Inter-microscope comparability of dental microwear texture data obtained from different optical profilometers.

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Abstract

Dental microware texture analysis has become a well-established approach for dietary inference and reconstruction of mammals and other tetrapods, both extant and extinct. As the amount of available data grows immensely, researchers could benefit from combining data gathered by others to perform meta-analyses. However, different devices used to capture three-dimensional surface scans for DMTA are known to produce variation even when measuring the same surface. Here we compare DMTA data of 36 guinea pigs that received different diets in a controlled feeding experiment, measured on a confocal disc-scanning and a confocal laser-scanning microscope. We are testing different pre-analysis filtering protocols to mitigate differences. We find inter-microscopes and filter-related differences for the majority of analysed 41 DMTA parameters. Certain microscope-specific filter routines resulted in less differences than other pre-analysis protocols. We further identify DMTA parameters which were stable regardless of microscope or pre-analysis treatment. Overall, the results obtained on both microscopes show the same dietary differentiation between guinea pig feeding groups, which supports that DMTA is a suitable method to obtain repeatable, objective dietary inferences. We finally propose a roadmap to enhance data exchange and inter-lab comparability and collaboration in the future.

Introduction

The analysis of microscopic wear features on teeth to infer dietary preferences of extant and extinct vertebrates, as well as use-wear analysis to infer the function of man-made tools, is of great interest to a broad community of biologists, palaeontologists, and archaeologists. Methods have undergone distinct modifications since microwear analysis was developed Baker et al. (1959) and gained popularity since Walker et al. (1972), with a recent trend to capture three-dimensional surface data in semi-automated, repeatable, and less observer-biased approaches commonly summarized under the moniker dental microwear texture analysis (DMTA). Within DMTA, different algorithms are applied to quantify surface texture patterns according to standardized scale-sensitive fractal analysis (SSFA) (e.g., Ungar et al. 2003, 2007; Scott et al. 2005, 2012) or International Organization for Standardization (ISO) surface roughness parameters (Schulz et al. 2010, 2013). The market standard for parameter computation is currently the software MountainsMap (DigitalSurf, France). More recently, MountainsMap-specific surface parameters such as mean depth and density of furrows, mean height, mean area, or motif analysis were also found indicative of dietary preferences and are frequently included into DMTA (e.g., Schulz 2013; Schulz-Kornas et al. 2019; Winkler et al. 2019a, b, 2020, 2021).

DMTA is relatively easily accessible through interferometry, confocal and laser-scanning microscopes of different price ranges, and several labs have established their own workflow, based on their specific devices. Thus, large amounts of data are being generated, and would likely enable meta-analyses if made available. However, the problem of availability is currently an important debate not only in the narrower DMTA community, but across disciplines. Most scientific journal encourage (or demand) all raw data to be made available through online repositories, but this leads to two problems:

1. Not all researchers agree to make raw data accessible at the moment of publication. This could be due to lax journal data availability policies, unwillingness to pay for storage services such as Dryad, or because they want exclusive access to their data for follow-up studies.

2. Data is being scattered across platforms, archives, and repositories (e.g., Zenodo, Dryad, github), which makes it hard to corroborate which data are available and which not.

The solution could be a joint initiative to store raw DMTA data, comparable to resources such as MorphoSource (www.morhposource.org) or the Paleobiology database (paleobiodb.org). However, if we achieve raw data availability, there is another problem to face:

How comparable are data obtained under different conditions? Data quality can potentially be affected on three levels. First, by whether scans have been obtained directly from original specimens (teeth, bones, artifacts) or from casts. Mihlbachler et al. (2019) found that scans obtained from casts gave significantly different DMTA results than scans obtained from original enamel surfaces, and that discrimination between dietary groups was thus diminished. Second, Goodall et al. (2015) highlighted not only differences between scans obtained from originals and casts, but also a second obstacle, that some moulding silicones are more accurate than others. It is therefore crucial to know how data were obtained (from original versus mould/cast), and to be aware of potential variation in fidelity of surface reproduction from different moulding compounds. Still, such methodological problems can be easily tackled by extending comparative studies of the widely available moulding materials and urging the community towards using the highest precision material available.

The third level of data quality differences, however, may potentially be the largest hindrance in making available data comparable. Data gathered from different confocal (blue and white light) profilers and laser-scanning microscopes has been found to differ for the same specimens. Even within the same product line (5 different Sensofar *PL*μ or 2 different Olympus LEXT), the same surface scan obtained on different microscopes produced different DMTA data (Arman et al. 2016, 2019). The authors found that differences could be reduced by using an automated pre-analysis filter-routine to mitigate measurement noise. Similarly, for two confocal laser-scanning microscopes of the same product line (Keyence VK), Kubo et al. (2017) found that application of different pre-analysis filter routines had a stronger effect on DMTA data than the microscope used. These are, up to now, the only studies comparing inter-microscope differences when generating DMTA data from biological surfaces. Besides Sensofar, Olympus, and Keyence, also confocal (laser and light) microscopes made by Zeiss (LSM 800) and NanoFocus (μsurf) are frequently used to obtain DMTA data. The different scanning methods and specifications (numerical aperture, spatial and lateral resolution etc.) will likely result in different results when scanning the same sample

(Calandra et al. 2019a), a problem that has often been discussed by metrologists (see Arman et al. 2016 and references within). Moreover, manufacturers do not share how data is processed from capture to output, thus we cannot assume that data will be comparable when captured on these different devices. We can streamline post-scanning filtering protocols (and statistical approaches for analysis) and usage of moulding material, but we cannot easily understand and accommodate for the differences in data processing right after scanning, which is essentially a black box.

Consequently, if we want to achieve comparability, and make use of the massive amounts of data generated by the community in the future, we need to establish protocols to increase comparability and follow up on the approaches by Arman et al. (2016) and Kubo et al. (2017).

The aim of this study is to perform a cross-device, and cross-scanning method comparison by compiling an extensive dataset of the same individuals on both a confocal (blue) light microscope (μsurf Custom) and a confocal (violet) laser-scanning microscope (Keyence VK-9700). Both devices are equipped with a comparable 100x long-distance objective, but the scanning field differs. Spatial and vertical resolution and other specifications are similar between both devices (Table 1).

We scanned the same dental moulds of 36 guinea pigs who received 6 homogenous diets in groups of 6 individuals during a 3-week feeding experiment (Winkler et al. 2019a, 2021), on both microscopes. Results are being compared in terms of relative differences between diet groups, but also in terms of absolute parameter values.

Under the given conditions, scanned areas are impossible to match perfectly when scanned on both devices, as the original scans were not taken with a repeated-measurement design in mind (see Boehm et al. 2019 and Calandra et al. 2019b for best practice how to prepare samples and the measuring system in order to match scans in a repeated-measurement design). Thus, we also test how well results of dietary differences can be reproduced when re-scanning the same individuals, and slightly varying the scanning positions.

Material and Methods

Silicone moulds of the upper right dentition for 36 guinea pigs were made using high-resolution silicone (Provil novo Light C.D.2 fast set EN ISO 4823, type 3 light, Heraeus Kulzer GmbH, Dormagen, Germany). We decided to measure moulds, because the jugular bone would contact

the microscope's objective and obstruct the desired measuring position. The measurements took place almost 3 years apart, on the same silicone moulds. They were first scanned in spring 2018 on a μsurf Custom confocal disc-scanning microscope, in the Center of Natural History, University of Hamburg. Further details can be found in Winkler et al. (2019), the original raw surface scans are published in Winkler et al. (2021).

The second time, the moulds were scanned on a Keyence confocal laser-scanning microscope VK-9700, at The Graduate School of Frontier Sciences, University of Tokyo, in spring 2021. Technical specifications of both microscopes are given in Table 1.

Description of scanning and filtering procedure

In both instances, the anteriormost enamel band of the upper right fourth premolar was scanned, and up to four non-overlapping scans taken. We were unable to match the exact same position of the previous scans. However, as the enamel band is narrow and short, it is very plausible that the re-scanned areas represent have a huge overlap with the previously scanned areas. Following Winkler et al. (2019), scans were then manually cropped in MountainsMap 8.2 to 60x60 µm, because enamel bands in guinea pigs are generally smaller in width than the default scanning areas (μsurf Custom: 160x160, Keyence VK-9700: 141x106um). The positioning of individual cut-outs will likely differ between scans obtained on μsurf Custom and Keyence VK-9700. As an exact matching cannot be ensured, the study also serves as a test of how well results can be reproduced when re-scanning the same individuals, and slightly varying the scanning positions.

Further data processing was conducted in MountainsMap 8.2, including re-analysis of the data obtained from the μsurf Custom at the University of Hamburg. Data were treated in two ways:

1. Applying a slightly modified version of the standard protocol established for the Keyence VK-9700 at the Kubo lab (Aiba et al. 2019, Kubo and Fujita 2021) which includes mirroring all surfaces in x and z (to compensate for the moulding procedure), levelling (least-square plane by subtraction), spatial filtering (robust Gaussian filter with a cut-off value of 0.8 µm), filling of non-measured points using the smoothing function of Mountains Map, noise-reduction by thresholding (upper and lower 0.5 %), removal of outliers (maximum slope of 85%) and form removal (polynomial of increasing power = 2). This protocol will hereafter be termed Filter A.

2. Following the standard protocol established for the μsurf Custom at the Kaiser Lab (Schulz et al. 2010, 2013) which includes mirroring all surfaces in x and z (to compensate for the moulding procedure), levelling (least-square plane by subtraction), spatial filtering (denoising median 5 x 5 filter size and Gaussian 3 x 3 filter size; default cut-offs are used), filling of non-measured points using the smoothing function of Mountains Map, noise-reduction by thresholding (upper and lower 0.5 %), removal of outliers (maximum slope of 85%) and form removal (polynomial of increasing power = 2). This protocol will hereafter be termed Filter B.

Both filtering protocols were applied for both datasets, resulting in four datasets:

Keyence*Filter A - μsurf*Filter A

Keyence*Filter A - μsurf*Filter B

Keyence*Filter B - μsurf*Filter A

Keyence*Filter B - μsurf*Filter B

We computed a total of 41 surface texture parameters that are frequently applied on biological surfaces (citations). There are some differences to the parameter set published in Winkler et al. (2019). We did not include the four ISO-12871 flatness parameters, as they are directly derived from the ISO-25178 height parameters *Sa*, *Sq*, *Sv* and *Sz* and thus redundant. Similarly, we excluded the ISO-25178 volume parameter *Vmp* because it is identical to *Vm* when using default cutoff settings. We additionally included the SSFA parameters *Asfc* (area-scale surface complexity) and *epLsar* (anisotropy) as these are among the most frequently applied measures of DMTA in other studies (Ungar et al. 2003, 2007; Scott et al. 2005, 2012; Merceron 2010; Schubert et al. 2010).

Statistics

All statistical analyses were carried out in JMP Pro v.16. For each specimen, median values per parameter were calculated from up to 4 (at least 3) non-overlapping scans (compare Winkler et al. 2019a). Because of the repeated-measurement design, i.e. the same specimens were analysed four times through a combination of two microscopes and two filtering protocols, we performed a

t-test for paired samples combined with a Wilcoxon signed-rank test to test for significant differences between the repeated measurements.

Additionally, we ranked data of each DMTA parameter within the same dietary group in order to standardize the difference between the dietary groups and applied a non-parametric Steel-Dwass test using the ranked dataset for all pairs.

Results

Generally, absolute parameter values were shifted between both microscopes, but we found a good matching of dietary differences on both devices, and regardless of filtering protocols. The groups lucerne fresh, lucerne dry and grass fresh showed similar parameter values on both microscopes for most parameters (Fig. S1, Tab. S1). Grass dry fell between these three previous groups and the two remaining groups, bamboo fresh and bamboo dry.

Pairwise t-tests showed that all filtering routines performed very similar. The least significant differences between datasets were obtained for three filtering routines: either using Filter A on both datasets, or Keyence* Filter A and μsurf* Filter B, or Keyence*Filter B and μsurf*Filter A. Each resulted in 25 out of 41 significantly different parameters. The combination Keyence*Filter B and μsurf*Filter B showed 27 significantly different parameters. According to the Wilcoxon signed-rank test, differences between filtering routines were even smaller, with either 28 or 29 significantly different parameters reported (compare Tab. S2). The Steel-Dwass test on ranked data identified least significant differences for the filter combination Keyence* Filter A and μsurf* Filter B (20), followed by Keyence* Filter A and μsurf* Filter A (22) (Tab. S3).

The Keyence VK-9700 showed less outliers on for bamboo groups in height parameters. Generally, the Keyence VK-9700 produced slightly lower height and volume values on lucerne and fresh grass on than the µsurf Custom.

Individual parameter groups

Area parameters

Data obtained using the μsurf Custom generally showed higher area values as data obtained on the Keyence VK-9700 (Tab. S1, Fig. S1). Least differences were found for *Sda* (standard dale area),

193 which was the only not significantly different area parameter when using the filter routine
194 Keyence*A – μsurf*B.

195 Both lucerne and the fresh grass group had the lowest area parameter values for both
196 microscopes and filter combination. The dry grass group showed higher values than lucerne and
197 fresh grass on μsurf Custom for all area parameter, and only for *Sda* on Keyence VK-9700. Both
198 bamboo groups consistently showed the largest values for all area parameters. For data from
199 μsurf Custom, the fresh bamboo group had higher values than the dry bamboo group, while for
200 Keyence VK-9700 there was either no difference or dry bamboo had slightly higher values than
201 fresh bamboo.

202 *Complexity parameters*

203 Complexity parameter values were generally higher when obtained on the Keyence VK-9700.
204 While the shift was similar for all diet groups and the parameters *Sdr* and *Asfc*, the parameter
205 *nMotif* (number of motifs) showed a larger offset for the dry grass group. Significant differences
206 between microscopes were not detected for *Sdr* and *Asfc* when using the filter routine Keyence*A
207 – μsurf*B.

208 For *Sdr* and Asfc, both lucerne groups and the fresh grass group had similarly low values on both
209 microscopes. Dry lucerne showed slightly higher values when measured on the Keyence VK-9700.
210 Dry grass and both bamboo groups showed higher values than the previous three groups. All three
211 had comparable values when measured on μsurf custom (with fresh bamboo having highest
212 variability), while on the Keyence VK-97000 dry grass and fresh bamboo had higher *Sdr* and *Asfc*
213 values than dry bamboo. For *nMotif*, the dry grass group displayed strong differences between
214 both microscopes. While on μsurf Custom it showed intermediate values between the lucerne
215 groups (high) and the bamboo groups (low), it had higher values than all other diet groups when
216 measured on VK-97000 (Figs. 1B, S1).

217 *Density parameters*

218 While for *Sal* (autocorrelation length) the μsurf Custom produced higher values, for *Spd* (density of
219 peaks) and *medf* (density of furrows), the Keyence VK-9700 recorded significantly higher

220 parameter values. Significant differences could not be eliminated by any of the applied filtering
221 routine combinations.

222 The general pattern of diet groups was maintained on both microscopes for the parameters *SaI*
223 and *medf*. For the parameter *Spd* (Density of furrows), the dry grass group showed low parameter
224 values like both bamboo groups when measured on the μsurf Custom, and high parameter values
225 similar to both lucerne and the fresh grass group when measured on the Keyence VK-9700 (Fig. S1).

226 *Direction parameters*

227 Measures of absolute texture direction (*Std*, *Tr1R*, *Tr2R*, Tr3R*)* were highly variable on both
228 microscopes and cannot be compared. For measures of isotropy (*Str*, *IsT*), the μsurf Custom
229 showed higher values, and consequently for anisotropy (*epLsar*), lower values as compared to the
230 Keyence VK-9700.

231 For the anisotropy and isotropy parameters (*Str*, *epLsar*, *IsT*), both microscopes showed the same
232 general pattern of diet groups. The lucerne and grass groups showed higher isotropy (larger *Str*,
233 *IsT*), while the bamboo groups showed larger anisotropy (larger *epLsar*) (Fig. S1).

234 *Height parameters*

235 Overall, the match between both microscopes was good for height parameters. Data from μsurf
236 Custom shows higher variability for the Bamboo dry group than data captured on the Keyence VK-
237 9700. Nine out of 14 height parameters were not significantly different for the filter combination
238 Keyence*A – μsurf*A. For DMTA parameters with significant differences between the microscopes,
239 data obtained on the μsurf Custom had higher values than data obtained on the Keyence VK-9700
240 (compare *S5v*, *Sa*, *Sq*, *Sv*, *Sxp*) (Figs. 1B, S1).

241 The general pattern of parameter values for the diet groups was the same on both microscopes
242 and for all filter routines. Both lucerne groups and grass fresh had the lowest height parameter
243 values, followed by dry grass with intermediate values, while both bamboo groups had the largest
244 height parameter values (Fig. S1).

245 *Peak sharpness*

246 The only peak sharpness parameter *Spc* was significantly higher when measured on Keyence VK-
247 9700. The divergence could not be corrected by any of the applied filter routines. The general
248 pattern, however, was the same on both microscopes, with lucerne fresh/dry and fresh grass
249 showing lower parameter values than dry grass and bamboo fresh/dry (Fig. S1).

250 *Plateau size parameters*

251 Values for both plateau size parameters were comparable, however, most filter routines resulted
252 in significant differences between microscopes. Data obtained on the μsurf Custom were larger for
253 *Smr* and *Smc* (except for the dry bamboo group).

254 The general parameter values pattern of all diet groups was the same for both microscopes and all
255 filter routines (Fig. S1).

256 *Slope*

257 The one slope parameter *Sdq* was larger when measured on the Keyence VK-9700. By applying the
258 filter routine Keyence*A – μsurf*B, the differences were no longer significant.

259 Both microscopes showed similar patterns for all diet groups (Fig. S1).

260 *Volume*

261 Volume parameters showed higher values for data from the μsurf Custom, with some exceptions
262 for the dry bamboo group. Filtering reduced differences, with Keyence*A – μsurf*A being the best
263 routine.

264 The general pattern of low volume parameter values for both lucerne groups and the fresh grass
265 group, intermediate values for the dry grass group, and highest values for both bamboo groups
266 was found on both microscopes and using all filter routines (Fig. S1).

267 *Exemplary correction*

268 By performing linear regression, it would be possible to obtain correction factors for each
269 individual DMTA parameter, and thus facilitate comparison between microscopes. Exemplarily,
270 the result of such a correction can be seen in Figure 2 for the parameter *medf* (mean density of

271 furrows). Here, data obtained on Keyence VK-9700 (Filter A) has been corrected according to data
272 obtained on μsurf Custom (Filter B) by the equation:

273 *medf* corrected = 1151.6965 + *medf* from Keyence*0.6161424.

274 **Discussion**

275 *Inter-microscope differences*

276 Not all parameters are suitable for direct comparison of absolute parameter values when data is
277 obtained on different devices. Our results clearly show that peak and furrow density-related
278 parameters are significantly different and should not be used for immediate comparison. The
279 source of this strong variation is likely due to the different scanning techniques and peak-
280 detection algorithms of the two microscopes. Just by visually comparing, it is evident that scans
281 from μsurf Custom appear slightly blurred, while scans from Keyence VK-9700 show sharper peaks
282 (Figs. 1, S2). Through introduction of a correction factor, however, these microscope specific
283 differences can be mitigated, and results become well comparable (Fig. 2).

284 Several DMTA parameters were similar in absolute values and could be compared with higher
285 confidence (Tab. 2). Microscope-specific filtering protocols can thus help to minimize inter-
286 microscope differences and account for device-specific characteristics. With the best filtering
287 protocol, the congruence between data was 16 out of 41 (t-test) or 21 out of 41 (Steel-Dwass test)
288 parameters, while the worst performing filtering protocol resulted in 14 or 15 out of 41
289 comparable parameters, respectively.

290 These results show that there is not one filter combination that yields unequivocally better results
291 than the other. When considering results from all statistical tests, and for which parameters better
292 comparability was achieved, we would favour the combination Keyence* Filter A and μsurf* Filter
293 B. This conclusion is mainly based on the fact that frequently used parameters such as *Asfc*, *Sdr*,
294 *Sda* and several height and volume parameters were comparable under this protocol. No other
295 routine resulted in comparable results for the SSFA parameter *Asfc*, but as it is of great importance
296 and generally applied, congruence should have high priority. Additionally, the applied filters were
297 those originally developed for the specific microscopic devices (Schulz et al. 2010, 2013; Aiba et al.

298 2019; Kubo and Fujita 2021), which is additionally beneficial when considering data comparability
299 of already published datasets.

300 *Comparability of results*

301 The dietary differences between experimentally fed guinea pigs originally described by Winkler et
302 al. (2019a) were confirmed for the newly obtained data from the Keyence confocal laser-scanning
303 microscope, and by all filtering protocols. Both lucerne diets and the fresh grass diet were very
304 similar in complexity, height, volume, area, and slope parameters. Especially low parameter values
305 for height, volume, and the complexity parameters *Sdr* and *Asfc* are interpreted as related to low
306 abrasive feeds (Kubo et al. 2017, Kubo and Fujita 2021; Schulz et al 2010, 2013; Winkler et al.
307 2019a, b, 2020). On the contrary, higher parameter values would indicate a more abrasive diet.
308 Even though slightly different enamel areas were scanned, as matching of the original areas was
309 not possible, the re-analysis gave the same result of increasing abrasiveness of experimental feeds
310 in the order: Lucerne fresh, lucerne dry, grass fresh < grass dry < bamboo fresh, bamboo dry (Figs.
311 1B, S1). This shows that the previously derived interpretation of Winkler et al. (2019a) holds true,
312 there is a significant effect of both silica content and hydration state of the plant tissue on
313 observed microwear texture pattern, with dry and more siliceous diets resulting in more abrasion.
314 Therefore, our study also provides a test for repeatability and reliability of DMTA results and
315 strengthens the robusticity of the method.

316 *Outlook and suggested best practice*

317 By finding the most stable (i.e., most comparable) parameters between the two microscopes, this
318 study might help in the identification of 3D microwear texture parameters to focus on, as the huge
319 availability has caused confusion and made it difficult to agree on a set of relevant parameters. By
320 choosing the most stable ones, this controversy can be advanced. At least for comparative studies,
321 where data is gathered in different labs, and on different machines, we suggest concentrating on
322 the parameters listed in Table 2. The height parameters *S10z*, *Sku*, *Sp*, *meh* and *metf* did not show
323 significant differences between microscopes, regardless of filter routines. Thus, we would consider
324 these parameters as most stable and most suitable for comparative data analysis without adapted
325 filtering routine, or introduction of a correction factor. For the parameters *nMotif* (number of

motifs) and *Spd* (density of furrows), the dry grass group fell into a different position among the overall pattern when measured on the two different microscopes. Therefore, these parameters should be excluded from comparative analyses, and maybe also avoided generally, as the results were not reproductible. For parameters which showed a general shift, but could not be adjusted through filtering, a correction factor should be introduced as exemplarily shown here for the parameter *medf* (Fig. 2) if one desires to include them into a comparative study. Nevertheless, it must be noted that even after correction, results obtained from different microscopes need to be discussed as such, and the possibility of persisting inter-microscope differences needs to be discussed.

Such comparative studies should be conducted between more DMTA labs to understand specific characteristics of each microscope used, and to find best-practice filtering protocols that facilitate inter-microscope (and thus inter-lab) data comparability. This will lead to a massive increase of comparative data available for future studies and avoid unnecessary data-recollection. We highly encourage striving for a shared data repository to which DMTA research labs worldwide can contribute. As an initiative to promote such comparability, we propose to compile a standard set of moulds from different typical specimens (e.g., ungulate, reptile, carnivore) and a few standardized flat surfaces (e.g., polished enamel) that are mounted on a microtiter plate with incision marks that can be aligned within a microscope-specific coordinate system. Such specimens shall be exchanged between DMTA labs, with each research group re-scanning the same areas, and processing the data according to their own preferred pre-analysis protocol, and the published protocols of other researchers. Subsequently this data can be used to obtain accepted “correction equations” for each device, so that data can be shared and used between labs.

Conclusion

Repeatability and less observer-biased interpretation of results are two key advantages often cited when comparing DMTA to classical microwear analysis. Our study supports these claims, as data gathered in two different laboratories, on two different microscopes, and 3 years apart resulted in the same dietary discrimination between experimentally fed guinea pigs.

This study also highlights that inter-microscope comparison can only be done without correction for a few DMTA parameter. The majority of often applied parameters needs to be corrected through a microscope-specific filter-routine, or a correction factor. Such correction factors could be obtained through a joint community effort which includes scanning of the same surfaces in multiple labs, which we propose here to our colleagues. Through our collaboration, we might achieve data comparability, and advance research in our field.

Data availability

All original, unfiltered surface texture scans used in this study are available online. Data from Winkler et al. (2019a, 2021) has been published under doi:10.25592/uhhfdm.9163. The comparative scans obtained for this study are deposited under doi.org/10.5061/dryad.7wm37pvw3.

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Tables and Figures

Table 1. Technical specifications of the two confocal microscopes employed in this study.

	Model	
	μsurf Custom	Keyence VK-9700
Scanning mode	Confocal disc-scanning	Confocal laser scanning
Vertical (z) resolution (µm)	0.002 – 0.06	0.001
Light source	Blue LED (470 nm)	Violet laser (408 nm)
CCD camera resolution	984 x 984 pixel	1024 x 768 pixel
Objective	100 xL	100 xL
Scan size (µm)	160 x 160	140 x 105
Spatial (x, y) resolution (µm)	0.16	0.137
Numerical aperture	0.8	0.95

Table 2. Comparison of stable, i.e., not significantly different parameters, for the best performing filter combinations (based on t-test). Parameters that showed no significant differences regardless of filter protocol are given in bold. Directional parameters (*Std*, *Tr2R*, *Tr3R*) are interpreted as not diet-informative, but mostly related to chewing mechanics. Therefore, these are greyed out.

Filter combination		
Keyence*A – μsurf*A	Keyence*A – μsurf*B	Keyence*B – μsurf*A
	Sda	
	Sdr	
	Asfc	
Std	Std	Std
Tr2R	Tr2R	Tr2R
Tr3R	Tr3R	Tr3R
S10z	**S10z**	**S10z**
S5p	S5p	
Sku	**Sku**	**Sku**
Sp	**Sp**	**Sp**
		Sa
		Sq
Ssk	Ssk	
Sz		Sv
		Sz
meh	**meh**	**meh**
madf		madf
metf	**metf**	**metf**
		Smc
Smr	Smr	
	Sdq	
Vm	Vm	
Vv		Vv
Vvc		Vvc

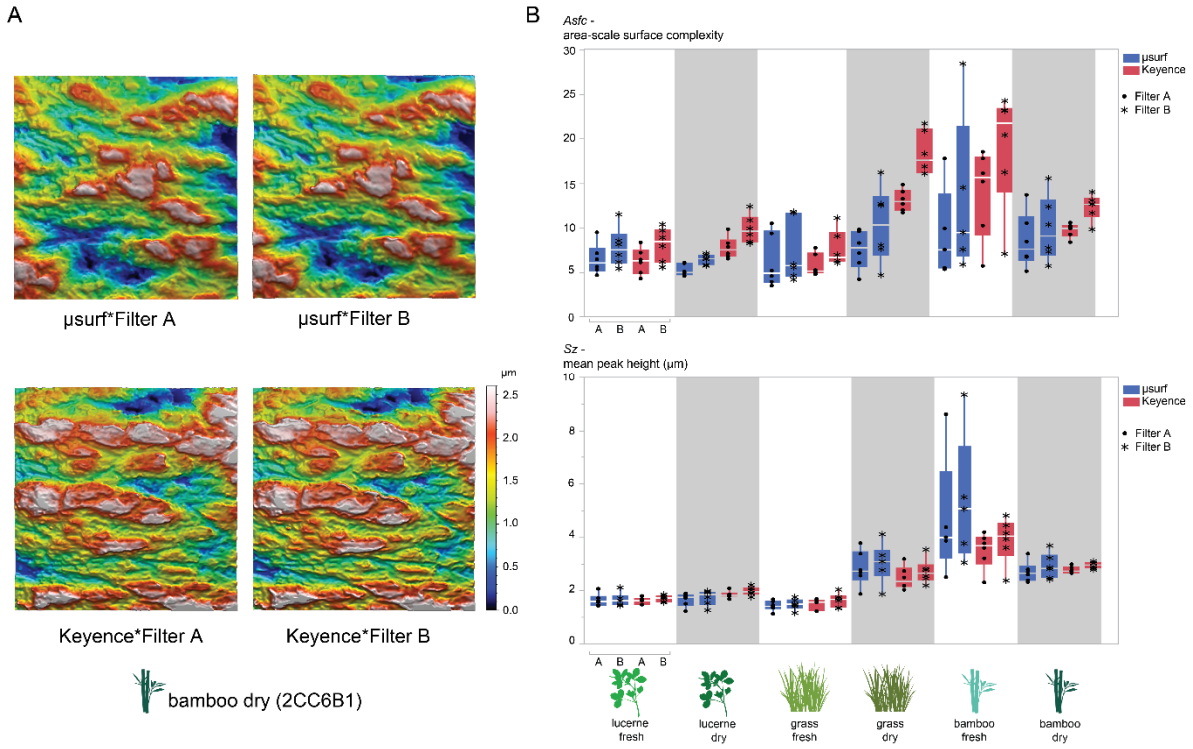

Figure 1. Comparison of data derived from either *µsurf Custom* or Keyence VK-9700. A) Exemplary 3D photosimulations of surfaces from one individual of the bamboo dry group, scanned on both microscopes and processed with Filter A and Filter B. Note that the scanning area does not match between microscopes. Scans are to the same scale (µm). B) Exemplary DMTA parameter (upper: *Asfc*, lower: *Sz*) calculated for all individuals, with data derived from *µ*surf Custom (blue) and Keyence VK-9700 (red) and different filter routines (Filter A: circle, Filter B: asterisk).

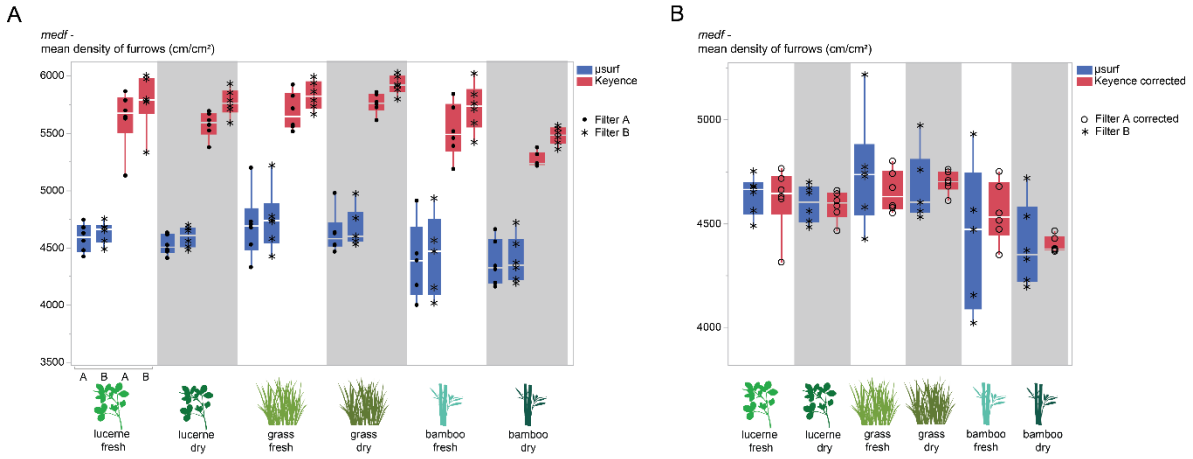

Figure 2. Comparison of results for the DMTA parameter *medf*. Scans were captured on either *µ*surf Custom (blue) or Keyence VK-9700 (red) and processed with different filter routines (Filter A: circle, Filter B: asterisk). A) All filter and microscope combinations. B) Data from Keyence*Filter A corrected according to linear regression equation and compared to *µ*surf*Filter B. Note that the data is well comparable after the correction.