11 Microscopes

One fact about medium-size theoretical entities is so compelling an argument for medium-size scientific realism that philosophers blush to discuss it: Microscopes. First we guess there is such and such a gene, say, and then we develop instruments to let us see it. Should not even the positivist accept this evidence? Not so: the positivist says that only theory makes us suppose that what the lens teaches rings true. The reality in which we believe is only a photograph of what came out of the microscope, not any credible real tiny thing.

Such realism/anti-realism confrontations pale beside the metaphysics of serious research workers. One of my teachers, chiefly a technician trying to make better microscopes, could casually remark: 'X-ray diffraction microscopy is now the main interface between atomic structure and the human mind.' Philosophers of science who discuss realism and anti-realism have to know a little about the microscopes that inspire such eloquence. Even the light microscope is a marvel of marvels. It does not work in the way that most untutored people suppose. But why should a philosopher care how it works? Because it is one way to find out about the real world. The question is: How does it do it? The microscopist has far more amazing tricks than the most imaginative of armchair students of the philosophy of perception. We ought to have some understanding of those astounding physical systems 'by whose augmenting power we now see more/than all the world has ever done before'.[1]

The great chain of being

Philosophers have written dramatically about telescopes. Galileo himself invited philosophizing when he claimed to see the moons of Jupiter, assuming the laws of vision in the celestial sphere are the

1 From a poem, 'In commendation of the microscope', by Henry Powers, 1664. Quoted in the excellent historical survey by Saville Bradbury, *The Microscope, Past and Present*, Oxford, 1968.

same as those on earth. Paul Feyerabend has used that very case to urge that great science proceeds as much by propaganda as by reason: Galileo was a con man, not an experimental reasoner. Pierre Duhem used the telescope to present his famous thesis that no theory need ever be rejected, for phenomena that don't fit can always be accommodated by changing auxiliary hypotheses (if the stars aren't where theory predicts, blame the telescope, not the heavens). By comparison the microscope has played a humble role, seldom used to generate philosophical paradox. Perhaps this is because everyone expected to find worlds within worlds here on earth. Shakespeare is merely an articulate poet of the great chain of being when he writes in *Romeo and Juliet* of Queen Mab and her minute coach 'drawn with a team of little atomies . . . her waggoner, a small grey coated gnat not half so big as a round little worm prick'd from the lazy finger of a maid'. One expected tiny creatures beneath the scope of human vision. When dioptric glasses were to hand, the laws of direct vision and refraction went unquestioned. That was a mistake. I suppose no one understood how a microscope works before Ernst Abbe (1840–1905). One immediate reaction, by a president of the Royal Microscopical Society, and quoted for years in many editions of Gage's *The Microscope* – long the standard American textbook on microscopy – was that we do not, after all, see through a microscope. The theoretical limit of resolution

> [A] Becomes explicable by the research of Abbe. It is demonstrated that microscopic vision is *sui generis*. There is and there can be *no* comparison between microscopic and macroscopic vision. The images of minute objects are not delineated microscopically by means of the ordinary laws of refraction; they are not dioptical results, but depend entirely on the laws of diffraction.

I think that this quotation, which I simply call [A] below, means that we do not see, in any ordinary sense of the word, with a microscope.

Philosophers of the microscope

Every twenty years or so a philosopher has said something about microscopes. As the spirit of logical positivism came to America, one could read Gustav Bergman telling us that as he used philosophical terminology, 'microscopic objects are not physical

things in a literal sense, but merely by courtesy of language and pictorial imagination. . . . When I look through a microscope, all I see is a patch of color which creeps through the field like a shadow over a wall.'[2] In due course Grover Maxwell, denying that there is any fundamental distinction between observational and theoretical entities, urged a continuum of vision: 'looking through a window pane, looking through glasses, looking through binoculars, looking through a low power microscope, looking through a high power microscope, etc.'[3] Some entities may be invisible at one time and later, thanks to a new trick of technology, they become observable. The distinction between the observable and the merely theoretical is of no interest for ontology.

Grover Maxwell was urging a form of scientific realism. He rejected any anti-realism that holds that we are to believe in the existence of only the observable entities that are entailed by our theories. In *The Scientific Image* van Fraassen strongly disagrees. As we have seen in Part A above, he calls his philosophy constructive empiricism, and he holds that '*Science aims to give us theories which are empirically adequate; and acceptance of a theory involves as belief only that it is empirically adequate*' (p. 12). Six pages later he attempts this gloss: 'To accept a theory is (for us) to believe that it is empirically adequate – that what the theory says *about what is observable* (by us) is true.' Clearly then it is essential for van Fraassen to restore the distinction between observable and unobservable. But it is not essential to him, exactly where we should draw it. He grants that 'observable' is a vague term whose extension itself may be determined by our theories. At the same time he wants the line to be drawn in the place which is, for him, most readily defensible, so that even if he should be pushed back a bit in the course of debate, he will still have lots left on the 'unobservable' side of the fence. He distrusts Grover Maxwell's continuum and tries to stop the slide from seen to inferred entities as early as possible. He quite rejects the idea of a continuum.

There are, says van Fraassen, two quite distinct kinds of case arising from Grover Maxwell's list. You can open the window and see the fir tree directly. You can walk up to at least some of the

2 G. Bergman, 'Outline of an empiricist philosophy of physics', *American Journal of Physics* 11 (1943), pp. 248–58, 335–42.

3 G. Maxwell, 'The ontological status of theoretical entities', in *Minnesota Studies in the Philosophy of Science* 3 (1962), pp. 3–27.

objects you see through binoculars, and see them in the round, with the naked eye. (Evidently he is not a bird watcher.) But there is no way to see a blood platelet with the naked eye. The passage from a magnifying glass to even a low powered microscope is the passage from what we might be able to observe with the eye unaided, to what we could not observe except with instruments. Van Fraassen concludes that we do not see through a microscope. Yet we see through some telescopes. We can go to Jupiter and look at the moons, but we cannot shrink to the size of a paramecium and look at it. He also compares the vapour trail made by a jet and the ionization track of an electron in a cloud chamber. Both result from similar physical processes, but you can point ahead of the trail and spot the jet, or at least wait for it to land, but you can never wait for the electron to land and be seen.

Don't just peer: interfere

Philosophers tend to regard microscopes as black boxes with a light source at one end and a hole to peer through at the other. There are, as Grover Maxwell puts it, low power and high power microscopes, more and more of the same kind of thing. That's not right, nor are microscopes just for looking through. In fact a philosopher will certainly not see through a microscope until he has learned to use several of them. Asked to draw what he sees he may, like James Thurber, draw his own reflected eyeball, or, like Gustav Bergman, see only 'a patch of color which creeps through the field like a shadow over a wall'. He will certainly not be able to tell a dust particle from a fruit fly's salivary gland until he has started to dissect a fruit fly under a microscope of modest magnification.

That is the first lesson: you learn to see through a microscope by doing, not just by looking. There is a parallel to Berkeley's *New Theory of Vision* of 1710, according to which we have three-dimensional vision only after learning what it is like to move around in the world and intervene in it. Tactile sense is correlated with our allegedly two-dimensional retinal image, and this learned cueing produces three-dimensional perception. Likewise a scuba diver learns to see in the new medium of the oceans only by swimming around. Whether or not Berkeley was right about primary vision, new ways of seeing, acquired after infancy, involve learning by doing, not just passive looking. The conviction that a particular part

of a cell is there as imaged is, to say the least, reinforced when, using straightforward physical means, you microinject a fluid into just that part of the cell. We see the tiny glass needle – a tool that we have ourselves hand crafted under the microscope – jerk through the cell wall. We see the lipid oozing out of the end of the needle as we gently turn the micrometer screw on a large, thoroughly macroscopic, plunger. Blast! Inept as I am, I have just burst the cell wall, and must try again on another specimen. John Dewey's jeers at the 'spectator theory of knowledge' are equally germane for the spectator theory of microscopy.

This is not to say that practical microscopists are free from philosophical perplexity. Let us have a second quotation, [B], from the most thorough of available textbooks intended for biologists, E.M. Slayter's *Optical Methods in Biology*:

> [B] The microscopist can observe a familiar object in a low power microscope and see a slightly enlarged image which is 'the same as' the object. Increase of magnification may reveal details in the object which are invisible to the naked eye; it is natural to assume that they, also, are 'the same as' the object. (At this stage it is necessary to establish that detail is not a consequence of damage to the specimen during preparation for microscopy.) But what is actually implied by the statement that 'the image is the same as the object?'
>
> Obviously the image is a purely optical effect. . . . The 'sameness' of object and image in fact implies that the physical interactions with the light beam that render the object visible to the eye (or which would render it visible, if large enough) are identical with those that lead to the formation of an image in the microscope. . . .
>
> Suppose however, that the radiation used to form the image is a beam of ultraviolet light, x-rays, or electrons, or that the microscope employs some device which converts differences in phase to changes in intensity. The image then cannot possibly be 'the same' as the object, even in the limited sense just defined! The eye is unable to perceive ultraviolet, x-ray, or electron radiation, or to detect shifts of phase between light beams. . . .
>
> This line of thinking reveals that the image must be *a map of interactions between the specimen and the imaging radiation* (pp. 261–3).

The author goes on to say that all of the methods she has mentioned, and more, 'can produce "true" images which are, in some sense, "like" the specimen'. She also remarks that in a technique like the radioautogram 'one obtains an "image" of the specimen . . .

obtained exclusively from the point of view of the location of radioactive atoms. This type of "image" is so specialized as to be, generally, uninterpretable without the aid of an additional image, the photomicrograph, upon which it is superposed.'

This microscopist is happy to say that we see through a microscope only when the physical interactions of specimen and light beam are 'identical' for image formation in the microscope and in the eye. Contrast my quotation [A] from an earlier generation, and which holds that since the ordinary light microscope works by diffraction even it is not the same as ordinary vision but is *sui generis*. Can microscopists [A] and [B] who disagree about the simplest light microscope possibly be on the right philosophical track about 'seeing'? The scare quotes around 'image' and 'true' suggest more ambivalence in [B]. One should be especially wary of the word 'image' in microscopy. Sometimes it denotes something at which you can point, a shape cast on a screen, a micrograph, or whatever; but on other occasions it denotes as it were the input to the eye itself. The conflation results from geometrical optics, in which one diagrams the system with a specimen in focus and an 'image' in the other focal plane, where the 'image' indicates what you will see if you place your eye there. I do resist one inference that might be drawn even from quotation [B]. It may seem that any statement about what is seen with a microscope is theory-loaded: loaded with the theory of optics or other radiation. I disagree. One needs theory to make a microscope. You do not need theory to use one. Theory may help to understand why objects perceived with an interference-contrast microscope have asymmetric fringes around them, but you can learn to disregard that effect quite empirically. Hardly any biologists know enough optics to satisfy a physicist. Practice – and I mean in general doing, not looking – creates the ability to distinguish between visible artifacts of the preparation or the instrument, and the real structure that is seen with the microscope. This practical ability breeds conviction. The ability may require some understanding of biology, although one can find first class technicians who don't even know biology. At any rate physics is simply irrelevant to the biologist's sense of microscopic reality. The observations and manipulations seldom bear any load of physical theory at all, and what is there is entirely independent of the cells or crystals being studied.

Bad microscopes

I have encountered the impression that Leeuwenhoek invented the microscope, and that since then people have gone on to make better and better versions of the same kind of thing. I would like to correct that idea.

Leeuwenhoek, hardly the first microscopist, was a technician of genius. His microscopes had a single lens, and he made a lens for each specimen to be examined. The object was mounted upon a pin at just the right distance. We don't quite know how he made such marvellously accurate drawings of his specimens. The most representative collection of his lenses-plus-specimen was given to the Royal Society in London, which lost the entire set after a century or so in what are politely referred to as suspicious circumstances. But even by that time the glue for his specimens had lost its strength and the objects had begun to fall off their pins. Almost certainly Leeuwenhoek got his marvellous results thanks to a secret of illumination rather than lens manufacture, and he seems never to have taught the public his technique. Perhaps Leeuwenhoek invented dark field illumination, rather than the microscope. That guess should serve as the first of a long series of possible reminders that many of the chief advances in microscopy have had nothing to do with optics. We have needed microtomes to slice specimens thinner, aniline dyes for staining, pure light sources, and, at more modest levels, the screw micrometer for adjusting focus, fixatives and centrifuges.

Although the first microscopes did create a terrific popular stir by showing worlds within worlds, it is important to note that after Hooke's compound microscope, the technology did not markedly improve. Nor did much new knowledge follow after the excitement of the initial observations. The microscope became a toy for English ladies and gentlemen. The toy would consist of a microscope and a box of mounted specimens from the plant and animal kingdom. Note that a box of mounted slides might well cost more than the purchase of the microscope itself. You did not just put a drop of pond water on a slip of glass and look at it. All but the most expert would require a ready mounted slide to see *anything*. Indeed considering the optical aberrations it is amazing that anyone ever did see anything through a compound microscope, although in fact,

as always in experimental science, a really skilful technician can do wonders with awful equipment.

There are about eight chief aberrations in bare-bones light microscopy. Two important ones are spherical and chromatic. The former is the result of the fact that you polish a lens by random rubbing. That, as can be proven, gives you a spherical surface. A light ray travelling at a small angle to the axis will not focus at the same point as a ray closer to the axis. For angles i for which sin i differs at all from i we get no common focus of the light rays, and so a point on the specimen can be seen only as a smear through the microscope. This was well understood by Huygens who also knew how to correct it in principle, but practical combinations of concave and convex lenses to avoid spherical aberration were a long time in the making.

Chromatic aberrations are caused by differences in wave length between light of different colours. Hence red and blue light emanating from the same point on the specimen will come to focus at different points. A sharp red image is superimposed on a blue smear or vice versa. Although rich people liked to have a microscope about the house for entertainments, it is no wonder that serious science had nothing to do with the instrument. We often regard Xavier Bichat as the founder of histology, the study of living tissues. In 1800 he would not allow a microscope in his lab. In the introduction to his *General Anatomy* he wrote that: 'When people observe in conditions of obscurity each sees in his own way and according as he is affected. It is, therefore, observation of the vital properties that must guide us', rather than the blurred images provided by the best of microscopes.

No one tried very hard to make achromatic microscopes, because Newton had written that they are physically impossible. They were made possible by the advent of flint glass, with refractive indices different from that of ordinary glass. A doublet of two lenses of different refractive indices can be made to cancel out the aberration perfectly for a given pair of red and blue wave lengths, and although the solution is imperfect over the whole spectrum, the result can be improved by a triplet of lenses. The first person to get the right ideas was so secretive that he sent the specifications for the lenses of different kinds of glass to two different contractors. They both subcontracted with the same artisan who then formed a shrewd

guess that the lenses were for the same device. Hence, in 1758, the idea was pirated. A court case for the patent rights was decided in favour of the pirate, John Dolland. The High Court Judge ruled: 'It was not the person who locked the invention in his scritoire that ought to profit by a patent for such an invention, but he who brought it forth for the benefit of the public.'[4] The public did not benefit all that much. Even up into the 1860s there were serious debates as to whether globules seen through a microscope were artifacts of the instrument or genuine elements of living material. (They were artifacts.) Microscopes did get better and aids to microscopy improved at rather a greater rate. If we draw a graph of development we get a first high around 1660, then a slowly ascending plateau until a great leap around 1870; the next great period, which is still with us, commences about 1945. An historian has plotted this graph with great precision, using as a scale the limits of resolution of surviving instruments of different epochs. Making a subjective assessment of great applications of the microscope, we would draw a similar graph, except that the 1870/1660 contrast would be greater. Few truly memorable facts were found out with a microscope until after 1860. The surge of new microscopy is partly due to Abbe, but the most immediate cause of advance was the availability of aniline dyes for staining. Living matter is mostly transparent. The new aniline dyes made it possible for us to see microbes and much else.

Abbe and diffraction

How do we 'normally' see? Mostly we see reflected light. But if we are using a magnifying glass to look at a specimen illumined from behind, then it is transmission, or absorption, that we are 'seeing'. So we have the following idea: to see something through a light microscope is to see patches of dark and light corresponding to the proportions of light transmitted or absorbed. We see changes in the amplitude of light rays. I think that even Huygens knew there is something wrong with this conception, but not until 1873 did Abbe explain how a microscope works.

Ernst Abbe provides the happiest example of a rags to riches story. Son of a spinning-mill workman, he learned mathematics and

4 Quoted in Bradbury, *The Microscope, Past and Present*, p. 130.

was sponsored through the Gymnasium. He became a lecturer in mathematics, physics and astronomy. His optical work led him to be taken on by the small firm of Carl Zeiss in Jena, and when Zeiss died he became an owner; he retired to a life of philanthropy. Innumerable mathematical and practical innovations by Abbe turned Carl Zeiss into the greatest of optical firms. Here I consider only one.

Abbe was interested in resolution. Magnification is worthless if it 'magnifies' two distinct dots into one big blur. One needs to resolve the dots into two distinct images. It is a matter of diffraction. The most familiar example of diffraction is the fact that shadows of objects with sharp boundaries are fuzzy. This is a consequence of the wave character of light. When light travels between two narrow slits, some of the beam may go straight through, but some of it will bend off at an angle to the main beam, and some more will bend off at a larger angle: these are the first-order, second-order, etc., diffracted rays.

Abbe took as his problem how to resolve (i.e., visibly distinguish) parallel lines on a diatom (the tiny oceanic creatures that whales eat by the billion). These lines are very close together and of almost uniform separation and width. He was soon able to take advantage of even more regular artificial diffraction gratings. His analysis is an interesting example of the way in which pure science is applied, for he worked out the theory for the pure case of looking at a diatom or diffraction grating, and inferred that this represents the infinite complexity of the physics of seeing a heterogeneous object with a microscope.

When light hits a diffraction grating most of it is diffracted rather than transmitted. It is emitted from the grating at the angle of first-, second-, or third-order diffractions, where the angles of the diffracted rays are in part a function of the distances between the lines on the grating. Abbe realized that in order to see the slits on the grating, one must pick up not only the transmitted light, but also at least the first-order diffracted ray. What you see, in fact, is best represented as a Fourier synthesis of the transmitted and the diffracted rays. Thus according to Abbe the image of the object is produced by the interference of the light waves emitted by the principal image, and the secondary images of the light source which are the result of diffraction.

Practical applications abound. Evidently you will pick up more diffracted rays by having a wider aperture for the objective lens, but then you obtain vastly more spherical aberration as well. Instead you can change the medium between the specimen and the lens. With something denser than air, as in the oil-immersion microscope, you capture more of the diffracted rays within a given aperture and so increase the resolution of the microscope.

Although the first Abbe–Zeiss microscopes were good, the theory was resisted for a number of years, particularly in England and America, who had enjoyed a century of dominating the market. Even by 1910 the very best English microscopes, built on purely empirical experience, although stealing a few ideas from Abbe, could resolve as well or better than the Zeiss equipment. This is not entirely unusual. Although sailing ships have been part of human culture almost for ever, the greatest improvements in the sailing ship were made between 1870 and 1900, when the steamboat had made them obsolete. It was just at that time that craftsmanship peaked. Likewise with the microscope, but of course the expensive untheoretical English craftsmen of microscopy were as doomed as the sailing ship.

It was not, however, only commercial or national rivalry which made some people hesitate to believe in Abbe. I noted above that quotation [A] is used in Gage's *The Microscope*. In the ninth edition (1901) of that textbook the author refers to the alternative theory that microscopic vision is the same 'with the unaided eye, the telescope and the photographic camera. This is the original view, and the one which many are favoring at the present day.' In the 11th edition (1916) this is modified: 'Certain very striking experiments have been devised to show the accuracy of Abbe's hypothesis, but as pointed out by many, the ordinary use of the microscope never involves the conditions realized in these experiments.' This is a fine example of what Lakatos calls a degenerating research programme. The passage remains the same, in essentials, even in the 17th edition (1941). Thus there was a truly deep-seated repugnance to Abbe's doctrine which, as quotation [A] has it, says 'there is and can be *no* comparison between microscopic and macroscopic vision'.

If you hold (as my more modern quotation [B] still seems to hold), that what we see is essentially a matter of a certain sort of physical processing in the eye, then everything else must be more in

the domain of optical illusion or at best of mapping. On that account, the systems of Leeuwenhoek and of Hooke do allow you to see. After Abbe even the conventional light microscope is essentially a Fourier synthesizer of first- or even second-order diffractions. Hence you must modify your notion of seeing or hold that you never see through a serious microscope. Before reaching a conclusion on this question, we had best examine some more recent instruments.

A plethora of microscopes

We move on to after World War II. Most of the ideas had been around during the interwar years, but did not get beyond prototypes until later. One invention is a good deal older, but it was not properly exploited for a while.

The first practical problem for the cell biologist is that most living material does not show up under an ordinary light microscope because it is transparent. To see anything you have to stain the specimen. Most aniline dyes are number one poisons, so what you will see is usually a very dead cell, which is also quite likely to be a structurally damaged cell, exhibiting structures that are an artifact of the preparation. However it turns out that living material varies in its birefringent (polarizing) properties. So let us incorporate into our microscope a polarizer and an analyser. The polarizer transmits to the specimen only polarized light of certain properties. In the simplest case, let the analyser be placed at right angles to the polarizer, so as to transmit only light of polarization opposite to that of the polarizer. The result is total darkness. But suppose the specimen is itself birefringent; it may then change the plane of polarization of the incident light, and so a visible image may be formed by the analyser. Transparent fibers of striated muscle may be observed in this way, without any staining, and relying solely on certain properties of light that we do not normally 'see'.

Abbe's theory of diffraction, augmented by the polarizing microscope, leads to something of a conceptual revolution. We do not need the 'normal' physics of seeing in order to perceive structures in living material. In fact we seldom use it. Even in the standard case we synthesize diffracted rays rather than seeing the specimen by way of 'normal' visual physics. The polarizing microscope reminds us that there is more to light than refraction, absorption and diffraction. We could use any property of light that

interacts with a specimen in order to study the structure of the specimen. Indeed we could use any property of *any kind of wave* at all.

Even when we stick to light there is lots to do. Ultraviolet microscopy doubles resolving power, although its chief interest lies in noting the specific ultraviolet absorptions that are typical of certain biologically important substances. In fluorescence microscopy the incident illumination is cancelled out, and one observes only light re-emitted at different wave lengths by natural or induced phosphorescence or fluorescence. This is an invaluable histological technique for certain kinds of living matter. More interesting, however, than using unusual modes of light transmission or emission, are the games we can play with light itself: the Zernicke phase contrast microscope and the Nomarski interference microscope.

A specimen that is transparent is uniform with respect to light absorption. It may still possess invisible differences in refractive index in various parts of its structure. The phase contrast microscope converts these into visible differences of intensity in the image of the specimen. In an ordinary microscope the image is synthesized from the diffracted waves D and the directly transmitted waves U. In the phase contrast microscope the U and D waves are physically separated in an ingenious although physically simple way, and one or the other kind of wave is then subject to a standard phase delay which has the effect of producing in focus phase contrasts corresponding to the differences in refractive index in the specimen.

The interference contrast microscope is perhaps easier to understand. The light source is simply split by a half silvered mirror, and half the light goes through the specimen while half is kept as an unaffected reference wave to be recombined for the output image. Changes in optical path due to different refractive indices within the specimen thus produce interference effects with the reference beam.

The interference microscope is attended by illusory fringes but is particularly valuable because it provides a quantitative determination of refractive indices within the specimen. Naturally once we have such devices in hand, endless variations may be constructed, such as polarizing interference microscopes, multiple beam interference, phase modulated interference and so forth.

Theory and grounds for belief

Some theory of light is of course essential for building a new kind of microscope, and is usually important for improving an old kind. Interference or phase contrast microscopes could hardly have been invented without a wave theory of light. The theory of diffraction helped Abbe and his company make better microscopes. We should not, however, underestimate the pre-theoretical role of invention and fiddling around. For a couple of decades the old empirical microscope manufacturers made better microscopes than Zeiss. When the idea of an electron microscope was put into practice, it was a long shot, because people were convinced, on theoretical grounds, that the specimen would almost instantly be fried and then burnt out. The X-ray microscope has been a theoretical possibility for ages, but can effectively be built only in the next few years using high quality beams that can be bought from a linear accelerator. Likewise the acoustic microscope described below has long been an obvious possibility, but only in the last 10 years has one had the fast electronics to produce good high frequency sound and quality scanners. Theory has had only a modest amount to do with building these ingenious devices. The theory involved is mostly of the sort you learn in Physics I at college. It is the engineering that counts.

Theory may seem to enter at another level. Why do we believe the pictures we construct using a microscope? Is it not because we have a theory according to which we are producing a truthful picture? Is this not yet another case of Shapere's remark, that what we call observation is itself determined by theory? Only partially. Despite Bichat, people rightly believed much of what they saw through pre-Abbe microscopes, although they had only the most inadequate and commonplace theory to back them up (wrongly, as it happened). Visual displays are curiously robust under changes of theory. You produce a display, and have a theory about why a tiny specimen looks like that. Later you reverse the theory of your microscope, and you still believe the representation. Can theory really be the source of our confidence that what we are seeing is the way things are?

In correspondence Heinz Post told me that long ago he had discussed the field emission microscope in order to illustrate the importance of producing visual representations of large molecules. (His example concerned anthracene rings.) At the time, this device was taken to confirm what F.A. Kekule (1829–96) had postulated in

1865, that the benzene molecules are rings involving six carbon atoms. The original theory about the field emission microscope was that one was seeing essentially shadows of the molecules, that is, that we were observing an absorption phenomenon. Post learned much later that the underlying theory had been reversed. One was observing diffraction phenomena. It made no whit of difference. People kept on regarding the micrographs of the molecules as genuinely correct representations. Is this all mumbo-jumbo, a sort of confidence trick? Only a theory-dominated philosophy would make one think so. The experimental life of microscopy uses non-theory to sort out artifacts from the real thing. Let us see how it goes.

Truth in microscopy

The differential interference-contrast technique is distinguished by the following characteristics: Both clearly visible outlines (edges) within the object and continuous structures (striations) are imaged in their true profile.

So says a Carl Zeiss sales catalogue to hand. What makes the enthusiastic sales person suppose that the images produced by these several optical systems are 'true'? Of course, the images are true only when one has learned to put aside distortions. There are many grounds for the conviction that a perceived bit of structure is real or true. One of the most natural is the most important. I shall illustrate it with my own first experience in the laboratory. Low powered electron microscopy reveals small dots in red blood platelets. These are called dense bodies: that means simply that they are electron dense, and show up on a transmission electron microscope without any preparation or staining whatsoever. On the basis of the movements and densities of these bodies in various stages of cell development or disease, it is guessed that they may have an important part to play in blood biology. On the other hand they may simply be artifacts of the electron microscope. One test is obvious: can one see these selfsame bodies using quite different physical techniques? In this case the problem is fairly readily solved. The low resolution electron microscope is about the same power as a high resolution light microscope. The dense bodies do not show up under every technique, but are revealed by fluorescent staining and subsequent observation by the fluorescent microscope.

Slices of red blood platelets are fixed upon a microscopic grid.

This is literally a grid: when seen through the microscope one sees a grid each of whose squares is labelled with a capital letter. Electron micrographs are made of the slices mounted upon such grids. Specimens with particularly striking configurations of dense bodies are then prepared for fluorescence microscopy. Finally one compares the electron micrographs and the fluorescence micrographs. One knows that the micrographs show the same bit of the cell, because this bit is clearly in the square of the grid labelled *P*, say. In the fluorescence micrographs there is exactly the same arrangement of grid, general cell structure and of the 'bodies' seen in the electron micrograph. It is inferred that the bodies are not an artifact of the electron microscope.

Two physical processes – electron transmission and fluorescent re-emission – are used to detect the bodies. These processes have virtually nothing in common between them. They are essentially unrelated chunks of physics. It would be a preposterous coincidence if, time and again, two completely different physical processes produced identical visual configurations which were, however, artifacts of the physical processes rather than real structures in the cell.

Note that no one actually produces this 'argument from coincidence' in real life. One simply looks at the two (or preferably more) sets of micrographs from different physical systems, and sees that the dense bodies occur in exactly the same place in each pair of micrographs. That settles the matter in a moment. My mentor, Richard Skaer, had in fact expected to prove that dense bodies are artifacts. Five minutes after examining his completed experimental micrographs he knew he was wrong.

Note also that no one need have any ideas what the dense bodies *are*. All we know is that there are some structural features of the cell rendered visible by several techniques. Microscopy itself will never tell all about these bodies (if indeed there is anything important to tell). Biochemistry must be called in. Also, instant spectroscopic analysis of the dense body into constituent elements is now available, by combining an electron microscope and a spectroscopic analyser. This works much like spectroscopic analyses of the stars.

Coincidence and explanation

This argument from coincidence may seem like a special case of the cosmic accident argument mentioned at the end of Chapter 3.

Theories explain diverse phenomena, and it would be a cosmic accident if a theory were false and yet correctly predicted the phenomena. We 'infer to the best explanation' that the theory is true. The common cause of the phenomena must be the theoretical entities postulated by the theory. As an argument for scientific realism this idea has produced much debate. So it may seem as if my talk of coincidence puts me in the midst of an ongoing feud. Not so! My argument is much more localized.

First of all such arguments are often put in terms of an observational vocabulary and a theoretical one. ('Innumerable lucky accidents bringing about the behaviour mentioned in the observational vocabulary, *as if* they were brought about by the nonexistent things talked about in the theoretical vocabulary.') Well, we are not concerned with an observational and theoretical vocabulary. There may well be no theoretical vocabulary for the things seen under the microscope – 'dense body' means nothing else than something dense, that is, something that shows up under the electron microscope without any staining or other preparation. Secondly we are not concerned with explanation. We see the same constellations of dots whether we use an electron microscope or fluorescent staining, and it is no 'explanation' of this to say that some definite kind of thing (whose nature is as yet unknown) is responsible for the persistent arrangements of dots. Thirdly we have no theory which predicts some wide range of phenomena. The fourth and perhaps most important difference is this: we are concerned to distinguish artifacts from real objects. In the metaphysical disputes about realism, the contrast is between 'real although unobservable entity' and 'not a real entity, but rather a tool of thought'. With the microscope we know there are dots on the micrograph. The question is, are they artifacts of the physical system or are they structure present in the specimen itself? My argument from coincidence says simply that it would be a preposterous coincidence if two totally different kinds of physical systems were to produce exactly the same arrangements of dots on micrographs.

The argument of the grid

I now venture a philosopher's aside on the topic of scientific realism. Van Fraassen says we can see through a telescope because although we need the telescope to see the moons of Jupiter when we

are positioned on earth, we could go out there and look at the moons with the naked eye. That is not so fanciful as it sounds, for there is a very small number of people living today who, it appears, can distinguish Jupiter's moons with the naked eye from here. For those of us with less acuity it is, for the moment however, science fiction. The microscopist avoids fantasy. Instead of flying to Jupiter we shrink the visible world. Consider the grids used to re-identify dense bodies. The tiny grids are made of metal; they are barely visible to the naked eye. They are made by drawing a very large grid with pen and ink. Letters are neatly inscribed at the corner of each square on the grid. Then the grid is reduced photographically. Using what are now standard techniques, metal is deposited on the resulting micrograph. Grids are sold in packets, or rather tubes, of 100, 250 and 1000. The procedures for making such grids are entirely well understood, and as reliable as any other high quality mass production system.

In short, rather than disporting ourselves to Jupiter in an imaginary space ship, we are routinely shrinking a grid. Then we look at the tiny disc through almost any kind of microscope and see exactly the same shapes and letters as were originally drawn on a large scale. It is impossible seriously to entertain the thought that the minute disc, which I am holding by a pair of tweezers, does not in fact have the structure of a labelled grid. I know that what I see through the microscope is veridical because we *made* the grid to be just that way. I know that the process of manufacture is reliable, because we can check the results with the microscope. Moreover we can check the results with any kind of microscope, using any of a dozen unrelated physical processes to produce an image. Can we entertain the possibility that, all the same, this is some gigantic coincidence? Is it false that the disc is, microscopically, in the shape of a labelled grid? Is it a gigantic conspiracy of 13 totally unrelated physical processes that the large scale grid was shrunk into some non-grid which when viewed using 12 different kinds of microscopes still looks like a grid? To be an anti-realist about that grid you would have to invoke a malign Cartesian demon of the microscope.

The argument of the grid requires a healthy recognition of the disunity of science, at least at the phenomenological level. Light microscopes, trivially, all use light, but interference, polarizing, phase contrast, direct transmission, fluorescence and so forth

exploit essentially unrelated phenomenological aspects of light. If the same structure can be discerned using many of these different aspects of light waves, we cannot seriously suppose that the structure is an artifact of all the different physical systems. Moreover I emphasize that all these physical systems are made by people. We purify some aspect of nature, isolating, say, the phase interference character of light. We design an instrument knowing in principle exactly how it will work, just because optics is so well understood a science. We spend a number of years debugging several prototypes, and finally have an off-the-shelf instrument, through which we discern a particular structure. Several other off-the-shelf instruments, built upon entirely different principles, reveal the same structure. No one short of the Cartesian sceptic can suppose that the structure is made by the instruments rather than inherent in the specimen.

In 1800 it was not only possible but perfectly sensible to ban the microscope from the histology lab on the plain grounds that it chiefly revealed artifacts of the optical system rather than the structure of fibres. That is no longer the case. It is always a problem in innovative microscopy to become convinced that what you are seeing is really in the specimen rather than an artifact of the preparation of the optics. But in 1983, as opposed to 1800, we have a vast arsenal of ways of gaining such conviction. I emphasize only the 'visual' side. Even there I am simplistic. I say that if you can see the same fundamental features of structure using several different physical systems, you have excellent reason for saying, 'that's real' rather than, 'that's an artifact'. It is not conclusive reason. But the situation is no different from ordinary vision. If black patches on the tarmac road are seen, on a hot day, from a number of different perspectives, but always in the same location, one concludes that one is seeing puddles rather than the familiar illusion. One may still be wrong. One is wrong, from time to time, in microscopy too. Indeed the sheer similarity of the kinds of mistakes made in macroscopic and microscopic perception may increase the inclination to say, simply, that one sees through a microscope.

I must repeat that just as in large scale vision, the actual images or micrographs are only one small part of the confidence in reality. In a recent lecture the molecular biologist G.S. Stent recalled that in the late forties *Life* magazine had a full colour cover of an electron micrograph, labelled, excitedly, 'the first photograph of the gene' (March 17 1947). Given the theory, or lack of theory, of the gene at that time, said Stent, the title did not make any sense. Only a greater understanding of what a gene is can bring the conviction of what the micrograph shows. We become convinced of the reality of bands and interbands on chromosomes not just because we see them, but because we formulate conceptions of what they do, what they are for. But in this respect too, microscopic and macroscopic vision are not different: a Laplander in the Congo won't see much in the bizarre new environment until he starts to get some idea what is in the jungle.

Thus I do not advance the argument from coincidence as the sole basis of our conviction that we see true through the microscope. It is one element, a compelling visual element, that combines with more intellectual modes of understanding, and with other kinds of experimental work. Biological microscopy without practical biochemistry is as blind as Kant's intuitions in the absence of concepts.

The acoustic microscope

I here avoid the electron microscope. There is no more 'the' electron microscope than 'the' light microscope: all sorts of different properties of electron beams are used. This is not the place to explain all that, but in case we have in mind too slender a diet of examples based upon the properties of visible light, let us briefly consider the most disparate kind of radiation imaginable: sound.[5]

Radar, invented for aerial warfare, and sonar, invented for war at sea, remind us that longitudinal and transverse wave fronts can be put to the same kinds of purpose. Ultrasound is 'sound' of very high frequency. Ultrasound examination of the foetus in the mother's womb has recently won well deserved publicity. Over 40 years ago Soviet scientists suggested a microscope using sound of frequency 1000 times greater than audible noise. Technology has only recently caught up to this idea. Useful prototypes are just now in operation.

The acoustic part of the microscope is relatively simple. Electric signals are converted into sound signals and then, after interaction with the specimen, are reconverted into electricity. The subtlety of

5 See, for example, C.F. Quate, 'The acoustic microscope', *Scientific American* 241 (Oct. 1979), pp. 62–9.

present instruments lies in the electronics rather than the acoustics. The acoustic microscope is a scanning device. It produces its images by converting the signals into a spatial display on a television screen, a micrograph, or, when studying a large number of cells, a videotape.

As always a new kind of microscope is interesting because of the new aspects of a specimen that it may reveal. Changes in refractive index are vastly greater for sound than for light. Moreover sound is transmitted through objects that are completely opaque. Thus one of the first applications of the acoustic microscope is in metallurgy, and also in detecting defects in silicon chips. For the biologist, the prospects are also striking. The acoustic microscope is sensitive to density, viscosity and flexibility of living matter. Moreover the very short bursts of sound used by the scanner do not immediately damage the cell. Hence one may study the life of a cell in a quite literal way: one will be able to observe changes in viscosity and flexibility as the cell goes about its business.

The rapid development of acoustic microscopy leaves us uncertain where it will lead. A couple of years ago the research reports carefully denied any competition with electron microscopes; they were glad to give resolution at about the level of light microscopes. Now, using the properties of sound in supercooled solids one can emulate the resolution of electron microscopes, although that is not much help to the student of living tissue!

Do we see with an acoustic microscope?

Looking with a microscope

Looking through a lens was the first step in technology. Then came peering through the tube of a compound microscope, but looking 'through' the instrument is immaterial. We study photographs taken with a microscope. Thanks to the enormous depth of focus of an electron microscope it is natural to view the image on a large flat surface so everyone can stand around and point to what's interesting. Scanning microscopes necessarily constitute the image on a screen or plate. Any image can be digitized and retransmitted on a television display or whatever. Moreover, digitization is marvellous for censoring noise and even reconstituting lost information. Do not, however, become awed by technology. In the study of crystal structure, one good way to get rid of noise is to cut up a micrograph

in a systematic way, paste it back together, and rephotograph it for interference contrast. Thus we do not in general see through a microscope; we see with one. But do we *see* with a microscope? It would be silly to debate the ordinary use of the word 'see', especially given the usages quoted at the end of the last chapter, where we 'see' most of the fermions, or 'observe' the sun's core with neutrinos. Consider a device for low-flying jet planes, laden with nuclear weapons, skimming a few dozen yards from the surface of the earth in order to evade radar detection. The vertical and horizontal scale are both of interest to the pilot who needs both to see a few hundred feet down and miles and miles away. The visual information is digitized, processed, and cast on a head-up display on the windscreen. The distances are condensed and the altitude is expanded. Does the pilot see the terrain? Yes. Note that this case is not one in which the pilot could have seen the terrain by getting off the plane and taking a good look. There is no way to look at that much landscape without an instrument.

Consider the electron diffraction microscope with which I produce images of crystals in either conventional or reciprocal space – nowadays, at the flick of a switch. Because the dots of an electron diffraction pattern are reciprocal to the atomic structure of a crystal, reciprocal space is, roughly speaking, conventional space turned inside out. Near is far and far is near. Crystallographers often find it most natural to study their specimens in reciprocal space. Do they see them in reciprocal space? They certainly say so, and thereby call in question the Kantian doctrine of the uniqueness of perceptual space.

How far could one push the concept of seeing? Suppose I take an electronic paint brush and paint, on a television screen, an accurate picture (a) of a cell that I have previously studied, say, by using a digitized and reconstituted image (b). Even if I am 'looking at the cell' in case (b), in (a) I am only looking at a drawing of the cell. What is the difference? The important feature is that in (b) there is a direct interaction between a wave source, an object, and a series of physical events that end up in an image of the object. To use quotation [B] once again, in case (b) we have a map of interactions between the specimen and the imaging radiation. If the map is a good one, then (b) is seeing with a microscope.

This is doubtless a liberal extension of the notion of seeing. We

see with an acoustic microscope. We see with television, of course. We do not say that we saw an attempted assassination *with* the television, but *on* the television. That is mere idiom, inherited from 'I heard it on the radio.' We distinguish between seeing the television broadcast live or not. We have endless distinctions to be made with various adverbs, adjectives and even prepositions. I know of no confusion that will result from talk of seeing with a microscope.

Scientific realism

When an image is a map of interactions between the specimen and the image of radiation, and the map is a good one, then we are seeing with a microscope. What is a good map? After discarding or disregarding aberrations or artifacts, the map should represent some structure in the specimen in essentially the same two- or three-dimensional set of relationships as are actually present in the specimen.

Does this bear on scientific realism? First let us be clear that it can bear in only the modest way. Imagine a reader initially attracted by van Fraassen, and who thought that objects seen only with light microscopes do not count as observable. That reader could change his mind, and admit such objects into the class of observable entities. This would still leave intact all the main philosophical positions of van Fraassen's anti-realism.

But if we conclude that we see with the light microscopes, does it follow that the objects we report seeing are real? No. For I have said only that we should not be stuck in the nineteenth-century rut of positivism-cum-phenomenology, and that we should allow ourselves to talk of seeing with a microscope. Such a recommendation implies a strong commitment to realism about microscopy, but it begs the question at issue. This is clear from my quotation from high-energy physics, with its cheerful talk of our having seen electron neutrinos and so forth. The physicist is a realist too, and he shows this by using the word 'see', but his usage is no *argument* that there are such things.

Does microscopy then beg the question of realism? No. We *are* convinced of the structures that we observe using various kinds of microscopes. Our conviction arises partly from our success at systematically removing aberrations and artifacts. In 1800 there

was no such success. Bichat banned the microscope from his dissecting rooms, for one did not, then, observe structures that could be confirmed to exist in the specimens. But now we have by and large got rid of aberrations; we have removed many artifacts, disregard others, and are always on the lookout for undetected frauds. We are convinced about the structures we seem to see because we can interfere with them in quite physical ways, say by microinjecting. We are convinced because instruments using entirely different physical principles lead us to observe pretty much the same structures in the same specimen. We are convinced by our clear understanding of most of the physics used to build the instruments that enable us to see, but this theoretical conviction plays a relatively small part. We are more convinced by the admirable intersections with biochemistry, which confirm that the structures that we discern with the microscope are individuated by distinct chemical properties too. We are convinced not by a high powered deductive theory about the cell – there is none – but because of a large number of interlocking low level generalizations that enable us to control and create phenomena in the microscope. In short, we learn to move around in the microscopic world. Berkeley's *New Theory of Vision* may not be the whole truth about infantile binocular three-dimensional vision, but is surely on the right lines when we enter the new worlds within worlds that the microscope reveals to us.