

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

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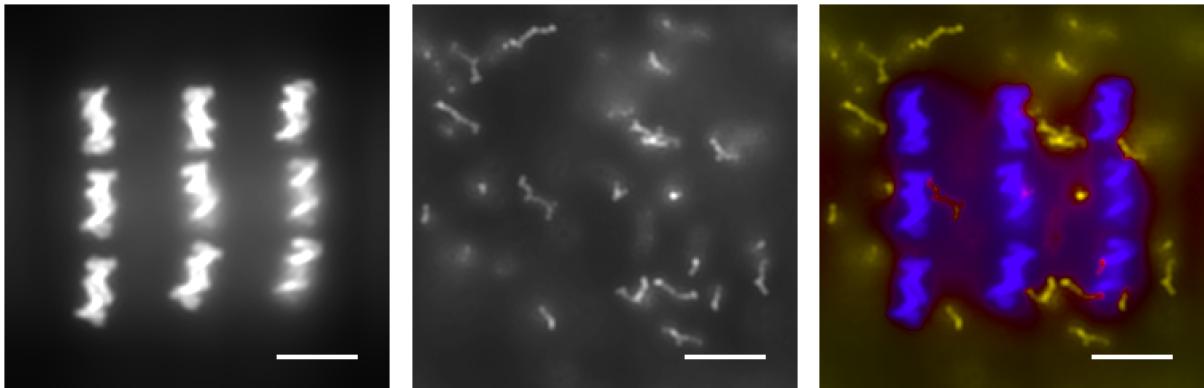
In the past **Tutorials**, images are only generated in JPEG format. This tutorial will cover generation of images in PNG and TIFF format. While using JPEG or PNG only 2D images (**slice**) can be generated, TIFF format allows the generation of 2DT, 3D, and 3D images, where D stands for dimensions (l,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

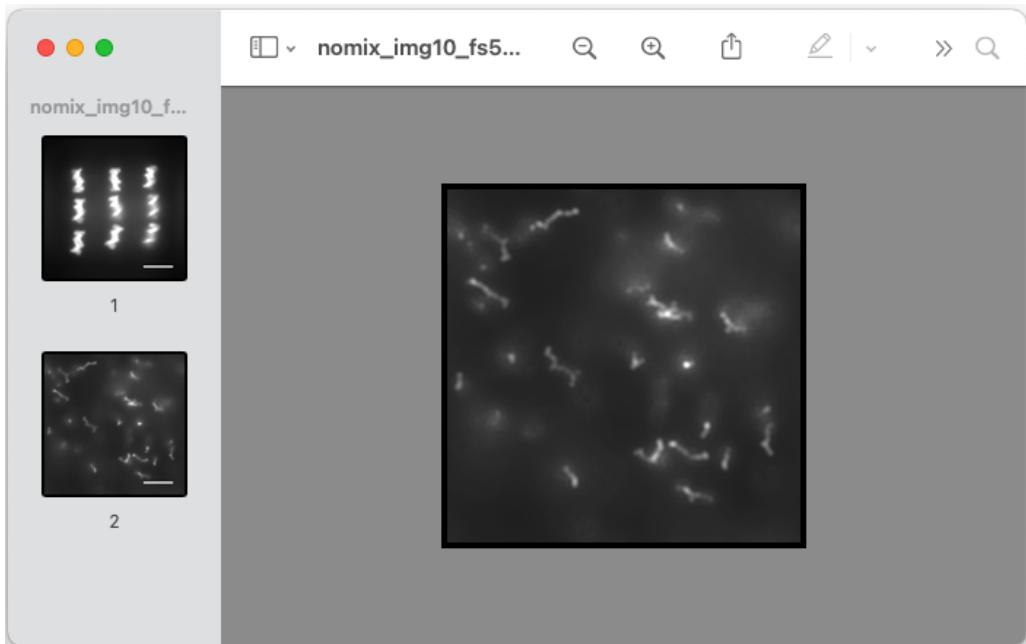
```
Tut2$ tar -xzf Intensities.tar.gz
Tut2$ siliscopy plot --file img --paramfile parameters.dat
    --method mono --timestep 10 --type png --output img --calc
    specific
Tut2$ siliscopy plot --file img --paramfile parameters.dat
    --method color --timestep 10 --type png --output img --calc
    specific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

This generates three images (identical to JPEG).



The same images can be generated in TIFF format using `--type tiff8` (for 8-bit 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\\
```



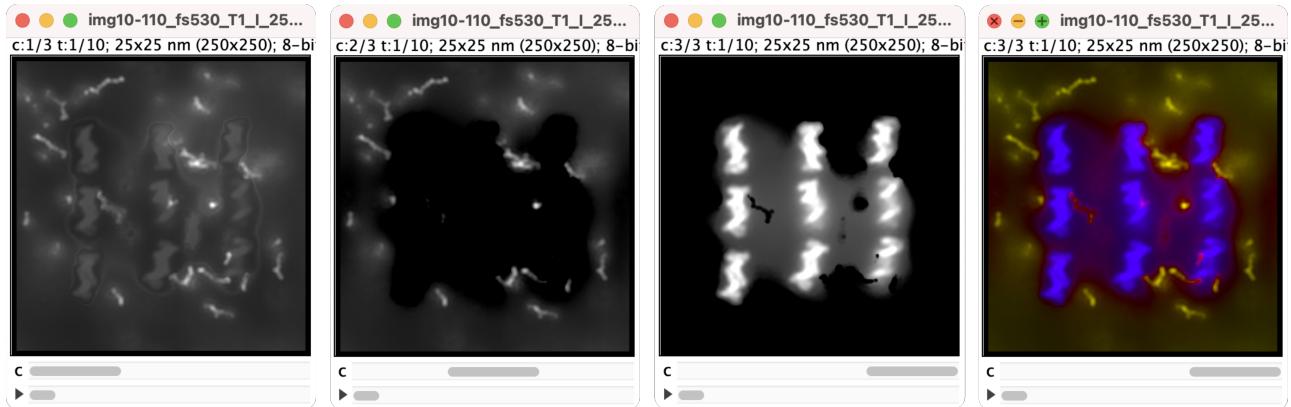
2 Generating 2DT images

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

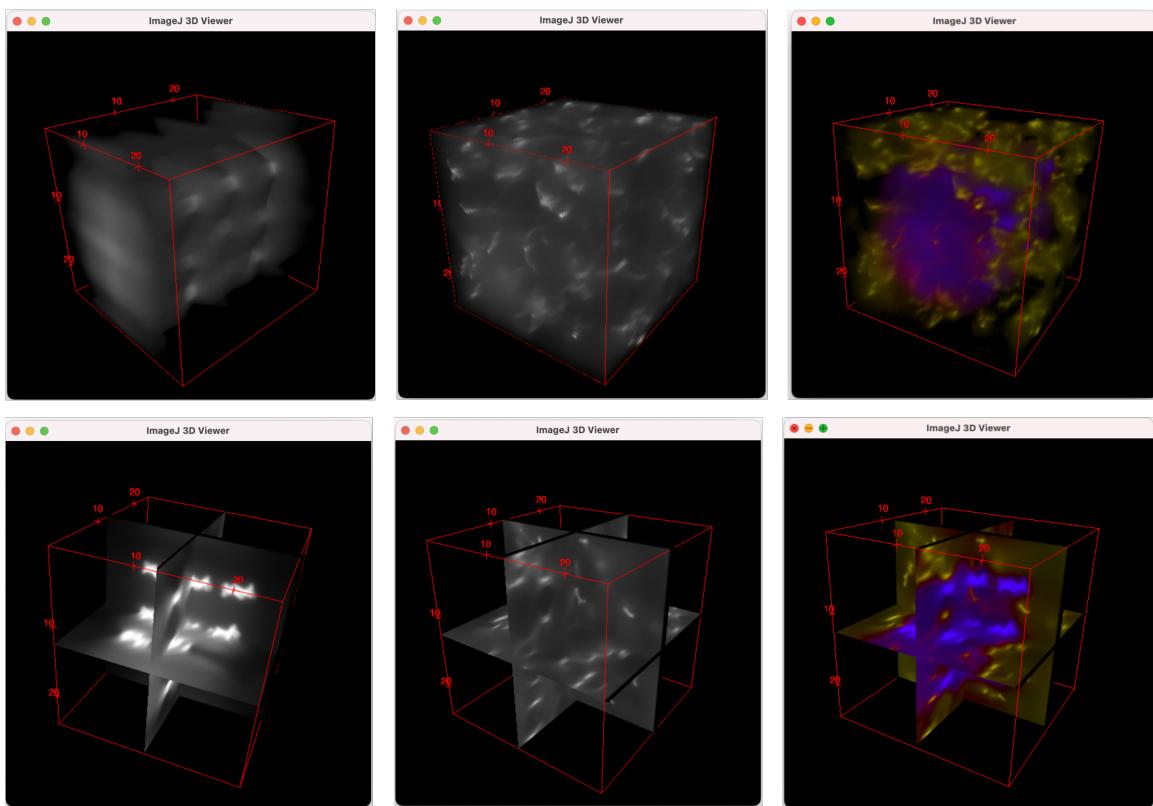
```
1 dp10.gro,parameters.dat,PSF_gandy,img,volume
2 dp20.gro,parameters.dat,PSF_gandy,img,volume
3 dp30.gro,parameters.dat,PSF_gandy,img,volume
4 ...
```

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

per volume image ($\text{int}(25/0.2)=124$). 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**).



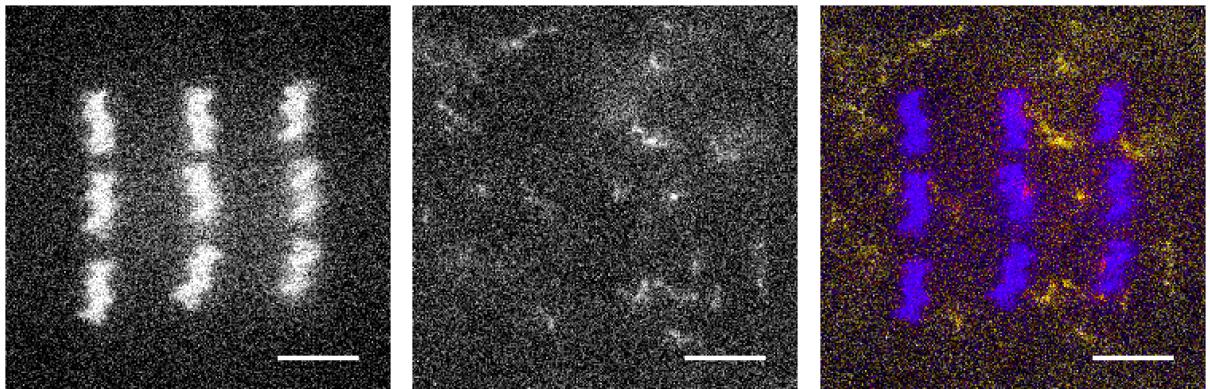
Similar steps can be followed for 3DT images.

```
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