Optical Projection Tomography with a Tissue-Clearing Agent for Developmental and Reproductive Toxicology Studies

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Abstract

Developmental and reproductive toxicology (DART) testing is one of the most expensive and time-consuming stages in determining the toxicological profile of new chemical entities. Within DART studies, gross morphological evaluation of fetal animal skeletons for developmental abnormalities presents a major bottleneck. Current processing techniques involve digestion of soft tissue using a strong base, followed by qualitative assessment of the remaining skeletal tissue by a pathologist. Micro computed tomography (microCT) has been proposed as a non-destructive image-based alternative. Such methods eliminate the need for extensive tissue processing and can be paired with quantitative analysis algorithms. However, due to the significant capital and operational expenses required for microCT imaging, this approach has yet to gain widespread traction. Here, we propose a cost effective optical imaging alternative. A novel tissue clearing agent was used in 1-week old rats to temporarily render soft tissue optically transparent. Alizarin red was used to stain skeletal tissues. High dynamic range (HDR) optical trans-illumination images were then acquired with a low-cost optical-CT imaging system and rendered as 3D images of skeletal structure. HDR-based optical-CT imaging of chemically cleared tissues can rapidly generate high resolution (50–250 um) reconstructions of whole skeletons. This study demonstrates that the combination of tissue clearing, optical imaging, and novel reconstruction algorithms may present a new paradigm for low-cost, high-throughput evaluation of tissues in DART testing.

Introduction

DART studies currently rely upon a human-based qualitative characterization approach for assessing skeletal morphology in these studies. This qualitative assessment approach is tedious, time-consuming and is susceptible to inter/intra pathologist variability. Due to the qualitative nature of these evaluations and inherent variability, regulatory bodies have encouraged a shift of this paradigm towards a quantitative and digital approach. Therefore, the contract research organizations that run these studies have begun to evaluate digital quantitative approaches for skeletal evaluation such as microCT. However, microCT has been slow to be adopted for these studies for a number of reasons including costs and the inability to achieve optimal radiometric density contrast in the small rodent bones typically used in DART studies.

Limitations of MicroCT for DART Studies

One of the major challenges with microCT for DART studies is the cost of a microCT device. Outside of the upfront capital cost of approx. $1 million, there is a significant ongoing cost for maintenance as well the salary for the highly trained medical physicist required to operate the device. Additionally, significant EHS oversight and building modifications may be required which adds to costs. The other main problem with microCT, or any quantitative digital analysis pipeline, is that the development and validation of the skeletal analysis software. These combined factors put digital skeletal evaluation out of reach for most contract research organizations as it presents a significant expense and requires competencies that they may not have access to. There is also a technical shortcoming limiting the applicability of microCT to DART studies because while these devices can image fetal rabbits and rats very well, they are limited in their ability to image GD18 fetal mice. The reason for this is that these models have limited skeletal ossification and the radiometric contrast between cartridge and bone for these animals is minimal.

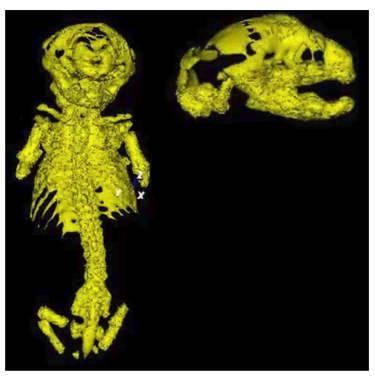

Figure 1. GD 18 mouse fetus imaged with an Albira microCT scanner.

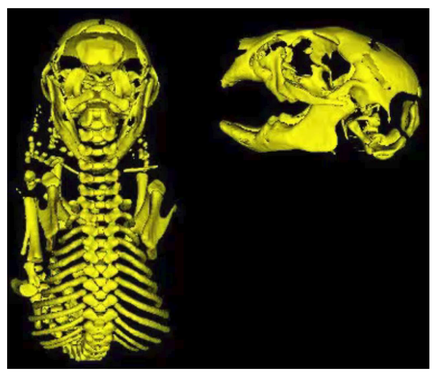

Figure 2. GD 28 rabbit fetus imaged with an Albira microCT scanner.

Optical CT for use in DART Studies

The current paradigm for skeletal evaluation in DART studies involves the use of reagents that render tissues transparent combined with alizarin bone staining. This processing paradigm allows for visual investigation of samples as soft tissues are rendered transparent and bones are rendered opaque. In this context, projection tomography which is typically used with entirely opaque specimens can be used with light to reconstruct skeletal features as light will pass through all features with the exception of bone.

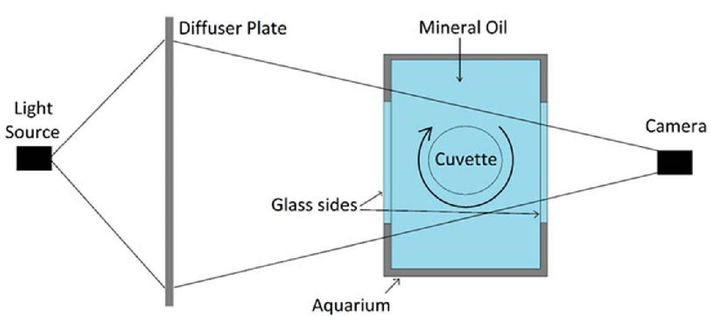

Figure 3. Schematic diagram of the Clara optical-CT scanner (viewed from above). Trans-illumination images of the specimen are acquired by the camera as the cuvette is rotated about an axis perpendicular to the plane of the diagram.

High Dynamic Range Imaging

One of the main challenges with Optical CT for use with fetal rats, mice and rabbits is that light is a much lower energy radiation source than X-rays and thus attenuates rapidly as it passes through samples. This is problematic as complete attenuation of light through samples will prevent accurate reconstructions and will manifest itself as significant artifacts such as bone fusions and amorphous bone reconstructions.

The approach that we have adopted to overcome this problem is to utilize a high dynamic range imaging approach where multiple image sets are combined to achieve optimal contrast between soft tissue and all bones. The reason why this high dynamic range technique is crucial for success in this space is that while high light intensity allows for reconstruction of optically dense features such as the skull, it washes out optically diffuse features such as the digits in the paw. Therefore, multiple image sets needs to be combined to achieve a wider dynamic range of imaging as shown in **Figure 4**.

Optical-CT Optimization

Because high dynamic range imaging requires specific imaging parameters based upon a specimens specific features, each specimen needs to have its imaging parameters optimized. Imaging fetal specimens is relatively simple with mice and rats as they are relatively small animals with little optical attenuation. However, for large specimens like fetal rabbits optical-CT becomes challenging as light begins to attenuate significantly which is the inverse problem seen in X-ray CT. To overcome this problem, a hyperspectral imaging approach was evaluated where infrared light was used as it penetrates soft tissue more readily than lower wavelengths of light.

Optical-CT for DART Studies

The current focus of our work has been on optimizing the image acquisition parameters of our optical-CT device for use in DART studies as these parameters need to be optimized for each animal model. However, for routine DART use it is essential that these images are then used to automatically evaluate skeletal morphology for abnormalities. The approach that we will use to address this problem is to segment three-dimensional reconstructions into individual bones and then extract quantitative features from each bone such as surface area, volume and key dimensions. By doing this for a reference library of specimens with known defects, we can develop a library that can be used to define “in range” and “out of range” criteria on a bone-by-bone basis. This will ultimately allow for the automatic evaluation of samples and for the device itself to identify specifically which bones are abnormal and why.

The main barrier towards technical feasibility at this point is image acquisition for optical-CT and ensuring that the data used for segmentation is indicative of the actual features of the specimen. We are currently conducting a comprehensive side-by-side evaluation of optical-CT against MicroCT and traditional evaluation to determine the accuracy of each method.

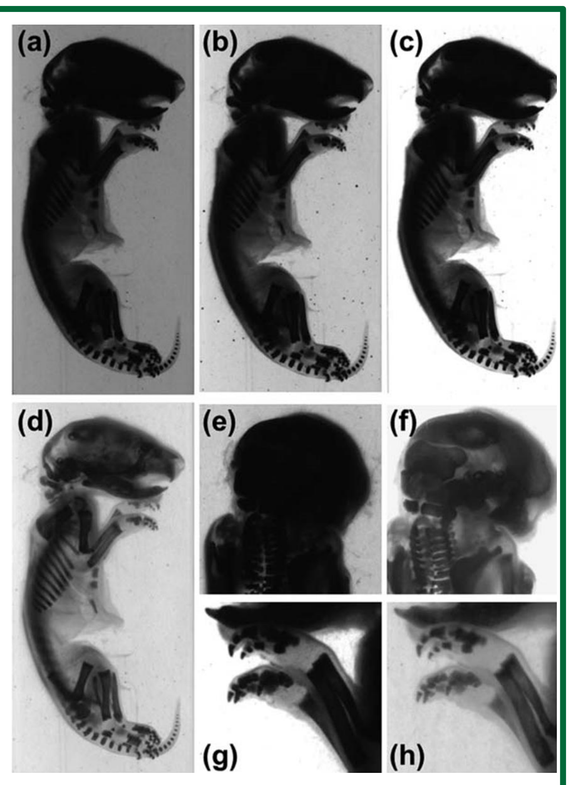

Figure 4. The 2D trans-illumination images of a 1-week-old rat acquired with an exposure time of 25 msec (a), 35 msec (b), and 45 msec (c). (d) HDR image generated from five individual images with exposure times ranging from 25 to 45 msec. (e,f) Close-up view of the skull region from 35 msec and HDR images, respectively. (g,h) Close-up view of the hands from 45 msec and HDR images, respectively.

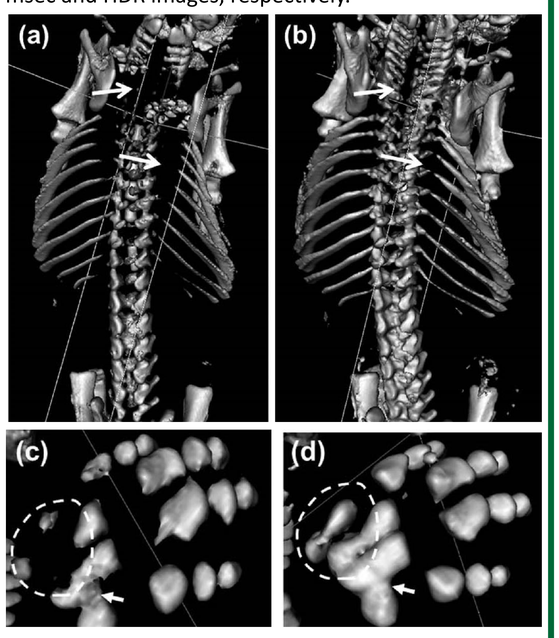

Figure 5. The 3D iso-surfaces reconstructed from 500 individual 2D trans-illumination images acquired at a fixed exposure time of 25 msec (a) and 500 HDR 2D trans-illumination images (b). Arrows illustrate regions of improved reconstruction accuracy with HDR input images. (c,d) Close-up regions of the right hand bone generated from fixed-exposure 2D images (c) and HDR images (d). Improved 3D reconstruction with HDR input images is illustrated by missing bone structure (outlined in (c)). Regions of fused bone structure (arrows in (c) and (d)) remain current limitations faced by optical –CT.

Future Direction

The future direction of our work is to optimize image acquisition and to demonstrate that optical-CT can routinely and repeatably evaluate specimens for abnormalities in a digital and automated workflow. Once we demonstrate and validate this capability, we will launch an optical-CT scanner alongside quantitative 21 CFR Part 11 compliant software for skeletal evaluation. In parallel, we are focused on developing an automated sample processing workflow that will be capable of scanning hundreds of samples per day and conducting analysis.

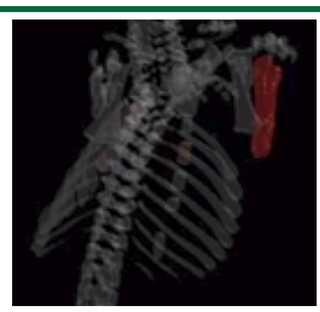

Figure 6. Segmentation algorithms have been developed that can rapidly differentiate different bones and extract quantitative data for analysis.