# DEVELOPMENT OF A MICROSCOPIC IRRADIATION TECHNIQUE FOR DELIVERING VIS-FELS TO SINGLE CELLS THROUGH A FINE-TAPERED GLASS ROD

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*Abstract*

This paper describes a microscopic irradiation system for delivering visible-free electron lasers (VIS-FELs) to single cells through a fine-tapered glass rod. The VIS-FELs of the Laboratory for Electron Beam Research and Application of Nihon University are of the higher harmonics generated by non-linear optical crystals and cover VIS to near infrared (NIR) spectra encompassing 0.35 to 6.5 microns. When using fine-tapered glass rods, we usually coat the whole surface with Ag-film using the Tollens' mirror reaction. The FEL power at the tip of the tapered glass rods depends on the form and diameter of the tip, wavelength, and FEL energy at the exit port. However, the current power at the tip is enough to stimulate the cell function; for example, the power of a 540-nm FEL in the microscopic irradiation system is 2.5-5.5 μJ/pulse with a 5-μm tip, which is equivalent to the energy density used in low-energy laser experiments of VIS and NIR. By using this technique, two preliminary irradiation experiments were carried out: one for single cells (in the resting stage) of green algae stimulated at 420 nm, 540 nm, or 660 nm, and the other for medaka (a bony fish: *Oryzias latipes*) embryos. When irradiating late-developing embryos (two days before hatching) at 540 nm, the guanophores and the margin of nerve tissue reflected strongly. At this time, however, we have not been able to attribute a significant biological meaning to the reflection observed in the irradiation experiments on living organisms.

## INTRODUCTION

The first lasing at the Laboratory for Electron Beam Research and Application (LEBRA) of Nihon University was successfully conducted in 2001 to produce near infrared (NIR) free electron lasers (FELs) with tunable wavelengths from 0.82 to 6.5 microns [1, 2], in which the higher harmonics generated by non-linear optical crystals covered visible (VIS) and NIR spectra encompassing 0.35 to 6.5 microns [3]. The LEBRA-FEL has pulse parameters of: 200 fs full width at half maximum, 350 ps inter-pulse spacing, 20 μs long macro-pulse duration, 2 Hz repetition rate, and 10 MW peak power.

Among the many photochemical and photo-biological applications [4-7], VIS-FELs are of particular interest and are expected to reveal photo-biochemical reactions of cultured single cells, even those in living organisms. To achieve this, it is a prerequisite to develop a micro-irradiation technique for targeting single cells without inducing photochemical effects to neighbouring cells.

Recently, we established a microscopic irradiation technique with VIS-FELs through a fine-tapered glass rod [8]. The FEL delivered through the rod with a tip diameter of about 5 microns or less has two major advantages over conventional microscopic FEL systems using a FEL focused by mirrors and a $CaF_2$ lens [9]. The first advantage is the ability to deliver the tunable FEL directly to targeted single cells in accordance with micro-manipulator injection techniques used in fields such as developmental biology and reproductive medicine [10]. The second advantage is the ability to irradiate specific areas such as the cell-surface and the micro environment of the cytoplasm, including cellular organelles, without severely damaging the targeted cells. Our microscopic irradiation technique, therefore, is the most direct, reliable, and quantitatively reproducible technique of delivering EFLs of any wavelength into single cells of certain living organisms. We also report here the use of this technique for preliminary microscopic irradiation experiments on targeted single cells in living organisms.

## EXPERIMENTAL SETUP

### *FEL Irradiation System*

Figures 1-A and 1-B show a schematic and a photograph, respectively, of the FEL microscopic irradiation system set up on an optical bench at LEBRA. This system consists of five parts: (1) a focusing part consisting mirrors and a $CaF_2$ lens, (2) a FEL transporter delivering FEL using a 1000-μm jacketed plastic optical fibre (NT02-536, Edmund Optics Inc., NJ), (3) a connector of unjacketed plastic optical fibre and a 1-mm glass rod (G-1000, Narishige Croup, Tokyo, Japan) in a metal tube, (4) a micro-irradiator of a glass rod holder (H-7, Narishige Group) manipulated by two micro-manipulators (MN-153 and MMO-220A, Narishige Group), and (5) a microscope (MXZ-2B, Sigma-Koki, Tokyo, Japan) connected to a high-resolution video camera (DFK22BUC03, The Imaging Source Europe GmbH, Bremen, Germany), which is in turn connected to a computer. The viewer software is IC Capture (The Imaging Source Europe GmbH), an accessory of the video camera. The connector of the metal tube enclosing the plastic optical fibre and glass rod is filled with a small quantity of microscopic emersion oil (optical density:

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1.54), and the plastic fibre and glass rod connect together. Furthermore, the position of the tip can be moved in four ways: horizontally (sagittal [X] and transverse [Y]), vertical [Z] and tilt angle [T]. When the tip is inserted into targeted single cells to irradiate them with FEL, the glass rod is held to the microscope stage at an angle of 60-70 degrees and penetrates the outer membrane of the targeted cells by vertical ([Z] axis) movement using the MMO-220A manipulator.

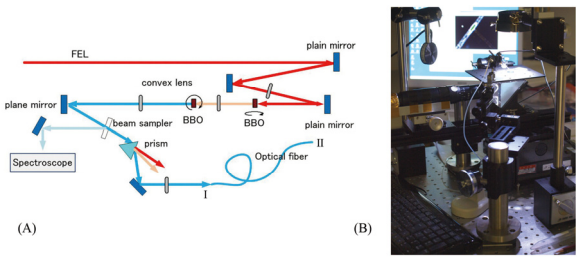


Figure 1: Schematic (A) and photo (B) of the microscopic irradiation system for VIS-FELs. The end of optical fibre (II) connects to the tapered-glass rod shown in (B). Details are described in the text.

## *Preparation of Fine-Tapered Glass Rods*

The tapered-glass rods were fabricated with a micro-pipette puller (PE-2 or PC-10, Narishige Group). Figure 2 shows the four parts of the two pulled tapered-glass rods: tip, shank, shoulder, and shaft. Each of the two tips is cut differently in diameter: one is at 5 microns, the other is at 10 microns. The shape (length and angle of shank) and diameter of the tip depend on current, pulling tension, and length of gravity fall in the case of a vertical puller (PE-2 or PC-10). A wide variety of glass-rod shapes can be achieved for microscopic irradiation experiments by empirically adjusting values for the various parameters in the pulling sequence, two of which are depicted in Figure 2.

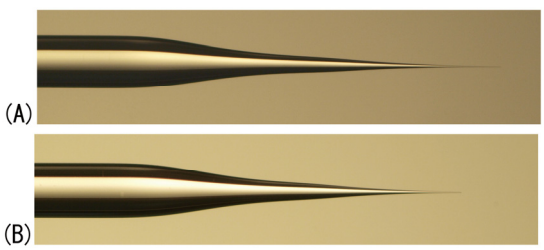


Figure 2: Tapered glass rods. (A) 5-μm tip, (B) 10-μm tip. Both rods were fabricated under the same conditions, and the cut was done at a diameter of 5-μm or 10-μm, respectively.

After pulling, the tapered-glass rods are usually coated with Ag film using Tollens' reagent and glucose (a reducing reagent) described elsewhere [11]. The Ag-coated tips are fairly good at preventing light transmission through the glass rods. When we used them, the Ag-coated tips were flexible enough to provide a delivery of FEL.

## *FEL Power at the Ag-Coated Tips*

When measured, the FEL energy delivered from the Ag-coated tip was very low compared with that of initial FEL measurement (5 mJ/pulse) at the exit port. Values are summarized in Table 1, indicating that the FEL power at the tip is about 3-5 μJ/pulse when used at 540 nm. The huge decrease in FEL power occurs: each ten-percent power loss was detected at three points: I, II, and the ejection site at the tip of the tapered-glass rod (Fig. 1). The FEL power also decreased when a new high harmonic generation was processed. Hence, we need improve the FEL transporting lines; however, the power at the tip might not be too weak for FEL irradiation experiments because low-energy laser (LEL, [12]) irradiation experiments using conventional lasers have been done at a laser power of 0.42-15 J/cm$^2$ (488-515 nm from an Argon laser, [13]), 5 J/ cm$^2$ (660 nm from an Argon laser, [14]) or 0.5 J/cm$^2$ (632 nm from a He-Ne laser, [15]), and consequently these conventional lasers could stimulate biological functions of cellular sources such as human tumor cells, peripheral blood lymphocytes, yeast, sperm, and fibroblasts.

Table 1: FEL Power at the tip of the Fine-Tapered Glass Rods

| FEL | FEL power (μJ/pulse)[1,2] |
|---|---|
| 420 nm | 0.5-1.0 |
| 440 nm | 0.5-1.0 |
| 540 nm | 2.5-4.5 |
| 660 nm | 9.0-12.0 |

[1] The FEL power at the tip was measured by a power meter (Field Max II, Coherent, Inc.)

[2] The initial FEL power at the exit port in the user's laboratory is about 5 mJ/pulse.

## *FEL Tip Irradiation Patterns*

In order to record the FEL patterns, a small piece of tracing paper (80g/m$^2$) was laid flat over the hole of the

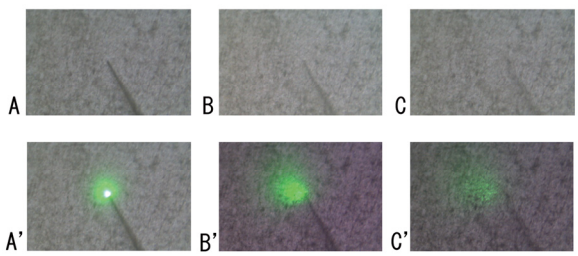


Figure 3: FEL (540 nm) power patterns at the tip of the fine-tapered glass rods. A and A', 0-mm from the tracing paper; B and B', 1-mm from the tracing paper; C and C', 2-mm from the tracing paper. Details are described in the text.

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mechanical stage and above the objective lens of the microscope. The FEL irradiation-key was switched on and the tip of glass rod was placed on the surface of the tracing paper (Fig. 3-A') and then, FEL patterns at 1-mm intervals from the surface of the tracing paper were recorded onto video. The fine vertical movement of the [Z] axis was accomplished by the micro-manipulator (MN-153, Narishige Group). The obtained FEL irradiation patterns are shown in Figure 3: it is difficult to see the figure when the tip moved more than 3-mm over the surface of the tracing paper (the target).

## FEL IRRADIATION EXPERIMENTS

In general, it is difficult to insert the fine tapered-tip of glass rods into plant cells, which have cell walls; therefore we first tried to irradiate the targeted cells by touching the surface of the cell wall. An example is shown in Figure 4 (2 through 4). Each of the targeted cells was irradiated by the FEL at three wavelengths (420 nm, 540 nm, and 660 nm), At 540 nm, part of the resting bud strongly and clearly reflected the FEL. In control experiments without FEL irradiation, the green structures started budding within two weeks and developed new stems (data not shown). To date, we have not demonstrated any significant change in the growth of buds after FEL irradiation, even at 420 nm (Fig. 4-2) and 660 nm (Fig. 4-4) which are known to be the absorption maxima wavelengths of chlorophyll.

The second preliminary experiment was carried out using medaka (a bony fish: *Oryzias latipes*) embryos. Figure 4-7 shows the 540-nm FEL microscopic irradiation experiment of a young embryo at stage 35 according to the description of Iwamatsu [16]. Some areas are reflected strongly, in particular guanophores and the margin of nerve tissue.

We were also able to deliver a FEL at 540-nm and other wavelengths into the cytoplasm of the embryos by inserting the tip through the chorion, the outer membrane surrounding the embryo, to irradiate the organelles in the medaka embryo (Fig. 4-8) at 540 nm. We detected several structures with strong reflection; however, we have not yet deduced any significant meaning. We are planning to analysis gene expression of the medaka embryos after irradiation with tunable VIS-FEL.

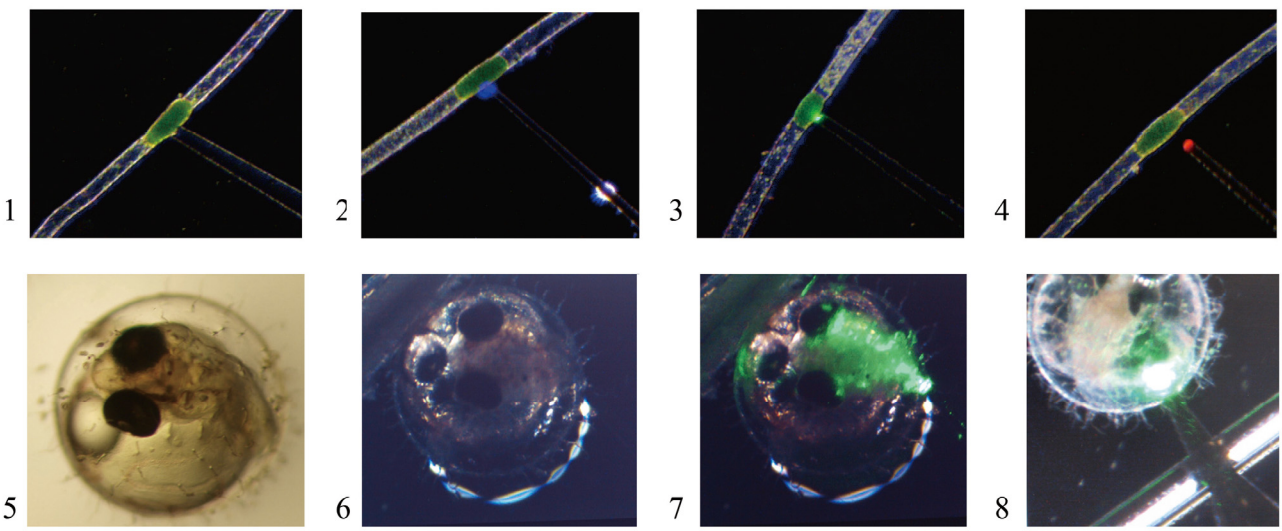


Figure 4: FEL microscopic irradiation experiments (1 through 4: single cells of green algae, 5 through 8: medaka embryos). 1, control; 2, irradiation at 420 nm (blue laser); 3, irradiation at 540 nm (green laser); 4, irradiation at 660 nm (red laser), 5, control under a dissection microscope; 6, control under the irradiation system microscope; 7, irradiation at 540 nm (green laser); 8, irradiation at 540 nm (green laser) by inserting the tip through the chorion. Note that some strongly reflected areas (green) were observed: the guanophores and the margin of nerve tissue.

## CONCLUSION

We developed and improved a microscopic irradiation technique for delivering VIS-FELs onto or into targeted single cells through a fine-tapered glass rod. At present, this technique is the most reliable and direct method of delivering tunable wavelengths of VIS-FELs into living organisms within a precisely controlled space and with a precisely controlled energy. However, there are several disadvantages with this technique: one is physical damage of the targeted cells, particularly when they are small. The two preliminary experiments indicated that strongly reflected sites were observed in the resting buds of green algae and the guanophores of medaka embryos and might stimulate the biological function of these structures. We are in the process of analysing the present findings.

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