Quantitative Imaging Checklist

☐ Stable light source

☐ Intensity control sample (fluorescent plastic slide) – measure intensity before, during and after experiments

☐ Unlabelled cells – measure autofluorescence

☐ Cells with each dye alone – check for excitation and emission cross-talk

☐ Antibody labelling controls

☐ Instrument settings kept constant

☐ Nyquist-Shannon sampling in x, y, z and t (maximum resolution divided by 2.3)

☐ Shading correction image

☐ Background intensity image or ROI measurement area within images

☐ No pixels reading zero

☐ No pixels saturated

☐ Laser powers as low as possible – no fluorophore saturation (LSM confocals)