Referee comment on “Technical note: TRACKFlow, a new versatile microscope system for fission track analysis” by Gerben Van Ranst et al..

GENERAL

The authors have developed a new microscope system for FT analysis, named TRACKFlow. The most notable improvements are as follows: 1) Obtaining an etched grain image can increase the degree of freedom of microscopic work and reduce stress. 2) Multiple samples can be placed on the stage and scanned easily without replacement. 3) This system has protocols that support both EDM and LAFT, and 4) has tools designed for large single crystal and U-doped glass external detectors. 5) In the case of EDM, the mirror image of ED can be converted into the same as the crystal. 6) Apatite D-Par can be automatically measured

The system that is based on new technology realizes something that was not possible 20 years ago. Such improvement of the system can be expected to reduce human work and reduce mistakes. However, improvements (1) to (5) are automation before measurement. On the other hand, there is only improvement (6) related to measurement of D-Par, but it cannot be used for automated fission track counting.

I have never used a standard TrackWORK system, but it is well known that the TrackWORK system has been used in many laboratories and made important researches. And automatic FT counting is also possible. I'm a Nikon user, using the Eclipse E1000 microscope installed twenty years ago. I admit that the performance of Nikon microscope is wonderful. In our laboratory, by combining with a touch sensitive monitor, we can superimpose a small red mark on each counted tracks and measure the number of tracks semi-automatically during FT counting on a PC monitor. This tool can reduce the burden on FT analysts (Danhara et al., 2007: Jour. Geol. Soc. Japan vol.113. 77-81.) Taking the above existing FT systems into account, it is expected that the authors focus on what’s NEW improvement in your system. This MS may give readers an impression of a Nikon product catalog or something like that. That is not preferred. However, publishing a new and improved FT system is important information for FT researchers in the world and is suitable as a technical note.

In general, the technical note (Short communication) should be shorter and more compact. This MS uses more than 6,500 words, so should be simplified further. I propose a major revision that at least halves the volume. I’d like to propose a simplified MS below only by deleting parts that were too detailed without technical corrections (because I am not a native. Sorry for it):

Technical note: TRACKFlow, a new versatile microscope system for fission track analysis

Abstract.
We here present TRACKFlow, a new system with dedicated modules for the fission track laboratory. TRACKFlow decouples image acquisition from analysis to decrease schedule stress of the microscope based on a number of user-friendly protocols: (1) the well plate design that allows sequential scanning of multiple samples without the need of replacing the slide on the stage, (2) two protocols that are designed for the External Detector Method and the LA–ICP–MS apatite fission track approach, with tools for repositioning and calibration to the external detector. (3) Two other tools are designed for large crystals, such as the Durango age standard and U-doped glass external detectors. (4) One more protocol is included for the measurement of etch pit diameters and (5) one last protocol prepares a list of coordinates for correlative microscopy.

1 Introduction

The fission track (FT) method distinguishes itself from most other methods of geochronology by its main 'sensors': the human eye or image analysis algorithms (e.g. Gleadow et al., 2009; de Siqueira et al., 2014). Being of this nature, the FT method is known to be tedious and time-consuming as an analyst needs to correctly identify and count thousands of tracks and measure the length and angles of hundreds of tracks at high (500–1000x) magnification (by optical microscope) for each sample. Furthermore, this puts a high schedule stress on the system since every user needs to spend long times at the microscope. Traditionally, FT analysis was done by one analyst, observing and counting tracks through the eyepieces of the optical microscope. Length measurements were either performed using a micrometre eyepiece or with a drawing tube and digitization tablet. With the arrival of CCD and CMOS microscope cameras, the eyepieces were gradually replaced by the PC screen. Conventional assessment of the remaining "parent" isotope ^{238}U occurs via the external detector method (EDM; Hurford and Green, 1982), in which induced tracks are counted in a co-irradiated external detector (ED), often muscovite. Matching of crystals and their according induced track clouds is done either by repositioning the ED after etching (Jonckheere et al., 2003) or with an automated stage (Dumitru, 1993; Smith and Leigh-Jones, 1985). More recently, direct measurement of the ^{238}U, rather than the indirect EDM, for fission track analysis can also be done using the LA–ICP–MS approach (LAFT) (e.g. Hasebe et al., 2004;

Chew and Spikings, 2015; Vermeesch, 2017). Besides the development of LAFT, new technology also allowed FT research to progress in terms of automated image acquisition and analysis (e.g. Gleadow et al., 2009, 2019; de Siqueira et al., 2014).

In this paper we introduce TRACKFlow, a novel microscope system developed and optimized for the FT laboratory, based on the Nikon Eclipse Ni-E motorised microscope (Fig. 1) and embedded within Nikon NIS-Elements software. We first discuss the rationale behind the microscope system, followed by a description of the system. We then shortly introduce the protocol modules that are contained in TRACKFlow at this time.

2. System philosophy

The main purpose of TRACKFlow is to obtain maximum efficiency from a single microscope system, as such releasing it from schedule pressure. This is mainly done by decoupling image acquisition from the analysis, as one microscope can acquire images that can then be analysed on different computers. FT analyses, which are most time-consuming, can thus be moved to other systems that are not under high schedule stress. The focus on image acquisition also comes from the need and nowadays also the possibility to make data available in its most raw form (e.g. FAIR Data Commitment Statement). At the same time, samples are archived for the research database of the institution and can be made available for students. Furthermore, with imaging the tracks are digitally preserved for destructive LAFT analysis.

3 Microscope system

The system is based on the Nikon Eclipse Ni-E motorised upright microscope (Fig. 1), with the dedicated modules for FT research embedded within the Nikon NIS-Elements Advanced Research (AR) software package with JOBS smart imaging design interface. This system is composed of the following four components.

3.1 Motorised components

The Nikon Eclipse Ni-E microscope is developed for its automated capabilities. Motorised components include shutters for both dia- and episcopic illumination, field- and aperture diaphragms, optical zoom, condenser, objective and filter cube turrets. The motorised nature of the optical path allows for a very reproducible and user friendly way of imaging, working with conditioned optical path settings,

3.2 Optical components

The diascopic (transmitted) light path is equipped with the Nikon fly-eye lens, which reduces inhomogeneity of the light source. Remaining inhomogeneity can be corrected for in the software by using shading correction images. Light then passes through the polarizer and the NI-CUD-E motorised universal condenser, which has a numerical aperture of 0.88 and houses several positions for additional optical components. In the TRACKFlow system, these include a 2–4x auxiliary lens and a custom quarter-lambda plate. The motorised cube turret contains an EPI-Brightfield, an EPI-DIC cube and a custom cube containing a quarter-lambda plate and an analyser. The Ni-E microscope is further equipped with the NI-RPZ-E Motorised DSC zooming camera port, which performs a continuous optical secondary magnification of 0.6 to 2.0 times (Fig. 11).

3.3 Camera

As the camera has largely replaced the function of the eyepieces, it carries high importance in terms of image quality, contrast and speed. Image acquisition is performed by the mounted Nikon DS-Ri2 colour camera. The 36.0 x 23.9 mm CMOS full frame sensor contains 16.25 (4908 x 3264) megapixels of 7.3 µm pixel size.

3.4 Stage and focal mechanism

For FT analysis, high precision and reproducibility of the XY stage and focal mechanism are required. The standard configuration comes with the Nikon NI-S-E motorised XY stage, which has a resolution of 0.1 µm. The focal mechanism is of the focusing stage type (opposed to focusing nosepiece type) and has a resolution of 0.025 µm. It has a built-in linear encoder to guarantee correct readout of the z-axis. The z-drive is thus well-suited for non-horizontal confined track measurements.

4 Protocols

FT method is very specific and thus requires specialised protocols. We present here the main protocols that are all based on the practical workflow for apatite FT (AFT) and include a large degree of adaptability. An overview flowchart of all imaging protocols can be found in the TRACKFlowChart in Supplement. Figure 9 displays a condensed version of the flowchart.

4.1 Well plate approach

Automation is most efficient when part of the protocol can be standardised, such as the locations of interest on a microscope slide. As such the automated microscope has a primary indication and knows where it needs to ‘look’ for apatite grains or mount-ED homologous points. Although wells are often physical pits, the well positions here are simple markings on a glass slide, which can easily be made with a stencil, or obtained from the fission track laboratory of Ghent University (Fig. 2). TRACKFlow comes with a standard well plate for four EDM mounts and four EDs and a well plate of either two or three wells for LAFT mounts. It is however still possible to generate new well plates, fitting the standard protocol of different laboratories. Geometrical limitations of the wells are set by the XY-limits of the stage and the diameter of the high-magnification objective lenses in case a thin ED is placed on the stage at the same time as the 1 mm thick mount. The problem of difference of thickness can be solved by fixing small 1 mm thick glass slides on the main glass slide at the position of the EDs.

4.2 List operator

The list operator is a small tool to convert between operator preference styles of coordinate storage. The function merges or splits lists of points stored in xml or csv format. Mainly it involves recoordination points and the target grains or positions.
The tool also provides the means for coordinate flipping as preparation for correlative microscopy for when the other stage has different X and/or Y directions.

4.3 Transformation engine

One of the main features in TRACKFlow is coordinate transformation or micro-georeferencing. Coordinate transformation is most of all necessary to accurately link the positions of apatite grains with their according induced track clouds but is also essential for correlative microscopy. It is also convenient to accurately retrieve points after removing the slide of the stage, e.g. for step etching or other experiments with repetitive imaging. TRACKFlow makes use of a basic 2D Helmert transformation with a least-squares calculation including a rotation, a translation and an isotrope scaling factor. From classical georeferencing we learn that more points often lead to better transformations. For this reason TRACKFlow gives the operator the option to select up to ten homologous point couples, which can be stored for later use. The system offers the option to perform a secondary selection of homologous points, which is often used when working with EDM and pinprick points (Fig. 5).

4.4 Scout pre-protocol

Sometimes it is convenient to inspect the mount(s) before scanning the grains. The scout pre-protocol can be enabled at the start of each scanning protocol to explore the sample area. The tool herewith provides a large image zoomable ‘map’ of each mount of the slide or well plate, which can be used to navigate each sample (Fig. 8). A detailed section at the current optical configuration can be added to this map at any moment. Double-clicking on the XYZ Overview moves the stage to the designated position, such as a certain grain or calibration point. Positions that are marked as targets during a protocol are also indicated on the XYZ Overview map (Fig. 8).

4.5 Imaging protocols

4.5.1 Durango (single large crystal?) tool

The main function of the Durango tool is to create a regular grid of points inside large apatite crystals, such as the Durango age standard (Fig. 8). These points represent digital, non-overlapping fragments of the grain, i.e. samples from the entire extent of the grain. The Durango tool first scans a large image of the mount and calculates the extent of the bounding box around the crystal. Optionally this bounding box can be set manually. The system then creates a regular grid inside the bounding box (Fig. 8). The density of the grid can be set manually, based on the number of generated points. If the set spacing between points is too low, which would cause overlap of images to occur, the system resets the spacing to prevent overlap and prompts a warning. At this point the operator can choose to manually inspect whether the generated points are in epoxy or in the crystal, or start the automatic inspection. The automatic inspection grabs an image in circularly polarized light of each point and through image intensity analysis decides whether it is epoxy (dark) or apatite (light). Only points in apatite are retained and exported as a list. This makes that zones crosscut by a large fracture and which are unsuitable for analysis are also not retained (Fig. 8). Optionally the operator can enable imaging, so that a z-stack image is acquired of each apatite point immediately after inspection. The Durango tool also lets the operator select a number of recoordination points, so that the same grid can be used for a second scan or for transformation to the ED.

4.5.2 U-glass tool

The glass tool is similar to the Durango tool, though optimized for the EDs of dosimeter glasses used in the EDM approach. A main difference is that the bounding box around the induced tracks cannot be determined automatically and must be set manually. The glass tool then also creates a regular grid with density of choice (within the limits allowed by overlap protection) and inspects whether there are induced tracks present at the location of each generated point. Only points with induced tracks are retained for export. With imaging enabled, the microscope captures a thin z-stack image of each retained point. It is also 10 possible to include recoordination points for each glass ED. As such, each generated grid of points inside the glass region can be reloaded for the scanning of the same glass EDs in other irradiation packages. Coordinate transformation is for this purpose included in the glass tool.

4.5.3 Mount-ED (EDM) tool

The mount-ED or EDM tool first guides the operator through grain selection and the selection of coarse calibration points (usually pin punctures through the ED into the apatite mount). If secondary calibration is enabled, the operator can then manually choose a number of grains for fine calibration or enable automatic random selection of a chosen number of grains from the target list. Based on the fine calibration the microscope will obtain z-stack images from all target apatite grains, followed by their according positions on the ED. The images are then available in two separate nd2 files (Fig. 10). It is also possible to disable imaging and to only export the grain and/or transformed ED coordinates.

4.5.4 LAFT tool

The LAFT tool is created for the imaging of apatite (or zircon) grains before and after LA–ICP–MS analysis of the mount. For new samples it first guides the user through the selection of recoordination points and target grains. For existing lists the protocol first asks to import the data, either as separate files for recoordination mark locations and targets or as a single file in which recoordination marks are stored as the first set of points.

4.5.5 Dpar measurement tool.

For thermochronology modelling a kinetic parameter is required to account for the effect of mineral chemistry on track annealing (Carlson et al., 1999; Ketcham et al., 1999, 2007). One approach is to use the etch pit diameter parallel to the c-axis, termed Dpar. As these features typically are small and in the order of 1 to 3 µm length (5.5 mol L-1 HNO3, 21 °C, 20 s etch), measurement accuracy and reproducibility are a challenge. The Dpar tool provides a means to measure large numbers of Dpars (dependent on track density) at reproducible imaging and contrast settings. The Dpar measurement tool takes images of predefined positions in episcopic light at 2000x magnification. It then uses image analysis to separate and measure etch pit diameters, both along (Dpar; maximum Feret diameter) and perpendicular to (Dper; minimum Feret diameter) the c-axis (Fig. 11). As an example, for an annealed Durango with induced tracks we

obtained a median Dpar of (1.45 ± 0.02) µm from 103 automated measurements in three spots (5.5 mol/L, 20 °C, 20 s etch). Fifteen manual measurements on the same locations resulted in a median Dpar of (1.44 ± 0.03) µm. Although this tool also automatically counts the number of withheld Dpars measured, it cannot be used for automated fission track counting in its current state.
(2,453 words)

SPECIFIC
1. Caption of Figure 1 should be corrected as follows:
Whole view of TRACKFlow system which consists of a motorized upright microscope with a color camera mounted and PC displays for track counting and storing data.
2. Figure 3 should be deleted to shorten the MS.
3. Figure 4 should be combined into one in Figure 1. Explanation for g) is missing.
4. Figure 5 should be deleted because it is not a new development.
5. Figures 6 and 7 should be deleted to shorten the MS.
6. Explanation for abbreviations in Figure 9 should be added.
7. DUR in text and Fig.9 seems strange and not general to readers. Large Single Crystal (LSC)?

With best regards,
Hideki Iwano
Iwano_hide@zeus.eonet.ne.jp