

# Operating Manual

VisionWorks®

Capture and Analysis Software



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## 1.0 Introduction

VisionWorks® capture and analysis software brings imaging workflow automation into a touch screen interface. Customizable one-touch actions will allow you to automate your imaging experiments for repeatable imaging results. This guide will outline all the tools included within VisionWorks® and provide guidance on best imaging practices using VisionWorks®.

VisionWorks® is compatible with the following Analytik Jena systems:

- UVP GelSolo
- UVP SoloElite
- UVP GelStudio
- UVP GelStudio PLUS
- UVP ChemStudio
- UVP ChemStudio PLUS
- UVP iBox Studio
- UVP iBox Scientia
- UVP iBox Explorer2

VisionWorks® is license-free and may be installed on any desktop or laptop system running Windows 10. Please reach out to Analytik Jena US Technical Support for installation instructions.

Note: VisionWorks® may be installed on Mac computers but will need Windows 10 to be installed through Apple Boot Camp, VMware Fusion, Parallels or any other virtual machine software.

System Requirement	
Operating System	Microsoft Windows 7, 8, 10
Processor	Intel Pentium N4200 or AMD A9-9420
Memory	4GB RAM
Disk Storage	2GB available free space

## 2.0 Major Updates for VisionWorks

This section will provide a quick overview of the new changes in VisionWorks

Change Type	Release Summary
F	Added VisionWorks Secure Mode ( <a href="#">Section 13.2</a> )
F	Added Audit Log Viewer ( <a href="#">Section 13.2.9</a> )
F	Increased Series Capture Frames ( <a href="#">Section 4.3</a> )
F	Increased Number of Area Density Grid ROI tool
B	Fixed Create Action Crash on UVP SoloElite
B	Fixed Auto Save for CHM and BLI Capture Mode
S	Updated the Prolific Driver

Legend	
Abbreviation	Description
F	Feature addition/modification
UI	User interface change
B	Bug fix
S	Service change

## 3.0 Getting Started with VisionWorks®

This section outlines the major interface elements of VisionWorks® and how to navigate around the software.

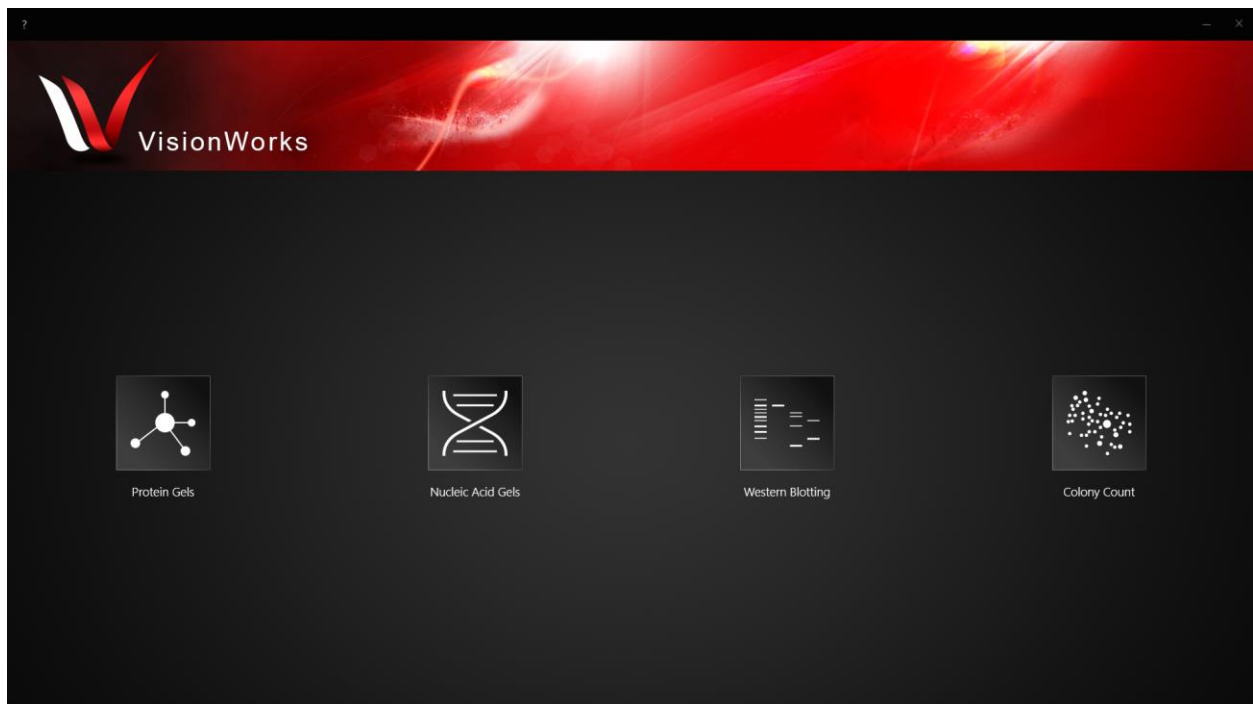
### 3.1 VisionWorks® Interface

#### 3.1.1 Standard Home Screen

Upon start-up, VisionWorks® will display up to four icons with each corresponding to the available applications for your system.

The home screen below will be present on the following systems:

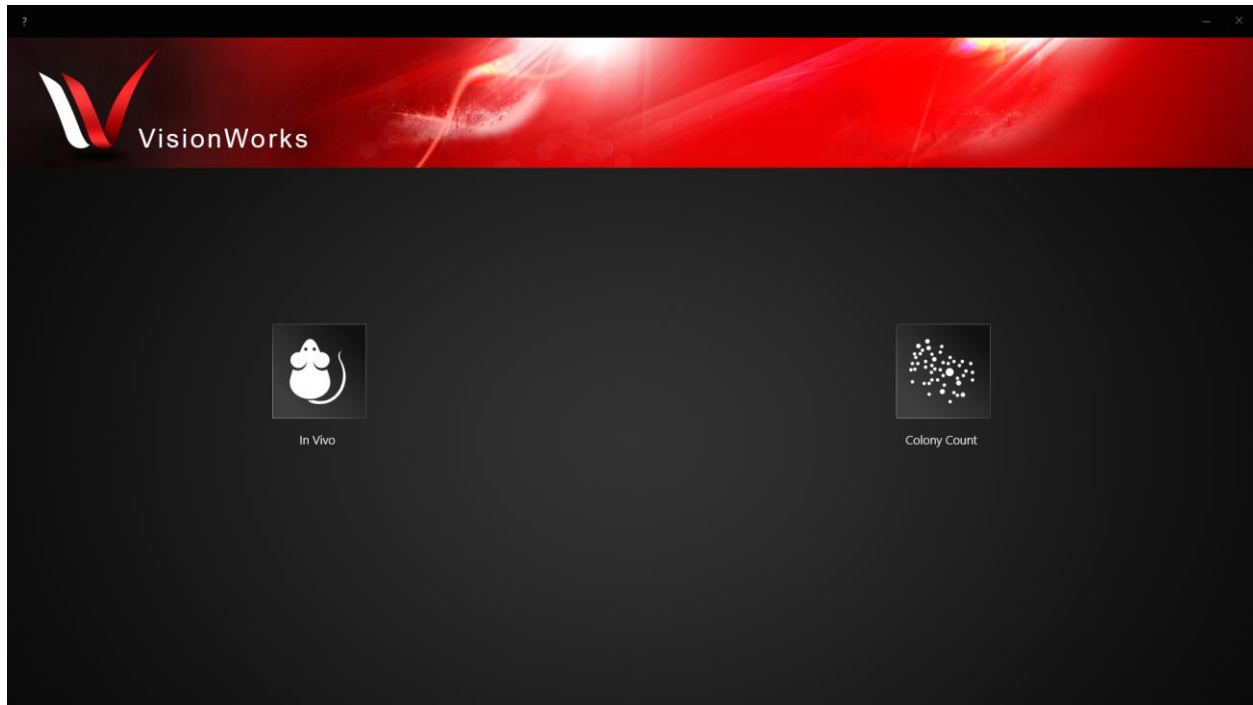
- UVP GelSolo
- UVP SoloElite
- UVP GelStudio
- UVP GelStudio PLUS
- UVP ChemStudio
- UVP ChemStudio PLUS



### 3.1.2 VisionWorks® *in vivo* Home Screen

The home screen below will be present on the following *in vivo* imaging systems:






- UVP iBox Studio
- UVP iBox Scientia
- UVP iBox Explorer2



## 3.2 List of VisionWorks® Application Icons

Once you select an application, the main interface of VisionWorks® will open up. The following sections will outline the major interface elements of VisionWorks® and provide an overview of image acquisition and data analysis.

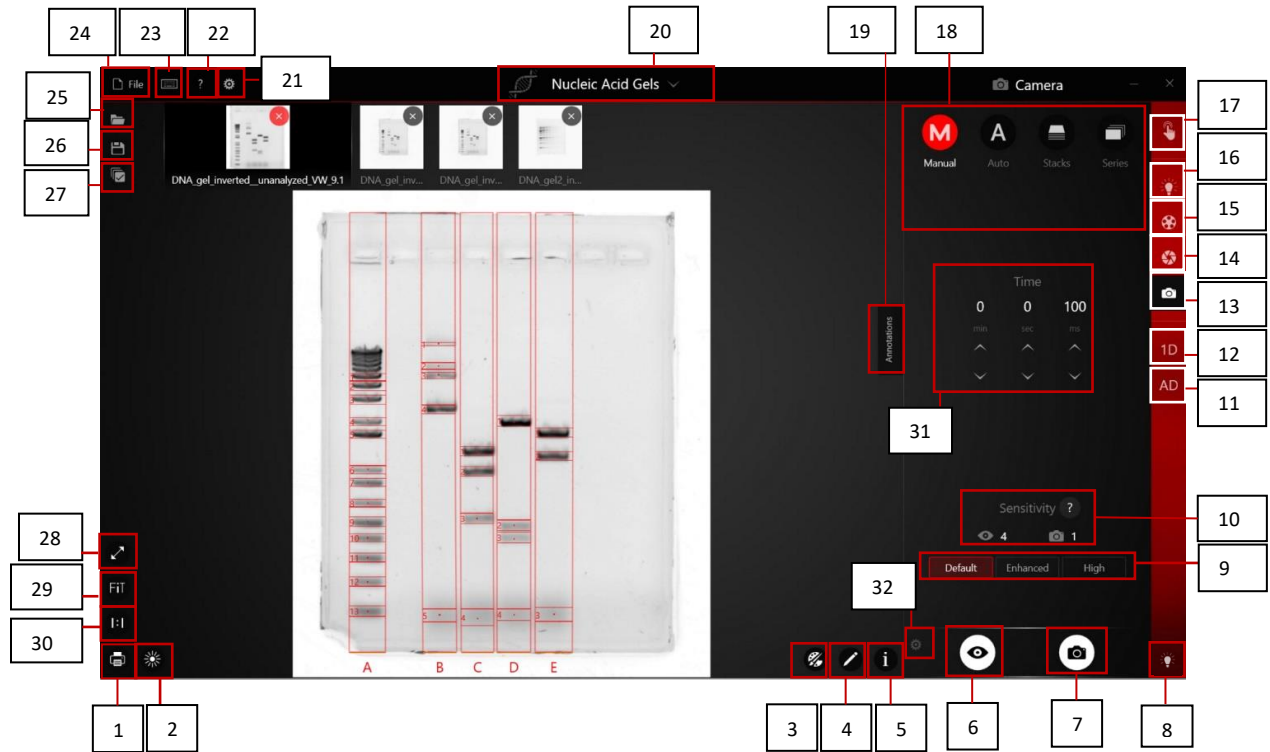
Based on your system configuration, the home screen will display application icons. The table below summarizes which icons should appear for your system:

Protein Gel	Nucleic Gel	Western Blotting	Colony Count	<i>in vivo</i>
				

The interface inside each application area is consistent, with access to similar features. To simplify workflows, VisionWorks® will show only certain features depending upon the application. For instance, if you purchased the iBox Studio *in vivo* imager, the 1D analysis feature is not displayed. This is because this analysis feature has little relevance for *in vivo* work. Should this feature become relevant, the user may change the available analysis options in the settings menu.

System	Protein Gels	Nucleic Acid Gels	Western Blotting	Colony Count	<i>in vivo</i>
UVP GelSolo	✓	✓		✓	
UVP GelStudio	✓	✓		✓	
UVP GelStudio PLUS	✓	✓		✓	
UVP ChemStudio	✓	✓	✓	✓	
UVP ChemStudio PLUS	✓	✓	✓	✓	
UVP iBox Studio				✓	✓
UVP iBox Scientia				✓	✓
UVP iBox Explorer2				✓	✓

### 3.3 Advanced Main Interface Elements



#### Main Interface Icon Descriptions

Icon	Description
1	Quick Print
2	Saturation Warning: only appears when the signal is saturated
3	Histogram adjustment and Pseudocolor
4	Image Editing
5	Image information
6	Preview
7	Capture
8	Lighting indicator: will blink to remind user the light is on
9	Preset binning settings <ol style="list-style-type: none"> <li>1) <b>Default:</b> 1x1 Capture binning</li> <li>2) <b>Enhanced:</b> 2x2 Capture binning</li> <li>3) <b>High:</b> 4x4 Capture Binning</li> </ol>
10	Capture and Preview Binning Manual Adjustment
11	Area Density Analysis
12	1D Analysis
13	Camera Settings

<b>14</b>	Lens Settings
<b>15</b>	Filter Settings
<b>16</b>	Lighting Options
<b>17</b>	One-touch Action
<b>18</b>	Capture Modes
<b>19</b>	Annotation Floating Bar
<b>20</b>	Application drop down list
<b>21</b>	Software Settings
<b>22</b>	Help
<b>23</b>	Keyboard
<b>24</b>	File: more open & save options and image processing options (e.g. Merge, Compositing, etc.)
<b>25</b>	Open Images
<b>26</b>	Save Images
<b>27</b>	Select multiple images to view, close or save
<b>28</b>	Maximize captured image
<b>29</b>	Zoom to fit
<b>30</b>	Display image at 1:1 actual pixel size
<b>31</b>	Manual Capture exposure time settings
<b>32</b>	Click to access pre-capture modification tools: <ol style="list-style-type: none"><li>1) <b>Auto Histogram</b> – System automatically determines histogram limits.</li><li>2) <b>Noise Reduction</b> – Apply level 1 Noise Reduction to image. Note: raw data will be modified if Noise Reduction in Pre-capture settings is turned on.</li><li>3) <b>Invert</b> – Inverts image colors</li><li>4) <b>Pre-capture pseudocolor</b> – Applies pseudocolor to Live View, carries over to capture.</li><li>5) <b>Crop</b> - Sets target percentage of original image size. Live View menu, replaces gallery only when Live View is on:</li><li>6) <b>Record</b> – Make a recording of the Live View.</li><li>7) <b>Guides</b> – Show patterns that can help for sample positioning.</li><li>8) <b>Intensity Range</b> – Intensity range of Live View image.</li><li>9) <b>Noise Baseline Adjustment</b> – Set baseline intensity, affects Live View image only. Helps to reduce “salt-and-pepper” noise in Live View.</li><li>10) <b>AE Size %</b> – Only available when Auto capture mode is selected for Live View. Set Region of interest to apply Auto Exposure.</li></ol>

---

## VisionWorks® Navigation

### Image Capture Icon Descriptions



**One touch Action** – This menu gives access to the preset actions



**Lighting** – Provides access to “*Transilluminator*” and “*Overhead lighting*” controls.



**Filters** – Provides access to the “*Filter Wheel*” control. Use the “*Filter*” control wheel to load your desired filters.



**Lens** – Provides access to “*Focusing*” and “*Brightness*” controls. Auto Focus will be available when EPI White light and Live View is on.



**Camera** – This tab contains all camera modes, settings and parameters for image capture.



**Micro** – Provides access to “*Microscope*” controls, will only appear if a UVP compatible microscope is connected.

### Analysis Features





















**1D Analysis** – Provides access to 1D lanes and bands identification and analysis tools.



**Area Density** – Provides access to area density identification and analysis tools.



**Colony Counting** – Provides access to colony counting/classification and analysis tools.

Annotation Floating Bar Icon Descriptions	
	<b>Hand Tool</b> – Deactivate selected annotation tool.
	<b>Pixel Intensity</b> – Click to activate pixel intensity tool.
	<b>Multi Selection</b> – Click to activate multi selection tool for annotation. Select multiple annotations to move, resize and edit.
	<b>Heatmap</b> – Click to add a heatmap.
	<b>Timestamp</b> – Click to add a timestamp on the captured image.
	<b>Export</b> – Export selected annotations.
	<b>Import</b> – Import previously saved annotations.
	<b>Copy</b> – Copy selected annotations.
	<b>Paste</b> – Paste previously copied annotations.
	<b>Delete</b> – Delete selected annotations.
	<b>Annotation menu</b> – Scroll up and down to select annotation tool: angle, standard bar, line, rectangle, text, circle, free shape.
	<b>Fill color</b> – Click to change the fill color of annotation.
	<b>Line color</b> – Click to change the outline color.
	<b>Text</b> – Click to change the color of text annotations.
	<b>Text size</b> – Scroll up and down to change the size of the selected text annotations.
	<b>Horizontal alignment</b> – Align selected annotations horizontally.
	<b>Vertical alignment</b> – Align selected annotations vertically.
	<b>Annotation transparency</b> – Scroll up and down to change the annotation transparency.

### Image Post-processing Icon Descriptions



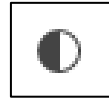
**Image Adjusting**— gives access to the following menu options:



**Histogram** – Provides access to auto and manual histogram adjustment.



**Image Brightness** – Provides access to adjust image brightness.



**Image Contrast** – Provides access to adjust image contrast.



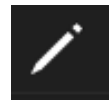
**Image Gray Level** – Provides access to adjust image gray level.



**Image Invert** – Click to invert the captured image.

Pseudocolor

**Image Pseudocolor** – Scroll up and down to change the pseudocolor of captured image.



**Edit** – Provides access to image editing tools, which impact the metadata of the image.



**Undo and Redo** – Undo and redo changes.

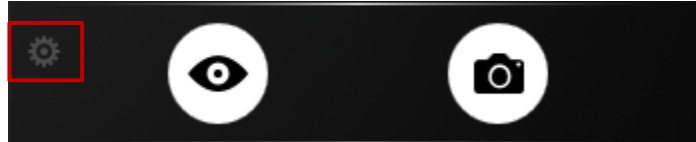


**Image Information** – Displays image history and image metadata for selected/active image in the thumbnail gallery.

## 4.0 VisionWorks® Acquisition

### 4.1 Pre-capture Settings

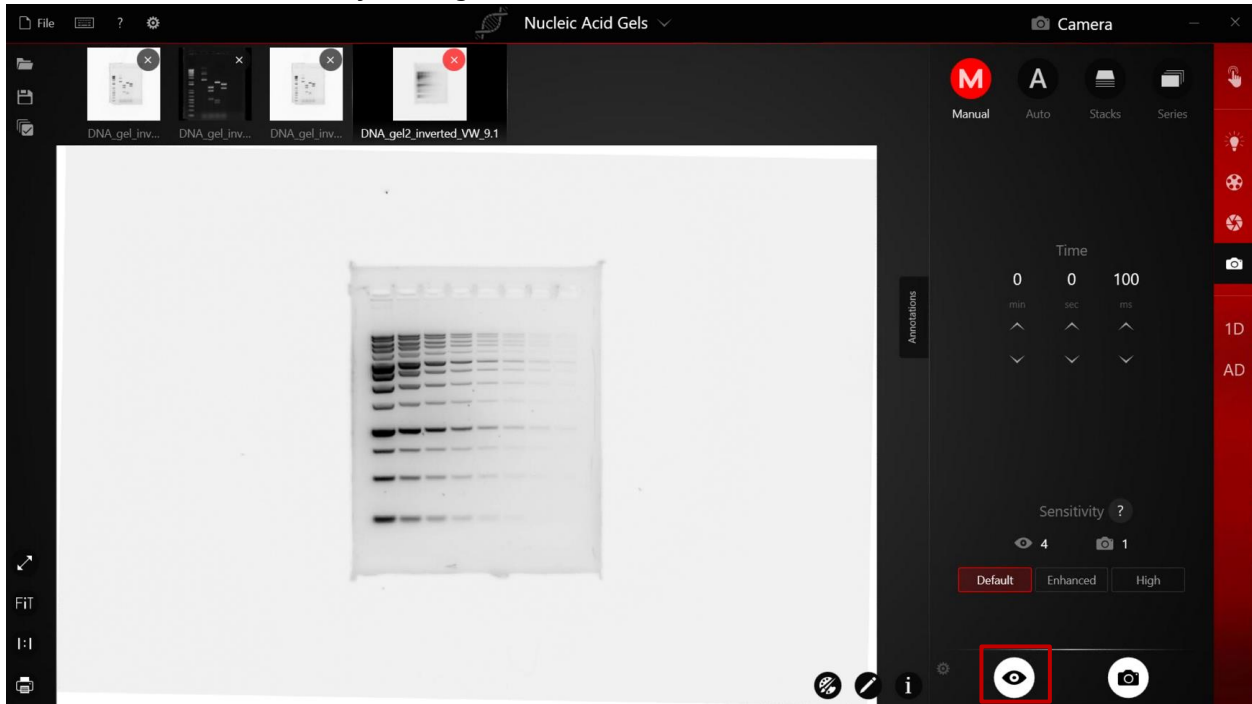
In Pre-capture settings you can activate Auto Histogram, select Invert or a Pseudocolor, and adjust the image crop size. Parameters set in pre-capture settings will be applied to captured image. These features are described in the table below:



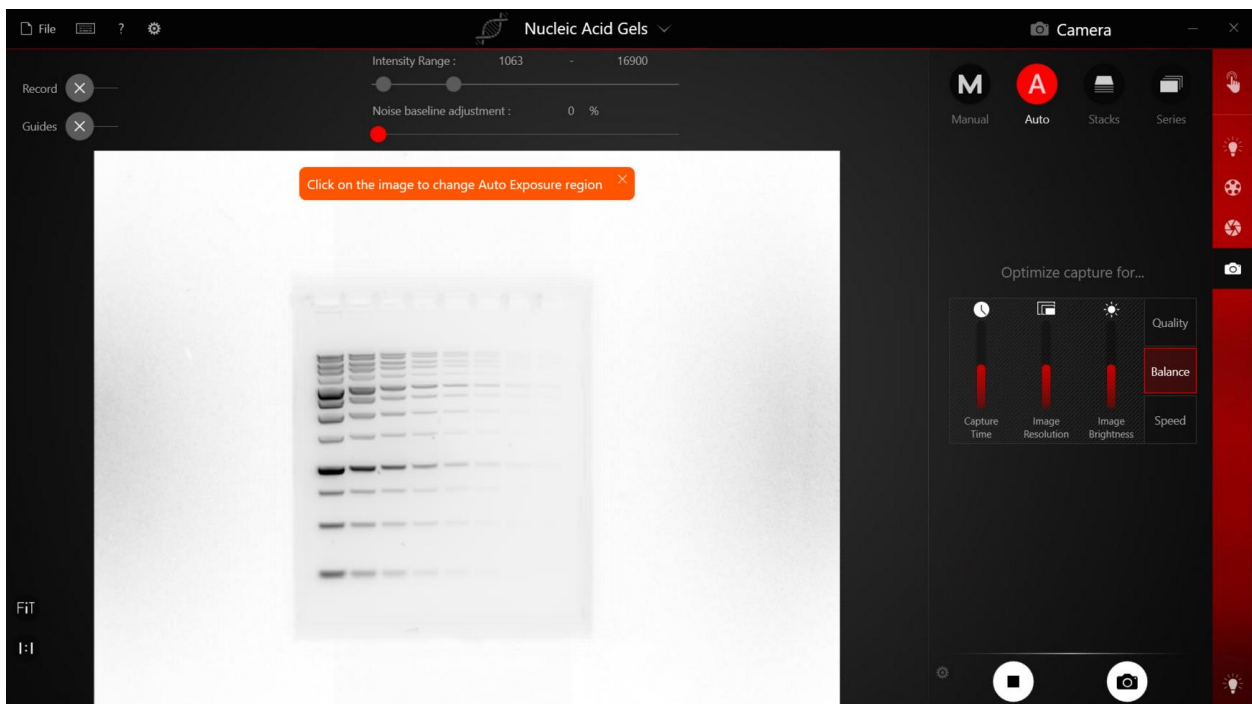
Pre-Capture Settings	Function
<b>Auto Histogram</b>	Automatically adjust histogram to make the image easier to view. <i>Note: Histogram can also be Adjusted under <b>Image</b> → <b>Hist</b>.</i>
<b>Noise Reduction</b>	Apply level 1 noise reduction to Live View only. <i>Note: the change will not be applied to the captured image. Noise reduction can also be performed under <b>Image</b> → <b>Edit</b> → <b>Remove Noise</b>.</i>
<b>Invert</b>	Invert Live View and captured image. <i>Note: Images can also be inverted under <b>Image</b> → <b>Adjust</b></i>
<b>Pseudocolor</b>	Choose pseudocolor to apply on your sample. <i>Note: Pseudocolor can be changed after capturing under <b>Image</b> → <b>PColor</b>.</i>
<b>Crop %</b>	Set Crop percent for your sample, the smallest crop size is 50%. <i>Note: the change will be permanent to captured image.</i>

## 4.2 Live View

1) Start Live View by clicking the Live View button under **Camera**.



2) When Live View starts, several features appear above the Live View window and are described in the table below:



Pre-capture Settings	
Live Window	Function
<b>Record</b>	Record Live View. <i>Note: files will be saved on your hard drive under Libraries→ Videos by default. Refer to <b>Section 9.2</b> for changing save location.</i>
<b>Guides</b>	Displays a grid guide during Live View to help positioning sample on the center of viewing tray.
<b>Intensity Range</b>	Displays the real-time intensity range during Live View. This value can be used to determine if a sample needs longer exposure time. <i>Note: it is best to use this feature in combination with Saturation Preview as not every pixel is accounted for in the intensity range display.</i>
<b>Noise Baseline Adjustment</b>	Changes the baseline value for the dark pixels in the image during Live View. This feature helps generate clean images in Live View recordings.
<b>Auto Exposure Region</b>	Click on the image to change the Auto Exposure region. The default is set to 50% for the whole image. Drag the auto exposure region outline to change the size of the auto exposure region.

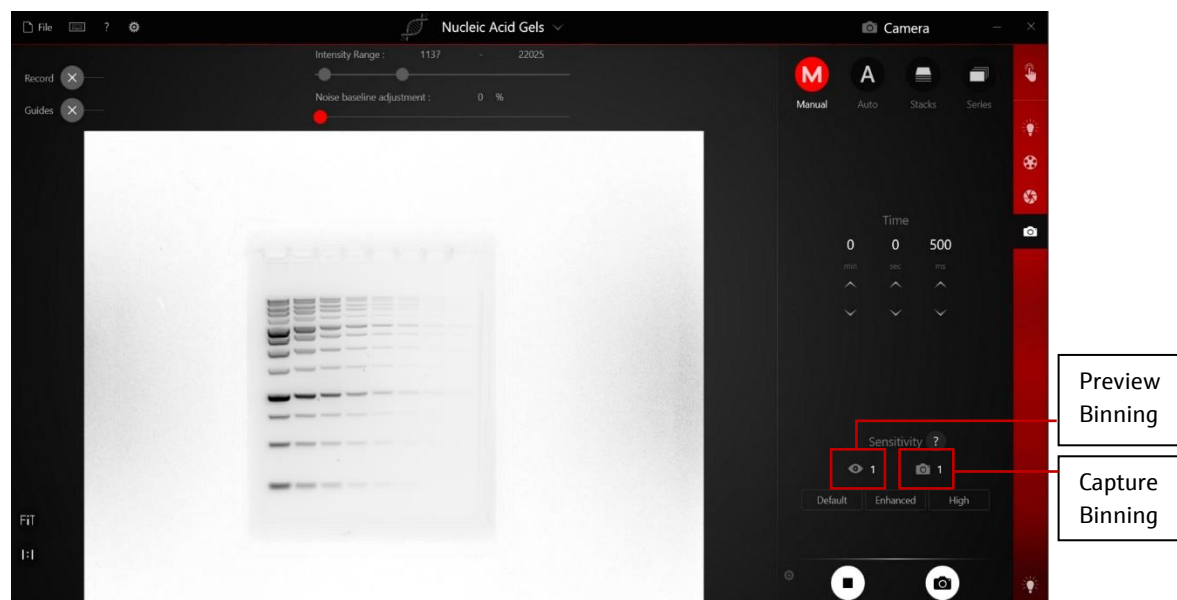
### 4.3 VisionWorks® Capture Workflow: Manual Set Up

- 1) Software will start with the Manual Capture panel. Four primary camera control modes are available. They are summarized in the table below.

Image Capture Options	
Camera Mode	Function
<b>Auto</b>	VisionWorks® automatically sets camera exposure.
	<b>Speed</b> — Start Auto Capture when the signal reaches 10% threshold with 4x4 capture binning. Image will be captured at short exposure time and lower resolution.
	<b>Balanced</b> – Start Auto Capture when the signal reaches 50% threshold with 2x2 capture binning. The exposure time and resolution are balanced for the captured image.
	<b>Quality</b> — Start Auto Capture when the signal reaches 75% threshold with 1x1 capture binning. Image will be captured at longer exposure time and higher resolution.

<b>M (Manual)</b>	This setting allows manual control over all camera settings.
<b>C (Chemi)</b>	Capture composited chemiluminescent image (only available under Western Blotting Application).
<b>B (BLI)</b>	Capture composited bioluminescent image (only available under in vivo Analysis Type).
<b>Series</b>	A series of images are taken. Users can set custom exposures and number of images to be taken.
<b>Stacks</b>	A series of images are taken and composited/summed as one image. Each successive image will be that image plus the previous. Stacks may be taken at a set exposure for a user defined length of time.
	<b>Continuous</b> – Generates only one image at the end. Capture won't be stopped until the user click Stop Capture.
	<b>Auto</b> – Generates only one image at the end. Capture will stop automatically when the image is saturated.
	<b>1,2,3</b> – Frame number is defined by the user. All images taken can be browsed with the stack navigator near the camera icon and the stack of images may be saved as a VisionWorks® Sequence (*.SQV) file.

2) Image intensity can be adjusted using camera binning and exposure time settings. These functions and their common uses are summarized below:



Camera Binning Options		
Button	Feature	Uses
<b>Live View Binning*</b>	Increase signal intensity in Live View. <i>Note: unavailable for GelSolo and Gel Studio.</i>	Best used for samples with weak signal.
<b>Capture Binning*</b>	Increase signal intensity in Captured image <i>Note: unavailable for GelSolo and Gel Studio.</i>	Best used for capturing sample with weak signal.
<b>Preset Binning</b>	<b>Default</b> – Capture binning 1x1, Preview binning 4x4. <b>Enhanced</b> – Capture binning 2x2, Preview binning 4x4. <b>High</b> – Capture binning 4x4, Preview binning 6x6 <i>Note: unavailable for GelSolo and Gel Studio</i>	Default for all samples. Enhanced for weak samples. High for Chemiluminescent samples.
<b>Exposure Settings</b>	Adjust exposure time to increase or decrease signal intensity.	For all applications.

\*More Binning Descriptions please refer to **Section 4.4 CCD Binning** and **Appendix B**

- 3) To display a live view of the dark room, select the **Live View** icon on the bottom of the right pane. A live view of the dark room will appear on screen. This live view can be used to adjust imaging parameters and see their effects in real time.



Starting Exposure Options		
Button	Feature	Uses
<b>Live View</b>	Activates live view to preview the sample in the darkroom.	Preview an image to adjust lighting, filters and brightness, etc.
<b>Capture</b>	Captures an image.	Best used for manually capturing an image.

- 4) Once parameters are set, select the **Capture** icon to begin the image capture according to your set parameters.

## 4.4 VisionWorks® Binning

The Binning setting in VisionWorks® refers to a special capability of CCD cameras to adjust the sensitivity of its sensor to light at the expense of resolution. The capture binning can be changed from a low setting (default at 1 x 1) to a higher binning (2 x 2 through 8 x 8), which increases its sensitivity, and lowers necessary exposure time to achieve a certain intensity, while combining the pixels of the output image to create a lower resolution image. Live view binning can be set to a higher setting as well to improve the refresh rate of the Live View image at the expense of resolution of the Live View image shown. For more information about binning, see **Appendix B**.

## 5.0 VisionWorks® Post Processing

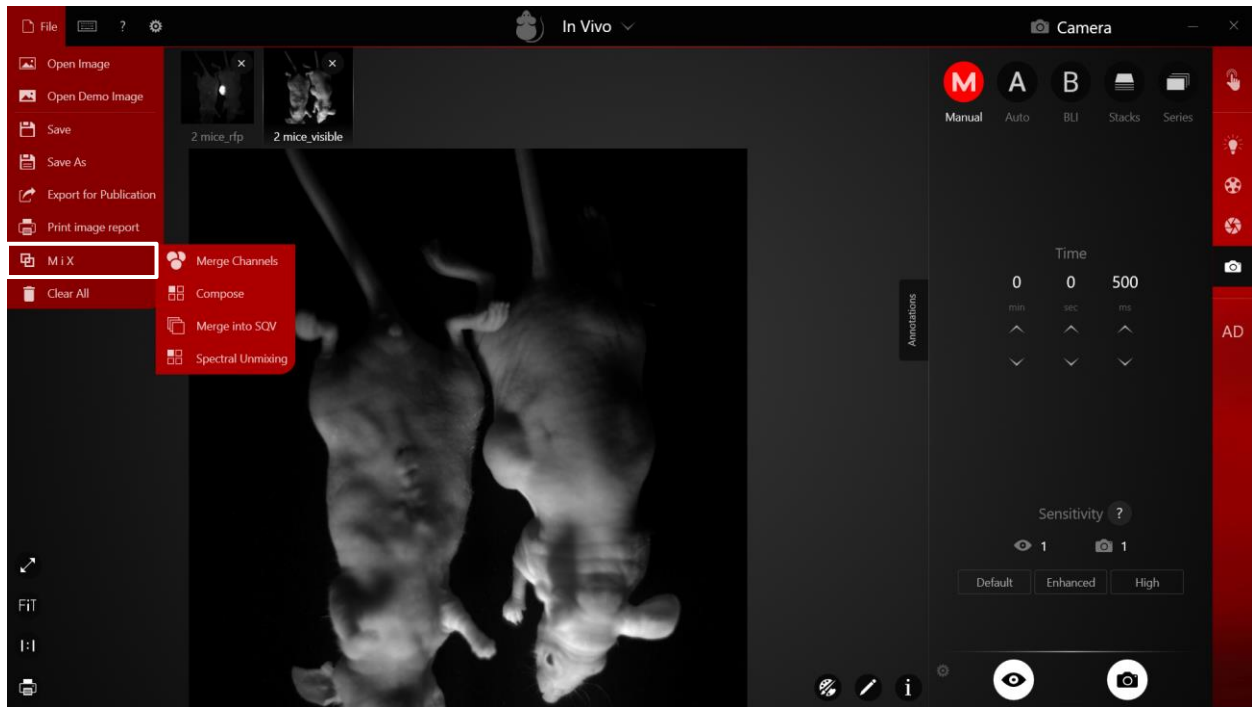
### 5.1 VisionWorks® Multi-Image

VisionWorks® includes a Mix tool that allows for captured images to be edited according to custom user preferences. The Mix feature provides four tools for image enhancement: Merge channels, Compositing, Merge to SQV, Spectral Unmixing. This section will give a brief overview of the functionality enabled by the new Mix feature.

Mix Menu Options		
Tool	Description	Application Example
<b>Merge Channels</b>	Combine up to four monochrome images into a single colored image by assigning each image a color channel.	Best used for multiplexing with different fluorophores.
<b>Compose</b>	Composite up to three images.	Combine different color channels onto a common base image. Recommended for creating an overlay for a chemiluminescent image and marker image ( <b>See section 5.2</b> ).
<b>Merge into SQV</b>	Save up to 25 of images as a single VisionWorks® Sequence (*.SQV) file.	Combining images together that are part of a time course study.
<b>Spectral Unmixing</b>	Correct for auto fluorescent background in an image.	Correction for auto fluorescent background for in vivo application.

1) Once you have taken your desired images, select the **"Mix"** icon under the **File** menu.

2) In the **Mix** window, four tools are presented:

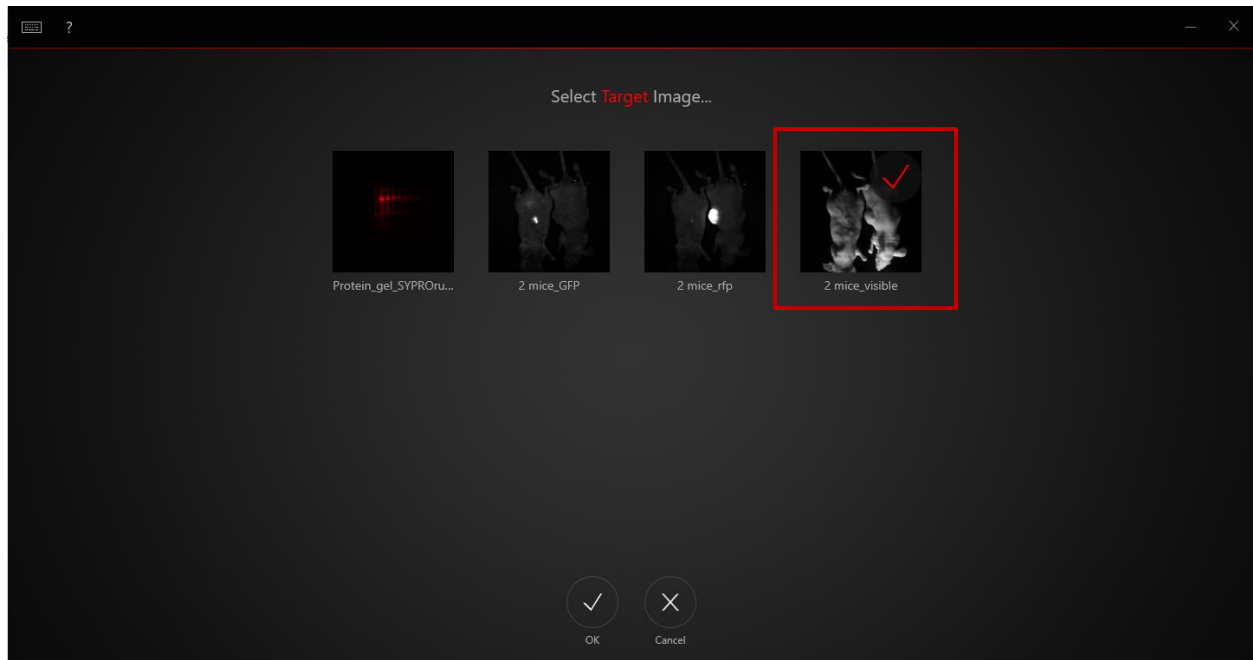


3) Select the desired tool then follow the onscreen instructions.

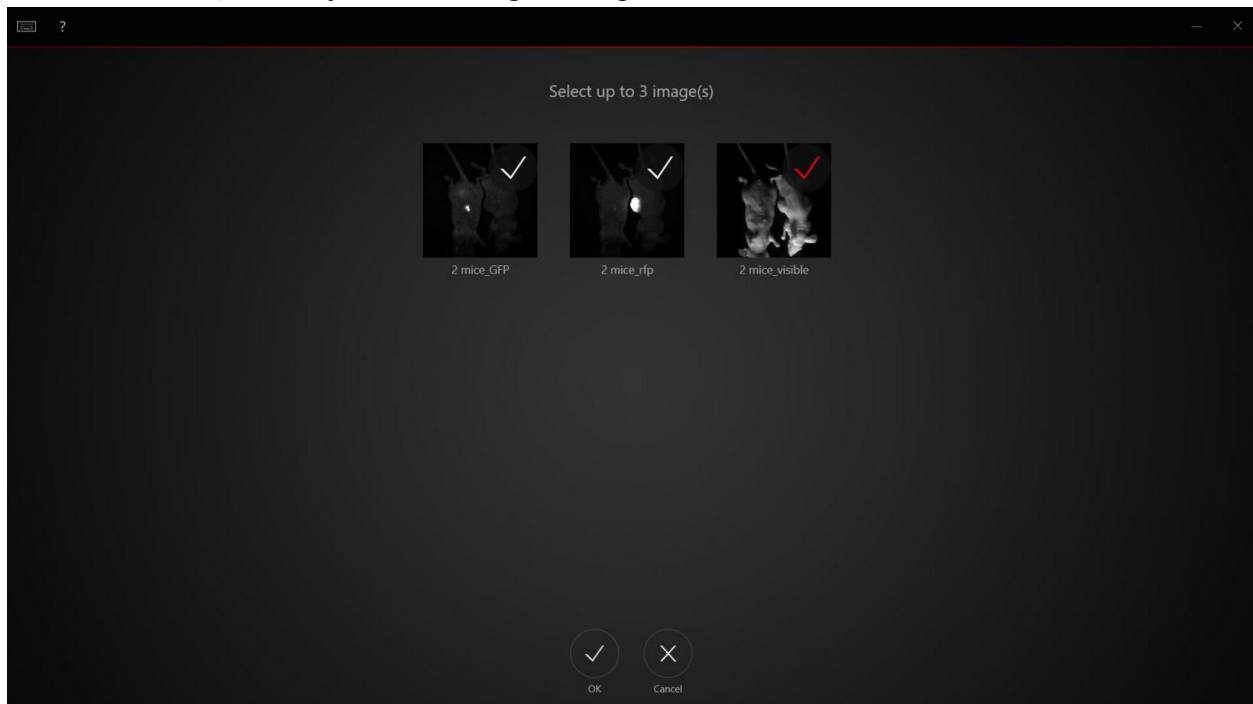
## 5.2 Compositing in VisionWorks®

1) From the **MiX** menu, select **“Compose”**.

2) In the Compose window, select your desired target image. This will be the image that all others will be composited onto.



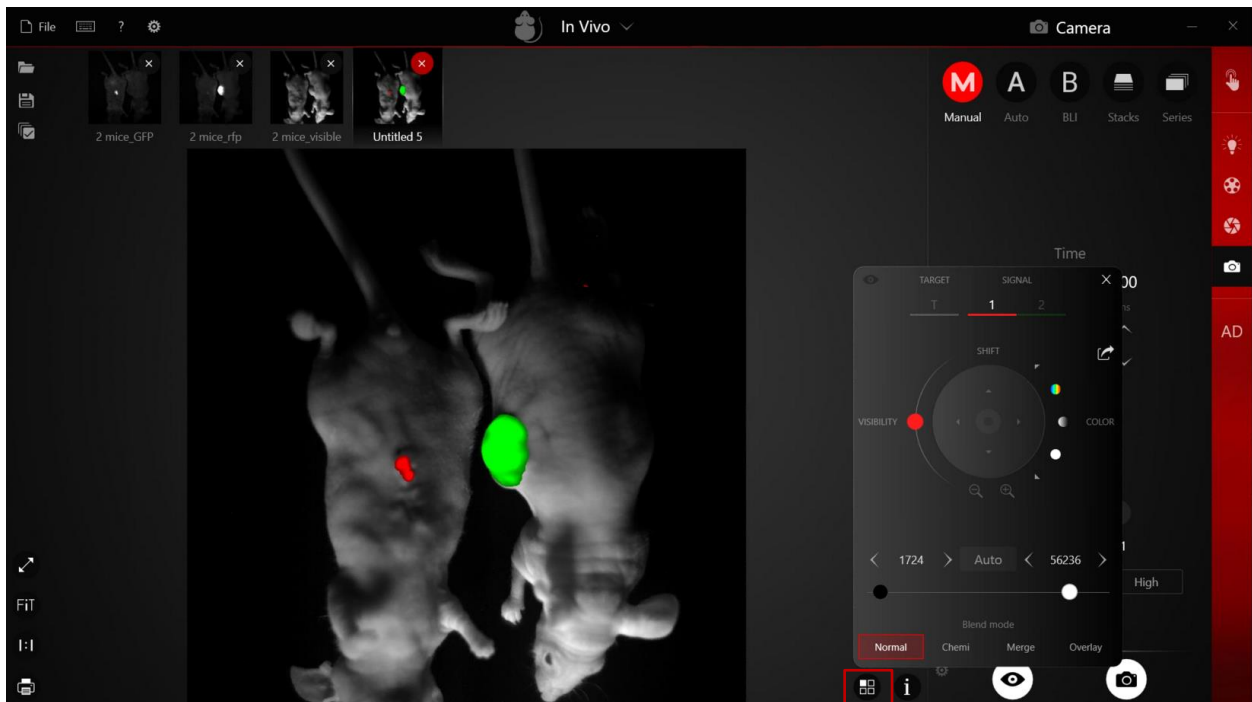
- 3) Select "OK" to confirm target image selection
- 4) Select your desired signal image(s)



- 5) Select "OK" to confirm signal image(s) selection

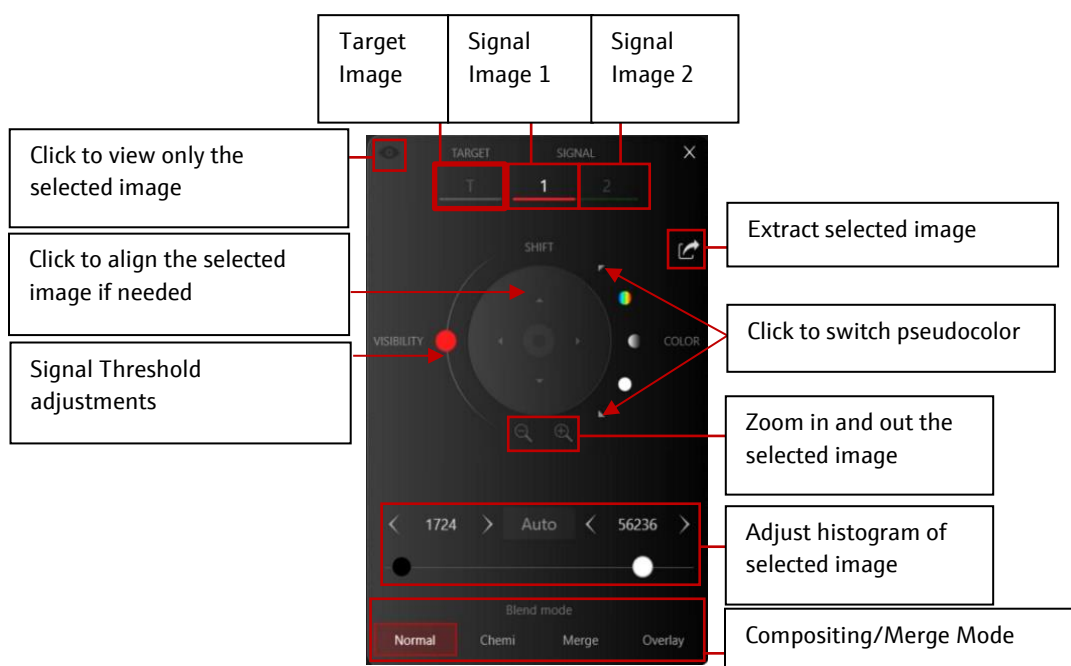


- 6) Use the Compositing Adjustment window and adjust signal threshold sliders to set the corresponding visibility of each signal on the resulting composited image.



Compositing Adjustments

- 7) Features available in the Compositing Adjustments are summarized in the table below:

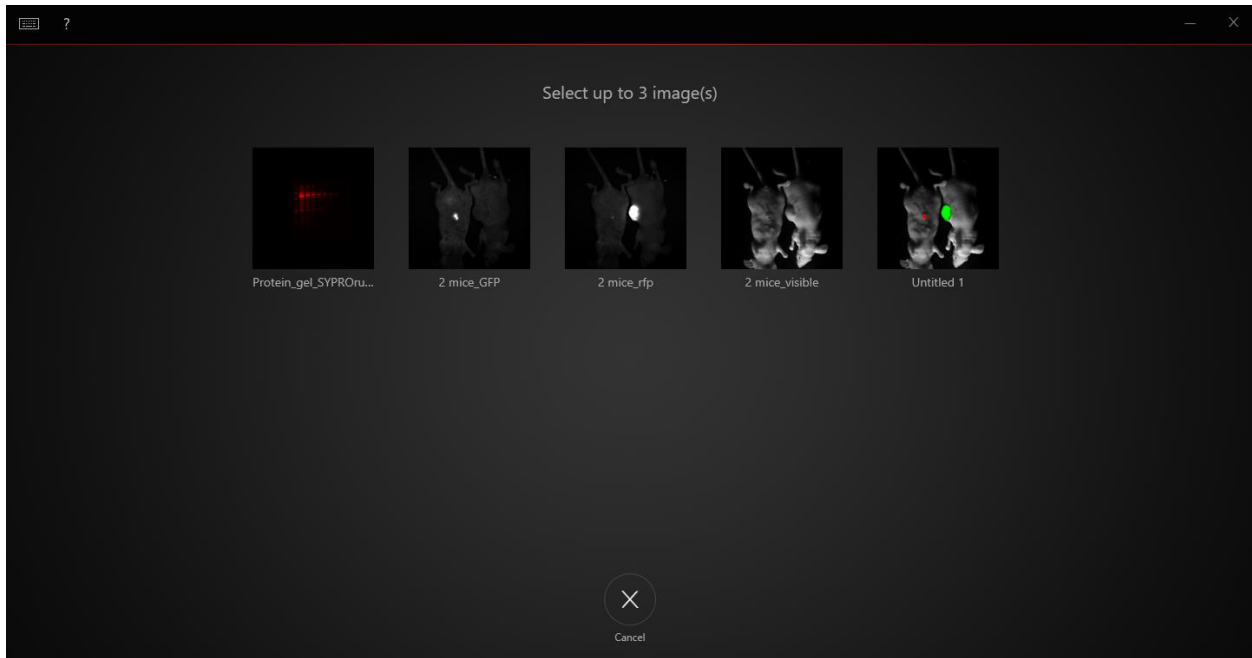


### Compositing Adjustments Features

Button	Feature
<b>Target</b>	Click to active and adjust the histogram of the target image
<b>Signal</b>	Click to active and adjust the histogram, color or signal threshold, etc.
<b>Extract</b>	Click to extract and view selected image.
<b>Pseudocolor</b>	Click to change pseudocolor for signal images.
<b>Zoom in&amp;out</b>	Allow better alignment for the images captured at different zoom level.
<b>Histogram</b>	Allow histogram adjustment for selected image.
<b>Blend Mode</b>	Extra blend mode available for composited and merged images: <b>Normal:</b> Default blend mode for Bioluminescent in vivo samples. <b>Chemi:</b> Default blend mode for Chemiluminescent samples <b>Merge:</b> Default blend mode for Merge samples <b>Overlay:</b> This feature is particularly useful for <i>in vivo</i> imaging if users want to visualize the fluorescent/ bioluminescent signal overlaid on a white light transparency, so the texture of the animal can still be observed
<b>Visibility</b>	Adjust to allow more or less signal to be composited or merged on target image
<b>Shift</b>	Adjust the signal position to help with signal alignment.
<b>View</b>	Turn off the compositing image and view only the selected target or signal image.

### 5.3 Multiplexing in VisionWorks®

- 1) From the **MiX** menu, select **"Merge Channels"**.
- 2) In the image selection window, select your desired fluorescent images and click **"OK"** to confirm the selection. You can select up to 3 fluorescent images to merge.



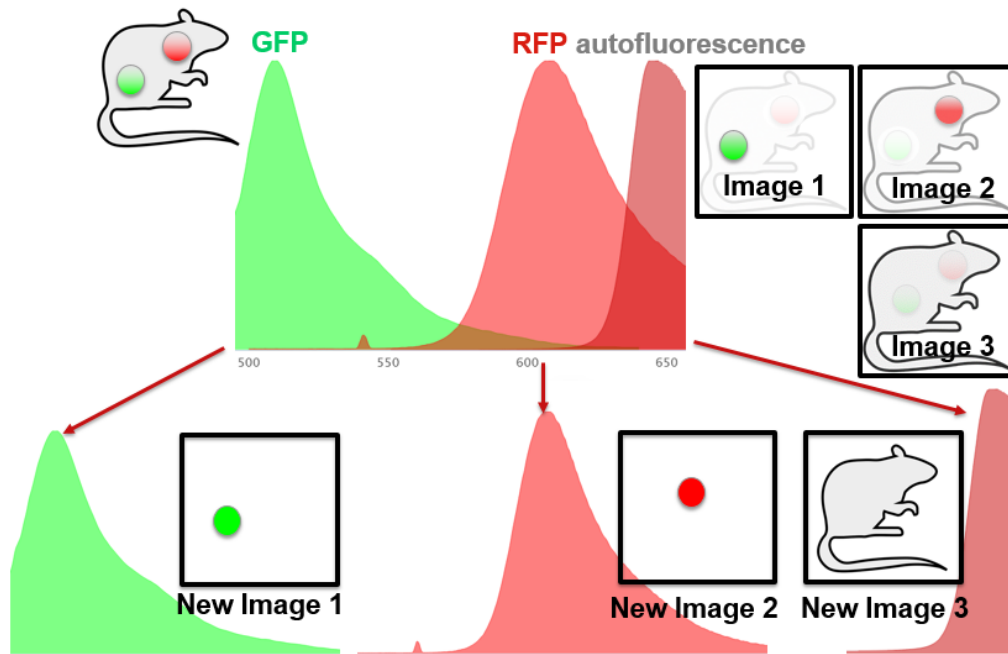
- 3) Software will add pseudocolor to each selected image automatically, the sequence of pseudocolor added is Red, Green, and Blue. If different pseudocolor needs to be applied, please change the color in the compositing adjustment window ([Section 5.2](#)).

### 5.4 Spectral Unmixing in Visionworks

VisionWorks® software contains a powerful spectral unmixing tool that will improve the separation of multiple fluorescent or bioluminescent reporters and autofluorescence to improve image quality in experiments involving overlapping spectra among these target reporters of interest. To begin, the user must first have emission filters to capture each target reporter and create an initial image dataset with some separation of the reporters by peak emission. With guidance from the user to isolate the regions of pure or relatively ideal reporters, the spectral unmixing feature will then further separate the signals in the separate images, reducing the overlapping signal from the spectra of each reporter, and create a merged image which the user can view for spatial location of each reporter or

extract channels from to view images of individual reporters.

In order to prepare for spectral unmixing, an area or ROI of ideal fluorescent or bioluminescent reporter should be selected on each image using the Area Density tool (see **Section 7.2** for more information on how to use the Area Density tool), as well as of autofluorescence if applicable. As an example, if the target data to be unmixed contains two fluorescent reporters and autofluorescence, there should be three images captured: one each for the peak emission of the two reporters and autofluorescence, using emission filters to capture the peak emission of each. Then, the Area Density tool should be used to draw an ROI around the highest signal reporter region that each image was captured for.



For example, if the target data has GFP with peak emission of 505 nm, RFP with peak emission of 610 nm, and autofluorescent tissue, the user would take Image 1 of the target with a 505 nm filter and appropriate excitation light for GFP, take Image 2 of the target with a 610 nm filter and appropriate excitation light for RFP, and take Image 3 to capture the autofluorescence using a longpass filter that blocks RFP and GFP emission, while using an excitation light. On Image 1, the user would draw an ROI using the Area Density tool around the peak GFP emitting region, on Image 2, an ROI around the peak RFP emitting region, and on Image 3, an ROI around a high autofluorescence emitting region on the target.

The below steps will walk through a simple example of separating one reporter from autofluorescence.

*Note: Darkframe subtraction should be turned ON for every spectral unmixing acquisition session, this is turned ON by default for all systems.*

- 1) Once images have been captured with emission filter for the signal and another image with emission filter for autofluorescence (or signals 2, 3...), use the area density tool (see **Section 7.2**) to draw an ROI around the signal regions of each image. There should only be one ROI drawn per image, and there should be as many images as there are signals and autofluorescence to unmix.

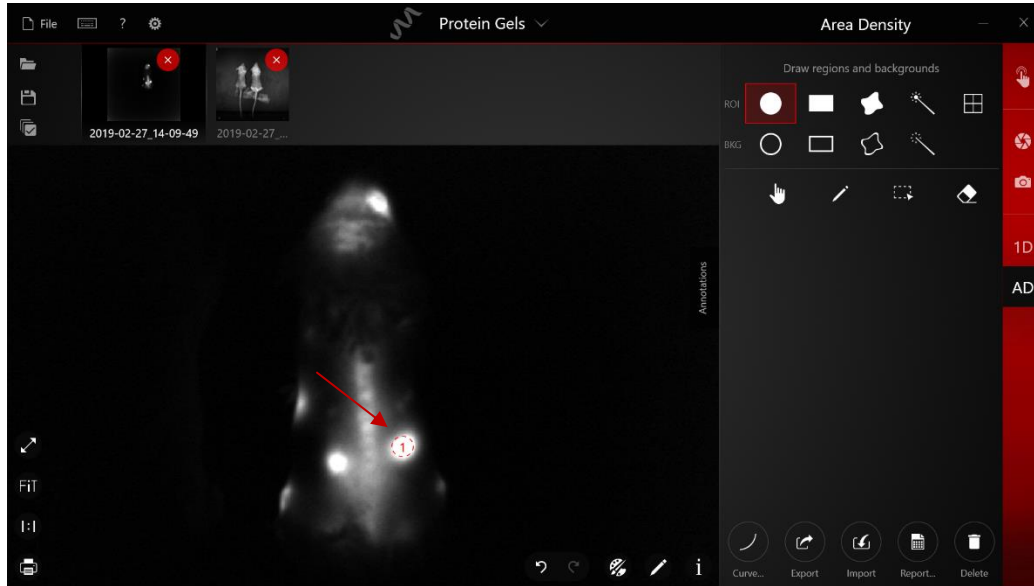


Image 1: signal ROI chosen using Area Density tool

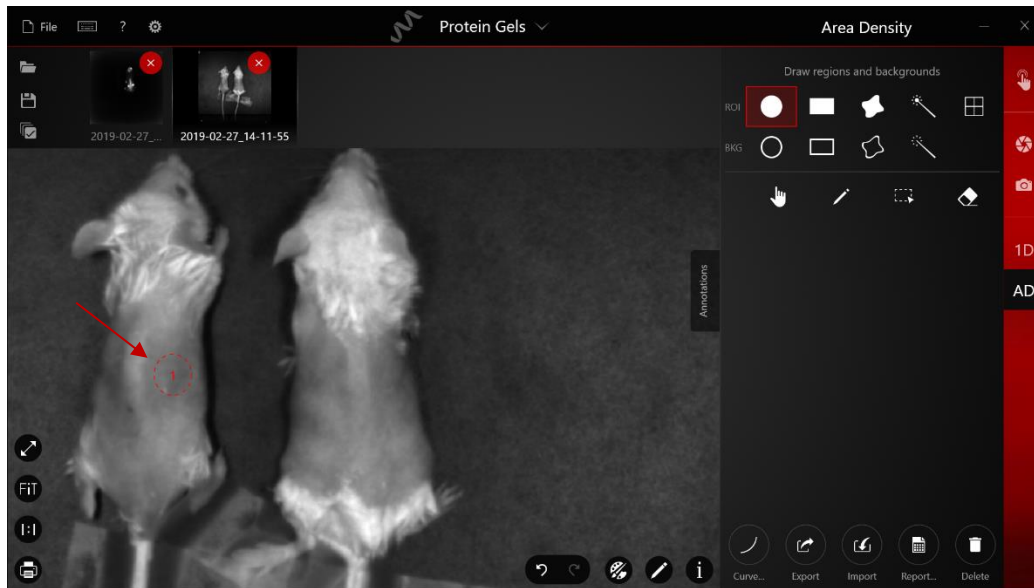
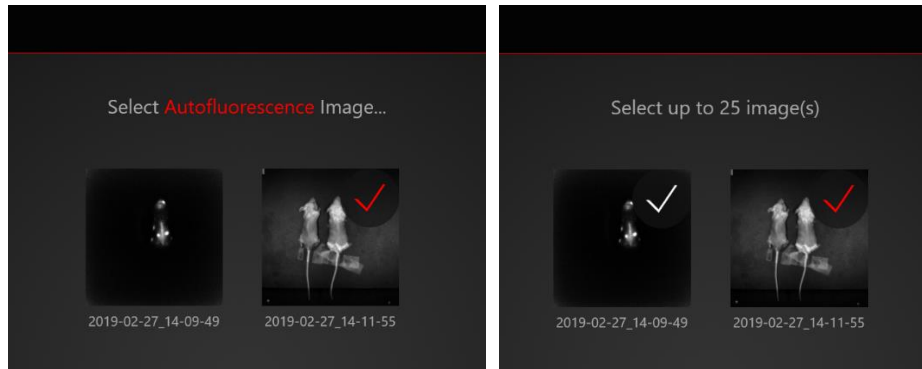
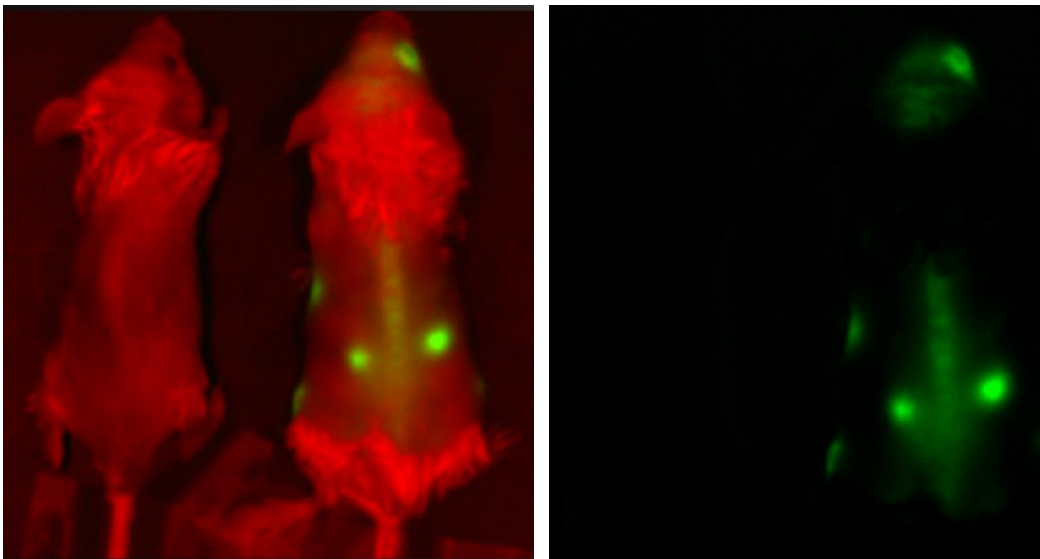


Image 2: autofluorescence ROI chosen using Area Density tool.

- 2) From the **MiX** menu, select **"Spectral Unmixing"**.
- 3) If the image set contains an autofluorescence image, select it and click OK. Otherwise, select **"Skip"**.
- 4) Select the signal images of the dataset, and click OK.



- 5) A merged image that can be viewed, adjusted, saved, or have individual channels extracted will appear in the gallery. Each channel of the merged image represents one of the reporters and autofluorescence.

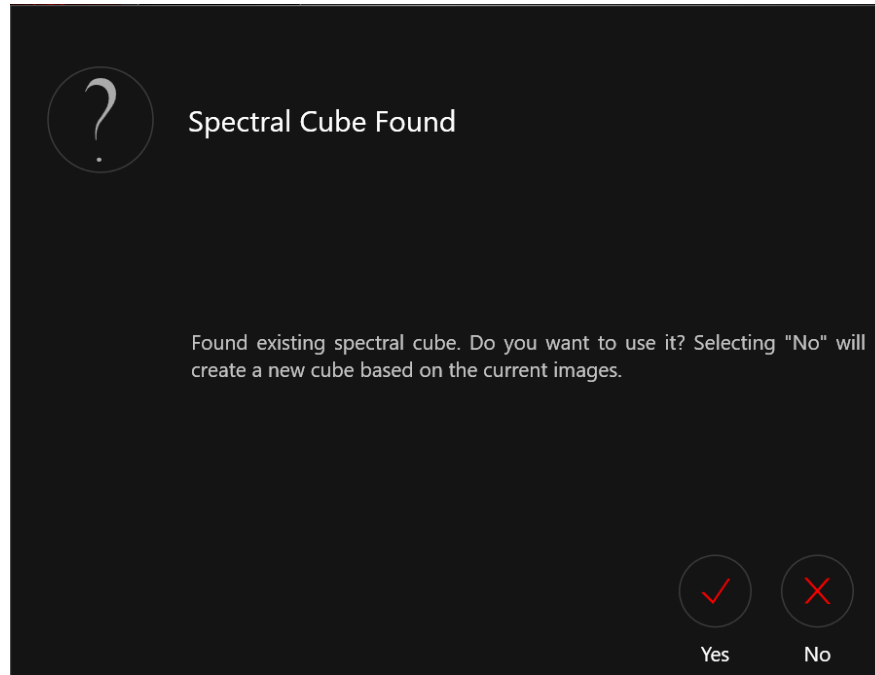


After spectral unmixing has been completed at least once in a session, the user has the option of re-using the same "spectral cube", or the identical calculated relative contribution of each reporter to each filtered image, on subsequent spectral unmixing selections. This is useful for several types of experiments:

(1) The study is first capturing images of pure reporters under each emission filter to compute the contribution of each spectra, then will capture the reporters in vivo, such as injected inside a mouse. In this case, they would select the ROI around the pure reporters for each emission filtered image. In their actual experimental images, they can capture the set in the same order of emission filters, and can just have images selected in same order in steps 3-4, without need to select ROIs again.

(2) The study is capturing many experimental subjects with the same reporters. In this case, they can run the spectral calculation (selecting the ROIs) for the first subject and image set


captured, but subsequent subjects and image sets no longer need the ROI selected and can just have the images selected in the same order in steps 3-4.



To repeat the spectral unmixing with the same spectral cube, the identical set of emission filters and excitation should be used on the new image set, and the images should be captured in the same order, and then selected in the same order during steps 3-4. It is no longer necessary to select an ROI in each image. The software will prompt the user if they would like to use the same spectral cube – select Yes, or select No if the images are now from a different experiment (i.e. different filters and reporters).

## 5.5 VisionWorks® Image Editing

VisionWorks® provides multiple image editing tools for users to modify images after capture. Some changes will change the raw data of the image, it is recommended users modify the image only after analysis or after having saved a copy of the original image. We indicate which image editing tools affect the raw data in the table below.

Image Post Processing and Editing Tools		
Icon	Description	Changes Raw Data
	<p><b>Histogram</b> – Provides access to auto and manual histogram adjustment.</p> <p><b>PColor</b> – Provides access to post capture coloring options. This pseudocolor list will only show up when a captured image is selected.</p>	<b>No</b>

**Image Adjust** – Provide access to image adjustment options.



Brightness



Gray Level



Contrast



Invert




**Edit**– Provides access to image editing tools.

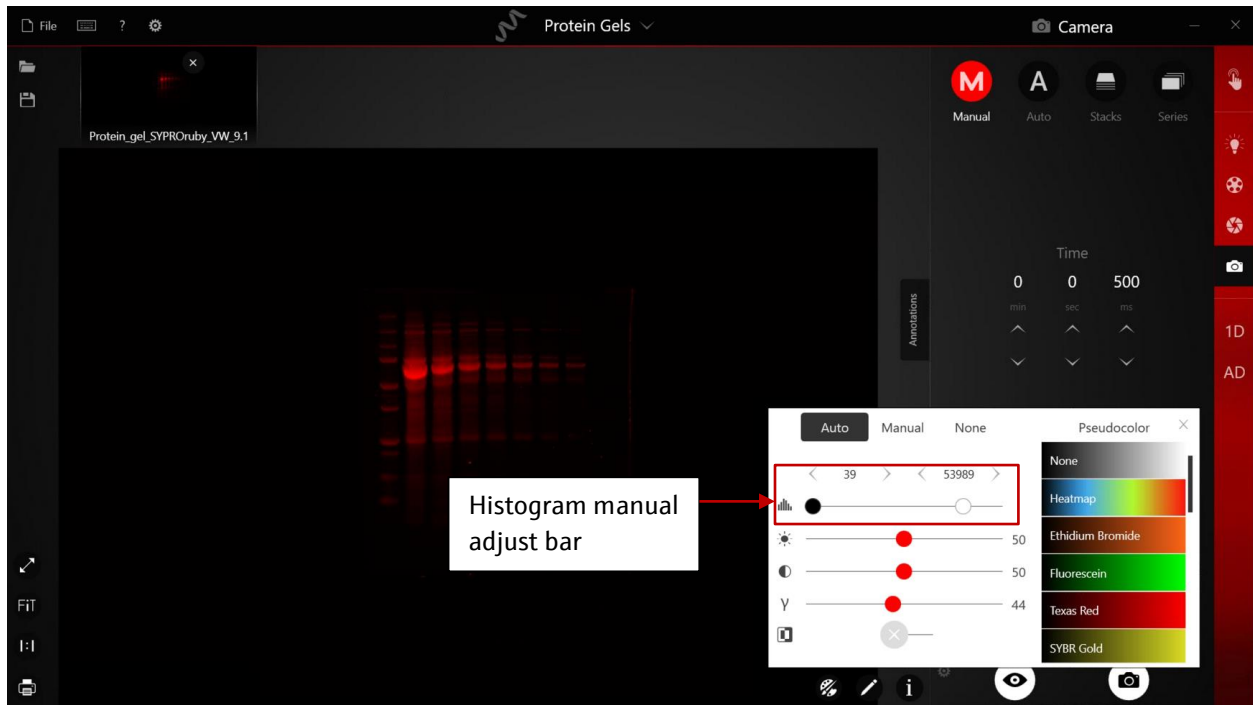
**Yes (all options)**

- Flip horizontal and vertical
  - Rotate
  - Remove Noise
  - Enhance Exposure
  - Sharpen
  - Resize
  - Crop
  - Black & White
  - Change depth
- 

## 5.6 Adjust Histogram

Histogram enables users to change the tonal information of an image. By default, the histogram displays the tonal range of the entire image. Histogram changes will not affect the raw data of the image.

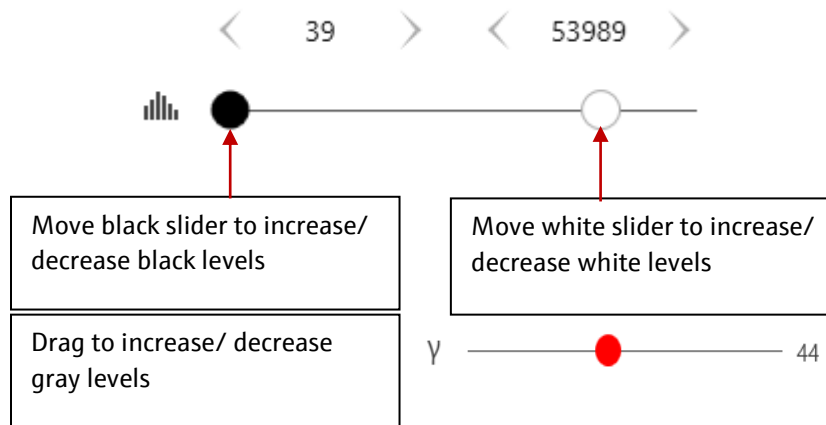
- 1) VisionWorks® enables users to modify the image histogram after capture. To access the Histogram menu, select 
- 2) From the top wheel of the Histogram menu, select desired Histogram mode.




### Histogram Modes

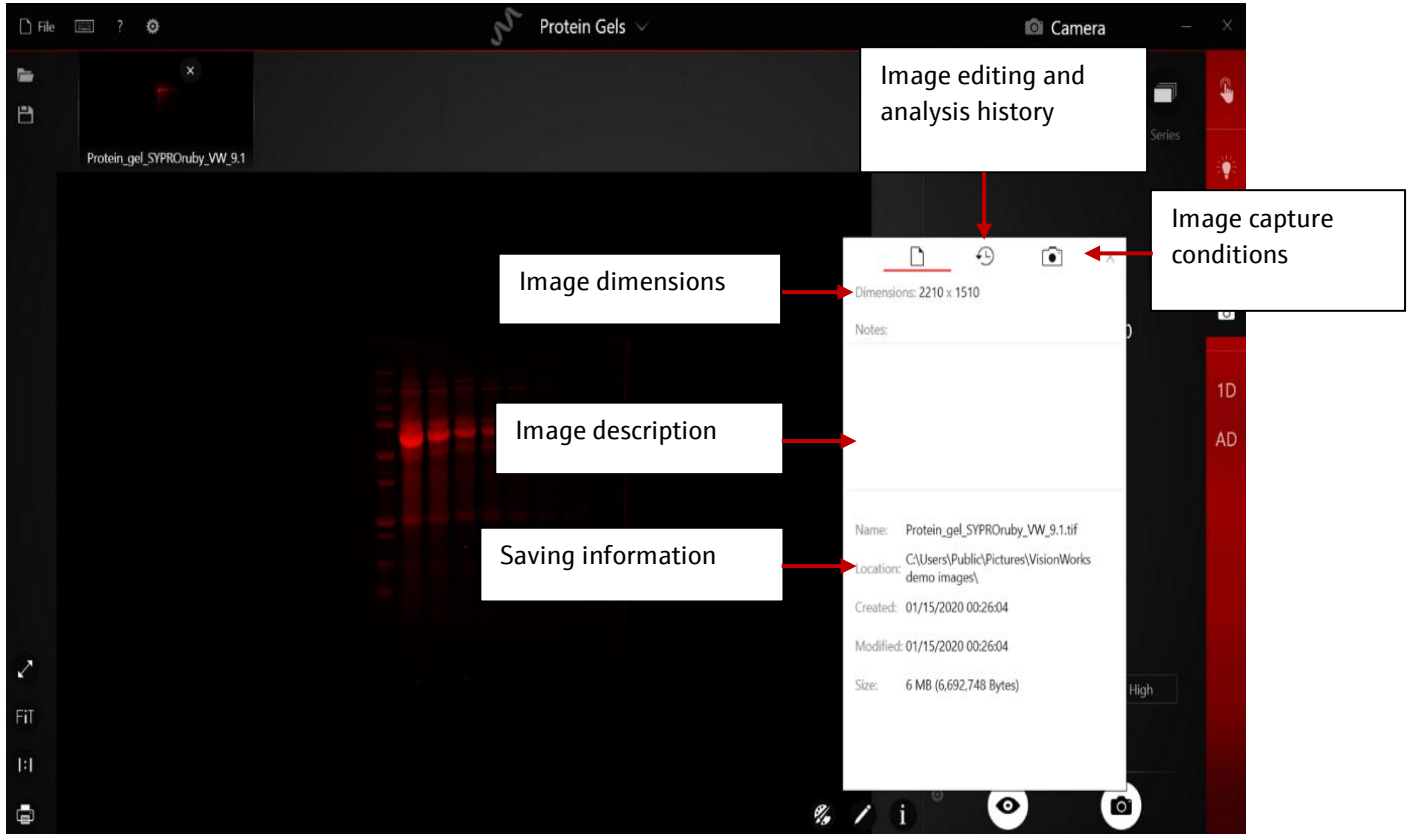
Feature	Description
<b>Auto (Default)</b>	Adjust histogram based on the whole image. Auto Histogram can be turned on and off in Pre-Capture settings (see <a href="#">Section 4.1</a> ).
<b>Manual</b>	Manually adjust histogram to change brightness of the image.
<b>No Adjustment</b>	No histogram adjustment.

3) Drag the black, white, and gray sliders left and right to adjust these levels.



## 5.7 Image Information

Image information can be accessed after capturing an image under  icon.


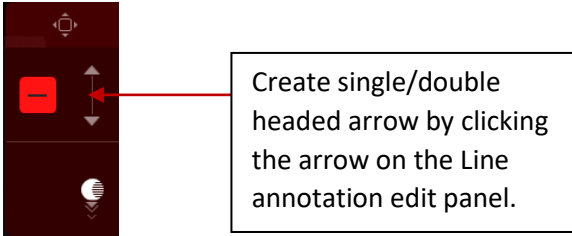



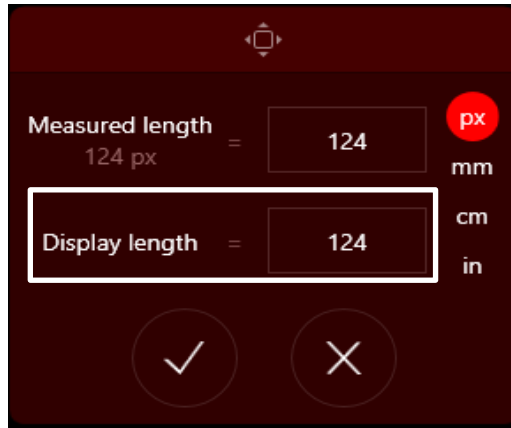
## 6.0 VisionWorks® Annotations

Annotations allow for images to be annotated without altering image data. This is to allow users the flexibility of highlighting points of interest within an image, in a non-destructive way. Annotations may be created, deleted or hidden at any time. Annotations may be exported, then later imported for repeat imaging applications.

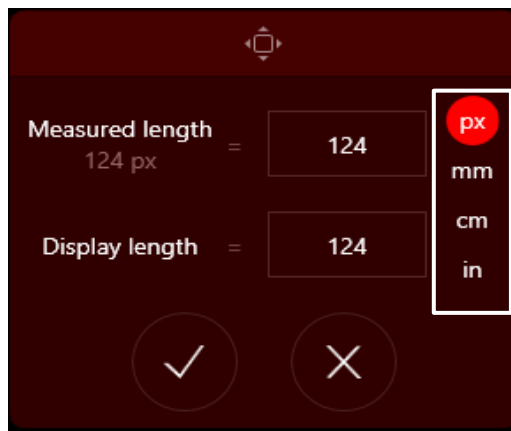
### 6.1 Annotation Tools

VisionWorks® provides access to 12 annotation tools. Once a tool is selected, tap/hold or click/drag on the image to draw annotation. The annotation tools are summarized and described in the table below:

Annotation Tools –	
Icon	Description
	<p><b>Line</b> – Draws a line from points A and B without quantitative analysis.</p> 
	<p><b>Standard Bar</b> – A calibration tool used to define standard distances in terms of pixels. To calibrate in other units, place a high accuracy ruler inside the instrument and capture an image and follow the steps below.</p> <ol style="list-style-type: none"> <li>1) Select the Standard Bar icon.</li> <li>2) Draw a line on the ruler. <i>Note: digitally zooming in may increase accuracy.</i> Press and hold Shift on keyboard to help create straight ruler easier.</li> <li>3) In the red pop up pane, the length of the line draw in pixels will be displayed. Change to the appropriate unit of measure by selecting the existing unit of measure and several options will appear. Calibration is complete.</li> <li>4) <i>Optional:</i> Users can change the size of the displayed Standard Bar by entering a value in the "Display" field.</li> </ol>




5) Define the unit of measure in terms of pixels.



- 6) All lines and area measures will now be based upon the definition that was just entered. To change or delete this definition, tap or click on the standard bar icon.
- 7) The "Display" field allows for your defined value to be multiplied and displayed according to a user's preferences. The line value displayed on-screen will be the user defined value times the display value.

	<b>Text Box</b> – Create a text box then type the desired text into the text field. Settings such as font, font size and color may be adjusted in the <b>Edit</b> sub-menu.
	<b>Rectangle</b> – Draw a rectangle around a region of interest.
	<b>Ellipse</b> – Draw an ellipse around a region of interest.
	<b>Free Form</b> – Draw a highlighting box around a region of interest. Colors can be changed in the <b>Edit</b> sub-menu.
	<b>Angle Measure</b> – Draw an angle on the image. Measurements are in degrees.



**Heat Map** – Displays a heatmap of the image that is calculated from pixel intensities in the image unless calibrated for photon flux as in our *in vivo* imagers (contact Technical Support for more information). The heatmap may be moved, resized or rescaled for best positioning on the image. To rescale high and low boundaries of Heatmap, navigate to  and adjust black, white, and gray sliders.

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## 7.0 VisionWorks® Analysis

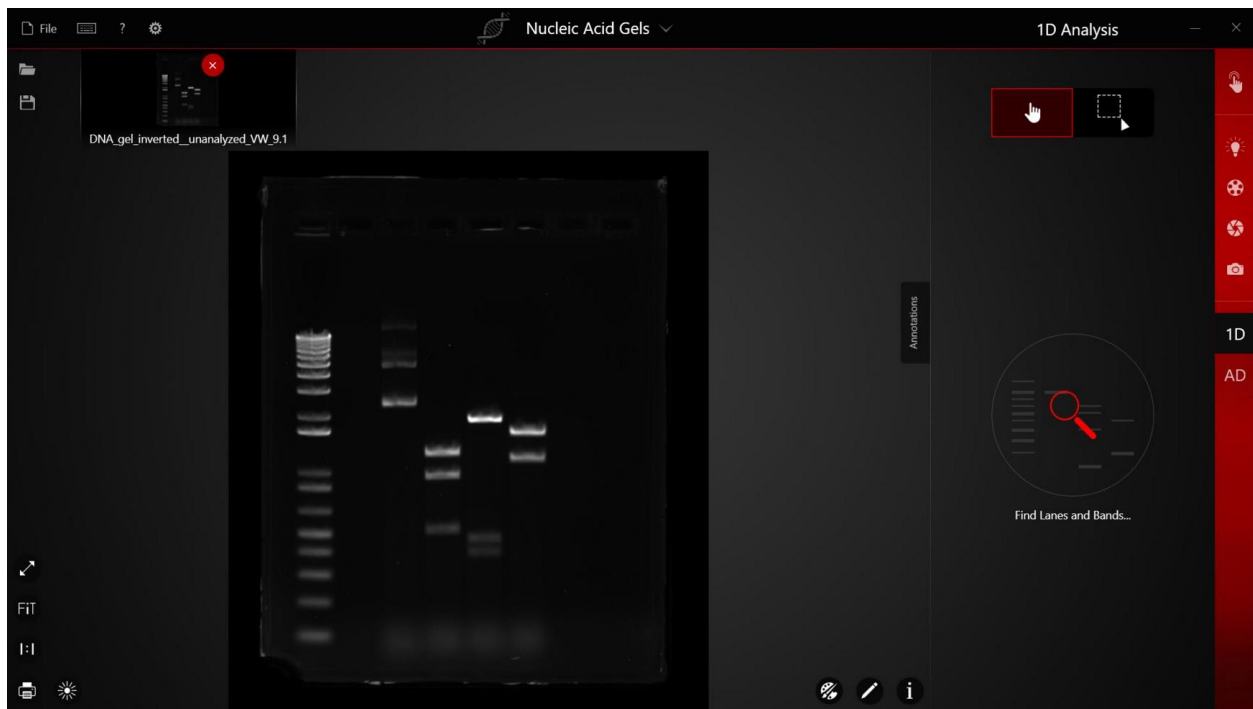
VisionWorks® contains advanced analysis features. These analysis features include: 1D Analysis, Colony Counting and Area Density. These powerful analytical tools allow for complex analysis to be performed immediately after imaging, and with the ability to export to Microsoft Excel or printed to a PDF or on paper.

### 7.1 1D Analysis

1D analysis includes identification and quantification of lanes and bands in protein/nucleic acid gels. Many of the functions within the 1D analysis are highlighted in the following section:

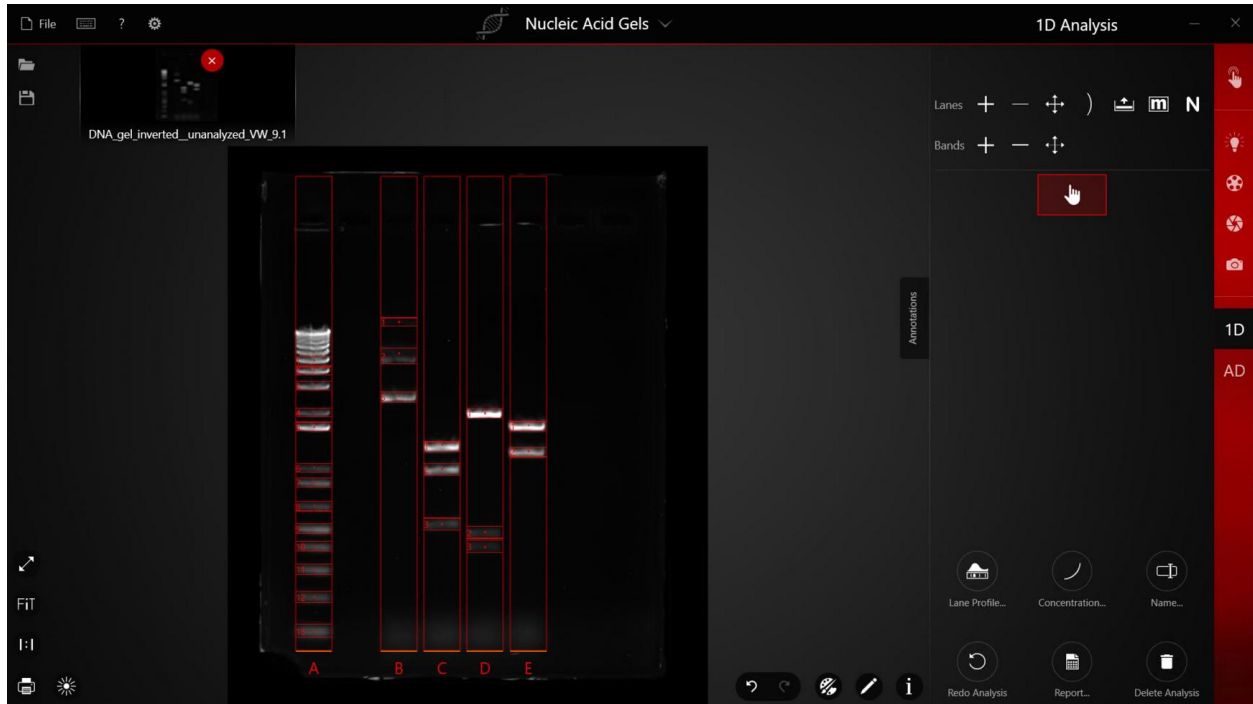
#### 7.1.1 Find Lanes and Bands

- 1) To have VisionWorks® automatically identify lanes and bands for a gel or a blot select **"Find Lanes and Bands"** illustrated below.



- 2) VisionWorks® will proceed to identify all lanes and bands within the entire image. Alternatively, select **"Define Region"** to select a region of interest to perform analysis.
- 3) Once satisfied, select **"OK"** to run the search in the defined region.

4) Lanes and bands identified by VisionWorks® will then be displayed.



### Summary of 1D Analysis Editing Tools

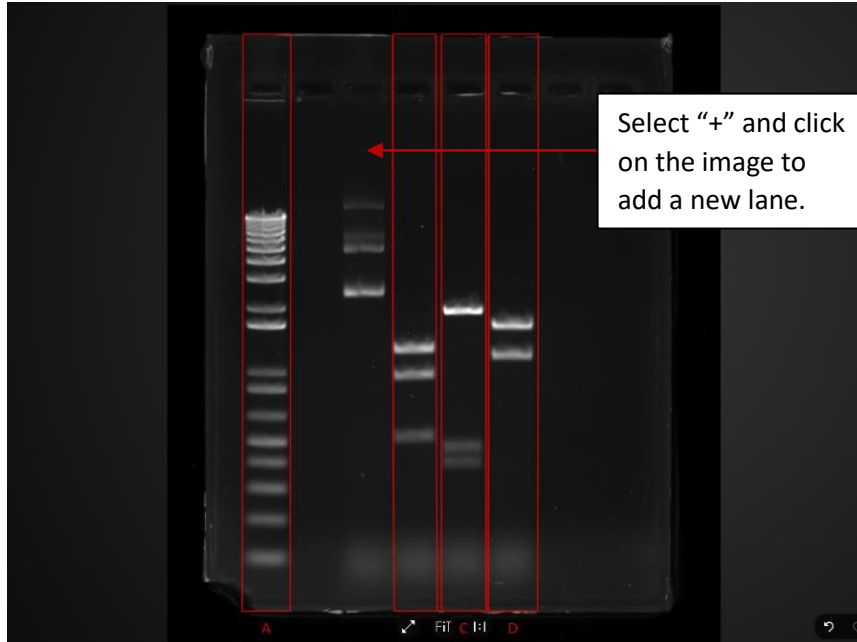
Settings

Function

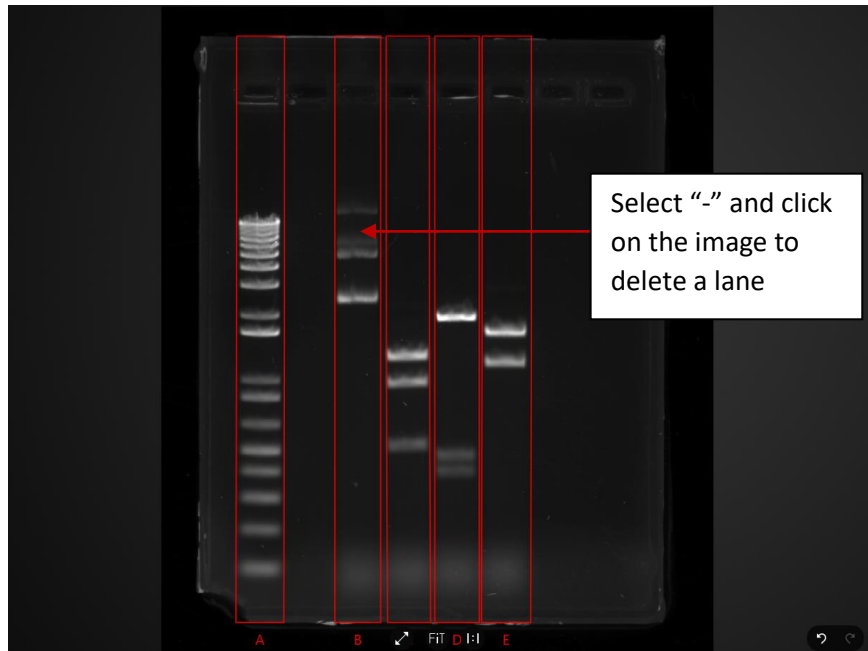
Lane



Click to activate the tool for adding an individual lane.

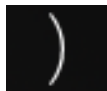


Click to activate the tool to delete an individual lane.

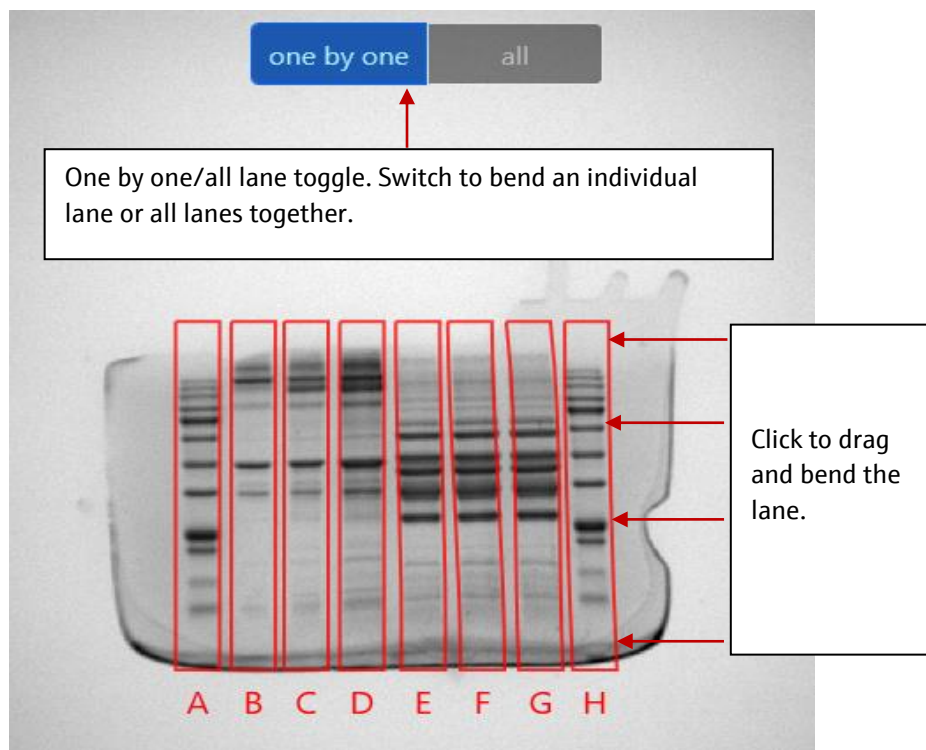




Click to activate the tool for moving and adjusting individual/all lanes.

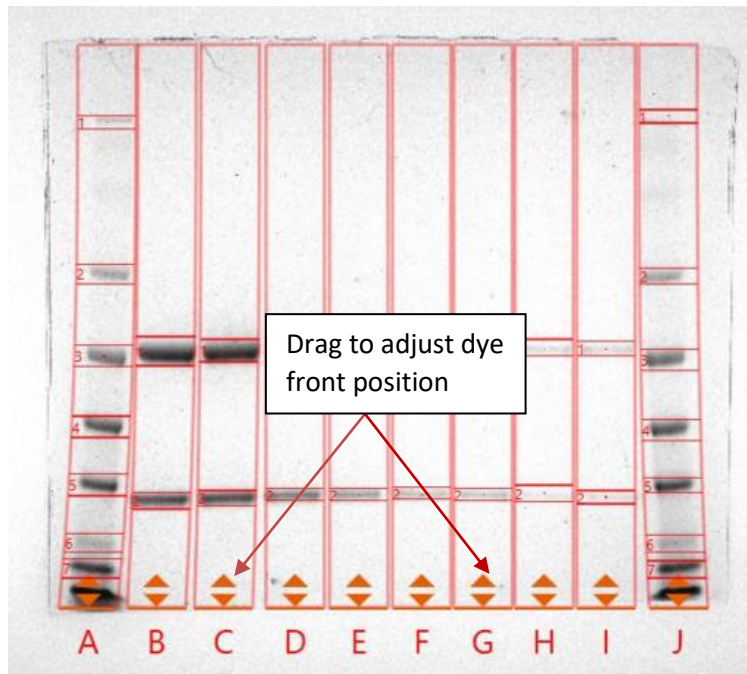


Click to activate the tool to bend an individual or all lanes.





Click to activate the tool to adjust dye front of each lane



**Band**



Individual bands can be added.

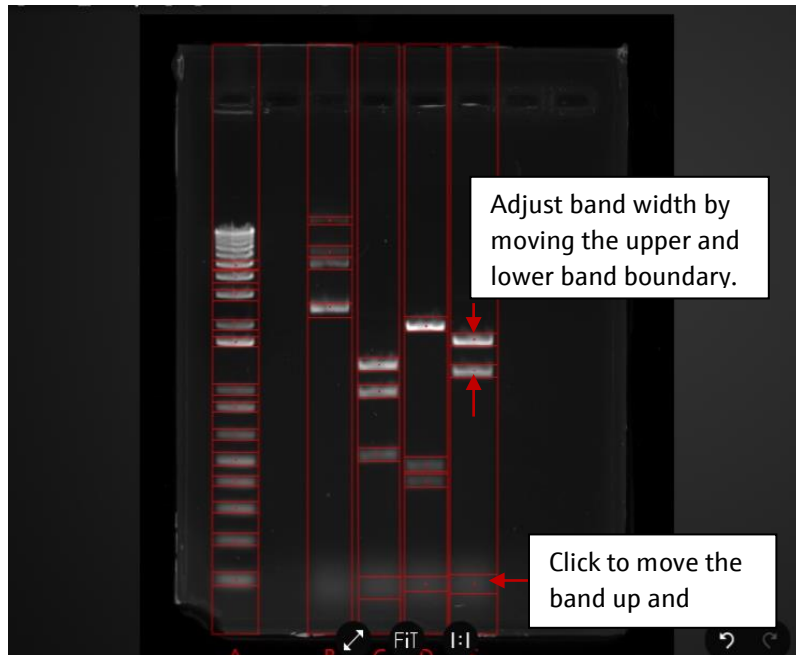




Allows for the deletion of individual bands.



Move and adjust individual band.



**Hand Tool**

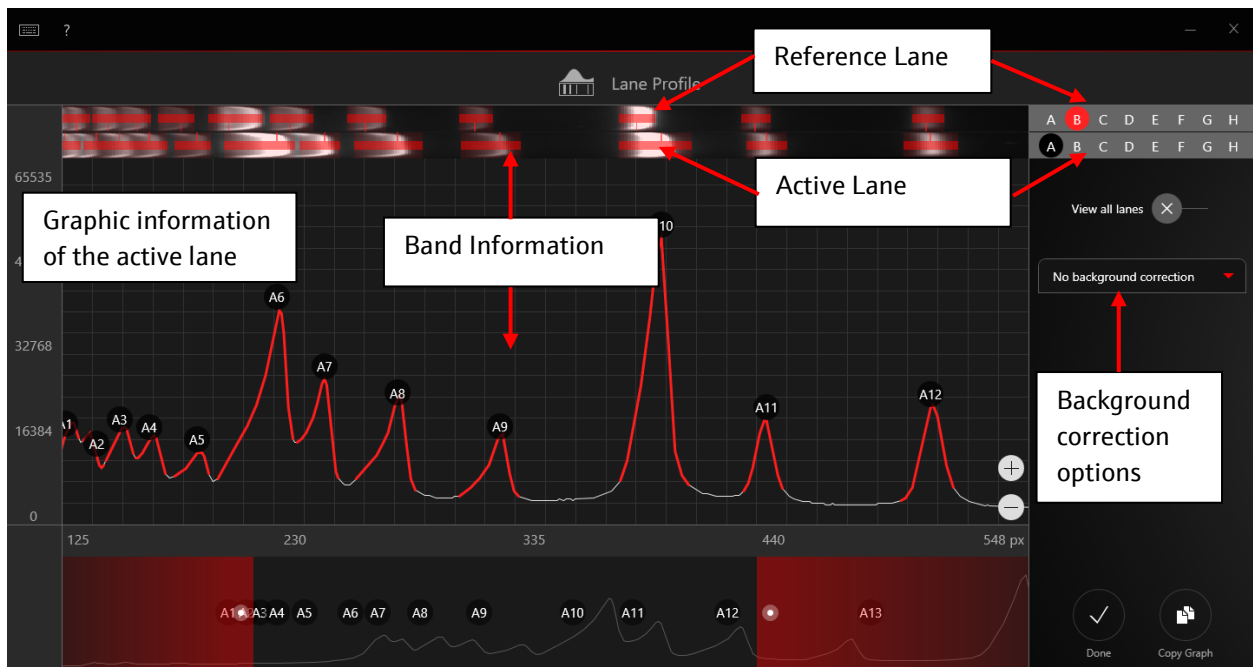
Select to inactivate editing tools to drag/ move the image.

- 5) More analysis tools become available after accepting 1D Lanes and Bands coarse adjustments. These tools are summarized in a table below.

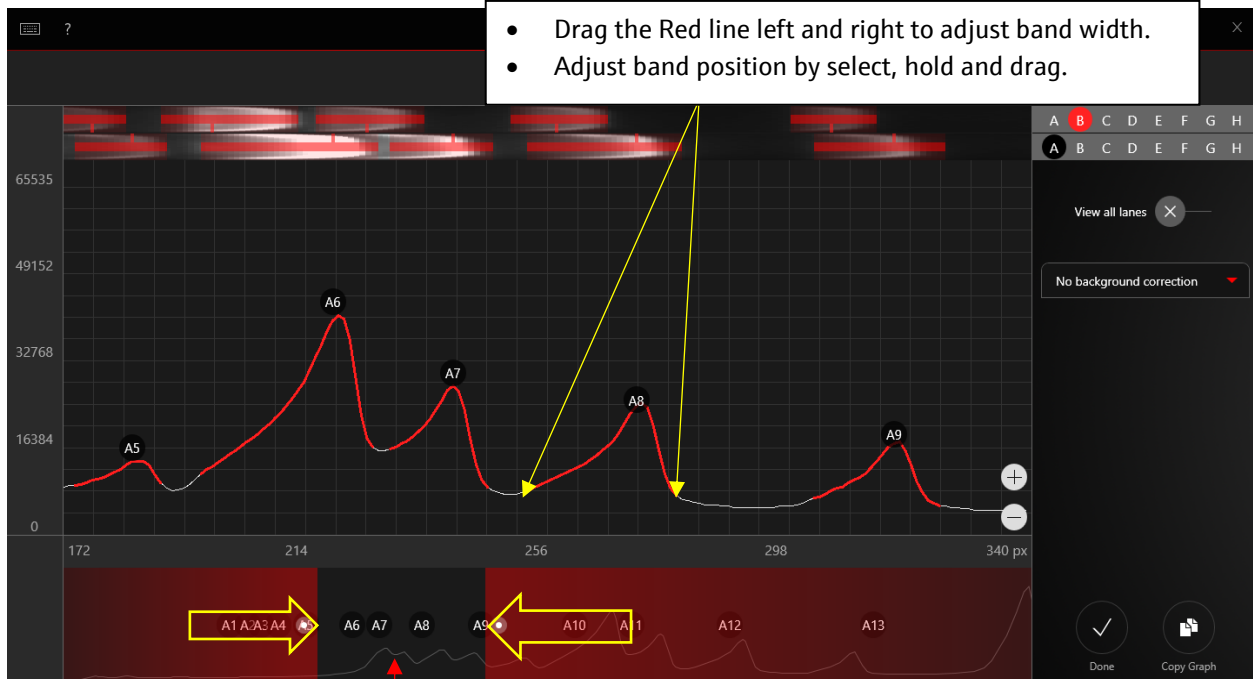
<b>Additional 1D Analysis Tools</b>	
Tool	Function
<b>Lane Profile</b>	Allows for band intensities to be displayed in a histogram for fine adjustments. Side by side lane viewing is possible for comparative analysis. Several background correction options are available. (See <b>Section 7.1.2</b> for more detail).
<b>Concentration Calibration</b>	Graph band intensity values with corresponding known concentrations and apply a best fit line. All band concentrations will be calibrated against this analysis and their concentrations will be available in the report, <b>1D Analysis → Report</b> . (See <b>Section 7.1.3</b> for more detail).
<b>Molecular Weight</b>	Graph band intensity values with corresponding molecular weight values from A) preset ladder or user defined ladders. To perform this analysis: <ol style="list-style-type: none"> <li>1) Select a lane to calibrate.</li> <li>2) Once selected, click "Calibrate".</li> <li>3) Select a preset ladder or create your own.</li> <li>4) Once selected, click "Next".</li> <li>5) Drag and drop MWT tiles to corresponding positions. <i>Note: We recommend selecting at least three values, including one tile each on the high and low end.</i></li> <li>6) Apply best fit line from the Curve dropdown.</li> <li>7) Click "Done". All band concentrations will be calibrated against this analysis and their concentrations will be available in the report, <b>1D Analysis → Report</b>. (See <b>Section 7.1.4</b> for more detail).</li> </ol>
<b>Protein Normalization</b>	Compare changes in protein expression using calculated normalization factor from the user defined reference sample <ol style="list-style-type: none"> <li>1) Select the "N" from the menu and follow the instruction appearing on the top of the menu.</li> <li>2) Perform background correction using Lane Profile.</li> <li>3) Select reference lane from the reference image.</li> <li>4) Click ✓ to confirm the normalization factor calculation.</li> <li>5) Open the signal image and apply the normalization factor.</li> <li>6) Open the report and select TPN or HKP from the result type drop down list.</li> </ol>
<b>Report</b>	Preview, Print, or Export Results to Microsoft Excel.
<b>Redo Analysis</b>	Redo analysis for the pre-selected region.
<b>Delete Analysis</b>	All analysis data is discarded, allowing for a new analysis to be started.

### 7.1.2 Lane Profile

Select Lane Profile to see graphically displayed lanes and bands information.

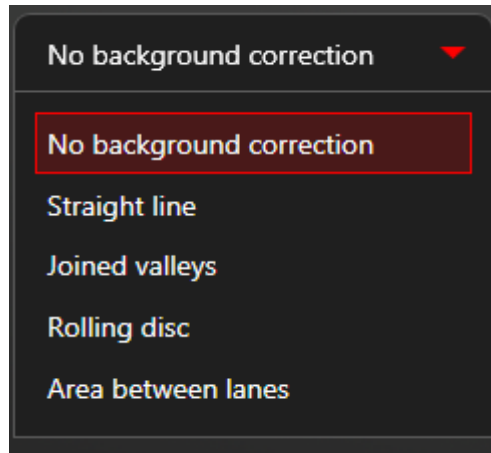


- 1) Select an active lane by clicking the lane ID, the image of selected lane will appear above the graph.

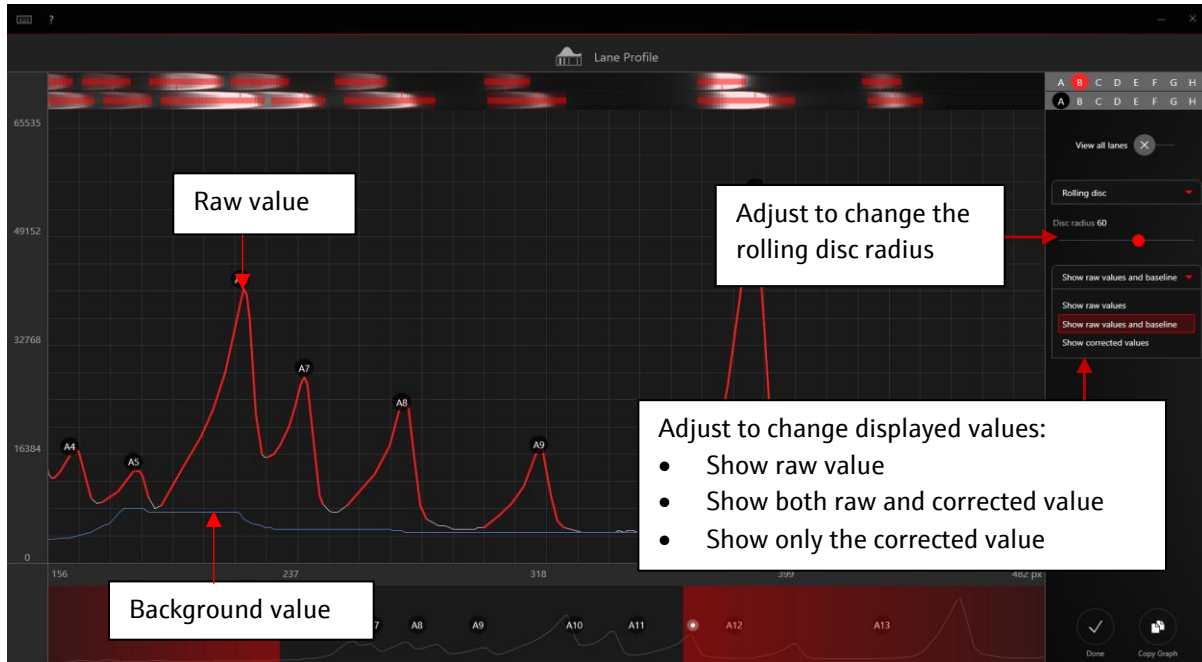


Drag the either dot to zoom in and out to define ROI for adjustment. Drag the gray box left and right to move the graph for adjustment.

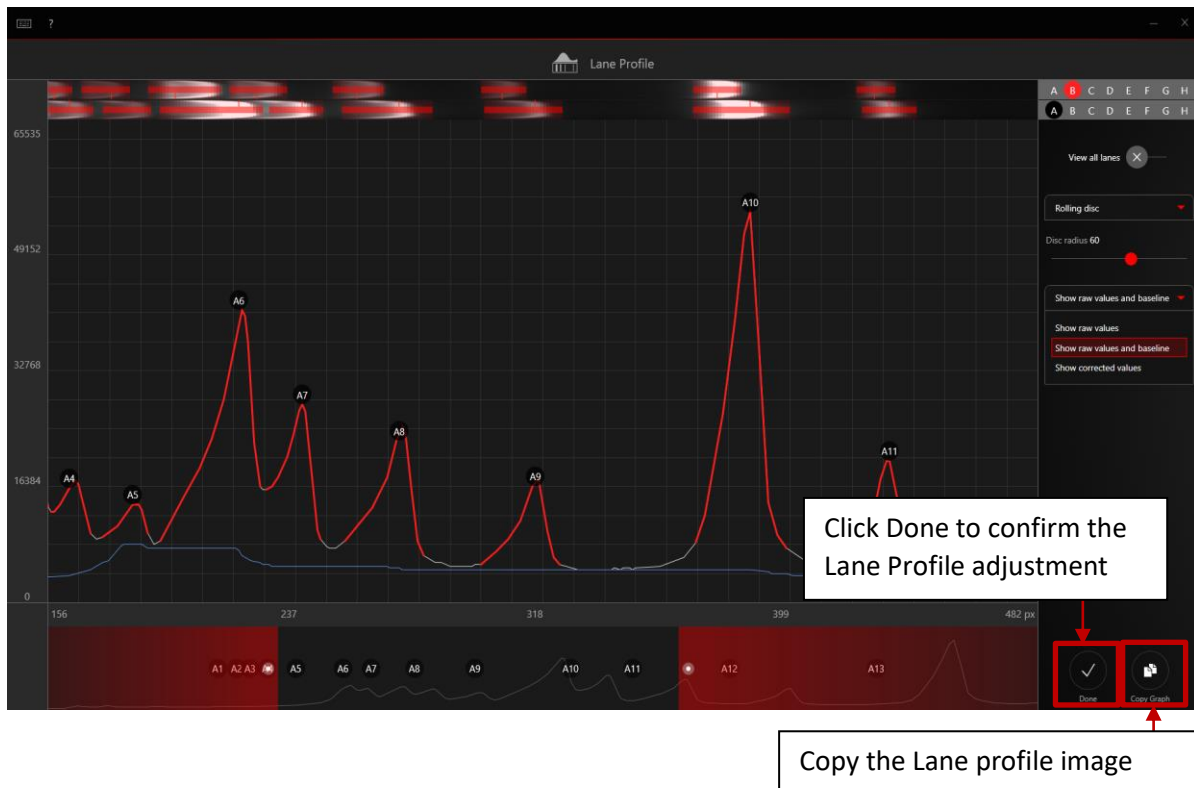
- 2) After adjusting the band width for selected lanes, choose a background correction option from the drop-down list:



- a. **Straight Line:** VisionWorks® will place a straight line (but not necessarily horizontal) under the lowest points at the beginning and end of each lane. VisionWorks® will remove the area of the graph under the straight line. Straight line correction tends to correct well for overexposure, and for variable illumination that is focused on an edge or corner of an image.
  - b. **Joined Valleys:** The valleys that bookend each peak will be joined. Joined Valleys can perform well in a variable illumination condition where the brightest spot is somewhere in the middle of the image, and where bands are sharply defined and quite distinct. Joined Valleys requires a sensitivity value to be designated.
  - c. **Rolling Disc:** A disc (circle) of a defined size is rolled across the bottom edge of the lane profile. If the disc is small, it dips into the valley, which will eliminate background as well as some signal. As the size is increased, the disc becomes too big to drop into the valley but can still effectively eliminate background. Rolling disc performs well in all background conditions, especially uneven illumination as seen with nucleic acid gels.
  - d. **Area between Lanes:** Part of the image may be overexposed, and there may be patterns of deformity between the lanes. This correction takes cross-sections between lanes and the "inter-lane" profiles. Area Between Lanes performs well in all variable illumination situations, providing lanes are distinct and there are clear gaps between them. It performs poorly if bands in adjacent lanes bleed together or touch, because it will tend to eliminate almost all band data in such a scenario.
- 3) After selecting the background correction options, the raw value and baseline value will display on the graph.



- 4) View all lanes can be selected to see a superimposed graph of all lanes and corrected values.
- 5) The corrected image can be viewed by clicking View Corrected Image.
- 6) Click **Done** to accept all the changes applied and exit the Lane Profile window.



### 7.1.3 Concentration Calibration

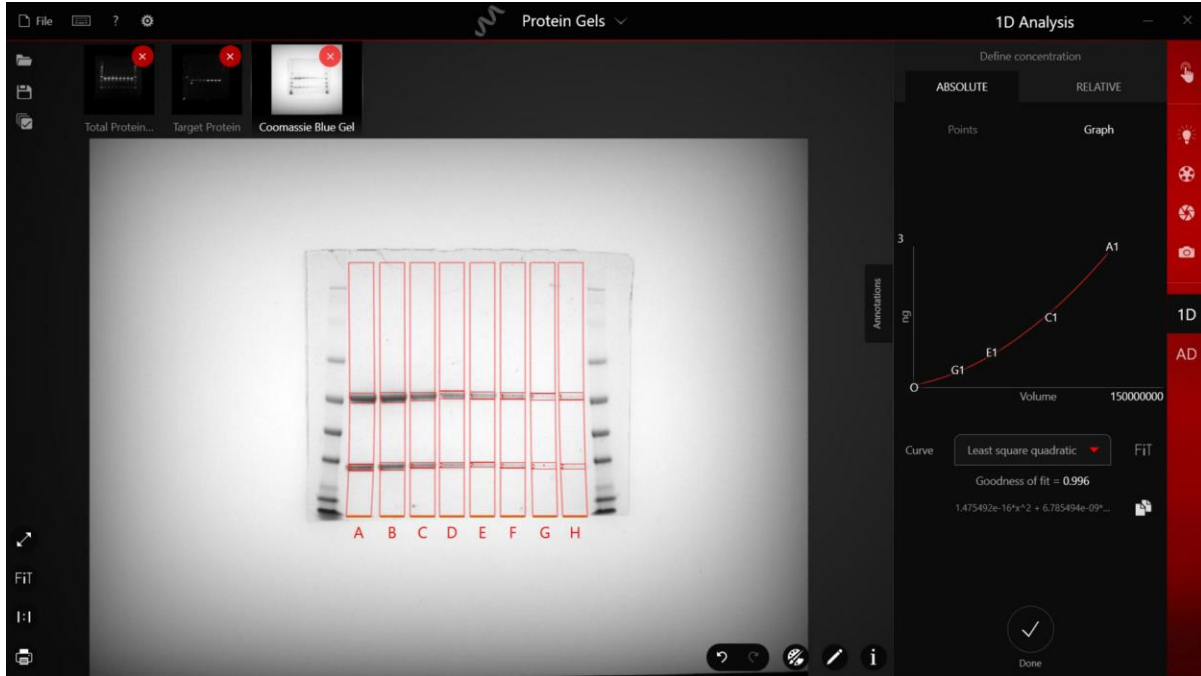
For more accurate results, we recommend users perform the background correction in Lane Profile tool prior to performing concentration calibration. With this tool, users select bands with known mass and assign these mass values to the selected bands in the Concentration Tool interface. A graph of intensity (I-Vol) versus mass (ng) will be generated and all other band masses will be extrapolated from a curve fitted to the provided data points.

#### 7.1.3.1 Absolute Concentration Calibration:

- 1) Select the Concentration calibration menu.
- 2) Select bands in the main viewing window—the band selection tool will turn green when a band is detected. The intensity value of the band will be added to the table on the right.

Define concentration			
ABSOLUTE		RELATIVE	
Points	Graph		
Volume	ng	Source	
1	0.00E+000	0.000	O
2	1.19E+008	3.000	A1
3	8.27E+007	1.500	C1
4	4.68E+007	0.750	E1
5	2.64E+007	0.375	G1

- 3) After adding all the bands of interest, input concentration values in the concentration column (e.g. default ng) in the table.



- 4) Select "Graph" to view a x-y plot of your data. A calibration curve can be applied to your data, by default least square line is displayed. Two additional lines are provided: *Least square line* and *least square quadratic*.
- 5) After selecting the appropriate curve, select "OK".
- 6) All band concentrations will be calibrated against this analysis and their concentrations will be available in the report, **1D Analysis** → **Report**.

Back

Coomassie Blue Gel

Export

Show Report on One Page

Image Information Lanes **Bands** Concentration Calibration Molecular Weight

Simplified

Bands

Lane ID	Band ID	Rf	I-Max	Volume	I-%	Absolute Mass (ng)	C-%
A	A1	0.54	49,425	1.194E+8	63.44	3E+0	71.28
A	A2	0.81	43,125	6.881E+7	36.56	1.209E+0	28.72
B	B1	0.53	48,934	1.213E+8	62.10	3.039E+0	69.17
B	B2	0.81	42,960	7.403E+7	37.90	1.355E+0	30.83
C	C1	0.53	40,813	8.271E+7	63.88	1.5E+0	68.70
C	C2	0.80	33,453	4.676E+7	36.12	6.834E-1	31.30
D	D1	0.52	33,151	6.086E+7	64.78	1.003E+0	70.01
D	D2	0.80	34,045	3.309E+7	35.22	4.296E-1	29.99
E	E1	0.52	28,532	4.677E+7	64.02	7.5E-1	69.84
E	E2	0.80	21,604	2.629E+7	35.98	3.239E-1	30.16
F	F1	0.53	28,470	4.036E+7	61.38	5.577E-1	64.20
F	F2	0.80	20,305	2.54E+7	38.62	3.11E-1	35.80
G	G1	0.53	16,974	2.643E+7	53.57	3.75E-1	57.57
G	G2	0.80	15,261	2.29E+7	46.43	2.764E-1	42.43
H	H1	0.53	18,324	2.976E+7	57.44	3.761E-1	58.68
H	H2	0.80	17,332	2.205E+7	42.56	2.649E-1	41.32

Image Information

Lanes

Bands

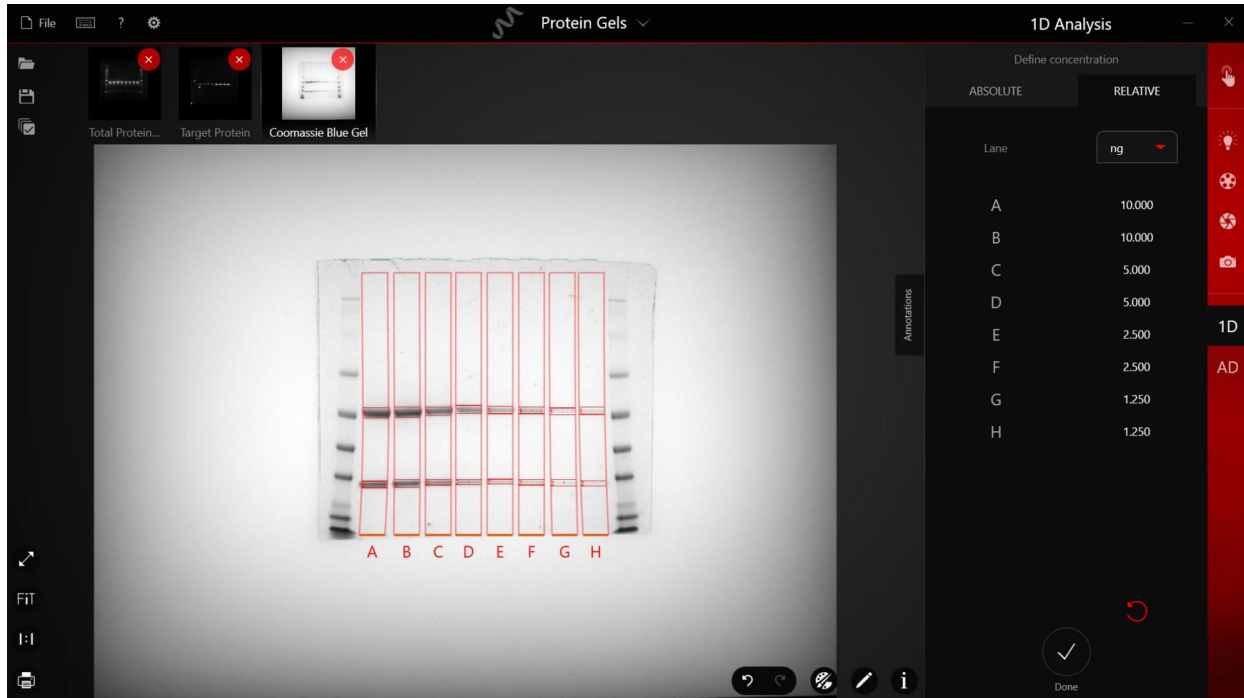
Concentration

Calibration

Molecular Weight

### 7.1.3.2 Relative Concentration Calibration:

1) Select **Concentration** Calibration menu and choose **Relative**.



- 2) Input protein concentration for each detected lane and click Done to confirm the concentration value.
- 3) The software will calculate relative concentration for each detected band based on the lane concentration and band intensity.
- 4) Result is available in 1D analysis Report.

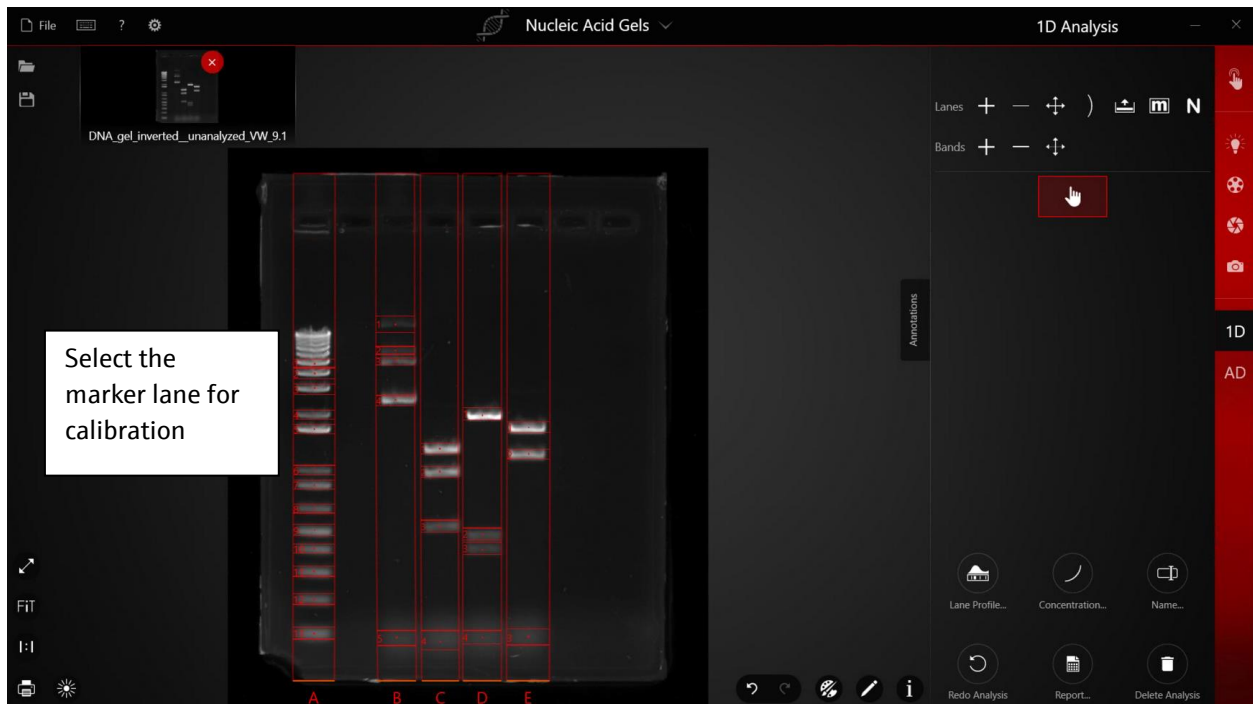
Lane ID	Band ID	Rf	I-Max	Volume	I-%	Relative Mass (ng)
A	A1	0.54	49,425	1.194E+8	63.44	6.34
A	A2	0.81	43,125	6.881E+7	36.56	3.66
B	B1	0.53	48,934	1.213E+8	62.10	6.21
B	B2	0.81	42,960	7.403E+7	37.90	3.79
C	C1	0.53	40,813	8.271E+7	63.88	3.19
C	C2	0.80	33,453	4.676E+7	36.12	1.81
D	D1	0.52	33,151	6.086E+7	64.78	3.24
D	D2	0.80	34,045	3.309E+7	35.22	1.76
E	E1	0.52	28,532	4.677E+7	64.02	1.60
E	E2	0.80	21,604	2.629E+7	35.98	0.90
F	F1	0.53	28,470	4.036E+7	61.38	1.53
F	F2	0.80	20,305	2.54E+7	38.62	0.97
G	G1	0.53	16,974	2.643E+7	53.57	0.67
G	G2	0.80	15,261	2.29E+7	46.43	0.58
H	H1	0.53	18,324	2.976E+7	57.44	0.72
H	H2	0.80	17,332	2.205E+7	42.56	0.53

### 7.1.4 Molecular Weight Calibration

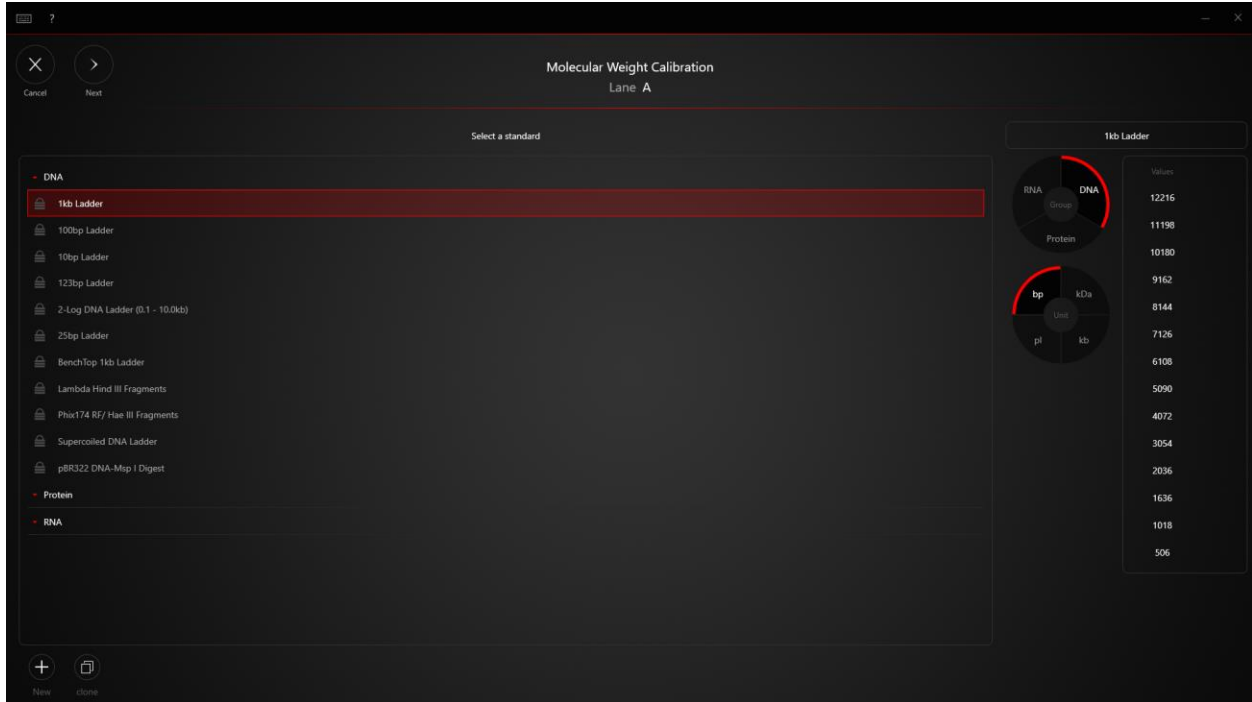
- 1) After finishing 1D analysis, proceed to Molecular Weight Calibration. Click **"M"** from the 1D analysis menu.



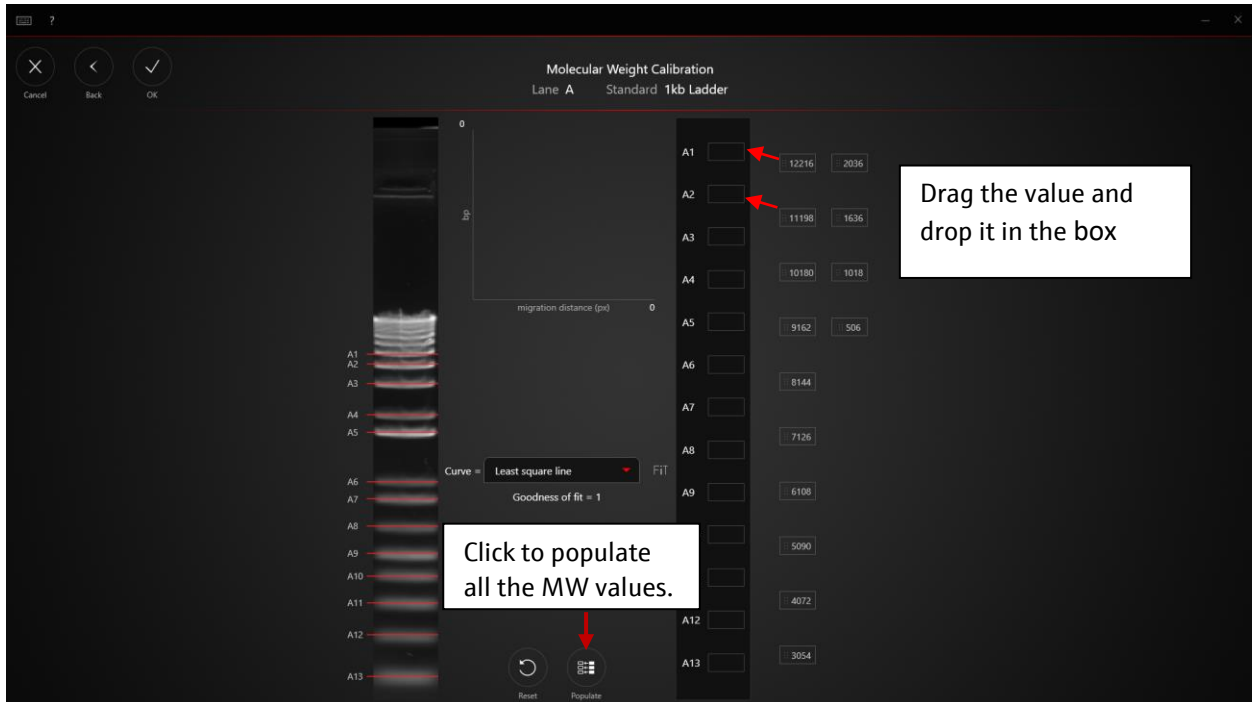
- 2) Select the marker lane from the image.



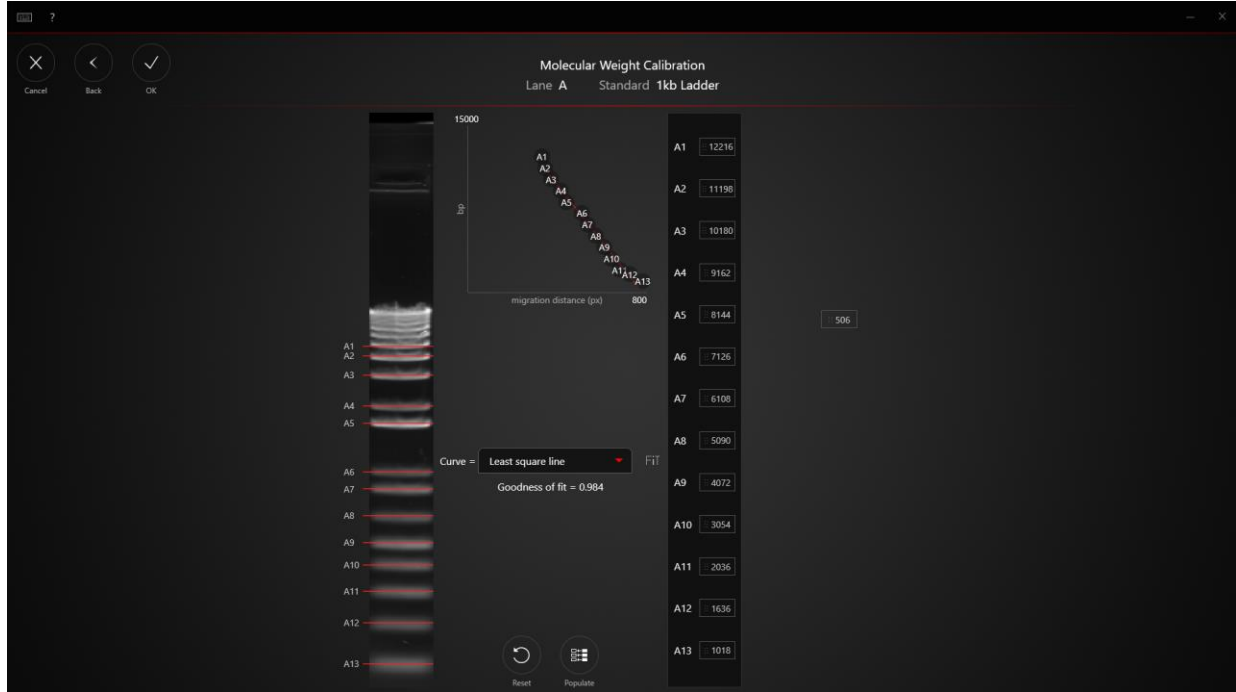
- 3) Choose a preset ladder from the list or click **"New"** to create a new ladder for your application. Enter values on the right side of the screen for a new ladder. Otherwise, click **"Next"** to proceed to assign molecular weights for each band.



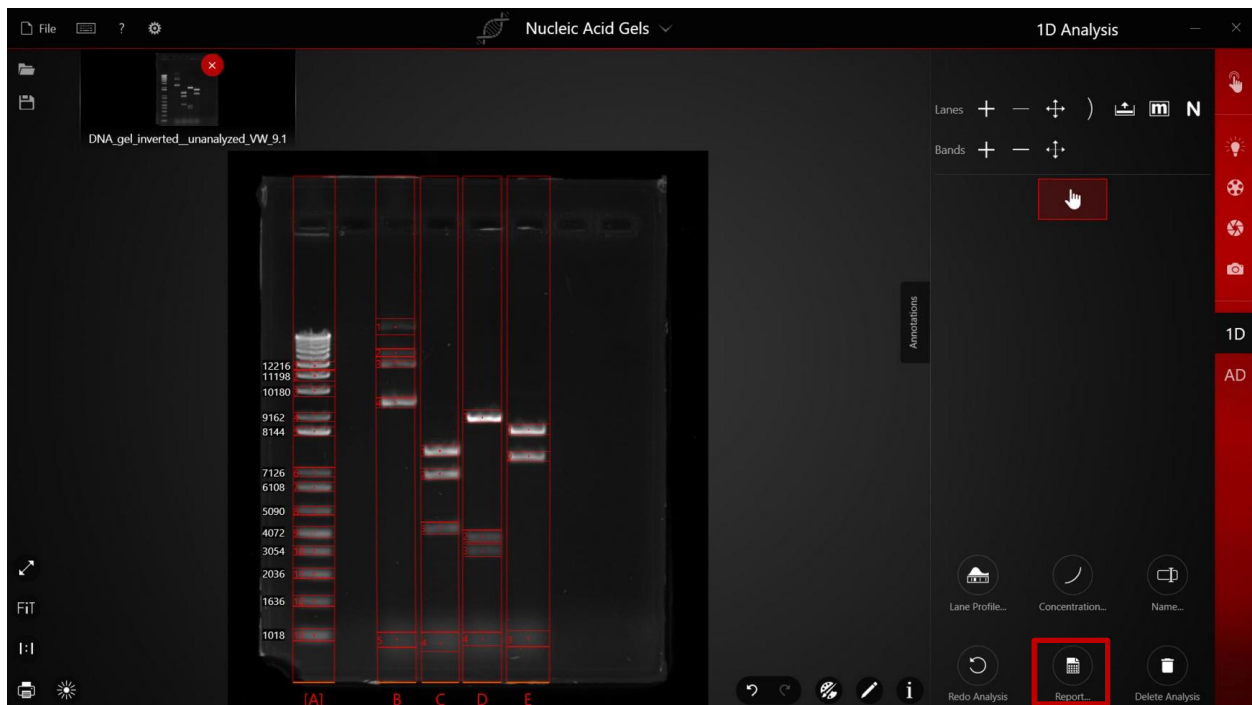
- 4) In the Molecular Weight Calibration window, drag the MWT value from the list to the placeholders next to the lane IDs or click "Populate" to populate all the MW values.



- 5) As more values are assigned to the marked bands, a calibration curve will be created. You can choose the curve type from the drop-down list under the graph. Click "OK" to accept the calibration and proceed to the next step. For more details about Molecular Weight Calibration, refer to the **How to Guide: Molecular Weight calibration**



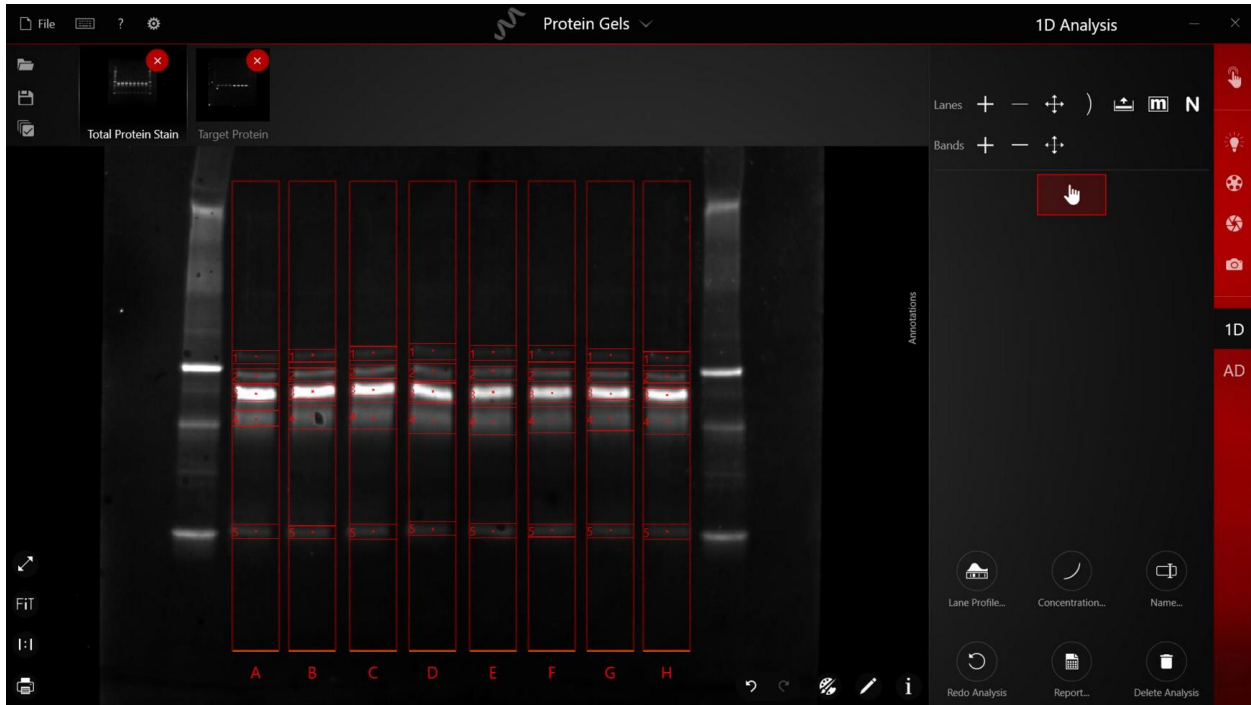
- 6) After accepting the Molecular weight calibration, software will return to the main window. Molecular weight calibration result can be accessible in **Report**.



### 7.1.5 Protein Normalization

Protein normalization allows user to compare the changes of protein expression using calculated total protein normalization factor or housekeeping protein normalization factor. The protein normalization factor is calculated from a user defined protein sample and applied to selected target image. Normalized area density result can be accessed from Report table.

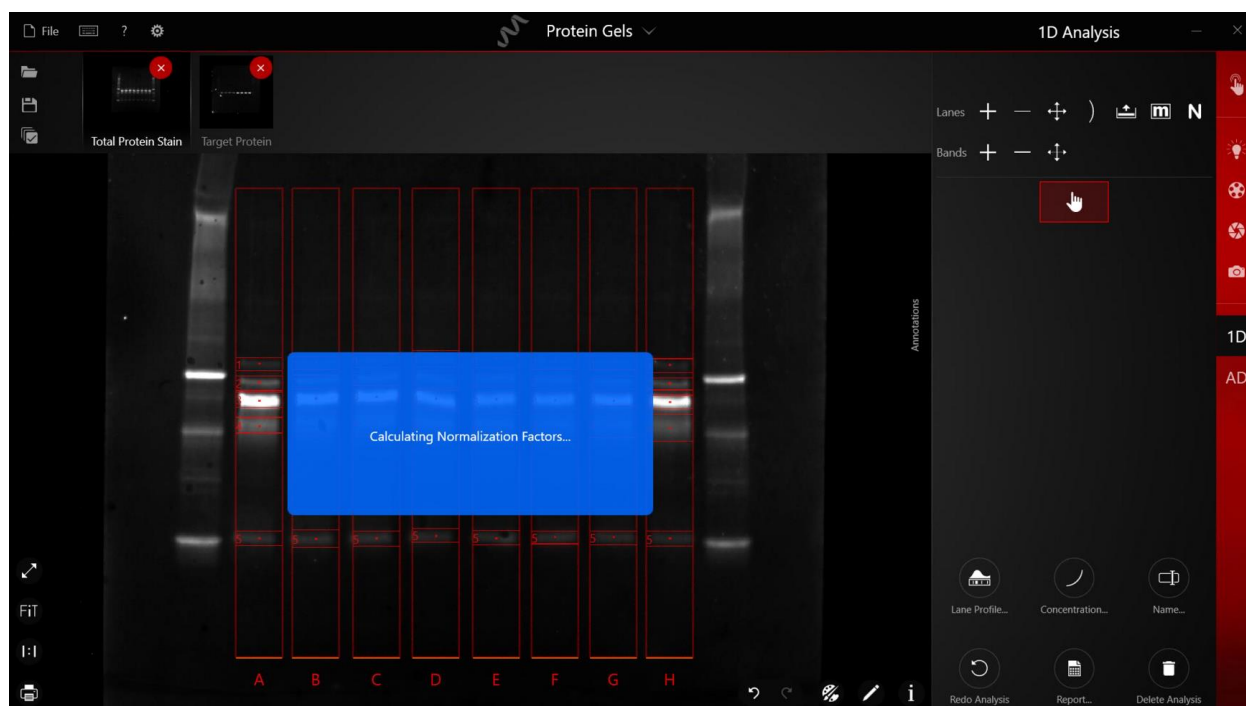
- 1) Open the reference lane and perform 1D analysis.



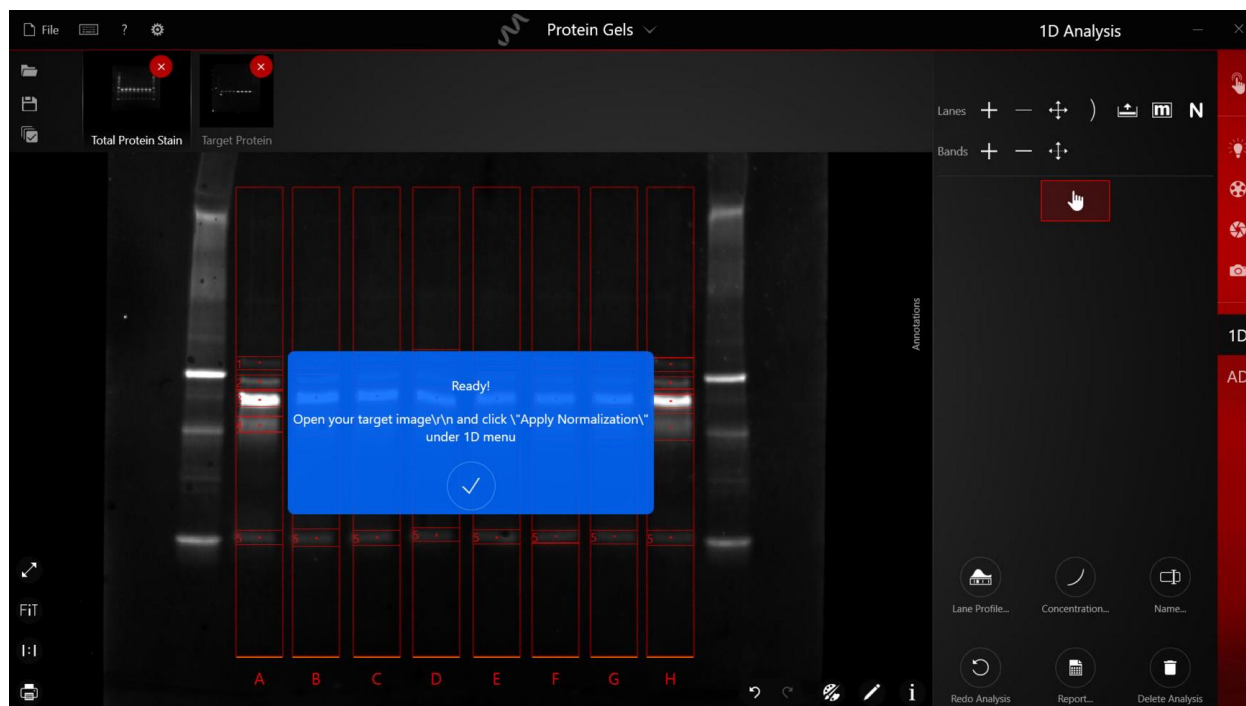
- 2) After finishing 1D analysis, click "N" from the menu to activate the protein normalization tool.



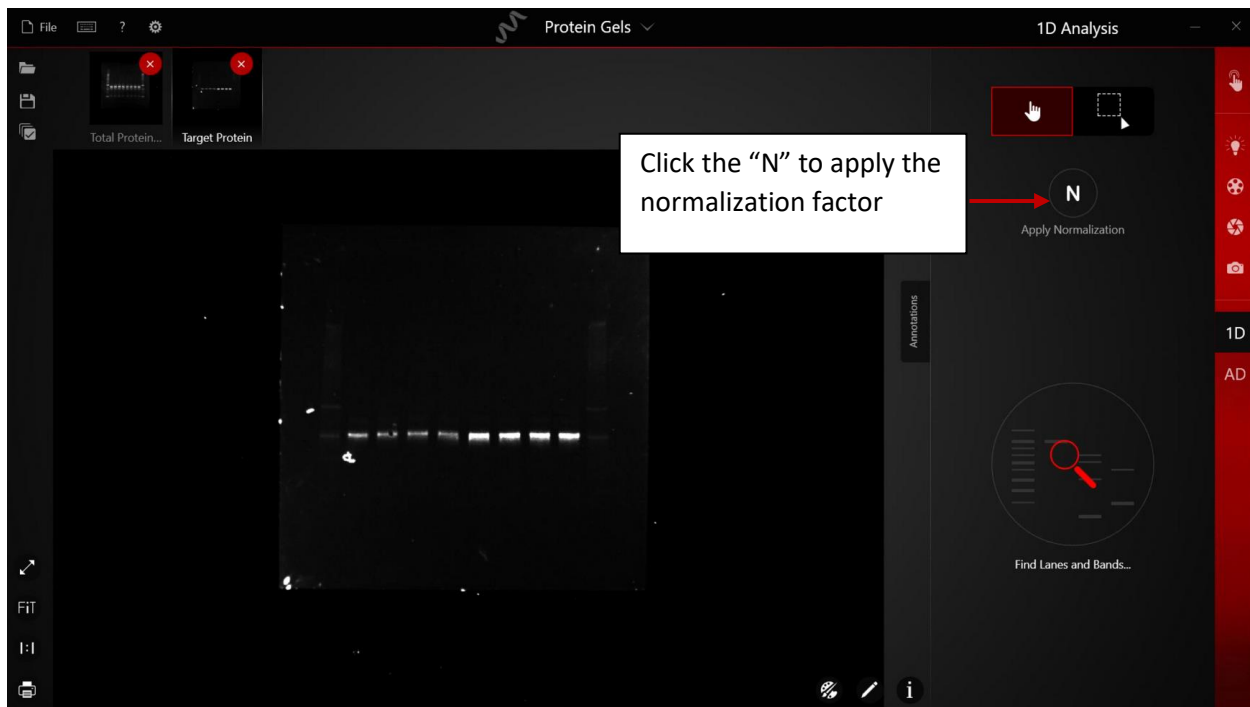
- 3) Perform the background correction follow the instruction in section 7.1.2 if the message "Check background correction" appears.
- 4) Click the reference lane on the image and wait for the Normalization factor to be available.



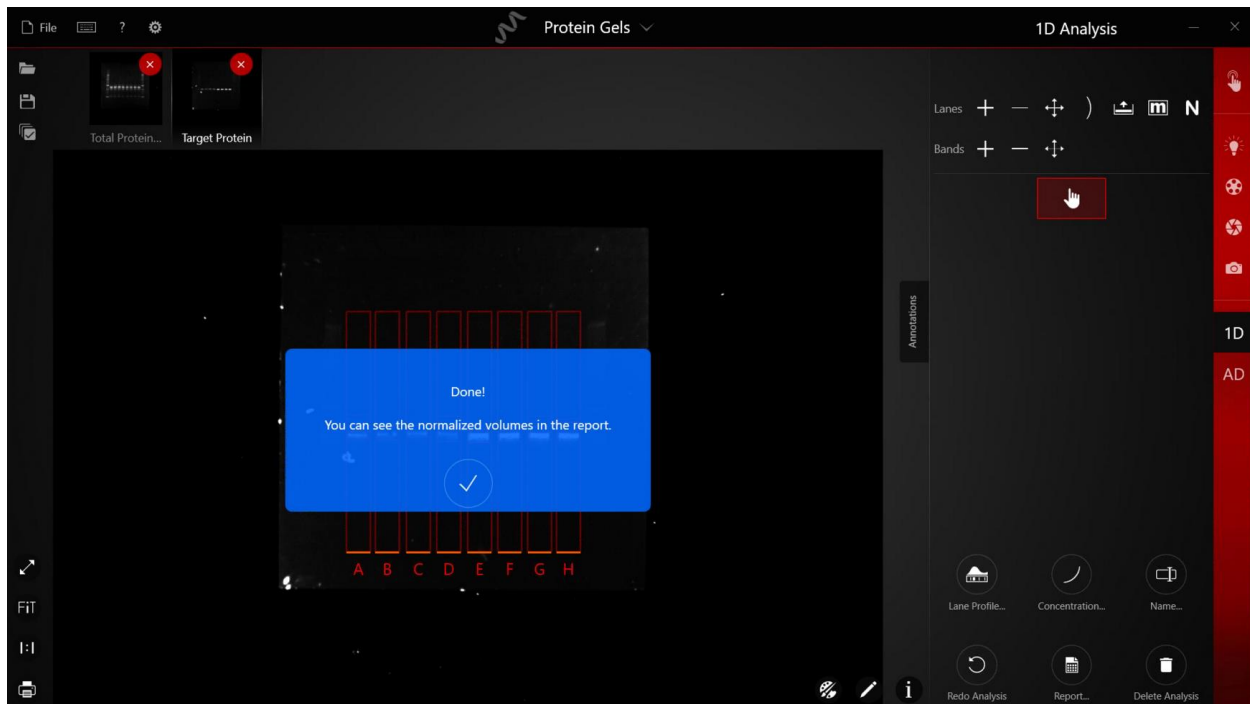
5) Click "OK" to accept calculated normalization factor.



6) Open the signal image and apply the normalization factor.



7) Click "OK" to confirm the protein normalization calculation.



8) Go to Lane profile to adjust the background correction, apply the same background correction type and value as the reference image to get more accurate result.

9) Go to Report to view the protein normalization result.

Select TPN to view total protein normalization value. Select HKP to view housekeeping protein normalization value.

Lane ID	Band ID	MW	Rf							
A	A1		0.57	950	3.197E+5	1E+0	3.197E+5	100.00		
B	B1		0.56	884	3.029E+5	1.011E+0	3.062E+5	100.00		
C	C1		0.56	808	2.902E+5	1.015E+0	2.945E+5	100.00		
D	D1		0.57	945	3.091E+5	1.034E+0	3.195E+5	100.00		
E	E1		0.56	1,598	6.253E+5	1.047E+0	6.549E+5	100.00		
F	F1		0.56	1,696	6.631E+5	1.047E+0	6.943E+5	100.00		
G	G1		0.56	1,637	7.265E+5	1.036E+0	7.527E+5	100.00	100.00	
H	H1		0.56	1,623	7.223E+5	1.028E+0	7.428E+5	100.00	100.00	

### 7.1.6 1D Analysis Reporting

- 1) Click **“Report”** to access analysis results. Click through each tab to view the data or select **“Show Report on One Page”** to view all the data on one page.

Click to enable One Page view

Select each tab to view analysis result

Click to view the gel image

Image name	Number of Lanes	Background Color	Background Correction	Diac radius	Lanes Constant	Lanes Straight
iBox 615_No Stain Total protein im.	8	Black	No background correction		False	True

- 2) The report is set to *Simplified* template by default. To create a customized report template with different measurements, select/deselect desired column item from the drop-down list next to each table title.

Click to add as customized template

Check desired data from the drop-down list

Lane ID	Width(px)	Start(px)	Length(px)	Mass (ng)	Full-Vol	I-Max	I-Vol	TPN Factor	TPN Vol	C-Max	C-Vol	Lanes Bands	Lane Image
A	63.00	346.79	712.04	100.00	6.36E+8	43.174	8.988E+7	1E+0	8.988E+7	0.00	0E+0	5	
B	63.00	346.79	712.04	100.00	6.291E+8	39.802	9.837E+7	1.011E+0	9.945E+7	0.00	0E+0	5	
C	63.00	346.79	712.04	100.00	6.266E+8	37.306	9.142E+7	1.015E+0	9.279E+7	0.00	0E+0	5	
D	63.00	346.79	712.04	100.00	6.152E+8	35.382	9.258E+7	1.034E+0	9.57E+7	0.00	0E+0	5	
E	63.00	346.79	712.04	100.00	6.072E+8	32.004	8.567E+7	1.047E+0	8.973E+7	0.00	0E+0	5	
F	63.00	346.79	712.04	100.00	6.074E+8	30.962	8.009E+7	1.047E+0	8.386E+7	0.00	0E+0	5	
G	63.00	346.79	712.04	100.00	6.138E+8	33.844	8.468E+7	1.036E+0	8.773E+7	0.00	0E+0	5	
H	63.00	346.79	712.04	100.00	6.184E+8	35.130	7.831E+7	1.028E+0	8.053E+7	0.00	0E+0	5	

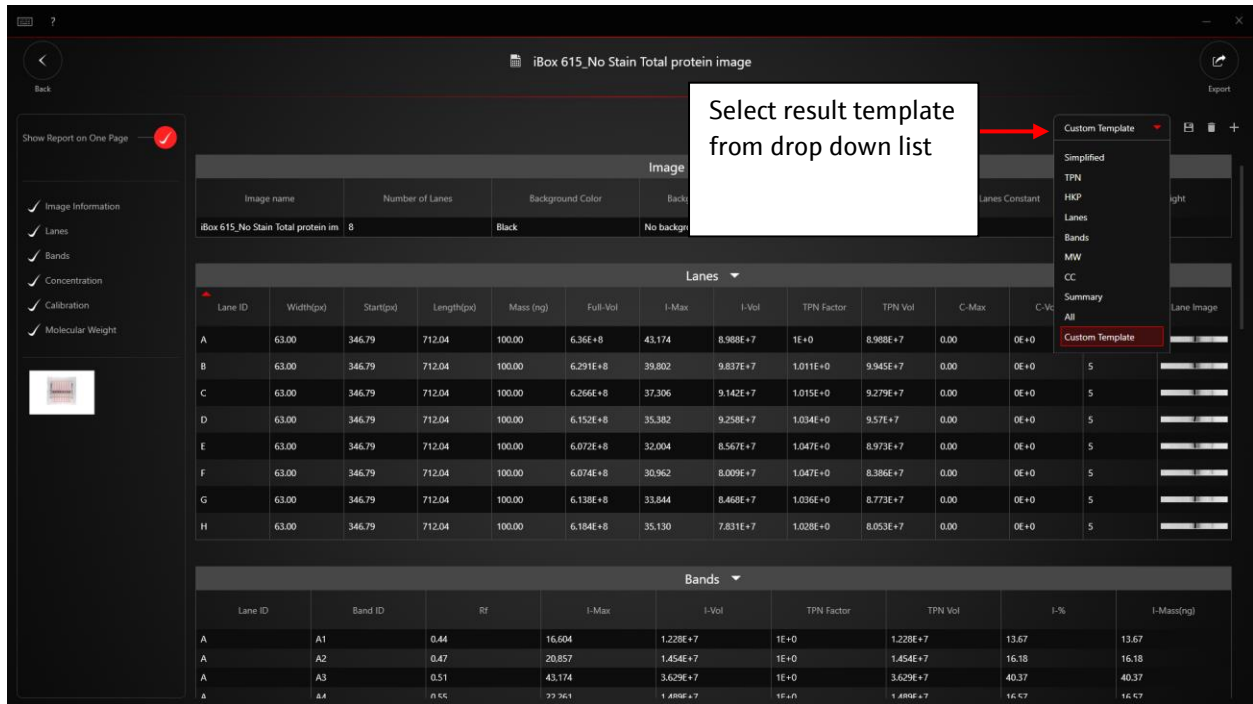
- 3) After selecting the analysis data for the table to display, click the plus symbol "+" to add the template as customized result template. Edit template name and select the check mark "✓" to save the template for later use.

Edit template name

Click to save the customized template

Lane ID	Width(px)	Start(px)	Length(px)	Mass (ng)	Full-Vol	I-Max	I-Vol	TPN Factor	TPN Vol	C-Max	C-Vol	Lanes Bands	Lane Image
A	63.00	346.79	712.04	100.00	6.36E+8	43.174	8.988E+7	1E+0	8.988E+7	0.00	0E+0	5	
B	63.00	346.79	712.04	100.00	6.291E+8	39.802	9.837E+7	1.011E+0	9.945E+7	0.00	0E+0	5	
C	63.00	346.79	712.04	100.00	6.266E+8	37.306	9.142E+7	1.015E+0	9.279E+7	0.00	0E+0	5	
D	63.00	346.79	712.04	100.00	6.152E+8	35.382	9.258E+7	1.034E+0	9.57E+7	0.00	0E+0	5	
E	63.00	346.79	712.04	100.00	6.072E+8	32.004	8.567E+7	1.047E+0	8.973E+7	0.00	0E+0	5	
F	63.00	346.79	712.04	100.00	6.074E+8	30.962	8.009E+7	1.047E+0	8.386E+7	0.00	0E+0	5	
G	63.00	346.79	712.04	100.00	6.138E+8	33.844	8.468E+7	1.036E+0	8.773E+7	0.00	0E+0	5	
H	63.00	346.79	712.04	100.00	6.184E+8	35.130	7.831E+7	1.028E+0	8.053E+7	0.00	0E+0	5	

- 4) To use the customized result template, select from the result template drop down menu.



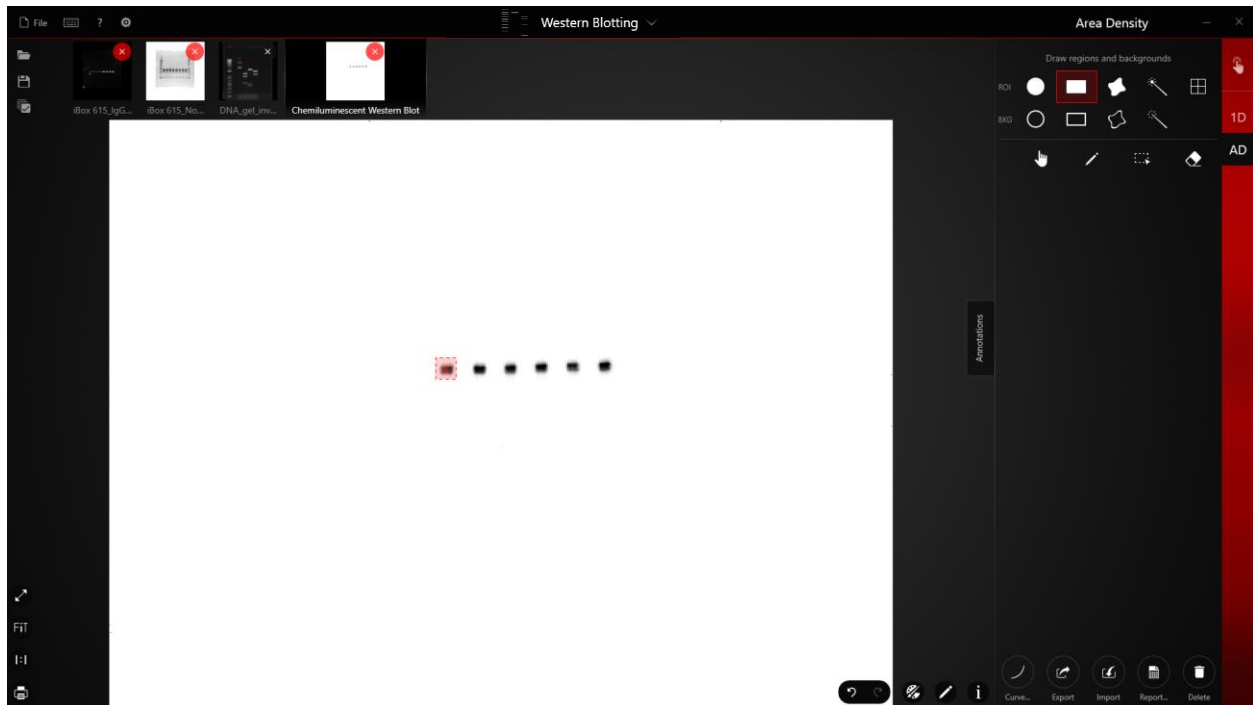
- 5) Click "Export" to print the report, or export to Excel file for further analysis.

## 7.2 Area Density

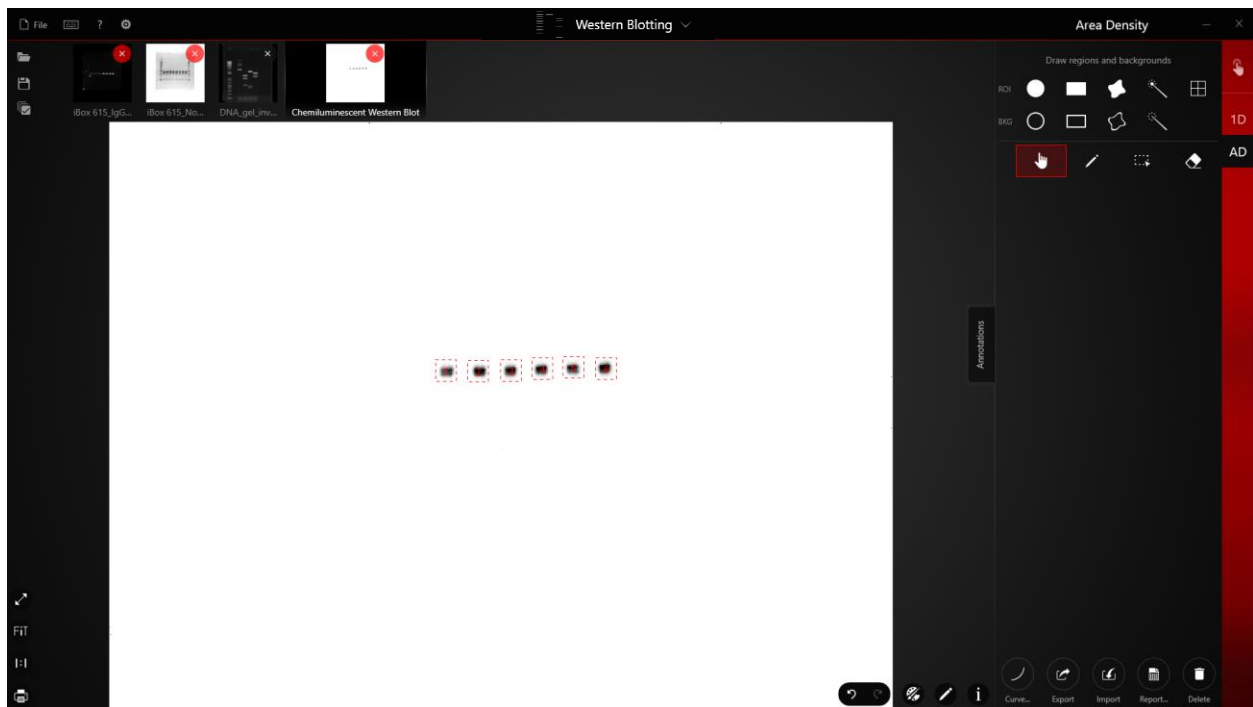
Similar to 1D analysis, Area Density allows for the quantification of intensities from a captured image. However, this feature is free form, which enables users to analyze objects not necessarily confined to lanes and bands (e.g. spot arrays). Area density still enables users to detect background and selectively subtract it out from an image.

### 7.2.1 Define Region and Background for Analysis

- 1) To begin using Area Density, navigate to **Analysis** → **AD (Area Density)** sub-menu and select the "Add Regions" button.
- 2) Select a shape tool to draw the region of interest for analysis.

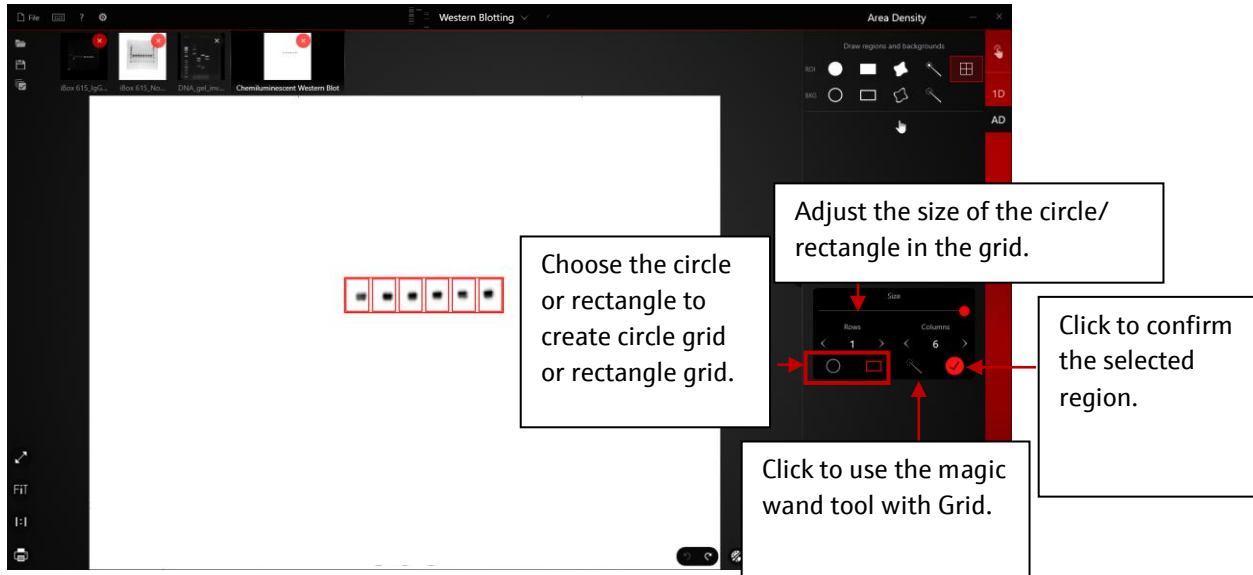


3) To draw additional ROIs of the same shape, simply click on the new region.

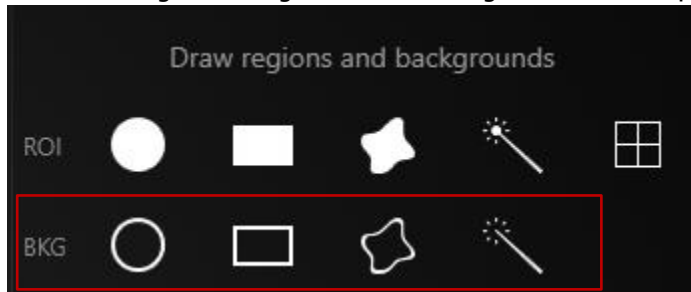


4) Click the Hand tool to deactivate the AD region tool.

- 5) The most commonly used region defining tool is rectangle (above). With relatively uniform spacing between lanes and bands, you can use Grid function to create multiple ROI boxes all at once (below). The size of grid boxes can be adjusted by slide the size bar. If you need to change the size of an individual grid box, you can do so under the Edit mode.



- 6) To add background regions, use the region selection options in the BKG section:



- 7) Follow **Step 2 to 3** to add background regions. The software will apply local background subtraction if the number of defined background regions is the same as the number of the ROI regions. If the number of selected background region is larger or smaller than the selected ROI region, the software will use the average value for the background subtraction.
- 8) After defining both the signal and background ROI, you can select **“Report”** to access the Area Density data.
- 9) After signal and background ROIs are selected, more tools become visible to customize the selected regions. A summary table of all the tools and their functions is included below:

---

<b>Tool</b>	<b>Function</b>
<b>Hand tool</b>	Deactivate the selected AD region tool.
<b>Edit</b>	Activate the edit mode.
<b>Multi Selection</b>	Select multiple regions to move or delete.
<b>Clean Region</b>	Click to delete selected AD ROI or background region.
<b>Delete Regions</b>	Allows for regions to be selectively deleted as needed.
<b>Curve</b>	Calibrated Value can be added to each Region # defined in the captured image. Mean Density and Total Density are not affected and depend only on the intensity curve, but the new Calibration value can be added to the Result Template.
<b>Import &amp; Export</b>	This tool allows for regions to be imported or exported. Useful for saving and loading regions for repetitive imaging applications.
<b>Report</b>	This tool displays all quantitative data from the selected regions. Options for print and export to Excel are available here. Refer to <b>Section 12.0 VisionWorks® Calculations</b> .
<b>Edit</b>	Allows for the relocation, resizing and adjustment of the overall image location.
<b>Delete Analysis</b>	This deletes the quantitative analysis data that was overlaid on the image allowing for a new analysis to be performed.

- 10)** Using the tools above, adjust all regions according to preference. Once satisfied, select the **"Report"** button to have all results displayed. From that window, select, your export preference.

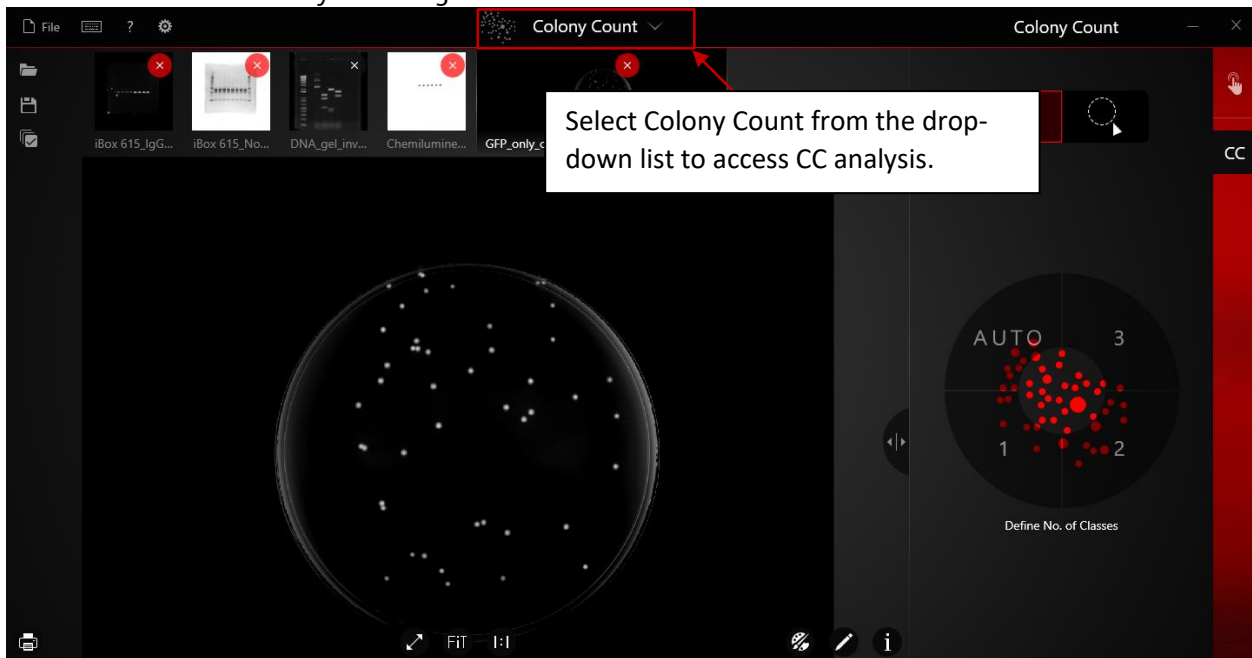
## 7.3 Colony Counting

Colony counting allows users to quantify intensities from colonies on a petri dish. VisionWorks® can identify these intensities automatically or users may define their own. Once identified results of the analysis may be displayed.

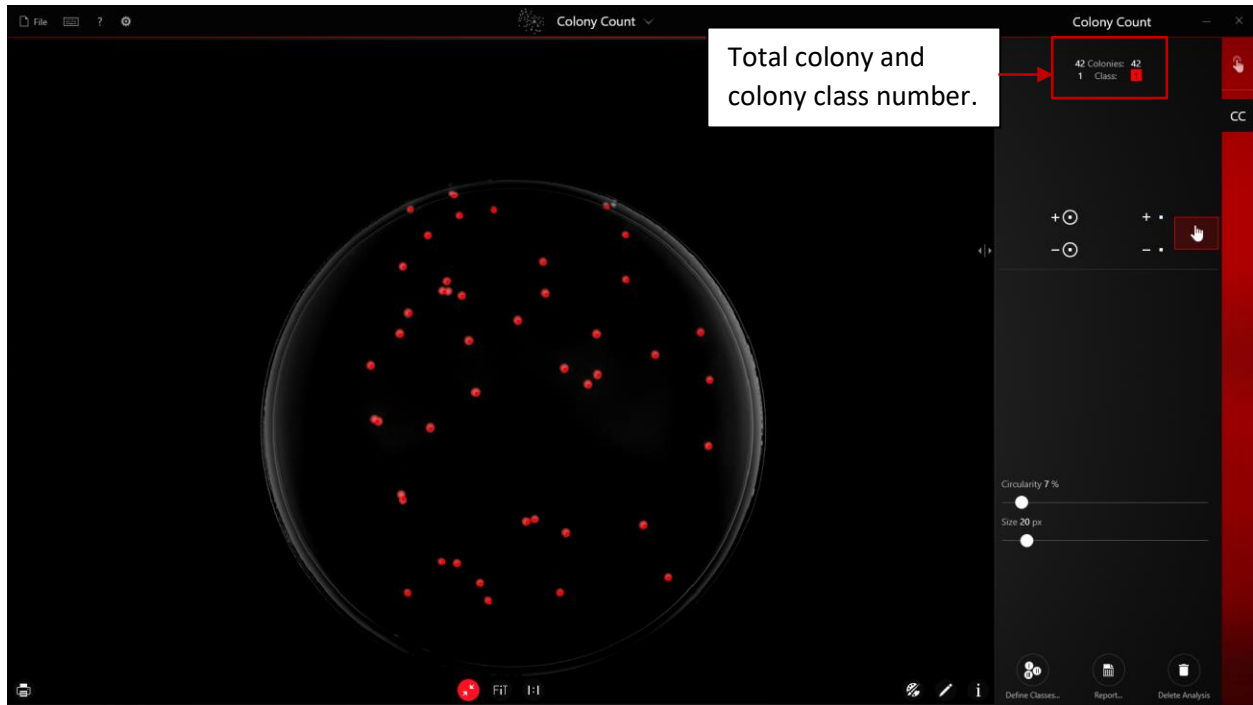
### 7.3.1 Colony Counting for Single Class Plate

To access the powerful colony counting tool, follow the steps indicated below:

- 1) Select the “CC” button in the top right corner of the screen, to be taken to the Colony Counting screen.



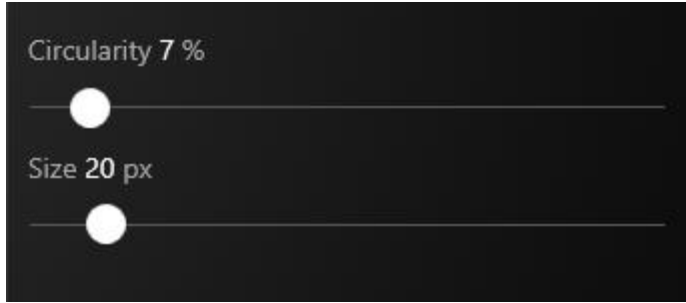
- 2) Within the “Colony Count” screen, select “Define Region” to draw a circle around the region of interest for your plate and click “OK” to confirm the region. Or simply click “1” from the lower wheel to start counting.



- 3) The total colony number will be displayed on the main interface in the menu after colony counting is done.
- 4) Tools available for editing defined colonies are listed below.

Tool	Function
------	----------

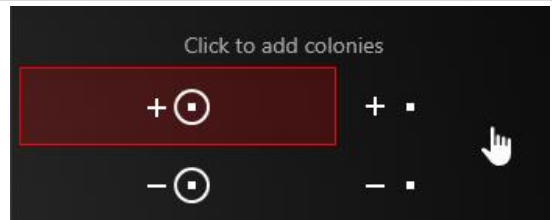
Available filters:  
**Circularity** and **Size**



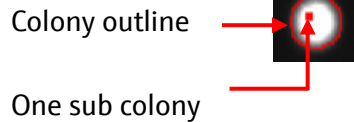
- Increase **Circularity** to remove irregular shaped colonies or decrease the circularity to pick up irregular shaped colonies
- Increase **Size** to remove small colonies or decrease size to pick up smaller colonies

**Edit** – Provides access to six tools to assist in the quantification of colonies

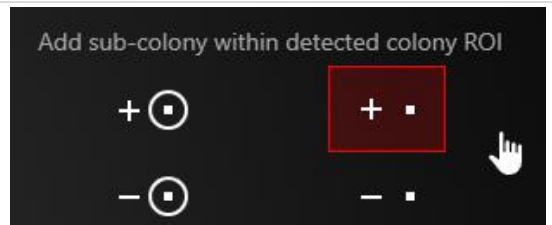
*Add Colony ROI*—Adds a colony ROI with a sub-colony



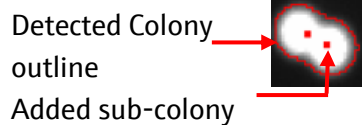
**Example of adding a Colony ROI:**



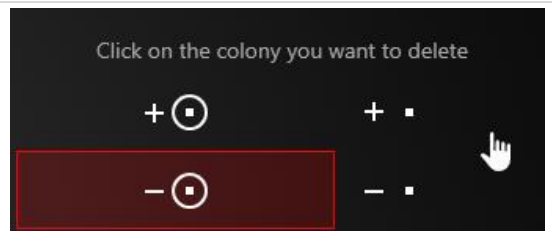
*Add Sub-colony*—Adds a sub-colony in a detected colony ROI



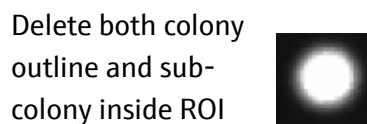
**Example of adding a sub-colony:**



*Delete Colony ROI*—Deletes a colony ROI with a sub-colony



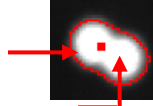
**Example of a deleted colony ROI:**



*Delete Sub-colony*—Deletes a sub-colony in a detected colony ROI

**Example of deleted sub-colony:**

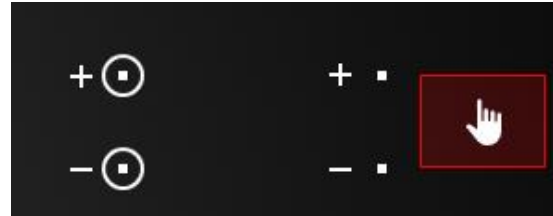
Colony outline remains



Sub-colony is deleted



*Hand tool* – Deselect any active editing tools and allows for the image to be move around the screen



- 5) After accepting all the edits to the detected colonies, total colony number and total colony area can be viewed under Report function.

The screenshot shows the 'Classes' report for 'Colony ROIs'. The table displays the following data:

Class Number	No of colonies	No of colony ROIs	Total Area (px)
1	42	41	12,067

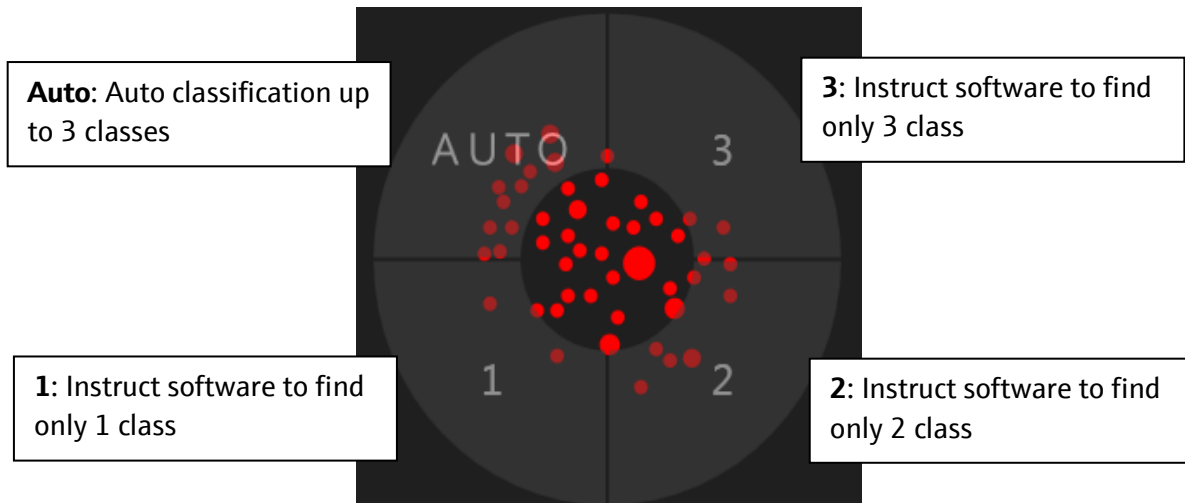
If No. of Colonies > No. of Colony ROIs:

- You may have two touching colonies recognized as 1 colony ROI with 2 sub-colonies. Refer to Section 7.3.1 for example of colony ROI and Sub-colonies.

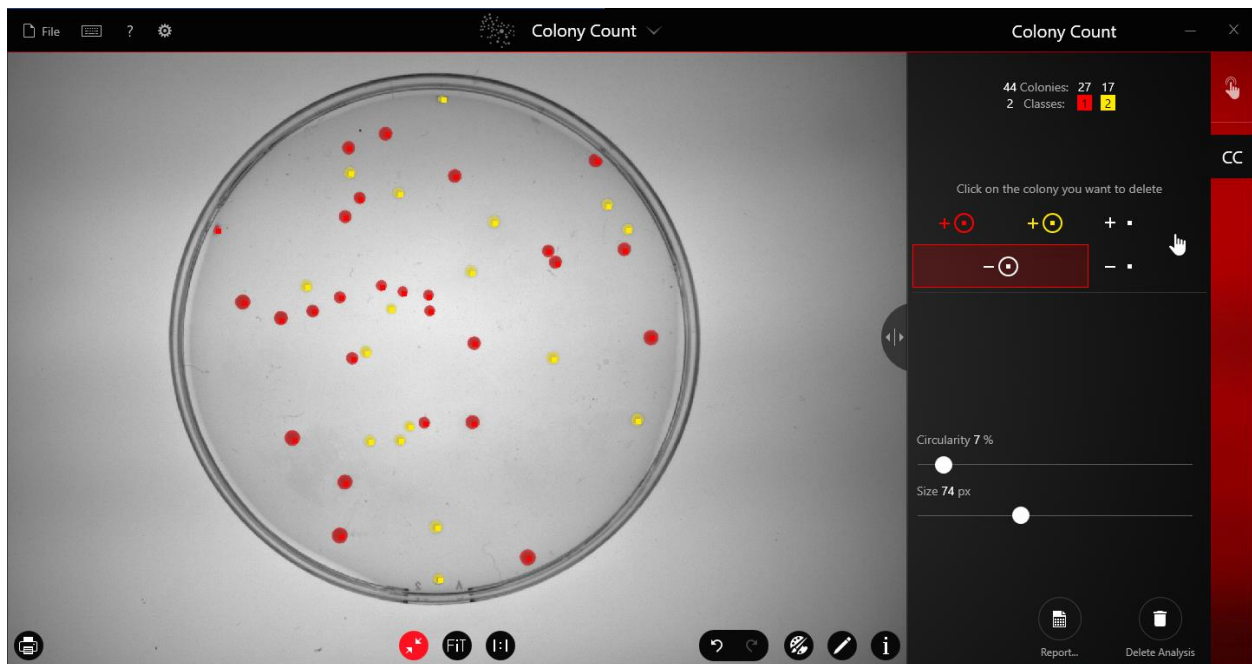
## 7.4 Colony Counting for More than One Class

VisionWorks 9.1 software offers multi-class auto classification up to three different classes. To access the Multi classification functions, follow the steps below:

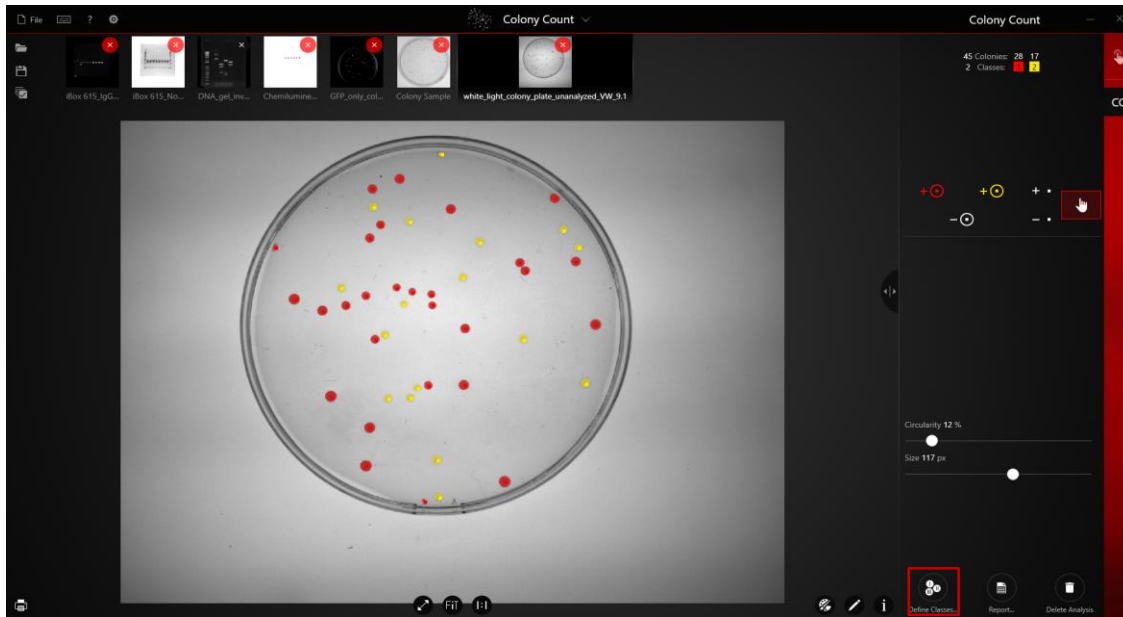
- 1) Within the "Colony Count" screen, select "**Define Region**" to draw a circle around the region of interest for your plate and click "**OK**" to confirm the region. Or simply click "**Auto**" if you don't know how many classes on your plate; "**2**" if you have two classes on the plate; "**3**" if you have three different classes on the plate from the lower wheel to start counting.



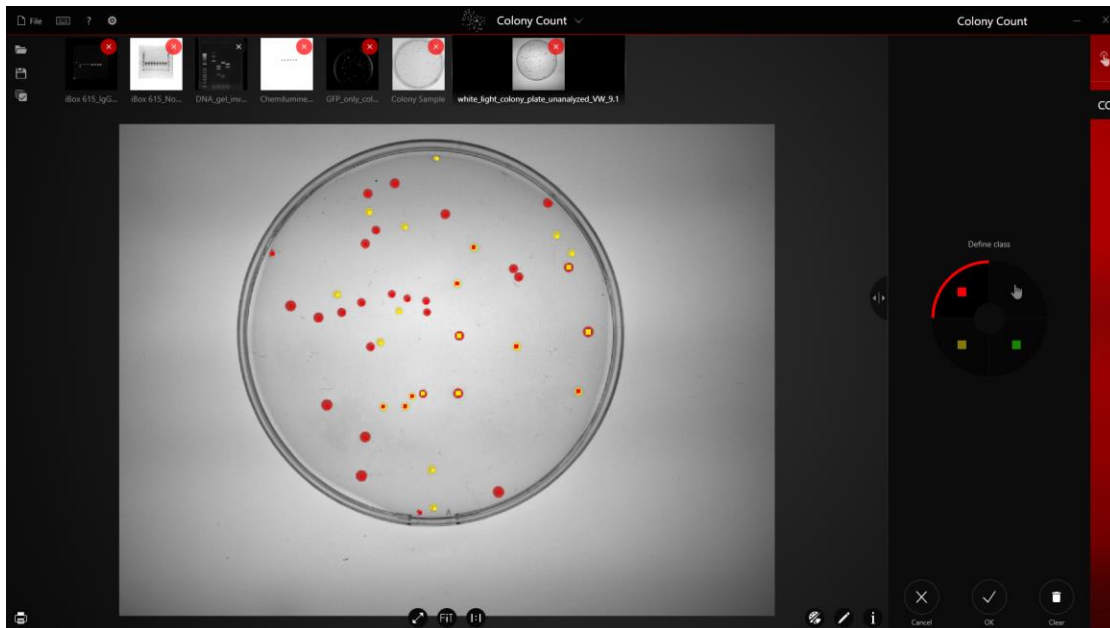
- 2) The total colony number will be displayed on the main interface in the menu after colony counting is done.



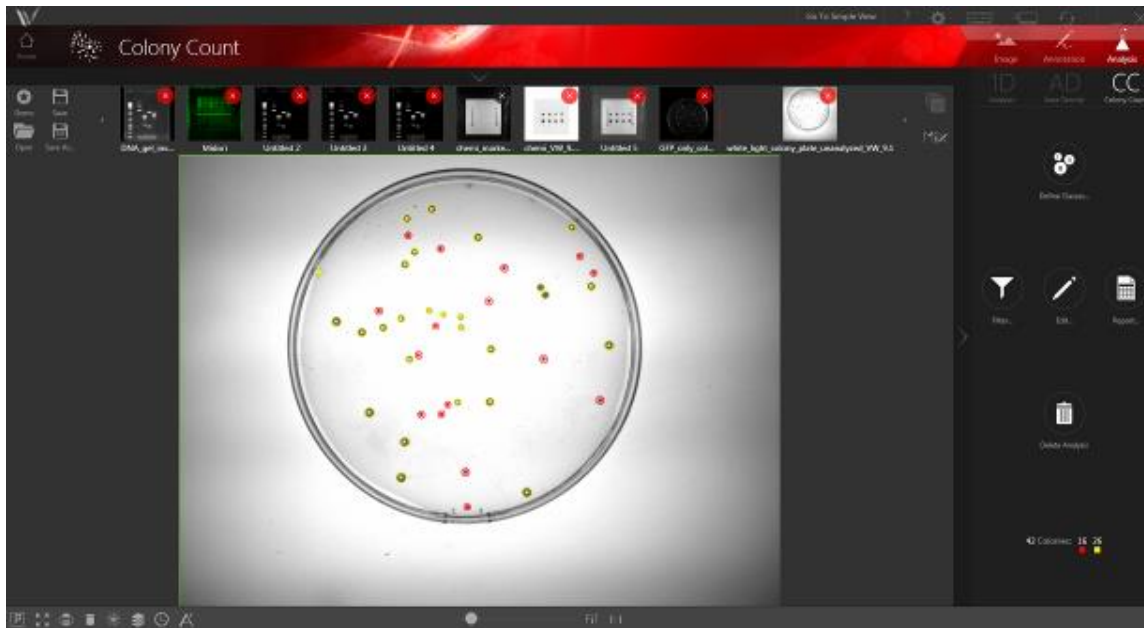
- 3) Use the **Size** filter under **Filter** tab to eliminate the small colonies. Click **Done** to accept the change.
- 4) After filtering the detected colonies, you can redefine classes by using **Define Classes** function.



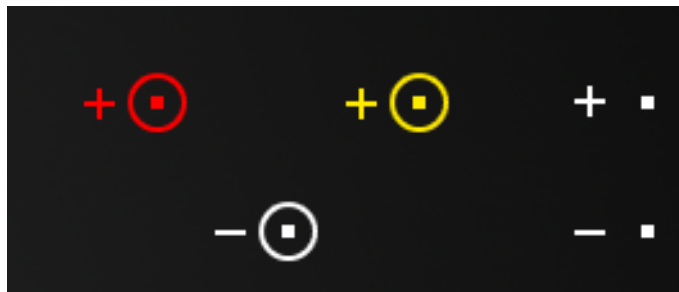
- 5) In the **Define Classes** menu, first choose class color, then click on the detected colonies to add them into appropriate classes. Click **OK** to accept selection and perform auto classification again. Please note, the Define classes should be performed before adding or delete any colonies using the editing tool.



6) Check the re-classified colony number from the main interface.



7) To edit detected colonies, choose edit tool from the menu below. When editing, choose class color first, then click on the missed colony to add to selected class.

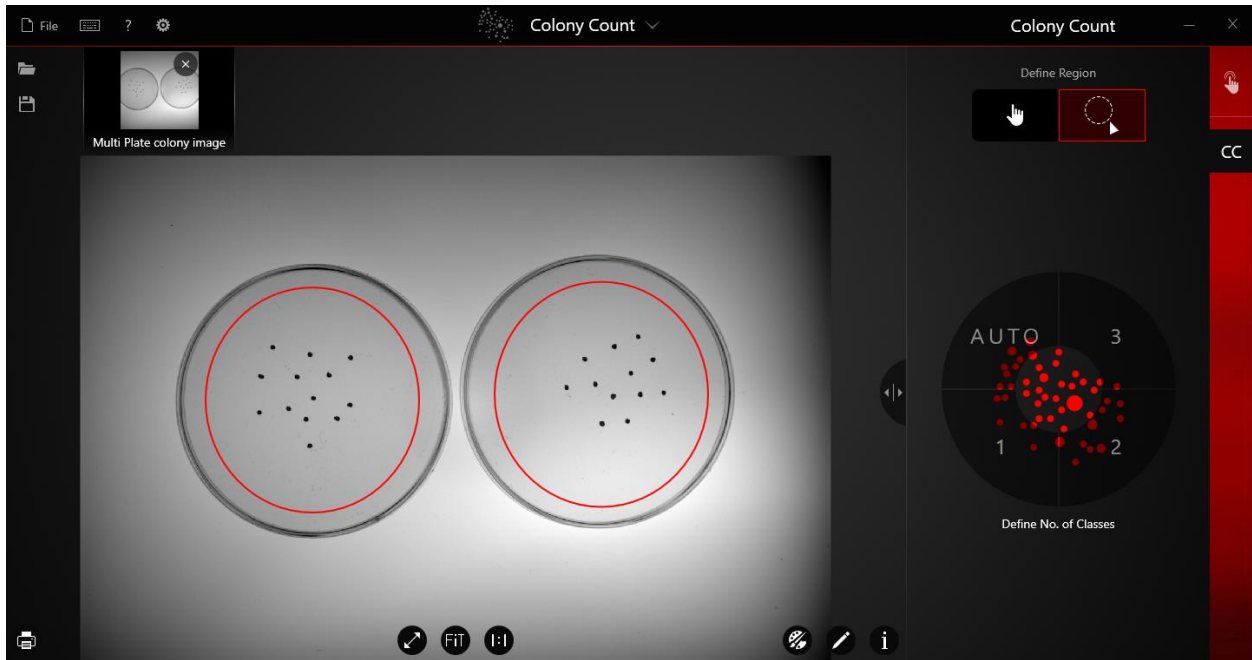


8) After finishing editing, access results by clicking "Report".

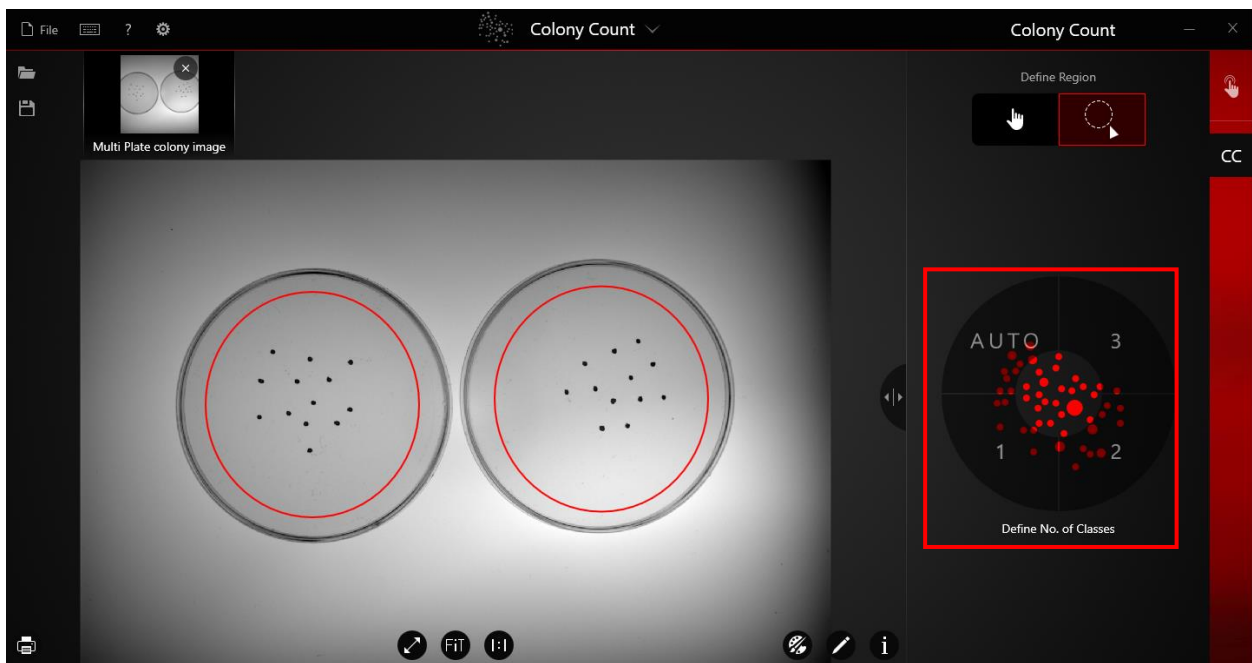
## 7.5 Colony Counting for Multiple Plates

VisionWorks software offers multi-class auto analysis for multiple selected regions. To access the multiple region analysis functions, follow the steps below:

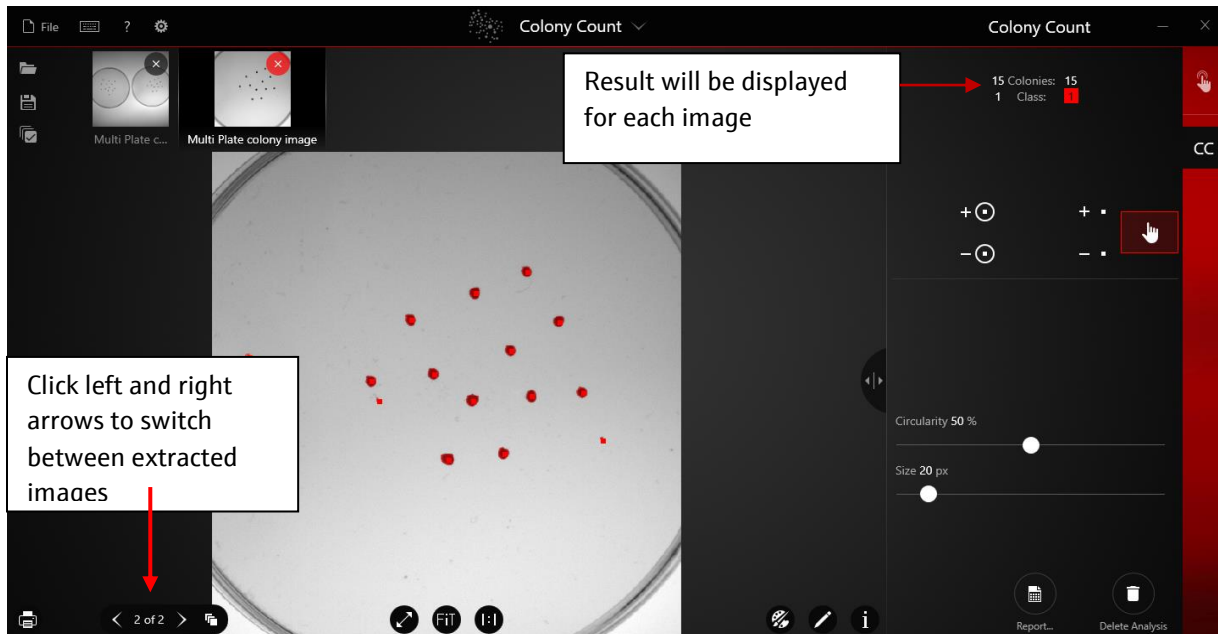
- 1) Within the "Colony Count" screen, select "Circle" tool to draw a Circle on the image, simply click on the second region to create a new ROI.



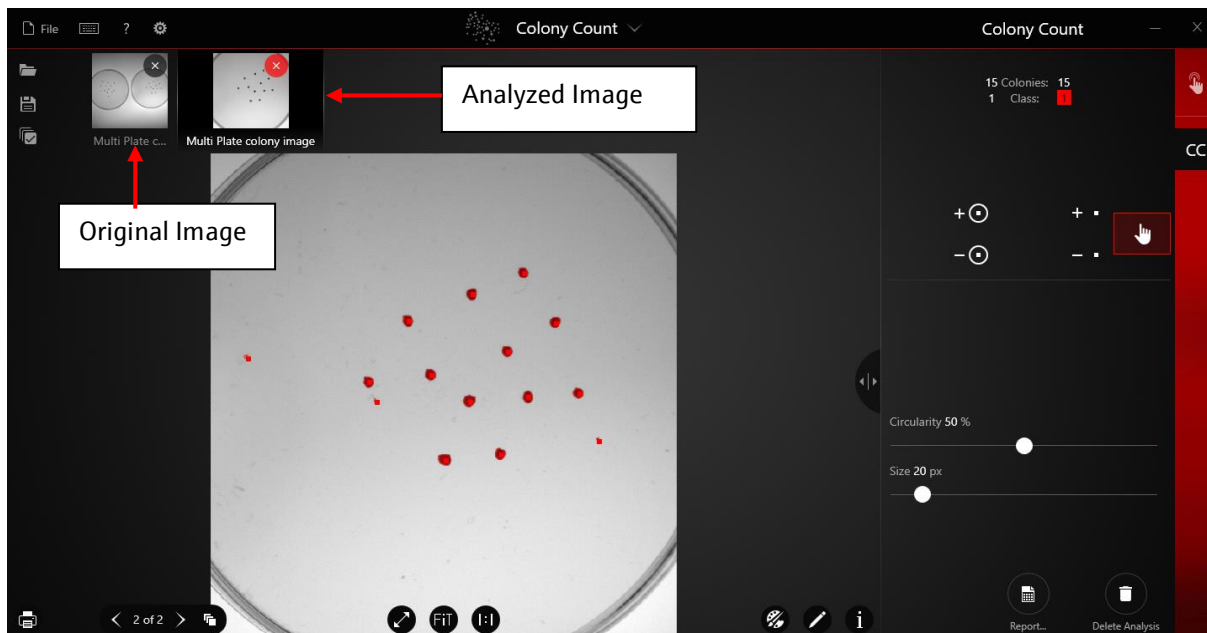
- 2) Depending on the sample type, choose colony counting options from the lower wheel.



- 3) Selected regions will be extracted into a series of images and select colony counting type will be applied to all the extracted images.
- 4) After the counting, you can use the **Size** and **Circularity filter** to edit detected colonies. Same filter value will be applied to all of the extracted images.



- 5) Click "Report" to view the full colony counting result for each extracted image.
- 6) Original image will be accessible from the thumbnail Gallery.



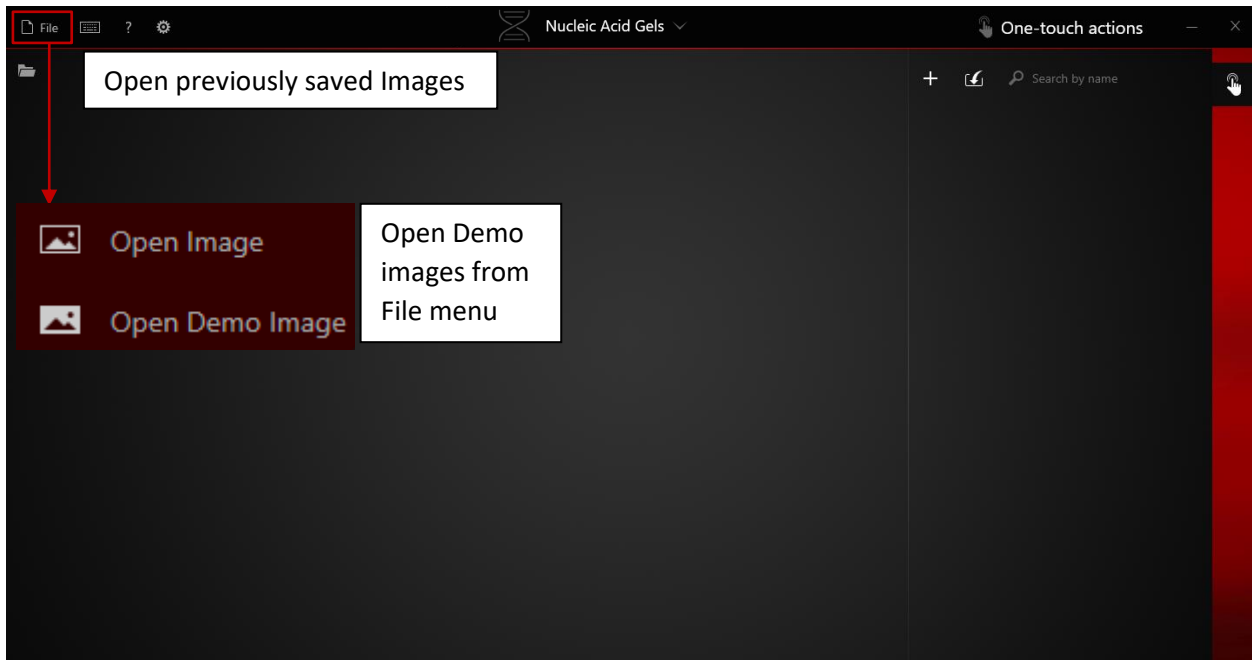
- 7) If you are working with clonogenic assays, follow **Step 1 to 6** to analyze the clonogenic assay sample.

## 8.0 VisionWorks® Open and Save Images

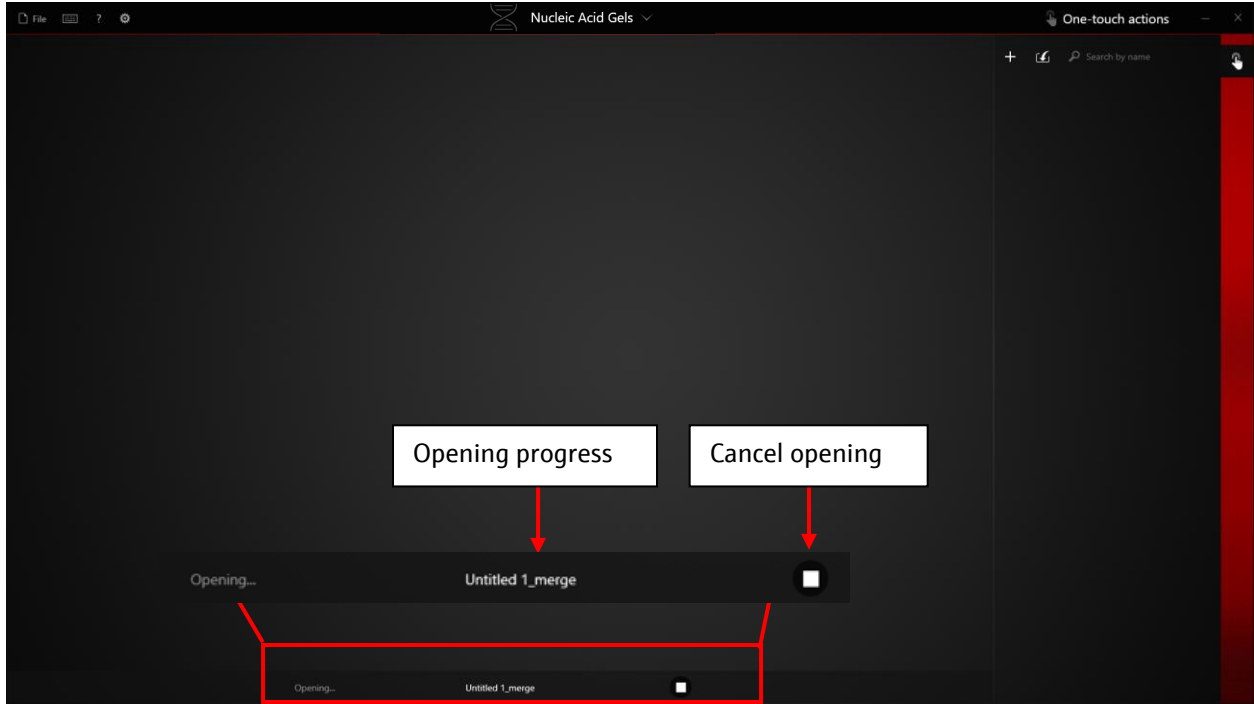
### 8.1 Open Images in VisionWorks®

VisionWorks® software will load images in popular formats, including Tiff, JPEG and PNG. It can also open a special multi-image sequence format called SQV. If the image was saved using VisionWorks® software package, then the image details such as the image history, annotation, and analysis will be load when the image is opened in VisionWorks®.

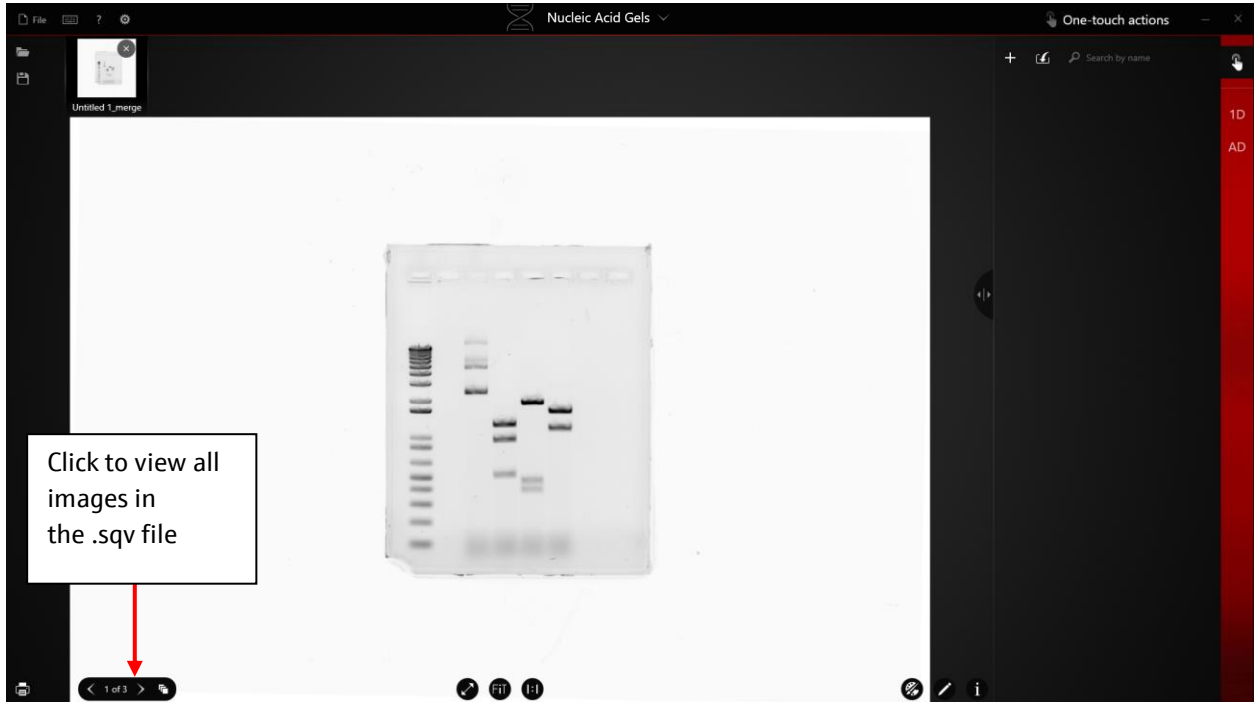
- 1) Open any application icon from the home screen.
- 2) Click Open to open previously saved image. Demo images are accessible from the File drop down menu.



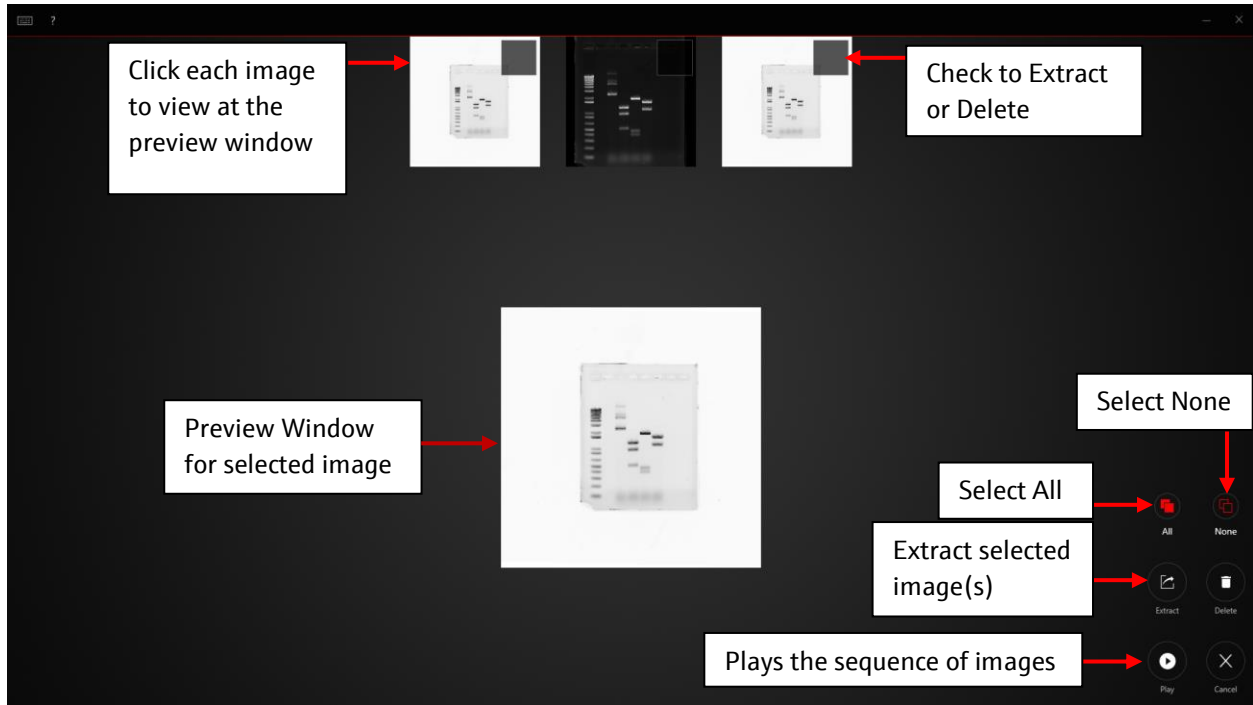
- 3) When opening a .SQV file, an open progress bar will be displayed at the bottom of the screen.



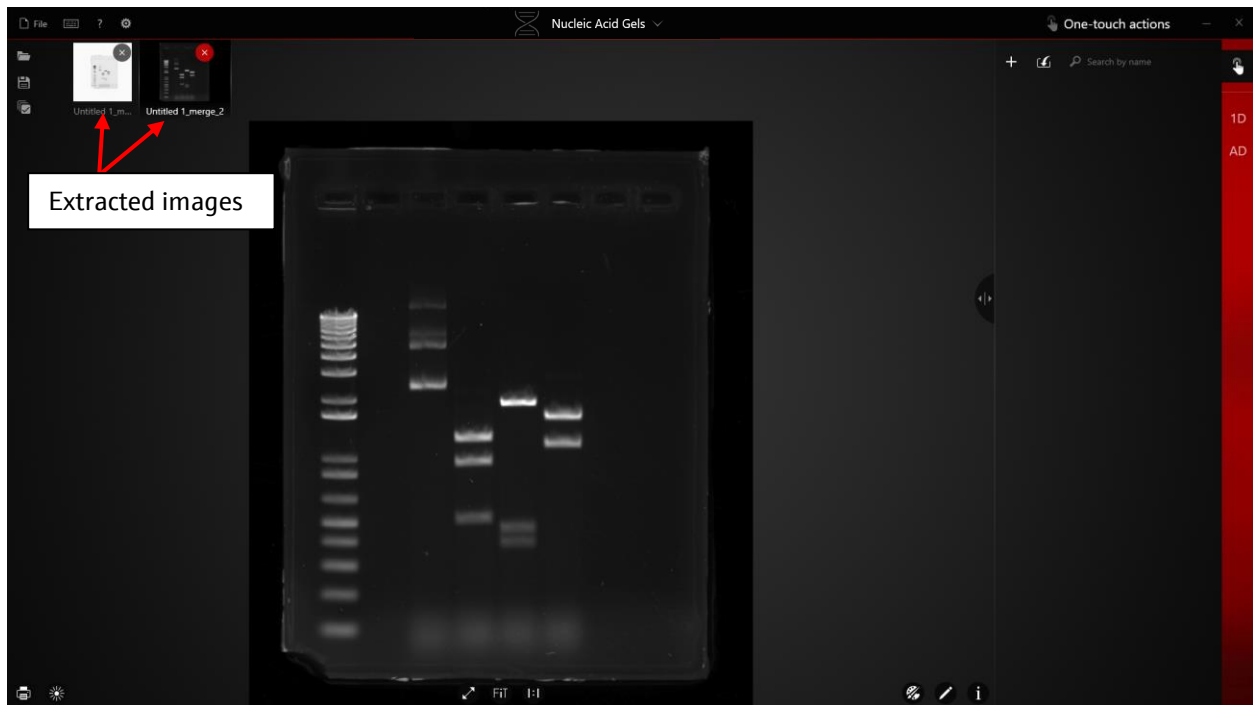
4) Once the .SQV file is opened, open all images in the thumbnail gallery by clicking View All.



5) More options will be available after opening .SQV file in View All window.



6) Extracted images can be viewed in thumbnail gallery.

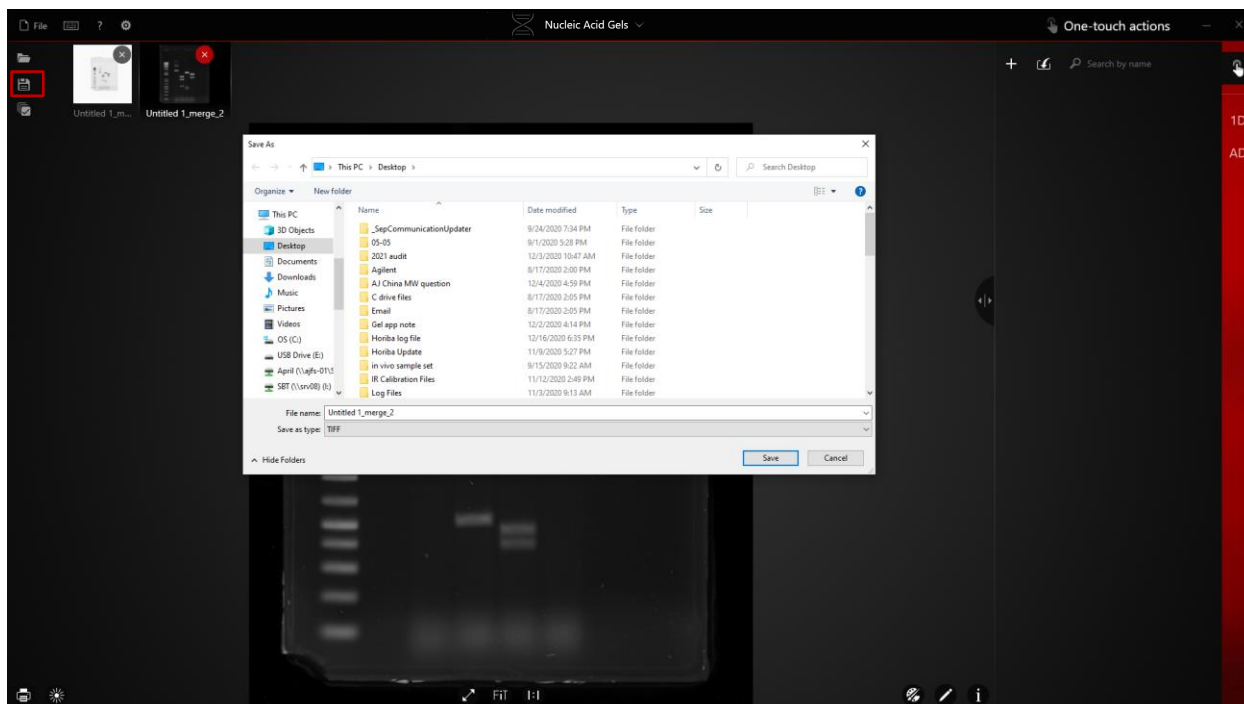


## 8.2 Save Captured Images in VisionWorks®

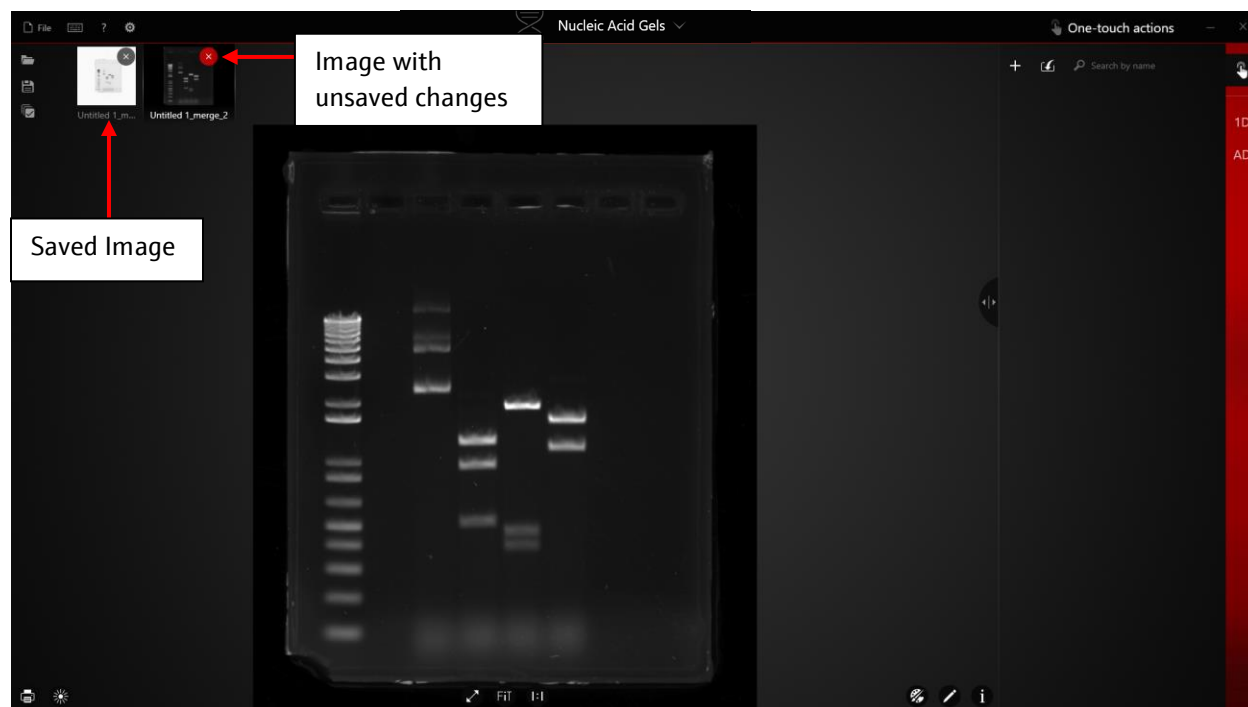
VisionWorks® provides multiple saving options. For analysis and publication purposes, we recommend to save the image in .Tiff format. Save images in JPEG format only for the purpose of being able to open it using other photo viewer and for using it for display.

### 8.2.1 Regular Save

- 1) After capturing an image, click the quick Save button in thumbnail gallery to save the image.



- 2) If the image contains unsaved changes, the close sign on top of each image in Thumbnail gallery will turn red.



3) Images saved in .tiff format will retain all of the original data and is good for analysis.

\*Note: .tiff format image with weak signal such as Chemiluminescent image may open as a very dark image in other photo viewers. You will need to open in VisionWorks to view the image or follow steps in **Section 8.2.2** to export the image for display purposes.

\*\*Note: The regular save function is to save as .Tiff only. If an image is opened from another format (JPG, PNG), only the "Save As" function (save to a new pathname) can be done on the image. To re-save the image in JPG or PNG format, use the Export function described below.

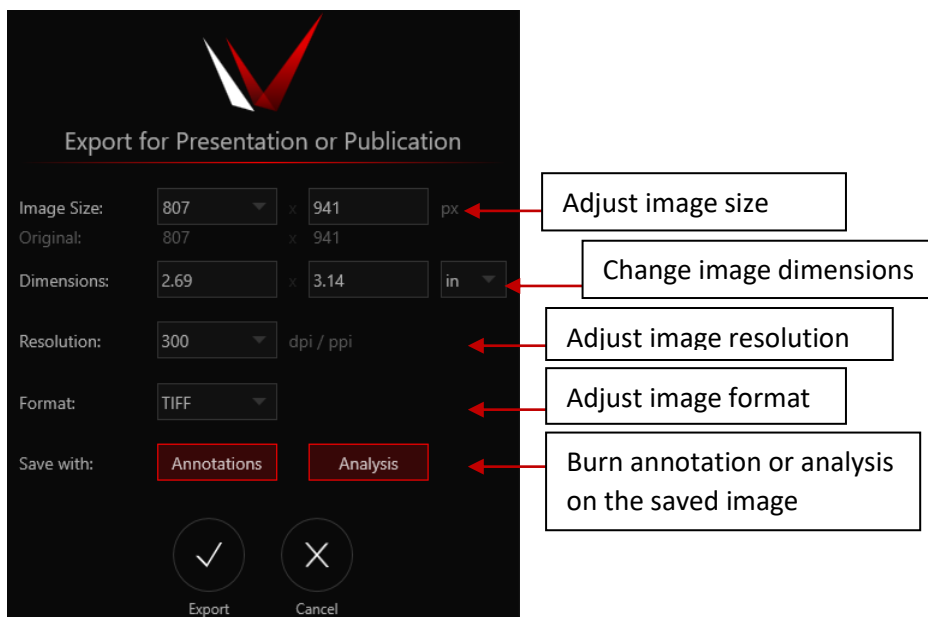
## 8.2.2 Export for Display

Annotations or analysis applied on a .tiff image is viewable when opening in VisionWorks®. If the image is needed for opening in other photo viewer, follow the steps below to export for display.

- 1) After saving the image for analysis in .tiff format, you can start to make modification to the image and export it for display purposes.
- 2) After finishing modifying your image, click Export for Publication to export images.



- 3) Adjust image parameters before saving.

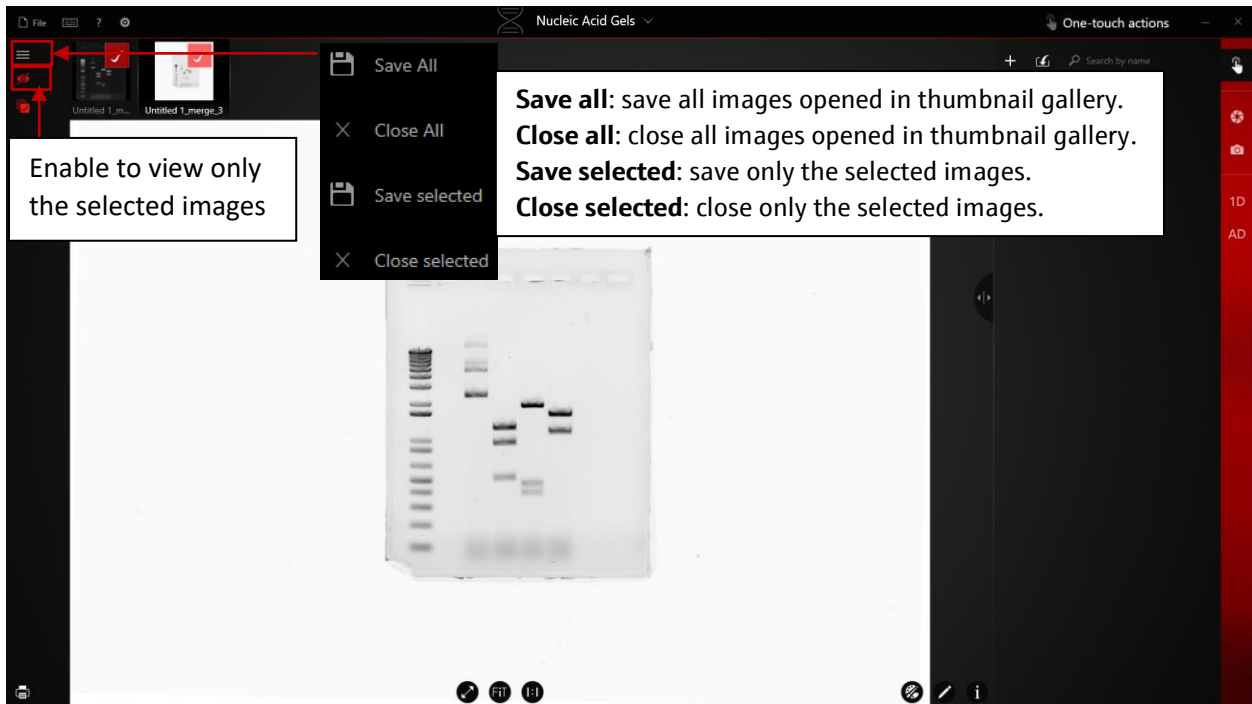


### 8.2.3 Saving Multiple Images

- 1) After capturing multiple images, enable multiple selection from the thumbnail gallery.



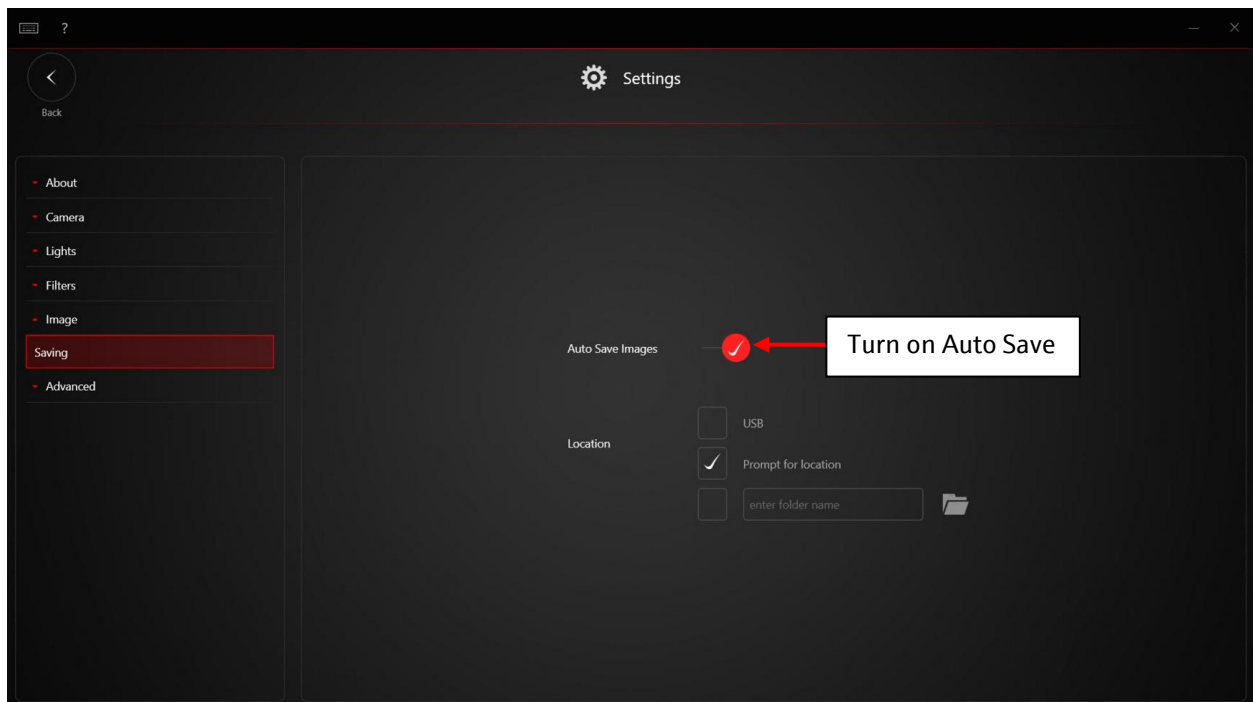
- 2) Select multiple images to view.



- 3)** Follow the message pop up after choosing the multi-image actions to Save or Close images. Saved images will use Capture date/time as the default name.

## 8.2.4 Auto Saving

1) To turn on Auto Save, go to Software Settings→ Image → turn on Auto Save.

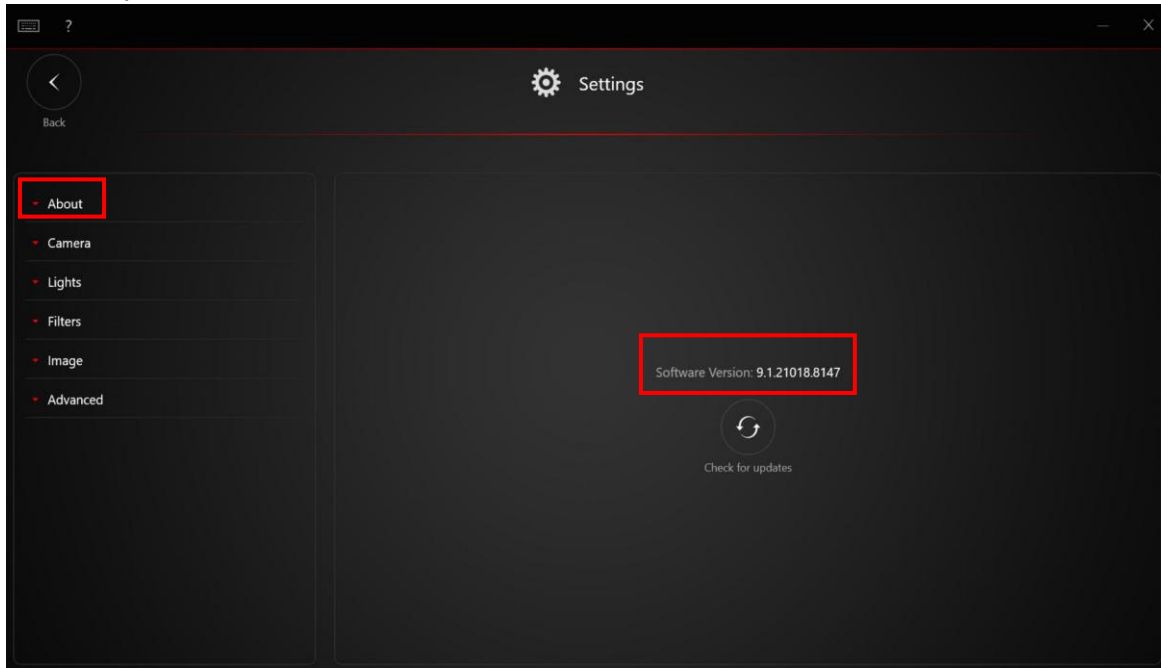


2) Choose auto save destination from the menu. Captured images will be saved as .tiff format automatically.

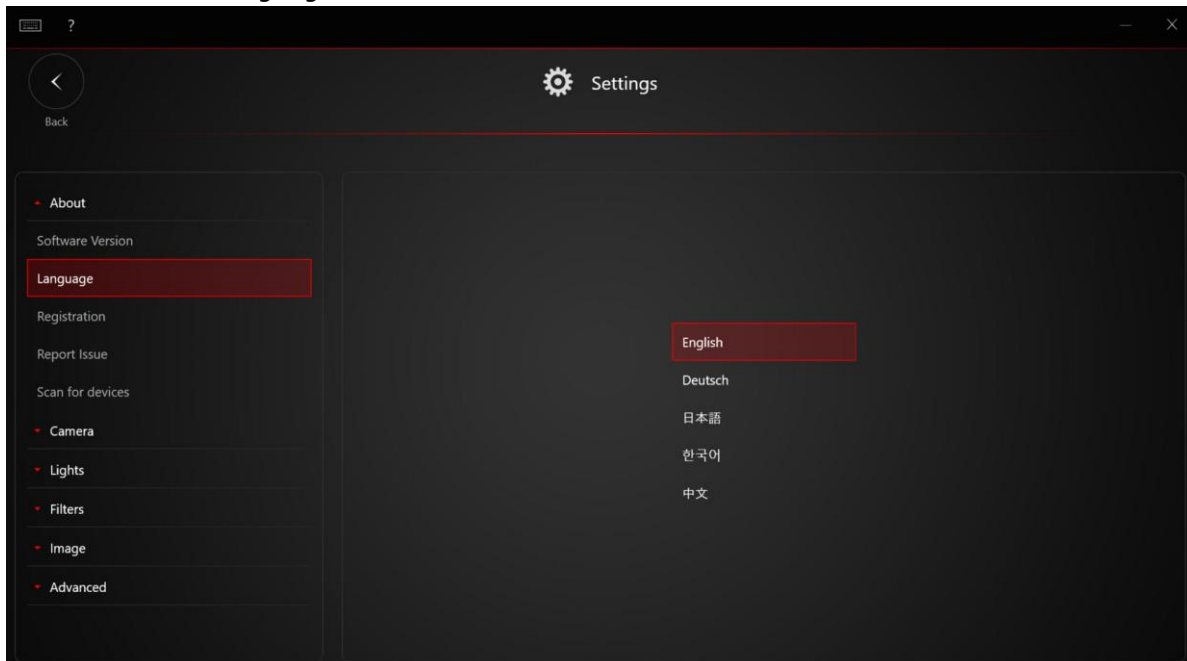
## 9.0 VisionWorks® Software Settings

### 9.1 General Settings

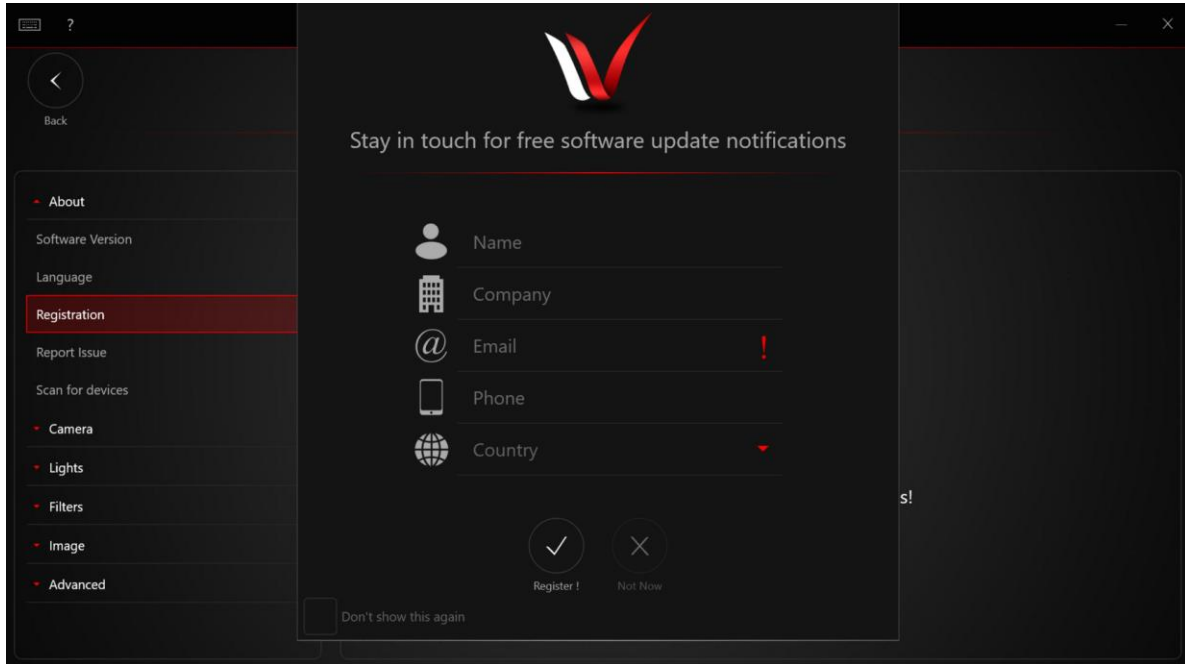
- 1) Software version information can be found under Settings → About → Software Version. This information is important when you need to contact tech support or checking for update.



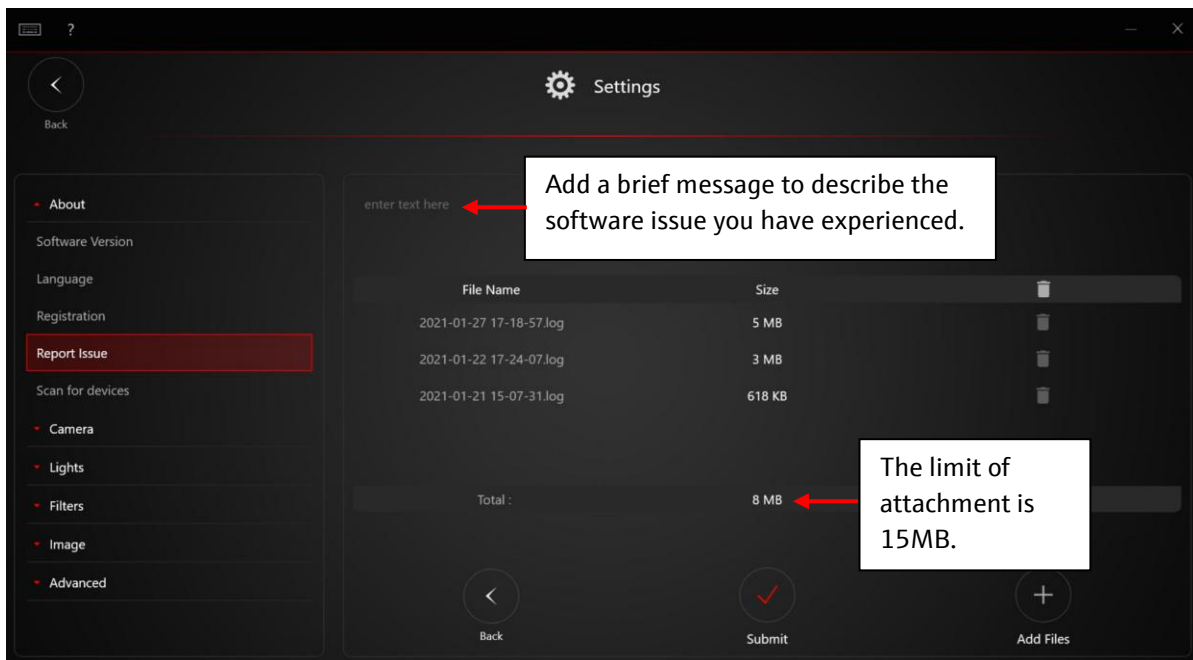
- 2) To change the language setting for VisionWorks®, click About → Language → Select desired language.



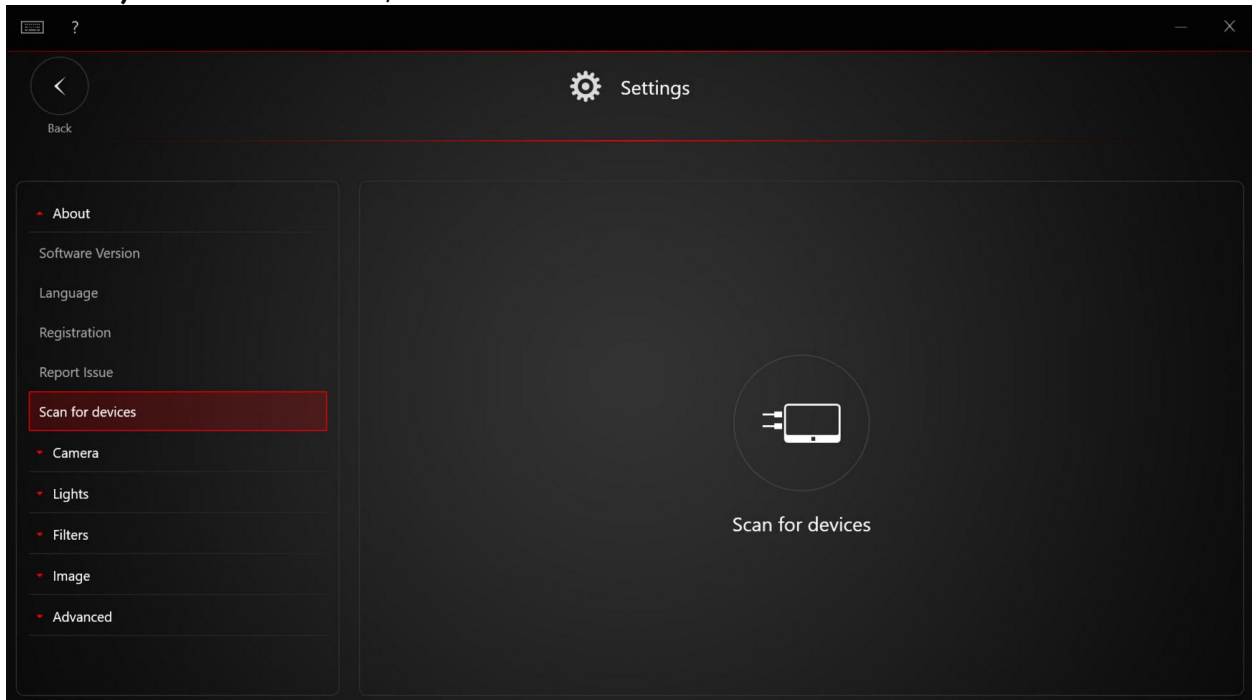
- 3) To register for updates, click About → Registration → Register for free update notification → Fill out the registration form → Register. You will be notified when the new software is available for download.



- 4) To report a software issue, click About → Report Issue → click New Issue → create a brief message to describe the software issue you have experienced and click Submit.

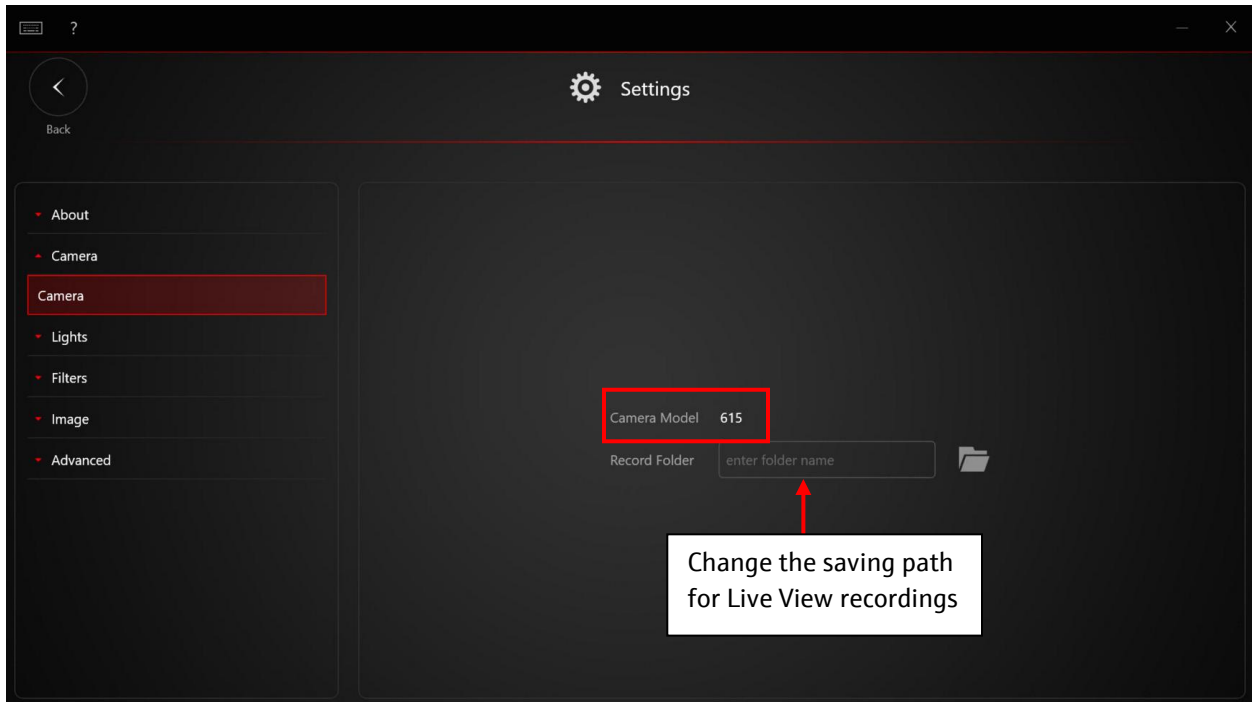


5) To scan for devices, click About → Scan for Devices.



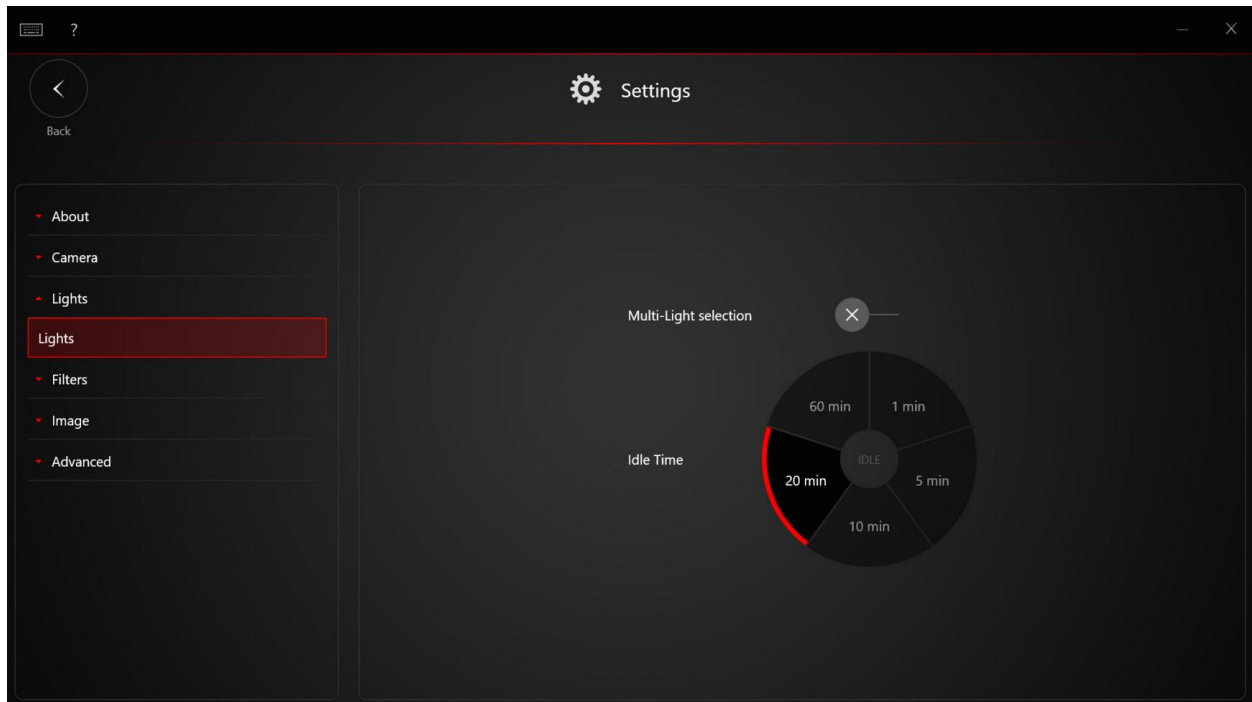
## 9.2 Camera information

- 1) Camera model information can be found under Settings → Camera tab.
- 2) You will also be able to change the Live View Record saving path under this tab.



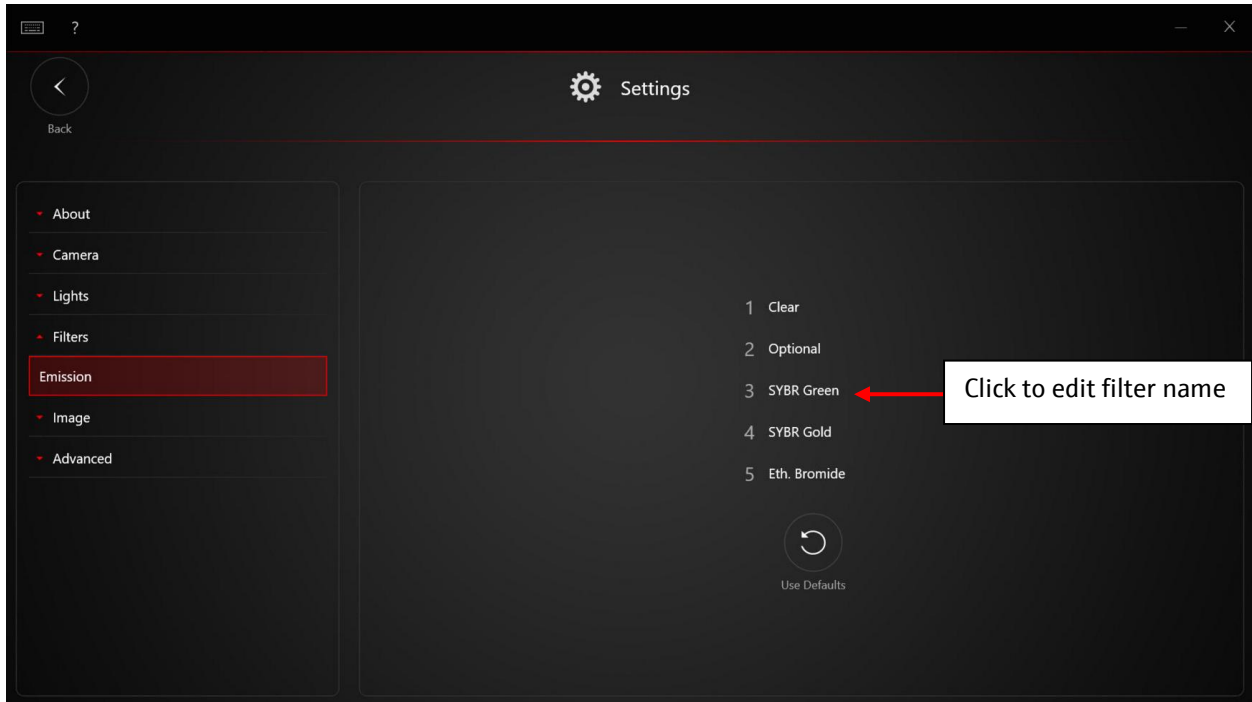
## 9.3 Lighting Settings

- 1) Multiple light toggle is available under the lighting setting. Once turned on, you can activate two or more excitation light sources at the same time.
- 2) Light idle time can be set, the default lighting idle time is set to 20 mins. A warning indicator will flash on the bottom screen as the idle shut-off time approaches. The lights will turn off once this time is reached.

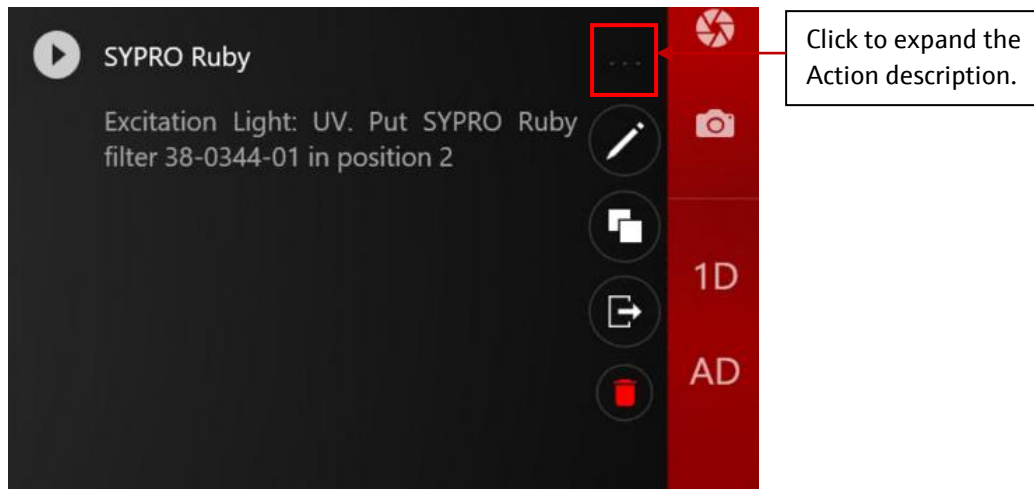


## 9.4 Filter Settings

1) Filter names can be edited under the Settings → Filter tab.

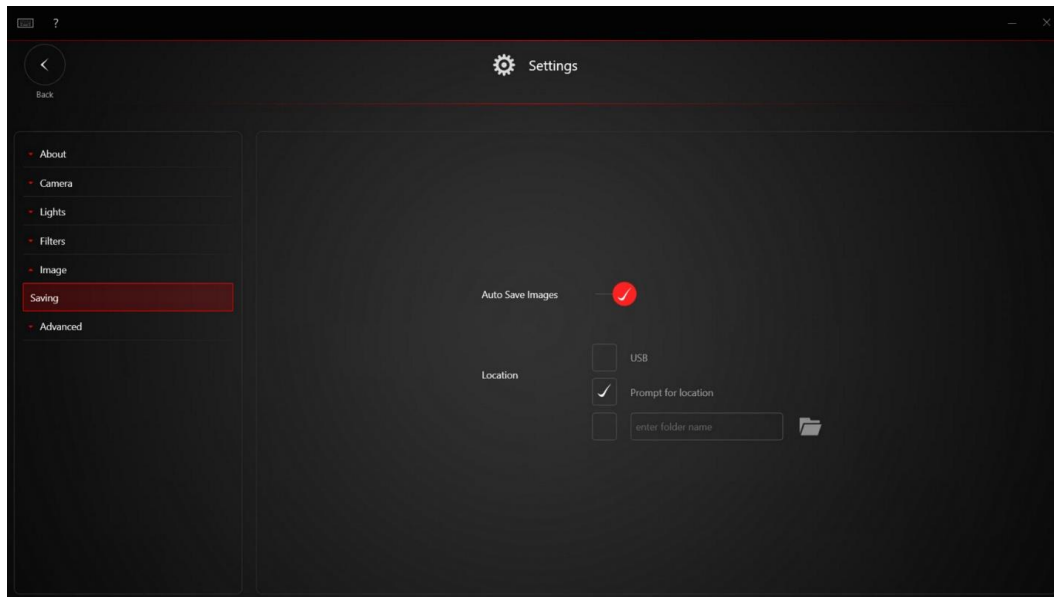


2) If you would like to use the preset actions, click to open the Action description for information about which filter position to put your filter.



## 9.5 Auto Saving Settings

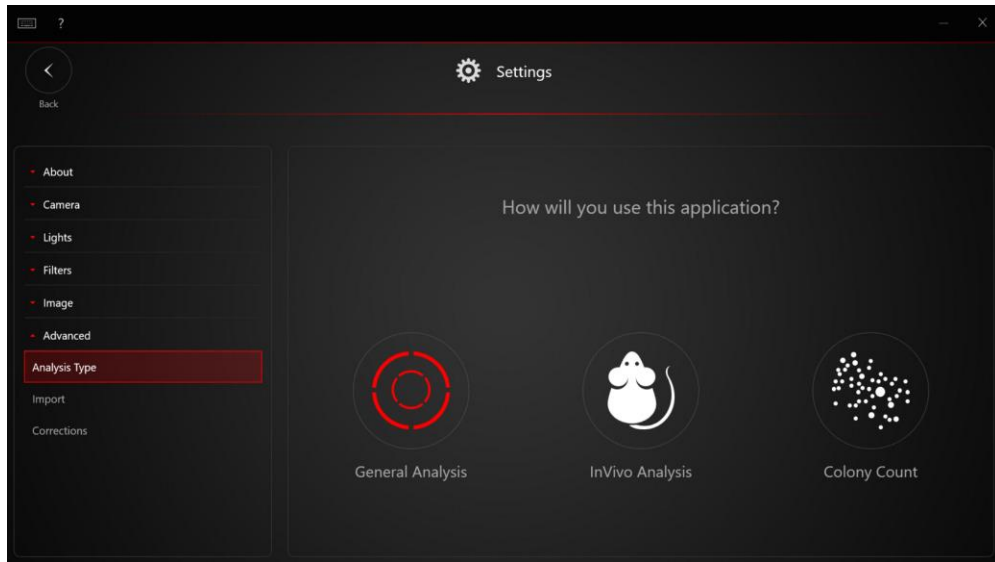
Turn on Auto Save toggle, choose saving format and saving location. See [Section 8.2.4](#).



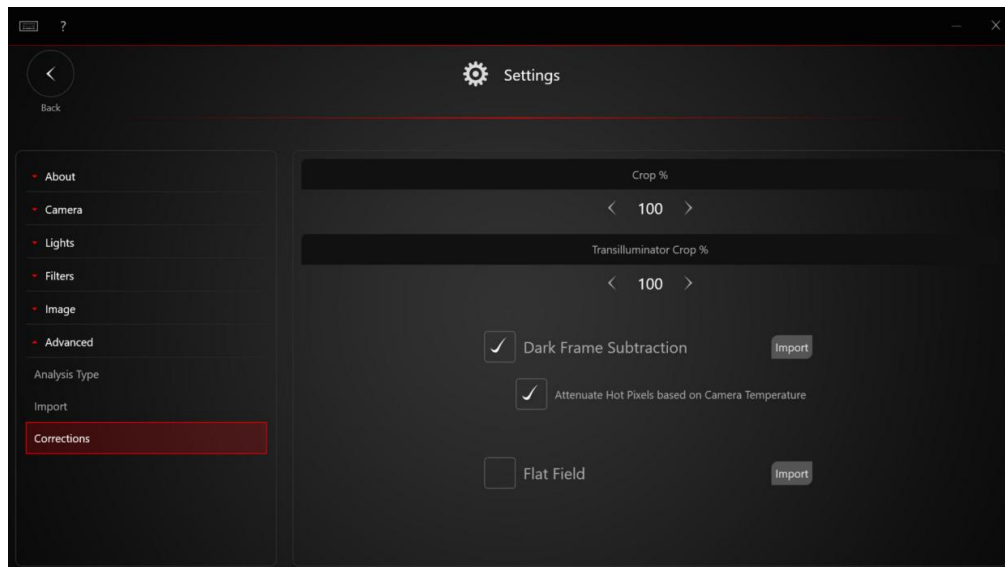
## 9.6 Advanced Settings

Here, you can change analysis type, import calibration images, and setup Dark Frame and Flat Field calibration.

- 1) Every system will come with the correct Analysis Type selected depending on the hardware detected by the software. To switch between analysis types, simply select the analysis type you want to use.



- 2) Import all the calibration images by clicking Import. Your instrument should be pre-calibrated and the Import function is only need to be run manually in special circumstances. For more details, see [Section 10.4](#).
- 3) Toggle on Calibration from the Corrections tab. Calibration will be turned ON by default. For more detail about setting up the calibration, see [Section 10.0](#).



## 10.0 VisionWorks® Calibration

In VisionWorks® software, the term Calibration refers to Dark Frame correction (built into all systems that come with the current software release), Flat Field correction (built into all systems that include a software-controlled lens), and Photon Flux calibration (for *in vivo* systems). Each calibration is applied automatically to any captured image once the appropriate setting is active in the settings, and their usage for an image is tracked in its metadata. The following table describes each calibration.

Instrument Calibration Options	
Calibration	Definition
<b>Dark Frame Correction</b>	Removes dark current or background intensity from the image, including hot pixels.
<b>Flat Field Correction</b>	Removes the effects of optical vignetting, where the image tends to be brighter at the center than the edges and corrects unevenness in Epi excitation lighting.
<b>Aperture Area Calibration (in vivo only)</b>	Calibration of the ratio of the lens aperture or brightness to the intensity of a standard light in the darkroom. Not used as a standalone calibration in the software but necessary for Photon Flux calibration.
<b>Photon Flux Calibration (in vivo only)</b>	NIST traceable calibration that converts the intensity of the image's pixels to the units photons/second/cm <sup>2</sup> /steradian. Allows comparison of luminescence between captures of different exposure times, binnings and apertures, and converts to a standard unit for comparison against data collected from other imaging methods or literature.

### 10.1 Calibration Availability:

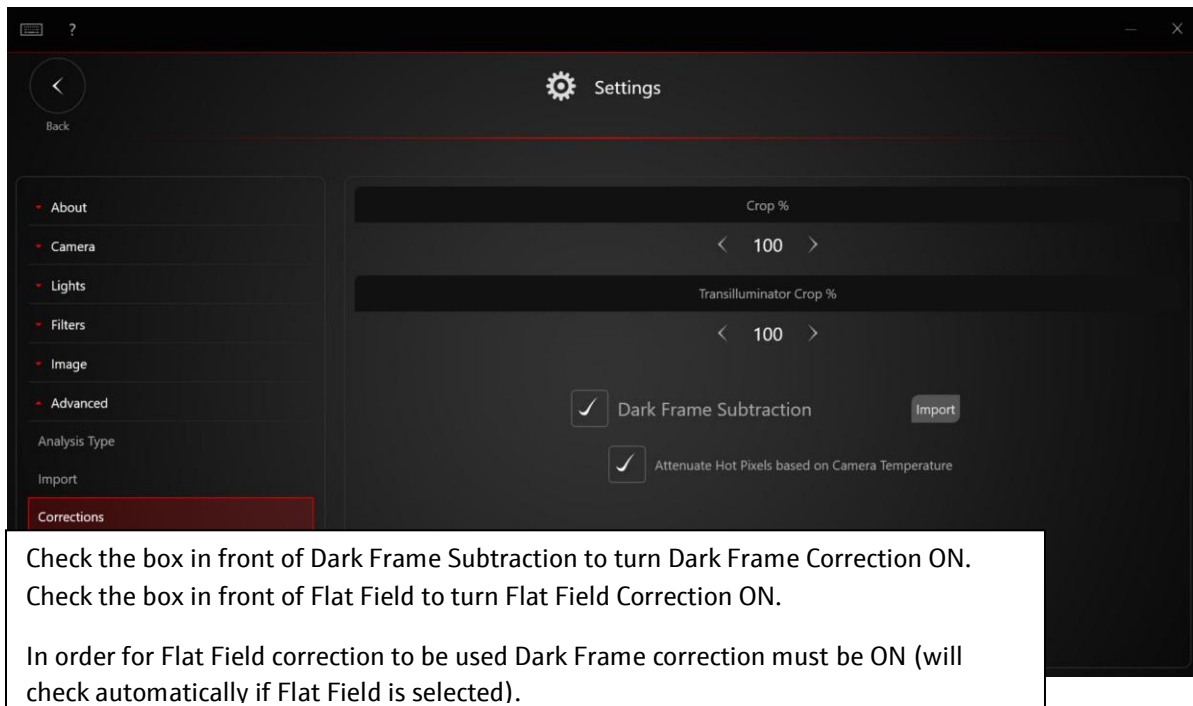
Calibration Options By Instrument				
System Type	Dark Frame Calibration	Flat Field Calibration	Photon Flux Calibration	Transilluminator Calibration*
UVP GelSolo	Y	X	X	X
UVP GelStudio/PLUS	Y	Y	X	Y
UVP ChemStudio/PLUS	Y	Y	X	Y
UVP iBox Studio	Y	Y	Y	Y
UVP Scientia 900	Y	Y	Y	Y

\*Transilluminator Calibration only applicable to the Visi-White Transilluminator, Visi Blue Transilluminator, UV-to-Blue and UV-to-White Converter plate. The transilluminator calibration is not applicable to the UV transilluminators.

## 10.2 Activating and Deactivating Corrections

Dark Frame and Flat Field correction can be switched on or off as necessary for the application. By default, the system will have Dark Frame and Flat Field correction ON, and can be turned off as necessary to the preference of the user.

To access the correction switches, go to the **Settings Panel**, and under the **Advanced tab**, select "**Corrections**".



\*Note: Calibration functions can be active only if there are the stored Calibration images available. If you need to import calibration images for Dark Frame and Flat Field calibration, please refer to [Section 10.4](#).

## 10.3 Calibration Setting Definitions

### 10.3.1 Calibration Crop Settings

Crop % is the percentage of the maximum dimensions of the sensor-output image that the correction will be applied to. In most systems this will be preconfigured and should not be modified, even when activating or deactivating either Dark Frame or Flat Field Corrections. If Dark Frame or Flat Field are activated, then the final image after applying the correction will be reduced from the maximum dimensions of the sensor-output in both height and length by at least this percentage (and by more if the user specifies a lower crop% value in the Pre-Capture settings), so that the entire image has the full calibration applied.

### **10.3.2 Attenuate Hot Pixels**

This is an optional setting when Dark Frame is ON, that reduces the intensity of hot pixels further from the originally applied Dark Frame correction, based on the camera temperature at capture. This only applies to cooled CCDs that track the camera temperature, and this setting will have no effect on the image for other types of cameras. This setting can be activated to correct issues with significant hot pixels on the image even after Dark Frame correction.

### **10.3.3 Lens Vignetting Correction**

“Ignore Lights, Correct for Vignetting Only” is an optional setting when Flat Field is ON. If this setting is activated, the software will ignore any correction for unevenness in excitation lighting and only correct for vignetting due to the effects of the lens.

This setting can be activated when using the fold down tray in the UVP ChemStudio PLUS system, or if the sample will otherwise be lifted far above the normal height (of the top of the transilluminator or sample tray) while using excitation lights.

## 10.4 Importing Dark and Flat Frames

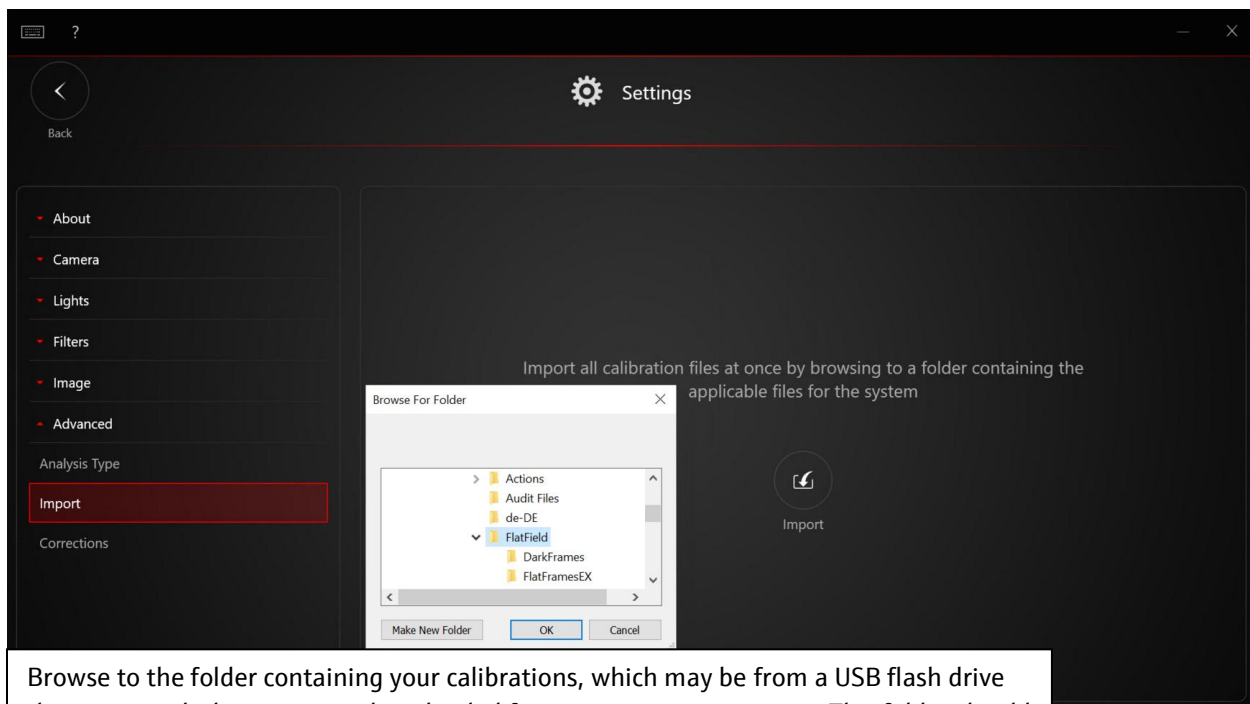
### 10.4.1 For a New System

The calibration images will be pre-installed on your system, there are no extra steps needed before activating the calibration function.

If you are using an external computer, the calibration images can be found in the USB flash drive that comes with the system. Please refer to **Section 10.4.2** for importing dark frame and flat field calibration before activating the calibration function.

### 10.4.2 Importing Calibration Images

To access the Import switches, go to the **Settings Panel**, and under the **Advanced tab**, select **"Import"**. In this tab, click the Import button to start importing calibration images.

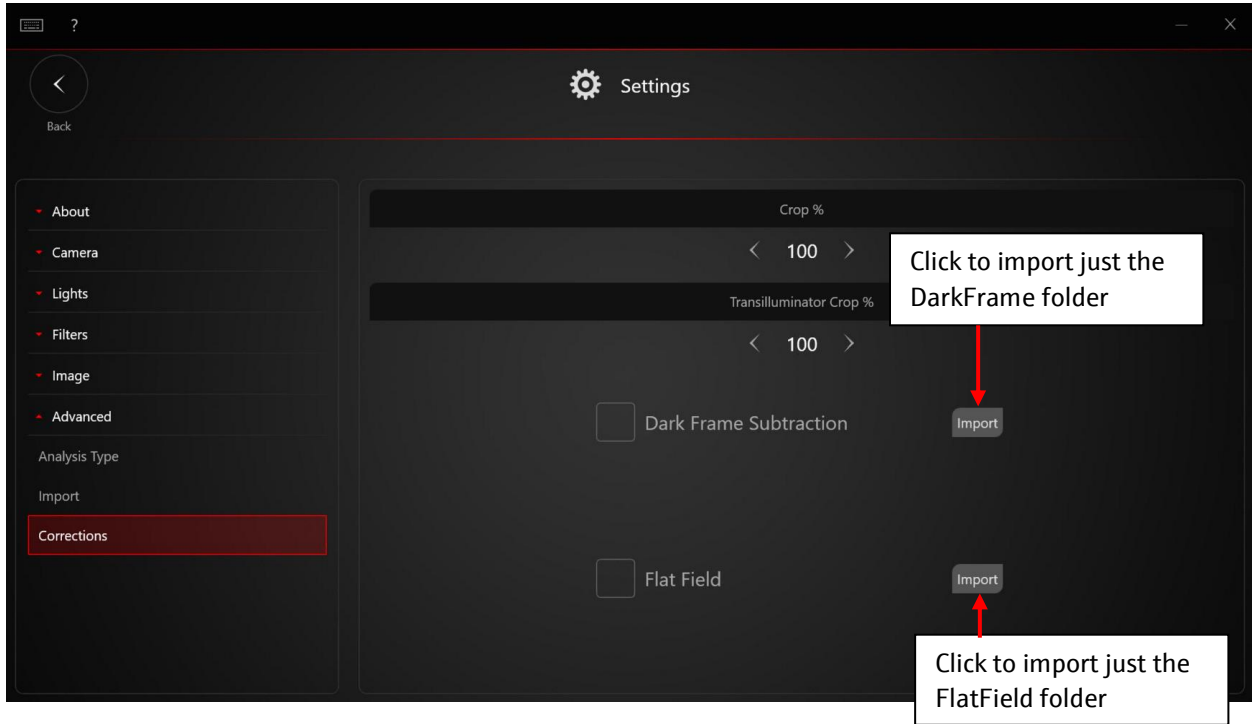


Browse to the folder containing your calibrations, which may be from a USB flash drive that came with the system or downloaded from an internet repository. This folder should contain two separate folders inside called "DarkFrames" and "FlatFramesEX". **Select (highlight) the outer folder** (i.e. not the DarkFrames or FlatFramesEX but the parent folder), then click OK.

\*Note: A message will appear to double check if it is okay to delete any pre-existing correction frames or calibrations. Select OK to proceed. The software will indicate whether the import was successful.

### 10.4.3 Importing Individual Calibration Folder

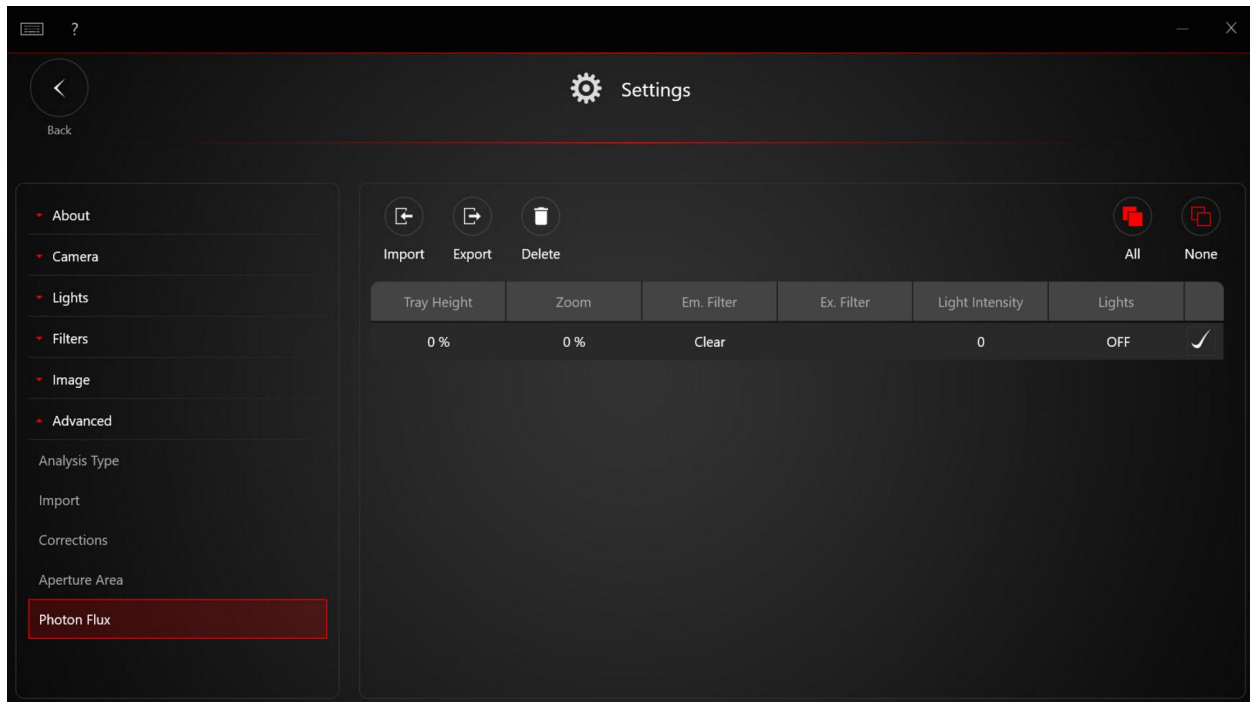
If you are updating the software and have some previously acquired calibration images, you can import selected files by going to **Correction Menu**. In the **Settings Panel**, and under the **Advanced tab**, select "**Corrections**". Note: Follow this section to import individual calibration file for systems containing part of the calibration images.



## 10.5 Calibration for *in vivo* Applications

### 10.5.1 Import *in vivo* Calibration Files

For *in vivo* systems, follow the steps described in **Section 10.4** to finish importing calibration images. These steps will import any Dark Frame, Flat Field, Aperture Area (.apr) and Photon Flux (.phf) calibration that were in the same folder. Check the **Photon Flux** settings window to make sure any calibrations that are needed were imported correctly.



If only the photon flux calibration needs to be imported, such as on a system that already had Dark Frame and Flat Field calibration, follow these instructions to import Aperture Area (.apr) and Photon Flux files (.phf) separately:

To import Aperture Area file (.apr), go to the **Advanced tab** in System Setting Window, select **Aperture Area** and click **Import** button. Browse to the .apr file on your system, then select **OK**.

To import Photon Flux files (.phf), go to the **Advanced tab** in System Setting Window, select **Photon Flux** tab and click **Import** button. Browse to the .phf file on your system, then select **OK**. If the .phf file contains any calibrations exactly identical to one already in the system under the **Photon Flux** settings window, this action will overwrite them.

**\*Note: The Photon Flux calibration (.phf) file alone will not work if the Aperture Area calibration file is not also in the system.**

## 10.5.2 Activate Photon Flux Calibration

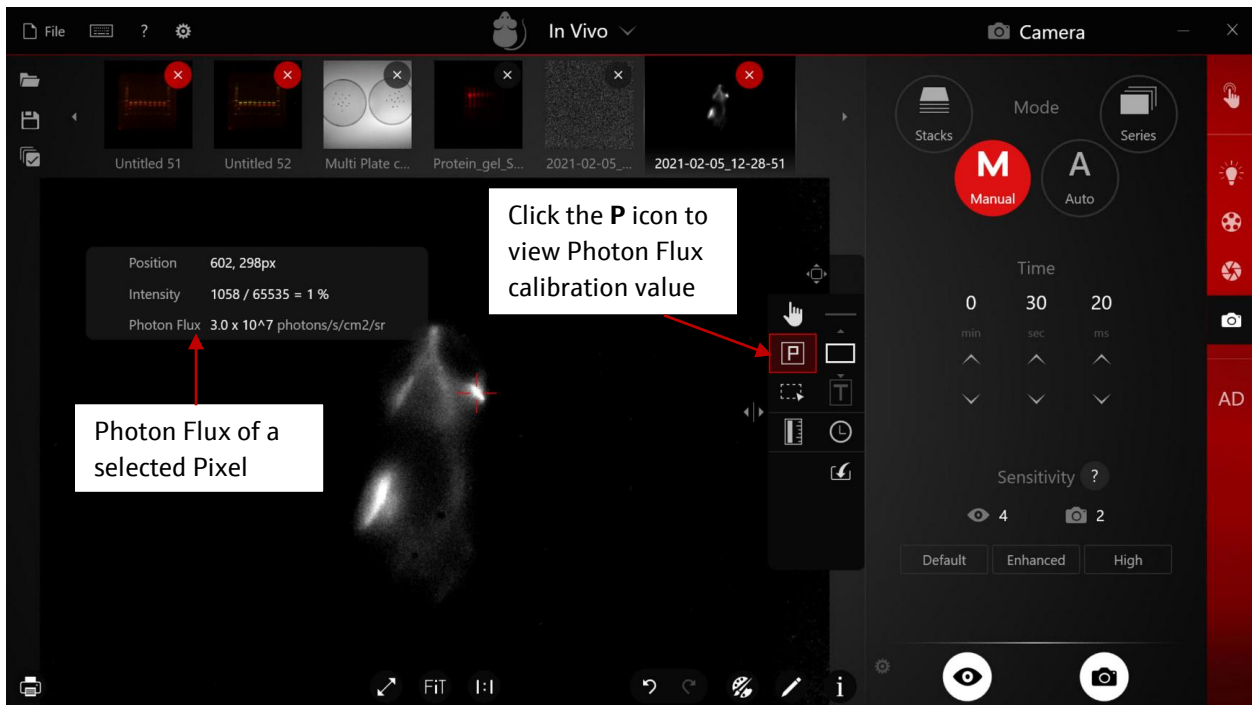
In most cases, *in vivo* systems will already be pre-calibrated for Photon Flux. To activate the calibration, the only steps that need to be done are: Flat Field should be turned ON (follow the steps in [Section 10.2](#)) and Analysis Type under the Advanced Tab of the Settings should be set to *in vivo*, which is the default for *in vivo* systems.

## 10.5.3 Photon Flux Calibration Result

If Photon Flux calibration is activated and working correctly, the Photon Flux calibrated data will be seen in three places:

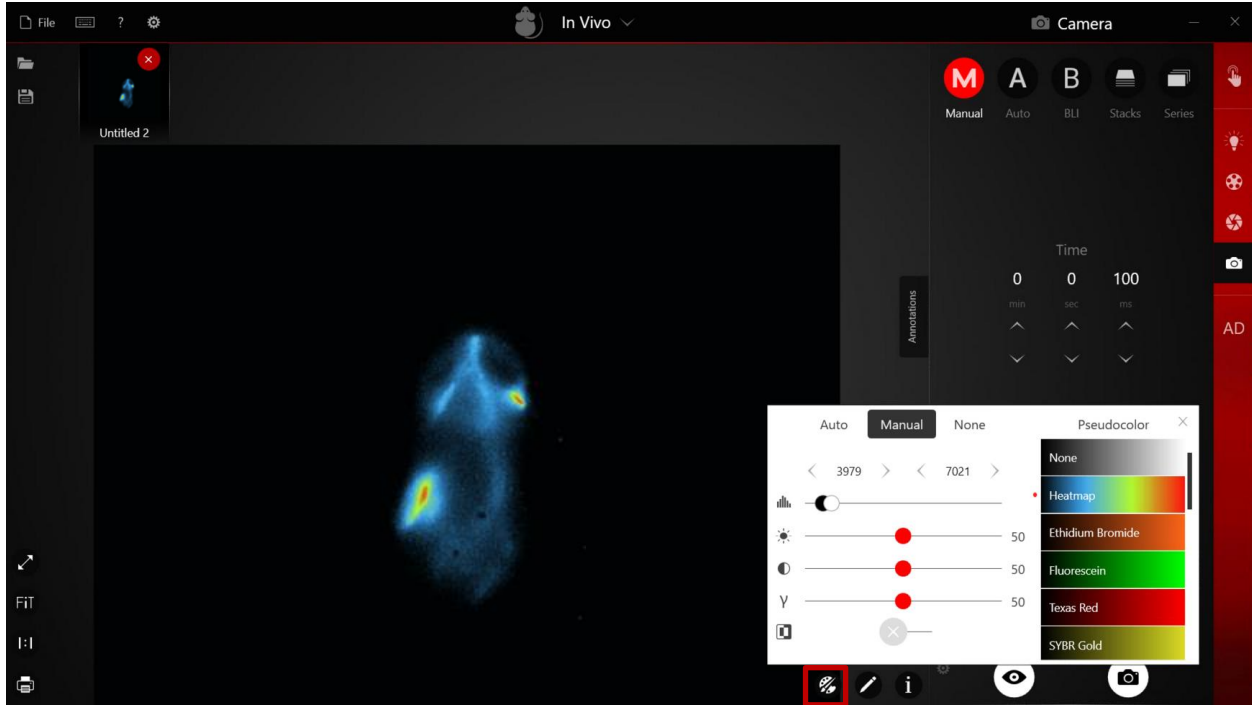
### 10.5.3.1 View Result in Captured Image

In a captured image, when Pixel Display Cursor is selected, highlighting or tapping a pixel on the image will display the photon flux value in photons/s/cm<sup>2</sup>/sr to the left of the intensity at the bottom of the gallery.

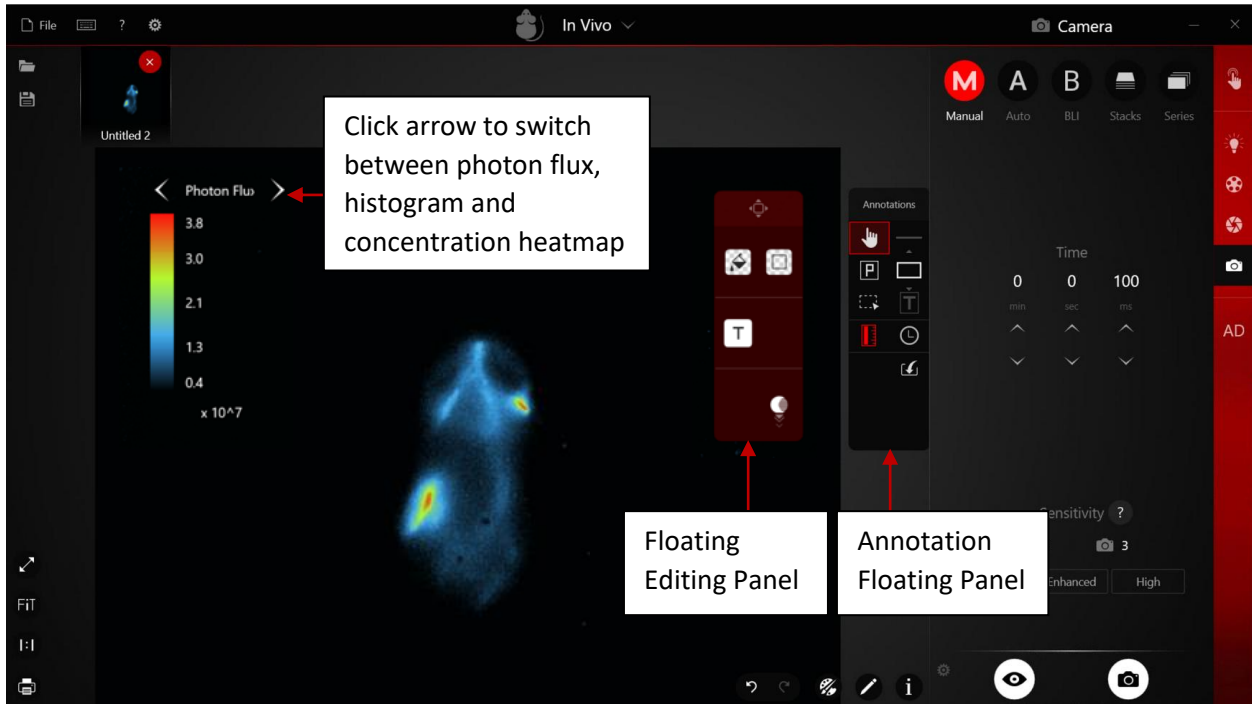


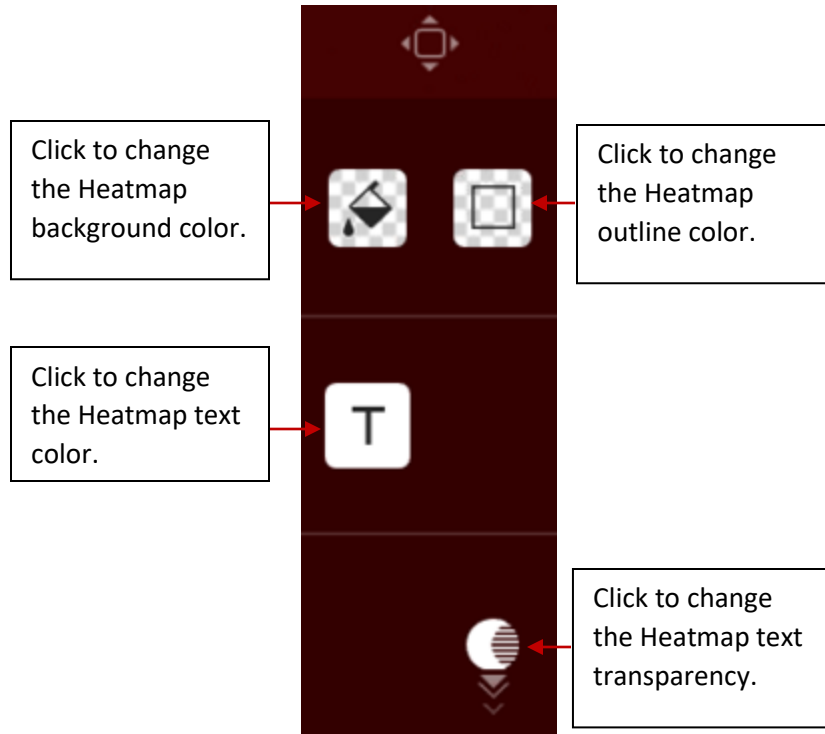
### 10.5.3.2 View Photon Flux Result in Annotation

- 1) Apply Pseudocolor to captured bioluminescent image by open **Histogram** icon.



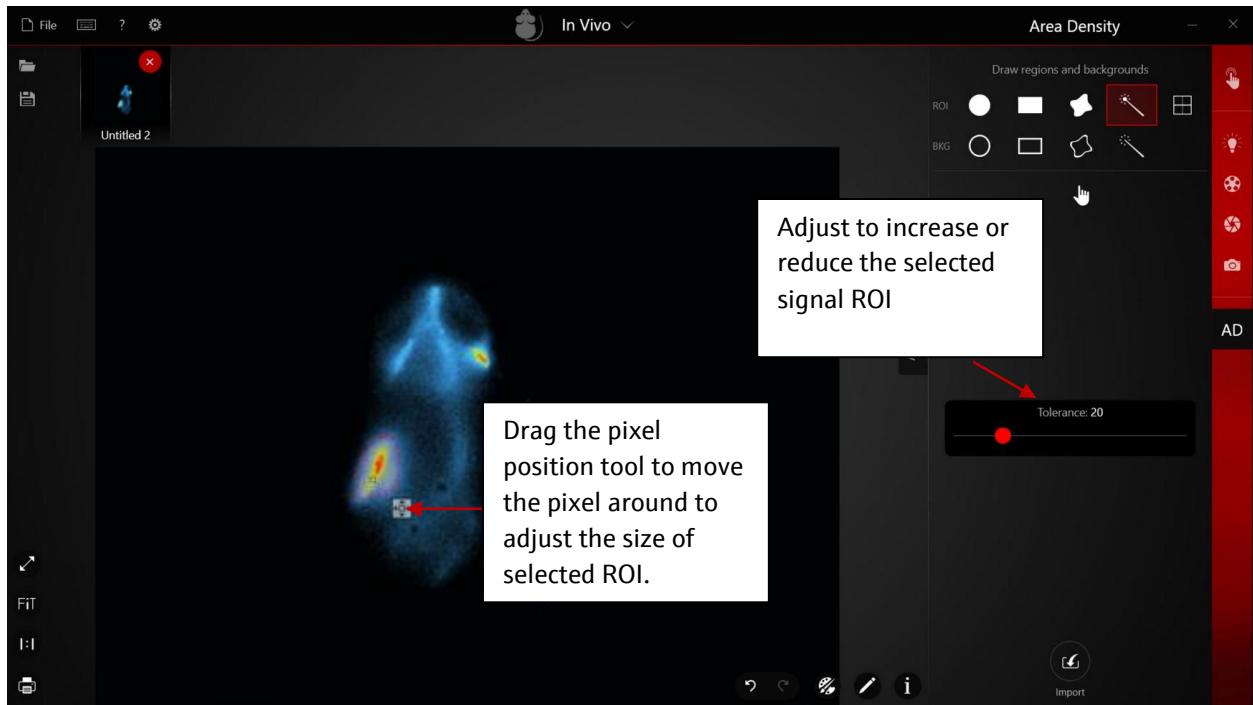
- 2) Open **Annotation** floating bar, then click on **Heatmap** icon to add Heatmap. Adjust Heatmap style in the red floating Editing Panel.



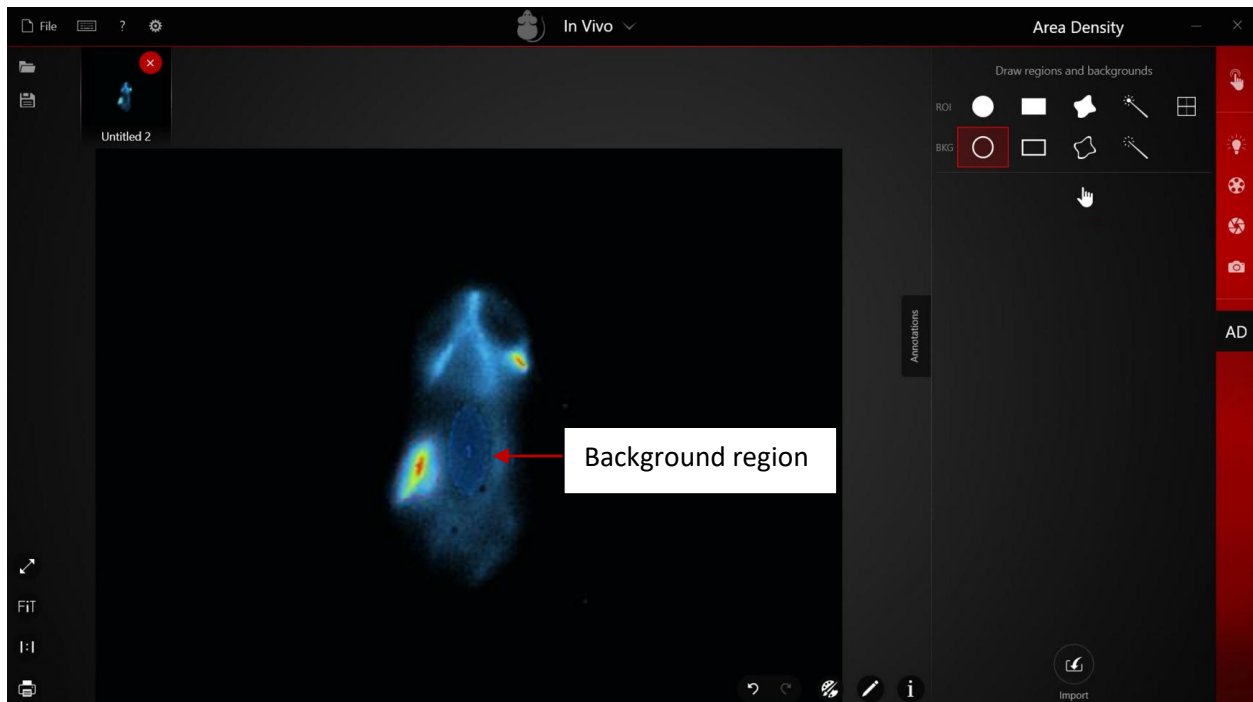


### 10.5.3.3 View Result in Area Density report

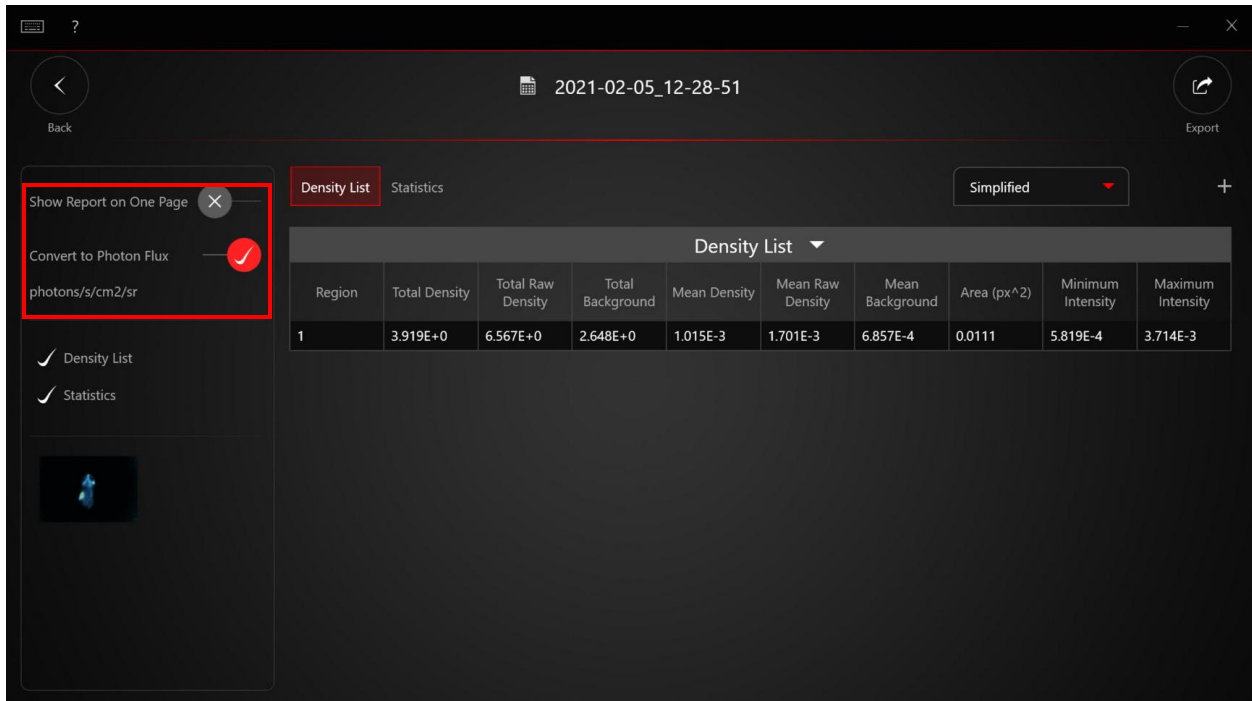
- 1) Open Area Density menu and define region. For details about defining Area Density region, refer to [Section 7.2](#).
- 2) Use Magic Wand tool to define the signal area. Then, use Tolerance to adjust the size of defined region (Magic wand will create a region based on the **Pixel** you choose, a quick way to adjust the signal region size is to click and drag the pixel position tool to change the selected pixel. To enlarge the region, drag and drop pixel position tool to a weaker pixel; to reduce the region, drag and drop pixel position tool to a stronger pixel).



3) Select the Background region using the background tool.



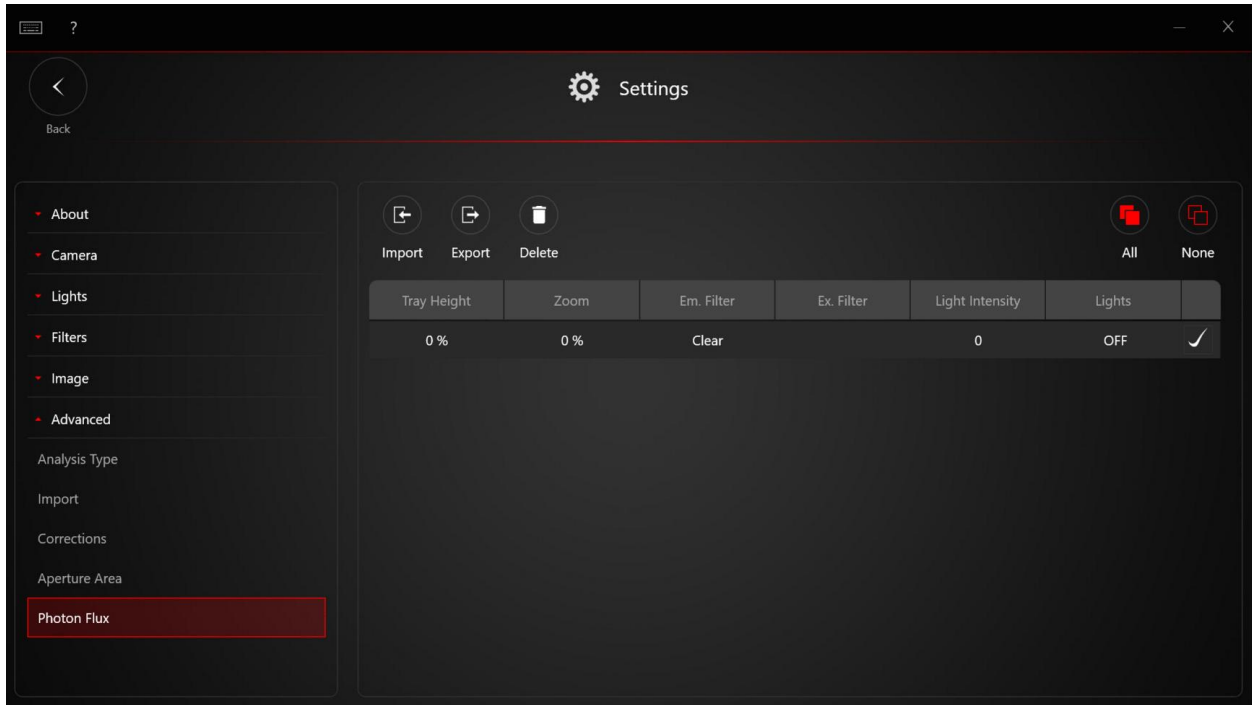
4) After selecting the signal ROI and background, click Report to access the Photon Flux data. In the **Report** Window, switch the toggle to convert intensity value to Photon Flux data (photons/s/cm<sup>2</sup>/sr).



#### 10.5.3.4 Missing Photon Flux Calibrated Data

If the Photon Flux calibrated data as outlined above does not appear as expected upon capture of an image, go to the Photon Flux menu by following steps below to check if Photon Flux calibration data is present:

Go to the **Settings** Panel, and under the **Advanced tab**, select "**Photon Flux**". The table will show the exact settings for which the system is Photon Flux calibrated; filter names should match exactly and are case sensitive, so the existing filter names may need to be adjusted under the Filters or Lights tabs in the Settings menu. If the appropriate calibration setting does not exist in this table, it will need to be imported or recalibrated.



\*Note: The calibration will only be active for the specific capture settings used to calibrate the system. This means that the Emission Filter name, Excitation Light, Excitation Filter and Intensity (if applicable, otherwise empty/0), and Tray Height or Zoom level (if applicable, otherwise 0%/0%), for which the system was calibrated for, must match the state of the current capture exactly.

## 10.6 Dark Frame Recalibration and Extended Calibration

### 10.6.1 Importing Dark Frame Recalibration Action

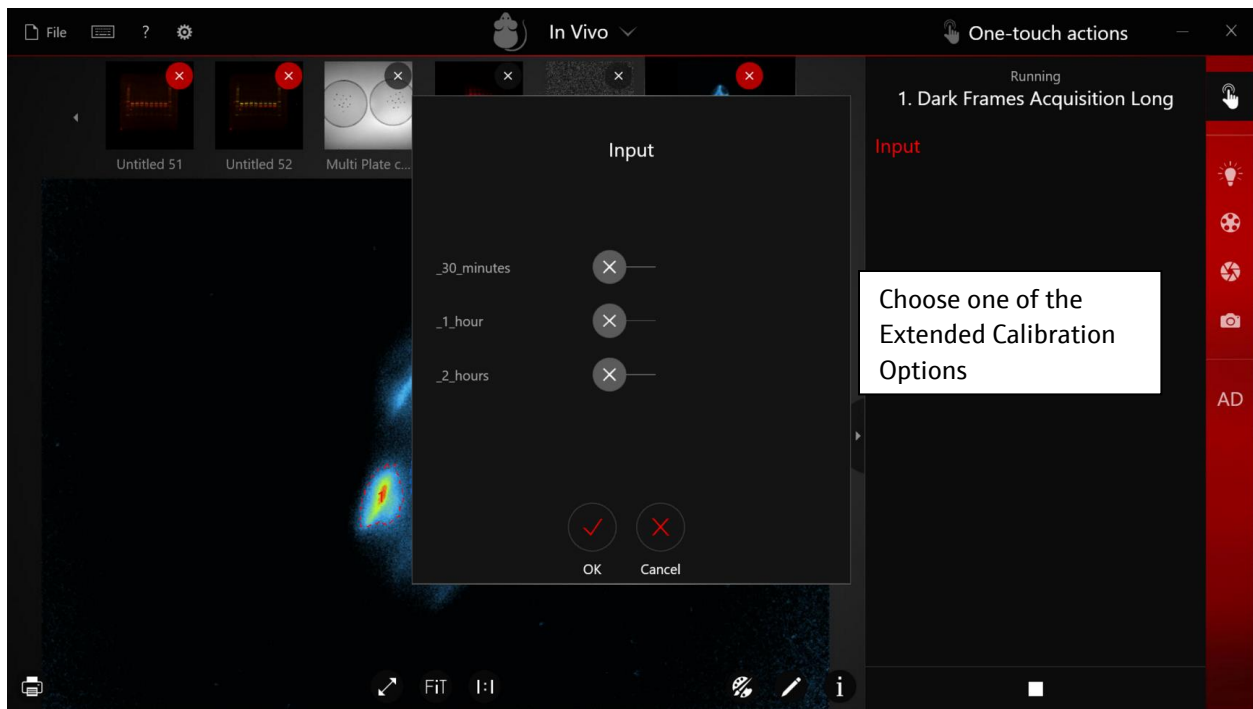
To perform Dark Frame Recalibration, you will need to first load the calibration actions from VisionWorks® Setup folder. Click the Import button under Action window. If the VisionWorks® Action folder is not displayed automatically, follow the steps to get access to the Calibration Action folder: C:\Program Files (x86)→ Analytik Jena→ Visionworks→ Actions→ All→ Calibration→ follow the table to load the appropriate action:

System Type	Actions for Recalibration
UVP GelSolo, UVP GelStudio, and UVP GelStudio PLUS	1. Dark Frames Acquisition Standard(Calibration time ~20min)
UVP ChemStudio, UVP ChemStudio PLUS, UVP iBox Studio and UVP Scientia 900	1. Dark Frames Acquisition Standard (Calibration time >~3hrs)

### 10.6.2 Importing Extended Dark Frame Calibration Action

All the systems will come with calibration images preinstalled. If you are using the UVP ChemStudio / ChemStudio PLUS, or UVP iBox Studio, the system will be pre-calibrated up to 10 minutes exposure time. If calibration for long exposure time (>10 mins) is needed, please load action **1. Dark Frame Acquisition Long**. When running the action, please select desired calibration limit depending on your application requirement. The approximate acquisition time can be found from the Table below:

Calibration Exposure Time for Each Extended Calibration Action	Approximate Acquisition Time
30 min (Calibrate for exposure time up to 30 mins)	~4 hours
1 hour (Calibrate for exposure time up to 1 hour)	~12 hours
2 hour (Calibrate for exposure time up to 2 hours)	~28 hours

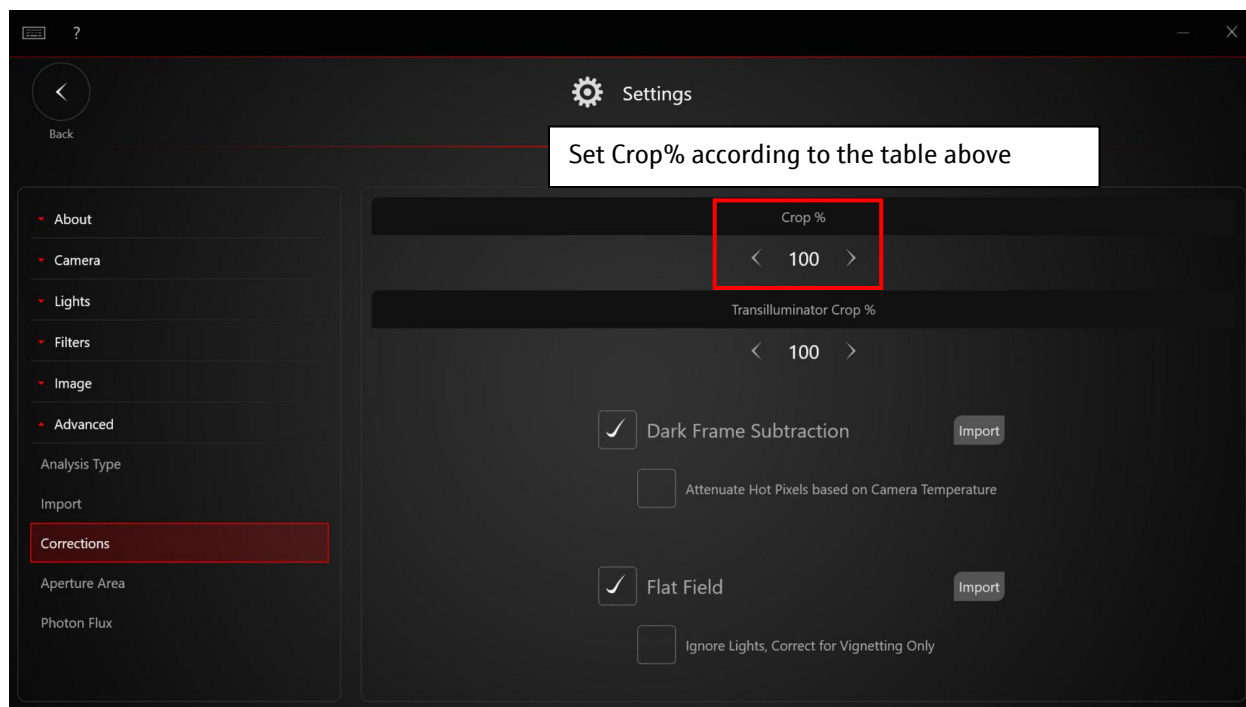


If Dark Frame Acquisition Long is used, the user needs to set whether dark frames should be acquired for 30 minutes, choose **\_30\_minutes**; 1 hour, choose **\_1\_hour**; and **\_2\_hours** by toggling these switches. Set this to the greatest time, rounded up, for which captures are expected to be used over the life of the system; for example, if the longest capture by users of the system will be up to 90 minutes, then switch on the 2-hour setting. Captures longer than 2 hours are not supported by Dark Frame correction.

## 10.7 Run Extended Calibration Actions

After loading the appropriate action, go to **Settings** → **Advance** → **Correction**. In Correction Window, set the **Crop%** to the correct value according to the table below. Contact technical support if the system is not listed.

System	Crop%
UVP ChemStudio (815 camera)	95
UVP ChemStudio (615 or 695 camera)	100
UVP ChemStudio PLUS	100
UVP iBox Studio	100
UVP GelStudio	100
UVP GelStudio PLUS (315 camera)	80
UVP GelStudio PLUS (12 MPx camera)	100
UVP iBox Scientia (900 Camera only)	80
UVP GelSolo	100



**Note:** Before running the Dark Frame calibration, please turn off all excitation lighting, remove any filters from filter slot #1, remove any objects from the darkroom, and close the darkroom door and viewing window if there is one. If the system uses a manual lens, manually set the Aperture to closed (C). Leave the darkroom door closed until the action shows the “Dark Frame Acquisition Successful” popup, then click **OK** to complete the calibration.

## 10.8 Transilluminator Calibrations

If you are using a white light transilluminator, blue light transilluminator, or a white or blue light converter plate on a UV or other transilluminator, you have the option of running the Transilluminator Calibration on your system to improve the image uniformity by correcting for unevenness in the transillumination, especially at the edges of the plate.

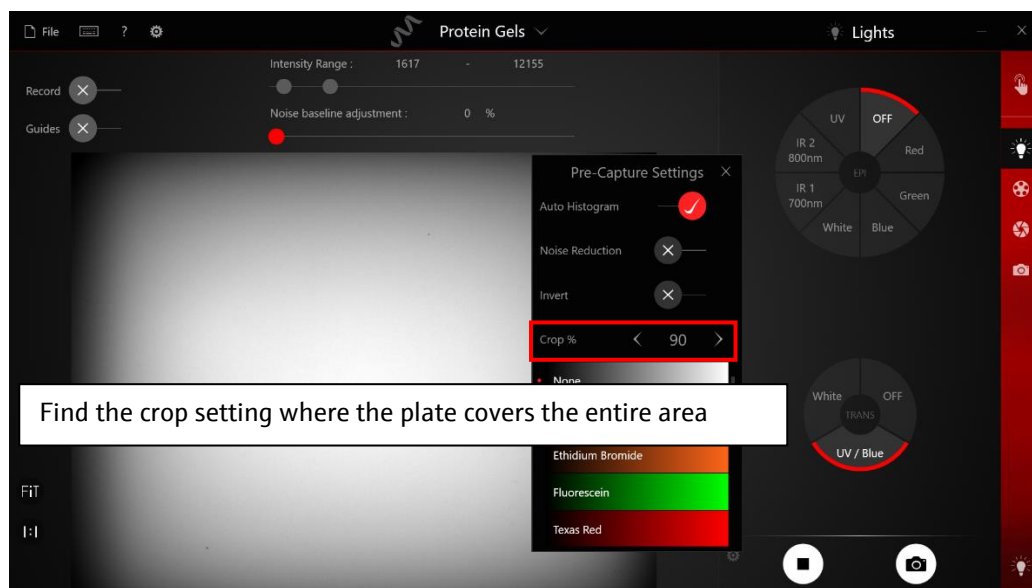
The Transilluminator Calibration is not recommended for the UV transilluminator used alone because the lighting from the bulbs will already lead to good uniformity with respect to the edges of the transilluminator. Instead, capture images without running the steps below and the software will automatically perform flat fielding correction for lens effects.

To run the Transilluminator Calibration, you will have to (1) Find the correct crop setting to use and set it in the software, (2) Import and (3) Run a premade one-touch action to acquire the calibration images. This procedure will take under 10 minutes.

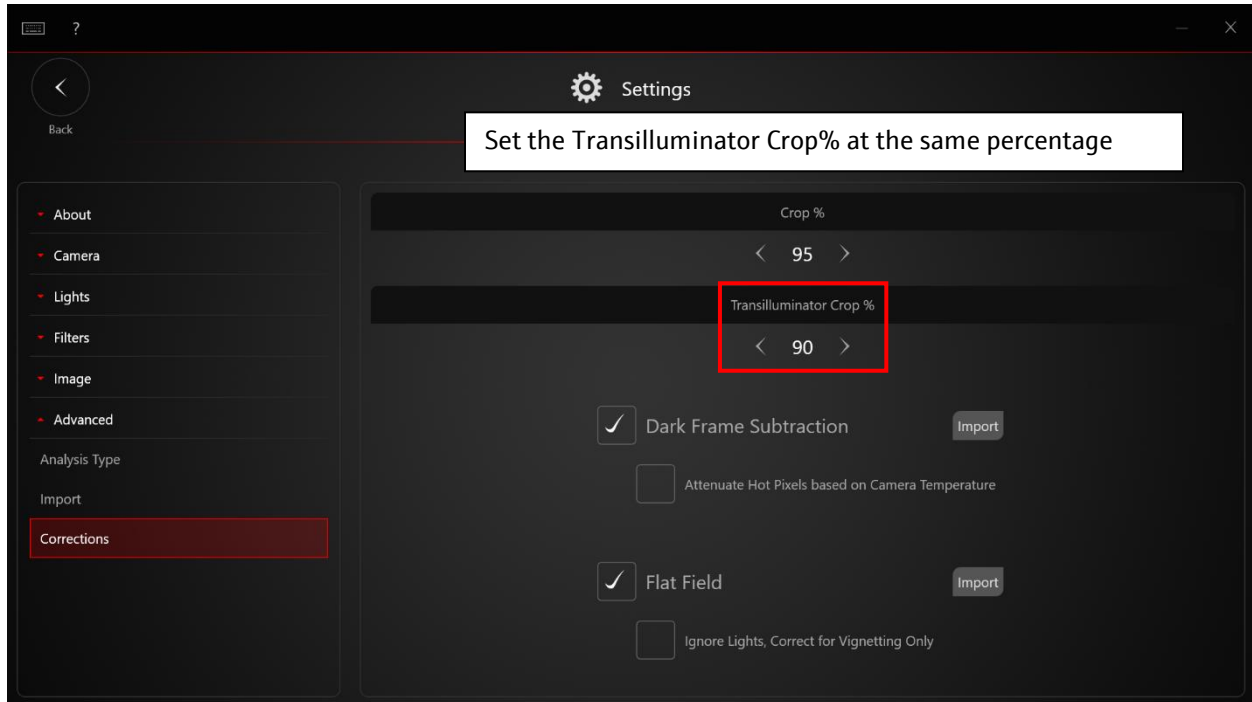
### 10.8.1 Find Correct Crop Setting for your Transilluminator

To find the correct crop setting for your transilluminator/converter plate, first make sure it is placed inside the system and is plugged in to the power. Go to the lighting tab and under the Trans wheel, select the appropriate lighting condition (White or UV/Blue/ (converter plate)) corresponding to your transilluminator. Start live view, and adjust the camera and lens settings (lens brightness, exposure time, live view binning) so that the image is focused on the surface of the transilluminator and the image is not saturated.

Open the pre-capture settings tab to the left of the live view button. Under Crop%, adjust downwards from 100% in increments of 5% until the transilluminator/converter plate covers the entire field of view and the metal edges of the plate are not visible in the live view image at all.



Go to **Settings > Advanced > Corrections**. Under Transilluminator Crop %, type in this value you just found, then exit the settings menu.



### 10.8.2 Importing Transilluminator Calibration Action

Go to the One-touch actions tab and select the Import button (square with downward arrow) to load an action.

Browse to C:\Program Files (x86)\Analytik Jena\Visionworks\Actions\All\2. Calibration\

and open 2. Transilluminator Flat Fielding.

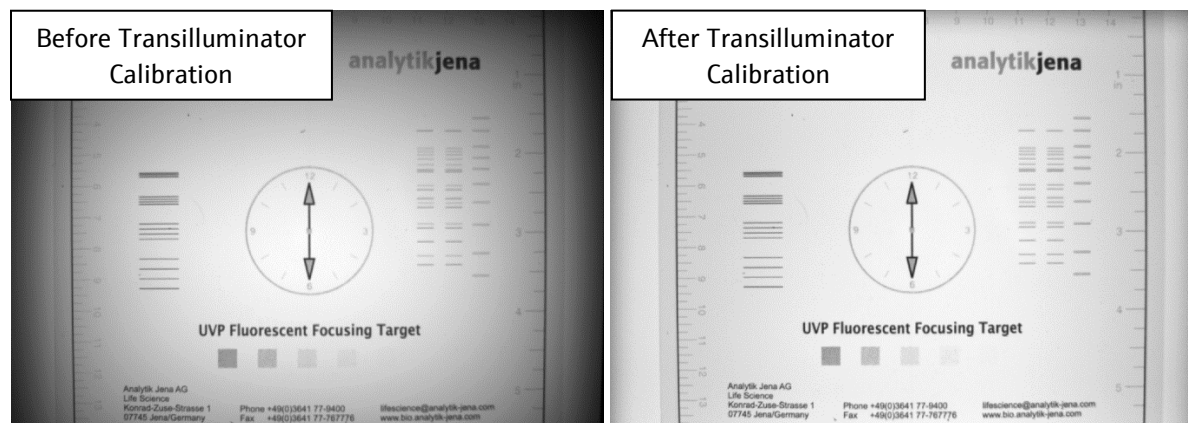
### 10.8.3 Run the Calibration Action and Validate

Prepare the darkroom to run the action: ensure the filter wheel is set to clear (no filter) and the transilluminator/converter plate is plugged in and placed in the correct position, and close the darkroom door.

In the actions menu, click the Run button next to 2. Transilluminator Flat Fielding.

A pop-up will appear asking whether your transilluminator is plugged into White or UV/Blue. Select the appropriate setting, and click OK, and allow the action to complete, which will take about 5 minutes, depending on your system.

When completed, go to **Settings > Advanced > Corrections**. Make sure “Flat Field” is checked. You can now capture an image, and verify that the brightness distribution is now more uniform to correct for unevenness in your transilluminator or converter plate.



*Note: If you are using multiple converter plates on the same switch (such as Blue converter plate and White converter plate on the UV table), you may first check if the calibration for one converter plate works well when it is switched to a different one, giving an evenly lit image. If not, you may run the calibration action every time the converter plate is switched. It is usually not necessary to change the calibration if you switch from converter plate to no converter plate (i.e. UV alone).*

## 10.9 For Other Calibrations

For Flat Frames, Photon Flux, and Aperture Area, the calibration requires special equipment. The user is advised to contact Technical Support if these files are missing and cannot be imported, or otherwise experiences difficulties with the calibration not covered in this section.

## 11.0 Actions within VisionWorks®

Actions in VisionWorks® allow for quick and easy imaging of repetitive experiments and may be accessed through the pane on the right of the screen under “**Actions**”. Preset actions come built into VisionWorks® to take images with minimal set up. Actions may be customized, created, or deleted to suit individual needs. This guide will provide a basic overview on how to get started with “**Actions**”.

### 11.1 Using Preset Actions within VisionWorks®

VisionWorks® includes numerous action scripts preinstalled for each home screen application. Preset actions allow users to quickly configure and access the most important and relevant features of the darkroom. This section includes a brief overview of preset actions, as well as, recommended settings for the darkroom.

#### 11.1.1 Setup Filter Position for Preset Actions

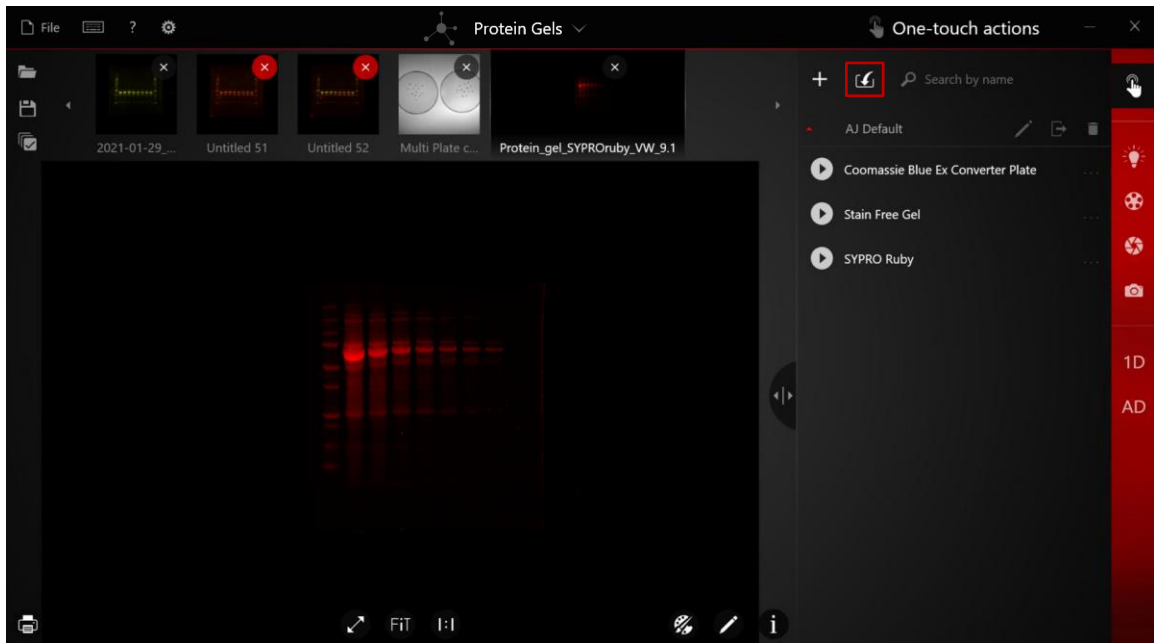
To use the VisionWorks® preset Actions, install filters according to the table below:

Filter Position Setup		
Filter Part Number	Dye Type	Filter Position
38-0220-01	EtBr, GelRed	5
38-0340-01	SYBR Green, SYBR Gold, SYBR Safe, GelGreen, Fluorescein, Stain Free Gel, Alexa 488, CF 488, Cy2, DyLight 488	3
38-0344-01	SYPRO Ruby	2
38-0369-02	Alexa 568, CF 568, Cy3, DyLight 550	2
38-0369-01	Alexa 647, 680, CF 647, 680, Cy5. 5.5, DyLight 680, IRDye 680	4
38-0417-01	Alexa 790, CF 770, Cy 7, DyLight 800, IRDye 800	5

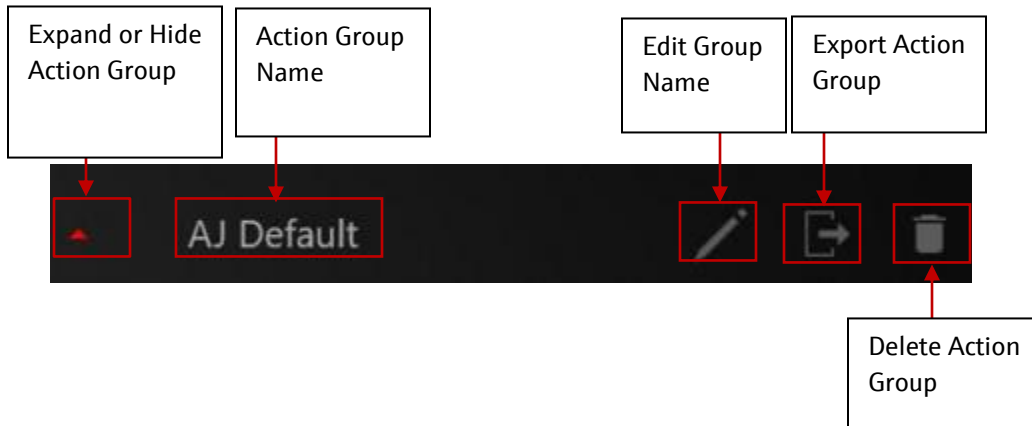
### 11.1.2 Use Preset Actions

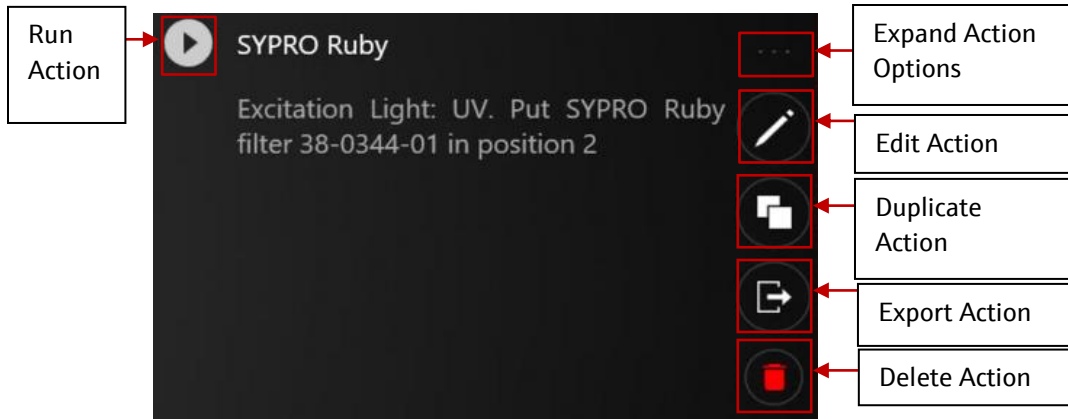
Action menu is available under Advance Interface. Use the Import function under the Action menu to import needed actions. All the default actions come with the Auto Focus step before capturing. If you wish to use the action without Auto Focus steps, the actions without Auto Focus are available under the folder:

C:\Program Files(x86)\Analytik Jena\VisionWorks\Action\All\Additional



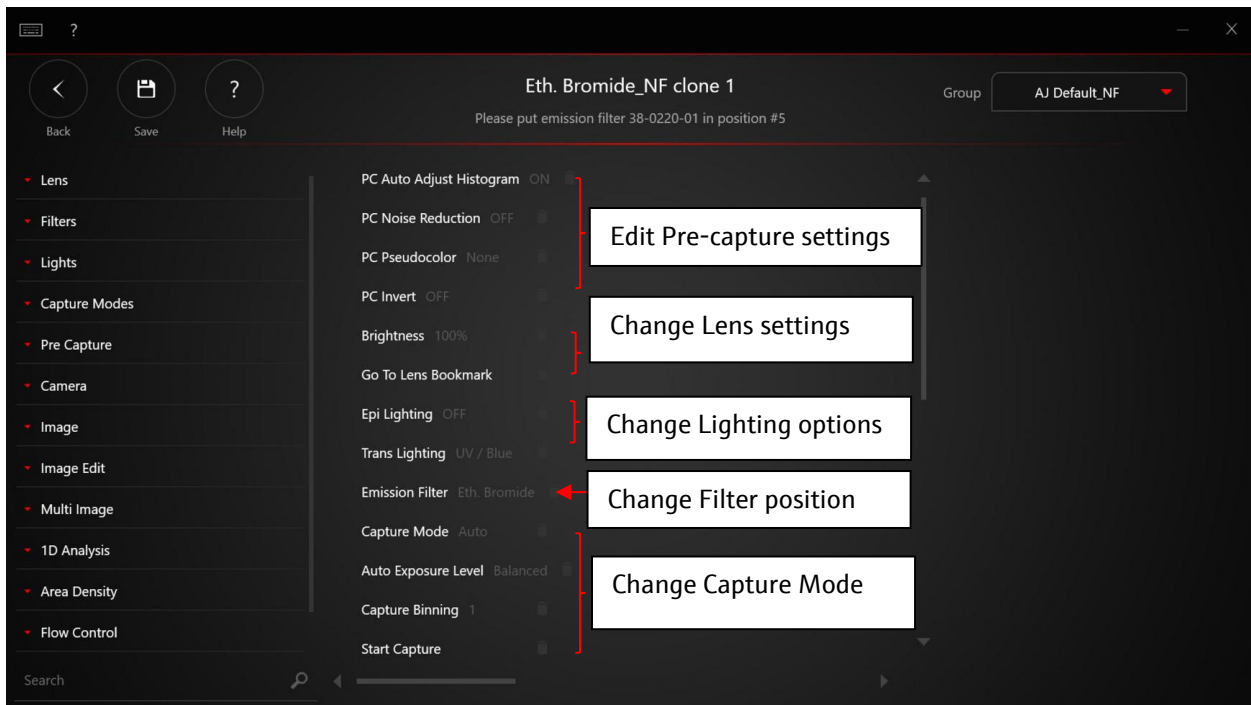
- 1) To execute an imported action, select the play icon next to each action description.
- 2) Tapping on the text of the action reveals a description of the action, as well as, the *edit* button, *duplicate action* button and *discard* button.





### 11.1.3 Edit Preset Actions

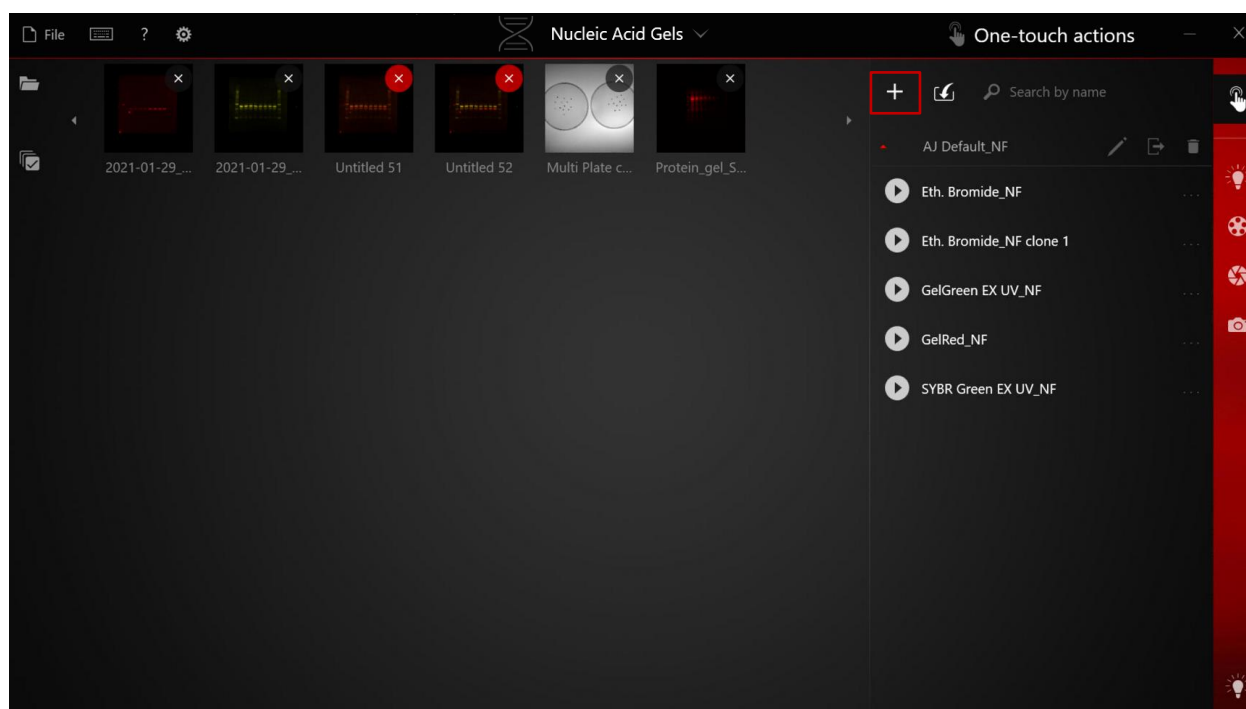
- 1) Create a copy of the Default action that you would like to edit by clicking the Duplicate icon in expanded action options.
- 2) In the action editor, you can change the Pre-capture settings, lens, filter, capture settings and also the post processing options for selected action.
- 3) Click the action command in preset actions to make modification.



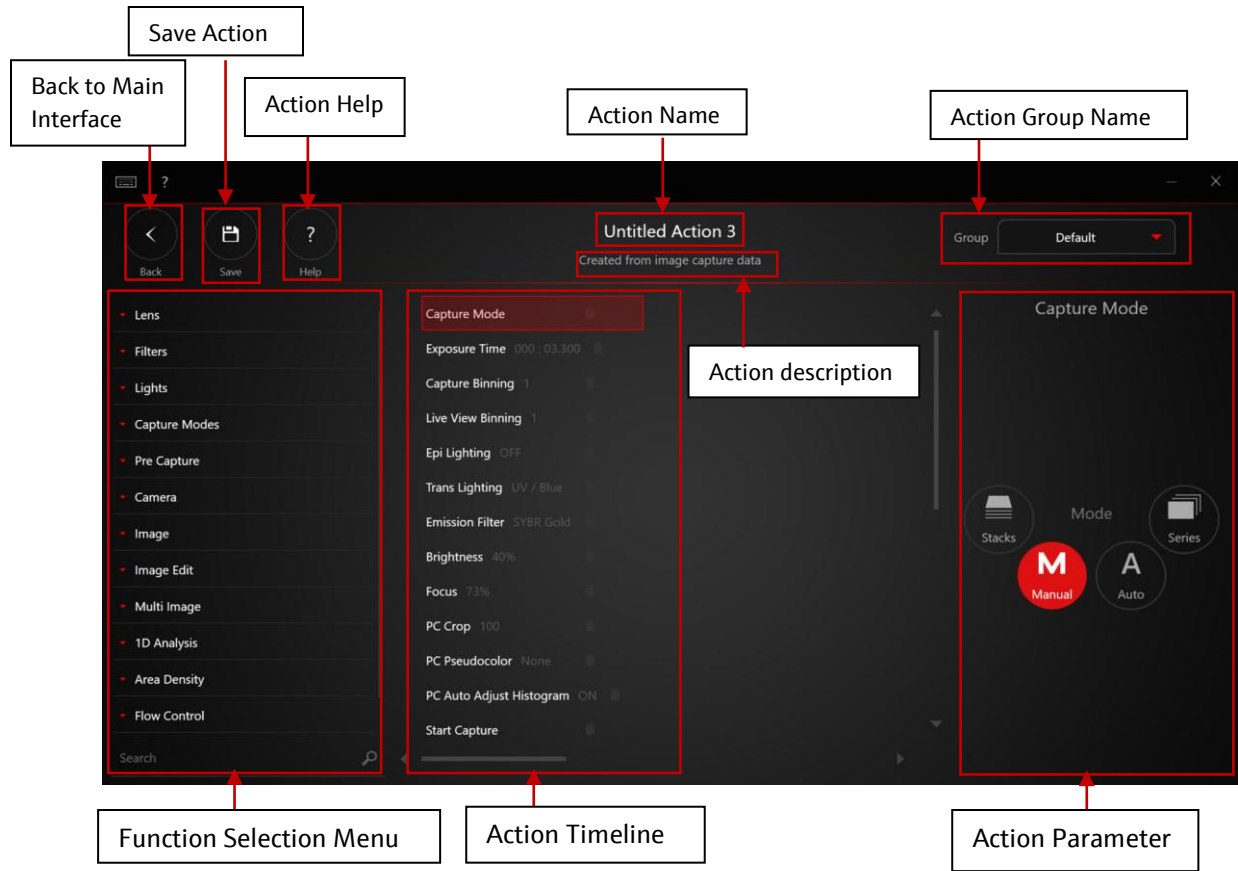
## 11.2 Creating and Customizing Actions

All actions within VisionWorks® may be customized to suit individual application needs. The action editor is designed to allow for the programming of a study into the unit for automatic, consistent, repeatable imaging. Actions scripts operate similarly to templates; however, action scripts allow for the execution of the device hardware in a prespecified order. This allows for deeper customization and application flexibility.

- 1) To create a new action, tap the **“New Action”** button on the bottom of the **“Actions”** pane. If you have a pre-captured image open, the New Action will automatically sync all the capture settings from the opened image. If no image opened, the New Action will start from scratch.



- 2) The action editor is brought up and displays three distinct interfaces on the screen.
  - a. The leftmost pane of the editor is the **Function Selection Menu** sorted by category. The action selection menu organizes all available functions/macros available to the system.
  - b. The center pane is the **Action Timeline**, this area allows you to select and rearrange action steps to your preferences.
  - c. The rightmost pane is the **Action Parameters** where device and imaging parameters are displayed for your selected action step.

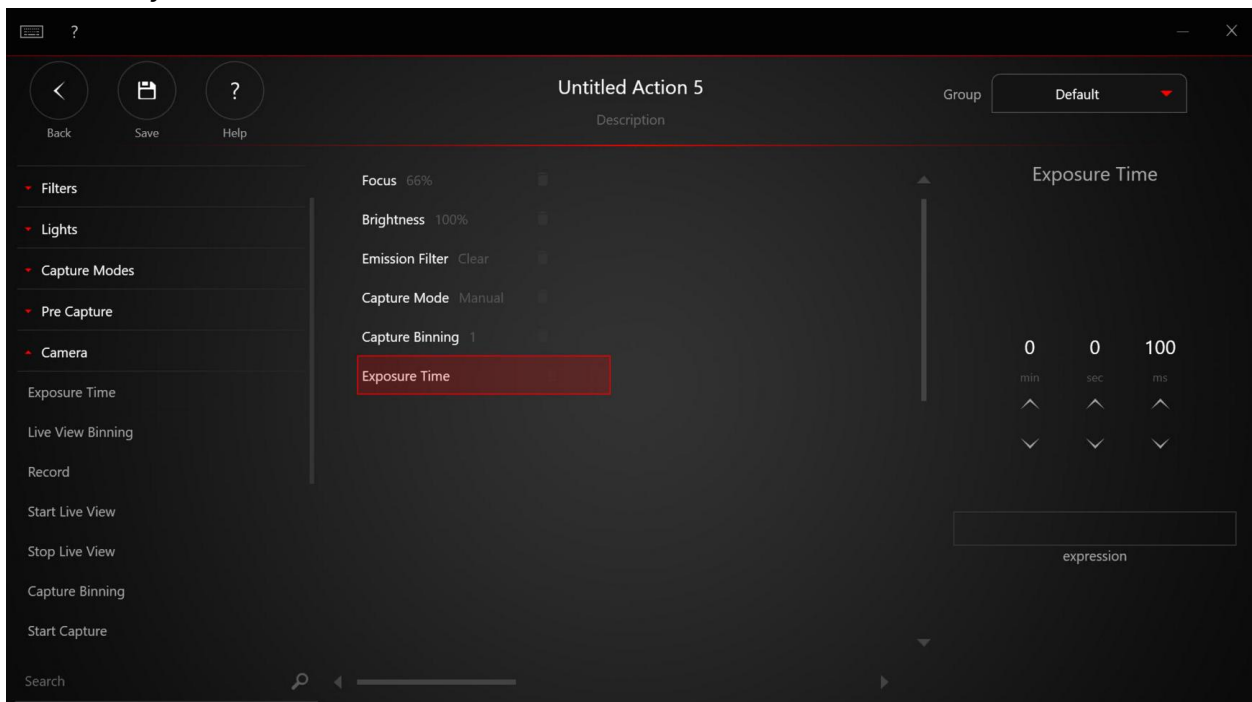


3) To create your first action, begin by selecting a function from the **Function Selection Menu**.



**Note:** It is most effective to begin constructing your action by starting at the top of the menu and working your way down. For instance, begin by defining the action steps under **“Lens”** then **“Filters”**. By working your way down the **Function Selection Menu**, you will ensure the best chance of creating the highest quality action.

- 4) Once selected, that action step will populate into the **Action Timeline**.
- 5) When selected in the **Action Timeline** the contextual **Action Parameters** will display all relevant parameters.
- 6) To change the order of your action steps, drag and drop the action steps into the desired order.
- 7) As you build your action, the **Action Timeline** will populate with all steps currently in your action.



- 8) Once completed, give your action a name by selecting the **Untitled Action** title on the top of the screen. Once you change the title, you can add a short description.
- 9) Select **“Save”** on the bottom to save your **Action**.

## 12.0 VisionWorks® Analysis Calculations

The following table summarizes the formulas that were used to calculate the quantities displayed in the analysis tables. The quantities calculated are contained in the columns of each report.

### 12.1 1D Analysis

Quantity	Definition
<b>I-Max (Intensity max)</b>	Maximum pixel intensity in given lane or band
<b>Volume (Intensity volume)</b>	Sum of all pixel intensities in the lane or band
<b>Lane Mass</b>	Mass of the lane (default is set to: 100 ng)
<b>I-% (Intensity percentage)</b>	$(\text{Band I-Vol} / \text{Lane I-Vol}) * 100$
<b>Relative Mass</b>	$\text{I-\%} * (\text{Lane Mass}/100)$
<b>C-% (Concentration percentage)</b>	$(\text{Band C-Vol} / \text{Lane C-Vol}) * 100$
<b>Top</b>	Top pixel coordinates
<b>Bottom</b>	Bottom pixel coordinates
<b>Left</b>	Left pixel coordinates
<b>Right</b>	Right pixel coordinates
<b>Peak</b>	Y-axis coordinate where maximum intensity is detected.

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## 12.2 Area Density Analysis

<b>Quantity</b>	<b>Definition</b>
<b>Region</b>	User-defined area of interest
<b>Total Density</b>	Sum total of intensities of all pixels with the region, minus background.
<b>Total Background</b>	Sum total of intensities of all pixels with the region marked as background.
<b>Mean Density</b>	Average of intensities of all pixels of the region, minus average intensity of background pixels.
<b>Mean Background</b>	Average intensity of background pixels.
<b>Total Raw Density</b>	Sum total of intensities of all pixels within the region.
<b>Mean Raw Density</b>	Average intensity of intensities of all pixels within the region.
<b>Area (Px)</b>	Total number of pixels in the region.
<b>Minimum Intensity</b>	Minimum intensity value among all pixels.
<b>Maximum Intensity</b>	Maximum intensity value among all pixels.
<b>Calibrated Value</b>	If an Amount Calibration curve exists, this displays the calibrated value based off that curve.

## 12.3 Colony Count Analysis

Quantity	Definition
<b>Class Number</b>	Classification type of colony.
<b>No of Colonies</b>	Number of colonies counted.
<b>% Colonies</b>	Percentage of colonies of the classification type.
<b>Total Area (px)</b>	Total area occupied by the colonies.
<b>% Area</b>	Percentage of area occupied by the colonies of the classification type / total area occupied by all colonies.
<b>Mean Area (px)</b>	Average area of a single colony.
<b>Std Dev Area</b>	Standard deviation of colony area.
<b>Min Area (Px)</b>	Smallest colony area found.
<b>Max Area (Px)</b>	Largest colony area found.
<b>Colony Number</b>	Identifier number given to specific colony.
<b>Area (px)</b>	Area of the specific colony.
<b>Perimeter (px)</b>	Perimeter length of the specific colony.
<b>Avg Diameter (px)</b>	Average diameter (distance from center to perimeter) for the specific colony.
<b>Mean Intensity</b>	Mean intensity across the area of the specific colony.
<b>Total Intensity</b>	Total intensity across the area of the specific colony.
<b>Circularity</b>	Circularity (closeness of fit to a perfect circle) of the specific colony, closer to 0 is more circular, higher indicates greater deviations from perfect circle.

## 13.0 Support for 21 CFR Part 11 Compliance

United States Food and Drug Administration (FDA) created and released Part 11 of Title 21 of the Code of Federal Regulations (CFR) in August 1997. The rules delineate the conditions under which the US-FDA considers electronic records and electronic signatures equivalent to paper records and paper signatures. The instructions for compliance span the entire organization and its practices. The software *supports* organizations with their 21 CFR Part 11 compliance by using both Windows User Management and the software features listed below. Please contact Tech Support for the VisionWorks Secure mode instructions.

**Note:** While software from Analytik Jena is an essential tool for assisting an organization to maintain CFR compliance, Analytik Jena cannot claim that this is the only tool needed to achieve overall CFR compliance. The organization must establish policies and procedures that work in conjunction with such efficient tools, to ensure total compliance with 21 CFR Part 11 regulations.

### 13.1 Features Supporting Compliance

VisionWorks® Secure mode supports two sections of part 11 compliance. The features are listed according to the sections they support:

**21 CFR 11.10 (e)** – VisionWorks® provides two features to support section 10(e), image history and a master audit file.

**Image History** records the username and timestamp of any modification to the original image data starting with image creation. The additions of pseudocolors, histogram and brightness/contrast adjustments are not recorded as they do not modify original image data. Image history is stored within the metadata of the captured image and will be updated by VisionWorks® as changes occur. If an image is modified by an external program, an error will be displayed under image history. Original capture data is also recorded; however, this is displayed under another tab and not needed for compliance.

**Master audit file** is the record keeping file of VisionWorks that will timestamp all activity within VisionWorks® during a session with the currently logged in user. A session is defined as the time from program execution to termination. The audit file will only be available through VisionWorks® and will not be recognized by any other program. This is to prevent unauthorized edits to the audit file.

**21 CFR 11.30** – VisionWorks® now supports application level user management. Users need to log on to the VisionWorks with assigned user name and password under the Secure mode. Three types of Visionworks accounts can be created: the administrator account, supervisor account and the regular user account. While Visionworks® ensures that the non-

administrator Visionworks accounts have limited access within the scope of the software, it is the responsibility of the administrator setting up the system to make sure that the Windows accounts used to log-in to the computer are always assigned the correct permissions, and to ensure that users intended to use the Visionworks supervisor or regular user accounts only log in to the operating system via a Windows standard accounts. Please see Visionworks® Secure Mode Start-up Guide for more details.

## 13.2 VisionWorks® Secure Mode

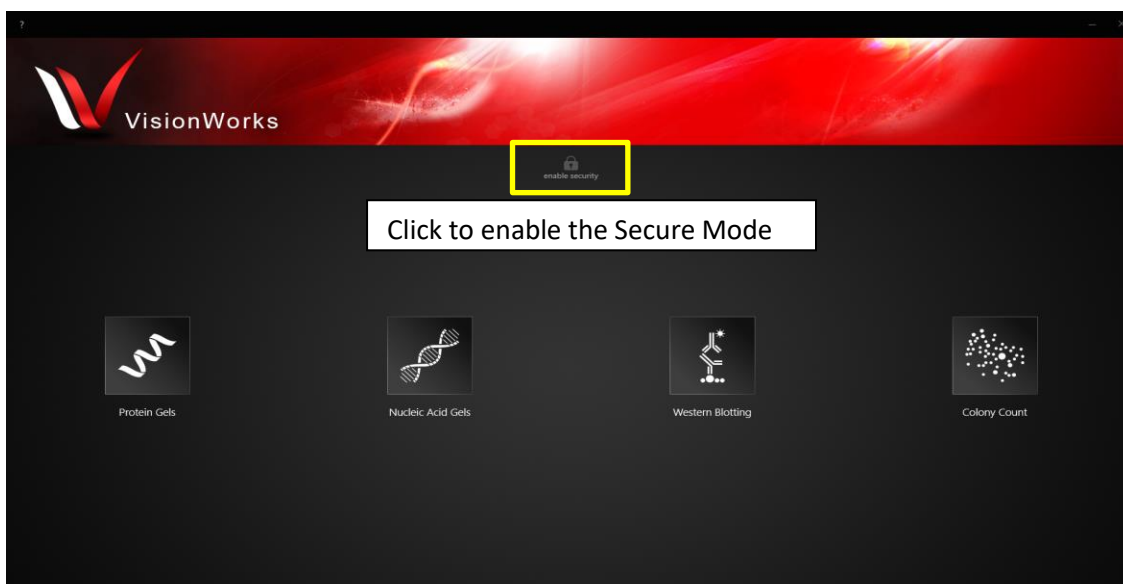
The VisionWorks® Secure mode provides a system of maintaining secure user accounts. The Administrator is required to properly setup the secure mode and assign unique usernames and passwords to all the users who will be using the software. Each account can also be configured to have different user permissions. Events generated in the audit trail are logged with the Windows and VisionWorks user name.

### 13.2.1 Introduction to VisionWorks® Secure Mode

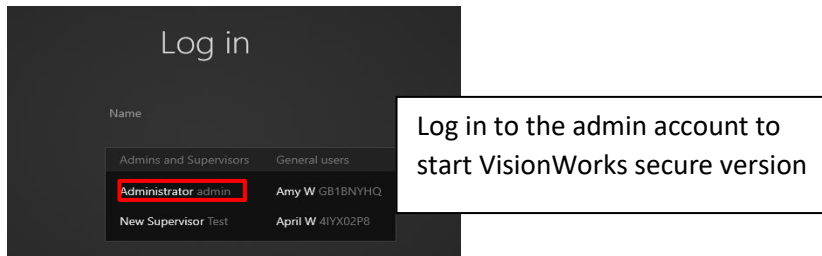
The VisionWorks can be run in either Standard Mode and Secure Mode. The software will be installed in Standard Mode by default. It continues to run in this mode until the Administrator enables the secure mode. The secure mode can only be activated by the administrator after completion of the steps in the Visionworks® Secure Mode Start-up Guide. Follow the instructions below to enable the secure mode after these steps have been completed.

1. Click Enable Security mode button from home screen.\*

\* Some set-up of the Windows permissions and program data files required for the button to be visible. Please see the Visionworks® Secure Mode Start-up Guide for more information.



2. Log in to the Admin account to activate the secure mode.



### 13.2.2 User Account Types

To use the VisionWorks under Secure Mode, users must log in with a user name and password. There are three types of user accounts can be created: Administrator account, Supervisor account and General User account. The administrator has full control of the software settings and features by default. The Supervisor has full control of the software features. Selected software features can be assigned to general users by administrator and supervisor account user.

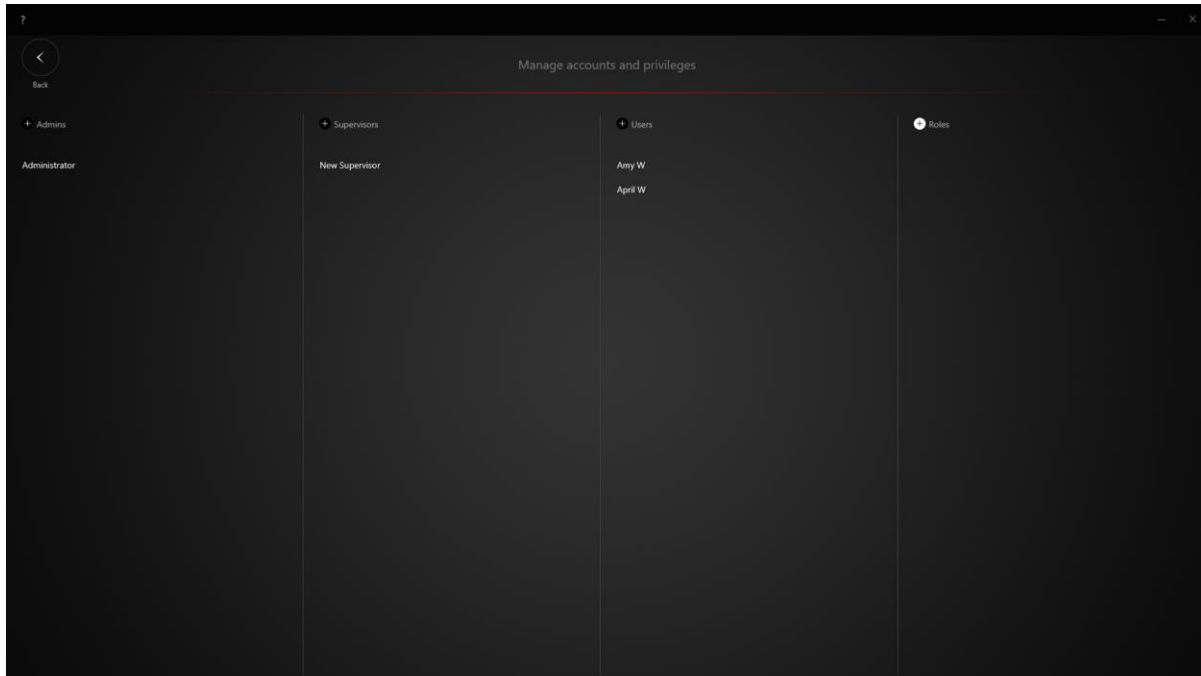
User Type	Description
<b>Administrator</b>	Administrator has full control of the software: enable/disable secure mode, create administrator, supervisor and general user account, view audit file, etc.
<b>Supervisor</b>	Supervisor has full control of all application features and functions.
<b>General User</b>	Has limited access to software features and functions.

Depending on the permissions enabled to the Windows account that has started Visionworks, only the accounts types applicable to the Windows user will be viewable in the Visionworks login screen. For example, if the Windows account is not the computer administrator or have specially assigned access to the Visionworks program data folders, that account will not see the Admin and/or Supervisors section as selectable when attempting to login to Visionworks.

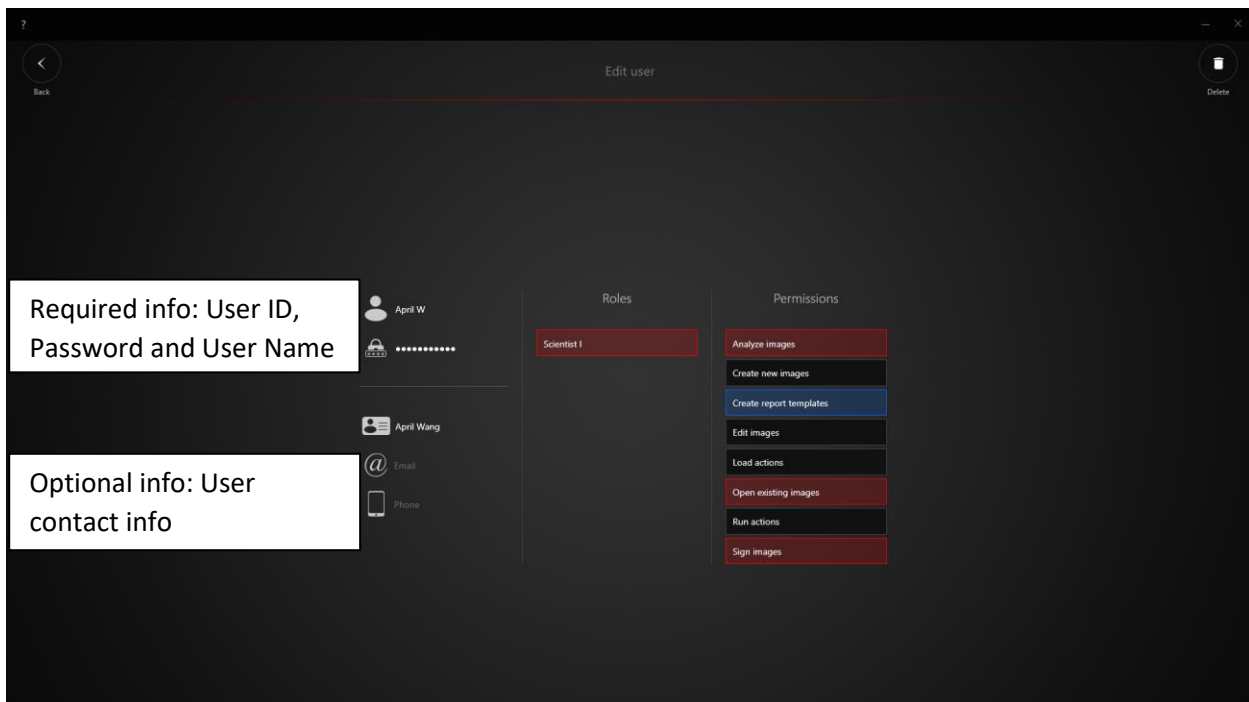
### 13.2.3 Create User Account

Follow the instructions below to create a new user account:

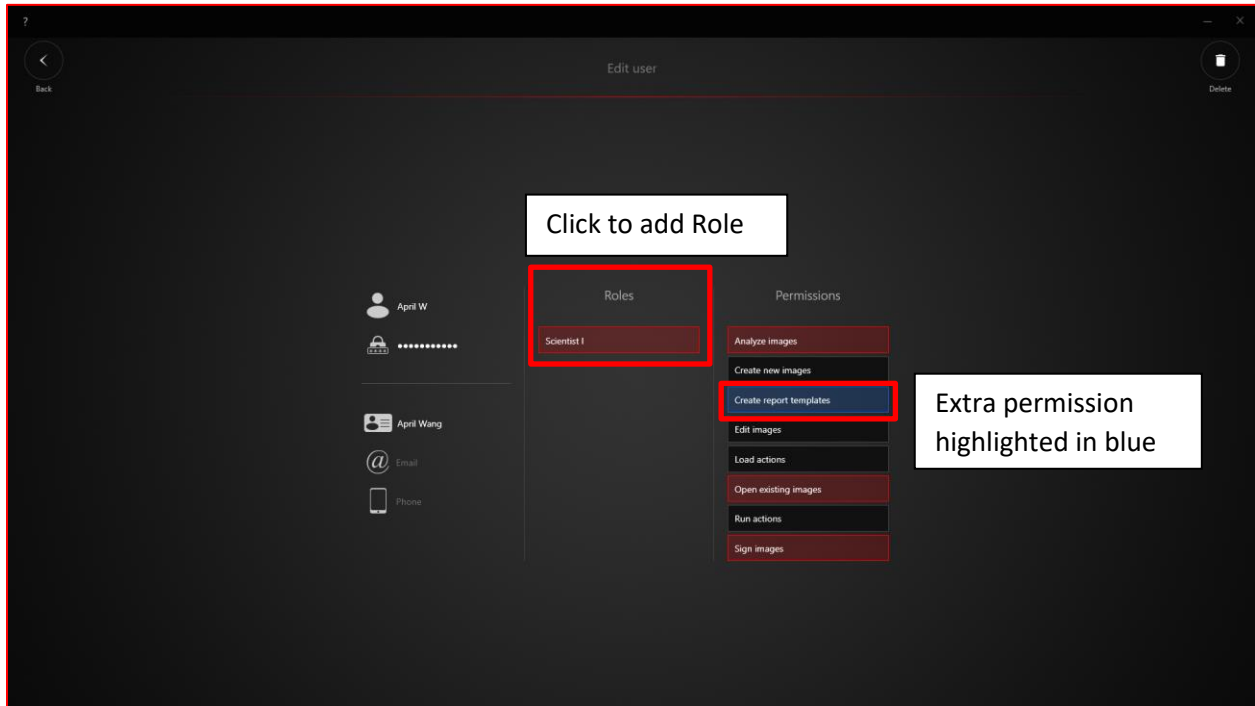
1. Navigate to the Manage Accounts window and choose the account type:



## 2. Add user information in the account window



3. Assign the Roles and permissions to the user: permissions included in the assigned Role will be highlighted in red. The extra permission selected will be highlighted in blue.

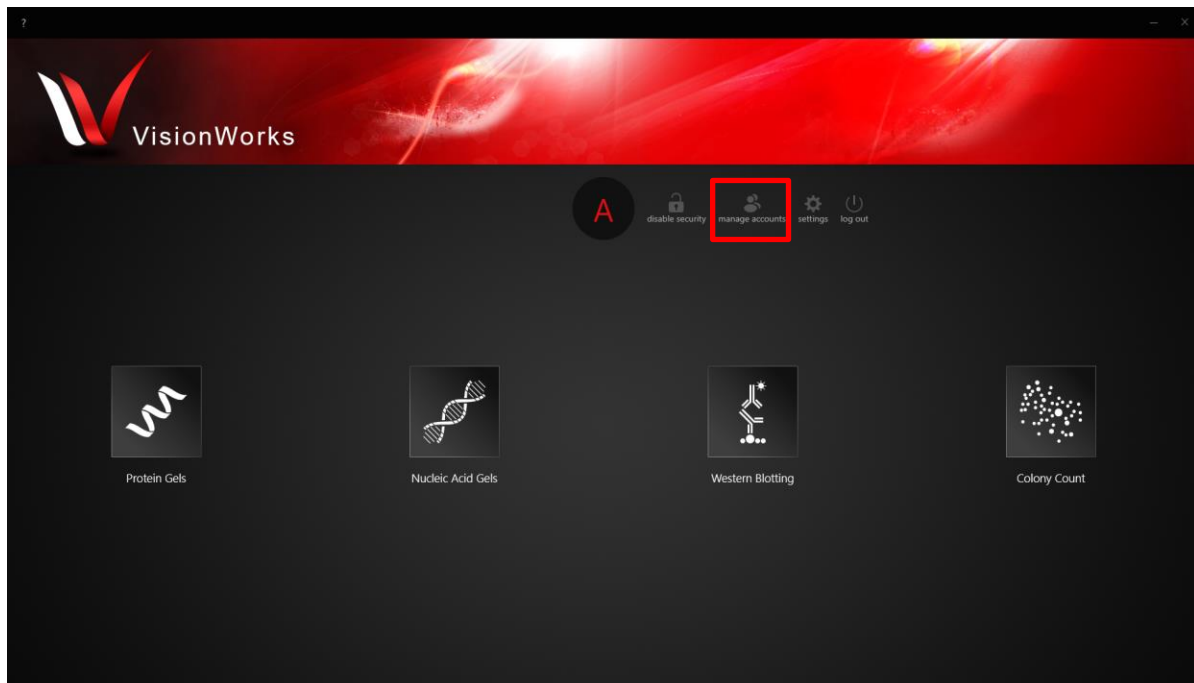


4. Click Back to confirm and save the user account.

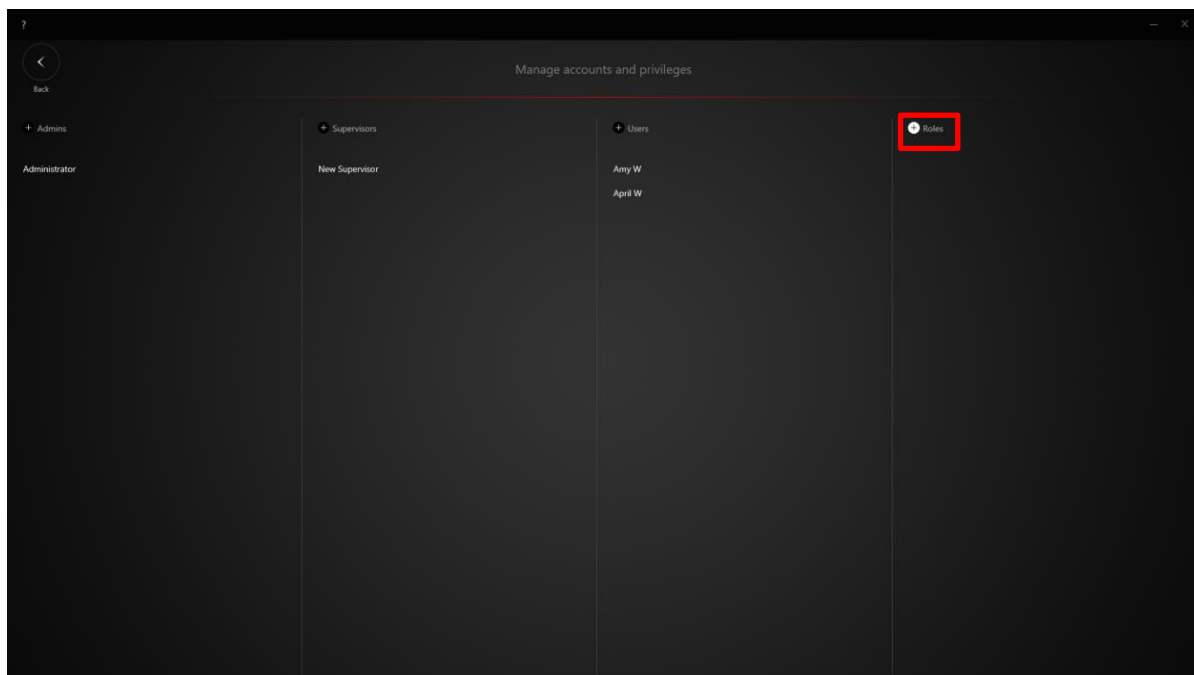
### 13.2.4 Create New Roles

Multiple user permissions can be grouped for a new role and apply to a new user. Follow the instructions below to create new roles:

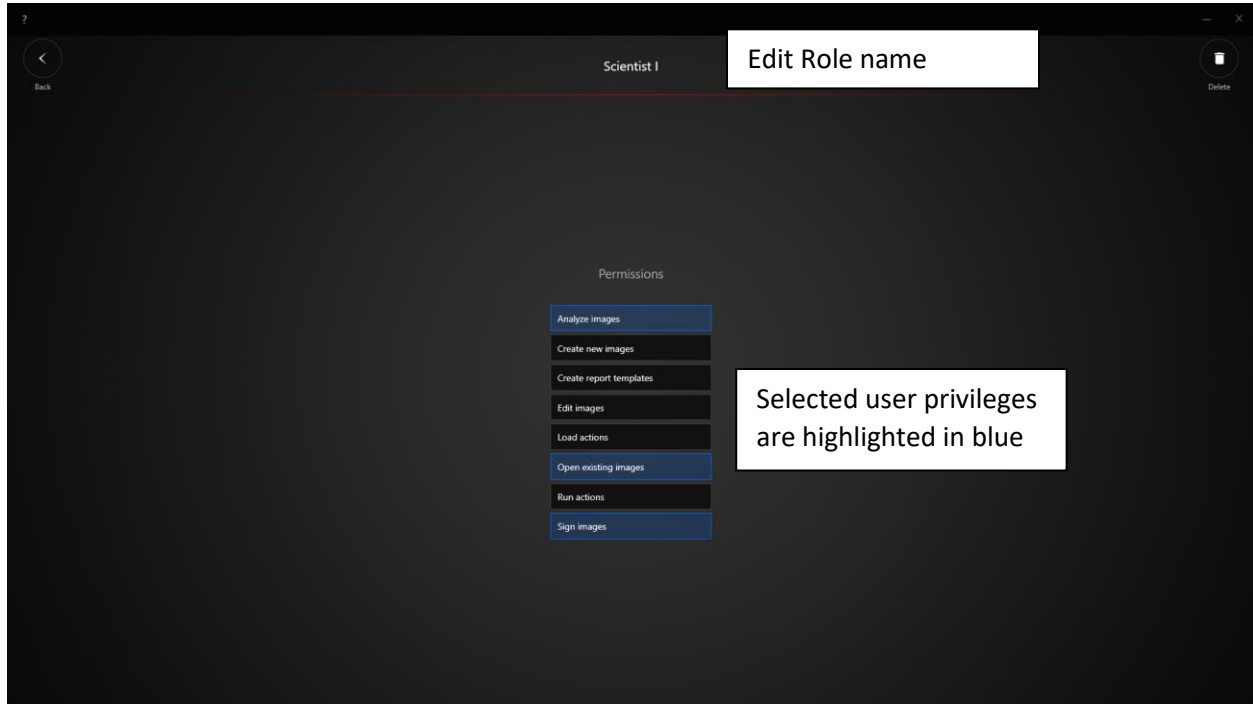
1. Navigate to the Manage Account from the home screen:



2. Select Role from the Manage Account window:



3. Select all the user privileges needed and create the Role name:



4. Click Back to confirm selection and exit the Role editing window.

### 13.2.5 User Restrictions

The User Account Type determines which software features are available in the VisionWorks Secure version. The software features maybe hidden or disabled if you are missing certain permissions in your role settings.

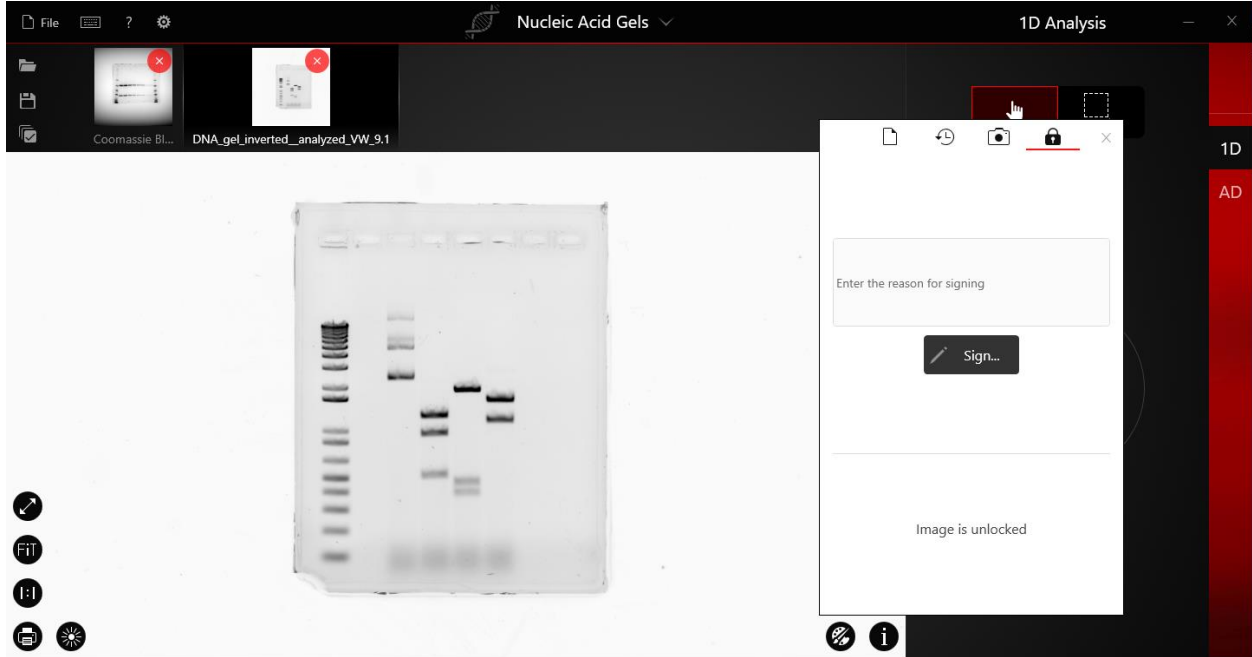
Software Function	User Type	Admin.	Supervisor	General	Description
Enable/Disable Secure mode		X			Only the Administrator can turn on and off the secure mode.
Create Admin account		X			Only the Administrator can create admin account.
Create Supervisor account		X			Only the Administrator can create Supervisor account.
Create General User account		X	X		Both the Administrator and Supervisor can create general user account.
Create new action		X	X		Only the Administrator and Supervisor can create new Actions.
Edit Action		X	X		Only the Administrator and Supervisor can Edit Action.

Export Action	X	X		Only the Administrator and Supervisor can export an Action.
Clone Action	X	X		Only the Administrator and Supervisor can clone an Action.
Lock/unlock Image	X	X		Only the Administrator and Supervisor can lock/unlock an image. No change can be applied to a locked image.
View Audit Trail	X	X		Only the Administrator and Supervisor can view the audit trail.
Load/Delete Action	X	X	X	Users with Load/Delete action permission can load and delete actions in the software.
Run Actions	X	X	X	Users with Run actions permission will also have the permission to save the image by default.
Create new images	X	X	X	Users with Create new image permission will also have the permission to save the image. Without this permission, the user cannot create new images by capture, extracting, merge and compositing, etc..
Open existing images	X	X	X	Users can open existing images only if they have this privilege.
Edit images	X	X	X	Users can Edit and save an image only if they have this privilege.
Analyze Images	X	X	X	Users can Analyze and save an image with this privilege.
Save images	X	X	X	Users with permissions to create a new image, edit an image, analyze an image, sign an image and run actions will have the Save image permission by default.
Create Report Template	X	X	X	Users can create new result report template with this privilege.
Sign Image	X	X	X	Users can sign the image with a reason and timestamp.

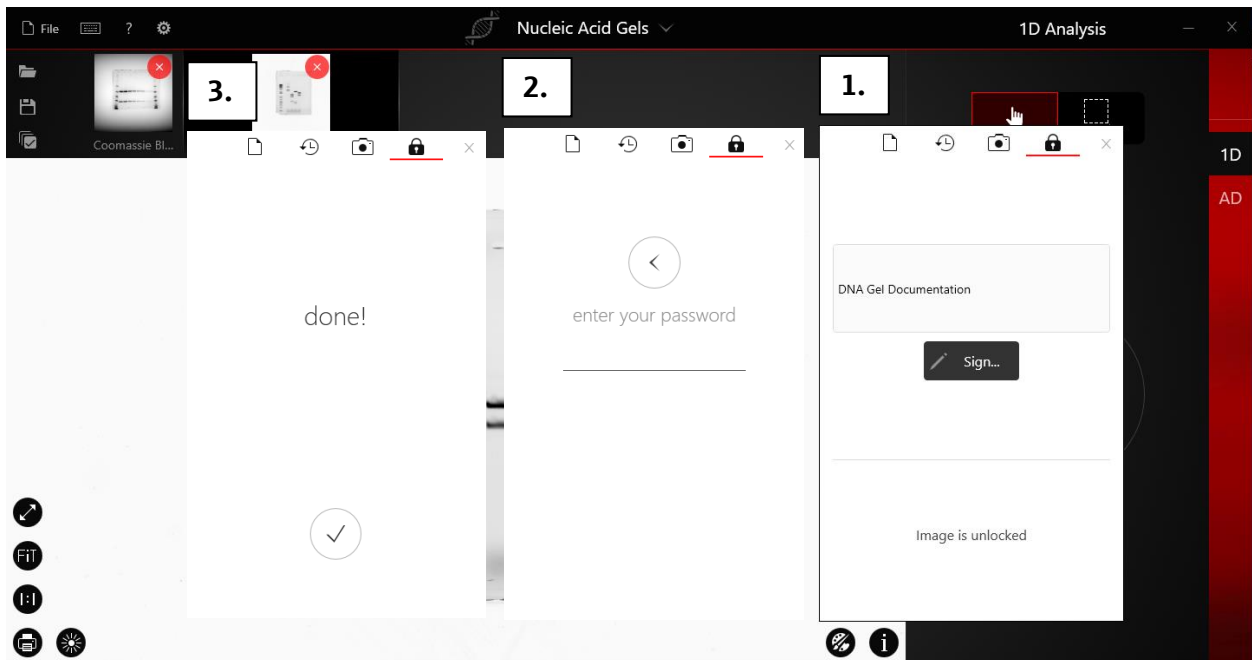
### 13.2.6 Signing Images

Follow the instructions below to sign the document:

1. After the image is captured, navigate to the image info to sign the image:



2. Enter the reason for signing and click Sign to re-enter the password:

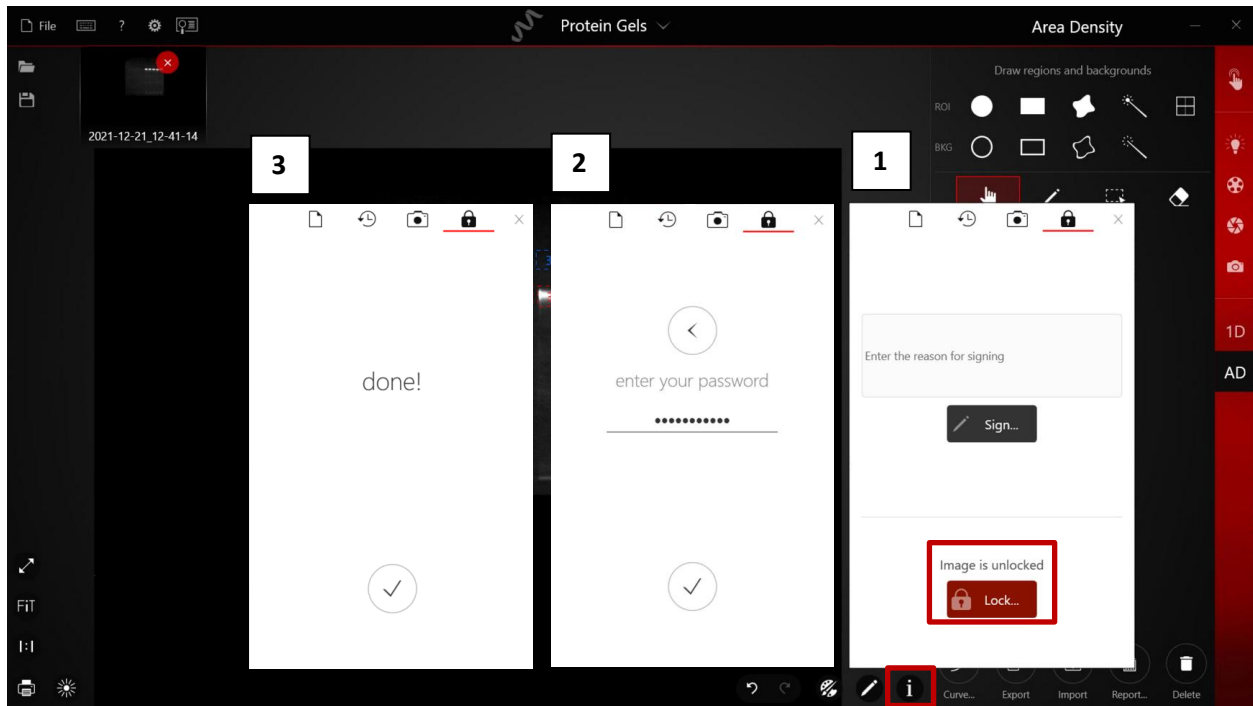


3. Click Done to confirm signing. The user name, reasons, date and time for signing will be available under the Image History.

### 13.2.7 Lock and Unlock Images

Images generated with the VisionWorks 10.3 can be saved as a secured and locked file. Only the administrator and supervisor user have the permission to lock an image. Once the image is locked, no changes can be added to the image. The locked image can be unlocked by the administrator and supervisor, new changes can be applied to an image after unlock. Please note, any changes applied to a saved image will create a new saving tag in the audit file. Only administrator and supervisor have the permission to view and export the audit file.

1. After finalizing the image editing and analysis, administrator and supervisor can navigate to the image info icon "i" and lock the image.



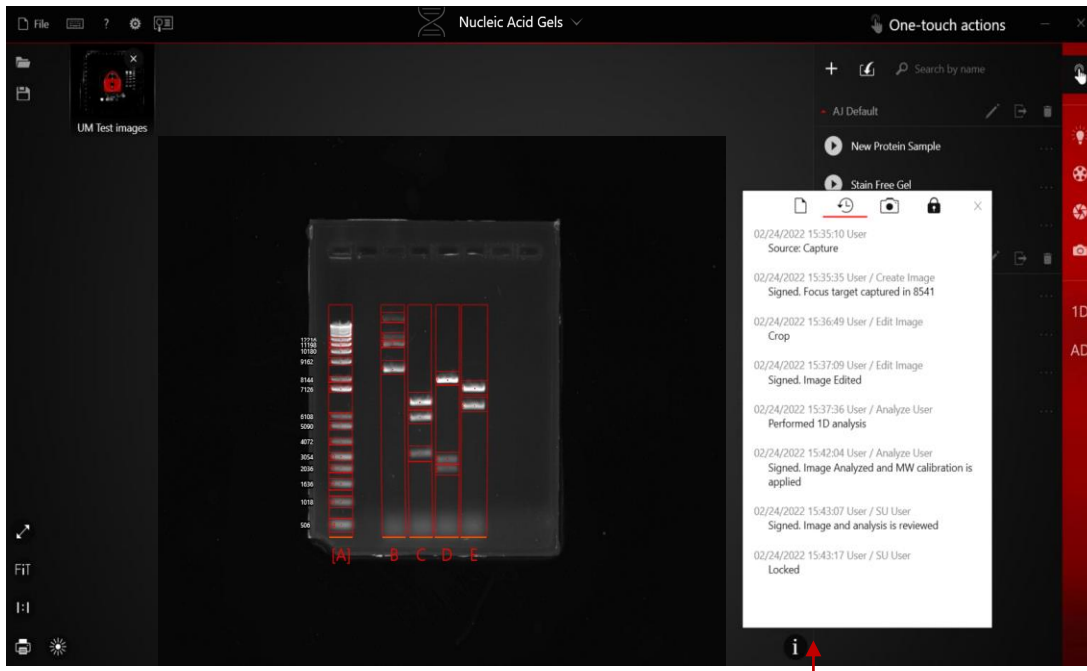
2. All editing and analysis tools will be hidden once the image is locked. A red lock icon will be added to the locked image in thumbnail gallery.



3. Image can be unlocked by Administrator and Supervisor if needed.

### 13.2.8 Image History

Image history is accessible by clicking the “i” icon. The screenshot below illustrates the basic layout of image history. Tapping or clicking on the timestamp entry reveals the saved action that was performed.

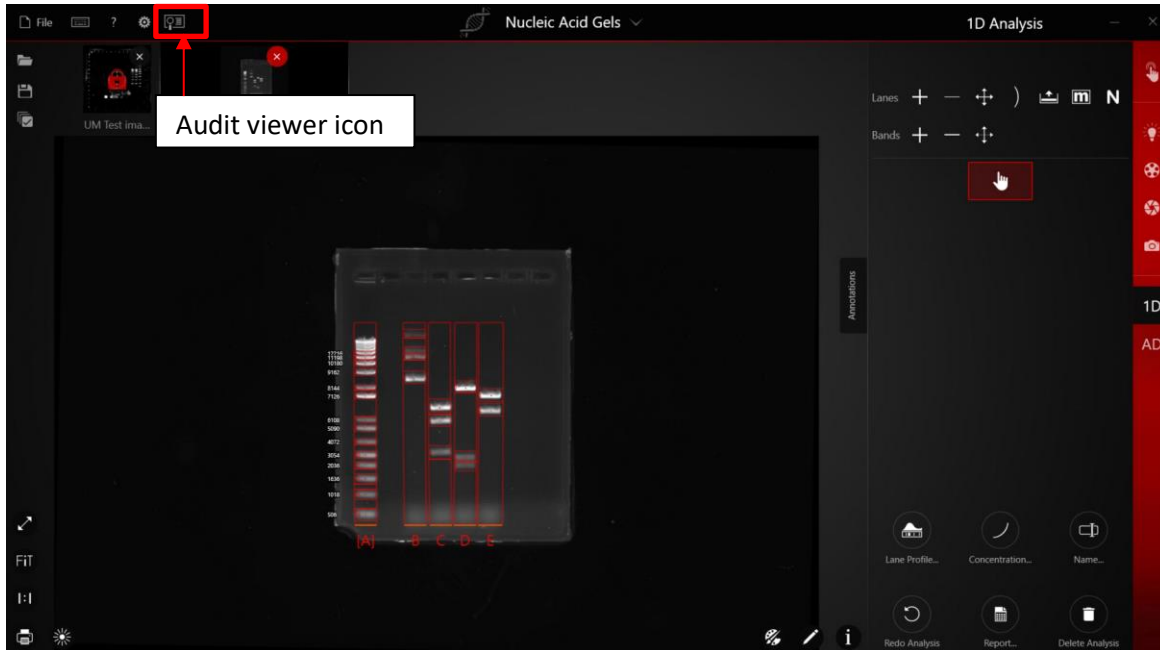


This image was created and edited in VisionWorks®.

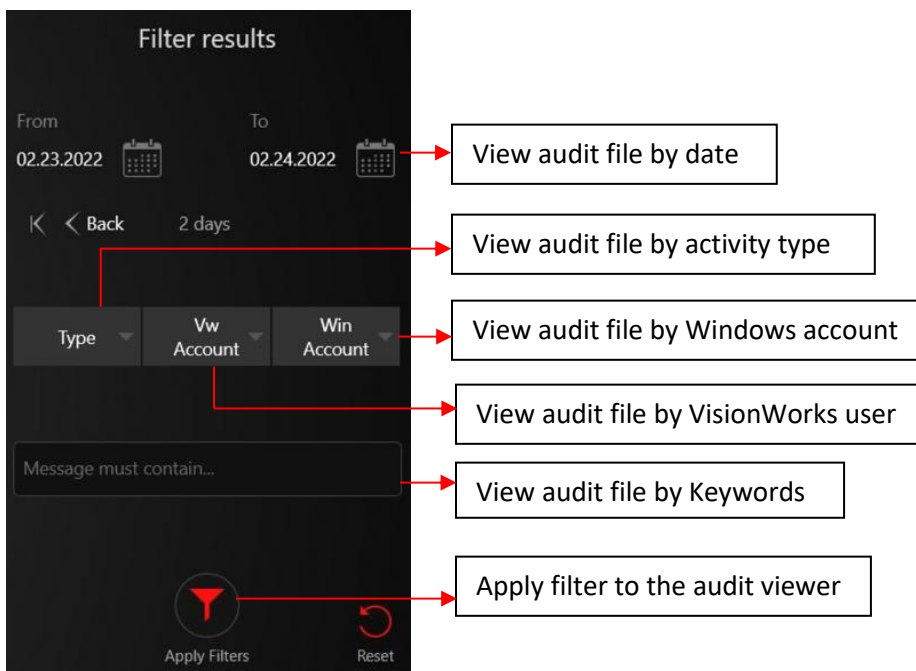
### 13.2.9 Master Audit Viewer

The Master Audit file timestamps all actions performed by the currently logged in user. This feature will provide an audit trail of a user per session.

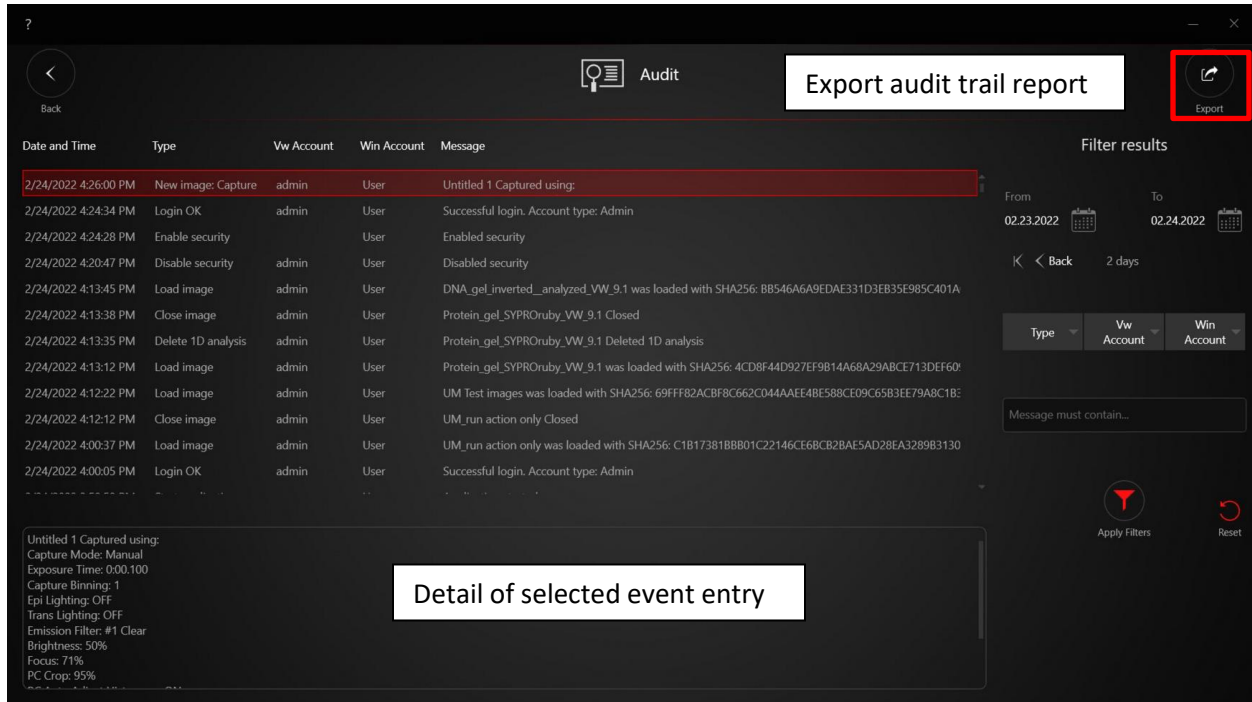
- 1) Navigate to the audit viewer icon on the top tool bar. The Audit Viewer will only be available for Administrator and Supervisor.



- 2) Audit trail can be filtered by date, type of activity, VisionWorks user, Windows Account and keywords.

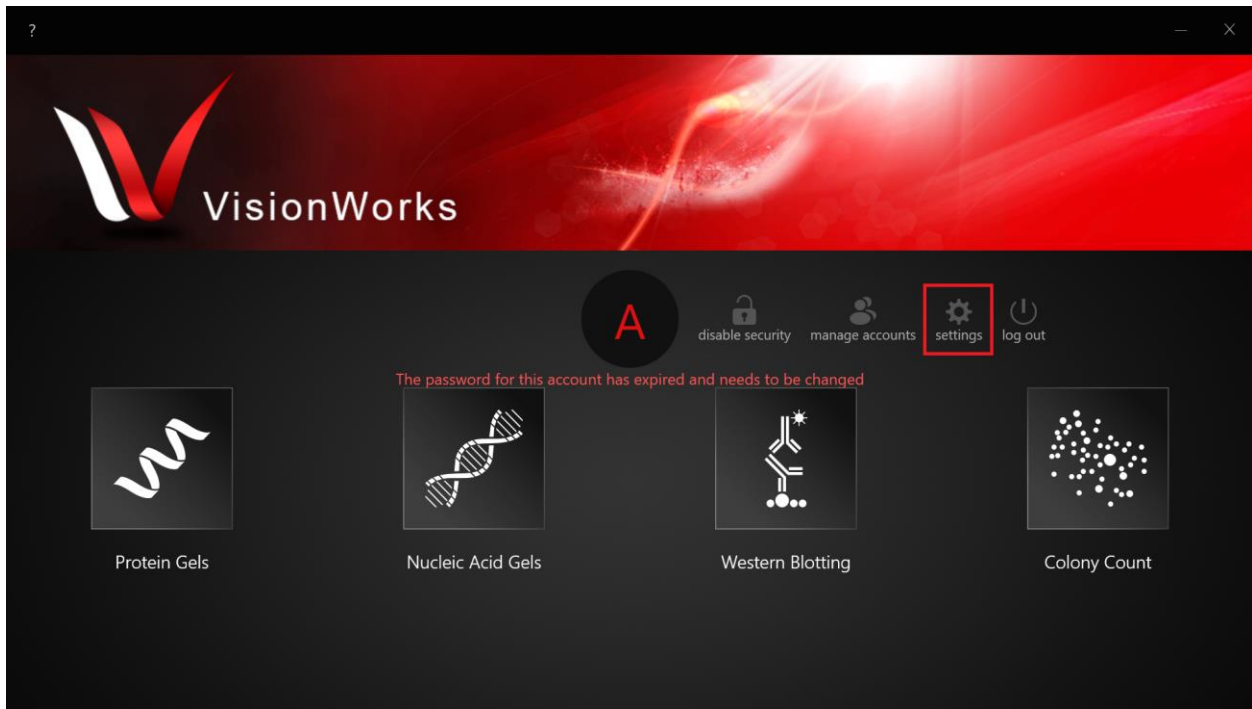


- The audit trail report can be printed or exported into PDF file from the audit viewer.

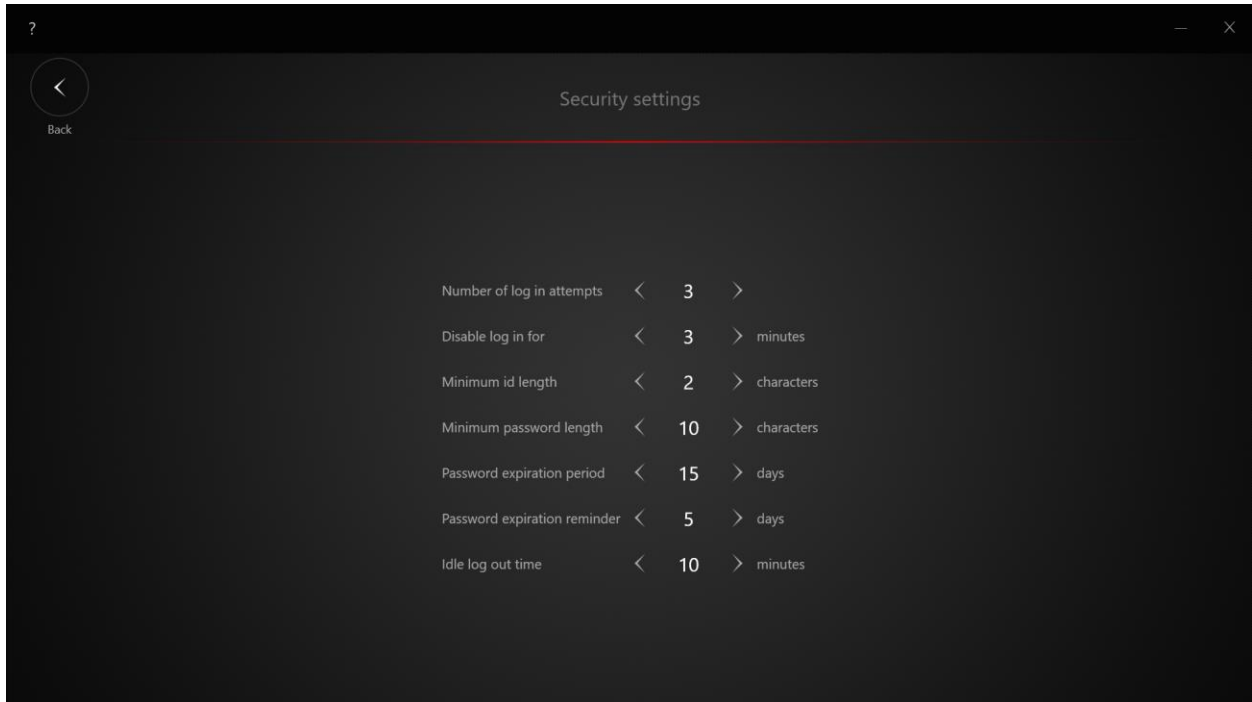


### 13.2.10 Managing Login Settings

Visionworks Secure Mode includes tools to have the password automatically expire after a certain number of days, remind the user to change their password, lock the user out for a set period after a certain number of failed login attempts, and also set restrictions on the password and user ID length. To access these settings, go to the Settings button in the home screen that is next to the security switch and manage account buttons.



The next screen will allow modification of the following settings as described below.



- 1) **Number of log-in attempts:** After the user fails to log in (enters the incorrect password) on any account in the login screen for the set number of times, the login screen will not allow any further login attempts at a time according to the next setting.

- 2) **Disable log in for:** Upon lockout, the user must wait at the login screen for the set number of minutes before they can attempt to log in again.
- 3) **Minimum ID length:** Minimum number of characters necessary for a valid user ID.
- 4) **Minimum password length:** Minimum number of characters necessary for a valid password.
- 5) **Password expiration period:** The number of days after which the user is required to change their password upon the next successful login.
- 6) **Password expiration reminder:** The number of days after which the user will be advised they should change their password.
- 7) **Idle log out time:** The number of minutes that passes with inactivity in VisionWorks before the screen locks and the user must re-enter their password to confirm their identity to continue use.

Note: For password and ID length requirements, changing the requirement after accounts have already been created and configured will not require the existing IDs and passwords to be modified until the next change of the given property. The administrator should set the requirement once establishing the system, before accounts are made.

## Appendix A – VisionWorks® One-Touch Action Function Definitions

### I. Image

- a. **Auto Adjust Histogram** – Applies the auto-adjusted histogram, after the image has been captured.
- b. **Pseudocolor** – Applies the selected pseudocolor to the captured image.
- c. **Invert** – Inverts the black and white pixels of a captured image.
- d. **Adjust Brightness** – Applies the selected brightness level to the image that is captured directly before this command.
- e. **Adjust Contrast** - Applies the selected contrast level to the image that is captured directly before this command.
- f. **Adjust Gamma** - Applies the selected gamma level to the image that is captured directly before this command.
- g. **Save Current Image** – Saves the image that is captured directly before or selected before this command.
- h. **Close Current Image** – After images are captured using actions, they will appear in the image gallery. This command will close that image from the gallery. This may be used for saving a large batch of images that were taken and it is not desirable for them to appear in the image gallery.

### II. Image Edit

- a. **Black & White** – Removes any color in an image and converts it to grayscale.
- b. **Flip Vertical** – Flips the previously selected or captured image 90 degrees to the vertical.
- c. **Flip Horizontal** - Flips the previously selected or captured image 90 degrees to the horizontal.
- d. **Enhance Exposure** – Automatically applies image lighting enhancement to the previously captured or selected image.
- e. **Change Depth** – Changes the bit-depth of the previously captured image. Best used when images of a certain bit depth are required.
- f. **Resize** – Applies a defined resize to the previously captured image.
- g. **Sharpen** – Applies the sharpening algorithm to the previously captured image.
- h. **Rotate** – Rotates the image a defined number of degrees in a direction.
- i. **Remove Noise** – Applies a defined noise removal filter to the image that is directly before this command.

### III. Multi-Image

- a. **Set Target for Multi Image** – Sets the image captured immediately before this command as the target for compositing. The target image will not have any pseudocolors so any pseudocolors applied to the target image will not have those pseudocolors. This is only applicable if images are being composited together.
- b. **Select for Multi Image** – Selects the image immediately before this command as a target for either compositing or mix. Place this command directly after the image is captured if you desire to select it for compositing or merge channels.
- c. **Compose** – Composites the target image with any selected “select for multimage” images previously.
- d. **Merge Channels** – Merges the images that have been selected “select for multi-Image”.
- e. **Background Correction** – Applies an automatic background correction to the image that was capture immediately before this command.
- f. **Background Subtraction** – Subtracts out the background of the image captured immediately before this command.

### IV. 1-D Analysis

- a. **Find Lanes and Bands** – Will run the 1D analysis “find lanes and bands” algorithm on the image captured immediately before this command.
- b. **Show 1D Results** – Shows the spreadsheet of intensity or photon flux values after the 1D analysis algorithm has been run.

### V. Area Density

- a. **Import AD Regions** – Imports previously saved regions to be used in the image that was captured directly before this command.
- b. **Show AD Regions** – Shows the AD results after the regions have been imported.

### VI. Flow Control

- a. **If** - The action will check if a mathematical comparison that you define is true, and run the corresponding statements below it if it is true.
- b. **Repeat** - The action will run through the corresponding statements below it the specified number of times.
- c. **While** - The action will check if a mathematical comparison that you define is true, run the corresponding statements below it, then check again if it is still true, run the statements below, etc., repeatedly until the comparison is false.

- d. **Break** - The action will terminate running the nearest While or Repeat statements that the Break line is a part of. The action will terminate running the nearest While or Repeat statements that the Break line is a part of.
- e. **End Action** - The action will terminate immediately (and not run any other lines in the action script).

## VII. Extra

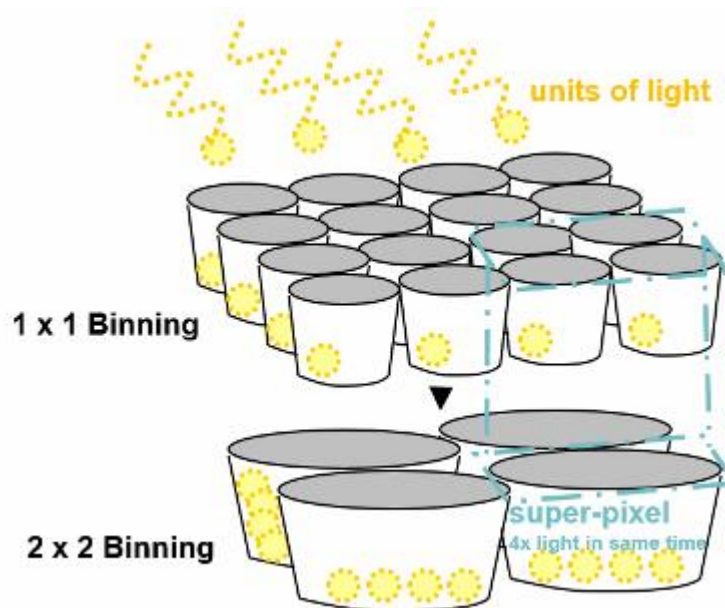
- a. **Number** - The action will define a number to a specific value or expression that you can set.
- b. **Text** - The action will define a text to a specific value or expression that you can set.
- c. **Input** - The action will pop up a box that allows whoever is running the action later to define a variable on the spot. Enter the Variable Name in the left-hand side and enter the type of variable that is being defined on the right-hand side, either Text, Number, Path (folder path), or Check (a direct true/false switch that can be used with If or While lines).
- d. **Wait** - When encountering this line, the action will pause (do nothing) for the time specified, to the nearest 100 milliseconds. It can also be set to an expression involving either numerical values or previously defined Number variables, this will be converted to milliseconds.
- e. **Message** - The action will pop up a box with a text or numerical message that you can set.

## Appendix B – VisionWorks® Camera Binning

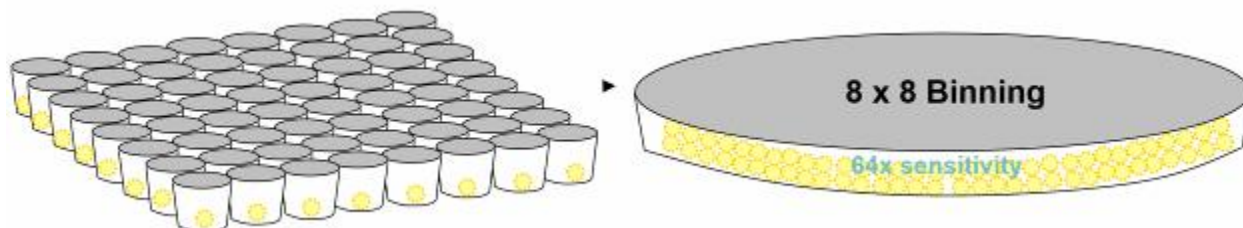
The Binning setting in Visionworks refers to a special capability of CCD cameras to adjust the sensitivity of its sensor to light at the expense of resolution. Through this setting, the camera can be changed to acquire images from very low light-intensity subjects at a faster exposure time, if resolution of the end capture image is not as important.

Binning works by combining multiple pixels in a sensor array. In a CCD sensor, the light collecting capacitors are arranged in an equivalent array to the output pixels of the end image: if the captured image is a 2500 x 2500 pixel (6 megapixel) image, these capacitors are arranged in a grid, such that there are 2500 rows and 2500 columns of the capacitors (also called pixels) arranged onto the sensor. Each of these pixels can be thought of as “buckets” that collect light. Because these buckets have a certain area of their opening, they can only collect a certain amount of light for a certain exposure time, which in this analogy is the time when the opening of the bucket is uncovered to allow light to collect.

The default binning is 1 x 1, where the bucket can collect light per exposure time as limited to the capability of the capacitor/pixel. If binning is 2 x 2, every square of two buckets in a row and two buckets in a column combine to form a “super-pixel”, or one larger bucket with the same area as the four buckets that comprise it. This can collect light 4x faster (equals  $2 * 2$  the combined area of each of the original buckets), so it is 4x more sensitive to light. Equivalently, it take 1/4 of the exposure time to collect the same amount of light as each individual bucket when 1 x 1 binning is used.



Similarly, if binning is 3 x 3, the super-pixels are 9x as sensitive and can collect the same amount of light in 1/9 the exposure time compared to default. The maximum possible binning on most Visionworks CCD cameras is 8 x 8, with 64x sensitivity and 1/64 of the required exposure time.



To set capture binning, use the small buttons below the exposure time in the Sensitivity section of the Camera tab. CMOS (GelStudio and Solo system) cameras do not have binning capability. If the system in use uses a CMOS camera, no binning will be available in VisionWorks capture settings.

A high binning allows high sensitivity to light at the expense of resolution and can be thought of as a “High Sensitivity” capture. A low binning gives high resolution but lower sensitivity to light or longer exposure time required to reach a target intensity.

**Example:** The user places one of many fluorescent DNA electrophoresis gels that they have prepared, which have a weak maximum signal with the normal excitation light. It would take 1 minute to reach their target intensity of 50% saturation at default (1 x 1) binning. The user does not care about image resolution of their captured image but only wants to take the images of all their gels quickly to detect presence/absence of a certain target band. The user can set capture binning to 2 x 2. Now the camera is 4x as sensitive to the fluorescent signal, with less resolution. They can take every capture at their target intensity in only 60 seconds / (2 x 2) = 15 seconds. The image capturing phase of their experiment has been sped up by 4 times.

Binning	Resolution (more pixels)	Sensitivity	Exposure Time (when best intensity at default = 1 min)
1 x 1 [Default]	- full resolution	- lowest	- longest (1 minute)
2 x 2 [Enhanced]	▲	- 4x	▲ - 15 seconds
3 x 3		- 9x	- 6.7 seconds
4 x 4 [High]		- 16x	- 3.8 seconds
5 x 5			
6 x 6			
7 x 7			
8 x 8 [Maximum]	- very low (64 times fewer pixels)	▼ - 64x	- fastest (0.9 seconds)

Binning of higher than 4 x 4 for capturing images is not recommended due to the extreme loss in

image resolution. Very high binnings such as 7 x 7 or 8 x 8 are available for custom applications such as super-long low intensity captures, or to set a faster live view binning, explained below.

VisionWorks software allows the live view binning to be set independently of the capture binning during capture preparation, while automatically compensating the Live View refresh time so that the intensity of the live view and end capture will be equivalent. In this way, the user can see the live view refresh much faster to reflect the current state of the darkroom, which would normally be restricted to be equal to the capture exposure time setting. Since the binning of the live view is set higher than capture binning, it can accurately reflect the intensity of the actual output image in a much shorter time, at the expense of resolution in the live view (but not in the capture).

**Example:** The user places a low intensity chemiluminescent sample in the darkroom, which will reach their target intensity of 20% saturation at an exposure time of 2 minutes at 1 x 1 capture binning. The user needs the highest possible resolution so they must use 1 x 1 capture binning. If live view binning was set the same as capture binning, the Live View image would refresh every 2 minutes, which would make sample positioning to align with the camera field of view time consuming as well as compromising signal intensity as ECL based luminescence decays rapidly. However, the user can set the live view binning to 6 x 6: the live view refresh rate is now  $120 \text{ seconds} / (6 \times 6) = 3.33 \text{ seconds}$ . The Live View image refreshes about every 3 seconds, and the user can position their sample quickly while still seeing an accurate intensity representation of the sample in the Live View.

Note that the live view will no longer accurately reflect the intensity of the end capture image if the compensated live view exposure time reaches below the fastest possible exposure time allowed for the camera (usually 1-10 ms, but can be higher for cameras with shutters). For example, if an intense fluorescent sample reaches target intensity at 50 ms with excitation lights at 1 x 1 capture binning and the user is using 4 x 4 live view binning, the compensated live view exposure time / refresh rate is calculated as  $50 \text{ ms} / (4 \times 4) = 3.1 \text{ ms}$ . If their camera only allows exposure times of 10 ms or greater (this can be checked by lowering the exposure time to the minimum possible during manual exposure setting), the actual refresh rate will be 10 ms, and the live view image will be about 3 times as intense as the actual captured image. If the user finds that the intensity of the live view image is brighter/saturated compared to the end capture image, they should lower the live view binning value.

## Appendix C – VisionWorks® How to Guide

### How to do 1D Analysis

#### Purpose

Below we instruct users how to perform 1D analysis using VisionWorks. This document will cover the major points below.

- I. **Adjust image size**
- II. **Adjust Image Appearance**
- III. **Perform 1D Analysis and a Simple Lane Annotation**
- IV. **Creating and Exporting a Report**




#### I. **Adjust Image Size**

After Capturing an image, your image will be viewable in the main viewing window. In this window there is a taskbar at the bottom that has multiple icons. In the lower taskbar, there are 4 tools to adjust the image size.



**Figure 1.** Main Viewing in VisionWorks

Main viewing window

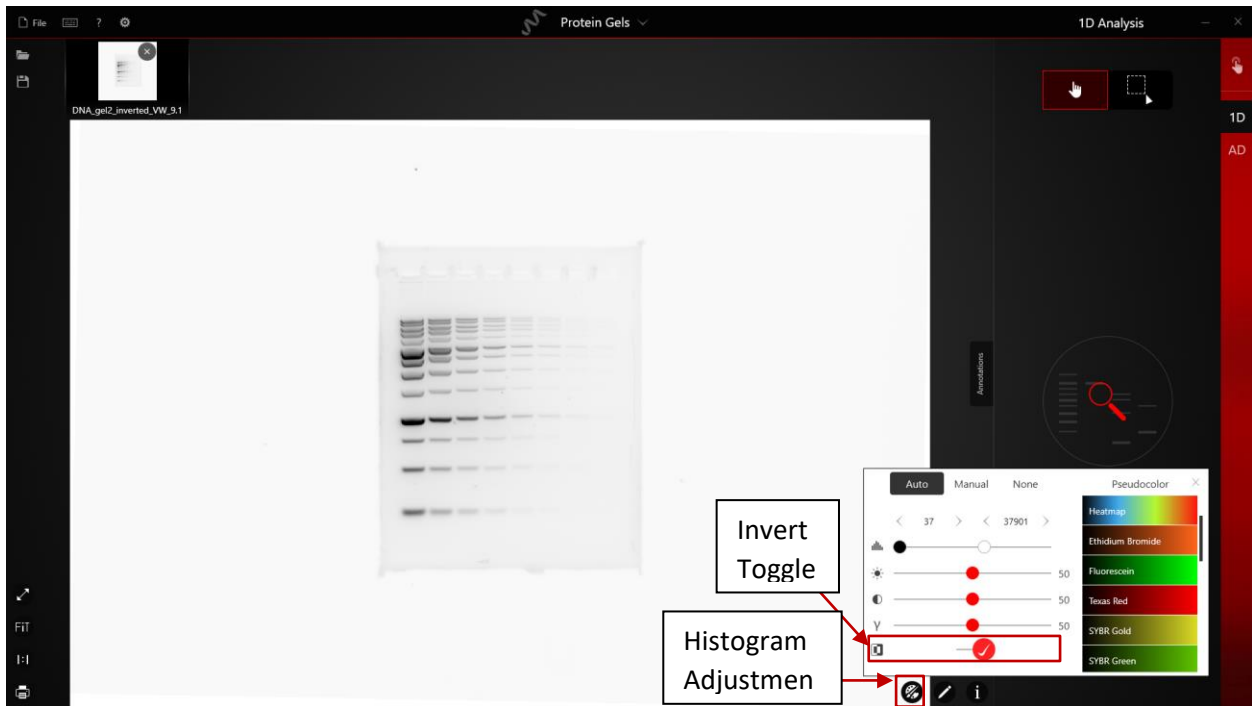
1. The **toggle icon**, , expands the image to the whole viewing window to see difficult-to-see features of your image.
2. If the mouse is attached, scroll up and down to zoom. Pinch-to-zoom can be used for touch screen computers.
3. The **FiT switch**, , adjust the image to fit the entire image in the screen
4. The **1-to-1 switch**, , adjust the image size to actual print size.

## II. Adjust Image Appearance

In VisionWorks, we have several features that aid in visualization, but do not modify the image metadata. Here we demonstrate **Invert** and **Pseudocolor**.

### a. Invert

- i. Navigate to the **Histogram Adjustment** and toggle **Invert**. This produces a traditional black-on-white, signal-to-background image (**Figure 2**).



**Figure 2.** Invert image in VisionWorks

### b. Pseudocolor

- i. Navigate to **Histogram Adjustment tab** and Select a pseudocolor. (**Figure 3**).

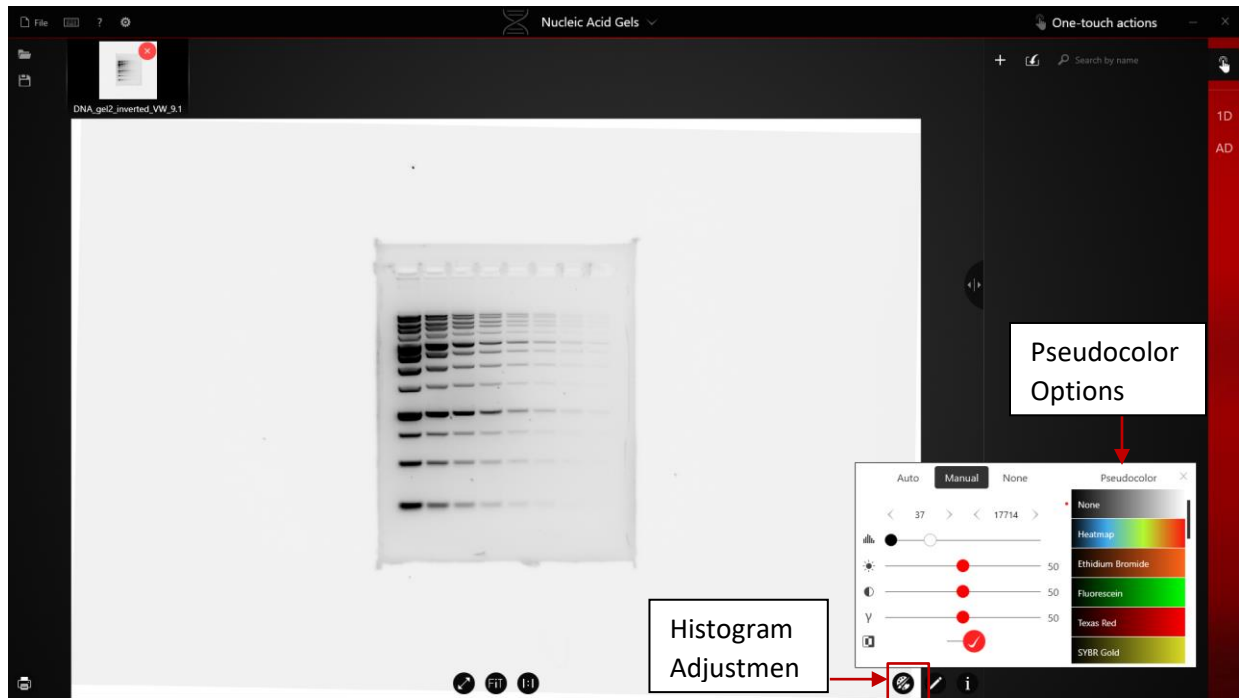


Figure 3. Pseudocoloring image in VisionWorks

### III. Perform 1D Analysis and a Simple Lane Annotation

Users can define their region of interest (ROI) or have the software automatically detect the ROI. Below we demonstrate a user-directed ROI and subsequent analysis.

#### User-directed Drawing of Region of Interest

1. Navigate to **1D analysis** → select drawing tool to **Define Region (Figure 4 and Figure 5)**

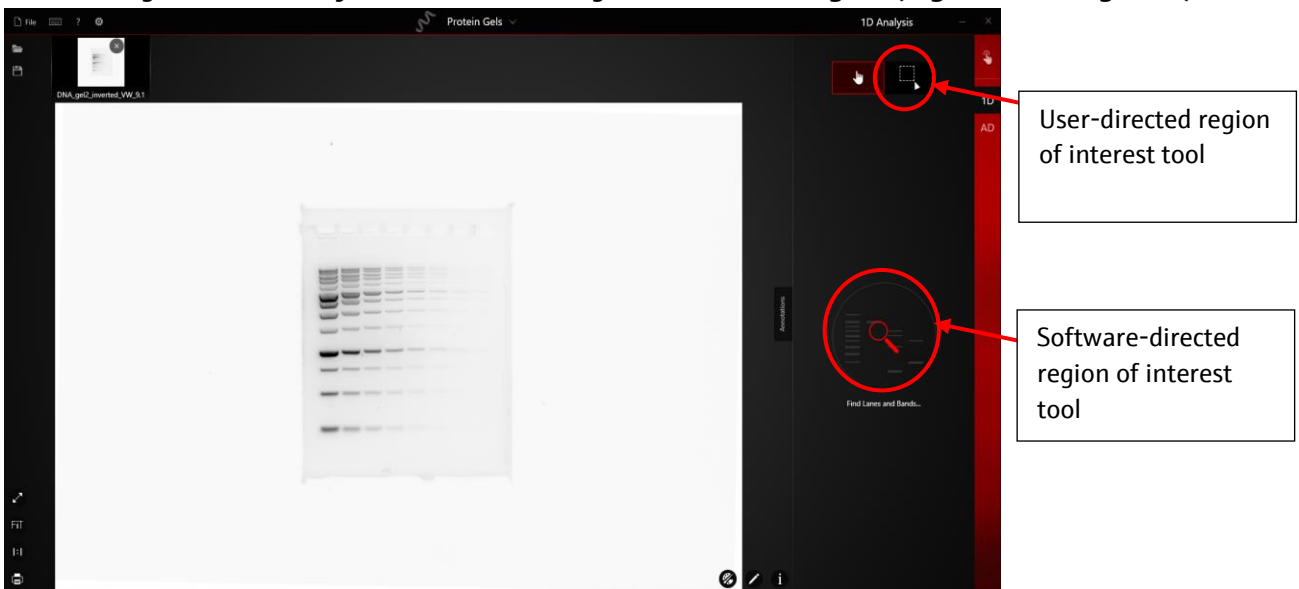


Figure 4. Defining an ROI for 1D Analysis with VisionWorks

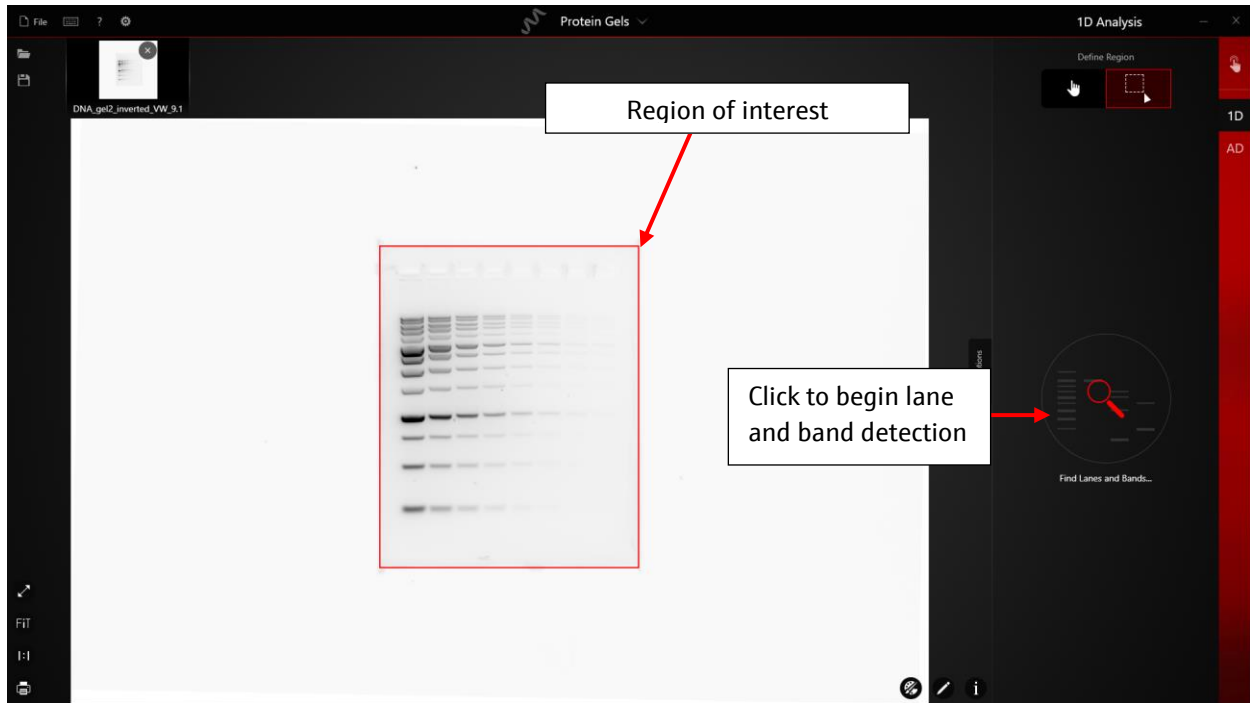


Figure 5. User-directed drawing ROI in VisionWorks

2. After drawing the ROI, the user can click Find Lanes and Bands... to start lanes and bands detection automatically. Lane and band markings will emerge in red after detection (Figure 6).



Figure 6. Detected Lanes and Bands in VisionWorks


Depending on your signal-to-noise, some manual lane and band detection may be required. Below we demonstrate how to add bands.

Adjusting Lanes and bands for 1D Analysis

1. Navigate to the Lanes and Bands editing tool menu (**Figure 7**):



**Figure 7.** Lanes and Bands Editing Tools

2. Use the lanes and bands editing tools to perform fine adjustment for detected lanes and bands.
3. Once complete, tap  to deactivate the selected editing tool.

#### IV. Creating and Exporting a Report

VisionWorks will create a report from the user input. The report can be adjusted to reflect the data most important to the user. *Note: We recommend using the template feature if there are multiple users with different data requirements. In this example we have pre-created a template called **Simplified**.*

1. Select **Report** (**Figure 8**)
2. Select **Export** on the top right (**Figure 9**)
3. And select **Print** or **Export to Excel** (**Figure 12**)

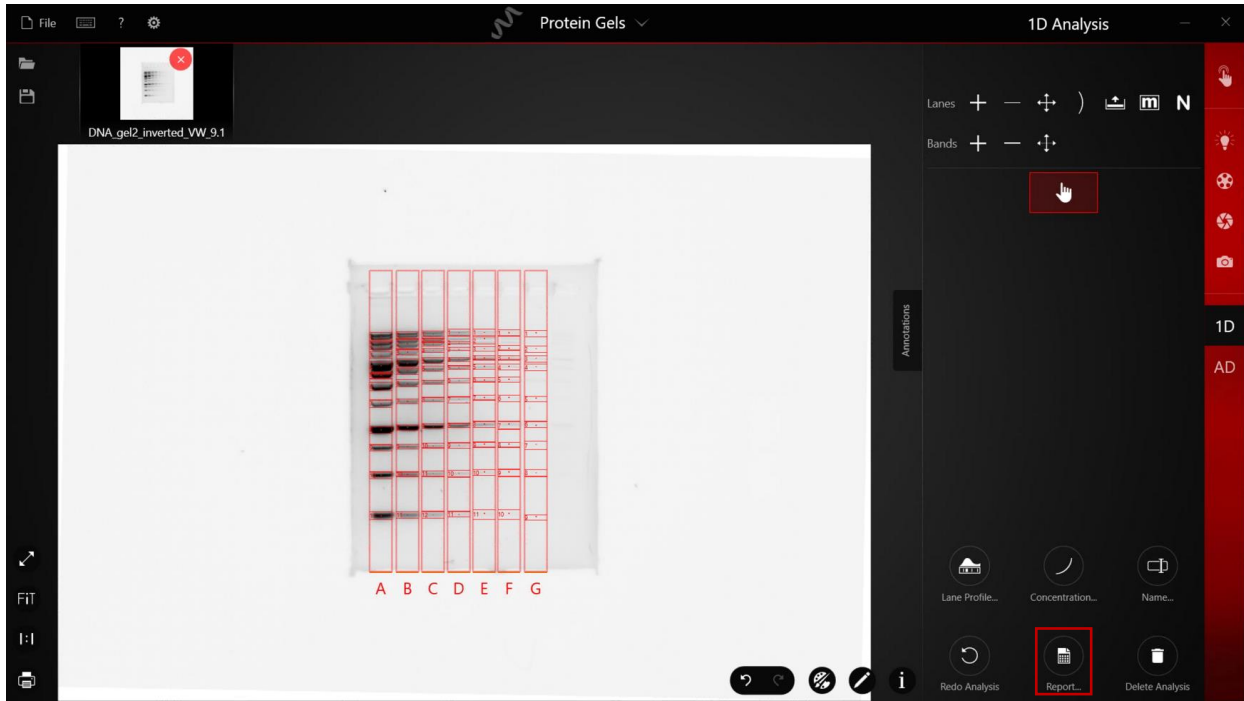


Figure 8. Creating report

**Export feature**

**Pre-created Simplified template**

Create a template by selecting columns of interest and custom naming template by selecting +. See 1D How to Guide for more info.

Image name	Number of Lanes	Background Color	Background Correction	Disc radius	Lanes Constant	Lanes Straight
DNA_gel2_inverted_VW_9.1	8	Black	No background correction		False	True

Lanes							
Lane ID	Width(px)	Start(px)	Length(px)	Mass (ng)	I-Max	I-Vol	C-Max
A	63.00	394.00	700.00	100.00	59,113	2.231E+8	0.00
B	63.00	394.00	700.00	100.00	35,370	1.494E+8	0.00
C	63.00	394.00	700.00	100.00	25,599	8.75E+7	0.00
D	63.00	394.00	700.00	100.00	15,420	4.829E+7	0.00
E	63.00	394.00	700.00	100.00	10,351	3.069E+7	0.00
F	63.00	394.00	700.00	100.00	6,288	2.507E+7	0.00
G	63.00	394.00	700.00	100.00	3,935	1.863E+7	0.00
H	63.00	394.00	700.00	100.00	3,011	5.951E+6	0.00

Bands								
Lane ID	Band ID	Rf	I-Max	I-Vol	I-%	I-Mass(ng)		
A	A1	0.18	26,700	2.183E+7	9.78	9.78		
A	A2	0.21	23,675	1.456E+7	6.53	6.53		
A	A3	0.26	16,054	1.143E+7	5.12	5.12		
A	A4	0.79	47,096	4.911E+7	19.13	19.13		

Figure 9. Report Tool

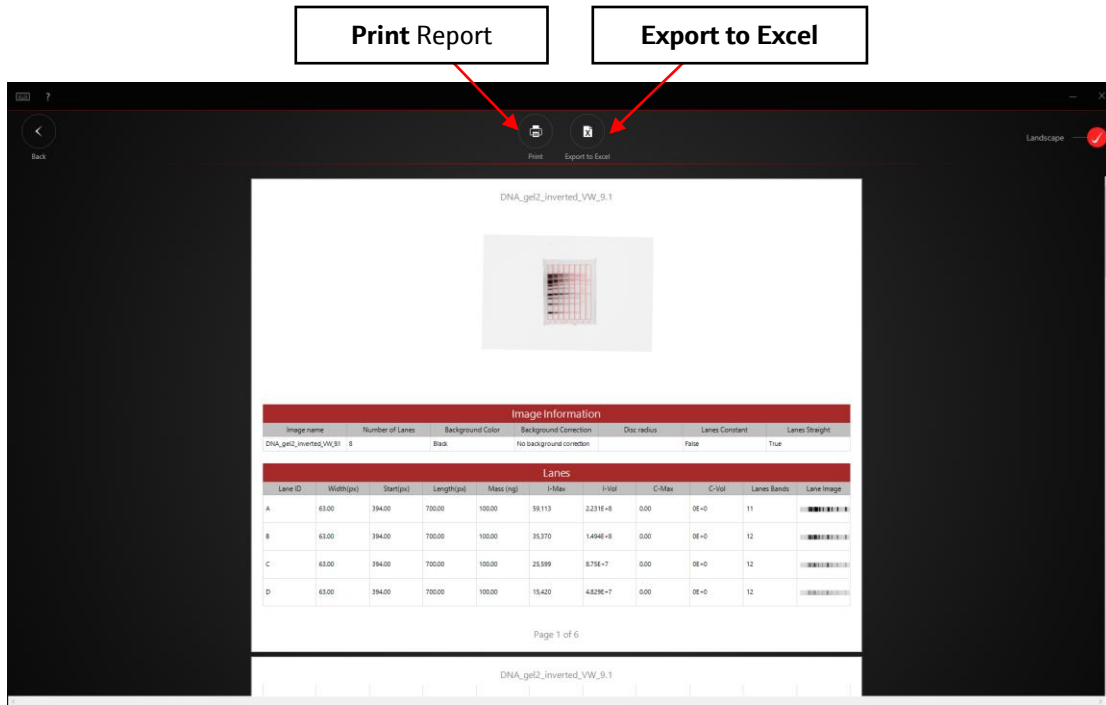


Figure 10. Report Preview

## Appendix C – VisionWorks® How to Guide

### How to do Molecular Weight Calibration

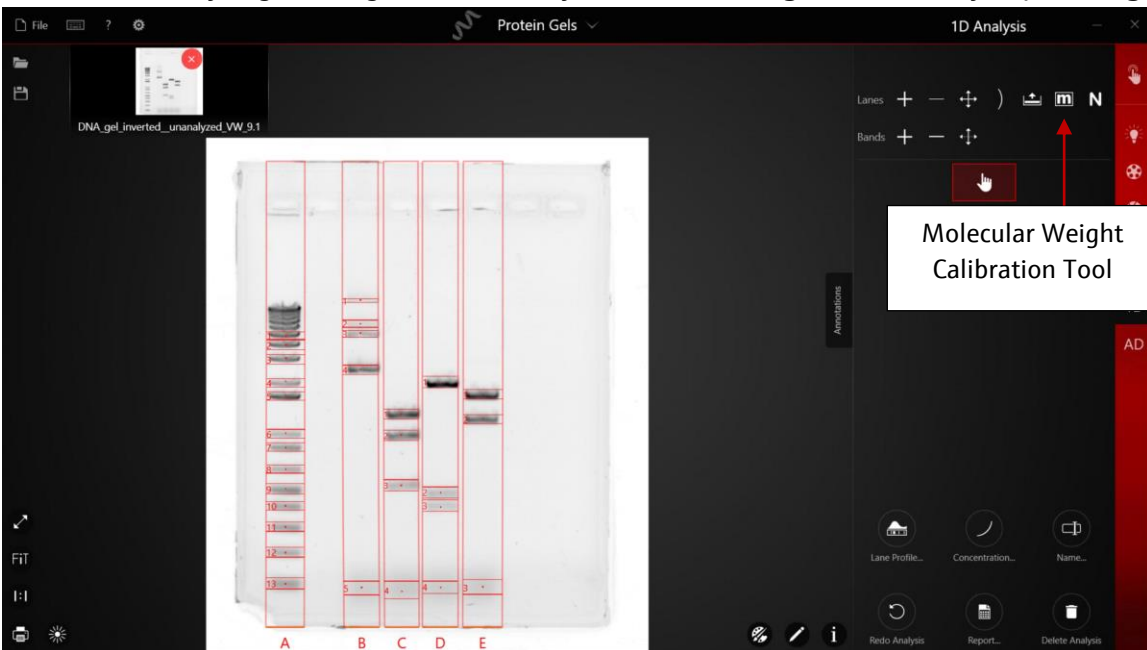
#### Purpose

Below we instruct users how to perform Molecular Weight Calibration on a DNA gel after 1D analysis. This document will cover the major points below.

- I. Identify marker lane
- II. Select desired marker
- III. Calibrate marker using drag and drop tiles and best-fit curve
- IV. View results in report.

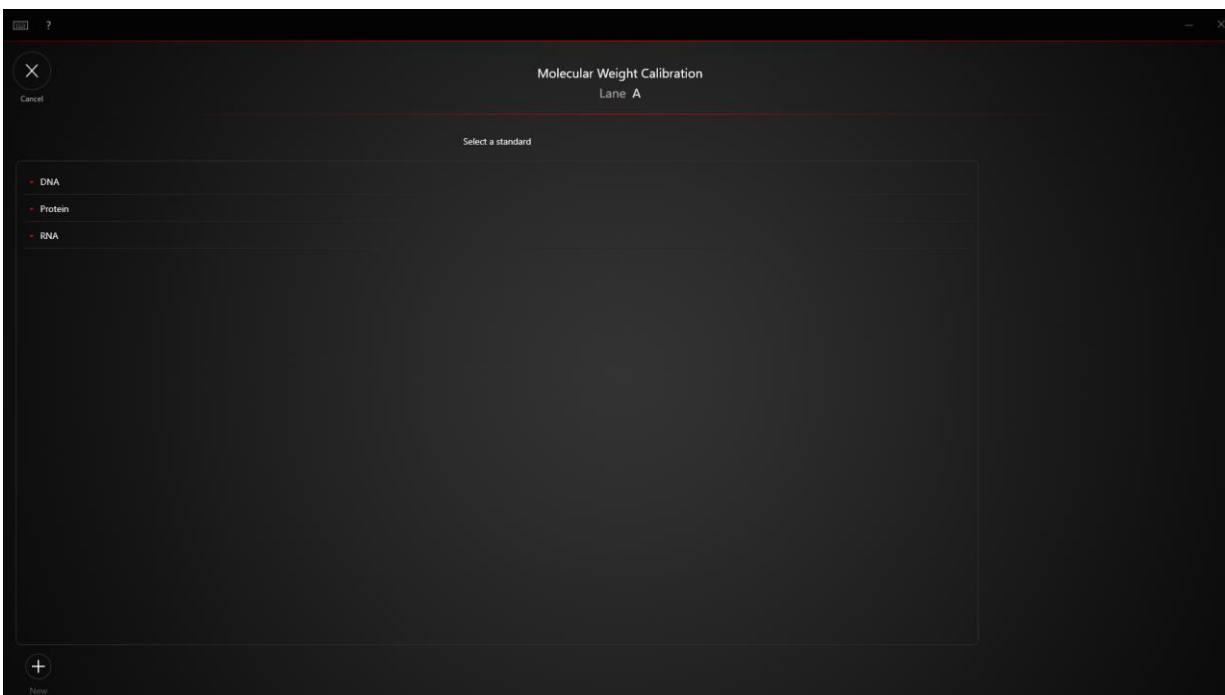
#### I. Identify marker lane

1. After analyzing an image with 1D analysis select **M. Weight** in the Analysis pane (**Figure 1**).



**Figure 1.** Main Viewing in VisionWorks with demo DNA gel image after 1D

2. Next, select your marker lane (**Figure 2**). Once selected, software will automatically switch to the marker selection window (**Figure 2**).
3. Select **Calibrate** (**Figure 2**).

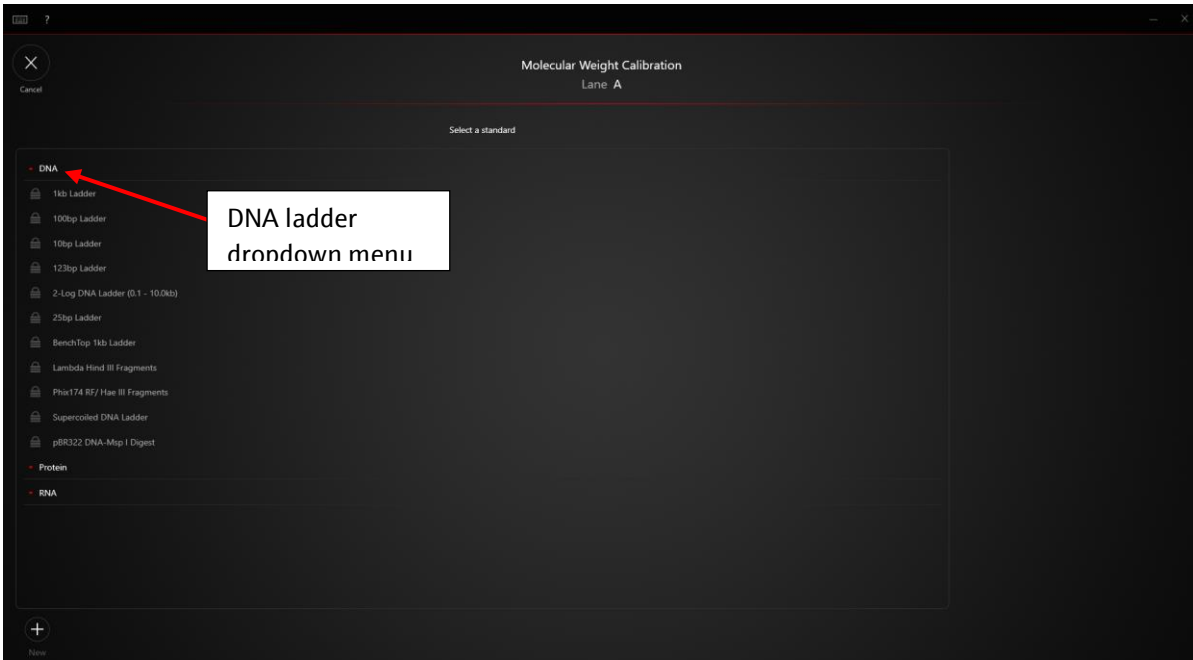


**Figure 2.** Marker selection window.

## II. Select desired marker

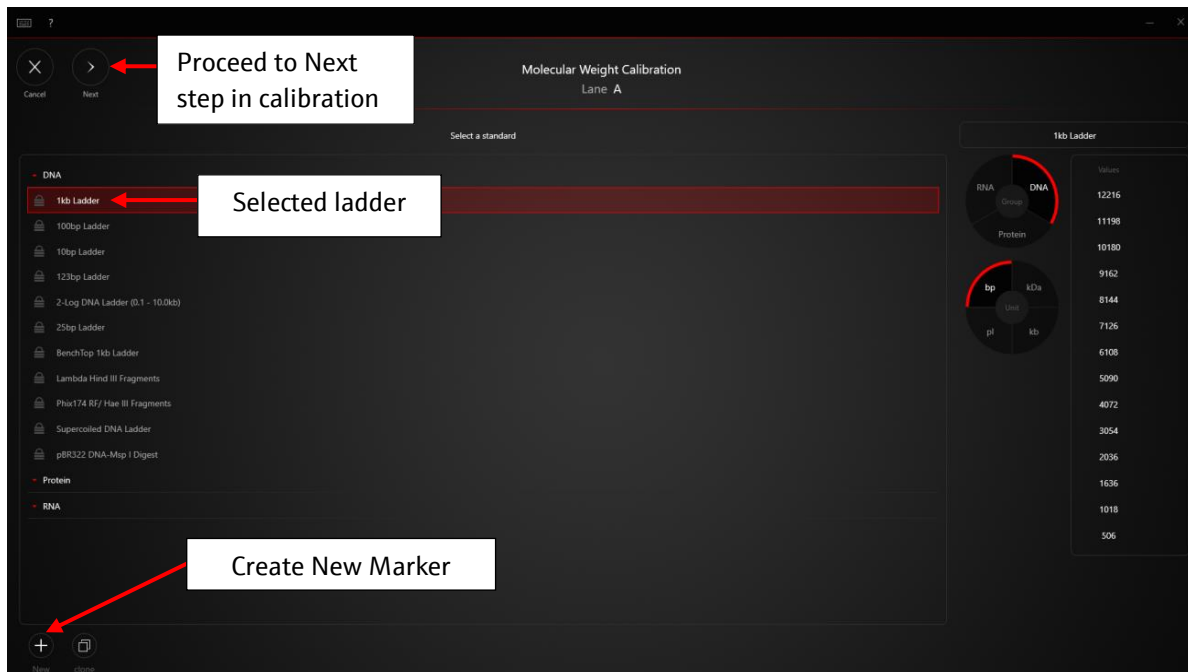
You can choose from several existing markers from the dropdown list in the marker selection window. Alternatively, you can create your own marker by selecting and New and manually entering the name and values of your ladder. Here we use an existing marker.

1. Select DNA dropdown menu (**Figure 3**).



**Figure 3.** Selecting marker for calibration in Marker Window.

2. Select *1Kb ladder* (**Figure 4**). Note: when using an existing marker, make sure the weight values match those listed in your reagent datasheet. Manufacturer's sometimes have similarly name markers. Selecting the incorrect marker will result in incorrect calibration and, in some cases, may cause an error during calibration.
3. Select **Next** (**Figure 4**).



**Figure 4.** Selecting marker for calibration in Marker Window.

4. Move the Drag and Drop tiles on the right side of the screen into the appropriate placeholder (Figure 5 and 6). A regression line will be applied once two Drag and Drop tiles have been placed. By default, a least square line (or linear regression) is applied *Note: While only two point are required to make a line, we recommend using as many bands as possible to get an accurate MWT estimation. For quick estimation we recommend the highest and lowest molecular weight bands as other selections may result in negative values in the case of a linear regression, or undefined values/error message in the case of a quadratic regression (also known as, nonlinear or second order regression).*

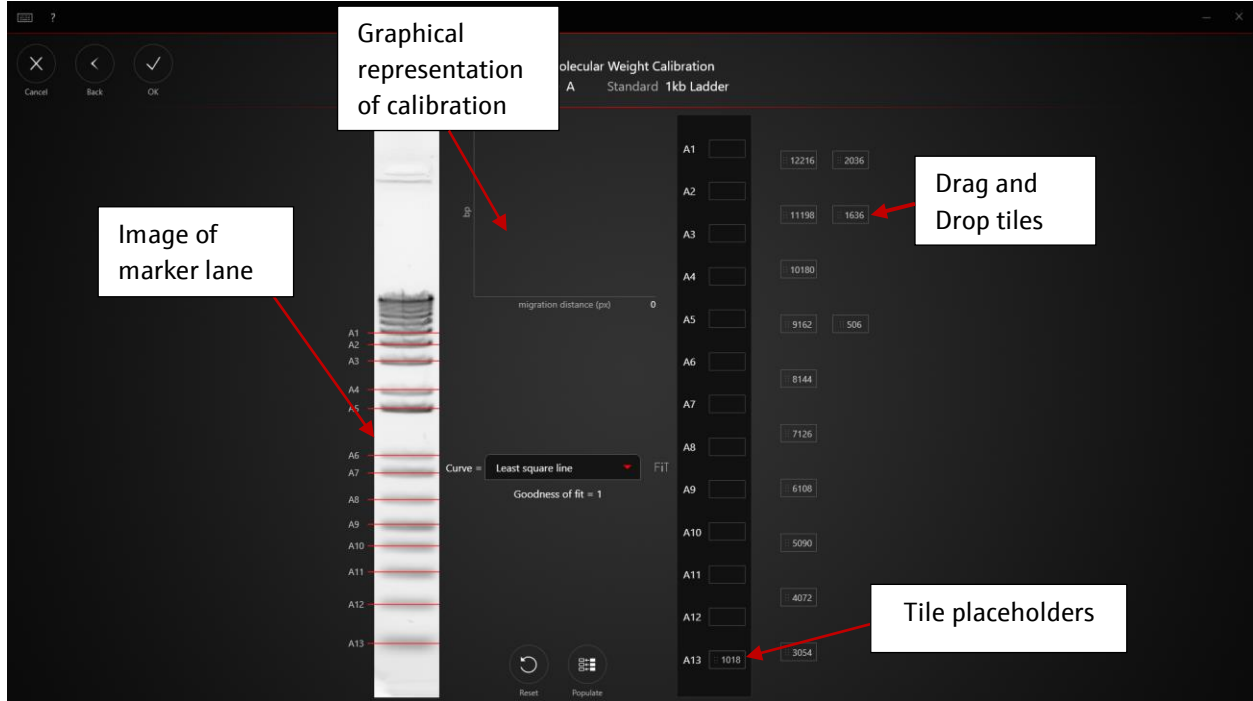
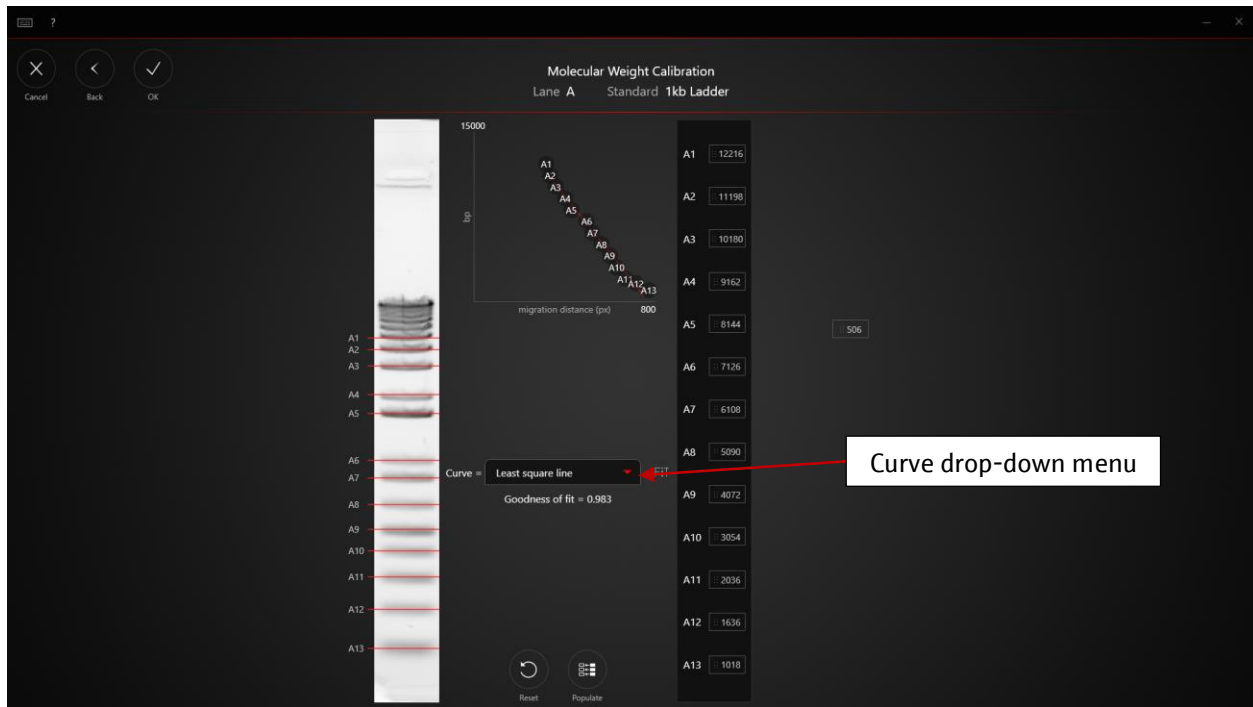
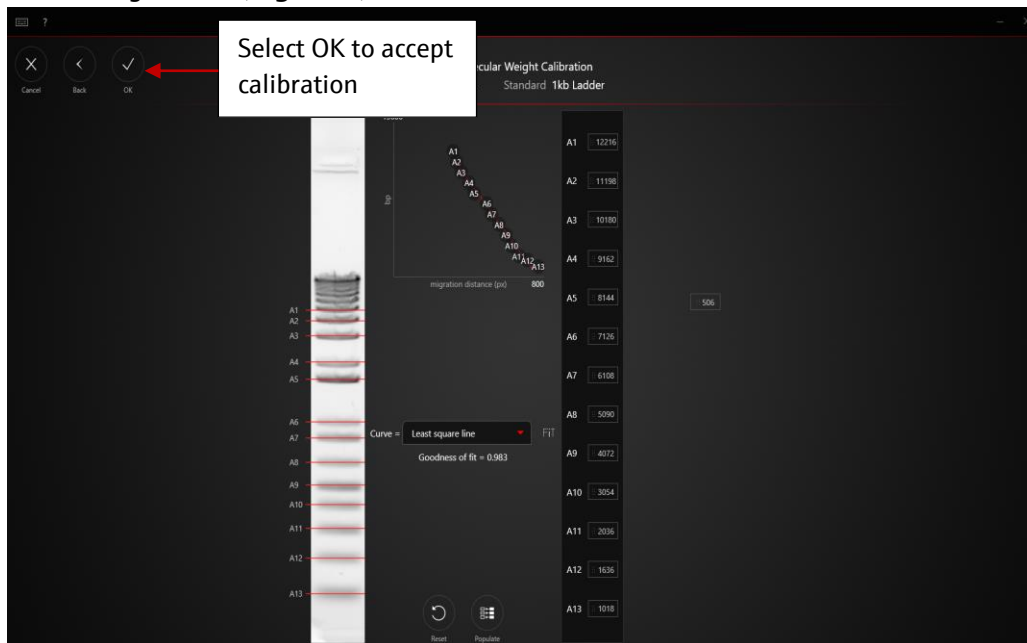


Figure 5. Using Drag and Drop tiles to set calibration in Calibration window.



**Figure 6.** Assigning Drag and Drop tiles to respective tile placeholders.

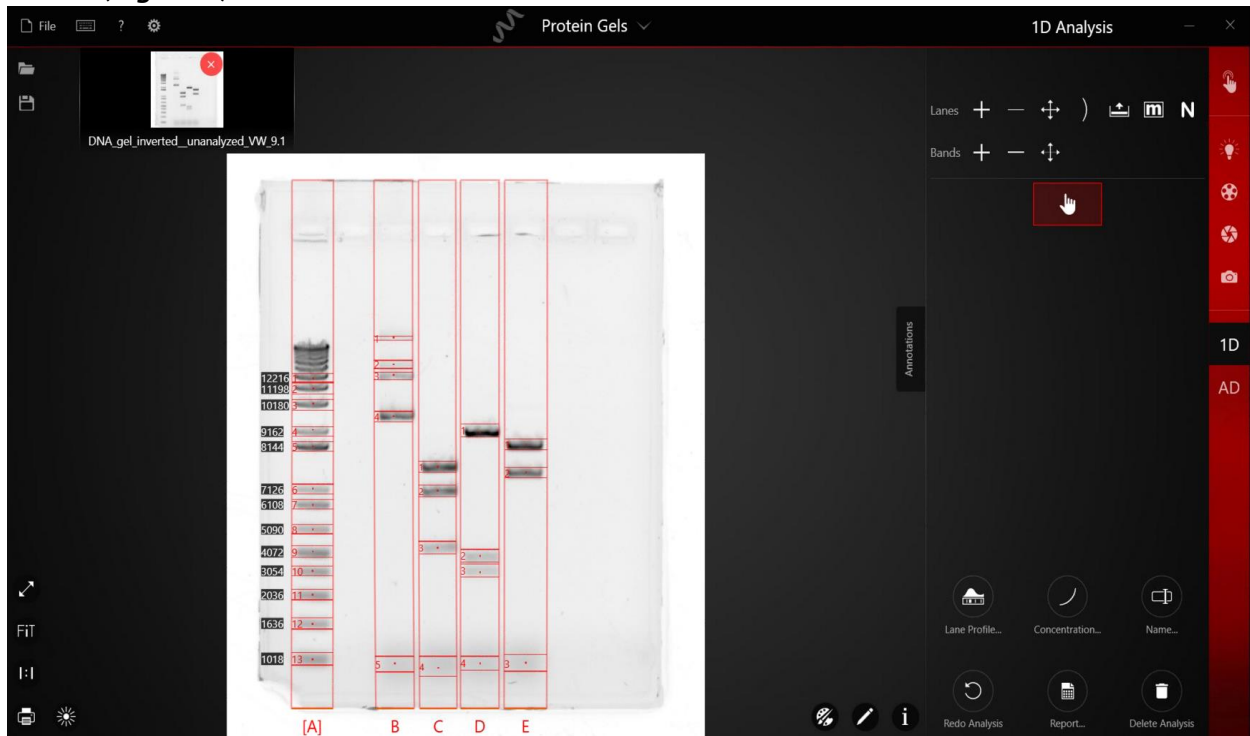
5. Assess the *Goodness of fit statistic*. In most cases, least square quadratic is the better choice for MWT calibration. Select the drop-down menu to apply the least square quadratic regression (**Figure 7**).



**Figure 7.** Changing regression model to apply to dataset.

6. Once you are satisfied with the analysis, select **OK**.

- After calibration, square [] and angle brackets <> will surround the calibrated lane name (Figure 8).



**Figure 8.** Exit to the Main 1D analysis menu after accepting M.Weight Calibration.

- Next, select **Report**. In the *Molecular Weight* tab of the report, all the values will now have calibrated molecular weight values.

Band	A	B	C	D	E
1	12,216.00 bp	17,037.85 bp	7,632.99 bp	9,003.52 bp	8,251.28 bp
2	11,198.00 bp	13,671.99 bp	7,000.36 bp	3,815.90 bp	7,514.01 bp
3	10,180.00 bp	12,532.66 bp	4,226.28 bp	2,989.52 bp	968.41 bp
4	9,162.00 bp	9,661.85 bp	906.01 bp	960.39 bp	
5	8,144.00 bp	960.34 bp			
6	7,126.00 bp				
7	6,108.00 bp				
8	5,090.00 bp				
9	4,072.00 bp				
10	3,054.00 bp				
11	2,036.00 bp				
12	1,636.00 bp				
13	1,018.00 bp				

## Appendix C – VisionWorks® How to Guide

### How to do Area Density Analysis

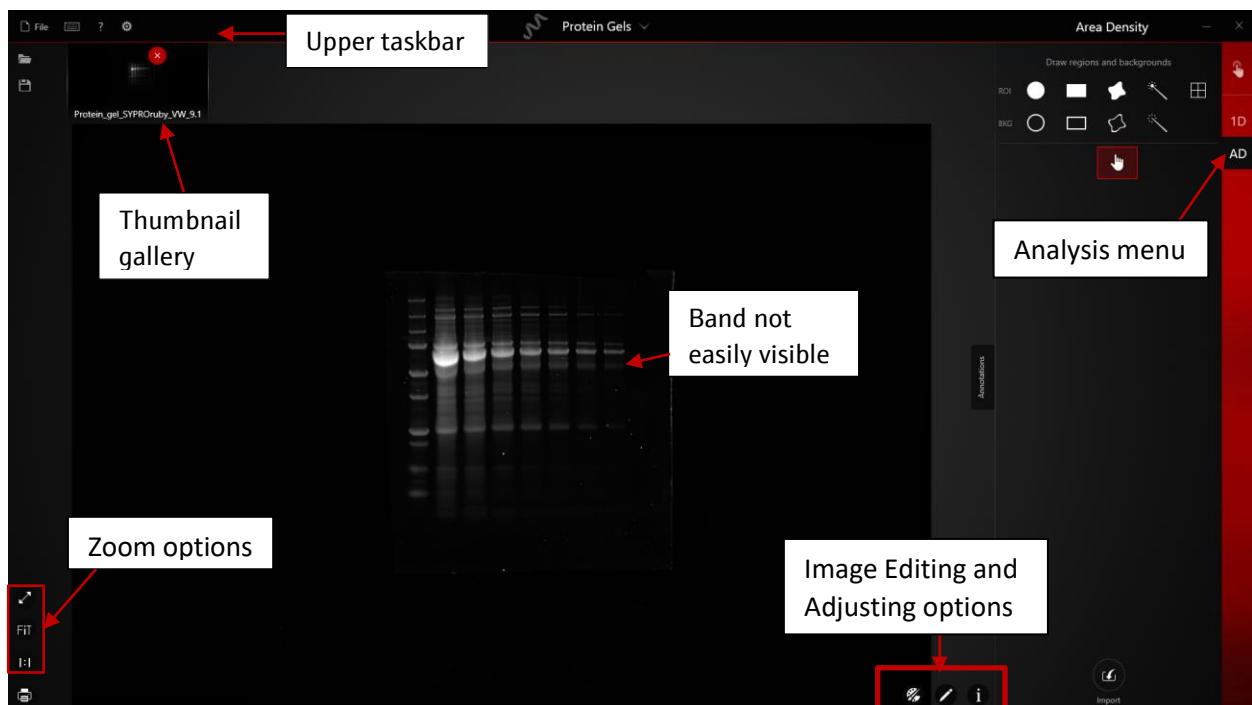
#### Purpose

Below we instruct users how to perform Area Density using VisionWorks Software. The following topics are covered.

- I. Adjust Image Appearance
- II. Perform Area Density
- III. Creating and Exporting a Report

#### I. Adjust Image Appearance

In VisionWorks, we have several features that aid in visualization, but do not modify the image metadata. After capture, your image will appear in the Main viewing window (**Figure 1**). From this viewing window you have access to all features of the software. Most image manipulation functions that aid in visualization are in the **Image Editing and Adjusting** options. Zoom features are accessible in the lower taskbar. Below, we demonstrate the pseudocolor feature, which is especially useful when trying to demarcate the margins of bands as well as seeing bands with very weak signals.



**Figure 1.** Main Viewing in VisionWorks

1. Navigate to the **Histogram Adjusting** tab → **PColor** options → and select your pseudocolor preference. Here we selected **Intensity Color Bar** (**Figure 2**).

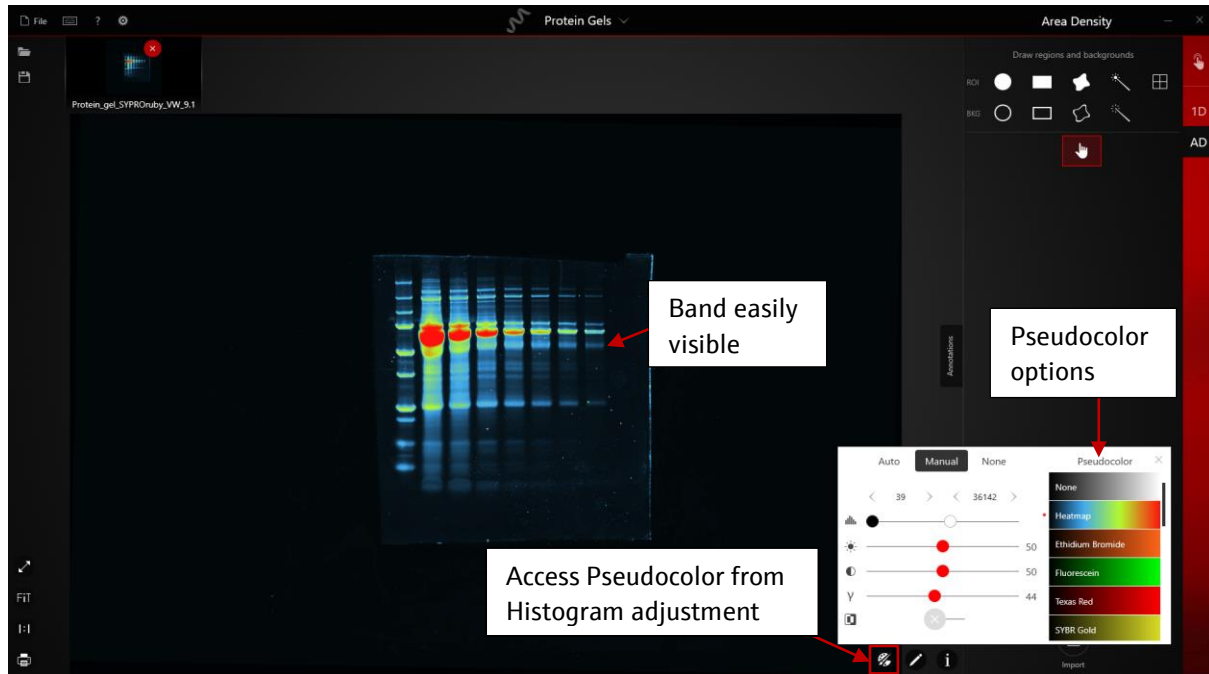


Figure 2. Pseudocolor image in VisionWorks

For additional image manipulation features to aid in visualization, see **1D analysis How-To Guide**.

## II. Perform Area Density

1. Navigate to **AD Analysis** → select drawing tool from the ROI menu to add new region.

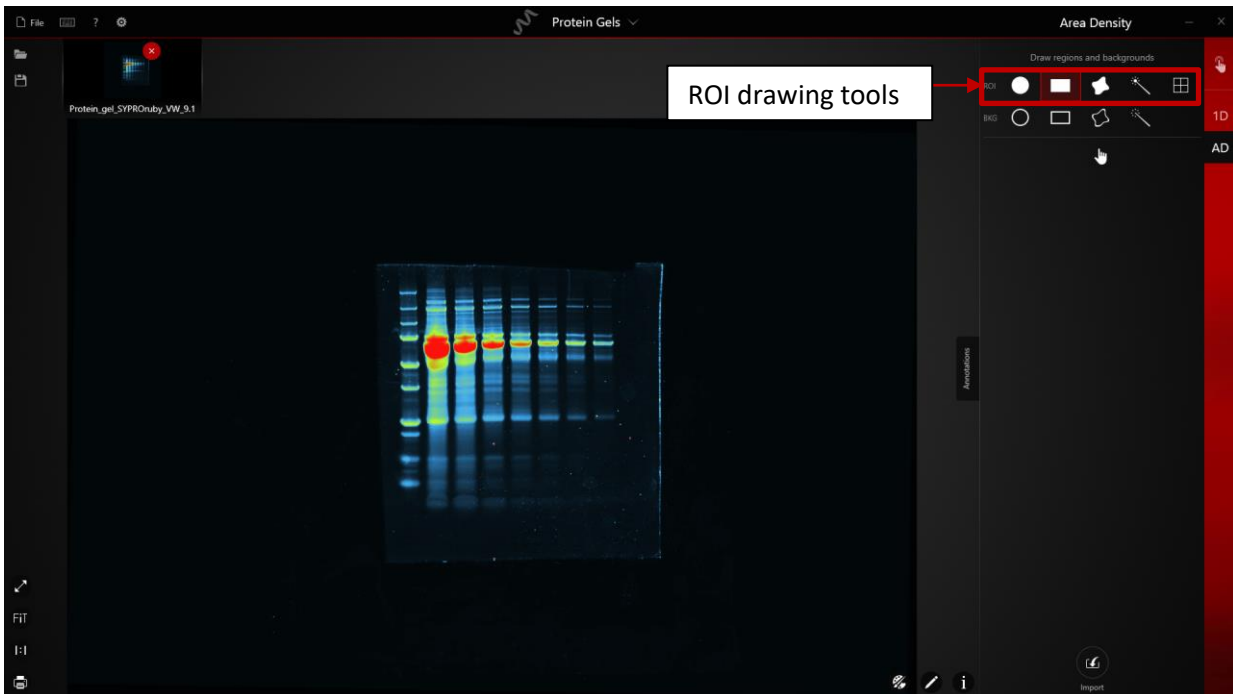
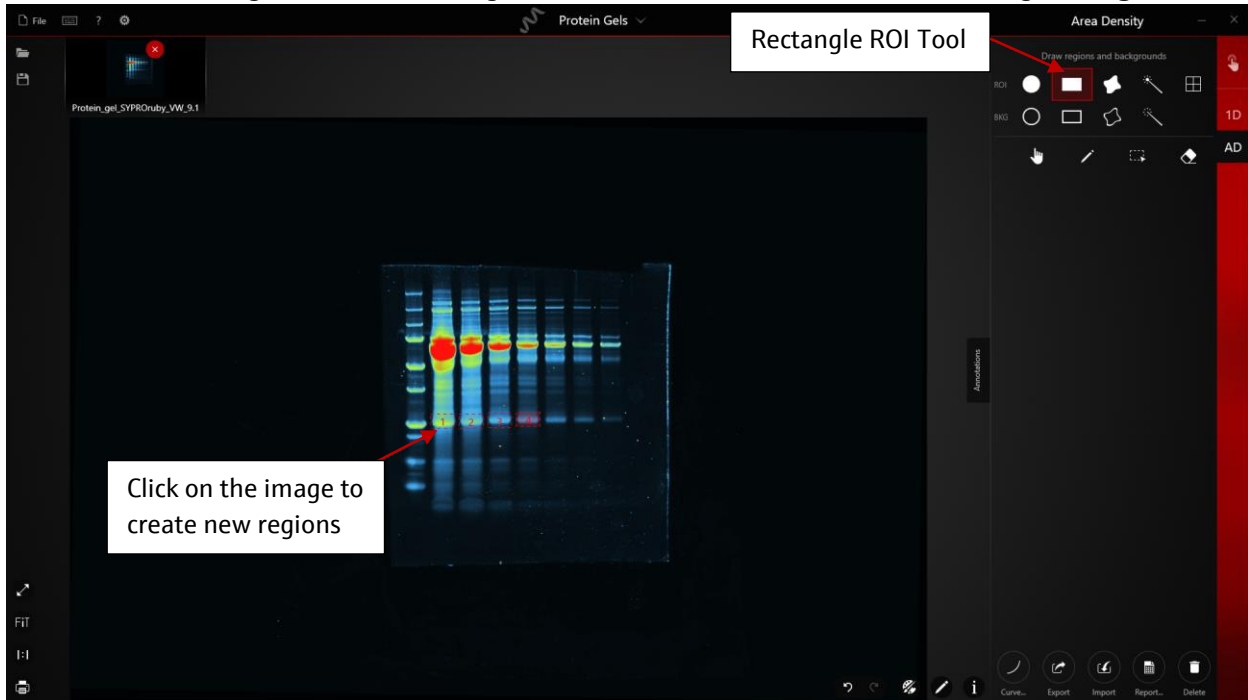


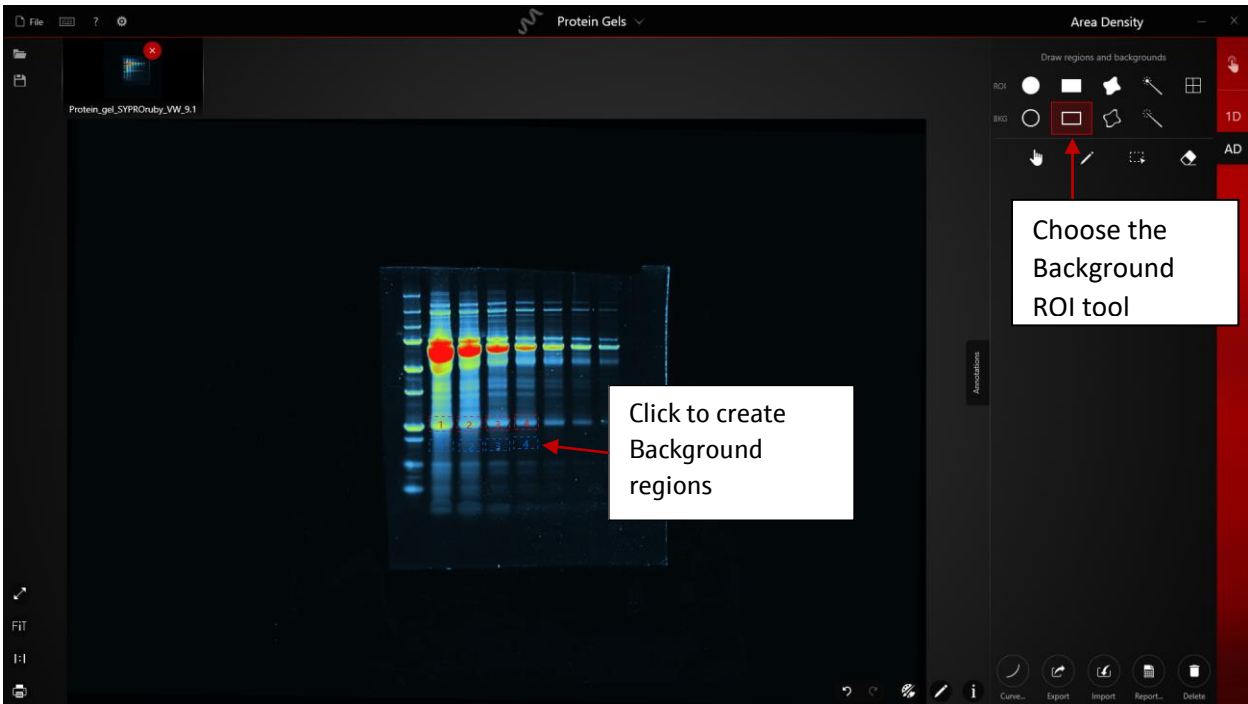
Figure 3. Performing Area Density Analysis

2. Click on the image to create new regions that is identical to the first created region (**Figure 4**).



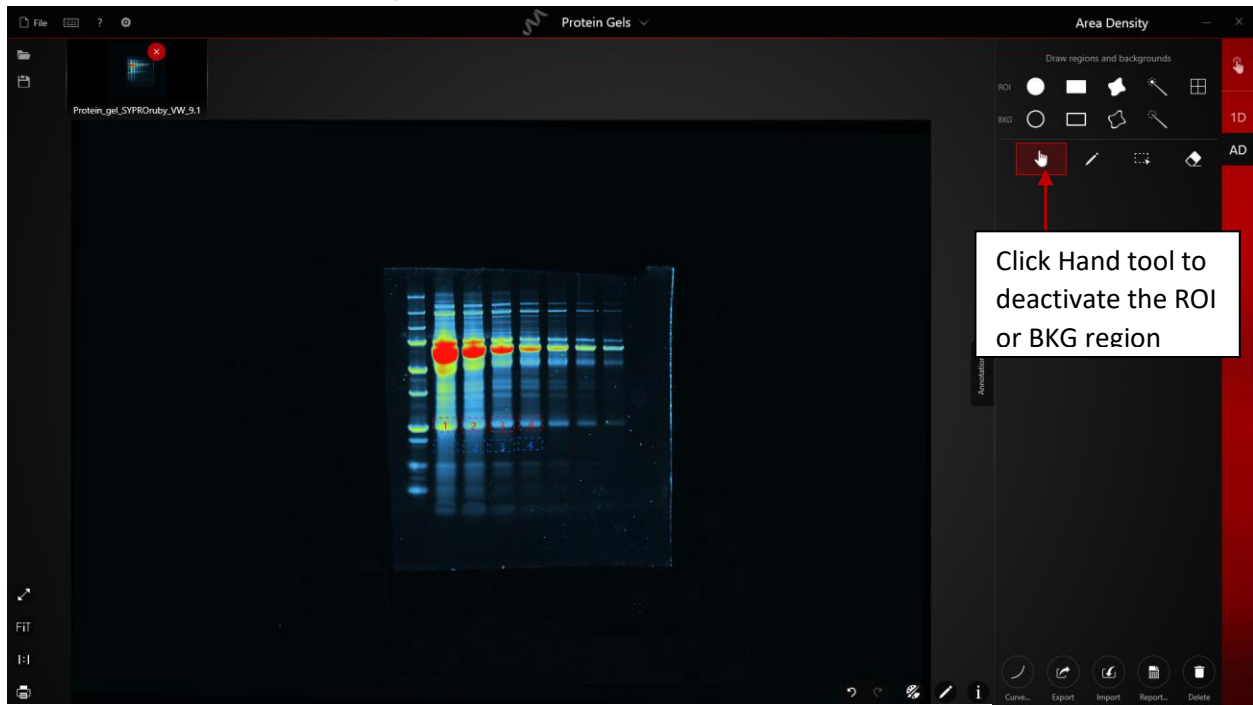
**Figure 4.** Defining an ROI for Area Density Analysis with VisionWorks

3. After you select the ROI, you can manually add a single global background ROI or several to get the local background subtraction (**Figure 5**).



**Figure 5.** Click/Tap Background ROI for Area Density Analysis in VisionWorks

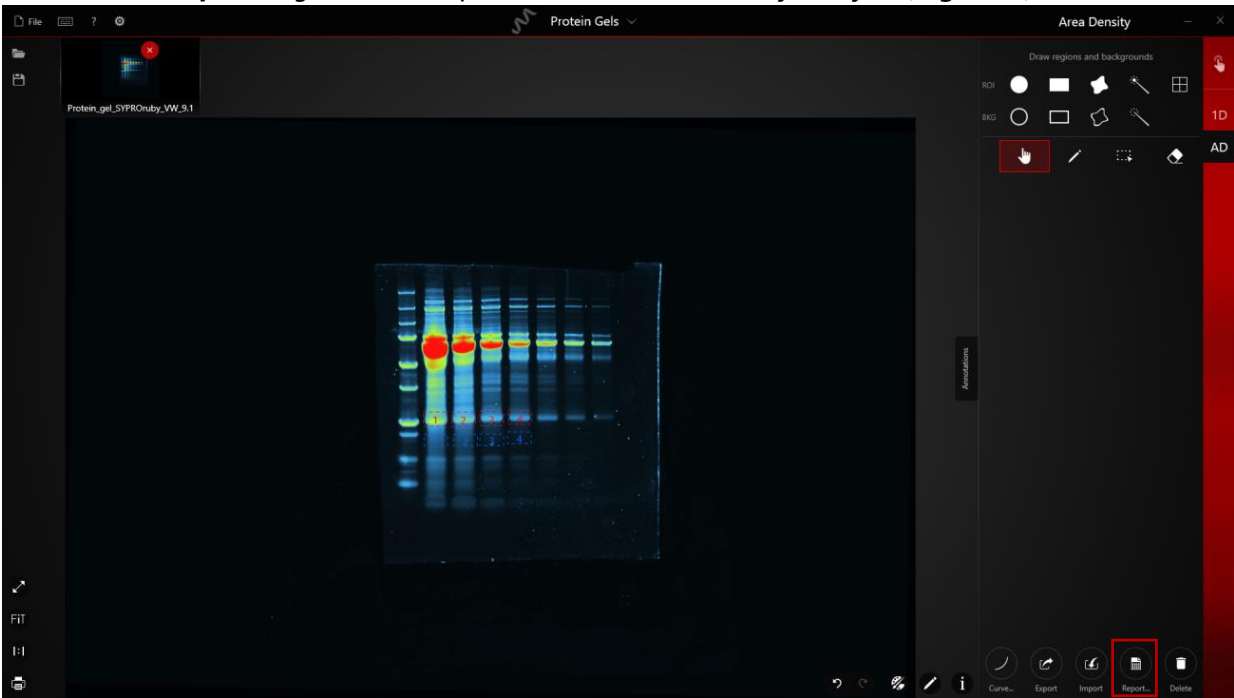
4. After the final ROI is drawn, click **Hand** tool to deselect the ROI tool.



**Figure 6.** Deactivate the ROI tools for Area Density Analysis in VisionWorks

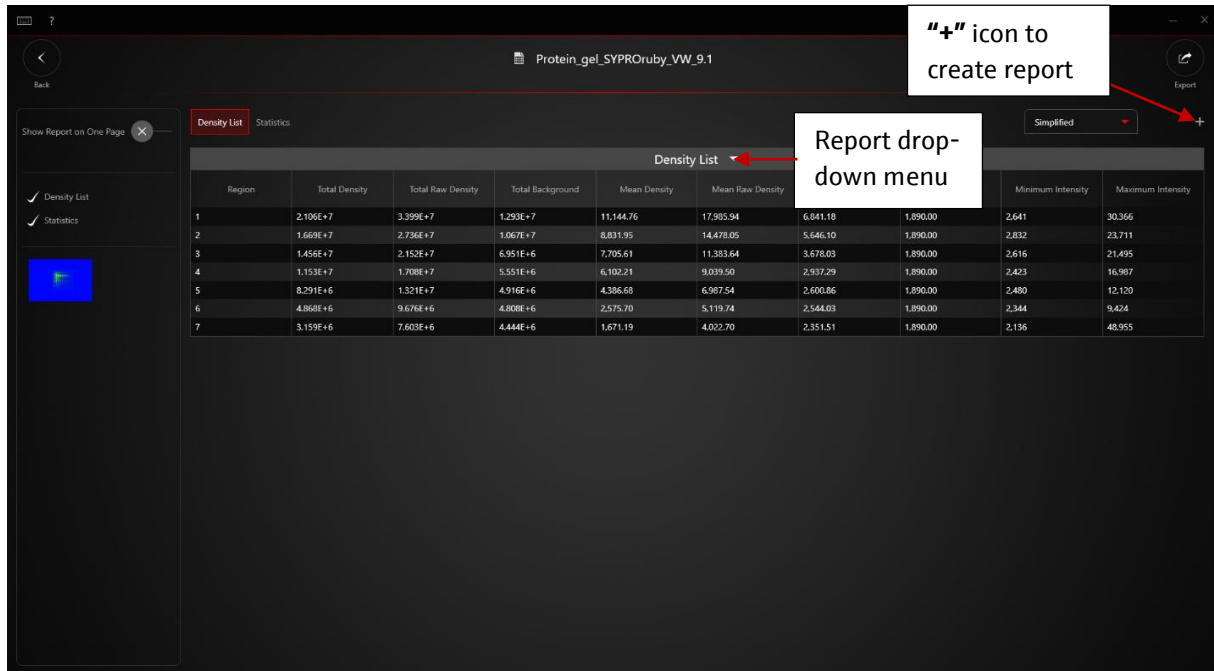
### III. Creating and Exporting a Report

1. Select **Report** to generate a report from the Area Density analysis (**Figure 6**).



**Figure 6.** Generating Area Density Report in VisionWorks

2. Select/Deselect columns of interest from the Report drop-down menu (**Figure 7**).



**Figure 7.** Lane and Band Detection in VisionWorks.

- Your preferences can be saved in a custom named template by selecting the **+** icon after selecting your columns of interest in step 2. Here we created a templated called *Simplified* (Figure 8).
- Lastly, you can export your report by selecting **Export** (Figure 8) and subsequently Print/Print to File/or export to Excel (Figure 9).

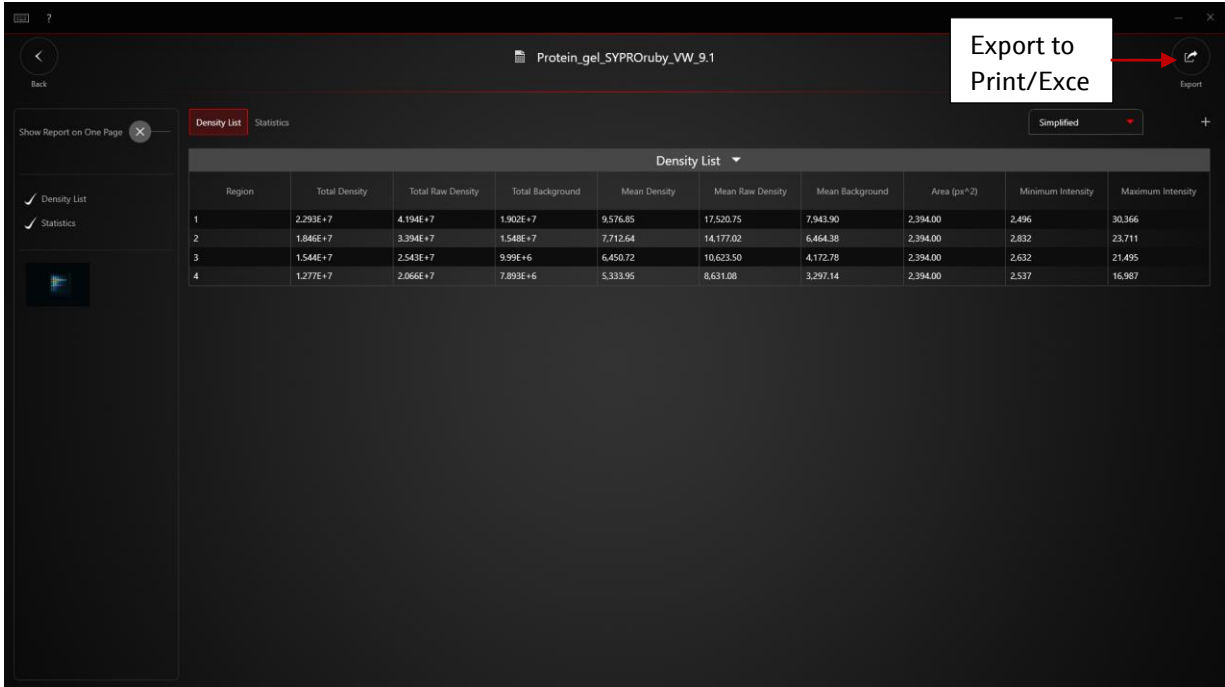


Figure 8. Exporting Report

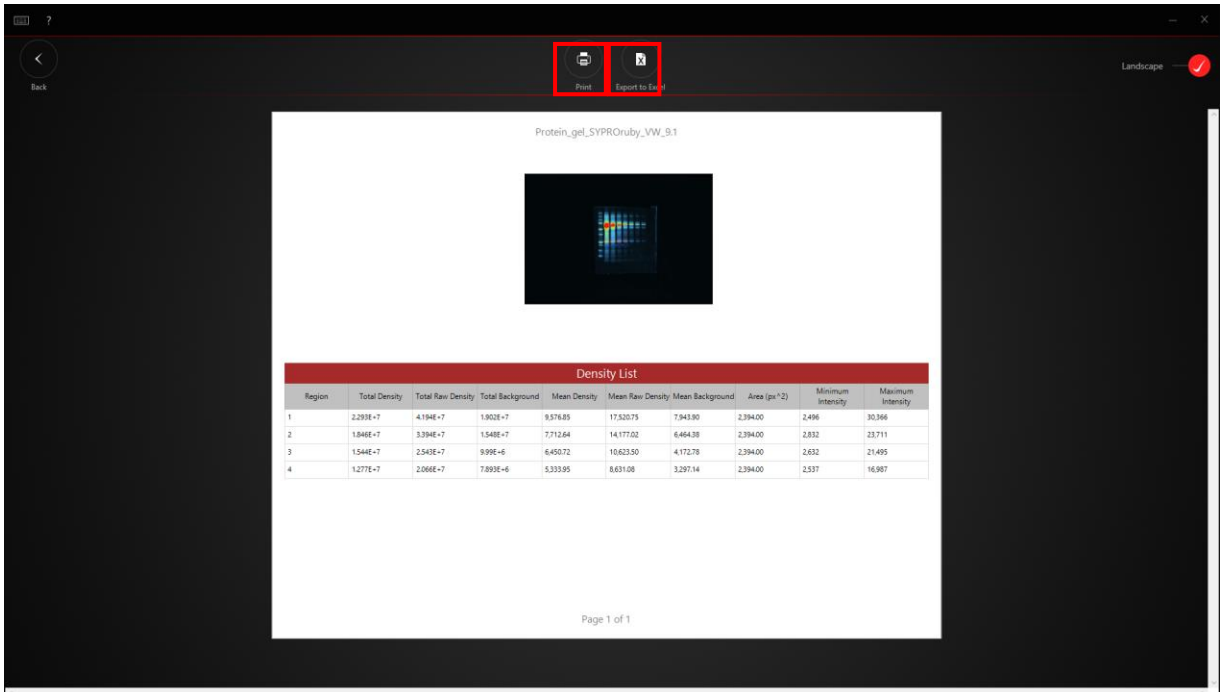


Figure 10. Print Report or Export to Excel

## Appendix C – VisionWorks® How to Guide

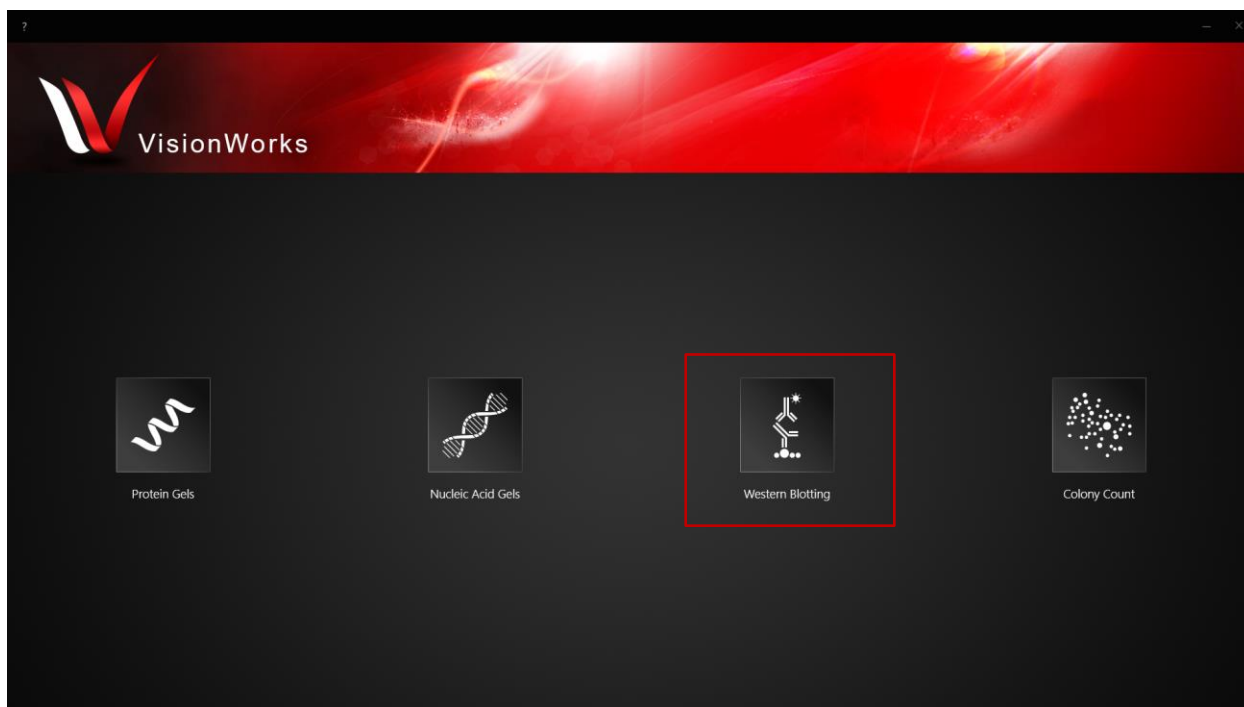
### How to Capture Blot Images

#### Purpose

Below we instruct users how to perform chemiluminescent blot imaging using VisionWorks Software.

#### I. Manual capture of chemiluminescent image

For Chemiluminescent Blot application, please choose the **Chemiluminescent Blot** icon from the Home Screen (**Figure 1**).



**Figure 1.** VisionWorks Home Screen

After selection, the camera will be set to higher sensitivity settings: 3x3 Capture binning and 6x6 Live View binning. If you wish to capture Chemiluminescent blot using manual settings, please access the Manual setting panel by clicking the sliding arrow on the right side (**Figure 2**).

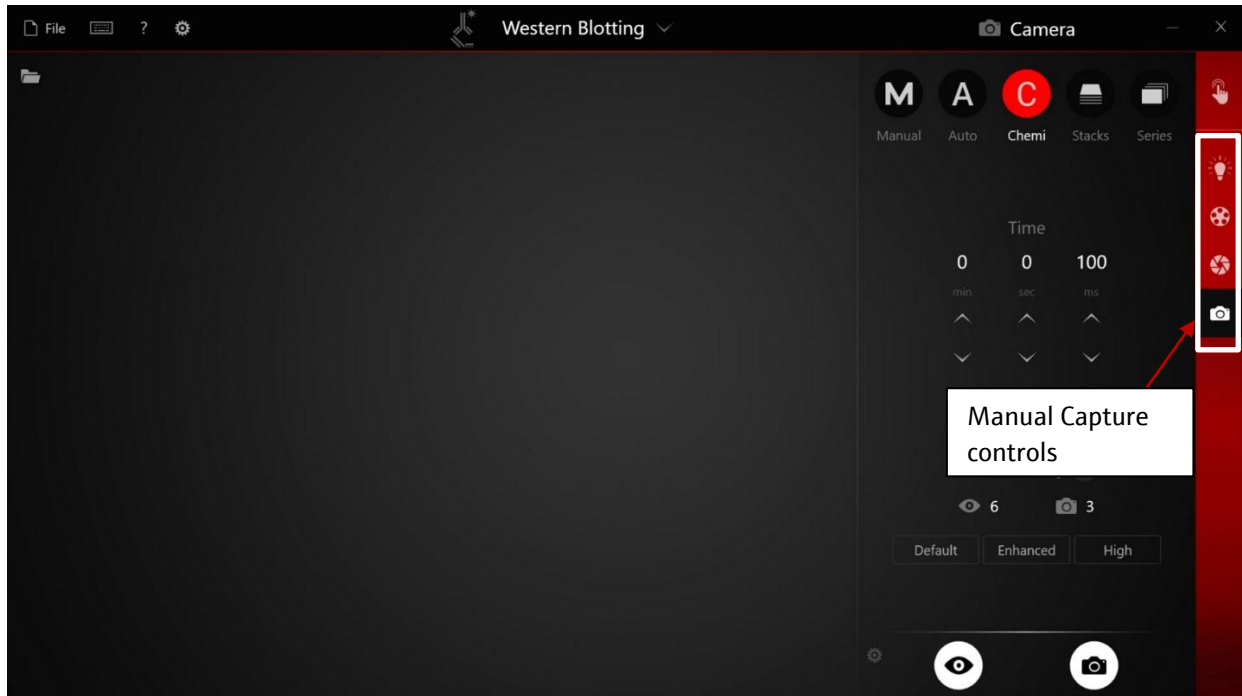


Figure 2. Chemiluminescent Blot Manual Capture Settings

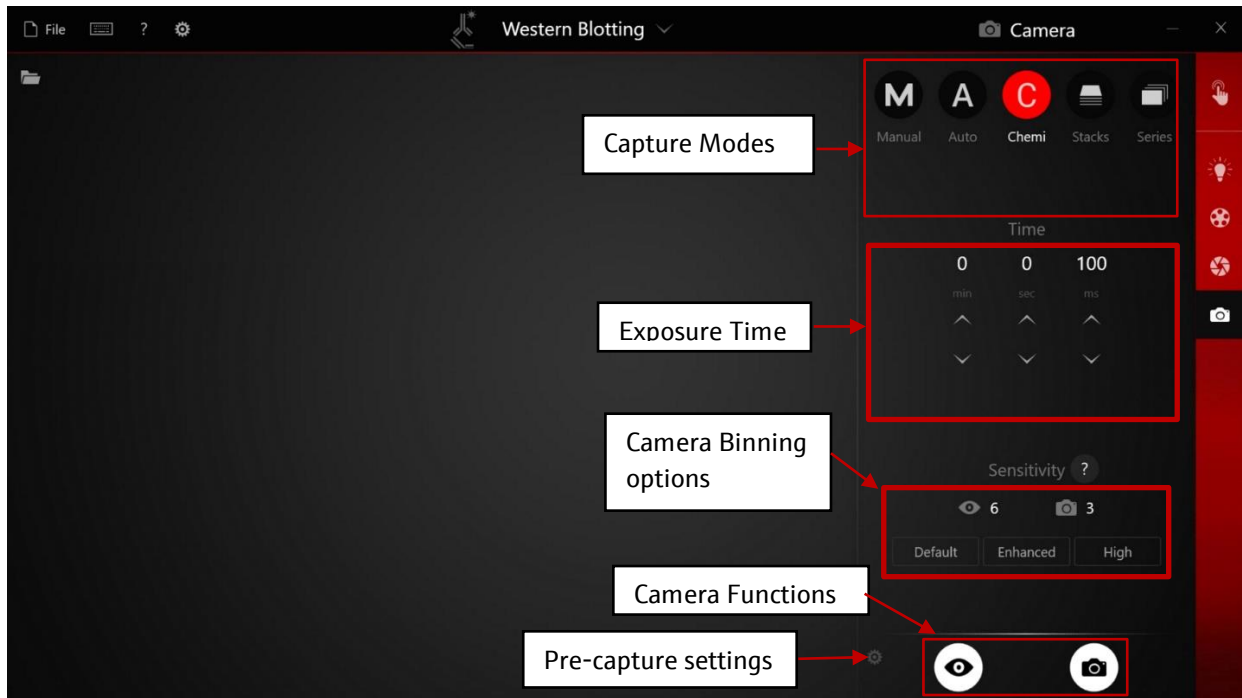


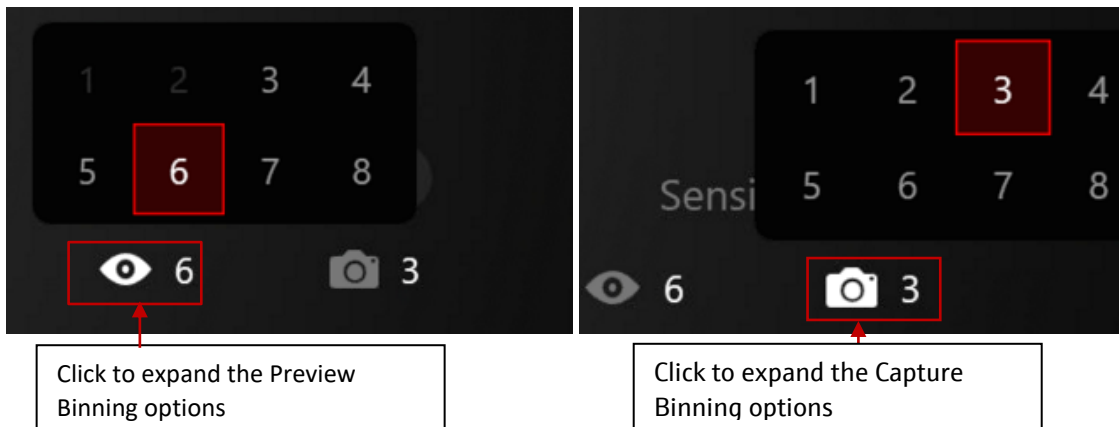


Figure 3. Manual Control Panel

Please follow the steps below to start adjusting hardware settings:

1. Select "C" (Chemi) capture mode from the Capture mode panel.
2. Start Preview by clicking 
3. Navigate to the Lens tab by clicking  if image is not focused.
4. Adjust exposure time to 30sec to see if Chemi Signal is visible.
5. If the signal is clear and bright in Live View, click **capture**.
6. If the signal is weak, increase exposure time to a higher number. Usually the Chemiluminescent image takes ~30sec to 5min to capture depending signal intensity.
7. Increase the Capture Binning to increase chemi signal intensity if needed (**Figure 4**).



**Figure 4.** Click to access the preview and capture binning options.

**Note:** Higher capture binning will increase the camera sensitivity, but lower the image resolution.

8. After clicking Capture, two images will be captured: Chemi Signal image and a White light Image. The composited Chemi image will be displayed in the image viewing window as shown in (**Figure 5**).
9. To adjust the blend mode and chemi signal threshold, please see **Section 5.2**.



Figure 5. Captured Chemiluminescent image