Two-photon photothermal microscopy: Biomolecular imaging using nonfluorescent nanoparticle labels

M. M. Bijeesh, P. K. Shakhi, Geetha K. Varier[1], and P. Nandakumar*
Department of Physics, Birla Institute of Technology and Science, Pilani
K K Birla Goa Campus, Zuarinagar, Goa – 403 726, India.
[1]Current Address: C-192, BITS Pilani K K Birla Goa Campus, Zuarinagar, Goa, India.
*E. mail: nandan@goa.bits-pilani.ac.in

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We report on the development of a nonlinear optical microscopic technique based on two-photon absorption induced photothermal effect capable of detecting individual nonfluorescent nanoparticles with high sensitivity. The method will allow the use of metal nanoparticles as well as dielectric nanoparticles as biomolecular imaging probes and is demonstrated by imaging single silver and $BaTiO_3$ nanoparticles with high sensitivity. These nanoparticles are potential biomolecular labels having high photo stability [1] and ultrafast response time. Two-photon photothermal microscope is inherently confocal and makes use of near infrared excitation at high repetition rates. A pump-probe detection scheme is employed to detect the temperature change in the nanoparticle environment due to two-photon absorption of the near infrared pump beam. A 120-femtosecond mode-locked Ti: sapphire laser (Mira 900, Coherent Inc., USA) operating at 76 M Hz is used as the pump laser and the pump induced temperature change is probed using a He-Ne (632 nm) laser. The images are acquired by raster scanning of laser beam at fast time scales with a pixel dwell time of 80 μs. The applicability of the technique in biomolecular imaging is demonstrated by imaging nanoparticle labelled HeLa cells and human cheek cells.

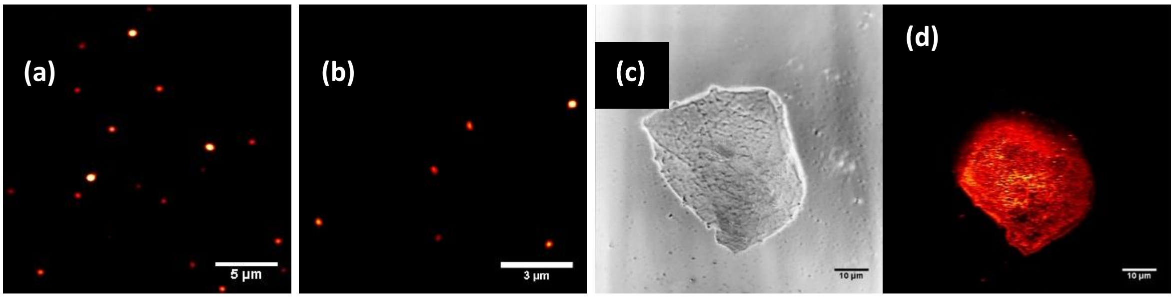

Fig .1: Two-photon photothermal images of (a) 25 nm Silver nanoparticles and (b) 20 nm $BaTiO_3$ *nanoparticles spin coated on a coverslip at 4000 rpm for 30 s. (c) Wide field image of the human cheek cell with 20 nm* $BaTiO_3$ *nanoparticles. (d) Corresponding image of human cheek cell taken with two-photon photothermal microscope.*

$BaTiO_3$ is a highly photostable and biocompatible material having femtosecond response time and could provide a potential label for imaging [2]. The near infrared excitation wavelengths employed by the microscope are less toxic to living cells. Further it provides a larger penetration depth and high SNR in imaging due to lower scattering and thus would be of interest in deep tissue imaging. The detection and thermal imaging of therapeutic silver nanoparticles could be of interest in biomedical research in selective imaging and treatment.

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