Digital image processing of confocal images

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In a confocal imaging system, the image is built up point by point and is therefore well suited for image digitization and processing. A confocal system also allows range information to be obtained which facilitates an understanding of the 3D structure of the object. The results of several digital image processing techniques applied to images from a confocal scanning optical microscope are presented.

Keywords: image processing, confocal imaging, algorithm

In order that an image may be processed accurately and effectively, it is necessary to know the properties of image formation of the particular system, i.e. whether the system is coherent or incoherent, the transfer function of the imaging system, etc. In many applications such information is unknown and it is therefore necessary to estimate the imaging properties. At other times the transfer function may be known but so complex that simplifying approximations are applied, e.g. for optical microscopy incoherent imaging may be assumed even though for high resolution the imaging is necessarily partially coherent.

In a confocal imaging system[1], the object is illuminated by focussing a coherent source and the signal is received by a point detector, the back projected image of which is arranged to coincide with the illumination on the object as shown in Figure 1. Confocal imaging has several desirable properties for digital image processing. These include

- purely coherent imaging
- range information of the object may be obtained, i.e. the relative distance of its surface from the observer
- improved resolution over a conventional imaging system using the same aperture and wavelength is attainable

A confocal system, by its very nature, must scan the object in order to produce an image. Thus the image may be digitized very simply.

This paper describes the results of applying simple digital processing algorithms to a confocal scanning optical microscope. In a confocal microscope the object is usually scanned relative to a finely focussed laser beam as shown in Figure 1 rather than vice versa[2]. Because the optical system is nonvarying, such mechanical scanning has the advantages of

- space-invariant imaging, including reduced aberrations since no off-axis imaging is involved
- high signal-to-noise ratios, e.g. signal-to-noise ratios of 1000 are attainable

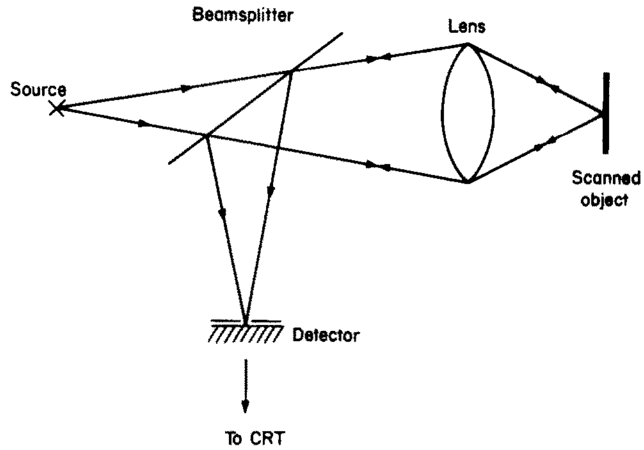

Figure 1. Diagram of a confocal scanning optical microscope

HARDWARE

A block diagram of the digital image processing system is shown in Figure 2. The LSI-11/23 microcomputer controls the mechanical scanning of the microscope and thus the data acquisition rate. The data is then stored in the $512 \times 512 \times 8$-bit framestore for display and processing purposes.

The field of view at such a microscope is approximately $50\ \mu m \times 50\ \mu m$ for a sample spacing of $0.1\ \mu m$. This field of view may, of course, be extended if the sample spacing is increased but at the cost of reduced resolution.

CONFOCAL IMAGE

Figures 3 and 4 are images of an integrated circuit obtained in a conventional and confocal microscope, respectively. It is immediately evident that the confocal image is both sharper and clearer due to the improved resolution of the system.

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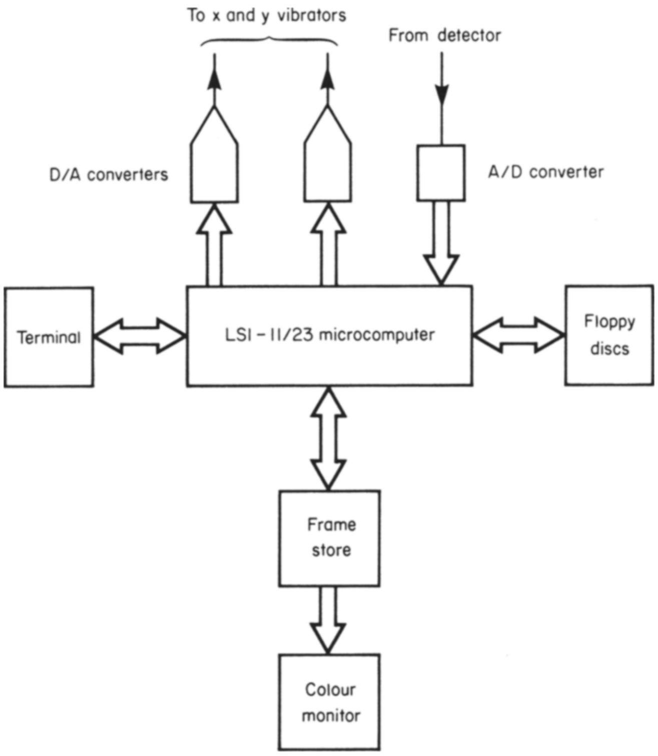

Figure 2. Block diagram of the digital image processing hardware

A further characteristic of the confocal image is to be seen on the aluminium strips. Notice that while the background intensity of the aluminium is constant in the conventional microscope, in the confocal case, the intensity alters at each step in height due to a novel depth discrimination property[3].

The depth discrimination effect is most easily explained with reference to Figure 5. The dashed line represents the imaging of a point in the focal plane while the solid line represents the imaging of an out-of-focus point. It is evident that much of the energy from the out-of-focus

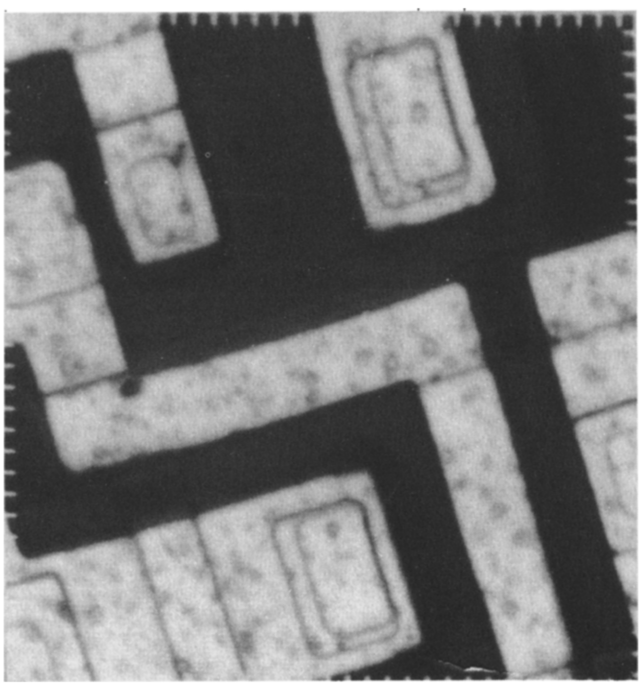

Figure 3. Conventional micrograph of an integrated circuit. Each division represents 4 μm

Figure 4. Confocal image of an integrated circuit

point is not detected and thus the signal strength falls off with distance from the focal plane.

AUTOFOCUS IMAGE

The variation in intensity with height in a confocal image may be advantageous if the object is of constant reflectivity but rough. This is because an image with good contrast will be formed. However, sometimes this contrast mechanism may be found confusing and therefore be regarded as an artefact.

If, at each point, the object is scanned in the axial direction, the focal position is easily recognized as this corresponds to the point of maximum detected signal. A confocal image in which each point is shifted into the focal plane before the intensity measurement is recorded is both simple to achieve and eliminates the depth discrimination effect. Figure 6 shows such an autofocus image. Notice that once again the aluminium strips are of uniform intensity.

CONTRAST AND EDGE ENHANCEMENT

Digital image processing may be used to enhance images. Since the image in a confocal microscope is always in the form of an electronic signal contrast enhancement is

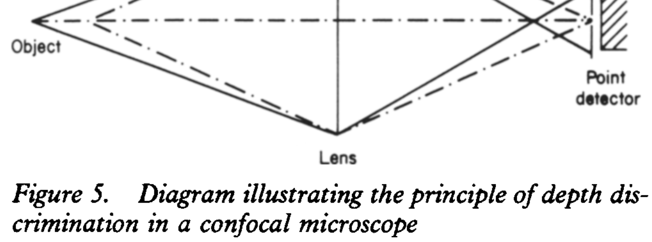

Figure 5. Diagram illustrating the principle of depth discrimination in a confocal microscope

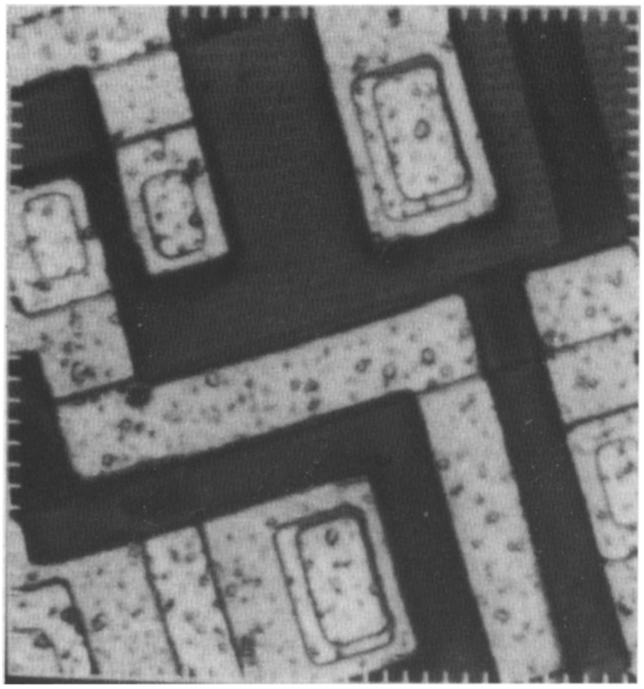

Figure 6. Autofocussed image of an integrated circuit.

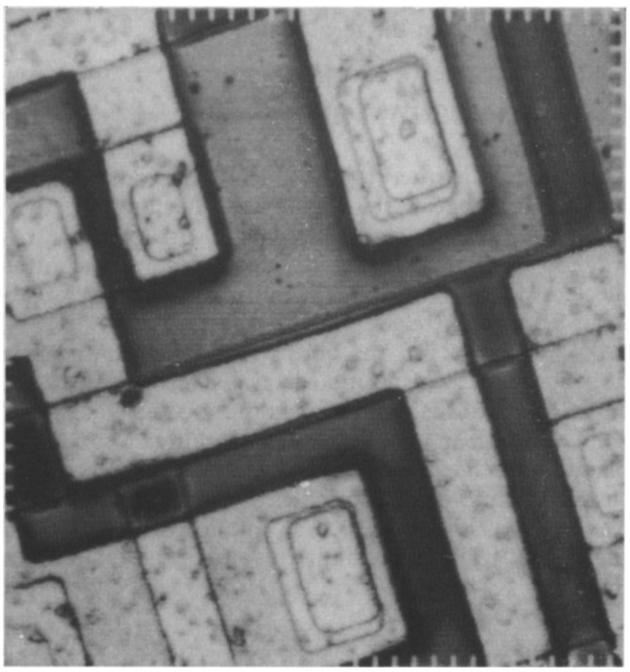

Figure 7. Contrast enhanced autofocus image of an integrated circuit

available even with an analogue system. However, digital image processing allows many more sophisticated contrast enhancement techniques to be applied[4].

Figure 7 shows the autofocus image after contrast enhancement by histogram equalization. A significant increase in perceived detail is evident which is especially striking when it is considered that the original image would normally be considered of high contrast.

Edge sharpening may also be performed on the image in order to enhance its appearance[5]. Figure 8 is an edge-enhanced autofocus image of the integrated circuit obtained by convolving the original image with a 3×3 Laplacian filter given by

$$h(x,y) = \frac{1}{8}\begin{vmatrix} -1 & -1 & -1 \\ -1 & 16 & -1 \\ -1 & -1 & -1 \end{vmatrix}$$

A remarkable improvement in image quality is noticeable between the edge-enhanced autofocus image and the image obtained in a conventional microscope, Figure 3, especially considering the simple nature of the image processing algorithms applied.

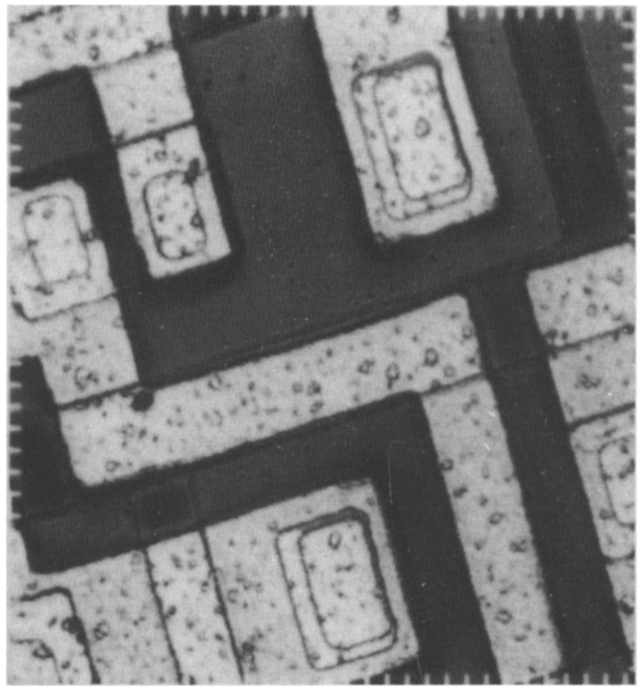

Figure 8. Edge enhanced autofocus image of an integrated circuit

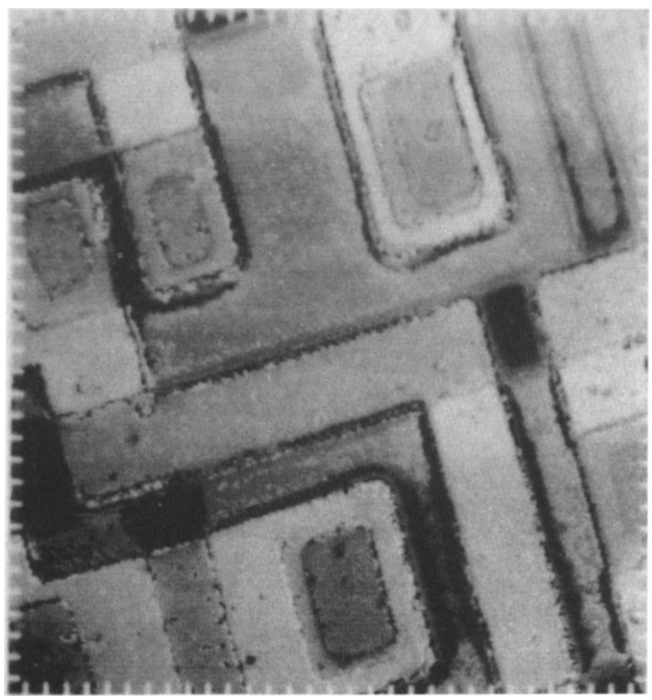

Figure 9. A range image of the integrated circuit. Lighter areas correspond to points closer to the observer

RANGE IMAGE AND STEREO PAIRS

While recording the autofocus image, it is also possible to record simultaneously the relative variations in height of the object in order to display a range image (Figure 9). Here, the lighter a particular point is, the closer it is to the observer. Such an image in association with the autofocus image allows the observer to acquire an understanding of the 3D structure of the object which would not be possible otherwise.

The data available in the autofocus and range images allow a stereoscopic image pair to be generated by computer. The theory behind such an image pair is relatively simple. Figure 10 illustrates the image forming process from which it is easily shown that the x-coordinates for the left and right eyes, x_1, x_r, respectively are given by

$$x_1 = x_s + \frac{DS}{2z}$$

$$x_r = x_s - \frac{DS}{2z}$$

and

$$y_1 = y_r$$

where the stereoscopic pair is positioned a distance D in front of the viewer's eyes, S is the interocular distance, z is the distance of the point from the observer and x_s, and y_s are the x and y coordinates for monocular viewing. Figure 11 shows a stereoscopic image pair, which may be viewed using conventional techniques.

It should be noted that in a confocal system the digital processing required to examine the 3D qualities of an object is considerably less than in a conventional system where autofocussing is more complex and image processing must be applied in order to remove the blurred out-of-focus planes at a specimen[6].

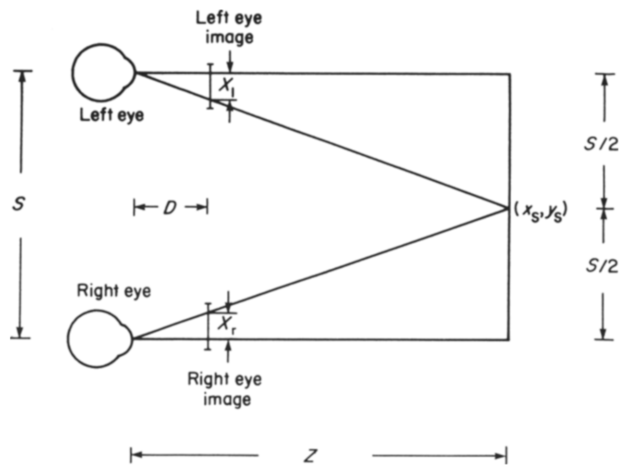

Figure 10. Stereo image forming process

CONCLUSIONS

This paper has described the results of applying simple digital image processing techniques to confocal optical microscope images. It has been shown that the imaging properties of such a confocal system are particularly well suited for digital processing.

Confocal imaging in association with digital imaging processing techniques such as autofocussing and contrast and edge enhancement provide images of a superior quality to that of a conventional microscope. Further, such a system allows the 3D structure of the object to be examined. Finally it should be noted that the techniques applied in this paper are applicable to a wide range of objects and other types of confocal imaging systems.

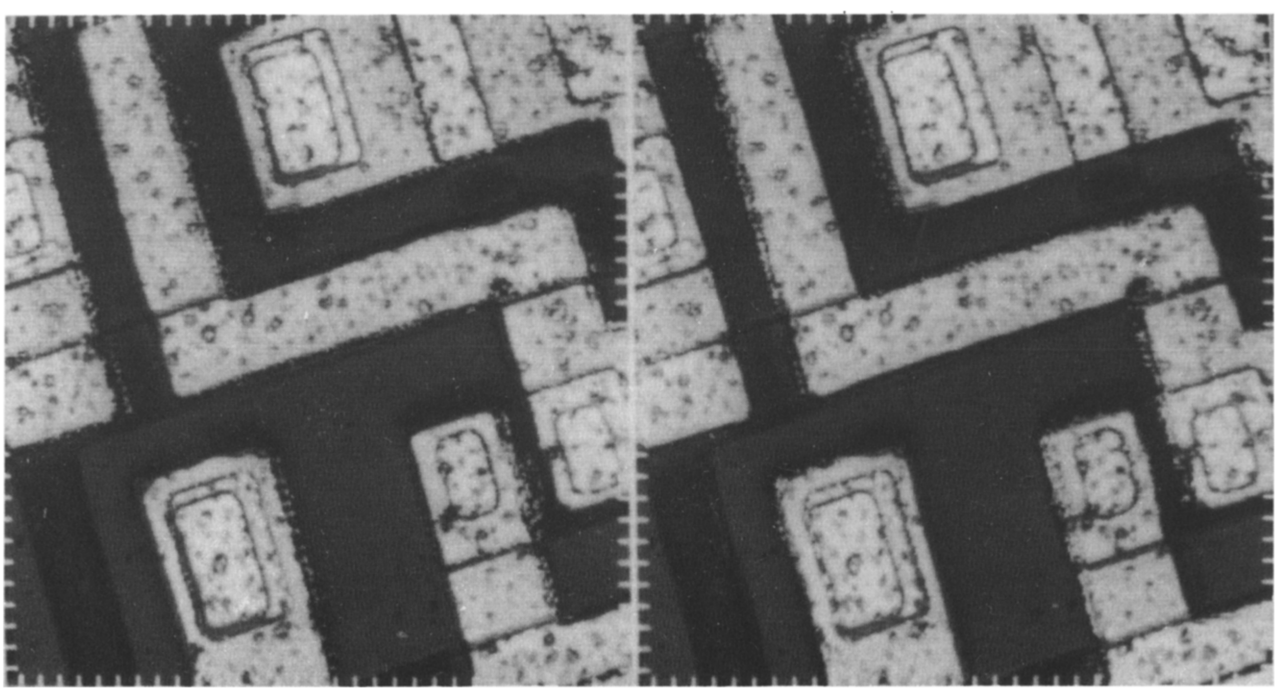

Figure 11. Stereo image pair of integrated circuit

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