# Generating simulated sphere data

To generate the simulated 3D spherical data, you can still use your original code and we just need to assign structured 3D position and 3D orientation to the validation emitters.

The code below will generate 3D spherical shape with orientations perpendicular to the surface for you.



Chart, scatter chart

Description automatically generated

# Analyze experimental data

## Location of the DPPC+chol data:

<https://doi.org/10.17605/OSF.IO/97GMV>

Graphical user interface, text, application, email

Description automatically generated

## Prepare the data:

1. **offset**: All the data is offset included. Offset is the value of the captured image when there are 0 photons.

To analyze the image, we have to subtract the offset from the image

1. **2\_beads\_w\_offset to 10\_beads\_w\_offset**: image data; each data set have 2000 frames. They are captured on a same sample. The final result is the concatenated estimations from 9 datasets.
2. **Bkg**: for each frame, I estimated a bkg image. You can train you network with background subtracted image. Then for experimental data, you can subtract the bkg from the raw image for estimating.
3. **AD count**: all the values in these images (bead\_w\_offset, offset, bkg) are **electron count, but not photon count**. The camera receives photon and converts the photon into electron. The electron counts are the value show on camera. In our camera, 1 electron count = 0.66 photon count.

In estimation, you will need to multiply images with 0.66 before putting into the neural network.

Graphical user interface, application

Description automatically generated

## Training the neural network for estimating the experimental data

1. **Phase mask:** any microscopy will introduce aberration into the image. We need to use calibrated phase mask for generating simulated data and train the network (pixOL paper, figure S27).

**Location of the calibrated phase mask:**

Graphical user interface, text, application, email

Description automatically generated



1. **NFP for DPPC chol data**: z=-350
2. **left\_to\_right\_trans\_ratio:** Our microscope splits the light into two channels: x polarization and y polarization channels. The splitting is not perfect. if ideally, the intensity in two channels is the same, in our microscope, we won’t get an exact same intensity, instead, we will get: y channel photon: x channel photon=1.1426.

To compensate this factor, when we are generating the training data, we can multiple 1.1426 to the y channel.

I will send you following code to implement the factor of **‘left\_to\_right\_trans\_ratio’** and **‘Phase mask’**

You can replace your original **forward\_model\_3D** with this **forward\_model\_3D\_retrieved** with



Text

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# Visualizing the estimation

I am sharing the code I used for generating the figures related to Fig.3. in pixOL paper.

The first 4 sections will give you all the image visualizations, and you can explore from this code first.





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