Autofluorescence of Electrophoresis Chip Grooved by Excimer Laser

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The autofluorescence of a chip is a serious problem for high sensitivity optical detection in on-chip electrophoresis. The autofluorescence spectra of a micro channel, which was grooved by an ArF excimer laser on an electrophoresis chip, were measured by a fluorophotometer equipped with a 532nm laser as an excitation source. A commercially available Poly(methylmethacrylate) (PMMA) plate was used as the specimen. The total fluorescence of the laser-grooved micro channel was effectively reduced by optimization of the beam shape, the pulse repetition rate and the traveling speed of the specimen. For further reduction of autofluorescence of the laser grooved micro channel, we used a solvent etching process. When etched by the solvent, the autofluorescence of the grooves was dramatically reduced to a level sufficient for practical electrophoresis. The electrophoresis signals of the chip processed by the combination of laser grooving and solvent etching compared favorably to that of a chip formed by conventional injection molding. The time required to make a micro fluidic chip by this method is only a few hours.

DOI: 10.2961/jlmn.2008.02.0006
Keywords: Excimer laser, PMMA, GG developer, autofluorescence, DNA electrophoresis

1. Introduction

Many kinds of microfluidic chips have been studied and some of them have been put on the market, especially for life sciences. Typically, they are made of fused silica, glass or several polymeric materials. To take advantage of their various optical, chemical, mechanical and thermal properties, as well as their cost benefits, some polymers are useful for microfluidic chips, for on chip electrophoresis and micro-analysis systems [1-4]. Above all, polymethylmethacrylate (PMMA) has superb optical and mechanical properties [5, 6]. For microfabrication from polymeric material, direct laser ablation is one of the well-known, excellent methods, especially for a one-off experimental or prototype chip [7, 8].

However, sometimes the roughness of the micro-channels is a problem. And for high sensitivity fluorescence measurement, the autofluorescence from the etched surface also may cause problems. A high-sensitivity fluorescence detector requires a smooth channel, with low-autofluorescence surface.

In this research, we combined two processes, UV laser ablation and etching by a solvent, in order to groove a channel with sufficient properties to function as an electrophoresis chip. By optimizing the process parameters, an electrophoresis chip with output signal almost the same as that of a commercially available chip was produced in a few hours.

2. Experimental method

2.1 PMMA substrate

A commercially available, transparent PMMA plate (CLAREX®, Nitto Jushi Kogyo Co., Ltd.) with little autofluorescence in its initial condition was used in this study.

2.2 UV LASER grooving

An ArF excimer laser (193nm), LPX-305i (Coherent Inc.), was used for processing. The specifications of the laser oscillator are shown in Table 1.

Table 1 Specifications of the laser

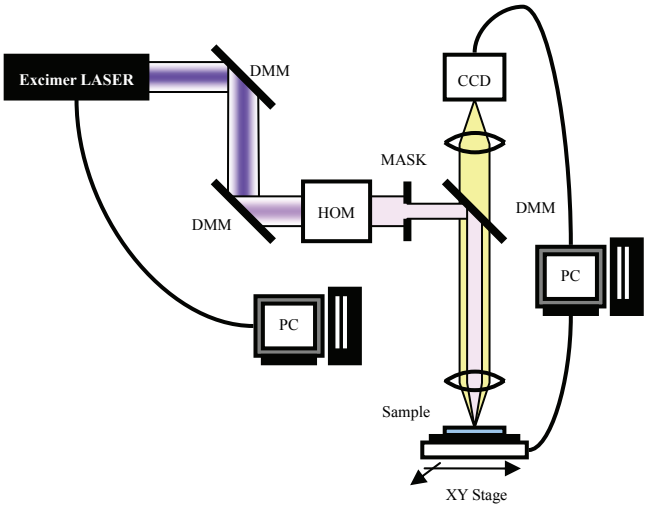
Max. pulse energy	500mJ
Max. power	25W
Max. repetition rate	50pps
Pulse width	14ns

The laser processing system for this study is shown schematically in Fig. 1. The magnification was 1/10 and the mask was oblong with apertures of 1×1mm, 1×10mm, etc. When the magnification is 1/10, the fluence at the processing surface is about 6.5 J/cm^2. (The fluence was calculated by dividing the beam power after the object lens by the dimension of the irradiated area.) The system has an x-y stage controlled by a computer. Laser repetition rate

was fixed at 5Hz in the experiment. The stage feed speed for grooving was calculated from the repetition rate of the lasers, the required number of pulses and the size of the laser beam. For a laser beam that is 100μm long in the feeding direction and operated at 5Hz, 50 laser pulses were required to overlap to obtain a channel 30μm deep, thus requiring a feed speed of 10μm/sec (Formula 1).

$$S_f = \frac{L_B R_L}{N} \quad \text{… Formula 1}$$

S_f: feed speed
L_B: Beam length on sample
R_L: Laser repetition rate
N: Number of laser pulses to overlap



DMM: Dielectric Multilayer Mirror
HOM: Homogenizer

Fig. 1 Schematic diagram of the laser and optics used for the experiment

2.3 GG solution etching

To evaluate the efficacy of the laser-affected surface layer, we used an organic solvent mixture known as "GG Developer (U.S. Patent No. 4393129)[9,10] to etch the groove.

Table 2 GG developer constituents

Developer solution

2-(2-n-butoxyethoxi)ethanol	60 wt%
Morpholine	20 wt%
2-aminoethanol	5 wt%
Water	15 wt%

Stopper solution

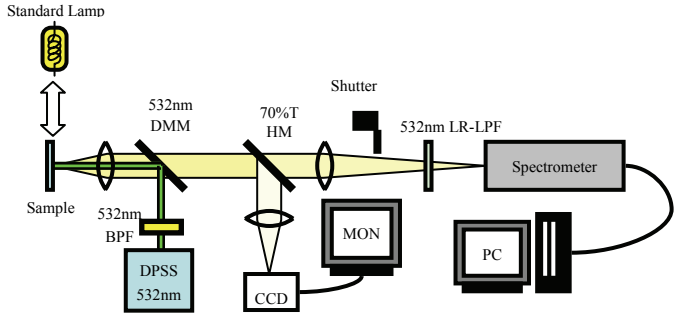
2-(2-n-butoxyethoxi)ethanol	80 wt%
Water	20 wt%

After laser grooving, the sample was placed in the GG Developer with ultrasonic agitation (US) for 1min, then into the stopper solution with US for 1min. The sample was then washed with deionized water.

2.4 Measurement of the fluorescence

Fluorescence was measured by an experimentally built confocal system, shown in Fig. 2. The second harmonic output (wavelength: 532nm) of a Nd:YAG laser was used for excitation. A 532nm band pass filter (CWL: 532nm, FWHM: 3nm) was used to eliminate the extra spectrum from the YAG laser. A spectrometer (M-25TP, Hamamatsu Photonics K.K.), equipped with a MOS optical sensor array and an image intensifier (C4564-010G, Hamamatsu Photonics K.K.), was used to measure the emission spectra during excitation. A high performance long-pass filter was set in front of the spectrometer to eliminate the reflection at 532nm, the excitation wavelength. Coaxial CCD imaging optics were used to align the measurement spot. The spectrum of a standard lamp with spectral radiation power ranging from 250nm to 2500nm, calibrated by JCSS (Japan Calibration Service System) in accordance with ISO/IEC 17025, was used to calibrate the spectral sensitivity distribution of the system before each measurement.

(a)



BPF: Band Pass Filter
HM: Half Mirror
LR-LPF: Low Pass Filter for Laser Raman spectroscopy

(b)

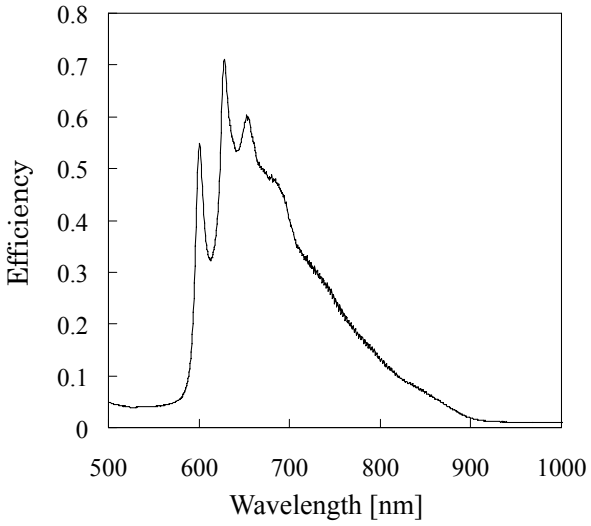


Fig. 2 (a) Schematic diagram of the confocal fluorescence spectrometer system, and (b) the total spectrum efficiency of the system

Based on the sensitivity curve shown in Fig. 2(b) and the spectral distribution of the fluorescence obtained, we integrated the signal from 600nm to 700nm in each spectrum to evaluate the autofluorescence.

First, we try to measure a quartz diffusion plate to check that there was no peak in the background.

2.5 DNA Electrophoresis

A chip with laser-grooved microchannels was evaluated by a chip-based electrophoresis device. SV1100 (HITACHI High-Technology Co., Ltd.) is a simple electrophoresis device using an excitation light at 468nm for the analysis of DNA and RNA. We made a compatible chip from PMMA with this device using laser ablation and solution etching. A standard DNA sample for SV1100 that included 100bp and 800bp DNA was used to compare the intensity and full width at half maximum of each peak.

The electrophoresis analysis was run in accordance with the conditions suggested by the provider of the SV1100 (750V 3min) with a commercial premixed gel recommended for SV1100.

3. Results and Discussion

3.1 Excimer grooving of PMMA

Under the grooving conditions described above, 50 overlaps with the laser irradiation produced a 30μm deep groove. The lines perpendicular to the feed direction on the bottom of the groove indicate the edges of each exposure.

Particles seen on the bottom and around the groove were generated while laser grooving. They are considered to be reattached coagulated particles that were vaporized from the PMMA by laser grooving [11, 12].

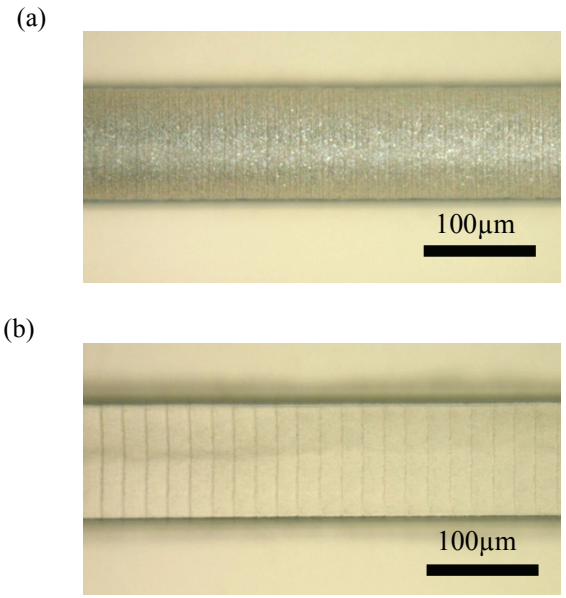


Fig. 3 Optical microscopic images of the groove made by ArF excimer laser on PMMA substrate. The laser beam sizes used for each exposed area of 100×100μm (a) and 100×1000μm (b) respectively.

3.2 Autofluorescence from UV Laser-Grooved PMMA substrate with and without GG development

The spectra of the PMMA substrate are shown in Fig. 6. Our fluorescence spectrometric system lacks sensitivity except in the 600nm to 700nm range, so there is a lot of noise. We therefore tried comparing the autofluorescence by integrating the intensity from 600nm to 700nm. Bulk PMMA has little autofluorescence (Fig. 6 (a)), but the grooves produced by the excimer laser processing (Fig.3 (a)) have strong autofluorescence (Fig. 6 (b)).

We can see the broad emission shoulder with a narrow peak at 630nm. It is feasible that the fluorescence was caused by some kind of functional group, such as a kind of cyclic compound, resulting from the UV laser ablation[13, 14], but it cannot be identified from the narrow spectral area only.

Following GG etching (Fig. 4 (a)), the autofluorescence was much diminished (Fig. 6 (c)). GG developer can etch the part of PMMA that consists of a low molecular weight polymeric chain produced, for example, by irradiation with UV [9]. The part exhibiting autofluorescence is on or in the area where the mass was irradiated with UV laser, and the polymeric chain subsequently became shorter and the fluorescent part removed as a result of treatment with GG developer.

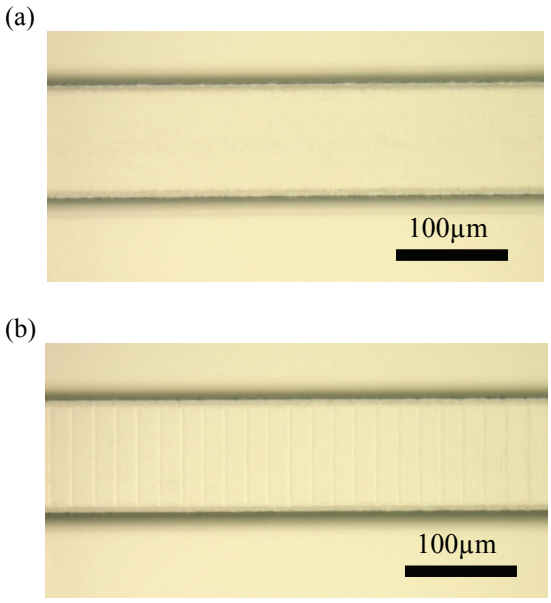
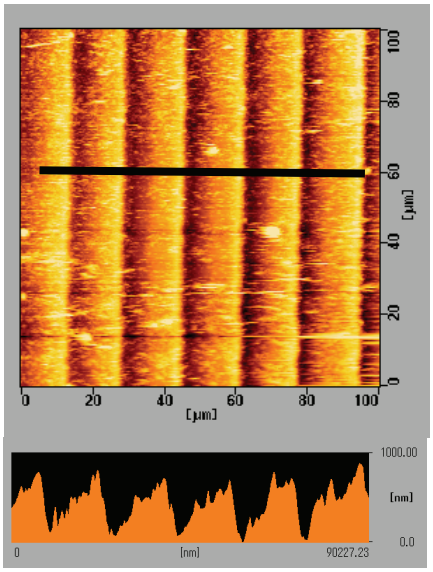


Fig. 4 Optical microscopic images of the groove after GG etching with 100×100μm (a) and 100×1000μm (b) exposed area.

(a)



(b)

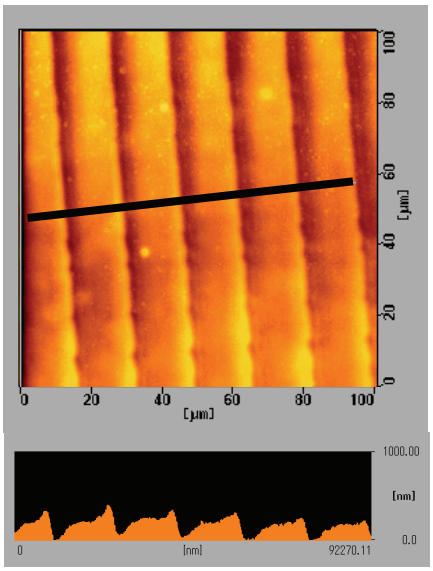
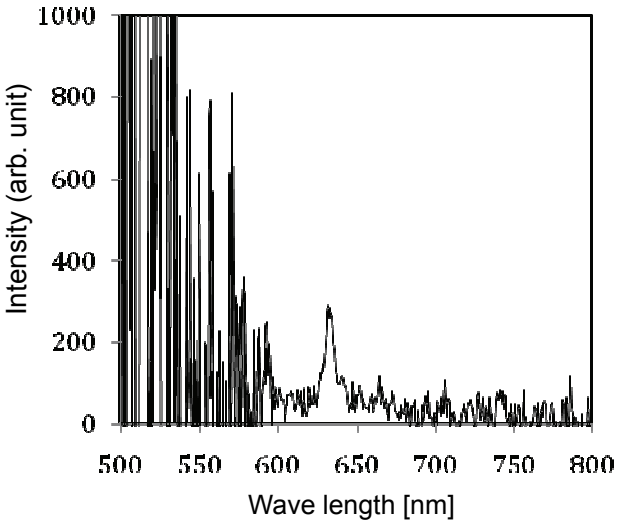


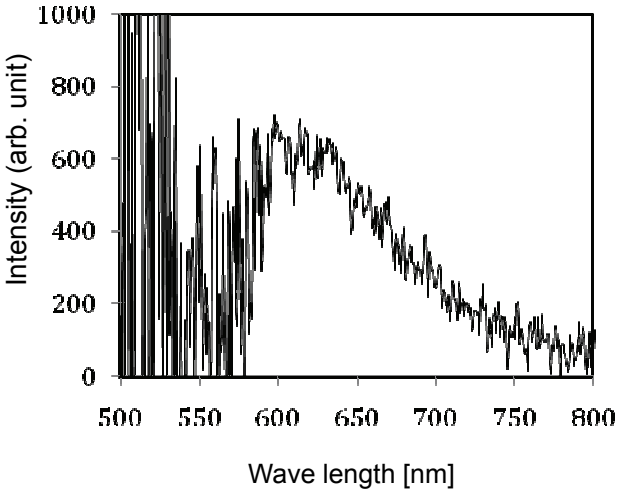
Fig. 5 AFM image of the bottom surface of the groove milled by UV Laser machined on PMMA. Surfaces are untreated (a) or etched by GG developer (b). The line in the upper part of each image indicates the viewing line for obtaining the cross-sectional image in the lower part.

With AFM, the edge of each beam creates steps (Fig. 5). Many particles with a diameter of about 10nm can be seen on the untreated surface (Fig. 5 (a)). After GG etching the particles were removed and the height of the steps reduced.

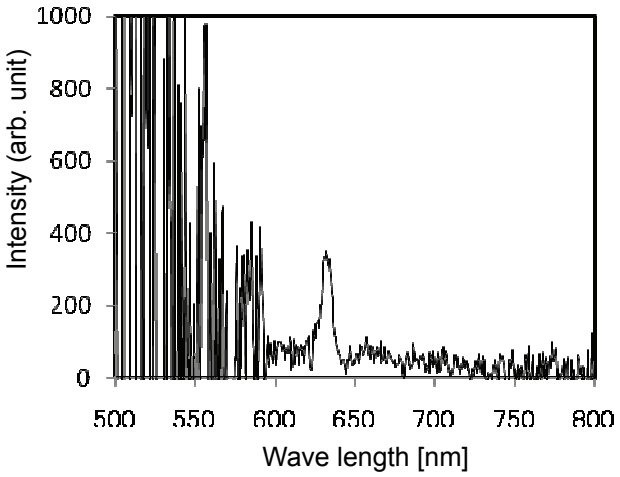
(a)



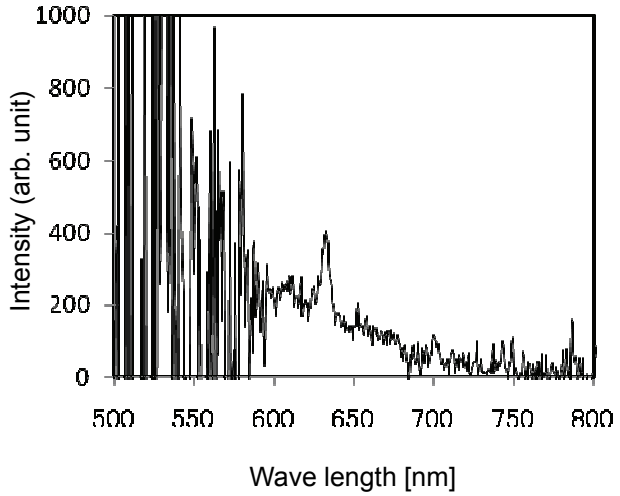
(b)



(c)



(d)



(e)

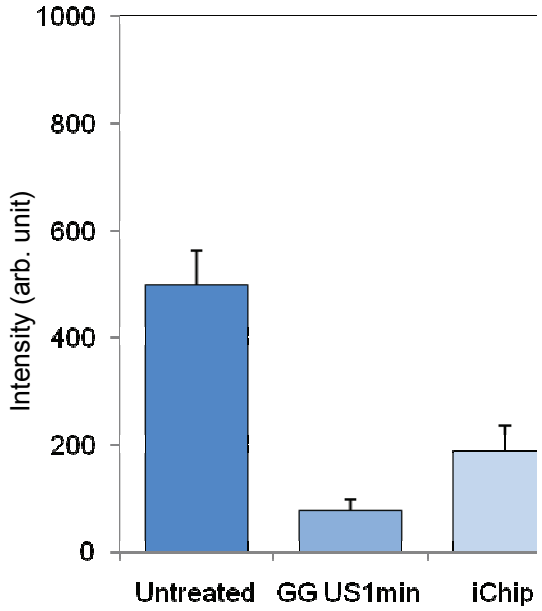
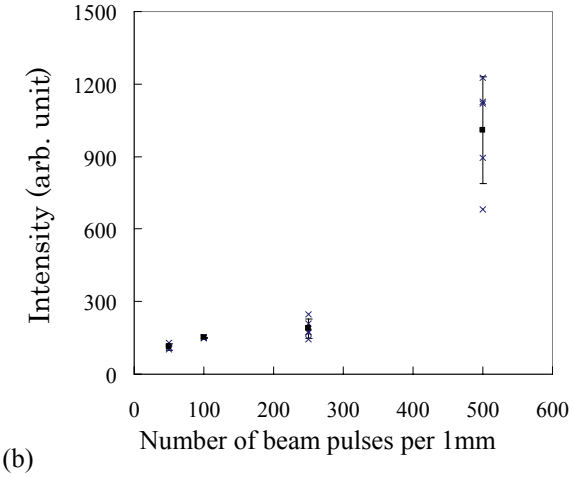


Fig. 6 Fluorescence from PMMA excited by a 532nm laser.
(a) Bulk PMMA shows little fluorescence. (b) PMMA etched by an excimer laser shows strong fluorescence. (c) The fluorescence was reduced after etching with GG Developer. (d) A commercially available PMMA injection molded chip for comparison. (e) The average and standard deviations of the integration of the signals from 600nm to 700nm in each spectrum.

We plotted the intensity of autofluorescence at the microchannel vs. the number of beam edges per 1 mm (Fig. 7). To produce samples with constant channel depths but at different beam lengths, the feed speed was changed, resulting in variations in the number of beam edges, as seen in Figure 3. Each laser pulse creates an edge around the exposed section.

The autofluorescence was seen to become stronger as the number of laser shots increased (Fig. 7 (a)). Because more beam pulses are applied to a section, a greater number of reattached particles are accumulated in same section. However this autofluorescence largely disappeared after GG etching (Fig. 7(b)). The intensity seen at around 50 is merely noise.

(a)



(b)

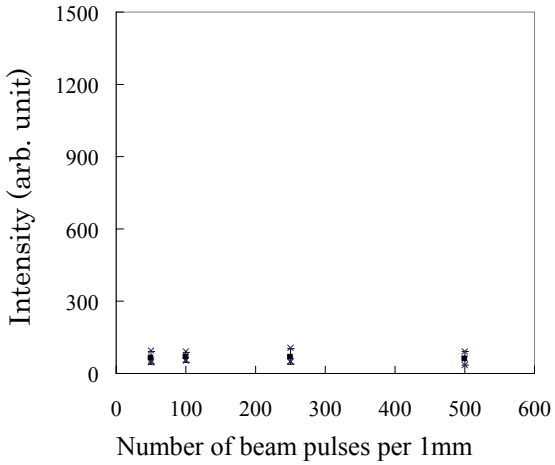


Fig. 7 Fluorescence vs. the number of section beam edges Immediately after grooving by excimer laser (a) and after further etching with GG Developer (b)

3.3 Particle effect

When we measured the autofluorescence from the surface adjacent to the groove that did not undergo surface modification with GG Developer, there was strong autofluorescence (Fig. 8). If only the layer produced by UV laser irradiation caused autofluorescence, there should be no autofluorescence except from the exposed area.

The grooved sample was placed in the deionized water and ultrasonicated to remove particles on the surface adjacent to the groove without causing chemical change, with the difference in autofluorescence measured before and after (Fig. 9)

After the ultrasonic cleaning, the autofluorescence became weaker than before. Optical microscopic images show that most of the particles around the groove were removed by the ultrasonication (Fig. 9). We concluded that the particles generated by UV laser milling around the groove have strong autofluorescence. This is supported by the relationship between the number of shots and the

strength of autofluorescence; more shots producing large amounts of particles in a given section.

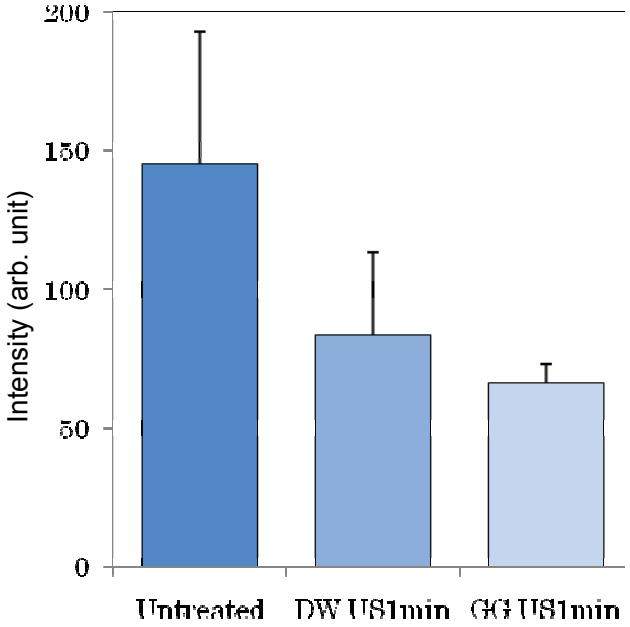


Fig. 8 Fluorescence from the surface around the groove immediately after UV laser grooving (left), after ultrasonication in deionized water (center) and after treatment with GG developer (right).

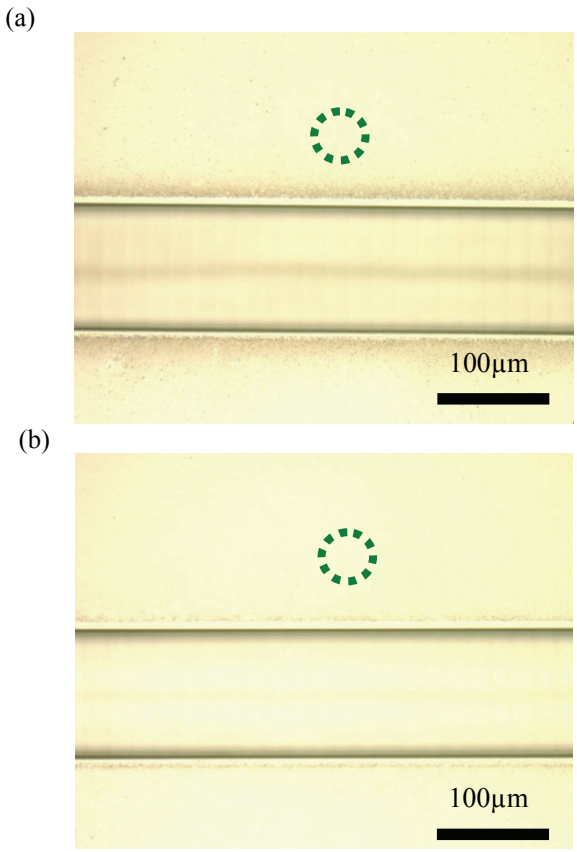
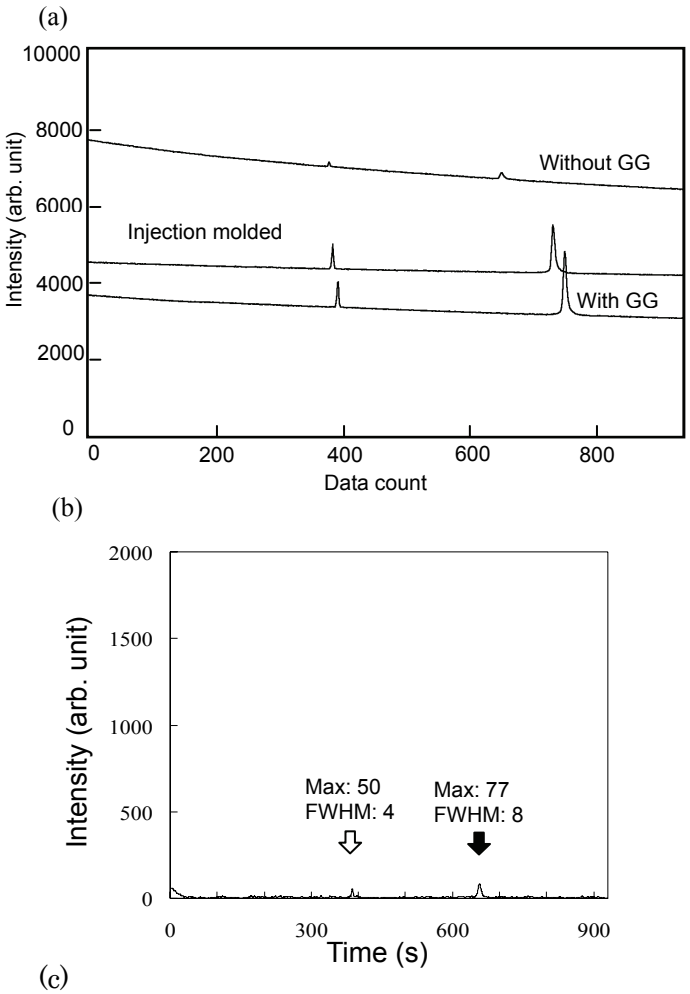


Fig. 9 Optical microscopic images of the surface adjacent to the groove before (a) and after (b) ultrasonication in deionized water. The green circle on the etch image shows the focus of the excitation light.

Nevertheless, the difference in the fluorescence shown in Fig. 9 is still not enough to explain all the autofluorescence from the groove. Therefore the layer affected by the UV laser must be one of the fluorescence sources.

3.4 DNA electrophoresis chip

We made a DNA electrophoresis chip using our technique. It has two intersecting channels, each 100μm wide and 30μm deep, that should be capable of making a 100 × 100μm injection plug. Fig. 10 shows the result of electrophoresis with a commercial electrophoresis chip system (SV1100 Hitachi Electronics Engineering Co., Ltd.). The system employed EtBr as a fluorescer.



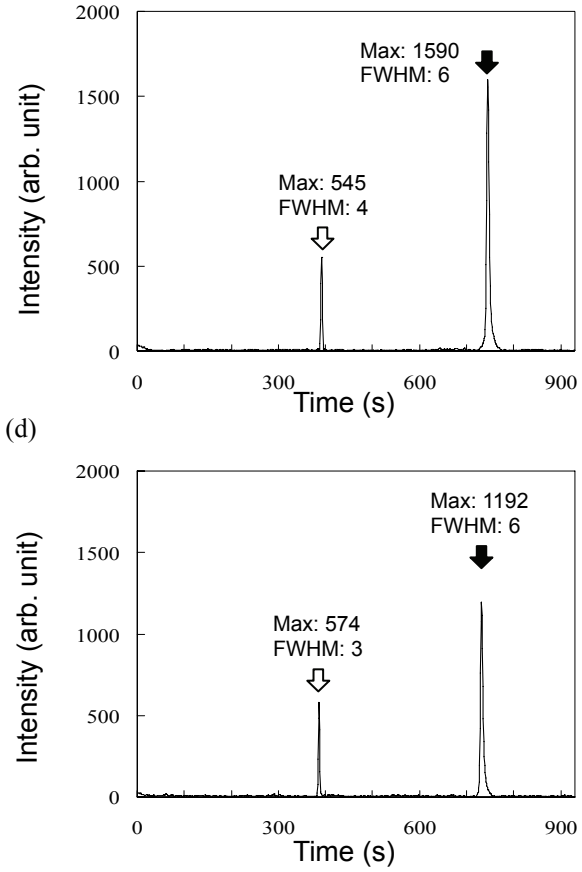


Fig. 10 DNA Electrophoresis chip test
(a) The data before signal processing. (Data in all other figures below was automatically optimized in SV1100). (b) Excimer laser grooved chip without GG development, (c) With GG development. (d) Commercially available chip (injection molded). The unfilled arrow shows the peak for 100bp DNA and the filled arrow shows the peak for 800bp. The maximal value (Max) and full width at half maximum (FWHM) of each peak is shown with each arrow.

The two peaks show that DNA of different sizes is included in the DNA sample. The left peak shows 100bp DNA while the right peak shows 800bp DNA.

In the raw data (Fig. 10 (a)), the signal from the chip without GG etching include high back ground and the back ground signal covered up the signal from DNA. And this data shows the back ground signal level from the chip with GG etching chip is lower than what from injection molded chip (iChip).

In the signal data automatically processed by SV1100, the peaks obtained from the untreated UV Laser prototype chip are extremely small (Fig. 10 (b)). This was because the noise level was so high but when the noise level was depressed, the signal was also depressed. DNA absorption to the channel walls may be another reason why the peaks were slightly wider and very low.

The result from the prototype chip etched with GG solution (Fig. 10 (c)) is comparable with the result from the commercial chip made by injection molding (Fig. 10 (d)).

This shows that while the untreated grooves in PMMA produced with UV Laser grooving are worse than the injection molded groove with regard to sensitivity in an electrophoresis system, the defect can be resolved with the subsequent etching technique we have developed. The technique is practical for making PMMA optical components applicable, for example, to optical sensing micro total analysis systems.

4. Conclusion

While injection molding is a practical method for producing large numbers of chips, it is very expensive for one-off chips.

We have shown that one-off chips that are comparable in performance to injection-molded chips can be produced satisfactorily by laser-milling commercially available PMMA plates. Although laser machining results in an unacceptable increase in autofluorescence, this can be mitigated by subsequently modifying the surface with an organic solvent such as GG Developer.

Acknowledgements

We are indebted to Prof. G. Hashiguchi and Ms. C. Nakata at Kagawa University for their helpful discussions and consent to use their MEMS facilities.

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(Received: April 24, 2007, Accepted: February 09, 2008)