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パラフィン切片の3次元解析への応用

The application for 3D analysis of paraffin section

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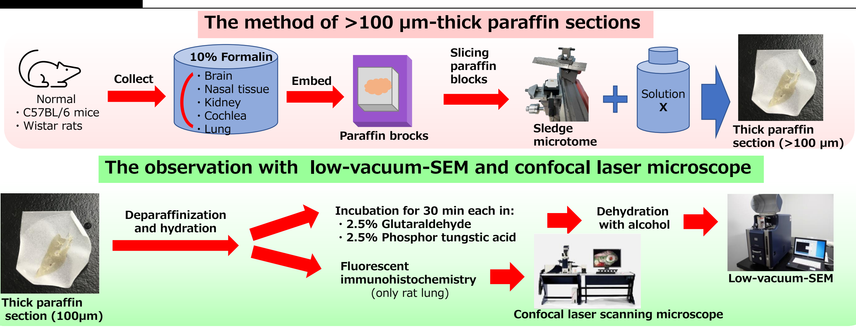
Background

Recently, 3-demintional (3D) analysis of organism tissue is increasing with the spread of confocal laser scanning microscope and simple scanning electric microscope (SEM) which need appropriate samples. Paraffin sections are popular and useful for histopathology. Some scientists have tried to apply paraffin sections for analysis using the 3D microscopes. However, the sections are too thin to efficiently get 3D information. Therefore, we developed a simple method for making paraffin sections at >100 μm in thickness and determined whether the thick sections were applicable for the 3D analysis.

Aim

The application of the thick paraffin section for 3D analysis

Material and Method



Result

The observation with low-vacuum-SEM

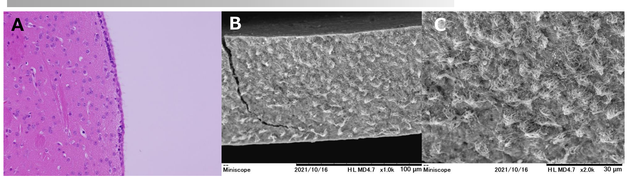


Fig.1 Cilia of ependymal cells.
A, an optical-microscope image of lateral ventricle (HE stain); **B**, a SEM image of the continuous thick paraffin section to the section of A; **C**, a magnified image of B.

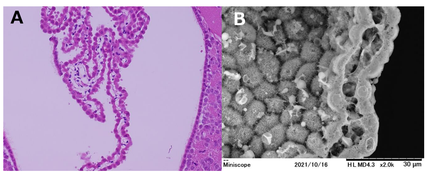


Fig.2 Choroid plexus.
A, an optical-microscope image of choroid plexus (HE satin); **B**, a SEM image of the continuous thick paraffin section to the section of A.

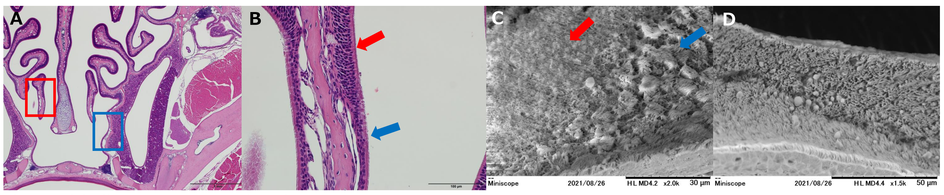


Fig.3 Epithelia of mouse nasal cavity.
A, an optical-microscope image of nasal cavity (HE stain) ; **B**, the magnified image of the red frame in the A. Red and blue arrows indicate olfactory and respiratory regions, respectively; **C**, a SEM image of the continuous thick paraffin section to the section of B. Red and blue arrows in C indicate the same regions of B; **D**, a SEM image of the blue-frame region in A, respiratory region. All SEM images show epithelial surfaces from cavity side.

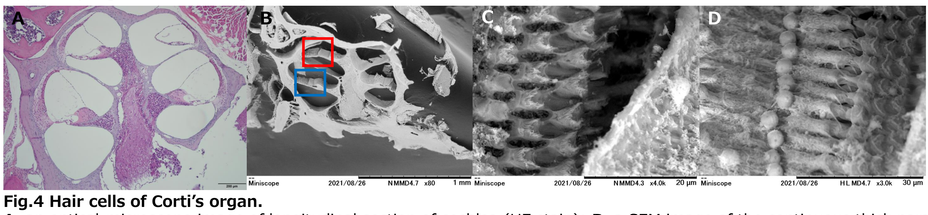


Fig.4 Hair cells of Corti's organ.
A, an optical-microscope image of longitudinal section of cochlea (HE stain); **B**, a SEM image of the continuous thick paraffin section to the section of A; **C**, the magnified image of the red frame in the B, showing auditory hairs of outer hair cells; **D**, the magnified image of the blue frame in the B. C and D shows auditory hairs of outer hair cells in Corti's organ.

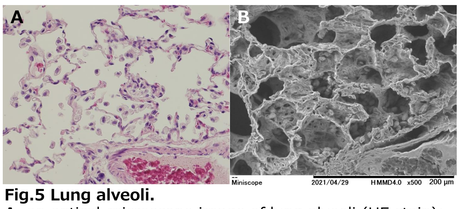


Fig.5 Lung alveoli.
A, an optical-microscope image of lung alveoli (HE stain); **B**, a SEM image of the continuous thick parafin section to the section of the A. Asterisks indicate the same arteriole.

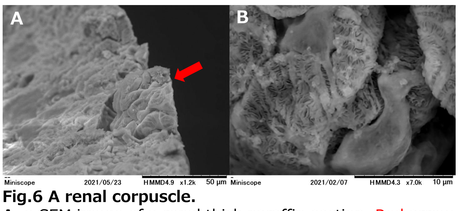


Fig.6 A renal corpuscle.
A, a SEM image of a renal thick paraffin section. Red arrow indicate a glomerulus. **B**, a SEM image of podocytes.

The observation with confocal laser maicroscope

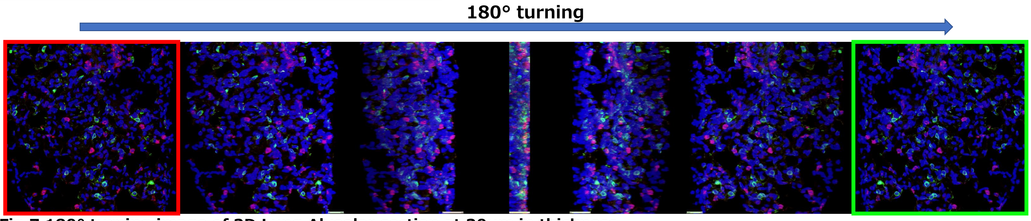


Fig.7 180° turning image of 3D Lung Alveolar section at 30μm in thickness
Blue, Cell nuclei (Hoechst); **Green**, immature type-1 epithelial cells (labeled with anti-HOPX antibody); **Magenta**, type-2 epithelial cells (labeled with anti-surfactant protein C antibody). The sequential figures show a turning section from red to green frames.

Discussion and Conclusion

- The observation by SEM
  - The thick paraffin sections in **continuous to the HE stained specimens** enabled to three-dimensionally observe cell surface structures on cavity sides of the thick paraffin sections even under x3000 magnification.
  - The image could be contrasted with images by an optical microscope.
- The observation by confocal laser scanning microscope
  - The thick paraffin sections enabled to observe and detect the expression of HOPX and surfactant protein C up to about 30 μm in depth from tissue surface.

Thick paraffin section is more useful in histopathological 3D analysis.