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A REAL TIME CONFOCAL MICROSCOPE

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Abstract. A confocal microscope with real time imaging capability has been developed. The success of this system is due to the effective application of an acousto-optical device (AOD) and a one-dimensional CCD image sensor.

The AOD is ideally suited for real time scanning applications, however the light reflected back to the AOD is of low efficiency and difficult to process. This new system utilizes a 1-D CCD so as to eliminate the necessity for light to pass through the AOD a second time. The use of a non-distorting CCD also increases the geometrical precision of the imaging system. The high speed scanning by the AOD enables confocal images to be obtained at a rate of several hundred times X faster than with the standard confocal microscope. With such a system it is possible to collect a series of images of in focus layers and combine them into a single three-dimensional image within seconds.

INTRODUCTION

In conventional high magnification microscope imaging design, the device consists of an imaging tube positioned on the real image side of the microscope. Such a system, while easy to construct, has many drawbacks including

(1) Low resolution
(2) Inferior color separation
(3) Geometric distortion
(4) Persistent image that makes the system unsuitable for real-time image processing.

By replacing the imaging tube with a two-dimensional CCD, problem (3) and (4) can be corrected. Never-the-less the limitation on the number of CCD elements that can be used leads to a lower resolution than that obtainable with either the imaging tube or photographic film. So the imaging device may impose limitations on the resolution. In addition the conventional microscope also has a low depth of focus at high magnification.

The confocal optical microscope offers greatly improved imaging properties over the conventional microscope. A confocal system will show only the in-focus portions of an image. Optical sectioning is therefore very easy to accomplish and it is

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possible to integrate sequential layered sections to obtain 3-D information.

Most confocal optic systems, however, cannot acquire a single image very quickly. To accumulate the necessary number of layers required for a 3-D image takes a considerable amount of time. The reason for this delay is that a fast and accurate scanning system is technically difficult to implement. We, however, have devised a solution to this problem and have successfully developed a real-time confocal microscope which is capable of obtaining a 1000×525 pixel image in 33.3 msec.

SCANNING SYSTEM

In addition to our scanning system there exist two other implementations for achieving a real-time confocal system that have been announced in publications. These microscopes either use an acousto-optical device (AOD) [1] through which both transmitted and reflected pass, or a Nipkow disk. [2] Advantages associated with these systems include high speed (real-time) scanning, purely confocal optics, and in the case of the Nipkow disk, observation is possible with the naked eye. Disadvantages include low image intensity, and possible geometric distortion. The linearity of the scanning and detection system largely determines the geometrical accuracy of the system. In most 7 applications involving an AOD there is some resulting geometrical distortion, especially along the horizontal axis. This effect is not negligible and has been fully documented in a published study by Hitachi. [3]

Our real time confocal system utilizes an AOD but in such a way that geometric distortion is almost non-existent. The key to this method is that the AOD is used only for illumination purposes while the detection system makes use of a one-dimensional CCD image sensor. This system is shown below in Fig. 0.

In our system the horizontal scanning is performed by an AO element, but the geometric dimensions in the horizontal direction are determined by the cell size of the CCD. Specifically, in horizontal scanning, the scanning by the illuminating light is synchronized with but independent of the reading of the image. This is because the optical data accumulated during the horizontal scanning period by the PIN photo diode array is loaded on the CCD transfer section instantaneously; in accordance with the read clock, pixel data are sequentially transmitted. As a result, the horizontal dimensional accuracy of the image, except for the distortion of the objective lens, is determined by the dimensional accuracy of the light-receiving window of the CCD image sensor and the accuracy of the read clock. Generally, these two factors are much more precise than the distortion of the objective lens, so no substantial distortion is produced by scanning.

In summary, the principal advantages of our scanning system are:

1) real-time scanning
2) use of a 1-D CCD allows the scanning system to be free from geometric distortion
3) because the reflected light does not pass through the AOD a second time chromatic aberration is minimized
4) the scanning system contains no large moving parts and is vibration free
5) imaging is without unwanted scattered light effects

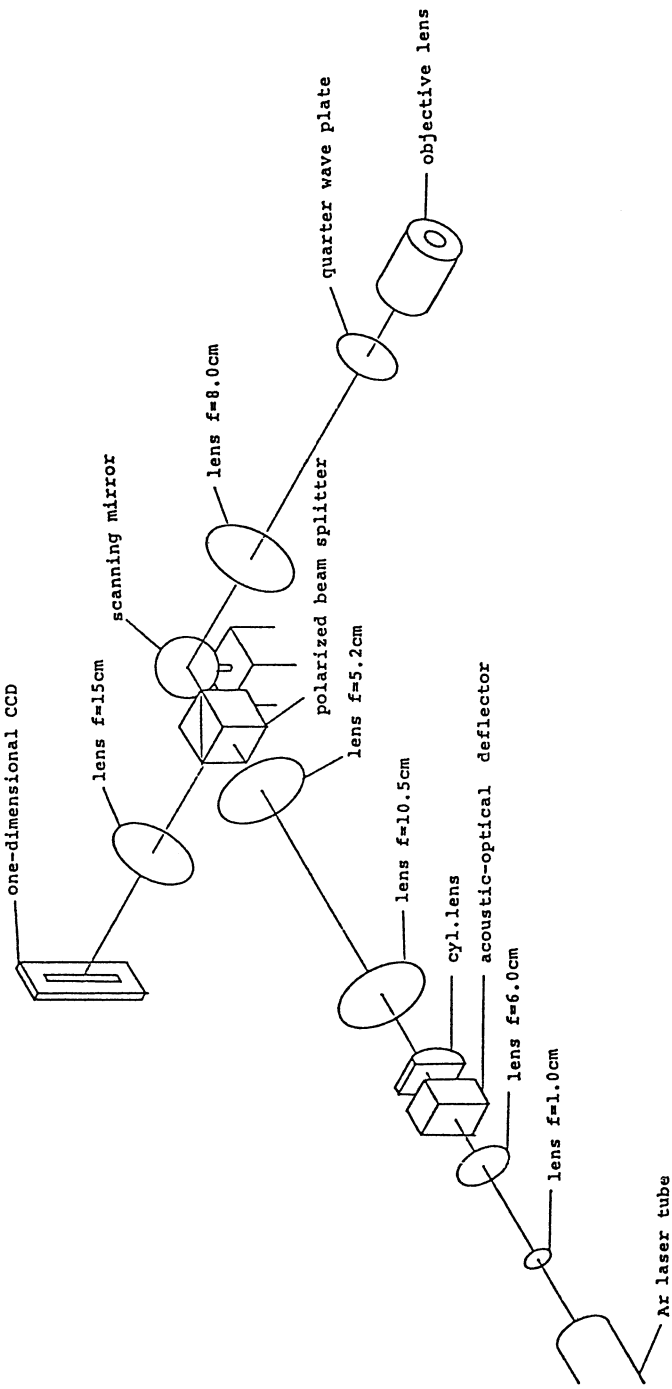


Fig. 0. Horizontal and vertical scanning system.

6) the optical system maintains its confocal characteristics

FOCUS SCAN MEMORY

One of the most practical applications of confocal optics has been to a system which we call the Focus Scan Memory (FSM). In this system the sample under observation is scanned along the *Z*-axis (Fig. 1).

During the scan points are collected as they come into focus and are stored in a video memory. When the process is finished a completely focused image with limitless focal depth is obtained.

Figure 2 shows an example of confocal optics with an in focus sample. Most of the light reflected from the sample's surface passes through the pinhole to the detector.

On the other hand, Figures 3 and 4 show samples which lie out of the plane of focus.

Notice that in both cases the amount of light reaching the detector is significantly reduced. The important property being demonstrated is that with

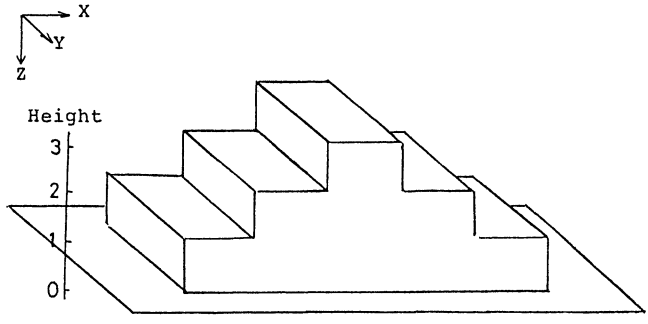


Fig. 1. Conceptual view of *Z*-axis scan.

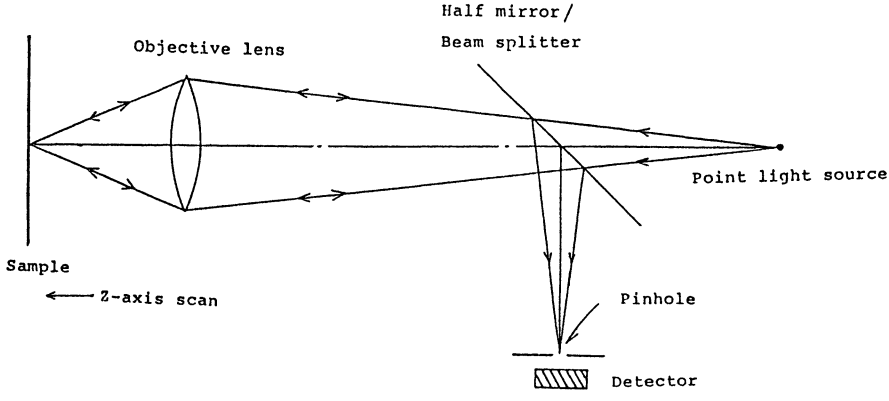


Fig. 2. Reflection type confocal optic.

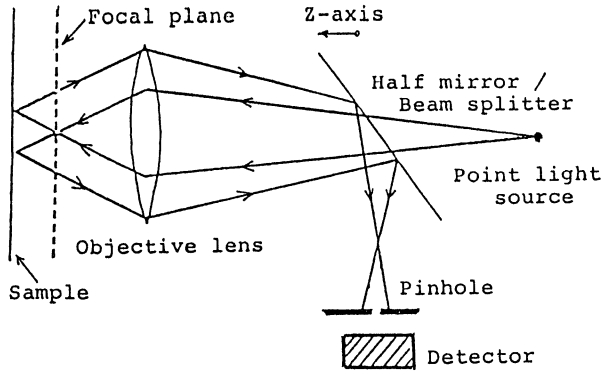


Fig. 3. Out of focus. (Sample behind the focal plane)

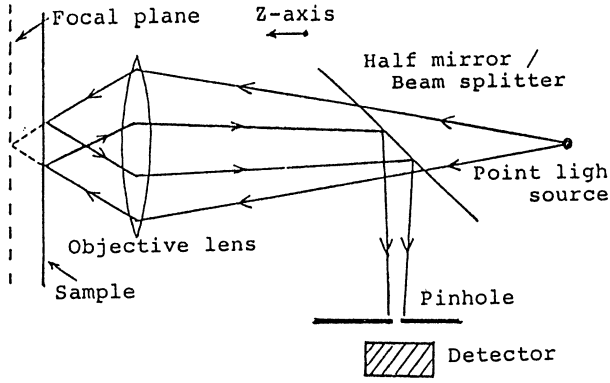


Fig. 4. Out of focus. (Sample in front of the focal plane)

confocal optics only when a point is in focus does it appear with maximum brightness.

The relationship between the intensity of light reaching the detector (I) and image resolution (R) versus Z, the distance separating the objective lens and the sample, is graphed in Figs. 5 and 6 for the confocal and ordinary microscopes respectively.

With the confocal microscope it can clearly be seen that resolution and brightness both peak sharply at the same point. In other words one only has to detect the peak intensity to determine when a point is in focus. This method would be difficult, if not impossible, for the ordinary microscope because the intensity remains relatively constant. With the ordinary microscope only the resolution, the ability to distinguish between two or more points, can be used to determine if an image is in focus. Thus if a single point image from an ordinary microscope were to be observed, it would be impossible to judge if that point were in focus. With a confocal microscope single point focusing is possible.

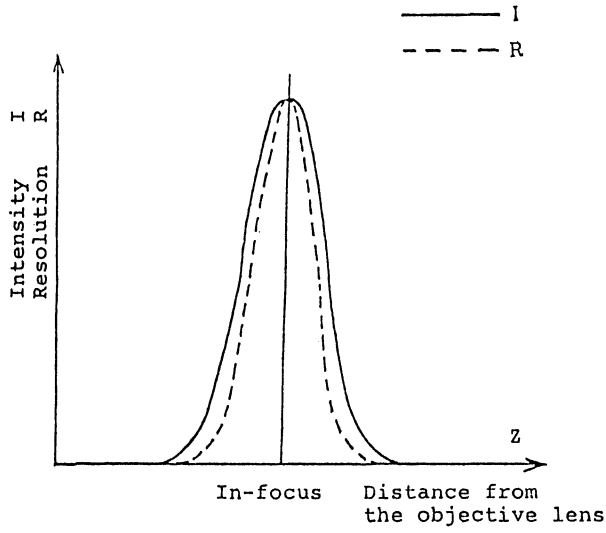


Fig. 5. Relationship between the intensity of light reaching the detector and resolution. (Confocal)

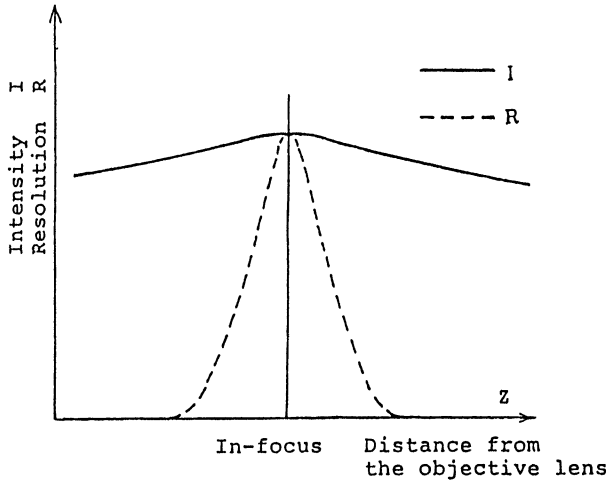


Fig. 6. Relationship between the intensity of light reaching the detector and resolution. (Ordinary)

A functional block diagram of the FSM system is shown in Fig. 7. Figure 8 shows conceptually the contents of the FSM at different stages in the collection process.

With FSM it is possible for a laser microscope to obtain a very deep depth of focus, similar to that of a scanning electron microscope (SEM). Figure 9 shows an actual image obtained from a Lasertech laser microscope with FSM.

See appendix I for further examples.

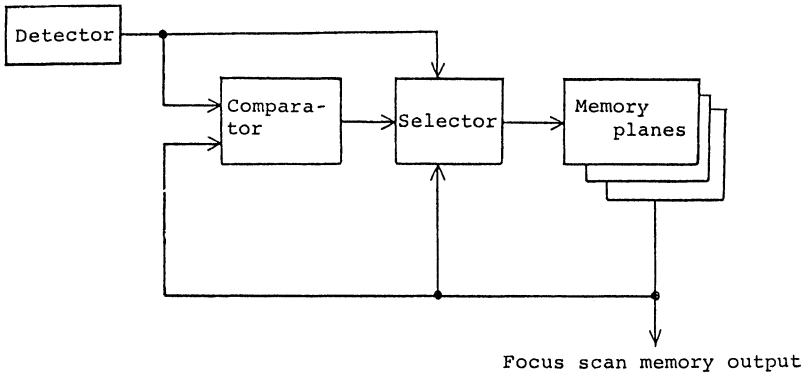


Fig. 7. Focus scan memory block diagram.

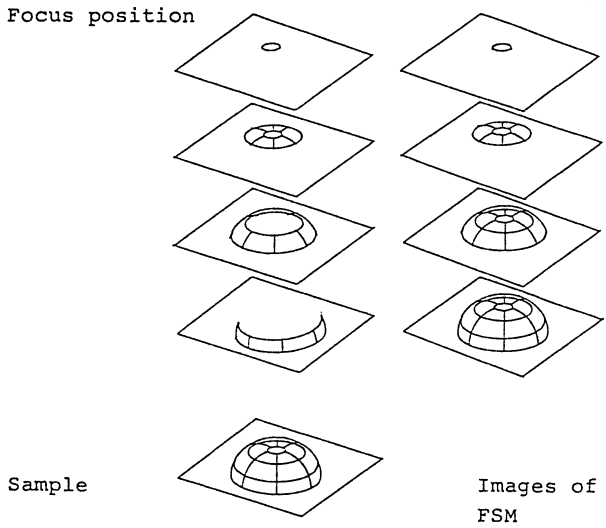


Fig. 8. Conceptual view of Focus Scan Memory.

SURFACE PROFILE MEASUREMENT SYSTEM

A surface profile measurement system based upon the same optical principles as the FSM has been developed. When data is stored in the FSM, the *Z*-axis coordinates of the in-focus data are simultaneously saved in a separate surface profile measurement system. A block diagram is shown in Fig. 10.

The stored coordinates can be plotted to yield a highly accurate surface profile (see Fig. 11).

There are many advantages associated with this method of surface measurement including:

1. non-contact

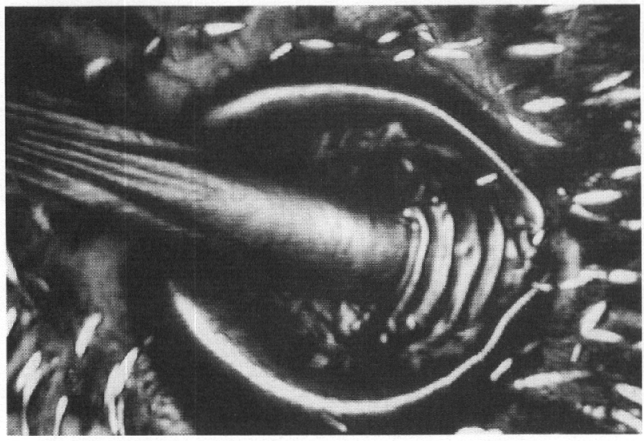


Fig. 9. Photograph of a fly's antenna from a Lasertech Focus Scan Memory Equipped laser microscope.

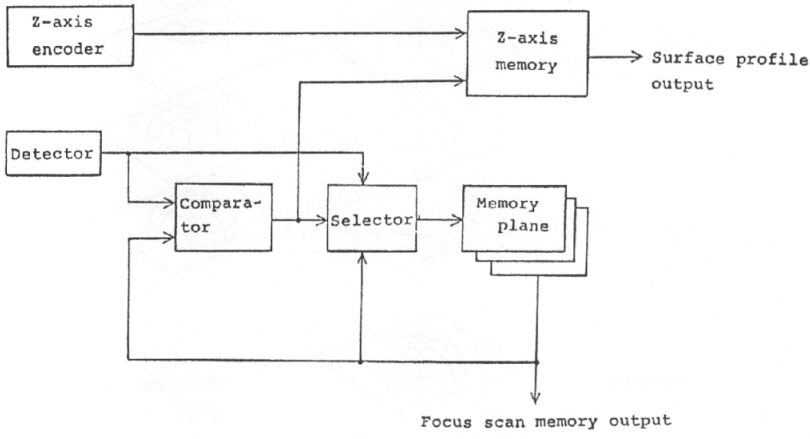


Fig. 10. Surface profile measurement system block diagram.

2. height information of point size data can be obtained
3. requires little time when compared to other surface profile measuring methods.

CRITICAL DIMENSION MEASUREMENT SYSTEM

For the measurement of critical dimensions (CD), use of the FSM provides a significant advantage. Figure 12 shows a sample and specifies three different planes in which measurements were taken. The corresponding video signals are shown in Fig. 13.

Because focal planes (a) and (c) do not lie in the best possible position the

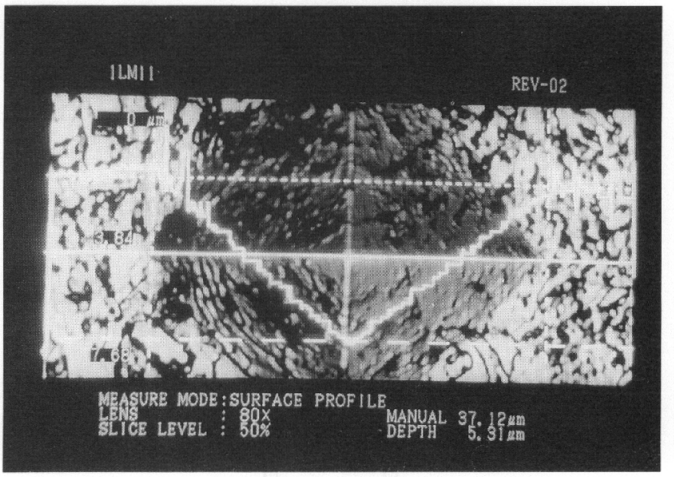


Fig. 11. Sample of hardness tester with superimposed surface profile waveform.

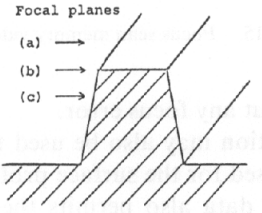


Fig. 12. Focal planes and a sample.

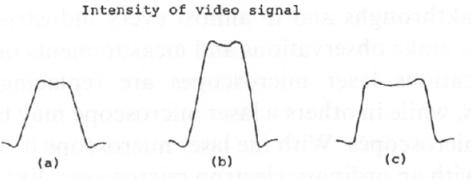


Fig. 13. Corresponding video signals of Fig. 12.

sample's image appears dark and the video signal is weak and inaccurate. Even with using a confocal system it can be difficult to determine the best possible plane of focus. The focusing error of (a) and (c) is a common problem in CD measurement systems. However, through the use of the FSM it is possible to eliminate this type of error.

Figure 14 shows the image obtained from the focus scan memory, and Figure 15 is the video signal.

Notice that because the light reflected by the steep edges does not reach the detector, they are represented as dark lines. Line width may be measured from the

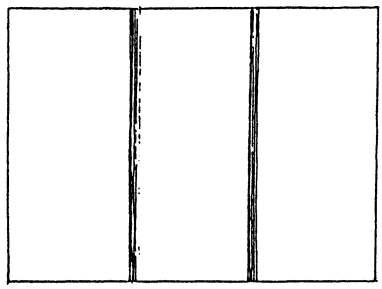


Fig. 14. Focus scan memory video image.

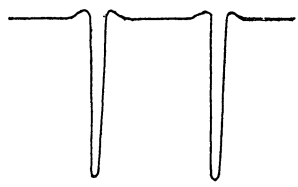


Fig. 15. Focus scan memory video signal.

video signal of Fig. 15 without any focus error.

Surface profile information may also be used to measure critical dimensions. Since only in-focus data is used for the surface profile, this method also eliminates focus error. Surface profile data also permits the measurement of objects with irregular line widths as shown in Fig. 16.

The scanning laser microscope has a history of over ten years but only recently has it begun finding a wide variety of practical uses. In recent years laser technology has made many breakthroughs and in almost every industrial field it is becoming essential to be able to make observations and measurements on the submicron level.

In some applications laser microscopes are replacing both ordinary and electron microscopes, while in others a laser microscope may be used in conjunction with other types of microscopes. With the laser microscope it is possible to see things that cannot be seen with an ordinary electron microscope. Examples include the fine wrinkles created during the cutting of high polymer films, the surface profile of powdered milk, an ion inplanted semiconductor wafer, and the ion inplant pattern in a bubble memory. In the future these types of applications will be further

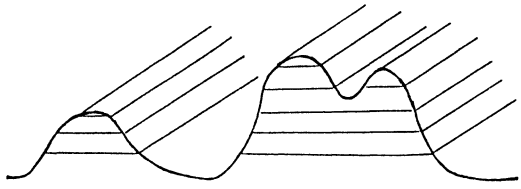
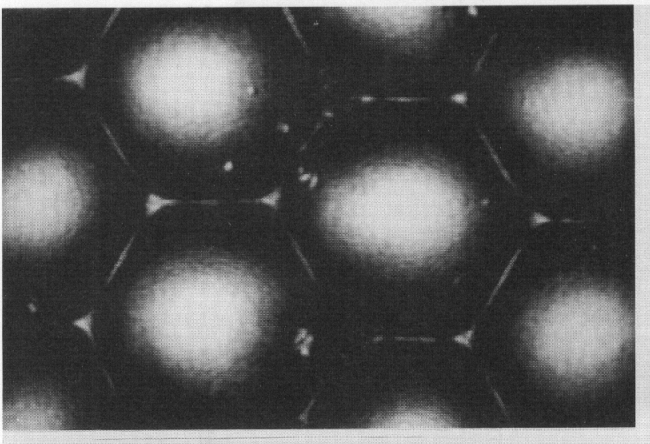


Fig. 16. Irregular shaped objects with varying widths.

expanded upon and developed. The laser microscope is destined to become a familiar tool in investigating the world of the micron.

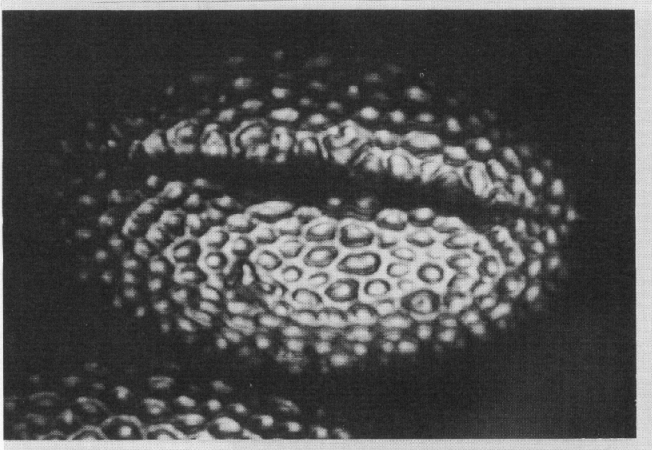
Appendix I: Focus Scan Memory



Fly's eye

Objective lens:100X

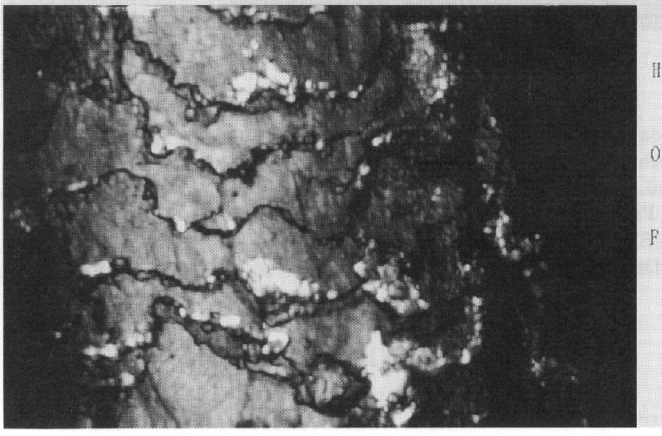
Fig.A



Pollen

Objective lens:100X

Fig.B

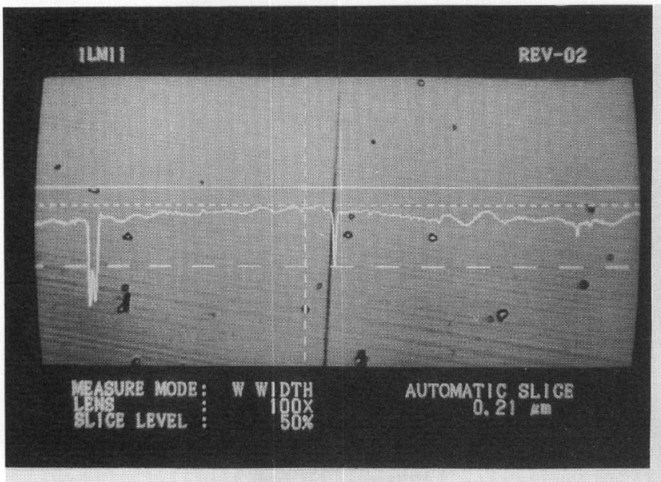


Human hair

Objective lens:100X

Fig.C

Appendix II: Critical Dimension and Surface Profile Measurement

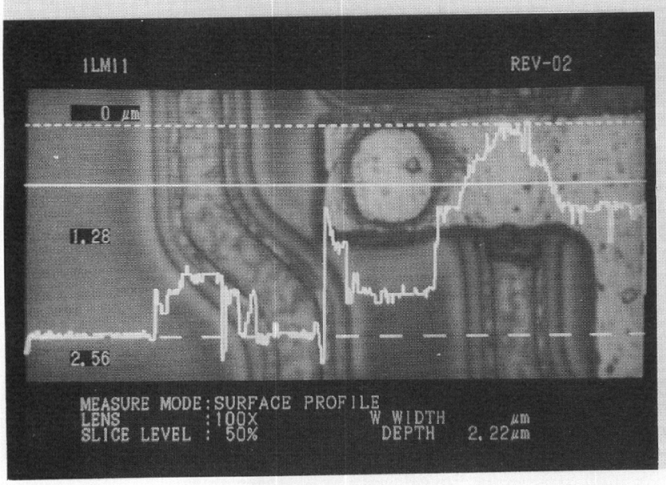


VHS magnetic tape head

Headgap = 0.21 μm

Objective lens:100X

Fig.D



LSI Wafer

Objective lens:100X

Fig.E

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1. Draaijer, A. and Houpt, P. M., "A real-time confocal laser scanning microscope (CLSM)," Scanning Imaging Technology, Proc. SPIE 809, 85–88, (1987).
2. Xiao, G. Q. and Kino, G. S., "A Real Time Confocal Scanning Optical Microscope," Scanning Imaging Technology, Proc. SPIE 809, 107–113, (1987).
3. Hosada, S. and Seya, E., Harada, T., and Takanashi, A., "High Speed Laser Beam Scanning Using an Acousto-Optical Deflector (AOD)," Japanese Journal of Applied Physics, Vol. 26, No. 7, 1026–1030, (1987).

DISCUSSION

Q. Does your microscope also work with fluorescence?

What do you expect the speed of the microscope to be in fluorescence?
(Brakenhoff, G. J.)

A. Our microscope does work with a fluorescent light source. However, fluorescent light is much weaker than normal reflected light. High speed fluorescent imaging depends upon the following:
1) development of a high efficiency fluorescence stain
2) use of a high power exciting light source
(should be as strong as possible without causing damage)
3) use of a high N. A. objective lens
4) development of a high S/N ratio detector

Comments on the above:
1) I am expecting the development of a new stain
2)–3) have limitations
3) will be our main project

The CCD is a device that can be a very low noise light detector when it is cooled to a temperature of -30 to 50°C. Doing so should enable us to obtain faster fluorescence imaging.

Q. To what extent did you have to sacrifice spatial resolution in order to obtain the high time resolution? What is the spatial resolution in your system?
(Kinosita, K.)

A. I am assuming that your phase "high time resolution" refers to the real time imaging property of our system.

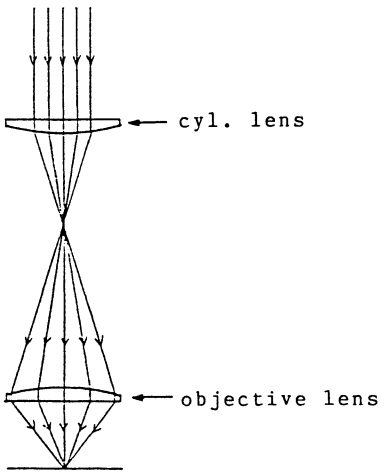
The resolution in the x and y directions do originally differ slightly. But by simple electrical enhancement of the x-direction signal, as in our system, the resolution in the x and y directions becomes almost the same. We have verified this by testing with the Siemens star chart.
The resolution of our system is 0.25 μm for both the x and y directions. This value was observed from an EB exposed semiconductor pattern.

Q1. Have you used synchronization of the CCD detector to obtain confocal performance in both x and y directions?
Q2. It is possible to obtain confocal performance in one direction only using illumination of a whole line of the object at a time. In this way you do not need an AOD, but need only scan in one direction. Have you tried this?
Q3. We have observed that when using a confocal system with a slit in front of the detector we see "streakiness" in the image when we defocus. Have you observed this? (Sheppard, C.)

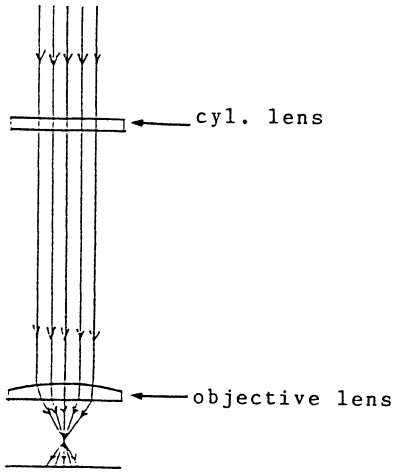
A1. Yes, there is confocal performance in both x and y directions. When using the OBIC imaging function, the CCD detector and laser beam must be synchro-

nized. If they and not, the superimposed reflected and OBIC images would not be dimensionally accurate.

A2. I have tried this method and have found two problems,
(a) Speckle noise appeared on the image.
(b) The resolution in the linear direction (CCD direction) decreased. The reason for (b) can be explained by the following diagrams.
A cylindrical lens is usually used to create one dimensional illumination.

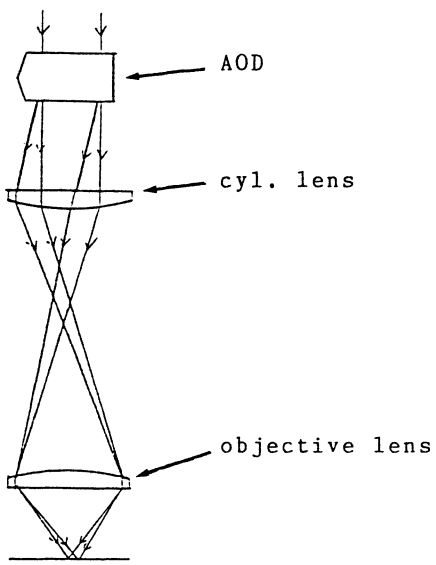


view: X
illuminating light has
enough N.A.



view: Y
illuminating light has
poor N.A.

Our method which utilizes an AOD is shown below.



view: Y
In this case the illuminating light has enough N.A.

A3. There has been negligible streakiness observed upon occasion.
The streakiness can be reduced by changing the slit size as appropriate.