Technical Note

The FluoVision System for Fluorescence Concentration Imaging

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Received: 07 September 2019
Accepted: 24 November 2019
DOI: 10.1039/x0xx00000x
http://FBT.tums.ac.ir

Keywords:
FluoVision;
Fluorescence Imaging;
Light Leakage;
Optical Imaging.

Abstract

Purpose: In this report the design concept and experimental evaluation of the performance of FluoVision have been illustrated.

Methods: The FluoVision system designed for fluorescence concentration imaging. In order to assess the capability of the system, results of reference design have been analyzed.

Results: Results of the FluoVision system are matched with the reference design.

Conclusion: The evaluation indicated that FluoVision is adequate as a fluorescence imaging system for fluorescence concentration imaging.

Technical Note

Cancer is one of the main causes of mortality in industrialized societies [1]. Presence or absence of lymph node metastases may be the most important factor in the prognosis of cancer patients that are treated as early as possible. In recent years, lung lymph node mapping has been obtained by optical imaging during surgery [2-5]. Fluorescence imaging is a way to diagnose the condition of lymph nodes and the presence of tumors in them.

Some commercial imaging systems have been developed to show the location of fluorescence substances in the body. Frangioni in 2003 [6] and Troyan in 2009 [7] proposed innovative fluorescence imaging systems for animal and human studies.

The essential components of any optical arrangement in fluorescence imaging are light source, lenses, filters and detector or camera. But the basic optical design leads to huge light leakage and aberrations [8].

Recent studies indicate that the excitation light leakage is the most important factor that can be influenced in signal to noise ratio and sensitivity of images acquired and will not lead to accurate diagnosis Sentinel lymph node [6, 8-10]. The FluoVision system is evaluated by the effect of light leakage and sensitivity on the point spread function of the surgical fluorescence imaging system in tissue-like phantom. Our developed system, the FluoVision system, has been designed based on ray tracing with ZEMAX software and developed reference design.

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Final design has a working distance of about 20 cm and a focal distance of 12 mm in primary state. The F# of final design is 2. Low F# means more open aperture and helps to increase the sensitivity of system.

Optimization is based on getting lowest Mean Squared Errors (RMS) in spot size at image plan.

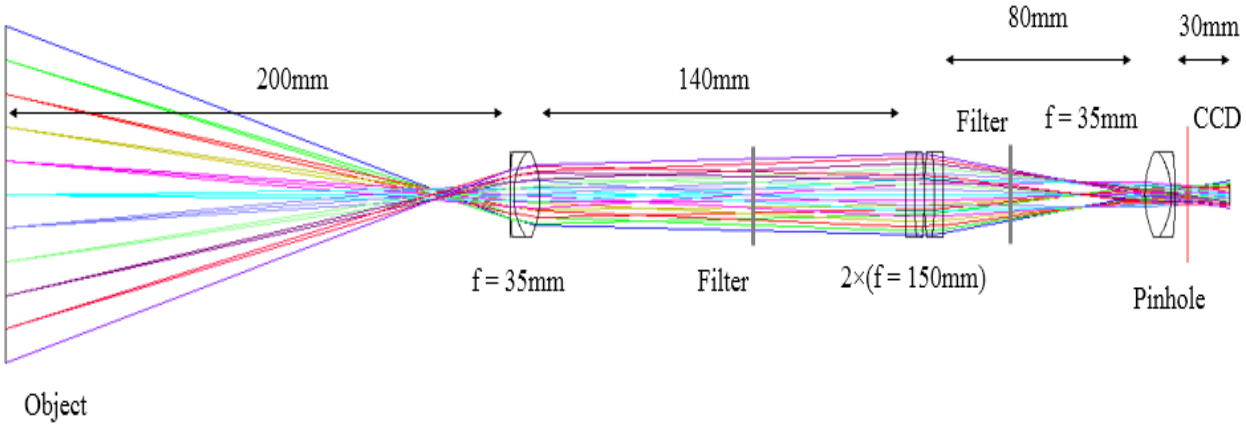

Figure 1. Adjusting the lenses using ZEXAX

Transmission ratio for excitation light leakage and PSF analysed for new design of FluoVision system by reference design (FLARE system) indicated evaluation of this system [9]. The backscattered light intensity includes the light source beam and fluorescent radiation which were collected by the CCD camera through filters and lenses arrangement as shown in Figure 2. To investigate the efficiency of the filter configurations for blocking backscattered excitation light and collecting propagated fluorescence signals, an agar phantom was employed [11, 12]. Excitation light leakage was defined as the signal S(λx), or average pixel intensity values over a Region Of Interest (ROI) of the scattering surface taken without fluorescence [6]. The light source beam leakage was obtained by calculating Transmission Ratio (TR) which can described by the following Equation: [9] TR= S(λx)/{ S(λm , λx) - S(λx)}. The S(λx) signals were called as the "out-band" transmission signals, whereas the differences, signal S(λm, λx) - S(λx), represent the "in-band" transmission signals [9]. The smaller value of the transmission ratio indicates the better performance for rejecting excitation and passing fluorescence light and the lower the noise floor.

The first design was included the focus lens, a 540 nm band pass interference filter and a CCD camera. The proposed optical arrangements were compared by obtaining the TR and Point Spread Function (PSF) which were analyzed using a student's t-test [7]. For measuring the PSF, we placed a tube filled with point fluorescence. and imaging with difference optical arrangements. Then, we measured FWHM of Gaussian curve profile to calculate the PSF [13]. The optimized optical arrangement was used to obtain non-invasive real time images from green fluorescent protein (GFP) transgenic mice (C57BL/6-Tg(ACTB-EGFP)1Osb/J, strain 003291) and BALB/c mouse. The Ethical approval was obtained from Ethics Committee of Tehran University of Medical Sciences.

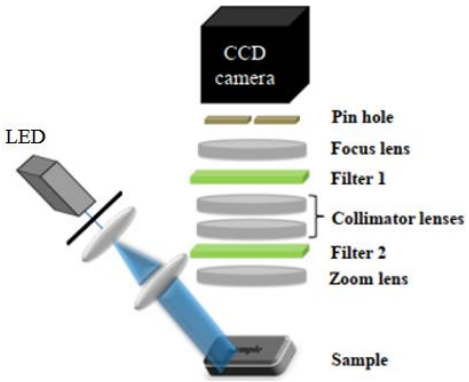

Figure 2. Schematic representation of optical arrangement (up to down: CCD camera, Pin hole setup, Focus lens, first Bandpass filter wheel, Collimator lenses setup, second Bandpass filter wheel, Zoom lens

Table 1 shows the calculated transmission ratio for the different experimental arrangement. The TRs of the reference design to final design were changed from 0.452 to 0.192, respectively ($p<0.0001$). Placing the collimator lens between two filters causes a remarkable increase for OD of filters and decreases the TR significantly. By comparing final design with reference design, TR amount decreased more than twice due to reducing blue shift effect.

Table 1. TR and PSF for diverse arrangements

Design	Transmission Ratio	PSF (Pixels)
Reference Design	0.452±0.007	49.9±0.3
Final Design	0.192±0.002	39.1±0.2

Table 1 presents the calculated PSF for all designs of the imaging system. As showed in final design, when the two 540-nm band pass filters are separated by the collimator lens, it improved the magnitude of the PSF significantly. Overall, we observed the PSF of the reference design to final design were changed from 49.9 pixel to 39.1 pixel, respectively ($p<0.0008$) (Where each pixel is equal to 0.1 mm). Figure 3 shows the image of the commercial device FluoVision.

Figure 3. FluoVision PreClinical system for fluorescence imaging

The detector head of this system has 6 degrees of freedom manually. The FluoVision system reported the results properly and the results were better than reference design. The results of the evaluation indicated that FluoVision system is adequate as a fluorescence imaging system for easy, simple and cheap non-invasive imaging for diagnosis and monitoring for treatment of cancer cells and has high sensitivity, specificity and accuracy. The effect of the two filters in the sequence was no longer the sum of their optical densities, because an interference cancellation is created by the reflection of light between two lenses. However, a small amount of loss inserted between two filters can efficiently cancel the multiple-path interference effects. When the angle of incidence to filter is non-normal, polarization occurs in transmission light which causes a decrease in the average intensity output light from the filter, so we should correct this plunge for output light.

In conclusion, the excitation light leakage in the surgical imaging system can be reduced dramatically by using the appropriate filter combinations and permutations as performed in design 4 by using two filters and four lenses as shown Figure. 1(d). Finally, this validation and qualification method to decrease and document excitation light leakage should be expanded to all fluorescence enhanced optical imaging systems which can potentially suffer from intensive excitation light leakage.

Acknowledgements

The authors would like to acknowledge Tajhiz Afarinan Nouri Parseh Co. for their technical support.

References

1- R. Pleijhuis *et al.*, "Identifying tumor markers in lymph node metastases for targeted imaging applications in breast cancer patients," *Relevance, targets, and clinical transla on of near-infrared fluorescence imaging in breast cancer*, vol. 159, 2014.

2- A. M. Wagner, J. M. Knipe, G. Orive, and N. A. Peppas, "Quantum dots in biomedical applications," *Acta biomaterialia*, 2019.

3- N. J. Harlaar, G. M. van Dam, and V. Ntziachristos, "Intraoperative optical imaging," in *Intraoperative Imaging and Image-Guided Therapy*: Springer, 2014, pp. 233-245.

4- J. Q. M. Nguyen *et al.*, "Development of a modular fluorescence overlay tissue imaging system for wide-field intraoperative surgical guidance," *Journal of Medical Imaging*, vol. 5, no. 2, p. 021220, 2018.

5- H. Dehghani *et al.*, "Near infrared optical tomography using NIRFAST: Algorithm for numerical model and image reconstruction," *Communications in numerical methods in engineering*, vol. 25, no. 6, pp. 711-732, 2009.

6- A. M. De Grand and J. V. Frangioni, "An operational near-infrared fluorescence imaging system prototype for large animal surgery," *Technology in cancer research & treatment*, vol. 2, no. 6, pp. 553-562, 2003.

7- S. L. Troyan *et al.*, "The FLARE™ intraoperative near-infrared fluorescence imaging system: a first-in-human clinical trial in breast cancer sentinel lymph node mapping," *Annals of surgical oncology*, vol. 16, no. 10, pp. 2943-2952, 2009.

8- B. Zhu and E. M. Sevick-Muraca, "Minimizing excitation light leakage and maximizing measurement sensitivity for molecular imaging with near-infrared fluorescence," *Journal of Innovative Optical Health Sciences*, vol. 4, no. 03, pp. 301-307, 2011.

9- P. K. Yalavarthy, B. W. Pogue, H. Dehghani, and K. D. Paulsen, "Weight-matrix structured regularization provides optimal generalized least-squares estimate in diffuse optical tomography," *Medical physics*, vol. 34, no. 6Part1, pp. 2085-2098, 2007.

10- S. Kim *et al.*, "Near-infrared fluorescent type II quantum dots for sentinel lymph node mapping," *Nature biotechnology*, vol. 22, no. 1, p. 93, 2004.

11- S. M. Hejazi, S. Sarkar, and Z. Darezereshki, "Fast multislice fluorescence molecular tomography using sparsity-inducing regularization," *Journal of Biomedical Optics*, vol. 21, no. 2, p. 026012, 2016.

12- M. Hejazi, F. Stuker, D. Vats, and M. Rudin, "Improving the accuracy of a solid spherical source radius and depth estimation using the diffusion equation in fluorescence reflectance mode," *Biomedical engineering online*, vol. 9, no. 1, p. 28, 2010.

13- R. W. Holt, F. L. Leblond, and B. W. Pogue, "Methodology to optimize detector geometry in fluorescence tomography of tissue using the minimized curvature of the summed diffuse sensitivity projections," *JOSA A*, vol. 30, no. 8, pp. 1613-1619, 2013.