**AUTOFLUORESCENCE CHAPTER**

**Autofluorescence in C elegans**

C elegans embryos have lots of autofluorescence. Can be seen by imaging N2s. Not only does this vary in intensity between embryos, but it varies spatially too. This contributes greatly to the spatial and inter-embryo signal variation seen for fluorophore-tagged proteins

Figure: SAIBR 1B

**Approaches to circumvent autofluorescence**

* Overexpression
* Chemical compounds, bleaching,
* Optimising the combination of fluorophores, excitation wavelengths and emission filters to reduce AF in images
* Red fluorophores: but lower brightness, which isn’t good for lowly expressed proteins

**Spectral imaging**

See <http://zeiss-campus.magnet.fsu.edu/articles/spectralimaging/introduction.html>

Autofluorescence correction

**SAIBR: a protocol for autofluorescence subtration**

*Analysis of unlabelled embryos reveals strong correlation between channels*

Figure:

- SAIBR 1E,F

- Possibly include S1 top row and discussion of noise/blur

*Subtracting autofluorescence from GFP labelled images*

Figure:

* SAIBR 2A-D
* Possibly include PAR-2 too

*Autofluorescence subtraction improves the ability to resolve membrane proteins*

Figure:

* SAIBR 2E-G

*Autofluorescence subtraction in two-colour samples*

Figure:

* SAIBR 4B, C, D

Figure:

* SAIBR 4F,G