

Rapid detection of invasive species in ballast water using molecular microfluidic technology

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Goal

- To develop a real time, portable microfluidic chip-based detection platform that is capable of detecting the presence of invasive species in ballast water with high sensitivity



Carcinus maenas
Europe and
N. Africa



Limnoperna fortunei
China and Southeast
Asia



Dreissena polymorpha
Eastern Europe

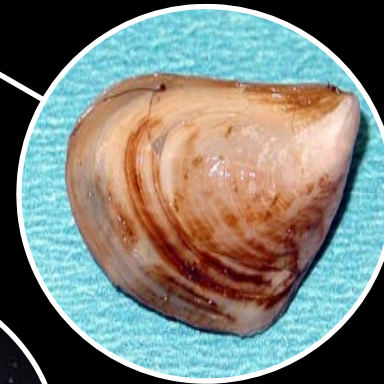


Target
organisms

Eriocheir sinensis
China



Dreissena bugensis
Eastern Europe



Dikerogammarus villosus
Ponto-Caspian region



Methods



Sample collection



DNA extraction



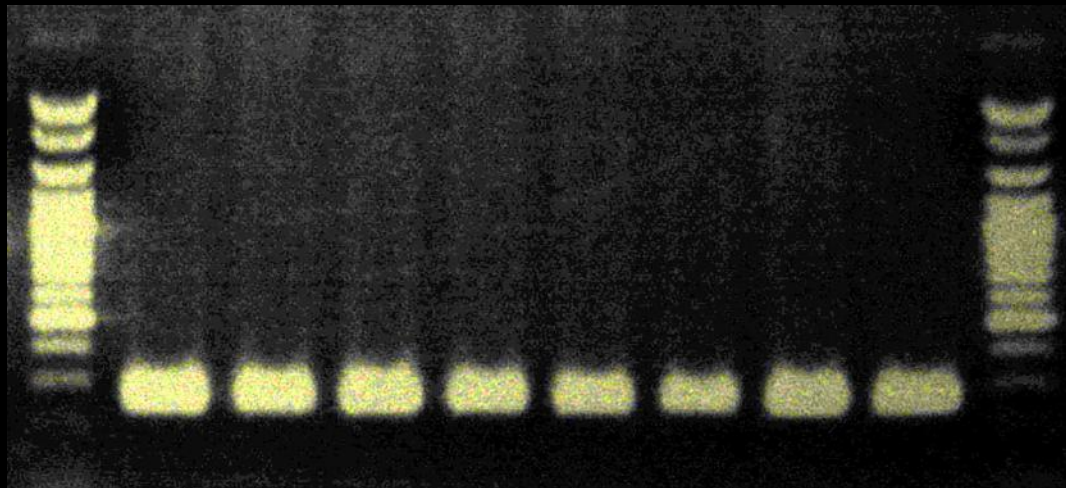
Amplification



Hybridization and
detection

Polymerase Chain Reaction (PCR) of ballast samples

- Target for amplification
 - Species specific
 - Cytochrome c oxidase subunit I (COI)





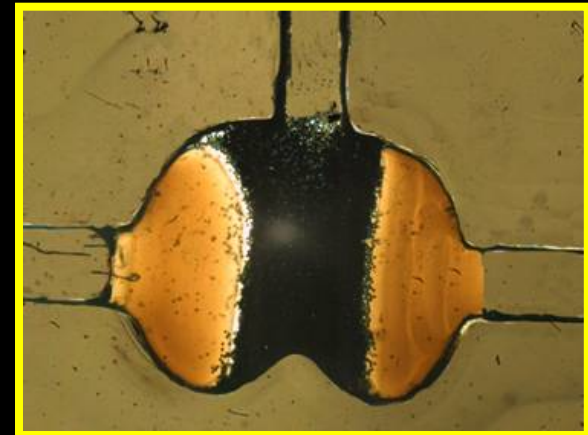
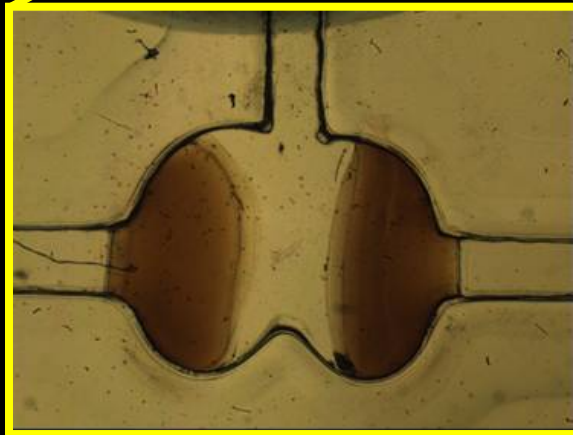
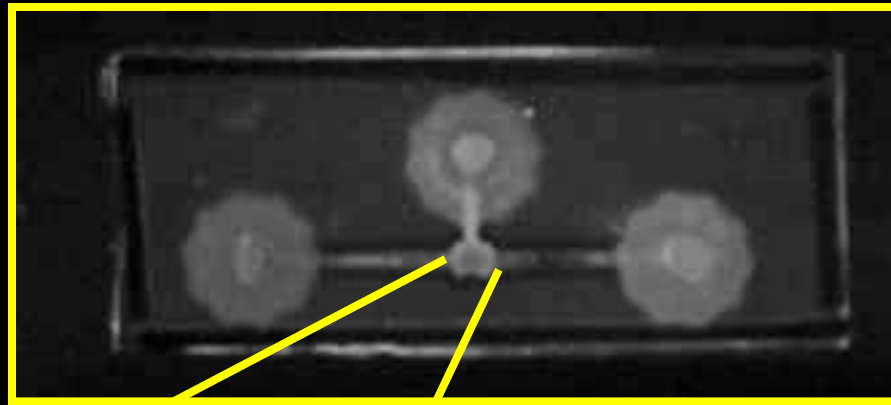
Hybridization and detection

- Two methods
 - Fluorescent bead based detection system
 - Carbon nanotube (CNT) detection system



Detection:

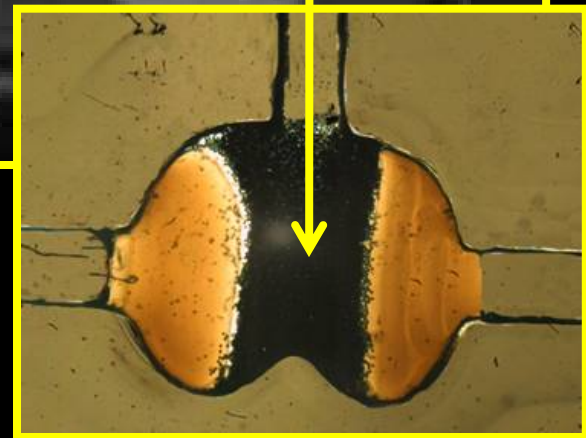
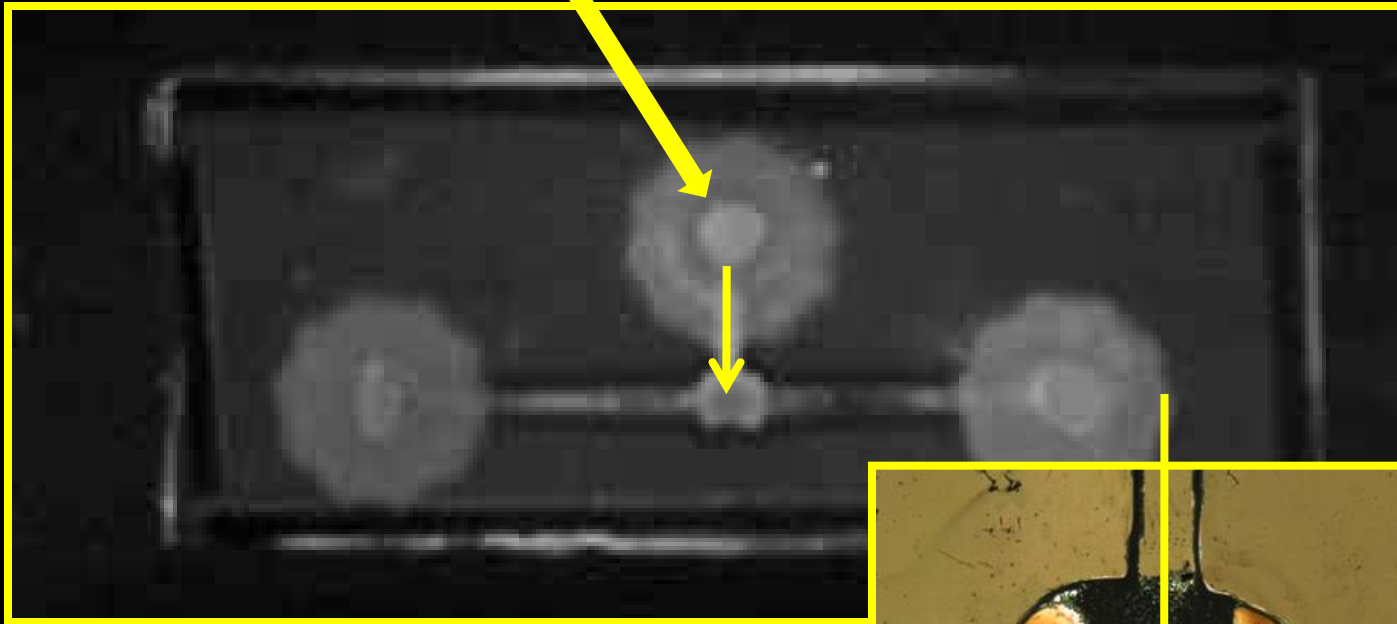
Fluorescent bead based detection



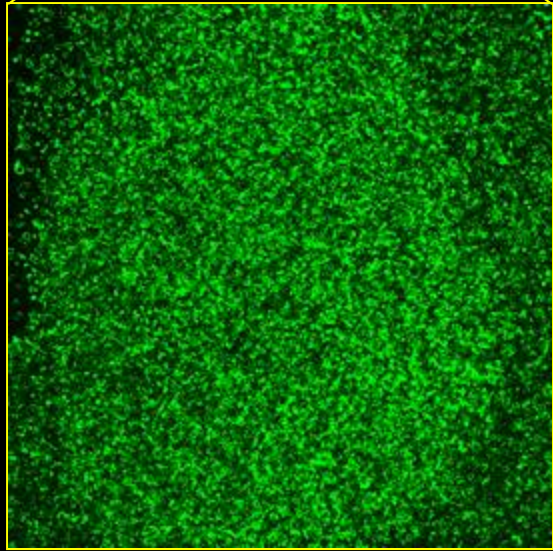
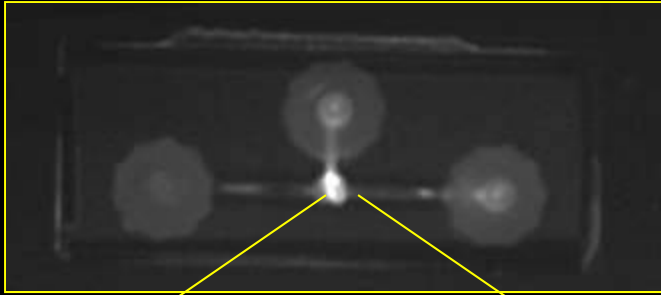


Detection: Fluorescent bead based detection

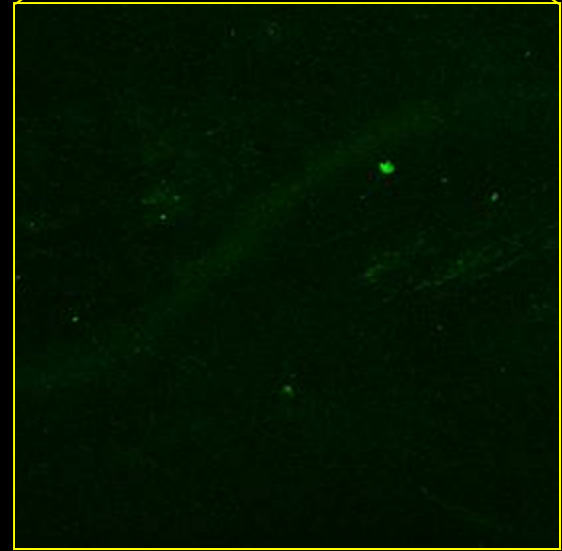
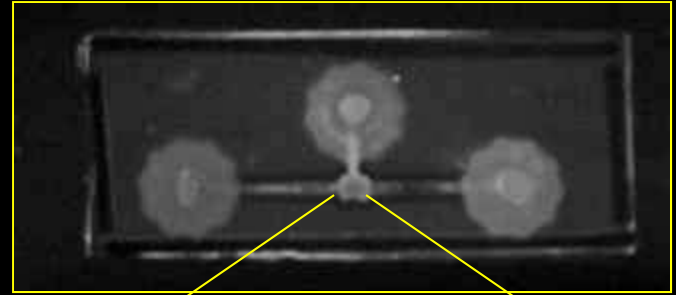
Sample injection port



Bead chamber



Carcinus maenas
Green crab target



Carcinus aestuarii
Congeneric species

Detection: Carbon nanotube (CNT) based system

- Methods:
 - Amplify (PCR) DNA as before
 - Apply PCR to chip containing tagged carbon nanotubes
 - Detection through electrochemical resistance rather than fluorescence
 - Quantitative measure

Carbon nanotube (CNT) based system



~ 5 centimeters in length

Made on a standard microscope slide

Carbon nanotube (CNT) based system



Current applied through this circuit

Carbon nanotube (CNT) based system



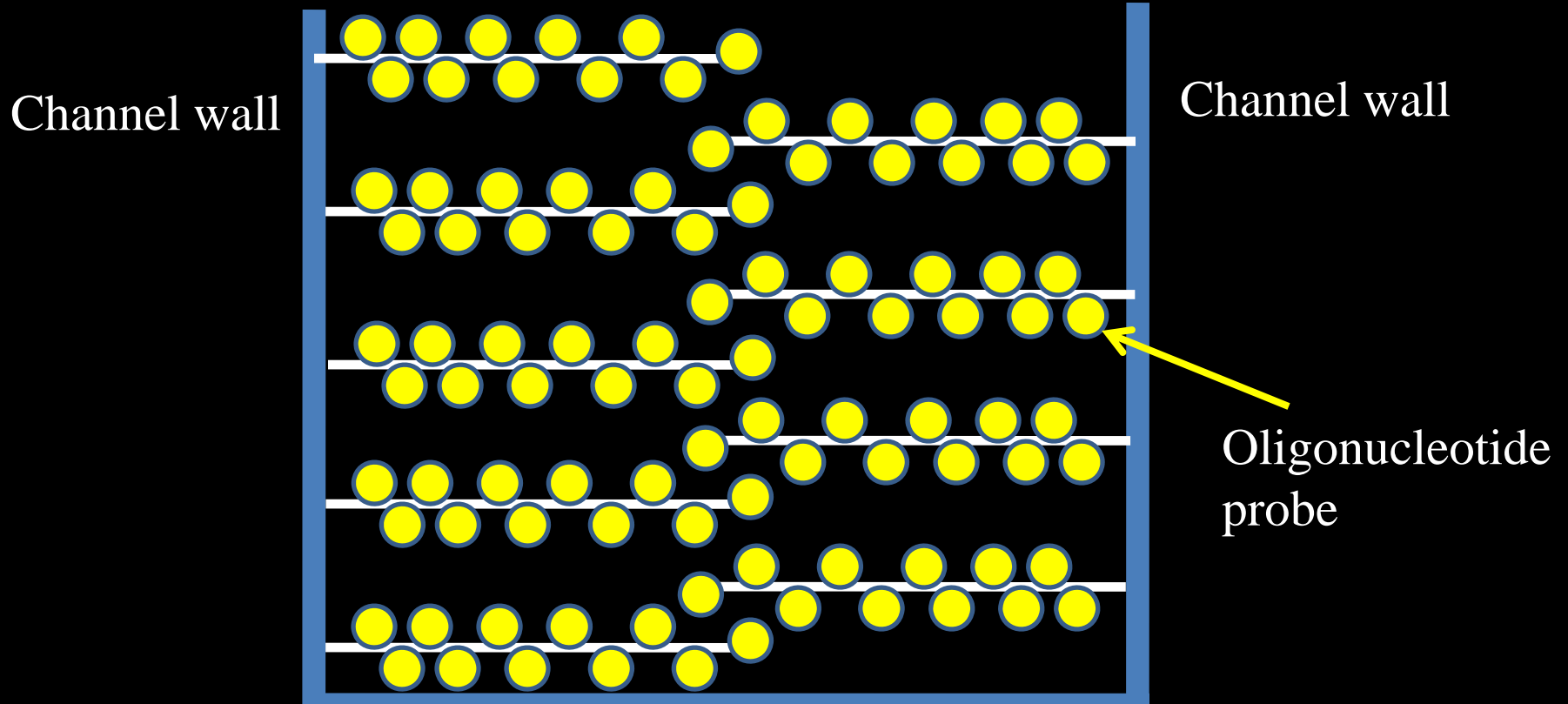
Sample introduced here

Carbon nanotube (CNT) based system



Sample flows through this channel

Looking through the CNT channel

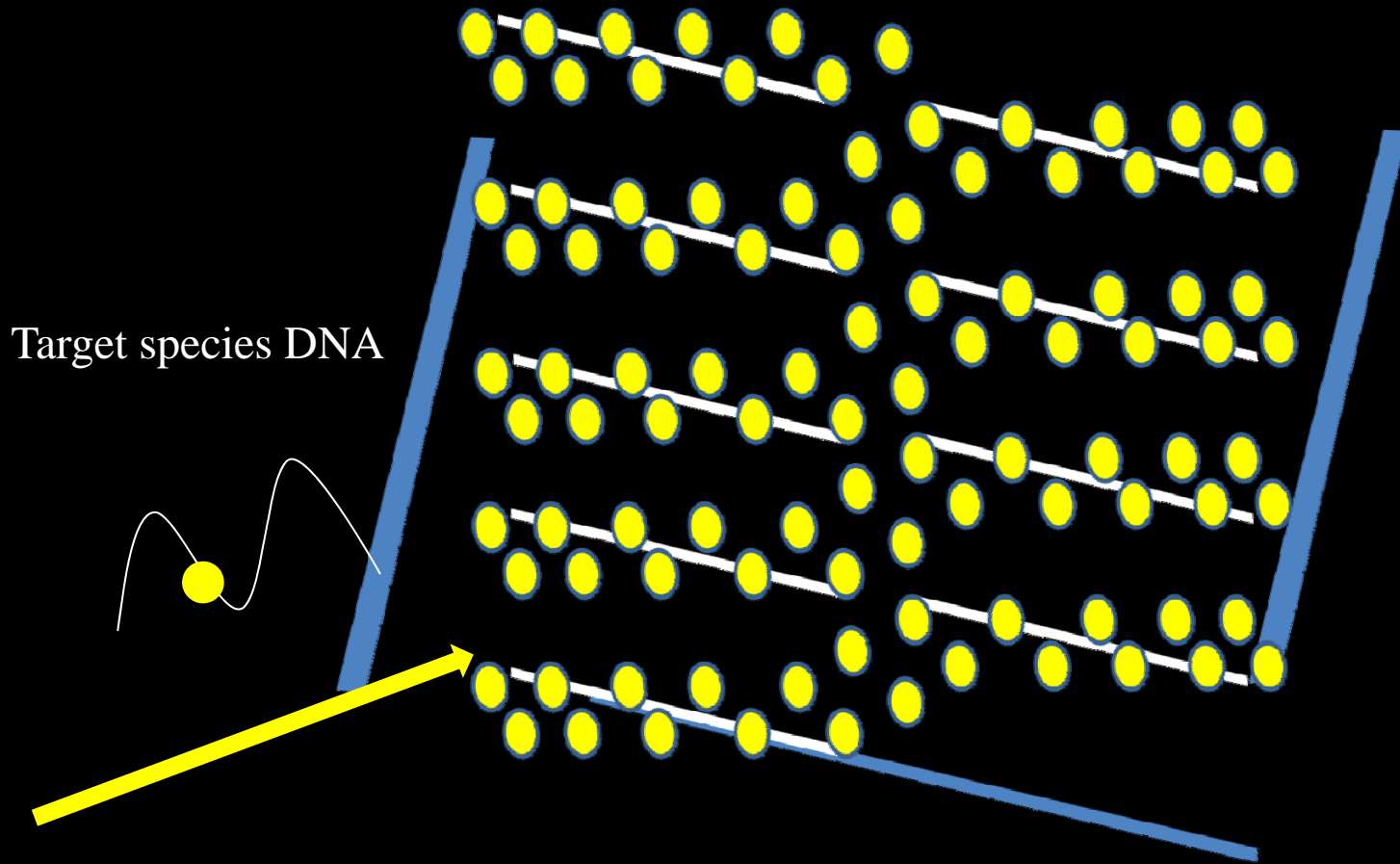


Detection:

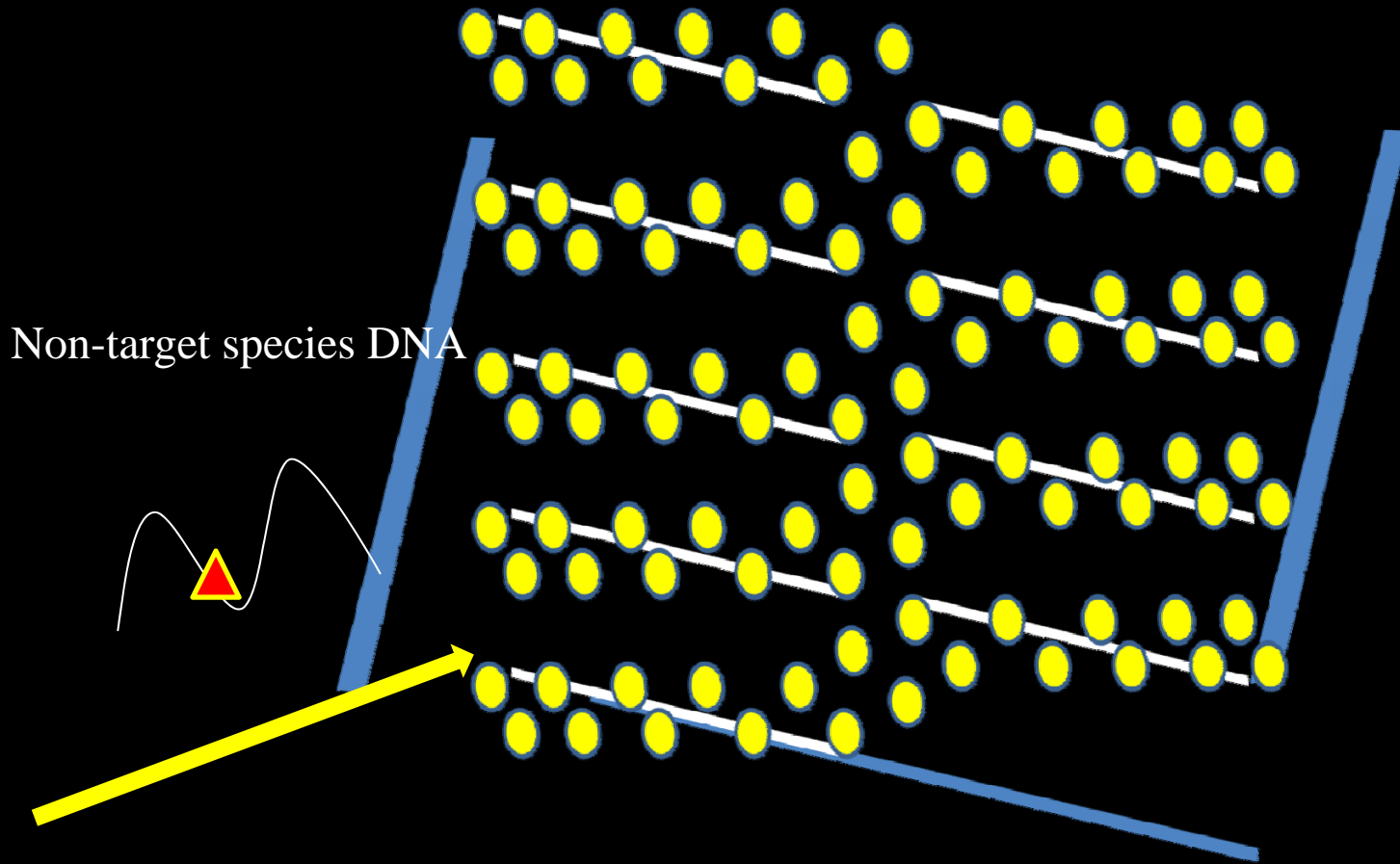
Carbon nanotube (CNT) based system

- How it works....
 - Samples passed through a channel containing carbon nanotubes that are tagged with a species specific (a.k.a. target species) primer
 - If the sample contains the specific DNA sequence that will bind to the species specific tag, it will cause the resistance to increase.
 - If the DNA is not the target species, it will not bind and no resistance increase will be detected.

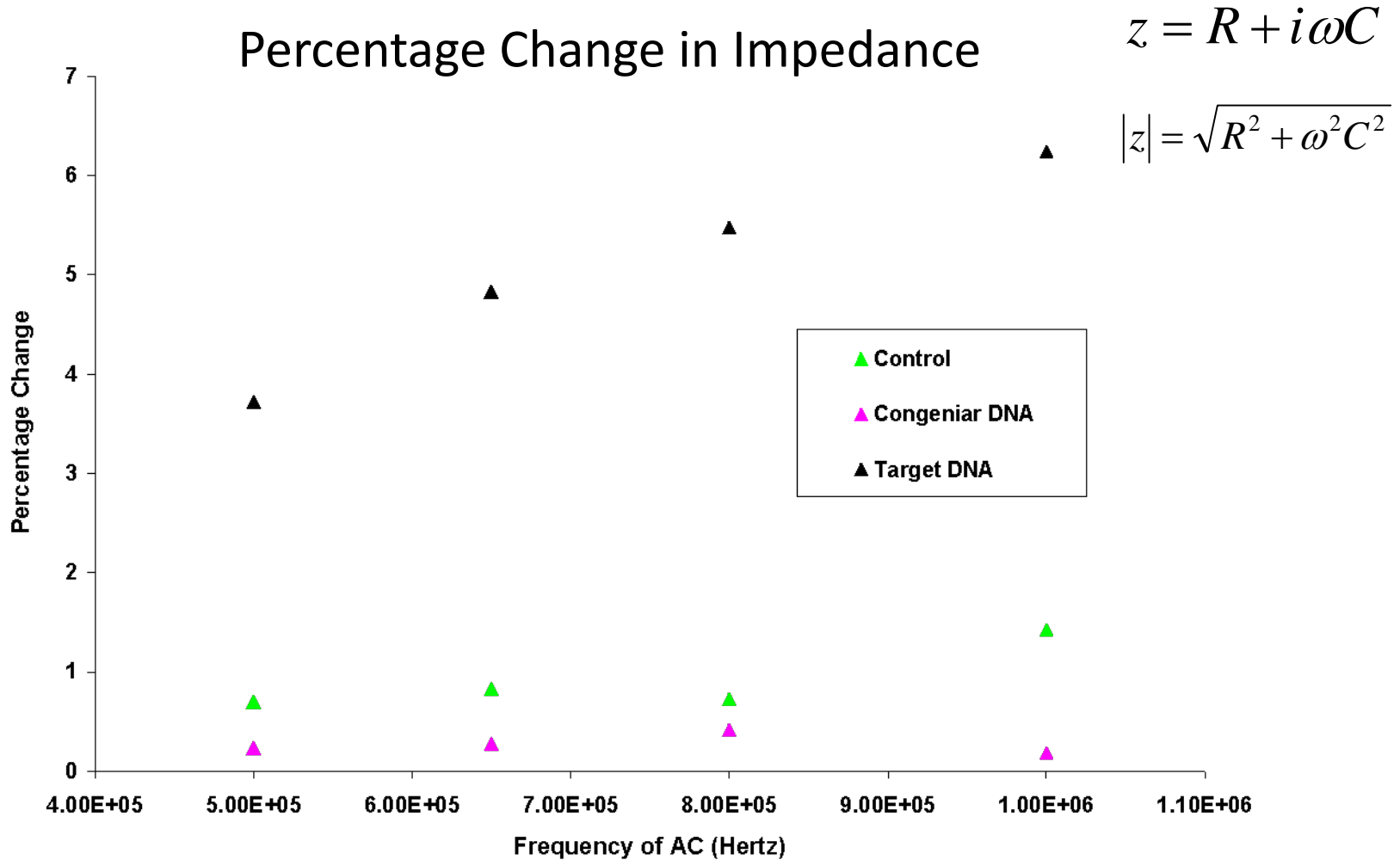
Looking through the CNT channel: Target species



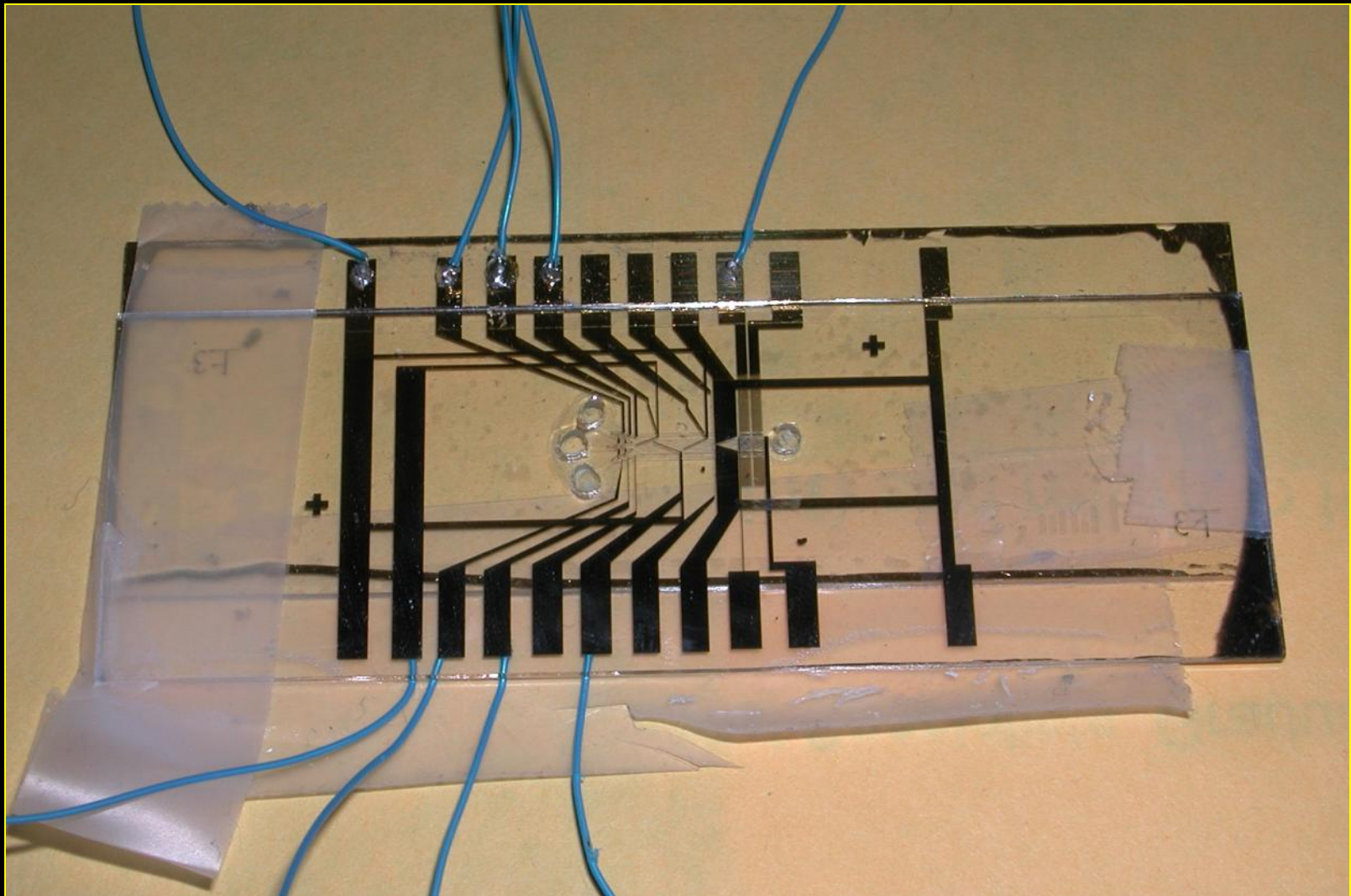
Looking through the CNT channel: Target species



Detection: Carbon nanotube (CNT) based system results



Carbon nanotube (CNT) based system: Detection of multiple species on a single chip



Results to date

- Target tag successfully developed for 5 of 6 target species
- Successful detection using fluorescent bead technology
- Successful detection using CNTs to 1/500,000 of an individual larvae

Results to date (cont.)

- Timing
 - Ballast filtering and DNA extraction = ~25 min
- PCR amplification: ~30 mins
- Hybridization and detection: ~15 minutes
- Total time from sample collection to detection:

70 minutes