Available online at www.sciencedirect.com
SCIENCE DIRECT®
Radiation Measurements 40 (2005) 311–315
Radiation Measurements
www.elsevier.com/locate/radmeas

Development of a high speed imaging microscope and new software for nuclear track detector analysis

N. Yasuda[a,*], K. Namiki[b], Y. Honma[c], Y. Umeshima[c], Y. Marumo[c], H. Ishii[d], E.R. Benton[e]

[a] *National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage, Chiba 263-8555, Japan*
[b] *Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan*
[c] *SEIKO Precision Inc., 1-1-1 Akanehama, Narashino, Chiba 275-8558, Japan*
[d] *K.I. Technology Inc., 19-1 Chigasaki-Chuuou, Tsuduki-ku, Yokohama, Kanagawa 224-0032, Japan*
[e] *Eril Research Inc., P.O. Box 150788, San Rafael, CA 94915-788, USA*

Received 27 August 2004; accepted 7 February 2005

Abstract

Automated digital imaging optical microscopy is widely used for diagnostic applications in the health care and biology fields and for routine inspection in industrial applications such as semiconductor fabrication. These applications require the imaging of large areas at high speed in order to obtain sufficient data for image processing with good statistics. Track detector analysis also benefits from the rapid acquisition of large areas on the detector surface. We have developed a new microscope system, the HSP-1000, for high speed image acquisition that uses a line sensor camera in place of a traditional CCD camera. Continuous, automatic focusing of the microscope is achieved by means of an optical pick-up system that provides fast feedback for control of distance between the objective and the image surface. Using transmitted light illumination, the microscope is able to digitize a 1 cm^2 area at 0.35 μm/pixel resolution in ∼ 20 s. As a result of continuous stage motion and continuous focusing, we have attained image acquisition speeds that are 50–100 times faster than conventional CCD-based microscope systems. In this paper, we describe a number of aspects of the microscope system including the use of the line sensor and the automatic focus system.
© 2005 Elsevier Ltd. All rights reserved.

Keywords: Line sensor; Microscope; Track detection; High speed imaging

1. Introduction

In recent years, automated digital imaging in optical microscopy has achieved widespread application not only for the analysis of nuclear track detectors (Trakowski et al., 1984; Ashaboglu et al., 1989; Price and Krischer, 1985; Fews, 1992; Espinosa et al., 1996; Dolleiser and Hasemi-Nezhad, 2002; Weaver and Westphal, 2002; Boukhair et al., 2000), but for a number of different fields (Inoue and Spring, 1997). Most often, an automated optical microscope system is equipped with a charge couple device (CCD) camera, a computer controlled stage and an autofocus drive. In such systems, the size of each image is limited by the area of CCD element and, although dependent on total magnification, typical image sizes are limited to several hundred micrometers square. In order to digitize substantially larger areas, the total image must be reconstructed out of a composite of many smaller images captured individually by the CCD camera. This method, often referred to as "image tiling," is illustrated on the left in Fig. 1. A major limitation of this method is the time consumed by the

* Corresponding author. Tel.: +81 43 206 3239; fax: +81 43 251 4531.
E-mail address: nyasuda@nirs.go.jp (N. Yasuda).

1350-4487/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.radmeas.2005.02.013

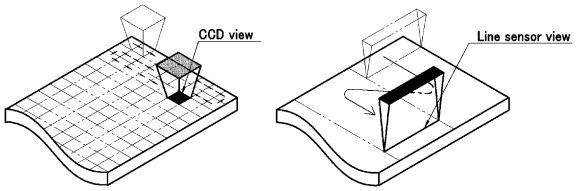

Fig. 1. Difference of imaging method using line sensor (left) and traditional imaging method (right).

mechanical movement of the stage, followed by autofocusing, between the capture of each individual image (Dolleiser and Hasemi-Nezhad, 2002).

In this paper, we describe the HSP-1000, a new microscope system for capturing large images ($> 1\,cm^2$) in relatively short periods of time (< 1 min). To achieve this high rate of image acquisition, the HSP-1000 microscope system makes use of a line sensor in place of the traditionally-used CCD camera and the microscope stage is constantly in motion. In this way, the microscope objective sweeps over the sample, continuously acquiring the image in much the same way as a desktop scanner or fax machine. As illustrated on the right in Fig. 1, the use of a line sensor permits not only the continuous acquisition of image data, but the image can have considerably greater width than that typically acquired using a CCD camera. A complete image of the entire sample surface can then be reconstructed from a relatively small number of long strips, rather than the much larger number of discrete square CCD images. In addition to its use in the analysis of nuclear track detectors, such a microscope system has many potential applications, especially in medicine, biology, high technology industry, or wherever large-scale acquisition of microscopic images is required.

2. Description of the microscope system

2.1. Overview

A dedicated controller is used to coordinate the various subsystems of the microscope (illumination, autofocus, stage movement and image capture) and to interface these subsystems with a personal computer. The microscope has light sources for both transmitted and incident light illumination. Image capture is achieved through use of an optical line sensor at the top of the microscope. For the monochrome system, the resolution is 256 greyscale values. A CCD camera is also included in the system, but only for real time monitoring of the image plane by a human operator during sample set-up. The linear motorized stage (X and Y movement) is mounted on a tilting table (Z movement and inclination correction of the sample surface). The autofocus unit uses an optical pick-up system consisting of a diode laser and photodiode array to detect the sample surface. This is the same type of system used for the reading of data from Compact Disks. The autofocus system controls the distance between the image plane and the objective, via feedback control, by continuous adjustment of the tilting table during image capture by the line sensor.

2.2. Image digitization

Currently the HSP-1000 microscope system uses a monochrome line sensor to acquire the image at a line rate of 32.6 kHz. The line sensor has a sensitive area of 4096×1 pixels and a resolution of $7\,\mu m^2$ per pixel. At a total magnification of $200\times$, each pixel corresponds to a $0.35\,\mu m \times 0.35\,\mu m$ field of view.

For image acquisition, we use a KIT1060 CLCB CameraLink™ capture board (K.I. Technology, Inc.) which is able to acquire image data at a rate of 320 MByte/s. The board has two 512 MByte memory buffers and a dedicated FPGA (Field Programmable Gate Array) for image processing. Image data is saved to the memory buffers before being permanently stored on the hard disk of the PC. Optionally, the user can use the FPGA for on-the-fly image processing, such as shading correction, noise reduction, or other simple image analysis.

2.3. Motorized stage

The HSP-1000 microscope uses a linear motor type stage ($120\,mm \times 120\,mm$, $0.1\,\mu m$ resolution) with linear scales as feedback encoders for X and Y movement. The stage is mounted on a tilting table equipped with three shafts that are independently controlled in the Z direction by three $0.25\,\mu m$ resolution ultrasonic stepping motors. The tilting table is used to correct the inclination of sample being imaged and, via the autofocus system, the focal distance between the microscope objective and the image plane.

2.4. Autofocus

The HSP-1000 microscope's autofocus system, illustrated in Fig. 2, uses the same type of optical pick-up mechanism used in compact disc (CD) and magneto-optical disc (MO) drives. The beam from a diode laser (780 nm) passes through a half mirror to the sample surface. The laser spot is located at the center of line sensor's field of view and provides information on the distance to the image plane as follows: (1) the reflected beam passes through a cylindrical lens and reaches a photodiode array, (2) a cylindrical lens slightly alters the horizontal and vertical focal distances of the resulting spot on the photodiode array. The laser spot on the photodiode array is perfectly circular only when the sample is positioned correctly. If the microscope objective is too close or too far from the image plane, the laser spot will be either elliptical or elongated. The laser spot will be elongated along a 45° axis when the image plane is too close and along a 135° axis when the image plane is too far away.

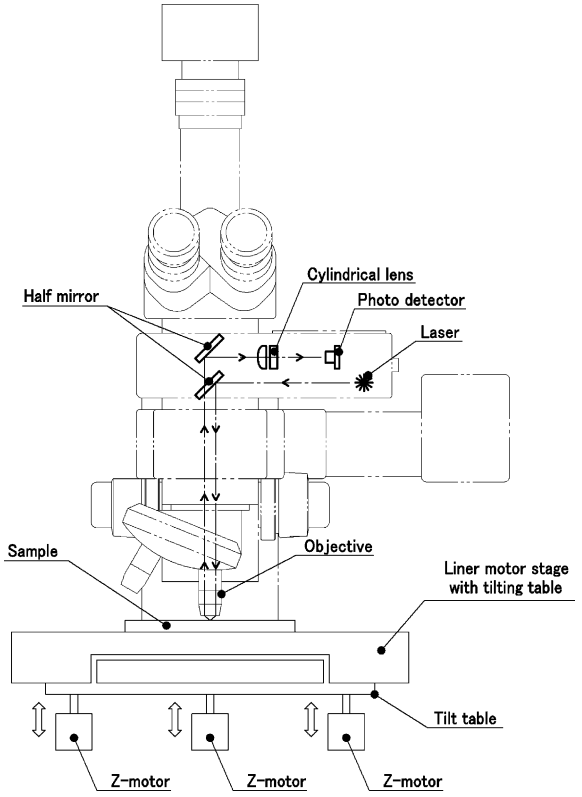

Fig. 2. Principle of surface detection for auto focusing using an optical pick-up.

The central part of the photodiode array is divided into 4 equal quadrants labelled A, B, C and D. Light intensities of each photodiode are converted into the digital values, then used as feedback signals for the autofocus (*Z*-movement). Focus is perfect when the signal, $AF = [(A + C) - (B + D)]/[A + B + C + D] = 0$. *Z*-axis control is performed independently from the *X* and *Y* stage movement. The digitized (A/D) signal from the autofocus (AF) unit is acquired in 65 μs cycles. During the movement of the stage, AF information is acquired following a trigger signal from the computer every 200 μm of movement along the *X*-axis. The digitized AF signal is then converted to height information and used as feedback to the ultrasonic *Z*-axis motors.

3. Results and discussion

3.1. Autofocus

In order to verify the autofocus performance, motion of the *XY* stage was fixed, and the change in value of the autofocus signal $AF = [(A + C) - (B + D)]/[A + B + C + D]$ was measured as a function of *Z* height (in 0.25 μm steps). The AF values, plotted as a function of *Z* height relative to the image plane, are shown in Fig. 3. The AF value provides information concerning both the direction and distance that the *Z*-axis motor must move to achieve sharp focus.

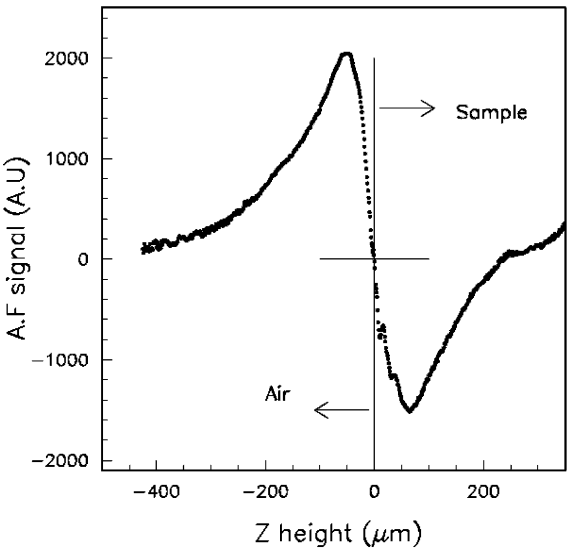

Fig. 3. Autofocus signal as a function of *Z*-height measured for the HSP-1000 microscope system.

The method of continuous focusing is responsible for some of the speed increase in image acquisition achieved by the HSP-1000 system. By contrast, other autofocus systems often employ a contrast detection method that requires capture of multiple images at a range of heights above and below the image plane and can take from several hundred msec to 1 s to properly readjust the focus.

The depth of focus, *d*, calculated using the relation $d = \lambda/2(NA)^2$ was found to be about 2 μm, using a 20× objective lens (NA = 0.45) and the 780 nm diode laser. In Fig. 3, the distance from the peak in air to the peak below the surface of the sample was measured to be 110 μm. When divided into 3500 digitized values (the maximum range of the A/D converter is 12 bits or 4096 values), the resolution of the height measurement is estimated to be 0.03 μm. This is a sufficient resolution given the depth of focus (∼ 2 μm) and the step size (0.25 μm) of the *Z*-motor.

3.2. Imaging speed

There is a close relationship between imaging speed and the intensity of the microscope light source due to the limitation in gain in the optical line sensor. This made necessary to use a relatively bright light source in order to obtain high speed image acquisition. We have verified the imaging speed using the upper surface of a glass microscope slide as a reference under the conditions of both transmitted and incident illumination. Using the central 3000 pixels of 4096 pixels/line, we acquired the image of one strip measuring

Table 1
Summary of typical imaging times for monochrome images under the various conditions measured using the HSP-1000 microscope system

Light source	Objective lens (resolution)	Processing time (μs)	Imaging time (s/cm^2)
Reflection	20 × (0.35 μm/pixel)	120	35
	10 × (0.7 μm/pixel)	70	10
Transmission	20 × (0.35 μm/pixel)	70	20
	10 × (0.7 μm/pixel)	50	6

1050 μm × 0.35 μm. With transmitted light and line sensor gain adjusted to a minimum value, a typical acquisition time required using an average brightness greyscale value of 200 (out of 256) is about 80 μs.

The times for capturing of single strips and a 1 cm^2 area under various conditions are summarized in Table 1. A total of 20 s are needed to acquire an area of 1 cm^2 at a resolution of 0.35 μm/pixel. The total image size is about 30,000 pixels × 30,000 pixels, translating into an uncompressed bitmap file of ∼ 1 Gbyte. The traditional image acquisition method using image tiling needs at least several tens of minutes to acquire an image of equal area, though of course this depends on the particulars of the microscope system.

There is no significant difference in the data acquisition rate for one pixel between CCD camera and line sensor. The difference in total time needed to acquire a 1 cm^2 image using image tiling versus the line sensor microscope system is due to the stop and go motion of the *XY* stage and the time needed for focusing by the contrast-based autofocus system. As a result of continuous stage motion and continuous focusing, we have attained image acquisition speeds that are 50–100 times faster than that of conventional CCD-based microscope systems.

4. Image analysis

Fig. 4 shows an example of a CR-39 nuclear track detector image reconstructed from three long image strips captured by the HSP-1000 microscope. The size of one strip is 1000 pixels (the center 1000 pixels of 4096 pixels/line) ×20,000 pixels and 3000 pixels × 20,000 pixels in total.

With the HSP-1000, acquisition of the detector image and the analysis of the image data for the location and measurement of nuclear track etch pits are carried out independently from one another. This approach lends greater flexibility to the system compared to previously developed interactive track detector analysis systems where a human operator must be present at the microscope system during both phases (image acquisition and image analysis) of detector analysis. Using the HSP-1000 approach, the entire detector area can be rapidly scanned into computer memory. The resultant bitmap image can then be transferred via DVD or network connection to other remote computers for subsequent interactive or automatic analysis. Because the image of the entire detector surface has been stored in permanent memory, it can be repeatedly analyzed using different threshold parameters or even different image analysis software.

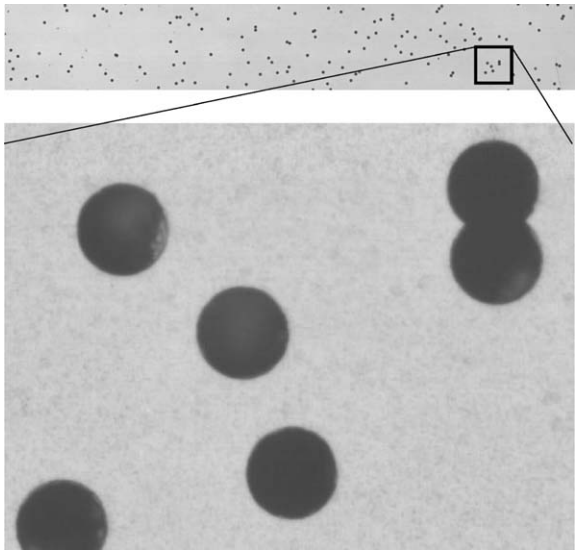

Fig. 4. An example of CR-39 image reconstructed from three image strips (above). Size of each strip is 1000 pixels (the center 1000 pixels of 4096 pixels/line) ×20,000 pixels and 3000 pixels × 20,000 pixels in total. Bottom is a real scale image of framed area in the above image (640 pixels × 480 pixels).

Software for the analysis of HSP-1000 image files acquired from nuclear track detectors is currently under development. At present, the software uses an approach similar to that used by other nuclear track detector analysis systems. The greyscale image is converted to a binary image based on a user-set greyscale threshold. The image is searched for features that possess the signature pattern of nuclear track etch pits. An ellipse is then fit to the opening of each etch pit.

There are a number of serious limitations in currently existing nuclear track detector analysis software. While such software is quite successful in measuring the high-contrast circular tracks produced by heavy ion accelerator exposures of nuclear track detectors at normal incidence, it is often unable to measure all track tracks in detectors exposed in isotropic, mixed radiation fields, such as those encountered aboard spacecraft and high altitude aircraft, nor the secondary proton and heavy ion recoil tracks produced in track detectors exposed in neutron and high energy proton fields. Most notably, this software is not able to accurately locate and measure highly elliptical, low contrast tracks pro-

duced by particles with shallow angles of incidence. At the same time, this type of image analysis software often locates many non-track features. These limitations have meant that a trained human operator must review all the measured track data in order to assure the accuracy of the analysis. This approach is generally referred to as "interactive" track detector analysis as opposed to "automatic" or "fully automatic" track detector analysis. While the software developed for the HSP-1000 microscope system has yet to overcome these limitations, the feature of separating image acquisition from interactive analysis inherent in the system lends itself to greater flexibility in both interactive and automatic track detector analysis and to easily incorporating improvements in the software. Because interactive analysis of the track detector can be carried out on nearly any computer at any remote location, the number of human operators can be increased nearly without limit. We are also attempting to incorporate an ergonomic design into the interactive analysis process in order to reduce operator fatigue, while increasing analysis speed.

Using the traditional methods such as noise reduction and binarization of the image after the setting of a greyscale threshold, the edge of each etch pit is detected. Image analysis algorithms then attempt to extract the size and shape information of each etch pit. We have adopted a least-square technique for a second order polynomial, $ax^2+bxy+cy^2+dx+ey+f=0$, with the constraint that $4ac-b^2=1$ (Fitzgibbon et al., 1999), approach in order to define the elliptical opening of each etch pit. The least-squares technique involves finding the set of parameters that minimizes the distance between the measured data points and the fitted ellipse, so that one can directly get above 6 parameters as a consequence the calculation of 6×6 matrix. The fitted ellipses are shown on the raw image as an overlay and numerical data for the etch pit position, major/minor axes, etc. are also displayed on the screen. After automated analysis, the user can modify the result, selecting the edge of the etch pit manually.

5. Conclusions

A new microscope for image digitizing, the HSP-1000, was developed using a monochrome line sensor camera instead of a CCD camera. An optical pick-up system is used in the autofocus unit for surface detection, enabling fast feedback for continuous height control above the image plane. This makes it possible for the microscope stage to always be in motion during the acquisition of the image. In case of transmission illumination, the HSP-1000 is able to digitize an area of $1\,cm^2$ within 20 s at $0.35\,\mu m$/pixel resolution. This speed is at least 50–100 times faster than conventional instruments. The microscope system is also able to acquire color images by replacing the monochrome sensor with a three color line sensor. For color image acquisition, a speed of $1\,cm^2/3$ min (typical value for pathology specimen) at $0.35\,\mu m$/pixel resolution and using transmitted light illumination has been achieved. In addition to nuclear track detector analysis, the HSP-1000 microscope system will be useful in many applications including medical pathology and laboratory diagnosis, and biological research.

Acknowledgements

We would like to extend our thanks to S. Inagaki, I. Kobayashi and S. Takayama of Matsudo Medical Laboratory for their valuable comments and for preparation of samples. We are grateful to K. Komata and I. Ishizuka in Opcell Inc. for theoretical aspects of optics for the autofocus unit. This work has been supported by the government-private sector joint research project in NIRS and was supported partially by Grants-in-Aid for Scientific Research from the Japan Ministry of Education, Culture, Sports, Science and Technology.

References

Ashaboglu, H., Kabage, J., Lui-Kwan, J., Mak, R., Mitsubichi, K., Poh, K.l., Tan, C., Vaidyanathan, S., Venegas, R., Nel, S., Wells, B., 1989. Radiation Track Project. San Francisco University.

Boukhair, A., Haessler, A., Adloff, J.C., Nourreddine, A., 2000. New code for digital imaging system for track measurements. Nucl. Instrum. Methods B 160, 550–555.

Dolleiser, M., Hasemi-Nezhad, S.R., 2002. A fully automated optical microscope for analysis of particle tracks in solids. Nucl. Instrum. Methods B 198, 98–107.

Espinosa, G., Gammage, R.B., Meyer, K.E., Dudney, C.S., 1996. Nuclear track analysis by digital imaging. Radiat. Prot. Dosim. 66, 363–366.

Fews, A.P., 1992. Fully automated image analysis of etched tracks in CR-39. Nucl. Instrum. Methods B 71, 465–478.

Fitzgibbon, A., Pilu, M., Fisher, R., 1999. Direct least-square fitting of Ellipses. IEEE PAMI.

Inoue, S., Spring, K.R., 1997. Video microscopy. The Fundamentals. Plenum Press, New York.

Price, P.B., Krischer, W., 1985. Semi-automated, three-dimensional measurement of etched tracks in solid-state nuclear track detectors. Nucl. Instrum. Methods A 234, 158–167.

Trakowski, W., Schofer, B., Dreute, J., Sonntag, S., Brechtmann, C., Beer, J., Drechsel, H., Heinrich, W., 1984. An automatic measuring system for particle tracks in plastic detector. Nucl. Instrum. Methods A 255, 92–100.

Weaver, B.A., Westphal, A.J., 2002. Extended analysis of the trek ultraheavy collector. Astron. Phys. J. 569, 493–500.