

AngioSys 2.0 Image Analysis Software Manual

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Introduction and ordering information

The AngioSys 2.0 image analysis software provides a semi-automated analysis of angiogenesis by measuring tubule area, length and number of tubule junctions. Angiosys 2.0 has been developed to quantify images of angiogenesis tubules obtained from conducting the Cellworks Angiogenesis Assay Kit: V2a Kit – Vasculogenesis to Angiogenesis (Product Code ZHA-4000). Image files can be conveniently grouped and processed to provide quantitative and repeatable measurements. Image processing methods can be saved and applied to groups of images to enable rapid analysis. The resulting data can be exported as a csv file in spreadsheet format.

AngioSys 2.0 software is available in 2 formats:

- A 21 day demonstration version, product code ZHA-5000D, that can be downloaded free of charge at: http://www.cellworks.co.uk/products/product_detail.php?CI_ID=14975



- Full licence, product code ZHA-5000, that can be downloaded at http://www.cellworks.co.uk/products/product_detail.php?CI_ID=14974.
- Activation of the full licence requires a serial key number which will be emailed to you upon receipt of a purchase order. Please submit an order to office@caltagmedsystems.co.uk or fax to +44 (0)1280 857466, containing the email address you wish the serial key number to be sent to.

Technical support is available via email from techsupport@caltagmedsystems.co.uk. Please use this email address to report any defects or requests for enhancements.

1. Activate your software

To activate the software and gain full access to all of the AngioSys features:

- 1.1 Open AngioSys 2.
- 1.2 Click on “Help” in the top panel
- 1.3 Click “Activate product”
- 1.4 Enter your unique serial key number in the box which you would have received via email
- 1.5 Click “Ok”

2. Analyse a new plate

To analyse a new plate:

- 2.1 Open AngioSys 2.0
- 2.2 Select “New” when the window opens
- 2.3 Enter a name for the plate
- 2.4 Enter the magnification of the objective lens used to capture the images (this can be left blank and completed later on in the process if the images were taken under different magnification).
- 2.5 Enter the magnification of the camera adapter used to capture the images (this can be left blank and completed later on in the process if the images were taken under different magnification). This field requires a value, please enter “0” if an adaptor has not been used.
- 2.6 The “Notes” can be used to record any general observations or details about the plate such as reagent lot numbers etc.
- 2.7 Click on “Create”
- 2.8 Now go to section 4 – “Add well details”.

3. Open a previous plate

To open an existing plate:

- 3.1 Open AngioSys 2.0.
- 3.2 Select “Open” when the window opens.
- 3.3 Double click on the appropriate file name.
- 3.4 The plate will open in AngioSys 2.0.
- 3.5 Now go to section 4 – “Add well details”.



4. Add well details

To add details of each individual well:

- 4.1 Click on the appropriate well e.g. A1.
- 4.2 Click on the treatment box. Enter details of the treatment used in the well, e.g. VEGF control, Suramin control, test compound concentration.
- 4.3 In the magnification box, enter the magnification of the objective lens used to capture the image (unless already completed in section 2.4).
- 4.4 In the adaptor box, enter the magnification of the camera adaptor used to capture the image. Leave this blank if no adaptor was used (unless was already completed in section 2.5).
- 4.5 Any additional notes required for this well can be added to the “Notes” box.
- 4.6 The well will turn red once the details have been filled in.
- 4.7 To complete the well details for the remainder of the plate you can either:
 - 4.7.1 Click on each individual well and add details if every well has different settings.
 - 4.7.2 Fill a row/column with the same details see section 6.
 - 4.7.3 Custom fill a row/column see section 7.
 - 4.7.4 To delete well details or row/column see section 5 and 8 respectively.
- 4.8 Proceed to section 9 to upload images.

5. Delete well details

To delete the details for a well:

- 5.1 Click on the well that you wish to remove the details from.
- 5.2 Click on “Delete well”.
- 5.3 A warning box will pop up, if you are sure that you want to delete the details click on “Ok”.

6. Fill a row/column

To fill a row or column with all of the same settings:

- 6.1 Click on the first appropriate well.
- 6.2 Enter all of the well details as described in section 4.
- 6.3 Click on “Well Details” in the top panel.
- 6.4 Click on either “fill row” or “fill column”.
- 6.5 The settings from the first well will be applied to all of the wells in the row/column.
- 6.6 The wells in the row/column will turn red.

7. Custom row/column fill

If only certain settings are required to be transferred to the row/column e.g. magnification only:

- 7.1 Click on the first appropriate well.
- 7.2 Enter all of the well details as above.
- 7.3 Click on “Well Details” in the top panel.
- 7.4 Click on “Custom fill”.
- 7.5 Select the tick box of the settings that you require to be transferred to the row/column.



7.6 Click on either “Fill row” or “Fill column”.

8. Delete a row/column

To delete the well settings in a row or column:

8.1 Click on a well in the appropriate row or column.

8.2 Click on “Well Details” in the top panel.

8.3 Click on either “Delete row” or “Delete Column”.

Note – You cannot undo this action, and will also delete all images which have been uploaded.

8.4 The wells in the row/column will turn grey.

9. Add images

Click on the appropriate well.

9.1 Select the grey “Choose image” box on right hand side of screen.

9.2 Select the appropriate image file.

9.3 Click “open”.

9.4 The well will turn light green in colour once an image has been added.

9.5 Repeat until an image has been added to each of the wells requiring analysis.

10. Process images

10.1 Click on the appropriate well.

10.2 Click on “Process well”.

***See section 16 for further information and examples of image processing steps 1 to 5 below.**

Step 1: Region of interest (ROI)

10.3 Specify the region/area of interest and prepare it for creation of a binary image that the software can understand.

10.3.1 The goal is to exclude shadows and black borders in the corners and edges by drawing a Region of Interest (ROI) that contains only the area we wish to analyse.

10.4 To select your region of interest, click and drag the mouse across the image to draw a rectangle.

10.4.1 If you wish to change your selection, simply draw a new rectangle and it will replace it.

10.4.2 Click “Next”.

Step 2: Optimise

10.5 Click “Smooth” to help increase the accuracy of the image analysis. (This greyscales and sharpens the images).

10.6 Click “Next”.

Step 3: Threshold



- 10.6.1 Thresholding is a critical step in the analysis. A new binary image is created based on the original image, which will then be used to detect and measure the tubules.
- 10.6.2 The objective of thresholding is to cover the tubules within the area of interest with a close fitting binary overlay.
- 10.7** Adjust the slider to the right to increase the threshold value and include more of the darkened pixels from the original image in the binary image.
- 10.8** Reduce the threshold value by moving the slider to the left which will exclude all but the darkest pixels from the original image.
- 10.9** Click “Next”.

Step 4: Skeleton (see section 16 for examples)

- 10.9.1 To measure the tubule lengths and number of junctions, the binary overlay image must be skeletonised. This is achieved by progressively stripping off layers of pixels until each tubule is rendered as a line of single pixels. The single pixel lines can be detected, measured, and split to locate junction points. White lines tracing the tubules should be visible on the image.
- 10.9.2 This process can result in some of the tubules becoming dis-jointed. These are re-connected later on in the process.
- 10.10** Click the 'toggle image' button to view the binary image on its own.
- 10.11** Enter a value in the “dilation” box. Try different dilation numbers e.g. start at 10 to see if the tubule lines are joined up again. NB. At this stage do not worry about the additional “V shaped” branches – these are pruned later.
- 10.11.1 Each dilation performed on the binary image progressively thickens the existing skeleton before performing the skeletonisation again on the new image. Increasing this number can help reconnect disjointed skeletons, whilst decreasing this number can help remove unwanted joins. More dilations will result in additional spurs appearing on the skeleton.
- 10.12** Enter a value in the “pruning iterations” box.
- 10.12.1 Pruning iterations thin the skeleton to reduce anomalous branches that may have been introduced at the thresholding stage or during dilations of the skeleton. Increase the number to trim more of the spurs from the skeleton but this may also reduce the length of other tubules. Hint: Start off with a similar number to the dilation value. If following pruning, loss of tubule length is observed, enter a value in the end-point dilations box.
- 10.13** Enter a value in the “end-point dilations” box.
- 10.13.1 End-point dilations attempt to preserve the full length of the skeleton during the Pruning process by identifying existing tubule end-points and re-combining them with the pruned binary image. Increasing this number should help if loss of tubule length is observed after pruning iterations.
- 10.14** Click “Next”.

Step 5: Clean

- 10.14.1 After thresholding and skeletonisation of the binary image some spurs may remain on the skeleton. Before measuring the lengths and splitting the junctions, it is useful to set an exclusion limit on the tubule length to remove left over spurs. This is effectively background noise.
- 10.15** To remove the left over spurs/background noise, specify the exclusion limit in the “min” box. E.g. 5 pixels maybe sufficient.
- 10.15.1 Branches or tubules with a length below this limit will be pruned from the binary image.
- 10.16** Click on “Results”.



- 10.16.1 The measured tubules from the binary image are now displayed as red lines over the original image and junctions displayed as blue dots on the red lines. A summary of results is visible on the right of the screen.
- 10.17 If the results appear satisfactory, click 'Finish'. If for example, the background noise is still present, return to the previous stage and enter a higher value.
- 10.18 Otherwise, click on any of the headings (from stage 1 to stage 5) to the left of the image to return to that point and amend the settings.

11. Results

To calibrate the results in SI units, please see section 14 below. To export the results:

- 11.1 Click on "Plate" in the top panel.
- 11.2 Select "Export results", and click to export.
- 11.3 Select where you would like to save the file.
- 11.4 Name the file.
- 11.5 Click "Save".
- 11.6 The data for the whole plate will be saved as one CSV format text file.

12. Saving Plate Details

To save the plate details:

- 12.1 Click on "Plate" in the top panel.
- 12.2 Select "Save".
- 12.3 Select where you would like to save the file.
- 12.4 Name the file.
- 12.5 Click "Save".

13. Close the software

- 13.1 Save the work if required using the details above.
- 13.2 Click on "Plate" in the top panel.
- 13.3 Select "Close".
- 13.4 If you do not want to save the work and exit the programme click on "Ok" when the warning box opens.

14. Calibrate the software

- 14.1 The default calibration for each image is pixels, however you can calibrate the system to measure objects in the measurement units you require. Calibration is achieved by drawing a line of known length on the image (perhaps along a calibration bar) and entering the true length of the line. The system then calculates the required calibration factors. Once the system has been calibrated, each subsequent image is assumed to have been captured under the same magnification so that the calibration factor is valid for each image. Calibrations for different magnifications can be saved and loaded when required.
- 14.2 To calibrate the software:
- 14.3 Open AngioSys 2.0.
- 14.4 Click on "Calibrate" in the top panel.



- 14.5 A file dialog is presented to allow you to load the required calibration image. Once the image is loaded the calibration dialog is presented.
- 14.6 Use the mouse to draw a calibration line, of a length which is known.
- 14.7 Enter the "Actual length" in the text box.
- 14.8 Select the appropriate measurement units from the drop down box.
- 14.9 Click "Save".
- 14.9.1 The system is now calibrated.
- 14.9.2 This calibration process needs to take place for every new microscope setup.

15. Select a calibration

To select a calibration which you have already saved:

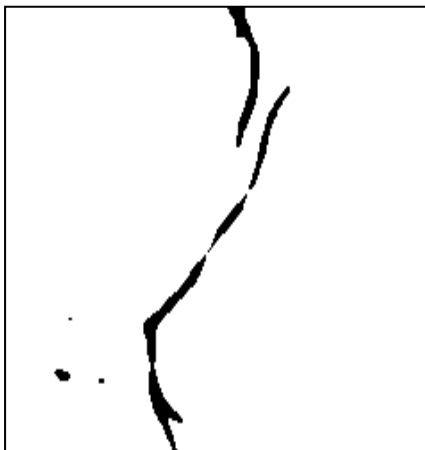
- 15.1 Click on "Calibration" in the top panel.
- 15.2 Select the appropriate calibration from the drop down list.

16. Skeletonisation functions

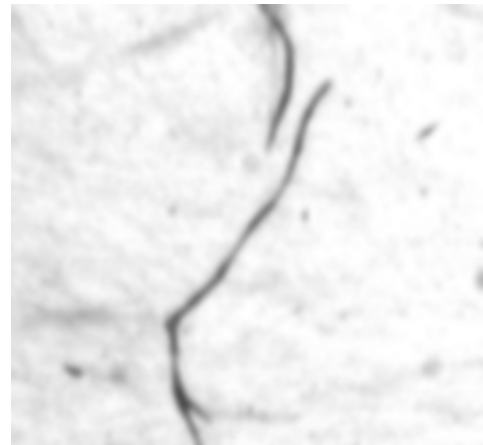
Further information and examples of use

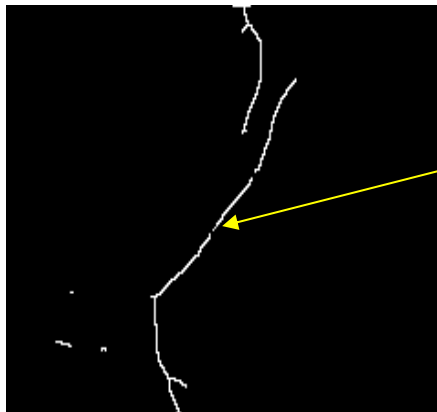
When thresholding an image, tubules can sometimes be left broken, due to lighter patches in the tubules but the threshold value cannot be increased any more without adding too much 'noise' to the binary image. For Example:

Using this image segment:



With a threshold of 179 gives:



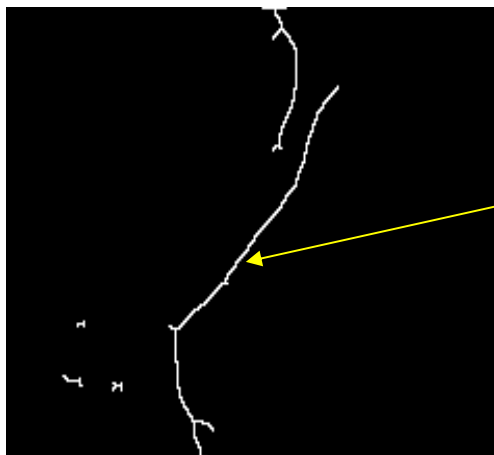


As there are gaps in the threshold, this leads to breaks in the resulting skeleton.

Dilation



Dilation is a morphological operation to progressively thicken a binary image. Applying a single iteration of dilation to the above threshold gives the following image.



This has now joined the tubule back together resulting in the following skeleton.

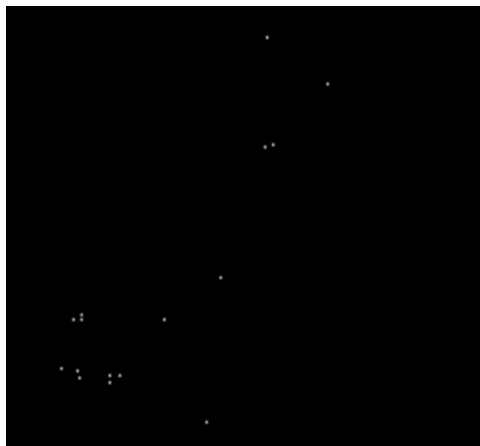
Pruning



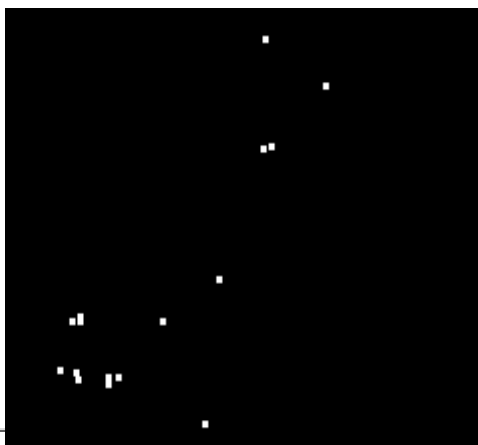
One of the downsides of the dilation operation is that extra 'spurs' can appear at the end of tubules. The Pruning function applies a morphological thinning operation on the skeleton aimed at reducing these extra 'spurs'. One iteration of this operation on the dilated skeleton above gives this result.

End Point Dilation

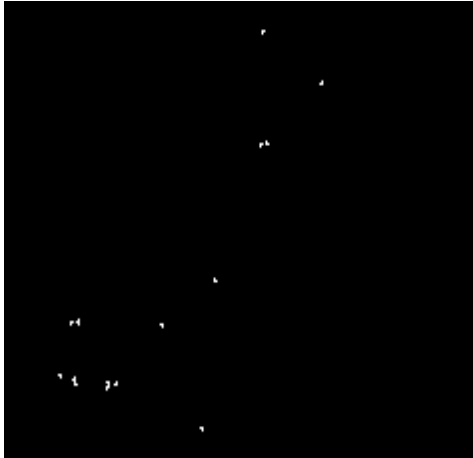
The Pruning operation can lead to shortening of the skeleton. The End Point Dilation operation attempts to limit this by trying to preserve the original end points of the skeleton.



First the end points of the skeleton are found.



A morphological dilation is applied to the end points; here is the above image with a single iteration.



Any pixels that were not already in the pre-pruned skeleton are subtracted.



This image is then combined with the pruned skeleton.

17. Check for updates

To check to see if there have been any updates to AngioSys 2.0:

- 17.1 Click on “Help” in the top panel.
- 17.2 Click on “Select for updates”.
- 17.3 If there have been updates, ensure all current work is saved and click “Ok”.

18. Image Formats

AngioSys will accept images in the following formats:

- TIFF
- BMP
- GIF



- JPEG
 - PNG
- Images should be resized to a maximum size of 1200 pixels x 800 pixels or approximately 300KB.

18.1 GFP Angiogenesis Analysis:

The software works on dark tubules on a light background. Photographs from the GFP Angiogenesis Assay (ZHA-4100) will need to be converted to binary images to allow the images to be processed.

19. Sample summary file

Well ID	Treatment	Image File	Magnification	Adaptor	Area	Junctions	Tubules	Total Length	Mean Length	Smoothing	Thresholding	Dilating	Pruning	End Point Dilations	Clean Length Threshold	Well Notes
A1	VEGF				378	378	11358	30	297.8	0	0	0	0	0	0	
A2	VEGF				1808	1808	28381	18	155.4	0	0	0	0	0	0	
A3	Media				81	128	3880	20	194.0	0	0	0	0	0	0	
A4	Media				90	108	3118	18	173.2	0	0	0	0	0	0	
A5	Surf				8	12	170	14	121.4	0	0	0	0	0	0	
A6	Surf				17	28	411	18	228.3	0	0	0	0	0	0	
B1	Wit				348	358	8288	21	394.7	0	0	0	0	0	0	
B2	Wit				338	308	8807	18	489.3	0	0	0	0	0	0	
B3	Wit				178470	118	381	4888	41	119.2	0	0	0	0	0	
B4	Wit				113888	338	8888	18	493.8	0	0	0	0	0	0	
B5	Wit				117888	187	187	8874	21	422.6	0	0	0	0	0	
B6	Wit				348888	378	378	7808	21	372.3	0	0	0	0	0	
C1	Cont				84	81	1108	11	100.7	0	0	0	0	0	0	
C2	Cont				48	71	888	18	493.8	0	0	0	0	0	0	
C3	Cont				88	88	1804	18	100.2	0	0	0	0	0	0	
C4	Cont				84	81	1108	11	100.7	0	0	0	0	0	0	
C5	Cont				80	114	3880	20	194.0	0	0	0	0	0	0	
C6	Cont				10	18	188	18	104.4	0	0	0	0	0	0	
D1	D5				188	180	4788	21	228.0	0	0	0	0	0	0	
D2	D5				811	404	7187	17	422.6	0	0	0	0	0	0	
D3	D5				371	308	8001	18	444.5	0	0	0	0	0	0	
D4	D5				381	388	8484	21	403.9	0	0	0	0	0	0	
D5	D5				434	438	7480	17	439.4	0	0	0	0	0	0	
D6	D5				338	371	8888	21	422.6	0	0	0	0	0	0	

The results CSV file states the following:

- Well ID, along with the treatment, image file name, magnification, and adaptor values
- The co-ordinates and area of the region of interest
- The number of junctions and tubules
- The total length of the tubules
- The mean tubule length
- The values that were entered for the smoothing, thresholding, dilating, pruning, end point dilations and clean length threshold
- The well notes



20. Getting help

If you require any assistance with the AngioSys software please contact the Cellworks technical support team and they will be happy to assist you.

Email: techsupport@caltagmedsystems.co.uk

Phone: +44 (0)1280 827460

21. System requirements

AngioSys 2.0 is able to operate on Windows XP, Vista, Windows 7, Windows 8, 32 bit, 64 bit

