

# Tutorial 6: Generating *in-silico* microscopy image with different thickness of excitation

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The thickness of excitation is controlled by the third float value of `Plmn`. To demonstrate this feature, the PSF generated in **Tutorial 1** (`PSF_gandy_lam518_fs800.dat` and `PSF_gandy_lam670_fs800.dat`) is used.

## 1 Generate *in-silico* monochrome image intensities

The monochrome image intensities are created for thickness of excitation of 1, 5, and 25 nm, using the command shown below. Since the PSF in Tutorial 1 was generated for a cuboidal box of  $15 \times 15 \times 25 \text{ nm}^3$ , the maximum possible thickness of excitation is 25 nm. (**Note:** For a higher thickness of excitation, PSF can be created with different `Plmn`).

```
Tut2$ bash gen_Pn.sh
```

The thickness of excitation in `parameters.dat` is changed using a for loop and replace function of `sed`,

```
3 for PN in 1 5 25
4 do
5     sed "s/Plmn\s*=.*Plmn = 15\.0 15\.0 ${PN}\.0/g" parameters.dat > foo.dat
6     siliscopy gen_mono --file dp100.gro --paramfile foo.dat --psf PSF_gandy \
7         --output Pn${PN}_img100
8 done
```

This reads the PSF files generated in **Tutorial 1** and reads the following variables from `parameters.dat`,

- `fs = 800`
- `lam[i] = 670, 518`
- `lam_names[i]`
- `dlnm = 0.1, 0.1, 0.2`

- $Plmn = 15, 15, 1$  or  $= 15, 15, 5$  or  $= 15, 15, 25$
- $maxlen = 25, 25, 25$
- $focus\_cor = 12.5$
- $opt\_axis = 2$
- $pbz = xyz$

This creates six files,

- |   |   |
|---|---|
| • <code>Pn1_img100_lam518_fs800.dat</code>  | • <code>Pn1_img100_lam670_fs800.dat</code>  |
| • <code>Pn5_img100_lam518_fs800.dat</code>  | • <code>Pn5_img100_lam670_fs800.dat</code>  |
| • <code>Pn25_img100_lam518_fs800.dat</code> | • <code>Pn25_img100_lam670_fs800.dat</code> |

## 2 Generate colored *in-silico* microscopy images

The colored images are created with the command,

```
Tut2$ bash gen_Pn_jpeg.sh
```

Since  $Plmn$  is not read during image generation, the images are created with the same `parameters.dat` using a for loop (without replacing anything with `sed`).

```
3 for PN in 1 5 25
4 do
5     siliscopy plot --file Pn${PN}_img --paramfile parameters.dat \
6                   --method color --timestep 100 --calc specific
7 done
```

This reads the image intensities generated in the previous step and the following variables from `parameters.dat`,

- |                              |                         |
|------------------------------|-------------------------|
| • $fs = 800$                 | • $maxlen = 25, 25, 25$ |
| • $lam[i] = 670, 518$        | • $T = 1$               |
| • $lam_{IO}[i] = 0.13, 0.25$ | • $scale = 5$           |
| • $lam_{hue}[i] = 255, 60$   | • $dpi = 600$           |
| • $dlnn = 0.1, 0.1, 0.2$     |                         |

**Thickness of excitation:** 1 nm (left), 5 nm (middle), and 25 nm (right).

