CapiScope HVCS Handheld Video Capillary Microscope

CapiScope HVCS

CapiScope User Manual

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Preface

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KK Research Technology Limited 4 Alliance Court Honiton Devon England EX14 1YL

Tel. (+44) 4040146242 email admin@kktechnology.com

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Introduction

The CapiScope Image Acquisition and Analysis software provides a simple and uncomplicated means to capture and store capillaroscopy images from video onto a Personal Computer. The video source can be a live signal direct from the KK Technology CAM1 Capillary Anemometer, KK Technology VCS Video Capillaroscopy System, KK Technology HVCS Handheld Video Capillaroscopy System, any analogue video camera, or pre-recorded video from a VCR. Previously digitised video sequences can also be imported from avi files and also read directly from a Cytometrics Cytoscan via a network connection. Live video can be displayed on the computer screen.

Input brightness and contrast can be controlled using slider controls to optimise the dynamic range of the image input signal. A single frame or short video sequences can be captured by a mouse click, keyboard, HVCS trigger, CAM1 marker or external trigger. By using the mouse, capillaries can be marked or dimensioned from live images, frozen images, or images previously saved to disk. Capillary density (either number or linear density) can be quickly calculated. Dimension and calculated data can be easily exported to other Window applications or spreadsheets, such as Excel, for further statistical analysis.

The optional Dynamic measurement version adds capillary blood cell velocity measurement, automatic movement correction, and continuous capillary width and densiometry measurements.

The actual CapiScope file (.cs1, single image, .dcs video sequence) contains the filename of the image data, together with the dimensions list, comments and capture time. The image data is saved in a proprietry raw binary file (.kkg, single image or .mve video sequence), or optionally, windows bitmap (bmp) or tif formats.

Video can be exported as an avi file.

The optical magnification can be calibrated seperately for the x axis and y axis. Dimensions can then be shown in either calibrated units or pixels.

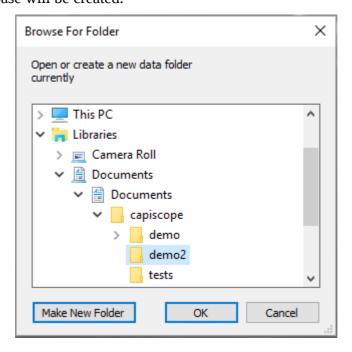
Getting Started

Set up Data Folder

Setting up the data folder

When first used, you will be asked to select an existing folder or create a new folder for storing the database and all data files.

If an existing folder is chosen, which also has an existing database, then this will be used. Otherwise a new database will be created.

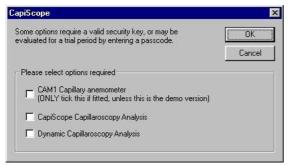


If recording video, then a local fast disc drive should be used. Network or external drives could result in missing frames in the video.

Start Up

When starting CapiScope, you may be presented with one or all three dialog boxes described below.

Selecting functions

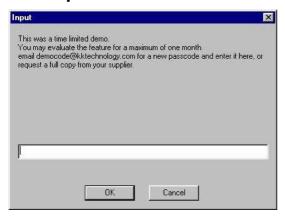


This dialog box appears when there are no profiles, or initialisation files (.ini), in the program directory, or no options have been selected.

- CAM1 capillary anemometer
 - *CAM1 Capillary Anemometer* Only select this item if this is the demonstration version, or if a CAM1 interface card is installed in the computer. Selecting it when the CAM1/PC interface card is not fitted, can cause the computer to lock up. It is ok to select this in the demonstration version, since the interface card and input data are simulated.
- Capiscope
 capillaroscopy analysis
 CapiScope Capillaroscopy Analysis If this is selected, and this is not the demo version, then
 a security key is required which fits into the parallel printer port of the computer. If there is
 not a security key, then you will be prompted for a passcode, which will enable this option
 for the current month. You can get a passcode from KKTechnology or your local KK
 Technology distributor.
- *Dynamic Capillaroscopy* Again, this option requires a security key, or an additional passcode to evaluate it for one month.

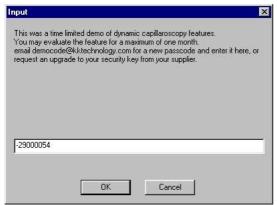
This dialog box should only appear once. The values chosen will be set in the profile. To change them you need to edit the profile (.ini file in program directory).

Demo passcode



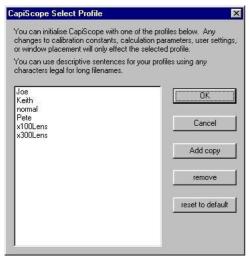
Enter the passcode number here. Include the minus sign if neccessary. The passcode is valid until the end of the calendar month.

Dynamic features passcode



An additional passcode is required if the dynamic features are enabled. Enter the passcode number here. Include the minus sign if neccessary. The passcode is valid until the end of the calendar month.

Select Profile



Different profiles can be selected. These store user settings and calculation parameters and magnification settings. Using different profiles could be used, for example, by different users, so that settings are not unexpectedly changed. Or they can be used to automatically provide the correct setings for different experimental setups.

Step by Step Guide

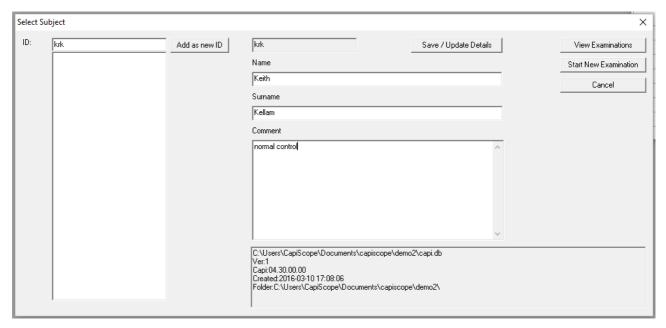
New Subject



To add a new subject, type a new subject ID. This can be any combination of numeric or alphabetic characters. There is no length restriction. Each ID must be unique.

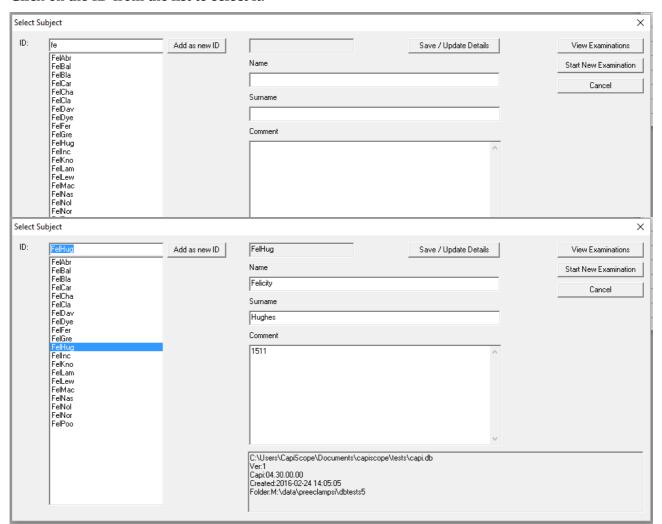
Click on the Add as new ID button.

Enter Name, Subject and comments fields as desired and click on the Save/Update Details button.



Select Subject

To select a subject, type in the subject ID. As you type the first 20 Ids that match will be listed. Click on the ID from the list to select it.

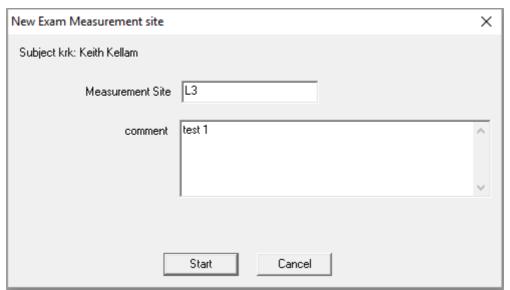


The selected Subject ID will be shown in the top greyed box and it's Name, Surname, and comments in the boxes below.

Start New Examination	To create a new examinations folder and start the measurement process. Files and results will be logged in the database.
View Examinations	To review previously recorded measurements and results.
Cancel	To carry on without using the database. Recordings and measurements wont be logged in the database.

Start New Examination

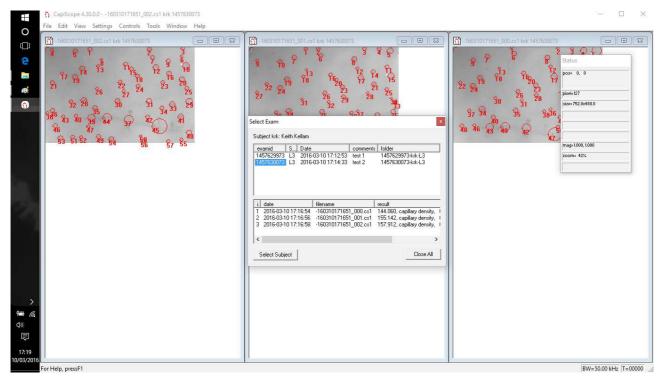
New Exam Measurement site



Enter any alphanumeric text for the measurement site. This is used as part of the name of the new folder used for storing files for this examination, and could be used for collating, sorting and analysing results.

There is no restriction on what is used, but it should be meaningful and consistent.

View Examinations



Click on an exam id in the top list to select it. The lower list will be updated to show all files associated with this examination. This will include original images and video and also any files and results produced from them. It also shows the last result calculated for that file.

To update the list after any changes or additions, click on another examid, then reselect this examid.

Click on the ID in the lower list to open that file.

The Close All button will close all document windows. You will be prompted to save any files that have been modified.

Use the Select Subject to go back to the first step and start again. Any open documents will be closed first.

Using CapiScope

Video Input Control

There are 4 or 5 input control sliders. From left to right they are:

LED Brightness

Adjusts the time on duration of the LED strobe. A lower value reduces image blurring due to movement. Units are in microseconds.

Contrast / Gain

Controls the input or sensor reference level or gain. On HVCS devices and CAM1 with IDS cameras, the best setting is to have this at zero if possible and adjust the GAIN in the dropdown box below. The slider may produce an uneven grey level histogram at some settings on some IDS camera modules.

Brightness / Black level

Controls the input or sensor black level. Adjusting this alters the image brightness.

Video 1992 0 0 5065 100 gamma 30 fps SET

Exposure

Controls the sensor exposure setting. A lower value reduces image blurring for those devices without LED strobe. A lower value will also have less sensor noise.

Setting to the bottom of the slider will give full maximum exposure possible at the current frame rate.

Exposure needs to be set to a higher value than the LED strobe brightness.

Units are in microseconds.

LUT Black level adjustments

Further black level adjustment is possible using the LUT. This may produce an uneven grey level histogram at some settings. The LUT is enabled by setting the check box below it. Not available on all devices.

GAIN

The drop down box below the sliders allows selection of additional input gain when available.

GAMMA

Check this to turn on gamma correction if available. (Not recommended).

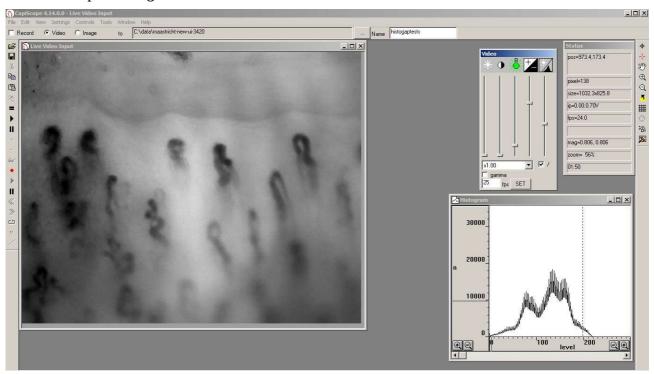
FPS

Use this to change the frame rate. Enter a new value and press the "Set" button. Video recording should be OFF and the video recording window closed. You might need to change the video sequence settings since the maximum buffer duration is saved as number of frames.

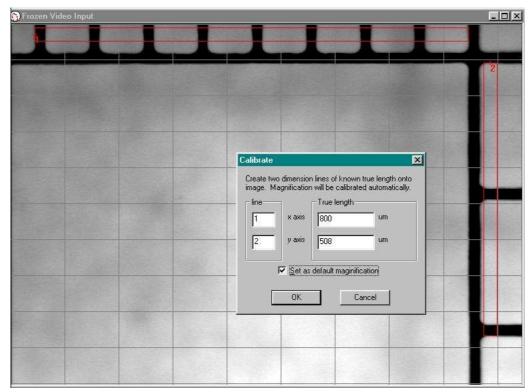
If you set too high a value, video frames will get dropped, or some frames will be out of sequence. The video grab image size will need to be reduced to get higher frame rates. Reducing the grab size will reduce the amount of data sent over the USB which will help increase frame rate when the USB transfer is the limiting factor. When aiming for very high frame rates, then reducing the grabbed height the sensor capture time. Changing the grabbed width will not reduce sensor capture time.

Histogram

Use the Windows menu "Histogram" option to show the grey levels. This will help in setting up the best video input settings.



Magnification Calibration



For image capture devices which have square pixels such as the CapiScope HVCS, then only one dimension needs to be calibrated.

Line up Graticule with Capiscope video output and focus as usual. Move the Graticule so as much of the 1/10 mm scale (x-axis) and the 1/100-inch scale (y-axis) is displayed on the output window.

Freeze the video output with the icon; click on the button (Measurement) and then on the button (Dimension line).

Click on the start of the furthest left black line on the mm (x-axis) scale and then on the start of the furthest right black line on the mm scale. This will be dimension line number 1.

For non-square pixel image capture devices or files, click on the bottom of the topmost black line on the inch (y-axis) scale and then on the bottom of the lowest black line on the inch scale. This will be dimension line number 2.

Alternatively, a single diagonal line can be drawn. The separate x and y components will be used for the calibration.

Click Calibrate Magnification to bring up the Calibration dialog box.

For square pixel images, enter the true length for just the x axis. Leave the y axis blank or enter zero for the y axis line.

For non-square images then enter the true length for both X and Y lines. Note that in the example above, the X scale has divisions of 0.1mm and the Y scale has divisions of 0.1, which is 254 um.

If using a single diagonal line, enter the same line number for both x and y. Enter the true x and true y distances.

Tick Set as default magnification and press OK.

The current magnification setting is shown in the status box.

Field of View (FOV)

Overview

The full Field of View for a particular device and lens combination is fixed. It depends on the optical magnification of the lens, the camera sensor size, video format, and the frame grabber digitisation.

Comparison of various CapiScope configurations is shown in the table below.

	mag (um/pixel)		pixels		FOV L300 6.3x	lens (um)	FOV L100 3x le	ens (um)	fps	filesize
	L300 6.3x lens	L100 3x lens	x y	,	x y	1	x y	1		Mbyte/second
HVCS std	0.925	1.93	752	480	696	444	1451	926	30	10.3
½ resolution	1.85	3.86	376	240	696	444	1451	926	30	2.6
HVCS hires	0.81	1.69	1280	1024	1037	829	2163	1731	25	31.3
½ resolution	1.62	3.38	640	512	1037	829	2163	1731	25	7.8
CAM1/VCS PULSAR										
CCIR (PAL)	1.20	3.40	768	512	922	614	2608	1739	25	9.4
RS170 (NTSC)	1.25	3.54	640	480	800	600	2264	1698	30	8.8
CAM1/VCS PX610										
CCIR (PAL)	1.20	3.40	768	572	922	686	2608	1943	25	10.5
RS170 (NTSC)	1.25	3.54	640	480	800	600	2264	1698	30	8.8
CAM1/VCS METEORII										
CCIR (PAL)	1.20	3.40	768	576	922	691	2608	1956	25	10.5
RS170 (NTSC)	1.25	3.54	640	480	800	600	2264	1698	30	8.8
CAM1/VCS FALCON										
CCIR (PAL)	1.20	3.40	768	576	922	691	2608	1956	25	10.5
RS170 (NTSC)	1.25	3.54	640	480	800	600	2264	1698	30	8.8
CAM1/VCS A602f										
	1.44	4.08	640	480	922	691	2608	1956	30	8.8
CAM1/VCS PIXELINK										
CCIR (PAL)	1.44	4.08	768	576	1106	829	3130	2347	25	10.5
RS170 (NTSC)	1.42	4.02	640	480	909	682	2572	1929	30	8.8

The FOV for capture display can be reduced changing the "grab image size" in the settings menu. Useful for producing recordings for comparing with another version of CapiScope or another manufacturers device.

The FOV for video recordings can be reduced further using the Region of Interest (ROI). Useful for the same reason above and also for reducing video file sizes and recording data rate, but still enabling the full FOV to be seen during recordings.

Video file sizes or recording data rate can also be reduced by using the "half resolution" option in the settings menu. Useful if you still want the full FOV.

Region of Interest (ROI)

The Region of Interest (ROI) is useful for performing image adjustments only only part of the image or for reducing the FOV used for video recordings and hence the video file size.

Click on the ROI toolbar button to draw the Region of Interest. Size is shown in the status box as you draw it.

The ROI rectangle can be dragged using the mouse.

A ROI drawn on the Live/Frozen Video window is saved between sessions.

Video recordings will only record the ROI. The whole image is recorded if there is no ROI. The ROI can be moved during video recordings if required.

When changing the resolution using "half resolution" in the settings menu, the ROI will keep the same pixel size. You'll need to redraw the ROI if you want the same actual tissue area.

Use the ROI button and one mouse click to clear the ROI.

Grab Image Size

The size of image captured and displayed can be reduced using the "Grab Image Size" option in the settings menu.

Enter the new size in pixels or "calibrated" units. This reduces the FOV and size of images and video but keeps the same optical resolution and magnification (um/pixel).

The grab image will be centred in the full sensor area.

A value of zero will use the whole sensor area.

The setting is saved between sessions.

The grab image size is stored as pixels, so changing the resolution using the "half resolution" setting will keep the same grab image size in pixels, but not the actual image size in um.

Half Resolution

The half resolution option in the settings menu turns on "binning" in the camera sensor. This will halve the x resolution and the y resolution. Therefore the area will be 25% of the full resolution. Each pixel will be the sum of four pixels so will have greater sensitivity and light level will need to be reduced.

Optical resolution will be reduced too and the magnification (um/pixel) setting will be automatically halved/doubled.

This is useful for reducing video file sizes and data bandwidth whilst maintaining the same FOV.

Zoom In 🖳 / Zoom Out 🔍

This changes the size of the image as displayed on the screen only. It doesn't have any effect on images or video recorded, or any measurements, calculations, or processing functions.

High Resolution Frame Rate Problems

Slow computers or using other USB devices may cause problems with displaying and capturing video using the full High Resolution HVCS. Slow hard disc drives may also cause dropped frames when recording video in high resolution. Video file sizes will be very large too in high resolution mode.

These problems can be reduced using one or more of the following:

- 1. Remove other USB devices.
- 2. Use half resolution mode.
- 3. Use the Grab Image Size option to reduce the image size and USB data bandwidth.
- 4. Use a ROI to reduce recording to video file sizes and/or dropped frames.
- 5. Use frame skip in the image sequence settings dialog box to reduce recorded video frame rate and hence video file sizes and/or dropped frames.

Dimension Lines

Overview

Dimension lines can be added manually or automatically for counting, or diameter, velocity or area measurement. There are several types of dimension lines from simple single points to polygonal areas.

Each image in CapiScope can have three separate dimension lists. Measurement output using the = key only include dimensions in the active Dimension List.

Dimension drawing tools



Click on the measurement toolbar button to reveal all the dimension drawing toolbar buttons.

Counting

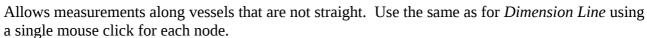
For manually counting capillary density. Position mouse over each capillary and click.

Dimension Line 🗹

For measuring vessel diameters, lengths and blood cell velocity.

Click (left mouse button down then up) for first node then second click for the end node. Line x and y length is shown in the status box as it is drawn.

Polyline 🕮



Freehand Line 🔼

Draw a freehand line along vessel. Click down and hold left mouse button down to draw along vessel.

Freehand area

Draw freehand line around capillary loop or other object. Use the same as *freehand line*. When mouse button is released, the area is automatically closed from last node to first node.

Polygon 🖸

For drawing straight edged areas. Click for each node. Double click or press **esc** key to end. Shape is automatically closed after final node.

Circles 🕒

For manually measuring areas of capillaries or capillary loops with user estimated circular area. Single mouse click at each end of diameter of circle.

Find Width 🛨

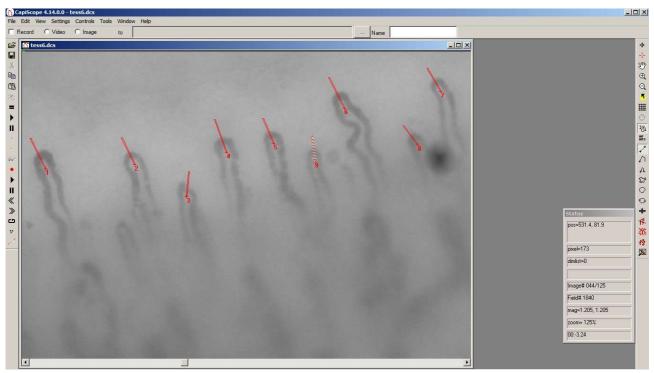
To automatically measure vessel diameter using active dimension line. Line must be perpendicular to vessel and start and end on background tissue.

Nailfold Calculations. Measure loops

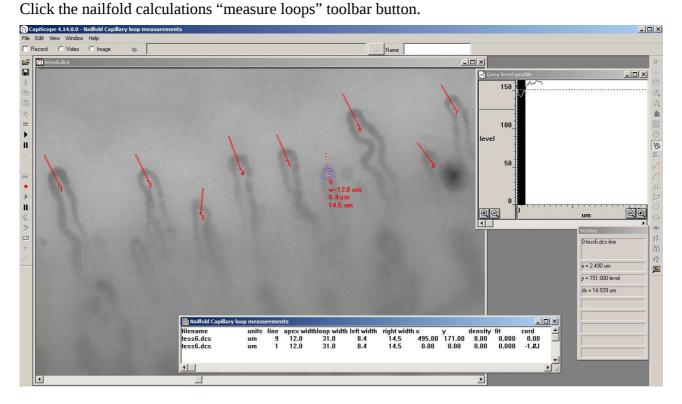
Draw dimension lines across the apex of each capillary loop in the final row of the nailfold.

The start point for each line should be inside the loop and will be the point where the capillary loop width and arterial and venous capillary diameters will be measured.

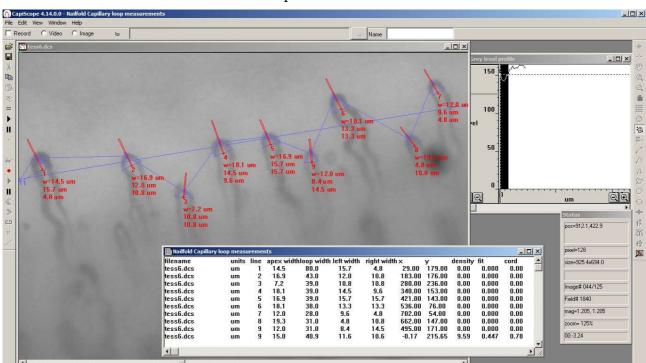
Any loops where the inside of the loop is not obvious, use the counting dimension toolbar button and mark the apex of these loops.



If a dimension line is selected (shown as red/white line) only that line will be measured.



Press escape to end the line drawing mode and click anywhere so that no lines are selected. With no dimension lines selected then all loops will be measured.



Click the nailfold calculations "measure loops" toolbar button.

The nailfold capillary loop measurements window shows the list of loop measurements. Individual capillary apex width, left and right limb widths and loop width are shown.

The final line shows average values for all loops. A straight line fit is drawn though the apex points in case the nailfold is not completely horizontal in the image, and the capillary/mm density is calculated along this line.

The Fit value shows how close the apex points are to a straight line. 1 = perfect fit.

Cord is the ratio of the length between leftmost and rightmost loops and the total length between each apex.

To reduce any spurious measurements, increase the dimension line widths or perform a low pass filter on the image.

Trace Capillaries 🌋

Automatically thresholds individual capillaries or vessels. Click on capillary to start thresholding.

Find Capillaries 🏂

Locates capillaries by iterative grey level thresholding in ROI or whole image if no ROI is set.

Use the = key or button to get capillary density results.

Image filtering and size limits can be set in *Settings*, *Processing*.

Dimension Lists

To step through the lists use the 'd' key. The currently active list is shown in the status box. Each list has different colour.

Selecting and Editing Dimension Lines

Activate a dimension line by clicking on the first node (small cross at start of line and next to the line number). The active dimension line will be shown in the status box together with its length or area.

The line can be dragged to a new position by holding the mouse down on the first node.

The line can be rotated by holding down the **ctrl** key then dragging the first node.

Activate the next, or previous dimension line using the TAB or SHIFT + TAB keys.

Pressing the DELETE key will delete the active dimension line.

To delete all dimension lines in the active list, use *Edit*, *Clear All dimensions*.

Dimension Line Width

To change line width, right click on first node and select *Set Line Width* from the popup menu. All new lines will also be drawn with the new line width. Width is in *pixels*.

Image Results Output

Image measurement results are produced by pressing the = key or button.

An "Image Results" window will be opened, or if already open, then measurement results will be added to the bottom of the list.

filename

Name of image or video that measurement was taken from.

units

User units of image and measurement results. i.e. pixels or μm .

ROI w

Width of area used for measurement. Width of ROI if set, or whole image. In *units*.

ROI h

Height of area used for measurement. Height of ROI if set, or whole image. In units.

ROI area

Area of image used for measurements. Area of ROI if set, otherwise area of image. In *units*².

Av. Level

Average grey level over measurement area. Range is 0-255.

min

Minimum grey level.

max

Maximum grey level.

list

Dimension list used for measurements.

Ν

Total number of dimensions in measurement area.

total L

Total length of all dimensions in measurement area. In *units*.

Av. L

Average length of all dimension lines in measurement area. i.e. $Total\ length\ /\ N$. Note that this will also include zero values from dimensions which do not have length. In *units*.

total Area

Sum of areas of all dimensions in measurement area. In *units*².

Av. Area

Average area of all dimensions in measurement area including dimensions which have zero area. In *units*².

density (%)

Dimension density in measurement area as a percentage. *Total area / ROI area*. Dimensionless.

Count density

Density of single point *counting* dimensions in measurement area. *Count / ROI area*. In number per 10^6 *units*². i.e. **mm**² if *units* is in **µm**.

Dim count density

Same as *Count density* above, except using all dimensions.

Linear density

Intended for measuring density of vascular networks. *total L / ROI area*. In *units* per 10^6 *units*². i.e. **mm** / **mm**² if *units* is in **µm**.

Tortuosity0

A simple tortuosity measure of the ratio of vessel length to chord length, where chord length is the shortest straight line length between the two end points. Dimensionless. See Tortuosity.

Tortuosity1

A more complex measure of tortuosity using a method described by *Enrico Grisan*. In 1/ units i.e. μm^{-1} if units is in μm .

Tort1 curves

Number of detected curves used in the *Tortuosity1* calculation. Dimensionless.

Velocity Measurement

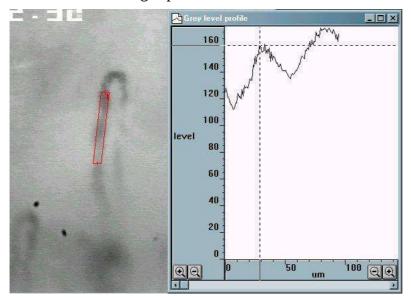
Theory

The dynamic capillaroscopy option enables capillary blood cell velocity to measured from live or recorded video using a spatial correlation technique.

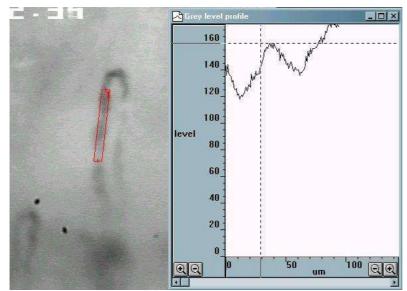
Measurements can be made in real time, directly from the subject. However, it is usually more convenient to record onto good quality video tape or, for best quality, directly into the computer memory, or for longer sequences, directly to the hard disk. This also allows more than one vessel to be measured from the same image sequence. Note that the digitised video is not compressed, and will use about 10 Mbyte per second for a full frame.

Velocity is measured by using the mouse to draw a line along the vessel. The vessel can have any orientation, and does not even need to be straight, although straight vessels are more likely to give good results. The line thickness can be adjusted so that it covers the width of the capillary. This gives an average of the pattern over the whole width. i.e. the image of the vessel is projected onto a 1 pixel thick line. Note that the image itself has already effectively projected 3D into a 2D image (thicker vessels appear darker), and the averaging across the width of the vessel reduces the 2D information down to 1 dimension.

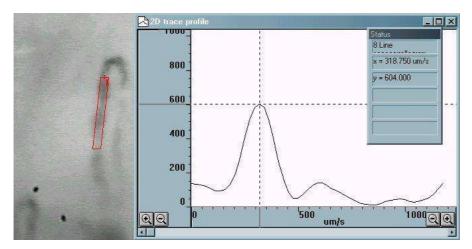
The grey level profile along the line is taken for each field every 1/50th of a second (1/60th second for NTSC based systems). Note that standard video formats have 25 (or 30) frames per second, but each frame is made from 2 interlaced fields. Even numbered lines make the even field, and odd numbered lines make the odd field. Usually, each field is captured seperately and then sent whilst the next field is being captured.



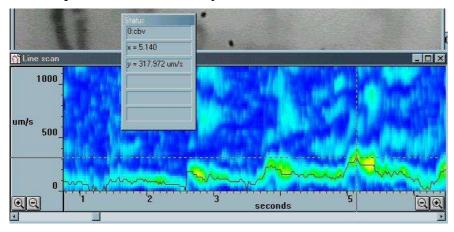
The grey level pattern along each line is compared to the pattern from the next field (or several fields later for very low velocities).



The comparison is performed by calculating the correlation coefficient for every possible shift of the previous grey level profile relative to the new profile. The shift which produces the highest correlation, and which can be seen as the peak in the correlation (the y scale is multiplied by 1000) in the following figure, indicates the distance that the pattern travelled between the two grey level profile measurements.



Since the time lapse between the two grey level profiles is known (ie 1/50th second) the velocity is easily calculated. CapiScope displays the correlation, along with the velocity trace (cbv) as a colour map, showing red for a high correlation through to blue for low correlation. If the correlation is below a preset limit then it is rejected, and a zero cbv value is set at that point.



Making Velocity Measurements

Measurement Line

The velocity calculation requires a measurement line. Most of the time, CapiScope can determine which line to use, but in the case of several dimension lines, or several video sequences open at the same time, you will need to specify which line to use.

Right click on the first node of the line. This should open a small menu. Click on "measurement line" to make this line the current measurement line.

Warning

All data in the linescan window will be lost without warning when switching to another line.

There are a few points to consider when creating measurement lines.

• Maximum velocity is half the line length multiplied by number of fields per second (i.e. 50 or 60). This is reduced if the <u>sample rate</u> or number of <u>linescan samples between</u>

correlations are not both 1.

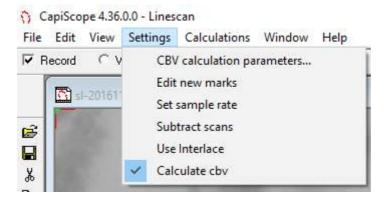
- A straight line avoids the distortions which occurs at bends.
- Horizontal lines have twice the resolution as vertical lines for interlaced video.
- Using a line which is wider than the vessel, might help when there are movement artifacts, but the signal, and hence correlation will be poorer, since it will be averaged with useless background information.
 - If the vessel is large (> 30 um) then make the line cover only the central third of the vessel, otherwise the correlation will tend to pick out the slower rolling leukocytes along the vessel wall.
- The line needs to be at least twice as long as typical pattern (erythrocyte gap or leukocyte). Note that the resolution of the cbv velocity measurement is half the length of the measurement line.

Movement

It is important to have no movement in the video sequence. If the subject moves, it is possible to reposition the line by dragging the first node of the measurement line using the mouse and left mouse button. This can be done whilst the sequence is running, or by stopping the video sequence, repositioning the measurement line, then resuming the sequence by clicking on the play button.

Linescan input settings

There are several settings which control and effect the capture of the video data for the velocity correlation measurements. These are found in the Settings menu whilst the Linescan window is active.



Calculate cbv

This menu item needs to be checked to enable cbv calculation in the linescan window. If it off, then only the grey level pattern along the line is recorded.

Subtract scans

This can be used for most measurements except very slow flows. When checked each grey level profile is subtracted from the grey level profile of the previous field. This removes any static pattern which might give an overwhelming zero velocity correlation. The newer high pass option in the CBV calculation parameters will give more options and also can be switched on/off without having to resample the video.

Set sample rate

The grey level profile along the line is, by default, sampled on every field of the video sequence. This setting allows a lower sampling rate for lower velocity measurements. It is recommended to leave this at the default value of 1 unless you are measuring for a long period (eg directly from the "live video" window with truly live video or from video tape) and so need to reduce the memory requirements of the linescan window.

Use Interlace

When ON, this option will sample the ODD lines, and then the EVEN lines so producing two linescans per video frame. This will effectively double the maximum velocity range when used with interlaced video.

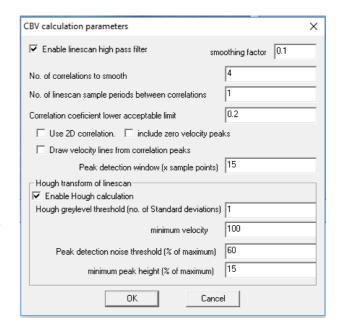
CBV calculation parameters

This dialog box enables fine tuning of the velocity measurement. Correlation and velocity will be recalculated when clicking on OK.

Number of correlations to smooth

This provides a smoothing function to the correlations. Without smoothing, the cbv trace would have many intermittent short spikes. Increasing the number of correlations smoothed, reduces the peak correlation coefficient. Therefore the correlation coefficient limit will need to be adjusted to a lower value.

Linescan sample periods between correlations



Increase this value for lower velocities, to improve resolution. Use an even number for interlaced video so that correlations are performed on the same ODD or EVEN field.

Correlation coefficient lower acceptable limit

Use this parameter to remove noise from low correlations. Values below this limit will give an invalid cbv value. Note that invalid values are not included in the average calculations (but this can

be changed see artefact filter).

Use 2D correlation

When OFF, the grey level across the width of the linescan dimension line is averaged to give a single value for each pixel along the dimension line. These are the values used for the high pass filter and the correlation.

When ON, the grey level for each pixel across the width is used in the high pass filter and the correlation. If the vessel is not straight, and the linescan dimension line is wider than the vessel, then there will be a detrimental effect on the correlation.

Include zero velocity peaks

When OFF then detection of the correlation peak will ignore the peak at zero velocity.

When ON, then the zero peak will also be included in detection of the maximum peak.

Draw velocity lines from correlation peaks

Enable automatic drawing of velocity slope lines. Peaks in the correllogram are used to draw the lines.

Enabling this option automatically disables the Hough transform.

Peak detection window

Window width used for detecting maxima in the correlation trace. Value is width in x data points. Only one velocity line will be drawn for the window width.

Hough Transform of linescan

This enables automatic detection of velocity slope patterns in the greylevel linescan. This creates a Hough transform from the high pass filter of the linescan. Peaks in the Hough transform correspond to potential lines detected in the linescan. Velocity slopes are created from peaks found in the Hough transform.

Automatically turns off velocity slope drawing using the correllogram.

Hough greylevel threshold

This determines which pixels from the difference trace are candidates for line detection. The threshold value is the greylevel value above the average in multiples of standard deviation of the difference trace greylevel. A lower value will increase the number of pixels used and increase sensitivity but could also increase false detections.

Minimum Velocity

Velocity slopes below this value are ignored and not drawn.

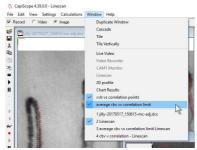
Peak Detection Threshold

This is the minimum level considered as a peak in the Hough transform. Value is percentage of the maximum peak found in the Hough transform.

Minimum Peak Height

This sets the minimum peak height above surroundings before a peak is considered as a velocity line.

Average cbv vs correlation limit



Use the average cbv vs Correlation limit to open a plot of average cbv, maximum cbv, minimum cbv, and data points used vs correlation limit.

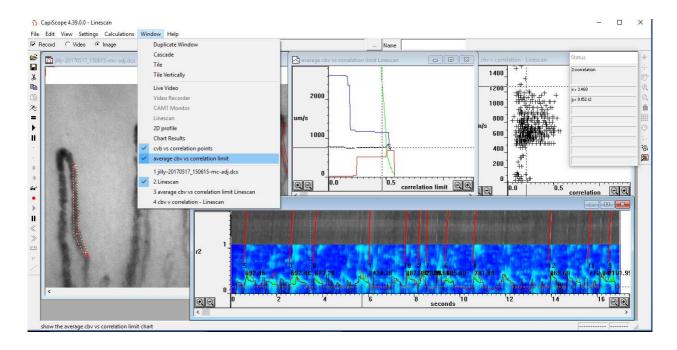
Black trace = average cbv

Blue trace = maximum cbv

red trace = minimum cbv

green trace = number of data points above correlation limit

Use this to help determine the best correlation coefficient limit.

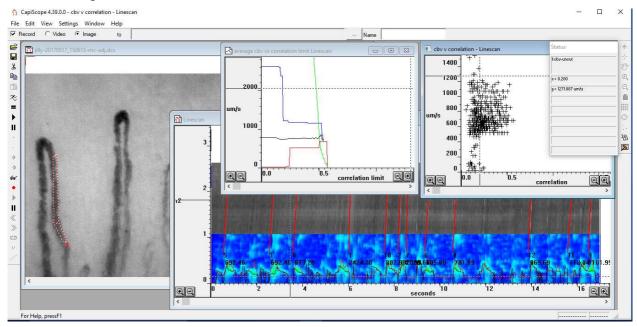


cby vs correlation



Use the cbv vs Correlation to open an x-y scatter plot of cbv vs correlation.

Use this to help determine the best correlation coefficient limit.



Movement Correction

Introduction

Movement artifacts in video sequences can be corrected either automatically, or manually by dragging the mouse. Individual images, selected images, or the whole video sequence can be corrected. These functions can be accessed using the Tools menu when a video sequence window is active.

Although good results can be obtained, it always much better to try and make sure there is no movement in the first place, especially if velocity measurements are going to be made. For fast movements it will probably be better to split the images from frame into fields. There will still be bluring of the image, but this can be reduced beforehand by increasing the shutter speed on the CCD and increasing the light level to compensate.

Automatic Movement Correction

This function will try to automatically correct any movements in the video sequence. It uses a least squares fit pixel by pixel so it can take quite some time processing full frame video. It is best to experiment on a short (say 5 second) video sequence to get a feel for the performance on your computer. When selected, a dialog box allows some parameters to be adjusted so that you can trade off between processing time and correction accuracy.

Manual Movement Correction

There are two methods of manually correcting movement, "Tracking" and "Dragging"

Tracking

This function allows you to track an identifying feature on the image using the left mouse button. It is better than the Dragging function for manually correcting movement in video sequences.

You may also want to slow down the playback speed to make it easier to react.

Move the video to your desired start position, then click down the left mouse button on some feature in the image that you can follow with the mouse. If you want to correct a sequence of images, keep the left button down, so that the total correction so far is remembered for following images, then press the 'P' key to play the sequence. Follow the movement with the left mouse button down as the video plays. Release the left mouse button as soon as you want to stop corrections. If the image has stopped moving around, you will probably still want to keep the left button depressed until you reach the end of the video so that the remaining images are corrected too.

Note that this method uses the distance of the mouse from the position of the left button click as an offset to adjust to the current image's offset. When the button is released, no offset is applied. When you click again, a new offset correction is started.

Dragging

This function allows you to drag the image into the correct position using the left mouse button. It is probably most useful for correcting individual images, but can be used on sequences. If using on a sequence, it helps to have a dimension mark or line marking a feature than then be draged back to the marking dimension line.

You may also want to slow down the playback speed to make it easier to react.

Move the video to your desired start position, then click down the left mouse button and drag the image to its corrected position. If you want to correct a sequence of images, keep the left button down, so that the total correction so far is remembered for following images, then press the 'P' key to play the sequence. Drag the image into position as the video plays. Release the left mouse button as soon as you want to stop corrections. If the image has stopped moving around, you will probably still want to keep the left button depressed until you reach the end of the video so that the remaining images are corrected too.

Note that this method uses the distance of the mouse from the position of the left button click as an offset to adjust to the current image's offset. When the button is released, no offset is applied. When you click again, a new offset correction is started.

Reset movement correction offsets

Use the "Reset offsets" function in the "Edit" menu to clear all offsets back to zero. This works wether the offsets were created by the automatic or manual methods.

To clear just a range of images, first set the start and end image numbers in the "Edit", "Select images...".

Video Tools

Selecting Video start and end

Many of the video sequence tools work either on the whole video sequence or only a selected part of the video.

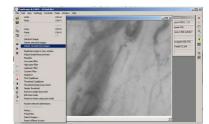
To select only part of the video, use *Edit*, *Select images*. Enter the image number of the first and last image.

Image numbers are displayed in the status box. Values of -1 will use first and last image for start and end respectively.



Trimming video (Deleting images)

Either the currently selected part of the video can be cut, or the video can be trimmed down to just the selected portion. Select the range as described above then use *Edit*, *Delete selected images*, or *Delete nonselected images*.



Average Whole sequence

Will create a new image with an average of the video sequence from START to END. If movement correction has been used, then the new image will be larger to include all movement.

Useful for sparsely filled capillaries.

Movement Correction

Movement artifacts in video sequences can be corrected either automatically, or manually by dragging the mouse. Individual images, selected images, or the whole video sequence can be corrected. These functions can be accessed using the Tools menu when a video sequence window is active.

Although good results can be obtained, it always much better to try and make sure there is no movement in the first place, especially if velocity measurements are going to be made. For fast movements it will probably be better to split the images from frame into fields.

Automatic movement correction

This function will try to automatically correct any movements in the video sequence. It uses a least squares fit pixel by pixel so it can take quite some time processing full frame video. It is best to experiment on a short (say 5 second) video sequence to get a feel for the performance on your computer. When selected, a dialog box allows some parameters to be adjusted so that you can trade off between processing time and correction accuracy.

Functional Capillary Density and Flow $\stackrel{\nu}{=}$

This is an experimental measurement which produces an image proportional to velocity. It is dependant on image contrast and relies on movement in the vessels such as gaps and white cells. It is calculated using similar processing as in laser Doppler blood flow monitors/imagers, but using the grey level fluctuation at each pixel position instead of laser Doppler shift.

This function uses either the whole video sequence or the selected image range.

You need very stable video for this to work well. Use the "tools" "automatic movement correction" first.

Threshold

Use the F3 and shift F3 function key to shift the threshold up and down.

To find a suitable threshold level, draw a small ROI (yellow box) say 35x35 (you can see the size in the status box as you draw). You can drag this box onto a vessel with flow then click =.

Note the conc value.

Drag the same ROI box into a obviously non-vascularised area. press =.

Note the new conc value.

Take the average of the 2 values.

Choose the menu "controls" "recalculate" and enter the average for the threshold and click ok.

Draw ROI and press "=" to get values for functional flow and density.

FCF = functional capillary flow

FCF = Flux / Conc in arbitrary units. This is calculated pixel by pixel where the conc value is greater than or equal to the threshold. Pixels where the conc is less than the threshold are set to zero.

FCD = functional capillary density

Density of detected functional capillaries / area of ROI as a percentage. Detected functional capillaries are where the conc value is greater or equal to the threshold value.

Flux

Calculated per pixel, this is

flux = rms of differential of grey level against time in arbitrary units

(as in the Watkins and Holloway laser Doppler flux monitor. Watkins, D. W. and Holloway, G. A. (1978) An instrument to measure cutaneous blood flow using the Doppler shift of laser light. *IEEE Trans.*, **BME-25**, 28–33.)

Conc

Calculated per pixel this is

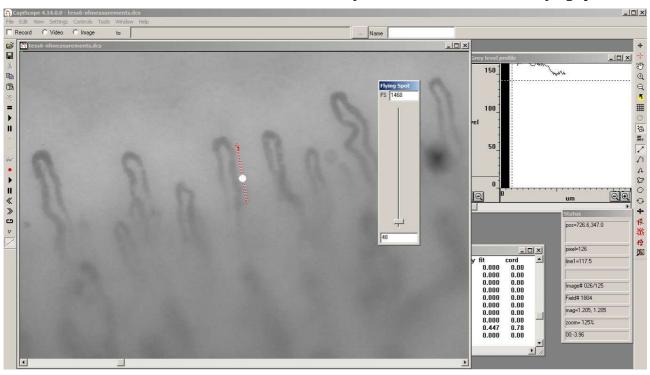
conc = rms of grey level against time in arbitrary units

Flying Spot

Use the loop play toolbar button to play the video in a continuous loop and draw a dimension line next to the vessel.

Click on the flying spot toolbar button.

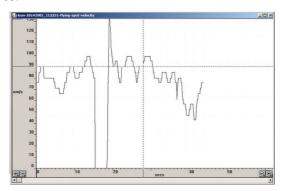
A dimension line needs to be selected, and the currently selected line will have the flying spot.



Use the flying spot slider to adjust the velocity of the flying spot. Whilst the slider is being adjusted, the flying spot velocity is saved for each frame. The flying spot velocity can be continuously adjusted throughout the video.

Corrective adjustments can be made by readjusting the slider at any point. If you "let go" of the slider it will use the previous velocity set for each frame (initially zero).

Use the Tools menu "Chart flying spot velocities" to show the flying spot velocity over time. The velocities are saved with the video sequence. There is only one velocity recording irrespective of the number of dimension lines.



Compress Video Output

In the Settings menu use the compress video output option to cause all video output to disk (live recordings, processing and file saves) to be compressed. This requires the x264vfw CODEC to be installed.

To avoid the first 90 odd frames from being blank, change the x264vfw CODEC default configuration. Check the Zero Latency check box.



You can also change the compression quality etc, but the default settings work very well.

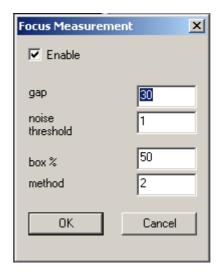
Focus Measurement Settings

Enable

Enable measurement of focus quality for each image.

Gap

For methods 2 and 3, this is the number of pixels to use between background pixels and current pixel. Not used for method 1.



Noise Threshold

When measuring the difference between background and current pixel, ignore any differences below this noise threshold value.

Box %

Specify the size of the measurement window to use as a percentage of the whole image. Window is centred. The average over the whole window is used.

The measurement window $+ 2 \times gap$ must be larger than the image size otherwise no measurement is made.

Method

Focus measurement method to use:

0: no measurement.

1: difference between adjacent pixels squared. $F = (p_x - p_{x-1})^2$

2: use two background pixels, at x-gap, and x+gap. $F = p_{(x-gap,y)} + p_{(x+gap,y)} - 2 p_{(x,y)}$

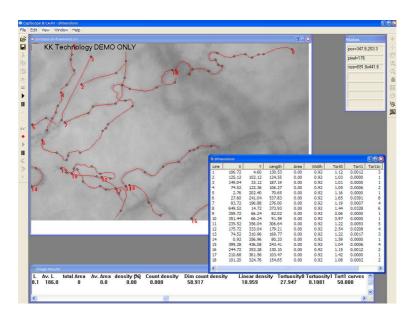
4: use four background pixels, at x-gap, x+gap, y-gap, and y+gap.

$$F = p_{(x-gap,y)} + p_{(x+gap,y)} + p_{(x,y-gap)} + p_{(x,y+gap)} - 4 p_{(x,y)}$$

Tortuosity

All freehand or polyline dimension lines with at least three points are analysed for tortuosity. Draw a line along the centre or edge of each vessel from end to end.

Two tortuosity methods are used:



Tort0

This is a simple tortuosity measure of the ratio of vessel length to chord length, where chord length is the shortest straight line length between the two end points.

$$\tau = \frac{L}{C}$$

This is a dimensionless measure.

Tort1

This uses a method described in: Enrico Grisan, Marco Foracchia, Alfredo Ruggeri. A novel method for automatic evaluation of retinal vessel tortuosity. Proceedings of the 25th Annual International Conference of the IEEE EMBS, Cancun, Mexico, 2003

$$\tau = \frac{N-1}{L} \cdot \sum_{i=1}^{N} \left(\frac{L_i}{S_i} - 1 \right)$$

The dimension line is split into N curves. The start/end of each curve is found by a change in curvature on the line. Then sum of the ratios of curve length to chord length (L_i/S_i) is used to determine total tortuosity.

Dimensions are 1/ length i.e. μm⁻¹

Configuration ini file settings are used to reduce noise:

[tortuosity]
minlength=10
minangle=5
mincurvelength=20.0
log=0

where:

minlength	Minimum length (in μm) before testing for change of angle
minangle	Minimum change of angle (in degrees). Only angle changes above this value are considered.
mincurvelength	Minimum curve length (in μm) between changes in curve.
log	0= no logging. 1= log angle and length calculations to the log file.

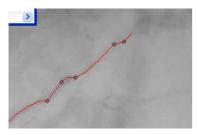
These configuration settings are read each time a Capiscope image file is opened. If you want to experiment changing these values, edit the ini file (same base filename and folder as the executable, e.g. c:\KKTechnolgy\CapiScope\kkcapiuSC.ini).

Result Output

Individual Tortuosity results are shown in the "Dimension List". Tort1n is the number of curves used in the tortuosity calculation. The Dimension List window is displayed by the menu "Windows", "Dimension List".

The Dimension list can be saved using "File", "Save". The file is a standard "csv" comma separated variable text file which can be opened in Excel or OpenOffice.

The total tortuosity for the whole image is calculated by using the toolbar button or the "=" key. Data in the "Image Results" window can be selected, copied and pasted into other applications such as Excel.

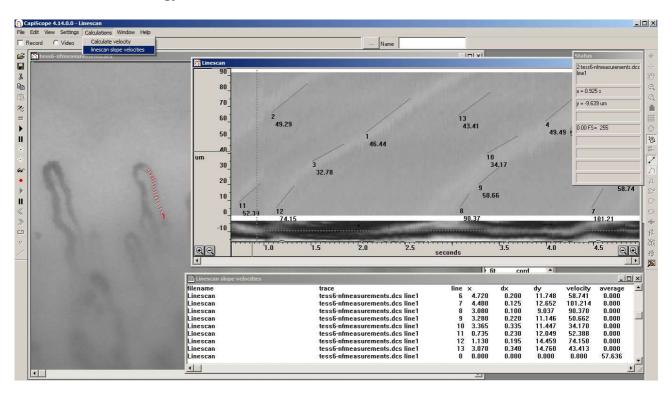


Changes in curvature direction are shown as black diamonds on the dimension lines. There might be more marks than indicated by the tort1n value since the curvelength value might be less than the configuration minimum for some of them.

Linescan Window

Velocity slope measuring lines

Use the Dimension Lines toolbar button to draw lines along slopes in the linescan trace. Use the menu Calculations linescan slope velocities function to output a list of velocities and time of the first point for each line. The final line shows the average of all velocity measurements (not dependant on the length of the measurement lines).



Lines will be deleted when restarting play of the video, or selecting a new measurement line. Lines will be saved with the linescan window. If inserting more than one linescan into a linescan chart, the lines use the scaling and offset values of the first linescan trace found (i.e. using ygroup 111).

Function Reference

Menu Commands

File Menu commands

New command (File menu)

Use this command to create a new Image, Video or Chart document in CapiScope. You can open an existing document with the <u>Open Command</u>.

Shortcuts Toolbar: Keys: CTRL+N

Open command (File menu)

Use this command to open an existing document in a new window. You can create new documents with the New Command.

Shortcuts Toolbar: Keys: CTRL+O

Close command (File Menu)

Use this command to close all windows containing the active document. CapiScope suggests that you save changes to your document before you close it. If you close a document without saving, you lose all changes made since the last time you saved it. Before closing an untitled document, CapiScope displays the <u>Save As dialog box</u> and suggests that you name and save the document.

Save command (File Menu)

Use this command to save the active document to its current name and directory. When you save a document for the first time, CapiScope displays the Save As dialog box so you can name your document. If you want to change the name and/or directory of an existing document before you save it, choose the <u>Save As Command</u>. By default the image will be saved with the same name as the CapiScope document, but with a kkg extension (a KK Technology format). If you want the image to be saved with a different filename and/or format then use the Edit, Notes... function to change the image filename before saving the document. Note only the kkg format is supported for saving the ROI.

See Export.

To always save in a different format, edit the profile(s) (ini files in the program folder) using notepad. Add the following line:

imagetype=.bmp

Currently, the available formats are kkg (default KK Technology), bmp (windows bitmap), and tif (tagged image format).

Video sequence data is saved with a .mve extension (a KK Technology format). To save in an avi format use the Export as avi command (File menu)

Shortcuts Toolbar: Keys: CTRL+S



Save As command (File menu)

Use this command to save and name the active document. CapiScope displays the Save As dialog box so you can name your document. To save a document with its existing name and directory, use the <u>Save command</u>. To save an image in a different format see the <u>Save command</u> and the <u>Export</u> command.

Export (Dimension List)

Use this function to save the dimension list as a TAB separated text file which can be easily opened as an Excel spreadsheet. see Also Export Image

Export (Image)

Use this function to save either the whole image, or if you currently have a Source ROI, just part of the image to disc. You will be prompted for a filename for the new image file. Only the kkg format image files are fully supported at present. TIFF and BMP files are only supported as whole images, NOT the ROI. If the currently active window is a chart or Dimension list, then the data from that

will be exported. See **Export Dimensions**

Export as avi

Use this function to export the video sequence as an avi format file. You will be prompted to select one of the available CODECs (COmpressor/DECompressor) available on your computer. Note that not all CODECs can compress, some are just decompressors, and also, some may not be available on other computers; they may have been installed with another application. For uncompressed output, click on cancel.

See COMPRESSION

Import avi

Use this to import avi files into a video sequence. The CODEC used to create the avi file needs to have been installed on the computer. If it runs in the Microsoft Mediaplayer, then it should load. Some avi files with compressed video may have incorrect headers and will not be decompressed. Mediaplayer probably guesses in these cases, but CapiScope will only correctly formatted files.

Import Image

Use this function to import an image into the currently active image. If the <u>ROI</u> is not empty, then it is possible to import into the destination ROI. The image size is not changed, or clipped by the destination ROI, but its top left corner is aligned with the destination ROI top left hand corner.

Open Cytoscan file

See the OPS Analysis User Guide for details.

Print Preview command (File menu)

Use this command to display the active document as it would appear when printed. When you choose this command, the main window will be replaced with a print preview window in which one or two pages will be displayed in their printed format. The print preview toolbar offers you options to view either one or two pages at a time; move back and forth through the document; zoom in and out of pages; and initiate a print job. Note the image will usually be printed with much better colour resolution than shown in preview mode.

Exit command (File menu)

Use this command to end your CapiScope session. You can also use the Close command on the application Control menu. CapiScope prompts you to save documents with unsaved changes.

Shortcuts Keys: ALT+F4

Edit menu

Cut command (Edit menu)

NOT IMPLEMENTED YET FOR IMAGES - only available for charts. Use this command to remove the currently selected data from the document and put it on the clipboard. This command is unavailable if there is no data currently selected. Cutting data to the clipboard replaces the contents previously stored there.

Shortcuts Toolbar: Keys: CTRL+X



Copy commad (Edit menu)

NOT IMPLEMENTED YET - only available for charts. Use this command to copy selected data onto the clipboard. This command is unavailable if there is no data currently selected. Copying data to the clipboard replaces the contents previously stored there.

Shortcuts Toolbar: Keys: CTRL+C



Paste command (Edit menu)

NOT IMPLEMENTED YET - only available for charts. Use this command to insert a copy of the clipboard contents at the insertion point. This command is unavailable if the clipboard is empty.

Shortcuts Toolbar: Keys: CTRL+V



Clear All Dimensions

Use this command to clear all dimensions from the current document.

Notes

Use this to add notes to your CapiScope document. Also allows the image filename to be changed. The notes are printed with the image.

Smooth Selection Menu

Use this command to smooth the resulting Resistance Index trace

View Menu

View Toolbar command

Use this command to display and hide the Toolbar, which includes buttons for some of the most common commands in CapiScope. A check mark appears next to the menu item when the Toolbar is displayed. See <u>Toolbar</u> for help on using the toolbar.

Video Input Control

Use this command to Hide/Show the video input control.

Show Crosshair

Use this command to Hide/Show the Target Crosshair. (Used for marking the CAM1 laser beam position). To reposition the target crosshair, double click the right mouse button.

Grid

Use this to toggle the grid on or off. See **Grid Settings** to adjust spacing, style and colour of the grid.

2D View Menu

Always on Top

Use this to make CapiScope always visible, even if another application is active.

Settings menu

Calibrate Magnification

Use this to calibrate the total optical magnification. Before calling this, create two <u>straight dimension lines</u>. Using something of a known length, create one horizontal line to calibrate the x axis, and one vertical line to calibrate the y axis. (You can use the same object, rotating it 90 degrees after marking one of the lines). In the Calibrate Magnification dialog box, enter the line numbers for the x axis and y axis calibration lines, and also their known true length, using your prefered <u>user units</u>. The magnification scaling will then be calculated. To see the resulting values, use the <u>Magnification...</u> option in the Settings menu.

Magnification

This opens a dialog box showing the current magnification scaling factors for the x and y axis. Usually these are set automatically using the <u>Calibrate Magnification</u> function.

Units

This allows one to choose between using calibrated user defined units, or pixels. All dimensions will be displayed using the current setting. The label for the user units is just a text label and has no other significance. It is recommended to use units so that dimensions and cursor position etc will be in the range of 1 to 1000. This is so that significant digits are displayed in the dimension list, and status bar. See Calibrating Magnification.

Timer

Use this to open the Timer Dialog and alter the timer settings. Number of images and time between images can be set. Also the base filename can be set. Each image will be saved using the base name

plus two digits for the number in the sequence. If a file with the same name already exists, it will be overwritten without warning. The <u>Start Timer</u> button is disabled until the number of images has been set. TIP: a quick way to view the saved images is to open the Windows Explorer, and position it next to the CapiScope window. Then the image files can be dragged onto CapiScope from the Windows Explorer using the left mouse button.

Grid Settings

Use this to set the Grid style (lines or points), colour and spacing. The Grid always passes through the <u>Origin</u>. Use the <u>Grid</u> command to hide/show the grid.

Tools Menu

Pan Image

Use this to pan the image in its window, by dragging with the left mouse button. Note that it not possible to pan when the window is maximised. Also it is not possible to pan the image beyond the actual screen area. e.g. the top left corner of the image cannot be panned beyond the top left of the screen, even though it cannot be seen because it is outside the CapiScope window.

Region of Interest (ROI)

Use this to create a region of interest (ROI). Click and drag with the left mouse button to create the ROI. To move the ROI drag by holding down the left mouse button. To remove the ROI, select this function and just click the left mouse button.

Origin

Use this to set the origin (0, 0) in the image. All dimension starting points are relative to the origin. Also the <u>Grid</u> passes through the origin.

Counting

Use this mode to count capillaries by clicking with the left mouse button. A cross will mark each capillary counted, and the dimension list will show the location of each count. A count of the number of dimensions is shown on the status bar.

Dimension Lines

Use this to create straight dimension lines. Click with the left mouse button to add another node, double click or press escape key to end the line. The start position and total calibrated, or pixel length is shown in the dimension list.

Freehand dimension lines

Use this to create freehand dimension lines. Click with the left mouse button and drag whilst holding the left mouse button. The start position and total length in calibrated units or pixels is shown in the dimension list.

Grab Live Video

Use this to start and stop live video. The current image will be destroyed if it has not been saved. WARNING: there is no prompt to warn about losing the current image if it has not already been saved!

Freeze Video Input

Use this to freeze and capture the video image. The image is NOT automatically saved to disc.

Start Timer

Use this to start the capture and saving of a timed sequence of images. It is disabled until a valid number of images has been set. See <u>Timer...</u> for setting the timer.

Window Menu

New command (Window menu)

Use this command to open a new window with the same contents as the active window. You can open multiple document windows to display different parts or views of a document at the same time. If you change the contents in one window, all other windows containing the same document reflect those changes. When you open a new window, it becomes the active window and is displayed on top of all other open windows.

Cascade command (Window menu)

Use this command to arrange multiple opened windows in an overlapped fashion.

Tile command (Window menu)

Use this command to arrange multiple opened windows in a non-overlapped fashion.

Window Arrange Icons Command

Use this command to arrange the icons for minimized windows at the bottom of the main window. If there is an open document window at the bottom of the main window, then some or all of the icons may not be visible because they will be underneath this document window.

Dimension List

Use this to Show/Hide the Dimension List.

Help Menu

Click on Help to open this User Guide.

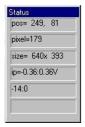
Click on About... to see what the current version of Capiscope you are using.

Toolbar

To hide or display the Toolbar, choose <u>Toolbar</u> from the View menu (ALT, V, T). The toolbar can be moved to any edge of the CapiScope window, or dragged away to become a floating toolbar. Click on the toolbar background with the left mouse button and drag to its new position. A floating toolbar can be resized by dragging its edge or corner.



Status Box



The status box displays information on the currently active window.

The indicators show (in order from top to bottom):

The cursor position in the currently selected coordinates. If not in pixels, then the coordinates will be in calibrated units.

Grey Level Image grey level (0-255) at current cursor position.

Object size, Image size, or ROI size in calibrated units or pixels.

Input voltage range. (black 0 to white 255).

Approximate Frame rate in frame per second.

Charts

Introduction to charts

Charts are documents that contain data in the form of <u>traces</u>. A chart can contain many traces. Traces can be moved between charts or duplicated, via the clipboard. TracecopyingTo copy a section of a trace to another chart, first select a section of the trace, copy to the clipboard, then paste into the other chart.

Traces can be smoothed (low pass filtered) and averaged. The results of average calculations are placed in *Results* documents. These can be saved to disc. Selected results from a Results document can be copied to a spreadsheet such as Excel via the clipboard.

Axes

Using the mouse

The x and y scales can be altered by clicking on the buttons on the respective axes.

The view of the data can be scrolled along the x axis by using the ScrollBar on the bottom of the Trace window.

The left and right arrow buttons on the scroll bar scroll all the traces by one pixel.

Clicking with the left button on either side of the scroll box, the traces are scrolled 3/4 of the visible view. Dragging the scroll box scrolls the traces accordingly.

The y scale can be offset by clicking on the y axis with the left mouse button and dragging up or down. The y axis controls only effects the traces in the same Y group as the currently active trace.

Using the Keyboard

The x axis scale can be increased or decreased by using the '+' and '-' keys.

Use the up and down cursor keys for the y axis scale.

Use the Page Up, Page Down, Home, End, cursor left and right keys to scroll along the x axis. See <u>Keyboard</u> below.

Traces

A trace window or file can contain more than one trace. Each trace has its own y axis, but only one y axis is visible at any one time. To activate a trace click on it using the left mouse button, or use the TAB key to activate the next trace. The currently active trace is indicated by its name displayed in the <u>Status</u>

Trace Properties

The trace properties can be altered by clicking the trace with the right mouse button or using the Trace Properties command in the edit menu. A <u>Trace Properties</u> Dialog box allows properties such as the trace name and trace colour to be altered. Note that some colours may not be printed on non-colour printers.

Selecting Trace Data

Click on the trace to select data by using the left mouse button. Drag the mouse whilst holding the left button down. Selected data will be highlighted.

Use the <u>Copy</u> Tool Bar button, <CTRL><Insert> keys or <CTRL>C keys to copy the data into the clipboard. In the current version this is stored in a private format which can only be used by CapiScope.

Use the '=' Tool Bar button to calculate <u>averages</u> etc. the results will be appended to the last activated Results window, or if no results windows are open then a new results file will be created.

Adding a Trace to a File

Use the <u>Paste</u> Tool Bar button or <SHIFT><Insert>, or <CTRL>V keys to insert data from the clipboard into the currently active trace window. If the window contains a marked selection then the data will be copied to that location, otherwise it is copied to the start.

Display Rate

Data can be displayed at any rate independent of the data rate. Additionally two or more windows can be used to show the data at different rates. For example use Window|Duplicate Window to produce two views of the CAM1 monitor window. One could be set to a slow display rate to show long term trends whilst using the other window at a fast display rate to show the pulsatile component.

Display rate is controlled by using the x axis magnify/minify buttons.

Edit

Edit Copy command

Use this command to copy selected data from the active trace onto the clipboard. This command is unavailable if there is no data currently selected.

Copying data to the clipboard replaces the contents previously stored there.

Note that when copying large 2D colour traces, there will be two copies in memory. Also if this is then pasted into another document, there could be three copies in memory. This could cause the system to become very sluggish. It is a good idea to copy a small section of a line trace into the clipboard to release at least one copy from memory.

Shortcuts: Tool Bar ; Keys: CTRLC, CTRLInsert

Edit Cut command

Use this command to remove the currently selected data from the active trace or the whole active trace if there is no data currently selected and put it on the clipboard.

The 'cut' data is set to zero in the trace if a selection was used (ie memory is still used).

The active trace is removed from the chart if no selection was made.

Cutting data to the clipboard replaces the contents previously stored there.

Shortcuts: Tool Bar .Keys: CTRLX; ShiftDelete

Edit Delete Command

Use this command to remove the currently active trace from the chart.

If part of a trace is selected, then the selection is set to zero, no memory is freed.

All unsaved data will be lost forever.

Not available in the monitor window.

Edit Paste command

Use this command to insert a copy of the clipboard contents at the insertion point or the beginning of the Chart (t=0) if there is no selection. This command is unavailable if the clipboard is empty.

Traces cannot be pasted into the Monitor window.

Shortcuts: Tool Bar: Keys: CTRLV, SHIFTInsert

Markers

Event Markers

Event Markers are small flags which can be used to mark events, artifacts, or Captured Images using CapiScope during a recording. Markers can also be added later to highlight a feature.

An Event Marker contains a label, which is shown on the flag, and a notelet, which can contain more detailed information. Event Markers are stored with the Chart, and are also Copied / Pasted to/from the clipboard together with the active trace.

<u>Image Markers</u> contain the filename of the captured image in the notelet.

Markers are added to a recording by pressing the space bar. This will generate a marker with a sequential number for the label, and will add the time and date to the notelet.

Alternatively, a marker can be created by pressing any key, the character from that key will be used for the label, and the time and date will be added to the notelet.

Image markers are added by pressing the F4 function key.

Markers can be added to a Chart by switching to Marker Mode and then clicking with the left mouse button to place a marker at the current cursor position.

Any marker can be edited by clicking on it with the right mouse button. This will open the Edit Marker box.

Markers can be edited as soon as they are created by enabling the Edit New Marks option in the Settings menu.

Edit Marker Dialog



This dialog allows the properties of the selected **Event Marker** to be edited.

The first line is the text displayed on the Event Marker flag, and the large edit box allows more descriptive notes to be added and saved with the marker. (Use <Ctrl> <Return> to add a newline to the note).

The marker is not updated until the Update button is clicked.

The dialog will show the properties of any newly selected marker if a different marker is selected whilst the Edit Marker Dialog is open. This makes it quicker to view the contents of many markers.

Edit New Marks

Enable this option to always open up the <u>Edit Markers</u> dialog box whenever a new marker is created. This is particularly useful if descriptive markers are required during recording. The suggested procedure is:

- (i) Press the spacebar to create the new marker. The markers position is set to the time at which the spacebar is pressed.
- (ii) Type in the label for the new marker and press return.

The only disadvantages using this method are that one or two data points may be lost whilst Windows creates the Edit Markers dialog box, and that the Doppler Spectrum trace is not redrawn when the dialog box is closed (WCAM1 disables the Doppler trace redrawing whilst recording to minimise lost data).

Note the new marker is not redrawn with the new label until recording is stopped.

Marker Mode

Use this to enable/disable the marker mode. When enabled, clicking the left mouse button will add an <u>Event Marker</u> to that position in the Chart.

Image Markers

If the KK Technology CapiScope imaging software is running, then images can be captured and saved to disc automatically from CAM1. A marker is also saved in the CAM1 chart, along with the unique filename of the captured image. Clicking on the marker with the left mouse button will reload the image into CapiScope automatically.

To create an image marker press the F4 function key.

The image will be saved in the current working directory of CapiScope.

The filename is created from the system clock which only has a resolution of 1 second. Therefore attempts to save more than one image in quick succession will result in the second image overwriting the first image.

Export data

Outputs trace data to a file in either binary or ASCII text. The ASCII text file can be loaded directly into Excel, the binary format is for users who wish to write their own data analysis programs.

Data

export (text)

TEXT

- 1. Output is in rows, each row corresponding to one pixel on the display x axis.
- 2. Each trace is output in a separate column(s).
- 3. Each Doppler spectrum is output in 256 columns (one column for each frequency)
- 4. Warning: Doppler spectrums will produce very large text files!
- 5. If 'no selection' is chosen, then data from left to right of the active view is used (even if the trace ends part way through).

Data

export (binary)

BINARY

- 1. Every point (within selection if applicable) is output irrespective of the x axis scale.
- 2. If a trace is not in the selection (if applicable) then it will not be output.
- 3. Each complete trace is output one after the other.
- 4. Data is always output as 'raw' values.
- 5. File Format: [HEADER] DATA [HEADER] DATA [HEADER] DATA
- 6. HEADER FORMAT (total 128 bytes):

type	bytes	description
long	4	no. of x points
long	4	no. of y points
int	2	no. of bytes per element
int	2	'?' (reserved for future versions)
double	8	x scaling factor
double	8	x offset
double	8	y scaling factor
double	8	y offset
double	8	z scaling factor
double	8	z offset
char[]	68	trace name (NULL terminated)

Export data Header Format

7. DATA FORMAT For line traces, the data consists of sequential array of elements, the first being the oldest point, the last is the youngest.

For 2D traces, the data is a sequential array of 256 or 64 point arrays.

Each 256/64 point array being one spectrum (0Hz - bandwidth).

FIR filter

Enables multi-tapped filtering of the current selection. A dialog box allows to enter the filename containing the weights for each tap.

For unity gain, the sum of the weights should equal 1.

For example, the following filter is similar to a 1 second RC filter but without the phase lag:

WEIGHT

0.06

0.09

0.1

0.15

0.2

0.15

0.1

0.09

0.06

FIR 1 second RC filter

This command destroys the original data in the selection of the trace!

Import data

Enables a new trace to be created from an arbitrary ASCII or binary source file. Once the data has been imported, use <u>the Trace Properties</u> editor to enter the correct scalings.

See also **Export Data**.

Remove Pulse

Use this function to filter the currently active line trace. (Does not work for Doppler spectrums). Designed to remove the pulsatile component. Does not introduce any phase lag.

This command destroys the original data in the selection of the trace!

Selecting Data

Select data for <u>Averaging</u> or <u>copying</u> to the clipboard, or for <u>Smoothing</u>, or other calculations, by pressing down the left mouse button and dragging, whilst holding down the left button. Selected data will be highlighted.

If the active trace changed when the left button was pressed, switch back to the desired active trace using the TAB key. Switching traces using the TAB key preserves the selection.

Smoothing Dialog

Set the time constant of the single pole rc filter to apply to the currently active trace.

Four common time constants are provided, or any other value can be set in the 'other' edit control.

Zero is a valid value. (No filtering will be applied, but the conditions set in the <u>Artifact Filter</u> Dialog will still apply.)

Status Box



Active Trace Name

The name of the currently active trace is shown here on the status box. Use the TAB key to change the active trace. Use the <u>Trace Properties</u> box to change the name

ToolBar

The Tool Bar is a feature common to many Windows applications. It allows quick access to most of the more commonly used menu commands.

The tool bar is displayed across the top of the application window, below the menu bar. The tool bar provides quick mouse access to many tools used in CAM1,

Sometimes some of the Tool Bar buttons will be inactive (dimmed). Usually this is when the button is not applicable to the currently active window.

To hide or display the Tool Bar, choose Toolbar from the View menu (ALT, V, T).

Calculating Averages

Use this command to recalculate averages. This will average the whole of the currently active trace or just the <u>selected</u> portion.

Averages, minimum and maximum are entered into the most recently active Results file. If no result file is open then a new one is created automatically. Each new average calculation is appended to the Result file.

Data is taken from the active trace, from the leftmost selected point up to the rightmost point. Zero points are excluded from the average, but this can be changed using the Artifact Filter.

Dropout and noise artifacts can be excluded from the result by using the <u>Artifact Filter</u>.

Data can be selected from the result file and copied to any other Windows application. Perhaps the primary use will be to copy results into a spreadsheet application such as Excel.

Hide Trace

Use this command to hide the currently active trace. The trace remains active although it is hidden from view. Activating a hidden trace automatically reveals it.

Increase Brightness

Use this command to increase the 'brightness' of the currently active Doppler spectrum.

This actually decreases the value associated with the darkest colour of the current palette.

This only effects the displaying of the spectrum in the current view, it does not alter the actual data.

Decrease Brightness

Use this command to reduce the 'brightness' of the currently active Doppler spectrum. This actually increases the value associated with the darkest colour of the current palette.

This only effects the displaying of the spectrum in the current view, it does not alter the actual data.

Increase Contrast

Use this command to increase the 'contrast' of the currently active Doppler spectrum.

This actually reduces the value associated with the brightest colour of the current palette.

This only effects the displaying of the spectrum in the current view, it does not alter the actual data.

Decrease Contrast

Use this command to reduce the 'contrast' of the currently active Doppler spectrum. This actually increases the value associated with the brightest colour of the current palette.

This only effects the displaying of the spectrum in the current view, it does not alter the actual data.

Smooth trace

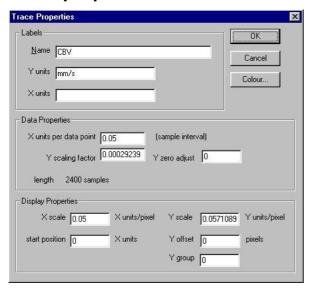
Use this command to apply a single pole RC filter to the <u>selection</u> of the currently active trace.

The **Smoothing** box allows the time constant to be selected.

The Artifact Filter controls the action to take for signal dropouts and noise spikes.

This command destroys the original data in the selection of the trace!

Trace properties



This dialog allows properties of the currently active trace to be altered.

This dialog can be invoked from the edit menu, or by clicking the right mouse button over a trace.

Trace Name

Name used to describe the active trace. Displayed in the <u>Status Box</u>.

Y units

Y units of the active trace. This is a text string which can be edited by the user.

X units

The x units label. This is a text string which can be edited by the user.

Sample Rate

This shows the interval between samples in x units. Normally this should not be altered since it would have been set when the trace was recorded. (Or in the original trace from which this trace has been derived).

See also Data Rate.

Trace length

This shows the number of samples in the active trace.

Axes X scale

The scaling of the x units to screen pixels.

Trace Start Position

Alter this to shift the trace start position. Don't change the start positions of traces in the Monitor window.

Screen Y scale

The scaling of y units to screen pixels. Note that the Doppler spectrum y scale has to result in the trace being displayed as a power of 2 pixels high.

Screen Y offset

The offset used to shift the active trace in the y axis, in screen pixels. This value does not change with y scale changes. Easier to change by clicking the left mouse button on the y axis and dragging. (Set Y Group to -1 to offset just this trace.)

Y scaling factor

The scaling factor used to scale the stored data to the y axis scale.

Trace Selection Dialog

Select desired trace from list of all traces available. Some traces may be empty (have been deleted using Edit command) and some may not be appropriate for the operation. The command should fail safely if you make a bad choice.

View

Colour / mono Doppler spectrum

Use this command to switch all Doppler traces in all views between colour and monochrome display.

Remember to switch to mono before printing onto a mono printer.

Inverse Colours

This function reverses the colour coding of the Doppler spectrum. Useful for printing out onto non-colour printers, either to save ink or toner, or to improve the visual appearance.

Minimum to background

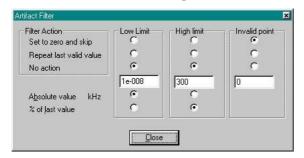
Use this set the colour of the lowest values to the same colour as the background. This is sometimes useful for printouts in order to either improve the appearance, or to conserve printer ink or toner.

Advanced Menu

This menu provides advanced functions, some of which are primarily for development purposes. To access this menu press [Ctrl]A.

WARNING: some of the undocumented functions do not have full error checking. Make sure important data is saved before using any of these commands.

Artifact Filter Dialog



The Artifact Filter dialog controls the action (if any) to take when the signal is above or below the specified levels. Used by the <u>Average</u> and <u>Smoothing</u> commands.

The low limit is primarily used for excluding dropout signals from average calculations. The default value, and 'Set to zero and skip' should be suitable for many cases. The dropouts may be from loss of signal due to tissue movement or gaps in the erythrocytes passing through the capillary.

The high limit is primarily used for excluding noise spikes generally caused by laser reflections from the tissue surface. This is not as effective as the low level filter, since it is difficult to distinguish between the sharp rise from the cardiac pulse and noise. Using an absolute value on small sections at a time may be most appropriate.

To apply only the artifact filter to a trace, Smooth the trace using a zero time constant.

Artifact Limit

Enter a value here for the limit of acceptable values. Either absolute values or percent of the last valid value can be used.

Absolute Value

Set the limit to an absolute value. Units of the currently active trace in the current view are used. Any values above or below this will be considered as artifact. Action to be taken is set by the <u>Filter Action</u>.

Percent

The percentage of the last valid value is used as the artifact limit. All values above or below this level are treated as artifact.

Filter Action

No Action

Ignore this artifact limit and calculate all points.

Repeat last valid value

The last value within the artifact limits is substituted for points falling outside the artifact limits.

Set to zero & skip

If the output of the operation is a trace then the result for any points falling above or below the limits will be set to zero.

In the Smoothing operation the artifact value is ignored and does not influence the smoothed trace. (No compensation for the missing time is made).

For the Average calculations the point is simply ignored.

Technical Details

Keyboard

The following list shows keyboard commands and shortcuts for using CapiScope.

F1 Help

shiftF1Context HelpF2Increase bandwidthshiftF2Decrease Bandwidth

F4 <u>Capture Image</u> and save to disc

F6 Next window
shift F6 Previous window
F8 <u>Calculate Average</u>
F9 Laser ON/OFF
F10 Start/Stop recording
TAB Activate next trace

up cursor magnify y scale (halve full scale) down cursor minify y scale (double full scale)

+ magnify x scale minify x scale

left cursor scroll one pixel right right cursor scroll one pixel left scroll one pixel left page up scroll left 3/4 screen page down scroll right 3/4 screen move to start of active trace End move to end of active trace

ctrlN File New ctrlO File Open ctrlS File Save ctrlP File Print ctrlC edit copy ctrlInsert edit copy ctrlV edit paste shiftInsert edit paste ctrlX edit cut shiftdelete edit cut

Keyboard commands and shortcuts

Database Technical Details

Database

The current database pathname is saved between sessions in the ini file.

If it not set, or doesn't exist then the user is prompted to select or create a new one.

The database name is always capi.db

It is a sqlite3 database.

The data folder

The folder the database is in, is also used as the root folder for all examination folders.

The name for each examination folder is created in the form

ddddddddd-patientid-site

where ddddddddd is the creation date in seconds since 1970. (unix timestamp).

ddddddddd is also used as the examid.

An examination folder stores all files for that examination.

Backup and Roaming

To backup or to save onto another drive for analysis on another PC, simply copy the entire data folder containing the capi.db database and all examination subfolders.

Files

Files (images and video) are saved into the current exam folder unless they are a copy, or derived from another exam, in which case they are saved there.

Files saved during recordings are named:

basename-yyMMddhhmmss

where yy=year,MM=month, etc.

Images saved using the timer have _000, _001, etc appended.

AVI Output compression

Velocity and quality comparison of a good quality video sequence (Demo.mve, 300 images, File Size: 86,020,804 bytes) using various video codecs. Tested with a 20um wide line from (304.8,116) to (296,199.2)

CODEC name	Quality of image	Velocity (um)	% GOOD	Velocity error (%)	% of Uncompressed file size
Cinpak Codec by Radius	Lowest	650.45	32	76.546	1.677
Cinpak Codec by Radius	Highest	650.45	32	76.546	1.677
Microsoft Video	Lowest	0	0	-100	0.862
Microsoft Video	Highest	337.69	87	-8.344	62.506
Microsoft RLE	Lowest	368.43	93	0	100.452
Microsoft RLE		368.43	93	0	100.452
Intel Indeo	Lowest	197.98	64	-46.264	0.676
Video 4.5	Lowest	137.30	04	40.204	0.070
Intel Indeo Video 4.5	Highest	177.39	61	-51.853	16.555
Intel Indeo	Lowest	299.59	97	-18.685	0.593
Video 5.04	Lowest	233.33	37	-10.005	0.555
Intel Indeo	Highest	349.76	91	-5.067	14.545
Video 5.04	Tilgilest	343.70	31	-3.007	14.545
Intel Indeo	Lowest	209.5	84	-43.137	12.812
Video 5.1	Lowest	203.3	04	-43.137	12.012
Intel Indeo	Highest	162.96	60	-55.769	14.545
Video 5.1	Tilglicst	102.50	00	55.705	14.040
Divx MPEG4	Lowest	0	0	-100	0.014
Low Motion	2011600			100	0.01.
Divx MPEG4	Highest	512.92	96	39.218	3.0127
Low Motion	111611000	01 2. 32	50	55.210	5.0127
Divx MPEG4	Lowest	0	0	-100	0.014
High Motion	2011600			100	0.01
Divx MPEG4	Highest	526.81	91	42.988	1.494
High Motion	8				
Brooktree YUV	Lowest	368.12	92	-0.084	149.993
411 Raw(16)					
Brooktree YUV	Highest	368.12	92	-0.084	149.993
411 Raw(16)	8				
Full Frames	Lowest	368.43	93	0	100
(uncompressed, no codec)					
Full Frames	Highest	368.43	93	0	100
(uncompressed,	111611600	550.45			100
(uncompressed,					

CODEC name Quality of Velocity (um) % GOOD Velocity error % of (%) Uncompressed file size

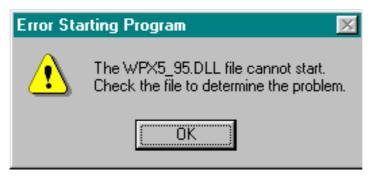
no codec)

Velocity results after video compression

Troubleshooting

CapiScope does not start

- Check that that CAM1 has not been enabled in the ini profile file when there is no CAM1 interface card fitted. Edit all .ini files in the program directory (usually c:\Program Files\KK Technology\CapiScope). Change the line: camEN=1 to camEN=0.
- If CapiScope does not terminate normally, the frame grabber may be not be freed properly. For the PX610, use the PXClear program in the PX5 program group on the start menu. For the Matrox, you may need to shutdown the computer.
- The Matrox and Microsoft DLL files have been installed correctly, in either the CapiScope directory or the Windows, or Windows\System directory.
- The correct SuperPro software key has been fitted.
- If using the USB superpro key, or WindowsNT with the parallel port key, install the superpro drivers. Select "Run..." from the start menu, and enter:
 D:\legacy\setup.exe /USB(replace 'D:' with your CDROM drive name).
- You are entering the correct codes when evaluating demo versions. A new code is required every month, and there are two codes: one to enable the demo version of the basic CapiScope then a second code to enable the optional dynamic features.
- Check enough memory has been reserved for the Imagenation drivers. Select "Run.." from the windows start menu, and type regedit and edit the key "HKEY_LOCAL_MACHINE\system\currentControlSet\services\Vxd\PX5_95\memory size". This should be set to 0x200000 (hexdecimal). You will need to reboot the computer for any changes to take effect. Alternatively, reinstall CapiScope, and make sure the default 0x200000 memory is set in the Imagenation setup program.
- For the Matrox MetoerII check memory allocation by right click on the "My computer" icon on the desktop. Select "properties", "Device Manager" tab. Select the MeteorII and click on Properties. In the Driver tab, it is possible to change the memory setting. Make sure it is at least 0x200000.
- If you get the following:



this is probably because of an old version of WPX5_95.DLL in the CapiScope folder. Copy the newer file from c:\PX5\bin\WPX5_95.DLL into C:\KK Technology\CapiScope\.

No video signal

- If no video is visable, check the frames per second indicator on the status box. It should give a value fluctuating around fps=25.0 (PAL European system) or fps=30.0 (NTSC US system).
- The video input is connected to the right input. For the ImagenationPX610, there are two BNC connectors. The lowest BNC is a trigger input, the top BNC (closest to the multipin 'D' connector) is the video input.
- The video source has been switched on. i.e. the IPS Isolator/Power supply (mains switch is on rear panel) and VCR.
- If using an LCD monitor, check the brightness and contrast settings. Sometimes the low contrast when out of focus just gives the impression of no video signal.