

**S A B I A**

**Version 3.0**

**Software**

**User Manual**

**EBIOGEN Inc.**

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# I. Introduction

## 1. Overview of SABIA (Solution for Automatic Bio-Image Analysis) Software

SABIA (Solution for Automatic Bio-Image Analysis) is an analysis software for cell and tissue image which comes from microscope and other scientific instruments. It calculates automatically cell confluency, cell number, a ratio of different characteristics, area, and length by using color difference. Numerical output makes it easier for researcher to distinguish accurately the experimental results.

## 2. Major Function

Function	Contents
Cell Confluency	Calculation automatically cell confluency from cell image Real time image capture and to analysis
Cell Counting	Counting automatically cell number Measurement of each cell length Calculation of aggregation ratio
Image Analysis	Comparison the ratio of characteristics of tissue Measurement of length and area of cell or tissue

## 3. Computer Specifications

- 1) Microsoft Window XP or above (recommend Window 7 or above)
- 2) Dual-core or higher processor and more than 4GB main memory
- 3) 1600\*900 or higher resolution
- 4) Necessity of Internet connection depending on version.
  - (1) License renewal version: check to connect internet every operating program.
  - (2) Free-trial version: check to connect internet every operating program

## 4. Purchase Software and Technical Support

**E BIOGEN Inc.**      Tel : 82-2-3141-0791      Email : service@e-biogen.com

## II. Install

### 1. Software Download

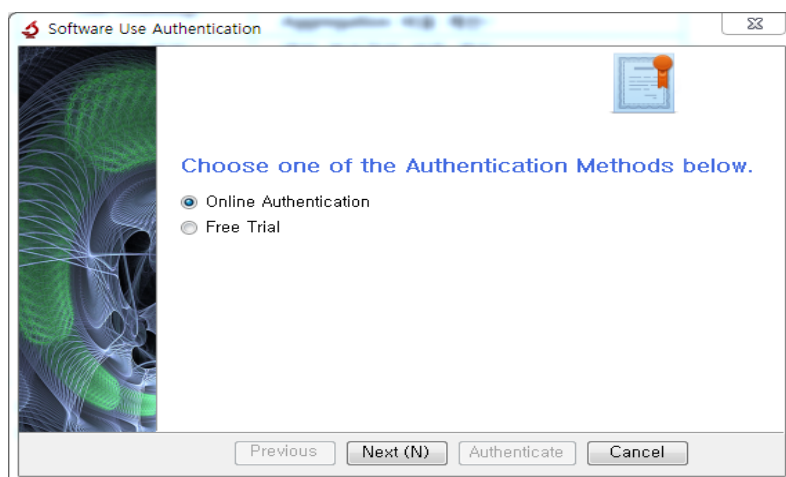
Visit EBIOGEN's homepage.

[www-e-biogen.com](http://www-e-biogen.com) > PRODUCTS > Software & Data Analysis > SABIA Software Download

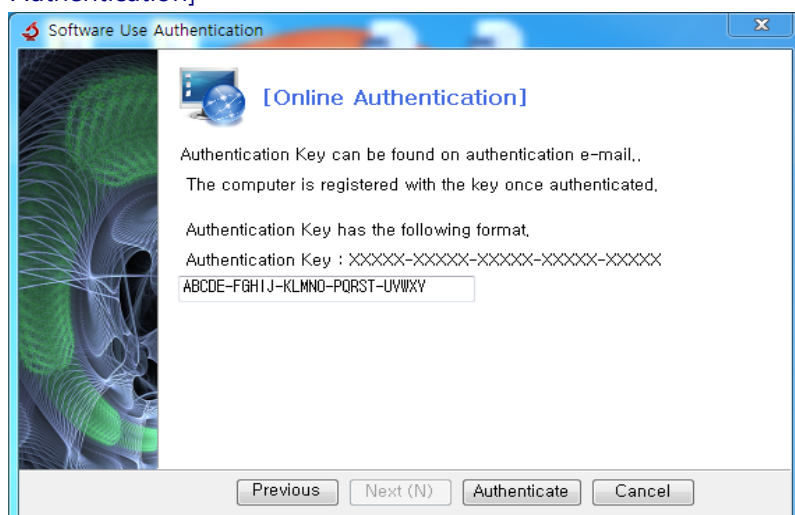
### 2. Installation and Authentication

Open an installation file, then execute 'exe'. Choose between **Online Authentication** and **Free Trial**. Click **Next**.

[Select Authentication Method]



[Online Authentication]



- 1) Enter 25 digits of authentication key that you received when you purchased the software. Click **Authenticate**.
- 2) Please check your Internet connection before Online Authentication.

[Free Trial]

Software Use Authentication

**FREE Trial** [Free Trial]

Company/Organization :

Name :  Tel :

E-mail :

Authentication Key :

✘ Email with Authentication Key will be sent to the email address above.

✘ MAC Address :

✘ If Free Trial Authentication is not available, copy the MAC Address above and send it to [service@e-biogen.com] with Name, E-mail, and Name of Company/Organization. We will promptly respond to it with Free Trial Authentication Key.

- 1) 15-day free trial is available before purchasing SABIA software
- 2) Enter the information about Company/Organization, Name, Tel, and E-mail. Click **Request an authentication key by e-mail**. Authentication key will be sent to e-mail address you entered above.

**[ATTENTION]** It might be fail to get the authentication key when using company or organization's e-mail because of a security. Try it again with a personal e-mail. If it is still fail despite trying above mentioned way, please contact us with copied MAC address and your information (service@e-biogen.com).

# III. Calculate Cell Confluency

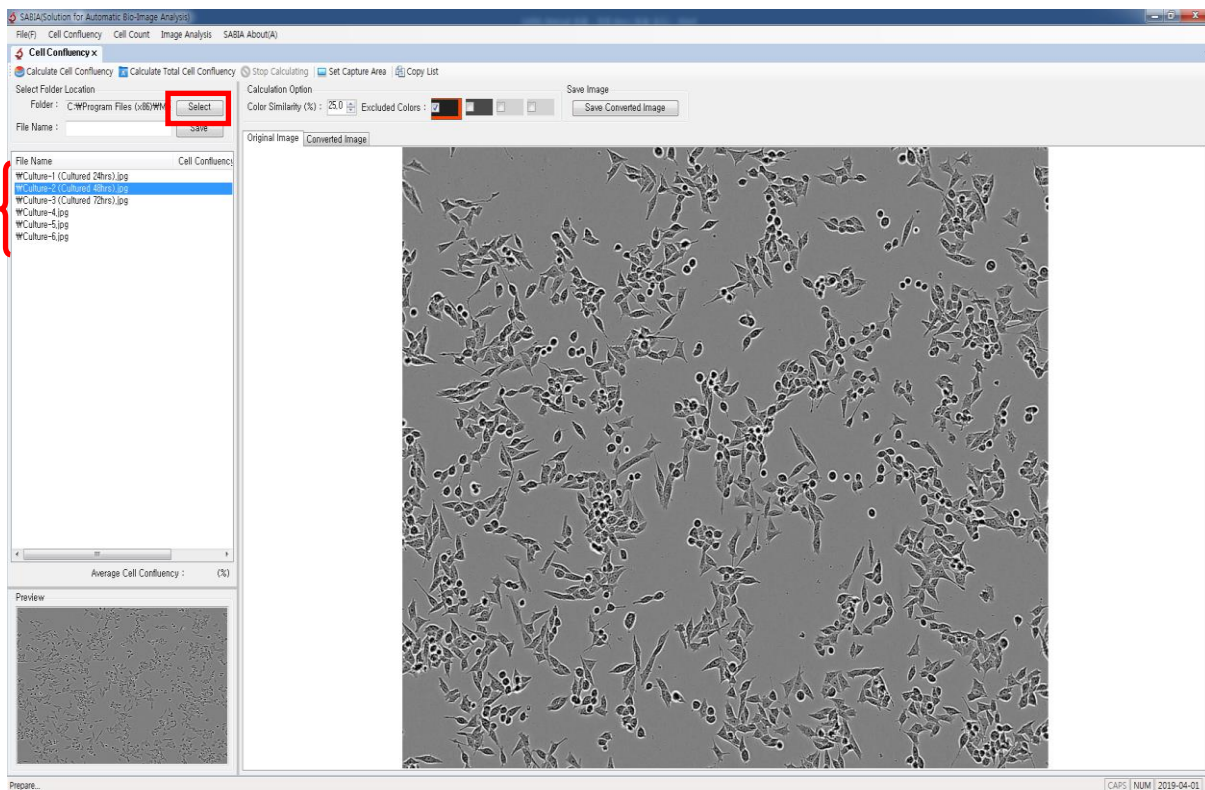
## 1. Overview

SABIA calculates automatically cell confluency on the cell culture dish by using color differences. It produces the numerical output and is helpful to achieve an optimal reproducibility of results. There are two ways for calculating cell confluency. Either saved images or captured images in real time can be used. A tool for capture image is useful when you use microscopy.

## 2. How It Works

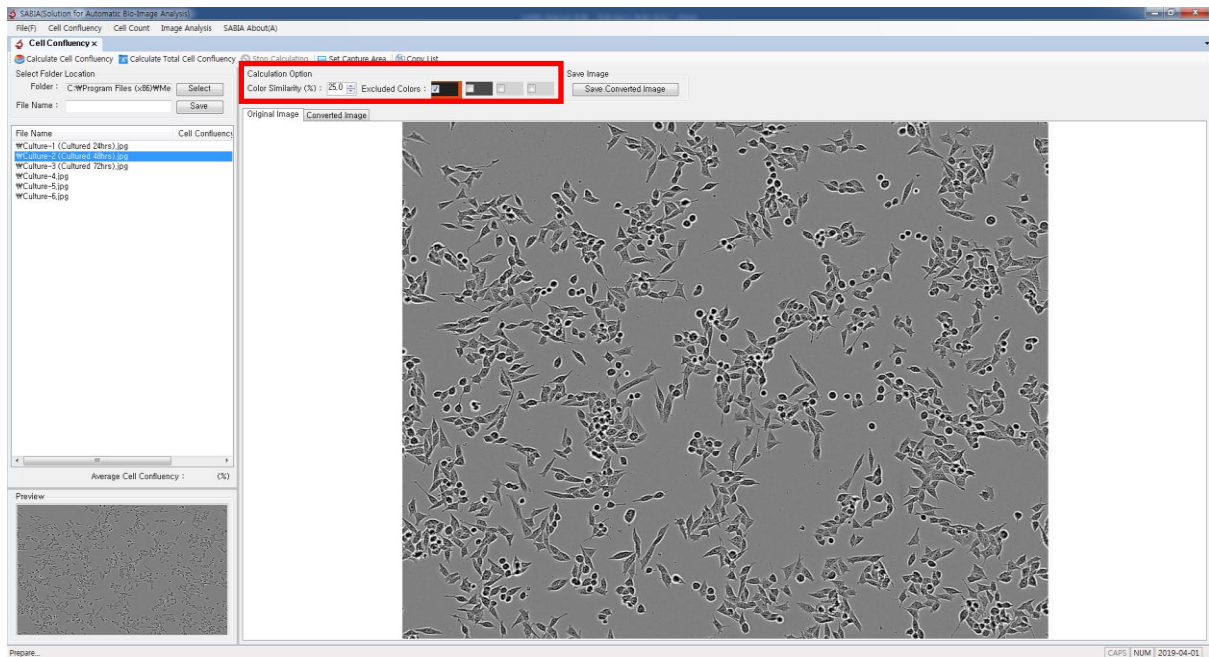
### 1) Select Folder

At the left side of the screen, select a folder which images that will be analyzed are located. Selected file can be confirmed using **Preview** and **Original Image** tab.



## 2) Set Calculation Option for Cell Confluency

It does not matter an order of setting between **Color Similarity** and **Excluded Colors**.



### (1) Select Excluded Colors

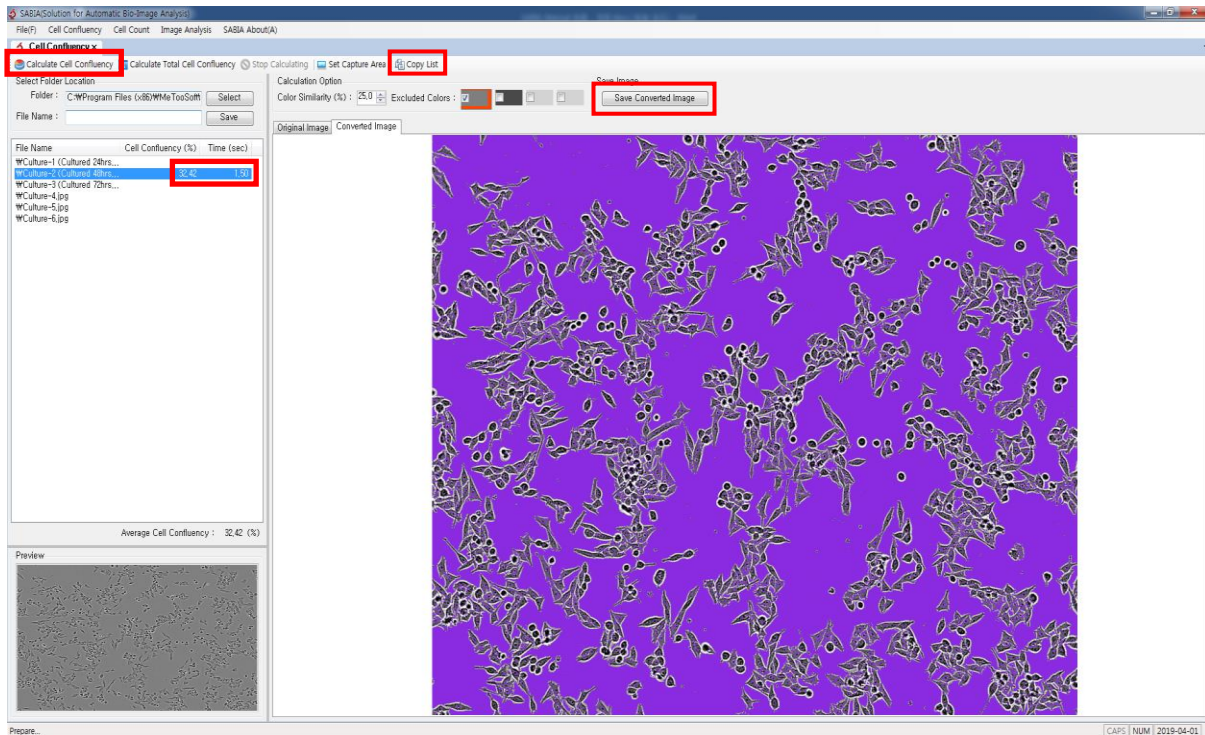
- ① Check any excluded color boxes (up to 4) and click checked color box for choosing a color.
- ② Touch the color from the original image. That color will be recognized as a background color and excluded from the calculation of cell confluency.
- ③ Unchecked color box will be excluded from the calculation even if you select color.

### (2) Set Color Similarity

- ① **Color Similarity** means a permissive range of color. The range of color similarity can be set from 0 to 100(%). Both using up & down button or inputting digits are available.
- ② Higher color similarity value, realize it more as a background color.
- ③ Appropriate range depends on image's characteristics or color. In first time, recommended color range is from 20 to 30%. Then, adjust it following the calculated value.

**[ATTENTION]** If value of color similarity is too big, all color would be the excluded color resulting in the color of cell would be recognized as the background.

3) Calculate Cell Confluency: Click **Calculate Cell Confluency** button on the menu bar.



- (1) Calculated cell confluency will be showed beside its file list. Excluded color that you selected will be changed to purple color. This means that it was recognized as a background and is not contain the result of cell confluency. **Converted Image** helps to divide clearly between cells and background.
- (2) If the result of cell confluency is not satisfied, follow next two steps.
  - ① Change the percentage of **Color Similarity**. Then, recalculate it.
  - ② Check **Excluded Colors** boxes and add more color following aforementioned protocol. It is recommended that choosing another color excepting purple color in converted image. Then recalculate it.
- (3) Setting of **Excluded Colors** and **Color Similarity** will be saved when existing the program and can use it next analysis of other image. (More than an image which came from the same experimental condition can be calculated by **Calculate Total Cell Confluency** with the calculation option.)
- (4) For saving the result of calculation, click **Copy List**, then paste it to Excel or Notepad.
- (5) For saving the converted image, press **Save Converted Image**. It will be saved in a sub-



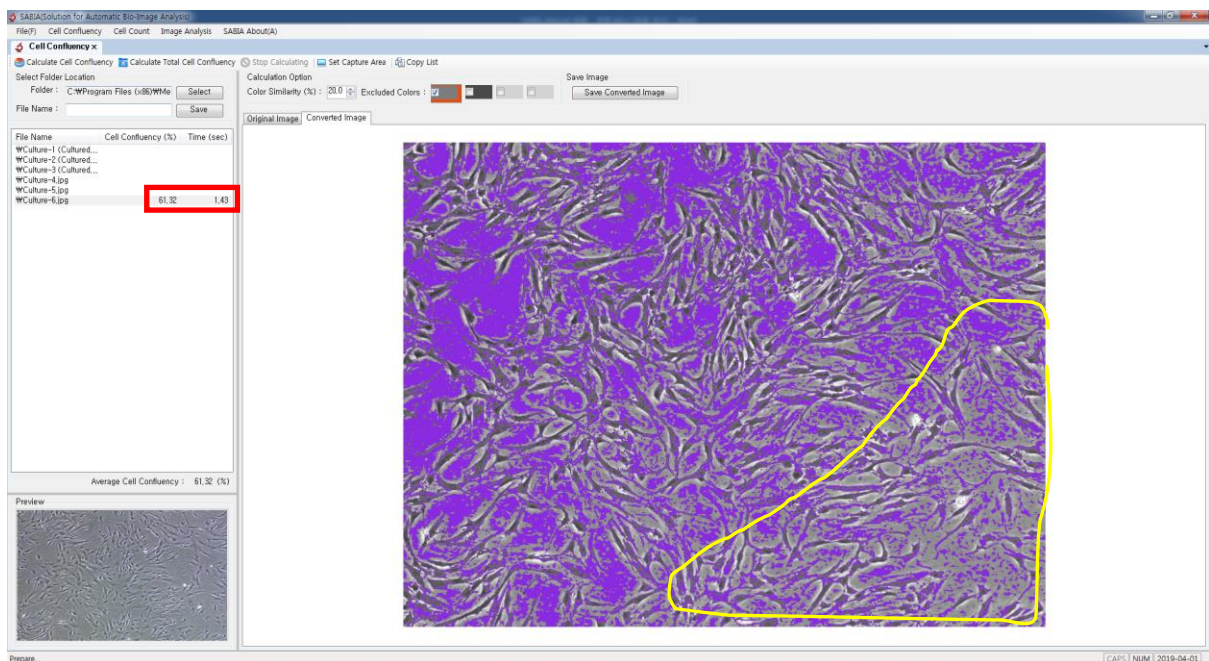
folder where the original image is located.

- (6) Click **Calculate Total Cell Confluency** to calculate simultaneously all images in the selected folder.

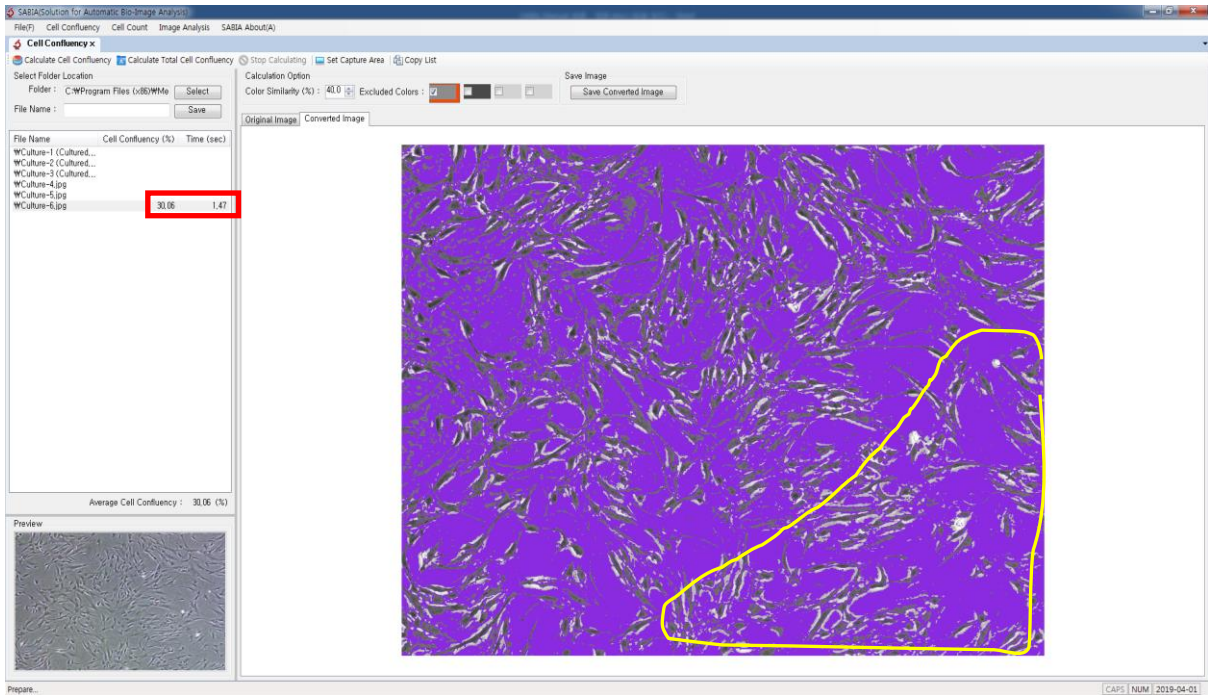
### 3. Example of Cell Confluency Calculation

Better result can be produced by only changing **Color Similarity**. 61.3% of cell confluency is calculated when select 'an **Excluded Colors**' and '20% of **Color Similarity**'.

- 1) For the deletion of background in the yellow part, the percentage of **Color Similarity** will be increased.



- 2) Only changing **Color Similarity** from 20% to 40%, 30% of cell confluency was obtained.
- 3) Higher **Color Similarity** makes the clearer delineation of cell in both areas, selected yellow area and whole area.



#### 4. Set Capture Area

General video, microscopic video, or any kind of image can be used directly by using capture function



##### 1) Image Capture

- (1) Click **Set Capture Area**, then follow a pop-up instruct on monitor.
- (2) Click and drag a certain area to be analyzed.

- (3) Press **E** to exist Set Capture Area. For switching monitor when using dual monitor Press **M**.
- (4) Press **Esc** to cancel of image capture.
- (5) Captured image would be appeared on **Preview** and it will be automatically used after saving image. To save image, enter an image name, then click **Save**.
- (6) Captured image will be saved on a subfolder of the current folder.

## IV. Cell Counting

### 1. Overview

A main function of cell count is an auto calculation of the number of cell (nuclear). Not only counting the number of cells in any kinds of cell images, it enables to measure the length of each cell. It provides reliable and reproducible data and disencumbers from both laborious and time-consuming tasks.

The basic concept of handling program is the same with the calculation of cell confluency. For cell counting, one of added options is Include Selected Color of which function works in opposition to Exclude Selected Color. Chosen color from Include Selected Color will be counted as cells, not be considered as background. Second option is to specify the area where cell counting takes place. Last option is to measure the cell length and aggregation.

### 2. How It Works

- 1) **Select Folder:** Open the folder which cell images are located by clicking **Select** in the upper-left side of screen. Select an image to be calculated.



- 2) Calculation Option for Cell Counting

#### (1) Set Color

There are two options, **Excluded Colors** and **Included Colors**. Excluded Colors is recommended when image's color is single. Included Colors is recommended when image's color is more than two.

- ① **Excluded Colors:** Choose a color that will be excluded from cell counting (up to 6 colors).
- ② **Included Colors:** Choose a color that will be included in cell counting (up to 6 colors)

## (2) Set Cell Counting Options

- ① **Color Similarity:** Color Similarity means the permissive range of color for cell counting
  - The range of color similarity can be set from 0 to 100(%). Both using up & down button or inputting digits are available.
  - When using Exclude Selected Color, try it first in the range of 10-20%
  - When using Include Selected Color, try it first in the range of 40-60%
- ② **Minimum Cell Size:** Set **min Cell Size** which is considered as a cell. The range of cell size can be set from 0 to 100 by using up & down button.
- ③ **Marking Color:** Click **Marking Color** and choose a color that will be used to mark counted cells.
- ④ **Set Analysis Area:** It is useful if you want to count cell on a certain part, not whole image. Details are described in p.14

(3) **Length Measurement Option:** Set **Length Measurement Option** to measure the length of counted cells. Details are described in p.15

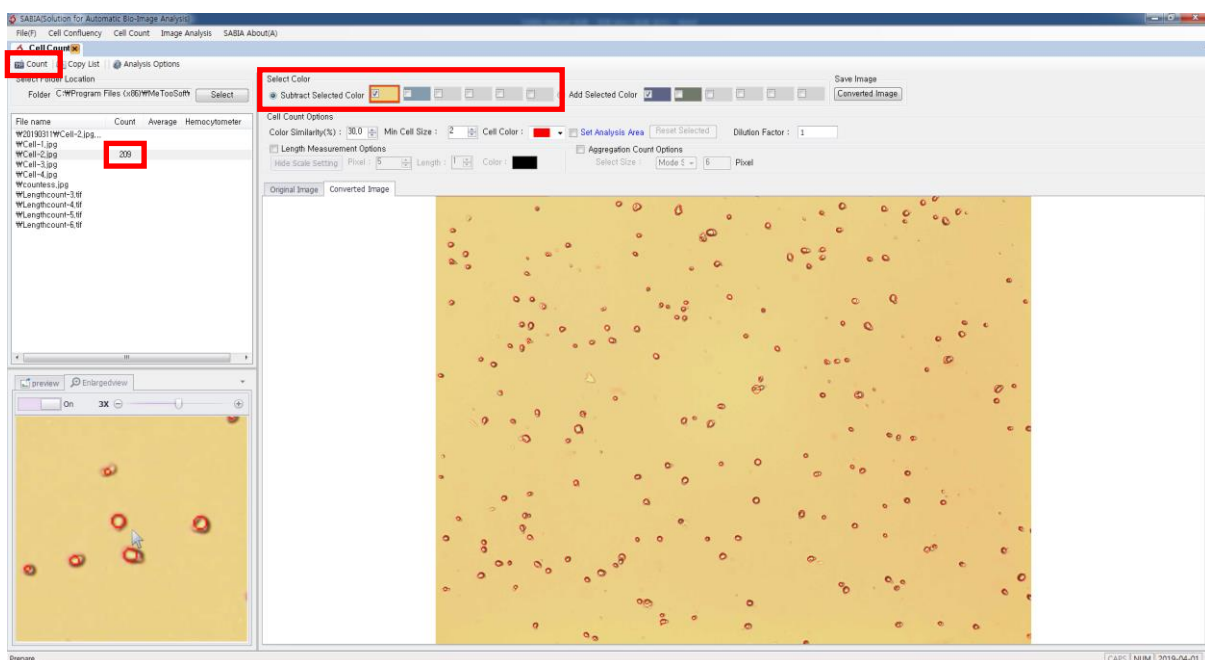
(4) **Aggregation Count Options:** Set Aggregation Count Options to calculate an estimation of aggregated cells. Details are described in p.17

(5) **Enlarged View:** It is a screening magnifier. The image could be magnified up to 4 times by using it. Details are described in p.18

## 3) Execute Cell Counting

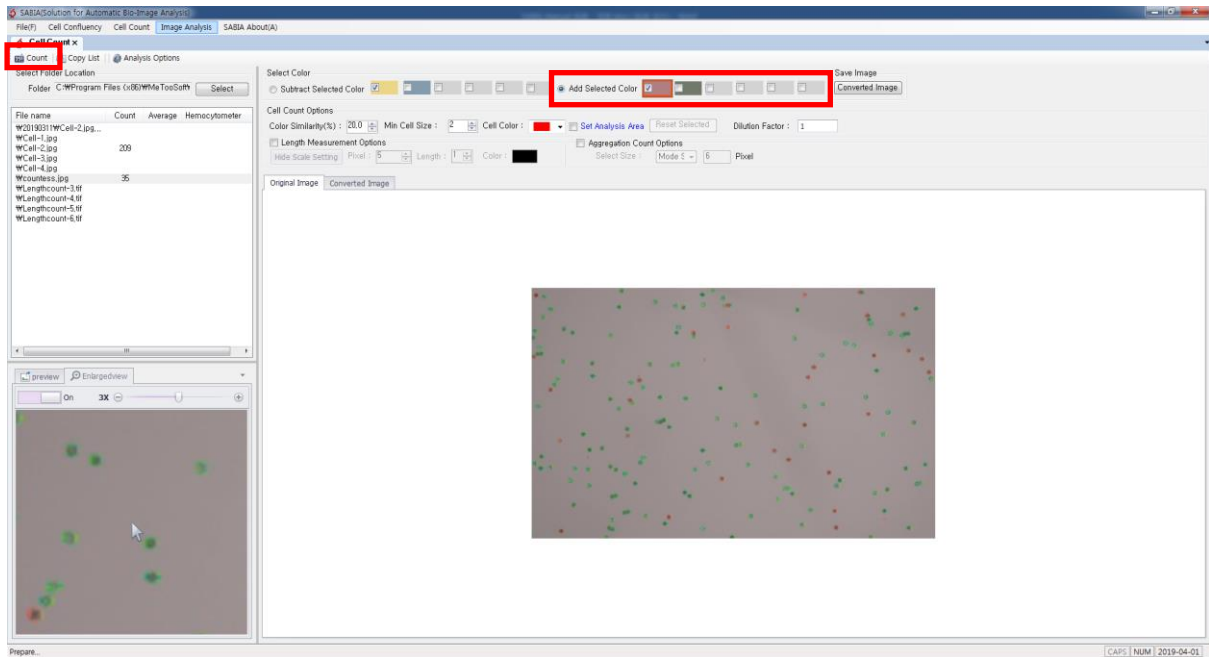
Click **Count**. The number of cells will be appeared beside the file name

### (1) Example of **Excluded Colors** Option.

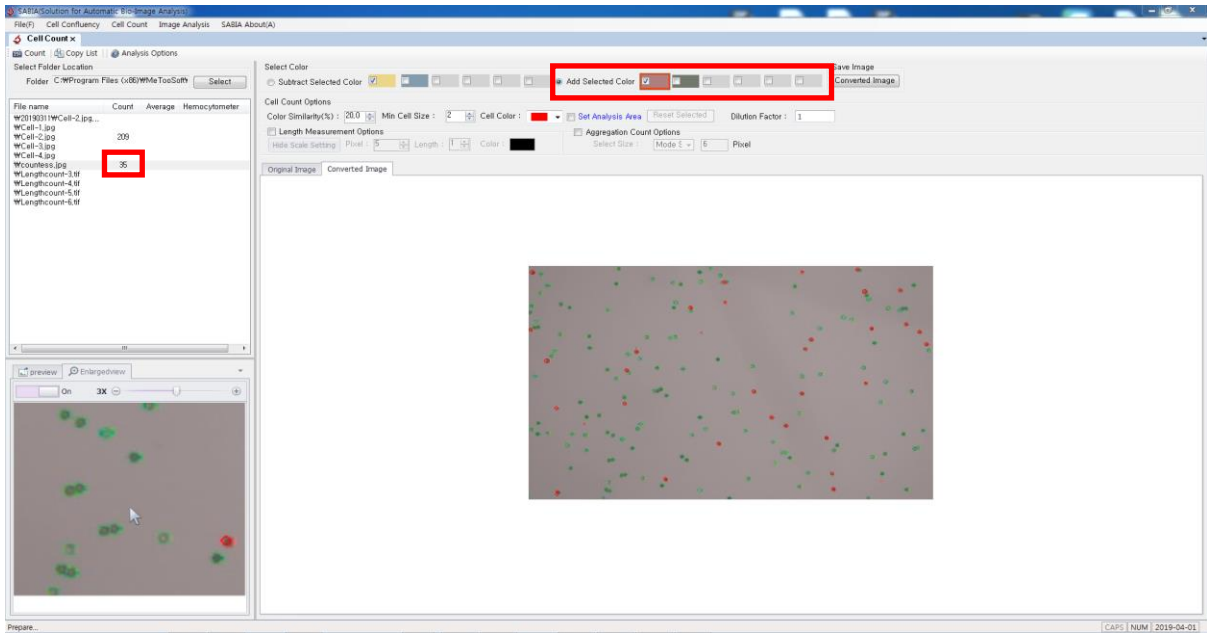


- ① Yellow background color was selected as **Excluded Colors** and **Color Similarity** was set at 30%.
- ② As a result, the number of cells are 209 which were marked with red color on the **Converted Image**.
- ③ **Enlarged View** on the below left helps to see details of the result.

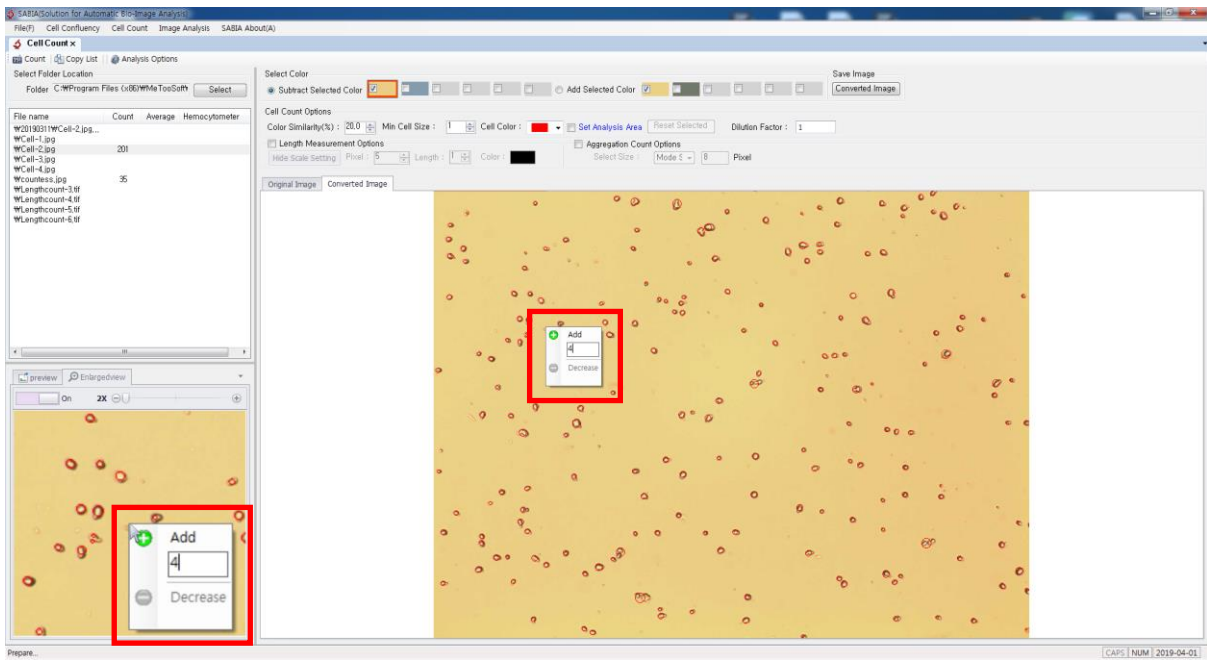
(2) Example of **Included Colors** options.



- ① **Included Colors** is useful when cells' colors are more than two. For example, live cells and dead cell in an image can be separately counted.
- ② Check **Included Colors** box, choose color from the image. Then, adjust **Color Similarity** and click **Count**.
- ③ Above example image has both red and blue colors cells. It shows that **Included Colors** is marked by red, resulting in only red color cell are counted.



### 3. Adjusting after Completing Cell Count (if necessary)



1) Adjusting-needed circumstance.

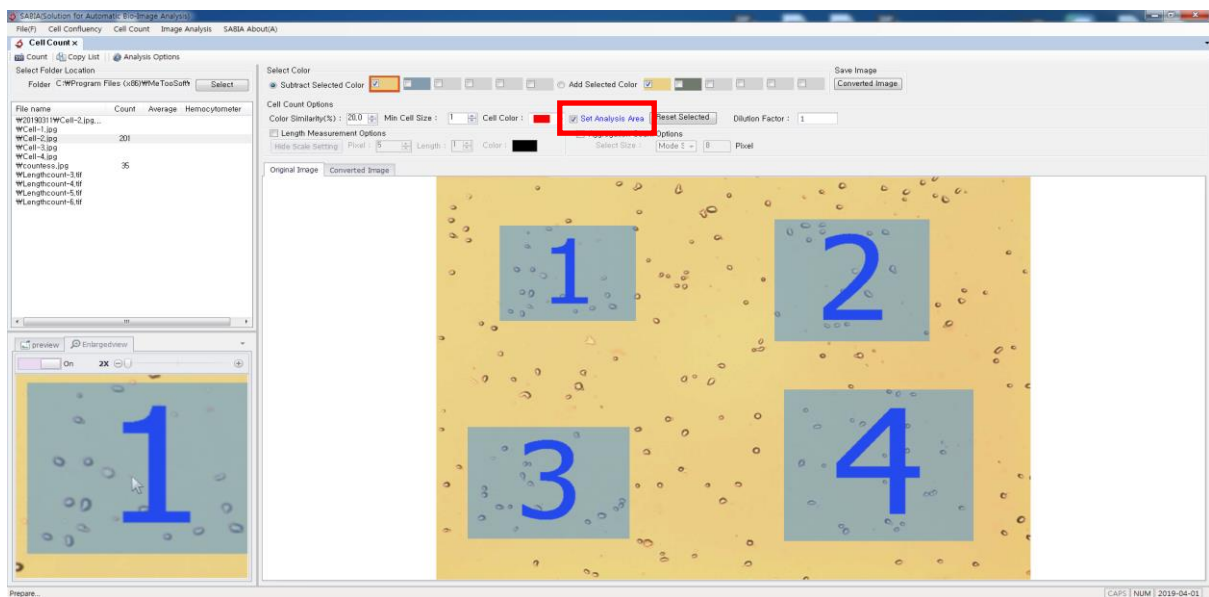
- (1) When actual cells were not counted.
- (2) When not actual cells were recognized as cells
- (3) When counted cells needs modification of its size
- (4) When the mark of counted cells were not matched with actual location.

2) Adjusting Ways

- (1) Click with the right button of mouse or double click cell which you want to adjust. Pop-up menu will be appeared.
- (2) **Add:** It makes a new cell which was not counted even if it is real cell.
- (3) **Increase:** it increases the number of the already counted cell.
- (4) **Decrease:** it decreases the number of duplicated cell which is actually a cell. If the number of cell is one, it will be showed Delete, not Decrease.
- (5) **Delete:** it deletes a cell which is not actually a cell.
- (6) **Number:** It means the size of cell. Default value is the same with Minimum Cell Size on the Cell Counting Option. Cell size can be adjusted by put an exact number.
- (7) **Move:** The spot of cell can be moved by clicking, dragging, and dropping.

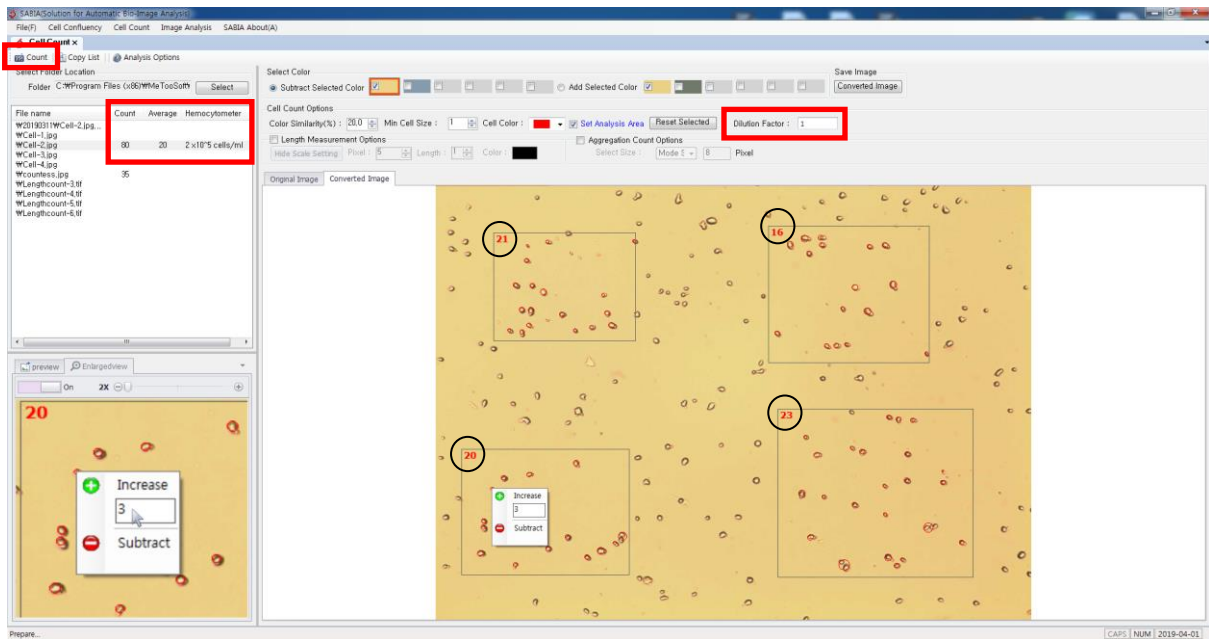
#### 4. Set Analysis Area

- 1) Check **Set Analysis Area**, then click and drag a certain area to be analyzed. Choosing multiple areas is available but overlapping areas is not allowed.



- 2) Set **Select Color** and **Cell Count Options**, then execute **Count**.



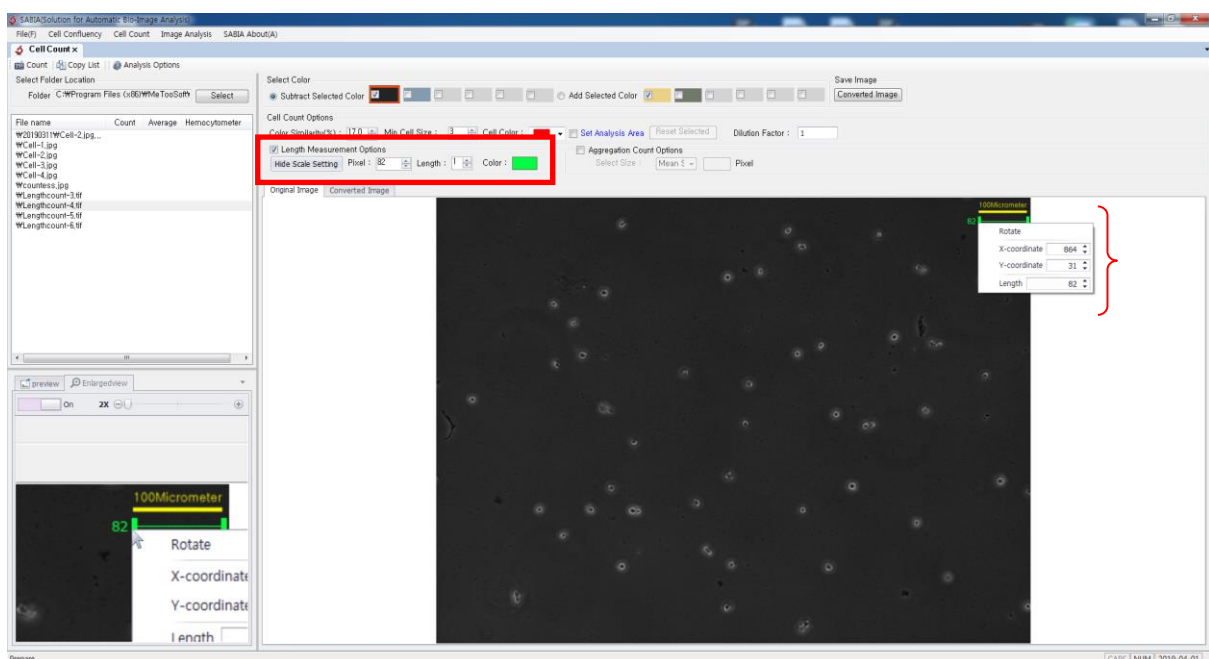


- (1) The number of cells in each area will be appeared in the black circle. An average of cells of all areas will be appeared beside the file list.
- (2) After completing cell count, the results like cells' number or size can be modified as above mentioned in **Adjusting Ways**.
- (3) **Dilution Factor**: This value is for the calculation of **Hemocytometer**.

## 5. Measurement of Length of Cell

Check Cell Length Option, then set Standard Pixel, Standard Length, and Standard Color. Cell length data will be automatically showed on the left side when counting cell.

**[ATTENTION]** Scale bar must be needed for this option.



## 1) Details of Length Measurement Options

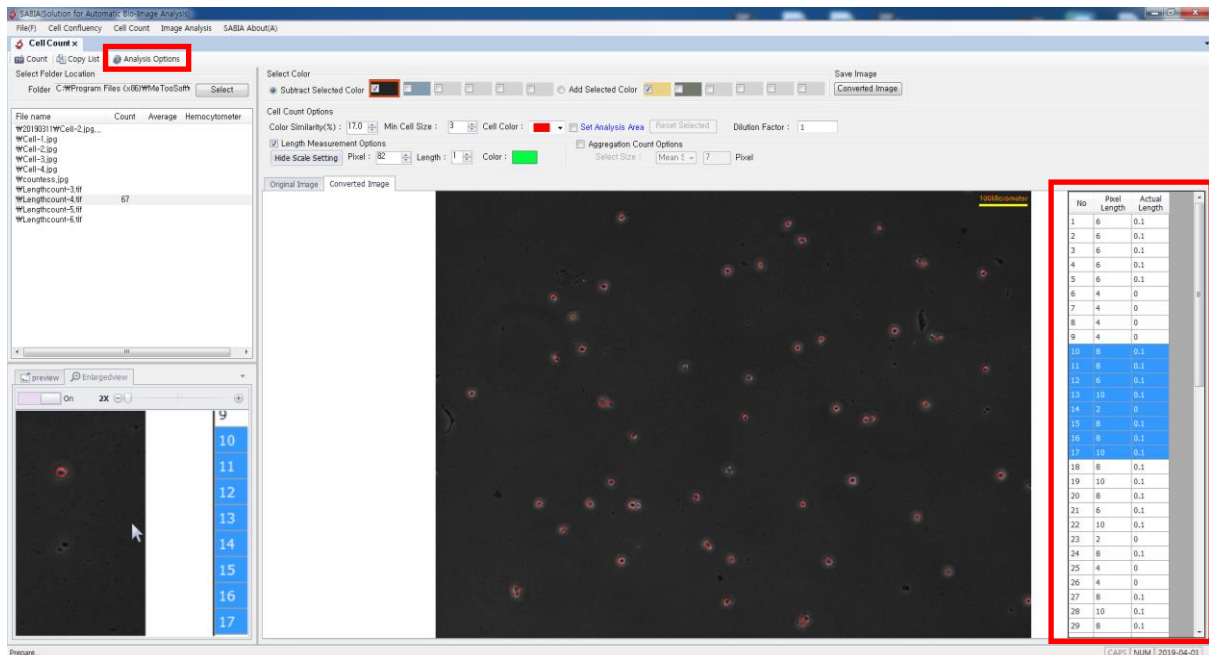
- (1) **Standard Pixel:** Pixel about the scale bar by using standard bar.
- (2) **Standard Length:** The value about the scale bar on the image.
- (3) **Unit :** Unit of scale bar (example image's unit is micrometer)
- (4) The example image indicates 100 micrometer is equal to 82 pixel. Using this pixel, the length of cell will be calculated and its unit will be micrometer.

## 2) Setting Standard Value for Length Measurement

- (3) Click **Display Scale Setting** to create a standard bar on the image.
- (4) Drag the standard bar and match exactly the end of standard bar and the end of scale bar on the image. Using **Enlarged View** on the bottom left side helps to adjust precisely.
- (5) Another way, click the right mouse button on the standard bar, then adjust the location of stand bar by using X and Y coordinates.
- (6) For adjusting the stand bar size, keep clicking an end of stand bar then drag it to extend or enter an exact digit into **Standard Pixel**.

## 3) Result of Cell Length Measurement

A result of measurement of cell length will be showed on the left side. It can be copied using **Copy List** or dragging and copy & paste directly the result on the left side to excel or notepad (see below).



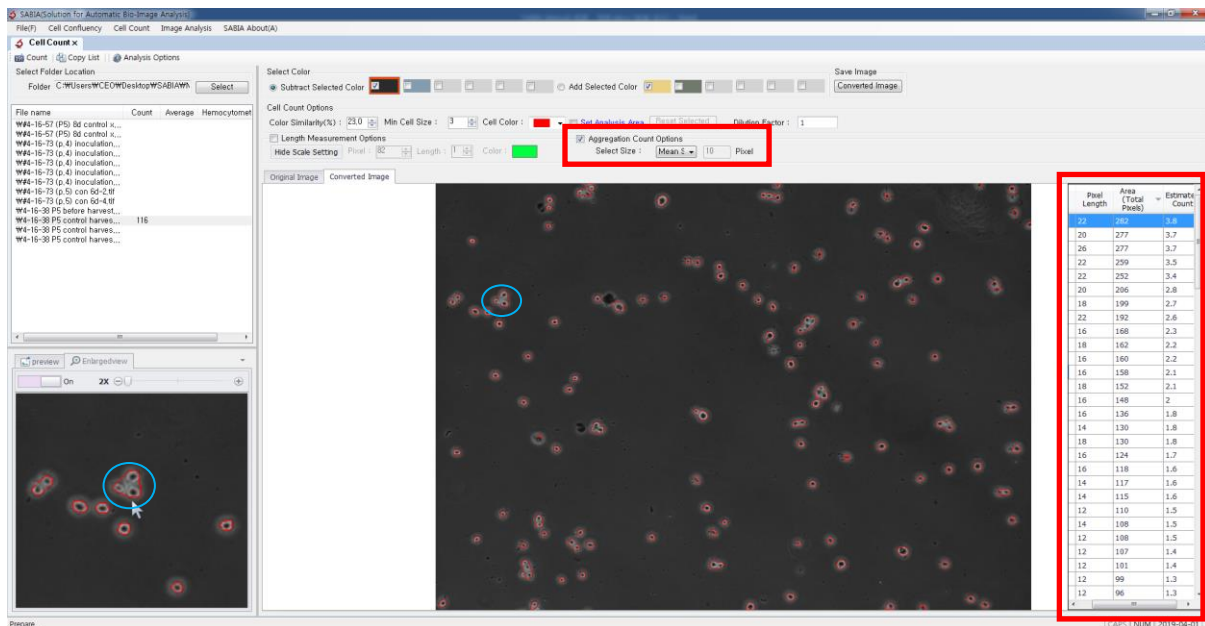
NO	Pixel Length	Actual Length
1	6	0.1
2	6	0.1
3	6	0.1
4	6	0.1
5	6	0.1
6	4	0
7	4	0
8	4	0
9	4	0
10	9	0.1
11	9	0.1
12	6	0.1
13	10	0.1
14	2	0
15	8	0.1
16	8	0.1
17	10	0.1
18	8	0.1
19	10	0.1
20	8	0.1
21	6	0.1
22	10	0.1
23	2	0
24	8	0.1
25	4	0
26	4	0
27	8	0.1
28	10	0.1
29	8	0.1

- (1) **Pixel Length:** the number of pixels which are occupied on the screen.

- (2) **Actual Length:** actual length of cell that was measured by the scale bar.
- (3) **Search Selected Cell's Length:** place mouse cursor on counted cell. Its length will be marked with blue color on the left list.
- (4) Search Cell's Location: double click the length data which you want to find the location of cell. Its location will be marked with white color on the converted image.

## 6. Calculation of Aggregation

Aggregation can be estimated by using the average size and by comparing to each size.



### 1) Details of Aggregation Count Options

- (1) **Mean Size:** the average size of all marked cell (automatically calculate)
- (2) **Mode Size:** most frequent size among all among marked cell (automatically calculate)
- (3) **Median Size:** middle value from all marked cell (automatically calculate)
- (4) **User Input:** the value that you want to input.

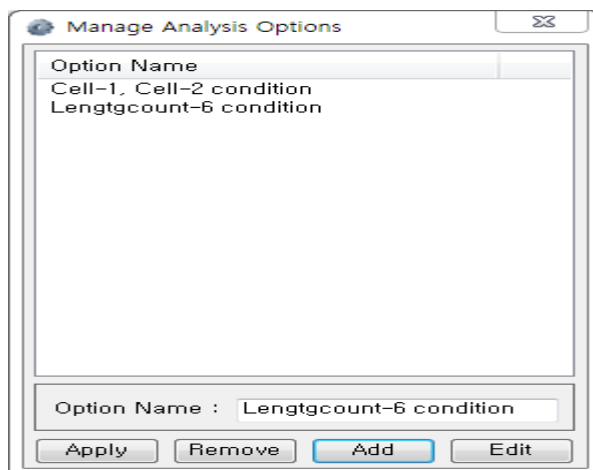
### 2) Interpretation of Aggregation Result (list on the right)

- (1) **Pixel Length:** the maximum length of marked cell
- (2) **Area:** total number of pixel marked cell. It indicates its area
- (3) **Estimated Number:** the estimated cell number from the counted cell. Estimated number =  $\text{area}(\text{total pixel}) / \text{area which is measured by Calculation Standard value}$ . It is able to sort list in ascending or descending order.

	Pixel Length	Area (Total Pixels)	Estimate Count
	22	282	3.8
	20	277	3.7
	26	277	3.7
	22	259	3.5
	22	252	3.4
	20	206	2.8
	18	199	2.7
	22	192	2.6
	16	168	2.3
	18	162	2.2
	16	160	2.2

## 7. Function of Analysis Options

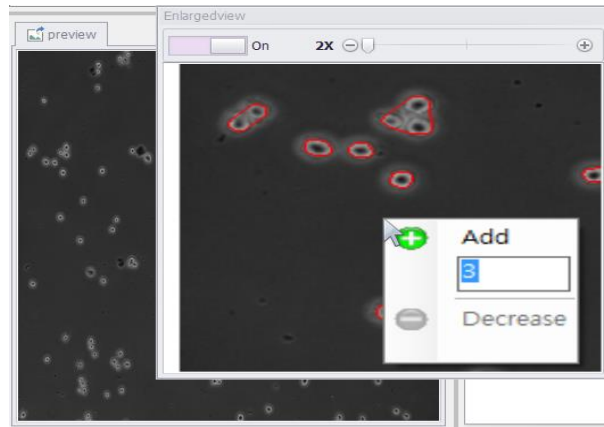
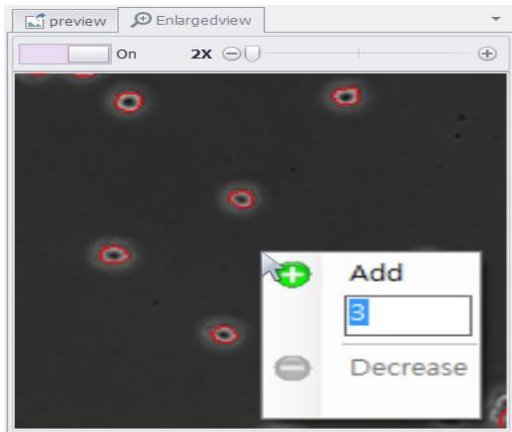
Cell counting options can be saved by **Analysis Options**. It is convenient and fast. Saved cell counting options will be retrieved and applied next cell counting tasks. Multiple cell counting options can be also saved.



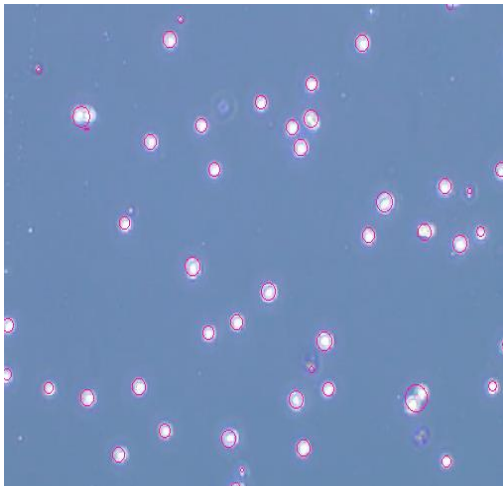
- 1) **Option Name:** easily rememberable name is better
- 2) **Apply:** apply the selected Analysis Option
- 3) **Delete:** delete the selected Analysis Option
- 4) **Add:** add a new Analysis Option
- 5) **Edit:** modify the name of selected Analysis Option

## 8. Enlarged View (Screen Magnifier)

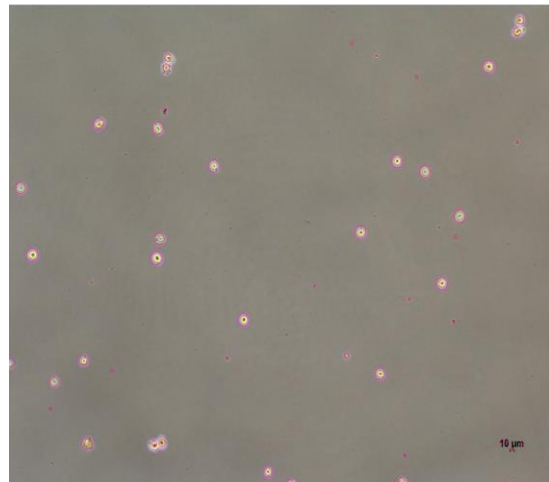
- 1) An image can be magnified up to 4 times.
- 2) For more big size, dragging Enlarged View window. For original location, drag Enlarged View to its original location or use mouse right button and click dock.



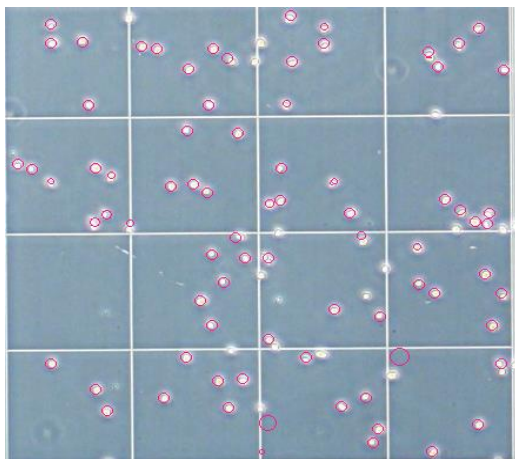
**9. Examples of Cell Count from Various Conditions.**



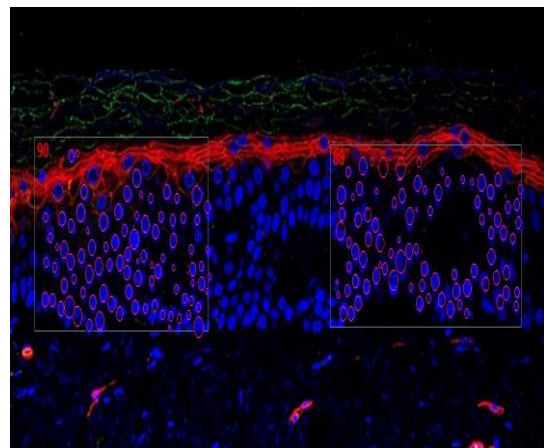
**[Single Color Background]**



**[Single Color Background 2]**



**[Grid Lines]**



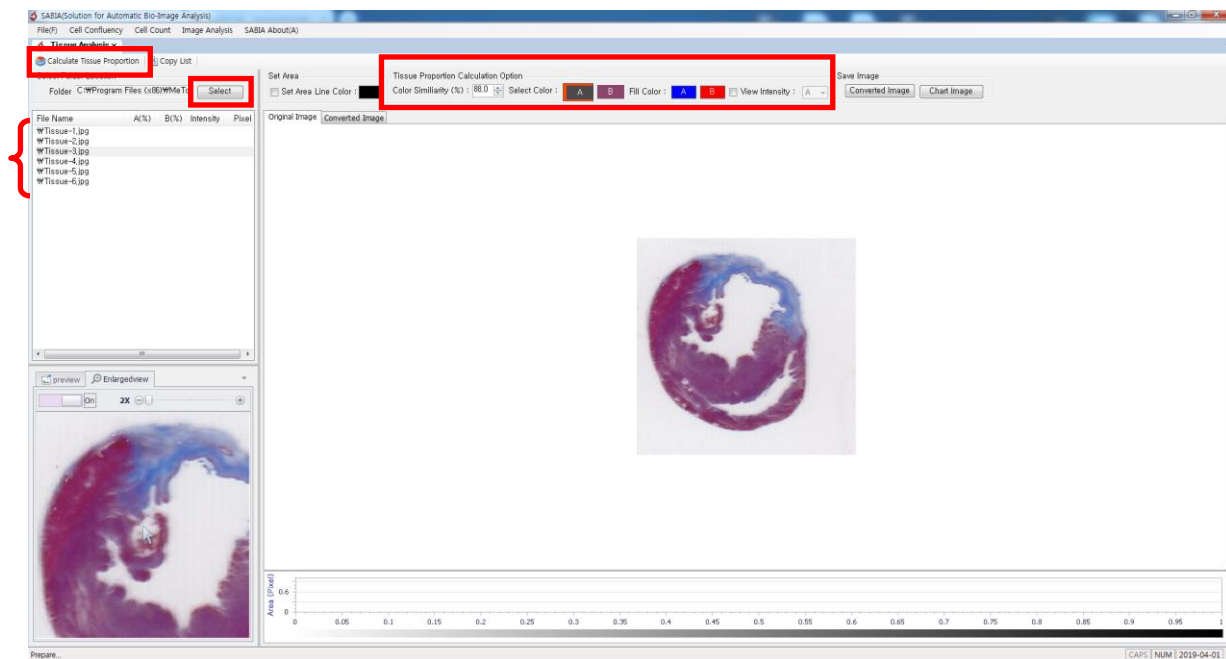
**[Set Analysis Area]**

## V. Image Analysis - Tissue Ratio

### 1. Overview

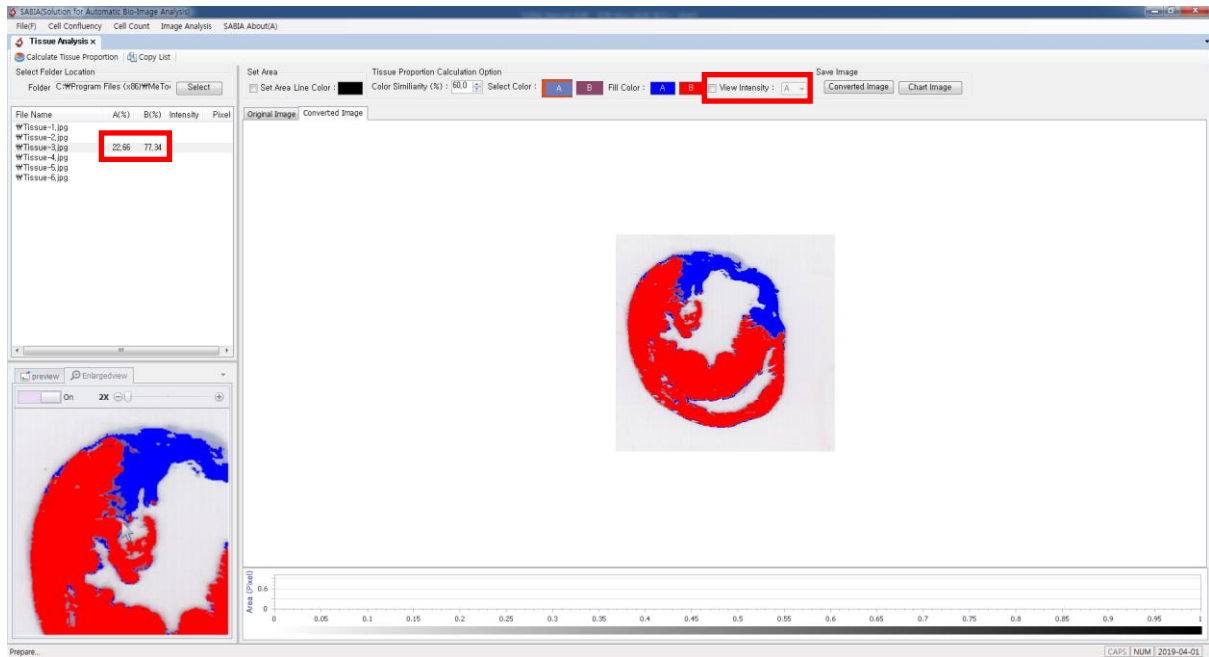
A purpose of Tissue Ratio analysis is to calculate the ratio of tissue characteristics (different colors) on an image. Not only the tissue's different characteristics can be verified, and also the thing how its characteristics are clear can be observed by using Color Intensity. A certain area can be specified as well.

### 2. How It Works



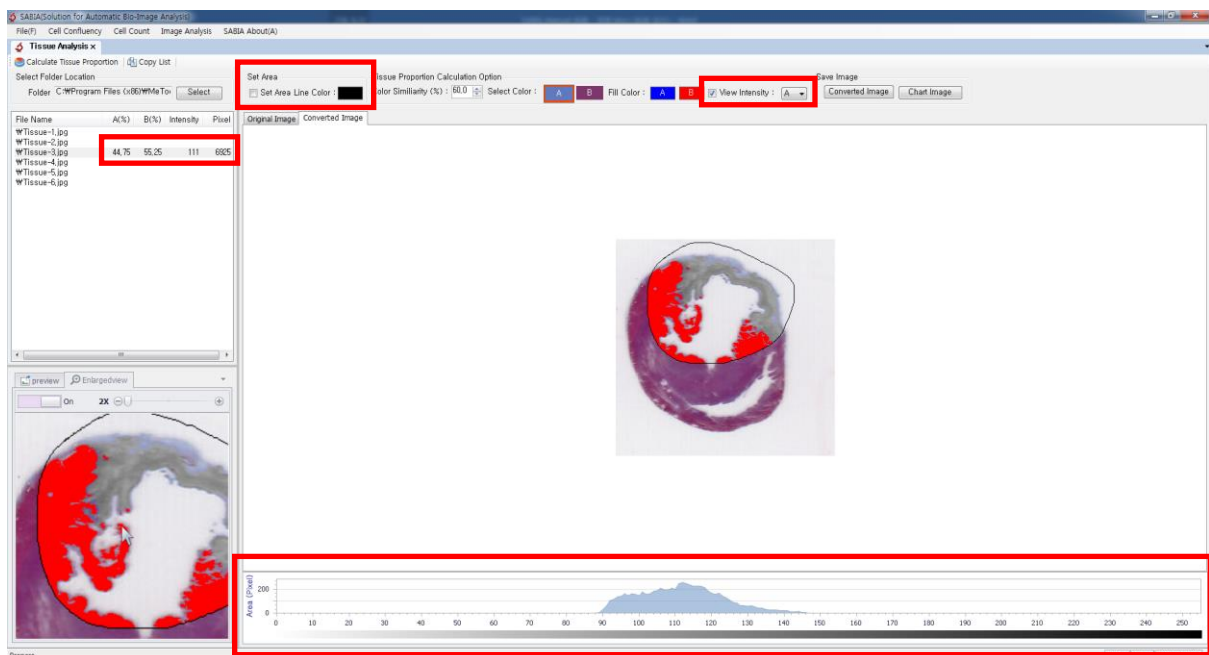
- 1) **Select Folder:** Open the folder which images are located by clicking **Select** in the upper-left side of screen. Select an image to be calculated.
- 2) **Set Tissue Ratio Calculation Option**
  - (1) **Select Color:** Select colors into **A** and **B** boxes to calculate the ratio of tissue's characteristics. Recommend **Color Similarity** starts at 50% then adjust it depending on the result.
  - (2) **Fill Color:** select colors into **A** and **B** boxes to mark calculated result (if necessary)
  - (3) **View Intensity:** check **View Intensity** and choose one A or B box. Its value will be showed on the bottom of image and the average intensity will be recorded on the left side(if necessary).
- 3) **Tissue Ratio Calculation:** Click **Calculate Tissue**. The ratio of two different colors will be calculated based on the **Tissue Ratio Calculation Option**.

### 3. Result of Tissue Ratio Calculation



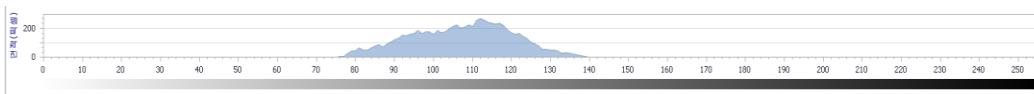
- 1) Based on **Select Color** and **Color Similarity**, the results of calculation will be showed on the **Converted Image** with Fill Color.
- 2) If color is not included in two colors, it will be excluded from the Tissue Ratio Calculation
- 3) The ratio of tissue characteristics will be appeared on the left side.

### 4. Calculation for A Specific Area



- 1) Check **Select Area**, then specify a certain area to be calculated. The ratio of tissue characteristics will be calculated within the selected area.
- 2) If check **View Intensity**, the intensity of A or B box will be showed on the bottom of image instead of Fill Color. Results of intensity value and pixel size will be appeared beside of file name.
- 3) **Chart for Color Intensity**

Color Intensity uses gray scale (difference of brightness) to calculate the degree of dyeing. Differences between same tissue, but the difference of the amount of dyeing or changes in tissues characteristics by time interval can be analyzed by using color intensity.



- (1) In chart of **View Intensity**, X-axis indicates the value of intensity and Y-axis indicates the area of calculated tissue(pixel).
- (2) Higher intensity value, closer to black. Smaller value, on the contrary, closer to white.
- (3) Results of Color Intensity can be copied to excel.

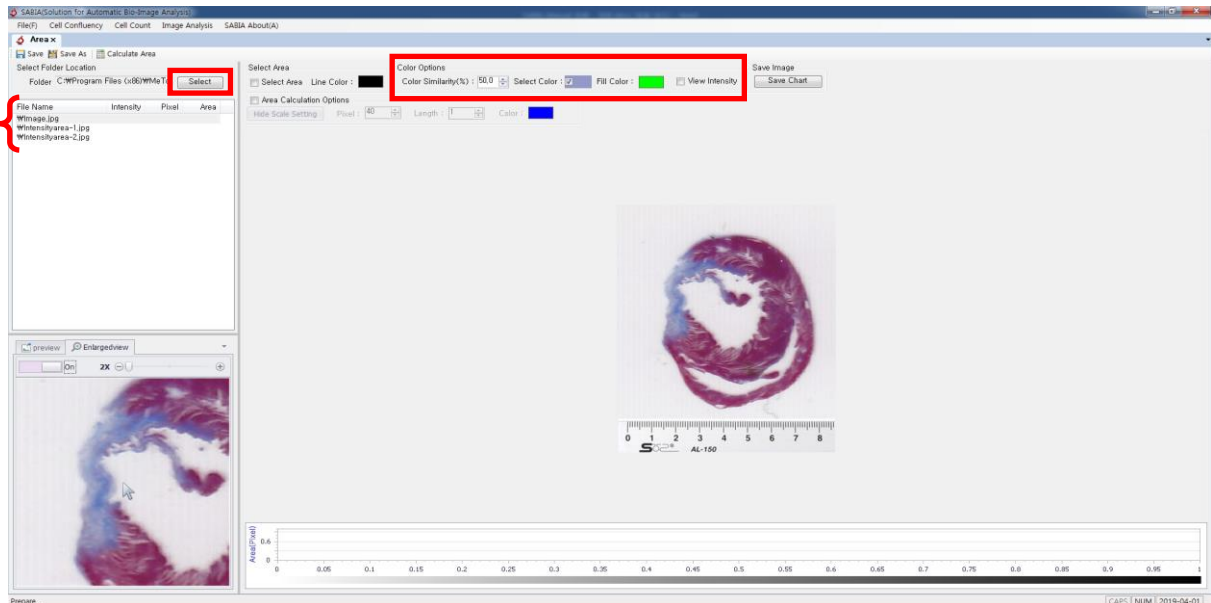


# V. Image Analysis - Area

## 1. Overview

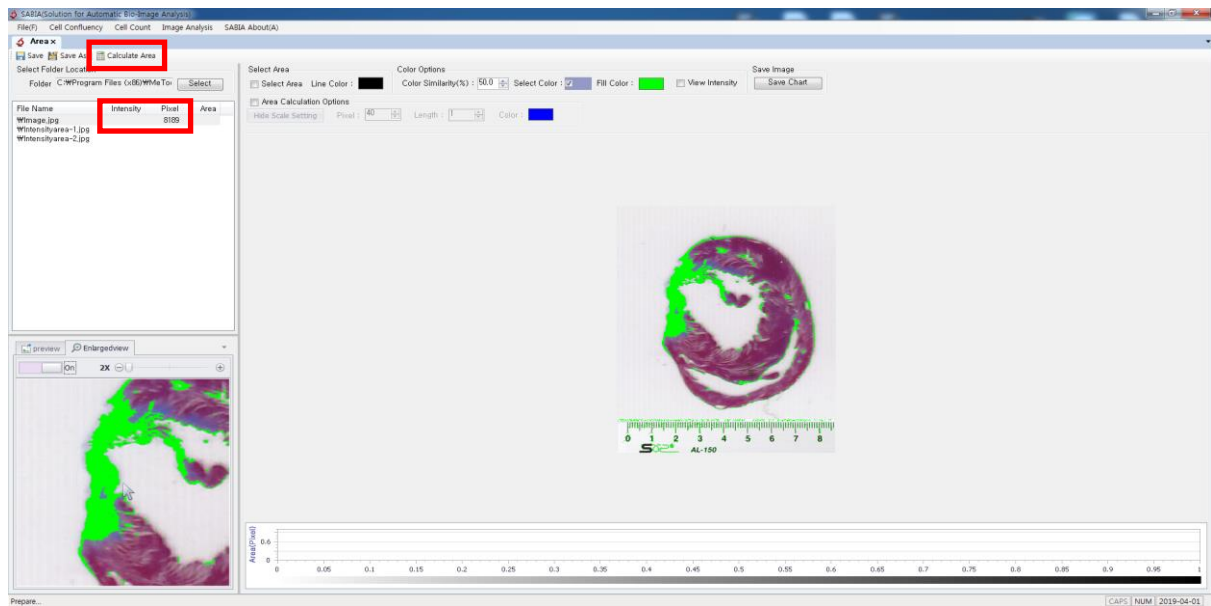
Image Analysis for area is to measure area. A measurement of area is calculated by color differences. A certain part is specified to measure area and Color Intensity is also available in this part. The basic concept is the same with other parts. An important thing is that scale bar is needed to measure area likewise Cell Counting.

## 2. How It Works



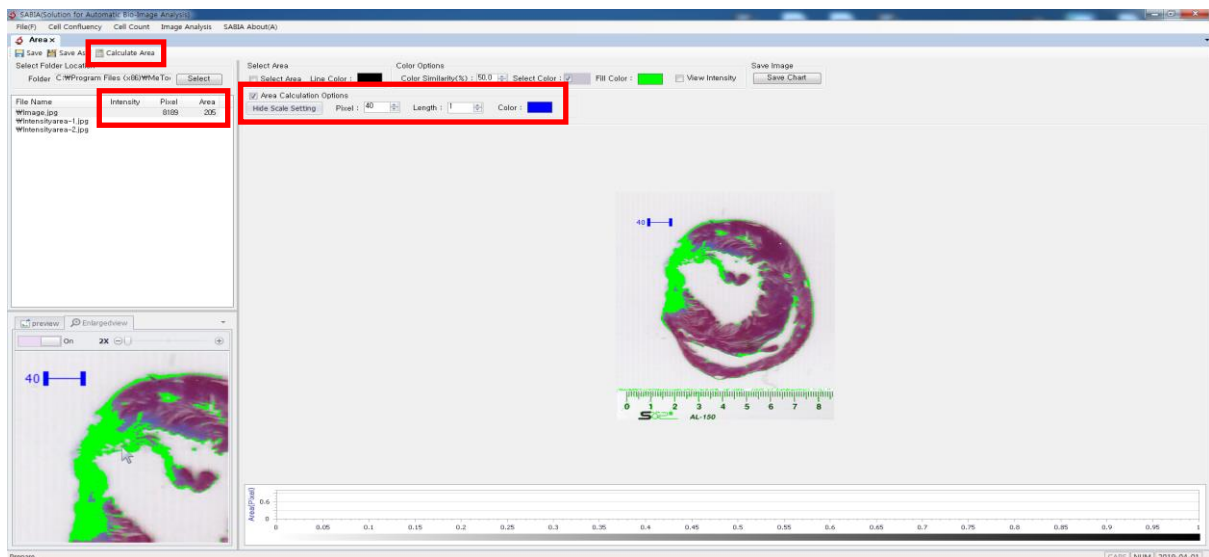
- 1) **Select Folder:** Open the folder which images are located by clicking **Select** in the upper-left side of screen. Select an image to be calculated.
- 2) **Set Color Options**
  - (1) **Select Color:** select a color from the image for measuring Area and Color Intensity.
  - (2) **Fill Color:** select color to represent area after measuring (if necessary).
  - (3) **View Intensity:** check **View Intensity**, color intensity and pixel will be showed instead of Fill Color (if necessary).
  - (4) **Color Similarity:** Recommendation is starting at 50%. Then adjust the range of Color Similarity base on the results.

### 3. Color Distribution and Color Intensity



After you execute **Calculate Area**, distribution of selected color will be changed with Fill Color and the sum of pixel will be appeared beside of file name. This is without the calculation of area.

### 4. Area Calculation



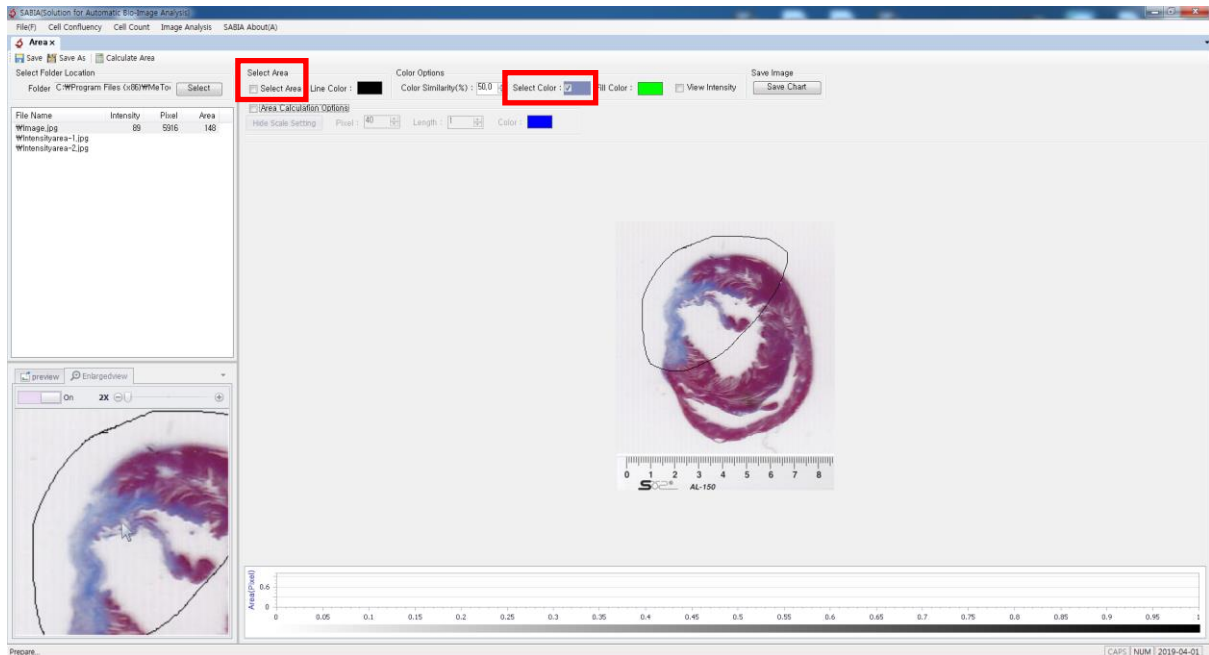
Check **Area Calculation Option** and **Display Scale Setting**. Set the standard bar options as described in chapter IV-5, Set Standard Bar.

Click **Calculate Area** resulting in measured area will be appeared beside of file name.

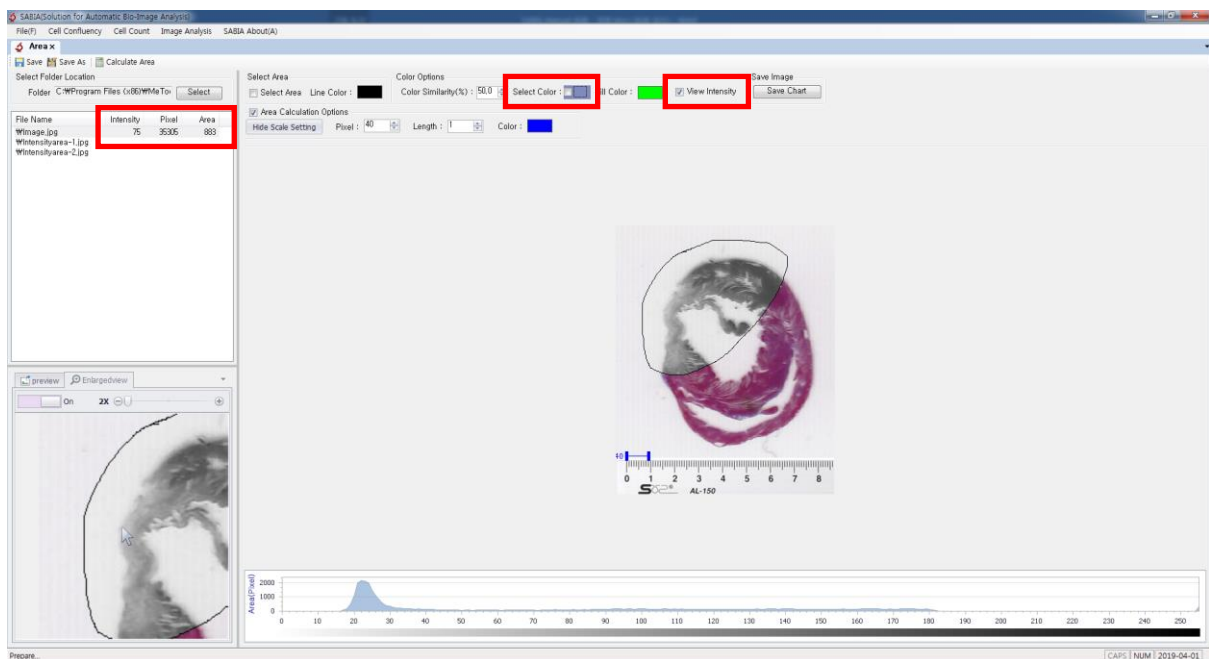
### 5. Calculate Area in A Specific Area.

Specifying a certain area is available to calculate area and color intensity. It has the same

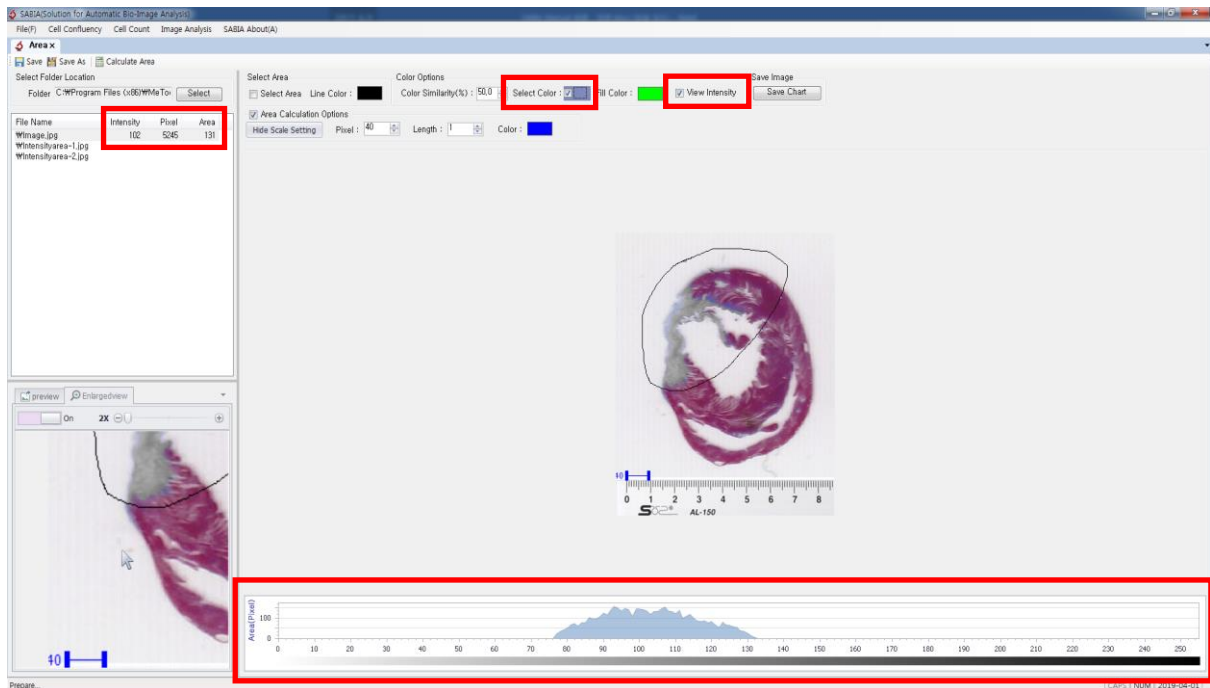
procedure to total area calculation.



- 1) Check **Select Area**, draw a part where you want to calculate area by using mouse.
- 2) If **Select Color** is unchecked, total area of selected part will be calculated. If **Select Color** is checked, selected color of selected part will be calculated. Either **Fill Color** or **View Intensity** is needed to check.
- 3) Click **Calculate Area**
- 4) If **Select Color** is unchecked (total area 883 with checking **View Intensity**)



- 5) If **Select Color** is checked (total area 131 with checking **Select Color**).

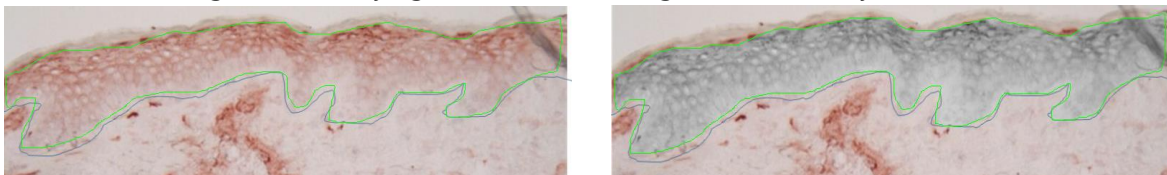


**6. Chart for Color Intensity:** It is the same as described in chapter V. Calculation Tissue Ratio 4-3

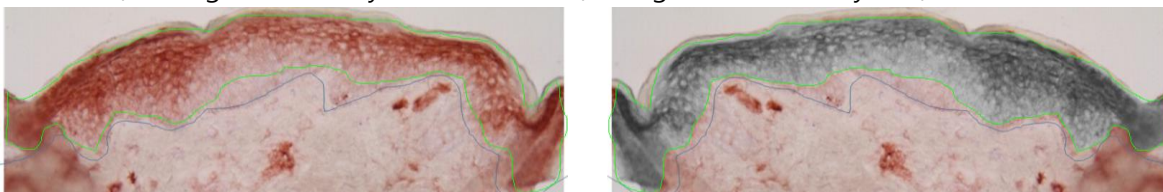
### 7. Example of Color Intensity

Area calculation can compare between the same sample with different time point or the same sample with different dyeing ways. The numerical value acquires objectivity and universality in comparison to manual effort.

1) Image A: relatively light condition (Average Color Intensity 125)



2) Image B: relatively dark condition (Average Color Intensity 167)

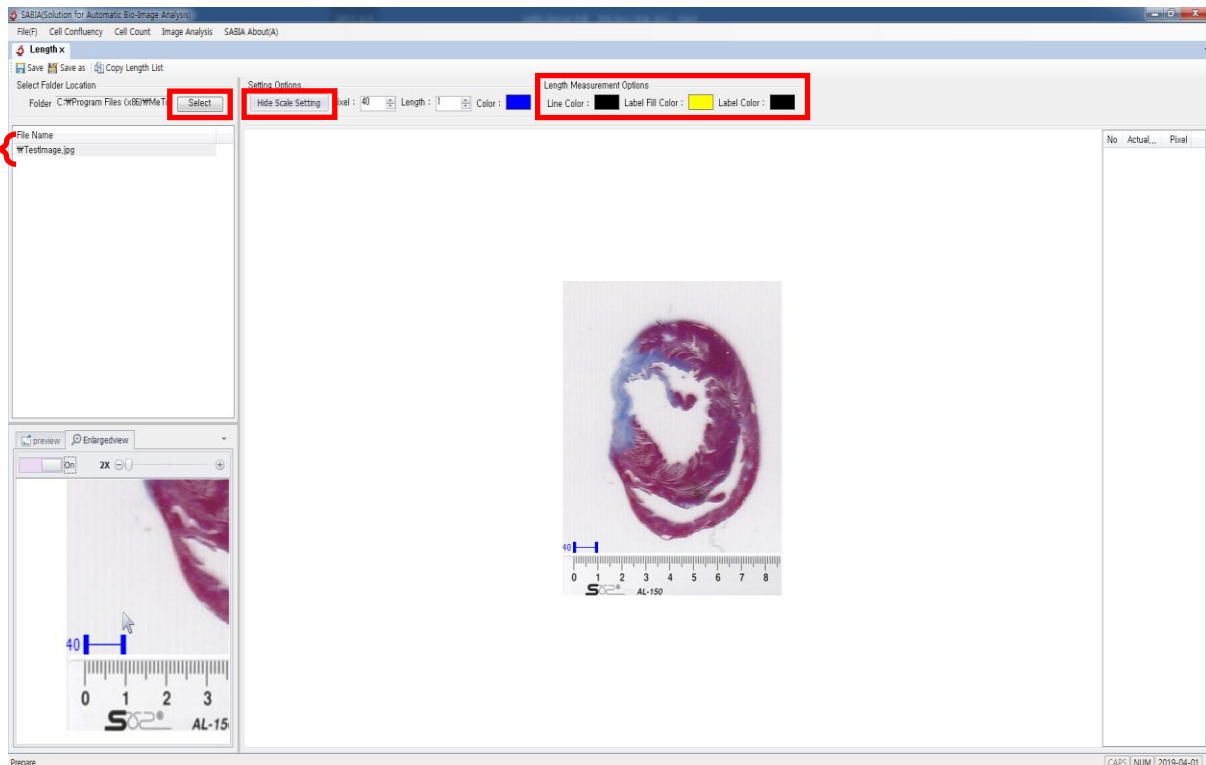


# V. Image Analysis - Length

## 1. Overview

An actual length can be measured when an image has scale bar and the measurement ways of length are various. Using straight line or adjustable curved line enable to measure the length. The basic concept is similar to other functions.

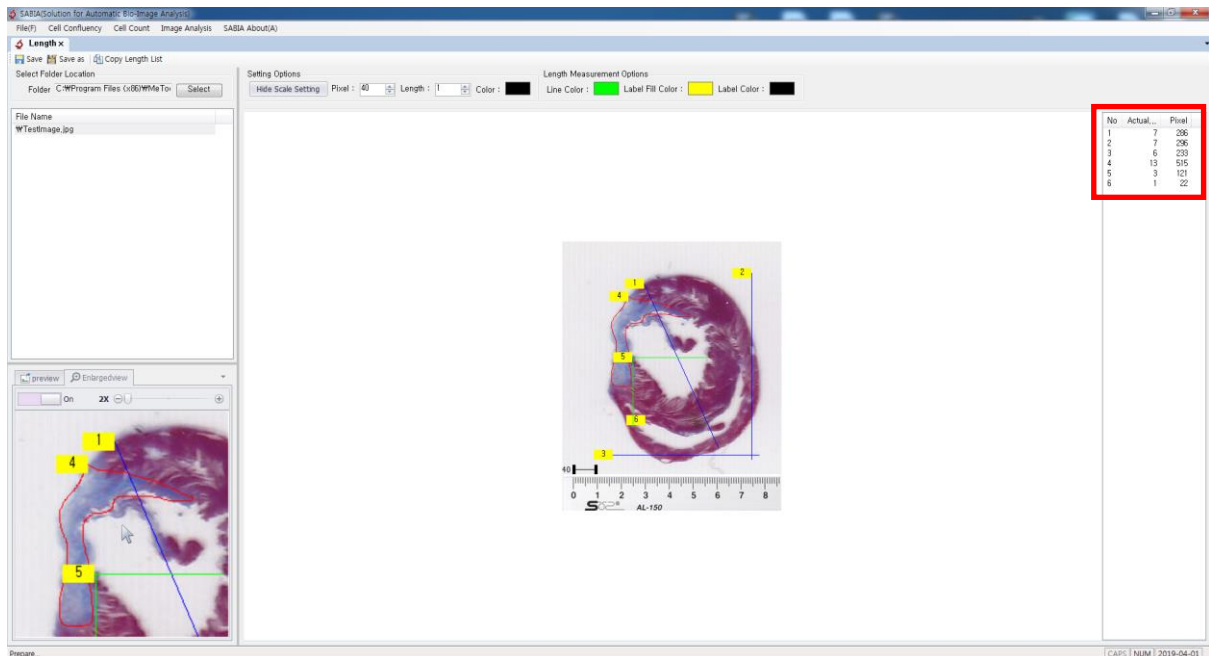
## 2. How It Works



- 1) **Select Folder:** Open the folder which images are located by clicking **Select** in the upper-left side of screen. Select an image to be calculated.
- 2) **Set Standard Bar:** Click **Display Standard Bar** and set **Length Measurement Option**. Handling options is the same to the calculation of standard bar (See IV-5 Cell Length Calculation).
- 3) **Other Options:** If necessary, **Line Color**, **Label Fill Color**, and **Font Color** can be changed by clicking the mouse right button.
- 4) **Unit of Length**  
Unit is equal to Scale bar's unit.

### 3. Methods of Length Measurement

The left button of mouse means the start of line setting and the right button of mouse means the end of line setting.



#### 1) Connect Two Points

- (1) Click two points which you want to measure length, then click the right mouse button to end setting.
- (2) Straight line will be appeared. Horizontal, vertical, and diagonal line can be drawn.

#### 2) Dragging Mouse

- (1) Keep clicking the left button of mouse and drawing the line. Then click the right button of mouse to finish setting.
- (2) It is useful when you want to draw the adjustable curved line.

#### 3) Using Ctrl key

- (1) Keep pressing **Ctrl** key and clicking the left mouse button, then move to where you want. Release the left mouse button and click the right mouse button to finish setting.
- (2) It is useful when you want to draw the straight line.

#### 4) Finish and Cancel Setting

- (1) To finish setting, click the right button of mouse. A label of line will be appeared at the point of line and the result will be added in the left side of screen.
- (2) To cancel line drawing, Press **Ctrl + Z** keys. Drawn lines and results will be disappeared.

5) Click **Copy Length List** to copy results of length measurement to clipboard.

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