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Photoresist Micro-Chamber for the Diffracted X-ray Tracking Method Recording Single-Molecule Conformational Changes

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Abstract

The diffracted X-ray tracking method has been established for capturing conformational changes of single KcsA potassium channels, which records position of X-ray diffraction spot from a gold nanocrystal attached to the channel. The conventional observation chamber setup could trace a diffraction spot; however relatively high background noise resulting from X-ray scattering degraded a spatial resolution. This paper report a novel low-noise microfabricated observation chamber consisting of SiN membrane window supported by Si and embedded microchannel in negative photoresist. The measured noise exhibited lower level compared to the conventional chamber, which almost reached the detection limit of the employed imaging system.

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Keywords: Microfluidics; DXT, diffracted X-ray tracking; Single molecules; Three-deimensional microfabrication, Photoresist

1. Introduction

Activity of ion channel is modulated by signals that tightly control the opening and closing of the channel, allowing ions to cross the membrane in response to cellular signals. Crystal structures of several kinds of potassium channels have revealed open and closed conformations, and the basic architecture was elucidated [1]. However, a mechanism underlying the gating of ion permeation pathways has not been well studied. Thus capturing the motion of ion channels at the single-molecule level is necessary for further understandings.

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Among the several imaging techniques to capture conformational changes of ion channels [2], the diffracted X-ray tracking (DXT) method is a promising approach since this method enables to record conformational changes of the channels with high spatial and temporal resolutions [3]. However, high background noise resulting from X-ray scattering of the observation chamber has limited the spatial resolution. In order to minimize background noise, leading to high-resolution imaging, this paper propose a novel microfabricated chamber consisting of SiN membrane window supported by Si and embedded microchannel fabricated by three-dimensional (3D) photolithography.

2. Diffracted X-ray Tracking Method

2.1. Principle

The DXT method (Fig. 1) has been established for capturing conformational changes of single KcsA potassium channels. In the observation chamber, KcsA potassium channels are fixed on a chemically modified substrate surface. A gold nanocrystal with an average size of 20-50 nm is then attached to a KcsA potassium channel and irradiated with white X-rays. Motion of the diffraction spots from the nanocrystal is recorded through a CCD camera located downstream.

The position of the diffraction spot on the image plane is expressed as the polar co-ordinate (Fig. 1). As the nanocrystal tilts by the angle θ from the channel axis, the diffraction spot moves along the radial direction (the angle 2θ). When the nanocrystal rotates around the beam direction or the channel axis, the diffraction spot moves circumferentially (the angle χ). Thus, the position and motion of the diffraction spot on the image plane represents posing orientation of the nanocrystal in the 3D space (the spherical polar co-ordinate). The trace locations are translated into movements of channel in real space.

2.2. Observation chamber

The DXT method has been conducted with the observation chamber consisting of Polydimethylsiloxane (PDMS) and glass substrate, and this setup successfully captured conformational changes of single KcsA potassium channels (Fig. 2); however, high background noise observed along the border of beam stopper's shadow has limited the record of the diffraction spots, and spatial resolution is not good enough even though the diffraction spot is detected. This background noise can be resulting from X-ray scattering of the observation chamber.

In order to minimize background noise, leading to high-resolution imaging, thinned observation chamber is one of the promising approach because the intensity of X-ray scattering depends on material and thickness. We have considered the potential of 3D microfabrication and propose a novel microfabricated chamber shown in Fig 3. The proposed chamber consists of two parts; SiN membrane window supported by Si and embedded microchannel in negative photoresist. The main advantage of this structure is the reduced thickness of both chamber ceiling and membrane substrate while keeping its mechanical strength for fluidics.

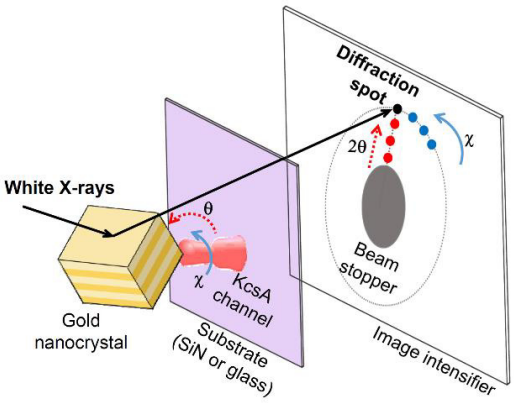

Fig. 1. The principle of the DXT method.

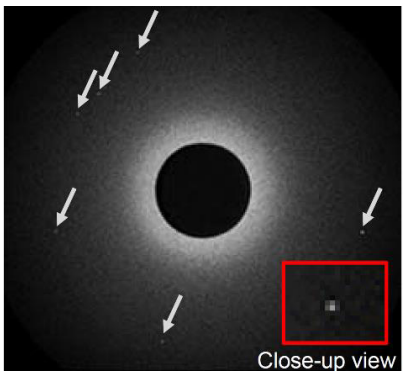

Fig. 2. The diffraction spots from the gold nanocrystal.

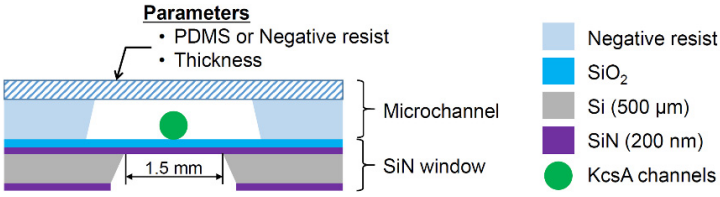

Fig. 3. Cross-sectional view of the proposed chamber.

3. Experimental

3.1. Fabrication

The chamber fabrication in Fig. 4 stars with, (a) SiN window was patterned by photolithography for subsequent KOH etching (b), which releases the SiN membrane having a thickness of 200 nm. (c) SiO_2 was deposited on the front side by plasma-enhanced chemical vapor deposition (PECVD) to fix KcsA potassium channels. (d) Microchannel embedded in photoresist were directly fabricated by moving-mask UV photolithography because this approach can fabricate a thin chamber ceiling exploiting negative photoresists (e.g. SU-8, TMMR/TMMF) properties. More details on the embossed microchannel fabrication and process parameters have been reported elsewhere [4]. The fabricated microchannel are utilized for sample preparations, such as surface modification, KcsA potassium channels introduction and gold nanocrystal attachment to the KcsA potassium channels. Further, in order to compare the background noise dependence on thickness for different materials, chamber with PDMS ceiling was fabricated using UV/O_3 treatment bonding. These fabrication process of chamber ceiling and its thickness are summarized in Table I.

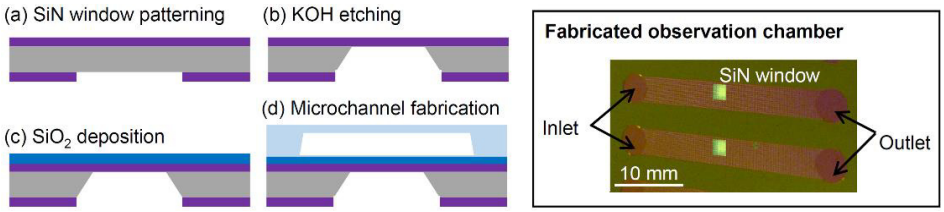

Fig. 4. Fabrication procedure of the proposed chamber structure. Inset is the optical image of the fabricated chamber.

Table I. Summary of the process for the chamber and the material and thickness of channel ceiling.

No.	Material	Method	Thickness	No.	Material	Method	Thickness
1	TMMR	3D lithography	7.4	4	PDMS	Bonding	221
2	TMMF	Lamination	53.8	5	PDMS	Bonding	696
3	TMMF	Lamination	102	6	PDMS	Bonding	1560

3.2. Noise evaluation

To evaluate the background noise of the chamber, fabricated chambers were irradiated with high flux white X-rays from the synchrotron radiation facility (the beamline BL28B2, SPring-8, Japan), and image was recorded with a 2D X-ray camera system for 0.02 sec at a video rate 5000 frames s^{-1}. The recorded images were further analysed statistically by fitting multivariate normal distribution. The noise level a can be expressed as:

$$g(x,y) = \frac{a}{\sqrt{2\pi\sigma^2}} \exp\left(-\frac{(x-x_c)^2 + (y-y_c)^2}{2\sigma^2} \right) \quad (1)$$

where x_c and y_c are the measured position on the image plane (the co-ordinate centred on the point of X-ray irradiation), and σ is the standard deviation.

4. Results and Discussion

Figure 5 shows the recorded images by comparing the conventional PDMS/glass chamber and the proposed photoresist/SiN one. The photoresist/SiN chamber indicated much lower background noise. The measured background noise a was further evaluated quantitatively by the average of 100 images captured. Figure 6 shows the noise level dependence on thickness for different materials of the chamber ceiling. When the thickness of the ceiling was thinned, the noise with both materials decreased and the noise of photoresist almost reached the detection limit of the imaging system. Furthermore, the noise of thin photoresist ceiling exhibited lower level compared at the same thickness of PDMS. These results demonstrated effectiveness of the proposed fabrication using negative photoresist, with respect to not only minimizing the noise by reducing the thickness of chamber ceiling but also keeping its mechanical strength due to higher Elastic modulus of negative photoresists.

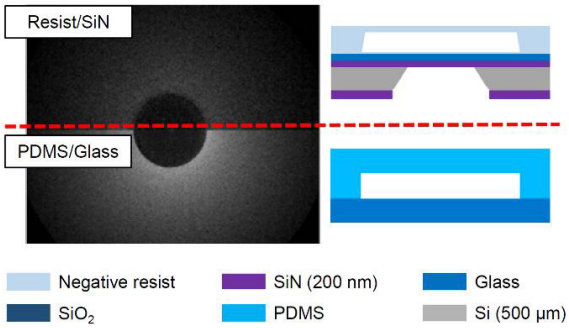

Fig. 5. Comparison of the background noise.

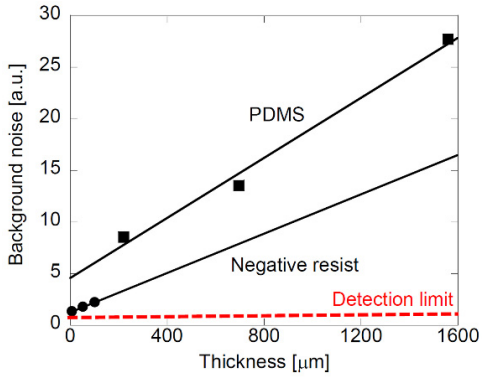

Fig. 6. Noise level dependence on thickness for different materials

5. Conclusion

We reported the low-noise observation chamber fabricated by 3D photolithography and an evaluation of its background noise. The noise level of the proposed chamber exhibited the lowest, which almost reached the detection limit of the employed imaging system. Therefore, we hope to continue our investigation of recording conformational changes of the KcsA potassium channels, particularly with respect to improvement of spatial resolution, in future research.

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References

[1] B. Hille, Ion channels of excitable membranes, third ed., Sinauer Associates, Sunderland, MA, 2001.
[2] S. Oiki, Channel function reconstitution and re-animation: a single-channel strategy in the postcrystal age. J. Physiol. 593 (2015) 2553–2573.
[3] H. Shimizu, M. Iwamoto, T. Konno, A. Nihei, Y. Sasaki, S. Oiki, Global twisting motion of single molecular KcsA potassium channel upon gating, Cell 132 (2008) 67–78.
[4] Y. Hirai, K. Sugano, T. Tsuchiya, O. Tabata, Embedded microstructure fabrication using developer-permeability of semi-crosslinked negative resist, J. Microelectromech. Syst. 19 (2010) 1058–1069.