Intraoperative fluorescence cerebral angiography by laser surgical microscopy: Comparison with xenon microscopy and simultaneous observation of cerebral blood flow and surrounding structures

学　位　論　文

Intraoperative fluorescence cerebral angiography by laser surgical microscopy: Comparison with xenon microscopy and simultaneous observation of cerebral blood flow and surrounding structures

（レーザ照影手術顕微鏡を用いたフルオレセイン術中蛍光脳血管撮影：キセノン照明との比較および血流画像と周囲構造物の同時観察の試み）

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論　文　内　容　要　旨

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学位論文題名	Intraoperative fluorescence cerebral angiography by laser surgical microscopy: Comparison with xenon microscopy and simultaneous observation of cerebral blood flow and surrounding structures (レーザ照影手術顕微鏡を用いたフルオレセイン術中蛍光脳血管撮影： キセノン照明との比較および血流画像と周囲構造物の同時観察の試み)

【目的】　現在使用されている脳神経外科手術顕微鏡の多くは、キセノンランプ照明を用いている。キセノン光は、波長構成が太陽光に近似していることから、術者にとって見慣れた色調で術野を観察することが可能である。その一方で、熱の発生による脳への影響や、紫外領域の波長を含むことによる術者の目への影響が危惧される。我々はこれまでにレーザ照明を用いた手術顕微鏡を開発し、その臨床応用を行っている。このレーザ照明手術顕微鏡は、従来のキセノン照明手術顕微鏡よりも少ないエネルギー光量で術野を均一に照射することが可能であると期待されている。今回、術中蛍光血管撮影におけるレーザ照明の有用性を検討した。【方法】蛍光色素は fluorescein sodium（Fluorescein）を使用した。レーザ照明装置が発するレーザ光を、光ファイバーを介して手術顕微鏡に引き入れ術野に照射した。レーザ光は 640nm の赤色光、532nm の緑色光、464nm の青色光の 3 波長で構成されている。第 1 に、蛍光撮影で得られる血流画像の明瞭度をレーザ照明とキセノン照明とで比較検討した。第 2 に、赤・緑・青 3 種のレーザ光の光量割合を変化させることで、蛍光血管撮影の際に血流画像のみならず周囲構造物も同時に観察することが可能な照明を求めた。さらに得られた励起光を使用して臨床例での有用性を検討した。【結果】青色単色のレーザ光を励起光として用いた蛍光撮影では、キセノンランプが発する青色光を励起光とした蛍光撮影よりも明瞭でコントラストの高い血流画像を観察することが可能であった。3 種類のレーザ光のうちの青色光の輝度を高め、緑色光をオフにし、赤色光の輝度を低めた組み合わせの励起光を照射すると、血流画像と同時に周囲構造物を、モニター画面のみならず術者が顕微鏡を介して直接観察することが可能となった。【結論】レーザ照明を用いた蛍光血管撮影では、キセノン照明に比較して観察される血流画像の明瞭度とコントラストが向上した。強い青色光と弱い赤色光の 2 色を組み合わせたレーザ光を励起光とすることにより、術者が顕微鏡を通して蛍光血流画像と周囲構造物とを同時に観察することが可能となった。レーザ照明手術顕微鏡は、通常の手術操作のみならず、蛍光血管撮影の際にも有用であった。

Intraoperative fluorescence cerebral angiography by laser surgical microscopy: Comparison with xenon microscopy and simultaneous observation of cerebral blood flow and surrounding structures

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Conflicts of interest disclosure

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ABSTRACT

BACKGROUND We have developed and marketed a laser illuminator as a light source for a surgical microscope**.** These laser surgical microscopes should enable uniform illumination of the operative field and require less luminous energy compared with existing xenon surgical microscopes.

OBJECTIVE In the present study, we examined the utility of laser illumination in fluorescence cerebral angiography.

METHODS Fluorescein sodium (fluorescein) was used as a fluorescent dye. We first compared the clarity of vascular image with blood flow collected by fluorescence angiography between the laser illumination and xenon illumination methods. We then assessed the use of the laser illuminator for simultaneous observation of vascular images with blood flow and surrounding structures during fluorescence angiography. Furthermore, the study was designed to evaluate the usefulness of the thus determined excitation light in clinical cases.

RESULTS Fluorescence angiography using blue light laser for excitation provided higher clarity and contrast vascular image with blood flow compared with using blue light generated from a xenon lamp. Further, illumination with excitation light consisting of a combination of three types of laser (higher level of blue light, no green light, and lower level of red light) enabled both vascular images with blood flow and surrounding structures to be observed through the microscope directly by the surgeon.

CONCLUSIONS Laser-illuminated fluorescence angiography provides high clarity and contrasts vascular image with blood flow. Further, a laser providing strong blue light and weak red light for excitation light enables simultaneous visual observation of fluorescent blood flow and surrounding structures by the surgeon using a surgical microscope. Overall, these data suggest that laser surgical microscopes are useful for both ordinary operative manipulations and fluorescence angiography.

Introduction

Many available surgical microscopes are fitted with a xenon lamp for illumination. Xenon light is close to sunlight in terms of wavelength composition, thus allowing surgeons to observe the operative field in familiar colors. However, xenon light generates heat that may affect the brain and contains ultraviolet range rays that may cause eye damage to the surgeon. We have developed and marketed a laser illuminator as a light source for a surgical microscope, which provides lower luminous energy to the operative field compared with conventional xenon microscopes, resulting in a smaller increase in brain temperature. Further, laser surgical microscopes can provide clear observation of deep operative fields with high contrast and avoid the potential for exposure to ultraviolet rays[1].

In the present study, we examined the use of laser microscopy for fluorescent imaging during surgery using fluorescein, a common fluorescent dye clinically used for fluorescence cerebral angiography. Our main aims were to; (1) compare the efficacy of laser microscopy with xenon microscopy in terms of contrast and clarity of vascular image with blood flow, and (2) determine whether laser surgical microscopy allows observation of vascular image with blood flow and surrounding structures within the same operative field.

Methods

Fluorescein was used as a fluorescent dye for fluorescence cerebral angiography. This dye emits green fluorescence (peak at 520 nm) in response to irradiation by blue excitation light (peak at 494 nm). Thus, if blue light is applied after intravenous administration of fluorescein, blood flow can be visualized as a flow of green light. Surgical microscopes (M500 OHS-1 and M530 OH6; Leica, Wetzlar, Germany) were combined with a laser illumination device (MML-

01; developed jointly by Mizuho Co., Ltd, Bunkyo-ku, Tokyo, Japan. and Mitsubishi Electric Engineering Co., Ltd, Nagaokakyo-shi, Kyoto, Japan.) The light generated from this device was guided through an optical fiber into the surgical microscope. The laser consisted of three wavelengths: 640 nm (red), 532 nm (green), and 464 nm (blue). The maximum output power of the laser light is about 12W at the output port of the device in the total sum of each laser light. For ordinary surgical manipulation, the operative field was illuminated with white light consisting of a mixture of the three wavelengths at an appropriate ratio. As the surgical microscope (M500-OHS1) did not have a fluorescence angiography mode, fluorescence angiography was performed by improving the fluorescent contrast using a filter (capable of blocking blue light) within the light path for microscopic observation.[2] Images were displayed at optimum clarity and contrast on the monitor using a camera capable of manual and automatic sensitivity adjustment and were saved to a computer for later analysis (MNIRC-2000K; developed jointly by Mizuho Co., Ltd., and Mitsubishi Electric Engineering Co., Ltd.).[3] The surgical microscope (M530 OH6) had a built-in fluorescence system for fluorescence imaging (FL560).

Comparison with xenon illumination

For laser-illuminated fluorescence angiography, the red and green light source of the laser illumination device was turned off, and only blue light was generated to excite the green fluorescence. During xenon-illuminated fluorescent imaging, a filter allowing passage of only blue light was added into the path of light illuminating the operative field. Thus, the operative field was only illuminated with blue light generated from the xenon light source.[4]

For the basic study, a 1:200 dilution of fluorescein solution was illuminated with either blue light from the xenon lamp or blue laser light of the same level (500 Lx at the output end of

the fiber), and the clarity and contrast of the fluorescence from the fluorescein solution compared. In the clinical study, fluorescence angiography with laser illumination was performed in patients undergoing craniotomy, and the fluorescence images were analyzed. We studied 5 patients who had undergone fluorescence angiography during craniotomy surgery.

Simultaneous observation of blood flow and surrounding structures

For the basic study, a plastic tube (inner diameter 2.4 mm) was arranged over a multi-colored still object, and a 1:200 dilution of fluorescein solution was passed through the tube. The levels of red, green, and blue laser lights were adjusted to achieve an optimum combination of the three light wavelengths, allowing simultaneous observation of fluorescein fluorescence and the still object.

In the clinical study, using the optimal combination of laser lights determined in the basic study, fluorescent cerebral angiography was performed in patients undergoing craniotomy. The ability to observe blood flow and surrounding structures (e.g., brain and nerve) within the same operative field was investigated. We studied 5 patients who had undergone fluorescence angiography during craniotomy surgery.

In each study, fluorescence clarity and contrast, as well as the effect on the actual surgical technique, were assessed by a consensus of three skilled neurosurgeons.

The local ethics committee approved this study, and all patients signed written informed consent forms permitting us to perform intraoperative cerebral angiography with fluorescein sodium dye using laser illumination.

Results

Comparison with xenon illumination

For the basic study, green fluorescence from the fluorescein solution, with lower blue fluorescence, was observed with the xenon microscope. By contrast, a sharp green fluorescence was observed after xenon illumination was switched to blue laser illumination (Figure1).

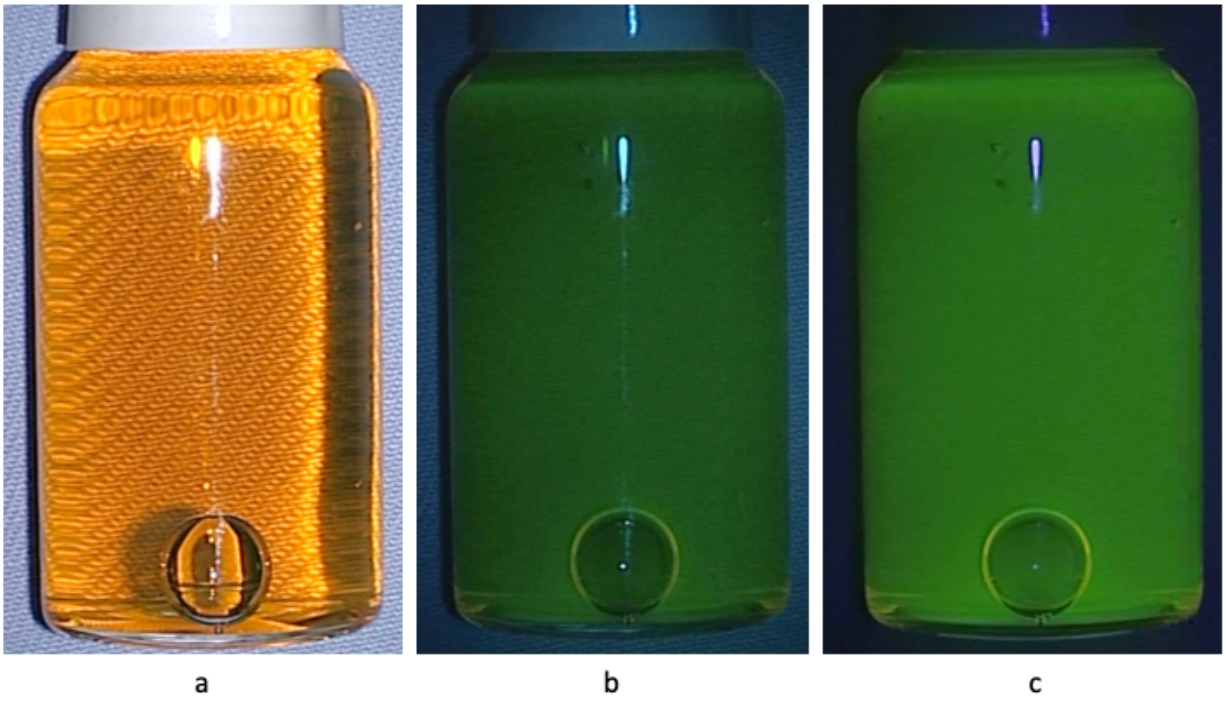

Figure1. A 1:200 diluted fluorescein solution was illuminated with white light (**a**), blue xenon lamp light (**b**), or blue laser (**c**), and fluorescence was examined after applying a blue light filter. Illumination with the blue laser improved the contrast and clarity of the fluorescent images.

A representative example from a 50-year-old woman from the clinical study is presented. Clipping of the unruptured distal anterior cerebral artery aneurysm was performed. After clipping, fluorescence cerebral angiography was performed using a laser surgical microscope (M500 OHS1). Obvious fluorescence was observed from the main arteries of the

brain, their branches, and superficial arterioles, confirming the absence of disturbed blood flow. The laser microscope also provided higher clarity and contrast fluorescent images compared with the xenon surgical microscope. Although the surgeon can only view the vascular image with blood flow through the eyepiece of the surgical microscope, the use of a high-resolution camera allowed the simultaneous presentation of vascular images with blood flow and surrounding structural images on the monitor display (Figure2).

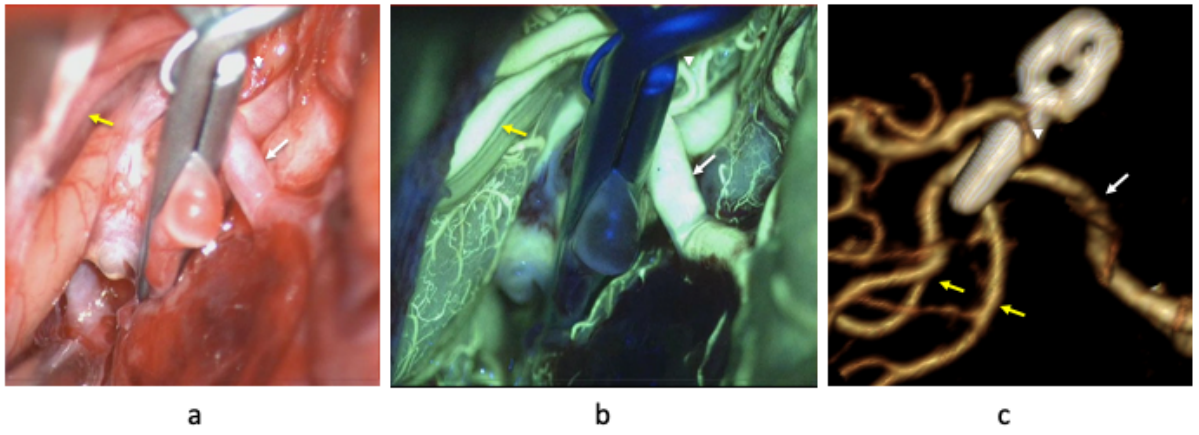

Figure2. Representative case 1: Intraoperative image with white light illumination (**a**), fluorescence angiogram recorded with a high sensitivity camera during blue laser illumination (**b**), and postoperative 3Dimensional Computed Tomography Angiography (**c**). White arrow: A2 portion of the anterior cerebral artery. Arrowhead: callosomarginal artery. Yellow arrows: pericallosal arteries.

Simultaneous observation of blood flow and surrounding structures

For the basic study, fluorescence was not identified when illuminating with a white laser, even after administration of fluorescein into the tube (Figure3a). The output intensity of red, blue, and green light was varied to find the optimal combination for simultaneous observation of the vascular image with blood flow and surrounding structures. When illuminated with a laser consisting of a high level of blue light and a low level (one-sixth the power intensity of the blue laser light) of green and red lights, we could observe both the still object and the flow of fluorescence in colors resembling those of ordinary white laser illumination; however, the clarity and contrast were lower (Figure3b). When illuminated with a laser consisting of a high level of blue light, no green light, and the lowest level (one-twelfth the power intensity of the blue laser light) of red light, the operative field became reddish and the brightness decreased. Nevertheless, observation of the structures was possible, and the brightness of the operative field was sufficient for continuation of operative manipulation. The flow of fluorescence was shown with high clarity and contrast, with visualization of the reddish still objects (Figure3c).

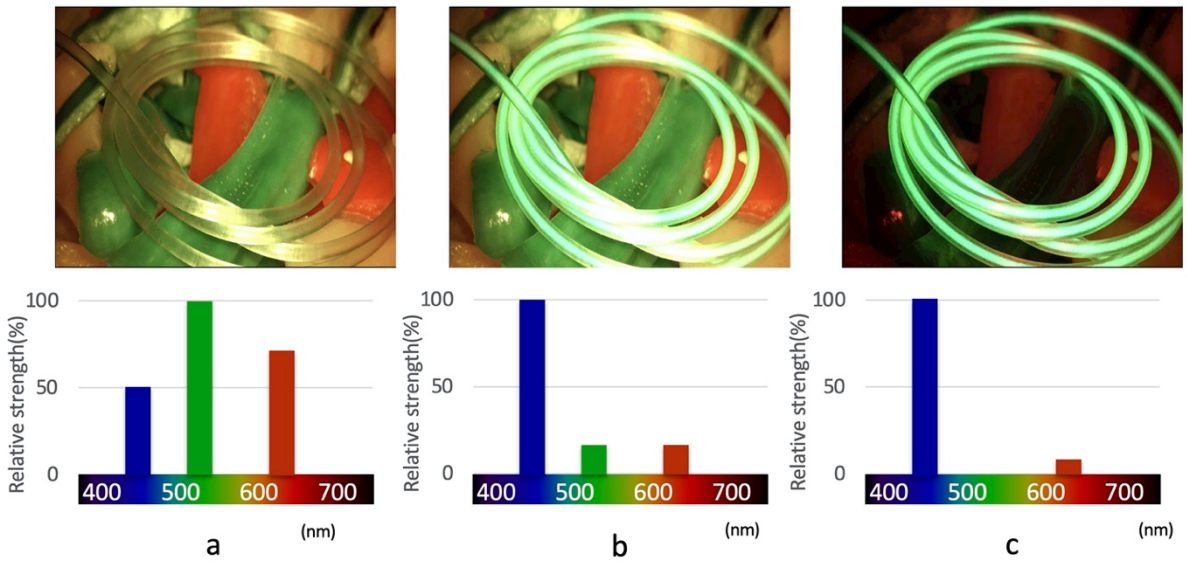

Figure3. A tube was placed on slices of bell pepper of three different colors, and a fluorescein solution (diluted 1:200) was applied through the tube. No fluorescence was noted when illuminated with white light consisting of a mixture of blue, green, and red light. (**a**). During illumination with light consisting of a higher level of blue light and the lowest level of green light and red light, the still object was observed in colors similar to those with white light illumination, although the clarity and contrast of fluorescence decreased (**b**). During illumination with light consisting of a high level of blue light, no green light, and the lowest level of red light, the still object became reddish, while the fluorescence images showed high clarity and contrast (**c**).

A representative example from a 77-year-old woman from the clinical study is presented. She was admitted to the Department of Neurology of our hospital with complaints of consciousness disturbance, dysuria, dysarthria, agnosia, and apraxia. Head magnetic resonance imaging revealed contrast-enhanced multiple lesions in the subcortical region of the left frontal

and parietal lobes. As definite diagnosis was not possible despite various examinations, we performed an open brain biopsy. Fluorescence imaging was performed before resection under illumination with the optimal combination of laser identified in the basic study (an intensity ratio of 100% blue light to 0% green light and about 8.33% red light.). With this laser combination, the operative field observed under the microscope became reddish and less bright, although it was sufficient to allow operative manipulation. When 10% fluorescein (2.5 ml) was administered via the venous line, a flow of green fluorescence appeared after approximately 20 sec, representing the blood flow through the main artery of the brain (3 mm in diameter), perforating branch (0.5 mm in diameter), and superficial small vessels (approximately 0.1 mm in diameter). There were no abnormal findings (Figure4). Thus, we resected subcortical tissues stained with fluorescein as pathological specimens, regarding anatomical landmarks and exercise-evoked potential monitoring findings.[5,6] The pathological diagnosis was primary intracerebral vasculitis. Her symptoms were alleviated following postoperative drug therapy.

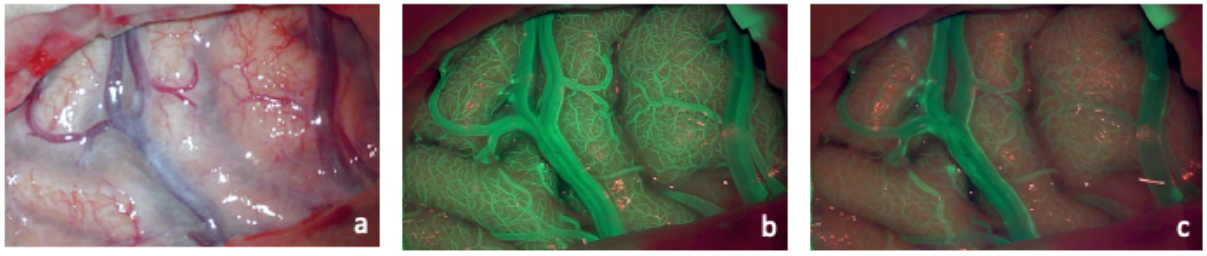

Figure4. Intraoperative pictures of representative case 2. The brain surface observed during illumination with white light consisting of a mixture of three colors (blue, green, and red) (**a**), and fluorescence cerebral angiogram arterial phase (**b**) and venous phase (**c**) during laser excitation (higher level of blue light, no green light, and the lowest level of red light). Simultaneous observation of circulation on the brain surface and the brain tissue (gyrus/sulcus) was achieved.

No complications arising from fluorescein injection or laser irradiation were observed in any of our cases where fluorescence angiography was performed with a laser microscope (n = 5).

Discussion

The use and efficacy of fluorescence angiography in neurosurgical procedures have been widely reported[2,3,7-10]. Indocyanine green (ICG) and fluorescein are the most commonly used fluorescent dyes in clinical practice. Both fluorescent dyes have the property of absorbing light energy of a specific wavelength and emitting light in a longer wavelength. The absorbed light is known as excitation light, and the emitted light is known as fluorescence. In both fluorescence angiography, the wavelengths other than fluorescence are blocked, causing an inability to simultaneously observe the surgical field structures with the vascular image with blood flow.

The ICG emits fluorescence with a wavelength's range of 780-950 nm when excited with a 700-850 nm wavelength's near-infrared light. The conventional ICG fluorescence angiography system confirms cerebral blood flow by observing the white fluorescence from cerebral blood vessels on a black screen. We have developed a camera system that allows simultaneous observation of the ICG fluorescence (near-infrared light) and the surrounding structures (visible light images). Furthermore, the images captured by this system are displayed on top of the surgical microscope view. Accordingly, it becomes possible for the surgeon to confirm the surrounding structure and vascular image with blood flow at the same time. We call this new system the "dual image video angiography" (DIVA)[11].

On the other hand, the fluorescein used in the present study emits green fluorescence (peak at 520 nm) in response to irradiation by blue excitation light (peak at 494 nm). Thus, if blue light

is applied after intravenous administration of fluorescein, blood flow can be visualized as a flow of green light. If a filter is placed within the light path used for observation under a surgical microscope and the blue excitation light is blocked, the contrast and clarity of the green fluorescence are improved. At present, fluorescence cerebral angiography is performed using a filter that allows only excitation light to pass, or a filter that removes excitation light but allows fluorescent light to pass.[2,12]

With current fluorescence angiography techniques, blue light from a xenon light source, or blue LED light, is used for excitation. Both the excitation light and the resulting emitted fluorescence have a range of wavelengths. The wavelength of the excitation light partially overlaps with the wavelength of the fluorescence (Figure5a). Thus, during fluorescence angiography, blocking the excitation light with a filter located within the observation light path improves the clarity of fluorescence. If a filter with a range enabling complete observation of fluorescence is used, then the fluorescence intensity increases, although the presence of excitation light reduces the fluorescent image contrast (Figure5b). However, the use of a filter with a range that completely removes the excitation light provides improved contrast and clarity but reduces the fluorescence intensity (Figure5c). Importantly, although the use of fluorescence cerebral angiography during neurosurgical operation is widely reported,[8,13,14] images of insufficient clarity can lead to surgical errors, resulting in postoperative complications because of unexpected disturbances in blood flow.[15] Thus, higher clarity and contrast fluorescent images are required to overcome these problems. As laser illumination provides a high-intensity excitation light, without overlap of excitation and fluorescence wavelengths (Figure5d), higher clarity and contrast fluorescent images can be obtained by completely blocking the excitation light.

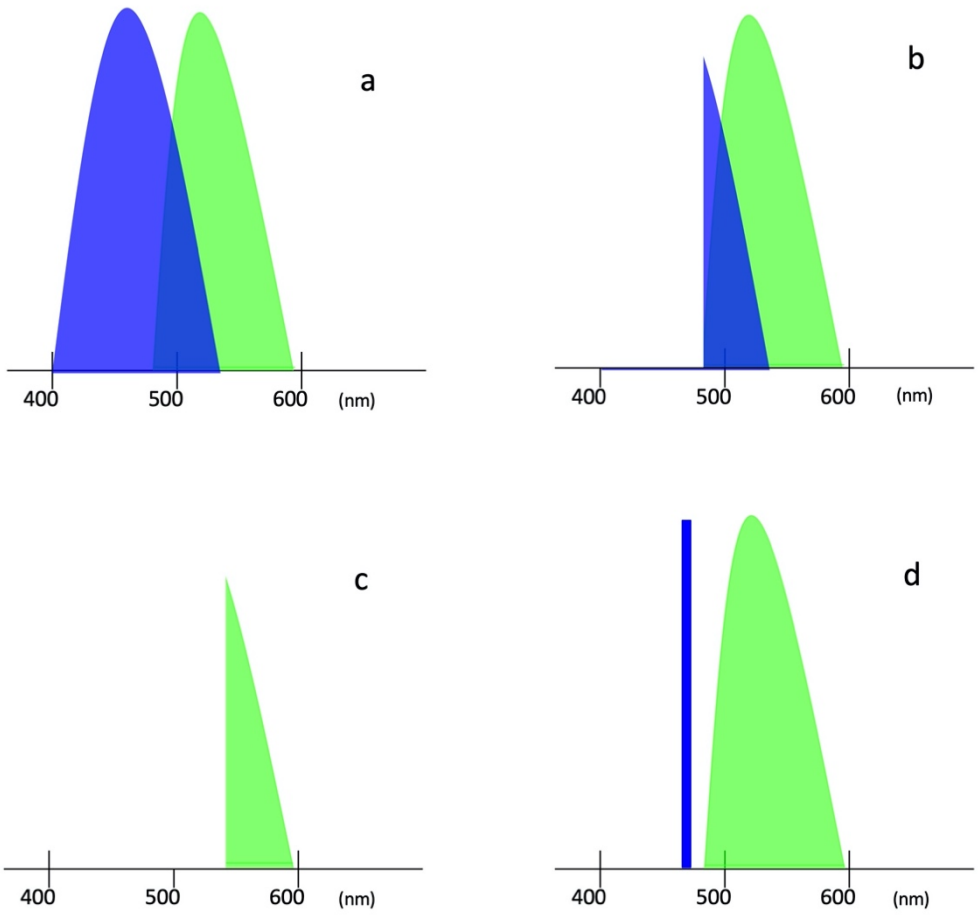

Figure5. When using light emitted from a lamp or light emitting diode (LED), there is a range of wavelengths for both excitation light (blue) and fluorescent light (green), which partially overlap (**a**). If a filter covering the complete range of emission fluorescence is used, the observed fluorescence intensity increases, but the contrast of the fluorescent images decreases because of the presence of excitation light (**b**). If a filter covering the complete range of excitation light is used, both the observed fluorescence intensity and the clarity decrease (**c**). If a blue laser with a wavelength that does not overlap with the fluorescence spectrum is used for excitation, the excitation light can be completely blocked, and the all the emission wavelengths can be observed (**d**). This provides fluorescent images of high contrast and clarity.

Current fluorescence cerebral angiography techniques do not allow simultaneous observation of brain parenchyma and nerves with the observation of vascular image with blood flow. However, simultaneous observation can help surgeons to determine the anatomical position of abnormalities with improved accuracy, and to assess blood flow disturbances. In the present study, illumination of the operative field with a low-intensity red light allowed the surgeon the directly observe the flow of green fluorescence with high clarity and contrast, while simultaneously observing the structures within the operative field.

Despite advances in fluorescence cerebral angiography such as the use of laser illumination, there are some limitations. For example, visualization of blood flow through vessels covered by cerebrospinal fluid or blood, or blood flow through the thick walls of atherosclerosis-affected arteries (e.g., the internal carotid artery), remains poor. This is primarily because of the attenuation of excitation light and fluorescence intensities during passage through the cerebrospinal fluid or the vascular wall. Unfortunately, in the present study, the use of a laser did not improve this problem. Thus, enabling better observation of fluorescence without attenuation remains an important area for future research. Another limitation of fluorescence cerebral angiography is that measurement of blood flow by fluorescence is not quantitative. Thus, concomitant use of other modalities such as laser speckle contrast imaging should be considered.[16] Finally, we only performed a subjective evaluation of the clarity and contrast of fluorescence in the present study. Further studies providing a quantitative assessment of fluorescence clarity and contrast using fluorescence cerebral angiography with laser illumination are required.

Conclusions

Fluorescence cerebral angiography using a laser microscope provides higher clarity and contrast fluorescent images of blood flow when compared with fluorescence angiography using a xenon surgical microscope. The use of particular combinations of blue, green, and red light also allowed simultaneous observation of vascular images with blood flow and surrounding tissues. Thus, laser microscopy can be used for observation with ordinary white light illumination and fluorescence cerebral angiography and is useful for neurosurgical surgery.

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