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Design and Implementation of Bioluminescence Signal Analysis Tool

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ABSTRACT

The term molecular imaging can be broadly defined as the in vivo characterization and measurement of biologic processes at the cellular and molecular level. Optical imaging that has highly reproducibility and repetition used in molecular imaging research. In the bioluminescence imaging, animals carrying the luciferase gene are imaged with a cooled CCD(Charge-Coupled Device) camera to pick up the small number of photons transmitted through tissues. Molecular imaging analysis will allow us to observe the incipience and progression of the disease. But hardware device for molecular imaging and software for molecular image analysis were dependent on imports. In this paper, we suggest image processing methods and designed software for bioluminescence signal analysis. And we demonstrated high correlation(r=0.99) between our software's photon counts and commercial software's photon counts. ROI function and processing functions were accomplished without error. This study have the importance of the development software for bioluminescence image processing and analysis. And this study built the foundations for creative development of analysis methods. We expected this study lead the development of image technology.

Keywords: Molecular imaging, Bioluminescence Imaging, Quantitative analysis, Photon Counts

1. INTRODUCTION

Molecular imaging is rapidly emerging and coordinating multidisciplinary fields in molecular cell biology, chemistry, pharmacology, genetics, biomedical engineering, and physics. Through the molecular imaging technique, information of normals as well as abnormal cellular processes at a molecular level could be obtained. Molecular imaging methods utilize the imaging signals derived from specific cellular or molecular events. Because molecular and genetic changes precede anatomical changes in the course of disease development, molecular imaging can detect events in disease progression earlier[1-2]. Molecular imaging analysis allows us observing the tracking and monitoring of the disease. These will make us easier to give a diagnosis in the early stage of intractable diseases such as cancer, neuro-degenerative disease, and immunological disorders[3].

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Several imaging technologies and new reporter genes are being studied for noninvasive imaging and quantitation of gene expression in living subjects. Reporter genes with optical signal are a low-cost alternative for real-time analysis of gene expression in small animal models[4]. Currently, the available molecular imaging modalities include CT, MR, PET, SPECT, optical imaging techniques, and US. Variations and subcategories of these modalities are also available, including optical coherence tomography, fluorescence or luminescence imaging, MR microscopy, photoacoustic US and US biomicroscopy. Efforts are under way to perform dual x-ray/gamma imaging, CT/PET, MR/PET, and other combinations of modalities[5]. In the fluorescence imaging, an illuminating light excites fluorescent reporters in the living subject, and a charged coupled device (CCD) camera collects emission light of shifted wavelength. In the bioluminescence imaging reporter genes code for the luciferase that is responsible for fireflies' glow. After the injection of the substrate luciferin, animals carrying the luciferase gene are imaged with a super-sensitive CCD camera to pick up the small number of photons transmitted through tissues [6-7]. Recently, several technical advances in developing highly sensitive CCD cameras are enable to image very low levels of visible light emitted from internal body organs of rodents[8-11]. Specialized optical imaging devices for small animal imaging have been developed. These have used either bioluminescence or fluorescence imaging techniques to monitor whole body, providing low cost and the versatility of optical spectroscopy.

In the current study, S. Bhaumik and S.S. Gambhir validate for the first time the ability to image bioluminescence from Renilla luciferase enzymeprotein (RL) by injecting the substrate coelenterazine to living mice. They show that both Rluc and Fluc expression can be imaged in the same living mouse and that the kinetics of light production are distinct. The validated approaches will have direct applications to various studies where two molecular events need to be tracked, including cell trafficking of two cell populations, two gene therapy vectors, and indirect monitoring of two endogenous genes through the use of two reporter genes[4]. In the other study, Noah Craft et al. researched about bioluminescent imaging of Melanoma in live mice. They have developed and characterized an accurate, sensitive, and reproducible bioluminescent B16 melanoma model that allows for serial and real-time analyses of tumor burden in live mice[12].

Most of medical images use 16 bit data set than 8 bit data. Because minute levels of medical image cannot be represented using 8 bit, 16 bit data set design for image acquisition is essential. However, a software for molecular image analysis and 16 bit image acquisition are dependent on hardware devices. In this study, we developed the quantitative analysis of bioluminescence image. This basic work for the development of molecular imaging analysis tool will contribute to advance of study-dependent analysis tool.

This paper is organized in the following manner. Materials and method for software design used in this paper are explained in section 2. Section 3 presents the results of the performance evaluation. Finally, conclusions from our study and areas for future research are given in section 4.

2. MATERIALS AND METHODS

2.1 Materials

Two mice were prepared for bioluminescence imaging study using light emitting bacteria. 1 X 10^8 host cell were injected subcutaneously in Balb/c nu-/nu- mouse using CT-26 mouse colon cancer cell line. After two weeks, DH5a strain were injected intravenously into the generated solid tumor. Amount of injected strain was $1x10^{10}$ cfu(colony form unit), $1x10^8$ cfu respectively.

2.2 Image acquisition

Twenty minutes later, bioluminescence images were acquired for 20 minutes from in vivo living mice. Image acquisition time was 1 minute. Eleven images were captured in first mouse in a day. Between 5th day and 9th day post injection, 9 images were obtained in second mouse.

The in vivo IVIS Imaging System (IVIS series 100, Xenogen Corporation, Alameda, CA) which is consist of a cooled CCD camera mounted on a light-tight specimen chamber (dark box), was used for data acquisition. The image was saved tiff file format with gray scale. The image matrix size was 240 X 240 pixels, and a pixel data was 16 bit data array. Data were analyzed based on total counts in the region of interest(ROI). Data analysis was performed at IVIS-100 and proposed software respectively.

2.3 Software design and implementation

Structure of analysis tool was composed visualization part and data processing part. Because the size of imaging signal was too high, 16 bit data array format was designed. The range of image data signal covers until 65536. 16 bit data set were scaled to 8 bit format for visualization. The Analysis software was implemented using Visual C++ 6.0. For the first, compatible image file was loaded. In the data processing part, after saving the image into 16 bit data set in array, feature was extracted from 16 bit data number set.

Several working steps were showed in Fig. 1 sequently. Description of each step was mentioned below.

a) Image loading

Two byte 'tiff' file format could be loaded into memory. After loading image, it storing in array for analysis.

b) Stored 16-bits data in array

Two byte data array represents image signal effectively. Optical signal have many signal level.

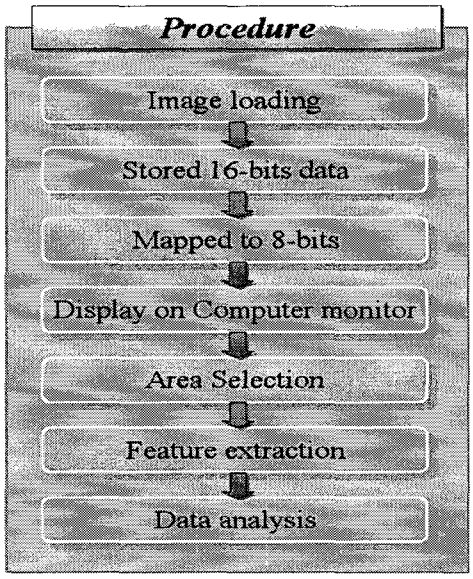


Fig. 1. Working flow of proposed analysis tool.

It stored and processed in 16 bit data array. 16bit images are represented using unsigned integers in the range 0 to 65,535. Count data also saved 16 bit data format using 2 byte array.

c) Mapped to 8-bits data in array

Sixteen-bit grayscale images are not directly displayable on computer monitors, which typically can show only 256 shades of gray. Therefore, the data are mapped to 8-bits by windowing. The window defines the range of gray values that are displayed: values below the window are made black, while values above the window are white.

d) Display on Computer monitor

Visual data need 8 bit for representation. Scaling factor is important in visualization part. Visual data and processing data works in different scale.

e) Area selection

For the ROI analysis, peak value was detected. Automatic ROI region was drown at 10 percent limit boundary from peak count.

f) Feature extraction

Total count and photon flux are two main factors. In this study, photon count changes were measured by ROI.

g) Data analysis

The quantity of the tumor marker was analyzed using total counts extracted from ROI range.

2.4 Calculation of the total counts

Counts are uncalibrated units that refer to the raw amplitude of the signal detected by the CCD camera. Because a signal measured in counts is related to the number of photons, the signal level varies, depending upon camera settings such as integration time, binning, f/stop and field-of-view. Thus, if the user changes these settings in the middle of an experiment to keep the signal below the digitizer saturation level of 65535 counts. Total counts refers to the sum of all counts for all pixels inside the ROI. The average counts (Avg) is the total counts divided by the number of pixels, or superpixels. The quantity of ROI pixels is the number of binned pixels inside the ROI, while Area (CCD pixels) is the number of unbinned CCD pixels inside the ROI. The range of count value about each pixel is from 0 to 65535 by 16bit CCD digitizer. Assume that ROI size is M by N, equation of total count is :

$$Total\ Counts = \sum_{i=0}^{M}\sum_{j=0}^{N} photoncount[i][j] \quad (1)$$

Photoncount[i][j] is pixel count in i-th column and j-th row.

2.5 Software for bioluminescence image analysis

Fig. 2(a) showed overlay image obtained by IVIS-100. Overlay image consist background field and color mapping image by luminescence signal. Fig. 2(b) and Fig. 2(c) showed color mapping image and background image. Fig. 2(d) showed bioluminescence image before color mapping. This

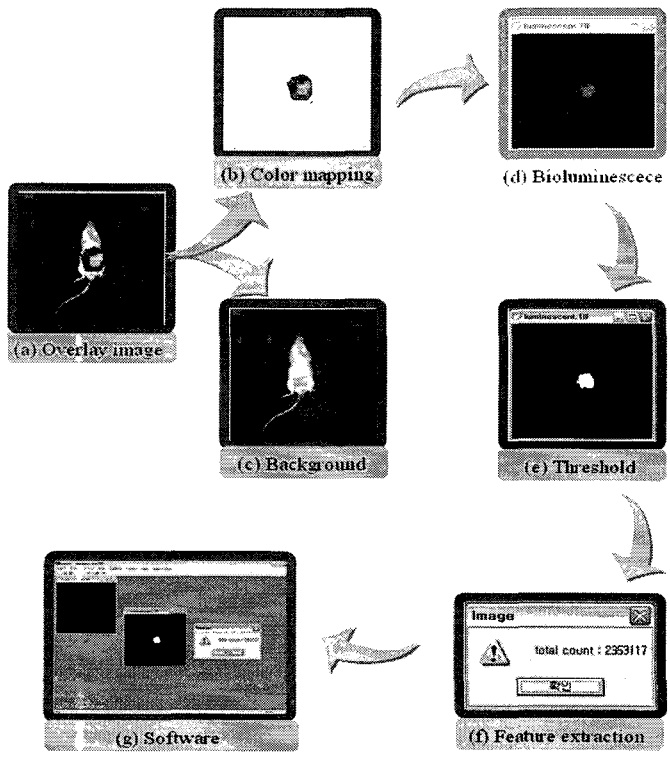


Fig. 2. Feature extraction from bioluminescence image analysis tool.

image contained only the luminescence light signal. Total count was measured by ROI in this image. Fig. 2(e) showed Threshold image. After computing peak value in whole image, ROI was calculated by 10 percent of peak value. Fig. 2(f) showed the message box for total count and Fig. 2(g) showed analysis tool.

Proposed analysis tools in Fig. 3(a) shows processing step for ROI analysis. Commercialized tool; LIVING IMAGE system of IVIS-100 shows quantified result in ROI on registered image. Color bar represent scaled level color as maximum photon counts.

3. IMPLEMENTED SOFTWARE AND RESULT DATA

3.1 Implemented software

Implemented software was executed organically. Visualization part that loading acquired image and feature extraction part that data processing are performed well. Image loading time was similar with commercial programs. Image processing functions for quantitative analysis such as ROI and count statistics were accomplished efficiently. Correlation between the first processing result and the second processing result using developed analysis tool showed high correlation coefficient (r=0.99).

3.2 Bioluminescence imaging experiment using mice

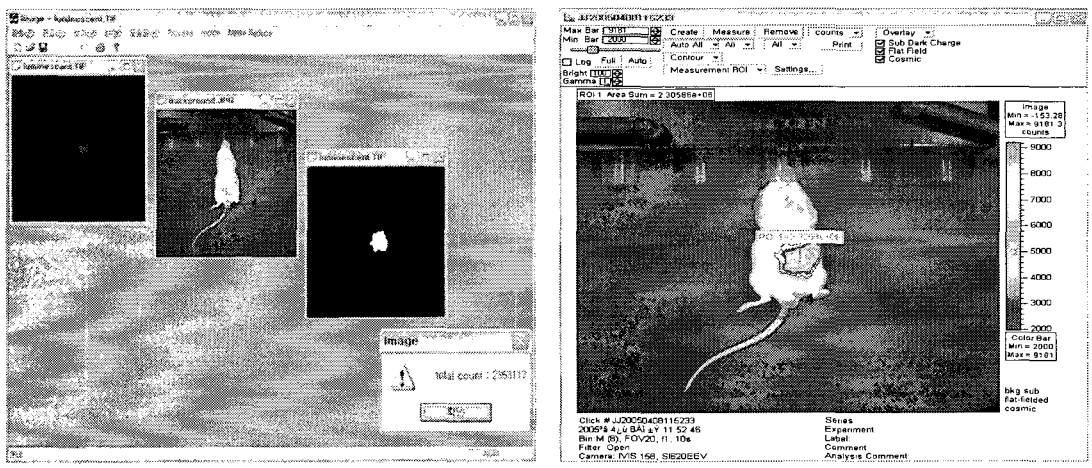
Designed software was implemented successfully. Image loading and analysis function were working without error. Data from two mice were compared between two software. Correlation coefficient was performed using MedCalc stochastic tool. Maximum photon count step was detected in the same step in the both software.

This table 1(a) showed results of eleven bioluminescence photon signals in the first mouse. And the maximum photon count was also detected in the same step in second mouse. Correlation of total photon counts of analysis tools was estimated.

Fig. 4 showed correlation plot. Correlation coefficient of first data set is 0.99($P<0.0001$) and second data set is 0.99($P<0.0001$).

4. CONCLUSIONS

In this paper, we developed a bioluminescence image analysis tool for quantitative analysis of luciferase reporter gene expression. Designed soft-



(a) Proposed analysis tool (b) Living Image in IVIS-100

Fig. 3. Bioluminescence image analysis tool. (a) shows proposed tool and (b) shows commercialized tool.

Table 1. Photon counts of bioluminescence imaging

m1	IVIS	developed S/W
1	12680000	12610000
2	13530000	13550000
3	14100000	14190000
4	14540000	14580000
5	14590000	14590000
6	13800000	13850000
7	12930000	13040000
8	12350000	12500000
9	13860000	14010000
10	13840000	13550000
11	12740000	12860000

(a) total counts of first data set

m2	IVIS	developed S/W
1	3810000	3830000
2	2860000	2890000
3	2300000	2350000
4	251400	243950
5	722500	705687
6	256600	242227
7	168300	177239
8	234800	226948
9	369500	340611

(b) total counts of second data set

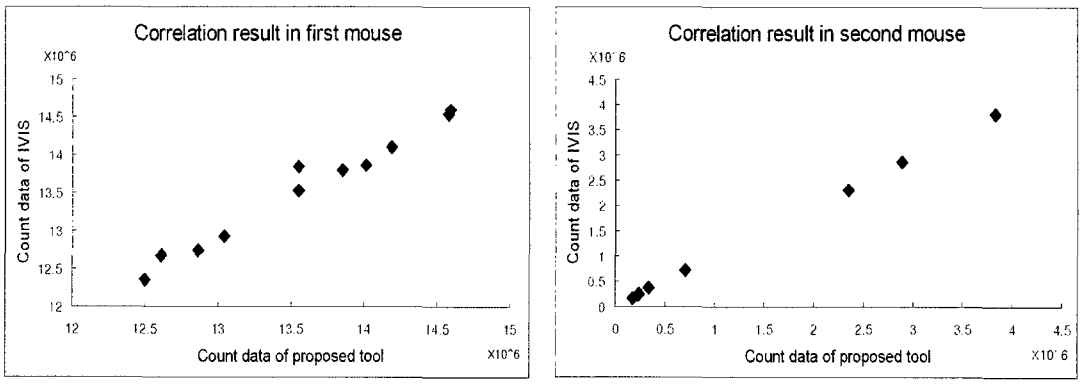


Fig. 4. Correlation results of each studies.

ware was executed successfully. Photon count signal was measured precisely. Comparison data of photon count between the designed analysis tool and commercialized tool of IVIS-100 shows high correlation. The photon propagation model and correction technologies are need in future study. We anticipate this tool will be a useful software for molecular imaging study.

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