

SUPPLEMENTAL MATERIAL

Title: Platelet-Rich Emboli in Cerebral Large Vessel Occlusion Are Associated with a Large Artery Atherosclerosis Source

Cover Title: Large Artery Emboli are Platelet-Rich

Authors: Seán Fitzgerald PhD^{1,2}, Daying Dai MD¹, Shunli Wang MD¹, Andrew Douglas MSc², Ramanathan Kadirvel PhD¹, Kenneth F. Layton MD³, Ike C. Thacker MD³, Matthew J. Gounis PhD⁴, Ju-Yu Chueh PhD⁴, Ajit S. Puri MD⁴, Mohammed Almekhlafi MD⁵, Andrew M. Demchuk MD⁵, Ricardo A. Hanel MD⁶, Eric Sauvageau MD⁶, Amin Aghaebrahim MD⁶, Albert J. Yoo MD⁷, Peter Kvamme MD⁸, Vitor M. Pereira MD⁹, Yasha Kayan MD¹⁰, Jossler E. Delgado Almandoz MD¹⁰, Raul G. Nogueira MD¹¹, Alejandro A. Rabinstein MD¹², David F. Kallmes MD¹, Karen M. Doyle PhD² and Waleed Brinjikji MD¹.

¹Department of Radiology, Mayo Clinic, Rochester, Minnesota, USA

²CÚRAM–Centre for Research in Medical Devices, National University of Ireland Galway, Galway, Ireland

³Department of Radiology, Baylor University Medical Center, Dallas, Texas, USA

⁴Department of Radiology, New England Center for Stroke Research, University of Massachusetts Medical School, Worcester, Massachusetts, USA

⁵Department of Radiology, Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta, Canada

⁶Stroke and Cerebrovascular Surgery, Lyerly Neurosurgery/Baptist Neurological Institute, Jacksonville, Florida

⁷Department of Radiology, Texas Stroke Institute, Dallas-Fort Worth, Texas, USA

⁸Department of Radiology, University of Tennessee Medical Center, Knoxville, Tennessee, USA

⁹Joint Department of Medical Imaging, Toronto Western Hospital, Toronto, Ontario, Canada

¹⁰Department of Radiology, Neuroscience Institute, Abbott Northwestern Hospital, Minneapolis, Minnesota, USA

¹¹Marcus Stroke and Neuroscience Center, Grady Memorial Hospital and Emory University, Atlanta, Georgia, USA

¹²Department of Neurology, Mayo Clinic, Rochester, Minnesota, USA

Corresponding Author:

Waleed Brinjikji

Address: Department of Radiology, Mayo Clinic, 200 1st St SW, Rochester, MN 55905

Email: Brinjikji.Waleed@mayo.edu

Telephone: 507-284-2511

Fax: 507-255-0706

Twitter: @WBrinjikji

Supplemental Methods:

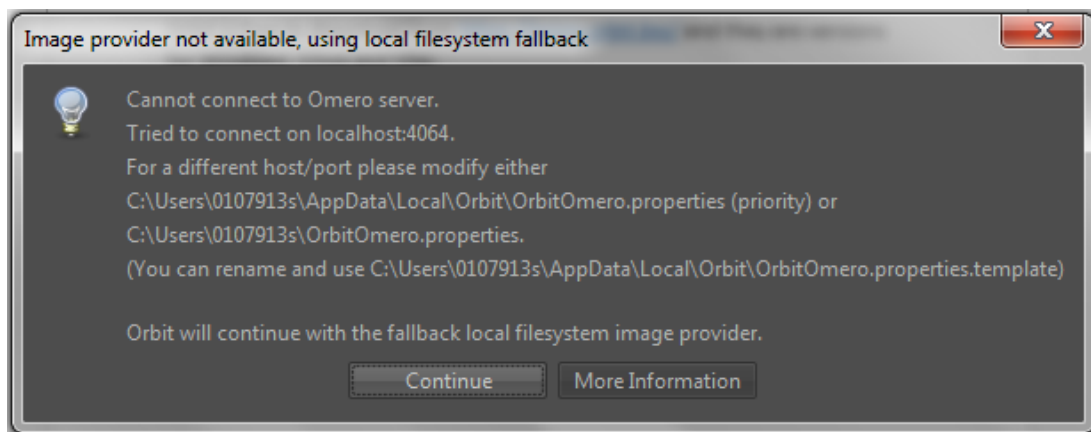
Orbit Image Analysis Standard Operating Procedure for Histology Quantification:

Orbit Image Analysis is free to download from <https://www.orbit.bio/> and there are versions available for Windows, Linux and Mac.

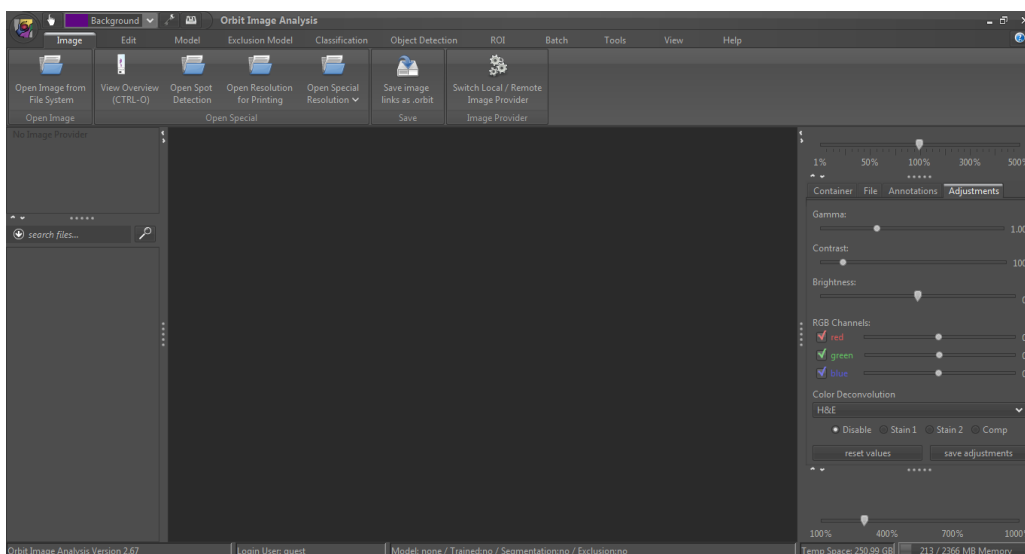
Permission to use the images from Orbit Image Analysis in this SOP has been granted by Manuel Stritt, the creator and author of Orbit Image Analysis.

For the purposes of this SOP I am going to use H&E as the stain and Red Blood Cells, White Blood Cells and Fibrin as the tissue types that I wish to quantify. However this can be adapted to different stains and different cell/tissue types as necessary.

- 1). Click on the desktop icon to Launch Orbit.
- 2). Sometimes a popup comes up and just click continue.

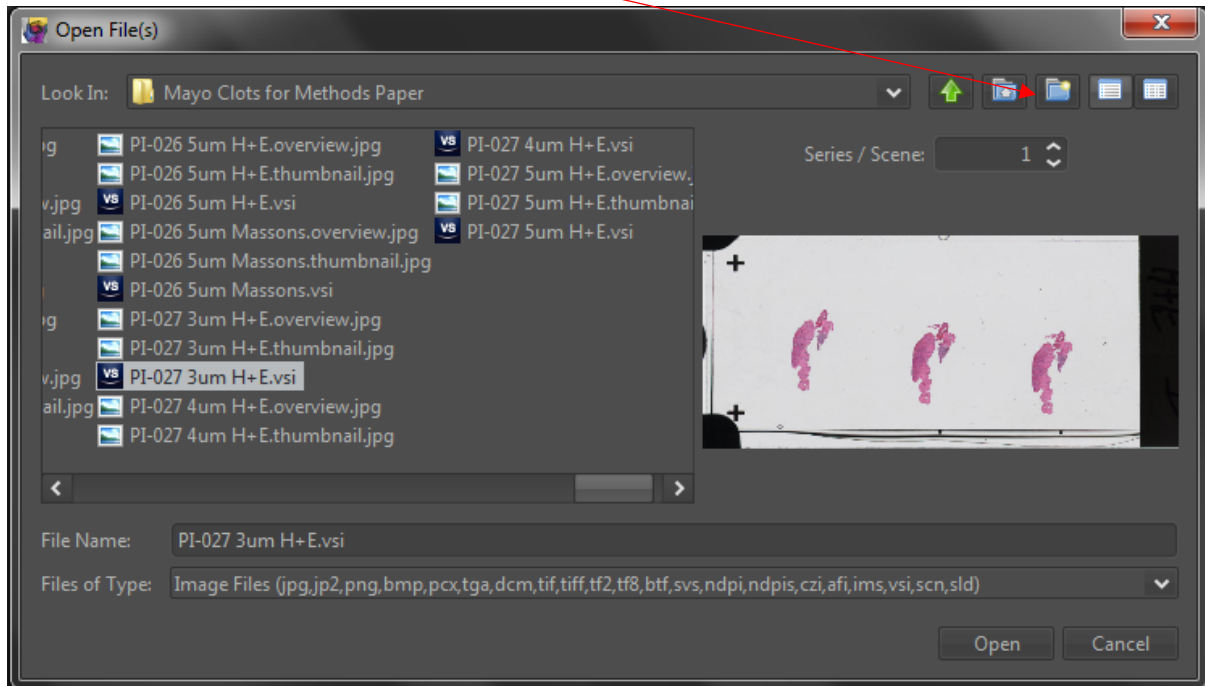


- 3). Click on the Image Tab and then click on Open Image from File System.



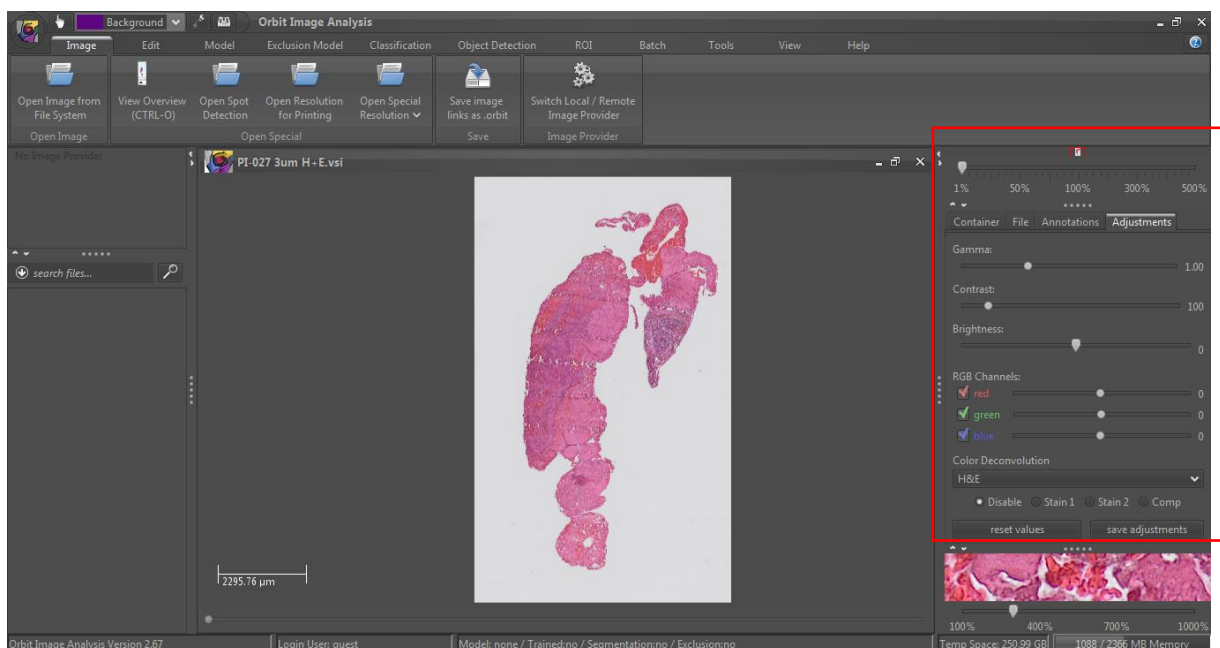
4). Browse to the folder that the file/image to be analysed is in. If you are working off a static Image (.jpeg, .tiff, .png, etc.) you can just select it and it will open.

*However if you are working off a whole slide scan file (.svi, .vsi, etc.) you need to use the Series tab to find the correct series for analysis. E.g. Series 1 is usually just the Label.




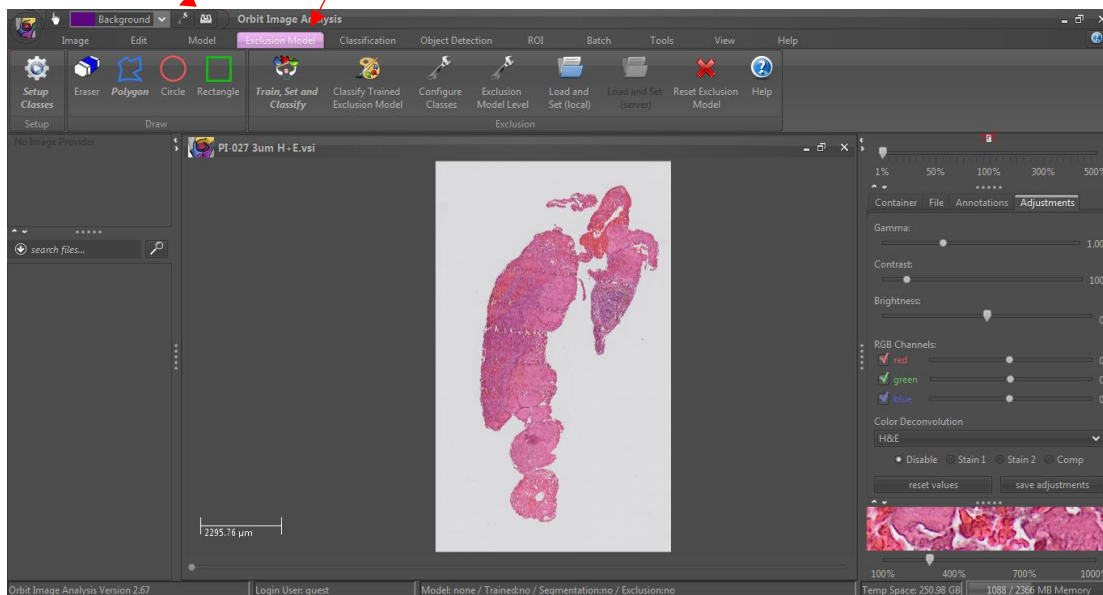
Click Open to open the image. Zoom in to make sure the definition is good.

5). The brightness and contrast of the image can be adjusted on the right if necessary.



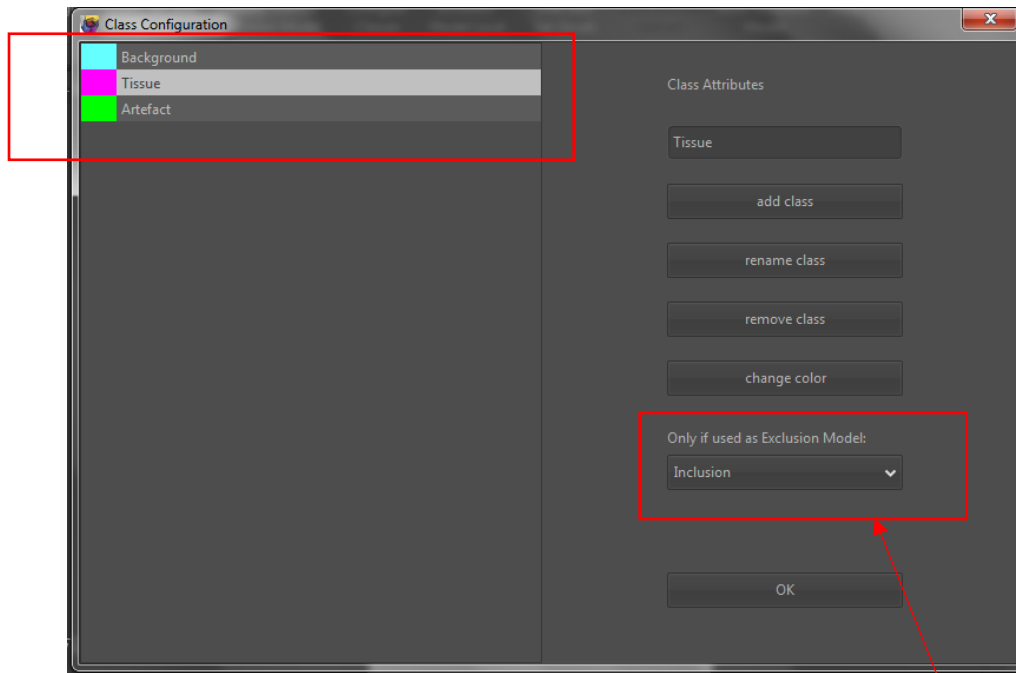
*6). **Exclusion Model:** This step allows you to define the tissue from background and also to mark areas of artefact which will be excluded from the quantification, increasing the accuracy of the model and quantification results.

Click on the Exclusion Model Tab, and then click on the spanner icon to setup classes 




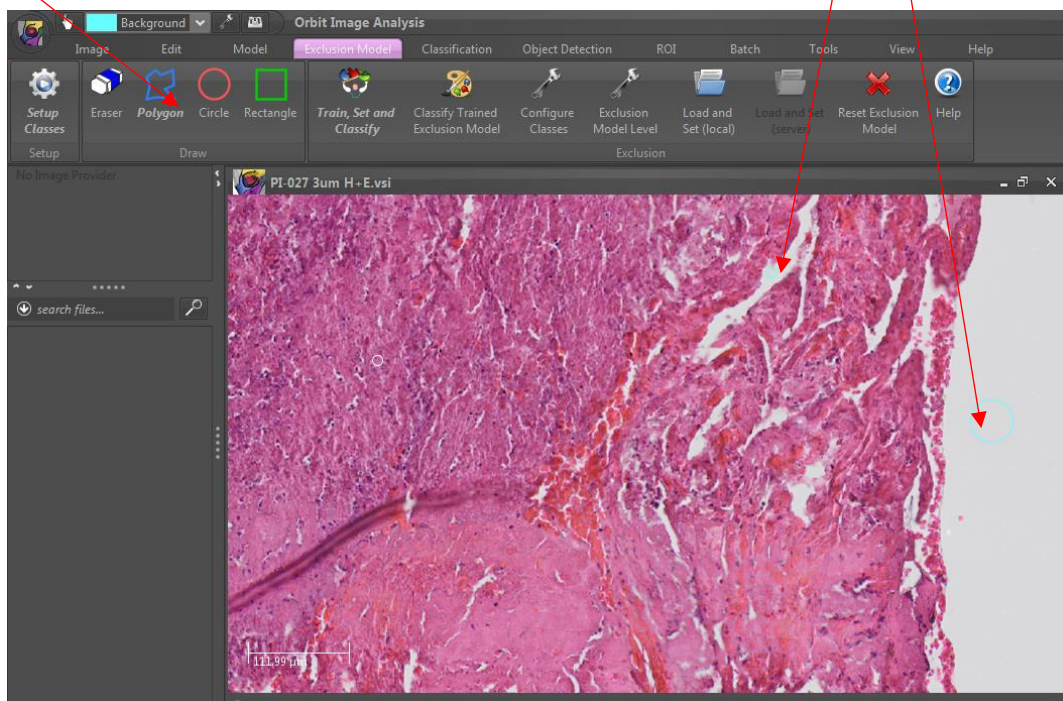
7). Rename the Classes as you wish. (E.g. Background, Tissue and Artefact). You can also adjust the colours that you use to represent each class.



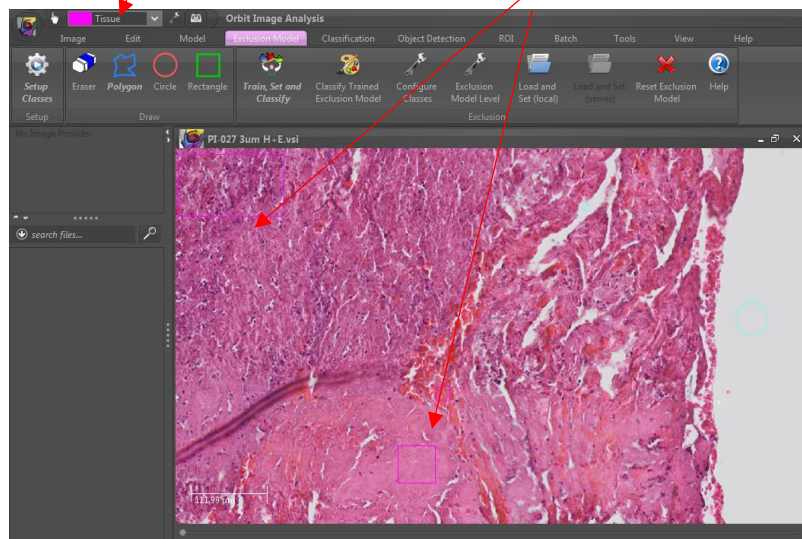


The important thing is to define each class as Inclusion or Exclusion. E.g. Background and Artefact are Exclusion because you don't want to quantify them. Tissue is Inclusion and you want to quantify the tissue. Click ok.

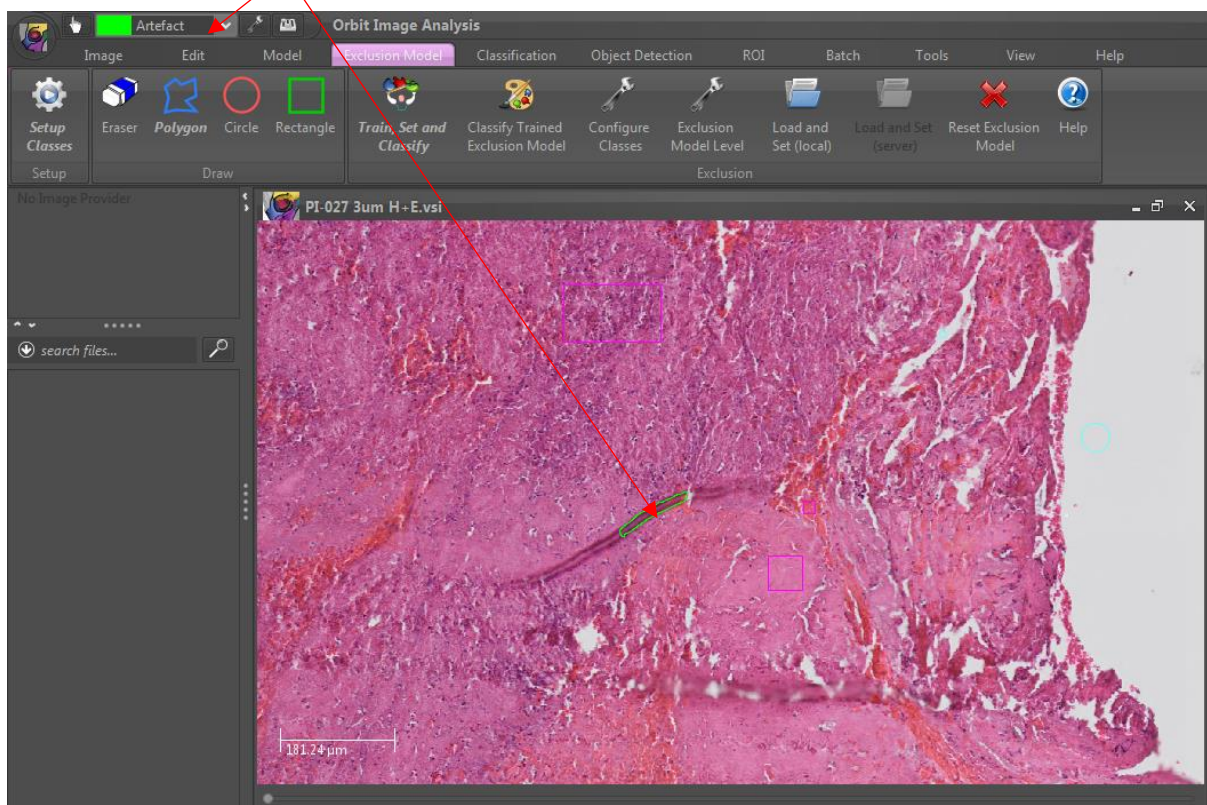
8). Whist on the Exclusion Tab, Select Background. Use the different tools (Circle, rectangle, Polygon) to mark areas, of background on your image. (You can zoom in and navigate around your image by clicking on the hand tool ). For example click on the Circle, move to an area of background and mark an area of background by left-clicking on the mouse.



9). Now Change to the Tissue tab and repeat the process to mark areas of tissues for inclusion. It is a good idea to mark different areas within the tissue.

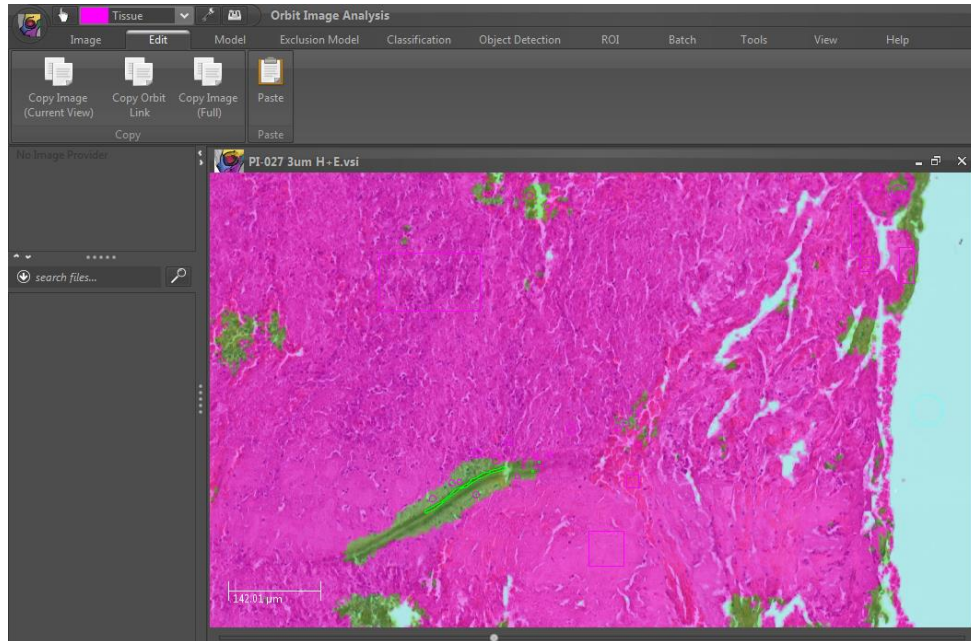


10). Now Change to the Artefact and repeat the process to mark areas of artefact for Exclusion. *Make sure not to touch areas of tissue or background as this confuses the software.



11). On the Exclusion Model tab, Click Train, Set and Classify.

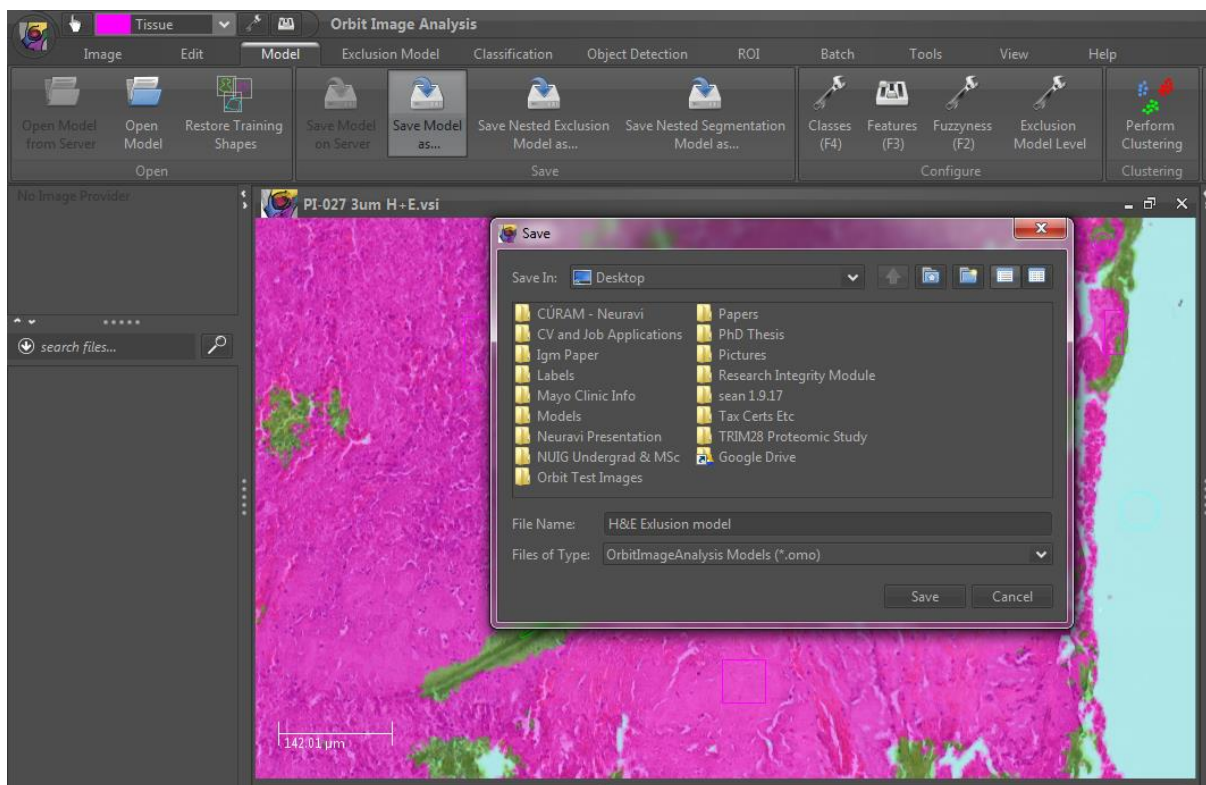
Once it has finished, use the toggle bar at the bottom of the image to see the Exclusion Map.



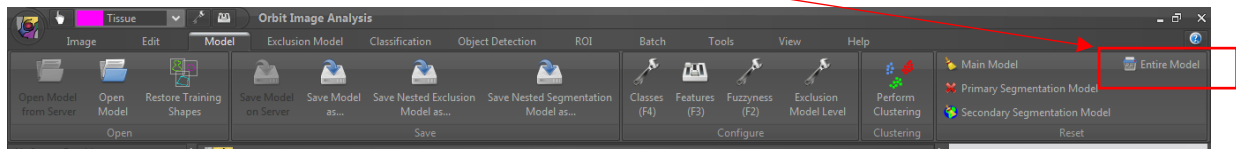
In this case Purple = Tissue (Inclusion), Blue = Background (Exclusion) and Green = Artefact (Exclusion). If you are not happy with your exclusion model, you can repeat steps 8-11 above marking more areas of tissue.

12). If you are happy, click on the Model Tab, then Save model As, select the folder that you wish to save your model in.

***Label this Model as Exclusion Model.**



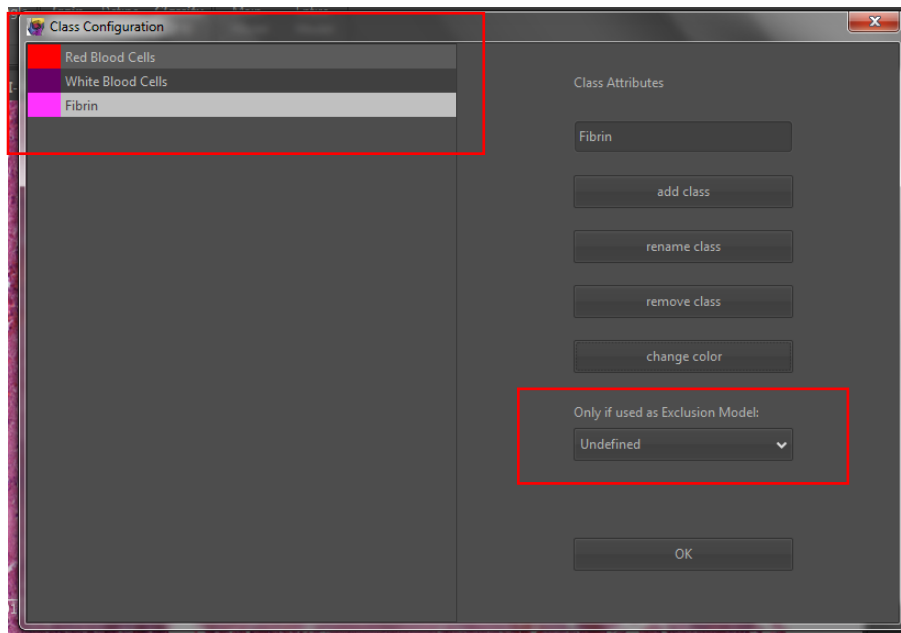
13). **Very Important:** Once the model is saved successfully, click on the Model Tab and then Click Delete Entire Model.



This will reset your classes in the setup classes (Spanner Icon) tab.

14). **Inclusion Model:** Click on the Spanner Icon again and rename the Classes as you wish. You can also change colours and can add or remove classes if necessary.

*All of these classes should be left as undefined. You only define the classes as Inclusion or Exclusion for the Exclusion Model. Click ok.

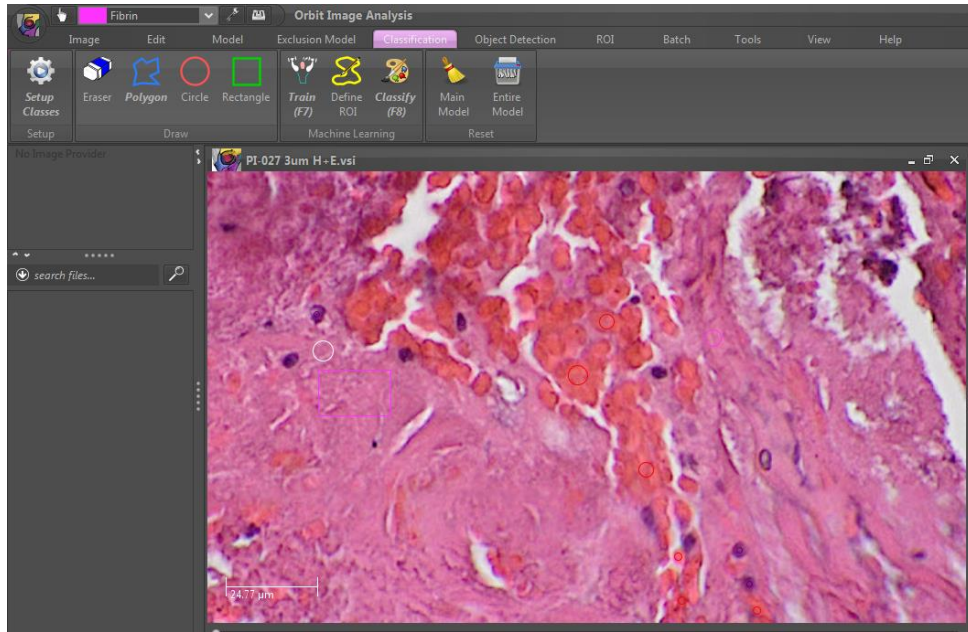


15). Click on the Classification Tab, Select Red Blood Cells and mark areas of Red Blood Cells using the circle, polygon or rectangle tools.

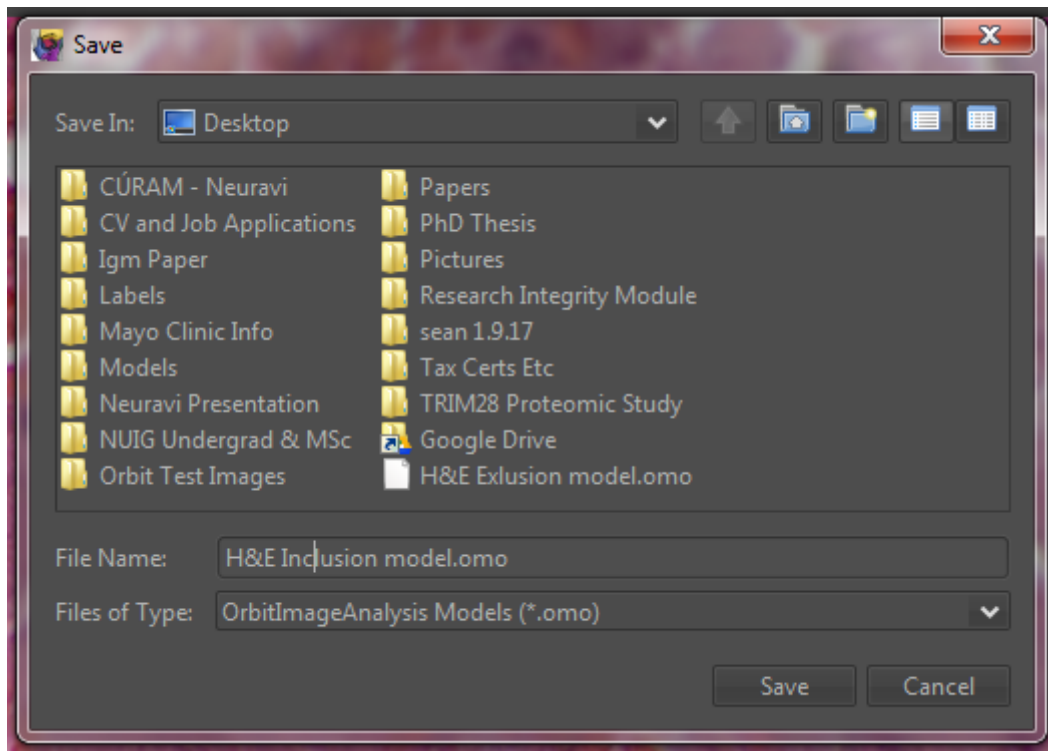
Repeat this for White Blood Cells and Fibrin. (Or whatever your cell types are).

*Make sure that you mark the areas carefully and don't overlap as this will confuse the system. It is important to navigate around the tissue and select cells in different areas as the colours can change slightly throughout the slide.

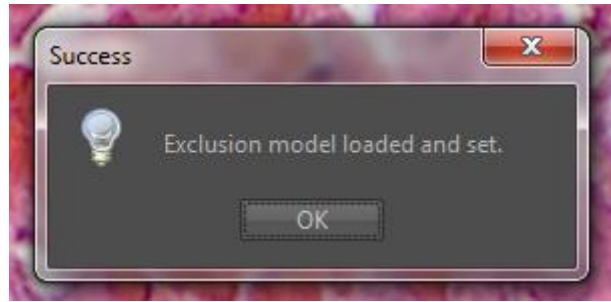




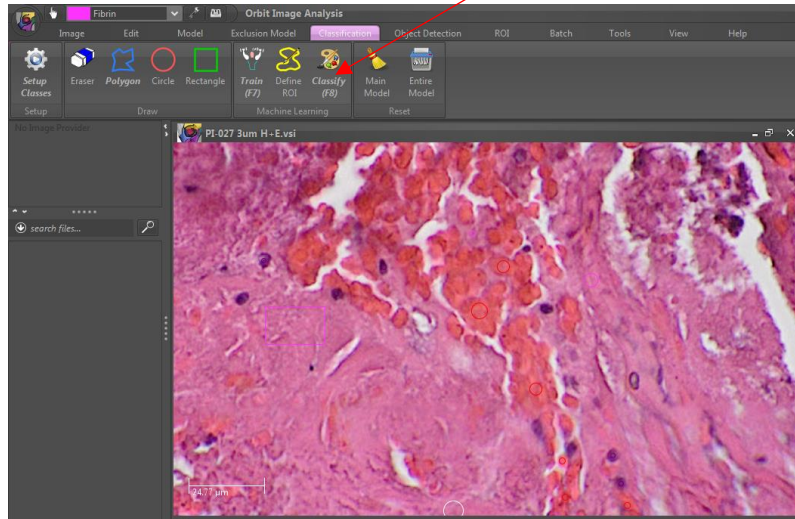
16). On the Classification tab, Click train. Then Click Save Model As, and Save it as Inclusion model.



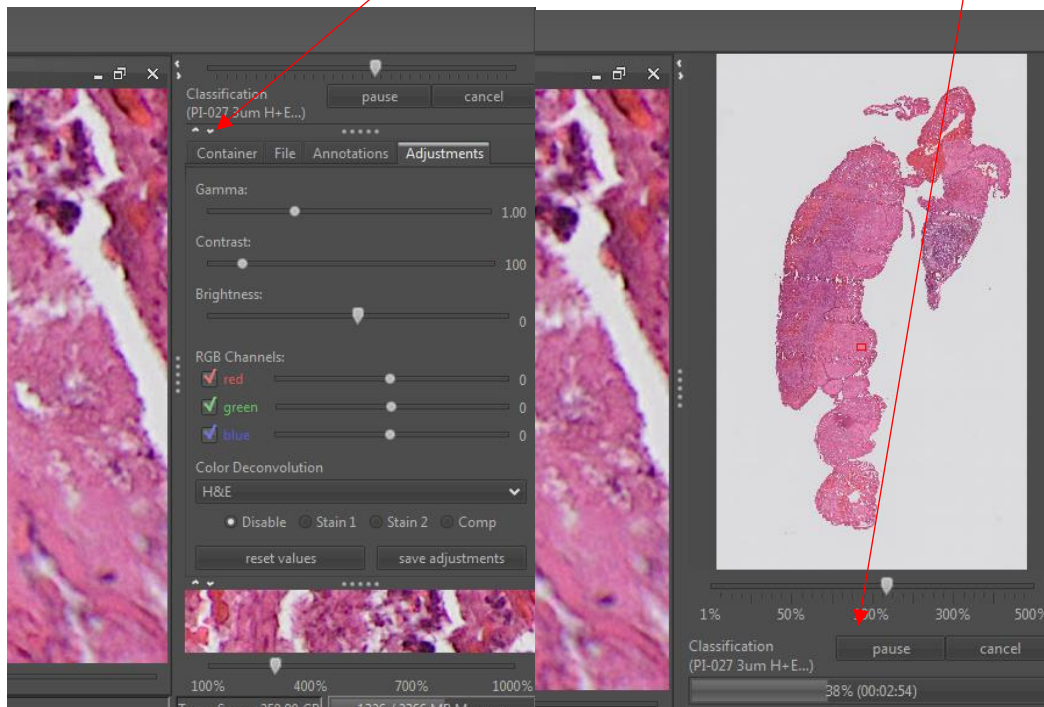
17). Next you need to Apply the Exclusion model that you trained earlier. Under the Exclusion Model Tab, Click on Load and Set (local). Find the exclusion model that you trained earlier and apply it.



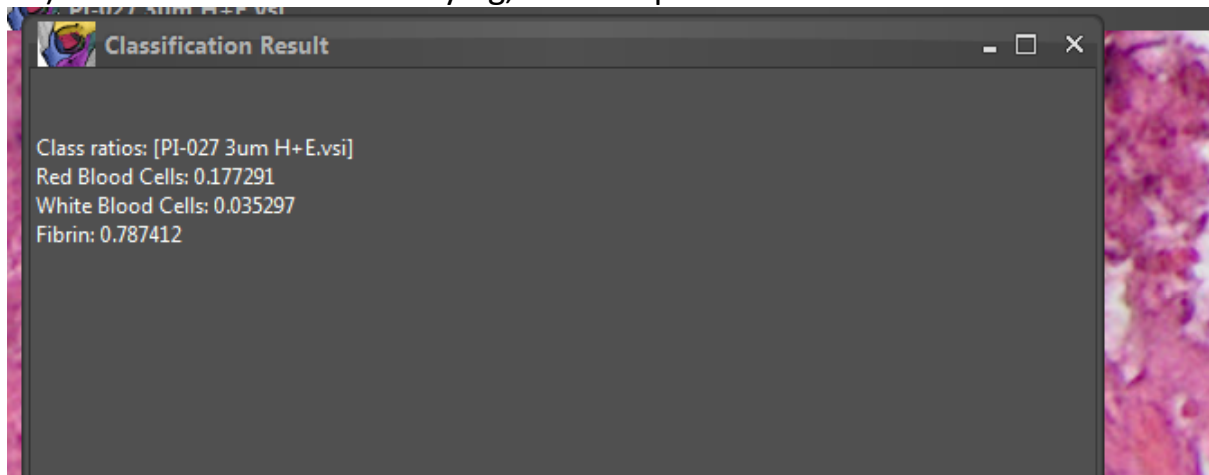
18). Navigate to the Classification Tab and Click on Classify.



By minimizing the colour adjustment options, you can see the progress of the classification.

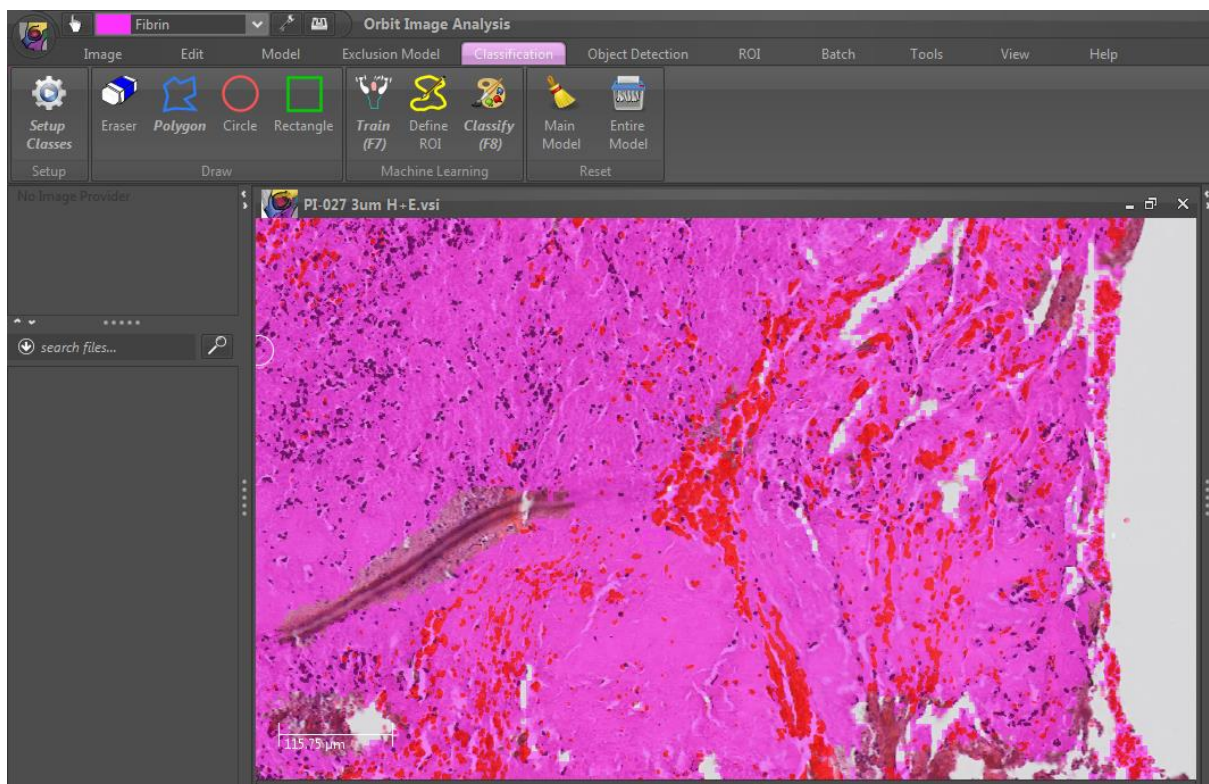


19). Once it is finished classifying, then output will look like this.

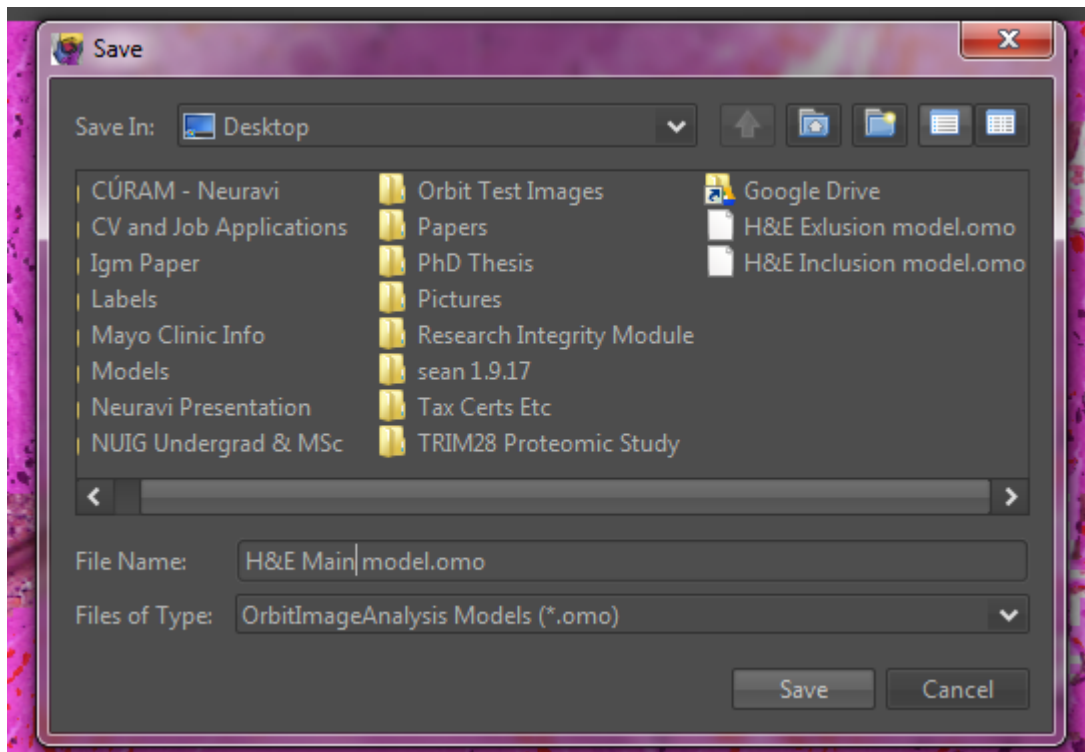


By multiplying each number by 100 you can convert it to a percentage.

You can use the togglebar at the bottom of the screen to assess how accurate your model was. Red = Red Blood Cells, Purple = White Blood Cells, Pink = Fibrin, White = Background and Grey = Artefact (both Background and Artefact are excluded from the quantification.)



20). If you are happy with your Model, Click Model, Save Model As, and Save it as Main Model. If you are not happy with the Inclusion model, you can repeat steps 15-19, marking more areas of tissue.



21). Now that you have this model saved and can apply this Main Model to other Slides. When you start the Orbit programme, Click on the model Tab, then open model and open your main model. This Main model includes both your exclusion and inclusion models. Under the classification tab, mark areas of each cell type on the new slide/image and click classify.