THE MICROSCOPE PAST
30 YEARS AGO

A Physicist Looks at Microscopy[1]

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When I am asked by people such as bankers, plumbers, Congressmen and the like what I am, I instantaneously answer "a physicist." But when they ask me what I *do*, my answer comes much more slowly and varies depending upon what, in fact, I have been doing. It would be no more helpful to answer "physics" than it would for a banker to answer "banking." So, when I look out over this well-fed assembly, I am aware that almost all of you are microscopists — why else are you here? — yet that tells me very little about what any of you do. "Microscopy," I suppose. But what, to a physicist, does "microscopy" appear to be? What can be abstracted, from that myriad of things that microscopists do, that sets them apart as a group from other scientists who may often do the same things? You and I both spend a lot of time looking through a microscope. Why do you think you are a microscopist, and why do I think I am a physicist?

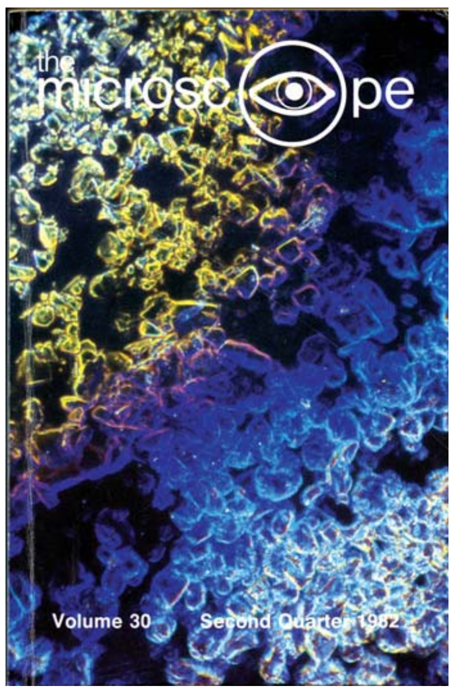

Figure 1. Cover image. This central stop dispersion staining shot of isotropic sodium bromate (nD = 1.617) shows a refractive index gradient of the liquid ranging from about 1.56 (upper left) to 1.68 (lower right), original at 50x.

On the theory that I am supposed to answer that question, I am first going to try to define some differences between the physicist and microscopist. (I will, on the whole, let the similarities take care of themselves.)

Then I will try to indicate the sources of these differences.

Finally, I will attempt to say something profound, subtle and full of insight about the subject as a whole. You would be well advised to doze off before I reach that point.

Now, I understand microscopists use a lot of slides — so, let's look at a picture. Here is Figure 1 (cover photo).

What is it? I don't know. I don't seem to have that written down here.

Well — no matter.

And that makes the point, I think. Like a microscopist, a physicist may not know what he is looking at when he looks through the microscope, but unlike a microscopist, he rarely cares. It is neither the identity of the object that concerns the physicist nor even the properties of the object illuminated, as it were, by the capacities of the instrument that interest him, but the *properties of the electromagnetic radiation thereby revealed* (1).

The mystery to be resolved is not the nature of the

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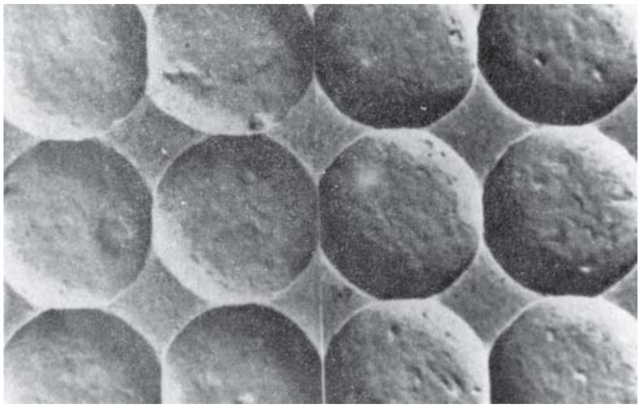

Figure 2. A reflected oblique-lighted micrograph of halftone dots was cut in half vertically and one half was inverted to the other half, original at 25x.

object, but the nature of the light that illuminates it!

What is light? What is the unique phenomenon of space and time, this manifestation of energy and momentum, this yardstick of the universe that lays down its length again and again with invariant speed, that carries news of the origins of the universe, and of the birth of a single cell, that is only perceived at the cost of its extinction?

Let us begin with the common experience of perception. When we *see*, something happens on two entirely different levels. First, there is the physical transport of momentum and energy distributed in space and time into the eye. This distribution of energy and momentum is then absorbed — used up, irrevocably changed — and in that moment of destruction arises again, transfigured and purified and stripped of physical attributes, as *information*. It is this second level, that of the image as *information* that leads us to say "I see!" (2).

I think we cannot fully appreciate what either the physicist or the microscopist experiences until we recognize the subtleties of this act of perception.

Let me play microscopist for a moment. Let's look again at Figure 1.

What do I *see*?

Well, colors, of course, and spatial variations in brightness. And physically, that's all. But let the transformation occur that orders these direct excitations into that sequence of events I call information, and I "see" shapes. I recognize — an act of the mind, possible only by comparison with previous perception — I recognize that these are crystals of some sort, and the colors are those characteristic of interference phenomena, a peculiar property that light can exhibit under certain predictable conditions. Again, I know this from previous experience. Further, I know from the dark field that light not passing through crystals does not become visible to me at all. I infer from this that the crystals are in one leg of a compound interferometer set to work with a phase shift of radians between the two beam paths. If that doesn't ring a bell, I'll just say for short that this is dispersion staining.(3)

Already you can see another difference between physics and microscopists. Physicists like to devise complicated explanations for things that go on around us, while microscopists like to name and use these things, usually for some other end.

But the real point of this example is that the image seen, in the human sense of the word, is an inextricable mix of external events that exist whether or not observed, with a stored matrix of information which exists only in the mind of the observer.

Will it surprise anyone if I maintain that, in the world of the microscopist, almost all of the meaning he gleans from looking through that little tube is already present in his own brain? That all the image does is help him select, from that vast catalog of the mind, the identifying label of asbestos, or blood, or iron oxide, or fly ash? That in the hands of one *without* such a catalog at his command — *me*, for instance — all these images are just more or less pretty?

But you can see how this is so by finding, just once, something under the microscope that you have never seen before. *You cannot recognize it.* And no amount of staring will help. You can describe it, but you can't say what it *is*. The determination of identity, the act of recognition, must be made by other means, using other information.

Let me show you the evidence for this statement — not by showing the unknown, but the unknowable.

Look at Figure 2. Which are depressions? Which are elevations? With a little effort you can make them look either way, right? Just turn the page upside down. But which *are* they? Alas — I don't know. You don't know. Nobody knows. The information that would settle the matter was not recorded by the film. In the kingdom of the one-eyed, the depth dimension *does not exist*. And the attempt to impose depth upon a picture that does not have it may give ambiguous results — or worse!

For example, Figure 3 shows a sketch of a triangle. Would you like to have one in your home? What is the matter with the drawing? Why, nothing. It's a perfectly good drawing. Shown to a member of a culture in which perspective drawing has not yet emerged, this sketch would elicit no special confusion. It will

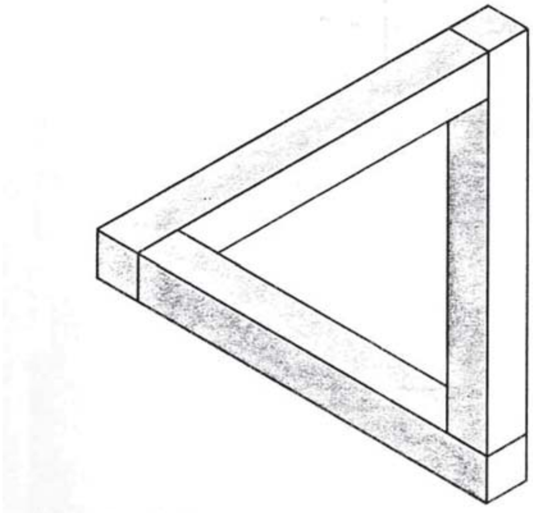

Figure 3. An "impossible triangle" derived by L.S. Penrose and R. Penrose of Imperial College, London.

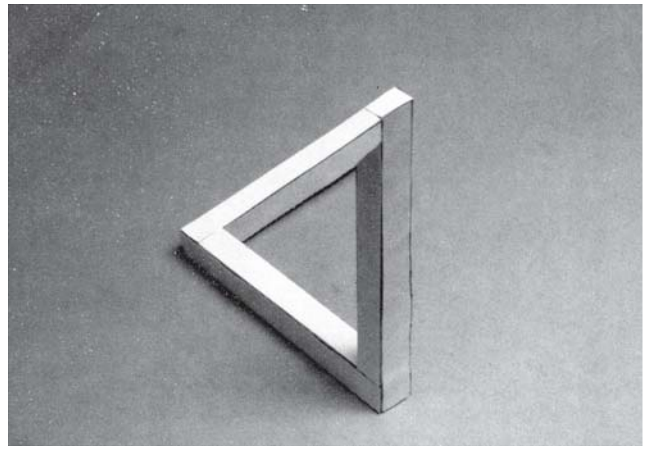

Figure 4. A model of the "impossible triangle."

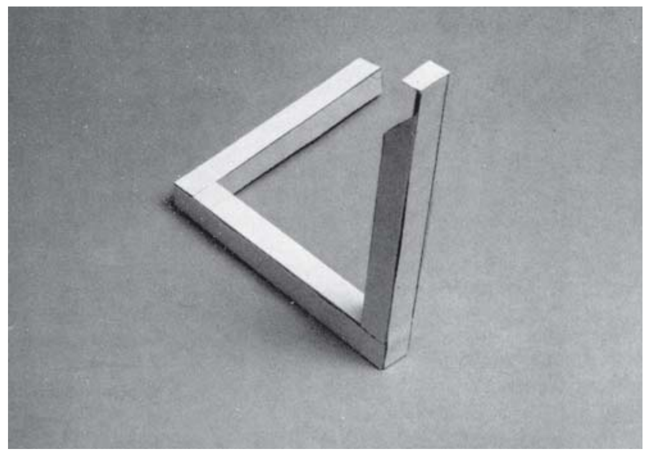

Figure 5. A slightly different view of the model in Figure 4.

look exactly like what it is — some lines on a piece of paper. Can you see it that way? I can't. But, to paraphrase Cassius, the fault, dear friends, lies not in the image, but in our interpretation of it! We insist upon trying to identify these lines with something else, something that exists only in our minds, something belonging to the class of solid objects we have known. And it doesn't belong (4).

Or does it? Figure 4 shows a *photograph* of one. How about that?

Figure 5 show the same object form a different camera angle. Like the hills and valleys, the secret of *this* optical illusion is in the *suppression of information* — in this case, too, of the depth dimension.

Now look at Figure 6. It is quite evidently a picture of a shadowed three-dimensional object, right? Well, of course you know better. The light variations are the result of differential interference contrast microscopy, and generally have very little to do with the shape of the object. Yet, for the life of me, I see that third dimension *and in fact from the picture alone* you can't prove that the dimension isn't really there. Either interpretation is possible: It might be a Nomarski differential interference contrast image or it might be a sidelighted three-dimensional object. The information needed to decide which is not in the picture. It has to be in your mind, or the picture is truly ambiguous. Figure 7 shows Hoffman modulation contrast, which produces a similar ambiguity.

An artist named Escher has played with the depth dimension in striking ways.

Give Figure 8 to your local carpenter to build.

Or, look at Figure 9. Before you get up on that ladder, check your insurance!

And in Figure 10 we have a scene right from the Wizard of Oz.

A more subtle ambiguity is shown in Figure 11 by Paul Fischer. Where does the face become the girl?

Figure 12 is another Escher. Where do the birds become fish? Again, the key to these paradoxical ambiguities is not in the pictures, but in the mind — in the eye of the beholder. Neither bird nor fish exists on this piece of paper, but only shadings of light and dark. The rest is in you and me. The stairs in Figure 10 don't lead up or down; there are no stairs, only streaks of light and dark.

A microscopist is first, last and always an observer, and what he brings to his profession is proportional to his catalog of recognizable patterns. Everything

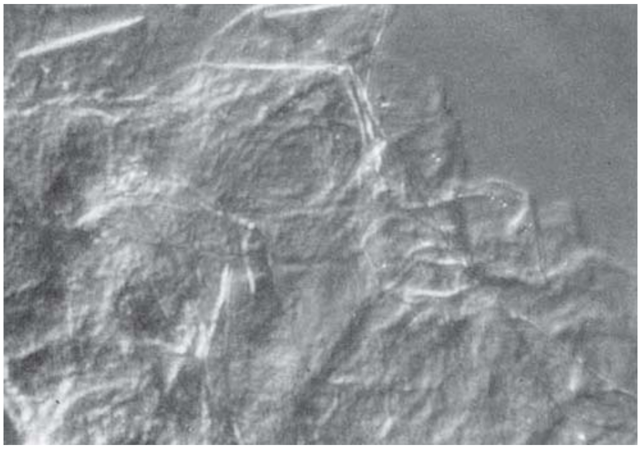

Figure 6. A dandruff flake with Nomarski differential interference contrast, original at 40x; by John G. Delly of McCrone Associates.

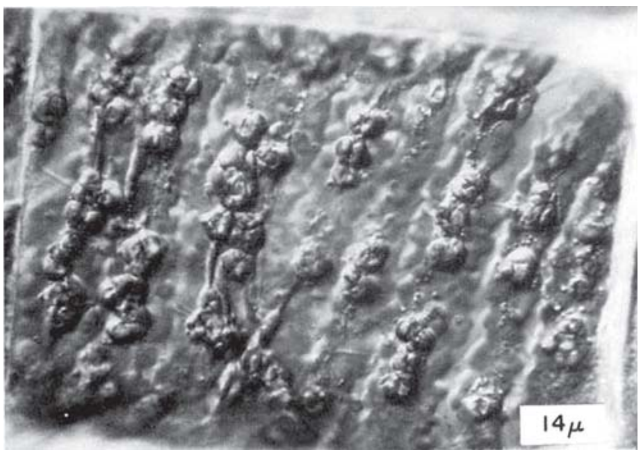

Figure 7. Cellular detail of spirogyra.

springs to from this. But the information presented to the microscopist is always partial, never complete. *He* must add the missing bits from the catalog of his mind. And it is not necessarily true that everything he thereby "sees" exists!

We have seen examples of ambiguity. Now let's look at something that's just plain wrong. Figure 13 (color plate) is a photomicrograph of a microelectronics component. You see colors. They are *not* real. These are colors created by arbitrarily assigning colors to X-ray wavelengths generated by the microprobe. Any other assignment would be equally acceptable. That this photograph is not "true" is something we accept without a qualm. In fact, information unobtainable from an ordinary photograph is presented clearly in this artificial manner and so we readily accept the distortions.

Now, how does all of this appear to a physicist?

First, let me remind you that to me the object of study is not what one puts on the microscope slide, but what one puts *through* the slide; the light. As far as I am concerned, the object on the slide is but another processing tool. I am interested in how it works, but not what it is. The light — or electron, or ultrasonic waves — but especially the *light* draws the physicist as a candle flame draws a moth. The more I learn about electromagnetic energy the more fascinating and challenging the topic becomes. From one point of view, light is an especially simple manifestation of a boson field theory. For light only two polarizations exist, the third polarization being suppressed by the Lorentz transformation's limiting properties at $v = c$. In this particular picture, light has zero rest mass: the electromagnetic field is of infinite range and is renormalizable. The fine structure constant is less than unity, so that perturbation theory always converges and so on and so on. I see your eyes becoming glassy. I understand; I feel the same way about the microscopical differentiation of serpentine and amphibole asbestos.

From another viewpoint, light is a mechanical system with a certain number of degrees of freedom, that number being proportional to the numerical aperture of the beam and to the time of exposure. How an object encodes its nature on that system has been completely understood for only about 20 years, and even now remarkable new theoretical and experimental results are still emerging. Only two years ago, Emil Wolf first proved that, by spatial filtering alone, it is possible to generate a beam of light of *any desired degree of coherence* — as coherent in space and time as any laser. Subsequently, this was accomplished experimentally and a laser-like beam was constructed from a diffuse thermal source (5, 6).

Within the last few years, remarkable advances have been made in image reconstruction from the incoherent superposition of image slices (7). These advances have been fueled by the development of the body scanner, and the new field of medical tomography has lead to the burgeoning of million-dollar instruments which will either save all of our lives or bankrupt medical services in the nation — maybe both. Yet did you know that these image reconstructions are not known to be correct? That they have not been proved to be unique, not in even a single case? This is a special case of the general unsolved problem of the reconstruction of the complete far field from its magnitude only. This is called the *phase* problem, and is of practical importance in such diverse applications as

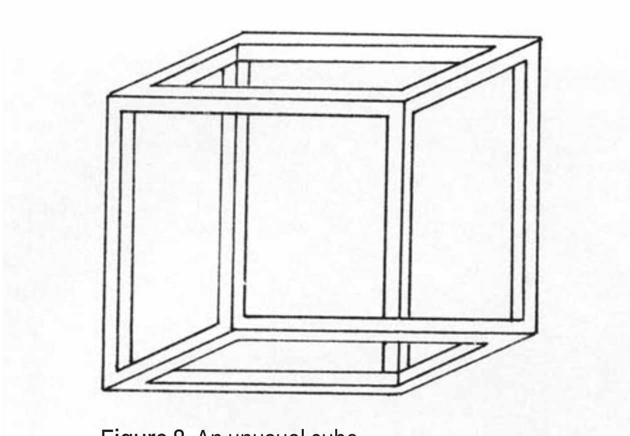

Figure 8. An unusual cube.

Figure 9. Check your insurance before ascending this ladder. Copyright © Beeldrecht, Amsterdam/VAGA, N.Y. Collection Gemeentemuseum, The Hague, 1981.

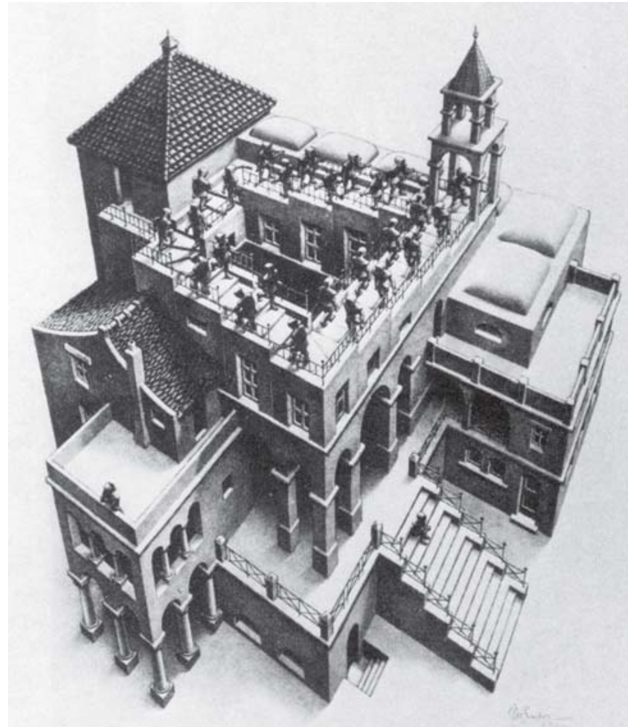

Figure 10. Ascending? Or descending? Copyright © Beeldrecht, Amsterdam/VAGA, N.Y. Collection Gemeentemuseum, The Hague, 1981.

imaging through a turbulent atmosphere or through a translucent mounting medium, imaging of crystal lattice from an X-ray diffraction pattern, and, of course, tomography.

It is almost certain that the phase problem is truly insoluble in the sense that the phase of a wave, once discarded, cannot generally be resurrected from the amplitude distribution alone. Of course, that doesn't stop us from trying (8). Yet if that is true, then a tomographic reconstruction is only one of an infinite number of possible images, all consistent with a single set of input data. Think of that the next time your doctor wants to give you a brain transplant on the basis of a single tomographic scan!

But if these reconstructions are not unique, then how is it that different scans of the same object do in fact lead to practically identical reconstructions? Is it that the infinity of *possible* reconstructions occupy such a narrow slice of image space that differences among them are almost sure to be negligible? And, how sure is "almost" sure?

Recently, a new development in imaging theory has appeared which fascinates me. I want to share with you the excitement I feel at the novelty and prom-

Figure 11. Man ↔ girl. Copyright © Beeldrecht, Amsterdam/VAGA, N.Y. Collection Gemeentemuseum, The Hague, 1981.

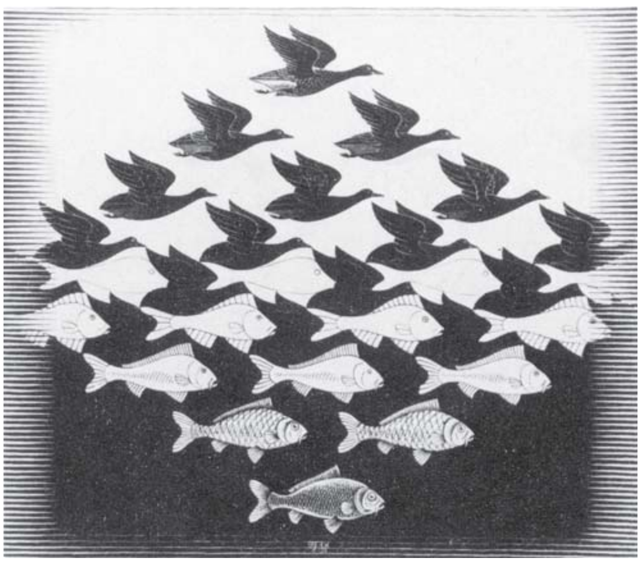

Figure 12. Birds ↔ fish. Copyright © Beeldrecht, Amsterdam/VAGA, N.Y. Collection Gemeentemuseum, The Hague, 1981.

ise of this departure from conventional lenses and mirrors.

Imagine an image illuminated with a plane coherent wave. In the far field we find the diffraction pattern of the object which, as we all know, contains all of the information out of which we may construct an image. Conventionally, we capture this information by suitable detectors distributed over the far field. But suppose we can place only two fixed detectors out there. Is there any way to reconstruct the image form the output of these detectors only?

Well, it turns out that if one records coherently their output using illumination whose wavelength can be changed over a suitable range, then an image can be constructed from the data set. The numerical aperture of the detector *does not matter*. The detector can be a point, for all that. A wavelength range of the 100 nm centered about 500 nm will, in principle, enable image resolution of 2.9 nm. And this may all be done without a single lens or mirror anywhere in the system! Think of it! Microscopy without lenses! All you need is a tunable laser, a couple of coherent photodetectors, a large digital computer, and — oh yes, a few megabucks.

Well, I told you I was no microscopist.

But — to a physicist, the study of light is the study of physical reality in its purest and simplest form. Almost anything one does with such an entity is certain to be informative. The basic laws of physics are best displayed by simple systems which can be exhaustively analyzed. And to a physicist, each experiment and each theoretical construct is carried out for that purpose; to display, to *illuminate* — pun intended — the basic laws of physics.

If the goal of the microscopist is to expand his knowledge so that he can identify at a glance anything small enough to fit between the objective and the stage of a microscope, then it would have to be said that the goal of the physicist must be to write a theory of the universe so comprehensive that anything the microscopist sees when he looks through the microscope is contained within that theory as a predictable special case.

We both have some way to go, and it is evident that we need each other's help.

I have now gone on a long time without a picture. That's not fair to microscopists, is it?

So look at Figure 14 (color plate). What is it? I don't know — don't care — but let me tell you how it was made….

[Editor's note: I'm sure you'd like to know how Jack took this picture. His interest in schlieren techniques induced him to copy a normal 2 x 2 transparency with a copy camera modified to give a schlieren image. If you look at the non-shiny side of most color transparencies you will see small surface height variations positioned along image details. The optical discontinuities will scatter the otherwise undeviated beams creating a unique schlieren image.]

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Figure 13. An integrated circuit — triple exposure with the electron microprobe analyzer — showing aluminum (red), silicon (blue) and gold (yellow); original at 50x. Micrograph by John Gavrilovic of McCrone Associates.

Figure 14. A 35 mm transparency copied 1:1 with a schlieren camera.