A Fast full-body fluorescence/bioluminescence imaging system for small animals

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ABSTRACT

Whole body in vivo optical imaging of small animals has widened its applications and increased the capabilities for preclinical researches. However, most commercial and prototype optical imaging systems are camera-based systems using epi- or trans- illumination mode, with limited views of small animals. And for more accurate tomographic image reconstruction, additional data and information of a target animal is necessary. To overcome these issues, researchers have suggested several approaches such as maximizing the detection area or using the information of other high-resolution modalities such as CT, MRI or Ultrasound, or using multi-spectral signals. As one of ways to maximizing the detection area of a target animal, we present a new fluorescence and bioluminescence imaging system for small animals, which can image entire surface of a target animal simultaneously. This system uses double mirror reflection scheme and it consists of input unit, imaging unit with two conical mirrors, the source illumination part and the surface scanner, and the detection unit with an intensified CCD camera system. Two conical mirrors are configured that a larger size mirror captures a target animal surface, and a smaller size mirror projects this captured image onto a CCD camera with one acquisition. With this scheme, we could capture entire surface of a target animal simultaneously and improve back reflection issue between a mirror and an animal surface of a single conical mirror scheme. Additionally, we could increase accessibility to an animal for multi-modality integration by providing unobstructed space around a target animal.

Keywords: Optical tomography, fluorescence molecular tomography, bioluminescence tomography, small animal imaging, instrumentation

1. INTRODUCTION

Fiber based systems with the flexibility of viewing directions have been widely used for tomographic imaging systems [1]. However, those systems have provided limited number of source and detector pairs and for higher spatial resolution of tomographic reconstruction results, more source and detector pairs have been required. In this respect, camera systems as a detector have increased the detectable area on a target animal innovatively. Nevertheless, a camera has limited viewing angle and this made the imaging geometry of most prototype or commercial camera based systems provide only planar imaging for reflectance or transmittance measurement or employ rotational systems or multiple cameras [2, 3]. Mirror reflection schemes for increasing the directional views of one camera also have been suggested [4, 5]. Among these methods, a single conical mirror scheme showed higher efficiency in 360° directional view detection and the camera pixel use. But using mirrors in the optical paths between an animal and a camera can generate unwanted light reflections. If these reflection phenomena are not modeled in the tomographic reconstruction algorithm, to reduce the reconstruction errors, unwanted light reflections, which may disturb precise signal measurements, should be minimized in terms of instrumentation design.

In this work, we developed the double conical mirror scheme for detecting the entire surface of an animal simultaneously. We also designed a source illumination unit and a surface scanner, which are integrated into the system without obstructing the camera's view of a target animal. With this scheme, we minimized the back reflections between a mirror surface and a target animal surface without losing the 360° detection capability of a single conical mirror scheme. For proving the improvement, the back reflection degree in the double conical mirror scheme was compared with that in a single conical mirror scheme by using Monte Carlo ray tracing simulation.

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2. METHODOLOGY

2.1 System overview

The imaging system was designed to measure amplitude modulation and phase shift for the frequency domain detection. It consists of three main units (input unit, imaging unit, and detection unit) and a host computer like figure 1. Input unit provides light sources to the imaging unit and reference signals to the detection unit. Imaging unit project the signal from an entire animal surface onto a camera in the detection unit and generates the surface geometry of a target animal.

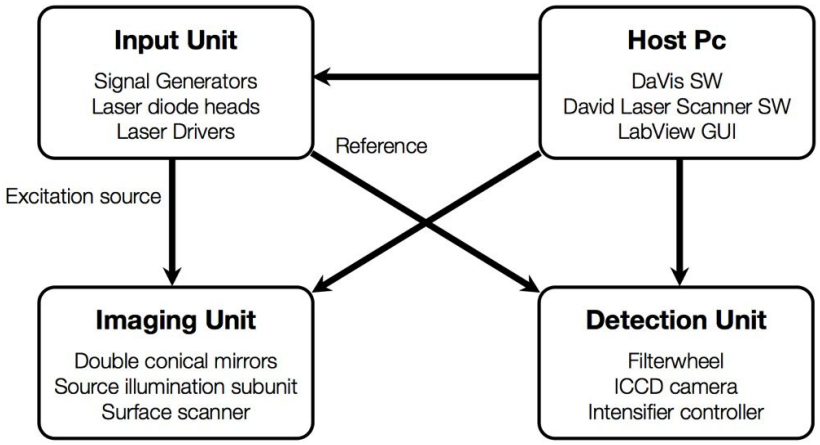

Figure 1. System overview; input unit, imaging unit, detection unit, a host computer and their subunits

2.2 Input unit and detection unit

Input unit provides intensity modulated laser sources to the source illumination subunit and the reference signal to the intensifier of the intensified CCD camera system. It employs two signal generators (2023A, Aeroflex Incorporated, NY), five laser diode heads (475, 661, 757, 828, 926 nm) with peltier cooling and three drivers (PicoQuant GmbH Germany). The laser heads are pigtailed into a multimode optical fiber with FC/APC connectors. The spot size of the laser beam is 1~2 mm diameter on a target animal surface by using a collimator (Schäfter + Kirchhoff GmbH, Germany).

Detection unit for frequency domain data acquisition uses the intensified CCD camera system (PicoStar HR 12, LaVision GmbH, Germany). It consists of an optical lens, an intensifier, and a CCD camera. Photons, passing through the filter wheel (AB302-T, Spectral Products, NM) and a lens, are incident on the intensifier, which consists of a photocathode, a single micro channel plate, and a phosphor screen for the photon amplification. The amplified photons are focused on the CCD sensor (1376×1024, 6.5 μm pixel size) lastly. DaVis imaging software (LaVision, Germany) controls all functions of signal generators and the camera system.

2.3 Imaging unit

Imaging unit is the main unit of the system for projecting the entire surface onto a detection camera and generating the surface geometry of a target animal. It is comprised of double conical mirrors, a source illumination subunit, and a surface scanner.

2.3.1 Double conical mirrors

The basic idea of capturing multi-directional views simultaneously is to use double consecutive mirror reflection scheme like figure 2. In this scheme, by using two mirrors, a camera A has the field of view of a camera B. The first mirror facing a target animal captures a surface of a target and the second mirror facing a detection camera reflects and projects the captured images by the first mirror onto the detection camera. Depending on the shape of a target, the shape of first and second mirrors can vary like flat or conical or oval or two combined shape. For our system, since the target is a

small animal like mice, we choose a conical shape to capture whole body surface of a small animal. The conical mirror size was designed to cover a 40 mm diameter, 80 mm length cylinder, the size of which is enough to cover a small animal.

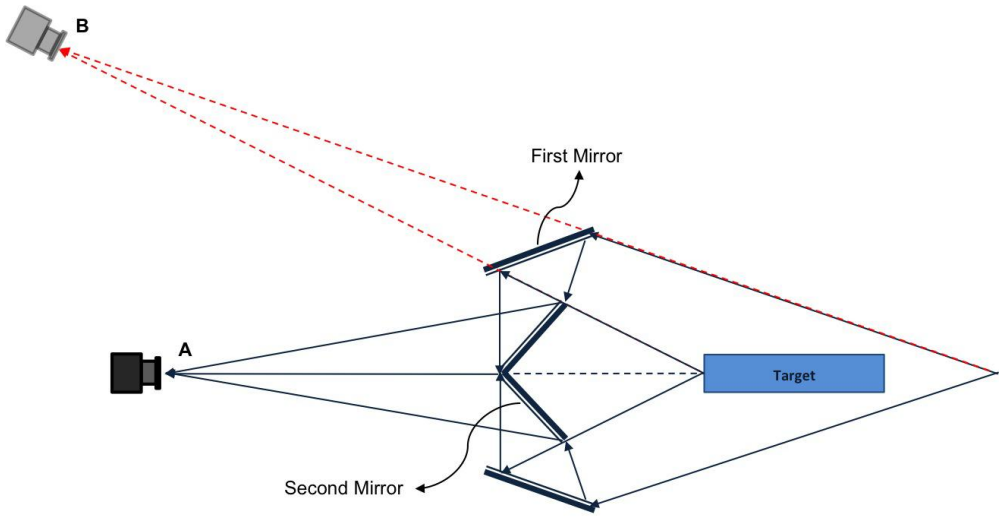

Figure 2. Double conical mirror scheme: the first mirror and the second mirror were designed to cover a 40 mm diameter 80 mm length cylinder target.

Figure 3 shows the positioning of a coke can as a target in the imaging unit and the taken image by a camera. As shown in the captured image, because of the conical shape of mirrors, there is an image distortion and to do tomographic reconstruction, we need to know where the photons in the captured image came from on the target. Thus we also developed the ray transfer-mapping operator [6].

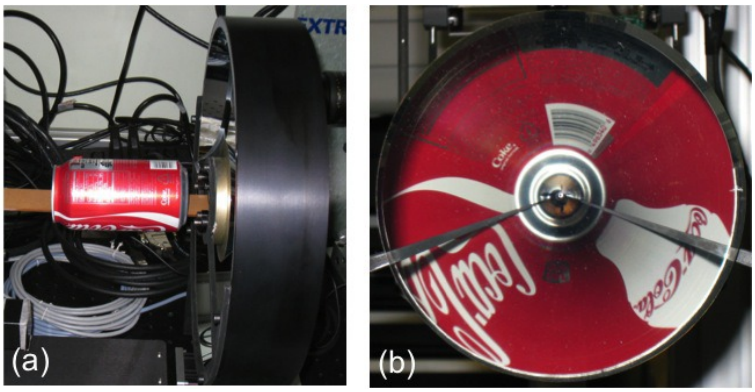

Figure 3. (a) Positioning of a coke can in the imaging unit (b) the captured image on the camera

2.3.2 Source illumination subunit

The design constraint of a source illumination subunit was the precise illumination of a laser source on a target animal surface without obstructing the camera's view of an animal. In figure 4 (a), the pink area is the field of view of a camera where should not be obstructed. Thus, we used out of field of view area (the blue area) and developed the source illumination subunit like figure 4 (b). This subunit is comprised of two components, a rotational gantry system and a motorized linear translation stage. A rotational gantry system, which employs gears and a DC brushless motor (Maxon

precision motors Inc., MA), provides 360° source illumination around a target animal. And the displacement of this gantry system by the translational stage (Aerotech Inc., PA) provides the illumination of pinpoint laser sources on the any spot of an animal surface. The developed LabView GUI controls all motions of motorized components.

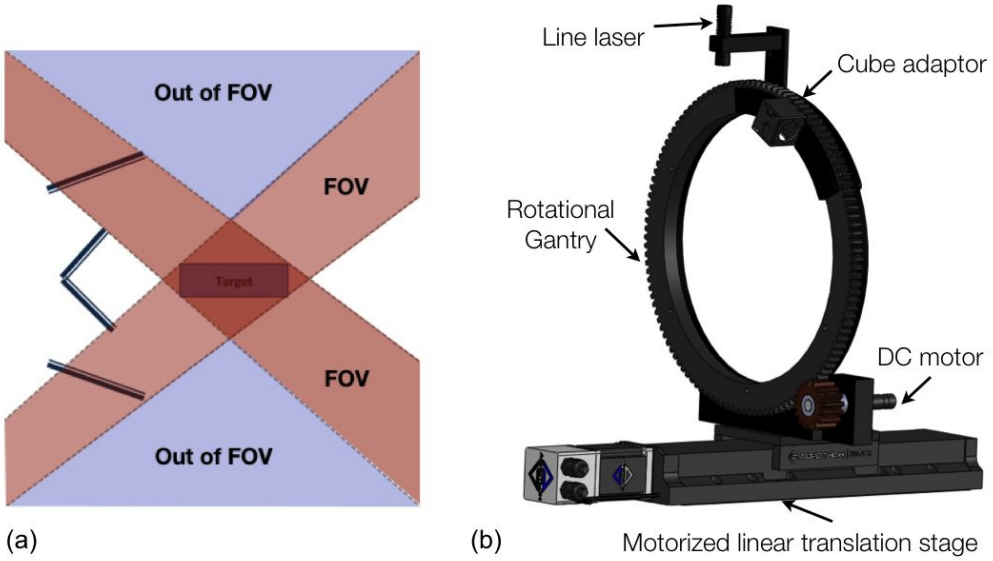

Figure 4. (a) Field of view of a camera (b) the developed source illumination subunit

2.3.3 Surface scanner

The system does not use a container with a matching liquid for simple surface geometry extraction. Instead, we developed a surface scanner to extract the surface geometry of a target animal. It consists of two webcams (Quickcam Pro 9000, Logitech, CA) and two angled first flat mirrors on a platform. The scan procedure is like figure 5. While a focusable green line laser, which is fixed on the linear translation stage, illuminates a line laser along the length direction of an animal like figure 5 (b), as shown in figure 5 (c) each webcam captures three different directional views. Then, total six partial surfaces are combined as one surface mesh like figure 5 (d) by David Laser scanner software (DAVID Vision Systems GmbH, Germany).

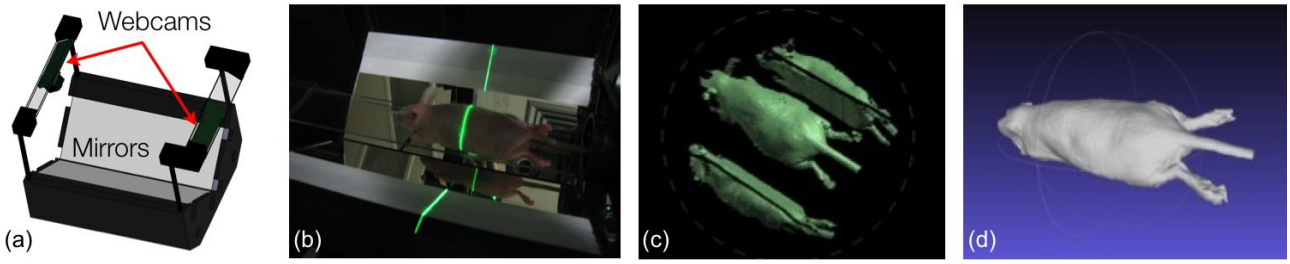

Figure 5. (a) Surface scanner (b) scan with a line laser (c) three directional views by one webcam (d) a final surface mesh

3. RESULTS

3.1 Back reflection simulation

To prove the improvement of back reflection issue between a mirror surface and a target animal surface in double conical mirror scheme, compared with a published single conical mirror scheme, we ran Monte Carlo ray tracing simulation by using commercial software, LightTools (Synopsys, CA). In the simulation, the specification of a single

conical mirror was the same in the paper of Changqing Li et al [5] and a 30 mm diameter, 80 mm length cylinder was chosen as a target. A one-Watt point source moved along the line of 90° circumference angle on the cylinder surface by 10 mm step and it illuminated the upper hemisphere isotropically. Because of complexity of mimicking multiple reflections between a mirror surface and a real animal surface, among the lights from a source, only one time mirror reflected lights to a target surface were traced by assuming the cylinder surface as an optical absorber and total 50,000,000 rays were traced at each point source position. The simulation results showed that, in the single conical mirror scheme, around 4~10% of light from a source returned to a target surface depending on the source position and in the double conical mirror scheme, around 0~2% of light returned to a target surface like figure 6. Additionally, while the returned lights on a target surface in a single mirror scheme were distributed broadly, in the double conical mirror scheme, the distribution of returned light were only concentrated on the area near the mirrors. As a result, this area will be projected onto the near vertex area of a second conical mirror and its effects on the entire measurement can be minimized additionally.

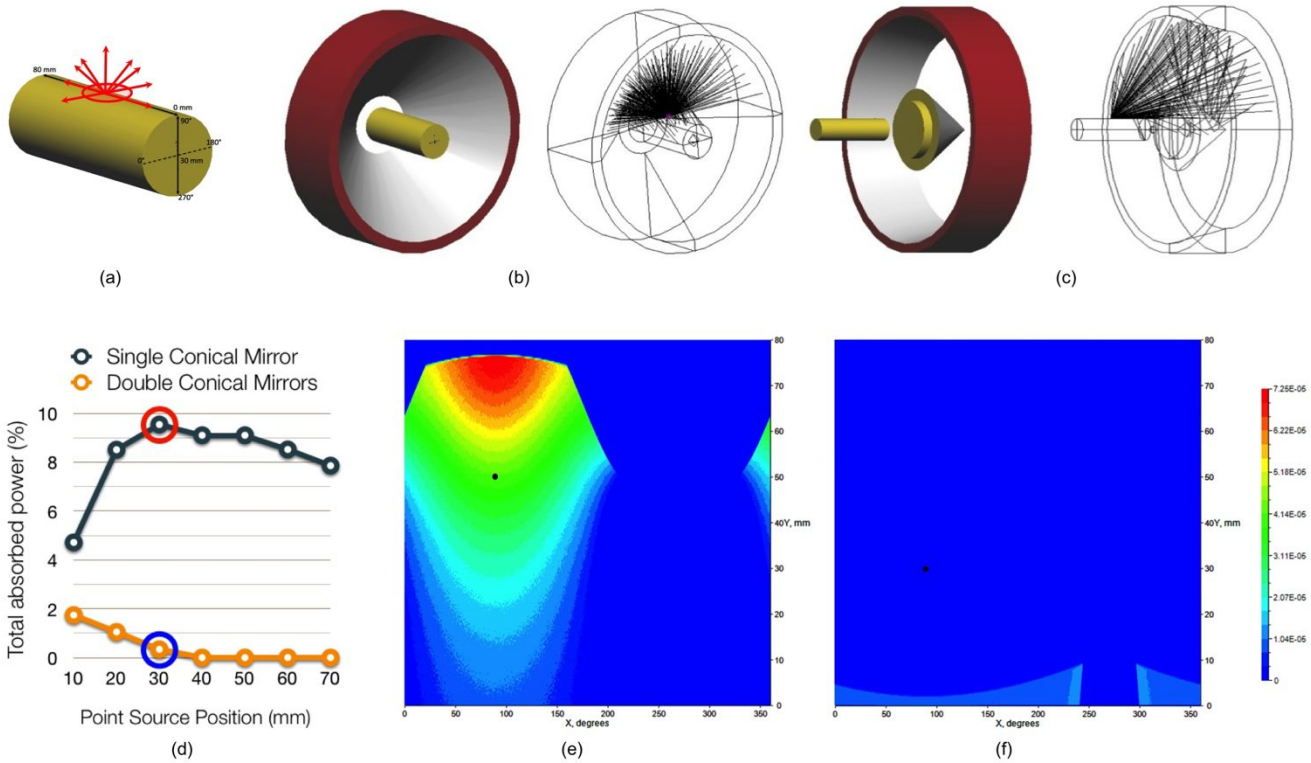

Figure 6. (a) A cylinder target for Monte Carlo ray tracing simulation and a 1 Watt point source, (b) single conical mirror scheme model and its ray tracing, (c) double conical mirror scheme model and its ray tracing, (d) total returned and absorbed light on a target surface (green: a single conical scheme, yellow: a double conical mirror scheme), (e) the detail simulation result of the red circle in (d), the rectangle shows the unwrapped cylinder target surface (x axis: circumference of a cylinder target by angle from 0° to 360°, y axis: length of a cylinder target from 0 mm to 80 mm), The black dot is the position of a 1 Watt light source, (f) the detail simulation result of the blue circle in (d). In (e), top side (80 mm side) is the near side of a conical mirror and in (f), bottom side (0 mm side) is the near side of a double conical mirror pairs.

3.2 System Assembly and operation

To block the ambient light, an imaging chamber was made like figure 7 (a) and all subunits of the imaging unit and the detection unit were assembled and positioned inside of an imaging chamber. In the operation of the system, the surface scanner moves forward and positions around a target animal for extracting the surface geometry of an animal, and then it moves backward and positions like in figure 7 (b) not to bother the camera view of a target animal.

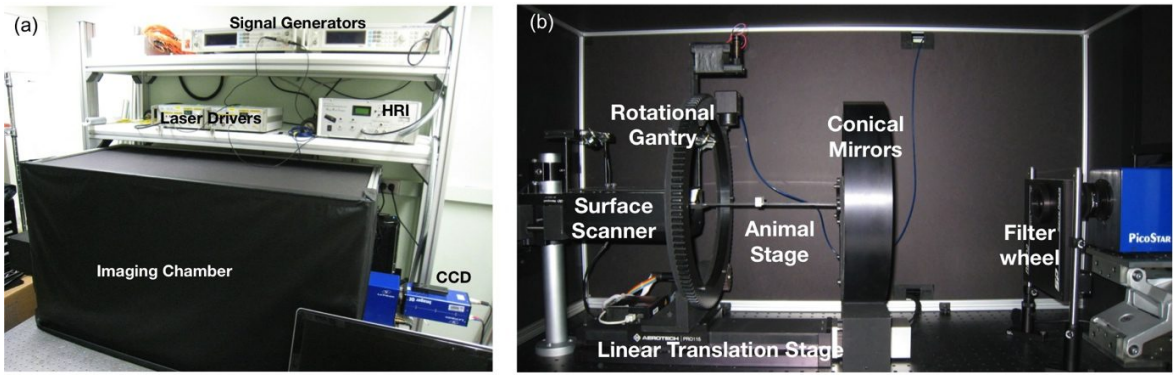

Figure 7. (a) Picture of the developed system (b) the view of inside the imaging chamber

4. DISCUSSION

For the optical tomographic imaging system, we presented double consecutive mirror reflection scheme as a general multi directional imaging geometry. With the conical shape mirrors, we can detect the entire surface of a target animal simultaneously and compared with a single conical mirror scheme, we can minimize the back reflection between a mirror surface and a target animal surface. In addition, by using the integrated surface scanner, the surface geometry of an animal can be extracted without changing the position and posture of an animal. Finally, we increased the accessibility to a target animal by providing unobstructed space around a target animal and the flexibility for multi-modality system development.

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