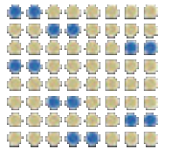


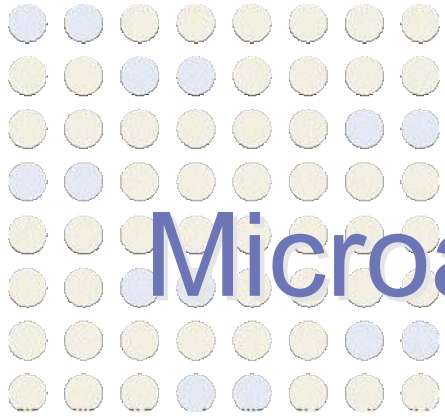
Microarrays: Where do we go from here?

Neil Winegarden

*BioMEMS & Biomedical NANOftech World
2002*

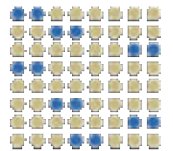


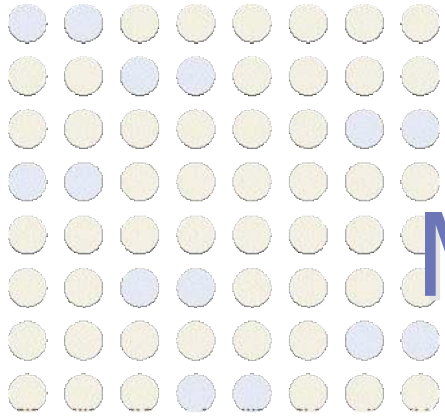
UHN Microarray Centre



Microarrays – A mixed blessing

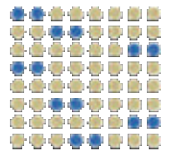
- Microarrays have revolutionized genomics research – *in fact they have made genomics research a reality.*
- With the advent of microarrays, researchers are able to study gene expression, mutation and function faster than ever before
- BUT - microarrays have provided more *headaches* than ever before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

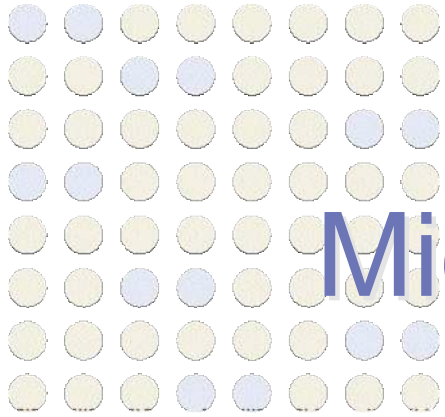




Microarrays of Today

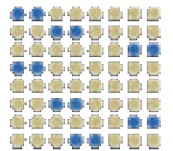
- Currently, spotted microarrays have specifications similar to:
 - 20,000-40,000 spots per slide
 - 50-100 μm diameter features
- And Microarray Labs are able to produce:
 - 100-2000 arrays per week
- And a typical microarray experiment requires
 - 10-200 arrays

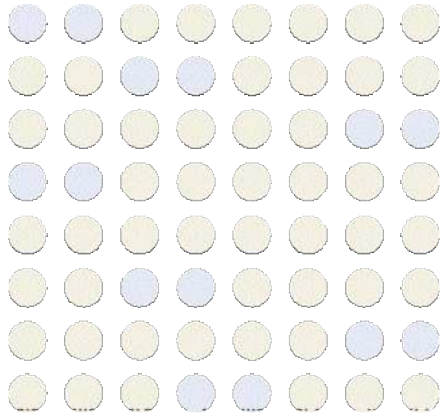




Microarrays of Tomorrow

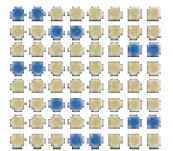
- Future spotted microarrays may require:
 - 100,000+ features per slide *or*
 - 10+ arrays of 30,000 features each per slide
 - 10 – 50 μm diameter features
- And array labs may need to produce:
 - 10,000+ arrays per week
- Because array experiments may require
 - 10,000 – 100,000 arrays

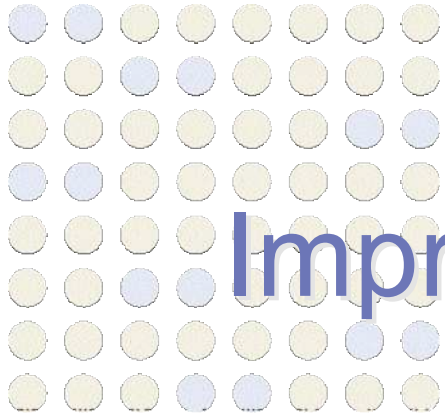




Needs Improvement

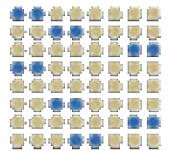
- Array Technology has come a long way, but, there are still areas that need improvement
- Just about each part of the process of the manufacture and use of microarrays can be improved
- Overall, the wish-list includes:
 - Higher Throughput
 - Greater Sensitivity
 - Improved Reproducibility

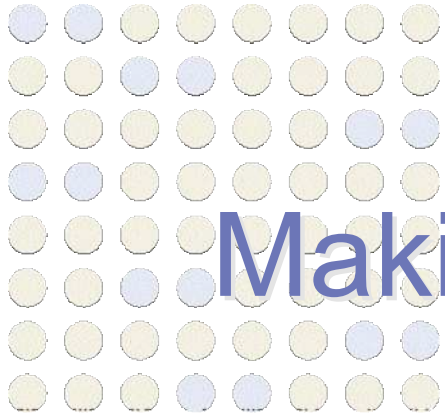




Improved Array Manufacture

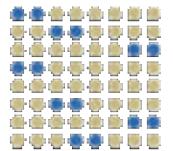
- Current technologies lack the throughput and robustness required for tomorrow's applications
- Advances in printing technologies are still required
- Perhaps a departure from 2D microarrays is required

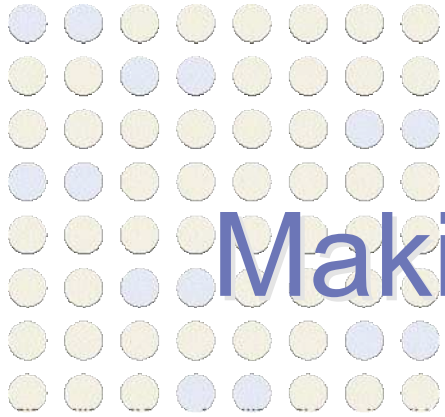




Making a Better Microarrayer

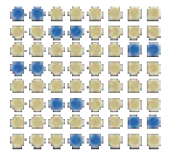
- Pin based arrayers are mostly limited to 48 applicators
 - 48 spots per slide, 100-150 slides per arrayer, 3 minutes per print cycle
 - Approximately 30 hours for 150 slides with 30,000 features = 12 minutes per slide (singlet printing).
 - Time is consumed by the need for physical deposition of the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

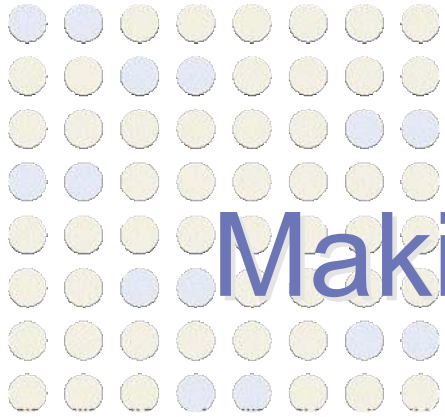




Making a Better Microarrayer

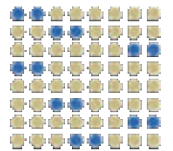
- Inkjet arrayers can provide similar throughput with only 8-16 applicators
 - Due to larger spot sizes, there are limitations to overall density.
 - “On the fly” printing allows for equivalent throughput with smaller number of applicators
 - Time is still consumed cleaning the applicators
 - Number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

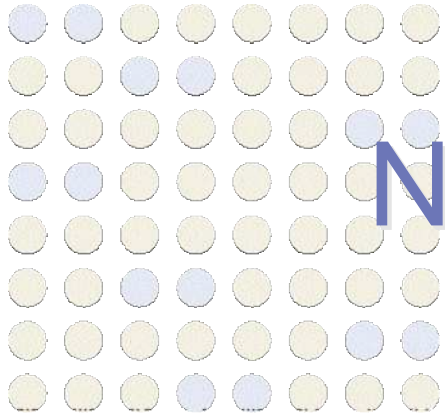




Making a Better Microarrayer

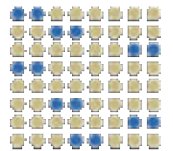
- Higher throughput could be obtained by:
 - Finer pins, allowing 192 applicators at a time (1536 well plates - 4X higher throughput)
 - Many challenges – and very costly
 - Further miniaturization of inkjet devices to allow higher number of applicators
 - 48 inkjet nozzles leads to 3X+ increase in throughput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





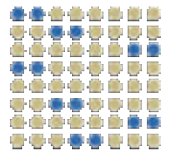
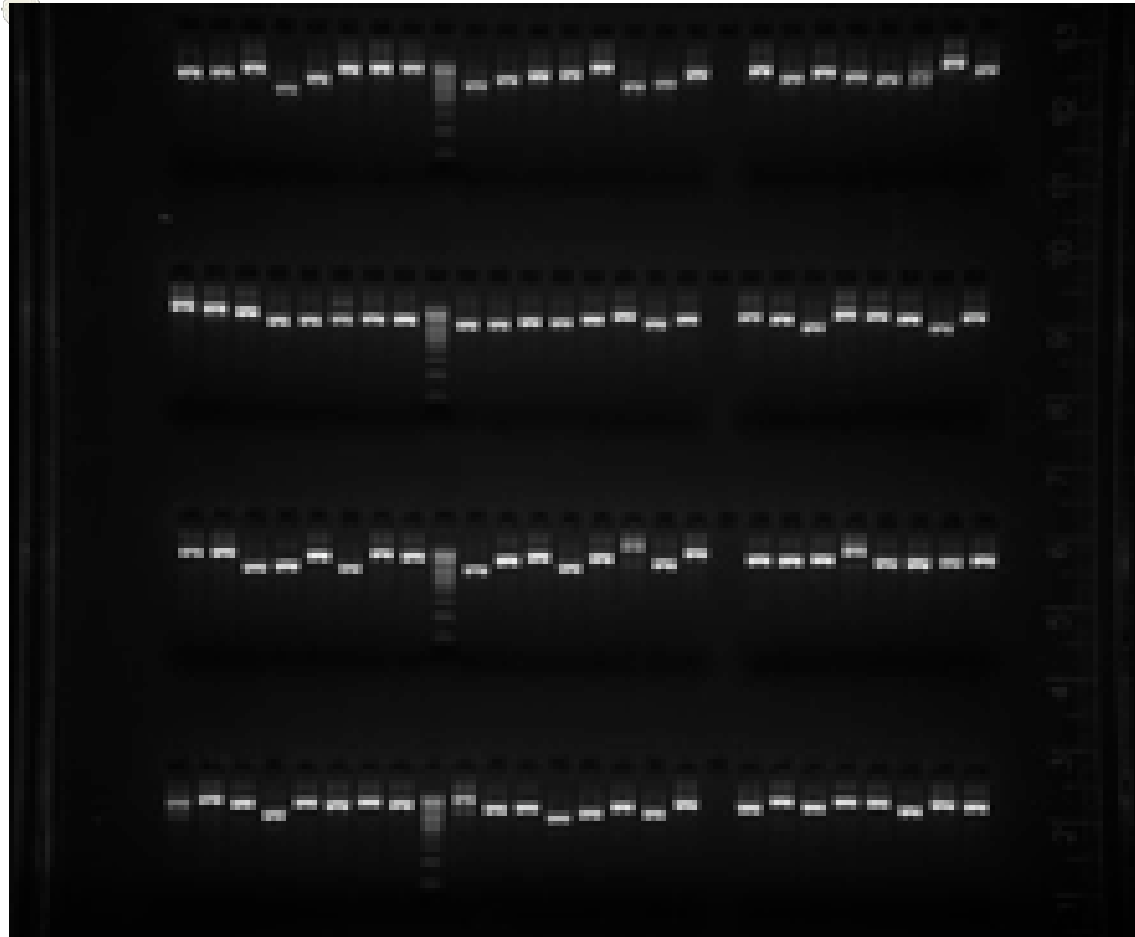
Not Just More Arrays – More Good Arrays

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



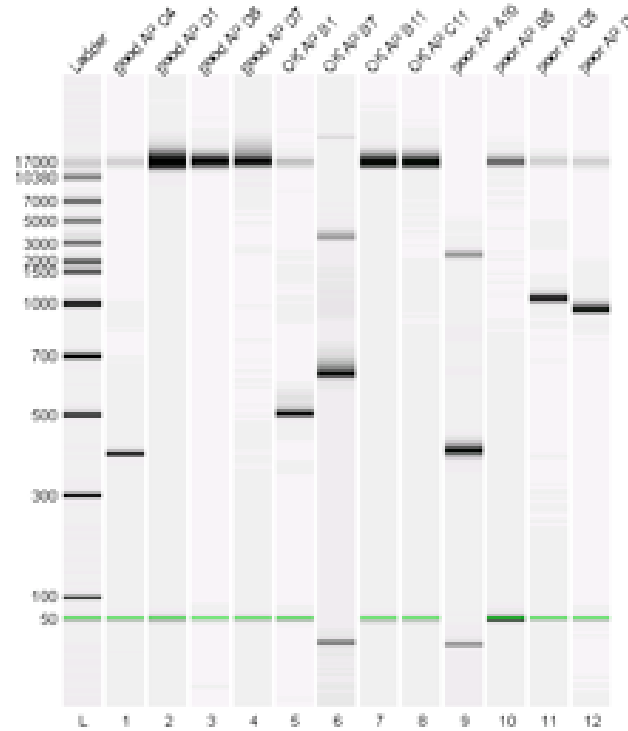
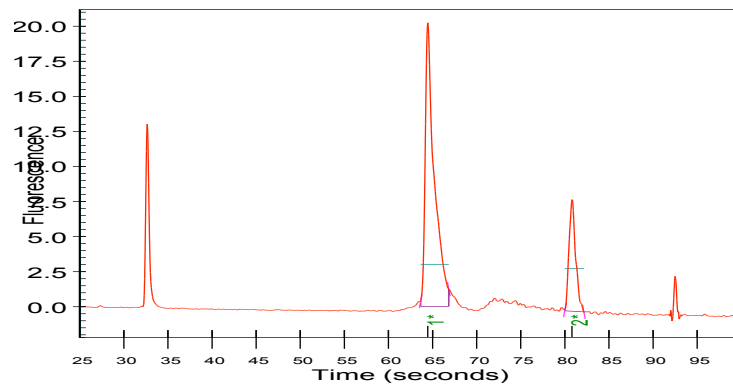
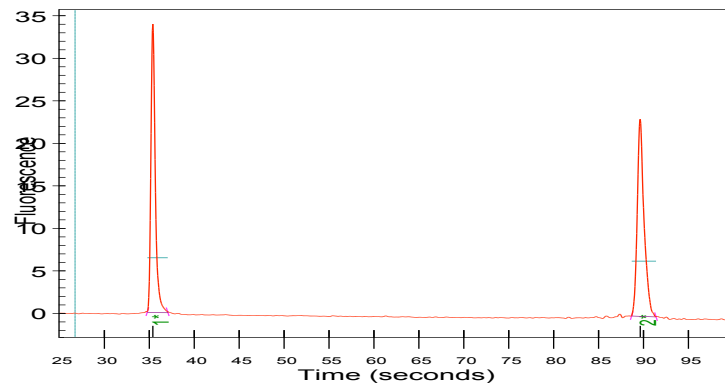
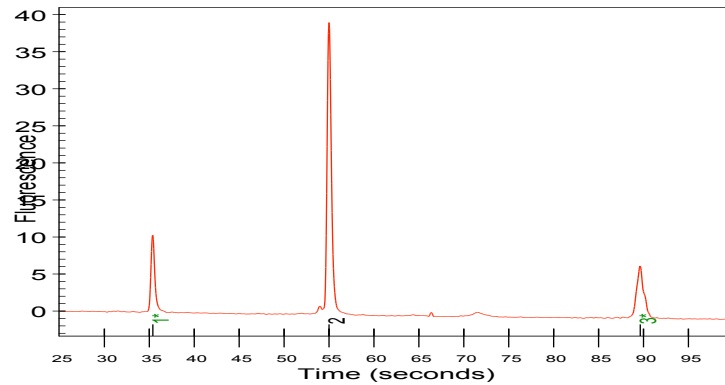


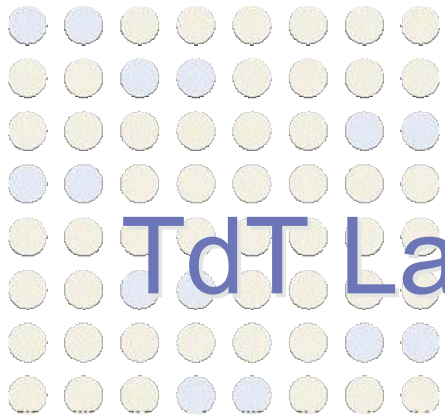
Agarose Gels for QC of Clone Production



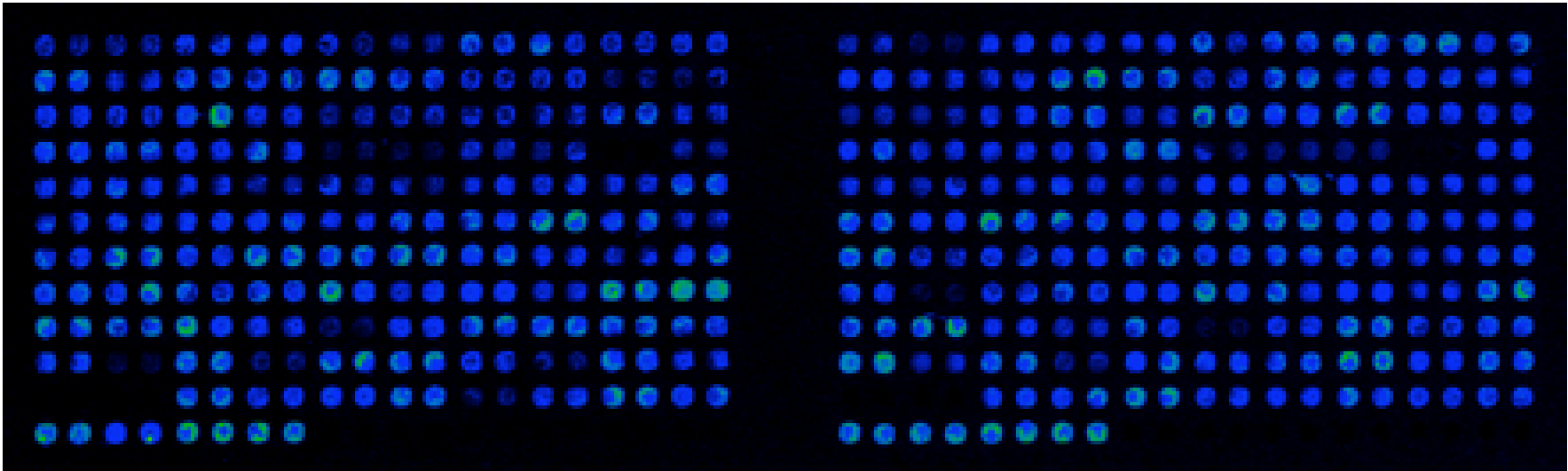
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CE for QC of Clone Production

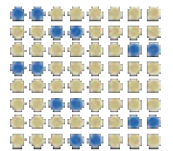


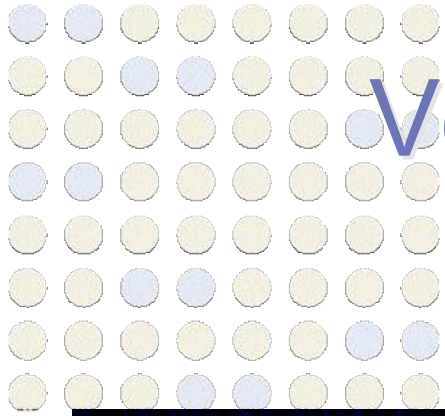


TdT Labelling to QC Array Printing

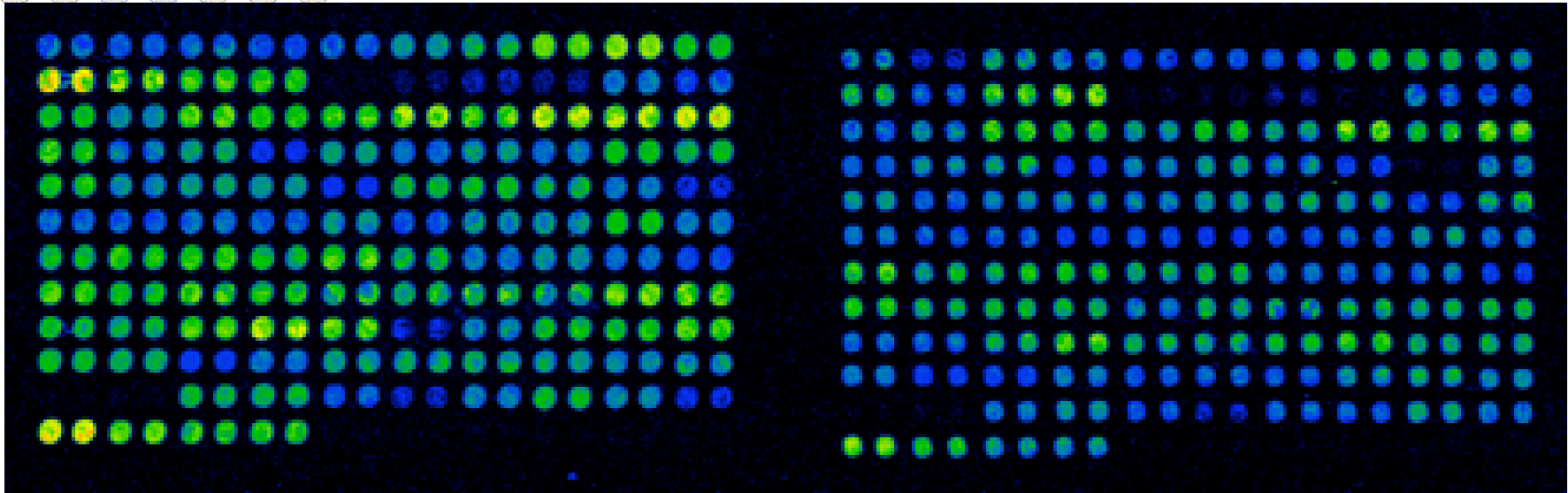


- Terminal Deoxynucleotidyl Transferase can add Cyanine 3 or Cyanine 5 labelled dCTP to the 3' ends of spotted cDNAs *or* oligos.

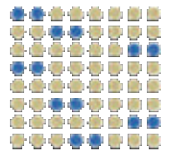




Vector Probe Labelling to QC Array Printing



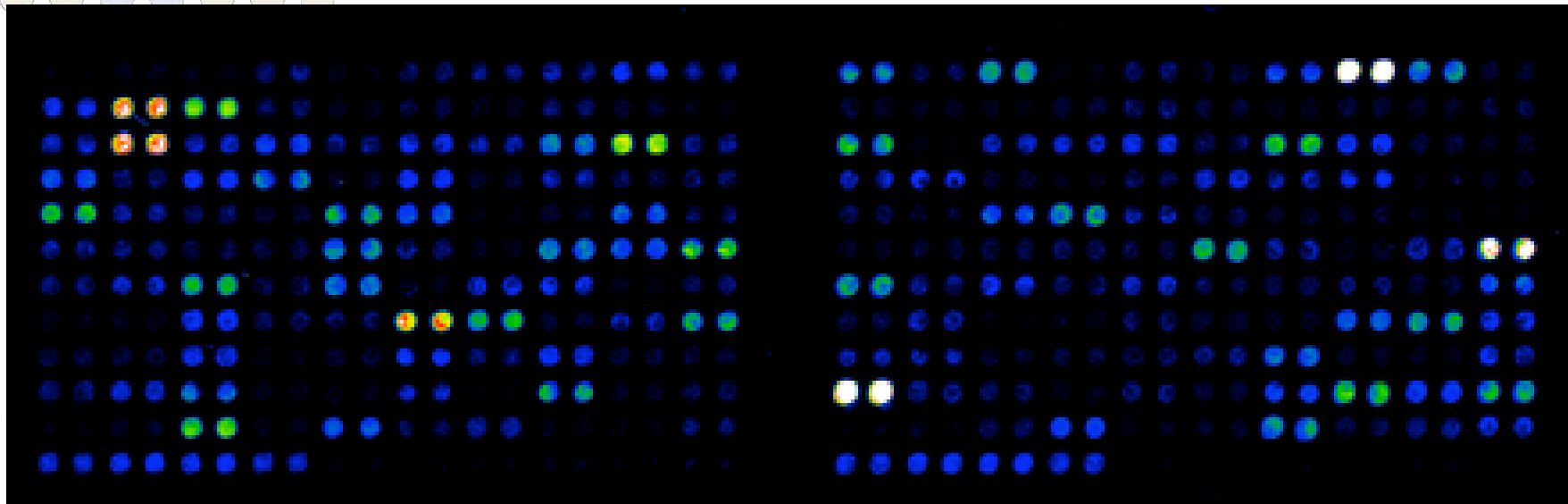
- By labelling an oligo complimentary to the universal primer, you can light up all the spots printed on the array.



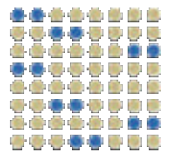
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Hybridization to QC Array Processing

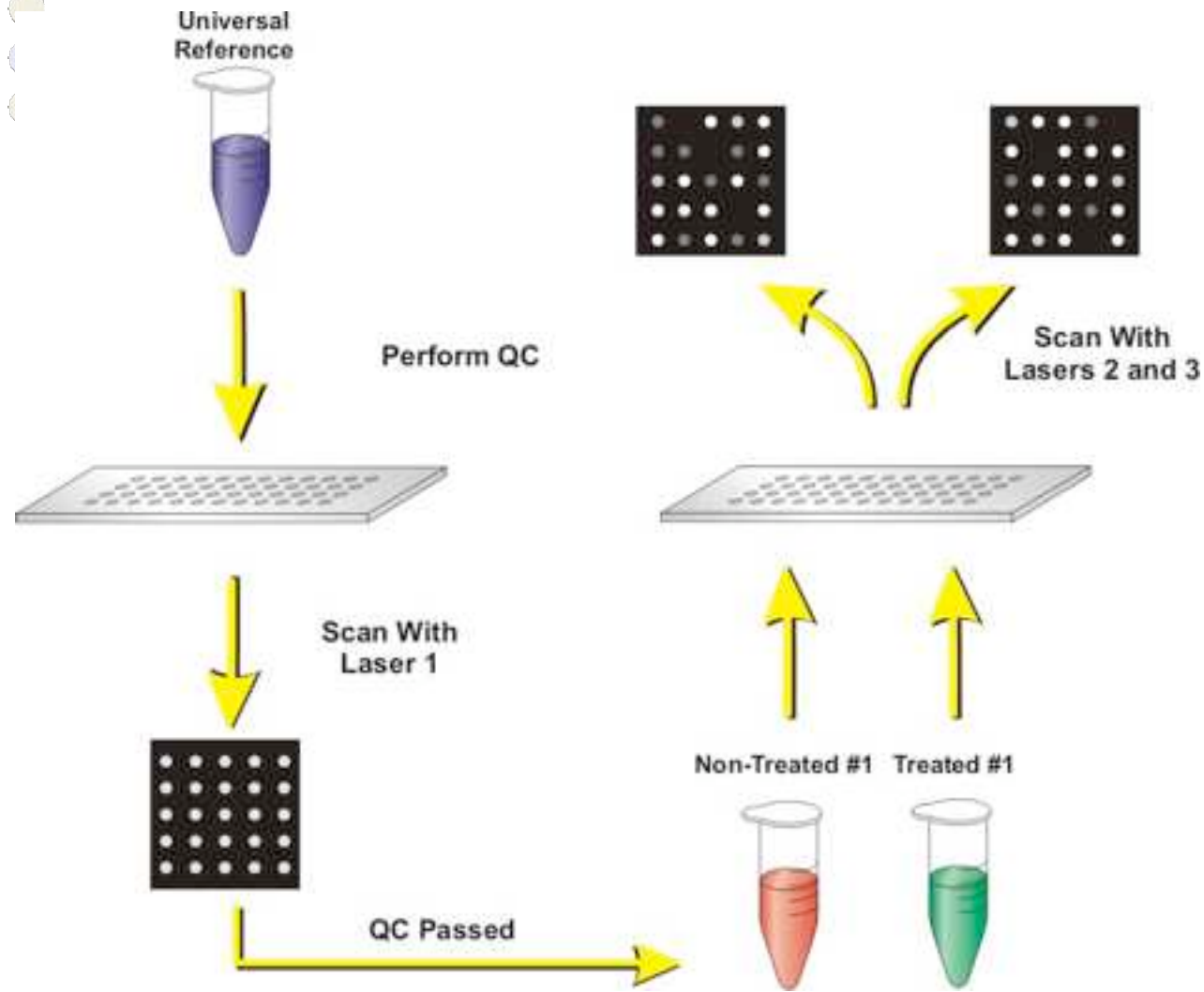


- Hybridization with a known and validated RNA, such as Stratagene® Universal Reference RNA (shown above) helps to assess the quality of the array post processing.



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Third Colour As A QC Channel

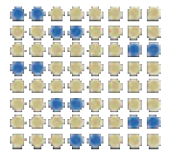


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Now you have the arrays – how are you going to use them?

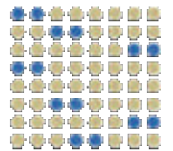
- There are five main steps in the use of a microarray
 - Labelling of sample
 - Hybridization
 - Washing
 - Scanning
 - Data Analysis
- A good technician can handle around 24-36 arrays per week using standard technologies
- Obviously to process 10,000+ arrays will require increases in throughput

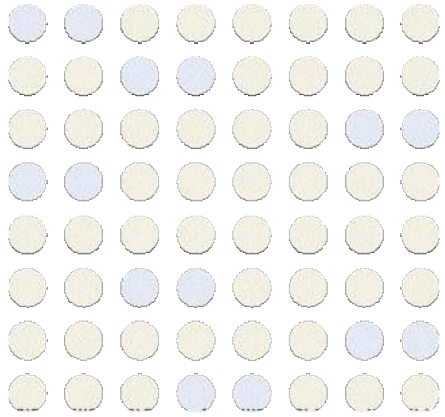




Increasing the throughput – using microarrays efficiently

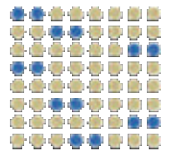
- Labelling
 - Throughput can be increased via automation – RNA extraction and labelling can be done with robotics (theoretically)
- Hybridization and Washing
 - Currently the majority of labs use the “Tupperware” solution for hybridizations
 - Automated hyb stations are available, but do not increase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

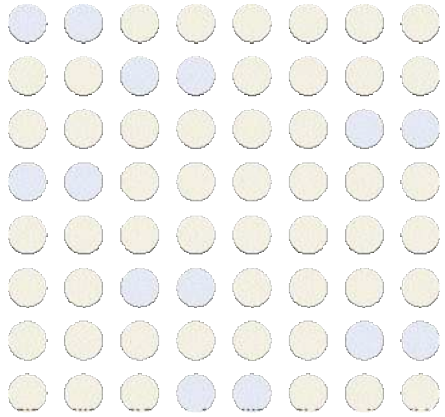




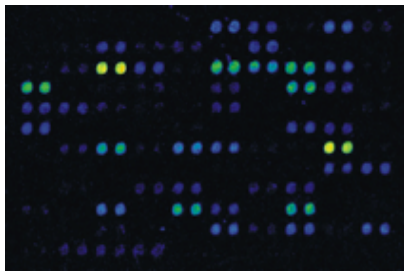
More Hybridizations

- 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

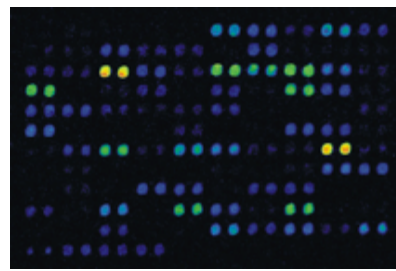




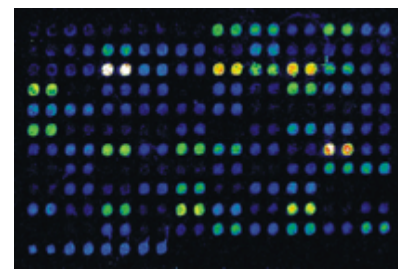
It's all about time



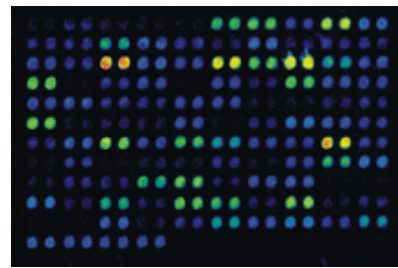
1 hour



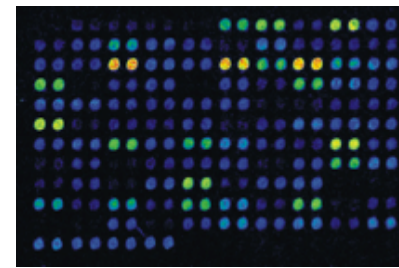
2 hours



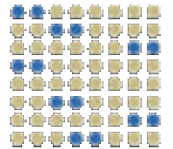
4 hours

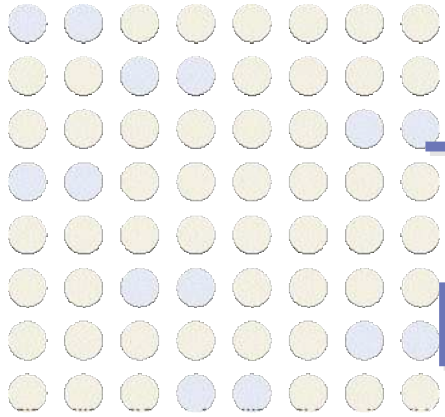


6.5 hours



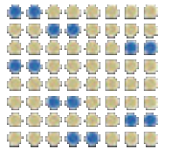
Overnight





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

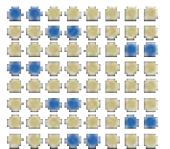


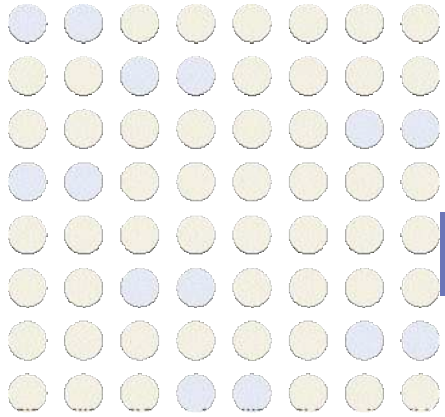
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Increasing the throughput – using microarrays efficiently

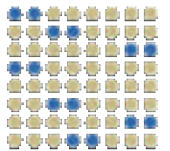
- Scanning
 - Automatic slide loading is a start
 - Some scanners have 10-48 slide capacity
 - Additional slides will be required
 - 100+ slides
 - Faster scanning
 - < 1 minute per scan
 - Requires brighter signals
 - Larger CCDs
 - Alternative Detection Schemes
 - Conductance
 - Non-Fluorescent labels

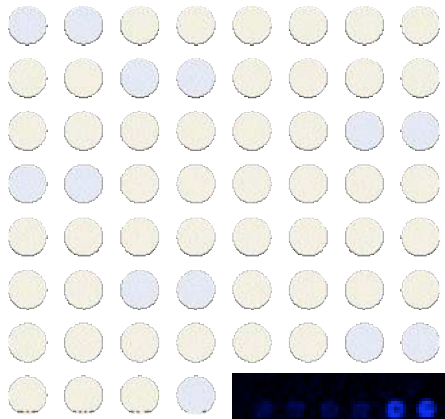




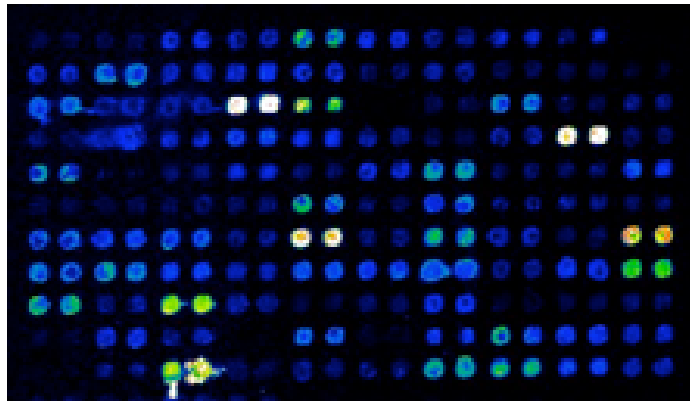
Improving Sensitivity

- Better fluors
- Non-fluorescent alternatives
- Amplification

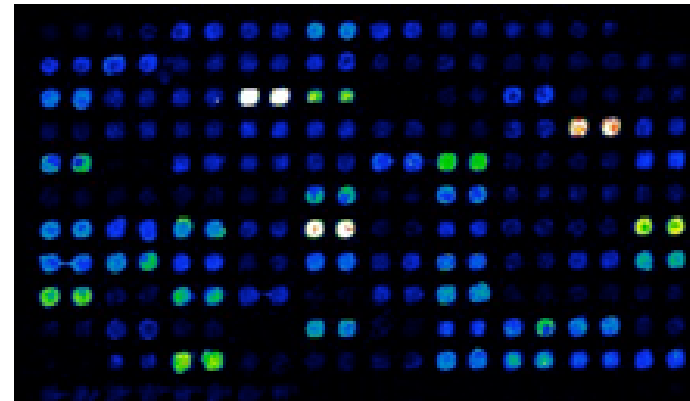




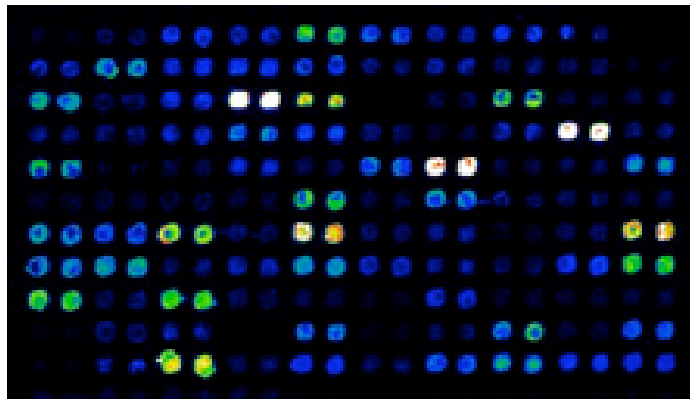
Amplification



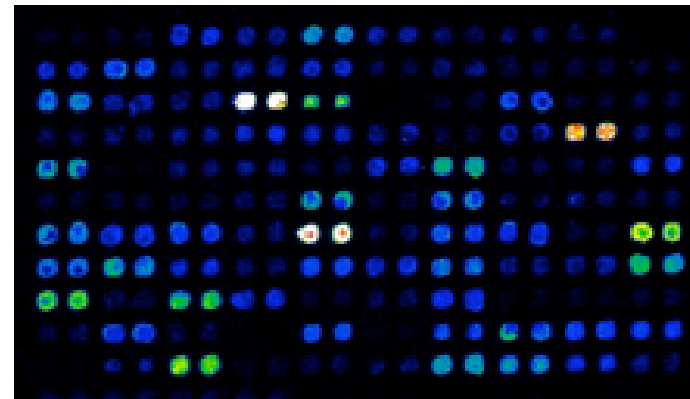
10 ng



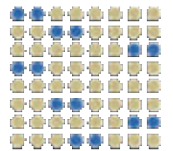
1 ng

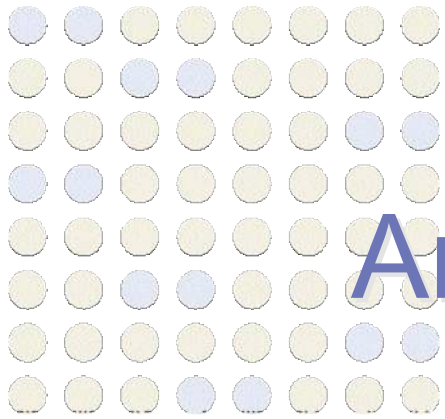


100 pg

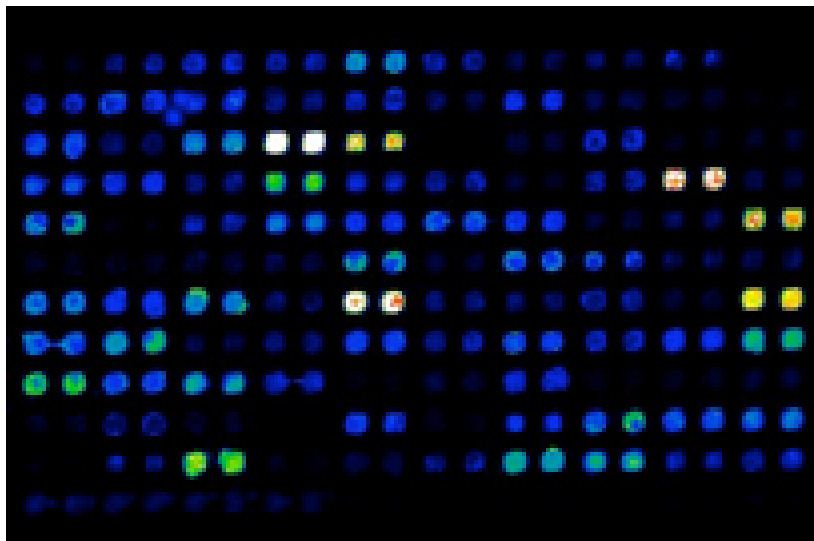


10 pg

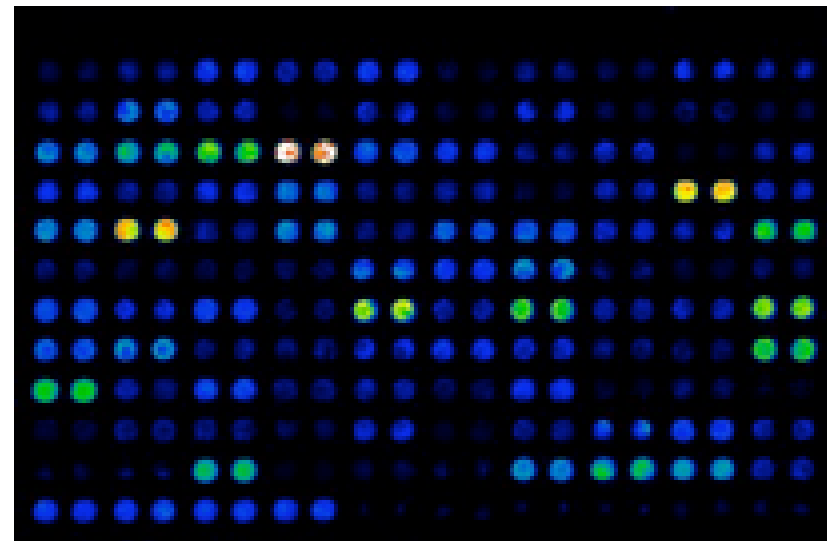




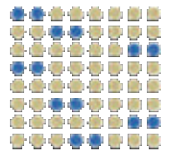
Amplification Continued

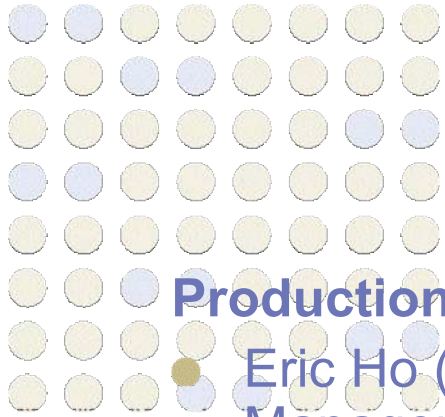


Amplified, 1 ng



Direct, 10 μ g





Acknowledgements

Production

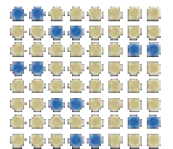
- Eric Ho (Clone Production Manager)
- Patrick Yau (Array Production Manager)
- Quyen Tran
- Tuyet Diep
- Robert Kardish
- Shani Mintzberg
- Elizabeth Sakac
- Gurbaksh Basi (Tech Support)

Engineering

- Sasan Raghbizadeh (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



Thank You

Please visit us at: www.microarrays.ca



The screenshot shows the homepage of the Microarray Centre. At the top, there is a navigation menu with links for 'about us', 'products & services', 'support', 'commercial', 'resources', and 'contact'. The main header features the Microarray Centre logo and the text 'University Health Network'. Below this is a large banner image of a microarray chip with the text 'A LEADER IN MICROARRAY TECHNOLOGY'. The page is divided into three columns: 'NEWS', 'MICROARRAY CENTRE', and 'NEWSLETTER'. The 'NEWS' column contains two articles: 'Human 1.7M arrays released' and 'Microarray Centre's new website is up!'. The 'MICROARRAY CENTRE' column features a photograph of a microarray chip and text describing the centre's role as a leader in Canadian microarray technology. The 'NEWSLETTER' column contains a message about being under construction and a 'SUBSCRIBE TO OUR NEWSLETTER' button. A decorative grid of colored dots is visible in the top left and bottom right corners of the page.

NEWS

3/5/02
Human 1.7M arrays released.
We are now sending out H1.7M arrays. GeneLists and sequence can be downloaded from our "support" section.

26/4/02
Microarray Centre's new website is up!
Our newly designed user friendly site is now up. Please look around and see what we have to offer. If you are currently a user of our microarrays, please sign up to our news letter so we can provide relevant information to you efficiently.

MICROARRAY CENTRE

 The Microarray Centre at The Ontario Cancer Institute, University Health Network is a leader in Canadian microarray technology. We are dedicated to providing high quality microarrays, technical support and service to Canadian researchers. At least 10 high quality microarrays will allow our Canadian researchers to be on the cutting edge of genetic research.

[Read more ▶](#)

NEWSLETTER

Under construction and testing. Do not use yet!
On occasions we may need to distribute new and important information to our users. If you use our microarrays, please sign up to our newsletter, it is the most effective and efficient way to receive information from us.

[SUBSCRIBE TO OUR NEWSLETTER](#)

UHN Microarray Centre