

# 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 Lpsf parameter used in gen\_mono. The PSF generated in Tutorial 1 is used.

## 1. Generate *in-silico* monochrome image data files

The image data files are created for thickness of excitation of 1, 10, 20, 30, 40, and 50 nm. Since the PSF in Tutorial 1 was generated for a cuboidal box of  $15 \times 15 \times 50 \text{ nm}^3$ , the maximum possible thickness of excitation is 50 nm. These image data files are generated with the command,

```
term$ bash gen_Pn.sh
```

The thickness of excitation is changed using a for loop and replace function of sed,

```
for PN in 1 10 20 30 40 50
do
    sed "s/Lpsf = ./Lpsf = 15\.0 15\.0 ${PN}\.0/g" parameters.dat > foo.dat
    ../../gen_mono -f dp100.gro -p foo.dat -o Pn${PN}_img100
done
```

This creates files, Pn1\_img100\_lam518\_fs800.dat, Pn1\_img100\_lam670\_fs800.dat for thickness of excitation of 1 nm. Similar files were generated for 10, 20, 30, 40, and 50 nm.

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

The colored images are created with the command,

```
term$ bash gen_Pn_png.sh
```

The images can be created with the same “png\_param.dat” using a for loop,

```
for PN in 1 10 20 30 40 50
do
    python ../../mono2color.py -f Pn${PN}_img -p png_param.dat -t 100
done
```

Images with different thickness of excitation  $P_n$ : 1 nm (top, left), 10 nm (top, middle), 20 nm (top, right), 30 nm (bottom, left), 40 nm (bottom, middle), 50 nm (bottom, right).

