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Characterisation of hydrogenated silicon–carbon alloy filters with different carbon composition for on-chip fluorescence detection of biomolecules

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ABSTRACT

Absorption filters based on hydrogenated amorphous silicon–carbon alloys are developed for application as fluorescence filters in microarray and lab-on-a-chip systems. The carbon content of the thin film is varied by changing the amount of ethylene added to the ethylene/silane gas mixture during plasma-enhanced chemical vapour deposition. The optical properties of the films are characterised by transmittance measurements to obtain the refractive index, the optical bandgap, and the energy E_{04} of the films as a function of their carbon content. A set of filters that is appropriate for the detection of naturally fluorescent biomolecules is indicated. The system is used to demonstrate the detection of one of these naturally fluorescent biomolecules, the reduced form of nicotinamide adenine dinucleotide (NADH).

1. Introduction

The detection of biological species using microarrays and lab-on-a-chip systems is a powerful diagnostic tool that enables the acquisition of genetic, proteomic, and cellular information [1–3]. Such approaches allow massively parallel, highly sensitive and rapid analysis for disease diagnostics, drug discovery, or food and environmental analysis. In microarray applications, each pixel in the array is functionalised with well-defined probe molecules, and a molecular recognition reaction occurs between the probe and the target molecules to be detected. Fluorescence is one of the most commonly used methods for the detection of proteins, cells and DNA in microarrays [4–6]. The method uses an external light source, which excites the fluorophores that label the entities of interest. The integration of a fluorophore sensor at each pixel of a microarray would allow for a fast and real-time detection of the biological recognition event, while potentially increasing sensitivity and portability [7], and reducing costs. A number of molecules occurring naturally in cells present autofluorescence, and hence do not require labelling for detection. These include aromatic amino acids and some of the vitamins and enzyme cofactors (Table 1). Monitoring the intracellular concentration of these species with time can give valuable information about the cell condition and metabolism. Such autofluorescence measurements are used in industrial scale cell-based production of biotechnological products, in food quality control and in the identification of tumor cells [8–14].

Fluorescence detection, however, requires the development of efficient light management to prevent the excitation light from reaching the detector and at the same time allow the emission light to be transmitted to the detector. In previous publications [7], a layer of hydrogenated amorphous silicon carbide (a-SiC:H) was proposed as a suitable optical filter which can be easily integrated with a-Si:H photosensors for on-chip detection of biomolecules labelled with Alexa Fluor 430 or PyMPO. Simple fabrication of absorbing a-SiC:H filters (single-layer, low cost, with low dependence on the incidence angle) presents an important advantage compared to other filtering solutions, such as interference filters [15] where a large number of layers need to be tuned accurately during deposition. Although transmittance characteristics of absorbing filters are generally less steep than those of state-of-the-art interference filters, the ease of fabrication and the possibility of their integration in larger photodetector arrays for multiplex analysis in microfluidic systems makes them attractive candidates for integrated filters used either by themselves or in combination with interference filters [16]. The challenge is, however, to optimise the filtering characteristics of the a-SiC:H filter in order to match the excitation/emission wavelength fingerprint of any selected fluorophore. Therefore, a-SiC:H filters of different carbon content resulting in appropriate optical properties have to be designed. The relation between the carbon content in the a-SiC:H

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Table 1
List of biologically relevant fluorescent molecules with the corresponding excitation and emission wavelengths. For each molecule, the best candidate from the a-SiC:H filters is suggested that would yield the highest rejection ratio of the emission/excitation-light filtering characteristics.

Molecule	λ excitation	λ emission	Suggested filter
Amino acids [22]			
Phenylalanine	260	280	SC 2973
Tyrosine	275	300	SC 2974
Tryptophan	280	350	SC 2974/SC 2927
Coenzymes [23]			
FAD	450	525	SC 2889/SC 2924
NADH	290; 340	440; 460	SC 2926
HADPH	336	464	SC 2926
Vitamins [24]			
A	327	510	SC 2926/SC 2925
B6	330	400	SC 2926
B12	275	305	SC 2974
D	390	480	SC 2925
K	335	480	SC 2926

film and its optical filtering characteristics must be determined to enable the fabrication of the optimal filter for detection of a selected fluorophore.

In this paper, a series of a-SiC:H films are deposited by means of plasma-enhanced chemical vapour deposition (PE-CVD) and optically characterised. By controlling only the flow of the precursor gases (silane, SiH_4, and ethylene, C_2H_4), the carbon content in the films is varied, resulting in different optical properties and transmittance characteristics of the filters. Optical parameters, such as the refractive index, the optical bandgap, and the energy E_{04} at which the absorption coefficient is $\alpha = 10^4\ cm^{-1}$ are determined for each filter, and their dependence on the carbon content is investigated. All of the samples are also tested for photoluminescence. Finally, the filtering characteristics of all filters are compared to the excitation/emission properties of numerous biologically relevant natural fluorophores. For each fluorophore, the most suitable a-SiC:H filter is indicated that would assure the highest rejection ratio between the transmitted emission/excitation light, thus achieving the optimal sensitivity of the fluorescence measuring system. As a proof-of-concept, one of the filters is tested to demonstrate the detection of the reduced form of nicotinamide adenine dinucleotide (NADH)—an enzyme cofactor and an important marker for cell metabolic activity [7].

2. Experimental procedures

Hydrogenated amorphous silicon carbide films were deposited on Schott AF 45 glass substrate using radio frequency (RF) plasma-enhanced chemical vapour deposition (PE-CVD) at a power density of 30 mW/cm^2, substrate temperature 100 °C, and process pressure of 0.1 Torr. Films were deposited from a $[SiH_4 + C_2H_4]$ gas mixture. While the total gas flow rate was kept constant at 10.8 standard cubic centimetres per minute (sccm), the flow rates of SiH_4 and C_2H_4 were changed in each deposition run by means of mass flow controllers. Thus, the ethylene gas flow ratio, which is defined as $[C_2H_4]/[C_2H_4 + SiH_4]$, was varied between 0% and 99%, achieving a-SiC:H films with different levels of carbon content. The duration of each deposition run was 3 h 16 min.

The thickness of the films was measured using a profilometer (Dektak 3030ST). All the a-SiC:H film samples have roughly the same thickness (~2 μm, see Table 2), except for the two samples with the highest carbon content, which have smaller thickness. The decrease of the deposition rate is caused by the ethylene-rich environment. Details about the analysed samples are summarised in Table 2.

The transmittance of the samples was measured with a spectrophotometer (PerkinElmer Lambda 950) in the wavelength range of 270–1600 nm. The illumination was incident on the a-SiC:H film side. By employing reference beam attenuation, the spectrophotometer allows accurate measurements of highly absorptive samples exhibiting transmittances below 10^{-6}.

Photoluminescence measurements were carried out using a double-monochromator system. All a-SiC:H samples were measured at room temperature under identical configuration and conditions. Excitation spectra were measured in the range of 300–400 nm and emission spectra in the range of 320–700 nm.

Autofluorescence of an aqueous solution of NADH (Sigma) was measured using an amorphous silicon (a-Si:H) p-i-n photodiode. A glass slide coated with the SC2925 a-SiC:H filter (29% ethylene flow, Table 2) was placed on top of the photodiode. All solutions were measured in polydimethylsiloxane (PDMS) wells with a 100 μm thick bottom layer (see inset in Fig. 5). A tungsten–halogen lamp coupled with a monochromator set at 380 nm and chopped at 13 Hz was used to provide the excitation-light source. The photodiode output signal was measured using a lock-in amplifier.

3. Results and discussion

The transmittance spectra of a series of eight a-SiC:H films (filters), selected from Table 2, are presented in Fig. 1. The percentages denote the ethylene gas flow fraction ($[C_2H_4]/[C_2H_4 + SiH_4]$) during the deposition of the corresponding filter. The value of 0% represents pure a-Si:H, whereas the value of 99% corresponds to the filter with the highest carbon content. The results show that all filters exhibit distinct high-pass behaviour, with very low transmittance (high absorption) in the short-wavelength region and high transmittance (low absorption) in the long-wavelength region of the spectrum. The position of the absorption edge connecting the two regions depends on the optical bandgap, E_{opt}, of the material, which is related to the carbon content in the a-SiC:H film. With an increasing carbon concentration, E_{opt} increases and the absorption edge is thus gradually shifted towards shorter wavelengths.

The efficiency of a filter used for fluorescence detection reflects in its ability to suppress light at the excitation wavelength of the fluorophore used. Therefore, the region of the highest absorption (i.e.

Table 2
Summary of deposition and optical parameters of the a-SiC:H filters.

Filter	C_2H_4 flow ratio (%)	Dep. rate (nm/min)	d (μm)	n	E_{04} (eV)	E_{opt} (eV)	B (cm eV)$^{-1/2}$
SC 2890	0	9.5	1.87	3.33	1.98	1.78	707.1
SC 2924	8	9.4	1.85	3.04	2.25	2.04	682.4
SC 2889	17	10.9	2.13	2.27	2.44	2.22	643.4
SC 2925	29	11.0	2.16	2.13	2.64	2.35	532.7
SC 2926	49	11.3	2.22	2.09	3.02	2.69	493.3
SC 2927	69	10.4	2.03	2.02	3.49	2.93	347.6
SC 2974	80	10.6	2.08	1.94	3.69	3.02	284.5
SC 2973	90	8.2	1.61	1.72	3.81	3.03	247.5
SC 2975	99	4.1	0.80	1.91	4.00	3.19	247.6