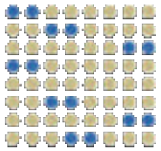
Microarrays: Where do we go from here?

Neil Winegarden

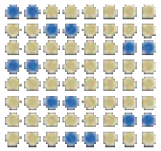
BioMEMS & Biomedical NANOtech World
2002



UHN Microarray Centre

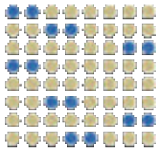
Microarrays – A mixed blessing

- Microarrays have revolutionized genomics research – *in fact they have made genomics research a reality.*
- With the advent of microarrays, researchers are able to study gene expression, mutation and function faster than ever before
- BUT - microarrays have provided more *headaches* than ever before too.



Microarrays of Today

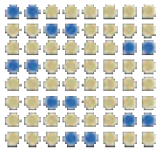
- Currently, spotted microarrays have specifications similar to:
  - 20,000-40,000 spots per slide
  - 50-100 μm diameter features
- And Microarray Labs are able to produce:
  - 100-2000 arrays per week
- And a typical microarray experiment requires
  - 10-200 arrays



UHN Microarray Centre

Microarrays of Tomorrow

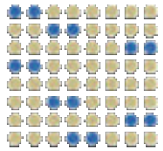
- Future spotted microarrays may require:
  - 100,000+ features per slide *or*
  - 10+ arrays of 30,000 features each per slide
  - 10 – 50 μm diameter features
- And array labs may need to produce:
  - 10,000+ arrays per week
- Because array experiments may require
  - 10,000 – 100,000 arrays



UHN Microarray Centre

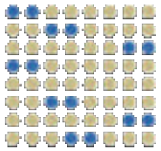
Needs Improvement

- Array Technology has come a long way, but, there are still areas that need improvement
- Just about each part of the process of the manufacture and use of microarrays can be improved
- Overall, the wish-list includes:
  - Higher Throughput
  - Greater Sensitivity
  - Improved Reproducibility



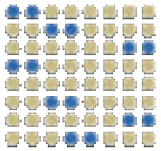
Improved Array Manufacture

- Current technologies lack the throughput and robustness required for tomorrow's applications
- Advances in printing technologies are still required
- Perhaps a departure from 2D microarrays is required



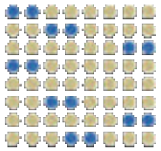
Making a Better Microarrayer

- Pin based arrayers are mostly limited to 48 applicators
  - 48 spots per slide, 100-150 slides per arrayer, 3 minutes per print cycle
  - Approximately 30 hours for 150 slides with 30,000 features = 12 minutes per slide (singlet printing).
  - Time is consumed by the need for physical deposition of the spot, and the need to clean the pins.



Making a Better Microarrayer

- Inkjet arrayers can provide similar throughput with only 8-16 applicators
  - Due to larger spot sizes, there are limitations to overall density.
  - “On the fly” printing allows for equivalent throughput with smaller number of applicators
  - Time is still consumed cleaning the applicators
  - Number of applicators is limited by the size of the inkjet devices



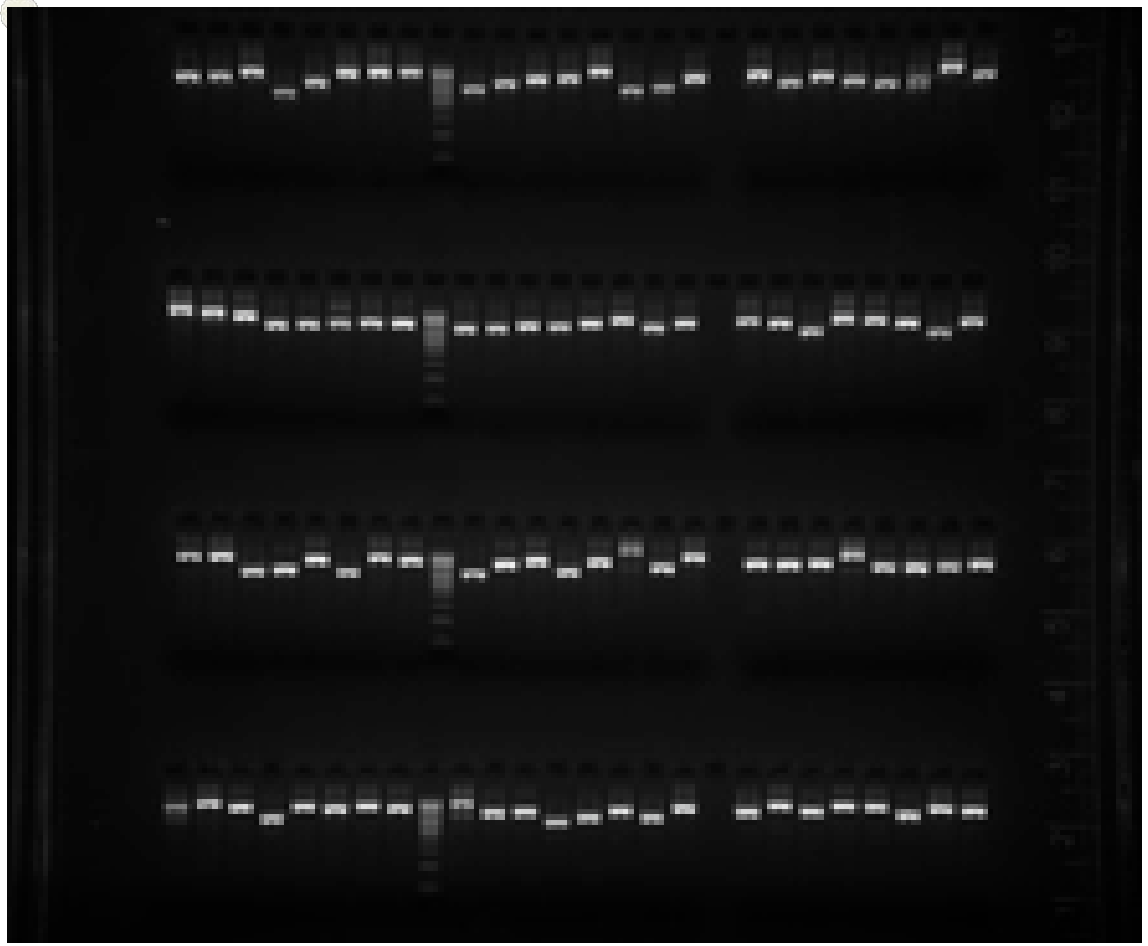
Making a Better Microarrayer

- Higher throughput could be obtained by:
  - Finer pins, allowing 192 applicators at a time (1536 well plates - 4X higher throughput)
    - Many challenges – and very costly
  - Further miniaturization of inkjet devices to allow higher number of applicators
    - 48 inkjet nozzles leads to 3X+ increase in throughput
  - A novel application device
    - Microfluidic applicator?

Not Just More Arrays – More Good Arrays

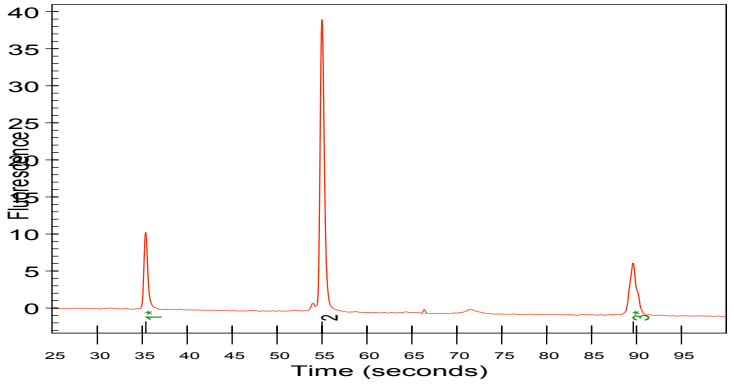
- Better QC methodologies are needed
  - Arrayers with vision systems
  - “Intelligent” substrates
  - Non-destructive QC
  - Additional fluors
  - Higher throughput, higher resolution clone production controls

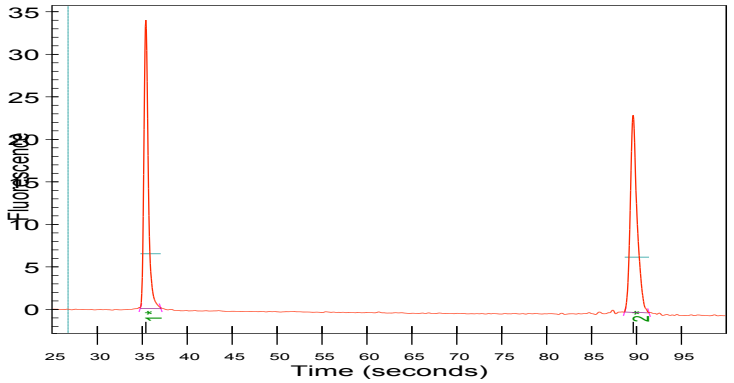
Agarose Gels for QC of Clone Production

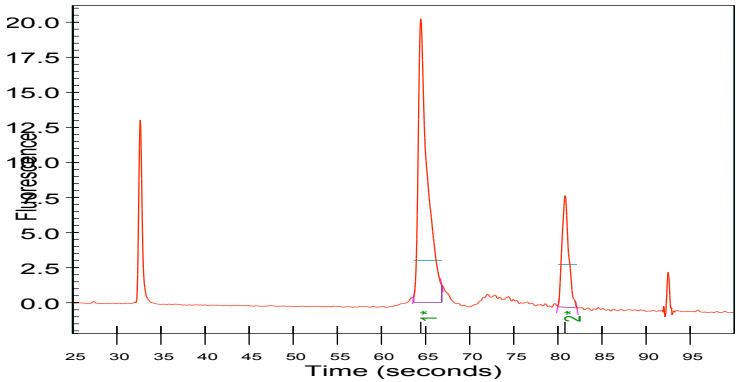


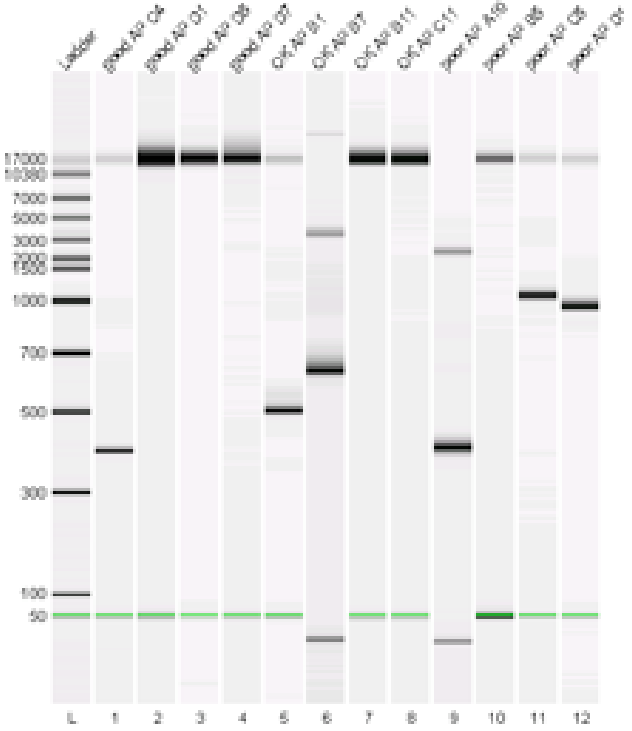
UHN Microarray Centre

CE for QC of Clone Production

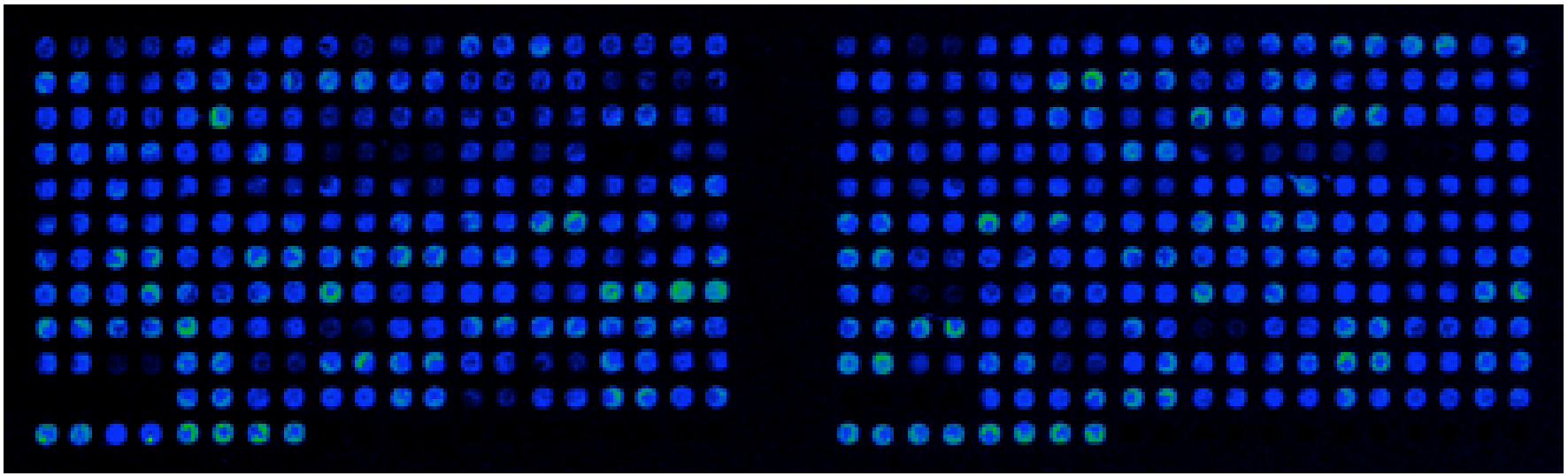








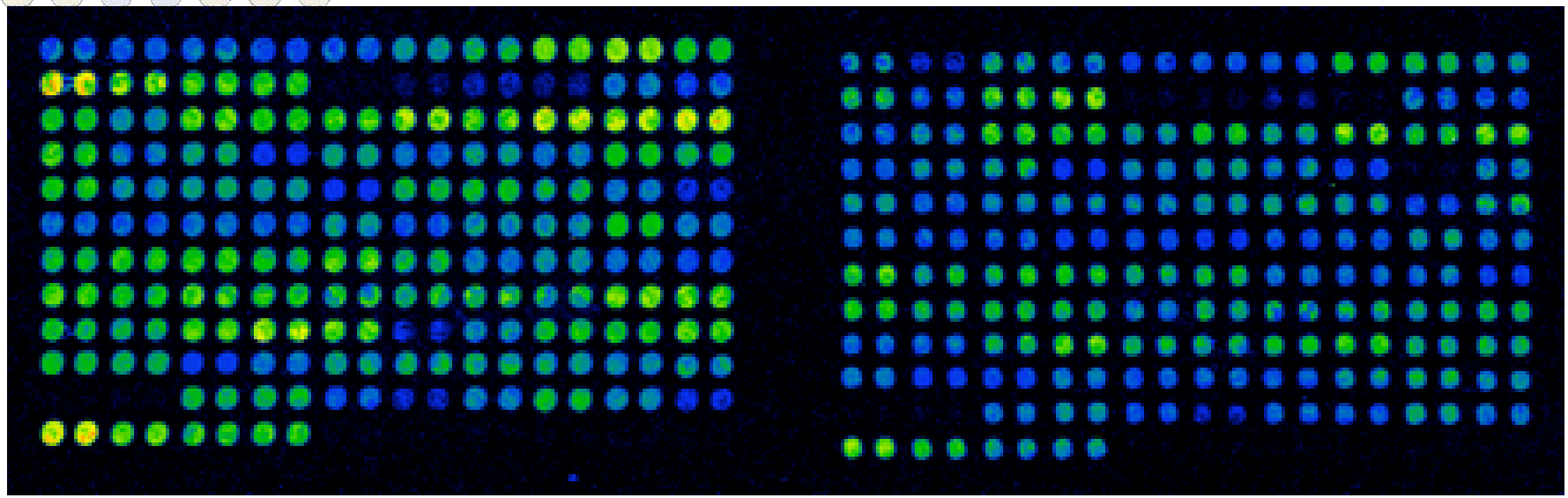
TdT Labelling to QC Array Printing



- Terminal Deoxynucleotidyl Transferase can add Cyanine 3 or Cyanine 5 labelled dCTP to the 3' ends of spotted cDNAs *or* oligos.

UHN Microarray Centre

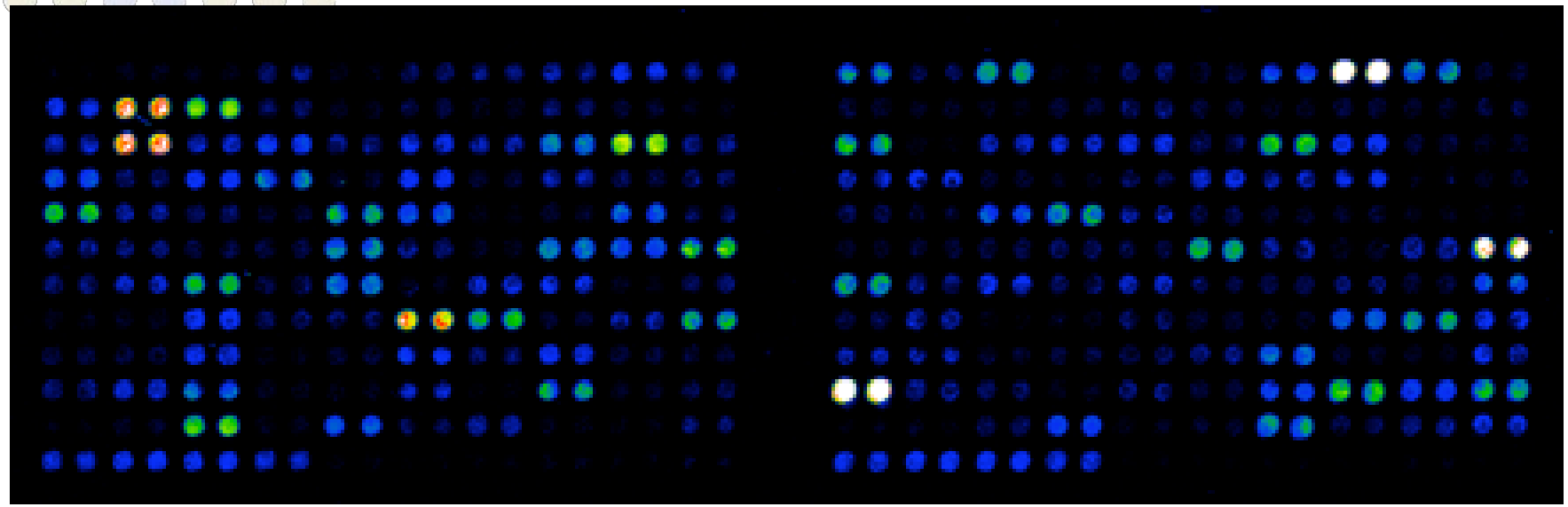
Vector Probe Labelling to QC Array Printing



- By labelling an oligo complimentary to the universal primer, you can light up all the spots printed on the array.

UHN Microarray Centre

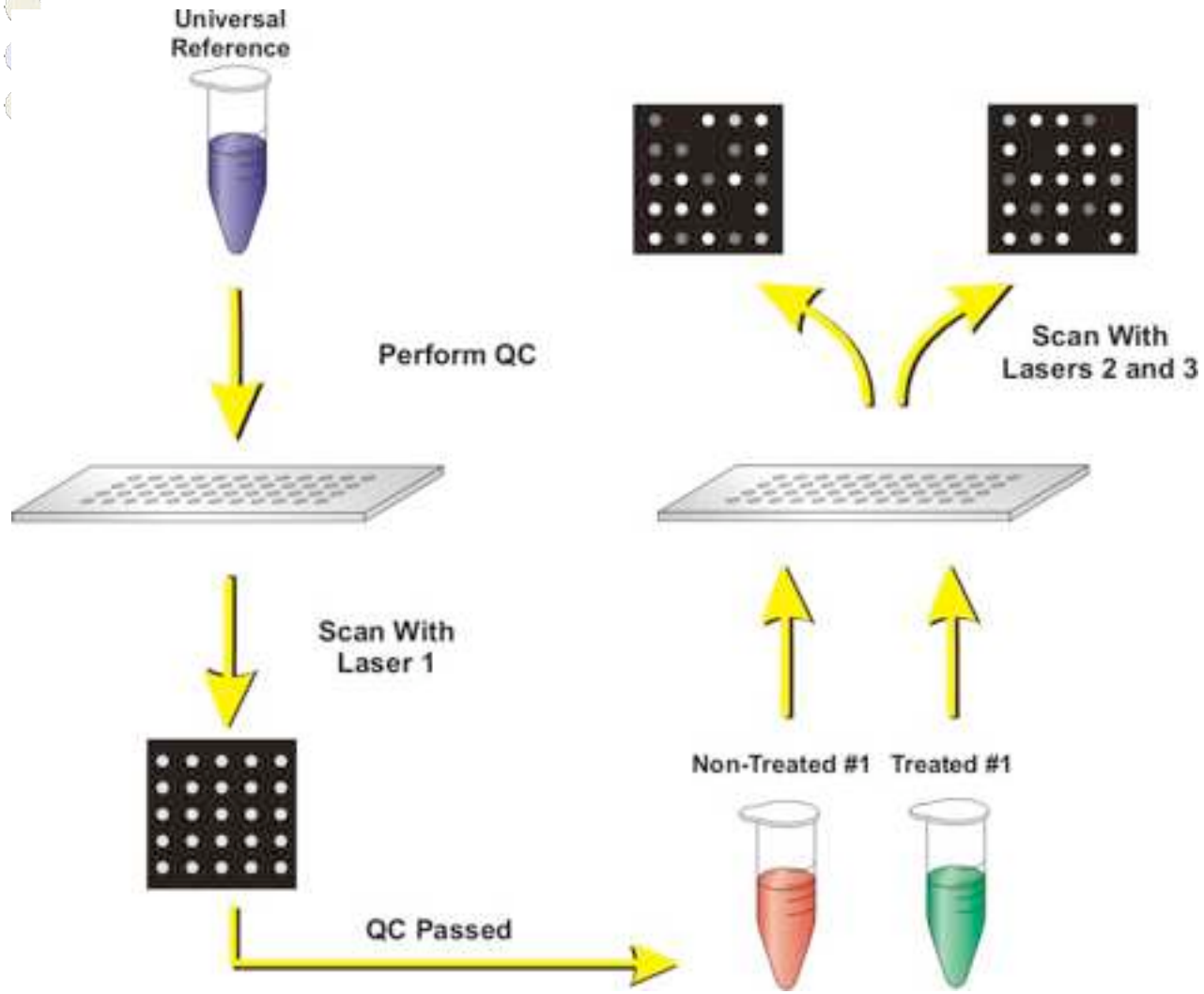
Hybridization to QC Array Processing



- Hybridization with a known and validated RNA, such as Stratagene® Universal Reference RNA (shown above) helps to assess the quality of the array post processing.

UHN Microarray Centre

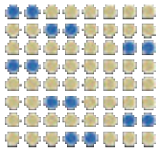
Third Colour As A QC Channel



UHN Microarray Centre

Now you have the arrays – how are you going to use them?

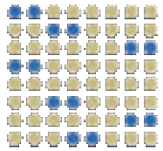
- There are five main steps in the use of a microarray
  - Labelling of sample
  - Hybridization
  - Washing
  - Scanning
  - Data Analysis
- A good technician can handle around 24-36 arrays per week using standard technologies
- Obviously to process 10,000+ arrays will require increases in throughput



UHN Microarray Centre

Increasing the throughput – using microarrays efficiently

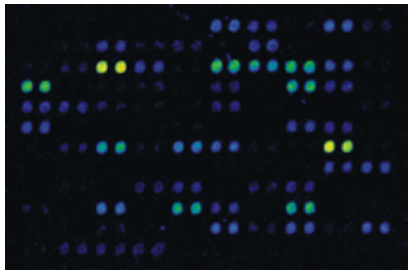
- Labelling
  - Throughput can be increased via automation – RNA extraction and labelling can be done with robotics (theoretically)
- Hybridization and Washing
  - Currently the majority of labs use the “Tupperware” solution for hybridizations
  - Automated hyb stations are available, but do not increase throughput much, if at all



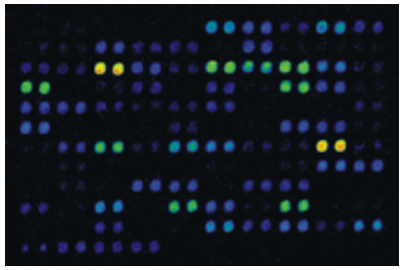
More Hybridizations

- Current hyb stations hold 12-48 arrays, and take similar amounts of time for a hybridization
- By multiplexing arrays, the number could be increased by 10 fold
- What is really required is increased automation and faster hybridizations

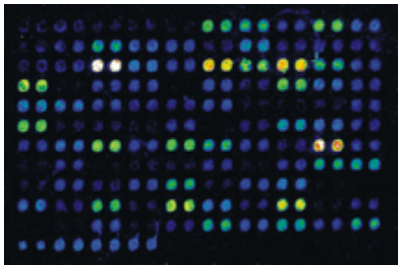
It’s all about time



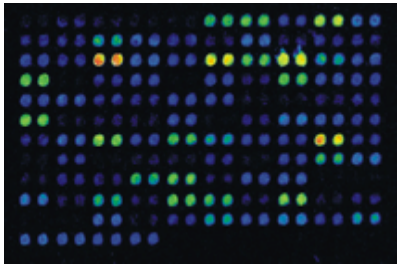
1 hour



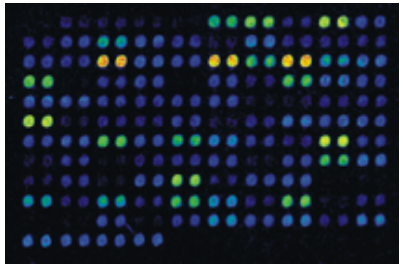
2 hours



4 hours



6.5 hours

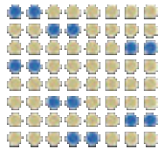


Overnight

UHN Microarray Centre

The Next Generation Hybridization Station

- Increased slide capacity – 96+ arrays
- Decreased cycle time – 1 to 2 hours
- Automatic slide handling (ability to stack slides – and automatically load them after first cycle complete)
- Liquid handling – auto-loading of samples, buffers etc…
- Also the possibility of incorporating microfluidics or BIOMEMs to incorporate the hybridization device on the array itself



UHN Microarray Centre

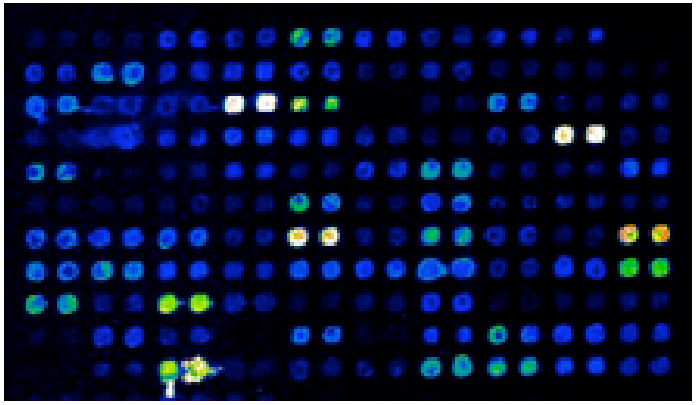
Increasing the throughput – using microarrays efficiently

- Scanning
  - Automatic slide loading is a start
    - Some scanners have 10-48 slide capacity
  - Additional slides will be required
    - 100+ slides
  - Faster scanning
    - < 1 minute per scan
    - Requires brighter signals
    - Larger CCDs
  - Alternative Detection Schemes
    - Conductance
    - Non-Fluorescent labels

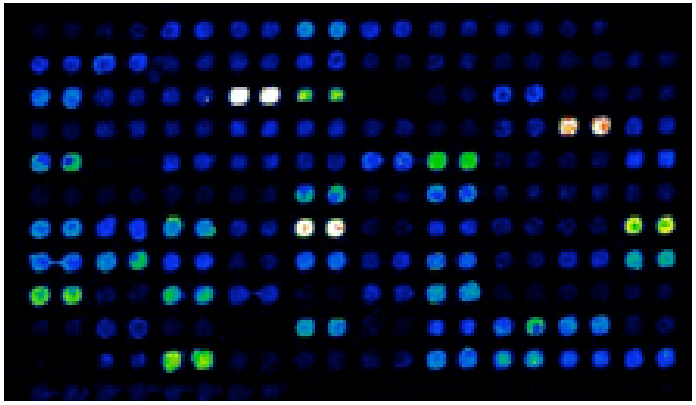
Improving Sensitivity

- Better fluors
- Non-fluorescent alternatives
- Amplifcation

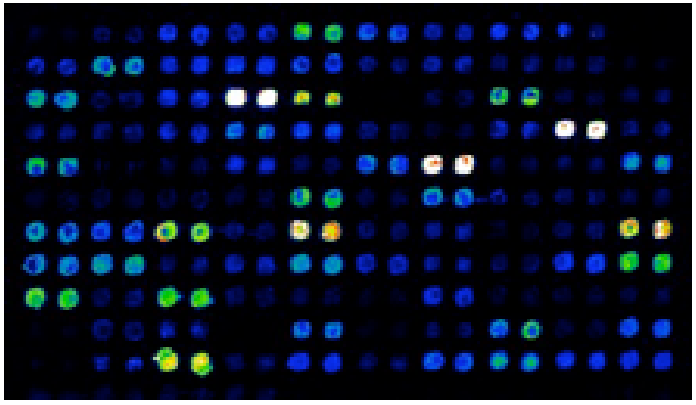
Amplification



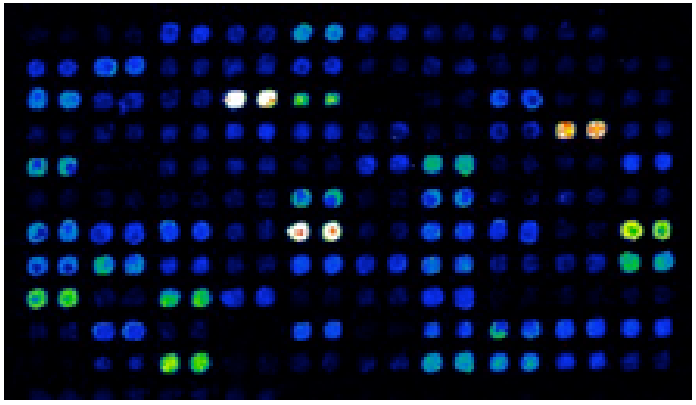
10 ng



1 ng



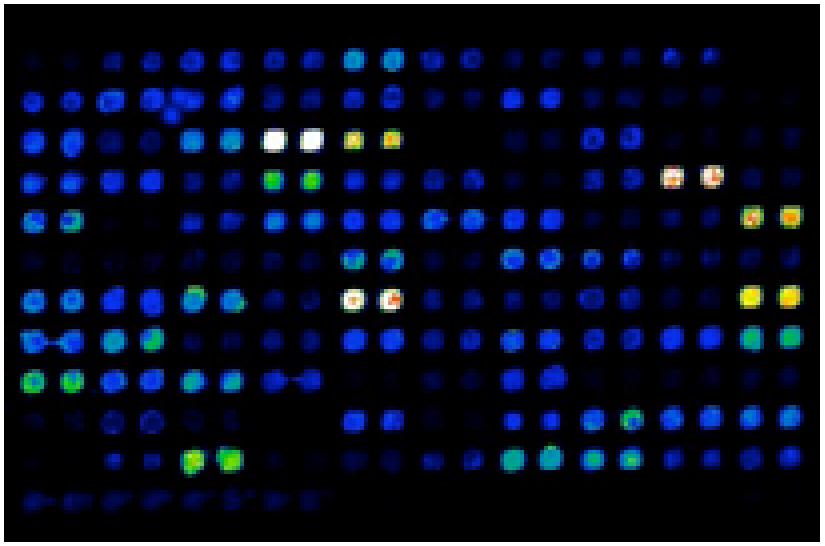
100 pg



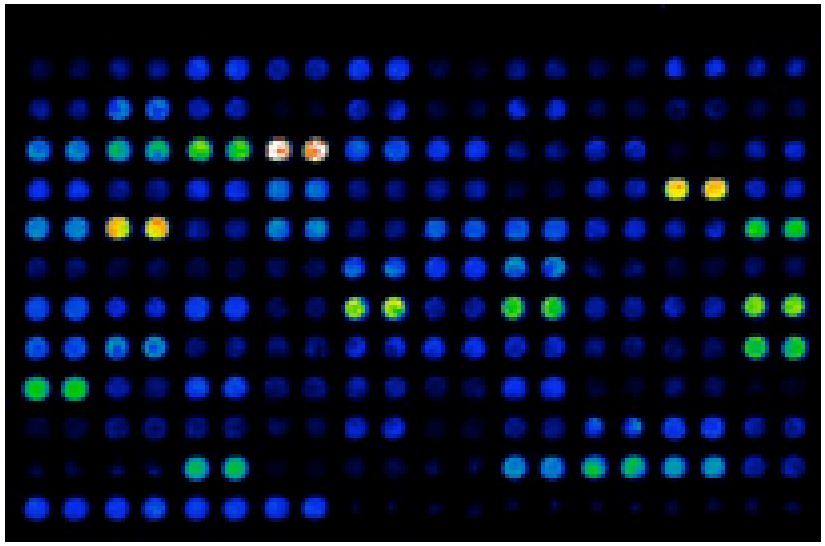
10 pg

UHN Microarray Centre

Amplification Continued



Amplified, 1 ng



Direct, 10 μg

UHN Microarray Centre

Acknowledgements

Production

- Eric Ho (Clone Production Manager)
- Patrick Yau (Array Production Manager)
- Quyen Tran
- Tuyet Diep
- Robert Kardish
- Shani Mintzberg
- Elizabeth Sakac
- Gurbaksh Basi (Tech Support)

Engineering

- Sasan Raghibizadeh (Bioengineering Manager)
- Yasin Bismilla
- Navid Nabavi

Research and Development

- Mark Takahashi (Research Scientist)
- Pascale Macgregor (Research Scientist)
- Elisabeth Tillier (Research Scientist)
- Carolyn Modi (Project Manager)
- Robert Clum
- Monika Sharma
- Natalie Stickle
- James Paris
- Monique Albert
- Thomas Liu

UHN Microarray Centre

Thank You

Please visit us at: *www.microarrays.ca*

UHN Microarray Centre