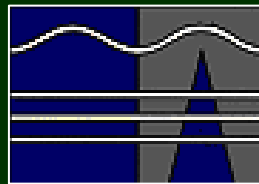
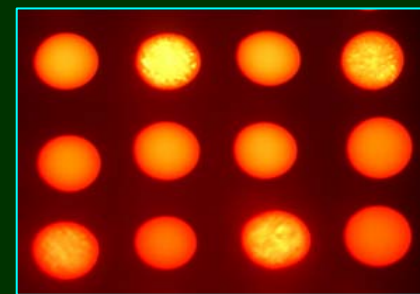


# DNA Microchip for detection of marine bivalve

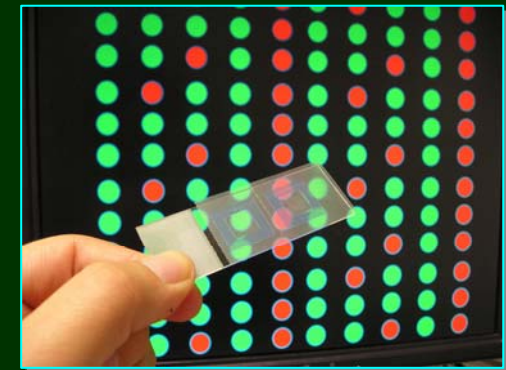
Dr Ivan F. Bendezu



Letterkenny Institute of Technology (LYIT)  
Science Department



700  $\mu\text{m}$



# Research Projects

- **NAP Tyndall.** DNA Microchip for detection of marine bivalves (I. Bendezu).
- **Strand III Project.** (HEA) Comparison of morphological and biotechnological identification of marine bivalve larvae. (I. Bendezu, B. Carney, J. Slater).
- **Proof of Concept (Enterprise Ireland).** Development of an immobilized molecular beacon DNA biosensor using electrochemical detection of hybridization. (D. McCrudden, I. Bendezu, J. Slater).
- **CAMBio.** Identification of Bivalve larvae using fluorescence *in-situ* hybridization (FISH) techniques and molecular beacons. (I. Bendezu, B. Carney, J. Slater).
- **EIRCSET.** Identification of bivalve larvae using fluorescence *in-situ* hybridization (FISH) techniques and linear probes. (S. Heaney, I. Bendezu, J. Slater).

# Objective

To develop a DNA microchip/Biochip for detection and/or identification of DNA from marine bivalves using Molecular beacons and glass slides.

## Characteristics:

easy implemented

robustness

consistency

automation

high speed of fabrication

versatility

repeatability

uniformity

accuracy

high precision

high resolution

cost effective

# Species used in this study

**King scallop**  
(*Pecten maximus* L.)



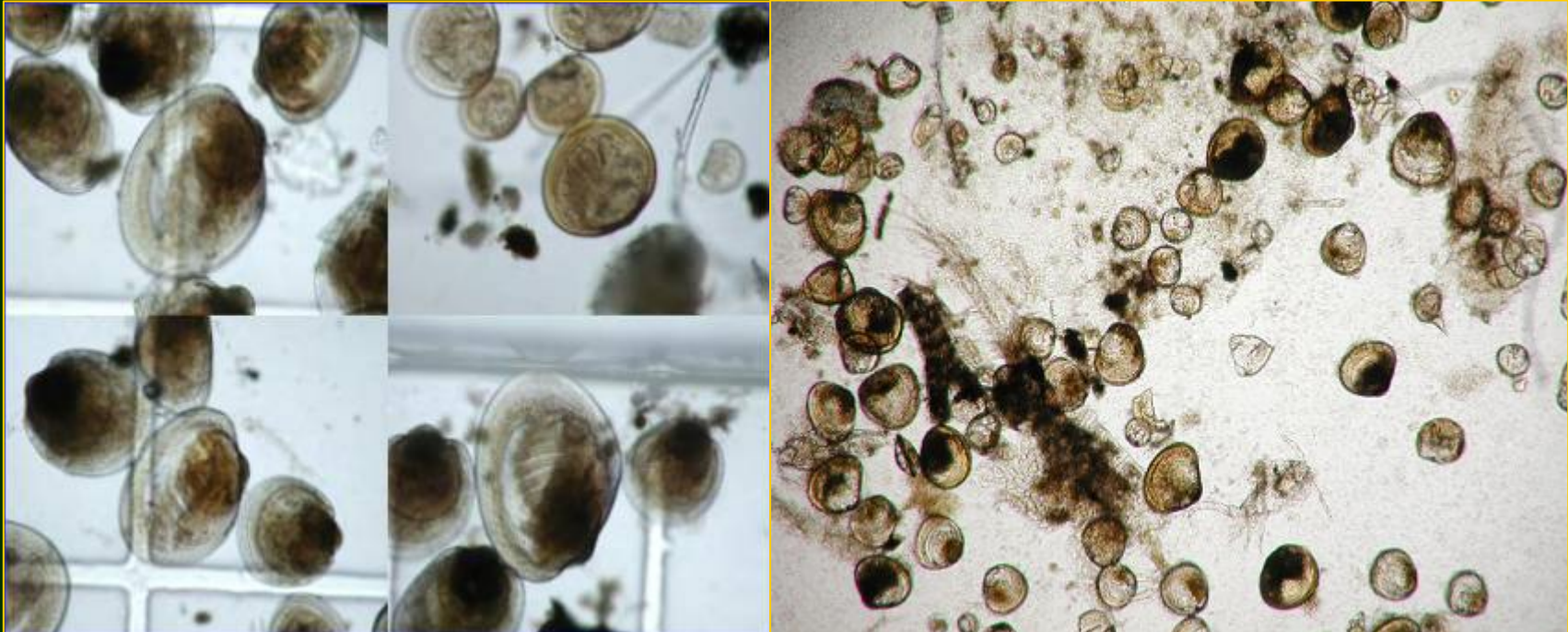
(negative control)

**Common mussel**  
(*Mytilus edulis/galloprovincialis* L.)



(target species)

# To help the shellfish industry

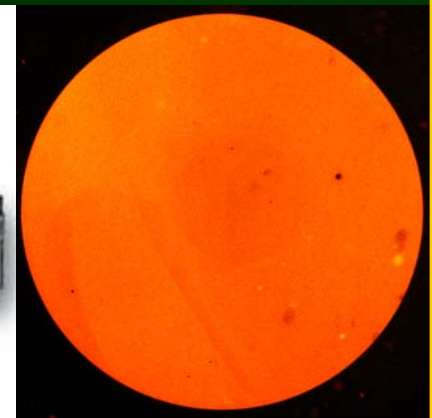


Mulroy Bay



# Strategies for the molecular detection and identification of marine bivalves

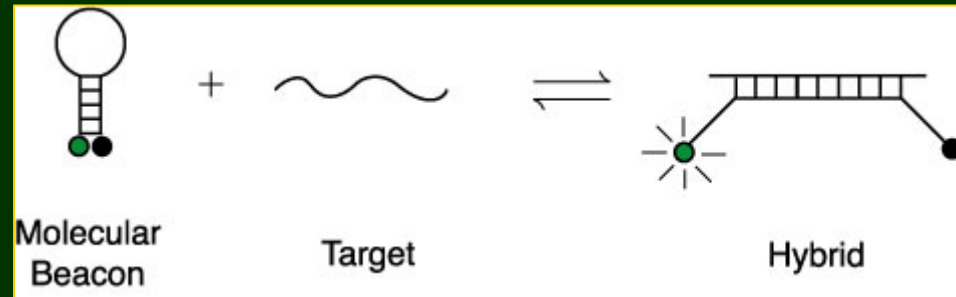
- Standard PCR
- Real time PCR
- Biosensors
- Fluorescence in-situ hybridization
- DNA Microchips



# The concept

## Molecular beacons

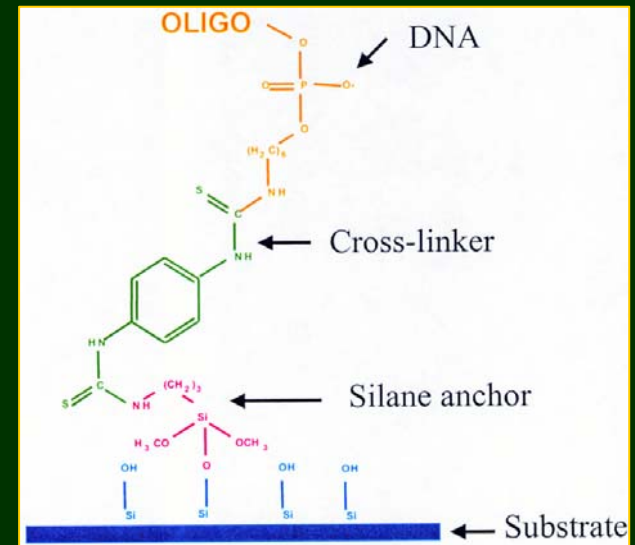
reusable single nucleotide stem and loop structures that allow the clear differentiation of positive and negative samples.



+

## Activated glass slide

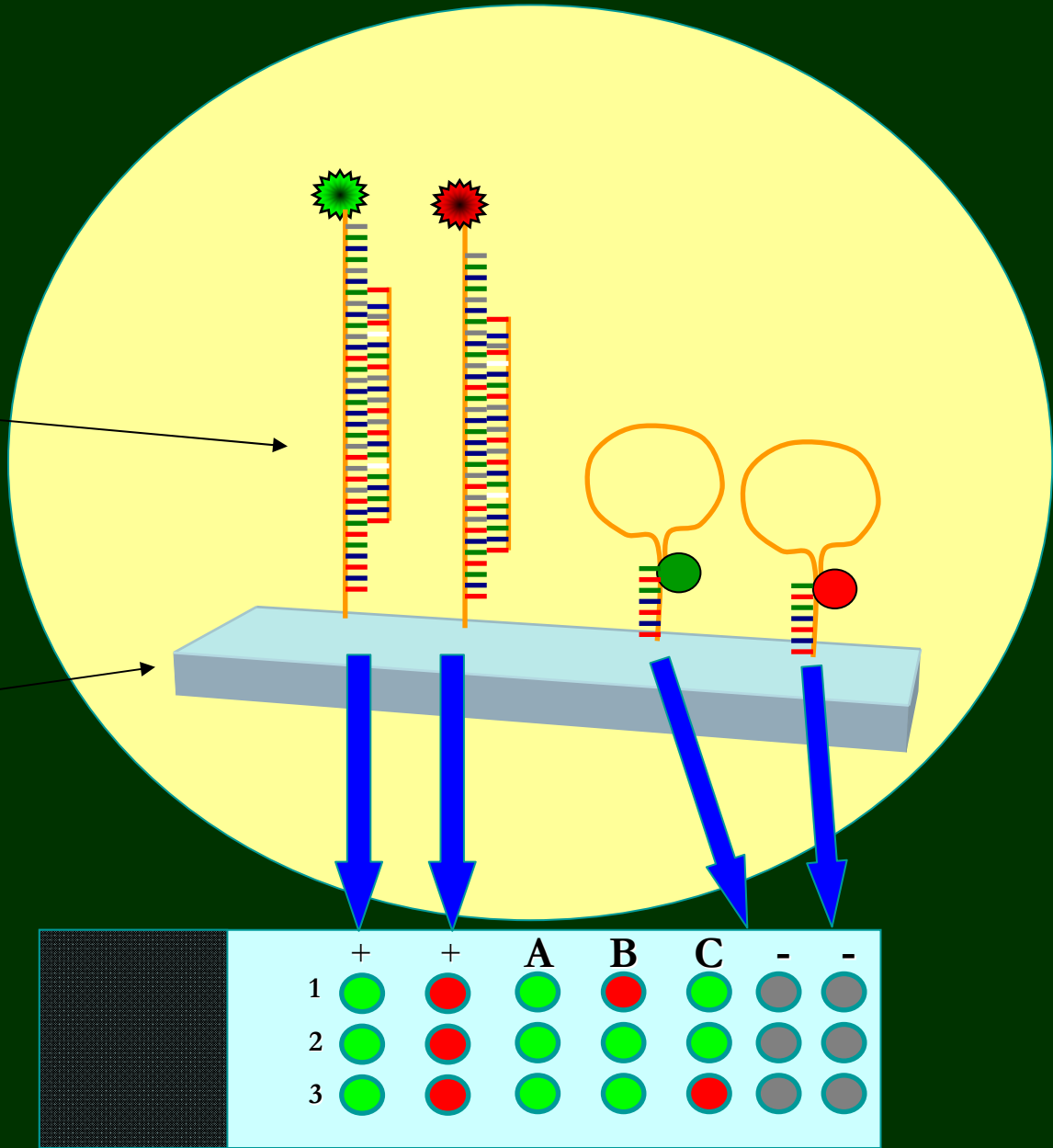
robust surface chemistry for immobilization of short strand of nucleic acids. (Manning, Galvin et al., 2002).



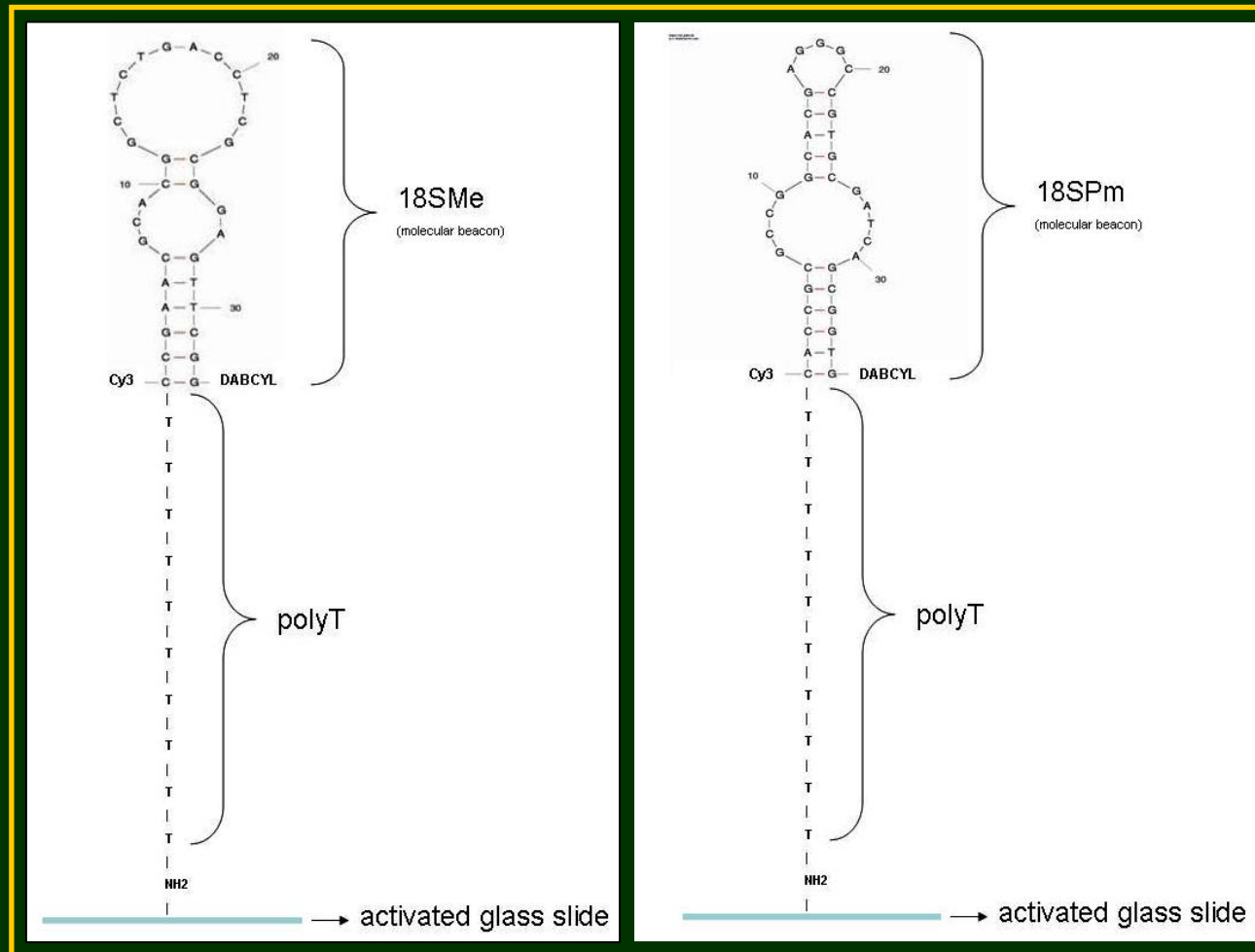
# The concept

Molecular beacons

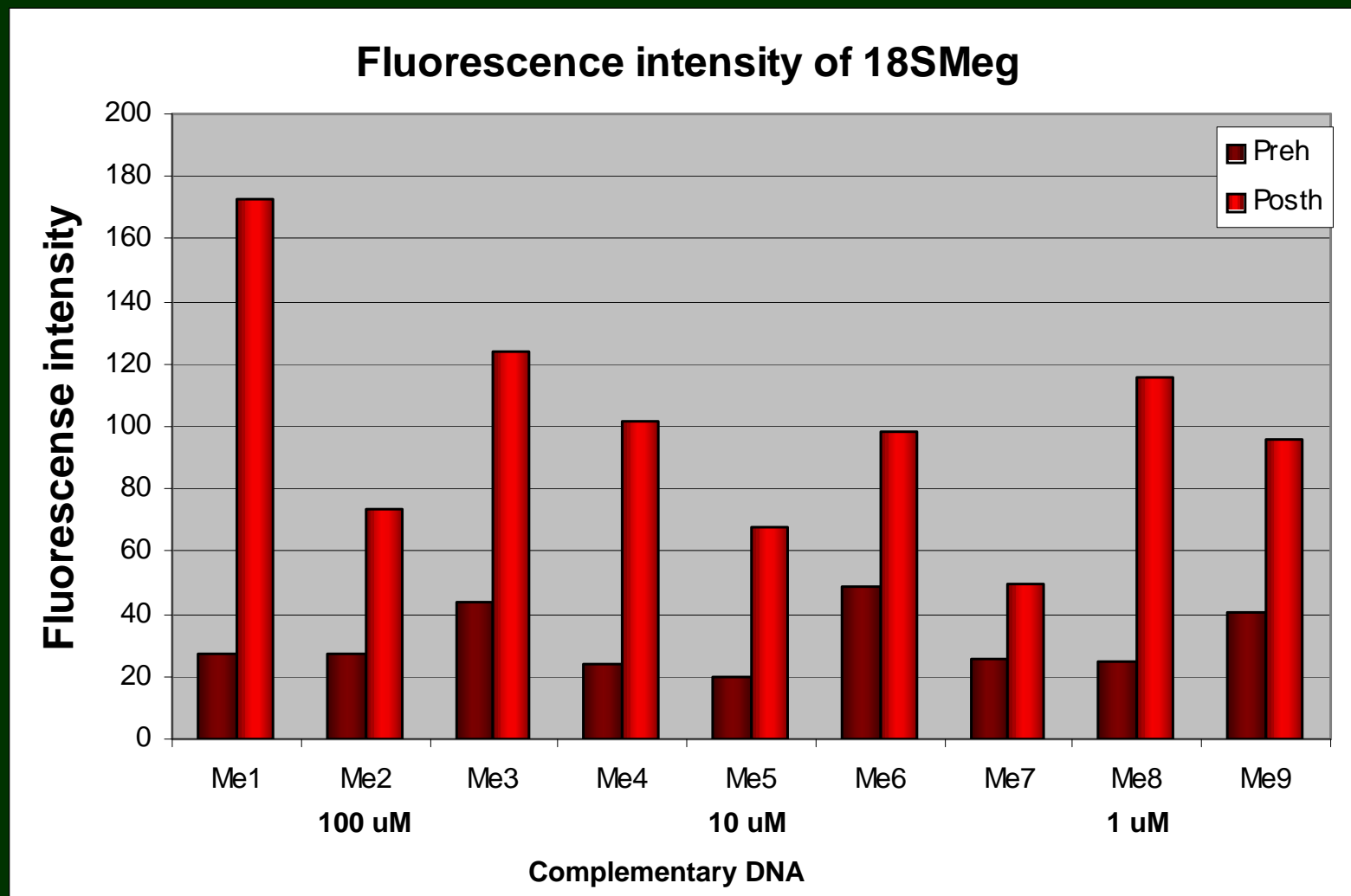
Activated glass slide



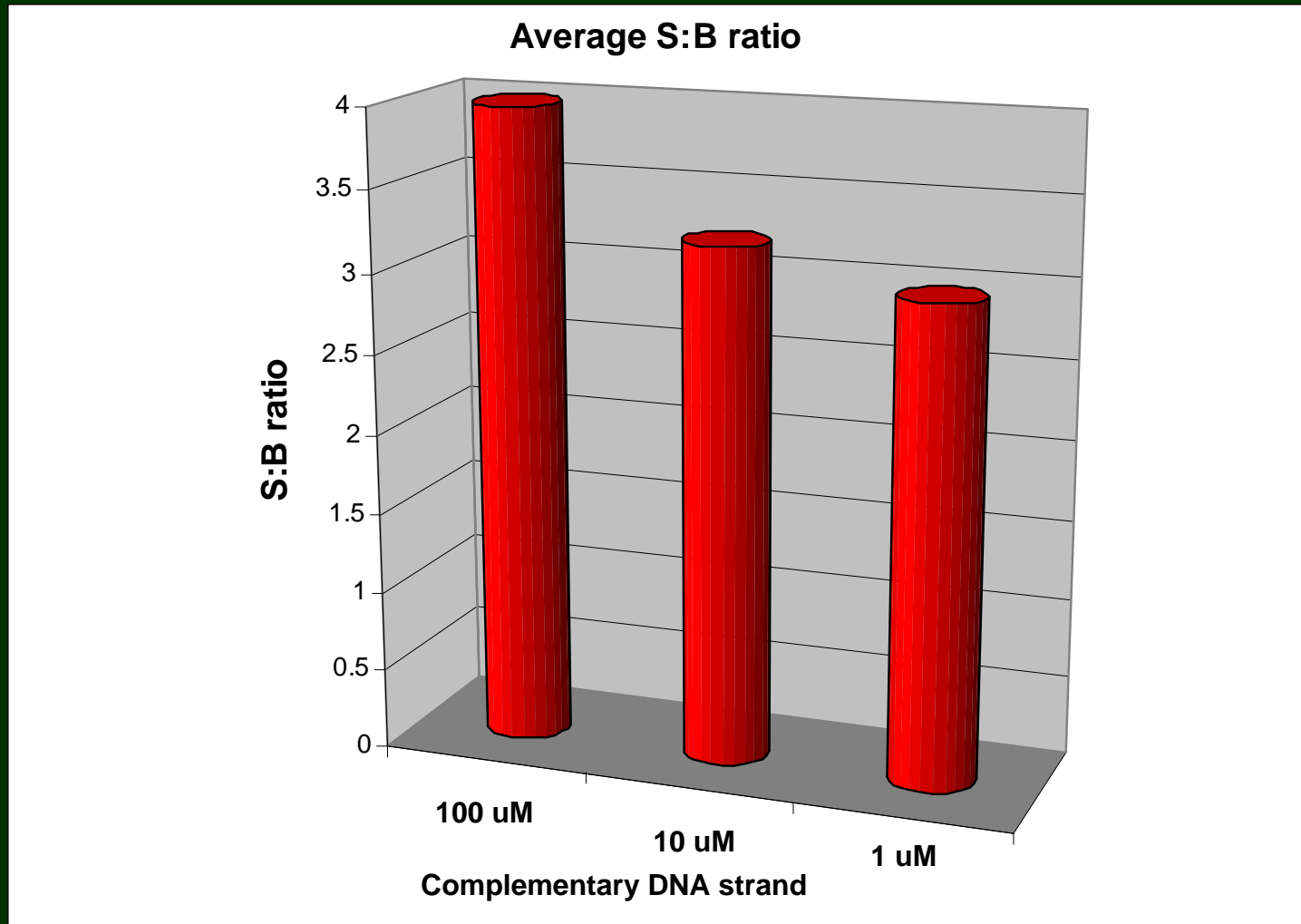
# The molecular beacons 18SMeg and 18SPm immobilized onto activated glass slides



# Fluorescence intensity of the modified molecular beacon 18SMeg pre- and post hybridization with 100, 10 and 1 uM of complementary single strand DNA (ssDNA)



# Signal to background ratios (S:B) of the modified molecular beacon 18SMeg when hybridized with 100, 10 and 1 $\mu\text{M}$ of complementary single strand DNA (ssDNA)



# Conclusions

- The modified molecular beacon 18SMeg positively hybridizes to the specific complementary ssDNA.
- The signal to background noise ratio (S:B) shown in the experiments range between 2.8 (1  $\mu$ M of complementary ssDNA) and 4 (100  $\mu$ M of complementary ssDNA).
- The optimal concentration of molecular beacon is 4  $\mu$ M.
- The optimal concentration of complementary ssDNA is 100  $\mu$ M.
- Slight modifications to the molecular beacon could improve significantly the S:B ratio.

# Plans for the Future

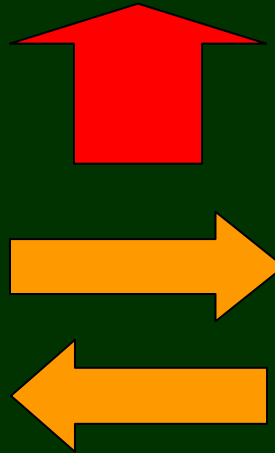
- Apply for additional funding to optimize the 18SMeg molecular beacon and increase the S:B ratio to the next order of magnitude (10s).
- Explore potential applications in other areas (medical diagnostics, agricultural pathology, etc.).

# How did the NAP programme work for LYIT/CamBio?

A working prototype platform for a DNA  
Biochip for detection of nucleic acids

Surface chemistry  
DNA microchip fabrication  
DNA molecules modification

Tyndall



Molecular beacons  
Real time PCR  
DNA/RNA technologies

LYIT/CamBio

## The team of collaborators

- Dr. Ivan F. Bendezu (LYIT/Cambio)
- Dr. Paul Galvin (Tyndall)
- Dr. Eric Moore (Tyndall)
- Dr. Maeve Curtin (Tyndall)



## Acknowledgements

Special thanks to Paul Roseingrave (NAP Coordinator-Tyndall)