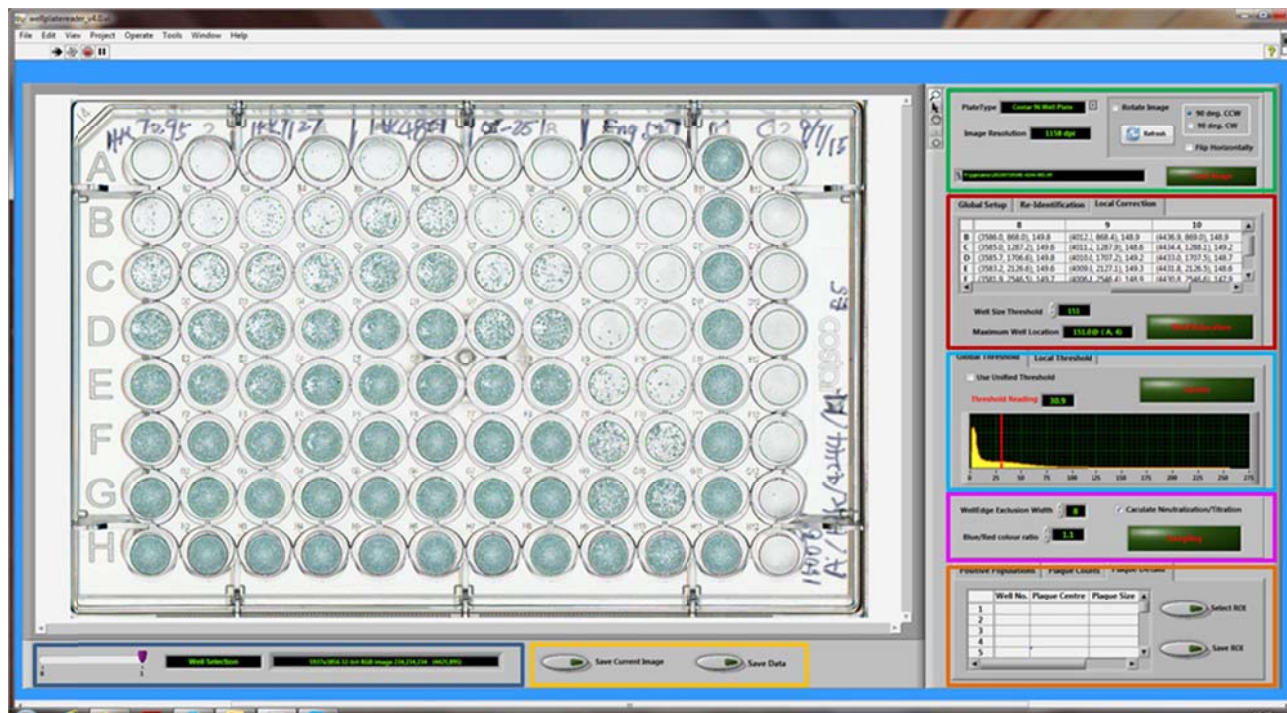


Neutralisation/Titration Quantification Software Help

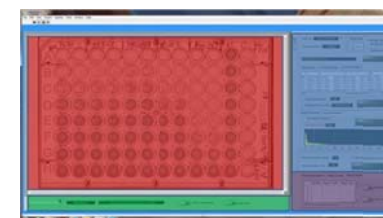
Version 1

Yan Gu

General Description



Desktop layout:



- Image display area
- Image process actions area
- Quantification results display area
- Information display area

The image process is designed into separate steps (in different colour blocks) with procedures running from top to bottom as follows.

Step 1: Load an image

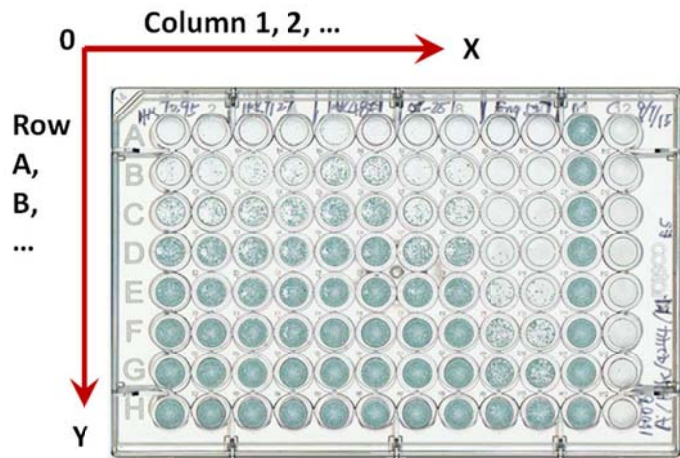
Step 2: Well identification

Step 3: Background thresholding

Step 4: Quantification

Step 5: Calculate Neutralisation/Titration

Step 1: Load an image



Wellplate orientation: During the quantification, the wellplate must be orientated as shown on the left. The top-left corner of the image is the zero coordinates for both X (horizontal) and Y (vertical). X corresponds to Column starts from 1 rightwards to 12 (for 96 wellplate) and Y corresponds to Row starts from A downwards to H (for 96 wellplate). The software expects the viral/serum dilution in a titration/neutralisation experiment goes from low to high from the top-left corner of a plate.

The image may be scanned in one of the two vertical orientations and must be rotated before quantification. The **Rotate Image** provides an option to alter image orientation during the loading process (referring to Rotate Image below for more details). Image can also be rotated using third party software (such as Photoshop) prior to the image load. In this case, **Rotate Image** must be unselected. When using Photoshop, it is helpful to add **Auto-contrast** process after image rotation.

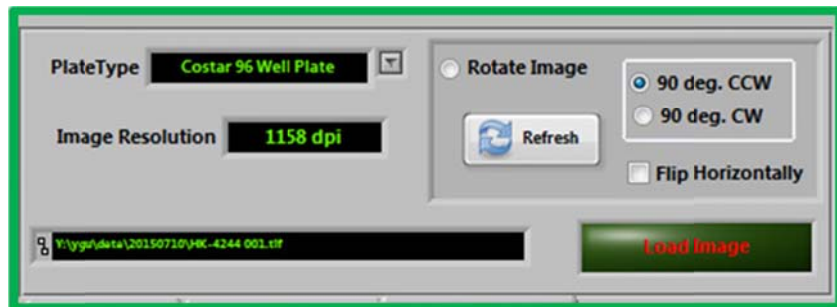


Plate Type: Costar 96 well plate is the default setting. If you change to other plates in the list, the change must be made before pressing **Load Image** button. Bearing in mind that even for same number of wells, different manufacturers use slightly different dimensions. The selection will influence the accuracy of well identification.

Image Resolution: Display of image resolution calculated from the loaded image.

Load Image (button): Start image loading process

Rotate Image: Select **Rotate Image** to activate the function. The activation should be done prior to the image load. It can also be corrected afterwards by pressing **Refresh** button. Depends on the scan orientation, a combination of **90deg. CCW** (counter clockwise) or **90deg. CW** with **Flip Horizontally** can be selected.

Image name and path: Display of file path where results are saved in default.

Step 2: Well identification

After image is loaded, the software does automatic well identification. The automatically identified well will be marked in green circle around well edge. Well locations that failed to be identified are interpreted using neighbouring identified wells. These wells are distinguished in red circles.

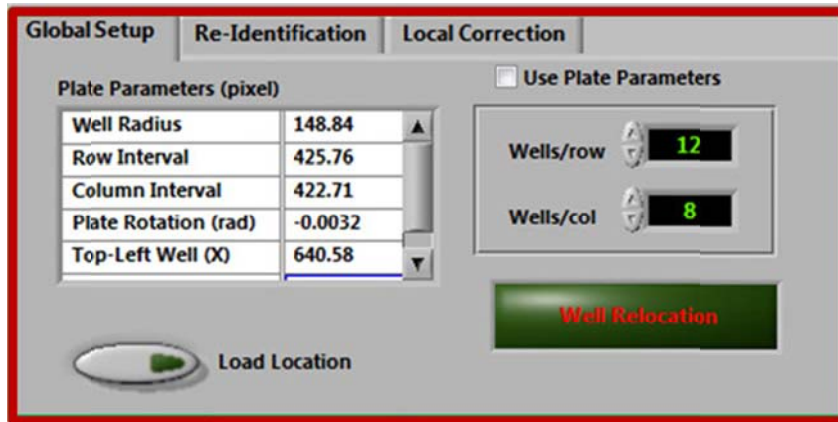


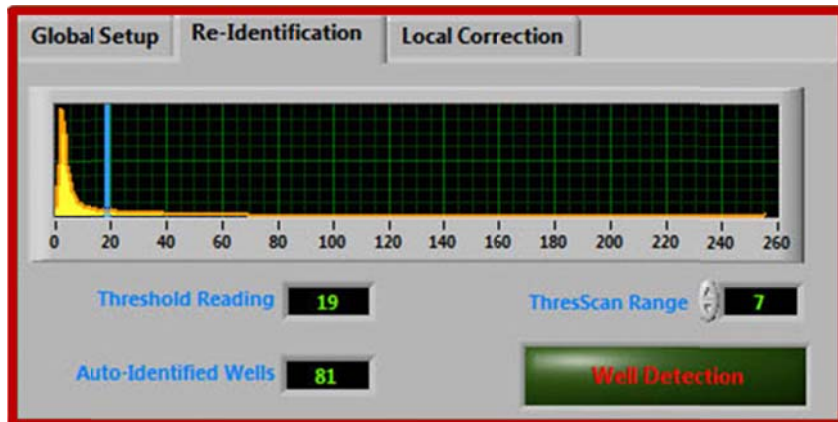
Plate Parameters (pixel): Information about plate parameters varies with number of wells and manufacturers. They are pre-set by administrator. Please do not change.

Use Plate Parameters: Click only when settings in **Plate Parameters (pixel)** are changed.

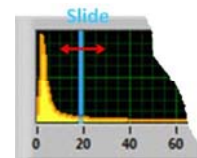
Wells/row & Wells/col: Change only when **Plate Parameters** is changed.

Load Location: Load saved **_Parameters.dat* file with identified well location from last process. This function may be useful when the quantification results needs to be revisited.

Well Relocation: Update any changes made above.



Auto-Identified Wells: Number of wells automatically detected. This is used to judge the quality of well identification. Low number (compared with total wells/plate) implies poor image quality.



Change threshold may improve the number of automatically detected wells. To change the threshold, slide the blue line across the histogram.

Threshold Reading: Display in number the threshold set by the blue line.

ThresScan Range: It defines the searching radius during the well identification. "7" is the optimised range.

Well Detection (button): Redo the well identification to validate any changes.

Global Setup		Re-Identification		Local Correction	
	8	9	10		
B	(3586.0, 868.0), 149.8	(4012.3, 868.4), 148.9	(4436.9, 869.0), 148.9		
C	(3585.0, 1287.2), 149.6	(4011.2, 1287.9), 148.6	(4434.4, 1288.1), 149.2		
D	(3585.7, 1706.6), 149.8	(4010.0, 1707.2), 149.2	(4433.0, 1707.5), 148.7		
E	(3583.2, 2126.6), 149.6	(4009.9, 2127.1), 149.3	(4431.8, 2126.5), 148.6		
F	(3581.9, 2546.5), 149.7	(4006.8, 2546.4), 148.9	(4430.8, 2546.6), 147.9		

Well Size Threshold

Maximum Well Location **151.0 @ (A, 4)**

Well Relocation

Well Size Threshold: Well radius up limit. Any bigger wells identified during well identification process are capped to this maximum.

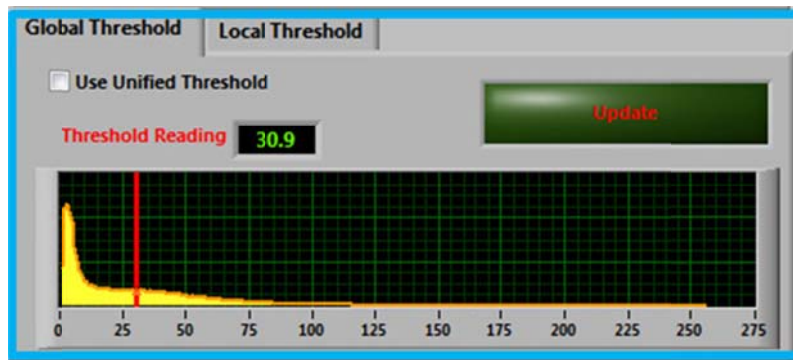
Maximum Well Location: Display of the biggest well radius detected on the plate.

Well Relocation (button): Redo the well identification to validate any changes.

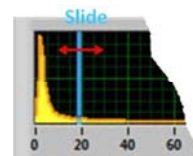
	8
B	(3586.0, 868.0), 149.8
C	(3585.0, 1287.2), 149.6
D	(3585.7, 1706.6), 149.8
E	(3583.2, 2126.6), 149.6
F	(3581.9, 2546.5), 149.7

This is a lookup table of the well plate with matched columns and rows. Data showed in each cell is the central coordinates and radius of identified well defined as “(x, y), radius”. To modify a well location, (1) Scroll the table, (2) highlight the corresponding well by mouse, (3) modify the coordinates manually, (4) click **Well Relocation** and (5) examine the correction visually on the image. The correction can only be validated by pressing **Well Relocation**.

Step 3: Background Thresholding



Use Unified Threshold: Ignore the lookup table of **Local Threshold**, and use global threshold shown in **Threshold Reading**. If no manual correction on **Local Threshold** is made, the selection makes no difference.



Adjust global threshold with the slide with mouse.

Threshold Reading: display the threshold changes made by the slide.

Update (button): Validate the threshold changes.

Global Threshold		Local Threshold						
	1	2	3	4	5	6	7	8
A	31.00	31.00	31.00	31.00	31.00	31.00	31.00	31.00
B	31.00	31.00	31.00	31.00	31.00	31.00	31.00	31.00
C	31.00	31.00	31.00	31.00	31.00	31.00	31.00	31.00
D	31.00	31.00	31.00	31.00	31.00	31.00	31.00	31.00
E	31.00	31.00	31.00	31.00	31.00	31.00	31.00	31.00
F	31.00	31.00	31.00	31.00	31.00	31.00	31.00	31.00

Well plate lookup table showing threshold in each well. Although not recommended in a typical neutralisation or titration experiment, threshold of individual well can be changed manually. The changes will only be validated when **Use Unified Threshold** is unselected.

Step 4: Quantification

WellEdge Exclusion Width ☒ Calculate Neutralization/Titration

Blue/Red colour ratio

Calculate Neutralization/Titration: Option of Neutralization and Titration calculation (details shown in Step 5)

Sampling: Button to trigger quantification.

WellEdge Exclusion Width: exclude a circular zone from identified well edge (green/red circles) from sampling in order to filter out uncertainty noise that is usually appeared near the well edge. 8 (pixels) is default value.

Blue/Red colour ratio: This ratio provides certain adjustment to cope with the staining colour variation. The typical value is 1.1.

Step 5: Calculate Neutrolization/Titration

NeuroV Titration Calculation

PlateSetup FilePath: []

Costar 96 Well Plate

Dilution in Column
Dilution in Row

	1	2	3	4	5	6	7	8	9	10	11	12
A	40											
B	80											
C	160											
D	320											
E	640											
F	1280											
G	2560											
H	5120											

VC - Viral Control
CC - Cell Control
BD - Bad Data

Populate Plate
Save Settings
Save Settings As
Cancel

Processing Procedures

Neutralization

1. Average duplicate wells
2. Normalization each well by VC with the influence of CC
3. Linear interpolation to find the titre at infection endpoints
4. Save the results

Normalized well = $\frac{\text{Sample well} - \text{CC}/\text{VC}-\text{CC}}{\text{VC}-\text{CC}}$

Titration

1. Average duplicate wells
2. Linearly divide non-saturated well
3. Split the results

Data Save Path: []

Neutralization: Infection Deduction (%) []
Titration: Optimal Virus Population (%) []

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.2	0.2	0.1
B	8.3	25.4	43.8	50.9	50.7	53.3	54.1	56.5	56.3	59.0	56.6	60.7
C	2.8	12.0	20.9	35.7	44.9	48.5	51.3	51.0	52.6	53.8	56.9	52.4
D	54.1	50.2	48.1	52.3	43.7	44.8	44.3	44.2	44.8	47.6	41.0	49.5
E	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.1
F	0.1	0.2	0.1	0.1	0.2	0.3	0.0	0.1	0.1	0.4	0.1	0.1
G	0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.1	0.2
H	0.3	0.2	0.2	0.2	0.1	0.2	0.1	0.0	0.1	0.1	0.2	0.2

Calculate Neutralization/Titration is split into top and bottom two parts.

The top part reflects the well plate settings of an experiment, including viral dilutions and variation of serum/virus. The settings has to be completed manually before calculation. It is easier to use the same well-plate setup in routine experiments.

The bottom part is related to quantification including Positive Population (%), Neutralization Process and Titration Process.

Positive Population (%): display the sampling results derived from above Sampling process (Step 4).

Neutralization Process: display neutralisation results including titre in steps.

Titration Process: display titration results in steps.

Step 5.1 define well plate setup

PlateSetup FilePath: []

Costar 96 Well Plate

Dilution in Column
Dilution in Row

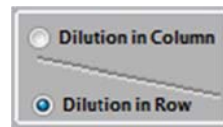
	1	2	3	4	5	6	7	8	9	10	11	12
A	40											
B	80											
C	160											
D	320											
E	640											
F	1280											
G	2560											
H	5120											

VC - Viral Control
CC - Cell Control
BD - Bad Data

Populate Plate
Save Settings
Save Settings As
Cancel



PlateSetup FilePath: Load saved wellplate setup file. If the current well plate setup is identical to a previous setup, it is quicker to load.

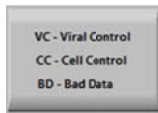


Dilution in Column: If viral dilution is set along column.

Dilution in Row: Select if viral dilution is set in row.



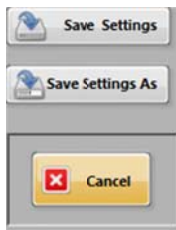
Fill in names and dilutions of virus/serum and finish with 'Enter'. Duplicated column/row must use the same name.



VC, CC and BD are abbreviations of Viral Control (VC), Cell Control (CC) and Bad Data (BD). Do not use other name for VC, CC and BD. BD is usually used to mark out unsatisfied wells after setting is populated. Every plate can only has one type of VC and one type of CC. No matter how many duplicates and how they are distributed, all VC (or CC) will be treated as the same type. BD labelled wells will be ignored during the process.

Vic@40	Vic@40	Florida@4	Florida@4	Flu@40	Flu@40	Peterburg	Peterburg	VC	VC	CC	CC
Vic@80	Vic@80	Florida@8	Florida@8	Flu@80	Flu@80	Peterburg	Peterburg	VC	VC	CC	CC
Vic@160	Vic@160	Florida@1	Florida@1	Flu@160	Flu@160	Peterburg	Peterburg	VC	VC	CC	CC
Vic@320	Vic@320	Florida@3	Florida@3	Flu@320	Flu@320	Peterburg	Peterburg	VC	VC	CC	CC
Vic@640	Vic@640	Florida@6	Florida@6	Flu@640	Flu@640	Peterburg	Peterburg	VC	VC	CC	CC
Vic@1280	Vic@1280	Florida@1	Florida@1	Flu@1280	Flu@1280	Peterburg	Peterburg	VC	VC	CC	CC
Vic@2560	Vic@2560	Florida@2	Florida@2	Flu@2560	Flu@2560	Peterburg	Peterburg	VC	VC	CC	CC
Vic@5120	Vic@5120	Florida@5	Florida@5	Flu@5120	Flu@5120	Peterburg	Peterburg	VC	VC	CC	CC

After the names and dilutions of virus/serum are filled in row and column, click **Populate Plate** to populate settings the plate map. Check the original image for unsatisfied wells. Label them with BD if they need to be marked out.



Save Settings (button): Save the current plate setting into data file with the same filename.

Save Settings As (button): Save the current plate setting into data file with a new filename.

Cancel (button): Cancel the whole neutrolization/titration process and close the window.

Step 5.2 Neutrolization/Titration Process

Positive Population (%) Neutrolization Process Titration Process

Data Save Path: Y:\ygu\data\20151217\experiment005

Neutrolization: Infection Deduction (%) 50
Titration: Optimal Virus Population (%) 50

Processing Procedures

Neutrolization

1. Average duplicate wells
2. Normalise each well by VC with the influence of CC

Normalised well = (Sample well - CC)/(VC-CC)

3. Linear interpretation to find the titer at infection criteria.
4. Save the results.

Titration

1. Average Duplicate wells.
2. Manually decide non-saturated well.
3. Save the results

Column Contents

	1	2	3	4	5	6	7	8	9	10	11	12
	Vic	Vic	Florida	Florida	Fisw	Fisw	Peterbur	Peterbur	VC	VC	CC	CC

Row Contents

	A	B	C	D	E	F	G	H
	40	80	160	320	640	1280	2560	5120

0.1	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.2	0.2	0.1
8.3	25.4	43.4	50.9	50.7	53.3	54.1	56.5	56.3	59.0	56.6	60.7
2.8	12.0	20.9	35.7	44.9	48.5	51.3	51.0	52.6	53.6	56.9	57.4
54.1	50.2	48.1	52.1	43.7	44.4	44.3	44.2	44.8	47.6	45.0	49.5
0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.1
0.1	0.2	0.1	0.1	0.2	0.3	0.0	0.1	0.1	0.4	0.1	0.1
0.1	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.1	0.3	0.2
0.3	0.2	0.2	0.2	0.1	0.2	0.1	0.0	0.1	0.1	0.2	0.2

Before the process, three parameters should be defined: **Data Save Path** and percentage of infection deduction where titre is set, and/or the viral concentration in a titration experiment.

Data Save Path: where results will be saved. The default path is the same as image path.

Neutrolization: Infection Deduction (%): titre point.
Infection Deduction = 1- viral positive population (%)

Titration: Optimal Virus Population (%): The ideal positive population of viral concentration during a titration.

The plate map shows the positive populations (%) calculated during sampling. **Processing Procedures** explains the details of the algorithm.

Positive Population (%) Neutrolization Process Titration Process

Duplicate Average

	Vic	Florida	Fisw	Peterbur
A	40	0.1	0.1	0.1
B	80	16.9	47.2	52.0
C	160	7.4	28.3	46.7
D	320	52.1	50.1	44.0
E	640	0.0	0.0	0.1
F	1280	0.2	0.1	0.3
G	2560	0.2	0.2	0.2
H	5120	0.3	0.2	0.2

Normalization

	Vic	Florida	Fisw	Peterbur
A	40	26.89	26.89	26.89
B	80	4.71	-35.29	-41.63
C	160	17.25	-10.34	-34.63
D	320	-41.76	-39.12	-31.07
E	640	27.02	27.02	26.89
F	1280	26.75	26.89	26.62
G	2560	26.75	26.75	26.75
H	5120	26.62	26.75	26.89

Virus Control 19.01
Cell Control 20.47

	Vic	Florida	Fisw	Peterburgh
Titre @ 50 %	87.6	57.0	55.4	54.5

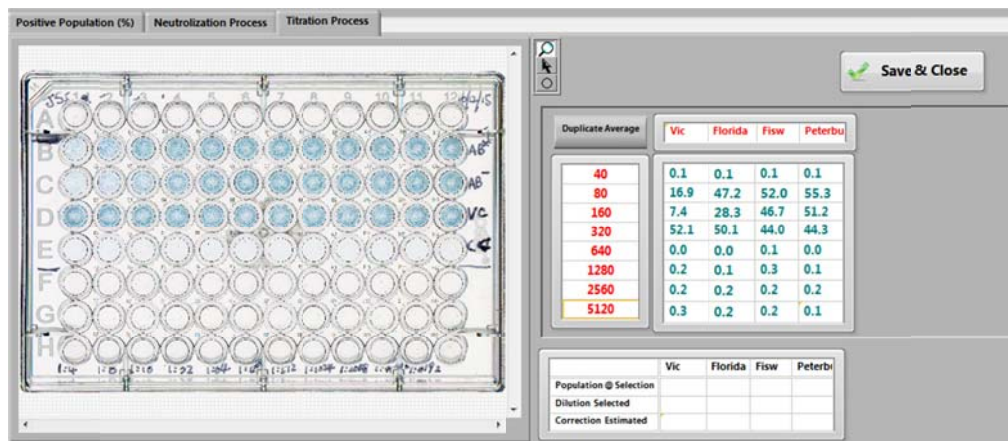
Save & Close

1. Duplicate Average: Average results of all non VC, CC wells. All wells with the same name according to the plate lookup table will be treated as the duplicates and averaged. All VC and CC will be averaged and shown in **Virus Control** and **Cell Control** windows. Only one type of VC and CC are allowed on each plate.

2. Normalization: All averaged wells are normalized against the difference between VC and CC.

3. Titre Estimation: Viral titre for each serum are interpreted according to the Normalization results.

4. Save & Close (button): Click the button to save all displayed data into a file *****_neutralization.txt** and close the whole neutrolisation process.



Titration quantification is a semi-automatic process. Operator examine the average positive population in conjunction with the well plate image to decide The best viral concentration. A typical viral concentration is 30% - 50%.

1. **Duplicate Average:** Average the duplicated wells of positive population.
2. Select the best wells and click the well in **Duplicate Average** with mouse. The estimation of viral dilution will show in the corresponding window as follows.

	Vic	Florida	Fisw	Peterbu
Population @ Selection	36.0	25.7	62.2	2.7
Dilution Selected	10XE-1	10XE-1	10XE-1	10XE-1
Correction Estimated	1.4x	1.9x	0.8x	18.5x

Population @ Selection: Positive population in the selected wells. Usually the selected well is the closest to the defined figure shown in **Titration: Optimal Virus Population (%)**.

Dilution Selected: Corresponding viral dilutions at the selected wells.

Correction Estimated: The correction ratio of dilution that reflects the ratio of positive population between the selected well and **Titration: Optimal Virus Population (%)**. "x" means the factor of correction to the **Dilution Selected**. For instance, in *Vic* column, as 36% positive population is the closest to 50% defined in **Titration: Optimal Virus Population (%)**. The **Dilution Selected** for 36% is 1.0×10^{-1} . To reach the optimal virus population (50%), the viral concentration should be 1.4x less diluted than 1.0×10^{-1} ($1.0 \times 10^{-1} / 1.4$).

Quantification results display

Positive Populations Plaque Counts Plaque Details

	Well No.	Plaque Centre	Plaque Size
1			
2			
3			
4			
5			

Select ROI

Save ROI

Positive Population: histogram of colour saturation of each well with predefined bins. Bins can be adjusted to meet experimental requirements.

Plaque Counts: histogram of plaque size of each well with predefined bins. The plaque quantification is only suitable for signal well microscopic imaging.

Plaque Details: Detailed list of plaques within each well. The list is updated by mouse click on the corresponding well in the image.

Information Display

Well Selection 5937x3856 32-bit RGB image 234,234,234 (4425,895) Save Current Image Save Data

Well Selection

Show information of the current displayed image. The slide increases with the progress of quantification. The window gives the name of the current image.

5937x3856 32-bit RGB image 234,234,234 (4425,895)

General image information including image size, image type, RGB pixel value and image coordinates of mouse cursor.

Save Current Image (button): Save the current displayed image .

Save Data (button): Save all the quantification results.