Airy-beam tomographic microscopy

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Abstract

We introduce Airy-beam tomographic microscopy (ATM) for high-resolution, volumetric, inertia-free imaging of biological specimens. The work exploits the highly adjustable Airy trajectories in the three-dimensional (3D) space, transforming the conventional telecentric wide-field imaging scheme that requires sample or focal-plane scanning to acquire 3D information. The results present a consistent near-diffraction-limited 3D resolution across a tenfold extended imaging depth compared to wide-field microscopy. We anticipate the strategy to not only offer a promising paradigm for 3D optical microscopy, but also be translated to other non-optical waveforms.

The development of self-accelerating Airy beams [1,2] in the past decade has triggered a resurgence of interest in nondiffracting waves [3]. As exact solutions of the paraxial wave equation, Airy beams can propagate over many Rayleigh lengths without appreciable diffraction, are self-healing after being obscured in scattering media, and undergo lateral displacement as they propagate, resulting in a curved self-accelerating trajectory [1,4,5]. These unique properties have rapidly drawn broad interests, in areas ranging from optical manipulation [6], laser filamentation [7], micromachining [8], and nonlinear optics [9], to the generation of varying nondiffracting waveforms, such as electron beams [10], plasmonic waves [11], acoustic waves [12], and quantum particles [13].

Notably, among many applications, recent years have witnessed the emergence of Airy-beam-enabled optical imaging, such as precise (3D) localization of single molecules in superresolution microscopy [14] and the enhanced field of view and image quality in light sheet microscopy [15,16]. However, these methods primarily implemented Airy beams as static point-spread functions (PSFs) of the microscopy systems, while it remains to be fully explored the highly adjustable Airy trajectories in the entire 3D space for volumetric imaging of biological specimens.

Here, we introduce Airy-beam tomographic microscopy (ATM), an approach that allows for mechanical-scanning-free, volumetric 3D cell and tissue imaging using nondiffracting, self-accelerating Airy beams. Conventional wide-field imaging techniques are telecentric, which produce orthographic views and thereby must acquire the 3D information in a scanning...
fashion. In contrast, the self-accelerating propagation trajectory of an Airy beam innately forms a perspective view of the object. Therefore, given sufficient perspective views by manipulating the Airy trajectories, the entire volume can be computationally synthesized in a tomographic manner (Fig. 1). Such an imaging scheme exploits the self-acceleration and maneuverability of Airy beams, circumventing the need for sample or focal-plane scanning as in many conventional wide-field systems. Furthermore, the nondiffraction of Airy beams effectively mitigates the trade-off between the axial Rayleigh length [i.e., the depth of focus (DOF)] and the lateral beam diffraction, thus offering a depth-invariant resolution across a substantially improved DOF [17].

In practice, each Airy beam [i.e., a 2D exponentially truncated Airy function \(Ai(x/a_0, y/a_0)\)], can be generated by the Fourier transform of a Gaussian beam \(A_0 \exp[-(k^2 x^2 + k^2 y^2)/w_0^2]\) modulated by a cubic spatial phase \((k_x h_0)^3 + (k_y h_0)^3\), where \(a_0, h_0, w_0\), and \(A_0\) are scale factors, and \((x, y)\) and \((k_x, k_y)\) are the spatial coordinates and the corresponding spatial-frequency components, respectively [1]. Experimentally, we used aspatial light modulator (SLM) to implement the cubic phase at the Fourier plane of the detection path of a wide-field microscope [Fig. 2(a) and Supplement 1]. The use of the SLM allows adjustment of the scale factor \(h_0\) to control the cubic phase and thus determine the self-accelerating property of the Airy beams.

Using the coherent model [6,7], the curved trajectory of an Airy main lobe can be described as \(\Delta l' = Az'^2 = \frac{1}{2k'}x'^2z'^2\), where \(\Delta l'\) and \(z'\) are the lateral displacement and the axial propagation distance, respectively, both in the image space; \(A\) is the acceleration coefficient; \(k = 2\pi/\lambda\) is the wavenumber; and \(x_0'\) is the lateral size of the main lobe.

By rotating the phase mask by azimuthal angles of \(\Delta \phi\) on the SLM, we are able to steer Airy beams into various accelerating orientations, thus generating views of the object from a full set of perspectives [Figs. 1 and 2(a)]. In this case, using the above equation, the relation between the accelerating trajectory of the Airy main lobe at the intermediate image plane and its projection on the camera plane can be described as

\[
\begin{bmatrix}
  x_c \\
  y_c
\end{bmatrix} =
\begin{bmatrix}
  1 & 0 & A \cos(\Delta \phi) \\
  0 & 1 & A \sin(\Delta \phi)
\end{bmatrix}
\begin{bmatrix}
  x' \\
  y' \\
  z'^2
\end{bmatrix},
\]

where \((x', y', z')\) and \((x_c, y_c)\) represent the coordinates of an emitter in the image space and at the camera plane (i.e., the intersection of the Airy trajectory at \(z_c = 0\)), respectively. Therefore, the 3D volume of the image space can be retrieved as an inverse problem using the tomographic camera recordings. Accordingly, the 3D volume of the object space can be attained as \((x, y) = (x', y')/M\) and \(z = z'/M^2\), where \(M\) is the magnification of the wide-field imaging system.

Practically, we introduced two main strategies to obtain optimum imaging performance. First, while essential for the nondiffracting and self-accelerating properties of the beam, the extended Airy side lobes considerably degrade the image contrast and resolution in dense samples, preventing precise computational reconstruction. In this case, we used a spatial filter (i.e., an additional phase modulation on the SLM), to suppress the Airy side lobes.
while preserving the property of the main lobe [14,18], as shown in Fig. 2(b) and Supplement 1. Second, the curved trajectory of the Airy beam is largely symmetric above and below the focal plane, causing ambiguity for volumetric reconstruction. To address this problem, we derived an additional phase chirp \( \exp(-ik_z \cdot z_0) \), where \( k_z = (k_x^2 + k_y^2)/2k \), and \( z_0 \) is empirically set to 3 μm, to control the ballistic trajectory of the Airy beam [19]. The phase chirp effectively elongates the unidirectional accelerating trajectory on one side of the focal plane to mitigate ambiguity [Figs. 2(c) and 2(d)]. It should be noted that the axial range (~20 μm) is mainly limited by the truncated Airy profiles adopted in practical realization, which can be enhanced by focus-adjusting (Supplement 1). Also, the numerical results in Figs. 2(c) and 2(d) were obtained without side-lobe apodization, thus showing a slight discrepancy with the experimental data near the focal plane.

To test the performance, we first used 200 nm, dark red fluorescent beads (T7280, Thermo Fisher Scientific, Waltham, MA, USA) and recorded their images using the Gaussian (or Airy disk to be exact), Airy, and chirped Airy beams at different depths [Fig. 2(e)]. As measured on one side below the focal plane, the peak intensity of the Gaussian beam exhibits an exponential decay with the half maximum at a depth of ~1–2 μm. Meanwhile, the Airy beam maintains the profile and a relatively constant intensity up to a depth of ~6 μm before noticeable decay. In contrast, the chirped Airy beam exhibits minimum diffraction with moderate, linear decay across an axial range of > 15 μm, close to an order of magnitude of improvement over the Gaussian beam.

We also measured the accelerating trajectory of the chirped Airy beams with respect to the axial positions [Fig. 2(f)]. The experimental results were fitted to a parabolic function \( \Delta l' = a(z+b)^2 + c \) with coefficients \( a = 0.80 \), \( b = 2.77 \), and \( c = -11.61 \). As seen, the accelerating property has largely been preserved, and the slight deviation of the experimental data from the theoretical model \( (A = 0.94 \mu m^{-1}) \) is mainly due to the suppression of the side lobes and the use of the phase chirp. The accelerated lateral displacement \( \Delta l' \), as a result, leads to a linearly enhanced axial sensitivity \( \Delta l'/\Delta z \), effectively counteracting the moderate diffraction and thus maintaining consistent resolving power as the Airy beam propagates along the axial direction [Fig. 2(g)]. Next, we recorded and overlaid the perspective images of a 200 nm fluorescent bead with phase masks rotated by every \( \Delta \psi = \pi/3 \), as well as at two different axial positions of 9 μm and 15 μm below the focal plane [Fig. 2(h)]. As seen, the images of the Airy beams were azimuthally oriented with respect to each \( \Delta \psi \), while the lateral displacements of the beams were differentiated by the two axial positions. The average radii of the displacements of all the azimuthal orientations for the axial positions of 9 μm and 15 μm were measured to be 104.7±4.4 μm and 241.9±6.2 μm in the image space, respectively, consistent with the respective values of 99.2 μm and 241.0 μm obtained from Fig. 2(f).

Using the perspective images, the 3D volume in the object space \((x, y, z)\) can be reconstructed as an inverse problem using tomography algorithms [20,21]. We first demonstrated ATM imaging of caliber structures. Using the 647 nm laser, we recorded 18 perspective images \((\Delta \psi = \pi/9)\) of 200 nm fluorescent beads and measured their 3D reconstructed images at varying depths [Figs. 3(a)–3(c)]. The full width at half-maximum (FWHM) values of these images at each depth were 400–700 nm and ~1–2 μm in the lateral
and axial dimensions, respectively, in agreement with the diffraction limit of \( \sim 450 \) nm and \( \sim 2 \) \( \mu \)m of corresponding wide-field microscopy. It is noticed that the FWHM values in \( y \) are slightly larger than those in \( x \), mainly due to the modest ellipticity of the Airy main lobe. As seen, these 3D FWHMs are well maintained across an axial range close to 15 \( \mu \)m, showing a tenfold improvement in the DOF over wide-field microscopy. Consistent measurements have been obtained using a varying amount of perspective images (Supplement 1). It should be noted that rotationally asymmetric or complex structures typically demand greater angular sampling.

To demonstrate the volumetric imaging capability of ATM, we imaged 200 nm fluorescent beads distributed in the 3D space of agarose gel [Fig. 3(d)]. To reduce epi-fluorescent background, we deconvolved individual perspective images with their corresponding experimental Airy PSFs [Fig. 3(e)]. As seen, the emitters located across an axial range of \( \sim 8 \) \( \mu \)m can be reconstructed without the need for axial scanning [Fig. 3(f)]. Notably, the perspective images of ATM are readily interpretable in their raw format, due to the side-lobe suppression. Therefore, the reconstruction remains consistent without deconvolution (Supplement 1). The reconstructed images exhibited FWHM values of 400–600 nm and 1.3–1.7 \( \mu \)m in the lateral and axial dimensions, respectively, agreeing with the measurements in Figs. 3(a)–3(c). Furthermore, we imaged a surface-stained, 6 \( \mu \)m fluorescent microsphere (F14807, Thermo Fisher Scientific, Waltham, MA, USA), and the entire hollow structure was clearly resolved using ATM [Fig. 3(g)]. The corresponding lateral and axial cross-sectional profiles showed the 3D FWHMs of the surface at \( \sim 500–600 \) nm and 1–2 \( \mu \)m in the lateral and axial dimensions, respectively, consistent with the results using 200 nm fluorescent beads [Figs. 3(a)–3(f)].

Finally, we demonstrated ATM by imaging a mouse kidney tissue slice (F24630, Thermo Fisher Scientific, Waltham, MA, USA). The 16 \( \mu \)m cryostat section was stained with Alexa Fluor 488 wheat germ agglutinin for elements of the glomeruli and convoluted tubules and with Alexa Fluor 568 phalloidin for filamentous actins in glomeruli and the brush border. Using the 488 nm laser, ATM recorded the perspective views of the convoluted tubules, which allows us to reconstruct and computationally synthesize the focal stacks of a significant volume of \( \sim 10 \) \( \mu \)m in the thickness of the tissue slice without deconvolution and the need for sample or focal plane scanning [Figs. 4(a)–4(d)]. As seen, the lining of the tubules of submicrometer profiles in the lateral dimension and structural variations of a few micrometers across the DOF can be clearly visualized, consistent with the axial stacks taken by scanning wide-field microscopy. We then performed two-color ATM imaging of the proximal tubules using the 561 nm and 488 nm lasers. The two spectral channels were first calibrated and registered using caliber markers to account for any deviations in the system or curved trajectories (Supplement 1). Next, ATM acquired the optical signals sequentially with the two lasers and reconstructed the volumetric object without deconvolution, showing a good agreement with the axial stacks recorded with scanning wide-field microscopy [Figs. 4(e) and 4(f)]. As observed, the two-color images revealed the structural relationship, where the prominent actin bundles in the apical domain of the brush border are in close contact with the tubular structures [Fig. 4(g)]. Furthermore, the profiles of individually separated tubular elements exhibited FWHM values of 600–800 nm and \( \sim 2 \) \( \mu \)m in the lateral and axial
dimensions, respectively, consistent with the measurements using the caliber structures [Figs. 4(h)–4(k)].

In summary, we have devised ATM based on engineered, tomographic Airy beams for high-resolution, volumetric imaging of 3D fluorescent samples. Exploiting highly adjustable Airy trajectories in the 3D space, ATM transforms the conventional imaging scheme in wide-field microscopy, presenting a consistent, near-diffraction-limited 3D resolution across a tenfold extended DOF. For future development to image thick samples, one can use overlapping acquisitions to oversample the dataset or control the intensity distribution along the accelerating trajectory [19] to reduce potential ambiguity generated outside the axial range. In addition, ATM can implement nonparaxial accelerating beams that exhibit highly tunable parabolic or elliptical trajectories beyond the paraxial limit to create an even greater degree of bending from the optical axis [3]. The imaging speed is mainly limited by the generation of all the perspective images, which can be further enhanced using faster devices or computational assays. As an approach to an extended DOF, ATM is also compatible with broad volumetric schemes to further enhance the imaging throughput and 3D capability [22–25]. Finally, such a scheme can be readily translated to other non-optical waveforms such as acoustic, plasmonic, and electronic waves.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Principle of ATM. The object is imaged with Airy beams of varying azimuthal angles of $\Delta \phi$ and tomographically reconstructed using the recorded perspective images.
Fig. 2.
Setup and system calibration of ATM. (a) Schematic diagram of the experimental setup for ATM. The objective lens (OL) and tube lens (TL) form an image of the sample at the intermediate image plane (black dashed line), which is relayed to the sCMOS camera by f/4 relay lenses (RLs). The spatial light modulator (SLM) situated at the Fourier plane of the RL imparts phase modulation that converts the light into Airy beams. NF: narrowband filter; P: polarizer; M: mirror; and DC: dichroic cube. The inset diagram illustrates the varying phase masks on the SLM that steer the Airy trajectories to form perspective views. (b) Transverse images of Airy beams generated using the phase masks without (left) and with (right) the additional phase modulation to remove the side lobes. (c) Simulated (top) and experimental (bottom) propagation trajectories of Airy beams. The image of the experimental results is composed of and illustrates the transverse images of the Airy beam at varying depths. Symmetric propagation above and below the focal plane is observed. (d) Same images of (c) generated using the phase chirp, showing enhanced propagation on one side of the focal plane to avoid ambiguity. Δ′ and z in (c) and (d) are measured in the image and object space, respectively. (e) Peak intensity of the Gaussian (or Airy-disk) beam, Airy beam, and Airy beam with the phase chirp as a function of the depth. (f) Lateral displacement of the Airy beam with the phase chirp as a function of the depth, in good agreement with the theoretical values. (g) Linear increase of the lateral displacement over a unit depth variation (Δz = 1 μm) as a function of the depth. (h) Merged image of a 200 nm fluorescent bead recorded at every Δφ = π/3, as well as at two different axial positions of 9 μm (green) and 15 μm (red) below the focal plane. Concentric dashed circles mark the average lateral displacements. Scale bars in (b, h) are characterized as in the object space. Scale bars: 5 μm (b, h).
Fig. 3.
Imaging caliber samples using ATM. (a) 3D view of a reconstructed 200 nm fluorescent bead located at $z = 10 \, \mu m$ below the focal plane. (b) Corresponding cross-sectional profiles of (a) in $x$–$y$, $y$–$z$, and $x$–$z$ across the center, exhibiting FWHM values of 0.58 $\mu m$, 0.63 $\mu m$, and 1.32 $\mu m$ in $x$, $y$, and $z$, respectively. (c) FWHM values of the cross-sectional profiles at varying depths over a 15-$\mu m$ axial range, showing 0.4–0.6 $\mu m$ in $x$, 0.5–0.7 $\mu m$ in $y$, and 1.2–1.7 $\mu m$ in $z$. (d)–(f) Raw, deconvolved, and 3D reconstructed images of seven fluorescent beads distributed in 3D agarose gel across a >8–$\mu m$ axial range. (d) and (e) show the perspective images acquired at $\Delta \phi = 0$. Projections of the 3D image in $y$–$z$ and $x$–$z$ were shown in the insets in (f). Depth information in (f) is coded as shown in the color-scale bar. (g) 3D reconstructed image of a surface-stained, 6 $\mu m$ fluorescent bead, whose entire hollow
structure was clearly observed. (h) The cross-sectional profiles across the center of (g),
exhibiting FWHM values of the left and right profiles of 0.67 μm and 0.52 μm in x, 0.69 μm
and 0.61 μm in y, and 1.51 μm and 1.68 μm in z, respectively. Scale bars: 5 μm (d)–(f).
Fig. 4. Imaging mouse kidney tissue using ATM. (a) Wide-field image of a cryostat section of mouse kidney stained with Alexa Fluor 488 on elements of the glomeruli and convoluted tubules acquired by overlaying 10 axial stacks at a step size of 1 μm over a 10 μm range. (b) 3D reconstructed image of the same region of (a) using ATM, showing consistent structural information. The insets in (a) and (b) display the x–z and y–z cross-sectional views along the corresponding dashed lines. (c) and (d) 3D perspective view and synthesized focal stacks of (b). The arrows in (d) show structural variations observed across different depths. (e) Two-color, wide-field images of the mouse kidney section stained with both Alexa Fluor 568 (top) on filamentous actins in glomeruli and the brush border and Alexa Fluor 488 (bottom) as in (a). The images were acquired by scanning and overlaying five axial stacks at a step size of 1 μm over a 5 μm range. (f) Two-color images of the same region of (e) taken using ATM. (g) Merged two-color top (red) and bottom (green) images of (f) at the depth of 4 μm, showing co-localized actin bundles in the apical domain of the brush border and tubular structures. The insets display the x–z and y–z cross-sectional views along the corresponding dashed lines. (h) 3D ATM image of a separated tubular element as marked by the arrows in (e)–(g). (i)–(k) Cross-sectional profiles of (h) across the center in x, y, and z, exhibiting FWHM values of 0.67 μm, 0.80 μm, and 2.07 μm, respectively. The depth information in (a), (b), (e), and (f) is coded as in the corresponding color-scale bars. Scale bars: 5 μm (a), (b), (d), (e)–(g), and 2.5 μm (g, inset).