Detection of Soybean Rust Spores Using Photonic Crystal Biosensors

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1. Abstract

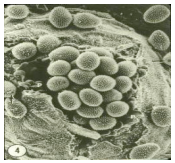
In this study, we report the initial efforts at detection of viable spores of the fungus *Phakopsora pachyrhizi* (the cause of soybean rust) using label-free photonic crystal biosensors. Attachment of the rust spores on the sensor surface results in a highly localized increase of the resonant peak wavelength value (PWV). The detection system enables imaging of the spores attached to the sensor surface without use of fluorescent labels or stains. Two kinds of surfaces are used to study the affinity of spores to the sensor. We believe our results to be important towards the realization of an economical, field deployable method for the detection of soybean rust spores.

2. Background and Motivation

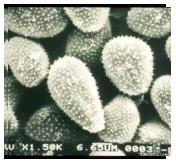
a) Soybean Rust

- Caused by a fungal pathogen *Phakopsora pachyrhizi*
- Most common symptom is the formation of gray to tan or brown lesions on the lower surface of the leaf[1] that eventually form pustules known as uredinia.
- Late symptoms include premature defoliation, early maturity, low seed weight, few pod and seed production.
- Losses can range from 10-80% depending upon the environmental conditions and it can be 100% under conductive field conditions.
- Early detection prior to visible symptoms is critical for timing fungicidal applications.
- Spreads primarily by wind-borne urediniospores formed from the uredinia
- Spores are obovoid to broadly ellipsoid and measure 18-34 to 15-24 microns[2].

Reddish brown lesions on leaf



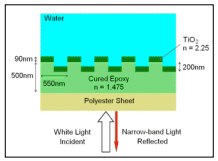
A uredinium with spores



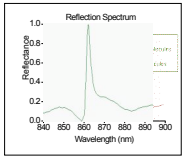
Echinulated urediniospores

b) Photonic Crystal Biosensor

Photonic crystal biosensor embedded into the bottom of a 96 - well microplate



Cross section of the photonic crystal biosensor structure

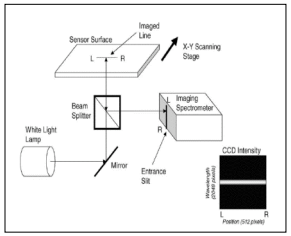


Reflectance before and after attachment of biomolecules

- Label-free photonic crystal biosensors have emerged as important tools for pharmaceutical research, diagnostic testing, and environmental monitoring[3].
- Such sensors can be mass-fabricated in a plastic-based manufacturing approach using nanoreplica molding and incorporated into standard microplate formats.
- Sensor structure is composed of a one dimensional grating incorporated with low and high refractive index materials and is designed to function as a narrow band reflectance filter[4].
- Attachment of biomolecules onto the sensor surface changes the local refractive index and results in a shift of the resonant peak wavelength to a longer value.

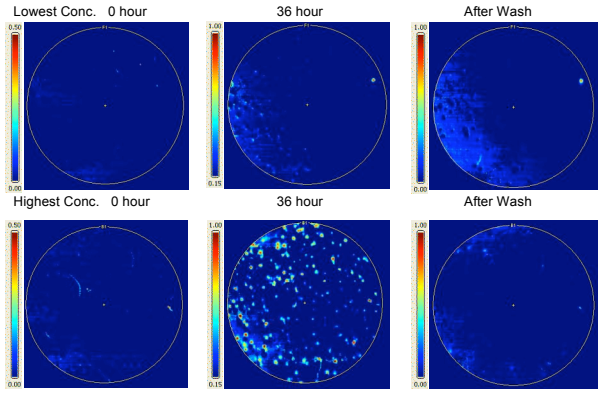
3. Instrumentation and Methodology

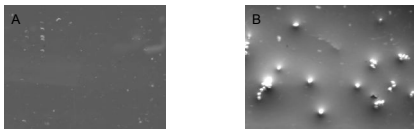
- The imaging instrument can generate a two dimensional map of the PWV on sensor surface.
- Unique design of the photonic crystal biosensor prevents lateral propagation of light and eliminates optical cross-talk.
- Resolution used in this study is 22.3 × 22.3 μm².
- Sensor surface is scanned before and after the attachment of spores and the two images are subtracted to generate a map of PWV shift.
- Two kinds of sensor surfaces are used: titanium dioxide and glutaraldehyde.
- Phosphate buffer saline (PBS, pH 7.5) solution is used to set up the baseline.
- Spores are suspended in 50μl PBS to an initial highest concentration of 1.3 × 10⁵ spores/ml followed by six 2-fold dilution series.
- All the experiments are performed in triplicates.



Imaging instrument used for this study

4. Results for Titanium Oxide Surface

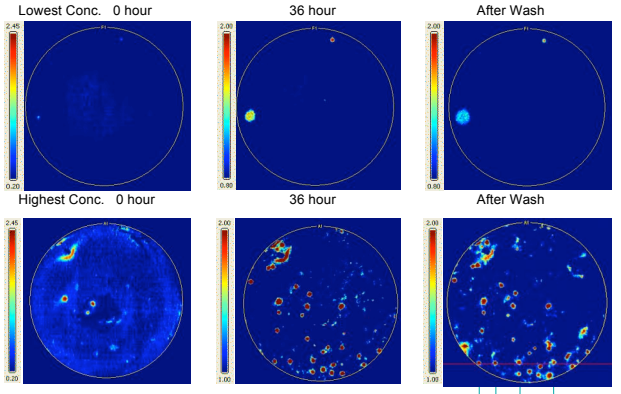




Optical microscope images of the titanium dioxide sensor surface (A) before and (B) after the attachment of spores.

- Attachment of the spores to sensor surface results in a highly localized increase of the PWV.
- Images from the biosensor and optical microscope indicate that the spores tend to form clusters with three to four spores in each.
- The number of spores attached to the sensor surface is very small compared to the concentration used and most of the spores could be seen suspended in the solution.
- Detection of almost no signal after the wash indicates the low affinity of the rust spores to titanium dioxide surface.

5. Results for Glutaraldehyde Surface



- Clusters of spores can have a local PWV shift of more than 4nm.
- The image acquired after wash shows that the rust spores have some affinity to glutaraldehyde.
- The number of spores attached to the sensor is still very small.

6. Discussion

- There is a need to develop a technique to precipitate the spores to sensor surface since most of them were seen suspended in solution.
- The better affinity of the spores to glutaraldehyde indicates the possible involvement of surface proteins, although they are not identified as of now.
- Future research will focus on spore detection using monoclonal and polyclonal antibodies.
- This platform has the potential to monitor spore germination on sensor surface and can provide essential information on spore viability.

7. Acknowledgements

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8. References

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