Detection of fungi using a long-period fibre grating

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Abstract: We present the first-time application of long-period fibre gratings written with a copper-vapour laser for detection of fungi in plants. The long-period gratings are used for identification of *Trichoderma* fungi species. A significance of our work lies in the facts that these bioagents protect plant roots against pathogens that can cause serious fungal diseases, resulting in great crop yield losses and, moreover, these can cause a lethal effect on human beings. We study such *Trichoderma* species as *T. Harzianum, T. Viride* and *T. Longibacterium*. They reveal characteristic attenuation peaks respectively at the resonance wavelengths 1524, 1520 and 1522 nm. The corresponding transmission dips change from 63.75 dB for the case of water to 54.85, 57.34 and 59.76 dB for the cases of water solutions of *T. Harzianum, T. Viride* and *T. Longibacterium*, respectively.

Keywords: long-period fibre gratings, surrounding refractive index, linearly polarized modes.

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1. Introduction

Long-period gratings are widely known as unique ultrafast and highly stable surrounding refractive-index sensors. Various applications of these gratings have been reported in the literature, including sensing of physical parameters [1–5], adulteration detection [6–10] and detection of harmful radiation [11]. Recently the sensors based on the long-period gratings have been used for studying *E. Coli* bacteria [12] and human serum [13].

Early detection of pathogens like bacteria, viruses and fungi in crops can reduce the damage caused to agriculture. Each of the traditional detection methods used for this aim (e.g., the standard methods termed as PCR, FISH and ALISA) reveals both benefits and limitations. Real-time processing, high sensitivity and capability to operate in harsh environments are still the important requirements in this area. All across the globe, crop infections causing food losses are among persistent issues. In contrast to chemical compounds, biofungicides prove to be useful bioagents for managing efficiently greenhouse vegetables and floriculture crops [14]. Since the biofungicides do not cure any pre-existing pathogen, they have to be applied before the onset of disease development. Their inherent benefits include stability, safety and a negligible toxic effect upon the host plants [15]. In spite of a number of benefits provided by utilization of biofungicides as bioagents, they also pose a serious, even lethal, threat to some immuno-compromised and immuno-suppressed humans. Moreover, a well-known *Trichoderma* species have been reported as a source causing a green-mould disease in plants [16–19]. As a consequence, fast and reliable detection of fungi can be a boon to the agriculture industry.

In the present paper, we have demonstrated a very reliable and fast method for detecting

fungi that employs the long-period gratings. Namely, we have identified three *Trichoderma* species such as *T. Harzianum*, *T. Viride* and *T. Longibacterium* with 2×10^7 cfu/gm, using so-called long-period fibre gratings (LPFGs).

2. Brief introduction of the method

Propagation of light in LPFGs can be simplified by assuming linearly polarized modes. Coupling of the fundamental guided mode LP_{01} and the cladding modes LP_{0m} ($m > 1$), which co-propagate in a LPFG, results in a series of resonant attenuation peaks in the optical transmission spectrum. It is governed by the phase-matching condition given as [6]

$$\lambda_{res} = \left[n_{effco}(\lambda) - n_{effcl,m}(\lambda) \right]. \tag{1}$$

Here λ_{res} is the resonance wavelength, $n_{effco}(\lambda)$ the effective refractive index of the fundamental core mode, $n_{effcl,m}(\lambda)$ the effective refractive index of the m-th cladding mode, and Λ the grating period. Note that the both parameters $n_{effco}(\lambda)$ and $n_{effcl,m}(\lambda)$ are functions of the light wavelength.

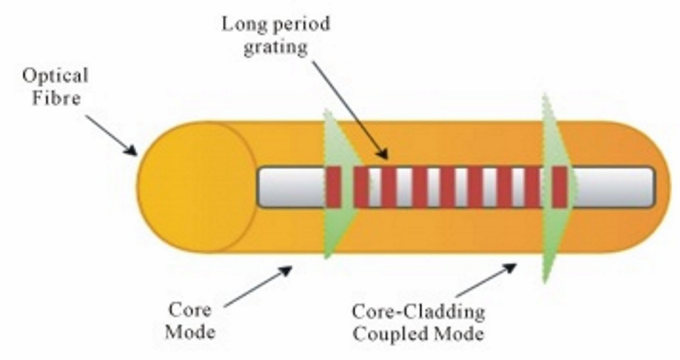

Fig. 1. Schematic illustration of LPFG used as a surrounding refractive-index sensor.

LPFGs are known to have high refractive-index sensitivity if compared to their counterparts, e.g. fibre Bragg gratings. Since the evanescent field of the coupled mode in a LPFG extends much further into the surrounding, the grating can be used as a good surrounding refractive-index sensor (see Fig. 1).

3. Experimental results

Fig. 2 shows a setup for writing LPFGs based on a point-by-point method. For this aim we used an ultraviolet beam (255 nm, 5.6 kHz, 30 ns and 40 μJ) generated from the second-harmonic conversion of a copper-vapour laser beam (510 nm) with a BBO crystal (Casix, $4\times4\times7$ mm^3 and a 51°-cut working at a type-I phase matching at $\lambda = 510$ nm) [20]. The second-harmonic beam was spatially separated and collimated to a diameter 2.5 mm. The profile of the ultraviolet beam was nearly 'top hat' and its divergence was equal to 120 μrad for the beam with a 10 mm diameter.

Our workstation incorporated computer-controlled translation stages capable to move along X, Y and Z directions, a magnetic clamped V-groove fibre holder, a beam-shaping optics, and an LPFG spectrum interrogation system. A microcontroller-based setup could be programmed to control both the displacement of a fibre (with the resolution 2 μm and the maximum translation

25 mm) and the ultraviolet-exposure time. In this way we fabricated long-period gratings with the period 289 μm. Then the fibre ends were fused with the patch cords and connected to a light source and an optical spectrum analyzer to monitor a real-time increase in the transmission minimum of the LPFG spectrum during fabrication.

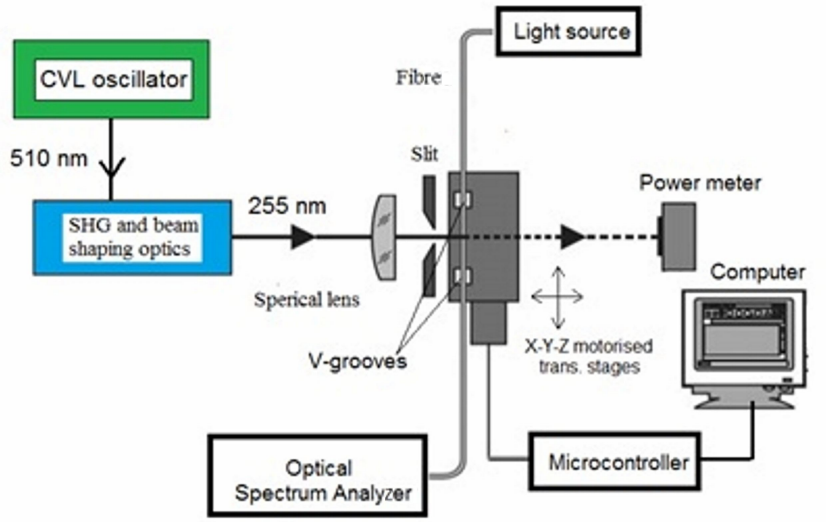

Fig. 2. Setup for recording LPFGs: CVL denotes the copper-vapour laser and SHG the second-harmonic generation.

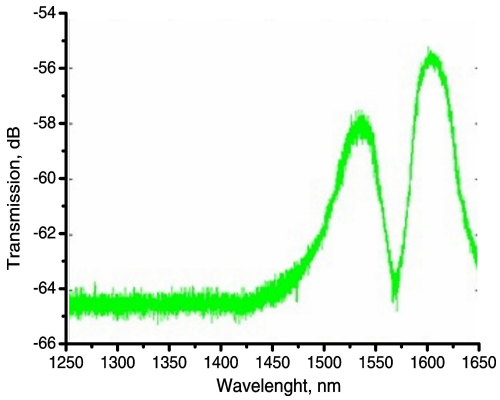

Fig. 3. Optical transmission spectrum of our LPFG with the period 289 μm written using a point-by-point method.

Fig. 3 shows a typical transmission spectrum of the LPFG written as described above. This LPFG written in a single-mode photosensitive fibre (Fibercore PS1250/1500) has been used for the qualitative analysis of *Trichoderma* species. The latter have been prepared after taking 1 g of *Trichoderma* sample taken in sterile distilled water and preparing a 10^{-6} dilution. 1 ml of this solution (10^{-6}) was transferred to sterile Petri plates and 15 ml of a molten cooled potato dextrose agar was added to these Petri plates. The plates were incubated for 5 days at the room temperature. The average population numbers of the colonies were counted. A minimum colony-forming unit with 2×10^{7} cfu/gm has been obtained. Solutions of the three samples mentioned above in deionized water were prepared and analyzed using our LPFGs. A superluminescent light-emitting diode was used as a broadband source covering the region from 1480 to 1600 nm. An optical spectrum analyzer Prolite-60 with the wavelength range 1250–1650 nm was used to monitor the transmission spectra of our LPFGs.

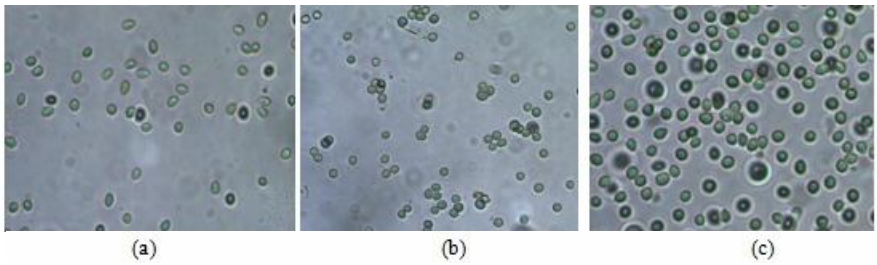

Fig. 4. Microscopic images of *T. Harzianum* (a), *T. Viride* (b) and *T. Longibacterium* (c).

Fig. 4 shows the images of our *T. Harzianum*, *T. Viride* and *T. Longibacterium* samples, as taken using the microscope. Our investigations have demonstrated that different attenuation losses are observed for different *Trichoderma* species. Fig. 5a shows the transmission spectrum of the LPFG immersed in deionized water. Fig. 5b displays the spectra obtained for the three *Trichoderma* species relative to the spectrum in water. The resonance wavelength located at 1526 nm is shifted towards 1524, 1520 and 1522 nm respectively for the cases of *T. Harzianum*, *T. Viride* and *T. Longibacterium*. The corresponding transmission dips reduce from 63.75 dB to 54.85, 57.34 and 59.76 dB, respectively. Table 1 summarizes the results for the three samples. The shifts of the peak wavelength in the transmission spectra are accompanied by the changes in the attenuation peaks. These can be attributed to the changes in the coupling coefficients due to changing surrounding refractive index. Notice that different LPFGs were used for the different samples to maintain biosafety and reduce the errors.

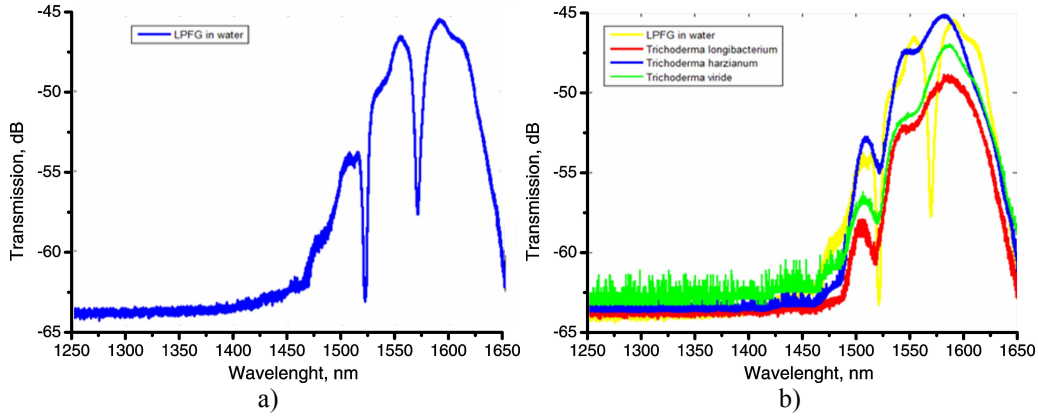

Fig. 5. Transmission spectra of LPFG immersed into water (a) and into water containing the *Trichoderma* samples (b).

Table 1. Transmission loss peaks and peak wavelengths found for our samples of *Trichoderma*.

Sample number	*Trichoderma* species	Peak, dB	Wavelength, nm
1	*T. Harzianum*	54.85	1524
2	*T. Viride*	57.34	1522
3	*T. Longibacterium*	59.76	1520

4. Conclusion

In conclusion, we have demonstrated for the first time that the LPFGs can be used for identification of the *Trichoderma* species *T. Harzianum, T. Viride and T. Longibacterium*, which work as useful bioagents in protecting plants from pathogens. It has been shown that the *T. Harzianum*, *T. Viride* and *T. Longibacterium* species reveal notable shifts in the loss peaks, when compared

with distilled water. These peaks shift from 63.75 dB to 54.85, 57.34 and 59.76 dB at the resonance wavelengths 1524, 1520 and 1522 nm, respectively for the three species mentioned. The present study can extend the scopes for identifying the other *Trichoderma* species with the LPFG-based sensors, including the species characterized by changing concentrations in samples.

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Анотація. *Вперше продемонстровано можливість застосування довгоперіодичних волоконних дифракційних ґраток, записаних за допомогою лазера на парах міді, для виявлення грибів у рослинах. ЦІ довгоперіодичні ґратки використано для ідентифікації видів грибів Trichoderma. Значимість наших об'єктів полягає в тому, що ці біоагенти захищають коріння рослин від патогенних мікроорганізмів, які можуть викликати серйозні грибкові захворювання, що призводять до значних втрат врожайності і, крім того, ці об'єкти можуть мати летальний вплив на людину. Такі види Trichoderma як T. Harzianum, T. Viride і T. Longibacterium виявляють характерні піки загасання відповідно на резонансних довжинах хвиль 1524, 1520 і 1522 нм. Відповідні провали в оптичному пропусканні зменшуються від 63,75 дБ для води до 54,85, 57,34 і 59,76 дБ для водних розчинів T. Harzianum, T. Viride і T. Longibacterium, відповідно.*