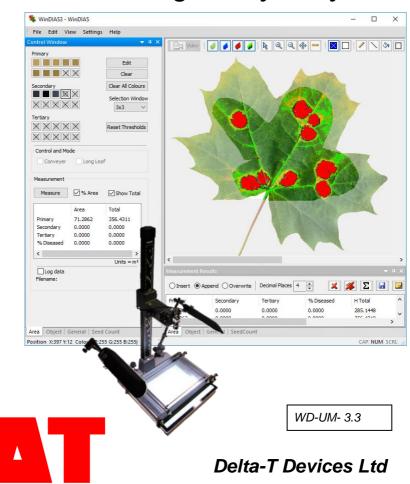
### User Manual for the

# WinDIAS 3 Image Analysis System



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### CE Conformity

The WinDIAS system conforms to EU regulations regarding electromagnetic emissions and susceptibility and is CE marked by Delta-T Devices Ltd.

For certificates look on our website at www.delta-t.co.uk ,

### Warnings

To maintain conformance to CE standards, the equipment must be used as described in this manual. Modifications to the equipment may invalidate CE certifications.

Delta-T Devices Ltd reserves the right to change the designs and specifications of its products at any time without prior notice. The information in this document is subject to change without notice.

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# Introduction

## **About WinDIAS 3**

This shows how to use the WinDIAS 3 Image Analysis system See also:

- WinDIAS 3.3 Quick Start Guide
- Online Help
- WinDIAS 3.3 Software Installation
- Video https://youtu.be/HbXFOPJ5Jrc

### Accessory manuals

• IDS µEye Camera Hardware Manual.

### To obtain manuals

• In Windows 10 Start Select WinDIAS 3, Documentation to display the complete set of WinDIAS manuals.

These are installed when you install WinDIAS 3 software on your PC.

These may be read using Adobe Reader obtainable online from adobe.com.

- Download them from our website at <u>www.delta-t.co.uk</u>
- Contact your distributor or contact us direct at Delta-T.

# **Overview**

### **About WinDIAS 3**

Designed for plant scientists, WinDIAS 3 uses an intuitive colour picking method for image analysis on a PC.

WinDIAS 3.3 can interface directly to an IDS UI 3270 CP USB3 digital video camera.

It is particularly good at measuring the area of healthy and diseased plant leaves.

Up to three different zones may be measured simultaneously (e.g. healty, diseased, necrotic) - each defined by up to ten colours.

Colours are selected intuitively by clicking on the image.

Rapid measurement of large quantities of leaves is possible with the video camera plus conveyor belt option.

Percentage diseased leaf area can be measured.

Simple shape analysis can be done on static images, including: length, width, perimeter, area, angles, centroid, average radial, shape factor, size distribution and object count.

Touching seeds can be counted using an average area method.

Images can be obtained directly from scanners and digital still cameras which support the TWAIN interface standard.

It can import jpeg, tiff or bitmap file format images

Image files can be quickly batch-processed for leaf area measurements or shape analysis.

The video input stream can be turned off. This can benefit scanner or still-camera users by freeing up central processor cycles on the PC.

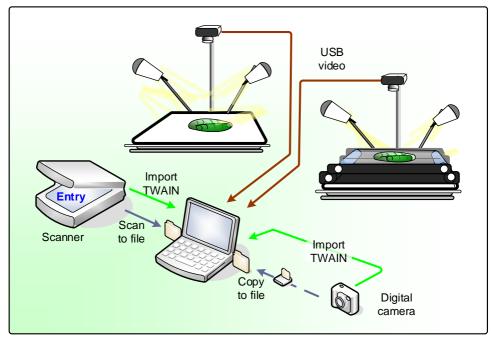
Simple image editing and drawing tools are provided.

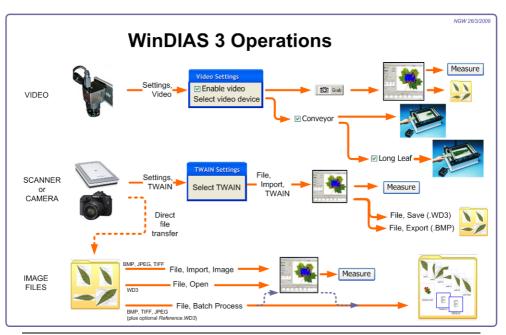
An Undo button is provided for colour picking and drawing actions.

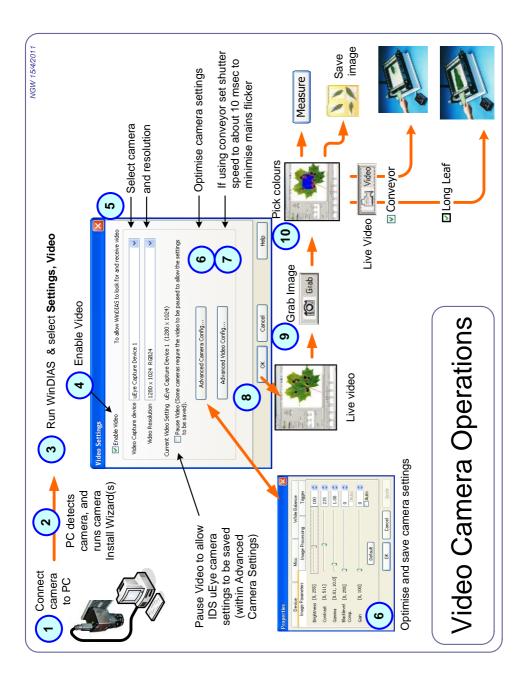
Complex Area of Interest regions may be drawn.

On exit WinDIAS can remember your configuration and restart the same way next time. The video camera software settings can also be saved and reloaded.

### **Systems and Operations**







# INSTALLATION

### Unpacking

A packing list is included in the box with the shipment. Check it against the options list below, and make sure you have what was ordered.

#### WinDIAS 3 Entry Level System WD-E3

WinDIAS v3.3 software and manual	WD-SW3
A4 Flatbed scanner	WD-FBS-1

#### WinDIAS 3 Standard System WD-S3-230/110

WinDIAS v3.3 software and manual	WD-SW3
USB digital video camera including mounting adaptor USB3 cable	WD-CAM-R2
Lens	WD-LEN-1
Light box	WD-LB2
Camera stand	WD-CS1
Overhead lights	WD-OL2-230 /110
Acrylic sheets	WD-AS1

#### WinDIAS 3 Rapid System WD-R3-230

WinDIAS v3.3 software and manual	WD-SW3
USB digital video camera including mounting adaptor and USB3 cable	WD-CAM-R1
Lens	WD-LEN1
Light box	WD-LB2
Camera stand	WD-CS1
Overhead lights	WD-OL2-230 /110
Acrylic sheets	WD-AS1
Conveyor (+10 spare belts)	CB-220/60

WinDIAS reference target set	WD-RTS-1
------------------------------	----------

#### **Spares**

Conveyor spare belt material (for 10 pairs of belts)	CBSP1
Conveyor spare belt material (for 50 pairs of belts)	CBSP2
Conveyor spares : "O" rings and drive bands	CBSP3

## WARNINGS

WinDIAS electrical components are equipped with a 3-wire power cable and plugs for the user's safety. Use these power cables in conjunction with a properly grounded electrical outlet to avoid electrical shock.

Refer to the computer electrical safety instructions in your computer user manual.

Electrical equipment, especially video equipment and monitors, can contain dangerous high voltages which may persist after the power is turned off.

Internal modifications and servicing should only be done by qualified staff in accordance with manufacturers' recommendations and whilst observing safe electrical working practices

### **PC Requirements and Software Installation**

Please refer to the WinDIAS 3 QuickStart Guide v 3.3

## Install Camera, Stand, Lights and Conveyor

Assemble the camera stand and lights.

Mount the light box and optional conveyor.

Connect the lighting and conveyor power cables to the separate power distribution board.

Mount the camera on the camera stand.

Cover nearby windows to ensure the lighting does not change.

The overhead light intensity is adjustable. If both lights are also connected together via the supplied cable with little square RJ11 phone-type connectors, then the two lights behave as one.



Figure 1 Standard System on left, Rapid System on right (cabling not shown).



Figure 2: Lighting considerations

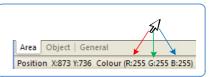
### Avoid variations in illumination

Cover the windows and lower or turn off the room lights - poorly controlled illumination can dominate the accuracy of your results.

Directional variations in lighting can also affect the accuracy of leaf images due finite thickness of the ribs and veins in a leaf. To evaluate this effect, scan a particular leaf at different orientations.

### Avoiding saturation:

Saturation is indicated when the RGB values associated with a pixel are 255,255,255. This is full scale.



Too much light can saturate a pixel, and this may help or hinder you depending on your application.

It can be helpful eliminating background shadows in conveyor mode. If you can safely ignore saturation in some pixels, then you can make better use of the remaining 256-1, 8-bit RGB levels by setting the illumination to best bracket the range of outputs from your leaves.

Saturation can cause glare and cross talk between pixels, preventing you from resolving thin targets e.g. carrot leaves, hairs or roots. It may also hinder you when setting the white balance See also page 19.

Controls for Illumination levels are provided for the overhead LED lights (via knobs) and underneath via the light box (via the touch sensitive button).

The lens aperture ring can and should be used.

In WinDIAS, changing the video camera settings integration time may help, but its usefulness is limited in conveyor mode or if mains flicker from the light box needs to be averaged out. (The overhead LED lights do not flicker)

<u>Do not</u> enable the video camera automatic gain control (AGC). (WinDIAS will not work correctly if AGC is enabled).

# **НОW ТО...**

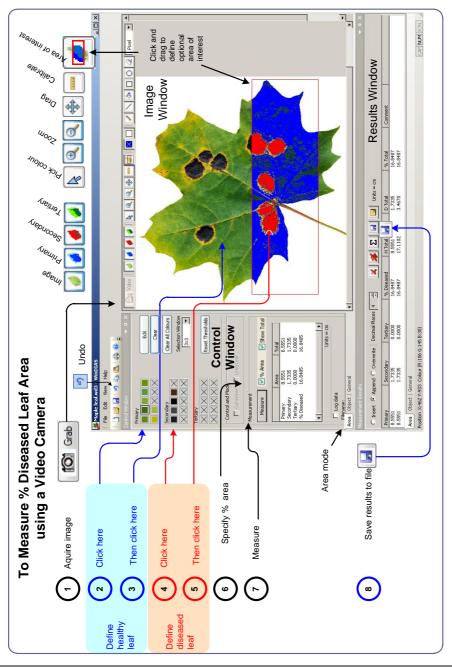
### How to Calibrate

- 1. Grab an image of a ruler.
- 2. Click on the Calibrate tool.
- 3. Drag the mouse cursor to draw a line a known distance along the ruler.
- 4. Enter its Value and Units.

Video   🥖				
- 2 				
Calibrate				
Line Length 568 (P	ixels)			
Equates to :-				
Value	80	Unit	mm	
			ОК	Cancel

Note: The long leaf mode is calibrated differently. See: *How to Measure Long Leaves* on page 39

## How to Measure Leaf Area using Video

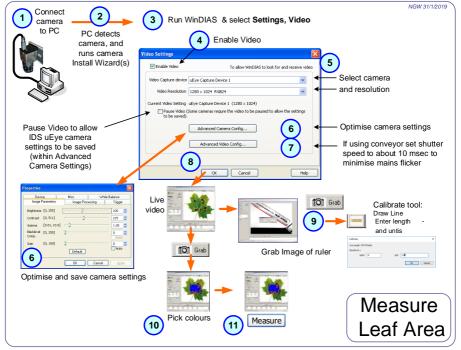


#### Summary

This assumes you have installed the software as described in the WinDIAS Quick Start Guide:

- Get a live video feed (steps 1-5 below)
- Optimise the image (steps 6-8)
- Calibrate (step 9)
- Set the colour threshold (step 10)
- Measure.

#### These instructions apply to the IDS video cameras supplied by Delta-T



#### 1. Set up the camera.

Mount camera vertically and square on the copy stand Connect to the PC using the IDS USB 3.0 cable.

#### 2. Windows detects the camera

On detecting the camera Windows will run two device connection Wizards, one after the other. Accept the default offerings.

#### See also WinDIAS 3 Software Installation.pdf

#### 3. Start WinDIAS 3

#### 4. Enable Live Video

In Settings, Video tick the Enable Video checkbox.

#### 5. Select the correct camera

In Video Capture Device select the **uEye Capture Device** and click **OK**.

If the camera is correctly installed a live video feed should appear after 2 or 3 seconds. If not see *Trouble Shooting: No Video?* on page 45.

### 6. Optimise the Camera Settings

In addition to the focus and aperture control on the lens, the video camera has many settings available via the software.

The following screens appear if using an IDS uEye camera. Other cameras will expose their settings to you differently



Focus Aperture

Select **Settings, Video Advanced Camera Config..** to display the Camera Properties:.

#### **Image Parameters**

Ensure that Automatic Gain Control (AGC) is OFF, i.e., unchecked.

Note: if Automatic Gain Control is turned on then the threshold value will change according to how dark the whole grabbed window is – which will make the readings inaccurate.

Properties				×
Color Settings Image Parameters	Device Image F	Misc Processing	Input/Output Trigger/Flash	
Gamma	1.0	2	1.00 +	
Brightness (Blacklevel Comp.)	0	25	4 🔹	
Contrast (Gain) The Auto Gain should	0	10	0 - 0 Auto	
<u>not</u> be used				

#### **Colour Correction**

In **Image Processing** select **Enable Color Correction** You may also need to select **Flip Frame vertical** or **horizontal** 

Properties			×
Color Settings Image Parameters	Device Ima	Misc ge Processing	Input/Output Trigger/Flash
Flip Frame vertica	ntal rection	Edge enhancen	

#### Setting the White Balance



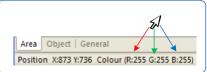
You want the image of a white target to appear white. This will require a <u>grey or black-and-white target</u>. There are several ways of doing this in **Color Settings:** We recommend you use the automatic white balance "One Shot" function.

#### Auto Whitebalance Method

1) Ensure that live video is looking at a grey or black and white target. For instance, looking at part of the black back of the Macbeth Colour Target along with some white paper.

2) Ensure at least part of the region of interest is not saturated. To tell if an image is saturated - hold the cursor over a pixel on a **grabbed** image, and look at its RGB values, displayed at the bottom of the WinDIAS window.

For the auto whitebalance function to work properly at least some of the individual Red, Green and Blue values should all be less than 255. At 255 a pixel is saturated - essentially blinded by bright light. Reduce the



light intensity and/or reduce the lens aperture and/or reduce the camera exposure time camera time (whatever is most convenient ) until most of the image is unsaturated.

....Continued on next page

3) With Live Video enabled, (don't "Pause video") in Whitebalance Mode select One Shot.

Properties						×
Image Parame	ters	-	e Processing		Trigger/Flash	
Color Settings		Device	Misc		Input/Output	
Whitebalancing						
Whitebalance	tebalance Mode: Disabled 🗸					
		Disabled				
Calas terment		Enabled				
Color temperat	One Shot					
RGB color	Manual	~	Presets	Morning s	sun 🗸	

# Figure: Showing the Colour settings in version 3.8 of the IDS camera driver software.

Note: the camera manufacturer company IDS have repeatedly changed this user interface in successive versions of their  $\mu$ Eye DirectShow software, so don't be surprised if this looks different on your setup!

The software takes an average over the whole of the active region of interest, automatically adjusts the RGB values, and displays **Disabled** when it has finished.

At this point the white parts of your image should now look white, and colours reproduced reasonably.

Note 1: It is sometimes beneficial to place one or more sheets of A3 white paper on the light box to prevent the camera saturating when looking directly at it.

This may be preferable to reducing the lens aperture, which will also cut down useful light reflected off the leaves. It depends of the type of leaf. For some leaves it may help to find an optimum balance between the amount of light transmitted from below and reflected from above, whilst maintaining a good effective dynamic range for good thresholding.

Note 2: Good colour reproduction in an on-screen image also depends on the good calibration of the monitor screen itself. There are devices you can buy that do this (e.g. from Spyder.com).

#### **Save Camera Settings**

To ensure the IDS camera software settings persist from one session to the next select: **Settings, Video**, and click on the **Pause Video** check box. This will interrupt the stream of live video from the camera to the PC.

#### In Advanced Camera Config, Device, select Save Settings.

The **Save Settings** dialog gives you a choice of locations where the settings information may be saved. (You need to have opened WinDIAS as an Administrator to do this.)

Properties			
Image Parameters Color Settings	lmag Device	e Processing Misc	Trigger/Flash Input/Outpu
Camera Camera model: Serial No.: Native resolution:	UI327xCP-C 4103181286 2056 x 1542		
Frames received:	41	Frames dropped:	0
Load/Save location	: display file	selection box	$\sim$
Save Settings	local registr EEPRom us display file		

Three save locations are offered, to the registry, to EEPROM, and to a normal file folder on your PC.

If in doubt, create a folder called My WinDIAS under My Documents and save the camera settings file there.

#### Then click OK.

Note



Save to "local registry" to ensure your camera setting will persist when closing and opening WinDIAS



You can only save the video camera settings when the video stream is paused.

#### 7. How to change the shutter speed

Select Settings, Video, Advanced Video Config...

IDS uEye Camera	
	9.978 ms
	ОК

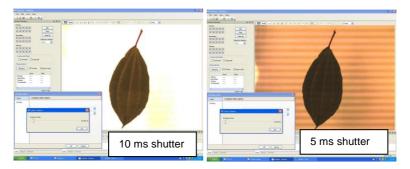
If you have an IDS camera connected the shutter speed control is a slider, as shown above.

Other cameras will expose their shutter speeds differently

The default shutter speed for the IDS cameras is about 200 ms (milliseconds)

If using the conveyor you should change this to about 10 ms. If you use a 200 ms shutter speed the image will blur on the conveyor.

Note as you reduce the shutter speed you will observe beating effects in the background illumination at some frequencies.



Compensate for reducing the shutter speed by opening the lens iris or changing the camera gain or brightness.

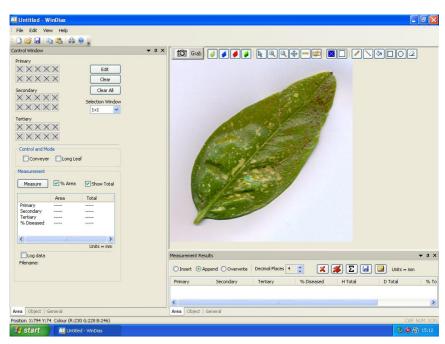
Note: In 2018 we replaced the tungsten filament overhead lights with flicker free LEDs.

However, the LED light box which replaced the light box on the camera stand *does* exhibit flicker, and so when using this you will still need to adjust the shutter speed.

#### 8. Get a Live Image

Having connected successfully to the video camera feed and optimised the video settings, select **OK** to return from the Video Settings dialog to the main WinDIAS window.

The following screen should appear, with your current image in.



The image on the right is live video from the camera.

Set the camera focus and aperture as best you can and, if necessary, configure the camera software settings as described above.

Note: Toggle between the **Area**, **Object**, **General** and **Seed Count** tabs at the bottom of the window to view the different types of measurement available.

Note that the **Control Window** and **Measurement Results** window are synchronised.

Note: All three Control, Measurement Results, and Image Windows can also be undocked, resized, dragged around and re-docked easily.

See the online help for instructions.

Select the Area measurement tab.

### 9. Calibrate using a ruler

Unlike scanners, video images can have any magnification, so calibrate before you measure. For area measurements you calibrate against a target of known length. In this exercise you will use a ruler. Use the mouse to mark a distance along the ruler. For best accuracy use marks on the ruler which are as far apart as possible.

1. Place a ruler diagonally on the light box and with live video displayed, **Grab** the image

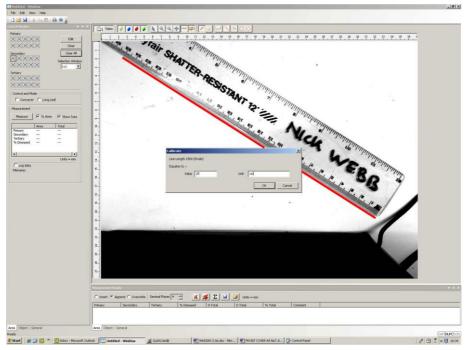


2. Select the Calibrate tool

			1
11	-	CC.	н
			н
1.0	_	_	а.

Then using the mouse, left click and drag, to draw a line of known length along the image of the ruler. On releasing the mouse a calibration dialog will appear.

Enter the known **Length** and **Units** in the calibration dialog box that now appears.

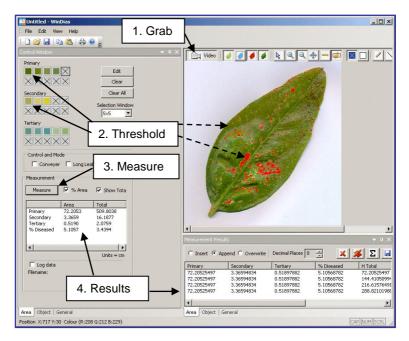


**Calibration using a ruler** showing the track created by dragging the mouse in red

### 10. Pick Healthy and Diseased Colours

To grab a leaf click on the Grab button





#### Alternately, Import a Leaf Image File

Note: For your convenience we have included some leaf images for you to play with. These were actually obtained with a scanner, and you can do the same if you wish.

Select File, Import Image and click on one of the leaf images.

In Windows 10 these may be found in C:\Program Files (x86)\Delta-T\WinDIAS 3.3\Samples. Once imported, an image can be measured just like the grabbed video image.

Calibrate for magnification as described on page 24.

Once you have got a good image, and calibrated, WinDIAS needs to be told what colours to include in its measurements.

(continued on next page)

Click on to the **Colour** picking tool



Examine the screen. There are three zones, each with a set of ten boxes in the on the **Control Window**. Every time you pick a colour it will appear in a box.

Primary boxes are for healthy leaf colours.

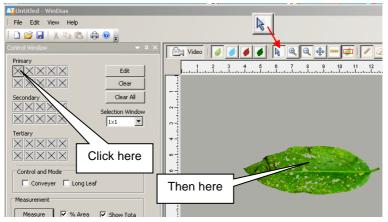
Secondary boxes are for diseased areas.

Tertiary boxes could be used for dead areas.

Pick representative healthy and diseased colours as follows.

Click the first Primary box to highlight it. Once selected, a small square will appear in it.

Then click on a healthy portion of the leaf.



WinDIAS will highlight all parts of the leaf close to the selected colour. It does this by drawing an overlay colour on top of the image window.

The default overlay colour is blue for healthy leaf (or primary zone) and red for the diseased area (secondary).

Each colour picking operation may take your PC a few seconds. When complete the chosen colour will appear in the box and as an overlay on top of the image.

It works best to alternately pick healthy and diseased zones, rather than pick all the healthy areas first.

Hint 1: Set the Selection Window size to 5x5 pixels to sample a larger number of pixels (25) in one go.

Hint 2: To select a greater area you may also **Edit** the width of the RGB colour zones. See **To Change the Colour Range** on page 29

#### **Use the Overlay Buttons**



Toggle the Overlay Buttons to see how they work.

These toggle the displays of the grabbed image, and of the Primary, Secondary and Tertiary regions - which appear as coloured overlays on top of the image.

When picking colours, not all the green areas in a leaf are selected in one go. For instance very dark green might be missed. Don't worry! You can pick up to 10 colours in each zone.

Each time you pick a colour, the focus moves automatically to the next box in the zone.

By now WinDIAS should have identified most of your leaf.

If not, continue picking colours until the whole leaf is identified.

#### 11. Measure

Having grabbed an image, calibrated and set the threshold.

- 1. Set % Area to on
- 2. Press the **Measure** button.

A result should appear in the results box in the units specified at calibration.

Congratulations! You have made your first measurement.

3. Repeat a few times to give yourself a simple set of data, and inspect the **Measurement Results** window.

#### 12. Save Image

Use **File**, **Save** or **Save As**, to save the image in WinDIAS **.WD3** format. The threshold settings and magnification are also saved - and reloaded when you re-open the image file.

Use **File**, **Export Image** to save the image as a **.TIFF** format, 24 bit colour bitmap.

# WARNING: Be sure to turn off the overlay colours before saving if you wish to measure a saved .TiFF image later.

If you don't do this, on reloading the image, only the overlay will be present, a green leaf may appear blue, and when you measure it you will probably get a zero result and wonder why!

#### 13. Exit

Use Exit to leave WinDIAS and return to Windows.

## How to Choose Colours

See also: 10. Pick Healthy and Diseased Colours on page 25

### About Zones, Ranges and RGB

#### Primary Zone

The Primary (Healthy) Zone is just an arbitrary name we use for the main part of the object. If you like, just call it the Healthy Zone or Healthy Leaf Zone. It has nothing to do with the concept of the primary colours, i.e. red blue and green etc. used in painting. Similarly, you can call the Secondary Zone the Diseased Leaf Zone if you wish.

#### **Colour Range**

Each time you pick a colour you actually pick the mid point of a small range of colours. The range is pre-set for you. You can alter the range, but you do not have to. You can instead just pick more colours. There is a trade off here between convenience and speed. The more colours you pick the slower the measurement.

### **RGB Colour Space**

Each picked colour is defined as ratio of Red: Green: Blue (RGB), occupying a small volume in a three dimensional colour space. Black is represented by the colour vector (1,1,1), white is represented by 255,255,255. All the other colours lie somewhere in between.

When you pick a particular pixel on the leaf it will have a precise RGB value. For instance it may have the value (100, 50, 60).

WinDIAS assumes a small range or variance of reds, greens and blues around each colour that you pick. You can **Edit** this range.

### **Editing Picked Colours**

The currently active colour (box) is surrounded by a thick border

**Edit:** Opens the Colour Boundary window where you can change the colour range (volume in RGB space) surrounding the currently active colour (box).

Clear: clears the current active colour box.

Clear All Colours: clears all the colour boxes.

**Selection Window**: defines the number of pixels surrounding the chosen pixel to be included.

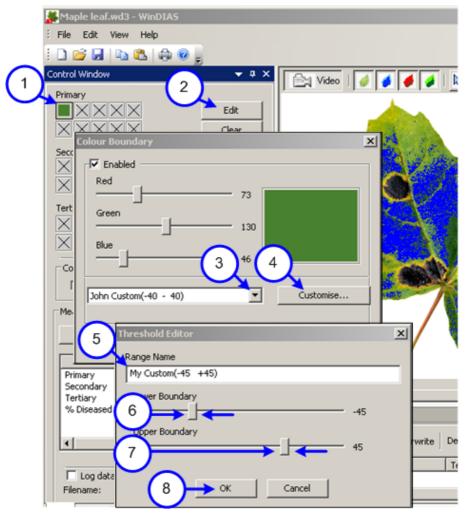
**Reset Threshold**: Resets the volume in RGB space of all picked colours back to the default value of ±20 RGB units

### To Change the Colour Range

This explains how to change the range of RGB colours associated with each selected colour.

This can be useful for fine tuning if you have difficulty separating the diseased and healthy zones in the normal way.

You should have grabbed or loaded an image and have selected at least one colour. So at least one of the colour selection boxes should have a colour in it.



- 1. Choose a colour to edit by clicking on the box containing that colour. The thin border around the perimeter of the box will turn into a heavy line and The **Edit** button will become available
- 2. Press Edit

The **Colour Boundary** window will appear, and the mid-point RGB values of the colour you have picked will be displayed on R, G and B sliders

A drop-down list with a default colour range setting is displayed in the bottom left hand corner of the dialogue box

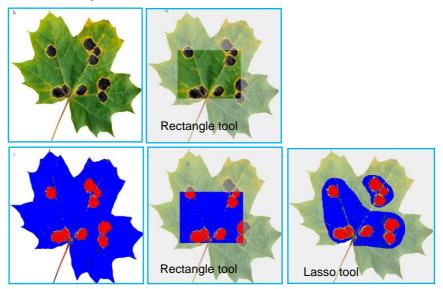
- 3. Press the drop-down arrow and a series of default range settings plus settings labelled "Custom" will appear
- 4. Select one of the Custom ranges and press Customise
- 5. The Threshold Editor dialogue box will appear
- 6. Rename the Range Name e.g. to My Custom (-45 to+45)
- 7. Drag to alter the Lower Boundary slider to, for example, -45
- 8. Drag to alter the Upper Boundary slider to +45.
- 9. Press OK.

Your custom range for the colour box picked will be adjusted accordingly when your PC re-calculates the video image

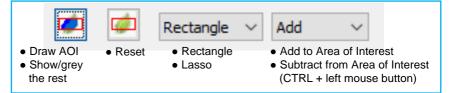
Try different settings to see the effect of changing the range.

## How to use the Area of Interest drawing tool

The Area of Interest defines the parts of the image that will be measured and within which the picked colours are seen. The region outside of the Area of Interest is indicated by a light grey overlay.



### **Drawing Tools for the Area of Interest**



• Click on the **Draw Area of Interest** button and drag (using left mouse button) to create an Area of Interest.



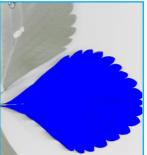
- To delete it click on **Reset**.
- Complex shapes may be created using the **Rectangle** or **Lasso** setting and the **Add** or **Subtract** mode.



The subtract mode can also be selected by holding own the CTRL button whilst using the left mouse.

The Lasso mode of the Area of Interest tool can be useful for isolating one leaf from an image containing several. This can occur when measuring leaves non-destructively.

For example: the raspberry leaf at right was acquired non-destructively with a DoxieFlip scanner, allowing its growth over time to be monitored.





Barley ear isolated using Area of Interest lasso drawing tool, Image acquired via flatbed scanner with no lid, so background is nice and black.

From lodging trials courtesy Helen Holmes ADAS UK

## **Batch Processing**

- Batch Processing quickly processes a large number of image files on your PC.
- It can measure BMP, JPEG or TIFF image file formats.
- It can be set to measure WinDIAS's area or shape measurement functions.
- You point WinDIAS at a folder full of image files. WinDIAS loads each image in turn, measures it, and saves the results back into the same folder.

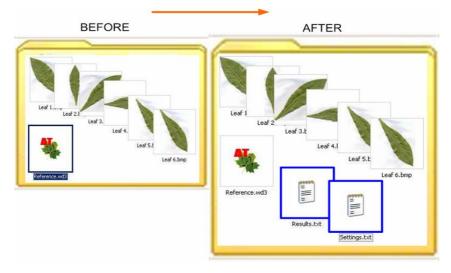


Illustration showing the contents of a folder before and after using the Batch Process command

- If you previously saved a WinDIAS (.wd3 format) image file to this folder with the name "Reference.wd3" then WinDIAS will find it and use the settings from that file to process all the other images in that folder.
- If no image file called Reference.wd3 is found in the folder, WinDIAS will use its current threshold settings and magnification.

#### Warnings:

If no threshold settings have been set, the results will all be zero! If no magnification is set, the units will be in pixels!

## How to Measure using Batch Processing

- 1. Using **File**, **Import Image**, load an image file into WinDIAS, calibrate it, and set the detection thresholds by picking colours for the Primary, Secondary or Tertiary regions, whatever you require.
- 2. Create a test folder for the tests and using **File**, **Save**, save the image as **Reference.wd3** to this folder.
- 3. Place all the image files you want processing into this folder.
- 4. Select File Batch Processing to display the Batch process dialog
- 5. Click on the browse button and select the test folder.
- Select Area or Object Measurement. These will measure according to the settings on your Area or Object Control windows in WinDIAS.
- 7. Click Start Processing.
- 8. Inspect the results as below.

tch process		
irectory C:\Documents and Settings\Ni	ck\My Documents\My Pictures\My	Leaves 4
Measurement Mode	C Object Measurement	Start Processing
Progress		
		Cancel

### **Batch Processing Results**

Results are saved to the same folder in a text file called "Results.txt" along with a second "Settings.txt" file

The Results.txt file is formatted as tab-separated values for ease of importing into spreadsheets such as Excel.

The Settings.txt file contains the reference file name and path, date and time, and the mid value and range for each of the colour zones picked.

Tip: To extract a record of the colour threshold settings from any .wd3 image file, save a copy, naming it "Reference.wd3" to an empty folder and run the batch process command on that folder. A Settings.txt file will be created with the colour threshold settings in.

## Use of the Doxie Flip portable A5 scanner



The DoxieFlip is a portable A5-format flatbed battery operated paper/copy scanner, with resolutions of 300 and 600dpi. It is supplied complete with a SD memory card and USB card reader.

Delta-T does not supply DoxieFlip scanners, but they are widely available online.

#### Limitations

Used in conjunction with WinDIAS it works quite well with small leaves with a petiole (leaf stem) like clover, but not so well with grasses and other monocotyledons due to both the small size and the inch-wide border around the scanning zone.

This scanner is a consumer product designed for indoor use and is not protected against water ingress.

Battery Life is short: ~200 scans at 300dpi.

#### Use for non-destructive leaf area measurement

Whilst limited in size, the DoxieFlip can be used for non-destructive leaf area measurement.



The photo above shows the DoxiFlip being offered up to scan an apple leaf. The parcel label was attached to help find and identify the leaf and

the subsequent image file.

A useful feature of the Doxie Flip is the transparent window at the back of the scanner. This is useful when aligning the leaf for scanning.

To minimise glare from sunlight, close the lid when scanning. We found it useful to add a foam strip to the lid (as shown in the photo) to prevent glare creeping in around the lid.

To minimise glare you also need to cover the transparent window at the back of the scanner. We have found that the travelling bag accessory for the Doxie Flip is ideal for this.



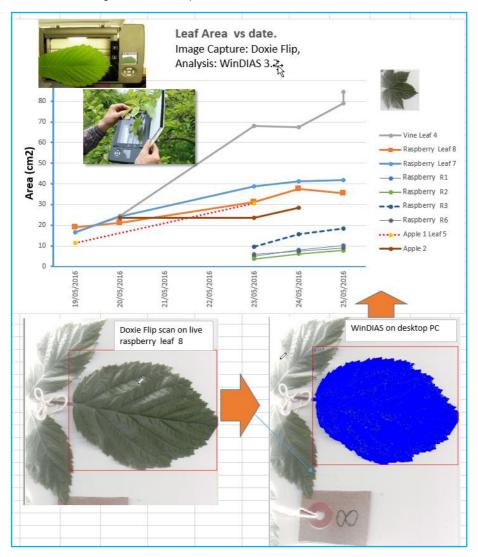
It took only 24 minutes to acquire these 20 images non-destructively, walking 120 m and visiting 16 plants in the Delta-T garden. Note the glare in some of the images, due to sunlight creeping in around the lid of the DoxieFlip scanner. With careful screening this can be prevented.

If you have more than one leaf captured in the image, then providing they are separate, you can use the WinDIAS Area of Interest drawing tools to isolate and measure an individual leaf. See also *How to use the Area of Interest drawing tool* on page 31



#### Leaf growth analysis example

In this example individual leaves were measured non-destructively every day for one week using a DoxieFlip scanner. The leaf area was obtained using WinDIAS and plotted in MS Excel.



## How to Measure Leaf Area by Conveyor

- 1. In **Settings, Video, Advanced Video Config**... set the video camera shutter speed to about 10ms. If the shutter speed is too slow the image blurs and accuracy is lost. If too fast, the illumination beats with the mains frequency.
- 2. In Settings, Video, Advanced Camera Config... optimise lens aperture and camera settings.
- 3. It may be necessary to repeat steps 1 & 2 to optimise the image.
- To save the camera settings first select Settings, Video, and tick the Pause Video check box.
   Then back in in Advanced Camera Config., Device, select Save Settings and press OK.
   Then back in Settings, Video untick the Pause Video check box.
- 5. Grab an image of a stationary ruler and Calibrate as described above.
- 6. **Grab** an image of a stationary leaf and pick healthy and diseased areas.
- 7. Enable **Conveyor** in the Area Control Window

Control and Mode		
Conveyer 🔲 Long Leaf		

- 8. Ensure that live video is being displayed.
- Turn on the Conveyor via its own Control Box. Note it can go forwards and backwards. When running correctly, each leaf is grabbed once, only when fully in the field of view (or Area of Interest, if defined).
- 10. Feed leaves in and check they are being fully thresholded. If necessary, adjust the colour picking thresholds until you are satisfied.
- 11. Clear any unwanted results using the delete button
- 12. Enable Log data in the Area Control Window, and name a results file in the Save As dialog.
- Feed leaves carefully into the conveyor, one at a time.
   See also the *online Help* and *WinDIAS 3.3 Quick Start Guide*..

Note: With the Conveyor measurement software switched on, the software will automatically grab and measure the area of any object in the field of view, whenever it completely enters the field of view from the "left". The Conveyor should run from "left" to "right". (We use quotes here for two reasons. Firstly, the camera's left may be opposite to yours. Secondly, some cameras (and software, not WinDIAS) can invert the image.

### How to Measure Long Leaves

Use this for leaves which are too long to be grabbed all at once.

The Long Leaf mode requires a leaf of known area for calibration.

Instead of grabbing each whole leaf once, the conveyor constantly measures a narrow vertical strip in the field of view, as the leaf continuously enters, producing a cumulative total proportional to the whole area. This is then scaled according to the known area of the reference leaf, which you have to determine independently.

- 1. Position a ruler the centre of the conveyor belt, (stop the conveyor), focus on it and grab an image of the stationary ruler and calibrate WinDIAS.
- 2. Cut a short section from your long leaf and feed it into view in the centre of the the conveyor, grab an image of the stationary leaf portion and measure its area.
- 3. Turn on the converyor.
- 4. In WinDIAS click on the Conveyor and Long Leaf check boxes to enable both modes

Control and Mode			
🔽 Conveyer 🔽 Long Leaf			

5. Feed through your reference leaf again, and enter its known area when asked.

The long leaf mode is now calibrated.

6. Feed long leaves carefully into the conveyor, one at a time.

## How to Measure Shapes.

#### **Object Measurement - Shapes**

WinDIAS can measure several simple shape measurement functions such as perimeter and object count.

For a full description of the shape analysis functions see the *Glossary* on page 75.

For convenience we have supplied a set of flat, black, target shapes on transparent A4 film. These can be found in the optional accessory called the **WinDIAS Colour Rendition Card and Target Set type WTS**.

The first film has accurate reproductions of rectangles, triangles and circles drawn on the film by laser lithography and has data about the objects printed beside them.

Alternatively, you may use coins or cut out pieces of paper as sample targets, or create and print an array of shape using an image editing application.

#### Calibrate

- Select **Object** control tab.
- Grab image and calibrate as described on page 24..

#### Grab Image

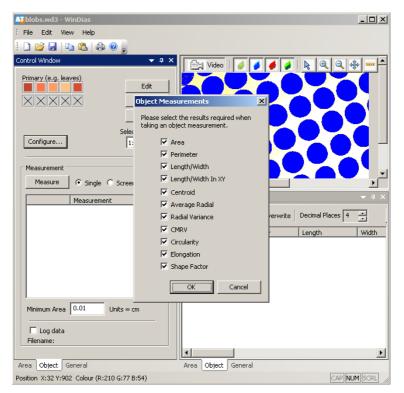
- Place your object target on the light box
- Optimise the illumination, camera aperture and focus to give a sharp well-defined image.

• Threshold the image as described in *How to Measure Leaf Area* on page **25** (depending on the lighting conditions, you have used you may need to pick more than one black area).

Continued on next page

#### Select Object Mode

Click on Configure to display the Object Measurement dialog



Click on the type of measurement(s) required and select OK.

• Set the **Minimum Area** less than the area of the smallest object: e.g. 0.01 cm or whatever is convenient. This stops small objects such as debris and dust from giving spurious readings.

#### Measure

You are now ready to measure the shapes. The Object Control gives a choice of **Single** and **Screen**.

Select Screen to measure all the blobs

After a few seconds the results will appear both in the **Control Window** and be appended to the **Measurement Results** window.

Note: The **Single** measurement mode will do the same analysis but on one object only, which you must click on to select.

## How to Analyse Plant Seeds

To count non-overlapping seeds, use **Count** in the **General** Control Window.

To analyse the shape of non-touching seeds, use the **Object** Control Window.

To count seeds which touch, use the **Seed Count** Control Window.

### How to Use Seed Count

The **Seed Count** Control Window provides a means for counting seeds which touch, using an average area method. For this to work, some seeds should not touch. Also, the degree of overlap of touching seeds should be small, for the overlap area will create a systematic error in the results.

- 1. Acquire seed image, ideally with minimal seed overlap, uniform background, good contrast and minimal shadow.
- 2. Set the threshold using the standard colour picking method.
- 3. Click on + Pick Seed and
- 4. Click on one single seed clearly separated from the rest.
- 5. Repeat steps 3 and 4 on several different isolated single seeds. Each time you do this the Control Window calculates the following:- area of the single seed, the running average area of the measured seeds so far, and the total area of all the seeds. From the running average and the total area of all seeds it estimates their number.

From the measured variance in the individual seed areas, and other parameters, it also estimates a statistic representing the uncertainty in the total count, shown as one standard deviation.

- 6. Continue repeating steps 3 and 4 until you either run out of separate seeds, or the statistic ceases to improve significantly.
- 7. Click on "Add results to the measurement window". This copies the last row of data across to the Measurement window.

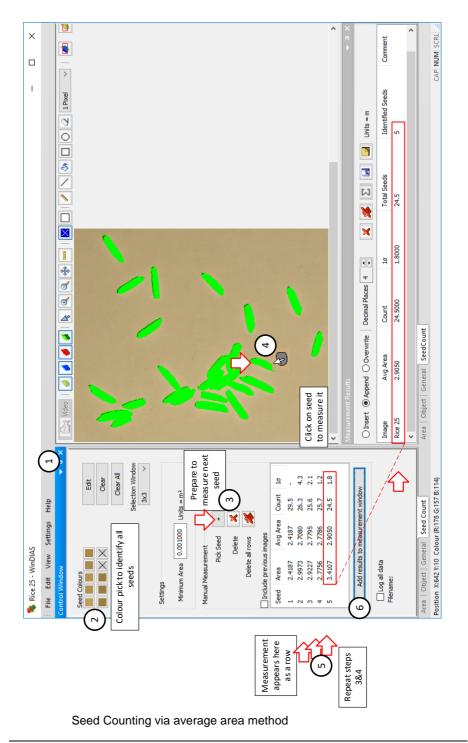
If you have another image of more of the same sample of seeds, then start again at step 1.

Your measured results when added, will then accumulate row by row in the Measurement Window.

#### Options

**Log data**. Saves a record of every line appearing in the Control Panel results window. Check this if you want a complete record of all the data. This might be appropriate for quality control.

**Include previous images**: Includes results from earlier images when calculating the average area.



# Accuracy of seed counting using the average area method

The average area method is similar to the use of weighing to count seeds, which uses the total weight divided by the average weight.

A key difference is that it does not matter if the weighed seeds overlap, whereas a systematic under-reporting error will be added to the average area method.

Given the above, WinDIAS uses a statistic developed by Steiner and Mackay (2001) for estimating the variance of seed counting accuracy based on weighing.

The standard deviation  $\sigma_r$  of the count is derived as follows:

$$\sigma_r^2 = n\gamma^2 \left\{ 1 + \frac{n}{p} \right\}$$

Where

$$\gamma = \frac{\sigma}{a}$$

 $\sigma\,$  = standard deviation of area of the individually measured seeds

 $\alpha$  = average area of the individually measured seeds

n =estimated total number of seeds = Total Area/a.

p = number of individual seeds identified and measured

Reference: S.H Steiner & R.J. Mackay (2001) *Seed Counting.* IIQP Research Report RR-01-07. Dept. of Statistics and Actuarial Sciences, University of Waterloo, N2L 3G1, Canada.

## **Trouble Shooting: No Video?**

📽 Untitled - WinDIAS				
File Edit View Settings Help				
i 🗋 💕 🛃 🖃 🗞 🕰 🕼 💮 💂				
Control Window + 4 ×				
Primary				
	Video source not detected			
XXXXX Clear				
Secondary Clear All Colours	Check			
Selection Window	1) Camera is plugged into PC			
	2) Camera is not is use by another application			
Tertiary           X         X         Reset Thresholds	2) Camera is not is use by another application			
	To Connect to Video Source			
Control and Mode	1) Select Settings, Video			
Conveyer Long Leaf	2) 🔽 Enable Video			
Measurement	3) Select Video capture device			
	4) Click <b>OK</b>			
Measure 🖌 % Area 🖌 Show Total	,			
Area Total	Troubleshooting			
Secondary	🎎 Remove and reconnect USB cable			
Tertiary % Diseased	🍇 See also on-line Help			
Linits = cm <sup>2</sup>				
Area Object   General				
Measurement Results	▼ 4 ×			
OInsert O Append O Overwrite Decimal Places 4 😋 🗶 🌋 🔽 🕞 🕝 Units = cm				
Primary Secondary Tertiary	% Diseased H Total D Total % Total Comment			
Area Object   General				
	CAP NUM SCRU ,;;			

The message above indicates that WinDIAS cannot find a camera.

(You may also see it temporarily when the image is resized.)

Select Settings, Video.

(continued on next page)

Video Settings			
	Enable Video	To allow WinDIAS to look for and receive video	
	Video Capture device	uEye Capture Device 1	
	Video Resolution	1280 x 1024 RGB24	
	Current Video Setting	(1280 × 1024)	
	Pause Video (Some cameras requre the video to be paused to allow the settings to be saved).		
		Advanced Camera Config	
		Advanced Video Config	
		OK Cancel Help	

## Video Configuration screen showing a IDS uEye 1465LE-C camera correctly found by WinDIAS.

Check to see that your camera is present. If it is available, select it and press **OK** to return to the main WinDIAS screen.

Note also that in the Video Resolution field the camera is correctly telling WinDIAS its resolution.

#### If no cameras are identified

Ensure the camera USB3 cable is correctly plugged into camera and PC.

Ensure you are not trying to run two copies of WinDIAS at once!

Exit WinDIAS, remove and reattach the camera USB cable, wait about 30 seconds for the hardware to redetect the camera, and restart WinDIAS.

Check the light on the back of the IDS 3270-CP camera turns from red to green when connected.

If the camera still does not appear in the Video Capture Device box it may be necessary to re-install the camera drivers.

#### See also: WinDIAS 3 Software Installation

#### See also: IDS uEye User Manual.

Note: A correctly installed USB camera will work with the demonstration software provided by the manufacturer.

For the IDS uEye cameras this is called uEye Demo or uEye Cockpit

This provides you with a way of checking that the camera is working independently of WinDIAS.

See also IDS uEye User Manual



#### If more than one camera present

This can happen!

More than one camera may be present if, for instance, you also have a web cam or microscope USB camera attached to your PC

Click on the Video Capture Device down arrow to select your camera if available. select the **uEye Capture Device 1.** 

# Camera Connection: New Hardware Found Wizard

After having already installed the camera drivers during the WinDIAS Setup, it may come as a surprise to find that when you plug the USB camera in to the PC another Windows wizard runs.

This links the physical camera to the camera driver software.

In fact two wizards run. First the IDS uEye boot installs, and then the particular camera type is installed.

These two Wizards may run whenever you disconnect and reconnect the video camera.

You will be asked to make two decisions when these wizards run:

Firstly, - on "Can Windows connect to Windows Update to search for software?" select the "No not this time" option.



Windows will then warn you that the uEye software has not passed Windows logo testing. Ignore this and select "Continue anyway"



See also pages 13 to 15 of *WinDIAS 3 Install Guide* –This pdf file is installed on your desktop, located under Start , WinDIAS 3, Documentation.

Note : Recent Windows upgrades do not display these New Hardware Detected messages when a USB video camera is disconnected and then reconnected. The user experience, on disconnecting and reconnecting a camera, now appears much smoother.

#### Image upside down or wrong way round

Select Settings, Video, Advanced Camera Config.., Image Processing and enable the Flip frame vertical or Flip frame horizontal check boxes.

#### Camera not found

Try removing and reconnecting the camera USB cable to your PC.

## CONVEYOR

## **Safety Instructions**

Read all these instructions before using the equipment. Retain this document for future reference.

If this equipment is not used in the manner described in this document, the protection provided by the equipment may be impaired.

#### **Equipment Ratings**

This equipment is rated for operation on only one voltage and frequency combination. Please ensure that the equipment you have received is rated for the voltage and frequency of your mains supply. This information is on the label attached to the conveyor controller. CB-CU-1.

Supply voltage: 230V 50Hz AC 250mA MAX

Mains input via IEC mains connector.

Fuse rating T200mA 250V

This appliance must be earthed.

Do not disconnect the safety earth.

#### **Environmental Requirements:**

The Conveyor is intended for indoor use only in the following normal environmental conditions:

- Temperature Range: 5 to 40 degrees C
- Maximum Relative Humidity: 80% for temperatures up to 31 degrees C decreasing linearly to 50% relative humidity at 40 degrees C
- Mains voltage fluctuations: +/- 10%
- Transient over voltages: typically present on mains supply (Cat II IEC 60364-4-443)
- Pollution: Degree II

This equipment should be located on a stable surface.

#### **Hazard Warning Symbols**



#### Caution, Risk of electric shock.

This symbol indicates a risk of electrical shock if the connector is disconnected. The connector marked with this symbol is not a user operable part. This connector should only be disconnected by a qualified electrician.



#### Protective conductor terminal.

This symbol indicates that the connection is a protective earth connection and the unit shall not be operated with this disconnected. This connection should only be disconnected by a qualified electrician and must be reconnected before the equipment is connected to the mains supply.

## **Cleaning the Conveyor Belt.**

- Before cleaning the conveyor belt or any other part of this product disconnect it from the mains supply.
- To clean the conveyor belt, wipe the surface with a damp cloth moistened with water and a small amount of detergent.
- To remove any traces of detergent, wipe the belt with a damp cloth moistened with just water.

Do not use any solvents or abrasive materials on the conveyor belt or its frame.

## **Conveyor Operation Summary**

#### Operation

- Place the Conveyor Belt on top of the light box
- Avoid bright ambient light and sunlight
- Adjust the belt tension
- Slide guide rods into position
- Run WinDIAS software, and set the focus aperture and threshold.
- Select Calibrate and calibrate with a ruler.
- Select Leaf Area, conveyor on option.

SLACKEN THE BELTS AT FINISH

#### **Belt Speed Selection**

- Choose the fastest speed that does not blur the image.
- Set the camera shutter to around 1-10 ms.
- The image should take about two seconds to cross the screen
- Drop the idlers pulley in its slot to release the drive band tension
- Remove the knurled nut from the motor shaft
- Fit the desired pulley and lock it in place
- Re-tension the drive band to eliminate slip. Do not over-tighten
- Keep all liquids away from the drive band and pulleys

### **Belt Removal**

- De-tension the belts
- Remove the line-guides
- Unlock and release the roller shafts
- Lift out each belt and its pair of rollers
- On re-assembly, locate the bottom roller inside the rubber drive band

#### **Belt Renewal and Cleaning**

- Cut the new material to length: top belt : 80 cm, bottom belt:104 cm
- Make a butt joint using clear adhesive tape on both sides
- Clean the belts with anti-static spray or a damp soft cloth.
- Avoid abrasive cleaners and solvents.

## Introduction

The Conveyor Belt Unit is an optional extra for WinDIAS. It is designed to permit the rapid measurement of the areas of plant leaves. It sits on top of the light box.

Advantages

- Easier handling of leaves which do not readily lie flat
- Automatic totalling of area measurements
- Faster throughput of material



The Conveyor Belt Unit on the Light Pad

## Description

#### Conveyor

Two highly transparent belts are mounted one above the other, on soft rubber O-rings, on pairs of counter-rotating rollers.

The rollers are staggered and spaced so that the upper and lower belts press gently against each other. This arrangement grips and flattens leaves and enables the Conveyor Belt to handle objects which differ considerably in thickness and texture.

The rollers at one end are driven by a synchronous AC mains motor, the drive being transmitted via a gearbox, a drive pulley, and a flexible rubber drive band.

The synchronous motor maintains a constant speed, which is geared down by the gearbox. A set of four interchangeable drive pulleys provide a selection of belt speeds. The flexible rubber drive band is sufficiently in-extensible to maintain a constant Conveyor Belt speed under reasonable loading conditions.

The Conveyor Belt can be run on both forward and reverse directions using switches on the Conveyor control box.

#### **Conveyor Control Box**



The control has two switches and a fuse holder as shown above.

The large red button is an emergency stop. Press it down to stop the conveyor and disconnect the power. Twist and lift to reset it.

The three-way switch is used to control the Conveyor. When centred it is off.

Twist the switch left or right to activate the conveyor to the left or right as appropriate.

#### Safety Warnings

Ensure the Emergency Stop button is readily accessible to the operator at all times when using the Conveyor.

Do not let the Conveyor or Controller get wet.

There are no user-serviceable parts inside the Control Box.

The fuse should only be replaced with one of the same type and rating.

### Software

WinDIAS works with the conveyor in the area measurement mode.

Toggle "on" the Conveyor check box in the in the Area Control Window.

With the Conveyor measurement software switched on, the software will automatically grab and measure the area of any object in the field of view, whenever an object completely enters the field of view from the "left". The Conveyor should run from "left" to "right". (We use quotes here for two reasons. Firstly, the camera's left may be opposite to yours. Secondly, some cameras (and software, not WinDIAS) can invert the image.

#### **Direction of Conveyor travel**

#### Conveyor on, Long Leaf mode OFF

Leaf must travel from left to right.

A leaf travelling the "wrong" way will give a zero result.

#### Conveyor ON, Long Leaf Mode ON

The Conveyor belt may travel in either direction.

We recommend you choose one direction for calibration and measurement and stick with it.

## WARNING :

Do not position the guide rails so that they appear in the measurement area otherwise WinDIAS will assume that the guide rail is an object entering the measurement area

The Conveyor option only measures area.

## **Safety Precautions**

Please refer to all safety precautions supplied with the Conveyor Belt documentation

## Setting up

#### Choice of site

Choose an appropriate location for the system. Avoid bright lights in the ceiling. The Conveyor Belts are so shiny that bright lights above the system can reflect back up into the camera. This can be a nuisance, so choose somewhere with diffuse lighting.

#### **Choice of illumination**

You have a choice here. In our judgement, leaves are best imaged in colour when illuminated both from above and below.

Lighting from above helps bring out the colour. To prevent reflection off the conveyor belt into the camera it needs to be diffuse and low on the horizon. A light box below can help provide a uniform background.

### Install WinDIAS first

Install WinDIAS first WITHOUT the Conveyor Belt.

We recommend you become familiar with the operation of the WinDIAS software before using the Conveyor.

#### Install the conveyor

1. SWITCH OFF THE POWER to the conveyor.

2. Place the Conveyor Belt on the light box - which should be under the camera, on the camera stand. The motor should be on the far side, away from the operator.

3. Set the Conveyor Control switches to OFF

4. Connect the Conveyor Control box to the Conveyor Belt.

5. Connect all power leads, using the distribution box if provided, and switch on the mains power.

6. Adjust the Conveyor Belt tension - see "Belt tension Adjustment" on page 59.

Slide guide rods into position: The guide rods can enable the operator to define the position of the measurement window without having to refer to the picture on the monitor. They should be positioned outside of the measurement window. Objects can then be positioned accurately on the Conveyor Belt before appearing on the monitor picture.
 Position the overhead illumination. For best effect this should be low on the horizon so that no direct reflection enters the camera. Four

tungsten lamps are provided for this purpose.

The Conveyor is now ready for use.

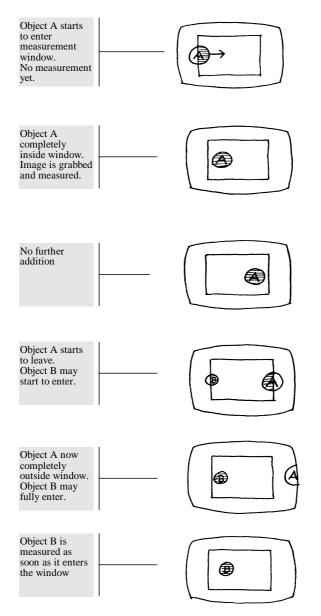
## After Use

IMPORTANT: ALWAYS slacken belts after use, or the soft rubber belt mounting rings may develop flats, which will cause a jerky belt movement.

Switch off and protect the system from dust.

## **Correct Operating Procedure**

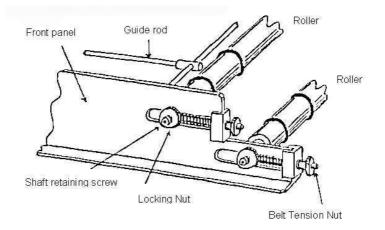
Objects should not be too close together. or they will be measured twice.



## **Belt** Servicing

Above all else, remember to slacken off the belts after use.

#### **Belt tension Adjustment**



#### Belt Tension Adjustment

The upper and lower conveyor belts are tensioned using the four belt tensioning nuts.

Initially the belt tension should be minimal.

Ensure that the tension is even and that the belts are centred.

Inspect the belts for ripples - this indicates uneven or excessive tension.

#### **Belt screeching!**

New belts tend to make a screeching noise if tensioned too much or unevenly

Adjust the belt tension

Belts become much less sensitive to the precise settings of the tension adjusters after some use.

#### Belt creeps to one side!

• Tighten the tension adjuster on the same side.

Take care not to over-tighten the belt by continuously tightening one side and then the other.

## Belt Speed

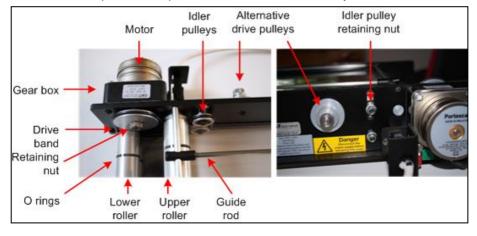
Use the fastest speed. The largest (5 cm diameter) pulley wheel goes fastest.

Cameras should be set to around 1 to 10 msec. shutter speed, with AGC off and any electronic iris circuit off as well.

. See also Blurred Image on page 71.

#### **Changing Belt Speed**

To change the belt speed you replace the drive pulley with one of a different size.



The spares are kept bolted to the side of the conveyor.

Figure 2 Changing belt speed

1. Slacken the idler pulley retaining screw, and drop the idler pulley in its slot.

- 2. Remove the knurled drive pulley retaining nut.
- 3. Pull off the drive pulley.

4. Fit another drive pulley on the drive shaft - note the locating pin in the drive shaft, which fits in the small hole in the drive pulley.

5. Replace the retaining nut and re-tension the drive band by sliding the idler pulley to an appropriate position in its slot. Avoid excessive tension in the drive band.

## **Changing Belts**

Refer to the diagrams "Belt Tension Adjustment" on page 59 and "Changing speed" on page 60.

- 1. De-tension the belts
- 2. Remove the line-guides
- 3. Unlock and release the roller shafts
- 4. Lift out each belt and its pair of rollers

5. On re-assembly, locate the bottom roller inside the rubber drive band

#### **New Belts**

1. Cut the new material to length :- top belt: 80 cm, bottom belt :104 cm

2. Make a butt joint using the clear adhesive tape supplied on both sides

- 3. Clean the belts with anti-static spray or a damp soft cloth.
- 4. Avoid abrasive cleaners and solvents.

#### Maintenance

#### Tension - Slacken off after use!

Slacken belts after use - otherwise the soft rubber wheels go flat and cause jerky belt movement.

#### Lubrication - none required.

None should be required as the rollers and idlers run on ball bearings.

Do not allow oil or other liquids to contaminate the drive band or any of the drive surfaces on the pulleys or rollers.

#### Cleaning

Belts may be cleaned with a soft damp cloth. Avoid abrasive cleaners and solvents. Anti-static spray may be used. but the belts should be removed to avoid contaminating the drive system.

### Spares and Consumables for the Conveyor

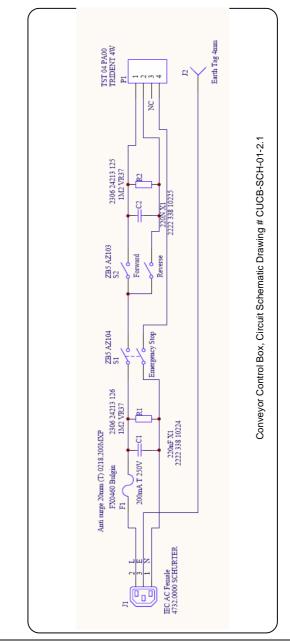
1  $\,$  x 20 m x 250 mm roll of melinex material to make about 10 sets of belts

1 x Roll of adhesive tape

- 2 x Spare drive bands
- 16 x O-rings for rollers

## **Control Box**

## Wiring Diagram



## PINE NEEDLE AND SMALL AREA MEASUREMENT

## Introduction

WinDIAS measures the projected area of pine needles, not their surface area. To obtain the actual surface area, multiply the measured area by a conversion factor, which depends on the shape of the needles.

### **Areas Conversion Factor**

Roberts, Pitman and Wallace (1982) obtained the total surface area of the foliage of Scots and Corsican pine by multiplying the projected area by 2.57. This was their estimate of the ratio of the projected to the total surface area determined by using a micrometer and a rule.

Cannel (1982) claims that "the all-sided needle areas of most pines are about 2.8 times their projected areas, and for most other conifers the ratio is about 2.3".

Flower-Ellis and Olsson found the ratio to be 2.758 (standard deviation 0.068) for Scots pine (*Pinus sylvestris L*). They found that fertilising or irrigating reduced this ratio significantly, to 2.680 (standard deviation 0.116) (private communication, 1986)

Other calculations on the surface area of pine needles based on measurement of their dimensions are given in Kozlowski and Schumacher (1943) and Ovington (1957) but these authors did not calculate the projected area.

### **Measurement of Small Areas**

With our standard camera you might use a zoom lens or close up lens adaptor for viewing small objects. WinDIAS3 may also work with a USB2 microscope.

If using an optical microscope your resolution cannot exceed either the resolution of the optics or of the camera.

Most users are much more likely to come up against the resolving power of the camera system first. If possible try to adjust your magnification so that the smallest dimension of the objects you are measuring is greater than about 10 pixels. Smaller than this will start to degrade accuracy.

The accuracy of the area of a single blob will be no better than half of the number of pixels on the perimeter times the area of one pixel. In the limit, when the object is no more than 1 pixel wide, the area could be in error by  $\pm 50\%$ 

Try to measure large numbers of non-overlapping objects simultaneously, to average out this error.

Accuracy will also be reduced where needles are joined together at their base.

#### References

Cannell MCR (1982) World Forest Biomass and Primary Production Data. Academic Press, London p3.

Kozlowskir T T and Schumacher F X (1943) Estimation of Stomated Foliar Surface of Pines. Plant Physiology, Vol. 18 pp 122-27.

Ovington J D (1957) Dry Matter Production by *Pinus sylvesties L.* Annals of Botany. NS Vol XXI, No 82 pp 288-313.

Roberts J, Pitman R M and Wallace J S (1982) A Comparison of Evaporation from Stands of Scots pine and Corsican pine in Thetford Chase, East Anglia. Journal of Applied Ecology, Vol 19 pp 859-72

## SPECIFICATIONS

## **Specifications**

	Entry Level System	Standard System	Rapid System
Throughput (leaves/hour)	~50 (typical, depends on leaf size)	~150	~800
Resolution	2400 dpi max	2048 x 1536 pixels	2048 x 1536 pixels
Minimum object size	~ 0.02 mm	1 pixel	1 pixel
Maximum sample area	297 x 210 mm (A4)	300 x 295 mm	250 x 290 mm (conveyor) 250 x >1000 mm (long leaf mode)
Accuracy			
area measurement1	± 1% typical	± 4% typical	± 4% typical
diseased/healthy area	contrast dependent	contrast dependent	contrast dependent
long leaf mode	not applicable	not applicable	± 5% typical
Colour depth	WinDIAS works in 24bit colour space (16 million colours)		
Image file formats	.jpg, .bmp and .tif		
Operating system and drivers	Windows XP, Vista and Windows 7,8 and 10 (32 and 64 bit)		
Calibration	against an object of known length, e.g. ruler not applicable against a target of known area		
static measurements			against a ruler
conveyor measurements			0
Conveyor belt speeds	not applicable	not applicable	60/100/140/190 mm/s (20% faster for 60Hz model)

Note 1: For very small areas, approx less than 200 pixels across, accuracy will become dominated by the pixel resolution error on the perimeter. This may be calculated as approximately one half the area of all pixels on the perimeter divided by the total number of pixels in the object.

### **Sample Dimensions**

The size of the field of view depends on several things:

- The focal length of the camera lens
- The magnification
- The size of the Area of Interest in WinDIAS
- The size of the light box. (430 x 310 mm)
- The size of the Conveyor, if used.

## **File Formats**

## WinDIAS .wd3 Format

WinDIAS3 files created using the **File**, **Save** command are binary files that contain a binary image copy of the grabbed image along with any picked colour threshold settings and calibration information.

The file is saved as filename.wd3

It includes the magnification and colour picking information. It does not include the camera settings.

## Old .wds format

Images previously saved in the private .wds format of earlier versions of WinDIAS are now not readable.

If this is a problem perhaps you could use your old version of WinDIAS to save them as a .bmp, .tif or .jpeg for processing by WinDIAS 3 – but the colour threshold settings and magnification may be lost.

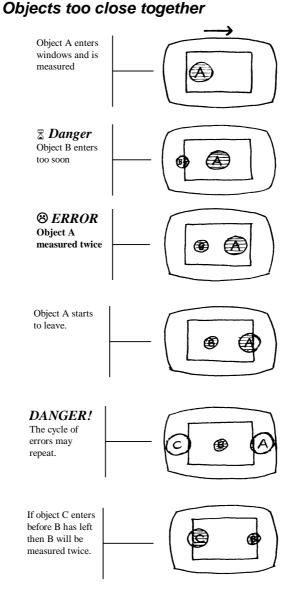
## TIFF, BMP and JPEG Images

Images can be Imported in these formats but they do not include colour picking and magnification information. Results from reloaded images could be unreliable if you change the colour picking thresholds or the magnification.

Hint: Save a reference leaf in.wd3 format with the filename **Reference.wd3** in the same folder as the saved image files. It will preserve the colour threshold settings and magnification, which may then be applied to other saved TIFF or BMP images reloaded into WinDIAS via the **File, Import Image** command.

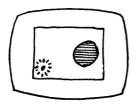
## **TROUBLE SHOOTING**

## Incorrect Conveyor Operation



#### Causes of spurious additions

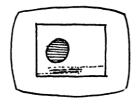
Dirt



A speck of dirt entering the window

Keep belts clean and limit the measurement window to the minimum size.

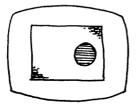
#### **Electrical Noise**



Electrical interference may cause a spurious addition. It can be caused by noisy mains power.

Try rearranging the mains wiring to the conveyor. Avoid switching other power sources on or off during measurements.

#### Threshold too high/ Colour range too broad



If the threshold settings are too high, or the aperture too low, and if the background illumination is not quite uniform, it may cause parts of the background to be interpreted as part of the object.

Reduce the range of colours detected, or try a different lens aperture, or provide a more even background.

#### Threshold too low/ colour range too small

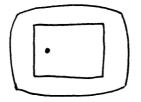


If the threshold is too low or the colour range insufficient, a complex shape may be detected as a sequence of small shapes. Each can separately trigger the grab/measurement cycle, causing multiple additions to occur, with spurious results.

Increase the number of colours or the colour range or individual selected colours.

#### Sources of error

#### **Object too small**



Objects are too small to trigger addition.

Currently the only way to deal with this if you are using the conveyor is to increase the magnification, move the camera closer to the object and refocus, or zoom in with the zoom lens

If you are not using the conveyor then you also may reduce the minimum object size.

This function does not work when the conveyor is running.

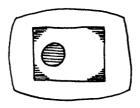
#### **Fixed object present**

$\square$		
	0	

Check that the conveyor guide rod, or other fixed objects, are not permanently intersecting the vertical edge of the video window

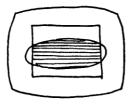
e.g. an incorrectly positioned guide rod.

#### Colour threshold or range too high



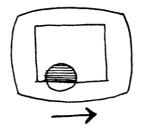
If shading appears in the corners of the field of view, pick a narrower range of colours. See if you can make the background illumination more uniform. Cheap camera lenses can cause this too.

#### Object too big



Raise the camera. Zoom out if you have the zoom lens, or cut the object into smaller pieces.

#### Object not fully in window



Position the leaf so that it appears completely inside the measurement window.

#### Blurred image, measured area too small

A moving image may appear too small if the image is blurred. A moving image could be blurred simply because it is out of focus, in which case check the focus with the conveyor stationary But the main thing to check is the shutter speed. Aim for around 10 milliseconds (ms).

Too slow and the image blurs, too fast and you see mains frequency beating in the illumination.

The default shutter speed of the IDS uEye 2240-C camera, at around 130ms, is too slow for use with the conveyor. On this setting you may lose 20% of your image area due to the blurred edges of the grabbed image.

One way to check if you have a blurring problem is to measure the area of the same leaf in the static and conveyor modes.

If the background illumination level changes cyclically you are witnessing a beating effect between the camera and mains frequencies.

See also: How to change the camera shutter speed on page 22.

#### Camera sensitivity "Bounce"

Camera design is constantly improving, using increasingly complex electronics and software for automatically compensating for variations in overall illumination. These may defeat the accuracy of WinDIAS threshold settings if enabled. In particular the Automatic Gain Control (AGC) should not be turned on.

In the past, with analogue Vidicon tubes and CCD cameras we found a further method to help prevent the camera sensitivity bouncing when an object appears and disappears. This was to place a fixed object, permanently outside of the measurement window, but within the camera's field of view. We used to recommend use of the two guide rods on the conveyor for this. This method is not now recommended, we prefer to simply disable the AGC-type camera settings..

### **Trouble shooting Camera Settings**

## Camera settings do not persist from one WinDIAS session to the next.

Open WindDIAS using Run as administrator.

Select Settings, Video, Advanced Camera Config, Device, choose the settings that work best for you, as described elsewhere in this manual. Then select OK to exit Advanced Camera Config Select Pause Video Reselect Advance Camera Config and select Device. In Load/Save location select local registry,

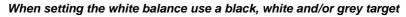
Select Save Settings and click OK

De-select **Pause Video**. Your camera setting should now load when you run WinDIAS.

#### Background is Coloured

With live video and a *black, white and/or grey* image on screen, select **Settings, Video, Advanced Camera Config, Color Settings, Whitebalamce Mode** and choose **One shot.** 

This will adjust the RGB values of the pixels and try to make them the same, (e.g. 30,30,30 or 200,200,200) so you need white, black or grey in the field of view. Once the colour balance is set you can look at a coloured subject and the whites should look white.



#### Background is too bright

You may find the light box so bright that it drives the camera into saturation – so that the background is over-exposed. This may degrade the precise edge of the image. The LED light panel has a touch sensitive button for adjusting the intensity.

Alternatively Insert clean sheets of A3 paper under the conveyor, onto the light panel, to reduce the illumination.

## Background is beating, pulsing or shows light and dark bands

Try altering the shutter speed.

Too slow and you lose image definition (if using the conveyor belt). Too fast and you see beating with the mains frequency.

Try different shutter speeds until you reach a good a good compromise. The overhead LED lights do not flicker. It is only the light panel (light box) that flickers, and of course any room lights turned on

# Light Reflected from the top of my leaf creates false colours

Angle the overhead lights so that they do not reflect directly into the camera

If a leaf is not flat you may still see glare from the overhead lights reflected in the curved surface of the leaf, changing its apparent colour. If so it may be necessary to shield the lights, move them further away, or shine through diffusers or polarising filters, or reflect the light from a diffuser. At the time of writing this we are looking into accessories to clip onto the overhead lights, to help solve this.

A double reflection image of the leaf may be discerned as a zone of higher saturation in the centre of the image when using the Conveyor. This is a reflection of the image in the upper conveyor belt.

It may help to move the camera further away from the image, but the root cause is inherent in the design of the double conveyor to hold the leaf flat.

We are looking into ways of improving this.

#### **Imaging Techniques**

To obtain the best results from WinDIAS, it is worth putting some effort into optimising the image quality. The following suggestions may help:

- WinDIAS will function best in diffuse light with no bright light sources or reflections nearby.
- Experiment with the lighting so that the optimum colour and contrast can be imaged by the camera (a combination of back-lighting and overhead lighting usually gives good results, but only overhead or back-lighting is sometimes necessary).
- The biggest source of error is often due to changes in illumination. Try to ensure that windows are screened. Give lights half an hour to stabilise.
- The image should not be too small or pixel resolution errors will dominate the accuracy. Try to have images larger than 200 pixels across.
- Use the acrylic sheets to reduce errors in area measurement due to "crinkling" of leaves (except when using the Conveyor)
- For small objects, add a zoom lens (remember to check the calibration if adjusting the zoom lens focus).
- For very small objects a USB2 microscope may be needed.
- For very large objects the camera stand can be extended with the aid of a suitable extension rod, or the camera can be mounted on an alternative mounting system.
- Image files of very large or very small objects may be imported using the **File**, **Import Image** command.
- Whilst flat-bed scanners can have very high resolution, accuracy may be dominated by the directionality of the scanner light source, which, combined with the leaf profile, e.g. non-flat leaf ribs, may change the thresholded area slightly, depending on the leaf orientation. To evaluate the magnitude of this error, scan the same leaf in at several orientations and compare the results.

#### My Computer keeps crashing!

If the mains power supply is erratic it may be desirable to use a line voltage conditioner or an uninterruptible power supply. If you have a special monitor with its own driver software check with the supplier to ensure that you have the latest version.

#### Image leaks across boundaries during fill

Boundaries 1 pixel thick or intermittent boundaries may cause the measured area to "leak" out into the surroundings and give a false result. Increase the boundary to 2 pixels wide to ensure that you have continuous boundaries.

## Glossary

#### Definitions

#### Aperture

The size of the iris in the lens. A measure of its light gathering capability. The lower the F number the more that light can be gathered in. The optimum setting maximises the dynamic range of the camera and image grabber, whereas too much light will tend to saturate the camera and reduce its ability to show differences.

#### Area of Interest

The area within the measurement window. The size of the measurement window can be changed using the Area of Interest drawing tool.

#### Average Radial

This is an average of all the distances measured from the centroid to each perimeter point.

$$\overline{R} = \frac{\sum R_J}{n}$$

Where Rj is the jth radius measured from the centroid to the jth perimeter point, and n is the total number of perimeter points.

#### Centroid

The centroid of an object is the most central point or centre of gravity of the object, (measured from the top left-hand corner of the screen). WinDIAS calculates it using an algorithm based on the following equation.

$$\overline{X} = \frac{\frac{1}{6} * \sum \left[ \left( X_{i+1}^2 + X_{i+1} * X_i + X_i^2 \right) (Y_{i+1} - Y_i) \right]}{\frac{1}{2} (Y_{i+1} + Y) (X_i - X_{i+1})}$$



$$\overline{Y} = \frac{\frac{1}{6} * \sum \left[ \left( Y_{i+1}^2 + Y_{i+1} * Y_i + Y_i^2 \right) \left( X_i - X_{i+1} \right) \right]}{\frac{1}{2} \left( X_{i+1} + X \right) \left( Y_i - Y_{i+1} \right)}$$

#### Circularity

Circularity is the square root of the ratio of the actual area of the object to the area of a circle with the same circumscribed diameter

$$C = \sqrt{\frac{A}{A_P}}$$

Where A is the actual area of the object and  $A_p$  is the area of a circle with a diameter equal to the circumscribed diameter or length of the object.

#### CMRV

This is the correlation of the Average Radial and the Radial Variance.

$$CMRV = \frac{RV*100}{\overline{R}}$$

#### **Colour Range**

Each time you pick a colour you actually pick the mid-point of a small range of colours. The range is pre-set for you. You can alter it, but you do not have to. You can instead just pick more colours. There is a trade-off here between convenience and analysis speed. The more colours you pick the slower the measurement.

#### **DPI** -Dots per Inch

Scanned image files are usually expressed in dots per inch.

Photographic camera resolution is expressed as the total number of pixels in the image.

Video camera resolution is given for the width and height separately:

The IDS UI-SS40-C camera has 1280 x 1024 pixels

The IDS UI-1465LE-C camera has 2048 x 1536 pixels

#### **Elongation-**

Elongation is the ratio of the length and width.

$$E = \frac{w}{l}$$

#### Length

Usages :

a) Distance between two points marked on screen using the mouse.

Used in the single and multi-step options for Length in the Measure Menu.

b) Diameter of the smallest circumscribed circle that will fit around an object

Used when WinDIAS calculates length in Objects option of Measure Menu.

#### **Overlay Plane**

This is a separate video memory area used to display the primary (e.g. healthy), secondary (e.g. diseased) and tertiary (e.g. dead) regions of the object.

#### Pixel

The smallest element of a digitised picture.

#### **RGB Colour Space**

Each picked colour is defined as ratio of Red: Green: Blue (RGB), occupying a small volume in a three-dimensional colour space. At the extremes black is represented by the colour vector (1,1,1), white is represented by 255,255,255.

When you pick a particular colour it will be some mixture of Red, Green and Blue. For instance, it may have the value (100, 50, 60). WinDIAS does not simply show these pixels on the overlay plane because it would take for ever to identify all the greens and greeny blues and greeny reds in your healthy leaf.

So WinDIAS assumes a small range or variance of reds, greens and blues around each point that you pick.

So every time you pick a point on the leaf, a small volume in 3-D colour space is selected, and allocated to either the Primary (Healthy) or Secondary (Diseased) or Tertiary (Senescent) Zones. All parts of the picture lying in those zones are identified as healthy or diseased.

#### **Shape Factor**

Shape Factor is the ratio of the actual perimeter to that of a circle with the same area.

$$S = \frac{P}{P_C}$$

Where P is the perimeter of the object and PC is the perimeter of a circle with the same area as the object. PC is calculated as follows

$$P_C = 2 \big(\Pi * A\big)^{0.5}$$

Where A is the actual area of the object.

The size of the smallest element in the image of the picture.

#### **Radial Variance**

Radial Variance is square of the standard deviation of all distances measured from the centroid to each perimeter point.

$$RV = \frac{\sum \left(R_J - \overline{R}\right)^2}{n - 1}$$

Where Rj is the jth radius measured form the centroid to the jth perimeter point, and n is the total number of perimeter points

#### Width

In Object shape analysis

Width of track traced out by dragging the object along in the direction of its length. Imagine a snail leaving a trail. What you get is the width of the track left behind.

Warning: for a square the length and width algorithms both give you the length of the diagonal!

#### **Colour Width**

When you pick a colour, for instance to represent the healthy part of a leaf, a band of closely related shades is also automatically selected. You have control over the range of shades associated with each colour. On a

linear scale this can be defined by its centre point, i.e. the colour you pick, and the width of the range of colours either side. See also "RGB Colour Space" on page 77.

#### Width in X

Width of thresholded object, measured along the horizontal, X axis.

#### Height in Y

Height of the thresholded object measured along the vertical, Y axis.

## **Technical Support**

#### **Terms and Conditions of sale**

Our Conditions of Sale (ref: COND: 06/14) set out Delta-T's legal obligations on these matters. The following paragraphs summarise Delta T's position but reference should always be made to the exact terms of our Conditions of Sale which will prevail over the following explanation.

Delta-T warrants that the goods will be free from defects arising out of the materials used or poor workmanship for a period of twenty four months from the date of delivery.

Delta-T shall be under no liability in respect of any defect arising from fair wear and tear, and the warranty does not cover damage through misuse or inexpert servicing, or other circumstances beyond their control.

If the buyer experiences problems with the goods they shall notify Delta-T (or Delta-T's local distributor) as soon as they become aware of such problem.

Delta-T may rectify the problem by replacing faulty parts free of charge, or by repairing the goods free of charge at Delta-T's premises in the UK during the warranty period.

If Delta-T requires that goods under warranty be returned to them from overseas for repair, Delta-T shall not be liable for the cost of carriage or for customs clearance in respect of such goods. However, Delta-T requires that such returns are discussed with them in advance and may at their discretion waive these charges.

Delta-T shall not be liable to supply products free of charge or repair any goods where the products or goods in question have been discontinued or have become obsolete, although Delta-T will endeavour to remedy the buyer's problem.

Delta-T shall not be liable to the buyer for any consequential loss, damage or compensation whatsoever (whether caused by the negligence of the Delta-T, their employees or distributors or otherwise) which arise from the supply of the goods and/or services, or their use or resale by the buyer.

Delta-T shall not be liable to the buyer by reason of any delay or failure to perform their obligations in relation to the goods and/or services if the delay or failure was due to any cause beyond the Delta-T's reasonable control.

#### **Service and Spares**

Users in countries that have a Delta-T distributor or technical representative should contact them in the first instance.

Spare parts for our own instruments can be supplied and can normally be despatched within a few working days of receiving an order.

Spare parts and accessories for products not manufactured by Delta-T may have to be obtained from our supplier, and a certain amount of additional delay is inevitable.

No goods or equipment should be returned to Delta-T without first obtaining the return authorisation from Delta-T or our distributor.

On receipt of the goods at Delta-T you will be given a reference number. Always refer to this reference number in any subsequent correspondence. The goods will be inspected and you will be informed of the likely cost and delay.

We normally expect to complete repairs within one or two weeks of receiving the equipment. However, if the equipment has to be forwarded to our original supplier for specialist repairs or recalibration, additional delays of a few weeks may be expected. For contact details see below.

#### **Technical Support**

Users in countries that have a Delta-T distributor or technical representative should contact them in the first instance.

Technical Support is available on Delta-T products and systems. Your initial enquiry will be acknowledged immediately with a reference number. Make sure to quote the reference number subsequently so that we can easily trace any earlier correspondence.

In your enquiry, always quote instrument serial numbers, software version numbers, and the approximate date and source of purchase where these are relevant.

**Contact Details** 

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