

How To Analyze Nodularity Using PAX-it Version 5.1 or Higher

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Goal of This Document

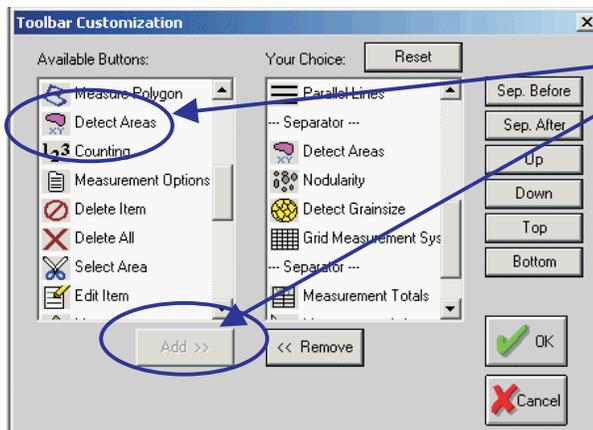
The goal of this document is to demonstrate how to configure the Nodularity feature in PAX-it and how to easily run it once it has been configured. One key advantage when using the PAX-it software is the ease at which repeatability and consistency can be maintained. The key pieces of data that will be returned are Nodularity value and number of objects per unit squared.

Getting Started—Assumptions

This document assumes that you have already configured your PAX-it measurement system and calibrated each objective. If you have not already done so, please consult your PAX-it manual for more information.

Configuring Your Toolbar

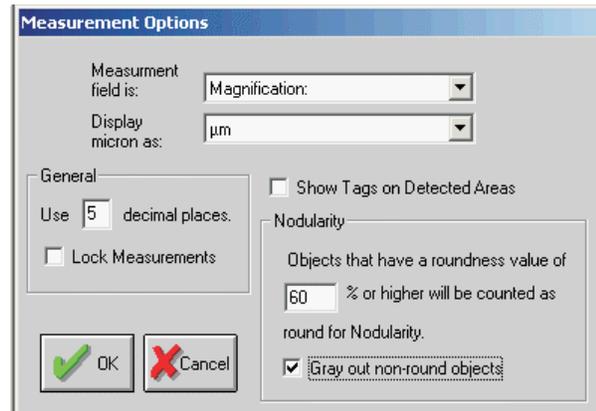
When upgrading from version 5.0 to 5.1, it may be possible that your DETECT AREAS and NODULARITY buttons are not available on your Analysis Toolbar. If this is the case, click your right mouse button over your Analysis Toolbar and then click on CUSTOMIZE ANALYZE BAR. You will then see the following screen. Add the buttons that are needed and click on OK.



- A. Select the DETECT AREAS and NODULARITY buttons.
B. Then click on ADD >> to add the buttons to your toolbar.

Setting Your Options

Go to OPTIONS then MEASUREMENTS and you will see the following screen.



It is suggested that you turn off the SHOW TAGS ON DETECTED AREAS feature as the large amount of tags may get in the way. By default, we recommend starting with a 60% roundness value. Any detected areas that have this percent roundness or higher will be counted as a "round" object when calculating roundness. This number may be adjusted to configure your system to get the appropriate value. If your resulting Nodularity value is too high, try increasing this number and if your value is too low, try lowering this number.

It is also suggested that you check GRAY OUT NON-ROUND OBJECTS. This will outline the detected areas that will not be counted as a "round object" in gray so you will visibly be able to see which objects will be counted as round and which ones are not.

When you have set all of these values, please click on OK.

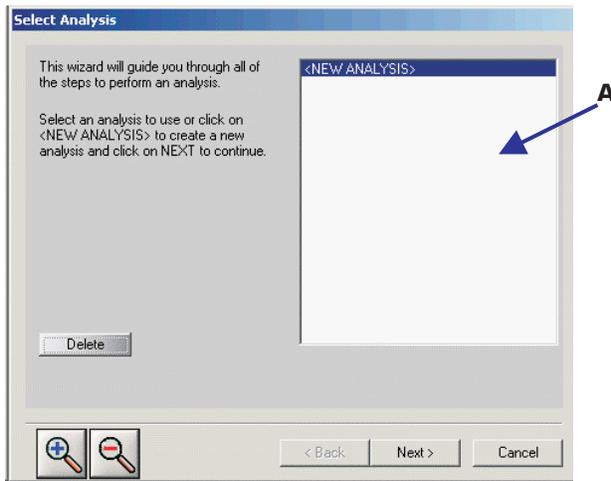
Doing the First Detection

The first time through doing Nodularity, you will have to manually configure the settings. After we save the settings, it can be performed automatically with a single click.

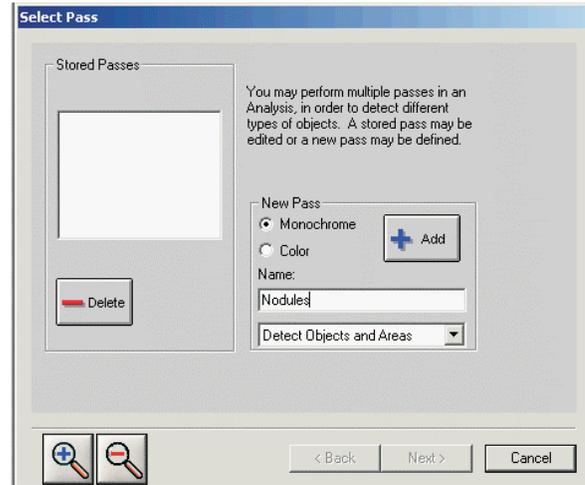
First, either capture and save an image, or open an image from your cabinets and folders to perform a detection routine.



Then, click on the DETECT AREAS button. You will see the following screen. Make sure that <NEW ANALYSIS> is selected and click on NEXT.



A. You can manage and delete different analysis settings here. For now, we will not be using it.



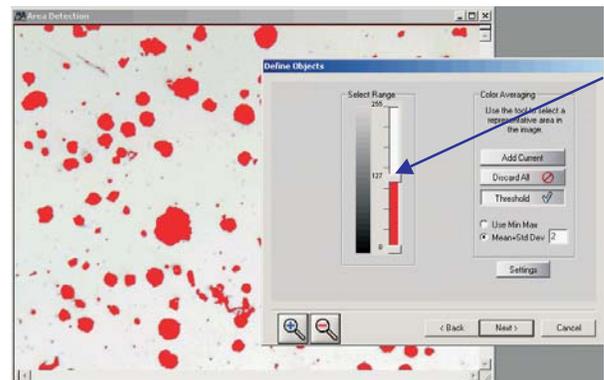
Click on SELECT FILE to pick a background corrector image (if you want to use one) and then click on NEXT.

PAX-it allows you to perform multiple analysis passes on an image (or, in other words, the PAX-it's Wizard allows you to use multiple bitplane operations, or multiple image measurement results for any single image). In the case of most Nodularity measurements, Nodularity requires just one pass per image since you are generally looking for a single result, i.e., a 95% Nodularity reading. Thus, for Nodularity, only one pass is needed. Click on MONOCHROME since Nodularity does not require a color analysis and enter in a title for the pass. The title is not important for this case, so enter something like "Nodules". Make sure that DETECT OBJECTS AND AREAS is selected and then click on ADD to create the pass. Click on NEXT.

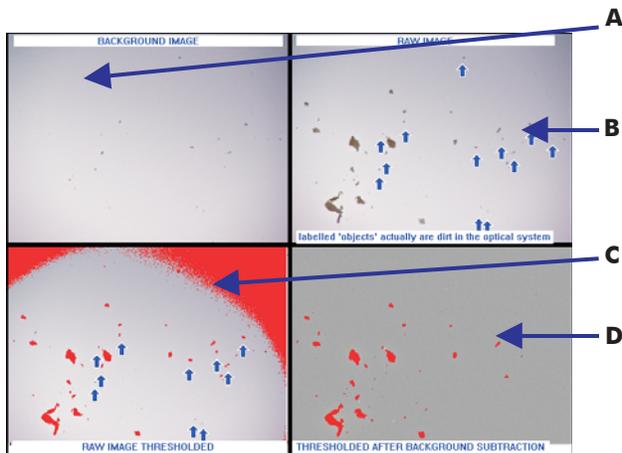
The next step will ask you to select a region of interest (ROI). In most cases, you will not need to do so. Click on NEXT.



You may select a background corrector. This background corrector is a previously stored image that will adjust your image for even illumination. Please refer to your PAX-it manual for more information.

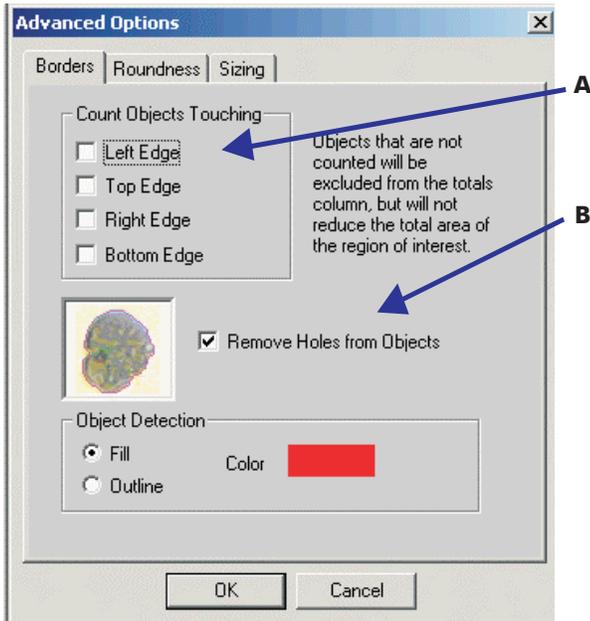


You can adjust your threshold level here. If you slide the range up, lighter objects will be detected.

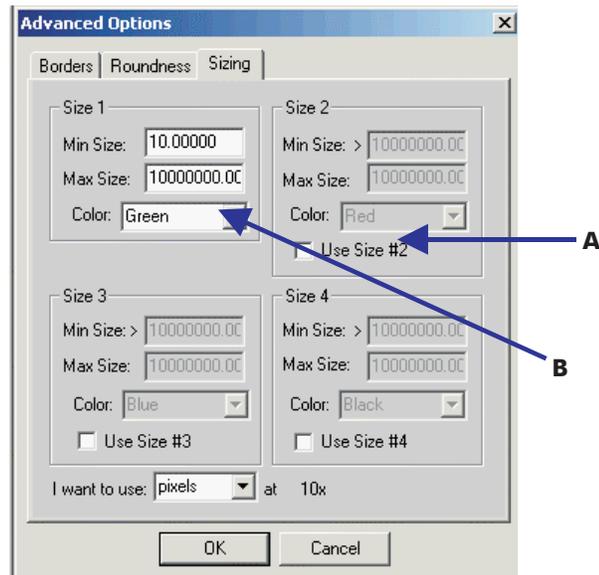


A. The blank image has uneven illumination and some noise in the optic path.
B. When you capture the image you have noise in your image.
C. When you try to threshold the image, the uneven illumination causes problems.
D. If you apply a background corrector, the thresholding works better.

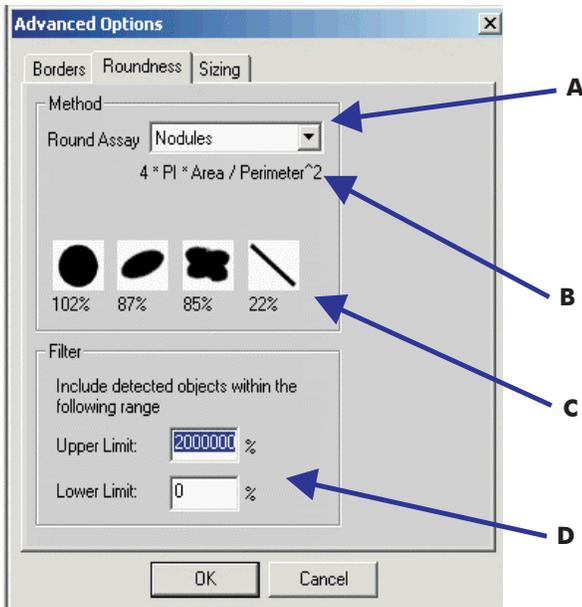
At this step in the wizard, you should adjust your threshold levels. You can see the results on your image. As you adjust the select range, you will see the results change on your image. Click on SETTINGS.



A. Uncheck the option to count objects touching different edges. If an object is half off of the screen, it will affect the roundness value.
B. Check REMOVE HOLES from Objects



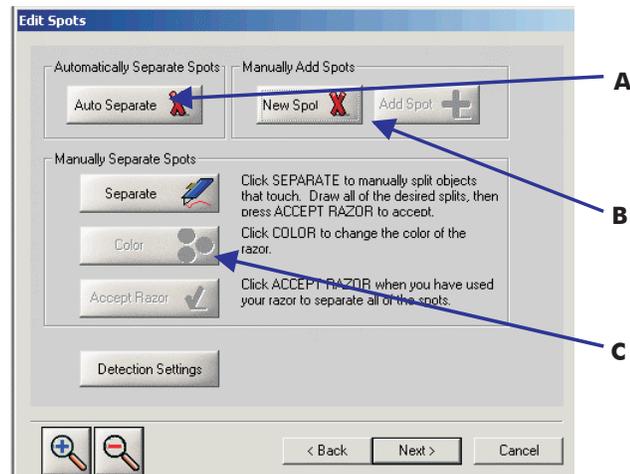
A. For detecting Nodules, turn off all of the size categories except for size category number one. You can filter out smaller objects (noise) by setting a minimum size of a value greater than zero. You want to see a high maximum size so that all of the valid Nodules are detected.
B. When you have configured all of your settings, click on OK.



A. PAX-it allows for different formulas to calculate Roundness. Select the type formula you would like to use here. For Nodularity, it is suggested that you use the "Nodules" roundness formula.
B. You can see the formula that is being used to calculate Roundness here.
C. You can see the expected values for the different shapes for the current Roundness formula here.
D. You can filter out objects (not detect them) if they have a Roundness value that is too high or too low here. This is a different Roundness operation than what is used in the Measurement Options screen. These settings are used to filter out objects that are not used to determine if an object should be counted as a "round" object. There are several methods for calculating Roundness; for more information, please see the calculating Roundness section at the end of this document.

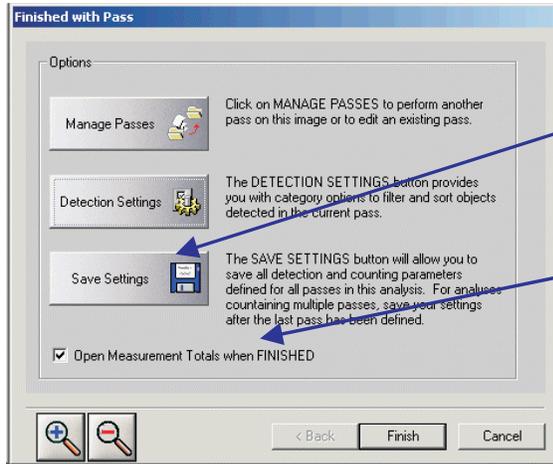
Click on NEXT in the wizard to advance to the next step.

On the Edit Spots page in the wizard, you may separate spots either manually or automatically. You can also manually add a spot that was not detected.



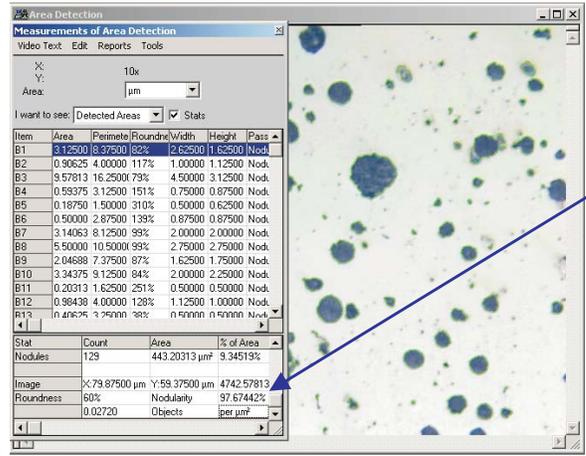
A. Click AUTO SEPARATE to have the system attempt to automatically separate spots.
B. You can outline a new object that the software did not detect and add it here.
C. This section will allow you to use a razorblade to manually cut separate objects.

Typically for Nodularity, you will not need to do anything on this step and can just click on NEXT to continue.



- A. Click on **SAVE SETTINGS** to save all of the settings that you have just configured.
- B. Make sure that **OPEN MEASUREMENT TOTALS WHEN FINISHED** is checked. This will display your measurement results each time you perform a measurement routine.

Once you have configured everything, click on SAVE SETTINGS to save all of the settings. When asked for a title, give it "Nodularity". It must be titled exactly "Nodularity" because later when you click on the NODULARITY button to run your saved settings, it searches for the "Nodularity" settings. Click on FINISH.



The Nodularity information will be display in the Stats area of the Measurement Totals window

When you have clicked on FINISH, you will see your objects outlined on your image and your data results in the Measurement Totals window. The Nodularity information is displayed in the Stats area.

The Measurement Totals window will have the following information. You may need to run through this process several times to configure your system for many different variables.

This section contains your individual object information Typically, this can be ignored.

This is the number of objects per micron squared information.

Measurements of Area Detection						
Video Text Edit Reports Tools						
X:	10x					
Y:						
Area:	µm					
I want to see:	Detected Areas		<input checked="" type="checkbox"/> Stats			
Item	Area	Perimeter	Roundness	Width	Height	Pass
B1	3.12500	8.37500 µm	82%	2.62500 µm	1.62500 µm	Nodules
B2	0.90625	4.00000 µm	117%	1.00000 µm	1.12500 µm	Nodules
B3	9.57813	16.25000	79%	4.50000 µm	3.12500 µm	Nodules
Stat	Count	Area				
Nodules	129	443.20313 µm²	9.34519%			
Image	X:79.87500 µm	Y:59.37500 µm	4742.5781 µm²			
Roundness	60%	Nodularity	97.67442%			
	0.02720	Objects	per µm²			

This is the number of Nodules detected.

This is your Roundness cut-off value that you set in the Measurement Options window.

This is your Nodularity value.

Performing Additional Detections

Once you have configured your system, you do not need to step through the wizard each time you want to do a Nodularity detection. Simply click on the NODULARITY button.



Once you click on the NODULARITY button, the software will run the wizard with all of your saved settings. You can go back and override the settings, but most of the time, you should not need to do so.

Further reports can be generated via the PAX-it Report Generation feature. Multiple fields can be added to a report and totals can be generated.

Multiple Calculations for Roundness

PAX-it allows you to choose from a variety of Roundness calculations. The following calculations are currently available.

Standard

1.0 Radius Standard Deviation/Radius Mean

This will produce a roundness value of 0–100. The closer to 100, the more round the object is.

Min/Max Radius

Min Radius/Max Radius

This will produce a roundness value of 0–100. The closer to 100, the more round the object is.

ISC

Max Radius/Min Radius

This will produce a roundness value of 100 infinity. The closer to 100, the more round the object is.

Perimeter

$\text{Perimeter} \wedge 2/4 * \text{PIE} * \text{Area}$

This will produce a roundness value of 100 infinity. The closer to 100, the more round the object is.

Nodules

$4 * \text{PIE} * \text{Area} / \text{Perimeter} \wedge 2$

This will produce a roundness value of 0 infinity. The closer to 100, the more round the object is.



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