

# New user project questions

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## **(1) "Experimental Hypothesis":**

- a) What is your experimental hypothesis?
- b) How will you test it - what info do you need?
- c) How will you get that info - what will you measure ?
- d) ...and how will you analyze the info you get in order to prove your hypothesis right or wrong?
- e) What controls will you need?
- f) What statistical test will you use to test your hypothesis?
- g) Are you interested in colocalization analysis?

## **(2) "Sample Preparation, Dyes and Mounting"**

- a) What is the sample? Organism? Tissue? Cell Type? Living? Fixed?
- b) How is it prepared? Grown on glass? Tissue section? Living Embryo?
- c) How is it mounted? Under a (proper exactly 0.17 mm) coverglass? Underwater in an agarose well? In an agarose column? On a filter/carrier/special chamber?
- d) What Staining / Dyes / contrast agents will you use? Fluorescence and/or transmitted light? Electron beams?

## **(3) Find appropriate microscope system**

- a) Do you need 2D (single focal plane) or 3D images (z stacks)?
- b) Do you need time series? If so, what time resolution / frames per seconds do you need?
- c) Do you need high Signal : Noise (see the difference in intensity accurately), or will somewhat noisy images work for you (just see where stuff probably is)
- d) Do you need high dynamic range (see very dim and very bright things in the same image at the same time)?
- e) What is the size of the field of view that you need?
- f) How big are the objects you are interested in? What (optical) resolution do you need (what should the pixel spacing at the sample be)? 5 nm? 200 nm? 1 micro meter? Bigger? (Hint: Theory says you need no more than 2.3-3 pixels across the object of interest in order to image it properly)

## **More hints to help you get started with your (new) imaging project**

### **Carefully define the imaging experiment with a testable hypothesis.**

- "I just want to see what it looks like" is not an experiment - you will be asked to go and think hard about that.
- It's very likely that you will need control samples and images of them.

### **Select appropriate microscope systems able to get the info you need from the samples to test the hypothesis**

- We have many tools at hand. The hammer (point scanning confocal) is a great general tool, but it's not always the most suitable.

### **Figure out sample preparation/mounting/environmental control**

- You need to know the spectral details of the dyes and understand the biochemistry you are using for your staining/preparation.
- High resolution images can only be gotten from well prepared and properly mounted samples
- Environmental control can be tricky to set up and optimize – don't underestimate the complexity.

### **Determine optimal imaging hardware settings to get the best image quality / speed tradeoff.**

- Your first images of a sample will hardly ever be THE final images
- Expect it to take some training, patience, and effort to become able to use a new microscope system properly. These are not point and shoot cameras.
- You are going to need to spend considerable time fine-tuning the setup to get optimal image data.
- DON'T RUSH - make an estimate of how long the imaging experiment will take, and then multiply it by at least 3x.

"But I need the images for my TAC on monday!" is a very bad way to attempt an imaging experiment!

Together we will find the best and/or most sensible way to get the image information you need to test your hypothesis. During the first meeting the answers you give to the below questions will be the basis of the discussion.