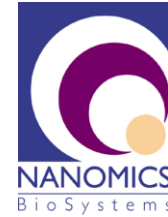




CRC **PLANT** biosecurity



Beyond ELISA:

High throughput plant virus detection via multiplexed bead-based immunoassays

Jill Meldrum^{1,2}, Kathleen Parmenter³, Darby Kozak¹,
Matt Trau¹, Andrew Geering³, Bronwyn Battersby³

¹Nanomics BioSystems Pty Ltd; ²Centre for Biomarker Research and Development, Australian Institute for Bioengineering & Nanotechnology, The University of Queensland; ³Cooperative Research Centre for National Plant Biosecurity and Queensland Primary Industries and Fisheries

Importance of Crop Protection

- Australian gross crop value in 2008 was \$23.8 billion¹
- For barley and wheat, losses from disease are 19.5% of the average annual crop value² (\$913 million for wheat, \$252 million for barley)

¹Australian Bureau of Statistics 7106.0, Farming in Brief, 2009

²Murray and Brennan (2009). Grains Research and Development Corporation

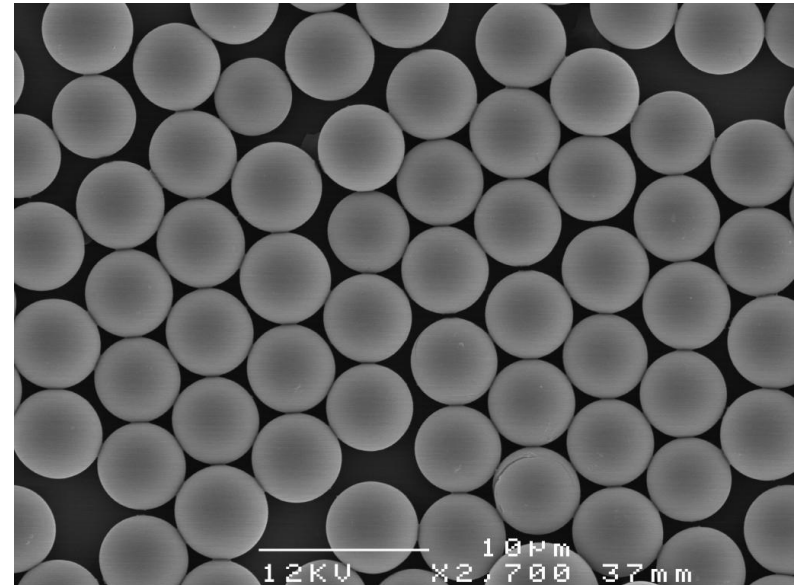
Diagnostic Challenges in Plant Biosecurity

- Pathogen and host diversity very large.
 - 44 horticultural industries recognized by Horticulture Australia Ltd
- Populations of plants are much larger than those of animals
 - national sheep flock = c. 100 million.
 - at a planting density of 200 plants/m², 50 ha of wheat contains 100 million plants).
- High throughput diagnostics are therefore needed



Project Aim

- To develop high throughput, multiplexed immunoassays using OptoPlex™ nanosensors (Nanomics Biosystems Pty Ltd).



OptoPlex™ nanosensors
– 5 µM silica spheres

Target Plant Viruses



AltMV-infected plant



PVY-infected plant



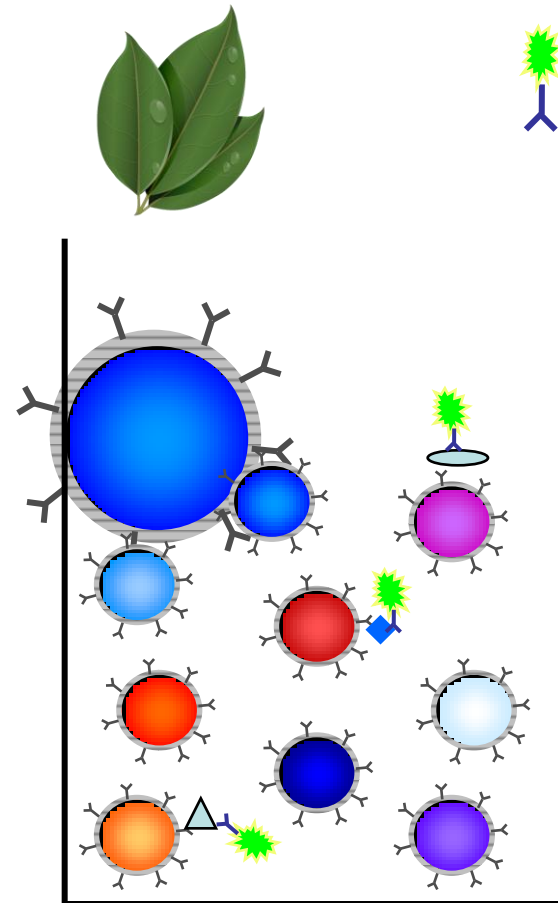
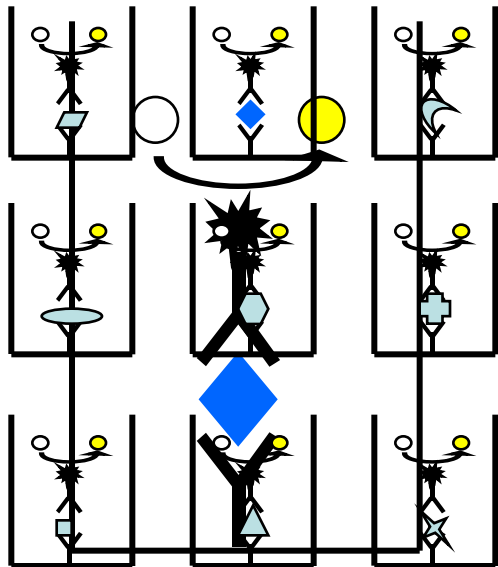
PLRV-infected plant

- *Alternanthera mosaic virus* (AltMV) – first identified in Queensland¹
- *Potato virus X* (PVX), *Potato virus Y* (PVY) and *Potato leafroll virus* (PLRV) – major pathogens of potato worldwide
- 2005/06 potato production gross value was \$465.1m*
- Viruses spread in infected potato 'seed' – diagnostics needed for seed certification schemes

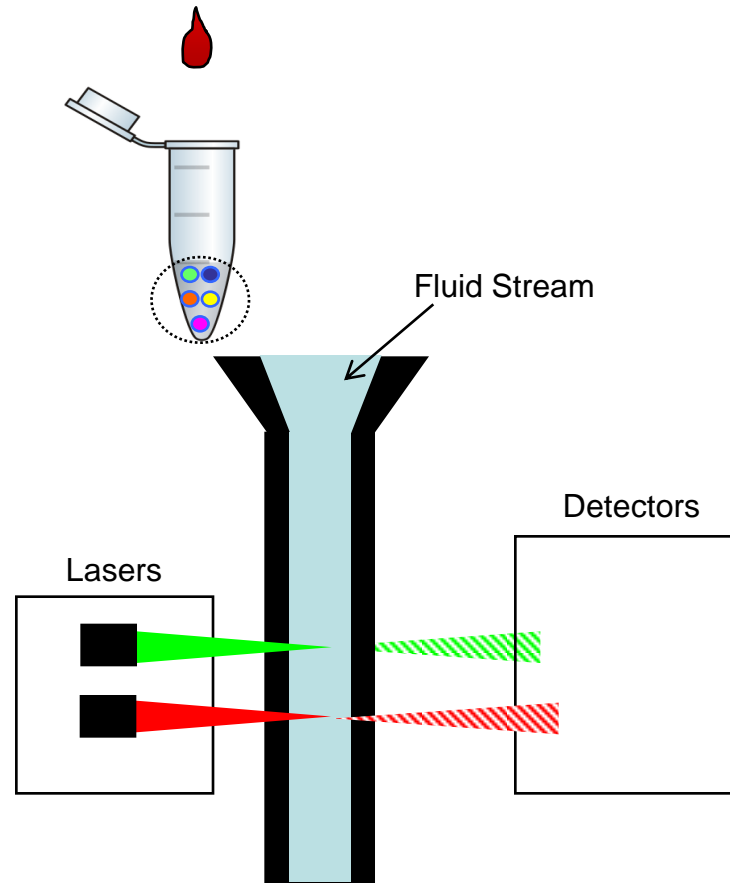
¹A. Geering, *et al.*, 1999, Archives of Virology 144: 477

* Vegetable Spotlight : 01-04-2008 , AusVeg

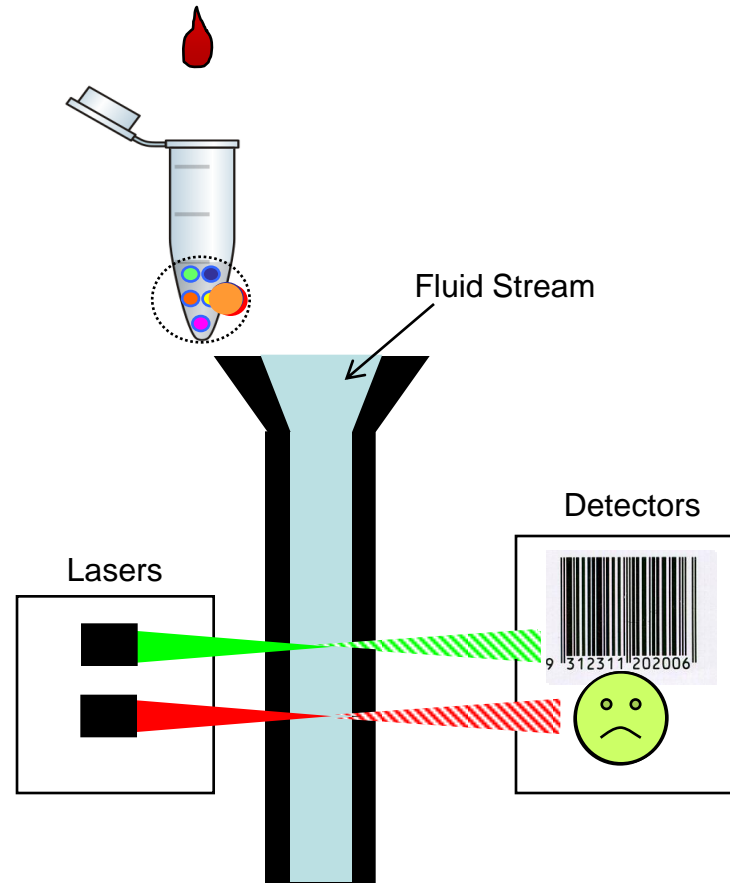
Molecular Diagnostics and the OptoPlex™ System

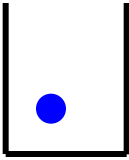


Flow Cytometry Analysis



Flow Cytometry Analysis



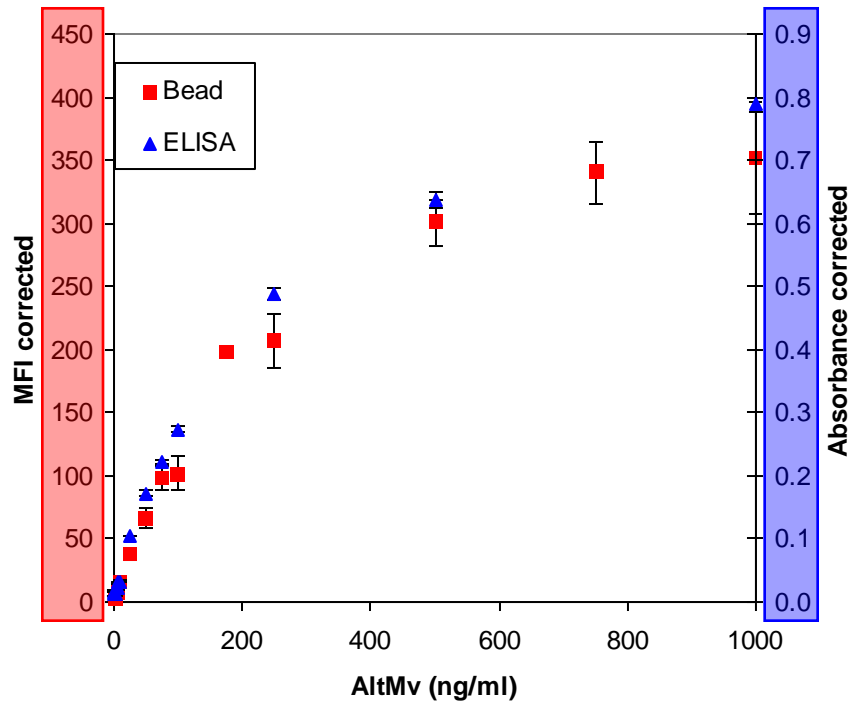


Plant Virus Assay

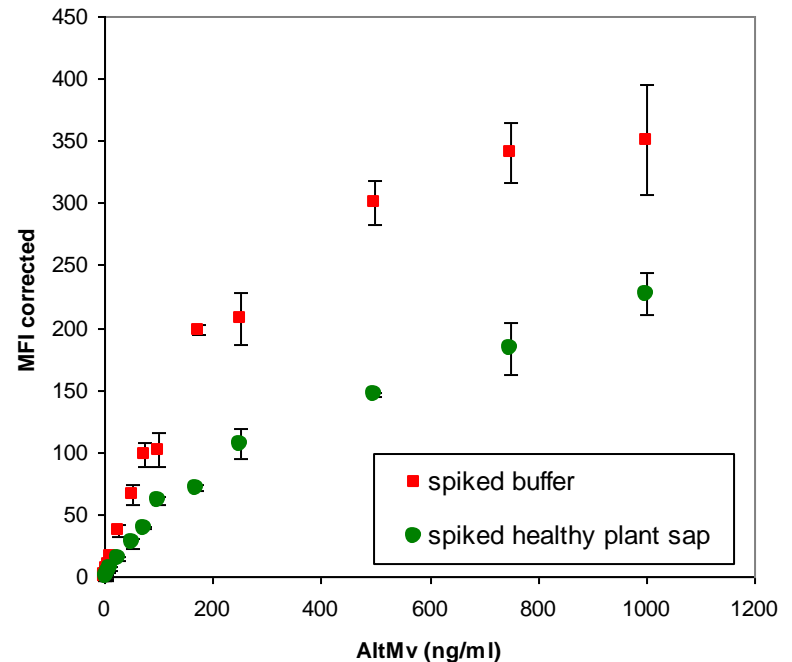


- Spiking with purified virus

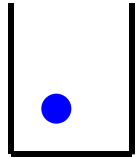
Bead and ELISA assays using AltMv spiked buffer



Assay results for AltMv spiked buffer and healthy plant tissue

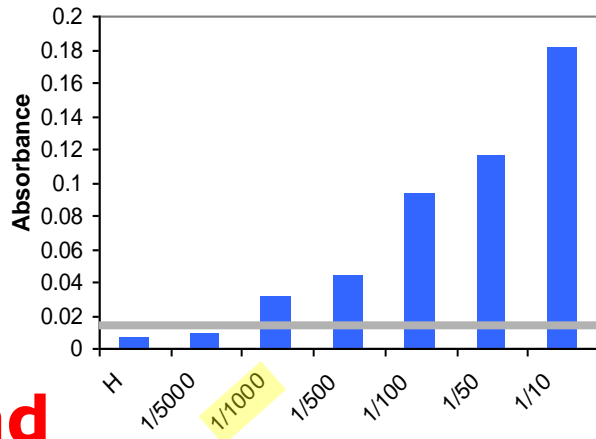


Potato Virus Singleplex

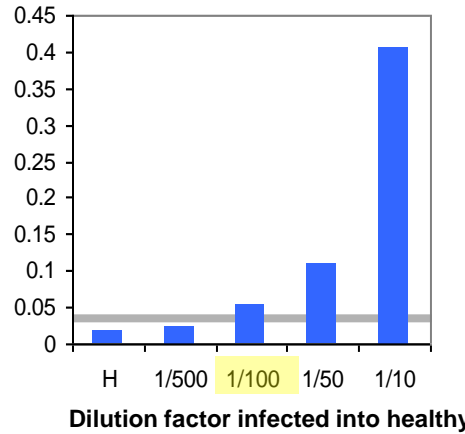


ELISA

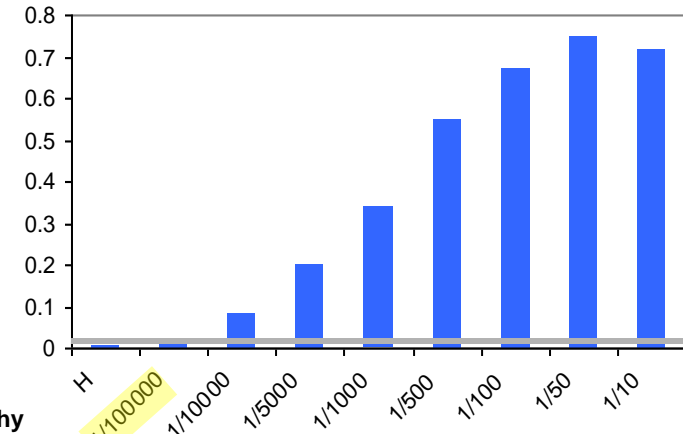
PVY



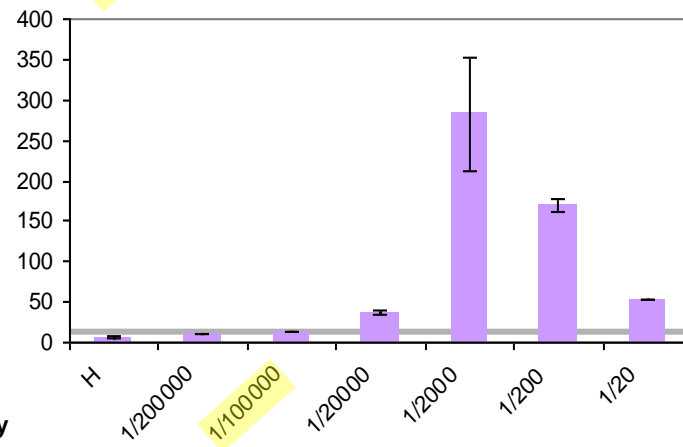
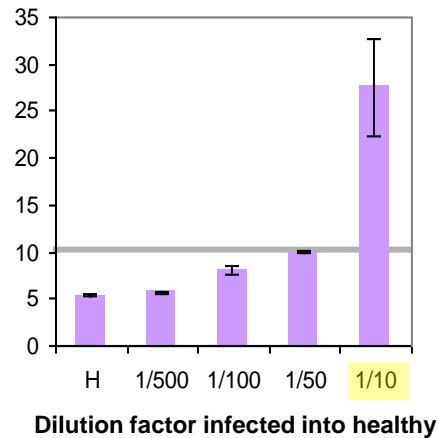
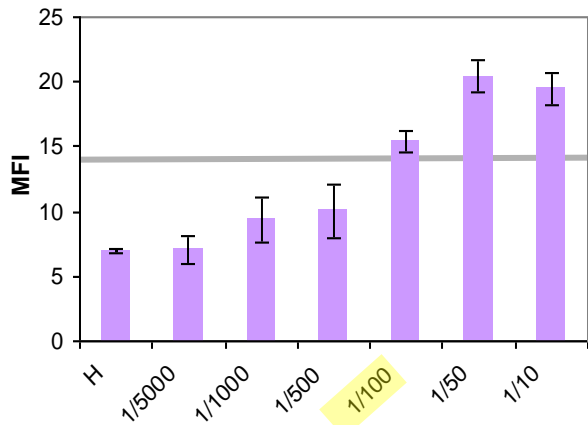
PLRV



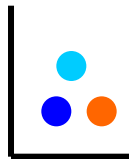
PVX



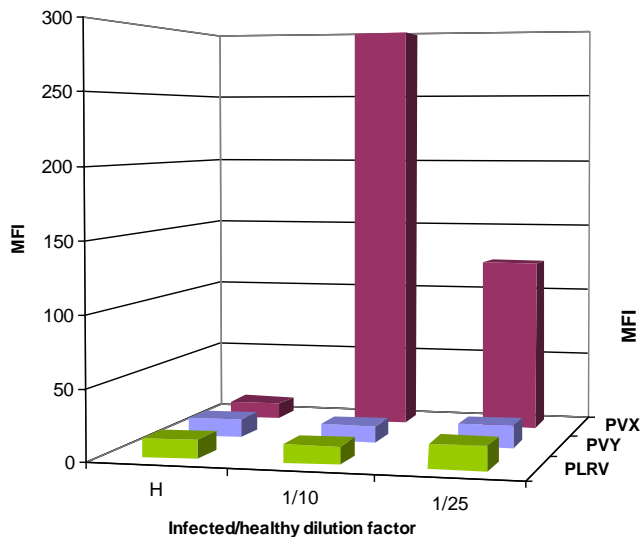
Bead



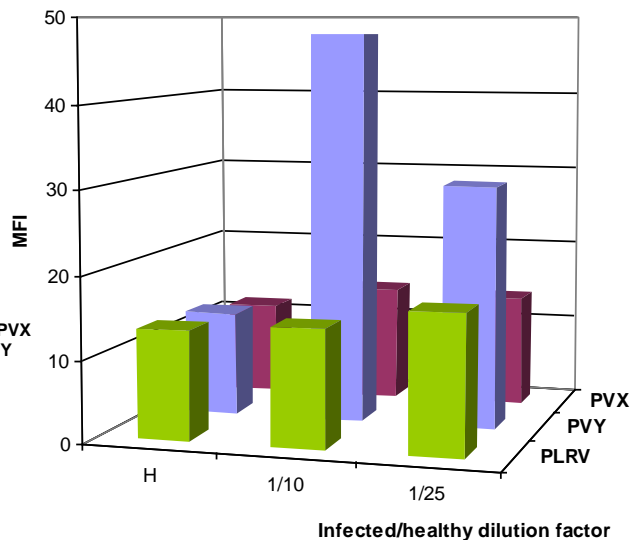
Potato Virus Multiplex



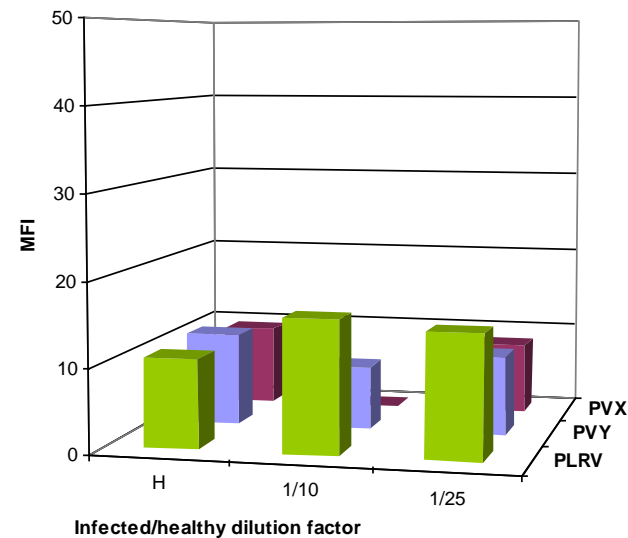
PVX infected plant material



PVY infected plant material



PLRV infected plant material



- PVX antibody beads
- PVY antibody beads
- PLRV antibody beads

Conclusions

- A high-throughput immunoassay for the multiplexed detection of potato viruses PVY, PLRV and PVX has been trialled
 - Remains in development
- The OptoPlex system is a versatile platform for the detection of a variety of plant pathogens
 - Suitable for protein, DNA or whole virus detection
- Future work investigating use of antibody single chain variable fragments (scFV) and orientation of scFvs on bead surface.



Thank you

- **Acknowledgments**

- Dr John Thomas for advice and antibodies.

- For more information, please email j.meldrum@uq.edu.au



Queensland Primary Industries
and Fisheries



Centre for Biomarker Research
and Development