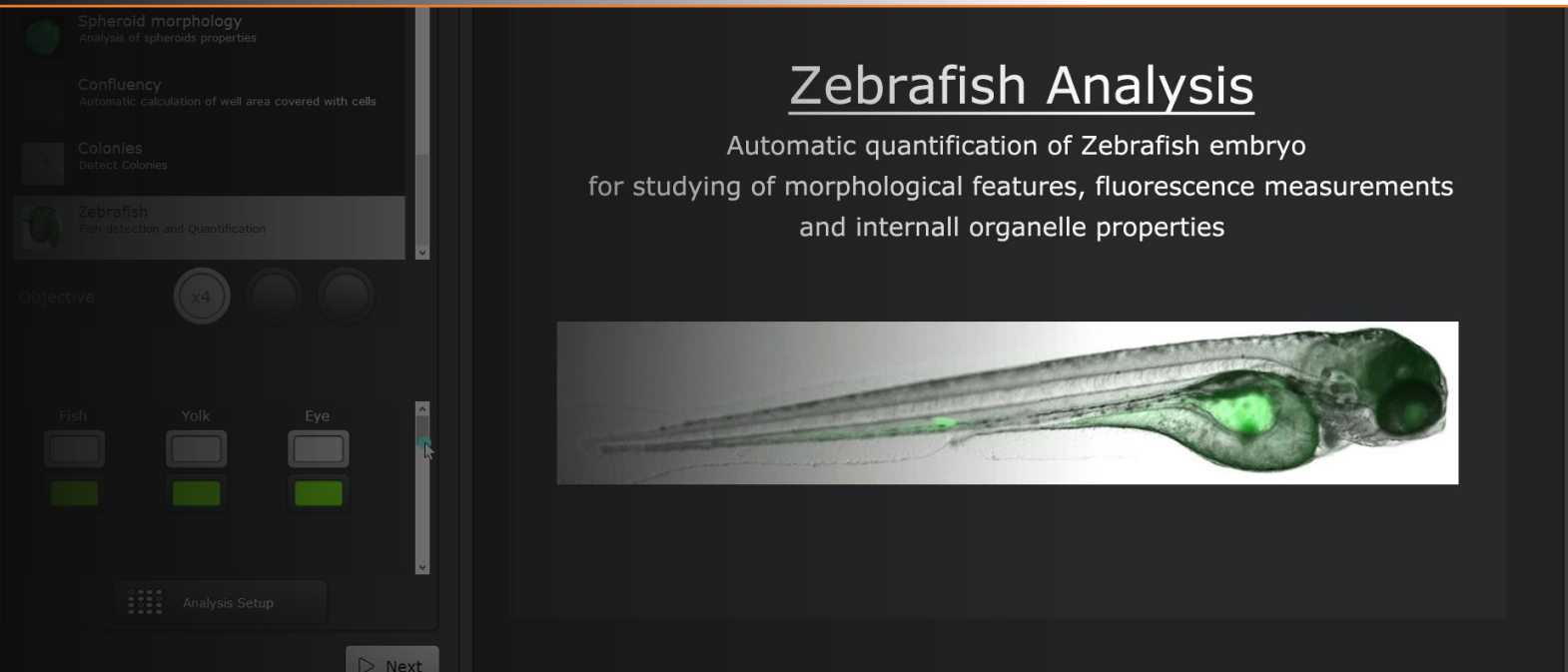
# Getting started with Athena Zebrafish software

IDEA Bio-Medical

Seeing Better



# Opening Athena

## *Shortcut from desktop*

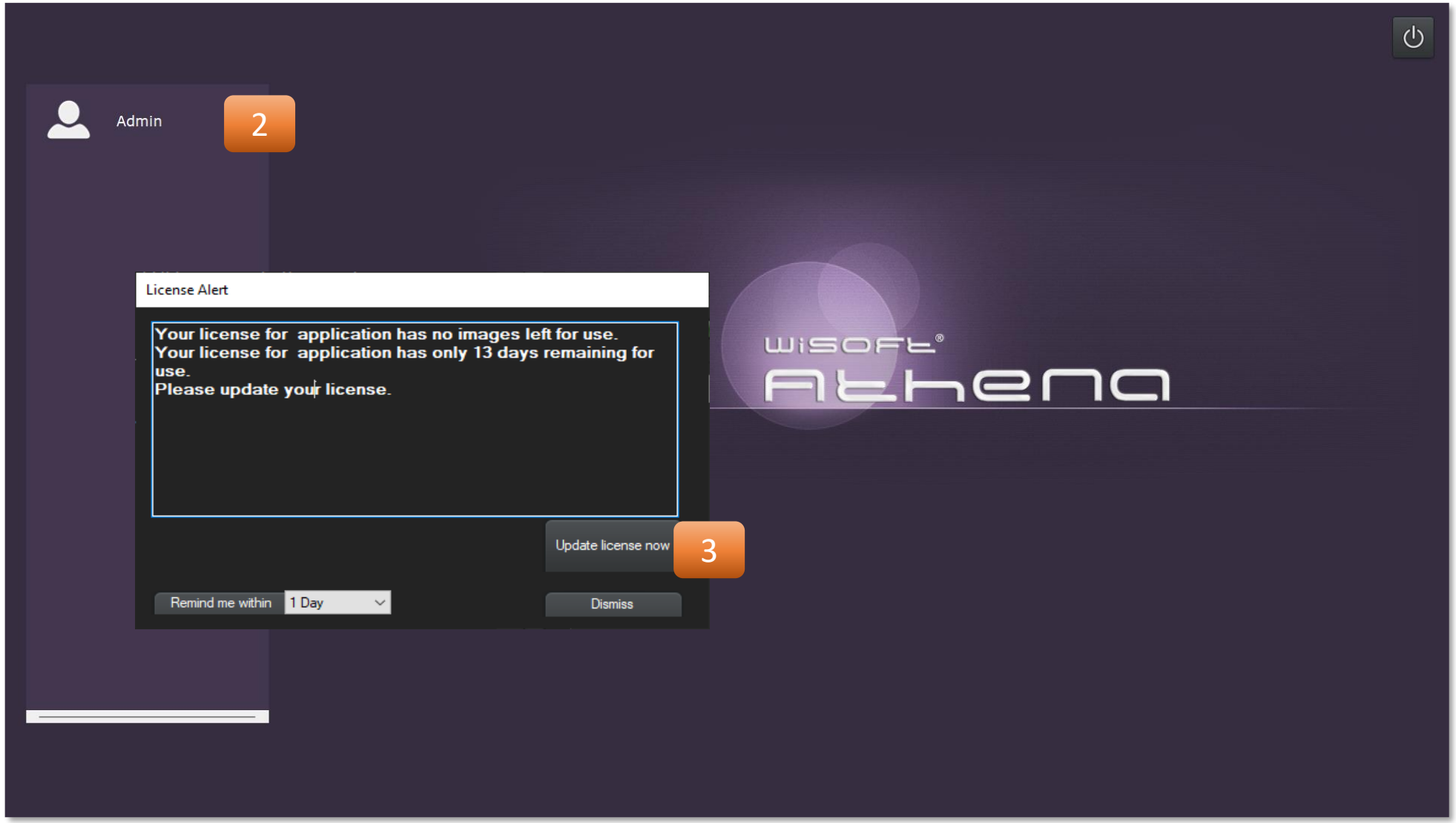
1. Open Athena using the shortcut on your desktop.

   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*

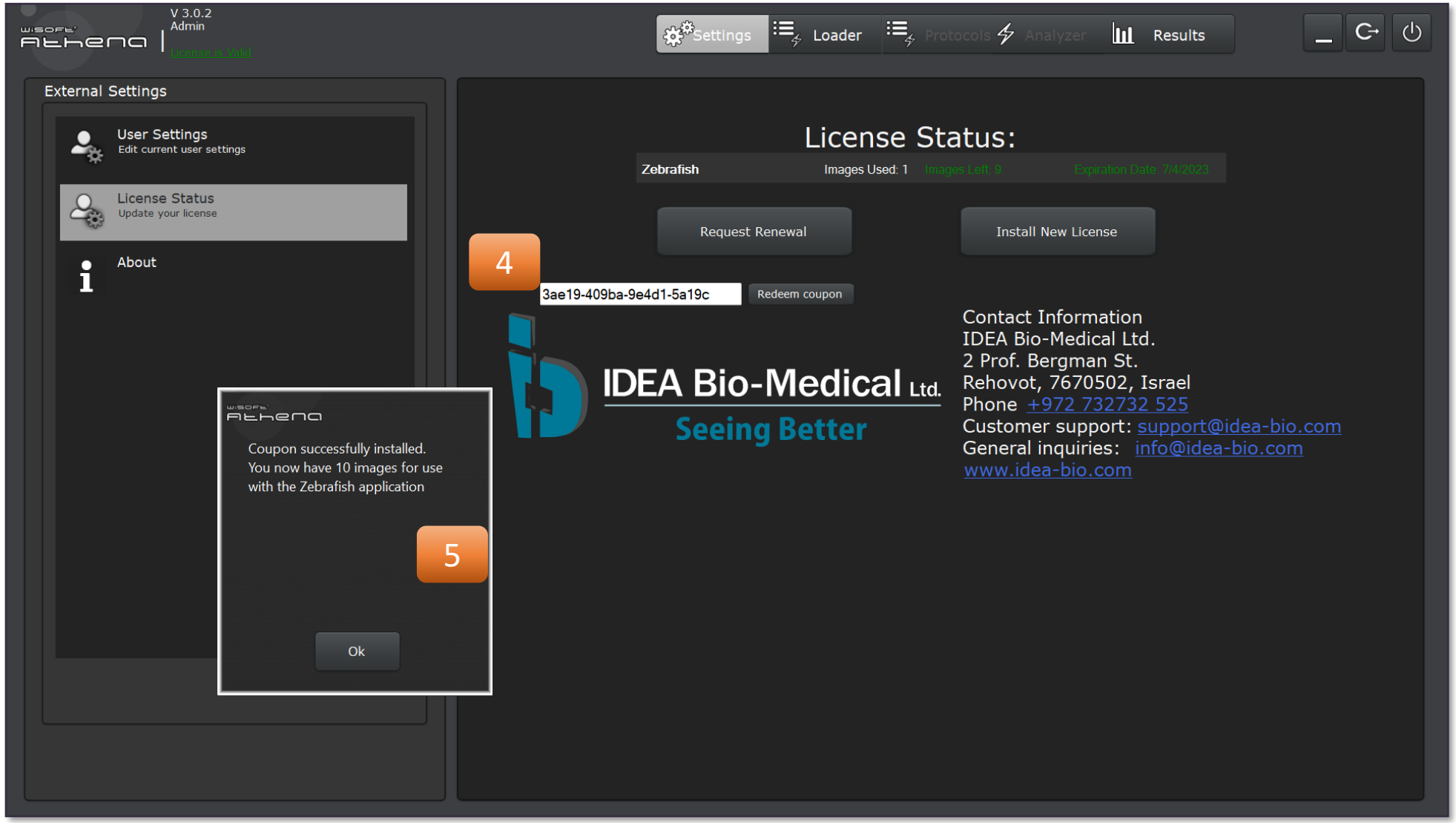
2. Select the default ‘Admin’ user from the menu.
3. A pop-up menu will appear.
   Click the “Update license now” button.



# Registering Athena

## *Using your coupon code*

4. Copy/paste the coupon code from your email into the empty, white box.
   Then, click “Redeem Coupon.”
5. The pop-up window will appear to confirm your coupon is accepted.
   You now have 10 analysis credits to use to analyze your own images. Click ‘Ok’ to close the window.
   First, analyze the free sample images.

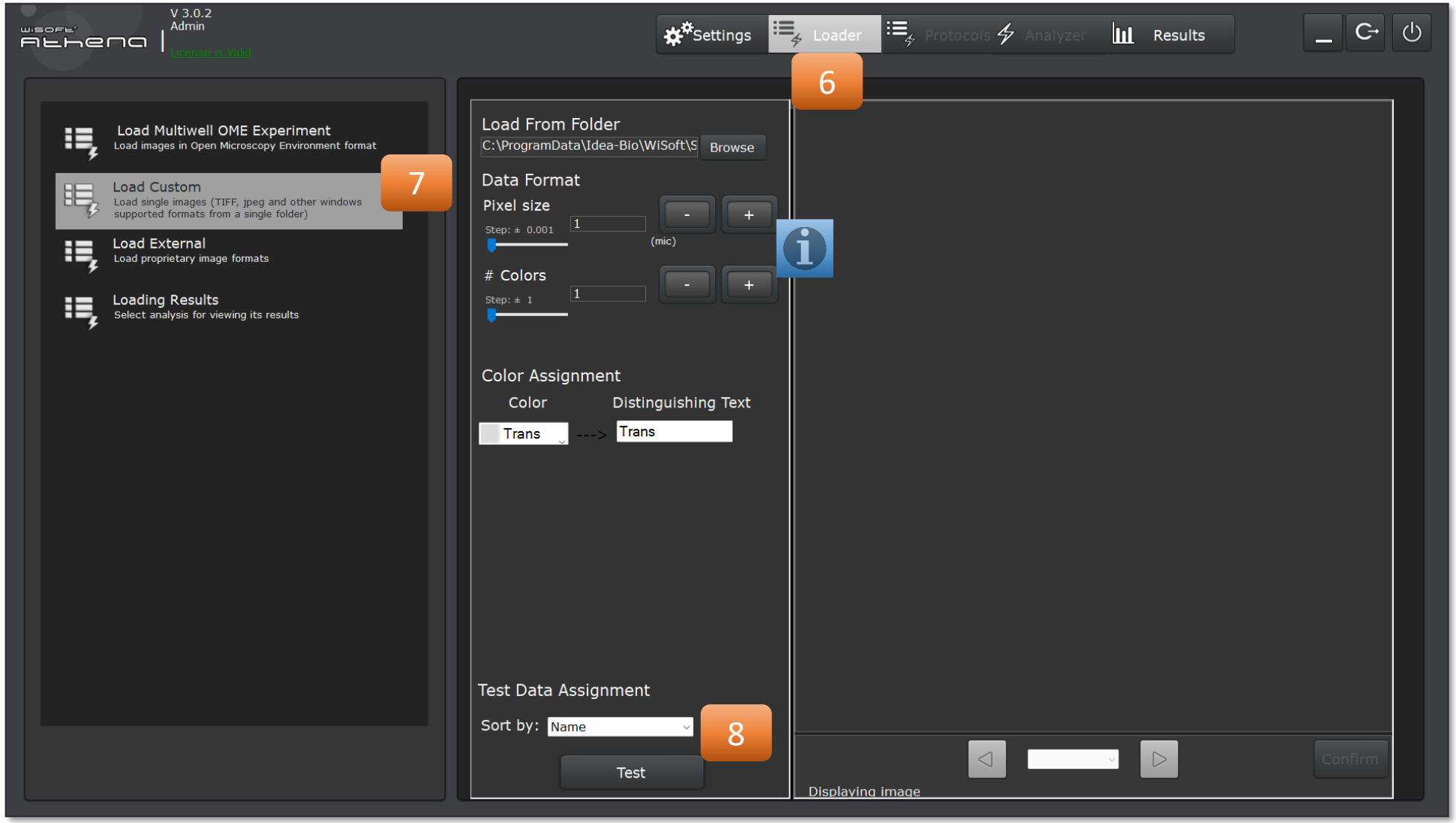


# Using Athena with Sample Images

## *Load the sample images*

6. Select the ‘Loader’ tab to open images.
7. Select the ‘Load Custom’ option from the menu on the left.
8. Press the ‘Test’ button to load sample images.

*Info:*
The folder path shown next to the browse button is pre-set to identify the freely analyzable sample images. Other parameters are also set to permit loading the sample images.

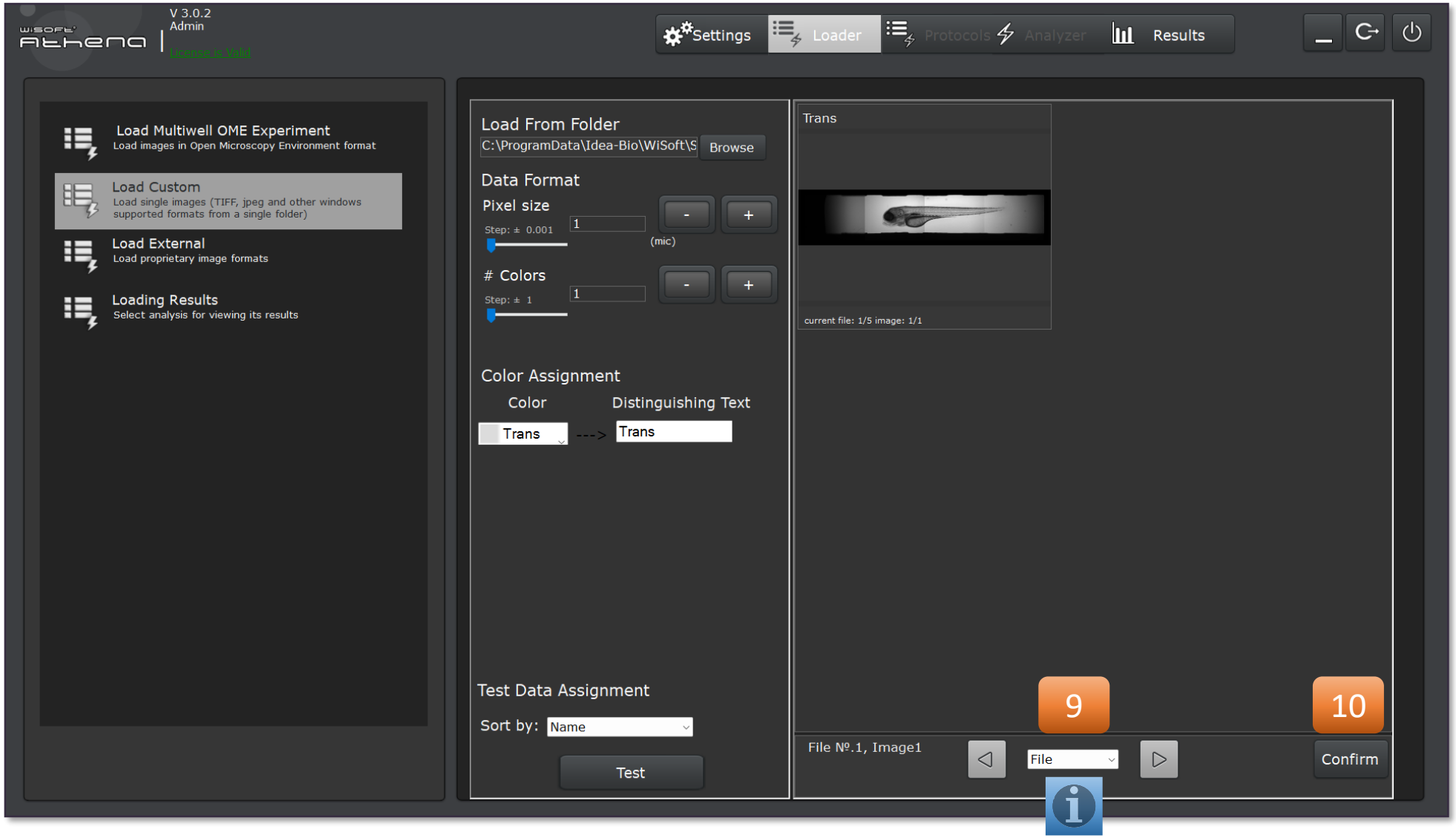


# Using Athena with Sample Images

## *Load the sample images*

9. Use the left & right arrow buttons to browse the sample images.
10. Click ‘Confirm’ when you are ready.

*Info:*
The menu in between the left & right buttons will permit moving through mutli-page .tif images, such as z-stack or time-lapse images.



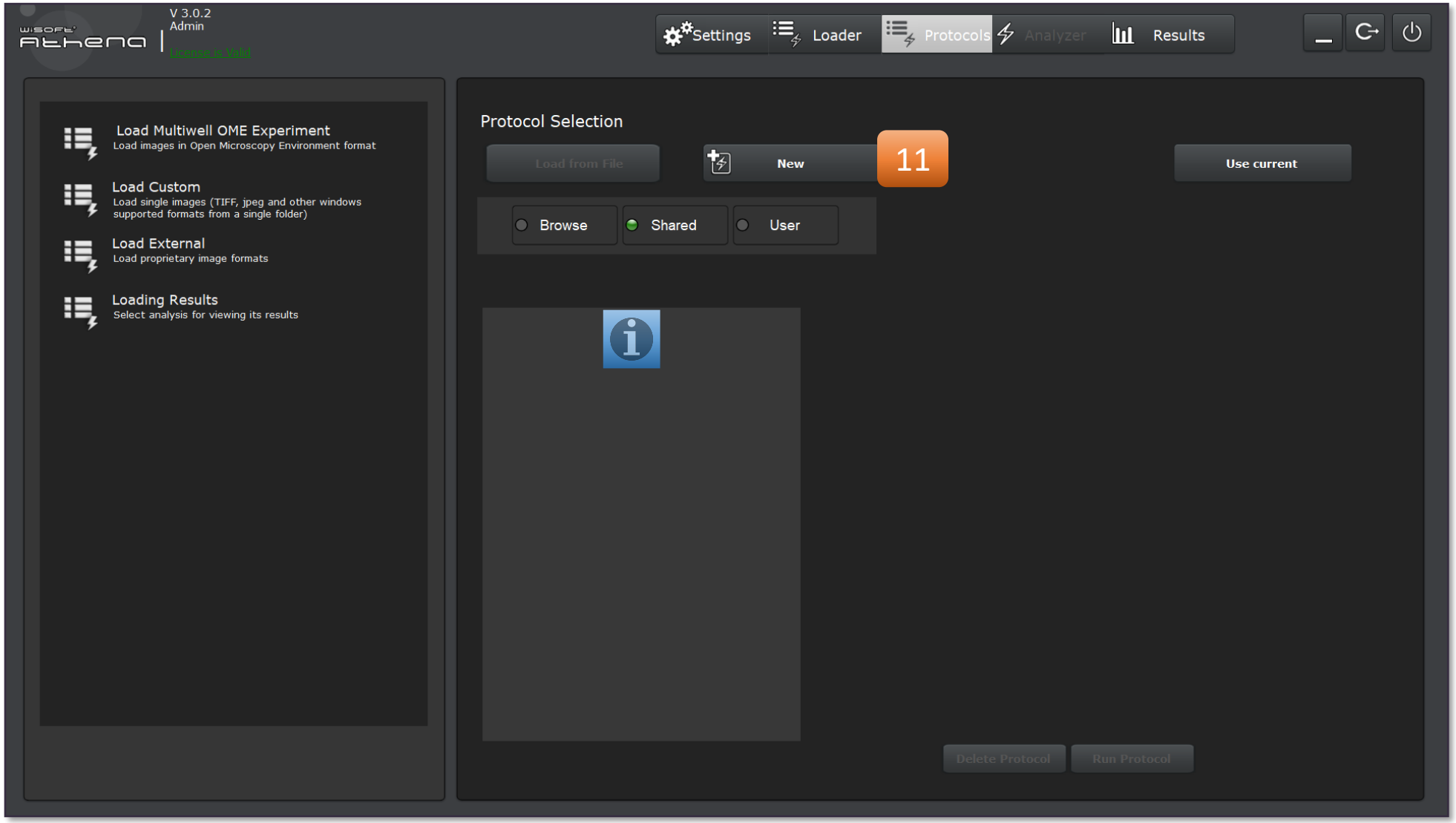
# Using Athena with Sample Images

## *Protocol menu*

11. 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.

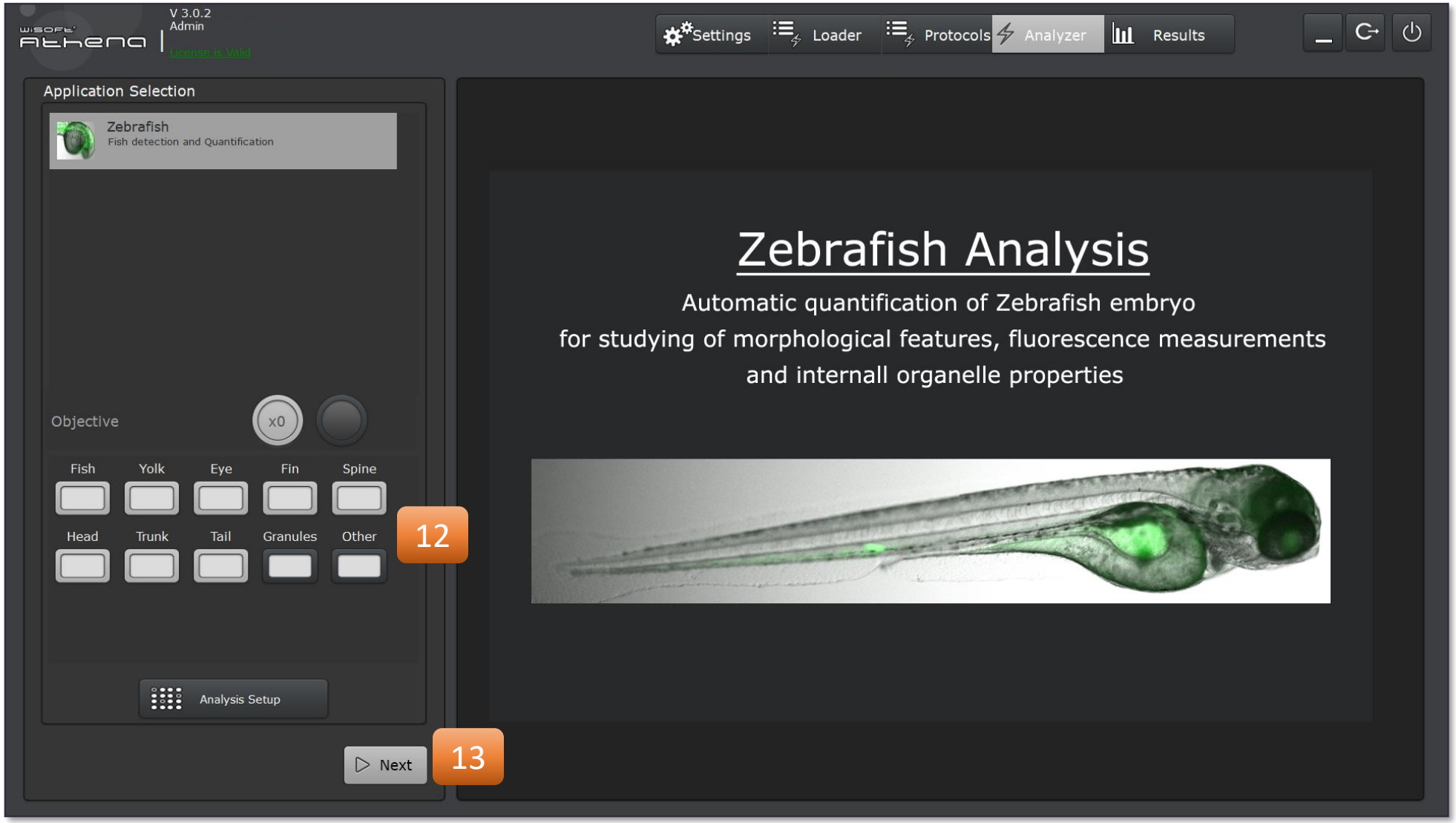
They are saved as files that can be loaded from a ‘Shared’ folder available to all users, a ‘User’ folder accessible to the user selected in step (2), or can be selected from the hard disk using the ‘Browse’ option.



# Using Athena with Sample Images

## *Select zebrafish application*

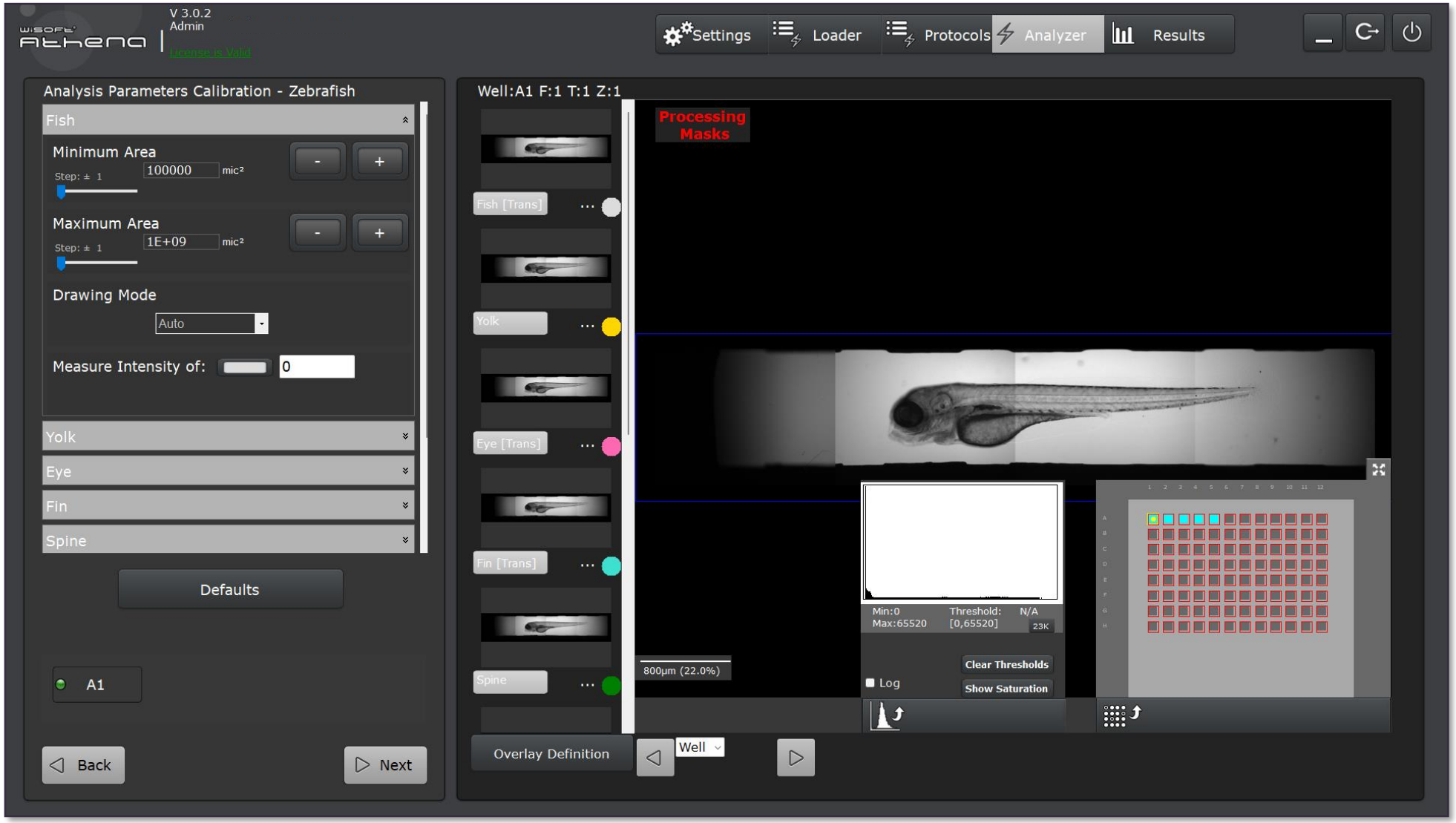
12. Select Anatomy to be identified.
    Light-grey = selected
    Dark-grey = omitted
13. Click ‘Next’ to advance.



# Using Athena with Sample Images

## *Parameter Definition*

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

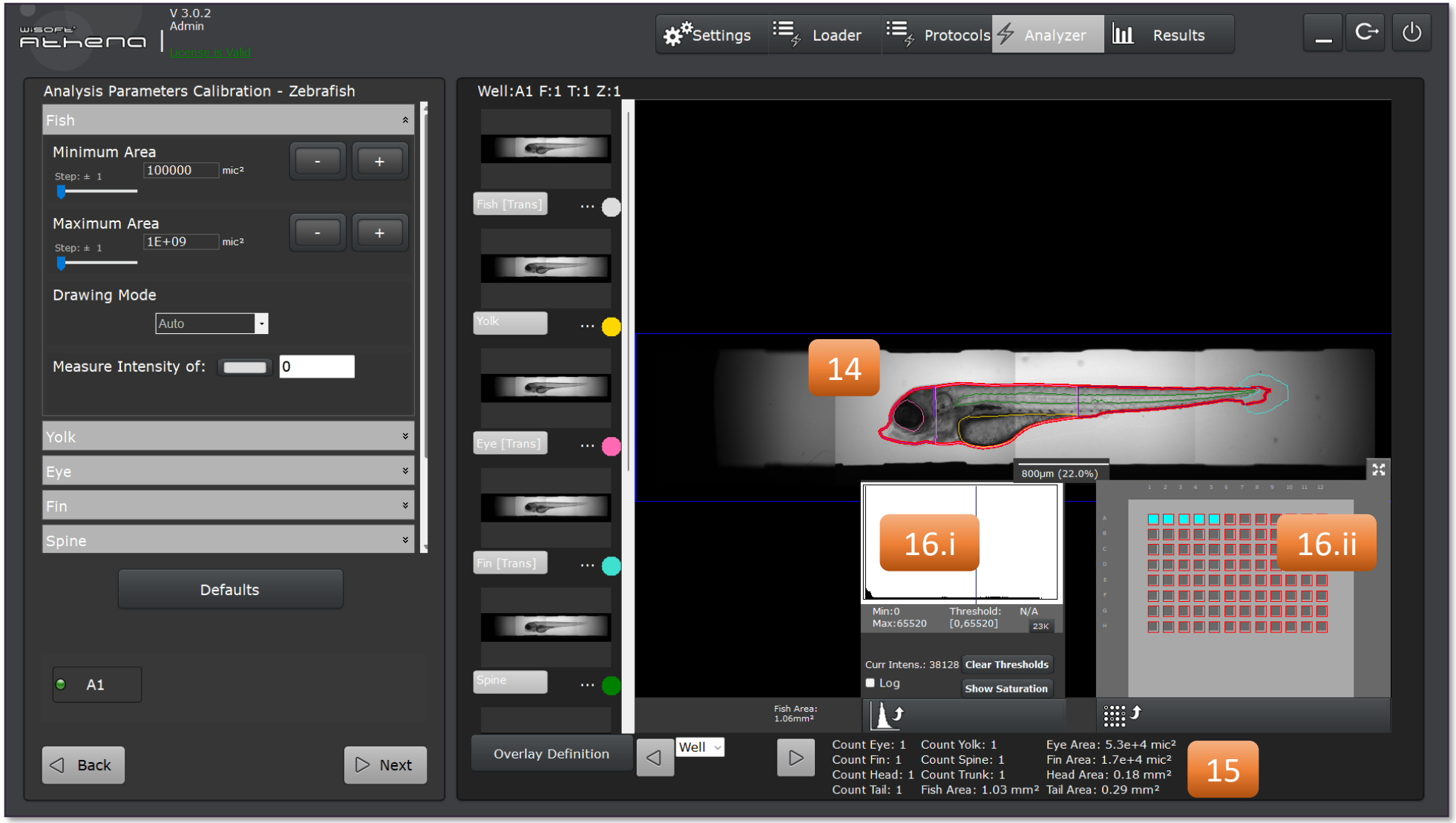


# Using Athena with Sample Images

## *Parameter Definition*

Identified structures are outlined in the image

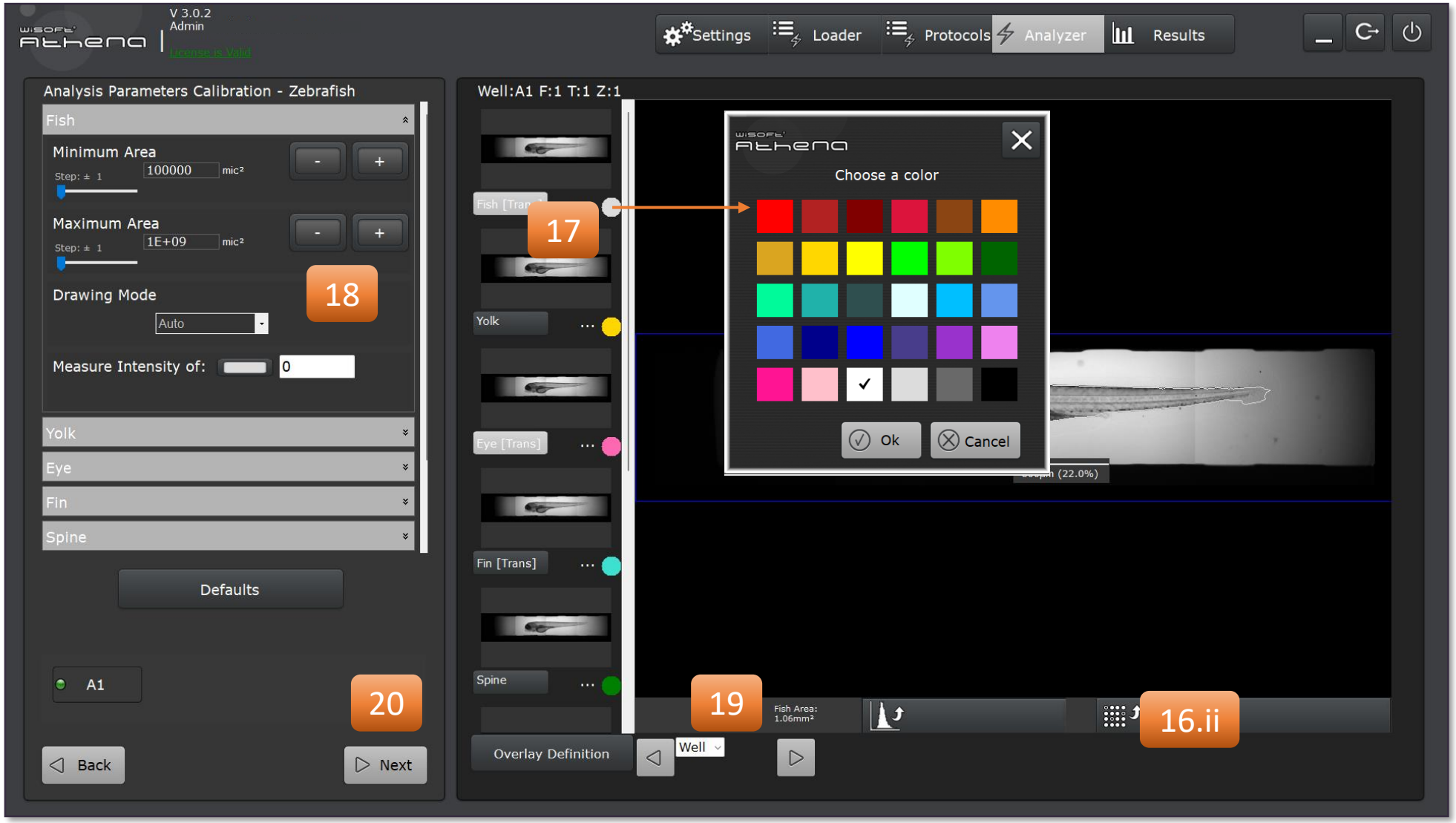
14. Hover the mouse over different anatomy detected in the image to see the outline. The outline will become thicker and red in color.
15. Quantification of the highlighted anatomical structure.
16. Intensity histogram (i) & navigation plate map (ii); minimize them with the arrow-buttons below them.



# Using Athena with Sample Images

## *Parameter Definition*

17. Click the name of each anatomical object below its thumbnails to toggle its visibility. Click on the colored dots to change the color of the outline.
18. Adjust the Min/Max area parameter to define what size of objects are permitted. (See point 15)
19. Move between different images using arrows or plate map (16.ii).
20. Click ‘Next’ to advance.



# Using Athena with Sample Images

## *Population Definition*

Optionally, fish can be selected into populations based on the metrics measured for each one.

Populations can also be defined after analysis

21. Click “All” (i) to select all fish, then click on the “+” button (ii).

# Using Athena with Sample Images

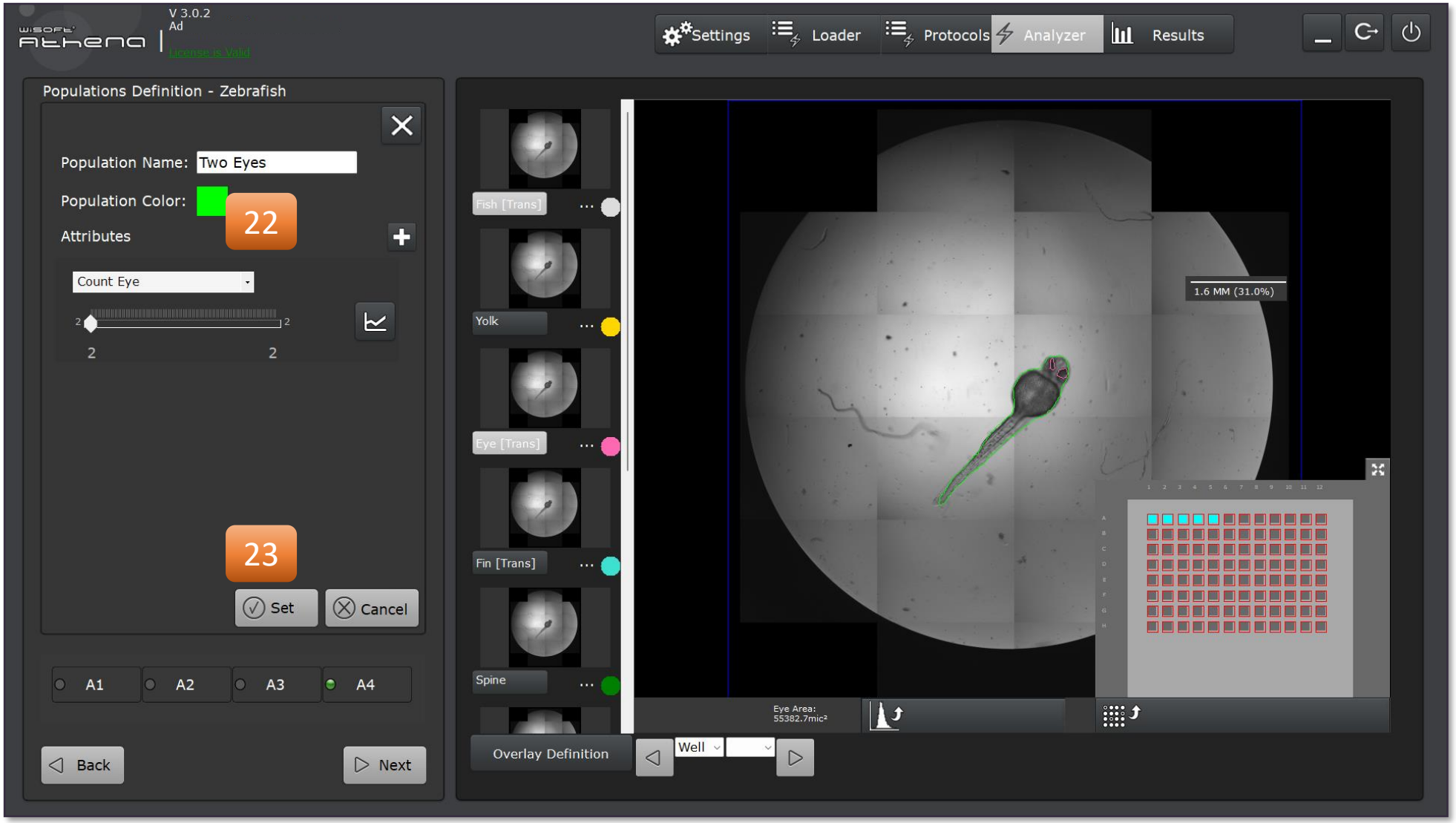
## *Population Definition*

Optionally, fish can be selected into populations based on the metrics measured for each one.

Populations can also be defined after analysis

22. Type the name of the population into the white text box.
    Select a color to identify the selected population (green square).
    Click on the “+” button to add an attribute to be used for defining a population.

    Select the desired attribute from the drop-down list and set upper/lower limits (both equal 2 here).
23. Click ‘Set’ to save.



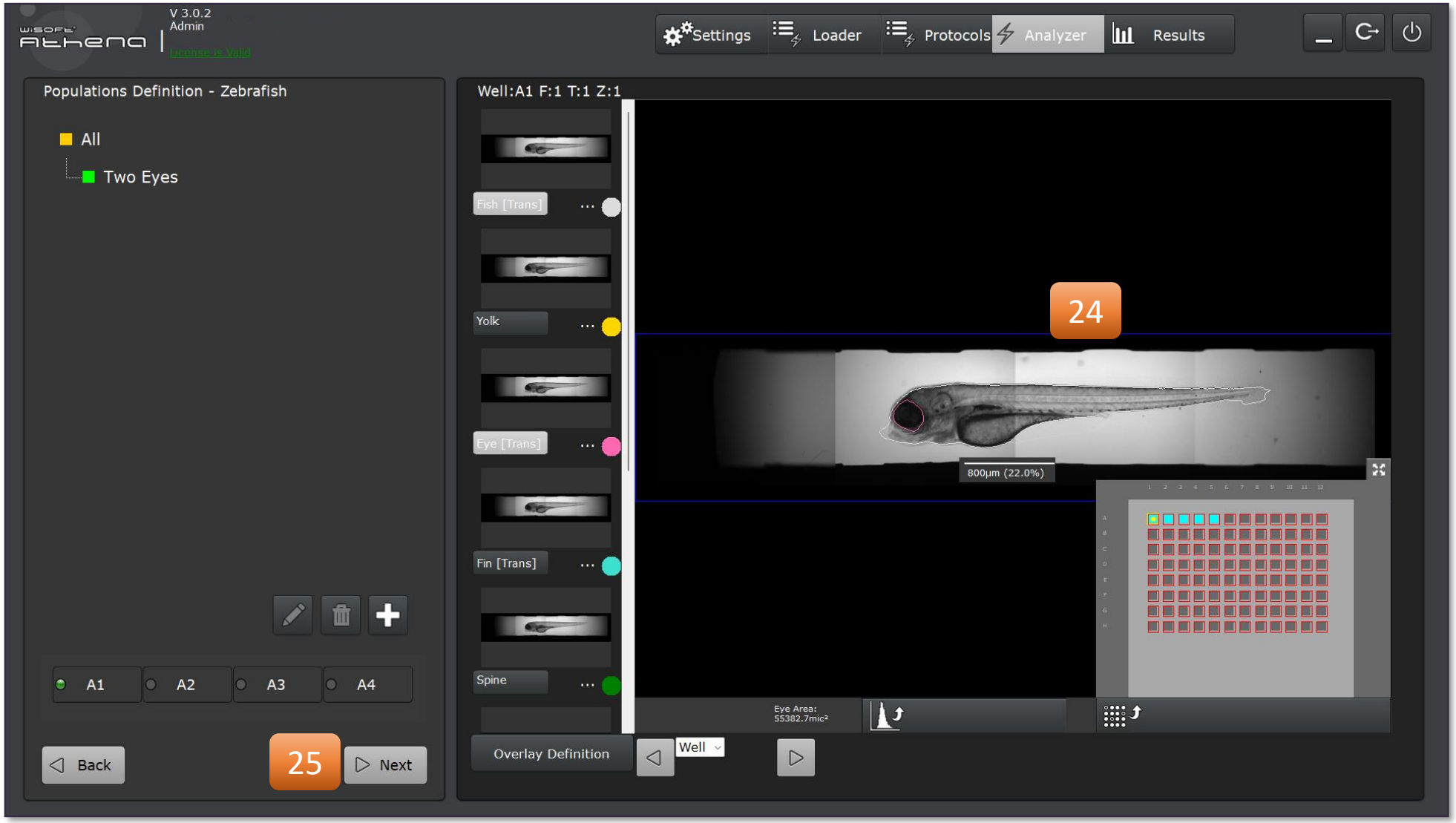
# Using Athena with Sample Images

## *Population Definition*

Optionally, fish can be selected into populations based on the metrics measured for each one.

Populations can also be defined after analysis

24. Fish not satisfying the population definition are outlined with the default color (white here).
25. Click ‘Next’ to advance.

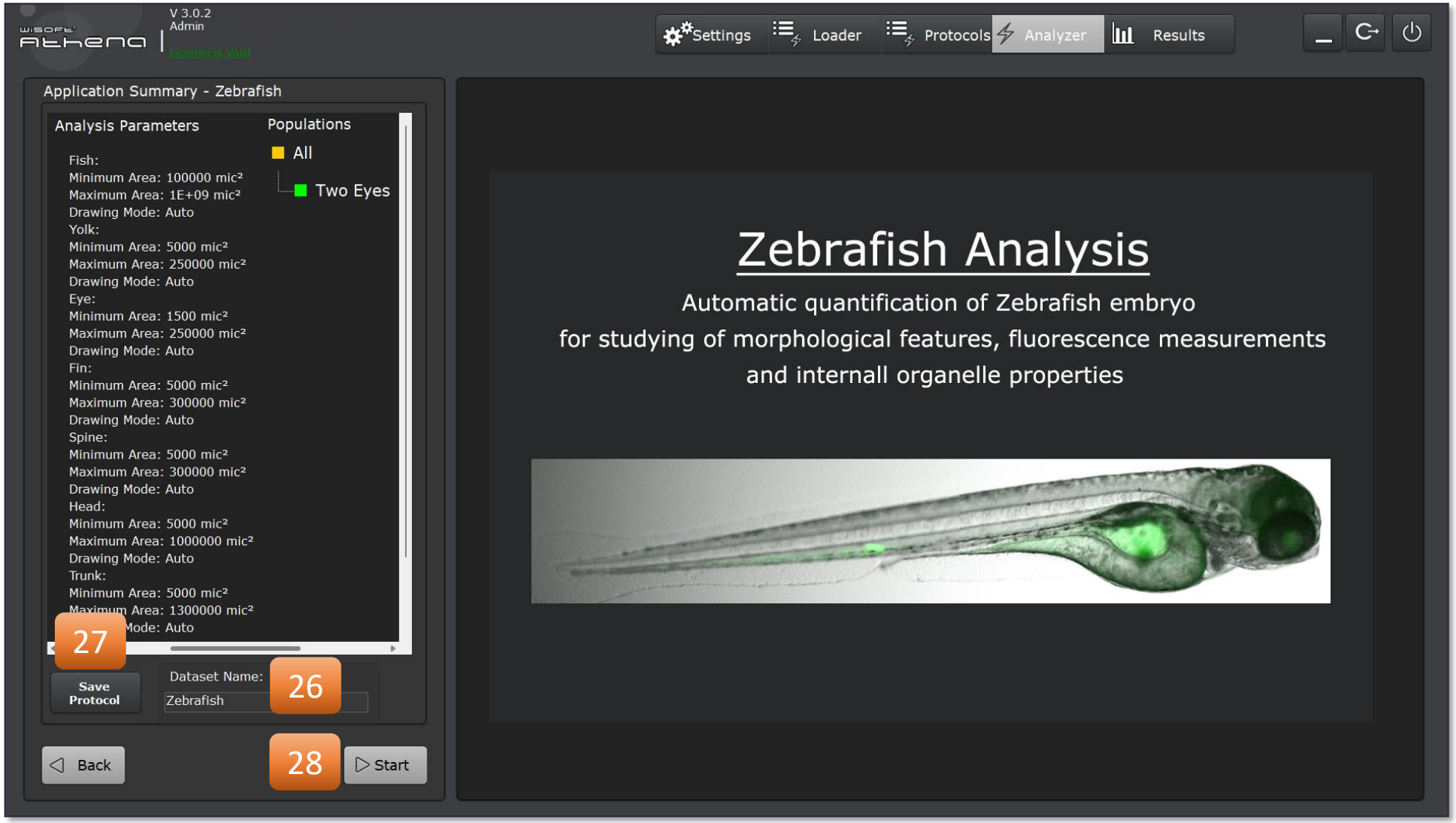


# Using Athena with Sample Images

## *Analysis Summary*

Scroll in the panel on the left-hand side of the screen to review the analysis parameters.

26. Define a name for the analysis folder; default is “Zebrafish”.
    Most parameter types are permitted.
    **Important:**
    **The analysis folder name cannot be changed afterward if data is to be re-loaded into Athena!**
27. Click ‘Save Protocol’ to save the analysis parameters for instant loading from the ‘Protocols’ screen.
28. Click ‘Start to begin analysis.

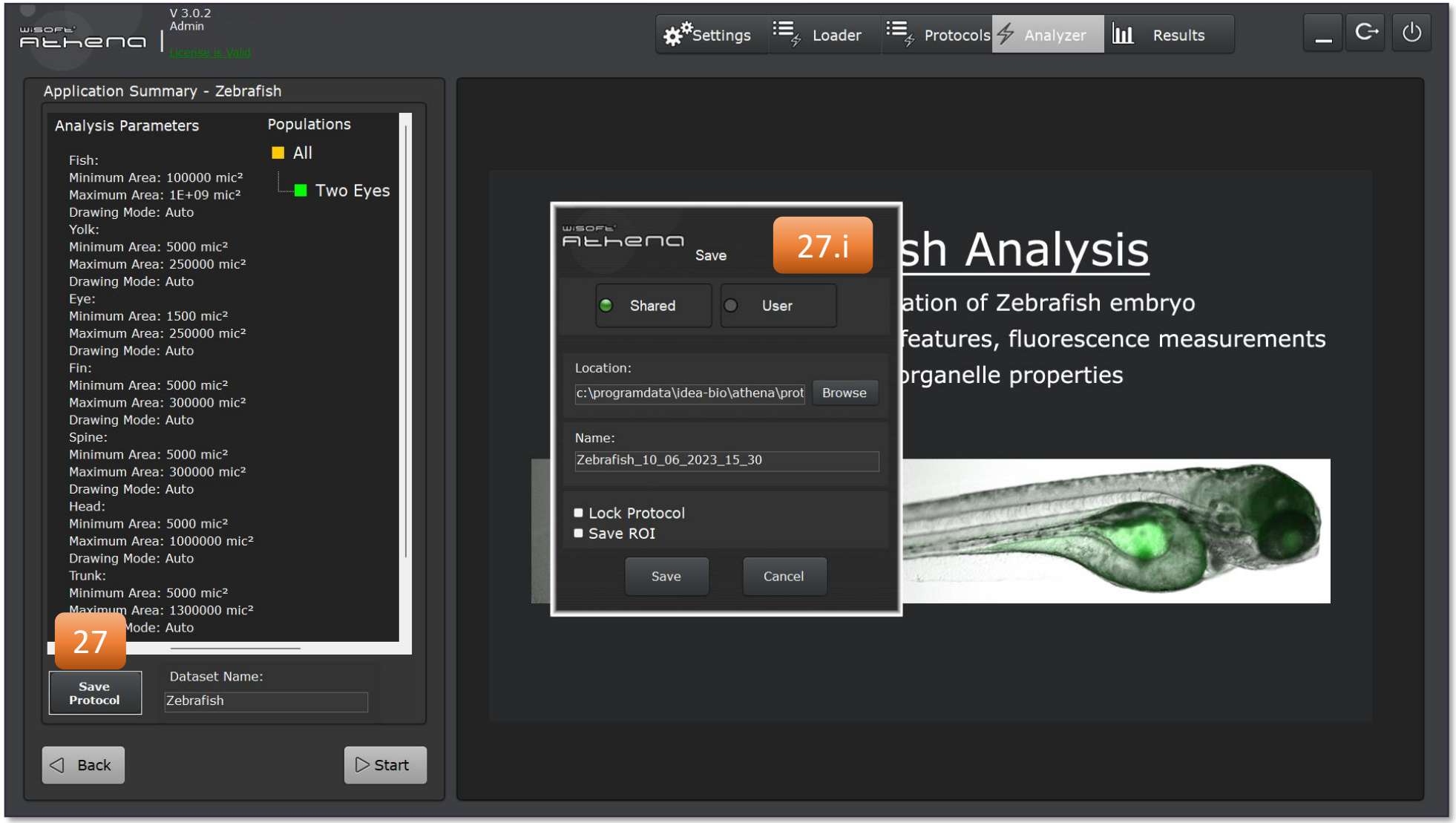


# Using Athena with Sample Images

## *Save a Protocol File*

Optional

27. Clicking the ‘Save Protocol’ button will open a pop-up window (i).
    Within this window, you can choose to save the protocol file in the ‘Shared’ folder accessible to all Athena users, in the ‘User’ folder using the two buttons near the top, or ‘Browse’ to a desired location.
    Re-name the protocol file as desired; default is the name of the application (zebrafish) with the date & time of creation.
    Lock protocol to prevent re-saving a protocol with the same file name (parameters can be adjusted when loaded).
    Save ROI not relevant here.



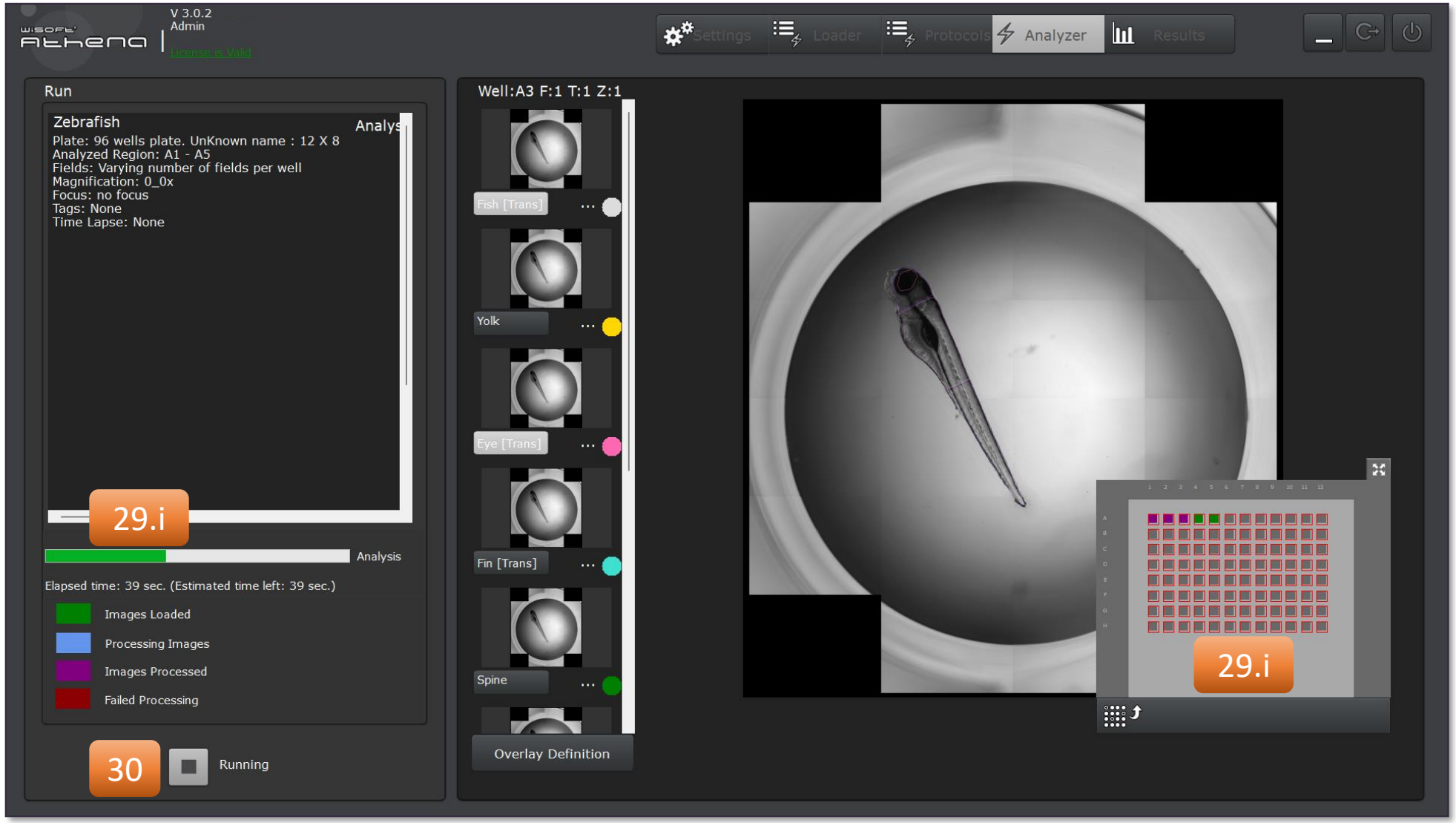
# Using Athena with Sample Images

## *Run Screen*

During the batch image analysis, the parameters used can be visualized and reviewed.

29. Progress is displayed in the status bar (i), along with estimated time, and in the plate map (ii). Wells/images yet analyzed are in green, completed ones are in purple.
30. Clicking the ‘Stop’ button to end analysis run.

Any images/wells that are already analyzed will have their data saved and displayed on the Results screen.

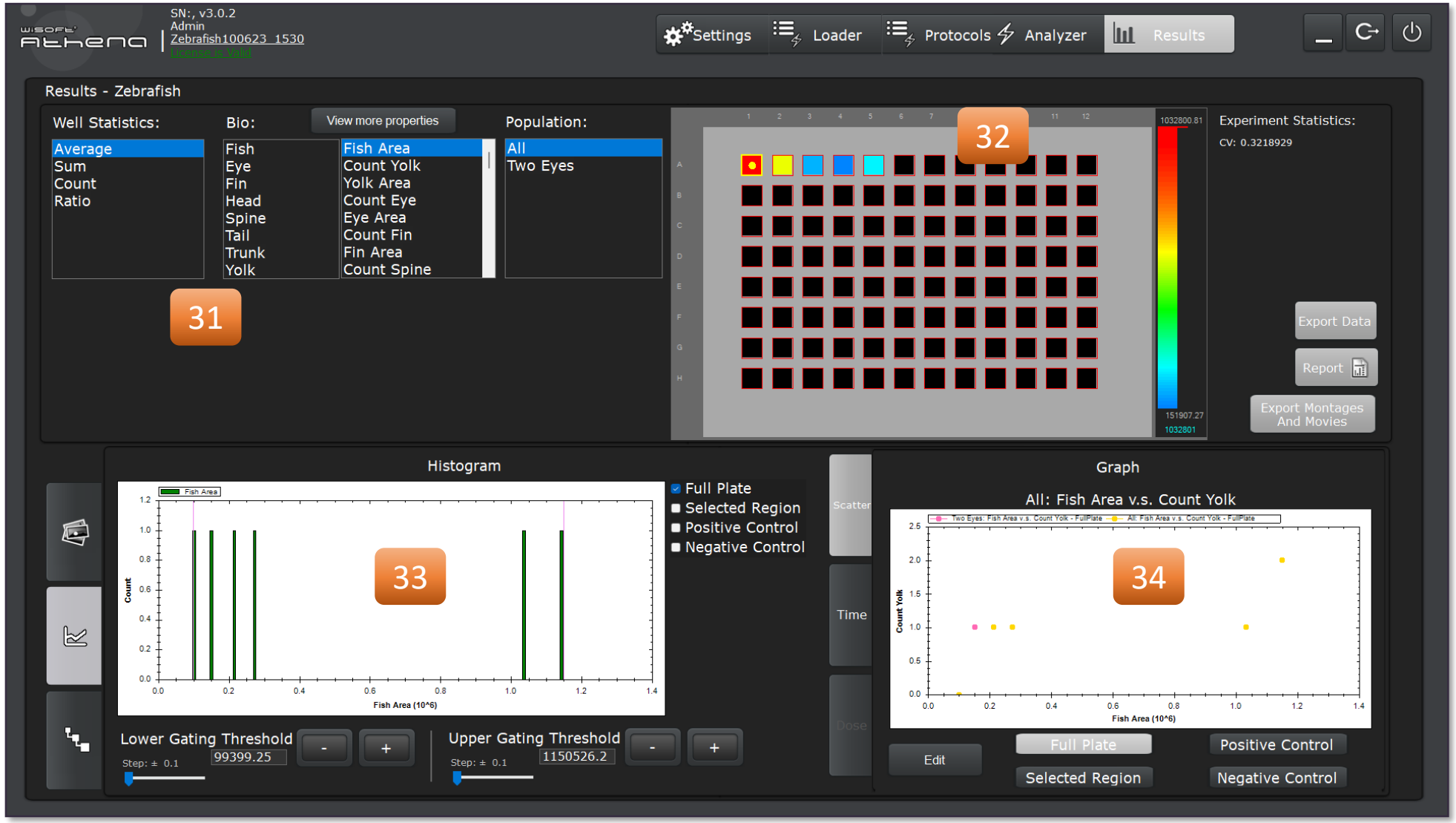


# Using Athena with Sample Images

## *Results Screen*

**Explore** the quantified results!

31. Choose the desired statistic, anatomy (Bio), property and population to view the data measured for each image (one image per well).
    Data selected here are shown in the following plots:
32. Heat map displaying color-scaled range of the data.
33. Histogram displaying the population distribution.
34. 2D scatter plot with one point for each fish.

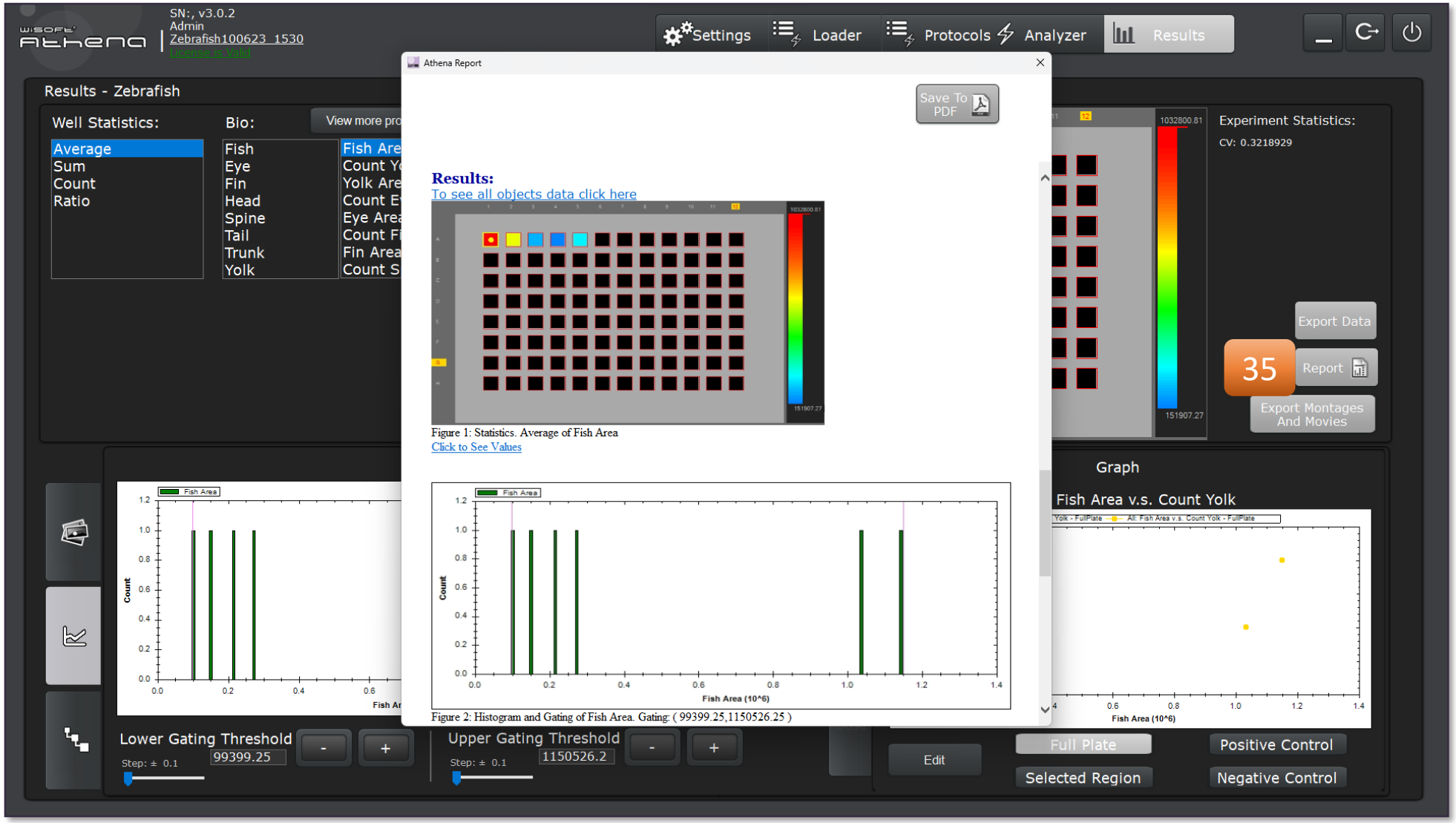


# Using Athena with Sample Images

## *Results Screen*

**Export** the quantified results!

35. Click the ‘Report’ button to create a PDF-exportable report of the data analysis.
    Scroll down to see the plots and click interactive hyperlinks to open the data present in each plot as a .csv file.



# Using Athena with Sample Images

## *Results Screen*

**Export** the quantified results!

36. Click the dataset name that is underlined text at the top left of the screen to open the location of the analyzed dataset in Windows Explorer.
    The Reports created by clicking the ‘Report’ button (35) are saved within the “Reports” folder.
    Report0 is default, all additional ‘ReportX’ folders are created each time the “Report” button (35) is pressed.
    All data plot images and data in .csv files are saved here for easy export and transport to other computers for further analysis.

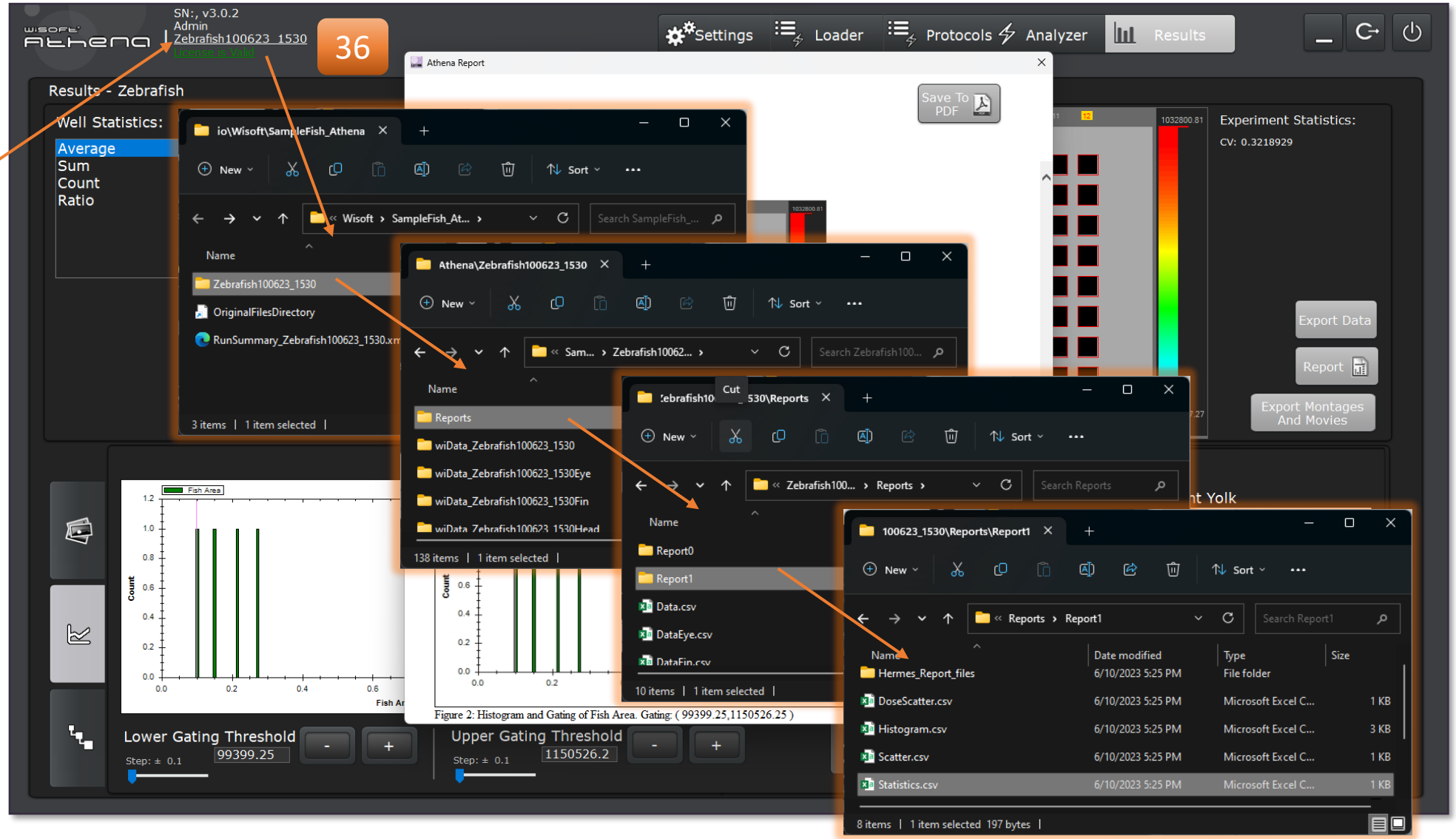


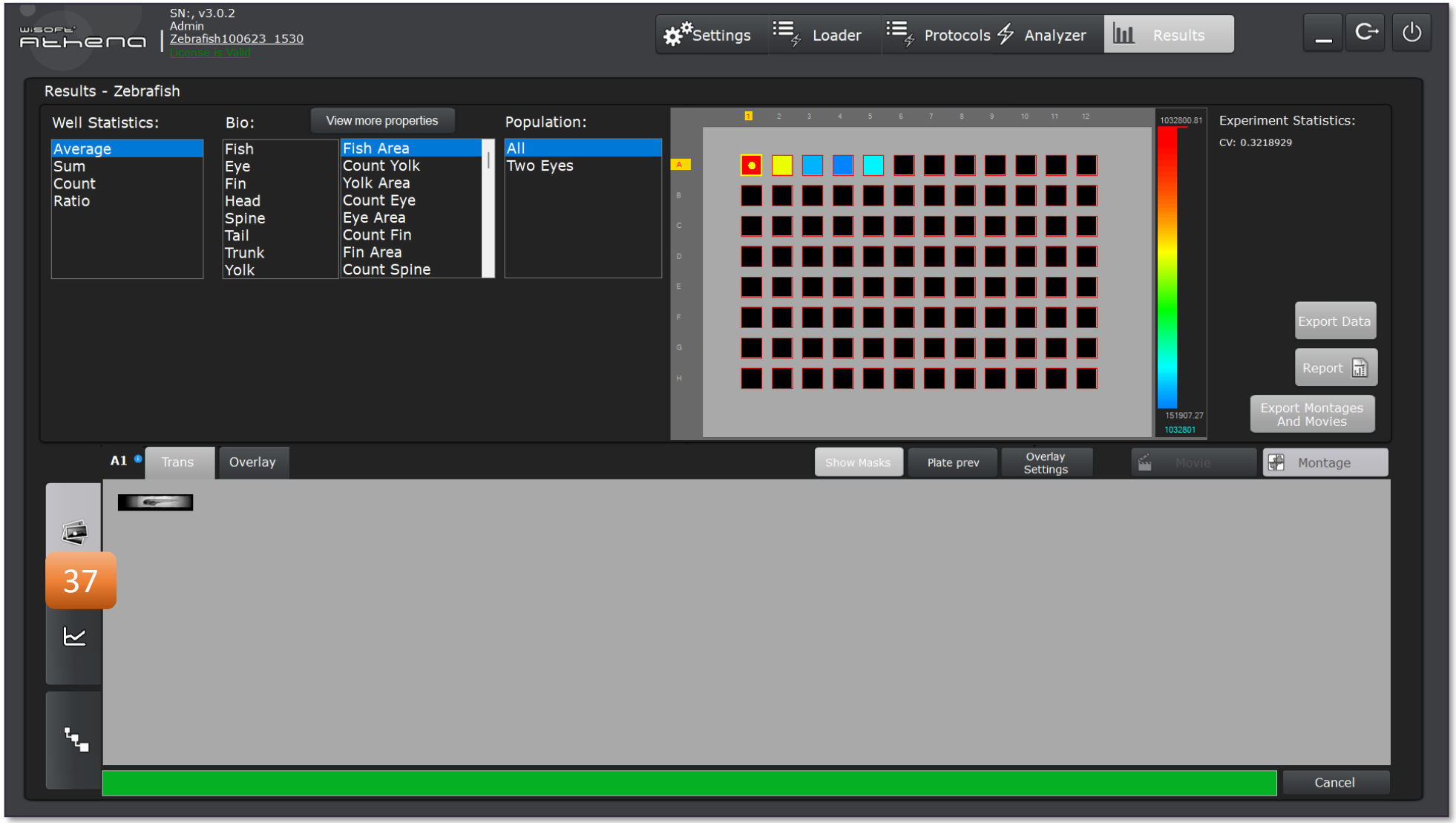
Figure 2: Histogram and Gating of Fish Area. Gating: ( 99399.25,1150526.25 )

# Using Athena with Sample Images

## *Results Screen*

View the images and outlines.

37. Click the top tab to view the images in a selected well.

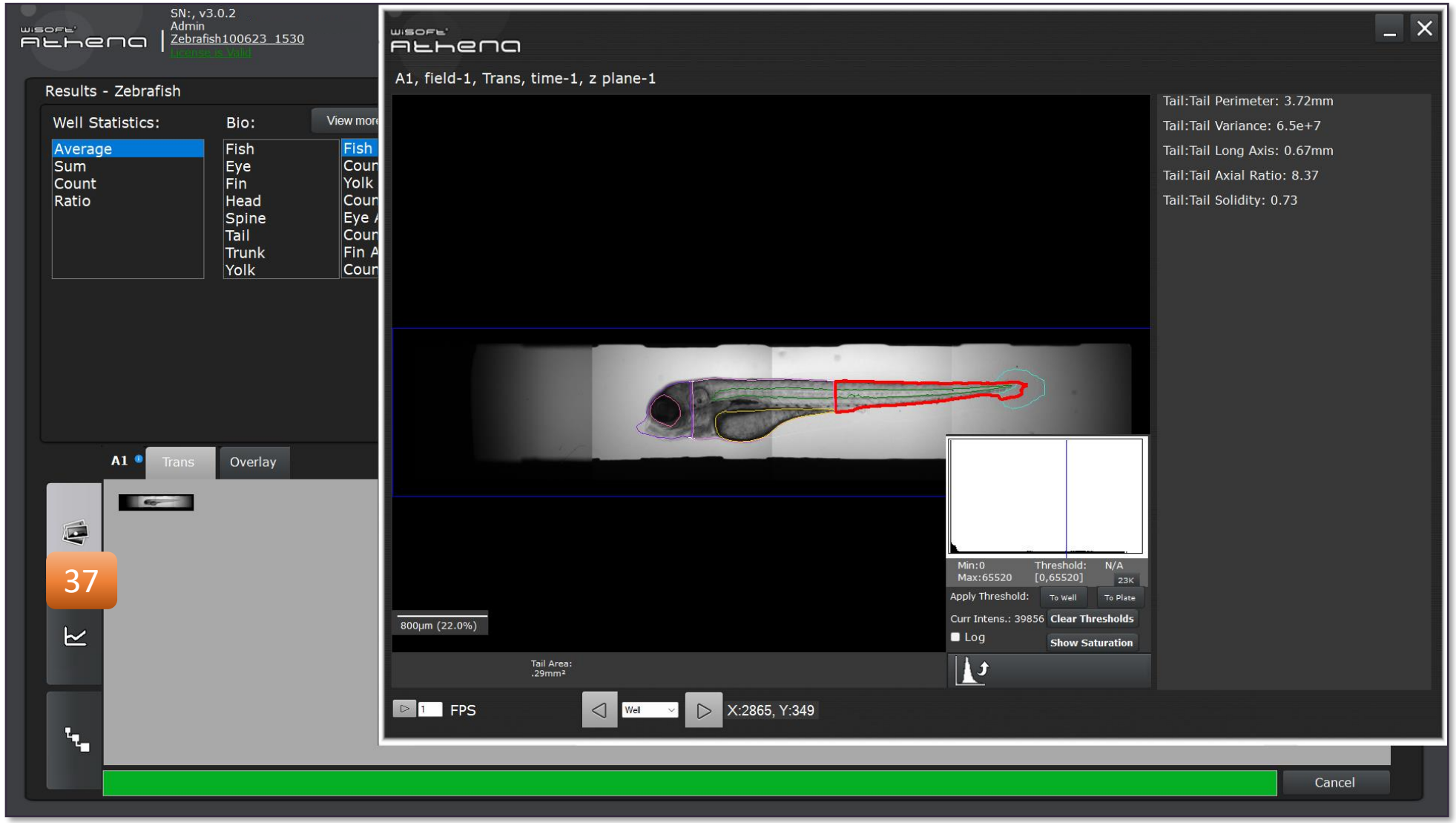


# Using Athena with Sample Images

## *Results Screen*

View the images and outlines.

37. Click the top tab to view the images in a selected well.
    Click the thumbnail to open the image and inspect the outlines presented.
    Quantified data is displayed on the right of the image.

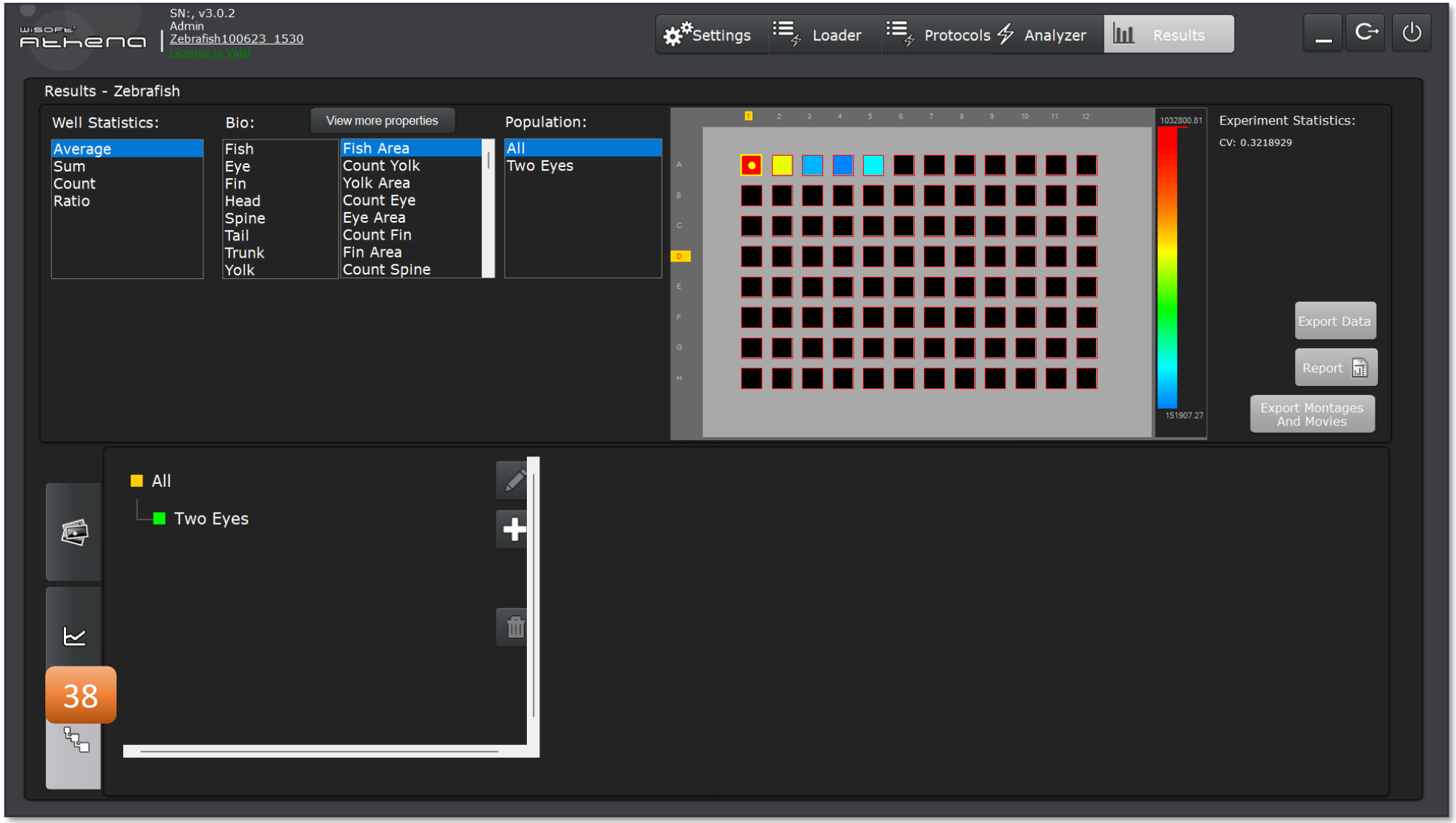


# Using Athena with Sample Images

## *Results Screen*

View the images and outlines.

38. Click the bottom tab to view review the populations that are defined.
    Click the ‘+’ to create a new one.
    Select an existing population and click the pencil button to edit, or the trash can to delete.



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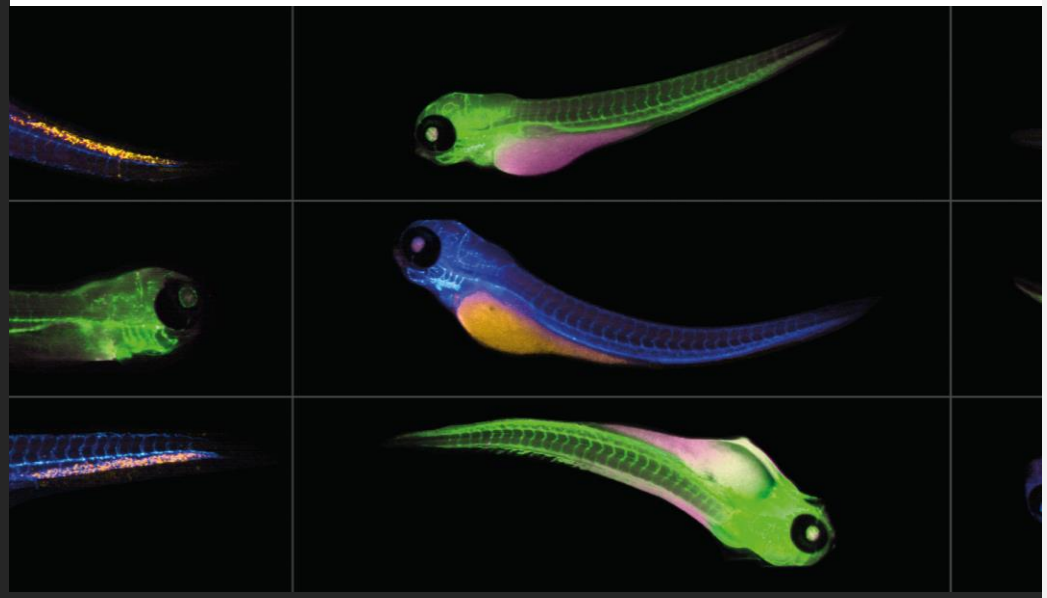

**Need some on-boarding support to get started?**

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

We'll be sure to contact you soon!