Edited by

John A. Reffner • Brooke W. Kammrath

Solving Problems with Microscopy

Real-life Examples in Forensic, Life and Chemical Sciences

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This edition first published 2024
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Library of Congress Cataloging-in-Publication Data
Names: Reffner, John A., editor. | Kammrath, Brooke W., editor. | John Wiley & Sons, publisher.
Title: Solving problems with microscopy : real-life examples in forensic, life and chemical sciences / edited by John A. Reffner, Brooke W. Kammrath.
Description: Hoboken, NJ : John Wiley, [2024] | Includes bibliographical references and index. | Summary: “Problem solving is essential to all progress. The scientific method and logical reasoning ensure that problem solving is systematic and based on factual data. One of the most significant and useful tools for solving problems is the microscope. There are many different types of microscopes, each having broad applications in a diversity of different disciplines”-- Provided by publisher.
Identifiers: LCCN 2023018048 (print) | LCCN 2023018049 (ebook) | ISBN 9781119788201 (hardback) | ISBN 9781119788218 (adobe pdf) | ISBN 9781119788225 (epub) | ISBN 9781119788232
Subjects: LCSH: Microscopy--Technique. | Microscopy--Case studies.
Classification: LCC QH207 .S65 2024 (print) | LCC QH207 (ebook) | DDC 570.28/2--dc23/eng/20230624
LC record available at https://lccn.loc.gov/2023018048
LC ebook record available at https://lccn.loc.gov/2023018049

Cover Design: Wiley
Cover Image: © Kuntalee Rangnoi/EyeEm/Getty Images

Set in 9.5/12.5pt STIXTwoText by Integra Software Services Pvt. Ltd, Pondicherry, India

Dedication

Our children, John R. Reffner, Elaine (Reffner) Teeters, Riley Kammrath, and Grayson Kammrath, and grandchildren, who inspire us to be our best selves, to make every day joyful, to think creatively, and to strive to use small (microscopical) things and thinking to make a big impact.

Our spouses (past and present), Sally E. Reffner, Phyllis Bender Reffner, and Matt Kammrath, who have provided much needed support and encouragement throughout our various scientific adventures.

Our mentors and mentees, too numerous to name, who have each left their traces on us as educators and life-long students. There is one person in the galaxy of individuals we have had the privilege of working with who shines brightest with her intelligence, integrity, creative problem solving, generosity of time and knowledge, and kindness – Dr. Pauline E. Leary – we are forever grateful for your friendship.

John A. Reffner and Brooke W. Kammrath

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Foreword

It would take some time to think of a technique of greater utility and practical value to the physical and biological sciences than microscopy. In spite of this, its full benefits to researchers and analysts alike remain, more often than not, unrecognized and underutilized. This rather surprising failure to fully exploit the capabilities offered by the microscope and its attendant methods is due, in no small part, to those practitioners who are content to merely use microscopes but never become proficient microscopists.

Since the microscope's earliest employment by curious amateurs, such as van Leeuwenhoek, the microscope has helped mankind expand human vision ever further down in scale to reveal the most minuscule secrets of nature in much the same way that the telescope made it possible to gaze into the sky and visualize the vast expanse of the cosmos. Almost immediately following its discovery, the microscope began to reveal the true nature of the previously unseen and unimagined world that has surrounded us for millennia, by providing direct visual evidence, which no amount of philosophical argument could refute.

In the years following its invention, some microscopes were built primarily as works of art and still others were fashioned for those rich enough to purchase one merely for their own amusement.[1] However, from nearly the time of its invention, the *raison d'être* for this instrument has been to extend human vision; primarily for the advancement of knowledge and more specifically that knowledge we regard today as scientific.

It should come as no surprise, therefore, that over the passage of centuries, the capabilities of both microscopes and microscopy, have improved dramatically. Far from being restricted exclusively to advances in design, magnification, and resolution, there have been developed entirely new types of microscopes and microscopies. With them the microscopist is no longer restricted to merely observing only color and minute morphological details. The instruments available to today's microscopists permit them to not only observe and probe microscopic specimens using ordinary white, polarized, and fluorescent light but also to monitor their interaction with electrons, other electromagnetic radiation (e.g., X-rays) and even sound. Thus, these new microscopes and their accessories allow us to analyze and not just observe the most minute specimens elementally, elucidate their chemical composition and even map the distribution of discrete phases of almost any type

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[1] For instance, Samuel Pepys recorded the purchase of a microscope in his famous diary, shortly after he bought a copy of Robert Hooke's then newly printed *Micrographia*.

of microscopic feature or particle, whether of chemical, biological, anthropogenic, or even extraterrestrial origin.

Concurrent with these developments in instrumentation have been dramatic improvements in the interpretation of the results obtained by means of these instruments. Prominent among these significant new developments are those relating to the interpretation of the observations and data resulting from microscopical study. Of particular value is the availability of computerized databases (based on and developed from authentic reference collections) that include almost every imaginable substance in existence. In spite of this, not every type of substance can be identified by its elemental, chemical, or crystallographic properties. Pollen grains, wood, cellulosic and natural protein fibers such as hairs, etc. are all, for the present at least, still best identified by their characteristic microscopic morphology, which may be by enhanced by the resolution of electron microscopy and then identified by comparing the salient microscopic features to images in the authoritative atlases (some old and some new) that now exist. The availability and practical importance of such non-hardware resources helps to simply substantiate the fact that not all of the improvements to the instruments and accessories employed by the microscopist are related to hardware.

If the identification of microscopic unknowns were the sole contribution of microscopy to the solution of real life problems, it would still be of sufficient importance to justify its place as one of the premier problem solving tools of modern science and engineering. I am speaking here of real problems, not those involving merely simple decision making. As those whose profession it is to solve serious problems know all too well, there commonly occur in real-life, questions of such importance, complexity, and difficulty (and quite often also secrecy and urgency) for which solutions must be obtained, at almost any cost. The key to solving problems such as these is to be found in the realm of the specialists, who have by training, experience, and inclination prepared themselves to attack and solve such enigmas when they arise within their area of expertise. While such specialists most often hold specific titles (e.g., cryptanalyst, analytical chemist,[2] consulting engineer, medical diagnostician, etc.) they may all be considered, at least for the purposes of this essay, as *analysts*.

Successful analytical microscopists bring not only their expertise (based on years of training and practical experience) to bear on the solution of the varied problems presented to them, but in addition, develop a special, nearly unique, insight into the way the world, and everything in it, appears and operates on the microscopic scale. This rare and most unusual perspective is one of the fundamental differences between microscopists and other analytical scientists. As a result, the analytical approach that has evolved from this uncommon perspective is, as far as I am aware, a unique and rarely appreciated or fully understood advantage enjoyed by the microscopist who works as a problem solver. I am speaking here in the pedagogical sense, since in everyday practice the expert microscopist thinks in this way without ever *thinking* about it, so to speak.

The extent to which microscopes, their accessories, and resources for interpretation have been brought to perfection in our day is difficult to comprehend even for those, such

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[2] Today, the analytical chemist seems to have become a rare specialist with most analytical scientists today identifying themselves as spectrographers, chromatographers, mass spectroscopists, etc.

as I, who have worked in this field for their entire professional career. During this time (and beginning even earlier in my home laboratories as a young boy) I have sought out, consulted, and acquired an extensive collection of books on practically every aspect of microscopy that might be of assistance to my work as an analytical microscopist – both antiquarian and modern as well as practical and theoretical without regard to the language in which they are written. While I cannot claim to have or even be aware of every book on the subjects bearing on the field of applied microscopy, I can state with some authority, that I have never stopped seeking out those potentially helpful titles, of which I am still unaware, for acquisition.

This is what makes the book you now hold in your hands so special and unusual and, I will add here, important to the aspiring adept. I am unaware of another book (old or new) whose authors have undertaken the task of exploring and attempting to explain the use of the microscope as an aid in the general solution of problems – period. There are books that explain how to use the microscope in fields ranging from the brew house to barber school and to how understand the underlying causes of glass breakage. T. E. Wallis in his excellent *Analytical Microscopy* (through three editions) attempted to broadly cover a range of topics that would help to solve the types of problems that might be encountered by the public analyst in Britain in the 20th century. The *Journal of Analytical Chemistry* published, some years ago, a series of articles (*The Analytical Approach*) by analytical chemists that described the processes of logical thought and details of analysis that went into solving a variety of real-life problems in the industrial and pharmaceutical industries, art forgery detection, and forensic science among others. Books and articles such as these are certainly better than nothing, but they only tease the imagination and make the absence of a comprehensive treatment of the subject more obvious – or so one would think. This work is, therefore, long overdue and the authors are to be congratulated on conceiving and bringing to print what will become, I believe, essential reading for anyone who uses or contemplates using microscopy to help solve problems that involve materials of any sort. It has been ably written by a team of two professional microscopists with quite different but complimentary backgrounds.

I have known and admired John Reffner for most of my adult life. He is the preeminent analytical microscopist of our age and has accumulated, quite literally, a lifetime of experience in applying the microscope, in all of its manifestations, to the solution of problems, many of which would never have been solved had it not been for his clever and resourceful intervention. He began his career in the microscopy laboratory of one of the big rubber companies,[3] before being recruited by Walter McCrone. There he gained experience and sharpened his microanalytical skills, before leaving to attend graduate school. This was followed by a period in academia and afterward as the head of the microscopy laboratory of a large chemical company. Since than his reputation for solving "insoluble" problems has continued to grow and his activities have ranged from providing expert witness testimony in criminal and civil proceedings to his personal involvement in the development of the first practical, commercial infrared microspectrophotometer. There are, I believe, very few

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[3] This was in the days when most manufacturing companies had laboratories devoted to both microscopy and analytical chemistry.

applications of microscopy with which he has not at one time or another been called upon to draw from in the solution of problems of the most diverse kinds.

His co-author, Brooke Kammrath, is a professor of forensic science at the University of New Haven, where she specializes in the application of new and newly developed microscopical and microanalytical instrumentation directed toward the improvement in and interpretation of microscopic trace evidence. She is, compared to John, relatively new to the field, but has the great advantage of having been his student. What she lacks in experience, however, she more than makes up for in energy and enthusiasm and, of course, her experience will only increase over time. It is fair to say that this book would not ever have been written if it had not been for her role in pushing it ahead. I must mention here that our first meeting was at a scientific conference where a colleague introduced her to me as an enthusiastic graduate student who had recently discovered the allure of microscopy as a practical problem-solving tool. I recall her excitement at the time, which was due to the fact that she had just purchased an elaborate polarizing microscope for her own personal use. She cheerfully relayed to me how she couldn’t wait to begin to learn how to use it so she could examine any and all microscopic objects she encountered whenever she had the urge to do so. In my experience, the best microscopists would never consider life complete without their own microscope(s). Little did I know at the time that she and John would come up with the idea for a book of great importance to the field of applied microscopy that had not yet been written, but should have been, long before now.

I would like to conclude by recommending this book as an essential resource for anyone who uses microscopes or contemplates using them to solve problems. By anyone, I mean not only those in the physical and biological sciences, engineering, and medicine but also anyone who might benefit from an understanding of how the information obtained with the aid of a microscope can be put to practical use. I only wish that this book had been written when I was just beginning my career in analytical microscopy.

Skip Palenik
President and Senior Research Microscopist
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Preface

Although a plethora of books exist about the science of microscopy, most focus on descriptions of microscopical methods and instrumentation. These “how-to” instructional books are exceedingly useful for learning how to achieve a quality magnified image, but there is something missing. With this book, we are addressing the “why-to” use a microscope to solve problems. Interpreting magnified images requires a knowledgeable understanding of not only how the image was achieved, its illumination and the morphological features present, but also an awareness of how the magnified image is related to solving the problem at hand. In many instances, this requires in-depth education, training, and experience to equip a scientist with a breadth of knowledge. Whether it is a question of identification or comparison, a microscope is a sophisticated tool that requires the user to understand ***how*** and ***why*** to recognize the meaningful features or differences in a magnified image.

In this book, specific case examples demonstrate the value of using the microscope to solve problems. These cases range from criminal and civil forensic investigations to industrial, environmental, cultural heritage, pharmaceutical, and biological problem solving.

John A. Reffner and Brooke W. Kammrath

Abbreviations

AFM	atomic force microscopy
API	active pharmaceutical ingredient
ASTM	American Society for Testing and Materials
ATR	attenuated total reflection
DLI	depolarized light intensity
DNA	deoxyribonucleic acid
DSC	differential scanning calorimetry
DTA	differential thermal analysis
EDX	energy dispersive X-ray spectroscopy
ELISA	enzyme-linked immunosorbent assay
fcc	face-centered cubic
FCC	Forensic Chemistry Center (US FDA)
FIB	focused ion beam
FID	flame ionization detector
FT-IR	Fourier transform-infrared
GC	gas chromatography
GPC	gel permeation chromatography
GRS	government rubber styrene
HPLC	high pressure liquid chromatography
IA	image analysis
IR	infrared
IRRFC	infrared reflectance false color
LIB	laser induced breakdown
Mn	Number Average Molecular Weight
MS	mass spectrometry
MW	molecular weight
Mw	Weight Average Molecular Weight
NAA	nucleic acid amplification
NCE	new-chemical-entity
O-PTIR	optical photothermal infrared spectroscopy
PCA	principal component analysis

PCR	polymerase chain reaction
PLM	polarized light microscopy
PMB	polymer modified bitumen
PMRB	postmortem root banding
qPCR	quantitative polymerase chain reaction
SBS	styrene-butadiene-styrene
SEM	scanning electron microscopy
SIMS	secondary ion mass spectrometry
TEM	transmission electron microscopy
TOA	thermal optical analysis
UV-Vis	ultraviolet-visible
UCL	upper confidence limits
XRD	X-ray diffraction
XRF	X-ray fluorescence
Y-STR	Y-chromosome short tandem repeat
ZBH	zero-background holder

Introduction

Mankind's progress is historically paced by the ability to solve problems. The discovery of fire, the wheel, the steam engine, etc. were important developments in the history of humanity because they provided solutions to problems. Fire supplied warmth and led to the development of cooking food. The wheel provided ancient Mesopotamians a method for doing work at an unprecedented pace and load. The steam engine started the industrial revolution. In today's technologically advanced society, solving problems is still essential to our progress.

How do problems get solved? History tells us that problem-solving requires applying knowledge to develop tools and methods specifically to accomplish a necessary task. The requisite knowledge is gained through education, training, and experience. The scientific method provides a systematic approach for problem solving successfully. The scientific method is an organized and iterative step-by-step pathway for answering questions and solving problems. Although there are many different descriptions of those steps, these authors have outlined the following nine steps for proper application of the scientific method: observation, documentation, preservation, examination, contemplation, speculation, verification, conclusion, and communication. Table 1 details these steps of the scientific method. A critical step of the scientific method is the verification stage, which includes a feedback loop from which hypotheses and conclusions can be refined.

Defining the problem is a critical first step to developing a solution. A poorly defined problem cannot be properly solved. Observation, the first step of the scientific method, is a paramount tool for understanding all elements of the problem. Contextual information (Chapter 11) is also required for success. Although this often goes unrecognized, successful problem solvers understand the importance of stating the problem.

Solving problems requires sophisticated and logical reasoning, often in the form of inferences. Three types of inferences, or thought processes, have been delineated by scientific philosophers: deduction, induction, and abduction. Deductive reasoning (also known as "top-down logic") is the determination of a conclusion based on known rules and premises (or preconditions). Inductive reasoning (also known as "bottom-up logic") is the determination of the rule based on specific premises, results, and/or conclusions. The third and lesser known inference is abduction, where the best or most likely explanation (or precondition) is determined given the rule and the results or conclusions. For more information on logical reasoning, we refer the reader to "The Sign of Three: Dupin, Holmes, Pierce"

Solving Problems with Microscopy: Real-life Examples in Forensic, Life and Chemical Sciences, First Edition.
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Table 1 The scientific method is a process that includes these steps.

The steps of the scientific method	Activities and actions
Observation	Seeing, listening, touching, smelling, tasting
Documentation	Taking notes, photographs, drawings
Preservation	Collecting, packing, labeling, recording
Examination	Inspecting, analyzing, measuring, experimenting
Contemplation	Thinking, organizing, correlating
Speculation	Developing hypotheses, brainstorming
Verification	Validating, testing, challenging
Conclusion	Establishing relationships, interpreting data, determining significance to the problem
Communication	Reporting, publishing, presenting

edited by Umberto Eco and Thomas A. Seabok (1983), which is an excellent collection of essays on critical thinking with examples from Edgar Allan Poe, Sir Arthur Conan Doyle, and Charles Sanders Pierce. The resolution to the problems presented in this book resulted from the use of each of these forms of logical reasoning, with the specific situation determining which was implemented. For example, in the case of the Yellow Rope (Chapter 10), deductive reasoning was used. In this case, the solution to the problem or the conclusion (the two ropes came from different batches) was logically inferred from the rule (different batches of polymer have different tacticity) and the premise (the known and questioned ropes had different tacticity). In the Polio Vaccine case (Chapter 2), inductive reasoning was used to determine the rule (all containers with high residual stress fail) after the scientist was provided with the precondition (these containers have high residual stress) and the result (these containers failed). The case detailed in "A Mouse, a Soft Drink Can … and a Felony" (Chapter 5) demonstrated the process of abductive reasoning to solve its problem. After being provided with a rule (a mouse cannot damage the outside of a soda can when inside of it during the manufacturing process) and the result (microscopic observation of damage from a mouse's teeth on the outside of the soda can), the precondition was determined (the mouse was put into the soda can after manufacturing). Deductive reasoning is the most algorithmic; therefore, it has the most guaranteed conclusions (or has the most certainty), but it is sometimes considered the least insightful. Abduction has the least certainty, but is the most insightful in that it has the potential to provide causal explanations from novel observations. Induction falls between the other two in its certainty and insightfulness. All methods of inference have value when solving problems.

One of the most significant and useful tools for solving problems is the microscope. Antoine Von Leeuwenhoek (1632–1723) is credited with being the father of microbiology due to his invention of the single lens microscope. What is often not recognized is that he developed this tool as a method for solving the problem of seeing fine threads to improve his work as a draper. Subsequently, he began looking at all kinds of materials, from bees and lice to mold and pond water. His discovery of microbes and the microstructure of materials opened the doors for numerous branches of scientific study. The microscope continues to be the symbol of science and scientific disciplines throughout the world.

Why use the microscope to solve problems? The answer lies in the first step of the scientific method: observation. Microscopes are the greatest tools for performing detailed observations about an item under investigation. In addition to imaging visual features, microscopes are measuring instruments. Microscopes have the ability to measure a huge variety of physical and optical properties. The imaging and measurement capabilities of microscopes are fundamental attributes that provide essential information which enable a microscopist to solve problems.

Microscopy is the art and science of producing, recording, and interpreting magnified images. Microscopy is both an art and a science. Art is defined as "the expression or application of human creative skill and imagination" (Oxford English Dictionary, 2022). The art of microscopy is in the skills required for successful microscopical investigations. From sample preparation to focusing the microscope to achieve the best image for the specific application, microscopy requires the art of the inquisitive mind. Science is defined as both the collection of knowledge about the physical and natural world and the method in which that information is systematically learned and studied. Microscopes are useful for both aspects of science, and are versatile tools for producing and recording magnified images that contain critical data for scientific examinations that are used for solving problems.

One of the microscope's early problems was the ability to record and communicate the image seen through the microscope to others. Although sketches were essential for this, they were laborious, required artistic abilities, and were not necessarily verifiable. The pairing of photography, both stills and motion picture, with the microscope was a major advance in the use of the microscope. This created the field of photomicrography. The ability to capture magnified images stimulated the growth of the acceptance of microscopy as it created a reviewable record that brought others into the microscope. Modern-day digital photography continues the advancement of the field of microscopy, not only making it easier to capture magnified images but also by being able to record dynamic events with video capabilities. However, the interpretation of the data demands both knowledge and a perceptive mind. Because of the failure to properly use the microscope, the incorrect or inappropriate interpretation of images remains microscopy's most pivotal problem.

The microscope has broad applications. The value of magnified images is not limited to a single discipline. The microscope is beneficial to many fields, ranging from biology, chemistry, forensic science, metallurgy, minerology, gemology, materials, and environmental science, etc. Microscopical methods are multifaceted and ubiquitous. Magnified images are useful data in all aspects of an investigation.

In addition to being a symbol of science, a microscope is a primary instrument for approaching the solution to numerous problems. The microscope is the means for taking us into the micro-world. There exists a range of complexity of microscopes, from the hand lens and children's plastic microscopes to sophisticated super-resolution electron microscopes. Their main function is to produce and record magnified images. These images are data that the scientist can use to answer questions. This will be further demonstrated in Chapter 3.

There is a vast array of different types of microscopes. One method for characterizing them is by their method of interaction with the sample of interest (Table 2). This creates

Table 2 Types of microscopy and microanalysis methods, classified by their manner of interaction with the sample of interest. Microscopical methods that can be configured with both transmitted and reflected light are designated with an asterisk (*).

Photons	Electrons	Ions	Probes
Brightfield*	Transmission(TEM)	Secondary Ion Mass Spectrometry (SIMS)	Scanning Tunneling
Darkfield*	Scanning(SEM)	Focused Ion Beam(FIB)	Atomic Force (AFM)
Stereomicroscopy*	Environmental SEM	Field Emission	AFM-Raman
Polarized Light*	Electron Microprobe	Field Ion	TERS
Dispersion Staining	Auger		AFM-IR
Phase Contrast	Scanning TEM		
Interference*	EDAX		
Modulation Contrast	EELS		
Rheinberg*	Cathodoluminescence		
Confocal*	Laser Induced Breakdown (LIB) Microspectroscopy		
Comparison*			
Microspectroscopy (UV-Vis,IR,Raman)*			
Fluorescence*			
Near-field Scanning X-Ray*			
Photo-electron*			
Optical-Photothermal Infrared (O-PTIR)			

four categories: photon (or light) microscopy, electron microscopy, ion microscopy, and probe microscopy. The largest and most common type of microscopy is photon microscopy. Although more commonly known as light microscopy, the term *photon microscopy* is more appropriate. Photons are the energy carrier of electromagnetic waves and are the subatomic particle that interacts with matter. Thus, the photon is used to probe a sample to create a magnified image. This is also more consistent with the terminology of the other three types of microscopies, where it is the electron, ion, or physical probe that interact with the sample to create the magnified image. When coupled with other technologies (e.g., vibrational or energy dispersive X-ray spectrometers), the capabilities of the microscope are further extended.

Every reader of this book is already aware of the value of interpreting images. We recognize our spouse, our children, our house, the street we live on, our workplace, etc. The eye forms an image and the brain interprets what we see. The human mind is exceptionally adept at recognizing similarities and differences in images. These are then used for pattern matching, identification, and interpretation. This is exemplified by the ability to identify familial relationships through a likeness in features shared between a parent and child or siblings (e.g., a break of the nose or shape of the eyes). Even in the presence of large variations, such as differing hair styles or ages, similarities are able to be interpreted to identify family members. Further, the brain's skill for differentiation by minute differences is demonstrated by the capacity to discern identical twins. These skills for image interpretation are vital for the successful use of a microscope to solve problems.

Image interpretation is a skill that requires practice, education, and training. As is true for all endeavors, there are some individuals with a natural propensity for recognizing similarities and differences in images. However, with dedication and quality education and training, skills in image analysis can be improved. For example, people can be taught to read and use a map, put jigsaw puzzles together, and navigate through mazes. The more you practice, the greater improvement you will see. In the book *Outliers*, Malcolm Gladwell proposed the 10,000-hour rule – it takes 10,000 hours of practice to become an expert in something. This equates to roughly 5 years of work (8 hours per day, 5 days per week, 50 weeks per year). Doctoral programs and apprenticeships are an excellent way to get this practice, through both education and training. On the other hand, a person can work for 40 years in a field, and have been doing it wrong that whole time. A person must practice the right thing. This is why quality education and training are vital.

For a microscope to function properly, one must have an appropriate sample. The three most important factors for good microscopical analysis are: (1) sample preparation, (2) sample preparation, and (3) sample preparation. If you do not properly prepare a sample for microscopical analysis, you will not be able to achieve the desired performance of the microscope. There are no optical tricks for compensating for poor sample preparation. The sample mounted on the microscope slide is an integral part of the optical system.

There is a union of the microscope with the observer or user that is required for good microscopical analysis. There is some fundamental knowledge that a microscope user (a.k.a., a microscopist) must have to use the microscope effectively. For example, the microscopist must focus the microscope to their eyes. This is not only to achieve a clear image with the fine and coarse focus, but when using a binocular microscope, the intraocular distance must be adjusted to fit their facial structure. This, and other necessary adjustments of the microscope and illumination system, is detailed in numerous microscopy books and websites; unfortunately they are often overlooked. A microscope is not a "plug-and-play" device; it must be customized for the microscopist. If you are using the microscope properly, you will be free from any stress or strain on your eyes. You will not get a headache or residual bright spots after using a correctly adjusted microscope. It should be like wearing glasses – the microscope should act as an extension of your eyes which enables the viewing of very small (or microscopic) objects and minute details.

There is not one "right way" to set up the microscope. Köhler (or Koehler) illumination is a good place to start, but there are reasons for making adjustments. The appropriate microscope alignment is sample and application dependent. For example, when making a relative refractive index determination, the condenser aperture should be reduced to increase contrast which enables a better observation of the Becke line of a sample. However, if you are more interested in examining the finer details at higher magnifications, the resolution of the microscope can be increased by opening the condenser aperture. There is not one way to achieve the highest quality image for every sample; the microscopist must adjust the microscope based on the desired use.

There is a need for awareness that the eye and the mind are not perfect. They can be misled. One must be careful about seeing things and making connections that do not actually exist. These may be due to cognitive bias, which has been defined as "a limitation in objective thinking that is caused by the tendency for the human brain to perceive information through a filter of personal experience and preferences" (Gillis & Bernstein, 2022). Pareidolia is a special type of bias, where one interprets an image as containing a meaningful pattern or structure that does not actually exist. This is demonstrated by the "seeing" of shapes in the clouds, such as a bunny, angel, or face (Figure 1). When using the microscope, if not properly alerted to the possibilities, one may easily fall victim to cognitive biases and pareidolia. The dangers of bias demonstrate the need for following the scientific method, validating the interpretation of images like any scientific analysis. The proper adherence to the scientific method is an excellent guard against bias.

Once the cause of a problem is discovered, an implementation strategy must be developed. In the case of the XB-70 Valkyrie Fuel Line (Chapter 11), microscopy proved essential. Microscopy was not only able to define the problem (the fuel line was failing due to the filters being blocked with debris), identify the source of the problem (two types of glass fibers: one from an exterior air filter and the second from fiberglass reinforced putty), and guide in the development of remediation and prevention efforts. In this case, and many others included in this book, the microscope proved to be essential for all aspects of solving problems.

Figure 1 A photograph of a cloud configuration appearing like the profile of a human face. Reproduced with permission from Barry Cord for Kieselstein-Cord ©Barry Cord 2021.

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Discovery with the Light Microscope

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We owe our understanding of the modern world to a single instrument above all others – the light microscope. These days, it is often assumed that the electron microscope is at the pinnacle of microscopy, and it seems to have eclipsed the light microscope. Indeed, there are informal reports of academics claiming that light microscopes should now be consigned to the broom-closet, since electron microscopes are so superior. The monochrome images offered by an electron microscope may provide unmatched resolution, but high resolution is not what we need for the great majority of investigations. Only the light microscope can show us the color of our specimens, and color is often crucial. And it is the light microscope alone that can reveal life as it is lived. A variable-pressure scanning electron microscope can briefly show us gray images of the movements of moribund arthropods, though only a light microscope can reveal the voluptuous twisting and turning of living cells as they pursue their complex little lives, the captivating colors of crystals under polarized light, or the selectively stained microorganisms we need to identify.

The majesty of the living cell is our current focus of attention and electron microscopes have no part to play in that. Light microscopes are among the most crucially important instruments in the world of science, and one of the few you will find in every field of investigation. Not only can they solve otherwise intractable problems, but the insights they provide influence the way we interpret the world. A trained and experienced forensic analyst can identify a particle, some strange fiber, a pollen grain, or a fragment of mineral, and solve a crime in an instant. These days, authorities rarely resort to images from light microscopes; they like to have analyses and fancy graphs to illustrate their reports, whereas microscopists recognize reality.

Microscopists are curious; we look differently at the world and are insatiably inquisitive. Indeed, this is how microscopy was born. It was on March 15, 1663, that young Robert Hooke, the 27-year-old curator of experiments at the newly formed Royal Society of London, was presented with a microscope constructed by Christopher Cock, an instrument maker from Long Acre in London (Figure 1.1). Cristopher Cock flourished in the middle 17th century. He pioneered the production of compound microscopes, the details of which appear in Robert Hooke's great work Micrographia, published by the Royal Society in 1665. They were provided with brass fittings and covered in polished shagreen

Solving Problems with Microscopy: Real-life Examples in Forensic, Life and Chemical Sciences, First Edition.
Edited by John A. Reffner and Brooke W. Kammrath.

Figure 1.1 Robert Hooke's published engraving of his compound microscope.

or embossed leather. Although impressive as possessions, they gave optical results greatly inferior to those obtained with a single lens.

The Society instructed Hooke to provide weekly demonstrations to the Society's fellows. A man given to enjoying the fine things in life, he sat there with his microscope, and toying with a cork from a bottle of wine. It's strange stuff, cork. It is incredibly light, buoyant, and compressible, yet it readily springs back to its original shape. For all its porous nature, it cannot absorb fluids, so it was unrivaled for stopping up bottles of liquid. Why – since it was so light and so porous – did it not leak? Hooke decided to solve the puzzle posed by cork and wrote: "I took a sharp pen-knife and cut a thin piece of it, placing it upon a black object Plate, because it was itself a white body, and casting the light on it with a deep *plano-convex Glass*, I could exceedingly plainly perceive it to be all perforated and porous, much like a Honey-comb ..." He reported that his microscope would "presently inform me" how cork was so light, why it would never "suck and drink in water," and how was it possible to take compression more than any other substance, before it is "found to extend it self [*sic*] again into the same space." These were unique attributes, and Hooke's meticulous investigations provided the explanation. He observed that cork was composed of little boxes, or cells, "altogether fill'd with Air." Cork contained mostly empty space, and very little solid substance. It was the microscope that had revealed the truth.

1.1 Hooke, Leeuwenhoek and the Single Lens

He published his coinage of the word “cells” in his great book Micrographia, published two years later (Hooke, 1665) and the term has come down to us today. But it was wrong. To us, a cell is a living, succulent, microscopic organism and not the empty box that Hooke observed. He was identifying the empty walls inside which living cells had once existed. Far more momentous (though dismissed at the time and ignored until my revelations (Ford, 1989) more than three centuries later) was his observation of living cells in the moss *Funaria hygrometrica*. Although he wisely compared the complexity of a moss plant with that of familiar plants – like a *Sempervivum*, the houseleek – he did not mention the delicate tracery of its component cells, even though they were accurately portrayed in his fine engraving of the moss. Hooke was also the first to document a microbe, when he presented an exhibit of mildew fungi growing on old leather and recorded the details in diligent drawings. One of the paradoxes about Hooke, which scholars missed for centuries, is that you cannot observe with his microscope the fine details that he published in his engravings (Figure 1.2). I have shown that he must have used a simple – i.e. single-lensed – microscope to fill in the details, and confirmation of his methods lurks in the unnumbered pages of the Preface to Micrographia. Hooke explains how to grind and polish a tiny plano-convex lens and mount it in a metal plate. These lenses offer far higher magnification and much improved resolution though, he

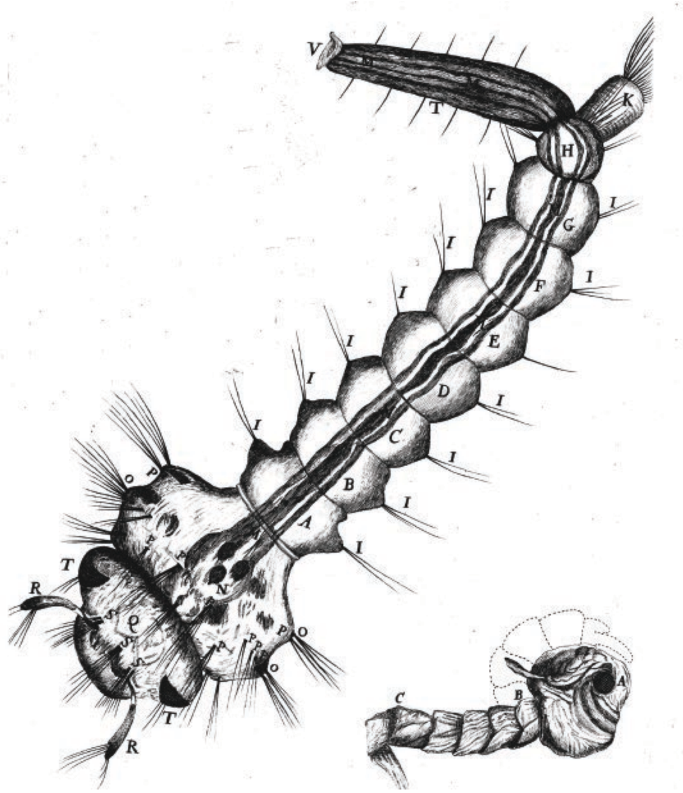

Figure 1.2 Robert Hooke’s detailed engraving of a mosquito larva.

admits, they are "very troublesome to be us'd, because of their smallness, and the nearness of the Object." (Lawson, 2016)

Robert Hooke's superbly detailed engravings were plagiarized over the centuries, and they reveal that he was an extraordinary draftsman. Here we see an aquatic larva of the mosquito Culex. Crucially, I have shown that the precise detail visible in this image cannot be seen with Hooke's microscope. Clearly, he used a single-lensed (simple) microscope – a design for which he published in his Preface – to observe fine structures which he incorporated into the final engraving.

It was this method of making a magnifier that caught the attention of the Dutch draper Thonis Leeuwenhoek, whom we know as Antony (and who added a "van" to his name in 1686, to give himself greater respectability). Although these simple microscopes were problematic, they were cheap and easy to make at home if you were a dedicated enthusiast, and Leeuwenhoek became single-minded in his quest for optical perfection. He had visited London in 1666, when he came across Hooke's great book. It inspired him to take up microscopy, and he was soon making his own little microscopes at home, based on Hooke's design (Figure 1.3).

Figure 1.3 Optical and SEM images of a newly discovered Leeuwenhoek microscope.