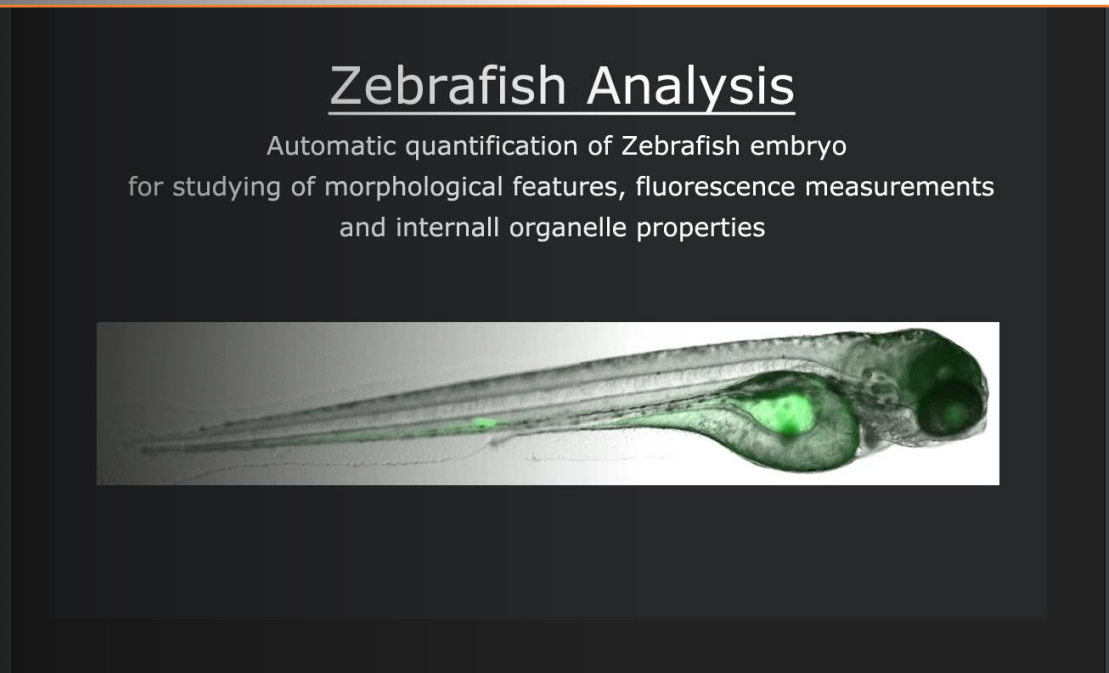
Loading your own images into Athena Zebrafish software

IDEA Bio-Medical

Seeing Better

Please begin, first, with our quick-start guide to analyze the sample images included with the software download.

Access the quick-start guide HERE.



Preparing Your Images for Analysis in Athena

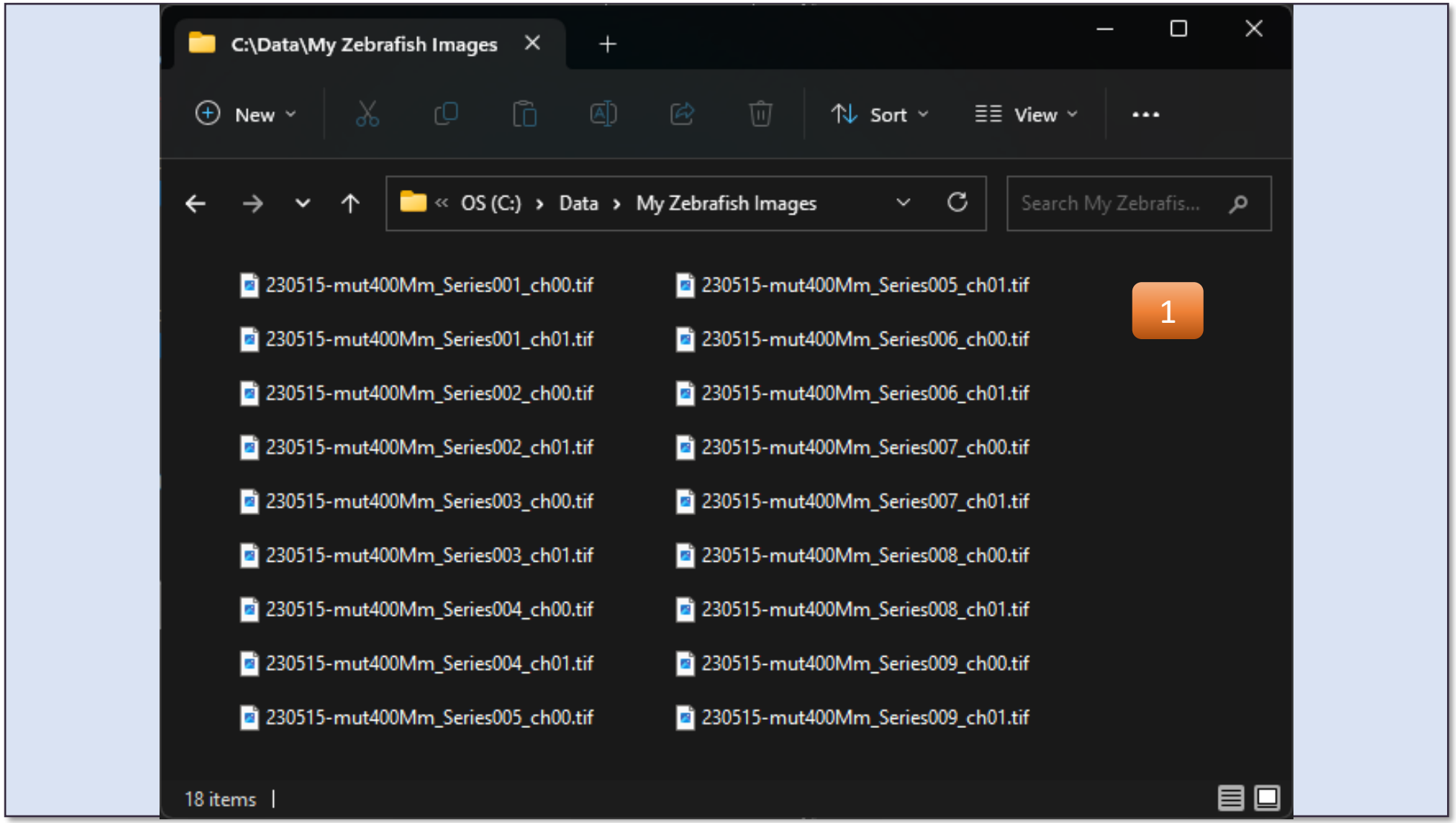
Two-channel tiff images example

1. In this example, standard .tif images are saved into a folder on the local hard drive; the path is displayed in the windows explorer.

   This dataset contains two color channels, each indicated in the filename with recognizable text:
   Brightfield = *ch01*
   Green fluor. = *ch00*

   Other text in the filename, as shown, will not affect loading the images into Athena.

   If images contain well-identifiers, such as A1, B2, C3, etc., then the images will be assigned to those wells.



Open Athena

Shortcut from desktop

2. Open Athena using the shortcut on your desktop.

   Sample images that can be analyzed for free are also accessible on the desktop from the ‘Samplefish’ folder shortcut.

Opening Athena

Selecting user & updating license

3. Select the default ‘Admin’ user, or other user profile, from the menu.

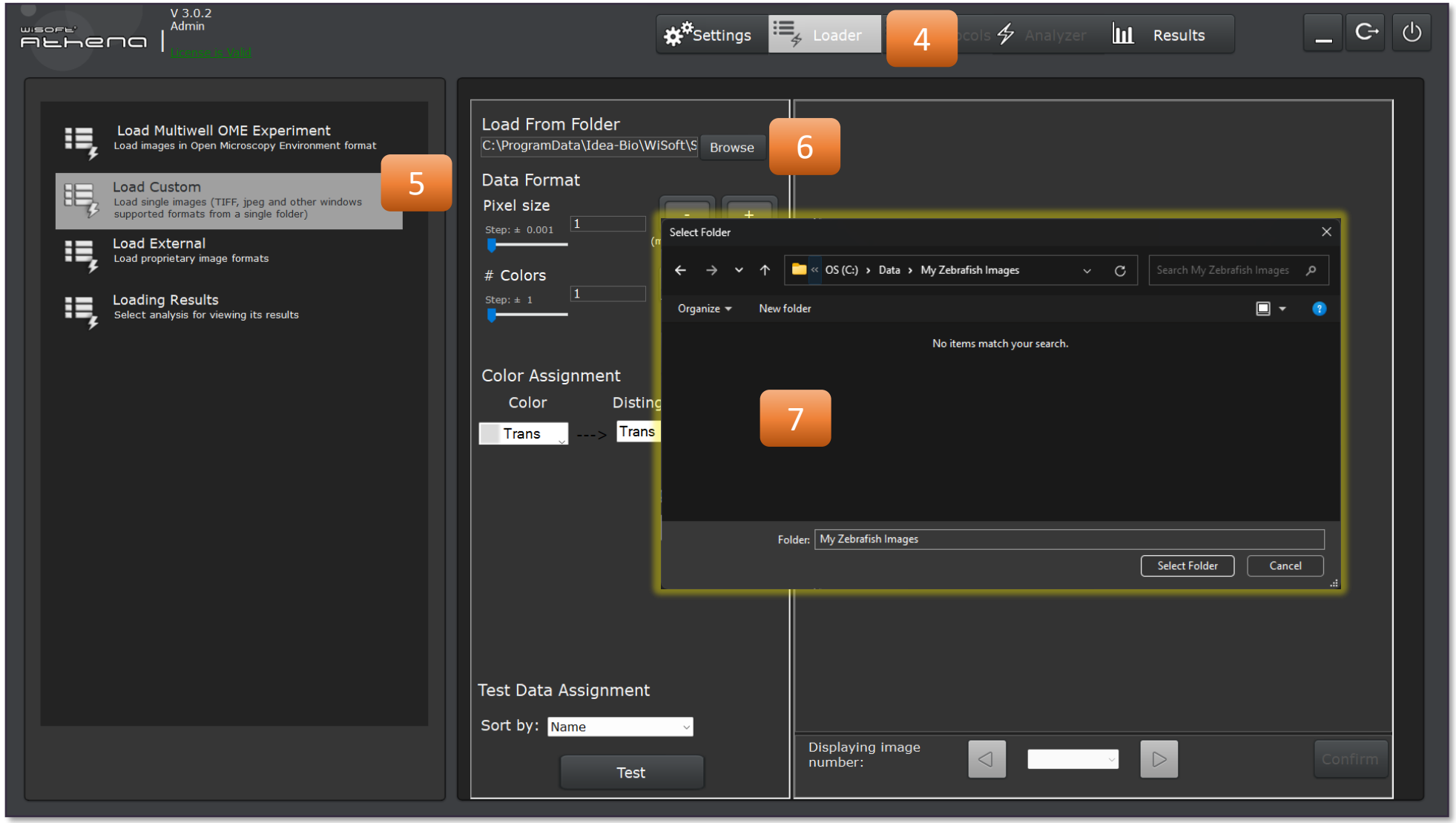
   If you need to insert a license code or coupon code, please see the quick-start guide by clicking **HERE**.

Using Athena with Your Own Images

Define where images are located

4. Select the ‘Loader’ tab to open images.
5. Select the ‘Load Custom’ option from the menu on the left.
6. Press the ‘Browse’ button to navigate to the **FOLDER** location where the tiff images you would like to load & analyze are located.
7. The file explorer window shows the currently selected folder.
   Navigate to your image storage location, then click the “Select Folder” button to choose this path.

Info:
The default folder path is pre-set to identify the freely analyzable sample images. Other parameters are also set to permit loading the sample images.



Using Athena with Your Own Images

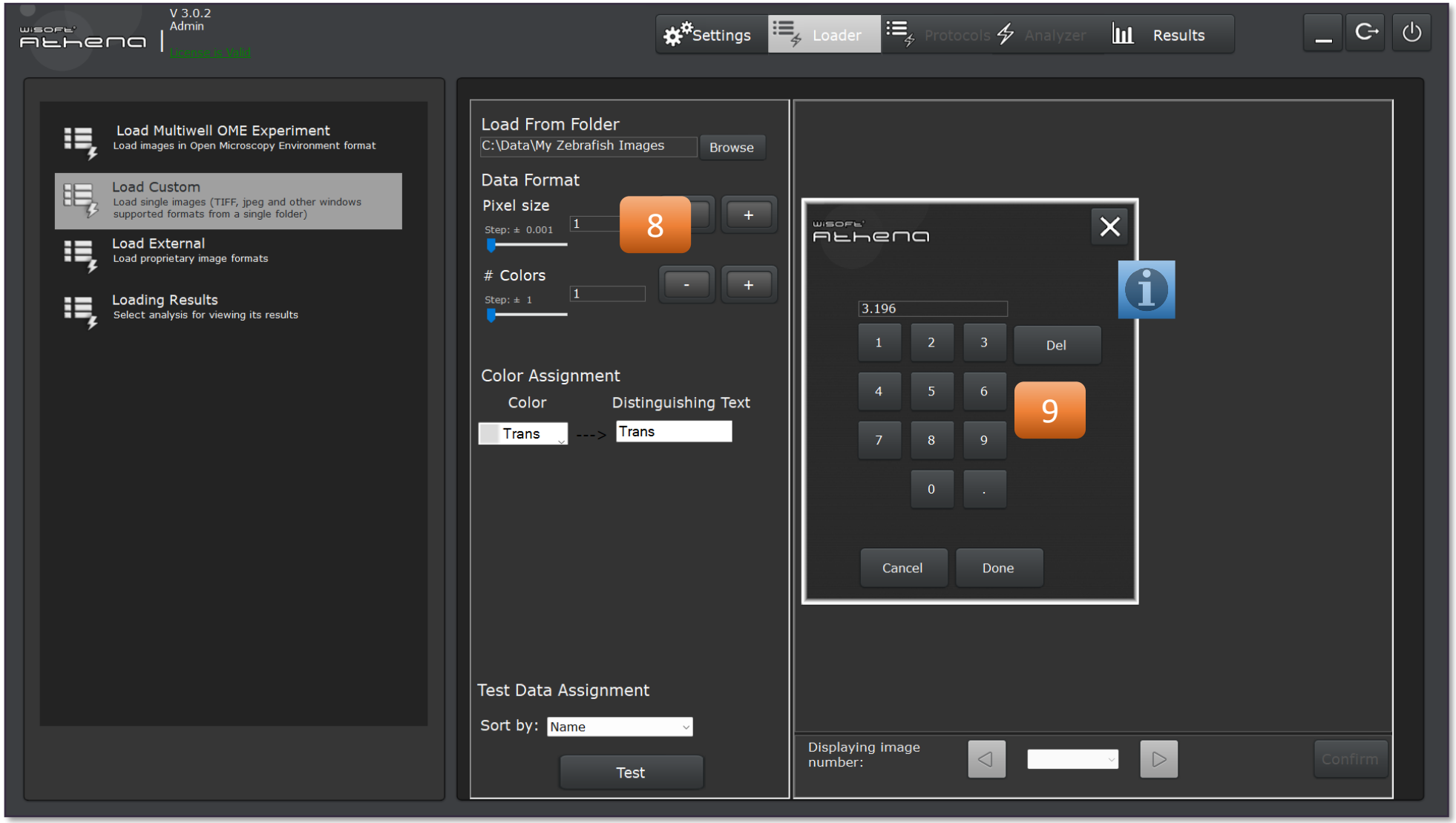
Define characteristics of your images

8. Define the correct pixel size for your images by clicking on the text field to open the number pad.
9. Use the numbers on screen or your keyboard to set the desired pixel size.

   Click ‘Done’ to set the pixel size.

   Using the default pixel size of 1 will return areas in dimensions of pixels, not microns.

Info:
Contact your microscope manufacturer if you have questions about how to find the pixel size for your images. In some cases, metadata such as pixel size may sometimes be saved in an associated .xml file, so can be found therein.

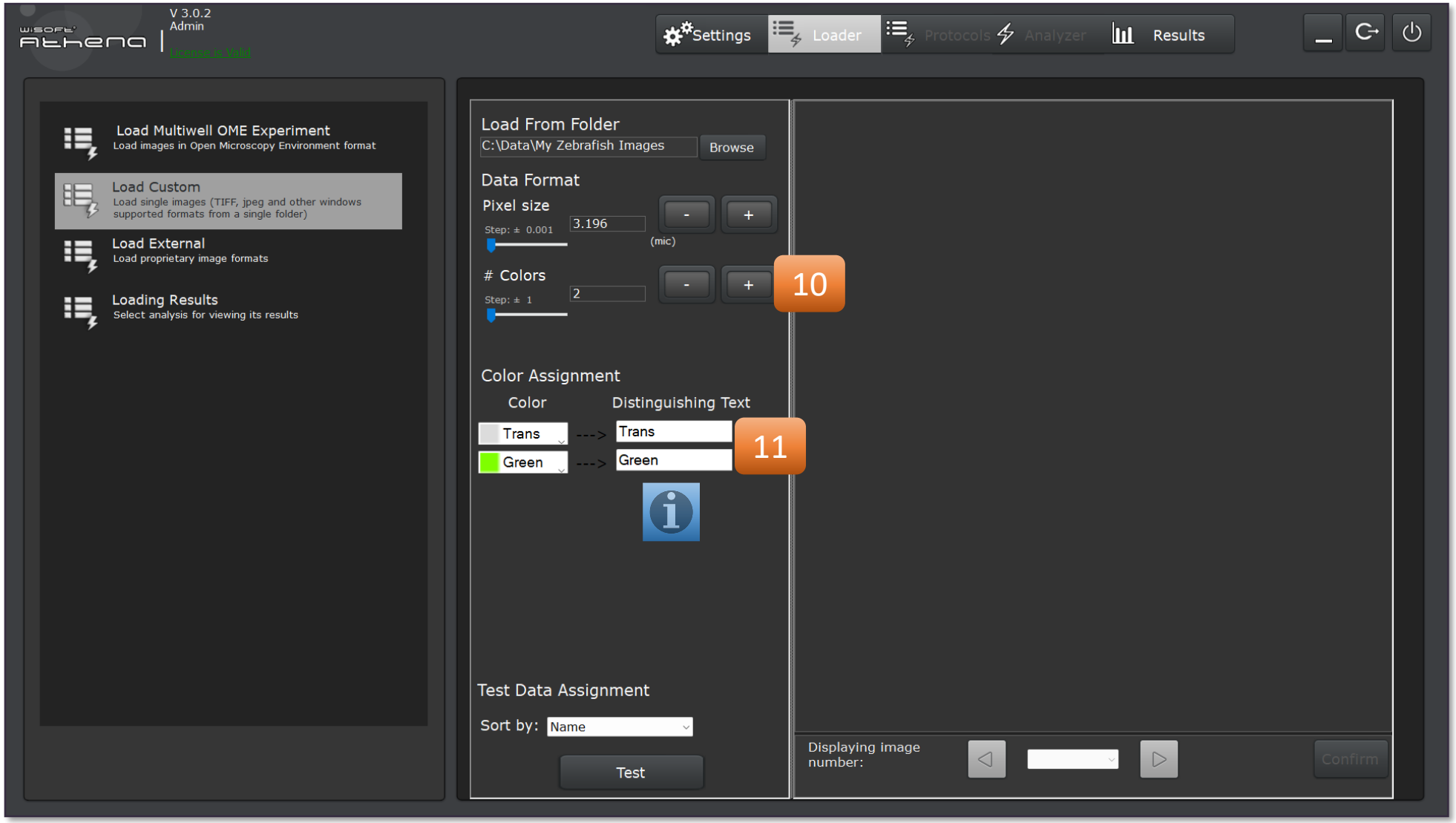


Using Athena with Your Own Images

Define characteristics of your images

10. Define the number of colors present in your dataset.
11. Define the channel that should be assigned to each of the colors present.

Info:
The “Distinguishing Text” editable fields are auto-filled with default values, as shown. These text strings are used to assign each image file in the selected data folder (steps 6 & 7) to one of the colors for analysis.

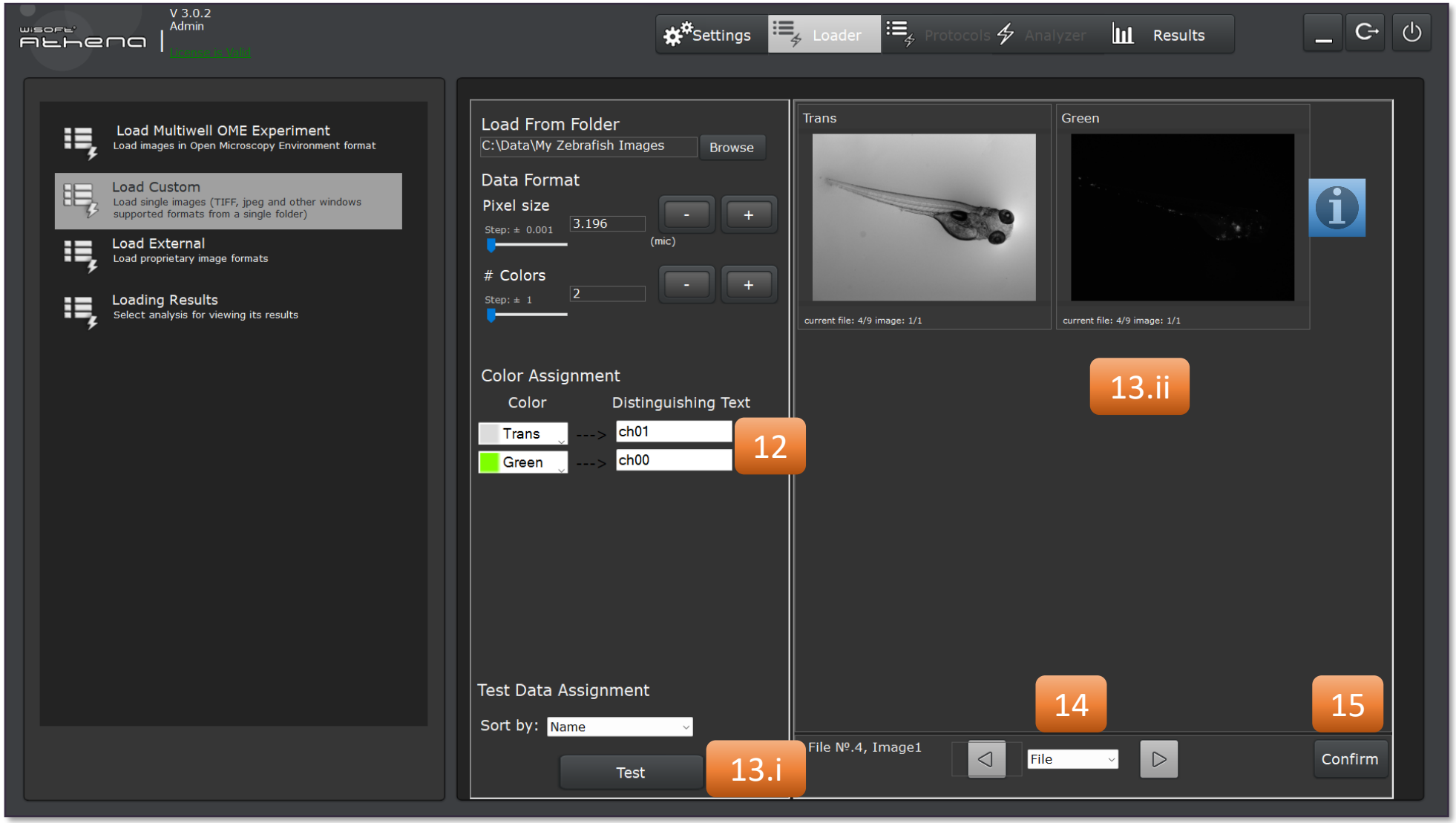


Using Athena with Your Own Images

Define characteristics of your images

12. Type the text strings identifying the images to be assigned to each color channel.
13. Click ‘Test’ (i) to load the images and visually confirm (ii) that the images are assigned to the color channels correctly.
14. Use the left & right arrows to move between each zebrafish image file. If the .tif images are multi-page, the menu will allow moving between pages.
15. Click ‘Confirm’ once you are finished.

Info:
The contrast settings are defined automatically and may not be optimized to visualize some fluorescence signal.



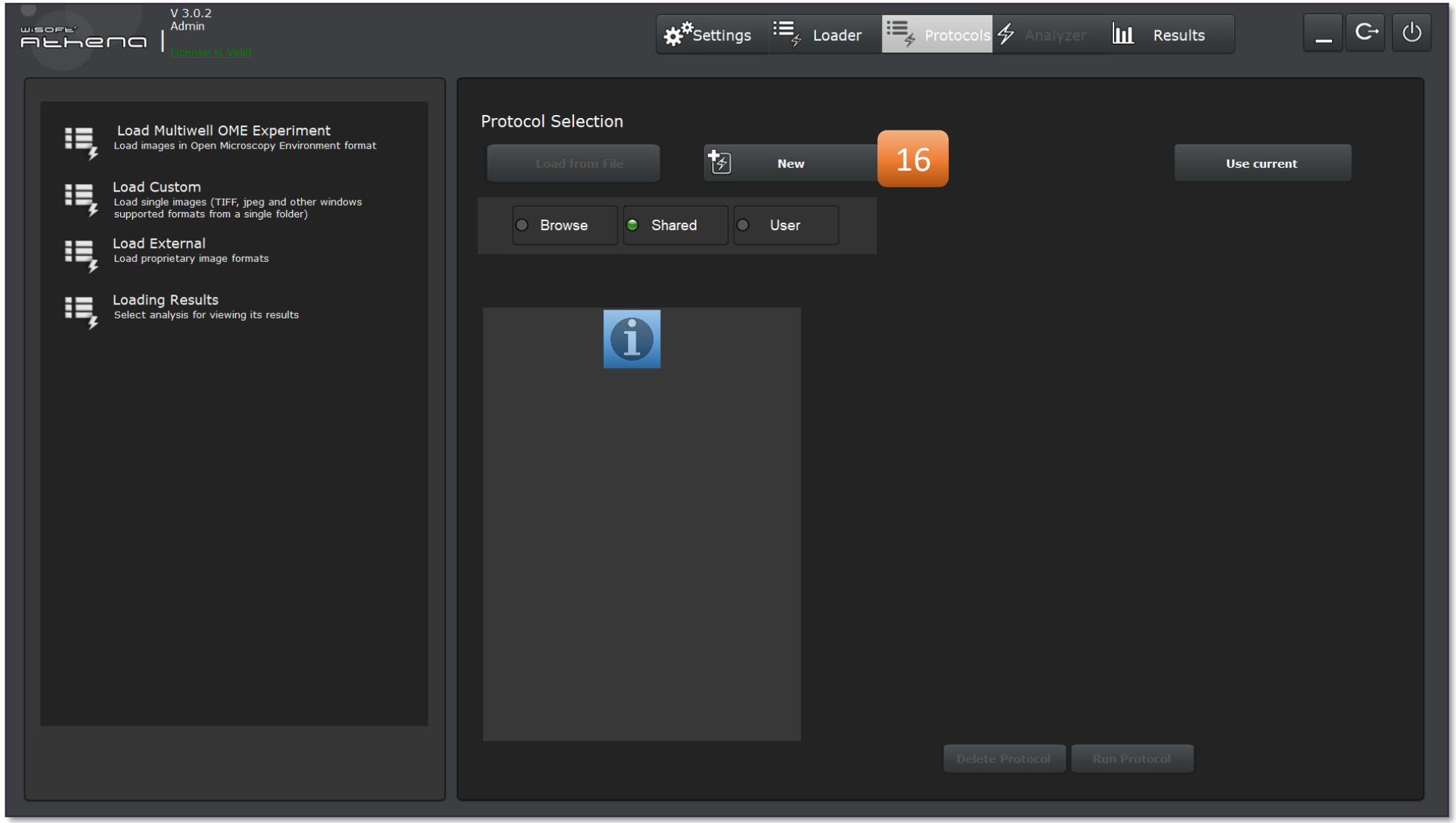
Using Athena with Your Own Images

Protocol menu

16. Click ‘New’ button to create a new image analysis protocol.

Info:
Analysis protocols allow for quick, reproducible analysis of images acquired in the same fashion for the same experiment.

They are saved as files that can be loaded from a ‘Shared’ folder available to all users, a ‘User’ folder accessible to the user selected in step (3), or can be selected from the hard disk using the ‘Browse’ option.



Using Athena with Your Own Images

Select zebrafish application

17. Select Anatomy to be identified in each color channel.
    Light-grey = selected
    Dark-grey = omitted
18. Click ‘Next’ to advance.

Info:
The “Other” structure is a flexible anatomical region, that can be manually outlined in brightfield channels or can be identified in a fluorescence channel.

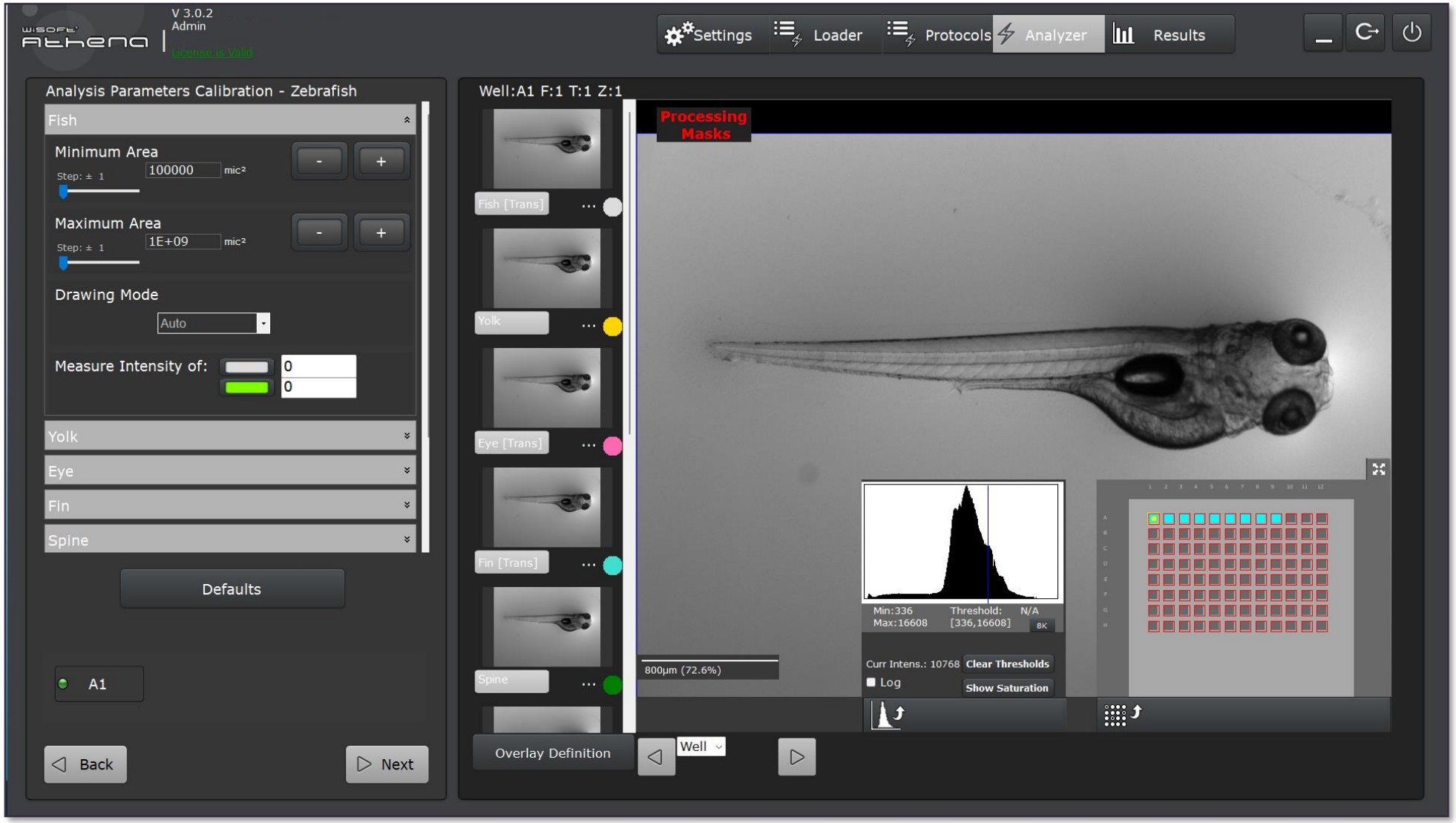
The “Granules” anatomy is intended to be for smaller, point-like structures, such as fluorescently labeled cells.

The “Other” structure can be larger structures, such as fluorescently labeled organs.

Using Athena with Your Own Images

Parameter Definition

Please be patient while the “Processing Masks” flashes, the software is working.

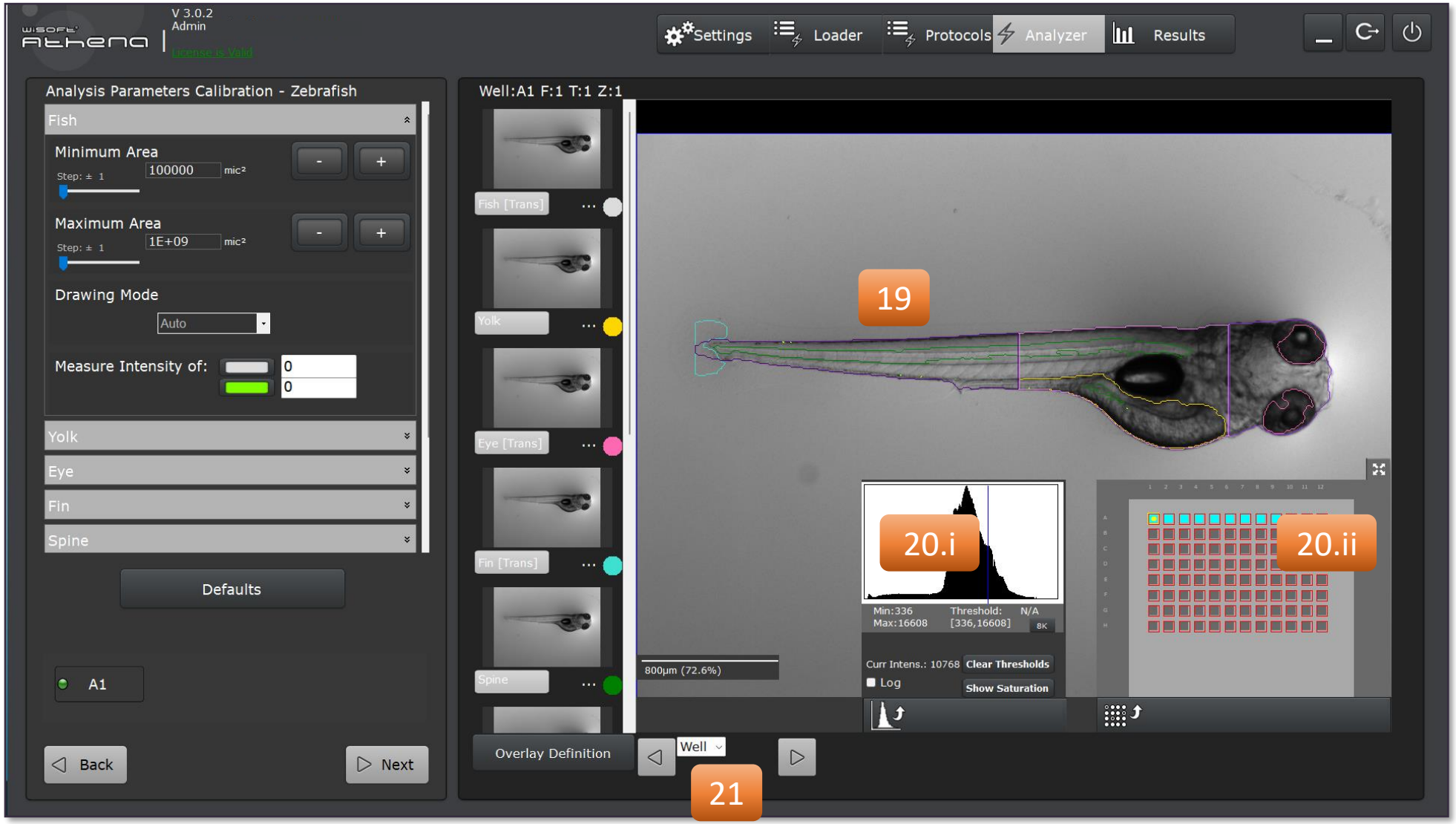


Using Athena with Your Own Images

Parameter Definition

Identified structures are outlined on the image

19. Anatomy detected in the image are displayed as masks, seen as outlines.
20. Intensity histogram (i) & navigation plate map (ii); minimize them with the arrow-buttons below them.
21. Scroll between different images, each assigned to one well, using the left & right arrow buttons. If T-stack or Z-stack data is loaded, you can also move between different time points or slices.



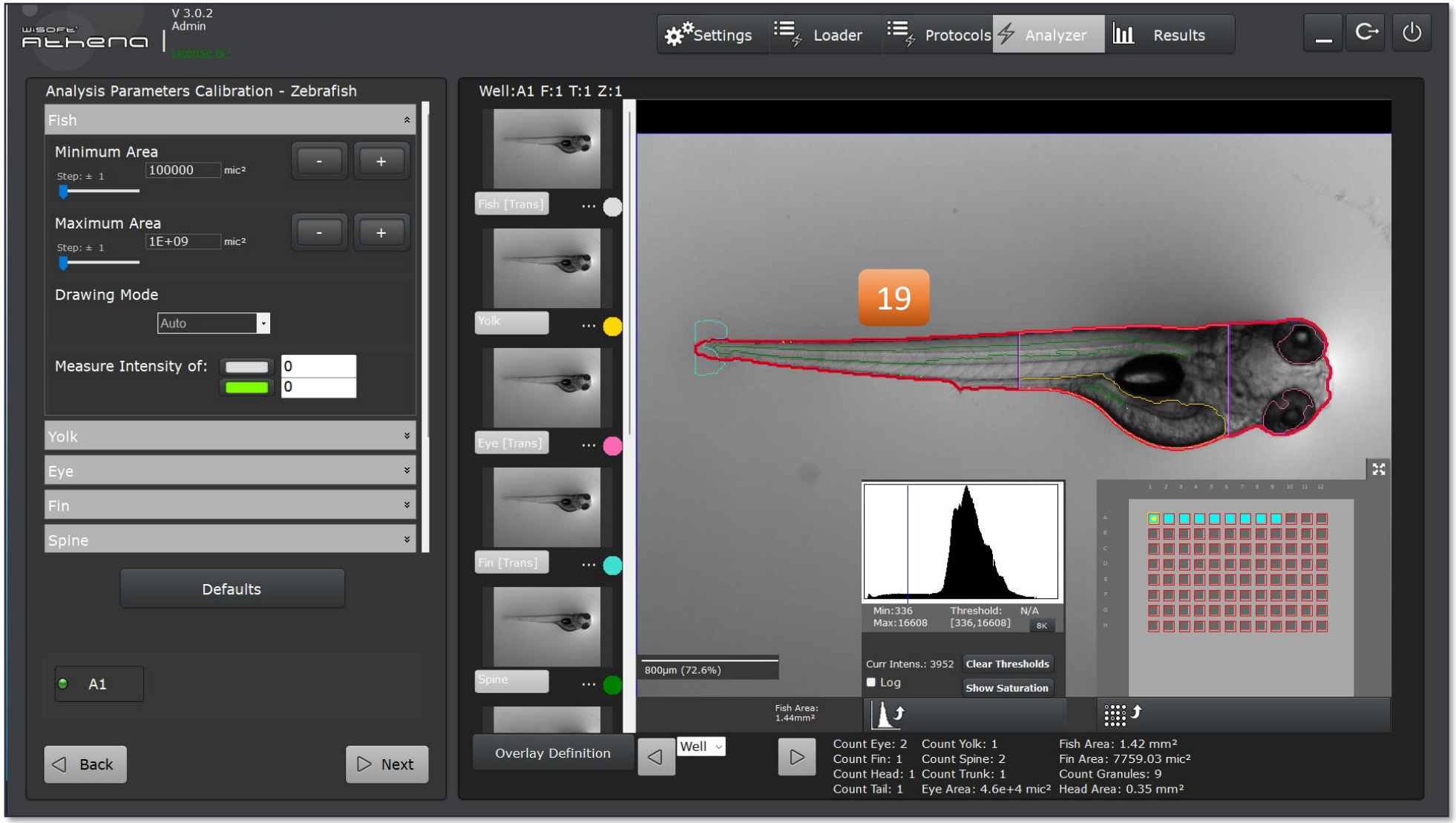
Using Athena with Your Own Images

License vs. Trial version

Identified structures are quantified

19. When highlighting a mask, as the fish here, it becomes selected.

    If you are using a **Licensed** version of the software, then quantitative data is made available and is displayed at the bottom of the screen.



Using Athena with Your Own Images

License vs. Trial version

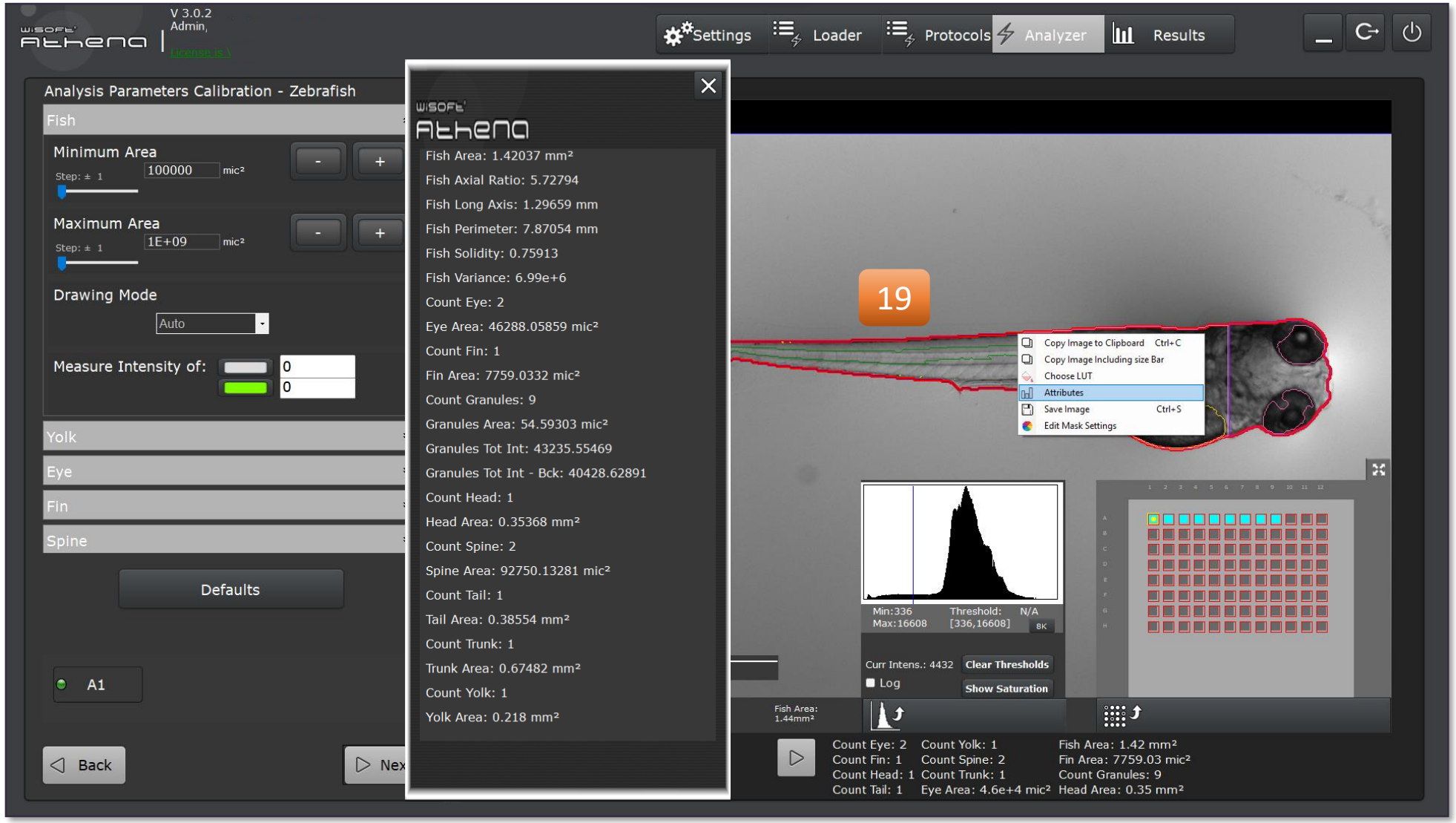
Identified structures are quantified

19. When highlighting a mask, as the fish here, it becomes selected.

    If you are using a **Licensed** version of the software, then quantitative data is made available and is displayed at the bottom of the screen.

    Quantitative data is also accessible via the ‘Attributes’ menu accessed by right-clicking on the image.

    More data is accessible on the final Results screen.



Using Athena with Your Own Images

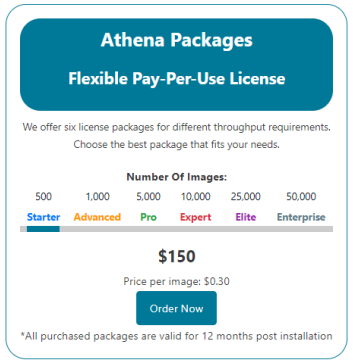
License vs. Trial version

Identified structures are quantified

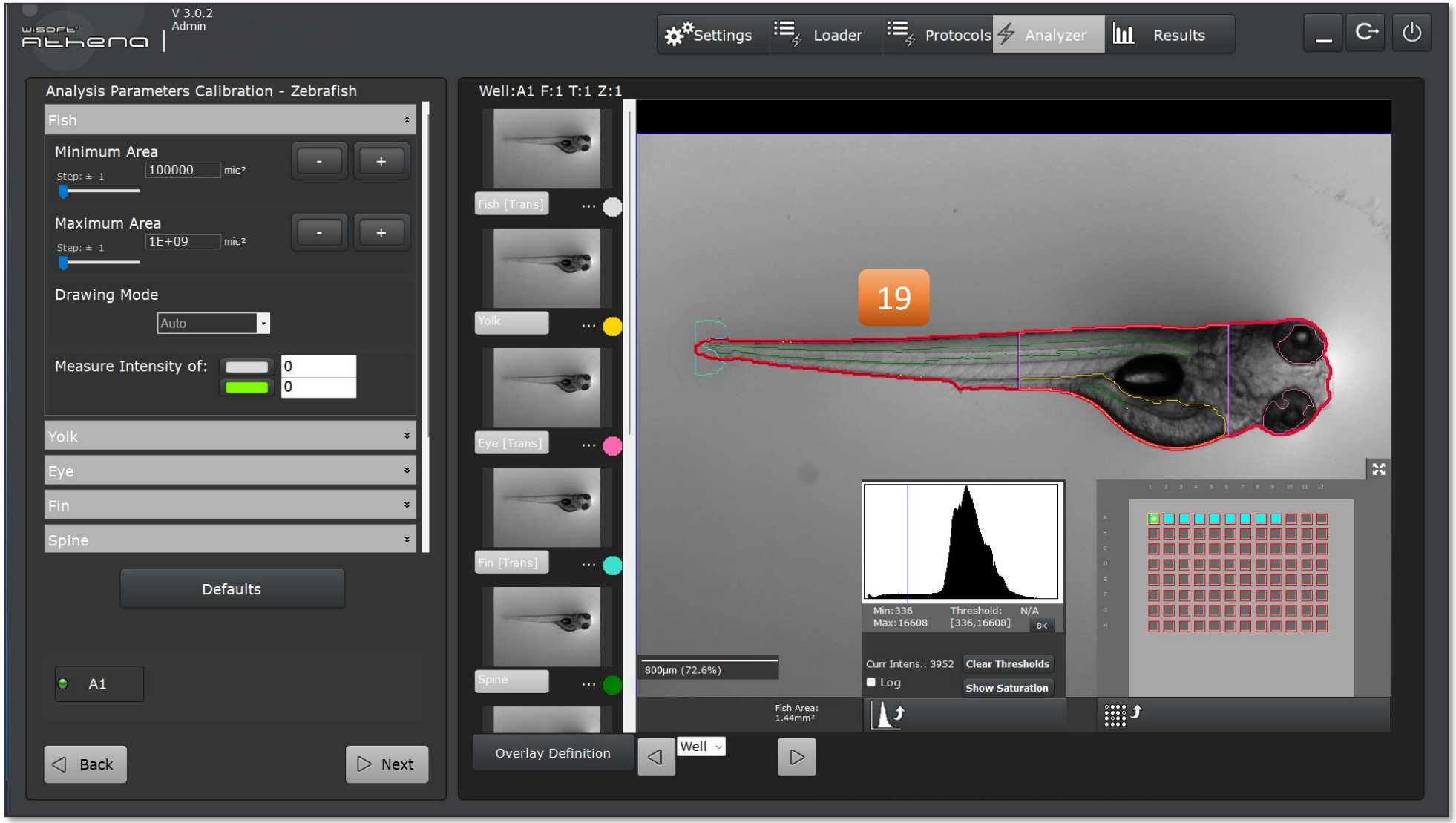
19. When highlighting a mask, as the fish here, it becomes selected.

    If you are using a **Trial** version of the software, then no quantitative data is made available.

    Email info@idea-bio.com to purchase a license package and get the data you need.



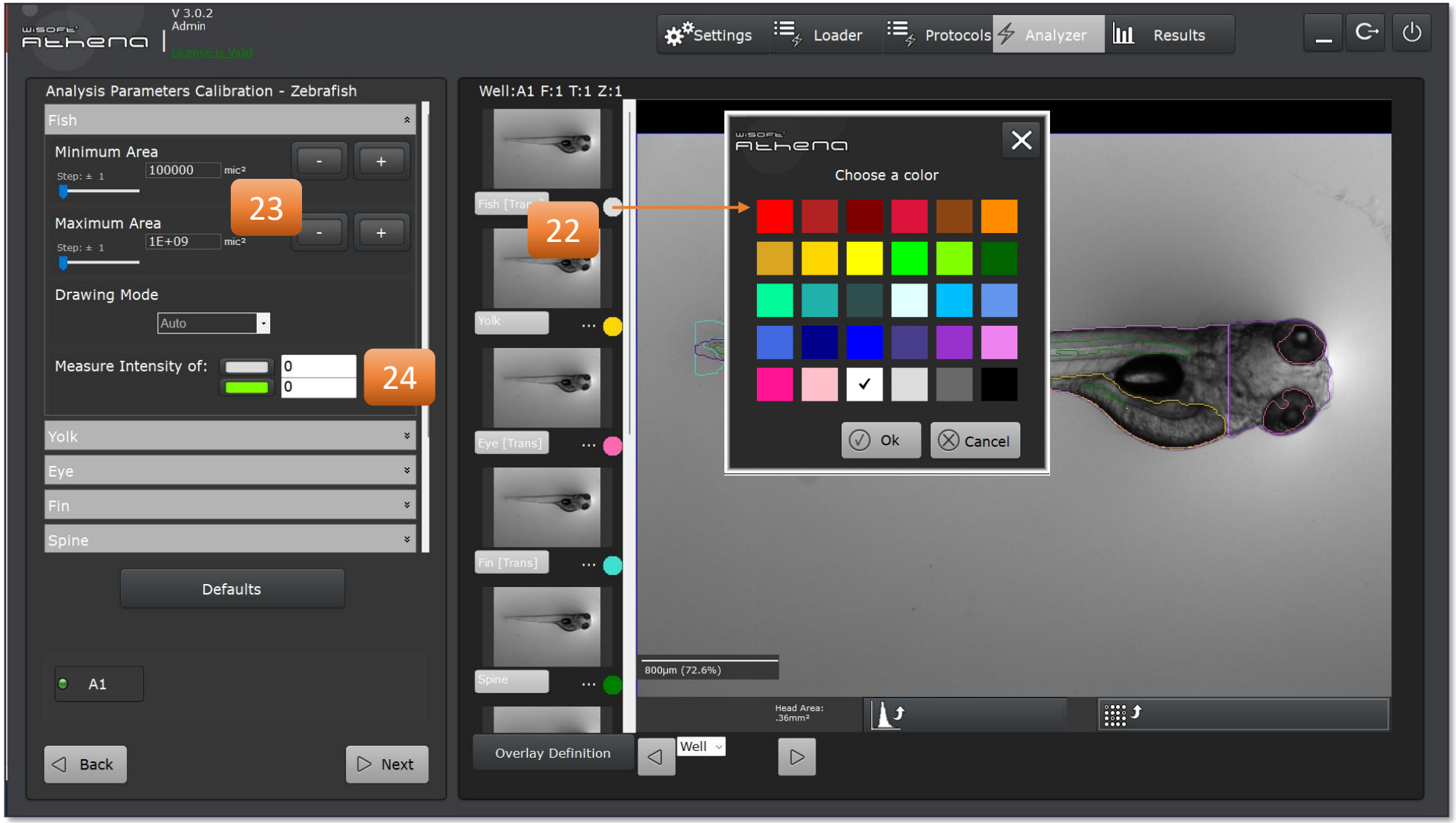
    Click above for more info.



Using Athena with Sample Images

Parameter Definition

22. Click the thumbnail for each anatomical structure to see the image it is associated with and access its analysis parameters.
    Click the name of each anatomical object below its thumbnails to toggle its mask visibility.
    Click on the colored dots to change the color of the outline.
23. Adjust the Min/Max area parameter to define what size of objects are permitted.
    (See point 19)
24. Define raw-intensity threshold values above which signal should be integrated and measured.
    See next page for continued definition…



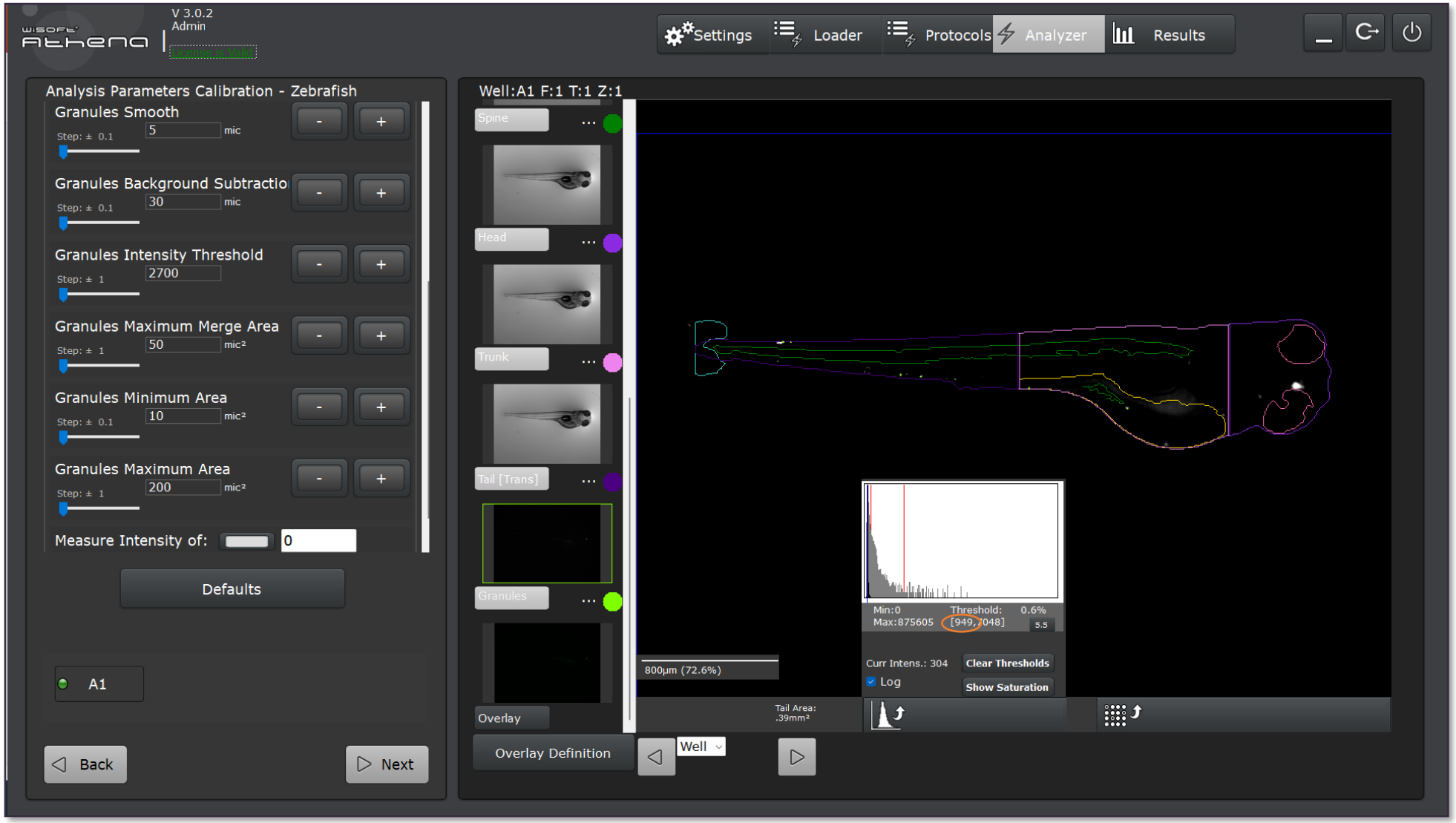
Using Athena with Sample Images

Signal Integration Within Structures

24. Using the pixel intensity histogram (20.i), drag left-to-right to define the visualized contrast levels.

    When all fluorescence objects of interest are visible in white or grey color, then the lower pixel intensity bound can be used for signal integration.

    In this example, the minimum value is 949 AU, highlighted with an orange circle.

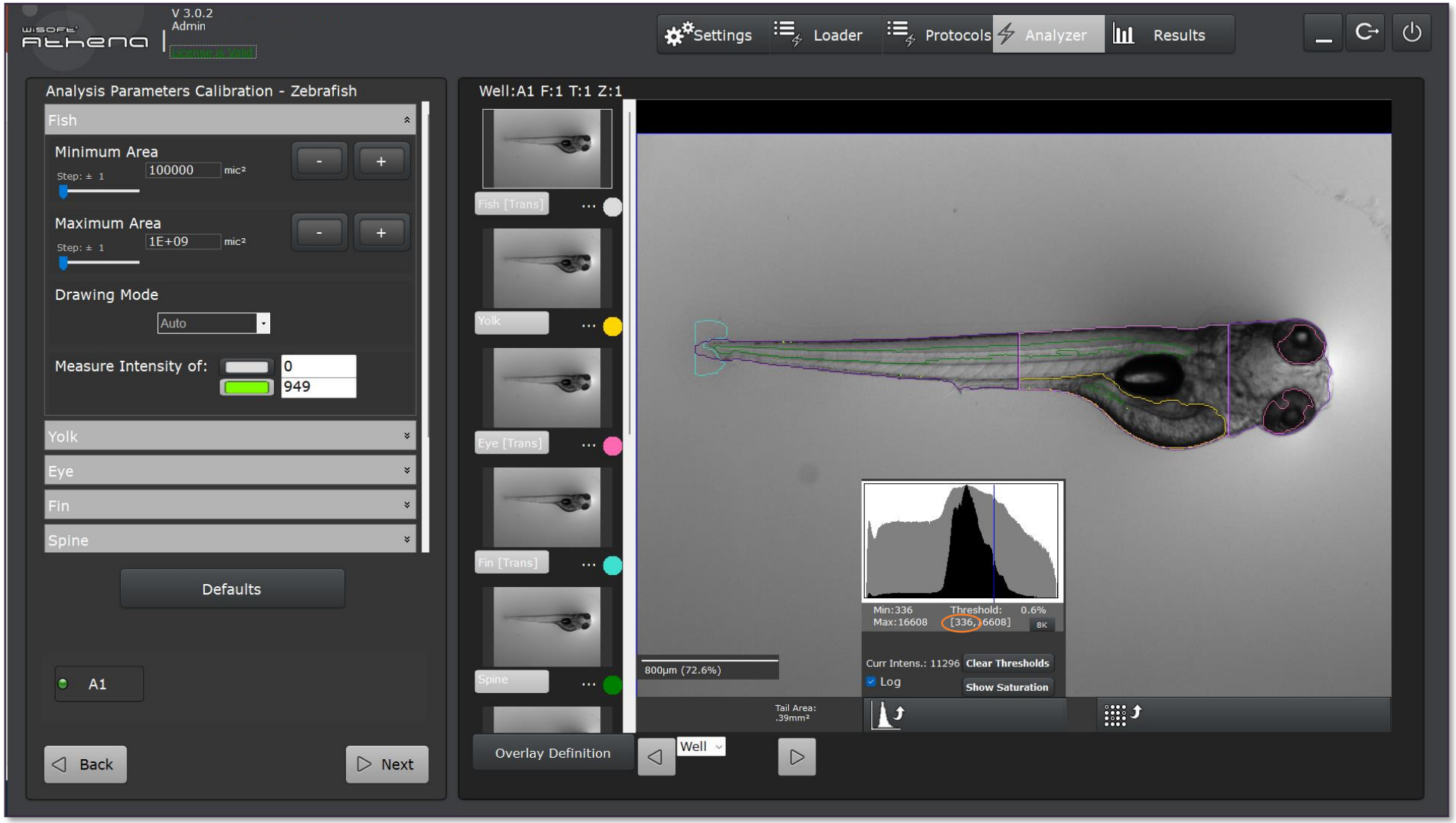


Using Athena with Sample Images

Signal Integration Within Structures

24. Insert this value into parameter (24) on the fish or other anatomy to integrate fluorescence intensity above this value

    The intensity integration can be included for all anatomical regions selected for analysis in the same fashion.



Using Athena with Sample Images

Identify Fluorescently-labeled Structures

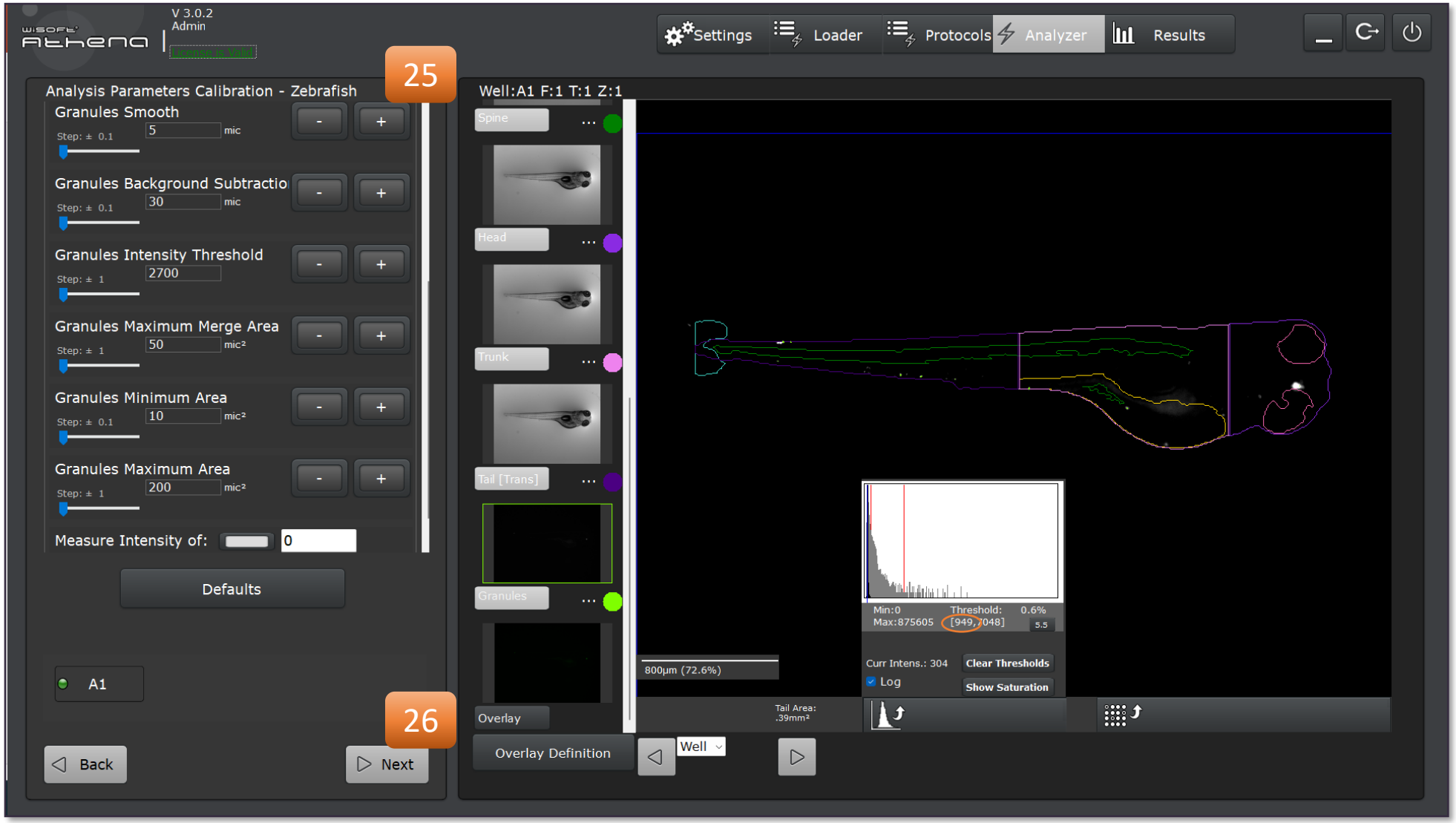
25. Use the 3 image analysis parameters and the 3 object selection parameters to identify fluorescent spots.

    <u>Image analysis</u>
    - Smooth
    - Background Subtraction
    - Thresholding

    <u>Object Selection</u>
    - Merge Area
      (joins touching objects)
    - Minimum Area
    - Maximum Area

26. Click ‘Next’ to advance.

Note:
Increasing the smooth or the background subtraction parameters tends to expand masks to detect larger objects.
Increasing the threshold parameter tends to shrink the masks and detecting smaller objects.



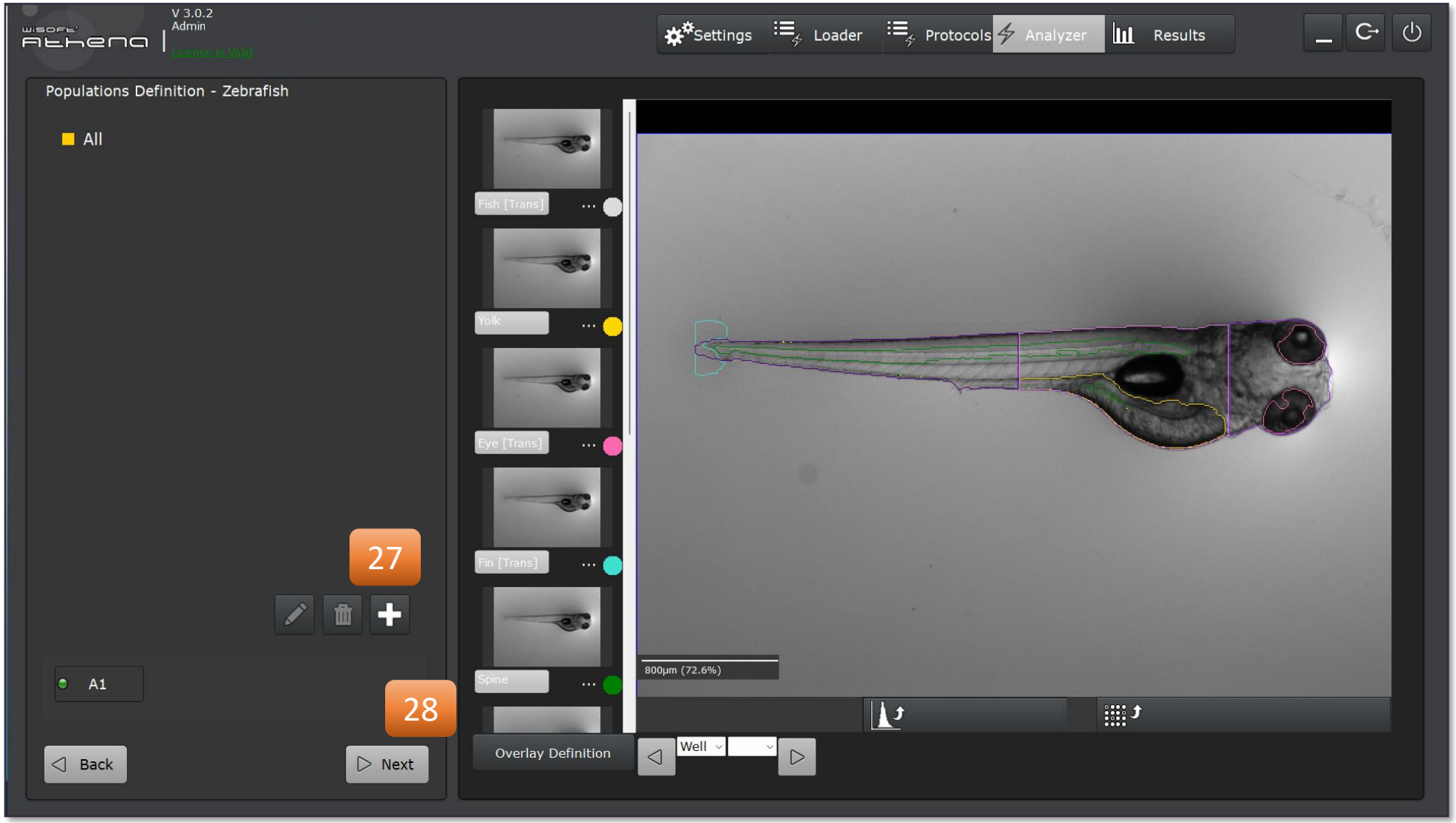
Using Athena with Sample Images

Identify Fluorescently-labeled Structures

27. Optionally, define populations to identify fish having desirable characteristics.

    Use of this feature is described in the Quick Reference Guide, so please refer to instructions there for clarification.

28. Click ‘Next’ to advance.



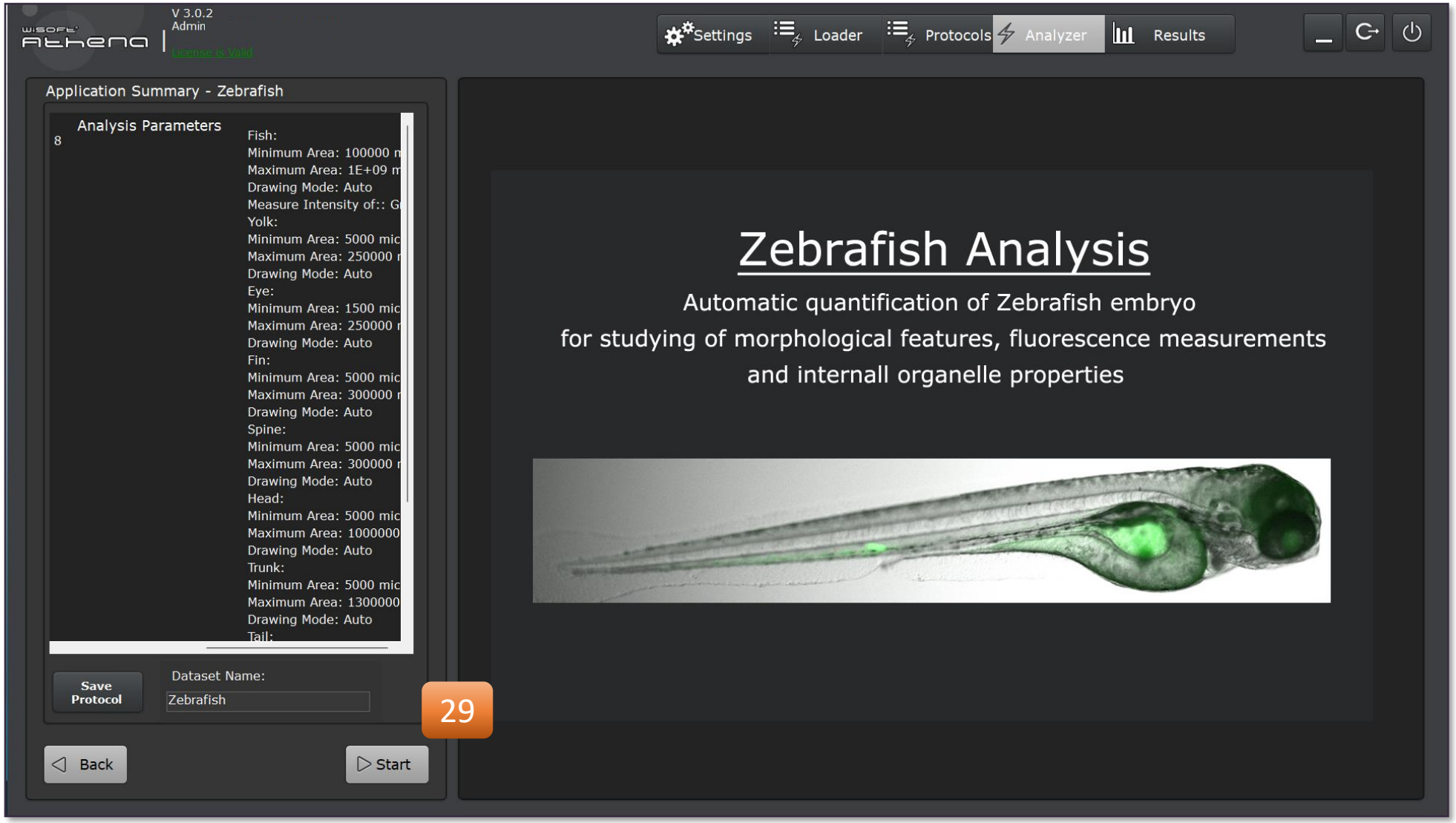
Using Athena with Sample Images

Identify Fluorescently-labeled Structures

29. The summary page allows for review of the analysis parameters defined on previous screens and setting the name of the analysis run.

    It also allows for saving of an analysis protocol to facilitate future analysis.

    Use of this feature is described in the Quick Reference Guide, so please refer to instructions there for clarification.



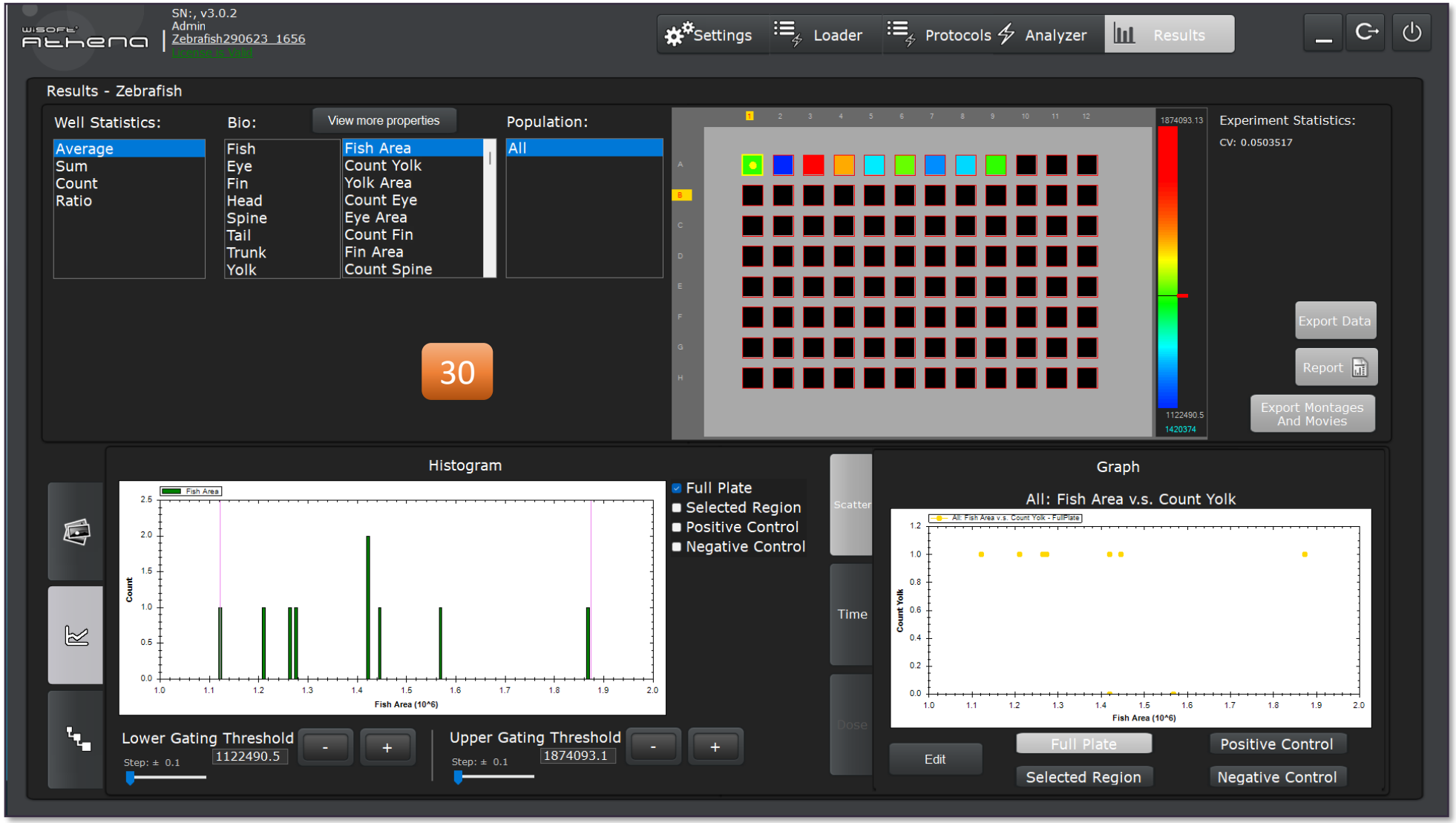
Using Athena with Sample Images

Identify Fluorescently-labeled Structures

30. The Results page allows for exploration of the data extracted from the zebrafish images (Licensed version only).

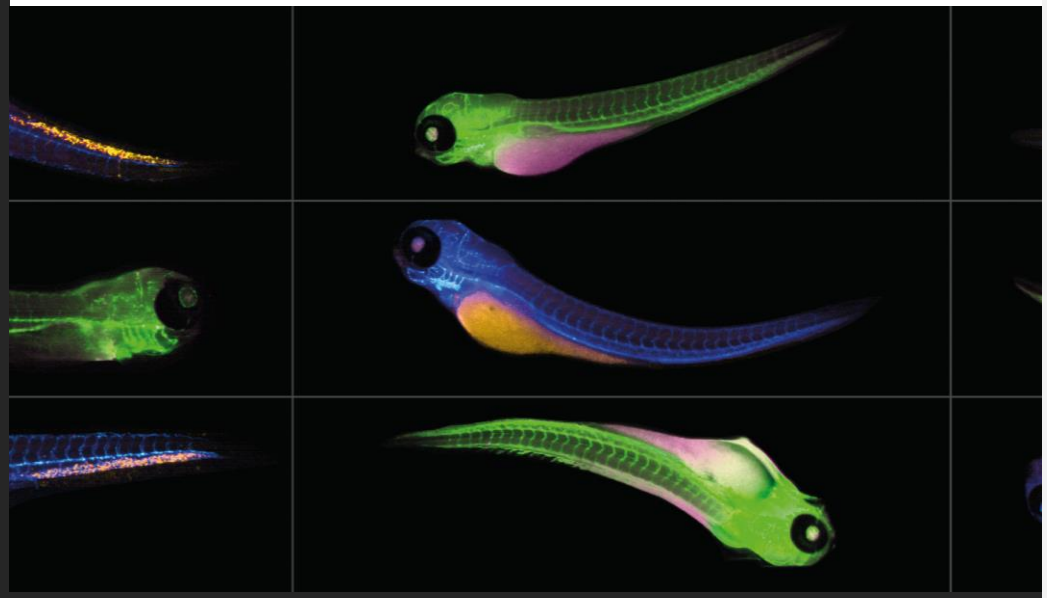
    It also permits review of the segmentation masks for each image analyzed.

    Use of this feature is described in the Quick Reference Guide, so please refer to instructions there for clarification.



IDEA Bio-Medical

Seeing Better



Need some on-boarding support to get started?

Just email us at info@idea-bio.com or fill up our contact form.

We'll be sure to contact you soon!