27.4 Planning an imaging experiment

Scientific images are not just 'pretty pictures'; they are spatial and/or temporal datasets of photon intensity. Without proper planning of your imaging experiment, time spent acquiring and analysing your images is wasted. Scientific investigation is based upon testable hypotheses, and **Figure 27-3** shows how this is an integral part of any imaging experiment, designed to explore observations and answer questions.

Statistical analysis is very often 'the bit tacked onto the end' of an experiment, whereas the experiment should rather be designed around the correct statistics required to answer the hypothesis. Dytham (2010) and van Emden (2008) are two extremely good books on statistics for biologists, while a series of articles on statistics has been published by physiological and pharmacological societies in the UK[1]. Aitken *et al* (2009) is helpful for those who wish to recap on basic mathematics as applied to scientific investigation.

Quantitative microscopy is a complete subject in its own right, and will not be covered here. In volume 123 of the *Methods in Cell Biology* series (Elsevier) there are three good chapters on quantitative microscopy. Waters & Wittman (2014) introduce the essential concepts of measuring fluorescence quantitatively; Jonkman *et al* (2014) apply these principles to confocal microscopy and Verdaasdonk *et al* (2014) and Coffman & Wu (2012) discuss how to detrmine absolute protein numbers using fluorescence microscopy.

When collecting data it is worth keeping in mind the difference between accuracy and precision (**Figure 27-4**), and the need to collect sufficient images for data extraction for the statistical analysis to be relevant. The following references are essential reading for anyone serious about extracting qualitative or quantitative data from their images. These are Waters (2009), Waters & Swedlow (2007) and Kedziora *et al* (2011). A worked example is given in Mutch *et al* (2011) and a further example in Terasaki (2006). For information on the subject of laboratory measurements, accuracy and precision, see Meah & Kebede-Westhead (2012). A useful paper with guidance on selecting sample sizes for analysis is Silcocks (1983).

The increasing ease of acquiring digital data coupled with the ability to capture many fields of view from many samples – 'high-throughput' analysis, bioimage informatics, or bioinformatics, has become a powerful tool for biologists and geneticists. Bioinformatics serves two purposes: it allows imaging data to be stored, and subsequently retrieved, with molecular biological data from other sources; it permits analysis of data holistically across previously disparate disciplines. Ultimately, the aim within biomedical research is to offer therapeutic solutions in combating disease. A themed issue on bioinformatics has been published in *Nature Methods* for July 2012; in particular refer to Eliceiri *et al* (2012) and for a *Nature Methods* course in practical statistics, see their Points of Significance series from September 2013 inwards.

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(contd)

[1] See: *Br. Jour. Pharmacol.* (2011) 163/2**:** 207 and *Br. Jour. Pharmacol.* (2012) 166/7**:** 1977. See also the Nature Methods series referred to in the text, below.

Figure 27-3 (Original by Michael Doube, Royal Veterinary College, London; used with permission)

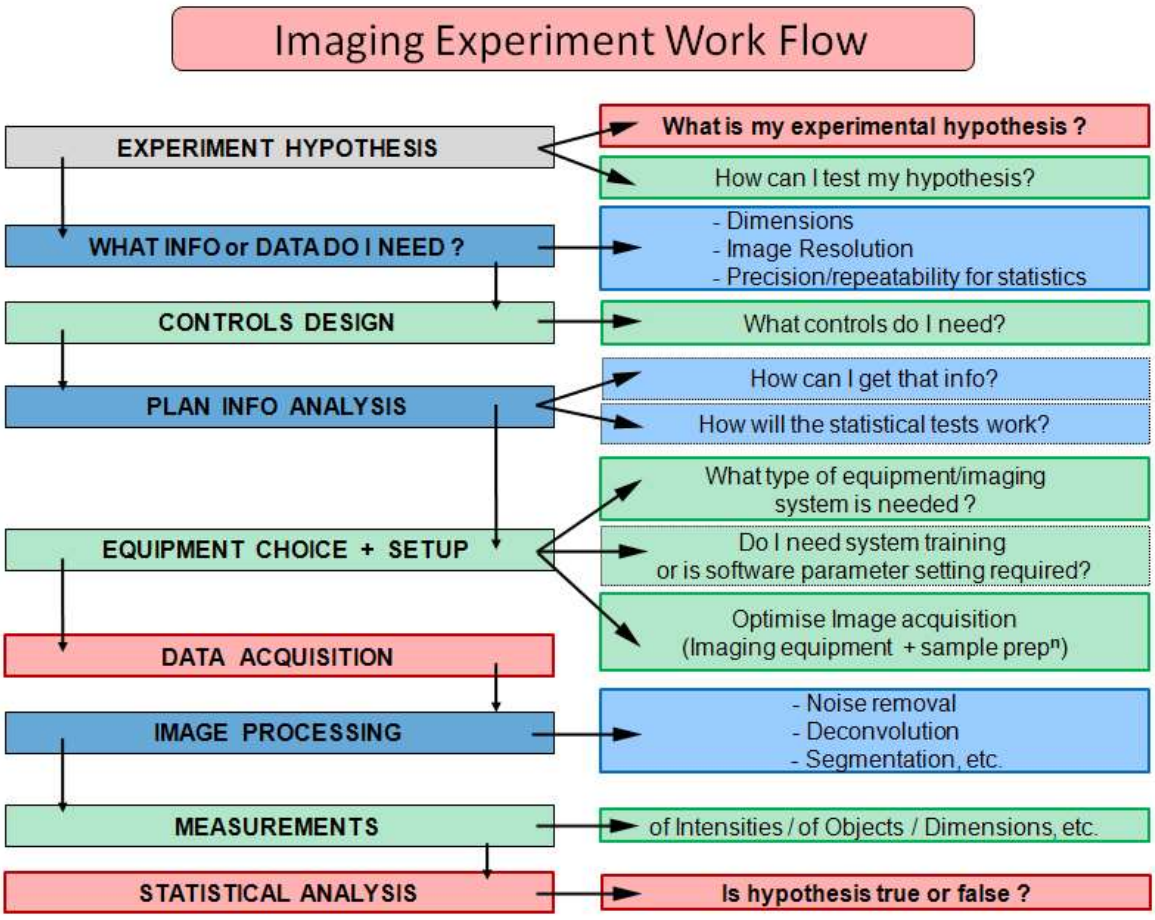
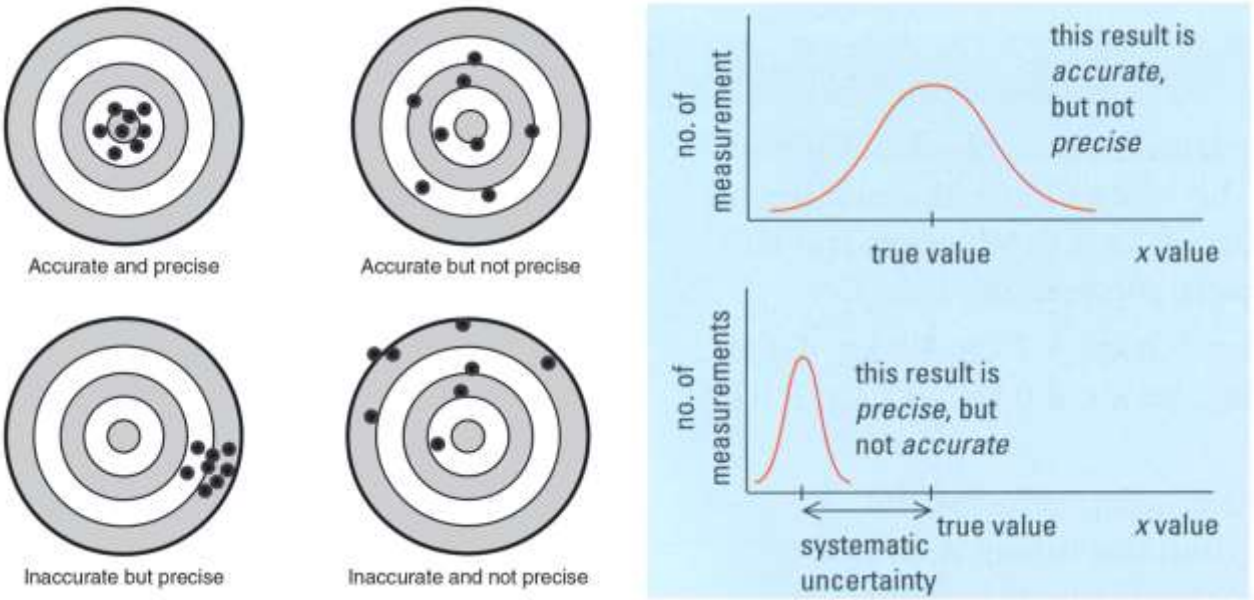


Figure 27-4 Precision and accuracy



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