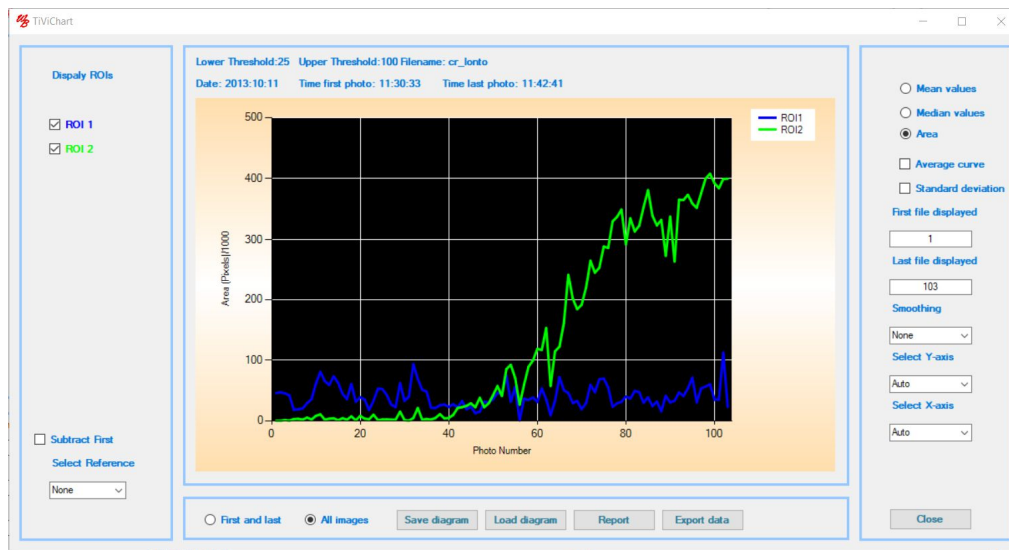
22. Select 'Major' in the 'Smoothing' combo-box.
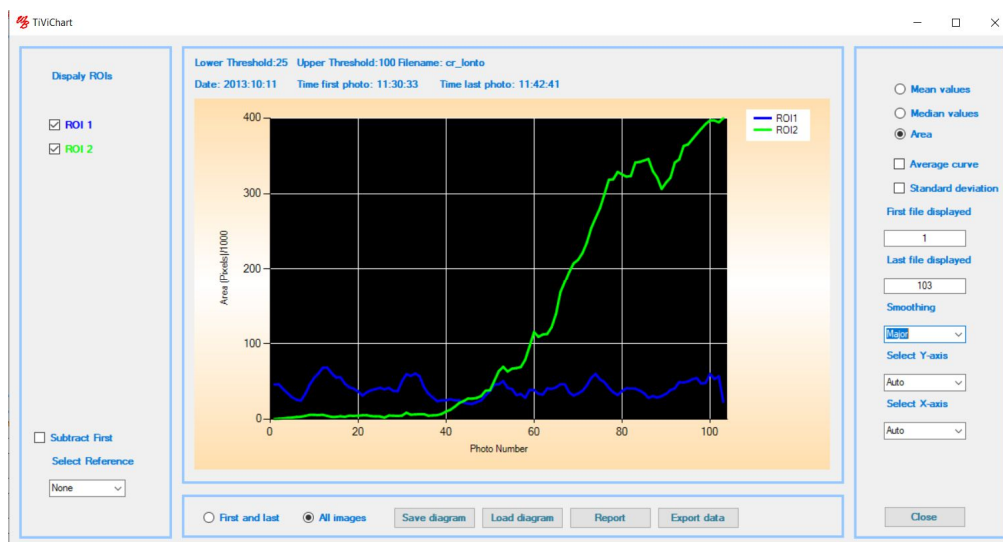
23. This completes the '*Iontophoresis of acetylcholine with active and reference electrode'* session.

Click the link below to play the video: How to analyze an iontophoresis experiment using TiVi.

http://media.wheelsbridge.se.loopiadns.com/2020/08/iontophoresis.mp4

Influence of pigmentation on calculated TiVi-values.

This session describes how different chromophores such as melanin (pigments) in addition to the haemoglobin molecules in the red blood cells influence the TiVi-values calculated.

A skin area with brown spots was exposed to topically applied methyl nicotinate. By investigating the erythema caused by the vaso-active substance in the brown spots and in normal skin in between the brown spots, the effect of melanin chromophores can be assessed.

1. Navigate to the folder *'Program Files(x86): Wheelsbridge: TiVi700 2.0: TiVi700: TiVi700demonstration: BrownSpots'* and double-click the photo file named *'MNA-0010'*. Click the *'Show First Photo'* radio-button.
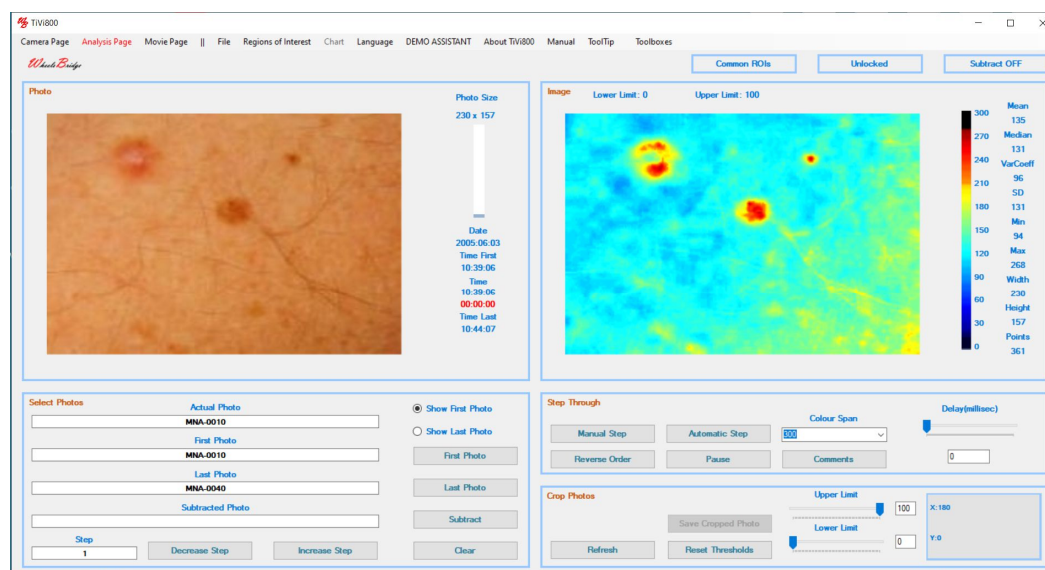
2. Click the '*Show Last Photo'* radio-button to display the last photo and image in the sequence.
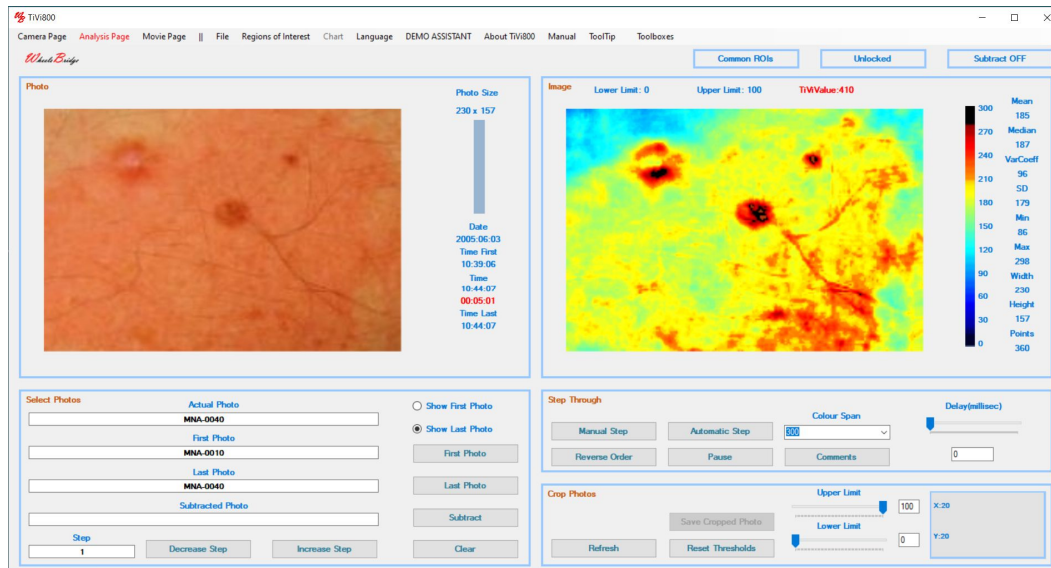
3. In the last photo the reddening of the skin is seen in the areas where the methyl nicotinate is applied. In the upper left corner is a skin area where no methyl nicotinate is applied which can be used as a normal reference area.

4. Draw three rectangular 'ROIs'; in the reference area, in normal skin (with methyl nicotinate) and in a brown spot (with methyl nicotinate).

5. Select 'Name ROIs' in the 'Regions of Interest' pulldown menu to open the '*ROI Naming'* window. Name '*ROI1*' as '*ref*', 'ROI2' as '*norm*', and 'ROI3' as '*brown*'.
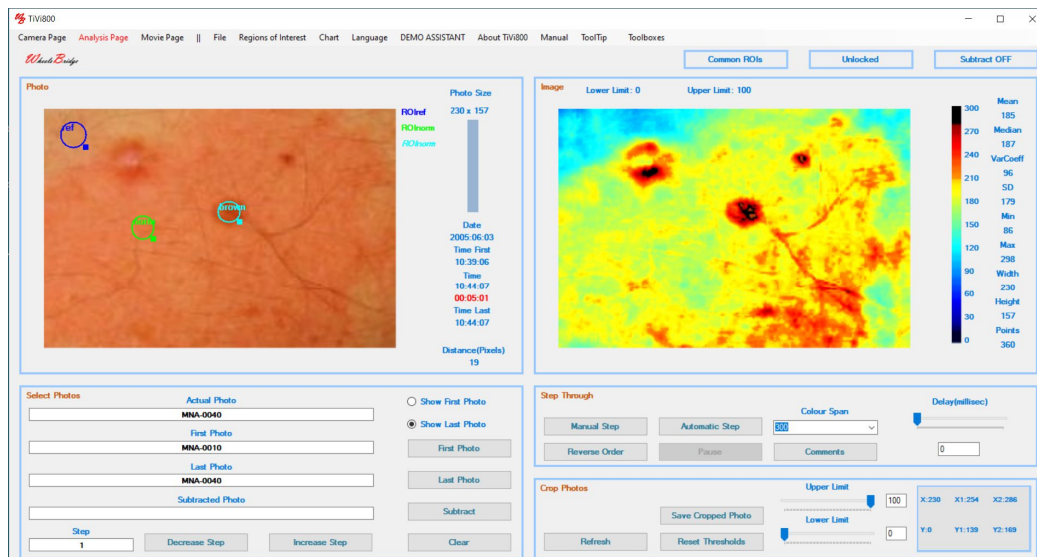
6. Click 'Chart' to display the chart.
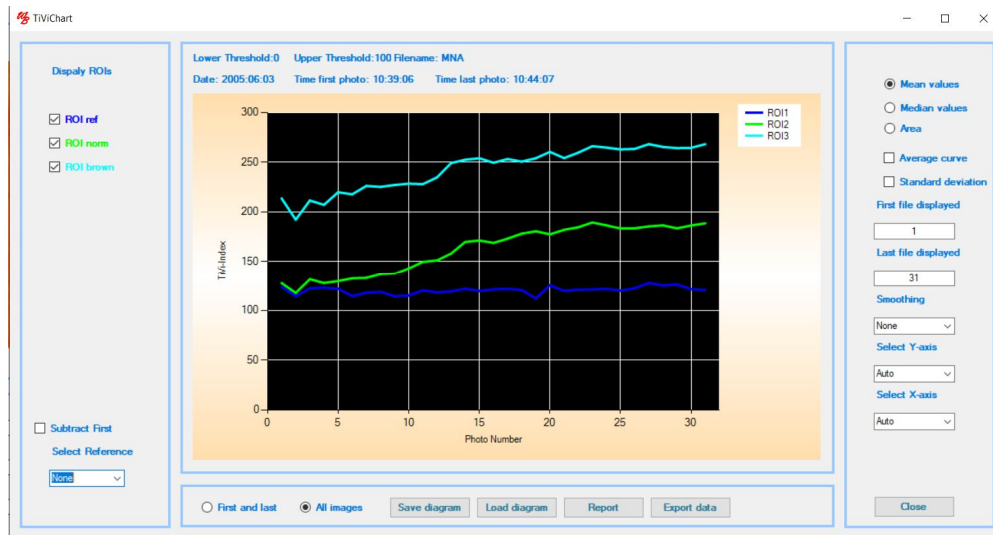
7. The upper turquoise curves represent the brown spot, the middle green curve the normal skin curve and the lower blue curve the reference area curve.

8. In the '*Select Reference'* combo-box select 'ROI1' (blue curve).

9. Check the '*Subtract First*' checkbox to subtract the baseline and the select '*Major*' in the '*Smoothing*' combo-box.
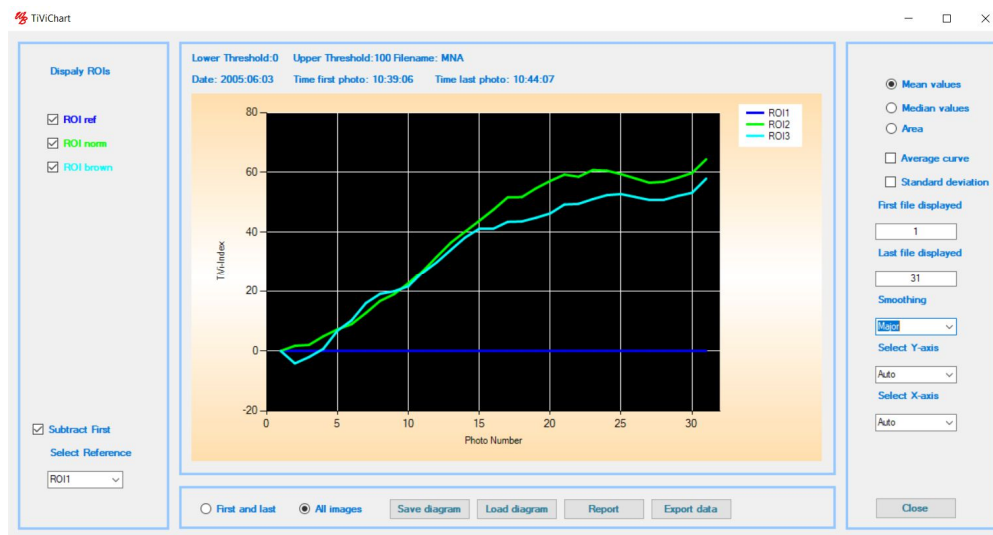
10. The interpretation of this chart is that the change in red blood cell concentration is about the same in the normal skin and in the brown spot. The baseline value is, however, higher in the brown spot than in normal skin because melanin is an absorber of green light, resulting in a higher recorded TiVi-value. Since the level of melanin in the skin does not change over a short period of time, the absolute TiVi-values will not influence the calculated changes in TiVi-values. Over a longer period of time – from summer to winter – the degree of pigmentation may, however, influence the result.

11. This completes the *'Influence of pigmentation on calculated TiVi-values'* session.

Analysing and comparing TiVi-images of photos captured at different points in time.

This session describes how to analyse and compare photos captured at different points in time, which is often the case when a skin care product is applied daily for several months by the test subjects. Prior to application of the product, baseline photos are captured and at the end of the test period end-point photos are captured. Since it is difficult to place the object exactly in the same place in the photo, region of interest (ROI) analysis must allow for movement of the ROIs to make the selected skin sites comparable.

1. Navigate to the folder *'Program Files(x86): Wheelsbridge: TiVi700 2.0: TiVi700: TiVi700demonstration: Skin Care'* and double-click the photo file named *'Sub-0001'*. Click the *Show First photo* radio-button. This photo and TiVi-image represent the baseline photo and image.
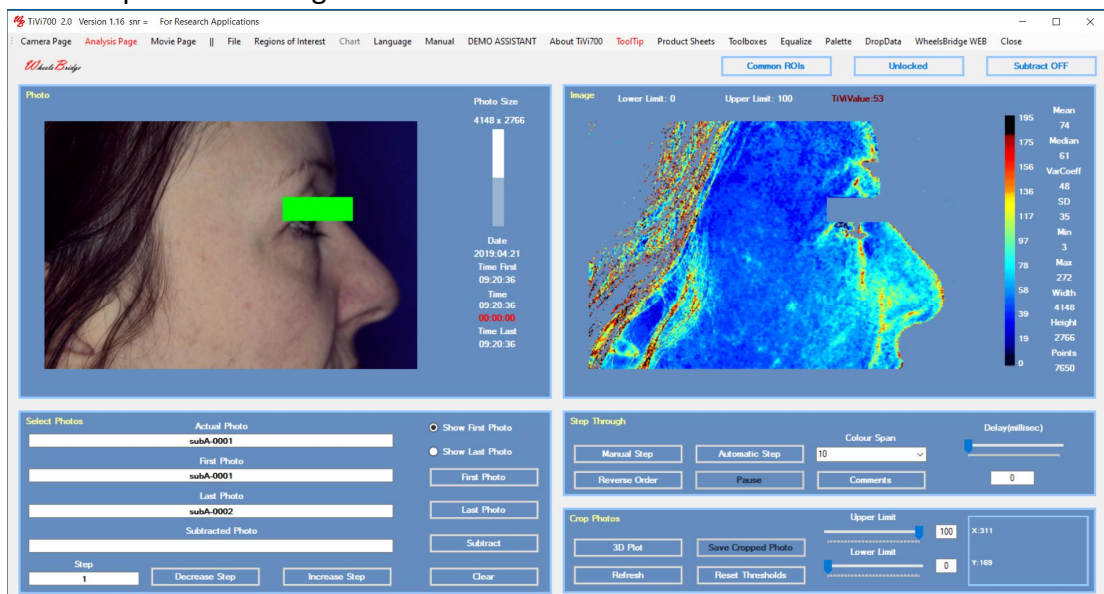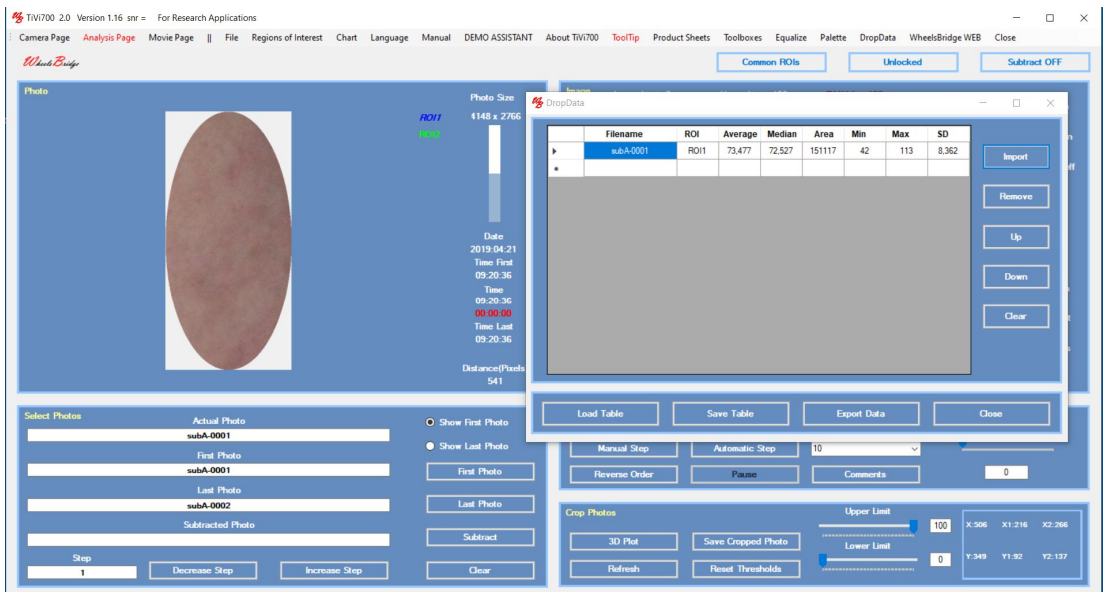

2. Click the *Show Last Photo* radio-button.
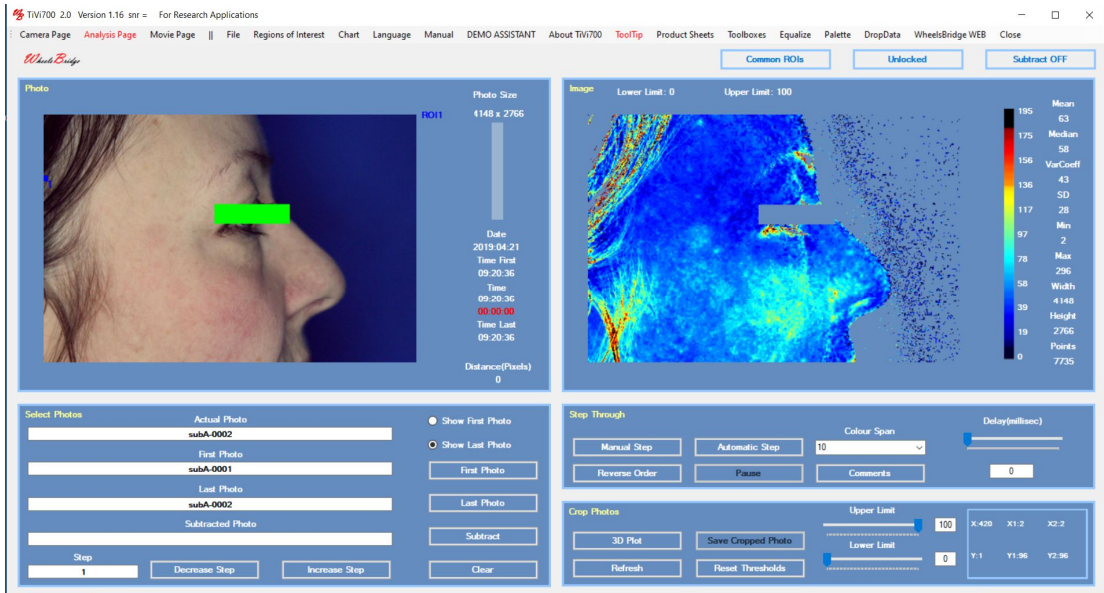
3. Since the object is not in the same place in the two photos the application of ROIs requires that the ROIs in the last photo are moved to cover the same anatomical skin area. Click the *Show First Photo* radio-button and draw two ROIs.
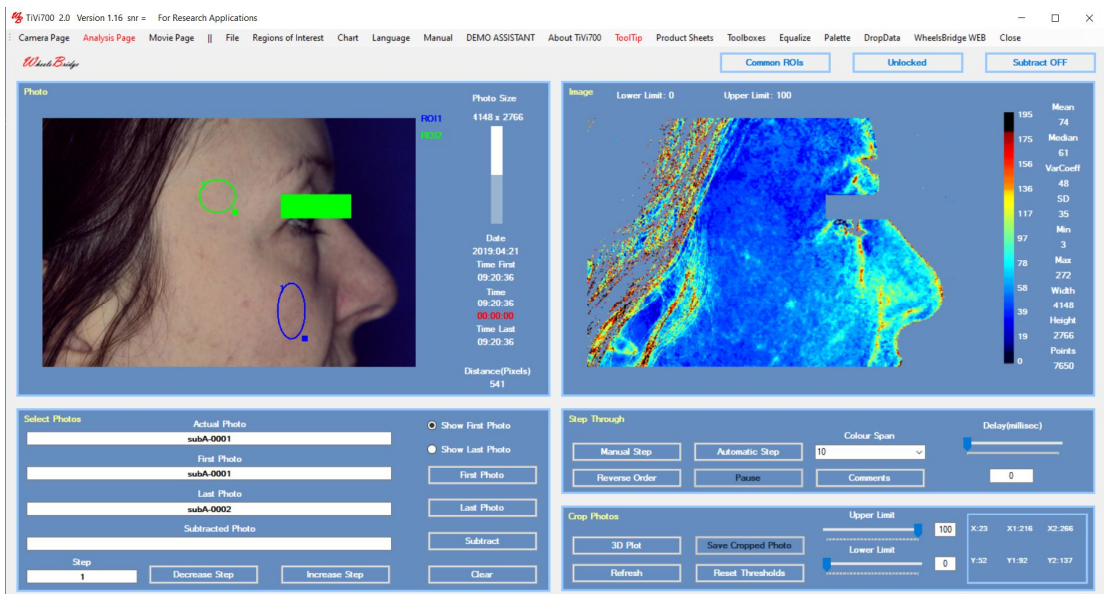

4. Select *File → Save ROI* and save the ROIs under the name "ROI1". Select *DropData* from the pulldown menu to open the *DropData* window.
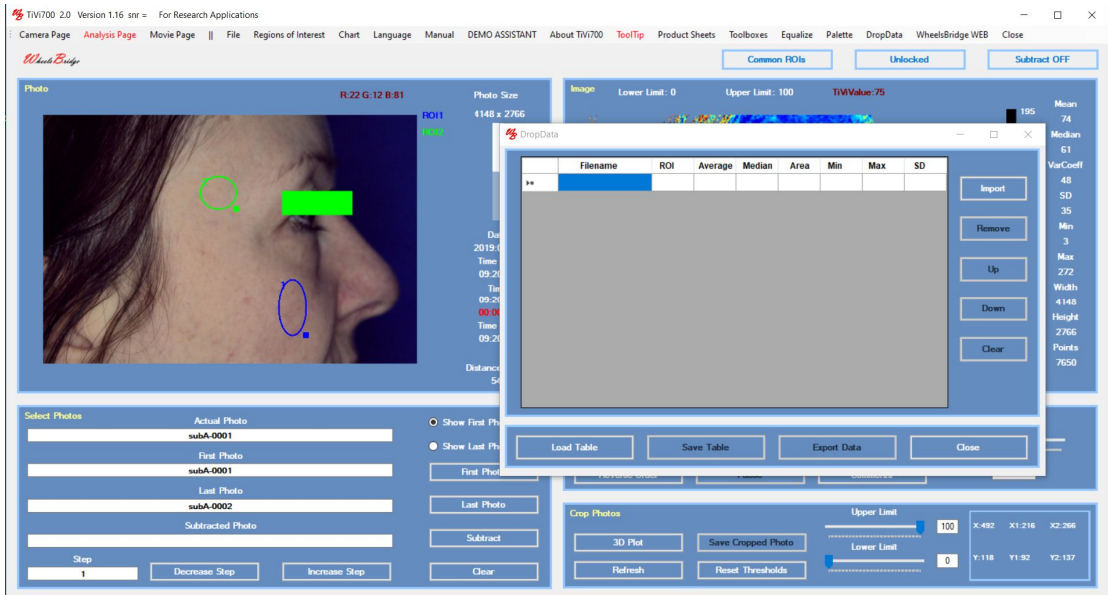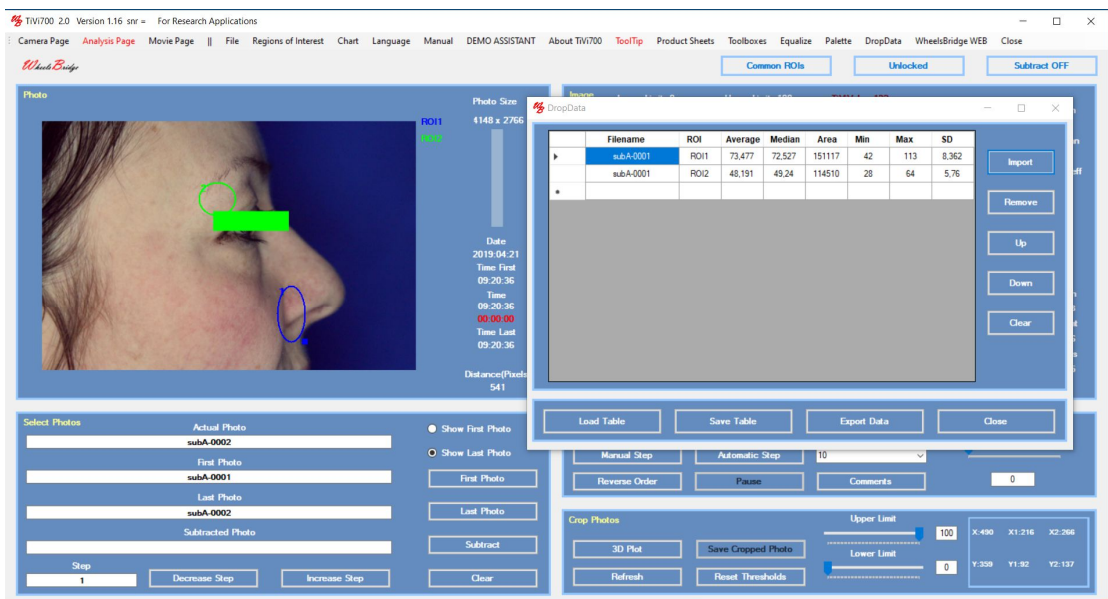
5. Click the ROI1 text to display ROI1 in zoomed in mode. Click the *Import* button in the *DropData* window to import the ROI1 values.
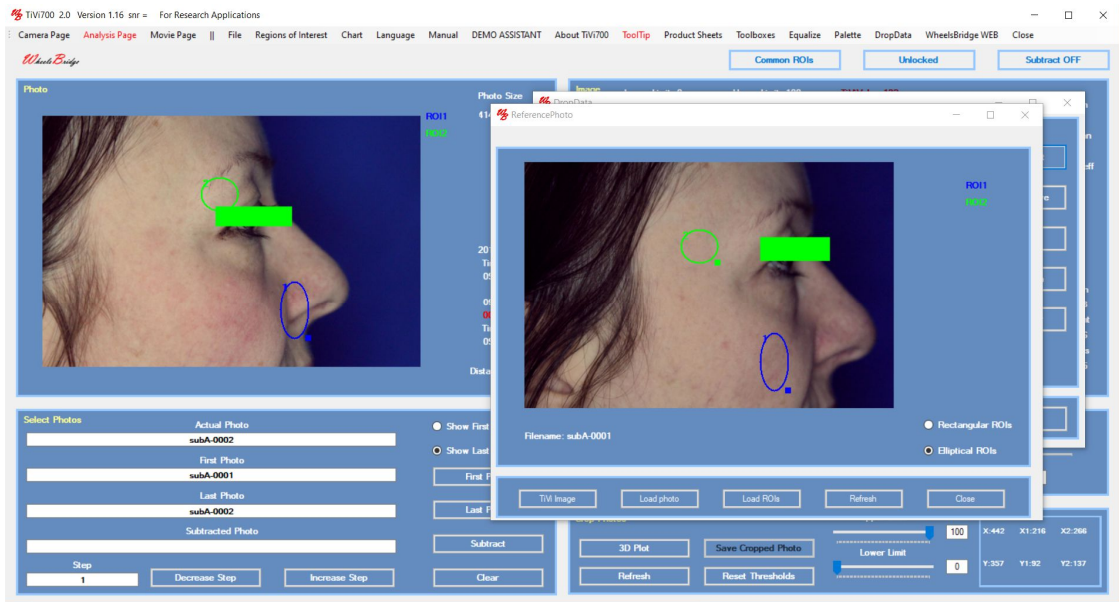

6. Click the ROI1 text again and then click the ROI2 text. Click the *Import* button in the *DropData* window to import the ROI2 values to the table.
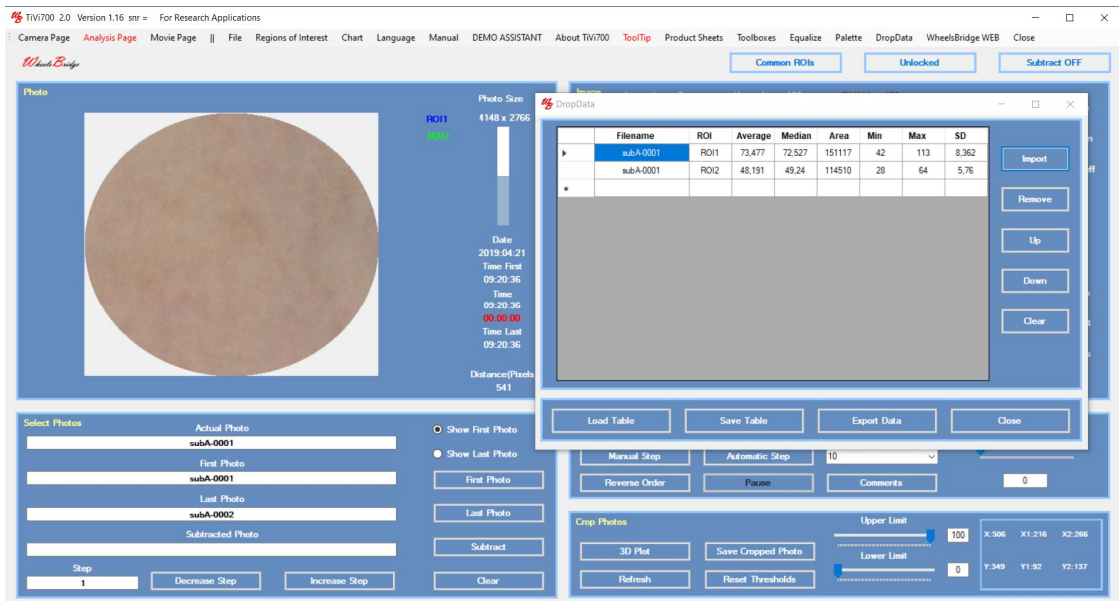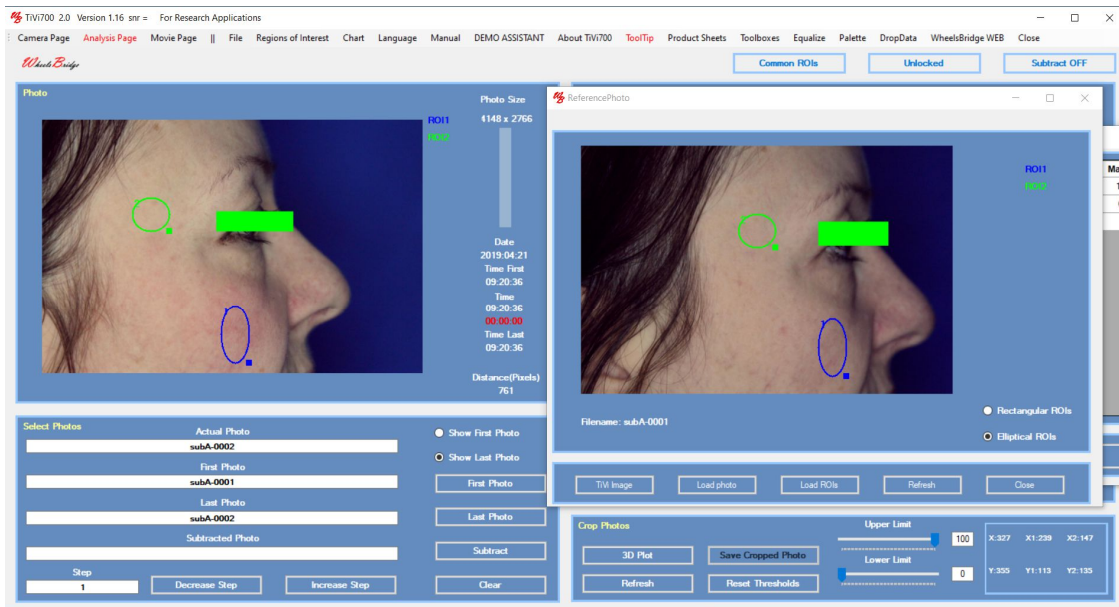
7. Click the ROI2 text to display the entire photo and then click the Show Last Photo radio-button to display the end-point photo.


8. Select File→ Reference photo to open the Reference Photo window. Click the *Load Photo* button and load the subA-0001.jpg photo. Then click the *Load ROIs* button and load the *ROI1_subA* file. Finally check the *Elliptic ROIs* radio-button.

9. In the main window move the two ROIs to the same anatomical positions on the skin as in the photo in *the Reference* Photo window.


10. Click the ROI1 text to display the ROI1 area only. Click the *Import* button in the *DropData* window to import the ROI1 values of photo subA-002. Repeat the procedure for ROI2.
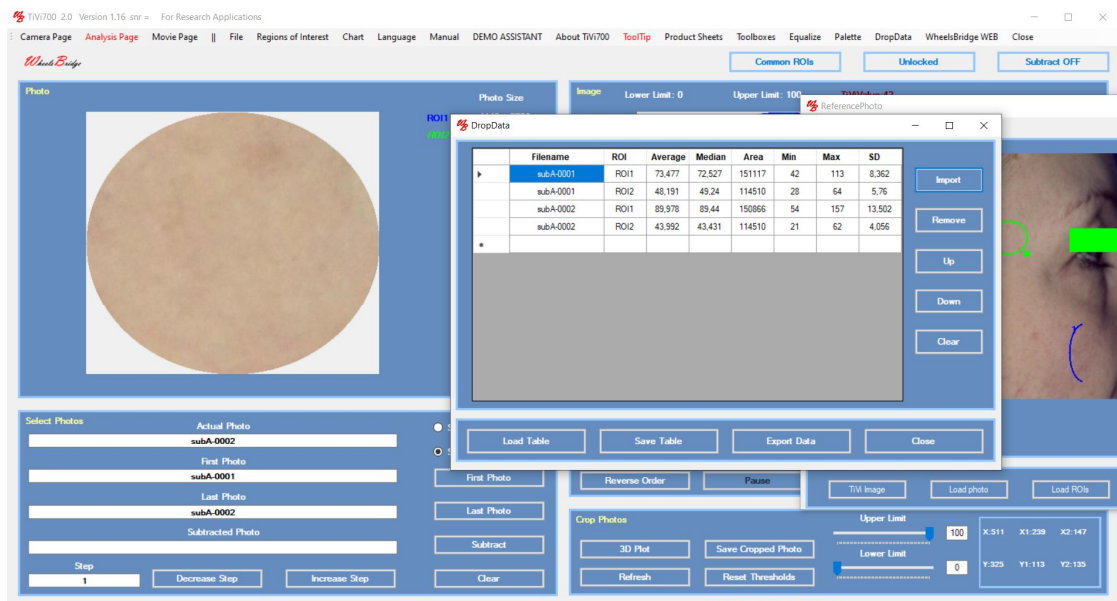
11. The *DropData* table now holds data for the two ROIs from the baseline and end-point photos.

12. Select the third raw in the *DropData* window and click the *Up* button once to move the line one step upwards.
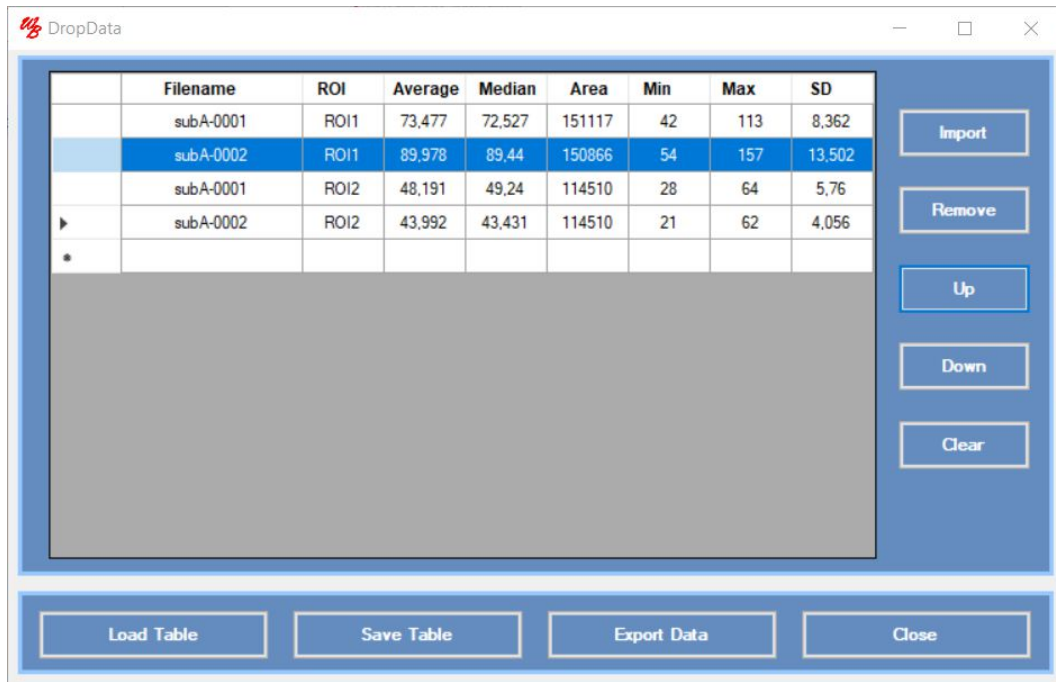
13. Click the *Save Table* button to save the table under a selected name.

14. Click the *Export Data* button to export the data to a spreadsheet.

	A	B	C	D	E	F	G	H	I	J	K	L
1	TiVi700 DropData			Date:2021-03-06 16:55:19								
2												
3												
4												
5				Filename	ROI	Average	Median	Area	Min	Max	SD	
6				subA-0001	ROI1	73,477	72,527	151117	42	113	8,362	
7				subA-0002	ROI1	89,978	89,44	150866	54	157	13,502	
8				subA-0001	ROI2	48,191	49,24	114510	28	64	5,76	
9				subA-0002	ROI2	43,992	43,431	114510	21	62	4,056	
10												
11												
12												
13												

15. More test subject data can be added to the table and the spreadsheet. Eventually baseline data and end-point data can be tested for potential difference in RBC concentration within the different ROIs. If a double-blind test is applied (recommended), the data in the spreadsheet can be rearranged after breaking the code and the hypothesis *"the substance tested does not result in any difference in RBC concentration"* can be tested and potentially rejected.

16. This completes the session *"Analysing and comparing TiVi-images and photos captured at different points in time"*.

Analysing and visualizing the pulse component of the RBC concentration.

This session describes how to use the *Pulse* window features to analyse and visualize the pulse component of the RBC concentration. Start with recording a HD video clip for 3 seconds with minimal relative movements between tissue and camera. Click the *HD Movie to Photos* button in the *Movie Page* to split up the HD video clip into individual photos and save these to file. Upload these photos (about 150 photos in total). Draw a ROI that includes the area of interest, click the *ROI1* text to display the zoomed in section and click the *Save Cropped Photo* button to save these cropped photos. Click the *First Photo* button and select the first of the cropped files to upload this sequence of cropped files. Select *Pulse* in the File pull-down menu. The system now scans through the photos and automatically finds the first minimum, the second minimum and the first maximum TiVi-value to identify a pulse cycle. If a pulse cycle is found the *Pulse* window is opened displaying the maximum pulse (as percentage of total RBC concentration) as a colour scaled image. To create a full video clip of a pulse cycle, click the *Create Video* button. The system now automatically subtracts the first minimum TiVi-value from the TiVi-value of each photo within the pulse cycle. Play this video repeatedly by clicking the *Start Video* button.

A pre-recorded HD Video clip (named *Pulse*) is found in the folder *'(x86: Wheelsbridge: TiVi70: TiVi700 2.0: TiVi700demonstration: Pulse').* This video clip is used for demonstration of the *Pulse* window features.

1. Start the TiVi700 program by clicking on the TiVi700 icon on the desktop. Select the *Movie Page*.
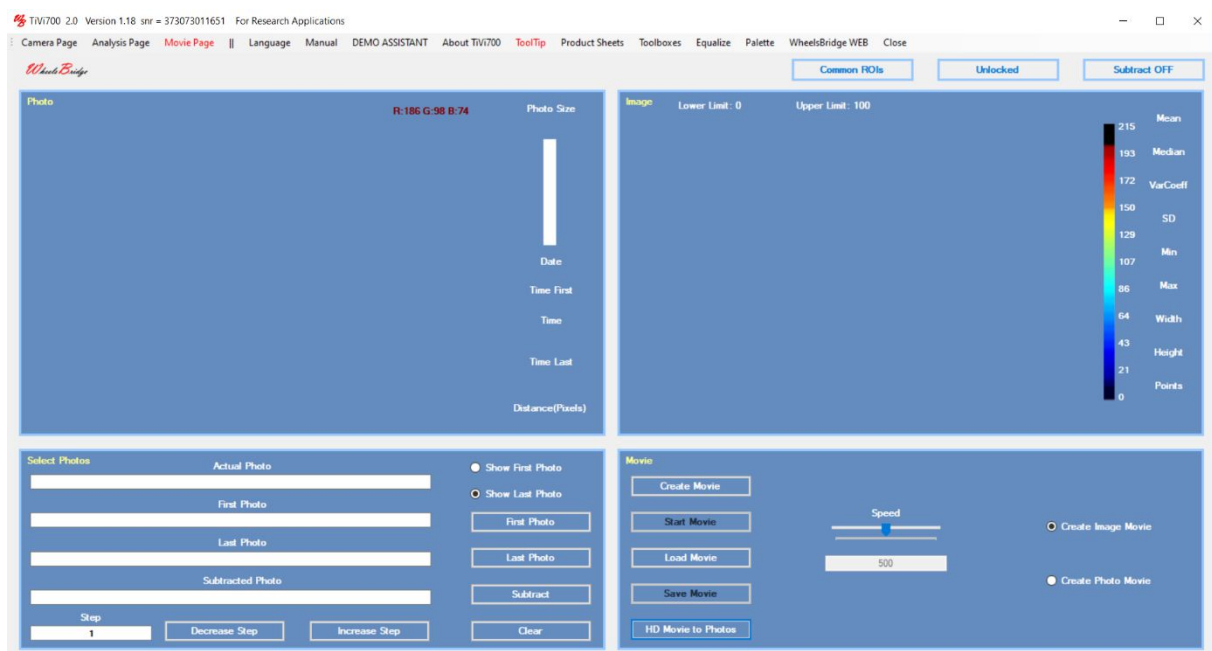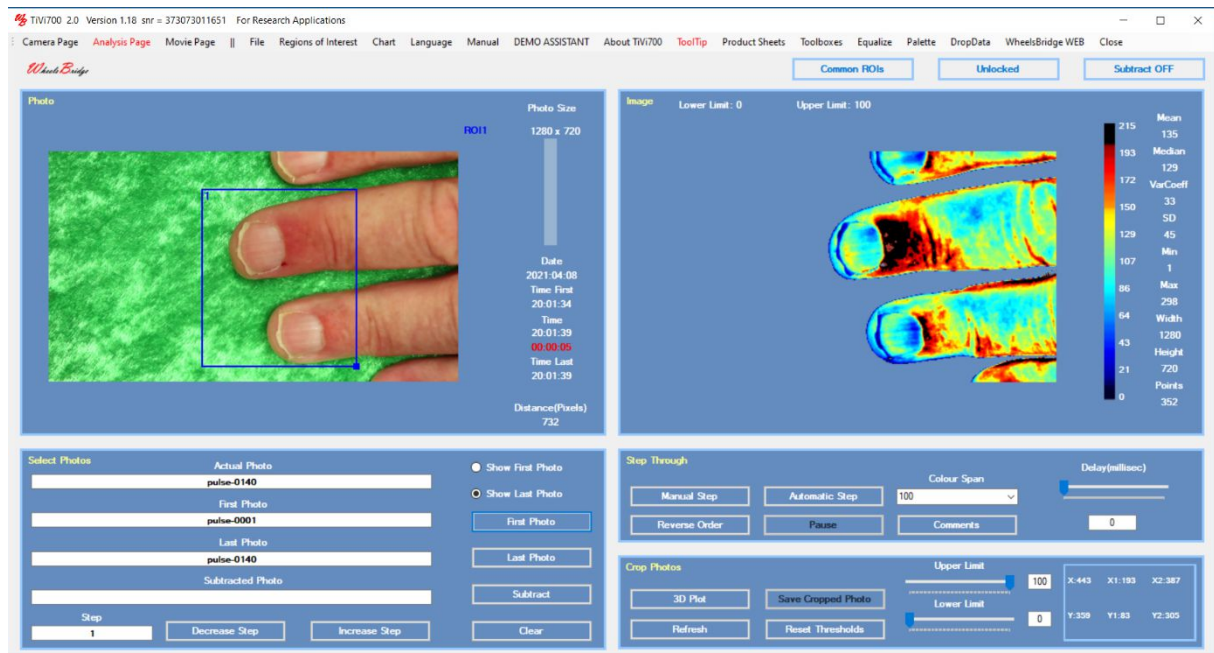

2. Click the *HD Movie to Photo* button and select the *Pulse* movie file located in the *x86: Wheelsbridge: TiVi70: TiVi700 2.0: TiVi700demonstration: Pulse'* folder.

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3. Click the *First Photo* button and select the *Pulse-0001* file to upload the photo files in the sequence.
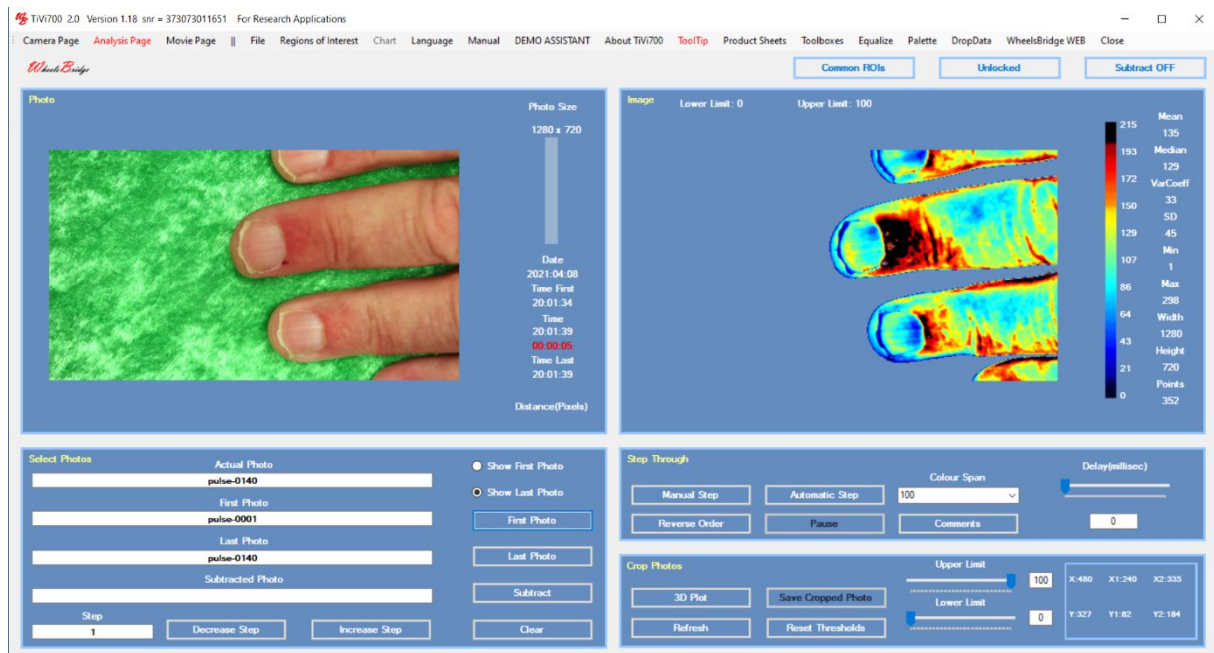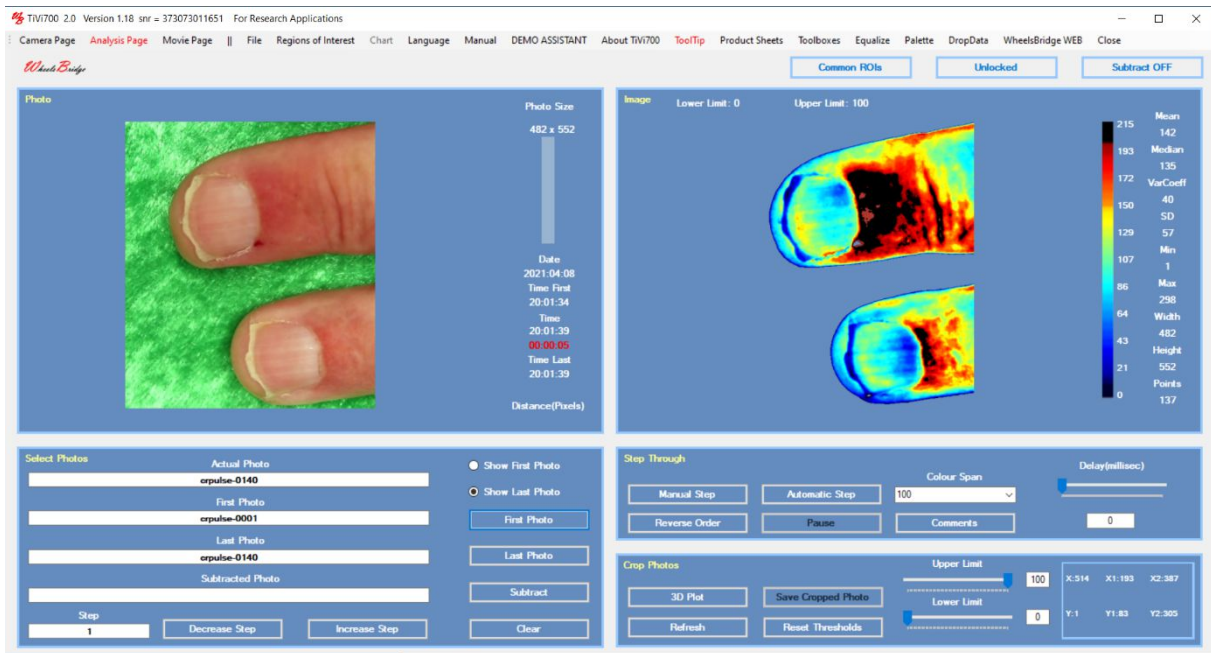

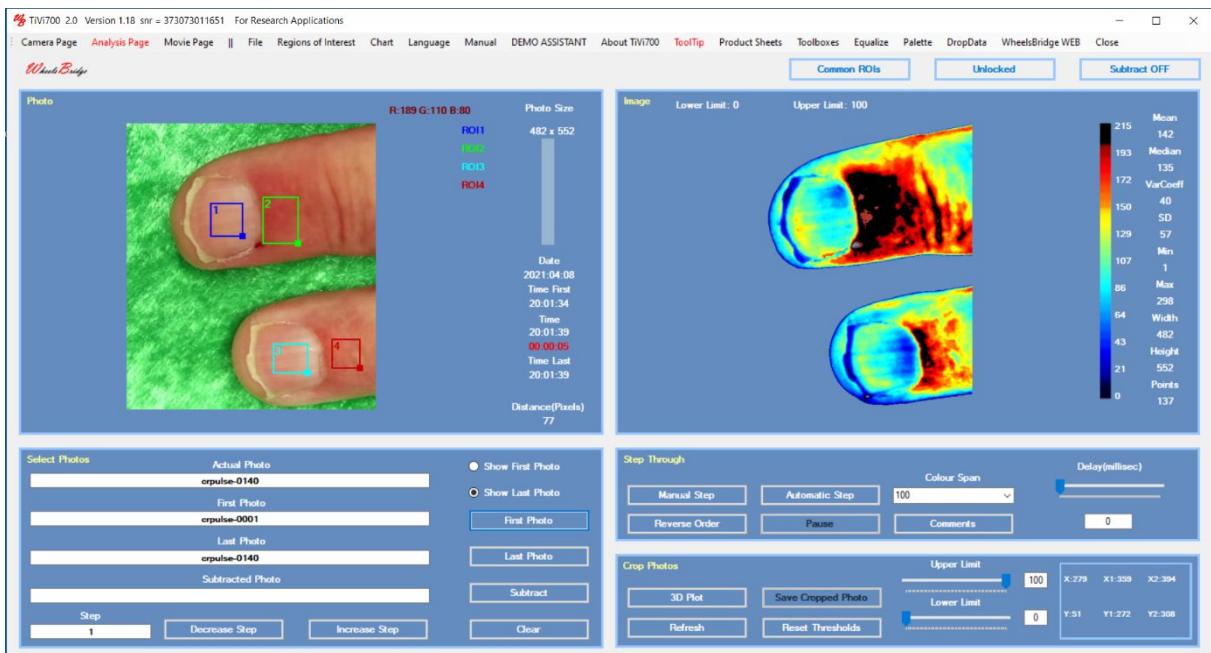4. Draw a ROI around the area to be further investigated.

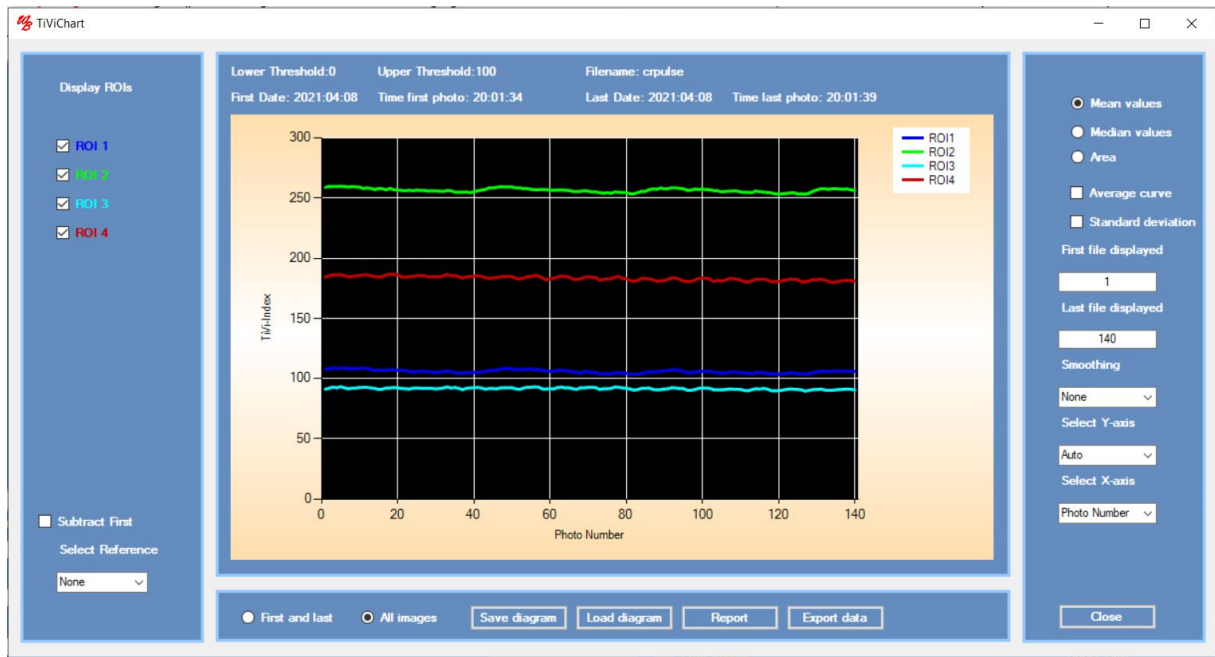5. Click the *Save Cropped Photo* button. Under filename insert "cr" and click the *Save* button.

6. Click the *First Photo button* and select the *crpulse-0001* file in the *crpulse* folder.
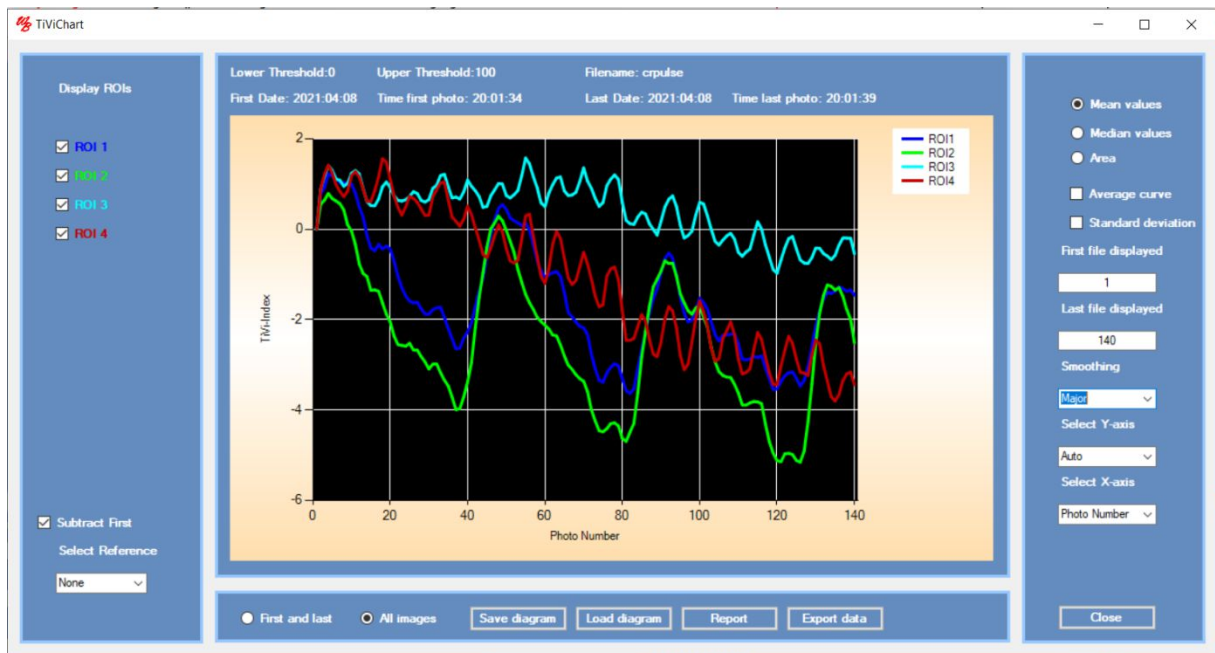
7. Draw some ROIs in the photo.

8. Click *Chart* to generate and display the *Chart* window.

9. Click the *Subtract First* button and set *Smoothing* to *Major*.

10. Pulsation in the TiVi-value is found within the green and blue ROIs. The cycles of higher frequency are due to shivering of the test subject. Close the *Chart* window.

11. In the *Main* window click the *Refresh* button to delete the ROIs. Draw a new ROI around the section to be further investigated.
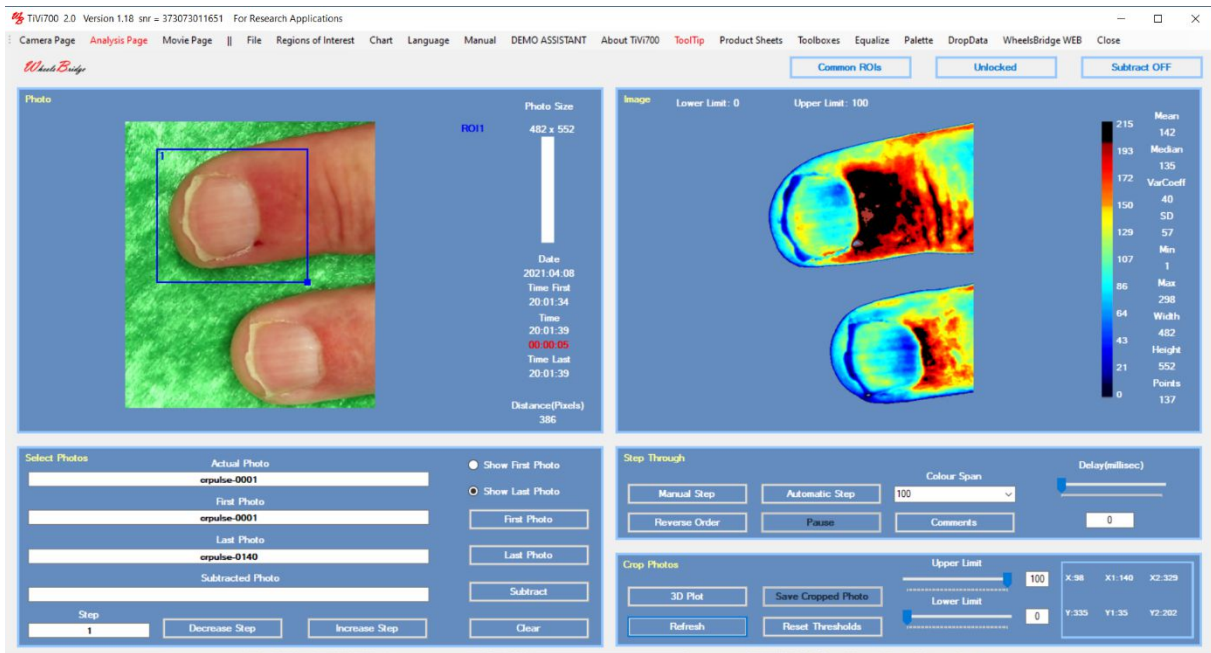
12. Click the ROI1 text and then click the *Save Cropped Photo* to save the cropped photo. Then upload this sequence of photos for further analysis.

13. Select *Pulse* under the *File* menu to start identification of the pulse cycle and the generation of the *pulse* image. If a pulse cycle can be identified the *Pulse* window is displayed.
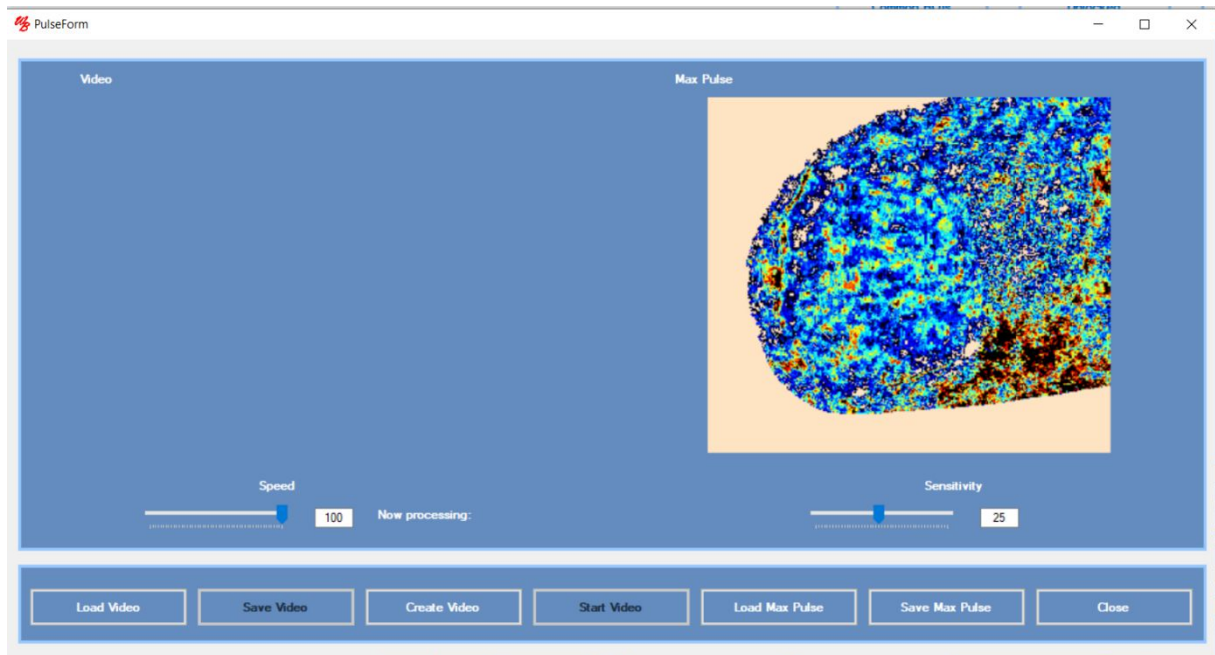
14. The *Pulse* window displays the *Max Pulse* amplitude image. Move the *Sensitivity* slider somewhat to the left to change the colour scale.
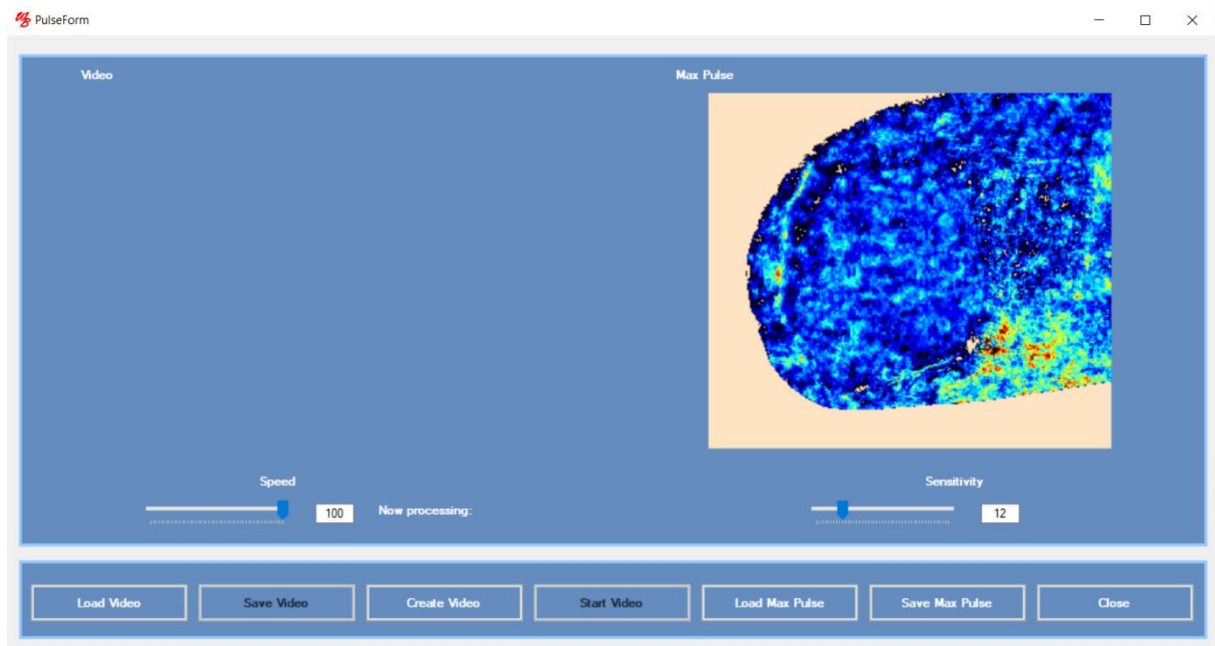
15. Click the *Create Video* button to create the pulse video clip. Then click the *Start Video* button to play the video 10 times. Set the *Speed* slider to 50 and click the *Start Video* again to play the video clip at a slower speed.
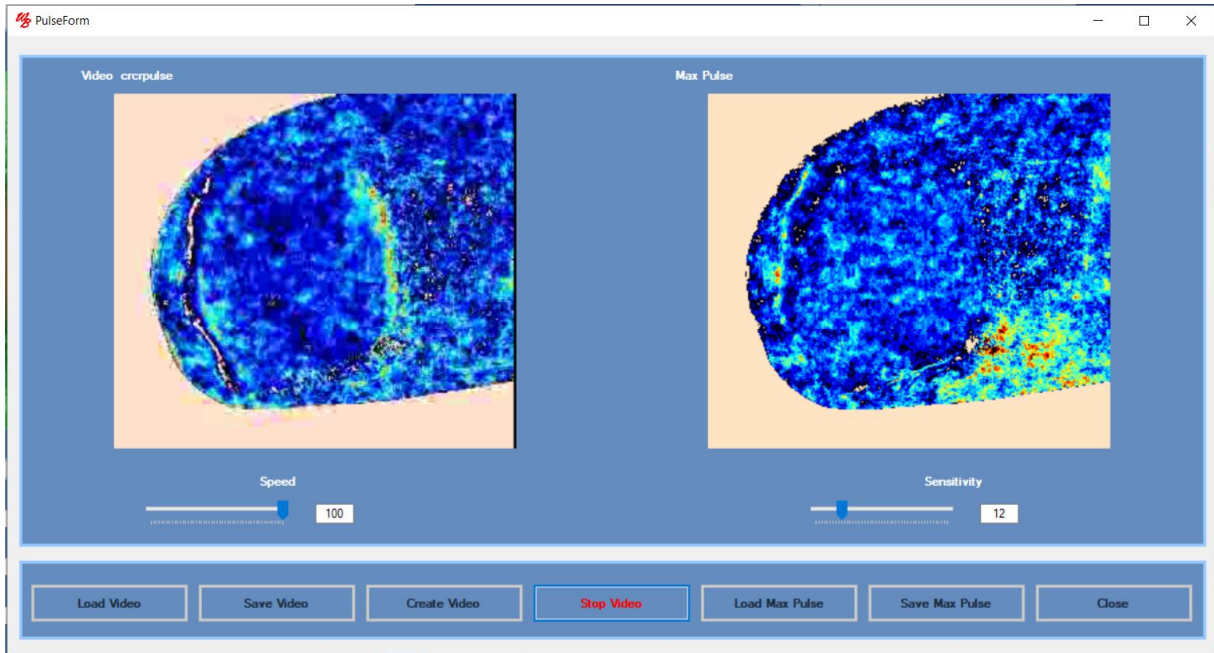
16. Click the *Save Video* and the *Save Max Pulse* buttons to save the video and photo under selected names. Then close the *Pulse* window.
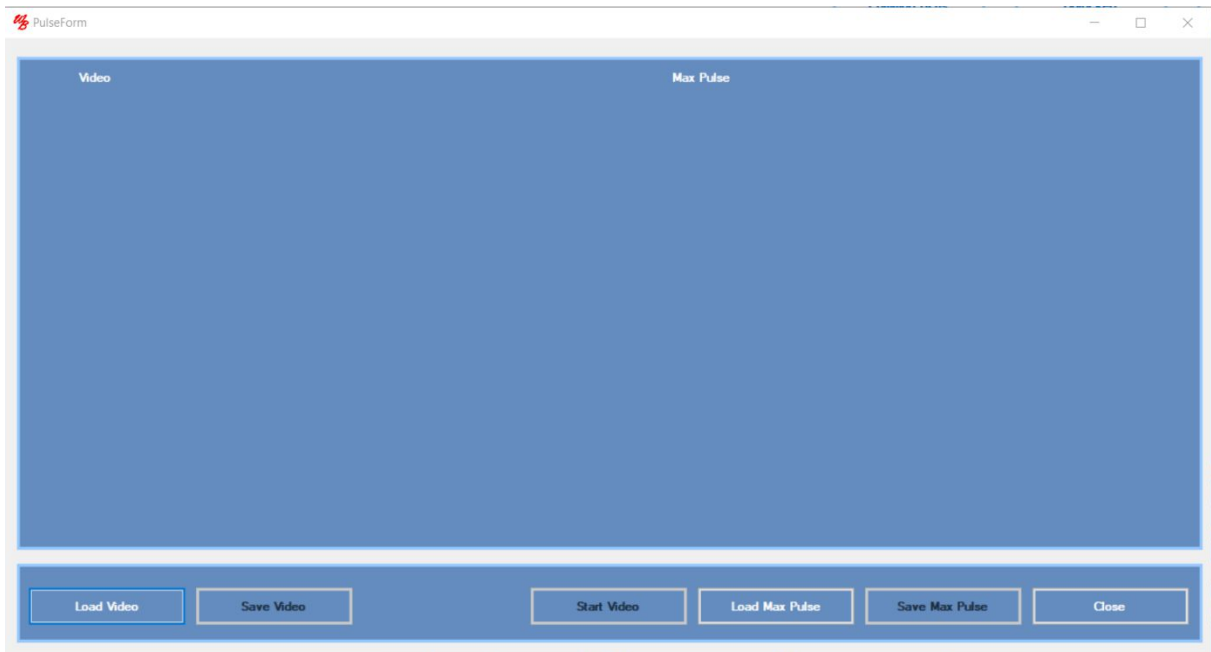
17. Click the *Clear* button in the *Main* window. Then select *Pulse* under the *File* pull-down menu to open a *Pulse* window.

18. Click the *Load Max Pulse* button to load the newly saved photos representing the *Max Pulse* image (*Max_p-0001* in this case). Click the *Load Video* and re-load the newly saved video (*PulseVidTiVi* in this case). Click the *Start Video* button to play the video.

19. Move the mouse pointe over the *Max Pulse* image to display the mouse pointer coordinates and the pulse amplitude as percentage of basic RBC concentration.

20. This completes the session *"Analysing and visualizing the pulse component of the RBC concentration".*

SPECIFICATIONS

Computer

Model	ThinkPad L590, Win10 Pro, Corei7, 15.6", OpSys: Eng. or equivalent.

Camera

Model	Canon EOS 800D
Mode dial setting	Av
ISOspeed	400
Metering mode	Partial Metering
White balance	Shade
Functions Setting	Auto Power off
Focus	AF (Auto Focus)
Stabilizer	On
Camera Power Supply	AC/DC Medical adaptor Model: GSM40A07

Illuminator	*Selens* 160 white light LEDs
Polarisation	Cross-polarisation (co-polarisation optional).
Output Power	10W
Luminous flux	1200LM
Illuminator Power Supply	AC/DC Medical adapter Model: GSM40A12

Camera & Illuminator Housing	Height: 20 cm, Diameter: 18 cm

Table mounted flexible Stand	Arm Length: about 70 cm

Photos

Large Fine	6000 x 9000 pixels	9.2MB
Large Normal	6000 x 9000 pixels	7.9MB
Large Photo maximum capture rate	12 photos / minute	

Medium Fine	3984 x 2656 pixels	4.5MB
Medium Normal	3984 x 2656 pixels	3.9MB
Medium Photo maximum capture rate	15 photos / minute	
Small Fine	2976 x 1984 pixels	2.8MB
Small Normal	2976 x 1984 pixels	2.4MB
Small Photo maximum capture rate	20 photos / minute	
Video mode	960 x 640 pixels	0.3KB
Video Mode maximum capture rate	10 photos / second	
HD Video Mode	1280 x 720 pixels	0.42KB
HD Video mode maximum capture rate	49 photos / second	

Resolution (with TiViMagnifier lens)	5 micrometers / pixel
Operating temperature	10 – 40 $^{\circ}$C

Values and Features saved and retrieved by the system between sessions:

1. Language.
2. Photo size (ImageQuality).
3. Shutter value.
4. Number of photos to be captured.
5. Delay time between two consecutive photos captured.
6. Voice for the "DEMO ASSISTANT".
7. Tooltips settings.
8. Voice Control for starting and stopping the capturing process, video recording or HD video recording.
9. Zoom In value for equalizer operation.
10. Equalizer set to "on" or "off".

All values are saved in the "initializer" file located in the "initializerDirectory". Factory settings are saved in the "initalizersFactory" file located in the same directory.

TIPS AND TROUBLE SHOOTING

When possible, operate the TiVi-system with the integrated zoom-lens set to zoom-in mode (zoom = 55) and change the field of view by altering the distance from the camera to the object. This will ascertain a uniform illumination of the object and thus a uniform sensitivity over the entire image. At a camera - object distance of 150 mm the field of view is about 110 mm x72 mm.

The possible adverse effect of ambient light on the TiVi-image is dependent on the intensity and spectral profile of the ambient light. Check this dependence by recording a TiVi-image of e.g. a hand with the ambient light switched on and off.

If the object does not fully cover the field of view, select a green coloured background, which results in negative TiVi-values displayed as grey background in the TiVi-image. Verify that the object covers the centre of the field of view where the camera meters the backscattered light intensity.

When the distance to the object is too short (less than about 50 mm) or the object does not contain any contrast, the camera cannot focus automatically. Increase the object-camera distance or make a mark on the object to create better contrast.

In case the camera does not display a video when in the *Camera Page*, click the "Click here to show/hide the photo video" under the photo in the *Camera Page*. If the video still does not display, remove the black top cover and check that the camera switch is set to "ON". The camera wheel should always be set to Av-mode. If it still does not work, check the camera power supply cable (disconnect and connect the cable again). When the camera is switched off or switched on a small indicator on the camera rear panel flashes red for a short period of time.

In the unlikely event that the software hangs up, click the clear button in *Analysis Page* or ultimately restart the software.

PART I I – TiVi700 2.0 Plus

## Including Pigmentation and Oxyhemoglobin mapping
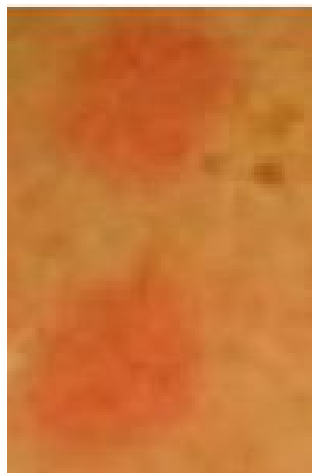
Pigmentation Mapping

INTRODUCTION

The perceived colour of the human skin is determined by the amount of melanin – a pigment residing primarily in the epidermal layer. Melanin gives the skin a colour ranging from almost black in appearance to white with a pinkish tinge due to the underlying microcirculation of the skin. Skin whitening and removal of pigmented spots are desired target results of modern skin care products and treatment. The *Pigmentation Mapper* is a software package that maps the result of such procedures and the effect of topically applied substances by analyzing the pigmentation of the skin with only minimal influence from the underlying microcirculation based on photos captured by the polarization spectroscopy camera technology.

OPERATING PRINCIPLE

While melanin absorbs light in the red wavelength region to a much higher degree than hemoglobin, the red plane in a true colour photo can be employed to create pigmentation maps. If a photo of the skin including both local erythema (produced by e.g. topical application of methyl nicotinate) and pigmented spots, the red plane (left) maps mainly the pigmented spots, while the green plane (right) maps both the erythema and the pigmented spots:
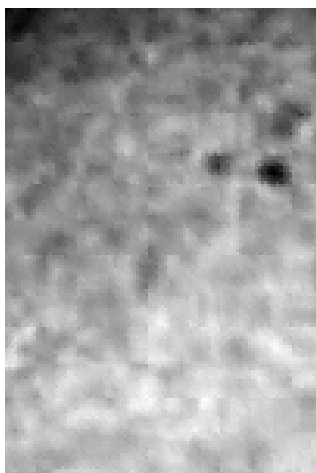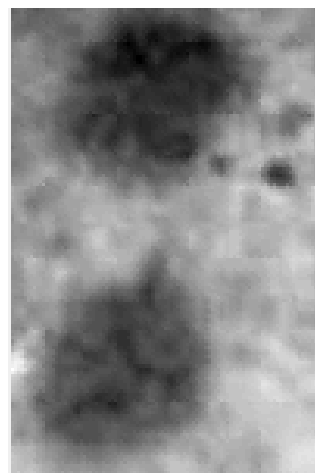

Red plane   Photo   Green plane

By applying an over-all gain-factor based on the difference between the normalized green plane average value and the red plane average value multiplied by the red plane information
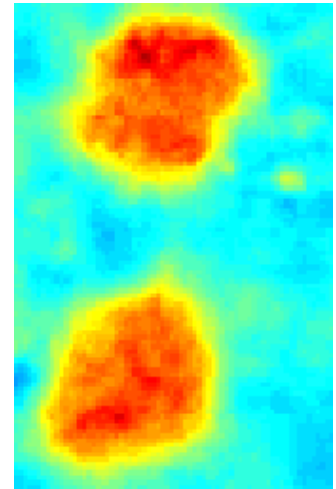
pixel by pixels a *Pigmentation Map* can be constructed in which the individual pixel values and colour scale with the degree of local pigmentation (left) independent of the amount of local erythema (brighter colour indicates a higher degree of pigmentation).
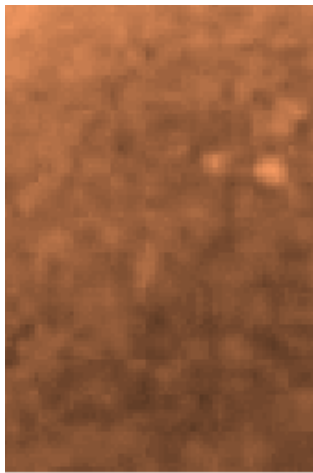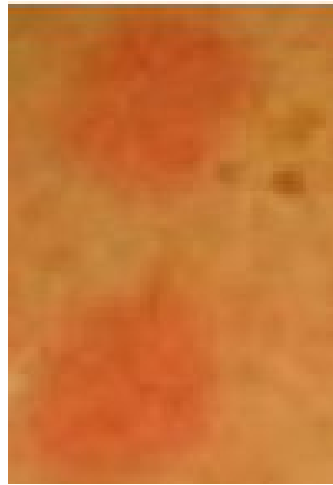


Pigmentation Map   Photo   *TiVi Map*

CALIBRATION

Calibration of the *Pigmentation Mapper* algorithm was performed by analyzing photos of different colours ranging from white-pinkish (mimicking the colour of Caucasian skin with a low degree of pigmentation) to dark brown (mimicking the colour of Afro-American skin with a high degree of pigmentation).
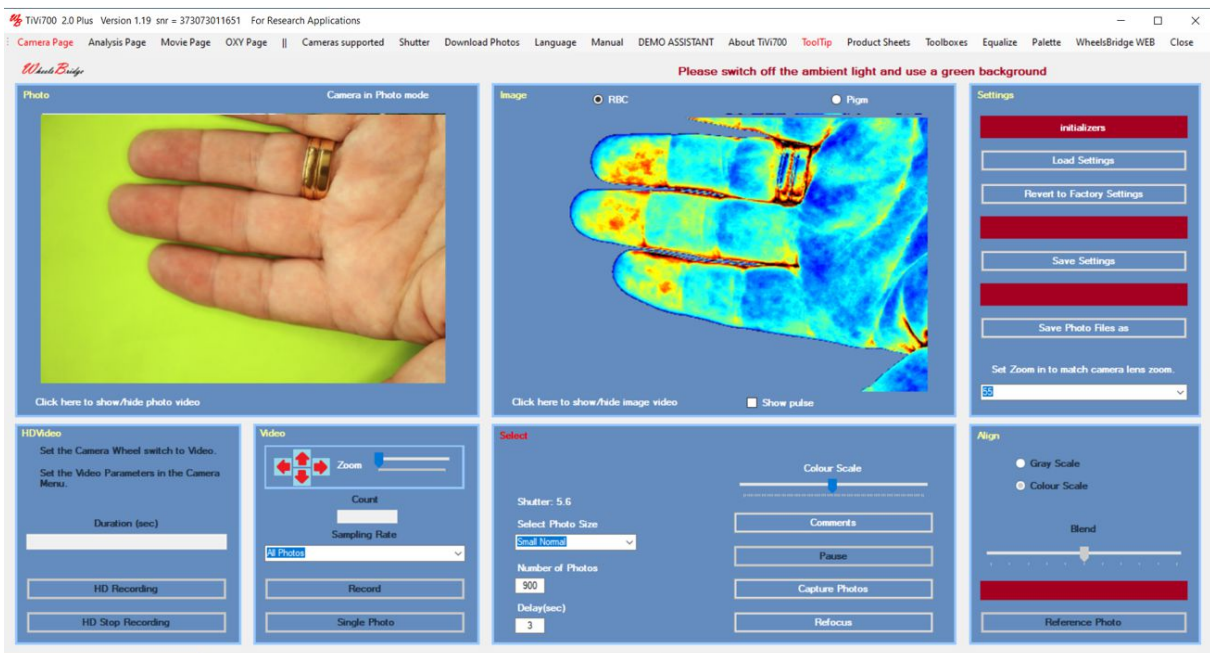
In the Figure below the calculated pigmentation value is displayed for the different colours analyzed.
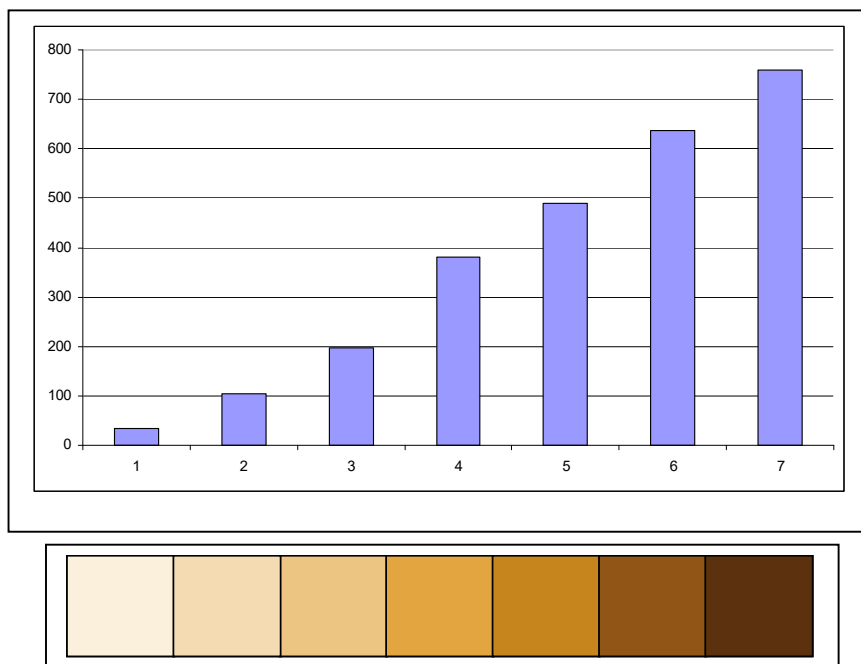
TiVi700 2.0 Plus Main window: Camera Page
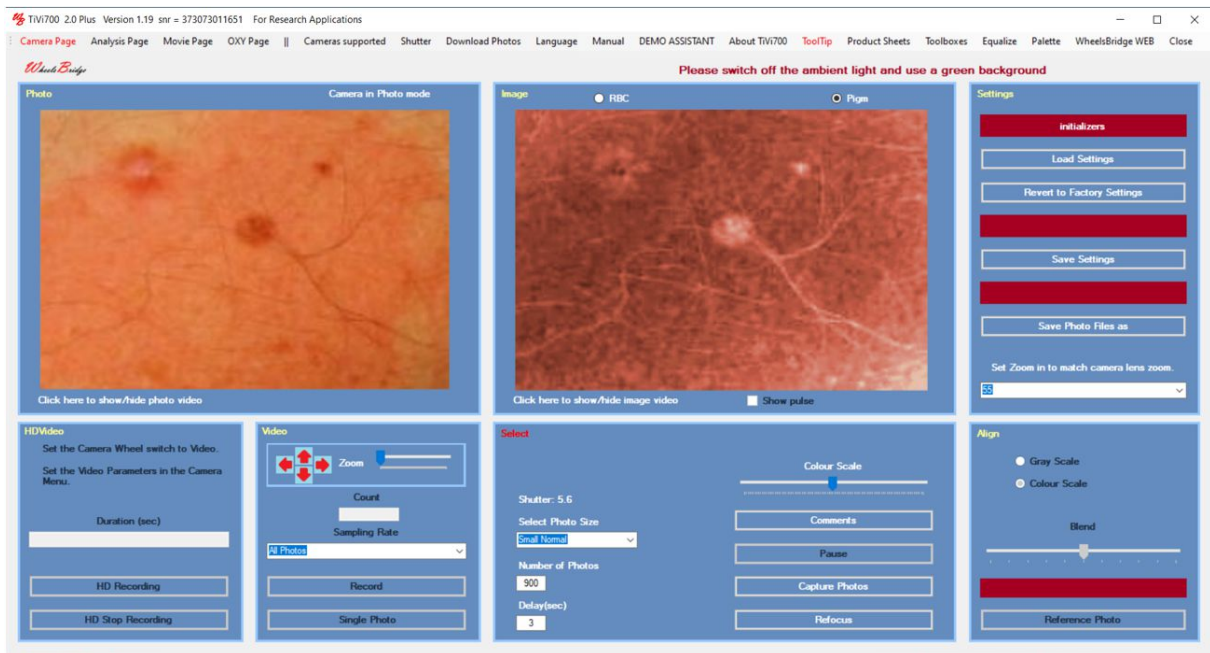
Check the *Pigmentation radio-button* to continuously display the pigmentation map.

Check the *Red Blood Cell radio-button* to continuously display the microcirculation map.

TiVi700 2.0 Plus Main window: Analysis Page
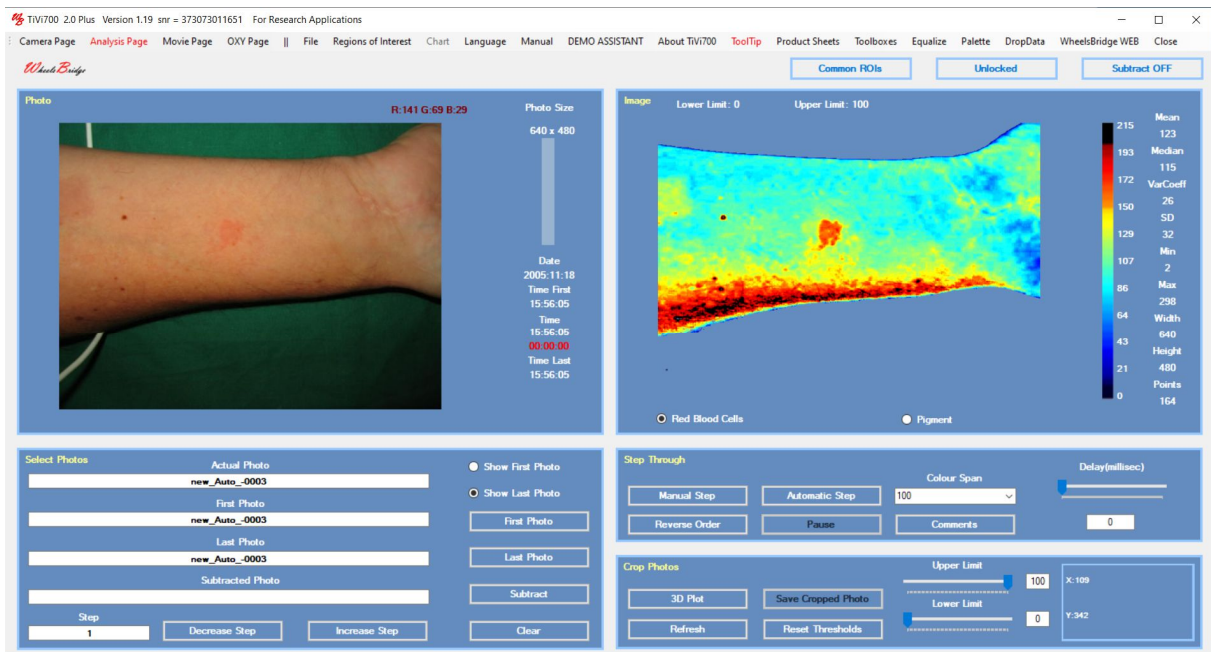
Check the *Red Blood Cell radio-button* to display the microcirculation map.

Check the *Pigment radio-button* to display the pigmentation map.

For instructions on how to operate the *Analysis Page, Movie Page, Chart* and *Project* windows please see *PART I – Basic System TiVi700 2.0.*

Oxy-hemoglobin Mapping

INTRODUCTION

The hemoglobin molecules in the red blood cells are responsible for transportation of oxygen from the lungs to the tissue. Light absorption properties of the hemoglobin molecules are dependent on the oxygen sat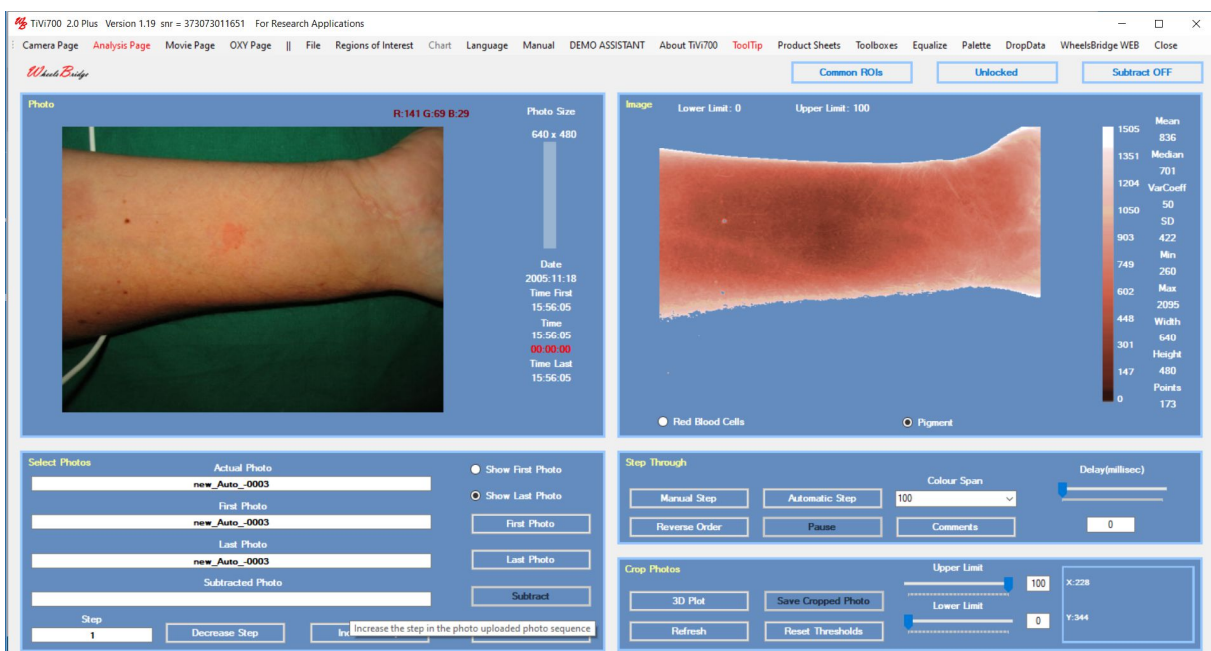uration status which can be assessed by analysis of diffused back-scattered light in the visible region. If the amount of light absorption in tissue within the red and green wavelength bands is analyzed, trend maps of the relative concentration of oxy- and deoxy-hemoglobin can be constructed and displayed.

From a technical point of view alterations in local concentration of oxy- and deoxy-hemoglobin can be assessed by use of polarization spectroscopy imaging provided by the *Tissue Viability Imaging system TiVi700* operating in cross-polarized mode.

The wave-length-dependent local alteration in Optical Density of diffusely backscattered light (ΔOD) is proportional to the sum of the products of the chromophore's extinction coefficient (ε) and the alterations in concentration of the different chromophores (Δ**C**). Given that – in addition to different chromophores in the basic tissue matrix - the most important dynamically changing chromophores in tissue are the oxy- and deoxy-hemoglobin molecules, the following equations can be set up for the red and the green wavelength bands:

$$\Delta OD(red) \sim \varepsilon_{oh}(red) * \Delta C_{oh} + \varepsilon_{doh}(red) * \Delta C_{doh} + \varepsilon_{tissue}(red) * \Delta C_{tissue}$$

$$\Delta OD(green) \sim \varepsilon_{oh}(green) * \Delta C_{oh} + \varepsilon_{doh}(green) * \Delta C_{doh} + \varepsilon_{tissue}(green) * \Delta C_{tissue}$$

where ε_{oh}, ε_{doh} and ε_{tissue} represent the extinction coefficients for oxy-hemoglobin, deoxy-hemoglobin and tissue respectively,

and

ΔC_{oh}, ΔC_{doh} and ΔC_{tissue} represent the changes in concentration (from a reference point in time or reference tissue area) respectively.

Under the assumption that the tissue matrix does not change in the short-term perspective, ΔC_{tissue} can be set to zero and the equation system above transforms into:

$$\Delta OD(red) \sim \varepsilon_{oh}(red) * \Delta C_{oh} + \varepsilon_{doh}(red) * \Delta C_{doh}$$

$$\Delta OD(green) \sim \varepsilon_{oh}(green) * \Delta C_{oh} + \varepsilon_{doh}(green) * \Delta C_{doh}$$

This new equation system is composed of two equations with two unknown variables (ΔC_{oh} and ΔC_{doh}) and can therefore be solved (ΔC_{oh} and ΔC_{doh} can be calculated) based on the extinction coefficient values and average path-lengths tabulated in the literature, the known sensitivity of the camera and spectral density of the illuminating light, and the measured difference in OD in the back-scattered light.

In practice the reference values can be obtained in one of three different ways.

1. The first photo in a stack of photos is used as the reference photo. The actual reference area is defined by the user-drawn region of interest in this photo (ROI M). Only a single region of interest is needed in this case and the region of interest in the first photo constitutes the basis for calculating the reference value. The benefit of this alternative is that the same skin site is used for reference and measurement. The drawback is that the reference value and the actual measurement value may be calculated from photos captured at different points in time.

2. The first photo in a stack of photos is used as the reference photo. The actual measurement area is defined by the user-drawn region of interest in this photo (ROI M). A second region of interest defines the reference area (ROI R). Two regions of interest are required in this case and the second region of interest in first photo constitutes the basis for calculating the reference value. The benefit of this alternative is that different skin sites can be used for the reference and measurement area respectively. The drawback is that the reference value and the actual measurement value are still calculated from photos captured at different points in time.

3. In an alternative arrangement the reference area is continuously updated and based on a region of interest in the same photo as the measurement region of interest (the photo currently displayed). The actual measurement area is defined by the region of interest drawn by the user in this photo (ROI M). A second region of interest defines the reference area (ROI R). Two regions of interest are required in this case. The benefit of this alternative is that different skin sites can be used for reference and measurement and that the actual reference area is calculated from the same photo as the measurement area (i.e. based on values captured at the same point in time). The drawback may be that the reference value and the actual measurement value are calculated from two separate sites on the skin.

The intended use of the *Oxygen Mapper* is to analyse changes in concentration of oxy- and deoxy-haemoglobin molecules in skin or other tissue in experimental and research applications. It is not yet approved for the diagnosis and treatment of disease.

OPERATING PRICIPLE

After uploading as single or a sequence of photos, a region of interest is drawn in the photo to define the measurement area (ROI M). The relative changes in oxy- and deoxy-hemoglobin within the region of interest are mapped and displayed. If only a single region of interest is employed, the reference oxy- and deoxy-hemoglobin maps are calculated as the average oxy- and deoxy- hemoglobin values within the region of interest in the first photo. The oxy- and deoxy-hemoglobin maps therefore display spatial variations around these average values with the azure color as the zero-deviation indicator color while pixels with values higher than the average value are displayed in colors towards red and pixels with values lower than the average value are displayed in colors towards blue. If as sequence of photos is uploaded, the reference map is always based on the average oxy- and deoxy-hemoglobin values within the first photo after the **Reference Photo ROI M** radio-button is clicked.

If a second region of interest (ROI R) is drawn, this region of interest defines the reference area. In case the **Reference Photo ROI R** radio-button is selected, the reference map is calculated based on the average values of the first photo and the associated reference map is used in all the photos in the sequence. In case the **Adaptive Reference ROI R** is selected, the reference map is continuously updated based on values within the region of interest in the currently displayed photo while scanning through the entire sequence of photo.

When all photos have been scanned through clicking **Show Diagram** button opens a new window that displays changes (in arbitrary units) of the average oxy- and deoxy-hemoglobin values within the measurement region of interest with reference to the selected reference values as selected by the **Select As Reference** radio-buttons.

TiVi700 2.0 Plus Main window: OXY Page (example)

1. Open the *OXY Page* by clicking on the *OXY Page* item in the main window pull-down window.
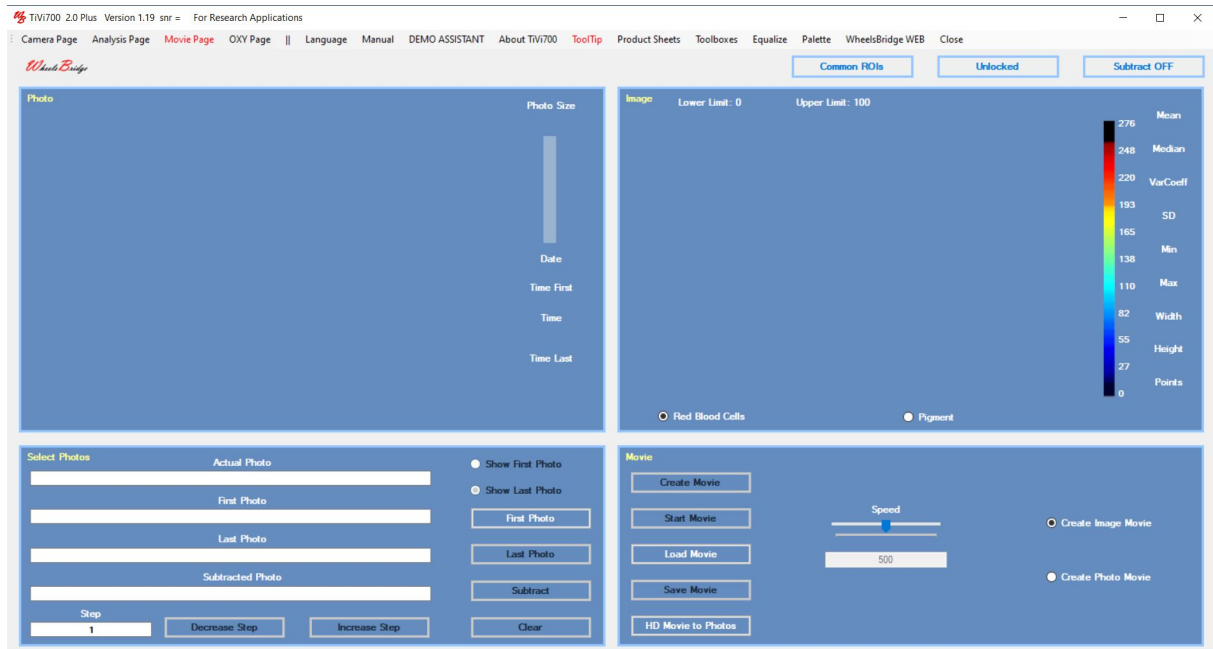

2. Select **First Photo** to upload a sequence of photos (in this example the photo named *c_artOccl-0001* located in the *Wheelsbrigde>TiVi700 2.0>TiVi106 Oxygen Mapper>TiVidemonstration* folder is used.
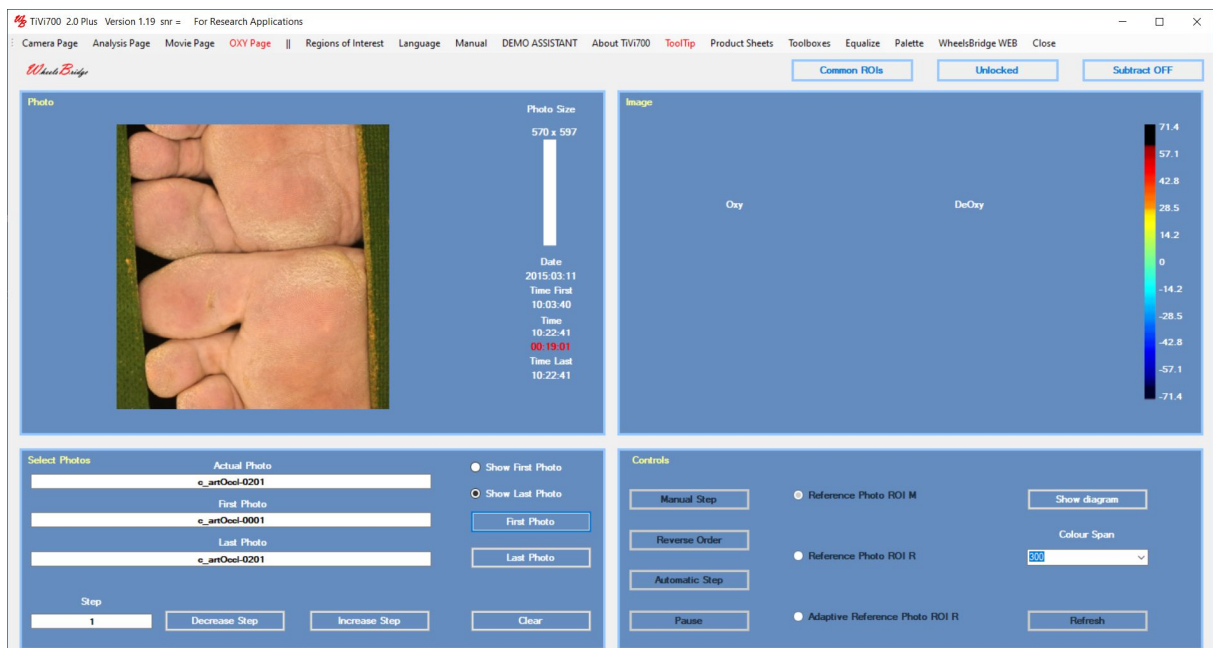

3. The lower foot is the measurement foot, and the upper foot is the reference foot. After about 4 minutes a pressure cuff applied around the calf of the measurement leg is inflated to above systolic pressure to produce arterial occlusion in the distal part of the measurement

leg. After yet another four minutes the cuff is released returning blood flow to the measurement foot string.

4. Draw a ROI on the lower (measurement) foot (ROI M) and check the Reference Photo ROI M radio-button.
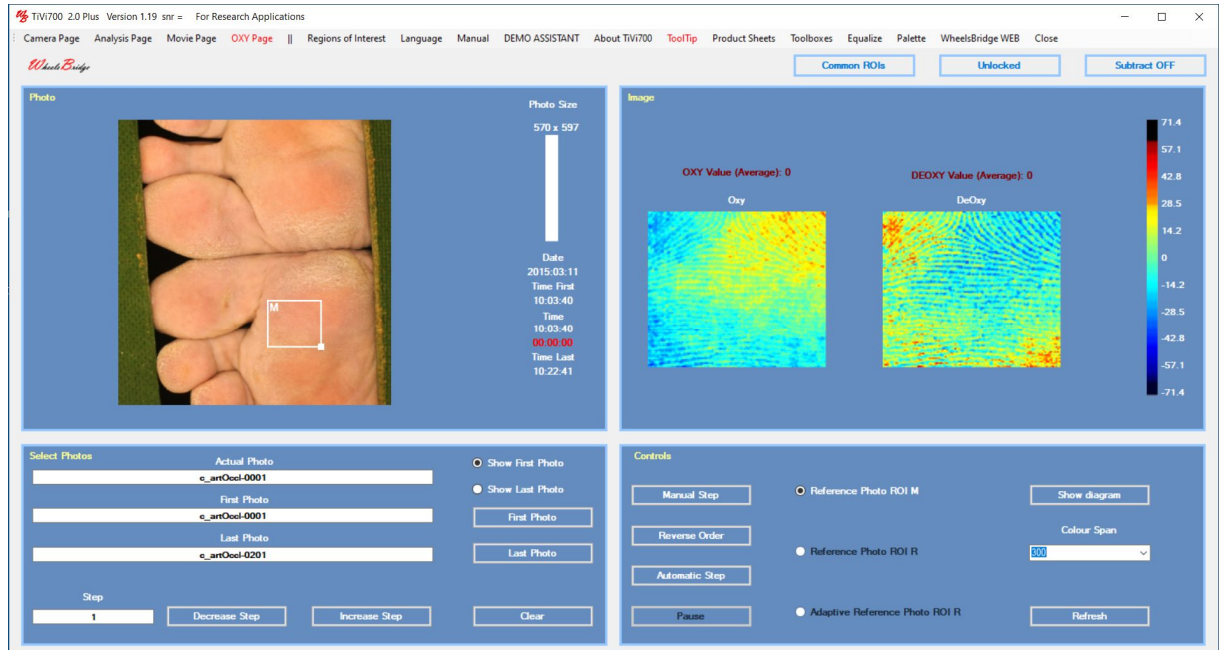
5. The relative distribution of oxy- and deoxy-hemoglobin is now displayed in the *Oxy* and *Deoxy* maps in the Image panel. The area from which the oxy and deoxy reference values are calculated is the ROI M area in the first photo.

6. Draw a second ROI in the reference foot (ROI R). The area from which the oxy and deoxy reference values are calculated is now the ROI area (ROI R) in the reference foot (in the first photo).
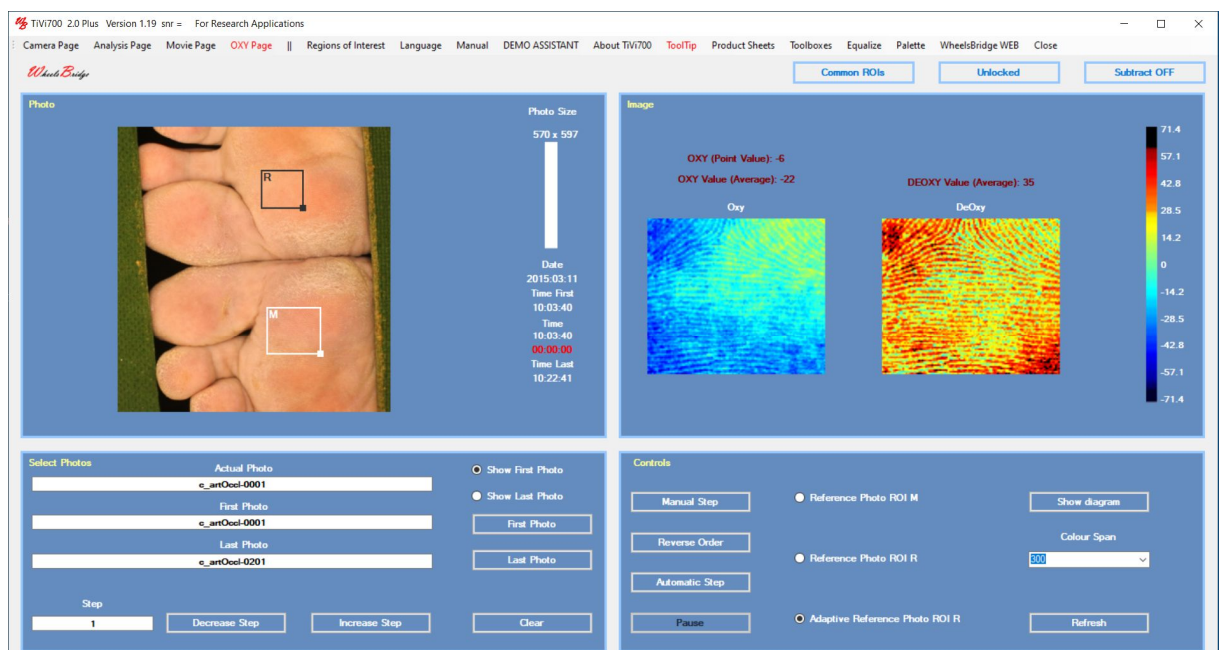
7. Check the **Adaptive Reference Photo ROI R**, to make ROI R in each new photo the basis for calculating the reference oxy and deoxy values.

8. Click the **Automatic Step** button to scan through the photos and calculated the oxy- and deoxy-hemoglobin curves. Click the **Show diagram** button to open the diagram and display the oxy and deoxy curves.
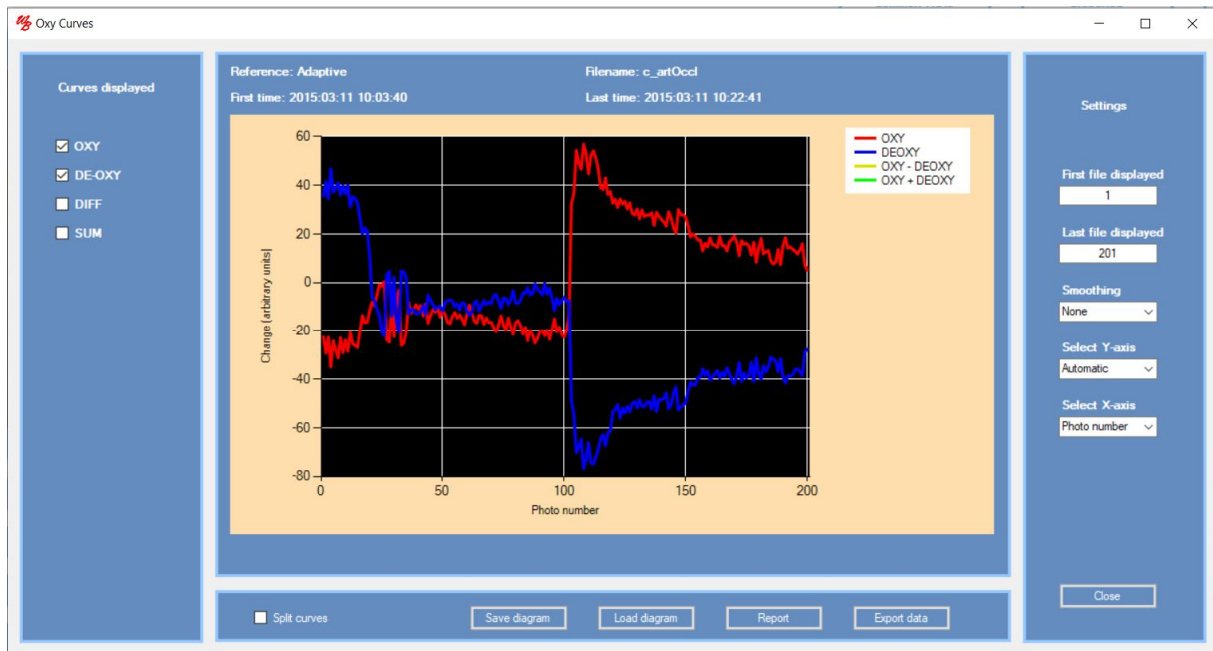

9. The red curve corresponds to the oxy-hemoglobin curve while the blue curve corresponds to the deoxy-hemoglobin curve. At about photo 25 the cuff pressure is inflated to above the systolic pressure causing the red curve to move in the negative direction and the blue curve in the positive direction because the red blood cells in the measurement foot successively becomes deoxygenated. At photo 100 the cuff pressure is released causing an inflow of oxygenated blood to the measurement foot (reactive hyperemia) which successively is reduced towards resting values.

10. Check the DIFF and SUM check-boxes to also display the difference and sum of the curves.
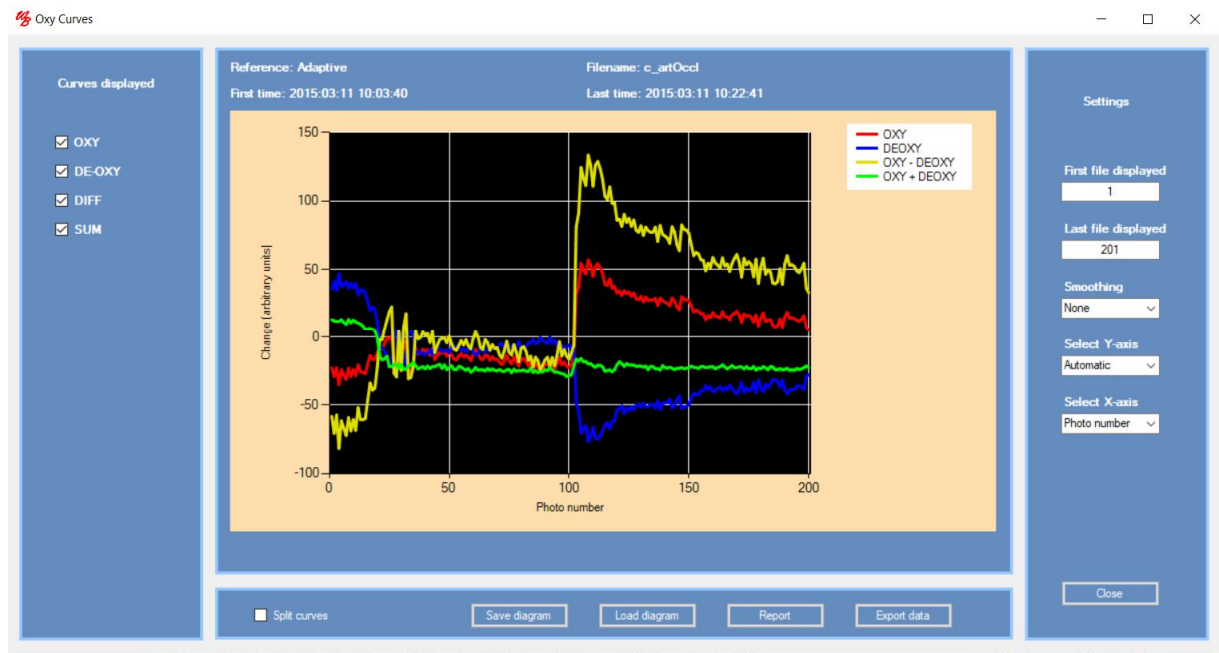
11. Observe that the total trapped volume of red blood cells (green curve) is not particularly affected, while the difference between oxygenated and deoxygenated blood (yellow curve) is profoundly affected by the release of arterial occlusion.

12. Check the **Split Curves** checkbox to display the curves in separate diagrams.
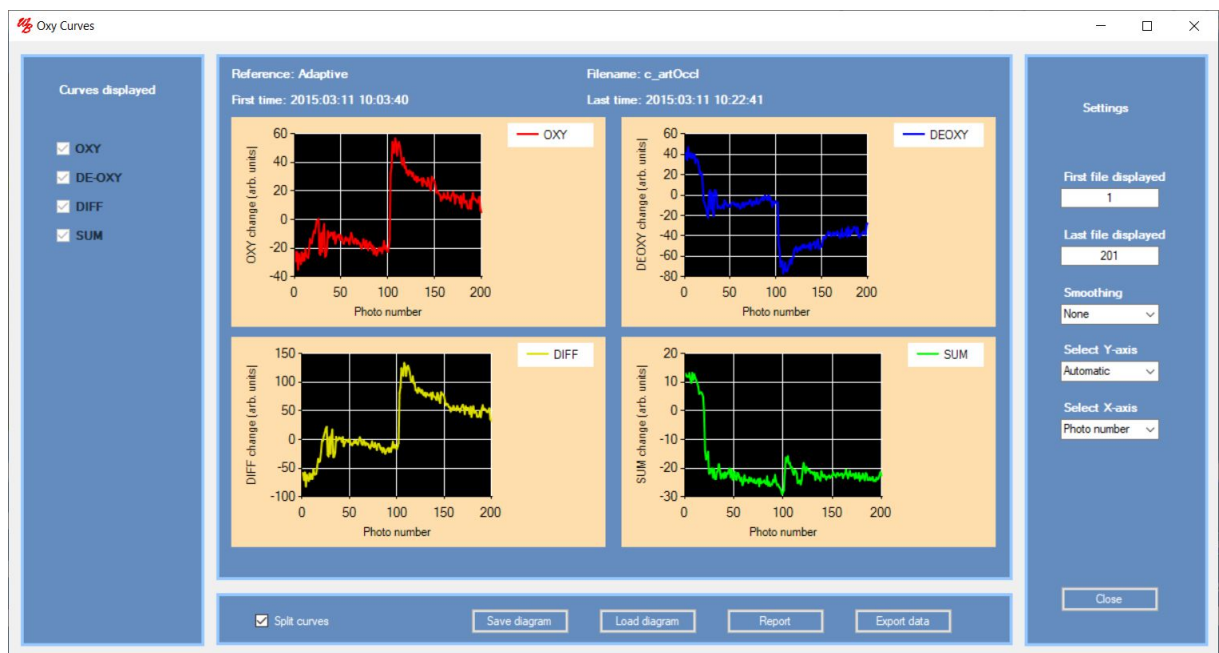
13. Click inside the upper left diagram to enlarge the *OXY diagram* (toggle switch).
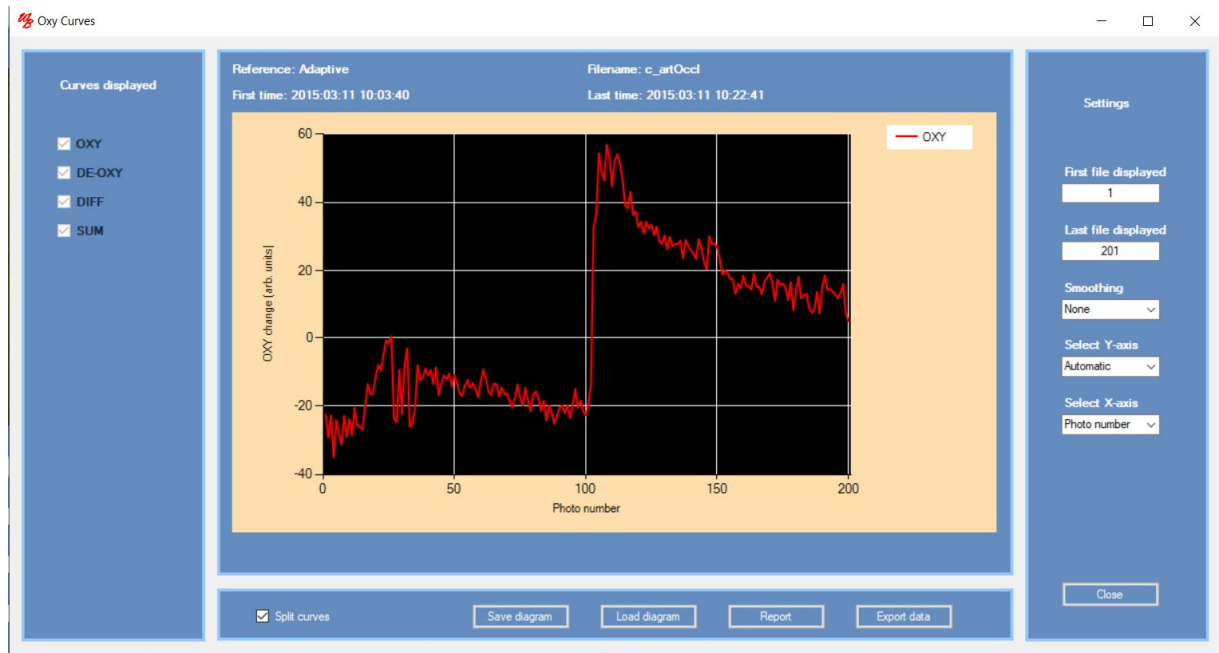
14. Select **Major** in the **Smoothing** combo-box to smooth the curve.
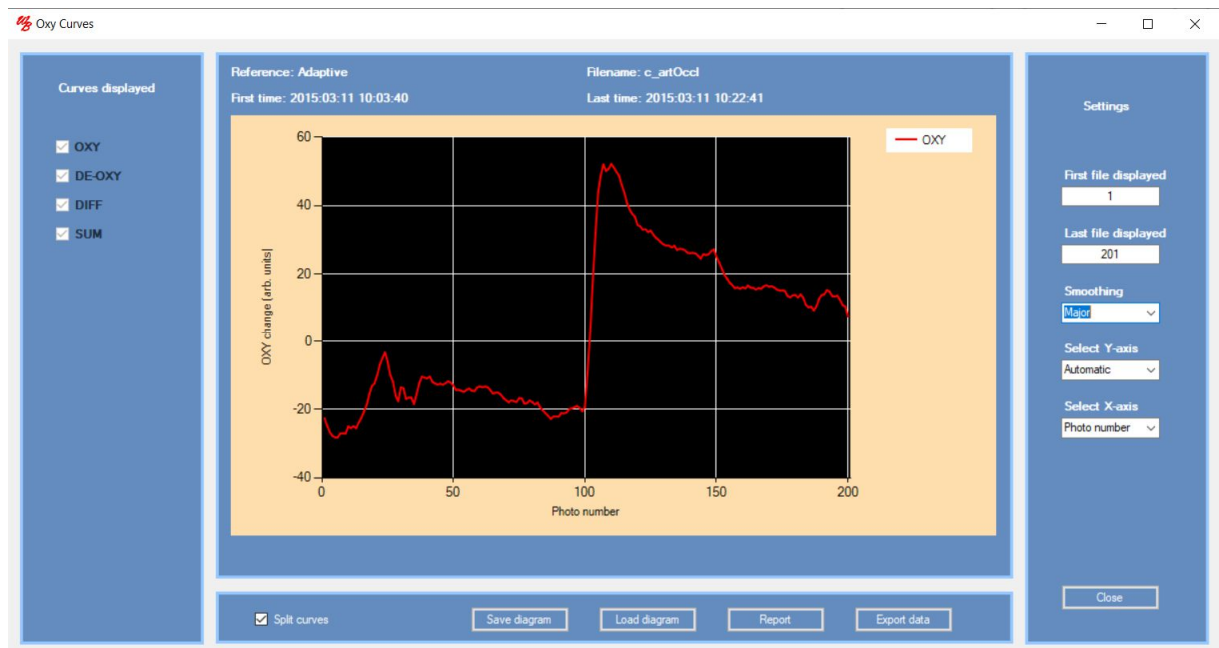
15. Click the **Save Diagram** and **Load Diagram** buttons to save and reload the diagram.

16. Click the **Report** and the **Export Data** buttons to generate a result pdf-file and an Excel document respectively (assuming that OFFICE LIBRE or MS EXCEL and a pdf-writer is installed).

17. Click the **Close** button to close the *OXY Curves* diagram.

TiVi700 2.0 Plus Main window: OXY Page – detailed description

Pull-down menu

Regions of Interest – Rectangular ROIs – click to use rectangular ROIs.

Regions of Interest – Elliptical ROIs – click to use elliptical ROIs.

Regions of Interest – Freehand ROIs – click to use freehand ROIs.

Photo panel

Photo Size – shows the size of the displayed photo in pixels.

The bar below the *Photo Size* indicator displays the relative position of the present photo in the sequence.

Below the bar the capturing date and the time for the first, present and last photo file is displayed. This information is saved as meta-data when the photo is captured and cannot be altered. The elapsed time (from the first photo capturing time) is displayed in red colour.

When a 'ROI' is drawn in the photo, the ROI is denoted *M* (Measurement) and the corresponding oxy and deoxy map are calculated and displayed in the Image panel. The Oxy and deoxy reference values are calculated as the average values within the *M* ROI in the first photo. If the relative oxy and deoxy maps for the entire photo are to be calculated and displayed draw an *M* ROI that covers the entire photo.

If a second ROI is drawn in the photo this ROI is denoted *R* (Reference) and the oxy and deoxy reference values are calculated as the average values within the *R* ROI.

Draw a ROI – draw a new 'ROI' by first placing the mouse pointer at the upper left corner of the 'ROI' to be drawn. Hold down the left mouse button while moving the mouse pointer to a new position. Release the mouse button. A new 'ROI' is now drawn, and the name of this 'ROI' is displayed to the upper right corner of the ROI (*M* or *R*).

Move a ROI – to move a 'ROI', move the mouse pointer to the centre of the 'ROI'. Hold down the left mouse button while moving the mouse pointer to a new position. Release the mouse button. The 'ROI' is now moved to the new position.

Stretch a ROI – place the mouse pointer at the lower right corner of the 'ROI' (indicated by a dot). The mouse pointer turns into a cross-shape pointer. Hold down the left mouse button while moving the mouse pointer to a new position. Release the mouse button. The 'ROI' will now appear as a stretched 'ROI'. Apply both *Stretch a '*ROI*'* and *Move a '*ROI*'* to change the shape of the 'ROI' and move it to a new position.
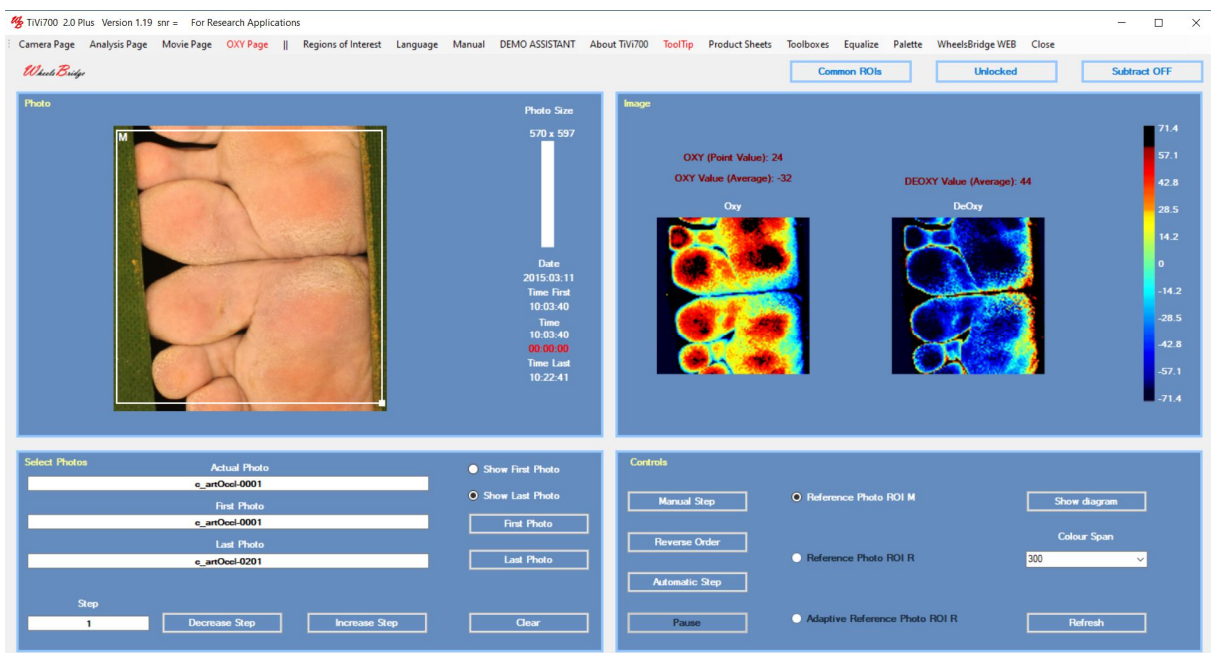

Image Panel

The Oxy and Deoxy maps for the area covered by the *M* ROI are displayed in the *Image* panel. The average values of the oxy and deoxy maps are displayed above the oxy and deoxy maps. When the mouse pointer points at a site inside the oxy or deoxy map, the local value is displayed.

Actual Photo textbox – displays the name of the photo currently displayed. The photo currently displayed can be altered manually to jump directly to a desired photo file in the sequence uploaded by inserting the name of the photo file in the *Actual Photo* textbox.

First Photo textbox – displays the name of the first photo in the sequence as selected by using the *First Photo* button.

Last Photo textbox – displays the name of the last photo in the sequence as selected by using the *Last Photo* button.

Show First Photo radio-button – click to display the first photo in the sequence.

Show Last Photo radio-button – click to display the last photo in the sequence.

First Photo button – click to select the first photo in the sequence to be uploaded. The last photo in the sequence and the sequence step is automatically identified. After selecting the first and last photo in the sequence by clicking this button, the last photo in the sequence is displayed.

Last Photo button – click to select the last photo in the sequence to be uploaded.

Step textbox – displays the actual step for the photos and images to be displayed.

Decrease Step – decreases the step.

Increase step – increases the step.

Clear – clears the photo and image picture boxes and makes the system ready for uploading a new photo sequence.

Controls

Manual Step – click to step through the photos and images sequentially.

Reverse Order button – changes the order in which the photos and images are stepped through.

Automatic Step button - click to step through the photos and images automatically. Click the same button to stop the step through process (button now labelled *Stop*)

Pause button – double-click to temporarily halt the step through process. Click the OK button in the continue window to proceed.

Reference Photo ROI M radio-button – check to use the *M* ROI in the first photo for calculation of the oxy and deoxy reference average values.

Reference Photo ROI R radio-button - check to use the *R* ROI in the first photo for calculation of the oxy and deoxy reference average values.
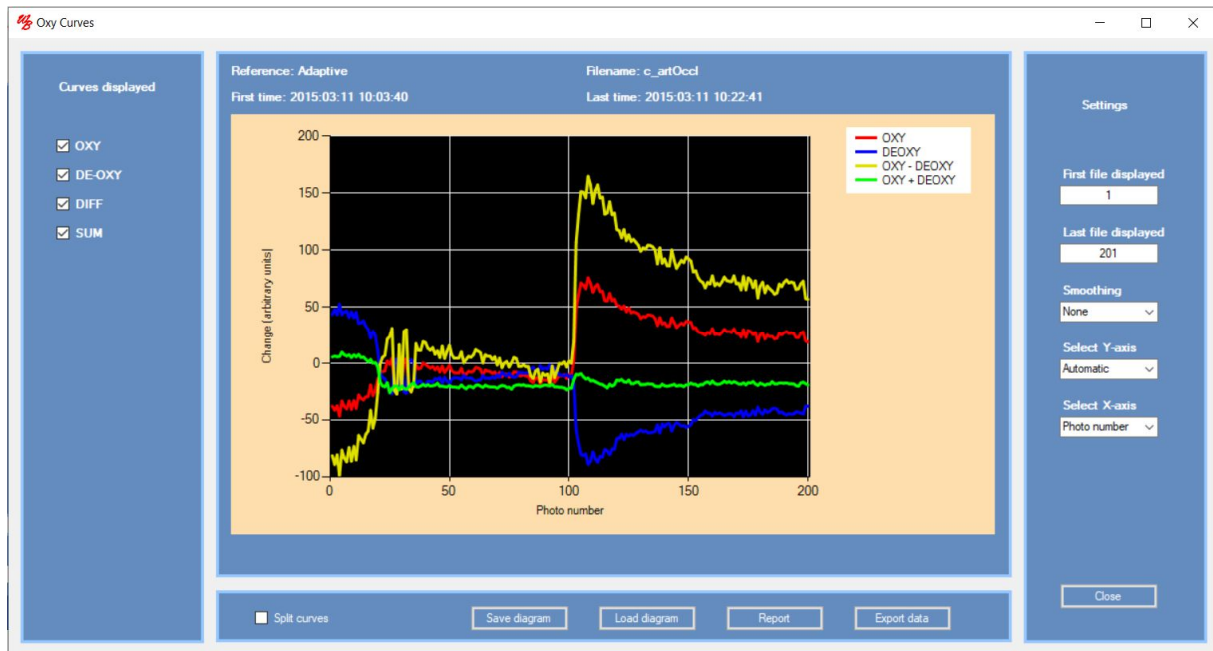
Adaptive Reference Photo ROI R radio-button - check to use the *R* ROI in the currently displayed photo for calculation of the oxy and deoxy reference average values.

Show diagram – click to display the OXY Curves diagram.

Colour Span – select an item to change the colour representation.

Refresh – click to remove the ROIs from the photo.

TiVi700 2.0 Plus OXY Curves diagram

Curves displayed

OXY checkbox – check to display the OXY curve in the diagram.

DE-OXY checkbox - check to display the DEOXY curve in the diagram.

DIFF checkbox - check to display the difference between the OXY and DEOXY curves in the diagram.

SUM checkbox - check to display the sum of the OXY and DEOXY curves in the diagram.

Settings

First File displayed – select the number of the first photo to be included in the diagram.

Last File displayed – select the number of the last photo to be included in the diagram.

Smoothing - select degree of curve smoothing.

Select Y-axis – select the scale of the Y-axis.

Select X-axis - select the scale of the X-axis (photo number or elapsed time).

Close – click this button to close the window.

Diagram

Reference Photo – reference photo method.

First time – time of capture for the first photo file.

Last time – time of capture for the last photo file.

Filename – name of the photo file sequence displayed.

Diagram – displays the curves.

Split curves checkbox – check to display the four curves separately. Click inside a diagram to expand this diagram (toggle switch).

Save diagram – click the actual diagram under a selected name.

Load diagram – click to load a diagram.

Report – click to generate a pdf report file.

Export data – click to generate an Excel diagram.