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FRACTAL ANALYSIS FOR BIOSURFACE COMPARISON AND BEHAVIOUR PREDICTION*

Fractal analysis was used in previous authors' researches for characterizations of grinded ceramics surface textures by surface profile fractal dimension. In this paper the "skyscrapers" method was chosen for calculating fractal dimension of surface, using the image processing toolbox, as well as a custom-developed algorithm of Matlab environment. This method entails recording the surface as an image, by using a scanning probe microscope. In the given contact lens case, fractal dimension values confirm changes of the surface roughness during the cleaning and wearing processes. Examination of real surface roughness could provide comparison and functional behaviour prediction.

Corrective rigid gas permeable contact lenses (RGP CL) are designed to improve vision. RGP materials are commonly composed of monomers containing silicone, fluorine and methylmethacrylate. This kind of lens is very durable and may last for several years without the need for replacement due to retention of polymer performance.

Moreover, every single RGP CL wearer provides unique ambient conditions in which these CL biosurfaces have to function. Since CL surfaces become significantly rougher after prolonged wear, they become more prone to bacterial adhesion and protein and lipid deposits. CL can lose functionality due to accumulated proteins, lipids, and other tear components on CL surface, despite routine cleaning activities.

The loss of RGP CL functionality has to be investtigated and related to measurable parameters in order to recommend replacement based on significant changes in surface properties. Thus, the paper describes usefulness of topography images analysis application for the RGP CL replacement.

There is no clear answer to the question as to what surface standard parameter should be used for critical limit determination. The result of the study also confirms the need for the replacement schedule of RGP CL [1]. The water contact angle, percentage of elemental surface composition and deposit rate of bacteria was related to standard average roughness parameter Ra. It was stated that surface roughness was the most influential lens surface property after 10 days of wear [2].

This paper focuses on the quantification of the textures of CL inner surface by applying a method differring from the standard parameters characterizations, because a single standard parameter fails to describe functional nature of the surface, and the use of more than one roughness parameter exhibits more shortcomings. This is mainly due to the partial information contained in each descriptor.

The authors belong to the group of researchers who prefer fractal parameter characterization that enables to distinguish surfaces, compare them and predict functional behaviour in use. Fractal analysis was used for quantitative characterizations of grinded ceramics surface textures by surface profile fractal dimension [3].

Fractal analysis of biomedical surface topography, as an extent of previous research efforts, is influenced by growing interest in biomaterials surface technology. Fractal geometry provides a useful tool for the analysis of complex and irregular structures such as biomedical surface topography based on image analysis methods that consider an image as a 3D surface.

Fractal dimension calculation based on "slit-island" and "skyscrapers" methods were proposed in [4]. Problem in adoption of "slit-island" method in contact lens case was reported in [4]. The "skyscrapers" method was range as appropriate one for this case. This method presupposes surface recording as an image, by using scanning probe microscopy (SPM).

PROBLEM FORMULATION

The samples are two RGP CLs made of ML 92 Siflufocon A. The first lens, shown in Figure 1, is the left one and was worn by a 37 year-old female with about 3 years of regular use and storage. The second lens, shown in Figure 2, is the right one and was worn by the same 37 year-old female over an extended period (in fact more than 5 years) of regular use and storage. Figures 1 and 2 are the captures from the video recording made by using an optometric device for cornea parameter measurement and contact lens control. The wearer reported an unpleasant sense during the wearing, namely the lens sliding across the right eye's cornea. The ophthalmologist pointed out that there was no vision deterioration but there is an obvious fast sliding process accompanied by occurrence of an air bubble, which can be observed in Figure 2.

The right RGP CL is "worn out" and replaced due to the change of some surface properties that caused low

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adhesion between inner surface and tear film. The adhesion force holds the RGP lens in the eye. The appropriate adhesion force amount obtained by manufacturing process, consisting of turning with polishing as finishing process, is shown in Figure 3.

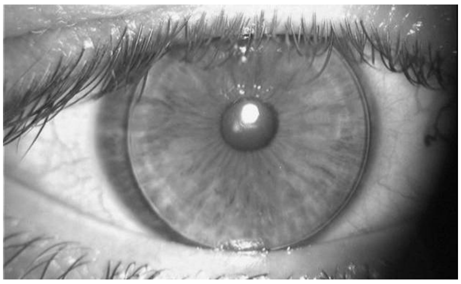


Figure 1. RGP CL placed on the left eye's cornea.

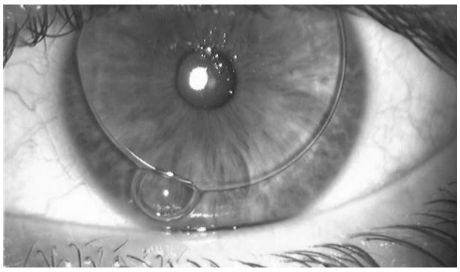


Figure 2. RGP CL placed on the right eye's cornea with air bubble on its edge.

Figure 3. Stages of RGP CL manufacturing process – diamond turning and polishing.

RGP CL design and material removal stages in the turning process are shown in Figure 4. The double side turning with diamond tools for coarse and fine operations also includes axial edge lift (AEL) operation. The side-cutting edge angle of diamond tool is $\psi = 60°$ and nose radius is $R = 0.25$ mm. The depth of cut for coarse turning and AEL operations is $a_c = 0.4$ mm and angular feed is $\theta_c = 6°/s$. Depth of cut for fine turning is $a_f = 0.05$ mm and angular feed is $\theta_f = 2°/s$. The rotational speed during the turning process is $n = 8000$ rev/min. The natural rubber (caoutchouc) polishing tool is moulded with sphere radius decreased by fibre patch thickness. The polishing paste contains aluminium oxide particles sized from 0.3 to 0.5 μm. The duration of polishing (10–15 s) determines surface quality, that is to say surface roughness, and consequently the adhesion.

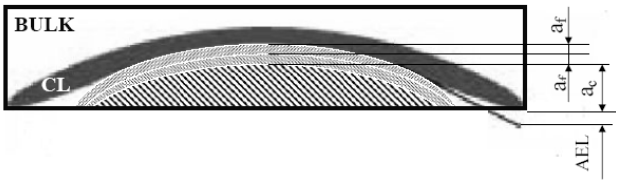


Figure 4. RGP CL design and material removal stages in the turning process.

Authors assume that the change of surface topography caused by extended wear, including protein and lipid deposits, is interesting for investigation.

EXPERIMENTAL WORK

AFM tapping mode recording

Experimental work is conducted on commercial JOEL scanning probe microscopy - JSPM 5200 (Figure 5) that can be configured by merely changing the tip. In general, JSPM 5200 has three different AFM modes used for topography imaging; these are the non-contact, contact and tapping mode, according to [5].

Figure 5. JOEL scanning probe microscopy - JSPM 5200 [5].

The tapping mode is used on the 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. In tapping mode AFM, the cantilever is excited into resonance oscillation with a piezoelectric driver in ambient air at or near its fundamental flexural resonance and with free air amplitudes. The interaction with the surface (tapping) leads to energy loss and reduced oscillation amplitude. The oscillation amplitude is used as a feedback signal to measure topographic variations of the sample, as explained in literature [6].

The contact lens inner surface topography image is recorded in tapping mode AFM in order to investigate changes in surface roughness. The measurement reports for both CLs are shown in Figures 6 and 7. RGP CLs were not clean before measurements, in order to acquire relevant information about disturbing factors on surface layers. In addition, the recorded areas are near to the CL diameter edge, since the bubble appears in that region.

topographic image recorded in tapping mode AFM is imported in Matlab as an image in tiff format accompanied by ASCII file. Image pixels are identified by their x and y position, while the grey-scale function is the z dimension. The image in tiff format consists of 512×512 pixels, shown in Figure 6 for the left and Figure 7 for the right lens, while the ASCII file contains 262144 five-digit numbers. That tiff image is considered as an intensity image type, and represents 512-by-512 matrix of 8-bit integers that are linearly scaled to produce colourmap indices in range [0,255].

In the second step, ASCII data is modified into 512-by-512 matrices using Matlab custom-made procedure for numbers conversion in 16-bit integers (step 2 shown in Figure 9). Such a matrix represents an inten-

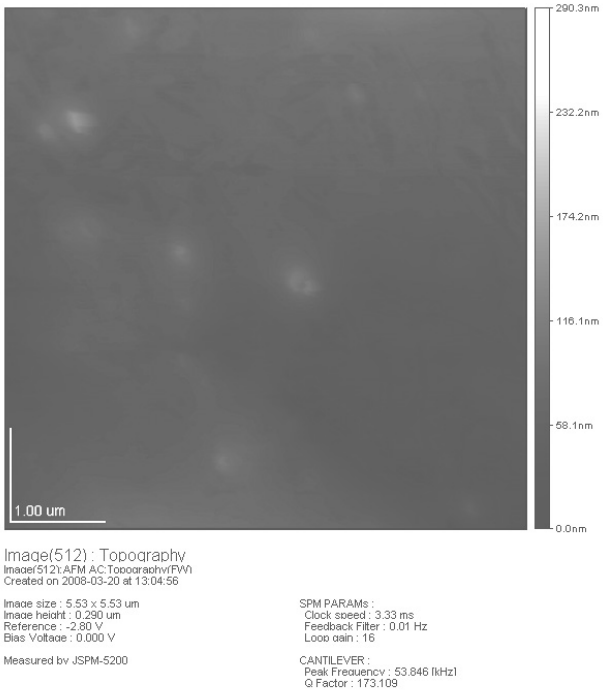


Figure 6. AFM tapping mode measurement report for the left CL.

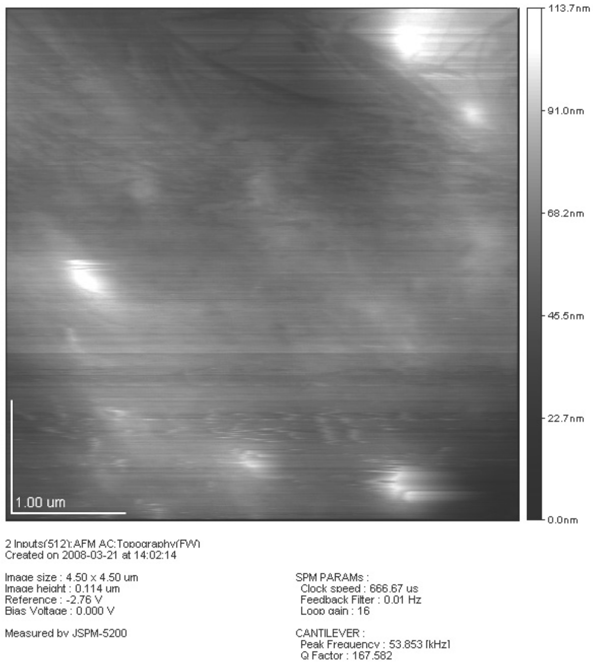


Figure 7. AFM tapping mode measurement report for the right CL.

There are visible distinctions between surface appearances. The left contact lens inner surface looks smoother with monotonous height change. The right contact lens inner surface looks coarser with intensive contrast in grey.

Fractal dimension as a measure of irregularities could provide additional information about surface roughness. In this paper, the “skyscrapers” method is chosen for calculating fractal dimension of surface [4].

Fractal analysis by “skyscrapers” method

Fractal analysis consists of six steps that are explained in this section and shown in Figure 8.

The first step consists of importing AFM recorded surface data in Matlab software for further analysis. The

sity-type image with a grey-scale colourmap, where the range of values is [0,65535]. The image generated from the ASCII file is more sensitive compared to the tiff image. The image of the left contact lens is shown in Figure 9 as well as the image’s area calculation procedure generated in Matlab as step three. This procedure adopts the well known “skyscrapers” method.

Skyscrapers analysis was originally suggested by Caldwell for fractal dimension calculation of digitized mammography. Pixels that constitute an image can be considered as skyscrapers, the height $z(x,y)$, represented by intensity of grey. The surface area of image A, referring to (1), is obtained by measuring the sum of top squares that represent skyscrapers roofs and the sum of exposed lateral sides of skyscrapers [7]. The roof of skyscrapers increases subsequently by adjacent pixel grouping and the intensities of grey are averaged. The square size ε is 2^n:

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

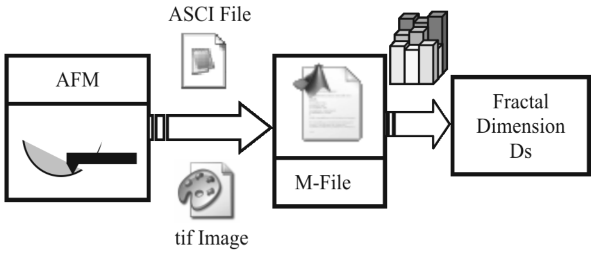
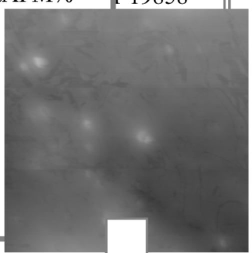


Figure 8. Scheme of data transition from AFM recording to fractal dimension calculation.

```
11512 11449 11469 11535 11625 11739
11896 11902 11881 1207  2154 12103
12108 12075 11854 117   1725 11788
11908 12154 12529 129   3096 13270
                        14336
                        15861
                        17820
                        19858
```

```
%% image from ASC file
dataAFM=load('cl005zoom.asc');
%matrix 512x512 from dataAFM%
rgbimg = zeros(512,512);
i=1;
for m = 1:512
for n = 1:512
rgbimg(m,n) = dataAFM(i);
i=i+1; end
end
img512=uint16(rgbimg);
```

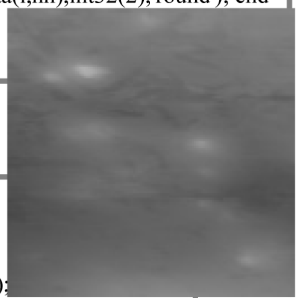


```
%% skyscrepers Area for square size 1
E=1;
%skyscrepers Area for img512 %
rightside512 = zeros(512,512);
downside512 = zeros(512,512);
ela512=zeros(512,512);
for m = 1:512
for n = 1:511
rightside512(m,n)=abs(img512(m,n)-
img512(m,n+1)); end
end
for n = 1:512
for m = 1:511
downside512(m,n)=abs(img512(m,n)-
img512(m+1,n)); end
end
for m=1:512
for n=1:512
ela512(m,n)=E^2 + E*(rightside512(m,n)
+ downside512(m,n)); end
end
area512 = sum(sum(ela512));
A(1,1)=area512;
```

Figure 9. Partial scene of ASCII file and Matlab code for skyscrapers method for left lens.

The grey-scale 16-bit image of left lens is modified for skyscrapers area calculation. In the fourth step, the surface area A was determined for square size $\varepsilon = 4$ of the left lens image referring to (1). The Matlab custom made procedure, shown in step 4 in Figure 10, results in pairs (A,ε).

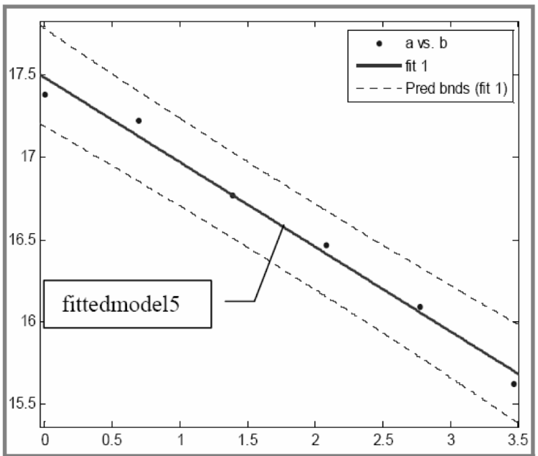
```
%% image from img256
%matrix 128x128 from matrix 256x256%
aaa = zeros(256,128); bbb = zeros(256,128);
for m = 1:256
for n = 1:2:256
i=idivide (n+2),int16(2),'floor');
aaa(m,i) = bb(m,n)+ bb(m,n+1);
bbb(m,i)= idivide(aaa(m,i),int32(2),'round'); end
end
aaaa = zeros(128,128); bbbb = zeros(128,128);
for nn = 1:128
for mm = 1:2:256
i=idivide((mm+2),int16(2),'floor');
aaaa(i,nn) = bb(mm,nn)+ bb(mm+1,nn);
bbbb(i,nn)= idivide(aaaa(i,nn),int32(2),'round'); end
end
img128=uint16(bbbb);
```



```
%% skyscrapers Area
for square size 4
E=4;
ela128=zeros(128,128);
rightside128 = zeros(128,128);
downside128 = zeros(128,128);
for m = 1:128
for n = 1:127
rightside128(m,n)=abs(img128(m,n)-
img128(m,n+1)); end
end
for n = 1:128
for m = 1:127
downside128(m,n)=abs(img128(m,n)-
img128(m+1,n)); end
end
for m=1:128
for n=1:128
ela128(m,n)=E^2 +
E*(rightside128(m,n)
+downside128(m,n)); end
end
area128 = sum(sum(ela128));
A(1,3)=area128;
```

Figure 10. Matlab code for skyscrapers method for square size $\varepsilon = 4$ for left lens.

Calculated values for left lens image area vs. square size (A,ε) are presented in a double-log graph as the fifth step. The dots are arranged along the straight line and shown in Figure 11. The custom-made procedure for fractal dimension calculation based on the "skyscrapers" method is generated using the image processing toolbox, as well as the custom-developed algorithm. The Matlab code is also shown in Figure 11.




```
%% fractal dim. calculation from loglog diagram
fitted linear slope
% area values
A=[area512,area256,area128,area64,area32,area16]
B=[1,2,4,8,16,32]; %B square size values
aCL5=log(A);
bCL5=log(B);
% Using "curve fitting tool" window for a vs.b data
set (95% confidence bounds)
% get polynominal linear fit: a=p1*(b)+p2
% Result for fitting process find in "fittedmodel5"
% Linear model Poly1:fittedmodel5(x) = p1*x + p2
% Calculation fitted values at every b=log(B) value
y=fittedmodel5.p1*b+fittedmodel5.p2;
%Plot the log-log values
%Real values in loglog plot
Y=exp(y);
figure, loglog(B,Y)
hold on
loglog(B,A,'ro')
title('loglog plot')
xlabel('B');
ylabel('A');
%fractal dimension calculation D
% slope p1=2-D
p1 =fittedmodel5.p1;
D=2-p1;
```

Figure 11. Data fitting using Matlab Curve fitting toolbox and custom made procedures for left lens.

Fractal dimension D can be generated from relation (2) for *Hausdorff–Besicovitch* dimension, where $N(\varepsilon)$ is the number of self-similar structures of linear size ε needed to cover the entire structure [3]:

$$D = \lim_{\varepsilon \to 0} \frac{\log N(\varepsilon)}{\log \frac{1}{\varepsilon}} \qquad (2)$$

Number $N(\varepsilon)$ can be represented as shown in Eq. (3):

$$N(\varepsilon) = c_1 \cdot \varepsilon^{-D} \qquad (3)$$

and used for the area *versus* square-size relationship (4):

$$A(\varepsilon) = N(\varepsilon)\varepsilon^2 \qquad (4)$$

resulting in Eq. (5):

$$A(\varepsilon) = c_1 \varepsilon^{2-Ds} \qquad (5)$$

The use of logarithmic rules on relation (5) results in a linear equation, expressed as (6):

$$\log A = (2 - Ds)\log \varepsilon + c \qquad (6)$$

Fractal dimension D is obtained from the slope, determined in step six by using relation (6) in the custom-made procedure for calculation shown in Figure 11.

The same algorithm was used for fractal dimension determination for the right lens inner surface. The double log plot for pairs (A,ε) and adequate fitted lines for left lens, label with CL5 and right lens, label with CL1, are shown in Figure 12.

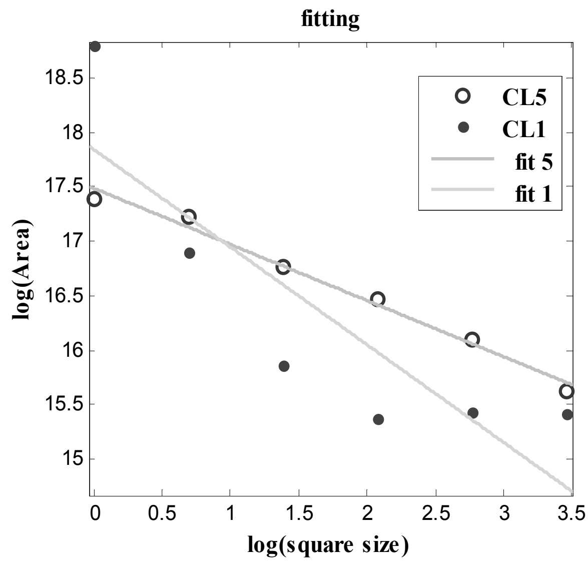


Figure 12. Log-log graph of image area vs. square size for left (CL5) and right (CL1) lens.

The fractal dimension generated by skyscrapers method for topography image shown in Figure 6, is Ds = 2.5150, for topography image shown in Figure 7 is Ds = 2.8961. The right lens inner surface has bigger fractal dimension than the left one.

This statement is in accordance with plot appearance. The steeper fitted line goes together with rougher surface.

RESULTS AND DISCUSSION

The appearance in double log plot for area and scale relationship indicates the existence of a power law between the two measures generated from the measured surface. The power law proves the fractal behaviour of the manufactured CL surface with the tear component on it.

The fractal dimension generated by the skyscrapers method for topography image offers additional and appropriate information about surface roughness. The comparison between surfaces was possible by using only one roughness parameter. Fractal analysis distinguished two contact lens surfaces using fractal dimension.

Fractal dimension, as roughness parameter, adequate explained surface functional behaviour, also [8]. Fractal dimension for new contact lens surface could be an adequate behaviour prediction parameter. That will be a topic for additional research.

CONCLUSION

Mandelbrot claimed that nature has a fractal face and scholars proved that engineering surfaces have fractal geometry. Compilations of a man-made surface with a tear component on it also show fractal behaviour, proven by power law of area versus scale relationship that is obvious in Figure 9. In this paper the “skyscrapers” method is applied for calculating the fractal dimension of such surface. The fractal dimension of RGP CL inner surface is chosen for observation, as an appropriate surface roughness parameter. Surface with fractal dimension 2.5 would be the optimum as an engineering surface for certain applications [9].

The left lens’ inner surface topography with deposits has a calculated fractal dimension Ds = 2.5150, and can be considered as appropriate for adhesion property. The right lens inner surface topography with deposits has a calculated fractal dimension Ds = 2.8961, and can be considered as too rough for adequate adhesion. This conclusion is in accordance with an ophthalmologist’s observation of low adhesion between the right inners RGP CL surface and tear film.

In this paper, in the case of worn-out right lens fractal dimension proved usefulness in surface characterization, comparison and behaviour prediction.

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ИЗВОД

ФРАКТАЛНА АНАЛИЗА БИОПОВРШИНА У ФУНКЦИЈИ ПОРЕЂЕЊА И ПРЕДИКЦИЈЕ ПОНАШАЊА У УПОТРЕБИ

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(Научни рад)

Фрактална димензија је коришћена у досадашњим истраживањима аутора, као параметар храпавости профила обрађене површине брушене плочице од алатне керамике. У раду је за прорачун фракталне димензије обрађене површине примењен «метод небодера». Наведени метод условљава снимање површине микроскопијом атомским силама и анализу слике коришћењем расположивих модула и развијених процедура у Матлаб окружењу. Поређењем вредности фракталне димензије ношеног, али и даље употребљивог са неупотребљивим (похабаним) сочивом потврђена је исправност фракталног приступа у компарацији биоповршина и предикцији њиховог понашања у употреби.

Кључне речи: Фрактали • Топографија • Површина• AFM • Слика
Key words: Fractals • Topography • Surface• AFM • Image