USE OF PARYLENE-C BONDING LAYER FLUORESCENCE AS REFERENCE FOR ON-CHIP IMAGING AND DETECTION APPLICATIONS

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ABSTRACT

We use the induced fluorescence ($F_{\text{ind}}$) in parylene-C, being a bonding layer on a microfluidic chip, for precisely quantifying the fluorescent dose received by a biological sample. Doing so, we can correct for the fluorescence loss in the biological sample, even after long term illumination, so that fluorescence read-out becomes much more quantitative than possible so far. We demonstrate the technique by recovering/correcting the fluorescent signal during photo-bleaching of diaminophenylindole (DAPI)-stained tissue sections.

KEYWORDS: Programmable fluorescence, Parylene bonding, Photo-bleached signal compensation, On-chip data storage

INTRODUCTION

Quantitative fluorescent imaging and detection require repeated microscope calibrations [1,2]. The more frequent these calibration steps take place, the more accurately can we account for signal variations due to photo-bleaching of the fluorophores. An ideal way to continuously measure the fluorescence exposure on a biological sample would involve the use of an in situ reference on-chip.

Recently, wafer bonding using parylene-C as intermediate layer to fabricate microfluidic channels was demonstrated [3,4]. Moreover, we have shown that intermediate parylene-C bonding layer fluorescence (iPBLF) can be programmed for fluorescent data storage on-chip by illuminating with UV or green light [5]. Here we demonstrate that the exposure energy-dependence of $F_{\text{ind}}$ of the parylene-C can be used to reproducibly determine the fluorescent illumination dose that is received on the chip by a biological sample and to correct the fluorescence emission signal of the latter for eventual photo-bleaching effects.

EXPERIMENTAL

Figure 1(a) is a schematic process flow of the fabrication of a microfluidic chip based on a Pyrex wafer, a micromachined Si wafer and an intermediate parylene-C bonding layer. A tissue chamber is realized, in which the fluorescence of a biological sample can be studied. Figure 1(b) shows the induced fluorescence in the parylene-C layer. Figure 2(a) shows the experimental set-up and Figure 2(b) indicates the two fluorescent excitation/emission channels (UV/blue and green/red), together with their filter wavelengths, which are used in this study.

Figure 1: Microfluidic devices fabricated using parylene-C to SiO$_2$ bonding. (a) Illustration of the fabrication process. 1) Si wafers with 2 μm thick wet SiO$_2$ were 2) etched using Deep Reactive Ion Etching (DRIE). 3) 2 μm thick parylene-C was patterned using RIE via an amorphous Si mask. 4) Wafers were bonded at 280 °C under vacuum during 40 minutes applying 1000 mbar tool pressure and back-side etching was performed to finally open the tissue chamber. 5) The bonded Pyrex-parylene-C-Si stack was reversibly assembled with a biological sample, in this case a cover-slipped tissue section on a glass slide. (b) Fluorescent image of a bonded device without a biological sample, observed with green excitation (537 - 562 nm)/red emission (570 - 640 nm) filters. The dark and clear regions correspond to the microfluidic channels/tissue chamber and the parylene-C bonding layer, showing intermediate fluorescence (iPBLF), respectively.
RESULTS AND DISCUSSION

We first characterized the behavior of the iPBLF and the fluorescence of a DAPI-stained tissue section under continuous UV illumination, as shown in Figure 3. Figure 3(a) explains the calculation of $F_{\text{ind}}$ for the parylene-C layer for a certain illumination dose, while Figures 3(b,c) show fluorescent image examples, taken before and after exposure to a 56 mJ/mm$^2$ dose of UV light of the parylene-C layer and a DAPI-stained tissue section, respectively.

The doses in Figure 3(d), obtained by increasing the exposure time of the mercury short arc lamp (HBO 50W/AC), and the parylene-C $F_{\text{ind}}$ are sufficiently regular to allow the iPBLF observed in the green/red channel to be used as a reference signal to calculate the dose received by the biological sample. To illustrate this, we also measured the DAPI-stained tissue, also shown in Figure 3(d). The fluorescent signal of the DAPI-stained tissue against $F_{\text{ind}}$ of the parylene-C, observed in the green/red channel, is plotted in Figure 3(e). By using an exponential fit, we can reconstruct the photobleached signal of the DAPI-stained tissue at different times of the experiment, as shown in Figure 4. The histograms of Figure 4(d) are obtained by dividing pixel-per-pixel DAPI images like shown in Figure 4(c), corrected following the exponential fit of Figure 3(e), by the initial image of Figure 4(b). The pixel distribution around 1 for each case indicates the good correlation between the average corrected DAPI fluorescent intensity and the original one. However, for the larger exposure doses, we note an increased broadening of the histogram, which is an artifact induced by the increased relative importance of pixels with initially less fluorescent intensity.
Figure 4: Use of the exposure energy dependence of $F_{\text{ind}}$ of parylene-C for correcting the photo-bleached fluorescence of the DAPI-stained tissue. Both the parylene-C layer and the DAPI-stained tissue were exposed during a variable time to UV light of constant power. Time-lapse images as observed in the green/red (a) and the UV/blue (b) channels were taken every minute during 120 ms observation. The intensity data recorded in (a) represent $F_{\text{ind}}$. Knowing $F_{\text{ind}}$ for a certain UV illumination dose, correction factors were obtained from the fitting curve shown in Figure 3(e) and applied to obtain the corrected images of Figure 4(c), starting from the as-observed fluorescent DAPI images of Figure 4(b). (d) Dividing pixel-per-pixel the intensity of the corrected images by the non-exposed image of Figure 4(b) shows a histogram with a distribution centered around a value of 1, indicating a strong correlation between the corrected/recovered DAPI signal and the original one.

CONCLUSION

Photo-bleaching due to continuous exposure of the sample during experiments can induce a significant reduction of the obtained fluorescent signal and, therefore, affect the possibility to compare different samples. We showed that having an on-chip reference of the dose received by the sample during experiments results can be of great use. Such reference, as determined by the iPBLF, can be employed to correct/recover fluorescent signals from common dyes during photo-bleaching, which will facilitate more quantitative on-chip fluorescence detection.

REFERENCES


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