APPLICATION NOTE

N-SIM S Super Resolution Microscope

# Quantitative analysis of lesions of renal foot processes and mitochondria, reusing optical microscopy specimens for clinical renal biopsies, made possible by structured illumination

Foot process fusion of renal glomerular epithelial cells and mitochondrial damage (morphologically fragmentation and swelling) of tubular epithelial cells are involved in the onset and progression of renal disease. Although an electron microscope is usually required to visualize these microstructural changes, most renal biopsy tissue is prepared as an optical microscope specimen, and the specimens observed with an electron microscope are so small that important lesions could be overlooked. Establishing a method for evaluating these lesions over the entire collected tissue using an optical microscope specimen has therefore been anticipated.
Although dyes for histopathological staining are optimized for visualization with brightfield observation, these dyes possess fluorescent properties peculiar to each dye. Dr. Ayumi Matsumoto, Dr. Isao Matsui and Dr. Yoshitaka Isaka of the Graduate School of Medicine, Osaka University et al. focused on the fluorescent properties of dyes for pathological tissue staining. They established a method for quantitative analysis of lesions of foot processes and mitochondria by observing pathological sections for the diagnosis of kidney disease with the N-SIM S structured illumination super-resolution microscope, without additional fluorescent staining. In this application note, we introduce examples of the N-SIM S being used as described in the report of their research.

Keywords: N-SIM S, super-resolution, structured lighting, kidney disease, renal biopsy, EMT stained sections, non-fluorescent staining

## Overview

In super-resolution microscope observation, fluorescent immunostaining sections are usually used, depending on the purpose of the observation. If it is a large tissue sample, a new fluorescent immunostaining section can be created for each observation purpose, but because a renal biopsy sample is a columnar tissue with a diameter of less than 1 mm, creating a new fluorescent immunostaining section may lead to sample loss. Therefore, it has been considered useful if glomerular epithelial cell foot process information and tubular epithelial cell mitochondrial morphological information can be directly detected from sections prepared for clinical practice.
Since dyes for pathological staining have fluorescence properties peculiar to each dye, paraffin-embedded sections prepared in daily clinical practice and stained with HE, PAS, PAM and EMT were observed with the N-SIM S structured illumination super-resolution microscope. As a result, it was found that the above-mentioned microstructure can be visualized by using Elastica-Masson trichrome (EMT) stained sections (Fig. 2a, 2b, Fig. 3a and Fig.4).
In this study, Dr. Matsumoto et al. established a method for quantifying observed microstructural changes using the Fourier transform. When human renal biopsy samples were evaluated using this method, the degree of disruption of glomerular epithelial cell foot processes was correlated with the amount of urinary protein (Fig. 2c). It was also found that the degree of mitochondrial structure disruption correlates with the rate of renal function decline (Fig. 3b).

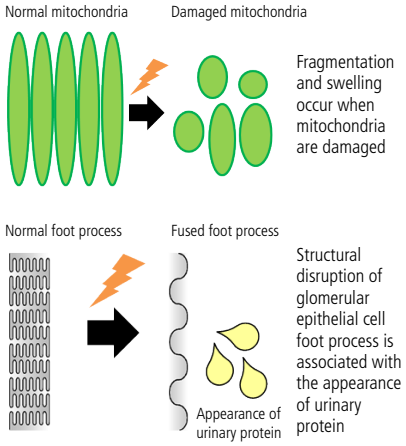


Fig. 1: Schematic diagram of disruption of mitochondrial structure and glomerular epithelial cell foot process structure

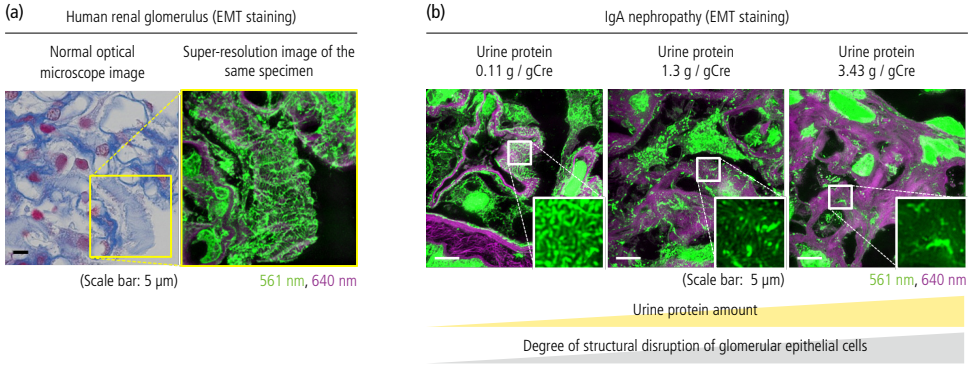


Fig. 2: Visualization and quantification of human glomerular epithelial cell structure disruption in EMT-stained sections, using the N-SIM S structured illumination super-resolution microscope
(a) Observation of EMT-stained human kidney tissue with the N-SIM S reveals glomerular epithelial cell foot processes (green) at 561 nm excitation and basement membrane (magenta) at 640 nm excitation.
(b) In patients with IgA nephropathy, it was observed that the higher the amount of urinary protein, the higher the degree of structural disruption of the foot process.
(c) There was a correlation between the degree of foot process disruption and amount of urinary protein in patients with minor glomerular abnormalities (cases that maintain a close-to-normal structure), minimal change nephrotic syndrome, and IgA nephropathy.

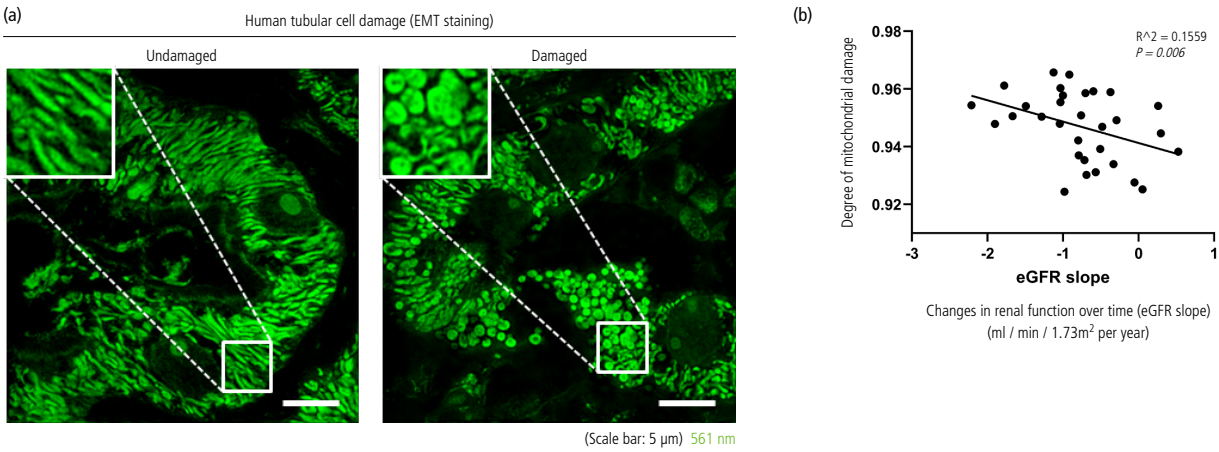


**Fig. 3: Visualization and quantification of human tubular cell mitochondrial damage in EMT-stained sections, using the N-SIM S structured illumination super-resolution microscope**

(a) When the tubular interstitial region of EMT-stained human renal tissue was observed with the N-SIM S, mitochondria were visualized by 561 nm excitation. Long tube-like structures of mitochondria were observed in the parts without cell damage, and fission and swelling of mitochondria were observed in the damaged parts.

(b) When the observed changes were quantified using the Fourier transform, a correlation was found between the degree of mitochondrial injury at the time of the renal biopsy and the rate of renal function decline after renal biopsy in patients with IgA nephropathy. This indicates that the degree of mitochondrial damage may predict future renal dysfunction.

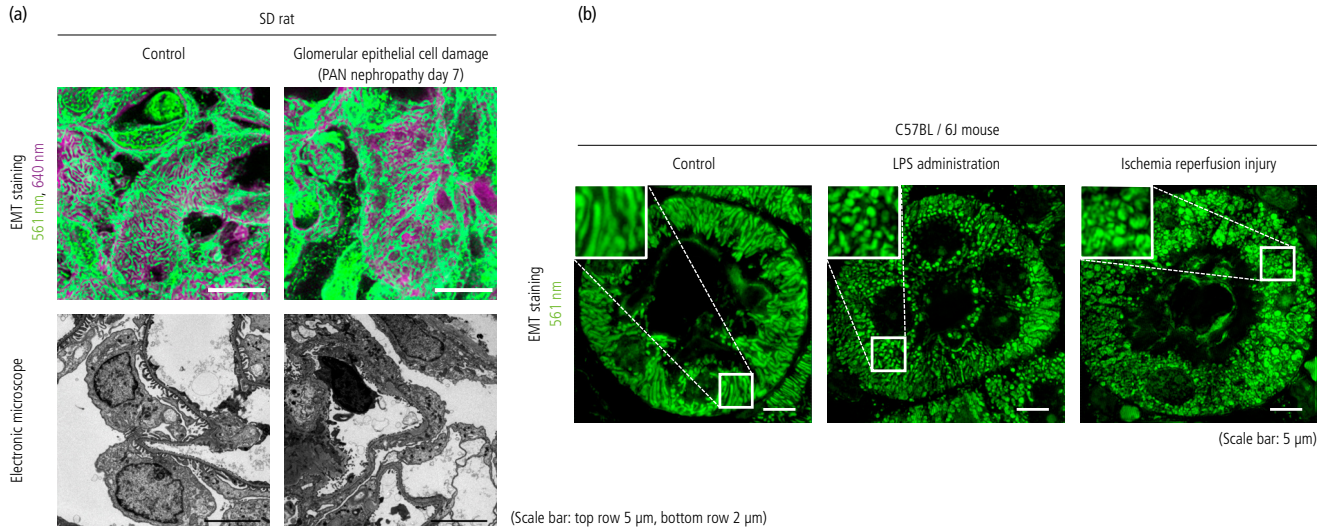


**Fig. 4: Visualization of glomerular epithelial cell structure disruption and mitochondrial damage in animal models, using the N-SIM S structured illumination super-resolution microscope**

Renal lesions in animal models can also be visualized using EMT-stained tissue.

(a) In the glomerular region of normal rats, the glomerular epithelial cell foot process structure was observable, and disruption of this structure was observed in the disorder model (PAN nephropathy). These microstructural changes were consistent with the findings obtained by electron microscopy.

(b) In a mouse model of tubular stromal disorders due to LPS administration or ischemia-reperfusion injury, mitochondrial fragmentation and swelling in tubular epithelial cells were observed.

## Summary

It was clarified that the degrees of glomerular epithelial cell foot process fusion and tubular epithelial cell mitochondrial damage in human renal biopsy tissue can be quantitatively evaluated using pathologically stained specimens for optical microscopy (Fig. 2 and Fig.3). It was also clarified that these microstructural changes correlate with clinical parameters. Disease-related changes in the structure of glomerular epithelial cell foot processes and mitochondria could also be observed in animal renal impairment models (Fig.4).

It is difficult to observe pathologically stained tissue with a STED microscope, because the dye for pathological staining is photobleached by strong light. However, using the N-SIM S structured illumination super-resolution microscope, it was possible to obtain a super-resolution image without photobleaching the dye of the pathologically stained specimen. With this method, it is expected that more useful information can be extracted from human biopsy tissues, and a detailed understanding of pathological conditions can be achieved.

## References

**Quantitative Analyses of Foot Processes, Mitochondria, and Basement Membranes by Structured Illumination**

*Kidney Int Rep*. 2021 Jul; 6 (7): 1923–1938.

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## Product Information

### N-SIM S Super Resolution Microscope

The N-SIM S utilizes Structured Illumination Microscopy (SIM) technology to capture the minute structures within a specimen at twice the resolution of conventional light microscopes.

- Lateral resolution: 115 nm (3D-SIM mode), 86 nm (TIRF-SIM mode)
- Axial resolution: 269 nm (3D-SIM mode)
- Field of view: Up to 66 μm x 66 μm (with a 100X objective)