REPORT:

A3.2.7: Documented example of surface roughness measurements

Workpackage 3

https://mfmet.eu

This report was written as part of activity 3.2.7 from the EMPIR Establishing Metrology Standards in Microfluidic Devices (MFMET) project. The three-year European project commenced on 1st June 2021 and focused on providing a generic methodology of accurate measurement of a particular quantity in a microfluidic device by utilising standardised methods and reference documents, e.g. VIM & GUM. For more details about this project, please visit https://.mfmet.eu

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Contents

1. Scope

This report addresses the activity A3.2.7 defined in the JRP protocol as:

Activity number	**Activity description**	**Partners (Lead in bold)**
A3.2.7 M20	LNE, IMTAG, IPQ, CEA and INESC MN will perform a documented example of the test protocol developed in A3.2.4. LNE with support from IMTAG, IPQ, CEA and INESC MN will write a technical report on the documented example of the test protocol for surface roughness. The report will be shared with the relevant standardisation group such as ISO/TC48/WG3 and WG5, ISO/TC229, CEN/TC332/WG7. This information will also be used for manufacturing the transfer standards in A2.4.2.	**LNE**, IMTAG, IPQ, CEA, INESC MN

2. Samples used in roughness measurements

The samples provided for this documented example were taken from existing, unbonded flow cells made of glass. The glass type is D263®bio and the substrate contains 100 µm deep wet etched channels. In order to make the flow cell design unrecognisable, the samples were cut into slides (ca. 35 mm x 52.6 mm) by laser dicing (see Figure 1).

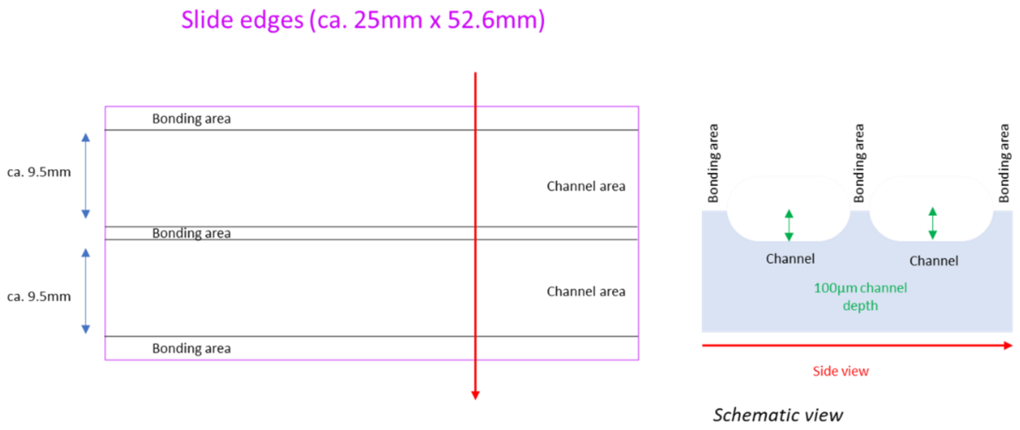


Figure 1: Slides provided for surface roughness measurements (view from the top on the left, sideview on the right).

The slides contain both parts of areas interesting for surface roughness measurements: the bonding area and channel area. The target for both areas is low roughness values: *i*) bonding can only be achieved with roughness below 1 nm and *ii*) a smooth surface is considered for the flow of fluids. It is assumed that the channel area is slightly rougher than the bonding area resulting from the wet etch process used to fabricate the channels. In addition, the roughness measurement in the channels should be carried out in the deeper part of the channel. Due to the isotropic nature of the wet etching of glass, a curved wall is obtained and in order to avoid measurement artefacts, especially for optical methods, this radial region must be avoided during the measurement. For any measurement method involving a tip, there is also a risk of tip breakage when measuring in this area.

The following sections present the 3 different methods used for surface roughness measurements in microchannels. Section 3 is dedicated to Confocal Microscopy, Section 4 to Atomic Force Microscopy and Section 5 to a Stylus-Type Instrument.

3. Roughness measurement using Confocal Microscopy

3.1. Material and method

Roughness measurements were performed using a VK-9700 from Keyence (Figure 2). An anti-vibration system was used to minimize the effect of natural vibrations on the measurement of the samples being examined. The measurement was conducted and saved using the VK Viewer software (version 2.1.0.2). The analysis of the measurement was done using the VK Analyzer software (version 2.1.0.0).

The documented example was conducted according to the test protocol described in task 3.2.4 for the confocal microscopy. Using the VK Viewer software, the focal adjustment of the test area was started with a 10x objective, continued with a 20x objective, and finished with a 50x objective. Laser adjustment was subsequently conducted at the position of the test area. The measurement was conducted at 50x magnification, which corresponds to a total scan area of 212µm x 282µm.

In total, three measurement per area were conducted and are described in Figure 3.

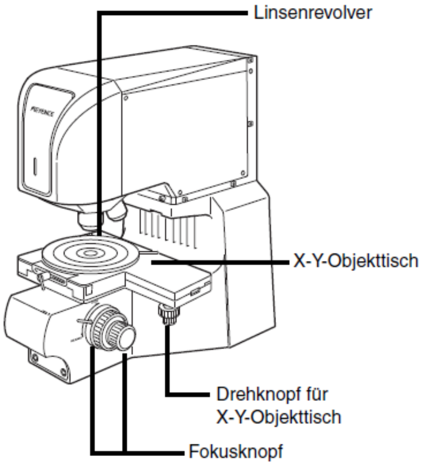


Figure 2: Measuring device VK-9700.

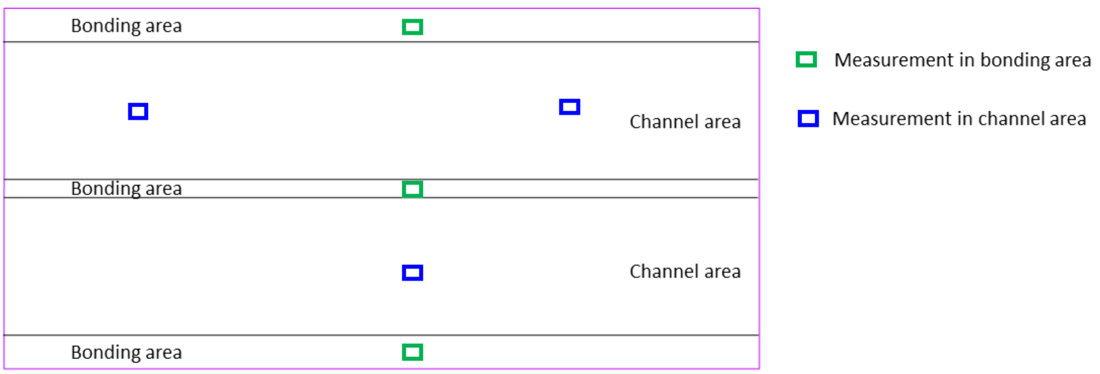
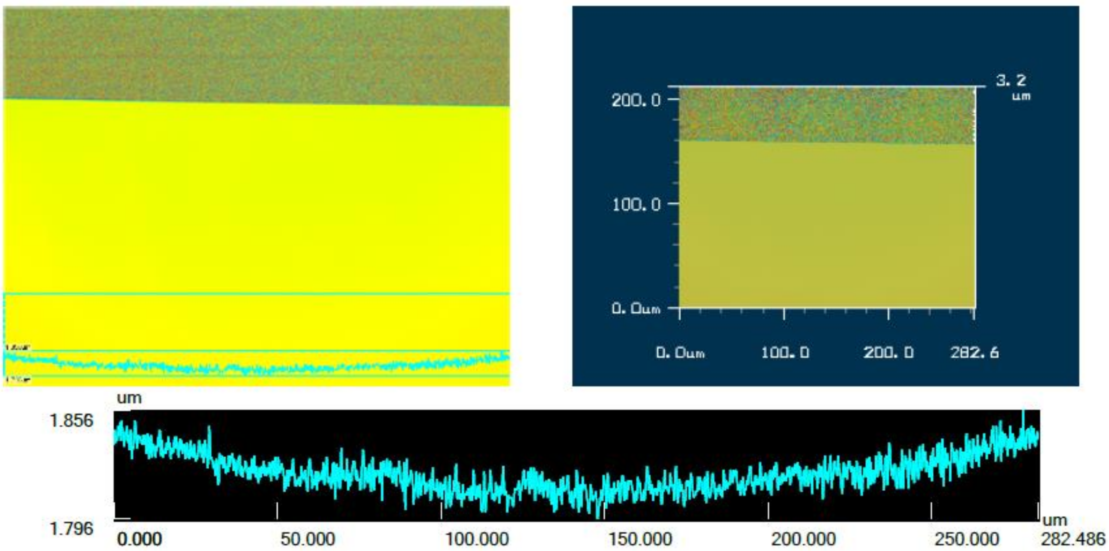


Figure 3: Schematic view of the measurements conducted on the test sample

The measurements were saved as *.vk3 files. These files were further processed in the analyzing software VK Analyzer software.

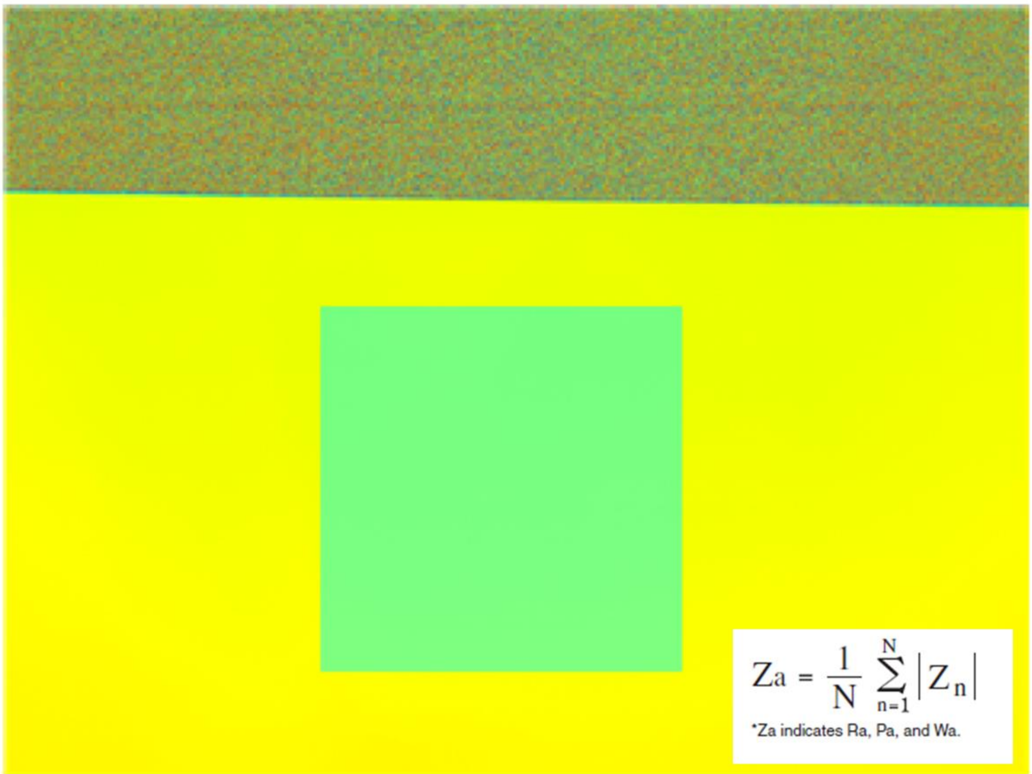
The following operations were performed on the *.vk3 measurement files:

- **Correcting the image tilt**: the sample is slightly tilted during the measurement. The entire image is corrected based on three points in the displayed image to create a standard plane on a triangle that connects those points. The reference plane is tilted until it is horizontal while retaining the average height of the standard plane.
- **Measuring line roughness (horizontally)**: the line roughness measurement sets a measurement line on the image and measures the line roughness along that measurement line. Filter settings were not applied to the analysis of the measurement. The measurement standard “JIS B 0601: 2001 (ISO 4287: 1997)” was selected and the results for various types of line roughness values are displayed in a table below. The relevant value from this line measurement is listed in the table as the **Ra value**, which is the arithmetic mean roughness of the average of the absolute value along the reference length (see Figure 4).
- **Measuring surface roughness**: the surface roughness of a specific area (or region of interest = ROI; here: 100µm x 100µm) is determined in this analysis. Filter settings were not applied to the analysis of the measurement. The measurement standard “JIS B 0601: 2001 (ISO 4287: 1997)” was selected and the results are displayed in a table. The relevant value from this surface measurement is listed in the table as the **Ra value**, and is defined as the average of the absolute value od the height of each point in the ROI (see Figure 5).



Profile 1	Horz. dist.	Rp	Rv	Rz(Rt)	Rc	Ra	Rq	Rsk	Rku	RΔq	RSm	RPc/cm	RLo	Rlr	R⊿a	Rλa	R⊿q	Rλq	Comment
All	282.486um	0.035um	0.025um	0.060um	0.036um	0.008um	0.010um	0.4465	2.6409	0.0295	211.968um	47.177	282.613um	1.0005	1.33°	2.233um	1.72°	2.129um	

Figure 4: Example of an analysed line roughness measurement for the bonding area. The line roughness values in this example is Ra = 0.008µm.



	Rp	Rv	Rz	Ra	Rq	Rsk	Rku
Seg.1	0.050um	0.038um	0.088um	0.012um	0.014um	0.3103	2.2392

Figure 5: Example of a surface roughness measurement of the bonding area. The surface roughness values in this example is Ra = 0.012µm for the specific area of 100µm x 100µm (marked in green).

3.2. Results of roughness measurements using VK-9000 confocal microscope

The results of the line and surface roughness measurements of the bonding area and channel area are displayed in Table 1.

Table 1: Results of the surface roughness analysis according to JIS B0601: 2001 (ISO 4287: 1997).

Roughness measurement & value	**Bonding area**			**Channel area**		
	1	**2**	**3**	**1**	**2**	**3**
Line roughness Ra	0.008µm	0.009µm	0.009µm	0.007µm	0.007µm	0.006µm
Surface roughness Ra	0.012µm	0.007µm	0.014µm	0.008µm	0.006µm	0.007µm

Confocal microscopy can be a suitable instrument for measuring surface roughness, including for glass etched channels. However, it is important to consider the resolution limit of the microscope and whether it is appropriate for the scale of the surface features being measured. This has already been mentioned in Task 3.2.4 (lateral resolution: ~ 0.2 µm; vertical resolution: ~ 0.5 µm).

The roughness values of the blank substrate before processing are specified (and confirmed by the supplier) to be below 0.5nm. The measured roughness values after processing are still extremely small (between 6nm and 14nm), which is very well below the resolution limit of many confocal laser microscopes.

The resolution limit of a microscope is determined by the diffraction limit, which is dependent on the wavelength of the light used and the numerical aperture of the objective lens. In general, the resolution limit of a confocal microscope is around half the wavelength of the laser light used (typically in the range of 400-700nm for visible light), which means that the VK-9000 microscope may not be able to accurately resolve features that are smaller than a few nm.

For the sake of completeness, measurements were carried out and evaluated. The measured values cannot be regarded as meaningful and do not allow any significant conclusions to be drawn about the actual surface roughness.

Therefore, it is important to consider the resolution limit of your confocal laser microscope and ensure that it is appropriate for the scale of the surface features you are trying to measure. If the microscope cannot resolve the surface features with sufficient accuracy, other instruments or techniques may need to be considered, such as atomic force microscopy.

4. Roughness measurement using Atomic Force Microscopy

4.1. Material and method

Roughness measurements were performed on a Veeco Dimension 3100 atomic force microscope (AFM) controlled by the NanoScope V software.

Bruker AFM probe OTESPA R3 (https://www.brukerafmprobes.com/p-3864-otespa-r3.aspx) was mounted on the AFM and used to perform the images in Tapping® mode.

The adjustment of the lever, i.e. its amplitude and its oscillation frequency, were adjusted according to the manufacturer recommendations. The gain parameters were adjusted to limit the noise on the AFM images while following the sample surface.

AFM images were acquired with the following parameters:

- 5 µm x 5 µm
- 1024 pixels x 1024 pixels (pixel size = 4.88 nm)
- Scan speed : 4 µm/s (scan rate = 0.498 Hz)

The raw images were then processed using MountainsLab software (DigitalSurf https://www.digitalsurf.com/software-solutions/multi-instrument/)

The following operations were performed on the raw images:

- Line to line flattening.
- Thresholding to exclude the possible dust/residue on the surface from roughness calculation.
- S_a value calculation in nm according to ISO 25178 standard.

Nine AFM images were taken of the samples as shown in Figure 6:

- Three images for the first channel (channel 1).
- Three images for the bonding area between the two channels.

- Three images for the second channel (channel 2).

The images are repeated with a regular interval of a few millimetres in order to observe the homogeneity of each area.

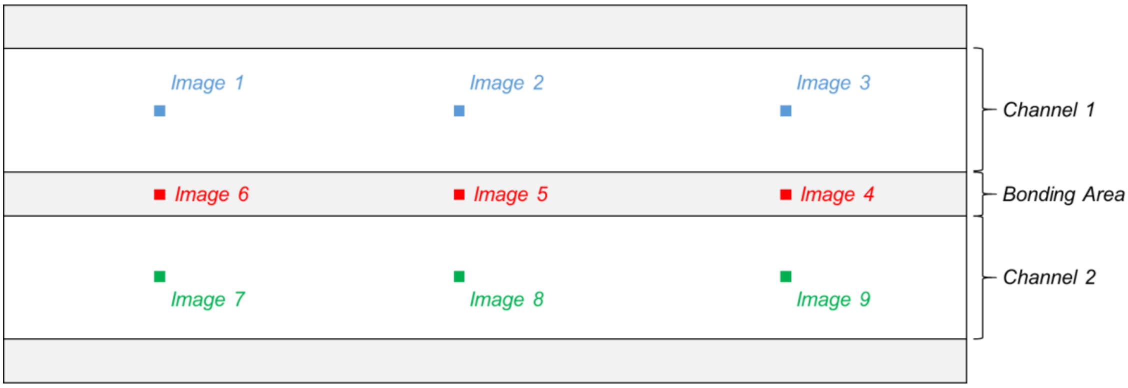


Figure 6 : Schematic view of AFM images made on the microfluidic sample.

In total, two microfluidic samples as shown in Figure 6 were measured by AFM. Moreover, three images were also taken of a third sample, which had no channels.

4.2. Results of roughness measurements using Veeco Dimension 3100 AFM

The results of the roughness measurements for the two samples analysed are shown in Figure 7 and Table 1.

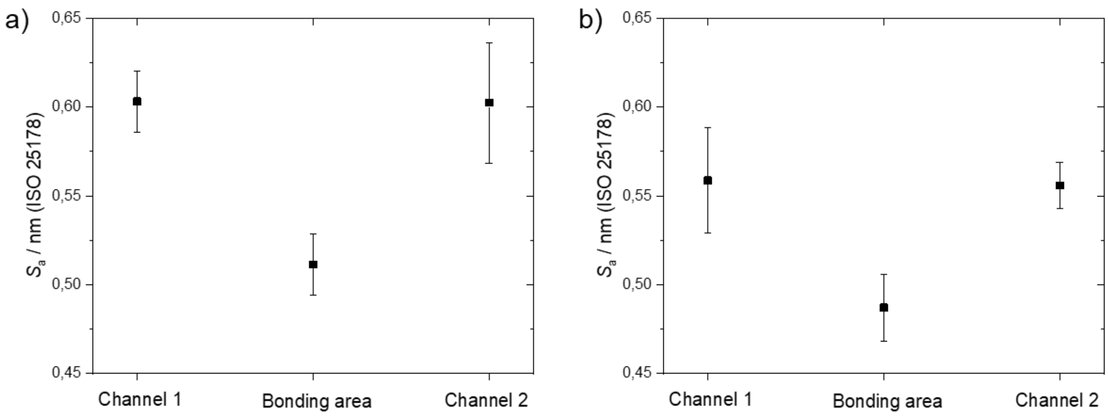


Figure 7 : Results of the roughness measurements for the two microfluidic samples. The results show the average of the three measurements for each area. The error bars represent the standard deviation between the three measurements for each area.

Table 2 : Results of the roughness measurements for the two microfluidic samples. The results after ± express the standard deviation between the three measurements for each area (k = 1).

	S_a (Channel 1) / nm	S_a (Bonding area) / nm	S_a (Channel 2) / nm
Sample 1	0.60 ± 0.02	0.51 ± 0.02	0.60 ± 0.03
Sample 2	0.56 ± 0.03	0.49 ± 0.02	0.56 ± 0.01
Sample 3	NA	0.46 ± 0.01	NA

NA : Not applicable.

For each sample, the measured roughness is slightly lower in the bonding area than in the channels. Furthermore, for each sample, the measured roughness is equivalent between the two channels. A slight shift is observed between the two samples, both for the measurements in the channels and those on the bonding area. This effect could be attributed to wear (different radius of curvature) of the AFM tip.

An example of an AFM image processed for each area with MountainsLab (Digital Surf) is shown in Figure 8.

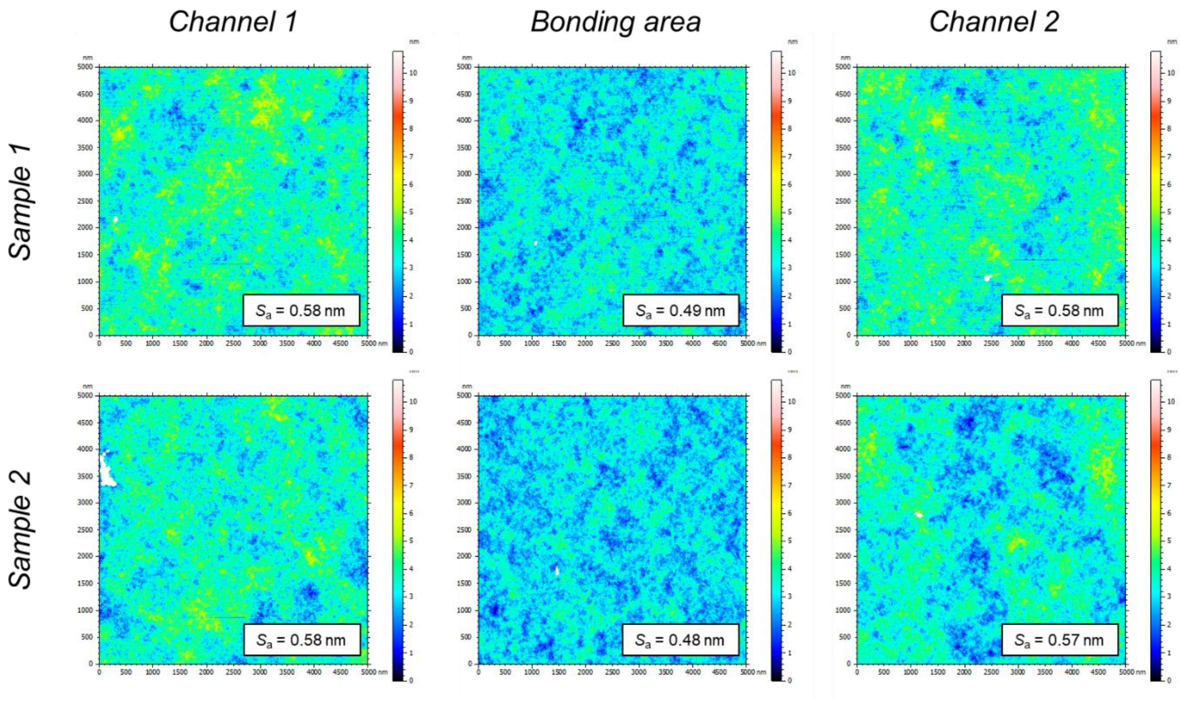


Figure 8 : AFM images processed with MountainsLab® (Digital Surf) for both samples. One image of each area is shown.

5. Roughness measurement using Stylus-Type Instruments.

5.1. Measuring instrument, samples, and method

For the roughness and waviness measurements the Mahr reference measuring station MarSurf GD 140 it was used, which includes a stylus instrument with a probe arm length of 45 mm (Figure 9). This instrument allows the scanning over the surface by contact at a constant velocity and force, given quantitative information on heights with respect to position. Subsequent data processing, (such as evaluations of surface roughness, profile, and waviness) the parameters calculations and graphic representation was performed using the MarWin EasyRoughness software, supply by Mahr.

Three of the five glass slides provided were tested. Each slide exhibits three bonding areas separated by two channels with an approximate depth of 100 µm between the bottom of the channels and the top of the bonding area. One slide exhibits no channels, only boding area. These slides samples are illustrated in the point 3. *Samples* of this document.

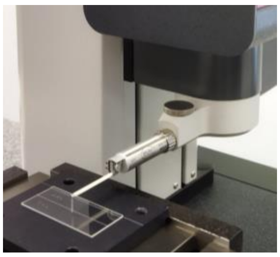


Figure 9 – MarSurf GD 140 stylus instrument and the glass slide (IPQ photo).

The measurement method was performed according to the test protocol for roughness measurement using stylus instrument described in the report MFMET A3.2.4.

As the slides were produced in a research framework there are not indications of their nominal length specifications, consequently the settings for the evaluation of the surface texture of the profile were based on the informative annex D of ISO 21920-3:2021. Therefore, based on the technical features and the measurement procedure to be applied in this study, the following roughness parameters of interest were identified, to define the settings:

- *Ra* – Arithmetic mean height
- *Rt* – Total height
- *Rz* – Maximum height
- *Rp* – Mean peak height
- *Rv* – Mean pit depth

Nominal tip radius, tracking speed and contact force were selected from the MarSurf GD 140 tip instrument specifications according to existing components. Therefore, data values were acquired with the following stylus instrument parameters:

- probe arm with a length of 45 mm;
- measuring force of 0.030 N;
- a spherical tip radius of 2 µm;
- X measuring traversing length from 0.1 mm to 140 mm;
- Z(x) measuring range of 500 µm (±250 µm) and a maximum resolution in Z of 0.2 nm;
- measuring speed of 0.10 mm/s.

Settings for the section length or evaluation length and number of sections to evaluate the surface topography were obtained by graphical analysis and from the estimated values of the parameters of interest (Ra, Rt, Rz, Rp, Rv) indicated in the table D.1 of ISO 21920-3:2021. The following steps were performed:

5.1.1 Measurement inside the channels

To identify the setting to be used, several scanning lines with different traveling length (*lt*) were measured inside of one of the slides (Figure 10). Vertical (Vert) and longitudinal (Hor) scanning lines were performed in both channels and the parameters of interest (*Ra, Rt, Rz, Rp, Rv*) defined above were evaluated.

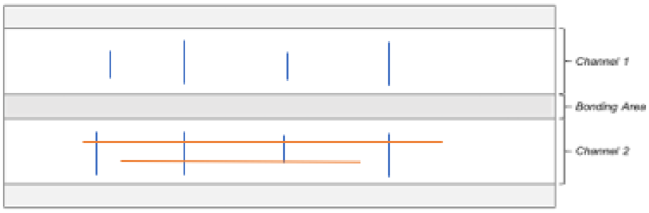


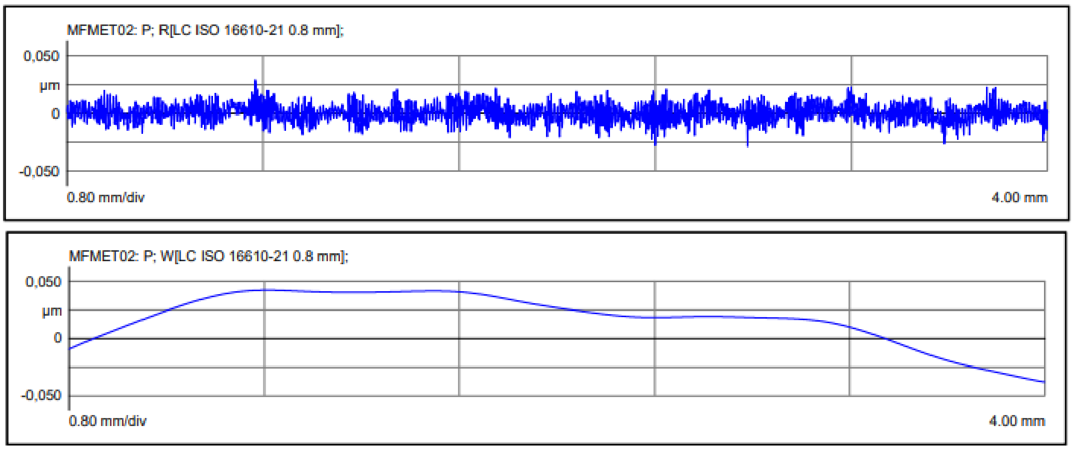
Figure 10 –Type of the scanning lines performed, vertical (blue line) and longitudinal (orange line).

The results for the roughness (*Rx*) and waviness (*Wx*) parameters (measured value (± standard deviation)) and graphics of the vertical scanning lines are present in the Table 3 and in the Figure 11.

Table 3: Results of the roughness and waviness evaluated from the transversal length lines (Vert).

Roughness parameters (Vert)						
	lt	***Ra***	***Rz***	***Rt***	***Rp***	***Rv***
Vert	5.6 mm	0.0059 µm (± 0.4 nm)	0.0471 µm (± 3 nm)	0.0571 µm (± 6 nm)	0.0242 µm (± 2 nm)	0.0229 µm (± 2 nm)
	0.56 mm	0.0016 µm (± 0.4 nm)	0.0102 µm (± 2 nm)	0.0200 µm (± 7 nm)	0.0048 µm (± 1 nm)	0.0054 µm (± 1 nm)
Waviness parameters (Vert)						
	lt	***Wa***	***Wt***	***Wv***		
Vert	5.6 mm	0.0318 µm (± 12 nm)	0.0994 µm (± 30 nm)	0.0293 µm (± 12 nm)		
	0.56 mm	0.0143 µm (± 7 nm)	0.0598 µm (± 30 nm)	0.0123 µm (± 6 nm)		

***lt* = 5.6 mm (Roughness (R) and waviness (W) – (Vert))**



***lt* = 0.56 mm (Roughness and waviness – (Vert))**

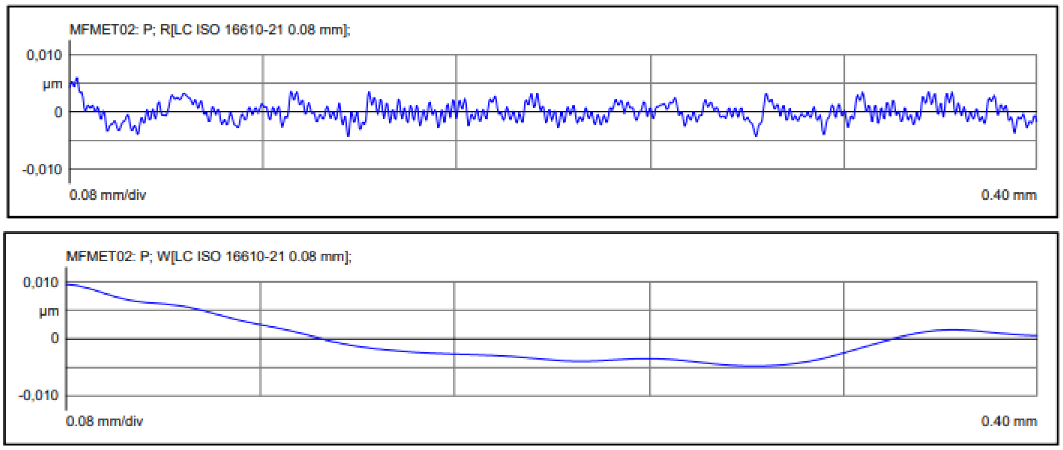


Figure 11 –Roughness R-profile and waviness W-profile from the transversal length lines (Vert)

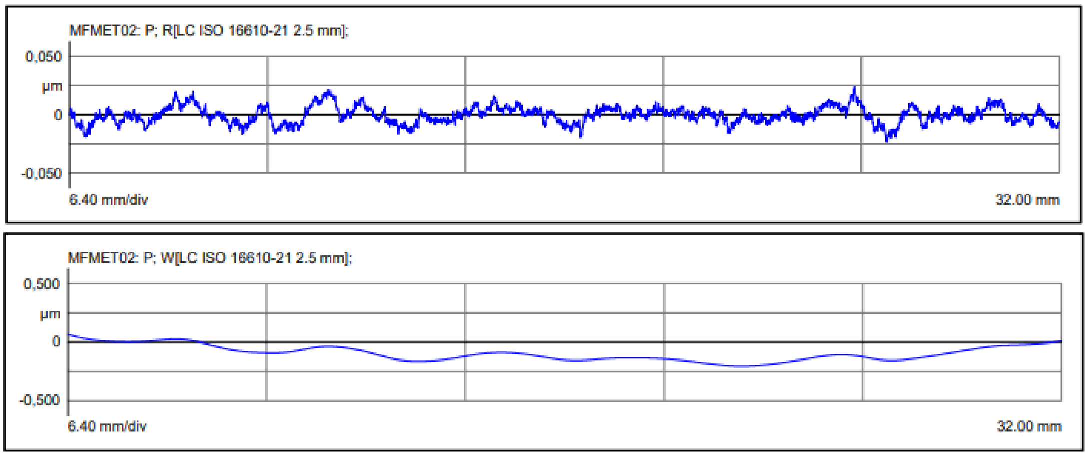
The additional measurements and the results and graphics for the longitudinal (Hor) scanning lines give us also information about the profile and undulation of the surface along the channel length axis. The results for this roughness (*Rx*) and waviness (*Wx*) measurements are presented in table 4 and in the Figure 12.

Table 4: Results of the roughness and waviness evaluated from the longitudinal lines (Hor).

Roughness parameters (Hor)						
	lt	*Ra*	*Rz*	*Rt*	*Rp*	*Rv*
Hor	44.8 mm	0.0057 µm	0.0383 µm	0.0471 µm	0.0192 µm	0.0191 µm
	17.5 mm	0.0073 µm	0.0411 µm	0.0538 µm	0.0221 µm	0.0190 µm

Waviness parameters (Hor)				
	lt	*Wa*	*Wt*	*Wv*
Hor	44.8 mm	0.0100 µm	0.2715 µm	0.1554 µm
	17.5 mm	0.0417 µm	0.0175 µm	0.0552 µm

***lt* = 44.8 mm (Roughness (R) and waviness (W) – (Hor))**



***lt* = 17.5 mm (Roughness (R) and waviness (W) - (Hor))**

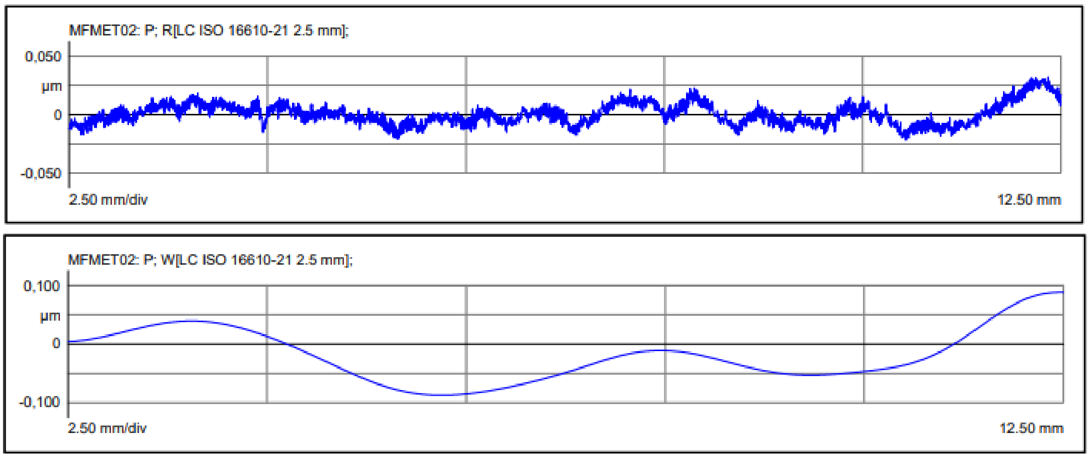


Figure 12 –Roughness R-profile and waviness W-profile along the longitudinal length of the channel (Hor).

5.1.2 Definition of settings to evaluate surface topography under test.

The estimated values (E) of the parameters of interest are defined from the roughness results obtained (Table 3 and Table 4). Throughout this estimated value (E) and from default settings specified in the ISO 21920-3:2021 (Table D.1), the measurement configuration has been defined with the following parameters:

- Total length *lt*: 0.56 mm;
- Number of sections n_{sc}: 5
- Section length l_{sc}: 0.08 mm
- Evaluation length l_e: 0.4 mm
- Maximum nominal tip radius: 2 µm

With a Gaussian filter in accordance with the ISO 16610-21 Geometrical product specifications (GPS) — Filtration — Part 21: Linear profile filters: Gaussian filters, indicated in the graphics.

With these specifications the specimens were measured. The results are presented in section 5.2.

5.2. Results of roughness measurements using MarSurf GD 140 stylus instrument.

Tests were performed according to the MFMET protocol 3.2.4. Each measurement of a profile line was carried out with a total length of *lt*: 0.56 mm as defined above. For slides with two channels (hereinafter referred to as I, II, III) six areas approximately 0.56 mm x 0.50 mm (Figure 13), were analysed. In each area, three profiles lines with spacing of 0.25 mm apart were scanned, recorded and evaluated.

Concerning the slide without channels (bonding area only), three areas were recorded with three profile lines in each area.

The roughness parameter, *Ra*, *Rz*, and *Rt* were selected for the present study, because these parameters, generally, characterize the roughness standards used to guarantee metrological traceability with stylus instruments. The *Rq*, *Rv*, and *Rp* parameters, are also evaluated due to their great use in the fabrication process.

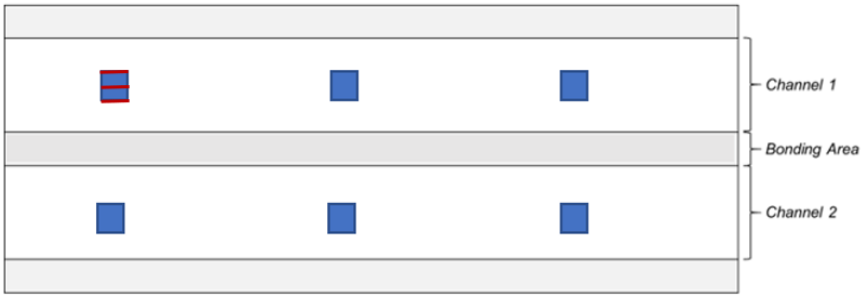


Figure 13: Schematic view of the areas (blue) and the example of the profile scanned lines (red).

The results of the roughness parameters evaluated in the slides with channel areas and in the slide without channels (bonding area) are displayed in Table 5. For each slide is reported the average of the roughness parameter measured at the six areas (Average I, Average II and Average III).

For all the roughness parameters, the result value (Average) and the standard deviation associated, from all the profile lines measured, is also displayed.

Table 5: Results of the roughness measurements parameters using the stylus instrument.

Slide		*Ra* /µm	*Rq* /µm	*Rz* /µm	*Rt* /µm	*Rp* /µm	*Rv* /µm	*Rsk* /µm	*Rku* /µm
I	channel 1	0.0014	0.0017	0.0082	0.0115	0.0042	0.0040	-0.0839	3,5714
	channel 2	0.0013	0.0016	0.0076	0.0099	0.0036	0.0040	-0.3813	3,2778
	Average I	0.0013	0.0017	0.0079	0.0107	0.0039	0.0040	-0.2326	3,4246
II	channel 1	0.0013	0.0016	0.0078	0.0107	0.0039	0.0039	-0.0746	3,5300
	channel 2	0.0013	0.0017	0.0084	0.0116	0.0041	0.0043	-0.2384	3,4846
	Average II	0.0013	0.0017	0.0081	0.0111	0.0040	0.0041	-0.1565	3,5073
III	channel 1	0.0014	0.0018	0.0082	0.0128	0.0042	0.0040	0.1422	4,6763
	channel 2	0.0015	0.0019	0.0086	0.0132	0.0044	0.0042	0.3650	5,0716
	Average III	0.0014	0.0019	0.0084	0.0130	0.0043	0.0041	0.2536	4,8739
Result value (Average)		**0,0013**	**0.0017**	**0.0081**	**0.0116**	**0.0041**	**0.0041**	**-0.0452**	**3.9353**

standard deviation	0,0001	0.0001	0.0002	0.0012	0.0002	0.0000	0.2615	0.8140

	***Ra* /µm**	***Rq* /µm**	***Rz* /µm**	***Rt* /µm**	***Rp* /µm**	***Rv* /µm**	***Rsk* /µm**	***Rku* /µm**
Bonding area	**0.0014**	**0.0018**	**0.0084**	**0.0113**	**0.0040**	**0.0044**	**-0.3830**	**3.2620**
standard deviation	0.0002	0.0003	0.0010	0.0014	0.0004	0.0006	0.1957	0.3045

No significative variations were found between the results of the roughness parameters measured, *Ra*, *Rz*, *Rt, Rq*, *Rv* and *Rp*, neither inside the channels nor in the bonding areas in all slides analized. This means that the manufacture process has a good reproducibility in both channels and bonding area for the studied parameters. The difference found for the *Rt* parameter can be explained by a possible random peak or valley scanned in one of the measured line profiles.

Skewness parameter (*Rsk*) is a measure of the asymmetry of an amplitude density curve. Negative skewness indicates a surface with good bearing properties. Furthermore, and according to Franz Konstantin Fussa [5], referred that a *Rsk* near zero indicate an equal distribution of peaks and valleys of the surface. “The larger the surface skewness *Rsk*, the “rougher” is the surface profile”,

Kurtosis parameter (*Rku*) is a measure of the slope of an amplitude density curve. For profile values with normal distribution, *Rku* = 3.

Figure 14 shows profiles with low and high values of *Rku*. This is a useful parameter in predicting component performance with respect to wear and lubrication retention.

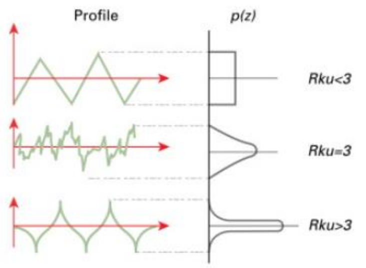


Figure 14: Example of profiles with low and high values of Rku (https://www.sciencedirect.com/topics/engineering/surface-height-distribution).

5.3. Additional measurements

Measurement of traversing lengths, one with 17.5 mm and another of 5.6 mm crossing the two channels and the central bonding area of a slide (Figure 15), to identify the topography of the surface were also performed.

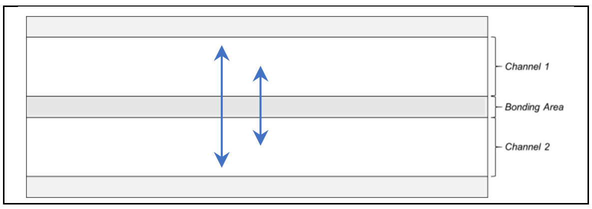


Figure 15 – Scanning of the surface crossing the channels.

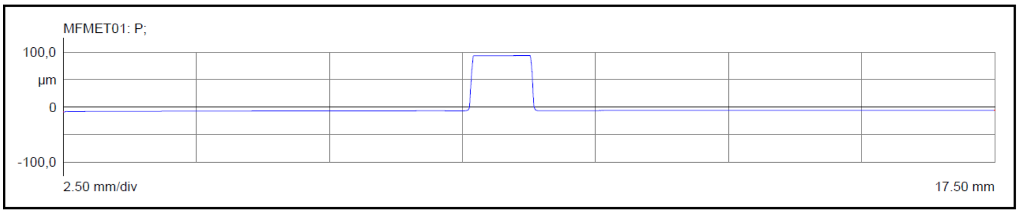


Figure 16 – Graphic of the profile registered

For the profile total height parameter *Pt* (the sum of the largest height with the largest pit) a measured value of 102.7364 µm was obtained with a standard deviation of 0.2968 µm.

6. Discussion and Conclusions

The specimens provided in this task were processed as follows (Figure 17).

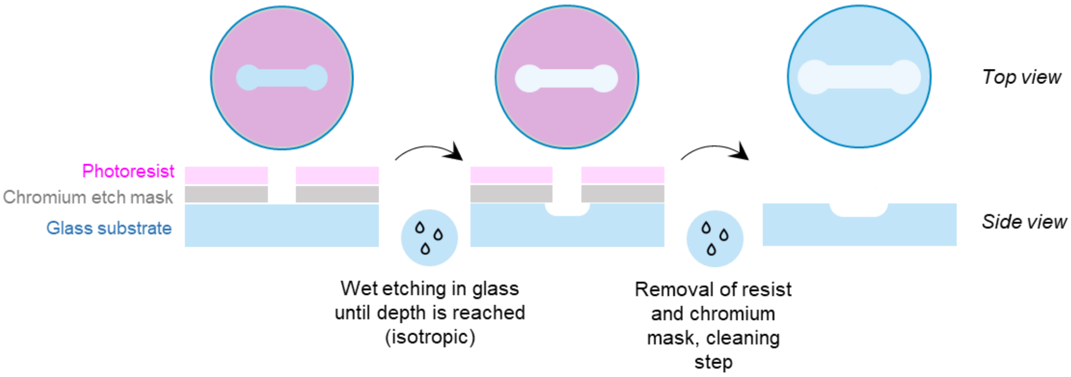


Figure 17: Schematic overview of the processing of the provided specimens for the creation of channels in glass. Process steps before that are not displayed here.

The glass wafer from the supplier exhibits a roughness specified below 0.5 nm. The roughness may increase during the various processing steps for microfluidic devices. So far, no precise information or value could be provided for the roughness in the channel and at the bonding area.

The confocal microscope can be used to measure roughness, but this is dependent on the resolution of the instrument or method. The method can be used for coarse roughness values in the range of several µm, but this is not the case with these specimens.

In the stylus roughness measurements, of an unknow surface it is important to define the default settings, to be used in the measurements before the tests are performed because the roughness parameters evaluation depends on the total length used. In this case the total length setting defined was 0.56 mm and no significant differences were found between the results of the measured roughness parameters *Ra*, *Rz*, *Rt*, *Rq*, *Rv* and *Rp*, neither inside the channels nor in the bonded areas with the Stylus instrument.

The results obtained for *Rku*, with both techniques (confocal and stylus) are similar and near the value 3.

AFM measurements showed that the values for the roughness of both areas of interests (0.46 nm - 0.60 nm) were in the range similar to the specified value (<0.5 nm) before the processing of the glass wafer. Interestingly, the surface roughness values for the channel are higher than for the bonding area. Therefore, it can be assumed that the wet etch process in glass does give a small increase to the initial surface roughness. It is also a good indication that the channels etched in glass do exhibit a very smooth surface, which is beneficial for the end application in microfluidics. Although only very small areas can be measured with AFM, the results are representative and give a good estimate of the roughness of all surfaces in a glass microfluidic.

Both measurement of macro-lines and micro-lines used for the roughness parameters determination are very important for the work in microfluidics as it allows a better understanding of the liquid surface path.

7. Bibliography

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