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Implementation and characterization of an absorption filter for on-chip fluorescent imaging

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ABSTRACT

Here we present fabrication and characterization of an absorption filter with superior roll-on properties and precisely tunable cut-off wavelengths for fluorescent imaging applications in lab-on-a-chip systems. The filters were fabricated by spinning dye doped photopolymer (Orasol Yellow in Norland Optical Adhesive 60) on glass substrates. The fabrication technique allowed us to precisely tune the cut-off wavelength of the filters. We showed that filters with different cut-off in the range of 386 nm–504 nm could be obtained simply by controlling the settling time before spinning. The filters exhibited a steep roll-on from stopband to passband at the cut-off. Transmission in the stopband was observed to be maximum 3% while it was almost constant at 100% in the passband within the range of 220 nm–620 nm. On-chip use of the filters was also demonstrated for imaging particular fluorescent beads.

1. Introduction

Optical detection based lab-on-a-chip devices, particularly the ones utilizing fluorescence, are very common since these devices are highly compatible with conventional laboratory equipment (e.g. fluorescent microscopes or plate readers) and practices.

In such applications, the analyte (which can be labeled cells or particles) is excited at a specific wavelength and absorbed light is emitted at a different wavelength based on the Stokes shift. Accordingly, the detection systems should be sensitive to emitted light, while being blind to excitation light to increase signal-to-noise ratio. This can normally be achieved by utilizing off-chip absorption filters which allows the emitted light to pass while filtering the excitation light. However, the spacing between the filter and the lab-on-a-chip device reduces the intensity of the optical signal, thus unfavorably affecting the detection sensitivity [1].

To improve the sensitivity, the researchers are forced to integrate the optical elements, such as filters, on chip. One of the early integration attempts was presented in [2]. Here an integrated interference filter, which was directly coated on the substrate by depositing alternating layers of SiO_2 and TiO_2, was utilized. Similar approaches were also presented in [3,4]. Although, utilizing such integrated interference filters resulted in improved efficiency of the devices, increased fabrication costs hindered their use [1].

As an alternative, Whitesides' group presented a stack of PDMS-sealed photodetector, a stand-alone polymeric absorption filter film, and a PDMS microchannel to detect separation of proteins by capillary electrophoresis [5]. Instead of utilizing a stand-alone filter, a monolithically integrated filter was proposed in [1]. In this work, microfluidic structure was fabricated by molding dye added PDMS resin, hence the structure itself acted as the filter. Based on the idea of fabricating an integrated filter by using dye-doped resin, absorption filters were fabricated by spinning dyed fish gelatine resin on glass substrates [6]. Similarly, an absorption filter fabricated by spin coating a photoresist-dye mixture on a substrate was presented in [7]. Ozcan's group utilized the same technique in [7] to fabricate on-chip absorption filters for wide-field fluorescent imaging [8]. In this method, Orasol dyes were dissolved in cyclopentanone and mixed with KMPR 1005 photoresist. The mixture was then spin coated on the substrate and baked.

Here, we present a cheap alternative of fabricating on-chip absorption filters based on spinning dye-doped resin. We used Orasol Yellow as dye and UV curable adhesive NOA60 as the photopolymeric host resin. We showed that filters with different cut-off wavelengths can be precisely fabricated by tuning single parameter during fabrication. We analyzed the effect of various fabrication parameters on filter properties. We also discussed and compared our filter with some other published on-chip filters.

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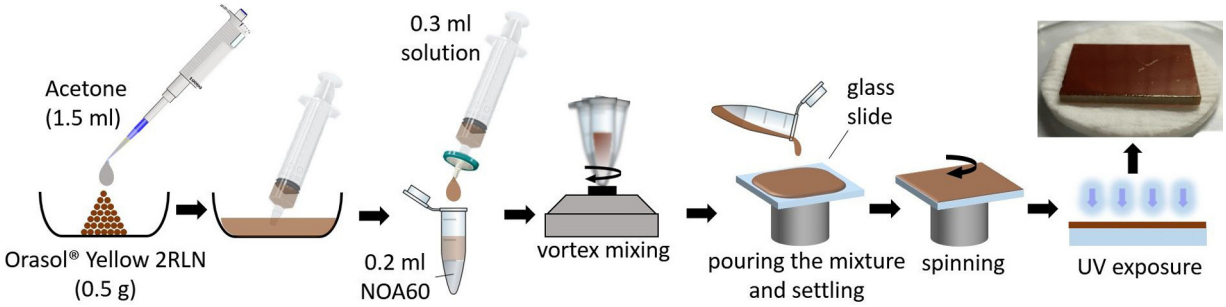

Fig. 1. Illustration of the process steps in fabrication of the filters.

Finally, on-chip use of the filter was demonstrated by detecting fluorescent beads suspended in a microfluidic channel.

2. Fabrication method

To fabricate the filters, first 0.5 g of Orasol® Yellow 2RLN soluble dye (KREMER Pigments, USA) was dissolved in 1.5 mL of acetone. The solution was then filtered by using polyester syringe filters with pore size of 0.45 μm to remove undissolved dye particles and other residues. Filtered dye solution was mixed with ultraviolet-curable photopolymer NOA60 (Norland Products, USA) at 1.5:1 solution:NOA60 volume ratio by using a vortex mixer. This mixture was spin coated on a cover glass to produce thin filter layer. Before spinning, a cover glass was placed on the chuck of the spin coater. To clean the surface of the cover glass, methanol was first spinned at 2500 rpm for 30 s. After spinning methanol, dyed photopolymer mixture was poured on the cover glass to cover the surface entirely. The mixture was kept on the cover glass without spinning for a while for the mixture to settle and for excess acetone to evaporate. We refer to the duration of such procedure as the settling time. After the settling time, a thin layer of dyed photopolymer layer was created by spinning the cover glass at 2500 rpm for 30 s. As the last step, spin coated layer was cured by exposing it to UV light at an intensity of 10 mW/cm^2 for 60 s in a UV exposure box. Fabrication process is illustrated in Fig. 1.

3. Results and discussion

During the optimization of this process, dye was dissolved in acetone to obtain a saturated mixture. Otherwise, the color tone of the filter came out to be so pale that it could not be possible to cut off the incident light during the tests. It was observed that UV curing time of the photopolymer had no considerable effect on the filter characteristics. Therefore, curing time was selected such that the photopolymer was not tacky after the process. Power was dictated by the UV source of the exposure box. Methanol spinning parameters had also no considerable effect on the result. Therefore, the main parameters affecting the filter properties were the dye solution-photopolymer mixing ratio, photopolymer mixture settling time and photopolymer mixture spinning parameters (spinning time and rate).

It was observed that when the spinning rate was kept relatively low, centrifugal forces were not high enough to obtain a uniform coating over glass substrate. Uniformity problems were also observed when the spinning rate was set to relatively high values. However, in this case uniformity could not be achieved because of excessively reduced coating thickness. Considering the spinning time, bubbles were observed on the surface when it was kept relatively short or long. We presume that this resulted from acetone evaporating from the photopolymer mixture. It is possible that the competition between the centrifugal forces and the interfacial forces on the surface of the acetone vapor bubbles was the main reason of this problem. Consequently, we found that a uniform bubble-free coating could be obtained when the spinning time and rate were set to 30 s and 2500 rpm respectively, while keeping the other parameters as defined in Table 1.

Table 1
Process parameters of filter fabrication.

Process	Parameter	Value
Dissolving dye in acetone	ratio	0.5 g:1.5 ml
Mixing dye solution with photopolymer	volume ratio	1.5:1
Spinning methanol	time	30 s
	rate	2500 rpm
Settling photopolymer mixture	settling time	15 s–120 s
Spinning photopolymer mixture	time	30 s
	rate	2500 rpm
UV curing	time	60 s
	power	10 mW/cm^2

During the experiments, we observed that dye solution and photopolymer mixing ratio significantly affected the result. Setting the volume of the dye solution relatively higher than that of the photopolymer resulted in filters with relatively higher cut off wavelengths. Similarly, keeping the volume of the dye solution low reduced the cut off value. However, tuning the cut off wavelength through modifying the volume ratio of the dye solution and the photopolymer was found to be infeasible. The reason was that the modification also affected the viscosity of the mixture, which in turn would require tuning of the spinning parameters for each filter.

On the other hand, we observed that a precise way to tune the dye solution photopolymer ratio was to modify the settling time before spinning. During settling of the photopolymer mixture, excess acetone in the mixture evaporates and increases relative dye content in the mixture. The effect of the settling time was first tested on non-methanol-treated cover glasses. Photopolymer mixture was poured on non-treated substrates and waited for durations ranging between 2 and 10 s. It was observed that the resulting filters have different characteristics, showing that the evaporation of acetone had a considerable effect. However, since the filter properties were highly sensitive to settling time, due to very short waiting durations, it was concluded this scheme was not feasible for tuning filter characteristics. To increase the settling time, hence to decrease the sensitivity of the process on the settling time, we proposed to treat the surface of the cover class with methanol before pouring the photopolymer mixture. As a result of methanol treatment, hydrogen in methanol makes weak hydrogen bonds with oxygen in acetone (Fig. 2a), thereby reducing the evaporation rate of acetone. We observed that the spectral characteristics of the filter fabricated with methanol treatment and 90 s of settling time were almost the same with those of non-methanol-treated sample with 60 s of settling time. This result shows that methanol has negligible or no effect on the optical properties of the filter. Another possible effect of methanol treatment can be related to the adhe-