Single-shot optical projection tomography for high-speed volumetric imaging of dynamic biological samples

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Abstract
A single-shot adaptation of Optical Projection Tomography (OPT) for high-speed volumetric snapshot imaging of dynamic mesoscopic biological samples is presented. Conventional OPT has been applied to in vivo imaging of animal models such as D. rerio, but the sequential acquisition of projection images typically requires samples to be immobilized during the acquisition. A proof-of-principle system capable of single-shot tomography of a ~1 mm³ volume is presented, demonstrating camera-limited rates of up to 62.5 volumes/s, which has been applied to 3D imaging of a freely swimming zebrafish embryo. This is achieved by recording eight projection views simultaneously on four low-cost CMOS cameras. With no stage required to rotate the sample, this single-shot OPT system can be implemented with a component cost of under £5000. The system design can be adapted to different sized fields of view and may be applied to a broad range of dynamic samples, including high throughput flow cytometry applied to model organisms and fluid dynamics studies.

KEYWORDS
mesoscopic imaging, optical projection, single-shot, tomography, tomography, video-rate volumetric, volumetric imaging

1 INTRODUCTION

3D optical imaging of intact samples at mesoscopic scales (mm–cm) has proved a valuable capability in biology and biomedicine, enabling structures to be observed in their native context. Furthermore, the use of nonionizing radiation and the wealth of fluorescent markers in the visible

Abbreviations: DoF, depth of field; dpf, days post fertilization; FBP, filtered back projection; FoV, field of view; LSM, light sheet microscopy; NA, numerical aperture; OPT, optical projection tomograph; ss-OPT, single-shot optical projection tomography.
region enables specific molecular imaging, including in vivo studies using small transparent model organisms. Mesoscopic volumetric imaging techniques include optical projection tomography (OPT) [1], and selective plane illumination microscopy [2], also described as light sheet microscopy (LSM) [3, 4] and these have been applied to a range of in vivo studies [5–9]. However, it is usually necessary to immobilize live samples since the final 3D volume is constructed from the sequential acquisition of multiple images. In the case of direct imaging techniques, like LSM, this is to ensure correct spatial correspondence of slices within the volume, while for tomographic techniques like OPT, the reconstruction is determined from all projection images so requires the sample to remain static during the whole acquisition period. Live organisms such as zebrafish embryos are therefore typically imaged under anesthetic and mounted in a gel such as agarose [6, 10, 11]. The total acquisition time, and therefore the physiological stress on the organism [12], can be minimized by reducing the integration time of the individual images [13], and/or reducing the total number of images required for 3D reconstruction, for example, using compressive sensing techniques [5, 14]. Nevertheless, all 3D imaging techniques that rely on sequential image acquisition can be prone to motion artifacts. Therefore, for highly dynamic samples such as fluids mixing, freely moving model organisms and 3D imaging flow cytometry, it would be desirable to simultaneously acquire a whole 3D image dataset at each time-point. To date, single-shot 3D volumetric imaging for biological/biomedical applications has only been demonstrated with holographic techniques [15], or parallax-based methods such as stereo microscopy or plenoptic imaging [16], where the combined image information is limited in angular coverage leading to significantly anisotropic spatial resolution. We note that simultaneous multi-channel imaging has been applied to flame and combustion tomography using mirror-based and fiber bundle-based multiplexed imaging, but these systems are, respectively, relatively complex and provide limited spatial resolution [17, 18].

OPT is the optical analogue of X-ray computed tomography for which a series of widefield projection views through a sample are recorded as it rotates through 360° [1]. The volumetric image is then reconstructed from the set of projection images, typically using filtered back projection (FBP) [1, 19]. Importantly, unlike LSM and holographic techniques, OPT can be applied both to transmitted and fluorescence light, making it applicable to a wide range of optical contrast agents and/or contrast mechanisms. Since the key components of an OPT system are a motorized rotation stage, camera, telecentric imaging system and light source (which can be an LED), OPT is low cost compared with most other volumetric imaging modalities. As wide-field imaging can easily be scaled to large fields of view using appropriate magnification, OPT has been applied to cm-scale chemically cleared tissue samples for preclinical studies [20], for example, whole mouse brains [21], mouse pancreas [22], adult zebrafish [23] and clinical pathology [24]. For such ex vivo applications, total acquisition time is not a critical parameter and highly sampled OPT projection data can be acquired on a timescale of minutes—noting that a fully Nyquist sampled OPT dataset requires πN equally angularly spaced projection images over 360° (assuming the front half of sample is covered by the depth of field), where N is the number of resolution elements across the projection image [19], and so 100's of images are typically required to realize Nyquist sampling.

OPT is also applicable to the imaging of transparent (weakly scattering) in vivo models, such as C. elegans [25, 26] and D. rerio [6, 8, 10], and can be minimally toxic through the use of nonionizing radiation. However, with OPT data acquisitions typically requiring several minutes per color channel, live samples may be compromised by phototoxicity and the physiological stress of being maintained under anesthesia, limiting the applicability for longitudinal studies. While the total OPT data acquisition time can be reduced by acquiring sparse (angularly under-sampled) OPT data on shorter timescales (~10 s) and applying compressed sensing techniques or machine learning to minimize “streak artifacts” in the reconstruction [5, 27, 28], any OPT approach that still requires the sequential acquisition of multiple projection images may be compromised by samples moving on the timescale of the complete OPT data acquisition.

We note that highly sampled time-lapse single-shot volumetric imaging could potentially be realized using OPT implemented with 10’s–100's of cameras, each acquiring separate projection images in parallel. This would be prohibitively expensive and practically challenging to implement in a fully sampled OPT system, and the size and readout rate of the resulting data itself (hundreds of projection images per second) would be challenging to record using a standard computer. By utilizing compressive sensing, however, it is possible to reduce the number of projections (and therefore cameras) required and so reduce the size of the acquired OPT datasets.

To the best of our knowledge, we present the first demonstration of single-shot volumetric imaging for biological applications using compressing sensing OPT that is capable of imaging a ~1 mm³ volume at the camera frame rate, here achieved by recording eight projection views simultaneously using four cameras. We demonstrate the 3D volumetric imaging of a free-swimming zebrafish embryo at 62.5 volumes/s (limited by the frame rate of the cameras used). As well as enabling imaging of
dynamic samples, single-shot OPT (ss-OPT) eliminates the need for sample rotation and so could also be applied to in situ long timescale imaging, for example, for applications in developmental biology or plant imaging. Using low-cost CMOS cameras, which feature a global shutter and the ability to receive a hardware trigger, the total component cost of the ss-OPT technique presented can be implemented for below £5000.

2 | EXPERIMENTAL SECTION

2.1 Angular multiplexed optical imaging

To reduce system cost and image data rate, and to avoid space limitations arising from camera bulk, we acquired two different projection views on each camera by implementing an angular multiplexing optical system in a compact novel configuration, depicted in Figure 1a. Each individual imaging arm incorporated a low-cost CMOS camera (Chameleon 3 CM3-U3-31S4M, Teledyne FLIR) with a sensor size of 7.07 × 5.30 mm. Therefore, to achieve a maximum field of view (FoV), the two adjacent images recorded by the CMOS sensor had their centers separated by ~3.6 mm. This separation was achieved using a compound tube lens constructed from two truncated 125 mm focal length doublet lenses (32-492, Edmund Optics) such that their centers were horizontally separated by 3.6 mm. A similar approach has been previously reported in an image relay used for quantitative phase microscopy [29].

This compound tube lens was used in conjunction with a single 4× infinity corrected objective lens (PF4X- INF, AmScope, effective focal length 45 mm) with a full numerical aperture (NA) of 0.13. To define the relative angular separation of the multiplexed images and to set the required depth of field (DoF), an aperture mask consisting of two adjacent 2.6 mm diameter holes separated by 3.6 mm (to align with the centers of the lenses in the compound tube lens) was positioned in the pupil plane of the objective lens, producing two imaging channels of NA 0.028, imaging at a relative angle of 3.8°. In order to prevent crosstalk between the multiplexed imaging channels, the compound tube lens was positioned directly behind the aperture mask and a baffle placed along the central axis of the system from the tube lens to the CMOS sensor. While the position of the compound tube lens immediately after the aperture mask means the system is not image side telecentric, the required object-side telecentricity is maintained since the mask is positioned in the pupil plane of the objective lens. This results in a multiplexed imaging system where each channel has a magnification of 2.8×, a FoV of 1.26 × 1.89 mm, diffraction-limited lateral resolution of 9.5 μm and DoF of ~1 mm. Figure 1b shows some representative projection images of sub-diffraction beads (1 μm diameter, F13081 ThermoFisher Scientific) positioned at the focal plane and 0.42 mm closer to and further away from the camera, respectively. The measured bead full width half maximum values were 9.3, 9.5 and 9.6 μm (±0.4 μm), respectively, demonstrating the telecentricity and extended depth of field of the multiplexed imaging system (Figure S1 further demonstrates the object-side...
telecentricity by imaging a grating at different positions along the optical axis).

2.2 Alignment of the angular multiplexed OPT system

Four of the multiplexed imaging arms described above were constructed, producing eight imaging channels for ss-OPT. Figure 2 shows a schematic of the ss-OPT configuration for transmitted light and fluorescence imaging. The four imaging arms were arranged around a water-filled octagonal cuvette such that the imaging arms were at 45° intervals (individual images acquired at 1.9° with respect to cuvette-window normal), covering ~135° on one side of the sample. For transmitted light imaging, four white-light LED panels were positioned opposite each of the four multiplexed systems. For fluorescence imaging, widefield excitation was provided by a 473 nm laser (Cobolt Blues, Hubner GmbH) directed through a rotating diffuser and collimated by a Fresnel lens. An emission filter with central wavelength 525 nm and bandwidth 50 nm was used in each imaging arm to block scattered excitation light. A complete list of system components is given in the Supplementary Information.

In standard OPT, which uses a motorized stage to rotate the sample, the minimum requirement for correct alignment is that the axis of rotation is orthogonal to the optical axis of the imaging system. Rigid image transformation (i.e., rotation and lateral shift) can then be applied post acquisition to align the axis of rotation vertically in the

**FIGURE 2** ss-OPT system operating in (A) transmitted light and (B) fluorescence modalities (see Figure S2 for system photograph). Transmitted light provided by white light LED panels, while time averaged laser excitation is provided through a spinning diffuser (SD) and Fresnel lens (FL), and fluorescence is imaged through emission filters (F). The sample (S) is placed in a cuvette (Cu), filled with refractive index matching fluid. Each imaging arm consists of an objective lens (O), an aperture plate (AP) (featuring two pinholes which define the view angle) and two tube lenses (TL). The eight images are recorded simultaneously on four synchronously triggered CMOS cameras (C1-4). Baffles (indicated as solid black line) are mounted between each camera and tube lens pair in order to minimize crosstalk.

**FIGURE 3** Demonstration of coordinate axes in ss-OPT. Global y-axis $y_0$ indicates the vertical axis in object space. $z_{1-4}$ are optical axes for each optical arm, respectively. $x_{1-4}$ and $y_{1-4}$ are the horizontal and vertical axes in each camera plane. $z_{1-4}$ must lie in parallel planes for the minimal system alignment condition. $y_{1-4}$ must also be parallel in order to avoid the need to rotate projection views after acquisition.
center of the projection images, allowing independent reconstruction of each row of pixels in the aligned data-set [30]. In the ss-OPT system, the minimum requirement for alignment is that the optical axes of all eight imaging channels lie in mutually parallel planes. To remove the need for rotation of the projection images before reconstruction, a further constraint of both the optical and lateral axes forming mutually parallel planes is required (labeled X1...4 and Z1...4 in Figure 3). This condition can be achieved using an appropriate test object that allows relative viewing angle and tilt to be determined for each individual imaging channel, for example two stacked cubes of different size with fiducial markers on their faces [31].

The ss-OPT system implemented here did include a motorized rotation stage (NM11AS-T4, Laser 2000 [UK] Ltd) mounted on a tip-tilt plate to allow direct comparison between standard OPT using a single imaging channel and ss-OPT using parallel acquisition from all eight imaging channels with no sample rotation. This enabled us to align each individual imaging channel to the axis of rotation of the stage, ensuring mutual alignment. Further, a standard OPT acquisition of a test object using all eight imaging channels, acquiring 1280 projections over 360°, was used to measure the angular separation between projection views and to determine the required image transformations (i.e., horizontal and vertical translations) to correctly register all eight channels pre-reconstruction. We note that if the measured magnification was different between imaging channels this could also be corrected during the registration process since object-side telecentricity ensures that parallel projections are acquired.

2.3 | Data Acquisition and Reconstruction

Based on the lateral resolution of 9.5 μm for a FoV of 1 mm width, a standard OPT acquisition would require ~165 projections over 180° rotation to fully sample the data [19]. Therefore, the eight projection images acquired in ss-OPT represent an under-sampling of 21×, but, crucially, they are acquired simultaneously rather than sequentially. The angular separation of 3.8° between projection views on each camera is greater than the minimum angular separation of 1.09° for full angular sampling, so each projection view contains independent information. Furthermore, using two imaging channels per objective lens doubles the amount of light collected by the system, making more efficient use of the available NA of the objective lenses and increasing the signal-to-noise ratio. This is illustrated in Figure S3.

Figure 4 shows the full calibration and acquisition workflow for an ss-OPT acquisition. The four cameras were triggered simultaneously by a pulse train form an Arduino Uno microcontroller, with acquisitions initiated and stopped by a manual hardware switch. The image data from the cameras was streamed directly to a PC and stored in a RAM buffer with ~17 GB available capacity, which was continually written to an NVMe SSD drive (with a sequential write speed of 1400 MB/s) using the software provided by the camera supplier (SpinView, Teledyne FLIR). The maximum acquisition duration before reaching the RAM capacity depends on the frame rate: for the exemplar (maximum) frame rate of 62.5 frames/s the acquisition duration was ~80 s, corresponding to ~5000 independent volumetric time-point measurements.

Before reconstruction, each set of eight simultaneously acquired projection images were registered and any illumination/detection variations corrected (based on calibration data). Reconstruction was then performed using an iterative approach. We previously reported that the TwIST algorithm can satisfactorily reconstruct under-sampled OPT data with as few as 20 projection images acquired with
conventional OPT instrumentation (i.e., sequential projection acquisition) [5]. Here we extend this approach applying TwIST to retrieve the volumetric information from our ss-OPT instrument using only eight projection images, but now acquired simultaneously. We note that other reconstruction methods may be used for under-sampled OPT image data, such as training a convolutional neural network to remove the streak artifacts from the reconstructed volumetric data [28].

Volume visualization and rendering were performed using 3DScript [32], an open-source plugin for ImageJ, with macros written to render multiple time-points from a ss-OPT dataset. Adobe Photoshop was used to color code and label some reconstructions for display.

3 | RESULTS AND DISCUSSION

To demonstrate that reconstructions of appropriate quality can be realized from just eight images, we performed a standard OPT acquisition consisting of 400 projections of a low concentration suspension of 1 μm fluorescent beads (projection data shown in Figure 1b) using the multiplexed imaging channels. Figure 5 shows cross-sectional reconstructions through two beads, one positioned (a–c) at the center of the object volume (i.e., close to the focal planes of the imaging channels) and (d–f) one displaced 0.42 mm from the center. Figure 5a, d shows FBP reconstruction using all 400 projection images, while (b, e) and (c, f) are reconstructed from only eight projection images, corresponding to the sparse angular sampling of the ss-OPT system, using FBP and the iterative TwIST approach, respectively.

Compared with the highly sampled reconstruction, the evident streak artifacts in the FBP reconstruction using only eight projection images clearly demonstrate the need for an alternative reconstruction approach for significantly undersampled data. The iterative TwIST algorithm reduces the erroneous “signal” contained in the streak artifacts by applying a total variation regularizer that assumes sparsity in the gradient domain. Using TwIST applied to eight projection images, the measured radial and tangential widths were 10.9 and 11.1 μm for the on-axis and 11.1 and 10.9 μm for the off-axis bead reconstructions. This compares favorably to 10.4 and 10.4 μm for the on-axis bead and 10.0 and 10.8 μm for the off-axis bead, respectively, in the highly sampled FBP reconstruction. We also note that the consistency in the radial and tangential directions confirms the extended DoF and telecentric properties of the multiplexed imaging arms.

Having demonstrated the efficacy of the ss-OPT approach, we applied it to in vivo imaging of nonanesthetized zebrafish embryos. We first performed transmitted light ss-OPT on a 4 days post fertilization (dpf) wild type zebrafish embryo mounted in a ~ 0.8 mm diameter tube (06406-60, Cole-Parmer Instrument Co Ltd) within the cuvette. This tube diameter kept the zebrafish within the DoF of the ss-OPT system but allowed movement along the tube axis. Eight simultaneous images were recorded every 16 ms with an integration time of 8 ms per frame, resulting in a 62.5 volumes/s volumetric image dataset, the limit achievable with the cameras used. Video S1 shows the recorded projection images for this acquisition from a single imaging channel (intensity inverted), indicating the rapid motion of the pectoral fin and the beating heart of the embryo.
Figure 6 shows the reconstruction of a single time-point viewed from four different directions. To aid visualization the contrast has been inverted, with higher voxel values corresponding to reduced light transmission. Video S2 shows 1.28 s of the volumetric time-lapse dataset, displayed from the four viewing angles seen in Figure 6, in which clear movement of the embryo’s tail can be seen.

To demonstrate ss-OPT applied to fluorescence, a 4 dpf transgenic Fli:GFP zebrafish embryo, expressing green fluorescence protein in endothelial cells, was imaged at 62.5 frames/s with an increased integration time of 15 ms. Again, to keep the nonanesthetized embryo in the DoF it was mounted in a ~0.8 mm diameter tube. Figure 7 shows reconstructions at time-points 0, 320, 400 and 640 ms, where the change in position of the pectoral fins is evident from a signal dominated by the vasculature in the head.

Video S3 shows a 1.3 s duration of the reconstructed dataset. The increased integration time leads to motion blurring in some frames compared with the equivalent transmitted light dataset, particularly as the zebrafish swims out of the FoV. To increase the acquired signal without increasing the excitation light intensity (possibly risking photobleaching/damage), larger apertures could be used to collect more light with a trade off in isotropic resolution with radial distance from the center of the volume of interest [33].

This demonstration of volumetric imaging at the camera frame rate suggests that ss-OPT could find applications in high-throughput 3D in vivo screening/phenotyping of mesoscopic model animals, such as zebrafish, where samples flow through the volume defined by the DoF.

To extend the application of transmitted light ss-OPT to freely swimming zebrafish embryos, the system was used to image a 4 dpf zebrafish embryo in a ~10 mm diameter tube (EW-06406-12, Cole-Parmer Instrument Co Ltd), allowing it to swim in any direction, including into and out of both the DoF and FoV of the system. Given the dynamic nature of this sample, the integration time was reduced to 5 ms to minimize potential motion blur and the frame rate was maintained at the maximum of 62.5 frames/s. To demonstrate the significant
movement frame-to-frame, Figure 8 displays 12 sequential volumetric reconstructions (a duration of 176 ms) with data from each time-point rendered in a different color, and up to four time-points displayed together. Panels displaying frames 2–5 and 6–9 show the significant movement of the zebrafish embryo on a timescale of 10’s of ms, but the simultaneous multi-camera acquisition employed in ss-OPT is able to provide whole-volume reconstructions at each time-point. Video S4 shows a 1.23 s interval of the volumetric time-lapse dataset, with false color encoding depth, again demonstrating the significant motion of the embryo.

In our implementation of ss-OPT we have demonstrated its practical application using cost-effective components, particularly the CMOS cameras. To implement the 2× multiplexed imaging on each camera requires specially modified tube lenses. This does increase the system complexity and having lenses truncated as we describe by a specialist company is estimated to cost £300. This complexity can be avoided by only recording a single projection image on each camera. However, the resulting OPT system would collect half the amount of light at each time-point and the reconstructed images would be degraded, as indicated in Figure S3.

We note that superior performance can be realized by making a number of adaptations. For example, improved signal-to-noise can be achieved by using (more expensive) scientific-grade camera technology and better reconstruction fidelity could be achieved by adding additional imaging arms (each adding a further two projection imaging channels).

We note that LSM has been demonstrated with compressed sensing techniques using spatial modulation of the excitation light over the sample volume, to achieve a maximum reported compression of ~10× [14, 34]. While this reduces the number of images required to reconstruct the volume, these images are still acquired sequentially. To replicate the temporal resolution demonstrated here with ss-OPT, such LSM systems would need to perform a full volumetric acquisition in 16 ms. To realize ~10 μm spatial resolution over the 1 mm FoV, approximately 10 sequential images would need to be acquired per time-point—
necessitating a camera framerate >600 frames/s, which would require a significantly more expensive camera.

4 CONCLUSION

We have presented a proof-of-principle single-shot optical projection tomography (ss-OPT) system capable of imaging ~1 mm$^3$ at up to 62.5 volumes/s with an isotropic resolution of ~10 μm. The system simultaneously acquires eight projection images using four low-cost CMOS cameras and 4× microscope objectives combined with compound tube lenses. The optical components of this ss-OPT system may be adapted to provide different performance in terms of magnification, field of view, imaging rate and image quality, for example, by changing the objective lens, tube lens and/or camera (sensor size, quantum efficiency, noise and frame rate), and by changing the number of imaging arms. It would also be possible to realize simultaneous transmitted light and fluorescence imaging by dividing the imaging channels as appropriate. The capability for rapid volumetric imaging—primarily limited only by the frame rate of the camera or the signal level—may make this a valuable approach for 3D flow cytometry and/or sorting, for example, of small organisms or 3D organoids, and may also be applied to study fluid dynamics. The ability to implement an OPT system without the need for sample rotation may also have applications in situations where sample rotation is not practical, such as developmental biology or plant imaging.

As well as improving the single-shot image quality by employing cameras with superior signal-to-noise properties or by increasing the number of imaging arms, it may also be possible to improve the image quality through advances in compressed sensing algorithms or in deep learning reconstruction methods. We note that CNN reconstruction provides faster data analysis once the training model is established, while iterative approaches (e.g., TwIST) can be used for any sample and may be more robust with respect to changes in sample conformation during the acquisition.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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