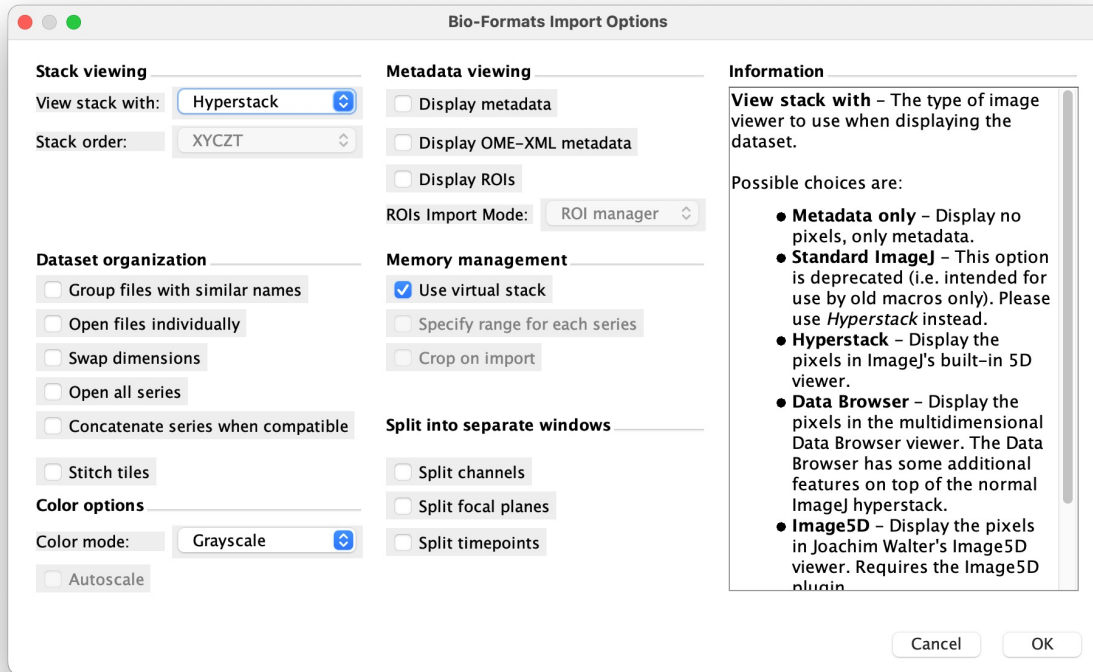




Acto3D Instruction

Convert images to TIFF format

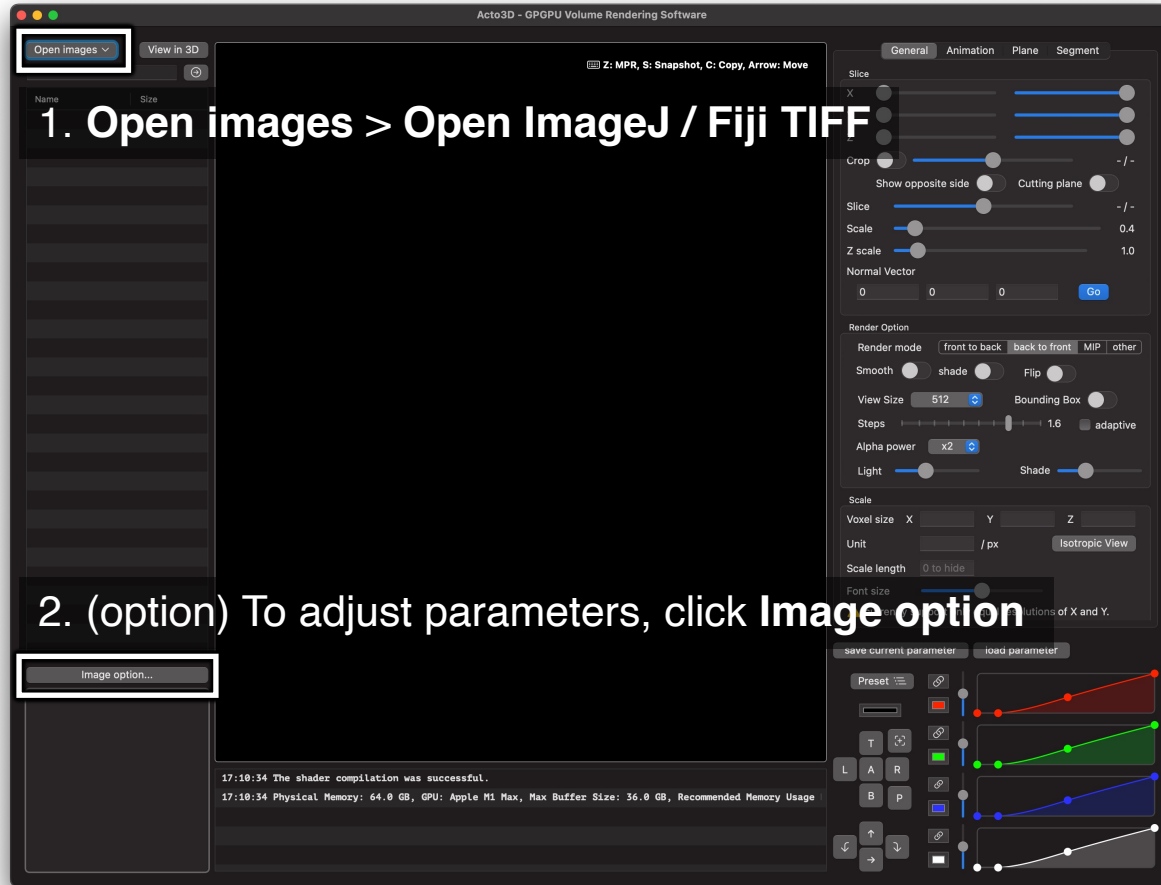
First, you need to convert manufactures format to TIFF using Fiji



Fiji
(version 2.3.0 / 1.53s)

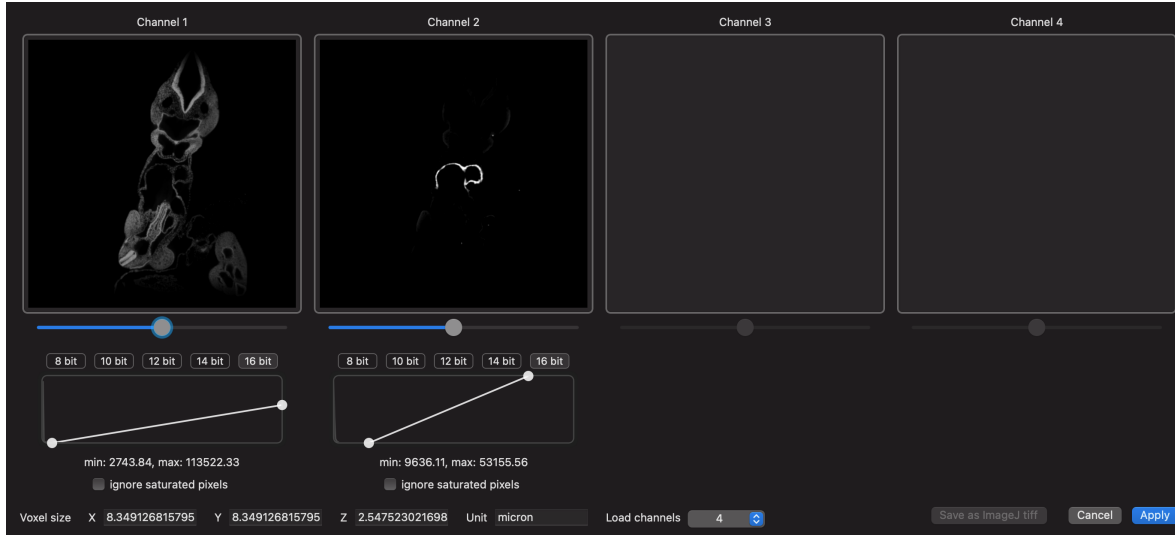
Click **File > Save As > Tiff....**

Load images in Acto3D



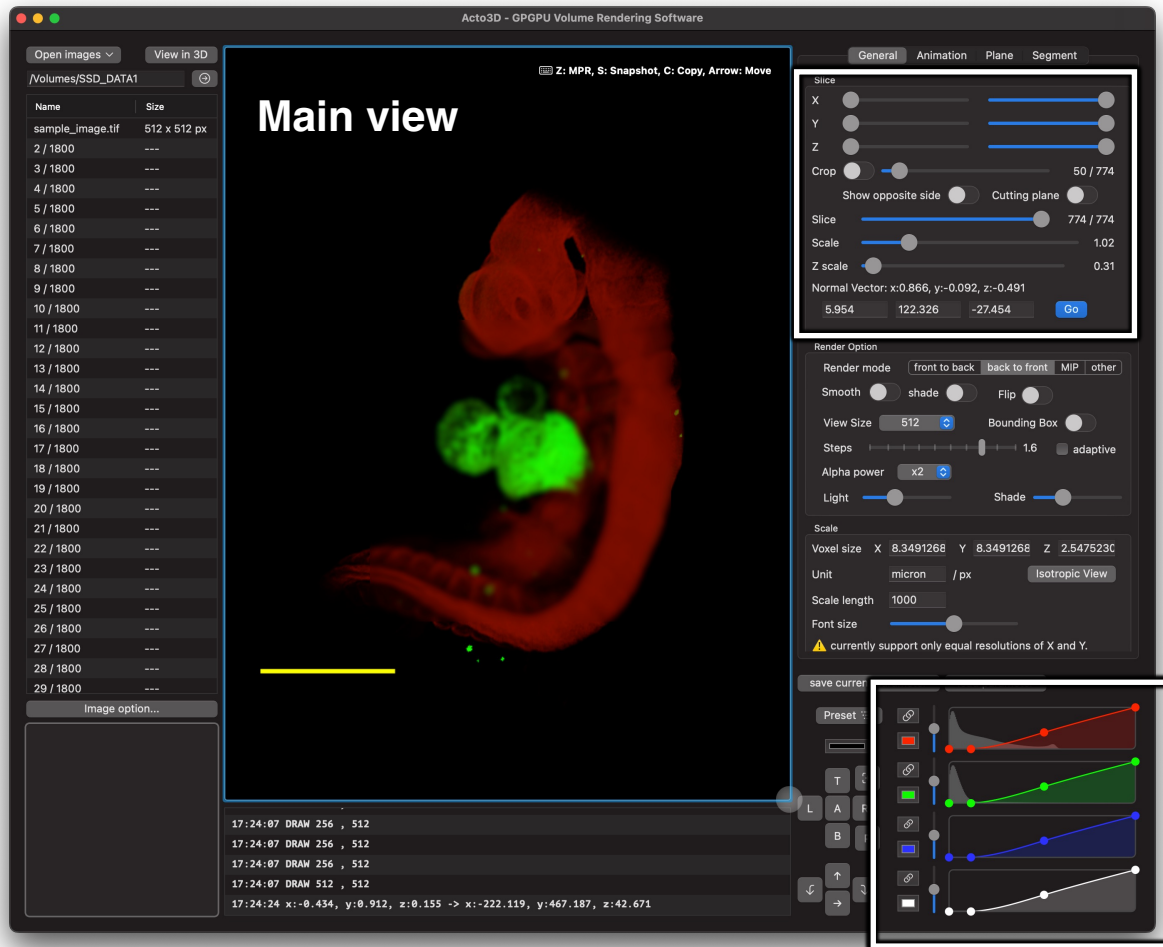
Physical Memory: 64.0 GB, GPU: Apple M1 Max, Max Buffer Size: 36.0 GB,

Adjust parameters



- Adjust display ranges (if you have adjusted this in Fiji, Acto3D uses the value)
- Setting the Voxel Size
 - When loading the microscope manufacturer's format in Fiji, it is automatically set in the TIFF metadata. If it is not set, you will need to determine it yourself based on the parameters at the time of imaging.
- Selecting the Number of Channels
 - If sufficient memory is available, select 4. If you need to conserve memory, select 1 if it's a single-channel image, 2 for a two-channel image, and 4 for a three or four-channel image.
- Ignore saturated pixels
 - If there is a strong non-specific signal and you want to ignore areas with extremely high pixel values, you can set the corresponding pixel areas to 0 by turning this ON.

Screen Layout

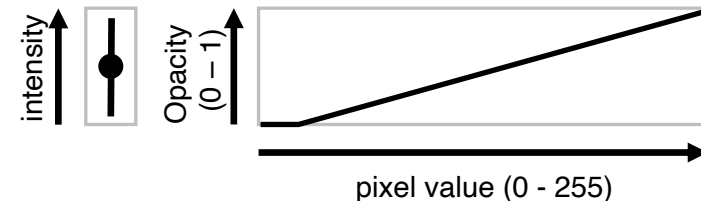


Slice setting

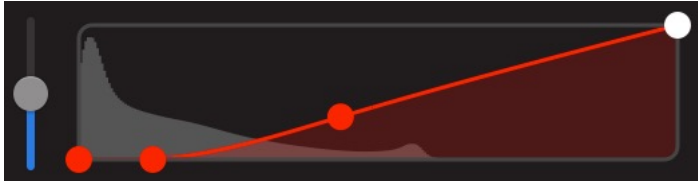
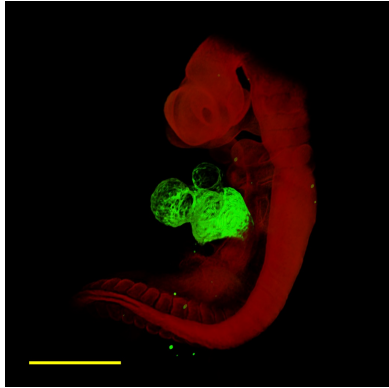
Set the Z scale as the Z voxel size divided by the XY voxel size.
For example, if XY is $2\mu\text{m}/\text{pixel}$ and Z is $5\mu\text{m}/\text{pixel}$, set it to 2.5. If it has already been set on page 3, the correct value will be set automatically.

Color tone and transfer function

In this section, you determine the opacity corresponding to each pixel value. Clicking allows you to freely increase the control points. Right-clicking on a control point allows you to delete it. Right-clicking elsewhere will display additional options.



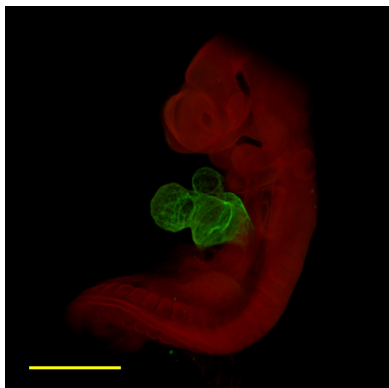
Adjust transfer function (adjust opacity) (1 / 2)



This setting makes parts with low pixel values transparent, which is effective when there is noise at a noticeably lower brightness than the original signal, such as non-specific signals.



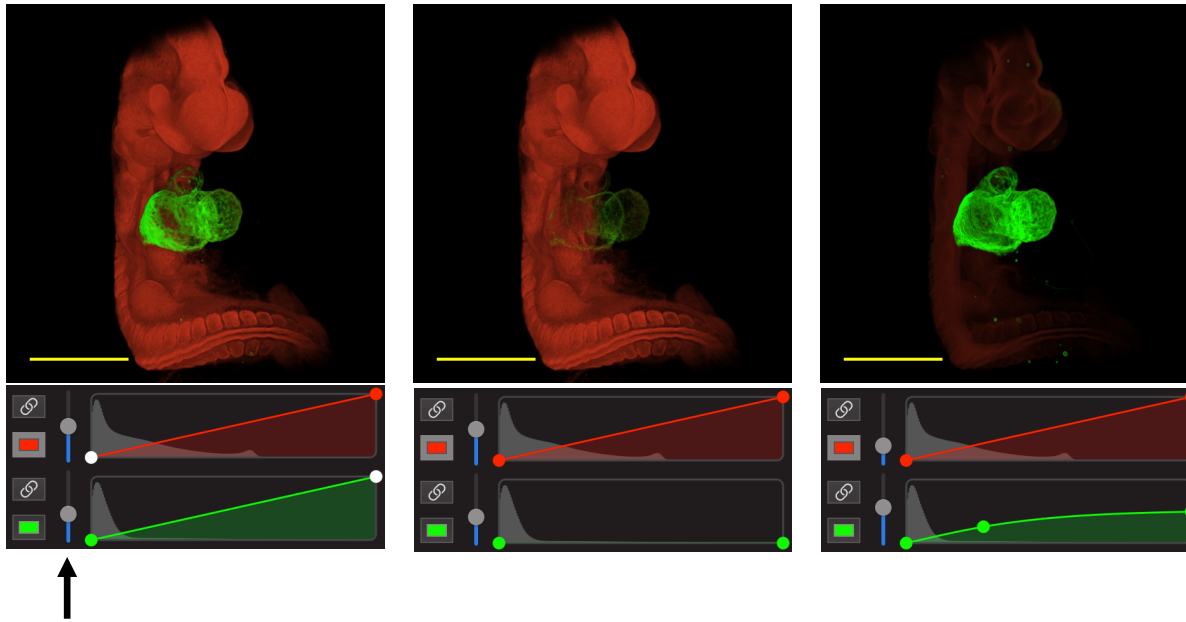
This setting makes parts with pixel values close to zero transparent, while still displaying areas of weak brightness. Areas where pixel values exist become opaque, making it easier to observe the overall object.



This setting allows parts with low pixel values to be transparent, and by giving a certain opacity to areas of strong brightness that are considered signals, it enables the construction of the interior further beyond the surface.

Based on these settings, make fine adjustments to construct your ideal image. Although it is possible to specify the opacity for each channel, by

Adjust transfer function (adjust opacity) (2 / 2)



The slider to determine pixel intensity.

Based on these settings, make fine adjustments to construct your ideal image.

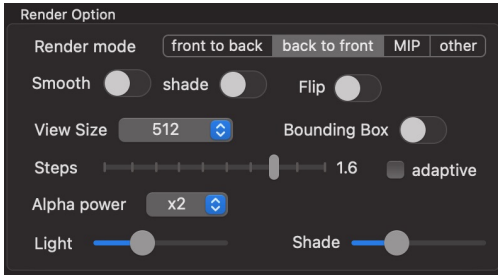
Although it is possible to specify the opacity for each channel, by default, in order to prevent unnatural displays (such as semi-transparent structures in the back being displayed even though there are opaque objects in the front), the opacity at a given sampling point is set to the maximum value among the four channels. If you want to make a certain channel more transparent, you can adjust its intensity using the slider.

Change color



You can specify the color tone for each channel.

Rendering options (1 / 2)



Render mode

Select the algorithm to use for volume rendering. The default selection is 'back to front', but 'front to back' requires fewer calculations and generally produces a similar image. MIP (Maximum Intensity Projection) is a method that adopts the pixel with the highest brightness in the line of sight. With a single image, the depth becomes indistinguishable.

Smooth

This setting uses linear interpolation to make the sampling along the line of sight smooth.

Shade

When sampling along the line of sight, this option takes into account the gradient with surrounding pixels.

Flip

This option allows you to change whether to arrange the image stack from back to front or from front to back, effectively allowing you to flip the image horizontally.

View size

Select the size of the image to be created.

Regardless of this value, the original image used for rendering is not resized.

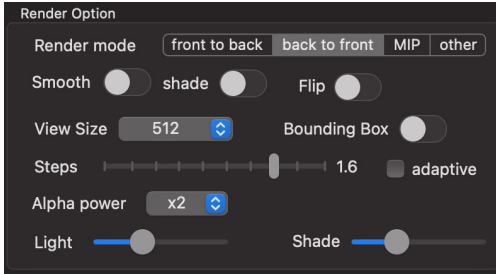
Bounding box

Display the outer frame of the 3D volume.

Steps

Specify the interval for sampling along the line of sight. Specify 1.0 for sampling at the same interval as 1 pixel. However, since this can drastically increase the cost of calculations, by default it is set to 1.6, with some degree of thinning. Note that when the number of images is small, or the resolution of the image is low, jagged edges may appear in the image, so setting it below 1.0 will smooth it out. Please note that in volume rendering, the final image changes depending on the number of times the same transparency level is overlaid.

Rendering options (2 / 2)



Adaptive

This is a setting related to the above view size and steps. When OFF, a 3D image is created from the original image and finally adjusted to the view size. When ON, the image size is adjusted (usually shrunk) to match the view size first, and the 3D image is constructed based on that image. Both methods do not change the isotopic display, but in many cases, the number of calculations drastically decreases when ON.

Alpha power

In volume rendering, many images are overlaid, so if the transparency is not set to a very low value, only the surface is often observed. Therefore, by default, the alpha value specified in the graph (in the range of 0.0-1.0) is squared. If it becomes too transparent, you can ignore this setting by setting it to x1. If you want to make it more transparent, you can set it to x3 to cube it.

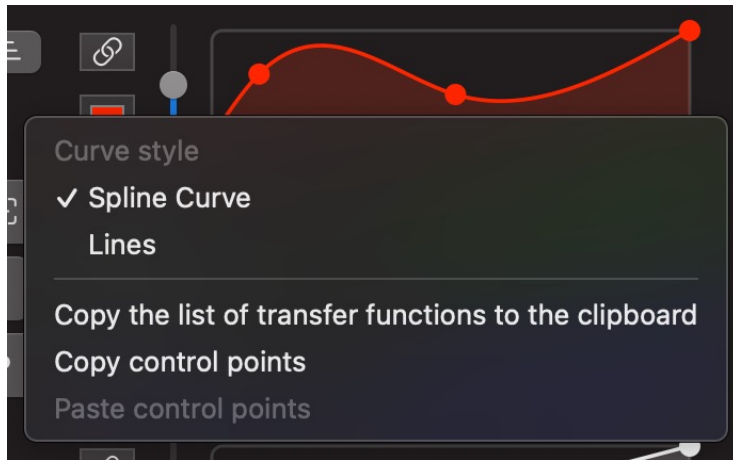
Light

Adjust the overall brightness.

Shade

If shade is turned on, it increases the effect of the gradient with surrounding pixels.

Check transfer function



To obtain the the transfer function:
Right click in the graph area
(in places other than control points)

	A	B
1	Pixel value	Opacity
2	0	0
3	0.1	0.00193923
4	0.2	0.00387845
5	0.3	0.00581763
6	0.4	0.00775676
7	0.5	0.00969583
8	0.6	0.01163481
9	0.7	0.01357369
10	0.8	0.01551245
11	0.9	0.01745107
12	1	0.01938955
13	1.1	0.02132785
14	1.2	0.02326598
15	1.3	0.02520389

These values can be pasted into Excel

Apply filter (Gaussian 3D filter)



Apply 3D Gaussian blur

Specify the parameters and target channels

Kernel Size: Sigma:

All channel

Channel 1

Channel 2

Channel 3

Channel 4

Cancel

In menu bar, click: **ImageProcess > Gaussian 3D**

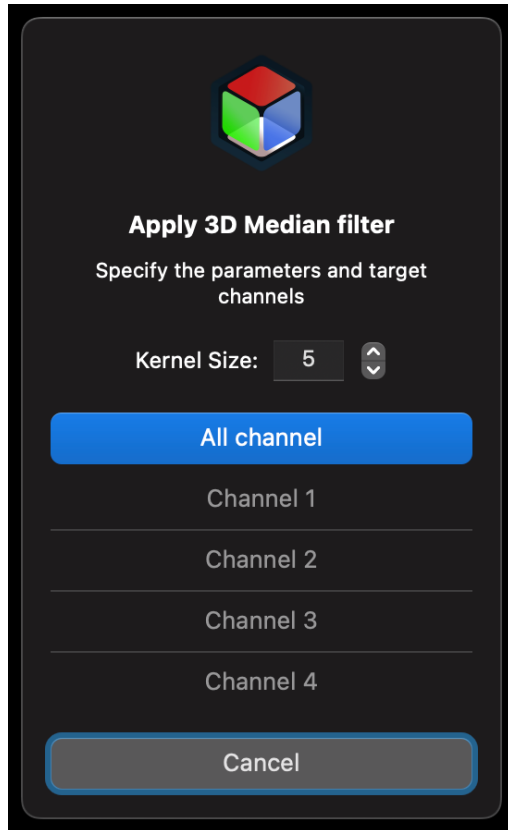
Specify the kernel size, sigma and target channels.
The kernel size **must be an odd integer**.

This process requires additional memory allocation.
When 'All' is selected, the size of the temporary buffer needed is 'width x height x depth x 4' bytes.
If a specific channel is selected, the size reduces to 'width x height x depth' bytes.

If there is insufficient memory available, the filter should be applied to each channel individually.

As the kernel size increases, the time required for the process also increases.

Apply filter (Median 3D filter)



In menu bar, click: **ImageProcess > Median 3D**

Specify the kernel size and target channels.
The kernel size **must be an odd integer**.

The median filter is time-consuming to process. Additionally, running the filter requires additional memory capacity.
The amount needed is
'width x height x 4 x (kernel size)³ bytes for each channel.

As the kernel size increases,
the time required for the process also significantly increases.