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3D Locomotion and Fractal Analysis of Goldfish for Acute Toxicity Bioassay

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***Abstract*—**Biological reactions of individuals of a testing animal to toxic substance are unique and can be used as an indication of the existing of toxic substance. However, to distinguish such phenomenon need a very complicate system and even more complicate to analyze data in 3 dimensional. In this paper, a system to evaluate in vitro biological activities to acute toxicity of stochastic self-affine non-stationary signal of 3D goldfish swimming by using fractal analysis is introduced. Regular digital camcorders are utilized by proposed algorithm 3DCCPC to effectively capture and construct 3D movements of the fish. A Critical Exponent Method (CEM) has been adopted as a fractal estimator. The hypothesis was that the swimming of goldfish to acute toxic would show the fractal property which related to the toxic concentration. The experimental results supported the hypothesis by showing that the swimming of goldfish under the different toxic concentration has fractal properties. It also shows that the fractal dimension of the swimming related to the pH value of FD ≈ 0.26pH + 0.05. With the proposed system, the fish is allowed to swim freely in all direction to react to the toxic. In addition, the trajectories are precisely evaluated by fractal analysis with critical exponent method and hence the results exhibit with much higher degree of confidence.

***Keywords*—**3D locomotion, bioassay, critical exponent method, CEM, fractal analysis, goldfish.

I. Introduction

SINCE the industrial revolution, a massive range of natural and synthetic products, including many toxins, have been created, a large quantities of these are released, deliberately, accidentally, or even as unmeasured waste products into the environment. Thus, the authors introduced a novel method which aims to quantitatively analyze acute toxicity bioassay data of 3D locomotion of goldfish (Carassius Auratus) by using Critical Exponent Method - Fractal Analysis.

Bioassay is a term for biological assay and is normally a type of in vitro experiment. They are conducted to measure the effects of a substance on a living organism. Bioassays may be qualitative analysis or quantitative analysis. Qualitative bioassays are often involving an estimation of the concentration or potency of a substance by measurement of the biological response that it produces. Quantitative bioassays are typically analyzed using the methods of biostatistics. Bioassays are essential in the development of new drugs or health products. The use of bioassays include measurement of the pharmacological activity of new or chemically undefined substances, investigation of the function of endogenous mediators, determination of the side-effect profile, including the degree of drug toxicity, and measurement of the concentration of known substances [1], [2].

An attempt to study the determination of the side-effect profile on the household bleach to the fish swimming behavior was revealed. Nowadays, bleaches are widely used to decolorize materials such as paper and wood in industry, disinfect drinking water in domestic water supply and whiten cloth in laundry. Bleach works by the process of oxidation or the alteration of a compound by the introduction of oxygen molecules. A stain is basically a chemical compound, and the addition of bleach breaks down the molecules into smaller elements so that it separates from the fabric. Conventional Bleach contains two types of bleaching agents: peroxygen bleaching agents such as hydrogen peroxide and sodium perborate, and chlorine-based bleaches, such as sodium hypochlorite [3]. Sodium hypochlorite (NaOCl) is a chemical compound consisting of sodium, oxygen, and chlorine [4]. 12% solution is widely used in waterworks and 30% solution high-test hypochlorite is commonly used in swimming pools. When sodium hypochlorite in the bleach dissolves in water, two substances form, which play an important role in for oxidation and disinfection. These are hypochlorous acid (HOCl) and the less active hypochlorite ion (OCl^-). The pH of the water determines how much hypochlorous acid is formed. Sodium Hypochlorite reacts with organic material which can cause skin burn, eye damage and severe respiratory distress [5], [6].

Household bleach used in laundering clothes contains 3-6% solution of sodium hypochlorite at the time of manufacture and gradually decreases when it was heated or after long storage [7]. The amount of 50 to 250 ml per load is usually recommended for a standard-size washer. Hot water increases the activity of the bleach, due to the thermal decomposition of hypochlorite, which ultimately generates environmentally undesirable corrosive gases including chlorine (International Chemical Safety Card 0482).

Among test organism, goldfish has long been used effectively in various expect of bioassays including

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toxicological studies [8]-[10]. Also, Guide for proper care of goldfish and disease diagnosis has been well published [11]. Its various biological activities, including swimming behavior and respiration, can be use as measuring indicator substances [12]. However, biological activity is very complex and irregular, therefore traditional method such as Euclidian geometry is insufficient to describe them.

Fractal geometry was introduced by Mandelbrot [13] to describe irregular and complex structure as in nature. There are various methods to evaluate fractal geometry such as Box-Counting Dimension, Capacity Dimension , Correlation Dimension, Information Dimension, Hausdorff Dimension, Hurst Exponent, Lyapunov Dimension [14], [15], q-Dimension [16]. Fractal information may have non-uniform scaling characteristic which difficult to uniquely determine the scaling index. Hence, Nakagawa [17], [18] was proposed a critical exponent method (CEM) to estimate the FD of self-affine time series information by obtain critical exponent of the moment of power spectral density to overcome such a problem. CEM has been successfully adopt to estimate FD in various application such as distinguish vocal sound [19] and motor imagery tasks [20].

Various methods to automate extract swimming trajectories were done in 2D where the water was shallow to prevent fish from swimming out of the camera's focal plane and to minimize errors due to changing swimming depth [21]. Instead, by allowing goldfish to swim freely in 3D, the swimming pattern exhibit more precise and provide improved results compare with 2D analysis [22]-[24]. To obtain 3D coordinate of an object along planar axis by using computer system, special 3D camera including stereoscopic camera, single optical sensor with Time-Of-Flight depth camera [25] is used. To enhance the use of ordinary digital video camcorder and reduce the cost from using special camera, a novel method to obtain 3D coordinate using two regular digital video camcorders was proposed. The camera were placed in front and beside of the transparency aquarium. Then, the 3D coordinate can be computed from the proposed equations. With this approach, it allow biologist to effectively adopt the regular digital video camcorder to efficiently measure the biological activities of a testing animal or substance.

II. Materials and Methods

A. Raising Fish

A couple of approximately 3 inch goldfishes were bought from local pet shop (Musashi Pet shop). They were maintained in 36L aquarium and treated following the guidelines [26]-[28]. They were fed twice a day with approximately the same time everyday at 10am and 5pm [29]. Aged tap water was used to fill the tank and it was replaced about 80% of the tank once a week. To prevent fish diseases such as ich and fungus, salt treatment [30] and chemical treatment [31] was used, along with the water was maintained at 26'C according to "Water Chemistry," an article written by Mike McEwan from Aquaria Central [32]. Power filter with mechanical and biological filter was used to maintain water quality. The 830 Lumens 60W incandescent light bulb was used to illuminate the tank. The light cycle was controlled by programmable timer for 14 hours of light and 10 hours of dark (LD 14:10) to simulate the daily light [33].

B. Toxicity Experiment

Prior to the experiment, a 400L (119x58x58 cm^3) experimental tank was filled with aged tab water. The aerated water temperature was maintained at 26'C by 1000w aquarium heater (FivePlan SX-K1). A bottle of 600mL household laundry bleach from local glossary store was use for the whole experiments. Its main active agent is Sodium hypochlorite with the pH value of 13.60.

The amount of 4mL of bleach was added into the experimental tank and it was stirred by using aquarium pump for 10 minutes. Then, the fish was introduced to the tank and acclimated for an hour before a trial; this will eliminate the effect of swimming behavior changes according to the change of the tank size and water. After the acclimation is done, the swimming was recorded for an hour in front (XZ) and beside (YZ) of the experimental tank; as illustrated in Fig. 1. The frame size is 720x480 pixels with the frame rate of 30 fps. After the record, the fish was immediately moved back to the aquarium. On the next day, another 4mL was added up to the experimental tank and repeat the same experiment. Each set of experiment take 5 controlled experiments (days) to investigate the response of the fish to the acute toxic load. The maximum accumulated poison of each set of experiment is 0.004% v/v which is about the amount of the solution of bleach recommended to use for normal size washing machine.

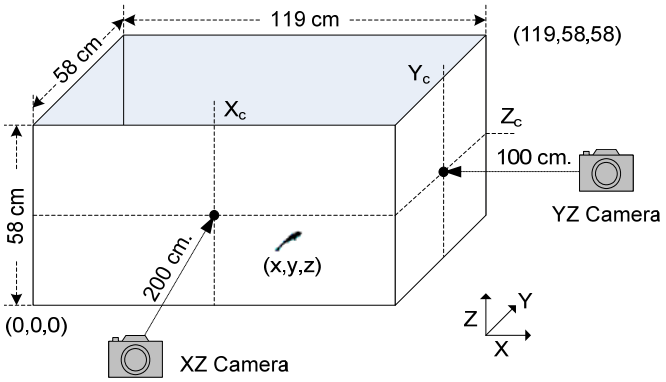


Fig. 1 Experimental setup, digital video camcorders (Sony PC-300K) were placed on front-side (XZ) and beside (YZ) of the 400L plexiglas experimental tank. The distance from the tank to the XZ camera was 200cm and YZ was 100cm. The experimental tank size was 119x58x58 cm^3. The origin's coordinate (0, 0, 0) was at the lower left of the experimental tank.

C. Swimming Trajectory Extraction

The swimming trajectories extraction scheme was shown in Fig. 2. The XZ and YZ videos were captured in MPEG format using Windows® Movie Maker via IEEE1394 connection. Then, the MPEG movies were fed into "Template matching" program to extract the swimming trajectories. Template image was manually obtained from the beginning of video file itself to ease the image registration in template matching process.

The extracted XZ and YZ coordinate, again, were fed into the "3D Coordinate Computation with Perspective Correction" (3DCCPC) to construct the XYZ coordinate to later compute for the fractal dimension.

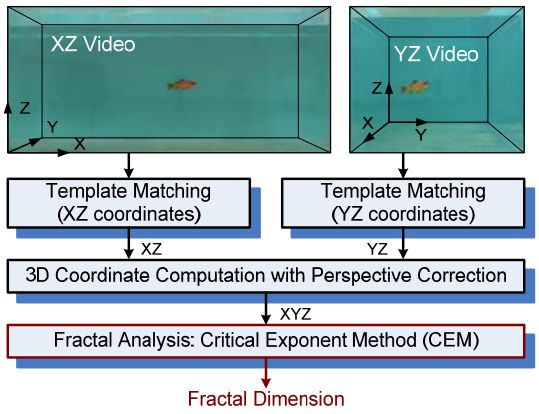
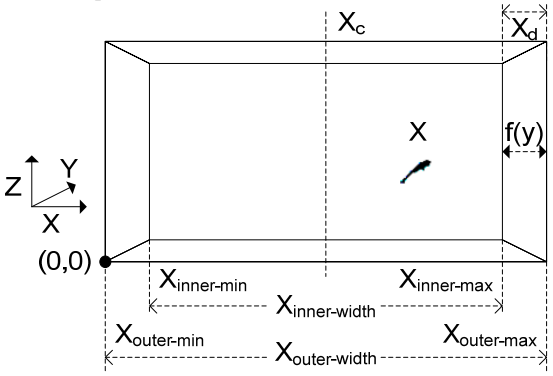
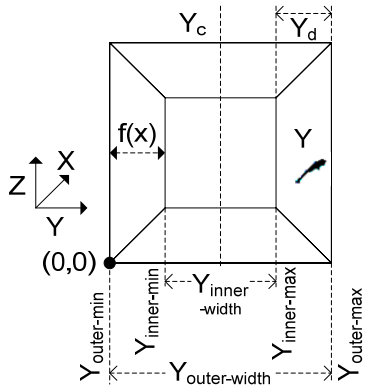


Fig. 2 The workflow of the proposed computation scheme. Fish movements were captured on the front-side and beside of the experimental tank (XZ, YZ videos). Then, the swimming was extracted by using template matching. 3DCCPC construct 3D coordinate from the given extracted XZ and YZ trajectories. Finally, the fractal dimension was quantified by using fractal analysis with critical exponent method as a fractal estimator.



Caption expression for the front-side (XZ) video.
(a)



Caption expression for the beside (YZ) video.
(b)

Fig. 3 Caption expression for the front-side and beside of the experimental tank for the 3DCCPC.

In Fig. 3(a) and 3(b), the captions of the 3DCCPC are shown. Hence, the equations to compute for the coordinates are as following:

$$x' = x + \Delta x,\ y' = y + \Delta y, \text{ and } z' = z + \Delta z \tag{1}$$

where x, y, and z are original coordinate and the x', y', and z' are the new coordinate.

$$\Delta x = \frac{x - x_c}{\left(\frac{x_{innerwidth}}{2}\right)} \cdot x_d \cdot \left(f(y)_{x->\max} + f(y)_{x->0}\right) \tag{2}$$

where

$$f(y)_{x->0} = \left(1 - \frac{x - x_{outer\,\min}}{x_{outerwidth}}\right) \cdot \left(\frac{y - y_{inner\,\min}}{y_{innerwidth}}\right) \tag{2.1}$$

$$f(y)_{x->\max} = \frac{x - x_{outer\,\min}}{x_{outerwidth}} \cdot \left(\frac{y - y_{outer\,\min}}{y_{outerwidth}}\right) \tag{2.2}$$

$$\Delta z = \frac{z - z_c}{\left(\frac{z_{innerwidth}}{2}\right)} \cdot z_d \cdot \left(1 - \frac{x'}{x_{outerwidth}}\right) \tag{3}$$

D. Fractal Analysis estimated by Critical Exponent Method

A critical exponent method (CEM) was proposed by Nakagawa [17] to uniquely estimate a scaling index of a function of fractional Brownian motion [34], [35] which associated to Hurst exponent [36]. The fractional Brownian motion (fBm) with Hurst function (H) is defined as follow:

$$\frac{d^{H+\frac{1}{2}}}{dt^{H+\frac{1}{2}}} B_H(t) \sim w(t). \tag{4}$$

where w(t) denotes a uncorrelated Gaussian noise, $B_H(t)$ is a fractional Brownian function, and t indicate the time. Then, the power spectral density (PSD), $P_H(v)$can be derived as follow:

$$P_H(v) \sim v^{-(2H+1)} = v^{-\beta}. \tag{5}$$

where β denotes a scaling index and H denotes Hurst exponent related to fractal dimension FD as follow:

$$D = 2 - H = (5 - \beta)/2, \text{ where } (0 < H < 1). \tag{6}$$

If the moment exponent is α, then the moment (I_α) of PSD can be defined as follows:

$$I_\alpha = \int_1^\infty P_H(v) v^\alpha dv, \tag{7}$$

$$\text{or, } I_\alpha = \sum_{v=1}^{\alpha} P_H(v) v^\alpha \text{ for discrete time.} \tag{8}$$

The critical exponent (α_c) can be evaluated from the zero crossing point by means of the least square method or third order derivation of log I_a. Then, the critical exponent (α_c) can be derived as follow:

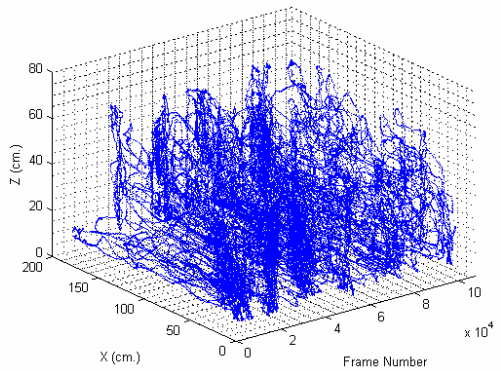
$$\alpha_c = 2H, \text{ where H is Hurst exponent.} \quad (9)$$

Finally, the fractal dimension can be derived as

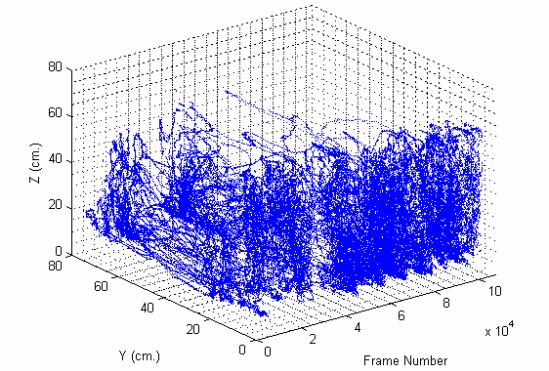
$$FD = 2\text{-}H = 2 - \alpha_c/2. \quad (10)$$

III. Experimental Results

The swimming trajectories of an approximately 3 inch goldfish, under 0 – 0.004% v/v solution of water and sodium hypochlorite, were recorded for an hour by using regular digital video camcorder placed in front and beside of 400L plexiglass experimental tank. With frame rate of 30fps of an hour video, approximately 108,000 frames of each video were extracted by the template matching, as also, 3D coordinate was constructed by the 3DCCPC.



Trajectories plot of front-side video (XZ) versus frame number.
(a)



Trajectories plot of beside video (YZ) versus frame number.
(b)

Fig. 4 The sample plot of about approximately 108,000 coordinate of front-side (a) and beside (b) extracted trajectories of the 0.003% v/v toxic concentration.

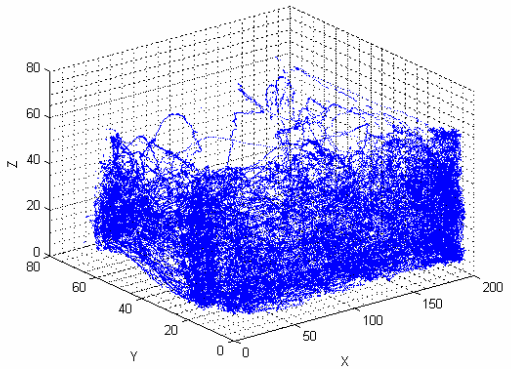
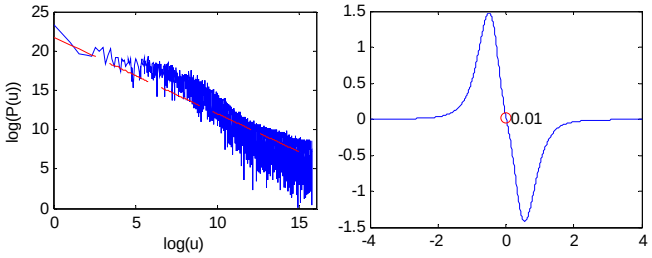


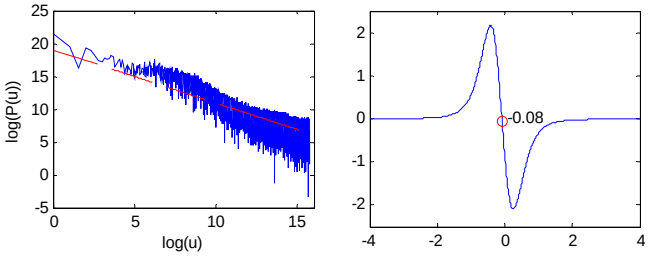
Fig. 5 The sample plot of about approximately 108,000 coordinates of constructed 3D trajectories of the 0.003% v/v toxic concentration.

Fig. 4(a) and 4(b) shows the sample plot of the extracted swimming trajectories of one hour videos under the toxic concentration of 0.003% v/v. Fig. 5 shows the constructed 3D swimming coordinates, results from 3DCCPC, of the swimming under the toxic concentration of 0.003% v/v. Hence, the plotted results show that the swimming trajectory is very complex and irregular. Moreover, there are higher density of swimming activities along the wall of the tank because of the turning movement of the fish; Fig. 4(a) where x is close to 0 and 200 and Fig. 4(b) where y is close to 0 and 80, while the other places exhibit lower density due to the fish swim slowly and calmly.



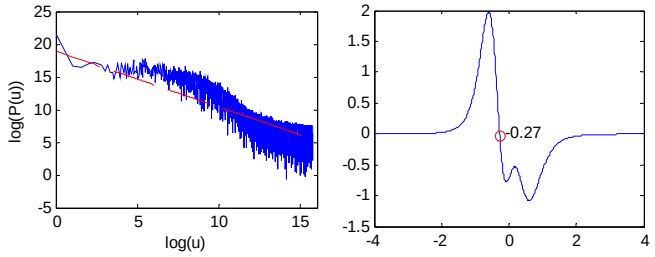
Power spectral density of X axis. (a) Critical exponent of X axis. (b)

Fig. 6 The power spectral density (a) and critical exponent, α_c (b) plots of X axis of the 0.003% v/v toxic concentration.



Power spectral density of Y axis. (a) Critical exponent of Y axis. (b)

Fig. 7 The power spectral density (a) and critical exponent, α_c (b) plots of Y axis of the 0.003% v/v toxic concentration.



Power spectral density of Z axis. (a)

Critical exponent of Z axis. (b)

Fig. 8 The power spectral density (a) and critical exponent, α_c (b) plots of Z axis of the 0.003% v/v toxic concentration.

The power spectral density $P_H(v)$,of the swimming trajectories was calculated by Fast Fourier Transform. The diagonal dash line plot in Fig. 6(a), Fig. 7(a), and Fig. 8(a) illustrate the slope of the log-log plot of the $P_H(v)$ which estimate by linear regression. Fig. 6(b), Fig. 7(b), and Fig. 8(b) show the moment of power spectral density (I_α), along with crossover point, which computed by third order derivation of log I_α. The critical exponent (α_c) which has the relationship with Hurst exponent (eq. 9.) was used to compute for the fractal dimension (eq. 10). As the results, the computed α_c of X, Y, and Z axis were plotted and estimated FD were shown in Fig. 9

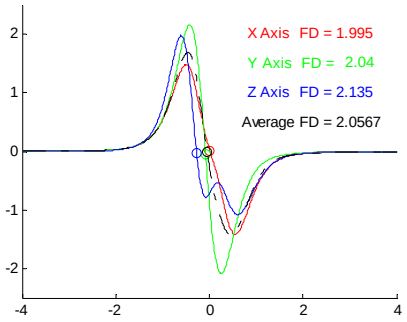


Fig. 9 Average fractal dimension of 2.0567 and cross over points by 3rd order derivation of log I_α (2.5) for the 0.003% v/v toxic concentration.

After a set of trails was completed, the results of pH value versus fractal dimension were plotted in Fig. 10. Also, the relative equation between the FD and pH value was computed by using polynomial fitting equation.

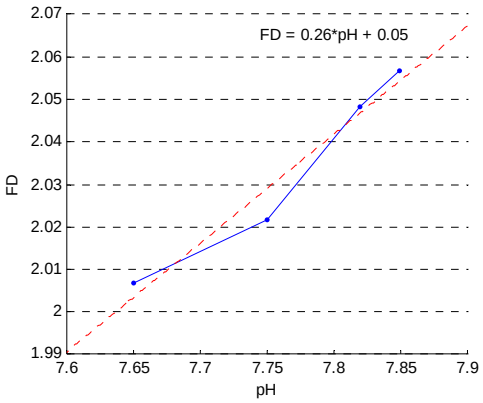


Fig. 10 Average fractal dimension (FD) and pH value of each percent toxic concentration.

IV. Discussion

The results confirmed that the composition of this approach allow us to effectively use regular digital video camcorder to efficiently obtain 3D locomotion and also measure the biological activities of a testing animal or substance. Moreover, the CEM serves as an excellent fractal estimator to uniquely determine the "Hurst exponent", as also, the fractal dimension. Beside, the results show that the locomotion of goldfish has a fractal structure and the change of fractal dimension is correlated to the pH value of the solution. However, different fish has dissimilar swimming pattern [21]. Therefore, the results on different fish are often different.

A. pH swing in toxicity Experiment

There are several reasons to use the pH value as the measuring index opposed to FD value. One is that aquatic organisms thrive only within a particular pH range. This range certainly varies from organism to organism, and it is not easy to justify that any particular range is best for an aquarium with many species. Thus, the experiment was conducted in such a way to control the free factor that will affect the pH value of the solution beside the bleach.

Although, the experimental procedures, equipments, and environment were controlled: exact time of the day to do the experiment, water temperature was set at 26'C ± 1'C, the aeration duration and rate was controlled, amount of sodium hypochlorite was precise measure. However, the pH swing still happened due to other factor such as the ambient temperature of the night before experiment, etc. However, the plotted results shows that the FD value exhibit correlated result to the pH value which indicate that the pH can be effectively use as a measuring index for acute toxicity bioassay of a fish.

B. Coordinate extraction

With the proposed scheme of using regular video camcorders and 3DCCPCP, ones can adopt their digital video camcorders to capture bioactivities of a substance or testing organism in the front-side and beside of a transparent experimental chamber (Fig. 3). Template matching was use to extract the swimming position of the fish.

However, the fish can cause a "noise" to template matching process by make ripple at water line when they swim up to get a breath, also, their body reflection when they swim along the wall of the tank and also when they swim on the most end of the both side of the tank. A correct swimming position can be obtained by selecting the object that closest to the center of the tank (Fig. 1. X_c and Y_c). Then, 3D coordination can be precisely constructed by 3DCCPC (eqs. 1-3).

C. Fractal Analysis estimated by Critical Exponent Method

Although, there are various methods to evaluate fractal geometry Dimension [14]-[16] but none can provide unique scaling index to estimate a fractal dimension. Thus, Nakagawa [17] proposed an innovative method to estimate fractal dimension using CEM.

By determine the slope or zero crossing point of the power spectrum of long-tail self-affine time series information, the scaling index; Critical exponent and Hurst exponent [36] is achieved.

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