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Femtosecond laser-fabricated biochip for studying symbiosis between *Phormidium* and seedling root

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Abstract We present the fabrication of a waveguide-like structure in a polydimethylsiloxane (PDMS) polymer substrate using a femtosecond laser to study the mechanism of symbiosis between filamentous cyanobacteria, *Phormidium*, and a seedling root. While symbiosis occurring underground promotes the growth of vegetable seedlings, the details of the mechanism remain unclear. Understanding the mechanisms of *Phormidium* gliding to the seedling root will facilitate improving the mat formation of *Phormidium*, which will lead to increased vegetable production. We assumed a symbiosis mechanism in which sunlight propagates through the seedling root and is scattered underground to guide the *Phormidium* gliding. Once attached to the root, *Phormidium* uses the scattered light for photosynthesis. Photosynthetic products, in turn, promote an increase in *Phormidium* mat formation and vegetable growth. To verify this assumption, the optical characteristics of the seedling root were investigated. A waveguide-like structure with the same optical characteristics of the root was subsequently fabricated by femtosecond laser in PDMS polymer to assess the light illumination effect on *Phormidium* behavior.

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1 Introduction

Phormidium is soil-dwelling unicellular and colonial cyanobacteria that form motile filaments. These filaments migrate from the main biomass to nearby seedling roots where they form a new mat. Formation of the mat around the root accelerates the growth of the seedling. Therefore, understanding the factors that induce *Phormidium* gliding and promote mat formation around the seedling roots is very important for developing a means to accelerate the growth of vegetable seedlings. The mechanism of the gliding movement has been studied for many years [1, 2] using conventional observation methods by fixing *Phormidium* and a seedling root on agar gel in a Petri dish. However, due to its gelatinous cell walls, *Phormidium* adheres strongly to the agar gel. Therefore, the time required to observe the gliding movement is typically a day or more. Moreover, as the seedling grows, its root penetrates into the semi-transparent agar gel, making it difficult to investigate the symbiosis mechanism.

The femtosecond (fs) laser is a promising tool for fabricating three-dimensional (3D) microstructures in transparent materials due to its ability to internally modify materials using multiphoton absorption [3, 4]. This feature can be used to directly fabricate optics such as waveguides and microfluidic structures in transparent materials in order to analyze biochemical reactions. Crespi et al. [5] employed a 3D unbalanced Mach–Zehnder interferometer comprising two waveguides in a commercial fused silica substrate to enhance the sensitivity of concentration measurements of biochemical liquids. Sugioka et al. [6] developed optofluidics comprising microfluidic channels, waveguides, microlenses, and optical filters in a single photostructurable glass substrate, using the fs laser direct-writing technique followed by hydrofluoric acid etching. Using the functional

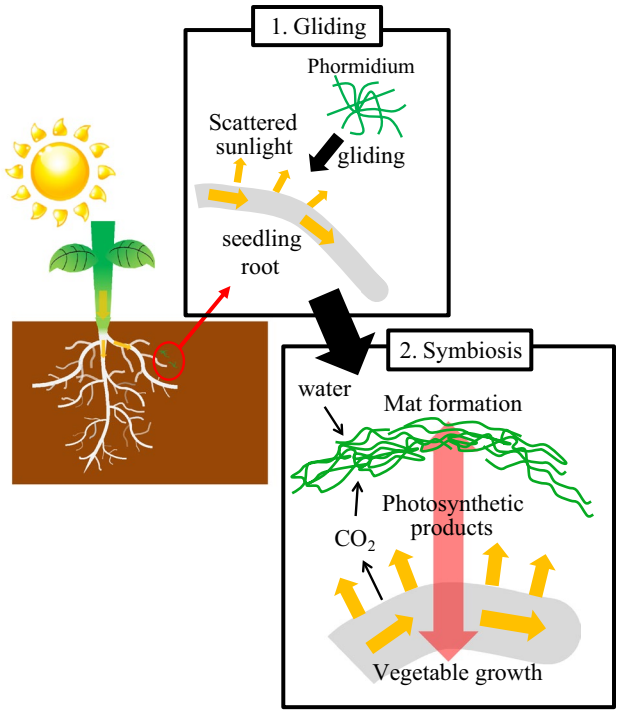

Fig. 1 Proposed underground symbiosis mechanism. After gliding, *Phormidium* undergoes photosynthesis around the root. Photosynthetic products increase mat formation by *Phormidium* and promote vegetable growth

glass biochip, they recently revealed that CO2 secreted from a seedling root is a possible attractant for *Phormidium* gliding. Moreover, they found that *Phormidium* only glides to the root under red-light illumination. However, how the light illuminates underground during symbiosis in nature and the light illumination effect on *Phormidium* behavior were not discussed in detail [7].

Therefore, in the present study, we used a transparent polydimethylsiloxane (PDMS) polymer substrate containing a waveguide-like structure fabricated by fs laser to investigate the light illumination effect on *Phormidium* behavior during symbiosis. The fabricated PDMS biochip reduces the time required to observe *Phormidium* gliding and its mat formation since on-chip observation can be carried out within the field of view of a microscope. In addition, *Phormidium* can glide more smoothly on the PDMS biochip than on conventional glass biochips due to hydrophobicity of a PDMS polymer (in Ref. 7, the glass microfluidic channel was treated with dimethyldichlorosilane in toluene to make the channel hydrophobic before the microscopic observation of *Phormidium* gliding). Furthermore, the fabricated waveguide-like structure has the same optical characteristics of a seedling root. Thus, the light illumination effect on *Phormidium* behavior can be the sole focus of the investigation. We propose that the following processes occur during symbiosis (Fig. 1): (1) First, sunlight propagates through the seedling root and is scattered underground. (2) Then, sunlight scattered by the root guides the gliding movement of *Phormidium*. (3) Once attached to the root, *Phormidium* uses the scattered light, CO2 secreted from the root, and water in the soil for photosynthesis. (4) Photosynthetic products, in turn, increase mat formation by *Phormidium* and promote vegetable production.

Although details of the photosynthetic products transferred between *Phormidium* and a seedling root during symbiosis remain unclear, in this paper, we optically investigated the symbiosis because light is required for photosynthesis even underground. We measured the optical characteristics of the seedling root and mimicked these characteristics in the waveguide-like structure in the PDMS substrate. We then assessed the light illumination effect at selected wavelengths on the gliding and mat formation behavior of *Phormidium* using the fabricated PDMS biochip.

2 Experimental

2.1 Optical characteristic measurement of a Komatsuna seedling root

To investigate the light scattering characteristics of a seedling root, we prepared a cut piece of Komatsuna seedling root after 3 days of germination. Selected wavelengths (465 ± 60, 620 ± 40, 710 ± 40 nm) from a white light source were introduced through dichroic filters and a ×20 microscope objective lens with a numerical aperture (NA) of 0.46 to the end facet of the cut root with a length of approximately 6 mm. Light at the selected wavelengths was introduced to the root at a constant power of 0.5 μW. The output power of the light scattered by the root was evaluated by passing a laser power meter along the root.

2.2 Fabrication of waveguide-like structure in PDMS polymer

Because the seedling root secretes CO2 that promotes the gliding movement of *Phormidium*, optics with the same light scattering characteristics as the seedling root are required to limit the investigation only to the light illumination effect on *Phormidium* behavior. To mimic the light scattering characteristics of the seedling root, a waveguide-like structure was fabricated in a PDMS polymer substrate using a commercial fs laser (800 nm wavelength, 180 fs pulse width, 1 kHz repetition rate) as a light source. The detailed configuration of the laser fabrication system has been described elsewhere [8]. The laser pulse energy was adjusted using a polarizer and neutral density filters. The 6 mm beam width of the output

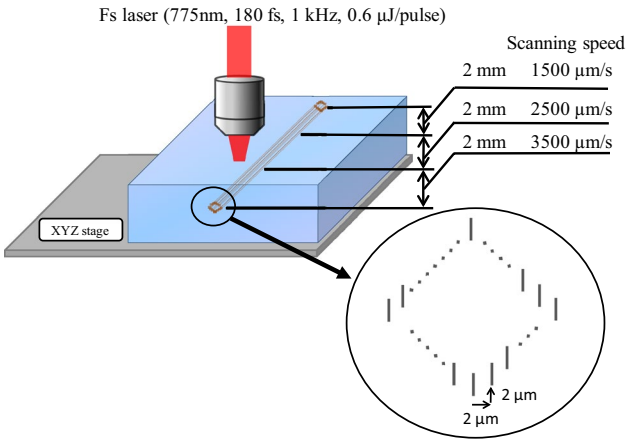

Fig. 2 Schematic illustration of waveguide-like structure in PDMS substrate and enlargement of laser-irradiated end facet

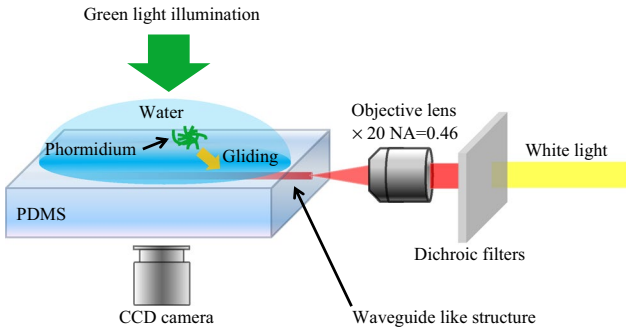

Fig. 3 Schematic illustration of *Phormidium* observation on PDMS biochip illuminated by selected wavelengths of light

laser was reduced to 3 mm by passing it through an aperture to improve the beam quality. The focusing system was a ×20 microscope objective with an NA of 0.46.

Figure 2 shows a schematic illustration of the fabricated waveguide-like structure and an enlargement of the laser-irradiated end facet. The structure was fabricated by translating the PDMS substrate perpendicularly to the laser beam axis using a computer-controlled xyz stage. The laser scanning speed was varied from 1500 to 3500 μm/s at a laser energy of 0.6 μJ/pulse before the objective lens. The waveguide-like structure was formed by a set of parallel tracks comprising a laser-induced damaged core and depressed cladding waveguides [9]. Thirty-eight tracks were formed in the structure to create a lozenge-shaped tube embedded 100 μm below the surface of the PDMS substrate.

To make a 2-mm-thick PDMS substrate, a commercial PDMS solution (SILPOT 184, Dow Corning Toray) was baked in a programmable furnace by ramping the temperature to 80 °C and maintaining it for 1 h. The substrate was then cut and mechanochemically polished along the cut edges to achieve an optical finish. After fabrication of the 3D waveguide-like structure in the PDMS substrate, light scattering measurements were carried out to optically characterize the structure.

2.3 Observation of *Phormidium* behavior using the fabricated PDMS biochip

To observe the gliding behavior, a drop of water containing *Phormidium* was placed on the PDMS biochip (Fig. 3). The *Phormidium* was fixed a distance of 2 mm from the center of the waveguide-like structure (see Fig. 7 for details). To investigate the light illumination effect on *Phormidium* gliding and mat formation, light at selected wavelengths was introduced at the end facet of the structure. The power of the light at each wavelength was set to 0.5 μW before the objective lens. Time-lapse images were acquired using a CCD camera placed beneath the PDMS biochip. During the observation, green light was used to illuminate the top of the biochip, since Hanada et al. [7] reported that green light does not affect the gliding movement of *Phormidium*. The behavior of *Phormidium* was recorded every 30 s for 24 h.

3 Results and discussion

3.1 Light scattering characteristics of Komatsuna seedling root

We first assumed that during symbiosis sunlight propagates through the root and is scattered underground. To confirm this, the optical characteristics of a seedling root were investigated. Selected wavelengths of light from a white light source as a model of sunlight were introduced to 6-mm-long cut pieces of almost straight seedling roots, and the output power of light scattered by the seedling root was measured as a function of the root length. Figure 4 shows the results for a wavelength of 620 nm. Three measurements were conducted for each seedling root, and the average was used for further analysis.

As shown in Fig. 4, the output power of the scattered light decreases as the length of the root increases. The results for wavelengths of 465 and 710 nm were almost the same as those for 620 nm light (See Fig. 6). Since 0.5 μW is very low compared with the power of sunlight, these results indicate that sunlight is very likely to propagate underground and be scattered.

3.2 Light scattering characteristics of fabricated waveguide-like structure

The light scattering characteristics of the waveguide-like structure were next measured under the same conditions.

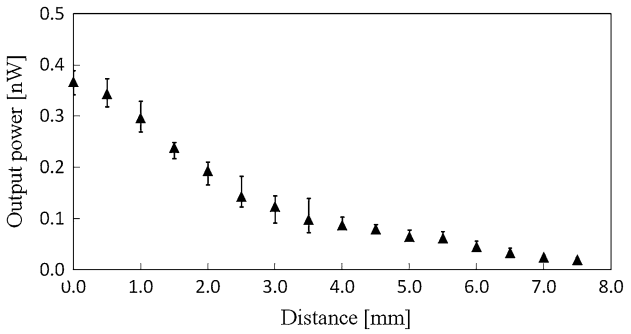

Fig. 4 Output power measurement of light scattered by a seedling root as a function of the root length. Light at 620 nm wavelength was introduced to the end facet of the root

The structure has a lozenge-shaped cross-sectional end facet with a 1000 μm2 area at the core. The size and shape were chosen by considering the light scattering conditions of the seedling root. The 6-mm-long waveguide-like structure comprises three parts fabricated using different laser scanning speeds to match the light scattering conditions of the root. In fact, the structure fabricated using the variety of constant laser scanning conditions could not precisely match the scattering conditions of the root. Figure 5 shows a schematic illustration of the fabricated waveguide-like structure and the propagation loss results for each part of the structure as a function of its length. The end facet of each part is also shown in the figure.

The part of the waveguide-like structure written at a laser scanning speed of 1500 μm/s contains the entire laser-induced damaged core that is formed by the mechanical stress induced in the vicinity of the surrounding depressed cladding waveguides with a reduced refractive index, resulting in a higher propagation loss. The part of the structure written at a laser scanning speed of 2500 μm/s contains the damaged core with a semi-transparent area at the center where less stress is induced and the inscribed surrounding depressed cladding waveguides. The part of the structure written at the speed of 3500 μm/s contains the core that is relatively intact domain of a PDMS polymer with a wider semi-transparent area at the center and the surrounding depressed cladding waveguides, resulting in a lower propagation loss than the other parts. Although the propagation losses for the fabricated waveguide-like structure are too high for general waveguide applications, the light scattering characteristics of the structure at the selected wavelengths match those of the seedling root well. Figure 6 shows the output power at the selected wavelengths for the waveguide-like structure and the seedling root as a function of length.

The structure exhibits light scattering characteristics at each wavelength similar to those for the seedling root. The

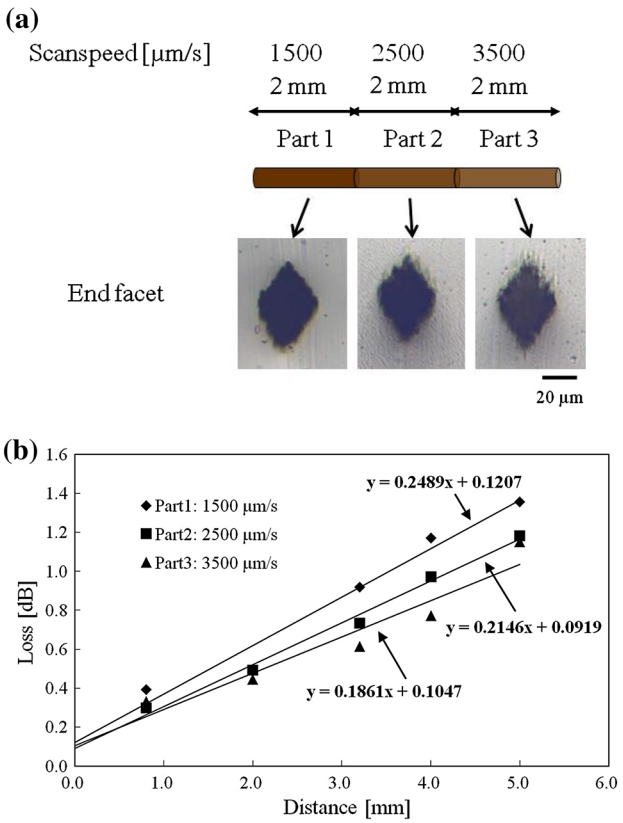

Fig. 5 **a** Schematic illustration of waveguide-like structure and images of end facets fabricated using different laser scanning speeds. **b** Measured propagation loss for each structure

scattered light decreases with increasing length. Therefore, the fabricated waveguide-like structure can replace the seedling root to investigate only the light illumination effect on *Phormidium* behavior.

3.3 Observation of light illumination effect on *Phormidium* gliding and mat formation using the PDMS biochip

Figure 7 shows a schematic illustration (a) of the *Phormidium* configuration on the biochip and sequential images and (b) of the gliding movement toward the waveguide-like structure illuminated at selected wavelengths. Under illumination at 465 nm (blue) and 620 nm (red), *Phormidium* formed a mat at the starting position and then glided to the structure within 12 h. After the gliding movement, *Phormidium* remained at and spread around the illuminated structure for another 12 h. On the other hand, when illuminated with 710 nm light, *Phormidium* formed colonies but did not glide to the structure. These results confirm that light at specific wavelengths guides the gliding movement of *Phormidium*. After the gliding movement, we did not observe any significant increase in the mat within 12 h, because the

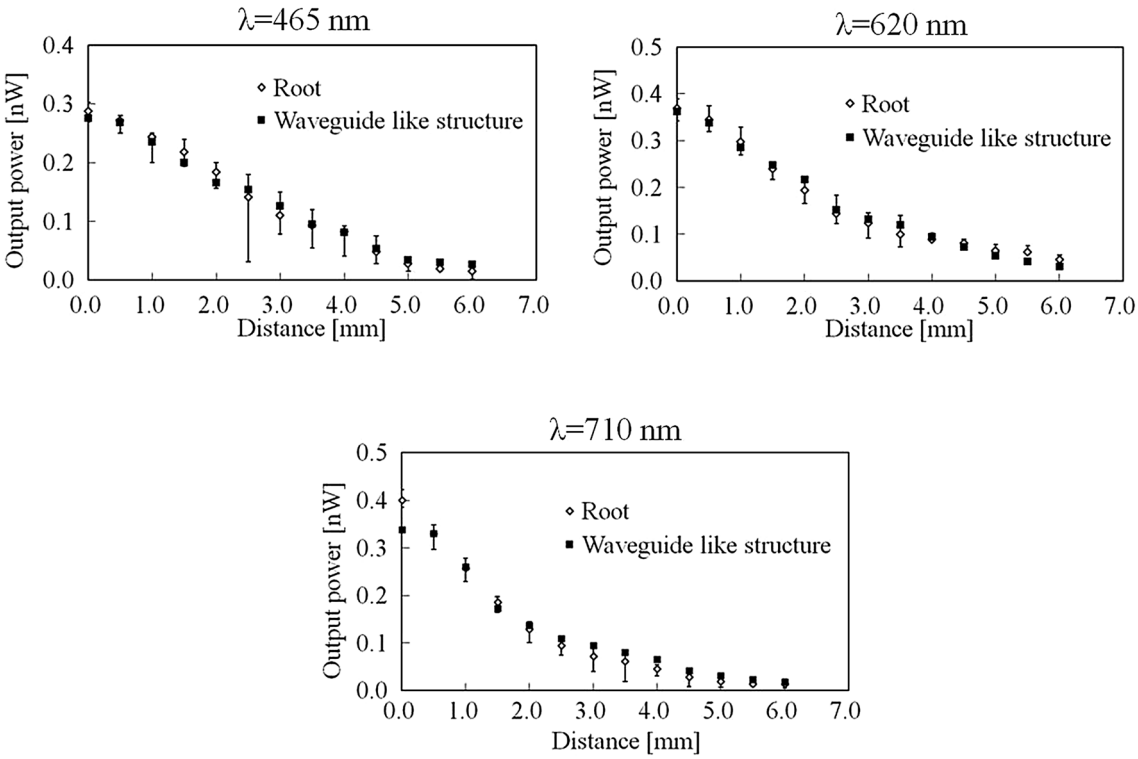

Fig. 6 Output power for waveguide-like structure and seedling roots as a function of length

low CO2 environment on the fabricated biochip inhibits its photosynthesis. However, as shown in Fig. 7b, for the last 12 h of the observation, the mat formation remained around the illuminated structure. Thus, blue and red lights increase *Phormidium* mat formation during photosynthesis in symbiosis.

The role of blue and red lights in symbiosis is not yet clear. However, the effect of specific wavelengths on the gliding movement and mat formation can be explained by the photosynthetic pigments of chlorophyll a in *Phormidium*. As described by Hanada et al. [7], chlorophyll a has two large absorption peaks around 430 and 660 nm, but little absorption in the green wavelength region. Additionally, there is no absorption from 700 nm in near-infrared wavelength regions. In the study by Hanada et al., *Phormidium* did not exhibit gliding movement under blue light; however, gliding movement and mat formation around the structure illuminated with blue light were clearly observed in the present study. Therefore, we speculate that during symbiosis underground, blue and red lights scattered from the seedling root promote the gliding movement of *Phormidium*. Once it reaches the illuminated root, the products of *Phormidium* photosynthesis increase mat formation and vegetable growth.

4 Conclusion

Using a PDMS biochip containing a waveguide-like structure fabricated by fs laser direct writing, we investigated the light illumination effect on the gliding movement and mat formation of *Phormidium*. The fabricated biochip is useful for studying the dynamics of microorganisms. Specifically, the observation and analysis time can be reduced, and only extremely small volumes of bio-sample are required. Moreover, optical analysis is possible. To confirm the light illumination effect on *Phormidium* behavior, we first revealed that light at specific wavelengths propagates through and is scattered by the seedling root. A waveguide-like structure with the same light scattering characteristics was then fabricated in a PDMS substrate to investigate only the light illumination effect on the gliding movement and mat formation of *Phormidium*. Scattered blue and red lights from the fabricated structure guide the gliding movement and promote mat formation. Therefore, during symbiosis underground, blue and red lights from sunlight scattered by the seedling root are required for the gliding movement and photosynthetic products to improve mat formation and to promote vegetable growth. Finally, we emphasize that the fs laser direct-writing technique in transparent material is a powerful tool for

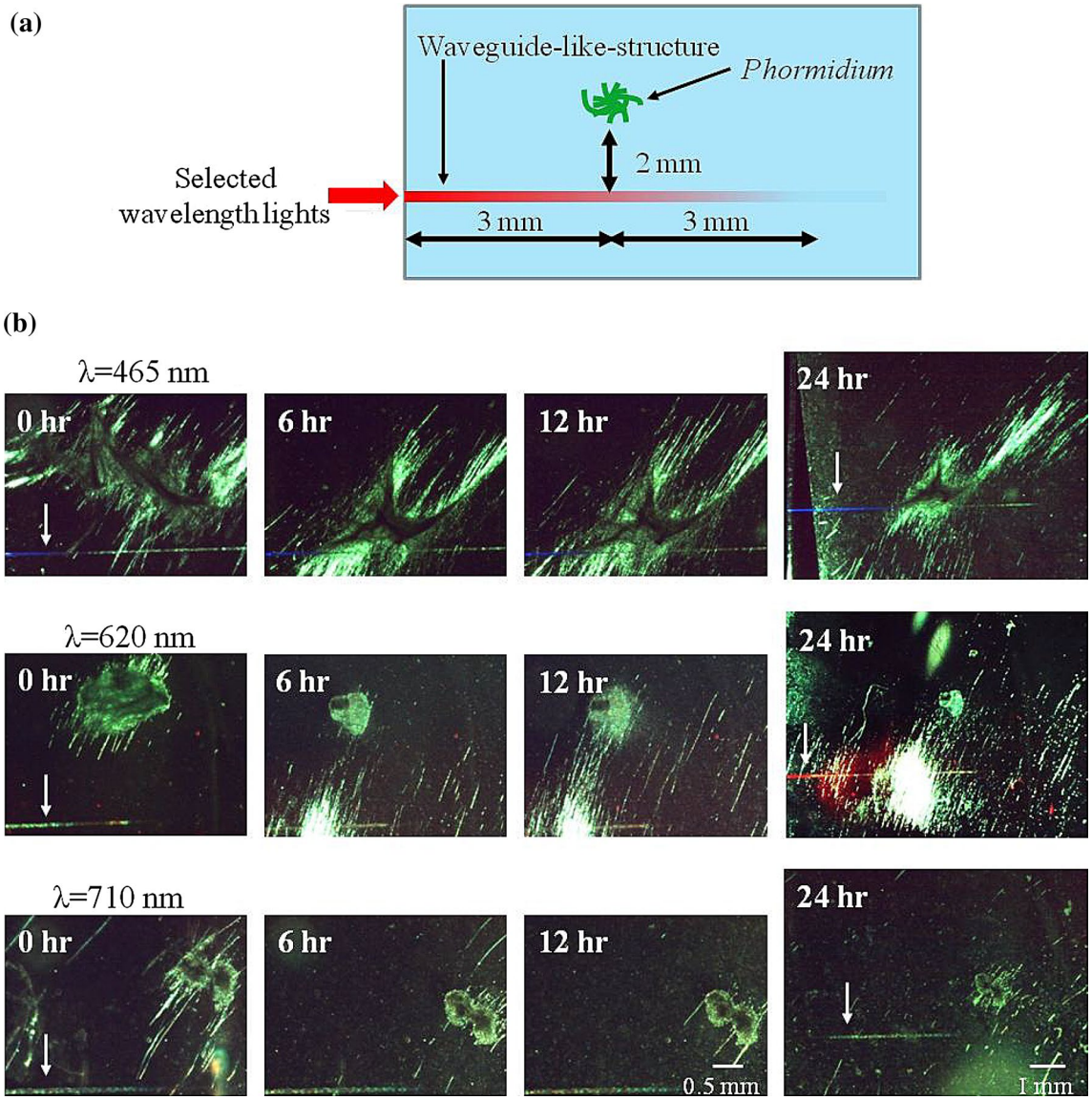

Fig. 7 **a** Schematic illustration of the *Phormidium* configuration on the biochip. **b** Light effect on gliding movement and mat formation of *Phormidium* (*enlarged image*). The *white arrows* indicate the waveguide-like structure

making prototypes of various biochips for functional analysis of microorganisms.

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