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A Synoptic View on Microstructure

Technical and Aesthetically Aspects on Multi-Detector Colour Imaging, Moving Images in the SEM and Development of the Software nanoflight.creator

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Introduction:

My name is Stefan Diller. I am a photographer and microscopist living at Wuerzburg, Germany.
Let`s talk what it takes to go flying with a scanning electron microscope...

My movie camera, a TESCAN MIRA3 FEG-SEM

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How it began:

In the year 2000 I had been in the Iceland pavilion at the Hannover World Expo, watching a Cineflex helicopter movie of the beautiful northern landscape.
I began to wonder how a similar multi-axes camera move might look like around microscopic specimen.

That seemed an idea worth pursuing and I decided to make this a project from 2010 on.

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My Credo:

The scientific community and with it every researcher should be committed to sharing the aesthetics of the microworld with as many people as possible.

This outreach to non-scientists is essential in helping communicate the importance of basic and applied research to the general public. An appreciation of the contribution of science to their everyday lives is essential if the tacit support for funding of your research is to be maintained.

In addition, the ability of an individual picture to inspire a child to inquire about a hitherto invisible world is an important first step in establishing future generations of researchers and microscopists.

Although, data from scanning electron microscopes (SEMs) is usually shown using grayscale images, colours can be added using additional detector signals and digital photo editing software. This procedure is widely accepted for communicating results on up-to-date research to newspapers, magazines and TV outside the scientific world and mostly comprise still shots of a specimen.

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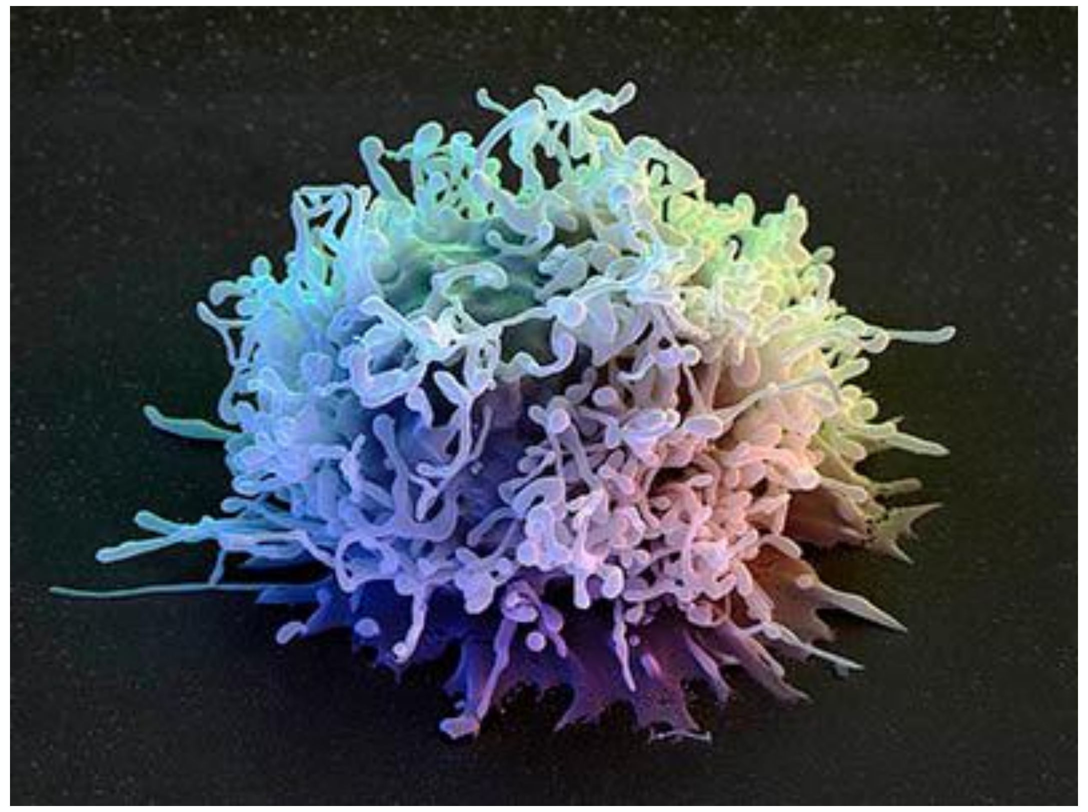
Why coloured SEM images ?

Using colours to enhance the perception of the specimen and structures on the specimen is a powerful tool when employed to communicate imaging from a scientific source to the general public.

A professional scientist or photographer must first search and acquire scientifically correct but also aesthetically appealing images.

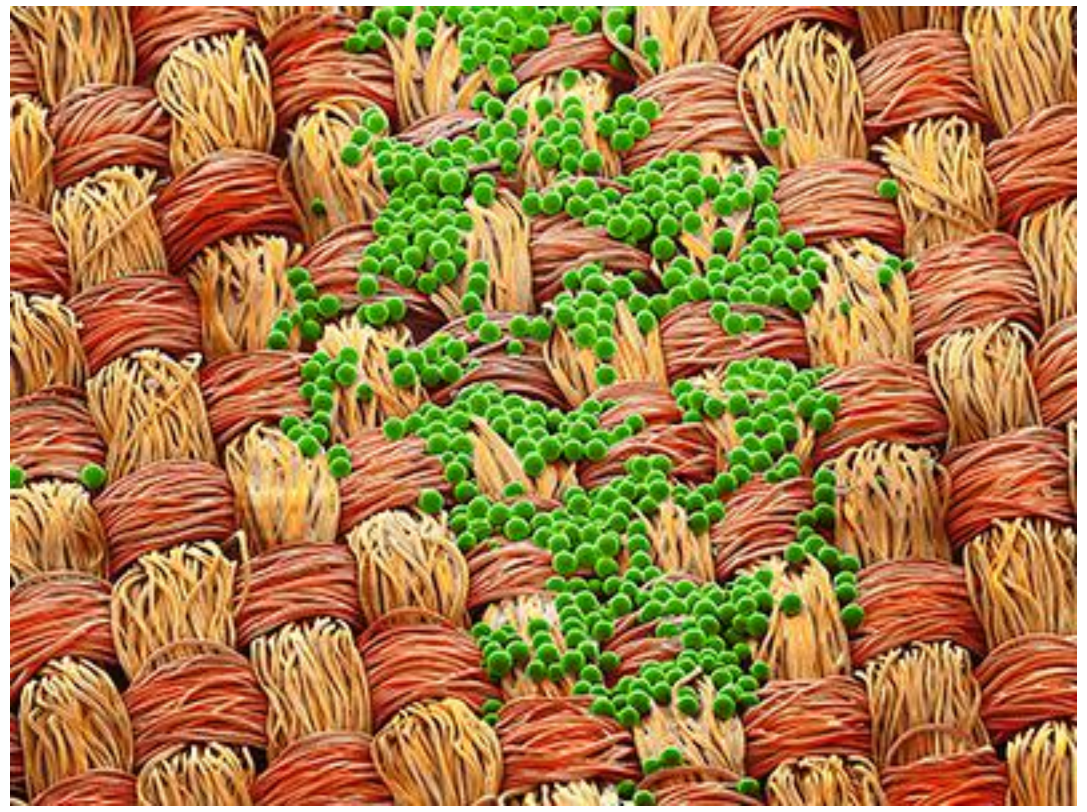
These two rules aid in making an image accessible to a non-expert viewer extending the impact of scientific imaging and aesthetics of the microworld to the wider public.



David Scharf: Lymphocyte cell, imaging with „Multi-Detector Colour Synthesizer System", coloured by „Solid angle" technique

eye of science: Tardigrade, imaging with two detectors and Photoshop retouching

Martin Oeggerli: Kohlweissling eggs, imaging with one detector, intensive Photoshop retouching



Steve Gschmeissner: Reflective material, normal SEM imaging, re-coloured with Photoshop techniques

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Why „nanoflights“ ?

In an increasingly digital world there is worth to bringing movement, colour and lighting effects into the microworld.

To achieve this I developed in collaboration with key instrument manufacturers a modular software called „nanoflight.creator“ to control the SEM, allowing sequential acquisition of specimen movements, detector values, focus, other important SEM parameters and „colours” of each detector channel to generate a series of slow scan RGB images.
These images may then be combined as a movie, yielding an impression like flying around the microstructure.

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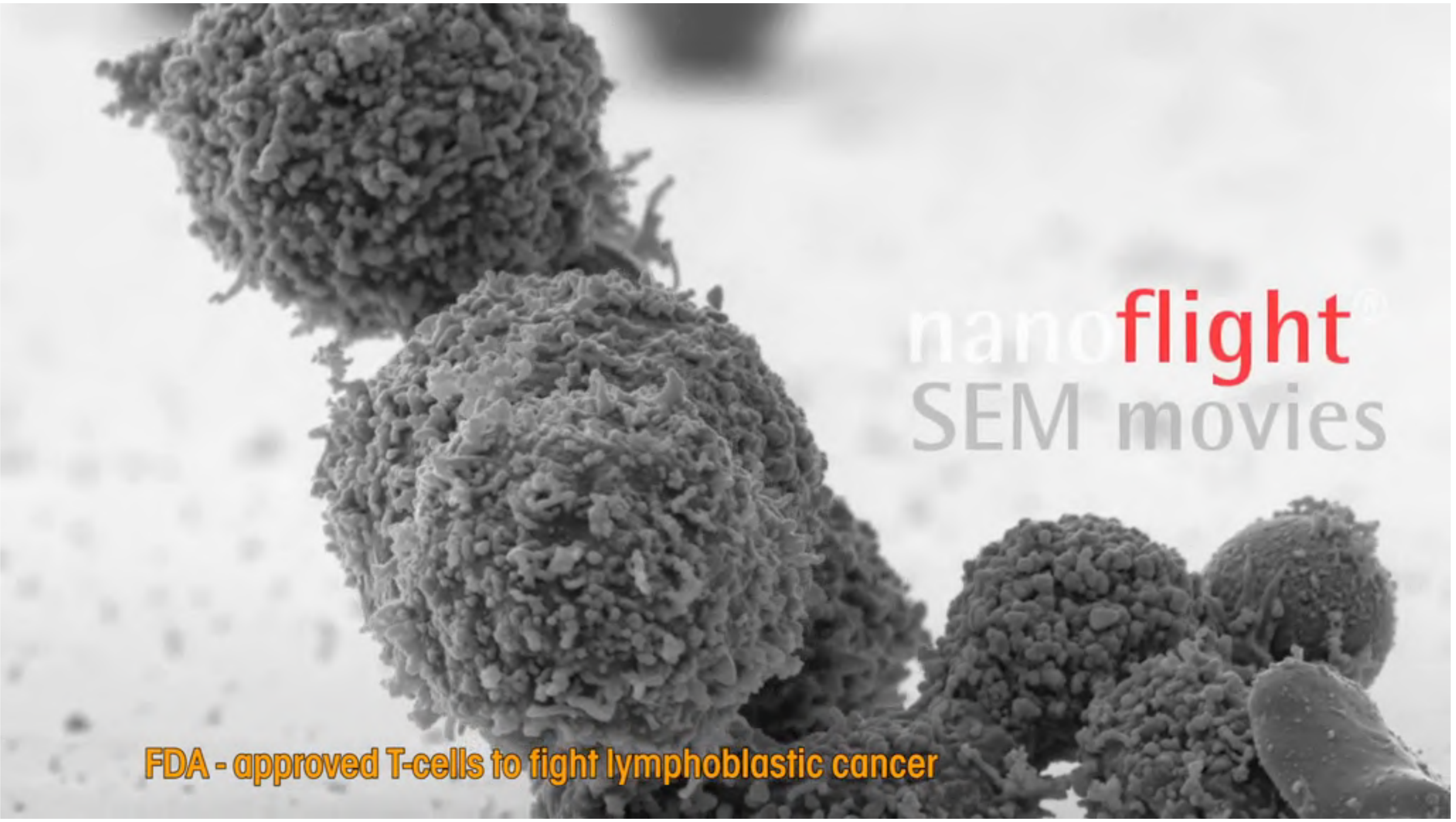
What does it look like ?

This is a short sequence what the technique is capable of:



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How is it done ?

Hardware needed to do „nanoflights“:

1. A large chamber scanning electron microscope
2. Remote capability of most of the SEMs functions
3. An up-to-date four channel image acquiring system specially modified to be remoted by script language
4. An eight axes piezo stage with a very good accuracy and repeatability of coordinates
5. A lot of detectors within the electron microscope: seven backscattered, one specimen current and one secondary electron detector to be able to choose the characteristic of „lighting“ and „coloring“ the specimen
6. The nanoflight.creator software package and specially adapted „extensions“ to read / write values from / to all connected hardware

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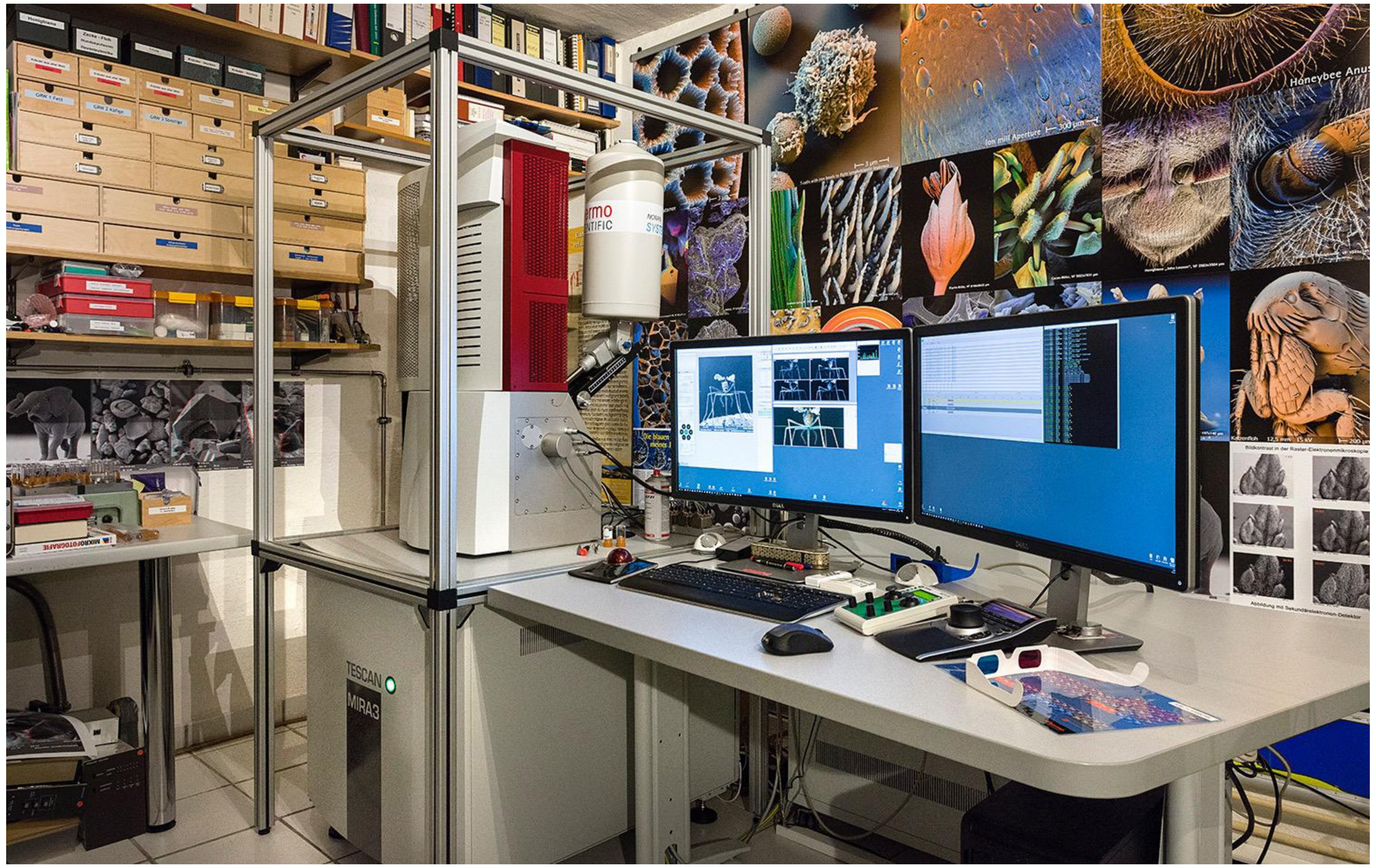
Hardware needed to do „nanoflights“

1. A large chamber scanning electron microscope:



New Field Emission FEG-SEM MIRA3 made by TESCAN

The TESCAN MIRA3 had been my long time wish to buy and it had been a real adventure to finally have it sitting in my lab...

Why did I choose it?

- Schottky field emission source: What shall I say more ?
- Very good resolution (1nm @ 30 kV) and adjustable beam intensity.
- Various imaging modes: Resolution, Depth, Field and Wide Field.
- Eight axes SmarAct piezo stage that had been specially developed to fit in the MIRA3 XMH chamber.
- Last but not least the possibility to either see „on the fly“ anaglyphic 2.5D imaging or acquire „left / right“ image pairs with a beam diversion angle between 0.1 and 3.0 degrees to capture „real“ 3D sequences.
- Extensive remote capabilities via the SharkRemote API for integration into my nanoflight.creator software.

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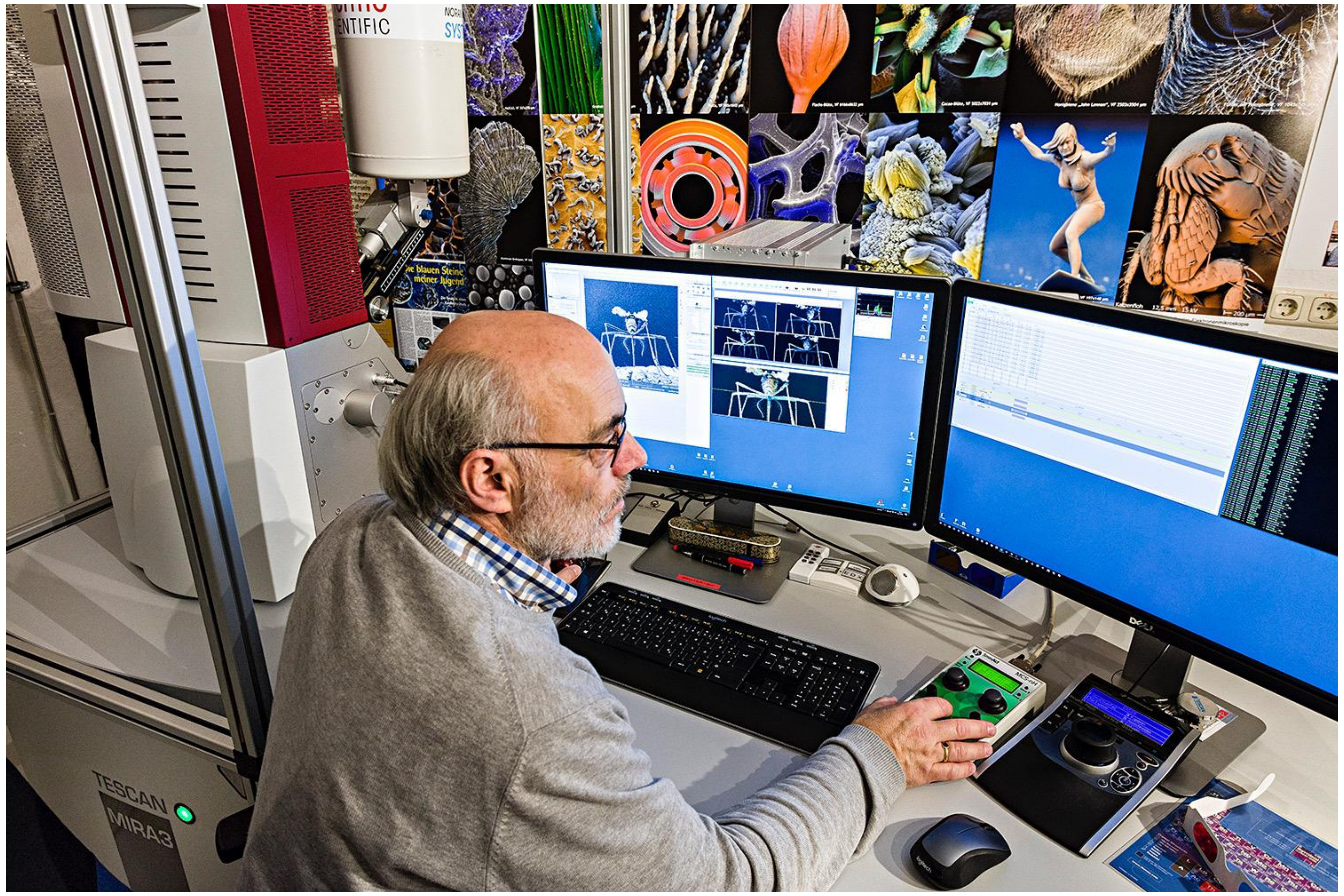
Hardware needed to do „nanoflights“

2. Remote capability of the most of the SEMs functions:



To make the „nanoflights“ possible, various parameters of the SEM need to be read, saved, interpolated and send again:

- Coordinates of the specimen table
- Focus value (in that case, „Working Distance“)
- Magnification (in that case, „View-field in microns“)
- Detector gain and black level (not implemented in MIRA extension up to now)
- Beam Shift X and Y for fine corrections at very small view-fields
- Scan Rotation for minor alignments
- 3D Beam angles X and Y for 3D sequences
- In development: Acquiring scans using TESCAN scan facilities (up to now DISS5 from point electronic is used to acquire the slow scan series)

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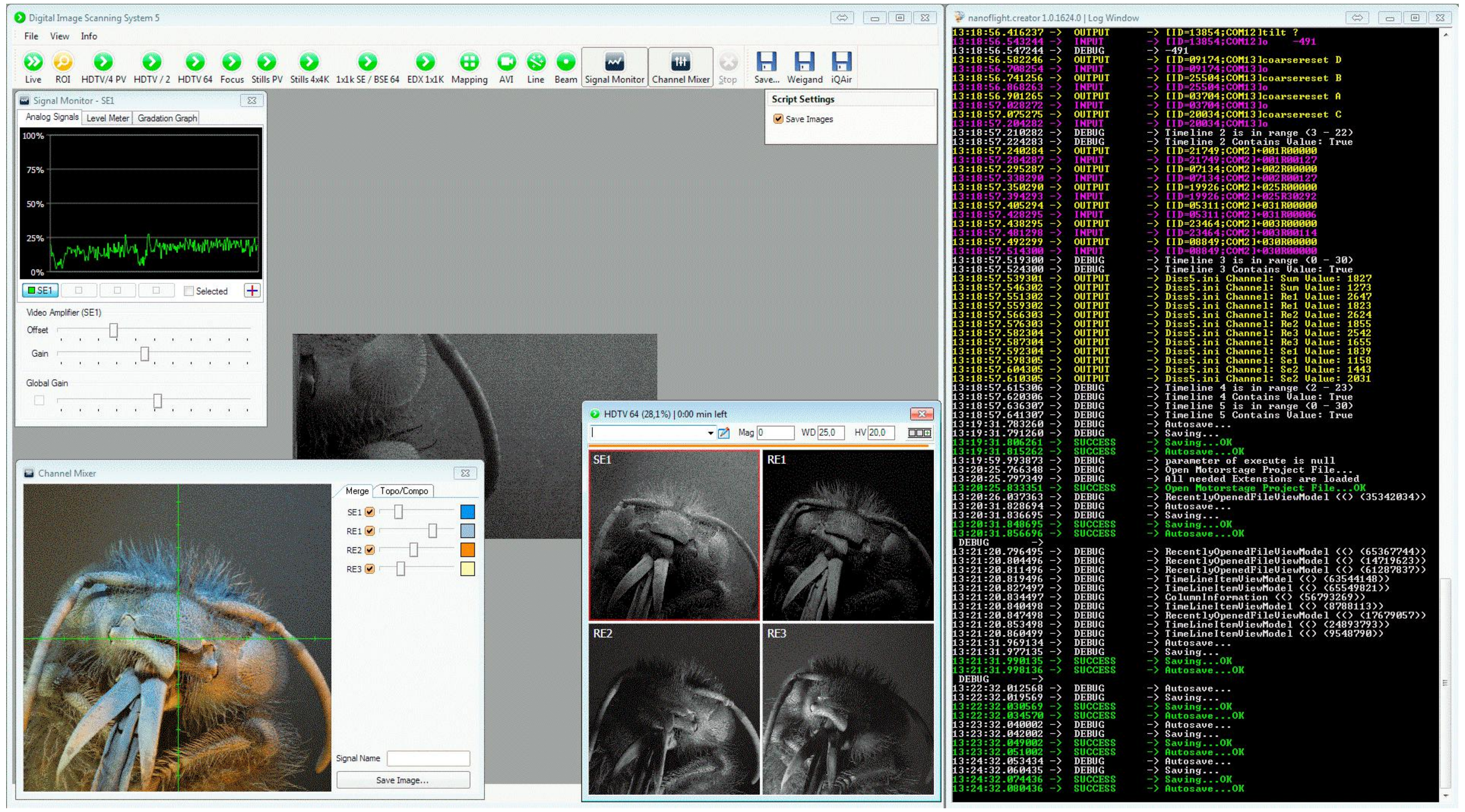
3. An up-to-date four channel image acquiring system specially modified to be remoted by script language:



The DISS5 scanning system made by Point electronic, Halle is a very sophisticated and versatile system to grab up to four detector channels in the SEM.

Within the nanoflight.creator DISS5 is used to deal with all the image data coming from the SEM:

- Scan field geometry and integration modes / times, max 7x7k px @ 4ch
- Selecting four out of eight detectors for imaging
- Gain and black-level of each detector used during the sequence
- Directly attributed 24bit RGB colours to each detector signal and possibility to change these values during the sequence
- Luminosity-adjustable „on the fly“ mixing of all coloured detectors to one final mixed colour SEM slow scan image
- Color images can be saved in 8bit RGB, detector channel images can be saved in 16bit gray value for later use

A new scanning and video pre-amplifier system in development at Point electronic will deal with some short-comings (for my kind of use...) of the existing system:

- 64x64k pixel scan size with minimum 20 nanoseconds dwell time per pixel
- simultaneously scanning of eight detector channels out of max. 32 signals

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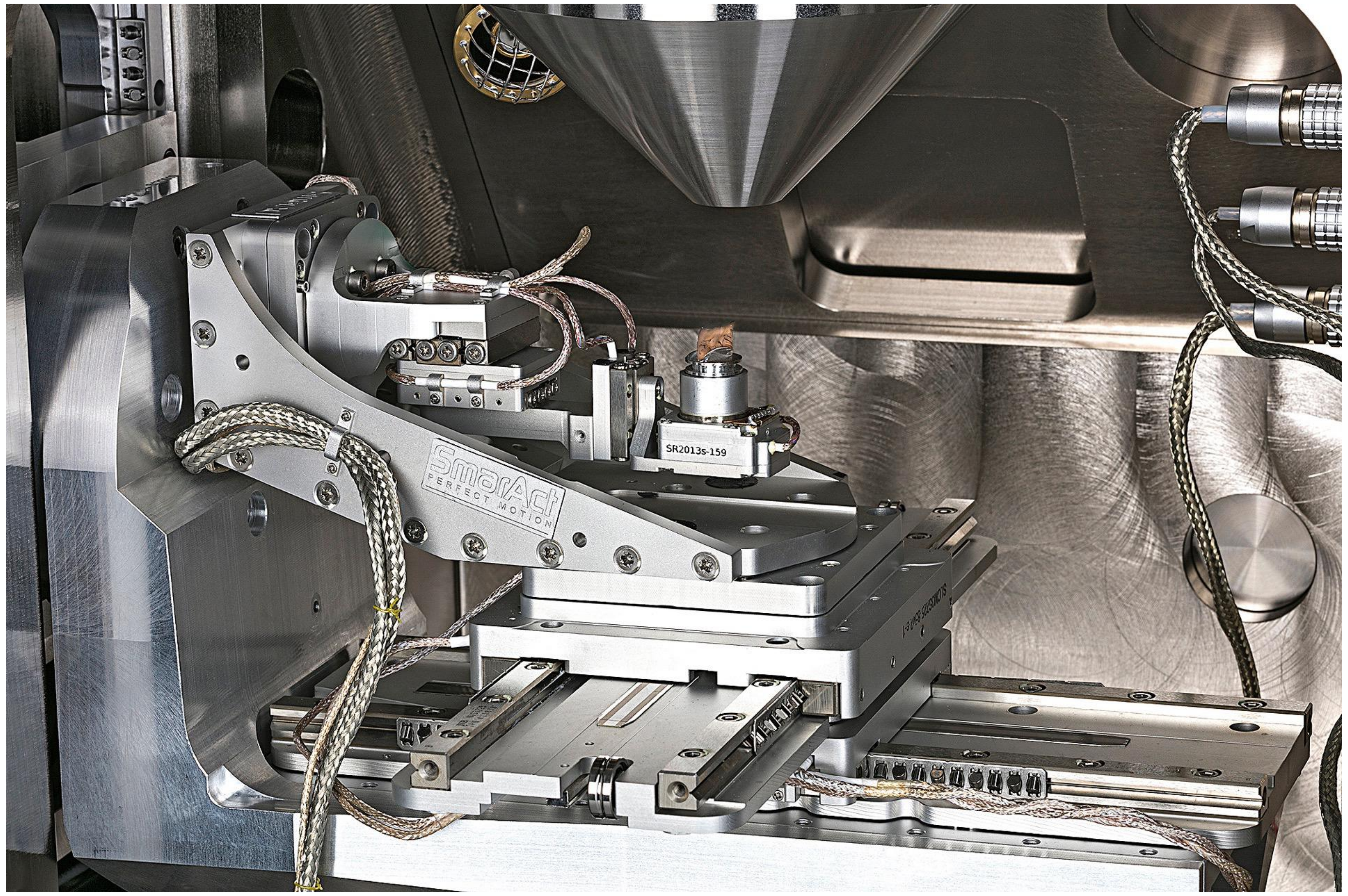
Hardware needed to do „nanoflights“

4.1. An eight axes piezo stage with a very good accuracy and repeatability of coordinates



To achieve an aesthetically pleasing movement of a specimen it is essential that the SEM is equipped with a very accurate (μ resolution) specimen stage controllable in very small steps (individual movements).

Accuracy needed is directly dependent on the view-field (VF) to be imaged. Most of the motorised stage in nowadays SEMs might not be able to deliver these specifications when going below a VF of some hundred microns wide. The piezo stage I am using had been specially built for me by SmarAct GmbH, Oldenburg.

The 3D X-Y-R lower stage is based on customised crossed roller bearings with integrated optical encoders. The closed loop resolution is about 1 nm, which leads to a repeatability of ca. ± 180 nm for the complete travel of ± 75 mm.

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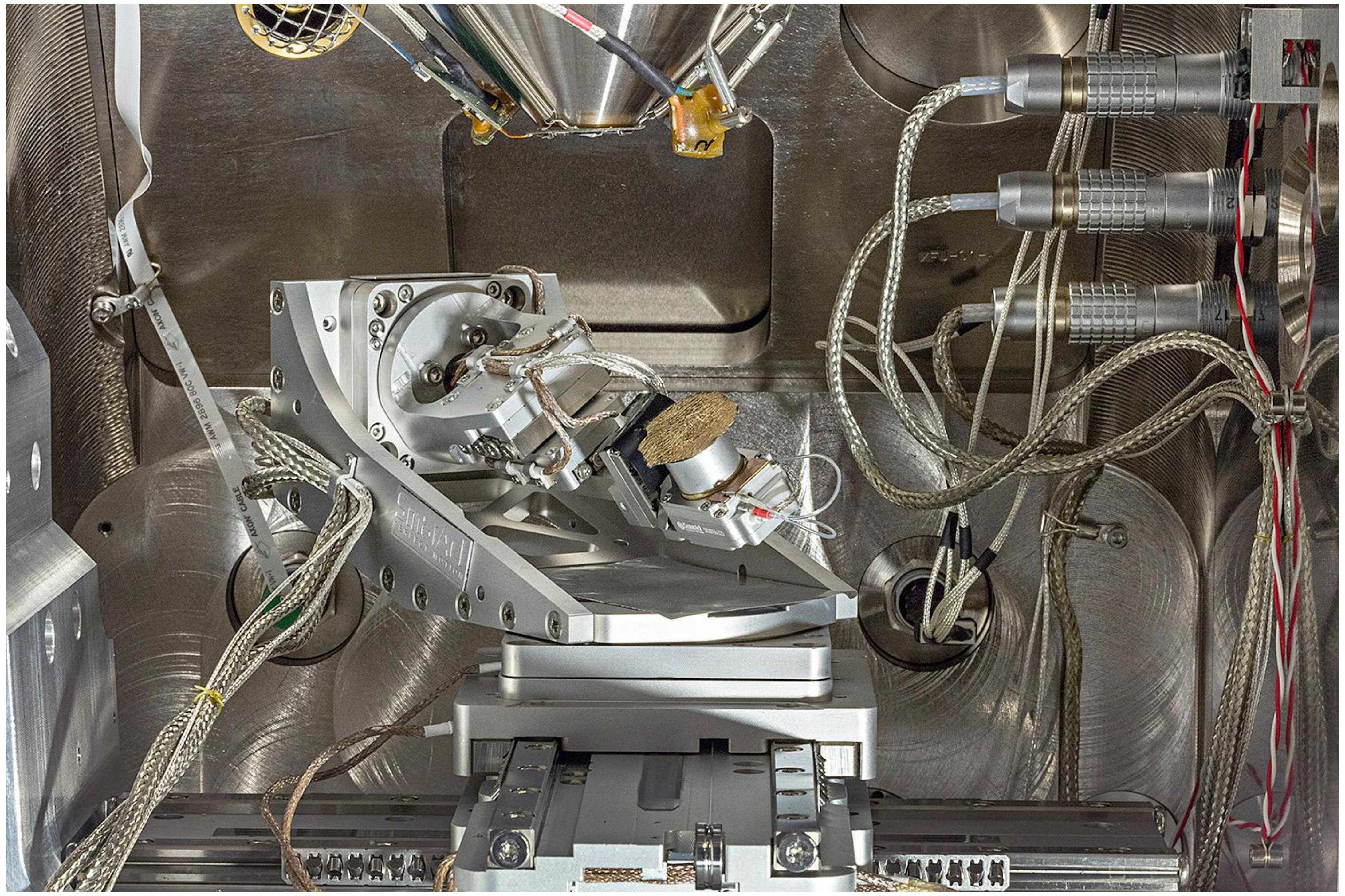
Hardware needed to do „nanoflights“

4.2. An eight axes piezo stage with a very good accuracy and repeatability of coordinates



On top of the 3D lower stage a compact 5D sample stage is mounted.

The first rotation is realised by a SR-4513 element with a closed-loop resolution of 15 µ°. The following linear positioners are based on SmarAct’s crossed roller bearings SLC series, providing a repeatability of ± 30 nm and a closed-loop resolution of about 1 nm.

The smallest closed-loop rotary positioner SR-2013 is equipped with a stub-holder for positioning the sample within the SEM beam focus and has a closed-loop resolution of about 25 µ°.

The available movements on the 5D substage are 360° for the first rotation, ± 8 mm for the Y axis, ± 10 mm for the X and ± 10 mm Z axis and unlimited travel for the final rotation. All axes can be controlled via IP protocol using the SmarAct API and manually, using the standard SmarAct MCS controller.

The exceptionally good repeatability in all stage coordinates will allow compucentrical movements, various translations of 3D paths for distinctive stereoscopic imaging and finally photogrammetric modelling of microstructures.

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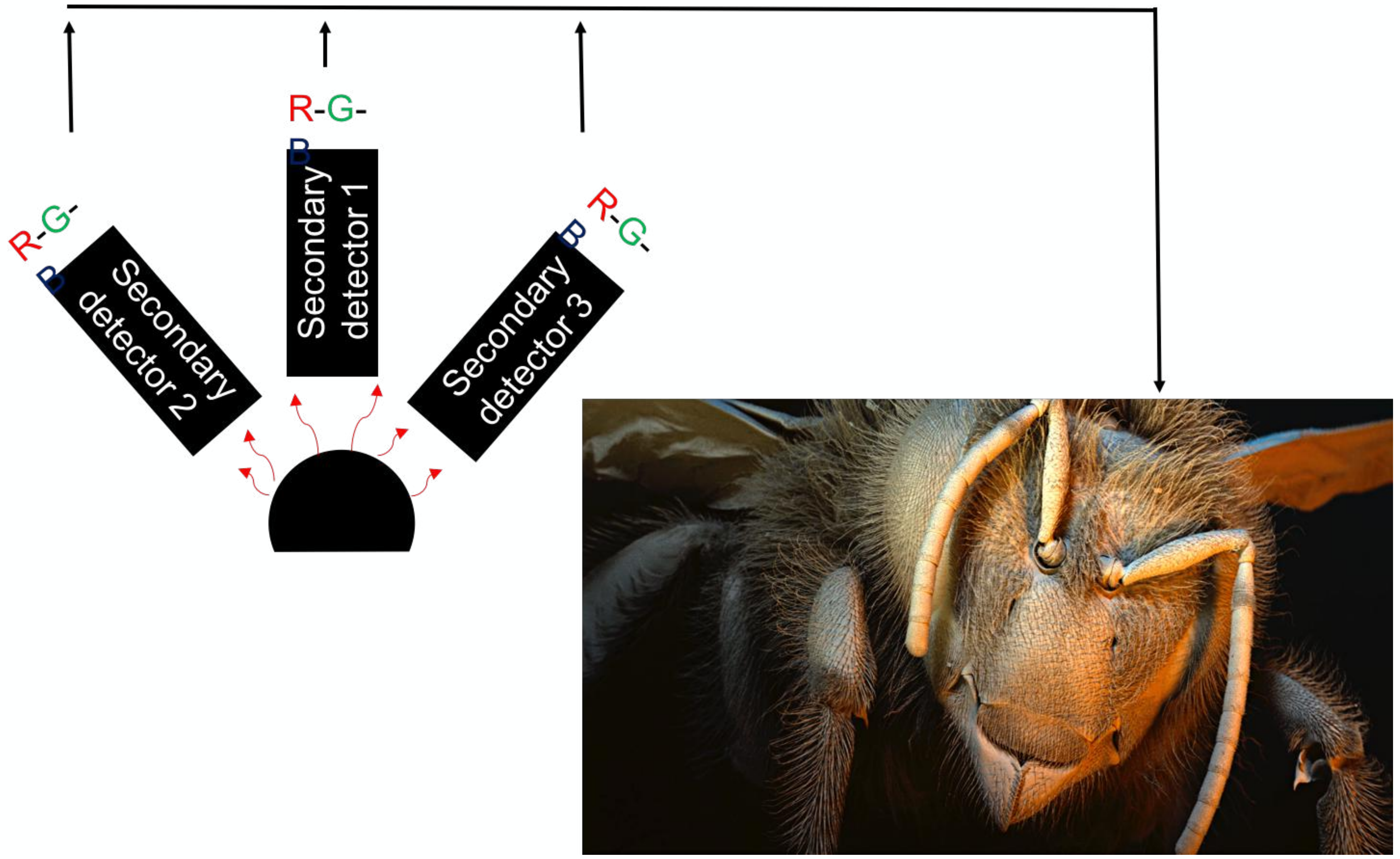
Hardware needed to do „nanoflights“

5. A lot of detectors within the electron microscope: seven backscattered, one specimen current and one secondary electron detector to be able to choose the characteristic of „lighting“ and „colouring“ the specimen



Nanoflights mostly use solid angle detector mixing with different SE and BSE detectors.

Presently, the instrument employs nine selectable detectors (1x SE / 1x SC / 7x BS).

Each detector is able to collect a signal which can be directly converted to a colour, which is then employed to colour small volumes (edges are populated with data from secondary electron signals and topography / flat areas with backscattered signals).

Three pairs of Si diodes around the final lens can be acquired like one single „ring light“ detector for nearly shadowless imaging (facilitates 2.5D reconstructions).

Three more Si diodes are far off-axis (ca. 30mm) to provide high contrast spot character signals.

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6. The nanoflight.creator software package and specially adapted „extensions“ to read / write values from / to all connected hardware

The „nanoflight.creator“ software is Windows-based and mostly written in C++.

First it had been intended as a proprietary software to work exclusively with the existing hardware at my lab but the software architecture allows also to use it with other remotely controllable instruments (for each new hardware an extension within nanoflight.creator needs to be written by the programmer) to take control of a lot of parameters in the microscope.

Since it is still „work-in-progress“ and not without errors, it is not presently commercially available but we are striving to support TESCAN SEMs using the SHARK Remote API from 2017 onwards.

The most demanding challenge for the development of the control software had been to make it modular, easily extendable for other remotely controllable SEMs or even stereoscopic light microscopy in natural colours. The main program handles only data and User Interface functions (GUI) and supplies a framework for all plug-ins used.

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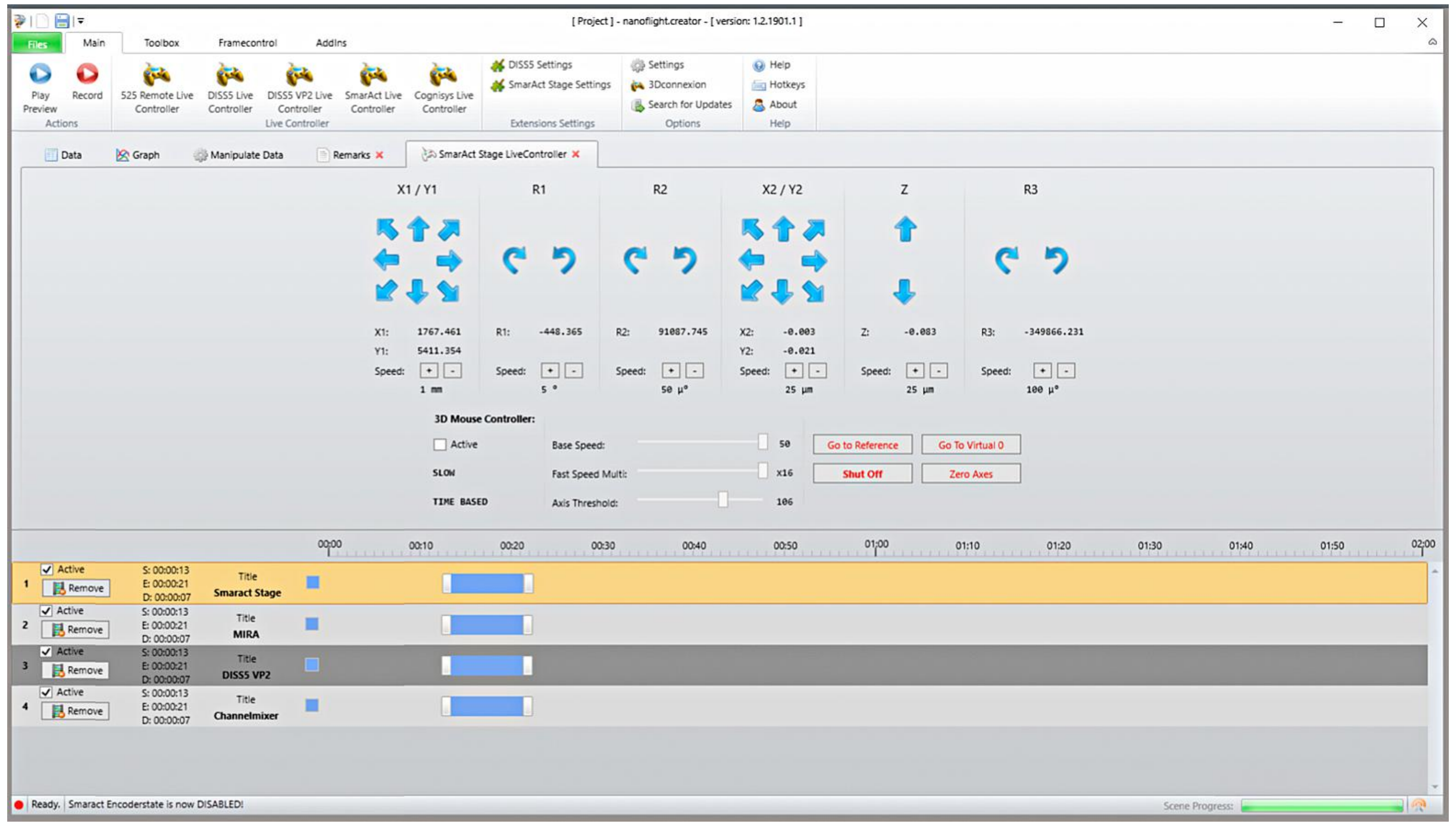
7. Different software modules in the nanoflight.creator package:



There are different levels of software modules:

- Extensions for proprietary hardware like specimen stages, remote controlled functions on the SEM and so on
- Live controllers to control this hardware, like the SmarAct controller shown in this image
- Add-ins like „Timelines“ to control the execution of programmed sequences within the overall time-schedule
- Pre-effect plug-ins like „Autofocus“ and after-effect plug-ins like „Colour-Correction“ or „Sharpness“

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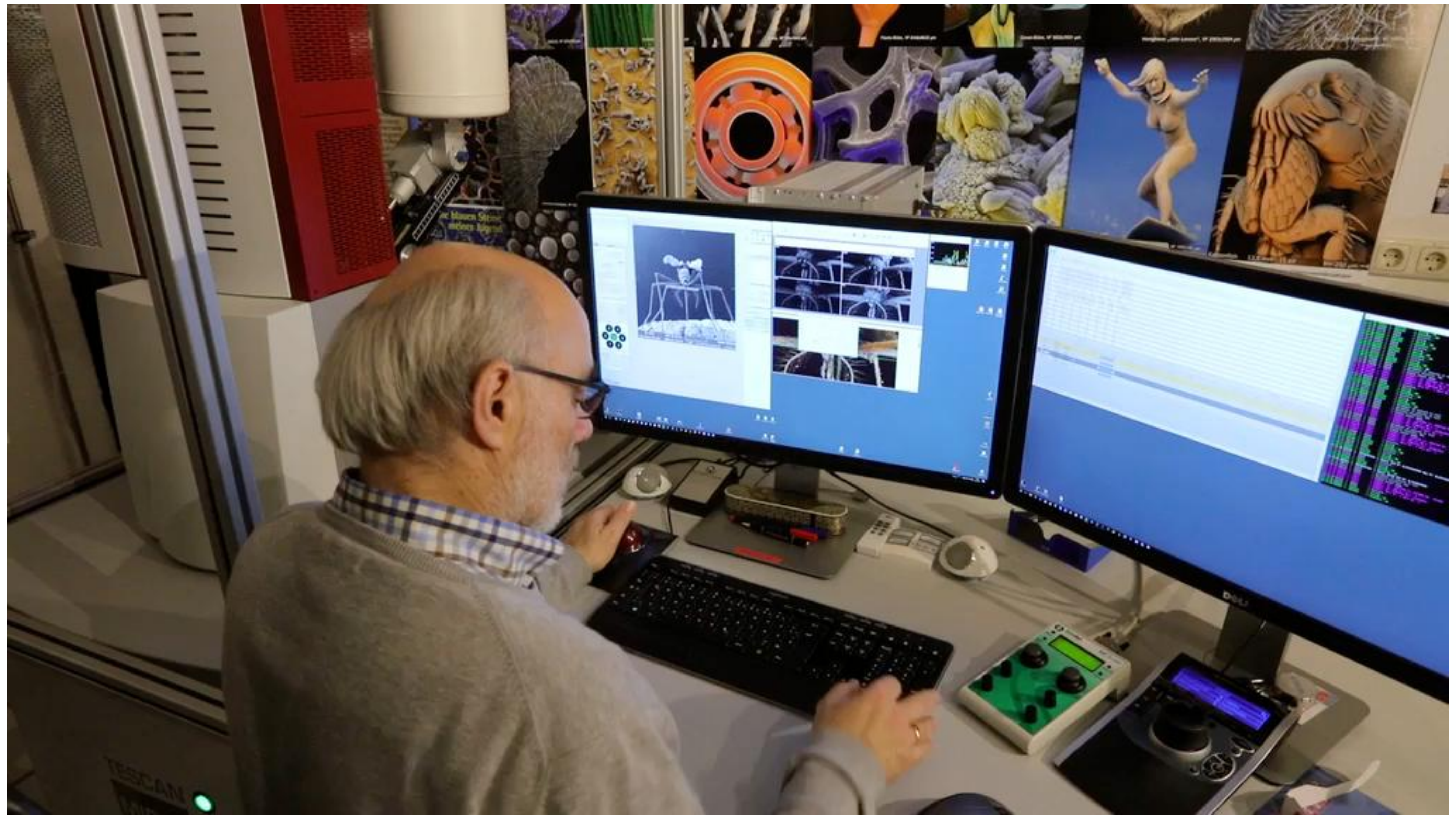
8.1. „nanoflight.creator“ takes control of a lot of parameters to be read from the attached hardware and to be sent back to this hardware during execution of the slow-scan frame sequence:

- Coordinates of the SmarAct specimen stage position in eight axes (five linear, three rotators)
- MIRA specimen stage coordinates
- Focus (Working Distance)
- Magnification (View-Field)
- Scan Rotation
- Beam-Shift and Beam Tilt X and Y for 3D imaging
- Detector gain and black level of up to eight detectors
- Detector colour and luminosity of up to eight detectors
- Number of frames between each acquired waypoint in the „flightpath“
- Slow-scan start and adjustable delay depending on scan parameters (integration time, resolution etc.)


Programming a 4 channel nanoflight

8.2. „nanoflight.creator“ takes control of a lot of parameters to be read from the attached hardware and to be sent back to this hardware during execution of the slow-scan frame sequence:

- Value interpolation between the waypoints for the number of frames can be either various types of Catmull-Rom or linear
- Resulting splines of read-in values can be corrected / smoothed by dragging the spline curves at a point in time
- Image data can be saved as a multichannel mixed colour image or as single grey scale images
- Preview function of the programmed sequence
- Stop / start new at a user defined frame-number
- Newly added Z-stack possibility
- Newly added 3D side-by-side sequences

All these waypoint function values are represented by vectors in a one- or multidimensional space that stand for the settings of all parameters used.

For example the software uses eight axes with the new piezo-controlled encoded stage and therefore an eight-dimensional spline interpolation routine is needed.

Within a normal image sequence more than forty hardware-specific parameters per image are thus send to the system.

9. Preparing the sequence setup:

When preparing the movie, different waypoints (stage coordinates) or function values are selected with the manual controller for the SmarAct piezo stage, the live controllers in the software graphic user interface (GUI) or the 3dconnexion (3Dconnexion, Waltham, MA, USA) six axes controller, which can control the substage movements, some of the remote values of the microscope and the scanning system (Figure 17a and b) or within the of the specially adapted scanning system DISS5 GUI.

The DISS5 brings with it a script language we used to integrate its functions within the „nanoflight.creator“ software (Point Electronic GmbH, Halle, Germany).

To get the impression of smooth translations in the final movie, the transitions between the waypoints have to be interpolated by using splines. For this purpose, a special cubic Hermite spline is used, the so called Catmull - Rom approach.

The basic principle of creating a smooth curve through all data points (normally in two dimensions or three dimensions) had been generalised to arbitrary dimensions. Tangents at all points are calculated with respect to adjacent points to obtain a continuous path.

In the „Framecontrol“ tab of the nanoflight.creator software between one and 500 frames can be interpolated within two spline waypoints.

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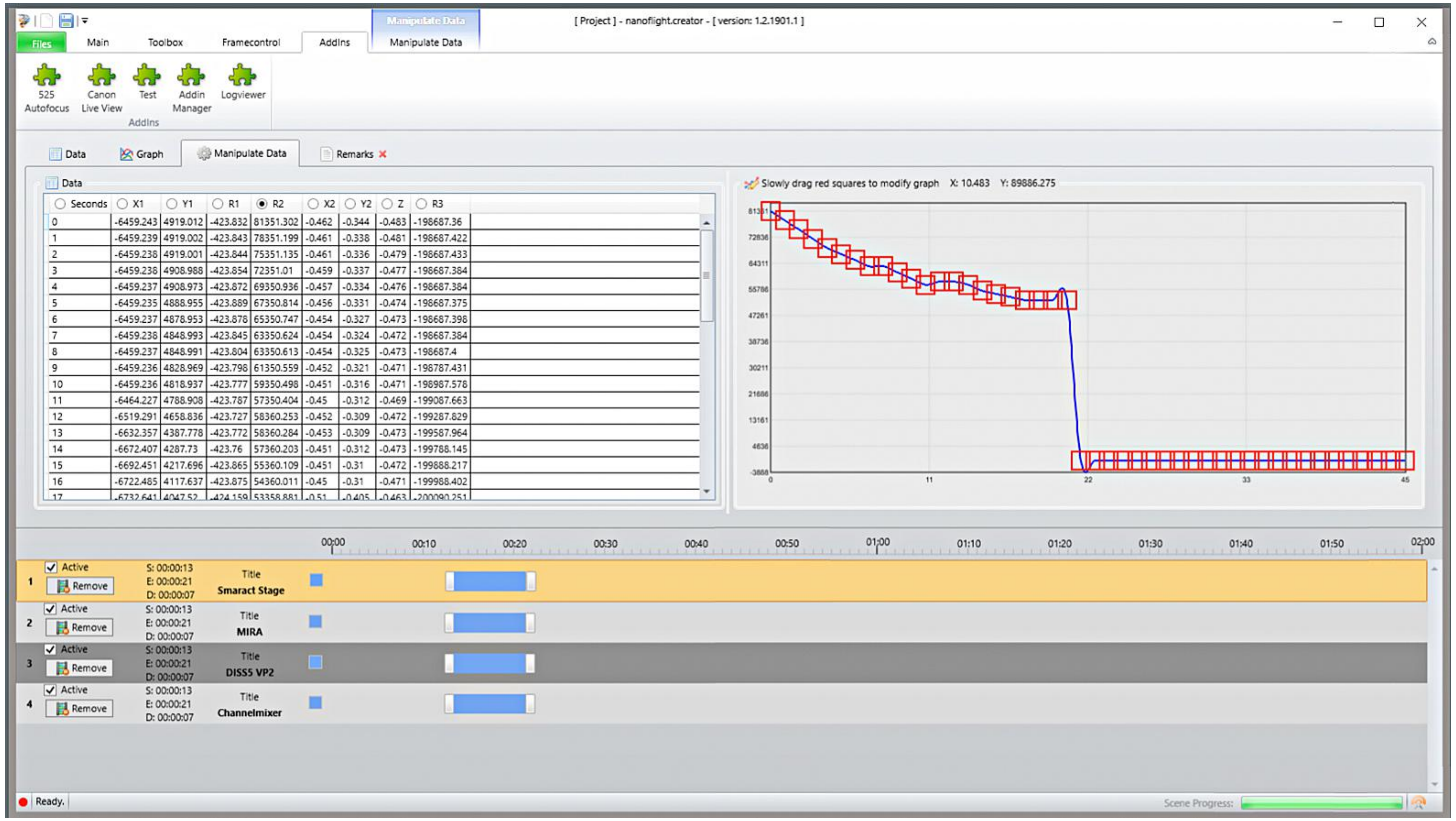
10. „nanoflight.creator“ example to adjust values graphically by dragging the spline waypoints:



Manipulation of spline values by dragging each waypoint

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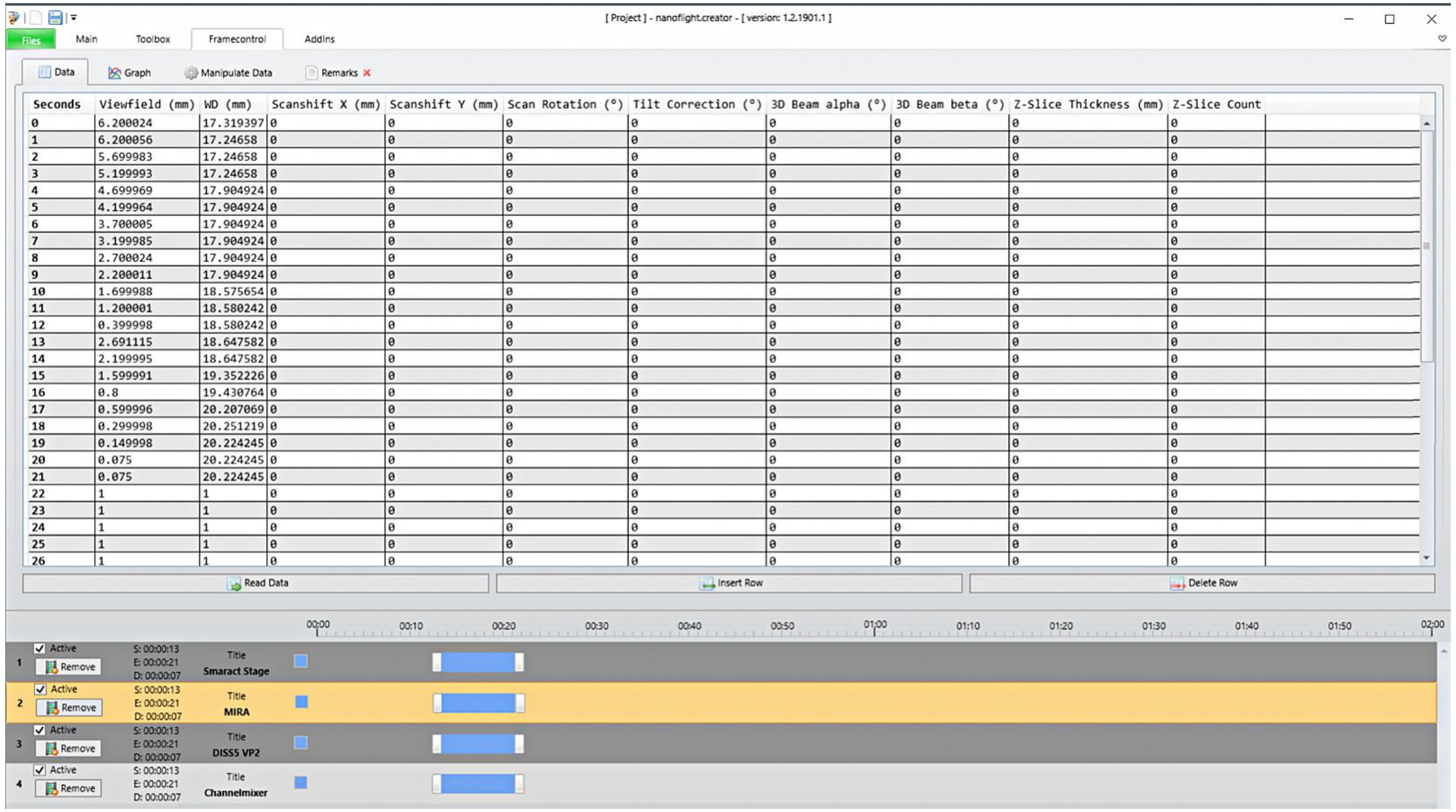
11. „nanoflight.creator“ MIRA extension with the parameters being read and send from / to the SEM:



Seconds	Viewfield (mm)	WD (mm)	Scanshift X (mm)	Scanshift Y (mm)	Scan Rotation (°)	Tilt Correction (°)	3D Beam alpha (°)	3D Beam beta (°)	Z-Slice Thickness (mm)	Z-Slice Count
0	6.200024	17.319397	0	0	0	0	0	0	0	0
1	6.200056	17.24658	0	0	0	0	0	0	0	0
2	5.699983	17.24658	0	0	0	0	0	0	0	0
3	5.199993	17.24658	0	0	0	0	0	0	0	0
4	4.699969	17.904924	0	0	0	0	0	0	0	0
5	4.199964	17.904924	0	0	0	0	0	0	0	0
6	3.700005	17.904924	0	0	0	0	0	0	0	0
7	3.199985	17.904924	0	0	0	0	0	0	0	0
8	2.700024	17.904924	0	0	0	0	0	0	0	0
9	2.200011	17.904924	0	0	0	0	0	0	0	0
10	1.699988	18.575654	0	0	0	0	0	0	0	0
11	1.200001	18.580242	0	0	0	0	0	0	0	0
12	0.399998	18.580242	0	0	0	0	0	0	0	0
13	2.691115	18.647582	0	0	0	0	0	0	0	0
14	2.199995	18.647582	0	0	0	0	0	0	0	0
15	1.599991	19.352226	0	0	0	0	0	0	0	0
16	0.8	19.430764	0	0	0	0	0	0	0	0
17	0.599996	20.207069	0	0	0	0	0	0	0	0
18	0.299998	20.251219	0	0	0	0	0	0	0	0
19	0.149998	20.224245	0	0	0	0	0	0	0	0
20	0.075	20.224245	0	0	0	0	0	0	0	0
21	0.075	20.224245	0	0	0	0	0	0	0	0
22	1	1	0	0	0	0	0	0	0	0
23	1	1	0	0	0	0	0	0	0	0
24	1	1	0	0	0	0	0	0	0	0
25	1	1	0	0	0	0	0	0	0	0
26	1	1	0	0	0	0	0	0	0	0

3D Beam angles for stereoscopic nanoflights and Z-stacks of the same frame are automated.

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12. Outlook to the future:

The „nanoflights“ are still a „project under construction“ depending on the availability of modern hardware for nearly-analogue viewing of the SEM sequences (e.g., high luminosity field emission microscopes with ultrafast scanning systems (DISS6 Scanning System - Point Electronic GmbH, Halle, Germany) / detectors (Ultrafast backscatter detectors specially to be build by PNDetector, Munich, and Point Electronic, Halle) and beam blanking during stage movements.

We intend to try to get a live stream of left / right image pairs out of the SEM, using them for immersive 3D SEM microscopy on-the-fly and even try to control the specimen and some microscope parameters like view-field by the integrated sensors available at high-end 3D glasses like OCULUS RIFT or HTC VIVE.

If any of you would like to contribute to this project, feel free to contact me.

13. Conclusions:

Film and movement are powerful tools to attract the attention of the general public, children and others to the importance of science at the nanoscale, however, the tools developed to create the movies can also support wider remote access to instruments.

The nanoflights software is not commercially available, however, can be accessed via the author. Future important developments include: developing of an inverse kinematics calculation routine for the existing eight axes piezo-stage, developing an on-the-fly routine to grab and smooth the „coordinate cloud“ of the six axes 3d connexion space mouse, nanoflight.creator GUI re-modelling, adding 2,5d modelling from 4 quadrant detectors and adding light microscopic imaging and Z-stacked movements with video or still-shot digital cameras.

However, as a software suite it has considerable potential in creating visually arresting movies of samples mounted in the SEM. In addition, its power as a SEM remote control software suite proffers nanoflights forming a foundation for an open public microscopy resource.

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14. References / Acknowledgements:

I am very grateful for constant help throughout the nanoflight project to my partners and sponsors:

Scan-Hard- and Software:	TESCAN, Brno / Point electronic, Halle
Specimen Stages:	SmarAct, Oldenburg / Kleindiek, Reutlingen
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Spline-Mathematics:	Prof. Dr. Hubert Mantz, Ulm
nanoflight.creator Software:	Max Holder / IRIS Software Solutions, Würzburg
Stacking Software:	Rik Littlefield, Zerene Systems

Concept, System Integration, Specimen Preparation:

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