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AN IMAGE ENHANCEMENT DEVICE FOR INCIDENT LIGHT MICROSCOPY

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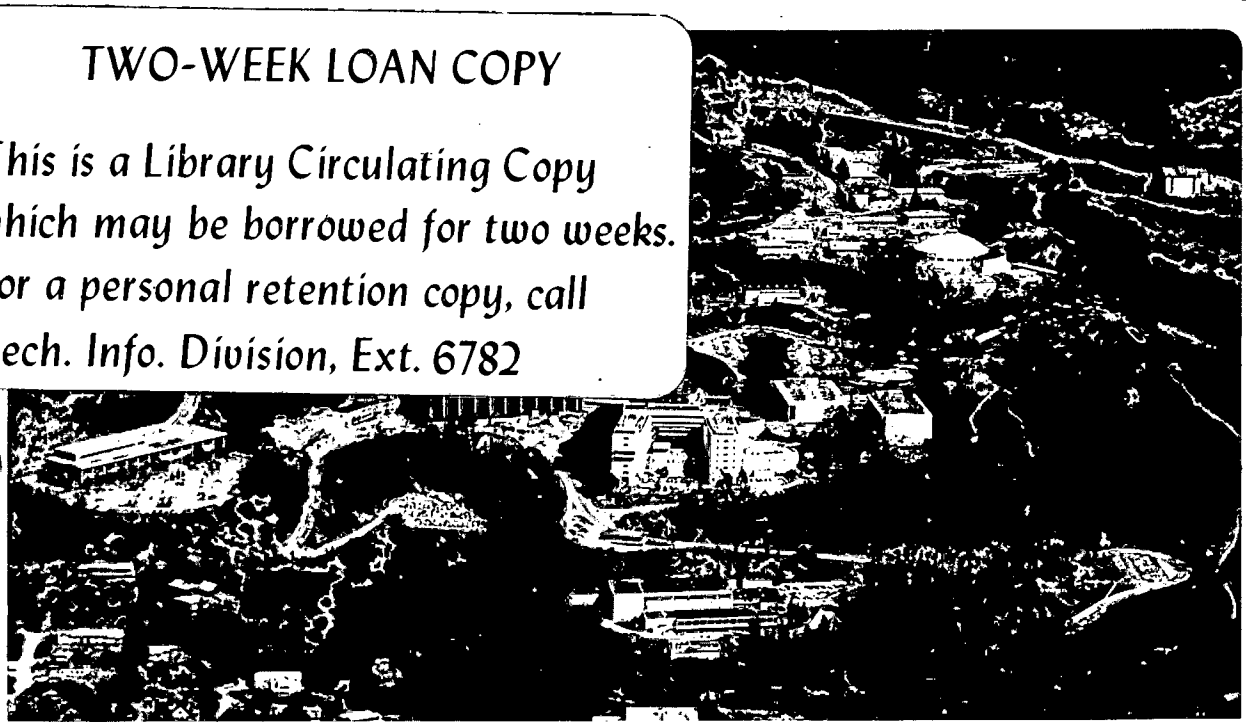

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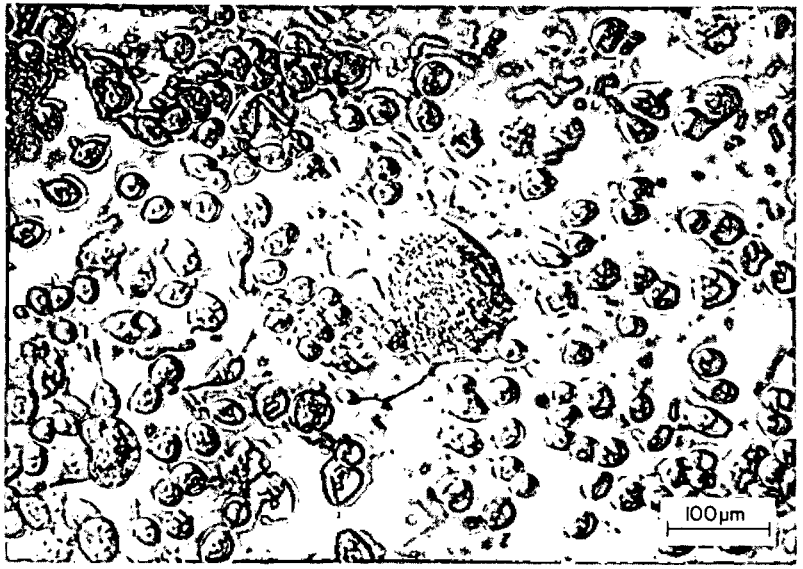

Figure 4 *Neuro blasts growing in a liquid medium inside a Petri dish viewed by incident Nomarski DIC.*

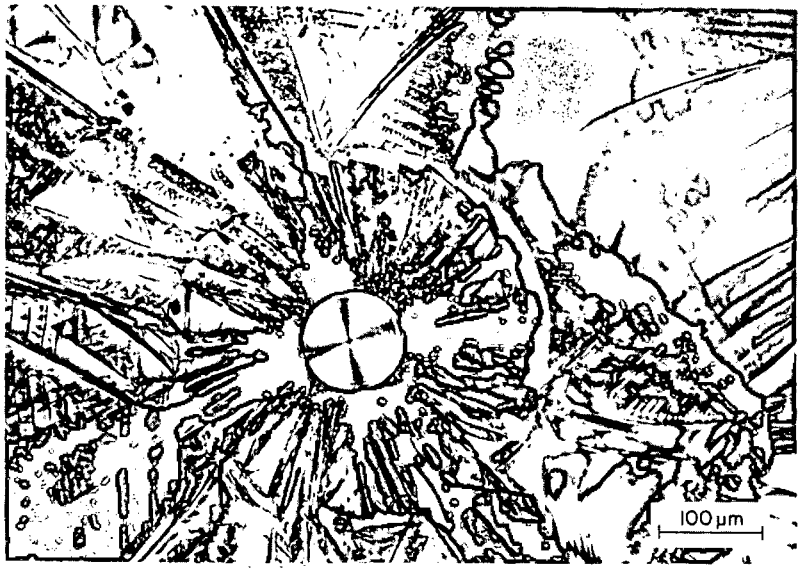

Figure 5 *Ascorbic acid crystals by incident polarized light and a red I compensator.*

The possibilities of the mirrored surface slide glass would seem to extend to the geological field microscope; one could eliminate the need for a condensing system and have a field instrument useful for either transparent or opaque specimens. Polarization colors are also observed if the incident light is polarized and if an analyzer is inserted in the bodytube (Figure 5).

A question arises as to numerical aperture, for instance, if one were to use a 45X objective, of 0.65 NA, on an incident light microscope using the image enhancement device, is the resultant NA equivalent to that of a condenser perfectly matching the objective?

Tests were made using a Reichert Zetopan microscope, Reichert incident illuminator set for brightfield, a Photovolt 200-M photometer set on low scale, with a blood smear on a microscope slide having half the slide mirrored, the other half clear glass (Figure 1). Measurements in foot candles were made examining the specimen on the mirrored side, and then on the clear side. As one would expect, the mirrored side produced a higher reading on the photometer, also yielded an excellent image, whereas on the nonmirrored side lower readings and poor image quality, especially so as one increases magnification and numerical aperture. The first series of tests were made using Reichert objectives, then objectives of other microscope manufacturers. In all cases, the results were comparable to the original Reichert readings.

Using the same test equipment, we have determined that having the specimen mounted directly on the mirrored surface is the optimum position when using objectives of over 0.8 NA; therefore, as numerical aperture and magnification increase, the distance between the specimen and the mirrored surface must decrease. In certain lower magnifications, higher light intensity is obtained by positioning the mirror below the slide containing the specimen (Table 1).

Table I. Light intensity for different mirror positions

Objective	Photovolt reading (foot-candles) Mirror at specimen plane	Mirror below slide
Reichert 23X; 0.65 NA	2.2	3.0
Reichert 45X; 0.65 NA	2.0	1.4
Reichert 85X; 0.95 NA	5.0	1.0

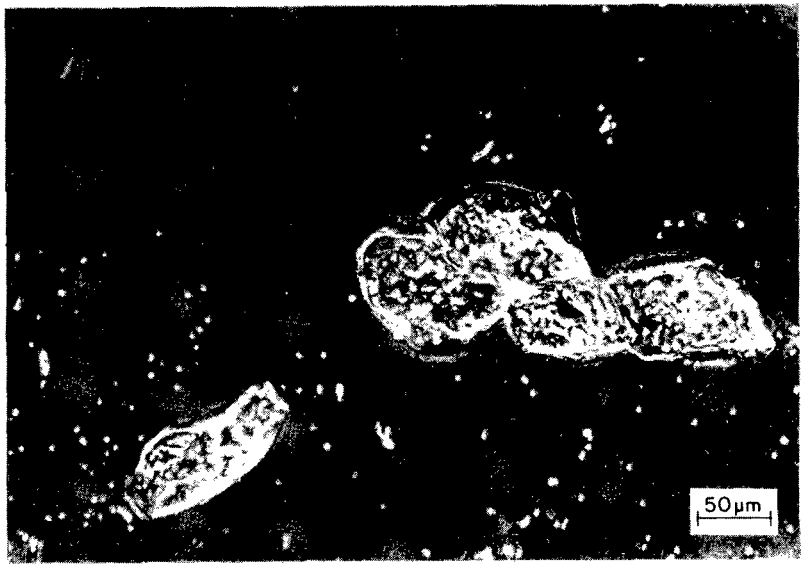

Figure 3 Epithelial cells by incident Zernike phase contrast.

with Nomarski in transmitted light (Figure 2). Blood platelet counting is facilitated when using a haemacytometer in incident light Nomarski, or incident light phase contrast, the mirrored surface of the haemacytometer behaves as does the mirrored surface on a microscope slide. Excellent images are also obtained with incident light Hoffman modulation contrast. Figure 3 shows epithelial cells in incident phase contrast.

The reader is probably familiar with the problems associated with viewing living cells growing in flasks, petri dishes, Leighton tubes or any container with an unusual configuration. By using incident light phase contrast, incident Hoffman or incident Nomarski on an inverted metallurgical microscope and placing a mirrored surface within the container and directly on the cells, the need for a condenser is eliminated and the cells may be viewed as with transmitted light (Figure 4). The use of plastic containers will reduce the enhancement of Nomarski due to the internal strain patterns of plastic, this particular example indicates the use of glass containers.

Etched tracks in plastic are more easily examined when the plastic is affixed to a mirrored surface and viewed with incident illumination. When using either transmitted or incident illumination the image of etched nuclear particle tracks in plastic is degraded by surface roughness of the plastic due to the etchant. With the plastic applied directly to the mirrored glass the surface roughness is far less detrimental to image quality. Cosmic ray tracks in large sheets of plastic might be more readily examined using a boom type micrscope and mounting the plastic on a mirrored surface.

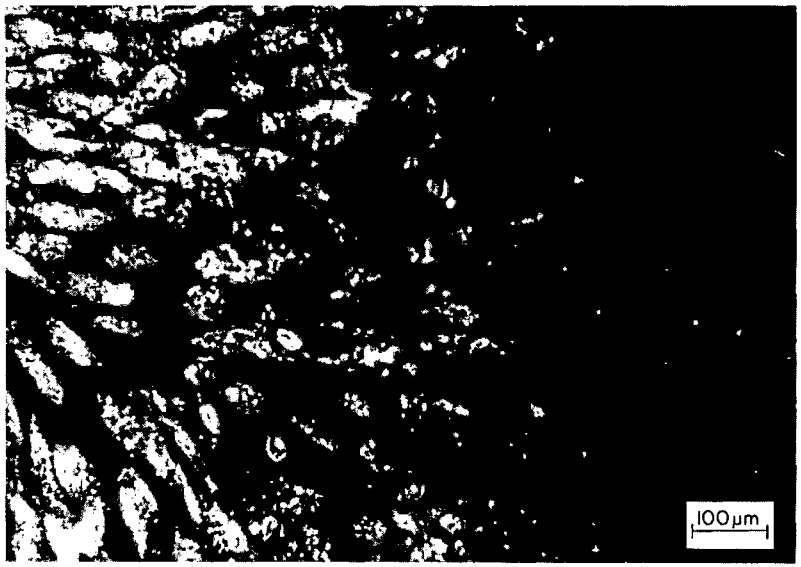

Figure 1 *Chick embryo fibroblasts with epi-fluorescence; cells mounted on a microscope slide, one end mirrored (brighter) and one end clear (darker).*

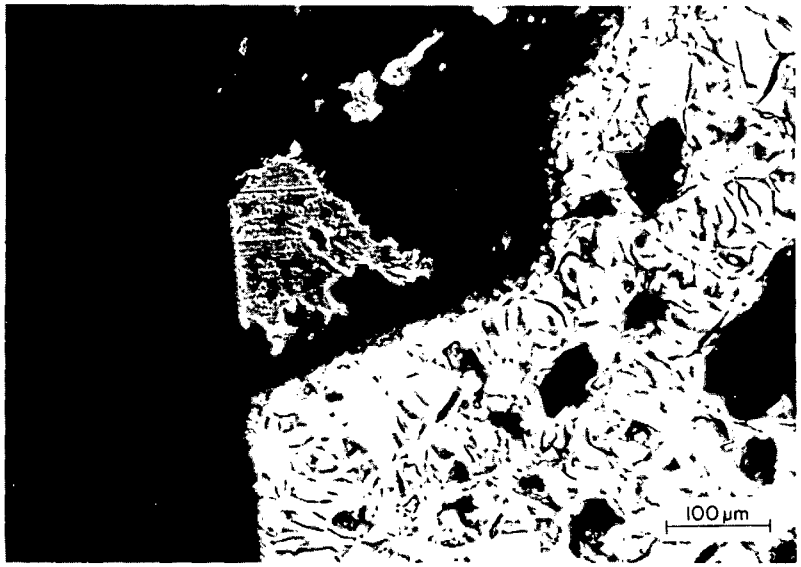

Figure 2 *Lung tissue section viewed by incident Nomarski DIC; the dark side was not mirrored.*

An Image Enhancement Device for Incident Light Microscopy*

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This image enhancement device usually consists of an aluminized or chromed surface of a microscope slide, in essence a first-surface mirror; although for certain applications, one does not necessarily adhere to the microscope slide configuration.

The metallized surface is positioned directly beneath an existing transparent, or semi-transparent, specimen mounted on a microscope slide; better results are obtained with mounts directly on the mirrored surface. The specimen is then observed as if in a transmitted light path by means of a microscope using incident illumination.

The chromed surface may be preferable due to its relative imperviousness to scratching and chemicals. An aluminum surface is, however, somewhat brighter than chrome and, with a protective coating of silicon monoxide, the aluminum coating may prove satisfactorily stable.

Many incident light techniques have proved to be quite satisfactory utilizing the image enhancement device.

In epi-fluoresence (Figure 1) the image on a mirrored surface is brighter, resulting in easier viewing by eye and shorter exposure time for photomicrography with no apparent loss of image quality.

In incident light Nomarski differential interference contrast (DIC) microscopy, transparent or semi-transparent specimens may be viewed as

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