Laser techniques are capable of removing the fuzziness from photographs. In its most spectacular application, this holographic apparatus improved the resolution of electron micrographs to 2.5 angstroms and revealed, for the first time, the internal, helical structure of a virus.

It can be very frustrating for a photographer to discover that a hard-earned shot is badly blurred, especially when the photographer was an astronaut taking pictures from orbit. Yet all need not be lost on such occasions. A few years ago I started to develop, with my students, a technique that is capable of turning a bad photograph into a good image in a great number of situations. In particular, we deblurred pictures taken by Lovell and Aldrin from the Gemini 12 satellite. Their hand-held Hasselblad camera was accidentally out of focus during a look at the coast of Arabia. Other cases suitable for our method include photos blurred by motion, atmospheric turbulence and instrumental defects. Furthermore we can improve dramatically the resolution of electron microscopes which have instrumental limitations.

Perhaps the most dramatic deblurring results obtained to date are shown in the figures. They illustrate the application of the powerful holographic image deblurring method which we first described in 1967 under the name of “holographic Fourier transform image deblurring method” (Physics Letters, vol 25A, p89). In contrast with ordinary holography, spectacular as it is in its 3-D imaging applications, the holographic image deblurring method requires very considerable photographic care. By means of a new type of “holographic filter” (Physics Letters, vol 33A, p 3) which we developed recently, the holographic image-deblurring arrangement is capable of carrying out the complex image-deblurring computation with a speed and data-capacity unattainable with even the most powerful digital computers, in their present state of development.

A blurred photograph may be “deblurred”, and a sharpened image extracted from it, because the “blurring” in the original photo did not cause an irretrievable loss of the high-resolution imaging information. An observer may assume that the information is lost when looking at an out-of-focus or motion blurred photo. But the sharp image actually is “encoded” in a decodable “convolution integral” form in the blurred photograph. The decoding operation may be readily carried out by means of the optical analogue computing arrangement shown in figure 1. The principles of optical image deblurring are highly mathematical and their details require considerable development beyond the scope of this introductory presentation. The principles may however be briefly sketched out with the aid of diagrams such as that of figure 1.

In many cases the deblurring may result in an almost perfectly “sharp” image. In other cases the blurred photograph is turned into an image which is much more easily interpreted by a human observer. In all cases the decoding “key” is contained in the defective image of a single point. since it is blurred by the same defects as the remainder of the photo; it is this blurred photo of a single point (commonly called the “point spread function” or “spread function” for short) which is used to produce the powerful Fourier-transform division filter, illustrated in figure 1. Details of the production of the holographic filter involve as much art (and technique) as they involve sophisticated theory and mathematical expertise. There are in fact very few examples in the recent history of optics, since the development of the laser, where a paraphrase of Charles H. Townes’s remark about the laser is perhaps more appropriate: the dramatic results recently achieved by means of holographic image deblurring truly form a part of the developments which “epitomize the great change that has recently come over the character of the technological frontiers”. These methods were worked out “almost entirely on the basis of theoretical ideas of a rather complex and abstract nature. These are not inventions or developments which could grow out of a basement workshop, or solely from the Edisonian approach of intuitive trial and error”.

In physical terms, the principle of optical image deblurring may be quite readily compared to electrical signal filter methods, such as those used in high-fidelity sound reproduction equipment. As in acoustical sound “frequency equalisation”, where the amplitude and phase of distorted frequency-components are restored by suitable networks of resistors, capacitors and inductors, we find that the optical image-deblurring procedure consists of suitable action on the spatial-frequency components in the blurred photograph, by means of the optical deblurring filter. The deblurring operation may be intuitively explained with the aid of figure 1. One of the inserts in the blurred photo illustrates the degradation in the image of a radial test-bar chart, when it is badly out of focus. In the original test-bar chart, all the radial spokes (bars) in the fan-like chart had the same intensity, either all black or all white, respectively. First of all, we notice that the overall contrast of the spokes in the out-of-focus images of the chart decreases with increasing closeness of the bars: the “closeness” of the bars is generally measured in terms of the number of white or black bars per linear millimetre at right angles to the bars. The number of bars per mm is called the “spatial frequency”. We thus notice that the overall contrast in the blurred photo of the radial-bar chart decreases with increasing spatial frequency of the bars.

A closer look at the blurred photograph reveals an even more striking phenomenon. The contrast drops completely to zero somewhere about one third from the top. At that spatial frequency the
image of the bars having that frequency is so badly blurred that there is no image at all. However, as we proceed towards still higher frequencies, the contrast gradually improves again, and this may well seem to be encouraging. However, if we closely examine the location of the black maxima in that region, compared to the location of the black maxima in the top third of the chart, we notice that the maxima are shifted by half a period (half an interval between two consecutive black bars). This shift is known as a “phase shift” for the bars having spatial frequencies for that domain (say the spatial frequencies for the middle third of the chart in this case). What this shift means, in terms of a more complicated image, is that regions in the original object with spatial frequency components in the shifted range will have their intensity incorrectly represented in the “blurred” photograph. As a result, regions which should be black may be white; and vice versa.

The importance of this remark must be additionally stressed. It is not readily possible to look at an electron micrograph, for example, which was recorded “blurred” because of unsurmountable instrumental imperfections (aberrations) and make definite conclusions from simple inspection. Some regions may be faithful representations of the generally unknown specimen, but other regions may have their intensity completely reversed! Clearly, erroneous interpretations of the specimens would result under these conditions. That this reversal of intensities really happens is illustrated by the letter “A” in the blurred photo of the word “DIFFRACTION”. The centre of the letter appears “black” where it should have been white!

The image deblurring results shown here may not be obtained by the several methods of photographic (or electronic) “contrast enhancement”, such as high-contrast printing, as one may perhaps be tempted to assume when first exposed to such results. Clearly no “high-contrast printing” alone could conceivably shift the incorrectly located bars back to their correct position (and to equalise the intensity throughout the radial chart). Nor could “high-contrast printing” help in the case of the reversed contrast in the letter “A”: in fact, high-contrast printing would have made the centre of the letter “A” even more “black”, compared to the rest, rather than restoring it to white.

These image “artifacts”, which were of course characteristic of all electron micrographs heretofore, clearly resulted in incorrect structure and image interpretation, in many cases. Even though this may not be readily apparent from a mere inspection of the electron micrograph of the virus of figure 2 (printed with its blurred circle considerably reduced, compared to figure 1 so as to show a correspondingly greater length of the virus) it is characterised by the same inversion of contrasts and shifts in component spatial frequencies as in the blurred photos of figure 1.

The restoration of the intensities in a blurred photograph to their correct contrast and location requires acting on two parameters: the amplitude (i.e. the intensity) and the phase (i.e. the location of the maxima of the different spatial frequency
components). The solution to this problem is provided by the holographic Fourier-transform division filter. It is illuminated by a collimated beam of laser light which passes through the blurred transparency. The filter is located in the focal plane of a lens which follows the blurred transparency. It can be shown that when a transparency consisting of a regular bar grating (say one of the spatial frequency regions in the radial bar chart) is illuminated with the collimated beam of laser light, then a similar grating-like image (spectrum) will appear in the “Fourier plane” where the filter is located. In effect, the lenses perform “Fourier transformations” on the images.

What is remarkable is that the spatial frequency of the spectrum in the Fourier plane is inversely proportional to that in the bar chart. In other words, the regions of the bar chart with coarse spatial frequency (widely spaced bars) will produce a spectrum with very high spatial frequency (closely spaced spectral ‘lines’), and vice versa. Moreover, no matter where in the “blurred” transparency a given spatial frequency component section is located its-spectrum will appear in the same place in the Fourier plane, provided only that the components have the same orientation. If the bar grating is rotated, its spectrum rotates with it. Thus the spectra of all bar components of same spatial frequency and orientation are all superposed in the Fourier plane. It is this fact which permits one to restore all the incorrectly imaged spatial frequency components in the blurred photograph with only one filter in the Fourier plane. It will become clear now also why the filter consists of two components, an amplitude component and a phase component.

The amplitude component, \( |H|^{-1} \), for the case of an out-of-focus photo consists of the ring system shown in figure 1 (it happens to be an Airy disk, with “gaussian” weighting). We can readily imagine that this ring system is formed by rotating a grating-like spectrum about the central axis. We note immediately that the amplitude component of the filter is darkest at the centre (the region corresponding to the lowest spatial frequencies in the out-of-focus test chart), and that it becomes increasingly more transparent away from the centre (the regions corresponding to the highest spatial frequencies in the test chart). Since the blurred photo of the test chart is characterised by a very weak intensity in the high-frequency regions (lower third of chart), compared to the low-frequency regions (upper third of chart), the restoration of correct intensity is performed by the amplitude component of the filter, which greatly enhances the high-frequency components as a result of its great attenuation of the low-frequency components by the dark-ring portion in its central region.

The restoration of the phase (correct location of the frequency components in the bar chart) is equally straightforward. A greatly enlarged section of the phase component, \( e^{-i\phi} \) of the filter is shown in figure 1. It is a part of the filter straddling two of the white rings, near the centre of the amplitude component. Close inspection of the phase component of the filter also reveals a grating-like structure: it is in fact the “carrier” grating of the hologram which forms the phase component of the filter. An even closer look reveals, moreover, that the carrier “fringes” in the holographic grating are displaced in one ring by exactly half a grating interval relative to the adjacent one. It may be shown that this half-interval displacement in the filter is exactly the displacement required to compensate for the half-interval displacement of the bars in the central third of the blurred radial test-chart photo. Similar fringe displacement characterises the corresponding phase shifts in the blurred images.

By far the most important results have been obtained within the last few months when we succeeded in holographically increasing the resolution of electron micrographs obtained by the most powerful electron microscopes available. This improved resolution is beyond the limit attained when the microscope is operating under its normal conditions. The results are shown in Figure 2. Figure 2a shows on the left the best electron micrograph (magnification 25 000x, resolution = 200 angstroms) that can be obtained with the type of commercial instrument used (the specimen shown consists of gold-palladium particles on collodion film). By using the same arrangement as that represented in figure 1, we extracted from the original micrograph the considerably sharpened images on the right: not only is the resolution increased (by a factor in excess of 3, to 70 angstroms), but the edge definition and contrast are also correspondingly increased—in keeping with theory. The arrows point to several details which would have been easily subject to incorrect interpretation in the original micrograph.

The reason why the holographic image sharpening method may be used to increase the resolution so considerably in the electron micrographs may not be immediately obvious. What may be shown is that the images (figure 2, left) which result from scanning with a
double-helical structure clearly revealing a specimen of the fd virus, shown in Figure 2b, of the same fd virus, recorded under similar conditions as that of Figure 2b. It is probably the first photograph of a virus clearly showing a structure resembling the double-helix of the famous DNA molecule. The length of the half period shown is approximately 150 angstroms. Because the virus was probably deformed during preparation, more work is required to confirm its structure. It is most important to note that an increase of resolution by factors of 2 or 3, as demonstrated here, are to be considered as “considerable” indeed, in biological applications of electron microscopy at these very high resolutions. This may perhaps be additionally emphasised with reference to the significance of a corresponding increase in bacteriological work using the visible light microscope in the one thousand magnification range. For instance, chromosome shape studies requiring a magnification (with full resolution) of 1000x are simply not possible with a magnification of only 500x. Similarly, the increase of resolution from say 3A to 1.5A in X-ray crystallography of macromolecules (using the methods of J. C. Kendrew, M. Perutz and co-workers at the MRC Laboratory of Molecular Biology in Cambridge) is a very formidable one indeed. Parenthetically, the work which we describe here now makes it possible to apply electron microscopy to high-resolution studies of amorphous specimens in the one A range. This should be of considerable help in unravelling the many questions which occupy the many researchers working in biology, especially perhaps in the cases where links between “virus” particles and certain forms of cancer have already become increasingly probable. The existence of electron microscopes capable of differentiating between viruses with the certainty and imaging faithfulness contributed by holography should most certainly help in these investigations.

In fact, a further improvement in the scanning electron microscope is made possible by the holographic method. By its ability to compensate for the geometrical aberrations a posteriori, it permits one to use even larger apertures in the recording of the original micrographs, and thus correspondingly to reduce even further the limits set by diffraction. When this is accomplished, new surprising discoveries will most certainly be made.