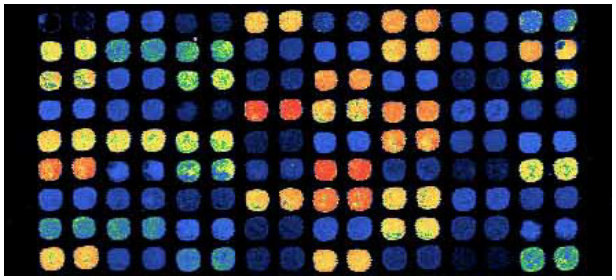
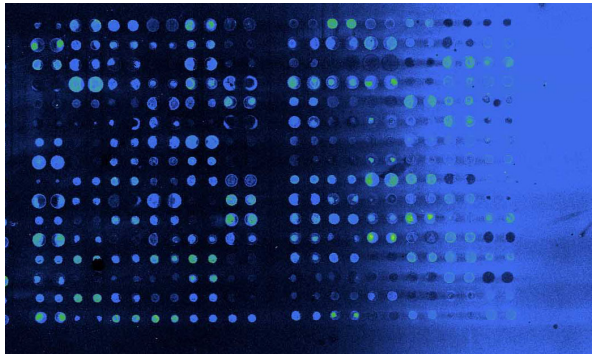
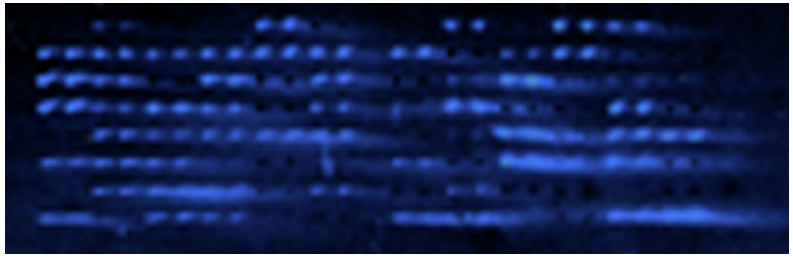
Troubleshooting



The Good



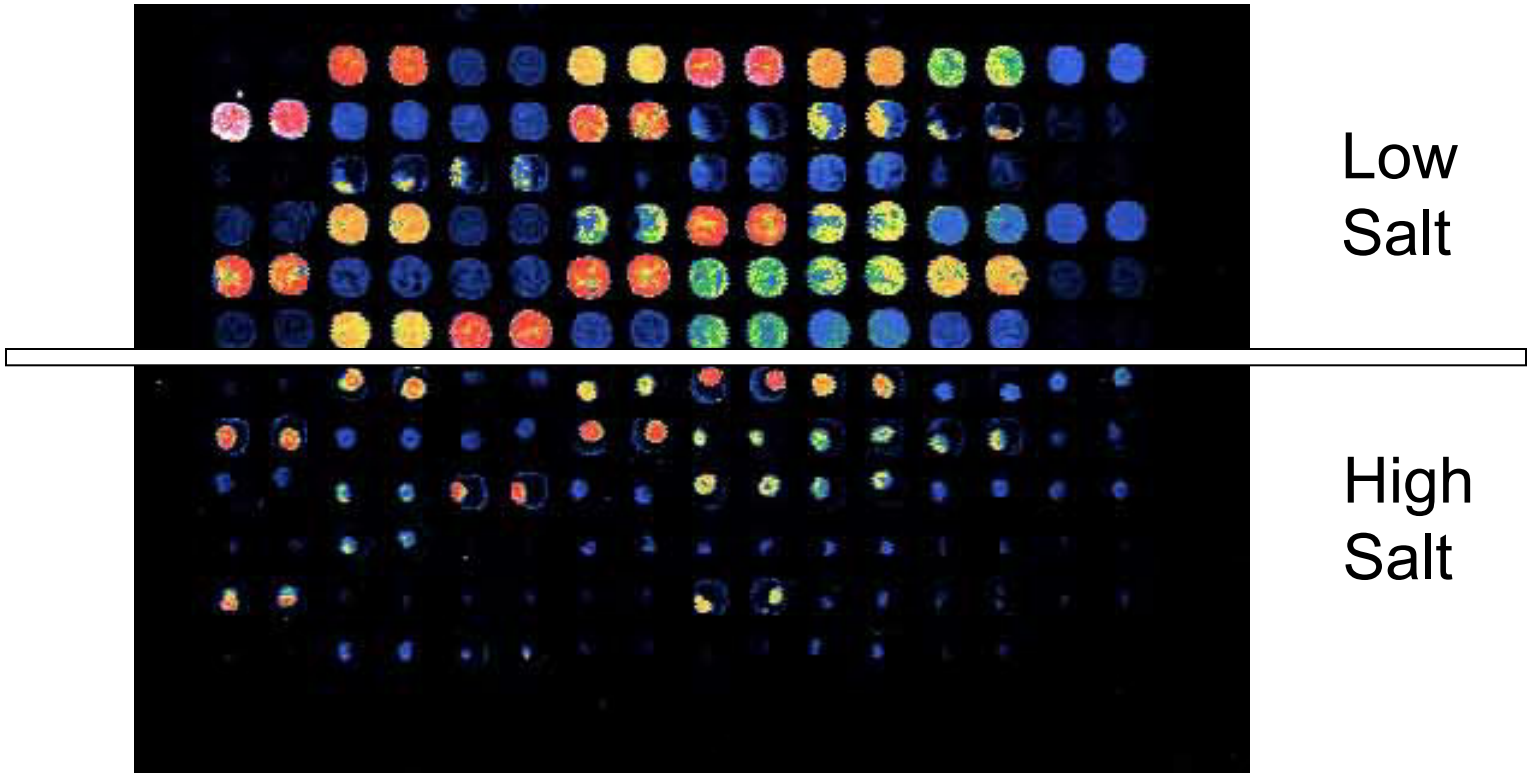
The Bad



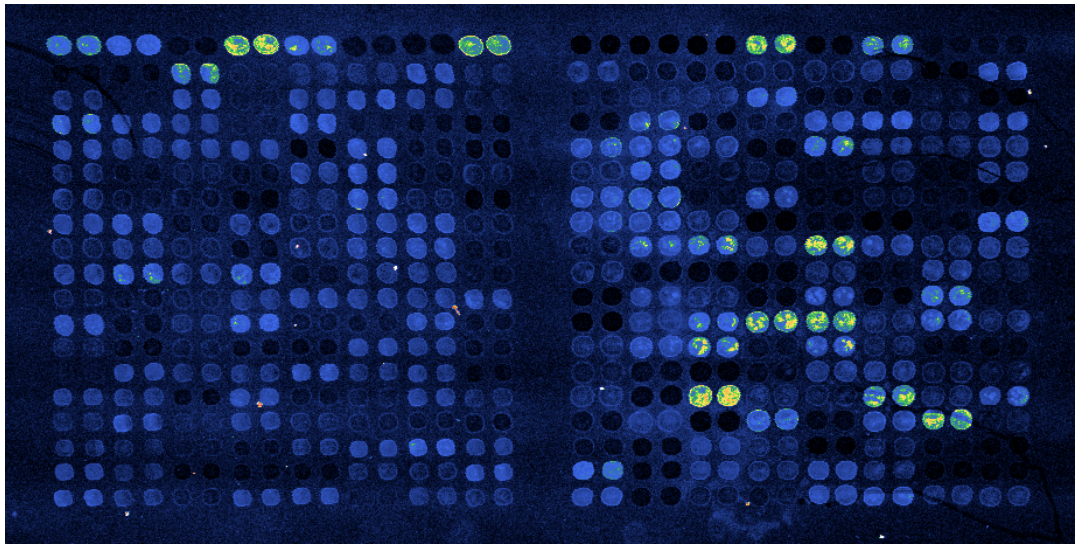
The Ugly

Spot size uniformity

Related to hydrophobic and ionic interactions between
DNA suspension and surface coating. Example, salt concentration.



Black Holes and Random Background

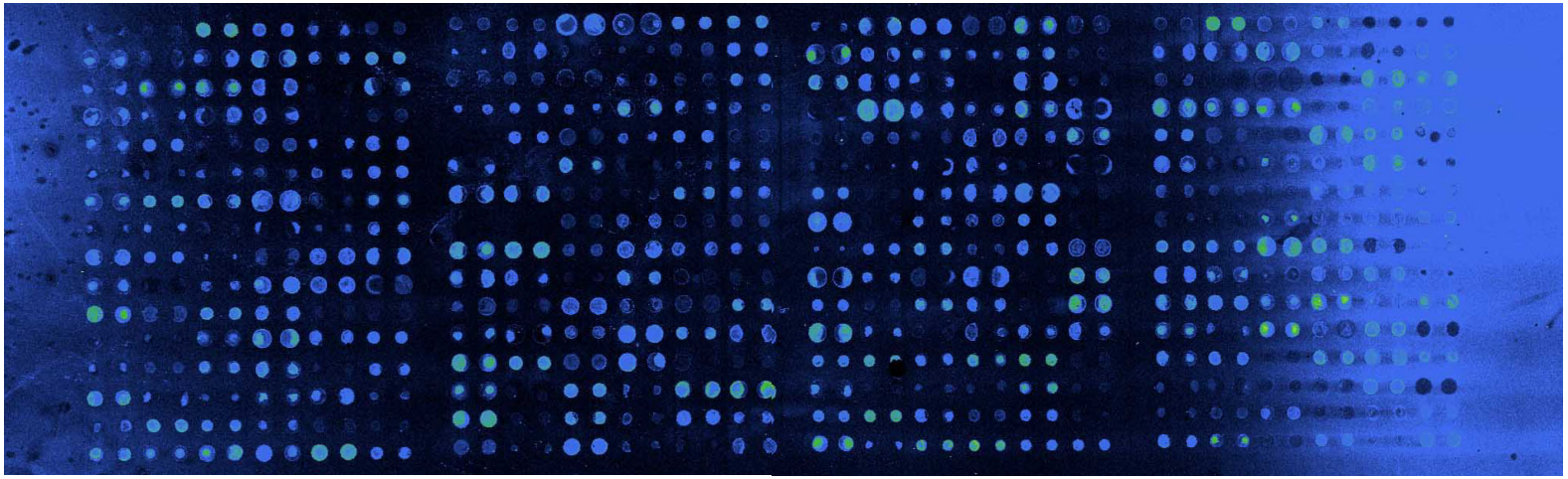


Solutions:

- Wash slide with SDS prior to chemical blocking step.
- Add ‘cold’ polyA (or polyT) and/or, tRNA, calf thymus, etc.
- Use a column-based purification kit (ie: Qiagen PCR cleanup) to clean-up probe after labelling.
- Use lower laser and/or PMT settings on scanner.

Probe Drying and/or Precipitating

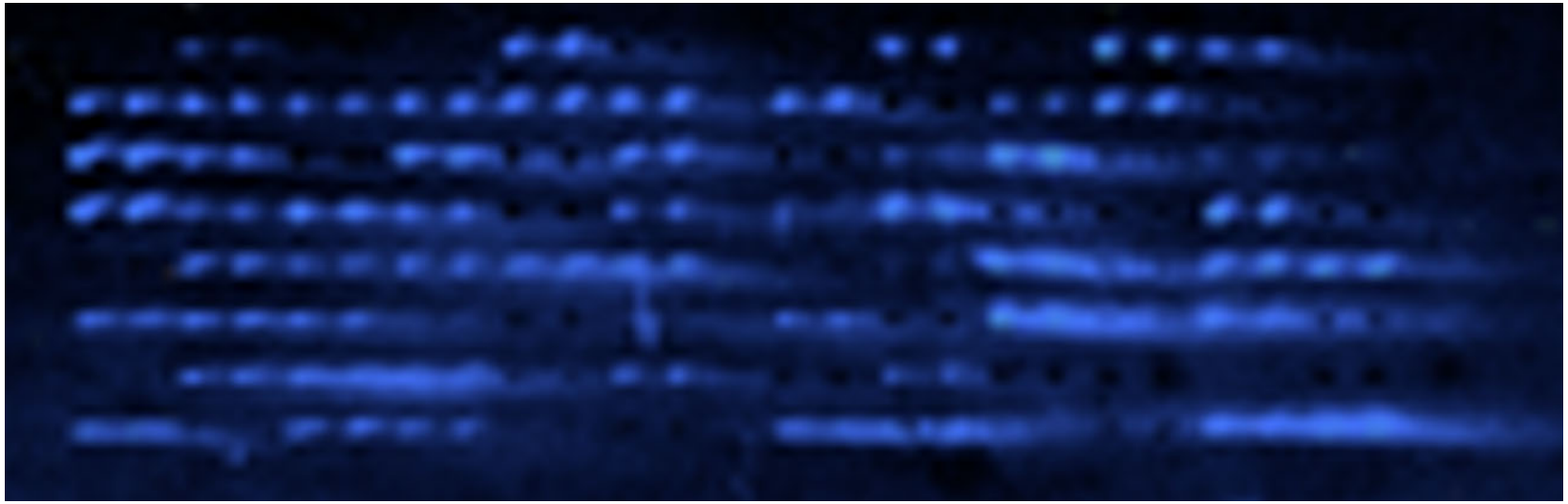
- Hybridization solution dried under coverslip at edges



Solutions:

- Ensure adequate humidity in hyb chamber. One can use moistened filter paper at ends of slide away from cover slip.
- Use coverslips (like LifterSlips) that are slightly raised to allow use of greater volume of hybridization solution.

Smeared spots (‘comets’)



Possible cause

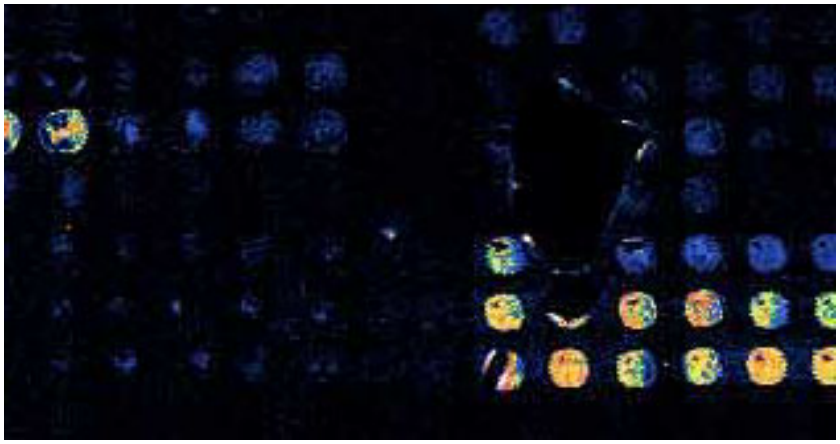
DNA spots smear across slide prior to adequate blocking of slide coating

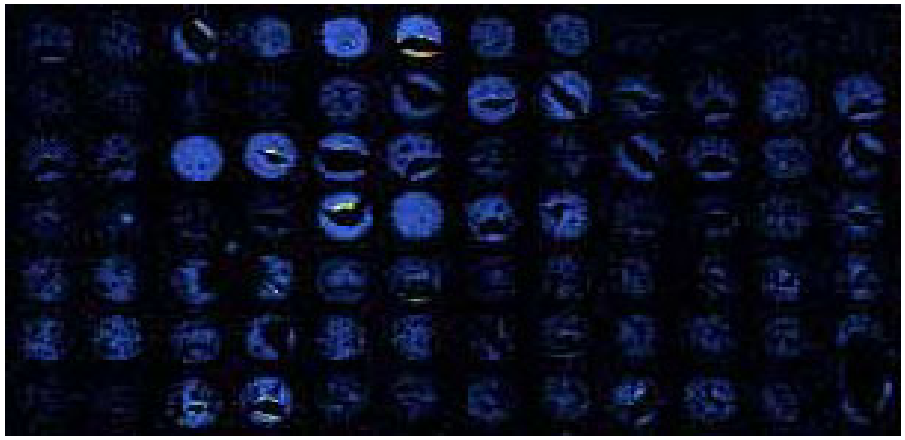
Solution

Make first blocking treatment a vigorous wash in 0.2% SDS

Cracking and Flaking of PL Coating

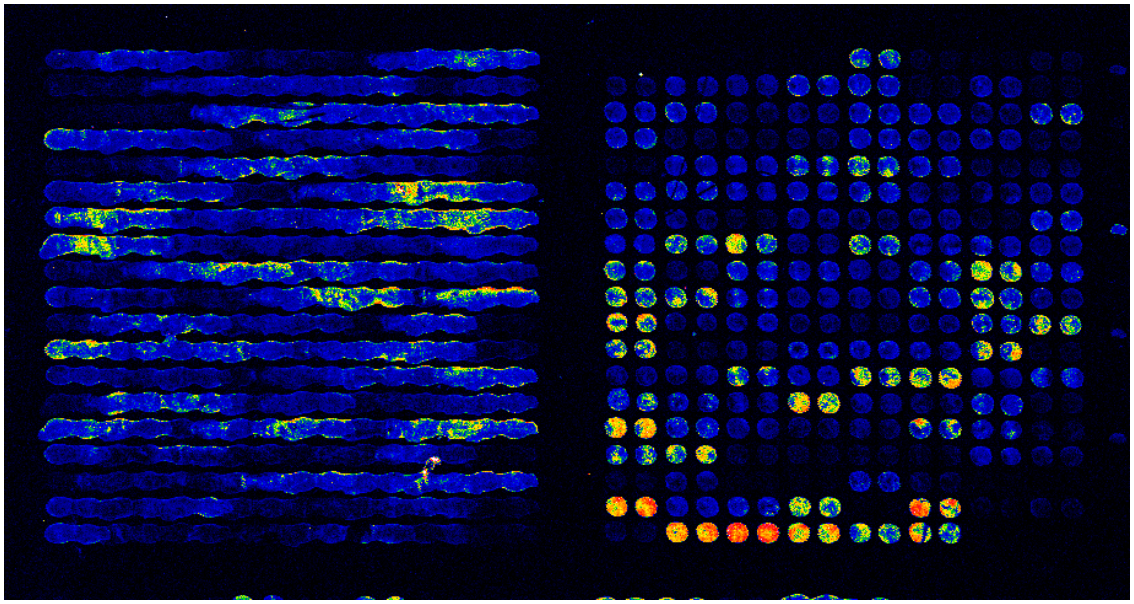
- Due to old or poorly made poly-L-lysine (PL) slides



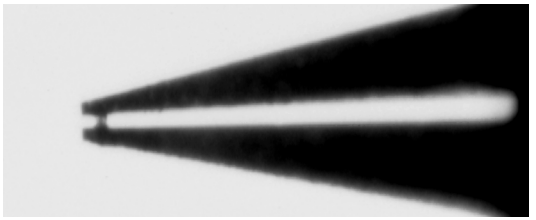


- Solution: Spot, UV-link, and hyb PL slides within 3-4 months.
  Test a few slides of each PL batch before
  investing too much time, money, and resources

Smeared spots within one grid



Due to clogged/damaged pin tip



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