# Endomicroscopic Two-Photon Luminescence Imaging of Cancer Cells Using Molecularly Targeted Gold Nanocages

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**Abstract:**

**Gold nanoparticles have been attractive as a new class of contrast agents for optical imaging of biological tissue. In this study, we demonstrate the use of gold nanocages with ~ 60 nm diameter as bright contrast agents for the two-photon luminescence (TPL) imaging of cancer cells. A breast cancer cell line, SK-BR-3, which overexpresses epidermal growth factor receptor HER2, was used to test the molecular specific binding of bioconjugated gold nanocages. Real-time TPL imaging of the monolayered SK-BR-3 cells was performed with a miniature nonlinear optical endomicroscope system. Overall, molecularly targeted gold nanocages are promising as contrast agents for *in vivo* optical cancer diagnostics combined with nonlinear optical endomicroscopy.**

## Introduction:

Gold nanoparticles have become a new class of contrast agents in biological imaging because they are biocompatible and their surface plasmon resonance peak can be precisely controlled over a broad spectrum by changing their sizes and shapes [1]. Gold (Au) nanocages, in particular, are being studied because their optical resonance wavelengths can extend more easily to the near-infrared spectral region. Our studies have shown that the nanocages can be used as contrast agents in spectroscopic OCT imaging [1]. Recent results have also demonstrated that the gold nanocages are effective photo-thermal transducers and can be used for cancer diagnosis and therapy [2].

In this study, we observed that gold nanocages exhibited strong two-photon luminescence signals. Similar to gold nanorods, the metal nanoparticles can absorb two photons simultaneously and generate electron-hole pairs; then the electron-hole pairs can be recombined, emitting new photons [3]. Furthermore, we demonstrated the application of gold nanocages in cancer cells with a homemade nonlinear endomicroscope system.

## Experimental Procedures:

First, gold nanocages were synthesized using a galvanic replacement reaction between silver nanocubes and chloroauric acid ($HAuCl_4$). The size and wall thick-ness of the nanocages was controlled by varying the amount of $HAuCl_4$ added. Figure 1(a) shows a typical scanning electron microscope (SEM) image of gold nanocages with 60 nm diameter.

Next, these nanocages were functionalized to succinimidyl propionyl poly (ethylene glycol) disulfide (NHS-activated PEG, M.W. = 1,109) by creating an Au-S linkage and then bonded to primary amine of the anti-HER2 antibody [4]. These molecularly targeted gold nanocages were then conjugated to SK-BR-3 breast cancer cells that overexpress the HER2 growth receptor. Figure 1(b) shows a typical SEM image of a SK-BR-3 breast cancer cell bioconjugated with nanocages.

## Microscope System:

Images were obtained using a homemade nonlinear optical endomicroscope system. Figure 2 shows the schematic of this system. Light centered at 810 nm was emitted from a femtosecond laser and directed through a double-clad fiber to the endomiscroscope probe with an outer diameter of 2.4 mm. The probe consisted of a double-clad fiber (for both delivery

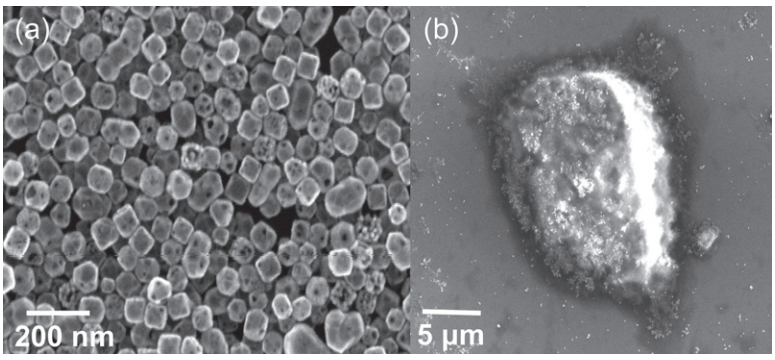


*Figure 1: SEM images of (a) gold nanocages and (b) SK-BR-3 cell targeted with bio-conjugated gold nanocages.*

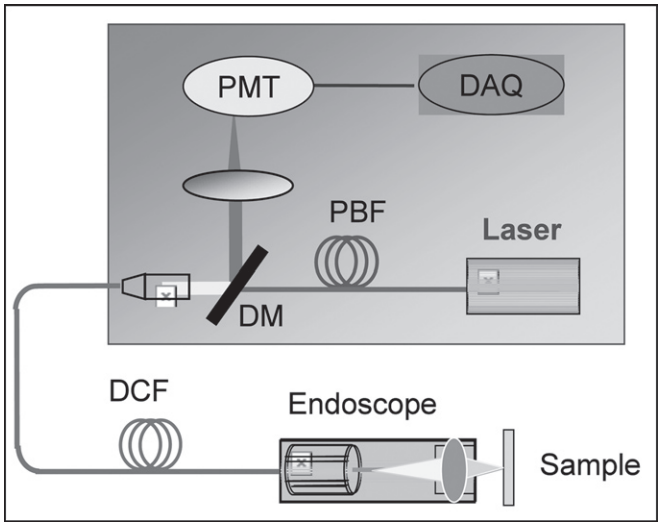


*Figure 2: Schematic of the fiber-optic scanning endomicroscope system. DCF: Double-clad fiber; DM: dichroic mirror; PBF: photonic bandgap fiber; PMT: photo multiplier tube; DAQ: Data acquisition system.*

of excitation light and collection of two-photon luminescence signals), a piezoelectric actuator (PZT) tube (for 2-D sweeping of the light on the sample), and a focusing lens (for focusing the light to the sample). In this endoscope, the frame rate was 3.3 Hz and about 160 $\mu$m scanning range on sample was achieved.

## Results:

Figure 3(a) shows a typical two-photon luminescence image of the gold nanocages in a water solution. It is obvious that single gold nanocages are randomly distributed. Since they are not molecularly targeted to any objects, they do not form any patterns in the solution. Figure 3(b) shows the endomicroscopic TPL images of the SK-BR-3 cells conjugated with nanocages tagged with anti-HER2 antibodies. As can be seen, most cell membranes can be clearly identified, indicating the successful conjugation. Overall, the gold nanocages show strong two-photon luminescence, but do not suffer photobleaching as organic dyes. Since they are more bio-compatible compared to quantum dots, the gold nanocages with the endomicroscopic TPL imaging are promising for *in vivo* application for early stage cancer diagnosis and treatment.

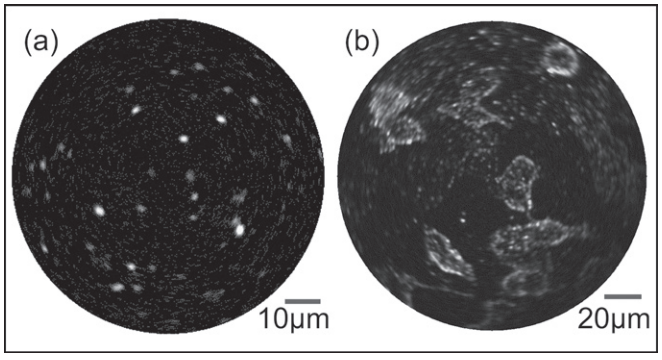


*Figure 3: Two-photon luminescence (TPL) images of (a) gold nanocages of a 50 nm edge-length in water and (b) SK-BR-3 breast cancer cells targeted with bio-conjugated gold nanocages.*

## Conclusion:

Gold nanocages are shown to be promising contrast agents for TPL imaging of cancer cells. Ongoing research is being done with three-dimensional imaging of phantom tissue made from collagen gel embedded with cells and bioconjugated nanocages. Future research will be focused on *in vivo* application of bioconjugated nanocages in tissue diagnosis by using the technology of nonlinear optical endomicroscopy.

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