

First Steps in ZEN Blue

Skill Builder Series: Image Processing



A Beginner's Guide to Automated Image Analysis in ZEN Blue



Seeing beyond

The ZEISS ZEN Blue software is designed around a set of powerful image analysis tools that can serve as building blocks for generating measurement tables, searching for rare morphological events, or even directing the image acquisition itself. When present, the optional ZEN Image Analysis module provides a user-friendly wizard for setting up segmentation routines across dozens of geometric and intensity-based parameters. The following guide reviews the steps involved for setting up a simple example of an automated cell count – and provides additional guidance for more complex approaches.

Overview and Purpose

Found in the main Analysis tab of the ZEN workspace, the Image Analysis window centralizes all setup options for building and executing measurements. Such measurements always occur on 2D images with no direct regard for other Z planes or time points. In other words, these basic tools are not intended for performing full 3D/volumetric segmentation or automated tracking of objects over time. These capabilities are available via a ZEISS partner known as arivis, whose powerful Vision4D software platform boasts a variety of analysis pipelines with an ever-increasing degree of integration in ZEN.

The tools of the ZEN Image Analysis window are ideal for quick screening when a simple “yes or no” question may be involved across a large number of image inputs. The user is often concerned with only a few measurements per object or field, not a slew of information that they are not sure will be needed later on. Some examples of the experimental framing that these tools can directly address:

“Of all the cells that I have imaged, does my treatment yield a morphology that is more round or oblong? Are my control groups different in certain levels of fluorescent signal? Are the nuclei, on average, larger or smaller after treatment? Are these puncta always found within the nucleus or outside it?”

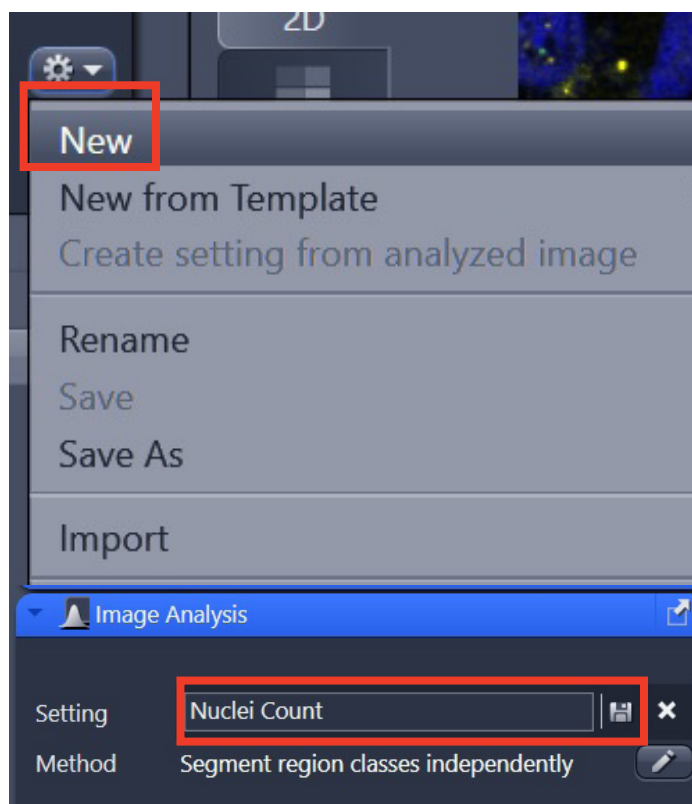
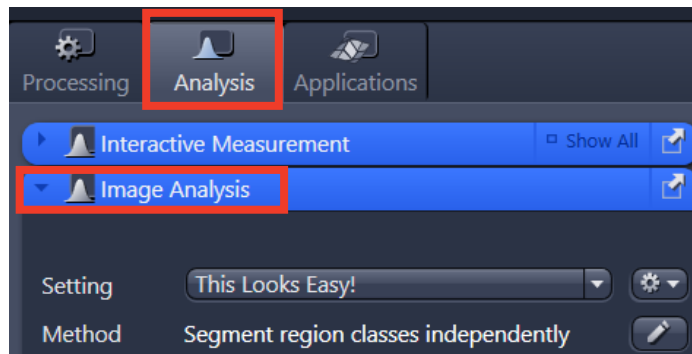
While setting up an analysis routine is always an iterative process, these questions should be characterized as much as possible before starting.

Working with the Wizard

Central to the Image Analysis window is a logic using so-called analysis Settings. These program-like Settings are self-contained, user-generated procedures for performing automated or semi-automated measurements. They populate a dropdown list and are refined when using the setup wizard.

There are two requirements in order to initialize a Setting:

1. A single or multichannel image must be open in ZEN.
This file should contain the same channel number and order that will be used for subsequent measurements.



2. A new Setting must be created using the gear icon to the right of the list. Once a name is typed and saved, the user can proceed.

By default, the newly-named Setting will display a list of default segmentation fields ("Classes 1," "Class 2"). These fields will ultimately summarize the organization of the analyzed objects.

To launch the wizard, click the large Setup Image Analysis button at the bottom of the Image Analysis window. (The same button also provides an entry point for modifying existing Settings if minor edits or updates are required.)

For this exercise, the DAPI channel of the FISH dataset below will be used to perform an automated cell count.

Wizard Step #1 - Classes

The setup wizard contains a total of 7 consecutive steps. Advancing through the steps is possible with the Back and Next buttons found at the bottom left. At any point, the Cancel button can also be used to leave the wizard while saving all progress to that point.

The first step of the wizard is intended for naming and organizing the segmented object hierarchies.

Note that all steps in the wizard can also be toggled as Interactive. This allows for creation of abridged wizards if certain analytical methods need to remain semi-automated (e.g. – for refining intensities for each image, manually removing outliers, etc.)

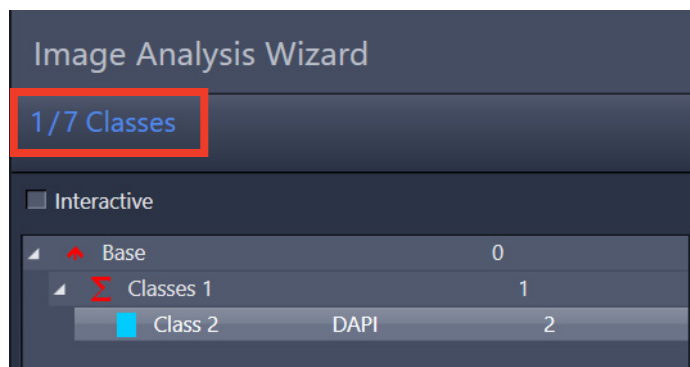
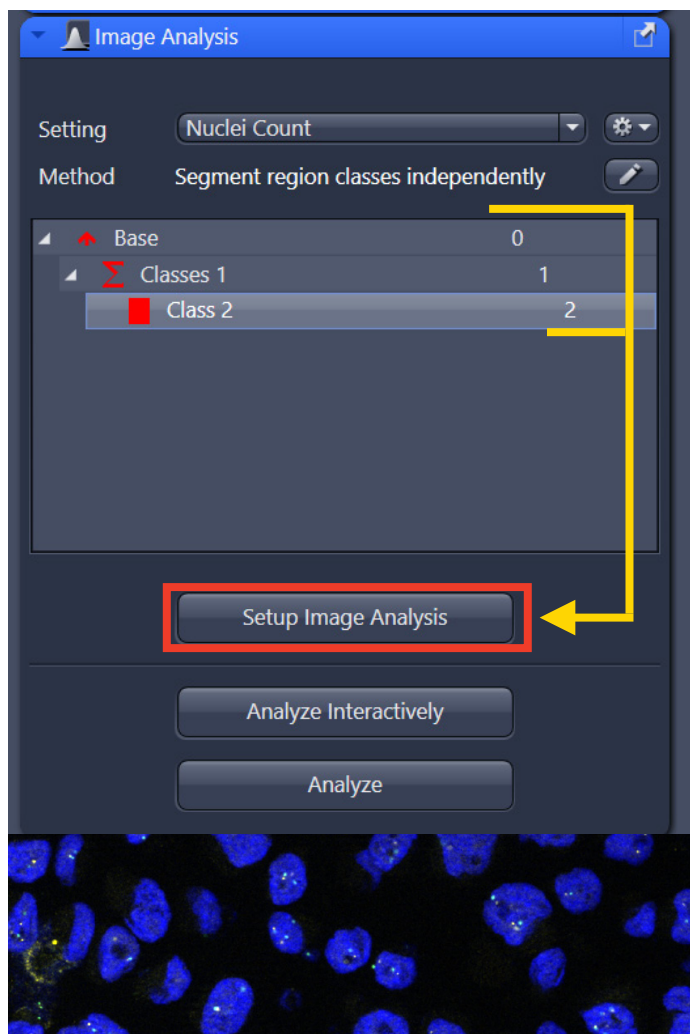
Each analysis entry contains two parts: an object-level row ("Class") and a summation parent row ("Classes"), which refers to all objects found in that group. Given the generic default naming scheme, it is highly recommended that the Name field is used to give each row a description, biological-friendly name. Rows of measurements are ultimately reported with these names, which makes combing through data much easier later on.

An easy way to think of the "Classes/Class" setup criteria is to imagine a room with several people in it:

"Classes": How many people total are in the room?

"Class": What are the individual heights of each person in the room?

Multiple independent object searches can be set up using the Add Class button, while nested searches (or "objects within objects") can be organized with the Add Subclass



button. (A more thorough example of the latter will be described later in this guide.)

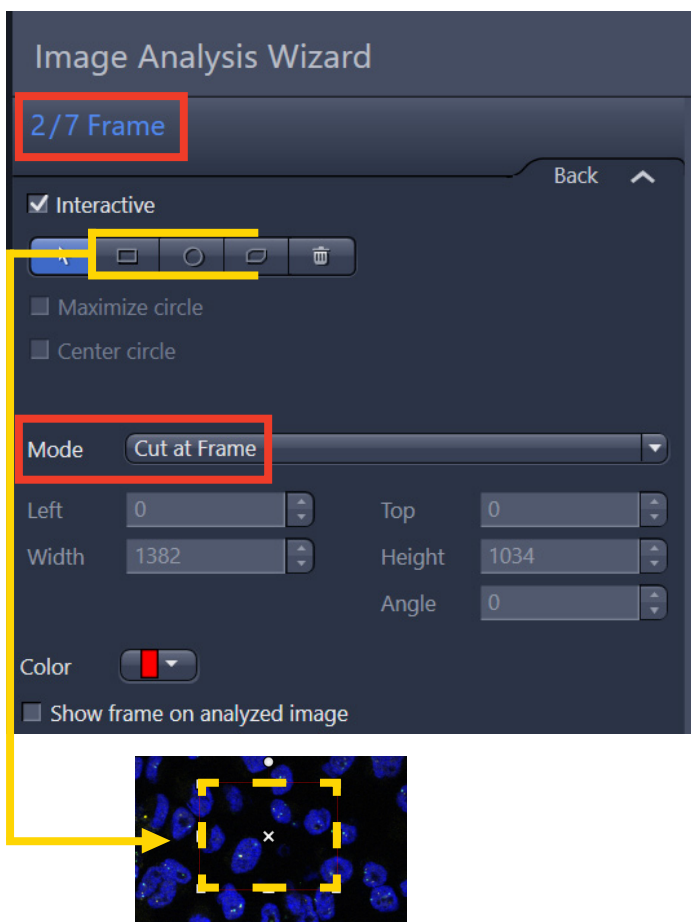
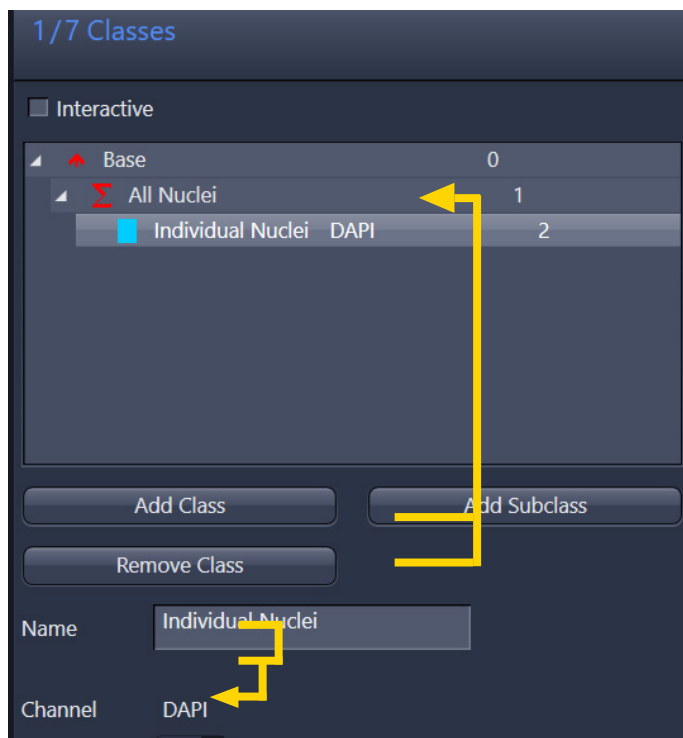
In the example above, the object-level class has been pointed to the blue DAPI label in the Channel section. To assist with the visualization of positively-identified nuclei, the Color has been changed to a slightly-different shade of blue so the two pseudocolors do not eventually obscure one another.

Once the naming, channel selection, and class organization is complete, click the Next button to proceed.

Wizard Step #2 – Frame

This step allows for the addition of one or more ROIs to be drawn onto the image to confine the measurement searches. Note that the placement of an ROI frame on an image will be applied in the same location for subsequent images, which may defeat the purpose of a fully-automated approach. For this reason, this is an excellent option for use with semi-automation and the Interactive checkbox.

In addition, the options of the Mode dropdown box (Cut at Frame, Inside Only) lets the user describe how objects on the edge of the frame are handled or excluded. (With no ROI drawn, the edge of the image field of view will be utilized as the basis for the selected mode.)



Wizard Step #3 – Automatic Segmentation

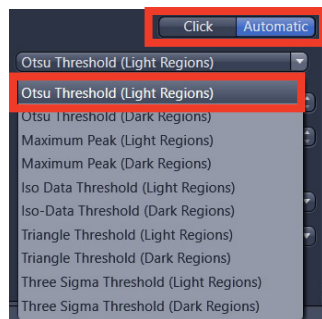
The heart of the entire wizard, this step contains all intensity-based threshold adjustments for identifying objects and refining their edges, minimum size, and overall aggregation. An object-level class must be select for the threshold options to appear. If multiple object-level classes are present, they can/must be adjusted independently.

This is the first instance of the Execute checkbox, which is ticked by default. Leave this setting on to permit automation of any set threshold parameters later on.

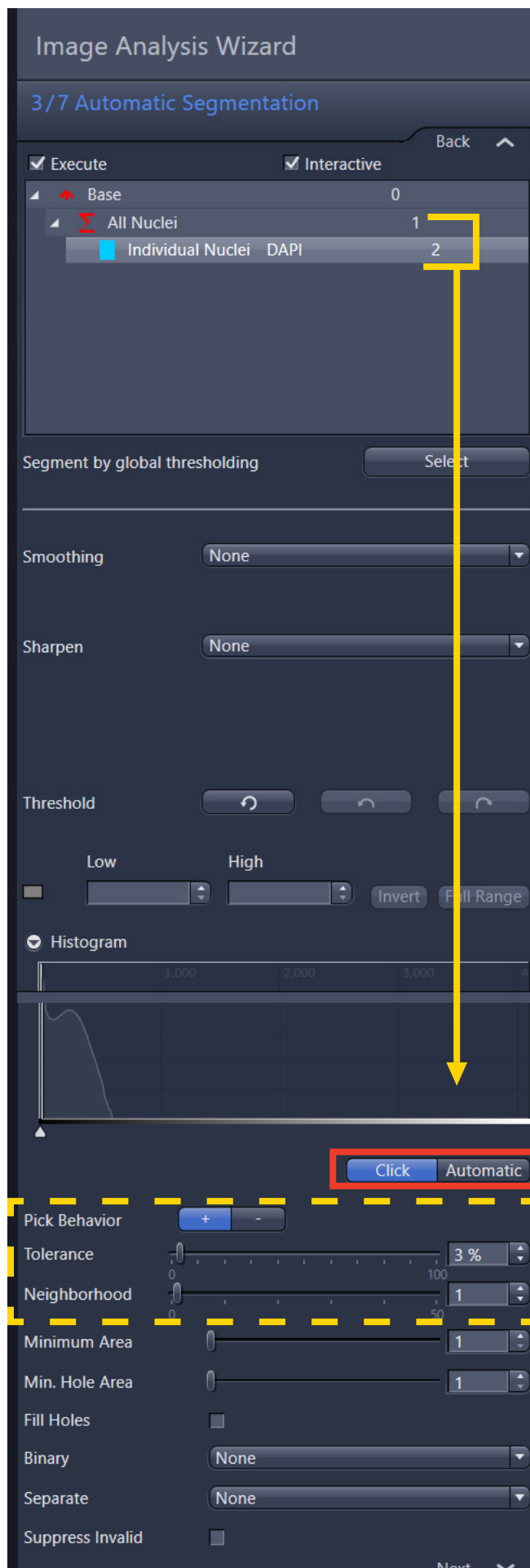
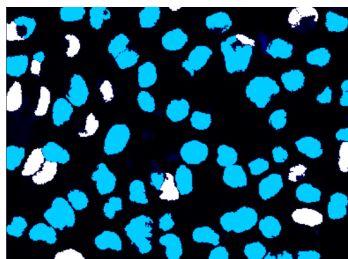
Once an object row is highlighted, jump to the Threshold section to begin the segmentation process. Two modes are available: Click or Automatic. The Click method requires the user to point the cursor at the image to expand/shrink the gray level cutoffs (depending on the Pick Behavior setting) with regards to the Histogram. The Tolerance and Neighborhood sliders will also influence the sensitivity and range of each click.

The Automatic methods include 5 main options/settings:

1. Otsu –foreground/background separation by sum of variances; excellent starting point
2. Maximum Peak – uses most frequent intensity value in the histogram for thresholding
3. Iso Data – threshold value is between two peaks
4. Triangle – threshold is between sum of averages
5. Three Sigma – threshold is three times the sigma value of histogram distribution



In general, the Click-based thresholding option is a better option if very little intensity variation is expected between images/slides, or if negative controls will be pooled. The Automatic thresholding routes can often be more forgiving for inconsistent labelling, but they will always be normalized to find objects, even in seemingly-empty fields. Such methods thus benefit from subsequent filtering steps in the wizard.



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For this DAPI example, we'll continue with the Automatic Otsu method, which yields the result on the left. Note the presence of holes and small debris at this stage.

To further optimize the identification of nuclei, other settings in this segmentation may be useful.

For example, the Minimum Area slider can be used eliminate small objects that do not represent a meaningful size. Units are expressed in raw pixels, ranging 1 – 1000.

The Fill Holes checkbox will any donut-shaped objects. As an alternative to this, the Min. Hole Area slider can be used to specifically restrict the size of holes that may be filled.

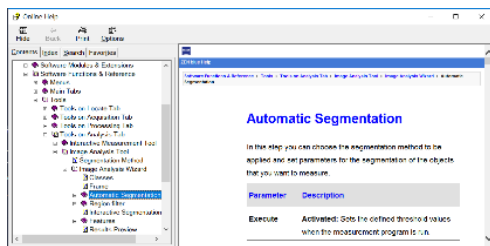
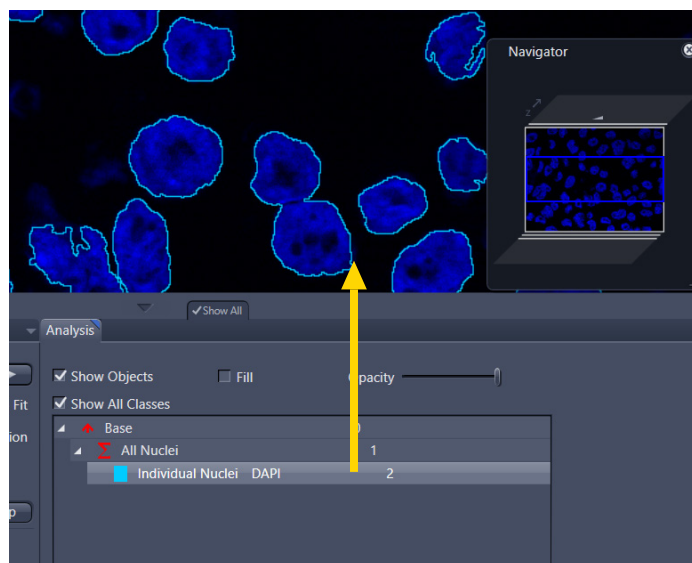
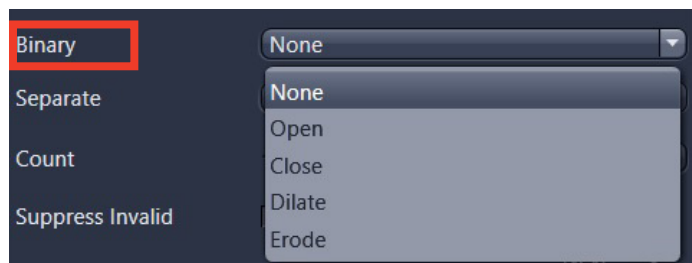
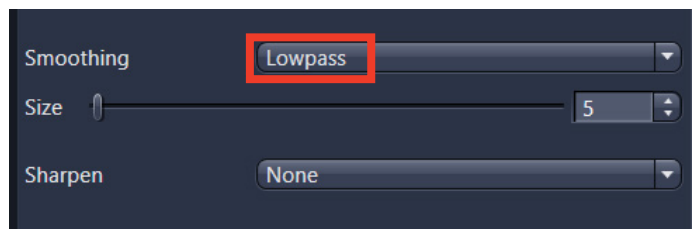
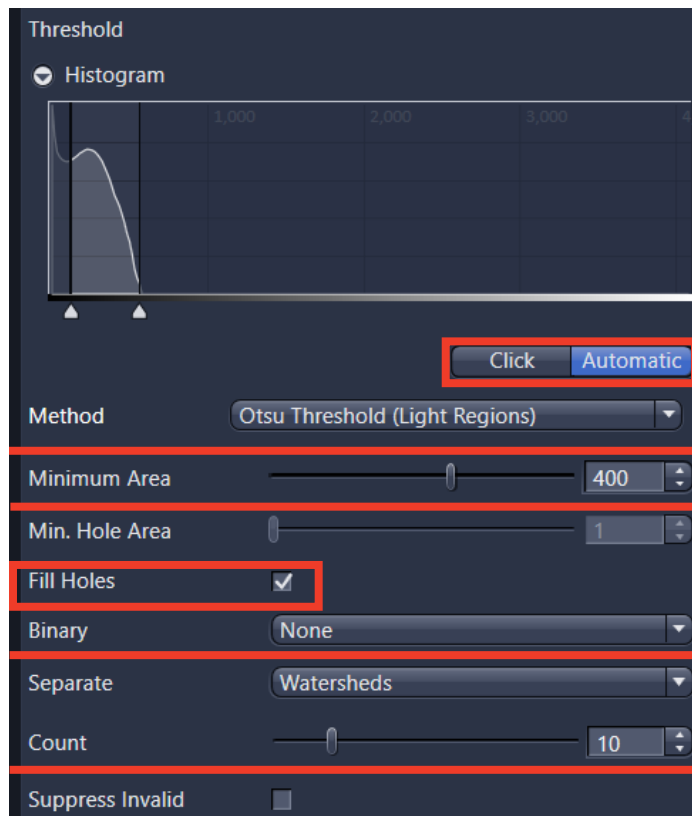
The Separate dropdown box allows for two options (Morphology, Watersheds) to iteratively separately two or more objects that may be touching or blooming into one. The Morphology option will reduce objects until they split (based on the value in the Count slider), then enlarge them. The Watershed option is ideal for separating round objects of similar size, as is the case for nuclei. However, it will tend to split elongated objects into equal halves.

Other useful parameters include the Smoothing (Lowpass, Gauss, Median) and Sharpen (Delineate, Unsharp Masking), which each have associated sliders to influence the degree to which pixels are softened/sharpened. The Lowpass or Median settings are particularly useful for dealing with noisier edges of membranes, thus rendering contours that mimic the true biological appearance.

The Binary dropdown box has several options (Open, Close, Dilate, Erode) for expanding or shrinking edges of objects. Open will automatically Erode, then Dilate; this can help remove isolated pixels. Close will automatically Dilate, then Erode; this can help fill small holes.

Some back-and-forth adjustments are normal when arriving at optimal settings. On the right pane, the Analysis mini-tab displays checkboxes for Show Objects, Fill, and Show All Classes. These tools permit the user to look behind or through the segmented objects to see the underlying image, if helpful.

Detailed descriptions of all options above are available within the Online Help of ZEN, accessed by pointing the cursor on a tool area and pressing F1 on the keyboard.



Wizard Step #4 – Region Filter

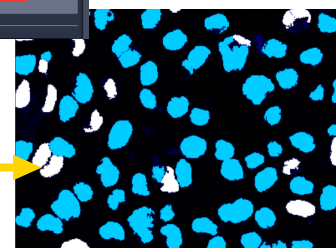
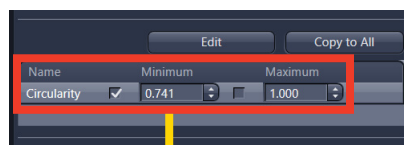
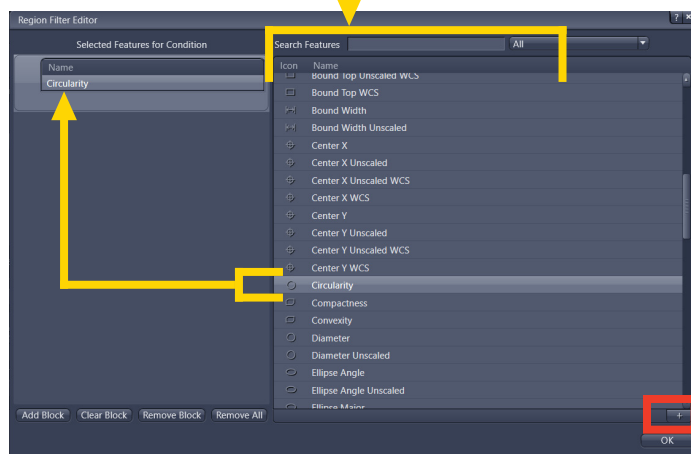
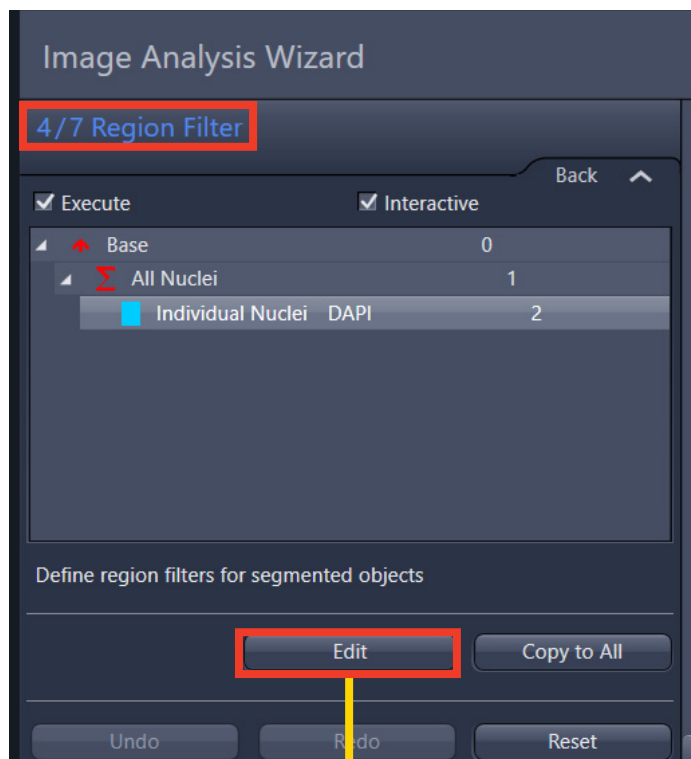
This step permits a powerful combination of user parameters for eliminating false positive or excess objects identified in the previous step. Therefore, it is often unproductive to spend considerable time perfecting the segmentation step if residual objects can be easily removed here.

To add a filter (essentially, a “rule”) to the wizard, first select the object-level row click the Edit button. A separate Region Filter Editor window will appear, which contains dozens of conditions that span Geometric, Intensity, Image, Position, and Statistical Features categories. To add an entry, highlight it and either double-click it or click the small + button on the bottom right; the filter will then populate the left panel.

For this exercise, we've selected the Circularity filter, a geometric parameter that is expressed from 0 to 1. Clicking the segmented image will dynamically update the Minimum and Maximum bounds of this filter. Alternatively, the fields can be manually defined. Any objects not meeting these criteria will appear white with the segmentation overlay and excluded from subsequent steps of the wizard.

In this DAPI example, we've set a Circularity parameter of at least ~0.7, which will eliminate the mitotically active or perhaps less viable cells in the field of view.

Note that it is also possible to combine multiple filters at once within the Region Filter Editor window, or even use the Add Block button to add an “or” statement between filter criteria.



Wizard Step #5 – Interactive Segmentation

This step is the last for making any corrections to the analysis settings, but requires the use of drawing tools to manually add, subtract, or modify objects. This step can be skipped entirely if the settings of the preceding steps were already effective. If, however, certain objects create consistent trouble spots for analysis, this step is a worthwhile example of the Interactive checkbox option.

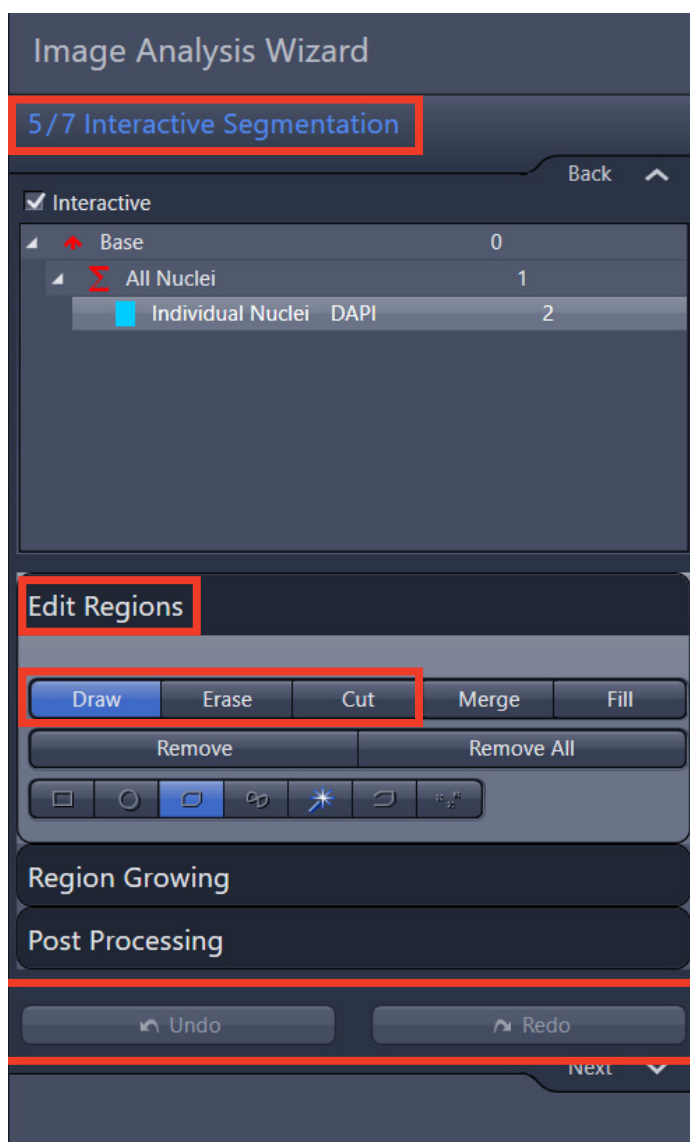
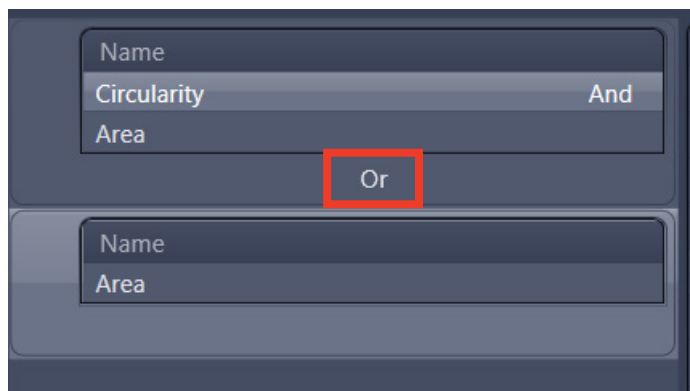
In the Edit Regions tray, the Draw button works in tandem with the available shape tools. A left-click on the image will start the drawing process. For contours or polygons, a right-click is required to close the shape.

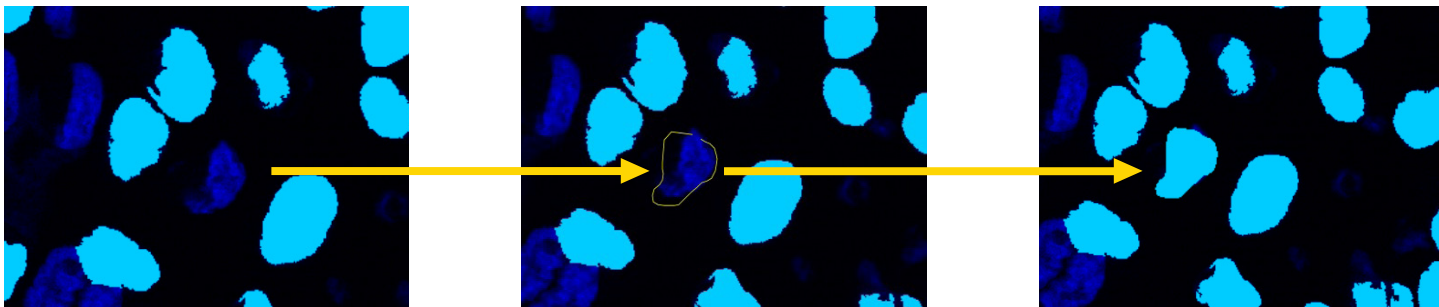
Existing objects can also be manually Cut or Erased with the corresponding button names.

The Remove and Remove All buttons are useful for clearing any existing elements, while the Undo and Redo buttons at the bottom help with any single click mistakes.

The Region Growing tray provides one additional chance for adding new objects via a click-based intensity search.

The Post Processing tray gives a simple option for applying newly-drawn objects through the previous step of the wizard (Step #4 – Region Filter).





Wizard Step #6 – Features

This step determines what is actually being reported with the measurements. The same options used for filtering earlier in the wizard can now be used to populate and organize data tables. In addition, the summation (“parent”) classes now have unique fields that pertain to the whole field of view (e.g. – Count, Area Percentage).

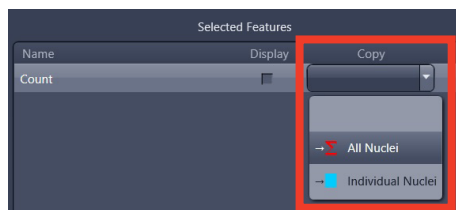
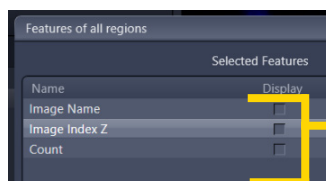
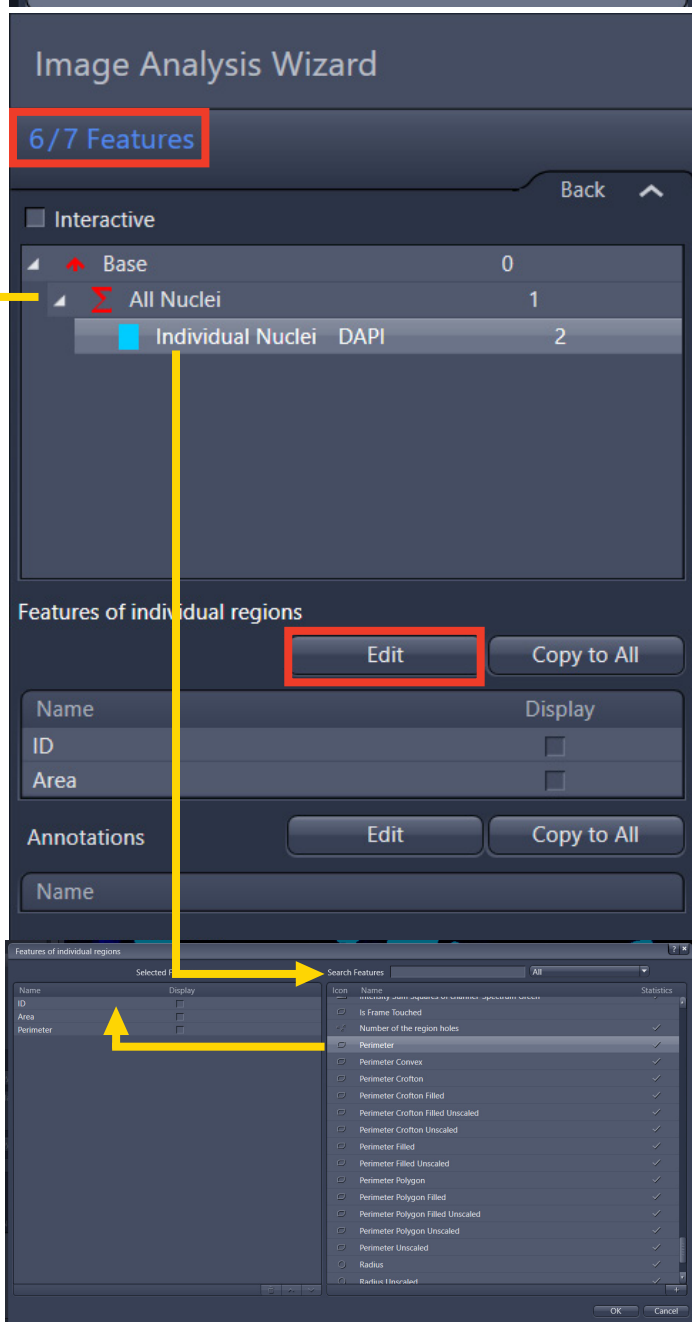
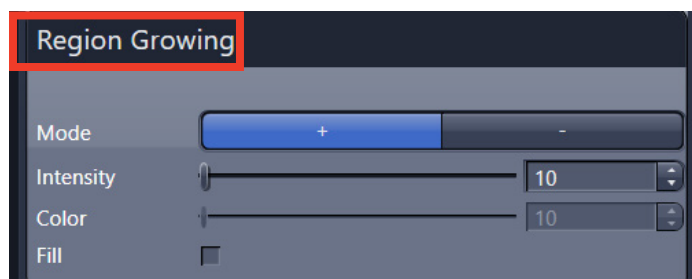
Once an object-level row or parent row is highlighted, click the Edit button to pop open the corresponding Features of Individual Regions or Features of All Regions windows, respectively.

To add an entry to a data table, double-click the item or click the + button at the bottom right. Note that the order of added features can be changed with arrow buttons; the final data tables will adopt the exact order displayed. By default, the object-level row will always include a generic ID value and the Area feature, while the parent row will have a Count feature.

For this exercise, we’ve added the Perimeter feature to each object-level measurement.

It is good practice to add certain metadata fields as additional features, such as Image Name, Image Index Z, or Image Index Time. This can make sifting through measurement tables easier when dealing with large multidimensional image sets.

Image analysis routines with multiple object classes can have their values bumped into other classes or hierarchies to help create a common data table. This Copy function is found as a column and dropdown box in the feature selection window(s).



Wizard Step #7 – Results Preview

This step simply shows what data to expect from experimental images. It is a quick way, without exiting the wizard, to see if the segmentation and the data table formatting look adequate.

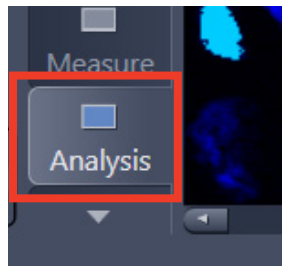
There is nothing to edit in this step; it is for preview purposes only. Data cannot be extracted in this step. Also note that certain data table values in the preview may be incomplete due to the partial analysis that occurs here.

Click the Finish button to close the wizard, which will automatically re-save the current image analysis Setting with the same name applied prior to the launch of the wizard.



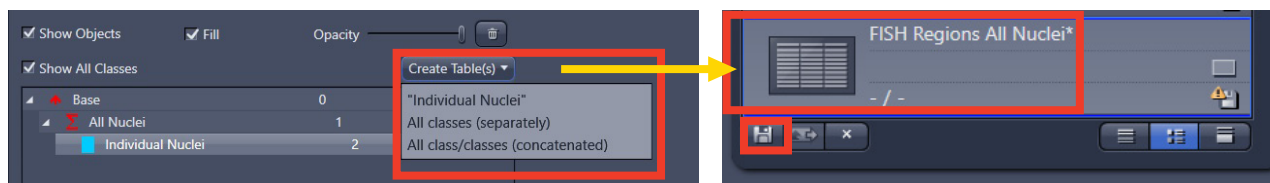
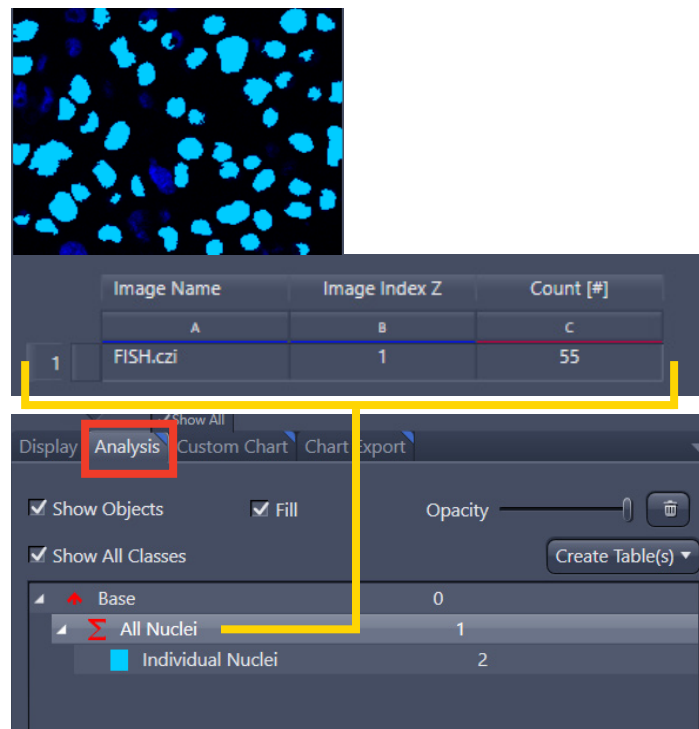
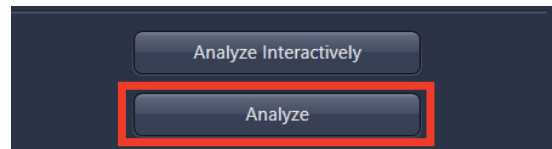
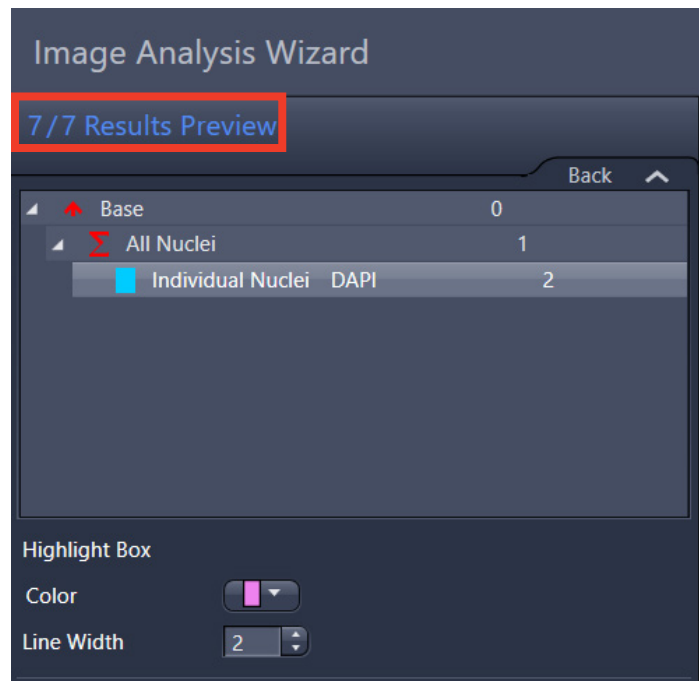
Running the Results!

Once the Setting is built, it can be executed within the Image Analysis window using any compatible active image visible in the ZEN workspace. This action is possible using either (1) the Analyze Interactively button, which will simply re-launch the wizard showing only the steps toggled to Interactive, or (2) the Analyze button, which will ignore any Interactive steps and completely automate the routine.



Depending on the image file size and processor speed, the analysis may take anywhere from a few seconds to a few minutes. When complete, the user will automatically be placed into a new Analysis view tab on the image. At the bottom right, an Analysis mini-tab reproduces the parent and object-level classes from the Setting file. Highlighting each will show the corresponding data tables above.

To extract the data tables from the Analysis view tab, highlight the desired class row and click the Create Table(s) dropdown box. One or all tables can be selected, which will cause a new file tab to appear in ZEN. Note the unique data table thumbnail icon that is displayed in the Images and Documents clipboard; clicking the disk icon below (Save) will permit .CSV and .XML file format options for easy importing into popular statistics and plotting packages.



That said, charts can be exported directly from ZEN using the Custom Chart and Chart Export mini-tabs that appear after running an Analysis routine.

Charts can be customized using a set of basic plotting options. The dropdown boxes for the X-Axis and Y-Axis contain entries automatically populated from the data tables managed in the image analysis Setting.

For Z-stacks or time series files, multiple dimensions can be plotted. The Z position or time point sliders within the Dimensions mini-tab have an interactive relationship on the chart itself.

All charts can be quickly exported as .PNG files of a given DPI setting within the Chart Export mini-tab.

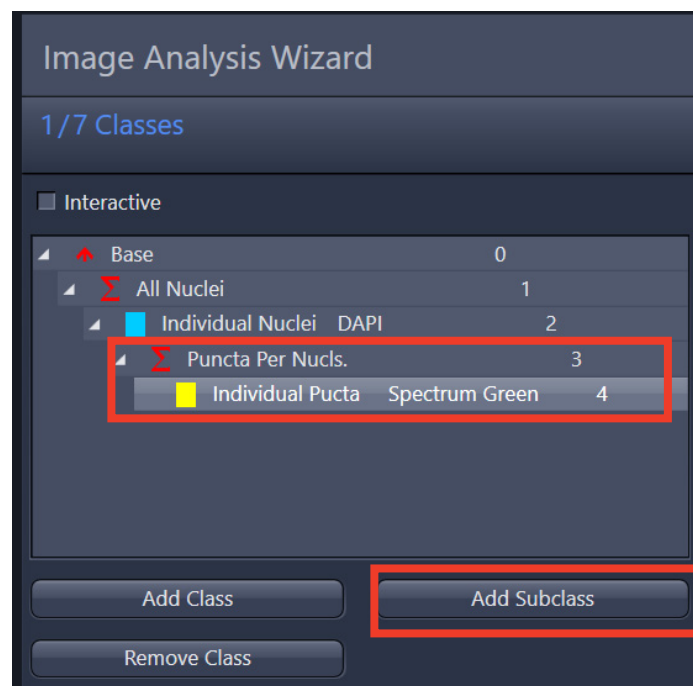
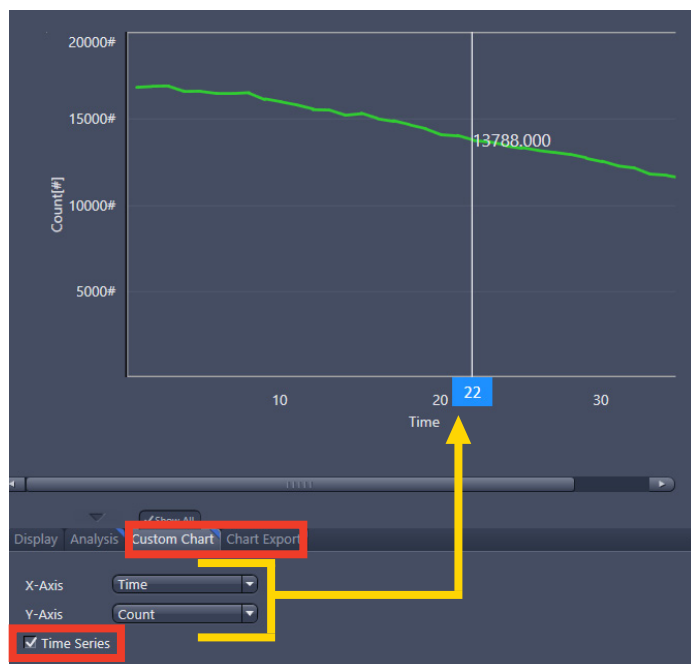
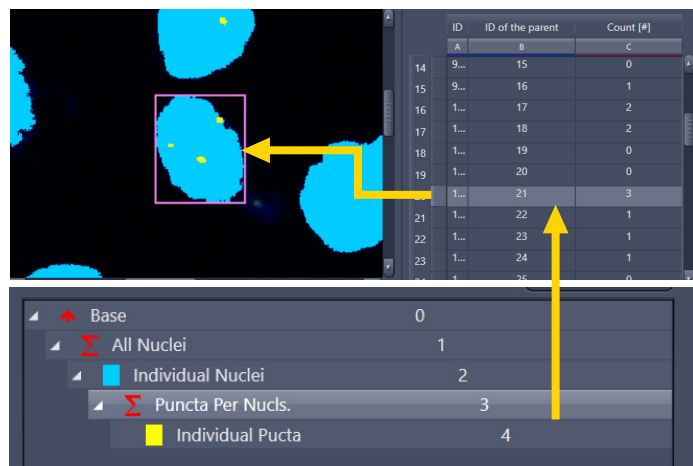
Advanced Tips and Tricks

A number of additional useful features exist both within and outside of the actual main Image Analysis window. Several of these tools – some advanced, some just well-hidden – are briefly summarized below.

Nested Classes

As mentioned earlier, it is possible to add “nested” sub-classes to existing object-level rows within the first step of the wizard. This permits isolating objects within objects for detection and counting. Simply highly the object row and click the Add Subclass button. As always, it is helpful to be descriptive with the naming used for such subclasses.

After running the analysis, the subclasses can be navigated as their own customized data tables. In this DAPI example, the FISH labels appear in yellow, and a count per nucleus is available. Clicking each row of the table will automatically move to the object on the image.



Note that at least 60% of the subclass object area must coincide with its segmented parent object to be counted. Other image analysis options – such as Zone of Influence (ZOI), not covered here – exist for analysis searches involving proximal or adjacent targets.



Color Camera Images

The Image Analysis window is not restricted to fluorescent image inputs. Color camera images with no multichannel information can also work well for certain colorimetric assays.

While the Automatic thresholding options in the wizard tend to be less useful for color images, the Click-based options work well for tuning RGB (red, green, blue) or HLS (hue, lightness, saturation) color models.

For this type of analysis, it is critical that hardware settings of the illuminator (LED, halogen bulb) and condenser yield consistent white balancing for all input images.

Processing Tool – Analyze Batch to File

Buried deep within the main Processing tab of ZEN is an extremely powerful batch analysis tool known as Analyze Batch to File. This function permits fully automated execution of an image analysis Setting across a single folder of image files.

While there is no fundamental limit to the number of images that are batched, they should all be compatible with the given Setting being run.

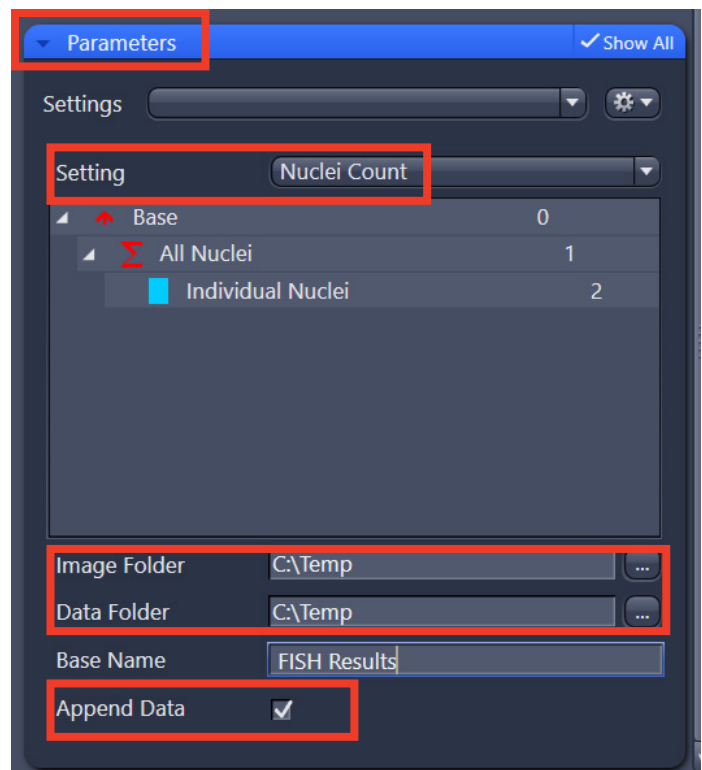
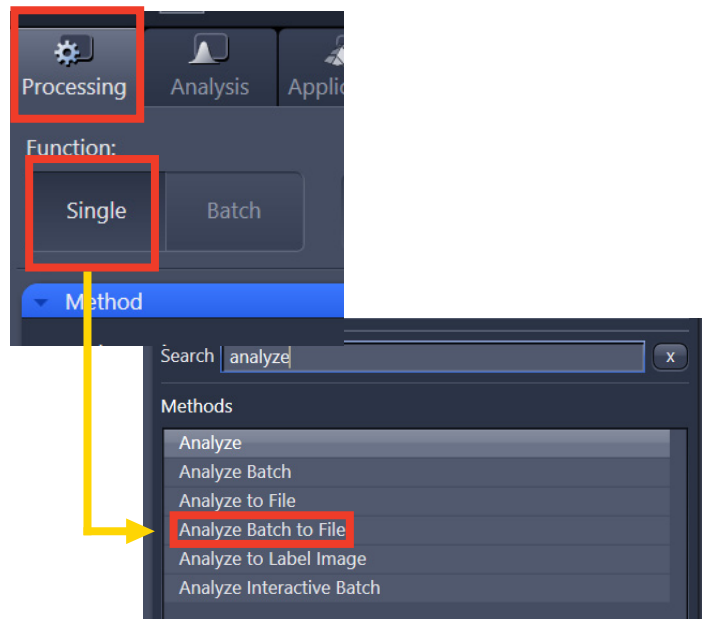
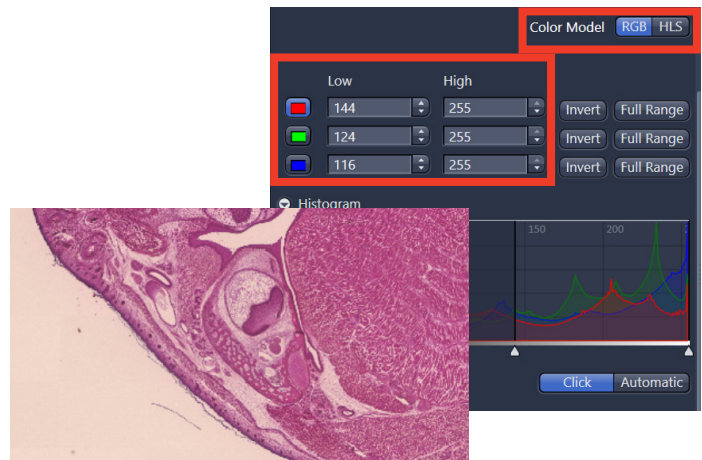
Simply search the Method field for “Analyze Batch to File;” locate and expand the associated Parameters window. The Parameters window for Analyze Batch to File is relatively straightforward, with options for loading the image analysis Setting, setting the Image Folder source, and the Data Folder output path.

An additional option for adding a specialized prefix Base Name to all generated measurements is also available. At the bottom, the Append Data can be extremely useful for compiling hundreds of image measurements into a single file. (Without this checkbox activated, each image will yield a separate results file.) This option is also why adding the Image Name metadata field in measurements tables can be helpful. All results are .CSV files.

A basic example of a 3 image batch output count is below.

| Name | Type | |
|--------------------------------|---------------------|--|
| FISH 1 | CZI File | |
| FISH 2 | CZI File | |
| FISH 3 | CZI File | |
| FISH Results_All Nuclei | CZI File | |
| FISH Results_Individual Nuclei | Microsoft Excel ... | |

| A | B | C |
|-------------|----------------------------|------------------------|
| ImageDocume | ImageIndexZ::Image Index Z | RegionsCount::Count!!! |
| | # | |
| FISH 1.czi | 1 | 55 |
| FISH 2.czi | 1 | 65 |
| FISH 3.czi | 1 | 66 |



Processing Tool – Analyze Label to Image

Another specialized tool found in the main Processing tab is the Analyze Label to Image function. Search for it in a similar manner as mentioned above.

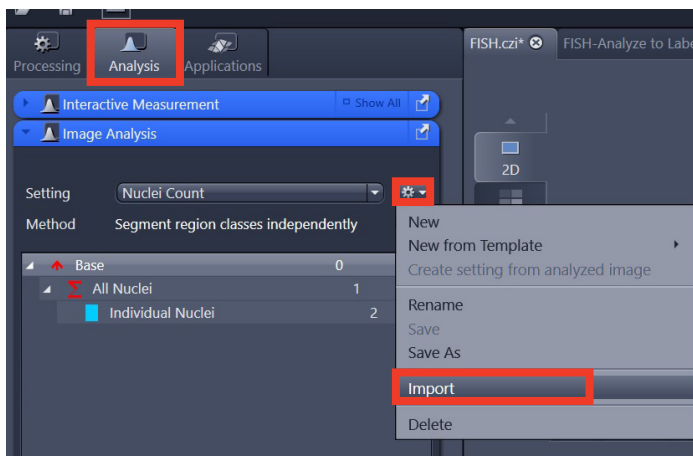
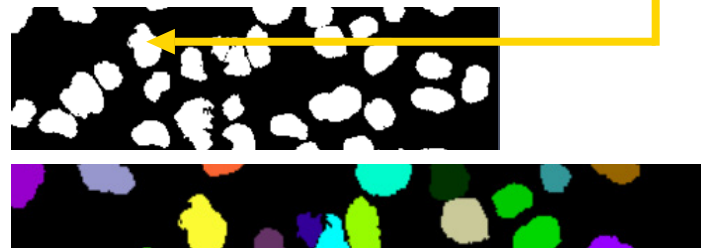
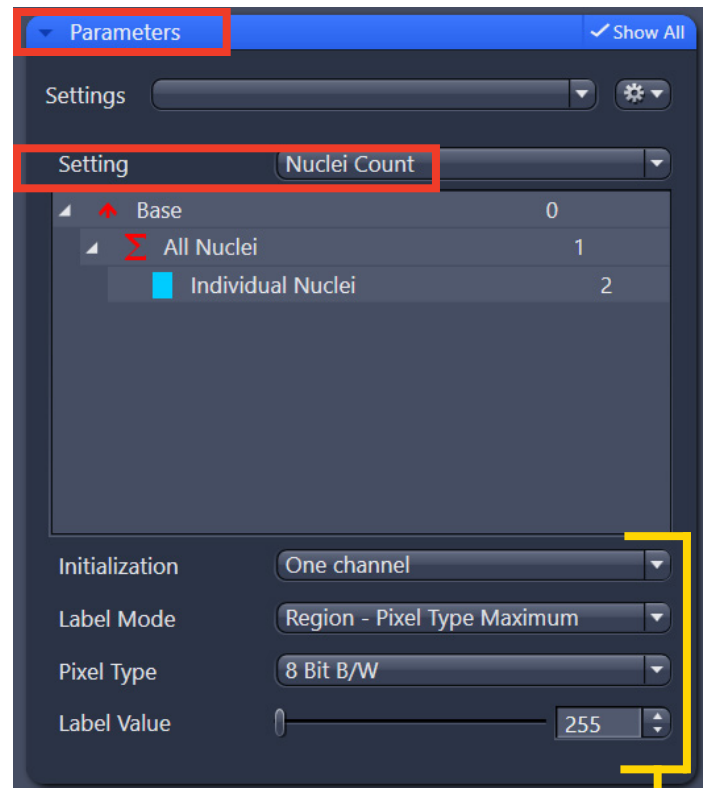
This tool allows for the segmentation mask(s) to be isolated as a new binary image file. Dozens of visualization options exist – but are beyond the intended scope of this guide. Two examples are shown below.

Collaborative Importing

One overlooked feature within the Image Analysis window is the ability to import the underlying analysis Setting (.CZIAS) file across versions of ZEN. This permits easy collaboration with colleagues or even a ZEISS imaging specialist as long as a test .CZI dataset can be freely shared or hosted.

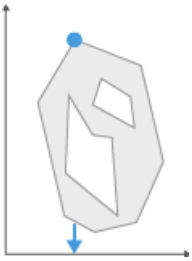
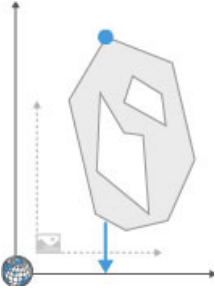
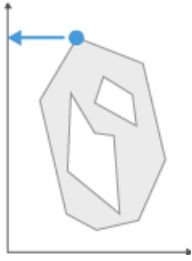
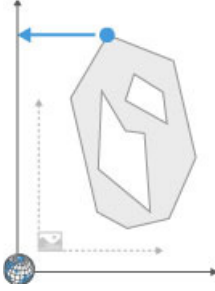
The .CZIAS files within the Setting list are located in the following directory:



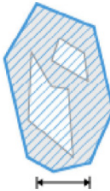
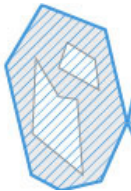

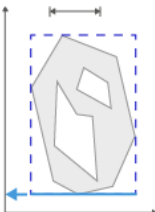
C:\Users\USER\Documents\Carl Zeiss\ZEN\Documents\Image Analysis Settings

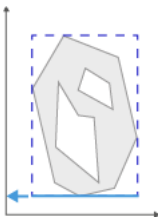
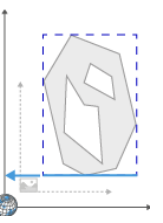
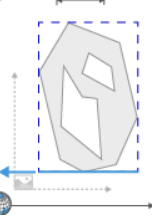
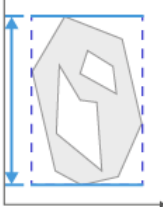
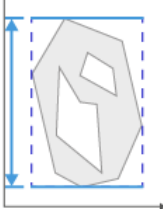
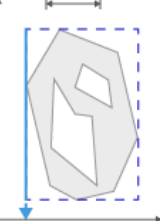
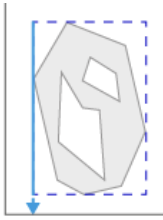


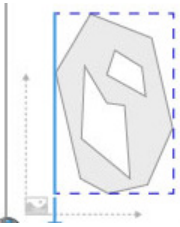
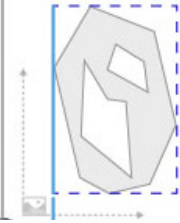
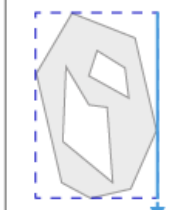
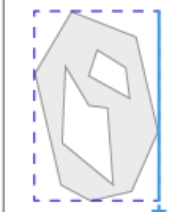
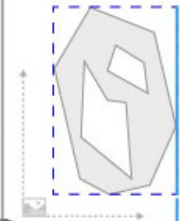
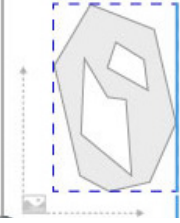
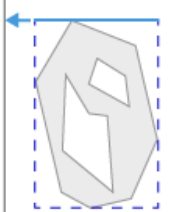
Appendix: Measurement Features


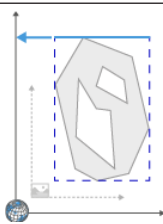
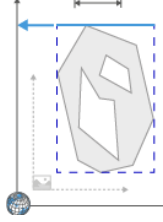

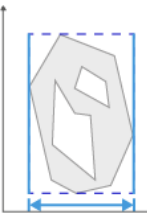

A detailed review of the Region Filters or Measurement Features available in ZEN are listed below:

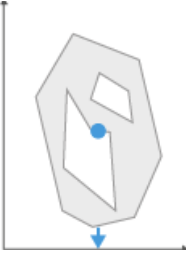
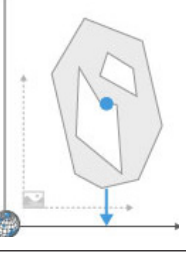
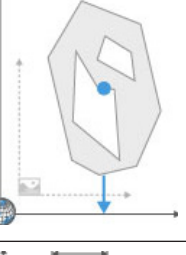
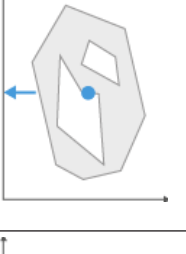
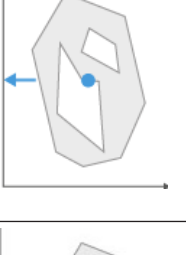
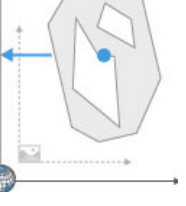
| Feature Name | Example | Description |
|--------------------|---|--|
| ACP X Unscaled |  | <p>The x coordinate of the first pixel in the first line of a region</p> <p>To identify measurement objects, the image is scanned from top left to bottom right. The so-called ACP (anti-coincidence point) is the first point that has been identified for a new object. The parameter Acp X indicates the x-coordinate of this point.</p> <ul style="list-style-type: none"> ■ Unit: pixels ■ Value range: 1 ... image size in x-direction |
| ACP X Unscaled WCS |  | <p>The x coordinate of the first pixel in the first line of a region</p> <p>To identify measurement objects, the image is scanned from top left to bottom right. The so-called ACP (anti-coincidence point) is the first point that has been identified for a new object. The parameter Acp X indicates the x-coordinate of this point in the world coordinate system (WCS).</p> <ul style="list-style-type: none"> ■ Unit: pixels ■ Value range: 1 ... image size in x-direction |
| ACP Y Unscaled |  | <p>The y coordinate of the first pixel in the first line of a region</p> <p>To identify measurement objects, the image is scanned from top left to bottom right. The so-called ACP (anti-coincidence point) is the first point that has been identified for a new object. The parameter Acp Y indicates the y-coordinate of this point.</p> <ul style="list-style-type: none"> ■ Unit: pixels ■ Value range: 1 ... image size in y-direction |
| ACP Y Unscaled WCS |  | <p>The y coordinate of the first pixel in the first line of a region</p> <p>To identify measurement objects, the image is scanned from top left to bottom right. The so-called ACP (anti-coincidence point) is the first point that has been identified for a new object. The parameter Acp Y indicates the y-coordinate of this point in the world coordinate system (WCS).</p> <ul style="list-style-type: none"> ■ Unit: pixels ■ Value range: 1 ... world coordinate size in y-direction |

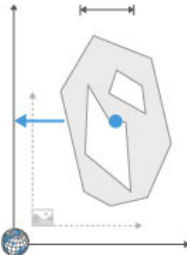

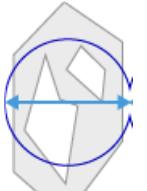
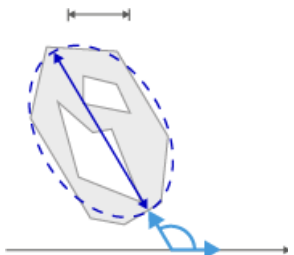
| | | |
|----------------------|---|---|
| Area |  | <p>Area of a region</p> <p>Area of a region excluding any holes it may contain. The areas of the holes are not included in the measurement. If you want to include them, use the Area filled parameter.</p> <ul style="list-style-type: none"> Unit: Unit of area of the scaling assigned to the image (e.g. μm^2) |
| Area Convex |  | <p>Area of convex hull of a region</p> <p>The current region is surrounded by a convex polyline. The (filled!) area of the resulting region is then measured.</p> <ul style="list-style-type: none"> Unit: Unit of area of the scaling assigned to the image (e.g. μm^2) |
| Area Filled |  | <p>Area of filled region</p> <p>Area of a region including any holes it contains. The holes are interpreted as belonging to the region or are filled prior to the measurement. If you do not want the holes to be measured, use the Area parameter.</p> <ul style="list-style-type: none"> Unit: Unit of area of the scaling assigned to the image (e.g. μm^2) |
| Area Filled Unscaled |  | <p>Area of filled region</p> <p>Area of a region including any holes it contains. The holes are interpreted as belonging to the region or are filled prior to the measurement. If you do not want the holes to be measured, use the Area parameter.</p> <ul style="list-style-type: none"> Unit: pixels² |
| Area Unscaled |  | <p>Area of a region unscaled</p> <p>The Area unscaled parameter corresponds to the Area parameter. However, the scaling of the image is not taken into account for the measurement. The (unfilled!) area of a region is displayed in pixels in each case.</p> <ul style="list-style-type: none"> Unit: pixels² |
| Bound Bottom |  | <p>Minimum y-coordinate of the bounding box of a region</p> <p>Indicates the y-coordinate of the bottom edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> Unit: Unit of the scaling assigned to the image (e.g. μm) |

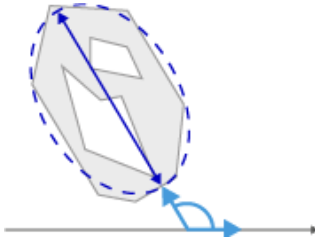




| | | |
|---------------------------|---|---|
| Bound Bottom Unscaled |  | <p>Minimum y-coordinate of the bounding box of a region</p> <p>Indicates the y-coordinate of the bottom edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> ■ Unit: pixels |
| Bound Bottom Unscaled WCS |  | <p>Minimum y-coordinate of the bounding box of a region</p> <p>Indicates the y-coordinate in the world coordinate system (WCS) of the bottom edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> ■ Unit: pixels |
| Bound Bottom WCS |  | <p>Minimum y-coordinate of the bounding box of a region</p> <p>Indicates the y-coordinate in the world coordinate system (WCS) of the bottom edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> ■ Unit: Unit of the scaling assigned to the image (e.g. μm) |
| Bound Height |  | <p>Indicates the height (size in y-direction) of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> ■ Unit: Unit of the scaling assigned to the image (e.g. μm) ■ Formula: Bound top - Bound bottom |
| Bound Height Unscaled |  | <p>Indicates the height (size in y-direction) of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> ■ Unit: pixels ■ Formula: Bound top - Bound bottom |
| Bound Left |  | <p>Minimum x-coordinate of the bounding box of a region</p> <p>Indicates the x coordinate of the left-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> ■ Unit: Unit of the scaling assigned to the image (e.g. μm) |
| Bound Left Unscaled |  | <p>Minimum x-coordinate of the bounding box of a region</p> <p>Indicates the x coordinate of the left-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> ■ Unit: pixels |

| | | |
|--------------------------|---|---|
| Bound Left Unscaled WCS |  | <p>Minimum x-coordinate of the bounding box of a region</p> <p>Indicates the x coordinate in the world coordinate system (WCS) of the left-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> Unit: pixels |
| Bound Left WCS |  | <p>Minimum x-coordinate of the bounding box of a region</p> <p>Indicates the x coordinate in the world coordinate system (WCS) of the left-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> Unit: Unit of the scaling assigned to the image (e.g. μm) |
| Bound Right |  | <p>Maximum x-coordinate of the bounding box of a region</p> <p>Indicates the x coordinate of the right-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> Unit: Unit of the scaling assigned to the image (e.g. μm) |
| Bound Right Unscaled |  | <p>Maximum x-coordinate of the bounding box of a region</p> <p>Indicates the x coordinate of the right-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> Unit: pixels |
| Bound Right Unscaled WCS |  | <p>Maximum x-coordinate of the bounding box of a region</p> <p>Indicates the x coordinate in the world coordinate system (WCS) of the right-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> Unit: pixels |
| Bound Right WCS |  | <p>Maximum x-coordinate of the bounding box of a region</p> <p>Indicates the x coordinate in the world coordinate system (WCS) of the right-hand edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> Unit: Unit of the scaling assigned to the image (e.g. μm) |
| Bound Top |  | <p>Maximum y-coordinate of the bounding box of a region</p> <p>Indicates the y coordinate of the top edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> Unit: Unit of the scaling assigned to the image (e.g. μm) |

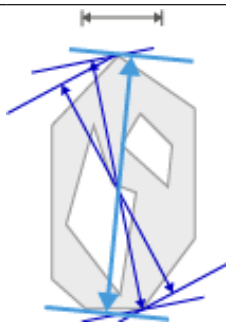
| | | |
|------------------------|---|--|
| Bound Top Unscaled |  | <p>Maximum y-coordinate of the bounding box of a region</p> <p>Indicates the y coordinate of the top edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> Unit: pixels |
| Bound Top Unscaled WCS |  | <p>Maximum y-coordinate of the bounding box of a region</p> <p>Indicates the y coordinate in the world coordinate system (WCS) of the top edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> Unit: pixels |
| Bound Top WCS |  | <p>Maximum y-coordinate of the bounding box of a region</p> <p>Indicates the y coordinate in the world coordinate system (WCS) of the top edge of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> Unit: Unit of the scaling assigned to the image (e.g. μm) |
| Bound Width |  | <p>Indicates the width (size in x-direction) of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> Formula: Bound right - Bound left Unit: Unit of the scaling assigned to the image (e.g. μm) |
| Bound Width Unscaled |  | <p>Indicates the width (size in x-direction) of a bounding box for a region. The box is drawn in parallel to the x and y axis.</p> <ul style="list-style-type: none"> Formula: Bound right - Bound left Unit: pixels |
| Center X |  | <p>The x coordinate of the geometric center of gravity of a region</p> <p>Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the Center Y parameter.</p> <ul style="list-style-type: none"> Unit: Unit of the scaling assigned to the image (e.g. μm) |

| | | |
|-----------------------|---|--|
| Center X Unscaled |  | <p>The x coordinate of the geometric center of gravity of a region</p> <p>Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the Center Y parameter.</p> <ul style="list-style-type: none"> Unit: pixels |
| Center X Unscaled WCS |  | <p>The x coordinate in the world coordinate system (WCS) of the geometric center of gravity of a region</p> <p>Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the Center Y parameter.</p> <ul style="list-style-type: none"> Unit: pixels |
| Center X WCS |  | <p>The x coordinate in the world coordinate system (WCS) of the geometric center of gravity of a region</p> <p>Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the Center Y parameter.</p> <ul style="list-style-type: none"> Unit: Unit of the scaling assigned to the image (e.g. μm) |
| Center Y |  | <p>The y coordinate of the geometric center of gravity of a region</p> <p>Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the Center X parameter.</p> <ul style="list-style-type: none"> Unit: Unit of the scaling assigned to the image (e.g. μm) |
| Center Y Unscaled |  | <p>The y coordinate of the geometric center of gravity of a region</p> <p>Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the Center X parameter.</p> <ul style="list-style-type: none"> Unit: pixels |
| Center Y Unscaled WCS |  | <p>The y coordinate in the world coordinate system (WCS) of the geometric center of gravity of a region</p> <p>Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the Center X parameter.</p> <ul style="list-style-type: none"> Unit: pixels |

| | | |
|-------------------|---|--|
| Center Y WCS |  | <p>The y coordinate in the world coordinate system (WCS) of the geometric center of gravity of a region</p> <p>Depending on the shape of the object, this point may also lie outside a region. The associated y-coordinate is determined via the Center X parameter.</p> <ul style="list-style-type: none"> Unit: Unit of the scaling assigned to the image (e.g. μm) |
| Circularity | | <p>$\text{Sqrt}(\text{Roundness})$</p> <p>$\text{Roundness} = 4 \times \text{Area} / \pi \times \text{FeretMax}^2$</p> |
| Compactness | | $4 \times \pi \times \text{Area} / \text{PerimeterConvex}^2$ |
| Convexity | | $\text{PerimeterConvex} / \text{PerimeterCrofton}$ |
| Diameter |  | <p>Diameter of a circle with an area equal to that of the object</p> <p>The object is measured using the Area) parameter. A circle with the same area as the object is created. The diameter of this circle is returned.</p> <ul style="list-style-type: none"> Formula: $\text{Sqrt}((4 / \pi) \times \text{Area})$ Unit: Unit of the scaling assigned to the image (e.g. μm) |
| Diameter Unscaled |  | <p>Diameter of a circle with an area equal to that of the object</p> <p>The object is measured using the Area) parameter. A circle with the same area as the object is created. The diameter of this circle is returned.</p> <ul style="list-style-type: none"> Formula: $\text{Sqrt}((4 / \pi) \times \text{Area1})$ Unit: pixels |
| Ellipse Angle |  | <p>Angle of the major axis of the ellipse</p> <p>The major axis of an ellipse with the same geometric moment of inertia as the current region is determined in accordance with the Ellipse major parameter. The angle to the x-axis is then determined. The indication of the angle always relates to a counterclockwise direction.</p> <ul style="list-style-type: none"> Unit: degrees Value range: 0 ... 180° |

| | | |
|------------------------|---|---|
| Ellipse Angle Unscaled |  | <p>Angle of the major axis of the ellipse</p> <p>The major axis of an ellipse with the same geometric moment of inertia as the current region is determined in accordance with the Ellipse major parameter. The angle to the x-axis is then determined. The indication of the angle always relates to a counterclockwise direction.</p> <ul style="list-style-type: none"> ■ Unit: degrees ■ Value range: 0 ... 180° <p>This tool uses unscaled pixels for calculating the angle. The results may differ from the results of Ellipse Angle.</p> |
| Ellipse Major |  | <p>Length of the major axis of the ellipse</p> <p>Length of the major axis of an ellipse with the same geometric moment of inertia as the region. The moment of inertia is calculated about the center of gravity of the region.</p> <ul style="list-style-type: none"> ■ Unit: Unit of the scaling assigned to the image (e.g. μm) |
| Ellipse Major Unscaled |  | <p>Length of the major axis of the ellipse</p> <p>Length of the major axis of an ellipse with the same geometric moment of inertia as the region. The moment of inertia is calculated about the center of gravity of the region.</p> <ul style="list-style-type: none"> ■ Unit: pixels |
| Ellipse Minor |  | <p>Length of the minor axis of the ellipse</p> <p>Length of the minor axis of an ellipse with the same geometric moment of inertia as the region. The moment of inertia is calculated about the center of gravity of the region.</p> <ul style="list-style-type: none"> ■ Unit: Unit of the scaling assigned to the image (e.g. μm) |
| Ellipse Minor Unscaled |  | <p>Length of the minor axis of the ellipse</p> <p>Length of the minor axis of an ellipse with the same geometric moment of inertia as the region. The moment of inertia is calculated about the center of gravity of the region.</p> <ul style="list-style-type: none"> ■ Unit: pixels |

Feret Maximum

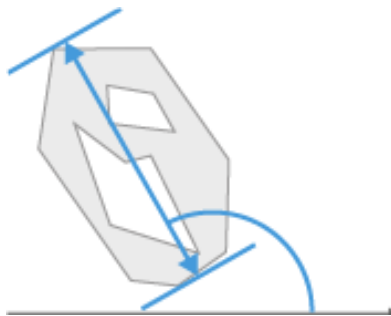


Maximum feret of a region

The maximum feret of a region is determined on the basis of distance measurements. Two straight lines are positioned on opposite sides of the object, like a sliding caliper, at 32 angle positions. The corresponding distance is measured for each angle position. The maximum value determined is the maximum feret.

- Unit: Unit of the scaling assigned to the image (e.g. μm)

Feret Maximum Angle

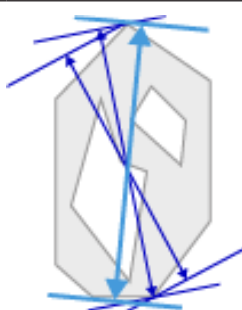


Angle of the maximum feret of a region in relation to the x-axis

The maximum feret is determined as described in Feret Maximum. The angle of the maximum feret in relation to the x-axis is then determined. The indication of the angle always relates to a counterclockwise direction.

- Unit: degrees
- Value range: 0 ... 180°

Feret Maximum Unscaled

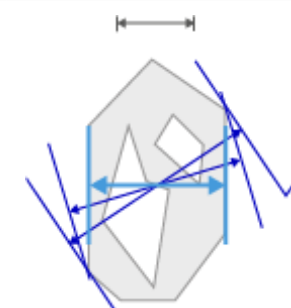


Maximum feret of a region

The maximum feret of a region is determined on the basis of distance measurements. Two straight lines are positioned on opposite sides of the object, like a sliding caliper, at 32 angle positions. The corresponding distance is measured for each angle position. The maximum value determined is the maximum feret.

- Unit: pixels

Feret Minimum

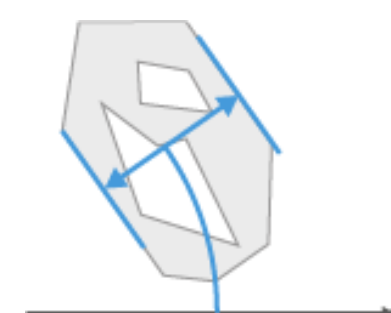


Minimum feret of a region

The minimum feret of a region is determined on the basis of distance measurements. Two straight lines are positioned on opposite sides of the object, like a sliding caliper, at 32 angle positions. The corresponding distance is measured for each angle position. The minimum value determined is the minimum feret.

- Unit: Unit of the scaling assigned to the image (e.g. μm)

Feret Minimum Angle



Angle of the minimum feret of a region in relation to the x-axis

The minimum feret is determined as described in Feret Minimum. The angle of the minimum feret in relation to the x-axis is then determined. The indication of the angle always relates to a counterclockwise direction.

- Unit: degrees
- Value range: 0 ... 180°



Feret Minimum Unscaled

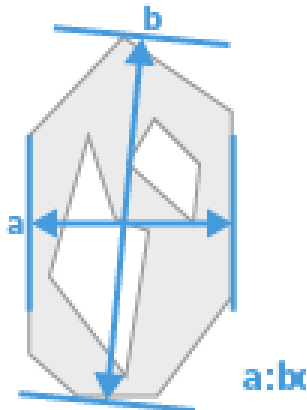


Minimum feret of a region

The minimum feret of a region is determined on the basis of distance measurements. Two straight lines are positioned on opposite sides of the object, like a sliding caliper, at 32 angle positions. The corresponding distance is measured for each angle position. The minimum value determined is the minimum feret.

- Unit: pixels

Feret Ratio

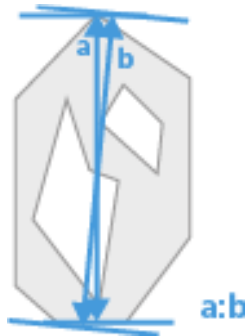


Feret ratio

The ratio of Feret Minimum to Feret Maximum is calculated. This ratio makes it possible to make statements on the form of the measured objects. If the feret ratio has a low value, long, elongated objects are present. Values approaching 1 indicate the presence of compact or circular objects, as in this case Feret Minimum and Feret Maximum have very similar values. The Form circle is also suitable for making statements on the circularity of an object.

- Formula: $\text{FeretMin} / \text{FeretMax}$ (a / b)
- Unit: none
- Value range: 0 ... 1

Feret Vertical to Maximum



The ratio between the feret distance at 0° and the Feret Maximum

- Unit: none

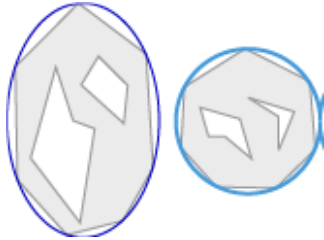
Fiber Length

Length of a fiber-like region

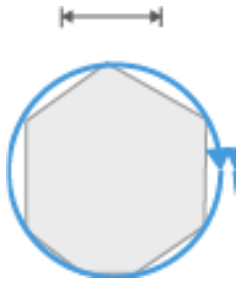
To calculate the fiber length, a structure that is actually similar to a fiber is required. Here it is not the distance between a start and end point that is determined. The check can be done using the Form circle, among other things.

- Formula: $\frac{1}{4} \times (\text{Perimeter} + (\text{Sqrt}(\text{Perimeter}^2 - 16 \times \text{Area})))$
- Unit: Unit of the scaling assigned to the image (e.g. μm)

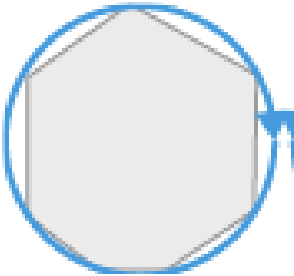




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| Fiber Length Unscaled | <div>Length of a fiber-like region</div> <div>To calculate the fiber length, a structure that is actually similar to a fiber is required. Here it is not the distance between a start and end point that is determined. The check can be done using the Form circle, among other things.</div> <div><ul style="list-style-type: none">Formula: $\frac{1}{4} \times (\text{Perimeter1} + (\text{Sqrt}(\text{Perimeter1}^2 - 16 \times \text{Area})))$Unit: pixels</div> |
| Form Circle | <div>Form factor of a region</div> <div></div> <div>Describes the form of a region on the basis of its circularity. A perfect circle is given the value 1. The more elongated the region is, the smaller the form factor. The calculation is based on the Area filled and Perimeter Crofton parameters.</div> <div><ul style="list-style-type: none">Formula: $4 \times \pi \times \text{Area} / \text{PerimeterCrofton}^2$Unit: noneValue range: 0...1</div> |
| ID | <div>Sequential ID of the object</div> |
| ID of the Parent | <div>Sequential ID of the object's parent</div> |
| Image Index Block | <div>Index for the individual image slices of a multi-block image. Multi-block images are composed of blocks of different dimensions.</div> |
| Image Index Position | <div>Index for the individual image slices of a Position List image. If the image was acquired using the Position List tool, the Image Index Position returns the number of the image that contains the object.</div> <div>In contrast, the ID parameter is a global counter, i.e. the number of the object.</div> <div>The Index Position can be used to guarantee unambiguous assignment between measured regions in the image and the individual lines of a data table, especially in cases where several images are measured, as automatic assignment is then no longer possible. To achieve this, the Index Region parameter must also be selected as a region feature and also be inserted into the graphics plane as an annotation (Draw Features). It is also advisable to activate the Image name parameter as a region feature so that the correct original image can be reloaded.</div> <div><ul style="list-style-type: none">Restriction: This value is only available for images that have previously been acquired with AxioVision or saved in AxioVision ZVI format.</div> |



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| Image Index Scene | Index for the individual image slices of a scene image |
| | Indicates the unambiguous number of the scene in an image. The word Scene refers to a coherent object on a slide that contains several objects for examination. The Index Scene can be used to guarantee unambiguous assignment between measured regions in the image and the individual lines of a data table, especially in cases where several images are measured, as automatic assignment is then no longer possible. To achieve this, the Index Region parameter must also be selected as a region parameter and must also be inserted into the graphics plane as an annotation (Draw Features). It is also advisable to activate the Image name parameter as a region parameter so that the correct original image can be reloaded. |
| Image Index Time | Index for the individual image slices of a time series image. |
| Image Index Z | Index for the individual image slices of a Z stack image. |
| Index | |
| Intensity Maximum of channel 'C1' | The pixel value of the brightest pixel in the object. |
| Intensity Mean Value of channel 'C1' | The average brightness (pixel value) of the pixels in the object. |
| Intensity Minimum of channel 'C1' | The pixel value of the darkest pixel in the object. |
| Intensity Range of channel 'C1' | The difference between the pixel value of the brightest and darkest pixels in the object, i.e. Intensity Maximum of channel 'C1'-Intensity Minimum of channel 'C1' |
| Intensity Standard Deviation of channel 'C1' | The standard deviation of the brightness (pixel value) of the pixels in the object. |
| Number of the region holes | Calculates the number of holes enclosed by the object. |
| Perimeter | <div>  </div> <p>Perimeter of a region</p> <p>This parameter is specially optimized for measuring the perimeters of circles. If the measured region contains holes, the total perimeter including the perimeters of the hole structures is determined. If you only want the perimeter of the outside contour to be determined, use the Perimeter filled parameter.</p> <ul style="list-style-type: none"> ■ Unit: Unit of the scaling assigned to the image (e.g. μm) |



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| Perimeter Unscaled |  | <p>Perimeter of a region</p> <p>This parameter is specially optimized for measuring the perimeters of circles. If the measured region contains holes, the total perimeter including the perimeters of the hole structures is determined. If you only want the perimeter of the outside contour to be determined, use the Perimeter filled parameter.</p> <ul style="list-style-type: none"> Unit: pixels |
| Radius |  | <p>Radius of a circle with an area equal to that of the object</p> <p>The object is measured using the Area) parameter. A circle with the same area as the object is created. The radius of this circle is returned.</p> <ul style="list-style-type: none"> Formula: $\text{Sqrt}((1 / \pi) \times \text{Area})$ Unit: Unit of the scaling assigned to the image (e.g. μm) |
| Radius Unscaled |  | <p>Radius of a circle with an area equal to that of the object</p> <p>The object is measured using the Area) parameter. A circle with the same area as the object is created. The radius of this circle is returned.</p> <ul style="list-style-type: none"> Formula: $\text{Sqrt}((1 / \pi) \times \text{Area1})$ Unit: pixels |
| Region Class Color | | Returns the color of the class to which the object is assigned |
| Region Class ID | | Returns the ID of the class to which the object is assigned |
| Region Class Name | | Returns the name of the class to which the object is assigned |
| Roundness | | Formula: $4 \times \text{Area}$ |

