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ACD Spot Studio 1.0

User Guide

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ACD Spot Studio 1.0

User Guide

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* * *

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1 System Requirements

Please ensure your workstation meets the following requirements. For production usage please adopt the recommended specifications. ACD Spot Studio performance will always benefit from the fastest processors, good CPU cache, and fast memory and disk access.

1.1 Minimum

- Operating System: Windows 7 Professional 64-Bit
- CPU: Intel or AMD Quad Core 64-Bit 2.6 GHz+ *
- Memory: 8 GB
- Disk space: 100 GB plus space for image storage
- Display: 1440 x 1080 +
- USB port for dongle

1.2 Recommended

- Operating System: Windows 7 Professional 64-Bit
- CPU: Intel Xeon/i7 or AMD 64-Bit 3.00 GHz+ with eight or more logical processing cores *
- Memory: 4 GB plus 1 GB per logical processing core
- Disk space: 200 GB plus space for image storage
- Display: 24", 1920 x 1200 +
- USB port for dongle

* ACD Spot Studio will benefit from additional CPU cores and higher processor speed.

2 Licensing and Installation

2.1 Things You Need Before Installing

2.1.1 ACD Dongle

The ACD dongle must be plugged into a USB port to validate the program – it will not function if the dongle is not present.

The licensing is based upon a fixed number of analyses, for example 3,000 over a one-year period. When the number of analyses reaches a certain point – typically ten per cent of the purchased number or one month before expiry – you will see a warning dialog.

If you have used up your quota of analyses, you can still open the application and preview slides, but will no longer be able to generate results.

2.1.2 The ACD Spot Studio License Manager

Figure 2.1. License Manager

The License Manager displays the following information:

- The product serial number
- The product expiry date
- Number of remaining analyses

Click on the Load License button to top up your allocation with a newly purchased license. The Export License button will export licensing information as a text file.

2.1.3 User Accounts and User Rights

Ensure you have a valid user account with local administrator rights. This confers read/write access to file storage for image data and results.

2.2 Running the Installer

Double-click on the installation (.exe) file to begin the installation process.

Follow the on-screen instructions – the installer will give you options to change the name and location of the installation directory.

3 Spot Studio Workflow

3.1 Overview

The basic data structure in Spot Studio is:

Project → Image → ROI Set → ROI

(where ROI is a region of interest)

At the start of an analysis you create a project into which you can import multiple images. Within the project you can create ROIs, which are in turn grouped into ROI sets. Spot Studio will detect cells, clusters and spots, as well as the number of estimated spots (for more details see *Results* on page 16).

When you have optimized the image recognition you can run the analysis and export the results – it is at this point that analyses are deducted from your license allocation (one license per ROI set).

3.2 The Workflow Pane

The steps necessary to perform your image analyses are organized in the Workflow tab (figure 3.1). Tasks are organized sequentially from left to right – when you have finished configuring a function, you can move on to the next tab.

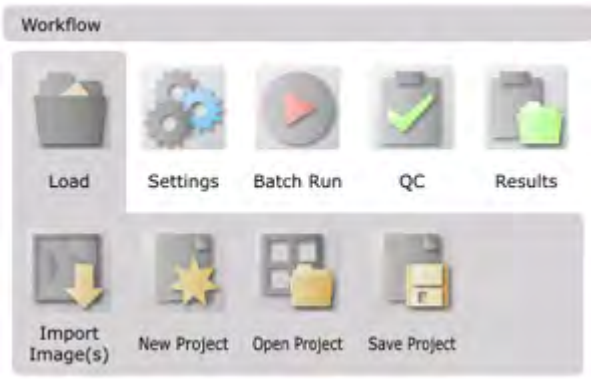

Figure 3.1. Workflow pane with Load tab active

- *Load* below functions let you import images and organize them into projects
- In *Settings* on page 9 you define regions of interest for your analysis and configure the thresholds for the image recognition
- *Batch Run* on page 14 functions analyze the images based on your configuration settings
- *QC* on page 15 lets you refine your image recognition and delete any unwanted cells
- The *Results* on page 16 tab lets you export your results (this tab will normally be inactive upon opening a new project)

3.3 Loading Images and Creating Projects

This section describes the functions available in the Load tab (figure 3.2).

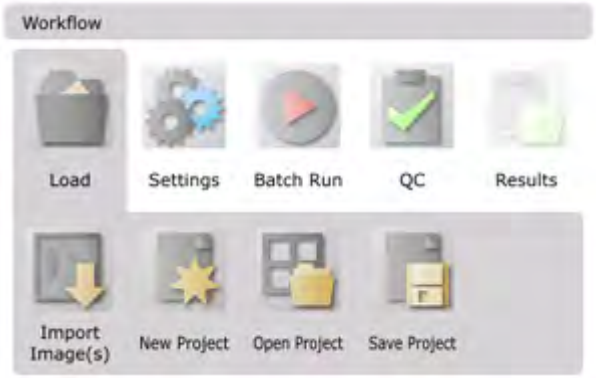

Figure 3.2. Functions available in the Load tab

3.3.1 Import Image(s)

Use the Import Image(s) button to import image files into Spot Studio. Multiple file import is supported – use the Shift or Ctrl keys to select multiple files in the Load Images dialog.

Spot Studio supports the following file formats:

- Aperio (.svs)
- Leica (.lif, .svn)
- Zeiss (.zvi)
- Generic formats supported by the GDAL driver (these include .jpg, .tif, .png, and .bmp – see the GDAL website[1] for a complete list).

When the Load tab is selected, the filenames of imported slides are listed in the left-hand pane (see figure 3.3). Double-click on a slide name to display the slide image (the currently active slide will be highlighted in bold), or right-click on the filename and

1. http://www.gdal.org/formats_list.html

select Open. To remove a slide from the list, press the Delete or Backspace key on your keyboard or right-click on it and delete it via the context menu.

The *State column* on page 14 displays image analysis information.

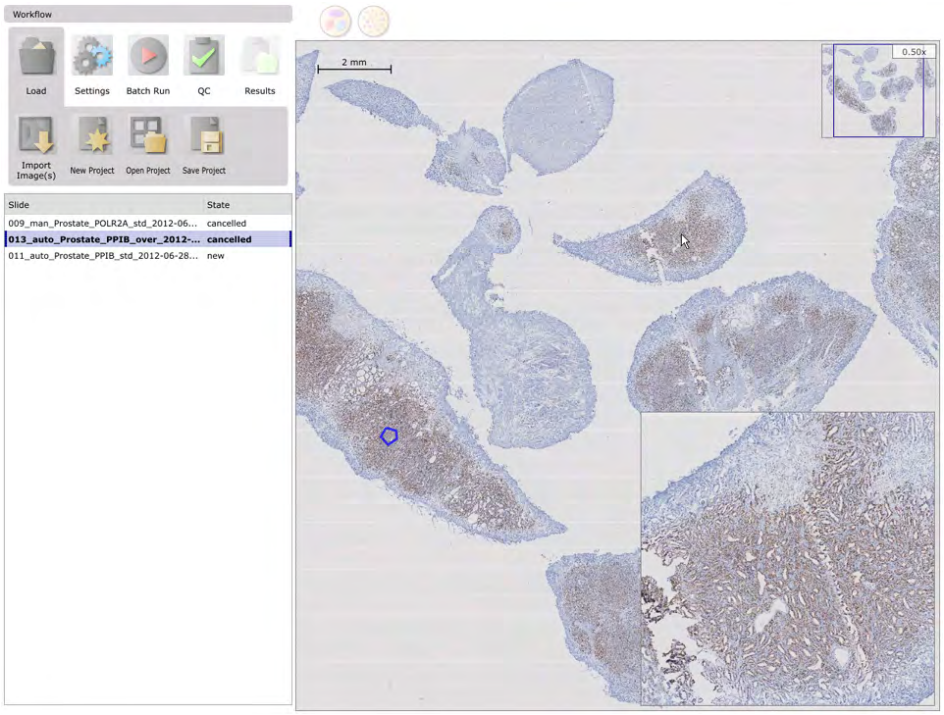

Figure 3.3. Open project with Overview (top-right) and Magnifier (bottom-right) activated

Viewing Options

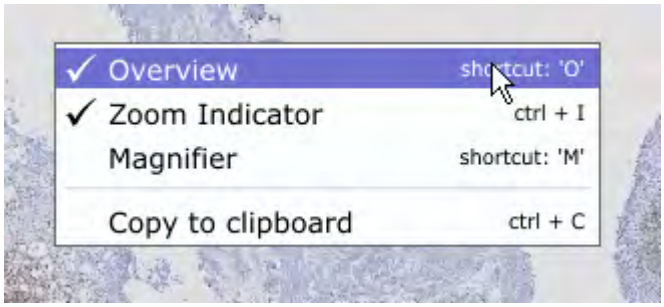

Figure 3.4. Context menu on right mouse-click

Most viewing options are available by right-clicking on the active slide (figure 3.4). The associated keyboard shortcuts are also displayed.

A scale bar is permanently visible in the top left of the slide view.

Moving the image To move the image, simply hold down the mouse button and drag it.

Overview By default, the Overview pane is visible at the top-right of the window, which displays a thumbnail of the whole slide and the current magnification. The rectangle on the Overview is the active view – you can drag this rectangle with the cursor to change the magnified view, or move the whole image by holding down the mouse button and dragging it.

Zoom Use the mouse wheel or the +/- keys to zoom in and out of the image.

Zoom Indicator When activated, displays the zoom level in the Overview pane.

Magnifier When activated, displays a pane in the lower right-hand corner, displaying an enlarged view of the area under the mouse pointer.

Copy to Clipboard Copies the current slide view to the clipboard, including any active panes (such as the Magnifier).

3.3.2 New Project

Creates a new project. A project name is generated automatically and displayed in the title bar of the window. If you press the New Project button while an unsaved project is already open, you will be prompted to save that project.

3.3.3 Open Project

Opens an existing project.

3.3.4 Save Project

Saves an open project.

> **NOTE**: Spot Studio project files have the extension .acd. A project saved with the filename `New Project (1).acd` will be automatically created inside a folder called `New Project (1)`. When you open a previously saved project, you will have to open its parent folder first.

List Options

Right-clicking on a list header (figure 3.5) displays various options depending on the list type, but essentially allows you to show or hide particular columns and provides auto-sizing options for them.

Figure 3.5. Header options on right mouse-click

3.4 Settings

In Settings you can define regions of interest (ROIs) for analysis and modify the threshold settings for image recognition.

Figure 3.6. Functions available in the Settings tab

The Settings tab (figure 3.7) in the Workflow pane has two buttons (see *Loading and Saving Threshold Settings* on page 14):

- *Load Settings* lets you load previously configured settings
- *Save Settings* saves your current settings.

The settings in this instance are the ones defined in the *Define Threshold Settings* on page 11 pane. (To save changes to the project, use the Save button.)

3.4.1 ROIs and ROI Sets

In ACD Spot Studio you define regions of interest (ROI) by drawing freehand shapes or polygons around regions you want to analyze. To organize your analyses, you group single or multiple ROIs into an ROI set.

In the Settings view, there are three available views – analogous to the slide list in the Load tab – that display ROI and slide information (figure 3.7).

In the ROI and ROI Set views, you can use the Prev and Next buttons to navigate between slides. The Slide view is the same as the feature in *Load* on page 6.

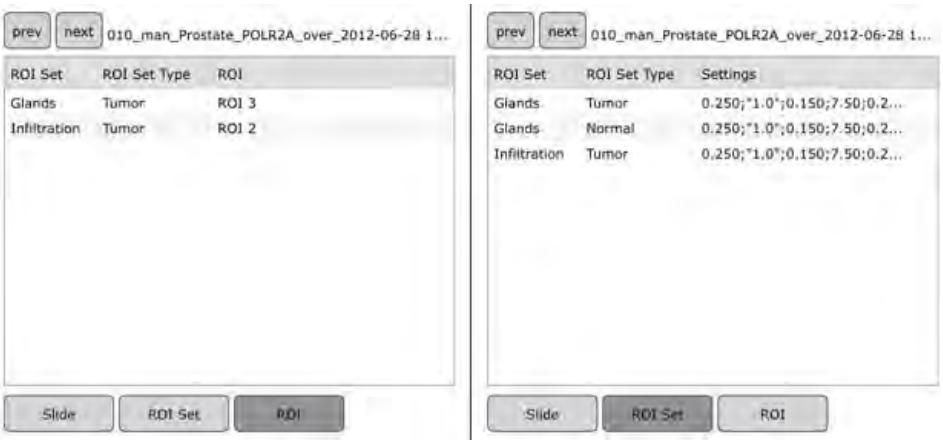

Figure 3.7. ROI Set and ROI views in the Settings tab

Slide View

Press the Slide button to display the list of slides (this is the same list as the one in the Load tab)

ROI Set View

Before you can draw a region of interest, you must first make a set – do this by pressing the Create ROI Set button. You can change the default ROI set name and add descriptive text in the ROI Set Type field if you wish. The Settings column displays the *threshold settings* on the facing page. When you draw an *ROI* below, it will be assigned to the currently active ROI set (in either ROI Set view or ROI view)

ROI View

ROI View displays individual regions of interest, along with the ROI set and set type. Selecting a ROI in this view will highlight it on the slide (and vice-versa).

3.4.2 Creating a ROI

Assuming you have already created a ROI Set, create a region of interest by clicking the Add ROI button and drawing a selection on the slide. There are two drawing options (figure 3.8):

1. Click on individual points to define the nodes of a polygon
2. Draw a freehand shape by moving he mouse while keeping the button held down.

It is possible to combine polygon lines and freehand paths in a single selection.

Spot Studio will close a shape automatically when the end-point meets the origin of your shape and you release the mouse button. Alternatively double-click to close the selection.

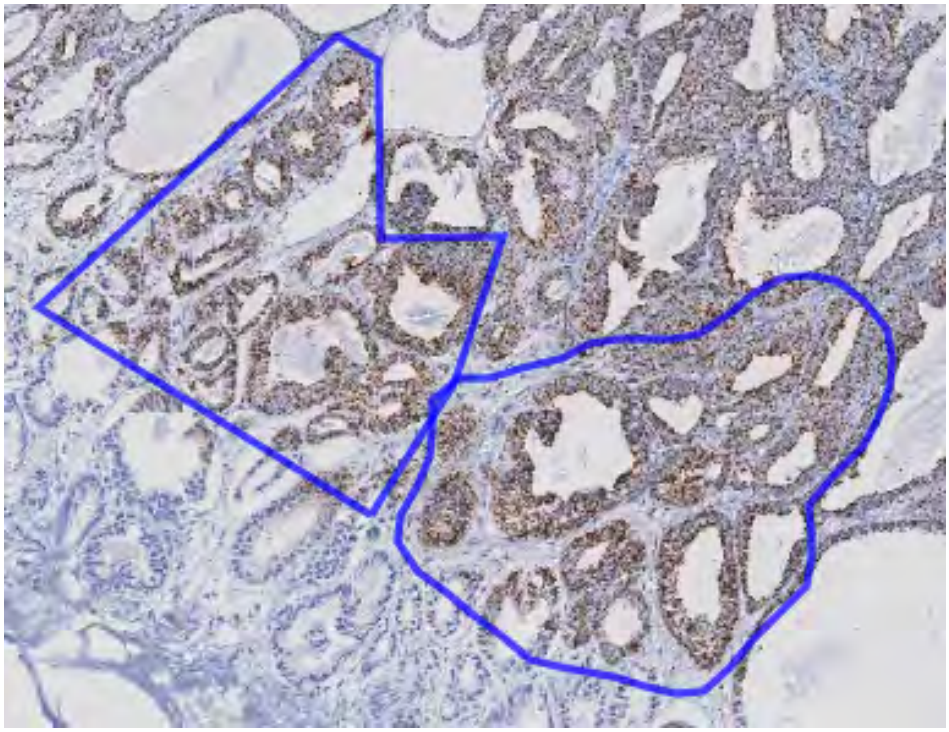

Figure 3.8. Polygon (left) and freehand (right) ROI selections

In the ROI view, clicking on an ROI in the list will highlight the shape on the slide; likewise, selecting a shape will highlight it in the list.

Editing ROIs

- To delete an ROI set, press the Delete ROI Set button while in ROI Set view (you can also right-click on a set and select this option from the context menu, or press the Delete or Backspace button on your keyboard). This will delete all the individual ROIs in the set
- To delete a single ROI, select the shape on the slide or highlight it in ROI view and press the Delete or Backspace key on your keyboard. A Delete function is also available in the context menu in ROI view
- To edit the shape of an ROI, right click it in the list in ROI view and select Edit. This will highlight the nodes, which you can then drag to new positions.

3.4.3 Threshold Settings

In Define Threshold Settings (figure 3.9), you can select the type of stain to be detected (DAB or FAST Red) and change the sensitivity of the detection criteria. Changes will directly be applied to the currently selected ROI set.

- To test your settings, press the Preview button and click on a region of the slide (the pointer will change to a crosshair) – the software will apply the settings to a small region around this seed point

Figure 3.9. Define Threshold Settings pane

- Use the Apply button to apply the current settings to other ROI sets on this or other slides. This will launch a dialog box containing combinations of open slides and ROI sets
- The Reset button returns all settings to system defaults
- The Undo button restores settings to previously applied settings.

Optimizing Settings

This section explains how to change the threshold settings to get the optimal result for your images.

In figure 3.10 on this page, all settings are optimal for the detection of cells in the image.

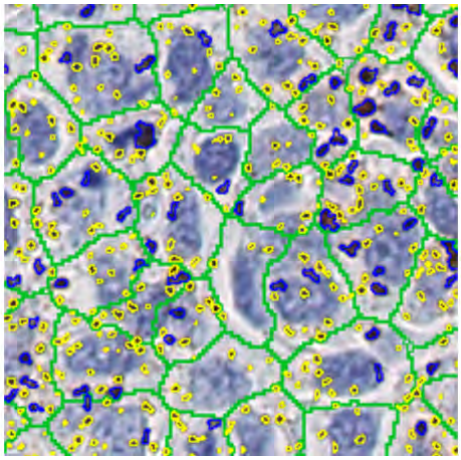

Figure 3.10. Optimal detection settings for nucleus diameter (9.0), spot stain level (0.2) and spot diameter (1.2)

Hematoxylin Level Settings The Hematoxylin Level should be lowered if cells with faint nuclei are not found, and should be raised if too many cells are detected.

Nucleus Diameter Settings The Nucleus Diameter will influence the size of the cells and how nuclei are split. Low values correspond to small nuclei and more splitting of hematoxylin clusters. Large values correspond to big nuclei or less splitting of hematoxylin clusters. Examples of too-low, and too-high settings are shown in figure 3.11 on the current page.

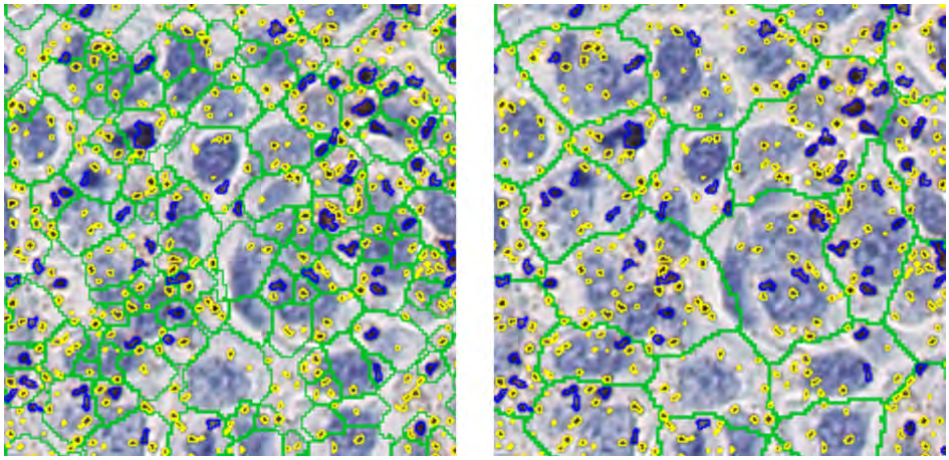

Figure 3.11. Detection for nucleus diameter settings that are set too low (left, 4.0) and too high (right, 18.0)

Spot Stain Level Settings The Spot Stain Level will define the intensity baseline for the RNA spot stain (DAB/Fast Red). Low values will find less intensely stained spots, high values will ignore them. Examples of too-low and too-high settings are shown in figure 3.12 on this page.

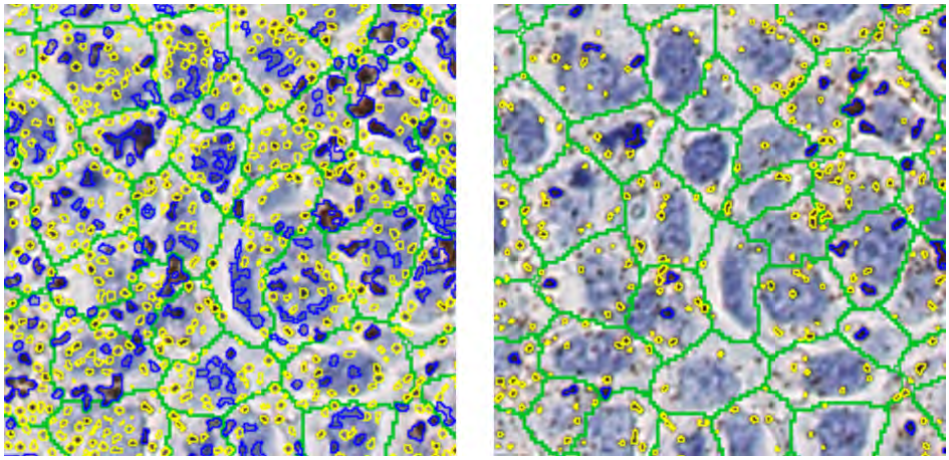

Figure 3.12. Detection for spot stain level settings that are set too low (left, 0.05) and too high (right, 0.8)

Spot Diameter Settings The Spot Diameter value will influence the minimum size of spots that will be detected and the size of spot stain that will be considered a cluster (rather than a single spot). Low values lead of smaller spots being detected and a smaller

spot stain regions being classified as cluster, and vice-versa for high values. Examples for too-low and too-high settings are shown in figure 3.13 on the current page.

Spot Diameter also influences the contribution of the cluster area to the number of estimated spots.

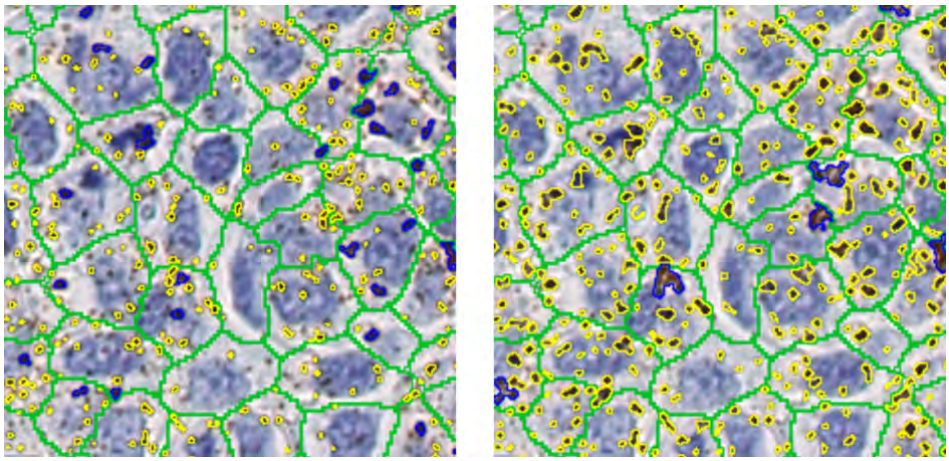

Figure 3.13. Detection for spot diameter settings that are set too low (left, 0.6) and too high (right, 2.4)

Saving and Loading Threshold Settings

Once you have optimized your threshold settings, you can save them to use in other projects. To do this, press the Save Settings button in the Settings tab, which saves settings as a *.stt file.

For housekeeping purposes, you may want to save the settings (*.stt) file into the project folder (if it is specific to that project) or to another convenient location if it will be used across multiple projects.

Use Load Settings to import previously saved threshold settings.

3.5 Batch Run

Once you have defined your settings, click on Batch Run to run the analysis. To analyze individual ROI sets, click on them in the list and press the Analyze Selected button. Alternatively you can analyze all open slides by clicking on Analyze All Images. To stop the analysis, press Cancel Processing.

The analysis may take some time, depending on your hardware and the size and number of regions. The following status messages may be displayed in the State column:

- Created
- New
- Processing
- Waiting (when no current analysis engine is available)

Figure 3.14. Functions available in the Batch Run tab

- Done
- Partially Processed (when subsets of images have been analyzed but others have not)
- Failed

> **NOTE**: Running the batch processing step does not use any of your license allocation.

3.6 QC

Figure 3.15. Functions available in the QC tab

The QC (quality control) tab lets you refine your image analysis by deleting cells. Double-click on an ROI set to display the segmentation. The two large buttons at the top of the screen let you display cell classification or spot classification. In figure 3.16 on the following page the following classification options are displayed (l-r):

- Spot and cell classification

- Spot classification only
- Cell classification only.

You can select image objects by clicking on them with Select Cell activated. You can also select multiple cells by holding down the mouse button and drawing a shape around them.

To remove cells, select them and press the Remove Cell button in the Edit Cells pane. Undo and Undo All options are also available. Press Save to update your saved project file.

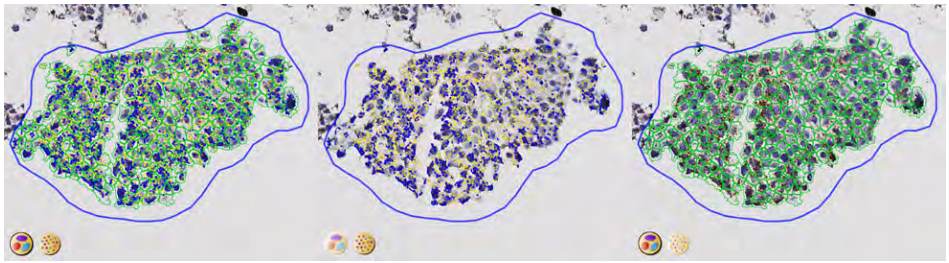

Figure 3.16. Spot classification views in Spot Studio

When you are happy with all your classifications, select the QC checkboxes by your ROI sets (figure 3.17).

After this step is complete, the Export options become active. To export results, select the relevant Export checkboxes then press the Export Results button. A Confirm Export dialog will appear, as each export will use up one of your allocated analysis runs.

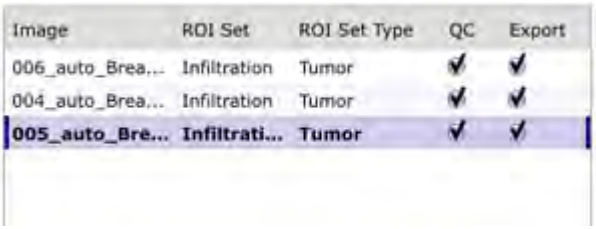

Figure 3.17. List view with QC and Export options

> **NOTE**: It is not necessary to select every QC or Export checkbox individually. Simply select multiple rows (Ctrl and Shift selection is supported) then tick one box to select all checkboxes.

3.7 Results

In the Results tab, the default view is Table View with a list of slides in the left-hand pane and analysis data in the right-hand pane (figure 3.19) – double-click on a slide or ROI set

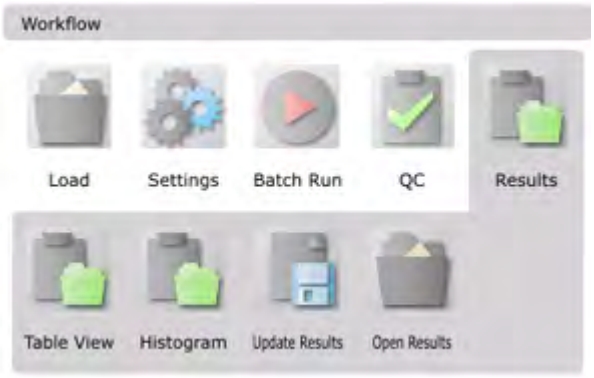

Figure 3.18. Functions available in the Results tab

to display the corresponding results. The Results tab is generally inactive until you have completed the analysis and QC steps.

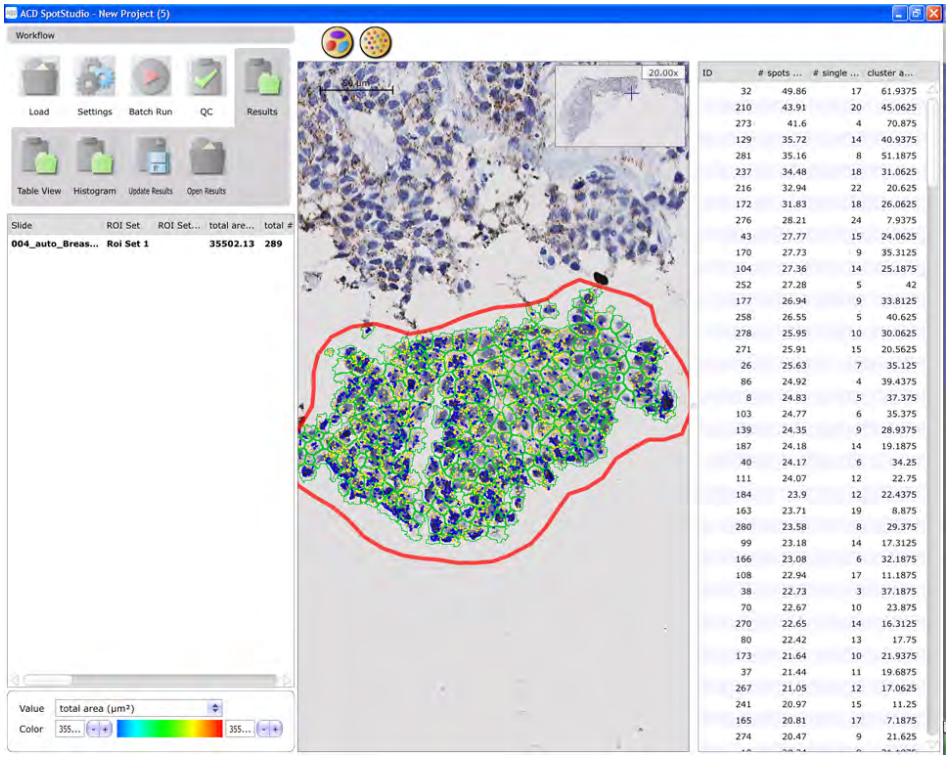

Figure 3.19. Results with Table View active

In Table View, data is displayed in a list. The colors of the list entries correspond to a heat map, with colors at the red end of the spectrum being the highest, and lowest at the blue end. Select the value of interest in the drop-down box and assign minimum and maximum values for each value.

Click Histogram to display your results as a vertical bar chart (figure 3.20). The Update

Results button updates the exported statistics based on the histogram settings.

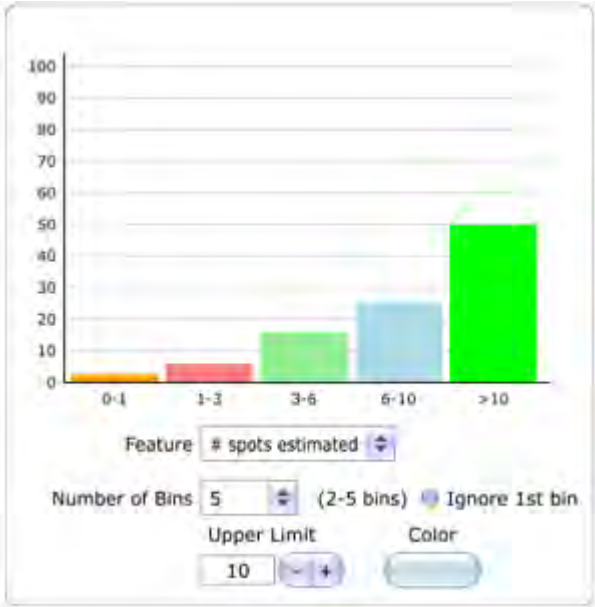

Figure 3.20. Histogram view

3.7.1 Histogram Options

Feature

Choose from:

- # Spots Estimated
- # Single Spots
- Cluster Area.

Number of Bins

Select a number between two and five.

Ignore 1st Bin

Check to remove the first column.

Upper Limit

Change this value to change upper limit of the distribution.

Color

Select a bin and change its color by pressing the Color lozenge.

3.7.2 Open Results

The Open Results button opens the containing folder where the results are stored – this folder is named 'results' and is inside the project folder. Inside this folder are two .csv files and a subfolder:

- The subfolder 'histograms' stores all generated charts as .jpg images
- The .csv file 'cell_statistics.csv' contains data for the following:
  - Slide
  - ROISet
  - ROISet Type
  - ID
  - # spots estimated
  - # single spots
  - cluster area
- The .csv file 'roi_set_statistics.csv' contains data for the following:[2]
  - Slide
  - ROISet
  - ROISet Type
  - total area (µm²)
  - total # cells
  - total # spots estimated
  - # spots estimated per µm²
  - average # spots estimated per cell
  - median # spots estimated per cell
  - total # single spots
  - # single spots per µm²
  - average # single spots per cell
  - median # single spots per cell
  - total cluster area (µm²)
  - relative cluster area
  - average cluster area per cell (µm²)
  - median cluster area per cell (µm²)
  - # cells in bin 1
  - # cells in bin 2
  - % cells in bin 1
  - % cells in bin 2

2. This example assumes the histogram contains two bins.

Acknowledgments

Portions of this product are based in part on the third-party software components. is required to include the following text, with software and distributions.

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