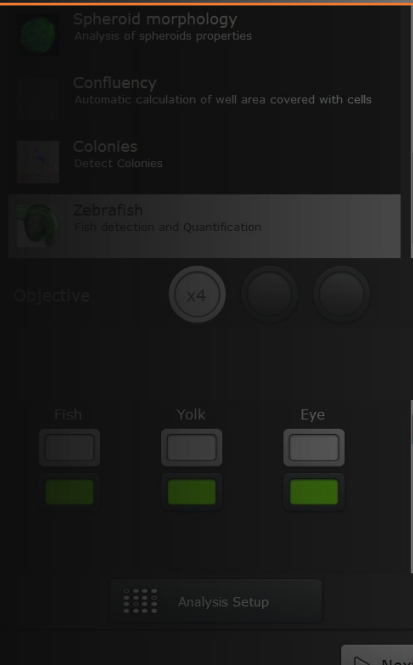


# Getting started with Athena Zebrafish software

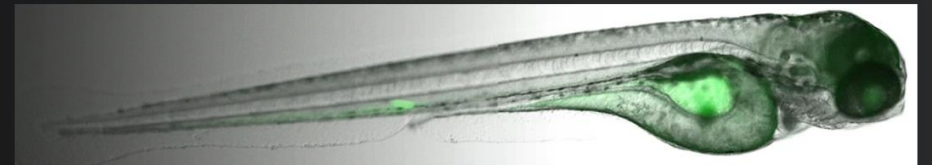


## IDEA Bio-Medical Seeing Better



### Zebrafish Analysis

Automatic quantification of Zebrafish embryo  
for studying of morphological features, fluorescence measurements  
and internal organelle properties



# Opening Athena

## *Shortcut from desktop*

1. Open Athena using the shortcut on your desktop.

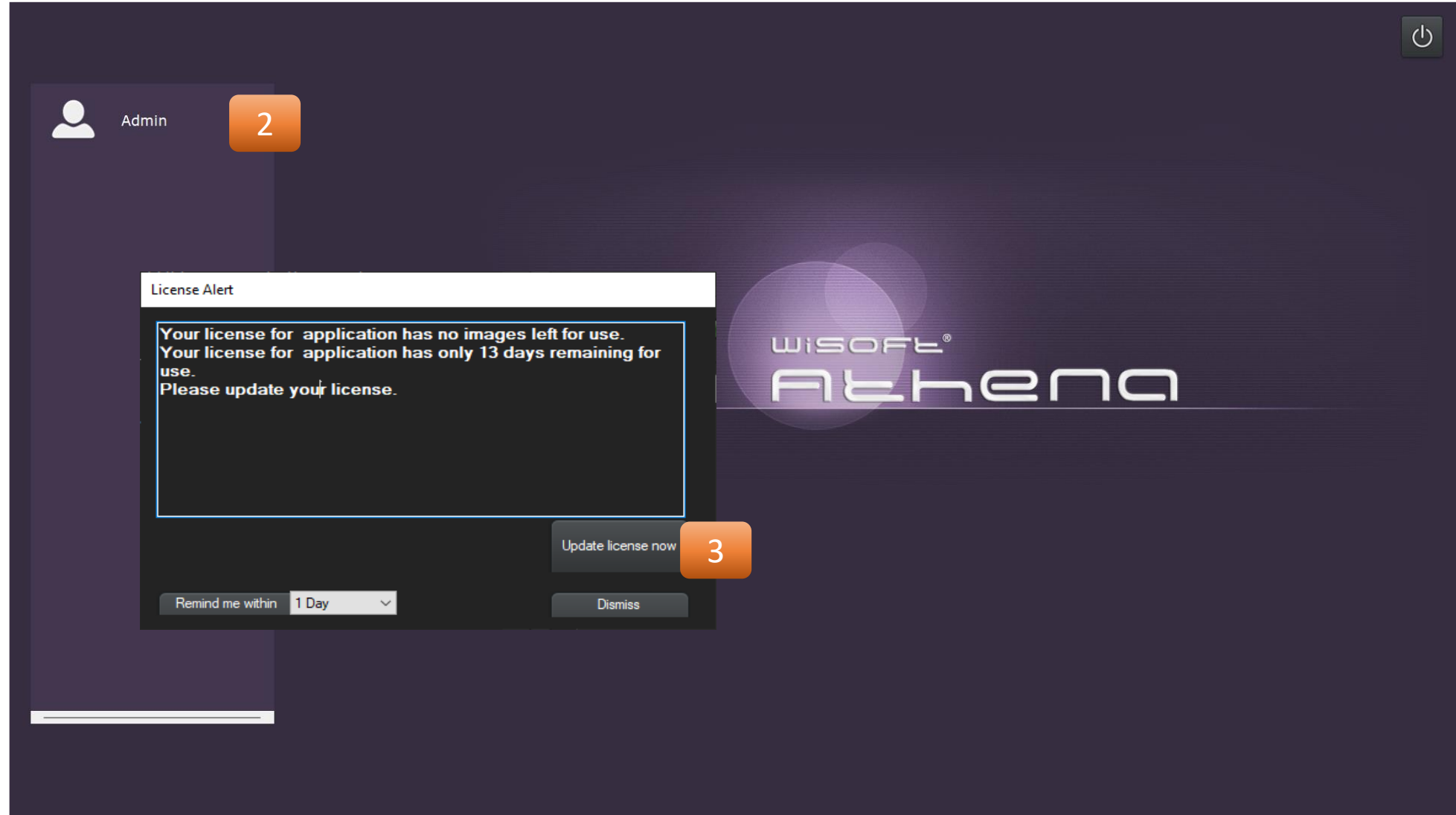
Sample images that can be analyzed for free are also accessible on the desktop from the 'Samplefish' folder shortcut.



# 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.

The screenshot displays the WISOFT Athena software interface. The top navigation bar includes 'Settings', 'Loader', 'Protocols', 'Analyzer', and 'Results'. The main content area is divided into two panels. The left panel, titled 'External Settings', contains 'User Settings', 'License Status', and 'About' sections. The right panel, titled 'License Status', shows 'Zebrafish' with 'Images Used: 1', 'Images Left: 9', and 'Expiration Date: 1/4/2023'. It features 'Request Renewal' and 'Install New License' buttons. A coupon code '3ae19-409ba-9e4d1-5a19c' is entered in a white box, with a 'Redeem coupon' button next to it. A pop-up window in the foreground displays the message: 'Coupon successfully installed. You now have 10 images for use with the Zebrafish application' and an 'Ok' button. The IDEA Bio-Medical Ltd. logo and contact information are visible in the bottom right corner.

**License Status:**

Zebrafish Images Used: 1 Images Left: 9 Expiration Date: 1/4/2023

Request Renewal Install New License

3ae19-409ba-9e4d1-5a19c Redeem coupon

**IDEA Bio-Medical Ltd.**  
Seeing Better

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# 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.

The screenshot displays the Athena software interface. At the top, the title bar shows 'WISOFT Athena V 3.0.2 Admin' and a navigation menu with 'Settings', 'Loader', 'Protocols', 'Analyzer', and 'Results'. The 'Loader' tab is active, showing a left sidebar with four options: 'Load Multiwell OME Experiment', 'Load Custom' (highlighted with an orange '7'), 'Load External', and 'Loading Results'. The main area is titled 'Load From Folder' and contains a 'Browse' button with a pre-set path 'C:\ProgramData\Idea-Bio\WiSoft\S'. Below this are 'Data Format' settings for 'Pixel size' (Step: ± 0.001, value: 1) and '# Colors' (Step: ± 1, value: 1), each with a slider and '+'/'-' buttons. An information icon 'i' is positioned next to these settings. The 'Color Assignment' section shows 'Color' and 'Distinguishing Text' both set to 'Trans'. At the bottom, the 'Test Data Assignment' section has a 'Sort by:' dropdown set to 'Name' and a 'Test' button (highlighted with an orange '8'). A 'Confirm' button is located at the bottom right. The status bar at the very bottom indicates 'Displaying image'.

# Using Athena with Sample Images

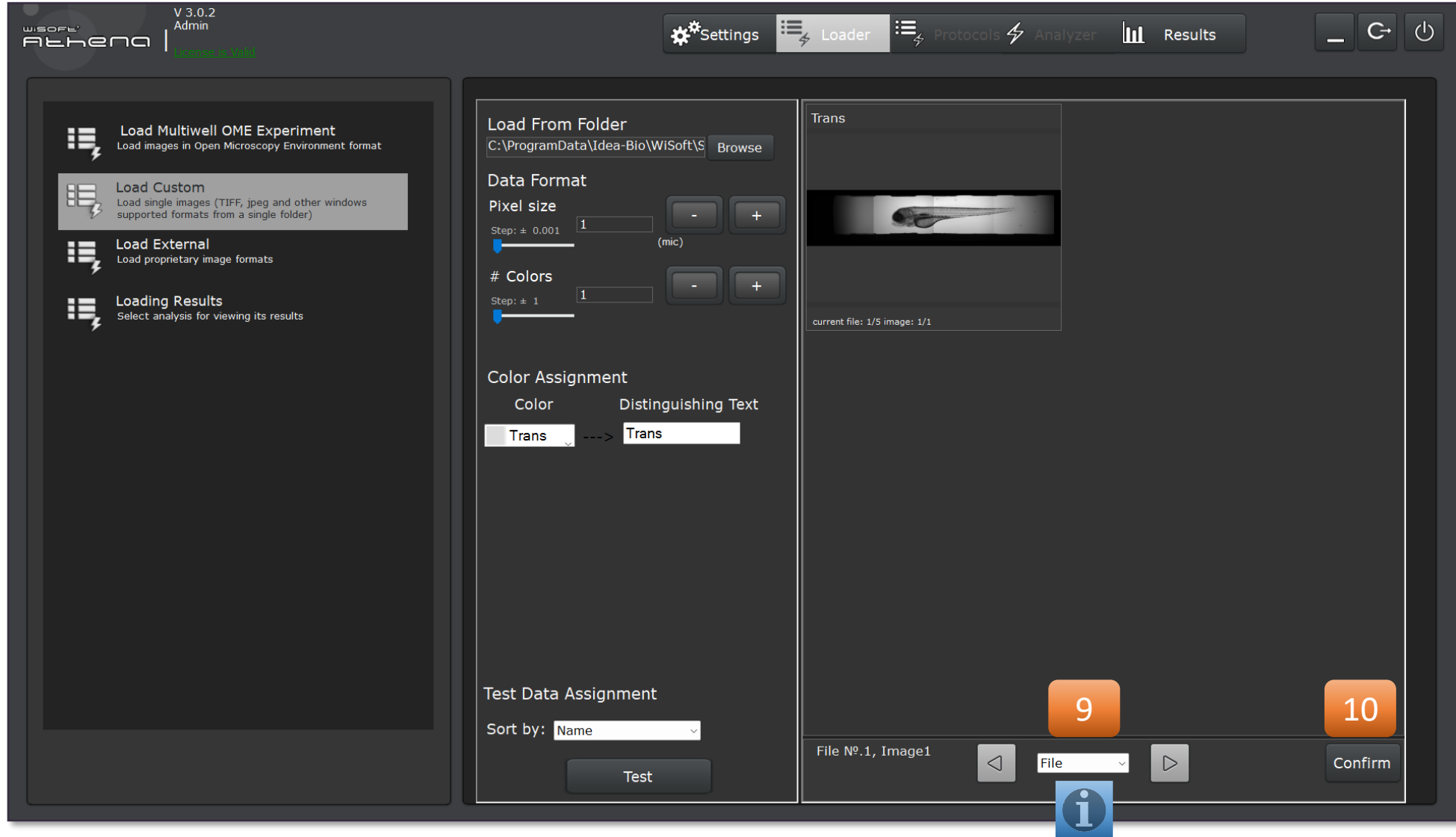
## *Load the sample images*

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

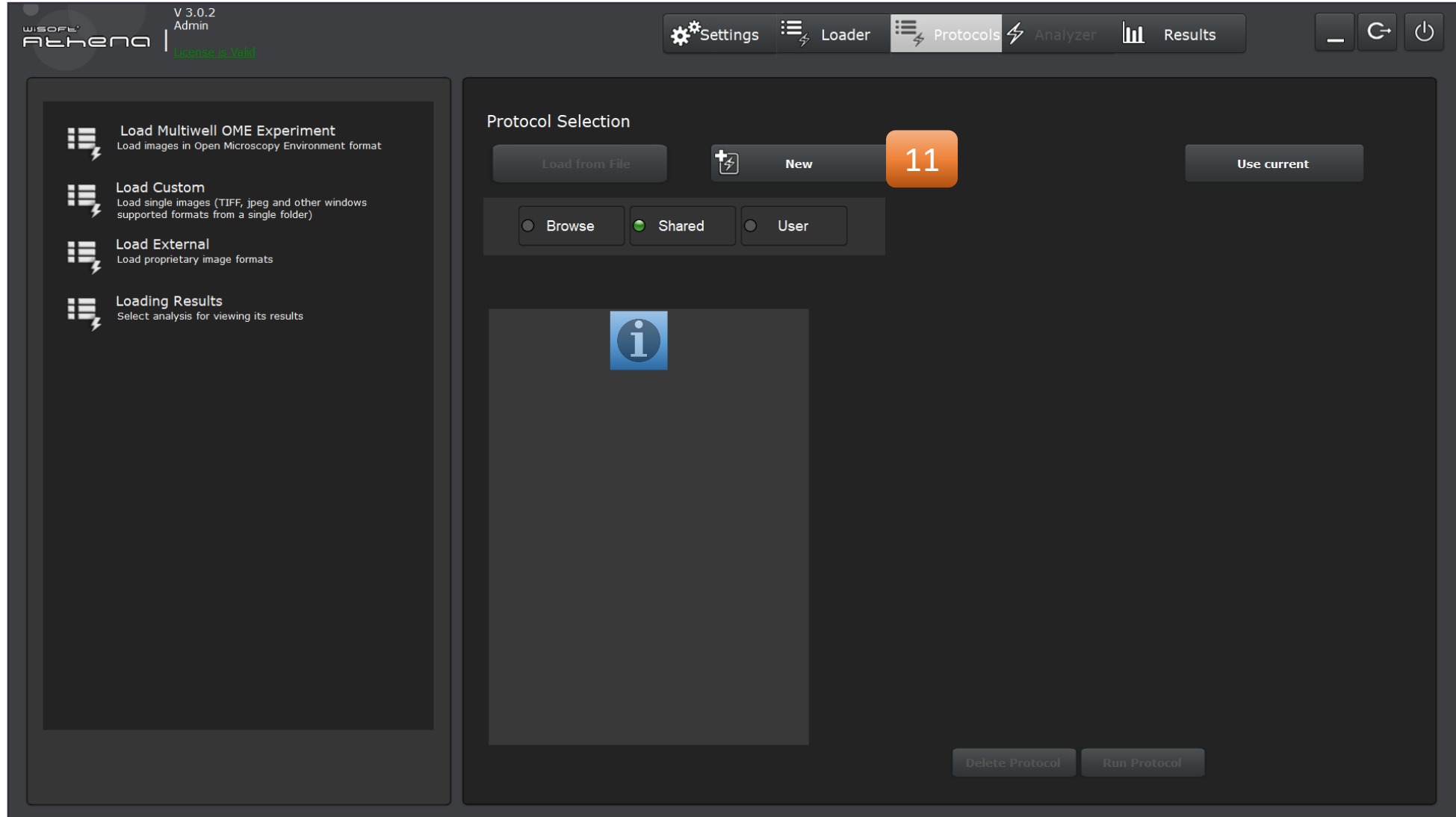
## Protocol menu

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.

The screenshot shows the Athena software interface. At the top, the version is V 3.0.2 and the user is Admin. The navigation bar includes Settings, Loader, Protocols, Analyzer (active), and Results. The main window is titled 'Application Selection' and shows the 'Zebrafish' application selected, with a description 'Fish detection and Quantification'. Below this, there is an 'Objective' section with a magnification control set to 'x0'. A grid of buttons allows selecting anatomical features: Fish, Yolk, Eye, Fin, Spine, Head, Trunk, Tail, Granules, and Other. The 'Fish' button is highlighted in light grey, indicating it is selected. An orange callout box with the number '12' points to this button. At the bottom of the interface, there is an 'Analysis Setup' button and a 'Next' button. An orange callout box with the number '13' points to the 'Next' button. The right side of the interface displays the title 'Zebrafish Analysis' and a description: 'Automatic quantification of Zebrafish embryo for studying of morphological features, fluorescence measurements and internal organelle properties'. Below the text is a grayscale image of a zebrafish embryo with a green fluorescent spot in its head region.



# Using Athena with Sample Images

## *Parameter Definition*

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

The screenshot displays the Athena software interface for Zebrafish analysis. The top navigation bar includes 'Settings', 'Loader', 'Protocols', 'Analyzer', and 'Results'. The main window is titled 'Analysis Parameters Calibration - Zebrafish' and is divided into several sections:

- Analysis Parameters Calibration - Zebrafish:**
  - Fish:** Minimum Area (100000 mic<sup>2</sup>), Maximum Area (1E+09 mic<sup>2</sup>), Drawing Mode (Auto), Measure Intensity of (0).
  - Yolk, Eye, Fin, Spine:** Each has a corresponding 'Trans' button and a color-coded indicator.
  - Defaults:** A button to reset parameters.
  - Well Selection:** A button for 'A1' is highlighted.
  - Navigation:** 'Back' and 'Next' buttons are at the bottom.
- Well: A1 F:1 T:1 Z:1:** A vertical strip of image thumbnails for different features.
- Processing Masks:** A large central area showing a zebrafish image with a red 'Processing Masks' overlay.
- Thresholding:** A grid of 12x12 cells with a color scale and a 'Clear Thresholds' button.
- Log and Saturation:** A 'Log' button and a 'Show Saturation' button.
- Overlay Definition:** A bottom bar with 'Well' dropdown and navigation arrows.

# 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.

The screenshot displays the Athena software interface for zebrafish analysis. The main window shows a zebrafish image with several anatomical structures outlined in different colors: Fish (red), Yolk (yellow), Eye (pink), Fin (cyan), and Spine (green). The interface includes a top navigation bar with tabs for Settings, Loader, Protocols, Analyzer, and Results. On the left, the 'Analysis Parameters Calibration - Zebrafish' panel is visible, showing settings for Minimum Area (100000 mic<sup>2</sup>), Maximum Area (1E+09 mic<sup>2</sup>), Drawing Mode (Auto), and Measure Intensity of (0). A central panel shows a list of structures with corresponding thumbnail images and color-coded buttons. At the bottom, a status bar displays 'Fish Area: 1.06mm<sup>2</sup>' and a table of counts and areas for various structures. Two tool windows are open: '16.i' (Intensity histogram) and '16.ii' (Navigation plate map). A '15' button is also present in the bottom right corner.

Structure	Count	Area
Eye	1	5.3e+4 mic <sup>2</sup>
Fin	1	1.7e+4 mic <sup>2</sup>
Head	1	0.18 mm <sup>2</sup>
Tail	1	0.29 mm <sup>2</sup>
Yolk	1	-
Spine	1	-
Fish (Total)	-	1.03 mm <sup>2</sup>

# 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.

The screenshot displays the Athena software interface for Zebrafish analysis. The top navigation bar includes 'Settings', 'Loader', 'Protocols', 'Analyzer', and 'Results'. The main window is titled 'Analysis Parameters Calibration - Zebrafish' and shows a list of anatomical objects: Fish, Yolk, Eye, Fin, and Spine. The 'Fish' object is selected, and its parameters are visible: Minimum Area (100000 mic²), Maximum Area (1E+09 mic²), Drawing Mode (Auto), and Measure Intensity of (0). A color selection dialog box is open, showing a grid of colors with a checkmark on the white color. The dialog box is titled 'Choose a color' and has 'OK' and 'Cancel' buttons. Orange callout boxes 17, 18, 19, and 20 highlight the 'Fish' object name, the 'Maximum Area' parameter, the 'Next' button, and the 'Next' button respectively.

# 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).



WISOFI Athena V 3.0.2 Admin  
Artemis@Well

Settings Loader Protocols Analyzer Results

Populations Definition - Zebrafish

All 21.i

Fish [Trans] ...

Yolk ...

Eye [Trans] ...

Fin [Trans] ...

Spine ...

21.ii

A1 A2 A3 A4

Back Next

Eye Area: 55382.7mic<sup>2</sup>

Well

1.6 MM (31.0%)

1 2 3 4 5 6 7 8 9 10 11 12

A B C D E F G H

# 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.

The screenshot displays the Athena software interface. On the left, a dialog box titled "Populations Definition - Zebrafish" is open. It contains the following fields and controls:

- Population Name:** A white text box containing "Two Eyes".
- Population Color:** A green square next to a large orange button with the number "22".
- Attributes:** A section with a plus sign button and a dropdown menu currently showing "Count Eye".
- Range:** A horizontal slider with two diamond-shaped handles, both positioned at the value "2".
- Buttons:** "Set" (with a checkmark) and "Cancel" (with an X).
- Well Selection:** A row of radio buttons labeled A1, A2, A3, and A4, with A4 selected.
- Navigation:** "Back" and "Next" buttons.

The main window shows a large circular field of view containing a zebrafish embryo. The embryo is highlighted with a green outline. A scale bar in the top right of the field indicates "1.6 MM (31.0%)". On the left side of the main window, there is a vertical "Overlay Definition" panel with several items, each with a small circular icon and a text label:

- Fish [Trans] (grey icon)
- Yolk (yellow icon)
- Eye [Trans] (pink icon)
- Fin [Trans] (cyan icon)
- Spine (green icon)

At the bottom of the main window, there is a status bar with "Eye Area: 55362.7mic²" and a "Well" dropdown menu.

# 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.

The screenshot displays the Athena software interface for population definition. The top navigation bar includes 'Settings', 'Loader', 'Protocols', 'Analyzer', and 'Results'. The main interface is divided into three panels:

- Left Panel:** 'Populations Definition - Zebrafish' with a legend showing 'All' (yellow square) and 'Two Eyes' (green square).
- Middle Panel:** A list of population categories: 'Fish [Trans]', 'Yolk', 'Eye [Trans]', 'Fin [Trans]', and 'Spine', each with a corresponding colored circle.
- Right Panel:** A large image of a zebrafish with a red outline on its eye. A large orange button with the number '24' is overlaid on the image. A grid of 12x12 wells is visible in the bottom right, with the top-left well highlighted in yellow.

At the bottom, there are buttons for 'Back', '25', and 'Next', along with a 'Well' dropdown menu.

# 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.

WISOFT Athena V 3.0.2 Admin

Application Summary - Zebrafish

Analysis Parameters

Fish:  
Minimum Area: 100000 mic<sup>2</sup>  
Maximum Area: 1E+09 mic<sup>2</sup>  
Drawing Mode: Auto

Yolk:  
Minimum Area: 5000 mic<sup>2</sup>  
Maximum Area: 250000 mic<sup>2</sup>  
Drawing Mode: Auto

Eye:  
Minimum Area: 1500 mic<sup>2</sup>  
Maximum Area: 250000 mic<sup>2</sup>  
Drawing Mode: Auto

Fin:  
Minimum Area: 5000 mic<sup>2</sup>  
Maximum Area: 300000 mic<sup>2</sup>  
Drawing Mode: Auto

Spine:  
Minimum Area: 5000 mic<sup>2</sup>  
Maximum Area: 300000 mic<sup>2</sup>  
Drawing Mode: Auto

Head:  
Minimum Area: 5000 mic<sup>2</sup>  
Maximum Area: 1000000 mic<sup>2</sup>  
Drawing Mode: Auto

Trunk:  
Minimum Area: 5000 mic<sup>2</sup>  
Maximum Area: 1300000 mic<sup>2</sup>  
Drawing Mode: Auto

Populations

All

Two Eyes

Dataset Name: Zebrafish

Save Protocol

Back Start

## Zebrafish Analysis

Automatic quantification of Zebrafish embryo  
for studying of morphological features, fluorescence measurements  
and internal organelle properties

# 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.

The screenshot displays the Athena software interface. The main window is titled 'Application Summary - Zebrafish' and shows various analysis parameters for different body parts of a zebrafish embryo, such as Fish, Yolk, Eye, Fin, Spine, Head, and Trunk. Each parameter has a 'Minimum Area' and 'Maximum Area' in mic<sup>2</sup>, and a 'Drawing Mode' set to 'Auto'. A 'Populations' section on the right shows 'All' selected with a yellow square and 'Two Eyes' with a green square. At the bottom of the main window, there is a 'Save Protocol' button and a 'Dataset Name' field containing 'Zebrafish'. A '27' badge is visible in the bottom left corner of the main window.

A 'Save' dialog box is open in the foreground, titled 'wISOFT Athena Save 27.i'. It has two radio buttons: 'Shared' (selected) and 'User'. Below these are fields for 'Location' (c:\programdata\idea-bio\athena\prot) with a 'Browse' button, and 'Name' (Zebrafish\_10\_06\_2023\_15\_30). At the bottom of the dialog are checkboxes for 'Lock Protocol' and 'Save ROI', and 'Save' and 'Cancel' buttons.

In the background, a slide titled 'Fish Analysis' is visible, listing 'ation of Zebrafish embryo', 'features, fluorescence measurements', and 'organelle properties'. Below the text is an image of a zebrafish embryo with a green fluorescent spot in its body.



# 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.

The screenshot shows the Athena software interface during a batch image analysis run. The top bar includes the Athena logo, version (V 3.0.2), and user (Admin). The main interface is divided into several sections:

- Run Panel (Left):** Displays analysis parameters for 'Zebrafish': Plate: 96 wells plate, UnKnown name : 12 X 8; Analyzed Region: A1 - A5; Fields: Varying number of fields per well; Magnification: 0\_0x; Focus: no focus; Tags: None; Time Lapse: None. A progress bar shows 29.i (orange) and a status bar shows 30 (orange) and a 'Running' button (grey square).
- Well List (Middle):** Shows a vertical list of wells with their analysis status: Fish [Trans] (grey), Yolk (yellow), Eye [Trans] (pink), Fin [Trans] (cyan), and Spine (green). Each well has a small thumbnail image and a color-coded dot.
- Main Image (Right):** A large grayscale image of a zebrafish embryo with a red bounding box around it.
- Plate Map (Bottom Right):** A 12x12 grid representing the 96-well plate. Wells A1-A5 are highlighted in green, indicating they have been analyzed. A '29.i' label is overlaid on the plate map.

# Using Athena with Sample Images

## Results Screen

**Explore** the quantified results!

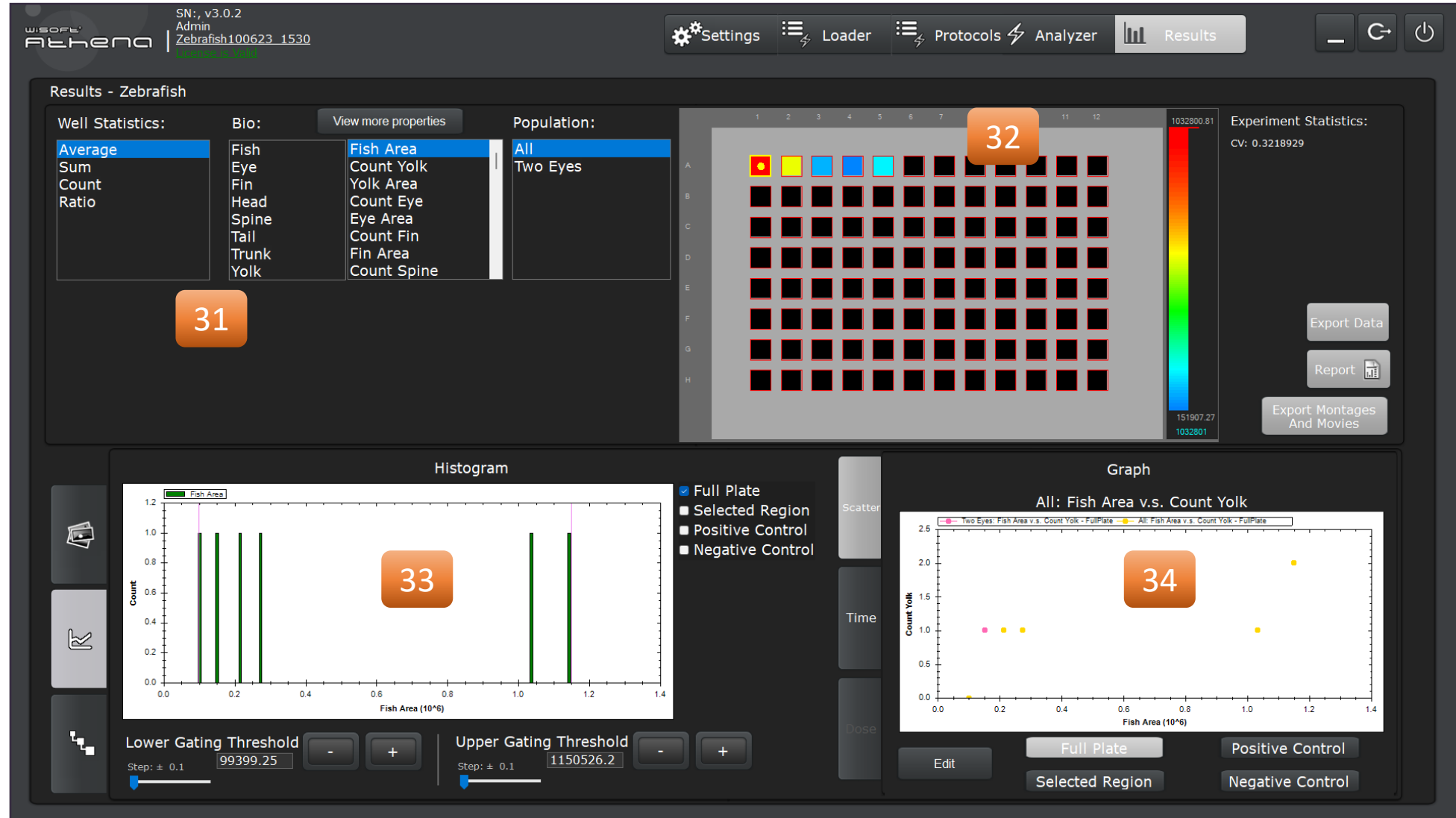
31. Choose the desired statistic, anatomy (Bio), property and population to view the data measured for each image (one image per well).

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.

WISOFI Athena SN: v3.0.2 Admin Zebrafish100623\_1530

Settings Loader Protocols Analyzer Results

Results - Zebrafish

Well Statistics: Average Sum Count Ratio

Bio: Fish Eye Fin Head Spine Tail Trunk Yolk

View more protocols

Fish Area Count Yolk Area Count Eye Area Count Fin Area Count Trunk Area Count Spine Area

Save To PDF

**Results:**  
To see all objects data click here

Figure 1: Statistics. Average of Fish Area  
[Click to See Values](#)

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

Graph  
Fish Area v.s. Count Yolk

Lower Gating Threshold Step: ± 0.1 99399.25

Upper Gating Threshold Step: ± 0.1 1150526.2

Edit Full Plate Selected Region Positive Control Negative Control

Experiment Statistics: CV: 0.3218929

Export Data Report Export Montages And Movies

# 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)

Name	Date modified	Type	Size
Hermes_Report_files	6/10/2023 5:25 PM	File folder	
DoseScatter.csv	6/10/2023 5:25 PM	Microsoft Excel C...	1 KB
Histogram.csv	6/10/2023 5:25 PM	Microsoft Excel C...	3 KB
Scatter.csv	6/10/2023 5:25 PM	Microsoft Excel C...	1 KB
Statistics.csv	6/10/2023 5:25 PM	Microsoft Excel C...	1 KB

# 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.

The screenshot displays the Athena software interface for Zebrafish results. The top navigation bar includes tabs for Settings, Loader, Protocols, Analyzer, and Results. The main window is titled "Results - Zebrafish" and features several panels:

- Well Statistics:** A list of statistical metrics including Average, Sum, Count, and Ratio.
- Bio:** A list of biological features such as Fish, Eye, Fin, Head, Spine, Tail, Trunk, and Yolk.
- Population:** A list of population categories including All and Two Eyes.
- Grid:** A 12x8 grid of wells (A-H, 1-12) with a color scale on the right ranging from 1032801 to 1032800.81.
- Experiment Statistics:** A panel showing CV: 0.3218929 and buttons for Export Data, Report, and Export Montages And Movies.

The "A1" well is highlighted in the grid, and the "Average" metric is selected in the Well Statistics panel. The "Fish Area" feature is selected in the Bio panel, and the "All" population is selected in the Population panel. The "A1" well is also selected in the Well Statistics panel. The "Bio" panel shows "Fish Area" selected. The "Population" panel shows "All" selected. The "Experiment Statistics" panel shows "CV: 0.3218929". The "A1" well is highlighted in the grid. The "A1" well is selected in the "Well Statistics" panel. The "Bio" panel shows "Fish Area" selected. The "Population" panel shows "All" selected. The "Experiment Statistics" panel shows "CV: 0.3218929".

# 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.

The screenshot displays the Athena software interface. At the top, it shows the user 'Admin' and the sample 'Zebrafish100623\_1530'. The main window is titled 'Results - Zebrafish' and contains a 'Well Statistics' table with 'Average' selected. Below this is a 'Bio:' section with a list of body parts like 'Fish', 'Eye', 'Fin', etc. The central image shows a zebrafish with various colored outlines (purple, green, red, yellow) representing different body segments. A scale bar indicates '800µm (22.0%)'. The right-hand panel displays quantified data for the tail, including perimeter, variance, long axis, axial ratio, and solidity. At the bottom, there are navigation controls and a 'Cancel' button.

Well Statistics:	Bio:	View more
Average	Fish	Fish
Sum	Eye	Count
Count	Fin	Yolk
Ratio	Head	Count
	Spine	Eye /
	Tail	Count
	Trunk	Fin A
	Yolk	Count

Tail:Tail Perimeter: 3.72mm  
Tail:Tail Variance: 6.5e+7  
Tail:Tail Long Axis: 0.67mm  
Tail:Tail Axial Ratio: 8.37  
Tail:Tail Solidity: 0.73

800µm (22.0%)  
Tail Area: .29mm<sup>2</sup>

Min:0 Threshold: N/A  
Max:65520 [0,65520] 23K  
Apply Threshold: To Well To Plate  
Curr Intens.: 39856 Clear Thresholds  
Log Show Saturation

X:2865, Y:349

# 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.

Results - Zebrafish

Well Statistics:

Average
Sum
Count
Ratio

Bio:

Fish
Eye
Fin
Head
Spine
Tail
Trunk
Yolk

Population:

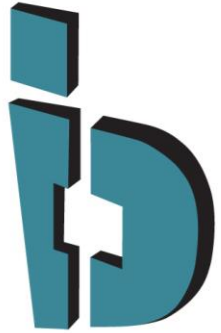
All
Two Eyes

Experiment Statistics:  
CV: 0.3218929

Export Data  
Report  
Export Montages And Movies

All  
Two Eyes

38



# IDEA Bio-Medical

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## Seeing Better



—  
Need some on-boarding support to get started?

Just email us at [info@idea-bio.com](mailto:info@idea-bio.com) or fill up our [contact form](#).

We'll be sure to contact you soon!