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Measurement of channel depth by using a general microscope based on depth of focus

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Abstract

This paper presents a simple and rapid method to measure the depth of the channel of microfluidic chip. With the depth of focus (DOF) low to few micro-meters, general microscopes show the strong axial (or longitudinal) resolving power. With the assistant of fine-adjustment, the objective was controlled accurately to focus on the channel side and channel bed respectively. Then the depth was calculated from the scales that fine adjustments turned. Experiments results demonstrated the possibilities of depth measurement with normal microscope. Compared with other methods, this method showed high degree of precision with a deviation less than 0.6μm while the magnification was 100X. This method is rapid, simple and reliable, and it is very suitable for monitoring the etching process to optimize the function of microfluidic chip.

Keywords: Microfluidic device / measurement/ depth/ microscope/ depth of focus

1. Introduction

Microfluidic chip was developed rapidly over the past decades due to the unique advantages of small reagent consumption, short analysis time, as well as small dimension, high integration[1-3]. With the improvements of fabrication technique, more and more components are integrated into microchip to perform complicated function (e.g., micro valves and pumps). The dimensions of microstructure fabricated are normally at micrometer levels. For some microstructures etched on glass, high dimensional accuracy is required to ensure

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the implementation of the function. So it is necessary to monitor and control the dimensions of structure during the etching process. The stylus profiler is usually employed in characterizing microfluidic structures. It is a high resolution tool which contacts the surface to be measured and then moves across the surface at a constant velocity to obtain surface height variation. However, some distortions occur in the profile curve at the near vertical channel edges because of the limited resolution and imperfections in the shape of the stylus tip. Distortions may also occur to samples fabricated from relatively soft substrates using the stylus profiler. And moreover, currently the stylus is not a common and cheap enough instrument for normal analytical laboratories. A new simple and indirect method was developed based on a combination of poly(dimethylsiloxane) (PDMS) replication and charge coupled device (CCD) imaging[4]. Despite reliability, non-destructivity, and cheapness and simpleness of this method, it is time-consuming and not convenient for checking the channel size while etching the glass mode. At least one and a half hours were taken on the preparation and polymerization of PDMS prepolymer. Fletcher et al. developed a method for the quantitative 3-dimensional profiling of micro channel networks within optically transparent microfluidic device by capturing digitized microscope images of the channel network filled with an optically absorbing dye[5]. Although this method is accurate and non-destructive technique, it has the limitation that the microfluidic chip must be transparent and the channels are solution-filled. And it is not convenient for a non-bonded chip, the non-bonded chip needs to be cleaned and covered with a cover plate to fill the solution.

Here, we introduced a simple, rapid, non-destructive method to measure the depth of channel only by using a common microscope without a requirement of optical transparency of microfluidic chip. For common analytical laboratories, measurements of depth of channel were realized conveniently and rapidly with this method. The measurement can be finished in one minute, so it is highly suitable for monitoring the depth of channel during wet etching. The principle was described in this paper, and this method was demonstrated to be reliable and effective by experiments results.

2. Principle

Since its invention, the microscope has been a valuable tool in the development of scientific theory. A compound microscope is composed of two elements; a primary magnifying lens and a secondary lens system, similar to a telescope. Light is caused to pass

through an object and then focused by the primary and secondary lens. The function of microscope is to enhance resolution. When considering resolution in optical microscopy, a majority of the emphasis is placed on point-to-point lateral resolution in the plane perpendicular to the optical axis (Fig.1). Another important aspect to resolution which was neglected all the times is the axial (or longitudinal) resolving power of an objective, which is measured parallel to the optical axis and is most often referred to as depth of focus (DOF). Depth of focus (DOF) is the range of object plane position at which the image can be viewed without appearing out of focus for a fixed position of object. The formula is:

$$DOF = \lambda[n^2 - (N.A.)^2]^{\frac{1}{2}} / (N.A.)^2 + (250/M^2) \qquad (1)$$

Where the λ is the wave length, n is the index of refraction, $N.A.$ is the numerical aperture of the object, M is the total magnification of the optical system.

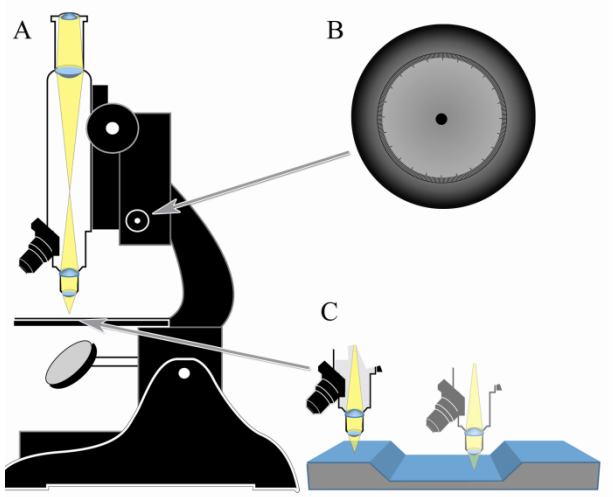

Fig.1 (A) General microscope structure; (B) Fine-adjustment with scales which was employed to calculate the distance that objectives moved down; (C) Focus on the channel side plane and channel bed plane respectively, the distance that objectives moved down corresponded to the depth of channels.

Table 1 presents calculated variations in the depth of focus (DOF) of microscope in our lab (XSJ-2, Chongqing Optical and Electrical Instruments Co., Ltd., Chongqing, China) in a series of objectives with increasing numerical aperture and magnification. With 100X magnification, the depth of focus is limited into only 0.48 μm. It means that only when the object plane was placed within this narrow range, the object can be viewed no loss of sharpness. Hence based on depth of focus, the microscope could be employed to measure the depth of micro channels. The pivotal procedure is that focus the microscope on the open

channel side plane and channel bed plane respectively. The distance that objectives moved down corresponded to the depth of channel.

Table 1. Depth of focus calculated in a series of objectives. (Using a eyepiece 10X, λ=550nm)

Magnification	Numerical Aperture	Depth of Focus (μm)
2.5X	0.07	511.97
4X	0.10	210.97
10X	0.25	33.52
25X	0.40	7.15
40X	0.65	2.55
100X	1.25	0.48

3. Experimental

A normal optical microscope (XSJ-2, Chongqing Optical and Electrical Instruments Co., Ltd., Chongqing, China) coupled with a CCD camera (SDC-313, Samsung, China) was employed for measuring structural dimensions. The glass chip was placed flat on the X–Y adjustable stage of the microscope and illuminated by a halogen lamp. Slowly turn the coarse adjustment until top layer comes into focus. Then use the fine adjustment for fine focusing. The sharp image can be estimated from oculars or CCD camera by human eyes. Then move down the objective, let the bottom come into focus. Calculate the scales the fine adjustment rotates. The depth was obtained by scale-to-length function.

The reliability of this method was studied by employing another direct method as comparison. The microfluidic chip was prepared by breaking across the channel and placed on the stage of the stereo zoom microscopes coupled with a digital camera (K700L, Motic China Group Co., Ltd., China). The images of cross section of channels were captured by digital camera as Fig.2 shows. The dimension data of channel was evaluated from the cross section image with the analysis software Motic Image Plus.

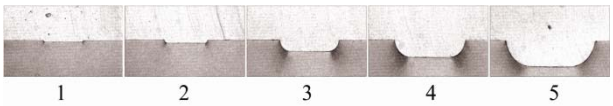

Fig.2 The cross view image of channel captured by digital camera while the chip was break cross the channel. With the assistant of the analysis software of microscope, the depth of channel was measured with high precision.

4. Results and discussion

4.1 Measurement of Channel depth based on low DOF

There is direct relationship between the scales that fine adjustment of microscope is turned and the distance which the objectives move down. The distance corresponds to channel depth d. The depth d is given by following equation:

$$d = \varepsilon \cdot \Delta A \qquad (2)$$

Where d is the depth, ΔA is the scales which fine adjustment turned, ε is the coefficient. To obtain ε, a series of samples were measured as follows. Glass chip were break across the channel. The depth of channels was obtained from cross section image with assistant of analysis software. The thickness of an about 110 μm cover glass was selected as a sample also whose thickness was measured using a micrometer. To obtain the scales which fine adjustment turned, a series of measurements based on DOF were carried out. Fig.3 shows plots of ΔA versus depth of the channel, the ΔA was proportional to depth of channel. From these results, we obtained the coefficient ε and the equation as follows:

$$d = 1.0037A + 0.3205$$

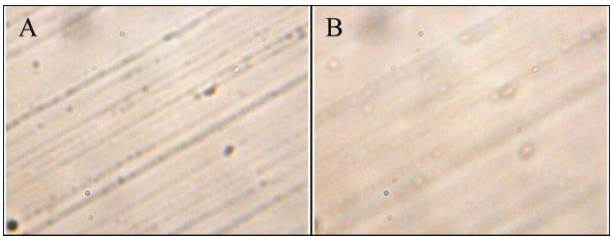

Fig. 3 (A) The micro dusts on the surface were sharply imaged while in the range of depth of focus with magnification of 100X. (B) The image became blurred obviously while objectives moved down 1μm more.

After the coefficient ε was evaluated as 1.0037, this method was applied to actual sample measurement to prove the reliability. A microfluidic chip with five channels had been etched for 5 min, 10 min, 30 min, 50 min, and 70 min respectively. Fig.2 is a CCD cross-sectional image of the glass chip prepared by breaking the chip across the channel. Results from channel depth measurements using the microscope based on DOF are listed in Table 2. Because the micro distance between the objective and the focus plane with high magnification, the magnification of 40X was selected to avoid objective contacting with the top layer while focusing on the channel bottom. In Table 2, relative standard deviations of the measurements were within 3%, with a clear tendency of the precision to improve at larger

dimensions. Results obtained for the same sample using a measuring microscope are also listed in the table for comparison. The results agreed well, with an absolute deviation of less than 0.9 μm. Although the precision of this method is slightly worse, the precision and accuracy is sufficient for the characterization of most microfluidic structures at micrometer levels.

Table 2. Comparison of measurements for the dimensions of sample channels using this method, and cross section view of channel method with digital camera.

Sample No.	This method(DOF)			Cross section		Deviation μm
	Magnification	μm	RSD	μm	RSD	
1	100X	7.1	2.8%	7.4	2.6%	0.3
2	100X	15.2	1.9%	14.6	1.0%	0.6
3	40X	43.6	2.0%	44.3	1.2%	0.7
4	40X	66.4	2.4%	67.5	0.4%	1.1
5	40X	105.4	1.7%	104.8	0.6%	0.6

4.2 Precision of this method

The precision depends on the procedure of focusing on the surface to obtain a sharp image. And the precision of the focus depends on the depth of the focus. In theory, the error of measurements was no more than DOF. With high power objectives high precision could be obtained up to 0.48 μm. However, normally the microfluidic chips are made of glass materials or other optically transparent material. It is difficult to distinguish in-focus and out-focus statuses. No sharp image could be obtained if the surface was smooth and clean absolutely. Fortunately the dust and the channel roughness helped us to determine the focus as Fig.3 shows. Fig.3 illustrated the difference of sharp image and blurred image, image A was taken by CCD under the 100X objectives while the object was placed in the range of DOF, image B was taken while the object was 1μm out of DOF range. It is evident that image B was less of sharpness. Normally, the dusts and roughness were with dimension less than 1μm. By selecting appropriate dusts, the precision of focusing could be enhanced near to the level of DOF.

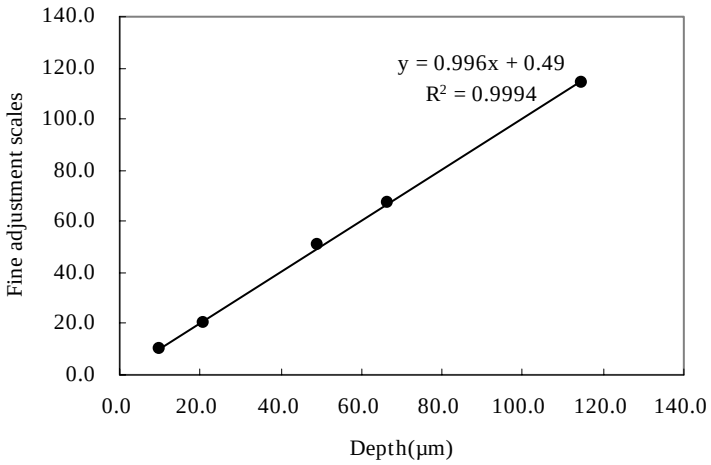

Fig.4 Scales fine adjustment turned while measuring depth the channel versus the depth of channel. There was good linearity in the range 10μm ~110μm (linearity coefficient R^2= 0.9994μm).

4.3 Monitor of etching process

Due to the property of rapidness and convenience, this method was applied in etching process monitoring. The variation of fitted depth of channel with etching time is shown in Fig.4 which indicates that the average etch rate is equal to 1.45 μm/min. The linearity is good in despite of a slight tendency of rate increasing, it indicates that the etch rate is approximately constant. The total monitoring procedure was simple, rapid and non-destructive.

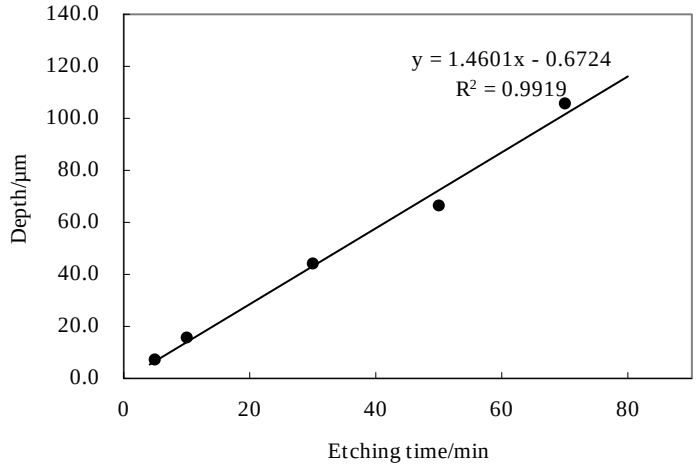

Fig.5 Variation of channel depth versus etching time. The etch rate was approximately a constant of 1.45 μm/min.

5. Conclusion

We have demonstrated a convenient, reliable and rapid method for measurement of depth of channels based on the resolution power of general microscope in axial direction. Each measurement could be accomplished in less than 1 min. Although the precision of this method was not better than other methods, it is nevertheless obvious that it is enough for application at micrometer level. It is very useful for common labs to optimize the function of channels.

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