Magnetic vortex nanodiscs for cancer cell destruction

Alfredo García-Arribas

Club Español de Magnetismo. Sevilla, septiembre 2017

Universidad del País Vasco Euskal Herriko Unibertsitatea

BASQUE CENTER FOR MATERIALS, APPLICATIONS & NANOSTRUCTURES

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Maite Goriena-Goikoetxea

Outline

- **Introduction**
  - Motivation
  - Magnetic vortex
  - Objetives of the work
- **Fabrication of the discs**
  - Hole-mask colloidal lithography
  - Morphological characterization
  - Release procedure
- **Magnetic properties and actuation**
  - Magnetization process and phase diagram
  - Large vortex core
  - Magneto-mechanical actuation
- **Discs in cancer cells**
  - Intracellular intake
  - Cytotoxicity
  - Magneto-mechanical treatment

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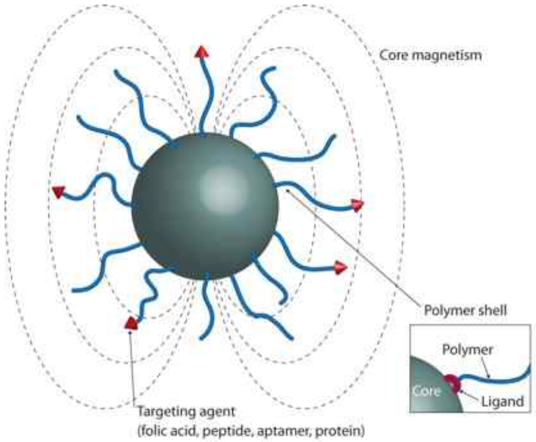
Introduction



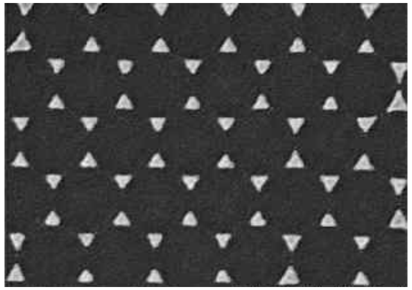
Magnetic nanoparticles for biomedicine (diagnosis and therapy)

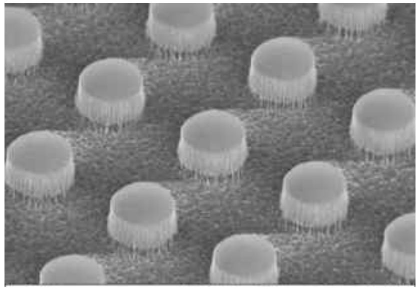
- Magnetic Resonance Imaging (MRI)
- Drug delivery
- Hyperthermia
- …

Iron oxides, chemically produced.

Patterned magnetic particles

- Great shape versatility
- Many different compositions
- Excellent reproducibility
- …

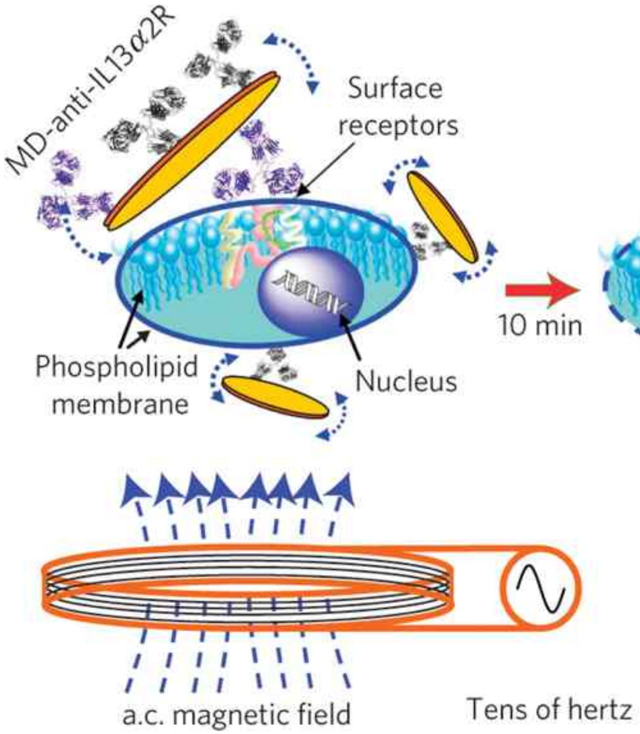


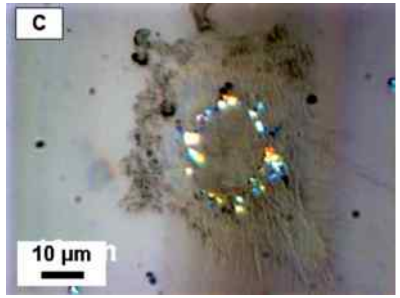


Produced by physical methods (vapor deposition, lithography, …)

Motivation

Magneto-mechanical actuation of Permalloy discs with vortex state





nature materials ARTICLES
PUBLISHED ONLINE: 29 NOVEMBER 2009 | DOI: 10.1038/NMAT2591

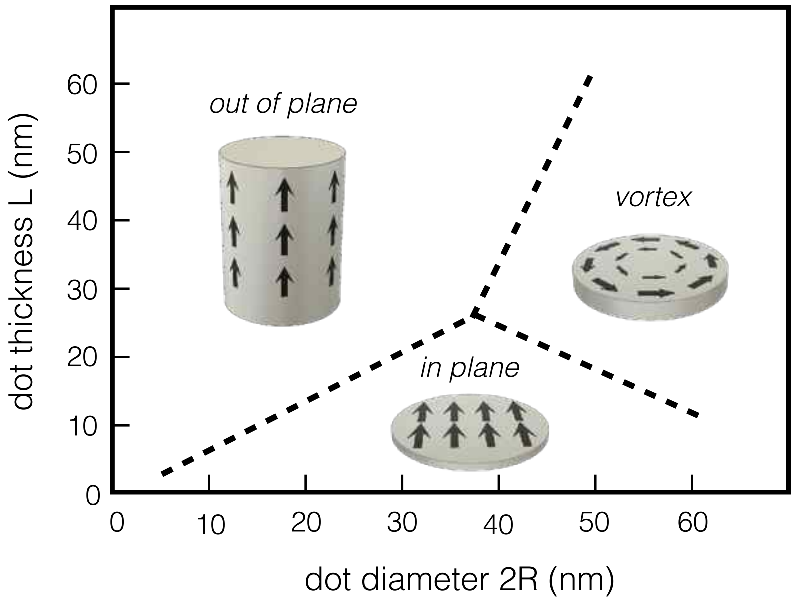
Biofunctionalized magnetic-vortex microdiscs for targeted cancer-cell destruction

Dong-Hyun Kim[1], Elena A. Rozhkova[2]*, Ilya V. Ulasov[3], Samuel D. Bader[1,2], Tijana Rajh[2], Maciej S. Lesniak[3] and Valentyn Novosad[1]*

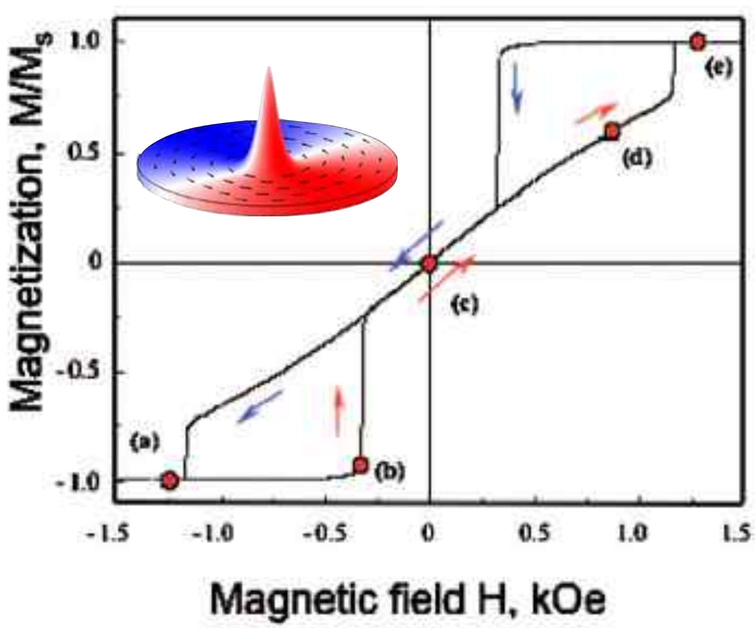
Kim et al, Nature Materials **9**, 165–171 (2010)

Magnetic Vortex state

Ground state



Magnetization process



V. Novosad et al. *Biomedical Engineering* (2011)

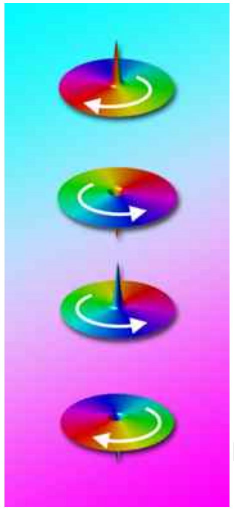
- Large permeability and magnetization → high actuation capability
- Null remanence → no particle agglomeration

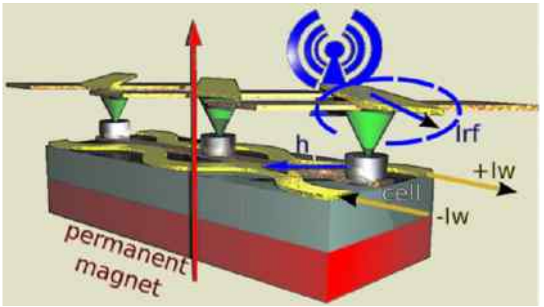
Magnetic Vortex state

Studied intensively in the last decades

R. P. Cowburn *et al*. PRL **83** (1999) 1042 T. Shinjo *et al*. Science **289** (2000) 930 K. Y. Guslienko *et al*. PRB **65** (2001) 024414

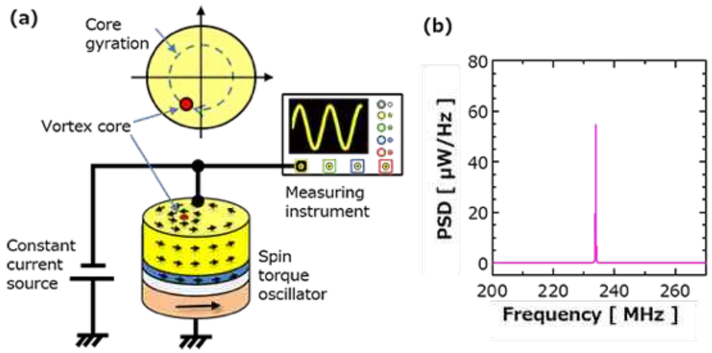
Information storage





B. Pigeau *et al.* Appl. Phys. Lett. **96**, 132506 (2010)

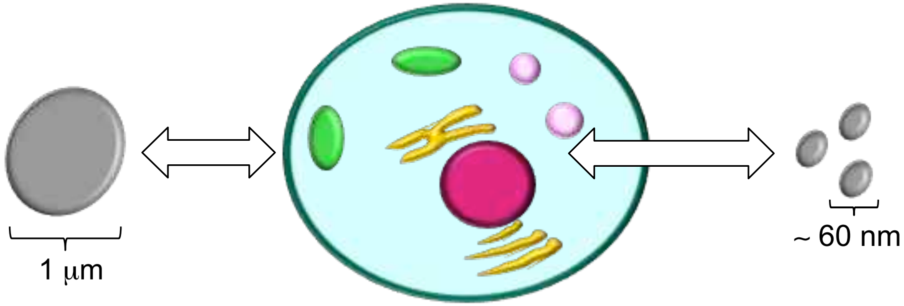
Spin torque oscillators



S. Tsunegi *et al.* Appl. Phys. Lett. **109**, 252402 (2016)

Objetives

- **Downsize Permalloy discs, maintaining the vortex state**



external actuation intra-cell actuation

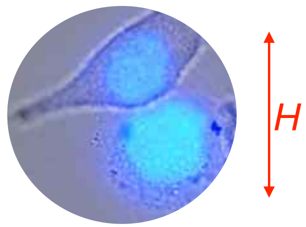
- **Explore the size limits of the vortex state**

Few works on magnetism of sub-100 nm discs

C.A. Ross *et al.* PRB **65**, 144417 (2002)

R.K. Dumas *et al.* PRB **75**, 134405 (2007) I.V. Roshchin *et al.* Euro. Phys. Lett. **86**, 67008 (2009)

- **In-vitro test of magneto mechanical actuation**



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Fabrication of the discs

Need a method:

- **relatively simple**. Reasonable preparation time.
- **moderate cost**. Affordable for a standard laboratory.
- **high yield**. Enough sample production for laboratory in-vitro assays.

- **Standard UV photolithography**

  👎 Minimum size ~1μm.
  👍 High yield and fast process.
  👍 Standard laboratory equipment (also used in this work).

- **Deep UV (DUV) lithography**

  👍 State of the art below 14 nm.
  👍 Standard in microelectronic industry. Suited for high volumen production.
  👎 Extremely expensive for research laboratories.

- **Electron beam lithography (EBL)**

  👍 Very small features (< 10 nm).
  👍 Expensive, but usual in research laboratories.
  👎 Small yield and slow process.

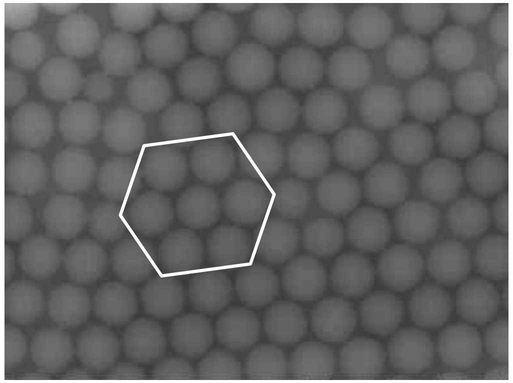
- **Others (nanoimprint lithography, etc ...)**

Fabrication of the discs

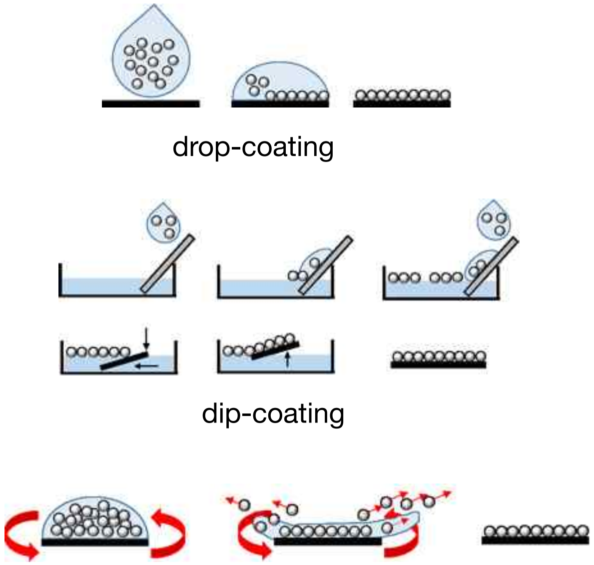
Nanosphere lithography

Use self-assembled latex spheres as template

- Low cost
- Relatively high yield



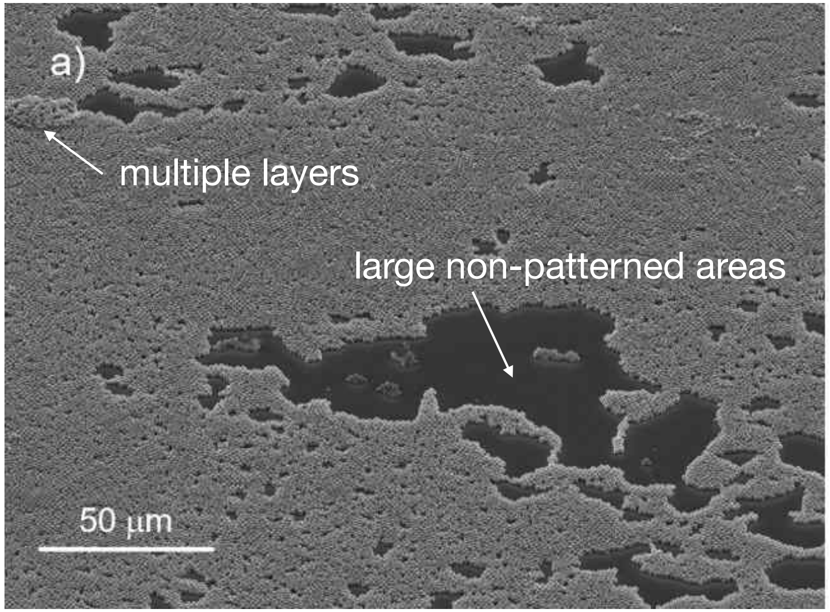
hexagonal closed-packed arrangement



drop-coating

dip-coating

spin-coating

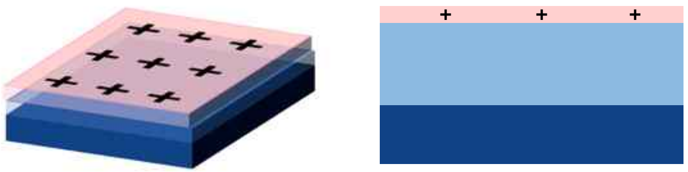


Hole-mask colloidal lithography

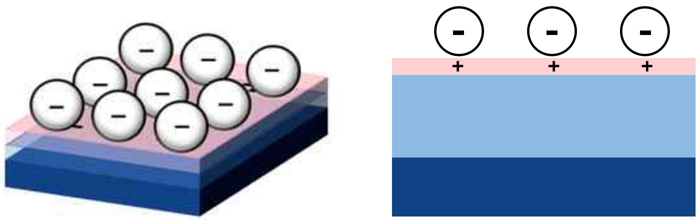
Use charged spheres to create a distribution of holes on a polymer

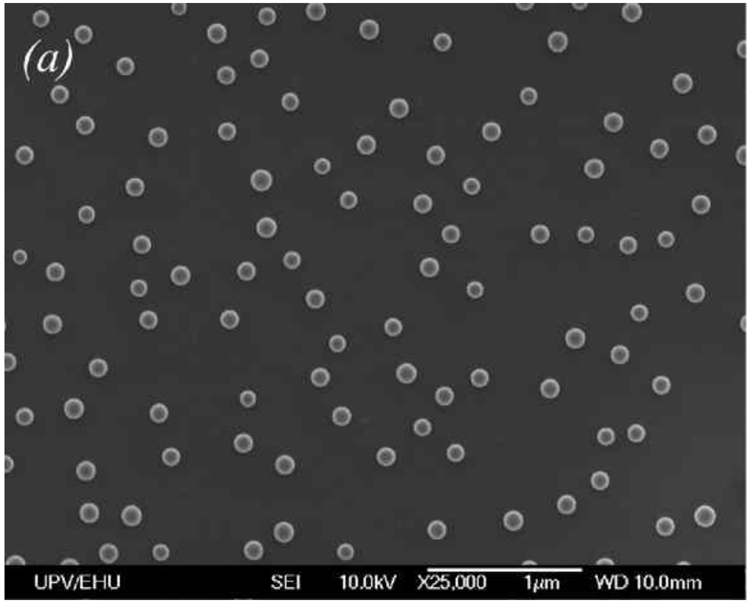
1) deposit a PMMA layer (spin coating)

2) charge surface with PDDA



3) deposit charged spheres



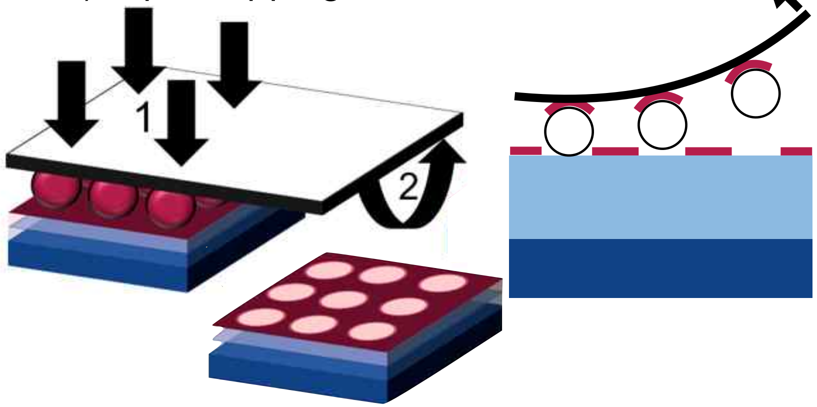


non-regular dense arrangement of nanospheres

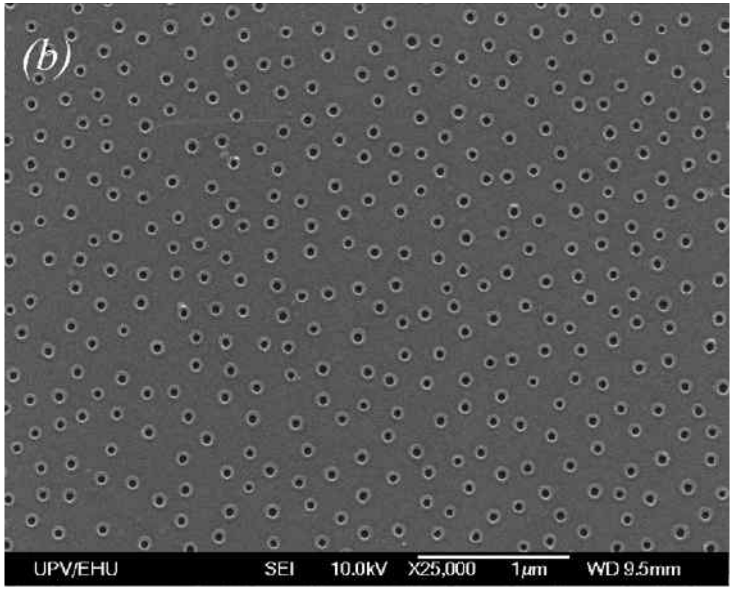
Hole-mask colloidal lithography

4) Ti sputtering

5) Tape stripping



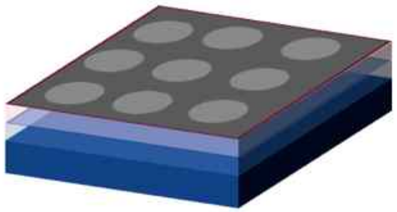
6) Oxygen plasma PMMA etching

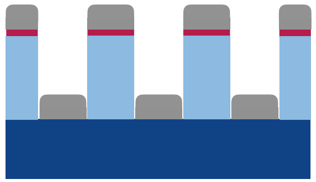


Ti template of holes

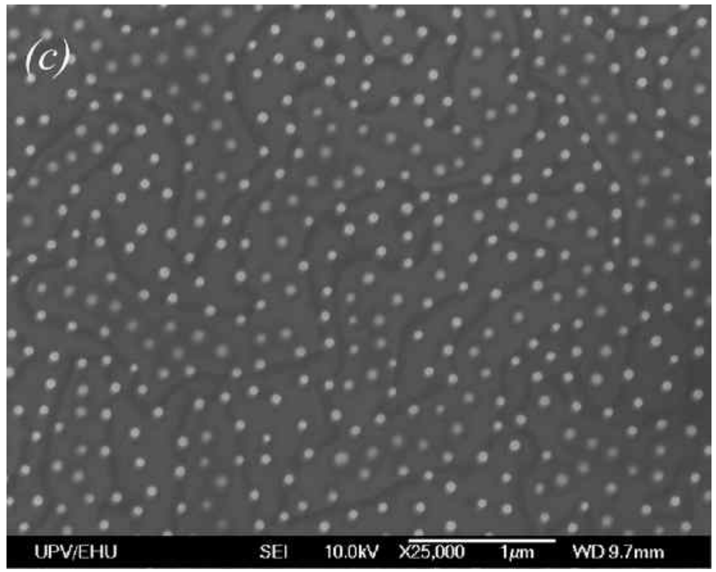
Hole-mask colloidal lithography

7) Py sputtering



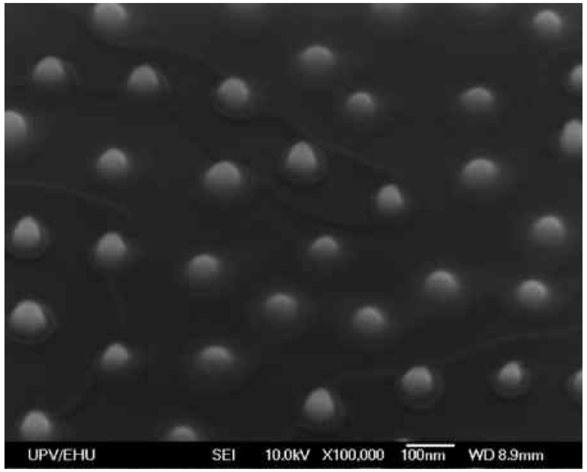


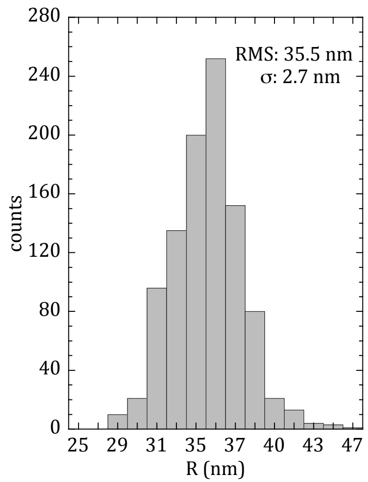
8) PMMA removal



Py nanodots on Si substrate

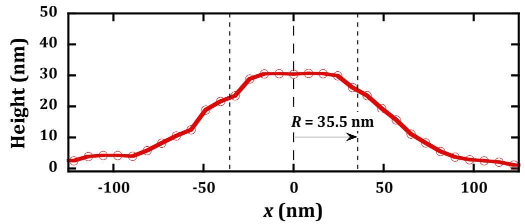
Morphology of the discs

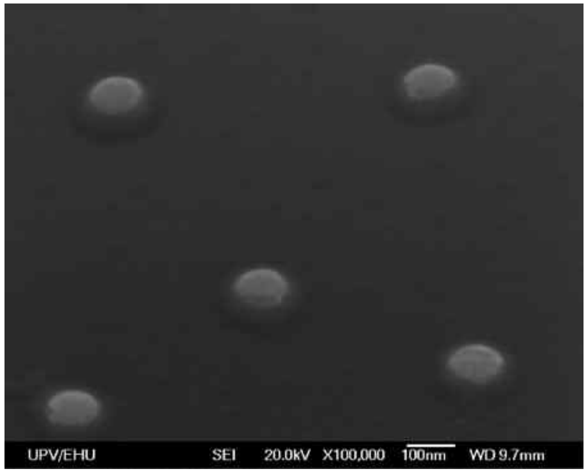


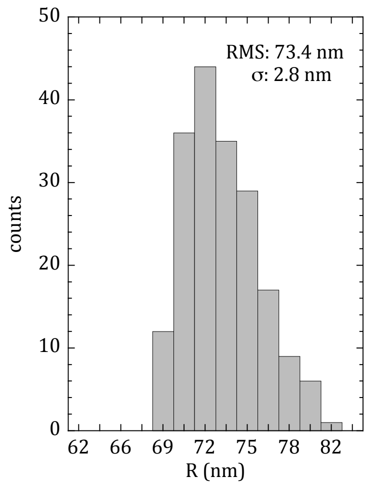


Radius = 30 nm

Thickness = 30 nm, 50 nm

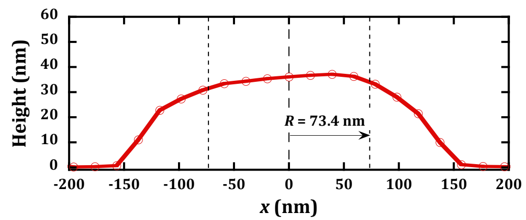






Radius = 70 nm

Thickness = 30 nm, 50 nm



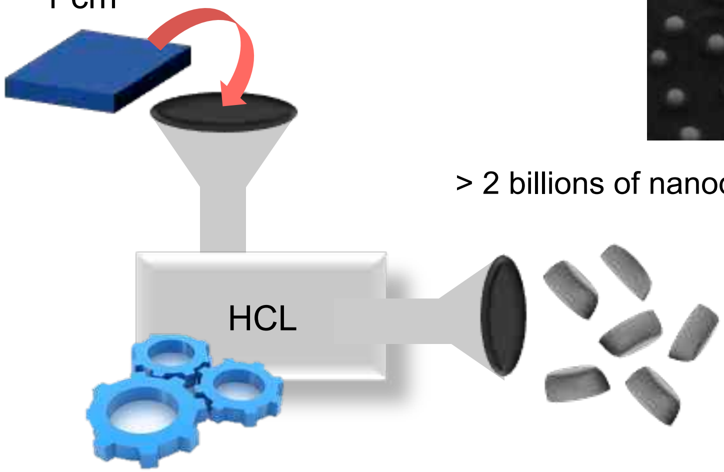
Production yield

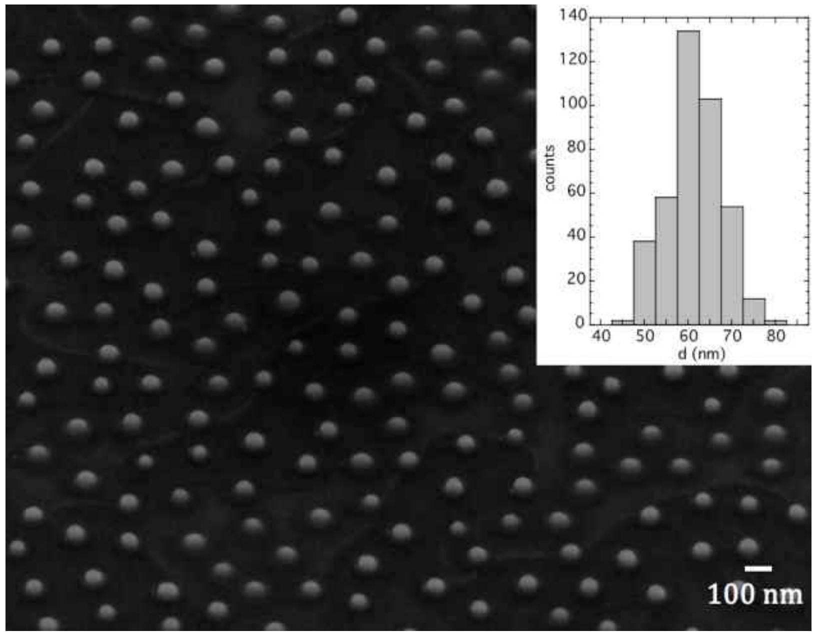
Estimated from SEM images

of discs

60 nm diameter, 50 nm thick

1 cm^2





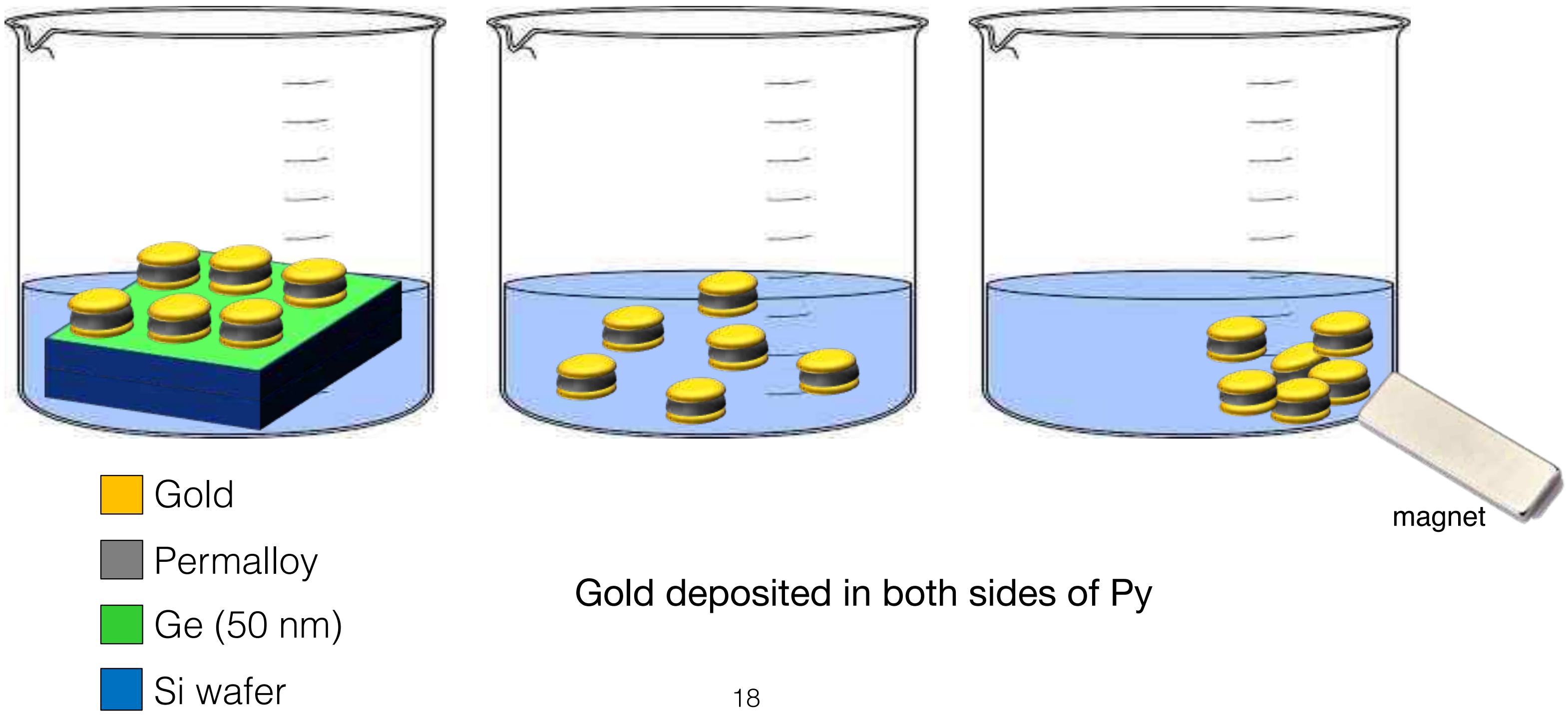
> 2 billions of nanodisks

0.1 mg
in a
2” wafer

enough for laboratory test!

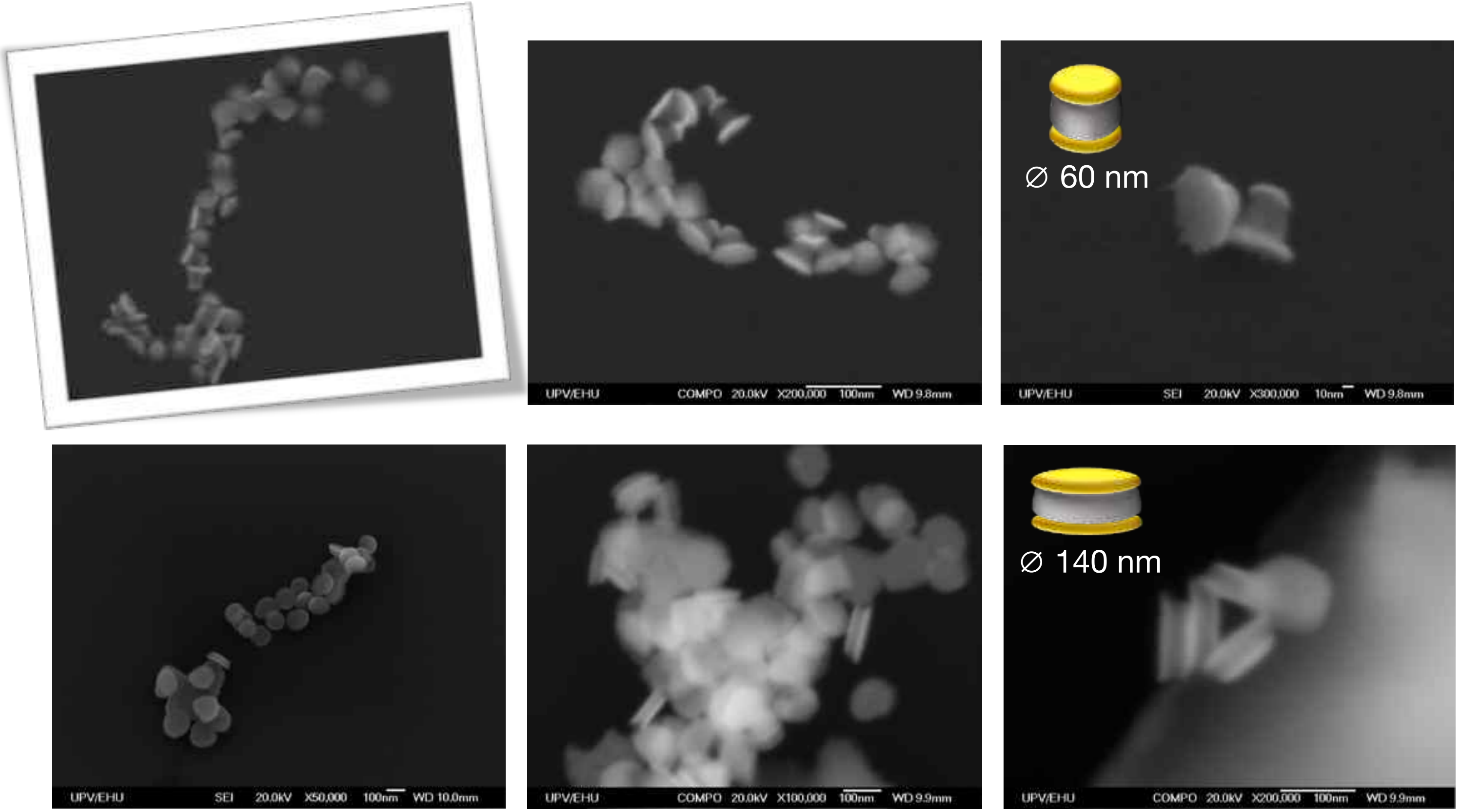
Substrate detachement

- HCL fabrication process performed onto a sacrificial layer
- Ge offers best results (Cu, SiO_2 also tested)
- Ge disolved in H_2O_2, releasing the discs
- Magnetic decantation for collecting them



Gold deposited in both sides of Py

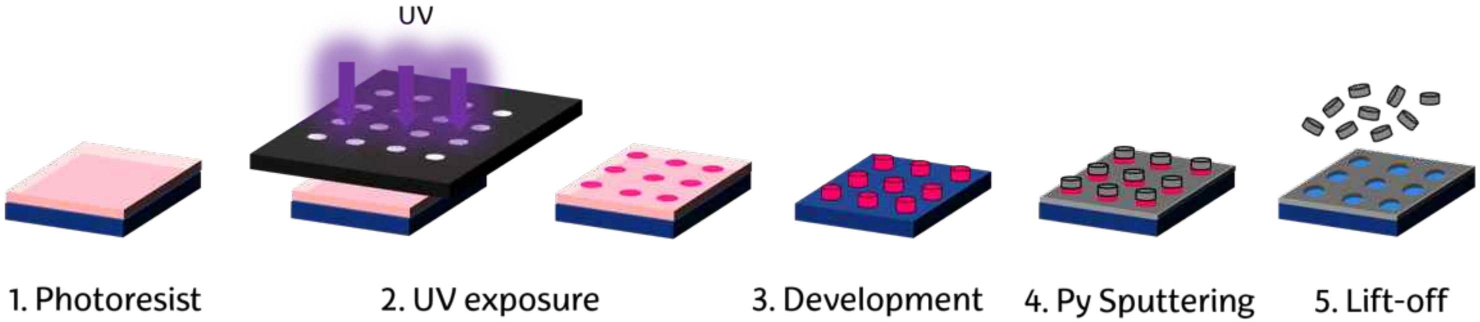
Substrate detachement

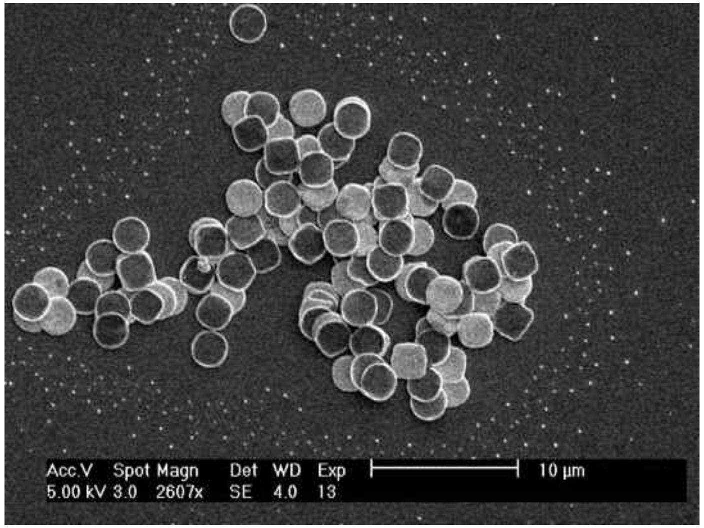


Micron-sized discs

Microdiscs (∅ 2 µm) Fabricated at Cambridge University (Prof. Cowburn)

Standard UV photolithography (lift-off)





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Magnetic Properties

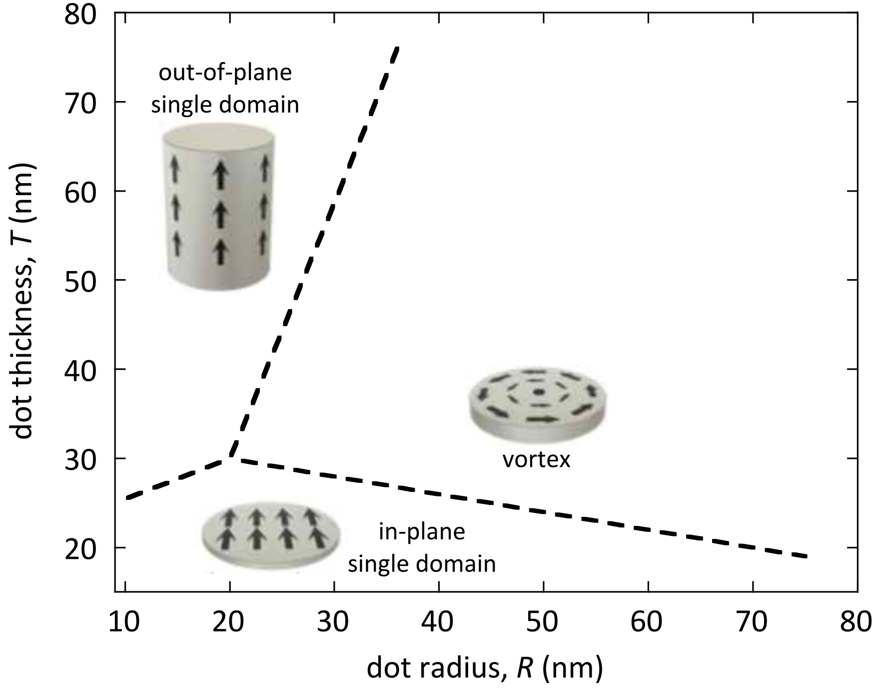
- **Magnetic phase diagram of Py discs**
- **Magnetization process and hysteresis loops**
- **Structure of the vortex in small dots**

Use of different techniques

- **MOKE** 20 μm spot size ➜ averages hundreds of discs
- **SQUID** average the whole sample
- **MFM** A. Asenjo, ICM-CSIC Madrid
- **Micromagentic simulations** OOMMF

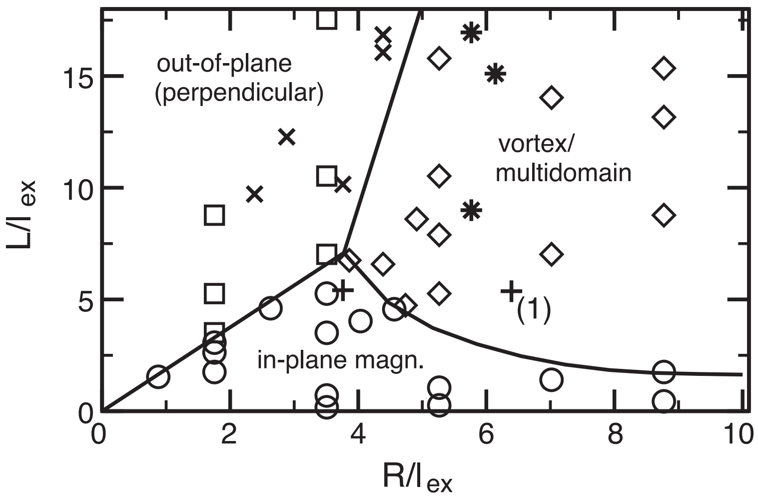
Magnetic phase diagram

The magnetic ground state of Py discs depends of the aspect ratio



Magnetic phase diagram

Experiment, theory and simulation

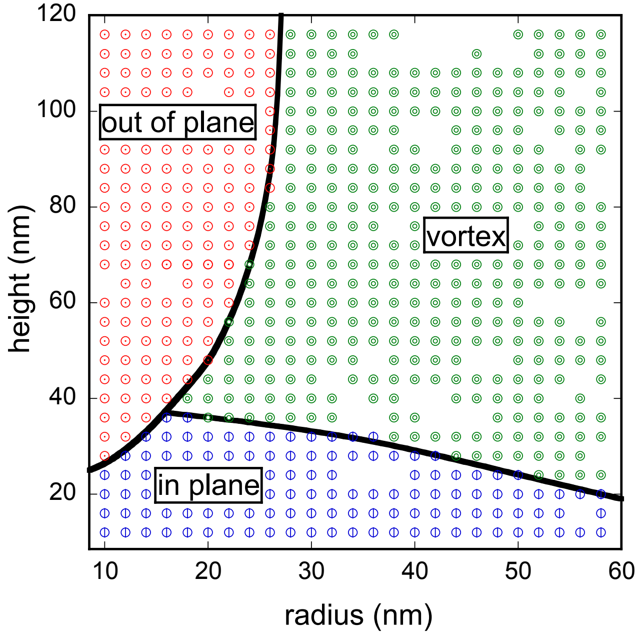


W. Scholtz *et al.* J. Magn. Magn. Matter. **266**, 155 (2003)

K.L. Metlov, K.Y. Guslienko J. Magn. Magn. Matter. **242-245**, 1015 (2002)

S.-H. Chung *et al.* PRB **81**, 024410 (2010)

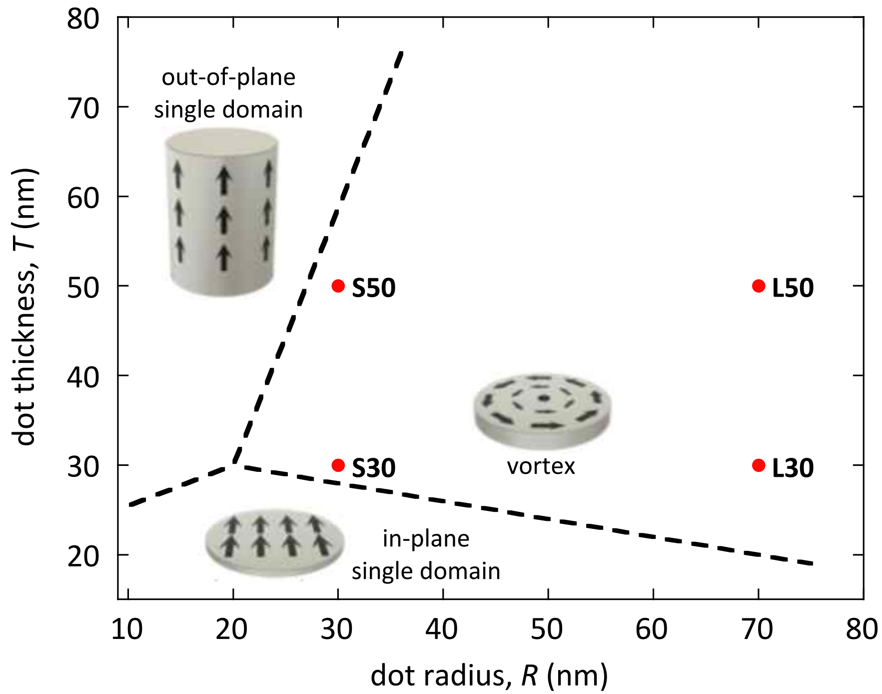
Micromagnetic simulation



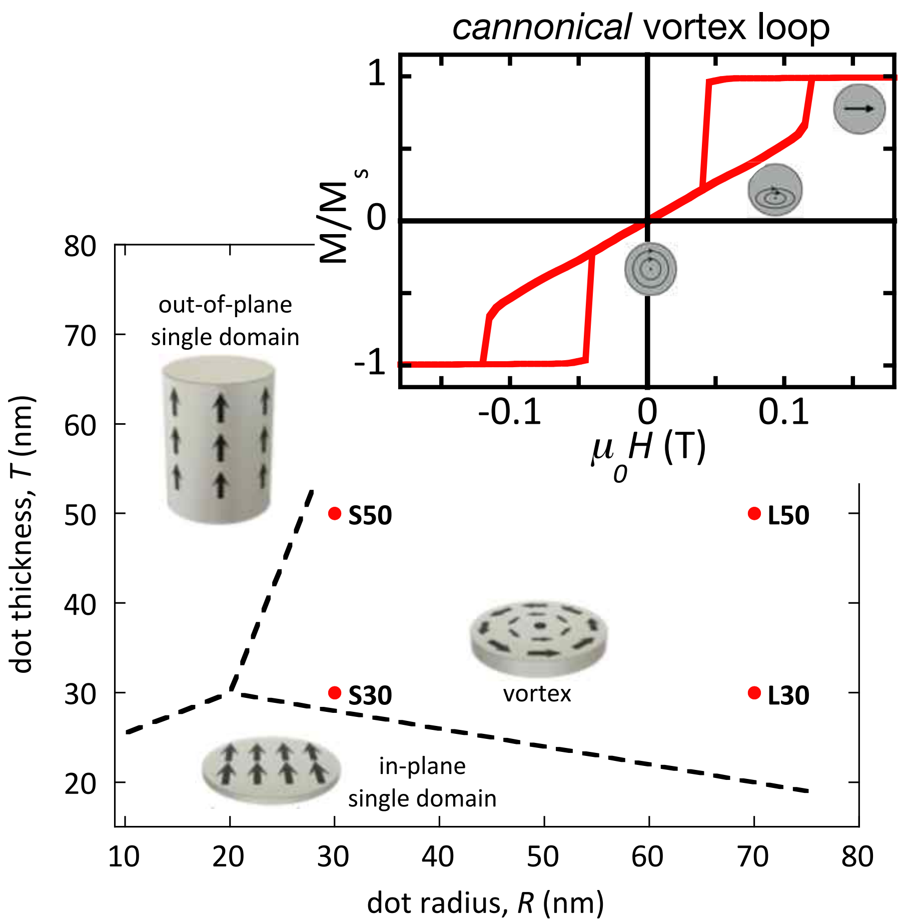
M. Goiriena-Goikoetxea *et al.* Nanotechnology **27**, 175302 (2016)

Magnetic phase diagram

Small Py discs studied in this work are close to boundaries



Magnetization process



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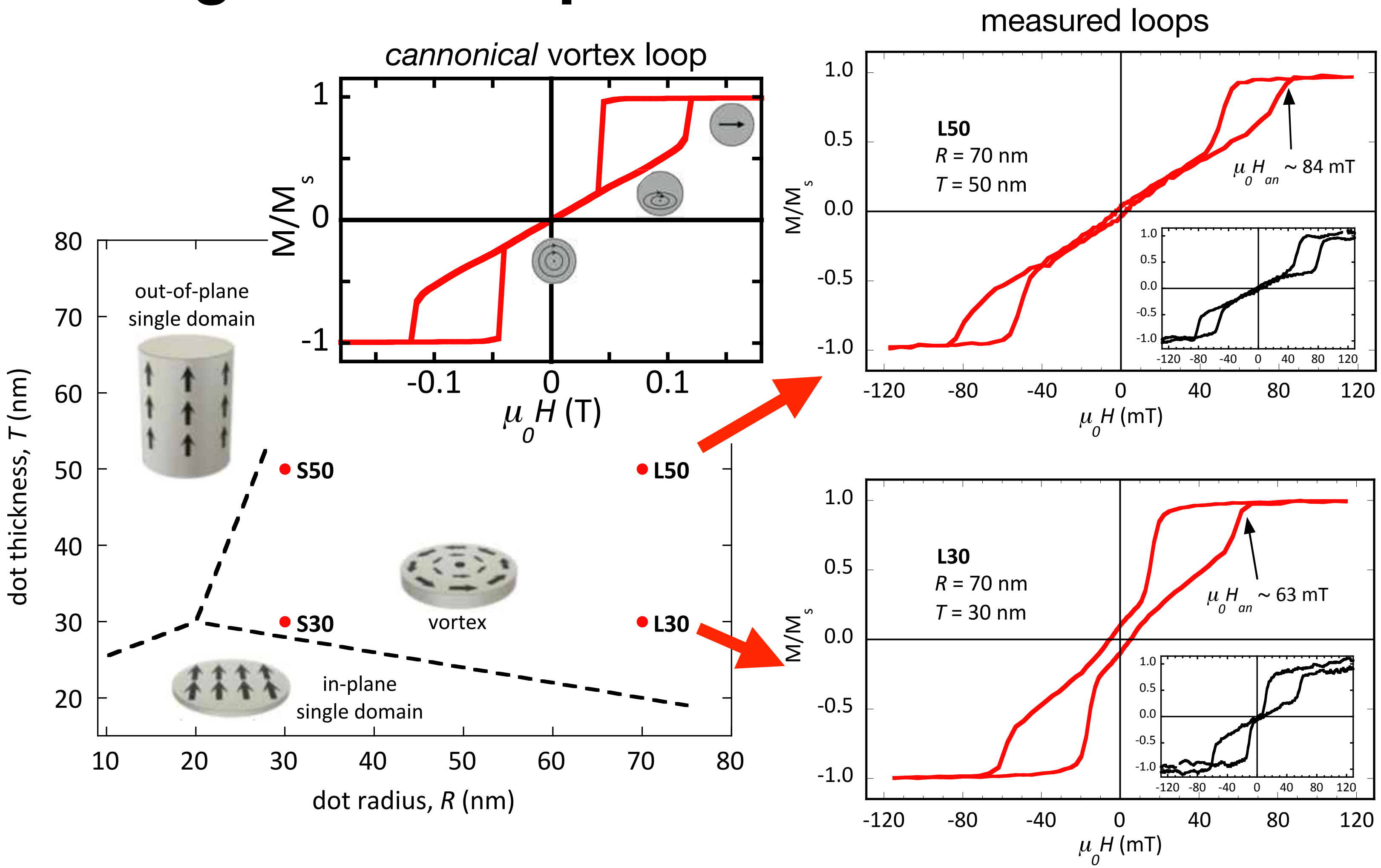
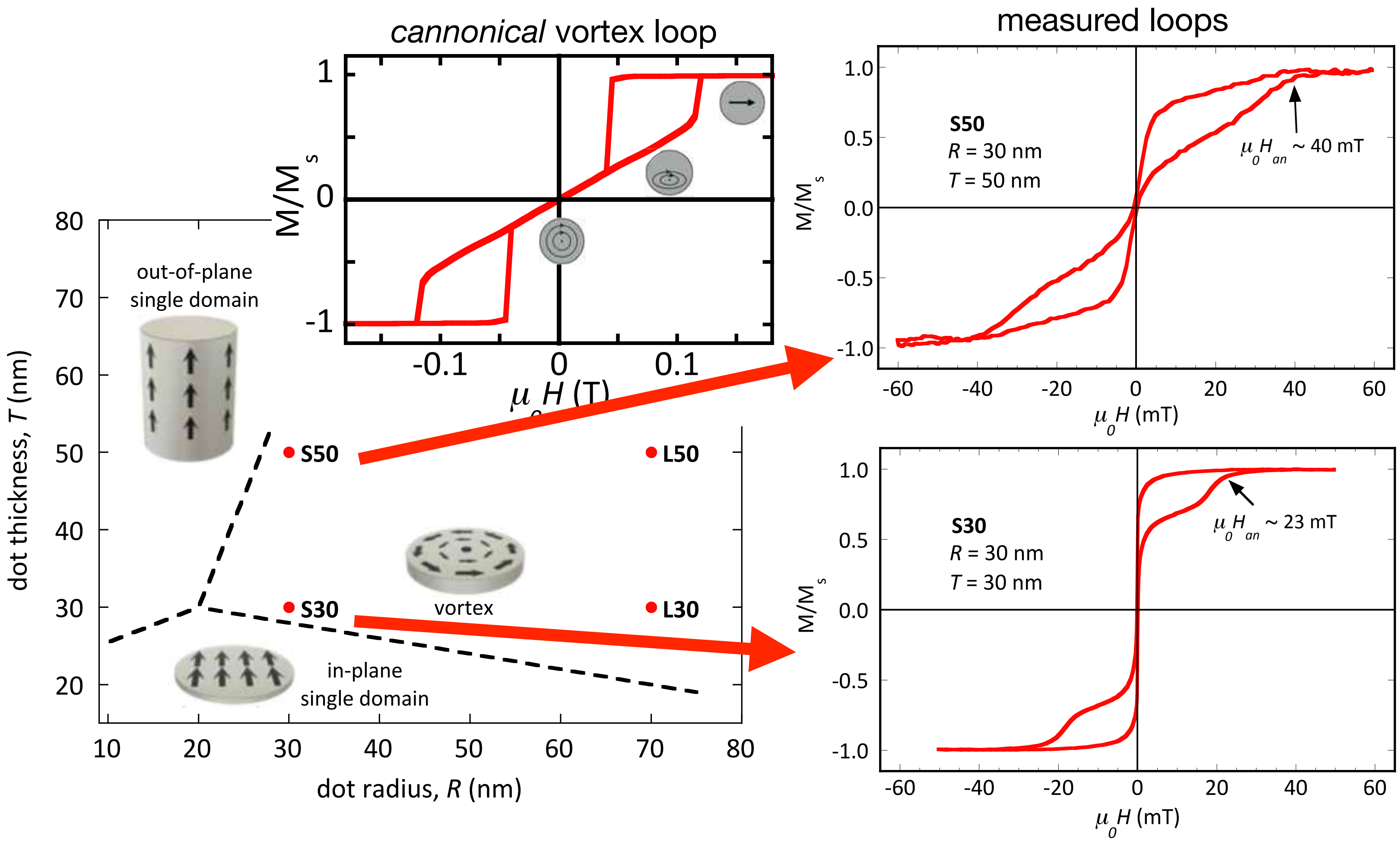
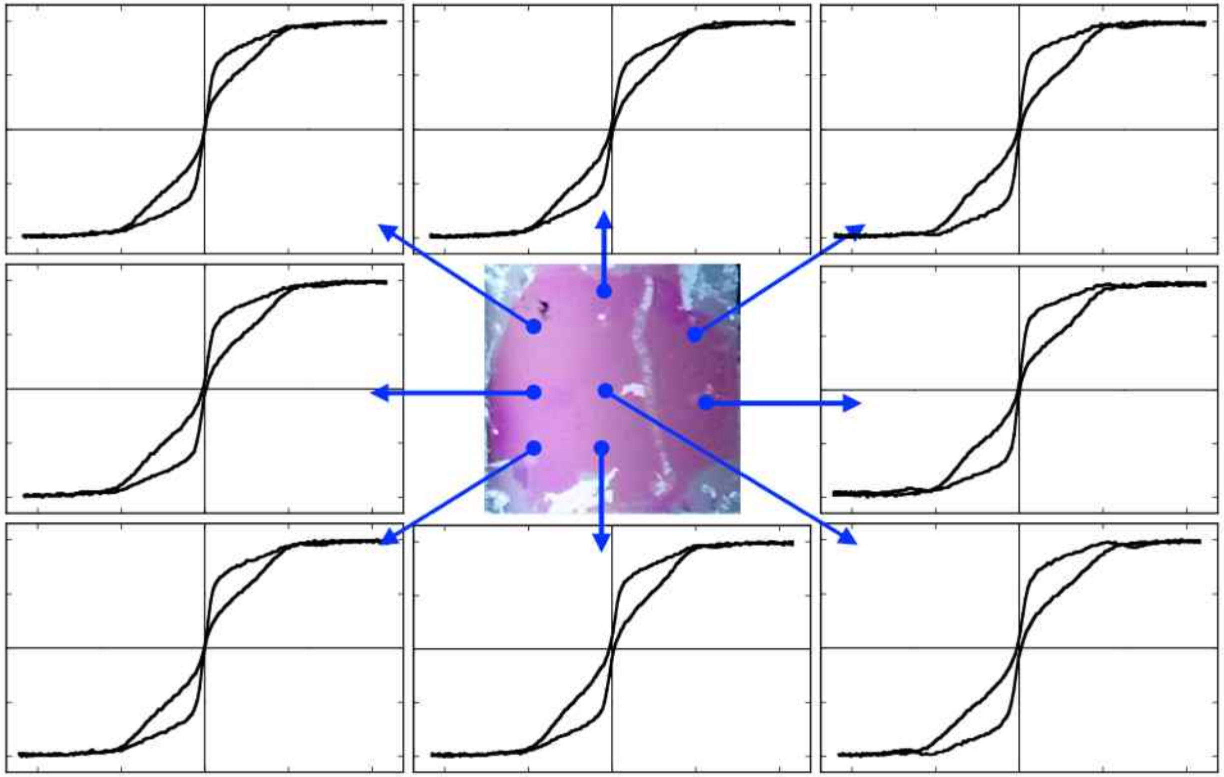


Magnetization process



Uniformity of patterning

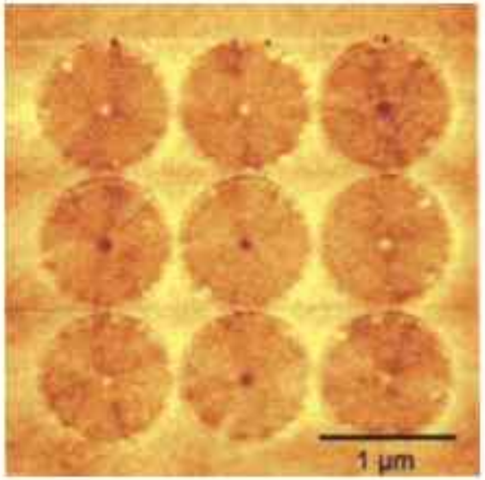
MOKE hysteresis loops



Large vortex core

Influence of the core size in the magnetization process

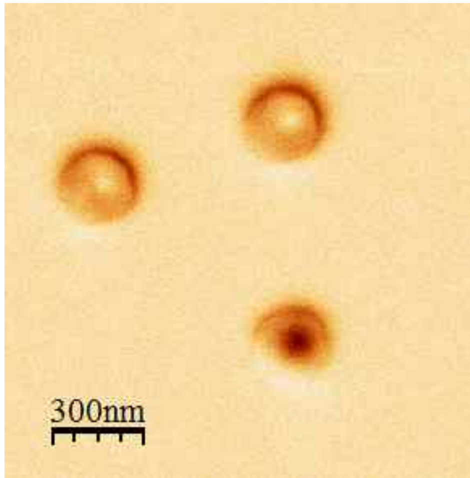
Py discs R = 500 nm



T. Shinjo *et al.* Science **289**, 930 (2000)

$c = R_c/R$

Py discs R = 70 nm



M. Goiriena-Goikoetxea *et al.* Nanoscale **9**, 11269 (2017)

R_c ~ 20-30 nm

c << 1, *classical* vortex

negligible core contribution to magnetization

R_c ~ 20-30 nm

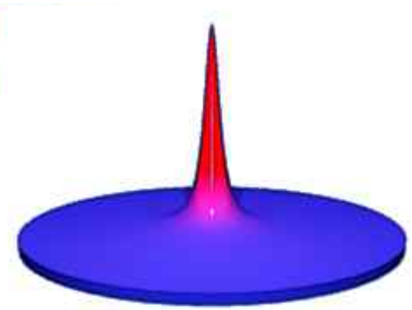
c < 1, *large* vortex core

non-negligible core contribution to magnetization

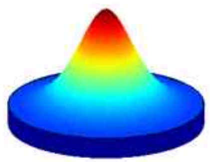
Large vortex core

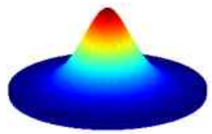
The size of the core can be determined by micromagnetic simulations

profiles of out-of-plane component of the magnetization

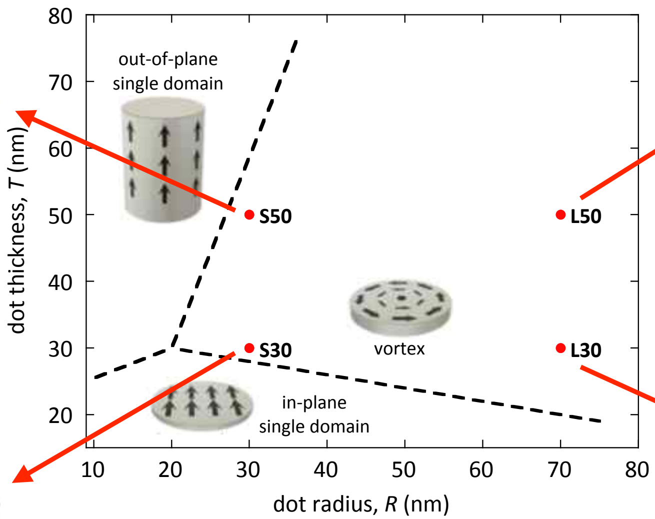


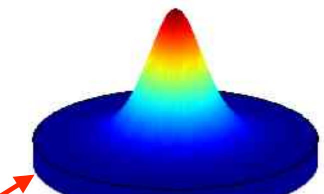
$c \ll 1$, *classical* vortex



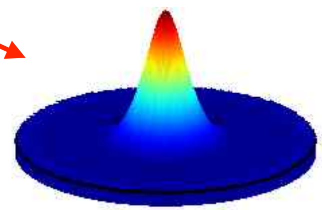


$c < 1$, *large* vortex core



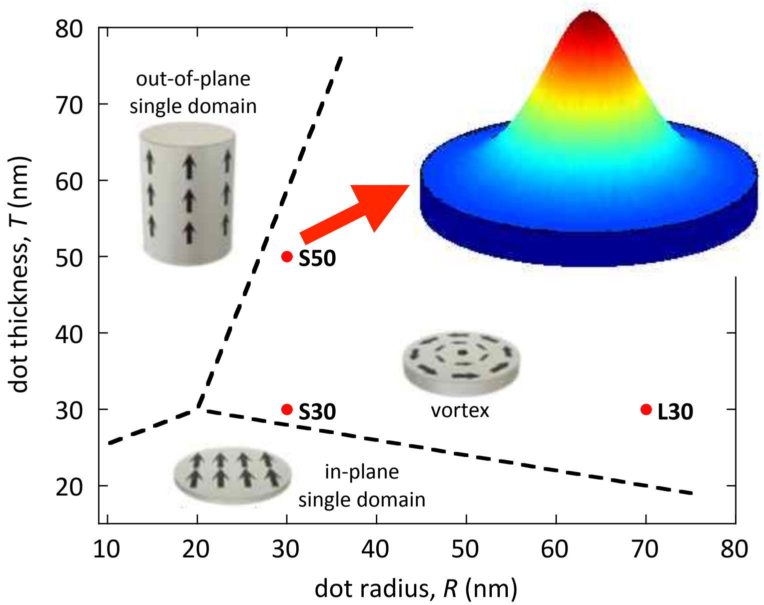


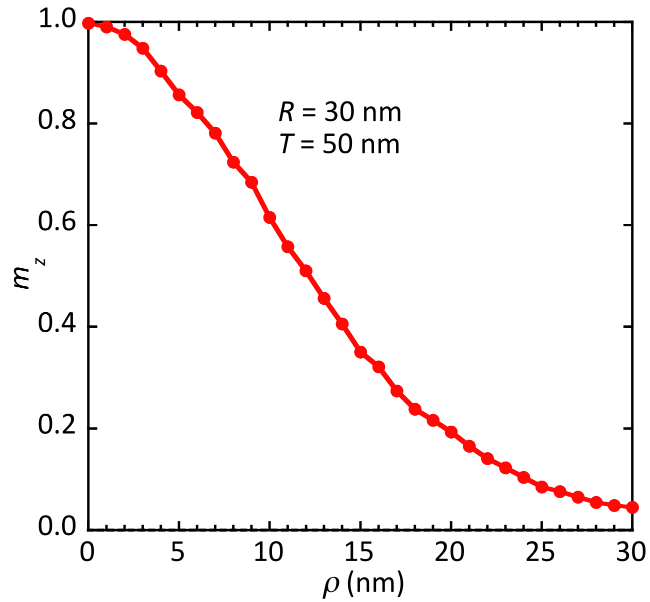
$c < 1$, *large* vortex core



$c < 1$, *large* vortex core

Large vortex core



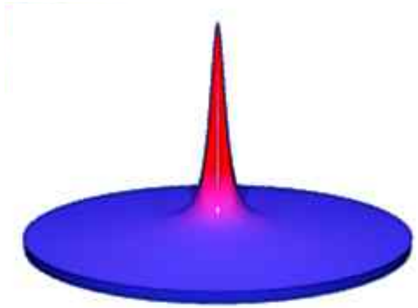


$R > R_c$

$c > 1$, *extra large* vortex core

Large vortex core

Analitical model of the magnetization process



c << 1, *classical* vortex

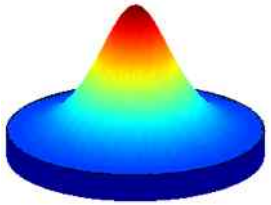
K. Y. Guslienko *et al.* PRB **65**, 024414 (2001)

Model neglects core magnetization

predicts the vortex annihilation field:

$$H_{an} = [4\pi F_1(\beta) - (l_{ex}/R)^2]M_s.$$

($\beta = T / R$)



c **>** 1, *extra large* vortex core

New analytical model,
including core magnetization

$$H_{an}(c, \beta, R) = \frac{(1+c^2)}{2c}\kappa(c, \beta, R)M_s$$

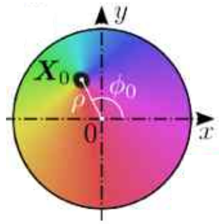
M. Goiriena-Goikoetxea, K. Y. Guslienko *et al.*
Nanoscale **9**, 11269 (2017)

Large vortex core

Summary of the calculation procedure

- Start from energy density $w = A(\nabla m_\alpha)^2 - \frac{1}{2}\mu_0 M_s \mathbf{m}\mathbf{H_m} + w_H$

  exchange + magnetostatic + Zeeman



- Model elaboration yields $w = w(c,s,H)$ $c = R_c/R > 1$ core size

  $s = \rho/R$ position of the core in the disc

- For each value of H, $\dfrac{\partial w}{\partial s} = 0$ gives the equilibrium position of the core s_0.

  - Annihilation field H_{an} is the field at which the core is at the border of the disc $s_0 = 1$.

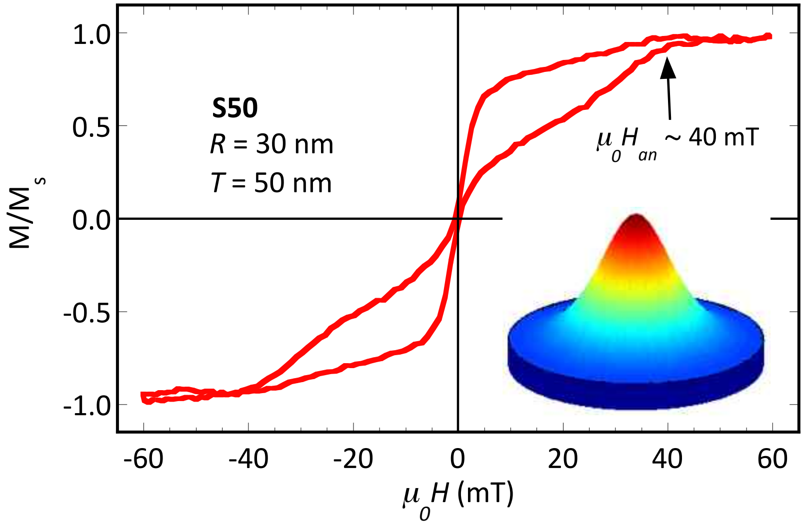
$$H_{an}(c, \beta, R) = \frac{(1+c^2)}{2c}\kappa(c, \beta, R) M_s$$

- The equilibrium core size c_0 is calculated from $\dfrac{\partial w}{\partial c} = 0$

  with vortex at the center of the disc $s = 0$.

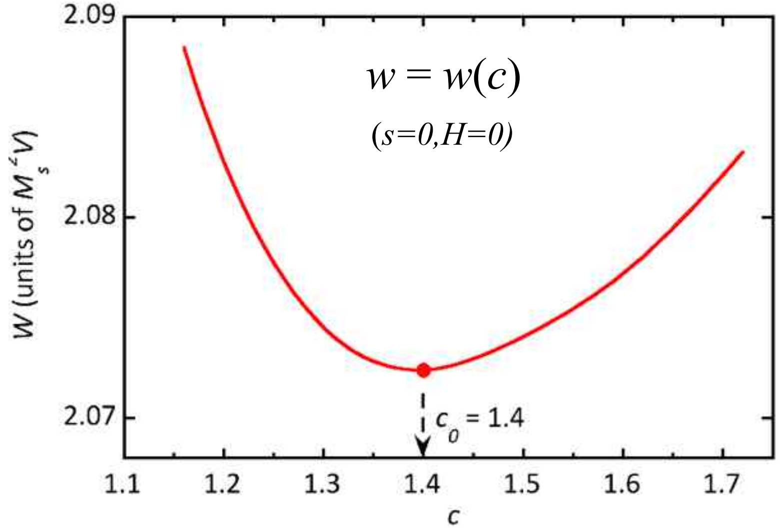
Large vortex core

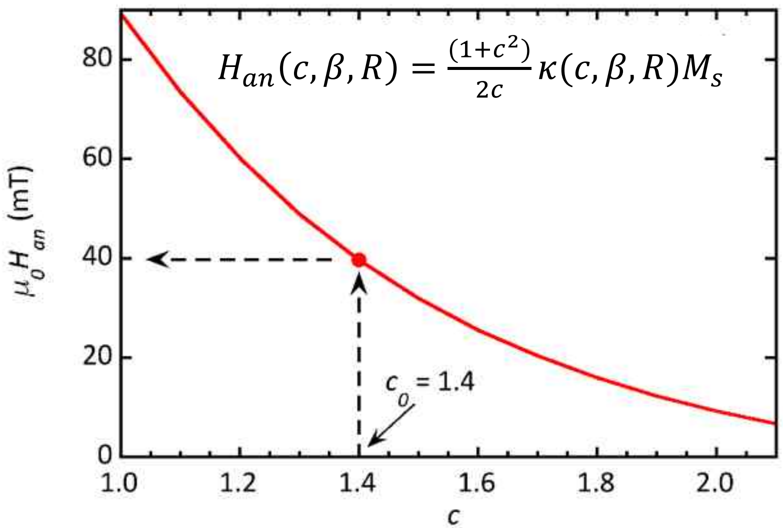
Comparison with experiment



$\mu_0 M_s = 0.75\ \mathrm{T}$

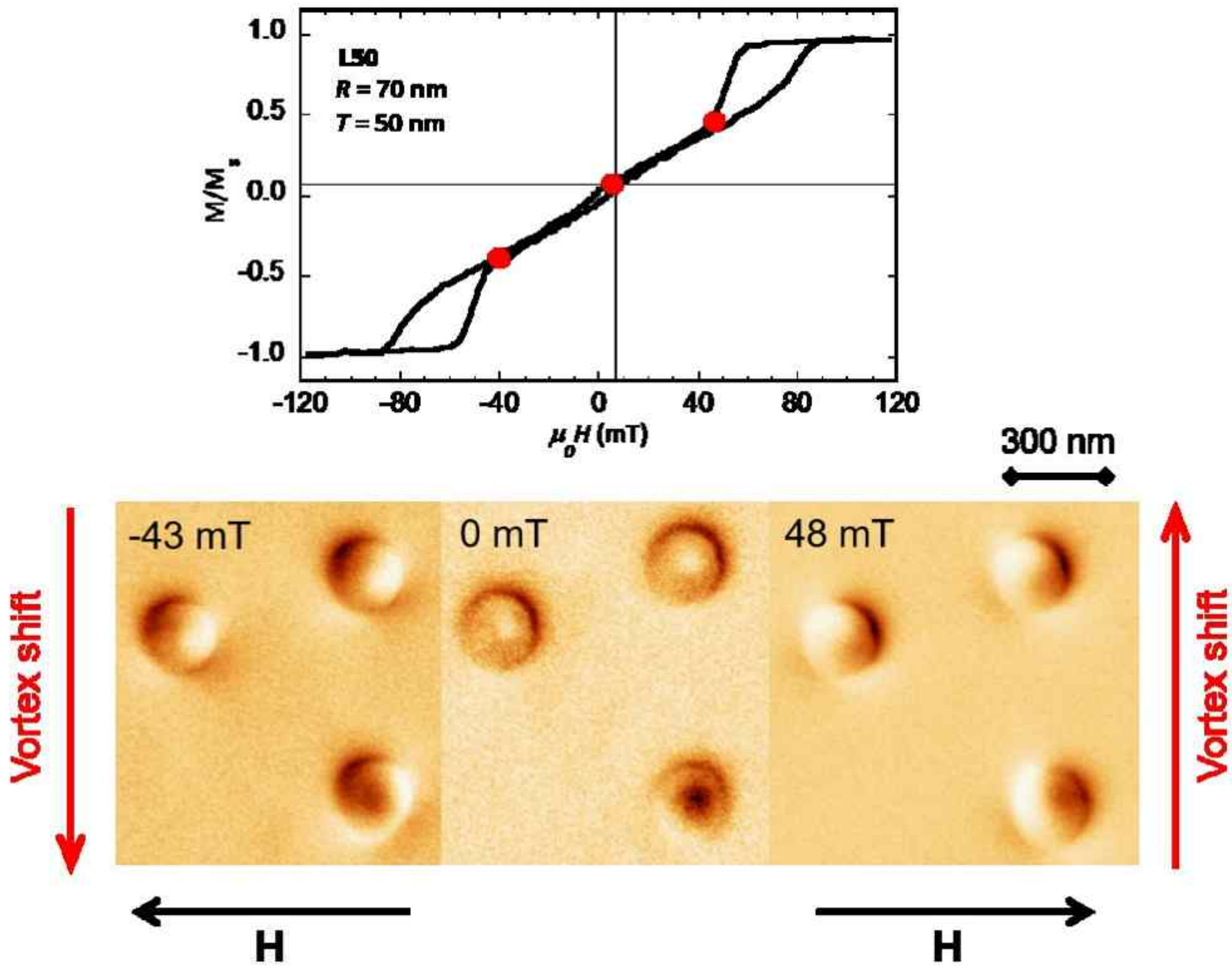
$\beta = 5/3$





Additional MFM results

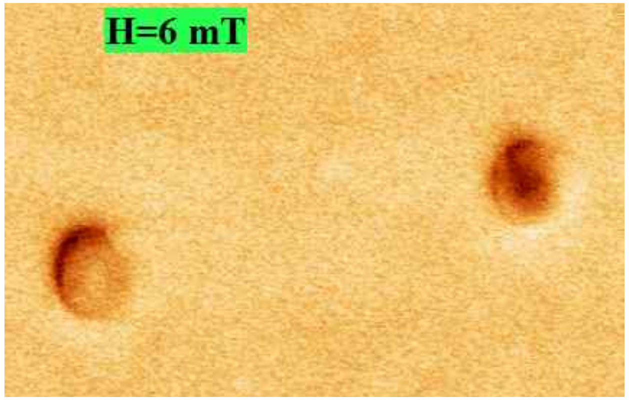
Movement of the vortex core

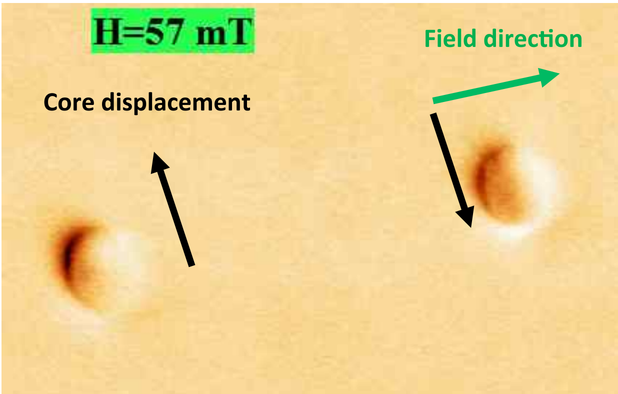


Additional MFM results

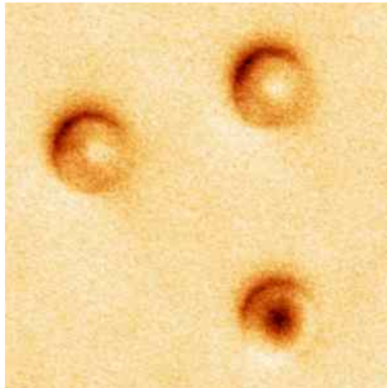
it is possible to distinguish the quirality and the polarity of the vortex

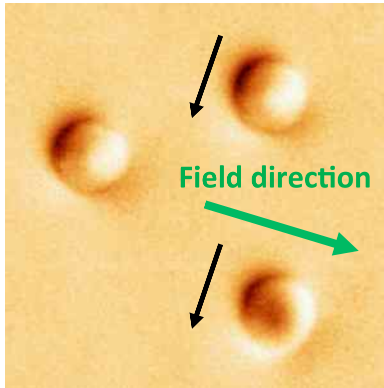
different polarity, diferent quirality





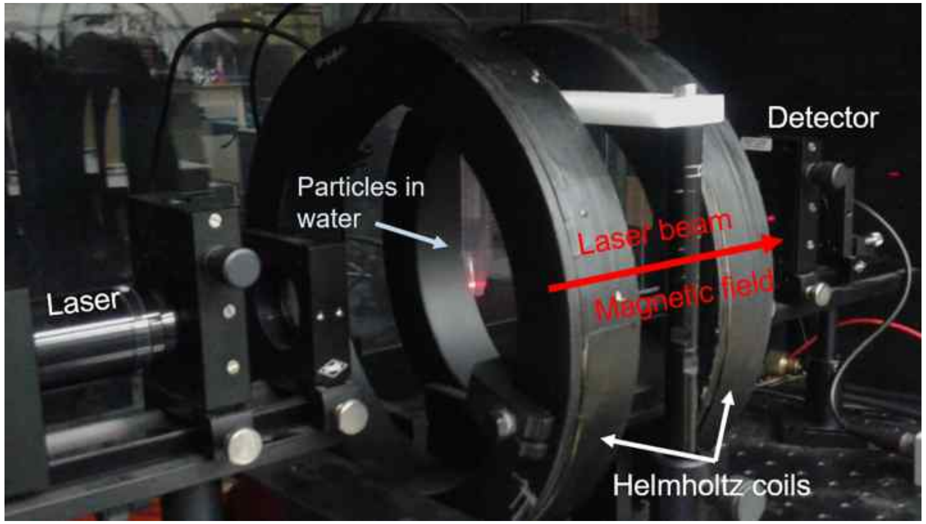
different polarity, same quirality

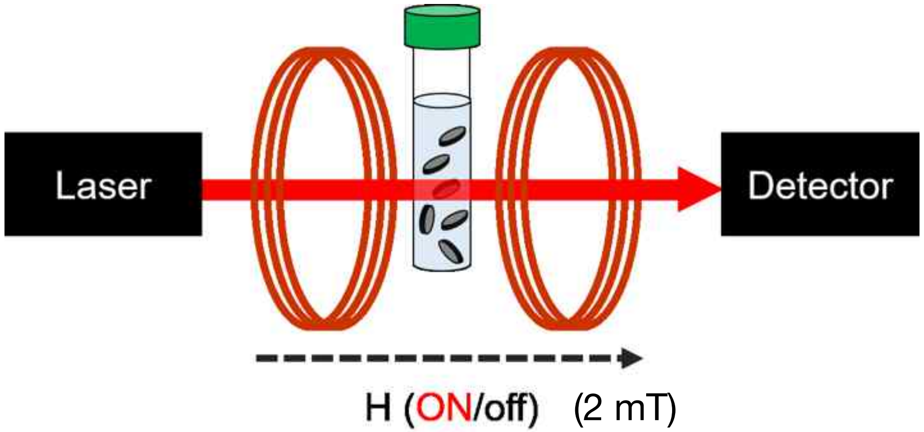




Magneto-mechanical actuation

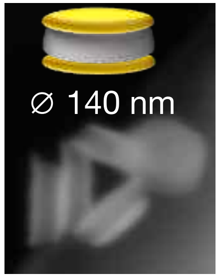
Light transmission experiments



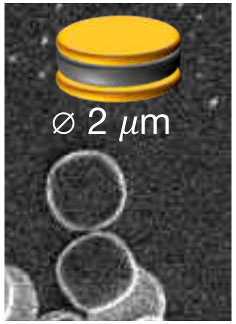


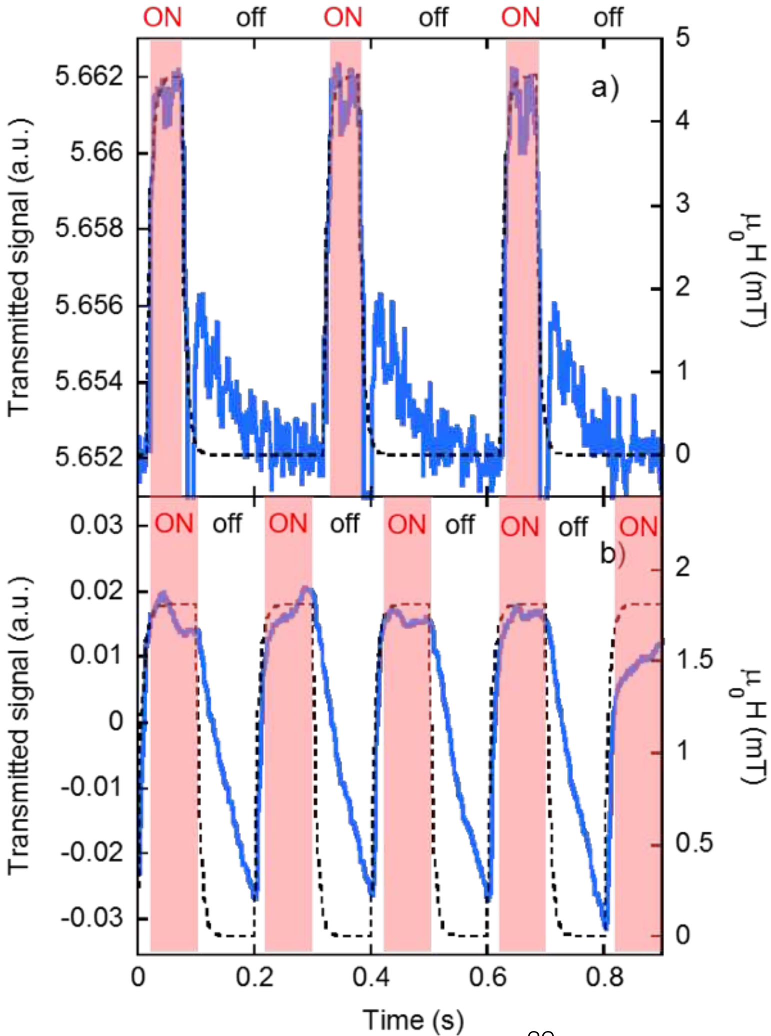
Magneto-mechanical actuation

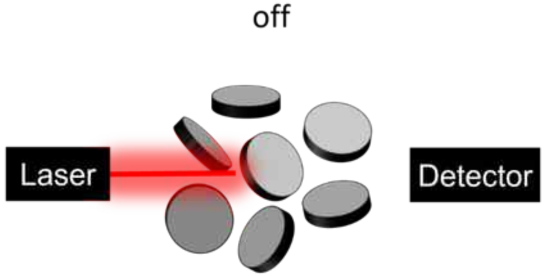
nanodiscs

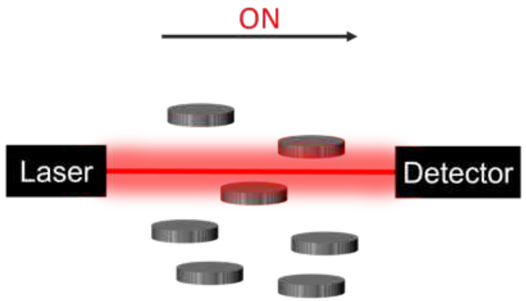


microdiscs







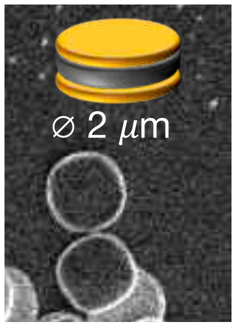


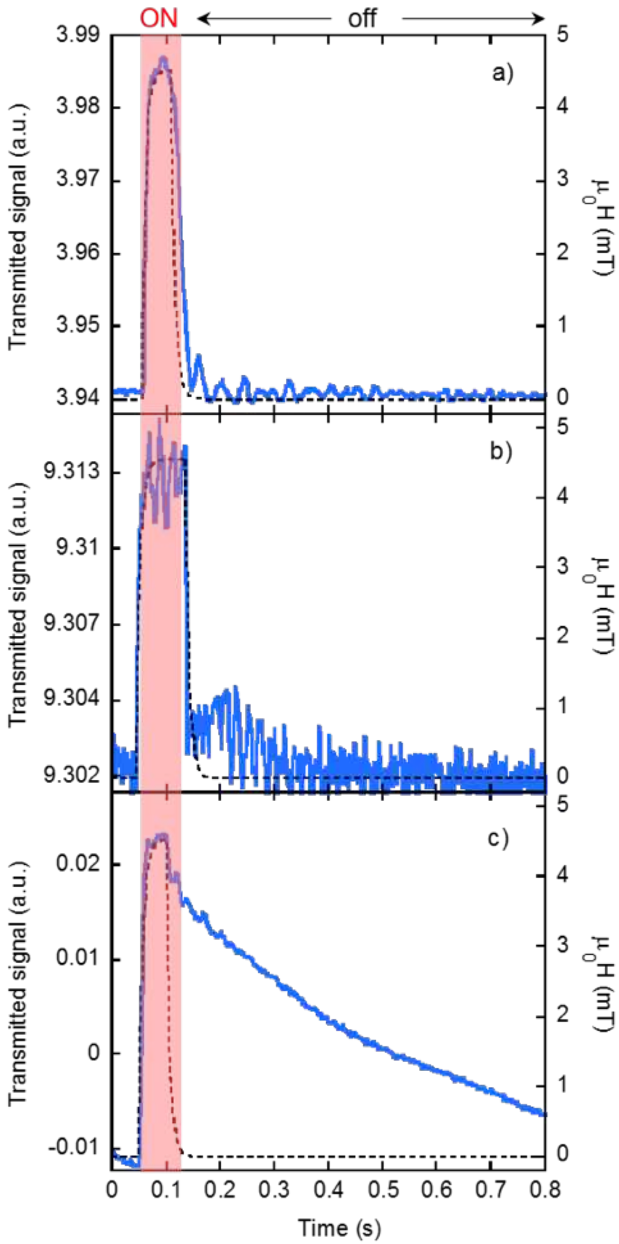
spherical Fe_3O_4 particles

∅ 35 nm

nanodiscs

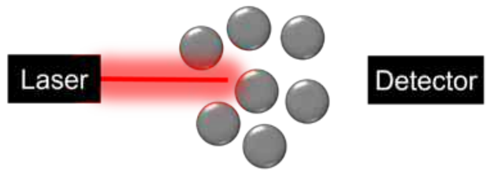
microdiscs



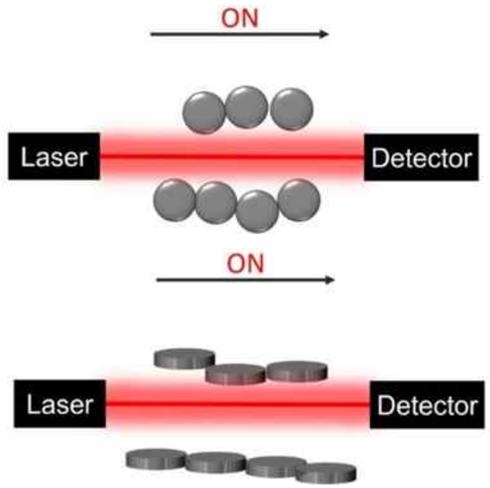


should be no difference!

off / ON



chaining effect



(already observed in microdics)

S. Leulmi *et al.* APL **97**, 253112 (2010)

2 μm discs in solution. Variable magnetic field.

Video courtesy of Selma Leulmi, University of Cambridge, UK.

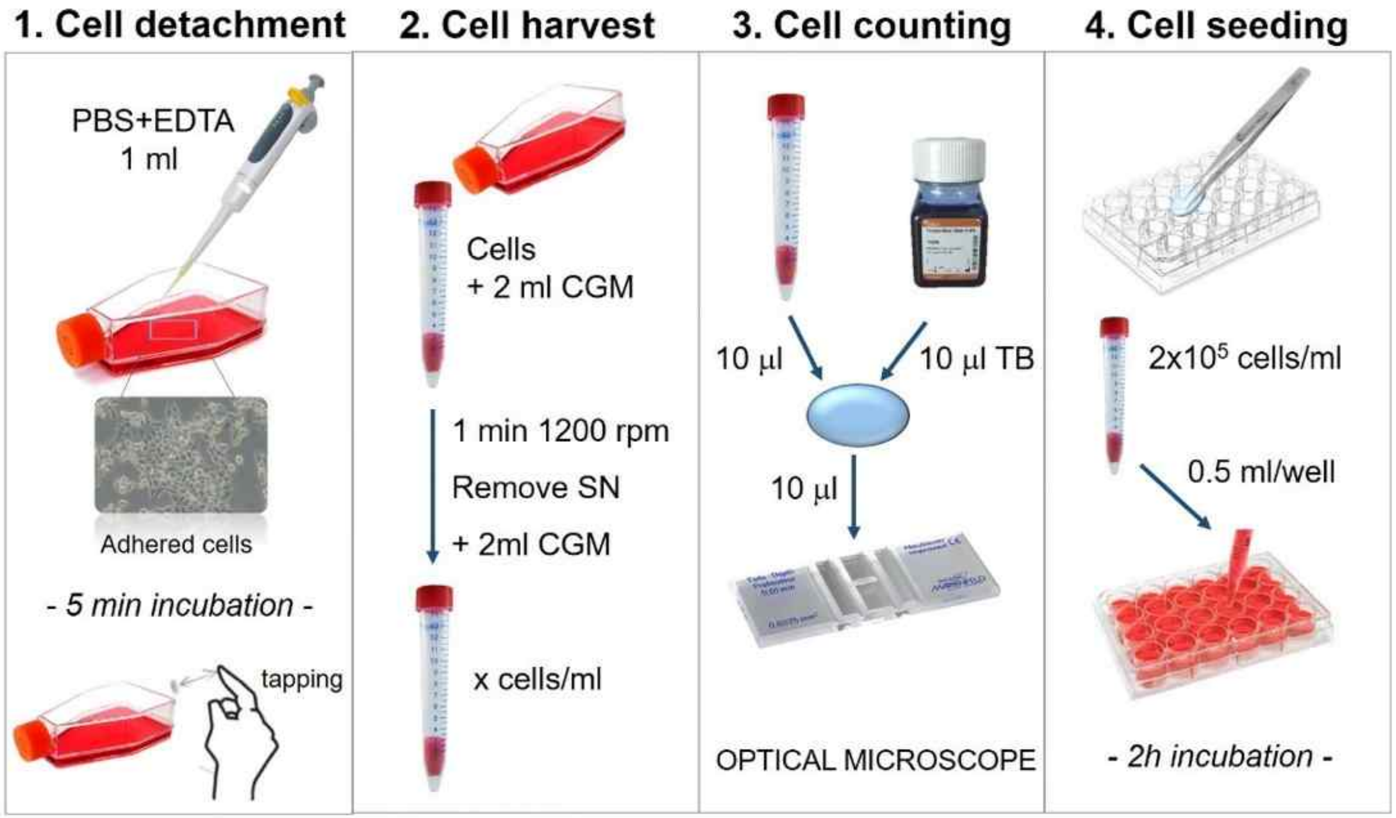
Outline

- **Introduction**
  - Motivation
  - Magnetic vortex
  - Objetives of the work
- **Fabrication of the discs**
  - Hole-mask colloidal lithography
  - Morphological characterization
  - Release procedure
- **Magnetic properties and actuation**
  - Magnetization process and phase diagram
  - Large vortex core
  - Magneto-mechanical actuation
- **Discs in cancer cells**
  - Intracellular intake
  - Cytotoxicity
  - Magneto-mechanical treatment

Discs in cancer cells

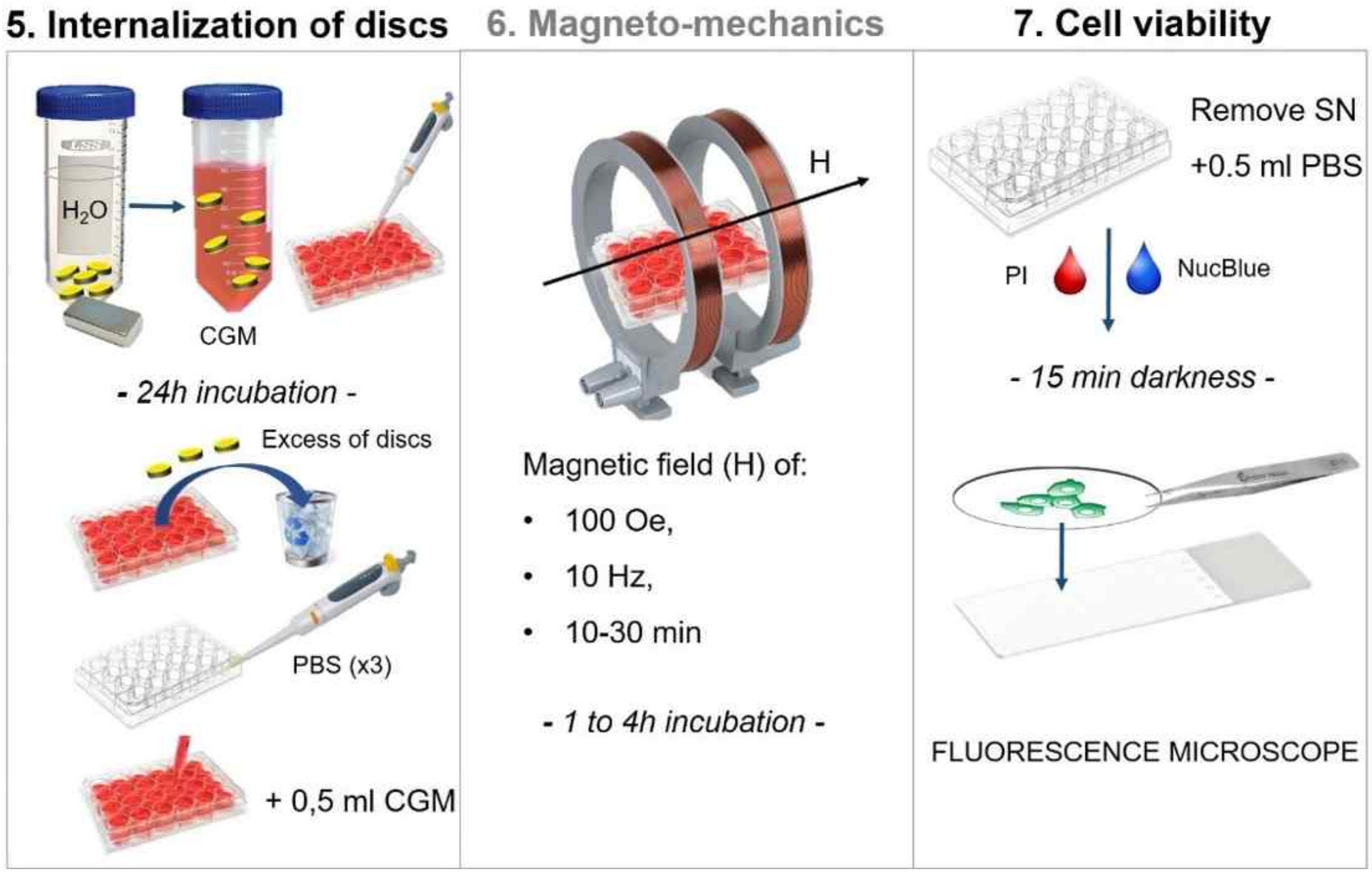
Interaction of { microdiscs (R = 1 μm, T = 60 nm) / nanodiscs (R = 70 nm, T = 50 nm) } with human lung carcinoma cells

Protocol of the in-vitro assays



Discs in cancer cells

Asses the effect of the discs and the alternating magnetic field

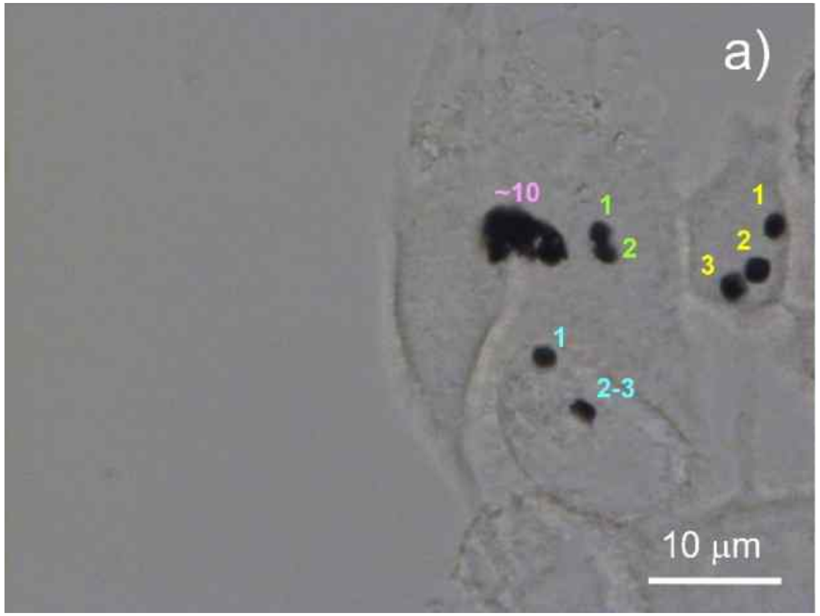


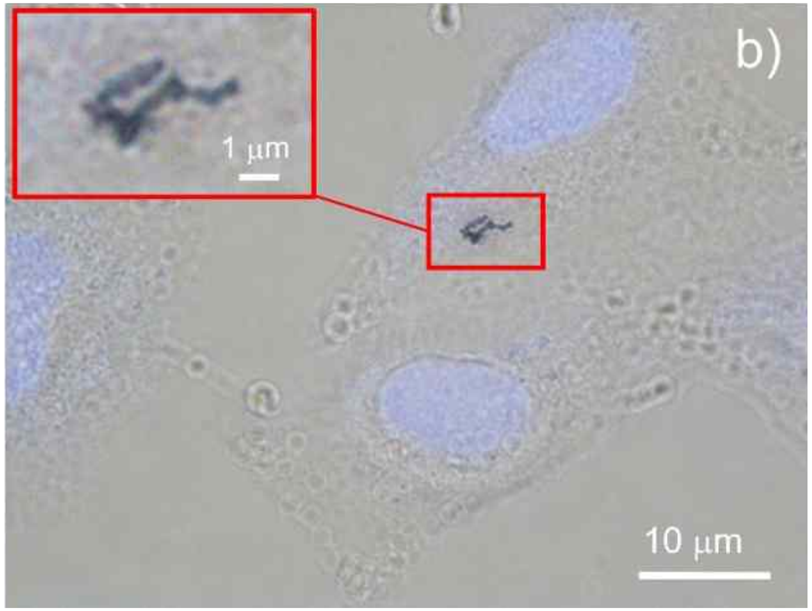
Intracellular intake of the discs

The same mass of disc is added to the wells

10 microdiscs/cells

2000 nanodiscs/cells



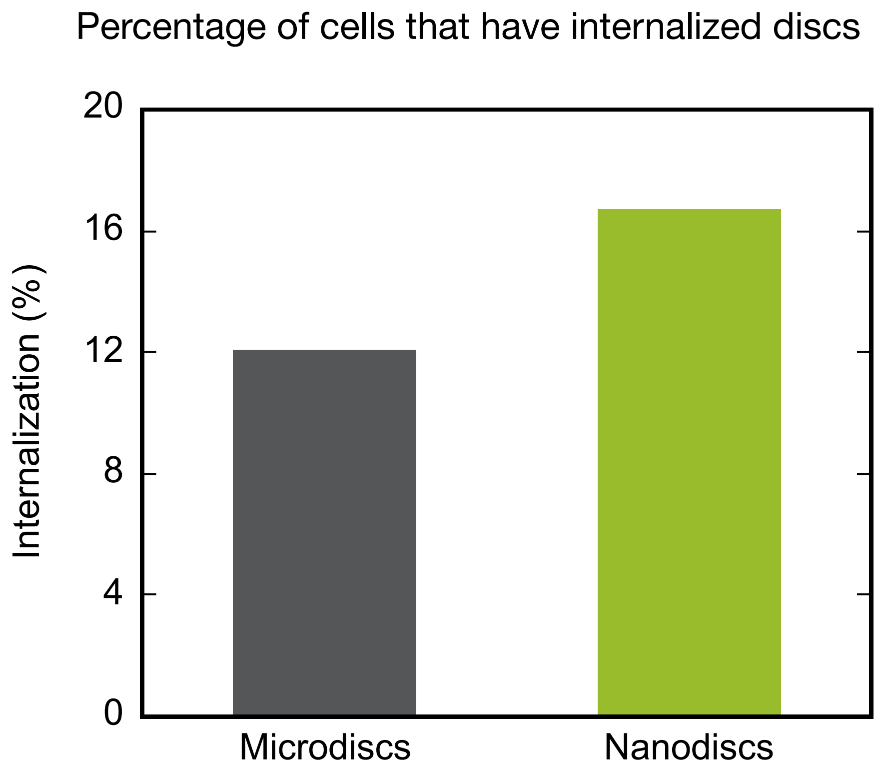


Even without functionalization, discs are internalized by the cells

mean count ~6 microdiscs/cells

mean count ~100 microdiscs/cells

Intracellular intake of the discs

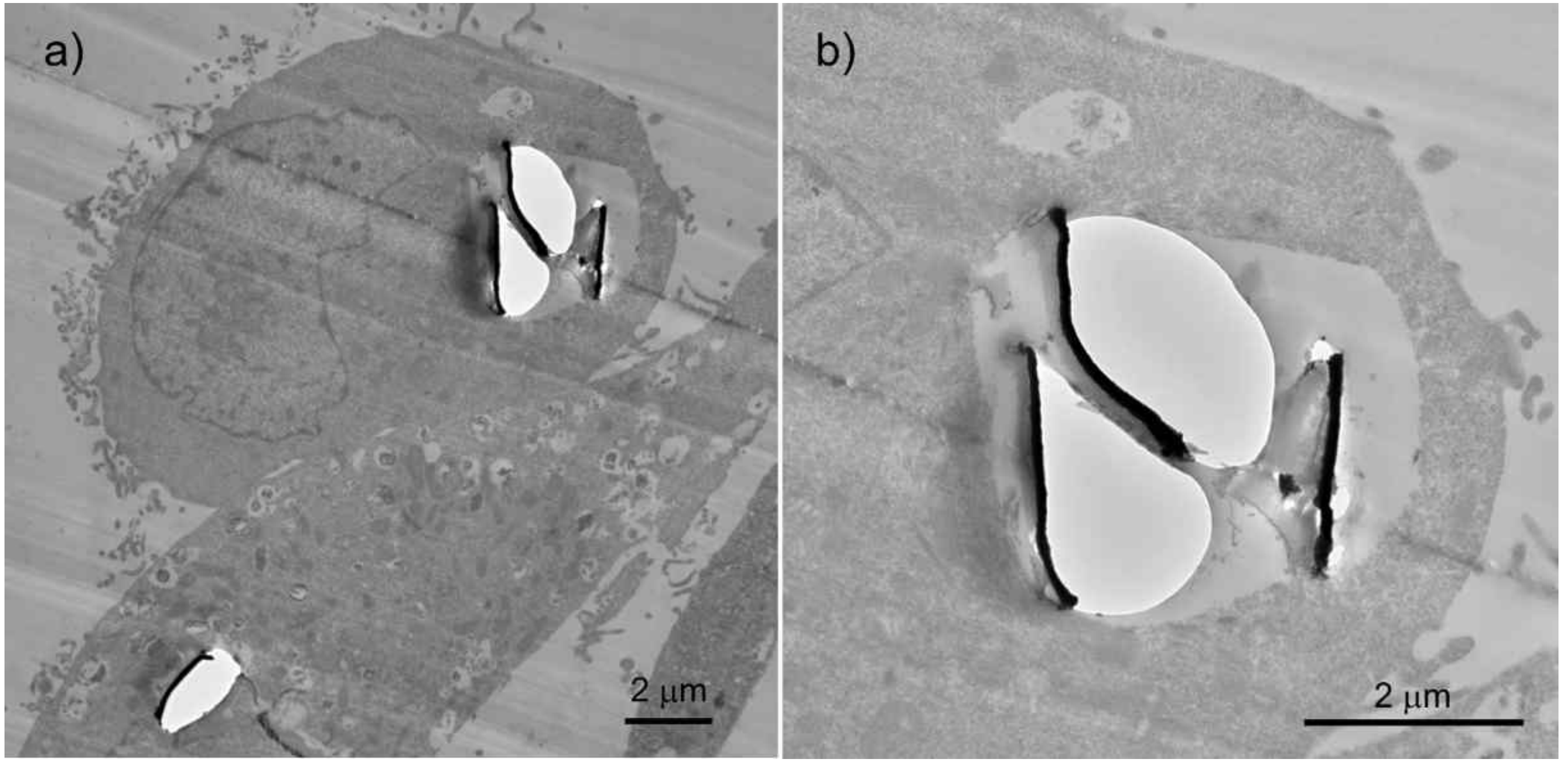


Nanodiscs seems to be easier in-taken by the cells, but

- nanodiscs are more difficult to count in the SEM images
- better distribution of the nanodiscs in the well

Intracellular intake of the discs

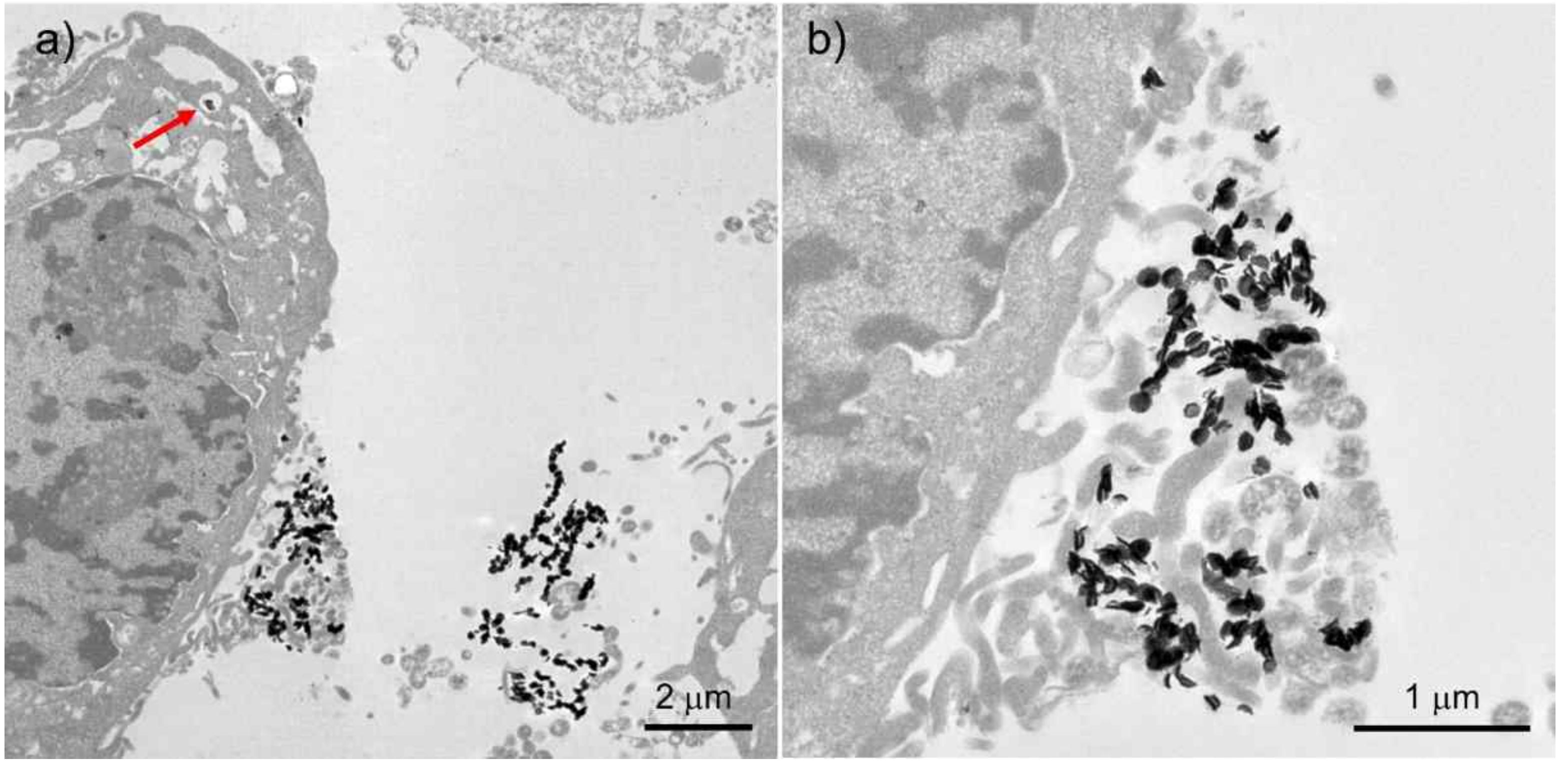
TEM images. Microdiscs inside cells.



Discs seems to be inside lysosomes.

Intracellular intake of the discs

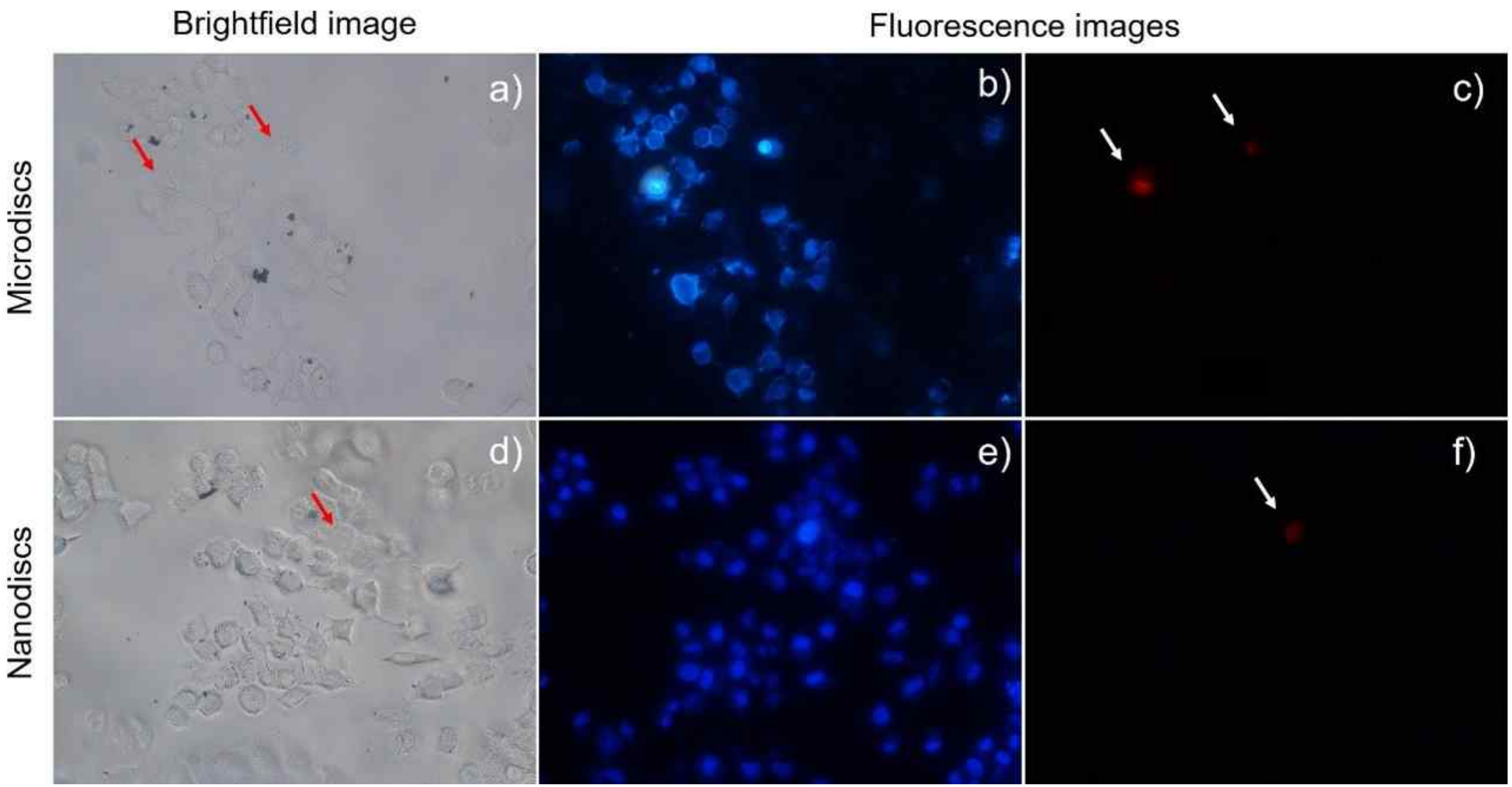
TEM images. Nanodiscs inside cells.



Discs inside a lysosome

Nanodiscs interacting with the membrane

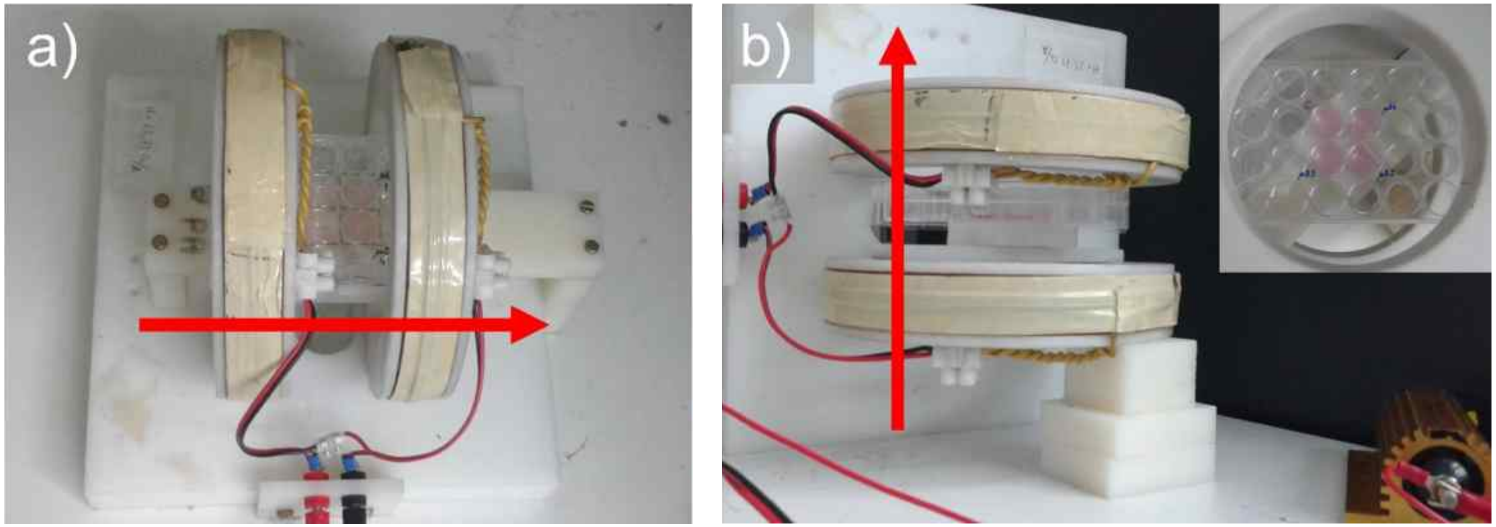
Cytotoxicity

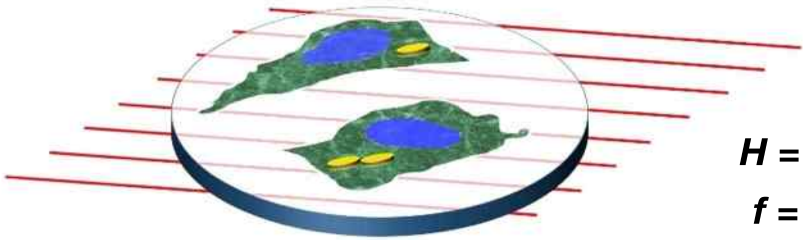


cells with discs all cells death cells

After 24 h incubation, nearly 100% of cells with discs survival

Magneto-mechanical treatment

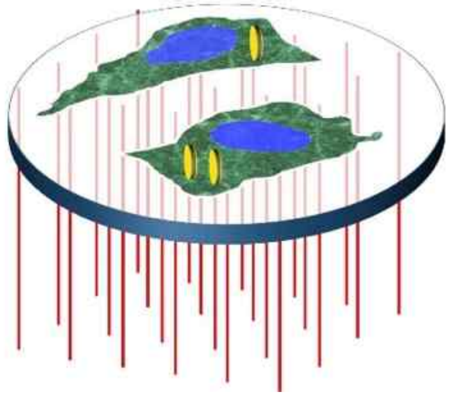




H = 10 mT

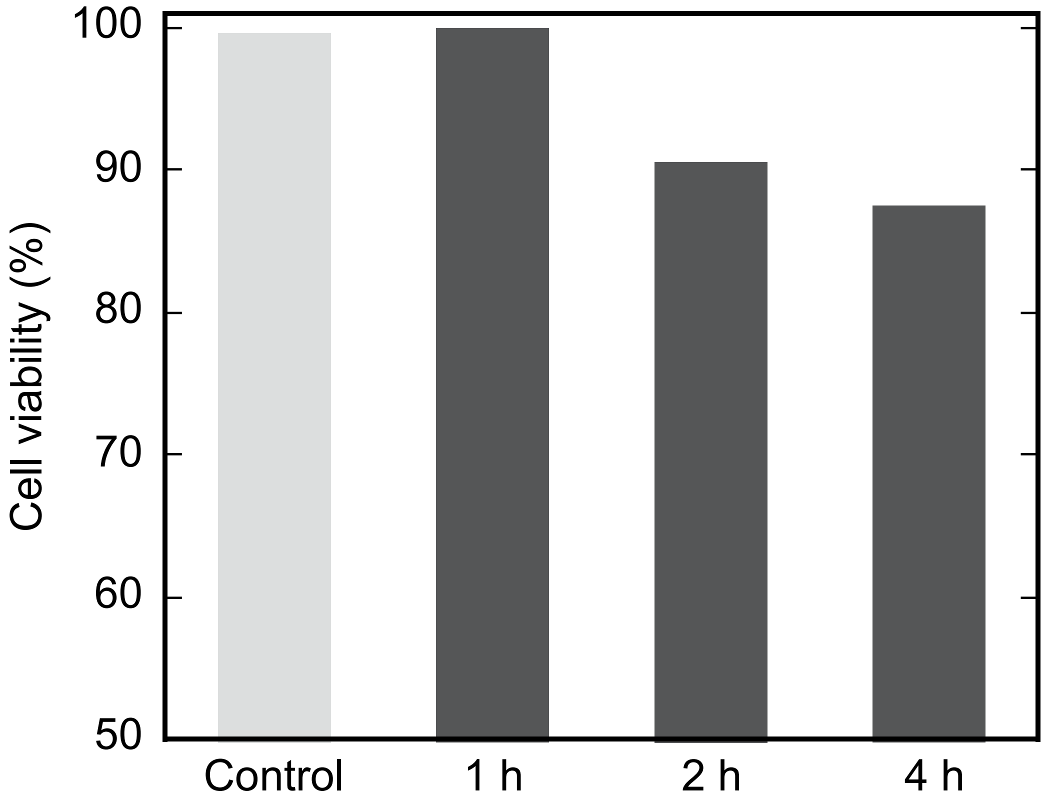
f = 10 Hz

t = 10, 30 min



Magneto-mechanical treatment

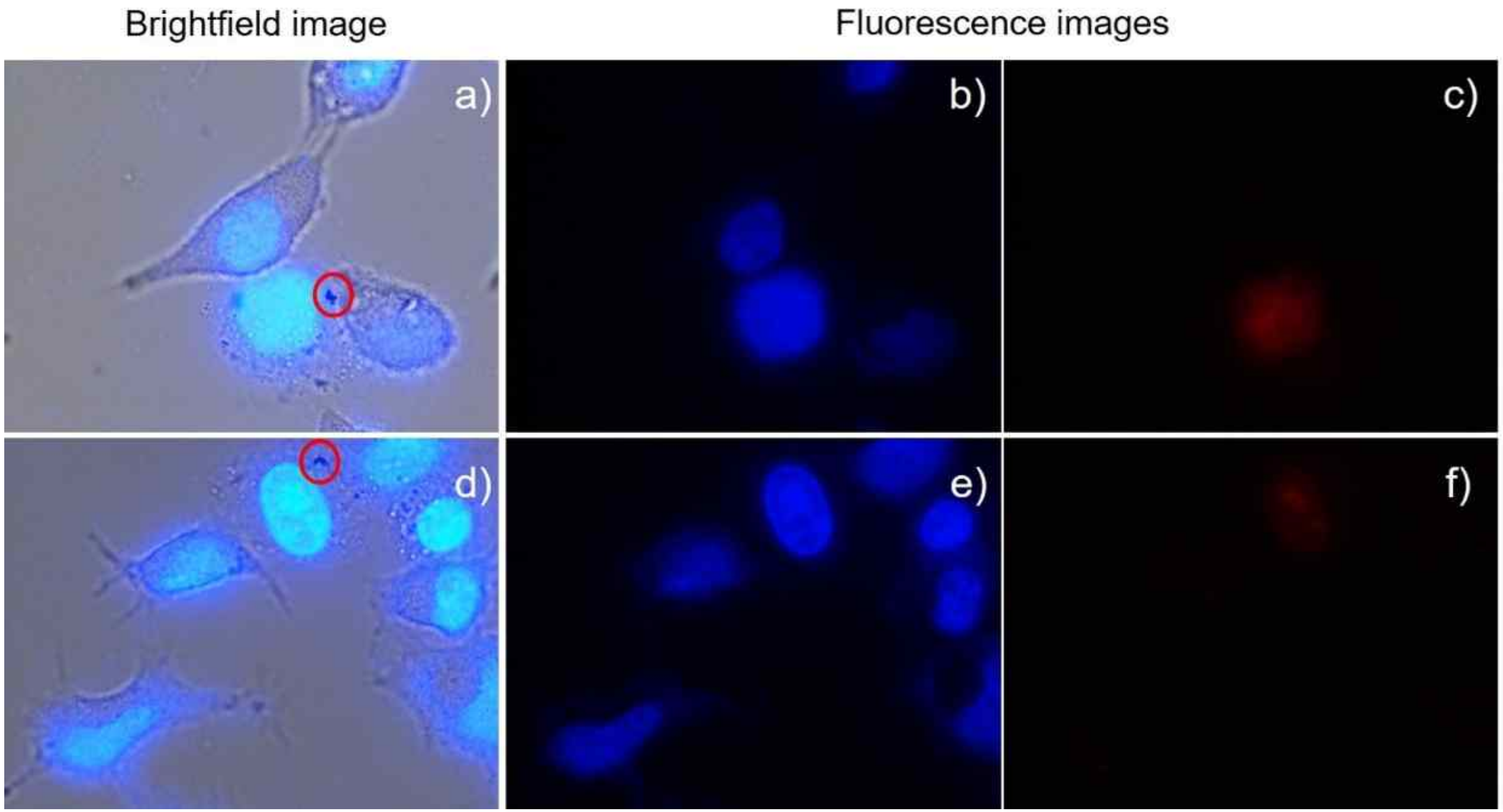
Cell viability evaluated 1, 2 and 4 H after the treatment



Typical result. Cells with microdiscs actuated for 10 min.

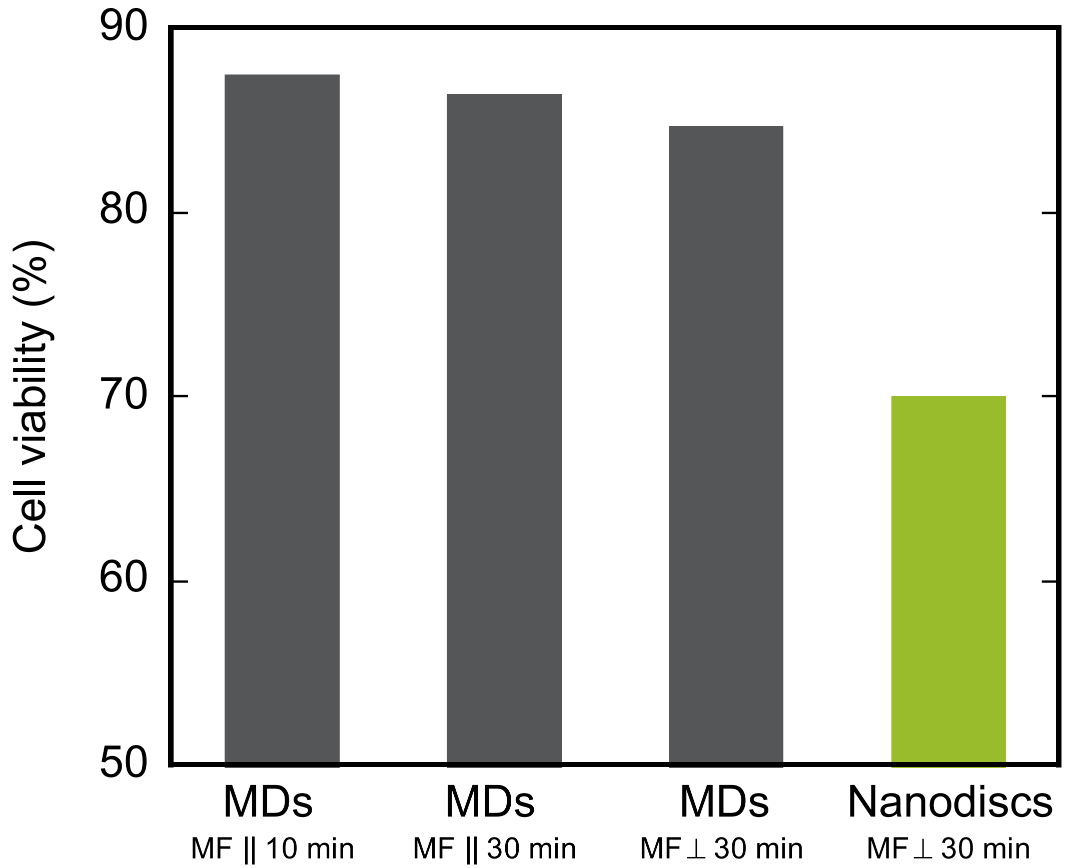
Magneto-mechanical treatment

Example: only cells with nanodiscs die after 30 min in perpendicular field



Magneto-mechanical treatment

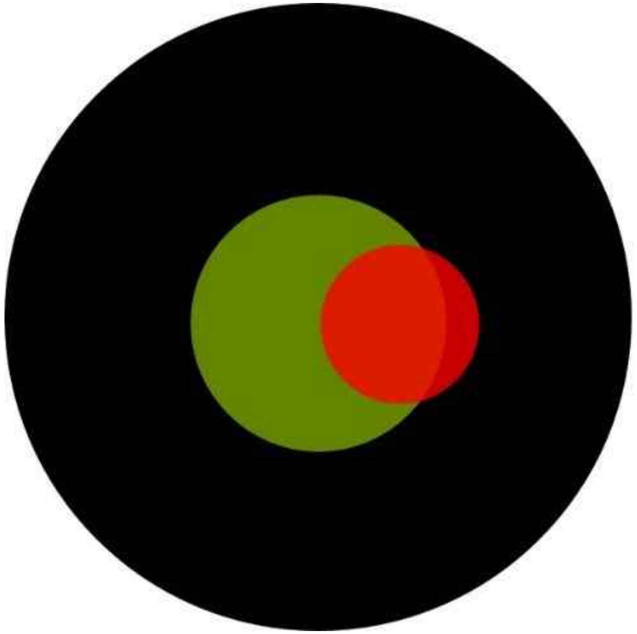
Comparison of the effectiveness of the mechanical treatment



Nanodiscs are more effective!

Magneto-mechanical treatment

Schematic overview of the results



100% of carcinoma cells

17% of the cells internalized nanodiscs

Dead cells: 7% of total cells

30% of the cells with nanodiscs

75% of the dead cells contained nanodiscs

Summary

- Discs with diameters down to 60 nm have been fabricated by Hole Mask Colloidal lithography with a satisfactory morphology and production yield.

- They display a well-defined magnetic vortex behavior, even being near the limits of the phase diagram.

- The size of the vortex core is comparable, or even greater, than the size of the discs. A new theory nicely matches the experimental results.

- *In vitro* assays reveal no cytotoxicity of the discs and give promising results for cancer cell destruction using the magneto-mechanical actuation

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