Biosensori bazîndu-se pe Magneto-resistența

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TFM

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Overview

- Bio-Assay Technology
- Magnetic Bio-Assay Technology
- Tunnelling Magneto-resistance sensor
- Magnetotactic Bacteria
- Targeting by MRI
- Conclusions

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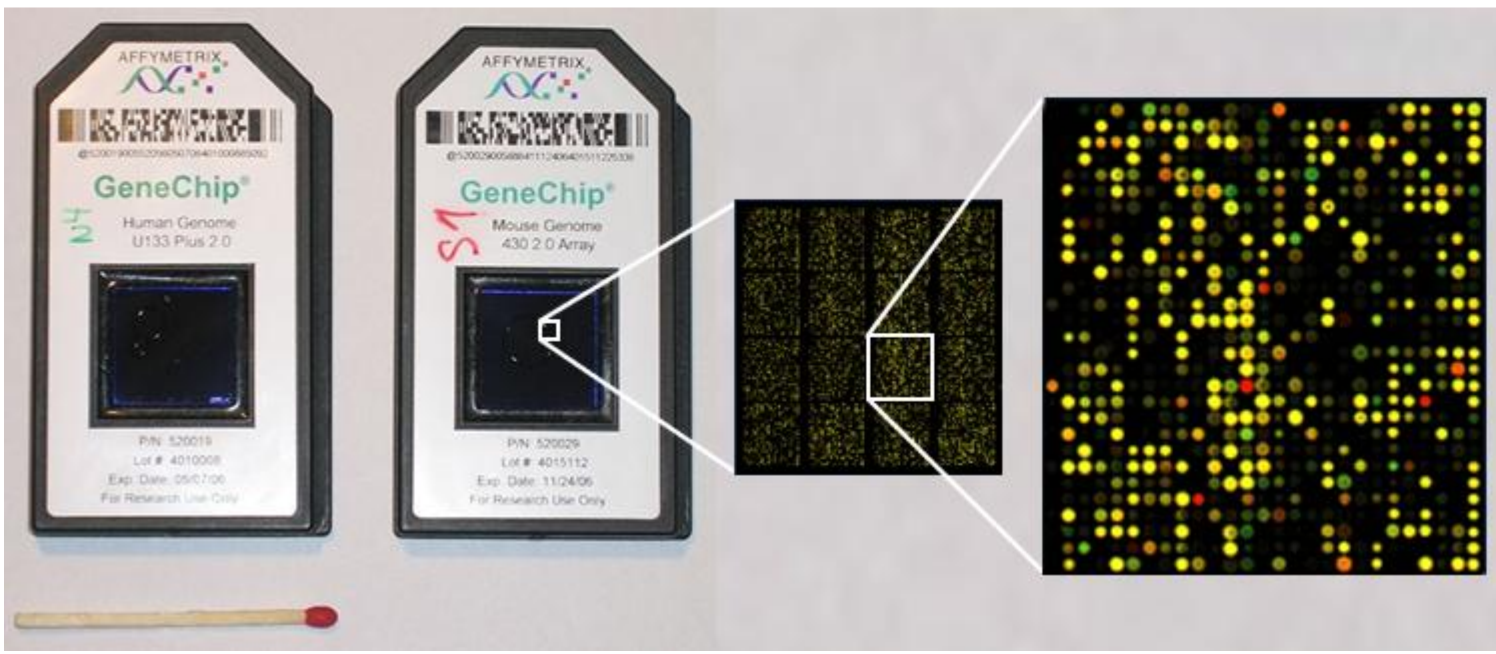
Microarrays

- Molecular probes immobilised on small spots on a substrate. Position is used to distinguish different probes.
- Spatially resolved fluorescence is used to detect hybridisation -> bulky and expensive optics needed.
- The GeneChip (Affymetrix) is used to search for millions of targets at once. But, arrays are expensive to customise and can not be modified post production.
- 2D kinetics limits sensitivity and increases run-time for multiplexed assays.



J. Llandro, J.J. Palfreyman , **A. Ionescu** and C.H.W. Barnes, Invited: "*Magnetic biosensor technologies for medical applications: a review*", in *Med. and Bio. Eng. and Comp.* (Springer, 2010) Doi:10.1007/s11517-010-0643-3.

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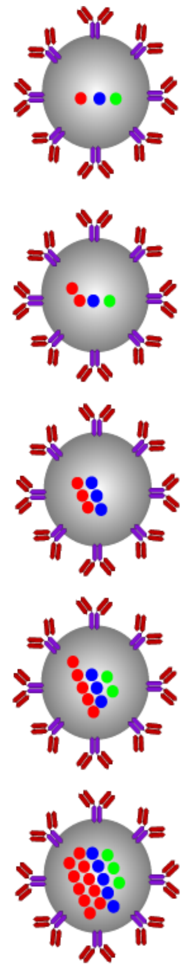
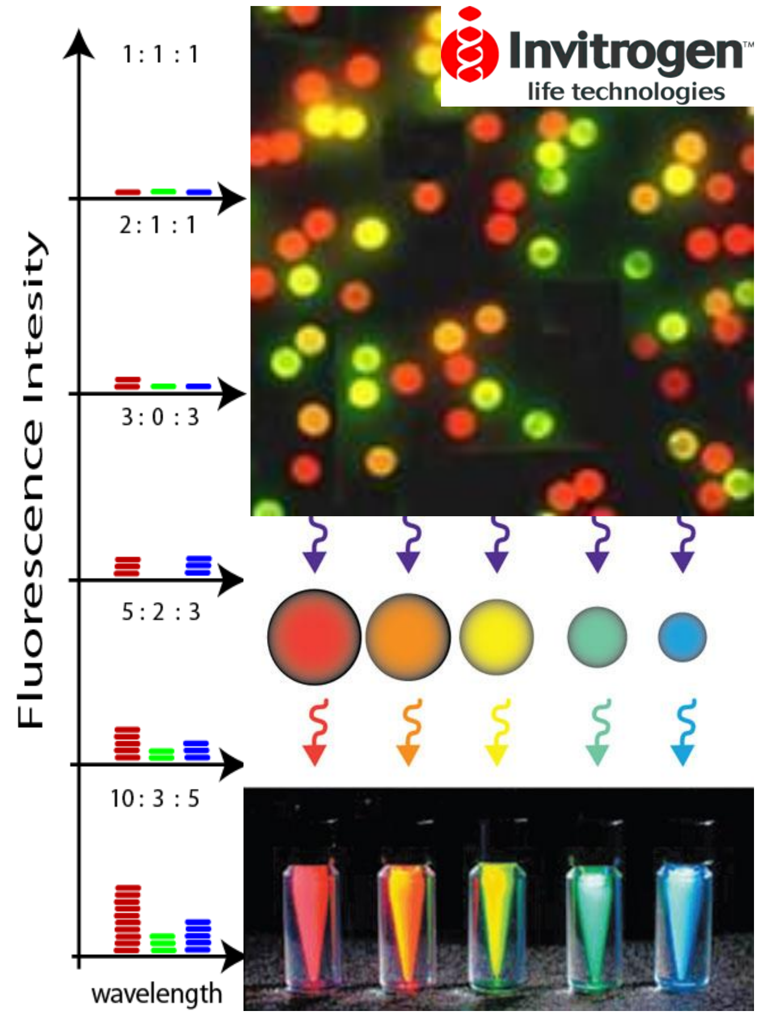
Suspension Assay Technology

- Molecular probes are attached to microcarriers which mix in a solution. 3D-Kinetics increases sensitivity and decreases run-time.
- For multiplexing, each particle is labelled in to uniquely identify the probe.
- Most encoding systems are graphical or fluorescent bead based, *e.g.* xMAP (Luminex) and QuantumDot (Invitrogen).
- SAT's are limited by the number of codes generated and expensive optics to read them.



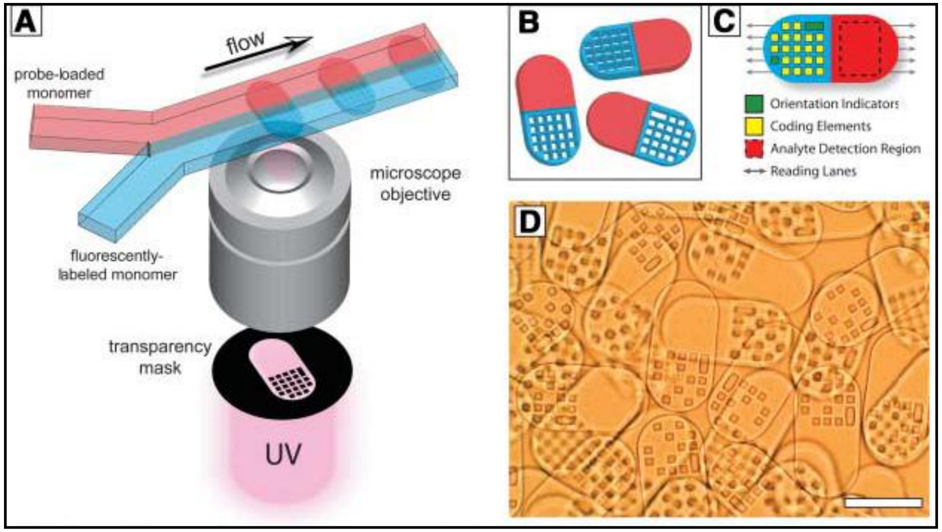


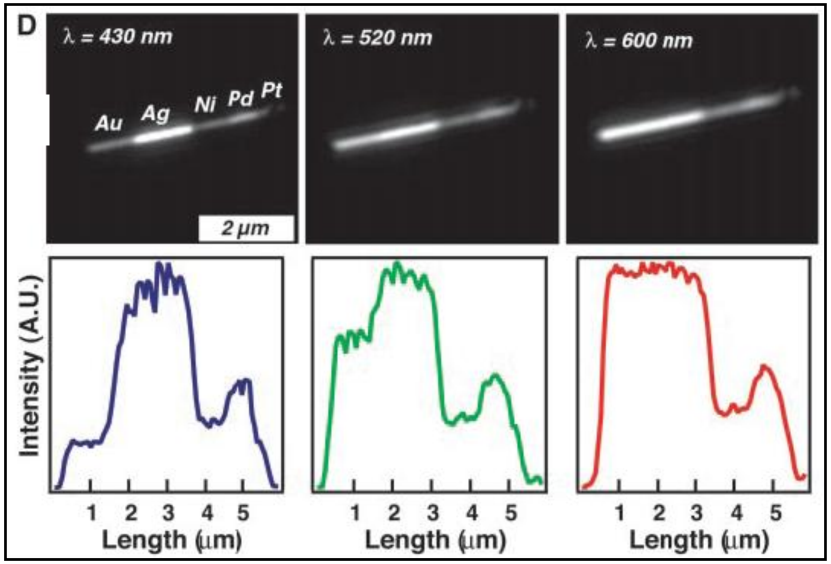
"Quantum-dot-tagged microbeads for multiplexed optical coding of biomolecules", M. Han, X. Gao, J. Z. Su and S. Nie, *Nature Biotechnology* **19,** 631 (2001).

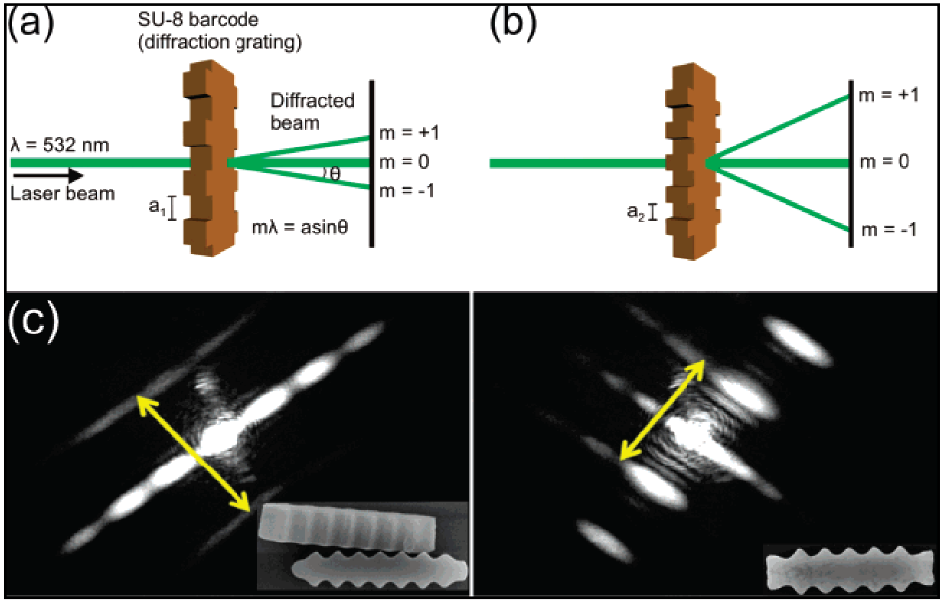
Multiplexing Technologies

Encoded Particles – Pregibon *et al.*, *Science* **315**, 1393 (2007).

Nanowires – Nicewarner-Peña *et al.*, *Science* **294**, 137 (2001).







Diffraction Gratings – Broder *et al., Anal. Chem.* **80** (6), 1902 (2008).

Optical or Magnetic

Optical labelling

- Reading the labels requires frequency or spatial resolution and so bulky and expensive optics are often necessary.
- Microcarriers are made with the labels pre-written.
- Multiplexing can be expensive due to the need for parallel fabrication.
- Number of labels available for fluorescence based techniques is limited due to the overlap of emission spectra.
- Autofluorescence in the sample can increase signal-to-noise ratio.

Magnetic labelling

- Codes may be read using integrated GMR/TMR devices, similar to those used in hard drives.
- The code can be written and rewritten using a magnetic field.
- All the microcarriers can be manufactured identically using MEMS techniques reducing costs.
- Scalable: number of available labels increases exponentially with each element in a digital architecture, *i.e.* each additional bit doubles the coding capacity.
- Biological samples have a very low magnetic background.

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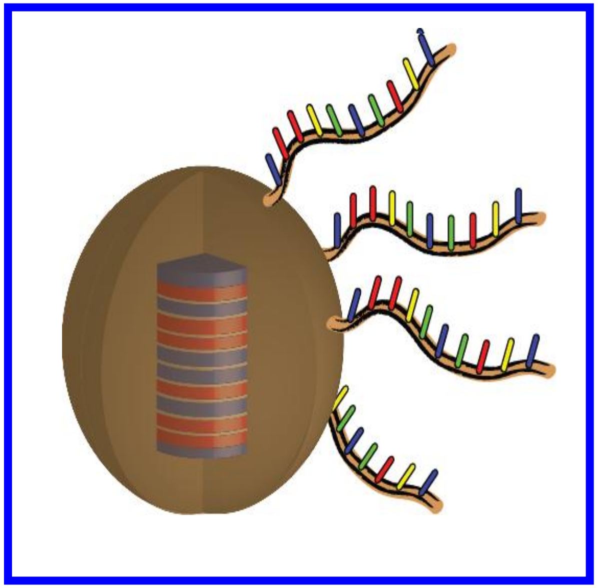
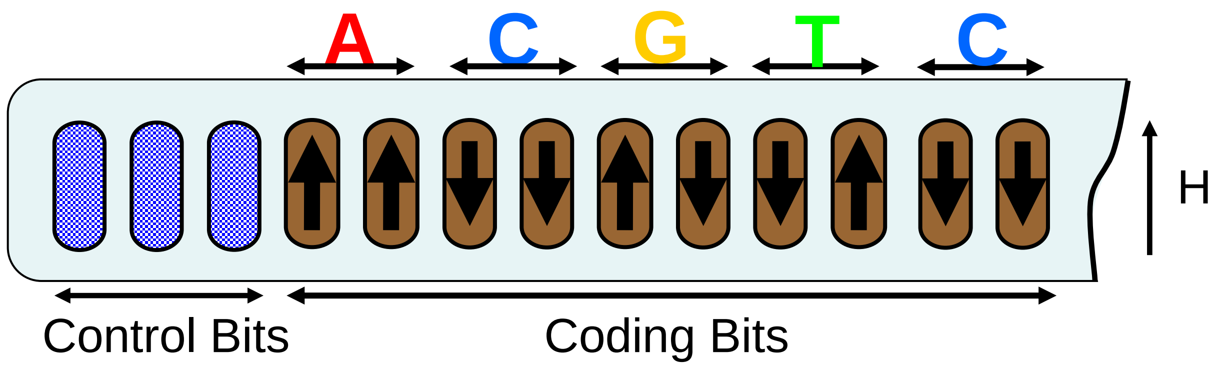
Magnetic Barcodes

How can magnetic tagging offer multiplexing potential?



Each magnetic element is magnetized in either of two stable states representing one bit of information – 2 bits code for each base.



...a linear increase in size offers an exponential increase in codes!

20 bits > 1 million
30 bits > 1 billion!

Tag Design & Fabrication

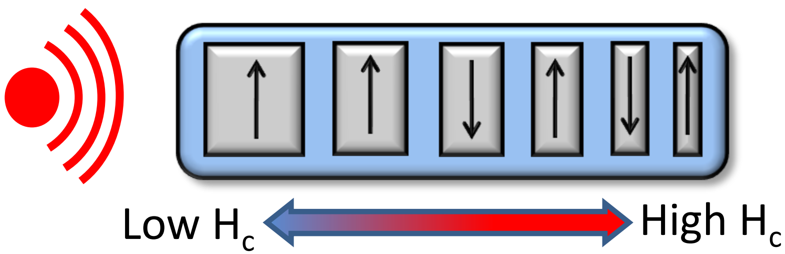
Magnetic Encoding

There are 4 key challenges

Biofunctionalisation

Detection & microfluidics

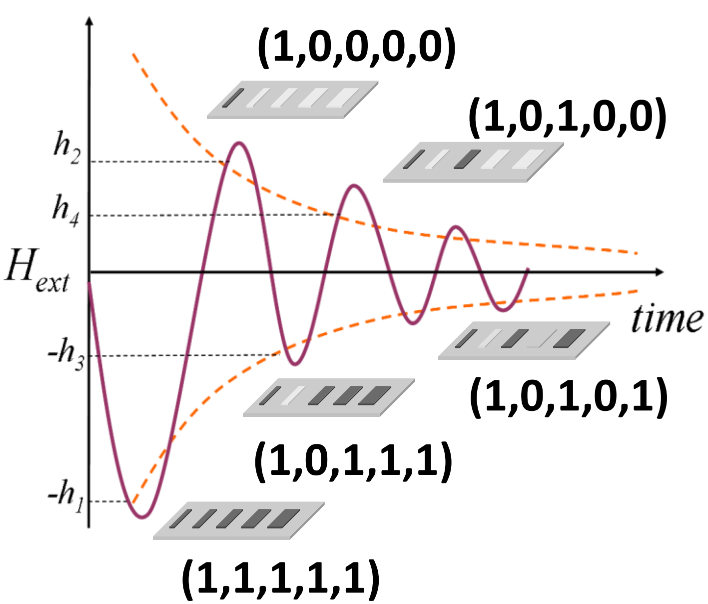
Writing the code

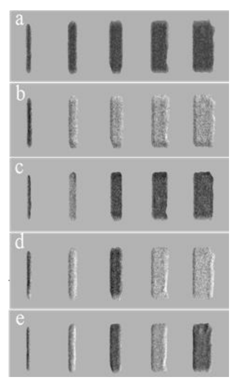


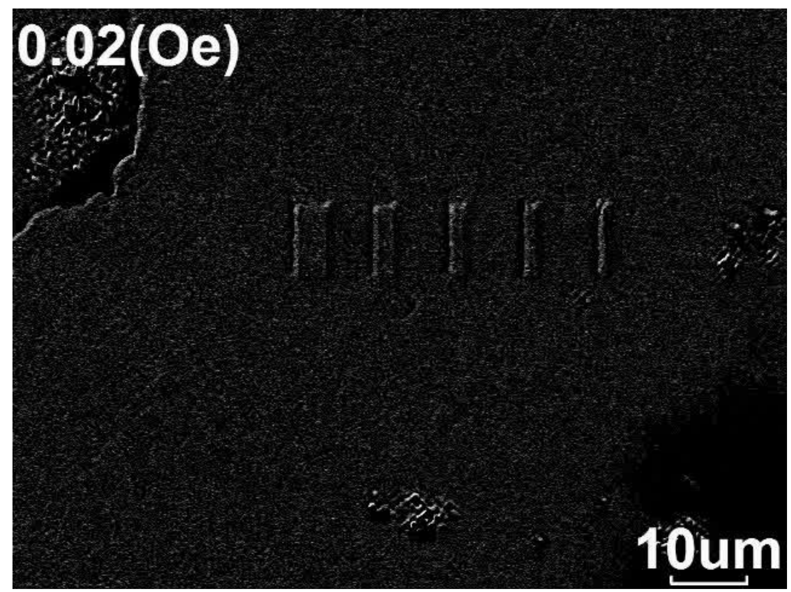
Tags with elements that switch at different field strengths can be written with a 'global' field.

- Enables to write a code on a large number of tags at any one time.
- However, the coercivity of magnetic elements must change for varying their switching fields.
- Shape anisotropy (different aspect ratio) can be used to tune the coercivity.

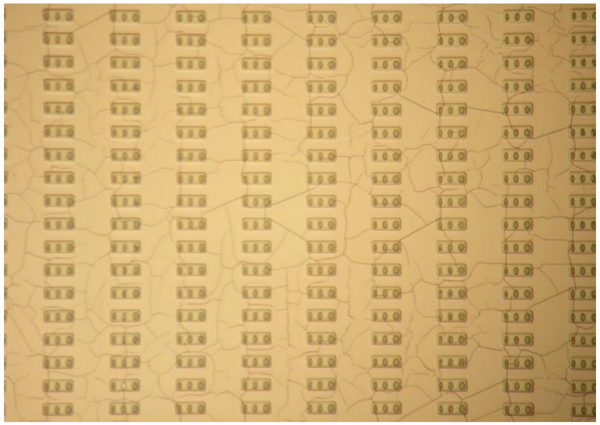
All unique codes can be written using a global field that varies in amplitude.

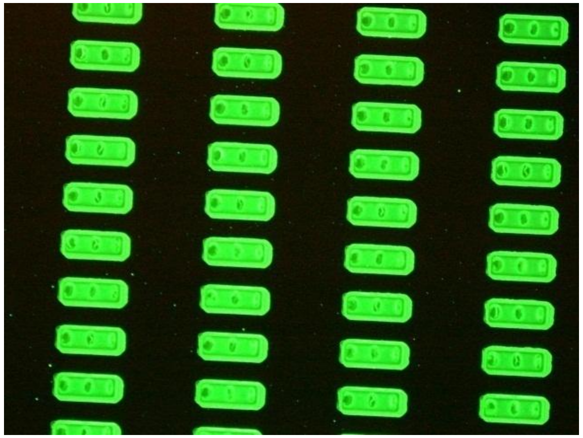




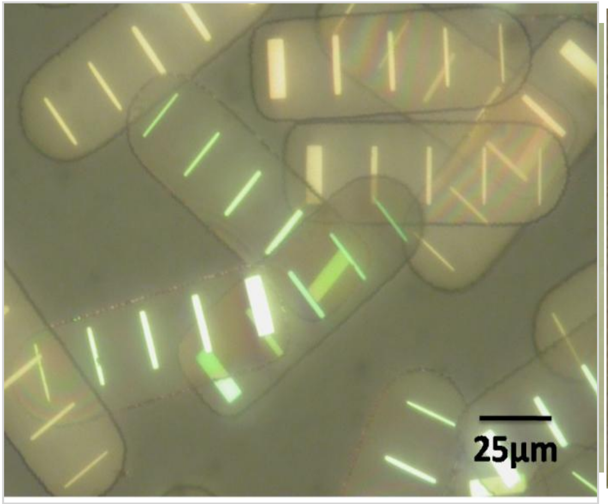


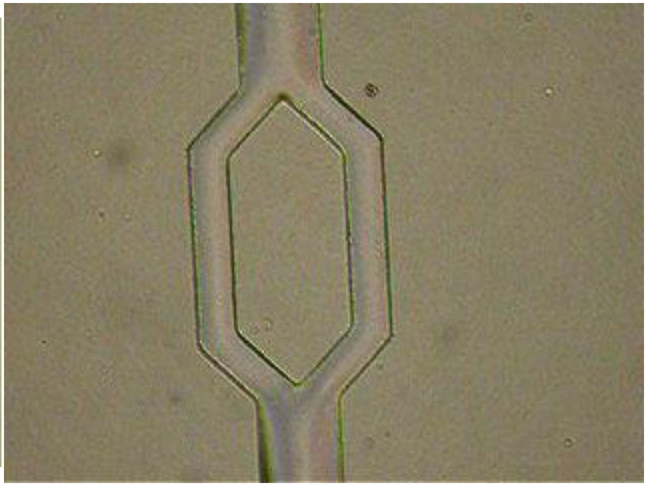
1st Generation Tags





The tags can be released from the substrate and biochemically functionalised…

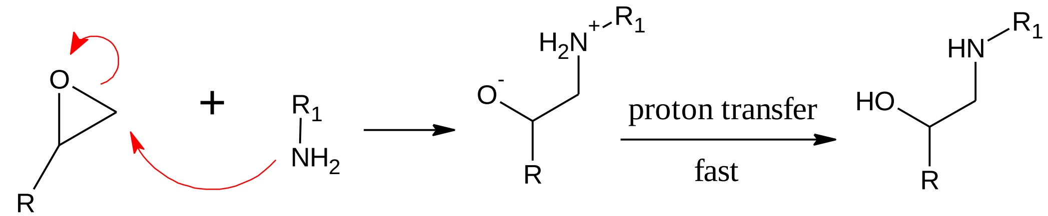


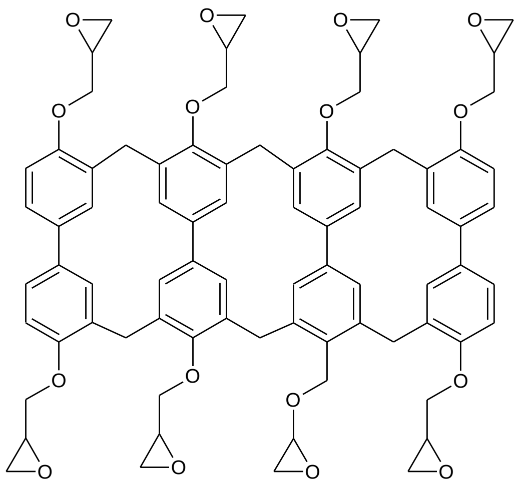


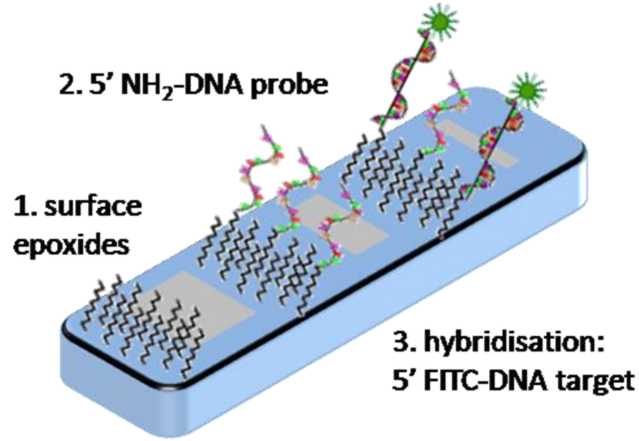
…and controllably flown (and sorted) within microfluidic channels over buried sensors.

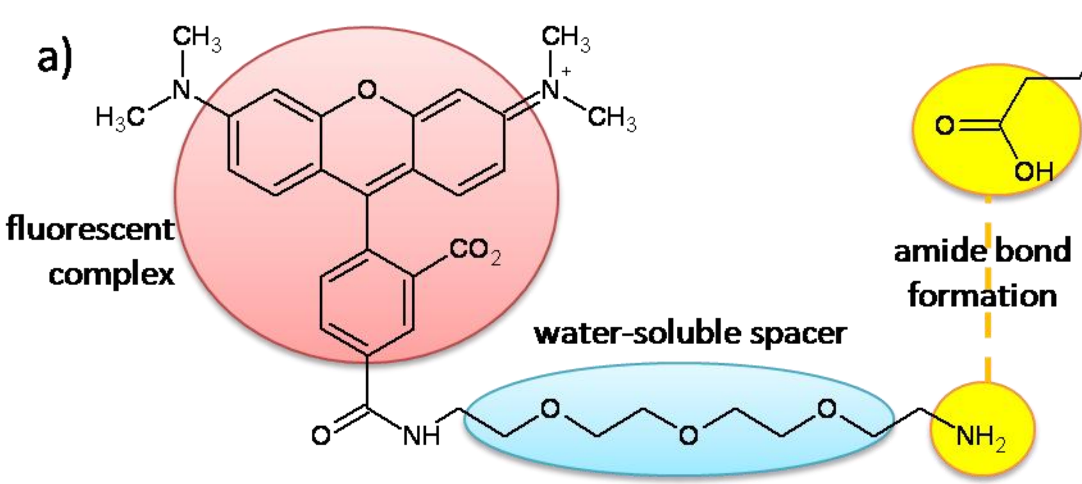
SU-8 Surface Chemistry

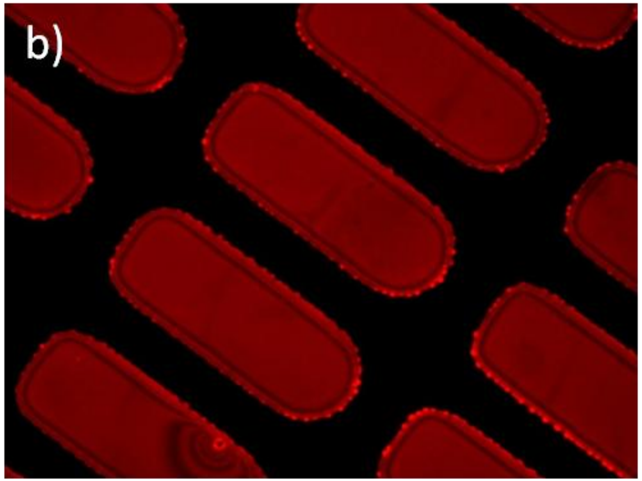
Simple epoxide ring opening under nucleophilic attack:





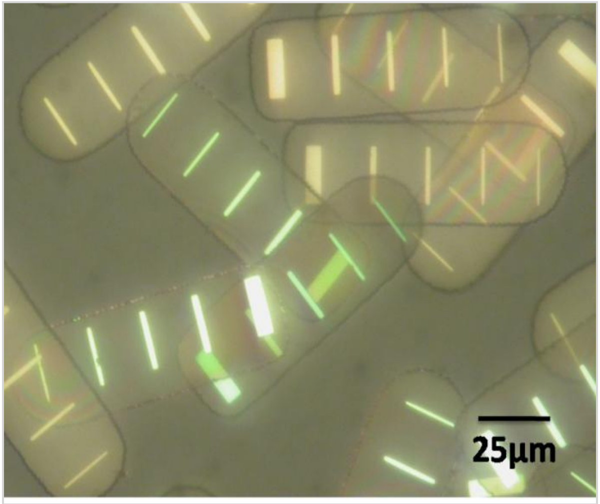






2nd Generation Tags

Better fluorescent marker (TAMRA vs FITC)



Simpler fabrication procedure:

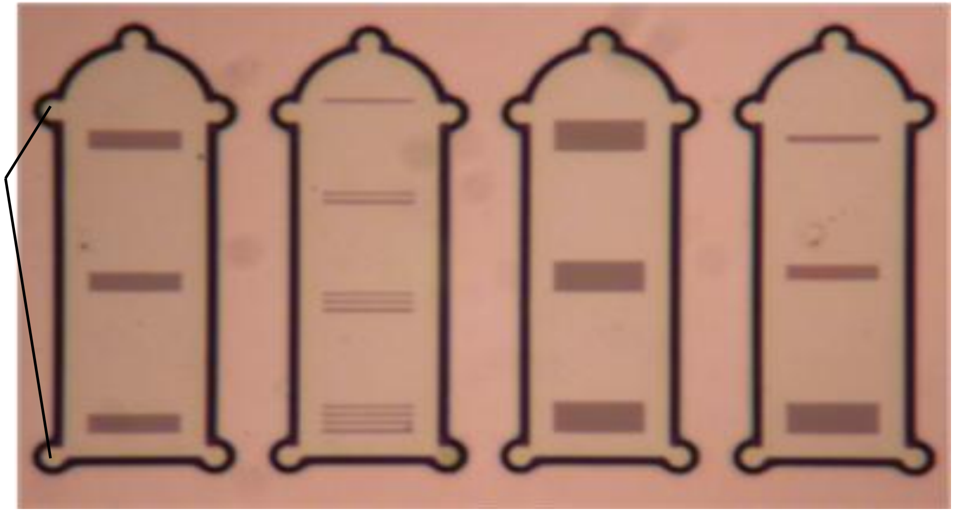
- Release layer is optional
- Only 1 alignment step (previously 2)

New chemistry (harder but more interesting):

- Novel SU8 etchant
- Chemical release from silicon wafer
- Spacer molecules

Microfluidic control:
optical trapping via “mickey mouse” ears

Dimensions: 100μm x 30μm x 3μm



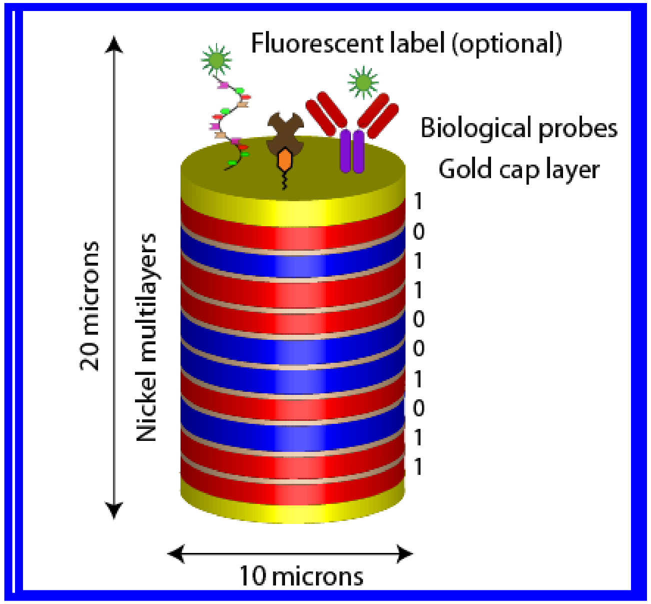
Multilayers

Micro-magnetic simulations suggest that a 200 μm long planar tag can currently accommodate 13 bits ⇒ 8000 codes (great for SNPs/gene expression)

Vacuum chambers and multiple photolithography steps add to the fabrication costs cheaper methods would allow for mass production of billions of tags

3D Electrodeposited multilayer pillars offers an answer to all three areas:

- More Coding Capacity
- More Compact
- More Cost Effective



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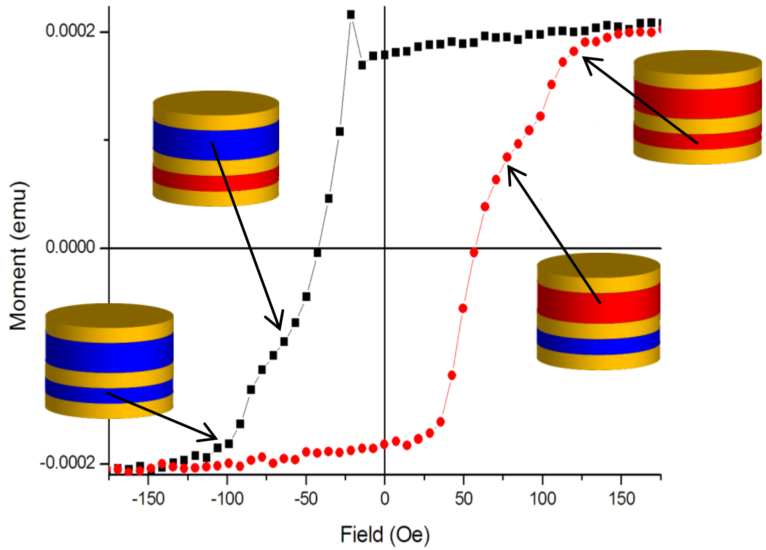
Multi-coercivity



❖ Height variation: thicknesses from 5nm to several μm can be grown by controlling the cut-off charge (electrodeposited).

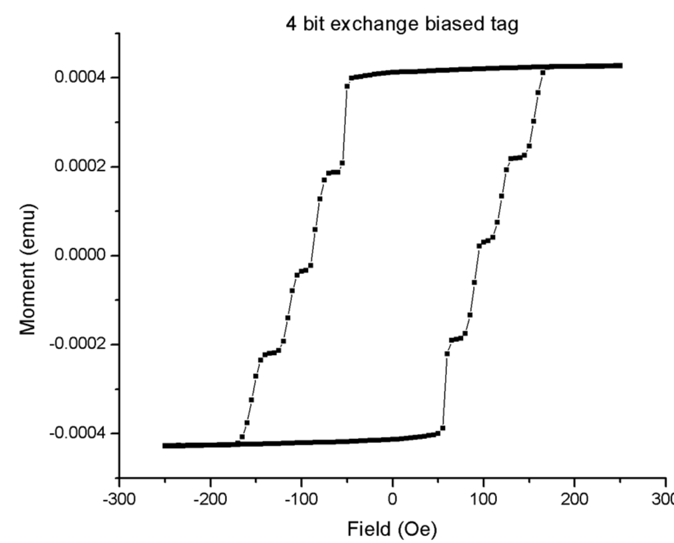
SQUID magnetometry of 2-bit multi-coercivity tags with structure:

Cu(50)/Co(50)/Cu(50)/Co(25)/Cu(25)

❖ Exchange-bias: magnetic elements are coupled to anti-ferromagnetic layers, thus changing the switching field.

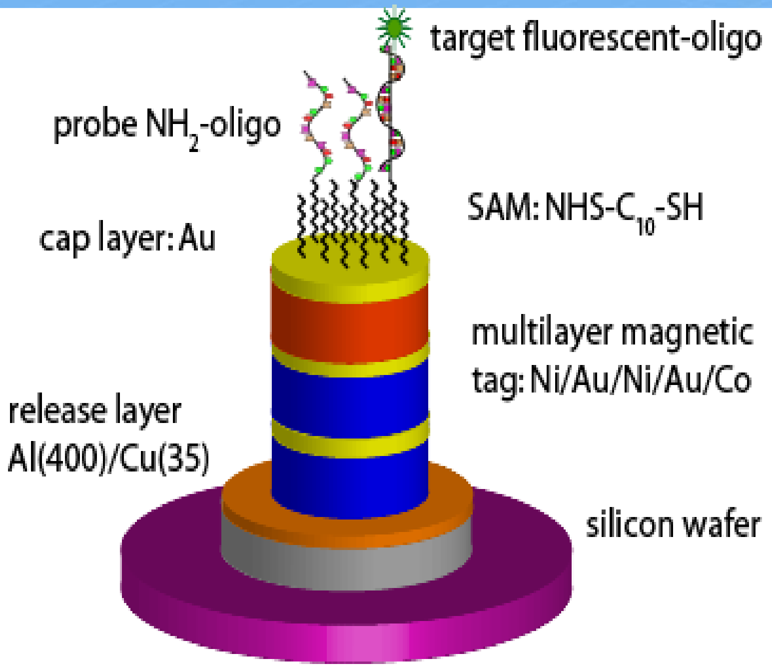
4-bit sputtered film with layers:

[Co(3)/PdMn(x)/Ta(5)]$_4$ where x = 13, 11, 9 and 7 nm from bottom to top of the loop.

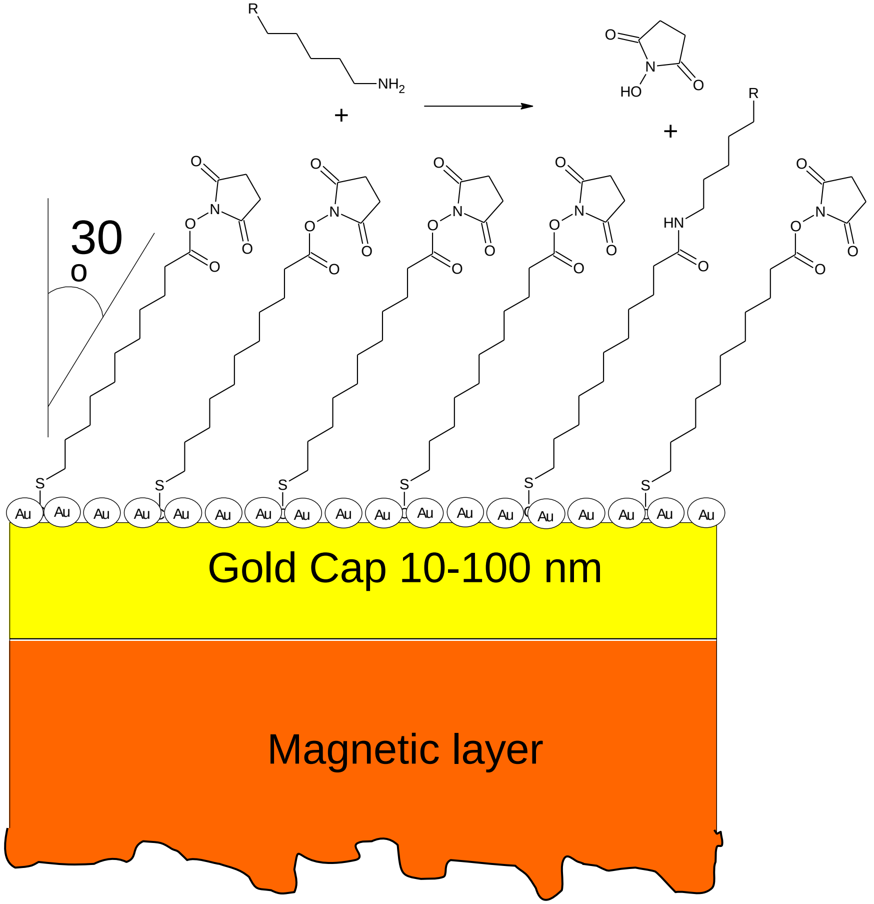


M. Barbagallo, F. van Belle, **A. Ionescu** and J.A.C. Bland, in *"Biomagnetism and Magnetic Biosystems based on Molecular recognition Processes"*, ed. **A. Ionescu** and J.A.C. Bland, *AIP Conf. Proc.* **1025**, 52 (2008) .

Chemistry on Metal



- A (di-)thiol based Self-Assembled Monolayer with a functional head group can be grown on the gold cap layer, enabling further chemistry...



- Spacer molecules or DNA modified with a nucleophilic group can bind covalently to the magnetic tag.

- The release layer can be dissolved to allow suspension based hybridisation and detection/sorting/reading steps.

DNA Hybridisation

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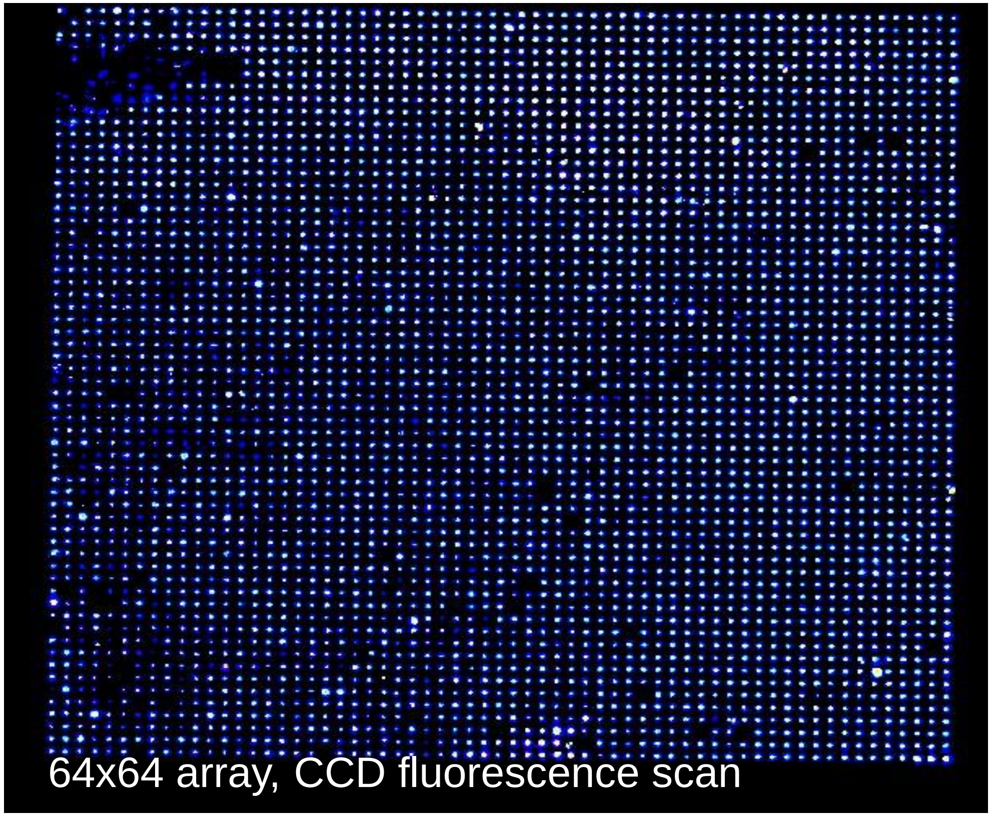
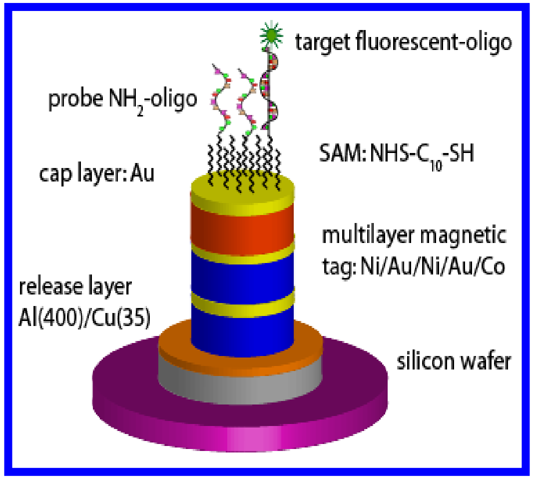
➢ Array of 15 µm diameter pillars hybridised to fluorescently-labelled DNA ...



...proof that all 7 steps are working:

- ✓ Release layer(s) evaporated
- ✓ Photolithographically patterned
- ✓ Multilayer pillars electrodeposited
- ✓ Gold cap layer electrodeposited
- ✓ SAM grown
- ✓ amino-DNA probe added
- ✓ hybridised to complementary fluorescent DNA.



However, this is just one DNA probe – how can we efficiently generate a large library?

J.J. Palfreyman, F. van Belle, J.A.C. Bland, M. Bradley et al. *IEEE Trans. Mag*. **43**, 2439 (2007).

Split ‘n’ Mix Synthesis

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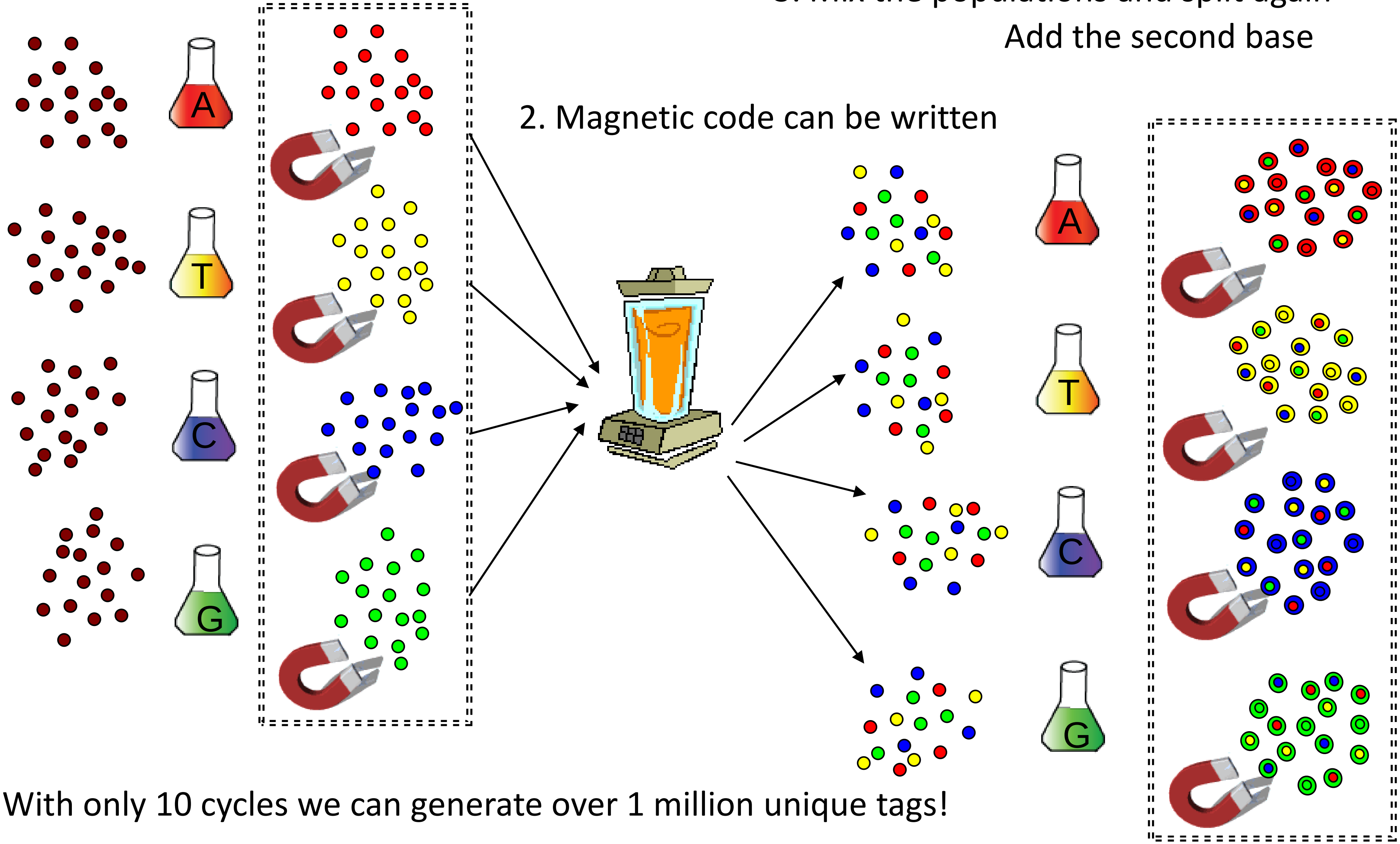
1. Split the tags into 4 populations
Add the first base

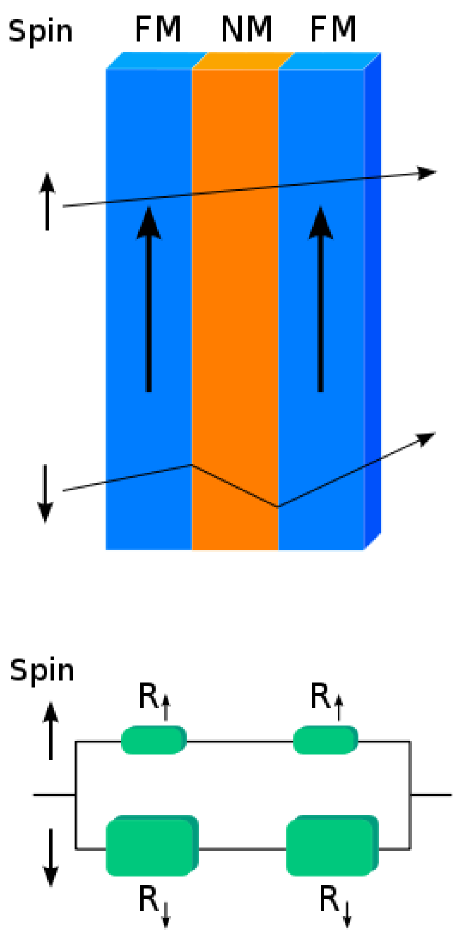
2. Magnetic code can be written

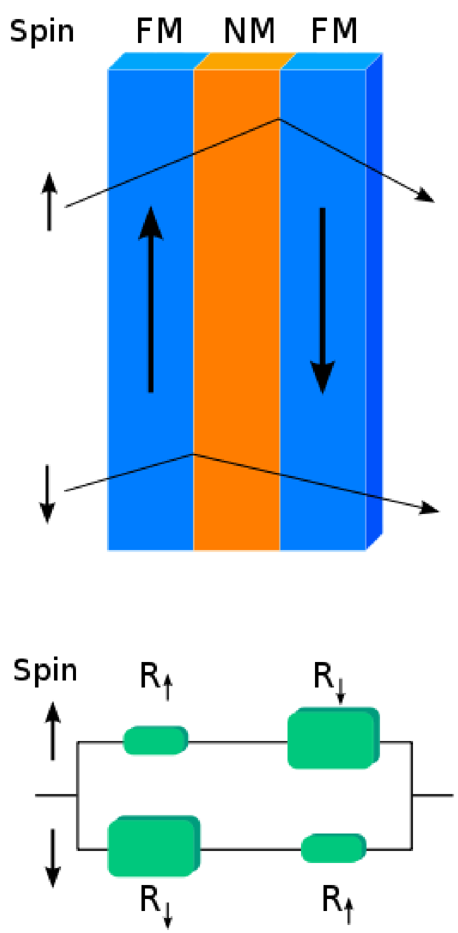
3. Mix the populations and split again
Add the second base



With only 10 cycles we can generate over 1 million unique tags!

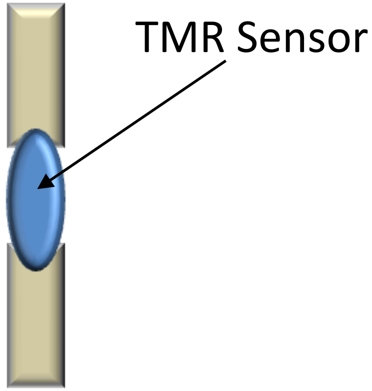
Giant/Tunnelling Magneto-resistance

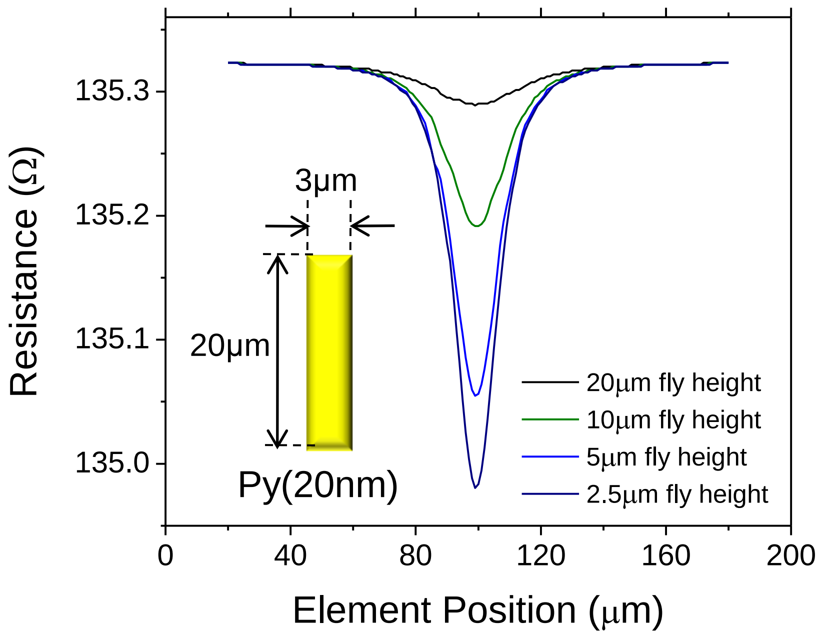


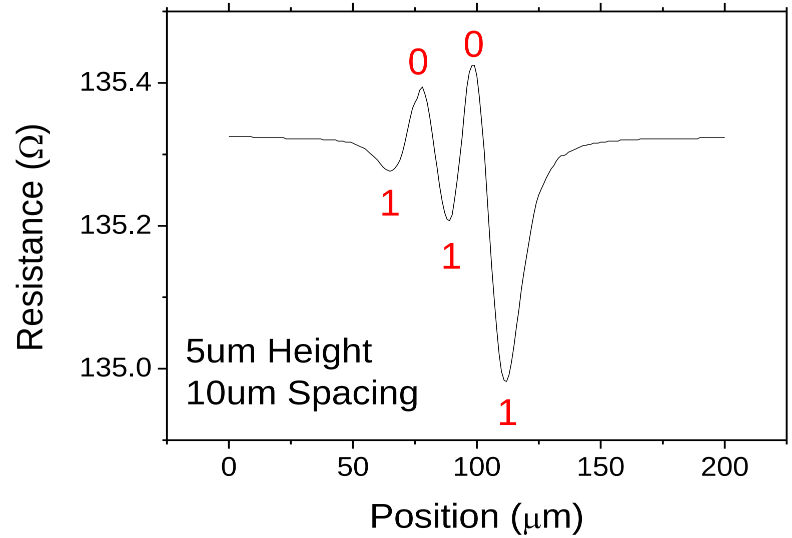


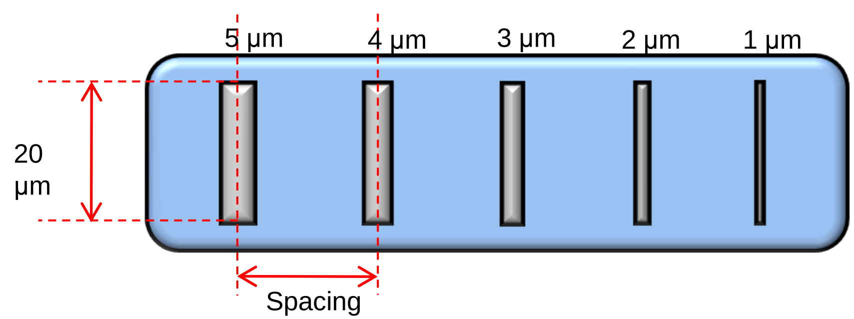
- GMR: 2007 Nobel Prize in Physics to Fert and Gruenberg
- "Spin-valve" formed of two ferromagnets (FM) separated by non-magnetic metal (NM) spacer
- TMR: Non-magnetic metal layer replaced with insulator
- Increased magnetic field sensitivity compared to GMR

Magnetic Detection



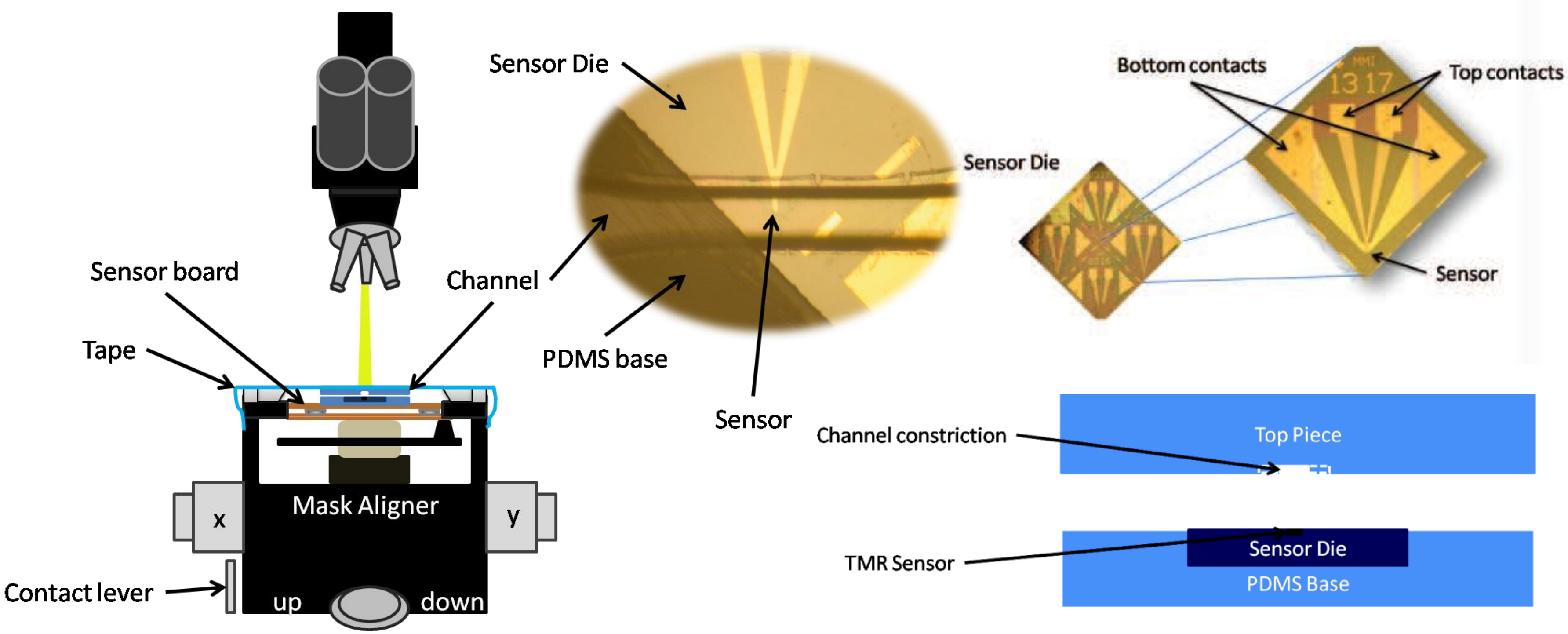






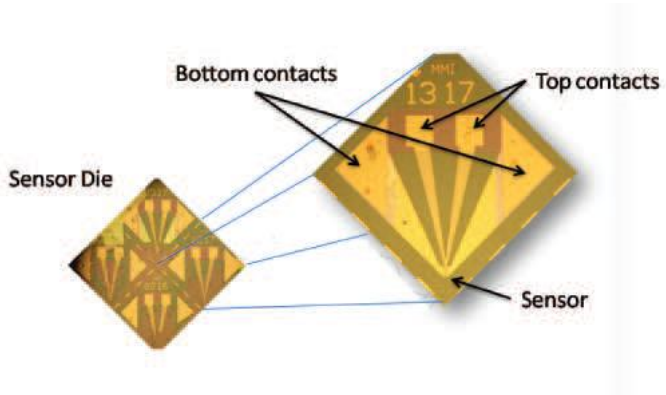
T.J. Hayward, J. Llandro, J.A.C. Bland, C.H.W. Barnes *et al.*, in *"Biomagnetism and Magnetic Biosystems based on Molecular recognition Processes"*, ed. **A. Ionescu** and J.A.C. Bland*, AIP Conference Proceedings* **1025**, 111 (2008).

Integrated TMR Sensor

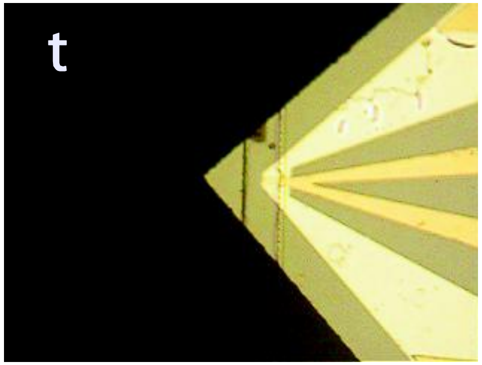


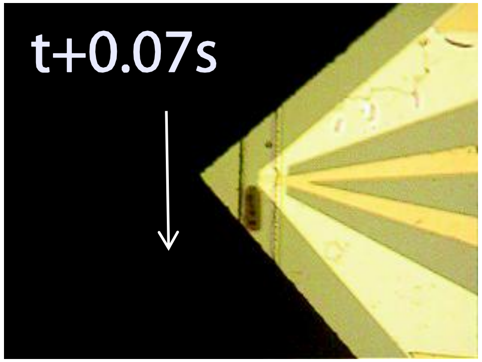
- ➢ TMR sensors (*Micromagnetics Inc.)* with an active area of 2x5µm.
- ➢ A dummy chip is used to mould a PDMS base with a cavity in which we can embed our TMR sensor chip.
- ➢ A PDMS channel is constructed using a lithographically defined SU-8 mould.
- ➢ After the sensor is wire bonded the channel is aligned accurately over the sensor using a customised mask aligner.

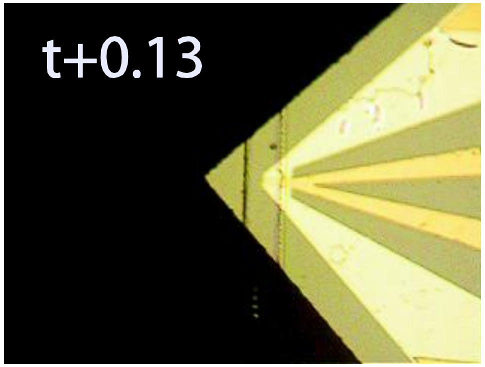
TMR Detection: Measurements

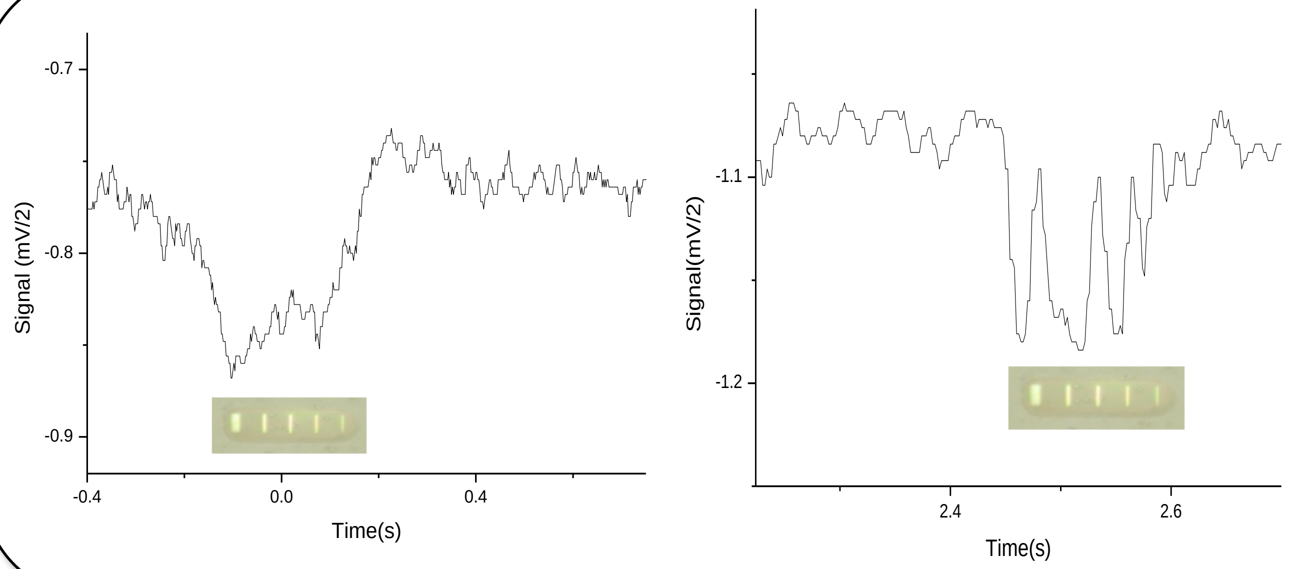


The response of 5-bit tags in the (1,1,1,1,1) orientation [hardest to distinguish] were recorded passing over a TMR sensor buried under a 50μm microfluidic channel.

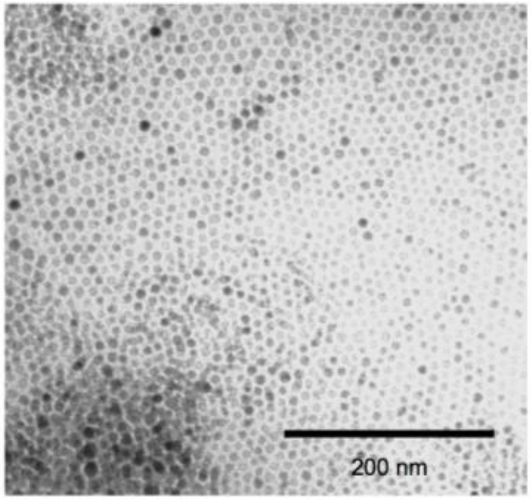




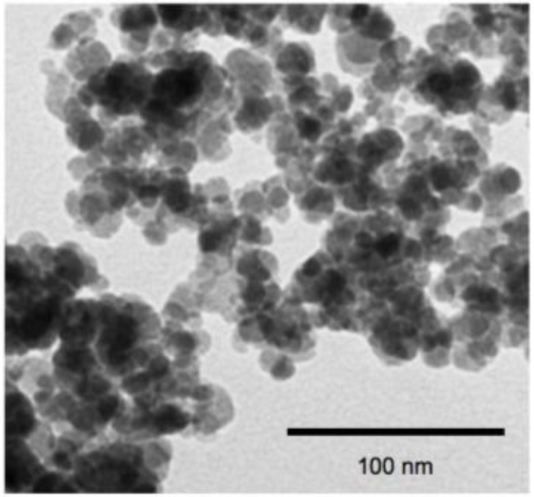




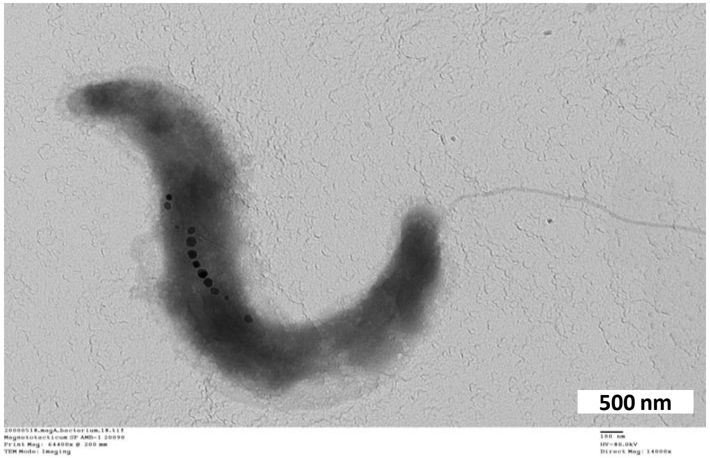
Magnetic Nanoparticles



Organic phase method



Aqueous precipitation method



Chemically synthesised magnetic nanoparticle inhomogeneity → Poor magnetic quantification

Magnetotactic bacteria produce magnetic nanoparticles → Better homogeneity?

N.J. Darton *et al.*, in *"Biomagnetism and Magnetic Biosystems based on Molecular recognition Processes"*, ed. **A. Ionescu** and J.A.C. Bland, *AIP Conference Proceedings* **1025**, 111 (2008).

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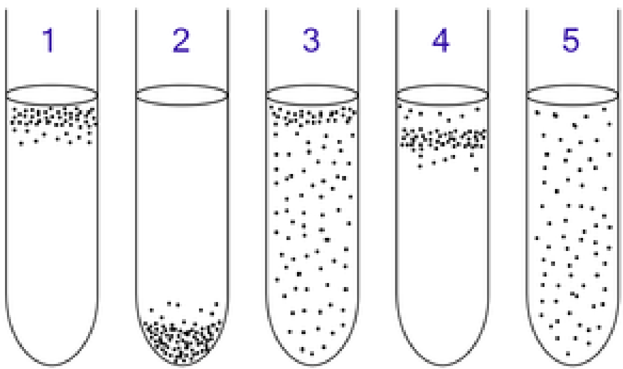
Culture of *Magnetospirillum sp.*



Magnetotactic bacteria found in the Cam!

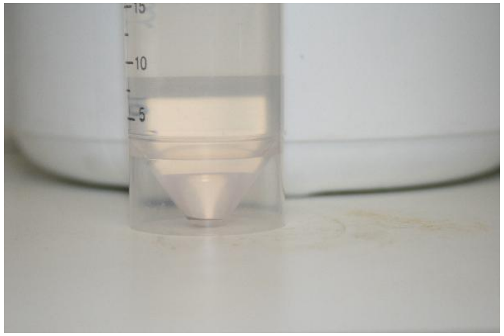
1: Obligate aerobe (oxygen-needing)

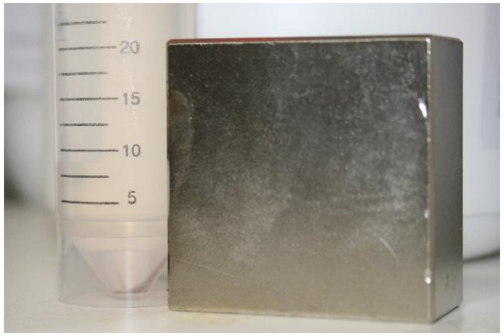
2: Obligate anaerobe (avoid oxygen)

3: Facultative bacteria (aerobic respiration preferred)

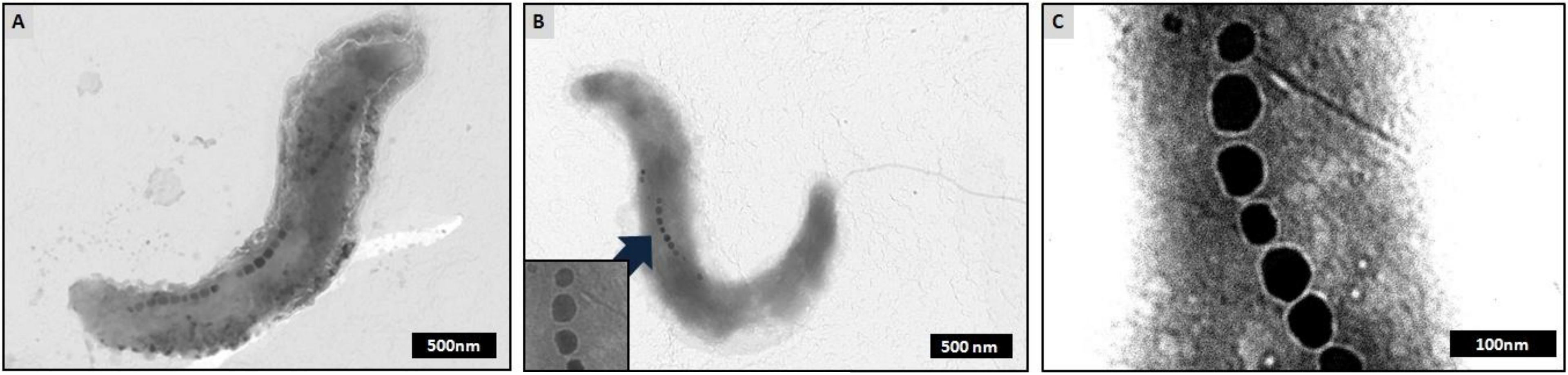
4: Microaerophiles (require oxygen at low concentration)

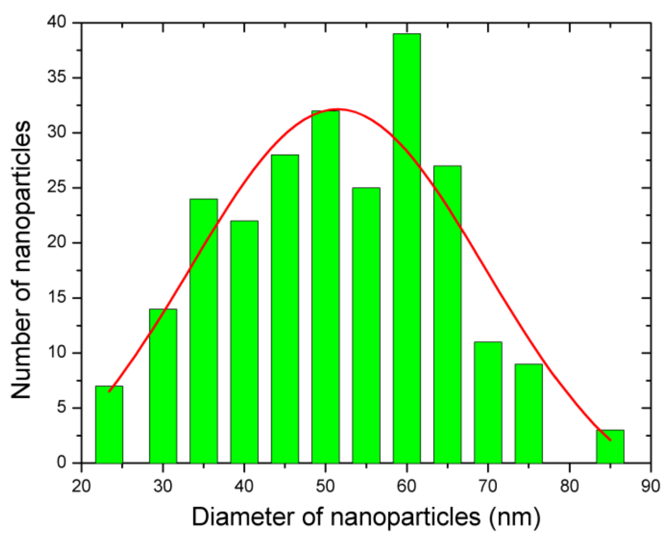
5: Aerotolerant bacteria (not affected by oxygen)





TEM of *Magnetospirillum sp.*

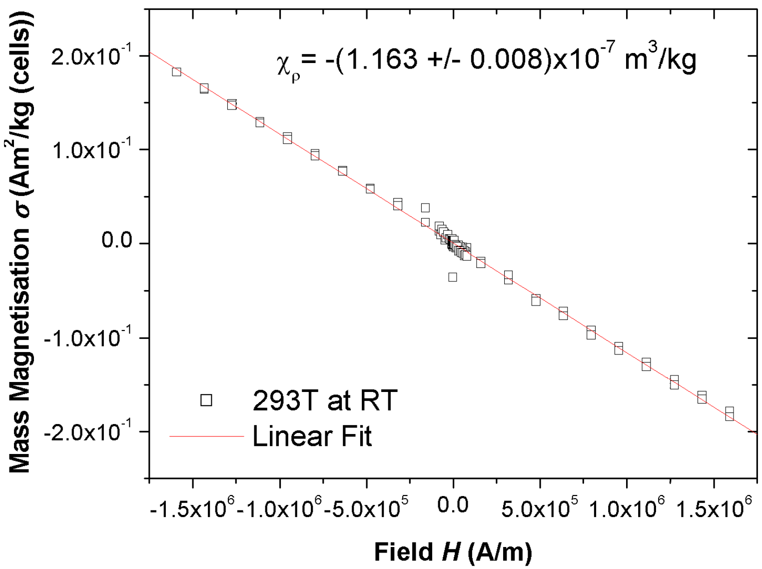


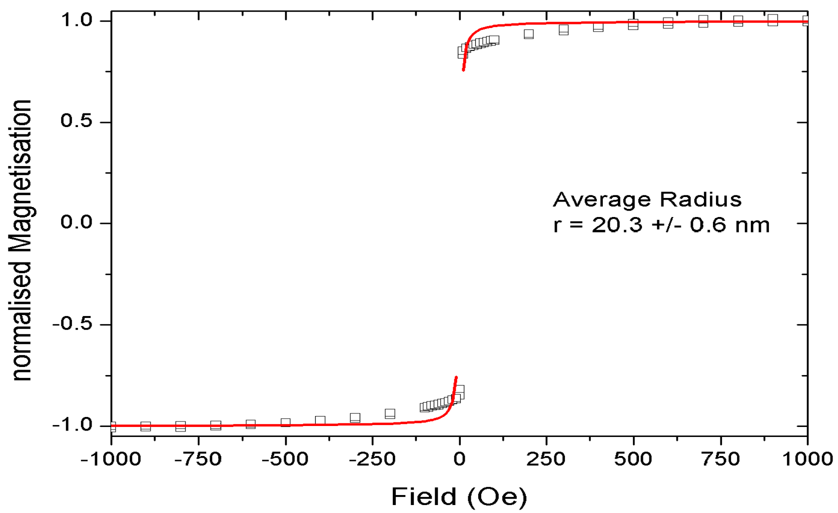


Mean nanoparticle size
from 237 measurements = 51 13 nm

A. Ionescu, N.J. Darton, J. Llandro and K. Vyas , Invited: *Philosophical Transactions of the Royal Society A* **368**, 4371 (2010).

SQUID Magnetometry

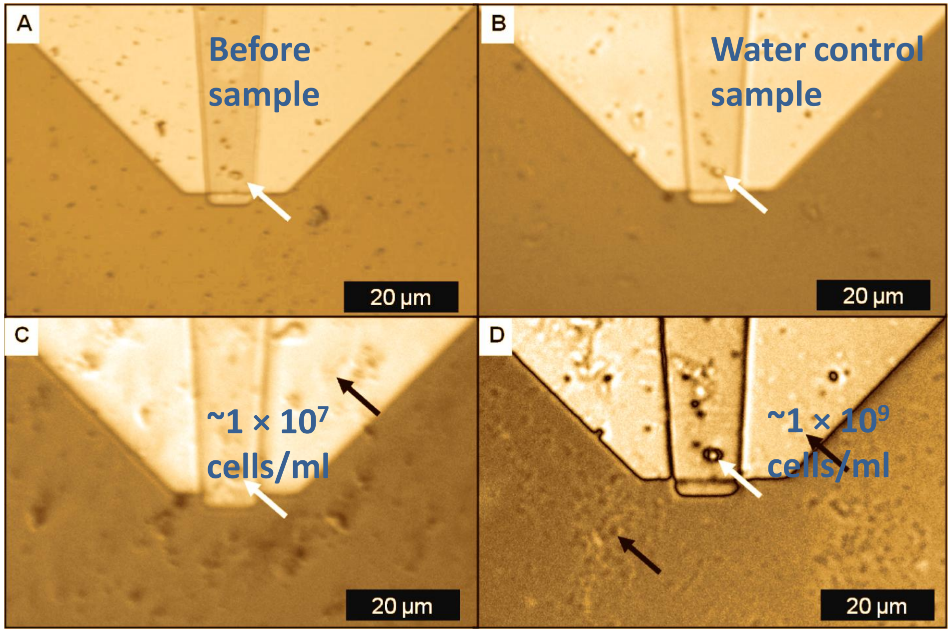


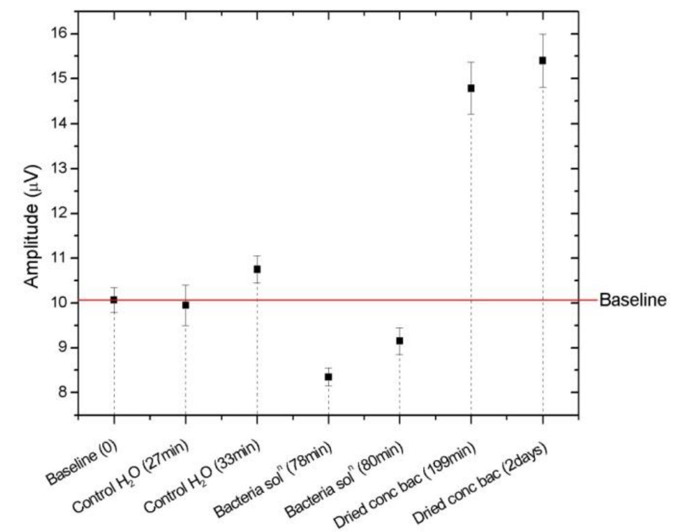


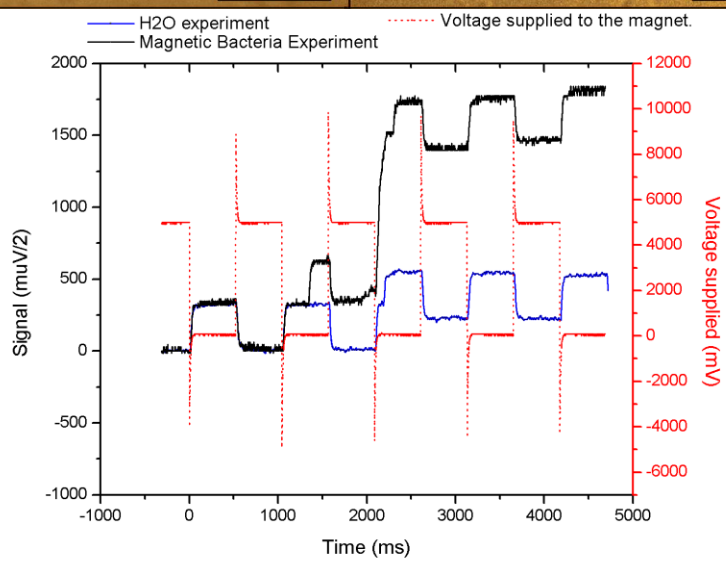
Langevin Equation:

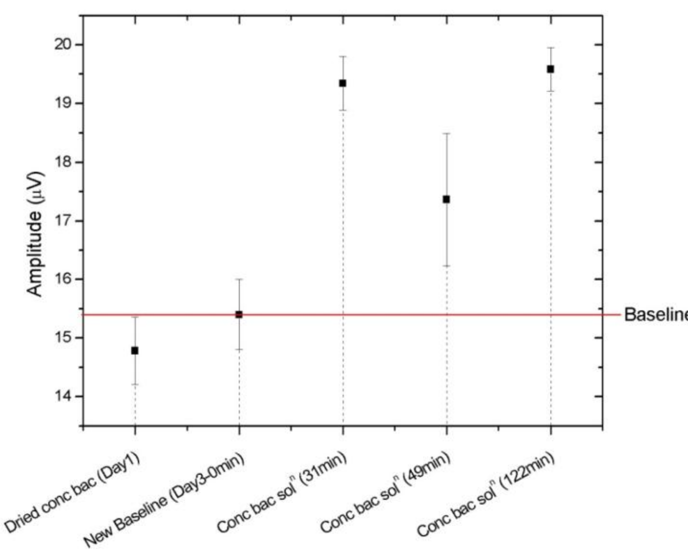
$$\sigma = N m \mu_B \left(\coth\left(\frac{m \mu_B H}{k_B T} \right) - \frac{k_B T}{m \mu_B H} \right) + \chi_p H$$

Static TMR Measurements

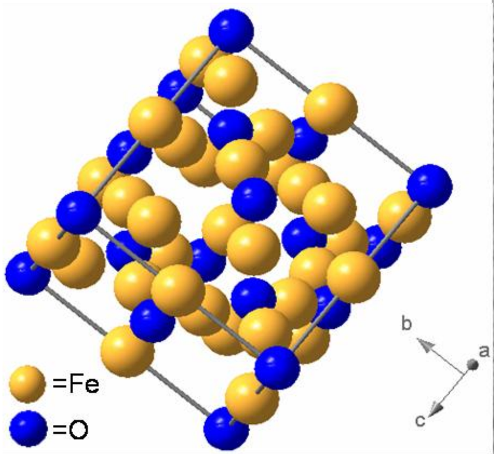


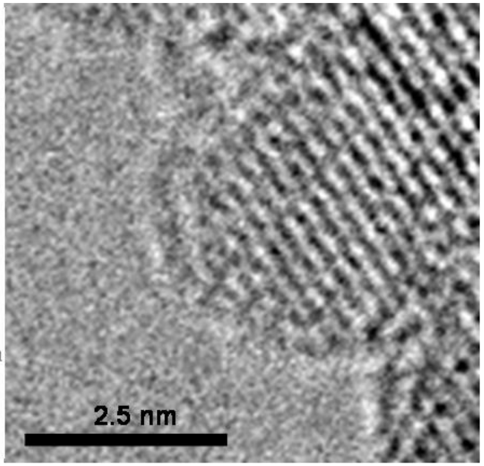






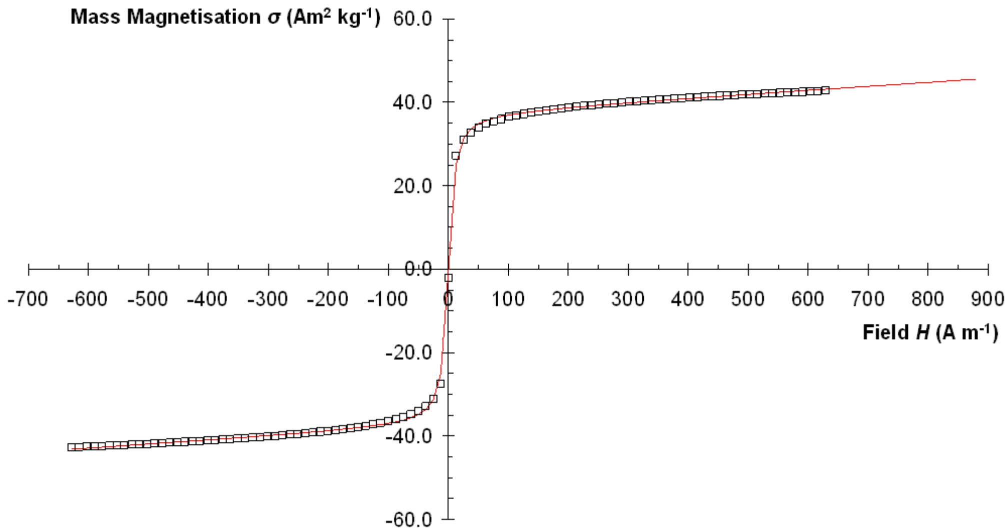
Manipulation & Tracking of SP Nanoparticles using MRI





Chemical Characterisation: High Resolution Transmission Electron Microscopy

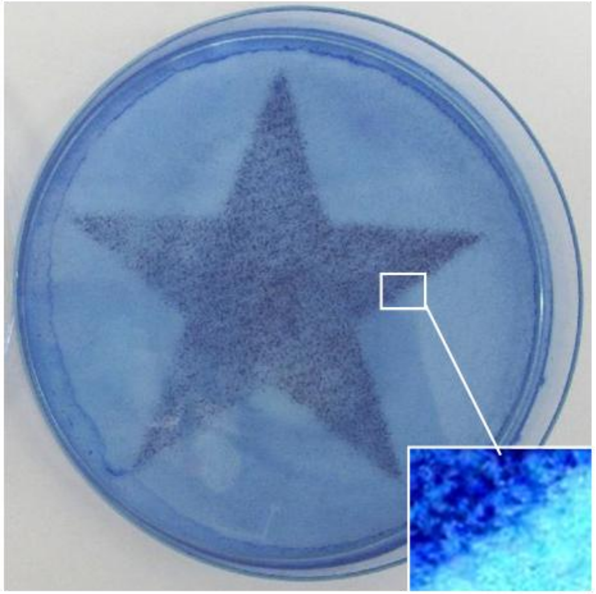
Magnetic Characterisation: SQUID measurement with Langevin Fit



N.J. Darton , R.D. Hallmark, **A. Ionescu** *et al.*, *Nanotechnology* **19**, 395102 (2008).

Targeting

Fridge magnet for targeting

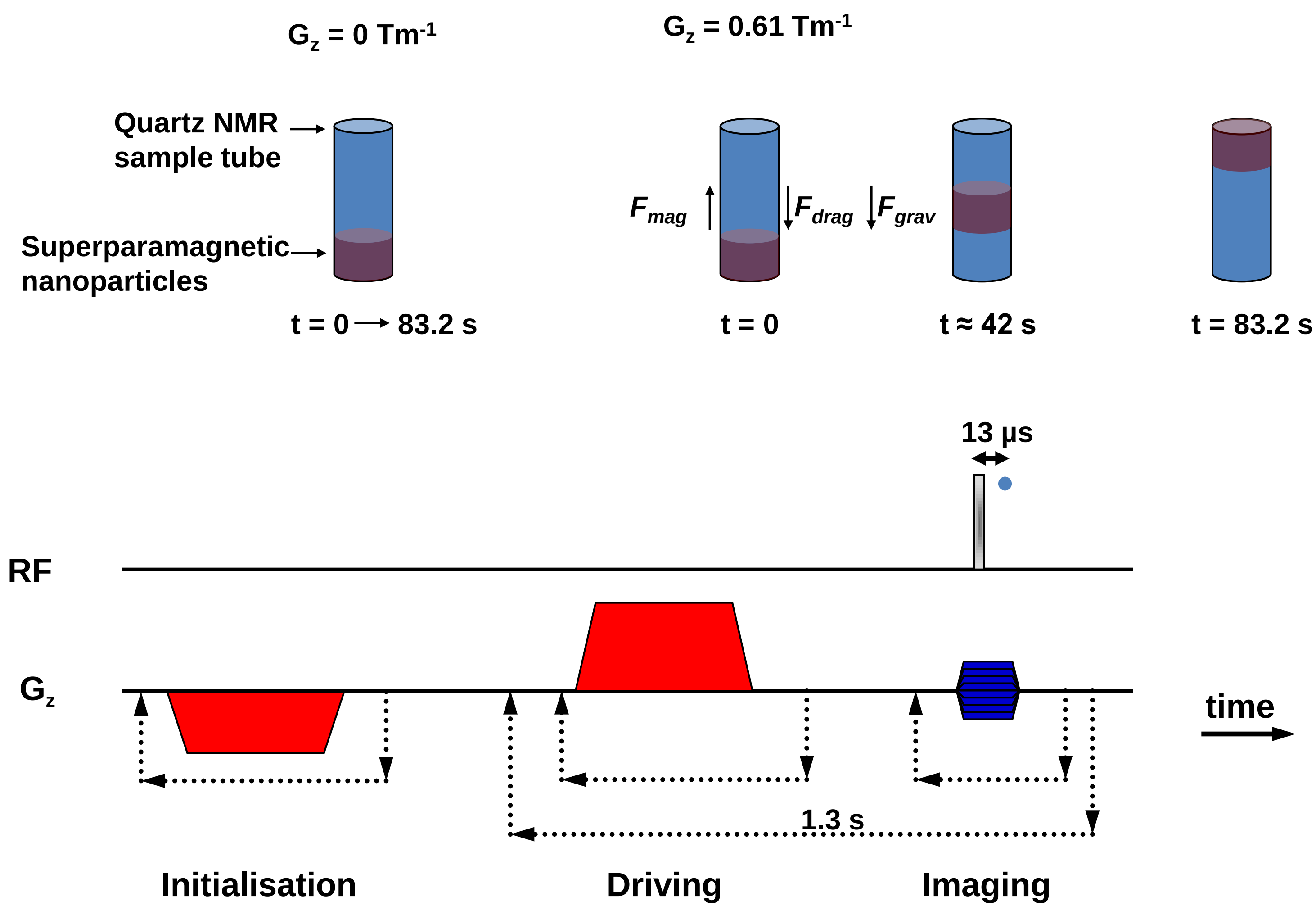


Targeted retroviral gene delivery

Targeted mammalian cell patterning

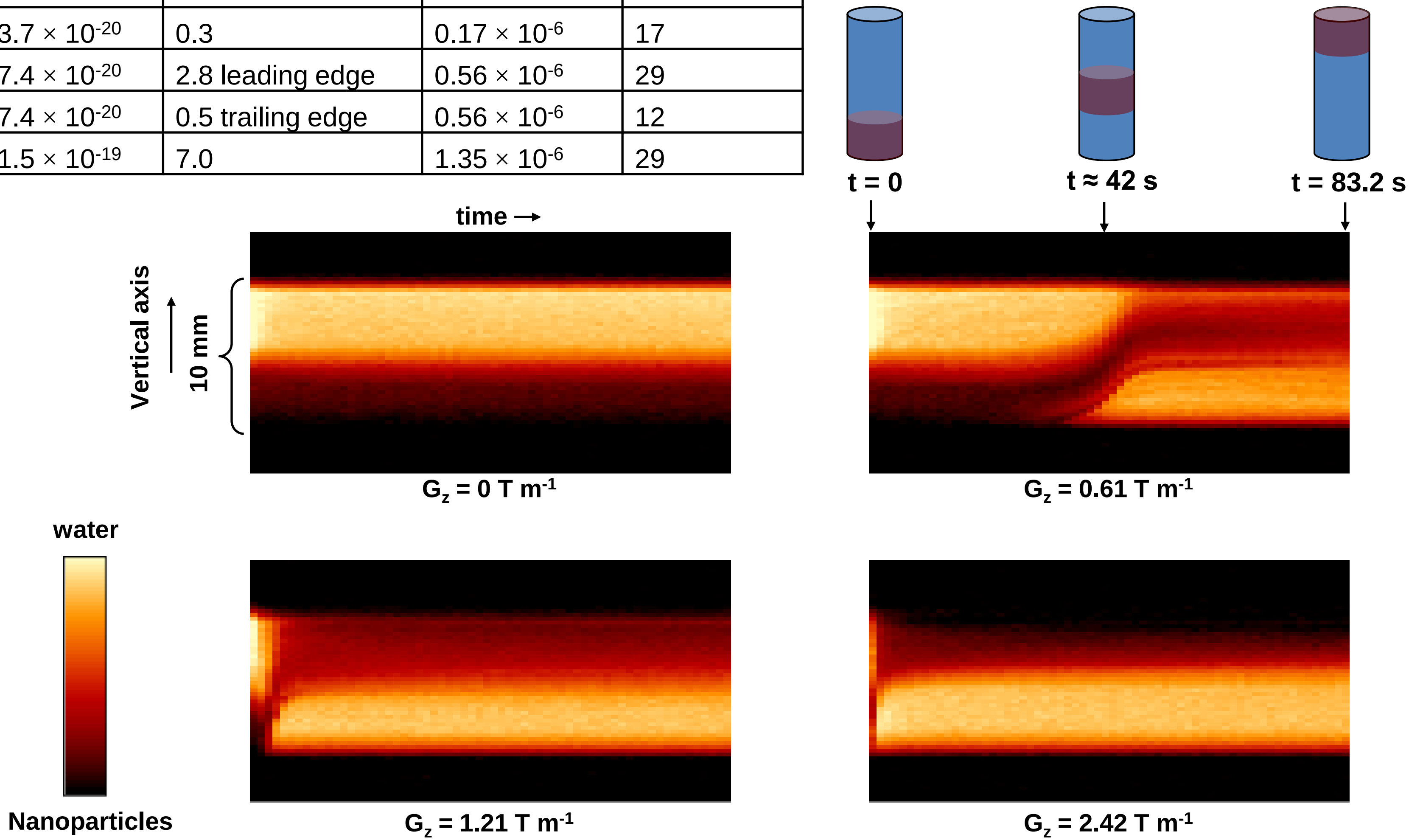
N.J. Darton *et al.,* in *"Biomagnetism and Magnetic Biosystems based on Molecular recognition Processes"*, ed. **A. Ionescu** and J.A.C. Bland*, AIP Conference Proceedings* **1025**, 111 (2008).

Targeting by MRI



Targeting by MRI

Magnetic field gradient G_z (T m^{-1})	Magnetic force F_{mag} (N)	Observed velocity (mm s^{-1})	Expected velocity v_E (mm s^{-1})	Predicted agglomerate size (µm)
0.61	3.7×10^{-20}	0.3	0.17×10^{-6}	17
1.21	7.4×10^{-20}	2.8 leading edge	0.56×10^{-6}	29
1.21	7.4×10^{-20}	0.5 trailing edge	0.56×10^{-6}	12
2.42	1.5×10^{-19}	7.0	1.35×10^{-6}	29



Conclusions

Magnetic Bio-Assay Technology offers real time measurements, multiplexing capability and higher sensitivity.

Detection of endogenous magnetic nanoparticles (static/dynamic mode) by TMR. Detection of smallest magnetic entity so far reported.

Magnetic targeting with Magnetic Resonance Imaging.

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Current Technologies

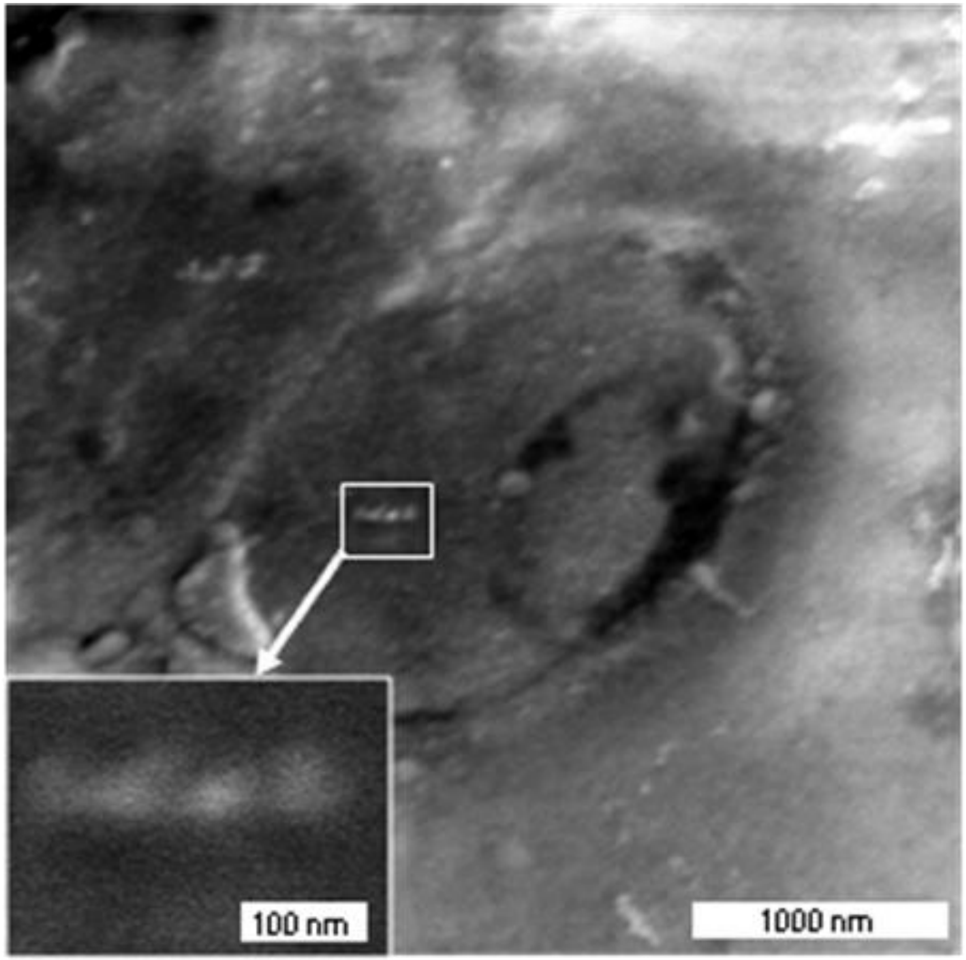
Microarrays

- Sensitivity limited by 2D geometry.
- Expensive to produce.
- Can not be tailored to the application after production.
- Inflexible requiring bulky and expensive optics to operate.
- Can be highly multiplexed.

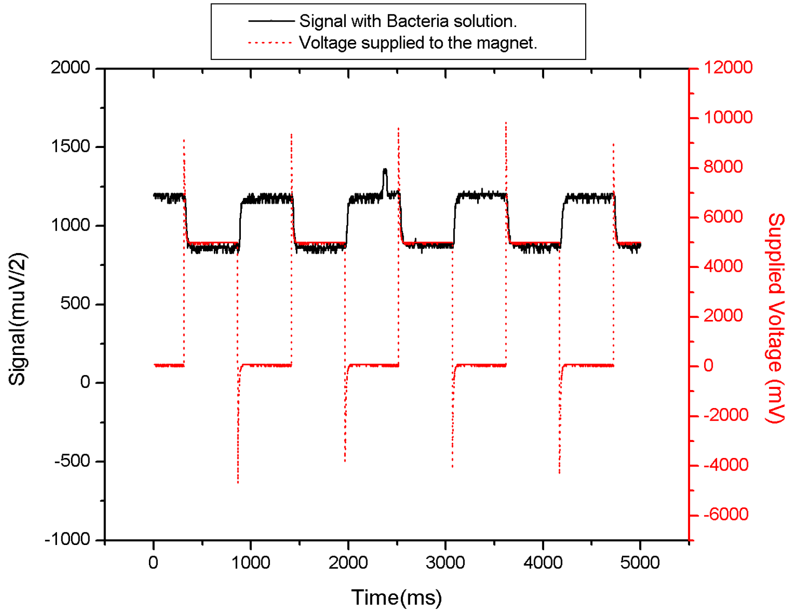
SAT’s

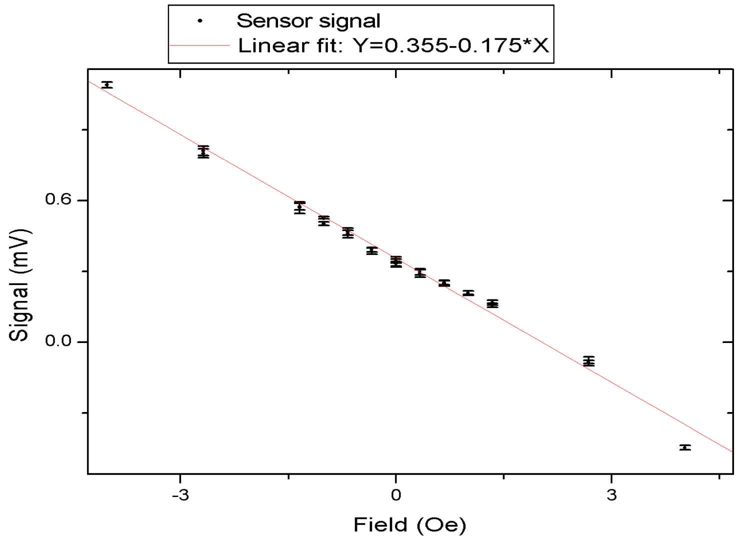
- Sensitivity is higher due to 3D microcarrier kinetics.
- Cost depends almost entirely on microcarrier design.
- Can be tailored somewhat after production.
- Equipment required depends on labelling architecture.
- Multiplexing is limited by number of labels generated.

Baseline Shift (SEM)



Dynamic TMR Measurements





$$H = \frac{m}{d^3} \times \left(1 - \frac{3l^2}{4d^2}\right)$$