

PLACING A STAIR STEP DEVICE AT THE OPTIMAL PUPIL PLANE IS CRITICAL FOR EXTENDED DEPTH OF FOCUS IMAGING

Yosuke Okita, Shunsuke Matsuda, Junpei Ohgita, Shigeo Watanabe, Katsuhide Ito and Hirotohi Terada

Systems Division, Hamamatsu Photonics K.K., Hamamatsu, Japan

Email: shigeo-w@sys.hpk.co.jp

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The stair step device (SSD, Figure 1) was originally developed for extended depth of focus (DOF) imaging by Abrahamsson et al [1]. This device has circular symmetric stair steps that divide the pupil plane into a series of annular zones. By introducing phase delays in the incident light for each zone this device creates a smooth and circularly symmetric point spread function, resulting in the extended DOF. The SSD extends the DOF five-fold, with 70% optical throughput, and a 20% degradation in the lateral resolution.

Regardless of the benefits of SSD, the practical application to microscopic imaging has previously been difficult. Proper function of the SSD requires placing the SSD at pupil plane, but is difficult in practice as the exact pupil plane is not precisely accessible due to the microscope architecture. Using simulation we investigate the effect of the axial location of SSD near the pupil plane on extended DOF imaging. The results show that placing the SSD at only 20 and 45 mm away from secondary pupil plane causes the point spread function (PSF) of point source at higher image height to become elliptically shaped and shifts the position (Figure 2). In commercial microscopes, the location of the pupil plane is both hard to estimate and control, therefore proper positioning on the SSD, while theoretically easy, is practically difficult.

These results indicate that the precise axial location of SSD at pupil plane is critical for high image quality with extended DOF over the entire field of view. The Hamamatsu W-VIEW GEMINI-2C can control the location of secondary pupil plane and SSD precisely, therefore this product could be useful for extended DOF imaging.

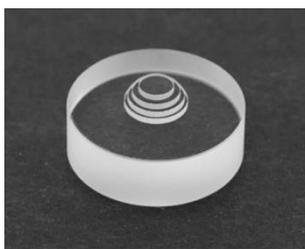


Figure 1: Stair Step Device (SSD)

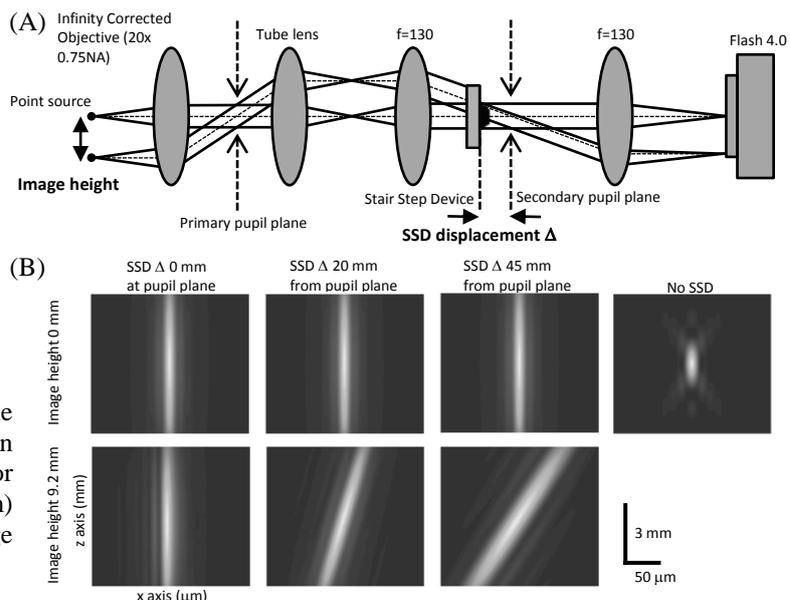


Figure 2: (A) Schematic diagram of the emission light path used for simulation study. (B) Sum of calculated PSFs for SSD displacement (0, 20 and 45 mm) and point sources at different image heights (0 and 9.2 mm)

[1] S. Abrahamsson et al, "A new approach to extended focus for high-speed, high-resolution biological microscopy" *Proceeding of SPIE* **6090**, 60900N (2006)