How to set the parameters on camera-based systems

1. Scientific question

Define the scientific question for this image. Usually 1 question per image. What metrics do you want to measure? How big are the differences you want to detect? Deduct the required image resolution.

2. Objective

Choose the objective that delivers this optical resolution (0.61*l/NA). Check that the working distance, field of view and immersion medium are also sufficient.

3. Fluorophores

Choose your fluorophore combination: match fluorophore spectra with the microscope excitation and emission specifications to optimize the efficiency of imaging while avoiding bleedthrough.

4. Display

- **Display the images to black and white to avoid colour bias.**
- **Use the LUT to push the brightness and contrast.**
- **Check the pixel intensity in and out of the region of interest to estimate the signal to background ratio.**
- **Always have the saturation and under exposure LUT on when adjusting the imaging parameters to ensure no saturation and no under exposure in or around the areas of interest.**

5. Binning

- **Use no binning by default.**
- **Add binning when the full resolution of the camera is not needed (e.g. overview).**
- **Faster acquisition, brighter images, smaller files (faster stitching).**

6. Extra magnification lens

Considering the camera pixel size, calculate if the objective magnification is sufficient to fulfill the Nyquist criterion (pixel size = 1/2 of the optical resolution) or if the extra magnification lens is needed to avoid artifacts in the undersampled details.

7. Camera area

Use the camera crop function so that the area of interest fills the image, minimize unnecessary information or black pixels.

8. Readout noise

- **Use low readout by default to avoid creating noise.**
- **If fast imaging is needed (e.g. fast timelapse for calcium imaging), increase the readout rate.**

9. Illumination power

If possible, increase the illumination power to decrease shot noise contribution.

10. Gain and EM gain

If these options are available, test different settings to get the best signal to noise ratio.

11. Averaging

Scan continuously without averaging.

See what changes from one scan to the next: this is random noise.

Does the random noise prevent you from answering your scientific question?

If yes, add some averaging.

12. Background correction

Acquire an image with a short exposure time but no illumination to find the camera offset.

Always subtract the camera offset to get the correct intensity values.

Use shading correction to correct for uneven illumination.