# Nanomaterials and biomedicine: activities, results and international collaborations of NIRDTP

**Dumitru Daniel Herea**

**Institutul National de Cercetare-Dezvoltare pentru Fizica Tehnica – IFT Iasi**
**47 Mangeron Boulevard, Iasi, RO-700050**
**E-mail: dherea@phys-iasi.ro**

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1. *INCDFT Iasi – activities in the area of nanomaterials intended for biomedical applications*
2. *Activities and results in the area of cancer therapy through magnetic nanomaterials*
   a. **Magnetic hyperthermia to kill cancerous cells in mouse models**
   b. **Killing cancerous cells through magnetic particles submitted to magnetic field vibrations**
3. *Collaboration of INCDFT Iasi in the framework of a FP7 project focused on magnetic nanomaterials and nanostructured materials for biomedical applications*

## 1. *INCDFT Iasi – several activities in the area of nanomaterials intended for biomedical applications*

- **Magnetic nanowires**
- **GMR sensors**
- **Magnetoelastic sensing device for pulse-wave detection and temperature sensors for biomedical and engineering applications**
- **Magneto-impedance sensor for quasi-noncontact monitoring of breathing, pulse rate and activity status**

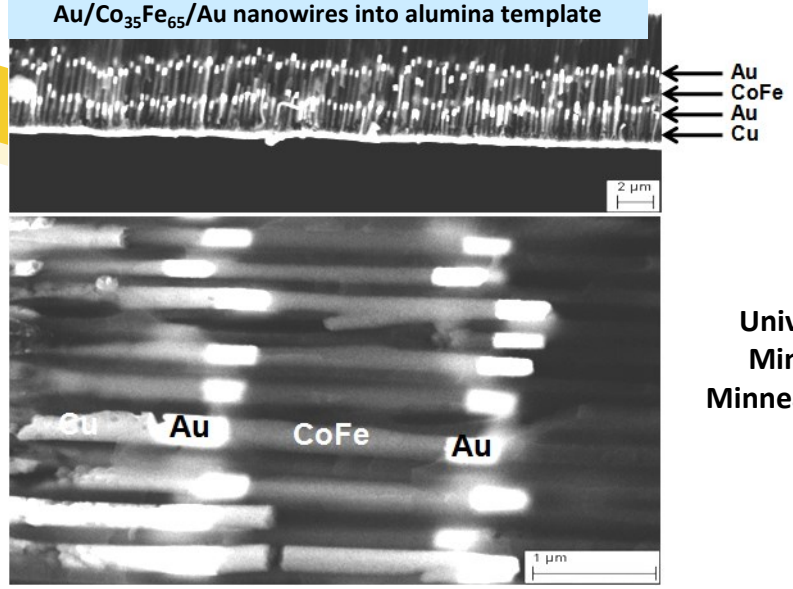
# Magnetic nanowires

## *Segmented Au/$Co_{35}Fe_{65}$/Au nanowires with the highest magnetic saturation for biomedical applications*

| Au tips (nm) | CoFe (μm) | NW diameter (nm) | $r_1$ ($mM^{-1}s^{-1}$) | $r_2$ ($mM^{-1}s^{-1}$) | $r_2/r_1$ |
|---|---|---|---|---|---|
| 500 | 12 | 200 | 0.42 | 3.12 | 7.42 |
| 500 | 4 | 200 | 0.2 | 0.12 | 0.6 |
| 500 | 2 | 200 | 0.89 | 4.13 | 4.64 |
| 700 | 0.7 | 200 | 0.05 | 3.71 | 74.2 |
| 700 | 0.25 | 200 | 0.12 | 4.57 | 38.08 |
| 700 | 0.1 | 200 | 0.39 | 8.11 | 20.79 |

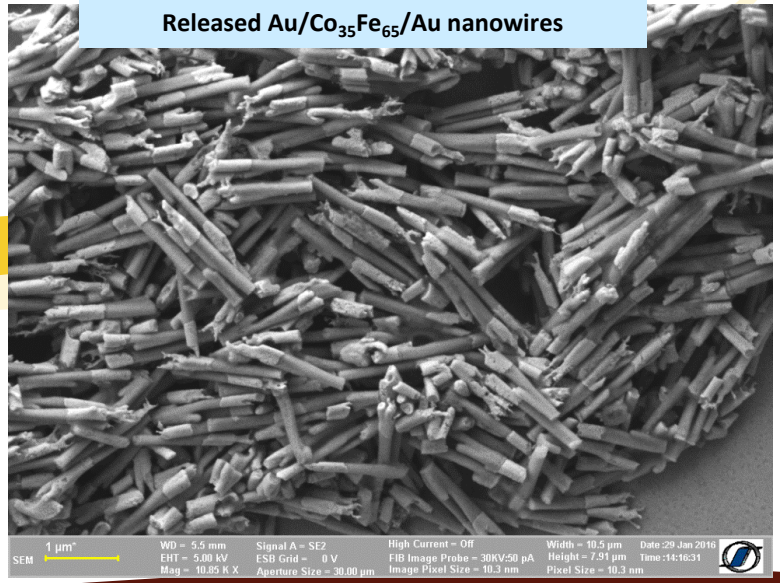
| Au tips (nm) | CoFe (μm) | SAR (W/g Fe) | [Fe] (μg/ml) |
|---|---|---|---|
| 500 | 0.1 | 951 | 44 |
| 500 | 0.25 | 1282 | 48 |
| 500 | 0.7 | 1398 | 47 |
| 700 | 2 | 387 | 40 |
| 700 | 4 | 308 | 38 |
| 700 | 12 | 132 | 114 |

Au/$Co_{35}Fe_{65}$/Au nanowires into alumina template

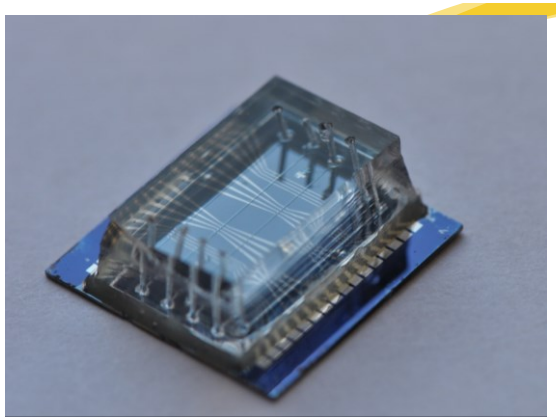



University of Minnesota, Minneapolis, USA

Released Au/$Co_{35}Fe_{65}$/Au nanowires

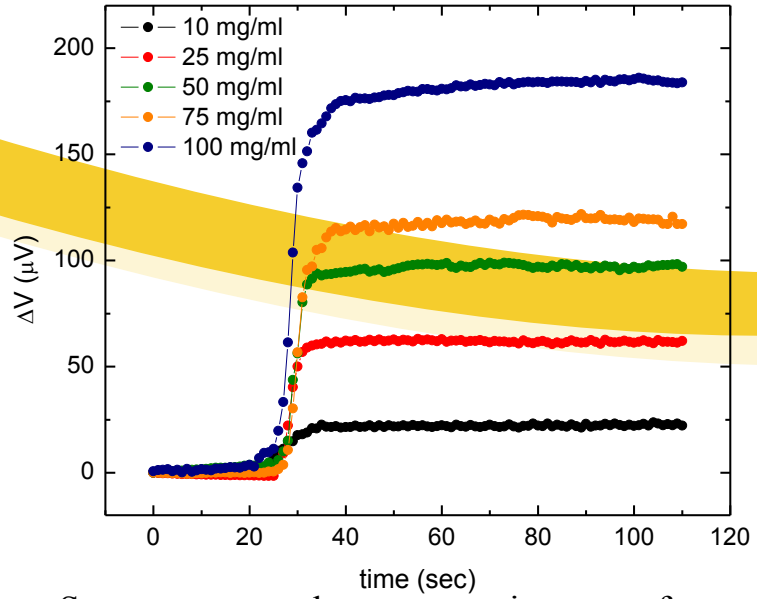


# GMR sensors and microfluidic devices for nanoparticle detection

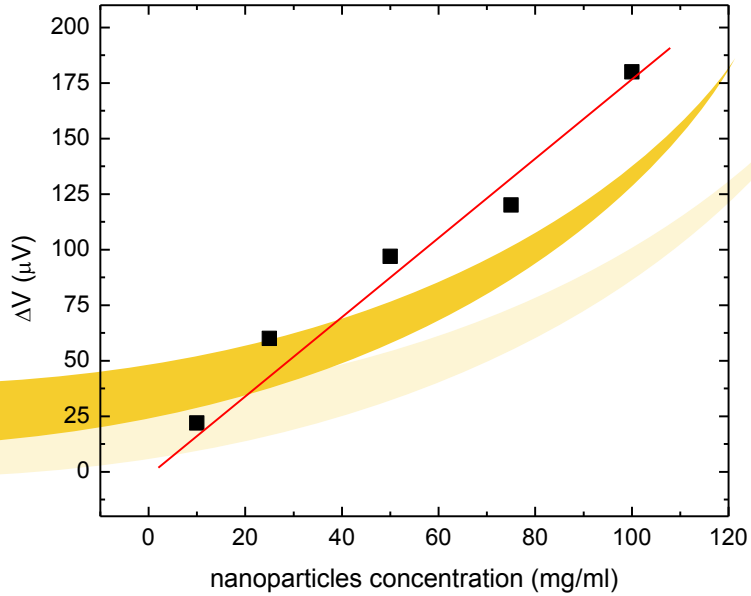


Photograph of the microfluidic device used for nanoparticles detection

- Fe-Cr-Nb-B magnetic nanoparticles were dispersed in DI water obtaining solutions with different concentrations of nanoparticles.
- The nanoparticles were injected through the microchannels and the output voltage of the sensor was recorded.



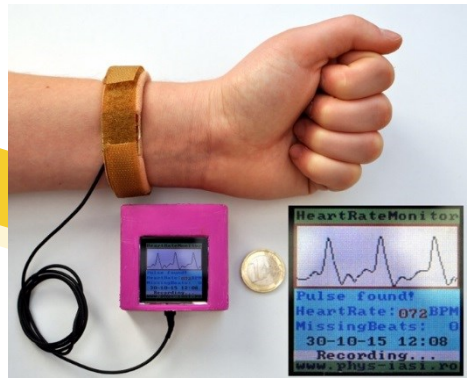

Sensor output voltage versus time trace for different nanoparticles concentration



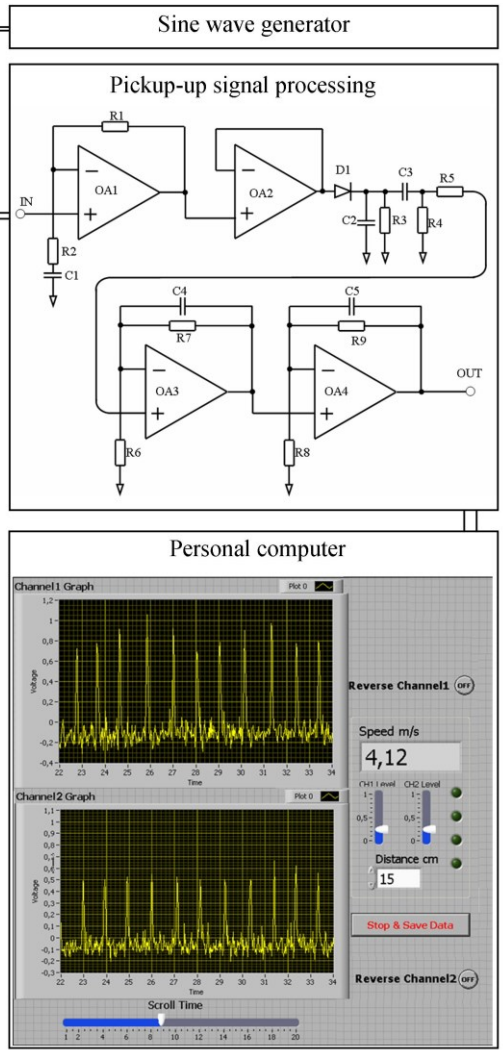

Sensor output voltage versus nanoparticles concentration

# Magnetoelastic sensing device for pulse-wave detection and temperature sensors for biomedical and engineering applications

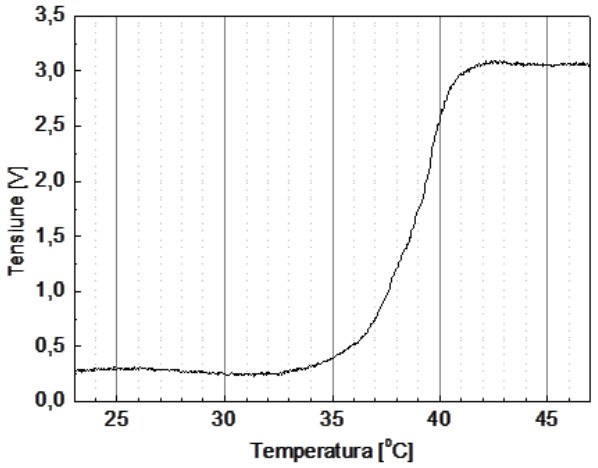
The sensing element (amorphous magnetic wire) follows the skin displacement due to the blood vessel expansion as a result of the blood pressure pulse

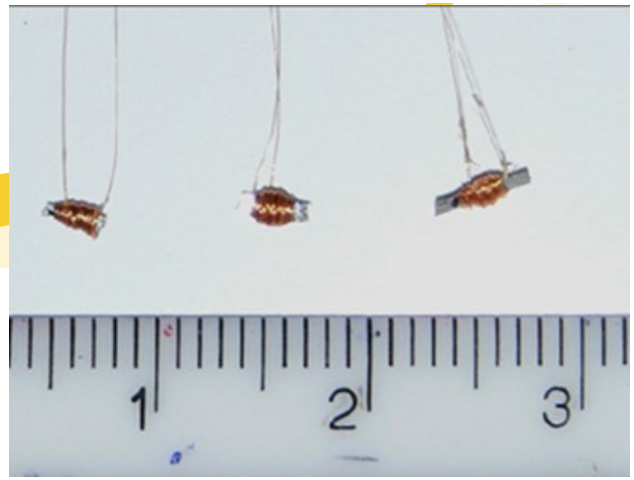



Magnetoelastic sensing device with the sensor embedded in the bracelet on the wrist of a patient.




Temperature sensors

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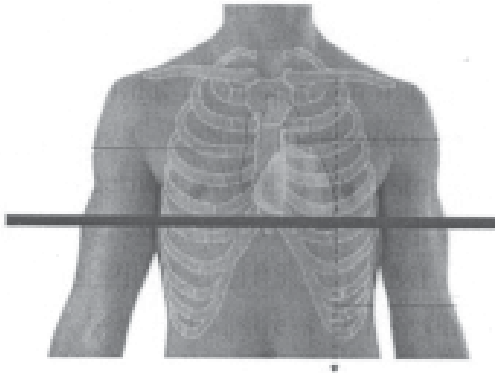
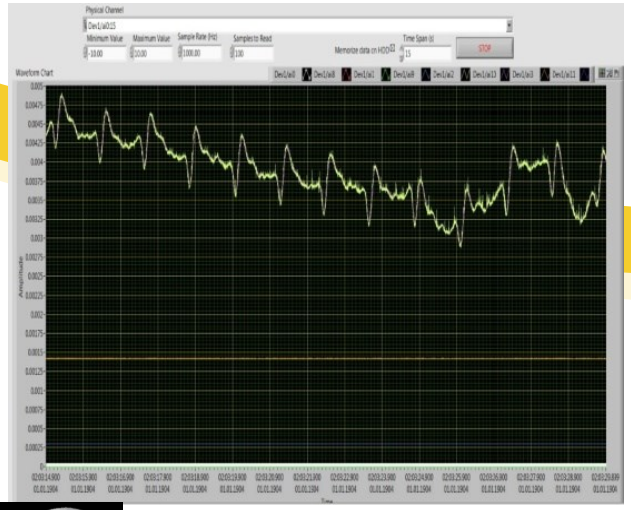
# Magneto-impedance sensor for quasi-noncontact monitoring of breathing, pulse rate and activity status

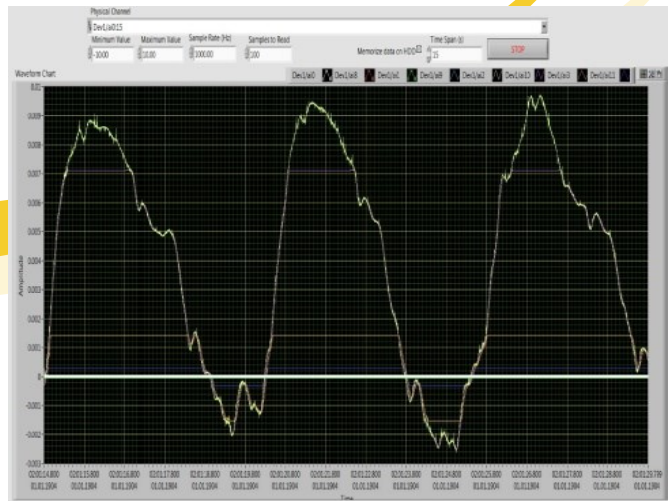


Position of the sensor along the 5th intercostal space(black line)

*Prototype of a robust and reliable MI sensor able to detect small movements associated with breathing and heart beat of a subject at rest.*



Pulse wave



Breathing signal

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   - **a. Magnetic hyperthermia to kill cancerous cells in mouse models**
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- Cancer is responsible for about 25-30% of deaths in developed countries, being the second cause of death after cardiovascular disease.
- Systemic therapies, including chemotherapy and radiotherapy have long been considered to be the main way of treatment but there is a tendency to reduce the negative effects of these methods by applying the treatment to a restricted area, where the tumor is located.
- Magnetic particles (MPs) have been lately used in:
  - biomedicine - for magnetic separation,
  - as magnetic carriers for drug delivery,
  - as thermoseeds in magnetic hyperthermia and
  - more recently, for their capability to induce apoptosis by mechanical forces.

*Y. Cheng et al.,* ***J. Control. Release 223****, 75 (2016)*

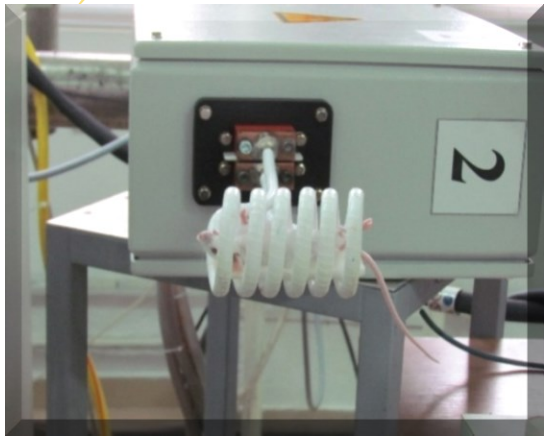
# Magnetic hyperthermia to kill cancerous cells in mouse models

## *About magnetic hyperthermia*

- Magnetic hyperthermia represents a physical process used to artificially increase temperature through alternating magnetic fields (AMF) and magnetic nanoparticles (MNPs)
- It has shown its high potential for curing mammalian malignancy.
- Moreover, it was demonstrated that it can successfully work by itself as an independent treatment method, revealing its promising curative potential in clinical applications against some cancerous processes such as prostate cancer or glioblastoma.
- The method is based on the principle that temperatures ranging between 42°C-46°C induce cancer cells apoptosis, leaving the normal cells alive.
- In this work, we have shown that on lab animals, the cytotoxic effects of MNPs coated with an antitumor drug is boosted by magnetic hyperthermia

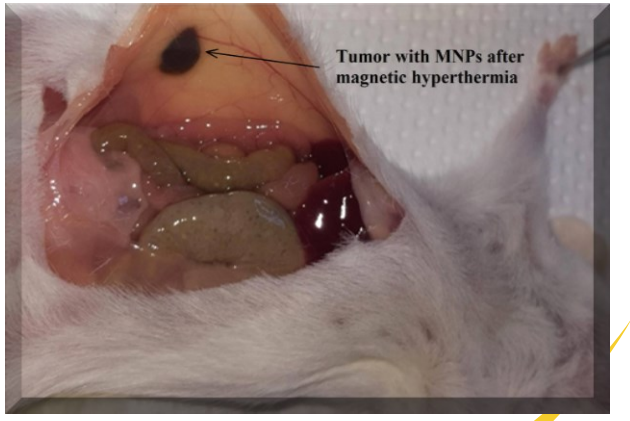
# Antitumor effect of the drug-coated MNPs on laboratory animals

- After AMF exposure, the mice were sacrificed. By analysis of the macroscopic morphology of the inner abdominal wall, a good tolerance following MNPs injection into the mammary tissue and a favourable cicatricial evolution were found.




*Mouse within the coil during the hyperthermia treatment*

- it was observed that mice underwent to hyperthermic treatment survived in significantly more favourable conditions than their counterparts who weren't under hyperthermic regime.




*Sacrificed mouse with an induced mammary tumor containing MNPs coated with mitoxantrone after hyperthermia treatment*

- Histological analysis showed no locoregional dissemination of the tumors in the abdominal wall of the mice submitted to magnetic hyperthermia. This may be due both to the cytostatic action in the cancerous area and boosting effect induced by the locoregional magnetic hyperthermia.

- Regarding the metastatic spread, we detected several certain metastasis, both intrapulmonary and intrahepatic, but not in kidneys. The appearance of the intrapulmonary metastasis was typical, with massive and multiple foci of alveolar parietal seeding, then intra-alveolar. However, in the case of hyperthermia treatment of the primary tumor, this picture, although present, was much diminished.

- The **decrease of the metastatic lung colonization was an unexpected favourable effect**. It is known that the lung has immunological suppressive atmosphere, and it is possible that through the effects of cytokines systemic effects induced by magnetic hyperthermia, this balance has been temporarily prone to cell inflammation that was unfavourable to metastasis expansion.

## Conclusions

- The MNPs- MIT had a positive anti-tumor effect in terms of cytotoxicity on cancerous cells.
- The heat generated by MNPs-MIT led to an important decrease of the tumor metastasis in mice, boosting the anti-tumor drug effect.
- In addition, the tested MNPS and MNPs-MIT do not determine, from a histological standpoint, systemic irritation action.
- Through combined chemotherapy and magnetic hyperthermia, we estimate the mice survival could be prolonged by 15-40%.

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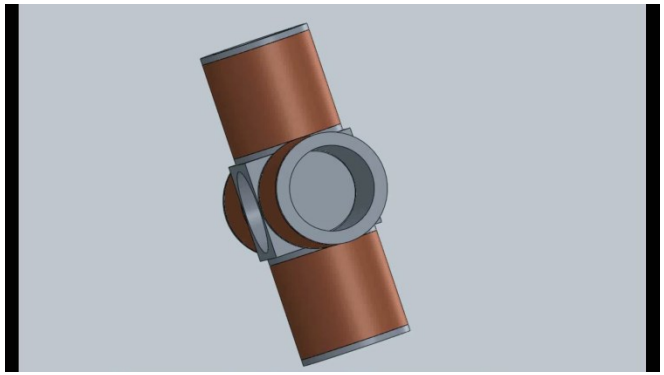
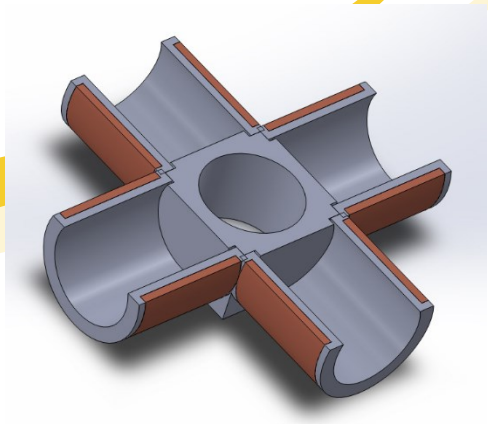
# Killing cancerous cells through magnetic particles submitted to magnetic field vibrations

- The aim of this work was to study **the effect of mechanical oscillations generated by Fe-Cr-Nb-B magnetic particles** (MNPs) introduced in a **variable gradient magnetic field** (VGMF) on the **mechanical destruction of osteosarcoma cancer cells**.
- **MPs of 40÷200 nm**, dispersed in cell culture medium or prepared as ferrofluid, were **accelerated with a VGMF** applied through a special laboratory-made coil system powered by an electronic system which **allows to apply a time and amplitude variable MF**.
- The Curie temperature of MNPs is adjustable in the temperature range of 40÷50°C by the Cr content, making them suitable for self-regulated magnetic hyperthermia, too.

# Experimental setup

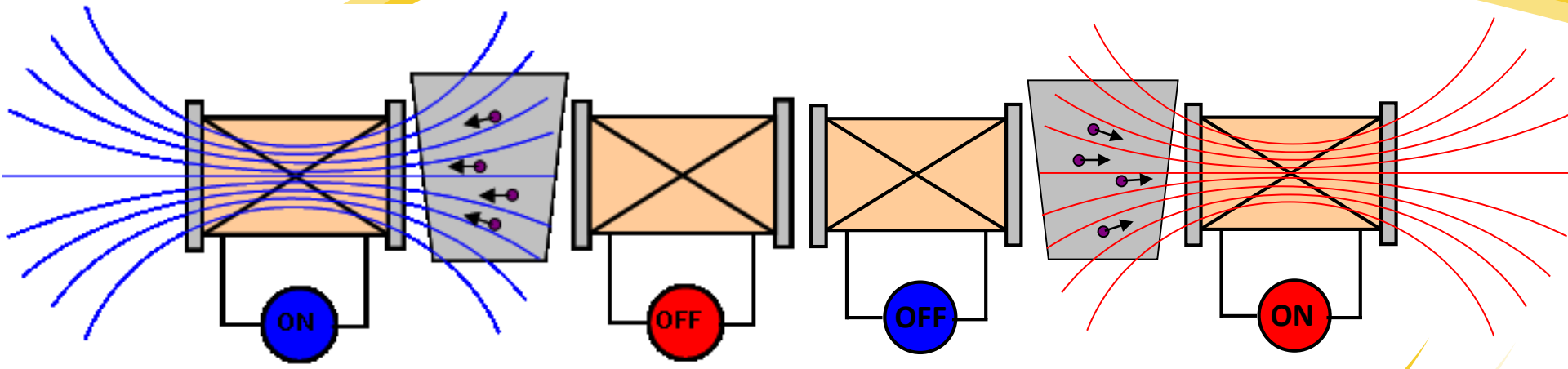
- In order to set up the experiments, we designed a system consisting of four coils placed in cross which can be used *either* to **produce a magnetic field variable in time providing a high gradient moving from one coil to another** *or* to **produce a rotating magnetic field**.
- We designed a special electronic system which allows us to **set the magnetic field intensity, its frequency and the time of exposure**.
- The center of the coil system presents a gap were a well containing cells in culture medium to be studied is placed. In this gap a device, which controls the temperature variation, can be inserted in order to modify and maintain the desired temperature in the well.

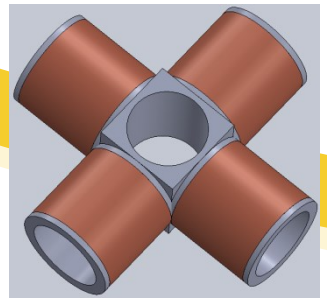


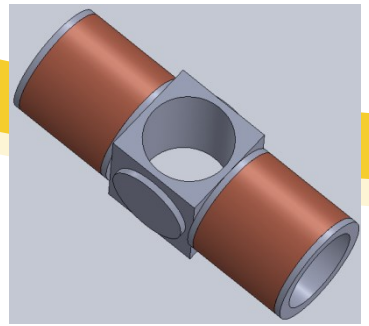


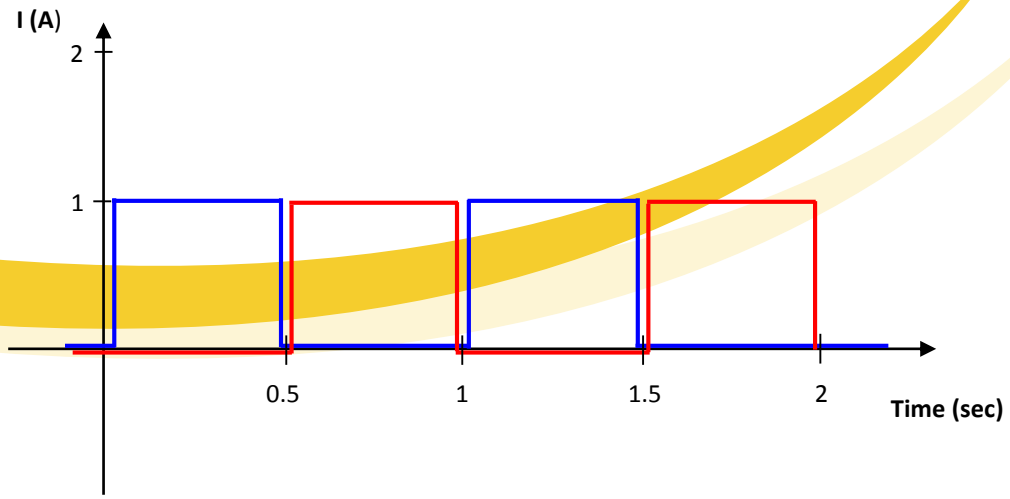
# Experimental setup

**Magnetic field variable in time providing a high gradient moving from one coil to another**

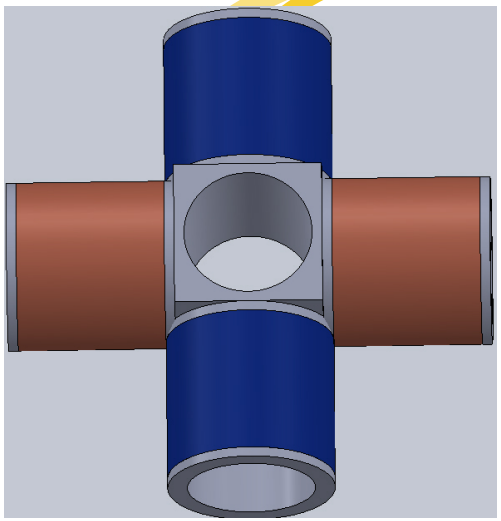


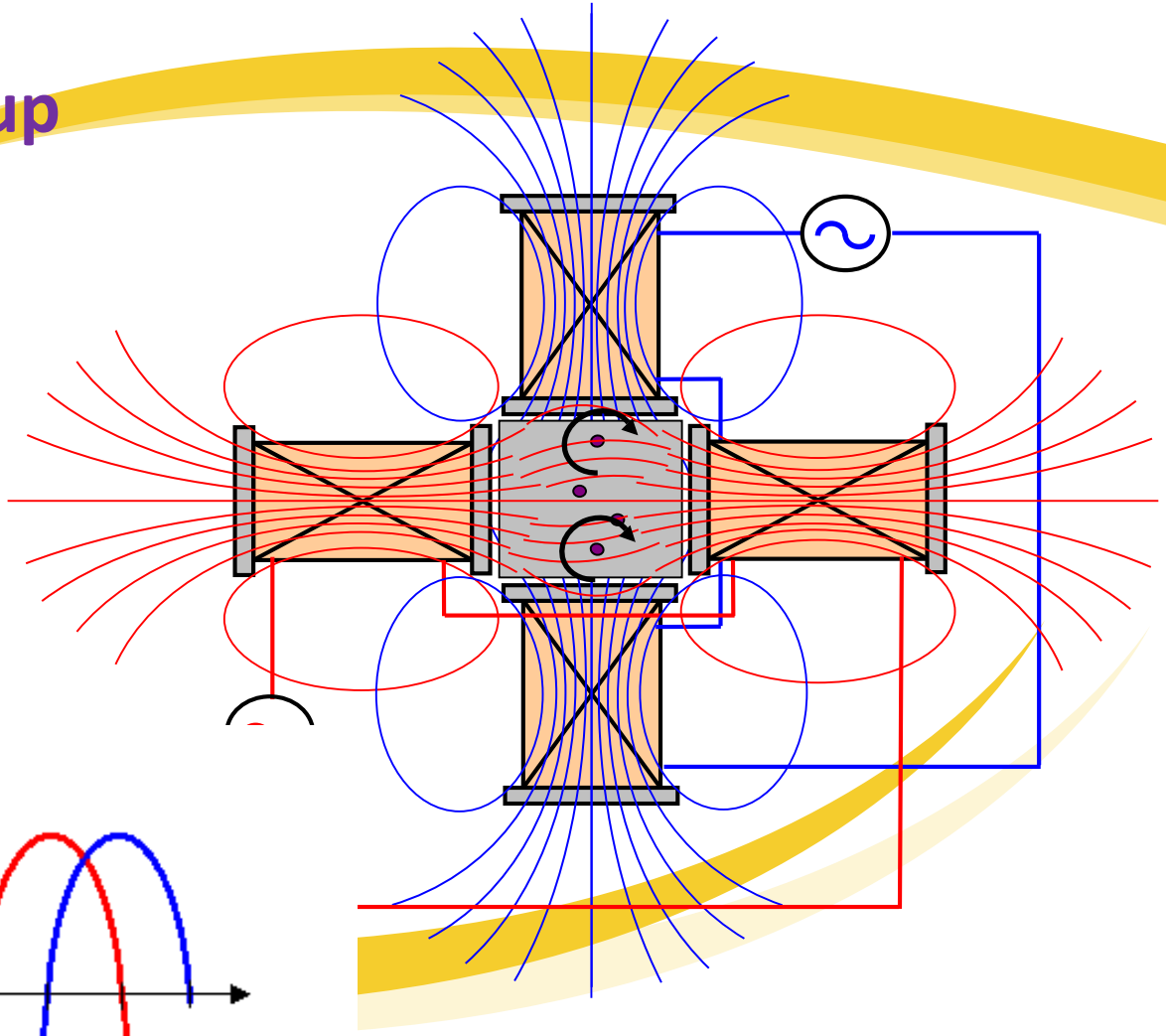



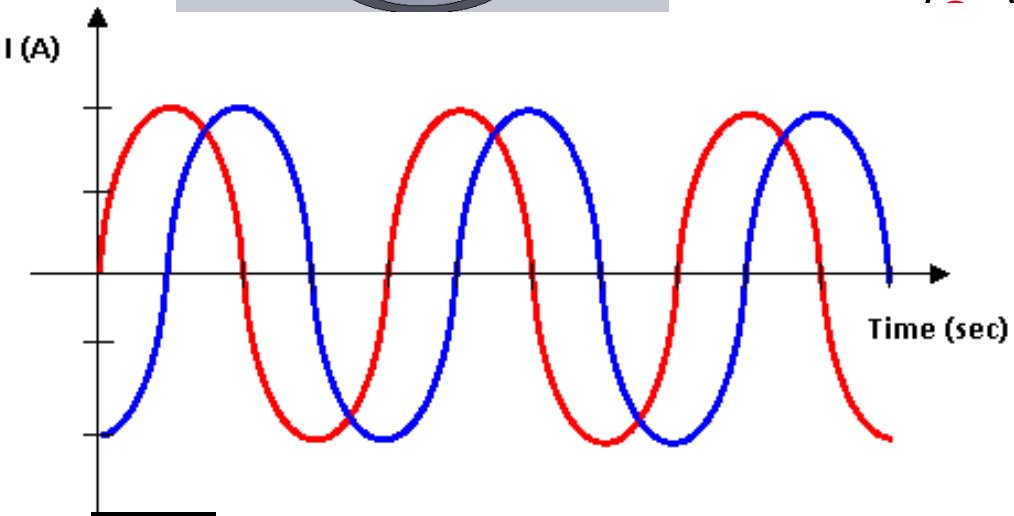




# Experimental setup

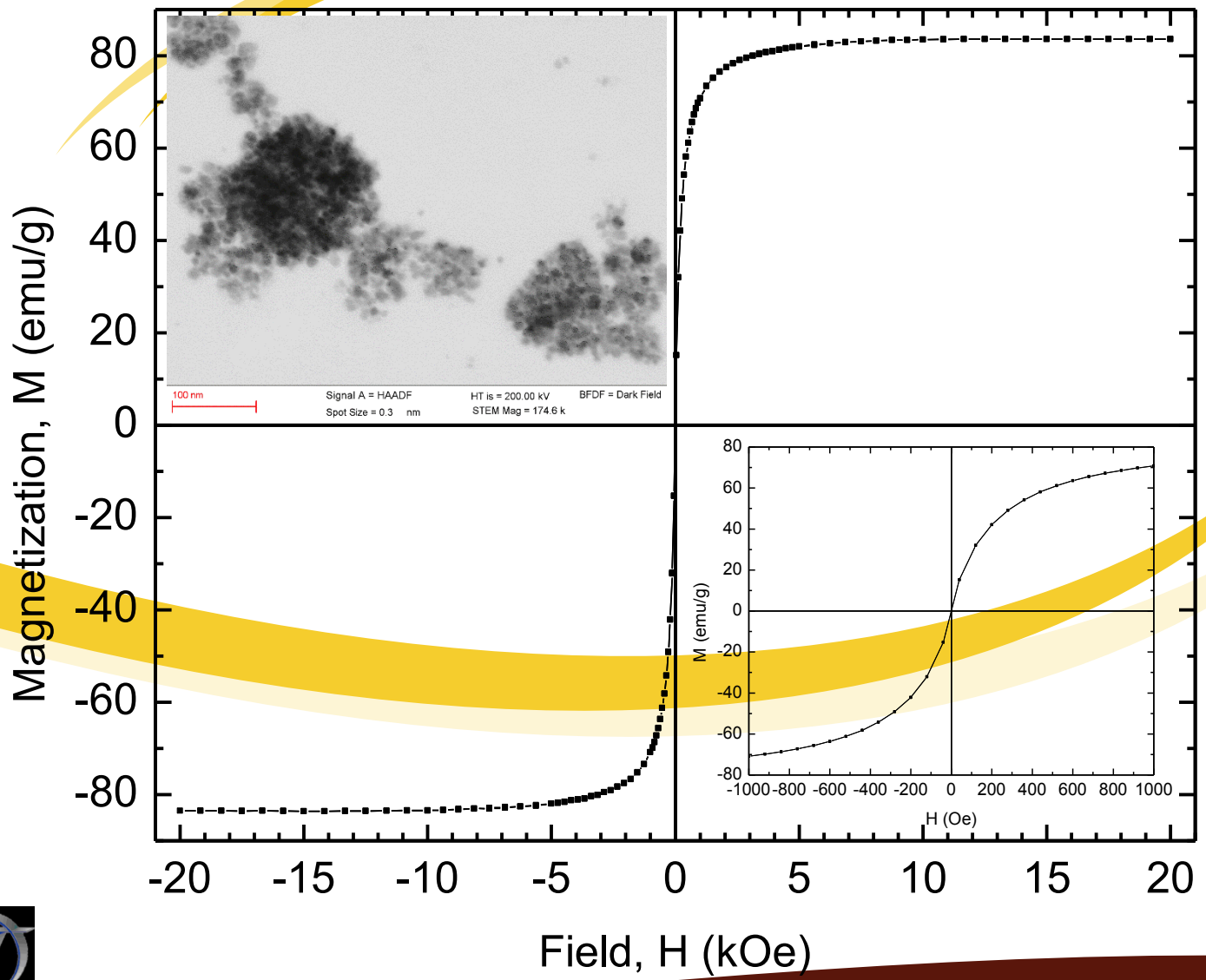






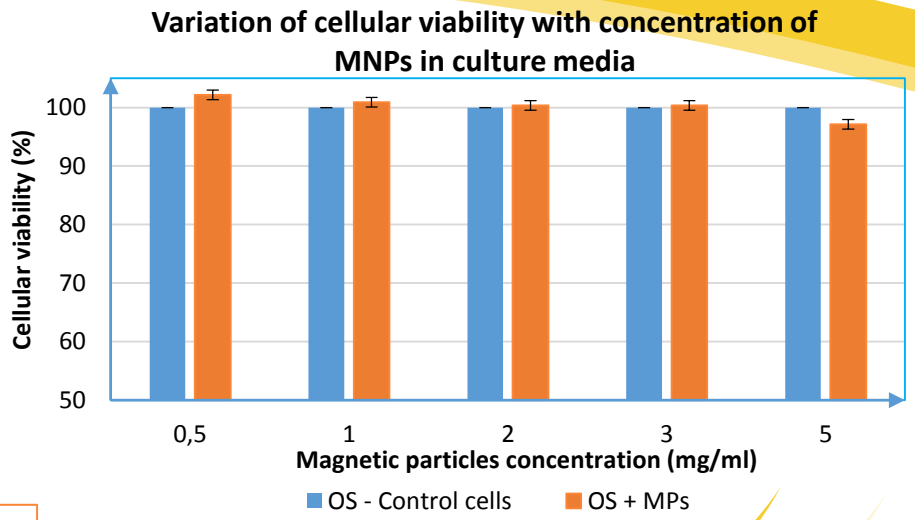

**Magnetic field variable in time producing a rotating magnetic field**

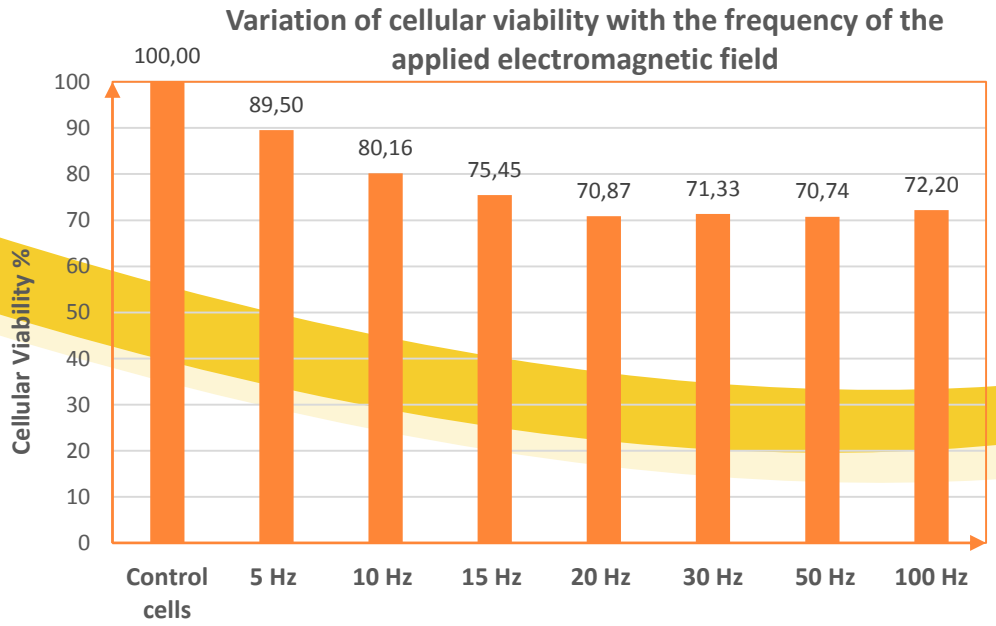
# Magnetic characteristics of Fe-Cr-Nb-B MPs



# Cellular viability vs. field frequency

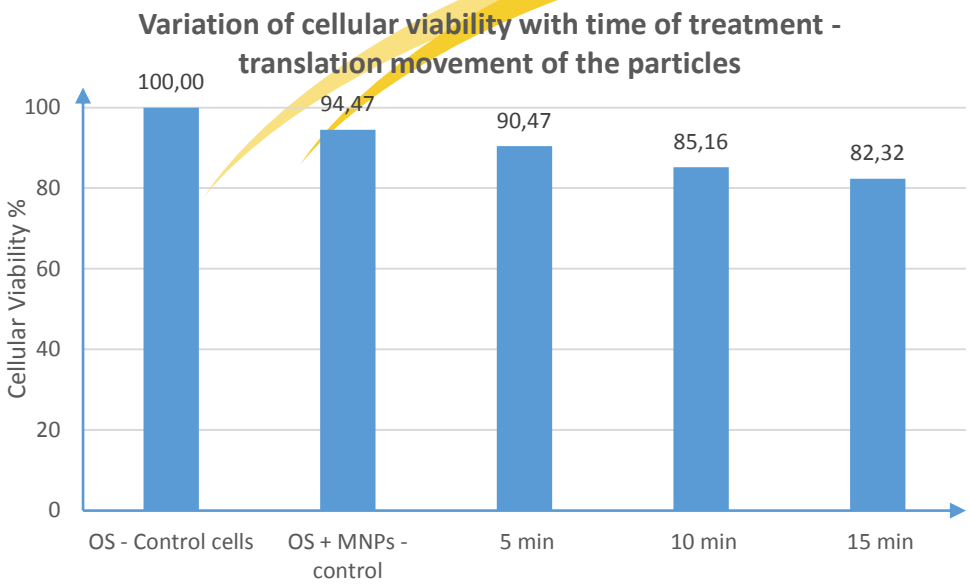
- Cells were seeded with a density of $3x10^4$ cells/well and left to adhere overnight.
- Then the culture media was removed and replaced with ferrofluid dispersed in culture media in a final concentration of 2 mg of magnetic material for 1 ml of medium.

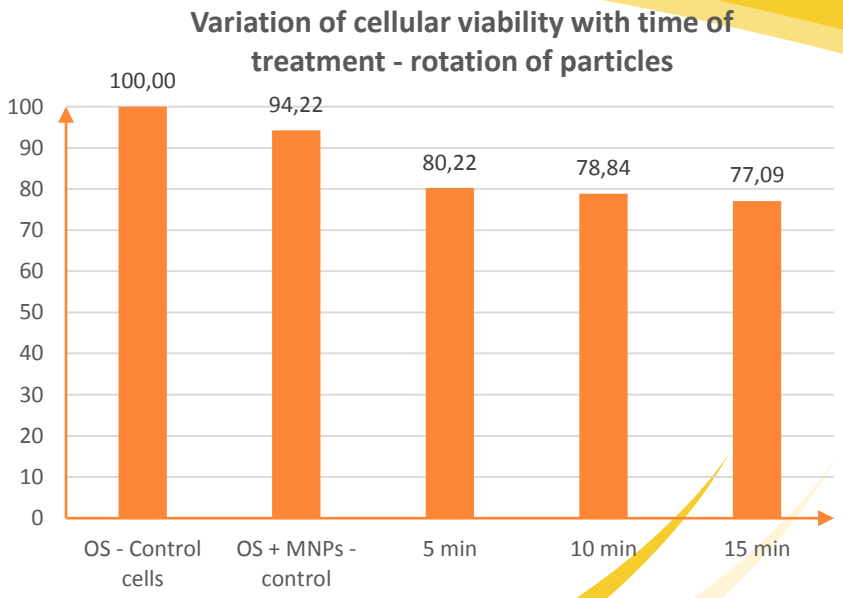


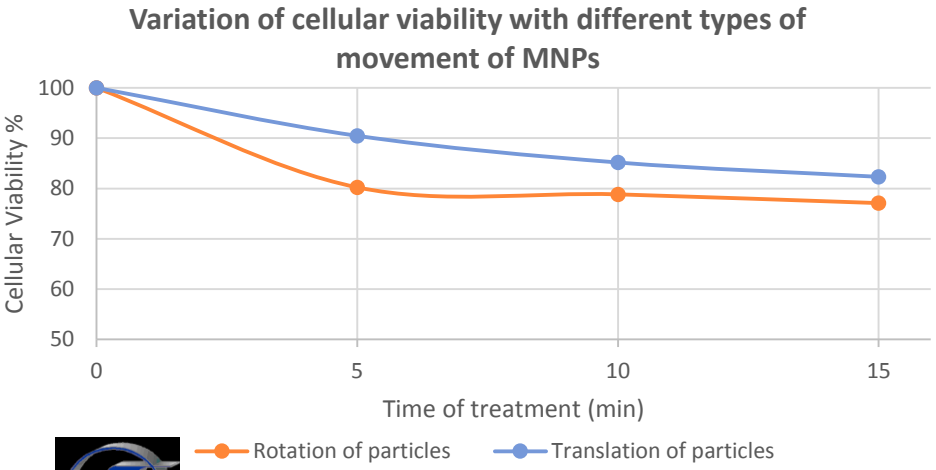




- After 30 minutes the wells were exposed to an electromagnetic field of 1.6 mT and different frequencies for 10 minutes.
- The MTT assay was applied to assess cellular viability.

# Cellular viability vs. time of treatment



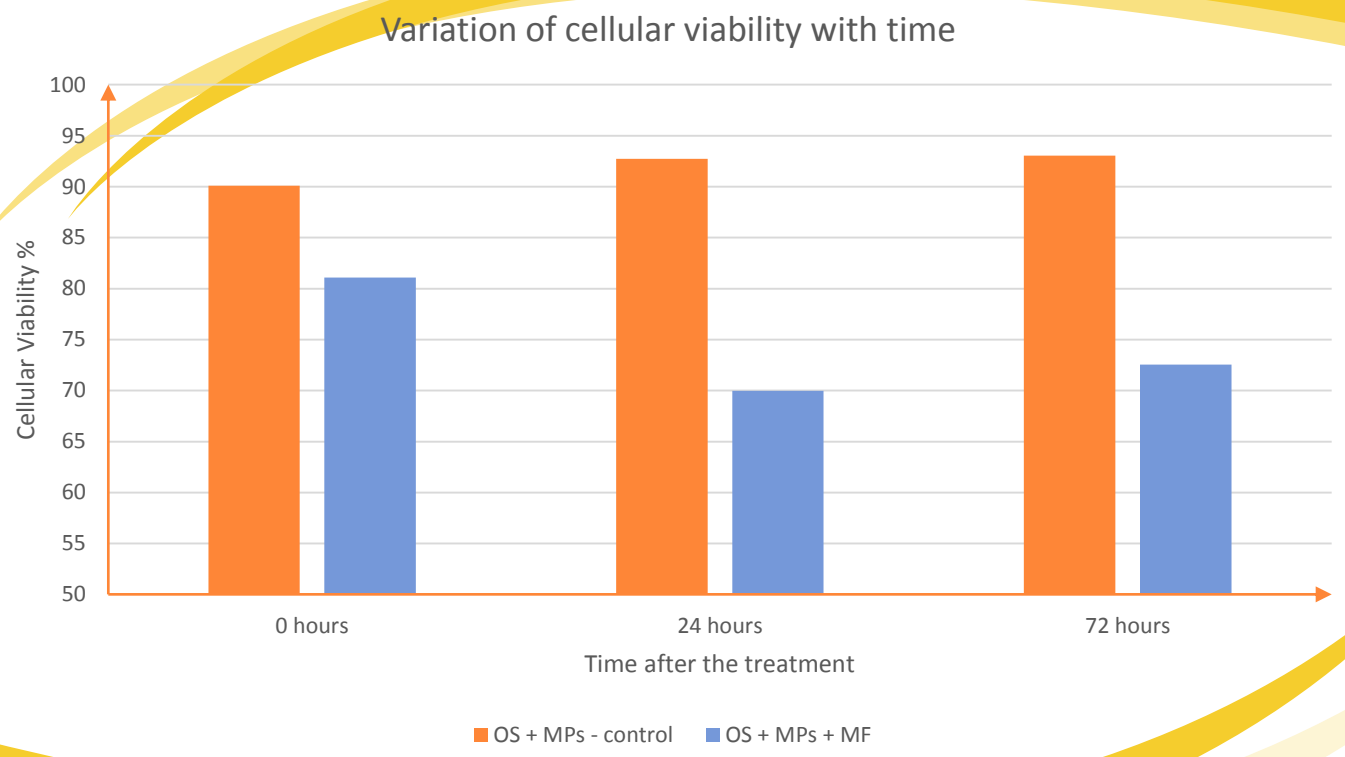




***Different movement of the particles***

Field intensity = 1.6 mT

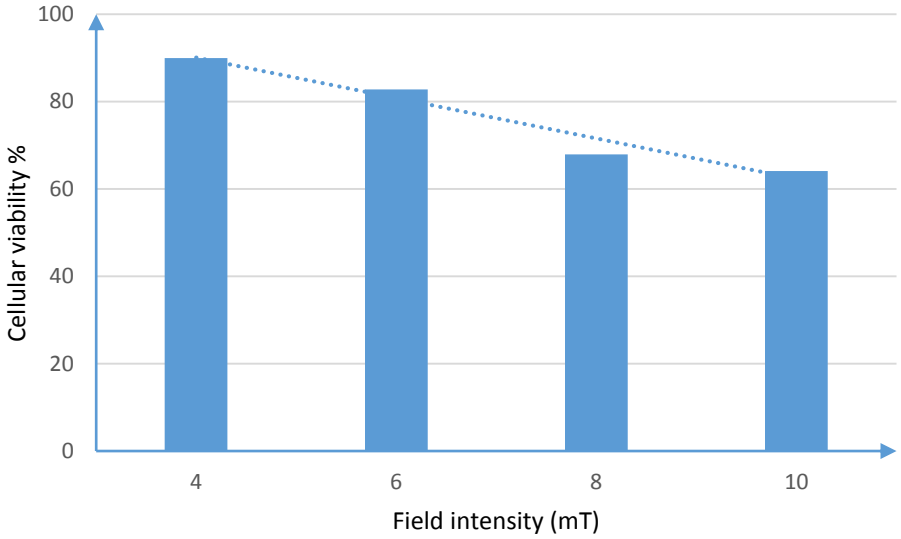
Field frequency = 1 Hz

Concentration of magnetic material in cell culture = 2 mg/ml



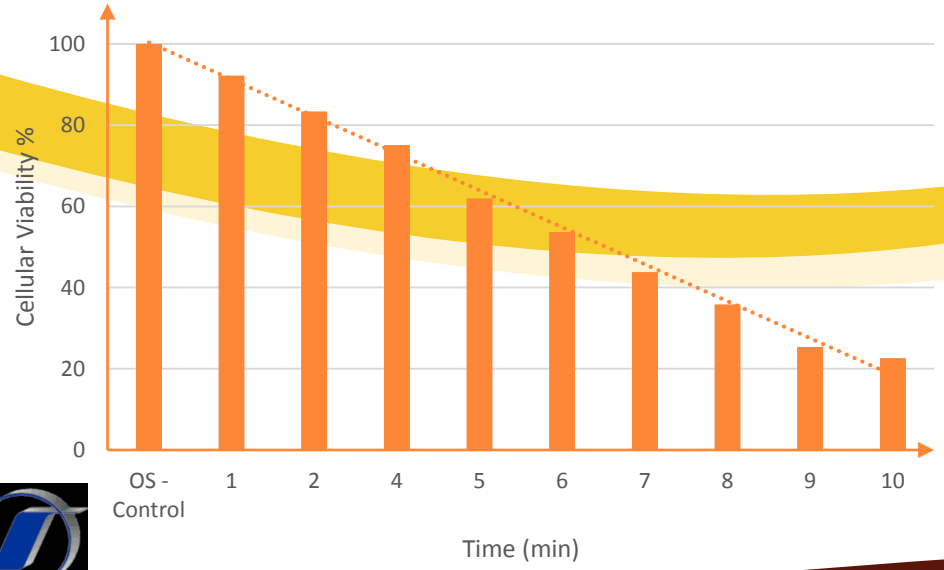

***Variation of cellular viability with the time***

- ❑ Field intensity = 1.6 mT
- ❑ Field frequency = 1 Hz
- ❑ Time of treatment = 10 minutes
- ❑ Concentration of magnetic material in cell culture = 2 mg/ml




## Cellular viability vs. field intensity

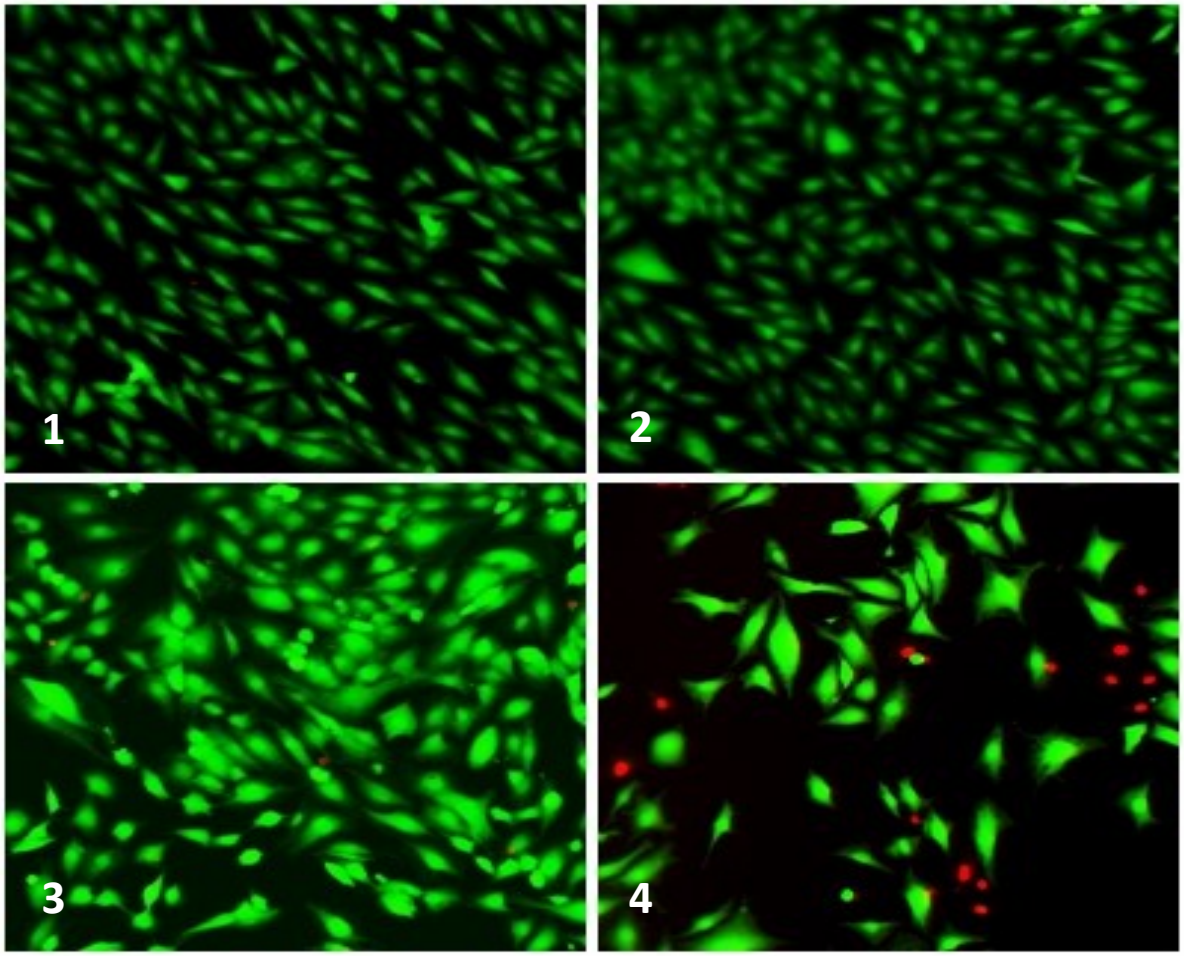
- ❑ Time = 1 min
- ❑ Field frequency = 1 Hz
- ❑ Concentration of magnetic material in cell culture = 2 mg/ml




## Variation of cellular viability with time of applied magnetic field

- ❑ Field intensity = 4 mT
- ❑ Field frequency = 1 Hz
- ❑ Concentration of magnetic material in cell culture = 2 mg/ml

# Cellular viability




***Cellular viability before and after magnetic field treatment assessed with Live/Dead Assay***

(1) OS cells, untreated
(2) OS cells exposed to MF
(3) OS cells loaded with MPs
(4) OS cells loaded with MPs and exposed to MF.

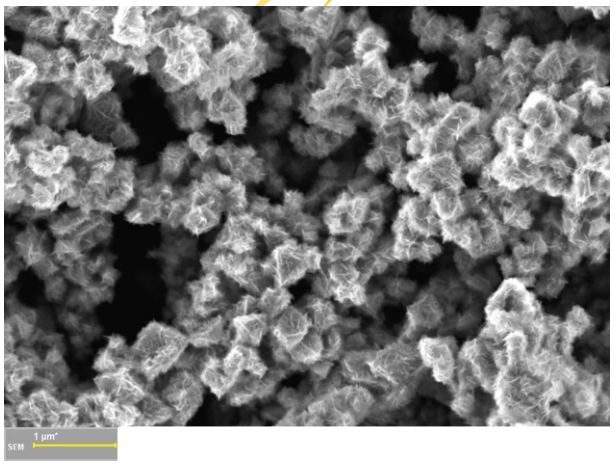
Live cells are colored in green.
Dead cells are colored in red.

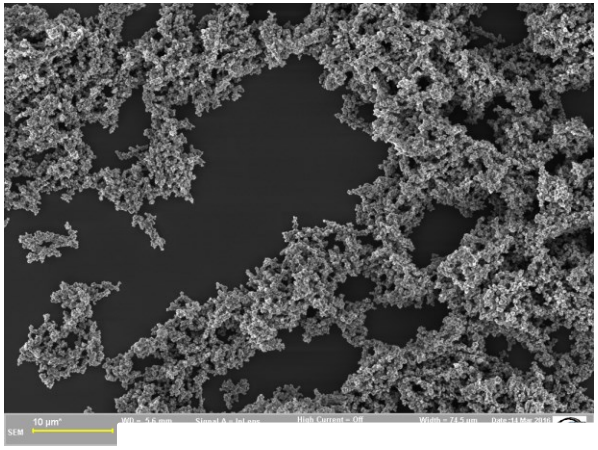
Cells without MNPs are not affected when exposed to the MF (2) and they are not affected when they are in contact with MNPs (3), but the cellular density decreases and many dead cells can be seen when the cells loaded with MNPs are exposed to MF.

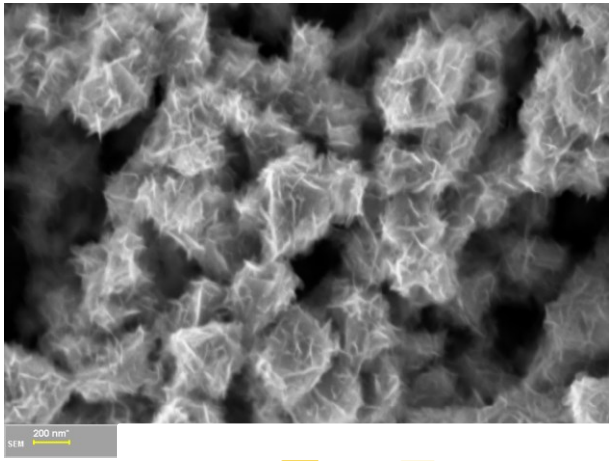
# Cellular destruction mechanisms

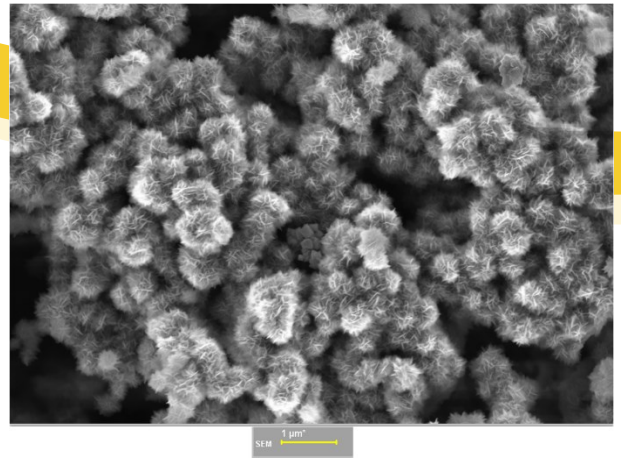
- The actual forces that act on the cells (applied through the magnetic particles) have been calculated considering the two main opposing actions:
  - the magnetic force that pulls the particles,
  - and the drag that acts against this pull.
- The resulting values are quite small, of the order of tens to hundreds of femtonewtons (fN).
- Thus, despite the small effect of the individual particles, their collective behavior seems to be more important, leading to a joint destructive effect of the particle ensemble on the living tissue.
- The exact mechanism is not yet fully understood, being one of the open issues that require further investigation.

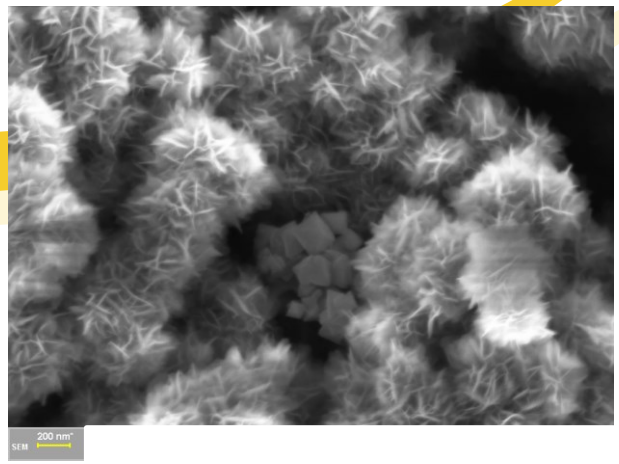
# *Further work related to this subject – Sharp cutting-edge magnetic nanoparticles for cancerous cells destruction*

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# Conclusions

- Magnetic nanoparticles are  good candidates for the destruction of cancerous cell by magnetomecanical effects, e.g., the movement of magnetic particles against the cells in the presence of a variable magnetic gradient field.
- The viability of the cancer (osteosarcoma) cells was found to be dependent on the magnetic field intensity, magnetic particles concentration, duration of the application of the magnetic field and the frequency of the alternating magnetic field.
- The healthy cells are not affected by the movement of the particles and this aspect should be clarified.
- The calculated mechanical energy induced by the variable magnetic field does not explain the magnitude of the effect, thus a different explanation should be considered.
- The mechanism of interaction between the moving magnetic particles and the cells needs further studies and clarifications.

# Content

1. *INCDFT Iasi – activities in the area of nanomaterials intended for biomedical applications*
2. *Activities and results in the area of cancer therapy through magnetic nanomaterials*

   **a. Magnetic hyperthermia to kill cancerous cells in mouse models**

   **b. Killing cancerous cells through magnetic particles submitted to magnetic field vibrations**

3. ***Collaboration of INCDFT Iasi in the framework of a FP7 project focused on magnetic nanomaterials and nanostructured materials for biomedical applications***

# Collaborations

- National institutes, public research institutions, hospitals, universities and partners from industry




INCDFT-IFT Iaşi

NIRDTP Iaşi

**Sheffield Centre for Advanced Magnetic Materials and Devices within the Department of Engineering Materials, University of Sheffield, UK (SCAMMD)**

SCAMMD provided training in the design of magnetostrictive materials for sensors, actuators and transducers. Technological development of acoustic microsensors for medical applications was done at SCAMMD with the participation of NIRDTP scientists. Through the twinning action, NIRDTP gained access to SCAMMD's transmission electron microscopy equipment and to their magnetometers and will develop scientific knowledge and skills on how to design and characterize magnetostrictive materials for sensors, actuators and transducers based on magnetostrictive nanowires.

**Department of Materials for Information Technologies in the Instituto de Ciencia de Materiales de Madrid, Spain (ICMM-CSIC)**

ICMM-CSIC provided training in the functionalization of the nanocrystalline magnetic microwires to make the implantable microsensors biocompatible and will train NIRDTP's young researchers to design and produce nanostructured magnetic microwires with improved characteristics. ICMM-CSIC will also helped NIRDTP researchers to develop and characterize new multilayered nanostructured microwires for medical applications.

**Instituto de Engenharia de Sistemas e Computadores para os Microsistemas e as Nanotecnologias (INESC MN), Portugal**

INESC provided training in the design and characterization of acoustic microsensors based on magnetostrictive nanowires and will train NIRDTP researchers in the characterization of the implantable microsensors for medical applications.

**Nanobioelectronics & Biosensors Group in the Institut Català de Nanotecnologia, Barcelona, Spain (ICN)**

ICN was in charge of training activities related to the design of novel electrochemical biosensors based on Quantum Dots and barcode nanowires /nanoparticles tracers.

**Solid State Physics group within the Department of Physics and Astronomy, University of Glasgow, UK (UGLA)**

The research is underpinned by advanced characterization and a long-standing reputation for the development of transmission electron microscopy techniques.

**Materials Science Electron Microscopy Department at the University of Ulm, Germany (UULM)**

Thanks to its interdisciplinary and cooperative working methods, the University has been able to establish numerous research concentrations and Collaborative Research Centres both in basic and applied research, achieving successful results.

In the cooperative model Science City Ulm, universities, research institutes and industrial companies work together intensively at a regional level on developing and using new technology.

Thank you for your attention!

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