Dynamic Tracking of a Nano-Particle in Fluids under Brownian Motions

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Abstract. Most previous studies on H$_2$S were devoted to its toxic effects. However, recently there have been increasing evidences which show that endogenously generated H$_2$S in specific mammalian tissues has certain significant positive physiological effects such as a neuromodulator and vasorelaxant in a membrane receptor-independent manner. In order to know the functions of endogenous H$_2$S, low concentration and high accuracy measurement of H$_2$S is a must. Furthermore, this measurement is desired to be real-time and non-invasive. It is reported that low concentration and nano quantity of H$_2$S can be detected in water solutions and sera using carbon nanotubes with the fluorescence spectrum by ZEISS LSM 510 Meta laser scanning microscopy. However, because of the Brownian motion of the small particle (carbon nanotube), a control system must be developed to track the movement of the particle in fluids. In this paper, we present a study to track a carbon nanotube which absorbs H$_2$S in water or serum using a Raman microscope or confocal laser scanning microscope. In particular, we developed a novel control system for this task. Simulation has shown that our system works very well.

1. Introduction
It was reported that low concentration and nano quantity of H$_2$S in water solution and sera [1,2] can be detected using carbon nanotubes with the fluorescence spectrum by ZEISS LSM 510 Meta laser scanning microscopy. These results have made it possible to develop a clinical approach using confocal laser scanning microscopy. Compared with existing approaches, this new approach requires only a very small amount of blood tissue (i.e., several µL). In addition, the new approach could be used for a real-time continuous measurement of H$_2$S in live cells.

In order to realize the new approach for a clinical application, a small (nano) particle (less than a laser point which is, typically, 5 µm in diameter) in a live cell should stay in the centre of the laser point during the sampling period – the period during which confocal laser scanning microscopy acquires information regarding the intensity of the fluorescence signal of the particle. The live cell contains fluids. As such, the nano particle will undergo Brownian motion and may move out of the laser point. For example, using a typical 5 µm diameter confocal laser point, there is a 90% chance that the nano particle, originally at the centre of the laser point, will move out of the laser point in 0.3 seconds (in a fluid with the diffusion coefficient of 5 µm$^2$/s). In order to track the movement of the nano particle in fluids, there is a need to develop a control system for both the laser and the stage that holds the live cell containing the nano particle.

A popular idea for such a control system is to apply the (external) vision system; however, the vision system has some difficulty in tracking a particle in a three dimensional space. For this reason, most of the techniques reported in the literature provide tracking only in a two dimensional space. Although
there are methods developed for tracking a particle in a three dimensional space [3, 4], they either have limitations in accuracy or are operated in an off-line manner. Cang et al. [5] reported a study to use confocal laser scanning microscopy to track a small particle between two cover slips with the edges sealed with wax. In their design, movements of the particle in three dimensions were measured and feedback to a controller that further commanded the stage but not the laser of a confocal laser scanning microscopy. Since the nano particle with the live cell is held by the stage, there may be significant perturbation on the cell and the particle due to the movement of the stage, which can degrade the accuracy of tracking. Although such disturbance may be rather small in Cang et al.’s case, their approach is not suitable for application to the situation, studied in this dissertation where the particle is allowed to freely move in the cell.

In this paper, a novel control system for tracking a nano-particle in fluid under confocal laser scanning microscopy is presented. In particular, Section 2 presents a conceptual design of the controller. Section 3 is a logical design of the controller. Section 4 describes the controller, which can be realized on an existing confocal laser scanning microscopy. Section 5 presents a simulation study for validation of the control system. Section 6 is a conclusion with some further discussion.

2. Conceptual Design of the Controller

Figure 1 gives the general configuration of the particle, laser scanning microscopy, and fluid. The laser point can be considered to be a sphere, with its trajectory in the XY plane being a circle. The particle can move in three dimensions in the fluid, and can emit the fluorescence signal only when it is within the laser sphere and is stroked by the excitation light of the laser. Further, the intensity of the fluorescence signal of the particle will be stronger if the particle is at the centre of the laser sphere. Ideally, we should maintain the particle at the centre of the laser sphere during the sampling period, which is therefore the goal the controller needs to achieve.

The control system usually comprises the following major components: (1) controller, (2) sensor, (3) actuator, and (4) target object to be tracked. The controller is essentially a software system that has input and output. In our case, the controller input is the position of the particle and the controller output is the updated position of the laser.

The objective of the sensor is to obtain the position of the particle at time t. The methodology for achieving this is as follows. First, the intensity of the fluorescence signal from the particle is acquired. This intensity depends on the distance between the particle and the centre of the laser point. Second, this intensity is converted or translated to the position of the particle, which can be achieved by adjusting the laser point in the X-Y plane and then up and down in the Z-axis direction to obtain the strongest intensity. With the pinhole in confocal laser scanning microscopy, there is one position in the
Z-axis direction that will excite the sample most or that will, in other words, give the strongest intensity. Therefore, change in the depth of the particle away from that unique position along the Z-axis direction will decrease the fluorescence intensity. In Figure 2, the method for finding the position of the particle at the strongest intensity of fluorescence signal in XY plane is shown. In Figure 2, d is the radius of the circle due to Brownian motion; point O is the original position of both particle and laser, and O’ is the updated position for the laser or for the position of the particle with the strongest intensity of the fluorescence signal.

![Figure 2: Obtaining the strongest intensity of the particle (O is the current centre of the laser point; d is the particle movement due to Brownian motion which can be calculated by knowing the signal intensity; O’ is the next centre of the laser point).](image)

The period of time for adjusting the laser to the position of the particle is called the adjusting period. The laser working period refers to the sum of the sampling period and the adjusting period. During the adjusting period, it is assumed that the particle does not move out of the laser point. This is made possible by properly controlling the adjusting period. It is also assumed that the movement of the particle is slower than the movement of the laser or the stage. Therefore, we assumed that during the working period, the particle was at rest.

The actuation system of the confocal laser scanning microscopy system is the motorized system for the laser (i.e., the X- and Y-axis movement) and the microscopy stage (i.e., the Z-axis movement). Ideally, the stage does not move during the working period because such movement may disturb the particle in addition to Brownian motion. This is an issue of control system implementation.

3. Logical Design of the Controller
The objective of the logical design of the control system is to develop mathematical equations that express the conceptual design. The most important task of this controller is to determine the position of the particle based on the intensity of that particle’s fluorescence signal. Let the diameter of the laser point be w. The relationship between this intensity and the position of the particle can be expressed as [5,6]:

\[
I'(t) = I_o e^{-\frac{2}{\tau^2} t^2} \left[ \epsilon \left( \frac{2 \Delta I}{\Delta x} \right) \right] + I_B
\]

(1)
where, $I_o$ is the photon count rate when the particle is in the centre of the laser sphere, $I_B$ is the background photon count rate and can be removed through calibration in the experiment, $z_B$ is the Rayleigh range ($z_B = \pi w^2 / \lambda$, where $\lambda$ is excitation wavelength), $d$ is the distance between the position of the particle in the XY plane and the centre of the laser sphere, and $z$ is the distance between the position of the particle and the centre of the laser sphere along the Z-axis direction. Further, in Equation 1, the term $d^2 = x_p^2 + y_p^2$ where $x_p$ and $y_p$ are the coordinates of point $O'$ in the coordinate system that takes $O$ as the origin and is in parallel with the coordinate system $O$-XY (see Figure 2).

At time $t=0$, the particle and the laser point are coincident at $O$ (see Figure 3). At time $t$, the particle moves to $O'$. Our goal now is to determine the coordinates of $O'$ based on the information of the intensity of the fluorescence signal of the particle. According to Figure 3, we have:

![Figure 3: Laser tracking of the particle in the XY coordinate plane.](image)

\[ d = \epsilon - r \]  
\[ x_p = \epsilon \cos(\eta) - r \cos(\omega_r t) \]  
\[ y_p = \epsilon \sin(\eta) - r \sin(\omega_r t) \]  

Then:

\[ d^2 = x_p^2 + y_p^2 = (\epsilon \cos(\eta) - r \cos(\omega_r t))^2 + (\epsilon \sin(\eta) - r \sin(\omega_r t))^2 \]
\[ = (\epsilon^2 + r^2) - 2r \epsilon \cos(\eta - \omega_r t) \]  

Substituting Equation 5 to Equation 1 (noticing that $I_B$ is removed) leads to:

\[ I(t) = I_o e^{-\frac{2}{\omega^2}(\epsilon^2 + r^2) - 2r \epsilon \cos(\eta - \omega_r t)} \]
This intensity follows a Gaussian distribution. Now considering the particle position by \( x_p(\varepsilon, \eta) \) and \( y_p(\varepsilon, \eta) \) (refer to coordinate with centre O), we apply the maximum likelihood estimator [6,7] to obtain:

\[
\dot{x}_p = \frac{2\pi}{w_0^2} \int_0^{2\pi} I(\tau) \cos(\omega_0 \tau) d\tau \cos(\eta - \alpha_0) = 2\pi I_0 e^{-\frac{1}{2} \left( \frac{x^2 + y^2}{w^2} \right)} I_1 \left( \frac{4re}{w^2} \right) \cos(\eta) \tag{7}
\]

\[
\dot{y}_p = \frac{2\pi}{w_0^2} \int_0^{2\pi} I(\tau) \sin(\omega_0 \tau) d\tau \sin(\eta - \alpha_0) = 2\pi I_0 e^{-\frac{1}{2} \left( \frac{x^2 + y^2}{w^2} \right)} I_1 \left( \frac{4re}{w^2} \right) \sin(\eta) \tag{8}
\]

where \( I_1 \) is a first order modified Bessel function. We also apply the maximum likelihood estimator for \( z \) to obtain:

\[
\dot{z}_p = \frac{\int I(\tau) e^{\frac{d(\tau)z(z)}{w^2 \alpha_0}} d\tau}{\int I(\tau) d\tau} \tag{9}
\]

In the following, we need to find the position of the particle, in particular \( \varepsilon, \eta, \) and \( z \). We consider the dynamic steady-state equation that describes \( \varepsilon, \eta, \) and \( z \) as follows [8]:

\[
\dot{\varepsilon} = -\alpha (\varepsilon(t) - \varepsilon_0) \tag{10}
\]

\[
\dot{\eta} = -\frac{\omega}{1 + \beta (\varepsilon(t) - \varepsilon_0)^2} \tag{11}
\]

\[
\dot{z} = -\alpha_c (z(t) - z_0) \tag{12}
\]

where \( \alpha, \alpha_c, \beta, \varepsilon_0, \) and \( \omega \) are given constants.
Equation 10 can also be expressed as:

\[
\frac{d(e(t)e^{\alpha t})}{dt} = \alpha e_0 e^{\alpha t}
\]  
(13)

or

\[
e(t)e^{\alpha t} - e(0) = e_\alpha e^{\alpha t} - e_0
\]  
(14)

\[
e(t) = (e(0) - e_\alpha)e^{-\alpha t} + e_0
\]  
(15)

It is noted that Equations 10 to 12 allow us to obtain the position of the particle. Suppose that \(\Delta t\) is the working period. We assume that \(\Delta t\) is the same for each working period. The time domain can then be expressed as \(t_k = k\Delta t \ (k=1, 2, \ldots, n; \ n\) is the number of the working periods), or the time series of the movement of the laser can be expressed by \(\{k\}\). It is further noted that the tracking for time \(k\) will be based on the working period \(k-1\). By applying Equation 15 to each time interval from \(k\) to \(k+1\), we obtain:

\[
e(k + 1) = (e(k) - e_\alpha)e^{-\alpha \Delta t} + e_0
\]  
(16)

By integration of Equation 11, we can obtain:

\[
\eta(t) - \eta(0) = \int_0^t \frac{\omega}{1 + \beta(e(\tau) - e_0)^2} d\tau
\]

\[
= \left(\frac{\omega}{2\alpha}\right) \ln(e^{2\alpha}(1 + \beta(e(0) - e_0)^2)) - \left(\frac{\omega}{2\alpha}\right) \ln(1 + \beta(e(0) - e_0)^2)
\]  
(17)

From Equation 15, we have:

\[
e(t) - e_0 = (e(0) - e_0)e^{-\alpha t}
\]  
(18)

Substituting Equation 18 to 17, we have:

\[
\eta(t) - \eta(0) = \left(\frac{\omega}{2\alpha}\right) \ln\left(\frac{e^{2\alpha t} + \beta(e(0) - e_0)^2}{(1 + \beta(e(0) - e_0)^2)}\right)
\]  
(19)

By applying Equation 19 to each time interval from \(k\) to \(k+1\), we obtain:

\[
\eta(k + 1) = \eta(k) + \left(\frac{\omega}{2\alpha}\right) \ln\left(\frac{e^{2\alpha \Delta t} + \beta(e(k) - e_0)^2}{(1 + \beta(e(k) - e_0)^2)}\right)
\]  
(20)

Using the same procedure as \(e\) as for \(Z\), we have:
At time $t$, the position of the centre of the focus point is then:

$$z(k+1) = (z(k) - z_0)e^{-\alpha \Delta t} + z_0 \quad (21)$$

Now let us determine the maximum working period denoted by $w_{t_{\text{max}}}$. The meaning of $w_{t_{\text{max}}}$ is such that when the particle moves for a period of time longer than $w_{t_{\text{max}}}$ from the centre of the laser point sphere, the particle will be out of the sphere. The $w_{t_{\text{max}}}$ is thus related to the radius $\left(\frac{W}{2}\right)$ of the laser point sphere. It is known that the Brownian motion of a particle in a fluid follows a Rayleigh distribution. Therefore, the probability ($P$) that the particle will move out of the laser point sphere can be expressed by:

$$P = \int_0^{\frac{W}{2}} Dw_{t_{\text{max}}} dq e^{\frac{q^2}{2w_{t_{\text{max}}}}} dq = 1 - e^{\frac{q^2}{2Dw_{t_{\text{max}}}}} \quad (26)$$

where $D$ is the diffusion coefficient of the fluid and $q$ is a distance variable for the movement of the particle.

From Equation 26, we can calculate the maximum working period:

$$w_{t_{\text{max}}} = \frac{\left(\frac{W}{2}\right)}{2D \ln \left(1 - P\right)} \quad (27)$$

### 4. Laser Scanning Microscopy Implementation

The implementation of the control system above can be done with a particular confocal laser scanning microscopy (Figure 4). This microscopy, which should be able to move the laser, is currently not commercially available.
Figure 4: Laser scanning microscopy design for small particle tracking.

The system can be built around an inverted microscopy. The 514 nm light is from argon lasers (Light Source). The filter is used to select a proper wavelength, specifically, 518 nm. Objective 1 is to focus the laser beam on the sample. The intensity of the laser beam should be adjusted for the best performance. The sample is placed on a piezoelectric translation stage. A 60x water immersion objective is placed beneath the sample. A part of the emission light, collected by the objective 1, is split by a 50/50 Beam Splitter 1 to a PMT that records the intensity of the particle fluorescence signal. Another part of the emission light meets a 50/50 Beam Splitter 2. The emission light that passes the Beam Splitter 2 can form an intensity that can be focused through Lens 3 and Lens 4, and detected by Detector 2 (an avalanche photodiode). The signal detected by Detector 2 is sent to the control system for actuating the laser movement in the XY plane. The emission light reflected by Beam Splitter 2 is collected by Objective 2. A pinhole is placed slightly behind the conjugate focal point of Objective 2. Lens 2 collects all the light coming out of the pinhole and focuses it at Detector 1 (an avalanche photodiode). It is noticed that when the particle moves in and out of the focus point, the intensity detected by Detector 1 is different from that of the particle on the focus point. The intensity signal that is collected by Detector 1 is sent to the control system actuator, which in turn controls the movement of the stage along the Z-axis direction.

5. Evaluation of the Control System Based on Simulation

Evaluation of the control system was done through simulation. In this simulation we used a common laser point diameter \( w = 5 \mu m \), assuming that initially the laser was at an original position such that \( z_o = 0, \eta = 0, \) and \( \varepsilon_o = 0; \omega, \alpha, \alpha_z, \) and \( \beta \) can be adjusted such that a suitable control system can be designed. In our simulation, \( \omega = \alpha = \alpha_z = 500, \) and \( \beta = 0.2. \) The diffusion coefficient of the fluid is 5 \( \mu m^2/s. \) For this diffusion coefficient, the maximum working period was found by Equation 27 to be about 0.3 s. In our simulation, the working period was 0.2 s.

It is difficult to simulate the process of getting the position based on the intensity of the fluorescence signal of the particle at this phase of study. Therefore, a set of random functions provided in
MATLAB was used for predicting the position of the particle. Figures 5–7 show the positions of both the laser point and the particle. Figure 8 shows the error of the tracking in the XY coordinate plane. In particular, the error is calculated by the following equation:

\[ E_{xy}(k) = |E_x(k)X(k)| + |E_y(k)Y(k)| \] (28)

where:

\[ E_x(k) = |X(k) - x(k)| \] (29)

\[ E_y(k) = |Y(k) - y(k)| \] (30)

X(k) and Y(k) are the positions of the laser point and the particle in the X and Y directions, respectively. x(k) and y(k) are the positions of the particle in the X and Y directions at working period k, respectively.

Figure 9 shows the error of the tracking in the Z direction, which is calculated by the following equation:

\[ E_z(k) = |Z(k) - z(k)| \] (31)

where Z(k) and z(k) are the positions of the laser point and particle in the Z direction at working period k, respectively.

From Figures 8–9, the maximum position error is less than 200 nm for either the laser in XY plane or the stage in Z direction. In the worst scenario, if the position of the particle is 200 nm away the centre of the laser point in both the XY plane and Z directions, about 99% of the particle’s can still be collected by using Equation 1 with a 514 nm excitation wavelength. Figure 10 shows the velocity of the laser movement in the XY plane, which is calculated by the following equation:

\[ v_{xy}(k) = \sqrt{v_x^2(k) + v_y^2(k)} \] (32)

where:

\[ v_x(k) = \frac{|X(k) - X(k-1)|}{t} \] (33)

\[ v_y(k) = \frac{|Y(k) - Y(k-1)|}{t} \] (34)

In Equations 27 and 28, t is the working period and X(k) and Y(k) are the positions of the laser point in X and Y directions at working period k, respectively.

Figure 11 shows the velocity of the stage movement in the Z direction which is calculated by the following equation:

\[ v_z(k) = \frac{|Z(k) - Z(k-1)|}{t} \] (35)
Figures 10 and 11 show the velocities in the XY plane and Z direction that are needed to track the particle at each working period. Both velocities are less than 100 µm/s, which are easily attainable by micro motors.

Figure 5: X-coordinate of the positions of the laser point and the particle (green curve: laser position; blue curve: particle position).

Figure 6: Y-coordinate of the positions of the laser point and the particle (green curve: laser position; blue curve: particle position).
Figure 7: Z-coordinate of the positions of the laser point and the particle (green curve: laser position; blue curve: particle position).

Figure 8: Position error of the control system in the XY plane (the maximum error is less than 200 nm).
Figure 9: The position error of the control system in the Z direction (the maximum error is less than 200 nm).

Figure 10: Velocity of the laser for tracking the particle in the XY plane.
6. Conclusion
The basic idea behind the control system by which the laser can track the particle that is in Brownian motion was to make use of the feedback of the intensity of the particle’s fluorescence signal to determine its position, and to update the position of the laser point. A proper working period need to be set up beforehand and the equation for computing the maximum working period must be developed. The selected working period should be less than the maximum working period. The controller that we designed in this paper can track a small particle in fluid with Brownian motion. The simulation of our control system shows that the position tracking error is less than 200 nm at any working period. The velocities needed by the laser and stage to track the particle are less than 100 µm / s. This velocity is easily attainable using micro motors.

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