A004-Unmixing of spectrally identical fluorescent proteins by lifetime imaging

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Abstract:
Fluorescence lifetime unmixing enables the independent imaging of two or three fluorophores that have identical emission spectra. The separation is based on differences in the excited state lifetime. This imaging method is of interest as it increases the options for multiplex imaging. (It aligns with my talk on the engineering of fluorescent proteins).

We have engineered fluorescent proteins by screening for lifetime variants (https://doi.org/10.1038/nmeth.1415). As a result, we have identified and characterized fluorescent proteins with different fluorescence lifetimes and have demonstrated that these can be used for lifetime unmixing (https://doi.org/10.1529/biophysj.107.125229). In this workshop, we will discuss the requirements for lifetime unmixing and the types of fluorescent probes that are available. A sample with two different Cyan Fluorescent Proteins (CFPs) that are targeted to two different cellular locations will be used (https://doi.org/10.1111/jmi.12168). The conditions for imaging and analysis will be discussed and the participants will have the opportunity to do the acquisition and tweak imaging conditions. The workshop has a focus on the data acquisition, but aims to explain and show the data analysis&visualization (using the software of the microscopy setup) as well. The outcome of the experiment is a set of two images with the distribution of the two cyan fluorescent proteins.

Educational goal:
The workshop will demonstrate the basics of unmixing on cyan fluorescent proteins, but this methods can be generally applied to fluorophores with different lifetimes.
At the end of this workshop the participants will be able to acquire and analyze data to perform the unmixing of (any) fluorophores based on lifetime.
The sample will depend on a publicly available plasmid (https://www.addgene.org/60491/), so the participants can reproduce the sample preparation in their own lab.
The participants will have sufficient knowledge to set up unmixing in their own lab and on their own samples (if they have access to a lifetime imaging setup).

Timeline:
Proposed time schedule 15 minutes Introduction (why is this important, how is this done in theory) 5 minutes Sample preparation 15 minutes Imaging (Instruction by the instructor, showing the data acquisition and on-the-fly analysis). 60 minutes Imaging by the participants. The participants will have the opportunity to look for suitable cells in the sample and optimize the imaging conditions 10 minutes Discussion. What are the advantages of this approach? What are the limitations/ How can you apply this in your own work?

Category:
None/None/Practical workshop