A DR-WFOI fusion system for the real-time molecular imaging in vivo

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Digital radiography (DR) and whole-body fluorescent optical imaging (WFOI) have been widely applied in the field of molecular imaging, with the advantages in tissues and functional imaging. The integration of them contributes to the development and discovery of medicine. We introduce an equipment, performance of which is better than that of another molecular imaging system manufactured by Kodak Corp. It can take real-time small animal imaging in vivo, with lower cost and shorter development cycle on the LabVIEW platform. At last, a paradigm experiment on a nude mouse with green fluorescent protein (GFP) transgenic tumor is given to present a real-time DR-WFOI fusion simultaneous image.

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Fluorescence technology is an emerging cost-effective approach for small animal imaging in recent ten years[1,2]. It can be used to take quantitative functional measurements. The activity of cancer can be quantified relaying on detectable fluorescein, when specific cancer markers "light on" tumors. It is possible to apply this method to analyze the process of cell in vivo on the molecular level. Meanwhile, the radiation imaging technology is prevalent on molecular imaging for indicating structure information. The whole-body fluorescent optical imaging (WFOI) system and the digital radiography (DR) system are the widespread imaging tools in the research of clinical medicine. The WFOI system is noninvasive. It has the capability of monitoring the growth and spread of tumors within intact animals. The WFOI has high spectral resolution, but it has defects in locating the tissue due to the lack of reference coordinate and proportion. On the contrary, the DR could make up the weakness of WFOI. It can easily image the bones of animals which can be nature reference coordinate and proportion, because the skeleton structure dose not change in a certain period, not like the tissue. Under the effect of the tumor or drug, the body probably becomes fatter or thinner. So we could infer not only the structure of animals but also the function of cells, such as how the cancer cell grows and spreads using anticancer drug, etc., in a DR-WFOI fusion system. The advantage of the fusion system with DR and WFOI is very obvious in the molecular imaging, which makes the disease research on early detecting, analyzing, evaluating, and treating much more quickly, easily, and accurately.

According to the priority of molecular imaging and its great market value, many companies such as Philips and GE have designed a certain molecular imaging instrument. The hot sell of the DR-WFOI fusion system in the market powerfully proves the importance and market prospect of this instrument. However, most of these instruments apply only one modality to detect tumor marked by fluorescence protein in small animals and are very expensive. Recently, Kodak Company pushes out a molecular imaging system with DR and WFOI module and the selling price is 125 thousand dollar in Chinese market as we know. However, the Kodak system can not gain real-time in vivo image for cause of the element of the phosphorus screen (the imaging component in DR module). It can only gain time-sharing image, either WFOI image or DR image seperately.

A fusion DR-WFOI system with ingenious structure, which can gain genuine real-time, in vivo, synchronous image, will be described in this letter. The DR module and the WFOI module can work together without crosstalk.

The system described in this letter consists of two modules — the direct DR and the WFOI. These two modules share a common optic path with addition of a plane mirror as shown in Fig. 1. This design makes sure that the WFOI and DR module can work synchronously without crosstalk.

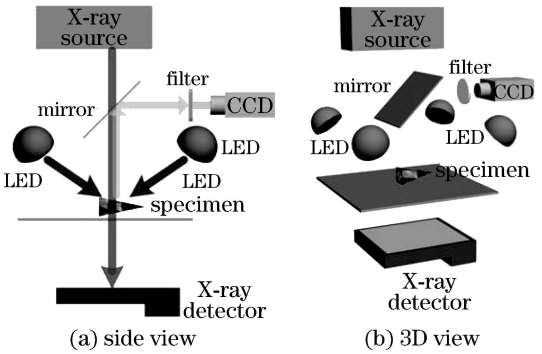

Fig. 1. System optical pathway and 3D layout. (a) The side view and the optical pathway diagram of DR and WFOI. The red line shows the X-ray's pathway; the blue line is the pathway of the blue light produced by the LED source; the cyan line shows the mixed light which contains the blue light and the green light excited from GFP. The green line expresses the green light remained after high pass filtering. (b) The 3D view of the system.

Radiography system is a highly common X-ray imaging apparatus. It contains an X-ray source and an X-ray detector, with the specimen lay on a stage between them. Background noise is removed by Gain calibration before DR imaging. X-ray is a sort of electromagnetic wave with ultra short wavelength, it is able to straightly penetrate general stuffs, like gas, organism, metal, etc. The projection of an object stage will interfere with that of target specimen on the stage. So it is necessary to Offset calibrate to remove the projection of the stage and materially choose a stage with low absorption of X-ray. Thus organic plastic stage (1 mm thickness) without any metal, such as lead, is chosen in our work.

In WFOI module, a specific light source (such as Hg lamp or laser) is used to excite fluorescent protein (such as green fluorescent protein (GFP)), absorption spectrum: $395-470$ nm, emission spectrum: $509-540$ nm) in the tumor in the specimen[3]. The wavelength can be selected, referring to emission spectrum of fluorescent protein. Recently, Light-emitting diode (LED) is used to substitute the ordinary excitation light sources such as Hg lamp and halogen lamp because of its longer service life: generally more than 30000 h. We choose the LED which produces blue light (power: 3 W, center wavelength: 470 nm, interval of wavelength: $460-480$ nm) as the light source. The active radiation (Q lm)[4−6] of the wavelength from λ_1 to λ_2 is given by

$$Q = L \times (\sum_{\lambda_i=\lambda_1}^{\lambda_2} P_{\lambda_i}) / \sum_{\lambda_i=0}^{\infty} P_{\lambda_i}, \quad (1)$$

where L represents the luminous flux (lm), P_{λ_i} represents the relative intension of radiation whose central wavelength is λ_i. The luminous flux used to excite green fluorescent protein is usually 2000 lm. From Eq. (1), the active radiation of the laser is 336.0 lm. The luminous flux of the LED selected for these experiments is 100.0 lm, so QLED is 89.5 lm. Accordingly, an array of four LEDs is used to give the same overall intensity as from a single laser or Hg lamp[7,8]. The light detector is a high quality charge coupled device (CCD) receiving the emission light from green fluorescent protein. A high pass filter (520 nm) is used to avoid background and other interfering light to promote contrast and reduce crosstalk.

The optic paths of the DR and WFOI should be identical to ensure the same filed of view, but it would lead to spatial conflict of the X-ray source and the CCD. A plane mirror is added to this integrated system to resolve this problem. It is placed between the X-ray source and the stage by the orientation of 45 degree to horizontal. Furthermore, the interferential production of this mirror can be removed by Offset calibration before DR imaging (shown in Fig. 1). In this way, the view filed of CCD is identical to that of DR's while its optic path does not entirely pass by that of X-ray detector.

The results from DR and WFOI module are in different modality. One is X-ray transmission imaging and another is fluorescent reflection imaging. So how to keep the two images correlative is a pending problem. Here, we resolve it by an image processing program. In this program, there are five or more recognizable positions chosen as reference points to keep the two images correlative in morphology. The fusion image is produced by the amalgamation arithmetic from the above adjusted result[9,10].

Within this system as shown in Fig. 1, the DR module consists of X-ray source and X-ray detector. The optical pathway diagram is as the red line in Fig. 1(a). The X-ray source produces the X-ray beam from the light-emitting windows on the side of the source and the X-ray beam crosses the 45 degree mirror and the specimen, stage, and reaches the X-ray detector at last. The X-ray detector should be offset and gain corrected before shooting specimen. The mirror and stage are the offset of the DR image. So the mirror before the specimen and the stage below the specimen will not affect the imaging quality. And the distance of the stage and the X-ray detector can be changed to adjust the DR enlargement ratio[11,12]. The X-ray source's spot focus is 50 μm in diameter. And its voltage range is from 30 to 90 kV, and current range is from 400 to 700 μA. The beam angle is about 24°. The X-ray detector is a PaxScan1313 produced by Varian Medical Systems, USA. The detecting area of the flat panel detector is 130×130 (mm) with 1280×1024 (pixel). The size of a pixel is 101 μm. The detecting voltage range is from 30 to 160 kV.

The WFOI module includes a quadruple LED light source, a plane mirror, a CCD, and a filter before the CCD lens. The four LEDs are distributed around the specimen uniformly to excite the GFP. An ingenious 45 degree mirror is located between the specimen and the X-ray source to solve the spatial conflict of the X-ray source and the CCD. The function of this mirror is to reflect fluorescent light to the CCD in horizontally. This solution ensures the WFOI and DR at the same degree, which is very important for the fusion imaging. It is shown as the blue line in Fig. 1(a) that the LED light source produces the blue light, whose central wavelength is 470 nm. The wavelength range is from 460 to 480 nm. And the power of each LED is 3 W. After the light reaches the specimen, the GFP is excited to emit the green light. The green light and the blue light mixed as the cyan line shown in Fig. 1(a). Then the mixed light is reflected by the mirror to transmit to the filter. The CCD in this system is a digital camera with 1280×1024 resolution (1.3×106 (pixel)), 10 bit per pixel and sampling at 48 MHz. The interface is standard cameral link. The optical filter is the crucial component of WFOI because its quality determines the quality of the image (captured the green line shown in Fig. 1(a)). Therefore, we used a high pass 520-nm filter finally.

The LabVIEW is chosen as the development environment. The program gains the DR and WFOI data from the X-ray detector and CCD synchronously. The software interface is shown in Fig. 2 below. We chose nude mice with GFP tumor under the leg skin as a specimen. And the fusion image is shown in Fig. 3.

By combining WFOI with DR, the different advantages of these two imaging processes are added together. The ingenious structure of the system makes the function and structure real-time in vivo imaging possible, which has not been implemented recently in the market.

Since the molecular imaging apparatus invented by Kodak Corp. adopt phosphorus screen as its X-ray imaging medium, the radial can be transformed into visible light and collected by CCD, so fluorescent imaging and radial

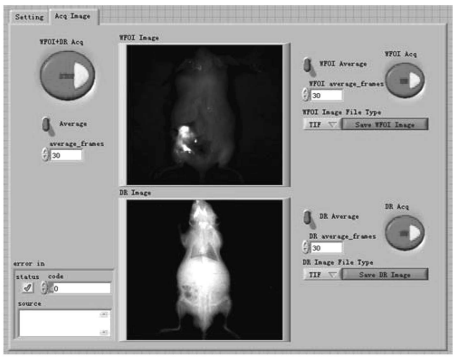

Fig. 2. Interface of software.

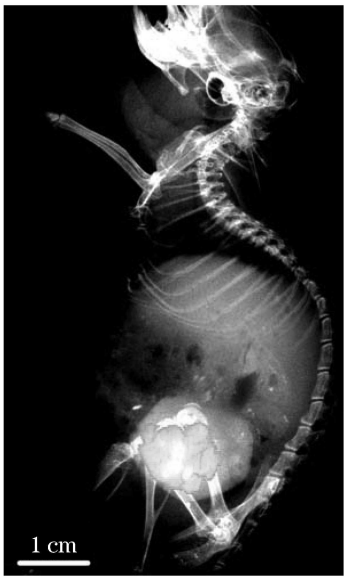

Fig. 3. Fusion image of a GFP tumor nude mice.

time-shared the CCD. However, comparing with the widely used flat panel detector, the quality of the image gained from phosphorus screen is not so high, but the price difference between flat panel detector and the CCD is trivial. So the scenario of this letter fulfills the demands of the imaging quality and the equipment cost is not increased. In this article, the staid plane mirror is added to the radial imaging beam and the absorption of the plane mirror to the radial can be calibrated as offset, so it has no influence on the final imaging. The equipment invented by Kodak Corp. uses the mechanical translation device to insert or withdraw the phosphorus screen in order to actualize the switch between the radial and fluorescent imaging. The apparatus of Kodak Corp. can not fulfill the requirement of the video imaging because of the restriction of weight of the phosphorus screen and the mechanical movement speed. There is no mechanical movement during imaging process in the configuration we proposed, so the X-ray detector and imaging speed of CCD are able to appease the demand of real time imaging on small animal in vivo. Furthermore, some parts of the system in the passage need to be perfected including fluorescent illuminate symmetrical calibration and more biologic examination, and these works above will be completed in subsequent labor.

Finally, the fusion image will be helpful for researches to develop and inspect much more easily and quickly.

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