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EFFECTS OF IMAGE SIZE AND SAMPLE AREAS ON COMPARISON OF CONTACT LENS SURFACES BASED ON FRACTAL DIMENSION

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Abstract: Methods used for calculation of fractal dimension demand large image resolution and adequate sample size, in terms of roughness threshold that defines spatial scope for rough surface fractal properties. Imaging device operators, on the one hand, recommend the image size and sample area based on experience and expertise, in order to minimize the imaging time. On the other hand, engineers make decisions based on their own requirements. To overcome these problems, in this paper we used ANOVA statistical approach (one-way and multi comparison) so as to establish significant image size and sample area. The conclusion made in this paper will enable decision guidelines on selection of parameters for new nanophotonic lenses imaging by scanning microscopes in near future.

Key words: fractal dimension, imaging, surface roughness, ANOVA.

1. INTRODUCTION

Topography of engineering surface created by one or more manufacturing processes can be characterized by widely used standard roughness parameters, as well as by a single and alternative fractal dimension. Gas-permeable (RGP) micro-machined contact lenses belong to a group of biomedical surfaces with finer grade of quality, called optical quality. ISO 1302:2002 Standard defines roughness parameters values for surface quality labeled with grade number N1 to N12, with R_a values of 0.025μm to 50μm, respectively. Although ISO standard has a certain recommendation for standard roughness parameter R_a values from 0.025μm to 0.008μm, there are not the values for sampling length. The fractal dimension is the intrinsic surface property as opposed to any standard roughness parameter that contains only partial information. Fractal dimension is a scale invariant parameter and, therefore is more suitable for texture characterization, especially in case of biomedical surfaces recorded by scanning microscopy. Methods used for fractal dimension calculation demand large image resolution, for example 512x512 pixels. Furthermore, the size of sample previously prepared from machined surface is very important in terms of roughness threshold defining the spatial scope for fractal dimension [1].

There are two practical problems faced by engineers during surface imaging in order to characterize machined surface. The first problem is to determine the values of the surface parameters that will characterize the desired intrinsic property and the second is to minimize time for imaging, both in terms of the image size and the sample area that have the main influence on it. The decision made by engineers is based not only on their own requirements but also on imaging device operators' experience and expertise. To overcome these problems, in this paper we used the well-known ANOVA test as a statistical approach to establishing significant image size and sample area. Relationships between various scans taken from different samples made of gas-permeable contact lens basic material are investigated using Matlab. Conclusion will be used for ongoing investigation that considers the new "nano-photonic" material for contact lens that is composed of basic material, doped with three types of nanomaterials.

2. MATERIALS AND METHODS

2.1. Sample Preparation

Samples are taken from contact lenses that are manufactured from fluorosilicone acrylate doped

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PMMA material. Rough surfaces of contact lens are formed using conventional machining by turning with a diamond tool and polishing as a finishing process. Polishing time as process parameter is different for contact lenses No 1, 5, 8 and 11. The polishing length affects the surface roughness and, consequently, the adhesion force that enables floating of rigid gas-permeable lens on tears film. Therefore, polishing time is spanned in recommended interval for that type of material. Intact contact lenses are cleaned and fixed in microscope for imaging. More than 60 images are scanned, but for this research we selected two spots for scanning three areas from each of contact lenses No 1, 8 and 11 and the four spots for scanning three areas from contact lens No 5.

2.2. Topography Scanning

Our research comprises contact lens surface measurement and analyses of topography images that are obtained by atomic force microscopy (AFM) Basically, AFM is a scanning probe microscopy technique based on point-to-point examination of a specimen made by a sharpened tip probe that can scan sample surface roughness with high precision. The AFM system used in this study is JSPM-5200, JEOL, Japan using tapping mode and PointProbe® Plus made by NANOSENSORS™ in ambient condition. This PointProbe® Plus probe is manufactured from highly doped, single-crystal silicon without any intrinsic mechanical stress. The monolithic tip is shaped as a polygon-based pyramid 10-15μm high with half-cone angles of 20° and tip radius finer than 7nm, according to the manufacturer [2].

The tapping mode is used on account of its ability of non-destructive high-resolution imaging of soft and fragile samples in ambient environment. The tip is alternately placed in contact with the surface, so as to provide high resolution, and then lifted off the surface in order to avoid dragging across the sample. Interaction with the surface leads to energy loss and reduced oscillation amplitude that is used as a feedback signal to measure topographic variations of the sample.

2.3. Fractal Analysis Method

The images of scanned surface are exported from WinSPM software as an image in tiff format and/or ASCII file. For further fractal analysis ASCII files are imported in Matlab software. The image in tiff format consists of either 512x512 or 256x256 pixels that are identified by their x and y positions, with the grey scale function as the z dimension. The ASCII file contains either five-digit numbers that are modified into 512-by-512 matrices using Matlab custom-made procedure or 65536 numbers modified into 256-by-256 matrices. Such matrix represents an intensity-type image with gray-scale color map, where the range of values is [0, 65535].

The skyscrapers analysis was originally suggested for fractal dimension calculation of digitized mammography [3]. Pixels that constitute an image can be considered as skyscrapers, the height z(x,y), of which is represented by the intensity of the gray colour. The surface area of the image A, referring to (1), is obtained by measuring the sum of the top squares, which represent roofs of skyscrapers and the sum of the exposed lateral sides of the skyscrapers, according to [2]. The square size ε is presented as 2^n and it increases consecutively (ε=1,2,4,8,16,32) for 512x512 image size and (ε=1,2,4,8,16) for 256x256 image size, by adjacent pixel grouping. The gray levels are averaged using Matlab custom-made procedure [1].

$$A(\varepsilon)=\sum\varepsilon^2+\sum\varepsilon\left[\left|z(x,y)-z(x+1,y)\right|+\left|z(x,y)-z(x,y+1)\right|\right] \quad (1)$$

The surface area A for each of images generated in the previous step is determined referring to (2) and resulting pairs (A,ε) is for images area vs. square size. The dots presented in double-log graph are arranged along the straight line. The linear regression is used for fitting the plot in Curve Fitting Toolbox in Matlab. The fitting process results in linear equation and the slope was determined from it. The fractal dimension D is in relation to the slope, according to the relation (2) and D is calculated using custom-made procedure.

$$\log A=(2-D)\log\varepsilon+c \quad (2)$$

2.4. Independent Samples Analysis

ANOVA is the most commonly used method tor testing significance and evaluating the differences in means between two and more groups. . Theoretically, one-way ANOVA assume homogeneity (variance between the groups should be equal) and normal distribution. If the assumptions of homogeneity or normality are violated, ANOVA can be conducted to the extent that the independence is not violated with equal sized groups. This is the main reason for choosing one-way ANOVA to compare the means of several groups to test the hypothesis that they are all the same, against the general alternative that they are not all the same [4]. Additional test that can provide multiple comparison information about which pairs of means are significantly different, and which are not is also performed.

ANOVA produces an F-value, the ratio of the variance calculated among the means to the variance within the samples. If the group means are drawn from the same population, the variance between the group means should be lower than the variance of the samples. The ANOVA returns the p-value under the null hypothesis according to which all samples in groups are drawn from populations with the same mean [4]. The multiple comparison function also displays a graph with each group mean represented by a symbol and an interval around the symbol. For one-way ANOVA and multiple comparison testing the commercial software Matlab with Statistics Toolbox and procedures are used.

3. RESULTS AND DISCUSSION

Topography images that represent surface roughness distribution are gathered from contact lens No5 spot 3 and contact lens No8 spot 4. Scanned surface areas are 15x15μm^2, 5x5μm^2 and 1x1μm^2 and the image size is 512x512. In this paper, we calculated fractal dimension as a roughness parameter for gathered topography images sized 512x512 and 256x256 pixels using skyscrapers analysis. In double logarithmic diagram the dots represent images area vs. square size and have a linear type of appearance. This kind of relationship indicates the existence of power law between the two measures generated from measured surface, which proves the fractal behavior of surface. Fractal dimensions for contact lens No5 spot 3 and contact lens No8 spot 4 are given in Table 1.

Table 1. Fractal dimension values for topography images

	Img./ Area size		1x1	5x5	15x15
Fract. Dim.	Sample No.5 spot 3	512x512	2.4903	2.5127	2.4683
		256x256	2.3312	2.5816	2.4584
	Sample No.8 spot 4	512x512	2.3180	2.3344	2.4543
		256x256	2.2697	2.3535	2.4326

In the ANOVA analysis, comparisons of means and measures of variation in the groups can be visualized in box plots. In order to test significance of fractal dimension values, one-way ANOVA is performed for topography images of contact lens No.5 spot 3 (labeled Cl53) and No.8 spot 4 (labeled Cl84). Results for different size of images for 512^2 vs. 256^2 pixels are shown in Fig 1 for two groups. The first group 'Cl536' represents fractal dimensions for topography images with 512^2 pixels and the second group 'Cl535' represents 256^2 for contact lens No.5 spot 3 in Fig 1-left hand side. In Fig 1-right hand side are shown box plots for topography images of contact lens No.8 spot 4 with labeled groups analog to the designation explained for No5. The small differences in center lines of box plots suggest that the groups belong to the same population.

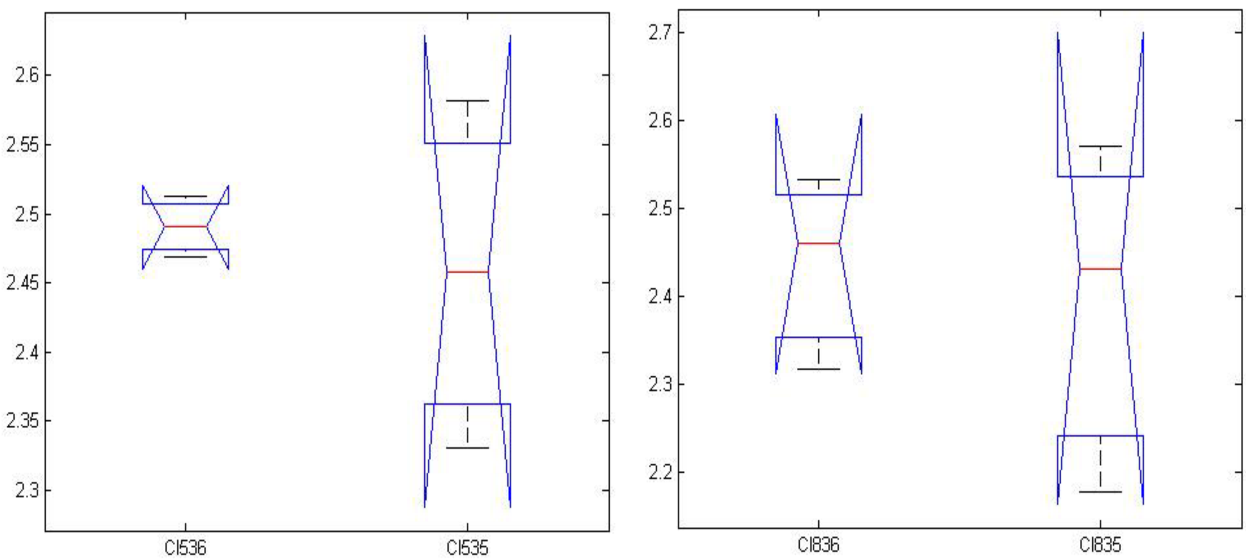

Figure 1. Box plot of two groups that are gathered from sample No5 (left-hand side), No8 (right-hand side). Images size in groups are combination of 512 vs. 256 pixels.

The results of one-way ANOVA for testing of null hypothesis that fractal dimension values for two groups 'Clxx6' (512^2 pixels) and 'Clxx5' (256^2 pixels) belong to the same population are shown in

Table 2. High p-values of p(536-535)=67.3% and p(846-845)=80.3% confirm the suggestion implied by box plots. It can be concluded that these images represented by fractal dimension belong to the same sample. This result also confirms lower image resolution for comparison of topography images.

Table 2. ANOVA p-values for topography images

Img. 512x512 vs. Img. 256x256		
Same population	536 - 535	846 - 845
p-value	0.673	0.803
Img. 512x512 vs. Img. 256x256		
Different population	536 - 846	535 - 845
p-value	0.053	0.289
Img. 512x512, 512x512, 256x256		
Different population	53 - 84	
p-value	0.033	

We perform another two ANOVA tests in order to confirm that topography images sized 256^2 pixels can be compared based on fractal dimension. According to the hypothesis, the groups consist of images size of 512^2 pixels and scanned from contact lens No.5 spot 3 (labeled Cl536) and No.8 spot 4 (labeled Cl846) belong to the same sample. ANOVA test gives for comparison '536–846' p-value is equal to 0.053 (close to type one error rate that is 0.05) and suggests to reject the null hypothesis. This is correct, but in case of '535–845' topography images size of 256^2 pixels ANOVA calculates p-value that is equal to 0.289. This result confirms the hypothesis that is incorrect. We conclude that the topography images with 256^2 pixels cannot be used for surface comparison, based on the results of ANOVA tests p-values that are shown in Table 2.

Anyway, we try intuitively a combination of images sized 256^2 pixels for surface areas 15x15μm^2 and 512^2 pixels for surface areas 5x5μm^2 and 1x1μm^2. ANOVA offers correct result, even better than all three images have 512^2 pixels. For analysis of variance for contact lens No5 and No8 that are labeled '536–846' p-value is equal to 0.033<0.05 and suggests that images do not belong to the same sample. The box plot of the two groups fractal dimensions suggests that big differences in the center lines of the boxes correspond to large values of F and correspondingly small values of p. Especially box plots on the right and left hand side in Fig 2 imply that the topography images are scanned from different contact lenses. The hypothesis is rejected which is a correct conclusion. Images sized 256^2 pixels belong to the same sample, according to box plot shown in Fig 2-center and that correspond to p-value. This result is incorrect. We use Matlab statistics 'multicompare' analysis that provides comparison graphs to confirm that low image resolution (256x256 pixels) are not good enough for contact lens surfaces that have optical quality.

Comparison graphs that correspond to box plots in Fig 2 are shown in Fig 3 and reject the use of lower image resolution for topography comparison, based on fractal dimension. Comparison graph in Fig 3-center shows that the group's means are not significantly different because their intervals overlap. This is an incorrect conclusion based on low resolution images. In Fig 3-left are shown groups comparison based on fractal dimension for three images with 512x512 pixels. Conclusion based on this comparison graph regarding intervals overlapping is the same as in the case of three images with 512^2-512^2-256^2 pixels that is shown in Fig 3-right-hand side. This is the reason why we take this combination for further investigations.

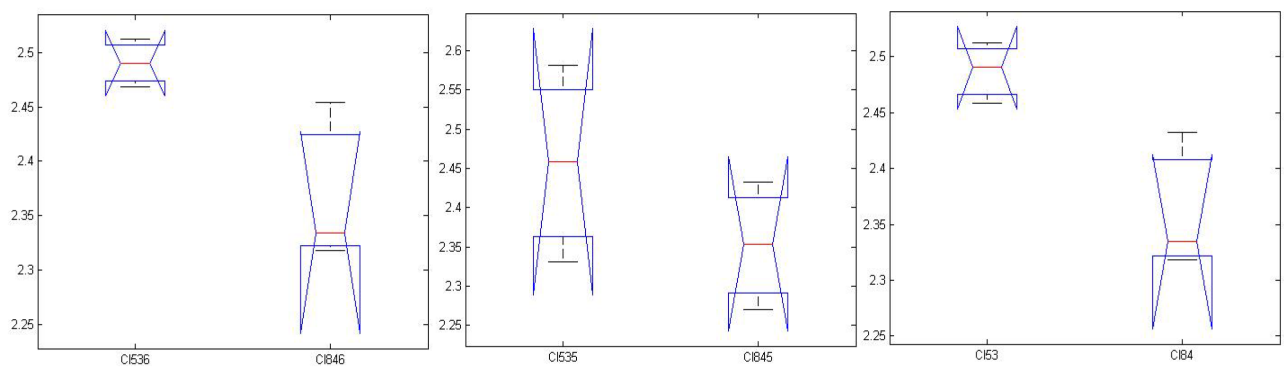

Figure 2. Box plot of two groups that are gathered from sample No 5 and No 8. Images size in groups are 512x512pixels (left hand side), 256x256pixels (center) and combination of 512^2-512^2-256^2 pixels (right hand side).

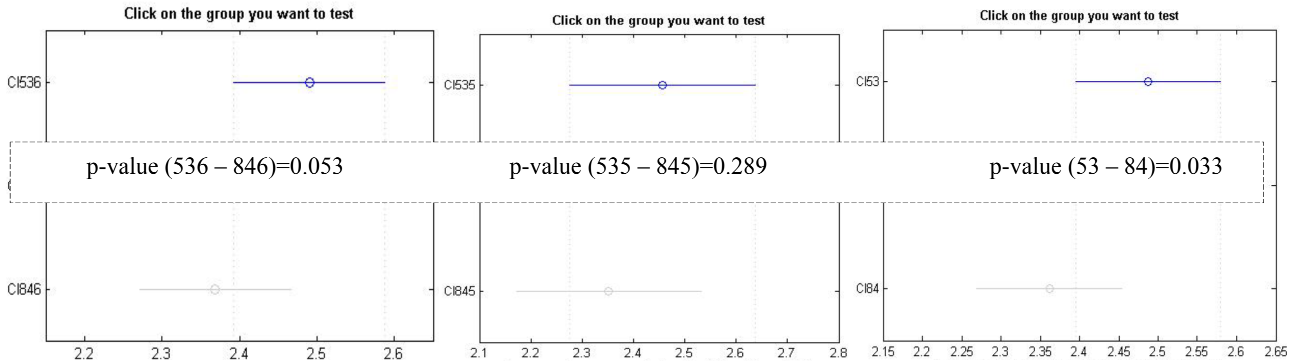

Figure 3. Comparison graphs of groups. Groups Cl53 (5/6) are gathered from sample No 5 and groups Cl84 (5/6) are from sample No 8. Images size in groups are 512x512 pixels (left hand side), 256x256 (center) and combination of 512^2-512^2-256^2 (right hand side).

Only in case of bigger surface areas this low resolution (256^2 pixels) is acceptable. That combination of image size is good concerning time consuming and offers us a quick view of target spot at lens, before we start to scan smaller areas with better resolution. This statement we test in case of contact lenses No 1, 8 and 11 where we select the two spots and scan three areas from each. Calculated fractal dimension for images arise shown in Table 3.

Table 3. Fractal dimension values for topography images

	Area size (Img size)		1x1 (512x512)	5x5 (512x512)	15x15 (256x256)
Sample No.1	Fract. Dim.	spot 3	2.5283	2.5511	2.5227
		spot 4	2.3987	2.5263	2.5333
Sample No.11	Fract. Dim.	spot 2	2.5048	2.4608	2.3990
		spot 3	2.5387	2.4863	2.5605
Sample No.8	Fract. Dim.	spot 3	2.3175	2.5334	2.4321
		spot 4	2.3180	2.3344	2.4326
Sample No.5	Fract. Dim.	spot 1	2.2795	2.5886	2.5441
		spot 2	2.3887	2.4289	2.4655
		spot 3	2.4903	2.5127	2.4584
		spot 4	2.4403	2.5189	2.4154

Groups of fractal dimension are tested by ANOVA and the test results are shown in Table 4. High values of p for each groups comparison p (13–14) = 34.3%, p(112-113) = 12.3% and p(83-84) = 41.1% proves that images belong to the same sample. Box plots for two groups of images gathered from two different spots of the same contact lenses No 1, 8 and 11 are shown in Fig 4. The small differences in center lines of box plots suggest that groups belong to the same contact lens.

Table 4. ANOVA p-values for topography images

Img. 256x256, 512x512, 512x512				
population	13 - 14	112 - 113	83 - 84	51-52-53-54
p-value	0.343	0.123	0.411	0.874

Number of spots where scans are performed, could increase the probability of accepting true hypothesis. In this case, it means that images belong to the same contact lens. For example, we scan three areas at each of four spots on the contact lens No 5. Results of ANOVA test is very high p-value p(51-52-53-54)=87.35% are shown in Table 4.

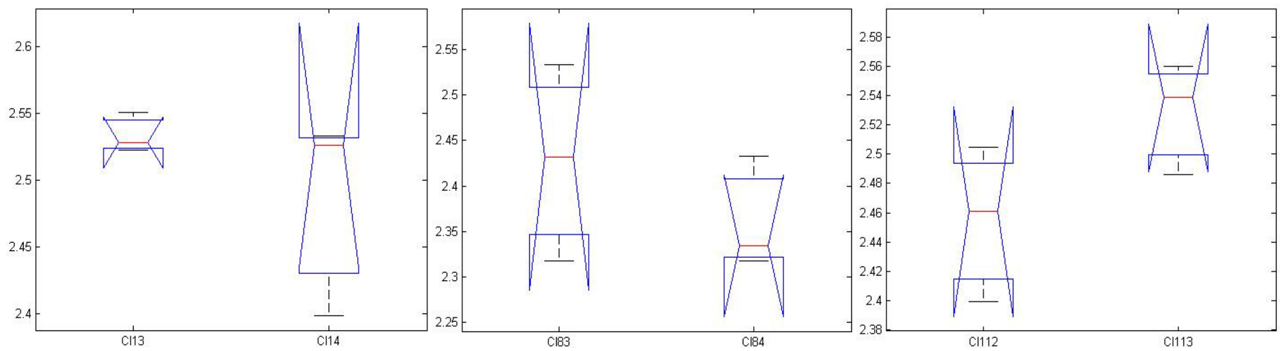

Figure 4. Box plot of two groups that are gathered from sample No 1 (left-hand side), No 8 (center) and No 11 groups (right- hand side). Images size in groups are combination of 512^2-512^2-256^2 pixels.

Figure 5-left hand side shows box plot and Figure 5-right hand side shows a comparison graph as a graphical result for one-way analysis of variance and multi comparison analysis, respectively. Box plots and comparison graph shows that four groups' means are significantly not different, because their intervals overlap. This statement agrees with the previously mentioned high p-value.

These results give us an argument to perform scan at single spot from sample and characterize topography image by fractal dimension. Therefore, fractal dimension, as numerical representation of surface roughness, could be used for contact lenses comparison. We already used the result of comparison as feedback for machining process optimization [1], for contact lens wear-out diagnostics [5] and for ongoing investigation that considers new "nano-photonic" material for gas permeable contact lenses [6].

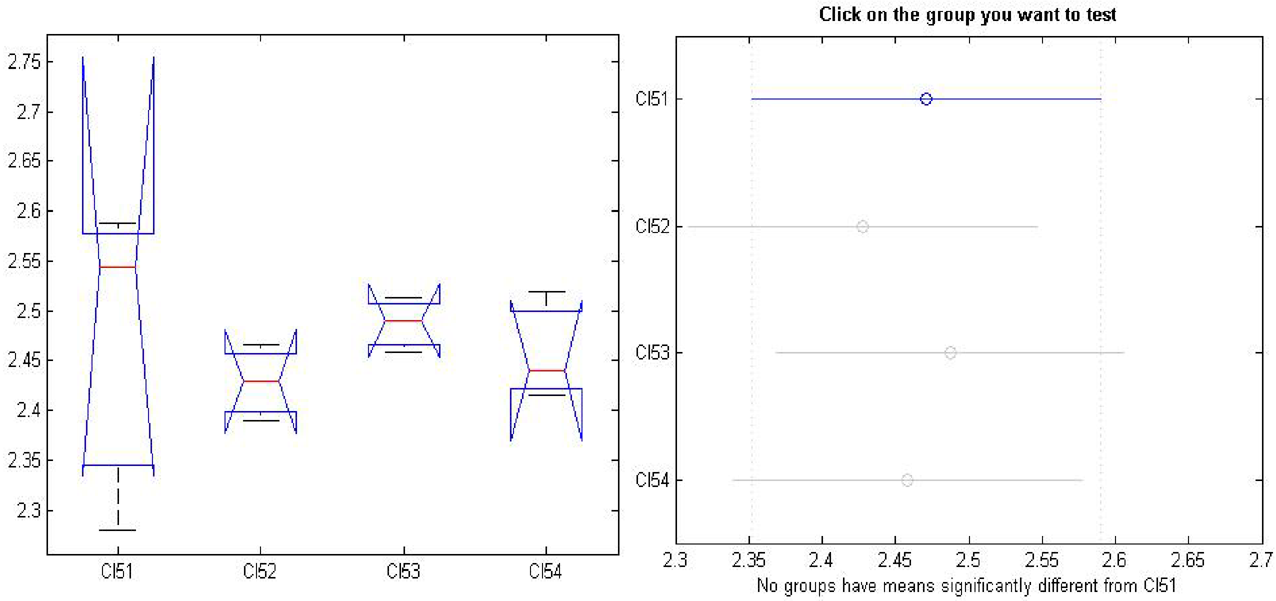

Figure 5. Comparison of four groups (Cl51, Cl52, Cl53 and Cl54) gathered from the same sample No5 are presented in box plot (left hand side) and comparison graph (right hand side).

4. CONCLUSION

Solutions proposed in this paper represent a way to overcome two practical problems that engineers have in surface imaging in order to characterize machined surface. The first is to determine the values of the fractal dimension and the second is to minimize time for imaging, both in terms of the image size and the sample area. In this paper we used the well-known ANOVA test as statistical approach for establishing significant image size and sample area.

First the fractal dimension of topography images previously gathered by AFM, are calculated by "skyscrapers" method. The comparison between engineering surfaces is possible by using an appropriate roughness parameter like fractal dimension. Therefore we apply fractal analysis in order to distinguish two contact lens surfaces using fractal dimension.

Afterward, several independent sample analyses were performed with the following results:

− First one-way ANOVA test confirmed that fractal dimension values for groups of topography images recorded in 512^2 pixels and 256^2 pixels size belong to the same sample. It was a correct conclusion.

− Another one-way ANOVA test implies that two groups of topography images recorded in 512^2 pixels size represented via fractal dimensions do not belong to the same sample. It was a correct conclusion, but in the case of topography images with 256^2 pixels ANOVA accepts the hypothesis that is incorrect. It is concluded that the 256^2 pixels resolution cannot be used for contact lens surface comparison.

− Matlab statistics 'multicompare' analysis also confirms that low imaging resolution is good enough for comparison of contact lens surfaces only in combination of three images area sizes 1x1μm^2, 5x5μm^2 and 15x15μm^2 with 512^2-512^2-256^2 pixels respectively. ANOVA verifies that the combination of image sizes if good enough concerning time consuming, because it offers quick view of target spot at lens, before imaging smaller areas with better resolution start.

− ANOVA test demonstrates that the number of spots where scans are performed increase the probability that images belong to the same contact lens.

The presented results are used for applied research of new contact lens materials, composed of poly methyl methacrylate, doped with three types of nanomaterials.

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УТИЦАЈИ ВЕЛИЧИНЕ И ПОВРШИНЕ СНИМАКА КОНТАКТНИХ СОЧИВА НА ПОРЕЂЕЊЕ ПОВРШИНА БАЗИРАНО НА ФРАКТАЛНОЈ ДИМЕНЗИЈИ

Сажетак: Методе прорачуна фракталне димензије захтевају снимке великих резолуција и адекватних величина скенираних површина, са становишта скале на којој се јавља фрактално понашање. Оператори на уређајима, с једне стране, постављају те параметре ради смањења времена скенирања на основу искуства и знања. С друге стране, инжењери траже параметре који ће задовољити њихове захтеве. У циљу превазилажења ових супростављених захтева, у овом раду је коришћена анализа варијанси, како једнострука тако и вишеструка, ради утврђивања сигнификантне величине и површине снимка. Закључак овде спроведеног истраживања пружа смернице на основу којих ће се донети одлука везана за адекватне параметре скенирања при будућим испитивањима нанофотонских материјала.

Кључне речи: фрактална димензија, снимање, површинска храпавост, анализа варијанси.