



Dee Lawlor

Introduction to Light Microscopy

Tips and Tricks for Beginners

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*For my mum, who is the reason I became a scientist.
Sorry for all the weird stuff you used to find in the fridge.*

Preface

I remember my first microscope—I still have it, in fact. It was a toy microscope that I got for Christmas when I was in primary school. This, of course, was back in the day when children's toys still came with scalpels and chemicals. I had hacked up an onion and lashed on some methylene blue. I squished down the coverslip and spilled the stain all over my mother's chopping board (note: methylene blue does not come out of wooden chopping boards). Under the battery light of my little microscope, there they were—the first cells I had ever seen. Throughout my education, I think I had only had two or three classes on microscopy. My knowledge of sample prep was pretty dire too. Not through any fault of my educators, but purely because—as a student—the goal was to get to the final image. The journey of how to get there didn't seem that important.

My journey into professional microscopy was born from panic. I had graduated my degree in zoology from the University of Aberdeen in 2010, after which I moved home to Dublin and was accepted into UCD to study my great love, evolutionary biology. Two weeks into my course, panic struck! At that time, Ireland was deep in recession and all thoughts were on what job you could get after graduation. Unfortunately, there were not many wanted ads looking for an evolutionary biologist. I hastily tore through the universities prospectus and found an MSc course in microscopy and image analysis. This was an entirely new subject to me. Like all biologists I had used microscopes, but like many people, I had never given them much thought.

I realised that microscopy was a skill, and it is a skill that is used in all areas of science. In the world of research, having good lab skills is invaluable. I figured I couldn't lose, and in fact, I gained so much more than I ever expected.

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About the Book

Microscopes are one of the most utilised and most ignored machines in the lab. Being easy and reliable to use, many researchers and scientists don't need to put too much thought into what they are doing or the machine they are using. That is, of course, until something goes wrong.

The goal of this book is to give microscope users a solid grounding in the technology they are using. With a strong knowledge of the basics, the user will be confident in approaching higher end systems and will be an invaluable resource in any lab.

Over the next few chapters we shall break the microscope down into its constituent parts, discuss how the part is assembled, how it works, and how to use it best. To cover as many bases as possible I have included traditional upright microscopes, geology microscopes, and inverted microscopes. I have included guides on sample preparation for brightfield microscopy and more general chapters such as consumables and what to consider when buying a microscope. As much as possible I have used clear and simple language. Where technical terms are required I have provided straightforward explanations. The troubleshooting chapter will cover a few extra notes and methods to assist in smooth imaging, but with a good introductory knowledge to microscopes, the user will be capable of troubleshooting many issues themselves.

One of the best things about microscopes is that they are accessible to everyone. No matter what the budget or requirement, there is a microscope that fits. Everyone has equal access to this microworld, and my goal for this book is to give every microscope user a clear field of view. Whether you are an undergrad in university, a teacher trying to introduce young students to science, or an armchair adventurer, I promise you this—once you take your first look down the microscope, there will be no looking back.

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About the Author

Dee Lawlor is a science writer and microscoper from Dublin, Ireland. She achieved her degree in zoology from the University of Aberdeen in 2010 and then pursued a master's in microscopy and image analysis from the University College Dublin in 2012. After graduation, Dee worked in microscopy sales and then spent several years working as a technical support engineer for digital imaging systems for Leica Biosystems.

Dee has a great passion for microscopy—both academically and in application. In her spare time, she collects and renovates antique microscopes and telescopes.

Her writing career started in 2016 when she started writing articles for a range of different scientific websites. Her specialist topics are microscopy, biology, and environmental sciences. She launched her own microscopy blog ‘What the Microscope Saw’ in November 2017, where she regularly publishes special interest articles. Her work is aimed at a wide variety of audiences, and her articles are written for the enjoyment of everyone—scientists and non-scientists alike.

After several years of problem-solving for scientists all over the world she has decided to put pen to paper, to share her knowledge and passion for microscopy in her first book *Introduction to light microscopy—tips and tricks for beginners*.



1

Introduction

The Microscope not only assists studies, and develops objects of profound interest, but it also opens up innumerable sources of entertainment and amusement, in the ordinary conventional acceptance of these terms; -it discloses to us peculiarities and attractions in abundance; -it impresses us with the wonderful and beautifully-skilful adaptation of all parts of creation, and fills our minds with additional reverence and admiration for the beneficent and Almighty Creator

Jabez Hogg [1], *The Microscope*.

Abstract The discoveries, advancements, and technologies that we enjoy today are the cumulative effort of millions of curious people over thousands of years. From the people who invented the wheel, the Greek philosophers, Renaissance explorers, instigators of the eighteenth-century enlightenment, Victorian taxonomists ... as a contemporary scientist, you are the latest link in a very long chain.

The history of science—and how we have arrived where we are today—is a fascinating topic. I have started this book with a brief history of the microscope. I have also included a brief history of glass, as this has had a direct impact on the development of microscopy.

While the history of science is not essential knowledge, I encourage all scientists to learn about the evolution of their field and to explore the path that has brought us to where we are today.

Nothing is a greater symbol of science and discovery than the microscope. Microscopes are an easily accessible technology and they have the power and potential to show us the world from a whole new point of view. Edward Tufte

said, ‘the commonality between science and art is in trying to see profoundly—to develop strategies of seeing and showing’. The microscope has been, and continues to be, a huge contributor to education and our understanding of the world.

1.1 History

Optics—the study of light—is one of the oldest branches of science. The word ‘optics’ was coined by the Greeks and comes from the Latin ‘ta optika’ which means ‘look’ or ‘appearance’. Light has been a source of great fascination for many of the great minds throughout history. In 300 BC, Euclid wrote one of the earliest known works on optics, in which he described his theories on the geometry of vision. In 350 BC Aristotle wrote in *Problemata* about the magnification of light and images. In 1021, Ibn al-Haytham, the Arab mathematician, astronomer and physicist, wrote the *Kitāb al-Manāẓir* (the *Book of Optics*). Many of the theories and ideas suggested by these great minds have today been proven to be correct. Interestingly, any the mistakes were mostly made on the biology side, but much of the physics was spot on.

The history of the microscope stretches back to 750 BC Assyria, where we find the earliest known examples of manufactured lenses. The Egyptians and Mesopotamians are credited with inventing glass; however, many cultures developed the technology independently for themselves. The Chinese, for example, are known to have been making glass from before 200 BC [2]. The lenses are the most important part of the microscope. Their quality, purity, precision, and alignment are essential for creating a high-quality image. The oldest known lens in the world is the Nimrud lens, which currently resides in the British Museum. Also known as the Layard lens, it is a 1.5 inch diameter piece of rock crystal, originating from modern-day Iraq. Research on the lens suggests that it has a focal length of 12 cm and a magnification of approximately 3×. There are several theories as to its original use—it was possibly used as a magnifying glass, used for concentrating sun light to start fire, or possibly even part of an ancient telescope, but it could also just have been for decorative purposes. It is made from rock crystal and thus the quality of the lens has not deteriorated over time. Early glass was full of impurities and imperfections and lenses made from crystal were considered to be superior for much of history. Quartz crystal was a popular choice as it is renowned for its clarity and strength, which makes it ideal for polishing and grinding. In fact, quartz crystal is still used to make the prisms in many microscopes manufactured today, and crystal is known to not degrade the way glass can over