ToF-SIMS Chemical State Imaging to Characterize DNA Microarray Surfaces

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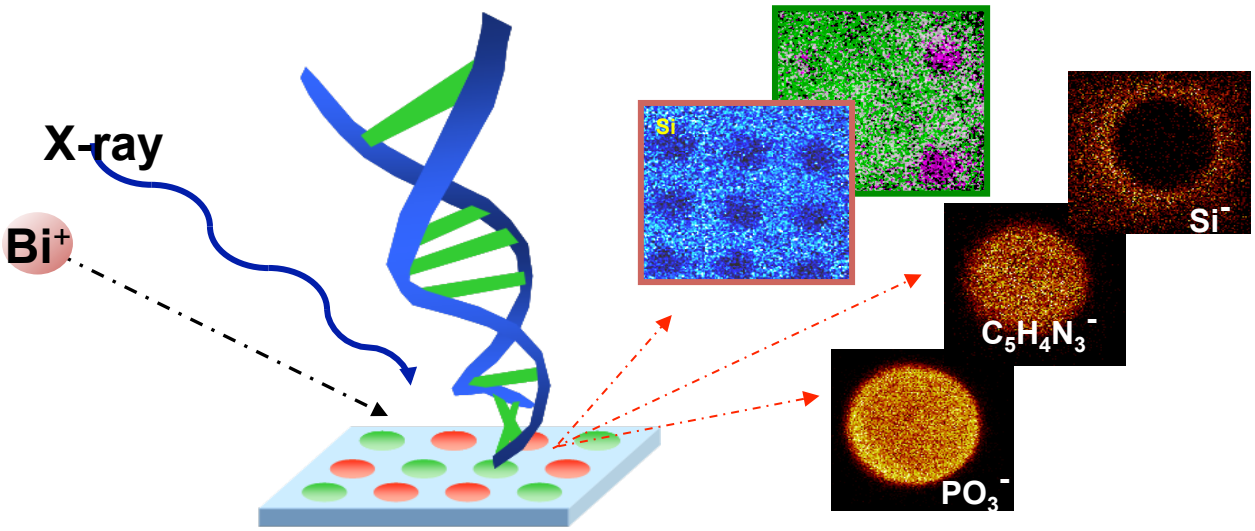
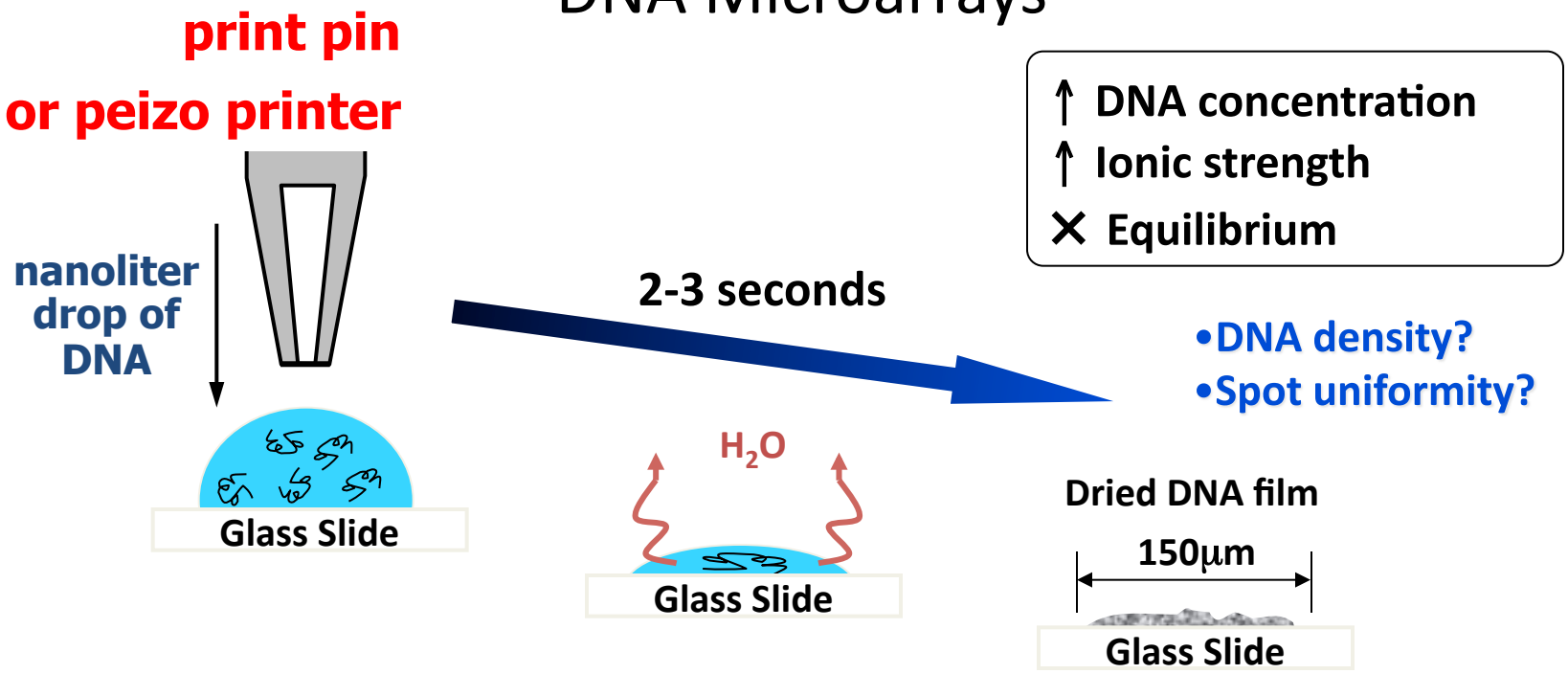


Surface Chemical State Imaging Analysis of DNA Microarrays



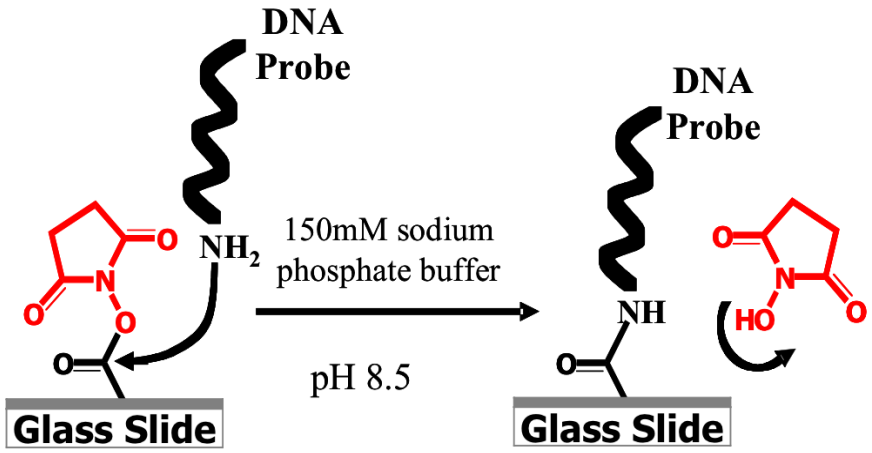
Objectives:

- Fluorescence imaging to determine DNA spot (Cy3) uniformity
- Imaging ToF-SIMS to determine surface distribution of different chemical species

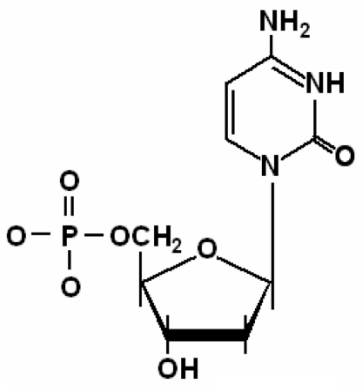
DNA Immobilization Chemistry on Commercial Polymer Slides

Commercial CodeLink™ microarray slides (Amersham), and Slide H Schott Nexterion

N-hydroxyl succinimide (NHS): active ester, amine-reactive

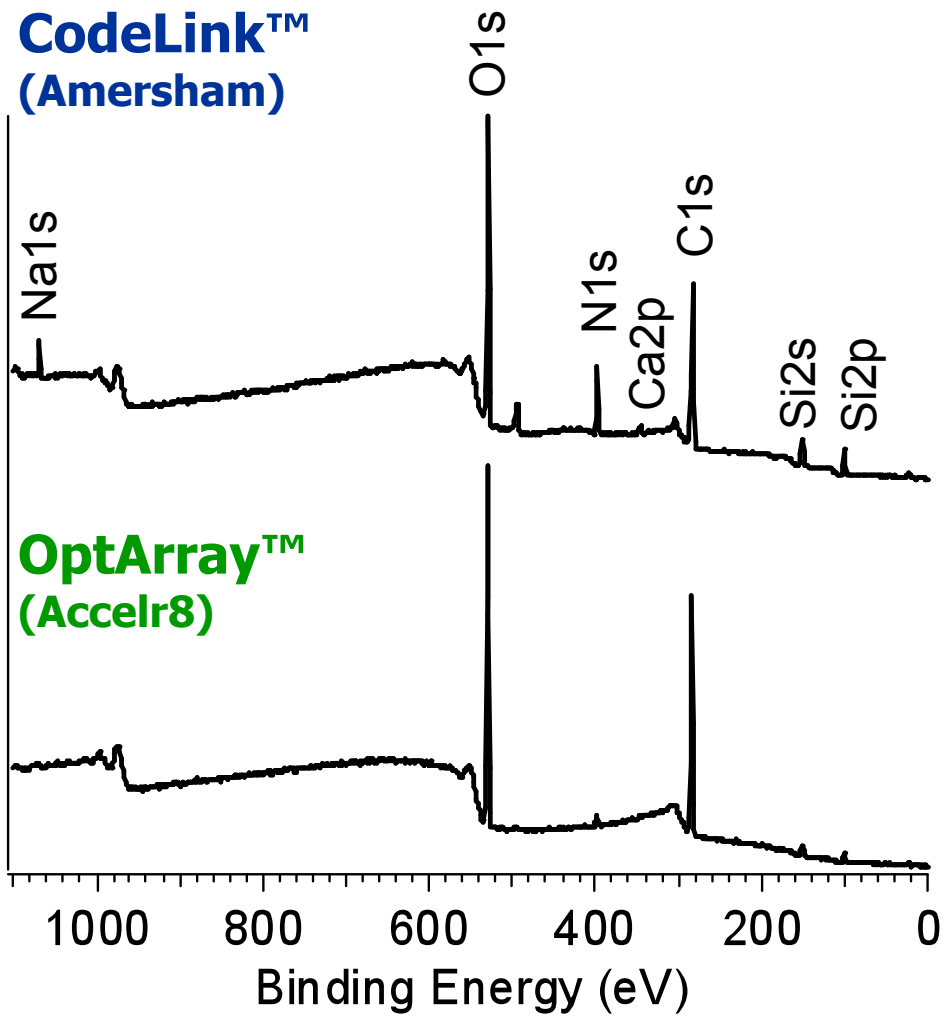


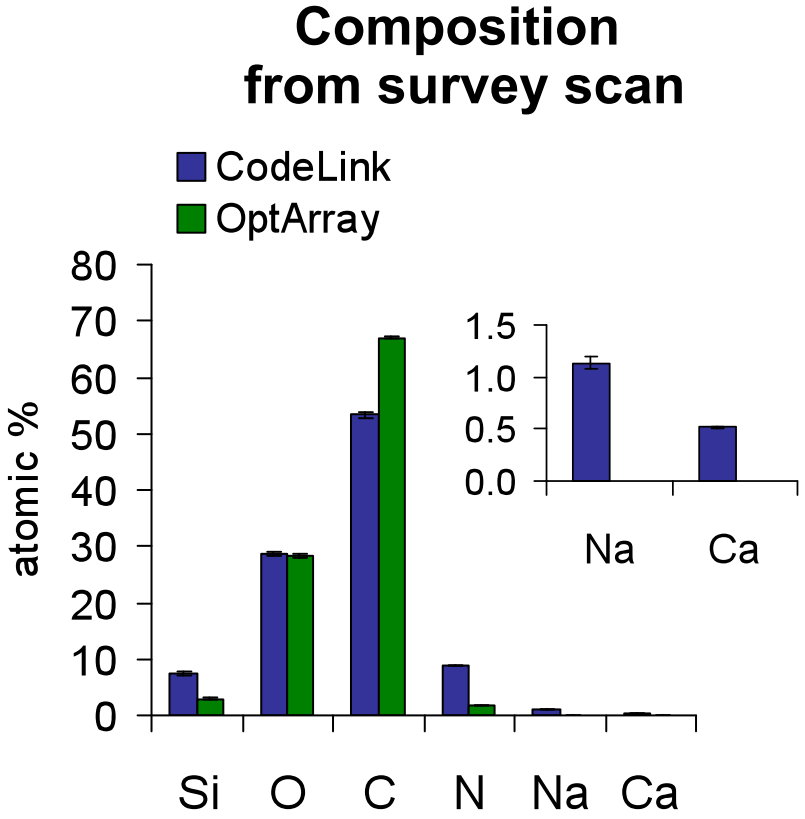
Covalent immobilization of DNA probe



5'Cy3-C_6-CTGAACGGTAGCATCTTGAC-C_6-NH_2

Surface Chemistry of Commercial Slides as Determined by X-ray Photelectron Spectroscopy (XPS)





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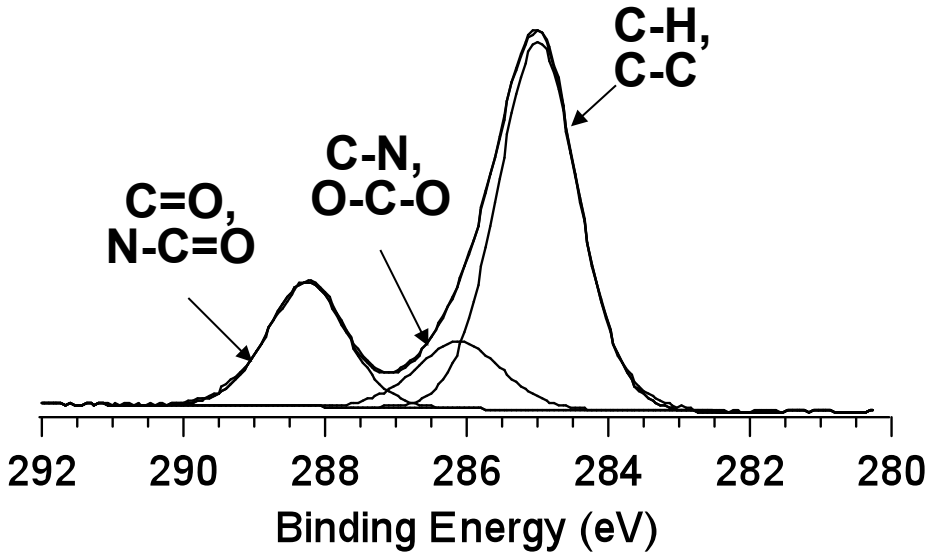
Surface Chemistry of Commercial Slides as Determined by XPS

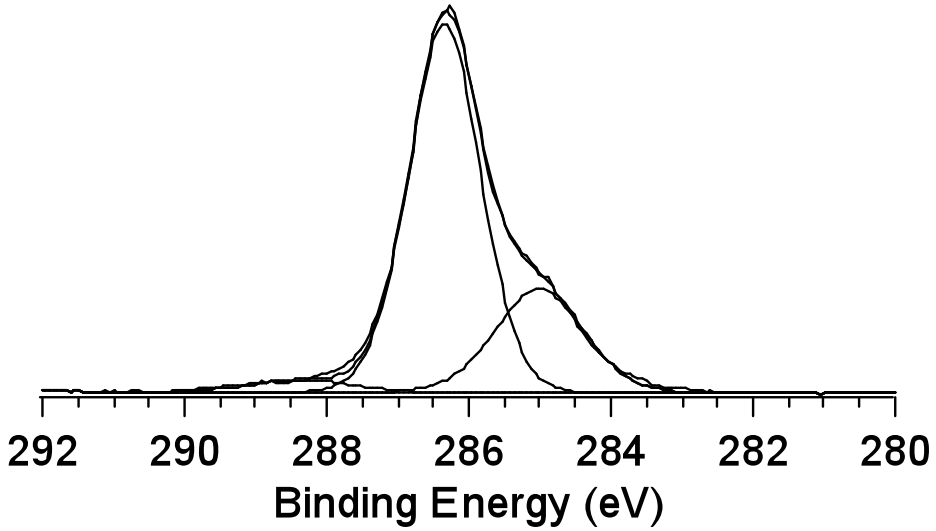
CodeLink™
(Amersham)

Acrylamide based



OptArray™
(Accelr8)

Poly ethyleneglycol (PEG) based



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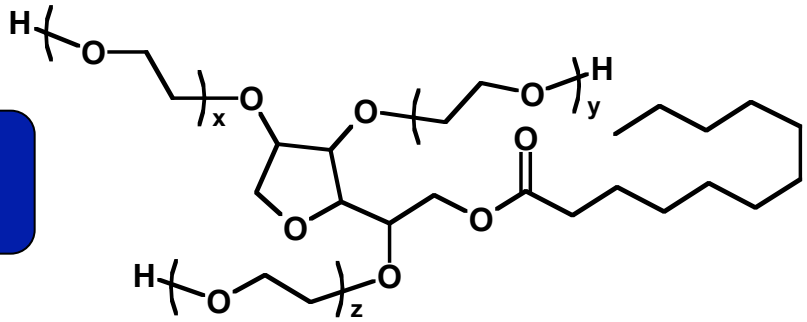
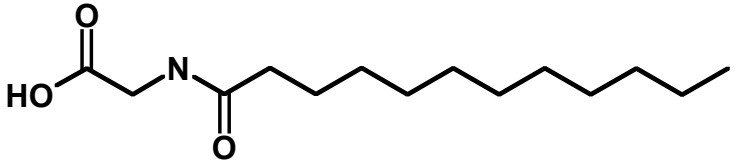
DNA Immobilization Chemistry on Commercial Polymer Slides

Printing

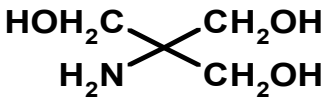


Tween20™



N-Lauroyl Sarcosine (Sarcosyl)

Blocking

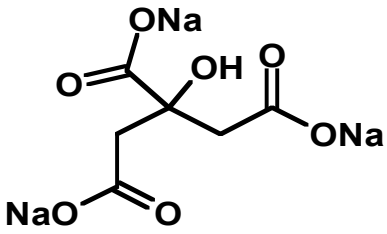


Tris(hydroxymethyl)aminomethane

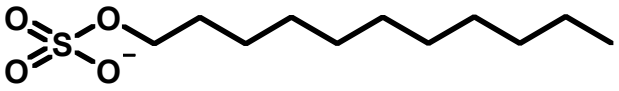
Washing

NaCl

Sodium Chloride



Sodium Citrate

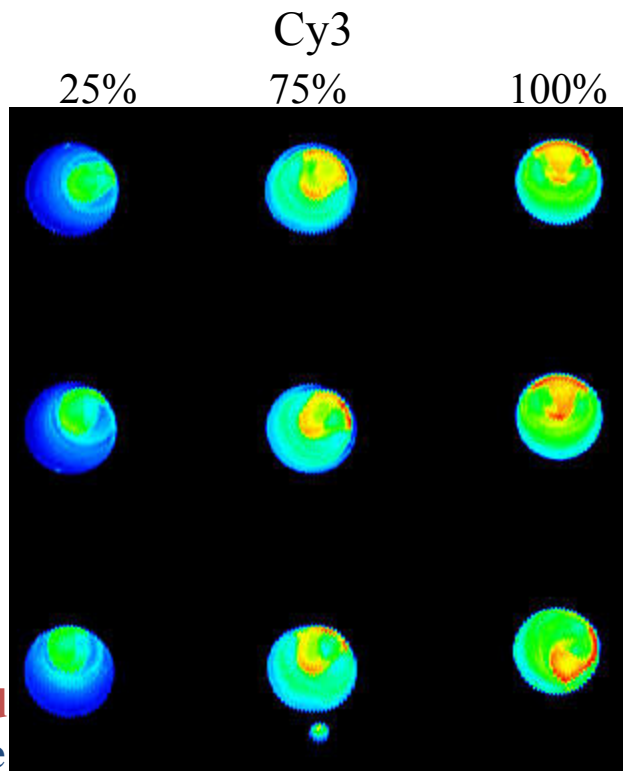


Sodium Dodecyl Sulfate (SDS)

*NESAC/BIO, University of Washington Seattle, WA. U.S.A.

DNA microarray fluorescence

- ✚ Fast and widely available
- ✚ High resolution (5μm)
- ➖ Non-quantitative
- ➖ Spot uniformity, reliability
- ➖ Spot bleeding into matrix



highest intensity -> **Red**
lowest intensity -> **Blue**

40 μM DNA probe conc.

Array fabrication

- Ink jet, contact pin
- Feature size ~100 microns diameter

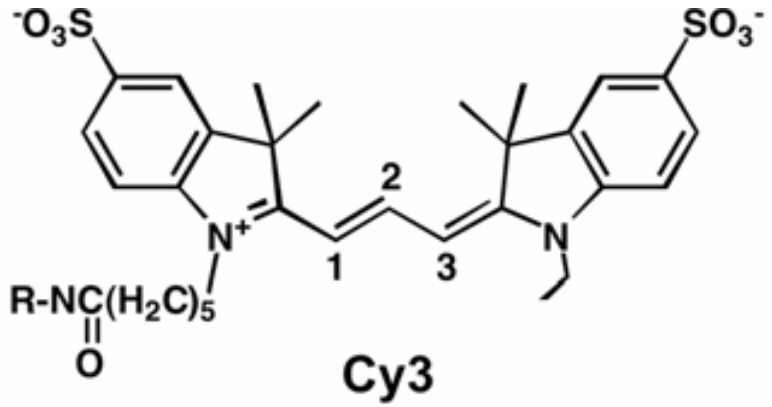
Objectives:

- Fluorescence imaging to determine DNA spot (Cy3) uniformity
- Imaging ToF-SIMS and statistical analysis to determine surface distribution of different chemical species corresponding to the fluorescence non-uniformity

Slide printing

Three different DNA concentrations: 10µm, 20µm, 40µm
Four different Cy3 labeled % for each concentrations: 0%, 25%, 75%, 100%
Ten replications of each spot.

Volume size, 333 pL and spot size ~315 µm



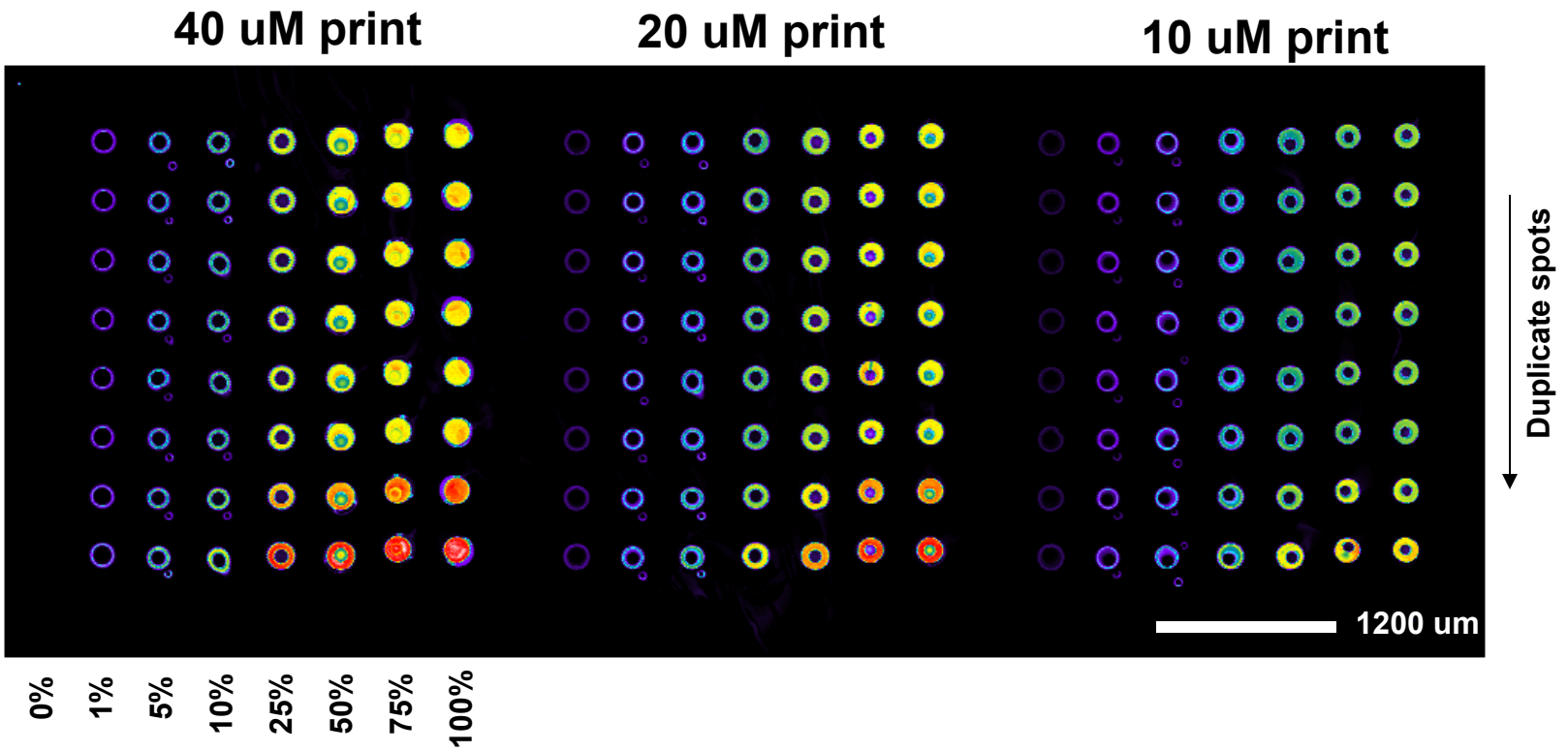
C_6-oligo-3' C_6-NH2

Examine distribution of chemical species within DNA microarray spots

Scanner Image showing 1 array

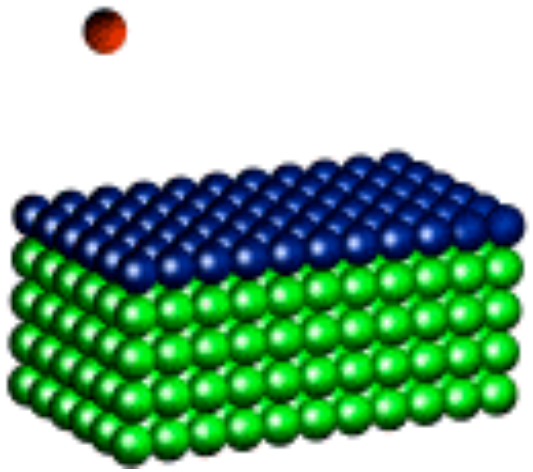
Effects of immobilizing Probe mixtures containing different amounts of Cy3-labeled probe

Scanned microarray image (ScanArray Express, Perkin Elmer)
Spots printed at 400 um center to center



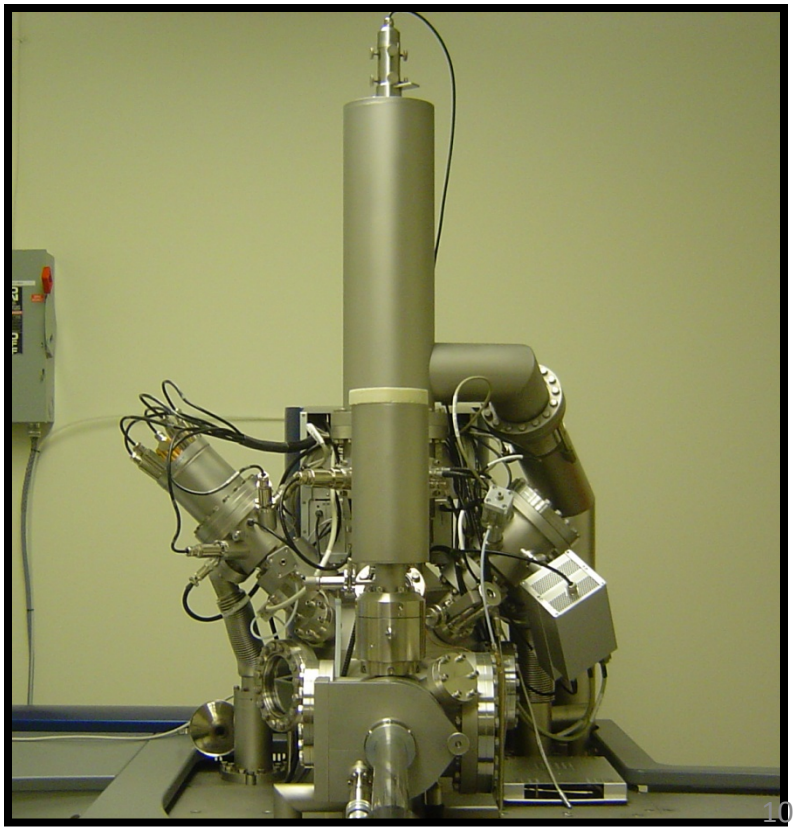
Hybridized with 1 uM Target (99:1 unlabeled:Cy5 labeled)

Time of Flight Secondary Ion Mass Spectrometry (ToF)- SIMS



http://www.iontof.com/technique-sims-IONTOF-TOF-SIMS-TIME-OF-FLIGHT-SURFACE-ANALYSIS.htm

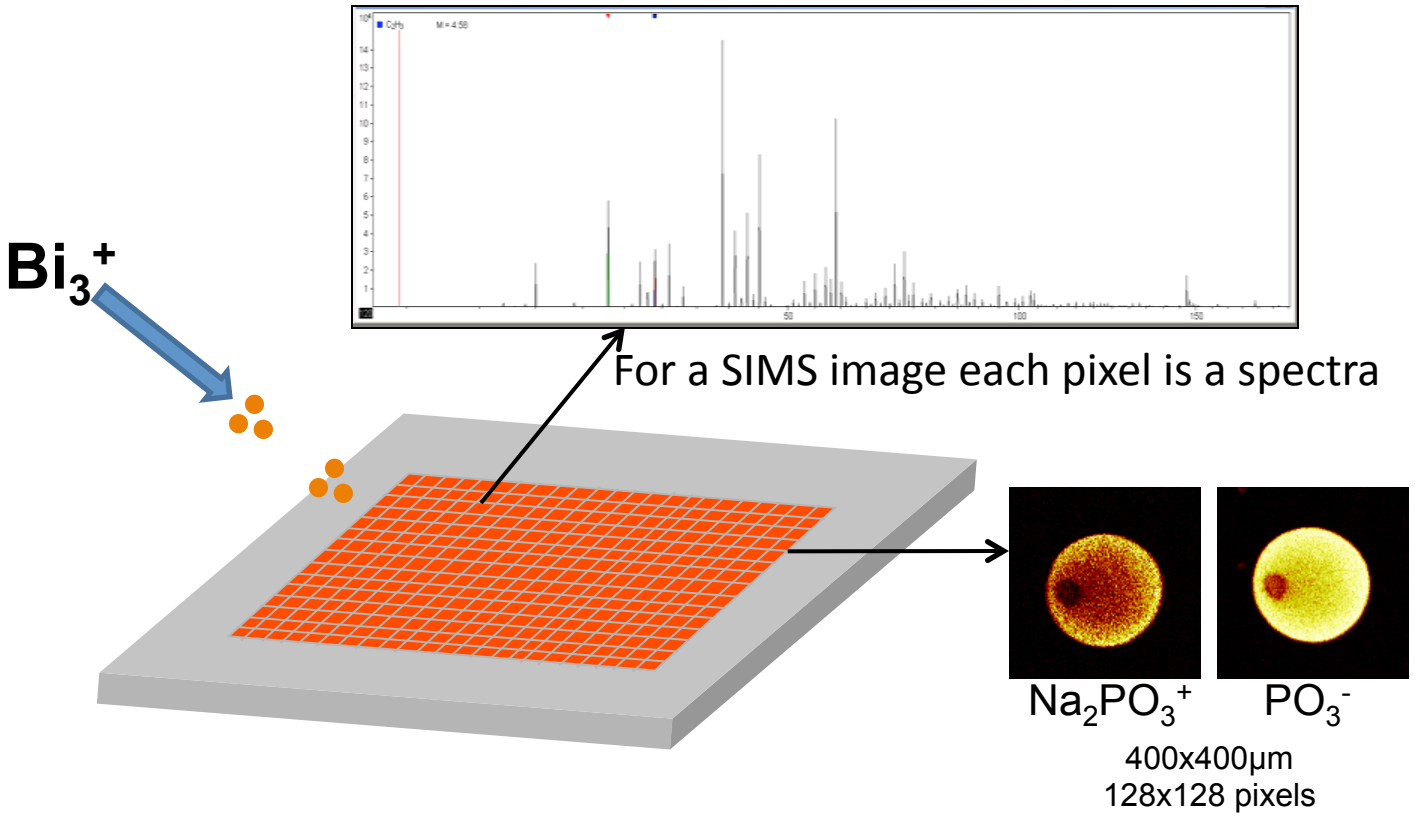
- Ultra High Vacuum (UHV)
- Primary ion impact causes collision cascade
- >99% ejected molecules are neutral, <1% are ions



ION-TOF ToF-SIMS 5-100:

- 25 kV Bi at 45° (Bi_3^+)
  - Analysis (Imaging) beam
  - High spatial resolution (< 1µm)
- 10 kV C_{60} at 45° (C_{60}^+ and C_{60}^{++})
  - High sputter yield
  - Sample etching

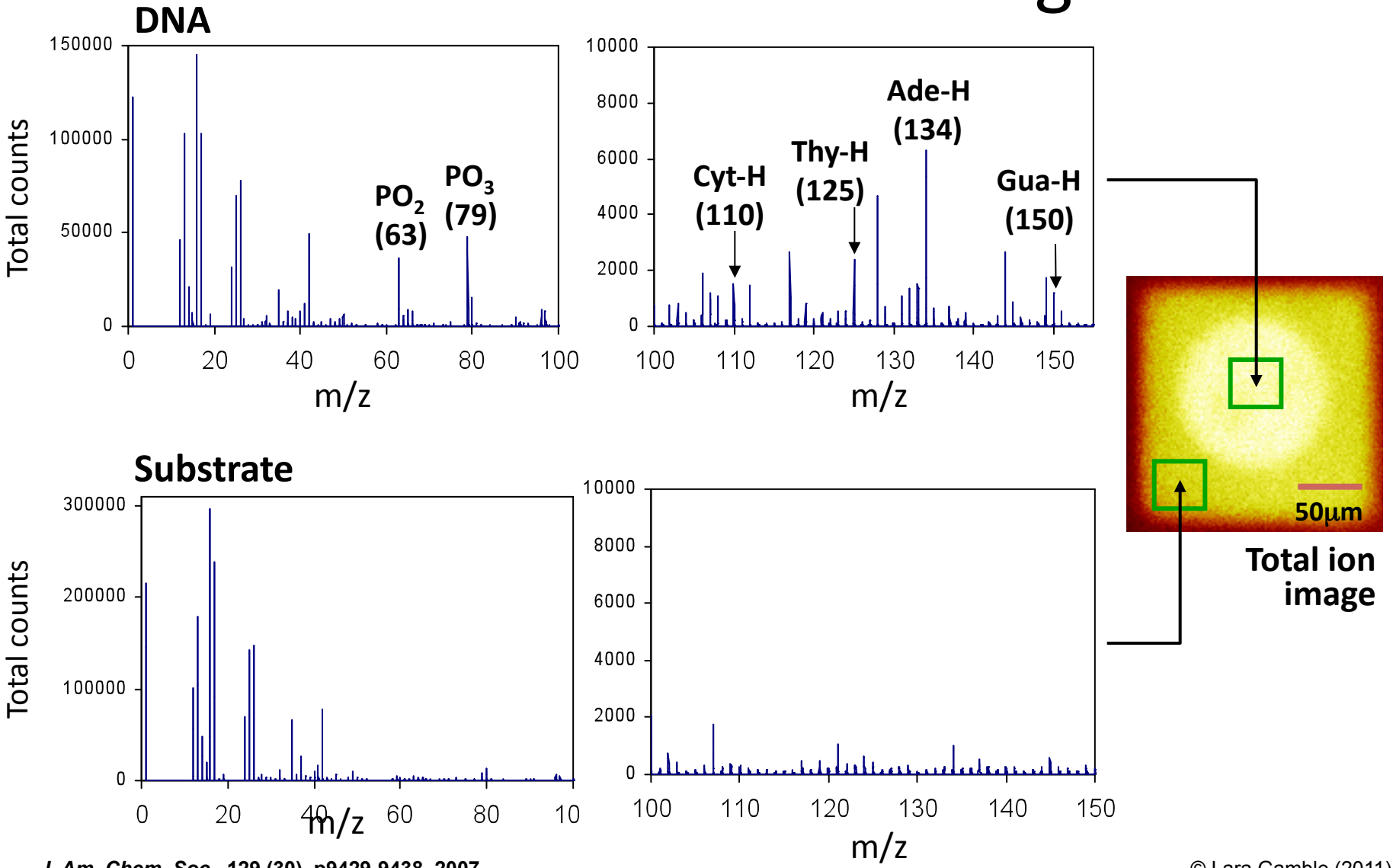
SIMS imaging



- Image resolution 128x128 pixel -> 16384 spectra

- Use Maximum Autocorrelation Factor (MAF) analysis to find the peaks corresponding to the largest variance between the different spectra.

Negative Ion ToF-SIMS ROI Spectra from DNA and Substrate Regions



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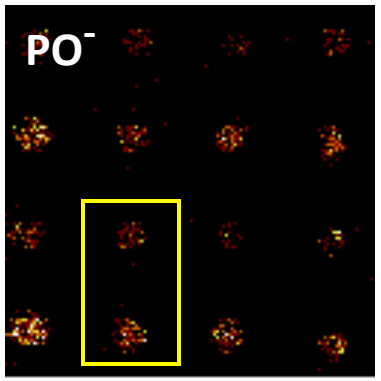
ToF-SIMS Analysis of Microarray Spot Uniformity

Field of view: 1.5 mm x 1.5 mm



Non-comp. probe

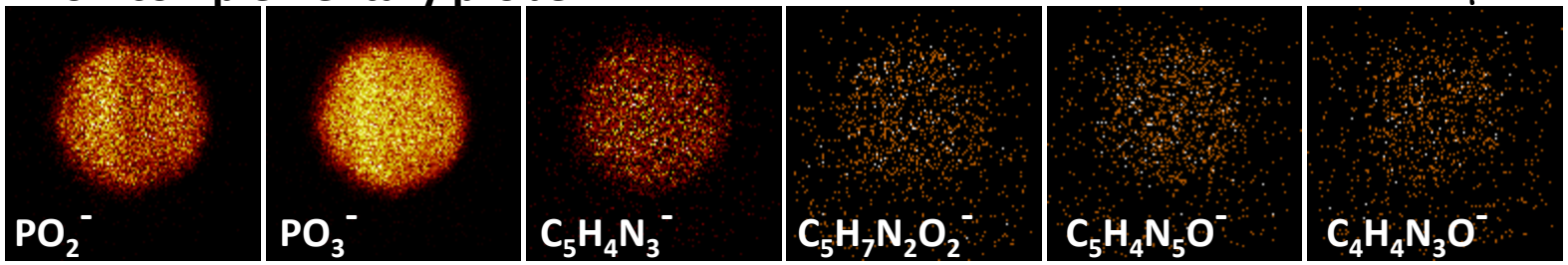
Comp. probe (hybridized)

Imaging parameters:

- IONTOF IV
- Bi^+ primary ion source
- Pulsed 25 keV primary ion beam at 1.3 pA

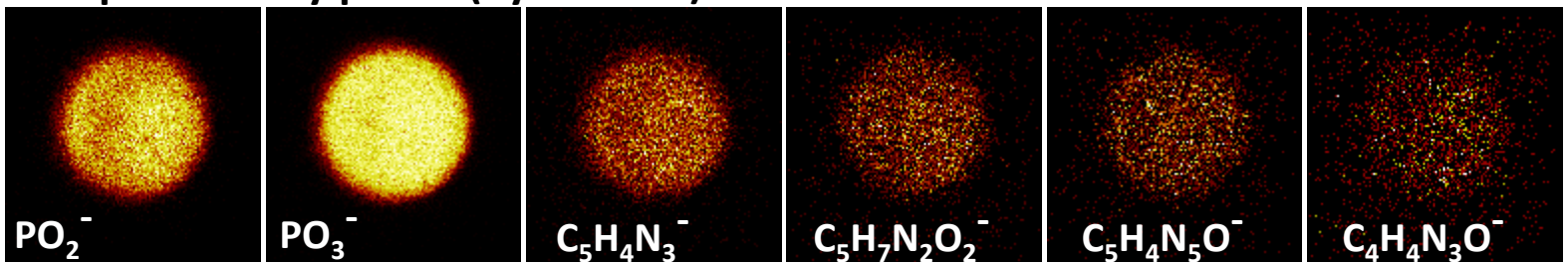
Non-complementary probe

Field of view: 200 μm x 200 μm



(adenine-H) (thymine-H) (guanine-H) (cytosine-H)

Complementary probe (hybridized)



(adenine-H) (thymine-H) (guanine-H) (cytosine-H)

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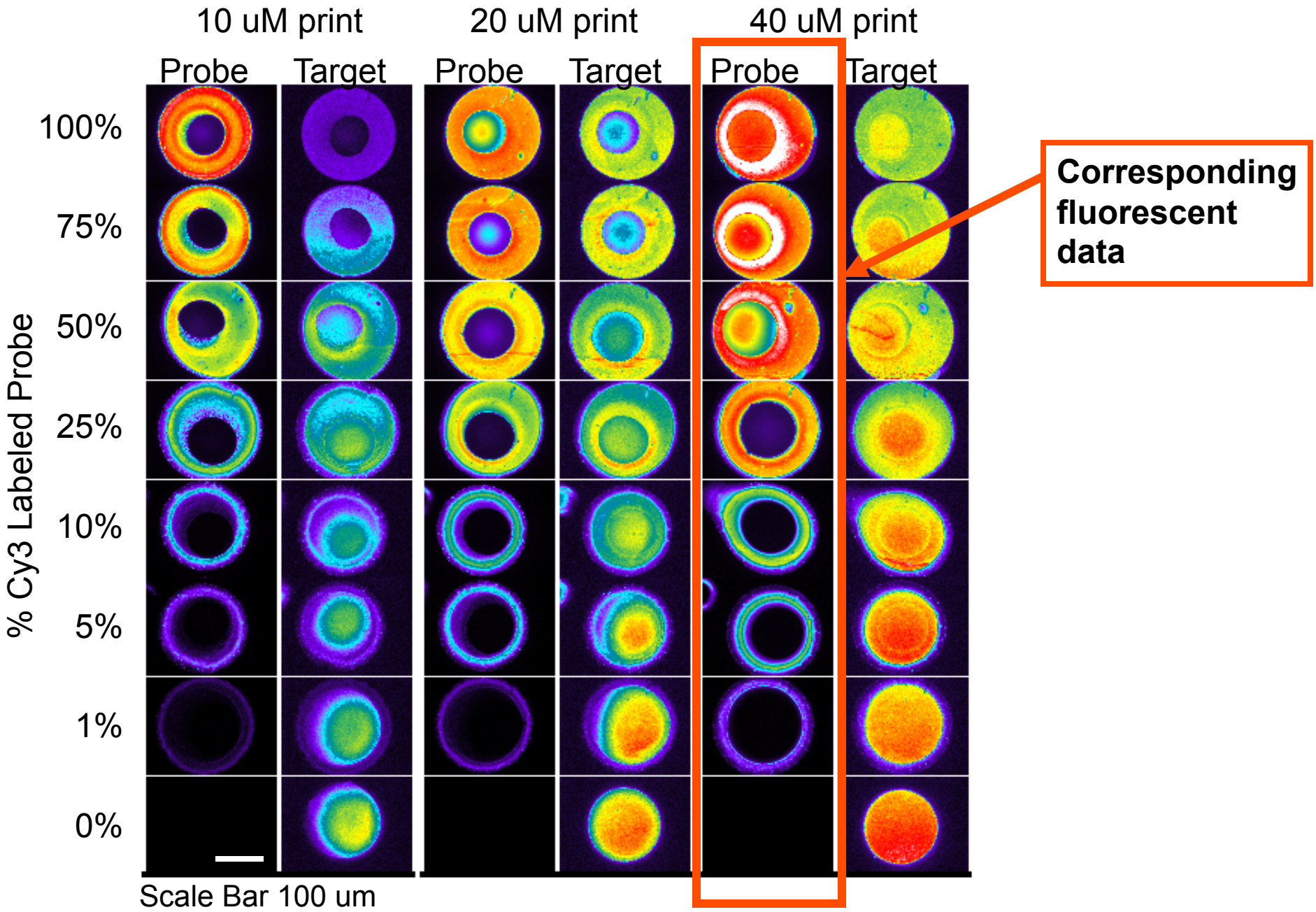
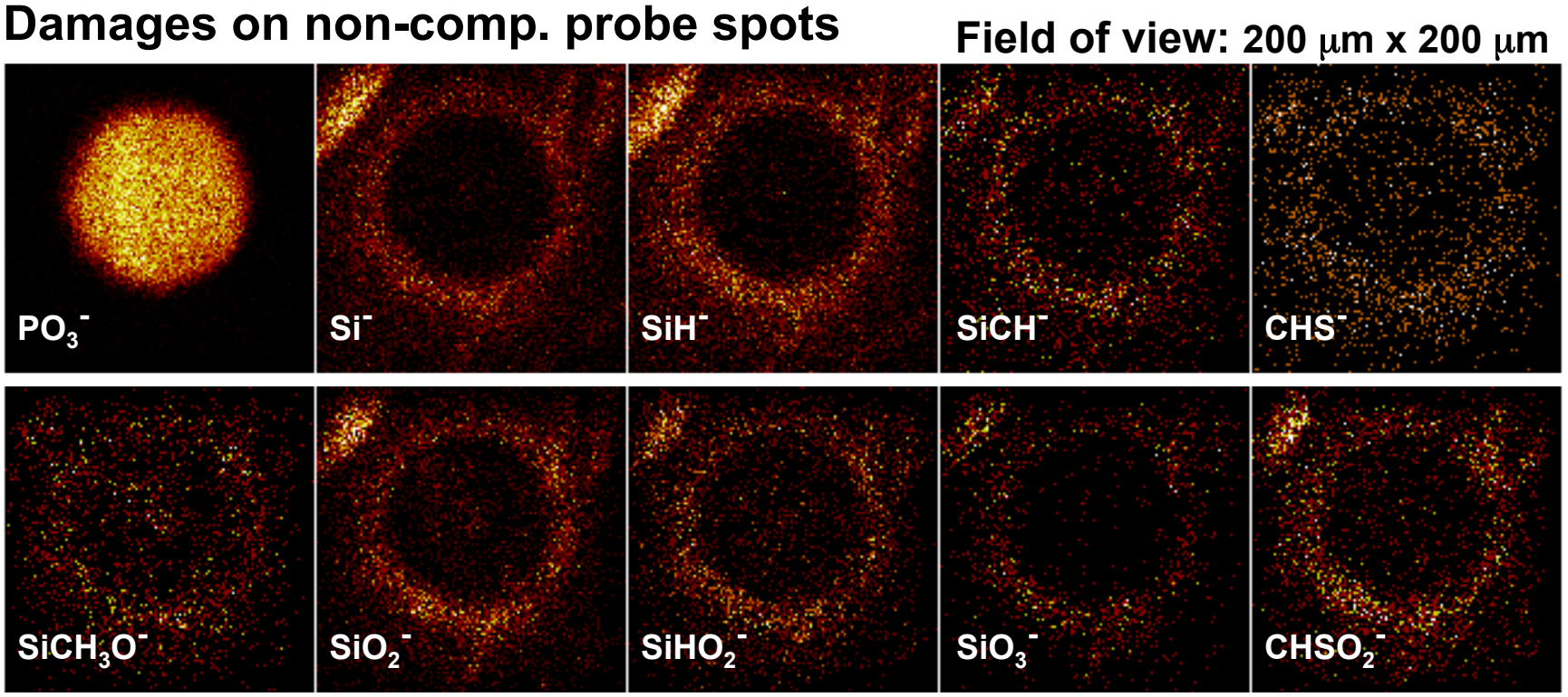


Hybridized with 1 uM Target (99:1 unlabeled:Cy5 labeled)

ToF-SIMS Indicate Similar Molecular Species Found on Ring Structure and Damaged Regions



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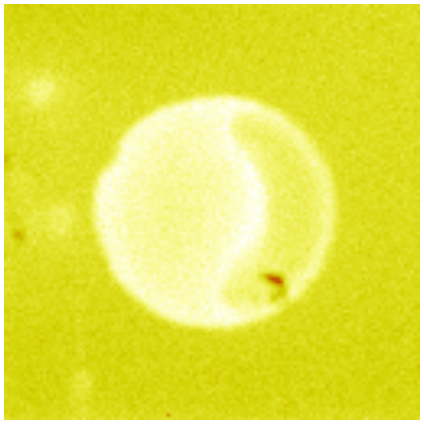
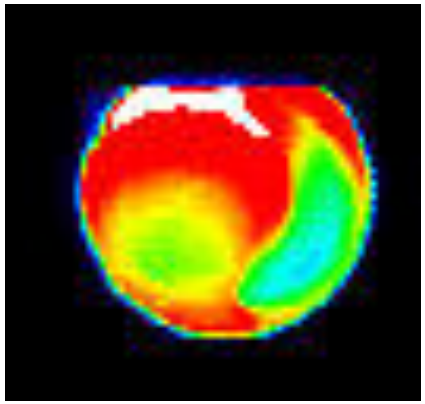
10 μM
Cy3 25%

Total ion Intensity



Fluorescence Intensity

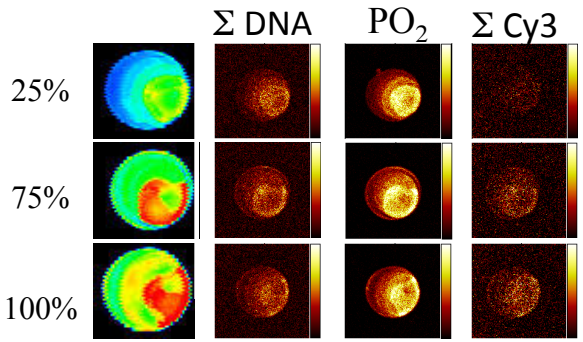


Maximum Autocorrelation Factors (MAF)

- Find linear combination of peaks that
  - –Maximize variance in image
  - –Minimize variance between neighboring pixels
- Improves image contrast
- Typically provides better separation of chemical constituents
- Scaling independent

Concerns

- Spot size “changes” with concentration of DNA
- Type of spotter ‘matters’
- Results differ with different substrates
- The non-uniformity is probably due to the rapid drying of the droplet rather then separation of labeled and unlabeled DNA on this surface.
- DNA purity?



Acknowledgments

- **University of Washington, NESAC/BIO**
  - **Prof. Dave Castner, Dr. Líney Árnadóttir, Dr. Nicolas Vandencasteel**
- **University of Utah (Prof. Dave Grainger)**
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