

# BIO-LUMINESCENCE/SCATTERING AND ITS DYNAMICS IN ENCHYTRAEUS JAPONENSIS BY IRRADIATION OF FREE ELECTRON LASER

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## Abstract

We report on the experiment results of behaviours of the Enchytraeus Japonensis (EJ) which was irradiated a free electron laser (FEL). The focused one shot of the FEL (Power: 300  $\mu$ J, Wavelength: 2.9  $\mu$ m, Micro pulse width: 200 fsec) which resonated with hydroxyl stretching vibration was irradiated to center of the EJ body and we observed it by change coupled device (CCD) camera. As a result, the reactive products from EJ were generated by FEL irradiation. The reactive products were increased in a few milliseconds. More shot of FEL irradiations, the EJ body was broken and we observed emission of laser ablation from the EJ body.

## INTRODUCTIONS

Earthworms are well known in whole globe which features are that there is the bottom of the food chain, and they work as the soil improvement agent. Exactly, the soil improvements by earthworms are reported on several papers [1-9]. Earthworms ingest the organic substances and microorganisms in the locality soil. Excretory products from earthworms are promoted effective use as plant growth and good farming land, generally. In addition, there are the earthworms which can ingest the heavy metal (Cd, Pb, Zn etc) [3-9]. These earthworms are expected to detoxifying source for infected soil.

Currently, earth worms which ingested the heavy metal are using for assessment of soil contamination. However, it is required the great care because this assessment method needs large amount of earthworms and processes. To assess the soil contamination by earthworms, first, these earthworms are made to be powders by drying and gridding. After, pollution materials in the soil are analysed by extracted solutions from those powders.

In order to simplify these processes, we focused on the laser ablation techniques [10-11]. The laser ablation is observed from laser irradiation area which wavelength is dependent on the laser irradiated materials. Moreover, laser irradiated materials are excited and emit photoluminescence. These emission spectra are used the qualitative analysis of laser irradiated materials. Therefore, we consider that it is effective method for soil contamination assessment to measure the emission spectra from the earthworm by laser irradiation.

A free electron laser (FEL) is generated by Laboratory for Electron Beam Research and Application (LEBRA), Nihon University [12-14]. This FEL light is irradiated to earthworm as a laser ablation source because this laser wavelength can tune up with the molecular vibration of the chemical component in earthworm, the pulse width of this laser is about a few hundred of femtosecond; Thermally damage is less affect by laser irradiation. The earthworms are used Enchytraeus Japonensis (EJ) [15-16].

In this paper, we report the bioluminescence behaviour from EJ body by LEBA-FEL irradiation.

## EXPERIMENTS

### *Selection of Free Electron Laser Wavelength for the Enchytraeus Japonensis by FT-IR*

Infrared light absorption of the EJ was measured by fourier transform infrared spectroscopy (FT-IR). Living EJs were gridded on sappier substrates. Those samples were annealed (Temperature: 200 degree, Time: 10 min) to evaporate the H<sub>2</sub>O in EJ. We conducted the FEL wavelength from FT-IR spectrum result.

### *Free Electron Laser Irradiation to the Echytraeus Japonensis*

Figure 1 shows the FEL irradiation system for EJ. A living EJ is put on the quartz substrate as a sample. This sample set up on the X-Y stage. The focused FEL (Power: 300  $\mu$ J, Wavelength: 2.9  $\mu$ m) was irradiated to the EJ. Number of FEL shot was 6 shots. Interval of time between FEL shots was 5 second. Behaviours of laser irradiating EJ were observed by change coupled device (CCD) camera. The quality of FEL is as shown below. Macro pulse interval: 500 ms, Macro pulse width: 7.4  $\mu$ s, Micro pulse interval: 350 ps, Micro pulse width: about 200 fs. (Micro pulse wide = 60  $\mu$ m/C  $\cong$  200 fs).

## RESULTS

### *Measurement results of Echytraeus Japonensis by FT-IR*

Figure 2 shows transmittance result of annealed EJ by FT-IR. Wavelengths about 3.42  $\mu$ m and 3.50  $\mu$ m showed clear absorption. These wavelengths were due to

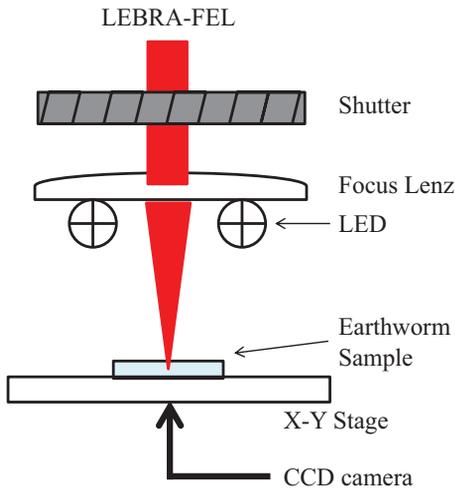


Figure 1: The FEL irradiation system for EJ. A focused LEBRA-FEL (Power: 300  $\mu$ J, Wavelength: 2.9  $\mu$ m, Micro pulse width: about 200 fs) was irradiated to the living EJ. Number of FEL shot was 6 shots. Interval time between FEL shots was 5 second Behaviours of laser irradiating EJ were observed by change coupled device (CCD) camera.

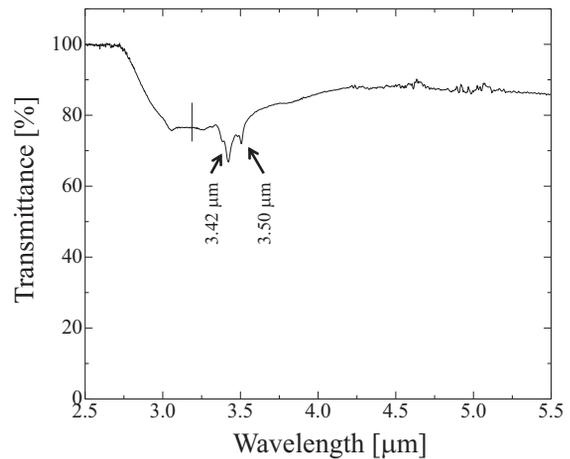


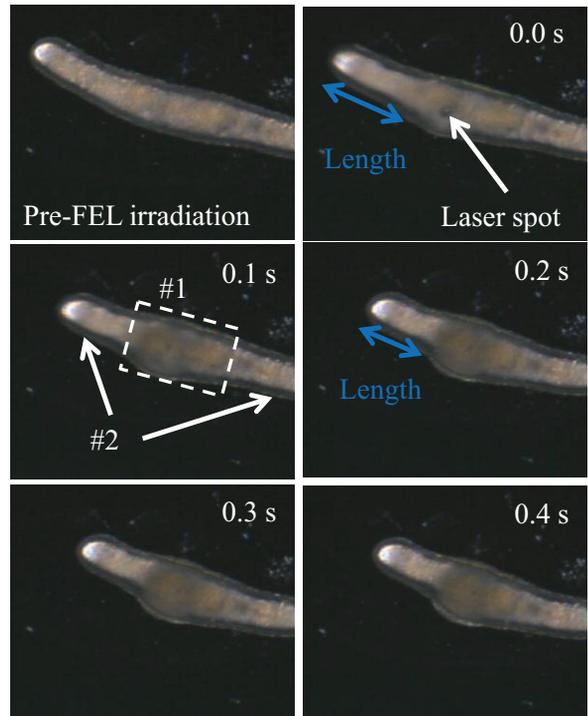
Figure 2: Infrared light transmittance of annealed EJ by FT-IR. This figure shows same absorption peak which due to the CH<sub>2</sub> vibrations. Region between 2.7 and 3.0 was decreasing the transmittance amounts.

CH<sub>2</sub> vibrations (3.42  $\mu$ m: antisymmetric stretching, 3.50  $\mu$ m: symmetric stretching). The wavelength region between 2.7 and 3.0 was broad absorbing. This region has several absorption modes such as -OH, -NH et al. We decided the FEL wavelength was 3.0  $\mu$ m because there is fear that the FEL irradiation of 3.42  $\mu$ m or 3.50  $\mu$ m were destroyed the EJ body.

### FEL Irradiation to Echytraeus Japonensis

Figures 3 show the CCD camera image of EJ which irradiated the one shot of FEL. We compared non laser irradiation sample (Pre-FEL irradiation) and 1 shot irradiated samples. 0 s means the moment of FEL irradiation. The 0 s sample observed FEL laser spot which size is about 50  $\mu$ m. The 0.1 s sample shows there was different of the pigment colors in EJ. Therefore, this image divided two regions. One is the reactive product region by FEL irradiation (#1). The other is the stability region (#2). #1 region of 0.1 s sample was larger than that of the 0 s sample. Moreover, #1 region became large after 0.1 s. Contrast, the length of #2 was shorter by laser irradiation. This indicated the EJ was reacted by FEL irradiation, and EJ was produced something around laser spot.

In order to clearly the variation of #1 region, we calculated the areas of #1 region and all of EJ.  $S_{\#1}$  means area of #1 region.  $S_{all}$  means all of EJ area. And  $S_{\#1}$  was divided by  $S_{all}$  [ $(S_{\#1}) / (S_{all})$ ]. Figure 4 shows relationship between amounts of  $(S_{\#1}) / (S_{all})$  and elapsed time of one shot of FEL. In case of from 0 s to 0.4 s,  $(S_{\#1}) / (S_{all})$  amounts was increasing. After 0.4 s,  $(S_{\#1}) / (S_{all})$  amounts



Figures 3: CCD camera image of the LEBRA-FEL irradiated EJ (Laser wavelength: 2.9  $\mu$ m, Power: 300  $\mu$ J). After one shot of FEL laser, reactive productions were appeared (#1 region).

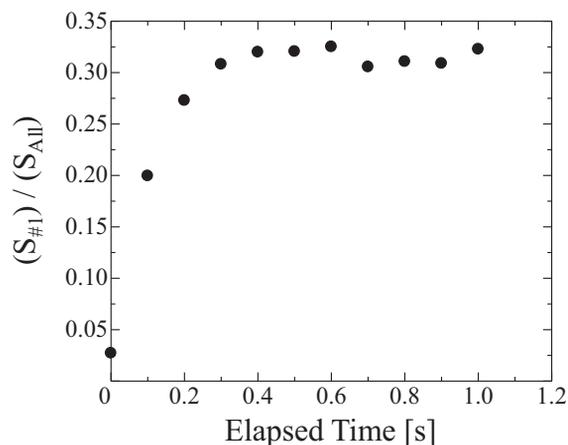
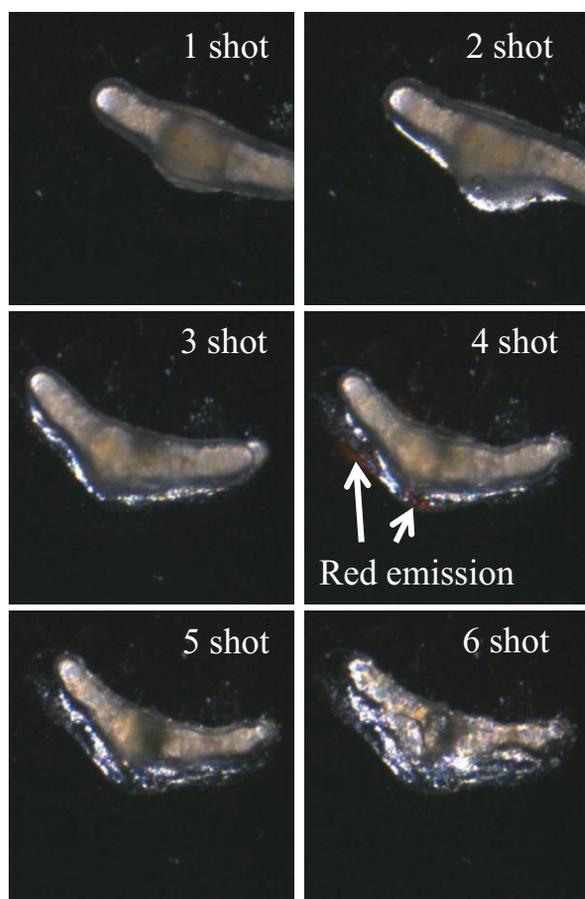


Figure 4: Relationship between amounts of  $(S_{\#1}) / (S_{all})$  and elapsed time of one shot of FEL.  $S_{\#1}$  is areas of #1 region in figure 3.  $S_{all}$  is all of EJ areas.  $(S_{\#1}) / (S_{all})$  amounts were increasing and saturating in a few milliseconds.



Figures 5: EJ behaviours by LEBRA-FEL irradiations. Over the 2 shots of FEL were observed white liquid around the edge of EJ. Red emission was observed in the 4 shot sample.

were saturated (about 0.32). These results indicated reactive products in EJ were not generated in a moment by laser irradiation, were slowly grown in a few milliseconds.

Figures 5 show the EJ behaviours of FEL shots by CCD camera. As noted previously, The 1 shot FEL irradiation sample had reactive production region. The 2 shot FEL irradiations sample observed white liquid at the edge of the EJ, and there was the reactive production region in this sample too. The 3, 4, 5 shot FEL irradiations samples were similar to 2 shot sample. However, the pigment colors of EJ body in 2, 3, 4 and 5 shots samples were whitely than that of the 1 shot sample. The 6 shots sample showed its all of body became white liquid status. This means number of laser irradiations could be broken the EJ body. Separately, red emission from the EJ was observed in 4 shot irradiations sample. We considered that this emission had possibility to study the qualitative analysis in the EJ. It is our future subject to measure this spectrum.

### CONCLUSIONS

We investigated the behaviour of the laser irradiated EJ using LEBRA-FEL. One shot of FEL (wavelength: 2.9  $\mu\text{m}$ , micro pulse width: 200 fsec) irradiation was generated the reactive products from EJ body. The reactive products were observed around the FEL irradiated spot and increased these products in a few milliseconds. Emission from EJ body was observed by 4 shots of FEL irradiated sample. More shot of FEL, the EJ body was broken. Our future issues are measurement of emission from EJ body for conducting components in this body.

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