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 the this feature, the PSF generated in **Tutorial 1** (`PSF_gandy_lam518_fs530.dat` and `PSF_gandy_lam670_fs530.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 $15 \times 15 \times 25$ 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`,

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

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

- `fs` $= 530$
- `lam1`, `lam2` $= 670, 518$
- `lam_names1`, `lam_names2`
- `dlmn` $= 0.1, 0.1, 0.2$

- `Plmn` $= 15, 15, 1$ or $= 15, 15, 5$ or $= 15, 15, 25$
- `maxlen` $= 25, 25, 25$
- `focus_cor` $= 12.5$
- `opt_axis` $= 2$
- `pbc` $=$ xyz

This creates six files,

- `Pn1_img100_lam518_fs530.dat`
- `Pn5_img100_lam518_fs530.dat`
- `Pn25_img100_lam518_fs530.dat`
- `Pn1_img100_lam670_fs530.dat`
- `Pn5_img100_lam670_fs530.dat`
- `Pn25_img100_lam670_fs530.dat`

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`).

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

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

- `fs` $= 530$
- `lam1`, `lam2` $= 670, 518$
- `lam_I0_1`, `lam_names2` $= 0.13, 0.25$
- `lam_hue1`, `lam_hue2` $= 255, 60$
- `dlmn` $= 0.1, 0.1, 0.2$
- `maxlen` $= 25, 25, 25$
- `T` $= 1$
- `scale` $= 5$
- `dpi` $= 600$

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