Plan Your Experiment

1. Plan your experiment from start to finish

The most important step. Getting this right will save you a lot of time!

Think carefully about exactly what you are trying to observe / measure.

Some examples:

1. Is protein X and protein Y found in the same cell/organelle?
2. Are there less X cells in the cortex when I treat with drug Y?
3. Does the expression pattern/intensity of protein X change in condition Y?

It can help to think about how you would display the final results of this experiment in a figure - what images would you need in each panel to show and convince a reviewer?

What you need to observe / measure will determine what techniques you should use.

If you are not sure what the best approach is at this point *you should ask for help*. Has someone previously carried out this experiment in your laboratory? How did they do it? Has a similar experiment been carried out in the literature? Check methods in publications where similar experiments have been carried out successfully.

Before commencing a project it is recommended you contact histology and microscopy to clarify the suitability of the technique you intend to use - doing so could save you time, unnecessary stress, troubleshooting later, as well as wasted money and resources.

Be aware of the time it will take to complete the imaging aspects of your project before you start so that you are not left desperate at the last minute - microscopy can be time consuming and instruments can be heavily booked.

For more information see page 5-7

2. Prepare your sample in the best way for the technique/s you intend to use

After you have decided what you are observing/measuring and what technique you will use, find out how your cells/tissue should be prepared *for best results*.

The right preparation will make your results easy to observe - the wrong preparation can mean lots of troubleshooting when it comes to imaging. Also, changing your method half-way through a study could make it difficult to compare your new results to older results - it is best to get this right the first time around.

Fixation: Choose an appropriate fixation method and don't over-fix - *see page 8*

Sectioning: Choose an appropriate method and thickness for sectioning your tissue - *see page 9*

40-50µm sections are often a good choice for most experiments. Any thicker and you can begin having trouble with staining and imaging. Confirm your choices with histology and microscopy.

3. Select the right markers/labels and staining method

Choose markers that will work well with your technique - there are many to choose from but you want to ***avoid marker combinations that may be difficult to tell apart*** (e.g. Heamatoxylin with nDAB or GFP with YFP).

You also need to choose a staining protocol. These may vary on the tissue or cells you are looking at.

for more information see page 10 and protocols

4. Choose the correct mounting media

DPX works well for brightfield sections to be imaged or scanned

Use **Mowiol** for fluorescence (or for brightfield sections to be used for stereology)

Use **prolong gold** if you are concerned about fluorescence fading or photobleaching

5. Use the right coverslips

Use ***0.17mm thick / No.1.5 coverslips***

Seal the coverslips onto the slide using **clear non-fluorescent nail-polish**

6. Choose the right objective and exposure time

20x or 63x objectives are often the best choice for most experiments.

When setting the exposure time avoid **oversaturation** which can prevent you from analysing changes in protein expression and localisation. Also, ensure your exposure time is not too long - excessive exposure times often mean you are imaging autofluorescence or bleed-through from another channel.

If you intend to compare your images **keep things consistent** by using the same objective and exposure time. If you want to analyse intensity changes you should image using the same microscope with all the same settings.

for more information see pages 11-13

7. Run a pilot study

In some cases running a small pilot study is recommended as this will assist in refining the methods without losing precious material. Positive and negative controls for the techniques should be included each time the procedure is done to validate the results. If you are not sure about controls ask for help.