GEOLOGICAL APPLICATIONS OF LASER SCANNING MICROSCOPY

By Dr Andrew C. Scott, Geology Department, Royal Holloway and Bedford New College, University of London, Egham, Surrey.

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Introduction

There is often a considerable time delay between the discovery of a new analytical technique and its widespread application and use. In part, this may be due to time for commercial equipment to become widely available. This is the case with Laser Scanning Microscopy (LSM). The development of scanning optical microscope systems and in particular, confocal scanning laser microscopy have recently been reviewed by several authors (1, 2, 3). For those wishing to consider using the technique, three main questions are often asked. 1. What does the microscope do and how does it work? 2. How easy is it to use and are any special preparatory techniques needed? 3. What advantage will I have over more widely known techniques including conventional light microscopy and Scanning Electron Microscopy?

Laser Scanning Microscopes are now made by several companies and each have major differences in operation and concept. Essentially, the laser-scan microscope is designed to optimise the performance of an optical microscope system. In most cases, a laser is connected to an optical system and, as in most scanning microscopes, the beam of coherent light is scanned point-to-point over the sample and an image is built up on a cathode ray tube. Additional magnification is achieved by reducing the scan size, angle and line length on the specimen (compared to the CRT line length) so a zoom factor may be added. In the case of the Zeiss instrument used in this study, a factor of up to x 160 is obtainable giving a screen magnification of 16,000 with a x 100 objective. The resolution is 1.4 times that in a conventional microscope. The microscope is driven by a computer console and is easily switchable between conventional, laser scanning and fluorescent light modes. In addition, images may be stored in a frame store and fed into an image analysis system which will show three dimensional reconstruction of images. The confocal mode which had been described in an earlier issue of this journal (1) gives a very shallow depth of field, but a sharp, high contrast, high magnification image. The significance, therefore, of this system is that high magnification may be obtained using light, and that special preparation and the use of SEM may not be necessary.

Equipment Used

This work was carried out on a Carl Zeiss (West Germany) Laser Scan Microscope based at their headquarters at Welwyn Garden City. The confocal Laser Scan research microscope comprises a universal research Axiotron with transmitted and reflected light modes using both white light and blue light fluorescence in conventional microscopy. In this study a 488 nm laser was used in reflected and transmitted mode, using laser light or blue light, confocal imaging or differential interference contrast. Hard copy was obtained via a videoprinter and a 35mm camera. Corrected neofluor objectives for both reflected and transmitted light were used in the range x 10 to x 100 (oil immersion).

Andrew C. Scott received his Ph.D. in geology from Birkbeck College, University of London, in 1976, for studying the Palaeoecology of Coal Measure Floras. He was appointed as Lecturer in Geology at Chelsea College, London, in 1978, and in 1985, transferred to the new Geology Department at Royal Holloway and Bedford New College, University of London. He has published over 60 papers on applied palaeobotany, palynology and coal geology and is currently working on oil source rocks. His recent research has utilized SEM, TEM as well as light microscopy together with image analysis systems. In addition, he has recently undertaken an applications project using the Scanning Acoustic Microscope for the study of geological materials.

Results

The major advantages of Laser Scanning Microscopy (LSM) over conventional microscopy is the high magnification achievable. The major advantage over the Scanning Electron Microscope (SEM) is that no special additional preparatory techniques are necessary.

In geology, microscopy is used to study a very broad range of materials from those of biological origin through to metallic ores. In the study of plant microfossils (palynology) light microscopy using transmitted white light is most commonly employed. In some cases, blue light fluoresence is also used. The spores, pollen, marine microplankton, etc. are extracted from the rock using acids and mounted on a slide with a suitable medium and covered with a coverslip. Such preparations are commonly used not only in academic laboratories, but widely in the oil industry where for example, the fossils are used to date rocks. Accurate identification of species is important and most workers employ light microscopical techniques using a x40 or x100 oil objective. The advent of the SEM allowed researchers to investigate the detailed ornamentation of the palynomorphs and more refined taxonomies were erected on the basis of the new results. This has caused a considerable dilemma for those using the occurrence of taxa for dating and correlating rocks. Some species have been erected on details only observable using SEM. To be sure, therefore, of identification, separate preparations have to be made for SEM and light microscopy but correlation between details seen may still be problematical.

The LSM offers the best of both worlds, as highly magnified images are obtainable from material prepared for conventional microscopy. Figure 1 shows an angiosperm pollen grain, *Spinizonocolpites*, probably from the palm *Nipa*, from Eocene rocks (40 million years) from the Isle of Wight, S. England. A conventional light image (Fig 1A) may be compared with the LSM images Fig 1B-E) where the detail is well displayed. The potential of this form of microscopy for many aspects of palynology is evident.

Plants may also accumulate as peat which when buried and subject to increased temperature and pressure, form coal. Plants comprise several different organic materials, woody tissues, cuticles, spores and pollen and these may retain some of their original form. The resulting coal macerals are usually studied in the form of polished blocks using reflected white light or by reflected blue-light fluorescence. For some macerals, contrast variation is not great and very high power is not often used. With low reflectance specimens the light intensity is a problem. The LSM can overcome some of the problems of light intensity and contrast and is of particular use in high magnification in the fluorescent mode. In the example shown here (Figure 2) the megaspores, microspores and cuticles show well in fluorescence. More importantly crisp, high magnification images, of the

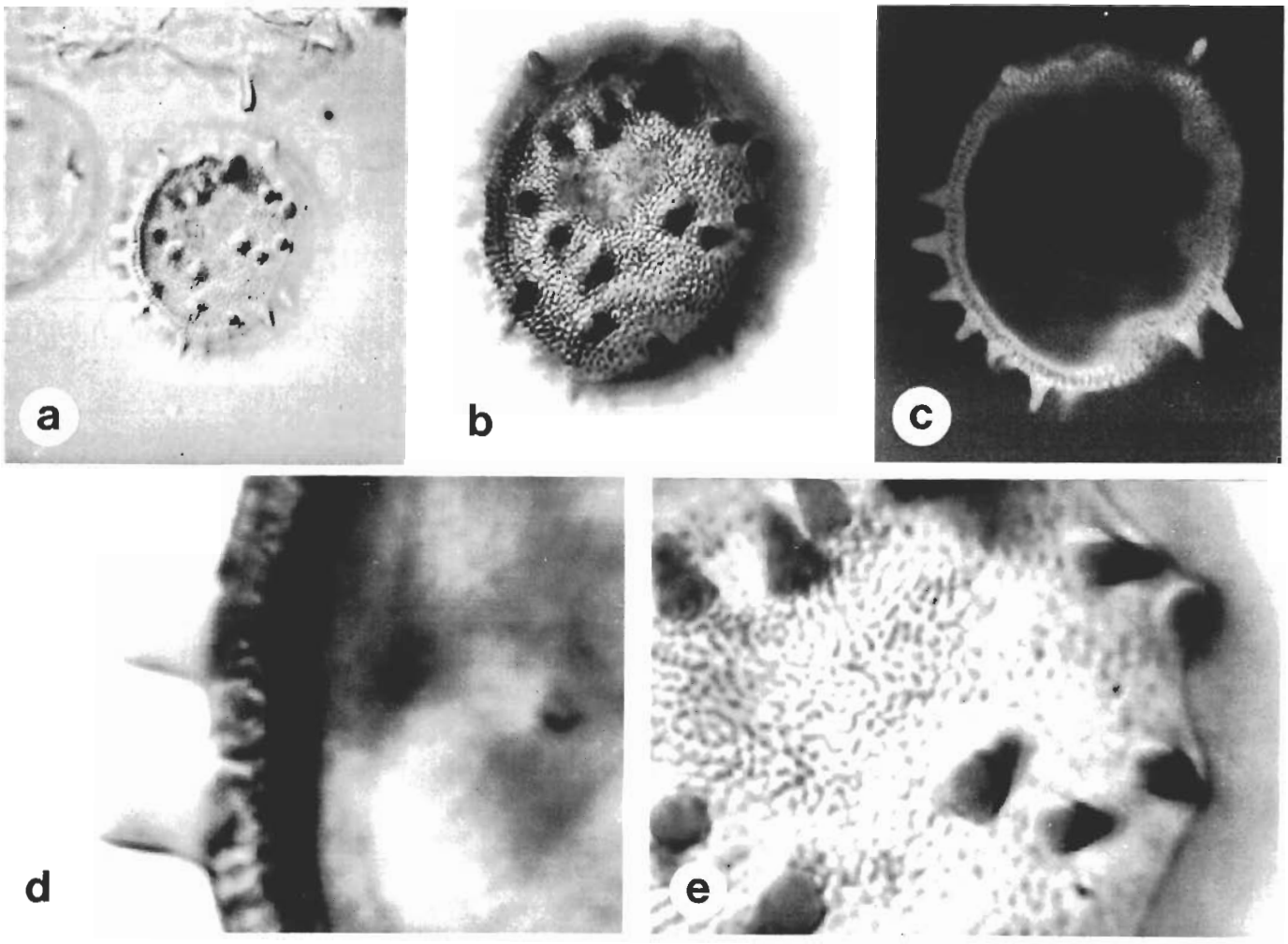

Figure 1 (above): Spinizonocolpites echinatus *Muller, an angiosperm pollen grain from the Bracklesham Beds, Eocene, Isle of Wight. All using a ×100 oil objective. a. transmitted white light, ×600. b-e LSM images. b. transmitted laser light, zoom ×40 (×1300). c. Fluorescence, confocal, zoom ×40 (×1300). d. transmitted laser light, zoom ×150 (×5000). e. transmitted laser light, zoom ×100 (×3000).*

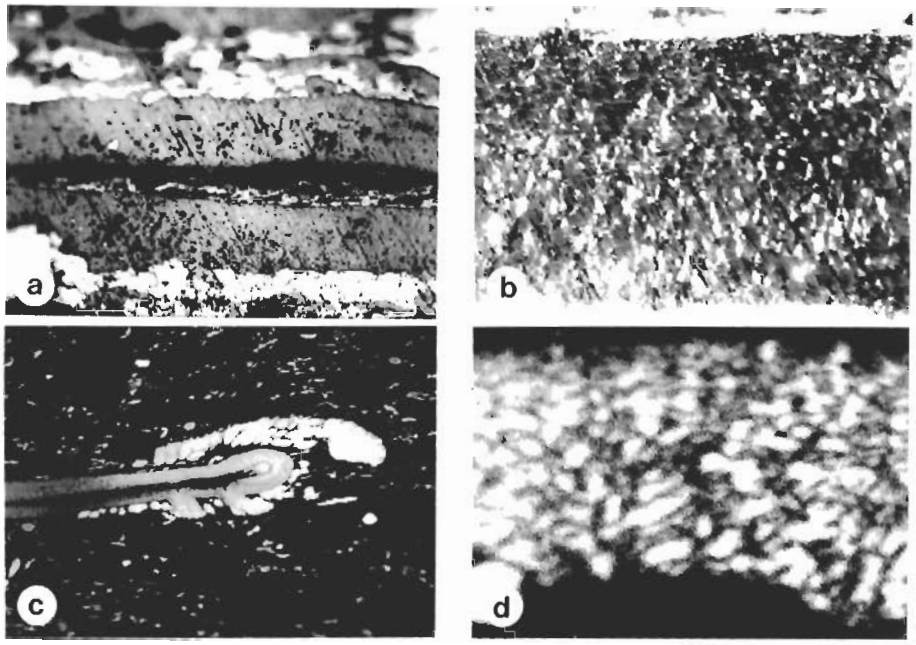

Figure 2 (right): Polished blocks of Barnsley Coal, Upper Carboniferous, Yorkshire. All except c. using ×100 oil objective. a. Megaspore in reflected white light (×430). b. Megaspore wall showing globular sporopollenin, reflected laser light, zoom ×40 (×850). c. coal showing brightly fluorescing microspores, megaspore and plant cuticle, reflected laser fluorescence, ×20 objective, zoom ×20 (×85). d. detail of megaspore wall showing ultrastructure, reflected laser fluorescence, zoom ×80 (×1735).

megaspore wall, for example, are obtained in fluorescent light which show ultrastructural detail illustrating the globular nature of sporopollenin. The potential of LSM for detailed coal maceral studies is very great Both of the above examples are of geological materials with a biological origin.

The study of rocks and minerals also benefit from the use of LSM. Three examples are illustrated below. Ores are usually studied as polished blocks in reflected light. The LSM not only increases magnification, but also enhances contrast. This is demonstrated in Figure 3 a & b where twinning and exsolution lamellae in magnetite have been emphasised.

Lack of contrast may also be a problem in other rock types such as in the sheered quartz in Figure 3 c & d. It is important to establish the direction of sheering in the crystals as this indicates the direction of pressure affecting the rock. Because the sheered quartz is transparent, imaging the cracks and recrystallization with white light is difficult. High power differential interference contrast using the LSM, however, allows the details of the microfractures and recrystallization to be seen. Image analysis of these will allow a statistical relationship of fracture direction to be established.

Many minerals have fluids trapped within them. These fluid inclusions are of wide interest as they may yield data on the origin of the minerals and their fluid history. Many are very small and their form cannot readily be studied by normal light microscopy. With the enhanced contrast and very high magnification which using the LSM (Figure 3 e & f) allows, the inclusions can be analysed and the occurrence of crystal growth within the in-

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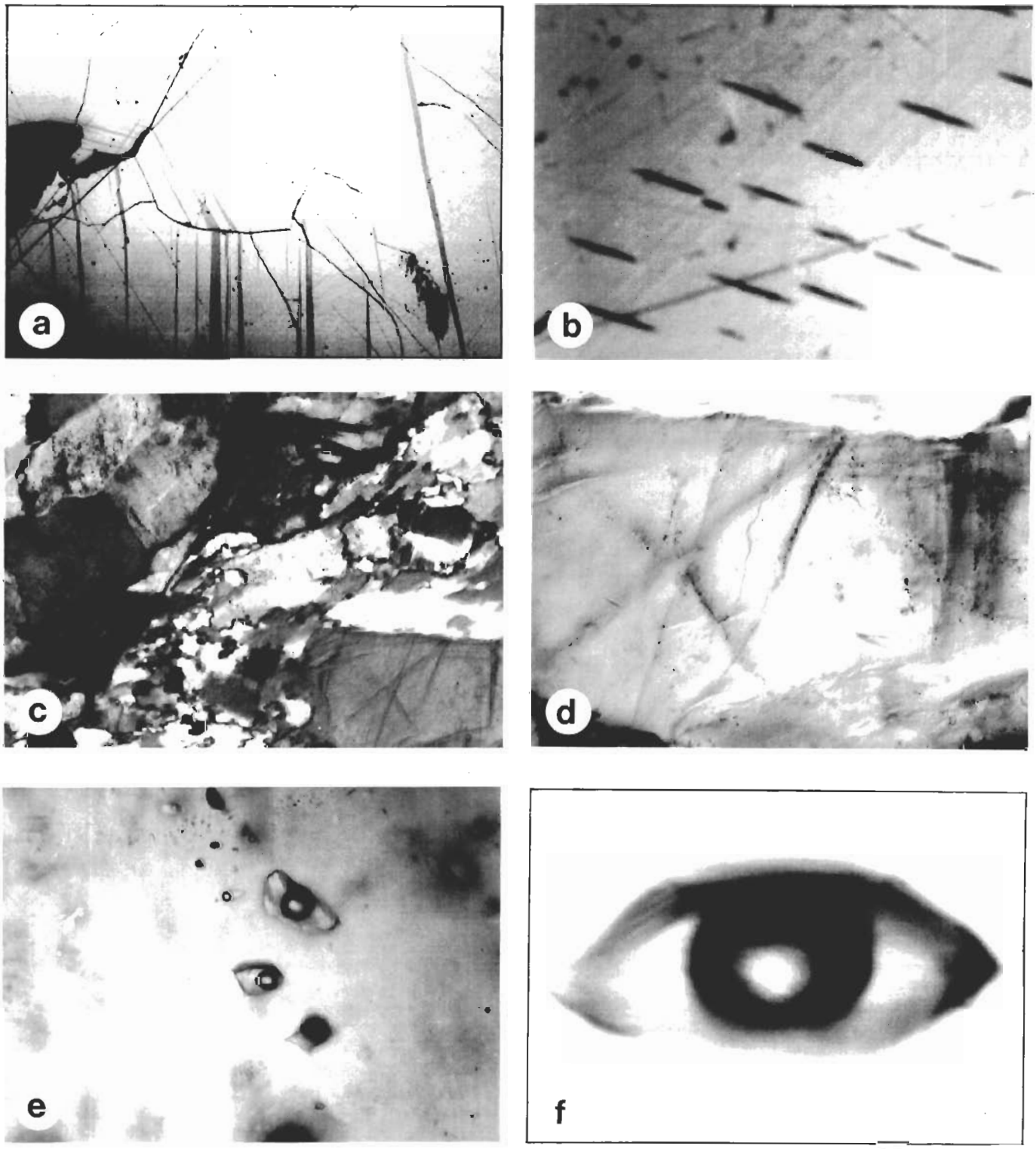

clusions noted.

There are numerous potential applications of LSM not illustrated here. These include studies of sedimentary rocks in sections where high magnification and high contrast are of major use, in the study of diagenesis, particularly with the growth of authigenic clay, through to the study of spore-wall and shell ultrastructure. The exact system you might choose will depend on several factors as discussed by Boyde(1) but microscopists studying geological materials should be encouraged to seek out and try Laser Scanning Microscopy for themselves - they will not be disappointed. LSM will become as common as abbreviation in geological publications in the next 15 years as SEM is now.

Acknowledgements

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Figure 3. a. & b. Magnetite twins, ×120 objective. a. Reflected laser, zoom ×20 (×130). b. Exsolution lamellae, reflected laser, ×100 oil objective zoom ×110 (×3650). c. & d. Brecciated quartz mylonite from W. Norway, thin section. ×20 neofluor objective. c. microbreccia zone, transmitted laser light, differential interference contrast, zoom ×20 (×130). d. detail of fractures in quartz grain, transmitted laser, differential interference contrast, ×50 neofluor objective, zoom ×20 (×330). e. & f. Fluid inclusions in quartz, polished slice, Donegal, Ireland. All ×100 oil objective. e. Transmitted laser light, zoom ×20 (×650). f. detail of inclusion showing gas bubble. Transmitted laser light, zoom ×80 (×2650).