Standard Operating Procedure:
Analyzing Imagery Using RosetteDetect™

Overview: This document summarizes how to use the RosetteDetect™ web platform using images gathered from RosetteArray® plates/kits.

Equipment Required:

- Computer(s) with Image Capture Software, internet access, and spreadsheet processing software

Materials Required:

- Immunostained 96-well RosetteArray plate (DAPI (405 nm), N-Cadherin (488 nm), Pax6 (561 nm) mounted with Glass Antifade Mountant Solution (Catalog #: P36984, Thermofisher) and cultured using Neurosetta's Standard Operating Procedure
- Image sets gathered using Neurosetta's Standard Operating Procedure
- Image Processing Credits purchased at: https://neurosetta.com/product/image-processing-credits/

Protocol Overview:

1. Buying image processing credits
2. Logging into the RosetteDetect web portal
3. Uploading and processing image sets with RosetteDetect
4. Downloading and interpreting results

Protocol:

1. Buying Image Processing Credits

Image processing credits are available for purchase at: https://neurosetta.com/product/image-processing-credits. One credit can be redeemed for the processing of one image set. Once purchased, an account for RosetteDetect will be automatically created using the email address input for the purchase.

NOTE: If you have gathered image sets according to Neurosetta's Standard Operating Procedure (Hyperlink) then there will be 2400 image sets for a single RosetteArray

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2. Logging into the RosetteDetect web portal

You will log in by accessing a magic link sent to your email account. Follow the steps below to get the link:

1. Navigate to https://rosettedetect.neurosetta.com/
2. Enter the email address used to purchase the image processing credits
3. A magic link will be sent to the email address you used above. Click the link and you will be automatically logged into the RosetteDetect portal. Once logged in, you can see purchased and available credits at the top of the screen. To buy more credits click the "Buy Credits" button in the top right corner.

3. Uploading and Processing Image Sets with RosetteDetect

Uploading and processing image sets using RosetteDetect is a user-friendly, intuitive process. Image sets must be captured using Neurosetta's Standard Operating Procedure. After upload, image sets will undergo a quality check to ensure reliable data. You will only be charged for image sets that pass the quality check. After the quality check is completed, the image sets will be analyzed using RosetteDetect AI-powered proprietary image analysis software.

1. To begin uploading and processing image sets, enter an image set name in the "Set Name" text box.
2. Next, adjust the color channel settings to whatever method was used for staining. To automatically quality-check and process image sets on upload, check the "Automatically process image sets after upload" box (this box will be referred to as the **"AutoBox"**). Alternatively, leave it unchecked and get quality check results before processing. To begin uploading, click the "Upload Images" button.
3. Click to upload or drag and drop image sets into the portal. Currently supported image set types are .nd2, .oib, .lif, .czi, and .tiff. All image files must have five slices and contain a single tissue.
4. **If the "AutoBox" is checked**, image sets will be automatically quality checked and processed.
5. **If the "AutoBox" is not checked**, then wait for all images to upload and click the "Finalize set" button. Once that is clicked, the set will be saved and ready for processing. Check the quality check status of image sets in the "IMAGES" column. If only a green checkmark appears, then all image sets have passed. If an image does not pass the quality check, then a red "X" mark will appear, showing the number of image sets that did not pass. Hover over this check mark to see which image sets have not passed the quality check and the reason for failure.
6. Click the "Begin Processing" button to process a dataset.

4. Downloading and interpreting results

Once processing is completed, a dataset will be saved and results can be downloaded by clicking the buttons under the "Actions" column. Clicking the first button ( ) will download tabular results showing rosette emergence and morphological data. Clicking the second button ( ) will download example result images that show the outlined rosette.

NOTE: You are only able to download 5 result images per 1000 images processed.

1. Tabular data (see Figure 1) includes:
    a. Name: Name of file uploaded
    b. Successfully Processed (True/False): Boolean value indicating is file was successfully processed. **If there is a "false" value please contact** customerservice_RD@neurosetta.com. **Include the corresponding "Message" text in the email.**
    c. Message: Error message if there is an unsuccessful image processed.
    d. Rosette Detected? (True/False): Boolean value indicating if there is a rosette detected in the image set
    e. Area (μm^2): 2D rosette area. This calculation uses the assumption that the image resolution is 0.62 microns/pixel and image sets are 512 pixels by 512 pixels
    f. X & Y Coordinates (pixels): Coordinates in pixels of rosette centroid (pixels)
    g. Eccentricity (0-1 double): Eccentricity of rosette
    h. Cells (integer): Count of thresholded DAPI+ segments
    i. NEC (integer): Count of neuroepithelial/radial glial cells from thresholded Pax6+ segments
    j. Sample Image Generated? (True/False): Boolean value indicating if a sample image was generated for this image set

Name	Successfully Processed?	Message	Rosette Detected?	Area	X Coordinate	Y Coordinate	Eccentricity	Cells	NEC	Sample Image Generated?
Tissue000.nd2	true		true	3927.4148	259.981079101563	245.690719604492	0.677985078441986	168	176	true
Tissue001.nd2	true		true	4848.8216	242.987930297852	247.679397583008	0.378221713862376	156	182	true
Tissue002.nd2	true		true	5342.0068	228.486831665039	308.845916748047	0.560860697153328	190	184	true
Tissue003.nd2	true		true	4966.448	285.927886962891	260.802734375	0.406463286127674	162	180	true
Tissue004.nd2	true		true	9373.9784	251.935882568359	271.897705078125	0.671957953223753	200	222	true
Tissue005.nd2	true		false					193	205	false
Tissue006.nd2	true		true	5880.5512	237.254867553711	264.045776367188	0.557649433380241	184	191	false
Tissue007.nd2	true		true	3489.5832	249.57502746582	247.908905029297	0.648022402044868	174	186	false
Tissue008.nd2	true		true	5378.5248	270.63427734375	264.305328369141	0.705611505772024	157	175	false
Tissue009.nd2	true		true	4349.8704	233.736785888672	275.19189453125	0.313819286225828	175	178	false
Tissue010.nd2	true		true	5897.4648	249.570251464844	244.081726074219	0.428003887850727	196	187	false

Figure 1. An example results table generated by the RosetteDetect web portal

2. Example result images showing an outlined rosette (Figure 2) are available at a rate of 5 examples per 1000 images processed. This is meant to be a check for your team to determine if data is being processed correctly. If there are any problems with your results please contact customerservice_RD@neurosetta.com.

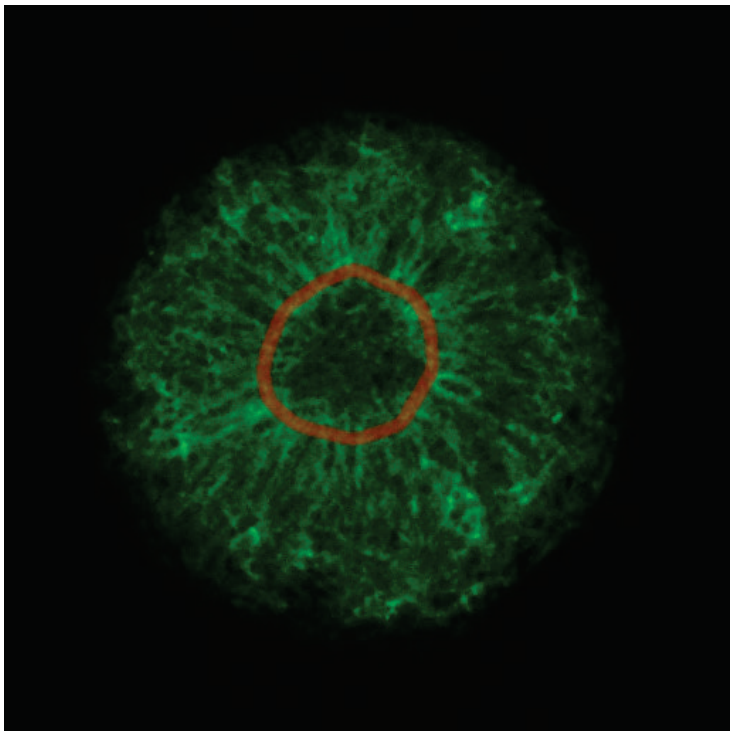

Figure 2. An example result image generated by the RosetteDetect web portal. The rosette is traced in red.