The infrared optical absorption spectra of the functionalized nanocrystalline diamond surface

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Abstract

We propose a new method of detecting the functional groups at the NCD surface based on the interference-free infrared reflection absorption spectroscopy of p-polarized IR light at Brewster’s angle of incidence (BA-IRRAS). We report IR absorbance spectra of a linker molecule monolayer (10-undecenoic acid) covalently bonded to the NCD surface with and without DNA fragments coupled to it, and IR spectra of organosilane polymer coatings deposited on NCD surface by spin coating technology. The homogeneity of the surface coatings was monitored by fluorescence microscopy.

1. Introduction

Low cost insulating (nominally undoped) and semiconducting (boron-doped) nanocrystalline diamond (NCD) films deposited on a large area substrates offer the unique mechanical, electrical and optical properties, as well as biocompatibility, chemical inertness, high affinity for covalent bonding with specific organic molecules and the ability to make the substrate electrically insulating or conducting [1,2]. The biomolecules can be covalently immobilized on the diamond surface via suitable linker molecules [3]. The main role of the linker molecules is to provide a high density of docking sites (the functional groups such as hydroxyl (–OH), carbonyl (–C=O), carboxyl (–COOH) or amino-group (–NH3)) for the specific attachment of biomolecules, and, at the same time, to maintain a sufficiently low density of electronic defects at the semiconductor surface [4]. This makes the functionalized NCD-coated substrates attractive for applications such as cells growth [5], DNA immobilization [6] or label-free, in-vitro, real-time, diamond-based DNA sensors [7].

Depending on the application and the desired physical and chemical properties of the surface, different strategies for NCD functionalization have been demonstrated. The experiments by Wenmackers et al. [8,6] demonstrated the repeated hybridization and denaturation after covalent immobilization of DNA on photochemically functionalized, hydrogenated NCD surfaces. Alternatively, the NCD surface can also be functionalized with several nm thick organosilane polymers [9,10,11]. In the organosilane polymer coatings a trivalent silane headgroup participates in multidirectional and interchangeable intra- and interlayer modes of coupling, both covalent and non-covalent (hydrogen bonds). Polymerization appears via rearranging –Si–O–Si– and –Si–OH moieties into a 3D network [12].

The IR absorbance spectra provide quantification of surface monolayers and thin coatings and direct evidence for the appearance and disappearance of the specific functional groups in the course of a chemical reaction. However, multireflections in a transparent film such as NCD introduce interference fringes in the IR spectra, making the identification of functional groups difficult. We propose a new method of detecting the functional groups at the NCD surface based on the interference-free infrared reflection absorption spectroscopy of p-polarized IR light at Brewster’s angle of incidence (BA-IRRAS). We report IR absorbance spectra of a linker molecule monolayer (10-undecenoic acid, UA) covalently bonded to the NCD surface with and without DNA fragments coupled to it, and IR spectra of ultra-thin organosilane polymer coatings deposited on NCD surface by spin coating technology.

2. Experimental

The optically smooth NCD films of size about 10×10 mm² and 200 nm thick, were deposited in 2.45 GHz plasma chemical vapor deposition (CVD) reactors on the one side polished Si wafer at the Institute of Physics of the ASCR, v.v.i., Prague, Czech Rep. [13,14] and at the Institute for Materials Research (IMO), Hasselt University, Belgium [15,16]. Prior to CVD growth, the Si substrates were seeded on the polished side by ultradispersed detonation nanodiamond powder yielding a very dense adsorbed diamond nanoparticle layer without damaging the polished Si surface.

Onto NCD samples prepared at IMO (#1, #2, #3), organic linkers and short DNA fragments were covalently coupled, according to the

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method in [16]. First, all samples were cleaned in boiling mixture of H2SO4 with KNO3, rinsed several times in ultra-pure water in ultrasonic bath and on hot plate in turn, and hydrogenated in H-plasma. Second, the organic linker 10-undecenoic acid (UA) was covalently coupled to the surface, using photochemistry. Inside a nitrogen flushed glovebox, a droplet of 100 µl UA (C11H20O2 99%, Acros Organics, Geel, Belgium) was pipetted on the sample surfaces. On top of the UA-covered surface, a UV-transparent quartz microscope slide was placed, leaving only a thin film of UA on the surface. For 20 h the samples were placed at a distance of about 2 cm under an intense UV lamp (>2.5 mW cm−2, “TUV G4T4 4W”, Philips, Eindhoven, The Netherlands) mounted in an EPROM eraser box (“ME5”, Lawtronics, Edenbridge, UK) to achieve a carboxyl-terminated surface. After this reaction, the samples are rinsed in pure acetic acid (C2H4O2 99% pure, Merck) at 100 °C (to remove non-covalently attached UA), followed by the same washing steps in ultra-pure water as after the reaction, the samples are rinsed in pure acetic acid (C2H4O2 99% pure, Merck) at 100 °C (to remove non-covalently attached UA), followed by two rinses for 5 min in 0.2× SSC buffer, at 75 °C and boiling ultra-pure water (20 min). The reference (background) spectrum of the substrate with clean surface. We applied a pinhole of 150 µm, a pixel time of 25.6 µs, and a band pass emission filter of 500 to 550 nm. A LD-Achromplan 40× objective with 0.6 numerical aperture was used for imaging in air.

3. Results and discussion

Since p-polarized light is not reflected at the Brewster’s angle of incidence at the diamond/air interface, the interference fringes in thin films are eliminated. The IR light is reflected only at the smooth film / substrate interface as it passes through the thin film, see Fig. 1. The reflectivity of the diamond/Si interface is only about 2%, but still the optical absorbance down to 10−3 (in the standard absorbance units) can be detected, except in the spectral region 2200–2400 cm−1 where residual CO2 absorbs. This spectral region was removed from all absorption spectra to improve the reproducibility.

The inverted laser-scanning confocal fluorescence microscope set-up was a “Zeiss LSM 510 META” Axiovert 200 M from Carl Zeiss (NV/SA, Zaventem, Belgium). The wavelength of 488 nm of an Ar-ion laser was used for excitation; an additional 488/10 nm excitation interference filter was inserted before the excitation fibre optics to guarantee spectral purity. We applied a pinhole of 150 µm, a pixel time of 25.6 µs, and a band pass emission filter of 500 to 550 nm. A LD-Achromplan 40× objective with 0.6 numerical aperture was used for imaging in air.

Fig. 1. The principle of the interference-free reflectance-absorbance spectroscopy of surface monolayer or ultra-thin coating (1) on optically smooth NCD film (2) deposited on one side polished Si substrate (3). The p-polarized IR light at Brewster’s angle of incidence passes two times the functionalized NCD surface where part of the light is absorbed at wavelengths characteristic for the functional groups vibrations. The reflectance at NCD/air interface is negligible; the NCD/Si interface reflects about 2% light. The bottom of Si substrate surface is optically rough and does not contribute to the reflected light.

Fig. 2. IR absorbance spectra of a linker molecule monolayer (10-undecenoic acid (UA), sample #1) covalently bonded to the NCD surface with and without covalently bonded DNA fragments (#2 and #3). The sample #2 contains 29 base pairs double stranded DNA (NCD-UA-29bpDNA), and the sample #3 single stranded DNA, consisting of 36 bases (NCD-UA-36bDNA).
The IR absorbance spectra can be used to estimate thickness of the surface coating. First, we applied spin coating technology to deposit AHAPS polymer coating (not yet diluted in toluene) on Au substrate (100 nm Au layer evaporated on glass substrate), and found the layer very homogeneous with thickness about 1 µm (deduced from the optical interference fringes). The intensity of the absorbance peaks at 2852 cm⁻¹ and 2921 cm⁻¹ was in the order of 1. The Au substrate was photobleached by the maximum laser intensity.

Regarding the samples #2 and #3, we note that the peaks at 2852 cm⁻¹ and 2921 cm⁻¹, assigned to the saturated hydrocarbons of the UA-chain, are essentially unchanged; this is to be expected, since only the end-group of the UA molecule is involved in the coupling to DNA. The peak at 1714 cm⁻¹, assigned to ν(C=O), is less clear in the spectra of samples with DNA as compared to sample with only the UA, probably overlapping with other peaks related to groups in the DNA (and possibly a shift of the C=O-related peak). In the involved wavenumber region, delocalised vibration modes are indeed expected in DNA [20,21]. The modes in the 1550–1750 cm⁻¹ range are attributed primarily to C=O stretching and N-H2 bending vibrations in the DNA bases [22]. In the spectra of samples #2 and #3, we attribute the absorbance peak at 1692 cm⁻¹ to Amide I [23]. Because we lack information below 1400 cm⁻¹, we were not able to confirm the presence of DNA-related peaks at 1550 cm⁻¹ (due to ‘Amide II’ [23]), or at 1220 cm⁻¹ and 1050 cm⁻¹ (attributed to stretching vibrations of the PO2⁻ in the DNA backbone [23,24]). IR spectra can in principle be used to distinguish between DNA in single and double stranded form: according to [22], the influence of hybridization on the C=O stretching and N-H2 bending vibrations due to Watson–Crick H-bonds can be observed in the 1550–1750 cm⁻¹ range. However, the spectra in Fig. 2 are not decisive on this point. A possible explanation is that after drying of the surface, boiling the sample in ultra-pure water is not effective in removing of the complementary strands. Two additional difficulties encountered here, are the limited spectral range (due to absorptions in NCD below 1400 cm⁻¹) and the overall low signal. Two weak peaks observed in the region 3250–3600 cm⁻¹ may be attributed to amino and hydroxyl groups [19], both present in the DNA bases. These peaks are more pronounced in the NCD-UA-29bpDNA (#2) spectrum than in the NCD-UA-36bDNA (#3) case, which is to be expected since more bases are present in the ds case than in the ss case.

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used as a substrate because it allows detecting absorbance spectra in wide spectral range (4000–800 cm$^{-1}$) and also because the reflectance at diamond Brewster’s angle of incidence is not interference-free when the surface polymer coating is thick. Fig. 3 shows that the intensity of absorbance peaks at 2852 cm$^{-1}$ and 2921 cm$^{-1}$ decreases proportionally with dilution of AHAPS in toluene being related to the total amount of polymer mass deposited on the surface. From here we estimate the polymer coating thickness just few nm using the dilution 1:200. The carbonyl peak at 1720 cm$^{-1}$ appeared in the absorbance spectra of the polymer on NCD in addition to the peaks observed in polymer on Au substrate. The origin of the carbonyl peak is not clear. Unfortunately, the FTIR spectra were not well reproducible and the fluorescence images revealed insulated polymer clusters, see Fig. 4A. The clusters appear as spots which emit fluorescence and there seems to be a continuous, fluorescenting background. After photobleaching, see Fig. 4B, spots as well as background became dark (photo-bleached). This may imply that the NCD surface is indeed covered with a continuous polymer film or with a dense layer of polymer clusters smaller than the optical resolution.

4. Conclusions

The IR absorbance spectra were measured by the interference-free reflectance–absorbance FTIR spectroscopy at Brewster’s angle. We have shown that FTIR absorbance spectroscopy can detect organic monolayers and coatings on NCD films down to few nm thickness and that the absorbance scales with the total mass of the organic molecules at the surface. In this paper we show for the first time the FTIR spectra of NCD surfaces functionalized covalently by a photochemically introduced 10-undecenoic acid monolayer with and without bonded DNA fragments, and IR spectra of organosilane polymer coatings deposited on NCD surfaces by spin coating technology to achieve continuous and stable surface functionalization. The photochemical functionalization (which takes about 20 h) can eventually be replaced by spin coating technology that takes just few minutes. In addition to the thin, continuous polymer coating, the polymer clusters have been also detected on the NCD surface coated by spin coating technology and these inhomogeneities need to be eliminated for further applications.

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