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Contact Lenses Characterization by AFM MFM, and OMF

Dušan Kojić, Božica Bojović, Dragomir Stamenković,
Nikola Jagodić and Đuro Koruga
University of Belgrade, Faculty of Mechanical Engineering,
Department of BioMedical Engineering
Serbia

1. Contact lense industry

The contact lens (CL) industry and market have displayed a high level of dynamism in the past few decades, and have evolved into a rapidly changing field in which science and everyday practice constantly interact, not only through broadening of material and product portfolio, but through innovative therapeutic and diagnostic solutions as well.

Stable market growth with numerous rearrangements in different product segments is constantly taking place, mainly stirred by innovative material and optical design. The standardly used hydrogel materials are being rapidly replaced by silicone doped hydrogel materials. The analyses of customer CL usage and satisfaction indicate continued market growth in future, however with many changes in product profile and significant increase in multifocal and daily disposable lenses market share.

The main impulse behind the dynamism of CL industry stems from results of scientific and technological improvements, which are enhancing medical field and reminding us that the focal point of sustainable development lies in scientific investigations.

2. Contact lenses in present, past and future

The technology of materials used in CL production has improved vastly in the past decades starting from glass and moving to polymer based materials (PMMA) with, eventually, major steps being taken in including hydrogel and doped-hydrogel based materials, shifting the functionality of CLs from rigid gas-impermeable (RGP) to soft gas-permeable materials represented by silicone hydrogel materials that are now in use.

This chapter will focus on multimodal applied research of rigid gas-permeable contact lenses (CL) that are manufactured from fluorosilicone acrylate based material. Our multimodal research comprises measurement of intermolecular interactions on the basis of optical, mechanical, morphological and magnetic properties of CL material.

The role of our research in such a complex system of CL industry was introduction of new diagnostic modalities through improved material characterization.

In the course of last decade, scientists have developed different possibilities of "on eye"CL application that are not related to its optical capabilities for which they were invented in the first place – correcting eye's refractive error. Furthermore, improvement in CL material manufacturing, both soft and rigid gas permeable, are mostly directed towards increasing oxygen permeability and wearing comfort. Rarely today, CL producers are dedicated to

improving CL material properties for the purpose of enhancing the quality of vision, on the contrary, by doping them with silicone, for example, the optical properties become even worse.

Apart from it's properties to correct eye's refractive anomaly (dioptric power), the most frequent factors influencing quality of vision while wearing RGP CL are those related to the fact that visible light, on it's way to the eye's "perception area"– the macula, must pass through CL material itself, and all it's characteristics can seriously modify it.

Geometrical optics and related functions of the eye (vision acuity etc.) should not be considered as the only one mechanism of light interaction with human organism. We also consider physical inputs that influence the functioning of the central nervous system (CNS) on the basis of optical-neuronal interactions, and point to perspectives of investigating the role of CLs in modifying the influence of light for therapeutic purposes, or using the CLs as a potential diagnostic tool in monitoring the state of other systemic parameters (such as serum glucose level etc). As a rule, when comparing "naked eye"vision with the vision aided by CL it is inevitable to conclude that vision with CLs is of lower quality, due to reduced contrast sensitivity, sub-normal color's perception, spherical and chromatic light aberrations. All these are considered mostly consequence of CL material imperfectness. The aim of our research is to organize a setup for development of a novel material for RGP CL production which should, after adequately lathe cut and polished, improve it's optical properties in transmitting visible and "near visible"light, while increasing contrast sensitivity for "on eye"usage and improving color perception with simulaneous reduction in both higher and lower order light aberrations. New, advanced CL materials, are still needed because, regarding biocompatibility and oxygen permeability, the advantages offered by high oxygen delivery have solved many hypoxia-related clinical problems, but the complications related to inflammation, infection and mechanical insult to the cornea still occur. The good news is that the industry continues to work on the next innovative materials and designs while our patients, and us, enjoy the silicone hydrogel lenses and the big step they represent toward safer and more effective extended (toward continuous) contact lens wear.

3. Physiological considerations related to contact lenses wearing

3.1 Tear layer considerations

Tear layer is constantly renewed tripartite film that covers conjunctival and corneal surface. All layers are separated:

- **Mucin layer** is the innermost and it is anchored to the corneal and conjunctival epithelial glycocalix of the microplicae.

- **Aqueous layer** is the middle one and represents 90% of the tear film thickness. It consists of water and salts dissolved in it, together with dissolved glucose, lysosime, for tear's specific pre-albumin, lactoferin and other.

- **Lipid layer** is the outermost, secreted by the Meibomian glands and it retards evaporation of the tears.

The tear layers have many different functions:

- Keeping the surface of cornea smooth and that way making it optically clear.

- During blinking tear film lubricates the friction area between lids and ocular surface.
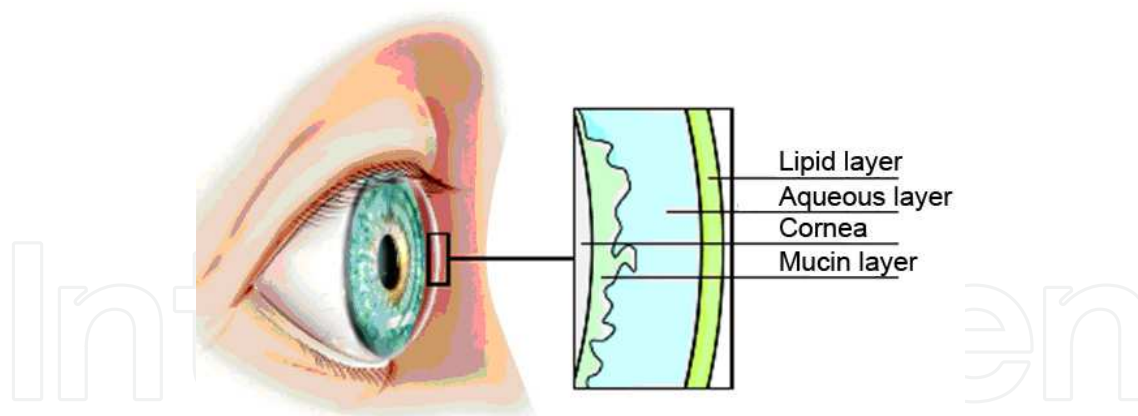
- Many of its constituents prevents ocular infection.

Fig. 1. Structure of sublayers that constitute tear layer.

Presence of any contact lens on the front surface of the eye influences physical and chemical properties of the tears and disturbs tear film stability. These changes in the healthy tear film are most likely caused by the following effects on the eye:

• Contact lens surface

• Contact lens design (mostly edges)

• Contact lens material

• Instillation of contact lens solutions with the lens

In comparison to the normal pre-ocular tear film, conventional (hydrogel) soft contact lens wearers have pre-lens tear film with all three layers of the tear film reduced. Tear film quickly deteriorates and renders poor surface wettability capability (weak attachment of ocular surface epithelium to the mucus layer). Pre-rigid gas permeable lens tear film is even thinner and more unstable then that of soft lens. Its lipid layer is often absent so the aqueous layer quickly dehydrates. In silicone-hydrogels, pre-lens tear film is something like combination of the previous two tear films although lipid layer is always present. As in the case of pre-ocular tear layer, good quality pre-lens tear film layer is *conditio sine qua non* for adequate optical properties of the eye.

Each eye consists of light receptor layer in the retina, optically active tissues that focuses the light on the receptor layer and nerve fiber system (retinal ganglion cell's fibers and optic nerve) which conducts electrical impulses created during electro-chemical reaction in the receptor layer (provoked by light absorption) all the way to the visual cortex and other parts of the brain.

Optically, eye functions very similar to photo-camera, inside the eye it is totally dark and this is provided by its outermost protection layer – the *sclera*, and pigmented parts of the middle layer – the *choroid*, which also provides most of the eye's blood supply. Light transmitting into the eye is absolutely controlled by the very agile diaphragm – the *pupil*. In order for light to be focused at the center of the eyes' reception layer it has to be refracted by *cornea*: absolutely transparent convex-concave lens and further refracted by another agile tissue in the eye - *the crystalline lens*. In order to prevent light reflection from behind the receptor layer to interfere with and disturb the primary light stimulation of the receptor cells, a pigmented cell layer is situated just behind the receptor layer and absorbs all the residual light.

Emmetropisation of the eye is a very sensitive process of dosing the eye's optical activity in relation to its axial growth. Any disturbances in this results in refractive errors of the eye

(*myopia*, *hyperopia* and *astigmatism*). Eye, just as any other optical device, is also prone to higher order light aberrations that influence the stimulation of the receptor cells therefore influencing the quality of vision.

3.2 Light perception and visual signal transmission

Eye's light receptor cells (rods and cones) have large amounts of photo-sensitive pigments (*opsin* and *retinen*). Once stimulated by visible light (397 – 723 nm) these photo-sensitive pigments are changing their structure which leads to the chain of events that are ending in nerve activity. Rods are much more sensitive to the smallest amounts of light stimulation and they are most active in the dark environment (*scotopic* conditions) when pupil (the diaphragm) is dilated in order to receive as much light as possible. On the other hand, cones are much less light-sensitive and are active only in *photopic* conditions when the pupil shrinks in order to prevent too much light entering the eye which can disturb highly sophisticated visual functioning like color vision and small detail discrimination. Light stimulation blocks Na^+/K^+ channels in the receptor cell and disturbs the balance of ions in the sense of hyper-polarization of the cell. This results in reduction of the amount of the synaptic neuro-transmitter (normally released in certain amounts without light stimulation) that triggers electrical activity in the retinal ganglion cell which is conducted to the brain. Before it reaches the optical nerve, electrical activity created in the receptor cells is changed by the activity of the modulation cells present in different retinal layers.

4. Structure-function relationship of conventional and novel CL materials

Newer, soft lenses (hydrogel) are made from polymers that are inherently flexible or may become flexible through absorption of fluid into the polymer matrix. In general, when speaking of rigid CLs, most commonly used polymers are poly(methyl methacrylate) or PMMA, polyacrylamide (PAA), cellulose acetate butyrate (CAB), and in order to make them gas-permeable various mixtures based on PMMA doped with silicone or fluorine are used. Also, many modification of PMMA based materials are also present in the market (such as poly (2-hydroxyethyl methacrylate (PHEMA), poly (2-hydroxypropyl methacrylate) (PHPMA) etc.).
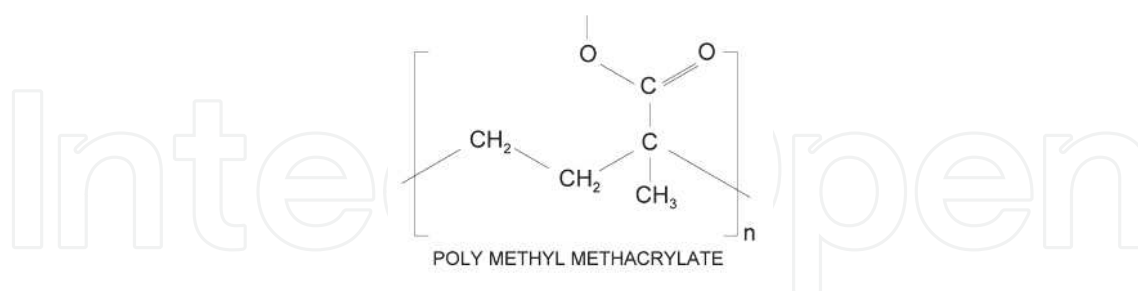

Fig. 2. Structure of poly methyl methacrylate.

The functions that contemporary contact lense materials are most often required to fulfill, encompass:

- **Dimensional stability** rendered through sufficient strenght and stiffnes – these properties are obtained by matrix of methylmethacrylate, which provides hardness and strength, and ethylene glycol dimethacrylate (EGDMA) which acts as a cross-linking agent adding to dimensional stability and stiffness but reducing water content.

- **Normoxia** rendered through material flexibility and gas permeability – these propeties are obtained by addition of several components, such as silicone (increases flexibility and gas

permeability through the material's silicon-oxygen bonds ($Si - O - Si$); however, it also brings in the disadvantage of poor wettability), fluorine (improves gas-permeability (less than Si) and improves wettability and deposit resistance in Si-containing lenses).

- **Proper adhesion** through controlled wettability – adequate level of adhesion is controlled by inclusion of hydroxyethyl-methacrylate – the basic water-absorbing monomer of most hydrogel-based soft lenses; methacrylic acid and n-vinyl pyrolidone (NVP) monomers are also added, both of which absorb high amounts of water and are usually adjuncts to hydroxyethyl methacrylate to increase lens water content.

Rigid contact lenses made of PMMA are recognized by excellent mechanical properties of dimensional stability (flexure resistance, stiffness and resistance to breakage) but practically have no oxygen permeability. Inclusion of Si and F has introduced gas-permeability properties but have compromised surface characteristics and stiffness. By chemically balancing silicone acrylate (SA) with stiff crosslinking monomers (esters) manufacturers have been able to achieve sufficieng gas-permeability without significantly compromising stiffness. Most contemporary gas-permeable lenses are composed of fluorosilicone acrylate that, due to oxygen's preference to dissolve into fluorinated materials, practically draws in the oxygen from the atmosphere and transports it towards the corneal surface, utilizing its $Si - O - Si$ component.

Hydrogel based lenses draw their advantage from the fact that hydrogels are materials that absorb and hold water inside their polymer matrices causing the spaces between the polymer chains to expand. Anything dissolved in the water can potentially enter the hydrogel matrix, depending on the molecular size and the matrix pore size. The pore size ranges from $0.5\mu m$ to $3.5\mu m$ for low and high-water content lenses, respectively. The oxygen permeability of hydrogel based contact lenses originates from their water content. Because various hydrogel polymers can greatly alter its chemical and physical properties, they may react differently to changes in pH, osmolarity, temperature and the components of the various lens care products that are used.

Silicone hydrogel based lenses use Si-doping which simultaneously decreases water content and increases permeability. Having in mind that the solubility of oxygen is very high in Si when compared with water, silicone doped hydrogel lenses permeability dramatically increases with lowering water content – which is a logical step towards improving functionality of CL materials. However optimal transport conditions for water and ion through a silicone lens, require an adequate amount of water. Synthesizing Si-hydrogel lenses is challenging since involves mixing non-polar (oxygen-rich silicone) and polar component (water) to produce lenses that renders high oxygen permeability, good wettability, flexibility, good optics, reasonable lens movement on the eye and general biocompatibility.

4.1 Conventional materials used in our investigation

We used two types of CL materials: gas-permeable CLs that are manufactured from fluorosilicone acrylate based material (Soleko $SP40^{TM}$) and standard non-doped PMMA CLs. The aim was to test the response of this material's surface roughness quality on the nano-level using standard nanotechnological methods and new nano-photonic method.

5. Characterization of contact lenses

Introduction of effective improvements in production quality as well as therapy efficiency requires the application of non-destructive surface analysis methods on the nanometer scale with minimal sample preparation. Many interesting studies have been performed on CL (Lim et al., 2001), (Kim et al., 2002), (Bruinsma et al., 2003), so that two research approaches can

be perceived from the literature: one aimed at surface modification and the other that was concerned with varying or simulating exploitation parameters. In both cases morphology of CL surface served as the main parameter used to describe behavior and quality of CL material under deterioration influences.

Production engineering aspects. Conformation states of polymers constituting CL surface are changed during final stages of manufacturing process (polishing) which presents a complex problem because surface molecules and their orientation influence the final state of surface quality. In general, over-polished internal surface renders a dysfunctional product since it prevents adequate adherence to corneal tissue. CL with over-polished surface slides over the cornea and cannot maintain its initial position therefore disrupting the geometry and function of the optical system (human eye + CL). However, too rough a surface will eventually lead to irritation and possibly damage of corneal tissue.

Exploitation aspects. During CL exploitation a lacrymal film is formed between inner CL surface and cornea. A good fit of CL and cornea depends on the adequacy of CL geometry and surface roughness. Moreover, every single RGP CL wearer provides a unique ambient conditions in which these CL biosurfaces have to function properly. 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 needs to be investigated and related to measurable parameters in order to recommend replacement based on significant changes in surface properties.

5.1 Multimodal, complementary and non-invasive approach

Answering the questions of optimal processing parameters directly influences surface quality of CLs which in turn reaches all aspects of its functionality: optical, medical and patient-comfort. Since our measurement confirmed that surface roughness of end-product ranges in the order of several tens of nanometers, the adequate tools for such samples must be derived from applied nanotechnology.

Experimental design, aimed at quantifying surface quality on the level of nano scale, utilized three methods of characterization that are meant to complement each other in interpreting and quantifying the measurement results. Moreover, we have applied a new method that is concerned with obtaining magnetic properties of samples from the interaction of material with visible light, named Opto-Magnetic Fingerprint of matter (OMF), with the aim to improve the speed and accuracy of analysis. Testing the product quality, reliably and accurately, in production environment is an essential characteristic of maintaining high level of product quality control.

We have chosen to investigate near-surface magnetic and optical properties of chosen CL materials since they are the physical quantities that most closely correlate with subtle modifications in material structure and composition. Therefore, we investigated five types of CL materials (two conventional and three nanophotonic materials) by selected three approaches (techniques):

1. **Classical methods**: spectroscopic examination in the region of ultraviolet and visible light (UV-Vis spectroscoscopy).

2. **Nanotechnology based methods**: Phase-Contrasted Atomic Force Microscopy (PC-AFM) with extended mode of Magnetic Force Microscopy (MFM).

3. **Nanomedicine based methods**: Novel method named Opto-Magnetic Fingerprint (OMF) that obtains magnetic properties of materials on the basis of interaction with visible light.

The experiment was designed to provide proofs for three interrelated phenomena:

- Change in topographical and conformation states of surface and polymer via PC-AFM,

- Related change in nanomagnetic behavior of near surface layers via MFM,

- The change of paramagnetic/diamagnetic and optomagnetic state that is recorded by OMF.

All three methods possess very high sensitivity (nanonewton for forces, nanotesla for magnetization), which is a necessity for this type of measurements.

5.2 Ultraviolet-visible spectroscopy
In order to investigate optical properties of RGP CLs, we have performed spectroscopic measurements in the range of ultraviolet-visible (UV-Vis) light in the range of wavelengths between 280–800 nm, using the UV-Vis scanner produced by Horiba JobinYvon, USA. The measurements were conducted in diffuse reflectance regime and the result is displayed as a graph of reflected energy vs. wavelength of emitted radiation. The spectroscopic measurements are used as a reference guide and were performed on two locations: the central point on the outer surface and the same point measured from inner surface in order to determine the difference and hence the degree of eye protection to different ranges of wavelengths and radiation intensities.

5.3 Phase-contrasted atomic force microscopy – PC-AFM
Morphology of CL surfaces was obtained by atomic force microscope (PC-AFM) that can measure sample surface roughness with high precision (less than $10^{-12}m$ or $\approx 1pm$ and confirm sample surface state as belonging to either group adequate or inadequate roughness. Topography of lens surface is important for determining the connection between surface morphology (conformational states of surface layer polymers) and corresponding optical properties that are influenced by the processing parameters.

Basically, AFM is a scanning probe microscopy technique based on point-to-point examination of the specimen made by a sharpened tip probe (Binnig et al., 1986). AFM probe is a micro-cantilever with sharpened conical or pyramidal tip whose radius can range from $2 - 90$ nm, depending on the application. All samples were imaged using phase-contrast technique in tapping mode and in ambient air. The AFM system used in this study was JSPM-5200, JEOL, Japan. The cantilever were type PPP-MFMR, produced by Nanosensors, Switzerland.

The principle of operation of AFM is shown in figure 3. In AFM, the probe (cantilever tip) is vibrated at near-resonant frequency and brought ino interaction with the sample by the mechanism of intermolecular attractive/repulsive forces that are distance-dependent. The cantilever is maintained in the close proximity from the sample so that probe tip is within reach of attractive/repulsive forces. A typical AFM system is able to detect intermolecular forces in the order of $10^{-11} - 10^{-13}N$ which makes it an extremely sensitive device. The intermolecular interactions belong to the class of van der Waals type forces, usually modeled by Lennard-Jones potentials. These forces cause the cantilever to deflect from the initial equilibrium position making it possible to derive the distance from the sample on the basis of force field gradient change that modulates the vibration of the cantilever.

A diode laser is directed to the back sided surface of the tip and is reflected to photo-diode detector. During the scanning movement, the angle of reflected beam is changed due to deflection of the cantilever (that is, in turn, due to interactions with the sample) and this movement is precisely recorded via photo-diode detector. A feedback loop is used to adjust the z-position of the sample so that a constant interaction is maintained as the tip is scanned across the sample in x and y directions. The tip-sample interaction equilibrium is constantly disrupted by changes in sample profile height which generates the control signal applied
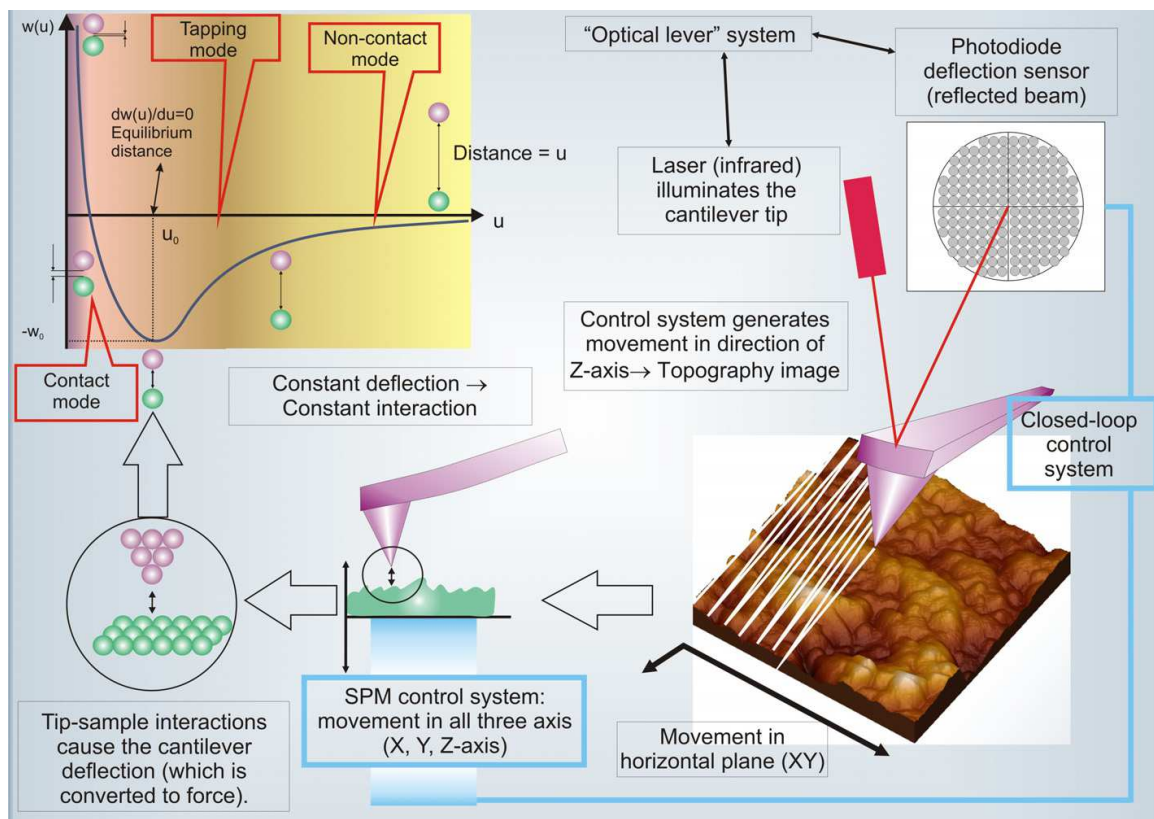
Fig. 3. Principle of operation of atomic force microscope.

to the z-scanner stage. This disturbance represents the changes in surface topography and the action of the control system is recorded and represents the actual data represented on topography image of the sample.

The PC-AFM imaging technique is based on the fact that intermolecular force gradients have certain physico-chemical specificity. The variations in the sample generate variations in the slopes of Lennard-Jones potential curves because of the different intermolecular forces acting on the AFM sensor tip. These differences modulate the vibration amplitude of the AFM cantilever, creating a higher-harmonics in the feedback signal of AFM system. By recording higher harmonics in the oscillation signal (see 3) we can detect different force-distance relationships, hence, different components of a material can be visualized. However, the PC-AFM signal correlation to separate chemical species is still unknown, which disables the AFM from making exact chemical characterization of the sample. The data obtained by PC-AFM are rather of a qualitative nature and are suitable for two or three component systems.

5.4 Magnetic Force Microscopy – MFM

Magnetic Force Microscopy is an extended operation mode of AFM that was used to obtain the distribution of magnetic properties of the surface that are previously imaged with topography mode. This tecnique was used to measure the magnetic properties in para- and diamagnetic range because we are also probing the magnetic properties in the same range with a novel technique that is introduced in the next section (Opto-Magnetic Fingerprint). MFM will is used as a comparative method for OMF. The MFM technique utilizes special cantilever sensors that are coated with a thin film of cobalt ($\approx 50nm$) that renders its ferromagnetic properties and ensures magnetic interaction with the sample.
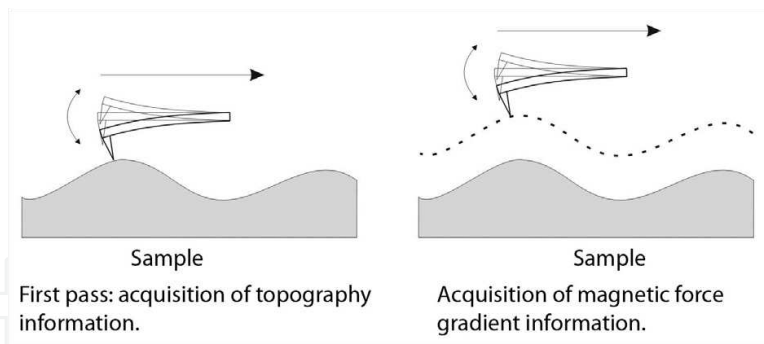
Fig. 4. Principle of operation of atomic force microscope.

This thin film creates magnetic interaction with the sample that is recorded via two-pass technique or "lift scanning"(see 4) during which the sample is scanned twice, with second scan being performed with a gap distance in order to physically filter the slowly decaying magnetic from all other more rapidly decaying intermolecular forces. The sensitivity of magnetic force measurements goes around $10^{-11} N$ which is able to detect very weak magnetic interaction that encompasses para- and diamagnetic range. The cantilevers which we used in this study are produced by Nanosensors (Switzerland), with a lateral resolution under 50 nm and coercivity of 300 Oe.

The cantilever has been magnetized previously to the measurement in order to ensure its magnetic interaction with the sample. Although MFM, in its current stage of development, still represents a qualitative measurement of magnetic properties, we attempted to improve the accuracy of this analysis by measuring the remnant magnetization of the cantilever, using JR-5 spinner magnetometer (AGICO, Brno, Czech Republic), and identified that our series of measurements fitted within the range of $72 \pm 2nT$, with a standard deviation of 0.3%. The measured axis of cantilever remnant magnetization was positioned in the vertical direction, so it was perpendicular to sample surface. The data on components of vectors of remnant magnetization enabled us to confirm that we have measured the magnetic field component that is perpendicular to the sample surface.

5.5 Opto-Magnetic Fingerprint – OMF

OptoMagnetic Fingerprint (OMF) is a novel method in investigating optical and magnetic properties of materials that is based on electron properties of matter (covalent bonds, hydrogen bonds, ion-electron interaction, van der Walls interaction) and its interaction with light (Koruga et al., 2008). The method was originally developed for early skin cancer and melanoma detection by MySkin, Inc., USA (Bandić et al., 2002). Bearing in mind that the orbital velocity of valence electron in atoms is about $10^6 m/s$, this gives the ratio between magnetic force (F_M) and electrical force (F_E) of matter, of around $\frac{F_M}{F_E} \approx 10^{-4}$. Since force (F) is directly related to quantum action – Planck's action – defined as:

$$h = F \times d \times t = 6.626 \times 10^{-34} Js \qquad (1)$$

where F is force, d is displacement and t is time of action) this means that the action of magnetic forces is four orders of magnitude closer to quantum action than the electrical ones. Since quantum state of matter is primarily responsible for conformational changes on the molecular level, this means that detecting differences between matter states is by far more likely to give greater sensitivity on the level of magnetic forces than it would be on the level of measurement of electrical forces (Koruga et al., 2002).

Picture of surface that is taken by classical optical microscope is based on electromagnetic property of light, while OMF is based on difference between diffuse white light (like that of daily light) and reflected polarized light. Reflected polarized light is produced when source of diffuse light irradiates the surface of matter under certain angle (Brewster's angle, see figure 5). Each type of matter has different angle value of light polarization. Since reflected polarized light contains electrical component of light-matter interaction, taking the difference between white light (electromagnetic) and reflected polarized light (electrical) yields magnetic properties of matter based on light-matter interaction. Because such measurement can identify the conformational state and change in tissue on molecular level we named this method the opto-magnetic fingerprint of matter (OMF).
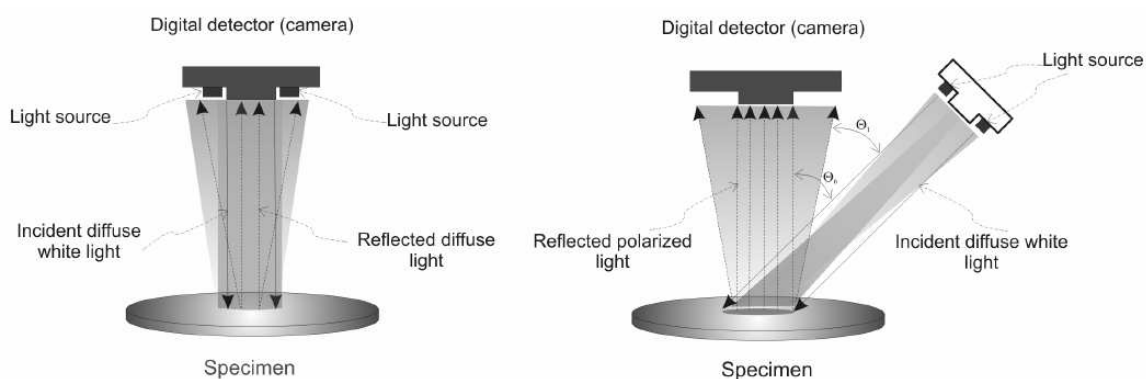
Fig. 5. Incident white light can give different information about thin layer of matter (surface) properties of sample depending on the angle of light incidence. When the incident white light is diffuse, the reflected white light is then composed of electrical and magnetic components, whereas diffuse incident light that is inclined under certain angle will produce reflected light which contains only electrical component of light. For each type of matter there is a characteristic angle of incidence (Bandić et al., 2002) for obtaining the appropriate reflected polarized light.

We used digital images in RGB (R-red, G-green, B-blue) system in our analysis, therefore we chose basic pixel data in red and blue channels for white diffuse light (W) and reflected polarized white light (P). Algorithm for data analysis is based on chromaticity diagram called "Maxwell's triangle"and spectral convolution operation according to ratio of (R-B)& (W-P) (Koruga et al., 2008). The abbreviated designation means that Red minus Blue wavelength of White light and reflected Polarized light are used in spectral convolution algorithm to calculate data for opto-magnetic fingerprint of matter. Therefore, method and algorithm for creating a unique spectral fingerprint are based on the convolution of RGB color channel spectral plots generated from digital images that have captured single and multi-wavelength light-matter interaction (Koruga et al., 2008). Preparation of digital pictures for OMF was made by usage of dermoscopic imaging device (MySkin, USA) that has previously been successfully used in biophysical skin characterization (skin photo type, moisture, conductivity, etc) (Bandić et al., 2002).

The final purpose of our research in applying OMF is the construction of quality control method which would be purely optical and able to, on the basis of digital image analysis and processing, detect both the morphology and functionality parameters in a quicker and more accurate manner. In order to do so we need to construct quantification parameters but, in this stage of research, primarily integrate results from morphological research and opto-magnetic properties.

6. Results

6.1 Topography measurement using Atomic Force Microscope – AFM

Topography measurements were routinely conducted in tapping mode in ambient air using uniform scanning surface of $5 \times 5\mu m$. The CL inner surface has been examined as shown in figure 6. The curvature of the surface prevents the AFM probe to reach its center, unless the sample is destroyed. Nevertheless, a good approach was able to the area that is approximately on the half distance of CLs radii. A total of four points were selected for scanning on each CL in two perpendicular directions. In each point two scans were conducted: one with $60 \times 60\mu m$ surface size and the other with the $5 \times 5\mu m$ surface size. The purpose of larger area scans were to confirm the uniform character of surface morphology while smaller scans were further analysed by fractal analysis.
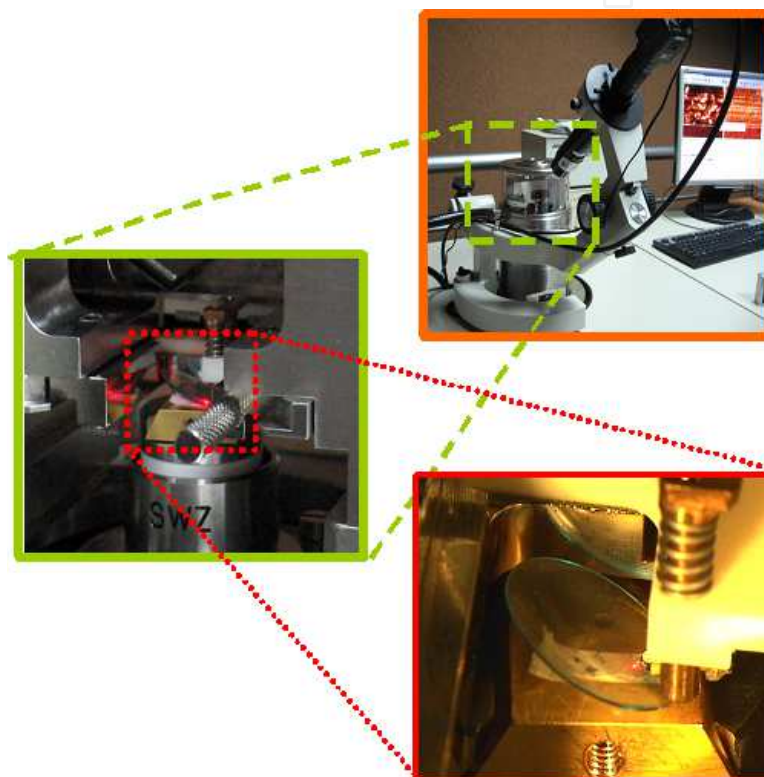
Fig. 6. Experimental setup used in AFM imaging of CL surfaces.

6.2 Phase-contrasted AFM

Phase contrast images are combined with topography images since these two measurements are performed simultaneously. Topography image yields information about surface shape and relative positions and dimensions. Phase contrast image enrichens that information by differentiating between different force gradients which in turn point to different conformational states of polymers, under the assumption that material constituents are homogenously distributed throughout the sample. Phase-Contrasted image of the CL is shown on figure 7.

6.3 Magnetic force microscopy

The AFM/MFM measurements display the features of surface morphology and magnetism on the nanometer scale and are shown on two images in figure 8. Since the sensitivity of
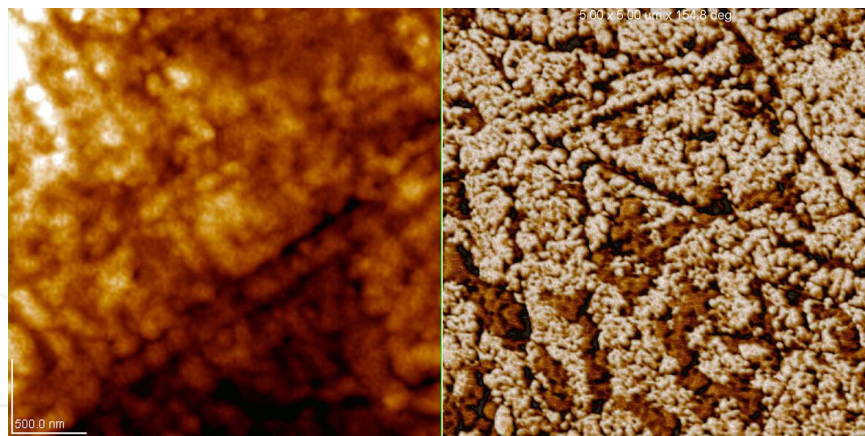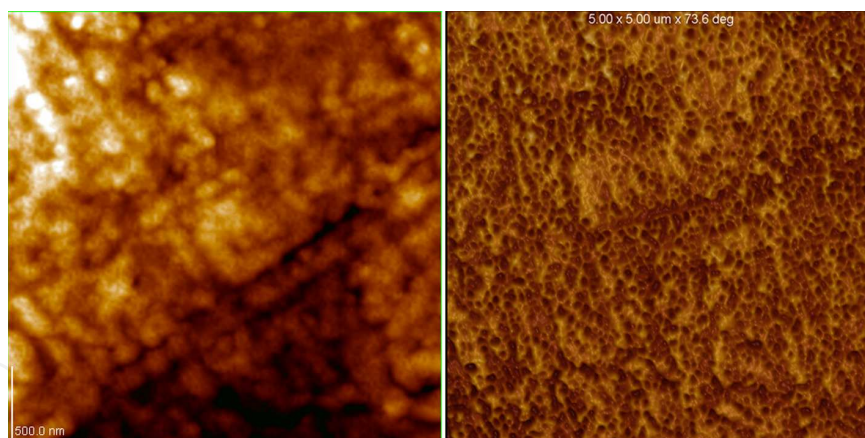
Fig. 7. Phase-Contrasted Atomic Force Microscopy: scan size is $5 \times 5\mu m$. Left: topography image with maximum profile height of 150.9 nm. Right: phase contrasted image for the same portion of the sample that is shown on the left image. Phase contrast image shows granular inhomogeneity in the sample that is synthesized as homogenous (on the nano-scale). The inhomogeneities may originate only as a consequence of processing, showing that certain parts of surface have polymers with altered conformation states, thereby expressing different slope of intermolecular interactions.

forces measured by cantilever go well below nanonewton range, this means that we are able to record paramagnetic and diamagnetic behavior of material. The brighter image areas mark more highly responsive magnetic behavior or paramagnetic areas while than darker areas correspond to diamagnetic areas of the sample.

Fig. 8. Magnetic Force Microscopy: scan size is $5 \times 5\mu m$. Left: topography image with maximum profile height of 150.9 nm. Right: the magnetic force gradient image of the same area. This image shows changes in magnetic force gradient, exhibiting that magnetic behaviour exist on the para- and diamagnetic levels and that they are inhomogenous in our samples (that are chemically homogenous).

6.4 Opto-magnetic fingerprint and UV-vis spectroscopy
Digital images of contact lenses were analyzed in terms of their separate color channels (red, blue and green color components) and subsequently processed by spectral convolution algorithm to give the final result – OMF diagram – which shows the intensity values of paramagnetic (positive) and diamagnetic (negative), properties in comparison to wavelength

difference. The diagrams on figure 9 show comparison of OMF diagrams with classical UV-Vis-NIR for two samples with different surface qualities.
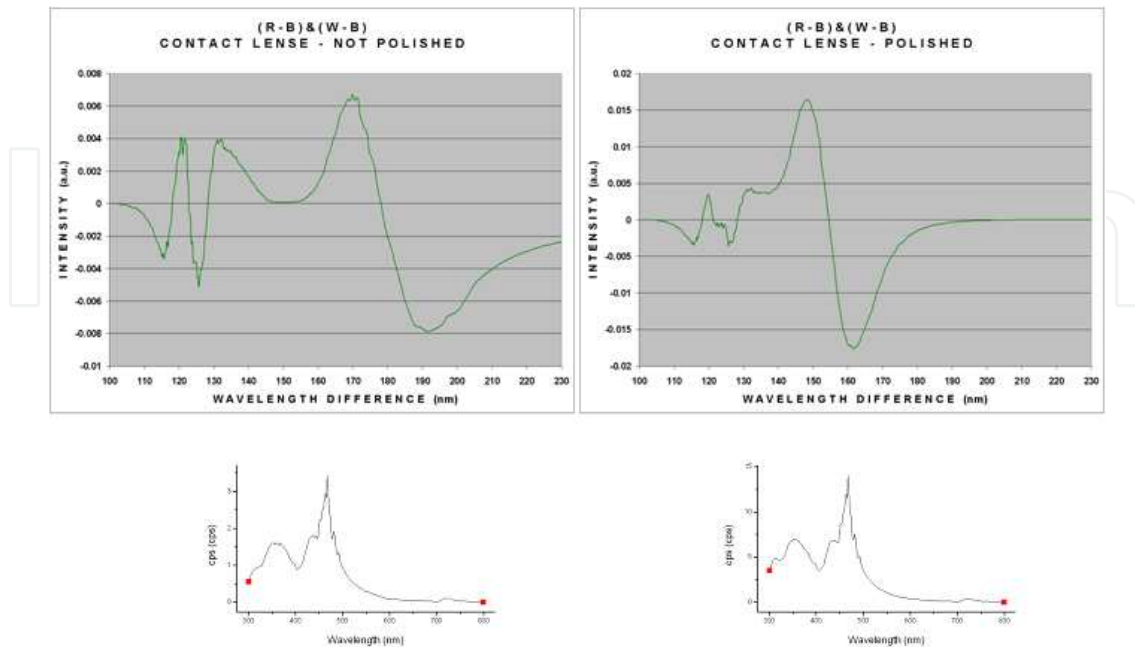

Fig. 9. Opto-Magnetic Fingerprint: comparisons of OMF (upper two diagrams) and UV-Vis spectras (lower two diagrams) for differently surface-treated contact lenses that were produced from the same, standard, material. The characteristic diagrams for lathe processed (left) and polished (right) CL surfaces. UV-Vis spectras show almost identical patterns of absorption while OMF diagrams show visible differences in the wavelength difference range between 100 and 150 nm. These differences are due to different processing and can be quantized by ratio of paramagnetic to diamagnetic properties.

The OMF diagrams show the distribution of energies of reflected light that are distributed over a wavelength difference range. We can observe a qualitatively same pattern, except in the subregion between 100–150 nm, which shows variations in wavelengths and intensities. These diagrams show very high sensitivity of OMF imaging and software analysis, which is the exact purpose this method was intended to achieve.
Moreover, we have tested the OMF response for two inner surfaces of RGP CLs of type $Boston^{TM}$ with respect to changes in the surface qualities. One pair of lenses was processed differently (one was while the other was not polished) while the other pair was processed identically (same polishing times).

6.5 Fractal analysis of contact lenses' roughness profiles
Currently we have no clear answer to the question what surface standard parameter should be used for critical limit determination. The result of the study, reported in (Bruinsma et al., 2003), also confirms the need for quantitatively establishing the replacement schedule of RGP CL. The water contact angle, percentage of elemental surface composition and deposit rate of bacteria was related to standard average roughness parameter R_a. It was stated in (Kim et al., 2002) that surface roughness was the most influential lens surface property after 10 days of wear.
Our research has utilized fractal analysis of roughness profiles to quantify the texture properties of CL inner surface. Standard surface parameters (roughness descriptors) fails
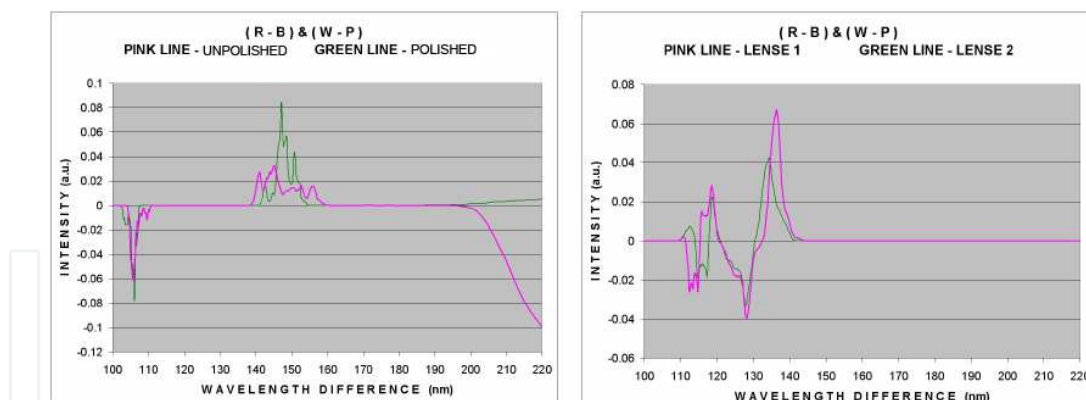
Fig. 10. Opto-Magnetic Fingerprint diagrams. Image on the left: overlapped OMF diagrams of two differently processed CLs that show different behaviour in the wavelength difference range of 130–150nm and sensitivity to changes in surface qualities. Image on the right: overlapped OMF diagrams of two identically processed CLs that show minor change in diagrams and surface qualities.

to describe functional nature of the surface. Moreover, use of more than one roughness parameter exhibits more shortcomings. This is mainly due to the partial information contained in each descriptor.

Fractal analysis of biomedical surface topography 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 is based on "slit-island"and "skyscrapers"methods that were proposed in (Bojović, 2008). This method stipulates that surface recording data is obtained as an image, by using scanning probe microscope. Fractal analysis consists of the following steps:

1. Conversion of AFM image to numerical data in the form of matrix with subsequent conversion of matrix with 256 levels to matrixs with 2^{16} levels of intensity $[0, 65535]$ needed for further calculations.

2. Calculation of image surface area by well known method called "skyscrapers"method. This method approximates surface area of image A with sum of top squares that represent skyscrapers' roofs and the sum of exposed lateral sides of skyscrapers, according to (Chappard et al., 1998). The roof of skyscrapers are increased subsequently by grouping of adjacent pixel grouping. Thus, the intensities of grey scale are averaged. The square size ε is 2^n and the formula is as follows:

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

3. According to (Bojović, 2008), the fractal dimension D can be generated from relation 3 for Hausdorff-Besicovitch dimension where $N(\varepsilon)$ is the number of self-similar structures of linear size ε needed to cover the entire structure. Number $N(\varepsilon)$ can be represented as shown in 4 and used for the area vs. square size relationship 5 resulting in equation 6. Using of logarithmic rules on relation 6 result in a linear equation, expressed as 7. Fractal dimension D is obtained as the slope of fitted line, determined by using relation 7 in the

custom-made procedure for calculation 11.

$$D = \lim_{\varepsilon \to \infty} \frac{\log N(\varepsilon)}{\log \frac{1}{\varepsilon}} \qquad (3)$$

$$N(\varepsilon) = c_1 \varepsilon^{-D} \qquad (4)$$

$$A(\varepsilon) = N(\varepsilon)\varepsilon^2 \qquad (5)$$

$$A(\varepsilon) = c_1 \varepsilon^{2-D_s} \qquad (6)$$

$$\log A = (2 - D_s)\log \varepsilon + c \qquad (7)$$

4. The log-log graph of surface vs. square size ε is then produced and line is fitted for each image (see figure 11).

5. Finally the fractal dimension is generated by skyscrapers method for AFM topography image as slope of the fitted line. This slope is steeper for rougher contact lens surface.
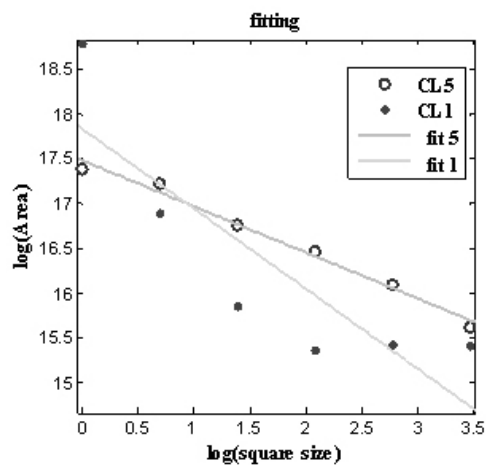
Fig. 11. The log-log graph of image area vs. square size for two CLs. Samples are two RGP CLs made of ML 92 Siflufocon A. The first lens (CL5) was worn for about 3 years while the second lens was worn over a period of more than 5 years (CL1). We can see that fractal dimensions can easily distinguish between two levels of surface roughness created by wear of CL surface.

The procedure is schematically presented in the figure 12 .

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 vs. scale relationship. According to (Russ, 1998) a surface with fractal dimension 2.5 would be the optimum as an engineering surface for certain applications.

The fractal dimension generated by skyscrapers method for topography image offers additional and appropriate information about surface roughness. Fractal dimension, as roughness parameter, adequately explains surface functional behaviour. Fractal dimension for new contact lens surface could be an adequate behaviour prediction parameter.

7. Discussion

By performing UV-Vis spectroscopy we have shown that UV-Vis spectra do not change with respect to changes in surface quality (see figure 9) because they measure bulk response of
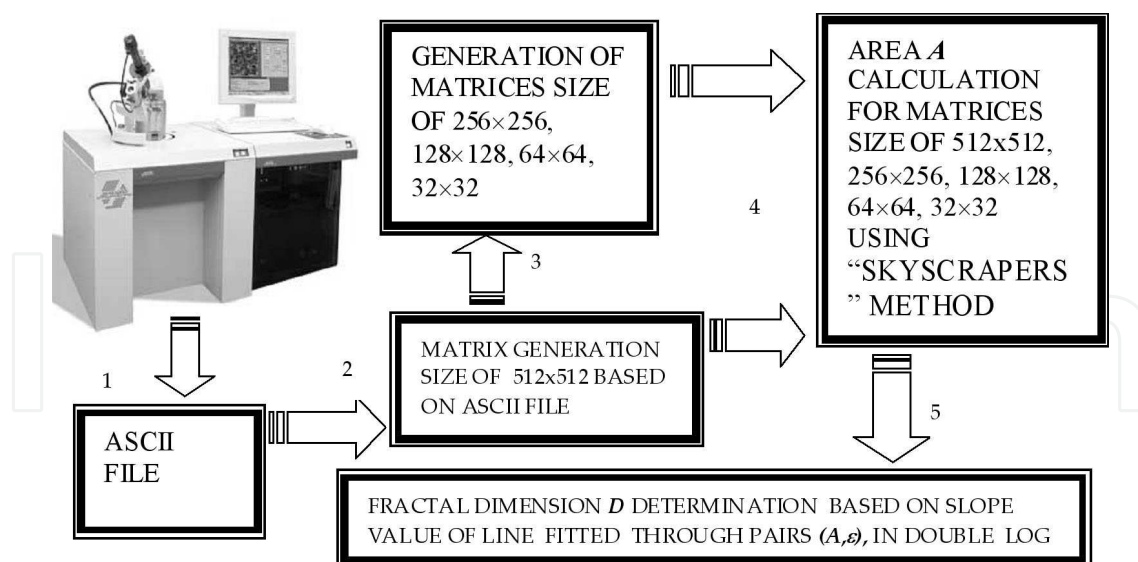
Fig. 12. Diagram of steps involved in fractal analysis of AFM scans.

contact lense materials while opto-magnetic diagrams (OMF) displayed informations that are more sensitive to changes in near-surface properties of material (which are primarily altered during contact lense production). OMF has shown as method that can detect very sensitive to differences in topographical features of contact lenses.

Since, conformation changes in near-surface polymer molecules generate quantum effects they might influence magnetic properties as well. Because of that, we have investigated near-surface regions of contact lenses samples from magnetic and optomagnetic point of view to see whether is there exist a measurable difference in surface magnetic and optical properties. Our aim was to explore the relationship between surface morphology on one side and optical and magnetic properties on the other side.

Measurements on the nano-scale have shown that phase-contrasted atomic force microscopy (PC AFM) and magnetic force microscopy imaging carry additional information that is not contained in morphology scans. However, PC AFM and MFM data need to be integrated with results of OMF in order to obtain quick quantitative assessment of changes in nano- and pico-magnetism that can be related to change in surface structure and its optical properties. Elucidating the origin of these kinds of surface behavior requires further investigations and inclusion of other polishing process parameters on one side and quantitative MFM measurements on the other side. It is our opinion that this kind of analysis will be able to precisely determine parameters of final shape and performance of CL surface.

Since conformation states of RGP CL surface determine paramagnetic diamagnetic properties that can be detected by novel OMF technique, we consider the OMF method and molecular level approach to investigation of optical properties of CL quality as a very promising field for both basic research and technology, with direct influence on application in biomedicine.

New methods of investigation require novel data processing techniques. Fractal analysis has offered more sophisticated tool that, on the basis of nano-scale precision information, generates finer estimate of surface roughness quality.

8. Conclusion

Light has influence on brain activity with very complex pathway (see figure 13). Since light is composed of electrical and magnetic spectra it is very important to know how light interact with contact lenses. These aspects of brain functioning have been thoroughly investigated;

vast amount of informations is available but the most subtle biophysical aspects are still not completely understood.
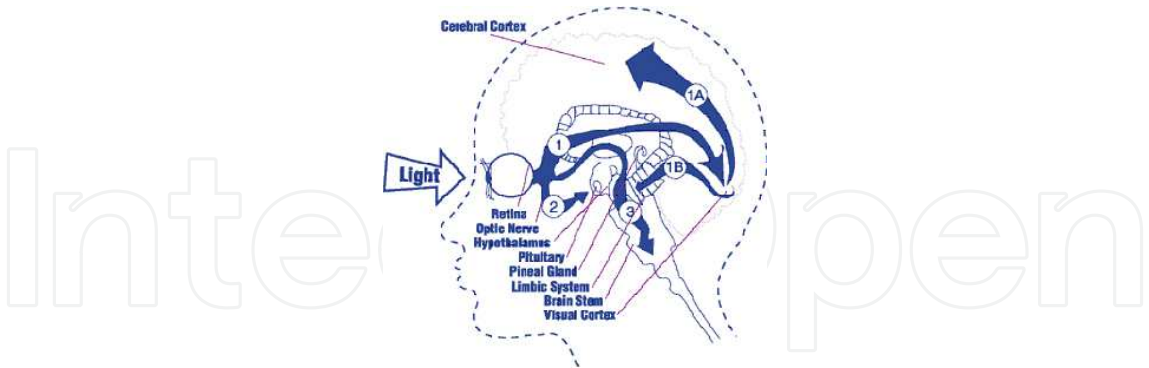

Fig. 13. Nerve pathways from the eyes to the brain goes not only to the visual cortex, but also to deeper brain areas, concerned with neurotransmiters, neurohormones, emotions, etc.

Visual perception is the ability to interpret information from information contained in visible light that reaches the eye. The act of seeing starts when the lens of the eye focuses an image of its surroundings onto a light-sensitive membrane in the back of the eye, called the retina. Since visible light is composed of electrical and magnetic spectra, which have different influence on brain activity (EEG and MEG signals, see figure 14), we investigated magnetic property of contact lenses, as optical material, which have influence on electrical and magnetic light signals properties.
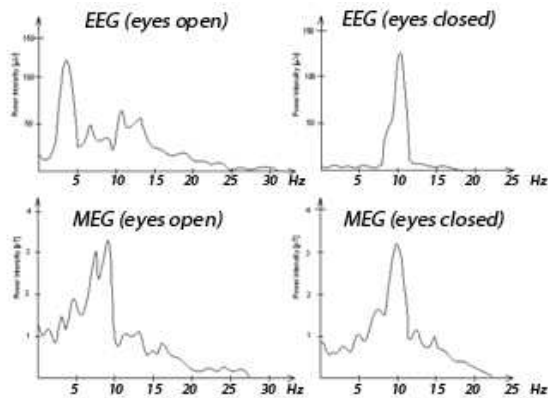
Fig. 14. Brain activity (EEG and MEG) under light influence when eyes are open and closed.

The findings of our measurements enable us to couple optical and magnetic behavior analysis in determination of mechanical properties of surfaces. The dynamical structure of CL materials and its behavior under dynamical mechanical and thermal load is expressed in changes in paramagnetic/diamagnetic behavior. Our results show that these changes are measurable and can be quantified by a simple, quick and accurate method of OMF.
We believe that this combination of intertwining methods could yield an optimal approach towards investigating phenomena in material synthesis and its behavior under mechanical and thermal stress that is still not well understood. The grounds for our propositions are proven relationships between optical, magnetic and mechanical properties of matter. It is our intention to further improve the application of all three used methods and customize their parameters in order to combine them into a new device for opto-magneto-spectroscopic characterization of matter.

Furthermore, our future research will involve nanomaterials as a new doping material influencing CL physical properties such as light transmission, these changes will be investigated by by UV-Vis-NIR spectroscopy as well as optomagnetism. The potential application of nanomaterials might bring significant biophysically based implications for contact lenses industry, biomedical application industry and applied optical science.

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Biomedical Science, Engineering and Technology
Edited by Prof. Dhanjoo N. Ghista

ISBN 978-953-307-471-9
Hard cover, 902 pages
Publisher InTech
Published online 20, January, 2012
Published in print edition January, 2012

This innovative book integrates the disciplines of biomedical science, biomedical engineering, biotechnology, physiological engineering, and hospital management technology. Herein, Biomedical science covers topics on disease pathways, models and treatment mechanisms, and the roles of red palm oil and phytomedicinal plants in reducing HIV and diabetes complications by enhancing antioxidant activity. Biomedical engineering coves topics of biomaterials (biodegradable polymers and magnetic nanomaterials), coronary stents, contact lenses, modelling of flows through tubes of varying cross-section, heart rate variability analysis of diabetic neuropathy, and EEG analysis in brain function assessment. Biotechnology covers the topics of hydrophobic interaction chromatography, protein scaffolds engineering, liposomes for construction of vaccines, induced pluripotent stem cells to fix genetic diseases by regenerative approaches, polymeric drug conjugates for improving the efficacy of anticancer drugs, and genetic modification of animals for agricultural use. Physiological engineering deals with mathematical modelling of physiological (cardiac, lung ventilation, glucose regulation) systems and formulation of indices for medical assessment (such as cardiac contractility, lung disease status, and diabetes risk). Finally, Hospital management science and technology involves the application of both biomedical engineering and industrial engineering for cost-effective operation of a hospital.

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