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The Dual-Mode Imaging of Nanogold-Labeled Cells by Photoacoustic Microscopy and Fluorescence Optical Microscopy

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Abstract

Photoacoustic microscopy is dominantly sensitive to the endogenous optical absorption, while a fluorescence optical microscopy can detect the fluorescence emission to obtain the image of a sample. To some extent, the physical processes of the 2 methods are opposite, one is absorption and another is emission, but both can be used to image cells. In this article, a simultaneous dual-mode imaging system of photoacoustic microscopy and fluorescence optical microscopy is set up to image tobacco cells. Furthermore, gold nanoparticles, which have a large absorption coefficient and enough fluorescence emission with wavelength of 512 nm, are used to label certain drugs and added to the tobacco cells. Then based on the simultaneous dual-mode microscopy imaging system, the photoacoustic microscopy and fluorescence optical microscopy images of gold nanoparticle-labeled tobacco cells are obtained. The final purpose of this experimental research is to detect if the labeled drugs can enter the cells by the positions of the gold nanoparticles. This will help the experts to deliver organic pesticide more accurately and effectively. The experimental results show that by gold nanoparticle labeling technology, the imaging quality of photoacoustic microscopy and fluorescence optical microscopy can be improved, which indicates that the drugs probably enter the tobacco cells successfully.

Keywords

photoacoustic, PAM, FOM, dual-mode imaging, optical biopsy

Abbreviations

CW, continuous wave; FOM, fluorescence optical microscopy; FOMI, fluorescence optical microscopy imaging; OMI, optical microscopy imaging; PA, photoacoustic; PAI, photoacoustic imaging; PAM, photoacoustic microscopy; PAMI, photoacoustic microscopy imaging; PMT, photomultiplier tube.

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Introduction

Photoacoustic imaging (PAI) concentrates on the endogenous optical absorption of samples. When pathological change appears in biological tissues, their absorption coefficients change correspondingly. If short-pulse laser illuminates tissues, the pressure transients generated by the tissues depend on the optical absorption distribution. So, PAI can provide a high lateral resolution for the structural and functional imaging of samples.[1-9] Photoacoustic imaging technique has characteristics of deep acoustic penetration depth and high optical contrast. It can be used to image multiscale samples. It can be achieved by illuminating the sample from one side and detecting the acoustic pressure signals at any directions.

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Photoacoustic microscopy (PAM) is a microscopically imaging mode implemented by the distribution of the optical energy deposition within samples.[4] So far, PAM images of red blood and anemia cells have achieved their structure and function with high resolution.[5,6] According to the principle of imaging, PAM can image most absorbent substances in cells. It has been widely used in the cancer cells research because cancer cells have stronger absorbance than normal cells, so PAM can be used to image local cancer cells for target therapy in clinical medicine.

Fluorescence imaging is a recent development in noninvasive biomedical imaging. It bases on the strong fluorescence intensity emitted by fluorescent material in sample. Under certain illuminating intensity of excitation, the specific fluorescence intensity is proportional to the amount of fluorescein in a sample, so it can be used to image spatial distribution of fluorescent material within sample. Imaging for specific mark proteins is its major advantage. But it cannot provide sample information of physiological process that has nothing to do with fluorescent material.

As microscopically imaging modes for cells, fluorescence microscope (FOM) and PAM have similar systems. The differences between them are the imaging mechanisms and principle—one is absorption and another is emission, so their reconstruction images can show different information of samples. But the light source, optical transportation, and optical scanning systems are similar to each other, which makes the dual-model imaging system of PAM and FOM feasible. The comprehensive and complementary structural and functional information of samples could be obtained by different contrast mechanisms by a PAM and FOM dual-model system.

In this article, PAM and FOM had been integrated together and the simultaneous dual-mode microscopy imaging system is set up. In the system, PAM and FOM share the same source and light transmitting path. Based on this dual-model imaging system, blank tobacco cells are imaged.

The final purpose of this experimental research is to detect if the drugs can enter the cells. Generally, drugs may not have enough absorptivity or fluorescence. To take the furthest advantages of FOM and PAM, drugs were marked by marker, which can produce photoacoustic (PA) signals and at the same time can emit fluorescent light when bathed in laser. As one of the 4 main immune labeling technologies, nanogold-labeling method is the first choice, for its strong absorptivity and fluorescence. The self-made gold nanoparticles with 512-nm emission wavelength had been made and used to label certain drugs. These gold nanoparticles have a large absorption coefficient and enough fluorescence emission. They are used to label certain drugs and are added to the tobacco cells. Then, by the FOM and PAM imaging results of the labeled tobacco cells, we can judge if the gold nanoparticles enter the cells, that is the same to the drugs. The experimental results show that by gold nanoparticle labeling technology, the imaging quality of PAM and FOM can be improved, which indicates that the drugs probably enter the tobacco cells successfully.

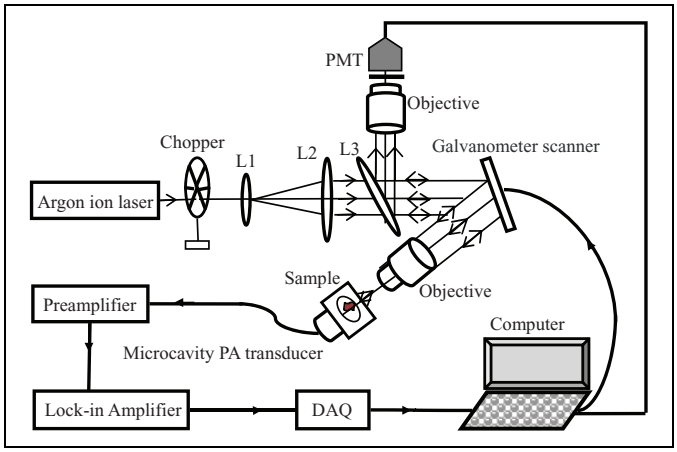

Figure 1. Schematic of dual-modal laser scanning imaging system with integrated photoacoustic microscopy imaging (PAM) and fluorescence optical microscopy (FOM) setup.

In general, in this article, FOM and PAM are combined together and dual-mode imaging for cells is achieved. Also, nanogold labeling technology is used to improve the quality of the sample image. All these will make the tracing of drugs or nutrition in plant cells more feasible.

Photoacoustic Microscopy and FOM Simultaneous Dual-Mode Imaging System

Both PAM and FOM have different imaging mechanisms and basic imaging principle. The former is based on absorption and the latter emission. So, the signals in PAM and FOM system are different. They are PA signals detected by PA transducer and light signals by photomultiplier tube (PMT), respectively. As shown in Figure 1, dual-modal laser scanning imaging system with PAM and FOM setup is integrated in a same system. But to match the 2 microscopes with each other, the fluorescence of sample is arranged to be detected by PMT backscattering in the system.

The amplification factor is determined by the size of the specific cell samples. The common radiation source is a continuous wave (CW) laser (argon ion laser, output wavelength is 514.5 nm) with the laser power about 10 mW. A chopper is used after the argon ion laser to change the continuous light to pulse one. A field flattening lens was used as the objective lens with the magnification of $\times 40$ or $\times 20$, which decide the resolution of the system. However, PA signals induced by modulated CW laser can hardly be detected by the commercial polyvinylidene fluoride needle hydrophone or piezoelectric ceramic transducer. Besides, the PA signals induced by modulated CW laser are much weaker than that of pulse laser. Thus, a PA transducer with microcavity based on a gas detection technique is used to strengthen the PA signals of samples. The microcavity PA transducer[6] can improve the signal to noise ratio of PA signals than an ordinary transducer. It is made by the 1-mm thick polymethyl. The diameter of both the microcavity and resonant cavity is 0.5 mm; thus, the volume of the

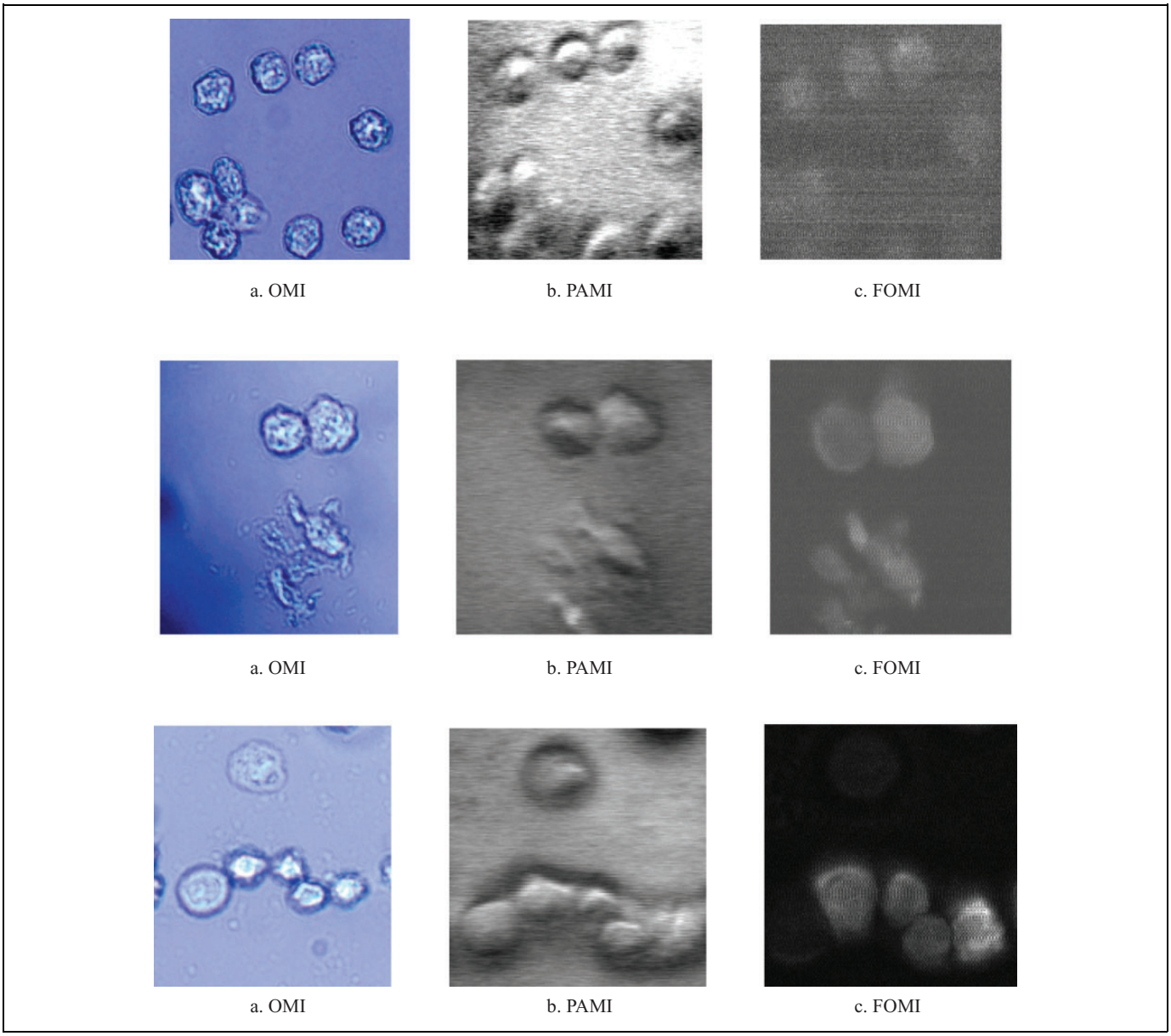

Figure 2. The results of ordinary optical microscopy imaging (OMI), photoacoustic microscopy imaging (PAMI), and fluorescence optical microscopy imaging (FOMI). (1) Images for blank tobacco cells; (2) images for tobacco cells labeled by gold nanoparticles and fixed by silica; and (3) images for tobacco cells labeled by gold nanoparticles and fixed by silica.

microcavity and resonant cavity is 0.2 mm^3. The response frequency of microcavity PA transducer is from 100 Hz to 3 kHz.[6]

The laser from argon ion laser (wavelength 514.5 nm, power 15 mW) becomes pulse laser after modulated by chopper. The modulated frequency is 2.5 kHz. Then the pulse laser is collimated by lens L1 and L2, passes through beam splitter L3, and then is shifted by galvanometer scanner. After focused by objective, the scanning pulse laser illuminates all parts of sample successively. At the same time, PA signals induced are recorded by microcavity PA transducer, amplified by preamplifier (Stanford Research Systems SR550, US) and lock-in amplifier (Stanford Research Systems SR830, US), collected by data acquisition card (PCI6115, National Instrument, US), and then transferred to computer. The PAM image of sample can be reconstructed by these PA signals.

Synchronized with the above procedure, the backscattered fluorescence from the sample backtracks through the galvanometer scanner, reflected by beam splitter L3 to another objective, focused on the pinhole and then arrives at the PMT (CR131; Beijing Hamamatsu). Then the FOM image reconstructed by these signals can be obtained.

Thus, a simultaneous dual-modal imaging technique can be achieved by one same laser source and optical system. Based on system shown in Figure 1, PAM and FOM images of cell

samples can both be achieved, and such combination will provide more comprehensive information for the cytological test. Images from the 2 modes are corresponding with each other, but functionally complementary.

Method was used before the objective lens to scan the probe beam in the x-y direction. With the beam diameter of 8 mm before the objective lens, the lateral resolution of the system, that is, the diffraction-limited optical focal diameter, was calculated to be 0.42 μm at 514 nm. The system lateral resolution was experimentally checked by the Guangzhou Municipal Standard Bureau, reporting a measured value of 1.25 μm.

Experimental Results and Discussions

Tobacco cells are selected as samples to image based on the dual-model imaging system as shown in Figure 1. The tobacco cells were divided into 3 groups.

Among the samples, 1 group remained as blank and certain drugs labeled by self-made gold nanoparticles with 512 nm (close to the irradiation beam wavelength 514 nm) emission wavelength were added to the other 2 groups. To prevent the cells become apoptotic and deformed during the experiment, silica was used to fix the tobacco cells.

The pictures in Figure 2 are the results of ordinary optical microscopy imaging (OMI), photoacoustic microscopy imaging (PAMI), and fluorescence optical microscopy imaging (FOMI). The pictures shown in Figure 2(1) A to C are the OMI, PAMI, and FOMI results of the blank tobacco cells. The second and third groups in A to C of Figure 2(2) and Figure 2(3) are the OMI, PAMI, and FOMI results for tobacco cells labeled by gold nanoparticles and fixed by silica.

Conclusion

In this article, tobacco cells are chosen as sample because tobacco cell has little chlorophyll which will cause large noise signals. Also, its size is big enough to image. Both nanogold labeling technology and microcavity PA transducer are the effective methods to improve the imaging quality. In addition, fixing the cells before experiment is a very important aspect to make sure it will not damage because we must ensure the stability of cells throughout the whole process. It is achieved by soaking the cells in silica.

But it can be seen from the results in Figure 2 that PAM and FOM dual-mode imaging system can obtain the sharp and exact images of blank tobacco cells. Comparing the first group in Figure 2(1) and the latter 2 groups in Figure 2(2) and Figure 2(3), it is obvious that the PAM and FOM images of blank cells have low contrast because of the low absorption of the blank cell itself, but after the tobacco cells were labeled by nanoparticles labeling technology, the quality of the PAM and FOM imaging results is improved, which indicates that the drugs probably entered the tobacco cells successfully. So, the PAM and FOM integrated imaging system can be used to judge if the labeled drugs can combine the cells by the positions of the gold nanoparticles, or even to monitor and trace the drugs. But it is difficult to determine whether the nanoparticles enter tobacco cells or probably absorbed on the surface membrane, unless the resolution is able to distinguish the internal structure of the cells.

All these will help the experts to deliver organic pesticide more accurately and effectively. The system and method proposed here can also be used to trace the medicine in the cell of human resource to monitor the medicine pathway and know much of the effect.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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