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Bioluminescence Tomography Improves Quantitative Accuracy for Pre-Clinical Imaging

James A. Guggenheim^a, Hector R. A. Basevi^a, Iain B. Styles^b, Jon Frampton^c, Hamid Dehghani^{a,b}

^aPhysical Science of Imaging in the Biomedical Sciences Doctoral Training Centre, College of Engineering and Physical Sciences, University of Birmingham, UK;

^bSchool of Computer Science, College of Engineering and Physical Sciences, University of Birmingham, UK;

^cSchool of Immunity and Infection, College of Medicine and Dentistry, University of Birmingham, UK

ABSTRACT

A study is presented that demonstrates that bioluminescence tomography can reconstruct accurate 3D images of internal light sources placed at a range of depths within a physical phantom and that it provides more reliable quantitative data than standard bioluminescence imaging. Specifically, it is shown that when imaging sources at depths ranging from 5 to 15mm, estimates of total source strength are stable to within $\pm 11\%$ using tomography whilst values deduced by traditional methods vary 10-fold. Additionally, the tomographic approach correctly localises sources to within 1.5mm error in all cases considered.

1. INTRODUCTION

Bioluminescence imaging (BLI) is used widely in *in vivo* pre-clinical biomedical studies in which the aim is to image distributed biological light sources, such as luciferase-tagged cancer cells, located inside a living animal. BLI images are used to estimate reporter concentrations and spatial distributions and thus to infer biological activity from images of the animal surface. However, the validity of direct quantification using BLI data directly is limited due to the highly attenuating and scattering nature of biological tissue which leads to ambiguous images.⁸

Practical bioluminescence tomography (BLT) studies have showed that in some cases individual bioluminescent sources can be reconstructed in 3D with height accuracy in terms of spatial and/or photon counting metrics, improving on BLI by presenting quantitative images of the source in context within the volume and allowing for better scientific analyses.^{2,5,9}

In this work, a physical phantom study is presented evaluating a tomographic approach² across several experiments involving an identical light source located at different positions within the same physical phantom. It is illustrated that the benefit of the approach over 2D planar imaging is maintained across a range of depths and positions that are representative of those encountered in pre-clinical studies. Critically, the estimated amount of luminescent source present, which is indicative of the cell-count that would be the quantitative end-point in the equivalent *in vivo* study, is shown to be significantly more stable with respect to increasing source depth when the tomographic, rather than the standard, bioluminescence imaging method is used.

2. MATERIALS AND METHODS

2.1 Imaging System

The imaging system used for this study, a schematic of which is shown in figure 1a, has been introduced previously along with a description of the protocol for bioluminescence tomography (BLT).⁷ Briefly, the system comprises a highly sensitive electron-multiplying charged-coupled device (EM-CCD) camera (ImagEM-1K, Hamamatsu,

Corresponding author James Guggenheim, jxg518@bham.ac.uk

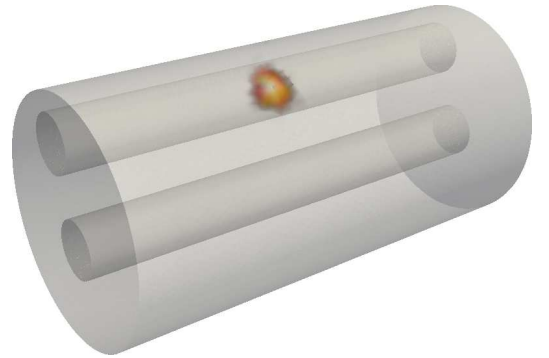
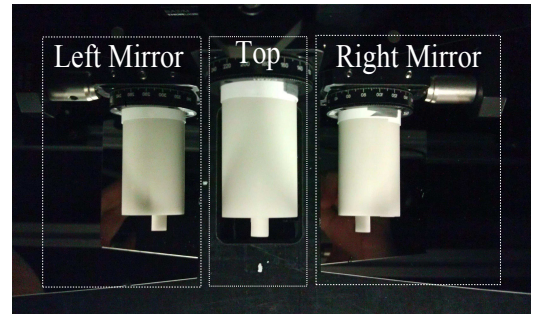
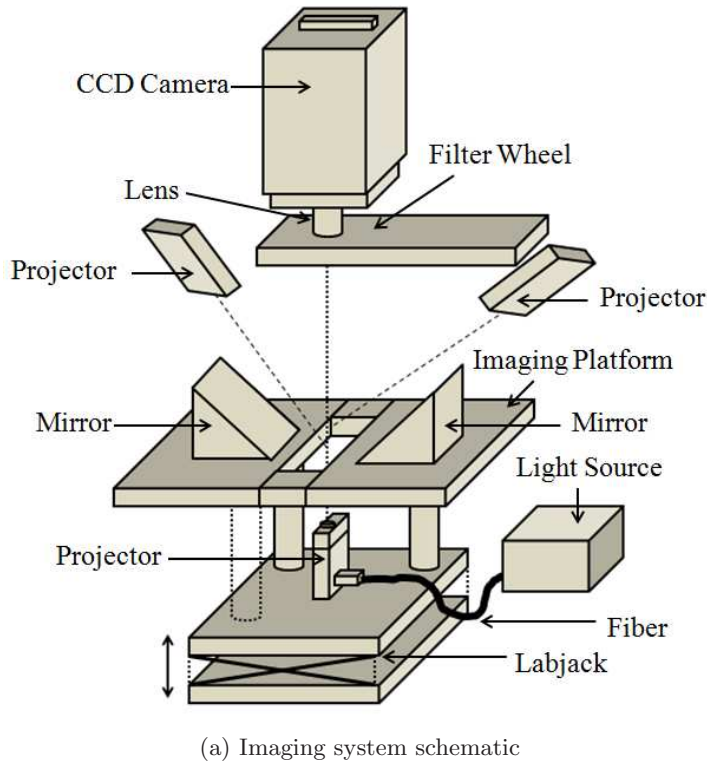


Figure 1: Overview of materials and methods with (a) a schematic of the imaging system, (b) a photo of the imaged cylindrical phantom in the system in which a direct view and two reflected mirror-views are visible, and (c) a visualisation of a tomographic bioluminescence source reconstruction in the cylinder, also showing tunnel locations. In the interest of clarity, note that whilst the imaging system contains a light source under the stage this is not used in the present study.

Japan) coupled to a 25mm lens (Techspec VIS-NIR, Edmund Optics, UK) and an automated filter wheel (FW102c, Thorlabs, UK) creating a multi-spectral optical detection system which can measure low-light luminescence signals, along with components for optical surface capture; a technique for the measurement of the shape of the animal using projected patterns also described in detail elsewhere.¹ The detection system is focused on a sample stage that supports two prism mirrors placed freely either side of the imaged subject to provide multiple views within single images as illustrated in figure 1b.

2.2 Cylindrical Phantom Imaging

A custom-made cylindrical phantom (Biomimic, INO, Canada; figures 1b and 1c) that is approximately the same size as a mouse (25mm in diameter and 50mm in length) and whose body is made of a solid plastic with spatially homogeneous but spectrally varying absorption and scattering properties that have been characterised by the manufacturer ($\mu_a \approx 0.01\text{mm}^{-1}$ and $\mu'_s \approx 1.8\text{mm}^{-1}$ in the range 500 to 850nm) is used. As shown in figure 1c, within the phantom body there are two tunnels (6mm in diameter) at depths of 5mm and 15mm into which rod inclusions can represent optical anomalies, such as organs or tumours, or can match the background. In this study a small ($0.9 \times 2.5\text{mm}$) self-sustained tritium-based light source (Trigalight Orange III, MB-Microtec, Switzerland) is held in a central position in one of the tunnels, supported between two rods with background-matching properties, whilst the other tunnel is filled by equivalent background-matching rods but with no light source. The effect is to create a luminescent source half-way along the length of the cylinder and at a depth of 5 or 15mm. By additionally using a 180° rotation with the source in the deeper tunnel, a total of three effective source depths are investigated as shown in figure 2.

In this study, luminescence images are acquired through 5 different 10nm full-width-half-maximum (FWHM) bandpass filters with central wavelengths of 500, 550, 600, 650, 700nm producing a multi-spectral image stack which is used as input data for BLT reconstruction.

2.3 Tomographic Reconstruction

Reconstruction is carried out using a compressed-sensing based optimisation algorithm² using a forward model of light transport through the phantom based on the diffusion approximation to the radiative transport equation supplied by the established software package NIRFAST.^{4,6} The phantom is represented by a finite element model (FEM) mesh with evenly spaced boundary detectors placed around the surface. The location of the phantom within the system is deduced by the surface capture sub-system (section 2.1) and FEM boundary data is subsequently related to CCD measurements by using the model of light propagation in free space developed by Chen et al.³ The *relative* spectral response of the imaging system is known and corrected for when reconstructing, thus all reconstruction data is presented in consistent but arbitrary units. Note that the whole volume is used in reconstruction, with no permissible source region or region-of-interest pre-defined. An example full 3D BLT source reconstruction is shown in figure 1c.

3. RESULTS AND DISCUSSION

Figure 2 shows cross-sectional schematics of the target source distributions in the studied scenarios along with BLI data (at 600nm) and slices through the corresponding 3D tomographic reconstructions. In the case of the BLI data, it can be seen that both the qualitative appearance of the image and the quantitative scale of the measurements changes dramatically as the source depth increases. This makes it effectively impossible to infer directly from the images where the source is, how large it is or how intense it is.

By contrast, it can be seen by visual inspection that in the case of the BLT results, reconstructed sources appear to be in approximately the correct locations in the volume; the source is clearly recognisable as a single entity within the expected region, though there is a tendency for it to become somewhat less compact as the depth increases. The reconstruction accuracy is quantified in table 1 where it can be seen that BLT reconstructed source locations are accurate to within less than 1.5mm across all cases. There is no comparable value for BLI data because it is not possible to deduce the position of the source directly from the images in a quantitative way. The table also shows the total intensity in the 3D reconstructed BLT images and in the single-view BLI images, which is representative of the cell-count that would be found in the equivalent *in vivo* study. Because the actual source intensity is not known, normalised values are shown under the assumption that a simple calibration could be performed in order to make the units physically relevant. It can be seen that over the range of depths investigated, the total source strength judged from BLT data is stable to within $\pm 11\%$ whilst the BLI values vary approximately 10-fold. This data is shown additionally in figure 3 in which the difference in stability with respect to depth is clearly visible.

Scenario	Depth (mm)	BLT Localisation Error (mm)	Total BLT Intensity	Total BLI Intensity
(a)	5	0.87	111%	201%
(b)	10	1.38	101%	67%
(c)	15	1.09	89%	23%

Table 1: Quantitative analysis of each scenario (figure 2). BLT localisation error is calculated as the Euclidean distance between the centre-of-mass of the reconstructed source distribution and the centre-point of the known target location in 3D. The last two columns show the total intensity in BLT reconstructions and in the raw 600nm BLI images (ROI in figure 2), in each set the values are shown as percentages of the mean value to emphasise the level of variability.

4. CONCLUSION

Practical experiments have shown that over a range of depths that are quite plausible in bioluminescence imaging (BLI) studies (being as the range was 5-15mm and the thickness of a mouse torso is around 20mm), estimates

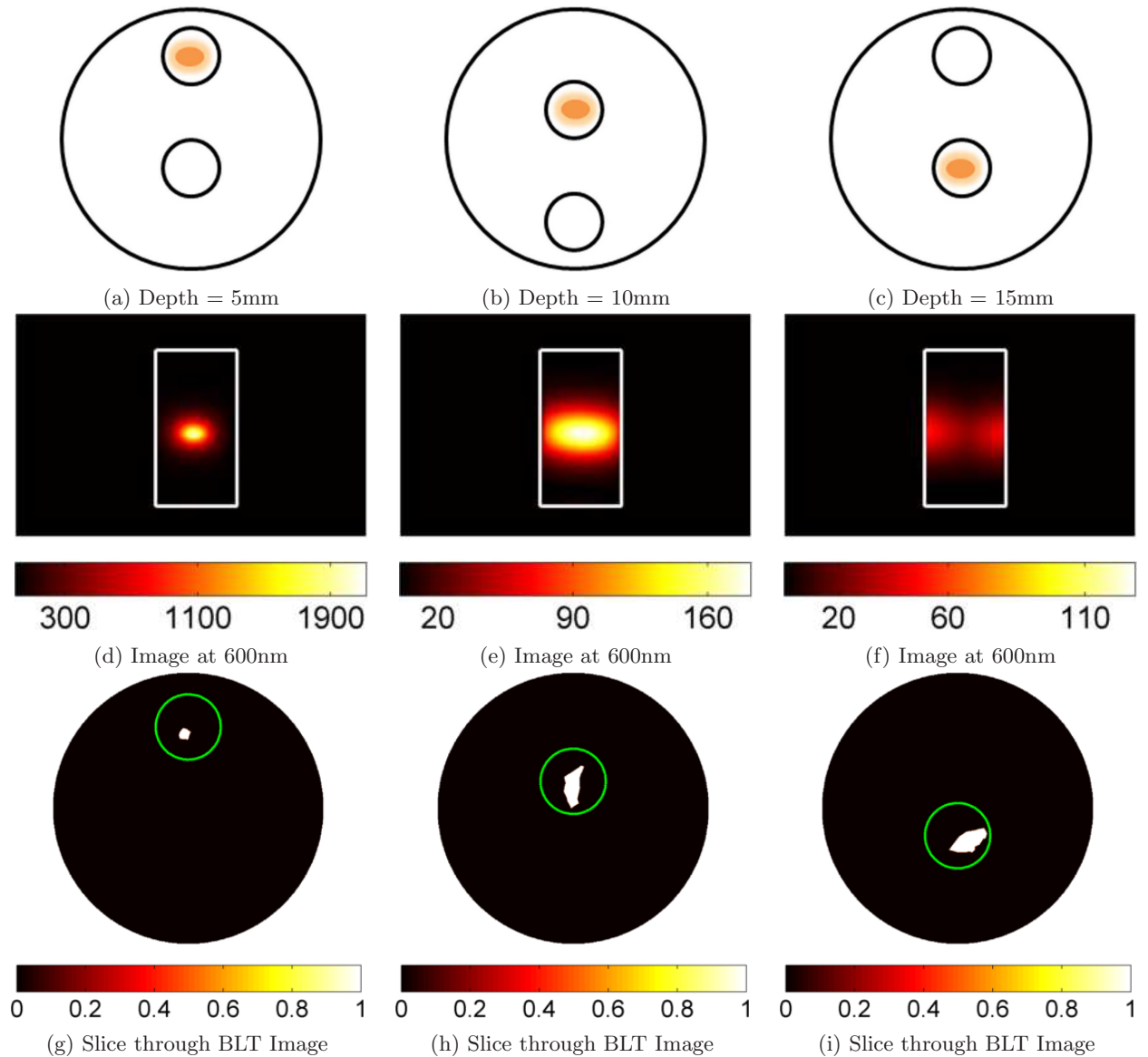


Figure 2: Overview of experimental results with (a-c) cross-sectional schematics of the actual internal luminescence, (d-f) BLI images (electrons/second/pixel on CCD) at 600nm with the outline of the cylinder drawn for reference, and (g-i) slices through reconstructed 3D BLT images taken at the axial offset corresponding to the centre-of-mass of the reconstructed luminescence distribution, thresholded at 75% of the maximum value per-image for visualisation in each imaging scenario.

of reporter strength made directly by summing intensities in BLI images vary 10-fold despite the fact that actual internal source was identical in all cases. This shows that this method is inadequate in any study where quantitative measurement of source is important.

In contrast, it is shown that using a recently developed bioluminescence tomography (BLT) system and method quantitative measurement of source estimates across the same set of problems can be improved to being stable to within $\pm 11\%$ of the mean value found. Thus the tomographic approach shows great promise for improving pre-clinical imaging. In addition to this striking improvement in source strength estimation, the tomographic approach has been shown to accurately locate the source inside the volume providing extra

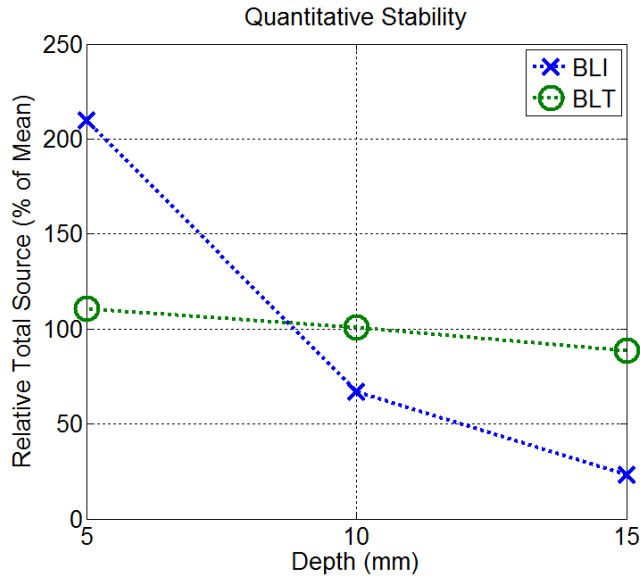


Figure 3: Graph of estimated source strength by BLI and BLT normalised to the mean of each set. This data is repeated from table 1

information.

The current investigation used a physical phantom in which the scattering dominates absorption and as such the diffusion approximation holds very well. Furthermore, the optical properties of the phantom are spatially invariant and known in advance. Tests in animals and in more realistic, i.e. heterogeneous, phantoms are therefore required in order to establish the applicability of the methods in the current form to more complex situations. It is also necessary to call on additional methods, such as diffuse optical tomography, to provide estimates of the optical properties of unknown subjects on-the-fly.

If the diffusion equation can work effectively in small animals, and optical properties can be discerned or estimated well, then the present study suggests that bioluminescence tomography could fundamentally improve the amount and the accuracy of quantitative data attained by luminescence imaging.

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