

The Need for Fission Track Data Transparency

Murat T. Tamer^{1,2}, Ling Chung³, Richard A. Ketcham², Andrew J. W. Gleadow³

¹State Key Laboratory of Earthquake Dynamics, Institute of Geology, China Earthquake Administration, Beijing 100029, China

²Jackson School of Geosciences, The University of Texas at Austin, 78712 Austin, Texas, U.S.A.

³School of Geography, Earth and Atmospheric Sciences, University of Melbourne, 3010 Melbourne, Victoria, Australia

Correspondence to: Murat T. Tamer (murat@ies.ac.cn)

Abstract. We report a new image-based inter-analyst study to investigate fission-track grain selection and analysis by 13 participants from an image data set that included grains of variable quality. Results suggest that participants with less experience show a higher rate of selecting unsuitable grains, while participants from the same laboratories generally provide similar results. Less analysis experience may result in the rejection of suitable grains, or inclusion of unsuitable ones. While inappropriate omission and inclusion can both bias results, the latter is more pernicious due to the standard practice of achieving a predecided number of analyses; particularly in difficult samples, there is a danger of “squeezing the rock” by weakening selection criteria. Juxtaposing selected regions of interest (ROIs) on the same grains indicates that zoned grains and grains with inclusions and defects yield varying track density estimates, indicating that ROI placement can be an influential factor. We propose developing image data repositories for global data transparency, a global guidance for fission-track analysis, digital teaching modules, and open science. We also point out the need for new approaches for zeta calibration that include consideration of grain quality, methods of uranium determination, and etching protocols.

1 Introduction

Fission-track dating and thermal history modeling are widely used for near-surface research in earth sciences, across a large spectrum of subjects such as landscape evolution (Reiners and Shuster 2009; Lemot et al., 2023; Gallen et al., 2023), climate change (Barnes et al., 2012; Qiu and Liu 2018; Yu et al., 2022), glacier-driven exhumation (Balestrieri et al., 1991; Fitzgerald and Goodge 2022; Karaođlan et al., 2023), natural resource exploration (Dumitru et al., 1991; Deng et al., 2015; Qiu et al., 2023; Gülyüz et al., 2024) and biodiversity (Kohn et al., 1992; Torres et al., 2013; Bernet et al., 2023). Five essential ‘ingredients’ are required for fission-track time-temperature modeling: the track densities calculated from (1) track counts over (2) a selected region of interest; (3) preferably more than a few tens of confined track lengths per sample; (4) average etch pit diameter (D_{par}) measurements per grain, or chemical information to infer kinetics; and (5) an estimate of the ^{238}U concentration (Tagami and O’Sullivan 2005). While laser ablation mass spectrometry has become an alternative (Hasebe et al., 2014) to the widely used external detector method (EDM) (Gleadow and Lovering 1977) for uranium content determination, the first four inputs are still largely analyst-driven, although recent developments in image analysis and AI have



35 contributed significant advances in auto-counting and auto-measurement (Gleadow et al., 2009, 2019; Nachtergaele and De Grave 2021; Li et al., 2022; Ren et al., 2023; Boone et al., 2023). Previous apatite fission-track inter-laboratory and inter-analyst experiments showed significant variation in measurements for the same samples and even standards (Naeser et al., 1981; Miller et al., 1985, 1990, 1993; Barbarand et al., 2003a; Ketcham et al., 2009; Sobel and Seward, 2010; Ketcham et al., 2015; Ketcham et al., 2018; Tamer et al., 2019). These variations have been attributed to a broad range of factors including instrumentation, analytical preferences, etching protocol, and analyst selection criteria.

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The process of selecting suitable grains, and regions of interest within those grains, has been relatively unexplored in these studies but may exert a first-order influence on the data quality and extractable thermal history information. From a given set of grains, grain selection influences results in several ways. Grains where oily fluids have penetrated into tracks may hinder the recognition of some surface tracks, causing confined tracks to appear shorter and thus more annealed (Ketcham et al., 45 2015), leading to underestimation of ages and overestimation of temperatures. Grains with excessive defects, such as polishing artefacts or etched dislocations, may cause misidentification of some spurious features as actual tracks and cause overestimation of ages. Track density can vary by up to 35% if the grain is oriented without the c-axis in the viewing plane (Aslanian et al., 2022), making both the resulting age and etch figure dimensions (e.g. Dpar) inaccurate, thereby affecting estimates of kinetics and initial track length. A perceived need to meet targets for the number of grains analyzed may cause an 50 analyst to select borderline-acceptable grains or tracks that may not have been selected otherwise (Tamer and Ketcham, 2023).

Whereas the area counted for fission-track density determinations has typically been defined by boxes in an eyepiece reticule, modern image-based systems allow the user to draw an arbitrarily shaped region of interest. In both cases, this process must be executed with care. Regions of interest need to be placed so that the grain surface they encompass is not biased with respect 55 to the ability to host detectable tracks. Regions of interest within one fission-fragment range of the grain edge will not sample tracks from a full 4π geometry (Donelick et al., 2005), and including sizable defects and cracks in the region of interest may result in uncountable areas; both effects will bias ages lower. Regions of interest that include zones with different U content complicate the accurate determination of U across the track-generating region (Vermeesch, 2017), and suffer edge effects from sampling a 4π region that is variable. This bias can result both from methods using laser ablation, where typically a smaller 60 area is sampled for the U-determination than for the spontaneous track count, and the external detector method, where perfect matching between spontaneous and induced track regions of interest can be difficult to achieve, especially where the track density is low.

The suggested number of grains for age measurements for igneous-type samples is typically ~20, or more if there is any 65 indication of kinetic variation (Donelick et al., 2005), while for detrital samples it is ~120 or more (Vermeesch 2004). If grains



are few or of low quality, an analyst may consciously or unconsciously “squeeze the rock” by adding some borderline-quality grains to provide the expected data quantity. Aiming for specific quantities of data may eventually cause a loss of quality.

We carried out a new inter-analyst experiment designed to investigate variability in grain selection and region of interest
70 definition criteria. Building upon a previous two-analyst study (Tamer et al., 2019), we also tested the identification of confined track lengths. Participants were asked to perform apatite FT analysis on a selection of grains drawn from an identical image set featuring variable grain quality using software that records all details of the analysis as overlays in a .xml file, thereby allowing for subsequent review. Analysts were also asked to fill out a questionnaire about their approach.

75 **2 Materials and Methods**

2.1 Image Data Repository

We created an image data repository consisting of 41 grain and 3 graticule images from the in-house fission-track data repositories at the University of Melbourne (UM) and the University of Texas at Austin (UT). Images from UM were captured by Ling Chung using a Zeiss Axio Imager M1m microscope with an IDS µEye camera and white balance correction, while
80 images from UT were taken by Sean Sanguinito and Murat Tamer, using a Zeiss Axio Imager M2m microscope with Olympus SYS UC30 camera and no white balance. Grains from UT were etched with 5.5M HNO₃ at 21 °C for 20s (Carlson et al., 1999), while the grains from UM were etched with 5M HNO₃ at 20 °C for 20s (Gleadow et al. 1986). 36 grains were selected for track density measurements and 5 for confined track length measurements. To test the self-reproducibility of the analytical results we repeated one grain image as two different grain areas (Grains 07 and 16). The grain descriptions are given in supplementary
85 file Table S1.

2.2 Announcement and Participant Instructions

The announcement of the study was made at the 17th International Conference on Thermochronology, 2021, Santa Fe (Tamer et al, 2021) and in relevant email lists. The participants were asked to perform track density and confined track length and Dpar measurements using their preferred approach, including any analytical software, manual measurement, or AI-based
90 analysis. The participants were not instructed to reach a given number of grains or confined track length analyses but were instructed to skip or accept grains for analysis according to their own judgement.

This experiment was made possible by Fission Track Studio, a dual software suite developed by the Melbourne Thermochronological Research Group (MTRG) that is capable of automatic grain stack-image acquisition (TrackWorks) and
95 image review and measurement (FastTracks). The FastTracks program offers manual and automated analytical tools for obtaining all essential parameters for FT dating as well as a cross-section tool for precise dip angle determination for length measurements. All analytical results were recorded in an .xml file that can be reloaded for a follow-up analysis and review.

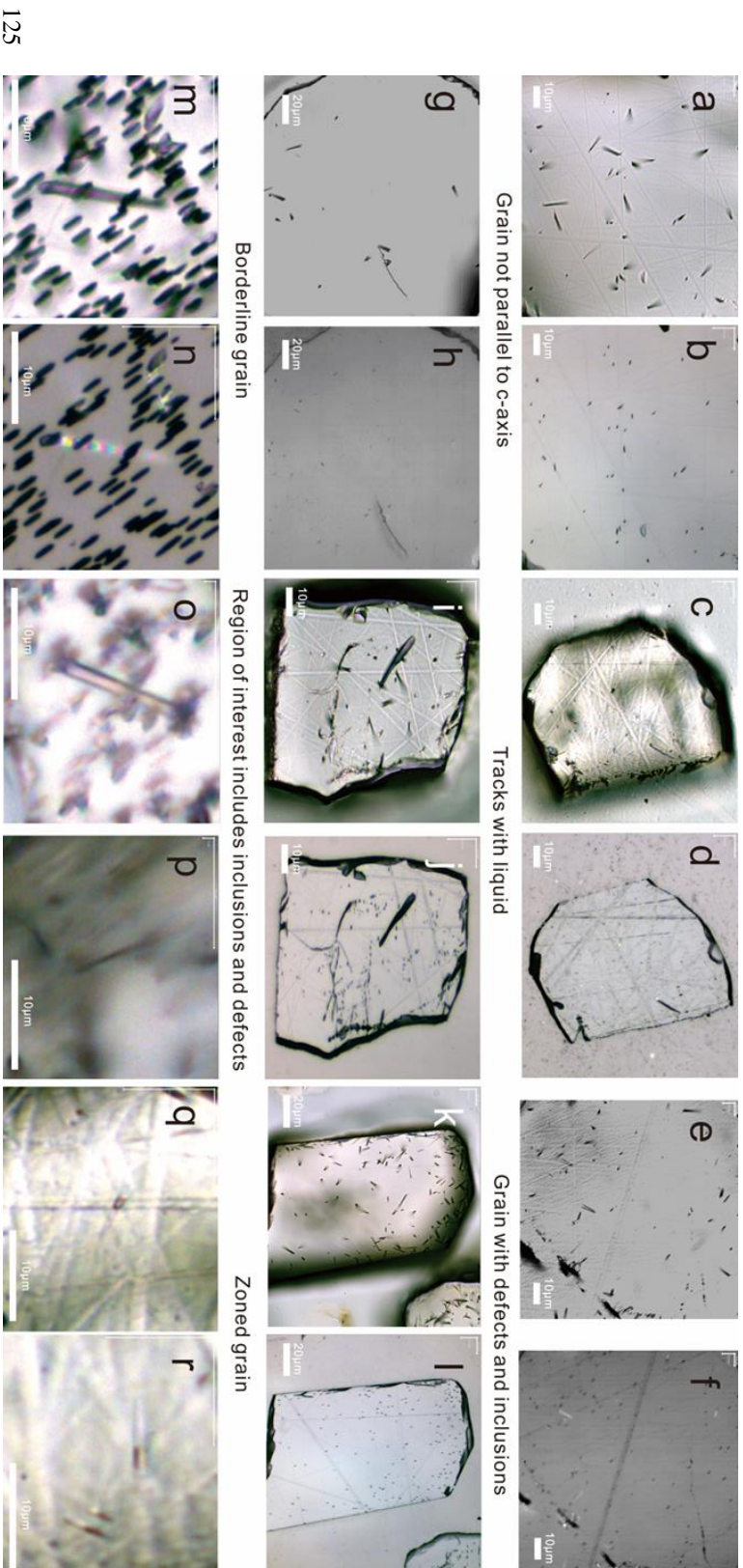


The University of Melbourne provided a temporary FastTracks license and a detailed user manual for those who wanted to participate in this study. The participants had the option to reveal their names and affiliations or to be anonymous. A
100 participant's submission was accepted only if the analysis was performed by a single analyst.

2.3 Reviewer Criteria

In the absence of absolute standards, we used the grain selection criteria of L. Chung and the confined track length measurement judgments of M. Tamer as reference points for the review of the participant results. However, no fission-track analyst can claim complete certainty in their judgments about track features and we do not suggest that these reference results
105 represent 'true' values. Rather they are simply used as reference values that are probably typical of reasonably experienced analysts. They were used as the starting point for a detailed grain-by-grain and track-by-track discussion with the participants to arrive at a consensus view and to ascertain which factors are most likely to lead to discrepancies between analysts. Such a detailed analysis has not previously been undertaken to our knowledge and would be all but impossible without the image-based approach used here. To test the objectivity of the reviewers, the participants were shown those of their selections and
110 measurements that the reviewers considered questionable or in some cases, erroneous, after which they could acknowledge or dispute the review.

Grains were judged to be suitable, unsuitable, or borderline. A grain having any of the three following properties was considered unsuitable: (1) the polished surface was not parallel to the c-axis (Fig. 1 a, b), (2) fluids were present in tracks (Fig.
115 1 c, d), (3) an excessive number of inclusions/defects/uncertain features was intermingled with actual tracks (Fig. 1 e, f). Additionally, heterogeneous U distribution within the grain, judging from the distribution of spontaneous tracks, can be a complicating factor, especially if LAICPMS spot analysis is used for U determination, but also from misalignment of the spontaneous and induced track regions of interest using the EDM. In samples with low abundance and/or low-quality of grains, some borderline-quality grains may be included in the resulting data sets (Fig. 1 g, h). Selection of the region of interest may
120 become a challenge for inclusion/defect-rich (Fig 1 i, j) and zoned grains (Fig 1 k, l). A confined track length is measurable as long as both ends are not exposed (Fig 1 m, n) at the surface, visible and unobscured by surrounding features (Fig 1 o, p). Moreover, it is important that confined tracks are not filled, fully or partially, with oily fluids, such as may result from fingerprints, which significantly affects their optical contrast within the host mineral (Fig 1 q, r). Any measurement that does not meet these criteria is considered invalid.



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Figure 1: Examples of grains and tracks that could cause erroneous grain and region of interest selection or invalid confined track length measurement for fission-track analyses. A grain not parallel to the c-axis appears to be acceptable in transmitted light (a), but varying etch pit orientations clearly show that the grain is not parallel to the c-axis in reflected light (b). Transmitted and reflected light images showing how a dirty grain surface with liquids in tracks can impair track recognition (c,d). Transmitted and reflected light images of a grain showing an excess of non-track features such as defect and inclusion that are difficult to distinguish from actual tracks (e, f). Transmitted and reflected light images of a grain with a low and uneven track density, suggesting the possible presence of zoning that would need to be accounted for (g, h). Transmitted and reflected light images of a grain showing large defects and inclusions occupying space obscuring fission tracks, resulting in underestimation of the fission-track density if these features are included in the region of interest (i,j). Transmitted and reflected light images of a zoned grain, for which different placements of the region of interest and location of ablation point(s) may yield divergent ages (k,l). Transmitted and reflected light images of a would-be confined track, where the reflected light image shows one tip may be exposed to the surface (m,n). Confined tracks may also be rendered invalid for measurement by obscuring features (o,p), or partial fluid fillings (q,r).



3 Results and Discussion

Thirteen analysts returned completed questionnaires, though one of them could not provide the .xml file due to technical
140 problems. Although L. Chung and M. Tamer evaluated each other's analyses, we consider them as reviewers in this study.
Two additional analysts from the same laboratory with different years of experience submitted answers in one combined .xml
file. Because they were unable to disentangle their results or reconduct them independently, their results are not included in
the analysis below. While some participants wished to remain anonymous, others chose to be transparent with their identities;
Table S2 provides the list of participants. Participants' overall and recent experience and their current fission-track setup, a
145 summary of the questionnaire, selection rates for grains, and validity rates for confined track lengths are provided in Table 1.
Excluding one of the repeated grains for checking self-reproducibility (Grain 16), of the 35 grain image sets, L. Chung
estimated that 22 are suitable and 13 are unsuitable for fission track analysis. In grain-by-grain checking, we counted how
many of the suitable grains were selected and how many of the unsuitable grains were rejected. The resulting suitable and
unsuitable grain selection rates are reported as percentages. M. Tamer examined every measured confined track length to check
150 validity as determined above. The percentage of valid measurements is reported as the confined track length measurement
validity rate. We did not evaluate how many valid tracks were excluded, as there was no way to determine whether such tracks
were intentionally omitted or simply missed.

3.1 Graticule Calibration

Graticule images taken by microscopes at UM and UT were included in the data set for calibration. Although calibration is an
155 essential step before performing an analysis, only five participants reported measuring them. Some omissions may have been
due to not fully understanding the terms of the experiment. To make the comparison of results easier, we used the default
graticule calibration for all analysts.

3.2 Self-Reproducibility

Grains 7 and 16 are duplicated images of the same grain in our data set. While some participants skipped Grain 16 after noticing
160 the repetition, some performed density measurements on both grains. Although these remeasurements demonstrated high self-
reproducibility (Fig 2), minimum and maximum densities vary by $\pm 30\%$. The difference can be traced to the varying region
of interest selection, light source preference, and track counting routines. Although the zeta method (Hurford and Green, 1983)
is intended to normalize some differences among analysts, the degree of variation shown here is more severe than that implied
by typical variation in zeta factors ($\sim 20\%$).

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Analyst	Total years of experience	Activity in the past two years	In-house fission-track setup	Auto c-axis assignment tool	Graticule measurement	Total number of grains selected as suitable	Suitable grain selection rate (%)	Unsuitable grain selection rate (%)	Number of measured confined track lengths	Confined track length measurement validity rate (%)
1	7	Yes	AS	Yes	No	22	100	8	16	69
2	14	Yes	AS	No	Yes	22	100	0	14	Reviewer
3	7	Yes	AS	No	Yes	29	95	62	31	32
4	6	Yes	AS	Yes	No	23	91	23	8	88
5	5	Yes	AS	Yes	No	11	50	0	19	63
6	4	Yes	C	Yes	No	26	95	39	5	80
7	6	Yes	C	No	No	26	100	31	10	60
8	4	Yes	AS	No	No	35	100	100	16	56
9	5	Yes	AS	No	No	34	100	93	33	55
10	17	Yes	AS	Yes	Yes	22	Reviewer	Reviewer	18	94
11	30	No	D	No	Yes	22	95	8	16	81
12	2	Yes	AS	No	No	31	95	77	4	25
13	5	Yes	AS	No	No	28	95	54	49	30
14	9	Yes	AS	No	Yes	24	100	15	18	78
15	2	Yes	AS	No	No	22	86	23	22	59
16*	40	Yes	C	N/A	N/A	22	100	0	N/A	N/A

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Table 1: Summary of the questionnaire, rates of grain selections, and confined track length measurement validities.

AS: Autosecan, C: Custom; D: Dumitru System. *: The analyst did not participate in the experiment but evaluated the grains as suitable and unsuitable.

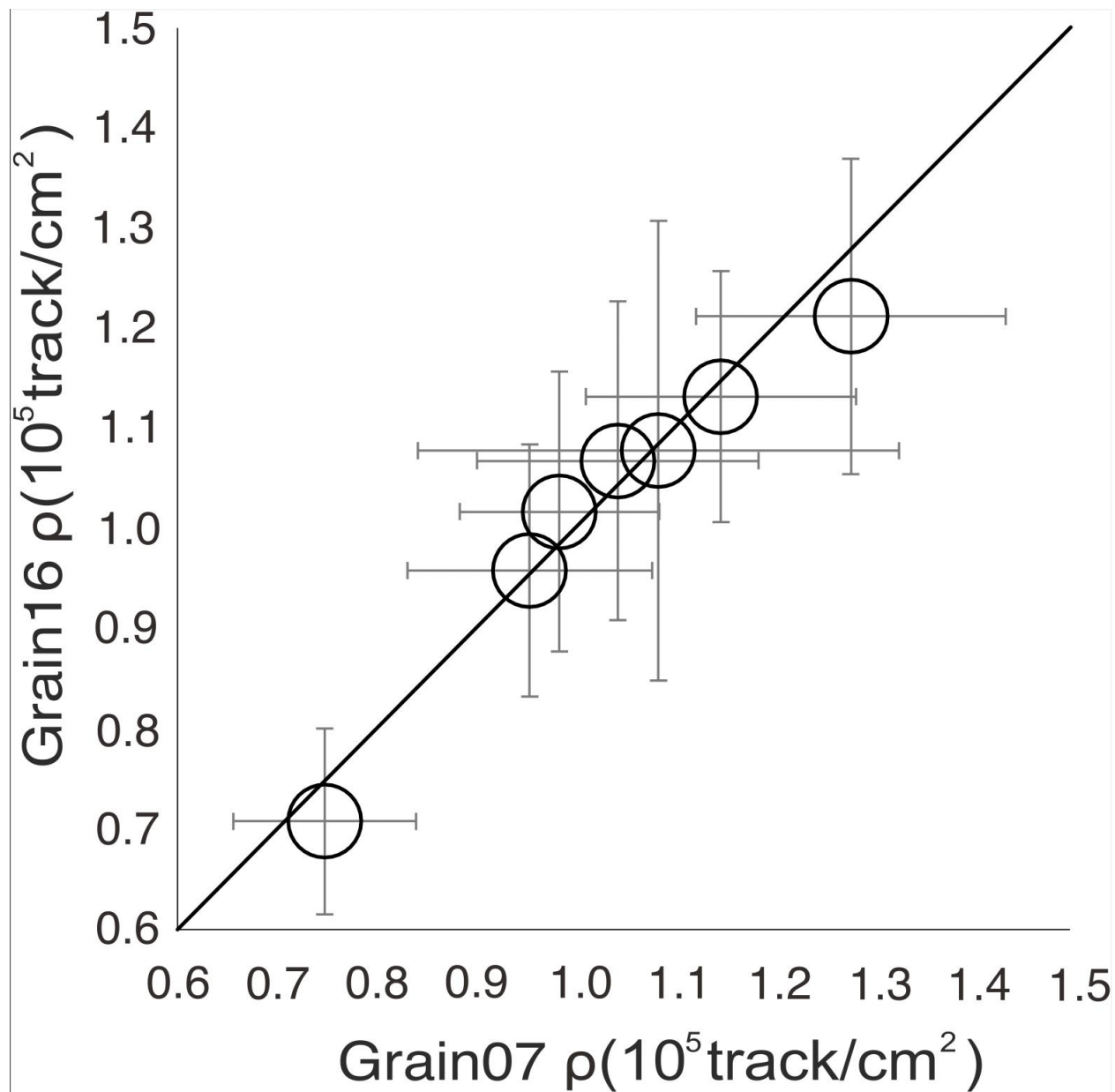


Figure 2: Self-reproducibility of track density (ρ) determinations on replicated grain images for seven analysts.

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3.3 Post-review follow-up and objectivity of the review

After the initial review of grain and confined track length measurements, a follow-up meeting with each participant was conducted to discuss each judgment deemed questionable or unsuitable by the reviewers. Virtually all participants



acknowledged all inappropriate grain selections and confined track length measurements, except Participant 6 considered
180 Grain 08 and Grain 31 as borderline instead of unsuitable. This high rate of acknowledgment by the participants supports the
soundness of the criteria utilized by the reviewers. According to the participants, inappropriate selection and measurements
stemmed from different factors. While some participants cited a lack of attention to details (e.g. poor identification of track
ends), others stated that they have been choosing some unsuitable grains in their routine fission-track studies since their
training. Some of the participants mentioned that they knowingly added unsuitable grains to the data sets in the past to meet
185 the expected number of grains. Some of the participants used FastTracks' automatic tools for c-axis orientation and dpar length
measurements.

3.4 Track Density and Confined Track Length Distributions

The track density and confined track length distributions of each participant show several patterns (Fig 3). The density
190 distributions of suitable grains appear to be somewhat more relatable and consistent than for unsuitable grains, and the inclusion
of unsuitable grains in all cases skewed the track density distribution to lower values. A similar effect tends to be present in
the confined track length distributions, but not in all cases. The confined track length distributions show that the analysts
observed and measured different numbers of confined track lengths with a varying number of measurements on valid tracks.
Combining all valid tracks yields a histogram with confined track lengths ranging from 8 to 17 μm . An analyst adopting
195 different selection criteria may select confined tracks that were exposed to etchant for different amounts of time (Tamer and
Ketcham 2023). Participants 1 and 10 and Participants 8 and 9 are from the same two laboratories and show similarities in
their respective track density results. This may be related to the shared training and/or analytical routine in counting, though
for length measurements participants 8 and 9 had more divergent results, possibly due to different personal selection criteria.
Dpar measurements on suitable grains tend to be more similar with some outliers, while the Dpars of unsuitable grains provide
200 higher dispersion (Fig S1). Post-experiment interviews with participants suggested that the dispersion of Dpars on suitable
grains may have stemmed from different levels of zoom applied.

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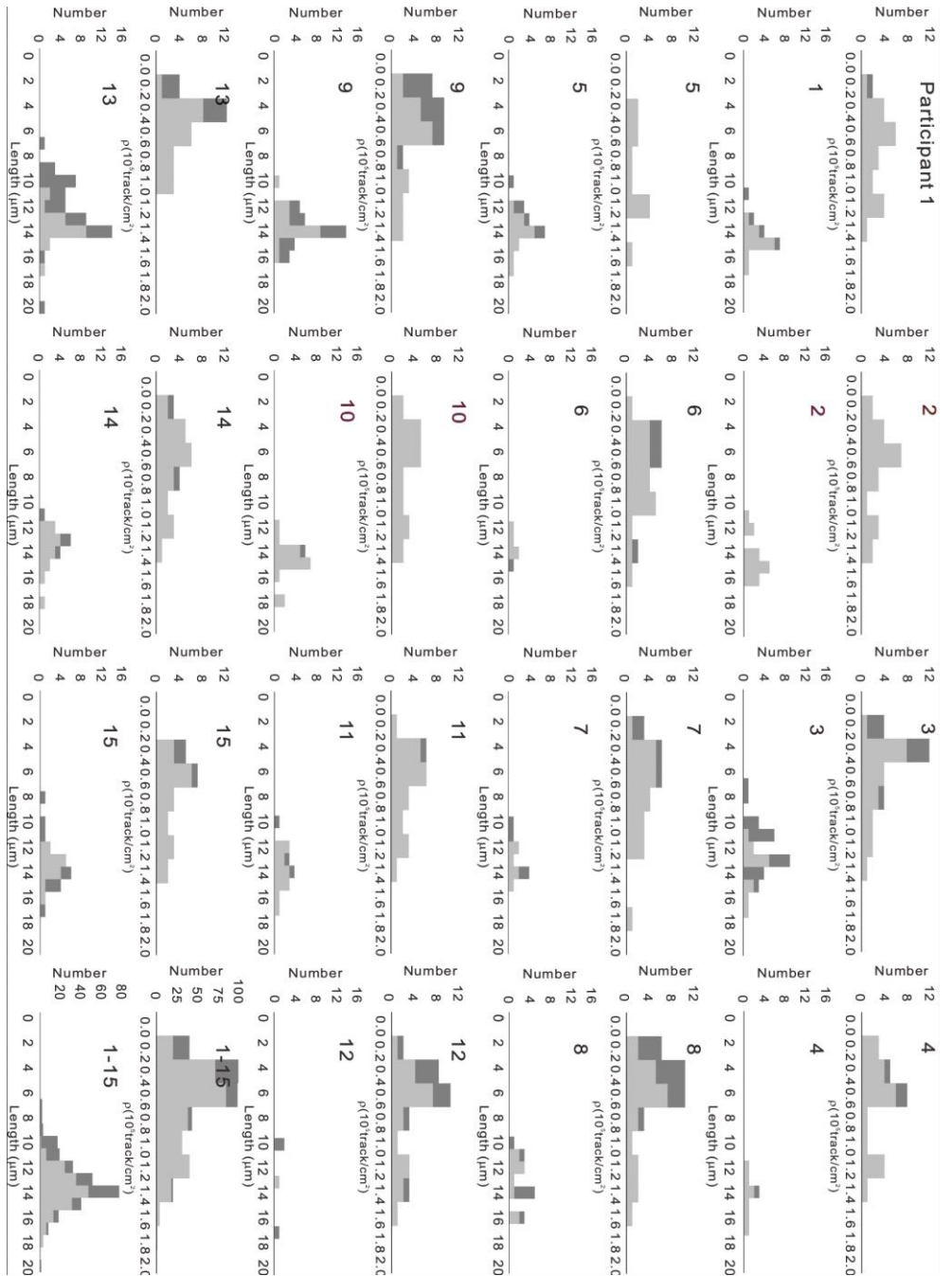


Figure 3: Track density (p) and confined track length distributions of each participant and the reviewers (2 and 10, in red). Participant numbers are indicated at the top left. The cumulative result for all participants is shown at the bottom right (1-15). Dark grey shows measurements of grains and confined tracks assessed to be suitable, and light grey displays the measurements of suitable selections.



215 3.5 Impact of Experience

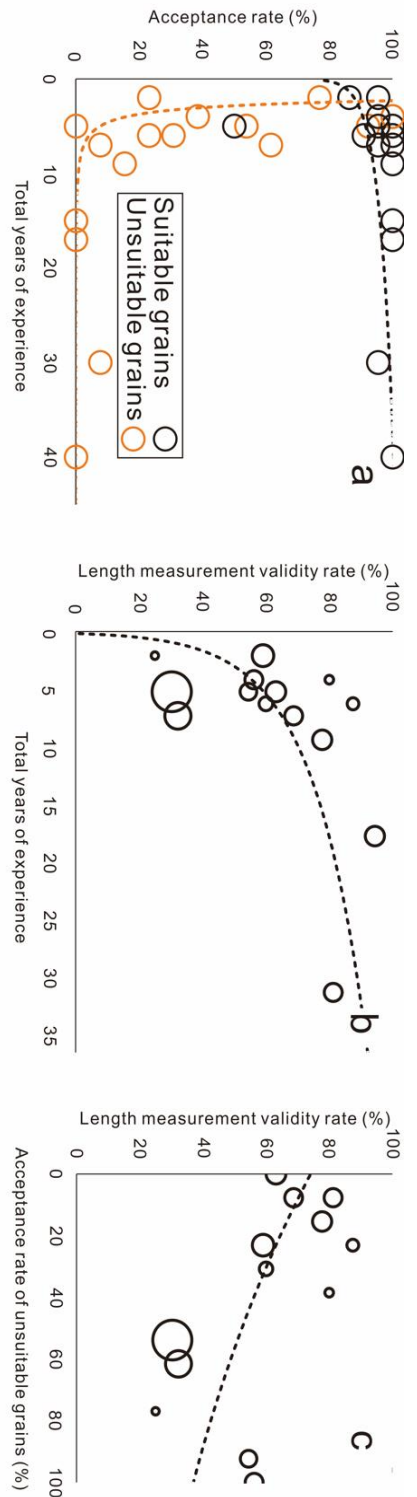
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3.6 Region of Interest (ROI) Selection

230 Track density measurements on defect/inclusion-free grains with homogeneous track distributions may not be greatly affected by different region of interest (ROI) selections, but inclusion of large defects in the ROI can cause an underestimation of density by obscuring tracks (Fig 4d). Similarly, the selection of high and low track density areas within a zoned grain can yield widely varying density determinations (Fig 4e). A single-spot or even dual-spot laser ablation approach on such grains may result in a significant dispersion of dates depending on the analyst's ROI selection. Several participants placed ROIs in too-
235 close proximity to the mineral border ($< \sim 10 \mu\text{m}$, Fig 4 d,e), where track registration is below the required 4π geometry (Donelick et al., 2005).

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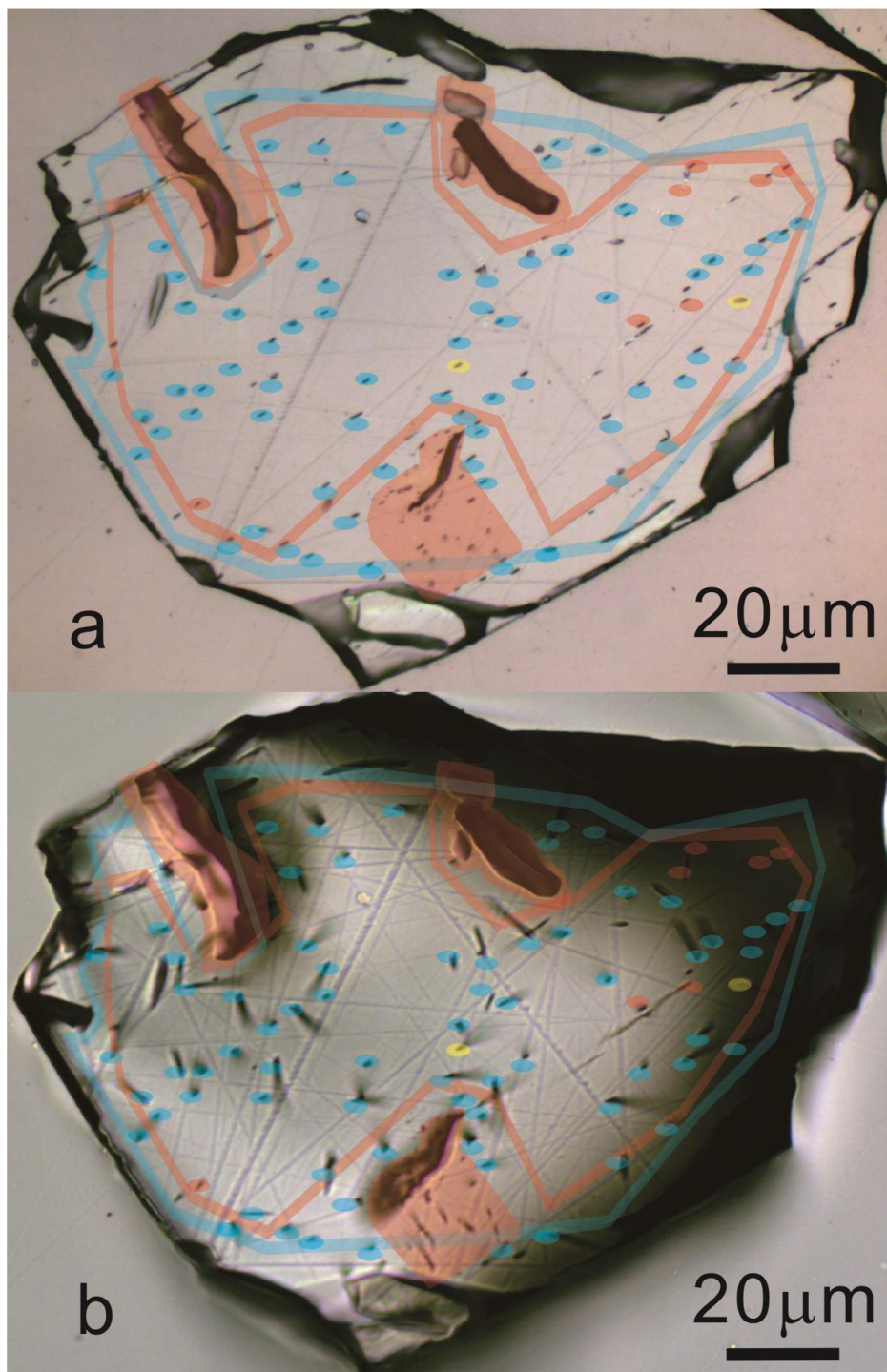


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Figure 4: Correlation of years of fission-track track experience against acceptance rate of suitable and unsuitable grains for density measurement (a) and confined track length measurement validity rate (b). The confined track length measurement validity rate against the acceptance rate of unsuitable grains is shown in c. The size of the circles in b and c reflect the number of confined track length measurements. Participant selections of the region of interest are juxtaposed in a defect/inclusion-rich grain (d) and a zoned grain (e).



3.7 Region of Interest (ROI) Selection

To demonstrate some effects of the light source and ROI specification on density measurement, at the request of one participant, we reanalyzed their track density for Grain 07, which was significantly lower than most of the group. Figure 5 shows the comparison on a track-by-track basis. Perhaps importantly, this analyst revealed a preference for counting tracks in transmitted light only. However, counting tracks solely in transmitted-light images can cause an underestimation of the track density (Aslanian et al., 2022; Tamer and Ketcham 2023). Figure 5 a and b show the transmitted and reflected light image for this particular grain, with the participant analysis in blue and re-analysis using images from both light sources in red with excluded counts in green. This reanalysis suggested that some genuine tracks had been overlooked, especially in the region on the right that is shaded in transmitted light. After adjustment of the ROI, the addition of the overlooked tracks in reflected light, and the exclusion of a track showing a defect-like pattern, the track density increases by ~35%.



265 **Figure 5: Transmitted and reflected light images of Grain 7 (a, b) with the analysis of one participant (blue) and a reviewer's proposed correction (red). Excluded defect areas and tracks.**



3.8. Highlights of participant comments on the data set

Participants 1 and 5 found the images taken with no white balance to be not ideal for counting, and participants 5 and 7 mentioned that the grains etched with the 5.0 M HNO₃ 20s 20°C (Gleadow et al. 1986) protocol appear to be under-etched.

4 Implications, Suggestions and Conclusions

270 Analysts may consider unsuitable grains and/or conduct invalid confined track length measurements depending on their years
of experience, training, and the difficulty in finding sufficient grains to meet analytical goals. “Squeezing the rock”, or indeed
“the grain”, to extract sufficient data in this way can lead to improper thermal history information. Comprehensive laboratory
training and calibrations are essential for fission-track analysts to avoid these problems. Results of graticule and confined track
length calibrations and the identity of the analyst should be stated in publications.

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ROI selection may cause varying track density determinations, especially in zoned and defect-rich grains. While a single-spot
laser ablation analysis is a time-efficient approach, its application on such grains may result in varying U determinations unless
the laser spot covers a high proportion of the counted area (e.g., Cogné et al. 2020). Laser uranium mapping (Ansberque et al.,
2021) or EDM (Gleadow and Lovering 1977) approaches require more work, but in ideal cases may better represent the
280 selected region of interest. However, precise matching of spontaneous and induced track areas in the EDM can also be difficult
in some cases. These approaches may also be more effective in identifying zoned grains when the spontaneous track density
is low.

Zeta calibration (ζ) against a set of age standards is intended to normalize for uncertainties in some parameters in the age
285 equation, such as thermal neutron fluence (ϕ) and the spontaneous fission decay constant (λ_f), and to account for varying
counting efficiencies of different analysts (Hurford and Green 1983). This method assumes, however, that a calibration derived
from measurements on near-ideal standard samples with minimal inclusions and defects (e.g. Durango, Fish Canyon Tuff
apatites) also reflects analyst judgements in unknown samples, which may yield any quality of grains. Zoning, high levels of
inclusions and defects, and variable ROI selection may cause significant divergences unaccounted for by zeta calibration,
290 particularly for a less-experienced analyst. These have been studied partially (Vermeesch 2017; Cogné and Gallagher 2021)
but further work is needed on this matter.

Among the available confined tracks, analysts can select tracks with different effective etch times based on their individual
perceptions and criteria. Some participants highlighted that the grains etched with 5.0 M HNO₃ at 20°C for 20s (e.g. Gleadow
295 et al. 1986) appear under-etched, which agrees with a previous inter-analyst comparison experiment (Tamer et al., 2019). A



proposed two-step etching protocol (5.5 HNO₃ 21°C 20+10s) allows analysts to select any suitable track but ensures that the final confined track length data set does not contain under-etched tracks (Tamer and Ketcham 2023).

300 The application of AI and machine learning methods have become popular topics in various research fields in earth sciences including fission-track counting and confined track length measurements. The quality of any automated analyses will be defined by not only sophisticated algorithms but also the fission-track analysis experience of the initial “teacher” of the AI, including hardware preferences during image acquisition and the resulting image quality. Determining which grains and tracks are acceptable for measurement represents an additional challenge for AI method development; training cannot be based on good images alone but must also include features to avoid based on sometimes subtle indications.

305 The exposure of the “ingredients” of thermal history modeling is limited to data summary tables and sometimes raw data as supplementary files to research articles. Although fission-track data have generally fared well in inter-laboratory age comparisons in recent years, these have tended to utilize relatively straightforward samples. This study illustrates some of the potential hazards of fission-track analysis of more challenging materials, but also pathways toward improving data reliability. In particular, the opportunity is coming into view for the fission-track community to share data on a new level, allowing analysts to see, learn from, and discuss each others’ image data.

Recent developments in data repositories and metadata reporting are healthy signs of an emerging open science culture and up-to-date reporting in low-T thermochronology. However, these are currently limited to collecting and presenting the data in their corresponding geo-locations (Boone et al., 2022; Boone et al., 2023) and data reporting formats and table contents (Kohn et al., 2024). Given the continuing relevance of fission-track data, we recommend building toward a global infrastructure and culture enabling and encouraging data transparency through the formation of online digital image repositories (such as geochron@home (Vermeesch et al., 2023)), which can accommodate fission-track image data. Furthermore, proper analyst training and reconsideration of laboratory routines for image acquisition are needed. It has been over 60 years since the beginning of the fission-track dating method (Price and Walker, 1962), and no clear guidelines have been formulated on “musts” and “cans” in fission-track practice. While existing fission-track laboratories develop their own preferences and routines, new laboratories often represent branching points, which can be a source of necessary and beneficial innovation but also undocumented and undesirable divergence. A global community repository housing guidelines for best-practice fission track analyses and fission track training modules are needed, as well as reference libraries of interpreted image sets. Adoption of an open science culture will ultimately benefit every fission-track laboratory and increase data quality.

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Code and data availability. The image data used for this study, and the analysis results, have been uploaded to geochron@home (Vermeesch 2024). The analyst numbers in Table S2 are replaced with random letters in geochron@home to preserve anonymity.



330 *Author contributions.* Conceptualisation: MTT, LC, RAK, AJWG. Data curation: MTT, LC. Formal analysis: MTT, LC. Software: LC, AJWG. Investigation: MTT, LC. Methodology: MTT, LC, RAK, AJWG. Resources - MTT, LC, RAK, AJWG. Writing – original draft preparation: MTT, LC, RAK, AJWG. Writing – review & editing: MTT, LC, RAK, AJWG.

335 *Competing interests.* The authors have no competing interests to declare

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