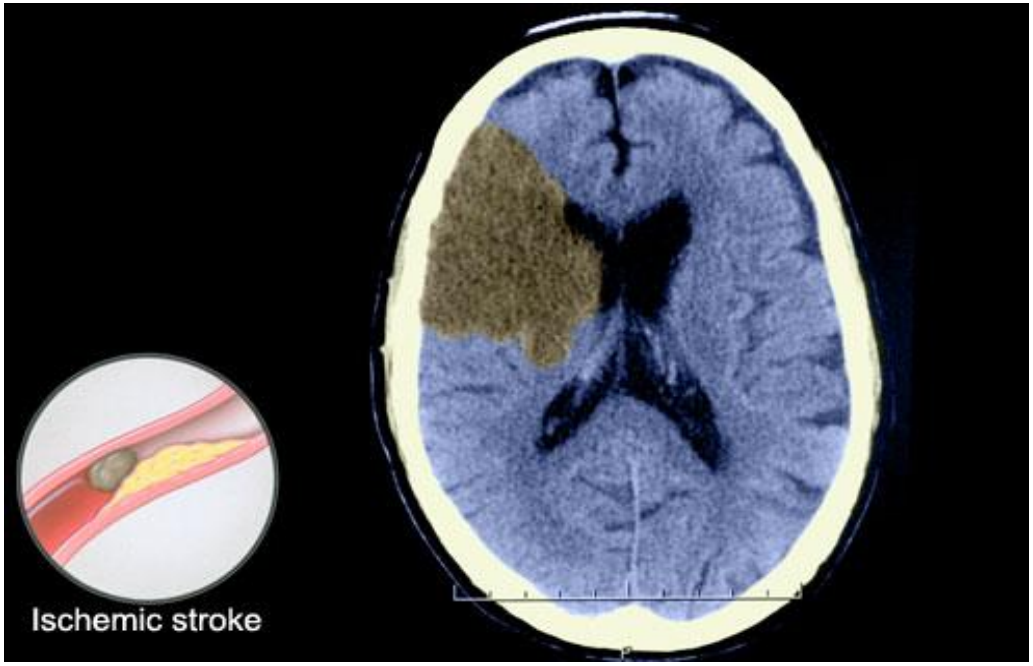
3-Multiplexed detection of ischemic stroke (ICTUS) related biomarkers Invennta Project, with Santiago de Compostela Hospital

Elisabete Fernandes

Biomarkers might aid physicians in several steps of stroke evaluation

Selection of patients at higher risk of hemorrhagic transformation

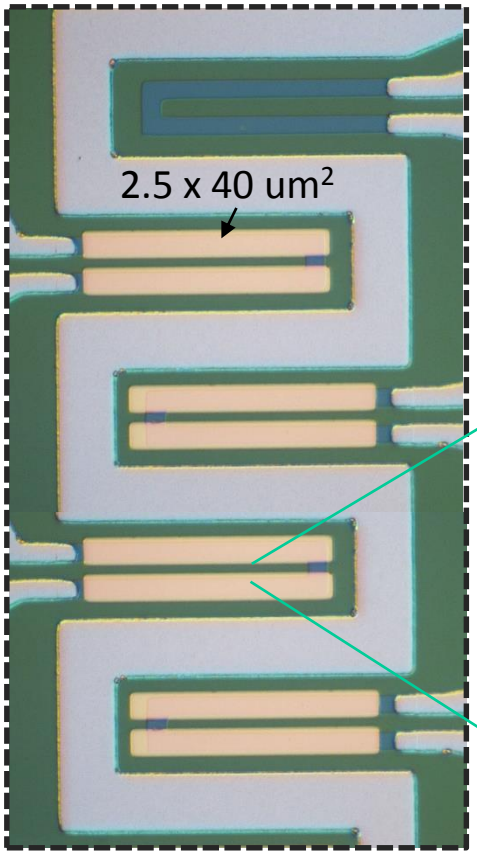


biomarkers

1. **Cellular Fibronectin**
2. **S100 Calcium Binding Protein B**
3. **Angiopoietin-1**
4. **PDGF-CC**
5. **Matrix Metallopeptidase 9 - MMP9**
6. **Neuroserpin**

Requirement: fast analysis (< 1h) of patient to select treatment

Sandwich immunoassay using MNP labels and magnetoresistive detection



Biotinilated antibody

magnetic label (FeOx)

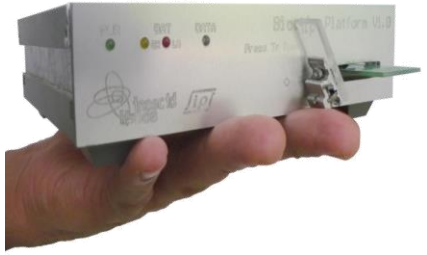
Labeling antibody

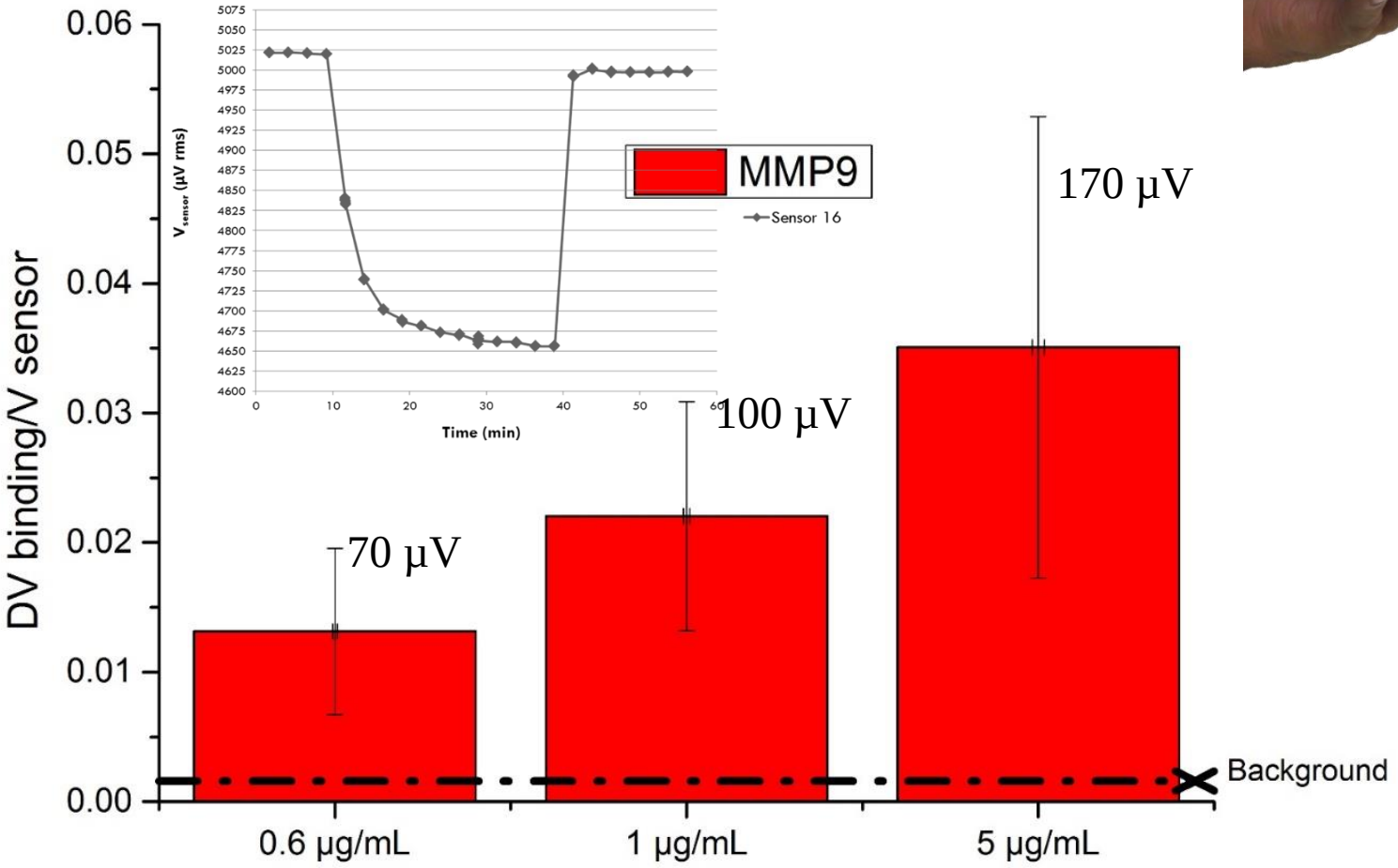
proteic biomarker

probe antibody

MR sensor

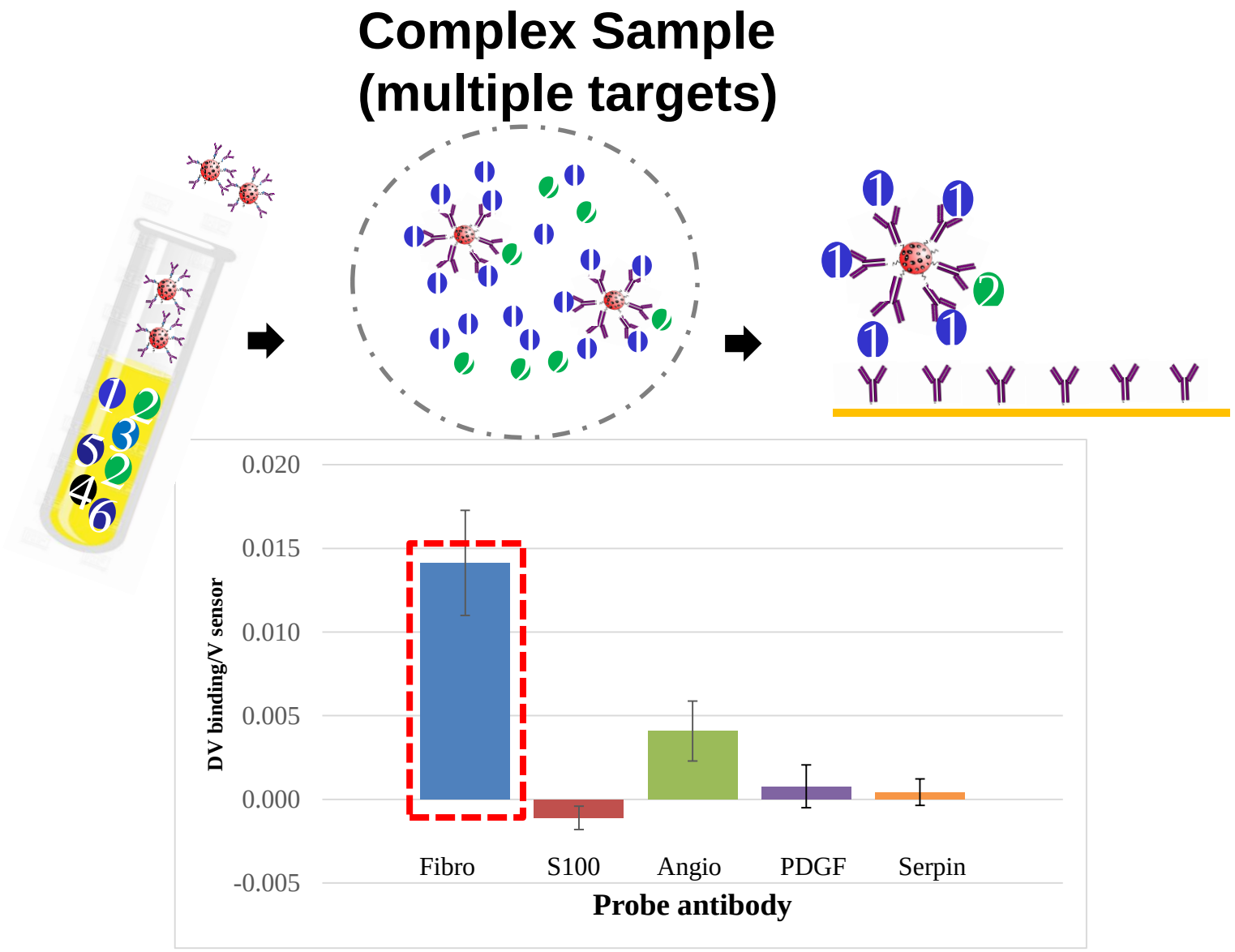
Matrix Metallopeptidase 9 detection





MMP9 present detection limit <1ng/mL
Fibronection < 0.5 ug/mL

Detection of Fibronectin in a complex sample matrix



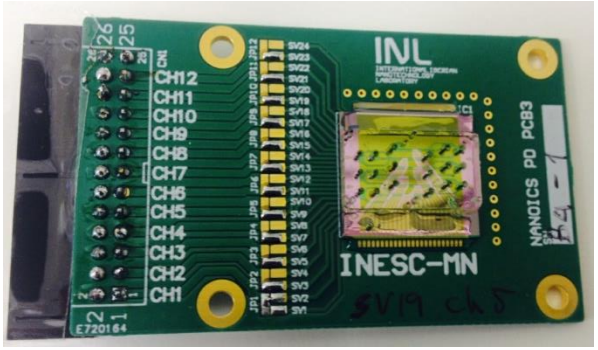
New projects already started (GAIN funding)

-detecting biomarkers for colo-rectal cancer In feces (with J Cubiella, Sergas, Ourense)

-detecting biomarkers for peritoneal fibrosis (with Africa Gonzalez, CINBIO, U Vigo)

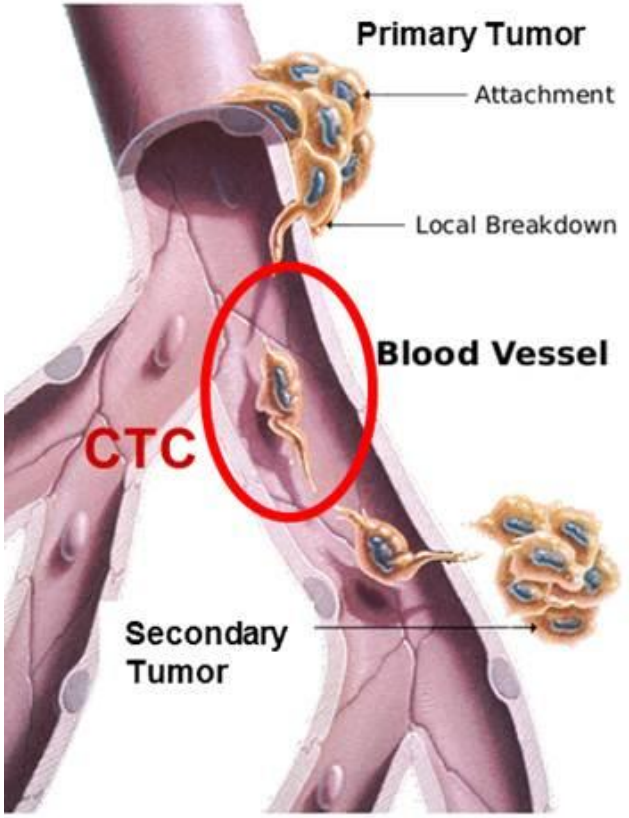
4-Integrated CTC's detection in blood

A. Chicharo, L.Dieguez, M.Oliveira, S.Cardoso, J.Piteira
R. Lopez, M.Abal, Clotilde
INL, INESC MN, IDIS, Hospital Santiago Compostela



- **Detection & Counting of Circulating Tumor Cells (CTCs) as a means of studying the process of metastasis, using an electronic, automated platform**

- **CTCs phenotype identification: EpCAM (+) vs EpCAM (-)**

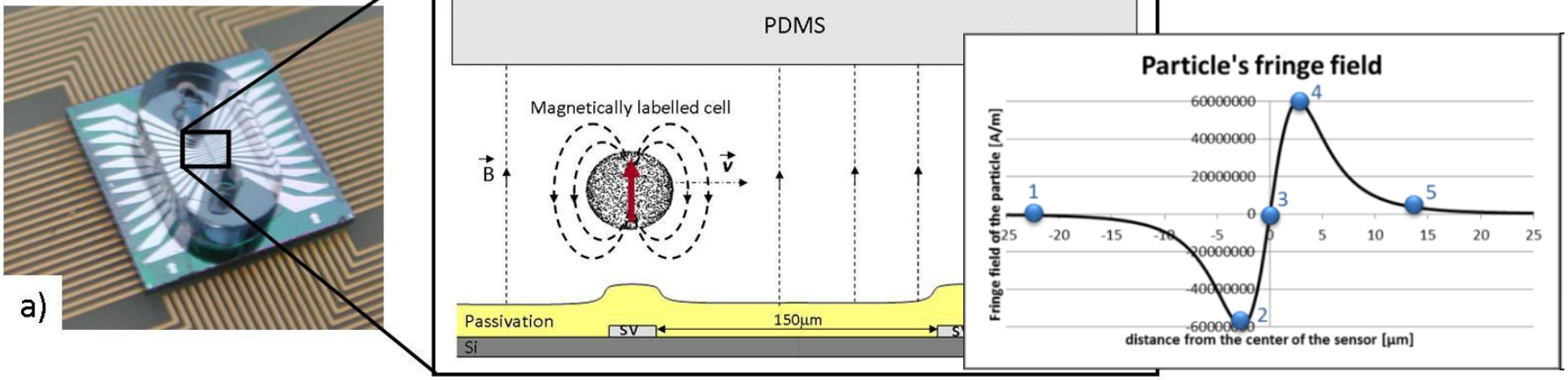


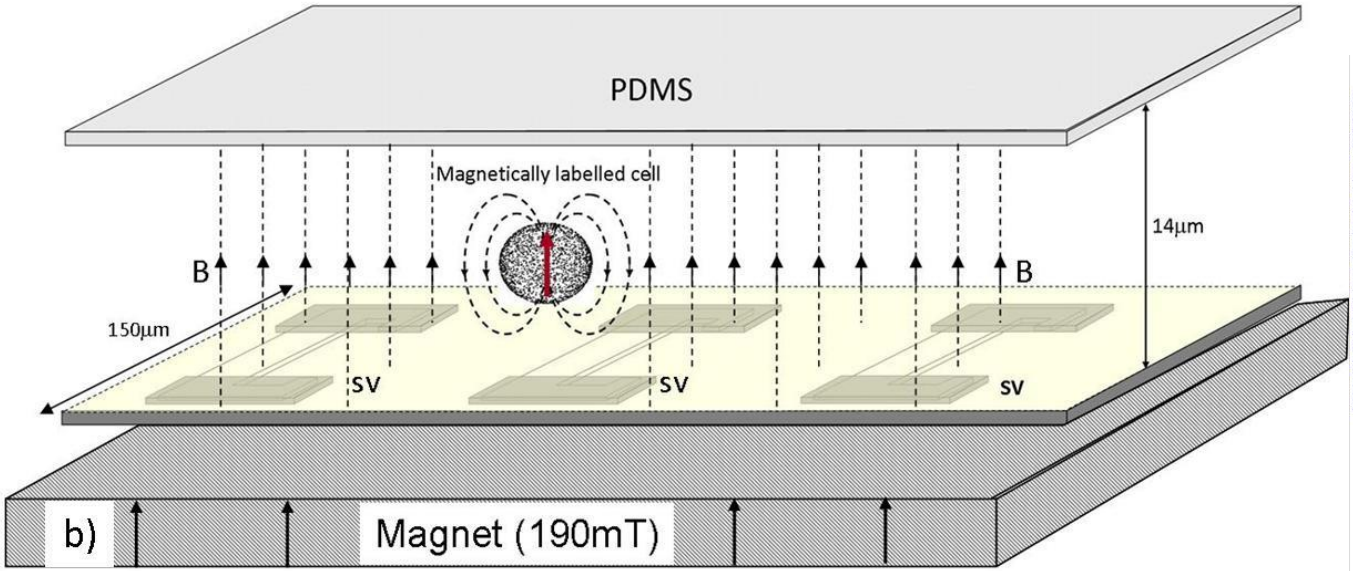
Adapted from National Cancer Institute

Invasive CTCs were identified as cells exhibiting CAM invasion (CAM+) and expressing standard epithelial markers (Epi+).

Clin Chem. 2011 Sep;57(9):1242-55. doi: 10.1373/clinchem.2011.165068. Epub 2011 Jul 22.

Previous work: detecting labeled cells in flow
(INESC MN)






Cite this: *Lab Chip*, 2011, **11**, 2255

www.rsc.org/loc

PAPER

Magnetoresistive chip cytometer

J. Loureiro,[a,b] P. Z. Andrade,[b] S. Cardoso,[a] C. L. da Silva,[b] J. M. Cabral[b] and P. P. Freitas[a]

Received 23rd August 2010, Accepted 6th April 2011
DOI: 10.1039/c0lc00324g

Although conventional state-of-the-art flow cytometry systems provide rapid and reliable analytical capacities, they are bulky, expensive and complex. To overcome these drawbacks modern flow cytometers have been developed with enhanced portability for on-site measurements. Unlike external fluorescent/optical detectors, magnetoresistive sensors are micro-fabricated, can be integrated within microfluidic channels, and can detect magnetically labelled cells. This work describes the real-time detection of single magnetically labelled cells with a magnetoresistive based cell cytometer. For Kg1-a cells magnetically labelled with 50 nm CD34 microbeads (Miltenyi) flowing through a 150 µm wide, 14 µm high microchannel, with speeds around 1 cm s^{-1}, bipolar signals with an average amplitude of 10–20 µV were observed corresponding to cell events. The number of cells counted by the spin valve cytometer has been compared with that obtained with a hemocytometer. Both methods agree within the respective error bars.

Introduction

Over the past decade, the drawbacks of conventional flow cytometers have encouraged efforts to take advantage of microfabrication technologies and advanced microfluidics to achieve smaller, simpler, more innovative and less expensive instrumentation. Most of these microfabricated systems still make use of external solid-state devices (diode laser, PIN photodiodes) for the detection and enumeration of cells/particles.[1] Magnetoresistive sensors can be used to replace these technologies and be easily integrated within microfluidic channels to detect/count magnetically labelled cells. These sensors have been integrated in biochip platforms used for biomolecular recognition detection, measuring the fringe field created by magnetic particles (MP) used as labels for different biomolecular targets (DNA, cells) that recognize immobilized probes.[2–4] These experiments involve the static detection of the fringe field emanating from the immobilized labels. First attempts at MP dynamic detection were made either with ferrofluidic droplets (dozens of microns long) moving inside microfluidic channels with cm s^{-1} velocities,[4] or with single beads manipulated over the sensor using current lines actuation involving slowly moving particles (µm s^{-1}).[5] Recently the detection of single micrometre size magnetic beads moving fast (cm s^{-1} velocities) through microfluidic channels was demonstrated using magnetoresistive sensors integrated on the channel bottom[6,7] but the work presented in this paper is the first demonstration of this technique for cytometer application.

An advantage of this approach is the magnetic labelling of the cells that can be used, within the same device, for magnetic separation which has several advantages in comparison with other techniques. It allows target cells to be isolated directly from crude samples such as blood, bone marrow, water, *etc.* Furthermore, the large differences between magnetic permeabilities of the magnetic and non-magnetic materials can be exploited in developing highly selective separation methods.[8–10]

From a technological point of view, one of the crucial issues in developing such a chip-cytometer is to demonstrate the real-time detection of magnetically labelled cells that are moving fast in a microfluidic channel using magnetoresistive sensing elements.

In this paper the detection of Kg1-a cells labelled with 50 nm magnetic particles in rapid flow (cm s^{-1}), by spin-valve (SV) sensors, through a microchannel is addressed. The counting capability is achieved with home-made software which validates the counting each time the absolute signal coming from the SV sensors rises above the threshold level established by the noise background amplitude.

Theory

Magnetoresistive detection

SV sensors are linear magnetic field transducers based on the giant magnetoresistance effect.[11] By patterning the SV with a large aspect ratio with the long direction perpendicular to the

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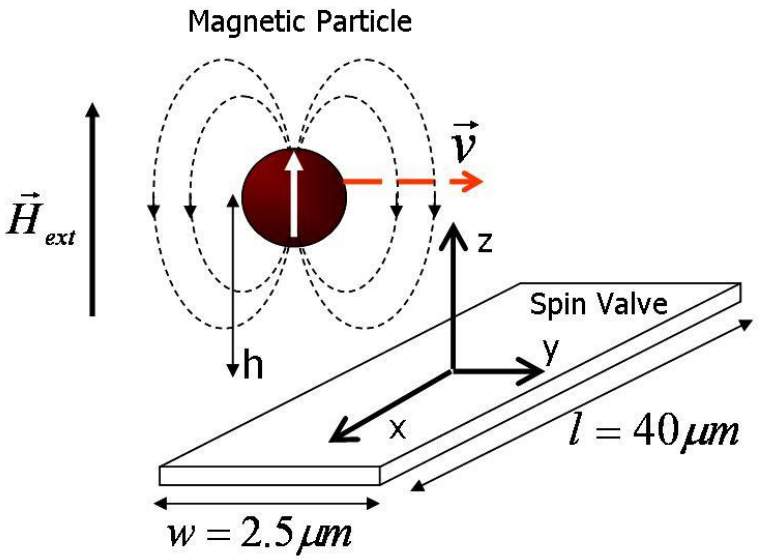
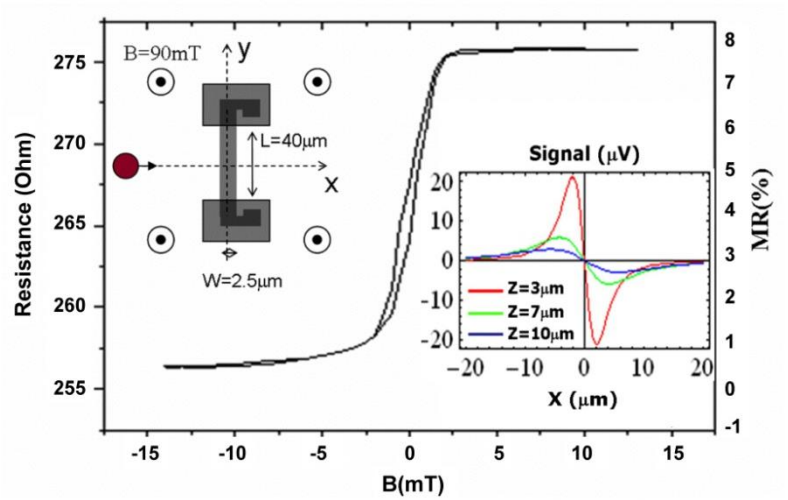
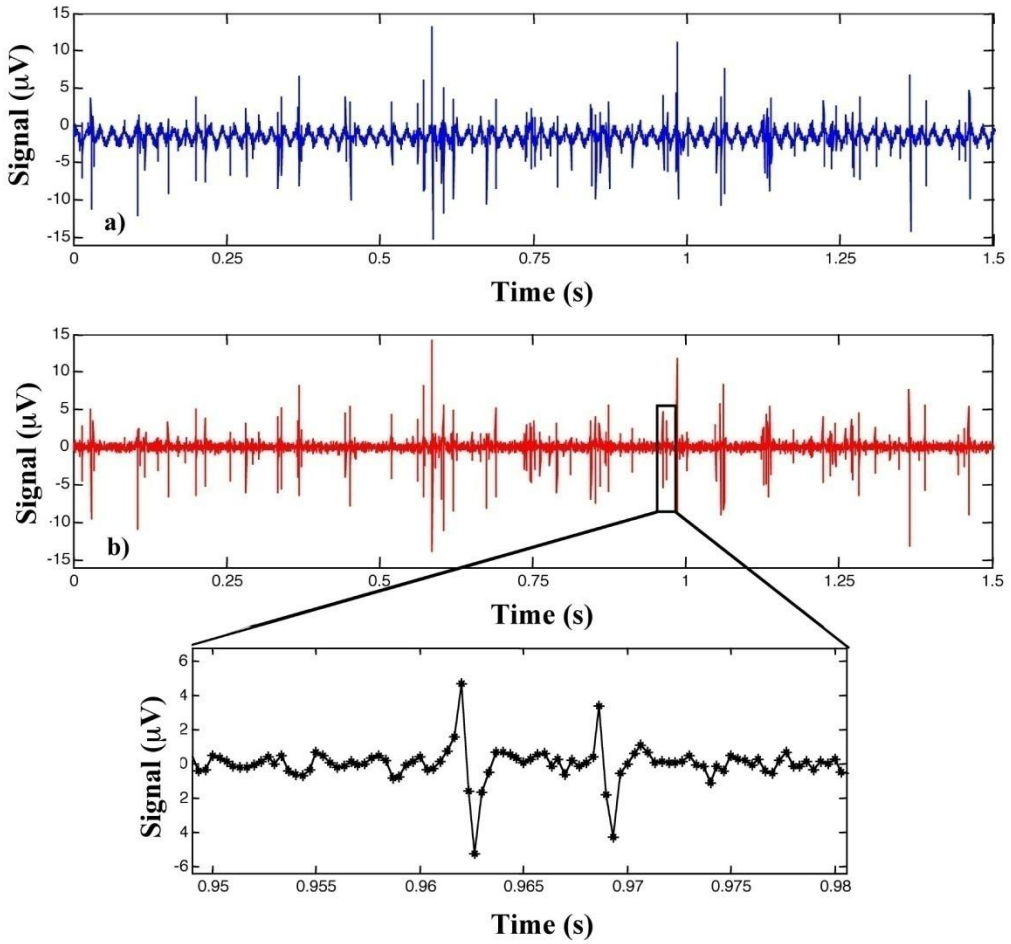
[b]Instituto Superior Técnico/Universidade Técnica de Lisboa, Av. Rovisco Pais, 1, 1049-001 Lisbon, Portugal. Fax: +351 218499242; Tel: +351 218417000
[c]IBB-Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, Lisbon, Portugal. E-mail: bergi@ist.utl.pt; Fax: +351 218419062; Tel: +351 218419065

Detecting 1 um magn. beads



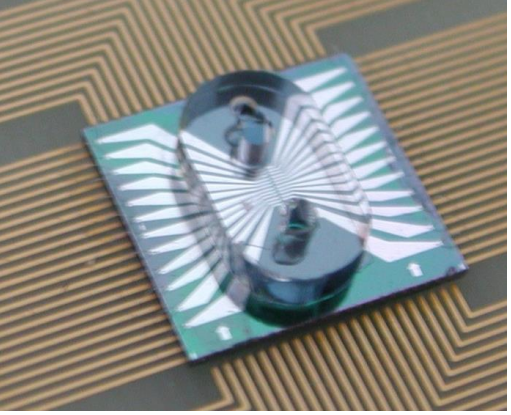


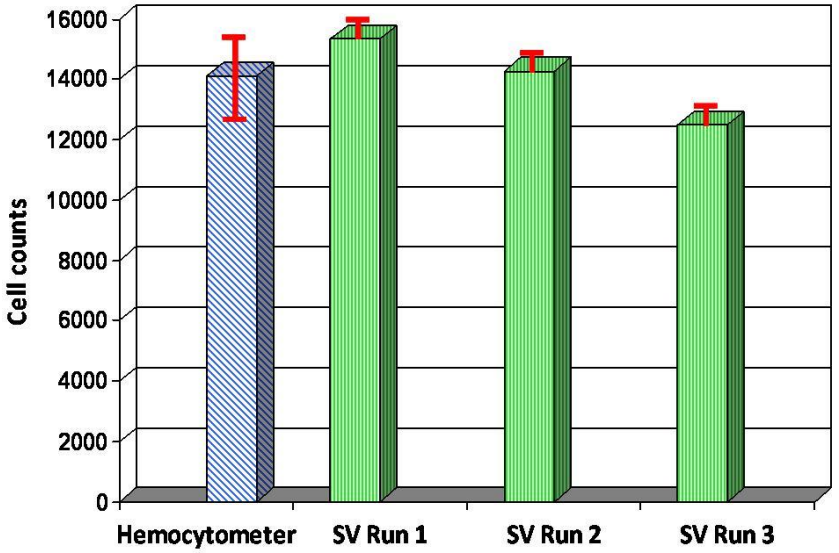


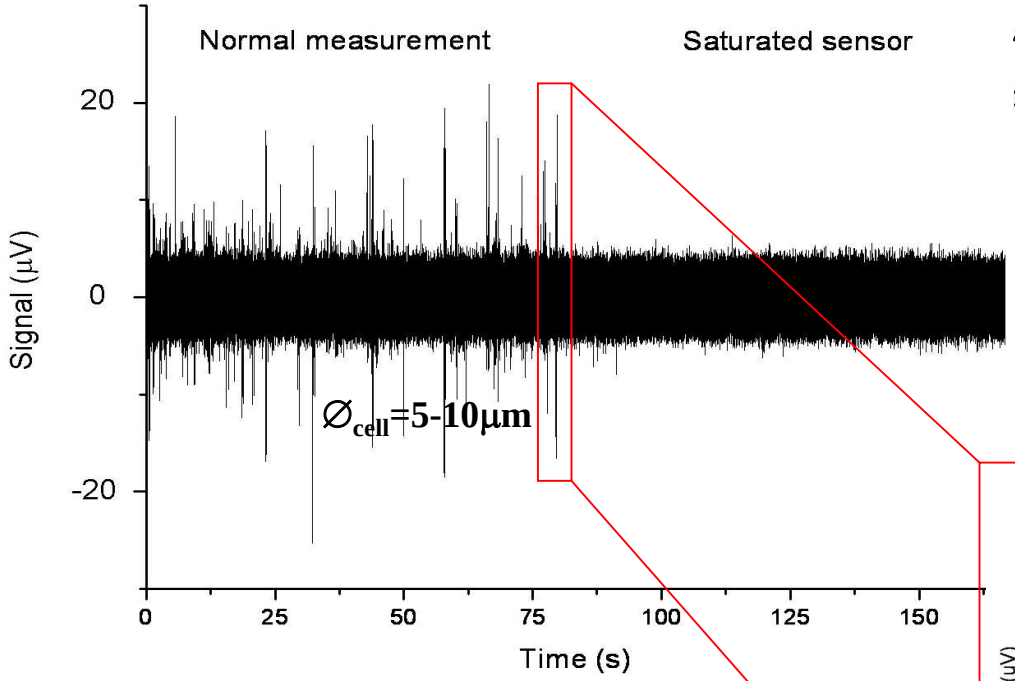
With C Fermon, and M Pannetier

APL 2009

Cell detection – Kg1a cells

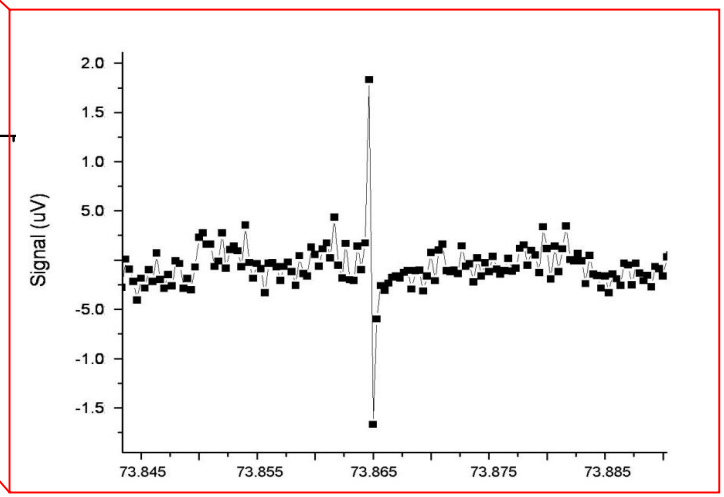






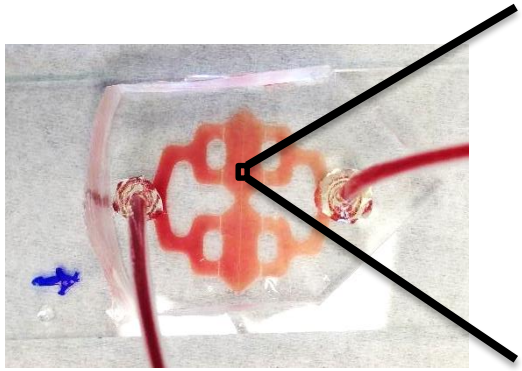
Lab on Chip (2011)

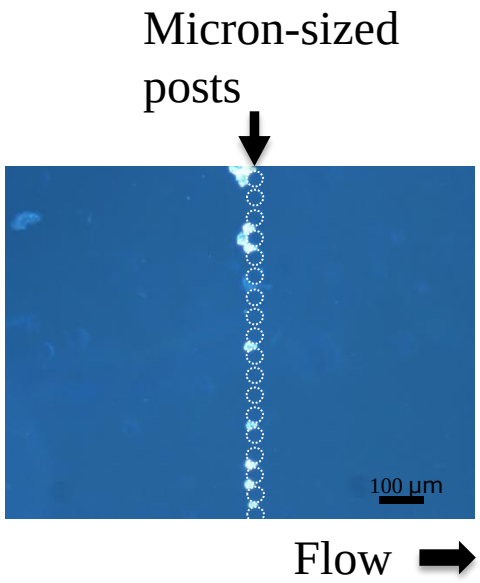
Cells marked with 50nm FeOx particles



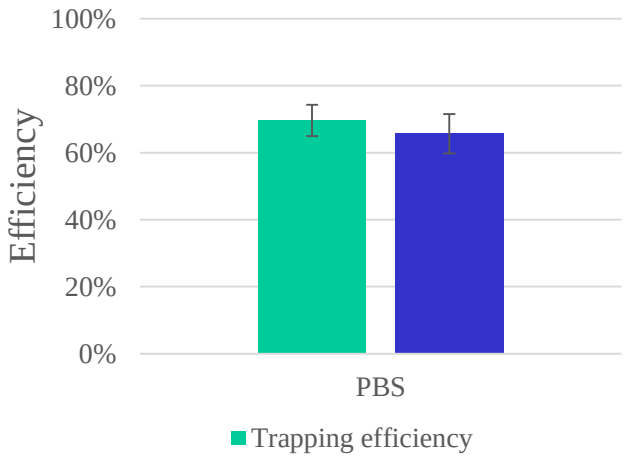
Trapping cancer cells (CTCs) in microfluidic device

- 50 cells SW480 labelled with magnetic beads
- Loaded in the microfluidic device
- Cells are suspended in PBS-BSA2%



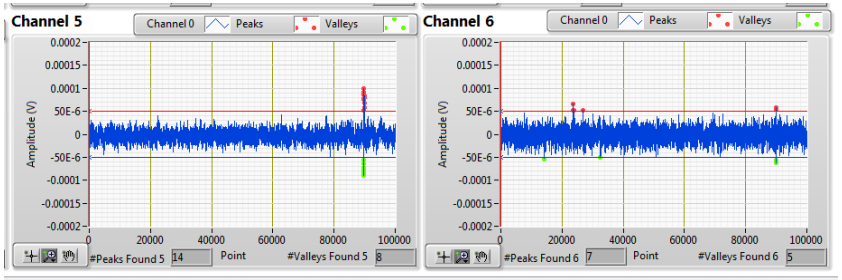


	Estimate #of cells Pipetted	Initial #of cells counted parallel	Traped #of cells in the filter	Recovered #cells	Trapping efficiency	Recovery effiency
Run #1	50	48	34	40	71%	83%
Run #2	50	44	35	31	80%	70%
Run #3	50	62	49	43	79%	69%
Results					70%	66%

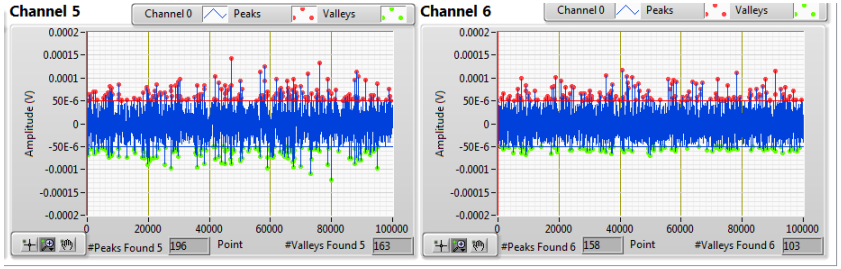


Alexandre Chicharo, INL/IST

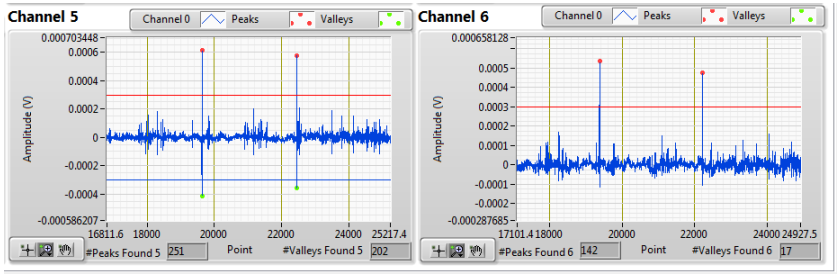
Baseline



Beads + Ab

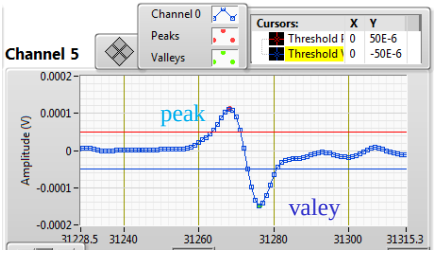


Cells + Beads + Ab

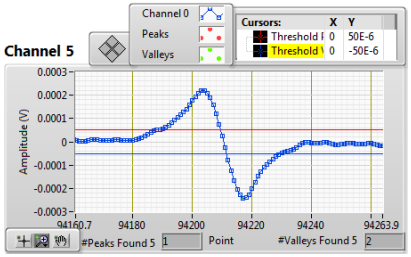


By direct measurements: the sample's velocity is 12.5mm/s

Bead



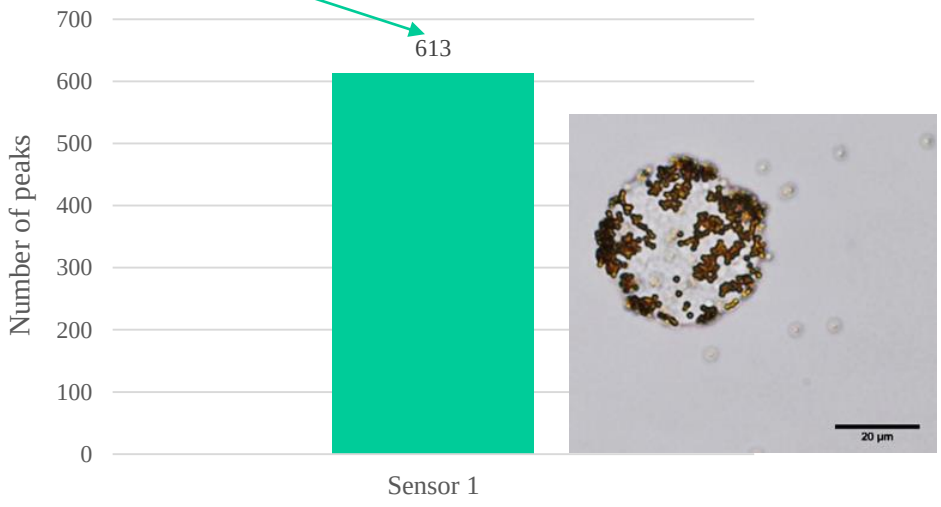
Cell



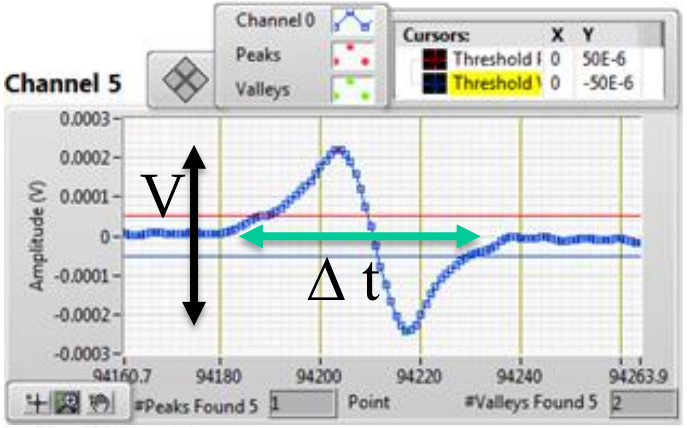
At a flow rate of 50µl/min:

	Amplitude	Time-of-flight
Bead	120µVpp	2-3 ms
Cell	240µVpp	15-30ms

- Baseline
- Sample of beads with control Antibody
- 500 Sw480 cells spiked in buffy coat+ beads with EpCam Antibody

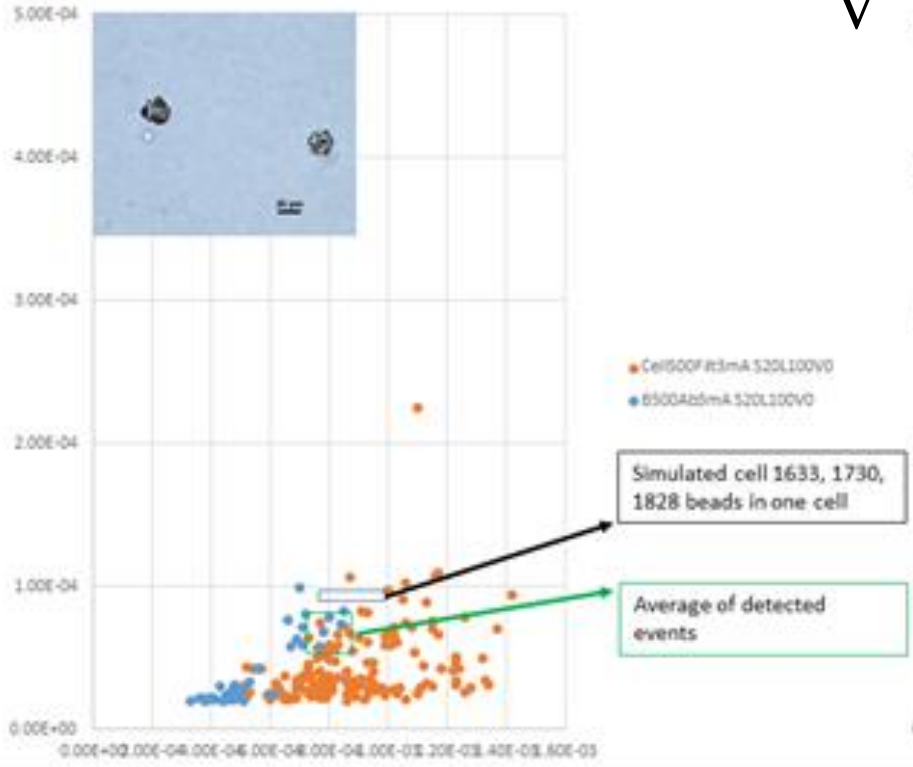


Separating labelled CTC cells from particle clusters



With no Vertical Focusing
S20L100V0

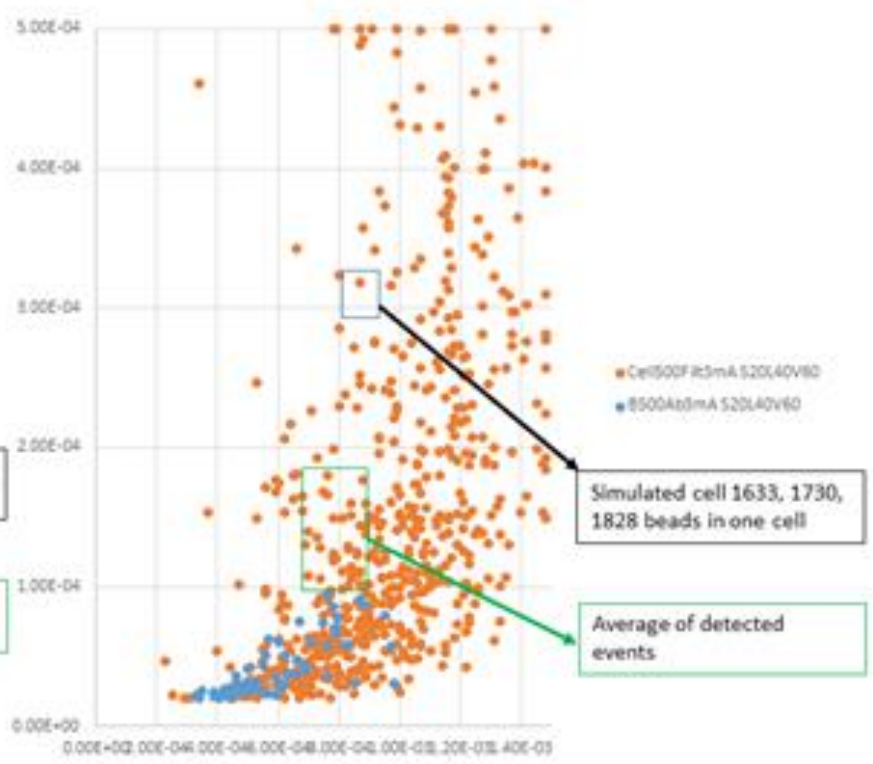
V



Δ t

With Vertical Focusing
S20L40V60

V

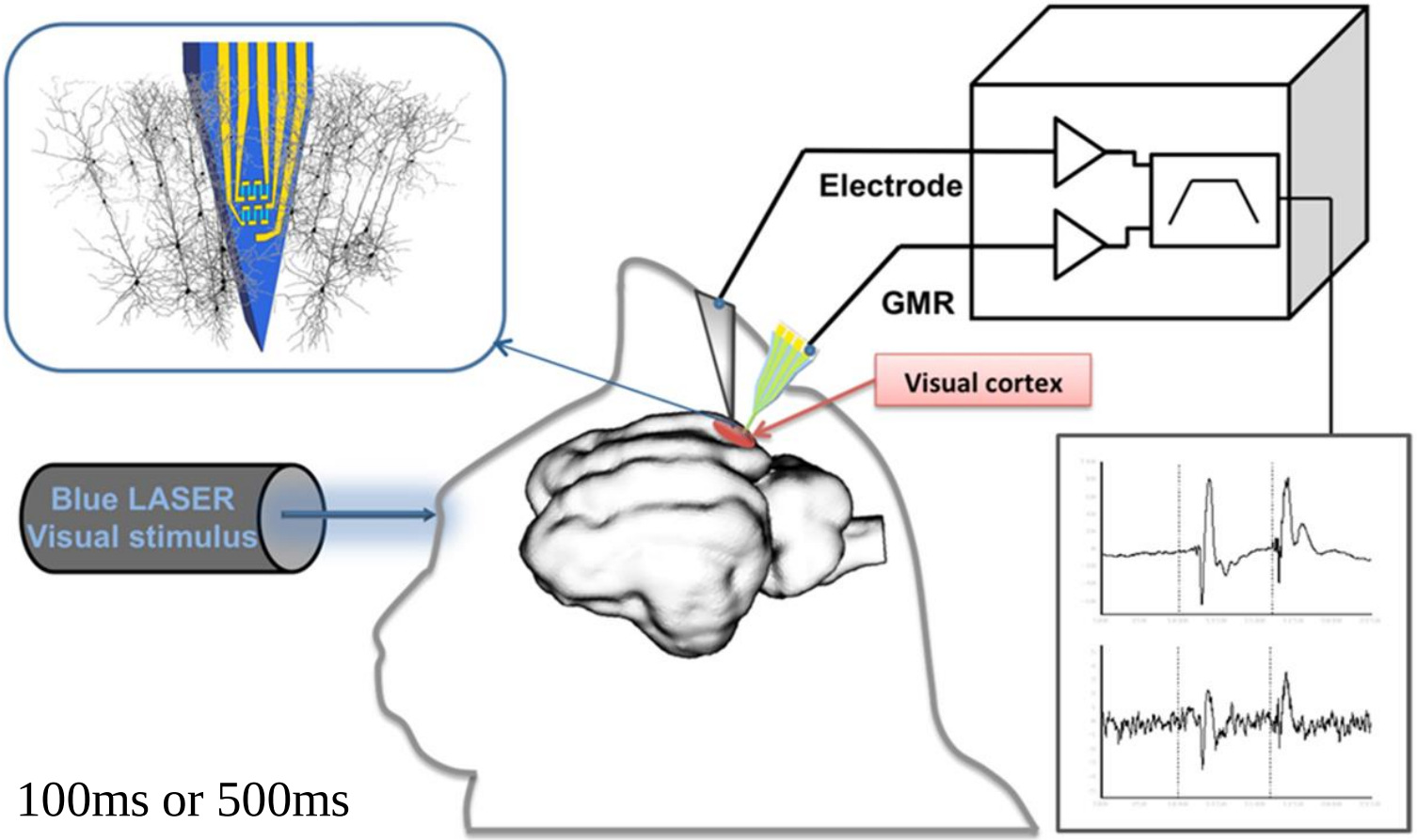


Δ t

Neuronal magnetic fields: In-vivo experiments

Laure Caruso[1], Thomas Wunderle[2], Christopher M. Lewis[2], Joao Valadeiro[3], Vincent Trauchessec[1], Josué Trejo Rosillo[1], José Pedro Amaral[3], Jianguang Ni[2], Claude Fermon[1], Susana Cardoso[3], Paulo Freitas[3], Pascal Fries[2,4], Myriam Pannetier-Lecoeur[1]*.

2014-2016

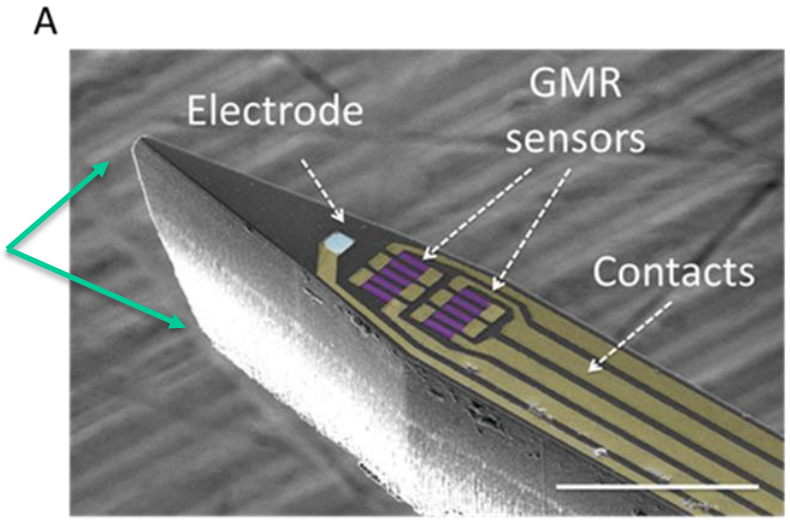


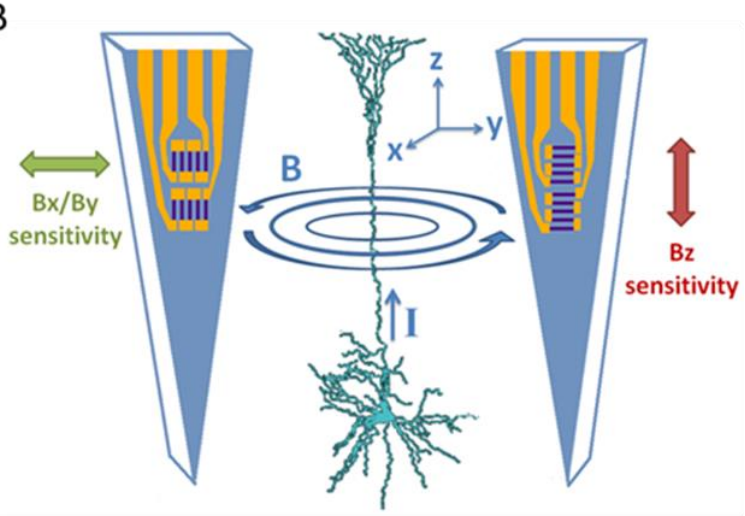
Stimuli: 100ms or 500ms
Light pulses, with inter-stimulus
Time around 1s

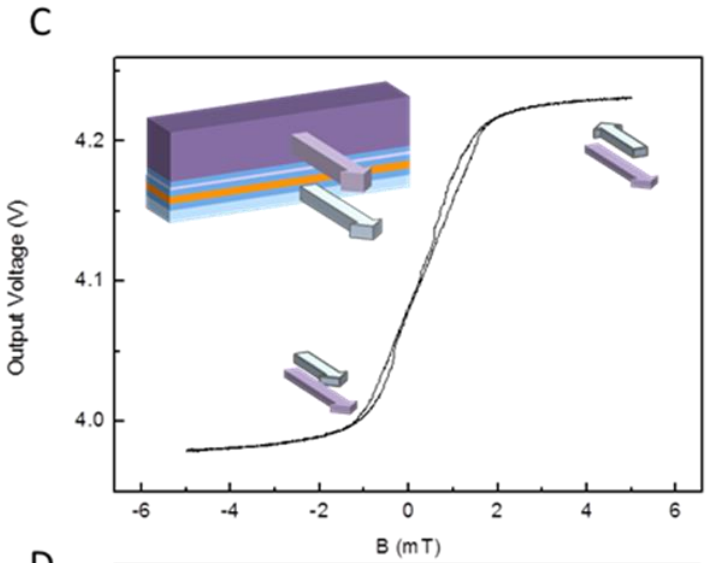
http://dx.doi.org/10.1101/092569 Neuron 95, 1–9, September 13, 2017

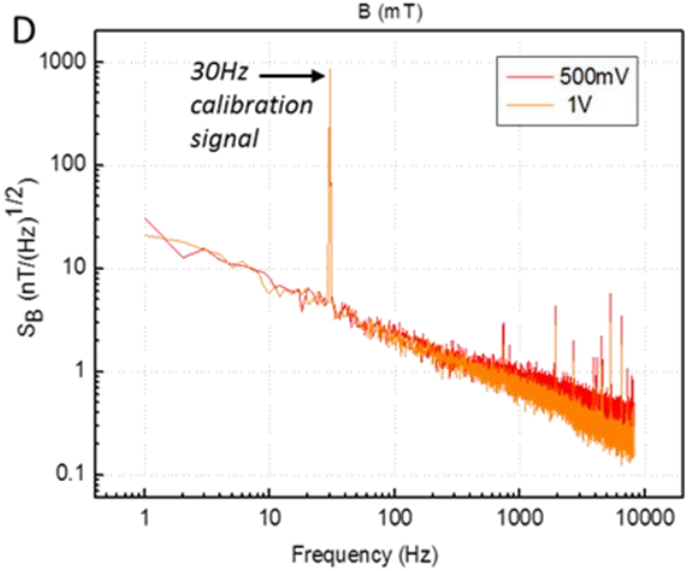
Sensors

200 µm thick Si



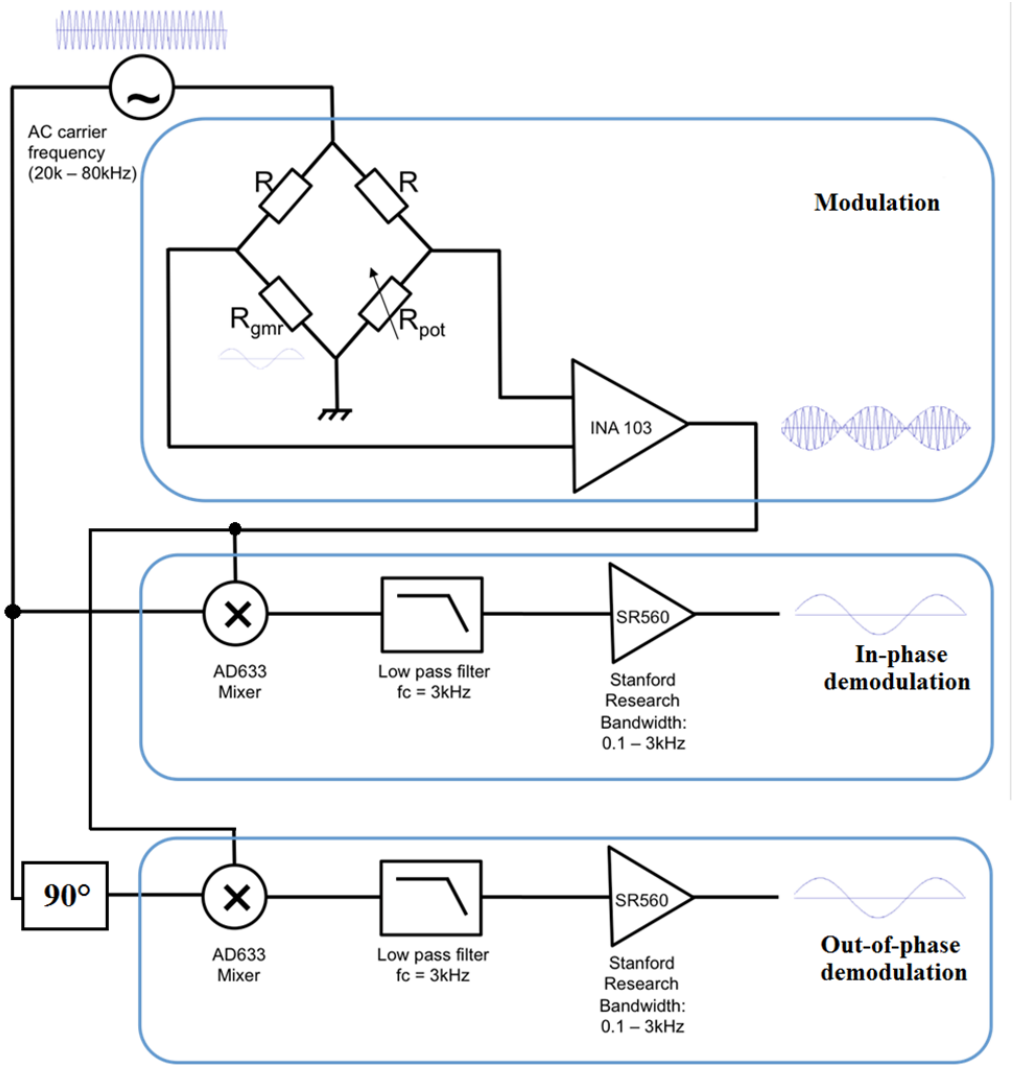






Problems: shield sensor from light (affecting Si substrate)

Signal modulation and demodulation

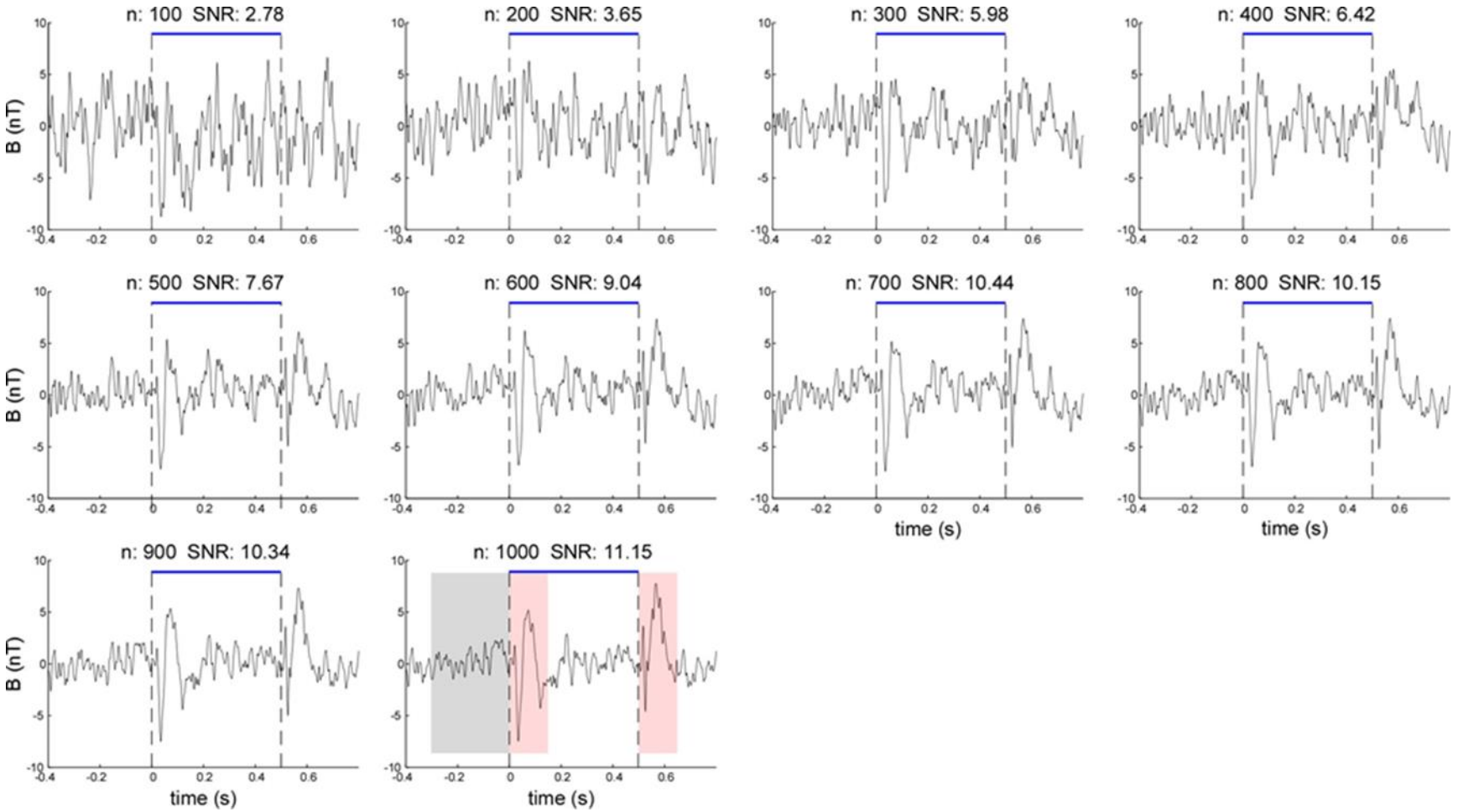


Total gain 500-1000

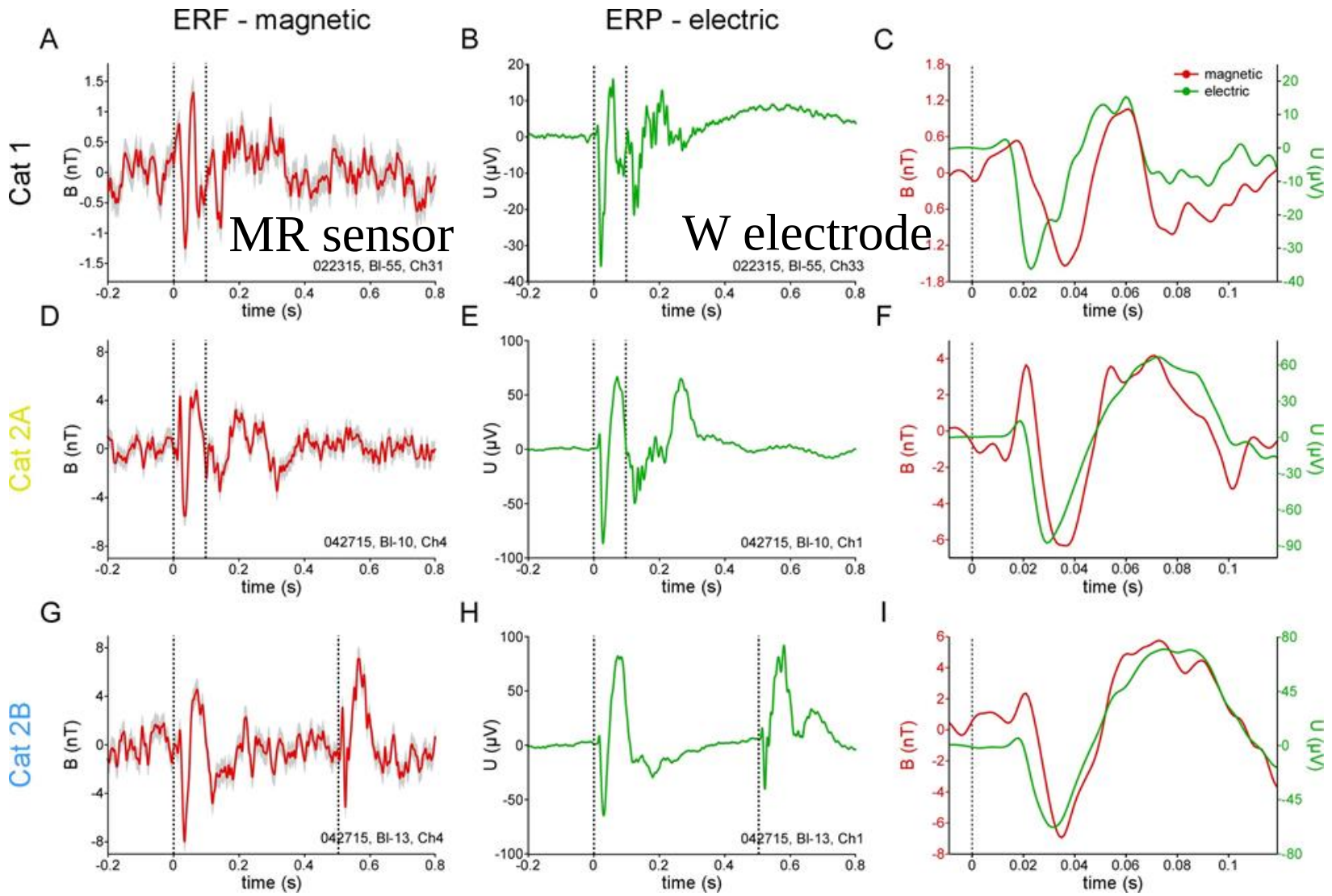
Magnetic signal

Electrical signal (capacitive couplin

6 months later



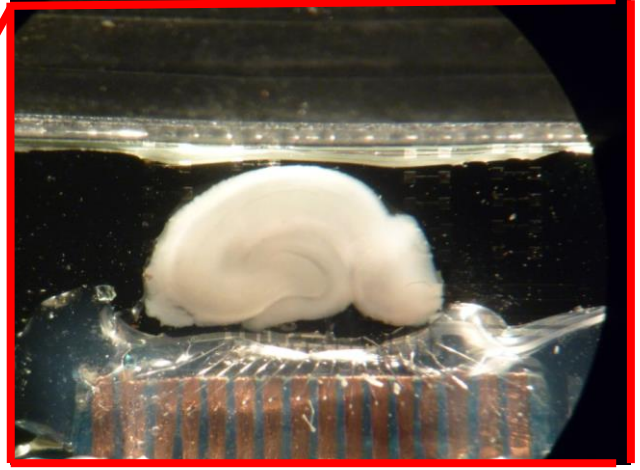
Comparison of magnetic and electrical signals: ERF and ERP

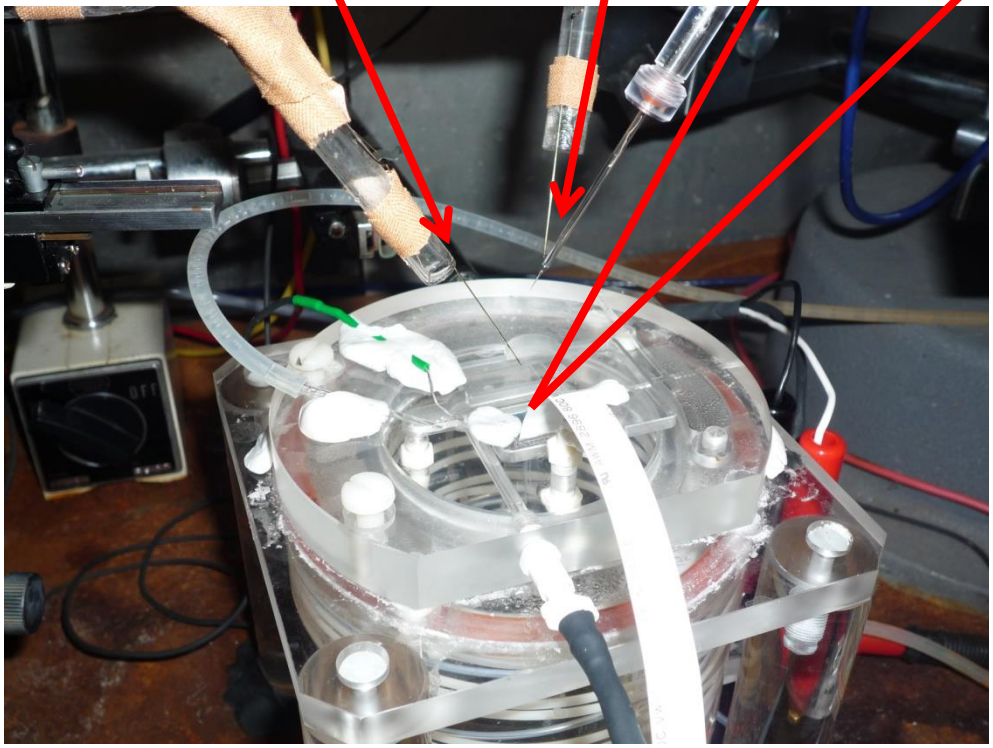


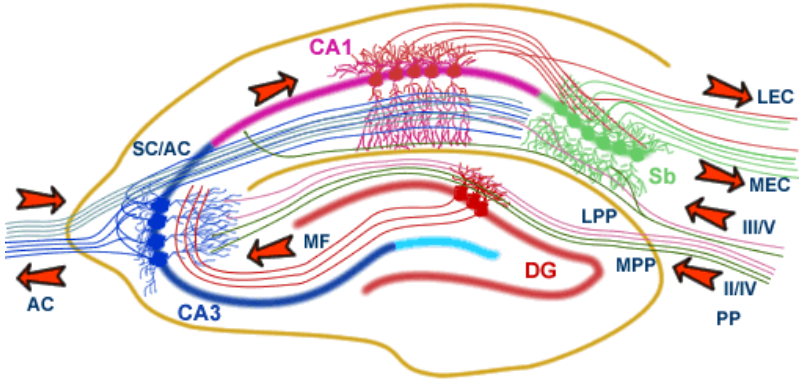
Synaptic current monitoring with high
Spatial resolution (with A.Sebastiao, IMM, V.Santos, ICVS)

Stimulation electrode

Recording electrode







INESC MN and IMM

Rat hippocampus

MAGNETRODES, FP7 (2013-2016)

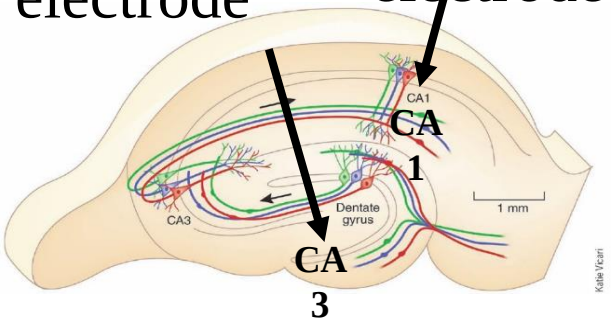
INESC MN

Recording Chamber

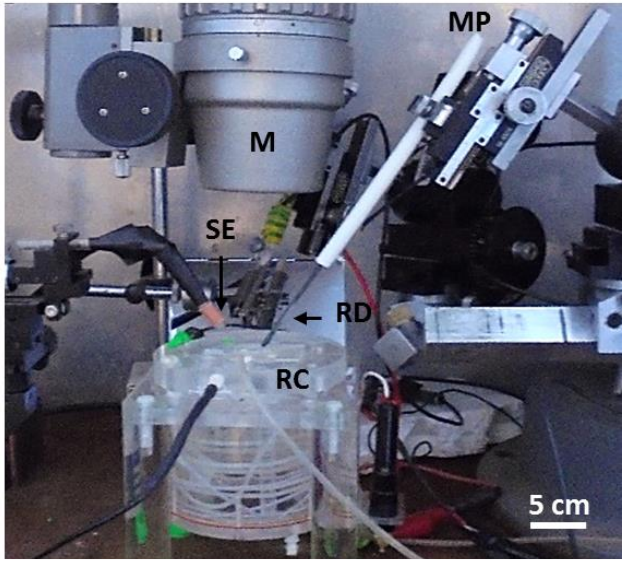
IMM INL INTERNATIONAL IBERIAN NANOTECHNOLOGY LABORATORY

Stimulus electrode

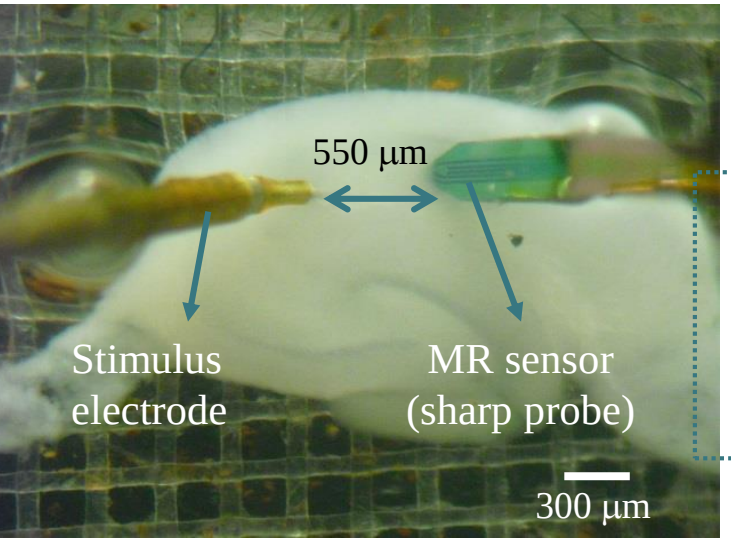
Recording electrode



Electrical Stimulus:
Frequency : 0.5 Hz
Amplitude: 300 µA
Duration : 0.3 ms

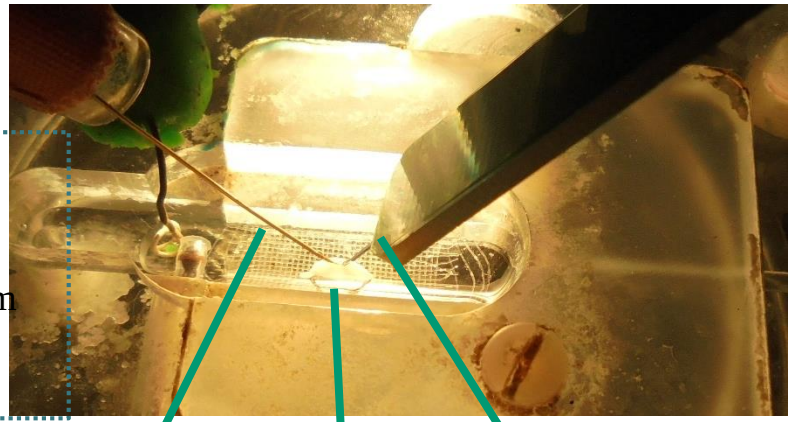


Setup facilities at IMM, Lisbon



Hippocampus Brain Slice dimensions:

- thickness: 300- 400 µm
- width: 1.6 mm
- length: 3 mm



Stimulus electrode

Rat brain slice

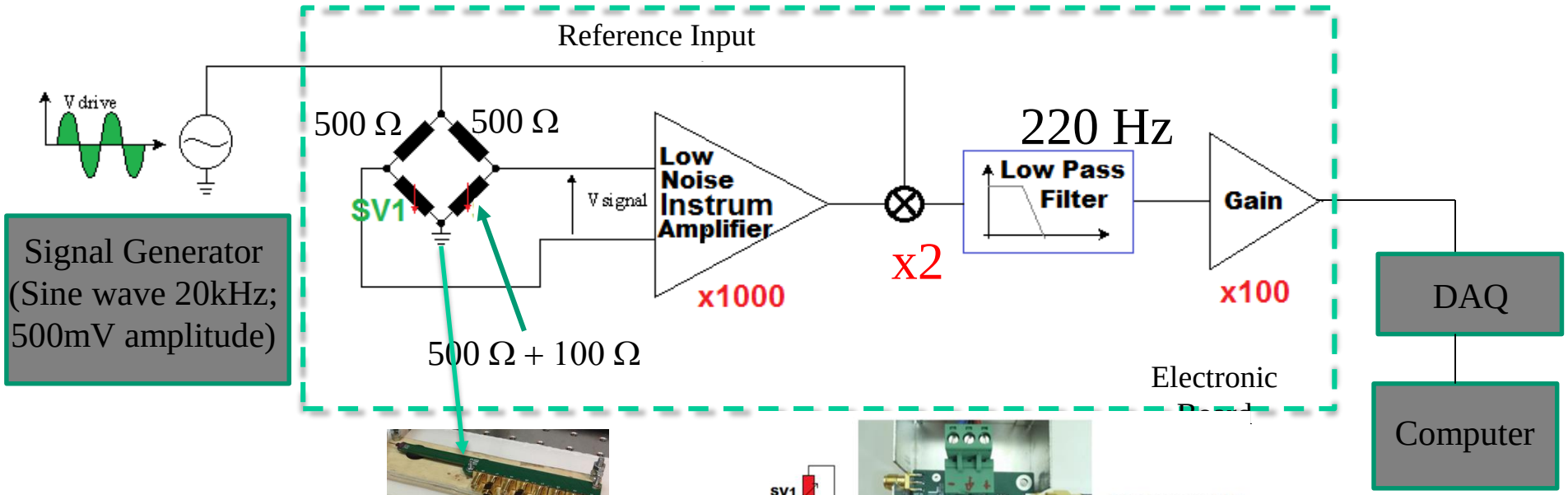
MR sensor (sharp probe)

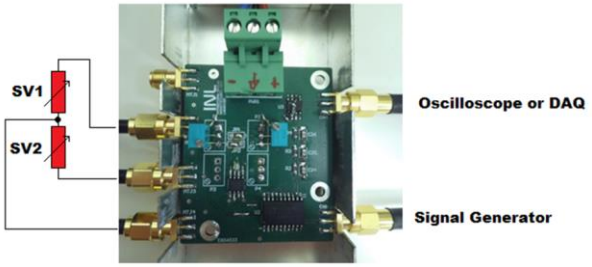
In collaboration with A Sebastiao, IMM, Lisboa

Measurement setup

ac measurements

AC measurement with modulation and demodulation





Total gain = 200 000

Wheatstone bridge:

- two fixed resistances (500 Ω)
- one adjustable resistance (fixed resistance 500 Ω in series with a potentiometer of 100 Ω)
- one variable resistance (Magnetoresistive Sensor)