<u>Title:</u> Machine learning techniques accurately quantify the histological composition of Acute Ischemic Stroke blood clots

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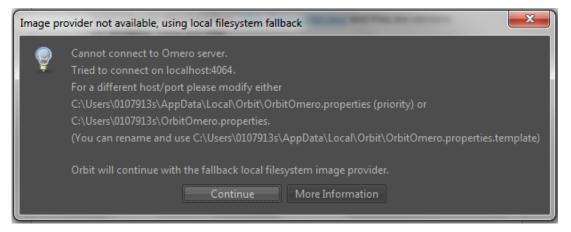
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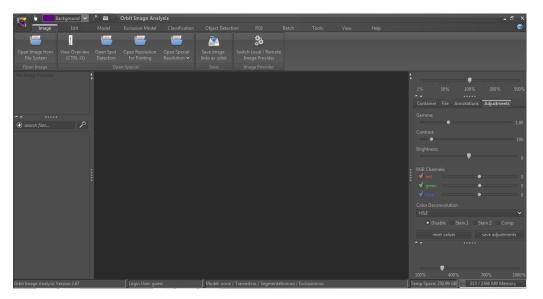
16 Supplementary File 1:

- 17 Orbit Image Analysis Standard Operating Procedure for Histological
- 18 Quantification.

- **1** Orbit Image Analysis Standard Operating Procedure for Histology
- 2 **Quantification:**
- Orbit is free to download from <u>https://www.orbit.bio/</u> and there are versions
 available for Windows, Linux and Mac.
- 5 For the purposes of this SOP I am going to use H&E as the stain and Red Blood
- 6 Cells, White Blood Cells and Fibrin as the tissue types that I wish to quantify.
- 7 However this can be adapted to different stains and different cell/tissue types
- 8 as necessary.
- 9 1). Click on the desktop icon to Launch Orbit.
- 10 2). Sometimes a popup comes up and just click continue.



- 11
- 12 3). Click on the Image Tab and then click on Open Image from File System.

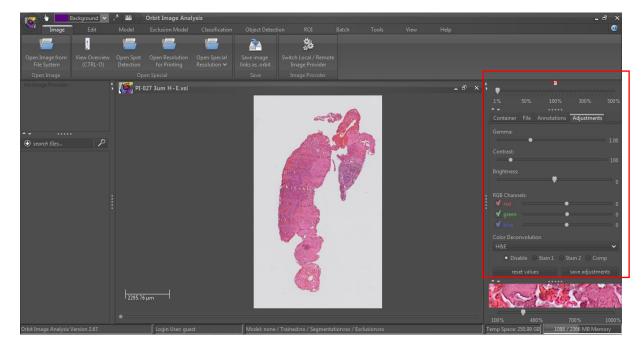


- 13
- 14 4). Browse to the folder that the file/image to be analysed is in. If you are
- working off a static Image (.jpeg, .tiff, .png, etc.) you can just select it and it willopen.

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

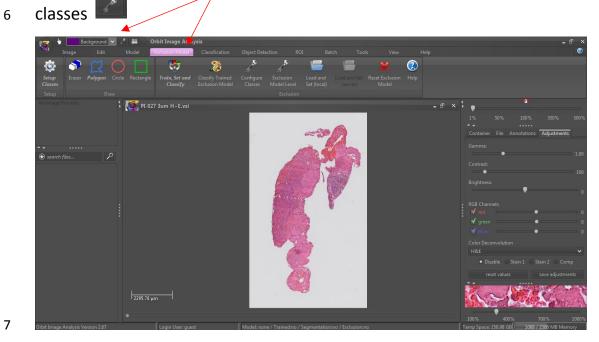
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- 5 Click Open to open the image. Zoom in to make sure the definition is good.
- 6 5). The brightness and contrast of the image can be adjusted on the right if
- 7 necessary.



9

- 1 *6). Exclusion Model: This step allows you to define the tissue from
- 2 background and also to mark areas of artefact which will be excluded from the
- 3 quantification, increasing the accuracy of the model and quantification results.
- 4
- 5 Click on the Exclusion Model Tab, and then click on the spanner icon to setup



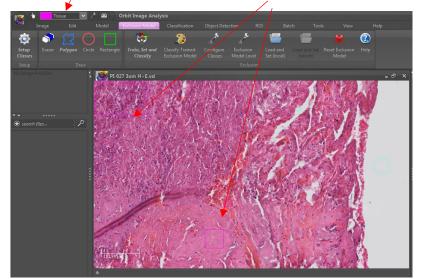
- 8
- 9 7). Rename the Classes as you wish. (E.g. Background, Tissue and Artefact). You
- 10 can also adjust the colours that you use to represent each class.

🥵 Class Configuration	
Background Tissue Bat Artefact	Class Attributes Tissue
	add class rename class
	remove class change color
	Only if used as Exclusion Model:
	ок

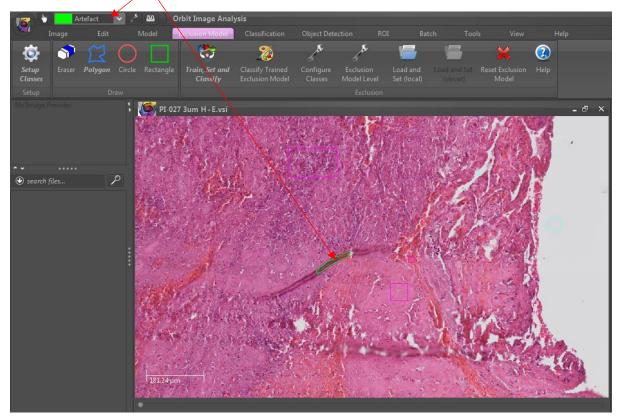
- 1 The important thing is to define each class as Inclusion or Exclusion. E.g.
- 2 Background and Artefact are Exclusion because you don't want to quantify
- them. Tissue is Inclusion and you want to quantify the tissue. Click ok.
- 4 8). Whist on the Exclusion Tab, Select Background. Use the different tools
- 5 (Circle, rectangle, Polygon) to mark areas, of background on your image. (You
- 6 can zoom in and navigate around your image by clicking on the hand tool
- 7 For example click on the Circle, move to an area of background and mark an
- 8 area of background by left-clicking on the mouse.



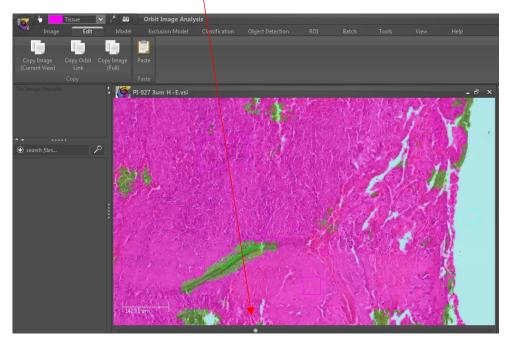
- 10 9). Now Change to the Tissue tab and repeat the process to mark areas of
- 11 tissues for inclusion. It is a good idea to mark different areas within the tissue.



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



- 5 11). On the Exclusion Model tab, Click Train, Set and Classify.
- 6 Once it has finished, use the toggle bar at the bottom of the image to see the
- 7 Exclusion Map.



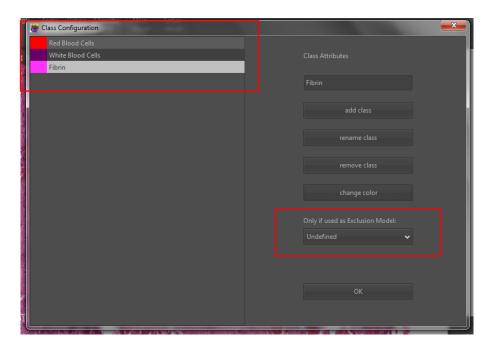
- 1 In this case Purple = Tissue (Inclusion), Blue = Background (Exclusion) and
- 2 Green = Artefact (Exclusion). If you are not happy with your exclusion model,
- 3 you can repeat steps 8-11 above marking more areas of tissue.
- 4 12). If you are happy, click on the Model Tab, then Save model As, select the
- 5 folder that you wish to save your model in.
- 6 *Label this Model as Exclusion Model.

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- 7
- 13). Very Important: Once the model is saved successfully, click on the Model
- 9 Tab and then Click Delete Entire Model. -



- 10
- 11 This will reset your classes in the setup classes (Spanner Icon) tab.
- 12 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
- 14 necessary.
- ¹⁵ *All of these classes should be left as undefined. You only define the classes as
- 16 Inclusion or Exclusion for the Exclusion Model. Click ok.

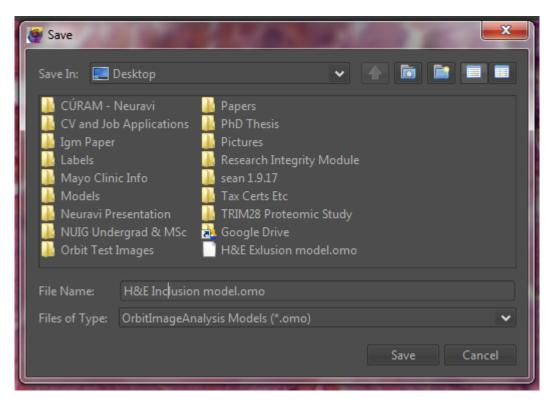


- 2 15). Click on the Classification Tab, Select Red Blood Cells and mark areas of
- 3 Red Blood Cells using the circle, polygon or rectangle tools.
- 4 Repeat this for White Blood Cells and Fibrin/(Or whatever your cell types are).
- ⁵ *Make sure that you mark the areas carefully and don't overlap as this will
- 6 confuse the system. It is important to navigate around the tissue and select
- 7 cells in different areas as the colours can change slightly throughout the slide.

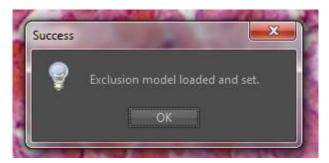


8

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



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



- 5
- 6 18). Navigate to the Classification Tab and Click on Classify.

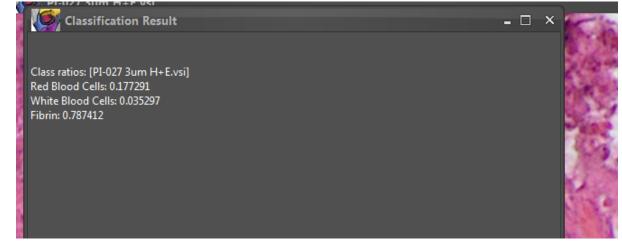


- 1 By minimizing the colour adjustment options, you can see the progress of the
- 2 classification.

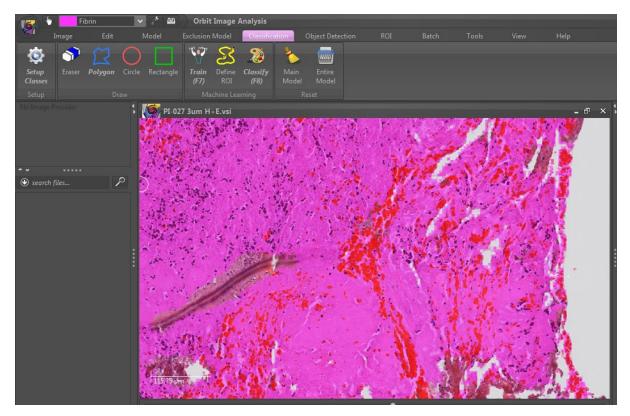
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4 19). Once it is finished classifying, then output will look like this.



- 6 By multiplying each number by 100 you can convert it to a percentage.
- 7 You can use the tooglebar at the bottom of the screen to assess how accurate
- 8 your model was. Red = Red Blood Cells, Purple = White Blood Cells, Pink =
- 9 Fibrin, White = Background and Grey = Artefact (both Background and Artefact
- 10 are excluded from the quantification.)



2 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

4 steps 15-19, marking more areas of tissue.

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21). Now that you have this model saved and can apply this Main Model to 1 other Slides. When you start the Orbit programme, Click on the model Tab, 2 then open model and open your main model. This Main model includes both 3 your exclusion and inclusion models. Under the classification tab, mark areas 4 of each cell type on the new slide/image and click classify. 5 6 7 Other Important things to note: Under the Classification tab you can use the ROI tool to mark regions of 8 interest that you want to analyse instead of analysing the whole 9 slide/image. 10 11 If your Main Model is working well, you can analysing multiple images 12 using Batch mode. First open your main model, then Click on the Batch 13

- using Batch mode. First open your main model, then Click on the Batch
 tab, then Local Area Execution and then select the files that you wish to
 process.
- 16