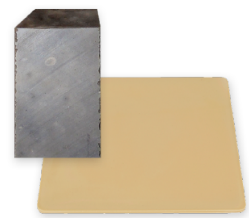
Oct 20, 2022 Version 2

The role of artificial contact materials in experimental use-wear studies - "artificial vs. natural experiment" V.2



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We use this protocol and it's working

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Keywords: controlled experiments, surface texture analysis, traceology, contact material, variable control, standardisation, edge angle, role of artificial contact material, artificial contact material, soft contact material, unidirectional cutting movement, experimental use, wear study, wear formation, mechanical device, experimental design, investigating use, natural experiment, experiment, different raw material, experimental

Abstract

The sequential "artificial vs. natural experiment" described here aimed at investigating use-wear formation on two different raw materials when unidirectional cutting movements were performed on hard as well as on soft contact materials. Simultaneously, artificial contact materials were tested as a controlled proxy within the experimental design
To limit the number of confounding factors, the samples are standardised and the experiment was performed with a mechanical device.

Protocol materials

- Plurafac LF 901 **BTC Europe GmbH, Rheinpromenade 1, D-40789 Monheim Catalog #**-
- Modified bone-like polyurethane coated with rubber skin **SYNBONE® Catalog #**PR0114.G
- soft tissue pad with matrix **SYNBONE® Catalog #**PR1043.10
- AccuTrans AB, brown **Coltene Catalog #**3700
- Acetone ≥99.5 % **Carl Roth Catalog #**5025.6
- Acetone ≥99.5 % **Carl Roth Catalog #**5025.6
- aloe vera sensitive detergent **Frosch (R) Catalog #**-
- Isopropanol 70% **Carl Roth Catalog #**CN09.4
- Hydrogen peroxide solution 3% **Otto Fischar GmbH & Co. Kg**
- Plurafac LF 901 **BTC Europe GmbH, Rheinpromenade 1, D-40789 Monheim Catalog #**-
- Technovit Provil Light regular **Kulzer GmbH Catalog #**66009333

Troubleshooting

Sample preparation

10m

1 **Standardised tools**

12 x 60 ° Baltic flint sample

12 x 60 ° silicified schist sample

1.1 **Raw material**

Baltic flint:
Southern Sweden (secondary deposit):
55.945852 N, 12.767851 E

Silicified schist:
Balver Höhle (secondary deposit)
51.339167 N, 7.871944 E
Buhlen (secondary deposit)
51.191022 N, 9.086585 E

1.2 **Blanks**

Raw material nodules (step #1.1) were first cut into rectangular blanks of the following dimensions:

10 mm thickness

25 mm width

60 mm minimum length

Equipment

Goliath 450	NAME
Lapidary rock saw	TYPE
Steinschleifmaschinen & Lapidary tools Ltd.	BRAND
-	SKU
https://www.steinschleifmaschinen.at/index.php?main_page=product_info&cPath=1_17&products_id=184	LINK
-	SPECIFICATIONS

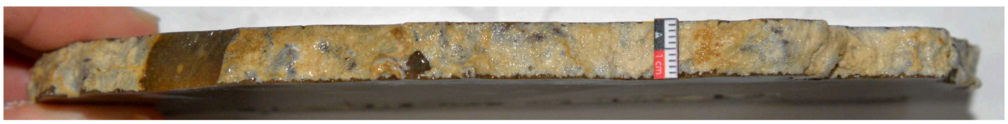
a) cut 10 mm slices

Cutting slice (here: flint).

Cutting slice, with protective cover lifted for photo (here: flint).

First cut to provide level surface, photo with protective cover lifted (here: flint).

Slice, side view (here: flint).



Slice, top view (here: flint).

b) cut slices into blanks

Blank, top view (here: flint).

Blank, lateral view (here: flint).

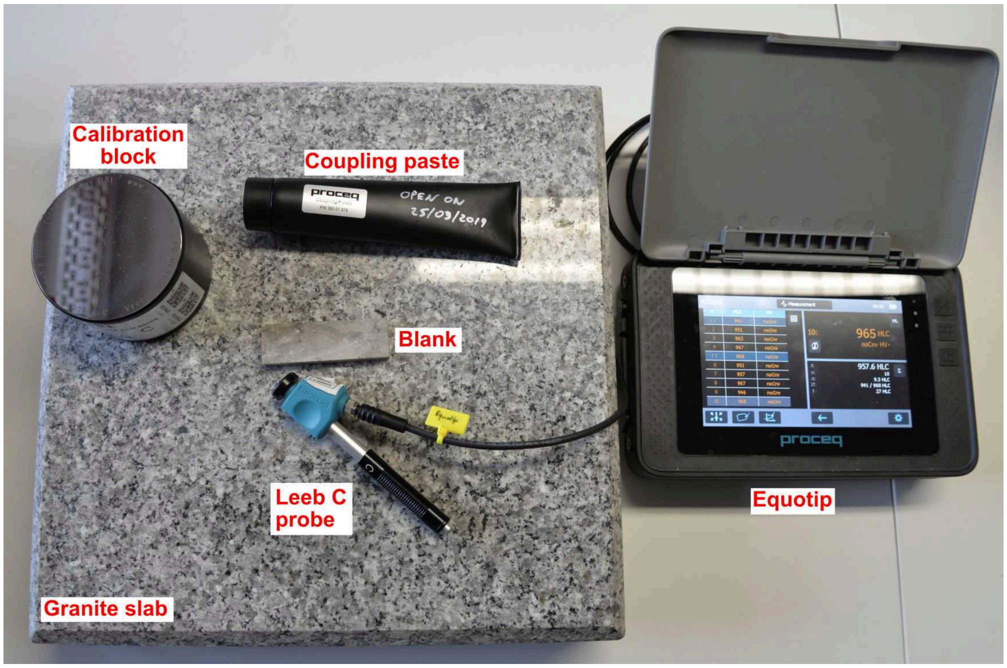
1.3 **Hardness measurement**

The hardness of the blanks (step #1.2) was measured with a Leeb rebound hardness tester.

Equipment

Equotip 550	NAME
Portable hardness tester	TYPE
Proceq	BRAND
-	SKU
https://www.proceq.com/products/list/Category/equotip-portable-hardness-testing/	LINK
Leeb C probe	SPECIFICATIONS

The blanks were placed on a stable base (here a flat rock slab of about 20 kg). Since the samples did not fulfill the requirements for minimum sample size and weight, an additional coupling paste was used to connect the sample with the base. Each blank was measured ten times to insure and test intra-blank variability.



Setup for measuring Leeb rebound hardness with the devices/components labeled.

Measuring Leeb rebound hardness.

1.4 **Edge angle**

One end of the blanks (step #1.2) was cut to produce tools with a 60 ° edge angle , with the following dimensions:

10 mm thickness

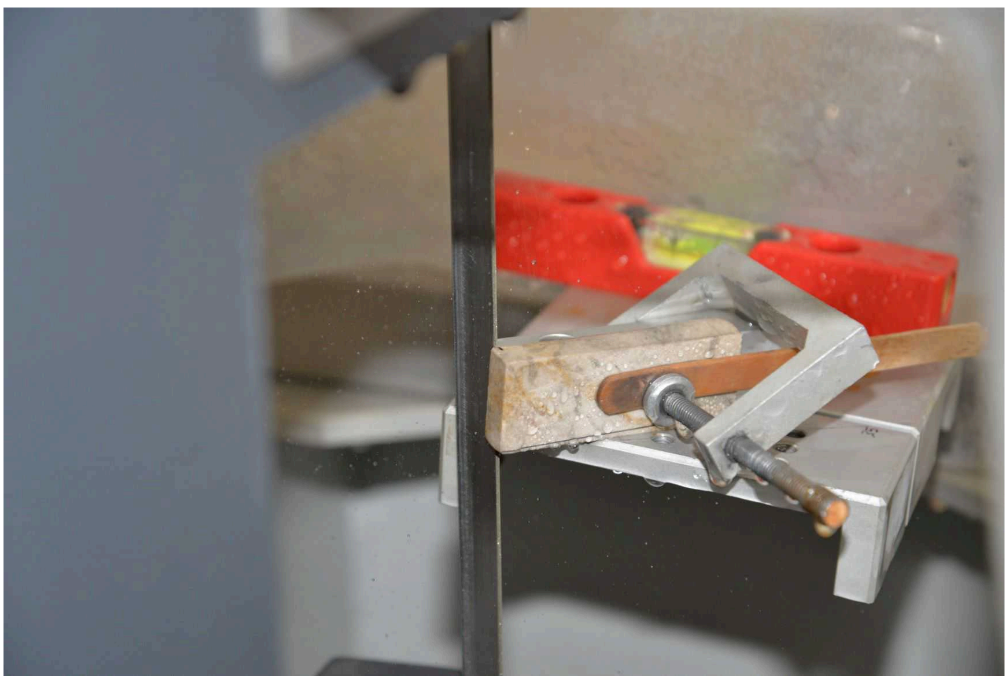
25 mm width

30 mm minimum length

Equipment	
310 CP	NAME
Diamond band saw	TYPE
Exact	BRAND
-	SKU
https://www.exakt.de/en/products/thin-section/industry.html	LINK

Cutting edge angle.

Positioning blank, close-up (here: flint).



Cutting edge angle, close-up (here: flint).

Sample, view of side A (corresponding to the 'dorsal' side of bifacial lithic artefact; here: flint).

Sample, view of side D (corresponding to the 'lateral' side of bifacial lithic artefact; here: flint).

1.5 **Chamfered edge**

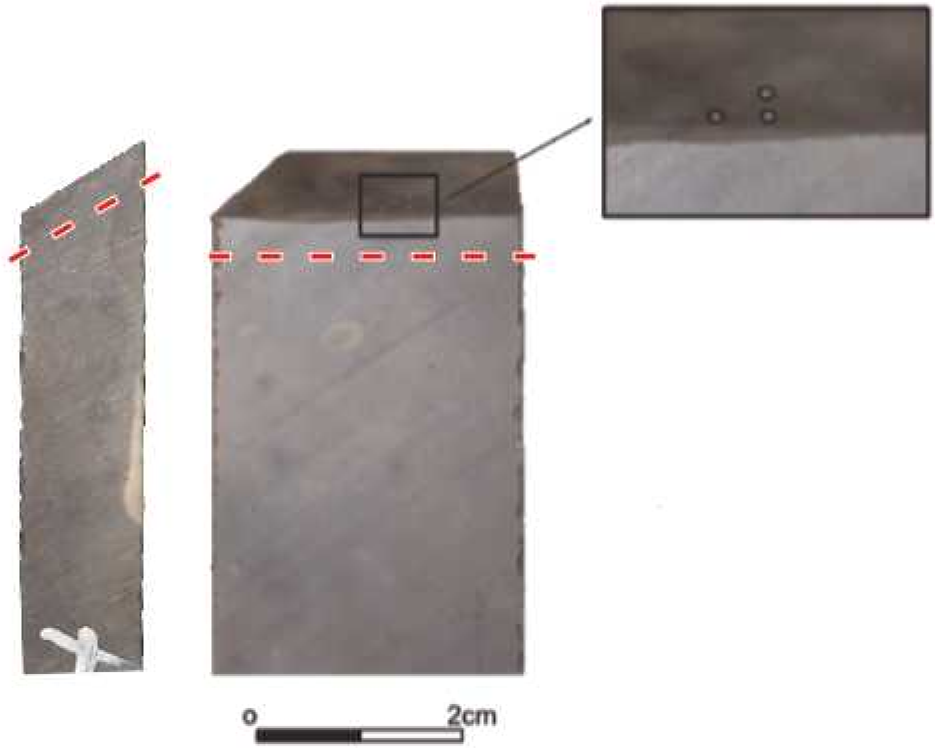
To avoid catastrophic breakage of the edge during experiments, the leading edge of the samples (step #1.4) was chamfered at 45 ° edge angle .

Equipment	
310 CP	NAME
Diamond band saw	TYPE
Exact	BRAND
-	SKU
https://www.exakt.de/en/products/thin-section/industry.html	LINK

Cutting chamfered edge (here: flint).

The cut with the band saw left a small burr between the two adjacent surfaces at the edge of the chamfered surface and the lateral side D. This burr was manually removed with a mini diamond drill bit.

1.6 Not all 24 experimental standard samples were produced immediately. After the experiment was conducted with half of the samples, the length of the sample was reduced by removing a ~1 cm slice along the 60° leading edge to reveal a fresh, unused surface for a new cutting edge by repeating step 1.4 - 1.5. This way, 12 new samples could be produced without increasing raw material intra-variability.



Hatched line in red shows cutting plane to reveal new cutting edge and to preserve the previously used one, including coordinate beads on either side.

1.7 **Coordinate system**

3 ceramic beads were adhered onto each side of the cutting edge to produce a coordinate system on either side.

Final sample with beads, view of side A (corresponding to the 'dorsal' side of bifacial lithic artefact; here: flint).

Final sample with beads, view of side C (corresponding to the 'ventral' side of bifacial lithic artefact; here: flint).

For details, see:

Citation

Calandra I, Schunk L, Rodriguez A, Gneisinger W, Pedergnana A, Paixao E, Pereira T, Iovita R & Marreiros J (2019). Back to the edge: relative coordinate system for use-wear analysis. Archaeological and Anthropological Sciences.

https://doi.org/10.1007/s12520-019-00801-y LINK

1.8 Cleaning

10m

100 mL Cleaning solution

1 Mass / % volume

Plurafac LF 901 **BTC Europe GmbH, Rheinpromenade 1, D-40789 Monheim Catalog #-**

Equipment	
Sonorex Digitec DT255H	NAME
Heated ultrasonic bath	TYPE
Bandelin	BRAND
-	SKU
https://ultraschall-welt.de/ultraschallreiniger/serien/bandelin-geraete/269/bandelin-sonorex-digitec-dt-255-h-5-5?c=0	LINK
4.5 L	SPECIFICATIONS

45 °C

00:10:00

Contact materials

10m

2 **Hard and soft contact material**

Four different contact material were used: two natural and two artificial contact materials.

2.1 **Natural contact material**

Cow scapula (*Bos primigenius)* was provided by a butcher in a fresh state. The periosteum and small pieces of flesh were still attached to the bone. The cutting experiment was carried out in a laboratory under ambient room temperature conditions. During the course of the experiment, the bone started to dry out but apart from desiccation no other degradation processes were visible. The bone was used as hard contact material.

Cow scapula horizontally fixed with three screws on the table.

Fresh pig skin (*Sus scrofa domesticus*) provided by a butcher by separating the skin from the flesh below. Experiments on the skin were conducted at room temperature. Overnight storage was executed in a refrigerator. Two pieces of skin were needed during the course of the experiment. The skin was used as soft contact material.

Pig skin fixed with metal strips on the table.

2.2 **Artificial contact material**

Synthetic bone plate with a rubber skin layer (imitating periosteum)

Modified bone-like polyurethane coated with rubber skin **SYNBONE® Catalog** #PR0114.G

with the following dimensions:

250 mm length

250 mm width

6 mm thickness

Shore hardness (D): 78 ± 5 %

Bone plate coated with rubber skin.

Artificial skin soft tissue pad with matrix **SYNBONE® Catalog** #PR1043.10 made of silicone ecoflex. The skin pad has the following dimensions:

140 mm length

130 mm width

4.2 mm thickness

Shore hardness (A): 0-3

Soft tissue pad.

Experimental setup

10m

3 **General settings**

Linear unidirectional cutting movements: 2000 strokes split in 4 cycles .

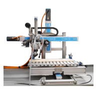
The cycles are defined by the follwing number of strokes:
1 strokes to 50 strokes
51 strokes to 250 strokes
251 strokes to 1000 strokes
1001 strokes to 2000 strokes

Note: the stroke movement length on the soft contact material (and for some samples on the fresh cow scapula) was half the length compared to the stroke movement length on the hard contact material. In thoses cases, the number of strokes was doubled.

4 **Experimental setup**

Equipment

SMARTTESTER	NAME
Modular material tester	TYPE
Inotec AP	BRAND
-	SKU
https://www.smarttester.info/	LINK
recorded values with the time stamps: - for each drive: position, speed, acceleration, force (as measured through the drive's current usage) - penetration depth with extra sensor - apllied force with extra sensor - friction with extra sensor	SPECIFICATIONS



Linear movement from start point to end point:

2 kg dead weights (soft contatct material) /

6 kg dead weights (hard contact materials)

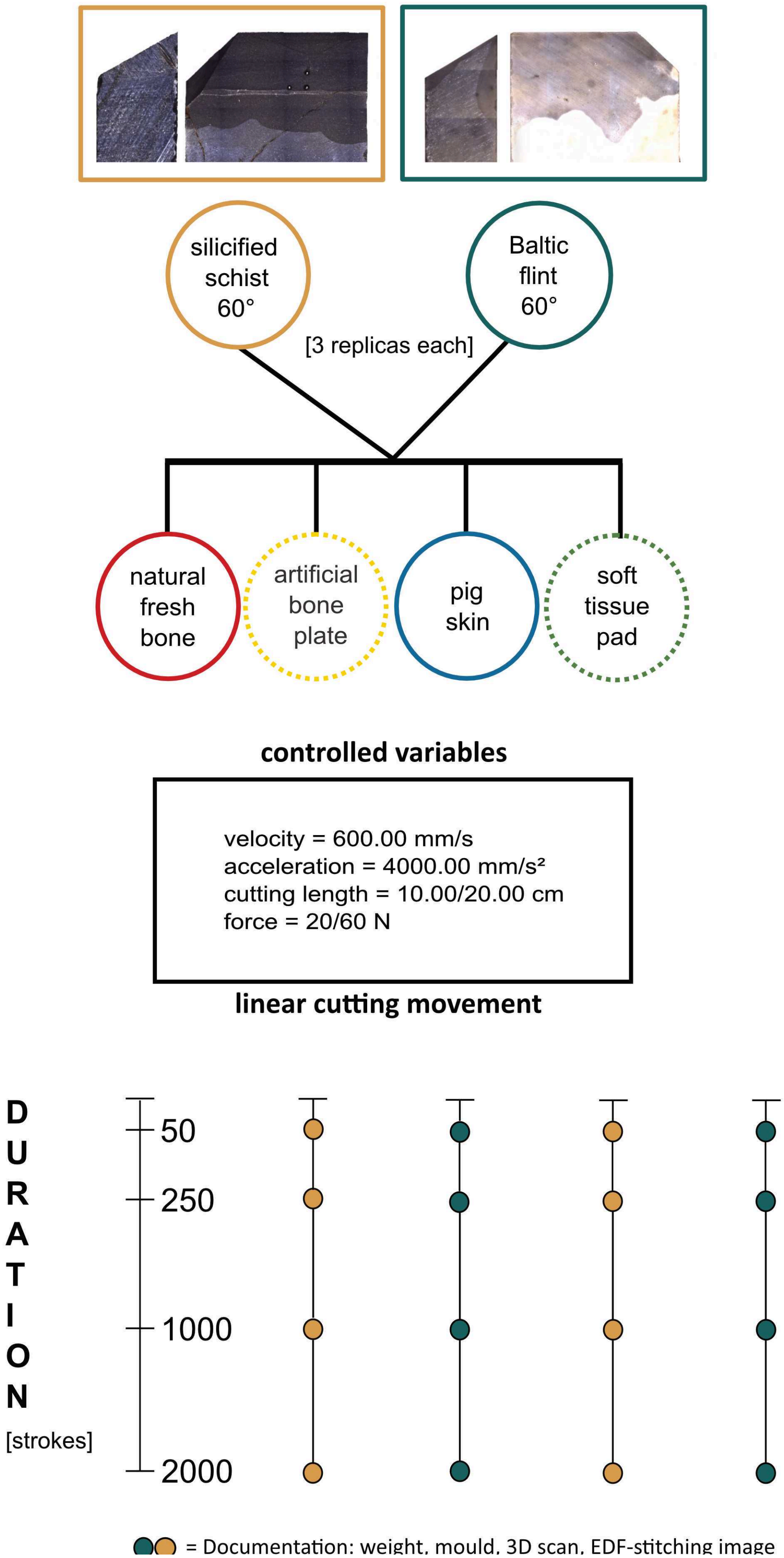
100 mm movement length (soft contact materials) /

200 mm movement length (hard contact materials)

600 mm/s movement speed

4000 mm/s2 movement acceleration

10 Hz reading frequency (for each data channel)

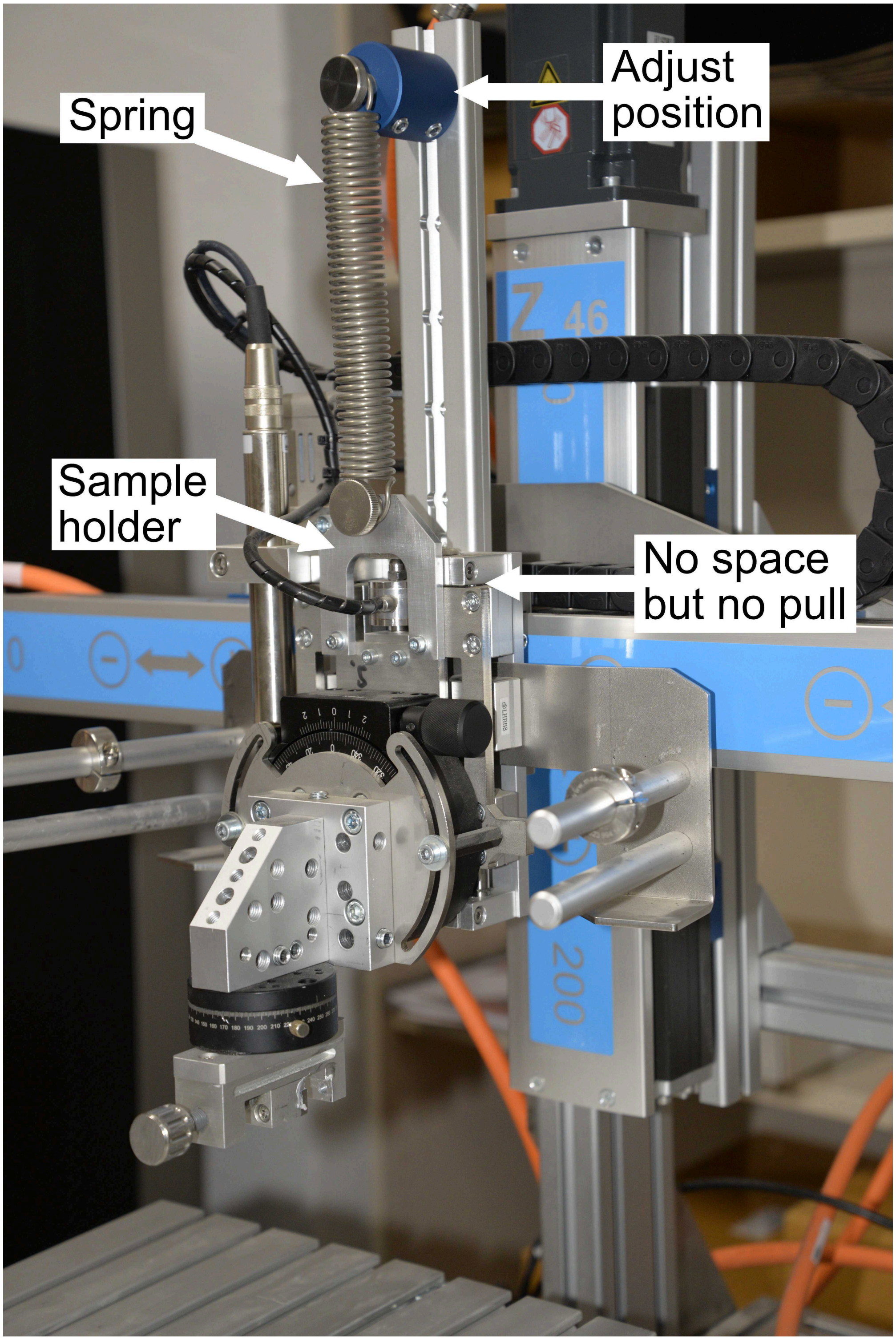

silicified schist 60°
Baltic flint 60°
[3 replicas each]
natural fresh bone
artificial bone plate
pig skin
soft tissue pad
controlled variables
velocity = 600.00 mm/s
acceleration = 4000.00 mm/s²
cutting length = 10.00/20.00 cm
force = 20/60 N
linear cutting movement
DURATION
[strokes]
50
250
1000
2000
= Documentation: weight, mould, 3D scan, EDF-stitching image

Experimental design.

4.1 **Setup sample holder**

The length of the spring on the sample holder was adjusted to compensate for the weight of the sample holder itself (without dead weights and sample).

Adjust the position of the spring attachment on the rail, so that:
1. the sample holder almost touches the frame, and
2. at the same time, there should be no pull from the spring against the sample holder frame.



Zeroing sample holder.

4.2 **Setup sample**

The sample (step #1) was clamped in the sample holder (SMARTTESTER) and manually orientated (i.e. angles corrected and standardised) in all directions.
The cutting edge was parallel to the plane of the contact material (
90 ° cutting angle)

Sample positioned for the experiment to begin.

4.3 **Setup contact material**

The contact materials were fixed onto the table in different ways. Each method guaranteed that the contact material could not move during the experiment. Additionally, after removing the contact material for scanning or overnight storage, repeated identical positioning was possible. The materials were aligned with the X-axis.

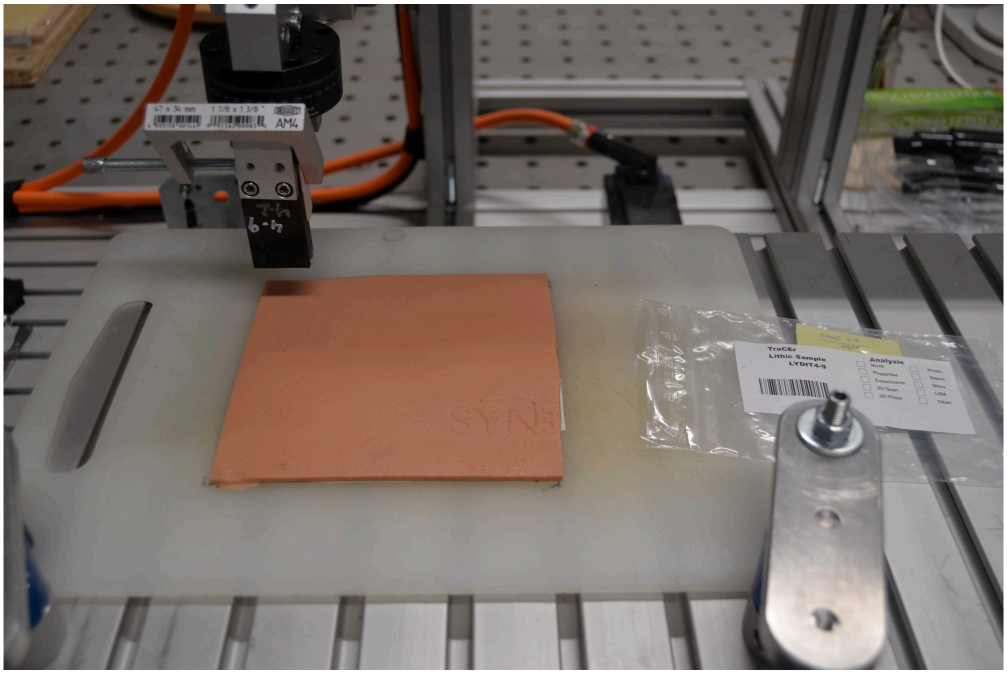
a) cow scapula
The cow scapula was aligned horizontally and fixed with three bolts on a wooden base (see images in steps #2.1 and #4.2). The wood itself was fixed with screw clamps to the table.

b) pig skin
The pig skin was fixed with two perforated metal strips on a wooden base (see image in step #2.1), which was fixed with screw clamps to the table.

c) bone plate
The bone plate (step #2) was clamped around the edges in a custom-made sample holder and fixed with a screw in the middle of the bone plate.

d) soft tissue pad
The soft tissue pad was attached with a double-sided adhesive tape to a plastic cutting board. The board itself was fixed with screw clamps to the table.

Bone plate with the sample holder (wooden frame).



Soft tissue pad clued to the cutting board.

4.4 **Program cutting movement**

For each sample, a new template was created (named with the sample ID and the stroke number).

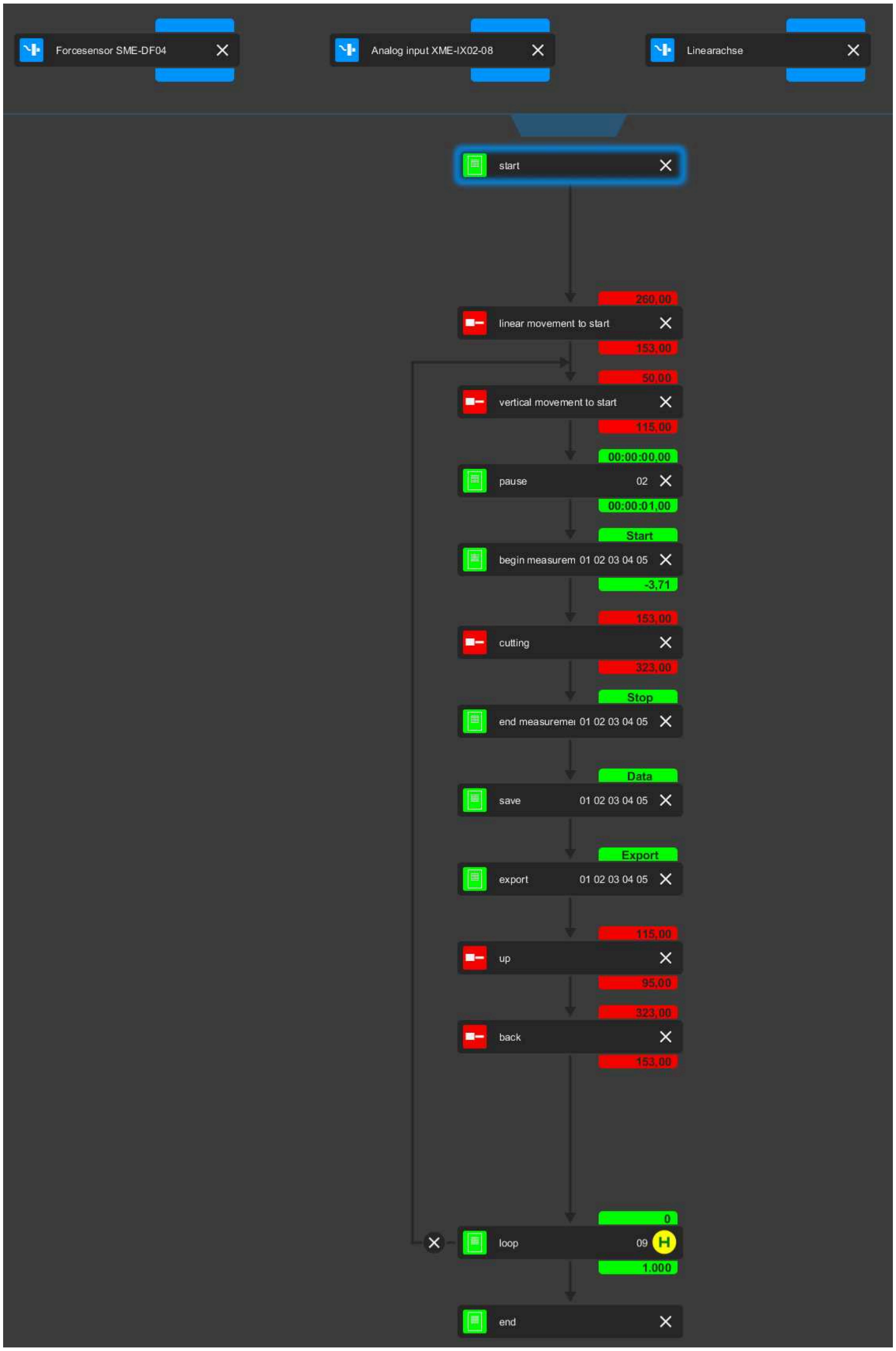
a) Move down in Z-direction to start position
The z-value of the starting point was defined as follows: the z-drive was moved down slowly until the edge of the tool was in contact with the contact material. A few extra mm (e.g. 5 mm for the bone plate) were added to the position of the z-drive in order to give the tool the possibility to penetrate into the contact material without cutting through it.

b) Move forward in X-direction 100 mm / 200 mm (linear movement)
c) Move up in Z-direction approx. 20 mm (above bone plate; no contact between contact material and tool)
d) Move backward in X-direction to starting point
e) Loop 50 times over steps #4.1a-4.1d and export data to CSV
f) Loop 200 times over steps #4.1a-4.1d and export data to CSV
g) Loop 750 times over steps #4.1a-4.1d and export data to CSV
h) Loop 1000 times over steps #4.1a-4.1d and export data to CSV

The experiment was planned as a sequential experiment. The 2000 strokes were therefore split into four sequences (steps #4.1e-h). The tools were documented after each sequence (step #5).

For each sequence, five CSV files were exported, one for each recorded data channel:

- Sample penetration depth into contact material as measured by the distance sensor in the sample holder.
- Force applied (Z-direction) as measured by the force sensor in the sample holder with affixed dead weights.
- Friction as measured by the force sensor in x-direction between the stage and the fixed stage frame (between mounted contact material and sample).
- Position of the X-drive (travel range).
- Velocity of the X-drive.



Program as seen in the GUI. Blue = sensors, red = actuators (drives) and green = control flow elements.

cutting_template.Smart

4.5 **Run program**

- Each sample was used over a duration of ~ 07:00:00 (= running time SMARTTESTER)

The following samples were used for the experiment:

a) cow scapula
FLT4-6
FLT4-14
FLT4-15
LYDIT4-5
LYDIT4-7
LYDIT4-12

b) pig skin
FLT4-4
FLT4-12
FLT4-13
LYDIT4-1
LYDIT4-4
LYDIT4-6

c) bone plate
FLT4-10
FLT4-7
FLT4-5
LYDIT4-2
LYDIT4-3
LYDIT4-8

d) soft tissue pad
FLT4-11
FLT4-12
FLT4-13
LYDIT4-9
LYDIT4-10
LYDIT4-11

Sample documentation

10m

5 **Sample documentation** 45m

Before the experiment, as well as after each cycle, all 24 samples were documented in an identical way following these steps:

- cleaning with tap water and comercial washing up liquid
- weight measurement (threefold repetition)

Equipment	
Kern PCB 3500.2	NAME
weighing scale	TYPE
Kern	BRAND
-	SKU
https://www.kern-sohn.com/	LINK
accuracy of 0.1g	SPECIFICATIONS

- 3D scanning (identical settings for all scans)

Equipment

smartScan-HE R8 NAME

3D structured light scanner TYPE

AICON BRAND

- SKU

https://www.hexagonmi.com/products/structured-light-scanners/aicon-smartscan LINK

S-150 FOV, resolution of 33 µm SPECIFICATIONS

- cleaning with tap water and comercial washing up liquid
- optical documentation of three of the four surfaces per sample (one lateral and the two main surfaces)

Equipment

Smartzoom 5 NAME

digital microscope TYPE

ZEISS BRAND

- SKU

https://www.zeiss.de/mikroskopie/produkte/imaging-systems/smartzoom-5.html LINK

1.6x objective; EDF-stitching images SPECIFICATIONS

- cleaning the area of interest with a cotton stick with
  Isopropanol 70% **Carl Roth Catalog** #CN09.4
- cleaning the area of interest with a cotton stick with
  Acetone ≥99.5 % **Carl Roth Catalog** #5025.6

Note

The cleaning protocol differed for samples used on fresh natural bone. The presumed presence of
lipids and collagen in addition to abraded mineral particulates was considered to act as a binder with adhesive properties. Therefore the use of a cleaning agent containing enzymes such as lipase and protease was considered instead of harsher treatments such as alkalies or acid hydrolysis.

Additional cleaning for the samples used on natural bone only:

- 100 mL Cleaning solution
  1 Mass / % volume

Plurafac LF 901 **BTC Europe GmbH, Rheinpromenade 1, D-40789 Monheim Catalog** #-

Equipment

Sonorex Digitec DT255H — NAME

Heated ultrasonic bath — TYPE

Bandelin — BRAND

- — SKU

https://ultraschall-welt.de/ultraschallreiniger/serien/bandelin-geraete/269/bandelin-sonorex-digitec-dt-255-h-5-5?c=0 — LINK

4.5 L — SPECIFICATIONS

45 °C

00:15:00

- 3x 100 µL tap water to rinse
- 1x 100 µL destilled water to rinse
- air-drying using compressed air from oil-free compressor
- localised brushing (soft bristle brush) with Acetone ≥99.5 % **Carl Roth Catalog #**5025.6
- localised brushing with Hydrogen peroxide solution 3% **Otto Fischar GmbH & Co. Kg**
- 14.5 g aloe vera sensitive detergent **Frosch (R) Catalog #**- (This was a readily available commercial substitute for more difficult to obtain enzymes and should not serve as a general product recommendation as it contains other ingredients tailored to general household applications)

Equipment

Sonorex Digitec DT255H	NAME
Heated ultrasonic bath	TYPE
Bandelin	BRAND
-	SKU
https://ultraschall-welt.de/ultraschallreiniger/serien/bandelin-geraete/269/bandelin-sonorex-digitec-dt-255-h-5-5?c=0	LINK
4.5 L	SPECIFICATIONS

45 °C

00:30:00

- 3x 100 µL tap water to rinse
- 1x 100 µL destilled water to rinse
- air-drying using compressed air from oil-free compressor

It should be noted that this cleaning procedure was used as an alternative to a more extreme procedure (e.g., use of acid), since the effects of certain chemical on the micro surface texture are not fully known yet (especially not for silicified schist).
To verify that no residues were attached to the sample surface anymore, selected samples used on natural bone were analysed with an SEM/EDX for elemental composition.

Equipment

SEM ZEISS EVO 25 + Bruker Quantax XFlash 6|30M NAME

scanning electron microscope + EDX detector TYPE

ZEISS BRAND

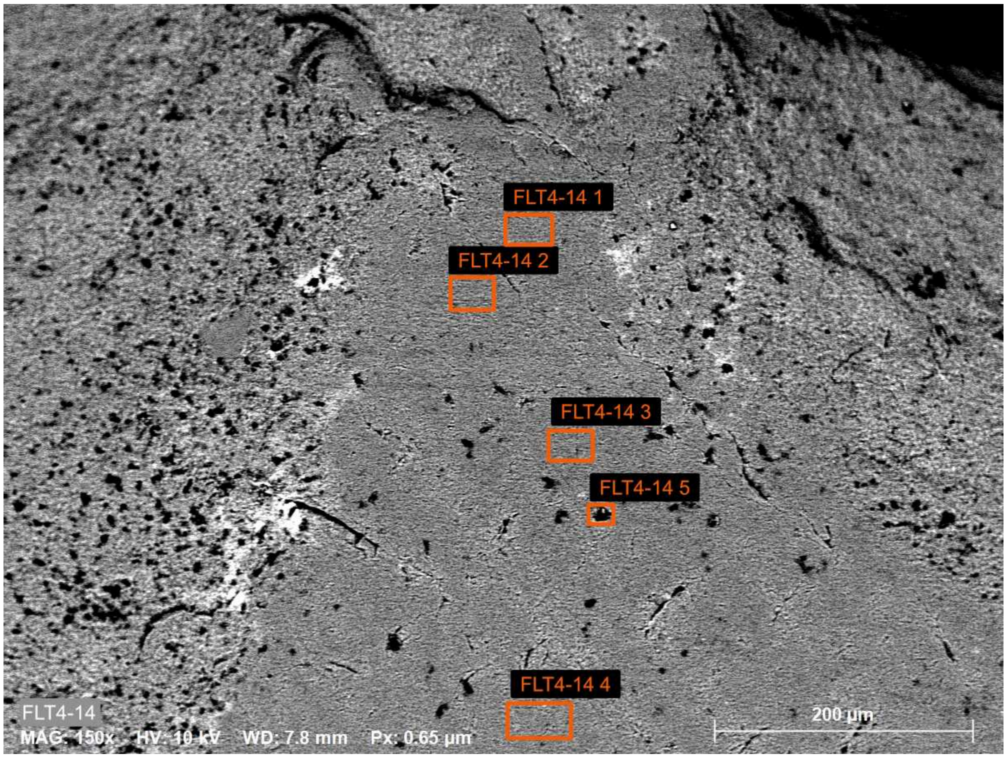
- SKU

https://www.zeiss.com/microscopy/en/home.html LINK

Per sample and spot, several measurements covering the different phases were acquired with the EDX detector where use-wear was visible, using the *Objects* workspace in the Bruker Esprit 2.3 software.

The images were acquired with the following settings:

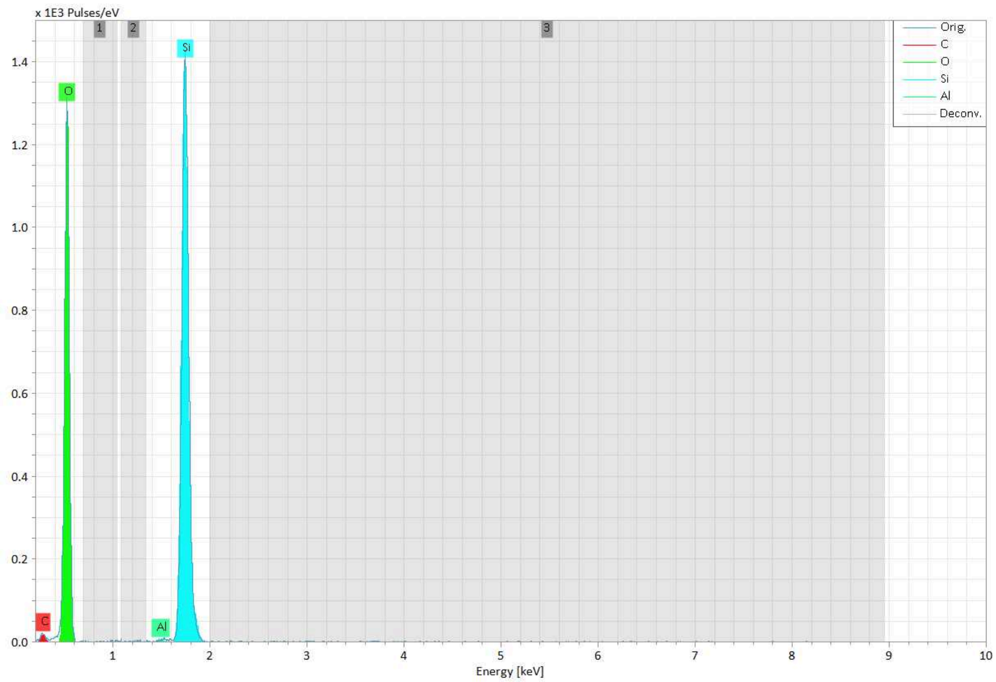
- Detector: HDBSD (back-scattered electron detector).
- High vacuum.
- Magnification: 100 or 150×, as shown on the image. The value is relative to the Polaroid 545 reference.
- Accelerating voltage (*EHT*): 10 kV, as shown on the image.
- Working distance: 7.8-8 mm, as shown on the image.
- Image size: 1200 × 900 pixels.
- Pixel size: 0.97 µm.
- Dwell time: 8 µs.
- Beam current (*I Probe*): 200 pA.



BSD image of sample FLT4-14. Locations of the individual EDX measurements are indicated in orange.

EDS spectra were acquired with the following settings:

- EDS calibration was first checked on a piece of copper tape. Deviation was less than +/- 5 eV.
- Rectangular objects were drawn at the regions of interest on the BSD image.
- Duration: the *Precise* setting was used, i.e. each location was measured until at least 250,000 counts were measured.
- The input count rate was around and pulse throughput was set at 60 kcps (the lowest available), resulting in dead time ≤ 3 % and live time 45-60 s.
- Maximum energy was set to 20 keV.



Results of the EDX analysis of sample FLT4-14 measurement 1. Neither calcium nor phosphorus have been detected on the surface, indicating that the surface is not contaminated with bone residues.

EDS spectra were quantified with the following procedure:

1. An automatic identification of the peaks was performed.
2. Background fit regions were automatically selected.
3. The X and Y axes were expanded to check if unidentified peaks are still present, starting from the highest accelerating voltage. If yes, the region around the peak is selected and the element finder is used to identify the peak. The fit is visually examined to check if the identified element correspond to the peak.
4. Background fit regions were automatically selected.
5. Background fit was visually examined to check that it fits the spectrum.

EDS spectra were quantified with the following settings:

- Carbon and oxygen were used for peak deconvolution only, i.e. their concentrations are not included in the final quantitative elementar compositions.
- Background fit model: physical (*SEM*).
- Deconvolution settings: *Series fit*, i.e. the line intensity ratios within a line family based on physical properties are used for the deconvolution.
- Quantification model: P/B-ZAF.
- No charging was observed: counts were measured across the whole range of accelerating voltage (this was checked visually using the logarithmic scaling of the spectra).

Spectrum	Carbon	Oxygen	Sodium	Magnesium	Aluminium	Silicon	Phosphorus	Sulfur	Calcium
FLT4-14 1	0	0			0.18913372	99.81087			
FLT4-14 2	0	0			0.12395776	99.87604			
FLT4-14 3	0	0			0.15600224	99.844			
FLT4-14 4	0	0				100			
FLT4-14 5	0	0	0.266794	0.420569711		75.71087	3.518854095	1.974716	18.10819

Exemplarily, the results of the quantification of sample FLT4-14.

After cleaning, all samples were documented with moulding material:

- moulding of the two main sample surfaces

Technovit Provil Light regular **Kulzer GmbH Catalog #**66009333 ; on some occasions,

AccuTrans AB, brown **Coltene Catalog #**3700 was used additionally

Moulding of one of the two surfaces (here: flint).

Data acquisition

10m

6 **Data acquisition: edge angle**

The 3D models (samples + contact material) were imported as STL files into GOM and existing mesh holes were closed. Based on the closed models, the volume could be calculated.

Software	
GOM Inspect	NAME
Hotfix 2, Rev. 111729, build 2018-08-22	OS
GOM	DEVELOPER

Additionally, the edge angles of the samples were calculated by means of GOM.

- the edge of each sample was defined by a digital line
- the "2-lines" procedure of the edge angle calculation method was applied

Citation

Schunk et al., in prep.
. A new semi-automated 3D digital method to quantify stone tool edge angle and design.

7 **Data acquisition: qualitative use-wear analysis**

a) data acquisition on all 24 samples

All samples were checked for use-wear on both sample sides (A and C; see section #1.7), respectively. First, each sample exposed to 2000 strokes was checked to localise areas of wear, then the corresponding areas on the sample in the state before the experiment was conducted.

b) data acquisition on eight selected samples

In a second step, four flint and four silicified schist samples were selected. Each of the four samples per raw material was tested on a different contact material: bone cow scapula, bone plate, pig skin and skin pad. This time the samples were analysed before and after each cycle.

7.1 **Acquisition settings**

The qualitative use-wear analysis was done in a high-power approach by means of an upright light microscope (ZEISS Axio Scope.A1 MAT). The samples were studied using EC-Epiplan 5x/0.13, 10x/0.25 and 20x/0.40 objectives. Traces were documented as an EDF black and white image.

Equipment

Axio Scope.A1 MAT

Upright light microscope RL

ZEISS

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4ck4F_DwUYMvMvDjQluN6Rw&q&adurl&ved=2ahUKEwivlLrfson4AhVAwAIH

Category	Setting	Value
Microscope	Manufacturer	Carl Zeiss Microscopy GmbH
	Model	Axio Scope.A1 MAT
Location	Laboratory	TraCEr, MONREPOS, Germany
	Floor	Basement (-1)
	Setup	Standard office desk
Acquisition	Software	Zen core 2.7
	Mode	Bright field
Objective	Manufacturer	Carl Zeiss Microscopy GmbH
	Objective	EC Epiplan 5×/NA = 0.13/WD = 11.8 mm
		EC Epiplan 10×/NA = 0.25/WD = 11.0 mm
		EC Epiplan 20×/NA = 0.40/WD = 3.2 mm
Illumination	Source	White LED, reflected co-axial light **
	Wavelength	550 nm
Size and resolution	Field of view	1.70 × 1.42 mm (5× obj.) / 850.08 × 709.32 µm (10× obj.) / 425.04 × 354.66 µm (20× obj.)
	Frame size	2464 × 2056 pixels

8 **Data acquisition: quantitative use-wear analysis**

a) data acquisition on all 24 samples

First, the last state of the samples (after 2000 cutting strokes) was analysed. The samples were analysed for use-wear and within the use-wear, 3D surface data was acquired. Those exact three spots were analysed then also on the samples from before the first cycle (0 strokes), respectively.
Per sample, three surfaces per tool were acquired on the same use-wear area, but on non identical areas. Here, "surface" refers to the result of the acquisition, i.e. a 2.5 D image. The thee surfaces per sample are called "spot A", "spot B" and "spot C".
All surfaces were acquired on the flat side of the tools, i.e. side C (see section #1.7).

3 surfaces per tool +
× 24 tools
x 2 cycles (before + 2000 strokes)
= 144 surfaces acquired

b) data acquisition on eight selected samples

In a second step, the same eight samples as described in step #7 b) were quantitatively analysed. The data acquisition was identical to #a). However, this time the samples were analysed before and after each cycle.

3 surfaces per tool +
× 8 tools
x 5 cycles (before + 50 + 250 + 1000 + 2000 strokes)
= 120 surfaces acquired

The majority of samples was studied as moulds (see step #5).

8.1 **Acquisition settings**

Equipment	
LSM 800 MAT	NAME
Confocal microscope	TYPE
Carl Zeiss Microscopy	BRAND
-	SKU
https://www.zeiss.com/microscopy/int/products/confocal-microscopes/lsm-900-for-materials-non-contact-surface-topography-in-3d.html	LINK
Mounted on an Axio Imager.Z2 Vario	SPECIFICATIONS

Category	Setting	Value
Microscope	Manufacturer	Carl Zeiss Microscopy GmbH
	Model	Axio Imager.Z2 Vario + LSM 800 MAT
Location	Laboratory	TraCEr, MONREPOS, Germany
	Floor	Basement (-1)
	Setup	Passive anti-vibration table on solid concrete base
Acquisition	Software	ZEN blue 2.3 with Shuttle&Find module
	Mode	LSM (laser scanning confocal microscopy) or WF (wide field)
Objectives	Manufacturer	Carl Zeiss Microscopy GmbH
	Coordinate system (WF)	C Epiplan-Apochromat 10×/NA = 0.40/WD = 5.4 mm
	Surface topography (LSM)	C Epiplan-Apochromat 20× /NA = 0.70/WD = 1.30 mm; C Epiplan-Apochromat 50×/NA = 0.95/WD = 0.22 mm
	Black and white EDF image (WF)	C Epiplan-Apochromat 10×/NA = 0.40/WD = 5.4 mm; C Epiplan-Apochromat 20× /NA = 0.70/WD = 1.30 mm; C Epiplan-Apochromat 50×/NA = 0.95/WD = 0.22 mm
Illumination for surface topography (LSM)	Source	Laser
	Wavelength	405 nm (violet)
	Intensity	4%
Scanning for surface topography (LSM)	Direction	Bidirectional (no correction, line step =1)
	Speed	8 (max)
	Bit depth	16 bits
	Master Gain	245 V
	Pinhole diameter	54 µm (50× obj.) / 34 µm (20× obj.) (1 AU lateral optical resolution)
Size and resolution for surface topography (LSM)	Zoom	0.5× (50× obj.) / 1.2× (20× obj.)
	Field of view	255.56 × 255.56 µm (50× obj.) / 266.21 × 266.21 µm (20× obj.)
	Frame size	3000 × 3000 pixels (50× obj.) / 3125 × 3125 pixels (20× obj.)
	Setp size	0.25 µm
	Data quality	No noise cut (0–65335 levels, post-processing)
Black and white EDF image (WF)	Bit depth	12 bits per channel (36 bits in total)
	Stitching	2 x 2 tiles
	Field of view	671.20 × 671.20 µm (20× obj.) / 706.56 x 706.56 µm (10x obj.)
	Frame size	3891 × 3891 pixels (20× obj.) / 2048 x 2048 (10x obj.)
	Step size	1 µm

AU = Airy Unit, NA = numerical aperture, WD = working distance

8.2 **Acquisition workflow**

For each sample:

a) Calibrate coordinate system with 10× objective in WF mode, see:

Citation

Calandra I, Schunk L, Rodriguez A, Gneisinger W, Pedergnana A, Paixao E, Pereira T, Iovita R & Marreiros J (2019). Back to the edge: relative coordinate system for use-wear analysis. Archaeological and Anthropological Sciences.

https://doi.org/10.1007/s12520-019-00801-y LINK

b) Find location using 10× and 20× objectives in WF mode.

c) Acquire z-stack for topography with the 50×/0.95 objective in LSM mode (alternative: 20×/0.75 objective). Process z-stack for topography without noise cut. Save the result as a SUR surface (= "Spot A").

d) Repeat steps b) and c) two more times to measure non-identical spots within the same use-wear trace on the sample (= "Spot B" and "Spot C").

d) Acquire z-stack for black and white image at the same location with the same objective in WF mode and defining a 2×2 tile region (because the field of view is smaller in WF than in LSM mode, due to the 0.5× zoom). Process z-stack into EDF image and stitch it. Save the result as a PNG image.

e) Acquire z-stack for black and white image of the same location (entire use-wear) with the 10× objective and 20× in WF mode and defining a 2×2 / 2×1 tile region. Process z-stack into EDF image and stitch it. Save the result as a PNG image.

Processing for step #8.2d/e has been done in batch with a macro in ZEN blue.

Data analysis

10m

9

Processing workflow quantitative use-wear analysis

The data acquired with the confocal microscope was processed in batch with templates in ConfoMap. For the analysed samples described in step #8a) the templates desribes as a), b) and d) have been used; for the samples described in step #8b) the templates a), b) c) and d) have been used instead.

Software	
ConfoMap (MountainsMap Imaging Tophography)	NAME
Digital Surf, Besançon, France	DEVELOPER

a) "mirroring template"
For the data acquired on moulds, an intital template was needed. Moulds always just display a mirrored version of the original surfaces. Therefore, the acquired surfaces needed to be mirrored in order to match the original surface again.

mirroring_FLT4-7.pdf

b) “aligning template”

A series of surfaces was built using the module "4D series" for ConfoMap. The coordinate system using the three beads (see step #1.7) repositioned the sample after each experimental cycle in order to acquire the same surface; nevertheless, this repositioning is not exact. This template allowed an exact repositioning and perfectly complements the coordinate system: the alignment in ConfoMap works only if the surfaces are similar enough and the more similar, the better. This way, the surfaces from identical samples measured after varying cycles within the experiment were compiled and aligned as precisely as possible.

aligning_FLT4-7.pdf

c) “resampling template”

A resampling in x and y on all 144 + 120 surfaces was performed, leading to an identical spacing. The template was necessary due to the different objectives used during data acquisition. After applying this template, all surfaces had the same size of 254.0 x 253.9 µm and 1198 × 1198 pixels.

resampling_FLT4-7.pdf

d) “processing template”

In a final step, the data was processed. The following procedure was performed:
I. Levelling (least squares method by subtraction)
II. Form removal (polynomial of degree3)
III. Outliers removal (maximum slope of 80°)
IV. Thresholding the surface between 0.1 and 99.9 % material ratio to remove the aberrant positive and negative
spikes
V. Applying a robust Gaussian low-pass S-filter (S1 nesting index = 0.425 µm, corresponding to about 5 pixels, end
effects managed) to remove noise
VI. Filling-in the non-measured points (NMP), VII.) Analysis: Calculation of 21 ISO 25178-2:2012 (International
Organization for Standardization, 2012) parameters, 3 furrow parameters, 3 texture direction parameters, 1

texture isotropy parameter and the scale-sensitive fractal analysis (SSFA) parameters epLsar, NewEplsar, Asfc,
Smfc, HAsfc9 and HAsfc81

processing _FLT4-7.pdf

The ConfoMap templates for each surface in MNT and PDF formats are available on Zenodo (10.5281/zenodo.6642970). This also includes all original and processed surfaces, as well as the results.

9.1 **Data analysis**

The processed data was statistically analysed with R.

Software	
R Studio Desktop	NAME
The R Studio, Inc.	DEVELOPER

The analysis was separated into the two datasets:

a) all 24 samples from before and after 2000 strokes (called "analysis_before.after")
b) selected eight samples from all cycles (called "analysis_all.cylces")

Available in open access on Zenodo: **10.5281/zenodo.7229814**

9.2 **Results**

Available in open access on Zenodo: **10.5281/zenodo.7229779**

Citations

Step 1.7

Calandra I, Schunk L, Rodriguez A, Gneisinger W, Pedergnana A, Paixao E, Pereira T, Iovita R & Marreiros J. Back to the edge: relative coordinate system for use-wear analysis
https://doi.org/10.1007/s12520-019-00801-y

Step 6

Schunk et al., in prep.. A new semi-automated 3D digital method to quantify stone tool edge angle and design

Step 8.2

Calandra I, Schunk L, Rodriguez A, Gneisinger W, Pedergnana A, Paixao E, Pereira T, Iovita R & Marreiros J. Back to the edge: relative coordinate system for use-wear analysis
https://doi.org/10.1007/s12520-019-00801-y