Development of a Pulsed-Transmission Electron Microscope and Observation Technique for Capturing Sub-Millisecond Dynamics in Solution

Ryoya Katayama[1, 2], Tomohiro Nishitani[2], Yohei Otsuka[2], Yuta Arakawa[2], Daiki Sato[2], Tomoharu Matsumoto[1], Akihiro Narita[1]

[1] *Grad. Sch. Science, Nagoya Univ., 464-8601, Japan.*

[2] *Photo electron Soul Inc., 464-0858, Japan.*

The development of cryo-electron microscopy has revolutionized structural biology. With recent advancements in computational technologies, atomic-resolution observation of biological specimens has become more accessible. However, visualizing the rapid dynamics of proteins moving freely in aqueous solutions remains a significant challenge. To address this issue, we developed a new pulsed transmission electron microscope (pulsed-TEM) using GaN-type photocathode technologies and a solution cell for biological samples.

In our pulsed-TEM, the conventional thermionic electron gun of a commercially available TEM was replaced with a photoelectron-based gun. Unlike conventional sources, this gun can generate a high-peak-current electron beam by irradiating a GaN-based semiconductor photocathode with a pulsed laser [1]. This capability allows us to maintain the total electron dose per frame equivalent to that of a continuous beam, even with a short duration. The solution cell consists of two 2.1 mm square Si substrates featuring electron-transparent windows made of SiN or SiC. This cell can maintain liquid specimens, such as colloidal gold or protein solutions, within a layer thickness of approximately 50-100 nm under high-vacuum conditions [2]. In this study, specimens were observed using a 40 keV pulsed electron beam with irradiation durations as short as 30 μs. Images were acquired using a Gatan Ultrascan 4000 CCD camera.

Figure 1 shows TEM images of a holey carbon grid captured using standard continuous-beam (DC) and pulsed electron beam. When observing the grid moving laterally at a speed of 3 μm/s, the DC beam resulted in significant motion blur. In contrast, the pulsed beam (300 μs pulse width) yielded sharp images despite the motion. Crucially, the high flux of the pulsed beam ensured sufficient signal-to-noise ratios, confirming that the image quality obtained with the pulsed beam during motion is comparable to that of a stationary specimen. We will also report on the observation of gold colloidal particles in liquid using the solution cell and discuss the application to the protein dynamics observation.

[1] D. Sato, H. Shikano, A. Koizumi, and T. Nishitani, J. Vac. Sci. Technol. B 40, 064204 (2022).

[2] R. Katayama, T. Yamasaki, T. Matsumoto, A. Narita, SEIBUTSU BUTSURI 63, Supp. 1-2, p.S439 (2023).

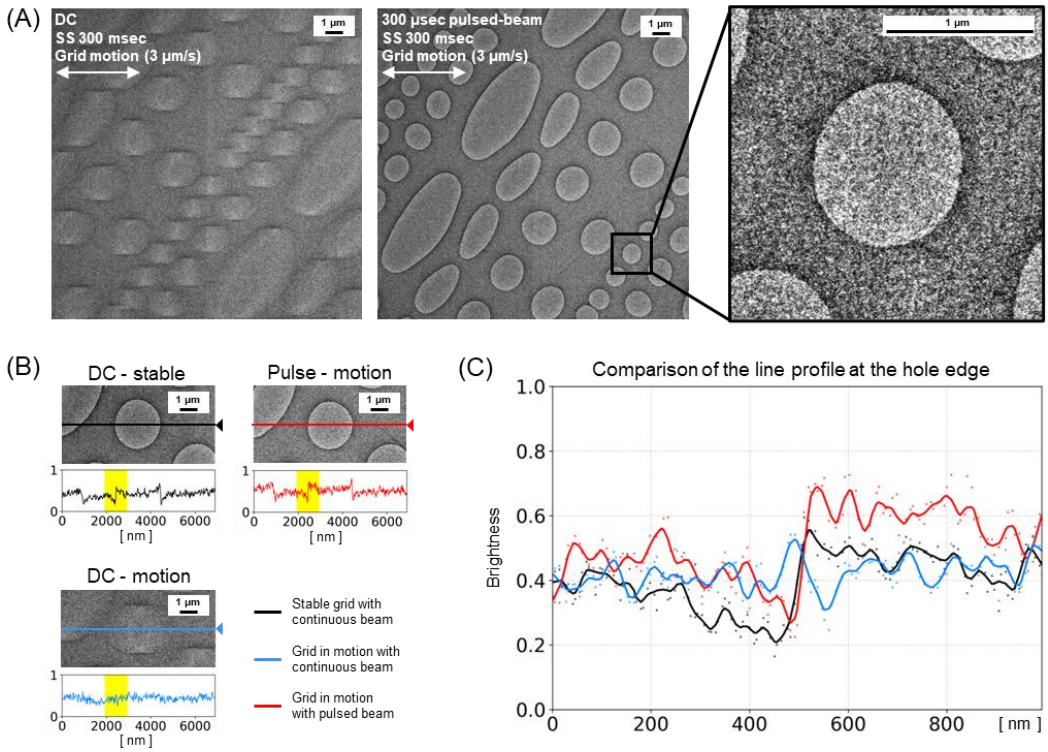

Figure 1: Suppression of motion blur using the pulsed-TEM.
(A) Imaging of a moving carbon grid. The grid was moved laterally at 3 µm/s during a 300 ms exposure. **Left:** Image acquired with a continuous (DC) electron beam. **Center:** Image acquired with a 300 µs pulsed electron beam. **Right:** Magnified view of the boxed area in the center image. The pulsed beam was synchronized with the camera exposure (single pulse per frame). **(B)** Brightness profiles along the horizontal lines in each condition were averaged over the line width (approx. 90 nm) and plotted as relative values. **(C)** Overlay of the dot style plots of the yellow-color-highlighted regions in Fig.1(B). Solid lines represent smoothed curves fitted by LOWESS using a data span of 50 nm. In the black and red profiles, the hole edges are distinct. This demonstrates both the coherence of the pulsed beam and the effective suppression of motion blur by pulsed illumination.