Mie solution of melanin particles and its stepwise multi-photon activation fluorescence

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Last, I would like to thank my parents for their support and encouragement, and thank all professors and friends at Northeastern University.
In this thesis, the author’s work is divided into two aspects: the experimental study of stepwise multi-photon activation fluorescence (SMPAF) of melanin, and a Mie analysis to predict fluorescence activation in melanin particles.

Melanin is a group of natural biological pigments that exist in most organisms with varied potential biological functions. The stepwise multi-photon activation fluorescence of melanin is a melanin specific phenomenon and has potential application to low-cost melanin detection. It can be activated by a pulsed laser or continuous wave (CW) in near infrared spectrum. We show the melanin can generate three-photon fluorescence through a stepwise multi-photon absorption process. However, melanin must be activated by optical, chemical or other methods. We also conducted several experiments to qualitatively and quantitatively analyze the activation process in SMPAF.

The Mie solution is a mathematical calculation for absorption and scattering coefficients of an electromagnetic plane wave by a homogeneous sphere. In this thesis, we do analytic equations and computer simulations are developed for the internal field of homogeneous and uniform spheres with the optical properties of melanin. From the analysis, we verify some experiment results and obtain some characteristics of SMPAF process. The work aims to offer theoretical study for future research on SMPAF of melanin.
Chapter 1

Overview

The author’s work contains two major aspects: experiments on stepwise multi-photon activation of melanin (SMPAF), and Mie scattering analysis of melanin.

Chapter 2 gives an introduction to the Keck multimodal microscope which was used in experiments. It is a powerful tool for researchers to image samples with different modalities. Confocal reflectance microscope (CRM) and Multi-photon fluorescence microscope (MPFM) are two important microscope which are integrated on the Keck.

Chapter 3 discusses a melanin specific process: stepwise multi-photon activation (SMPAF) of melanin. melanin fluorescence can be obtained and enhanced by a stepwise multi-photon excitation process in near infrared radiation but it must be activated first. This is a melanin specific process with potential application to low cost melanin detection.

Chapter 4 discusses the Mie analysis result to melanin particles. We hypothesize that activation threshold depends on volume density of power. Size and shape of melanin affect the power density inside the sphere with given irradiance. As a brief test of hypothesis, we assume the melanin is a uniform sphere. Based on the experimental activation threshold, we calculate the power density threshold for melanin particles. Mie analysis can offer clues in theoretical study and understanding experimental conclusion on melanin SMPAF.
Chapter 2

Multimodal Microscopy

2.1 Introduction to Keck Multimodal Microscopy

Multimodal microscopy is a powerful tool for people to investigate biomedical tissues and it is widely used in biomedical optics. Different imaging modes can provide complementary information about a specimen. At present, the major application of multimodal microscopy involves fluorescence microscopy, either alone or in combination with bright field microscope. This chapter gives a brief introduction to the Keck 3D fusion multimodal microscope (3DFM) which is used by author in the experiment. The author also did routine maintenance, alignment and robustness regulation.

The Keck 3D fusion multimodal microscope in the Optical Science Laboratory (OSL) at Northeastern University is a powerful tool for researcher to image samples with multiple modalities based on different contrast mechanisms on the same stage. However, different hardware, interference and requirements make merging different modalities a challenging task. The 3DFM was first built by Daniel J. Townsend in 2004. It underwent a major upgrade in the year 2013 led by Zhenhua Lai and assisted by Stephen Karasek, James Mclean and Zetong Gu. The control system was redesigned and programmed in Labview. Control platform, performance and resolution of the optical system was highly improved. The modalities on 3DFM include: Bright field Microscopy, Differential Interference Contrast Microscopy, Epifluorescence Microscopy (EFM), Confocal Reflectance Microscopy (CRM), Confocal Fluorescence Microscopy, Multi-Photon Fluorescence Microscopy (MPFM) and Second Harmonic Generation (SHG). In the next two sections, we will discuss the two modes relevant to the author’s work.


2.2 Confocal Reflectance Microscopy (CRM)

Confocal microscopy is an optical imaging technique for increasing optical resolution and contrast of a micrograph. Marvin Minsky patented the confocal microscope in 1957. Compared to conventional wide-field microscope, confocal microscope is able to provide better signal to noise ratio (SNR), higher resolution and better imaging by means of rejection of out of focus light and collecting optical sections from a thick specimen.

The technique to reject out of focus light is achieved by adding a spatial filter. Usually a pinhole in front of the specimen can eliminate most of the light which doesn’t come from the focal plane. Figure 2.1 shows the typical layout of a confocal reflectance microscopy. The laser is collimated by a lens and separated by a beam splitter and focuses to the specimen through the objective. The light (green line) which is reflected from the focal plane, can be received by the detector in the figure. However, the out of focus light, which is denoted by red and blue line in the figure, comes from the plane in front or behind the focal plane. This light is mostly blocked by the aperture.
A confocal reflectance microscope typically uses polarization schemes to separate the illumination and detection paths and maximize the throughput of the system. If the light source is polarized, the beam splitter should be polarized, a quarter-wave plate must be put between the beam splitter and the objective. In Figure 2.1, the input laser is reflected by the beam splitter, the backward light from the specimen goes through the beam splitter and is measured by the detector. Assume the input power of light is $P$ and the power of the detected signal is $P_d$, the transmittance of the beam splitter is $T$ and reflectance is $R$. Ignoring the other loss, we have

$$P_d \propto T \cdot R \cdot P \tag{2.1}$$

Since $T + R <= 1$, that is $T \cdot R <= 0.25$, means the throughput will be smaller than $1/4$ of possible maximum. In contrast, if we assume the input light is P-polarized, that is the electrical field is parallel to the incident plane, it will pass the quarter-wave plate twice and change to S-polarized. The transmittances to P and S polarized light of the polarized beam splitter are $T_p$ and $T_s$, the reflectance of P and S polarized light are $R_p$ and $R_s$. So we have

$$P_d \propto T_p \cdot R_s \cdot P \tag{2.2}$$

Since $T_p + R_p <= 1$ and $T_s + R_s <= 1$, so both $T_p$ and $R_s$ in equation 2.2 can be close to 1. In this way, the application to polarized light as the source of confocal reflectance microscopy optimizes the throughput of the system.

Figure 2.2 shows a group of images of a business card taken by the CRM mode on Keck. The focal planes of those images are in different depth of the sample. A near infrared laser with a 800nm wavelength is used as the light source. We can clearly see the paper fibers in the business card. Bright signal is from the focal plane of that image according to the principle of CRM. The out of focus light is effectively eliminated. This is frequently used to verify sectioning. Different focal planes result to different images in the same mode. So confocal reflectance microscopy is convenient to observe tissues under the skin since it can get optical sections from specimen that is relatively thick.

### 2.3 Multi Photon Fluorescence Microscopy (MPFM)

The other important modality in Keck used by the author is the Multi photon fluorescence microscope (MPFM) which is developed based on the confocal fluorescence microscope. Muti-photon
excitation is a nonlinear optical process first predicted by Maria Goppert-Mayer in 1931. Usually, fluorescence is excited by one photon when a electron in the ground state absorbs a higher energy photon to reach the excited state. After a short decay, the electron emits a photon with lower energy from the excited state and falls back to the ground state. This process is illustrated in Figure 2.3.

The subfigures (a) (b) and (c) of Figure 2.3 are the schematics of one-photon excitation, two-photon excitation and three-photon excitation process. In the common situation, one photon excitation, the emission energy is smaller than absorbed. Emission of photons from atoms in various
excited states leads to an electromagnetic spectrum showing a series of characteristic emission lines. In contrast, excitation by two or more photons occurs when an electron absorbs multiple lower energy photons simultaneously, while one of the photon can’t be absorbed individually because its energy is not enough to reach the excited state. Then it emits a photon with higher energy than any of the input individual photons.

Since the lifetime of virtual states is about $10^{-16}$ s, the probability of multi-photon excitation is very low. Therefore, a femtosecond pulsed laser is used to meet the requirement of high peak photon density.\[1\]. The typical layout of MPFM shown in Figure 2.4.

The layout of the Muti-photon fluorescence microscopy is similar to confocal reflectance microscope, but the light source is replaced by a femtosecond laser. Dichroic beamsplitter, multi layer coating placed on glass by using a vacuum deposition process, is used instead of a polarized beamsplitter. It has the ability to reflect the excitation light and transmit the emitted fluorescence. The efficiency of the transmission or reflectivity can be up to 95%.\[10\]. The signal is filtered by an emission filter before it reaches to the detector. MPFM do not need aperture to reject out of focus light since the fluorescence is only generated at the focal point. Emission filter selects fluorescence with specified spectrum.
2.4 Layout of the Keck microscope and its control system

In Zetong Gu and Zhenhua Lai’s thesis, the detailed optical layout of Keck microscope has been presented.\[1\][2]. The base of the microscope is a Nikon inverted TE2000U microscope shown in the right part of Figure 2.5. The femtosecond pulsed titanium-sapphire laser (Ti:Sapphire Laser) operates within near-infrared spectrum from 700nm to 1000nm, the red beam in Figure 2.5. The maximum average power is 1.5 watts when pumped with 8 watts pump laser from Millenia XsJ (Spectra Physics). A Krypton-Argon Laser (purple beam) is used as the illumination source of fluorescence microscopy. P1 and P2, the two Glan-Thompson polarizers are served as an adjuster to the Ti:Sapphire Laser.

Both the Titanium-sapphire laser and Krypton-Argon Laser are combined at the dichroic beam splitter D1. A small portion of the beams reaches the beamsplitter BS1 and majority of the power reaches the periscope. The Ocean Optics spectrometer monitors the spectrum and power meter displays the immediate power of the beam separated by the beam splitter BS1. Therefore, there is a conversion factor which equals to ratio of the actual power received by the sample and the value on the power meter. If the laser has 800nm wavelength and the objective’s amplification is 20 and its numerical aperture is 0.75, the conversion factor is 4.11. The reflected light (Dark red line) from specimen comes back to BS2. 150um pinhole rejects out of focus light, and light from focal plane is received by the avalanche photodiode (APD), the detector of CRM. Fluorescence (Light blue line) goes to the photomultiplier tube (PMT), the detector of MPM and CFM.
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Figure 2.5: Optical layout of Keck multi-modal microscopy [1][2]
Most of the laser passes through the polarized beam splitter BS2 and reaches the polygon mirror scanner. The 32 facets polygon mirror scanner is driven by a 356.7Hz control signal generated by NI USB-6431, a data acquisition device as well as a programmable signal generator and has a speed of 200 rotations per second. A photoconductive resistance receives light reflected from an LED by each facet of the polygon mirror to provide a synchronization signal at about 6400Hz (initial signal). The initial signal is filtered by a bandpass filter and serves as the trigger (Filtered Initial Signal) for a NI USB-6341 to generate Horizontal synchronization signal (HSync) square wave with a 6400 Hz frequency.

The relation of the signals can be described by Figure 2.6. HSync signal also triggers NI USB-6431 to generate Vertical Synchronization signal (VSync) which synchronizes the acquisition of each image; Pixel Clock Signal (PCLK) which synchronizes the acquisition of each pixel of the image; the sawtooth control signal which controls the galvanometric mirror scanner. The frequency of VSync is 10Hz and PCLK is 7.14MHz. The PCLK signal has been redesigned by Lai [1] which improves the resolution from 640*480 to 800*600. HSync, VSync and PCLK are sent to NI IMAQ PCI-1408, a 4 channel image acquisition module which can converts analog signal to 8-bit gray scale.
CHAPTER 2. MULTIMODAL MICROSCOPY

images. Separate detectors are used for Confocal reflectance Microscopy (CRM), Confocal Fluorescence Microscopy (CFM), Multi-photon Fluorescence Microscopy (MPFM) and Second Harmonic Generation Microscopy (SHG) as the 4 acquisition channels.

Figure 2.7: Image acquisition by HSync, VSync and PCLK signal

A schematic diagram of image acquisition is shown by Figure 2.7. Each red spot stands for a data acquisition sample for one pixel, triggered by the PCLK signal. At every rising edge of HSync, the data acquisition begins with a new line of pixels. The sawtooth signal controls the galvanometer mirror to move up and down as the vertical scanning with a 10 Hz frequency. The scanning of a picture is finished in one period of VSync signal. The Sawtooth control signal with the same frequency as VSync signal, makes the mirror back to the beginning place of scanning. This allows NI IMAQ PCI-1408 to output images at 10 frames per second. However, because of the rotating polygon mirror, this image acquisition method can’t provide precise positioning. So Keck is not able to focus the laser to fixed pixels and target small particles.

2.5 Conclusion

Multimodal microscopy is a powerful tool in biomedical optics. In the study of melanin SMPAF, the Keck multimodal microscope plays a very important role in capturing images with different modalities. Based on those images, a significant characteristic of melanin was found. It is
Confocal reflectance microscopy (CRM) and Multi-photon fluorescence microscopy (MPFM) are the two models used in the author’s work. CRM can photograph the physical structure of sample. It is an optical imaging technique for increasing optical resolution and contrast of a micrograph. By adding a spatial filter, CRM can reject out of focus light and collect optical sections in a thick specimen. Besides, using polarized light as illumination source can increase the throughput of the system. MPFM uses fluorescence generated by sample to create image. It is designed based on the principle of multi-photon excitation. The process comes up when electrons in the ground state absorb multi photons simultaneously to reach the excited state. MPFM has a similar layout to CRM, but spatial filter is not necessary since fluorescence is only generated at the focal plane of specimen. Emission filter helps to select the fluorescence with specific spectrum.

The Keck microscope’s history, optical layout, control system and functions are presents in this chapter. It underwent an important upgrade by Lai and Gu at 2013. More modalities were added and the control system was rewritten by LabVIEW instead of C#. The pixel resolution of laser scanning is increased. CRM and MPFM modalities can be acquired simultaneously for observing the fluorescence of melanin. The stability and flexibility of the system has improved. The system can output images at 10 frames per second. The only disadvantage is that Keck microscope can’t provide precise positioning.
Chapter 3

Stepwise Multi-Photon Activation Fluorescence of Melanin (SMPAF)

3.1 Introduction to Melanin

Melanin is an irregular light-absorbing polymer containing indoles and other intermediate products derived from the oxidation of tyrosine. It is a group of natural biological pigments which exists in most organisms and is produced by melanocytes, a specialized group of cell. For people, it is the characteristic chromophore of human skin with many potential biological functions. Despite its importance, melanin is poorly understood because it is an insoluble polymer without a well-defined structure, which is difficult to isolate and study.

Melanin, which is found in human skin, can be classified in two forms: eumelanin and pheomelanin. Eumelanin is more common in black and brown hair or skin, while pheomelanin is the red and yellow pigments. Except hair and skins, melanin-containing tissues have been located in various parts of the human body, including heart, lungs, liver, brain, lymphocytes and inner ear. The melanin which exists in human brain are considered as another basic type. Its name is neuromelanin and its function is still obscure.

The process of generating melanin which is called melanogenesis happens after exposure to solar radiation and changes skin to visibly tan. Melanin is an effective absorber of light. It protects human skins from harmful ultraviolet radiation and convert 99.9% of the radiation to heat. Due to
CHAPTER 3. STEPWISE MULTI-PHOTON ACTIVATION FLUORESCENCE OF MELANIN (SMPAF)

that property, melanin is thought to have the ability to reduce skin cancer from ultraviolet radiation. In addition to this, melanin has other biological functions including antioxidant defense and camouflage. Melanin is also involved in skin disease such as malignant melanoma, the most aggressive skin cancer with high metastatic potential; vitiligo, a long term skin condition characterized by patches of skin losing their pigment. Therefore, melanin plays a very important role in many potential biological functions and is related to many diseases including deadly skin cancer.

Since eumelanin is the most common type of melanin, melanin in this thesis stands for eumelanin without special declaration. The experimental melanin samples are produced by Sigma-Aldrich Inc.

3.2 Stepwise Multi-Photon Activation Fluorescence (SMPAF)

Melanin is considered a weak emitter with a very low quantum yield of fluorescence. In 2001, Josef Kerimo et al in our lab (OSL) found that melanin fluorescence can be enhanced by stepwise three-photon excitation. In most cases, the muti-photon fluorescence is a simultaneous excitation process and Figure 2.3 describes how it happens. This process requires a femtosecond pulsed laser to satisfy high peak photon density. However, the fluorescence of melanin is activated and enhanced by exposure to near-infrared radiation (NIR) with a three orders magnitude increase. The fluorescence can be activated through a low-cost continuous wave (CW) laser or femtosecond pulsed laser with same average power.

As mentioned in the Figure 2.3 about the simultaneous excitation, the lifetime of the virtual states are quite short. For comparasion, the lifetime of the intermediate states are much longer, usually $10^{-9}$s. That’s the reason why SMPAF can be activated by a low cost continuous wave laser. The relation of the electron density in each state will be discussed in the next chapter. Besides, this phenomenon is melanin specific technique as far as we know, so stepwise multi-photon activation fluorescence can be a new method of melanin detection.

3.3 Activation

Activation is an unique process for melanin SMPAF. To obtain the melanin SMPAF, melanin should be activated first. Kerimo states in his paper that the near infrared irradiation can
cause obvious changes to the melanin and the changing can be seen either by fluorescence and bright field image. My experiment confuse this conclusion since NIR may cause the melanin powder to melt, or other physical changes. Figure 3.1 shows the part of brightfield image of melanin under different intensity of Near infrared radiation. Subfigure (a) presents melanin without NIR, taken at the time \( t = 0 \) s, Subfigure (b) presents melanin under the intensity of activation threshold, in other words, we can observe SMPAF on the screen. It is taken at time \( t = 30 \) s. Subfigure (c) is under high intensity, about 7 times of activation threshold. The measurement of activation threshold will be given later in this section. It is taken at time \( t = 60 \) s and most of the melanin has been activated. We can see the obvious changes to the melanin in the red circle. However, the principle of melanin activation is still unclear, we can’t prove that the activation process can cause obvious physical changes to melanin.

According to my experiment, this activation process is only related to the irradiance of the sample and it is not wavelength dependent, wave type dependent (CW or pulsed laser) or polarization dependent. In other words, the melanin should reach the activation threshold first. Activation threshold varies by the type of the melanin and surrounding medium. It is usually around \( 10^5 \sim 10^6 \) W/cm\(^2\). The irradiance of the melanin is calculated by the power at the sample, \( P_s \), and the image spot area. According to Rayleigh criteria, the minimum distance of two point sources observed in the microscope is determined by the diffraction limit, which is

\[
r = \frac{0.61\lambda}{NA},
\]

(3.1)

where \( \lambda \) is the wavelength of the source, in our experiment \( \lambda = 800 \) nm. NA is the numerical aperture of the objective. The area of the spot can be regarded as a circle so we have

\[
I_{\text{act}} = \frac{P_s}{\pi r^2},
\]

(3.2)

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CHAPTER 3. STEPWISE MULTI-PHOTON ACTIVATION FLUORESCENCE OF MELANIN (SMPAF)

Figure 3.2: CRM(a) and MPFM(b) images under $2.05 \cdot 10^5 W/cm^2$ irradiance before activation

where $I_{act}$ is activation threshold irradiance. To obtain $P_s$, the equation is:

$$P_s = f \cdot P_m, \quad (3.3)$$

$f$ is a coefficient and measured by power meter at sample and power meter at the place shown in Figure 2.5. The activation threshold of the Synthetic Melanin is about $2.19 \pm 0.1 \cdot 10^5 W/cm^2$.  

After the activation, SMPAF can be detected below this threshold. Experiment results demonstrate a 10% $\sim$ 15% deduction of the power compared to the melanin activation threshold. That means we can see the fluorescence by exposing melanin under $1.86 \sim 1.97 \cdot 10^5 W/cm^2$ radiation. Note that this process is irreversible since activation causes changes of the melanin samples. The changes can be physical and chemical. To protect samples from permanent damage, the common activation time is less than 30 seconds and could be smaller if a laser with larger power is in use. Figure 3.2 shows the CRM and MPFM images under $2.05 \cdot 10^5 W/cm^2$ irradiance, below the activation threshold. The images were taken before the melanin was activated. So there is no fluorescence in the MPFM image.

From Figure 3.2 we can see there is no SMPAF below the activation threshold for non-activated melanin. However, we can see clearly SMPAF at even lower irradiance for a activated melanin sample. Figure 3.3 shows the CRM and MPFM images for activated melanin under $1.91 \cdot 10^5 W/cm^2$ irradiance.

The melanin has been exposed under $1.52 \cdot 10^6 W/cm^2$ irradiance for 30 seconds. The
CHAPTER 3. STEPWISE MULTI-PHOTON ACTIVATION FLUORESCENCE OF MELANIN (SMPAF)

Figure 3.3: CRM(a) and MPFM(b) images under $1.91 \cdot 10^5 W/cm^2$ irradiance after activation. The CRM image is darker than Figure 3.2 since the input laser power is decreased. However, we can see some SMPAF in MPFM image. If we increase the laser power, we can observe more SMPAF as shown in Figure 3.4.

Figure 3.4: CRM(a) and MPFM(b) images under $3.12 \cdot 10^5 W/cm^2$ irradiance after activation. Images are taken under $1.91 \cdot 10^5 W/cm^2$. The CRM image is darker than Figure 3.2 since the input laser power is decreased. However, we can see some SMPAF in MPFM image. If we increase the laser power, we can observe more SMPAF as shown in Figure 3.4.

By increasing the input laser, the CRM image becomes brighter and we can observe more and stronger SMPAF through MPFM image. Figure 3.5 demonstrates the strongest SMPAF our lab can reach.

Figure 3.5 are the CRM and MPFM images of a sample (different from the sample above) exposes in $1.95 \cdot 10^6 W/cm^2$ radiation. The sample will undergo SMPAF and soon it is affected by photo bleaching. SMPAF signal will decrease to a relative lower level. Figure 3.6 shows MPFM
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Figure 3.5: CRM(a) and MPFM(b) images with $1.95 \cdot 10^6 W/cm^2$ irradiance images of melanin under $1.95 \cdot 10^6 W/cm^2$ at different times, from $t = 0s$ to $t = 60s$. We can clearly see that the signal intensity decreases quickly in the first 30 seconds and then maintains at a lower level. Those images are gray scale with pixel values varying from 0, totally black to 255, totally white, so the average greyscale value are proportionally to the signal intensity. By calculating the average grey-scale value of each image, Figure 3.7 presents the SMPAF signal intensity as a function of time.

The Blue curve in Figure 3.7 is plotted with the original data. The red curves are function fitted curves. We need to discard the saturated picture and choose a part of image with fluorescence to do the average. The fitted curve can be expressed as:

$$y = 123 e^{-0.04231x} + 27, \quad (3.4)$$

It has a good fit with an exponential decay curve. So the photo bleaching decreases the SMPAF intensity exponentially with time. We can see the curve accords with our observation. The curve decreases rapidly from 0s to 30s. After 60s, it nearly becomes flat. The experiment is repeated 2 days later by using the same sample, we can still see the fluorescence keeps at a low level. That means activation process is irreversible and photo bleaching causes damage to melanin samples by exposing them to high radiation.

In the experiment, we can’t see that all the melanin particles in the field of view (FOV) have stepwise multi photon activation fluorescence when the input radiation is not very strong. More SMPAF of melanin can be obtain through increasing input irradiance. Most of the melanin has
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Figure 3.6: MPFM images of melanin with $1.95 \cdot 10^6 \text{W/cm}^2$ in one minute.

Figure 3.7: SMPAF signal intensity as a function of time with input irradiance $1.95 \cdot 10^6 \text{W/cm}^2$. 

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Figure 3.8: SMPAF signal intensity as a function of irradiance

SMPAF in high irradiance. This may due to the different radius and other factors. The irradiance of activation threshold is the macroscopic behavior of melanin SMPAF. We hypothesize that the activation is determined by power density inside the melanin. We present a simulation in Chapter 4. Hopefully, it will help to reveal the cause of activation of melanin for future research, though intrinsic reason for melanin activation is still unknown and melanin is still poorly understood.

3.4 SMPAF signal intensity as a function of irradiance

Figure 3.8 shows the SMPAF signal intensity of a non-activated melanin sample SMPAF as a function of input irradiance. Input laser is a 800 nm continuous wave. The activation happens at $2.19 \cdot 10^5 \text{W/m}^2$. The red curve in the picture shows the fitted polynomial equation (except for non-activated points):

$$P_f = 0.035(\frac{P_i}{1.012})^3,$$

where $P_f$ is the intensity of fluorescence and $P_i$ is the irradiance of incident laser. This shows that the SMPAF is three-photon process since the signal intensity fits well with cubic input fluorescence.
3.5 Conclusion

Melanin is a group of natural biological pigments which exists in most organisms. For people, it is the characteristic chromophore of human skin with various potential biological functions. The fluorescence of melanin can be activated and enhanced by a process called stepwise multi-photon activation fluorescence (SMPAF). Multi-photon exciations require femtosecond pulsed laser to absorb photons simultaneously. However, since SMPAF is a stepwise process, the fluorescence of melanin can be activated though either a pulsed laser or a low cost, continuous wave in near infrared radiation. This phenomenon is a melanin specific technique as far as we know, so SMPAF has potential application to low-cost melanin detection in vivo cells.

Melanin must be activated first to obtain melanin SMPAF. The activation threshold of synthetic melanin is about \(2.19 \times 10^5 \text{W/cm}^2\). After activation, SMPAF can be detected below the threshold. Activation is an irreversible process and makes chemical changes to melanin. SMPAF signal intensity increases with the laser irradiance. Meanwhile, photo bleaching effect can reduce the SMPAF signal intensity to a relatively lower level. The fitted curve shows that it is a three-photon absorption process.
Chapter 4

Mie scattering analysis of Melanin

This section is a description of the Mie scattering and Mie absorption algorithm and its application to simulating the activation process of Melanin particles. The Mie solution is a mathematical calculation for absorption and scattering coefficients of an electromagnetic plane wave by a homogeneous sphere. Derivation of Mie scattering was given by Bohren and Huffman in 1983[18]. Christian Matzler also gives Mie scattering Matlab code for the scattering functions and scattering field(electrical field outside the sphere) in his research report.[19][20]. In this section, analytic equations and computer simulated pictures are developed for the internal field of homogeneous and uniform spheres with the optical properties of melanin.

4.1 Mie scattering algorithm

4.1.1 Scattering functions

We are interested in the field at any point inside a sphere which is illuminated by a polarized monochromatic wave. The electrical and magnetic field pair of the incident wave can be denoted as $(E_i, H_i)$, where

\[ E_i = E_0 \exp(i \mathbf{k} \cdot \mathbf{x} - i \omega t), \quad H_i = H_0 \exp(i \mathbf{k} \cdot \mathbf{x} - i \omega t), \quad (4.1) \]

$E_0$ and $H_0$ are the amplitudes of electrical and magnetic field of the incident wave. Spherical polar coordinates are used in the calculation. Incident laser light is along the Z-axis direction as shown in Figure 4.1.
The key functions in Mie scattering algorithm are the Mie functions $a_n$, $b_n$ for the scattered field and $c_n$, $d_n$ for the internal field,

\[
\begin{align*}
    a_n &= \frac{\mu m^2 j_n(mx) [x j_n(x)]' - \mu_1 j_n(x) [mx j_n(mx)]'}{\mu m^2 j_n(mx) [x h_1^{(1)}(x)]' - \mu_1 h_1^{(1)}(x) [mx j_n(mx)]'}, \\
    b_n &= \frac{\mu_1 j_n(mx) [x j_n(x)]' - \mu j_n(x) [mx j_n(mx)]'}{\mu_1 j_n(mx) [x h_1^{(1)}(x)]' - \mu h_1^{(1)}(x) [mx j_n(mx)]'}, \\
    c_n &= \frac{\mu_1 j_n(x) [x h_1^{(1)}(x)]' - \mu_1 h_1^{(1)}(x) [x j_n(x)]'}{\mu_1 j_n(mx) [x h_1^{(1)}(x)]' - \mu h_1^{(1)}(x) [mx j_n(mx)]'}, \\
    d_n &= \frac{\mu_1 m^2 j_n(mx) [x h_1^{(1)}(x)]' - \mu_1 m h_1^{(1)}(x) [x j_n(x)]'}{\mu m^2 j_n(mx) [x h_1^{(1)}(x)]' - \mu_1 m h_1^{(1)}(x) [mx j_n(mx)]'}.
\end{align*}
\]

These functions are given in the book *Absorption and Scattering of Light by Small Particles* in Chapter 4, *Absorption and Scattering by a Sphere*. $\mu$ and $\mu_1$ are the permeabilities of the surrounding medium and sphere. $m$ is the relative refractive index of the sphere, given by

\[
m = \frac{k_1}{k} = \frac{N_1}{N},
\]

where $N_1$ is the refractive index of the sphere and $N$ is the refractive index of the surrounding medium. $x$ is denoted as the size parameter, defined by

\[
x = ka = \frac{2\pi Na}{\lambda},
\]
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where \( a \) is the radius of the sphere and \( k \) is the wave number. The relation of \( h_n^{(1)}(z) \) and \( j_n(z) \) is given by

\[
h_n^{(1)}(z) = j_n(z) + iy_n(z). \tag{4.5}
\]

\( j_n \) and \( y_n \) are spherical Bessel functions of order \( n \) (\( n=1,2,3... \)), which satisfy the recurrence relations:

\[
f_{n-1}(z) + f_{n+1}(z) = \frac{2n+1}{z} f_n(z), \tag{4.6}
\]

where \( f_n \) is either \( j_n \) or \( y_n \). The first two orders of \( j_n \) and \( y_n \) are:

\[
\begin{align*}
j_0(z) &= \frac{\sin z}{z}, & j_1(z) &= \frac{\sin z}{z^2} - \frac{\cos z}{z}, \\
y_0(z) &= -\frac{\cos z}{z}, & y_1(z) &= -\frac{\cos z}{z^2} - \frac{\sin z}{z}. \tag{4.7}
\end{align*}
\]

4.1.2 Electric and magnetic field inside the sphere

Assume the electrical and magnetic field inside the sphere are \((E_1, H_1)\), the expansion of the internal field is given in equation \[4.8\]

\[
\begin{align*}
E_1 &= \sum_{n=1}^{\infty} i^n E_0 \frac{2n+1}{n(n+1)} (c_n M_{o1n}^{(1)} - id_n N_{e1n}^{(1)}) \\
H_1 &= -\frac{k_1}{\omega \mu_1} \sum_{n=1}^{\infty} i^n E_0 \frac{2n+1}{n(n+1)} (d_n M_{o1n}^{(1)} - ic_n N_{e1n}^{(1)}). \tag{4.8}
\end{align*}
\]

The expansions are infinite series, the calculation can be stopped after enough accumulation \( n_{\text{max}} \), equals to the closest integer of

\[
x + 4x^{1/3} + 2. \tag{4.9}
\]
The vector wave function $M$ and $N$ are given in the spherical coordinates $(r, \theta, \phi)$ by

$$M_{o1n} = \begin{pmatrix} 0 \\ \cos \phi \pi_n(\cos \theta) j_n(rmx) \\ -\sin \phi \tau_n(\cos \theta) j_n(rmx) \end{pmatrix},$$

$$M_{e1n} = \begin{pmatrix} 0 \\ -\sin \phi \pi_n(\cos \theta) j_n(rmx) \\ -\cos \phi \tau_n(\cos \theta) j_n(rmx) \end{pmatrix},$$

$$N_{o1n} = \begin{pmatrix} \sin \phi \pi_n(n+1) \sin \theta \pi_n(\cos \theta) j_n(rmx) \\ \sin \phi \tau_n(\cos \theta) \frac{rnxj_n(rmx)}{rnx} \\ \cos \phi \pi_n(\cos \theta) \frac{rnxj_n(rmx)}{rnx} \end{pmatrix},$$

$$N_{e1n} = \begin{pmatrix} \cos \phi \pi_n(n+1) \sin \theta \pi_n(\cos \theta) j_n(rmx) \\ \cos \phi \tau_n(\cos \theta) \frac{rnxj_n(rmx)}{rnx} \\ -\sin \phi \pi_n(\cos \theta) \frac{rnxj_n(rmx)}{rnx} \end{pmatrix}. \tag{4.10}$$

The function $\pi_n(\cos \theta)$ and $\tau_n(\cos \theta)$ follow the recurrence relations below:

$$\pi_n = \frac{2n-1}{n-1} \cos \theta \pi_{n-1} - \frac{n}{n-1} \pi_{n-2},$$

$$\tau_n = n \cos \theta \pi_n - (n+1) \pi_{n-1}, \tag{4.11}$$

beginning with $\pi_0 = 0, \pi_1 = 1$.  

4.1.3 Absorption of electromagnetic energy

The Poynting vector, denoted as $S$, defines the rate of energy transfer per unit area in $W/m^2$. Consider an electromagnetic field $(E, H)$,

$$S = E \times H. \tag{4.12}$$

In this chapter, the incident wave is a harmonic field and the time average is

$$\langle S \rangle = \frac{1}{2} Re \{ E_c \times H_c^* \}. \tag{4.13}$$

For the homogeneous wave in its propagating direction,

$$S = \frac{1}{2} Re \left\{ \sqrt{\frac{\epsilon}{\mu}} E_0^2 \epsilon \exp \left( -\frac{4\pi k z}{\lambda} \right) \hat{e} \right\}. \tag{4.14}$$
The magnitude of $\mathbf{S}$ is called irradiance and denote by the symbol $I$, its dimensions are energy per unit area and time ($W/m^2$). As the wave passes the medium, the irradiance is exponentially attenuated:

$$I = I_0 e^{-\alpha z},$$

(4.15)

where the absorption coefficient $\alpha = 4\pi k/\lambda$. The optical power absorbed per unit volume, denoted as $P_v$, from a monochromatic source at angular frequency $\omega$ can be calculated from the divergence of the Poynting vector.

$$P_v = \frac{1}{2} Re \{ \nabla \cdot \mathbf{S} \} = \frac{1}{2} \omega |\mathbf{E}|^2 Im \{ \epsilon \}.$$

(4.16)

Since $\mathbf{E}$ has a sinusoidal shape, we have

$$\langle |\mathbf{E}|^2 \rangle = \frac{1}{2\pi} \int_0^{2\pi} |E_0 \sin(\alpha)|^2 d\alpha = \frac{1}{2} E_0^2,$$

(4.17)

the average absorbed power is

$$\langle P_v \rangle = \frac{1}{4} \omega |E_0|^2 Im \{ \epsilon \}.$$

(4.18)

### 4.2 Calculation result

#### 4.2.1 Simulation model and assumptions

The computer simulation of the internal field is based on the assumption that melanin particles are uniform and homogeneous spheres with all optical properties of bulk melanin. Since melanin can be activated through a step-wise three photon activation process, we assume that there exists an average power density activation threshold for melanin denoted as $\langle P_{act} \rangle$, if $P_v > \langle P_{act} \rangle$ for a small volume $\Delta V$, melanin at $\Delta V$ will be considered as being activated, has SMPAF and absorbs the photons and emits fluorescence. For a certain input irradiance, denoted as $I_0$, if the maximum of the power density distribution inside the sphere, $\text{max}(P_v)$, is smaller than the threshold, that is $\text{max}(P_v) < \langle P_{act} \rangle$, we will say that this melanin is not activated. If $\text{max}(P_v) > \langle P_{act} \rangle$, we will say that the melanin begins to activate.

The optical attributes used in the calculation are


\[ \lambda = 800 \text{nm}, \]
\[ m = 1.687 + 0.0018i, \]
\[ \mu = \mu_0, \]
\[ n_{\text{air}} = 1, \]
\[ r_0 = 50 \text{nm} \quad \text{or} \quad 100 \text{nm}, \]

where \( m \) is the refractive index of melanin, \( \lambda \) is the wavelength of the incident laser, \( \mu \) is the permeability of melanin, equal to \( \mu_0 \), the permeability of vacuum, because the wavelength of near-infrared light is too small to change the magnetism of melanin, and \( n_{\text{air}} \) is the refractive index of air. The radius of melanin particle, \( r_0 \), we choose 50nm and 100nm, based on a statistical result of synthetic melanin particles on a paper by Alexey in 2000.[21]

### 4.2.2 Theoretical power of fluorescence signal

The energy diagram of step-wise three photon absorption are shown in Figure 4.2.

![Figure 4.2: State diagram of three photons stepwise excitation](image)

In the energy diagram \( N_0,N_1,N_2,N_3 \) are the electron densities in the ground state 0, excited states 1, 2 and 3. \( \beta_1, \beta_2 \) and \( \beta_3 \) are the transition rates for electrons from lower states 0,1,2 to the
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higher states 1,2,3 respectively. \( \gamma_1, \gamma_2 \) and \( \gamma_3 \) are the transition rates for electrons from excited states 1, 2 and 3, to the ground state 0. We ignore the decay at the excited state 3 in the calculation.

Since Boltzmann distribution predicts that electrons have very small probability to go up to a higher state in equilibrium, we can assume:

\[
N_0 \gg N_1 \gg N_2 \gg N_3, \quad (4.20)
\]

We can express the density variation as a set of rate equation:

\[
\begin{align*}
\frac{dN_1}{dt} &= \beta_1 N_0 - \gamma_1 N_1 - \beta_1 N_1 \\
\frac{dN_2}{dt} &= \beta_2 N_1 - \gamma_2 N_2 - \beta_2 N_2 \\
\frac{dN_3}{dt} &= \beta_3 N_2 - \gamma_3 N_3. \\
\end{align*}
\]

(4.21)

In steady state, the system reaches the equilibrium and we have

\[
\begin{align*}
\frac{dN_1}{dt} = \frac{dN_2}{dt} = \frac{dN_3}{dt} = 0. \\
\end{align*}
\]

(4.22)

Thus

\[
\begin{align*}
\frac{N_1}{N_0 - N_1} &= \frac{\gamma_1}{\beta_1} \\
\frac{N_2}{N_1 - N_2} &= \frac{\gamma_2}{\beta_2} \\
\frac{N_3}{N_2} &= \frac{\gamma_3}{\beta_3}. \\
\end{align*}
\]

(4.23)

With the assumption in Equation [4.20] Equation [4.23] can be written as:

\[
\begin{align*}
\frac{N_1}{N_0} &= \frac{\gamma_1}{\beta_1} \\
\frac{N_2}{N_1} &= \frac{\gamma_2}{\beta_2} \\
\frac{N_3}{N_2} &= \frac{\gamma_3}{\beta_3}. \\
\end{align*}
\]

(4.24)

so the ratio of electron density of state \( N_3 \) and \( N_0 \) is

\[
\frac{N_3}{N_0} = \frac{\beta_1 \beta_2 \beta_3}{\gamma_1 \gamma_2 \gamma_3}. \\
\]

(4.25)

We can treat the probabilities that an electron goes up to an adjacent higher level in any state are same, denoted as \( \beta \), thus

\[
\beta_1 = \beta_2 = \beta_3 = \beta. \\
\]

(4.26)
So Equation \(4.25\) can be rewritten
\[
\frac{N_3}{N_0} \propto \beta^3
\]  
(4.27)

Since the magnitude of the fluorescence signal, denoted as \(P_f\), depends on the number of the electrons in state 3 for a three photon absorption, \(P_f \propto N_3\). In quantum mechanics, the transition rate \(\beta\) is proportional to the power density absorbed, \(\beta \propto P_v\). Hence,
\[
P_f \propto P_v^3.
\]  
(4.28)

Since power density \(P_v \propto E_0^2\), irradiance \(I \propto E_0^2\), thus:
\[
P_f \propto I^3.
\]  
(4.29)

However, fluorescence occurs in the activated volume, for multi-photon excited fluorescence,
\[
P_f \propto \int P_v^N dV_{act},
\]  
(4.30)

where \(N\) is the number of photons absorbed by one electron and \(V_{act}\) is the activated volume of melanin. For three-photon absorption, \(N = 3\).

To calculate power density threshold \(\langle P_{act}\rangle\), since
\[
m^2 = \frac{\epsilon \mu}{\epsilon_0 \mu_0}
\]  
(4.31)

and
\[
\mu = \mu_0,
\]
so we get
\[
I m(\epsilon) = \epsilon_0 (2 Re(m) Im(m)).
\]  
(4.32)

Substituting into Equation \(4.18\) we get
\[
\langle P_{act}\rangle = \frac{1}{2} \omega \epsilon_0 Re(m) Im(m) E_{0act}^2,
\]  
(4.34)

where \(E_{0act}\) is electric field at \(\delta V\) inside the sphere if the power density in \(\delta V\) reaches the power density activation threshold.

For incident irradiance \(I\), according to Equation \(4.14\) and Equation \(4.15\)
\[
I = \frac{1}{2} Re \left\{ \sqrt{\frac{\epsilon}{\mu}} \right\} E_0^2.
\]  
(4.35)

In the air we have \(\epsilon = \epsilon_0, \mu = \mu_0\), so
\[
I = \frac{1}{2} \sqrt{\frac{\epsilon_0}{\mu_0}} E_0^2.
\]  
(4.36)
4.2.3 Result and analysis

Figure 4.3 shows the power density $P_v$ distribution along the $z$ axis, while $x = y = 0$. Subfigure (a) is plotted for 50nm radius particle and the (b) is for 100nm. The incident laser has unit amplitude of electrical field in the calculation, in other words, $E_0 = 1 \text{V/m}$, mentioned in the above section. From Equation 4.36 $I_i = 1.327 \cdot 10^{-3} \text{W/m}^2$.

We can see that the power density is concentrated on the right of the center. The minimum power density appears at $z = -r_0$. The power density increases by 6.4% for 50nm radius particle. However, the maximum power density is about 50% higher than subfigure (a) if the radius of the particle changes to 100nm. Besides, the peak of subfigure (b) moves to the position $z = 31\text{nm}$, away from the center ($z=0$).
Similarly, Figure 4.4 shows the power density $P_v$ distribution along $x$ axis, while $y = z = 0$. The peak appears at the center for both the two situation. The power density increases by 4.4% from its minimum to maximum value for 50nm radius particle. In contrast, the power density increases by 20% for 100nm radius particle. The minimum and maximum power density are also larger if $r = 100\text{nm}$.

Figure 4.5: Power density distribution along $y$ axis, (a)$r=50\text{nm}$, (b)$r=100\text{nm}$
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Figure 4.5 shows the power density $P_v$ distribution along $y$ axis, while $x = z = 0$. The distribution curve looks similar to figure 4.4. The power density increases by 9.1% in subfigure (a). It increases by 46.4% in subfigure (b). The maximum values appear at the center and they are same in both Figure 4.4 and Figure 4.5. However, for same radius in both pictures, the minimum value decreases from $13.52\, W/m^2$ in Figure 4.4 to $12.95\, W/m^2$ in Figure 4.5. This is due to the direction of the polarization of the laser.

![Figure 4.6](image1.png)  
**Figure 4.6**: power density gray-scale in the xy plane, a) r=50nm, b) r=100nm

![Figure 4.7](image2.png)  
**Figure 4.7**: power density gray-scale in xz plane, a) r=50, b) r=100nm

Figure 4.6 and 4.7 are visualizations of the power density gray-scale distribution in the x-y plane (z=0) and x-z plane (y=0). Figure (a) is for 50nm radius melanin particle and figure (b) is for 100nm radius melanin particle. The brighter places represents higher power density. In Figure 4.6, we can see the maximum power density appears at the center and decays along the axis from the center to the both ends, which matches the result in figure above. Note that that due to the direction
Figure 4.8: Maximum power density as a function of radius (a) original data (b) fitted curve

of polarization, the power density at \( y = -r_0 \) or \( y = r_0 \) is smaller than the density at \( x = -r_0 \) or \( x = r_0 \) for both the subfigures a and b. The color bar of subfigure b has a range from 14 to 20 while the range of color bar of subfigure a changes from 13 to 14. The variation between minimum and maximum power density increases from 50nm radius to 100nm radius.

Figure 4.8 demonstrates the maximum power density of the melanin particle as a function of its radius (blue line). The input irradiance is \( I_i = I_{act} = 2.19 \cdot 10^5 \text{W/cm}^2 \). The range of radius is from 30nm to 150nm, which is the radius of common melanin particles. Subfigure (b) compares the curve with a fitted quadratic equation (red line). From the curve, the maximum power density inside the sphere increases with the radius since larger particle can absorb more energy within the same input irradiance. The equation of red curve is:

\[
y = 2462x^2 - 1.83 \cdot 10^5 x + 2.52 \cdot 10^7,
\]

We can see that the \( \max(P_v) \) increases approximately quadratically with the radius. So we can have the conclusion that in the melanin radius scale, the bigger particle requires lower irradiance to activate. Experimental results have shown the irradiance activation threshold is \( I_{act} = 2.19 \cdot 10^5 \text{W/cm}^2 \). If we consider the radius as the only fact that affects the activation and the melanin’s radius is from 30nm to 150nm, we can infer that 150nm radius particles begin to activate if \( I_i = I_{act} \). According to our assumption, the \( \max(P_v) \) at 150nm radius particle reaches the power density threshold \( \langle P_{act} \rangle \), which is \( \langle P_{act} \rangle = 5.44 \cdot 10^7 \text{W/cm}^3 \).
Figure 4.9 presents activated areas for the 150nm melanin particle with different irradiance. Green represents the activated area. Figure (1), (2), (3), (4) demonstrate the activated area in the x-z plane (y=0), and figure (5), (6), (7), (8) demonstrates the activated area in the x-y plane (z=0). The input irradiance are \( I_i = I_{act} = 2.19 \cdot 10^5 \text{W/cm}^2 \), \( I_i = 1.2I_{act} = 2.63 \cdot 10^5 \text{W/cm}^2 \), \( I_i = 1.4I_{act} = 3.06 \cdot 10^5 \text{W/cm}^2 \), \( I_i = 1.6I_{act} = 3.5 \cdot 10^5 \text{W/cm}^2 \), from left to right. At activation threshold, the volume \( \Delta V \) with power density \( \text{max}(P_v) \) is activated. It appears at xz plane, the green area at subfigure (1). But there is no activation area at xy plane. The activated area increases with the input irradiance. 150nm particle starts to activate at activation threshold \( I_{act} \).

Figure 4.10 presents activated areas for the 50nm melanin particle with different irradiance. Green represents the activated area. Figure (1), (2), (3), (4) demonstrate the activated area in the x-z plane (y=0), and figure (5), (6), (7), (8) demonstrates the activated area in the x-y plane (z=0). The input irradiance are \( I_i = I_{act} = 2.19 \cdot 10^5 \text{W/cm}^2 \), \( I_i = 2.35I_{act} = 5.15 \cdot 10^5 \text{W/cm}^2 \), \( I_i = 2.4I_{act} = 5.26 \cdot 10^5 \text{W/cm}^2 \), \( I_i = 2.45I_{act} = 5.37 \cdot 10^5 \text{W/cm}^2 \), from left to right. We can clearly see that in both xy plane or xz plane, the activated area increases with the input irradiance.
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x-z plane (y=0)

(1) $I_i = I_{act}$  
(2) $I_i = 2.35I_{act}$  
(3) $I_i = 2.4I_{act}$  
(4) $I_i = 2.45I_{act}$

x-y plane (z=0)

(5) $I_i = I_{act}$  
(6) $I_i = 2.35I_{act}$  
(7) $I_i = 2.4I_{act}$  
(8) $I_i = 2.45I_{act}$

Figure 4.10: Visualized 50nm melanin activated part in x-y (z=0) and x-z (y=0) plane in different irradiance

No activated area if $I = I_{act}$. Compared to 150nm radius, we need twice more irradiance to activate the melanin.

Figure 4.11 demonstrates the activated area for 100nm radius particles with different irradiance. Figure (1), (2), (3), (4) demonstrate the activated area in the x-z plane (y=0), and figure (5), (6), (7), (8) demonstrates the activated area in the x-y plane (z=0). The input irradiance are $I_i = I_{act} = 2.19 \cdot 10^5 W/cm^2$, $I_i = 1.6I_{act} = 3.5 \cdot 10^5 W/cm^2$, $I_i = 1.8I_{act} = 3.94 \cdot 10^5 W/cm^2$, $I_i = 2I_{act} = 4.38 \cdot 10^5 W/cm^2$, from left to right. Similar to Figure 4.10, activated area increases with higher input irradiance. No activated area if $I_i = I_{act}$. To make 100nm radius particle begin to activate, about 1.6 times irradiance threshold is necessary, which is less than the number in 50nm radius.

Figure 4.12 shows simulation curves describing the power of fluorescence signal as a function of the input irradiance. Figure (a) uses actual irradiance value. Figure (b) uses ratio of $I_i$ and $I_{act}$ as variable. The red curve in the picture represents the fluorescence signal before melanin is
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**x-z plane (y=0)**

1. \[ I_i = I_{act} \]
2. \[ I_i = 1.6I_{act} \]
3. \[ I_i = 1.8I_{act} \]
4. \[ I_i = 2I_{act} \]

**x-y plane (z=0)**

5. \[ I_i = I_{act} \]
6. \[ I_i = 1.6I_{act} \]
7. \[ I_i = 1.8I_{act} \]
8. \[ I_i = 2I_{act} \]

Figure 4.11: Visualized 100nm melanin activated part in x-z (y=0) and x-y (z=0) plane in different irradiance

![Visualized 100nm melanin activated part in x-z (y=0) and x-y (z=0) plane in different irradiance](image)

Figure 4.12: Simulated fluorescence signal as a function of the irradiance of input laser (50nm radius particle)

![Simulated fluorescence signal as a function of the irradiance of input laser](image)

activated. The blue curve is the situation that the melanin has been activated by \( 1.32 \cdot 10^6 W/cm^2 \). So there is no activation threshold for all the activated area. The dotted line represents the experimental activation threshold. For 50nm melanin particle, the activation begins after about \( I > 2.33I_{act} = 5.1 \cdot 10^5 W/cm^2 \). For activated melanin, we can assume all activated area has SMPAF. The blue
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curve has a cubic shape,

\[ y = 1.161 \cdot 10^{-18}x^3, \tag{4.38} \]

activated melanin has larger fluorescence intensity than non-activated melanin in same irradiance. They converge to each other at \( I = 1.32 \cdot 10^6 W/cm^2 \) and overlap after that.

4.3 Conclusion

In this chapter, we use Mie algorithm as well as experimental results to simulate the internal power density distribution of melanin particles. Melanin has no well-defined structure and it is completely difficult to do simulation and study. We have to simplify our model with some assumptions. Our model and analysis are based on the assumptions: Melanin particles are uniform and homogeneous spheres with all optical properties of bulk melanin; there exists a power density activation threshold \( \langle P_{act} \rangle \) to determine where and how much volume inside the sphere is activated. Besides, we limit the radius of melanin particles from 30nm to 150nm.

First we calculate the internal power density distribution for both 50nm and 100nm radius particle. Result shows that in the same irradiance, the \( \max(Pv) \) in 100nm radius particle is larger than the value in 50nm particle. The differences between \( \min(Pv) \) and \( \max(Pv) \) are increased from 50nm particle to 100nm radius particle. Then we get the \( \max(Pv) \) as a function of radius of particles from 30nm to 150nm in \( I_i = I_{act} \) irradiance. Result indicates that the \( \max(Pv) \) in 150nm radius particle is the largest among all different sizes.

The Mie solution is a mathematical calculation for absorption and scattering coefficients of an electromagnetic plane wave by a homogeneous sphere. Mie scattering can’t get the power density activation threshold since it is a special characteristic to melanin. However, we can have a rough estimation for \( P_{act} \) if we think 150nm radius particle is the first activated melanin. Combined with experimental irradiance threshold \( I_{act} = 2.19 \cdot 10^5 W/cm^2 \), we estimates \( P_{act} \) is \( 5.44 \cdot 10^5 W/cm^3 \). Then we show a branch of visualized pictures of activated area within different input irradiance. Compared to 150nm particle, 50nm and 100nm particles need more energy to begin to activate.

At last, the intensity of fluorescence signal is calculated as a function of input irradiance for 50nm radius particle. It begins to activate at about \( I = 2.33I_{act} = 5.1 \cdot 10^5 W/cm^2 \). Activated
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melanin have higher power density in same irradiance. The intensity of power has a cubic relation with the input irradiance. Two curves overlap at the end. In real case, melanin can be aggregated to a particle, the radius can be larger than 150nm. The melanin at the surface of the sphere has similar field distribution inside to our discussion. And the melanin inside receives less laser and hard to activate. The model is more complex and Finite-difference time-domain (FDTD) is a good method to simulate. Our model offers a simulation of activation process and explains that larger particle is easier to activate.
Chapter 5

Conclusion

The Keck multi modal microscope is a powerful tool for biomedical research, especially the experimental study of melanin SMPAF. Melanin plays a very important roles in organisms with varies potential biological functions. Different from multi-photon excitation, fluorescence of melanin can be generated by a stepwise process which is called SMPAF. Activation is a unique, irreversible and prerequisite process to obtain melanin SMPAF. Melanin can be activated through a pulsed laser or continuous wave in a near infrared wavelength. The activation threshold has been measured. Different CRM and MPFM images of melanin are shown in the thesis. The thesis also provides research on photon absorption number of SMPAF, photo bleaching effect on melanin and SMPAF intensity as a function of input irradiance. Since SMPAF is a melanin specific process, it has potential application to low-cost melanin detection in vivo cells.

The Mie algorithm as well as experimental results are used to simulate the power density distribution inside melanin particles. Power density distribution analysis inside the sphere is done on both 50nm and 100nm particles. Result indicates that the larger particles have higher maximum power density inside the sphere if the input irradiance is same. Maximum power density on 150nm particle is used to calculate the power density activation threshold. We also show images on visualization of activated area within different input irradiance. At last, the intensity of the fluorescence signal is calculated as a function of input irradiance for 50nm radius particle. Mie analysis can offer clues in theoretical study and understanding experimental conclusion on melanin SMPAF.
Bibliography


BIBLIOGRAPHY


