

**Stable FP single molecule simulations on mEos3.2, assumed to be immobilized with
SpyTag/SpyCatcher**

Simulations made as a tool to assess the capability of processing pipelines to extract accurate photophysical parameters.

Raw data in: Z:\DOMINIQUE\SMIS\SIMULATIONS\STABLE_FP_FEBRUARY_2023

Common parameters:

- ⇒ **Data sets : 30,000 frames (but only 5000 frames on Resana for size considerations ...)**
- ⇒ **pixel size: 130 nm**
- ⇒ **frame time: 50 ms**
- ⇒ **561 nm laser: 500 W/cm² at center, Gaussian beam**
- ⇒ **Background: 100, very slowly decaying**
- ⇒ **EMCCD gain: 200**

Simulations 1&2: discard

Simulation 3: medium density

10000 molecules, 405 nm laser: 10 W/cm²

Simulation 4: high density

50000 molecules, 405 nm laser: 2W/cm²

Simulation 5: low density

2000 molecules, 405 nm laser: 10 W/cm²