Quantitative surface radiance mapping using multiview images of light-emitting turbid media

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A novel method is presented for accurately reconstructing a spatially resolved map of diffuse light flux on a surface using images of the surface and a model of the imaging system. This is achieved by applying a model-based reconstruction algorithm with an existing forward model of light propagation through free space that accounts for the effects of perspective, focus, and imaging geometry. It is shown that flux can be mapped reliably and quantitatively accurately with very low error, <3% with modest signal-to-noise ratio. Simulation shows that the method is generalizable to the case in which mirrors are used in the system and therefore multiple views can be combined in reconstruction. Validation experiments show that physical diffuse phantom surface fluxes can also be reconstructed accurately with variability <3% for a range of object positions, variable states of focus, and different orientations. The method provides a new way of making quantitatively accurate noncontact measurements of the amount of light leaving a diffusive medium, such as a small animal containing fluorescent or bioluminescent markers, that is independent of the imaging system configuration and surface position. © 2013 Optical Society of America

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1. INTRODUCTION

Biomedical studies involving noncontact imaging of light-emitting turbid media, such as bioluminescently or fluorescently labeled small animal models, are very popular as a mechanism for probing various biological phenomena, in vivo, on a macroscopic scale. Examples of applications include imaging cancer/tumor growth and response to therapy [1–4], immune cell responses and trafficking [5,6], and stem cell trafficking and differentiation [7]. These preclinical studies now represent an essential part of the transitional stage between in vitro biology and human in vivo studies.

Most bioluminescence imaging studies involve imaging the light flux on the surface of an animal in a noncontact geometry with a highly sensitive camera at several time points during a longitudinal study. A particular advantage of this approach to monitoring disease progression is that the same animal is imaged at each time point, meaning that interexperiment variability across time points is reduced and the animal can act as its own control. In addition, fewer animals need to be used, which is ethically and economically beneficial.

In all cases it is intended that the measurement of the light flux distribution on the surface should be representative of internal luminescent activity (which may then be related back to the biology). However, most current luminescence imaging systems and studies neglect a very important effect, which is that of the lensing and relative perspectives of the system and imaged surface; the same surface imaged from a different perspective or placed in a different position or orientation within the same imaging system, or imaged with a different lens, can appear very different to the imaging system and therefore will provide different measurements.

By way of example, consider Fig. 1, which shows two images of an identical physical bioluminescence phantom seen from two different orientations within the same imaging system. It can be seen that while the light flux distribution on the surface is identical in each case, the changing perspective changes the intensity measured by the camera. This is representative of having a mouse positioned slightly differently within an imaging system when performing an in vivo imaging experiment. Clearly, it is important that this effect be recognized and accounted for in order to perform accurate quantitative measurement of the surface flux.

While simple subject-specific calibrations can be performed to convert image counts to quantitative radiance or flux measurements for well-known imaging domains with known imaging parameters (see, for example, Troy et al. [8]), this cannot be generalized to all surface geometries, orientations and positions, and distances from the focal plane without
more advanced techniques. In order to perform quantitatively accurate surface imaging, the relationship between the camera and the lens system must therefore be modeled along with its relation to the particular surface being viewed.

This represents a serious current limitation of bioluminescence imaging because different surfaces and different parts of the same surfaces cannot be compared effectively. Also, when performing longitudinal studies, imaging the same animal multiple times, it is not practically possible to place the animal in exactly the same position within the system (including both the global position of the body and the local orientation of every part of the surface, e.g., the limbs). As such, the complicated function relating the surface flux to the measurements on the detector is different and therefore quantitative surface imaging is not accurate or comparable throughout the study.

Work has been done in modeling noncontact detection schemes to account for these variations. Ripoll and co-workers [9,10] provided a rigorous analysis of the noncontact imaging scenario and implemented a method for the modeling of both externally introduced (e.g., laser excitation) and internal light sources within turbid volumes to diffuse boundary fluxes and the propagation of those fluxes to noncontact detector measurements. Cases considered included those of optical fiber detection schemes, and in- and out-of-focus charge-coupled device (CCD) camera-based systems. The authors tested an implementation of the derived model in which the focal plane of the CCD camera was modeled as an array of virtual detectors with an empirically determined numerical aperture term accounting for the angular sensitivity of the detection system [9]. By combining the internal light propagation model and the external free-space model into a single transformation matrix, Ripoll and Ntziachristos [10] went on to perform model inversion and thereby reconstruct internal fluorescence inclusions from experimental fluorescence imaging data by use of an algebraic reconstruction technique (ART). Thus it was shown that internal fluorescence distributions could be reconstructed directly from noncontact measurements.

Chen et al. [11] extended the above work by reforming the free-space forward model to include explicitly a model of a lens, treated as a thin lens. This initially comprised the inclusion of a binary visibility coefficient, which was calculated for many discrete positions on an emitting surface and receiving detector. The discretization and visibility calculation is computationally expensive but provides a conceptually correct description of the lensing system. The same group went on to improve the model by also accounting for additional apertures present within the system [12].

Given this forward model relating surface flux to CCD measurements, in the form of a linear matrix transformation applied to surface data, and by taking advantage of the principle of optical path reversibility, it was shown that a simple model inversion method (based on transfer matrix backprojection, i.e., multiplication by the transpose of the model matrix; see Section 2.B) could be used to map boundary fluxes in the domain of bioluminescence tomography imaging in a qualitatively accurate fashion [13–15]. Quantitative accuracy was not demonstrated however, and is not generally obtainable using this method. The same group showed that the reconstructed flux could be used to perform reconstruction of internal luminescent sources within phantoms and animals demonstrating the usefulness to bioluminescence tomography [14,15].

In this paper, a modification is made to Chen et al. [12] forward model that allows more flexible imaging geometry; namely, a limitation is overcome in that the focal plane that was previously constrained to lie above the animal can now be positioned freely. A new inversion method is then proposed for reconstructing boundary fluxes from noncontact CCD measurements, based on an iterative, regularized nonnegative least-squares algorithm [16]. Results of flux mapping using this method are compared with those obtained using simple backprojection, and it is found that the new approach is quantitatively far superior. Simulation studies demonstrate the robustness of the new approach across several imaging scenarios, and experimental multiview luminescence phantom imaging studies are used to validate the findings.

The proposed method provides an accurate way of performing quantitative imaging of diffusely radiating surfaces that can be directly applied to bioluminescence and fluorescence imaging problems to improve on the current standard. A notable feature of the method is that, being general, it can be applied to virtual detectors created by mirrors; it is demonstrated that signals received via mirrors as well as directly can be treated as part of the same model and inverted accurately despite the complication that there are strongly varying optical path lengths within the scene and multiple focal planes. This is an important feature because multiview imaging systems are widely used in small animal imaging studies because they provide tomographic (multiangle) imaging at little extra cost.

2. THEORY

A. Forward Problem

An imaging system is considered in which a thin lens facilitates the imaging of a diffuse light-emitting surface as shown graphically in Fig. 2. It has been shown that this thin-lens-based setup can accurately model more complex lensing systems when it is constructed such that the distance from the lens to its front focal plane and the focal length are both maintained (with the effective position of the detection plane then being calculated according to the thin-lens equation) [11,12].

Chen et al. [12] presented a forward model for diffuse imaging problems in this scenario, i.e., mapping surface fluxes onto the lensed detector. Under the assumption that the imaged surface is acting as a Lambertian source at all points, the measured intensity can be described as
The power received by a differential detector point resides in the part of the focal plane that is visible through the lens on the detector; in other words, the ray traced from the surface point through the virtual detector point intersects the plane containing the modeled thin lens within the bounds of the lens. The second condition is that the line segment joining the surface point to the virtual detector point does not intersect the surface at any point other than the originating position; i.e., there exists a line of sight from the surface point to the virtual detector point without any physical obstacles being in the way. The $\beta$ term has the same test condition as the first $\alpha$ term except that the traced ray must pass within the area of an aperture rather than the lens. Note that this term is very useful if a further aperture is present in a different plane from the modeled lens; in this work the $\beta$ term is utilized to model the mechanical vignetting present in an imaging system by placing the aperture at the entrance to the first optical component met by incoming rays, which is at some distance in front of the lens. By doing some simple ray tracing within a model of the system, it is therefore possible to establish visibility or nonvisibility via these terms.

While this model of the imaging system visibility is adequate for the situation in which the focal plane lies between the imaged surface and the lens, it works incorrectly when the focal plane is behind or passes through the object. This is because there is then a part of the volume lying between the focal plane point $r_{vd}$ and the surface point $r$ that violates the second condition of the $\alpha$ term creating false negatives. There is additionally no obstruction between the focal plane and points on the underside of the object, creating false positives. These situations are illustrated graphically in Figs. 3(a) and 3(c). In this work, a modification to the first visibility factor is proposed to overcome this issue:

![Fig. 2. Schematic of imaging system model in which the position and behavior of the lens, detector, and focal plane adhere to the thin-lens equation. Emission points on the surface are either visible or invisible to detection points on the CCD dependant upon whether or not the ray passing through the corresponding virtual detection (focal plane) point location intersects the lens plane within the bounds of the lens or aperture and is not obscured by any part of the surface. In the example shown, the surface point is visible at the detection point.](Image)

\[
\beta(r, r_{vd}; \Omega_{D}) = \begin{cases} 
1 & \text{If } (s_{r-r_{vd}} \cap \Omega_{D} \neq \emptyset) \\
0 & \text{If } (s_{r-r_{vd}} \cap \Omega_{D} = \emptyset),
\end{cases}
\]

(4)

where $s_{r-r_{vd}}$ is the line segment connecting $r$ and $r_{vd}$, $\Omega_{E}$ is the detection area constructed by the lens, and $\Omega_{D}$ is the detection area constructed by the aperture. For a graphical illustration of these parameters, see Chen et al. [12]. The $\alpha$ term evaluates to 1 (visible) given two conditions: the first is that the virtual detector point resides in the part of the focal plane that is visible through the lens on the detector; in other words, the ray traced from the surface point through the virtual detector point intersects the plane containing the modeled thin lens within the bounds of the lens. The second condition is that the line segment joining the surface point to the virtual detector point does not intersect the surface at any point other than the originating position; i.e., there exists a line of sight from the surface point to the virtual detector point without any physical obstacles being in the way. The $\beta$ term has the same test condition as the first $\alpha$ term except that the traced ray must pass within the area of an aperture rather than the lens. Note that this term is very useful if a further aperture is present in a different plane from the modeled lens; in this work the $\beta$ term is utilized to model the mechanical vignetting present in an imaging system by placing the aperture at the entrance to the first optical component met by incoming rays, which is at some distance in front of the lens. By doing some simple ray tracing within a model of the system, it is therefore possible to establish visibility or nonvisibility via these terms.

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\[
\alpha(r, r_{vd}; \Omega_{D}) = \begin{cases} 
1 & \text{If } (r_{vd} \in \Omega_{E}) \text{ AND } (s_{r-r_{vd}} \cap S = \{r\}) \\
0 & \text{Otherwise}
\end{cases}
\]

(5)

where $r_{i}$ is introduced as the point of intersection between the ray $s_{r-r_{vd}}$ and the plane containing the thin lens. This term is now a test for free line of sight between the surface and the lens plane rather than the surface and the focal plane and therefore corrects the treatment of the situation in which the focal plane is past the object, as shown in Figs. 3(b) and 3(d). The modified forward model can now be expressed as

\[
\beta(r, r_{vd}; \Omega_{D}) = \begin{cases} 
1 & \text{If } (r_{vd} \in \Omega_{E}) \text{ AND } (s_{r-r_{vd}} \cap S = \{r\}) \\
0 & \text{Otherwise}
\end{cases}
\]
The forward problem here is the computation of CCD measurements given an imaged diffuse surface flux; the inverse problem is the calculation of the originating surface flux from the CCD measurements.

Using the reciprocity theorem and the reversibility of light paths through an imaging system, Chen et al. [15] proposed a model relating CCD measurements to surface measurements using the previously proposed forward model [12] with the surface flux and detected power terms reversed. It was proposed that within this model the imaging detector could be viewed as a diffusive light source and the surface as a detector. The relationship was then

\[ J(r) = \frac{1}{\pi} \int_A P(r_d) \alpha(r_{vd}, r) \beta(r_d, r, \Omega_d) \frac{\cos \theta_x \cos \theta_d}{|r - r_{vd}|} dA. \tag{10} \]

This uses the principle of the reversibility of light paths in that the same visibility terms are used as in the forward model [12]. By updating the conditions for visibility to include the correction introduced above, this can be written in discrete matrix form as

\[ \tilde{b} = T^T \tilde{a}. \tag{11} \]

Thus this approach is a simple backprojection via the forward free-space transfer matrix and will hereafter be referred to as the backprojection method. While this approach has been shown to be effective in some experimental cases, specifically when dealing only with normalized results [13–15], it is not a quantitatively accurate model (see Section 4).

In this work an alternative approach is presented for recovering the boundary data from the CCD measurements \(\tilde{b}\) by instead solving the inversion

\[ \tilde{b} = T^{-1} \tilde{a}. \tag{12} \]

This cannot generally be computed directly because \(T\) is typically nonsquare and therefore without an inverse. To overcome this, an inversion method that has been applied to many problems, such as bioluminescence tomography image reconstruction in which boundary data are used to reconstruct three-dimensional (3D) source distributions within the volume in an ill-posed scenario [16–18], is used. The problem is formulated as a least-squares problem:

\[ \min_{\tilde{b}} \|T \tilde{b} - a\|_2^2. \tag{13} \]

and is solved using a single-step inversion that is regularized using Tikhonov regularization so that the solution takes the form

\[ \tilde{b} = (T^T T + \lambda I)^{-1} T^T a. \tag{14} \]

where \(\lambda\) is a regularization parameter defined in this study as the product of a chosen value and the maximum of the diagonal of the Hessian matrix: \(\lambda = \lambda \times \max(\text{diag}(T^T T))\). The inversion equation is solved once; then any elements of the solution vector \(\tilde{b}\) that have negative values are identified and set to zero. The residual is then recalculated and solved for again using the above equation. Reconstruction continues in this manner, iteratively solving the equation and setting negative values to zero, until the stopping criterion is reached. In this study, a stopping criterion was implemented whereby the reconstruction will terminate if the relative improvement in the solution fit is less than 1% between iterations. Additionally, iterations are limited to a chosen maximum value, which is typically five iterations.

3. MATERIALS AND METHODS

In order to perform the light modeling described above, the transfer matrix \(T\) describing the sensitivity of finely discretized detection elements to finely discretized surface elements.
must be calculated. Thus in all cases the imaging system and surface geometry must be known and the detection system focal plane and object surface must be represented in terms of many small discrete elements.

A. Simulations

Simulations were carried out first in a two-dimensional (2D) plane in which a minimalist version of the imaging system presented in Section 3.B.1 was modeled and second in full 3D modeling the same system just as when dealing with real data. All computation was performed using 64-bit MATLAB 2011b (7.13.0.564; MathWorks, Cambridgeshire, UK) on a computer (Viglen Genie; Viglen, Hertfordshire, UK) with a quad-core processor (Intel Q9550; 12 M Cache, 2.83 GHz, 1333 MHz FSB), and 8 GB of RAM, running 64-bit Windows 7 Enterprise Service Pack 1 (Microsoft, Redmond, Wash., USA). Additionally, this hardware and software were used when performing calculations on experimental data.

In all cases, when working with simulated data, two versions of the T matrix were calculated based on different levels of discretization to avoid an inverse crime.

Simulated surface fluxes were calculated using NIRFAST [19] software, which models light propagation through tissue using the diffusion approximation to the radiative transport equation. Within this modeling framework, the volume of a test object was represented by a tetrahedral mesh containing an internal light source and boundary data were calculated at the centroids of the surface elements used in the free-space calculations.

B. Phantom Imaging

1. Imaging System

Physical imaging experiments were carried out with a previously reported small animal optical tomography system [20,21]. The system, shown in Fig. 4, comprises an electron-multiplying CCD (EMCCD) camera (ImagEM-1K, Hamamatsu, Japan), along with a 25 mm fixed-focal-length lens (Techspec VIS-NIR, Edmund Optics, UK) and an automated filter wheel (FW102c, Thorlabs, Cambridgeshire, UK) containing six interference-based bandpass filters [with full width-half-maximum (FWHM) of 10 nm and central wavelengths in the range 500–850 nm] within a light tight box and pointing at a sample stage. The sample stage supports small animal or phantom subjects between two right-angle prism mirrors that provide multiview data in single images; i.e., the imaged surface is visible directly and through two mirrors. The system also contains two small optical projectors (MPro120; 3 M, Bracknell, UK) that facilitate the use of an optical surface capture technique [22] to measure the shape of small object surfaces with an accuracy of approximately 100 μm. This technique is used within the current study to recover the object surface.

The sample stage is supported by an automated high-precision vertical translation stage (L490MZ, Thorlabs, Cambridgeshire, UK) that can move the sample stage vertically through a range of approximately 5 cm with submicrometer resolution.

2. Luminescence Phantom

A custom-made cylindrical phantom (Biomimic, INO, Quebec, Canada), which is approximately the same size as a mouse (25 mm in diameter and 50 mm in length), was used in experiments (Fig. 5). The phantom is made of a solid plastic with spatially homogeneous but spectrally varying absorption and scattering properties that have been characterized within the range 500–850 nm in terms of the absorption coefficient, μa ∈ [0.007, 0.12] mm⁻¹, and the reduced scattering coefficient, μ's ∈ [1.63, 1.79] mm⁻¹. Scattering therefore dominates absorption as in most bulk biological tissues, and as such light traveling through the phantom quickly becomes diffuse and is well-modeled by the diffusion approximation [19].

In the phantom there are two tunnels (diameter 6 mm) at depths of 5 and 15 mm from the surface. In this study, bioluminescence is modeled by placing a self-sustained, tritium-based light source (Trigalight Orange III; mb-microtec, Switzerland) halfway along a tunnel enclosed between two rods of background-matching material, while the other tunnel is filled with background-matching material but without a source. Figures 5(b) and 5(c) show the resultant configurations.

C. System Geometric Calibration

Using a thin-lens model for both the camera and the projectors in the imaging system, the locations and directions of all rays (one per pixel) were calculated using a geometric calibration method. The particular method was developed in-house, but it
is conceptually similar to many published calibration methods that exist for cameras and projectors [23–25]. The method is based on first imaging a flat regular grid at multiple heights (using the translation stage) and using image processing to automatically extract the grid point locations in images. Camera parameters are then solved for under the assumption that the optical axis is perpendicular to the imaged grid.

Once the camera is calibrated using this method, each of the projectors projects a regular grid onto the stage at several different heights, the camera model is used to extract absolute coordinates of the projections, and the resultant ray traces are used to solve for the projector parameters.

Note that the resulting camera model is sufficient to uniquely define the focal length of the lens system that is nominally 25 mm but was found by this method to be 23.9 mm. The model also yields the effective lens and detector locations as well as the magnification of the system. All of these parameters are needed for performing free-space calculations.

4. RESULTS

A. Two-Dimensional Simulation

In a first simulation study, a 2D slice through the imaging system is considered (Fig. 6); detectors, lenses, mirrors, and surfaces are considered as 3D objects, but the surface and detection element arrays are each one element thick. This has the effect of creating a 2D-like problem domain for which all of the complexity can be visualized accurately with 2D plots (see below) but still a functioning 3D environment so that the same equations and functions can be used as in the full 3D model.

A line detector consisting of 1024×1 square detection elements of size 13×13 μm was considered. A 23.9 mm focal-length thin lens was placed ≈25.8 mm in front of the detector, creating a front focal plane positioned ≈317 mm past the lens at z = 21 mm. A circular surface with radius 25 mm (approximately 160 mm in circumference), 1 mm thick and consisting of 200 elements, was placed on a flat stage near the focal plane. The detection elements were oriented perpendicular to the optical axis, as was the top-most element of the circular surface. The surface and the detector were then discretized 18× and 6×, respectively (in a single direction only—in keeping with the 2D-like representation), and the transfer matrix T was calculated based on the resultant 6144 discretized detection elements and 3600 discretized surface elements. The height of the surface was determined by the position of a sample stage, which was placed at z = h, where h ∈ [−120, −80, −40, 0, 40] mm. The transfer matrix calculation was carried out for each stage height considered. For simplicity in simulation, signal modifications due to lens transmittance, camera quantum efficiency, and image digitization were not considered.

Figure 7 shows the total sensitivity of the whole detector to each discrete surface element for the range of stage heights investigated. It can be seen that only approximately half of the surface elements are at all visible (have nonzero sensitivity), which is to be expected given the geometry under consideration; the underside of the surface is pointing away from the detection system and obscured by the upper half. It can then be seen that the surface sensitivity follows a curve that shows the most sensitivity in the center of the field of view (the top of the circular surface) and falls off to either side. This is due mostly to the increasing curvature of the surface but is influenced to some extent by all of the factors present in Eq. (6), namely the angles the surface and detector normals make with the rays that connect the surface and detector points, the distance of surface points from focal plane points, and the visibility of the surface to the detector.

It can be seen that the peak height of the sensitivity is strongly influenced by the position of the surface relative to the focal plane and that the total response broadens in cases in which the object is more out of focus. This is indicative of the blurring that occurs in the system.

A Gaussian-shaped surface flux was simulated on the top boundary of the 2D cylinder slice, in order to represent a typical flux in a diffuse imaging experiment (Fig. 1, for example). The peak output at the surface was 50 million photons. This was then multiplied by each of the calculated free-space transfer matrices to simulate the measurement process by the detector in each height-resolved setup. Figure 8 shows the resultant detector measurements along with the initial flux. It can be seen that the flux is imaged on the detector producing a qualitatively accurate depiction of what is on the surface as in a real imaging system. This is best-focused in the case in which the upper half of the surface is nearest to the focal plane (h = −40). It can be seen that, as expected, the signal blurs more and more the farther from the focal plane it becomes. The detection system captures less than 0.1% of the light emitted by the surface, with the better focused scenario leading to the most light collected.

In order to more accurately model a practical imaging scenario, shot noise was added to the simulated signals (normally distributed noise with σ(Ii) = √(P(Ii))). The T matrix calculation was then repeated using a coarser discretization level (9× for the surface and 3× for the detector) and flux was then reconstructed using backprojection and the nonnegative iterative least-squares algorithm. In the case of the latter,
the regularization parameter was fixed at $\lambda = 10$, and the maximum allowed number of iterations was 10.

Figure 9 shows sample results for the case in which the stage height was $h = -40$ mm and the maximum photon emission on the surface elements was 50 million. It can be seen that while the normalized result of the backprojection appears roughly qualitatively accurate in that the flux is centered on the correct location and the distribution is smooth, consistent with the literature [15], the backprojection without normalization is not accurate. The peak value is approximately 8.5 orders of magnitude weaker than the original signal. The reason that the backprojected signal is so much lower than it should be is that the measurement matrix has the effect of reducing the signal by approximately 4 orders of magnitude and this is effectively applied twice in this approach (once forward and once back). In contrast, the proposed method of iterative, regularized nonnegative least-squares reconstruction provides qualitatively and quantitatively accurate results without normalization.

Further reconstructions were performed for the other stage heights and in addition for several signal strengths $\in [5 \times 10^7, 5 \times 10^6, 5 \times 10^5, 5 \times 10^4]$. To quantify the accuracy of the reconstructions, the percentage total error in the reconstructed source was considered with reference to the known ground truth:

$$\text{error}(b, b^\prime) = \frac{\sum_{j=1}^{n} |b_j - b_j^\prime|}{\sum_{j=1}^{n} b_j}.$$  

(15)

where $b$ represents the known ground truth values and $b^\prime$ represents the reconstructed values of the surface flux. Each reconstruction was performed 50 times with a different instance of simulated shot noise added. The results are shown in Fig. 10.

It can be seen that regardless of the signal strength, the backprojection method lacks quantitative accuracy being so far from the ground truth as to have an error of 100% in all cases. In contrast, the inversion method presented here shows very low errors where there is high signal-to-noise ratio (SNR) (the maximum SNR is listed in Table 1). It can be seen that the error generally increases as the SNR gets lower. Specifically, in all cases where the maximum SNR is better than 10:1, the mean reconstruction error is less than 10%. In all cases where the maximum SNR is greater than 30:1, the mean reconstruction error is less than 5%. In the highest signal case (with SNR $\approx$95:1), the mean error is less than 3% with a standard deviation that is less than 5% of the mean value. Even when the maximum SNR is less than 10:1, it can be seen that in some cases the mean error is less than 30%. To put these results in context, a maximum SNR of around 200:1 would be achievable in practice (see Section 5) and therefore the test cases are difficult problems.

B. Three-Dimensional Simulation of Multiview Imaging System

A fully 3D simulation was undertaken in which the physical imaging system (Section 3.B.1) and physical phantom (Section 3.B.2) were modeled. The imaged cylindrical phantom was represented by a finite element mesh created using NIRFAST. A luminescence source was simulated within the mesh at a depth of 5 mm [Fig. 5(b)], and boundary data were calculated with NIRFAST at a single wavelength (580 nm).

A feature of the modeled system is that it uses mirrors to expand the field of view of the CCD camera [Figs. 4 and 11(a)].

Fig. 8. (a) Simulated surface flux (ground truth) and (b) measurement on line detector simulated in each height-resolved setup.

Fig. 9. Sample reconstructed fluxes overlaid on ground truth values for $h = -40$ mm and 50 million photons: (a), (b) backprojection approach with absolute or normalized comparison; (c), (d) proposed approach with absolute and normalized comparisons. Note particularly the $8.5x$ factor indicated in (a).

Fig. 10. Errors in total flux reconstructed on the surface across 50 repeats for a range of stage heights affecting imaging geometry and for a range of signal strengths affecting signal-to-noise ratio (SNR). Note that only one data set is plotted for the backprojection method because the quantitative error was practically 100% in all cases.
Table 1. Maximum SNR in Simulated Noisy Measurements Used for Reconstructions

<table>
<thead>
<tr>
<th>Peak Flux (Photons)</th>
<th>SNR as a Function of Stage Height ( h ) (mm)</th>
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</thead>
<tbody>
<tr>
<td>( 5 \times 10^7 )</td>
<td>( h = -120 )</td>
</tr>
<tr>
<td></td>
<td>32.93:1</td>
</tr>
<tr>
<td>( 5 \times 10^8 )</td>
<td>10.41:1</td>
</tr>
<tr>
<td>( 5 \times 10^9 )</td>
<td>3.20:1</td>
</tr>
<tr>
<td>( 5 \times 10^{10} )</td>
<td>1.04:1</td>
</tr>
</tbody>
</table>

Noise was added as shot noise so SNR is a function of signal; the listed values are therefore \( \sqrt{m} \), where \( m \) is the maximum-valued simulated measurement.

There are now three times more detection elements (the original and those reflected in each of the two mirrors), but once the geometry is established, i.e., the positions and normals of all components, the free-space modeling proceeds exactly as before. Multiview images are constructed by the addition of all fluxes incident on direct or reflected incarnations of each detection element.

In the system model, the detector was represented by a 256 × 256 array of elements (one for each physical pixel in this case, the 1 MP detector being binned 4 × 4 for imaging), and the cylindrical surface was represented by 1600 equally sized elements corresponding to a 50× angular discretization and a 32× axial discretization. For computation of forward data, a further discretization was applied of 26 × 26, 41 × 41, and 21 × 21 to the surface for the direct view and left and right mirror views, respectively. For the detection areas, discretizations of 2 × 2, 4 × 4, and 2 × 2 were applied. A higher discretization was applied to the surface as compared to the detector because the initial size of surface elements is approximately 10× that of virtual detector (focal plane) elements. Different levels of discretization were applied for each of the views because the different focal plane positions (see Fig. 11) resulted in different distances between the focal plane and the surface, and it was observed that higher discretization is required to get accurate results when the focal plane is nearer to the surface. Optimal selection of discretization levels is a subject for further study, and it is expected that the levels used here are higher than required.

Simulated forward data were obtained for seven different cylinder positions, corresponding to seven different rotations (with steps of approximately 30°) of the cylinder about its axis along with very slight movements in global position. In all other ways the imaging system parameters were unchanged. Simulated images are shown in Fig. 12. Note that 100% mirror reflectance was assumed for the simulation and mechanical vignetting was not modeled (this is addressed in Section 4.C). The surface flux was then reconstructed using the simulated forward data for each scenario. In this reconstruction no

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Fig. 11. Plot showing the position of the cylindrical phantom, mirrors, and focal planes (the direct focal plane of the lens-camera system and the two focal planes of the virtual systems through the mirrors).

Fig. 12. Simulated forward images with the phantom at different orientations. Approximate phantom outlines shown for reference in direct and mirror views (recall the mirror positions in the simulated imaging system; Fig. 4). Some empty space has been cropped.
noise was added, the regularization parameter was set to 0.1, and the reconstruction was terminated at the fifth iteration. For reconstruction, the discretization levels used were the same as for the forward model for detectors and \( n - 1 \) times for the surfaces where \( n \) was the value used in the forward model.

A single mapped flux [corresponding to the third scenario; Fig. 12(c); \( \theta = 60^\circ \)] is shown alongside the known ground truth in Fig. 13 along with a single slice taken around the central plane (axially) of the surface plotted for reference. It can be seen that the reconstructed flux is effectively indistinguishable from the ground truth in this case. Slice plots for the remaining six data sets are shown in Fig. 14. Note that the slices are taken through the mesh following its registration to a common orientation so that in all cases the target and reconstructed fluxes should appear in the same place. It can be seen that the quantitative accuracy is excellent across all data sets although it breaks down in those instances where the relevant part of the surface is physically invisible to the detection system [see Figs. 12(f) and 12(g)], which is unavoidable.

### C. Practical Validation with Phantom Luminescence Imaging Data

In a practical validation experiment, the luminescence phantom was prepared with the internal light source at a depth of 5 mm from the surface [Fig. 5(b)]. The phantom was fixed just above the sample stage in a rotation mount such that it could be rotated small amounts and without otherwise moving appreciably. It was turned by approximately 30° six times and imaged at 580 nm in each position so as to produce the seven images shown in Fig. 15. While the intention was to turn the phantom 30°, there was some error in the turning and the actual angle turned was established based on the results of surface capture. As can be seen in the images, a single rod was left protruding from the phantom, and surface capture points from this were used to establish the angle of rotation.

![Fig. 13. (a) Target and (b) reconstructed fluxes for set 3 in the simulation study. (c) Illustration of the axial plane at which cross-sectional data were extracted (i.e., flux values around the circumference) and plotted for this set (d) and others (see Fig. 14).](image1)

![Flux slice location](image2)

Fig. 13. (a) Target flux (\( \theta = 60^\circ \)), (b) Reconstructed flux (\( \theta = 60^\circ \)), (c) Flux slice location, (d) Reconstruction slice (\( \theta = 60^\circ \)).

Flux reconstruction was then performed using each of the practical data sets. For these reconstructions, \( \lambda \) was set to 1 and the maximum number of iterations allowed was 5. The same discretization levels were used as in the 3D simulation reconstructions (listed in the previous section). Mechanical vignetting (present in the system owing to the filter wheel; Section 3.B.1 and Fig. 4) was modeled by considering an additional aperture [modeled with the \( \beta \) term in Eq. (6)] located at the opening to the filter wheel with appropriate diameter (25 mm); this has the effect of dimming the mirror views as can be seen in practical images (Fig. 15) as compared to the corresponding simulated images (Fig. 12). Additionally, a heuristic scaling factor was applied to all measurements made via the mirrors to compensate for the differing
transmittance properties of the interference filter given different angles of incidence [28]. Note that this should not be necessary in cases in which colored glass filters are used, or in cases in which there is no filter.

Figure 16 shows each reconstruction in 3D with the surfaces rotated back into a common orientation for ease of comparison. It can be seen that the flux distribution is reconstructed in the correct location with approximately the correct shape in all cases. As per the analysis of the simulation results, a slice was taken through the cylinder and the results are plotted in Fig. 17. It can be seen that the practical data show very consistent results across the range of orientations considered; the values are remarkably similar. At high angles, larger gaps are seen than in the simulation due to lack of surface visibility in any view [Figs. 17(f) and 17(g)], which is because in simulation the mirrors were modeled as infinite, whereas in practice they stop at the sample stage. Despite not being completely visible in some cases, it can be seen that the values that are present are still good. Beyond this, similar results are found as in simulation although there is slightly more quantitative variability as might be expected when the errors associated with modeling the real system are introduced. Figure 18(a) shows a quantitative comparison of total signal measurements based on the results of the new mapping method, of backprojection, and of quantification of total light output direct from single-view bioluminescence images (i.e., simply summing the image intensity in the background-subtracted top-view region). The graph only shows results for sets 1–5 because beyond this point a large amount of the surface flux was physically invisible to the camera. It can be seen that the direct method of quantification produces results that vary 10-fold and that the backprojection method improves on this by very little. In contrast, the presented method of flux reconstruction produces a value that varies by less than ±3% making it a far more reliable metric of flux output on the surface.

Figure 18(b) shows results of a final experiment in which the cylinder was prepared with the source at a depth of 10 mm [Fig. 5(c)]. With the system in the same setup as previously, the cylinder was then imaged (without mirrors) resting directly on the stage at four different heights. This was achieved by moving the stage in increments of 10 mm; the cylinder started with the top-most surface point being 14 mm above the focal plane and then moved nearer to the lens at each point; thus it moved through the focal plane and was in varying states of focus in the experiments. The results show that the mapping method produces a height and therefore a focus-independent, as well as an angularly independent, measurement of total signal on the surface that again varies by less than 3%. In contrast, the direct quantification and the backprojection approaches both show a clear height dependence, and the signal changes by up to 25%.

While it has been shown here that the reconstructed flux obtained using the backprojection method is quantitatively poor, it is noted that the results of backprojection appeared qualitatively (i.e., spatially, judged by visual inspection, and comparing with normalized targets) accurate, as reported in the literature [12].
5. DISCUSSION

In total four distinct experiments have been presented, two simulation and two experimental, testing various aspects of the proposed method for mapping surface fluxes from CCD images.

In the first experiment, a 2D slice through the imaging system presented in Section 3.B.1 was considered in the case of imaging a circular surface—representing a slice through a cylinder or a small animal model. Across the 50 repeats of several scenarios in which the imaged surface was moved vertically through the focal plane, it was shown that in cases in which there was adequate SNR the presented method performed well—with errors as low as 3% and small standard deviations as shown in Fig. 10. It was shown that quantitatively the method of backprojection was not effective and so the presented method represents a substantial improvement.

It is worth noting that the maximum SNR in the best-case in this study was set at approximately 95:1 for the highest-valued measurement, which is a realistically achievable SNR value for a real system. Consider a 16-bit detector being used in a bioluminescence imaging study; if a detected signal reaches 50% of the full-well capacity of the detector, then it will be read out at $2^{16}/2 = 32768$ counts—on this measurements the shot noise could be expected to contribute approximately 181 counts, making the SNR 181:1, which is even higher than the highest maximum SNR considered in this simulation study. Thus the experiment has demonstrated that even in fairly moderate SNR levels, compared to those realistically attainable, the method is accurate in the presence of noise.

In the second simulation experiment, it was shown that a full 3D system could be modeled to provide full 2D simulated images as has been shown previously [11,29] and that the presented inversion method could map fluxes effectively invariant of the turning angle of the imaged 3D cylinder. This is significant because it is representative of the small animal imaging scenario in which a mouse is imaged from a slightly
different perspective in, for example, successive longitudinal study imaging sessions. The simulation showed that where the surface was physically visible, the flux could be mapped effectively and from multiple views.

It is worth emphasizing that the multiple views within the model were dealt with simultaneously in the inversion (by adding successive system matrices and measurements before reconstruction), and as such multiple observations (i.e., from different perspectives) can be used simultaneously to overcome noise. The differing geometry introduced to the system by the mirrored versions creates multiple focal planes, and if these are arranged so as to be best-focused on average it is likely that the focal planes will fall on either side or at least through the surface; this highlights the importance of a model that can deal with the focal plane being positioned arbitrarily with respect to the surface. It was demonstrated that this was not the case before the adaptation made to the visibility terms, and the multiview simulation experiment has demonstrated, having focal planes positioned on either side of the surface with respect to the detector, that the new method is robust to the placement of the focal plane.

The practical validation experiments have then shown that the findings in simulation are in keeping with those found practically. As the physical phantom was turned and imaged, it was shown that the maximum intensity in images decreased dramatically as a function of angle, and it is seen that there is a 10-fold drop in total top-view intensity in images over the range in which the majority of the flux was physically visible (sets 1 to 5). This is a dramatic change given that this method is presently used for quantification in biomedical studies [15,30] and the actual surface flux was the same in each case.

Applying backprojection did not improve results, but when applying the proposed method the reconstructed flux was stable as a function of angle. This is an important result as it suggests that if an animal is imaged in an arbitrary position in, say, a bioluminescence imaging study, the presented method could reconstruct the flux on the surface accurately whereas no other method currently accounts for this scenario. Note that although all quantitative measurements shown in this paper have been given in arbitrary units, they have always been the same arbitrary units so that a single experiment with a calibrated light source could trivially make all the measurements correct in physical units. This is clearly distinct from the other case presented (in Fig. 9, for example), where case-by-case normalized results are compared.

The final experiment from which results were presented was that in which the cylinder was imaged through a range of heights; this further tested the robustness of the presented method with respect to changing surface properties, and it showed that the method worked effectively whereas existing approaches did not.

Overall the experiments have shown that the new technique is robust to realistic levels of noise, changing imaging geometry (e.g., the changing relative position of the focal plane, the mirrors), and changing imaged surface orientation.

The levels of discretization used in all cases were chosen empirically based on comparison of performance in forward models (i.e., an analysis of forward images in qualitative terms), and it is expected that they were higher than is required to effectively map data. A full study of the effects of varying discretization is considered to be an interesting topic of future work. An observation that has been made is that finer discretization is necessary when the object is near to the focal plane in order to obtain good results, as the sampling of the angular collection of the lens is done via rays cast through pairs of points, which, if closer together, change the angle more sharply and therefore sample the space near to the lens more rarely. Further study is required on this, but it is suggested that a variable discretization approach, taking into account the distance between surface and virtual detector points, may work well.

The choice of regularization parameter has also been observed to affect results significantly, and an automated or reliable heuristic approach for its choice is worthy of future study. Specifically, optimization techniques such as L-curve [31], maximum likelihood (ML), and generalized cross validation (GCV) [32], as well as other recent developments such as the U-curve method [33], could be investigated for the automatic, optimal determination of such parameters. It is possible that novel optimization methods (possibly with fewer free variables) could effectively overcome this problem, and it would be useful to evaluate other algorithms applied to the current problem.

6. CONCLUSION

The wide and growing popularity of noncontact imaging of small animals in fluorescence and bioluminescence imaging scenarios necessitates an accurate method for measuring distributions of light exiting a surface: surface fluxes. It has been demonstrated in this paper with explicit examples that relative surface measurements will generally vary significantly if the surface point under observation is not identical to those to which it is compared. However, a method has been presented herein that solves this problem by reconstructing accurately the flux distributions on arbitrary surfaces requiring only images, knowledge of the system, measurements of the surface, and the appropriate computations.

Specifically, it has been shown that the flux reconstructed in practical imaging scenarios can be reconstructed with a repeatable result (to within 3%) independent of its position in the system and its orientation. This has been supported by simulation studies that have shown the same trends and additionally shown that the results are robust to noise by repeated experiment. This builds significantly on existing methods such as the discussed backprojection method [15], which works well qualitatively but not quantitatively, as has been shown in this paper.

Given that biomedical studies currently rely on metrics based on summed regions of interest direct from images to quantify surface fluxes, the presented method makes a marked improvement that can immediately find useful application in many domains.

It is important that the method be applied to real animal studies so that the performance on biological samples can be evaluated. It is expected that, for the case of deep light sources within nude or shaved mice, the cylinder phantom used in this work is an effective analogue to the real animal case and therefore good results are anticipated. A remaining open challenge for quantitative optical imaging is making measurements through hair on animals that are not shaved or nude, and this will form the basis of future studies.
A further extension of the presented work could be the application of the proposed method to the modeling of noncontact excitation as used in fluorescence tomography or diffuse optical tomography systems. This problem is related closely to the work presented here since the light paths considered, for example from an optical source originating from a digital micromirror device (DMD) through a lens, are identical to those considered here, but reversed as the problem is now one of projection rather than collection of light. This will require further experimental studies.

It is intended that the source code used in the calculations of this paper will be made openly available online in due course as part of the NIRFAST software package [10]; until then the author will provide the code upon request.

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