Technical note: Nikon–TRACK*Flow*, a new versatile microscope system for fission track analysis

Gerben Van Ranst[1], Philippe Baert[2], Ana Clara Fernandes[2], Johan De Grave[1]

[1]Department of Geology, Ghent University, Ghent, 9000, Belgium
[2]Nikon Belux, Groot-Bijgaarden, 1702, Belgium

Correspondence to: Gerben Van Ranst (gerben.vanranst@ugent.be)

Abstract.

We present Nikon–TRACK*Flow*, a new system with dedicated modules for automated microscope control and imaging for the fission track laboratory. It serves as a Nikon alternative for the Zeiss-based TrackWorks package from Autoscan Systems. Nikon–TRACK*Flow* is based on the Nikon Eclipse Ni-E motorised upright microscope and is embedded within Nikon NIS-Elements software. The system decouples image acquisition from analysis to decrease schedule stress of the microscope based on a number of automated user-friendly designs and 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 fission track approach with tools for repositioning and calibration of the external detector, (3) two other tools are designed for automated point selection and scanning of large crystals, such as the Durango age standard and U-doped glass external detectors. In future versions, Nikon–TRACK*Flow* aims to step away from the dedicated system for fission track imaging, towards a general high-throughput imaging system for Earth Sciences and other material-oriented sciences.

1 Introduction

The fission track (FT) method (e.g. Malusá and Fitzgerald, 2019) places high requirements on its optical instruments and is further characterised by a high need for specific protocols and equipment when regarding image acquisition and analysis, which are not always readily available from microscope producers. The FT method can thus benefit strongly from the implementation of technological improvements and automation of the process (Gleadow et al., 2019). The University of Melbourne was the first to develop a complete and comprehensive automated microscope system based on Zeiss microscopes and offers the only complete system of its kind (Gleadow et al., 2019). Their effort, which is commercialised as Autoscan Systems Pty Ltd, have become well-established in many fission track laboratories around the world. However, while this readily-available system thus exists, it must be noted that the market choice is limited for fission track laboratories. Specific preferences, such as the microscope brand, software specifications, style and specific protocols can thus often not be (fully) met due to the limited options available on the market.

In this paper we introduce Nikon–TRACK*Flow*, a novel microscope system developed and optimised for the fission track laboratory, based on the Nikon Eclipse Ni-E motorised upright microscope (Fig. 1) and embedded within Nikon NIS-Elements software. As it was developed for the same purpose, it serves as a Nikon-based alternative for AutoScan TrackWorks, which is the microscope control and imaging package of the Autoscan Fission Track Studio Suite. Both systems, among others, have the aim to obtain maximum efficiency from a single microscope system as, amongst others, to reduce 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. Although these systems pursue a similar goal and consequently contain similar automated tasks, they differ, amongst others, in microscope brand, style and specific approach.

In this note we will first present a brief description of the system, after which we introduce the protocols which are implemented within Nikon–TRACK*Flow*. We finalise with a short conclusion and future perspectives. For a visual representation of the system, we refer to the short demonstration video (Van Ranst, 2020).

2 System components

The system is based on the Nikon Eclipse Ni-E motorised upright microscope (Fig. 1; Nikon Corporation, 2019c), with the dedicated modules for AFT research embedded within the Nikon NIS-Elements Advanced Research (AR) software package (Nikon Corporation, 2019b) with JOBS smart imaging design interface. NIS-Elements AR contains, amongst others, basic microscopy features and advanced image analysis functions (Supplementary material). Nikon–TRACK*Flow* is embedded within NIS-Elements as to retain access to its versatile functionality on top of the specific features for fission track research. We equipped our prototype with the Nikon DS-Ri2 camera (Nikon Corporation, 2019a), which has both a resolution to cover high to low-magnifications and has a large pixel size to obtain a high signal-to-noise ratio. High speed can be retained at high magnifications by changing analog gain without introducing too much noise (Fig. 2). Although we recommend this camera to be combined with the system, it is also possible to equip the microscope with any other camera compatible with Nikon NIS-Elements (Nikon Corporation, 2020).

3 Nikon–TRACK*Flow* Protocols

All protocols of Nikon–TRACK*Flow* are created using the JOBS module of the Nikon NIS-Elements package. Each protocol is built from a sequence of combined Nikon pre-defined tasks and newly implemented tasks programmed in the C-based Nikon API. These protocols are all based on the practical workflow for apatite FT (AFT) and include a large degree of adaptability, such as sample shape and size (Section 3.2), degree of automation or illumination settings (Section 3.1). An overview of the available protocols and their workflow is provided in the Nikon–TRACK*Flow*Chart (Electronic supplement). Figure 3 displays a condensed version of this flowchart.

3.1 Valve-based set-and-go protocol design

As can be deduced from the Nikon–TRACK*Flow*Chart (Supplementary material), each main protocol contains a workflow for a specific task. Structuring the modules around these work*flow*s for fission track research in the Nikon environment forms the rationale behind the name of Nikon–TRACK*Flow*. Each main protocol in itself is built out of both serial and parallel sub-protocols, separated by on/off switches or ‘valves’ that direct the flow (Supplementary material). In this branched design, the operator starts the required main protocol, after which a choice menu is prompted. This menu also contains ‘valves’ that direct the workflow by activating or deactivating sub-protocols according to the operator’s preferences. Examples are the thickness of the mount, the number of calibration points (3–10), imaging/location storage, etc. Once the desired workflow is set, the system can operate without the need for operator intervention. An exception is the N–TF–EDM_s tool, which is designed for mount and external detector (ED) being attached to separate microscope slides, which thus requires replacing the slide during the protocol.

3.2 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. Microscope slides with such standardised positions or wells are frequently used in Life

Sciences, where they are known as well plates. In this view, each apatite mount or external detector occupies the place of a well, the microscope slide on which they are attached serving as a well plate.

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 FT laboratory of Ghent University (Fig. 4). Nikon–TRACK*Flow* comes with a standard well plate for four EDM mounts and four EDs and a well plate of either two or three wells for LA–ICP–MS FT (LAFT) mounts. It is however still possible to generate new well plates, fitting the standard preparation style 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 however be solved by fixing small 1 mm thick glass slides on the main glass slide at the position of the EDs (e.g. Kohn et al., 2019).

3.3 Transformation engine

Coordinate transformation is an essential tool for AFT, when using the ED method (EDM; Fleischer et al., 1964, 1975) by means of an automated microscope stage (Dumitru, 1993; Gleadow et al., 1982; Smith and Leigh-Jones, 1985). Homologous points to calculate transformation parameters are usually produced by pin pricks, co-embedded zircons or cupper TEM grids (Smith and Leigh-Jones, 1985).

Nikon–TRACK*Flow* makes use of a basic 2D Helmert transformation with a least-squares regression. This Helmert transformation is preceded by a reflection along a vertical axis to deal with the flipping of the ED as to place it in tracks-up position. The Helmert transformation can cover most coordinate differences that are met in the application.

Nikon–TRACK*Flow* gives the operator the option to select up to ten homologous point couples, which can be stored for later use. After transformation the system displays the average X- and Y-residuals as a metric for the transformation parameter quality. As it is common practice to refine the primary alignment using mineral grains (Kohn et al., 2019), the system offers the option to perform a secondary selection of homologous points. The primary calibration is then used as a guide to find homologous points of manually pre-selected points, such as zircons or apatite grains or automatically generated points selected from the apatite target list. In this case images are acquired from these fine calibration points on the mount, which allow the operator to select the homologous position inside the induced track cloud. We state however that grains with U-zonation or low-U content may still be problematic for this grain-based approach (e.g. Jonckheere et al., 2003).

The order in which two homologous sets of calibration points are selected can be arbitrary, so the operator is not required to retain a same order. This is due to the sorting algorithm in Nikon–TRACK*Flow* that is executed before the calculation of transformation parameters. All points are first sorted according to a scanning line that crosscuts the barycentre of the calibration points and rotates in counter clockwise direction. As this line segment starts horizontally in the direction of the x-axis, calibration points should not be chosen in the middle left section of the mount. On the ED the scanning sweep is reflected horizontally. Allowing an arbitrary order of selecting points is also convenient for when these points are retrieved automatically through image analysis.

3.4 Scout pre-protocol

The scout pre-protocol embeds the Nikon NIS-Elements Large Image Stitching function as a sub-protocol. The tool provides a zoomable ‘map’ of each mount of the slide or well plate, which can be used to navigate each sample (Fig. 5).

3.5 Imaging protocols

3.5.1 Large Single Crystal tool (N–TF–LSC)

The main function of the Large Single Crystal (LSC) tool is to automatically create a regular grid of points inside large apatite crystals, such as the Durango age standard (Fig. 5). These points represent digital, non-overlapping fragments of the grain, i.e. samples from the entire extent of the grain. The tool automatically defines the bounding box of the crystal, into which a regular grid is created according to the operator's pre-set specifications. A warning is prompted if these specifications would produce overlap. The automatic inspection verifies whether the generated points are either crystal, epoxy or contain large cracks. Only clear crystal points are retained for imaging and their coordinates exported. Both setting the bounding box and inspection can be performed manually as well. The tool can both be used for the standard zeta calibration (Hurford, 1990; Hurford and Green, 1983) and for the absolute calibration (Jonckheere, 2003). As an indication, our prototype system had a run of 55 minutes for a Durango scan, including set-up, automated crystal detection, grid generation (148 points), point-by-point-inspection and imaging (EPI + DIA z-stack). Using recoordination, a next scan of the same crystal does not require a new crystal detection, point generation or point inspection. Processing and image storage speed is also dependent on the attached computer.

3.5.2 Glass tool (N–TF–GL)

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 it is not possible to determine the bounding box around the induced tracks automatically and that this action needs to be performed manually on a presented large image. Automatic inspection of whether a generated point contains tracks is embedded in the protocol. It is also 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.

3.5.3 Mount–ED tool (N–TF–EDM_s/m)

The mount–ED or EDM tool comes in two versions: single mount (EDM_s) or multi-mount (EDM_m). The only difference between the two is that the single mount version is designed for the scanning of one mount and ED (mounted on the same or different glass slides) at arbitrary locations, and the multi-mount version is intended for mounts and their EDs mounted on a well plate. The latter can thus scan up to four samples in a single run. A single mount–mica couple (20 points) was imaged in 24 minutes, including protocol set-up and autofocus for each point. It should also be noted that automated imaging systems, in contrast to a human analyst, can work 24/7.

3.5.4 LAFT tool (N–TF–LAFT)

The LAFT tool is intended for the scanning of grains for the LA–ICP–MS based method (e.g. Cogné et al., 2019; Hasebe et al., 2004). It allows the registration of recoordination points, recoordination of existing coordinates and the scanning of thin or thick (~5 mm) mounts locked in a well plate. If the option is selected, illumination is automatically adapted for thick mounts as to deal with the mismatch of the focal plain level with the optimal distance of the condenser lens for this type of samples.

4 Concluding remarks and further developments

Nikon–TRACK*Flow* is a new system for (semi-)automated microscopy, which broadens the market for fission track laboratories. It comes with user-friendly modules for specific tasks, which exhibit adaptable degrees of automation.

The system presented here is the current working version of Nikon–TRACK*Flow*. Continuing developments are however still being made for a next version of the system. These mainly focus on a more extensive automation and upscaling to high-throughput imaging, e.g. by making use of the Märzhäuser Slide Express 2. Further automation is achieved by training image analysis recipes to be applicable to different samples. These recipes can then be embedded into the current protocols and enable the system to make decisions without intervention of the operator. A future objective is also to reach beyond the application of fission track thermochronology and to evolve to a general high-throughput system for Earth Sciences and other material-oriented sciences.

Author contribution

Philippe Baert: Technical support, Software support, writing – reviewing & editing.
Ana Clara Fernandes: Technical support.
Johan De Grave: Supervision, funding acquisition, resources, writing – reviewing & editing.

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Availability

Nikon–TRACK*Flow* is licensed from Ghent University to Nikon. The full software package for Nikon–TRACK*Flow* is based on the NIS-Elements AR software suite (Nikon) and can be purchased through Nikon.

Conflict of interest

The authors declare the following potential conflicts of interest:

- Nikon–TRACK*Flow* is licensed from Ghent University to Nikon as a commercial product.
- Philippe Baert and Ana Clara Fernandez are employed at Nikon.

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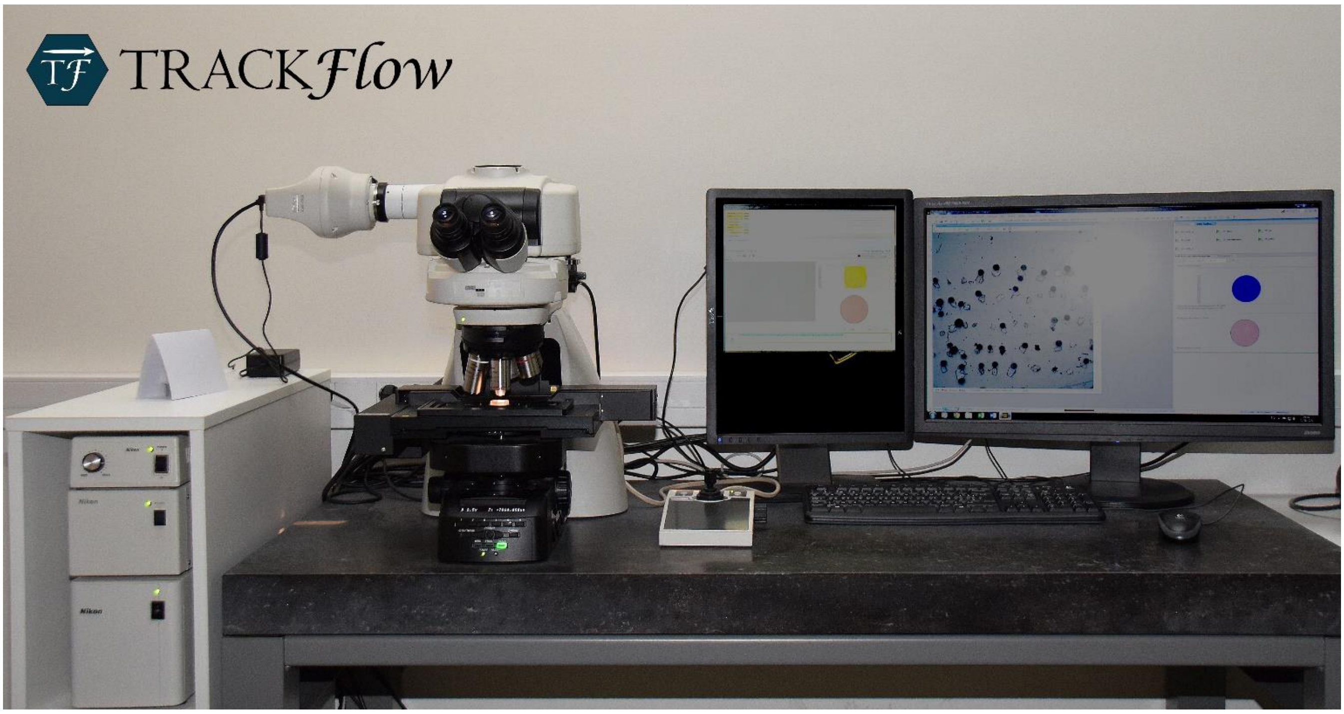

Figure 1: Whole view of Nikon–*TRACKFlow* system, which consists of the Nikon Eclipse Ni-E motorised upright microscope with DSC zoom body and Nikon DS-Ri2 camera. A PC/workstation and double displays are included for system management and data storage.

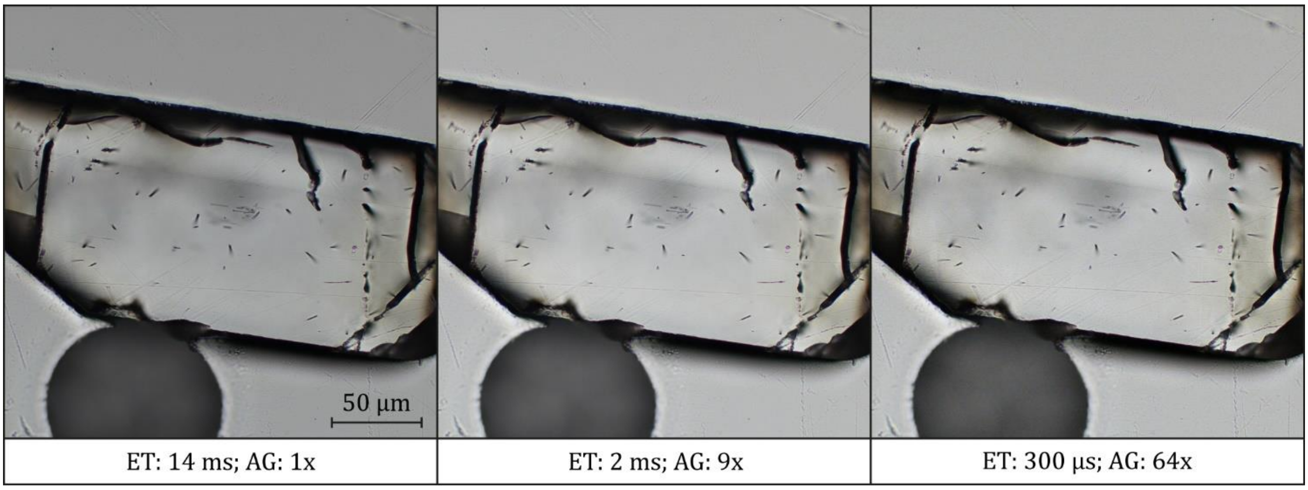

Figure 2: Images created with the Nikon Eclipse Ni-E microscope and the DS-Ri2 camera, illustrating the effect of camera exposure time (ET) and analog gain (AG). It can be observed that although an increase of the noise with higher AG the image quality remains within acceptable values, in combination with shorter exposure times and thus faster refresh rates. All images were obtained using the 50x objective and diascopic illumination.

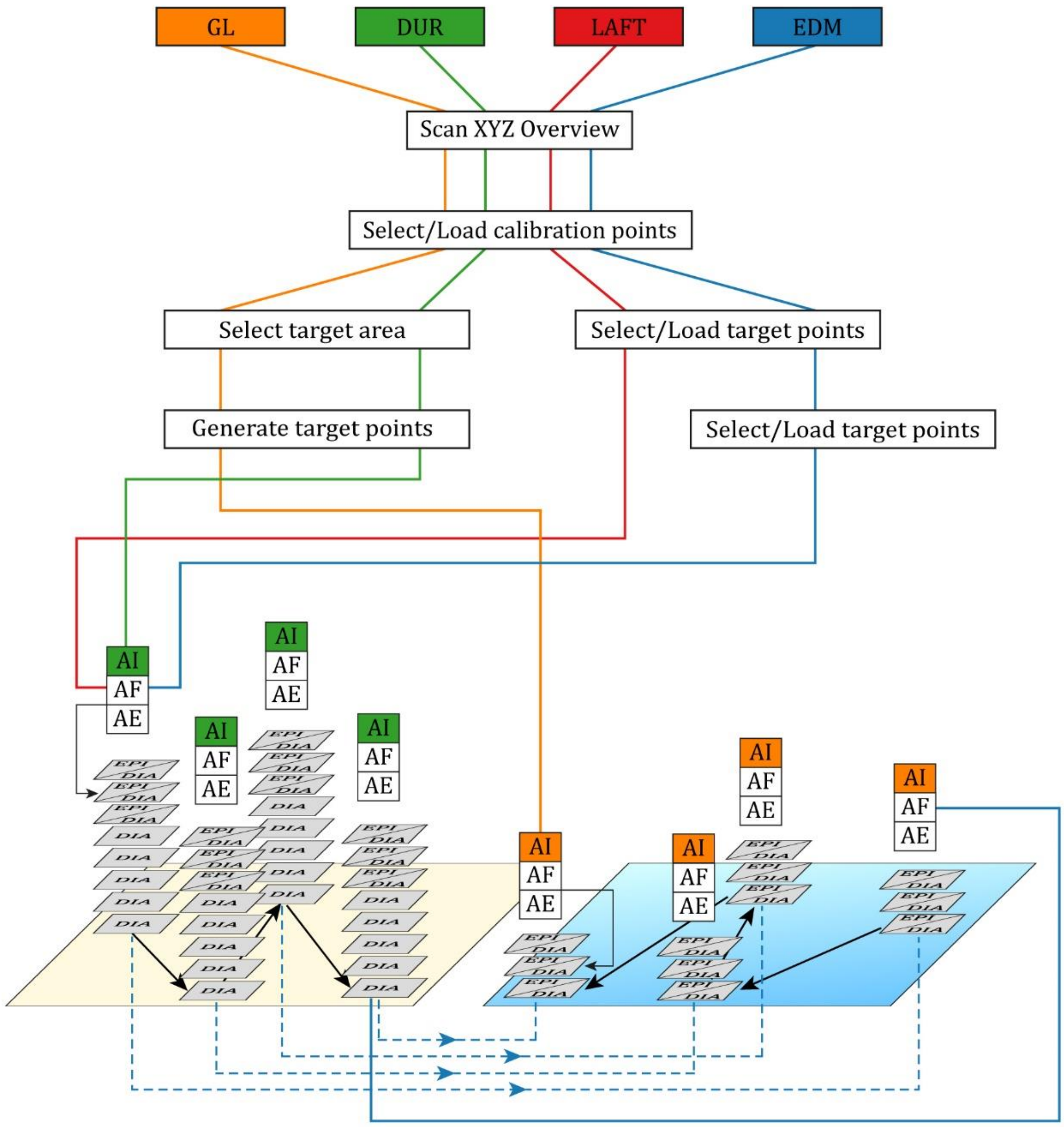

Figure 3: Condensed version of the Nikon–TRACK*Flow*Chart demonstrating the major steps of the different protocols. GL: Glass tool, LSC: Large Single Crystal tool, LAFT: LA–ICP–MS fission track tool, EDM: external detector method tool. AI: Auto-inspection, AF: autofocus, AE: auto-exposure, EPI: episcopic, DIA: diascopic.

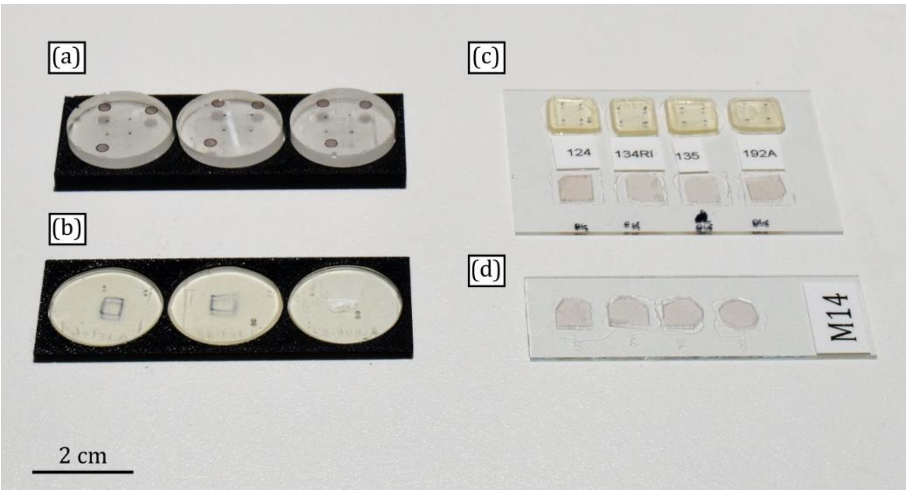

Figure 4: Standard Nikon–TRACK*Flow* microscopy slides. (a) Thick 3-mount adaptor slide for thick (~0.5 mm) 25 mm mounts, usually for LAFT. (b) Thin 3-mount adaptor slide for thin (1 mm) 25 mm mounts. 25 mm Mounts can be placed in the adaptor slides for imaging and are removed after. (c) Standard EDM Nikon–TRACK*Flow* (52x76 mm) glass slide. It is possible to raise thin micas

to the level of the apatite mounts by attaching a standard microscopy glass slide (26x76 mm) to the bottom of the main slide using nail polish. (d) Standard EDM Nikon–TRACK*Flow* (26x76 mm) glass slide. Used for U-doped glass EDs or separate mounting of apatite mounts and EDs.

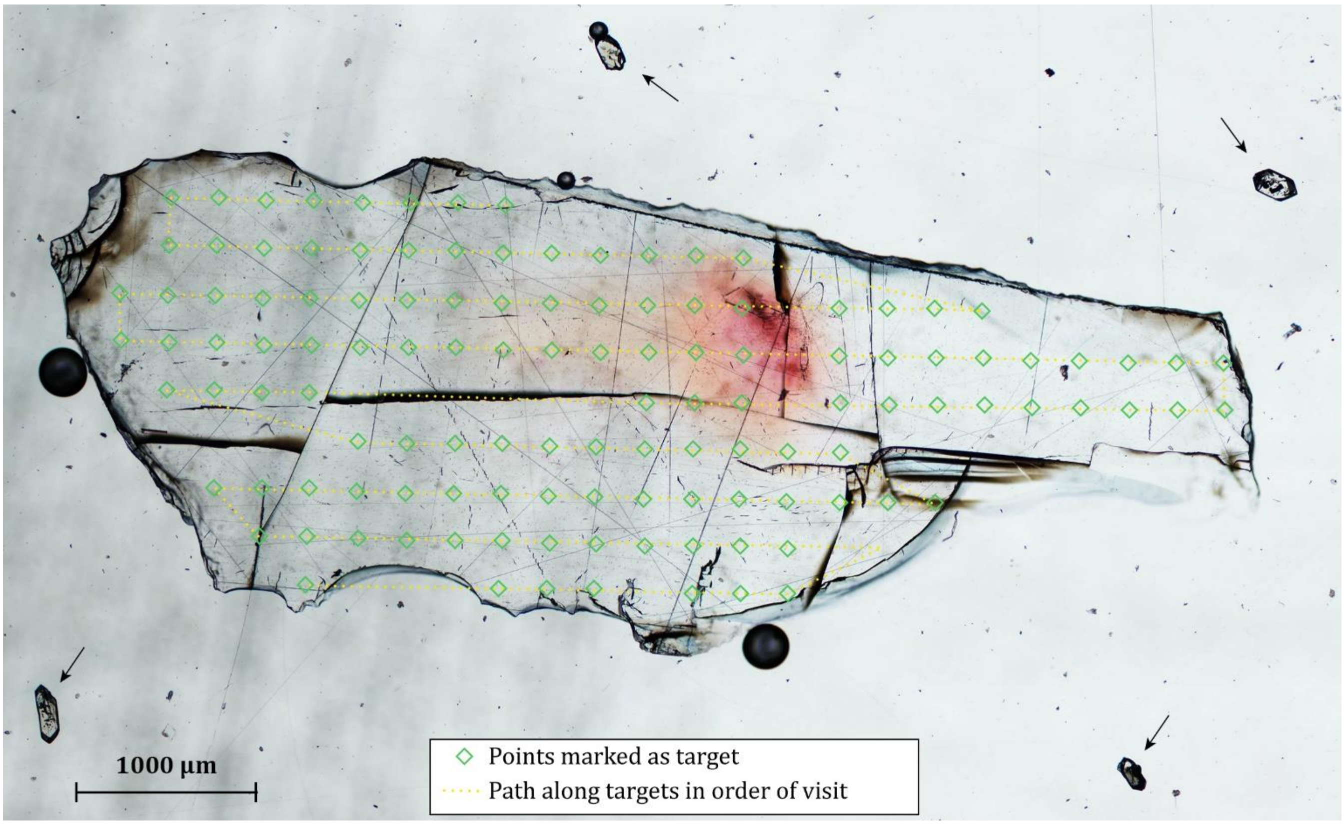

Figure 5: Illustration of the XYZ Overview with a large image composed of stitched tiles. The image contains a thin slab of a Durango crystal and four co-embedded zircons as calibration points (indicated with arrows). The green diamonds are positions automatically generated by the N–TF–LSC protocol with automatic crystal detection and automatic inspection enabled. Points near the crack in the centre were not retained by the automatic inspection due to insufficient luminosity caused by the crack.