

A Nano-biosensor for Sclerotinia Stem Rot Forecasting

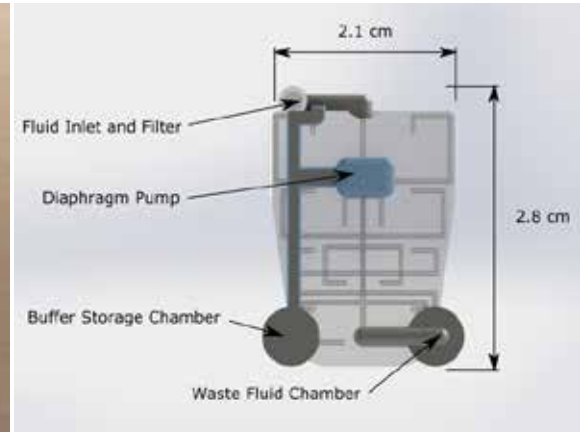
Xiujie Susie Li, Jian Yang, Jie Chen

The Design



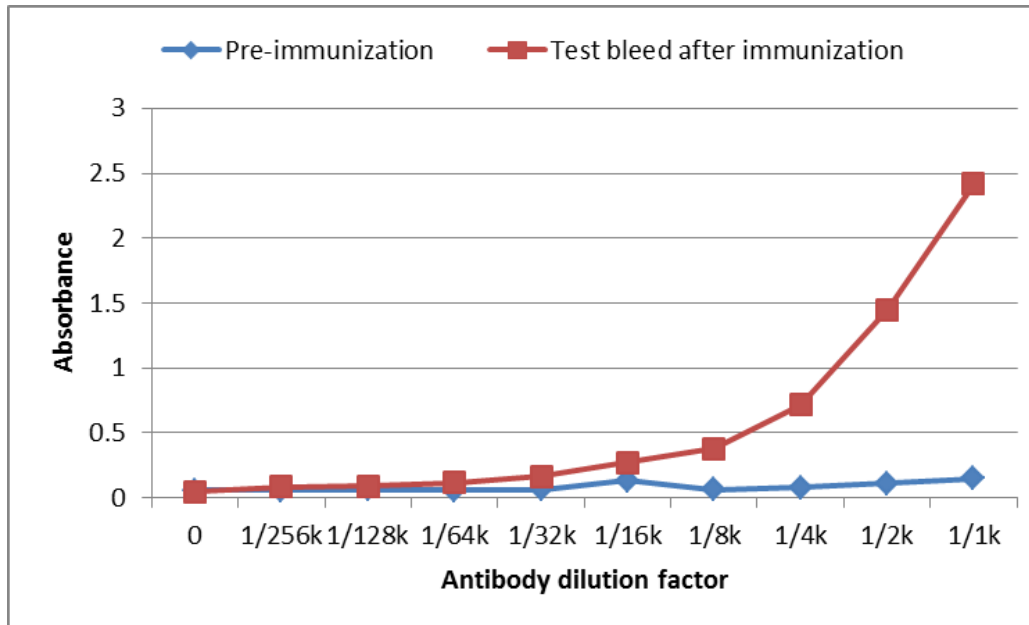
Nano-biosensor

An extremely small device, with a dimension on the order of one billionth of a meter, capable of detecting and responding to physical stimuli (e.g. spores)

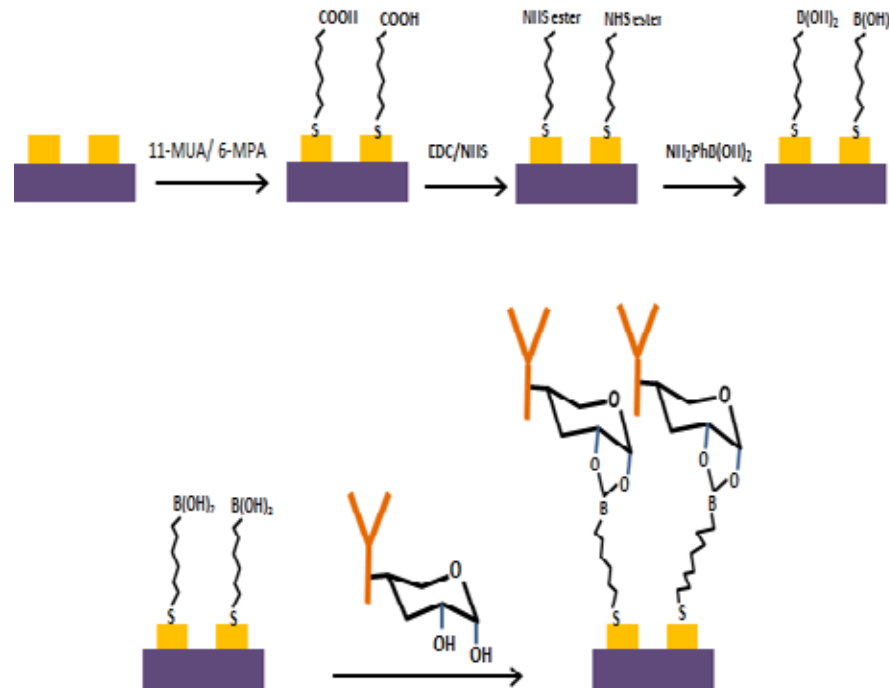


Antibody production

§ Polyclonal antibody production

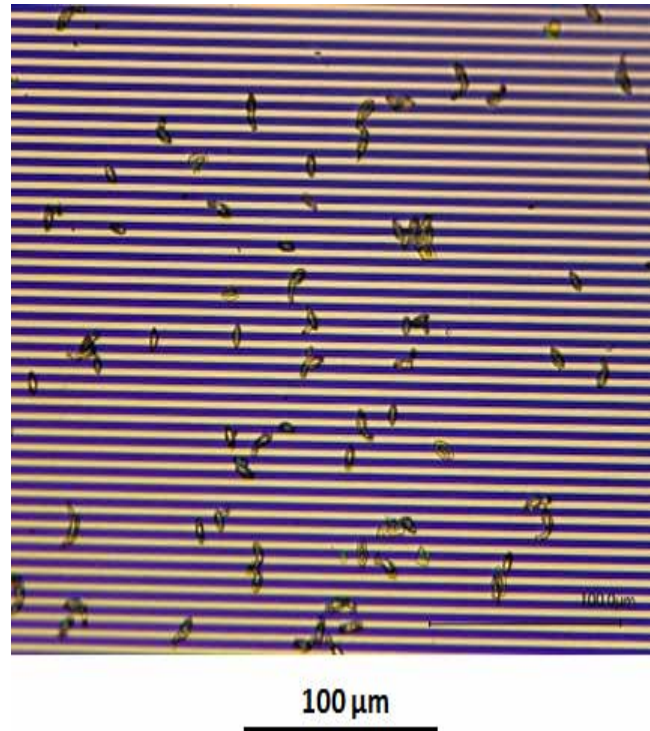


Priming Sensor Chip for Canola Ascospores Detection



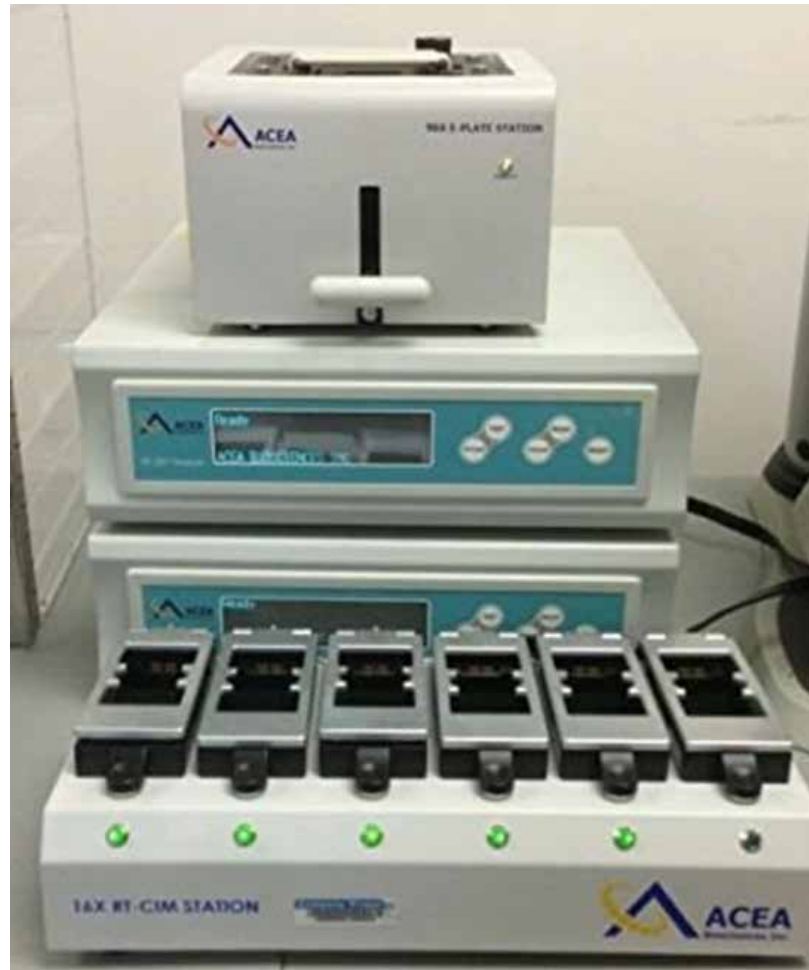
Schematic illustration of surface functionalization of gold IDE surface to covalently attach an insulating SAM and to achieve oriented immobilization of the anti-*S. sclerotiorum* antibody by boronate ester conjugation.

Microscope Images of Ascospores Captured on Sensor Chip



A typical optical image of ascospores selectively captured by immobilized anti-*S. sclerotiorum* antibody on SAM modified IDE surface. The number of ascospores on 3 mm x 3 mm IDE is 9700 or 1.1×10^5 ascospores/cm²

Real-time cell electronic sensing system

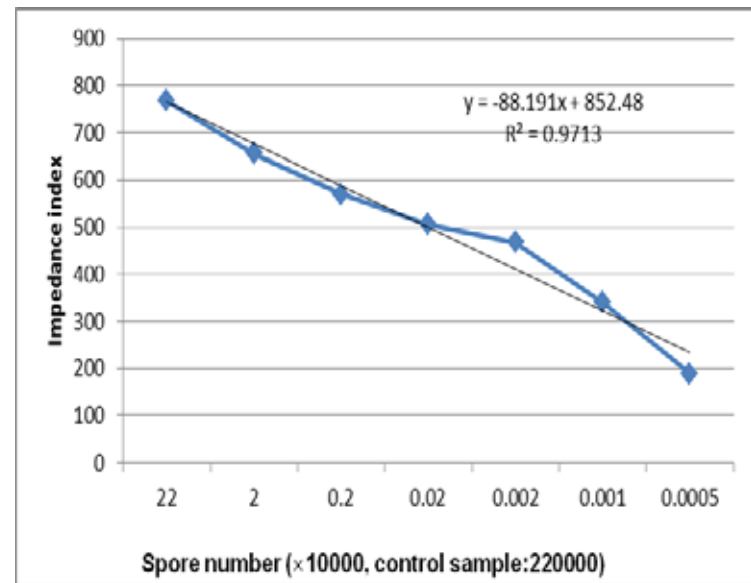
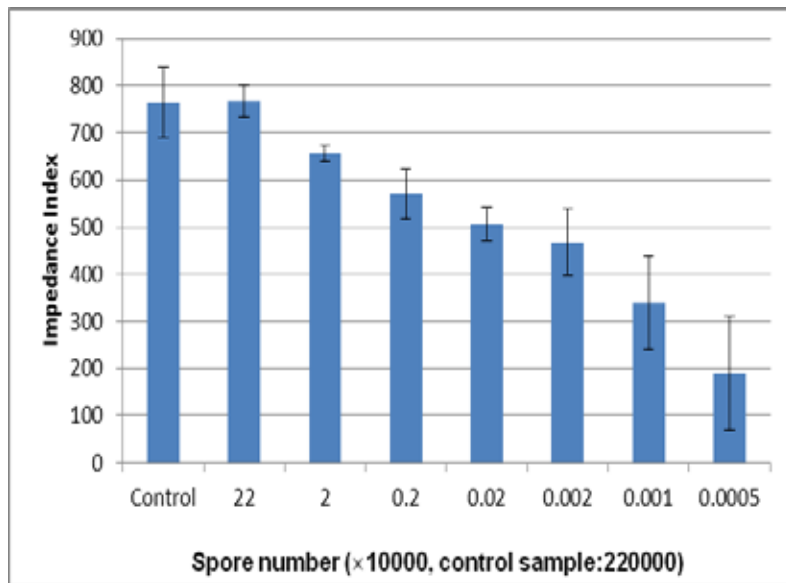


In Vitro Detection

§ Correlation and Accuracy

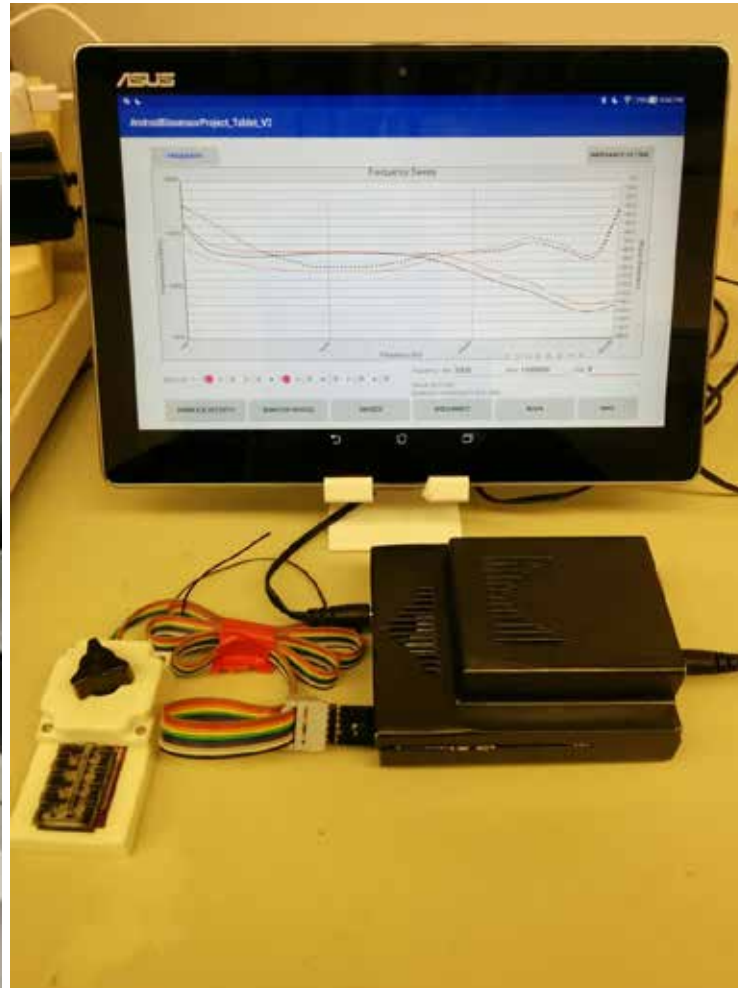
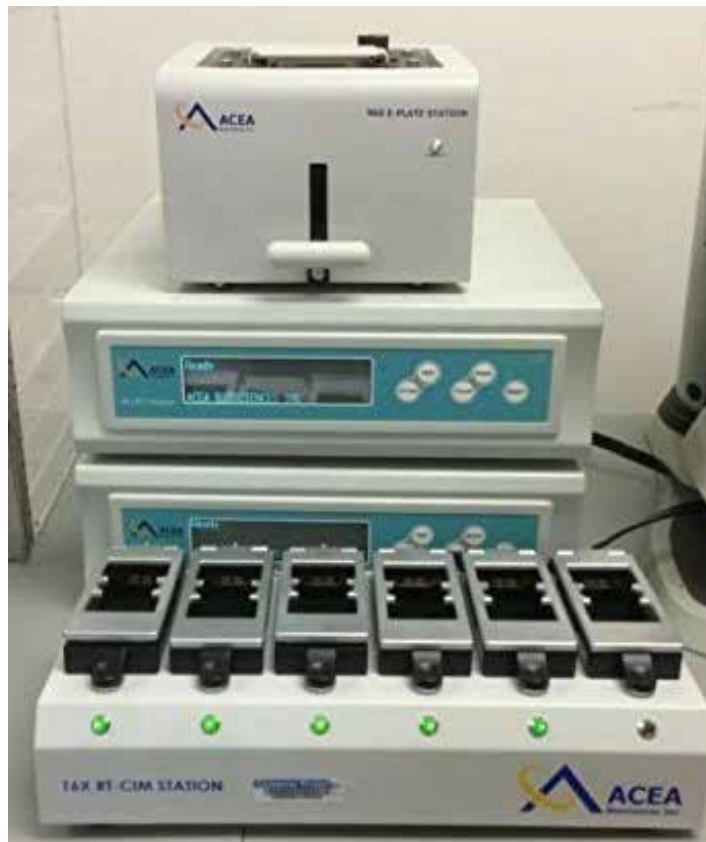
§ With the decrease of spore numbers there is a decrease of the impedance

§ As low as 5 spores could be detected

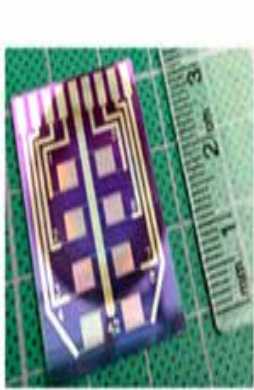


Instrument vs sensor

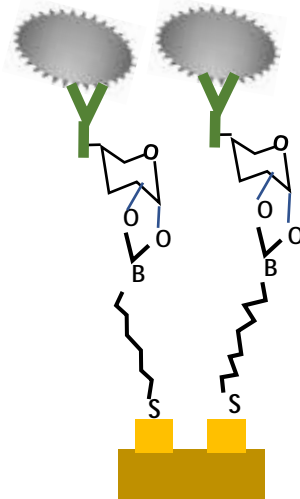
Real-time cell electronic sensing (RT-CES) system



Nano-biosensor



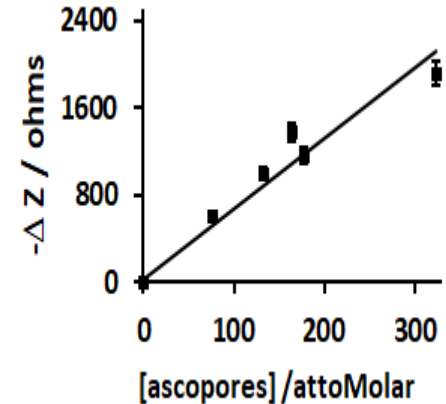
(a)



(b)



(c)

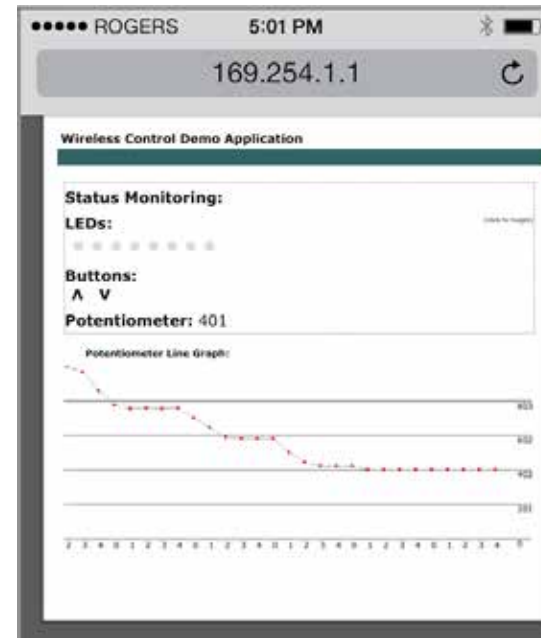


(e)

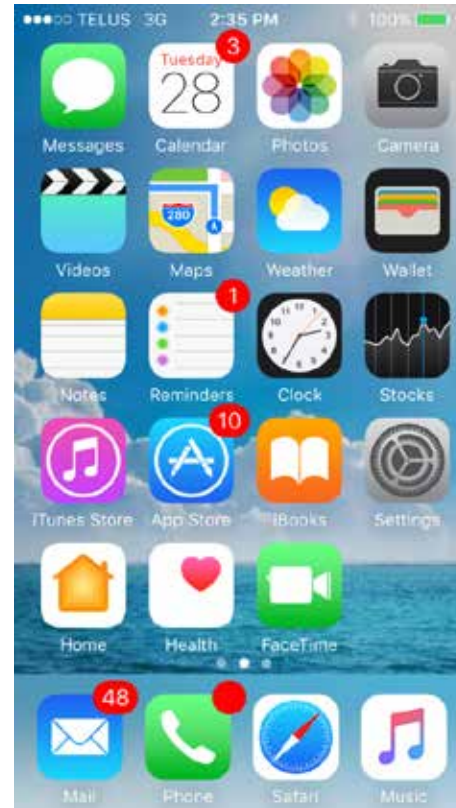
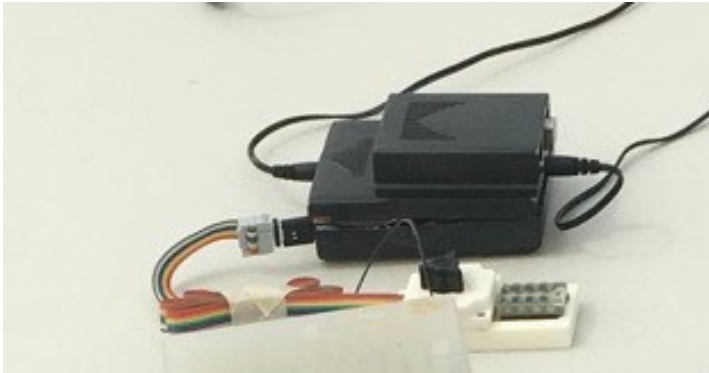
Schematic illustration of immuno-impedimetric biosensor. Biosensor chip and accessories (a), covalently bonded SAM, oriented immobilization of antibodies and ascospores captured on IDE surface (b), optical image of ascospores selectively captured on IDE surface (c), calibration curve (e). As the number of ascospores on the IDE surface can be determined from the optical microscopic images, the experimental results provide a direct correlation between the number of ascospores on the sensor surface and their impedance response.

App for data delivery

- § Signal transmission
 - § WiFi / iPhone app development
 - § Bluetooth, designed the user interface as a web application so that cross-platform devices/computers can access the user interface



The sensor



Growth Chamber Study

§ Methods

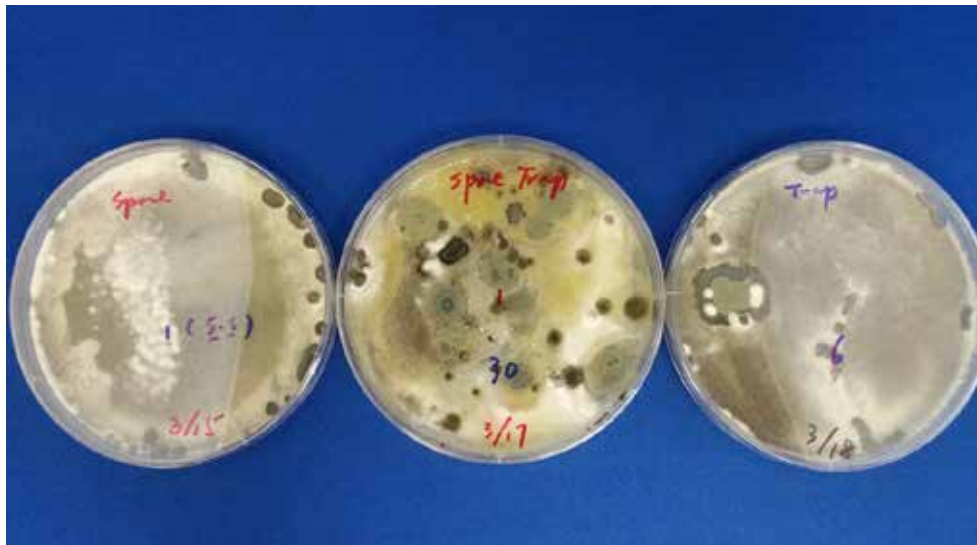
- § Canola plants (46) were grown in a growth chamber till flowering stage
- § A jar of sand containing germinating sclerotia was placed into the chamber
- § A spore trap device was placed in the growth chamber, run continuously starting at flowering stage
- § Monitor the plant for disease at the same time

Growth Chamber Study – cont.



Growth Chamber Study – cont.

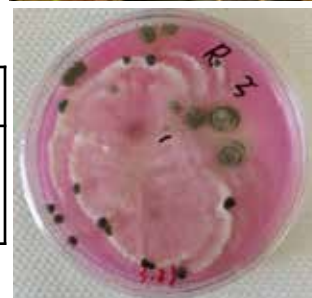
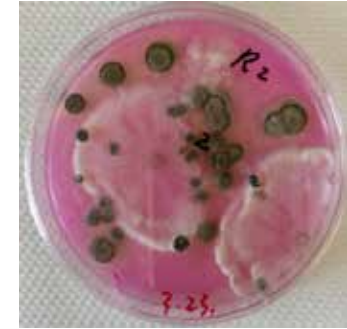
- § Liquid sample was taken and plated on culture medium every 24 h, 0.1 mL/plate, duplicated
- § Count fungal colonies 5 days after incubating at room temperature
- § Identify *S. sclerotiorum* colonies.



Growth Chamber Study – cont.

§ Spore numbers and canola disease correlation

§ 10 *S. sclerotiorum* spores per mL collected in 24h in 3.3 sq. meter growth chamber start to cause leaf infection



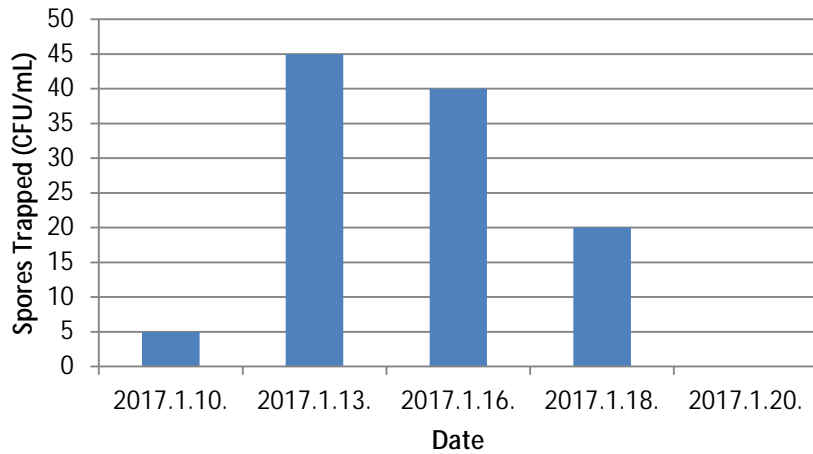
Days	1	2	3	4	5	6
Spore No.	3	6	7	13	35	48

Greenhouse study

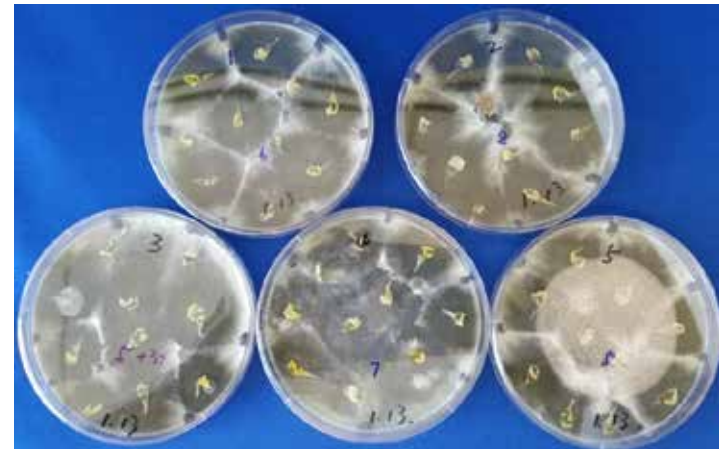
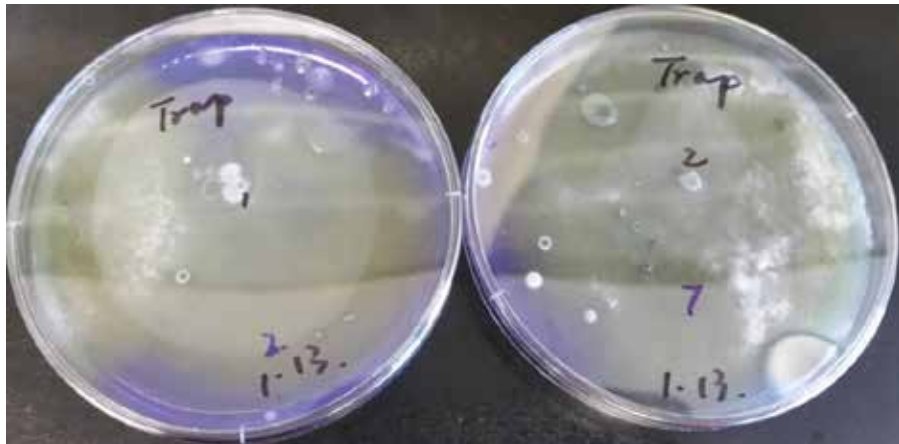
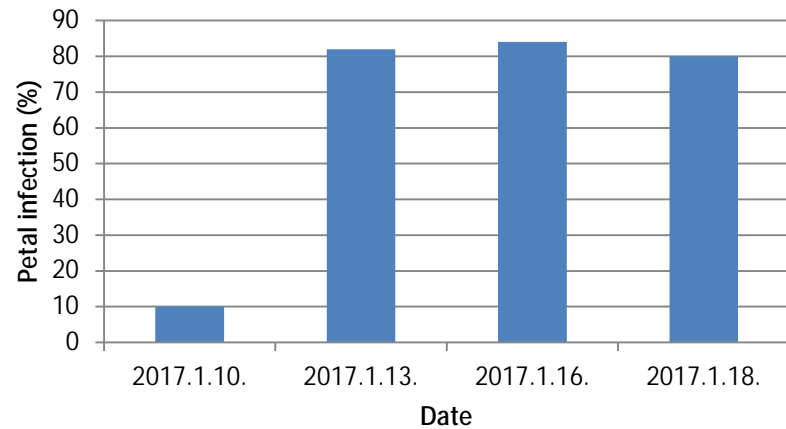


Greenhouse spore level and petal infection correlation

Spore population (CFU/mL)



Petal infection (%)



Field study

§ Disease incidence 2016, 33%

§ Disease incidence 2017, 6%



Next Steps

- ∅ Improve sensitivity by reducing the signal-to-noise ratio
- ∅ Further determine and confirm the area one biosensor can cover
- ∅ Multi-location field studies
- ∅ Verify the results

Acknowledgements

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Thank You!