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RGB Camera-Based Imaging of Oxygen Saturation and Hemoglobin Concentration in Ocular Fundus

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ABSTRACT We propose a red, green, blue (RGB)-based oximetry to assess the ocular fundus and determine its oxygen saturation (SO2) and hemoglobin concentration. The oxygenated hemoglobin concentration, deoxygenated hemoglobin concentration, and SO2 were estimated employing a method that combines Monte Carlo simulation of light transport in the fundus tissue with a multiple regression analysis. In this study, a single-layer model of the ocular fundus was employed for the Monte Carlo simulation. We constructed an experimental apparatus for measuring the fundus of a rat’s eye using an RGB detector and investigated the physiological response that occurs upon a change in the fraction of inspired oxygen (FiO2). The resultant images of oxygenated hemoglobin concentration, deoxygenated hemoglobin concentration, total hemoglobin concentration, and SO2 indicated that the response was caused by the defective oxygenation of the blood. The results of the present study indicate the possibility of oximetry based on the RGB images of a fundus.

INDEX TERMS Digital cameras, biomedical optical imaging, image color analysis.

I. INTRODUCTION Observations of the ocular fundus are useful for diagnosing fundus diseases such as age-related macular degeneration (AMD) [1], diabetic retinopathy [2], [3], retinal pigmentary degeneration [4], and retinal vascular occlusions [5]. Fig. 1 shows the ocular fundus of a rat that was used in this study. We can observe the vessels in the ocular fundus directly because some parts of the eyeball, such as the vitreous body and the crystalline lens, are transparent. Therefore, observations of vessels in the fundus facilitate the diagnoses of fundus disease and various other diseases related to blood vessels in the eye, such as hypertension [6] and kidney disease [7]. Currently, doctors diagnose these diseases qualitatively by observing pictures taken with a fundus camera. The fundus has various pigments that absorb light of different wavelengths [8]. Some studies have reported that target pigments or tissues of the retina can be emphasized by selecting an optimal wavelength from multichannel spectral images of the retina [9], [10]. However, it is difficult to extract the specific information of the retina directly from the red, green, blue (RGB) image acquired by the fundus camera because each channel of the RGB image integrates over a broad spectral band based on the transmittance spectrum of each color filter. Moreover, fundus fluorescein angiography (FFA) [11], [12] or indocyanine green angiography (ICGA) [13], [14] has also been widely used to diagnose eye diseases, such as diabetic retinopathy and retinal vascular occlusions. However, the imaging agent may cause side effects, such as nausea, itchy red areas, cough, etc. Rare cases of anaphylactic shock have also been reported. Because of the heavy burden FFA places on patients, it is desirable to quantitatively evaluate the vessels of the ocular fundus for early detection of these diseases without administering an imaging agent to subjects. One of the signs of these diseases is the formation of new blood vessels in the ocular fundus caused by hypoxia [15]. Moreover, the retinal vessel occlusion causes decreased oxygen saturation (SO2) [16]. Therefore, SO2 in the ocular fundus allows for early detection of these diseases. Many researchers have proposed various retinal SO2 imaging methods. An initial study in this area was conducted by Hickam et al. [17], where fundus pictures were acquired through two filters, which passed light of wavelengths 640 nm and 800 nm. Then, the venous SO2 on the optic disc...
was estimated from a linear relationship between SO$_2$ and the ratio of the red to infrared optical density (OD) images. Additionally, other studies have reported two-wavelength oximetry [18]–[23]. Two commercial two-wavelength retinal oximetry systems, Vesselmap oxygen module (Imedos Systems GmbH, Jena, Germany) [21] and Oxymap T1 (Oxyma ehf., Reykjavik, Iceland) [22], have contributed to ophthalmological research. Recently, hyperspectral imaging (HSI) [24]–[26], [34], [35] and multispectral imaging (MSI) [25], [27]–[33], [36], [37], [34], [40]–[46] have also been used to estimate SO$_2$ in the fundus. HSI can be used to acquire spectral images with higher wavelength resolution than that of MSI but is limited to time-sequential measurements. Alternatively, MSI can be used to acquire images from a snapshot that can concurrently acquire images at multiple wavelengths or by time-sequential imaging. Either of the two band-pass interference filters, one a liquid-crystal tunable filter (LCTF) and the other an acousto-optical tunable filter (AOTF), was used in the time-sequential measurements. Seven-band MSI-based oximetry with LCTF, which has a wavelength sensitivity range from 522 to 586 nm, was proposed by Khooobei et al. [33]. HSI-based oximetry with LCTF for the human retina was developed later [24], [34], [35]. Five-band MSI-based oximetry techniques with AOTFs were also proposed [25], [36], and HSI-based oximetry with AOTFs have also been developed [25], [26]. AOTFs can switch wavelengths faster and have narrower bandwidths than LCTFs. Further, multispectral scanning laser ophthalmoscopes (SLO), which have higher contrast and spatial resolution than fundus cameras, were developed for oximetry [27]–[32], [37]. These techniques illuminate and scan the retina using multiple lasers of different wavelengths. However, these time-sequential oximetry methods are affected by involuntary eye movement, which can be classified as microsaccade, drift, and tremor [38]. Indeed, Hendargo et al. reported that the snapshot MSI could reduce the motion artifact that was caused by the blood flow in the blood vessels [39]. Moreover, MacKenzie et al. observed the rapid oxygen dynamics using the snapshot MSI [40]. Therefore, to eliminate the influence of involuntary eye movement during measurement, the snapshot MSI technique is more suitable for the estimation of SO$_2$ in the fundus, because this technique allows multiband images to be acquired simultaneously without switching wavelengths. Currently, there are two types of snapshot MSI that have been used for retinal oximetry. The first type uses beam-splitter multiplexing, which splits a single beam into two or more, then spectrally filters the split beams [41], [42]. While this approach is simple, it is optically inefficient because a beam is being split by multiple beam splitters. The second approach is the use of an image replicating imaging spectrometer (IRIS), which demultiplexes light with quarter wave plates and Wollaston prisms. Alabboud et al. first reported basic data on snapshot MSI oximetry using IRIS [34]. After that, applications including retinal oximetry that utilized IRIS were reported by other researchers [43]–[46]. Moreover, some researchers have reported that photoacoustic imaging (PAI) [47] and optical coherence tomography (OCT) [48] could also be used to estimate SO$_2$ in the retina. However, these systems require complex optical systems.

To address these problems, we propose a noninvasive imaging method for measuring SO$_2$ based on diffuse reflectance spectroscopy with an RGB color detector that is mounted on commercial fundus cameras. Therefore, our method requires no additional equipment and can be used to estimate SO$_2$ in the fundus from only an RGB image. Since the RGB color detector is less affected by the involuntary eye movement, it is suitable for fundus measurement. This technique is based on a method that combines a Monte Carlo simulation (MCS) of light transport in the fundus tissue with multiple regression analysis (MRA). In this study, a single-layer model of the ocular fundus was used for the MCS. Moreover, to evaluate the hemodynamics in the ocular fundus, we formed SO$_2$ images from the ocular fundus images of a rat by changing the fraction of inspired oxygen (FiO$_2$).

There are two previous studies of oximetry that have employed an RGB charge-coupled device (CCD) or complementary metal-oxide-semiconductor (CMOS) image sensor [21], [49]. Hammer et al. designed two monochromatic fundus cameras using specific dual wavelength transition filters (548, and 610 nm) and a color CCD detector [21]. Moreover, Putten et al. designed a multispectral microscope that combined LCTF and a digital single-lens reflex (SLR) camera [49]. However, our method is different from these studies. That is, our method requires only an RGB detector and no additional equipment, whereas these other systems require special filters. In the RGB image sensor, red, green, blue filters are arranged on the pixel array based on a Bayer arrangement [50]. The Bayer filter consists of 50% green filters, 25% red filters, and 25% blue filters [50].

Retinal oximetry based on the RGB image or MSI can detect the initial state of disease from oxygen dynamics. Alternatively, ICGA can detect the initial state of disease from oxygen dynamics.
hemoglobin only. The fundus has melanin and a yellow coloring matter (xanthophyll) [54]–[56], but the model in this study has none of these pigments. As Fig. 2 shows, incident light is scattered by the tissue and absorbed by the hemoglobin after incident light migrates into the tissue. Then, light exits from the tissue as diffuse reflected light. The diffuse reflectance of the retina is given by [57]

$$O(\lambda) = \frac{I}{I_0} = \int_0^\infty P(l; \mu_s, g) \exp \left\{ - \left( \mu_a,HbO(\lambda) + \mu_a,HBr(\lambda) \right) l \right\} dl,$$

(4)

where $I_0$ and $I$ represent the incident and reflected light intensities, respectively. $P(l; \mu_s, g)$ is the probability function of photon path length $l$, and it is determined by the scattering coefficient $\mu_s$ and anisotropy factor $g$. In addition, $\mu_a$, $C$, and $\varepsilon$ are the absorption coefficient, concentration, and extinction coefficient of the oxygenated and deoxygenated hemoglobin, respectively [58]. The subscripts, $r$, $HbO$, and $Hbr$ denote retina, oxygenated hemoglobin, and deoxygenated hemoglobin, respectively. Herein, the absorption coefficient $\mu_a$ can be expressed as the product of the concentration and extinction coefficient, i.e., $\mu_a = C \varepsilon$. Therefore, from Eq. (1) to (4), the RGB values are functions of the concentration $C$.

### B. ESTIMATION OF OCULAR FUNDUS CHROMOPHORE FROM RGB VALUES

If the path length probability function $P(l; \mu_s, g)$ can be measured, the concentration $C$ can be calculated from the RGB values in Eq. (1) through (4). However, it is difficult to measure the function directly. Therefore, in this study, the MCS of the light transport is used to estimate the diffuse reflectance spectrum of the fundus $O(\lambda)$.

Fig. 3 illustrates the procedure that was proposed by Nishidate et al. [60] for estimating $C_{HbO}$, $C_{Hbr}$, $C_{Hbr}$, and $SO_2$ from the red-green-blue image. In this method, we need to calculate two transformation matrices, defined as $N_1$ and $N_2$, in advance. The first matrix $N_1$ transforms RGB values into CIEXYZ tristimulus values, and the second matrix $N_2$ transforms CIEXYZ values into $C_{HbO}$ and $C_{Hbr}$. After the white balance of the RGB detector is calibrated using a standard white diffuser with 99% reflectance as a reference material, the images of both a standard color chart and a white chart are acquired using the RGB detector. Moreover, to correct illumination non-uniformity, the RGB values of each color chip are normalized by the RGB values of the white images.

The transformation matrix $N_1$ relates RGB and XYZ as

$$\begin{bmatrix} X \\ Y \\ Z \end{bmatrix} = N_1 \begin{bmatrix} R \\ G \\ B \end{bmatrix},$$

(5)
and is determined using the MRA between the measured RGB and the XYZ values. The XYZ values under specific illumination, which are supplied by the chart, are the response variables, while the measured RGB values are the predictor variables. The resulting regression coefficients are the elements of $N_1$. Even if the spectral characteristic of a color filter on the RGB detector and an illumination are unknown, any RGB values are transformed into the common XYZ values using the matrix $N_1$. Therefore, because of the matrix $N_1$, we do not need to consider the differences of individual devices. If we use another fundus camera system for estimation, our method requires measurement of the chart and calculation of $N_1$ on one occasion only.

Next, the second transformation matrix $N_2$ is also determined using the MRA between the XYZ values and the chromophore concentrations by

$$
\begin{bmatrix}
C_{\text{HBO}} \\
C_{\text{HBR}}
\end{bmatrix} = N_2
\begin{bmatrix}
X \\
Y \\
Z
\end{bmatrix}.
$$

(6)

The chromophore concentrations, i.e., the oxygenated hemoglobin concentration $C_{\text{HBO}}$ and deoxygenated hemoglobin concentration $C_{\text{HBR}}$, represent the light absorption coefficients of the fundus. The $C_{\text{HBO}}$ and $C_{\text{HBR}}$ are the response variables, while the XYZ values are the predictor variables. The resulting regression coefficients are the elements of $N_2$. Using various combinations of these hemoglobin concentrations, we employ the MCS model for light transport in the fundus to calculate the diffuse reflectance spectra of the fundus $O(\lambda)$. The parameters of the MCS are as follows. As mentioned before, the absorption coefficient $\mu_a(\lambda)$, which indicates the light absorption of the oxygenated and deoxygenated hemoglobin, is obtained from the concentrations $C_{\text{HBO}}$ and $C_{\text{HBR}}$ and the known extinction coefficient spectra of $\epsilon_{\text{HBO}}$ and $\epsilon_{\text{HBR}}$ [59]. Then, the reduced scattering coefficient $\mu'_s(\lambda)$ can be approximated by [61], [62]

$$
\mu'_s(\lambda) = a\lambda^{-b},
$$

(7)

where the two parameters $a$ and $b$ are defined as the scattering amplitude and scattering power, respectively. The measured scattering spectrum of biological tissue gives the typical parameters. The coefficients $\mu'_s(\lambda)$ are set by changing the typical parameters, $a$ and $b$, in five steps [63], [64]. Five different parameters are derived by multiplying the typical parameters of $a$ and $b$ by 0.5, 0.75, 1.0, 1.25, and 1.5, respectively. In addition, the refractive index [65] and layer thickness are set to be 1.4 and 5 cm, respectively. To determine matrix $N_2$, we use the simulation to calculate 300 diffuse reflectance spectra in the 400–700 nm wavelength range at 10 nm intervals. The XYZ values are calculated by integrating the diffuse reflectance spectra, obtained by the MCS.
Then, the transformation matrix $N_2$ is determined using the MRA. After the XYZ values are transformed from the measured RGB values using the first matrix $N_1$, the XYZ values are transformed into the chromophore concentrations $C_{HbO}$ and $C_{HbR}$ using matrix $N_2$. The total hemoglobin concentration $C_{HbT}$ and oxygen saturation $SO_2$ are then calculated as $C_{HbT} = C_{HbO} + C_{HbR}$ and $SO_2 = (C_{HbO} / C_{HbT}) \times 100$, respectively. The absorption coefficients of blood when $C_{HbT} = 100\%$ are set to those of blood with 44% hematocrit and 150 g/L of hemoglobin [59].

III. MATERIALS AND METHOD

Fig. 4 shows an experimental apparatus that consists of a white LED light source (Fiber-Lite Mi-LED A2, Dolan-Jenner industries Inc., MA, USA), a light guide, a beam splitter (CM1-BS013, Thorlabs Inc., NJ, USA), a zoom lens (VZM 600i, 1.0X-6.0X, Edmund Optics Inc., NJ, USA), a 24-bit RGB CCD camera (DFK23U618, Imaging Source LLC, NC, USA), which has a diagonal 4.5 mm interline CCD solid state image sensor (ICX618AQA, SONY, Tokyo, Japan), and a PC. Figure 5 shows the relative intensity of white LED light source, both the relative response of the red, green, and blue filters mounted on the CCD chips and the molar extinction coefficient of hemoglobin. These color filters are arranged in the Bayer array. An IR cut filter in the sensor rejects unnecessary light greater than 700 nm.

Male Wistar albino rats, which have no melanin and xanthophyll [54]-[56] in their fundus, were used as experimental samples, whereas the human fundus has various pigments,
such as melanin and xanthophyll and the importance of other pigments has been shown for the estimated accuracy of SO$_2$ \cite{4, 38}. In this system, after white light from the light source illuminates the rat eye through the light guide and the beam splitter, the RGB color detector measures both specularly reflected light from the surface of the eye and diffusely reflected light from the fundus that passes through the beam splitter and the zoom lens. The image size was 640 × 480 pixels. The image was saved as a bitmap (BMP) image. A mydriatic agent (Mydrin-P ophthalmic solution, Santen Pharmaceutical Co., Ltd., Osaka, Japan) was used to dilate the left pupil. In addition, high viscosity eye drops (New Rohto Dry Aid EX Eye Drop, ROHTO Pharmaceutical Co., Ltd., Osaka, Japan) were used to prevent the eye from drying. The drops also function as a coupling gel, and the eye was covered with a cover glass. A standard white diffuser with 99% reflectance (USRS-99-010, Labsphere Inc., NH, USA) was used to correct the spatial nonuniformity of illumination. The image of the white diffuser is acquired by the detector after the white diffuser is fixed by a flexible stand (MB-MX, SIGMA KOKI, Tokyo, Japan) at the same position as the eye.

Animal care and experimental procedures were approved by the Animal Research Committee of Tokyo University of Agriculture and Technology. In preparation for the experiment, the rats were put under anesthesia by inhalation of mixed isoflurane and air. Isoflurane was maintained at a

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<th>FiO$_2$ [%]</th>
<th>$C'_{\text{HbO}}$ [vol.%]</th>
<th>$C'_{\text{HbR}}$ [vol.%]</th>
<th>$C'_{\text{HbT}}$ [vol.%]</th>
<th>SO$_2$ [%]</th>
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**FIGURE 8.** Imaging of oxygenated hemoglobin $C'_{\text{HbO}}$, deoxygenated hemoglobin $C'_{\text{HbR}}$, total hemoglobin $C'_{\text{HbT}}$, and oxygen saturation SO$_2$ in rat fundus for various fraction of inspired oxygen (FiO$_2$) values (sample 1). The regions of interest (ROI) represent the optic disk (white frame) and the tissue area (black frame), respectively.
FIGURE 9. Imaging of oxygenated hemoglobin $C_{HbO}$, deoxygenated hemoglobin $C_{HbR}$, total hemoglobin $C_{HbT}$, and oxygen saturation $SO_2$ in rat fundus for various fraction of inspired oxygen (FiO$_2$) values (sample 2). The regions of interest (ROI) represent the vein area (blue frame) and the artery area (yellow frame), respectively.

<table>
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<tr>
<th>FiO$_2$ [%]</th>
<th>$C_{HbO}$ [vol.%]</th>
<th>$C_{HbR}$ [vol.%]</th>
<th>$C_{HbT}$ [vol.%]</th>
<th>$SO_2$ [%]</th>
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As shown in Fig. 7, we gradually varied the FiO$_2$ by adjusting the gas flowmeter and valve for 21 min. The x-axis and y-axis represent time and FiO$_2$, respectively. The percentage of O$_2$ and N$_2$ contained in the air at 0 m above sea level is 20.9% and 78.1%, respectively. Therefore, an FiO$_2$ value of 21% is similar to normal conditions. The RGB images of the rat’s fundus were captured after the rats breathed for 3 min at each FiO$_2$ step.

IV. RESULTS AND DISCUSSION

Fig. 8 and 9 show the RGB fundus images of two rats (sample 1 and sample 2) and the changes in the images of $C_{HbO}$, $C_{HbR}$, $C_{HbT}$, and oxygen saturation ($SO_2$), depending on the changes in FiO$_2$. To make the RGB images shown in Fig. 8 and 9 easy to examine, the contrast and brightness of the images were adjusted. The regions of interest (ROI)
shown in Fig. 8 represent the optic disk (white frame) and the tissue area (black frame), respectively. Moreover, the ROI shown in Fig. 9 represents the vein area (blue frame) and the artery area (yellow frame). Fig. 10 shows the change in the mean $C_{\text{HbO}}$, $C_{\text{HbR}}$, $C_{\text{HbT}}$, and $SO_2$ within the ROI, depending on the change in the FiO$_2$ value. The x-axis denotes FiO$_2$ and the y-axis represents the mean $C_{\text{HbO}}$, $C_{\text{HbR}}$, $C_{\text{HbT}}$, and $SO_2$ values within the ROI. The breath of the rats was stopped when FiO$_2$ value was 0%.

First, as Fig. 10 shows, $C_{\text{HbO}}$ and $SO_2$ decreased as FiO$_2$ decreased, whereas the values of $C_{\text{HbR}}$ and $C_{\text{HbT}}$ increased as FiO$_2$ decreased. These results correspond to the following physiological phenomena. Decreased partial oxygen pressure (PO$_2$) resulting from decreased FiO$_2$ causes defective oxygenation of the blood. Consequently, $C_{\text{HbO}}$ decreases and $C_{\text{HbR}}$ increases in the artery. This results in decreased $SO_2$ in the artery. Moreover, the low concentration of oxygen in the blood is consumed in the peripheral tissue, and $C_{\text{HbR}}$ shows a greater increase in the peripheral vein. This results in decreased $SO_2$ in the vein. $SO_2$ values in capillaries in the optic disk and the tissue area also decreased similarly. In comparison to the estimated $SO_2$ value shown in Fig. 10 between the artery area and the vein area, the $SO_2$ level in the artery area was higher than in the vein and, therefore, our method could distinguish between an artery and a vein.

Next, in Fig. 10, $C_{\text{HbT}}$ largely increased in a hypoxic state at less than 21% FiO$_2$. This phenomenon shows that defective blood oxygenation is compensated by either vasodilation or increased heartbeat. Then, $C_{\text{HbO}}$ and $SO_2$ increased in the samples when the FiO$_2$ was 12%. This occurred presumably because the involuntary motion of the eyeball under the anesthesia created variations in the photographic conditions. Moreover, the $SO_2$ decreased largely when FiO$_2$ decreased from 14% to 0%. The results indicate that the $SO_2$ decreases sharply as oxygen partial pressure becomes close to zero.

To analyze the temporal change in the $SO_2$ in detail, it is necessary to observe the change in fundus color at longer interval with high temporal resolution. Therefore, in future work, we will observe the temporal change in the fundus color by capturing the video.

The estimated $SO_2$ in the tissue area was lower than in the vein. However, the $SO_2$ in the tissue area should be higher than the vein because the tissue area includes both arteries and veins. This result shows that our fundus model does not...
match the actual fundus completely. For example, the fundus is not flat but a curved surface, whereas the white diffuser that was used for the calibration of our method had a flat surface. As mentioned before, to correct illuminance non-uniformity, the fundus images were normalized by the image of the white diffuser placed at the same position as the eye. As it goes away from the center of the image, the light intensity of a flat white diffuser is different than for the curved surface of the fundus. In this sense, the SO₂ in the optic disc was reasonable because it was located around the center of the fundus image.

The human fundus has various pigments, such as melanin and xanthophyll [54]–[56] and the importance of other pigments has been shown for the estimated accuracy of SO₂ [4], [38]. Therefore, this issue should be improved by proposing a fundus model that has these pigments in future work. Additionally, the model parameters of the MCS simulation rely on the assumptions made and are different from actual biological tissue. Therefore, our simulation will require a calibration using a phantom of the eye or the eyeball of an animal in future work.

Furthermore, our system measures not only diffusely reflected light from the fundus but also specularly reflected light from the surface of the eye. Thus, the saturated pixels shown in Fig. 9 indicate the specular reflection. Particularly for the case when the FiO₂ was 14%, the estimated values of artery and vein of sample 2 were affected by the specular reflection. In our future work, we plan to reduce the reflection by aligning two polarization plates in the crossed Nicols configuration. Moreover, we will compare the estimation accuracy of SO₂ between our method and other methods that use multiple wavelengths.

V. CONCLUSION

We proposed an RGB-based oximetry for the retina. First, we constructed a measuring system for a rat fundus and estimated the SO₂ in the rat fundus from the RGB image of the fundus. In addition, we investigated the change in CHBO, CHBR, CHBT, and SO₂ by controlling the FiO₂ and observing the physiological response caused by the defective oxygenation of the blood. Therefore, because these estimated results could be explained by physiological phenomena, the results indicate the possibility of oximetry based on the RGB images of a fundus. However, our method needs to correct for the influence of both the spatial non-uniformity of illumination by the curved surface of the fundus. In future studies, we will construct a multilayer model, of the ocular fundus, containing melanin and yellow coloring matter (xanthophyll) [54]–[56]. Moreover, we will estimate the SO₂ using the multilayer model and investigate its influence on the estimation of SO₂ across a range of tissue chromophores. In addition, we will compare the results from the single-layer and multilayer models.

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HIDEAKI HANEISHI received the M.S. and Ph.D. degrees from the Tokyo Institute of Technology, in 1987 and 1990, respectively. He joined Chiba University as a Research Associate, in 1990. He was a Visiting Research Scientist with the Department of Radiology, University of Arizona, from 1995 to 1996. He has been a Full Professor of the Center for Frontier Medical Engineering (CFME), since 2007. He is also the Director of CFME.