Detection of *Phakopsora pachyrhizi* Spores Using Photonic Crystal Biosensors

Ramya Vittal[1], Wei Zhang[3], Leo L. Chan[3], Brian T. Cunningham[3], and Glen L. Hartman[1,2]

[1]*National Soybean Research Laboratory, Department of Crop Sciences, University of Illinois at Urbana-Champaign*

[2]*USDA, Agricultural Research Services, Urbana, IL*

[3]*Nano Sensors Group, Department of Electrical & Computer Engineering, University of Illinois at Urbana-Champaign*

APS North Central Meeting

June 20, 2007

1. Abstract

This objective of this study was to detect urediniospores of the fungus *Phakopsora pachyrhizi* (causes soybean rust) using label-free photonic crystal biosensors. Two kinds of surfaces (titanium dioxide and glutaraldehyde) were used to study the affinity of spores to the sensor. Attachment of the spores to the sensor surface results in a highly localized increase of the resonant peak wavelength value (PWV). The detection system enabled imaging of the spores attached to the sensor surface without the use of fluorescent labels or stains. This study may represents the first use of photonic crystal biosensors for detection of rust spores. This may be the first step in reaching the goal towards developing an economical and field deployable detection system.

2. Background and Motivation

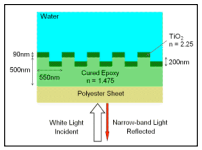
a) Soybean Rust

- Caused by a fungal pathogen *Phakopsora pachyrhizi*
- Most common symptom is 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.
- Early detection prior to visible symptoms may be critical for timing fungicide applications.
- Spreads primarily by wind-borne urediniospores which are obovoid to broadly ellipsoid and measure 12 to 24 x 16 to 31 µm[1].

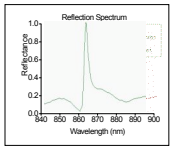
b) Photonic Crystal Biosensor

- Label-free photonic crystal biosensors have emerged as important tools for pharmaceutical research, diagnostic testing, and environmental monitoring[2].
- 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[3].
- 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.

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



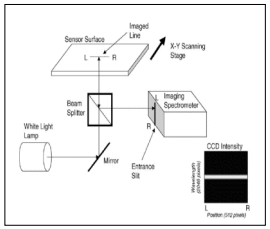
Cross section of the photonic crystal biosensor structure



Reflectance before and after attachment of biomolecules

3.1 Instrumentation and Methodology

- The imaging instrument generates 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 was 22.3 x 22.3 µm^2.
- Sensor surface was scanned before and after the attachment of spores and the two images were subtracted to generate a map of PWV shift.
- Affinity of the spores to the sensor was measured by two experiments



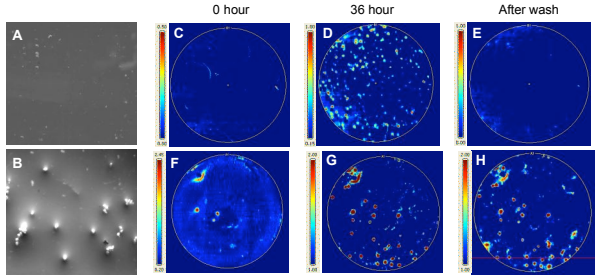
Imaging instrument used for this study

3.2 Instrumentation and Methodology

- Experiment 1: Using two sensor surfaces (titanium dioxide, TiO2 and glutaraldehyde, GA) with Phosphate buffered saline (PBS) as buffer.
- Experiment 2: Using two kinds of surfaces (TiO2 and GA) and four different buffers (water, PBS, 0.1% tween 20 in PBS and 0.1% glycerol in PBS).
- Experiment 3: Using three kinds of polyelectrolytes that confer a net charge on TiO2 surface: polyethylenimine (PEI, +ve), polystyrenesulfonate (PSS, -ve) and polyallylamine hydrochloride (PAH, +ve) with 0.9M NaCl as the buffer.
- Concentration of spores: 1.3 x 10^5/ml, 2.2 x 10^5 to 4.3 x 10^6 and 1.3 x 10^3 to 1.3 x 10^3 for all the three experiments respectively.
- A baseline was set up using respective buffer solutions followed by loading 50µl spores to measure the PWV shift.

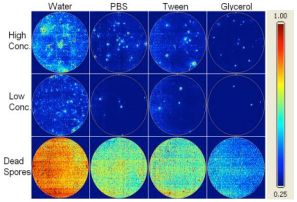
4.1 Results comparing the TiO2 and GA surfaces

- Attachment of the spores to sensor surface resulted 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 was small compared to the concentration.
- Detection of almost no signal after the wash indicates the affinity of the rust spores to TiO2.
- The image acquired after the wash shows that the rust spores have more affinity to GA.

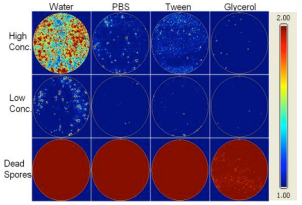


Optical microscope images of the TiO2 sensor surface (A) before and (B) after the attachment of spores. Images of the TiO2 plate at (C) 0 hour, (D) 36 hour and (E) after wash. Images of the GA plate at (F) 0 hour, (G) 36 hour and (H) after wash.

4.2 Results for the TiO2 and GA plates using various buffers

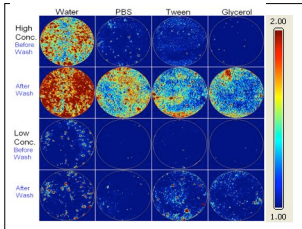


TiO2 Plate – Before wash

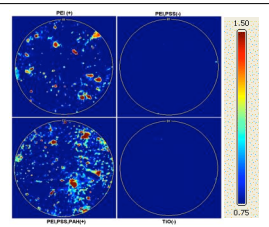


GA Plate – Before wash

- As in experiment 1, attachment of the spores to GA surface resulted in a higher shift compared to the TiO2 surface.
- Among all the buffers used, water resulted in the highest PWV shift irrespective of the surface.

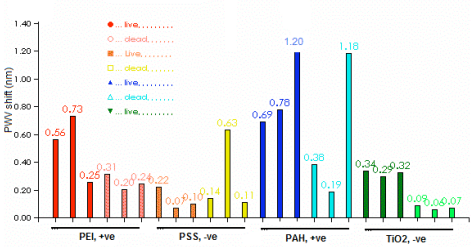


GA Plate – Spores before and after wash



TiO2 plate with polyelectrolytes – after wash

4.3 Results for TiO2 plate using various polyelectrolytes



Endpoint plot of PWV shift on TiO2 plate – After wash

- The postively charged polyelectrolytes resulted in a higher PWV shift compared to the negatively charged PSS and TiO2 surface.
- Spores on PSS layer recorded the highest shift on both the reader and imaging machine.

5. Discussion

- This study aimed at understanding the factors affecting sensor-spore interaction.
- Spores have a better affinity to glutaraldehyde indicating the possible involvement of surface proteins
- Buffer solutions have some effect on the affinity of the spores to the sensor with water recording the highest PWV shift.
- Charge on the sensor surface affect the binding of the spores considerably.
- This platform has the potential to monitor spore germination on the sensor surface and may provide essential information on spore viability.

6. Acknowledgements

The authors gratefully acknowledge Soybean Disease Biotechnology Center and Illinois Council on Food and Agricultural Research for providing financial support for this work. The authors also extend their gratitude to the support staff of the Micro and Nanotechnology Laboratory at the University of Illinois at Urbana-Champaign and all members of Professor Hartman and Cunningham's research groups.

7. References

[1] G. L. Hartman *et al.*, *Compendium of Soybean Diseases* 1999. APS Press.
[2] B. T. Cunningham *et al.*, *Journal of Biomolecular Screening* **9**, 481 (2004).
[3] B. T. Cunningham *et al.*, *Sensors and Actuators B, Chemical* **1**, 316 (2002).