Novel method to detect target specimens within endoscopic ultrasonography-guided fine-needle aspiration biopsy samples: an experimental study

Kazuya Matsumoto (matsumotokazuya@tottori-u.ac.jp)
Division of Medicine and Clinical Science, Department of Multidisciplinary Internal Medicine, Faculty of Medicine, Tottori University, Yonago 683-8504

Masaru Ueki
Advanced Medicine, Innovation and Clinical Research Center, Tottori University Hospital, Yonago 683-8504

Kunio Ichino
Department of Information and Electronics, Graduate School of Engineering, Tottori University, Tottori 680-8552

Takeshi Tsuka
Department of Veterinary Clinical Medicine, School of Veterinary Medicine, Tottori University, 4-101, Koyama-Minami, Tottori, Tottori, 680-8553

Kazutake Uehara
Advanced Medicine, Innovation and Clinical Research Center, Tottori University Hospital, Yonago 683-8504

Yohei Takeda
Division of Medicine and Clinical Science, Department of Multidisciplinary Internal Medicine, Faculty of Medicine, Tottori University, Yonago 683-8504

Takumi Onoyama
Division of Medicine and Clinical Science, Department of Multidisciplinary Internal Medicine, Faculty of Medicine, Tottori University, Yonago 683-8504

Soichiro Kawata
Division of Medicine and Clinical Science, Department of Multidisciplinary Internal Medicine, Faculty of Medicine, Tottori University, Yonago 683-8504

Hiroki Kurumi
Division of Medicine and Clinical Science, Department of Multidisciplinary Internal Medicine, Faculty of Medicine, Tottori University, Yonago 683-8504

Hiroki Koda
Division of Medicine and Clinical Science, Department of Multidisciplinary Internal Medicine, Faculty of Medicine, Tottori University, Yonago 683-8504

Taro Yamashita

Division of Medicine and Clinical Science, Department of Multidisciplinary Internal Medicine, Faculty of Medicine, Tottori University, Yonago 683-8504

Yoshiharu Okamoto

Department of Veterinary Clinical Medicine, School of Veterinary Medicine, Tottori University, 4-101, Koyama-Minami, Tottori, Tottori, 680-8553

Shigeo Sasaki

Organization for Research Initiative and Promotion, Tottori University, 4-101, Koyama-Minami, Tottori, Tottori, 680-8550

Hajime Isomoto

Division of Medicine and Clinical Science, Department of Multidisciplinary Internal Medicine, Faculty of Medicine, Tottori University, Yonago 683-8504

Research Article

Keywords: EUS-FNB, wavelength, transmitted light, endoscopic ultrasound-guided fine-needle aspiration, endoscopic ultrasound-guided fine-needle biopsy, pancreas, rapid on-site evaluation, target sample check illuminator

Posted Date: July 20th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1755558/v1

Abstract

Background

Endoscopic ultrasonography-guided fine-needle aspiration biopsy (EUS-FNB) enhances the diagnostic capabilities of EUS by providing additional pathological samples. However, detecting the target specimens within the collected samples can be challenging. This study determined the most appropriate wavelength for detecting target specimens within EUS-FNB samples in an animal experiment.

Methods

EUS-FNB pancreatic tissue samples were collected from a male beagle (weight, 10 kg). The samples were illuminated with monochromatic light of varying wavelengths at 5-nm intervals from 430 to 700 nm. Optimal wavelengths for distinguishing target specimens within samples were determined.

Results

Transmitted monochromatic light at a wavelength of 605 nm effectively enhanced the contrast between target specimens and blood in EUS-FNB samples. The strengths of the target specimen and blood samples were quantified and analyzed using densitometry in images obtained by irradiating at each wavelength. The contrast between target specimens and blood samples peaked at 600–605 nm.

Conclusions

Microscopical observations using transmitted light at a wavelength of 605 nm effectively identified target tissues within EUS-FNB samples.

Background

Endoscopic ultrasonography-guided fine-needle aspiration biopsy (EUS-FNB) has enhanced the diagnostic capabilities of EUS by providing additional pathological details [1]. The use of EUS-FNB guides treatment decisions. However, identifying a target specimen within a sample can be challenging because specimens collected using fine needles are microscopic and contain blood. While rapid on-site evaluation during EUS-FNB is beneficial, many healthcare facilities cannot provide this procedure due to insufficient cytopathologists, and EUS-FNB should be conducted promptly to avoid potential complications [2–7].

These limitations motivated the development of a device using transmitted light at a specific wavelength [8, 9]. However, it has not yet been tested in basic experiments. This study aimed to identify the optimal wavelength for detecting target specimens within samples collected using EUS-FNB in an animal experiment.

Methods

This study was approved by the institutional review board of Tottori University and was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki.

EUS-FNB pancreatic tissue samples were collected from a male beagle (weight, 10 kg) using a 19G biopsy needle (Boston Scientific, Marlborough, MA, USA). Monochromatic light was used to illuminate the samples at variable wavelengths to identify the optimal conditions for detecting the target specimens. The light (6-nm wavelength of half-power width) emitted at 150 W from a xenon lamp (model no. P-250, Nikon Corporation, Tokyo, Japan) was diffracted using a sweeping-type spectroscope.

The collected samples were irradiated with monochromatic light at 5-nm wavelength increments, from 430 to 700 nm, to identify the most appropriate wavelength to distinguish the target specimens within the samples. The aspirated samples were coated with a plastic petri-dish on a glass plate. After monochromatic light irradiation, observation revealed the presence of the target specimen in the sample with formalin, confirmed microscopically.

Results

The light in the wavelength range of 430–585 nm did not penetrate the blood drop. Therefore, the target specimens were not identifiable within the aspirated samples. With a wavelength of > 590 nm, the light penetration was enhanced at the margins of the accumulated blood due to the similarity in thickness. The target specimens in the samples were detectable at wavelengths of 595–635 nm. However, at a wavelength of ≥ 640 nm, the contrast between the target specimen and the blood drop decreased. Transmitted monochromatic light at a wavelength of 605 nm maximized the contrast between the target specimens and blood in the samples (Fig. 1). Observation with transmitted light at the 605-nm wavelength was more effective for distinguishing target specimens within EUS-FNB samples than reflected light observation using white light (Figs. 2a, 2b). The target specimen, detected using transmitted light at the 605-nm wavelength, was confirmed microscopically (Fig. 3). The strengths of both the target specimen and blood samples were quantified and analyzed by densitometry to obtain an image as a result of irradiation at each wavelength. The contrast between the target specimens and the blood samples peaked at 600–605 nm (Fig. 4).

Discussion

The absorption spectrum of visible light for deoxidized hemoglobin peaks at a wavelength of approximately 550 nm, while that of oxidized hemoglobin peaks at 540 and 585 nm [10].

Transmitted light is the most effective modality for distinguishing target specimens in EUS-FNB samples; this study aimed to identify the optimal irradiation method in an animal experiment. The target specimens in the blood drop of a EUS-FNB sample are similar to the watermarks in a bill. If the bill is set on a base impenetrable to light, the watermarks are invisible. This phenomenon is consistent with our

observations on transmitted light in the current study. When the target specimen was covered with a blood drop, it was difficult to identify using reflected light because blood absorbs it. Conversely, transmitted light allowed the specimen identification due to the enhanced contrast.

The white specimens in the EUS-FNB samples provided histological data, while the red fraction was the blood component. After collecting samples with a 19G needle, a histologic core was found in 78.9% of white and 9.3% of red specimens [11]. It is challenging to identify the target specimens using reflected light because the volume of the blood drop is greater than that of the aspirated specimen. The transmitted light observation is suitable when the target specimen could not be detected using normal reflected light.

In our observational experiment using various wavelengths, monochromatic light at 605 nm wavelength was the optimal method to identify target specimens. In contrast, previous studies reported that the optimal discrimination ability was obtained at 540 and 585 nm, the absorption spectra of hemoglobin [10]. The light at wavelengths of 540 and 585 nm was reportedly unable to penetrate the blood drop. Our results also indicated that light with longer wavelengths could equally penetrate blood and tissues in the EUS-FNB samples.

This study has some limitations. First, even if a white specimen was identified in the sample, the target sample check illuminator (TSCI) could not distinguish between a tumor component and non-tumorous tissue in the sample. The cost-effectiveness of this method should also be addressed in addition to accuracy and safety. In the future, subjective impressions could also be objectively assessed by scoring and evaluating the diagnostic accuracy with receiver operating characteristic analysis.

Conclusions

Transmitted light at a wavelength of 605 nm effectively maximized the contrast to identify the target tissues within EUS-FNB samples. Thanks to the feasibility of this method, we hope that the frequency of TSCI use in real practice will increase. This study will contribute to reducing the frequency of EUS-FNB procedures, their adverse events, and the burden on practitioners.

List Of Abbreviations

EUS, endoscopic ultrasonography; EUS-FNB, endoscopic ultrasonography-guided fine-needle aspiration biopsy; TSCI, target sample check illuminator

Declarations

Ethics approval and consent to participate

This study was approved by the institutional review board of Tottori University and was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki. Informed consent was

obtained from the owner of the beagle to participate in the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

All authors declare no conflict of interests for this article.

Funding

None

Author contributions: KM, MU, KI, KU, SS, and HI contributed to the original concept and design of the study. KM, MU, TT, YO, YT, TO, SK, HK, HK, and TY were involved in data collection and sample management. KM, MU, KI, KU, SS, and HI analysed and interpreted the data. All authors have critically revised this article and approved the final version for publication.

Acknowledgements

We would like to thank Editage (www.editage.jp) for English language editing.

References

1. Hewitt MJ, McPhail MW, Possamai L, Dhar A, Vlavianos P, Monahan KJ. EUS-guided FNA for diagnosis of solid pancreatic neoplasm: a meta-analysis. Gastrointest Endosc. 2012;75:319–31.
2. Matsumoto K, Yamao K, Ohashi K, Watanabe Y, Sawaki A, Nakamura T, et al. Acute portal vein thrombosis after EUS-guided FNA of pancreatic cancer: case report. Gastrointest Endosc. 2003;57:269–71.
3. Hirooka Y, Goto H, Itoh A, Hashimoto S, Niwa K, Ishikawa H, et al. Case of intraductal papillary mucinous tumor in which endosonography-guided fine needle aspiration biopsy caused dissemination. J Gastroenterol Hepatol. 2003;18:1323–4.
4. Matsumoto K, Hara K, Sawaki A, Mizuno N, Hijioka S, Imamura H, et al. Ruptured pseudoaneurysm of the splenic artery complicating endoscopic ultrasound-guided fine-needle aspiration biopsy for pancreatic cancer. Endoscopy. 2010;42 Suppl 2:E27-E8.
5. Wang KX, Ben QW, Jin ZD, Du YQ, Zou DW, Liao Z, et al. Assessment of morbidity and mortality associated with EUS-guided FNA: a systematic review. Gastrointest Endosc. 2011;201173:283–90.

6. Micames C, Jowell PS, White R, Paulson E, Nelson R, Morse M, et al. Lower frequency of peritoneal carcinomatosis in patients with pancreatic cancer diagnosed by EUS-guided FNA vs. percutaneous FNA. Gastrointest Endosc. 2003;58:690–5.
7. Ikezawa K, Uehara H, Sakai A, Fukutake N, Imanaka K, Ohkawa K, et al. Risk of peritoneal carcinomatosis by endoscopic ultrasound-guided fine needle aspiration for pancreatic cancer. J Gastroenterol. 2013;48:966–72.
8. Matsumoto K, Ueki M, Takeda Y, Harada K, Onoyama T, Kawata S, et al. Development of a device for detecting the target specimens from EUS-guided FNA samples. Endosc Int Open. 2015;3:E662-4.
9. Matsumoto K, Hara K, Yasuda I, Itoi T, Kurumi H, Matsumoto S, et al. Usefulness of a target sample check illuminator in the detection of target specimens in endoscopic ultrasound-guided fine-needle biopsy samples: Multicenter prospective study. Dig Endosc. 2021;33:970–6.
10. Zijlstra WG, Buursma A, Meeuwsen-van der Roest WP. Absorption spectra of human fetal and adult oxyhemoglobin, de-oxyhemoglobin, carboxyhemoglobin, and methemoglobin. Clin Chem 1991;37:1633–8.
11. Iwashita T, Yasuda I, Mukai T, Doi S, Nakashima M, Uemura S, et al. Macroscopic on-site quality evaluation of biopsy specimens to improve the diagnostic accuracy during EUS-guided FNA using a 19-gauge needle for solid lesions: a single-center prospective pilot study (MOSE study). Gastrointest Endosc. 2015;81:177–85.

Figures

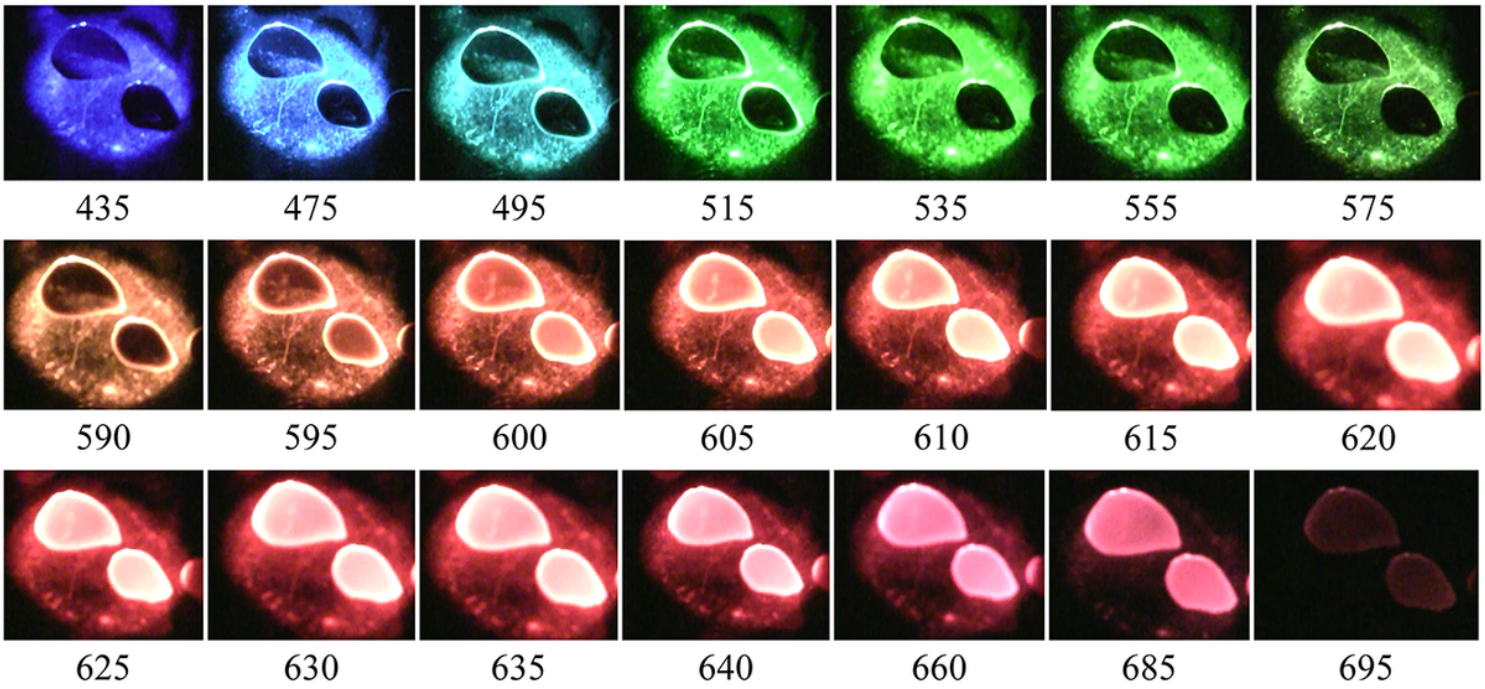

Figure 1

Visual inspection and discrimination of the target specimen in the samples, readily detectable at a 595–635-nm wavelength.

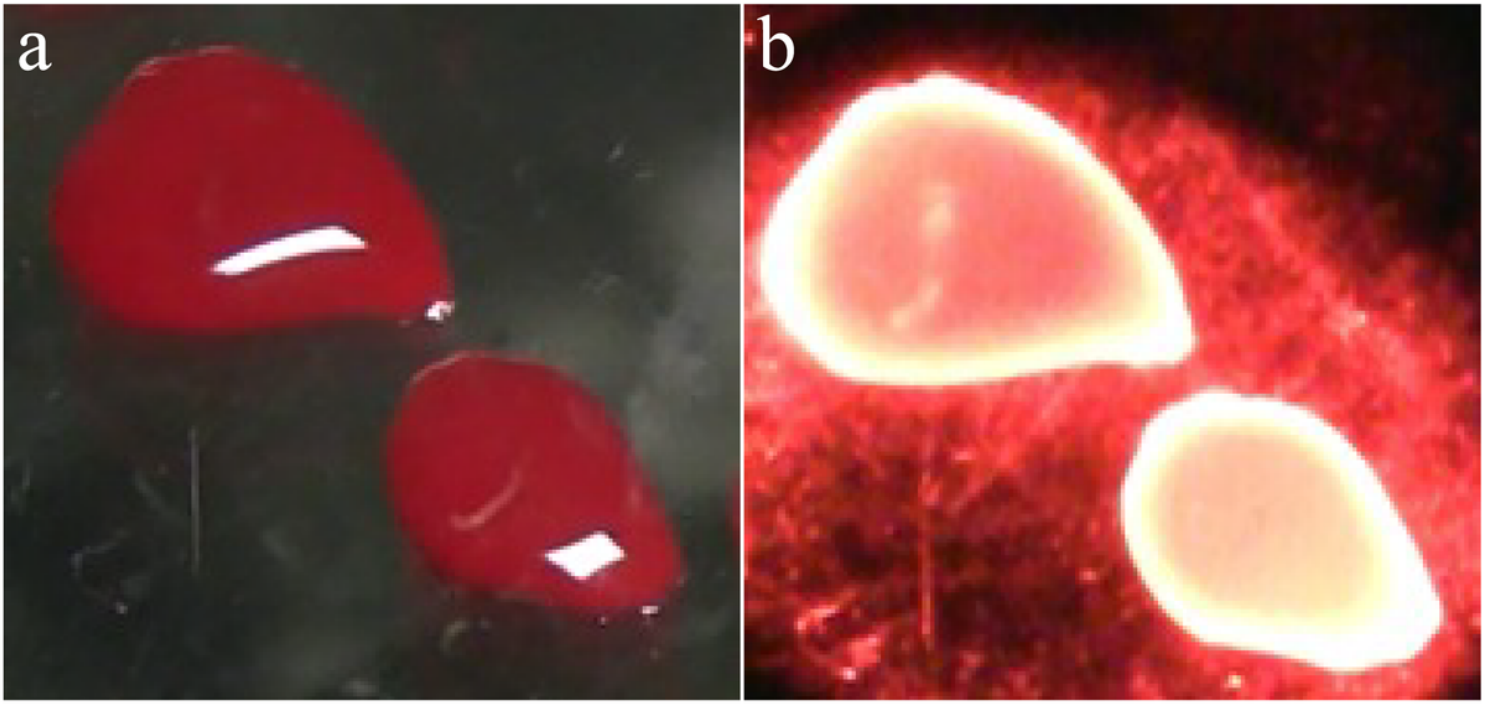

Figure 2

Transmitted light observation using a 605-nm wavelength was superior to reflected light observation using white light for identifying target specimens within endoscopic ultrasonography-guided fine-needle aspiration samples. a) Reflected light observation using white light.

b) Transmitted light observation at wavelength 605 nm.

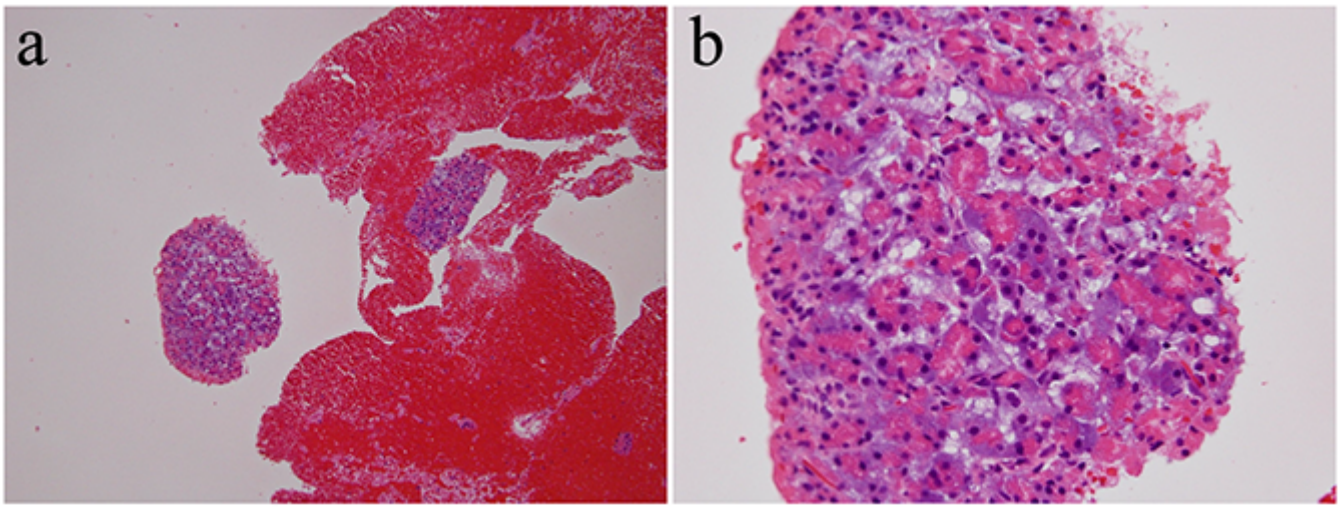

Figure 3

The target specimen detected by transmitted light at a 605-nm wavelength was confirmed as pancreatic tissue on microscopy.

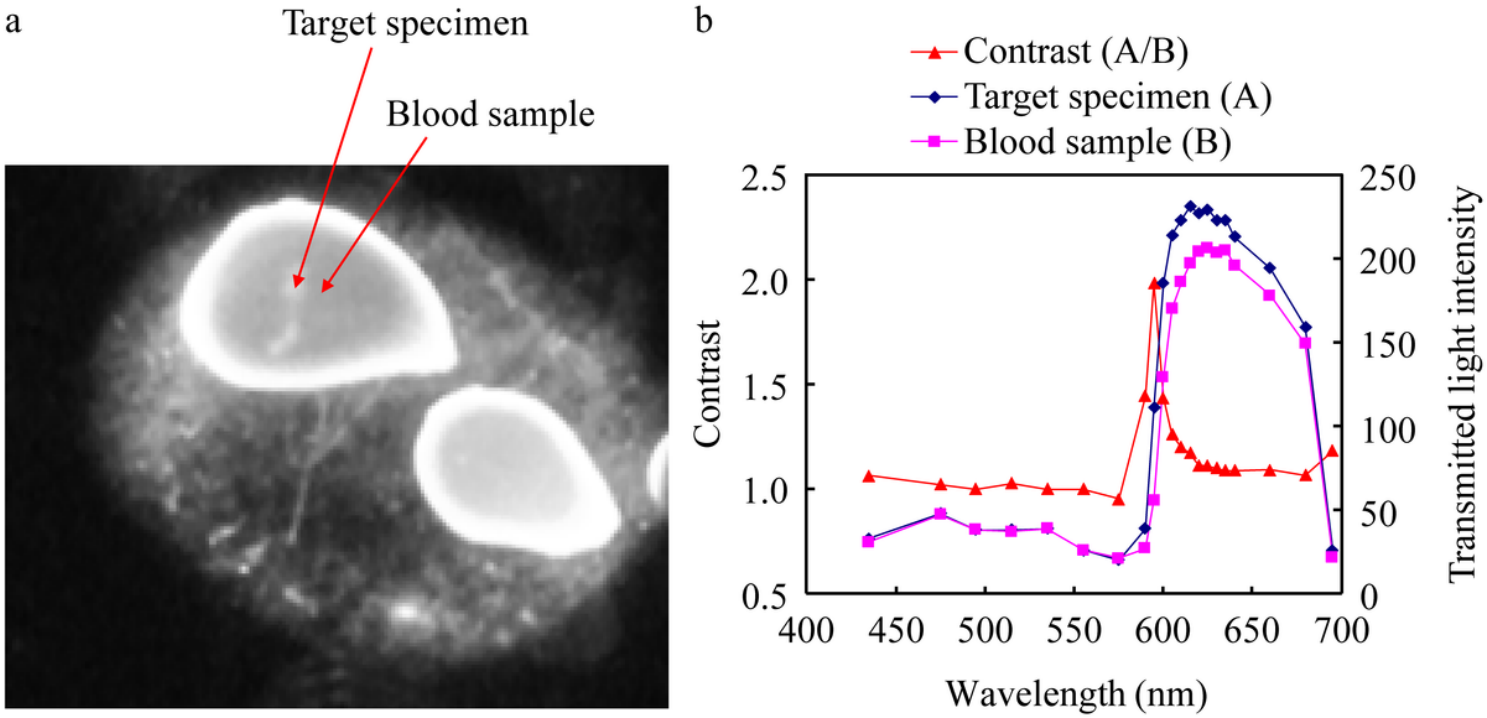

Figure 4

The contrast peaked at 600–605 nm because the absorbance of the tissue component increased, as shown by the contrast analysis between the target specimen and samples using densitometry.