Fluorescence correlation spectroscopy (FCS) in *C. elegans* - Tips & Tricks:

Fluorescence correlation spectroscopy (FCS) is a stochastic imaging technique that can be used to analyze the diffusive behavior, the concentration and interactions of proteins inside solutions.

The complexity of the technique should not be taken lightly and data acquisition and analysis should be carried out and discussed with experts to ensure interpretability of the obtained results.

Here I will give some Tips & Tricks for doing FCS in *C. elegans* embryos:

1. For a typical FCS measurement per embryo I measured 3x 8s in 3 different positions insight the embryo, yielding a total of 72s total measurement time.

2. *C. elegans* embryos are autofluorescent, especially in the GFP channel. This is important because FCS works best at low protein concentrations and thus low signal. Any pollution of the signal can severely impact the quality of the data, thus it is important to remove the autofluorescence.

   Solution: Prebleach with laser for 1 s.

3. *C. elegans* embryo cytoplasm is tightly packed with vesicle and other particles that can get in the way of the measurement. When such a particle diffuses through the small vocal volume the intensity will drop to zero and ruin your measurement.

   Solution: Do multiple short measurements instead of single long ones. This way you can discard measurements that show artifacts. For the analysis this does not make a difference as you can concatenate the short measurements into a single long one prior to data analysis. I typically did 3x 8s measurements.

4. *C. elegans* embryo are about 20-25 µm thick and the fluorescent signal will decay rapidly the further into the sample you go.

   Solution: For optimal signal and results perform the FCS measurements in the cytoplasm close to the objective.

5. Keep in mind: FCS works best with low concentrated fluorescent particles. Since the amplitude of the FCS curve inversely scales with protein concentration, the resolving power of FCS decreases with increasing protein concentration. Concentrations between 10-100 nM (1-10 molecules in the focal volume at every given time) are preferable. Many proteins are much higher concentrated, especially when contained in vesicles or other structures. In such situations it is probably best to use alternative techniques like FRAP.

6. Useful reads for FCS and FCCS:

- Heinze, K., & Schwille, P. (2007). Fluorescence correlation spectroscopy in living cells. *Nature Methods*.
- Langowski, J. (2008). Protein-protein interactions determined by fluorescence correlation spectroscopy. *Methods in Cell Biology*, *85*, 471–484.
- Bacia, K., Kim, S. A., & Schwille, P. (2006). Fluorescence cross-correlation spectroscopy in living cells. *Nature Methods*, *3*(2), 83–89.
- Bacia, K., & Schwille, P. (2007). Practical guidelines for dual-color fluorescence cross-correlation spectroscopy. *Nature Protocols*, *2*(11), 2842–2856.
- Wueseke, O., Bunkenborg, J., Hein, M. Y., Zinke, A., Viscardi, V., Woodruff, J. B., et al. (2014). The C. elegans pericentriolar material components SPD-2 and SPD-5 are monomeric in the cytoplasm prior to incorporation into the PCM matrix. *Mol Biol Cell*.

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