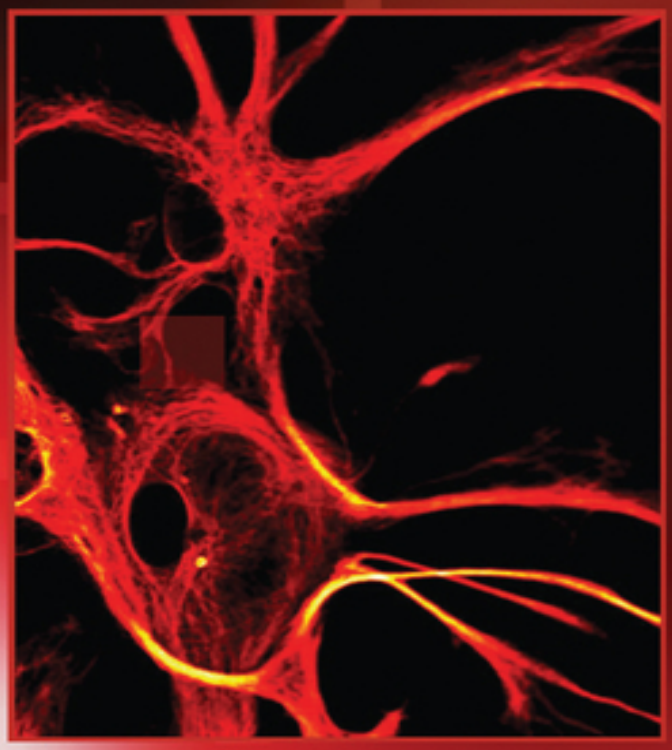
Editor

Philip D. Langton



Essential Guide to Reading Biomedical Papers

Recognising and Interpreting Best Practice

WILEY-BLACKWELL

Contents

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WILEY-BLACKWELL

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Foreword

Biological research is an experimental science in which the testing of hypotheses through experiments generates knowledge and ultimately, understanding. However, while the construction of a hypothesis is open to the imagination, the experiments that produce the data to be analysed in the light of the hypothesis are only useful if they are reliable, accurate, reproducible, and come from carefully designed and controlled experiments. In the acquisition of data, therefore, there is no room for the imagination and even less for wishful thinking – the vice that reads the data to fit the hypothesis and not the other way around. Therefore, although it is true that a hypothesis guides the gathering of information, the primacy of the data is obvious since without them all hypotheses remain in the "to be done" drawer.

Biological research nowadays can be approached using a variety of techniques, the majority of which have been developed in the last 50–60 years. The power of these new techniques is such that biologists often forget two fundamental things, (1) that the usefulness of the technique does not lie so much in its intrinsic power but in the way it is applied to a biological question and (2) that all techniques have been designed for a specific purpose and therefore have limitations. There is not a single technique that can inform us about the whole of a biological problem and therefore it is advisable, wherever possible, to use more than one complementary technique.

These are the reasons why the present book by Langton and colleagues is so important. It is not merely about techniques; it puts techniques into context – their potential, their limitations and possible pitfalls. This is accompanied

by useful primers on the philosophy of science, on experimental design and on statistics. As such, this volume will undoubtedly be of great interest and value, not only to the novice scientist but also to the experienced investigator and mentor.

I highly recommend this book to all practitioners of biological research.

Professor Sir Salvador Moncada

FMedSci, FRS

27th July, 2012

Preface

Imagine you are interested in buying a used car. Are you likely to be entirely trusting of the person selling the vehicle? Will you accept everything they tell you without question and without evidence? I suspect not. I suspect that you will have a *healthy scepticism*. The seller wants your money and it is your responsibility to ensure that you are satisfied with the trade – hence the phrase '*caveat emptor*', which means '*let the buyer beware*'.

I would argue that you should regard journal articles with the same healthy scepticism, but I imagine your reaction to this is either disbelief or a mixture of confusion and panic. If you are sceptical, good! I need to provide evidence for my argument. If you are confused, let me explain.

How is a journal article like a used car? It is easier to consider how the seller equates to the author(s) of a journal article. The seller wishes to get the best price and is unlikely to point out defects and flaws; certainly not with the same enthusiasm as they have for the *plus points*. The authors of a journal article are also selling something – their interpretation of their experiments, including their underlying assumptions. It is hugely important that the authors win the reader's confidence; that they convince *you* that their work is showing true facts (see Primer 1 for an expansion of this idea). Science is big business and careers depend on how widely and how securely the views of the authors are endorsed by the scientific community. In some ways, it is possible to regard journal articles as advertisements for a particular doctrine.

Some of you will have picked up a counter-argument in the shape of the *peer-review process*. Good for you, you are reading with scepticism. Surely the peer-review process means the reader can have confidence that an article in a

peer-reviewed journal is accurate and true – at least at the time it was published? Sadly, this would not be a safe assumption. Though it is arguably the best system we have to ensure the quality and validity of what is published, peer-review is very far from perfect and even the most prestigious journals make mistakes.

Science makes use of an approach that has become known as the Scientific Method (see Primers 1 and 2). It was developed into something we can recognize today in a process that can be traced back nearly 400 years to a succession of great thinkers and great philosophers. In 1601, Francis Bacon (1561–1626) wrote:

> *'Read not to contradict and confute; nor to believe and take for granted; nor to find talk and discourse; but to weigh and consider.'*
>
> From Bacon's essay *of Studies* (published in full in [1]Madden, 2007).

It is too easy to read and simply accept as facts those things that are offered as such. It is highly likely that excellence in your written work, which relies upon your interpretation of the academic literature, will be judged on your ability be demonstrate that you are '*critical of what you read*'.

Unless you have knowledge of the experimental techniques used in a study, however, you will find it difficult to discriminate between studies that are well designed and/or controlled and those that are not, and you will find it hard to be critical. Therefore, in essence, each primer in this book is intended to provide you with the means to be critical about studies described in journal articles.

Aims of each primer

It is important that you are aware of the aims of these primers. Each one is designed to:

- ✓ provide orientation and guidance to readers who have no experience of the technique;
- ✓ suggest reasons/motives for electing to use a specific technique;
- ✓ provide details of a method only where detailed knowledge is required;
- ✓ provide limits on what can 'reasonably' be claimed of data – specificity, selectivity, etc.;
- ✓ describe control experiments that *should* be included in a journal article;
- ✓ explain why particular controls are performed;
- ✓ list other techniques that are commonly used in conjunction;
- ✓ list common misconceptions about a technique or the data produced by a technique;
- ✓ list some caveats about interpretation of data [where appropriate].

However, they are *not*:

- ✘ intended to be encyclopaedic manuals or reviews;
- ✘ 'how to' guides;
- ✘ sufficient in themselves as resources (hence the further reading);
- ✘ likely to be useful to persons experienced with the technique.

I want to end this preface with another quotation from Francis Bacon:

> *"If a man will begin with certainties, he shall end in doubts; but if he will be content to begin with doubts, he shall end in certainties."*

From Bacon's [2] *The Advancement of Learning* (1605) book 1, primer 5, section 8

What I take from this quotation is that it is *not* healthy for scientists to believe too fiercely in what appears today to be true. We must be prepared to question anything; there should be no [3]dogma in science, because our current understanding reflects a continuum beginning with the tentative '*more probable than not*' and moving to greater and greater probability of being accurate or true – but *never reaching certainty*. Ultimately, nothing is ever proved.

Phil Langton

Notes

1. Madden, P. (Ed., 2007). *Quotidiana* (http://essays.quotidiana.org/bacon/studies/). Accessed 22 Apr 2012.

2. Available from: www.lifesmith.com/Berkeley%20Teaching/The%20Oxford%20Dictionary%20Of%20Quotations.pdf; accessed 22nd April, 2012.

3. ***Dogma*** – 'a principle or set of principles laid down by an authority as incontrovertibly true'. Source: Oxford Dictionaries.

Acknowledgements

This book can trace its origins back to about 10 years, to learning and teaching resources for undergraduate students studying anatomy, neuroscience or physiological science at the University of Bristol and this explains in large part why Bristol academics are involved with the majority of the primers you will find listed. The aim for the book is simple, as it was for the original resource; to communicate some of the practical wisdom that can only come with years of experience and scholarship in laboratory research. It does not pretend to make the reader an expert or to train the reader to use these research techniques but it does provide insight into the assumptions and issues that can lie beneath the surface of seemingly transparent and persuasive research reports. In editing the book, I have been surprised on a daily basis by the scope and significance of the aspects highlighted by my colleagues. Indeed, I have learned so much that I would now view with extreme scepticism the claim that one can read contemporary research reports without, as minimum, the sort of insight that this book aims to provide.

The original resource would not have been possible without the enthusiastic support of my colleagues in the University of Bristol and it is to them that I am most deeply grateful. I wish also to thank the team at Wiley-Blackwell who saw the potential for a book and the independent reviewers who reacted so positively to the preliminary outline and example primers. The first edition required a significant expansion from the original resource and had involved a large number of people from Universities in the United Kingdom, Australia and North America to whom I am enormously and sincerely grateful.

I wish to express my gratitude to the University of Bristol for its encouragement of excellence in learning and teaching as well as excellence in research. For some time the prevailing wind within UK HE has benefitted a focus on research above all else and yet Bristol has striven consistently to promote the interests of its students and the education they receive. This book reflects the ethos of enquiry and excellence that is so typical of the University of Bristol.

I need to acknowledge the support (and patience) of my partner, Rosie, without whom this project would have failed. Finally, I gratefully acknowledge my parents who taught me to respect the potential in dedication and hard work and the value of integrity; attributes that no research scientist should ignore. Lastly to Alice, Polly and Jess who just wanted to see their names in print.

Dr Phil Langton

August, 2012

Introduction

Phil Langton

This introduction explains the structure of most of this publication (Primers 4 to 35). The first three primers escape this structure, as their aim is different. These first three primers cover:

1. The philosophy of science
2. Experimental design
3. Statistics

These are topics of fundamental importance in science. Reading these first will allow maximum benefit from the other primers and indeed from every journal article you read in future. Switch on your scepticism!

As Claude Bernard said in his [1] textbook, "*L'expérimentateur doit douter, fuir les idées fixes et garder toujours sa liberté d'esprit*", which means "*the investigator should doubt, avoid preconceptions, and always keep an open mind*"

Primers 4 to 35 will have the following structure:

- **Basic 'how-to-do' and 'why-do' section**

  This section will cover what the technique is used for. For some techniques, the answer is far from obvious. If there are facets of the technique that require insight into how the experiments are done, then they will be included in this section. It will be basic – just enough to make sections on *required controls* and *pitfalls in execution or interpretation* intelligible and to help you tackle articles in the reference section.

- **Required controls**

If your eyes have at tendency to glaze over at the mere mention of '*experimental controls*', then give yourself a slap and read on. Without well-judged experimental design and comprehensive controls, the results of an experiment are next to useless. There will endless possible interpretations – so much so that the experiment will not advance our understanding. Each primer will list the controls that should be present, and particularly those that require special attention, because these are frequently absent or poorly designed in published articles. If appropriate, the section will indicate what failure of a particular control might indicate. For example, failure of the negative control in a PCR experiment suggests contamination of the RNA by genomic DNA – mRNA implies ongoing transcription, but the presence of genomic DNA says nothing about transcription.

- **Common problems or errors in literature**

The errors pointed out should be findable by undergraduate students who have little or no practical experience of the technique. This does not mean there are not others – just that you will not be able to recognise them without first-hand experience, i.e. without using the technique to perform experiments.

- **Pitfalls in execution or interpretation**

If you think that the peer-review process prevents the publication of studies that contain significant errors then, again, give yourself a slap – you're being naïve! Consider this example: a study uses immunocytochemistry (Primer 13) to provide evidence that a protein is expressed in sections of tissue or in isolated/cultured cells; it then goes on to do immunoblotting (Primer 15) to back up the immunocytochemistry (demonstrating that the protein bound by the antibody has the correct apparent mass), *but* uses different primary antibodies for

the immunocytochemistry and immunoblots. Yes, it does happen! If the significance of this logical error is lost on you, then you need to read Primers 12 to 15.

- **Complementary and/or adjunct techniques**

This will list techniques that are often used together.

- **Further reading and resources**

Some of the articles will be those cited in the text, while others will be suggestions for further reading. You need to keep in mind that these techniques require years of careful scholarship and training to master. The primers have been written by researchers who have developed true expertise with each technique but each primer is very short and of limited scope – hence the term, '[2]*primer*'.

Notes

1. '*Introduction à l'étude de la médecine expérimentale*', 1865.

2. **Primer**: a book (or text) that covers the basic elements of a subject. Source: OED.

Section A

Basic Principles