# EXTRACTION OF DEPTH INFORMATION USING AN AIRY BEAM IN LASER SCANNING MICROSCOPY WITH AN OPTICAL NEEDLE

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**KEY WORDS:** Laser scanning microscopy, volumetric imaging, structured light

Laser scanning microscopy utilizing detection with a confocal pinhole or a multi-photon excitation technique has a great advantage for acquiring three-dimensional (3D) images of specimen due to the ability of optical sectioning. Constructing a 3D image is, however, achieved by stacking multiple two-dimensional (2D) images with moving the focal position, which is, in general, a time-consuming process if the number of slices is large. Here, we propose a novel method that enables a 3D, volumetric imaging without moving an observed plane in a framework of fluorescent laser scanning microscopy.

Our method is based on a 2D scanning of a Bessel beam that produces an optical needle spot with a long depth of focus (DOF). The 2D scanning of the optical needle spot superimposes the whole 3D structure of a specimen within the DOF on a single image while its depth information is lost. To extract the depth information from such an image, we apply the non-diffracting and self-bending characteristics of an Airy beam [1,2] for detection. When a fluorescence signal is converted into an Airy beam, the centroid of the Airy beam shifts along the lateral direction at the image plane in accordance with the axial (depth) position of the fluorophore. Therefore, the intensity distribution along the lateral direction of the converted fluorescence signal at the image plane reflects the spatial distribution of fluorophores along the axial direction of the specimen at each scanning position of the optical needle spot excitation.

We verified the concept by building a laser scanning microscope equipped with a piezo stage. We focused a 532-nm laser with an annular amplitude mask to produce an optical needle at the focus of a water immersion objective lens with a numerical aperture of 1.15. An orange fluorescent bead with a diameter of 200 nm fixed on a cover glass was located at the focus. The fluorescence was converted into an Airy beam by a spatial light modulator and detected by a high-sensitive 2D array detector. As shown in Fig. 1, we confirmed the displacement along the lateral direction for the measured bead's image when the bead was moved along the axial direction within the needle spot. Hence, 3D image construction with depth discrimination can be expected by using Airy beam conversion for detection in laser scanning microscopy with optical needle spot scanning.

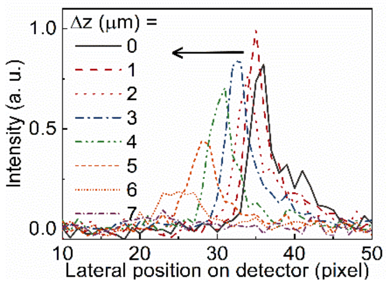


Fig. 1. Measured intensity profiles of an Airy beam emitted from a fluorescent bead at different axial positions ($\Delta z$).

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