Observations on three-dimensional measurement of confined fission track lengths in apatite using digital imagery

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ABSTRACT

We report the results of a comparative study to explore the usefulness of 3D measurements of confined fission track lengths (TINTs) relative to horizontal confined track length measurements (dips ≤10°), and evaluate their suitability for thermal history modelling.

Confined fission track lengths were measured in ten annealed Fish Canyon Tuff apatites containing synthetic mixtures of different length components, and two Durango apatites containing spontaneous fission tracks. Measurements were primarily carried out using a digital image-based microscope system, and compared to those from a regular optical drawing tube-digitizing tablet set-up and a confocal laser scanning microscope. The results indicate that 3D measurements of confined track lengths are closely comparable to
conventional horizontal track measurements, and the mean track lengths of inclined
(dips >10°) and horizontal (dips ≤10°) confined tracks from the one sample are equivalent
within the measurement uncertainty. A strong dip-bias was observed, so that almost all the
confined tracks measured were dipping at <30°, and the great majority (~70%) were dipping
at ≤10°, thereby qualifying as ‘horizontal’ confined tracks. Our results suggest that a useful
increase of more than 40% in sample size can be achieved from including dip- and
refraction-corrected 3D track length measurements. Some evidence was seen for a small bias
in favor of shorter tracks at higher dip angles but this has very little influence on the mean
lengths or length distributions up to the practical limit of dips (~30°) observed in these
measurements. Results obtained using the same measurement system by a single analyst over
time, and between six different observers in the one laboratory, show good reproducibility.
These results also agree well with conventional horizontal confined track length
measurements in the same samples in the second laboratory involved. We conclude that 3D
measurements of confined track lengths, including both horizontal and inclined tracks, are
suitable for use in current fission track annealing models derived from experiments using
horizontal confined tracks.

**Keywords:** Thermochronology, fission track dating, apatite, confined track lengths, 3D
measurement, digital imaging

**INTRODUCTION**

Apatite fission track (AFT) thermochronology is used for reconstructing geological thermal
histories through combining apparent age and confined track length measurements (e.g.
Gleadow et al. 2002; Gallagher 2012; Ketcham 2005). Fission tracks form continually over time, but the length of each track is subjected only to the subsequent thermal history since its formation. Thus, the distribution of confined track lengths in a particular sample is characteristic of its thermal history (Gleadow et al. 1986) since entering the partial annealing zone. Detailed thermal histories can be reconstructed from the combined fission track length and age data by using fission track annealing models (e.g. Ketcham et al., 1999; Laslett and Galbraith 1996; Laslett et al. 1987).

Laslett et al. (1982) pointed out that all practical schemes for sampling etched fission track lengths will be subject to various kinds of bias. They concluded that sampling horizontal confined fission tracks will be the least biased and provide the closest approximation to the underlying distribution of unetched track lengths. Since that work, and later empirical studies by Gleadow et al. (1986), standard practice has been to measure the projected lengths of such horizontal confined track (HCTs). In reality, ‘horizontal’ is taken to mean tracks dipping at up to ~10° (Donelick et al. 2005) or even ~15° (Laslett et al. 1982), for which the resulting errors introduced by measuring only the horizontal length component are relatively small, ~1.5% to ~3.4% respectively.

In the absence of actual dip measurements, it is obviously difficult to apply these criteria for a particular track to be horizontal in any rigorous sense. Mostly the operator makes a qualitative judgment based on the focus and appearance of the track in transmitted and/or reflected light, which will therefore depend to some extent on the microscope conditions and the level of experience. This judgment is made more complex by the fact that tracks in apatite...
appear to dip at significantly less than their true dips due to refraction effects when observed under air (Laslett et al. 1982).

Another consideration from those early studies was that, while it was simple to measure the horizontal component of a track length, it was more difficult and laborious to measure the vertical component required for measuring the dip with comparable precision, using typical microscopes available at that time. Such measurements were clearly possible (e.g. Dakowski, 1978), but have become much more straightforward and convenient with the current generation of fully motorized and digitally controlled microscopes (e.g. Gleadow et al, 2015).

A significant limitation caused by using only HCTs, however, is that this restriction reduces the potential sample size for measurements on features that are already rare events. In the case of young or low-uranium apatites, it is often difficult to locate enough HCTs to make thermal history modeling possible.

Measurement errors introduced by including tracks that are not strictly horizontal are likely to be small in most cases, but in standard practice will always be present, and potentially to different degrees between samples and observers. Such errors could contribute to some degree to the poor inter-laboratory reproducibility of HCT length data reported by Ketcham et al. (2009; 2015). In principle, it should always be better to correct for the dip and measure the true etched lengths of confined tracks in three dimensions, even for HCTs. Jonckheere and Ratschbacher (2010) reported an approach to measuring the lengths of non-horizontal tracks in apatite with the potential to significantly increase the available sample size, and pointed out that the more complex biases inherent in such measurements should not introduce
insurmountable problems. It is not the purpose here to evaluate the range of potential biases present, however, but rather to explore empirically just how significant their cumulative effect might be relative to standard HCT measurements. The primary aim is therefore to test the usefulness and practicality of including 3D measurements of non-horizontal track lengths in routine fission track analysis.

The measurements reported here utilize a fully motorized microscope system to capture multi-plane image sets (so-called z-stacks) at precisely controlled vertical intervals to digitally image the 3D structure of the etched fission tracks. A secondary aim of this study is therefore to establish the degree to which such image-based techniques produce measurements that are comparable to those from older optical drawing-tube systems attached to the microscope. The confined fission track lengths acquired using an image-based system, (here referred to simply as ‘3D lengths’) are automatically corrected for both dip and refraction, and represent the ‘true’ lengths of the etched tracks. The orientation of each track relative to the crystallographic c-axis is also automatically determined in this system. Including additional lengths for what are here termed inclined confined tracks, i.e. those with dip angles of >10°, can increase the sample size.

3D track length measurements are reported from twelve apatite samples, containing fission tracks with length distributions of varying degrees of complexity. We compare 3D track length measurements of dipping tracks with conventionally measured HCTs, and investigate the difference between horizontal and inclined track length measurements. We also examine whether the precision of 3D measurements can be improved by using more closely spaced
image stacks, and by using confocal laser scanning microscopy with its inherently higher image resolution (e.g. Petford and Miller 1992, 1993). Finally, we evaluate the consistency of 3D length measurements by comparing results obtained by a single analyst over time on several samples, and by different observers on one particular sample that was also part of the comparative study by Ketcham et al. (2015).

LENGTHS AND ORIENATIONS OF CONFINED FISSION TRACKS

The length of a fission track at any arbitrary orientation can be calculated from a simple set of geometric equations using a coordinate system that is defined relative to an observation surface and the crystallographic orientation (Fig. 1, after Galbraith and Laslett, 1988). The observation surface in an apatite grain is normally selected to be parallel to the crystallographic \( c \)-axis. The coordinate system consists of a plane \( XY \) that is parallel to this observation surface, where the \( X \)-axis is parallel to the crystallographic \( c \)-axis. The 3D length of a confined track \( l \), orientates from the origin \( O \) with dip angle \( \theta \), and has a projected length \( l_p \) on the observation plane \( XY \) and a depth \( D \) parallel to the \( Z \)-axis. The angle between \( l \) and the \( c \)-axis is denoted by \( \phi \) (Galbraith and Laslett 1988), while \( \omega \) represents the azimuth angle in the observation plane between \( l_p \) and the \( c \)-axis.

Once the \( XYZ \) coordinates of the end points of a confined track are known, \( l \), \( \theta \), and \( \phi \) can be calculated from \( l_p \), \( D \), and \( \omega \) using the following equations (after Jonckheere and Ratschbacher, 2010), assuming that the polished surface is perfectly flat:

\[
l_t = \sqrt{l_p^2 + D^2} \tag{1}
\]

\[
\theta = \cos^{-1} \frac{l_p}{l_t} \tag{2}
\]
Because of the contrasting refractive indices between apatite and the surrounding air, the apparent depth of the end point of the track ($d_a$ in Fig. 1) is shallower than the true depth ($d$) when observed using a dry objective lens. The apparent ‘true’ length of the tracks is given by $l_a$ in Fig. 1. The true depth is determined simply as the product of the apparent depth and the refractive index of apatite. While the difference between the ordinary and extraordinary rays in apatite is at its maximum on the prismatic observation surfaces used, the birefringence is so low that this can be ignored and an average refractive index applied. Here a refractive index of 1.634 was used, as a reasonable average for near fluorapatites (e.g. Deer et al., 1965, p 507).

This refraction effect can usually be ignored in projected track length measurements (typical HCTs), as the effect is small in this case, but can become significant when tracks that are not strictly horizontal are included. If uncorrected, this refraction effect means that a track which appears to dip at 10° is actually dipping at 16° and the 1.5% measurement error increases to 4%, whereas an apparent dip of 15° is actually 25°, for which the measurement error would be nearly 10%. In this study, the criterion for tracks to be ‘horizontal’ is taken to be ≤10° true dip after correction for the refractive index.

Most of the comparisons made here will be between different mean track lengths, with their respective standard errors (SE) and standard deviations (SD) of the distributions. When comparing confined tracks over different dip ranges, the terms HCT and ICT will be used to denote mean lengths of ‘horizontal’ and ‘inclined’ confined tracks dipping at ≤10° and > 10°.
respectively. We will also refer to mean lengths of ‘all’ confined tracks (ACT) over the full range of dips observed, and will differentiate between ‘projected’ lengths (i.e. projected onto the XY plane in Fig. 1) and ‘true’ lengths, corrected for dip, by using the subscripts ‘p’ and ‘t’ respectively.

SAMPLES AND METHODS

Sample details

A total of 12 samples from two well-known apatite reference materials, the Fish Canyon Tuff (FCT, 10 samples) and Durango (DUR, 2 samples) apatite, were used in this study. The Fish Canyon Tuff samples were prepared at the London Geochronology Centre at University College London (UCL) and consisted initially of twelve aliquots of separated apatite. All were first annealed at 600ºC for 24 hours to remove all pre-existing spontaneous fission tracks. The aliquots were then irradiated in the former HIFAR Reactor at Lucas Heights, Australia, with a total neutron fluence of $9 \times 10^{15}$ n/cm$^2$ to induce $^{235}$U fission tracks. One aliquot, containing only fresh induced tracks with a mean track length of ~16 µm, was set aside at this point as Control 1. Splits of the remaining 10 samples were reheated for 1 hour to temperatures of 300º, 350º or 370ºC to produce different degrees of partial annealing to mean track lengths of ~13, ~11 and ~8 µm represented by Controls 2, 3 and 4 respectively. The sample Control 2 (~13 µm) was not available to this study and is not considered further. The remaining seven FCT samples were then re-irradiated in the same reactor with various thermal neutron fluences between $1.1 \times 10^{15}$ and $2.5 \times 10^{16}$ n/cm$^2$ to produce two-component mixtures of one of the three annealed components and a new unannealed (~16 µm)
component in varying proportions as indicated in Table 1. This sample suite has other important calibration applications, but is used here purely to present a known range of track length distributions and mixtures of known length components.

Sample DUR was prepared at the Melbourne Thermochronology laboratory and consists of suitably sized crystal fragments (80–200 μm) that were obtained by crushing a single Durango apatite crystal. The sample was analyzed in its natural state, containing only spontaneous $^{238}$U fission tracks. Sample DUR-4 is a sliced ~1 mm thick plate of Durango apatite that was part of a previous inter-laboratory comparative fission track length study (Ketcham et al., 2015) and was prepared at UCL. The crystal was cut parallel to the $c$-axis before being heated to 500°C for 24 hours in order to fully anneal all spontaneous tracks. It was then irradiated at the same reactor to generate induced tracks, before being reheated again to 240°C for 10 hours in order to reduce tracks to lengths that would be expected in a rapidly cooled, natural volcanic sample.

All apatites were mounted in epoxy resin on glass slides, ground and polished to expose internal surfaces. At this stage, $^{252}$Cf fission tracks were implanted in the surface of samples DUR and DUR-4 in order to increase the number of confined track lengths for measurement (Donelick and Miller 1991). Etching conditions for all mounts were identical: 5M HNO$_3$ for 20 seconds at 20°C. FCT samples were mounted and etched at UCL, while DUR and DUR-4 were prepared at the University of Melbourne (UoM).

**Length measurements using conventional wide field microscopy**

Confined track lengths where measured by Analyst A from UCL and Analysts 1-6 from the
UoM group using different equipment and techniques. In both cases, mineral mounts were set-up under the microscope and referenced to the stage coordinate system. The operator then scanned grain mounts for confined tracks etched though surface-intersecting tracks (Track-in-Track features - TINTs; Lal et al. 1969, Green 1981, Donelick et al. 2005), marking the location of suitable tracks for analysis. The criteria for track selection were to only include those TINTs with distinct track ends, excluding tracks with blurred ends due to overlap with other features, or with thin or faint track ends that may not have been fully etched (Laslett et al. 1984). All measurements were made on prismatic surfaces of apatite grains with their c-axes in the plane of the microscope stage, as determined by observation under circular polarized light, or the sharpness of polishing scratches and parallel orientation of the long-axes of surface etch pits (Dpar; Donelick et al. 1999; Green et al. 1986; Gleadow et al. 2009a).

At UCL, horizontal confined track lengths were measured with a Zeiss Axioplan microscope using a 100x dry objective (total magnification 1250x), and a Calcomp Drawing Board III tablet with an LED attached to the cursor. The LED light is projected via a drawing tube into the microscope field of view where it produces a bright spot ~0.2 μm in diameter. The projected length of each track was determined by clicking the cursor at each end. Only tracks with estimated dips <15° (apparent) were measured. Calibration of the digitizing tablet used a certified stage micrometer with 2 μm divisions. Precision of the measuring system is estimated at ± 0.11μm.

At the UoM laboratory, track length measurements were carried out as refraction and
dip-corrected 3D lengths using an automated image acquisition and processing system
developed in house, which consists of a motorized Zeiss Axio-Imager M1m microscope
controlled by the TrackWorks software package (Gleadow et al. 2009a, 2009b; 2015;
Gleadow and Seiler 2015). The microscope is fitted with a motorized stage system with
vertical movements in 25 nm steps. For each track located by the operator, z-stacks of digital
images, at vertical spacings of 0.1, 0.2 or 0.3 μm between image planes, were captured
autonomously in transmitted and reflected light at previously marked locations. All images
were captured using a 100x dry objective and a 3.3 Megapixel Zeiss ICc3 camera on a 0.5x
C-mount adapter. At the time of acquisition the image sets were automatically cropped to a
box 35×35 μm in area around the location of each identified confined track. The captured
image sets were then archived to a local network storage array.

Archived fission track image sets were later retrieved and analyzed on a computer using the
fission track image analysis and measurement software FastTracks (Gleadow et al. 2009b;
2015; Gleadow and Seiler 2015). Track lengths were measured by focusing through the
digital image stacks on the monitor and clicking at each end of the confined track with the
cursor at a total effective magnification of ~6,000x - 10,000x. The point of this higher
magnification is not that it contains any further information than may be observed under the
microscope, but that it minimizes placement errors in positioning the cursor at the ends of a
track. Image scale was calculated to be 0.069 μm/pixel, based on total magnification to the
camera and the pixel spacing in the Sony CCD sensor (3.45 μm), and confirmed by direct
calibration against a Pyser-SGI S21 stage micrometer with divisions of 10 μm.
At least 170 confined tracks were located, imaged and measured on each sample, except for FCT G, where only 120 tracks could be found in the entire sample (Table 2). For FCT samples, an initial sampling of ~100 tracks were selected for measurement irrespective of their dip angle $\theta$. A second selection was then added until at least 100 HCTs were included in the measurement to make that component comparable to most conventional measurements. For samples DUR and DUR-4, track lengths were measured from 355 and 223 captured track image sets, respectively, each containing one or more confined tracks. The particular selection strategy for DUR-4 is described in more detail below under ‘Reproducibility between multiple analysts’.

All images were captured on $c$-axis parallel surfaces, and the $c$-axis direction in this surface plane was determined automatically by image analysis from the mean direction of the long-axes of the parallel track openings in reflected light, i.e. the orientation of the $D_{par}$ parameter. These automatically determined azimuth directions were adjusted manually if required. In addition to the calculated true and projected confined track lengths, the orientation angles $\theta$ and $\phi$ were also automatically determined using FastTracks. Analysts at UCL and UoM were unaware of each other’s results during analysis.

Confocal laser scanning microscopy (CLSM)

Confocal laser scanning microscope measurements were made using a Zeiss $LSM700$ materials science module utilizing a single 405 nm laser attached to a Zeiss $Axio Imager Z1m$ microscope, controlled by Zeiss $ZEN$ software. The fully motorized $Z1m$ microscope is fitted with a piezo drive $x$-$y$ scanning stage and motorized $z$-axis with a vertical resolution of 10
nm, monitored by a piezo encoding device.

CLSM length measurements were carried out on exactly the same tracks that had previously been selected and analyzed by conventional wide-field microscopy. Analysis was only carried out on the FCT samples as these cover a wide range of possible track length distributions. During capture, step sizes of the z-stack and objective magnification were the same as that used during the conventional microscopy.

**RESULTS AND DISCUSSION**

**Horizontal and 3D confined fission track lengths**

A comparison of the mean track length results from Analyst 1 at UoM and Analyst A at UCL is shown in Table 2 and individual track length ($l_t$) distributions for Analyst 1 are shown in Fig. 2. The 3D measurements of Analyst 1 for mean tracks lengths of confined tracks over all dip angles (ACT$_t$) range from 15.89(05) to 8.25(18) (±1 SE), from the unannealed Control 1 to the most highly annealed Control 4 sample. The spontaneous tracks in DUR and DUR-4 both contain a single length component with mean track lengths 14.13(05) and 14.24(06) µm, consistent with previous measurements on Durango apatite (e.g. Gleadow et al. 1986; Green 1988; Kohn et al. 2002). The FCT samples A-H have mixed length distributions with means from ~12-15 µm, which shorten in line with the ratio of unannealed to annealed track lengths, and the different components can be seen in the complex length distributions in Fig. 2. The distribution for DUR-4 is essentially identical to DUR and not illustrated.

The mean projected lengths of horizontal confined tracks (HCT$_p$) measured at UCL and UoM
agree closely, but the UCL lengths tend to be slightly longer in most samples, as shown in
Fig. 3A. The differences ranged from 0 to 1.22 µm, with an average 0.24 µm. Only the means
for Control 1 were significantly different at the 95% confidence level. These discrepancies
probably include typical inter-laboratory factors such as differences in microscope
configuration, system calibration and observer biases, but they are very small compared to
the range observed between different laboratories reported by Ketcham et al. (2015) and
Barbarand et al. (2003). The two largest deviations of 0.39 and 1.22 µm were found for
samples FCT F and G, both of which show complex two-component length distributions with
significant numbers of short tracks. This suggests that in these two cases differences in track
selection are the most significant factor.

Very little difference was observed between measurements of the projected lengths of
horizontal tracks (HCT_p) and the true, dip- and refraction-corrected, 3D lengths (ACT_t) of
confined tracks in all samples measured by Analyst 1 at UoM (Table 2, and Fig. 3B). These
measurements were all made by the same observer using an identical measurement system
and the mean lengths for tracks at all dips tend to be slightly shorter than the means projected
lengths of horizontal tracks. The differences in this case range from -0.18 to 0.20 µm with a
mean of -0.02 µm, and none are significant at the 95% confidence level. Once again, the two
largest deviations are for FCT F and G, again suggesting that small differences track selection
between the two length peaks have been a factor, although in this case, the effect is very
small.

The true 3D length measurements were further divided into horizontal (θ ≤10°) and inclined
(θ >10°) components to compare the mean lengths of the shallow (HCT₁) and more steeply
dipping (ICT₁) fractions. A summary of mean track length results for the two sub-groups is
shown in Table 3 and the differences between them in Fig. 3C. The results show that the
mean track lengths are systematically longer for horizontal than for inclined tracks in nearly
all cases, except for two most complex samples (FCT G and FCT H) where the difference is
reversed. The differences range from -0.13 to 0.82 μm with a mean value of 0.28 μm. Except
for the two extreme samples, where track selection is again likely to be the dominant factor,
the differences are small but consistently in one direction. This implies that 3D measurements
for confined tracks at higher dip angles are systematically shorter than those at lower dips,
but in most cases the differences between the means are still not significant at the 95%
confidence level.

Orientation analysis of individual 3D lengths

Individual 3D confined track length measurements \( l_i \) are plotted against dip angles \( \theta \) in Fig. 4.
The alignment of the measurements into sub-parallel ‘dot-curve’ arrays is due to the depth
measurements being quantized by the discrete layer planes sampled in the image z-stacks, as
illustrated in Fig. 5. Each of these dot-curves represents tracks observed to terminate in the
same image depth plane.

The four samples containing a single unannealed length component (Control 1, DUR), or
once-annealed fission tracks (Controls 3 and 4) show a relatively uniform distribution of
individual track lengths with increasing dip angle. The dispersion of lengths in each
component increases with the degree of annealing, as expected, due to the anisotropic
annealing of tracks in different crystallographic orientations (e.g. Green et al. 1986). All of
the two-component mixtures (FCT A-H) show fields of lengths belonging to each component,
with varying degrees of overlap depending on the degree of annealing. For samples
containing the most strongly annealed components, with means of ~11 and ~8 µm, the two
length components are essentially separate from each other (Fig. 4: FCT B and C, and
especially FCT F, G, H).

In most of the observed length components the maximum individual track lengths tend to
decrease, and the minimum lengths increase, with increasing dip angle, giving a tapering
field towards the higher dip angles. This is most obvious for the longer length components,
where the density of the field is greatest. Perhaps surprisingly, almost all of the measured
tracks lie at dips of 30° or less, with only a few outliers beyond this and only three beyond
40°. This may be due to the difficulty of identifying confined tracks at higher dips given the
limited depth of focus of the microscope at the high magnification used. There is a tendency
for the few tracks dipping at >30° to be shorter than the main group of HCTs at <10°, and it is
likely that these extreme outliers are lowering the mean lengths for ICTs to the small degree
observed in Table 3 and Fig. 3C.

The histogram of all dip angles in Fig. 4B shows even more strongly how the number of
tracks sampled decreases rapidly with increasing dip, so the apparent narrowing of the field
of lengths towards higher dip angles might reflect the more limited sampling in this region.
About 70% of the observed tracks dip at ≤10°, and would therefore qualify as HCTs in a
conventional measurement. Even more (~90%) would be HCTs if the threshold were set at
As indicated previously few confined tracks were observed at dip angles above ~30° and almost none beyond 40°.

These results suggest that there is a strong real or observational bias towards low angle tracks in 3D measurements of confined fission tracks. This dip-bias is probably due to the limited depth range over which the confined tracks are sampled. All the measurements in this study were made on track-in-track (TINT) features, where the lengths of the surface intersecting semi-tracks, from which they are etched, limits the intersection depth from which they can be revealed. This depth will range up to the maximum length of a confined track and will on average be about half of this length. Confined tracks that are intersected only a few micrometers below the surface must of necessity be almost horizontal because if the dip was greater they would intersect the surface and no longer qualify as confined tracks. Longer tracks at higher dip angles, must therefore be intersected further below the surface, on average, and might therefore be etched to a slightly lesser degree because of the finite time taken for the etchant to reach their ends. This might be another factor in the very slightly reduced length apparent for ICTs, compared to HCTs observed in Table 3.

In principle, it might be expected that shorter tracks would be observed to higher dip angles than longer tracks, as the longer tracks would be more likely to intersect the surface and so be excluded. This has been termed a surface-proximity bias by Galbraith (2005 p.157). However, the results for the mixed length components in Fig. 4(A) do not show any consistent trend in this regard, with both long and short track groups occurring over a similar angular range. It would appear then that this postulated proximity-bias is not a significant factor limiting the
use of 3D length measurements, at least over the limited dip range that is actually sampled in practice.

Sensitivity to step-size in the image stack

The quality of an image-based measurement of a fully etched fission track depends on the resolution of the input image in all three dimensions, as well as the precision with which the track ends can be defined. The image resolution is controlled by the magnification, the numerical aperture of the optics and the wavelength of the light source, as well as the pixel resolution of the image sensor. In our experiments, images were captured digitally at a pixel resolution of 70 nm, which exceeds the diffraction-limited resolution of the microscope optics for visible light (~280 nm with a 100x dry objective, numerical aperture NA=0.9). This satisfies the Nyquist limit in the image plane (~100 nm), which defines the sampling rate required to faithfully digitize an analog signal. The same does not apply to the z-direction, however, where the image spacing of typically ~300 nm (or ~490 nm after correction for the refractive index) exceeds the optical resolution in this direction and the stack is under-sampled. The effect of this limitation was tested first by reducing the step-size between image planes within the captured z-stacks until they were close to the Nyquist limit (~100 nm), and second by utilizing confocal laser-scanning microscopy, which achieves a higher resolution than is possible with conventional wide field microscopy.

The step-size of an image stack refers to the vertical interval between captured image planes of the stack, which is used to calculate the true (refraction-corrected) vertical distance between the two ends of a fission track when they are in focus. Reducing the step-size
increases the vertical sampling of images, thereby increasing the vertical resolution of the stack so that track ends can be measured more precisely, but at the expense of larger stack sizes.

To evaluate the effects of the step-size, repeat 3D length measurements were made on images of the same set of confined fission tracks in sample DUR captured using three different step-sizes: 0.1, 0.2 and 0.3 μm (corresponding to refraction-corrected step-sizes of 0.16, 0.33 and 0.49 μm). Measurements were made on totals of 105, 106 and 117 confined tracks respectively, but most of the tracks were common to two or three of the image sets. Results are shown for mean lengths of the 80 tracks common to all three step-sizes in Table 4, for all measurements in Fig. 6A and for the 88 individual lengths common to the 0.1 and 0.3 μm step-sizes in Fig. 6B. The maximum difference between the mean lengths is 0.06 μm, and none are statistically significant. The mean track lengths (ACT,) for the three step-sizes are essentially identical and show no systematic difference between the minimum and maximum increments.

Individual 3D track lengths plotted against dip angles for these three step-sizes are shown in Fig. 6A. The spacing between the dot-curves decreases with decreasing the step-size, but there is no systematic change in the overall field covered. A comparison of all paired lengths on the same tracks obtained by the largest (0.3 μm) against smallest (0.1 μm) step-sizes (Fig. 6B) shows that data points are tightly scattered around the 1:1 line (root mean square deviation: 1.6%). This finding is consistent with the small difference in the mean track lengths (<0.03 μm) arising from the three different step-sizes (Table 4), suggesting that the differences are negligible.
Confined track length measurements by Confocal Laser Scanning Microscopy

Track length measurements of FCT samples were repeated using CLSM in order to assess whether the increased resolution of CLSM is advantageous for 3D confined track length measurements (c.f. Petford and Miller, 1992, 1993). As can be seen in Fig. 7, the same tracks imaged using CLSM are more sharply resolved and the track ends better defined than in a conventional wide field image. Track lengths on the same individual tracks measured by both CLSM and conventional optical microscopy are highly correlated and essentially identical (Fig. 8). The mean track lengths, standard deviation and standard errors for all the samples obtained by both methods are in close agreement with each other and essentially indistinguishable within error (Table 5). On average, the CLSM lengths are very slightly longer than 3D lengths by wide field microscopy (by 0.10 μm), but the difference is insignificant (root mean square deviation: 0.94%).

A key restriction of CLSM, however, is that measurements can only be carried out on tracks with relatively low dip angles (Petford and Miller, 1993). The mean $\theta$ of tracks measured on the CLSM is between 2.4° to 4.1°, with the steepest track dip measured at 15.7° (Table 5). That is because confocal laser imaging is inherently an incident light method and only tracks with low $\theta$ reflect sufficient light to enable them to be detected and measured. In an effort to overcome this limitation, we attempted to enhance the visibility of inclined tracks using a fluorescent dye and captured images in fluorescence mode, but the number of measurable tracks did not increase due to the resulting overall poor illumination conditions.

Reproducibility of 3D measurements over time
Repeat measurements were made by Analyst 1 on the same captured image sets after an interval of ~2.5 years (Table 6) to assess the reproducibility of these results. Analyses were made on five of the FCT and DUR samples and the measurements were made on exactly the same tracks in most cases, although slightly more or less confined tracks were judged suitable in three of the samples (Table 6). Thus the selection of tracks was essentially identical for both cases and the only differences were in the measurements themselves. In all cases the replicates closely reproduce, and are statistically indistinguishable from, the original measurements. The initial measurements are very slightly, but consistently, higher than the second, with differences ranging from 0.03-0.12 μm, and a mean of 0.07 μm. On the other hand, the standard deviations from the repeat analyses are consistently slightly greater, with differences ranging from 0-0.12, with mean of 0.04 μm, but none of these differences are statistically significant. The reason for these slight systematic differences is attributed to a change in the magnification used on the monitor, leading to a subtle difference in defining the ends of tracks. However, the differences are insignificant and all the track length measurements are highly reproducible.

Reproducibility between multiple analysts

In order to assess the reproducibility of 3D length measurement results between different analysts, six experienced analysts in the UoM laboratory were requested to measure confined tracks on a set of archived images captured from sample DUR-4. This sample was part of the blind inter-laboratory comparison experiment reported by Ketcham et al. (2015). The measurements reported here were carried out before that study was published, so the
comparative results were unknown at the time of measurement. To allow for an element of individual selection of the tracks to be measured, a total of 400 TINTs were identified across 223 locations in the mount. Image stacks were acquired from all locations and distributed to the analysts. Each analyst was asked to select and measure at least 100 tracks from the entire set, based on personal criteria as to which tracks were satisfactory for measurement. The results are presented in Table 7 and Fig. 9. The latter also includes the comparative data from Ketcham et al. (2015), acquired from identically prepared apatite samples and measured by 55 analysts in 30 different laboratories.

Mean 3D lengths in DUR-4 from this study ranged from 14.12 to 14.29 μm between the different analysts with a mean of 14.20(03) μm (SE), and SDs ranged from 0.79 to 0.98 μm. The consistency of these measurements is excellent and even the maximum difference observed (0.17 μm) is not significant at the 95% confidence level. The mean value is also consistent with the mean of the international comparison. The variability is substantially lower than most of the measurements on the same sample from other laboratories in Ketcham et al. (2015) at both the inter-laboratory and intra-laboratory level (Fig. 9).

**IMPLICATIONS**

‘Horizontal’ confined track length measurements, which are a central component of apatite fission track thermochronology, in reality include tracks with a range of dips up to a threshold of usually ~10°. The resulting length measurements are projected lengths that will mostly be shorter than the true lengths by a small, and presumed negligible, amount. The discrepancies will increase with increasing dip angle, however, and this is compounded by
refraction in apatite, which makes track dips appear significantly less than they actually are. Where dips are not measured explicitly, it is difficult to rule out the possibility that tracks significantly above the dip threshold are being included in the measurement. Differing dip thresholds for apparently ‘horizontal’ tracks could therefore explain at least some of the previously observed variability between analysts, although the overall effect is likely to be relatively minor in most cases.

3D measurements of confined fission track lengths based on captured z-stack images, were used in this study to determine the true track lengths, corrected for both dip and refractive index, thereby overcoming the small but known errors associated with projected length measurements. Our observational results across apatite samples with a wide range of track length distributions, show that 3D length measurements are actually closely comparable to the commonly used ‘horizontal only’ projected track length measurements (Table 2, Fig. 3B). These 3D measurements showed excellent reproducibility between individual analysts, and between replicate measurements over time. In addition, the image-sets upon which they are based provide a permanent digital record of those measurements, which could assist in standardization between laboratories.

A major contributing factor to the very close agreement between the dip-corrected and projected measurements of confined track lengths is the presence of a strong dip-bias in the 3D measurements favoring shallow dipping tracks. The great majority of confined tracks in the 3D measurements (~70%) are thus dipping at low angles (≤10° true dip) and meet the criterion to be measured as HCTs. This natural control on the observed dip range is most
probably due to the limited depth over which TINTs can be sampled from surface-intersecting semi-tracks.

Our results also reveal a variable tendency for the mean length of inclined tracks dipping at >10° to be slightly shorter than those for horizontal tracks (dips ≤10°) in most samples, by an average of ~0.3 μm. This probably reflects a small bias in favor of shorter confined tracks at higher dip angles, where longer tracks might intersect the surface and therefore be excluded. Such a surface proximity-bias is not obvious, however, in the proportions of short and long tracks at higher dip angles in the individual track data in Fig. 4, but this does not rule out a small effect on the mean 3D length. Other factors, such as a possible lesser degree of etching for tracks with steeper dips due to their deeper location in the crystal, might also be involved in the small differences observed. However, these are likely to be subordinate to the influence of other factors, such as the selection of tracks for measurement. It is possible that any small deficit in the 3D lengths at high dip angles from these causes may actually contribute to the concordance of dip-corrected 3D measurements and projected HCT lengths, which are also subject to a very small underestimation of the true lengths due to the uncorrected dips.

One of the potential benefits of using 3D length measurements is the expected increase in the number of tracks available for measurement, and the results reported here show this increase to be typically about 40%. This increase was less than anticipated, and substantially less than the 3-4 times increase reported by Jonckheere and Ratschbacher (2010) using much deeper-penetrating implanted heavy ion tracks. The reason for this difference is probably that
the measurements reported here were made on confined TINTs etched from mostly relatively short semi-tracks allowing etchant penetration from the surface, which means that almost all measured tracks were at dips of $<30^\circ$, and the great majority were dipping at $<10^\circ$.

Sensitivity studies indicate that there is little potential to improve the quality and consistency of the 3D length results either by reducing the image spacing in the captured image $z$-stacks, or by using Confocal Laser Scanning Microscopy to increase the optical resolution. The statistically identical results obtained on the same tracks by conventional optical microscopy and CLSM mean that the latter has no advantages for track length measurement, and is impractical for routine use.

A distinct advantage of a digital image-based 3D measurement method is that it enables more consistent application of measurement protocols, which is realized principally by allowing for more precise cursor placement at greatly enlarged magnification. Digital image sets can also be shared easily between laboratories, providing an additional aid to standardization of procedures. However, it is also clear that strict control of other factors, such as etching, equipment conditions, sampling criteria, system calibration, etc., remain important in the effort to enhance compatibility of track length data sets. We suggest that including image-based 3D measurements with other endeavors to standardize measurement procedures, can contribute to the ongoing efforts to improve the reproducibility of track length data across different laboratories.

The results of this study indicate that 3D confined track length measurements on TINTs are directly comparable to conventional ‘horizontal only’ track length measurements and lead to
a moderate increase in the number of tracks available. As a result, it is concluded that 3D
confined track length measurements should be compatible with current annealing models
based on horizontal track length measurements, and therefore useful for thermal history
reconstruction. The moderate increase in the number of tracks available for measurement in
this way may be particularly useful for samples that lack sufficient horizontal tracks for
robust thermal history modeling.

ACKNOWLEDGEMENTS

This work has been supported by infrastructure grants through the AuScope Program under
the Australian National Collaborative Research Infrastructure Strategy (NCRIS). Development of the Automated Fission Track Analysis System at the University of
Melbourne was initially supported through an Australian Research Council Linkage Project
grant (LP0348767) in partnership with Autoscan Systems Pty Ltd. We would like to thank
Ling Chung and Abaz Alimanovic for technical support in the laboratory, and Ling Chung,
Vhairi Mackintosh, Matthew Barrand, Guangwei Li, Zhiyong Zhang, and Jianhui Liu for
assistance with the project. The manuscript was greatly improved by detailed reviews from
Raymond Jonckheere and Rich Ketcham, which were very much appreciated.

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Table 1. Annealing experiment details – Fish Canyon Tuff (FCT) apatites

<table>
<thead>
<tr>
<th>Slide</th>
<th>Primary fluence (n/cm²)</th>
<th>Annealing conditions</th>
<th>Secondary fluence (n/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1 (~16µm)</td>
<td>9 × 10¹⁵</td>
<td>unannealed</td>
<td></td>
</tr>
<tr>
<td>Control 2 (~13µm)*</td>
<td>9 × 10¹⁵</td>
<td>300°C for 60 mins</td>
<td>2.0 × 10¹⁵</td>
</tr>
<tr>
<td>Control 3 (~11µm)</td>
<td>9 × 10¹⁵</td>
<td>350°C for 60 mins</td>
<td>1.5 × 10¹⁵</td>
</tr>
<tr>
<td>Control 4 (~8µm)</td>
<td>9 × 10¹⁵</td>
<td>370°C for 60 mins</td>
<td>2.5 × 10¹⁶</td>
</tr>
<tr>
<td>FCT A (4:1 13µm, 16µm)</td>
<td>9 × 10¹⁵</td>
<td>300°C for 60 mins</td>
<td>8.0 × 10¹⁵</td>
</tr>
<tr>
<td>FCT B (4:1 11µm, 16µm)</td>
<td>9 × 10¹⁵</td>
<td>350°C for 60 mins</td>
<td>1.7 × 10¹⁶</td>
</tr>
<tr>
<td>FCT C (1:4 11µm, 16µm)</td>
<td>9 × 10¹⁵</td>
<td>370°C for 60 mins</td>
<td>1.1 × 10¹⁵</td>
</tr>
<tr>
<td>FCT E (1:1 13µm, 16µm)</td>
<td>9 × 10¹⁵</td>
<td>300°C for 60 mins</td>
<td>4.3 × 10¹⁵</td>
</tr>
<tr>
<td>FCT F (1:4 8µm, 16µm)</td>
<td>9 × 10¹⁵</td>
<td>370°C for 60 mins</td>
<td>1.7 × 10¹⁶</td>
</tr>
<tr>
<td>FCT G (4:1 8µm, 16µm)</td>
<td>9 × 10¹⁵</td>
<td>370°C for 60 mins</td>
<td>1.1 × 10¹⁵</td>
</tr>
<tr>
<td>FCT H (1:1 8µm, 16µm)</td>
<td>9 × 10¹⁵</td>
<td>370°C for 60 mins</td>
<td>4.3 × 10¹⁵</td>
</tr>
</tbody>
</table>

*Control 2 was not available for this study.

Controls 1–4 are single-irradiated samples representing three of the four discrete length components in the FCT mixtures. The remaining seven FCT samples contain two component mixtures of tracks following the second irradiation. Brackets show the ratio of the two components and their respective mean lengths in each case.

Table 2. Mean track length data for all samples

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Analyst 1 (UoM)</th>
<th>Analyst A (UCL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCTp(SE) (µm)</td>
<td>SD(µm)</td>
</tr>
<tr>
<td>Control 1</td>
<td>142</td>
<td>15.85(06)</td>
</tr>
<tr>
<td>Control 3</td>
<td>143</td>
<td>10.78(07)</td>
</tr>
<tr>
<td>Control 4</td>
<td>139</td>
<td>8.28(21)</td>
</tr>
<tr>
<td>FCT A</td>
<td>135</td>
<td>14.43(10)</td>
</tr>
<tr>
<td>FCT B</td>
<td>151</td>
<td>12.12(17)</td>
</tr>
<tr>
<td>FCT C</td>
<td>153</td>
<td>15.13(17)</td>
</tr>
<tr>
<td>FCT E</td>
<td>198</td>
<td>14.92(10)</td>
</tr>
<tr>
<td>FCT F</td>
<td>187</td>
<td>14.52(30)</td>
</tr>
<tr>
<td>FCT G</td>
<td>73</td>
<td>12.31(54)</td>
</tr>
<tr>
<td>FCT H</td>
<td>141</td>
<td>14.59(29)</td>
</tr>
<tr>
<td>DUR</td>
<td>162</td>
<td>14.14(07)</td>
</tr>
<tr>
<td>DUR-4</td>
<td>157</td>
<td>14.20(06)</td>
</tr>
</tbody>
</table>

UoM = University of Melbourne; UCL = University College London; N = number of confined fission tracks measured; HCTp = mean projected length for horizontal confined tracks (dip <10°); ACTt = mean true 3D length for tracks of all orientations; SE = standard error of the mean; SD = standard deviation.
**Table 3.** Mean 3D track length data for horizontal ($\theta \leq 10^\circ$) and inclined ($\theta > 10^\circ$) confined tracks

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>HCT length measurements ($\theta \leq 10^\circ$)</th>
<th>ICT length measurements ($\theta &gt; 10^\circ$)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N ($\mu$m)</td>
<td>SD ($\mu$m)</td>
<td>N ($\mu$m)</td>
</tr>
<tr>
<td>Control 1</td>
<td>143</td>
<td>15.91(06)</td>
<td>0.70</td>
</tr>
<tr>
<td>Control 3</td>
<td>143</td>
<td>10.81(08)</td>
<td>0.9</td>
</tr>
<tr>
<td>Control 4</td>
<td>139</td>
<td>8.30(21)</td>
<td>2.48</td>
</tr>
<tr>
<td>FCT A</td>
<td>135</td>
<td>14.47(10)</td>
<td>1.12</td>
</tr>
<tr>
<td>FCT B</td>
<td>151</td>
<td>12.16(17)</td>
<td>2.13</td>
</tr>
<tr>
<td>FCT C</td>
<td>153</td>
<td>15.18(17)</td>
<td>2.06</td>
</tr>
<tr>
<td>FCT E</td>
<td>198</td>
<td>14.97(10)</td>
<td>1.37</td>
</tr>
<tr>
<td>FCT F</td>
<td>187</td>
<td>14.56(30)</td>
<td>4.15</td>
</tr>
<tr>
<td>FCT G</td>
<td>73</td>
<td>12.36(54)</td>
<td>4.65</td>
</tr>
<tr>
<td>FCT H</td>
<td>141</td>
<td>14.64(29)</td>
<td>3.49</td>
</tr>
<tr>
<td>DUR</td>
<td>162</td>
<td>14.20(07)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

HCT$_t$ = mean 3D length of Horizontal Confined Tracks; ICT$_t$ = mean 3D length of Inclined Confined Tracks. Brackets show the standard error of the mean.

**Table 4.** Mean 3D track lengths for DUR measured using different vertical step-sizes

<table>
<thead>
<tr>
<th>Step-size ($\mu$m)*</th>
<th>N</th>
<th>ACT$_t$ (SE) ($\mu$m)</th>
<th>SD ($\mu$m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>80</td>
<td>14.08(09)</td>
<td>0.87</td>
</tr>
<tr>
<td>0.2</td>
<td>80</td>
<td>14.14(08)</td>
<td>0.89</td>
</tr>
<tr>
<td>0.3</td>
<td>80</td>
<td>14.10(08)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

* Distance between captured planes in the image stack.
Table 5. Comparison of conventional and confocal laser scanning microscopy measurements

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>Conventional wide field microscopy</th>
<th>Confocal laser scanning microscopy</th>
<th>Difference</th>
<th>Dip angle* (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ACT(_t) (SE) (µm)</td>
<td>SD (µm)</td>
<td>ACT(_t) (SE) (µm)</td>
<td>SD (µm)</td>
</tr>
<tr>
<td>Control 1</td>
<td>94</td>
<td>16.00(08)</td>
<td>0.80</td>
<td>16.12(09)</td>
<td>0.86</td>
</tr>
<tr>
<td>Control 3</td>
<td>96</td>
<td>10.83(09)</td>
<td>0.85</td>
<td>11.04(09)</td>
<td>0.84</td>
</tr>
<tr>
<td>Control 4</td>
<td>69</td>
<td>8.19(31)</td>
<td>2.57</td>
<td>8.41(30)</td>
<td>2.52</td>
</tr>
<tr>
<td>FCT A</td>
<td>73</td>
<td>14.41(13)</td>
<td>1.14</td>
<td>14.54(13)</td>
<td>1.15</td>
</tr>
<tr>
<td>FCT B</td>
<td>92</td>
<td>12.03(21)</td>
<td>2.01</td>
<td>12.20(21)</td>
<td>2.04</td>
</tr>
<tr>
<td>FCT C</td>
<td>61</td>
<td>15.02(28)</td>
<td>2.16</td>
<td>14.92(28)</td>
<td>2.18</td>
</tr>
<tr>
<td>FCT E</td>
<td>125</td>
<td>14.86(13)</td>
<td>1.43</td>
<td>14.96(13)</td>
<td>1.49</td>
</tr>
<tr>
<td>FCT F</td>
<td>106</td>
<td>15.43(30)</td>
<td>3.10</td>
<td>15.43(30)</td>
<td>3.11</td>
</tr>
<tr>
<td>FCT G</td>
<td>51</td>
<td>13.54(58)</td>
<td>4.16</td>
<td>13.63(59)</td>
<td>4.19</td>
</tr>
<tr>
<td>FCT H</td>
<td>82</td>
<td>15.23(30)</td>
<td>2.71</td>
<td>15.19(31)</td>
<td>2.78</td>
</tr>
</tbody>
</table>

*Dips determined from conventional wide field microscopy. All measurements are mean track lengths of measurements on the same tracks imaged by both methods.

Table 6. Replicate measurement of mean track lengths by Analyst 1 after a 2.5-year interval

<table>
<thead>
<tr>
<th>Sample</th>
<th>1st Analysis</th>
<th>2nd Analysis</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>ACT(_t) (SE) (µm)</td>
<td>SD (µm)</td>
</tr>
<tr>
<td>Control 1</td>
<td>171</td>
<td>15.91(06)</td>
<td>0.76</td>
</tr>
<tr>
<td>FCT A</td>
<td>191</td>
<td>14.34(09)</td>
<td>1.21</td>
</tr>
<tr>
<td>FCT B</td>
<td>213</td>
<td>11.99(14)</td>
<td>2.06</td>
</tr>
<tr>
<td>FCT G</td>
<td>120</td>
<td>12.51(40)</td>
<td>4.37</td>
</tr>
<tr>
<td>DUR</td>
<td>256</td>
<td>14.13(05)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Measurements made on the same captured image sets.
Table 7. Mean track length measurements for DUR-4 by six University of Melbourne analysts

<table>
<thead>
<tr>
<th>Analyst</th>
<th>N</th>
<th>ACT (_t) (SE) (µm)</th>
<th>SD (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>169</td>
<td>14.24(06)</td>
<td>0.79</td>
</tr>
<tr>
<td>2</td>
<td>120</td>
<td>14.20(07)</td>
<td>0.80</td>
</tr>
<tr>
<td>3</td>
<td>122</td>
<td>14.29(09)</td>
<td>0.98</td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>14.12(07)</td>
<td>0.82</td>
</tr>
<tr>
<td>5</td>
<td>130</td>
<td>14.14(07)</td>
<td>0.84</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>14.23(09)</td>
<td>0.94</td>
</tr>
</tbody>
</table>
Figure Captions

Figure 1. Three different measures of fission track length (colored lines) shown against a 3D coordinate system. These show the true track length \( l_t \) (red), the projected length \( l_p \) (green), and the apparent length (\( l_a \), uncorrected for refraction) in apatite. Apparent (measured) and true depths to the end of the track are represented by \( d_a \) (red), and \( d \) (blue) respectively, these being related by the refractive index. Angles shown are the azimuth to the c-axis \( \omega \), the true angle to the c-axis \( \phi \), and the dip angle \( \theta \). XOY is a plane parallel to the observation surface and contains one end of a confined fission track at the origin O. The X-axis is parallel to the crystallographic c-axis (modified after Galbraith and Laslett, 1988).

Figure 2. Confined fission track length distributions for all samples showing the ‘true’ 3D lengths (i.e. corrected for dip and refraction), \( l_t \), as grey histograms and red relative probability plots. Two sub-sets of these 3D length data are also shown for ‘horizontal’ (blue) \( (0 \leq 10^\circ, \text{blue}) \), and ‘inclined’ \( (\theta > 10^\circ, \text{green}) \) confined tracks as relative probability curves. Numbers in colors for each sample denote mean confined track lengths (ACT = All, HCT = Horizontal, and ICT = Inclined) and standard deviations for the three distributions. The number of tracks measured in each case is shown in brackets.

Figure 3. Residual differences between mean confined track lengths determined for ‘horizontal’ \( (0 \leq 10^\circ, \text{HCT}) \), inclined \( (\theta > 10^\circ, \text{ICT}) \), and all (ACT) fission tracks. Subscripts denote projected \( (l_p) \) and true \( (l_t) \) lengths as shown in Fig. 1. *Measured by Analyst A at UCL, all other measurements were made by Analyst 1 at UoM.
Figure 4. (A) 3D lengths of individual confined tracks plotted against dip angle, θ, in Control, FCT and DUR apatite samples. Alignment of the data into discrete dot-curve arrays is a result of the depth measurements being limited to specific image planes in the z-stack (Fig. 5). The vertical image spacing was 0.3 µm for all samples except DUR, for which it was 0.2 µm. The different length components can be clearly seen in the mixtures of unannealed and moderately to highly annealed tracks (FCT B, C, F, G, H). (B) Histogram of dip angles for all tracks measured showing that the number of observed tracks decreases rapidly with increasing dip angle θ, and that almost no confined tracks are observed at dips greater than 30°.

Figure 5. (A) Individual 3D fission track lengths plotted against dip angle in sample FCT C, measured in a transmitted light z-stack with a vertical image spacing of 0.3 µm. Colors show the depth component of each 3D length measurement and how the results align into discrete dot-curves corresponding to track lengths that cover the same fixed intervals between the image planes defining the ends of each track. (B) Simplified diagram illustrating how dip angles can increase continuously while the depth is limited to discrete intervals between two image planes. Dashed lines represent two planes in the image stacks where the track ends are located.

Figure 6. (A) Variation in lengths of individual confined tracks as a function of dip angle in apatite DUR, as summarized by the mean lengths in Table 4. Results are shown for measurements made on z-stack images with three different step-sizes between the image
planes. The spacing between the dot-curves varies according to the step-size, but in all three cases the overall range of variation is the same. (B) Comparison of paired 3D track lengths for 88 individual confined tracks measured in image stacks with step-sizes of 0.3 µm and 0.1 µm.

**Figure 7.** Comparison of images on the same horizontal track (dip ≈ 0°) captured by (A) confocal laser scanning microscopy ($l_t = 11.03$ µm) and (B) conventional wide field transmitted light microscopy ($l_t = 10.93$ µm). Confocal imaging increases the resolution enabling the ends of the track to be more clearly defined, but is only useful for relatively shallow dipping tracks.

**Figure 8.** Comparison of individual 3D track lengths on the same confined tracks measured by confocal laser scanning microscopy and conventional wide field transmitted light microscopy for ten samples studied, confirming a high degree of consistency between the two methods.

**Figure 9.** Mean confined track lengths and uncertainties for apatite sample DUR-4. (A) Summary of all track length results from 55 analysts in the inter-laboratory comparison of Ketcham et al., (2015) measured as projected lengths of horizontal confined tracks ($HCT_p$). Data shown in red are the mean 3D lengths ($ACT_t$) measured by six UoM analysts in this study (Table 7). Green dashed lines denote the 14.12 ± 0.08 µm mean value reported by Ketcham et al., (2015). (B) Expansion of the mean track length results from this study (Table
738 7). Blue dashed lines denote the mean value of $14.20 \pm 0.03 \mu m$ for the six measurements.
Figure 1 revised
<table>
<thead>
<tr>
<th></th>
<th>Control 1</th>
<th>Control 2</th>
<th>Control 3</th>
<th>Control 4</th>
<th>FCT A</th>
<th>FCT B</th>
<th>FCT C</th>
<th>FCT D</th>
<th>FCT E</th>
<th>FCT F</th>
<th>FCT G</th>
<th>FCT H</th>
<th>DUR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15.91 ± 0.76 (171)</td>
<td>15.94 ± 0.77 (143)</td>
<td>10.77 ± 0.98 (184)</td>
<td>10.81 ± 0.9 (143)</td>
<td>8.25 ± 2.36 (181)</td>
<td>14.34 ± 1.21 (191)</td>
<td>11.99 ± 2.06 (213)</td>
<td>12.16 ± 2.13 (151)</td>
<td>11.58 ± 1.81 (62)</td>
<td>15.01 ± 2.18 (198)</td>
<td>14.9 ± 2.06 (153)</td>
<td>14.42 ± 2.46 (45)</td>
<td>14.34 ± 4.3 (253)</td>
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<td>15.94 ± 0.77 (143)</td>
<td>15.77 ± 0.66 (28)</td>
<td>10.81 ± 0.9 (143)</td>
<td>10.62 ± 1.22 (41)</td>
<td>8.3 ± 2.48 (139)</td>
<td>14.47 ± 1.12 (135)</td>
<td>11.58 ± 2.06 (62)</td>
<td>11.58 ± 1.81 (62)</td>
<td>15.18 ± 1.37 (198)</td>
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**Legend:**
- $\text{ACT}_t \pm 1 \text{ SD (N)}$
- $\text{HCT}_t \pm 1 \text{ SD (N)}$
- $\text{ICT}_t \pm 1 \text{ SD (N)}$

Figure 2 revised
Figure 3 Revised
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Dip angle ($\theta^\circ$)

Track length (µm)

Figure 4 revised
Figure 5 revised
Figure 6 revised
Figure 7 revised
Figure 8 revised
Figure 9 revised

(A) 10.08±0.22
14.12±0.08

(B) 14.20±0.03

Data source:
- Ketcham et al., 2015
- This study