Tutorial 8: Generating *in-silico* microscopy image with different format and noise

Subhamoy Mahajan

13 Jul, 2021

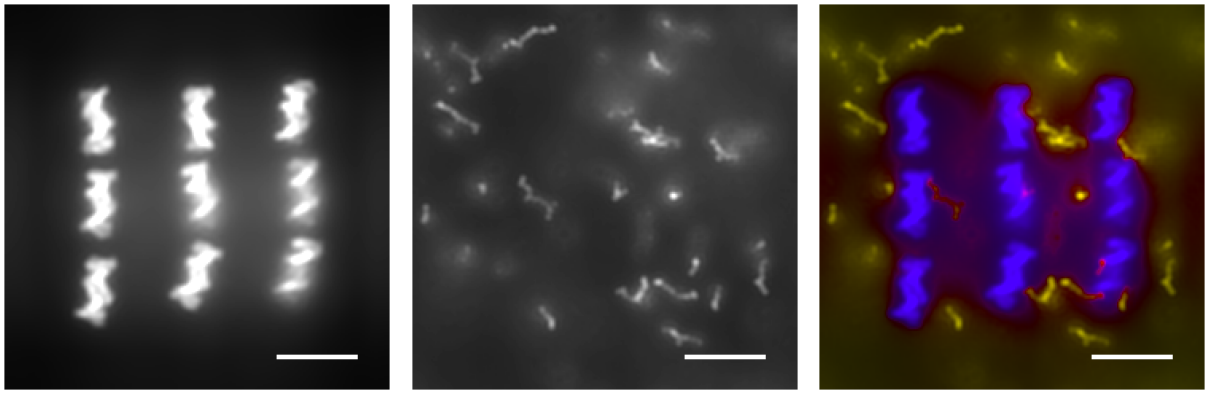
In the past **Tutorials**, images are only generated in JPEG format. This tutorial will cover generation of images in PNG and TIFF format. While using JPEG or PNG only 2D images (`slice`) can be generated, TIFF format allows the generation of 2DT, 3D, and 3D images, where D stands for dimensions (l, m,n) and T stands for time.

1 Generating 2D images

For 2D images, we would use image intensities generated from **Tutorial 2**, which is provided in `Intensities.tar.gz`. Generation of PNG image is similar to JPEG, where `--type png` has to be used. TIFF format does not have a scale because the dimensions of the system are embedded in the file.

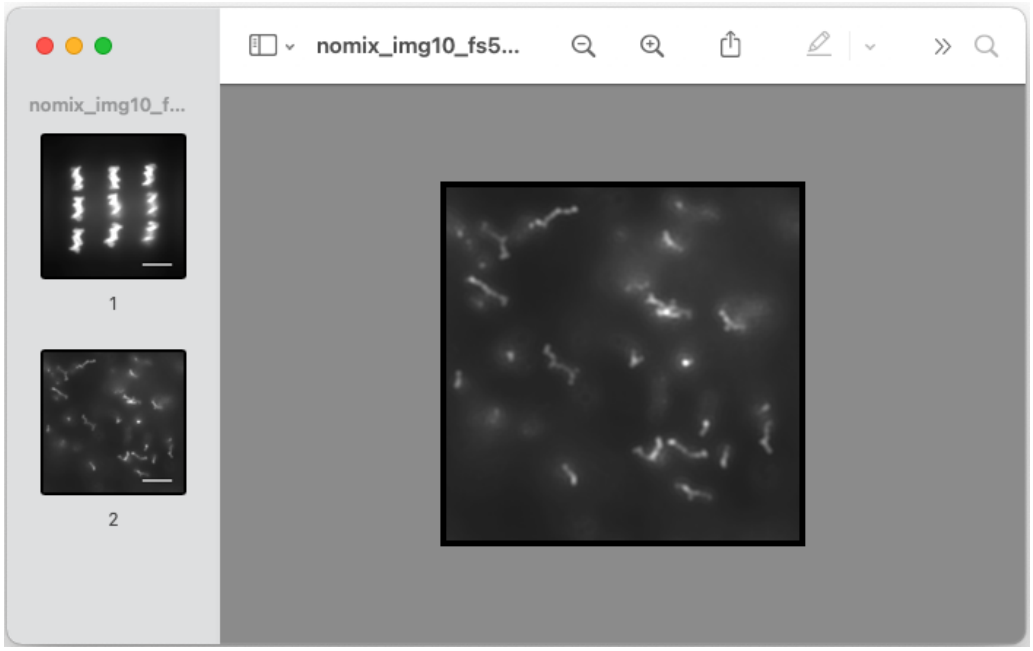
```
Tut2$ tar -xzf Intensities.tar.gz
Tut2$ siliscopy plot --file img --paramfile parameters.dat
   --method mono --timestep 10 --type png --output img --calc
   specific
Tut2$ siliscopy plot --file img --paramfile parameters.dat
   --method color --timestep 10 --type png --output img --calc
   specific
```

This generates three images (identical to JPEG).



The same images can be generated in TIFF format using `--type tiff8` (for 8-bin image; or `--type tiff16` for 16-bit image). Additionally, TIFF format can generate a single file containing all monochrome images, i.e., not mixing any color. This is done by using `mix_type = none` in `parameters2.dat` file and the command,

```
Tut2$ siliscopy plot --file img --paramfile parameters2.dat
   --method color --timestep 10 --type png --output nomix_img
   --calc specific\\
```



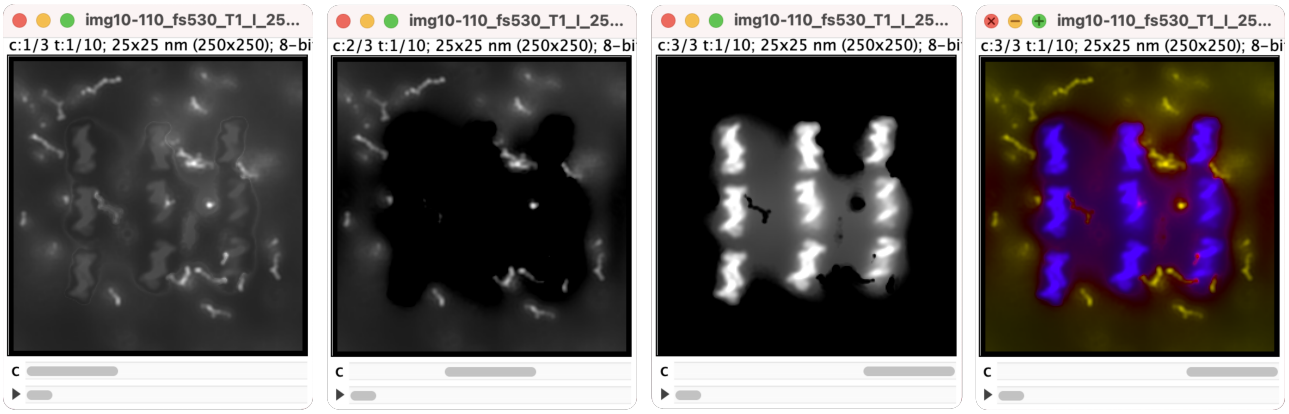
2 Generating 2DT images

These images are equivalent to 'videos' and can only be generated using TIFF format and are meant to be used with ImageJ or Fiji ImageJ (https://imagej.net). The structure files from

Tutorial 2 is used `Struct.tar.gz`. This image can be generated using the following command,

```
Tut2$ tar -xzf Struct.tar.gz
Tut2$ siliscopy plot --file img --paramfile parameters.dat
   --method mono2dt  --type tiff8 --output img --calc specific
Tut2$ siliscopy plot --file img --paramfile parameters.dat
   --method color2dt --type tiff8 --output img --calc specific
```

Similar to videos, it reads the parameters `tbegin`, `tdiff` and `tmax` from `parameters.dat` file. Since the image is generated using Mahajan and Tang [1] color mixing scheme, individual channel intensities shown in ImageJ does not reflect any physical quantity (left three images). To view color image (right) use `Image -> Color -> Channel Tool...` and in the dropdown menu choose `Composite`. Clicking the play icon, plays the movie.



3 Generating 3D and 3DT image

Generating 3D and 3DT images are computationally expensive. First, 3D image intensities are generated using the command,

```
Tut2$ siliscopy gen_mono --data imggen.dat --multiprocess
```

imggen.dat

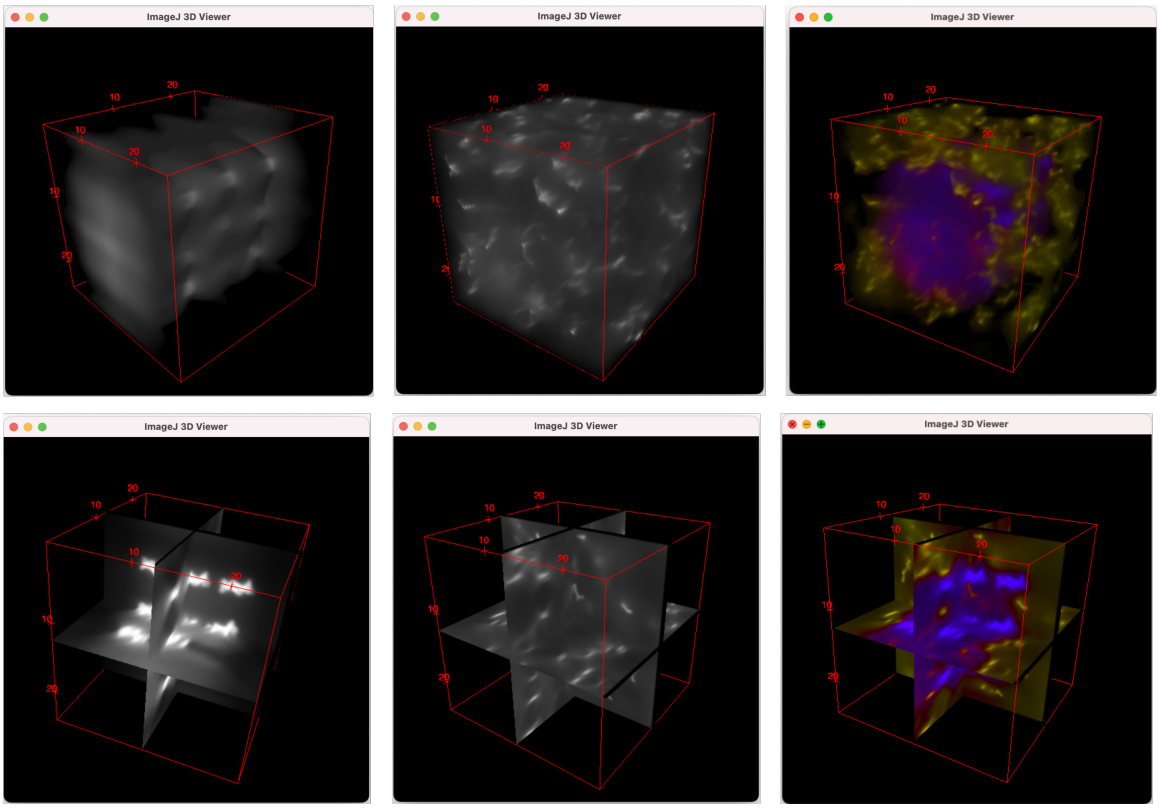
```
dp10.gro,parameters.dat,PSF_gandy,img,volume
dp20.gro,parameters.dat,PSF_gandy,img,volume
dp30.gro,parameters.dat,PSF_gandy,img,volume
...
```

Notice the method used is `volume` instead of `slice`. Since maximum length is 25 nm in z direction (optical axis) and `dlmn = 0.1 0.1 0.2`, 124 `slice` image intensities are generated

per `volume` image (`int(25/0.2)=124`). Next, 3D images are generated using the command,

```
Tut2$ siliscopy plot --file img --paramfile parameters.dat
    --method mono3d --timestep 10 --type tiff8 --output img
    --calc specific
Tut2$ siliscopy plot --file img --paramfile parameters.dat
    --method color3d --timestep 10 --type tiff8 --output img
    --calc specific
```

Default 2D view in imageJ is probably the most convenient representation (similar to **Section 2**), however, 3D view can be generated using `Plugin -> 3D viewer`, and then `OK`. This use the default `Volume` display settings (top panel). The display settings can be changed using `Edit -> Display as -> Orthoslice` (bottom panel). For color 3D images, `Composite` colors has to be generated (See **Section 2**).



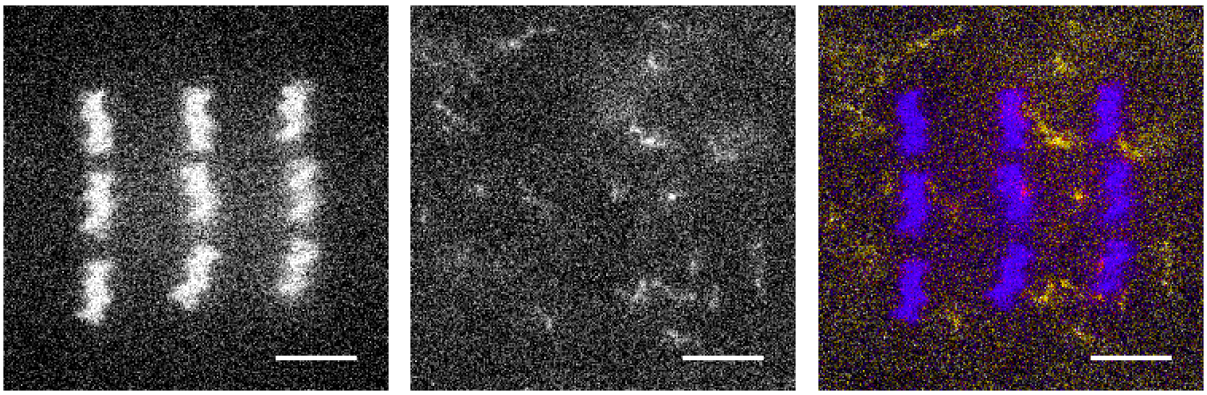
Similar steps can be followed for 3DT images.

```
Tut2$ siliscopy plot --file img --paramfile parameters.dat
   --method mono3dt --type tiff8 --output img --calc specific
Tut2$ siliscopy plot --file img --paramfile parameters.dat
   --method color3dt --type tiff8  --output img --calc specific
```

Note: 3D viewer in ImageJ is found to have bugs while showing timelapse of 3D images.

4 Images with noise

Images with noise can be generated with the `--method noise_mono` and `noise_color` for 2D images, `noise_mono2dt` and `noise_color2dt` for 2DT images and so on. Two noise parameters are read from the `parameters.dat` file, `poi` and `gauss`. `poi` a real number between 0 and 1, where 0 represents no Poisson noise and 1 represents Poisson noise based on Ref [2]. `gauss` is the variance of zero-centered Gaussian noise.



Other noise images are not shown.

References

[1] Mahajan S., and Tang T., Meeting experiments at the diffraction barrier: an in-silico widefield fluorescence microscopy, bioRxiv 2021.03.02.433395; DOI: 10.1101/2021.03.02.433395