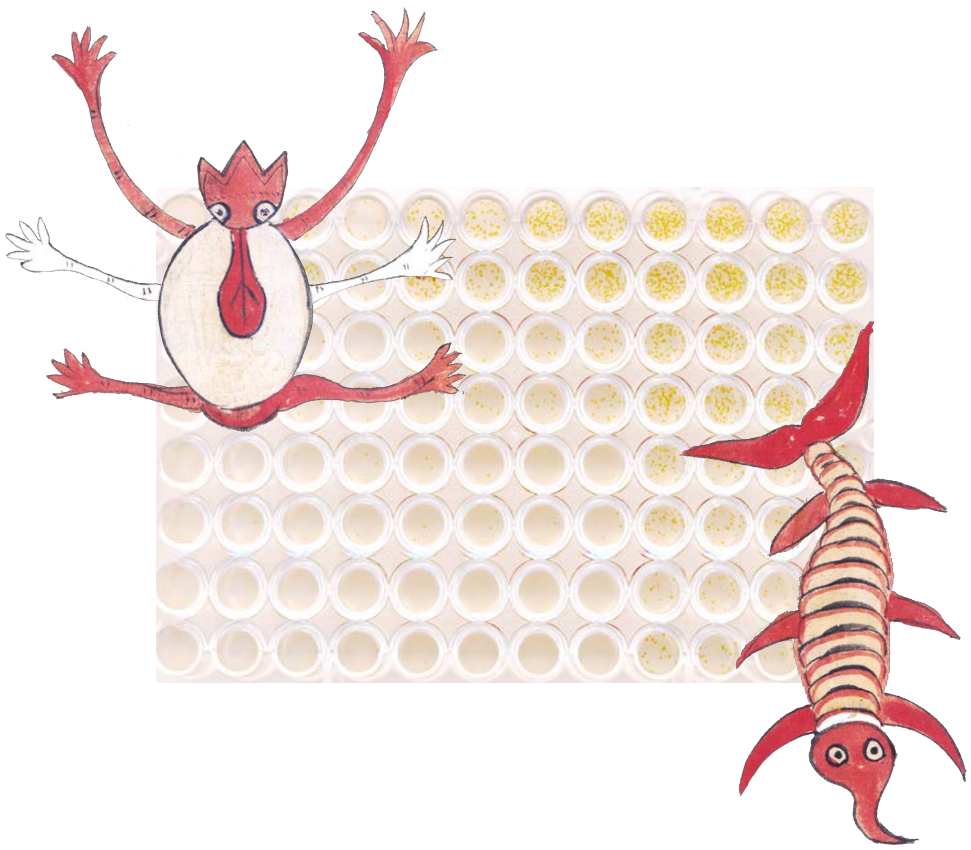
Sabayomikun

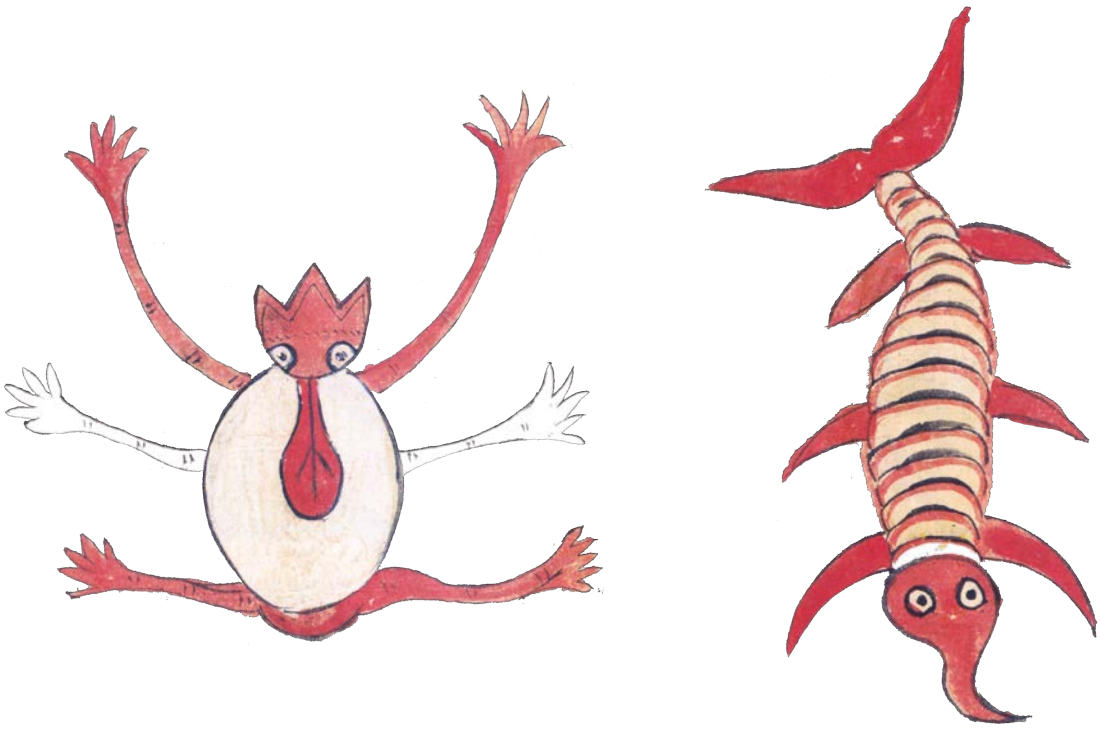
Semi-automatic viral foci counting system



Sabayomikun is a semi-automatic viral foci counting system.

The phrase, "Saba wo yomu" implies incorrect, or very roughly counted numbers. Therefore, "sabayomikun" means "Mr. Miscount".
The icons of the softwares come from a medical book "Harikikigaki" which published in Japan in the 15th century. These strange creatures are explained as pathogens. To see the original book, please visit Kyushu National Museum.
If you think this system is useful for your work, I will continue to improve and maintain this system.

Takeshi Nabeshima
E-Mail:mtmikami@tm.nagasaki-u.ac.jp



Prerequisite: Hardware

This workflow starts from scanned images of DAB stained 96-well plates. Therefore, prepare a scanner. This system was developed with using

EPSON GT-8200U

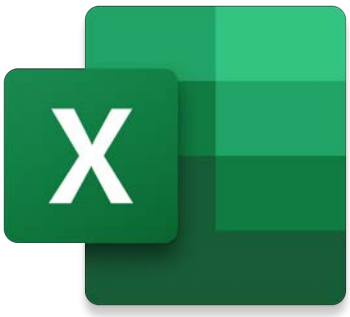
This system is tested on Mac (over 10.12), MS-Windows (over Windows 7, both 32bit and 64bit) and Linux on arm (Raspberry Pi 3B+, Raspbian Jessie).

Prerequisite: Software

The workflow requires 4 softwares.

MS-Excel

If you do not have MS-Office, you can use LibreOffice or Apache OpenOffice instead.



Katikati counter

This is an excellent hand counter software.

Sabayomikun & katisaba

Newly developed softwares for this system.

The Katikati counter is available free for charge from,

MS-Windows ver
https://www.vector.co.jp/download/file/win95/art/fh419524.html

Mac ver
https://www.vector.co.jp/download/file/mac/art/fh613400.html

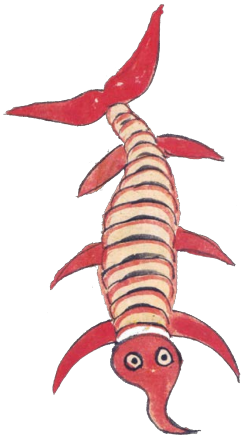
The Katikati counter requires Java8 (it did not work on recently provided Java11 or later). Java8 is available from
https://www.oracle.com/technetwork/java/javase/downloads/index.html

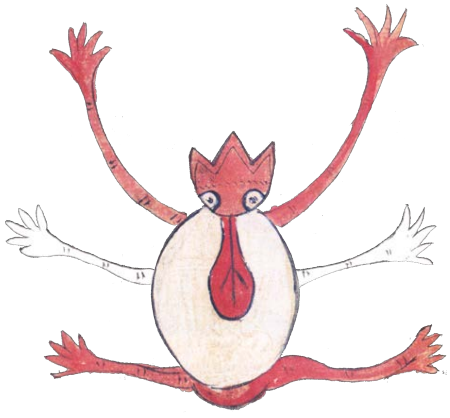
To obtain the executables of sabayomokun and katisaba, please contact to Nabeshima. These softwares are developed with using Python version 3.7.3.

For MS-Windows, these softwares are provided as executable binaries of file name extension “.exe”. You can execute them both from double clicking on icons of these softwares and command from console. Both 64bit and 32bit binaries are prepared. Choose adequate one for your system. De-compress the zip file and move the binaries to the “Desktop”, or somewhere you can access.

For Mac, they are provided as *.app bundles. De-compress the zip file for Mac, and put *.app bundles somewhere in “/Applications” folder.

For Linux, executable command binaries are provided. Put them in your command search $PATH.





This workflow has three steps.

First step:

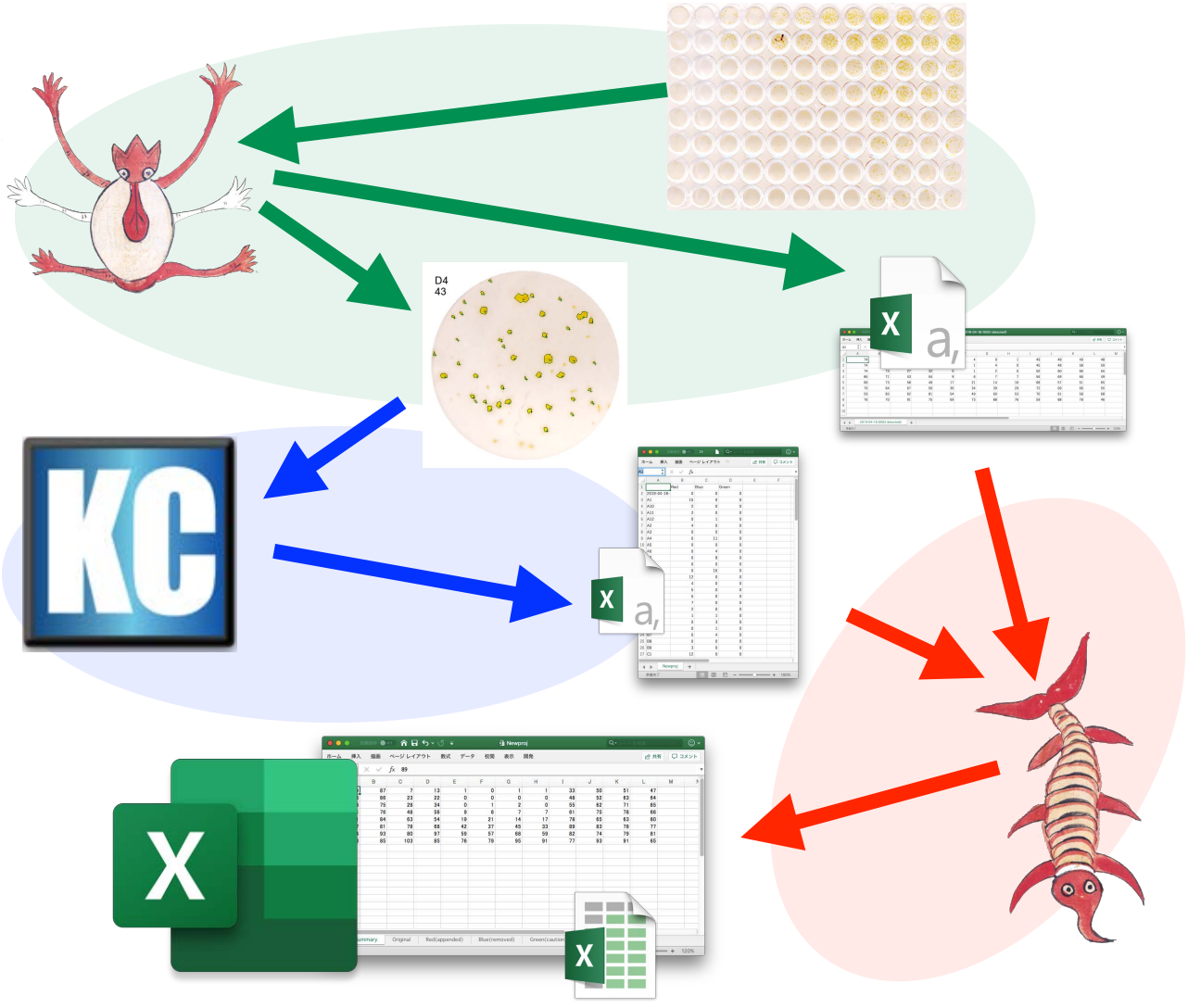
Crop each well from 96-plate images, and counts foci in each well automatically.

Second step:

Correct the mechanical counts from the first step by hand counting.

Last step:

Merge the mechanical count from the first step and the hand count from the second step.



Let’s start.

First step:

Crop each well from 96-plate images, and counts foci in each well automatically.

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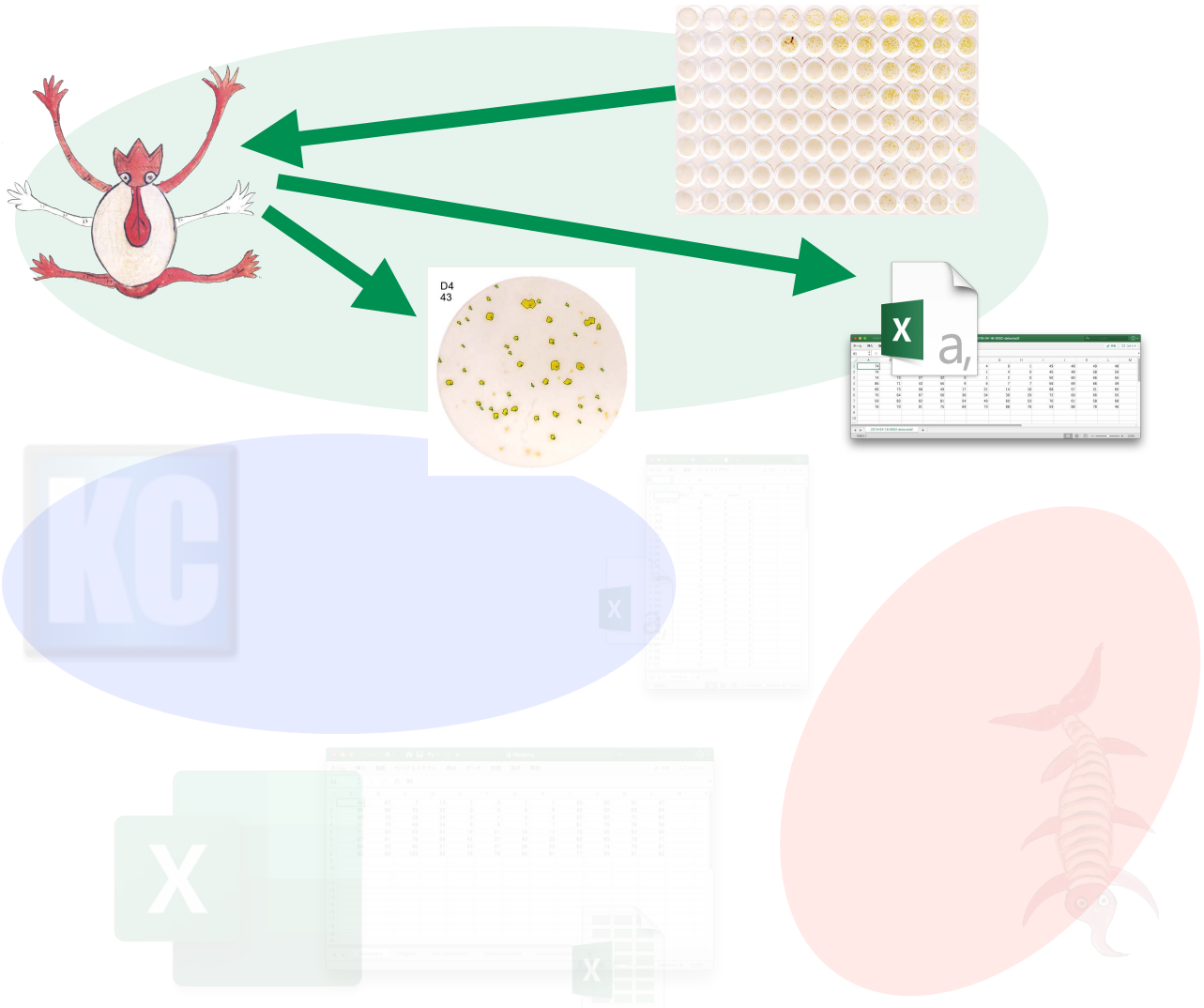
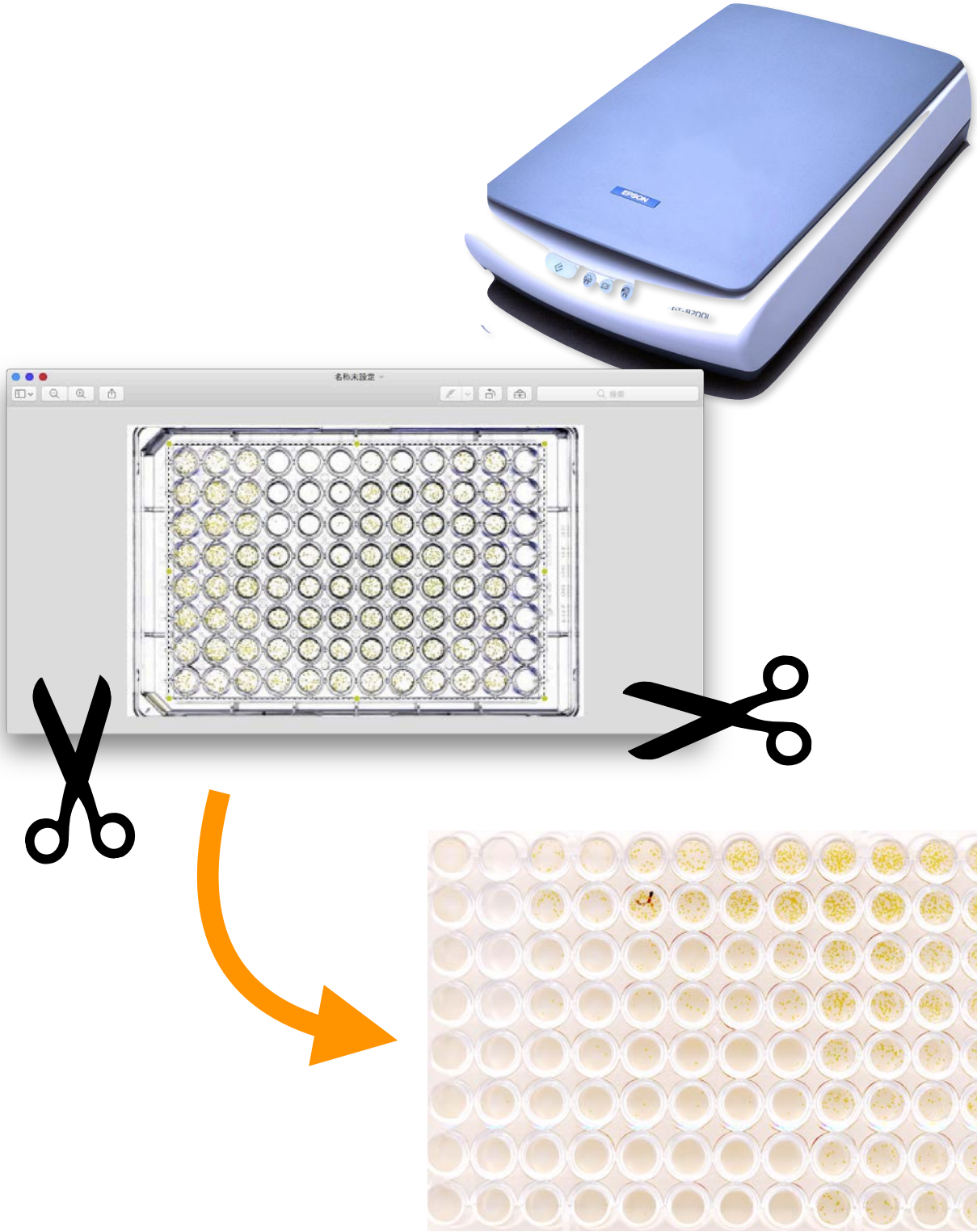
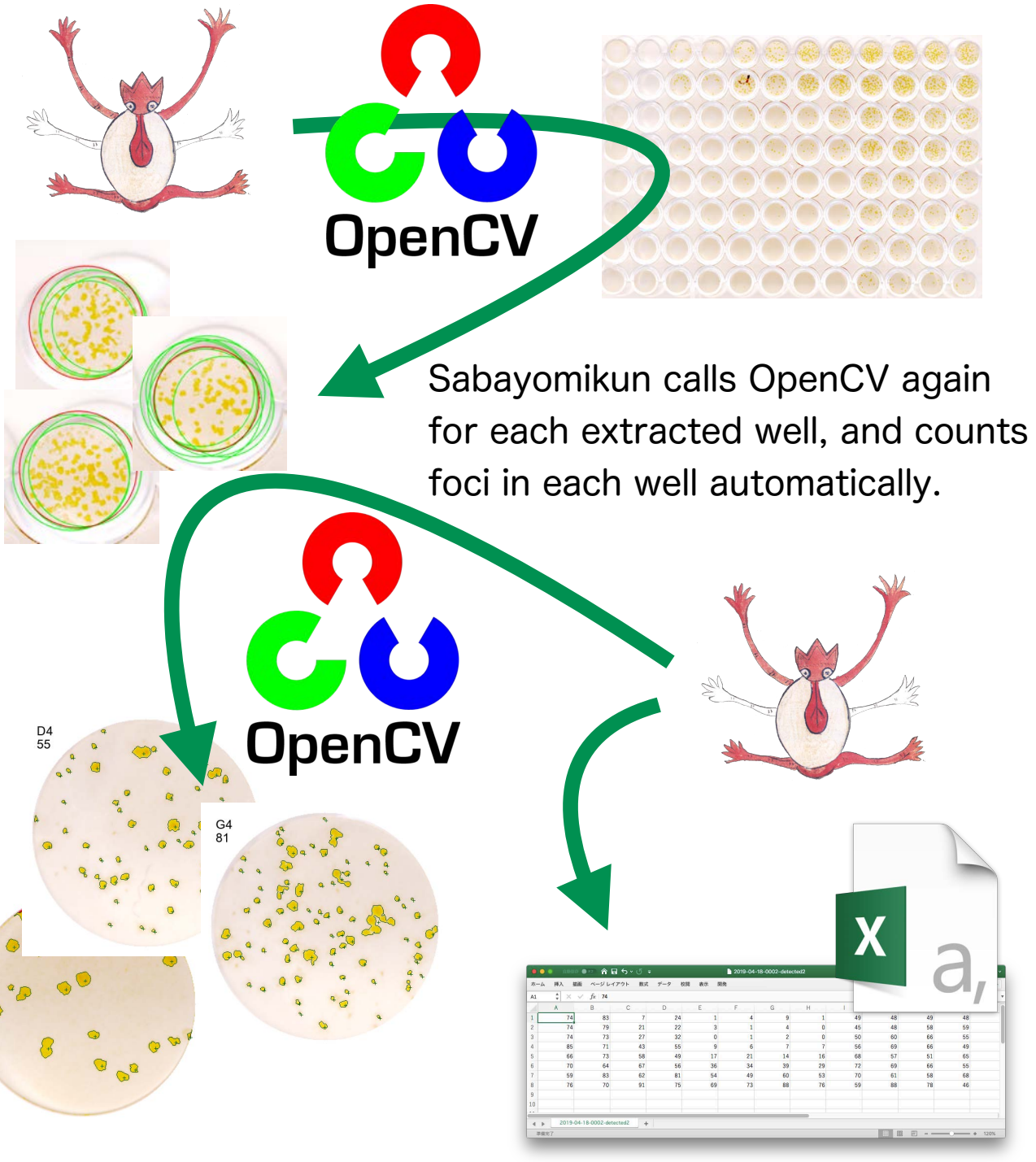


First, scan DAB stained 96-well plates in as high resolution as possible. Trim the area of wells with using some graphic editor software (“Paint” in MS-Windows, “Preview” in Mac?). And save the trimmed images in “tiff”, “jpeg” or “png” format.



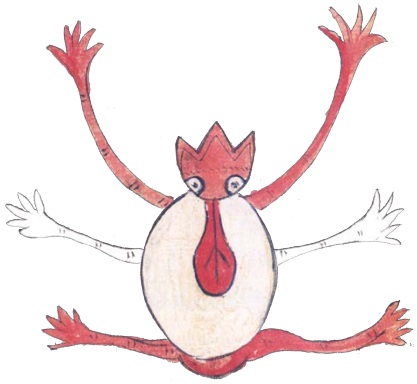
Sabayomikun calls OpenCV, and extracts each well area from 96-well plate images.

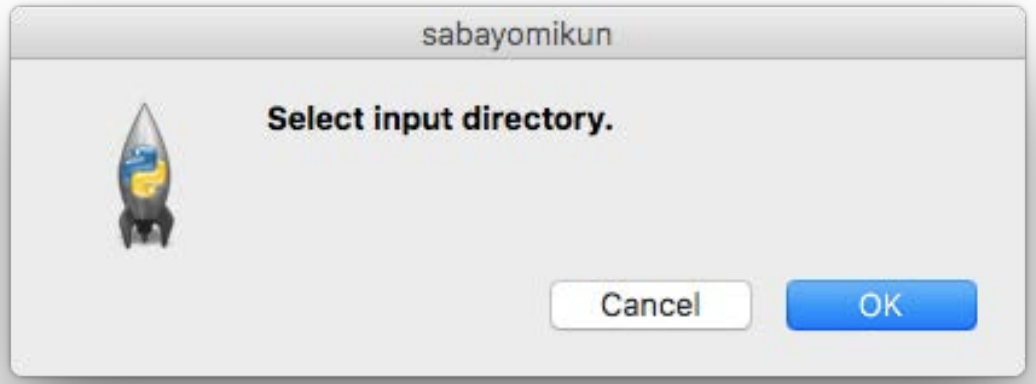


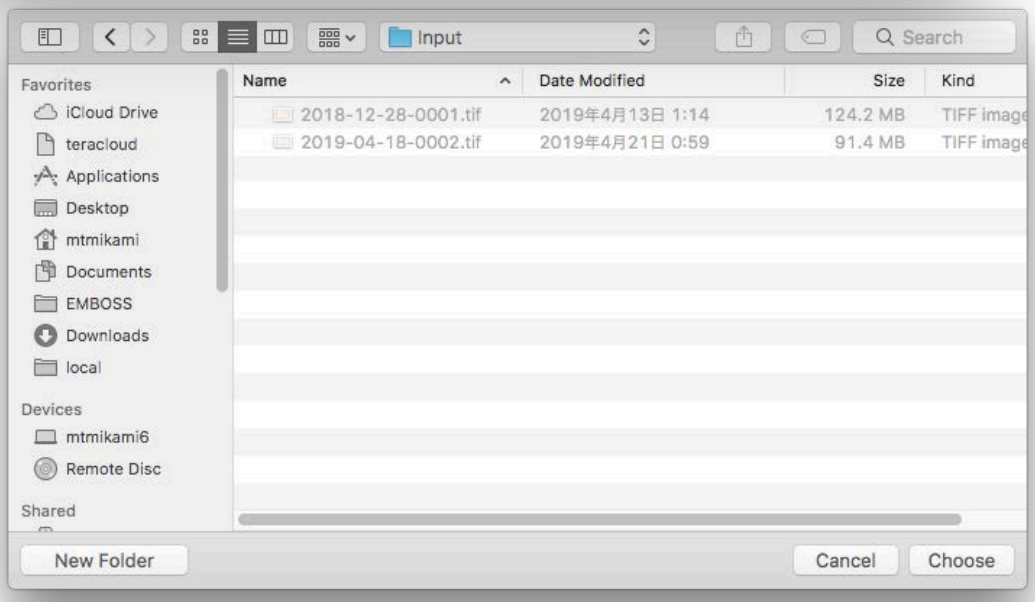
Sabayomikun draws image of each well with marks on the detected foci.

The count results are exported as a “csv” file.

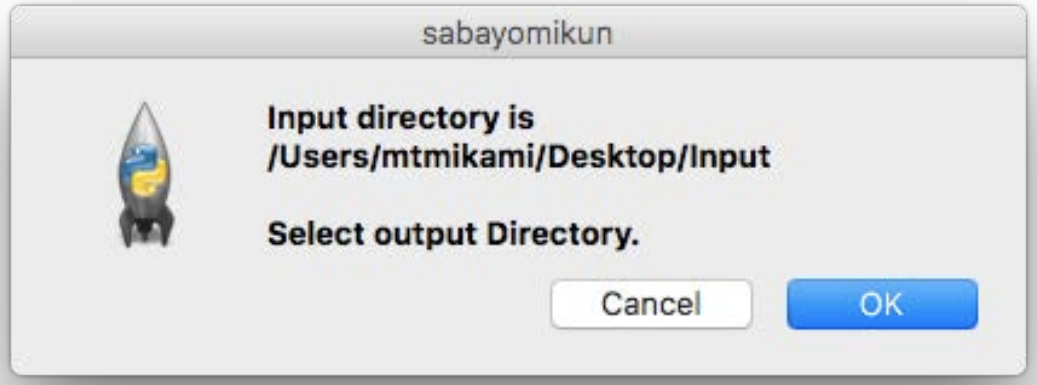
Double click the icon of sabayomikun.
Startup of sabayomikun is slow, so please wait patiently.
Sabayomikun asks the folder which contains plate images.
From file-chooser, select the input folder. In case of some version of MS-Windows, the contents of the input folder are invisible.

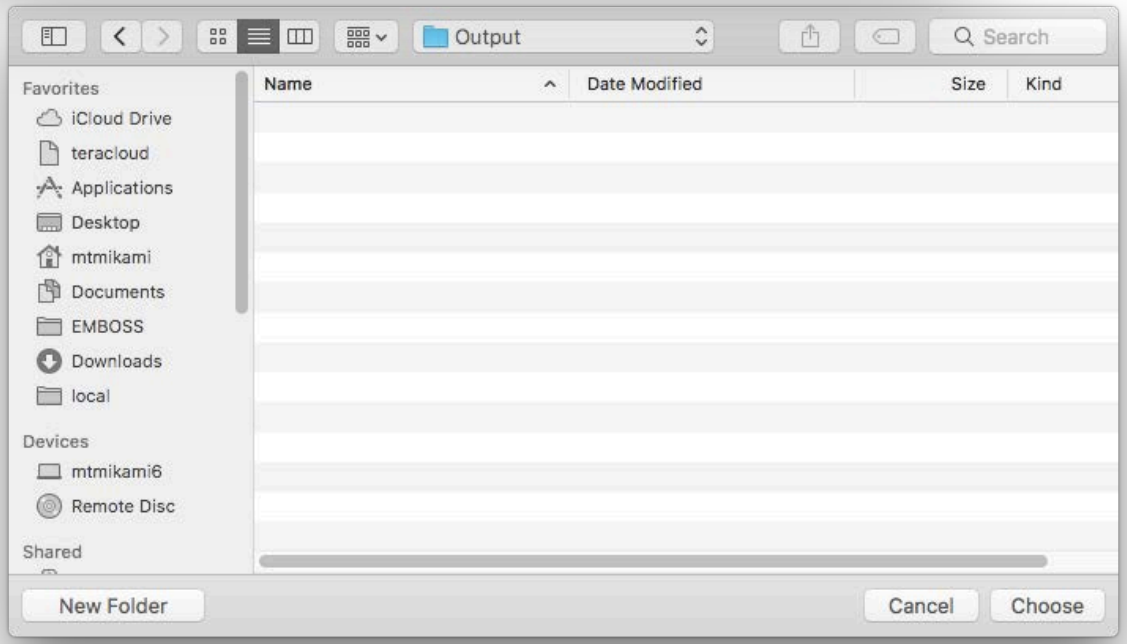




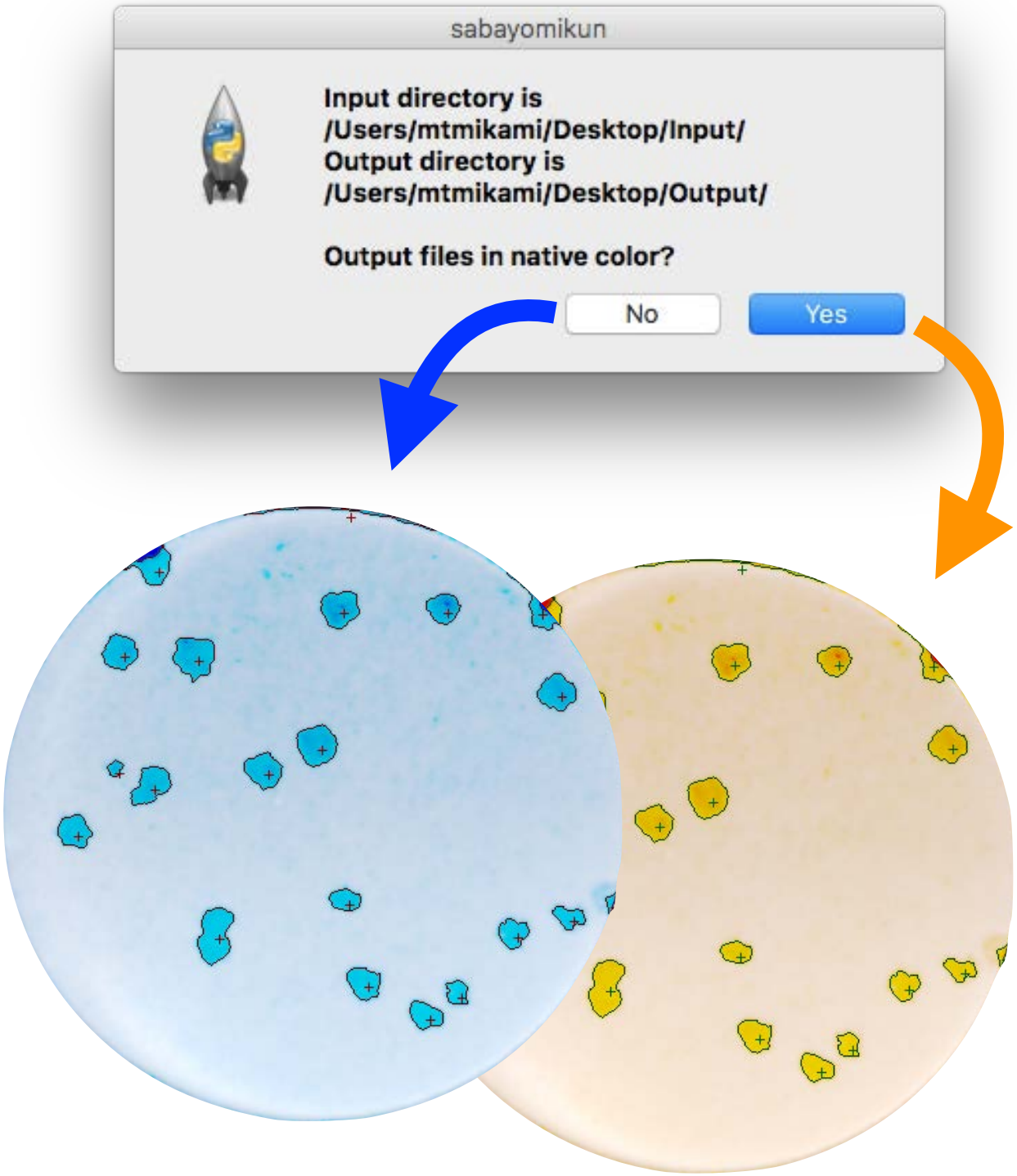


Next, sabayomikun asks the folder to where the output data exported. Select a folder.

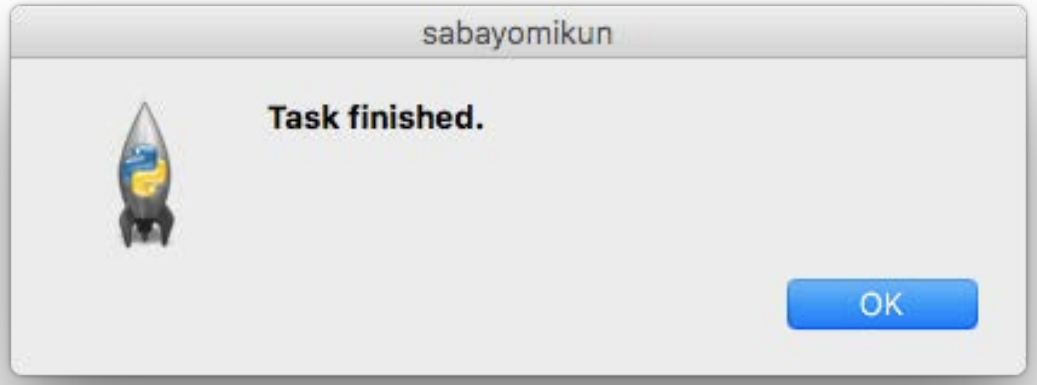


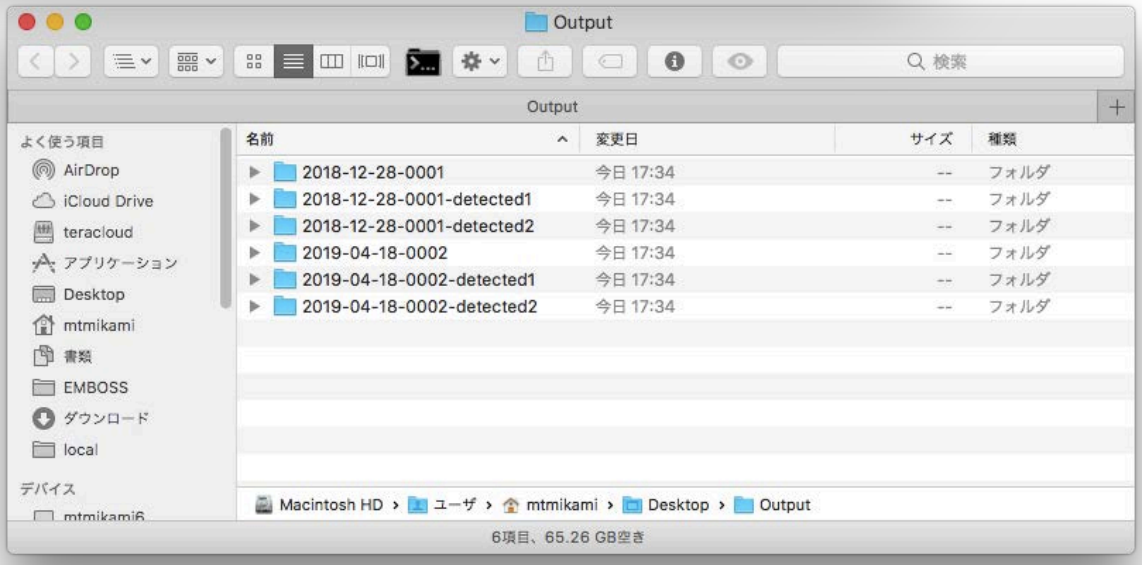


Our using DAB stains foci in light brown or yellow. If you want to get high contrast (blue) images, choose “No” at this step.

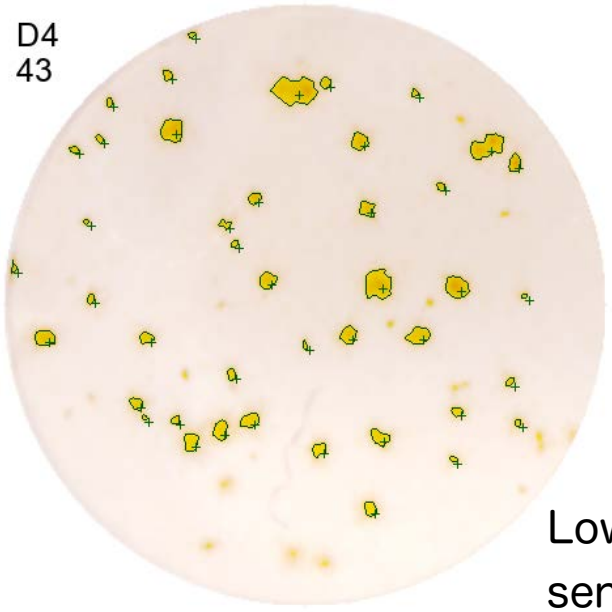


Sabayomikun automatically detects each well on the plate images, and crops them. And counts foci in low and high sensitivity modes.
The results of low and high sensitivity mode are exported in the folder named *-detected1 and *-detected2, respectively.

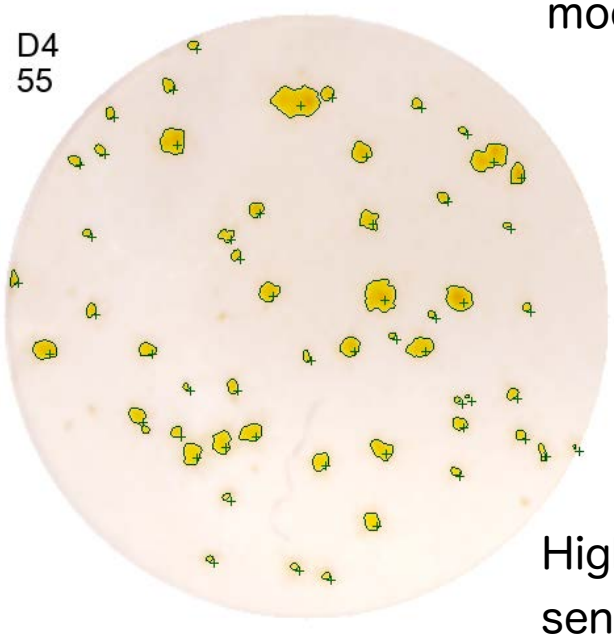




In each well image, the well position and counted number are shown in upper left corner.
The high sensitive mode aggressively detects small, or weakly stained spots. However, sometimes mis detects noises as foci. Look and compare the output images, and choose better one.

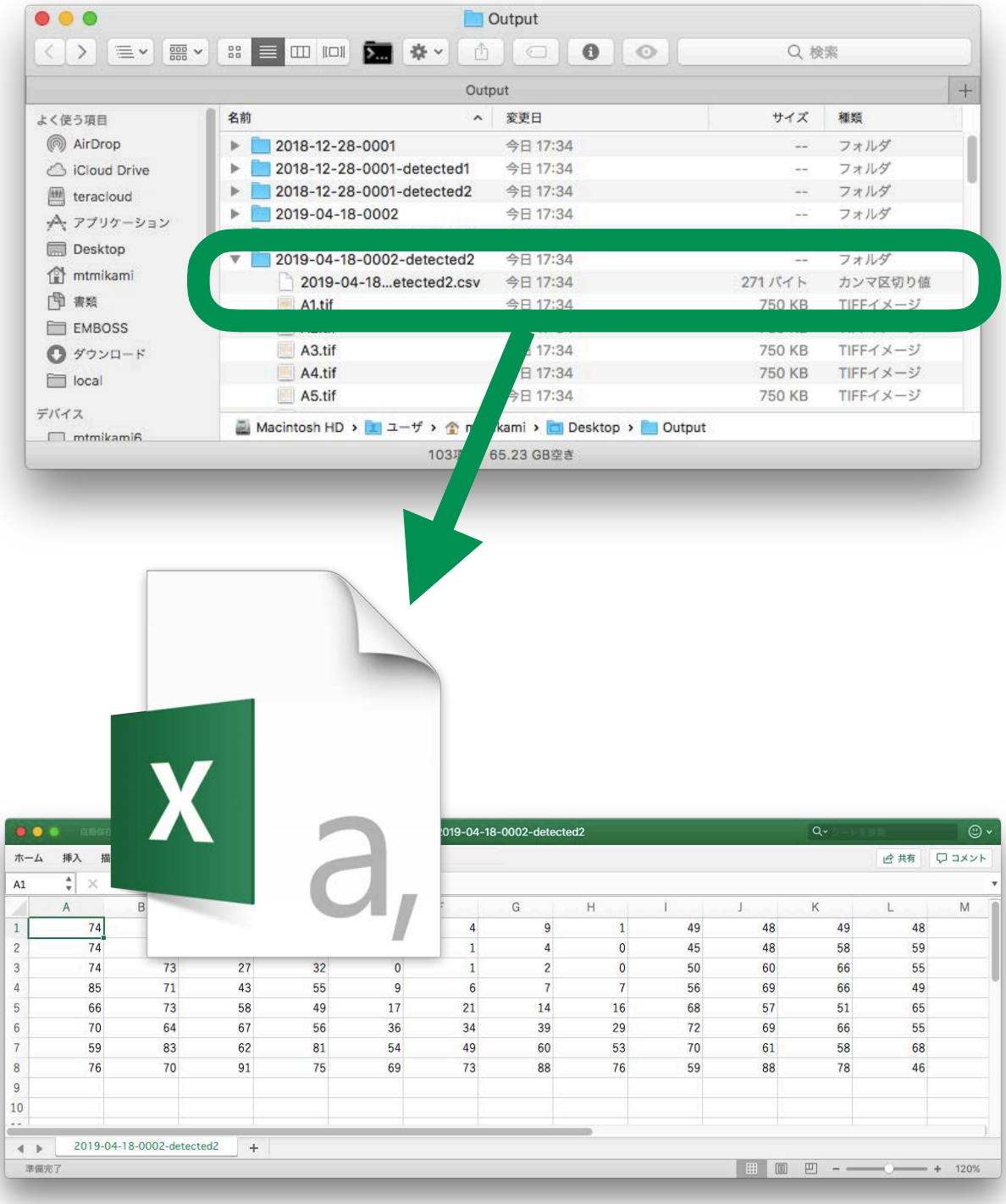


Low sensitive mode



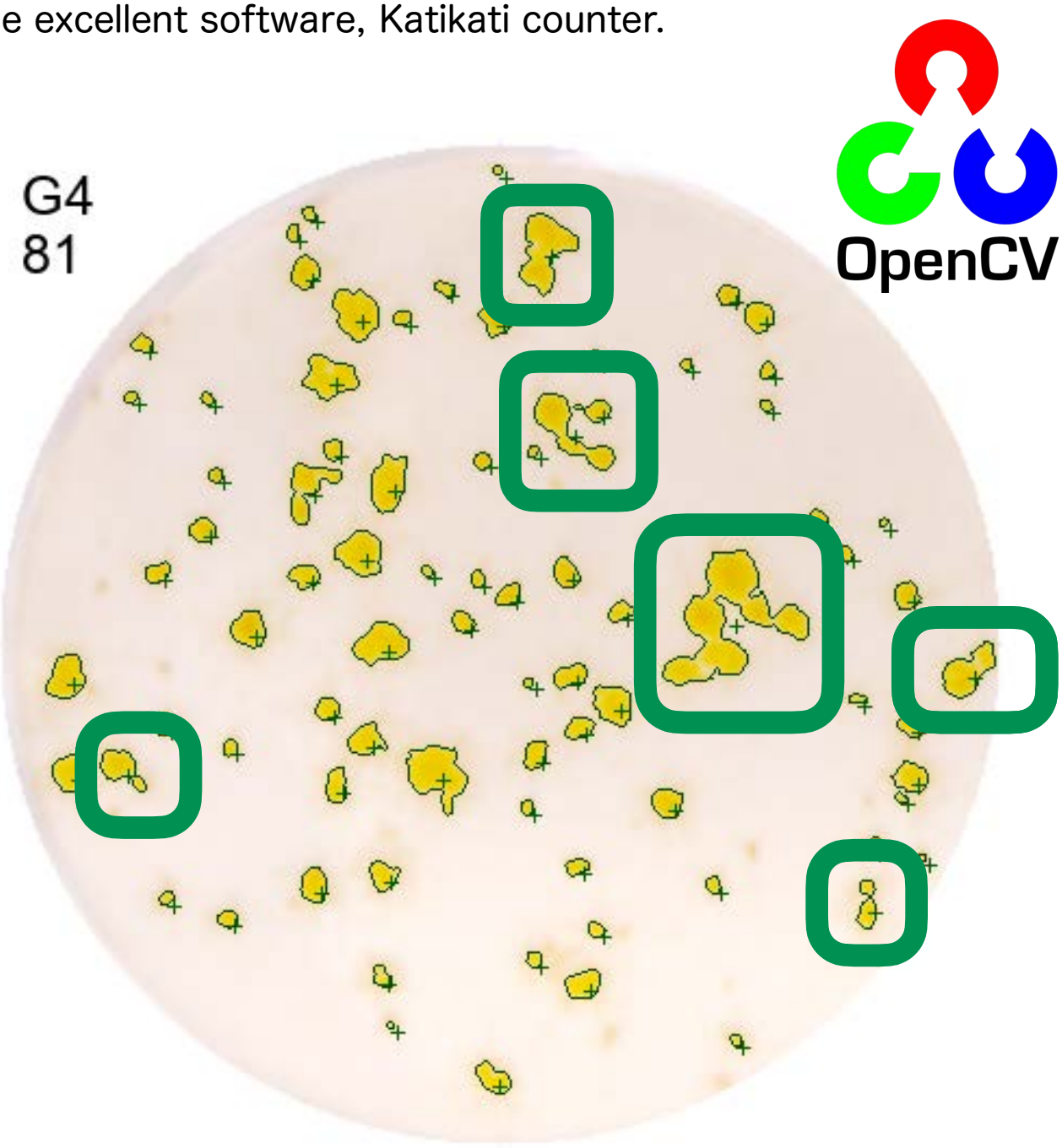
High sensitive mode

Sabayomikun outputs a “csv” format file which contains the number of the counted foci. You can open it with MS-Excel. Please check the result.



“csv” file

Sabayomikun uses findContours algorithm of OpenCV. By this function, sabayomikun detects independent area of specific color (yellow) as single focus and draws small cross for each detected area.
Overgrown foci sometimes join with neighboring ones. OpenCV cannot separate the each focus from the joined clusters. To correct such the miscounts, this workflow uses the excellent software, Katikati counter.



Let’s go to the next step.

First step:

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Last step:

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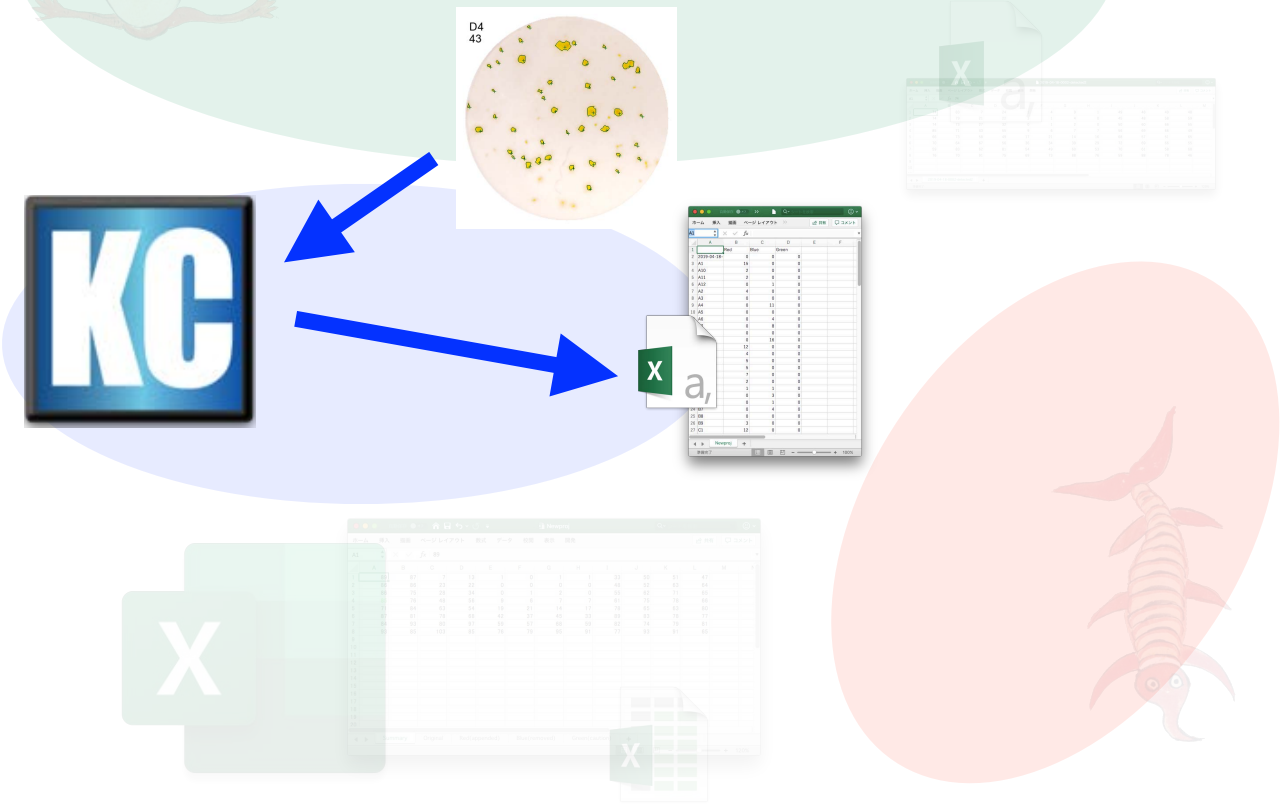
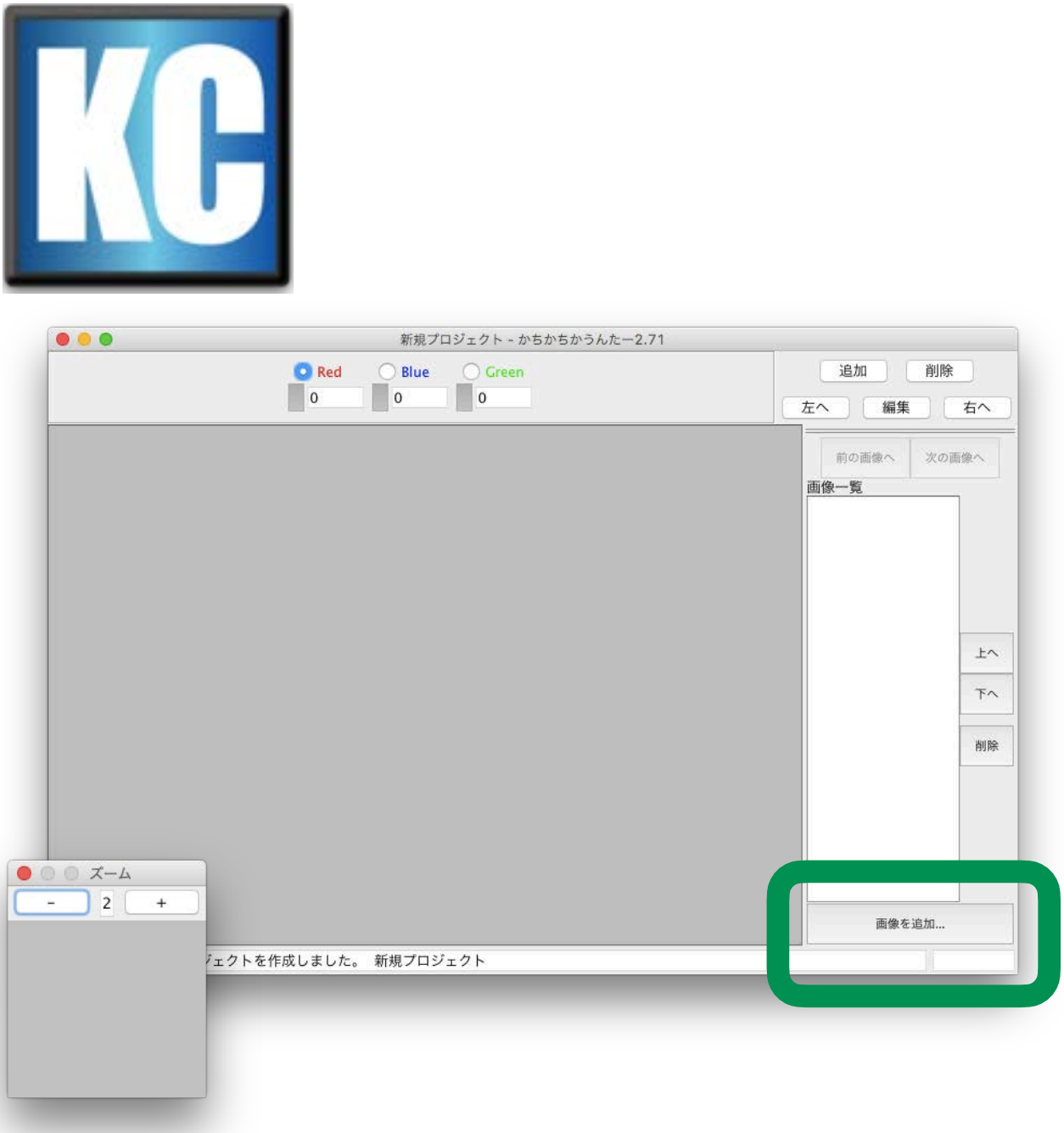
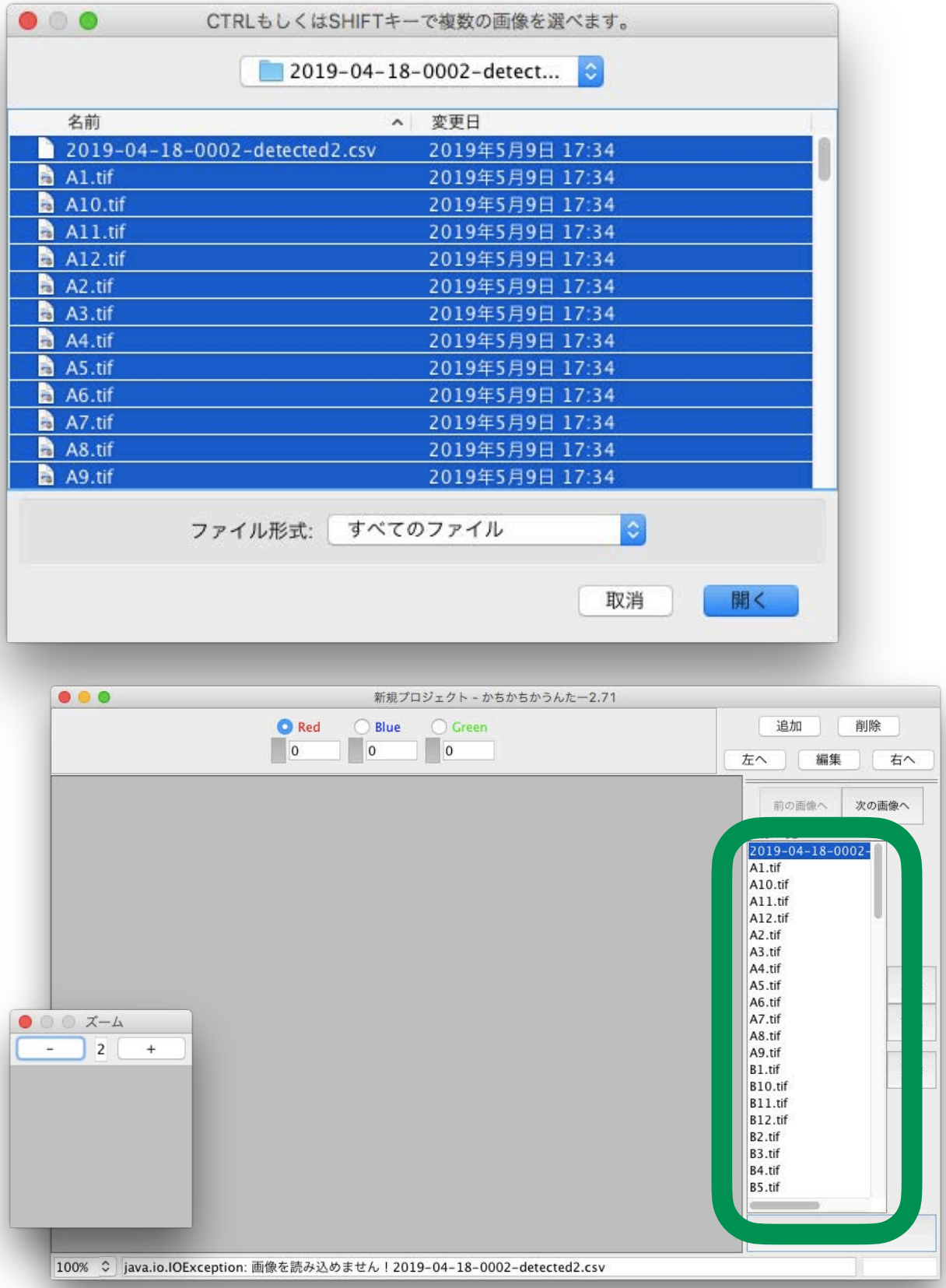


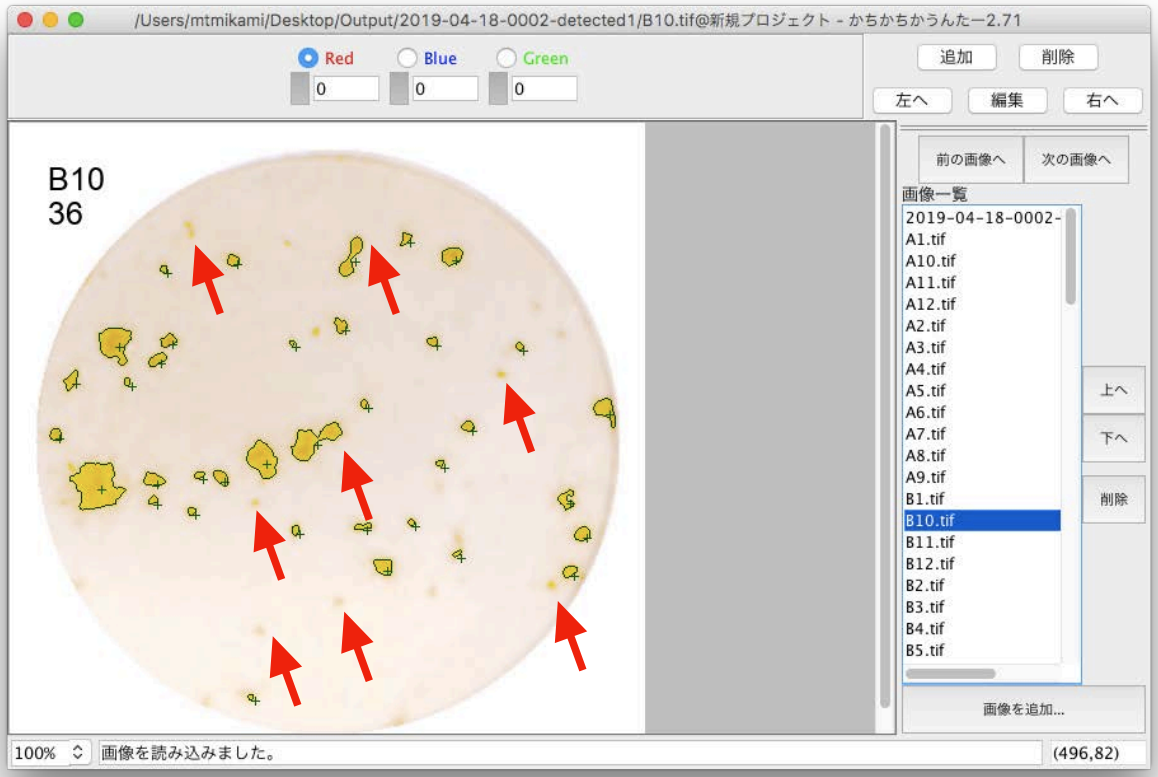
Execute the Katikati counter. Two windows appears.
Click the button at the right bottom corner, and choose the folder which contains output files of sabayomikun.

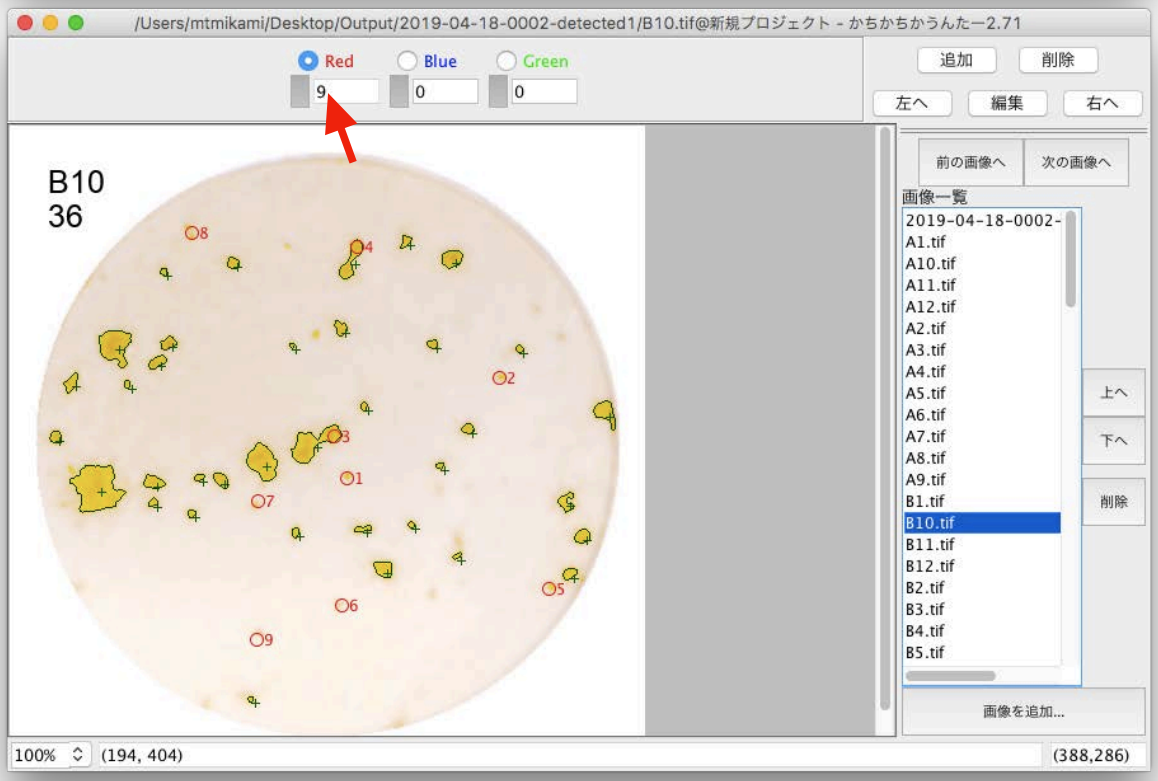


Select all the 96 images. The image names are appear in the panel shown in the right.

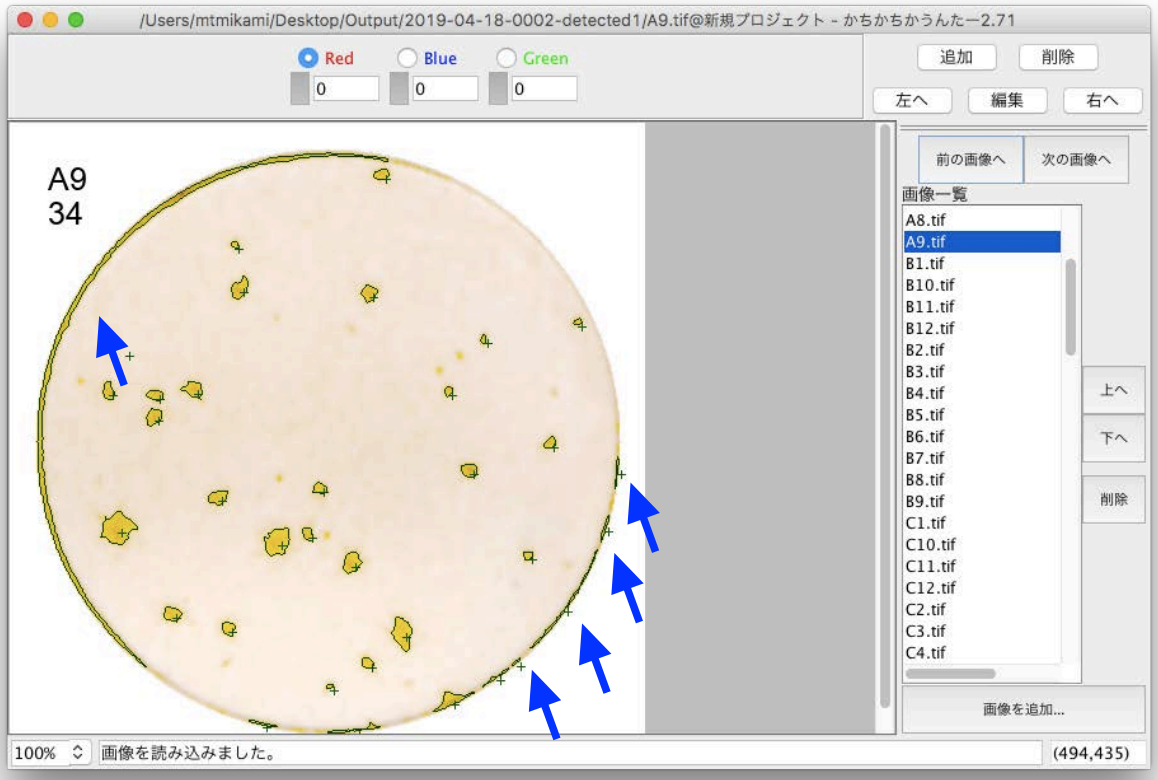


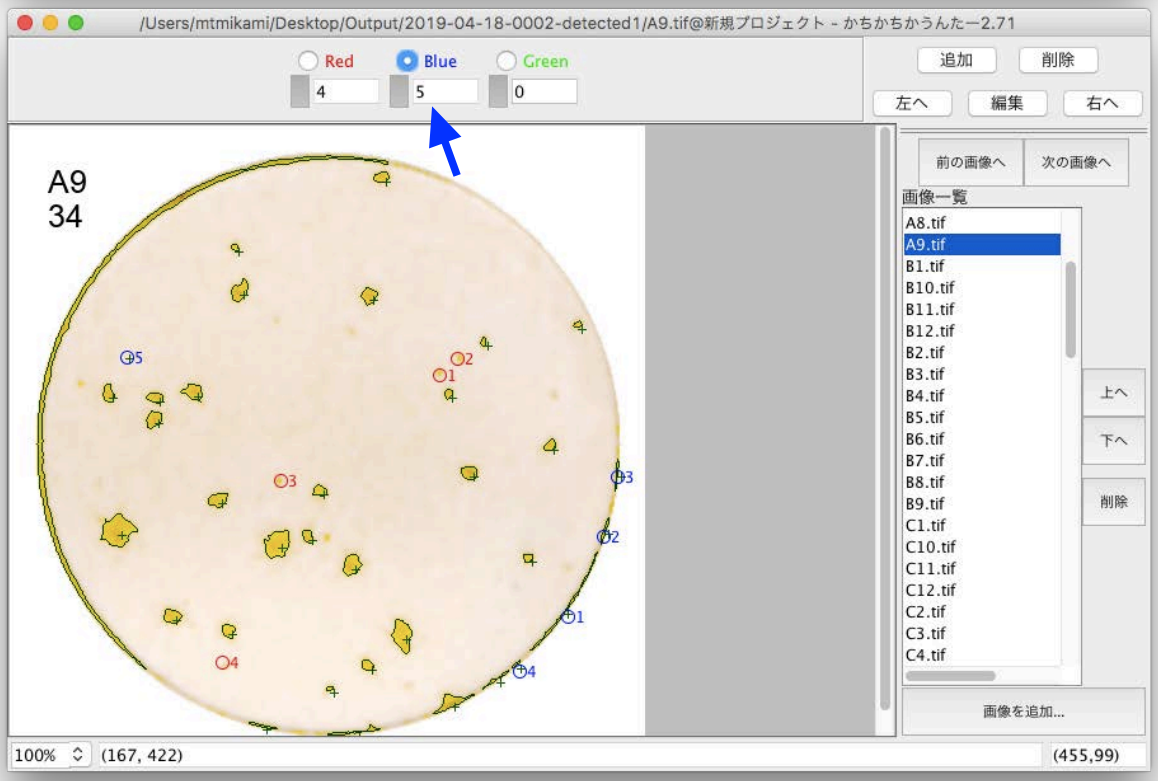
Check the well images. If there are foci, but sabayomikun did not detect them (false negative), put red markers on them. Also for jointed foci, put red markers on each focus.



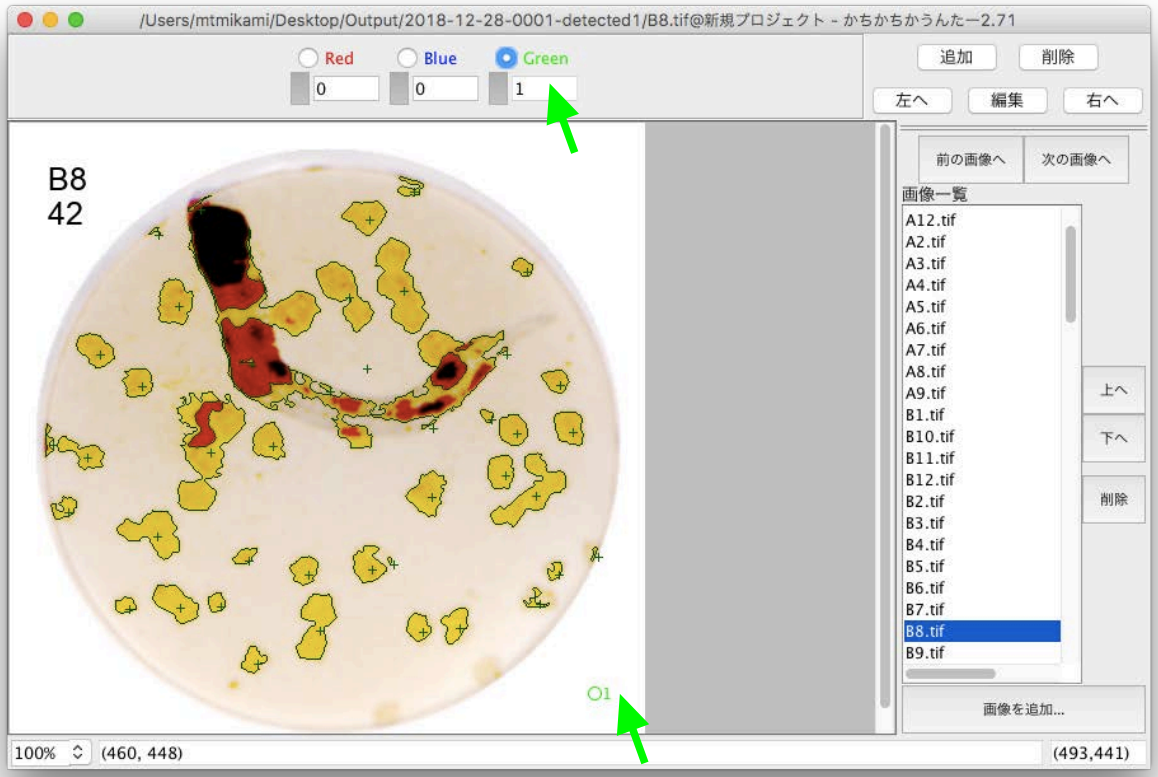


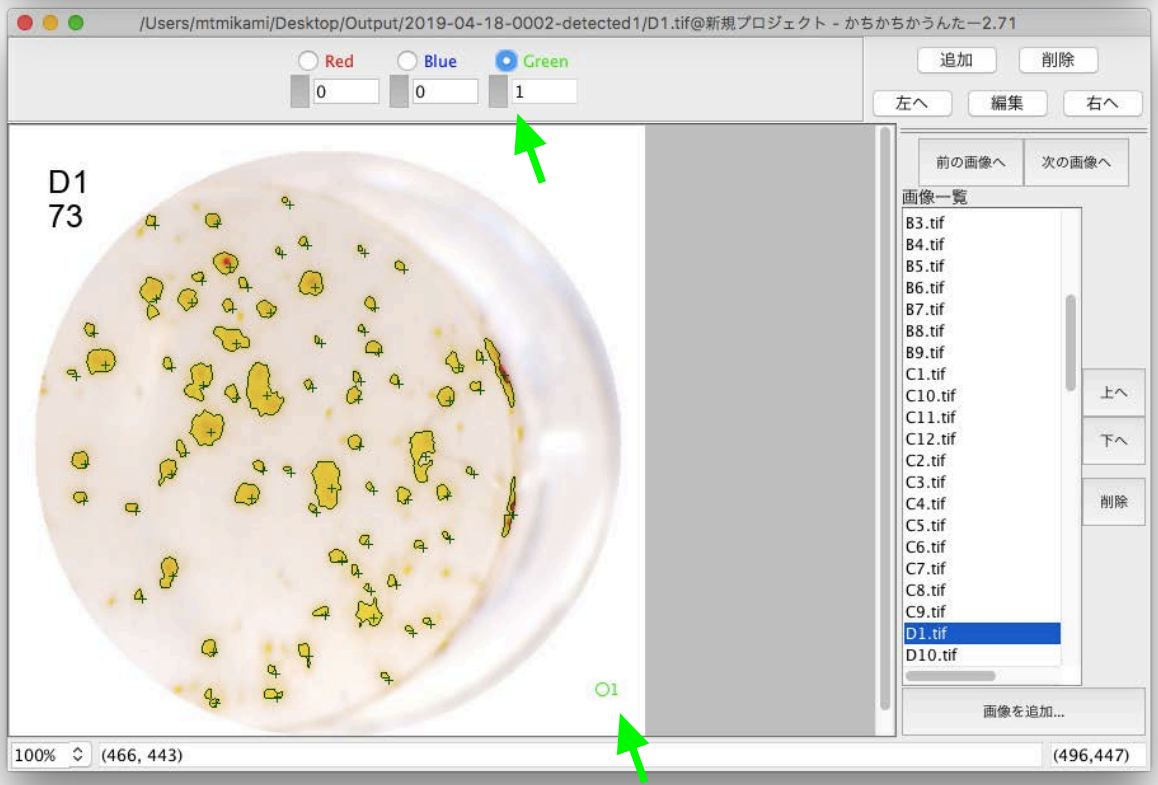
Sometimes edge of well were stained, but it is not focus.
For such false positive cases, put blue markers.



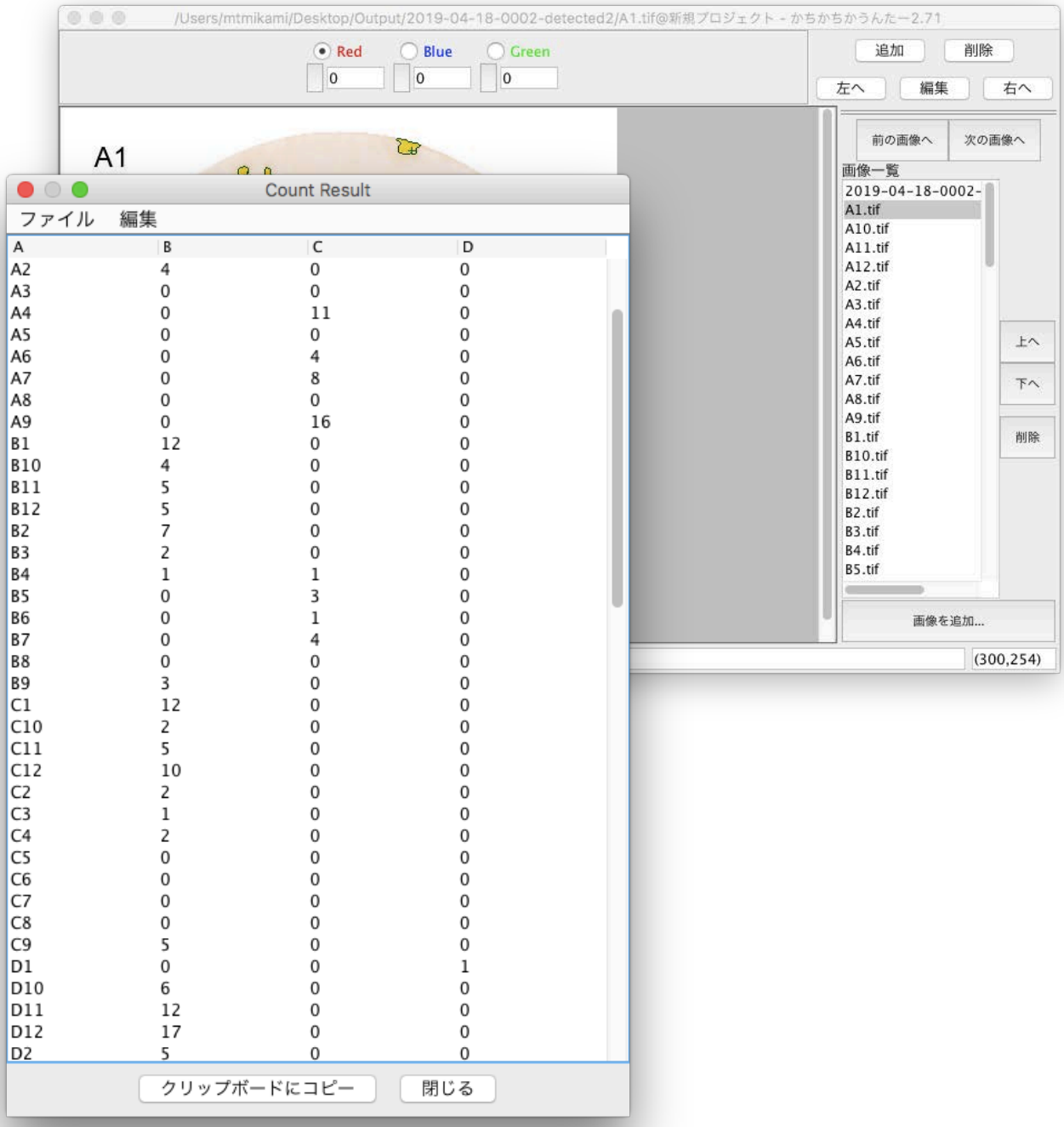


Dust found in the well! Sabayomikun failed to detect the edge of wells! Cells detached!
If you find any abnormality, put green marker.

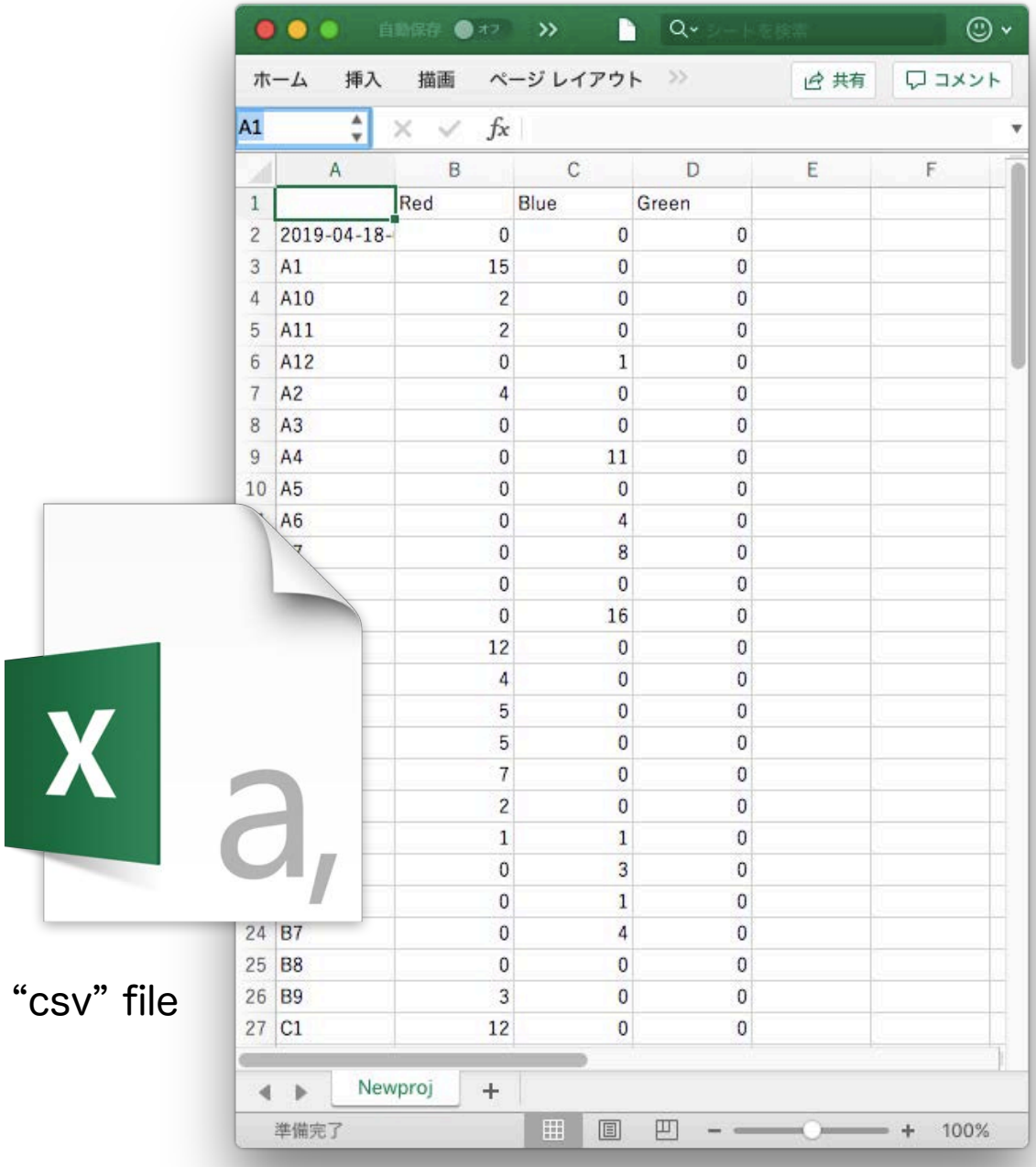




Export the count result. Press “ctrl + R” on MS-Windows, “cmd + R” on Mac. This key combination opens a window which shows the count result. Save the result as a “csv” format file.



You can open the “csv” format file with MS-Excel. To integrate this result with the automatically counted result of sabayomikun, I made the second software.



“csv” file

Let’s go to the last step.

First step:

Crop each well from 96-plate images, and counts foci in each well automatically.

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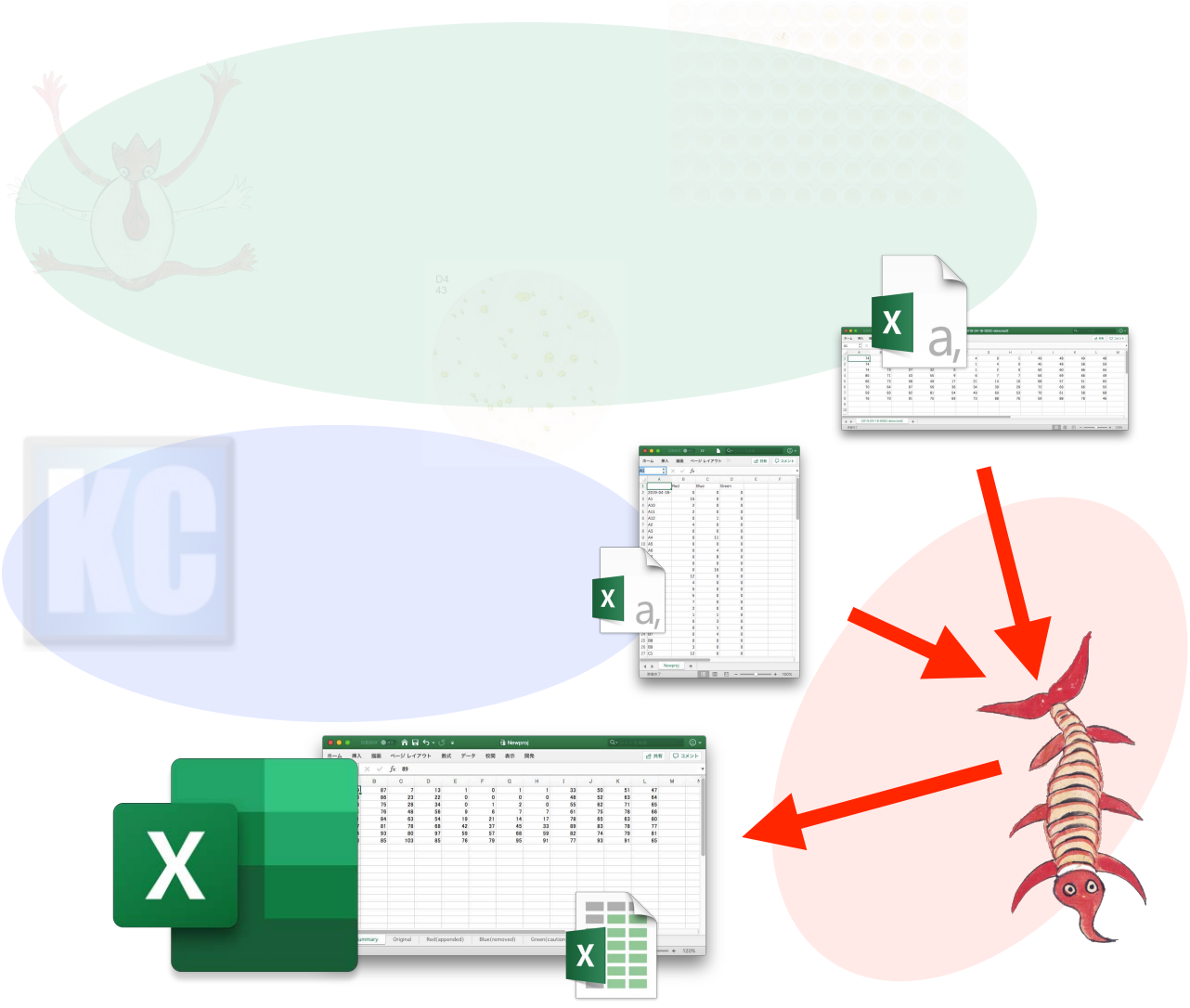
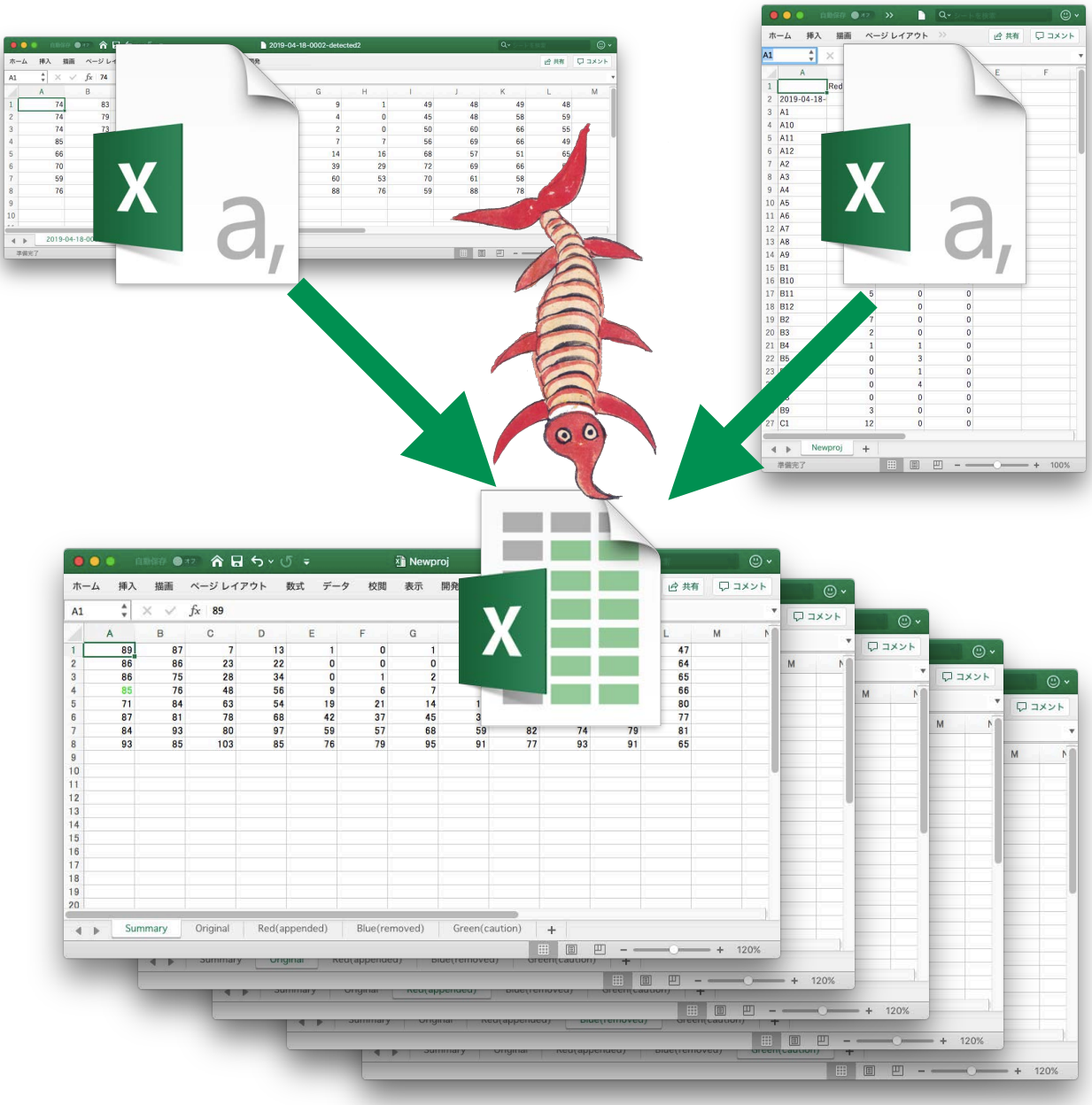
Last step:

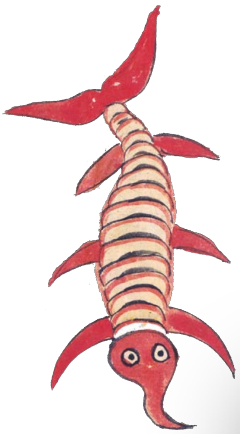
Merge the mechanical count from the first step and the hand count from the second step.

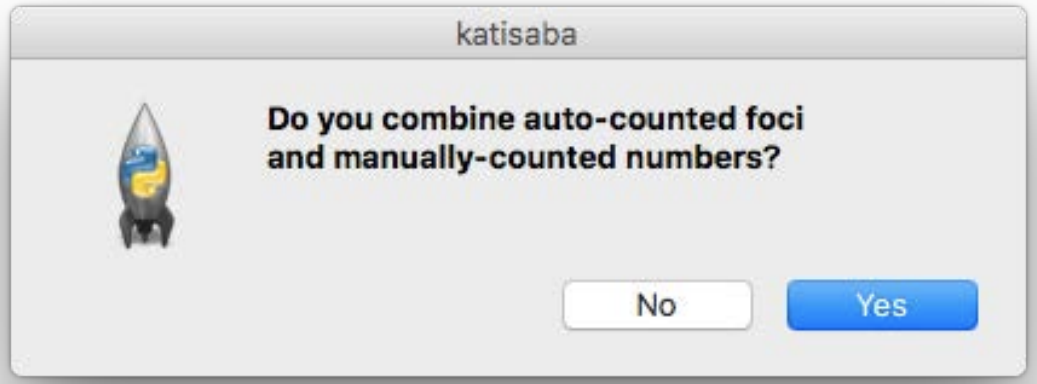


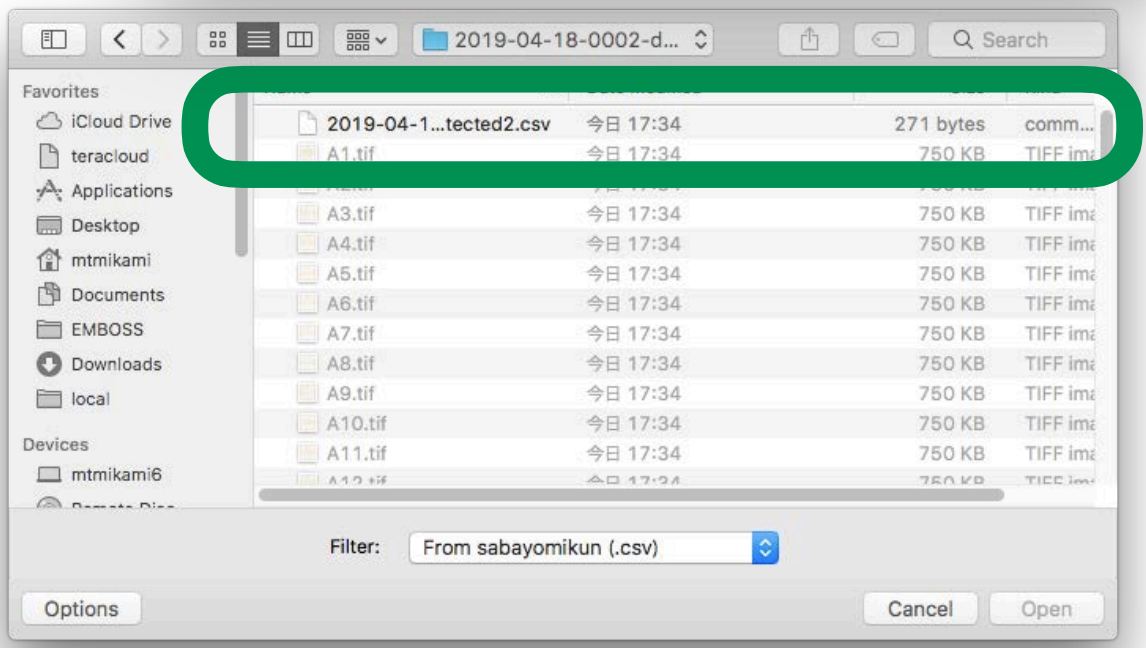
The second software does not have good name. I temporally call it katisaba. If you come up with a good name for it, please tell me.
The output “csv” files format are completely different between the first automatic count step and the second hand count step. Therefore, this specific tool was developed. Katisaba merges these two “csv” files and makes a new MS-Excel format file which contains 5 worksheets.



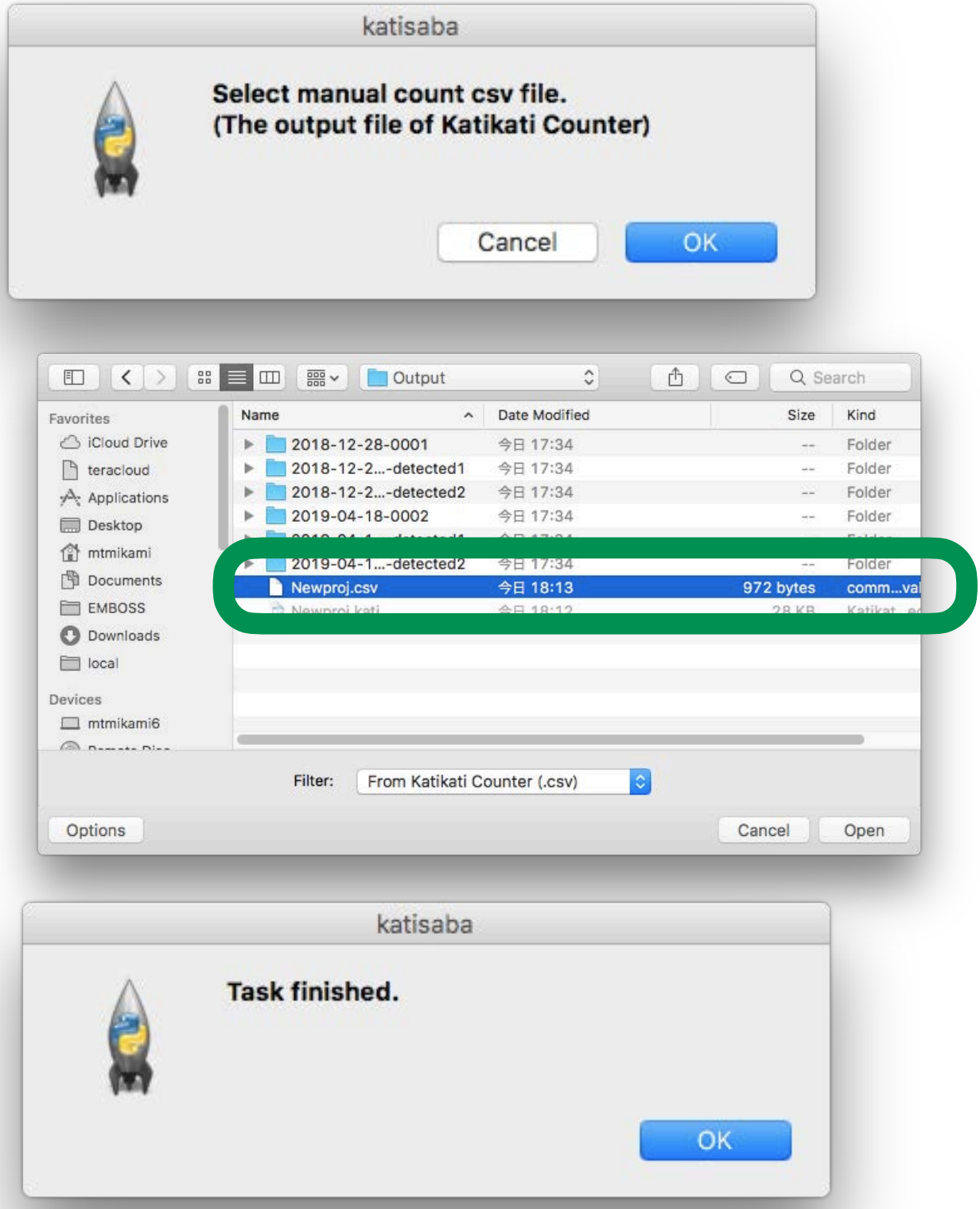
Execute katisaba. Katisaba asks whether you want to combine the auto-count and hand-count data.
Click “Yes” in the dialog window, and choose the first “csv” file which made by sabayomikun. The file might found in the *-detected* folder.



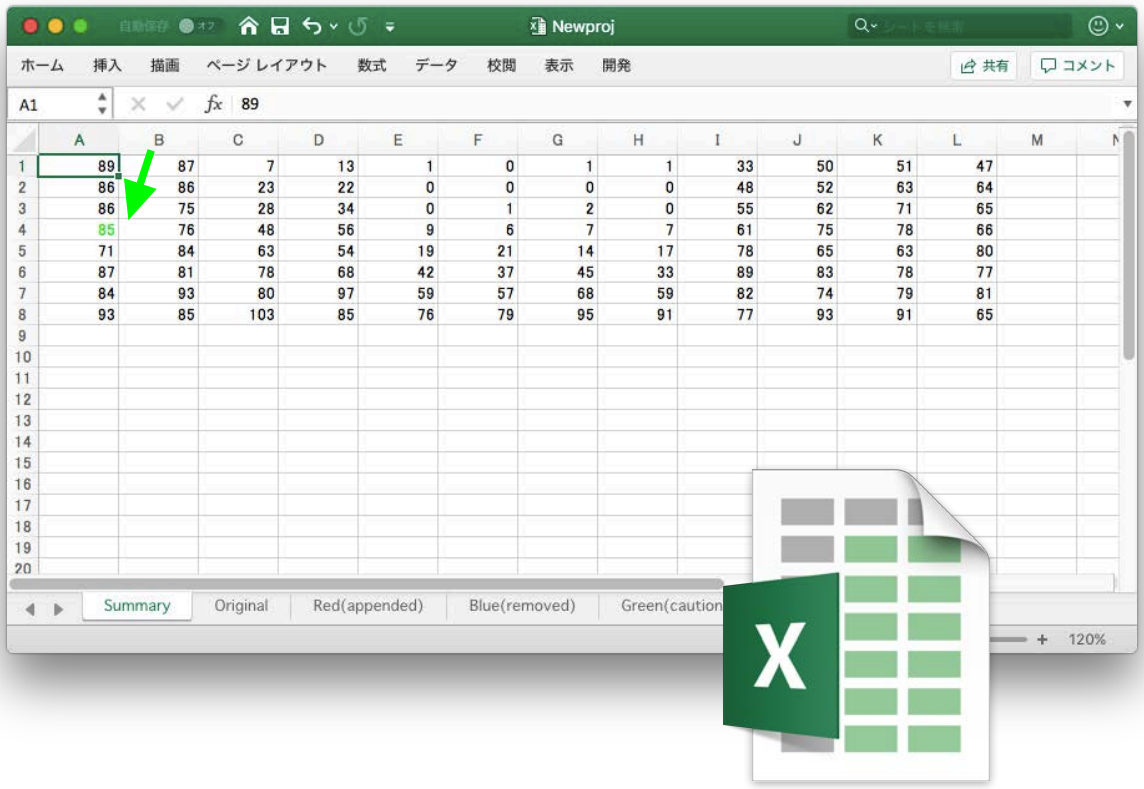




Select the second “csv” file which made by the Katikati counter. The task will finish within few seconds.



See the first “summary” worksheet.
Hand counted numbers of false negative (red markers) were add, false positive (blue markers) were removed from the automatically counted number. Abnormal well (green marker) is shown as green letter.



This is the all of this workflow. If you have any good idea to improve this system, please tell me.