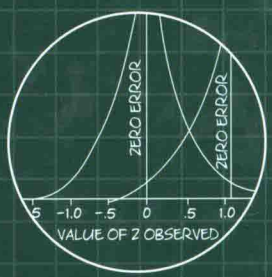
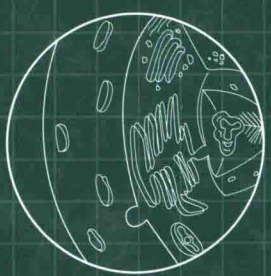
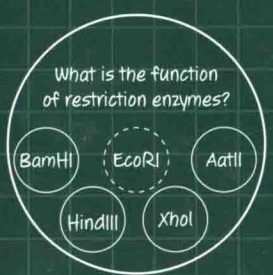
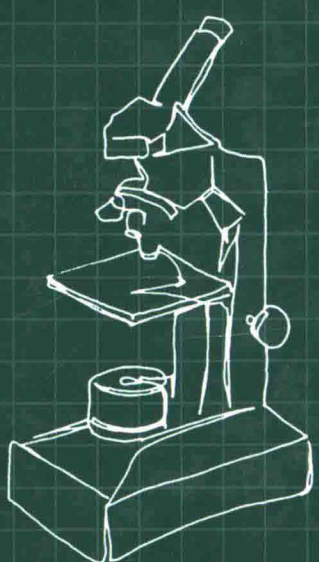
Experimental Design for Biologists

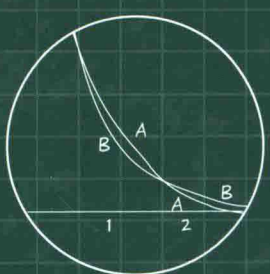
Second Edition

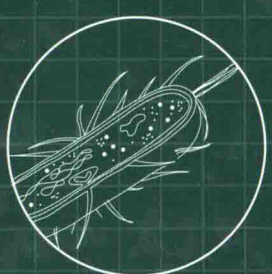


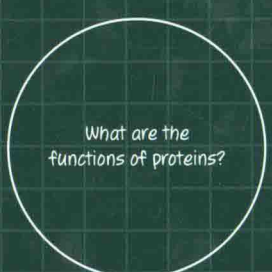


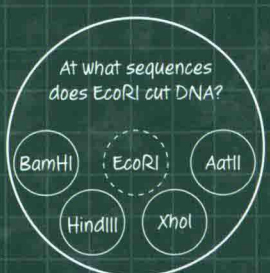












David J. Glass

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SECOND EDITION

David J. Glass

Novartis Institutes for Biomedical Research

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Second Edition

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Preface

AFTER THE FIRST EDITION WAS PUBLISHED, I had the opportunity to teach experimental design in a variety of settings. Every spring, Randy King of the Cell Biology Department at Harvard Medical School (HMS) and I offer a course of the same title as this book to graduate and MD/PhD students from the combined departments of HMS. In addition, I teach much more abbreviated versions of the material in the form of 5- to 10-hour "nanocourses," and these have been given at The Rockefeller University, Columbia University, Novartis, Tufts, and as a distinct course for post-doctoral fellows at HMS. All of this experience provided the basis for writing this text.

First, the students gave very helpful feedback. The first edition was somewhat informal and chatty in style, and it was clear that more structure would be helpful. Thus, the second edition is organized in the sections that comprise our course. In all, the text was substantially rewritten, with many new chapters added. Only a few chapters made it from the first edition virtually unscathed, those being the chapters on the various types of controls, and one prior chapter was moved to the addendum.

I would like to thank those who helped with this book. Thank you very much to Cold Spring Harbor Laboratory Press for their support at every stage. Michael Zierler capably edited the book. Inez Sialiano, the Project Manager, was quite patient and supportive throughout the process. Thank you very much also to William Karlson and Marc Egerman, who read every chapter and provided very useful edits. My assistant Sherry Rozek also read quite a bit of the book and was very helpful in giving feedback. Edits to distinct sections were provided by Joe Blitzstein from Harvard and Jens Praestgaard of Novartis. Individual chapters were reviewed by former students, including Eli Anders, Brad Bolman, Geoff Smith, Alicia Smart, and Daniel Taylor.

With the exception of one cartoon that is a carry-over from the first edition, all of the figures are new. These line drawings were executed by a young artist named Yunhan Xu. Thanks very much to her for her work. The minimalist style seemed to work well in representing the kind of hand-drawn representations that one might find in a lab notebook. The original cartoon was drawn by Woody Fu, as noted.

Of course, if despite all of the attempts at editing and improving the book, errors remain, those are purely my responsibility. If you find any such errors, please let me know; you can email me at david_glass@hms.harvard.edu.

My colleagues at the Novartis Institutes for Biomedical Research have been very supportive of my teaching efforts, for which I am quite grateful. Special thanks especially go to Mark Fishman for this. And thanks to Leslie Pond for helping me get the Novartis version of the course off the ground.

Harvard has been tremendously supportive of the course. Thanks especially go to Randy King, who has given excellent and consistent feedback over the years and who has co-taught our course since its inception. Thanks also to Catherine Dubreuil—an education fellow at HMS—who has helped with versions of the course. I also appreciate Joan Brugge's welcome and support for our efforts and for giving me a home in the Department of Cell Biology at HMS.

At Rockefeller, thanks to Sid Stickland, Winrich Freiwald, Marc Tessier-Lavigne, and Emily Harms for helping with the nanocourse there. At Columbia, thanks go to Fred Loweff, Ron Liem, and Steve Goff.

Ultimately, the success of a book such as this is whether the reader finds it helpful. It would therefore be great to hear from you with any suggestions for future editions.

Best of luck in your research!

David Glass
May 12, 2014

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SECOND EDITION

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SECTION ONE

PHILOSOPHY OF EXPERIMENTATION

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1

Why You Need to Read a Little Philosophy First

The Philosophy of Science Governs the Practice of Science

AT MEDICAL SCHOOL, STUDENTS ARE trained in the basics of the medical craft: how to perform a physical examination, interpret symptoms, approach a patient, and perform a differential diagnosis. At law school, the initial course load is designed to steep the novitiate in the forensic approach: how to write a brief, follow legal procedures and prepare contracts; how lawyers are expected to conduct themselves in various situations; and how to think like a lawyer.

Now, here is an amazing fact: At this writing, PhD and MD candidates receive very little formal instruction in the theory and practice guiding the design of experiments, despite the fact that the effective design, conduct, and interpretation of experiments is critical to their future success as basic and clinical scientists. In the curricula of universities, courses for PhD students in cell biology, biochemistry, genetics, physiology, and related disciplines are devoted almost entirely to substance: the facts gleaned from prior experimentation. Process—the way in which scientists must approach and conduct their experiments, the essential training as to how science should be performed—is often neglected.

Indeed, most graduate programs place very little emphasis on the formal processes required to perform experiments, nor do they discuss how these processes derive from various—and sometimes dissenting—theories of epistemology.[1] Throughout the history of biology, frank disagreements have arisen concerning ways of interpreting experimental data. Biologists must understand these dis-

[1] Epistemology is the study of knowledge—its limits and validity. Scientific epistemology is the subject of this book, addressed so as to be useful to the working scientist at the most practical level: How can we produce and interpret a data set that can be reproduced by others? How can we come to a conclusion that can be validated by its predictive power?

agreements so they can recognize different experimental designs that could be constructed to collect data and, from there, the distinct statistical methodologies that might be used in interpreting their data.[2] The scientist must be alerted as to how even the most common approaches to experimental design, data collection, and subsequent data analysis can unintentionally result in inappropriate interpretations and, most problematically, in representations of data that cannot subsequently be reproduced when the experiment is repeated by others.

The exception to this lack of procedural education is training in statistics. However, even this is sporadic, varying across scientific discipline and school. Moreover, in most statistics courses, there is little emphasis on inductive statistical methods, such as Bayesian statistics, that help the researcher to appreciate the probabilities of future outcomes. Furthermore, as is discussed later, a reductionist emphasis on the binary question of statistical significance ("binary" because an experiment is either statistically significant or it is not) sometimes clouds the equally important emphasis on the biological relevance of the effect seen. The premise for this book is that both the apprentice and more experienced scientists need a broader understanding of all of the steps that are required before, in concert with, and after consideration of statistical methods.

I wish I could report that there was a single, accepted, agreed-upon set of precepts that establish the basis for setting forward a scientific project. Unfortunately, there is instead a fundamental disagreement that has existed since at least the early 1700s as to what sort of conclusions are permissible from any prior knowledge. In this case, we mean, more pointedly, what we might conclude as a result of the data produced in a particular experiment, from a set of experiments, or even from an entire project. It is important for the scientist to understand this disagreement, because it explains the divergent schools of statistics, the use of terms such as "hypothesis falsification" versus "model verification," and the validity (or lack of validity) of predictive statements based on prior experience or, in the case of an experiment, prior data.

Before explaining this disagreement, we should discuss some basic premises. First, let us consider what scientific research is and what it can accomplish, because these concepts will help to establish parameters for what we seek to do in the laboratory.

[2] Statistics are outside the scope of this book, because that subject requires a text in itself. What is missing from most statistics books are the other issues that arise in formulating experiments, which create the demand for particular statistical approaches; it is those other issues that form the basis for this book.

2

Defining Scientific Research

TO START A SCIENTIFIC PROJECT, THE scientist must first decide what he or she wants from it. Why is the experiment being performed? What is to be accomplished?

Broadly speaking, most scientific research is performed either to (1) discover something that is not currently known, in response to some need to acquire that information, or (2) to test someone else's claim that is not yet fixed or confirmed. Thus, we might simply posit that the reason a scientist conducts research is in response to the need to acquire some information that we usually refer to as "data," with the goal that the research will result in the desired knowledge that is currently lacking. This "need to know" can be seen as constituting a set of seven distinct types of questions:

1. Does a particular thing *X* exist?
   Examples: Are there any planets besides Earth that could support life as we understand it? Is there a cell that can regenerate a hair follicle? Is there a drug that can stop a cancer cell from dividing?
2. What does *X* look like?
   Examples: What is the appearance of a mitochondrion under an electronic microscope? What is the structure of hemoglobin, to a resolution of at least 4 Å?
3. What is *X* made of? What are its constituent properties?
   Examples: What is the sequence of the human genome? How many mouse genes encode proteins? What are the sugar components of a plant cell?
4. What does *X* do? What is *X*'s function *Y*?
   Examples: What does the heart do? What does the protein myosin do? What functions do lion's whiskers serve?
5. What causes *X* to do *Y*? What causes *Z* results in *X* doing *Y*?
   Examples: What genetic mutation causes a liver cell to become cancerous? What is the cellular signal that makes skeletal muscle contract?

6. By what mechanism does *X* do *Y*?
   Examples: How does the release of calcium by the sarcoplasmic reticulum result in muscle contraction and what are calcium's downstream targets? How does a mutation in a particular chloride channel result in cystic fibrosis?

7. What can perturb *X* to perform its function differently? How does *W* affect *Z*? What factor *W* can cause *X* to do *Y′* instead of *Y*?
   Examples: What cellular signal can make a cancer cell die? What signaling pathways can dominantly suppress an oncogenic mutation in the ras protein?

We might condense these questions into a single definition of scientific research. Quite a large body of philosophical writing and discussion has been amassed to answer the question, *What is science?* But for the purpose of what I hope is a practical book for the working scientist, I skip all of the justifying exercises and simply posit that **scientific research is the process of determining some property *Y* about some thing *X*, to a degree of accuracy sufficient for another person to confirm this property *Y*.** There are many issues with this definition, but for the purpose of this book I defend it as providing the most useful umbrella for the sort of activities that must happen in the prosecution of a scientific project.

To illustrate one issue, our definition ignores René Descartes' claim, later repeated by David Hume, that one must find the cause of *X* doing *Y* for the activity to constitute science. That demand for "causality" (captured in questions 5 above) places observational or descriptive studies (captured in questions 1–4) outside of the realm of science—notwithstanding the fact that for many scientists, it is entirely valid to look into a microscope and describe what is observed, determine the structure of a protein to very high resolution, and label such activity as scientific research. The piece that our definition adds to this observational activity is that in order for this activity to be "scientific research," the scientist must describe what is being observed to a degree of accuracy and reproducibility that someone else can confirm the description.

A famous example of "descriptive science" was William Harvey's 17th-century study of the heart, from which he concluded that the heart's function was to pump blood throughout the body. Descartes championed the idea that the heart moved blood through the body, but in an effort to attach a cause to the process, he incorrectly opined that the heart heated the blood, causing it to expand through the vessels. From this example, we can see clearly that an incorrect mechanism or cause is not helpful for a piece of scientific research and also that a pure piece of accurate descriptive work, which reveals something that was previously unknown, can provide important scientific contributions on its own.

For more modern examples of purely descriptive work, consider the sequencing of human and other genomes or the determination of genetic markers that segregate with traits (phenotypes) of interest. Activities such as these often lay the groundwork for later efforts that determine cause and effect, but by itself this

work is purely descriptive. However, it is included as valid "scientific research" in this book.

In our discussion of what constitutes scientific research, we also must include the most basic activities that go on in the laboratory, such as accurate measurements of a sample's weight, its pH, or its location in a cell. If the scientist does not conduct such measurements accurately and reproducibly, the rest of the scientific project is doomed to fail, which brings us back to our definition: Scientific research is the process of determining some property *Y* about some thing *X*, to a degree of accuracy that another person can confirm this property *Y*.

Now that we have defined scientific research, it is necessary to devote a chapter to describe the purpose of scientific research, because this will help the reader to understand a fundamental disagreement about what can be concluded from a scientific study. Let us start with what research is for.

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