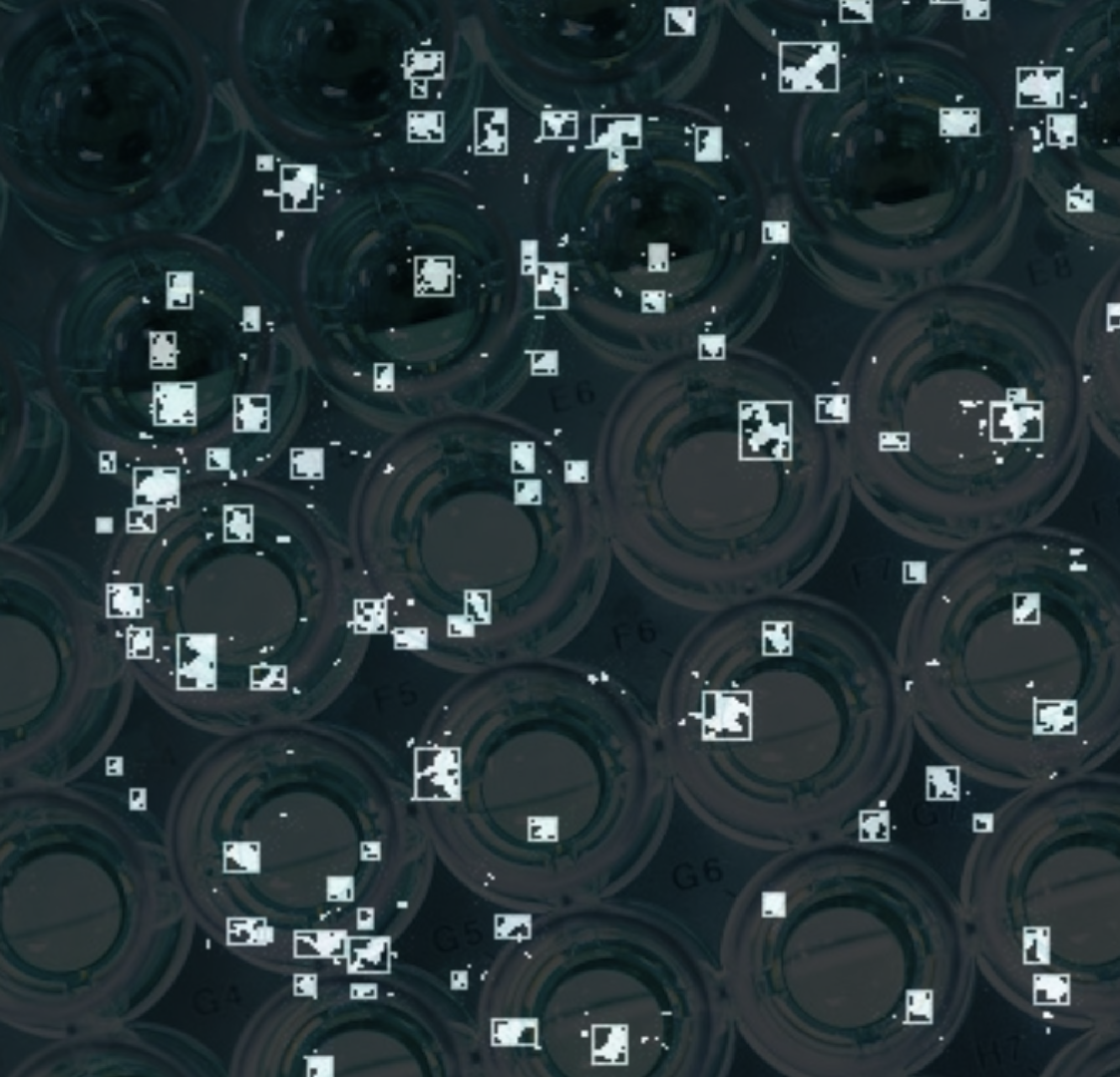


A close-up photograph of a 96-well plate, showing the circular wells and their arrangement in a grid. The image is overlaid with a semi-transparent teal rectangle that contains the project title.

# *Image segmentation* **DENV Foci Detection** *for Focus forming assay*

Image source:  
[https://www.freepik.com/premium-photo/close-up-view-96-well-plate-used-biology-research\\_2652535.htm#page=1&query=96 well plate&position=22](https://www.freepik.com/premium-photo/close-up-view-96-well-plate-used-biology-research_2652535.htm#page=1&query=96%20well%20plate&position=22)



A microscopic image showing numerous circular, dark, textured structures, likely cells or viral foci, arranged in a grid-like pattern. The image is overlaid with a dark, semi-transparent grid of small squares, some of which contain white, pixelated patterns, suggesting a segmentation or detection process.

*Image segmentation for dengue virus foci  
detection in forming focus assay*

---

**Introduction**

**Datasets**

**Program Pipeline**

**Evaluation**

**User manual**

---

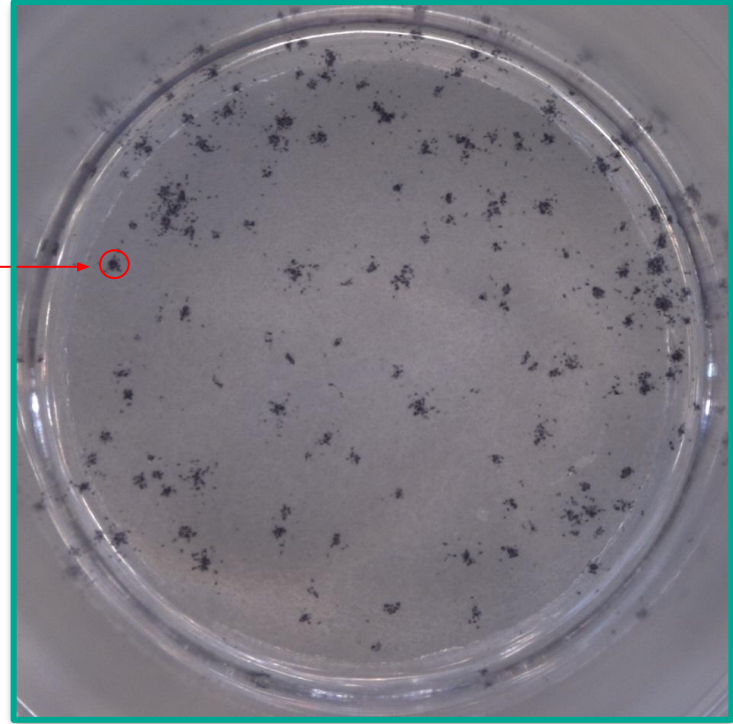
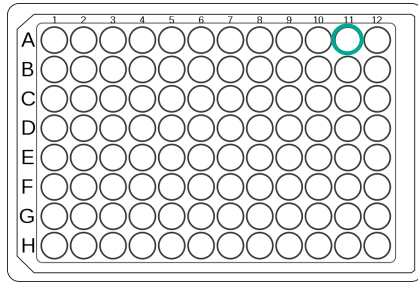
**OUTLINE**

# Introduction

## Focus forming assay

The focus forming assay (FFA) is an immunostaining technique and a variation of the **viral plaque assay**. Instead of detecting the plaque formation after virus-induced cell lysis these assays detect infected host cells and infectious virus particles before a plaque is formed.

## 96-well plate



# Datasets

## FFA: 4 plates

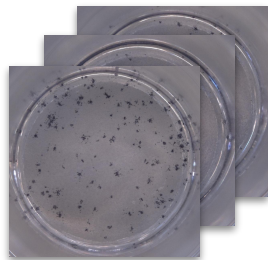
**Foci count**  
(by human)

336 rows

	id	Human count
0	P1_A1	0
1	P1_A2	33
2	P1_A3	41
3	P1_A4	40
4	P1_A5	66
...	...	...

**Raw image**

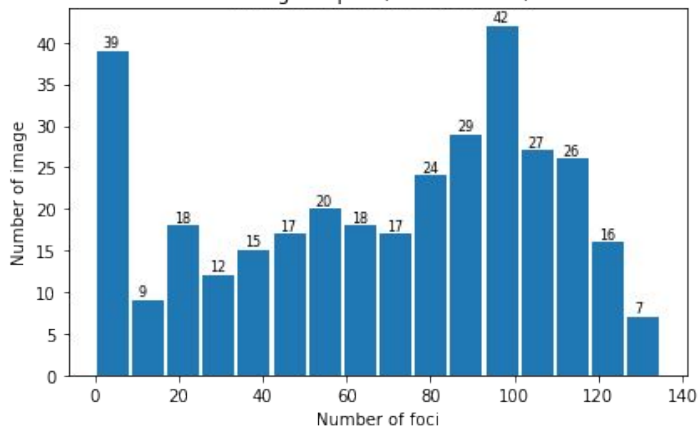
335 images



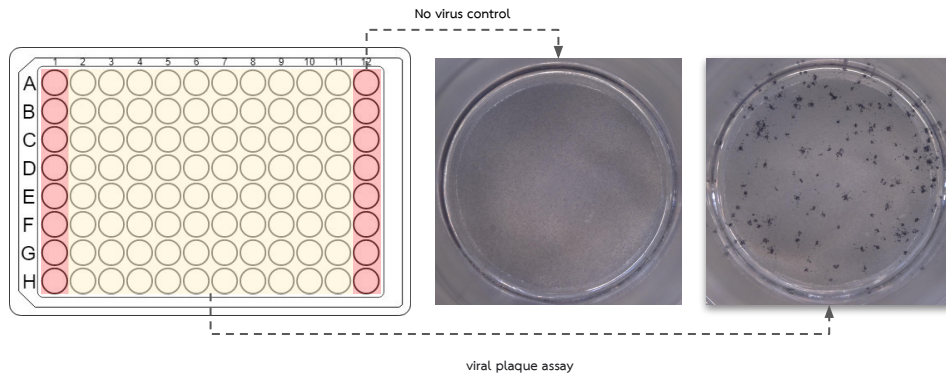
Foci negative: 20 images (control 16 images)

Foci positive: 315 images

Histogram plot (human count)



(n=336)



# Datasets

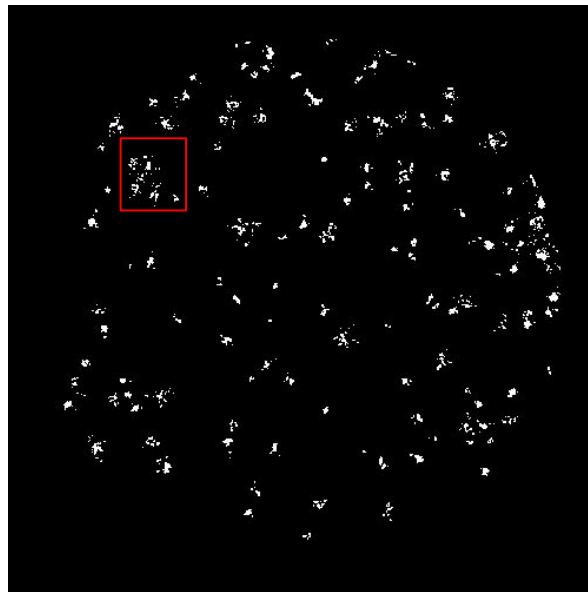
## Pixel mask

319 file

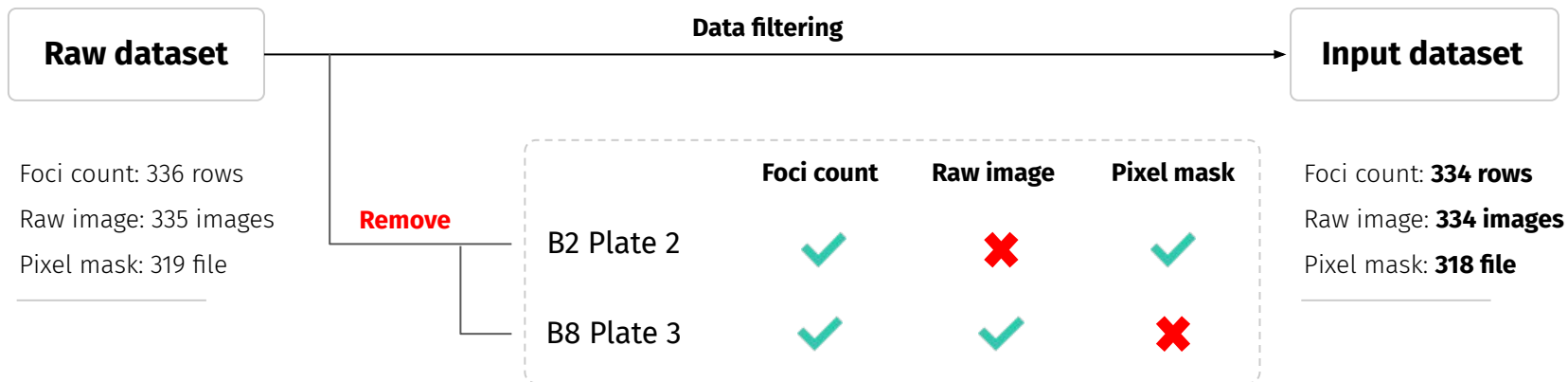
x	y	cluster
215	52	1
218	52	1
265	52	2
266	52	2
191	53	3
192	53	3
193	53	3
194	53	3



*Pixel mask*

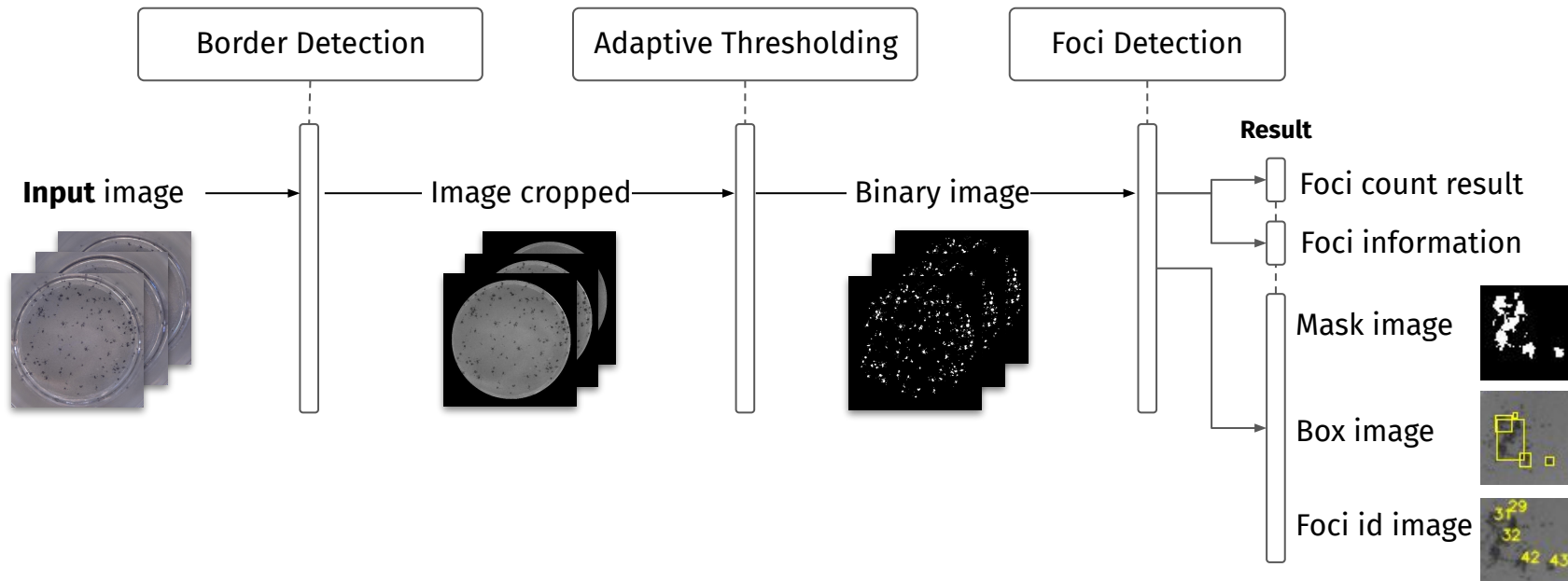


# Datasets





# Program pipeline



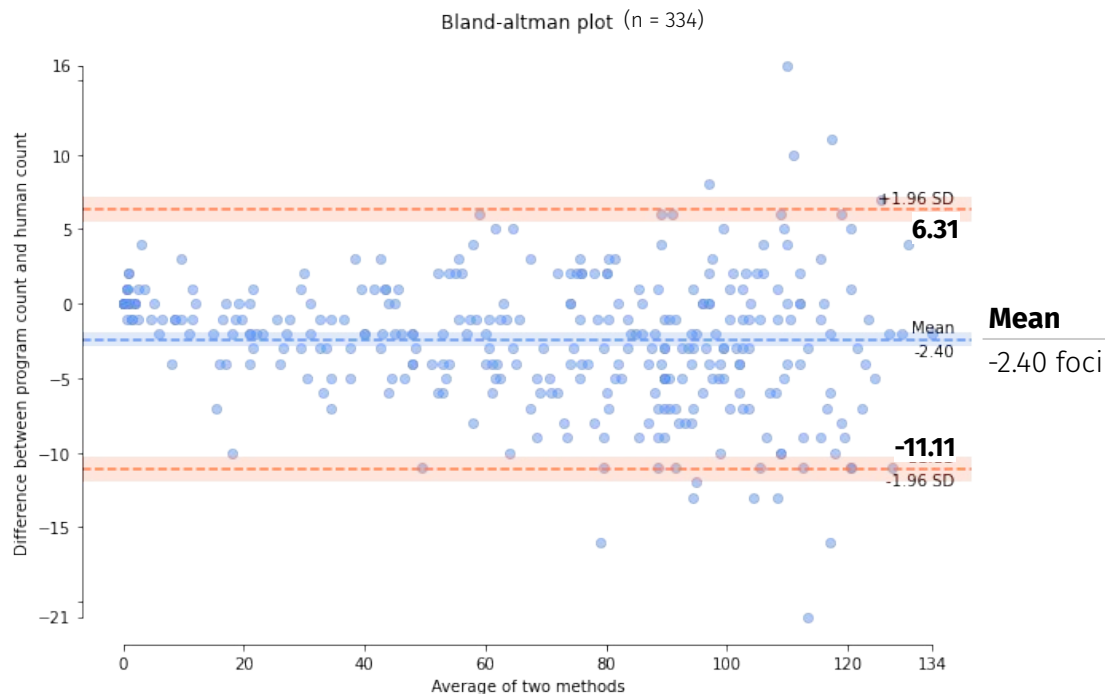
# Evaluation

## Bland-Altman

Bland-Altman plot is a method to describe agreement between two quantitative measurements. These statistical limits are calculated by using the mean and the standard deviation of the differences between two measurements.

### Program - Human

The average of Program-count less than Human-count is 2.4 foci





# Evaluation

## *t-test :Paired two sample for mean*

A paired t-test is used to compare two population means where you have two samples in which observations in one sample can be paired with observations in the other sample.

Hypothesized Mean Difference = 0

Alpha = 0.05

P-value =  $2.75 \times 10^{-21}$  (Reject  $H_0$ )

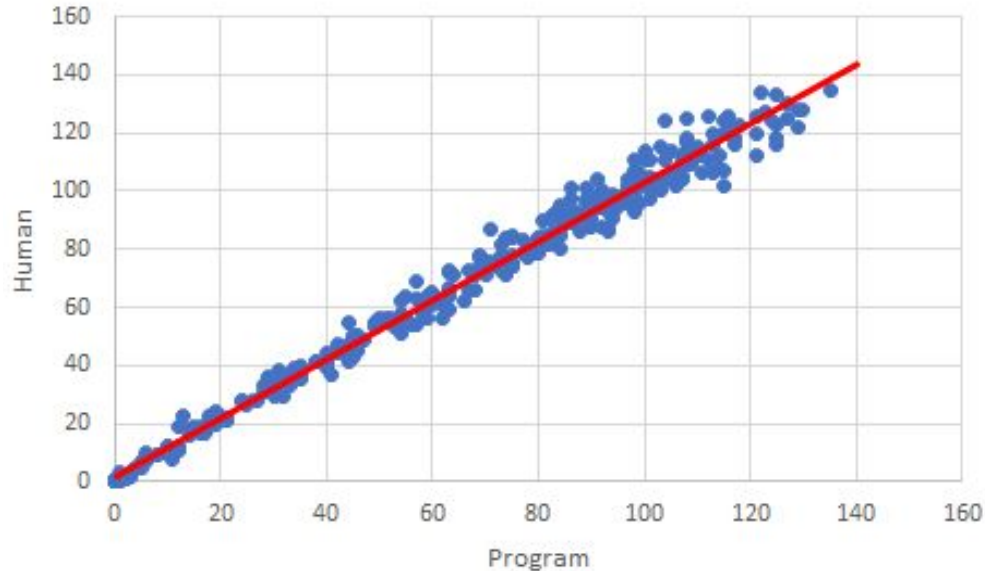
The program counted foci significantly difference human count (p-value < 0.05)

	Program	Human
Mean	65.655	68.149
Variance	1452.082	1507.365
Observations	334	334
Pearson Correlations	0.9933	
Hypothesized Mean Difference	0	
df (degree of freedom)	333	
t Stat	-10.1558	
P(T<=t) one-tail	$1.37 \times 10^{-21}$	
t Critical one-tail	1.649	
P(T<=t) two-tail	$2.75 \times 10^{-21}$	
t Critical two-tail	1.967	

# Evaluation

## Regression statistic

Regression analysis is a set of statistical methods used for the estimation of relationships between a dependent variable and one or more independent variables. It can be utilized to assess the strength of the relationship between variables and for modeling the future relationship between them.



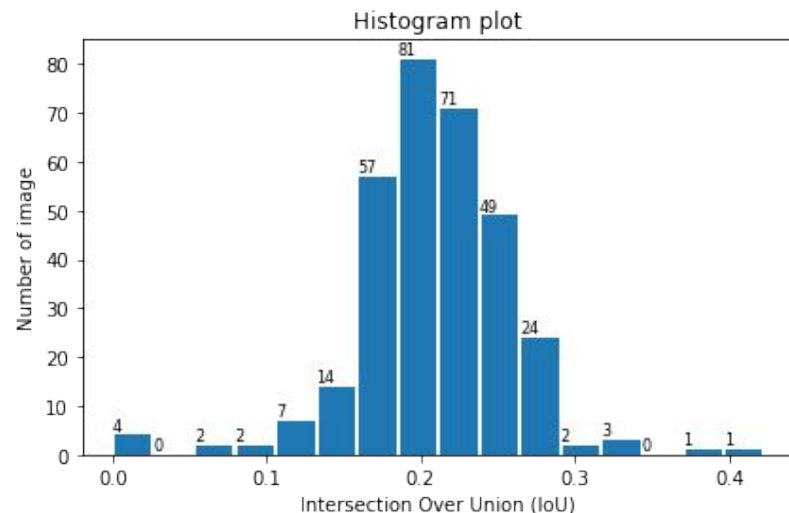
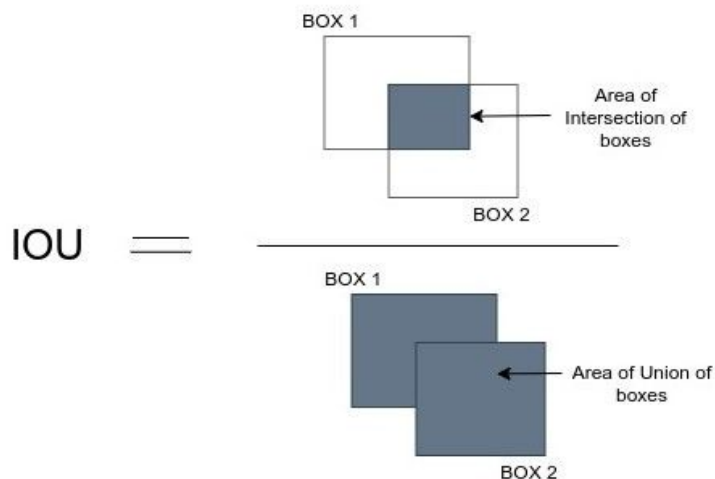
**Regression Statistics table**

Multiple R	0.993
R Square	0.986
Adjusted R Square	0.986
Standard Error	4.388
Observations	334

# Evaluation

## Intersection Over Union (IoU): pixel position

IOU(Intersection over Union) is a term used to describe the extent of overlap of two boxes. The greater the region of overlap, the greater the IOU.



Average IoU of pixel position for each foci from the program detected image and the original pixel position is 21%.

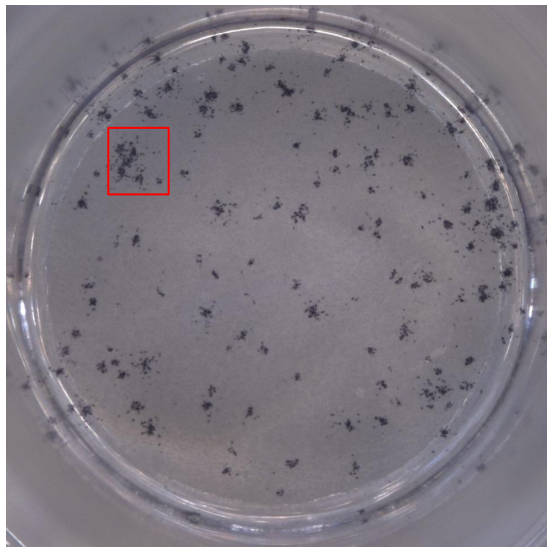
# Evaluation

*Intersection Over Union (IoU): pixel position*

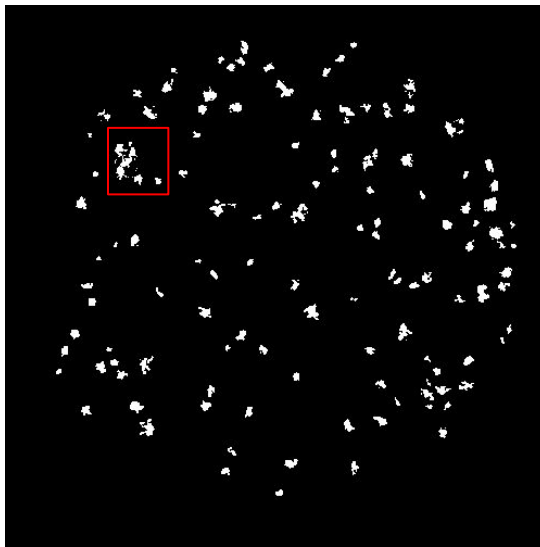


$\text{IoU} = 0.265$

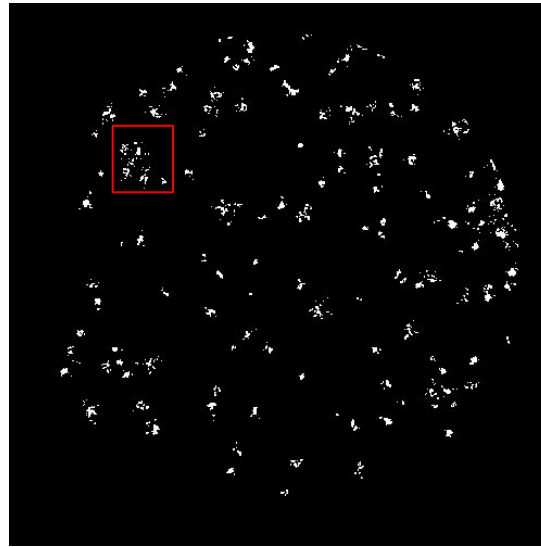
*A11 raw image*



*A11: Program*



*A11: Pixel mask*



# Evaluation

## Confusion matrix (Specificity)

Specificity measures a test's ability to correctly generate a negative result (also known as the "true negative" rate).

id	Program	Human
P1_A1	0	0
P1_A12	0	0
P1_B12	0	0
P1_B1	0	0
P1_C1	0	0
P1_C12	0	0
P1_D12	0	0
P1_D1	0	0
P1_E1	0	0
P1_E12	1	0
P1_F12	0	0
P1_F1	0	0
P1_G1	0	0
P1_G12	0	0
P1_H12	0	0
P1_H1	0	0

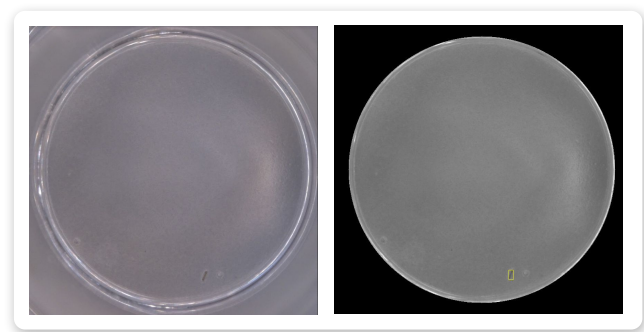


Image : P1\_E12





$$\text{Specificity} = \text{TN} / (\text{TN} + \text{FP})$$

$$\text{TN} = 15$$

$$\text{FP} = 1$$

$$\text{Specificity} = 15/16 = 0.9375$$

# User manual

 app_window.py	วันนี้ 00:37	4 KB	Python Script
 config.ini	วันนี้ 00:37	241 ไบต์	เอกสาร
 foci_detection.py	วันนี้ 00:37	5 KB	Python Script
 README.md	วันนี้ 00:37	190 ไบต์	Markdown File

## 1. config.ini file for set the parameters

config.txt - Notepad  
File Edit Format View Help

[Paths]

input\_image\_folder = D:\internship\code\Python\_configparser\input

output\_image\_folder = D:\internship\code\Python\_configparser\output

[Parameters]

; default parameter: margin = 0 pixel

border\_margin = 0

; set min pixel area for foci detection: 9 pixels is default parameter

min\_foci\_size = 9

1.1 input folder path

1.2 output folder path

1.3 margin setting

### Example

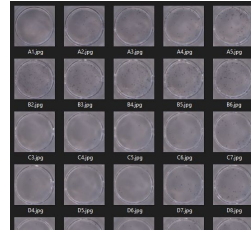
If you want to move the well into 10 pixels, specify that  
border\_margin = 10

If you want to move the well out of 10 pixels, specify that  
border\_margin = -10

1.4 Set the smallest pixel size to be identified as foci by default, 9 pixels.  
You can increase or decrease the minimum size.

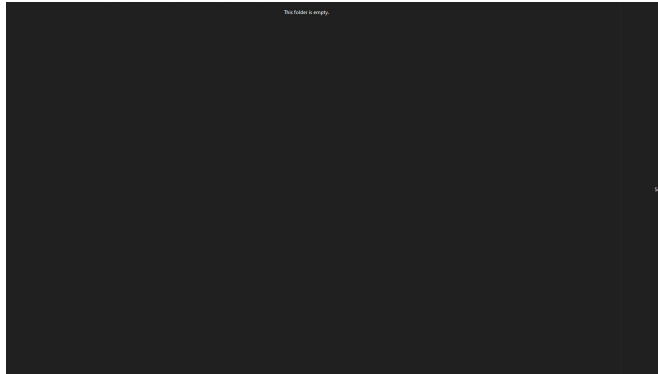
## Input folder

1-figure_P4-DV4-day60	19/7/2564 20:17	File folder
1-figure_P3-DV4-day60	19/7/2564 20:17	File folder
1-figure_P2-DV4-day60	19/7/2564 20:17	File folder
1-figure_P1-DV4-day60	19/7/2564 20:17	File folder



\*Image data in each folder

## Output folder





Example of data input folder by path given in step 1.1  
parameter : `input_image_folder`

Additionally, the input folder will contain a folder of image files (.jpg) of each plate you wish to use in the foci count.

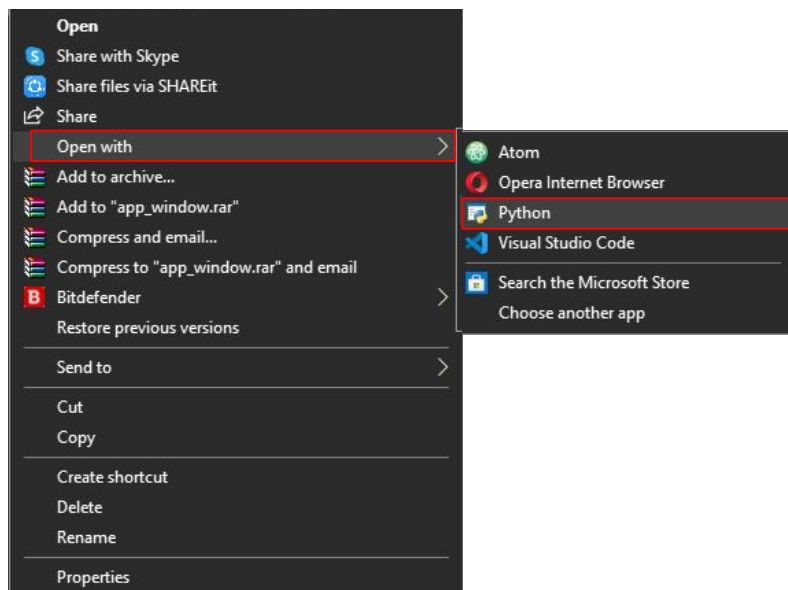
Example of data output folder by path given in step 1.2  
parameter : `output_image_folder`

Additionally, the output folders need to be created and set the name similarly in [Paths]  
`output_image_folder`








 app_window.py	00:37	4 KB	Python Script
 config.ini	00:37	241 ไบต์	เอกสาร
 foci_detection.py	00:37	5 KB	Python Script
 README.md	00:37	190 ไบต์	Markdown File

2. right click on the file:  
app\_window.py > Open with > Python  
For run it with python



## Output folder

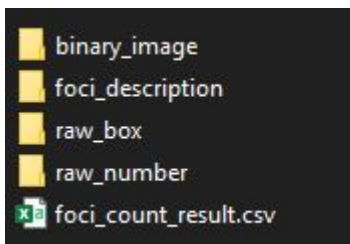
When the program finishes running, the output will be in the output folder with the specified path location.

 1-figure_P1-DV4-day60_2021-7-19_20_27_54	19/7/2564 20:28	File folder
 1-figure_P2-DV4-day60_2021-7-19_20_27_54	19/7/2564 20:28	File folder
 1-figure_P3-DV4-day60_2021-7-19_20_27_54	19/7/2564 20:28	File folder
 1-figure_P4-DV4-day60_2021-7-19_20_27_54	19/7/2564 20:28	File folder
 logfile.log	19/7/2564 20:28	Text Document

The results consist of “Folder by number of input folders” and “logfile”

Each folder contains:

- 1) folder “binary\_image”
- 2) folder “foci\_description”
- 3) folder “raw\_box”
- 4) folder “raw\_number”
- 5) “foci\_count\_result.csv”

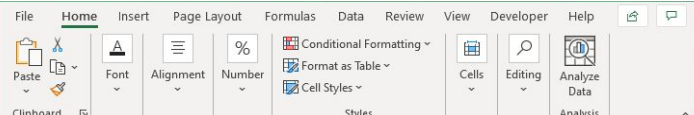


Additionally, 1-figure\_P1-DV4-day60\_2021-7-19\_20\_27\_54 : file name, year/month/day, hours/minutes/seconds respectively

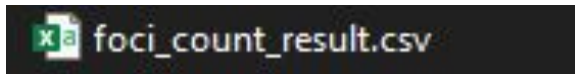
Sample data inside the folder:  
foci\_description

Name	Date modified	Type	Size
A1.csv	14/7/2564 15:25	Microsoft Excel C...	1 KB
A2.csv	14/7/2564 15:25	Microsoft Excel C...	1 KB
A3.csv	14/7/2564 15:25	Microsoft Excel C...	1 KB
A4.csv	14/7/2564 15:25	Microsoft Excel C...	1 KB
A5.csv	14/7/2564 15:25	Microsoft Excel C...	2 KB
A6.csv	14/7/2564 15:25	Microsoft Excel C...	3 KB
A7.csv	14/7/2564 15:25	Microsoft Excel C...	3 KB
A8.csv	14/7/2564 15:25	Microsoft Excel C...	3 KB
A9.csv	14/7/2564 15:25	Microsoft Excel C...	3 KB
A10.csv	14/7/2564 15:25	Microsoft Excel C...	4 KB
A11.csv	14/7/2564 15:25	Microsoft Excel C...	4 KB
A12.csv	14/7/2564 15:25	Microsoft Excel C...	1 KB
B1.csv	14/7/2564 15:25	Microsoft Excel C...	1 KB
B2.csv	14/7/2564 15:25	Microsoft Excel C...	3 KB
B3.csv	14/7/2564 15:25	Microsoft Excel C...	3 KB
B4.csv	14/7/2564 15:25	Microsoft Excel C...	3 KB
B5.csv	14/7/2564 15:25	Microsoft Excel C...	3 KB
B6.csv	14/7/2564 15:25	Microsoft Excel C...	3 KB
B7.csv	14/7/2564 15:25	Microsoft Excel C...	4 KB
B8.csv	14/7/2564 15:25	Microsoft Excel C...	3 KB
B9.csv	14/7/2564 15:25	Microsoft Excel C...	3 KB
B10.csv	14/7/2564 15:25	Microsoft Excel C...	4 KB
B11.csv	14/7/2564 15:25	Microsoft Excel C...	3 KB
B12.csv	14/7/2564 15:25	Microsoft Excel C...	1 KB
C1.csv	14/7/2564 15:25	Microsoft Excel C...	1 KB
C2.csv	14/7/2564 15:25	Microsoft Excel C...	1 KB
C3.csv	14/7/2564 15:25	Microsoft Excel C...	1 KB
C4.csv	14/7/2564 15:25	Microsoft Excel C...	1 KB
C5.csv	14/7/2564 15:25	Microsoft Excel C...	1 KB

Additionally, The folder contains a data file (.csv) with details of Center point (x,y) of each foci, foci size (pixels) and Normalize area (%).



	A	B	C	D	E	F	G	H	I
1		id	centroid_x	centroid_y	area_pixels	Normalize Area %			
2		0	1	190	51	12	0.007		
3		1	2	215	51	21	0.0122		
4		2	3	225	63	34	0.0198		
5		3	4	279	91	29	0.0169		
6		4	5	187	110	20	0.0116		
7		5	6	251	129	32	0.0186		
8		6	7	115	132	27	0.0157		
9		7	8	281	147	42	0.0244		
10		8	9	74	181	21	0.0122		
11		9	10	252	185	27	0.0157		
12		10	11	395	188	36	0.0209		
13		11	12	360	190	13	0.0076		
14		12	13	372	193	36	0.0209		
15		13	14	242	231	43	0.025		
16		14	15	33	245	18	0.0105		
17		15	16	33	267	30	0.0174		
18		16	17	282	268	27	0.0157		
19		17	18	351	291	22	0.0128		
20		18	19	376	312	39	0.0227		
21		19	20	356	317	49	0.0285		
22		20	21	364	335	19	0.0111		
23		21	22	337	338	31	0.018		
24		22	23	56	348	66	0.0384		
25		23	24	275	382	39	0.0227		
26		24	25	319	378	13	0.0076		



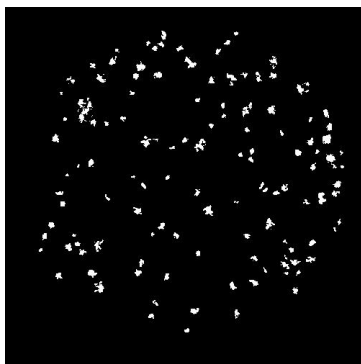
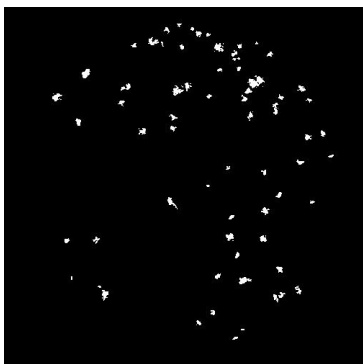
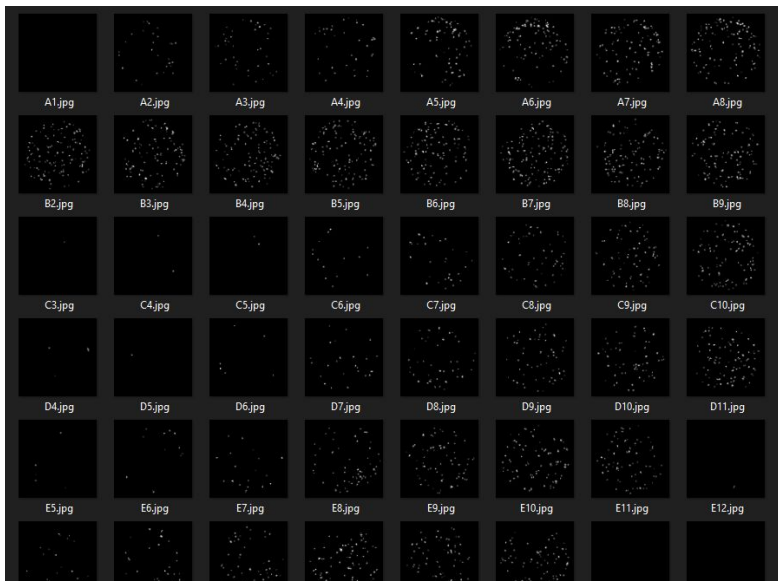
	id	foci count
0	P1_C8	52
1	P1_A1	0
2	P1_C9	68
3	P1_A3	40
4	P1_A2	28
5	P1_A6	92
6	P1_A7	84
7	P1_A5	67
8	P1_B9	105
9	P1_B8	108
10	P1_A4	35

Example, Inside the `foci_count_result.csv` is the result file of counting the number of foci in each image.



Example, Inside the `logfile.log` the result is about the program running status.

```
2021-07-14 14:30:38,838, INFO, app.py, P1, C8.jpg, foci detected: success
2021-07-14 14:30:38,911, INFO, app.py, P1, A1.jpg, foci detected: success
2021-07-14 14:30:39,002, INFO, app.py, P1, C9.jpg, foci detected: success
2021-07-14 14:30:39,058, INFO, app.py, P1, A3.jpg, foci detected: success
2021-07-14 14:30:39,128, INFO, app.py, P1, A2.jpg, foci detected: success
2021-07-14 14:30:39,271, INFO, app.py, P1, A6.jpg, foci detected: success
2021-07-14 14:30:39,381, INFO, app.py, P1, A7.jpg, foci detected: success
2021-07-14 14:30:39,453, INFO, app.py, P1, A5.jpg, foci detected: success
2021-07-14 14:30:39,549, INFO, app.py, P1, B9.jpg, foci detected: success
2021-07-14 14:30:39,637, INFO, app.py, P1, B8.jpg, foci detected: success
2021-07-14 14:30:39,703, INFO, app.py, P1, A4.jpg, foci detected: success
```



Sample data inside `binary_image` folder

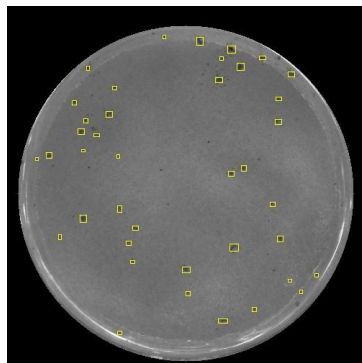
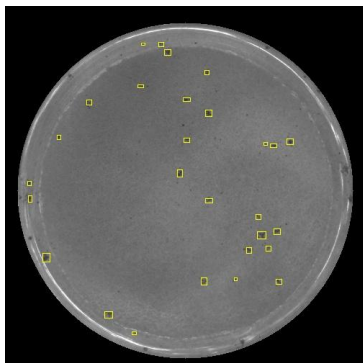
Additionally, This folder contains image files (.jpg) that are binary image (black:0, white:1).

- Black is Background.
- White is Foci.



Sample data inside raw\_box folder

Additionally, This folder contains image files (.jpg) that are raw image with a rectangular frame detected for each foci.





Sample data inside raw\_number folder

Additionally, This folder contains image files (.jpg) that has an id number for each foci. So, that connect to the information file in foci\_description folder.

