Supplementary Information

In-vivo Optical Tomography of Small Scattering Specimens: time-lapse 3D imaging of the head eversion process in Drosophila melanogaster

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Supplementary Discussion

Information Content of hOPT and OPT

Due to the fact that OPT and hOPT are based on *projection* measurements, each single image already contains information on the whole volume: it is the reconstruction algorithm dedicated to solve the inverse problem which will give us the actual 3D volume. That is, there is a non-linear relationship between the measurement and an actual point in space. This is in contrast with all other 3D optical sectioning techniques such as Light Sheet or Confocal microscopy, where each point in space has a direct relationship with the 3D volume imaged (see **Supplemental Fig. 1**). This means that even though hOPT requires an intermediate step between the measurement and the image, it has

higher information density than direct 3D imaging approaches. As mentioned in the main text, one of the main advantages of hOPT is its high throughput and its ability to image in 3D several specimens simultaneously. This, of course, has the potential of generating extremely large datasets, leading to a variety of problems, including data storage and handling. So as to showcase this in actual numbers, consider a simple cube of dimensions N_x x N_y x N_z. In an ideal case, we could have imaged this volume using Light Sheet Microscopy (LSM) yielding a data set of size N_x x N_y x N_z x 16bit where we have assumed we save data as 16bit unsigned integers. On the other hand, by using OPT we can store an equivalent amount of information in M_p projections, in which case the dataset would be N_x x N_y x M_p x 16bit in size but would still represent the same volume. Note that the lower the M_p number the more complex the reconstruction algorithm needs to be in order to recover the same volume with the same accuracy and quality. This effectively means that as long as $M_p<N_z$, hOPT will require less storage space than SLM, the penalty for this being that an intermediate setup with an image reconstruction process is needed. This becomes even more obvious in those cases where N_{ang} angular measurements are used in LSM (as in Ref [1,2], for example), in which case the raw data stored is N_x x N_y x N_z x N_{ang} x 16bit.

Irrespective of the space required for data handling, the most relevant feature of OPT is the fact that it relies on solving an inverse problem in order to provide an image. This indeed represents an extra step which might add artifacts to the reconstructed image (this issue will be further discussed in the next section), but it also provides the opportunity to model appropriately light propagation within the sample and account for it when reconstructing the data. To be more specific, within this reconstruction algorithm we have the chance implement the full Radiative Transfer Equation[3] or any

approximation to it, such as the low scattering approximation implemented through the Radon Transform and the Filtered Back-Projection [4], the Fokker-Plank approximation[5], or in those extreme cases where scattering dominates, the diffusion approximation[6]. The fact that we may improve how we model light propagation within our specimen showcases the great potential of OPT in those situations where the contribution of scattering is relevant. Future work in this respect needs to address the implementation of these complicated solutions so that the inverse problem is solved in a timely fashion, reducing computation times to a minimum.

Reconstruction Artifacts in OPT

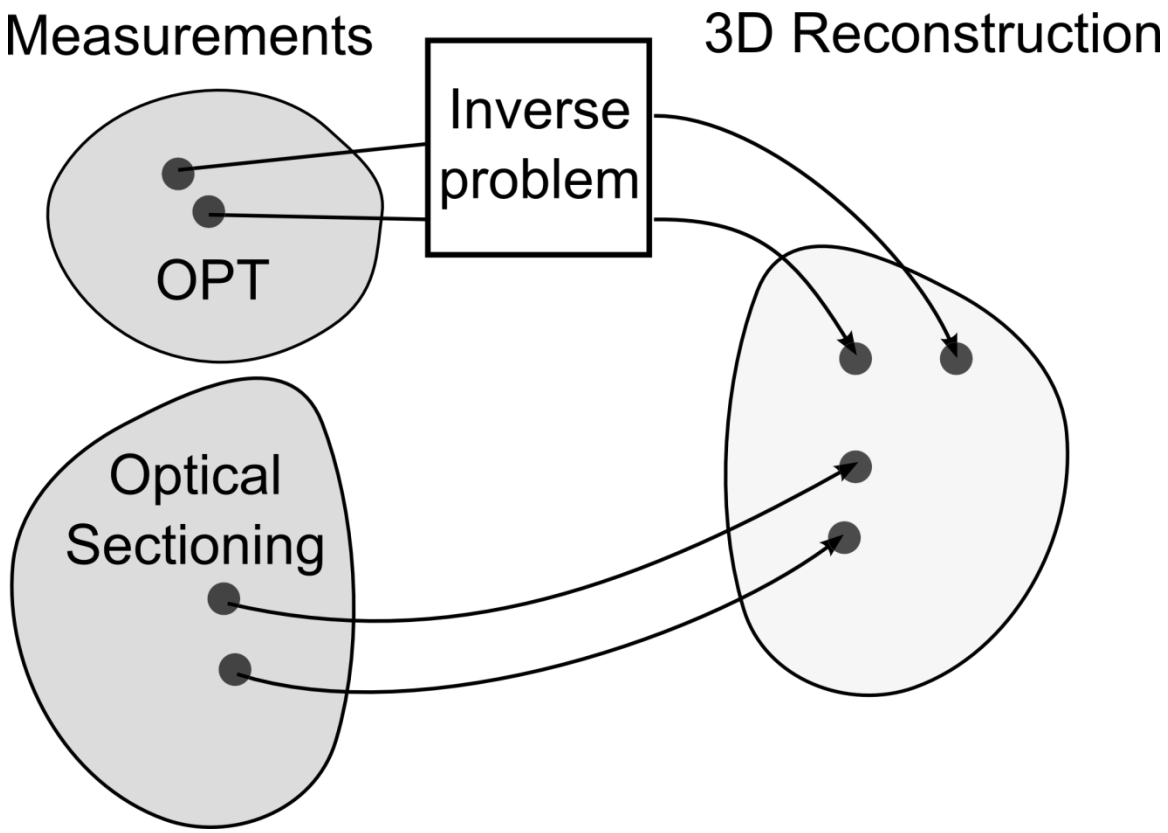
As any imaging approach which requires the solution of an inverse problem, OPT suffers from some of the typical reconstruction artifacts present in X-ray Computed Tomography such as ring artifacts (a defective detector element generates rings centered about the center of rotation), noise (the presence of noise results in random thin bright and dark streaks that appear preferentially along the direction of greatest attenuation), and truncation (during the 360^{o} rotation the sample exits the field of view). Fortunately, all these have been extensively covered and studied in X-ray Computed Tomography (see for example [7]), and a battery of reconstruction methods to alleviate these artifacts are publicly available.

Artifacts which appear specifically in OPT are mainly those related to its use in fluorescence mode, in particular photobleaching of the sample and the presence of movement while acquiring the data. The effect of prolonged light exposure and the consequent reduction in fluorescence intensity is studied in detail in [8,9], together with the main artifacts present in OPT. Reduction of the exposure times, as mentioned in the main

text of this article, is very important in order to reduce photobleaching and phototoxicity to a minimum. As an example, we saw that the exposure times (~0.02s) used in our study for *D. melanogaster development* making use of short imaging times for a full 360o rotation (less than 10s) did not affect development or fluorescence emission. We did experience a loss of fluorescence emission and even death of the developing pupa when continued exposures exceeded one minute and where repeated in short time-intervals.

Another important factor to consider when imaging development *in-vivo* is the speed at which changes take place, in which case the sample might move during image acquisition. In this sense, similarly to the new developments in Laser Sheet Microscopy which implement fast-scanning approaches[10–13], the use of several cameras simultaneously[14] further increases detection speed, enabling 3D imaging of fast processes.

Supplementary Figures



Supplementary Figure 1. Information content in hOPT data.

This figure shows how there is a direct relationship between spatial positions in the sample and measurements performed with an optical sectioning method such as Light Sheet Microscopy or Confocal Microscopy. Indirect imaging methods such as OPT and hOPT rely on high information content in the raw data and thus require smaller datasets, needing however an intermediate step requiring the solute ion of an inverse problem in order to reach the 3D reconstruction. The need for this intermediate step offers great flexibility and the opportunity to account for light propagation properties such as scattering in OPT measurements.

Supplementary Videos

Supplementary Video 1: Raw Fly Data at several angles for Control and Headless specimens

Supplementary Video 2a: OPT video of head eversion of wild type fly.

Supplementary Video 2b: Close-up of head eversion of wild type fly.

Supplementary Video 3: Examples of raw reconstructed data for pre- and post-eversion stages of the control specimen.

Supplementary Video 4a: OPT video of head eversion of headless mutant fly.

Supplementary Video 4b: Close-up of head eversion of headless mutant fly.

Supplementary Video 5: High Throughput Time-lapse Imaging of wild type fly.

Supplementary References

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