Wetting, Microarrays and Unilateral MRI

Glen McHale, Mike Newton, Martin Bencsik, Gareth W. Cave, Robert H. Morris, Hans Adriaensen and Stuart Brewer*

*Dstl, Porton Down

IoP P&G, London 4th July 2008

Overview

1. Microarrays

- Principles
- Print quality
- Wetting and droplet spreading

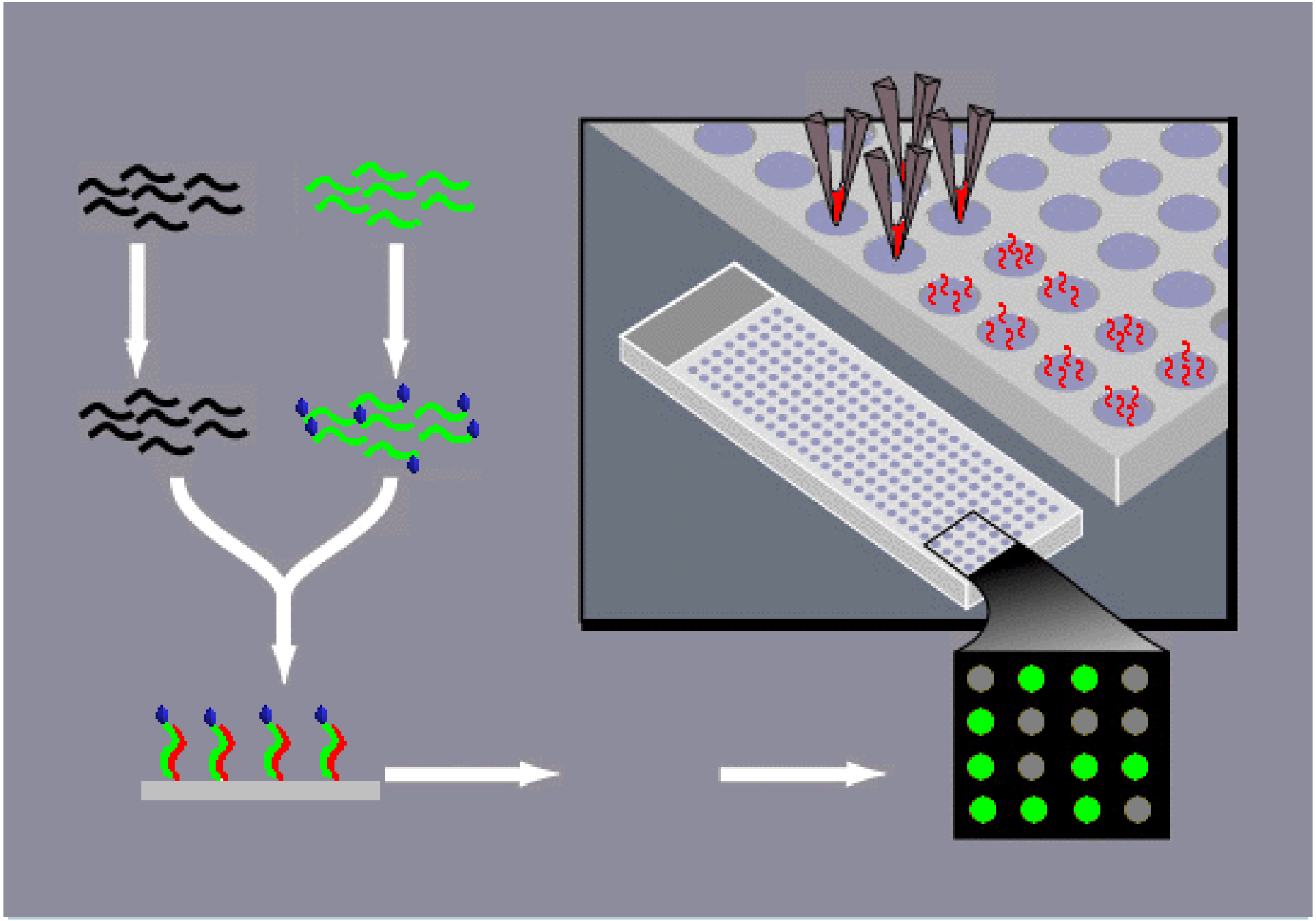
2. Unilateral Magnetic Resonance Imaging (MRI)

- Principles
- Use to study liquid penetration
- Application to spotted microarrays

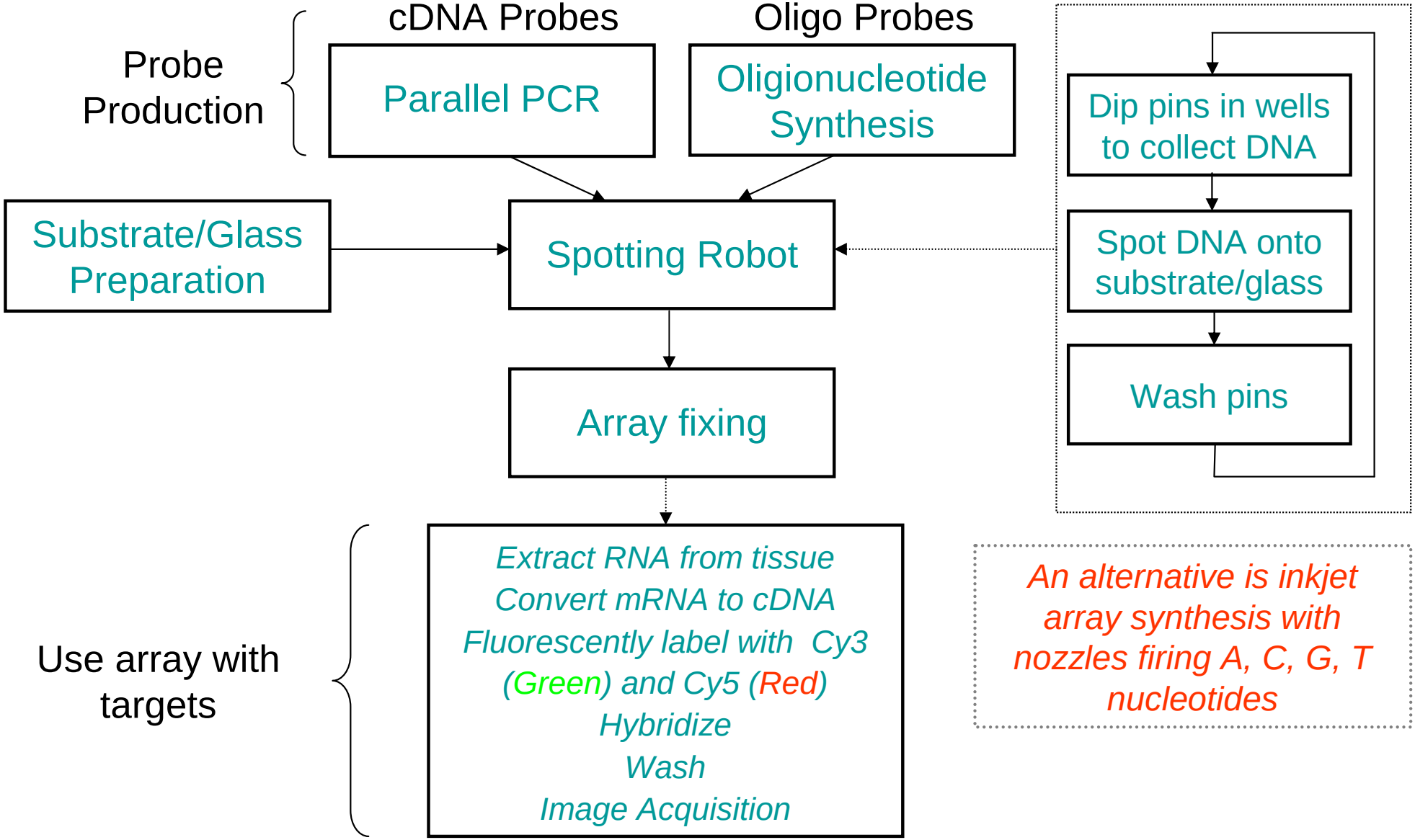
Microarrays

Combinatorial assessment of biomolecular interactions

Reference G. McHale. (2007), 132, 192-195.



Robotic Spotted Microarray Principles

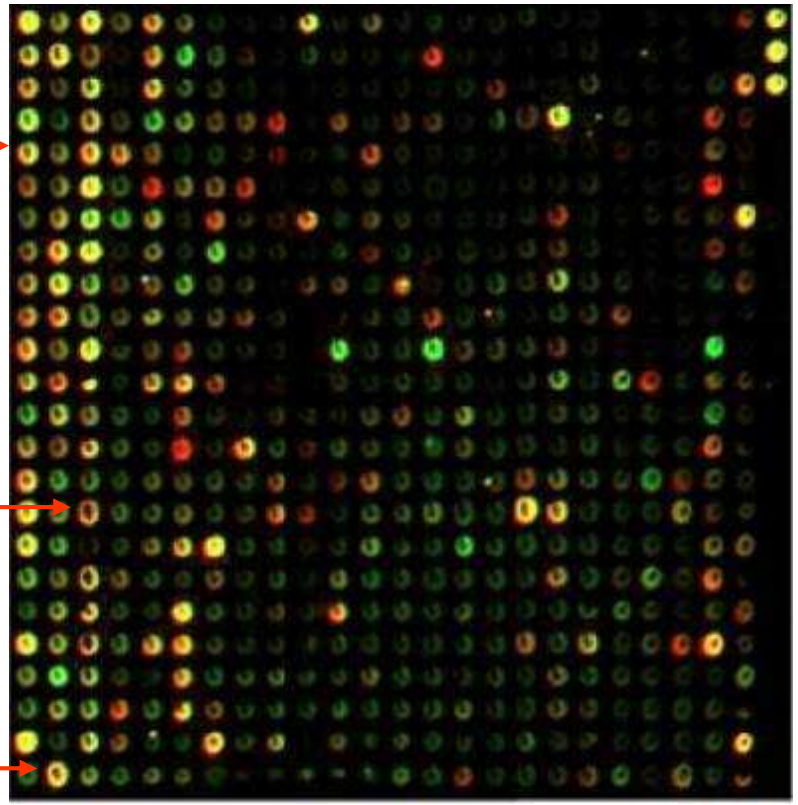


Spotted Fluorescent Microarrays

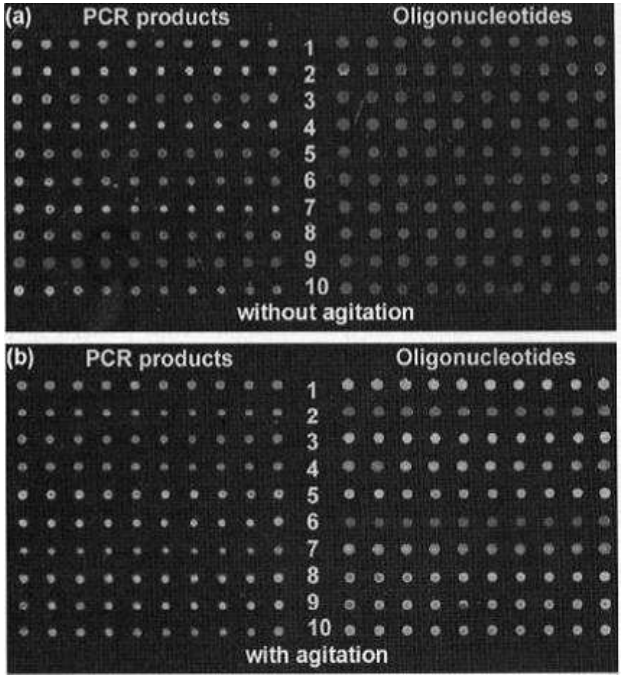
Donuts

Irregular sizes

Distorted shapes

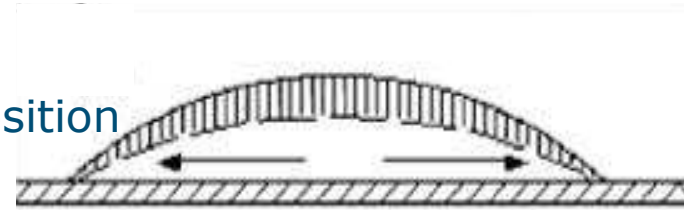


Micro-mixing improves intensity, but doesn't stop donut's etc:



Wetting Dominated Effects

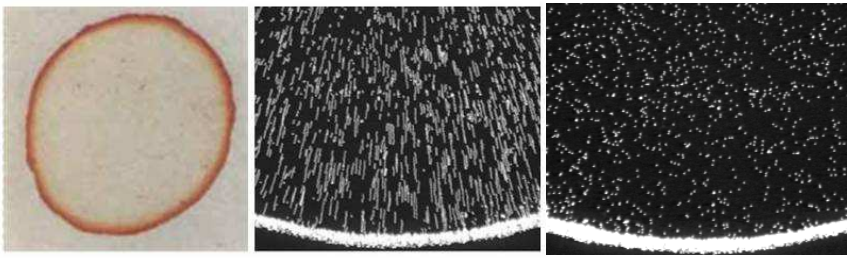
- Donut or "coffee/ring-stain" effect
- Size, shape, uniformity, reproducibility of deposition
- Drying effects (evaporative considerations)



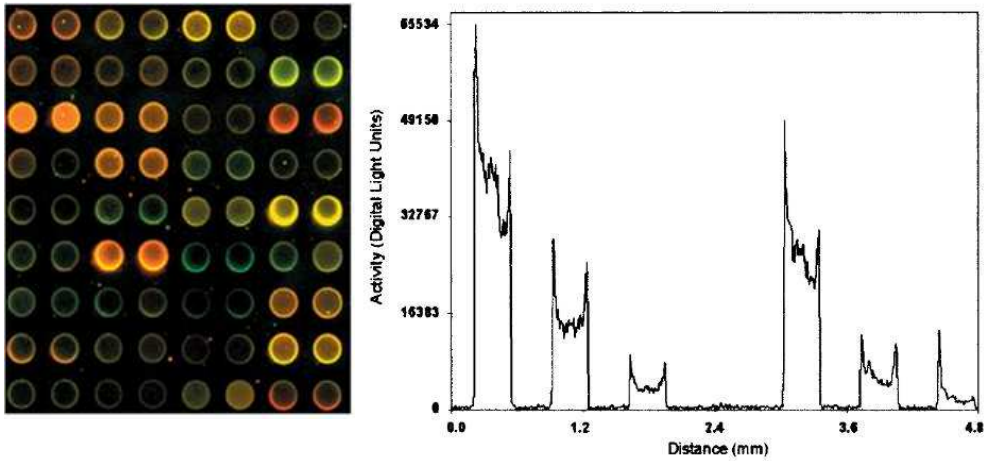
Acknowledgement
A. Wixforth, Superlattices & MicroStr. (2003), 33, 389;
Anal. Bioanal. Chem (2004), 379, 982.

Issues with Microarrays

- Technical Problems
  - DNA base pair length v spot density
  - Non-uniform fluorescence (replicates)
  - Porous substrates difficult for fluorescence
  - Dust contamination
  - Non-specific binding
  - Fluorescence quenching
  - Spectral overlap
  - Irregular spot shape and size
  - Ring stains/donuts



a) Coffee ring stain [R. D. Deegan, PRE, 2000, 61, 475], b) solute flow [R. D. Deegan, *et al*, Nature, 1997, 389, 827]



Non-uniform fluorescence across a spotted microarray due to ring-stain formation [R. Blossey & A. Bosio, Langmuir, 2002, 18, 2952]

- Printing Issues
  - Spotted array technologies: pins or needles, pin & ring, inkjet printing
  - Substrate: surface chemistry and hydrophobicity/wetting
  - Liquids: buffer viscosity, pH, evaporation, etc

Unilateral NMR/MRI

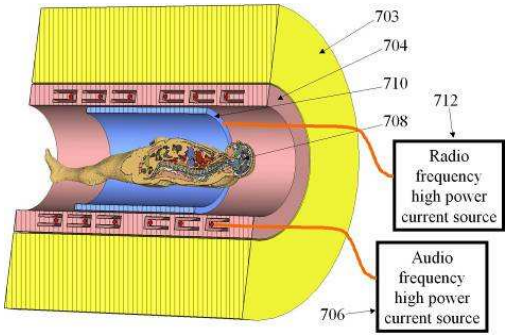
Liquid Penetration

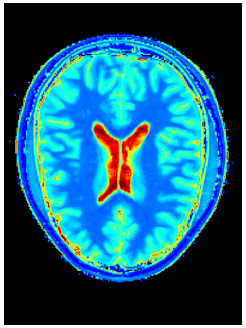
Reference M. Bencsik, H. Adriaensen, S.A. Brewer & G. McHale, J. Mag. Res. 193 (2008) 32.

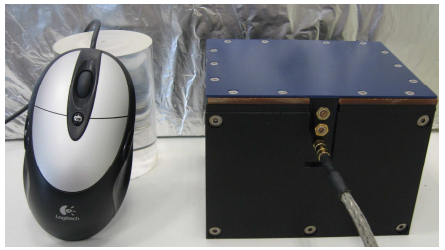
NTU

NMR, MRI and MR Microscopy

- Nuclear Magnetic Resonance (NMR)
  - Uses intrinsic magnetic moment of nucleus (e.g. proton in hydrogen)
  - Align with external field
  - "Kick-out" out of alignment using rf field
  - Monitor signal recovery
  - Recovery depends on environment
- Magnetic Resonance Imaging (MRI)
  - Use magnetic field gradients to resolve spatially
  - Signal can be deconvolved into an image
  - Most familiar from medical imaging applications
- Magnetic Resonance Microscopy
  - NMR and MRI made small (down to 10 microns)
  - Relatively inexpensive equipment (£30k-£60k)
  - Portable and benchtop use possible
  - Unilateral MRI (e.g. NMR MOUSE®)



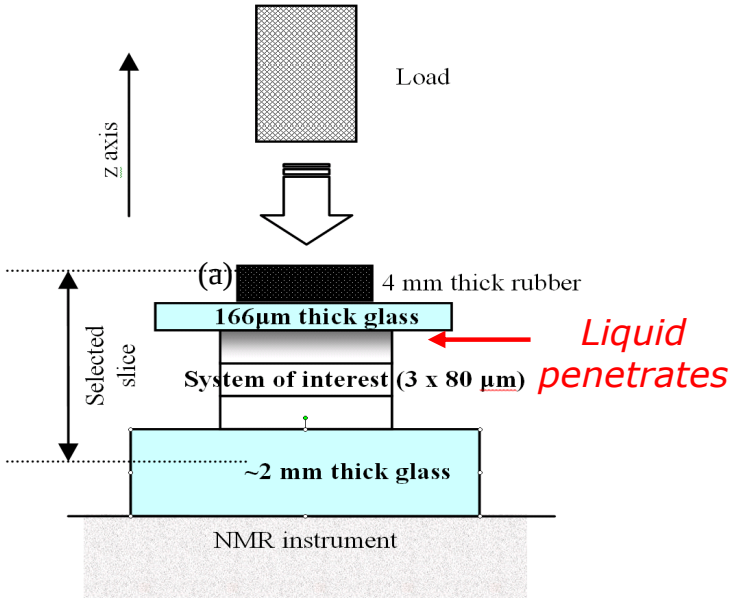


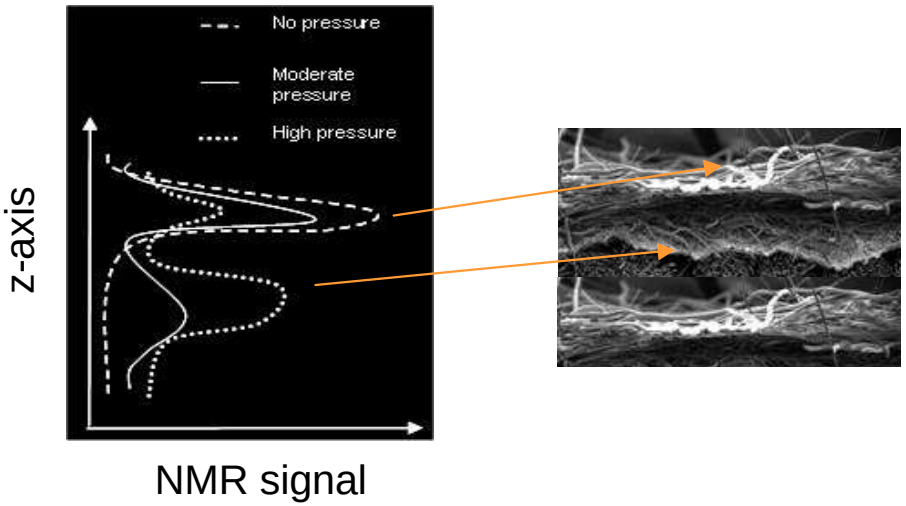


3D imaging of a small volume above it, with a region of interest 15 mm x 15 mm x 10 mm.

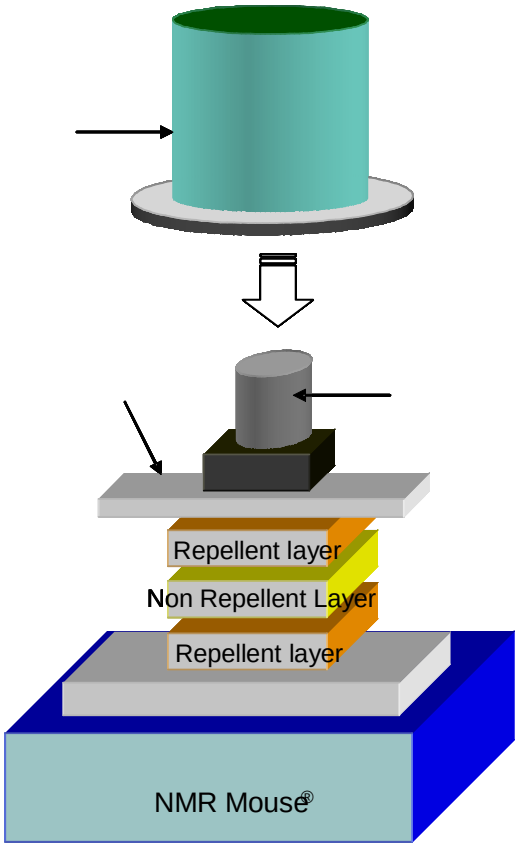
Application 1: Assessment of Textile Substrates

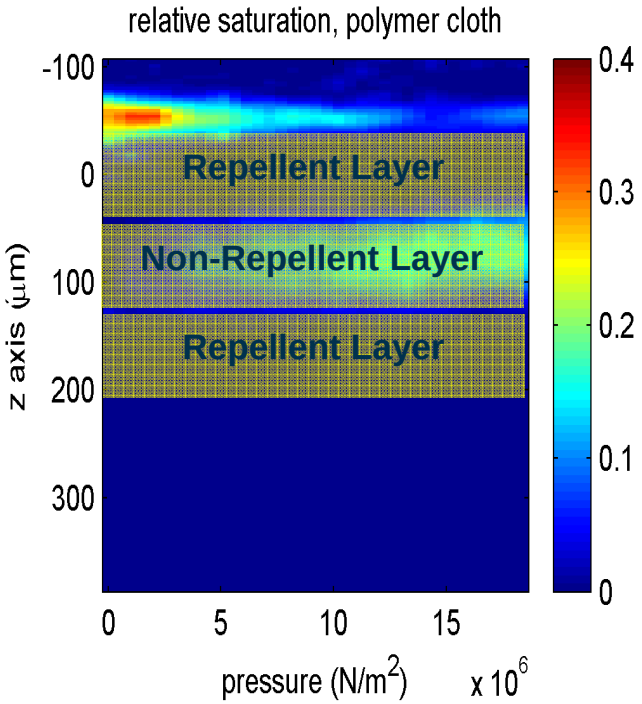
- Textiles that provide protection against toxic chemicals need to prevent the ingress of aerosols, vapours and liquids
  - Aerosols: particle capture
  - Liquid: repellent and wicking layers
  - Vapour: activated carbon
- Test methods that image a textile's performance are desirable

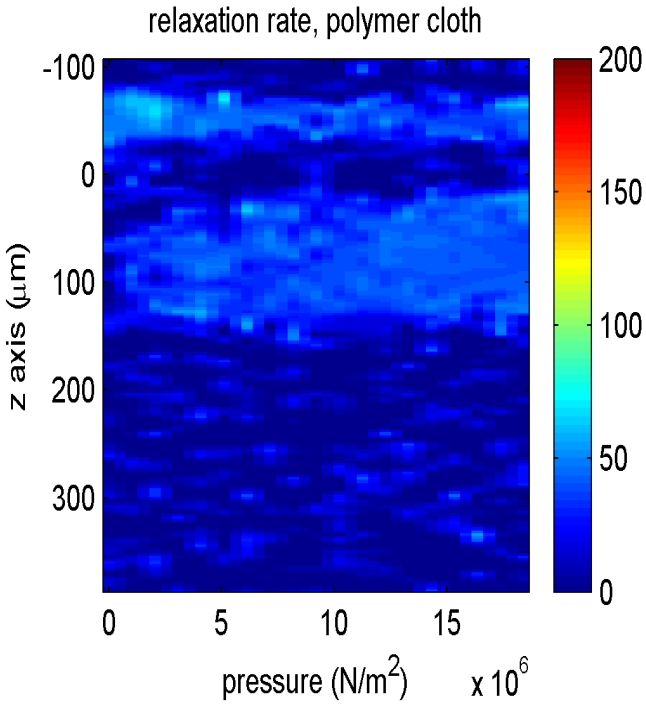




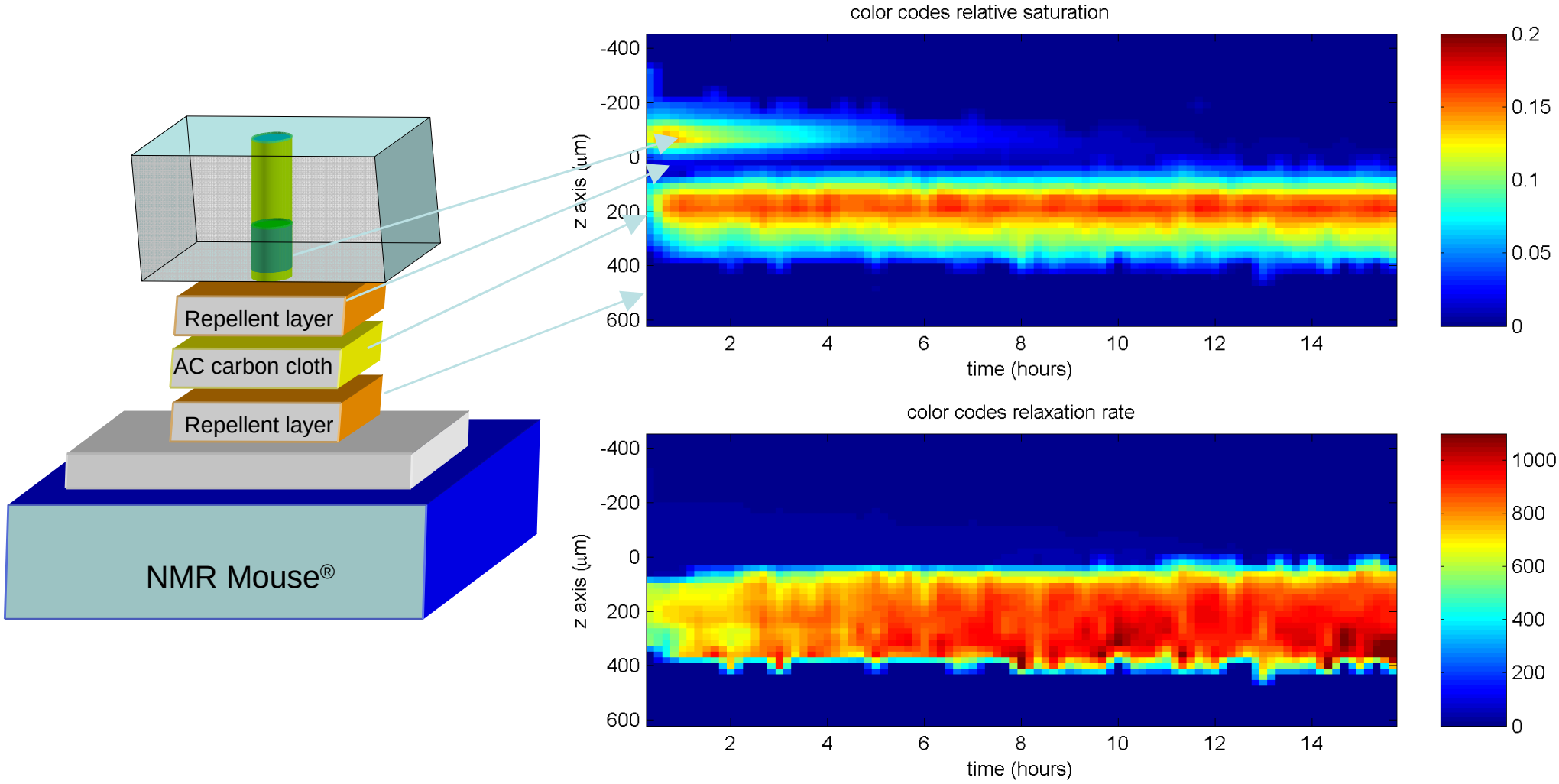
Spatially Resolved Measurement







Spatially Resolved Measurement of Vapour Uptake through a Repellent Layer



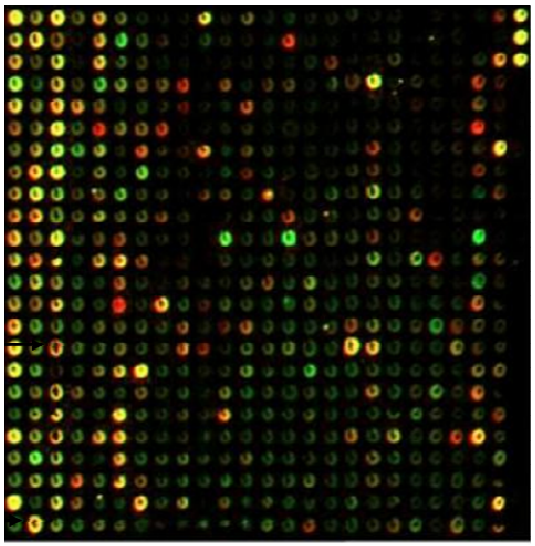
MRI-based Microarray

M-Ray

Reference McHale, Newton, Bencsik & Cave, "Methods of detecting interactions on a microarray using nuclear magnetic resonance" UKPA No. 0618514.4 (2006).

MRI Applied to Microarrays

- DNA, Protein & Chemical Microarrays
  - Interactions between surface immobilised probes and targets
  - Combinatorial method of screening for chemical & biochemical interactions
- Existing Technology
  - Immobilisation Techniques
    - Lithography (Affymetrix)
    - Beads (Illumina)
    - Robotic Spotting (i.e. Printing)
  - Detection Strategy
    - Fluorescence dominates
- Our Concept for Microarrays
  - Nuclear Magnetic Resonance (NMR)
    - → Magnetic Resonance Imaging (MRI)
      - → NMR Microscopy (MRM)



Spotted microarray (A. Wixforth, Superlattices & MicroStr, 2003, 33, 389.)

M-Ray Approach

- Basic Concept

  Replace fluorescent interrogation by NMR Microscopy

  Signal determined by fluid environment of magnetic carrier

  Make environment changes visible by,

  Magnetic nanoparticle labels, e.g. Superparamagnetic iron oxides (SPIOs)

  Or by using diffusion hindering labels, e.g. dendrimer molecules

  SPIO concept already used in molecularly targeted MRI

- Proof of Concept

  Immobilize molecule on glass

  Allow SPIO labelled molecule to bind

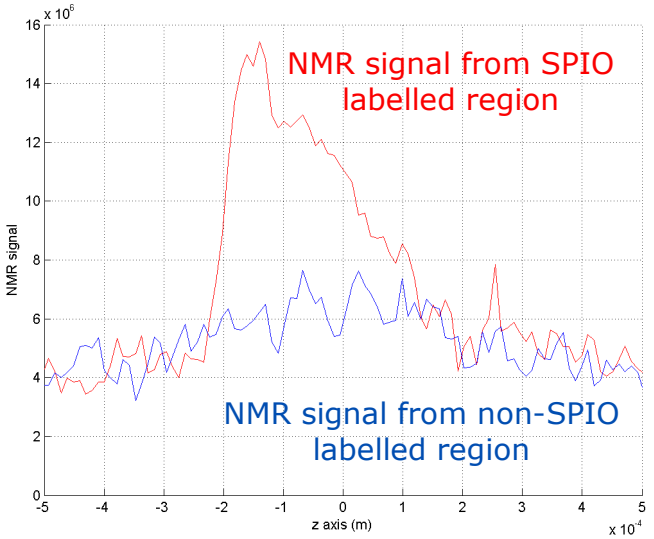
  Introduce NMR fluids and perform MRM

  Single spot case: water as imaging fluid

  Multispot case: contrast of labelled to non-labelled

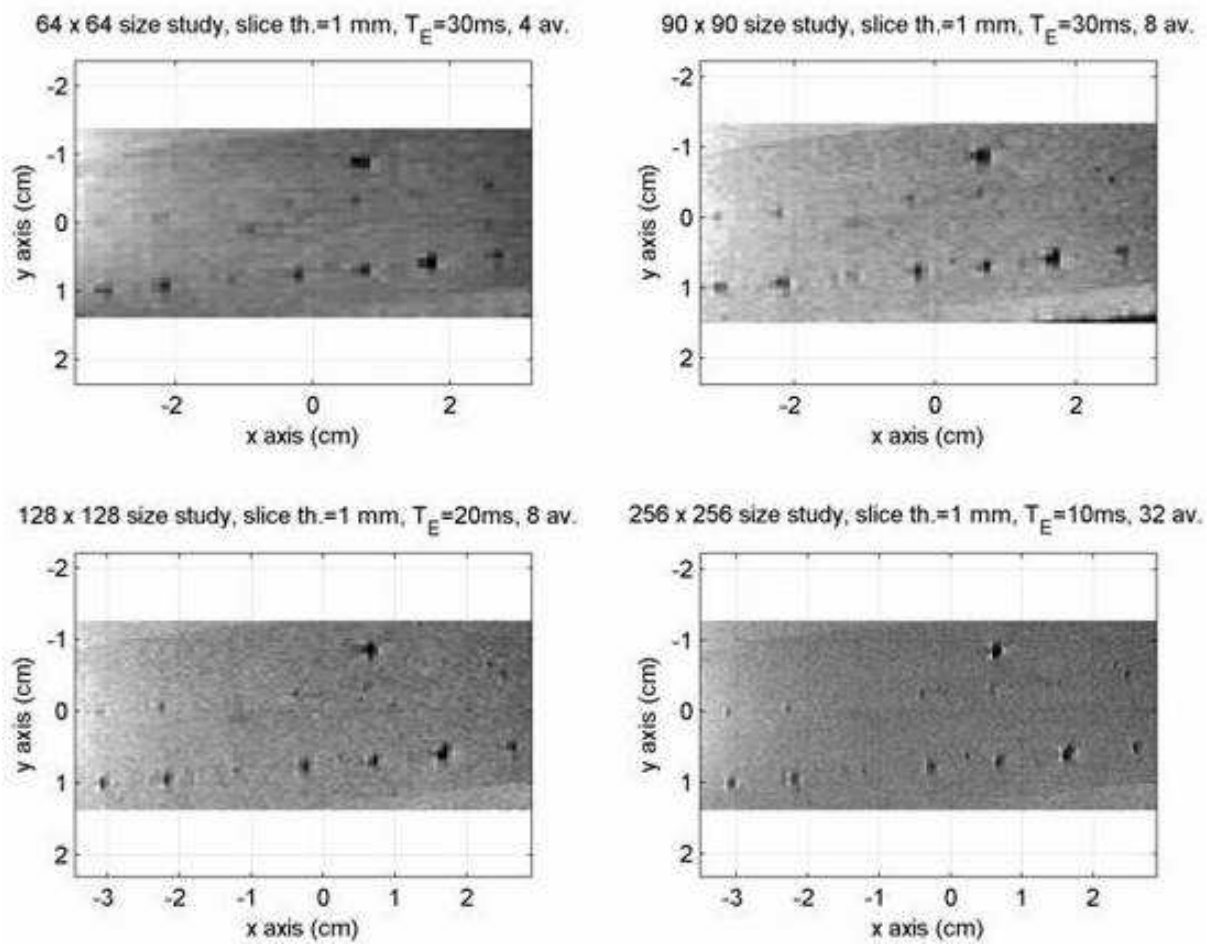
  Single spot case: basic reproducibility,

  effect of repetition time, imaging fluids



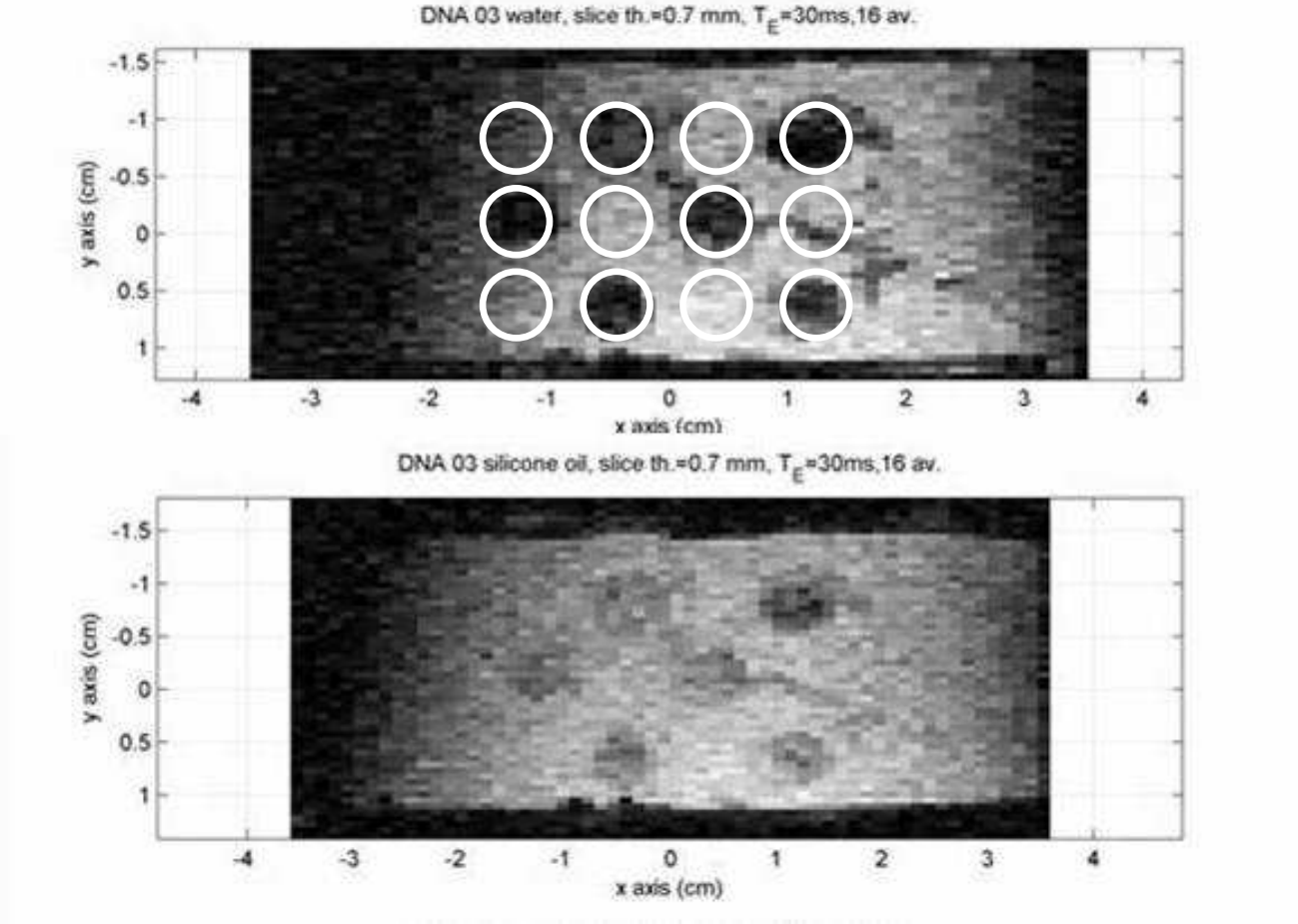
First ever experimental result (water as NMR fluid & SPIOs as positive contrast)

Imaging of a Chemical Array using Water



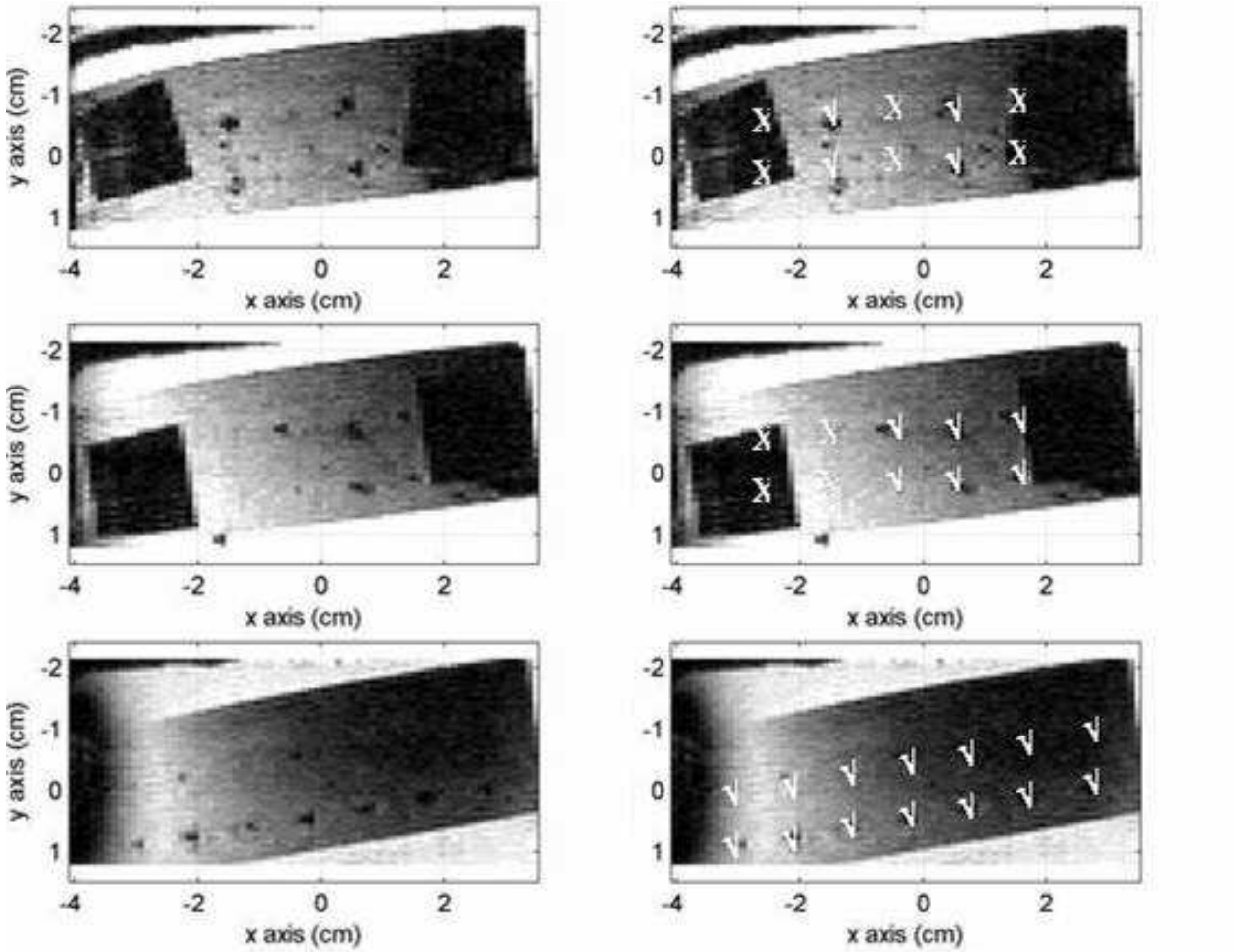
Glass slide possessing a line of 1 mm SPIO-labelled spots and a line of 0.5 mm SPIO-labelled spots. This was immersed in water and imaged with increasing spatial resolution – first figure is shortest time and has strongest contrast. Array of SPIO-labelled spots may be imaged in a single image acquisition sequence.

Imaging of a DNA Array using Different Fluids



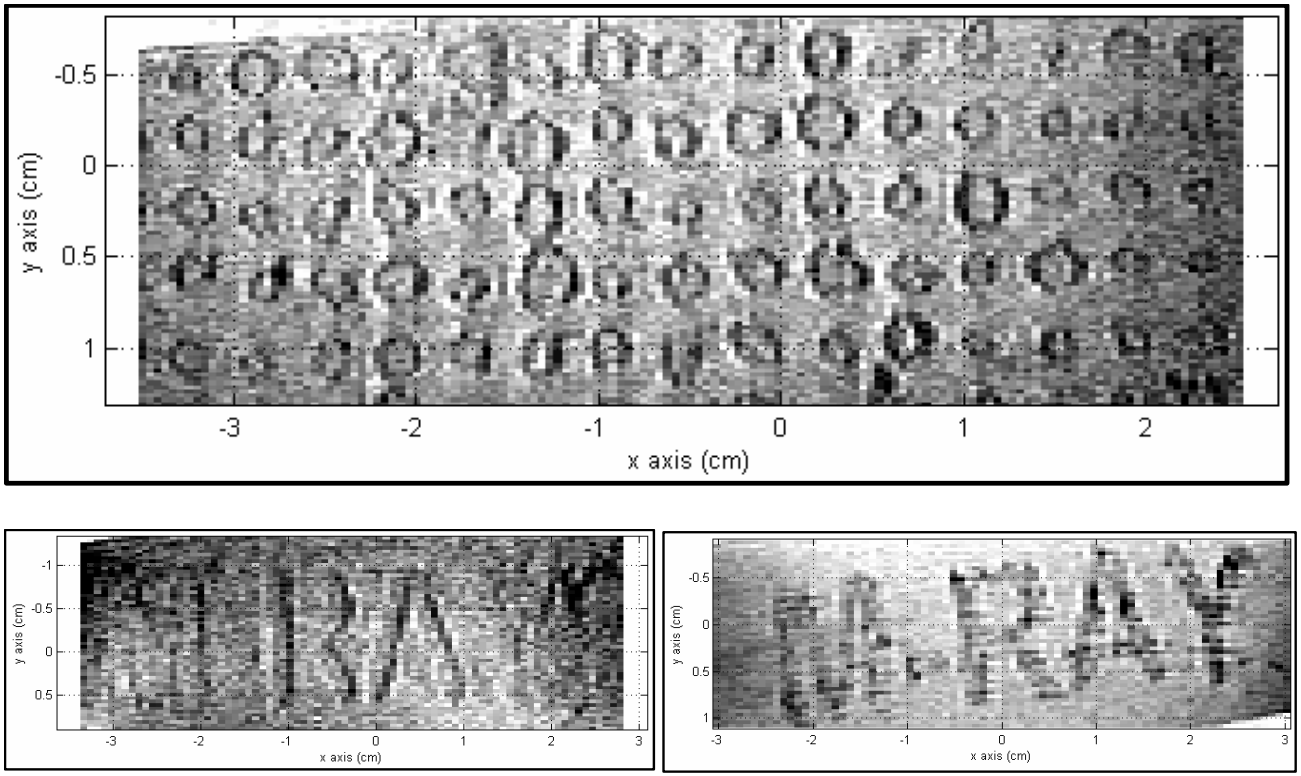
Glass slide possessing a 3x4 DNA array of alternate SPIO-labelled spots. This was imaged in i) water (upper figure), ii) ethanol (not shown) and iii) silicone oil (lower figure). Multiple fluids can be used and wetting properties of fluid can be optimised. Slice level relative to surface plane can be adjusted to remove impurities contributed by randomly non-specifically bound SPIO's on the surface.

Simultaneous Imaging of a Stack of Slides



2D images taken from a single 3D image acquisition of signal from a stack of chemical arrays. Different slices corresponding to glass slides in a stack. Ticks and crosses have been superimposed on the right hand side column of images to show expected positions of SPIO-labelled spots.

Simultaneous Imaging of a Stack of Slides



2D high spatial resolution and high density M-RAY (top), gold coated M-RAY (middle) and gold coated M-RAY (bottom).

Summary

1. Non-invasive spatially resolvable volume measurements
2. MRI can probe opaque substrates
3. Unilateral MRI can be used to look at liquid-solid interactions
4. Dynamics can be followed
5. Applications demonstrated include:
    - Quantitative monitoring of liquid penetration into textiles
    - Magnetic nanoparticle labelled microarray

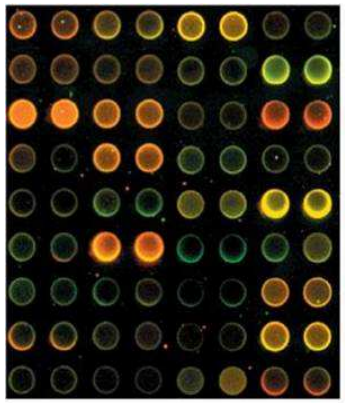
The End

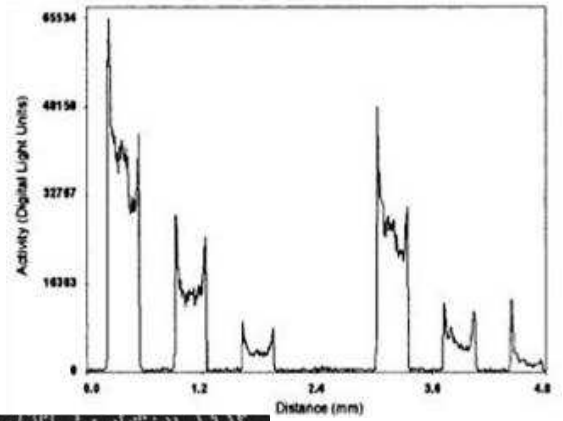
Appendices

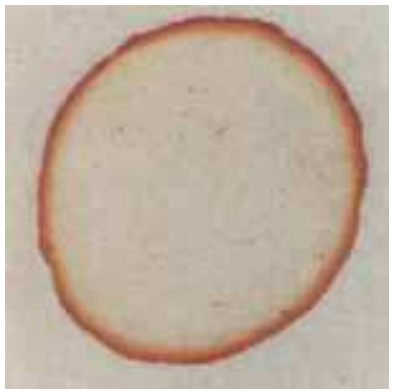
Donuts & Contact Line Pinning

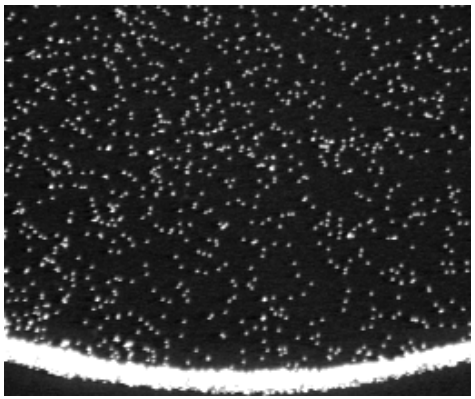
"Coffee" Ring Stains

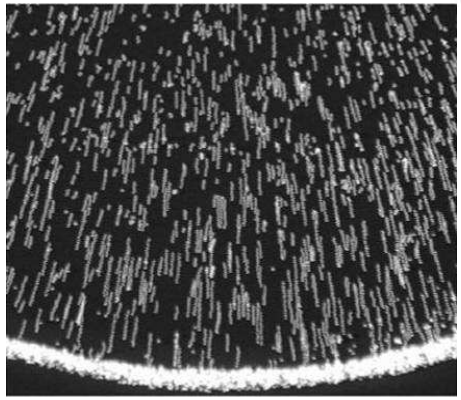
- Evaporation of droplet with solute
- Contact line is pinned
- Deposition often occurs at edge of droplet
- Relative humidity determines rate
  - low humidity ⇒ fast ring formation





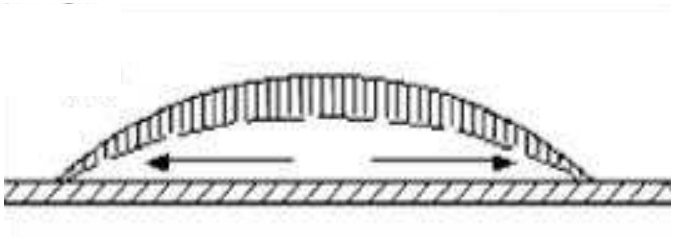






Suppression of Ring Stains (after Blossey)

- Initial solute concentration is high
- Contact angle is very small
- Reduce contact line pinning
- Initial deposition needs to be homogeneous - strong memory of initial distribution



Acknowledgement

Deegan *et al*, Nature 389 (1997) 827; Wixforth *et al*. Superl. Microstr. 33 (2003) 389; Anal. Bioanal. Chem. 379 (2004) 982. Blossey & Bosio, Langmuir 18 (2002) 292.

M-RAY Approach

Basic Concept

- Replace fluorescent interrogation by NMR, NMR Microscopy or MRI.
- Signal determined by fluid environment of magnetic carrier.

Make environment changes visible by:

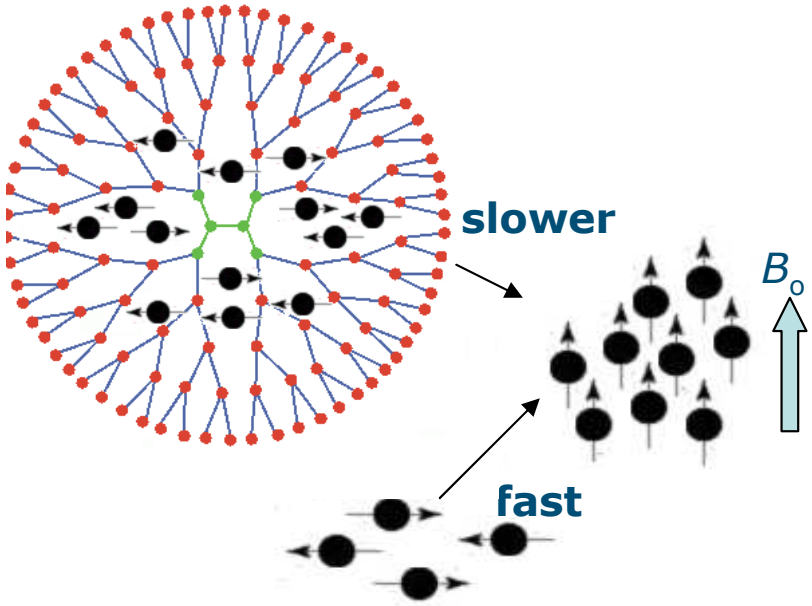
Magnetic nanoparticle labels

e.g. Superparamagnetic iron oxides (SPIOs)

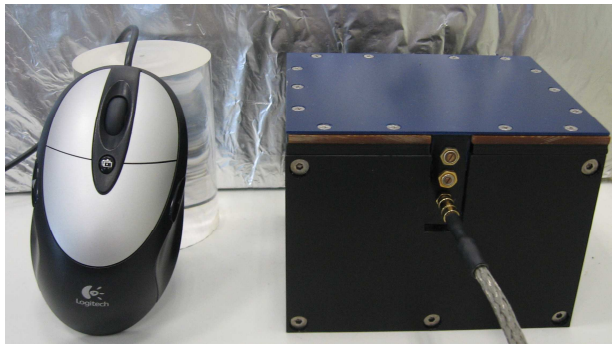
OR

by using **diffusion hindering labels**

e.g. dendrimer molecules



M-RAY – Proof of Concept

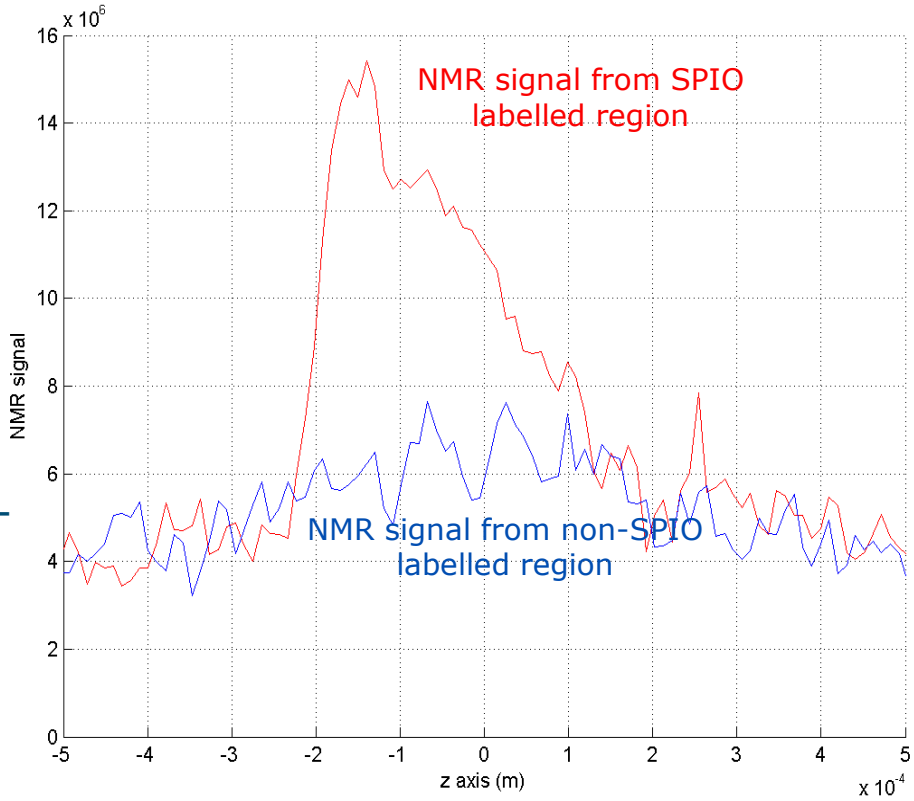


Unilateral MRI

- Immobilize molecule on glass
- Allow SPIO labelled molecule to bind
- Introduce NMR imaging fluids
- Perform MRI using a profile NMR-MOUSE® instrument

Examples 1-3

- Single spot case: water as imaging fluid
- Multispot case: contrast of labelled to non-labelled
- Single spot case: basic reproducibility and effect of repetition time



First ever experimental result with water as NMR fluid & SPIOs as positive contrast

M-RAY – Proof of Concept 2

- Small-bore MRI
  - Create chemical arrays on glass
  - Create DNA arrays on glass
  - Introduce NMR imaging fluids
  - Perform MRI using a Bruker® 2.35 T small-bore MRI scanner

Examples 4-6:

Line of spots: water as imaging fluid

DNA array (25 nucleotides): different imaging fluids

Stacks of arrays: 3D microarrays

The following data was for exemplification/proof-of-concept and so spatial resolution, time of acquisition, signal-to-noise, NMR capture parameters and methods have not been optimised

Advantages of NMR detection over fluorescence

- NMR does not require transparency.
- NMR can probe a volume, non-invasively, even if that volume is totally opaque to light.
- 3D/Volume ability allows cubic arrays or stack slide systems within seconds.
- MRI can spatially resolve the signal in 1-, 2- or 3-dimensions, without scanning motion.
- NMR does *not necessarily* require a label.
- Contrast agents such as SPIOs are FDA approved, inexpensive, readily available and offer multiple binding sites for enhanced labelling.
- The sensitivity can be 'tuned' with outstanding flexibility,
- The same array can be interrogated with different NMR fluids
- MRI could be combined simultaneously with fluorescence.