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Three-dimensional imaging of fission tracks using confocal scanning laser microscopy

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Abstract

Confocal scanning laser microscopy (SLM) is a relatively new technique designed to generate detailed three-dimensional images of the interiors of solid materials by probing them with laser light. Although used routinely in the life sciences, SLM appears to have escaped the attention of the mineralogical community. The method offers a nondestructive means of examining in detail the internal three-dimensional geometry and structure of crystalline defects at resolutions of less than 0.5 μm.

This paper reports the results of a study using confocal SLM to record and measure the three-dimensional orientation of selected fission tracks in apatite grains from the Fish Canyon Tuff. We show how individual SLM images can be electronically enhanced using image analysis software and then combined into three-dimensional stacks, where the true fission-track length and orientation can be accurately measured. Three-dimensional measurement of inclined fission tracks is not possible in transmitted light, the principal medium used in state-of-the-art fission track analysis systems. Confocal SLM provides one way of overcoming some of the limitations inherent in these systems and may ultimately lead to an improved computer-aided track-counting and -measuring system.

Introduction

Fission tracks are micrometer-sized damage trails that form in U-bearing minerals such as apatite, titanite, and zircon through the spontaneous decay of the radionuclide ^{238}U. Although initially the tracks were studied using transmitted electron microscopy (Silk and Barnes, 1959), fission-track analysis (FTA) is currently undertaken with conventional light microscopes on tracks that have been enlarged by chemical etching.

Fission-track data, which consist essentially of track-density and track-length measurements, can be used to date geological and archeological material (Fleischer et al., 1975) and to provide information about the tectonothermal histories of orogenic belts (Zeitler, 1985; Green, 1986). More recently, with the realization that fission tracks in apatite anneal over the hydrocarbon maturation window (ca. 70–120 °C), the technique has been used to help reconstruct the paleothermal environments in some sedimentary basins, with encouraging results (Naeser, 1981; Gleadow et al., 1983, 1986; Green, 1989).

The track-density and track-length measurements involved in FTA using conventional light microscopy can be extremely labor intensive, and for inexperienced observers there is the added complication of distinguishing tracks from spurious crystalline defects. Furthermore, paleothermal studies and the successful dating of geologically young material often require large amounts of material to be processed. In order to speed up the analytical procedure, a number of semiautomated measurement systems have been proposed recently (Birkholtz et al., 1989; Wadatsumi and Masumoto, 1989; Rebetez et al., in preparation). However, all these systems use transmitted light to view tracks and do not fully address the problem of track recognition. Perhaps more importantly, these two-dimensional systems are incapable of resolving how fission tracks lie in the third dimension, thus providing no useful information about track shape or spatial distribution. As real size distribution and shape measurements are only possible in three dimensions (De Hoff, 1983), a knowledge of these properties is an important prerequisite in any automated or semiautomated track-analysis system.

In this paper we expand on ideas first presented by Petford and Miller (1990) and show how the relatively new technique of confocal scanning laser microscopy (SLM), when combined with image-analysis software, can both enhance track recognition and show the true three-dimensional form of selected fission tracks in apatite.

Fission track measurements

Figure 1 shows the various types of track arrangements one can expect to find in a mineral such as apatite. Although fission tracks are distributed randomly throughout the crystal volume, some, known as surface tracks, will intersect the polished surface of the grain. During etching, any surface tracks that by chance happen to intersect confined tracks (i.e., those that do not cut the grain surface) may be enlarged by the etchant. Confined tracks will also be etched if they are cut by surface-intersecting fractures. For age determination, much of the data collection involves counting the number of surface fission tracks per unit area, regardless of their orientation. In

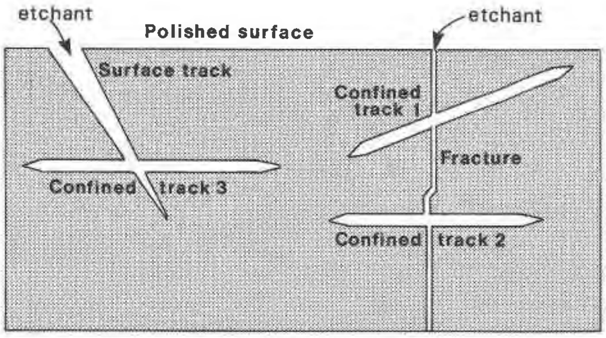


Fig. 1. Schematic diagram showing some of the internal geometries that fission tracks can make in a U-bearing mineral such as apatite. Although the confined tracks 1–3 do not intersect the polished surface of the grain, they can still be etched, and hence enlarged, if they are cut by surface-intersecting tracks or fractures (after Gleadow et al., 1983).

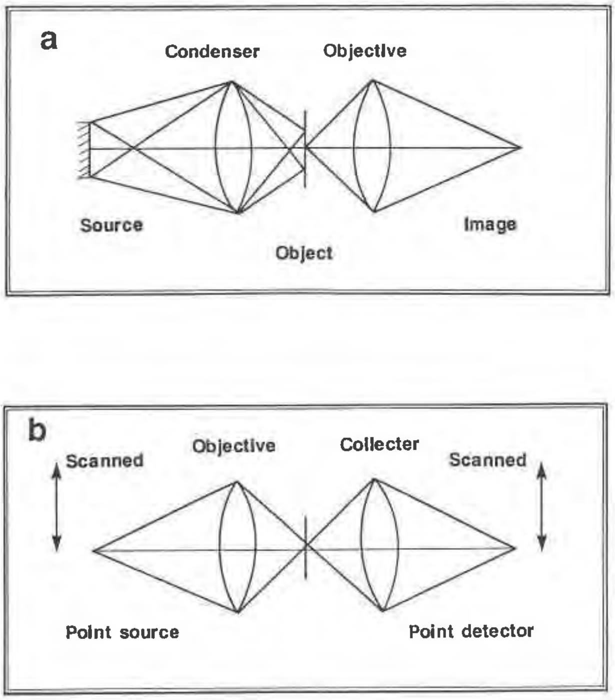


Fig. 2. Comparison of transmitted and confocal microscope arrangements. (**a**) The lens arrangement in a conventional light microscope. In transmission, light passing through the condenser lens consists of an in-focus component formed in the focal plane of the objective lens, and a blurred component from above and below the objective focal plane. Illumination (source) and detection (eyepiece) are undertaken separately. In contrast, with confocal microscopy (**b**), the point and detector source (the latter aperture known as the confocal pinhole), lie in conjugate focal planes. Light is brought to a focal point within the sample by the objective lens. The collector lens is positioned so that the back-projected image from the focal point coincides exactly with the point detector. Unlike the conventional illumination in **a**, the confocal lens arrangement restricts information above and below the focal plane to a very narrow depth of field. By scanning the laser beam across the specimen, a two-dimensional optical section is generated.

paleothermal studies, only individual track-length measurements are required. Although a seemingly trivial procedure, it highlights a major problem faced when transmitted light is used to study fission tracks—that only the length of confined tracks that are horizontal in the plane of observation (confined tracks 2 and 3 in Fig. 1) can be measured accurately. This means that although many confined tracks might be fully etched in a given volume (i.e., confined track 1), because of their unfavorable orientation they are effectively unmeasurable, and much potential data can be undetected. The power of SLM over transmitted light is that three-dimensional optical probing can quickly reveal how individual fission tracks are oriented in a crystal, thus allowing tracks that lie out of the plane of observation to be measured.

Confocal scanning laser microscopy

Confocal SLM as a technique for quantitative three-dimensional analysis is used routinely in disciplines such as histology and pathology, where the nondestructive nature of the technique enables three-dimensional measurements to be made in vivo (Pawley, 1990; Howard, 1990). The technique is an extension of scanning optical microscopy (SOM), first developed by Young and Roberts (1951), where sample imaging is performed by spot illumination in a raster (X and Y) scanning action. Surprisingly, in contrast to the life sciences, scanning confocal microscopy seems to have been largely overlooked in geology, although we consider that the technique has much to offer.

In confocal imaging, both illumination and detection are confined to the same spot on the sample (Fig. 2). The result is a sharp, high-resolution image with a very narrow depth of field, typically on the order of 100 nm (Wilson and Shepherd, 1984; Wilson, 1990). Unlike other techniques such as SEM and TEM, high magnifications can be achieved without special sample preparation.

The light source for this study was supplied by a 514- and 488-nm Ar ion laser with the beam set to scan point to point over a fixed sample, building up an image as it goes so that it can be viewed instantaneously on a TV monitor. The laser system is connected to an optical microscope, enabling a sample or target area to be selected before confocal probing. In the present study, suitable apatite grains were chosen in transmitted light using a 40× and 100× oil objective. The sample can also be viewed in phase contrast and fluorescence modes.

Confocal three-dimensional analysis is achieved by programming the microscope, by an attached computer console, to scan incrementally at 0.5-μm intervals down through the sample, starting at the grain surface. After the scanning has been completed, the individual (two-dimensional) confocal images from different levels are combined into a three-dimensional Z series to show the interior of the sample, whose whole depth is in focus. In this way, confocal SLM enables the observer to look "inside" objects at high resolution and to examine reflective internal features (Fig. 3). One drawback of confocal images compared with those viewed in transmitted light is a general reduction in image brightness; however, this can be easily overcome by electronically processing the stored confocal images.

Image processing

The controlling software for the SLM system is housed in an IBM-compatible microcomputer. Images recorded from inside the specimen are stored on disc as *X Y Z* pixel elements and can be viewed either separately (two-dimensional) or combined into a *Z* series for three-dimensional analysis. Each confocal image has a picture element width of 768 pixels and a height of 512 pixels. Image size is approximately 350 kilobytes. A camera attachment to the TV monitor allows hard copies, including stereo pairs, to be made. Fission-track length and density measurements can be made directly from the TV screen or from hard copy.

Although the raw confocal images are of considerable interest in themselves, the fission-track images have been further processed using image analysis software and an Apple Macintosh PC. After importing selected images from the IBM PC, the confocal images, reformatted as TIFF files, were processed using Image 1.3, an Apple Macintosh public domain image-analysis program. The images were first enhanced (segmented) in gray scale from their background to make track identification more easy. False color coding of the segmented images was found to be a particularly effective way of analyzing the fission-track population within the sample matrix and of studying track morphology.

Results

The sample material used in this study was an apatite fraction from the 27.8-m.y.-old Fish Canyon Tuff, an interlaboratory standard used for fission-track age calibration. Most of the tracks have formed through the spontaneous decay of ^{238}U and have been etched in 5N HNO_3 for 20 s to make them visible under an 800–1500× optical microscope. Figure 4a is a gray-scale-enhanced image of an etched surface track, similar to that shown schematically in Figure 1. The image is a composite *Z* series made up of eight two-dimensional optical sections. Figure 4b shows the same track in false color, with the body of the track (green) thickening upward from its rounded termination inside the apatite grain to a diamond-shaped exit hole at the grain surface (marked as e in Fig. 4a).

Although the apparent track length, in this case 8 μm, is easily measurable in transmitted light, the true track length can only be found if the vertical distance (*Z*) from the grain surface to the base of the track is known. Using SLM, *Z* was determined by simply following the track in a vertical plane (at step intervals of 0.5 μm) from the surface to its termination inside the grain. The true length of 8.9 μm was then calculated using the Pythagorean theorem.

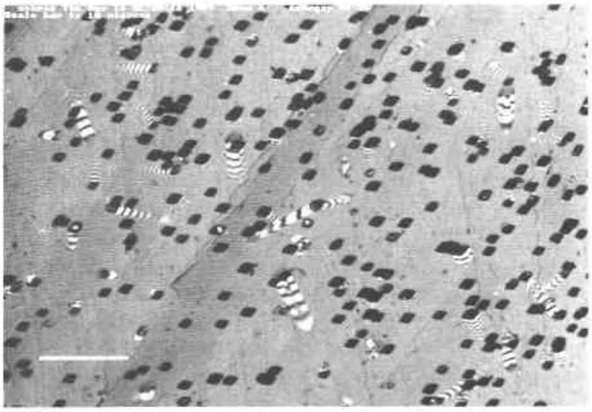
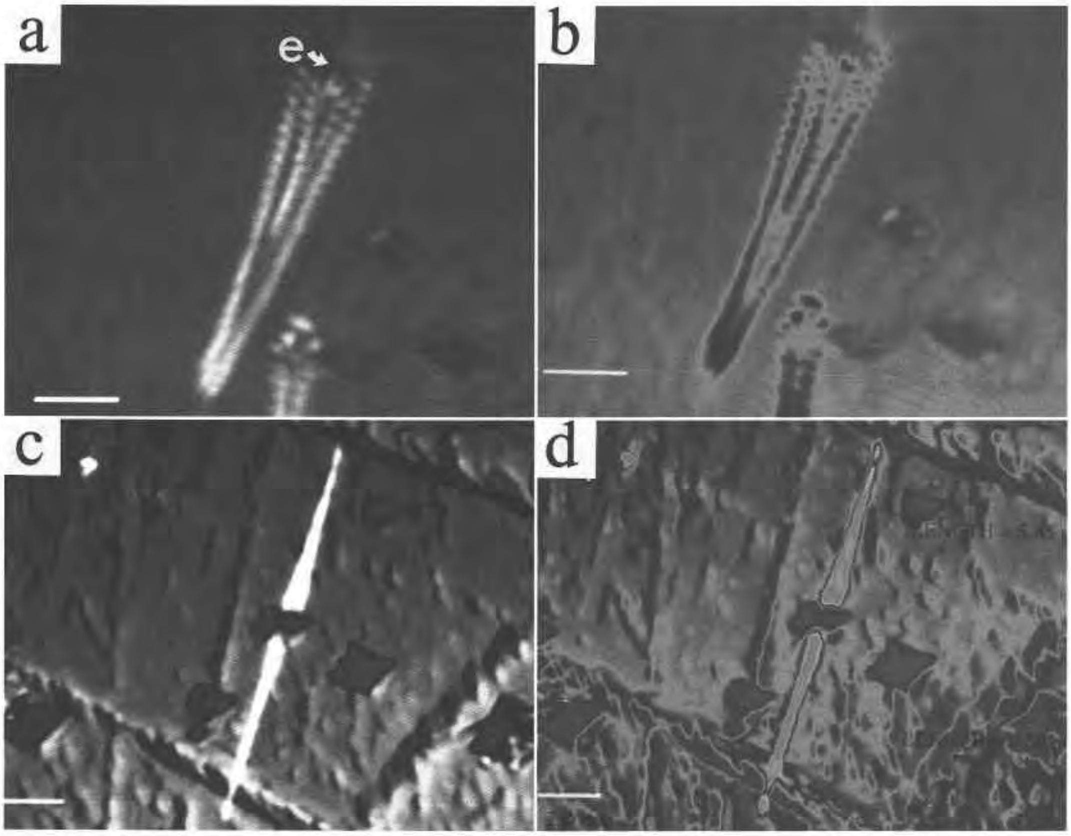


Fig. 3. Confocal SLM image of a mica detector used to measure the amount of fissioned ^{235}U produced during the thermal irradiation of apatite grains. Black, diamond-shaped particle entry holes (formed by fission fragments emanating from the apatite sample) mimic the crystal symmetry of the mica. Near-surface (<0.5 μm), low-angle fission tracks produce distinctive zebra interference patterns. Scale bar = 10 μm.

Figures 4c and 4d show a stacked *Z* series made up of 12 images at 0.5-μm step intervals. The *Z* series starts at the grain surface and extends to a depth of 6.0 μm inside the mineral. Both images show a brightly reflecting, horizontal fission track that has been segmented in gray scale (Fig. 4c) and processed in false color (Fig. 4d). The image is of interest, as the accurate measurement of confined horizontal fission tracks forms the basis of many paleothermal studies. Seven diamond-shaped etch holes can also be seen, each representing a subvertical fission track. Although the horizontal track is confined, it has been etched along its length by a vertical, surface-intersecting track (cf. confined tracks 2 and 3 in Fig. 1). The true length of the confined track is 12.3 μm. The topography seen in both images is an artifact caused by polishing grooves at the grain surface.

Once a *Z* series has been made, the position (depth from the grain surface) of any horizontal track inside a grain can be easily established. Figure 5 shows a series of four segmented false-color images that together represent a vertical slice through the horizontal track shown in Figure 4. The range of colors from deep blue to yellow through the sequence is related to increasing pixel intensity. The first image (Fig. 5a) is from 3.0 μm inside the grain, and shows the irregular upper surface of the track. Note the reflections from the surface polishing grooves are still visible as white streaks. Figure 5b is an image from 4.0 μm inside the grain, and 1.0 μm inside the fission track. Here pixel intensity (yellow) is at a maximum, and the well-developed track has a near-perfect symmetry, tapering away from the etch point near the track center. The upper (northern) section of the track has a length of 5.85 μm, whereas the lower (southern) section is slightly longer at 6.50 μm. In Figure 5c, taken at a depth of 4.5 μm, track definition, although still good, is starting to fade, and pix-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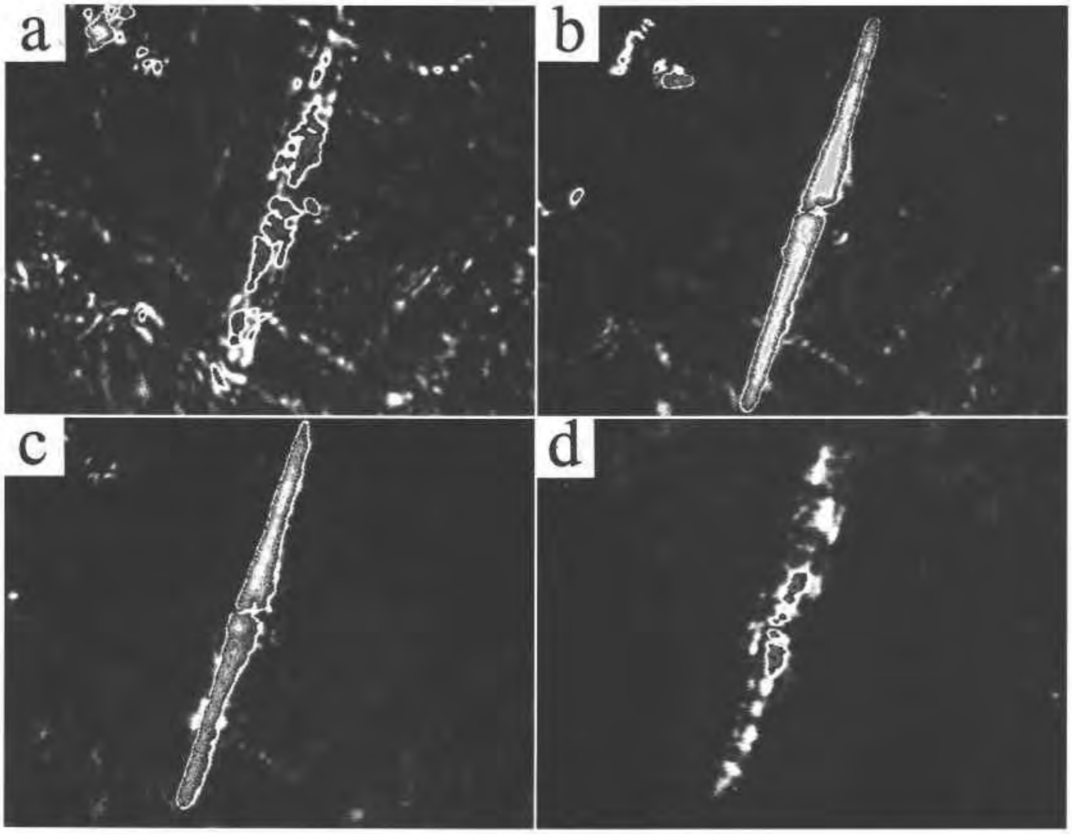
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Fig. 4. (**a** and **b**) Surface intersecting fission track in scanning confocal laser light projected through eight optical sections at 0.5-μm intervals. (**a**) Segmented 256 gray-scale fission-track image. (**b**) False color image of the same track. The structure within the track is probably the result of internal reflections and is similar, although not as pronounced, as the zebra interference fringes seen in low-angle tracks in Figure 3. The track length is 8.9 μm and has a dip of 28.9° from the grain surface, marked in **a** by a diamond-shaped etch pit (e). Depth of field, 4.0 μm. Scale bar = 2.0 μm. (**c** and **d**) Horizontal, confined fission track in scanning confocal laser light, projected through 12 optical sections at 0.5-μm intervals. (**c**) Segmented 256 gray scale image. Note the increased reflectance of the track compared to the inclined track in **a** and **b** and the lack of interference fringes. The parallel lines, marking the surface of the grain, are polishing grooves. (**d**) The same image in false color. The surface of the grain is clearly marked by a series of diamond-shaped etch pits. Depth of field = 6.0 μm. Scale bar = 2.0 μm.

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el intensity is lower. The final image (Fig. 5d) is from 5.5 μm inside the apatite grain, and represents more or less the base of the track with respect to the vertical Z series. Note that at this depth, surface reflections are absent, although some internal reflection from within the track is visible. The track width is ca. 2.5 μm.

Conclusions

SLM confocal microscopy, combined with image analysis software, can provide more detailed two-dimensional and, more importantly, three-dimensional information about both the morphology and internal geometry of fission tracks than is obtainable using conventional light techniques. Furthermore, the electronic nature of the stored images is ideal for computer-aided data measurement and manipulation. Although automatic measurement of three-dimensional images is beset with many of the problems associated with conventional two-dimensional images, serious efforts are now being made in the biological sciences to produce software that will analyze three-dimensional binary SLM images automatically (Gesbert et al., 1990). A similar approach to fission track analysis using SLM techniques will, we believe, provoke a second generation of more efficient semiautomated track analysis systems. The technique also has potential in other branches of mineralogy, for example, in fluid inclusion and microstructural studies, where three-dimensional analysis could provide useful information about the internal distribution of crystalline defects.

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Fig. 5. Selected Z series through the horizontal fission track shown in Figures **4c** and **4d**. (**a**) The vertical series starts 3.0 μm inside the apatite grain, where the top of the track is starting to take shape. (**b**) A fully defined track image is seen at a depth of 4.0 μm (i.e., 1.0 μm inside the fission track). (**c**) At a depth of 4.5 μm, track resolution is starting to be lost. (**d**) At a depth of 5.5 μm, the track is almost gone, although some internal reflection from above can still be seen. Depth of field, corresponding roughly to track width, is 2.5 μm.