Two-photon imaging of lymphoma cells targeted by gold nanoparticles

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Gold nanoparticles (NPs) have highly efficient multi-photon-induced luminescence. In this paper, we record the two-photon images of gold NPs, lymphoma cell line Karpas 299, and Karpas 299 incubated with 30-nm-diameter gold NPs and ACT-1 antibody conjugates (Au30-ACT-1 conjugates) by using a multi-photon microscopy system. Due to the specific conjugation of ACT-1 antibody and cell membrane receptor CD25, gold NPs are only bound to the surface of cell membrane of Karpas 299. The luminescence intensity of gold NPs is higher than that of cells at 750-nm laser excitation. By comparing the images of Karpas 299 cells incubated with and without gold NPs, it is found that by means of gold NPs, we can get clear cell images with lower excitation power. Their excellent optical and chemical properties make gold NPs an attractive contrast agent for cellular imaging.

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Recent research in biomedical imaging centers on non-invasive imaging of cancer with sub-millimeter spatial resolution and high signal intensity. In order to acquire more specific information, molecular specific contrast agents are used for imaging research widely. In optical imaging, one of the earlier contrast agents is conventional organic dyes. They can emit bright fluorescence and be used for both cytoplasmic and nuclear labeling, but undergo permanent photobleaching[1]. The fluorescent semiconductor nanoparticles (NPs), which are called quantum dots, have wide absorption ranges and unique size-dependent fluorescence properties, but potential cytotoxicity is the major problem for their application *in vitro* and *in vivo*[2,3].

Another kind of luminescent contrast agents is metal NPs which have high luminescent efficiency[4−7]. The metal NPs also exhibit the ability to resonantly scatter visible and near infrared light. Preliminary studies have reported their use as contrast agents for biomedical imaging using different optical imaging systems[7−10]. In this letter, we use a multi-photon microscope system to imaging colloidal gold, and lymphoma cell line Karpas 299 which is incubated with ACT-1 antibody conjugated gold NPs. Their imaging results using different experimental methods are compared.

Figure 1 presents the schematic of experimental setup. The multi-photon imaging system DermaInspect (JenLab, Germany), which was designed for deep-tissue optical tomography, uses a Ti:sapphire femtosecond laser (75 fs, 80 MHz) tunable from 710 to 920 nm (Mai Tai, Spectra Physics, Germany) with a mean laser output greater than 900 mW at 800 nm. The scanning module contains a motorized beam attenuator, a shutter, and a two-axis galvoscanner. A beamsplitter (640 DCSPXR, Chroma, USA) and a short-pass filter (BG39, Schott, Germany) separated the excitation power from the emission. Fluorescence light was detected in the wavelength range between 350 and 600 nm by a photon-counting photomultiplier tube module (PMH 100, Becker and Hickl, Germany). High spatial resolution was provided by a high numerical aperture (NA) oil immersion objective (Plan-Neofluar 40×, NA = 1.3, Zeiss, Germany) with 140-mm working distance.

In this study, the human lymphoma cell line Karpas 299 was provided by Forschungszentrum Borstel Leibniz-Zentrum für Medizin und Biowissenschaften, Germany. Cell suspension was centrifuged at 1400 rpm for 5 min at 20 °C and then resuspended in phosphate-buffered saline (PBS) with cell densities of 10^6 mL^{-1}. Then, 30-nm-diameter gold NPs and ACT-1 antibody conjugates (Au30-ACT-1 conjugates) were added to the cell suspension with conjuagates/cells ratio of 10^4. After incubation for 20 min, the cells were centrifuged and washed twice

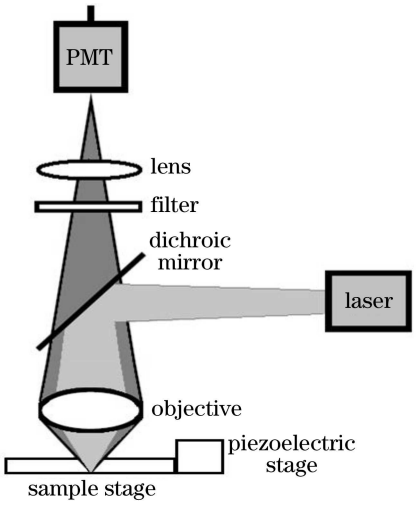

Fig. 1. Schematic of multi-photon microscope setup. The system consists of a Ti:sapphire femtosecond laser (710−920 nm), a scanning module, and a detector module.

and then resuspended in PBS.

It has been demonstrated that the absorption of multiple photons from a near-infrared laser can lead to efficient luminescence of gold NPs[11]. In the first experiment, we used gold NPs with 30-nm diameter to do the two-photon imaging. Figure 2 shows the intensity images of gold NPs under laser irradiation with different laser powers of 10, 15, 20, and 25 mW. Although it is difficult to quantify the luminescence difference precisely for a given gold NP under irradiation of different laser powers, we still observe that the luminescence intensity of gold NPs increases with the increase of irradiation power averagely. After experimental measurement of the luminescence efficiency for gold NPs under laser irradiation, we learn that gold NPs are a kind of optical probe with high emission efficiency.

Two-photon autofluorescence images of Karpas 299 cells without gold NPs are shown in Fig. 3. Karpas 299 cells were centrifuged and resuspended in PBS, then transferred into a small chamber on microscope glass slide, and covered by a cover glass. The images were recorded with different laser excitation powers. When the excitation power is about 12 mW, the autofluorescence signal of Karpas 299 is very week, and we cannot distinguish whether it is a cell. The profile of cells can be observed when the power arrives at 18 mW.

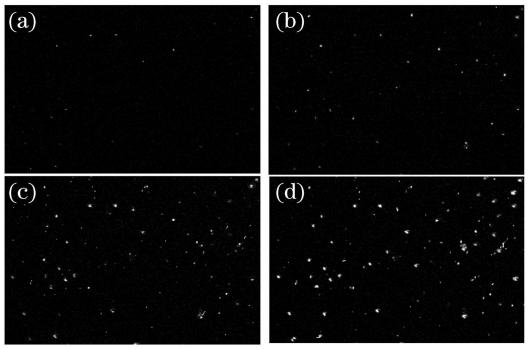

Fig. 2. Two-photon luminescence images (150×) of 30-nm gold NPs under 750-nm laser irradiation with different laser powers of (a) 10, (b) 15, (c) 20, and (d) 25 mW.

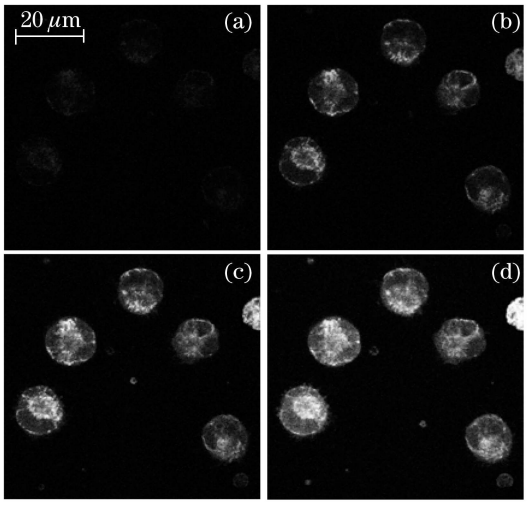

Fig. 3. Two-photon autofluorescence images of Karpas 299 cells under 750-nm laser irradiation with different laser powers of (a) 12, (b) 18, (c) 22, and (d) 28 mW.

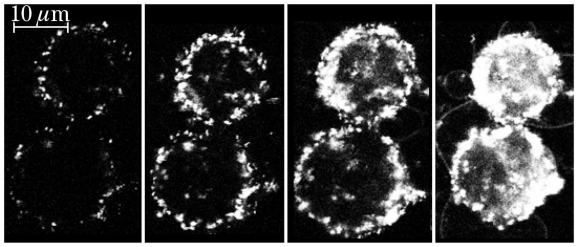

Fig. 4. Two-photon intensity images of Karpas 299 cells after incubation with Au30-ACT-1 conjugates under 750-nm laser irradiation with different laser powers of (a) 10, (b) 15, (c) 20, and (d) 22 mW.

With the increase of excitation power, the fluorescence intensity increases. While the power is approximately 28 mW, a very sharp cell imaging can be obtained. The clear intensity image delineated the cell structure, including cytoplasmic area and cellular nucleus area.

For Karpas 299 cells incubated with Au30-ACT-1 conjugates, the imaging results are shown in Fig. 4. ACT-1 is a specific antibody to the cell membrane receptor of CD25. Due to the binding of ACT-1 to CD25, gold NPs are only distributed on the surface of cell membrane of Karpas 299. While the excitation power is 10 mW, the profile of cells can be detected. As we can notice, the bright spots fasten on cell membrane, which is because of the strong luminescence of gold NPs. When the laser power increases to 20 mW, the whole cells are visible, but the signal of cell membrane area is still stronger than that of cellular structure. Therefore, under the same condition of laser excitation, the emission intensity of gold NPs is much stronger than that of Karpas 299 cells.

The other important finding is the photo-thermolysis of gold NPs in cell destruction. In Fig. 4, when the laser power increases to 22 mW, Karpas 299 cells with Au30-ACT-1 conjugates are damaged and become smaller. The effect of gold NP on cell necrosis is still unclear[12,13]. Future study will address gold NPs for imaging different kinds of cells and a study of photodamage to different kind of cells.

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