Photoacoustic flow measurements by use of laser-induced shape transitions of gold nanorods

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A quantitative technique for flow measurements based on a wash-in analysis is proposed. The technique makes use of the shape dependence of the optical absorption of gold nanorods and the transitions in their shape induced by pulsed laser irradiation. The photon-induced shape transition of gold nanorods involves mainly a rod-to-sphere conversion and a shift in the peak optical absorption wavelength. The application of a series of laser pulses will induce shape changes in gold nanorods as they flow through a region of interest, with quantitative flow information being derived from the photoacoustic signals from the irradiated gold nanorods measured as a function of time. To demonstrate the feasibility of the technique, a Nd:YAG laser operating at 1064 nm was used for irradiation and a 1 MHz ultrasonic transducer was used for acoustic detection. The flow velocity ranged from 0.35 to 2.83 mm/s. Excellent agreement between the measured velocities and the actual velocities was demonstrated, with a linear regression correlation coefficient of 0.93. This study is a pioneer work on wash-in flow estimation in photoacoustic imaging. © 2005 Optical Society of America

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Gold nanoparticles can be used as contrast agents for photoacoustic imaging,^{1,2} and when they are coupled with biomolecules they can be used for biological labeling.³ There are several technical advantages in using gold nanoparticles in these applications. First, a nanoparticle with a large surface-to-volume ratio is suitable for the covalent attachment of multiple ligands, which can generate a strong binding affinity between the nanoparticle conjugates and a specific binding target.⁴ Second, gold nanoparticles are biologically compatible and hence inherently safe. Third, gold nanoparticles exhibit a strong photoacoustic response in the visible-to-near-infrared region.¹ Thus such particles can potentially be used for photoacoustic molecular imaging.

It has also been demonstrated that gold nanoparticles can be used to assist quantitative flow estimation in photoacoustic imaging based on the indicator-dilution theory.² This theory provides a mathematical model for estimating hemodynamic parameters based on changes in the signal intensity as a function of time (also known as time-intensity measurements). In many applications, these time-intensity measurements are based on washout analysis,^{5,6} in which the mixing of particles in a chamber is modeled as a mixing process in a compartment based on dilution theory. Flow parameters can then be estimated by use of the time-intensity data measured at the output of the mixing compartment. Critical assumptions of washout analysis are that a bolus injection is used and that the injection site is sufficiently close to the measurement site,⁵ both of which limit its applications. In this Letter we investigate a different approach, based on a destruction-replenishment model, that is not subject to the above limitations. As

is shown below, the flow velocity is proportional to one of the model parameters. Such a method is also known as wash-in analysis, and it is based mainly on the nanorod-to-nanosphere shape transition that occurs in nanorods when they are irradiated by a laser pulse.

A gold nanosphere with a diameter of tens of nanometers exhibits a single absorbance peak near 550 nm.⁷ In contrast, a gold nanorod is a cylindrical particle whose optical absorption wavelength is affected by the aspect ratio (defined as the length of the major axis divided by the diameter).^{7,8} When the aspect ratio increases from 1.0 to 6.5, the absorbance peak moves from 550 to 1050 nm.⁸ Furthermore, because a nanosphere is more stable than a nanorod, irradiating gold nanorods with sufficient laser power tends to change them into nanospheres. This shape transition reduces the concentration of nanorods with the original shape and thus weakens the laser-induced photoacoustic signal. The concentration is also affected by the flow velocity. If the acoustic signal amplitude is linearly proportional to the concentration, it is then possible to estimate the flow velocity from the temporal changes in the acoustic intensity. Note that the linearity of the relationship between concentration and acoustic amplitude was established by a previous study.²

Figure 1 shows a photoacoustic measurement system with a vessel located along the x axis, the laser-beam axis along the y axis, and the ultrasonic-beam axis along the z axis. In this system the laser beam width is much larger than the diameter of the vessel and the ultrasonic beam width. Assume that the concentration of gold nanorods in the vessel reaches a constant n_0 before time $t=0$ and that the measure-

Fig. 1. Coordinates of the measurement system.

ment that involves irradiating the vessel with a series of laser pulses with a fixed pulse repetition frequency (PRF) starts at $t=0$. Let $n'(t)$ be the effective concentration of gold nanorods measured by the transducer; then $n'(0)=n_0$ and

$$1 - n'(t)|_{t=(k/\text{PRF})^-}/n_0 \cong \exp(-u/\text{PRF}) \times [1 - n'(t)|_{t=(k/\text{PRF})^+}/n_0], \quad (1)$$

where k is a nonnegative integer, the superscripts $-$ and $+$ in the subscripts stand for “immediately before” and “soon after,” respectively, and u is a parameter proportional to flow velocity v .⁹ Relation (1) indicates that between $t=(k/\text{PRF})^+$ and $t=[(k+1)/\text{PRF}]^-$ are the replenishment phases, during which $n'(t)$ increases toward n_0 with time as a result of the flow.

The short time periods between $t=(k/\text{PRF})^-$ and $t=(k/\text{PRF})^+$ are the destruction phases during which some gold nanorods change into spheres or other shapes. When $v=0$, $n'(t)$ will decrease asymptotically to a constant level n_∞ . Explicitly,

$$n'(t)|_{t=(k/\text{PRF})^-} \cong (n_0 - n_\infty)r^k + n_\infty, \quad k = 0, 1, 2, \dots, \quad (2)$$

where $r < 1$ is a constant that depends on the pulse energy. In general, $0 < n_\infty \ll n_0$ and

$$n'(t)|_{t=[(k+1)/\text{PRF}]^-} \cong rn'(t)|_{t=(k/\text{PRF})^-}. \quad (3)$$

Furthermore, when $v=0$, $n'(t)|_{t=[(k+1)/\text{PRF}]^-} \cong n'(t)|_{t=(k/\text{PRF})^+}$. Therefore

$$n'(t)|_{t=(k/\text{PRF})^+} \cong rn'(t)|_{t=(k/\text{PRF})^-}. \quad (4)$$

Relation (4) describes the effects of the destruction phases on $n'(t)$.

We denote $n'(t)/n_0|_{t=(k/\text{PRF})^-}$ as $n''(k)$. According to relations (1) and (4),

$$1 - n''(k+1) = s[1 - rn''(k)], \quad (5)$$

where $s = \exp(-u/\text{PRF})$. Solving Eq. (5) with $n''(0)=1$, we have

$$n''(k) = \left(\frac{1-s}{1-rs} \right) + \left[1 - \left(\frac{1-s}{1-rs} \right) \right] (rs)^k. \quad (6)$$

In other words, the concentration of gold nanorods tends to decrease exponentially to a constant determined by the flow velocity and the laser power level.

Note that $n''(k)=1$, and consequently the wash-in analysis does not work if no shape transition occurs. Fitting the measured time-intensity data to the following model:

$$n''(k) = n''_\infty + (1 - n''_\infty)w^k, \quad (7)$$

where

$$n''_\infty = \frac{1-s}{1-rs}$$

and $w=rs$, allows

$$s = 1 - (1-rs)n''_\infty = 1 - (1-w)n''_\infty \quad (8)$$

to be solved, and the flow velocity is

$$v \propto u = -\text{PRF} \log[1 - (1-w)n''_\infty]. \quad (9)$$

The ratio of u to v can be determined by means of phantom experiments. Note that, in this technique, each pulse is used to induce a shape transition and generate photoacoustic signals.

The method described above was experimentally verified with the setup shown in Fig. 2, which consists of an infusion pump (KDS100, Geneq), a laser system, and an ultrasonic receiver. A frequency-doubled Nd:YAG laser (LS-2132U; LOTIS TII) operating at 1064 nm with a pulse duration of 5 ns was used for optical illumination. The laser energy was 3.3 mJ/pulse, which is sufficient to transform the original nanorods into nanospheres or nanorods with a smaller aspect ratio (i.e., nanorods with less optical absorption at 1064 nm). An ultrasonic single-element transducer (V-303; Panametrics) with a center frequency of 1 MHz and a focal depth of 1.7 cm was employed to detect the photoacoustic signals. The acoustic waveforms were amplified by an ultrasonic receiver (5800PR; Panametrics) and then recorded by a data acquisition card (CompuScope 12100; Gage) at a sampling rate of 100 MHz. The acquired data were stored in a personal computer (PC, Fig. 2) for subsequent data analysis. Gold nanorods with an absorption peak at 1018 nm wavelength were injected into a 1 mm diameter vessel in a poly(vinyl alcohol) phantom. The shape transition and replenishment of nanorods occurred simultaneously as nanorods flowed into the region of interest, with a concentration equilibrium being achieved. Laser irradiation was from

Fig. 2. Schematic diagram of the experimental setup.

Fig. 3. Estimated flow velocity (vertical) versus actual flow velocity (horizontal) and the results of linear regression. The actual velocities measured were 0.35, 0.71, 1.06, 1.41, 2.12, and 2.83 mm/s.

the side of the vessel, and acoustic detection was from the top. To prevent recirculation of the nanorods into the phantom, the solution with nanorods was passed through the measurement site once only. The results are shown in Fig. 3, which demonstrates good agreement between the actual flow velocity and the estimated flow velocity, with a linear regression correlation coefficient of 0.93. The fitted line relates the measured velocities (y) to the actual velocities (x) by $y = 0.87x + 0.1$, which further indicates that good accuracy can be achieved with this technique (the ideal case is $y = x$).

This study has demonstrated the feasibility of photoacoustic flow estimation by use of gold nanorods. We intend to focus our future research on improving the sensitivity for *in vivo* applications for blood flow

measurements, and biocompatibility testing will also be performed according to standard ISO10993. Note that, for *in vivo* applications, the photoacoustic signals from the blood in the vessels and the surrounding tissues (i.e., the interferences) can be estimated before the injection of the gold nanorods and thus mostly removed in the subsequent measurement process.

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