Coordinating simultaneously tens of millions of intelligent autonomous robots, each being smaller that a red blood cell, for cancer therapy and beyond

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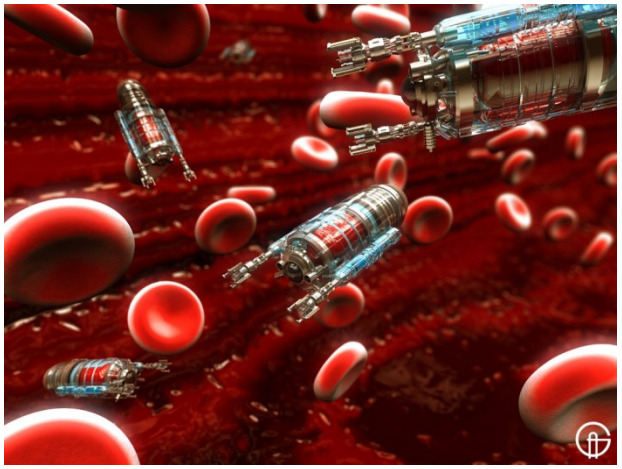
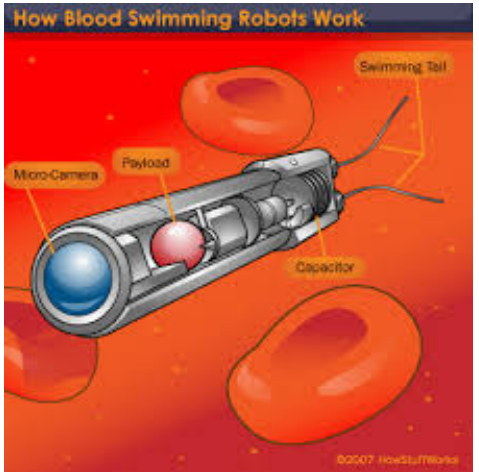
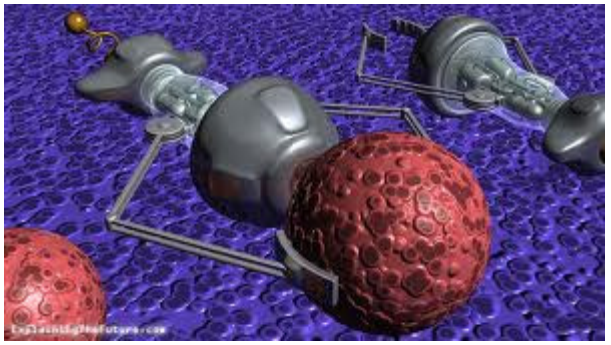
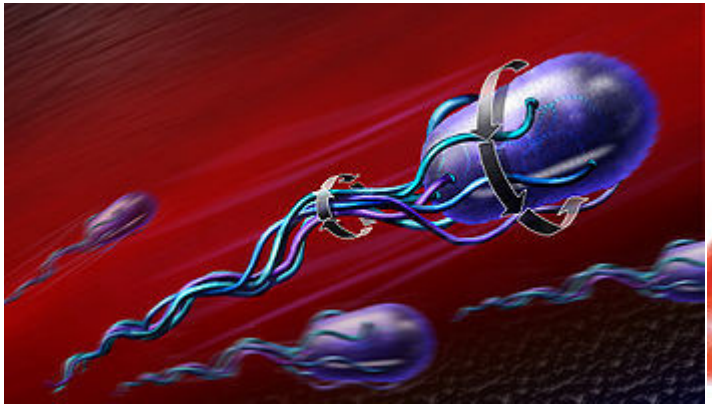
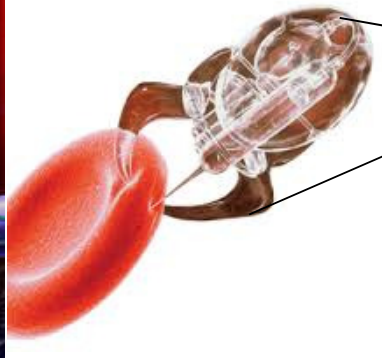
Nanorobots (Nanobots)
Popular Representations

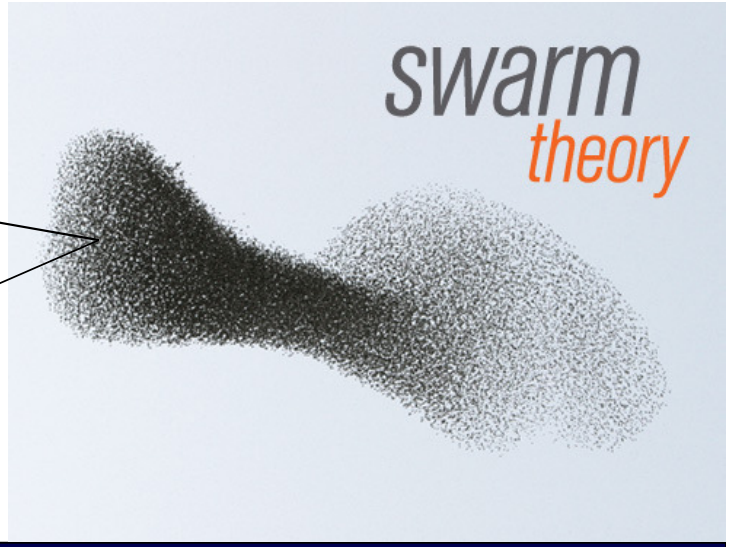








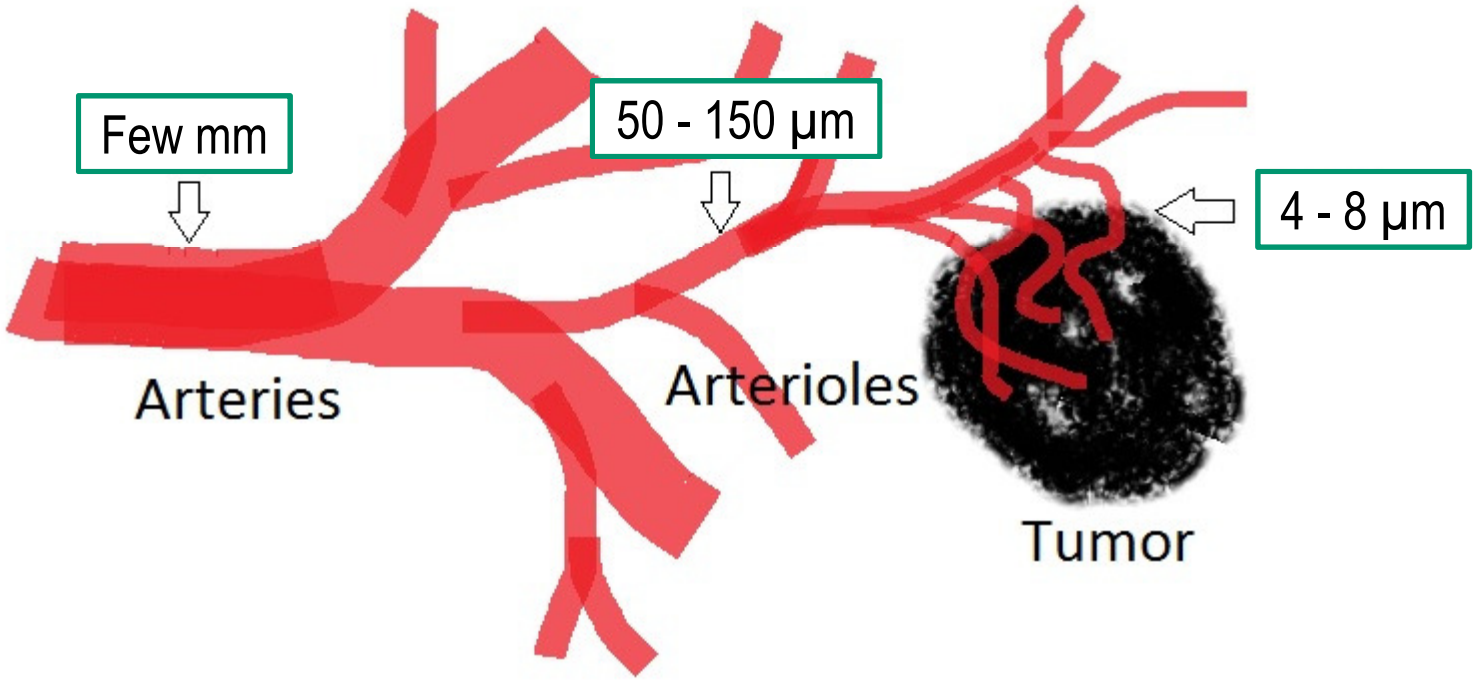




A first example of possible applications: Cancer therapy

- For 2013 in US alone: an estimated 1,660,290 new cancer cases.
- This results to approximately 580,350 deaths – almost 1,600 people per day or 1 every 54 seconds.
- With an estimated today's world population of 7.119 billion individuals with US population representing only 4.45%, one can easily realize the importance of finding new or improved treatment modalities.

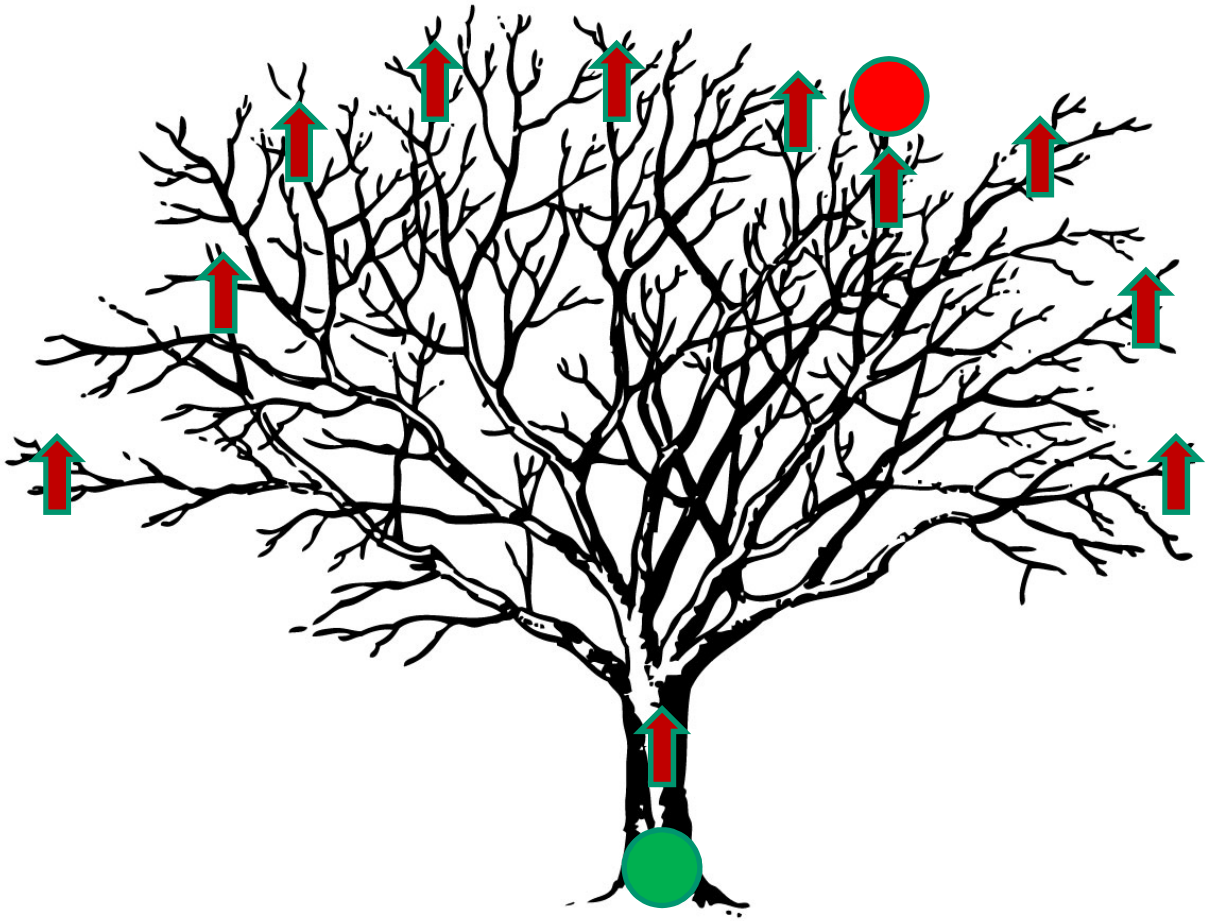
Solid Tumors
(more than 80% of all cancers)



Tumor Targeting

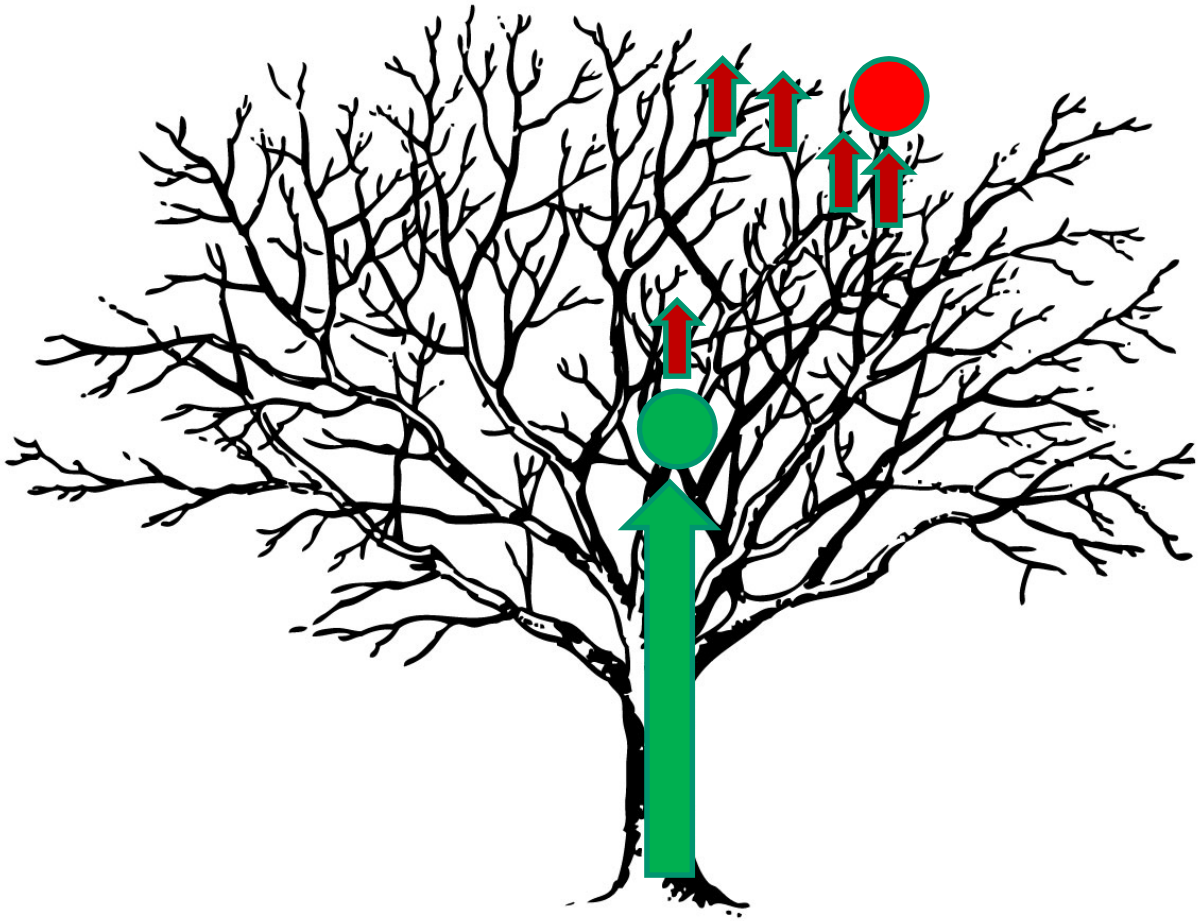
- Passive targeting
  - Accumulation in pathological sites with compromised vasculature
  - Systemic circulation: lower therapeutic index (increase toxicity while lowering therapeutic efficacy)
- Active targeting
  - Based on the attachment of specific ligands to the surface of pharmaceutical carriers to recognize and bind pathological cells
  - Systemic circulation
- **Direct targeting** (new concept)
  - Pharmaceutical carriers or agents (nanobots) being navigated directly from the injection site to the targeted area
  - Avoid or at least reduce systemic circulation
  - Can be combined with passive or active targeting

Without Direct Targeting



Low therapeutic index
(low therapeutic efficacy + high toxicity)

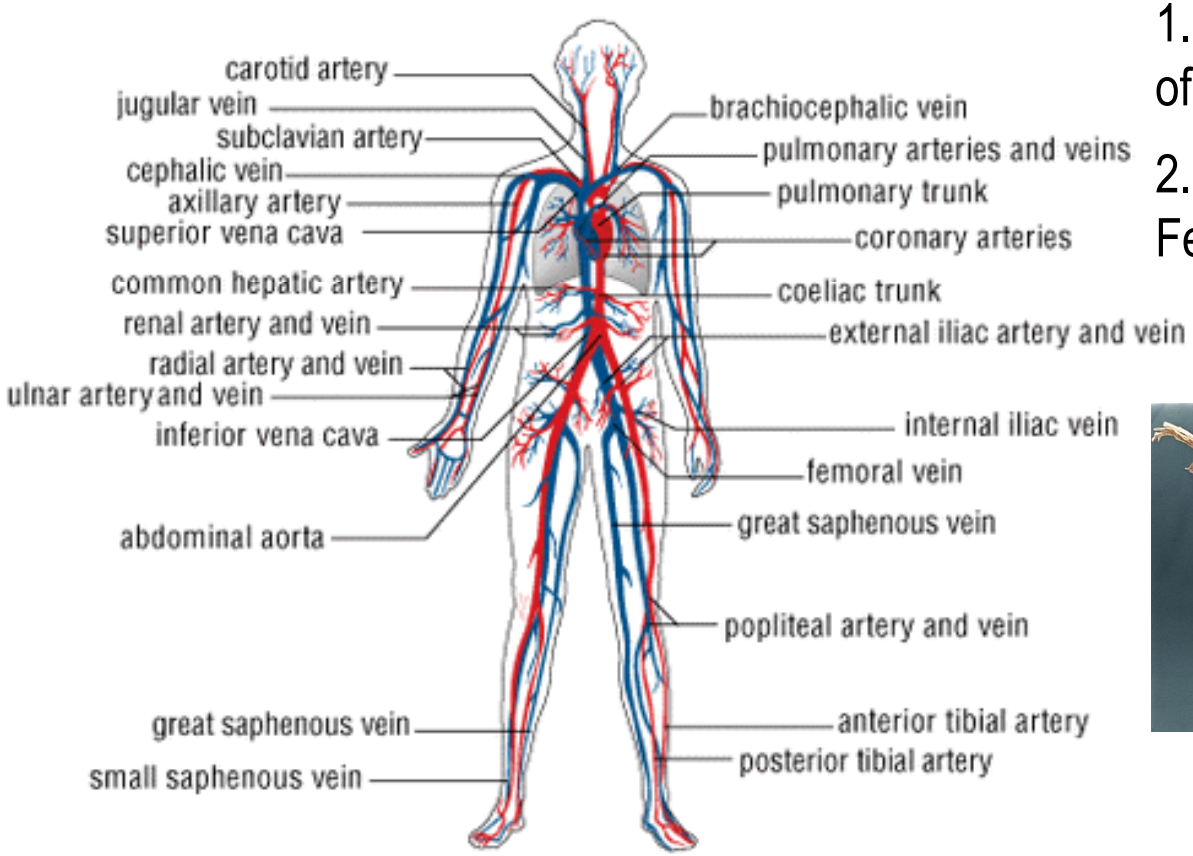
Increasing Direct Targeting



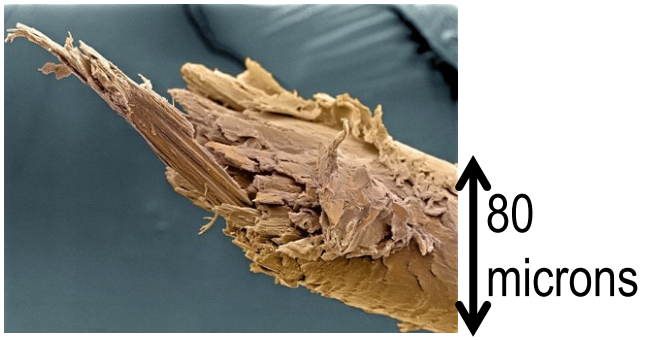
Reduced toxicity and improved targeting

The Major Accessible Routes for Medical Nanorobotics

(smaller vessels, e.g. capillaries not shown)



1. Near 100,000 km of blood vessels
2. Diameters from: Few mm to 4 microns



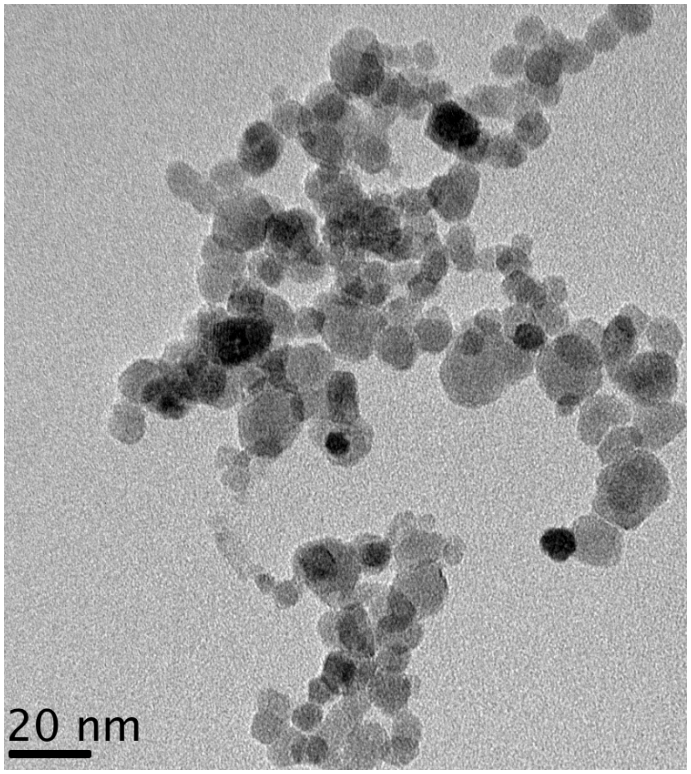
Human hair

NanoRobotics (Nano + Robotics)

Nanotechnology

How to exploit phenomena at the nanoscale in the context of drug delivery…

Nanoparticles as Propelling Systems



Maximizing Induced Directional Force on a Magnetic Agent

$$\vec{F}_{magnetic} = (\vec{m}.\nabla)\vec{B} = V_{ferro}.(\vec{M}.\nabla)\vec{B}$$

Embed maximum magnetic nanoparticles

Increase magnetic field strength and saturation magnetization

Increase magnetic gradient (variation of magnetic field over distance)

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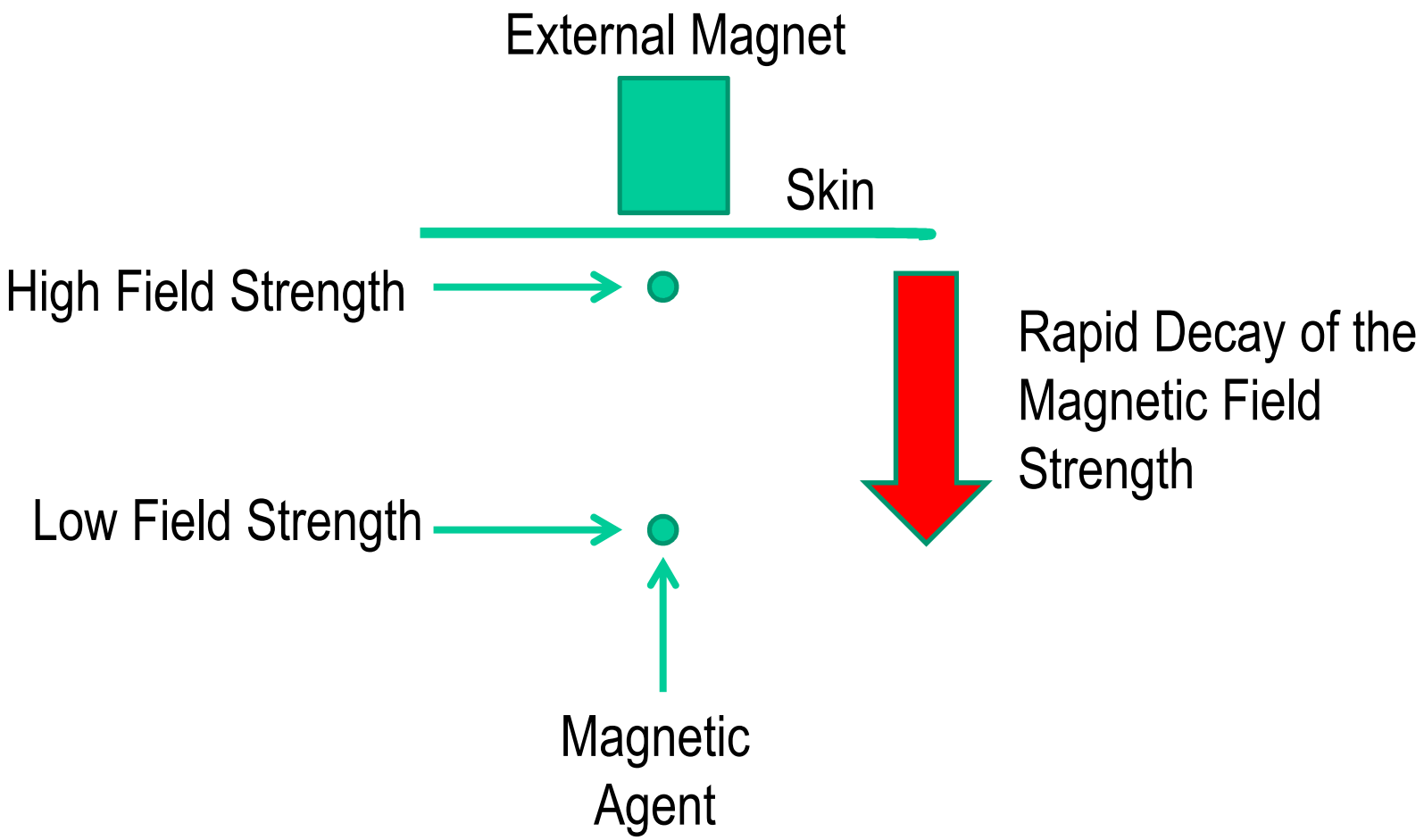
Use of an External Magnet



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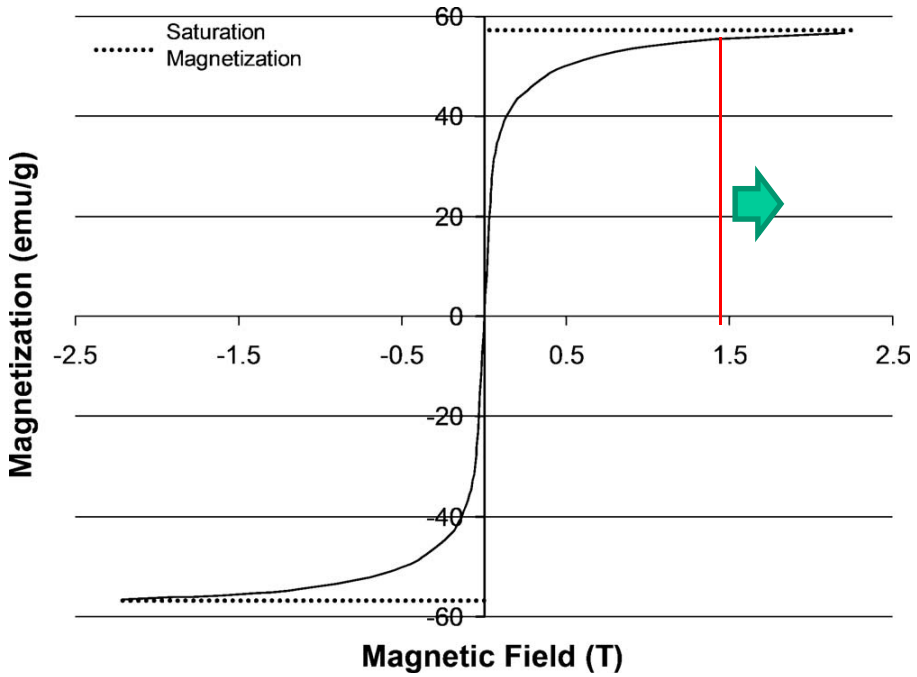
Solution: Immersing Magnetic Agents (Patient) in a High Strength Uniform Magnetic Field



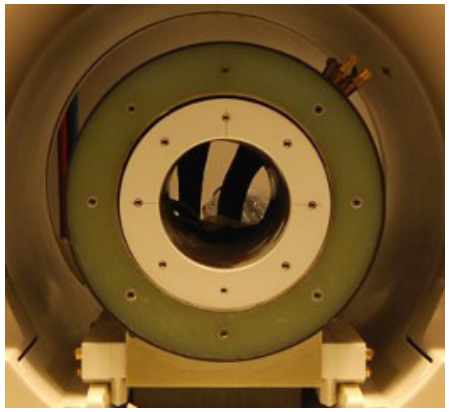
Magnetization curve of Fe_3O_4 magnetic suspension

Mathieu J-B. and Martel S., "Aggregation of magnetic microparticles in the context of targeted therapies actuated by a magnetic resonance imaging system," *Journal of Applied Physics.* 106, 044904-1 to 7, 2009

Implementation: Clinical MRI Scanner

1.5T to 3T inside the tunnel provides depth independent constant high magnetization

Directional Gradients to Move Magnetic Agents in any Directions



Optional Gradient Insert

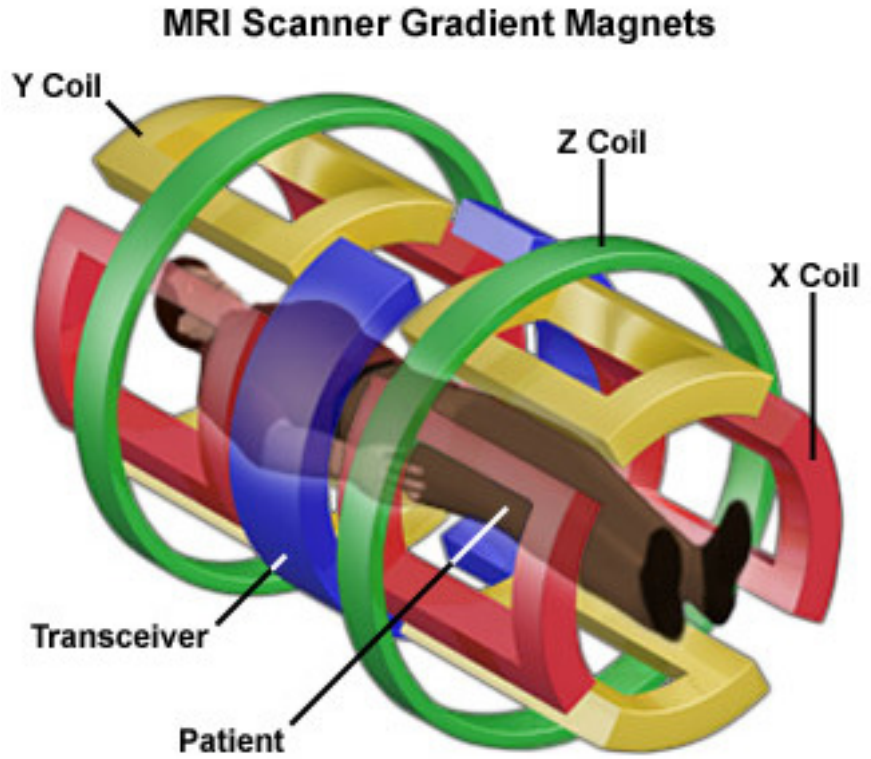


Image from: magnet.fsu.edu - National High Magnetic Field Laboratory

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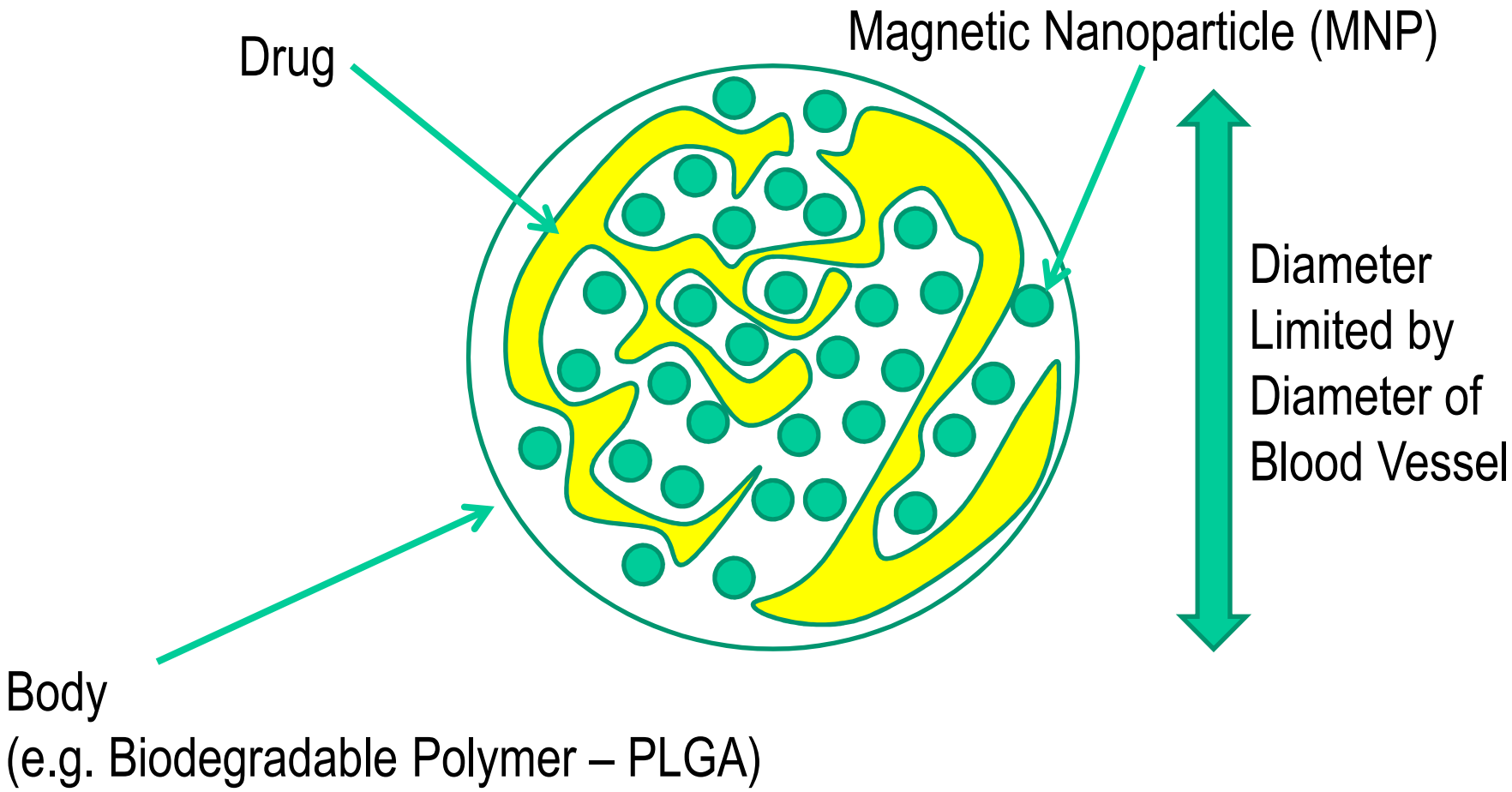
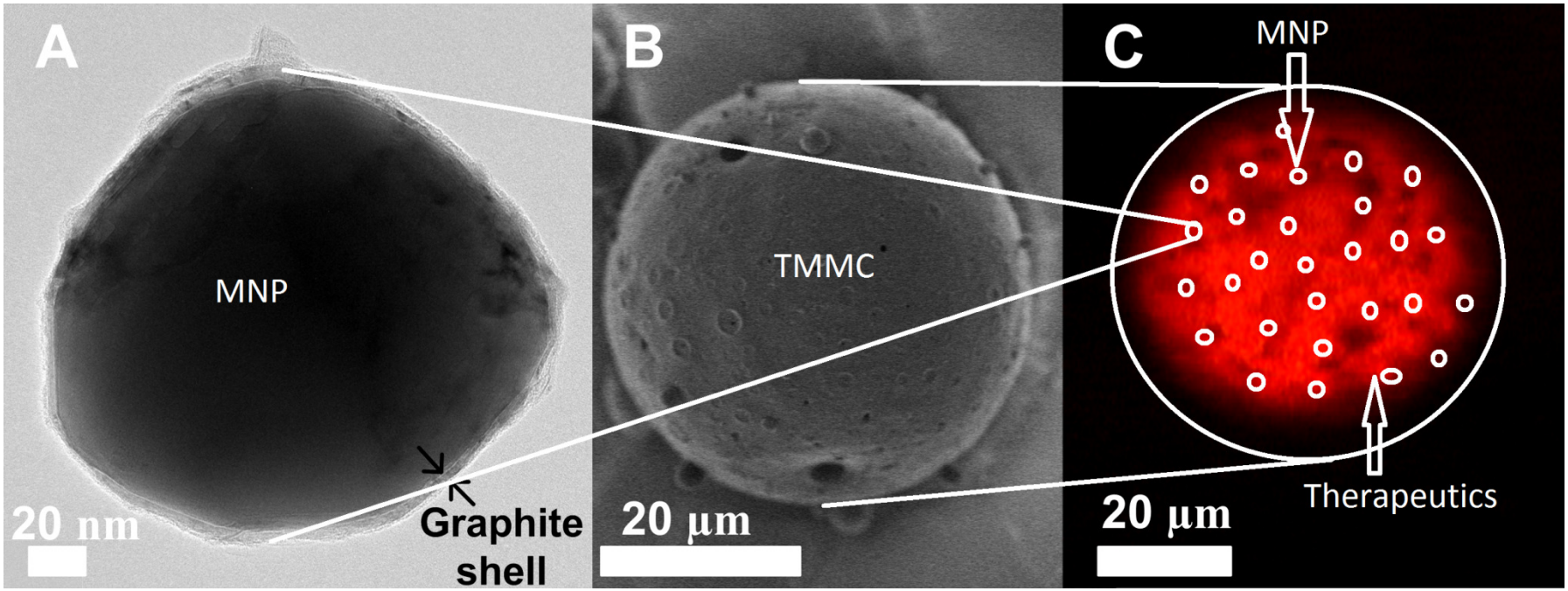
Navigable Delivery Agents

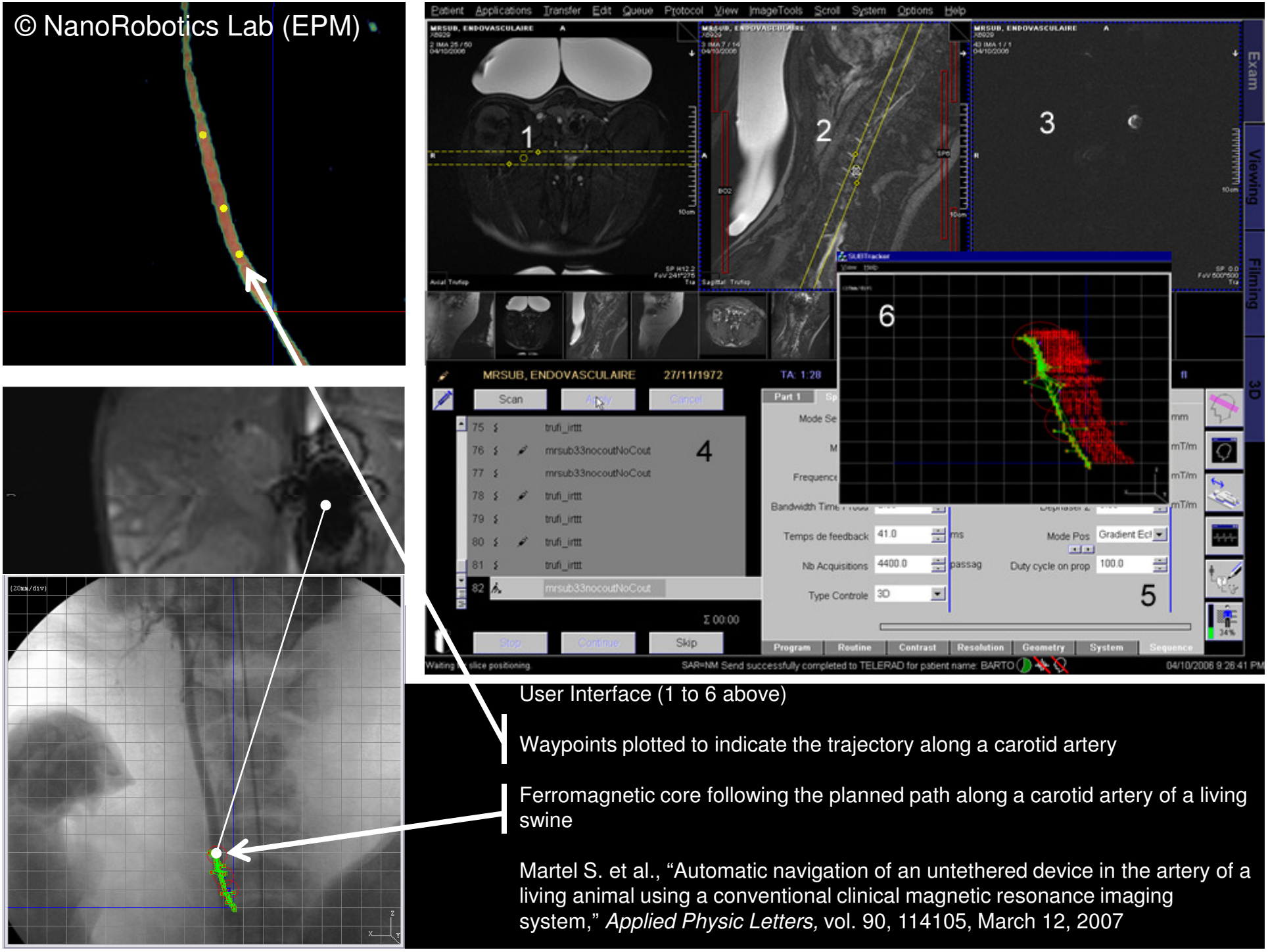
(Many MNP to maximize V_{ferro})



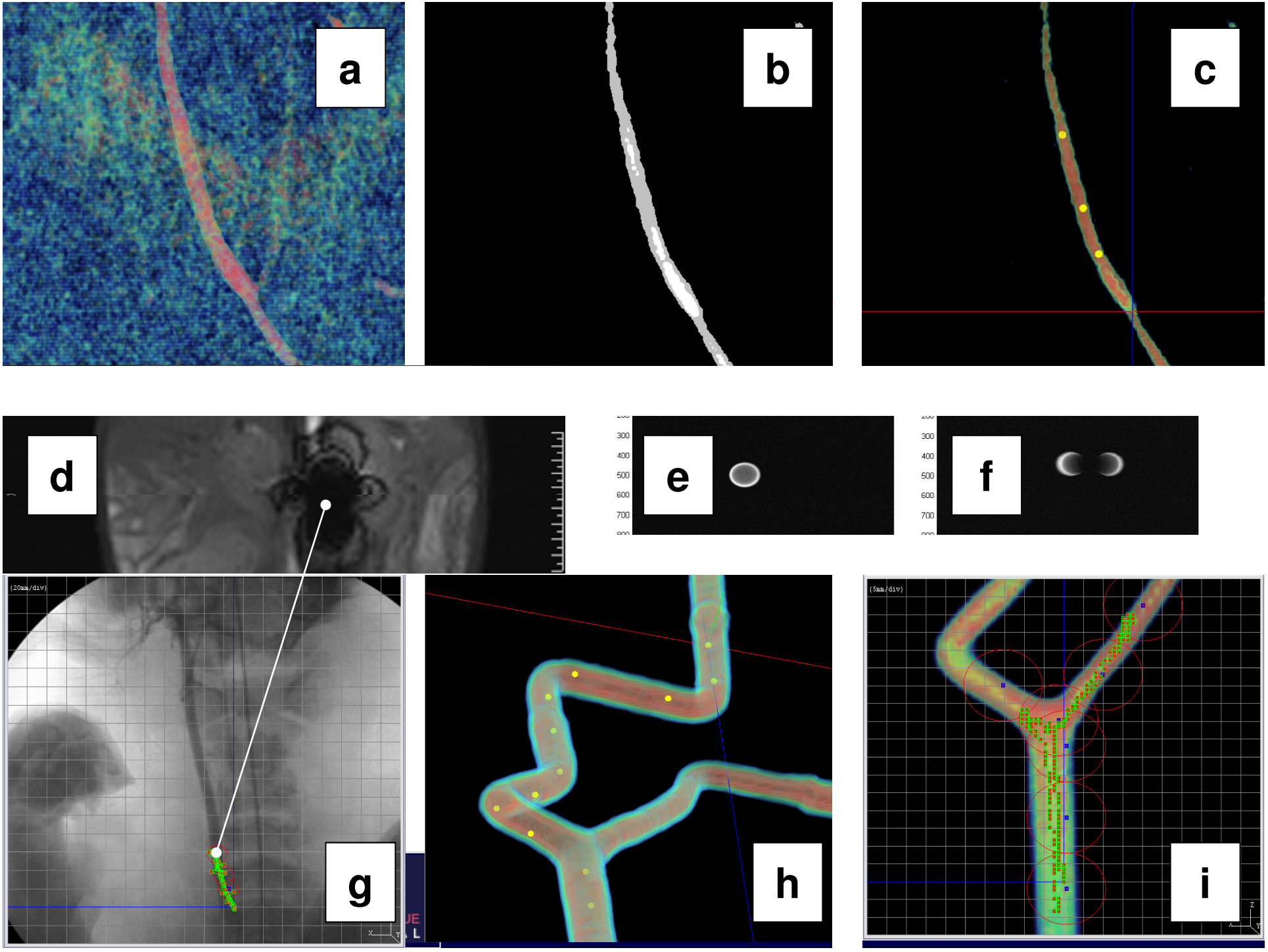
Therapeutic Magnetic Micro Carriers (TMMC)



Adapted from: Pouponneau, P., Leroux, J.-C., Soulez G., Gaboury L., and Martel S., "Co-encapsulation of magnetic nanoparticles and doxorubicin into biodegradable microcarriers for deep tissue targeting by vascular MRI navigation," *Biomaterials*, Vol. 32, Issue 13, pp. 3481-3486, May 2011



Martel S. et al., "Automatic navigation of an untethered device in the artery of a living animal using a conventional clinical magnetic resonance imaging system," *Applied Physic Letters,* vol. 90, 114105, March 12, 2007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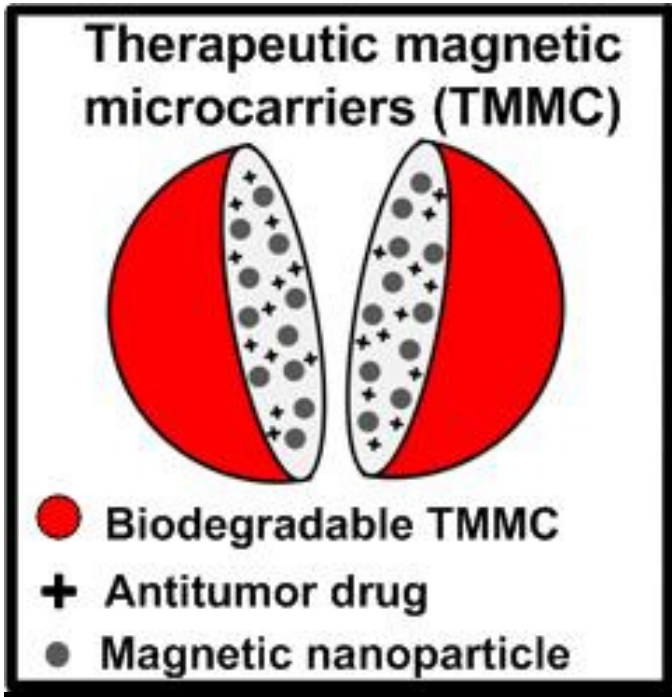
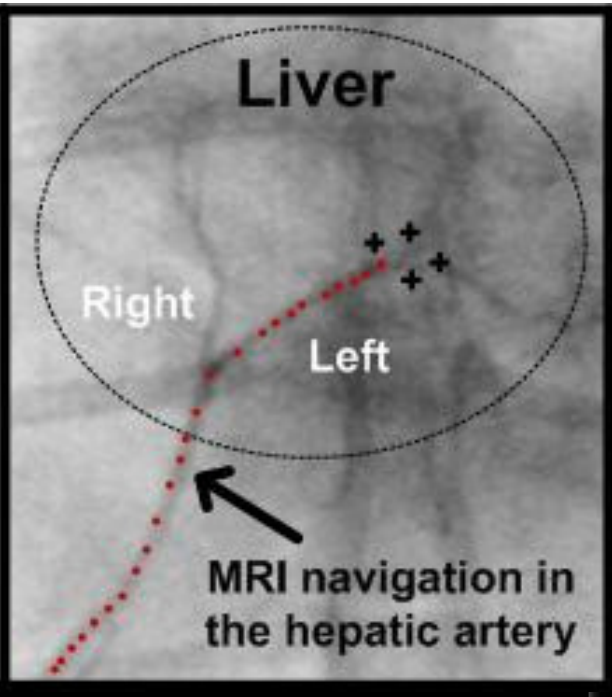
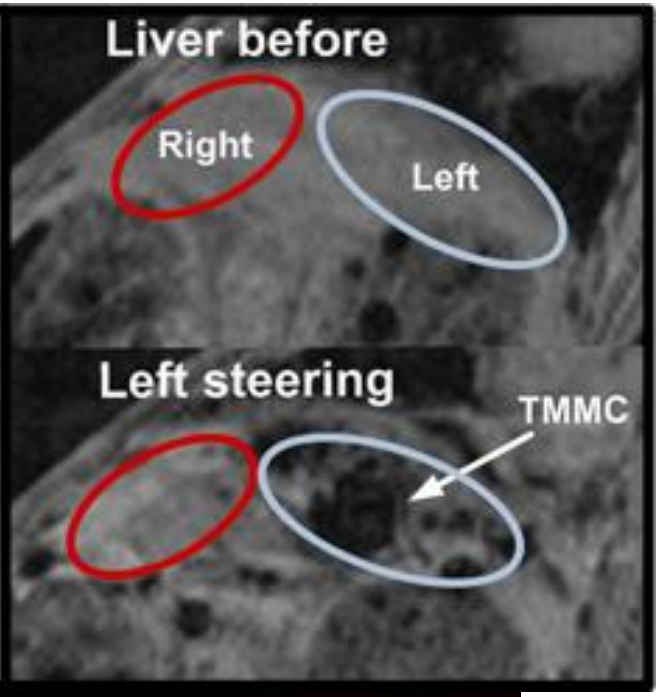
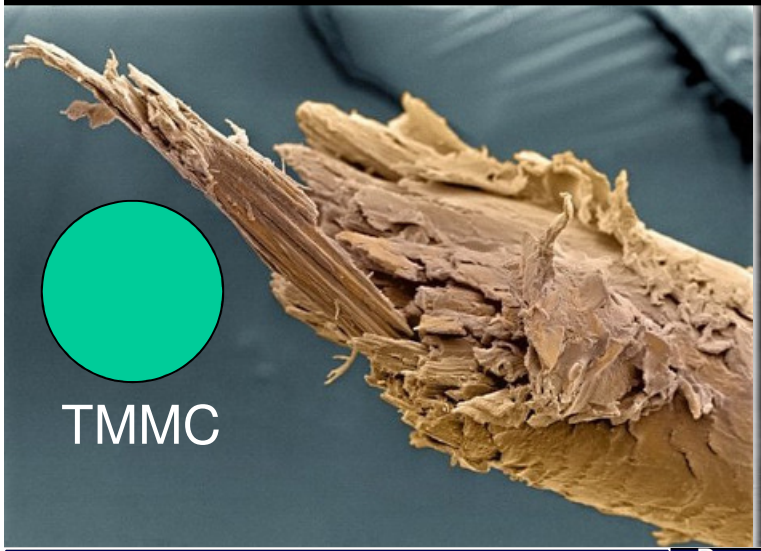


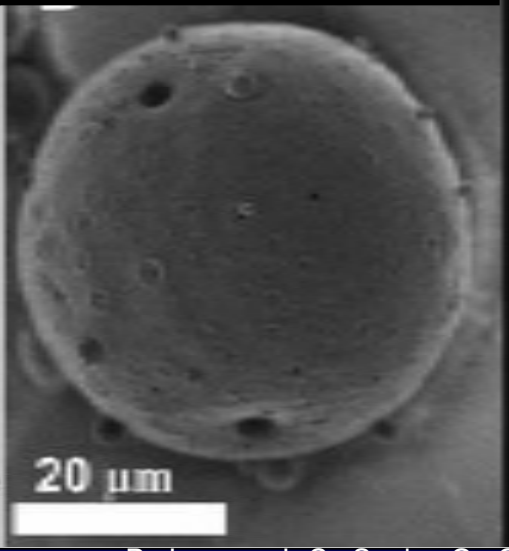




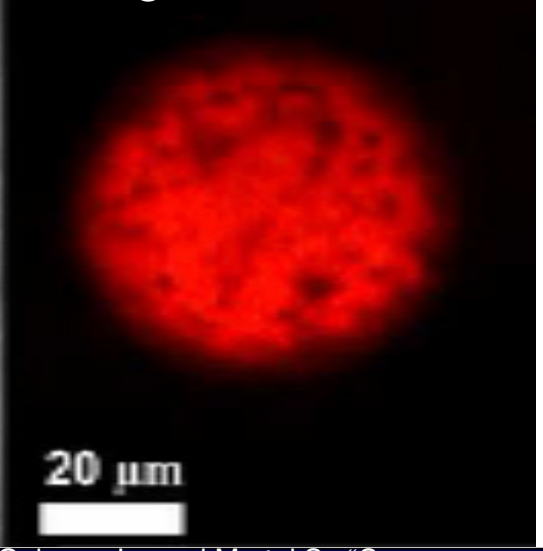
TMMC versus a human hair



TMMC



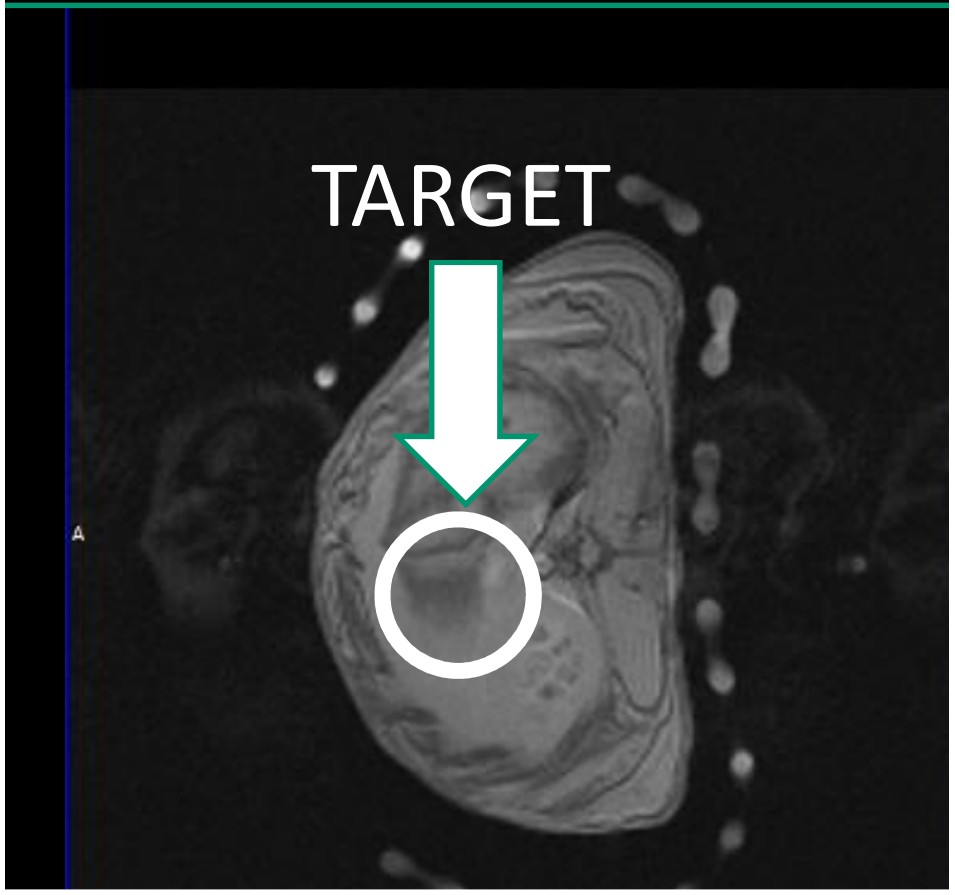
Drug inside TMMC

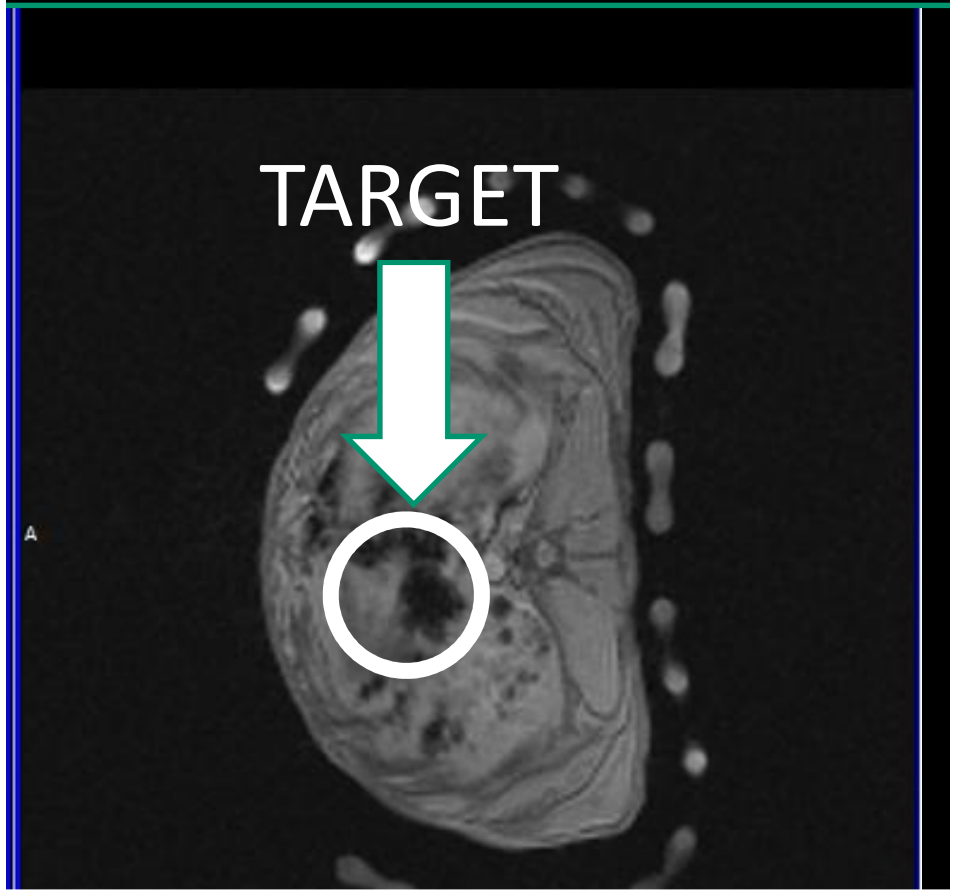


Pouponneau, P., Leroux, J.-C., Soulez G., Gaboury L., and Martel S., "Co-encapsulation of magnetic nanoparticles and doxorubicin into biodegradable microcarriers for deep tissue targeting by vascular MRI navigation," *Biomaterials*, vol. 32, Issue 13, pp. 3481-3486, 2011

BEFORE

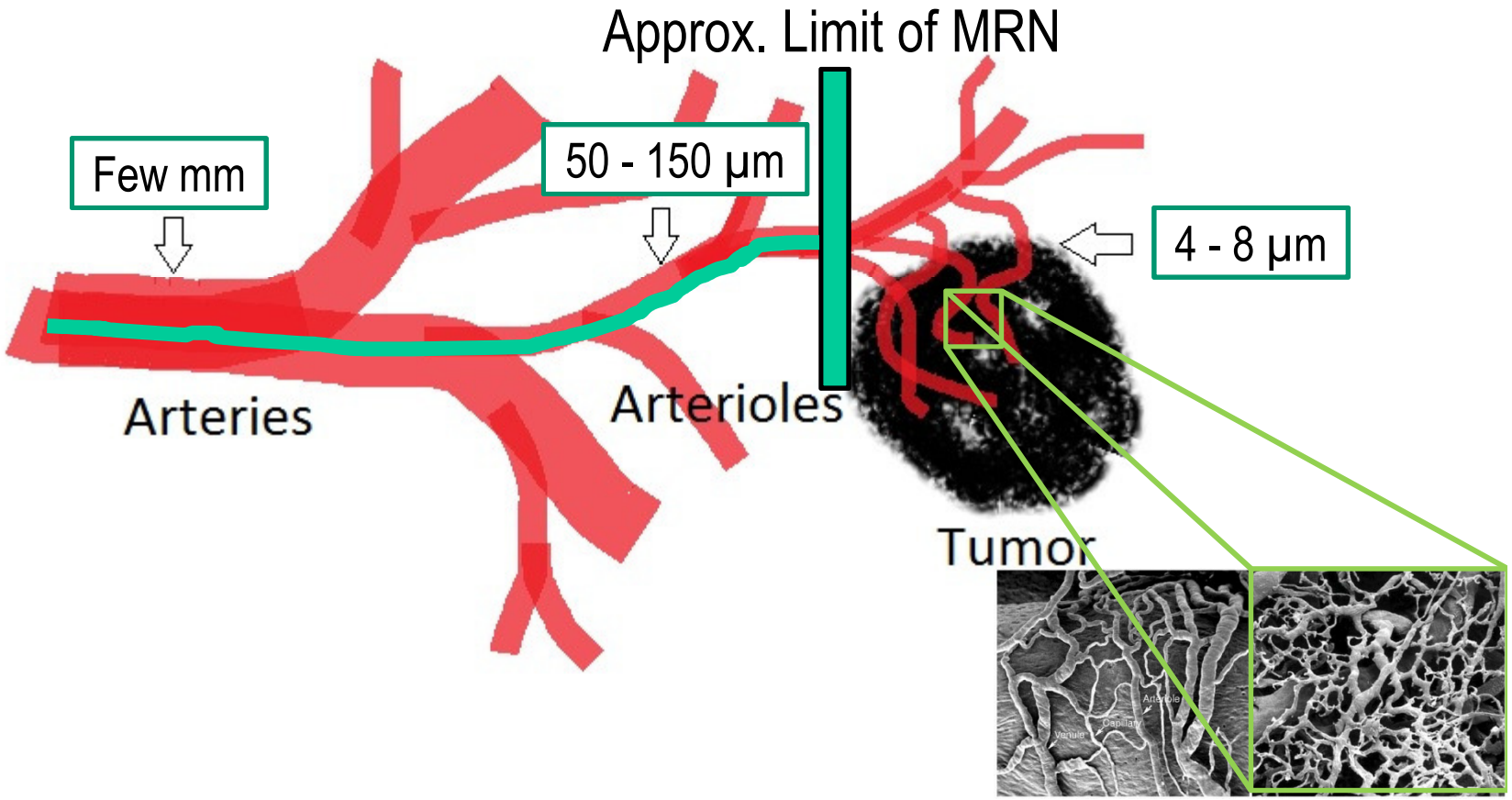
AFTER





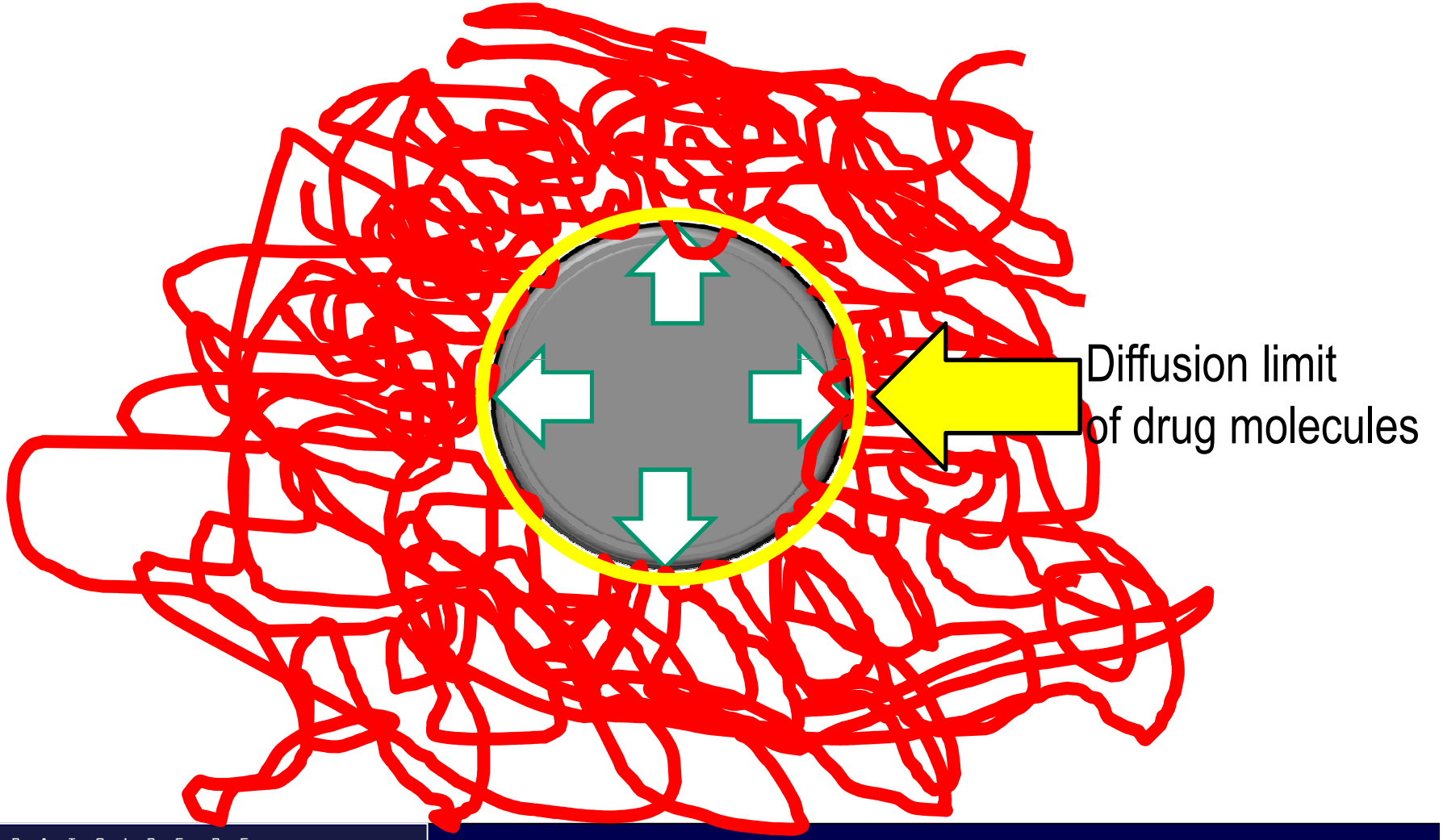
Theranostic agents – MNP used as propulsion and MR-tracking to assess targeting efficacy and dose being delivered

Magnetic Resonance Navigation (MRN) - Limitation



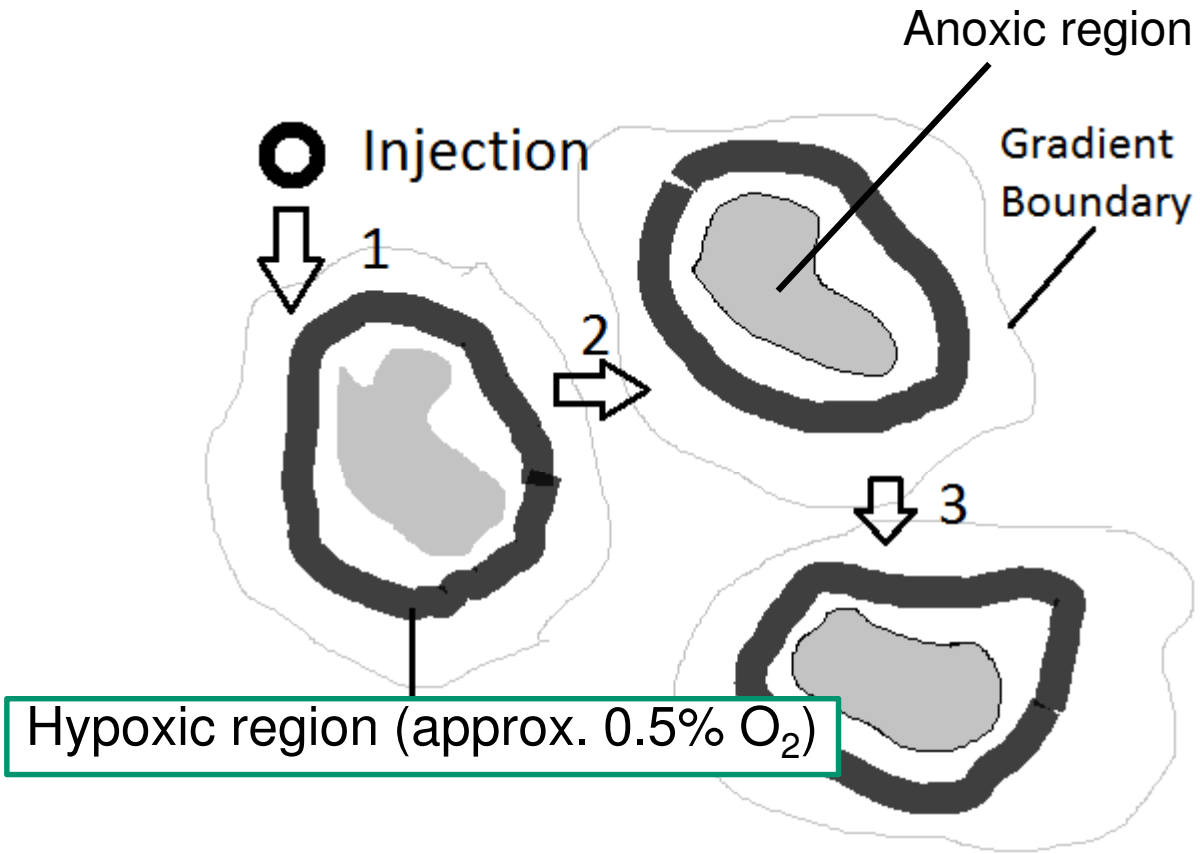
But there is more…

Tumor Interstitial Fluid Pressure (TIFP)



And there is much more…

Hypoxic Regions inside Solid Tumors



QUESTION:
How to increase the efficacy of drug beyond the development of new molecules

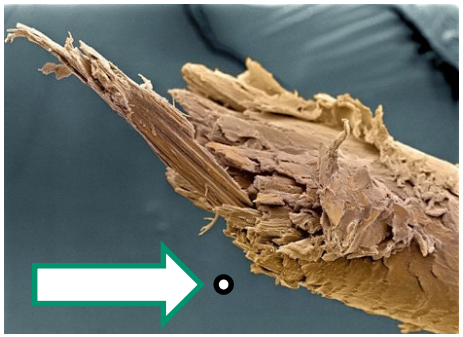
ANSWER:

Develop intelligent navigable agents (micro- nanorobots or microsystems) that support all of the following 10 specifications

(characteristics or functionalities)

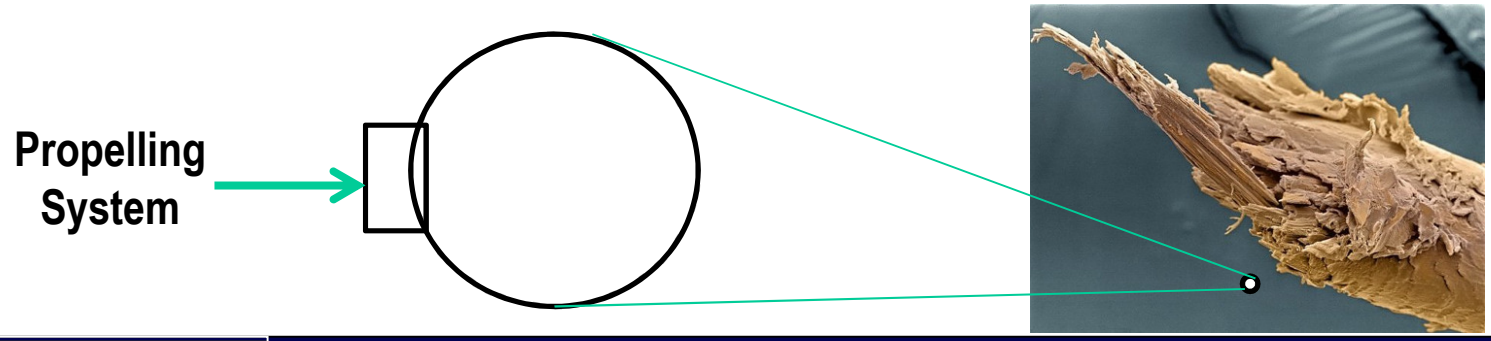
Specification 1

- Such intelligent navigable therapeutic agents or microsystems (agents) must be capable of following the same route as the oxygen and nutrients through the angiogenic network since the TIFP prevents direct injections to the tumor. This means that the maximum diameter of each agent is limited to a couple micrometers only.



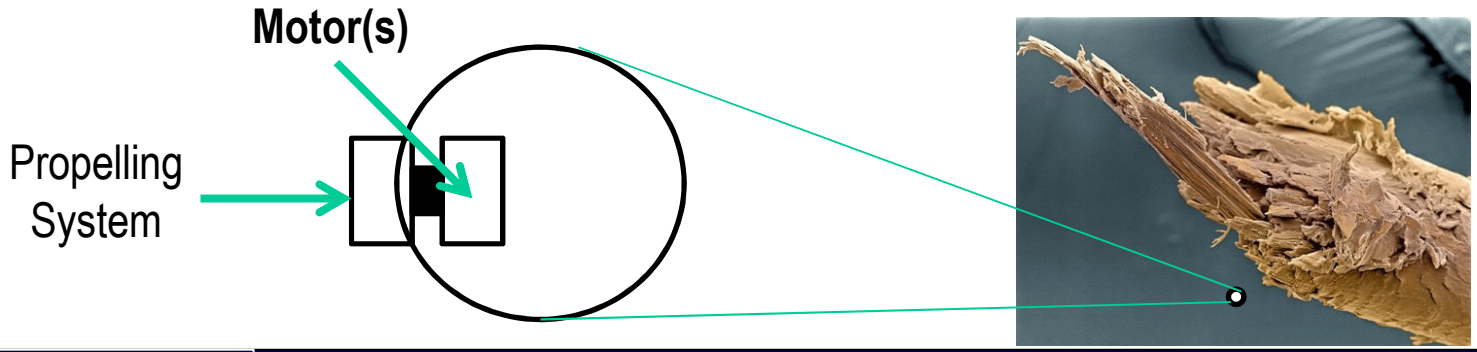
Specification 2

- Each agent must also have a propelling system that can perform effectively in low Reynolds hydrodynamic conditions (Reynolds number being in the order of 10^{-4}) as encountered when operating in the tumoral environment since effective induced 3D directional propelling force at such a scale is not possible.



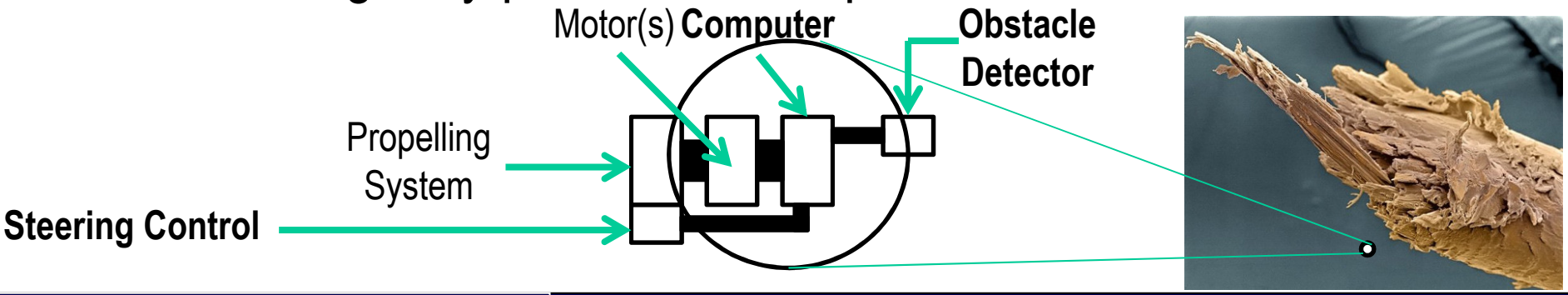
Specification 3

- The same propelling system must be actuated by one or more motors providing sufficient force to transit effectively through the angiogenic network and to propel each agent deep inside the tumor interstitial space well beyond the limit of conventional drugs that cannot generally diffuse deeper due to the TIFP.



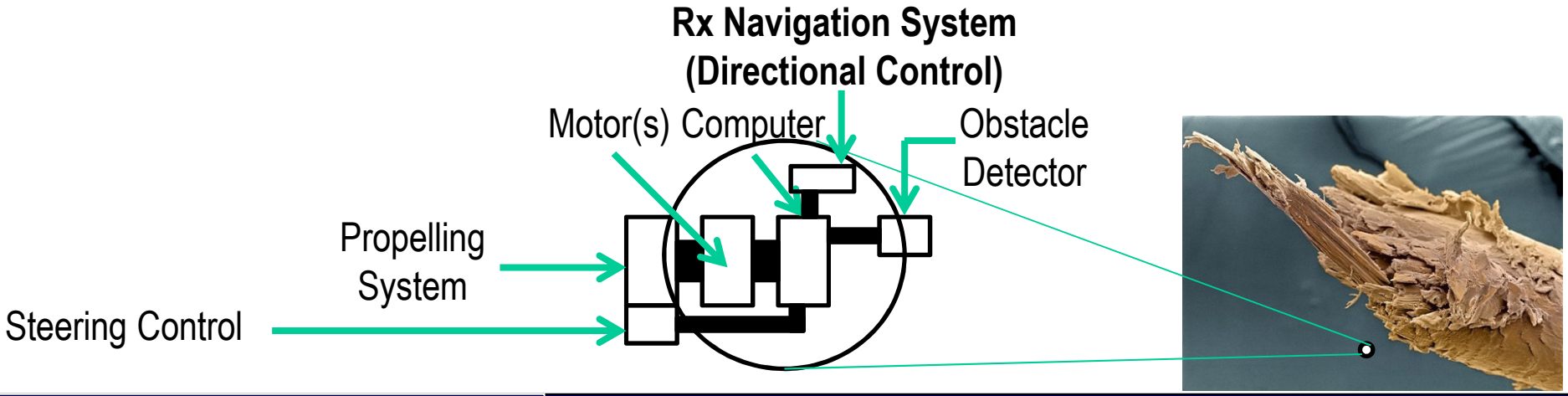
Specification 4

- Some level of autonomy embedded in each agent is also required to navigate through the chaotic angiogenesis network since without medical imaging modalities capable of imaging such tiny vessels for gathering the required information, navigation control from an external source to direct each agent around physiological obstacles, is not technologically possible at the present time.



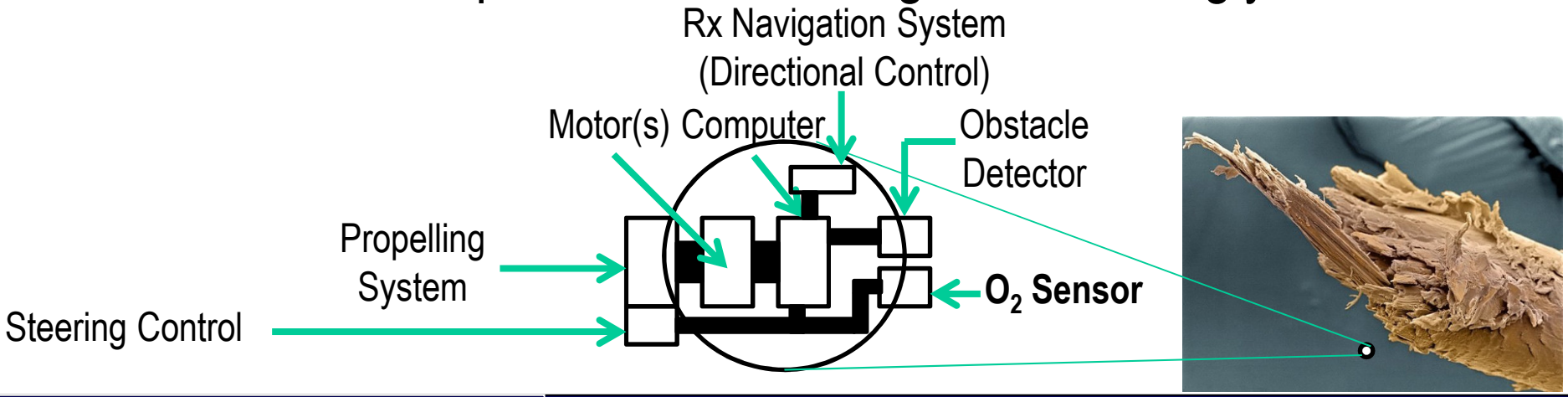
Specification 5

- Each agent must also have some sort of receiver (Rx) or navigation system to allow an external imaging modality platform to indicate to each agent the general direction of the tumor.



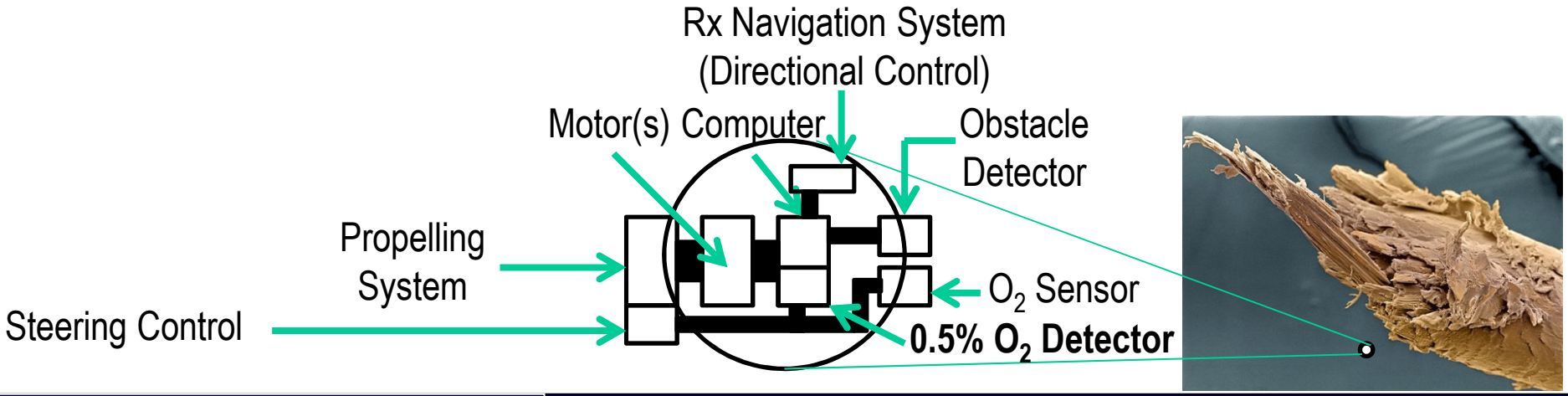
Specification 6

- At the entrance of the interstitial space of the tumor, since hypoxic regions cannot be visualized, each agent must have at least one onboard oxygen sensor capable of detecting decreasing oxygen gradients from the end of blood vessels to the hypoxic regions while influencing the direction of displacement of the agent accordingly.



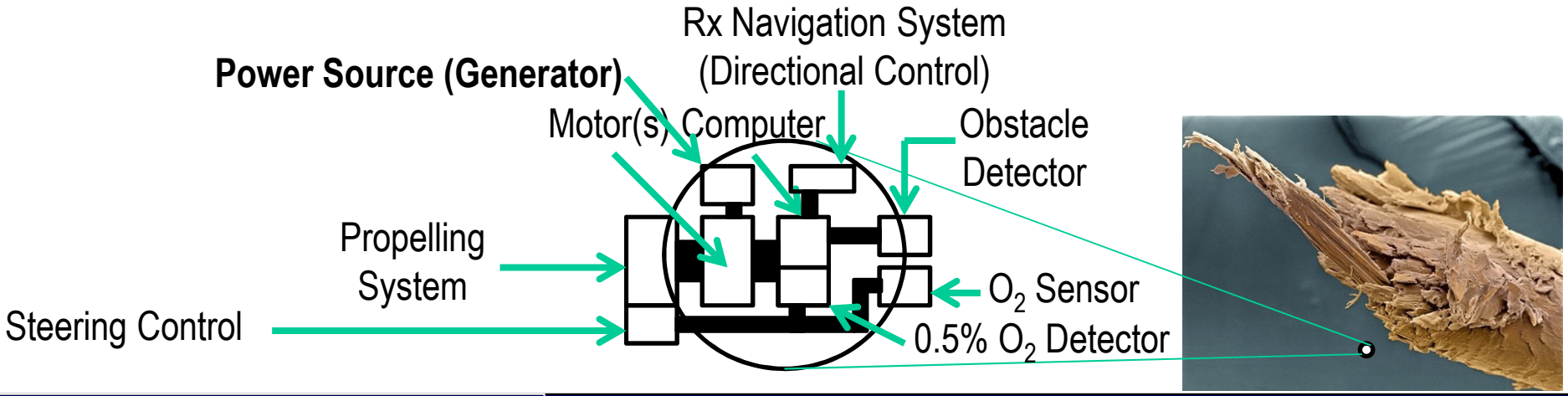
Specification 7

- In addition, the same oxygen sensor must be able to detect the appropriate lower oxygen concentration (approx. 0.5% O_2) of the hypoxic regions and to instruct the agent to maintain its position at such targeted sites until the therapeutics are being released.



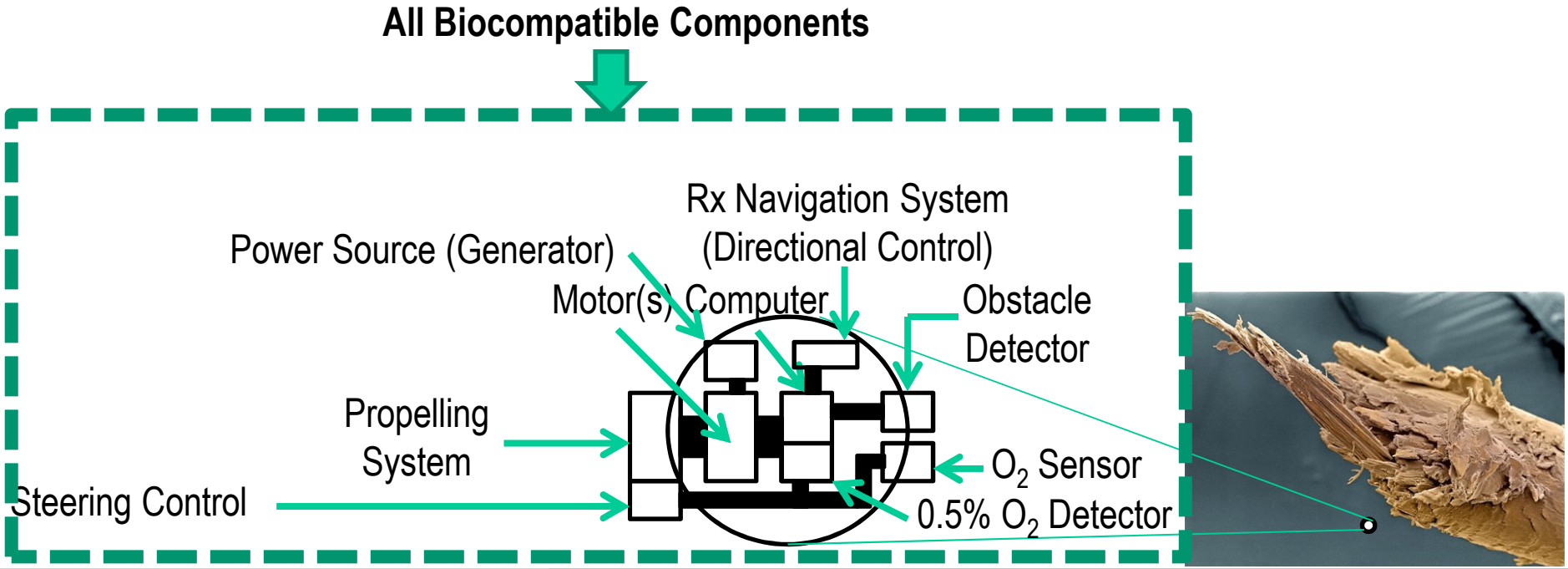
Specification 8

- Each agent must have sufficient power to maintain all these embedded functionalities operational for the period of time required for targeting without relying on known power technologies that cannot be implemented or induced at such a small scale.



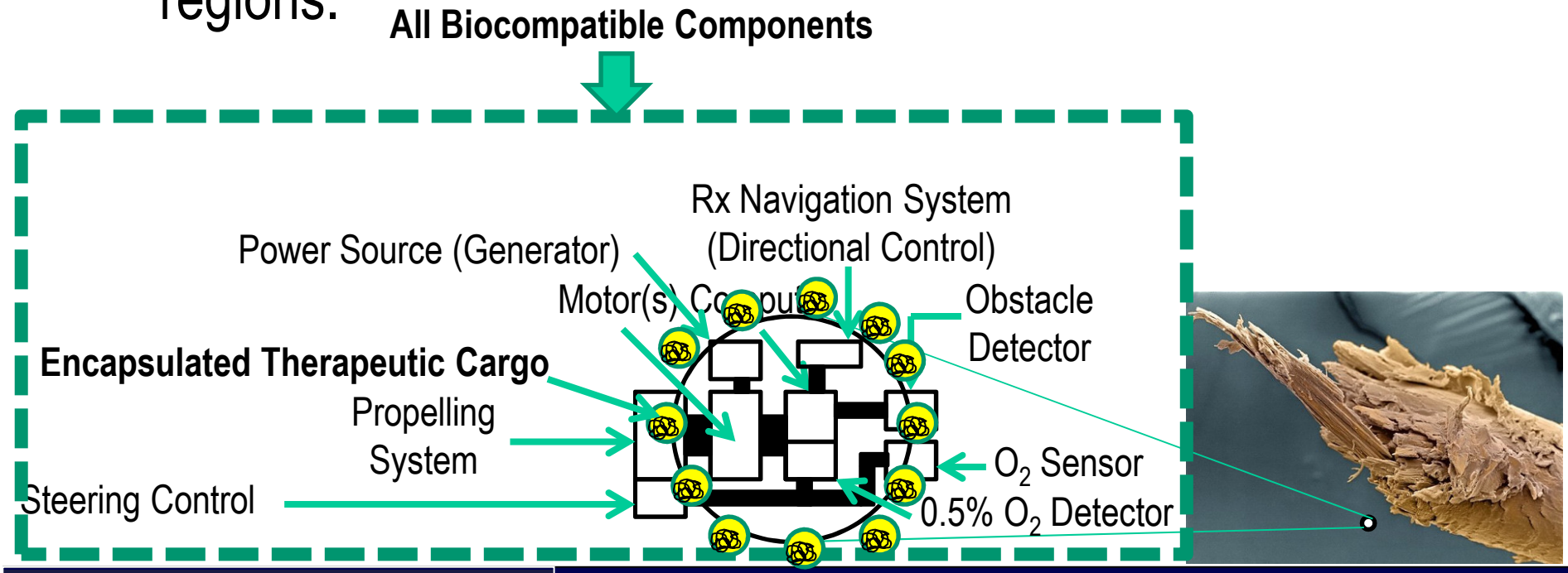
Specification 9

- Each agent must also meet both cytotoxicity and immune system response requirements for potential uses in humans.



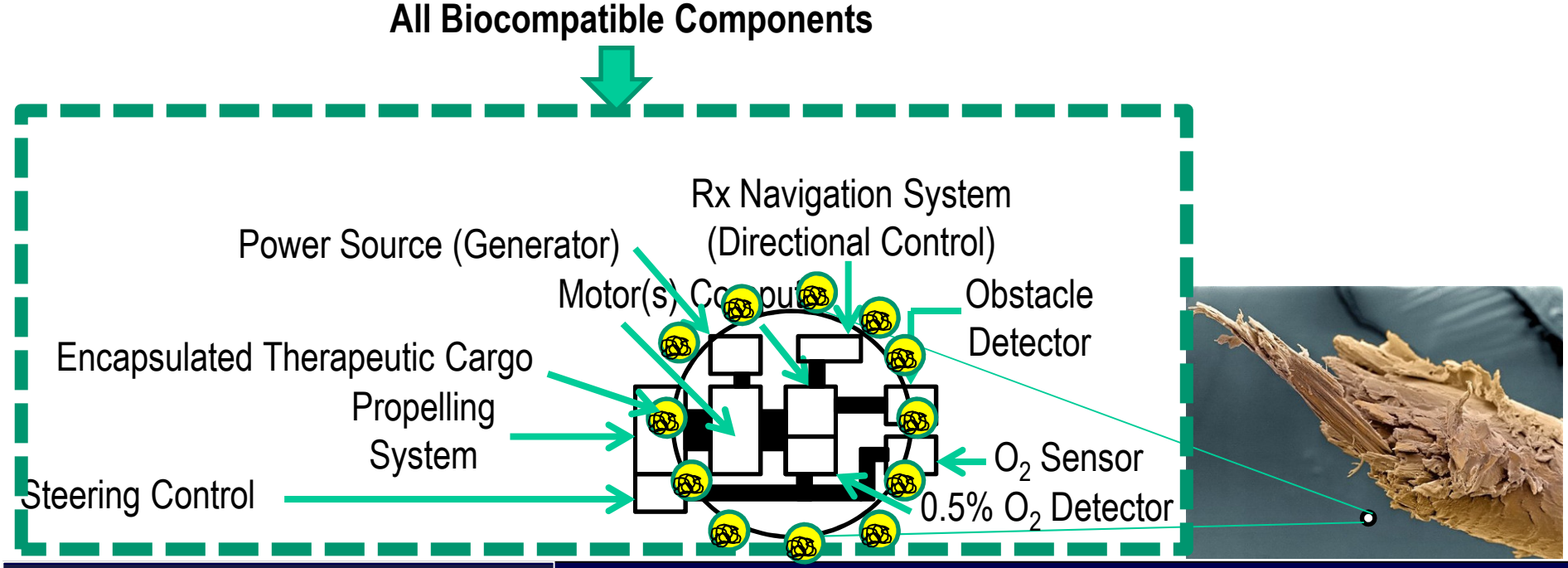
Specification 10

- Finally, each agent must be able to carry sufficient therapeutic payloads encased in special containers attached to the agent for controlled release in the hypoxic regions.

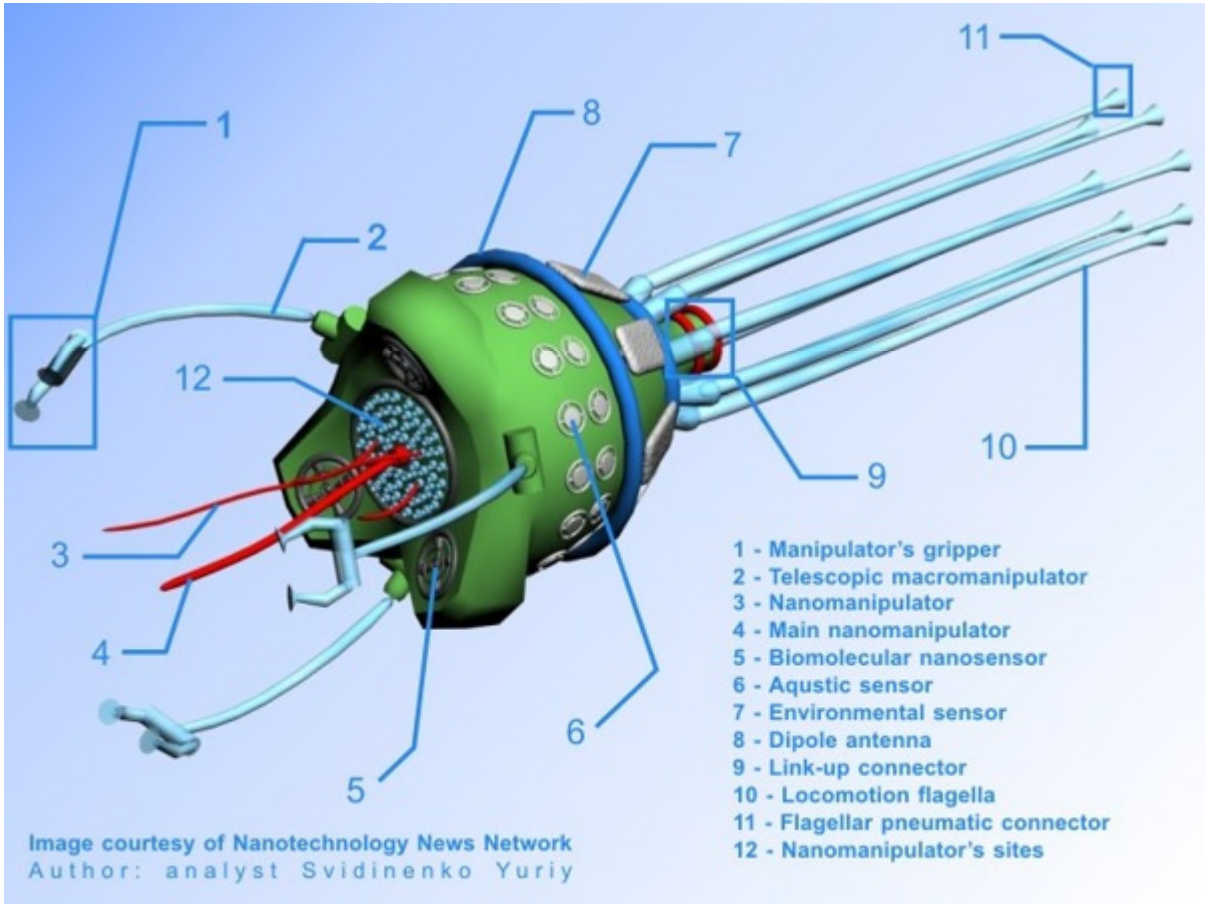


Extra Specifications

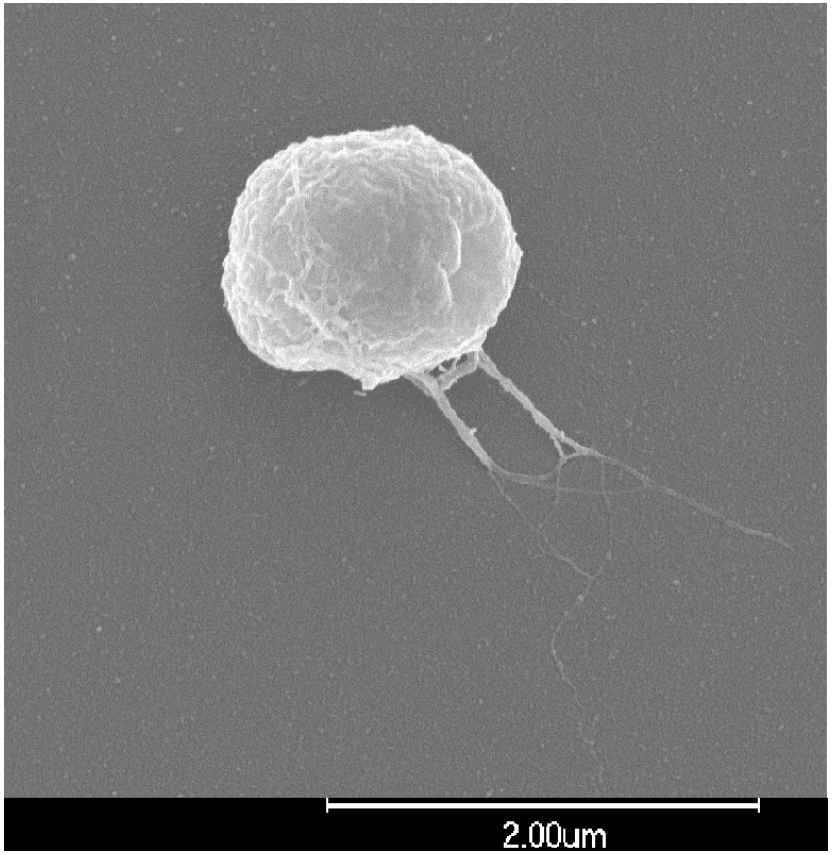
- In a practical point-of-view, since many agents are required considering their overall size to deliver sufficient therapeutic payloads for effective treatments, to maintain an affordable cost, such agents should be self-replicating while the therapeutic payload should be attached through a self-assembly process.



One potential solution…
in a far, far future

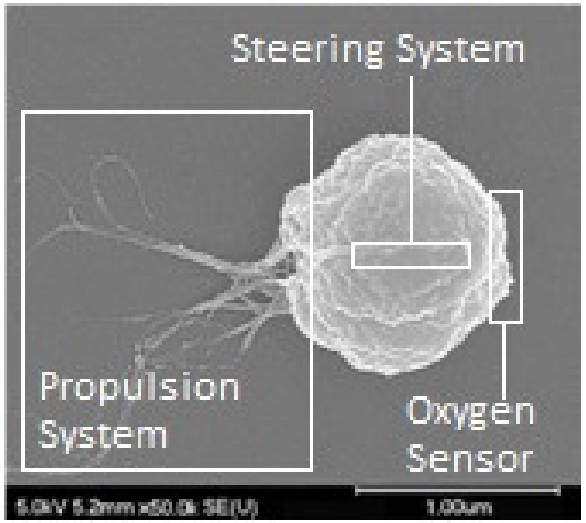


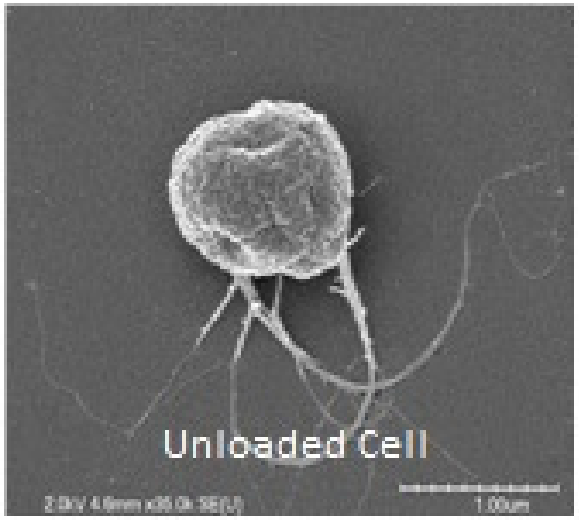
Since this was far beyond technological feasibility, a potential strategy is to identify a micro-organism that has all these specifications and to harness it to act as a nanorobot for drug delivery applications

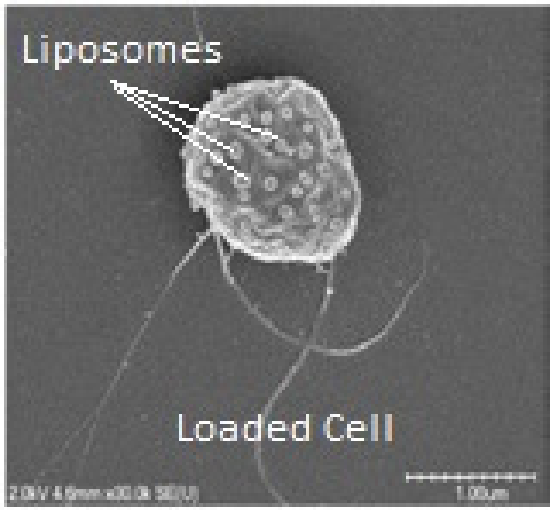


MC-1 Magnetotactic Bacterium (MTB)

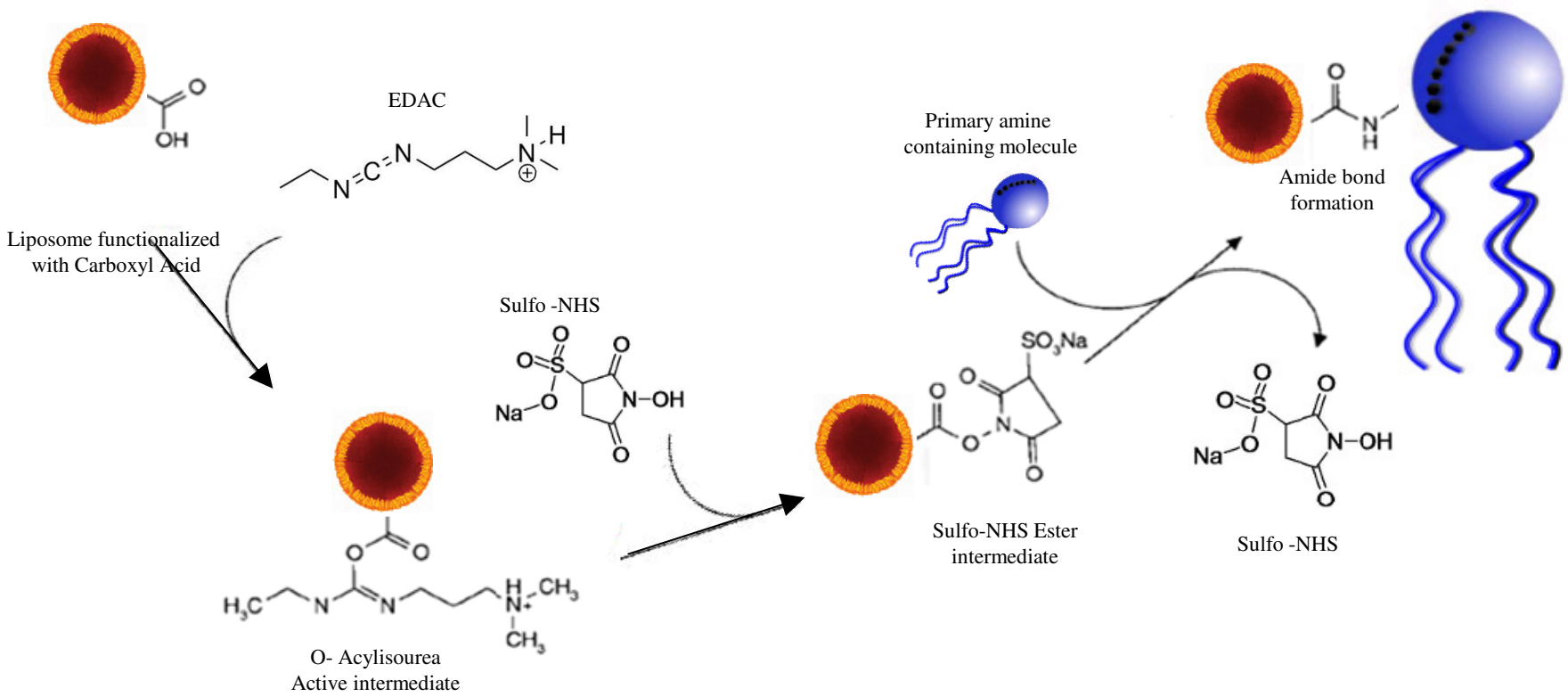
Harnessing What Nature Already Provides





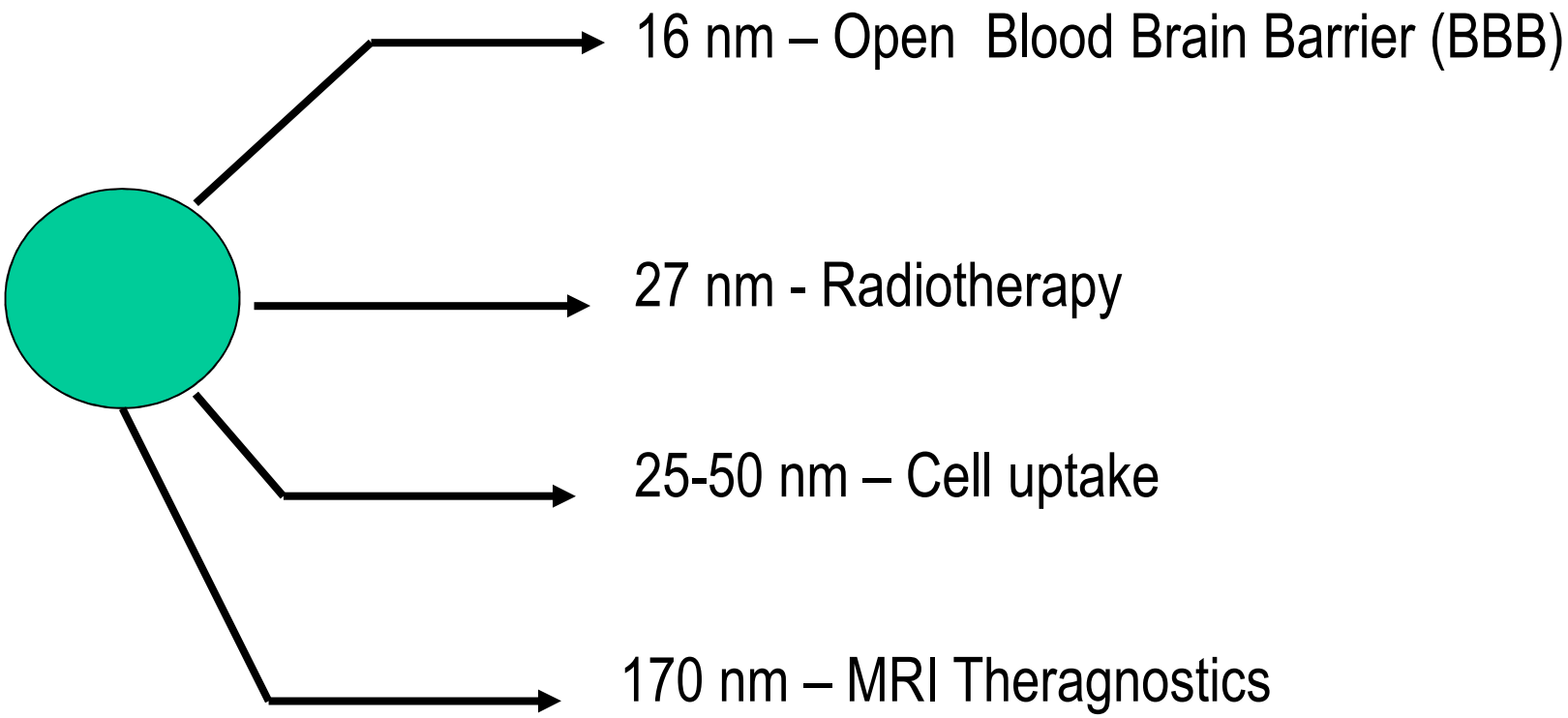


Attachment of activated drug-loaded liposomes to functional groups of MC-1 MTB using carbodiimide chemistry

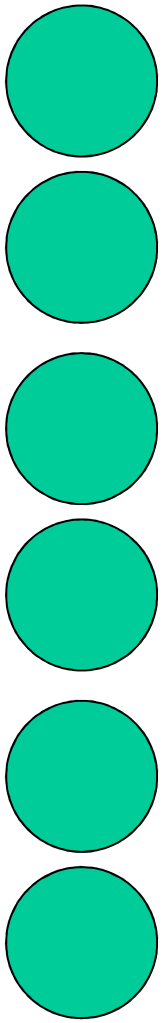


Taherkhani S., Mohammadi M., Daoud J., Martel S., and Tabrizian M., Covalent binding of nanoliposomes to the surface of magnetotactic bacteria acting as self-propelled target delivery agents," *ACS Nano,* 2014 (DOI: 10.1021/nn5011304)

Nanoparticles – Size Matters

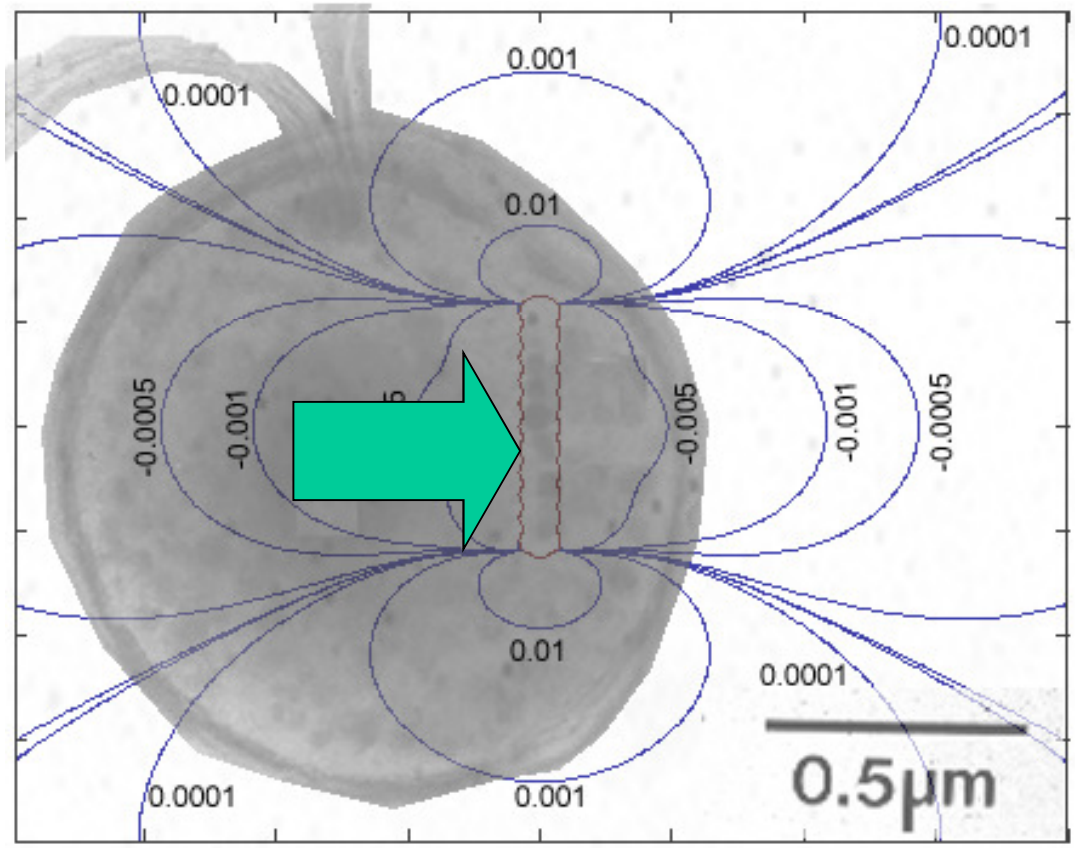


Nanoparticles – Size and Shape Matter

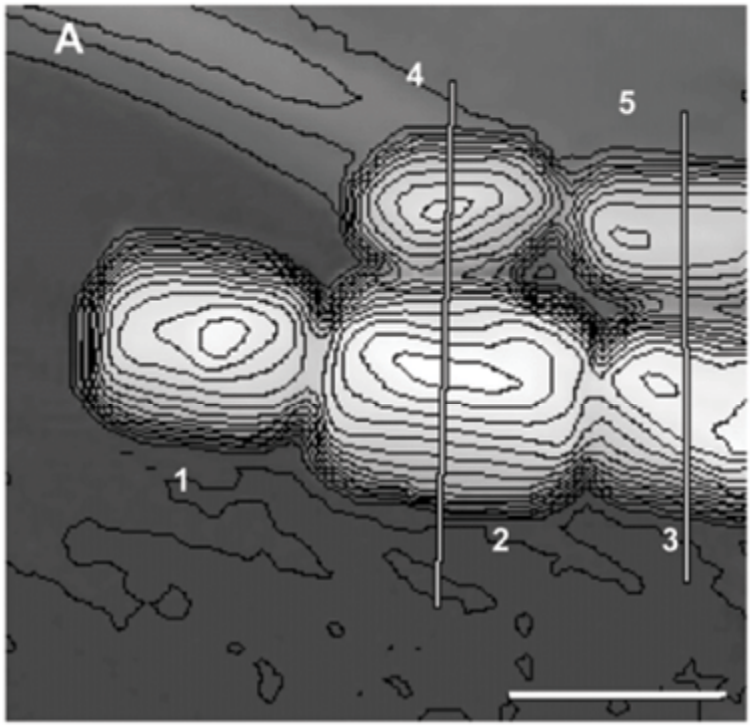


Diam: 70 nm

Rx Navigation System (Magnetotaxis Navigation Control)



Rx Navigation System (Magnetotaxis Navigation Control)



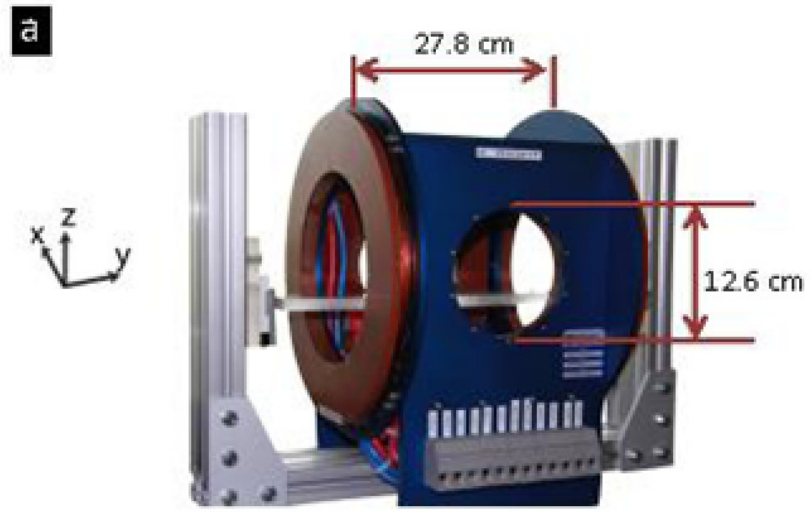
Dennis A. Bazylinski & Richard B. Frankel
Magnetosome formation in prokaryotes
Nature Reviews Microbiology 2, 217-23 (March 2004)

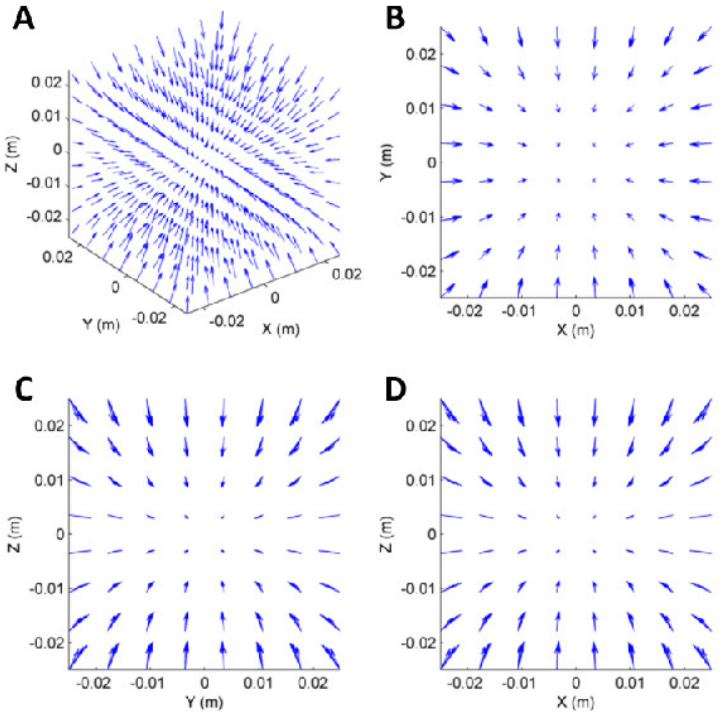
The alignment of the magnetosomes creates a cell magnetic dipole that when exposed to a magnetic field can be described by the Langevin function as

$$\cos\Theta = L\left(\frac{m\mu_0 H}{k_B T}\right)$$

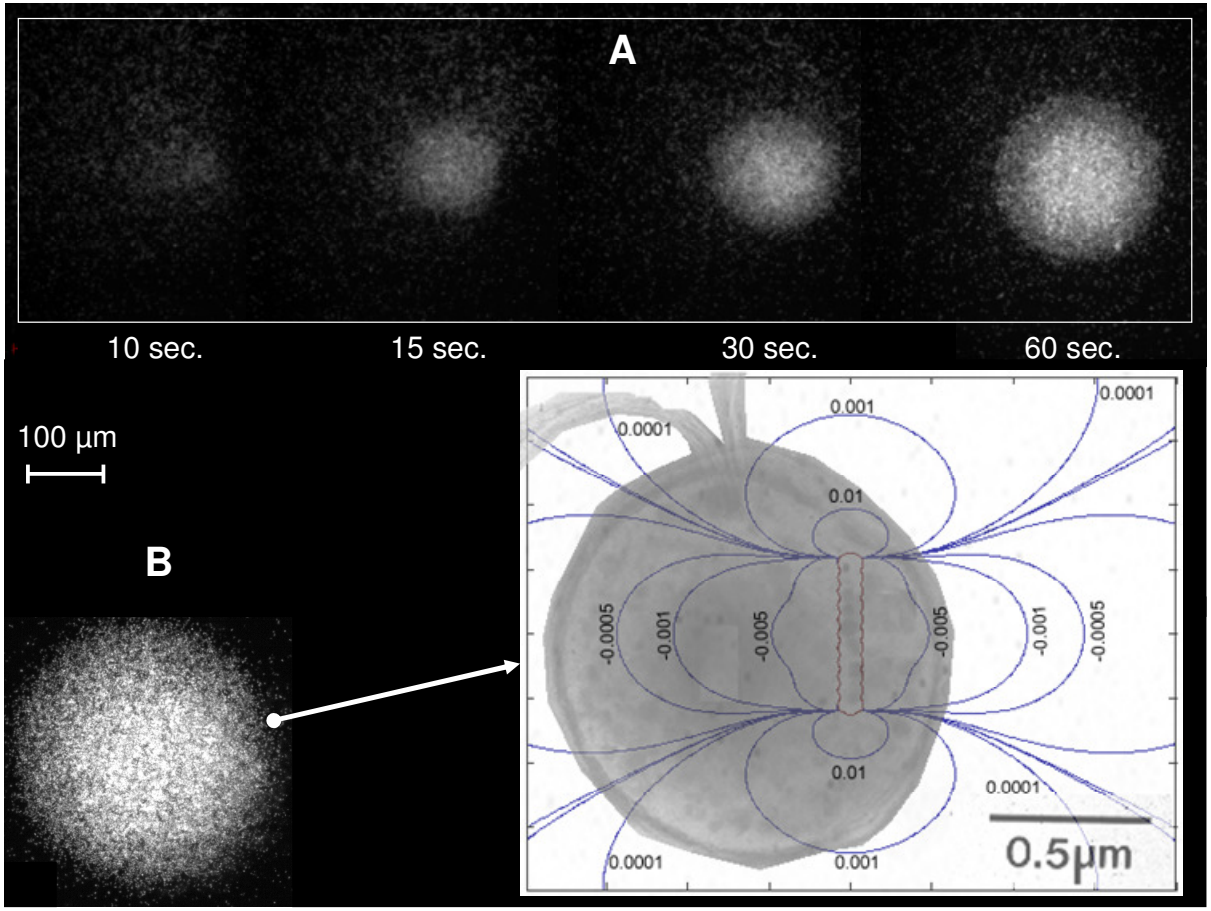
where Θ is the angle between the direction of the cell magnetic moment m and the ambient directional field $H = \mu_0^{-1} B$, B being the magnetic field density and μ_0 the permeability of free space. The Boltzmann constant and the temperature are represented by k_B and T respectively. Considering that the total magnetic energy of the magnetosome chain takes into account the contribution of the 10 to 15 closely spaced individual magnetite (Fe_3O_4) nanoparticles with a magnetic moment per MC-1 cell of $m = 10^{-16}$ (A m^2), a minimum magnetic field in the order of just a few Gauss (e.g. 4 Gauss (0.4 mT)) is sufficient to achieve proper magnetotactic directional response (90% efficiency)

The Magnetotaxis Platform

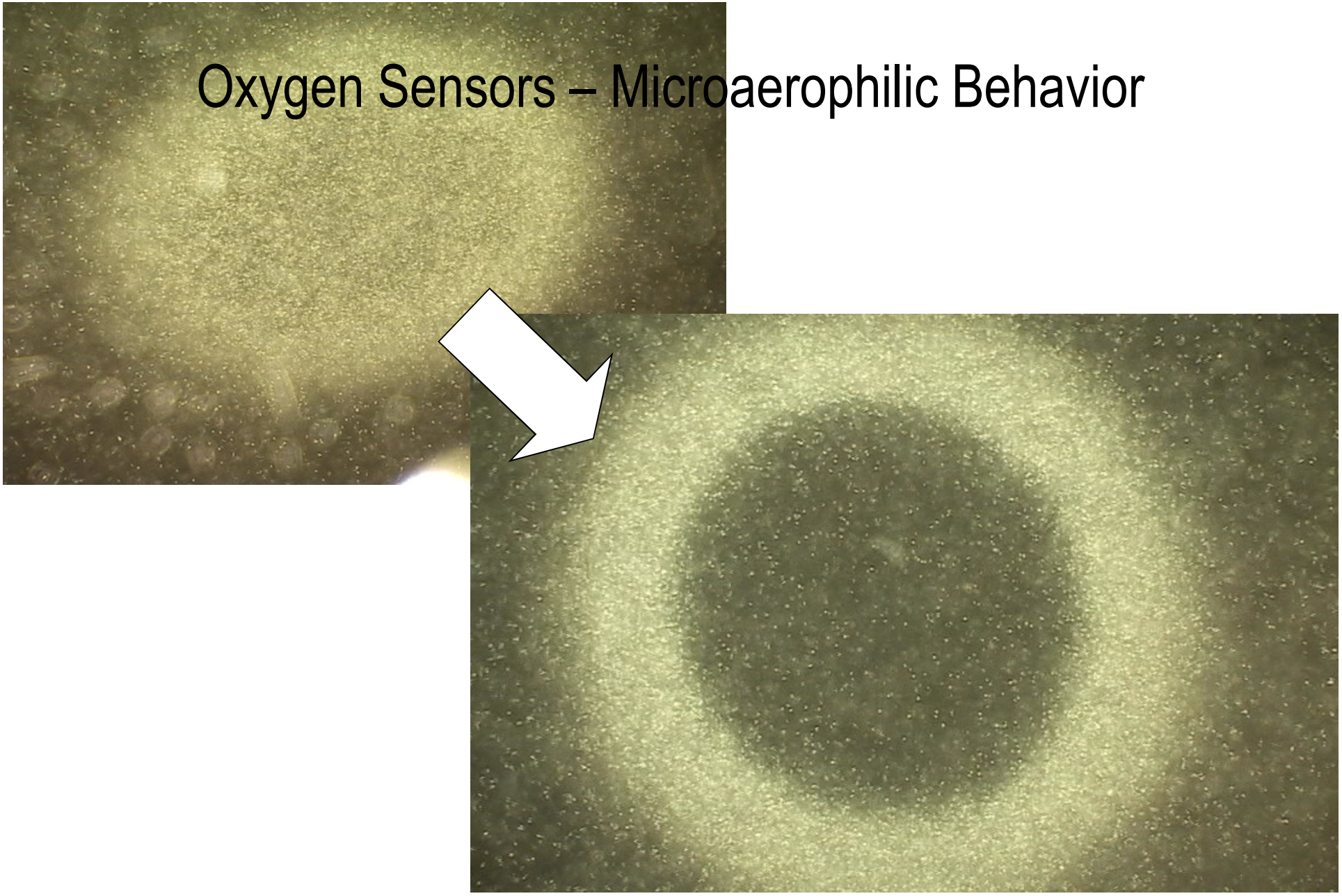




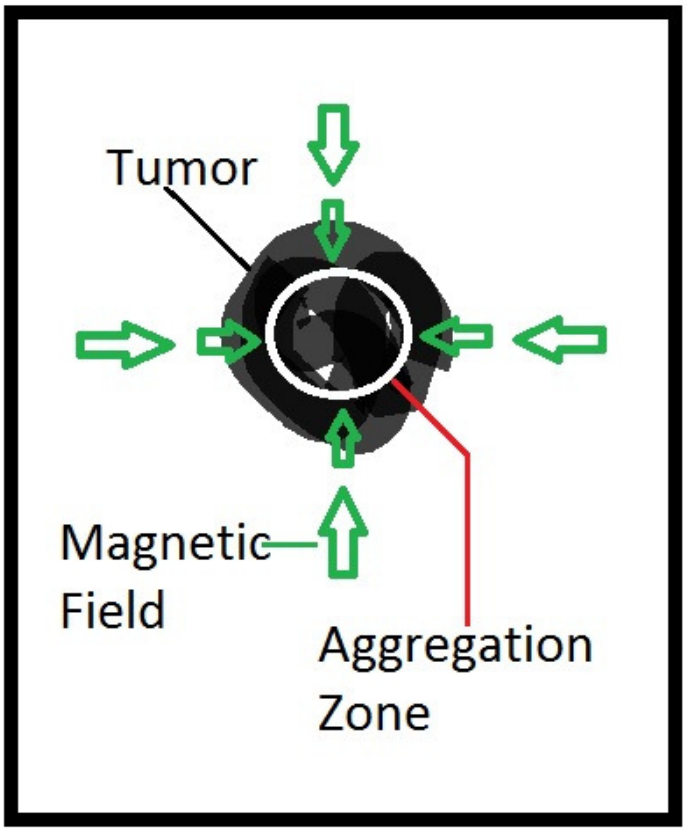
Aggregation at the Targeted Site with Number of Bacteria Corresponding to the Dosage Required

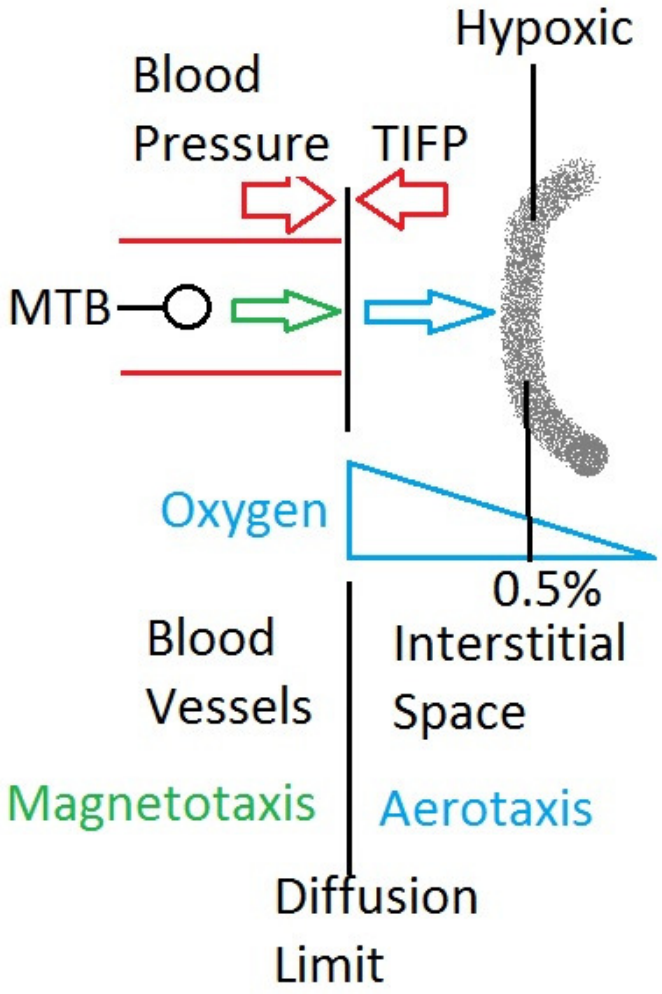


Oxygen Sensors – Microaerophilic Behavior

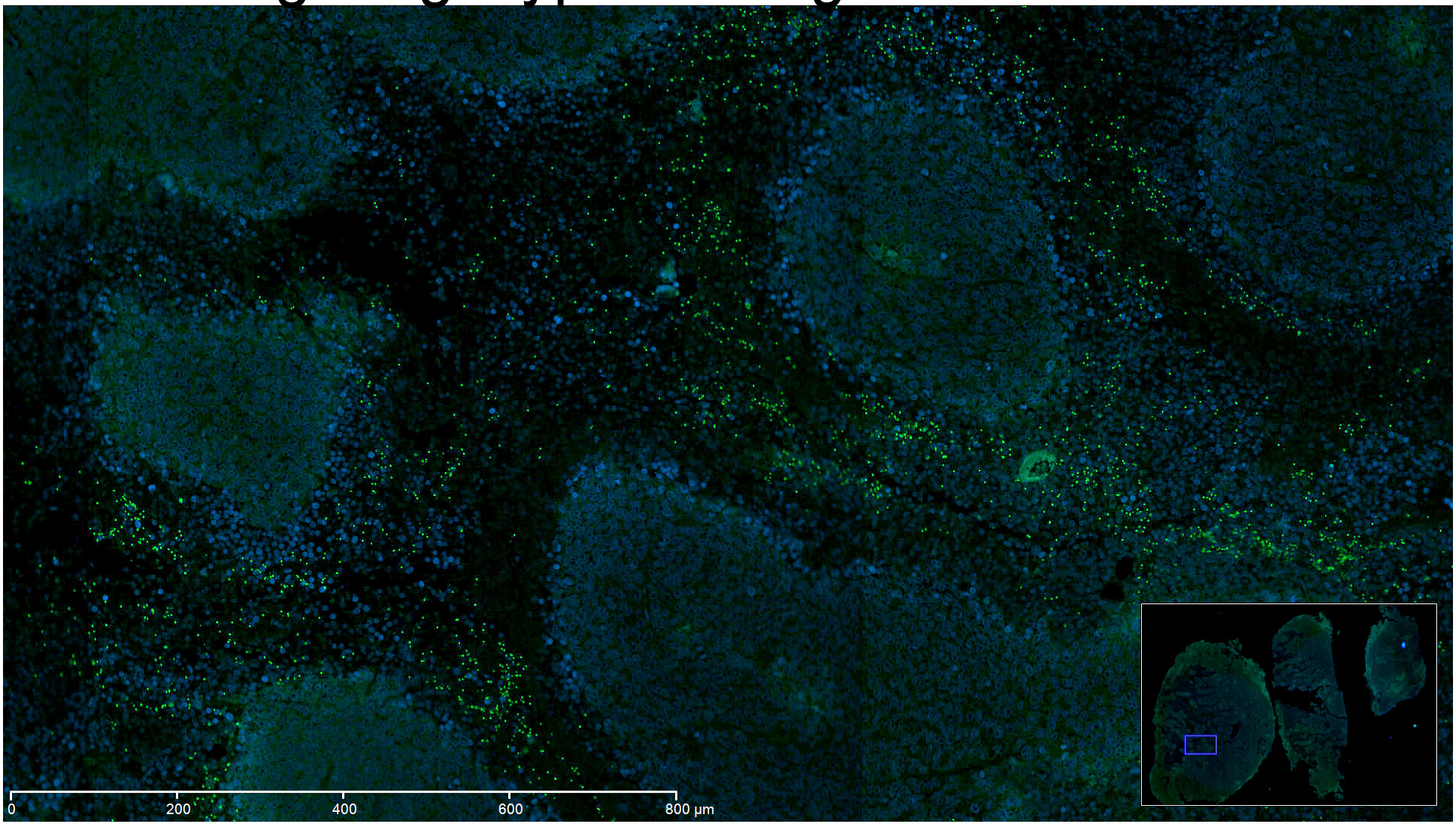


Basic Principle





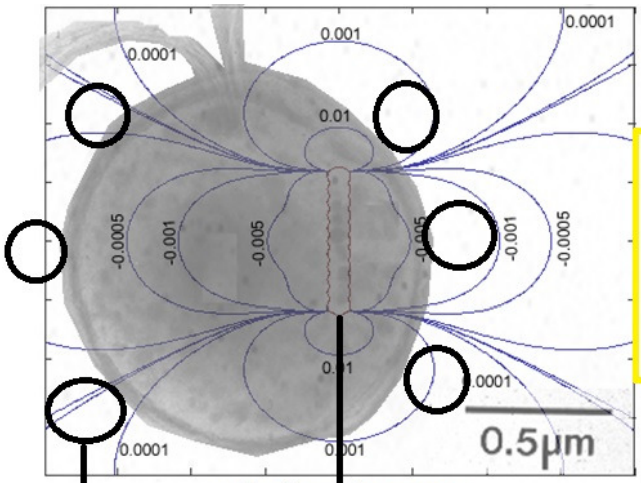
Targeting Hypoxic Regions in Tumors



Safety Tests

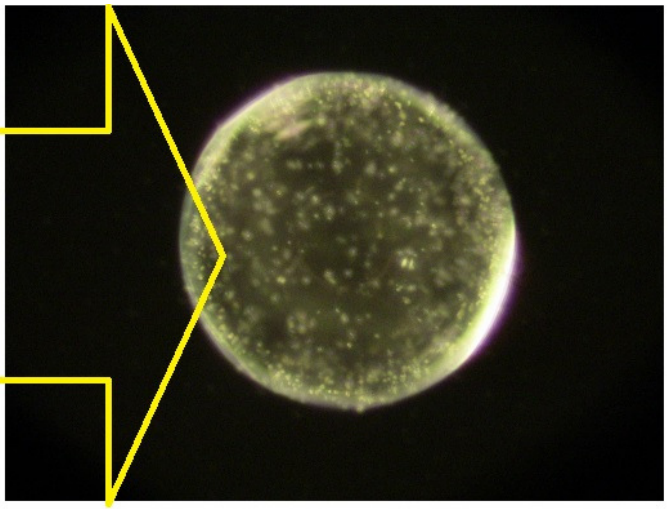
- No inflammatory cytokines could be observed in rodents injected with MC-1
- Infections with Pseudomonas causes increase in WBC, Neutrophils and decrease in platelets, whereas no significant changes observed following injection of the same amount of MC-1
- No alteration in the histology of organs (kidney, lungs, liver, spleen) was observed following MC-1 injection
- Presence of MC-1 can be monitored both by PCR using primers specific for MamC gene as well as by WB analysis

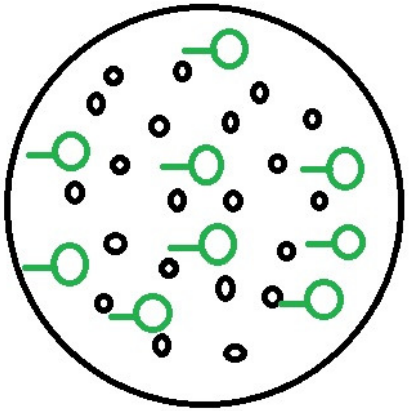
Linking Both Approaches



Chain of MNPs

Therapeutic Cargo



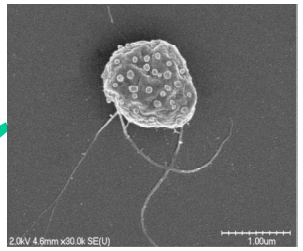


Drug-loaded Bacteria

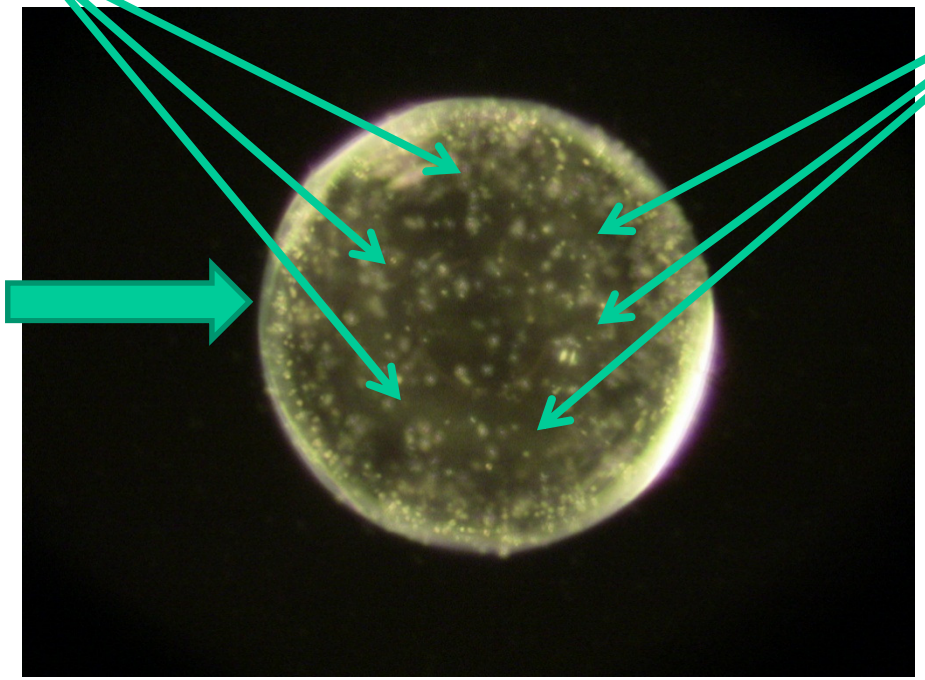
Magnetic Nanoparticles

Later: More Complex Direct Targeting Therapeutic Vectors

Magnetic Nanoparticles

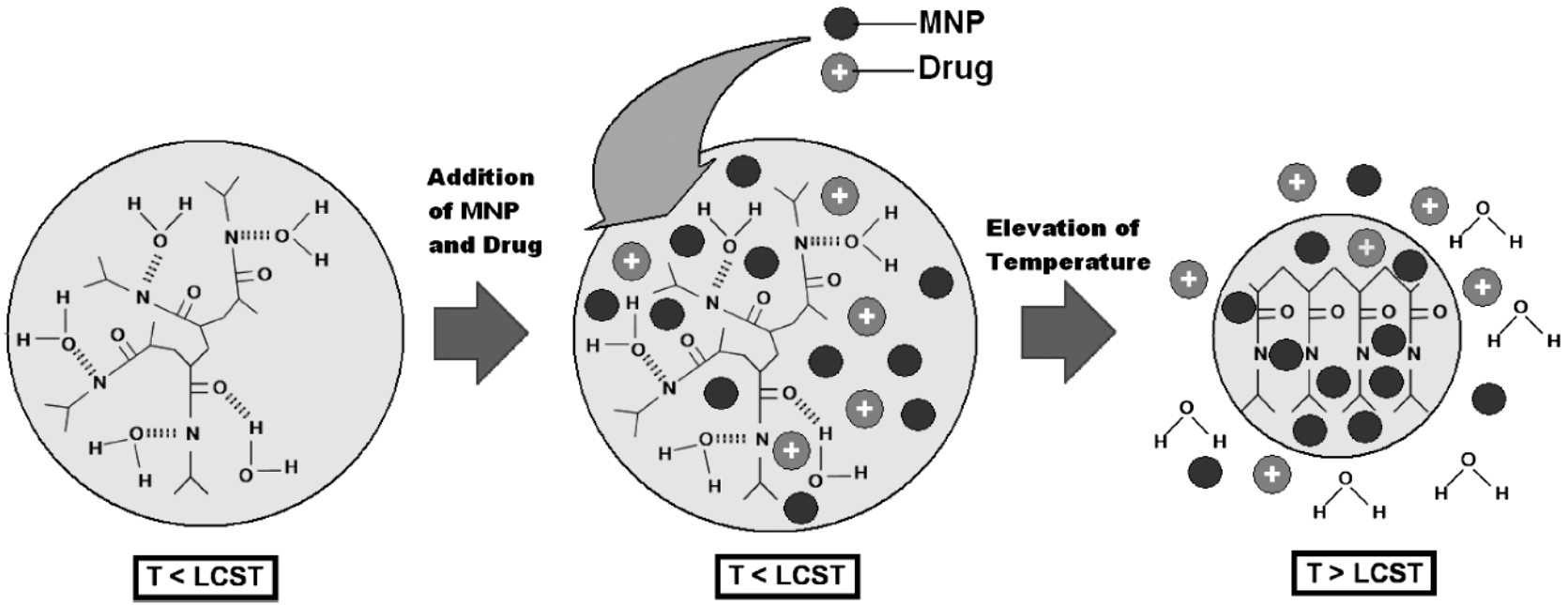


MRN-Compatible Micro-carrier



Martel S., Felfoul O., Mathieu J-B., Chanu A., Tamaz S., Mohammadi M., Mankiewicz M., and Tabatabaei N., "MRI-based nanorobotic platform for the control of magnetic nanoparticles and flagellated bacteria for target interventions in human capillaries," *International Journal of Robotics Research (IJRR)*, *Special Issue on Medical Robotics*, vol. 28, no. 9, pp. 1169-1182, Aug. 2009

Hyperthermia, Controlled Drug Release, Modifying the Shape of Agents, Etc… with the same MNP used for MRN



Transiting the Blood Brain Barrier

with the same MNP used for tracking and propulsion during MRN

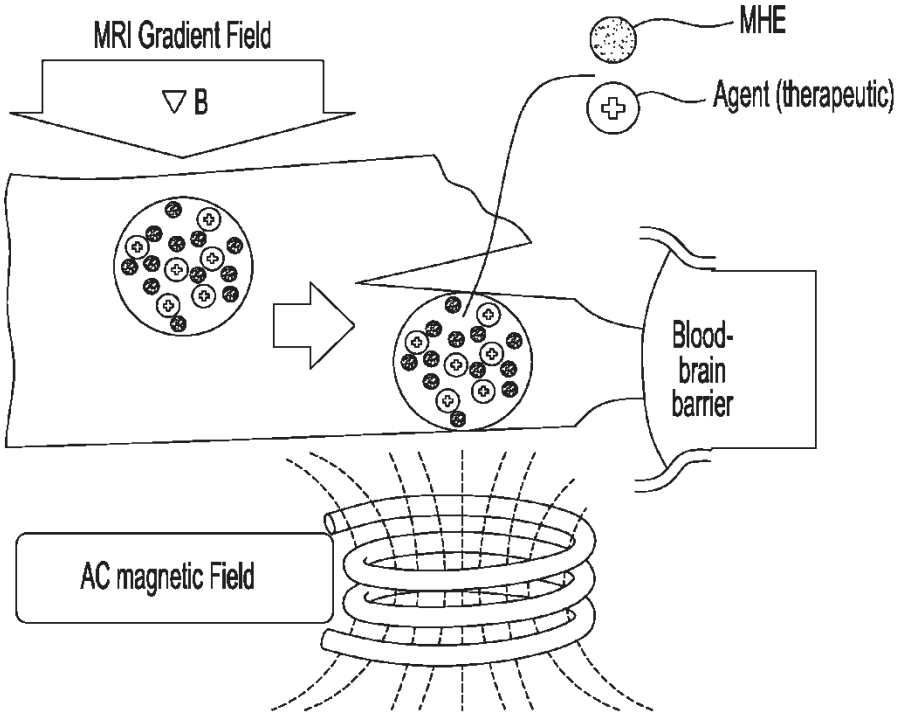
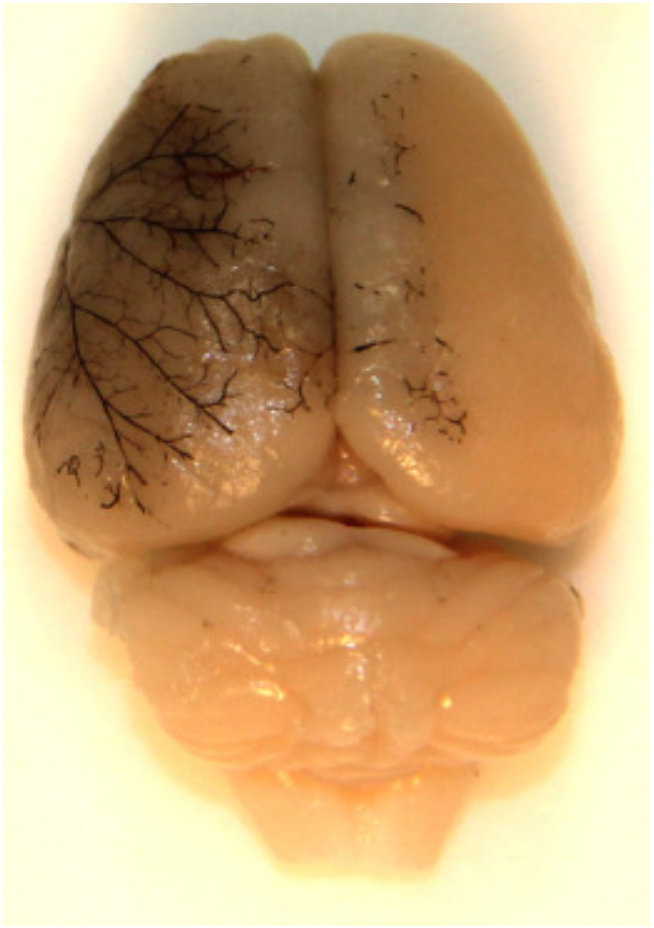
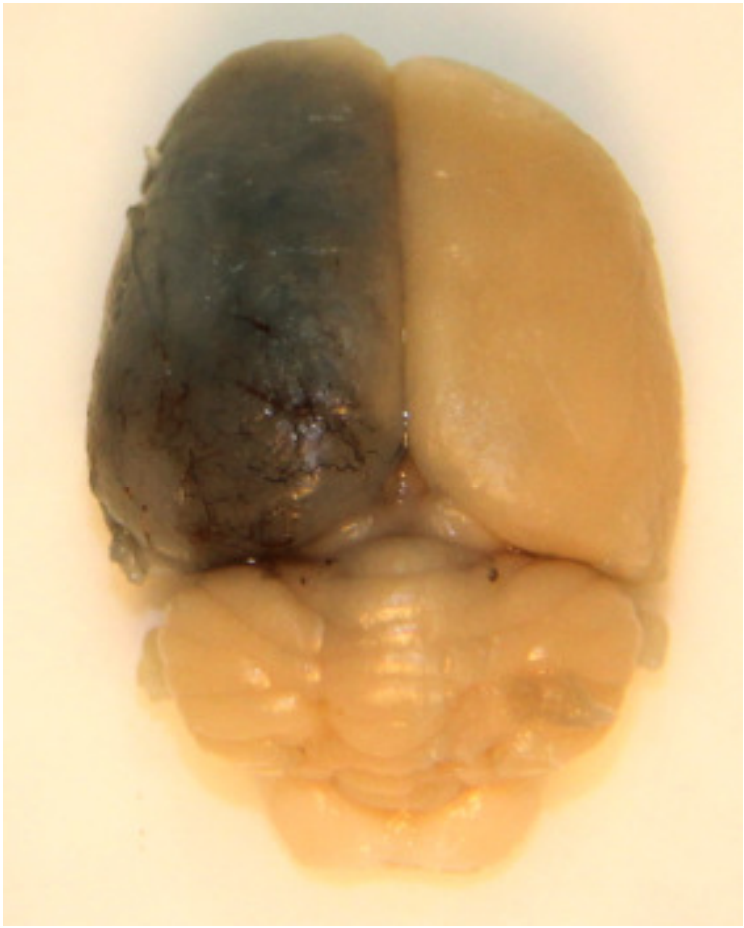

MRI Gradient Field
∇B
MHE
Agent (therapeutic)
Blood-
brain
barrier
AC magnetic Field

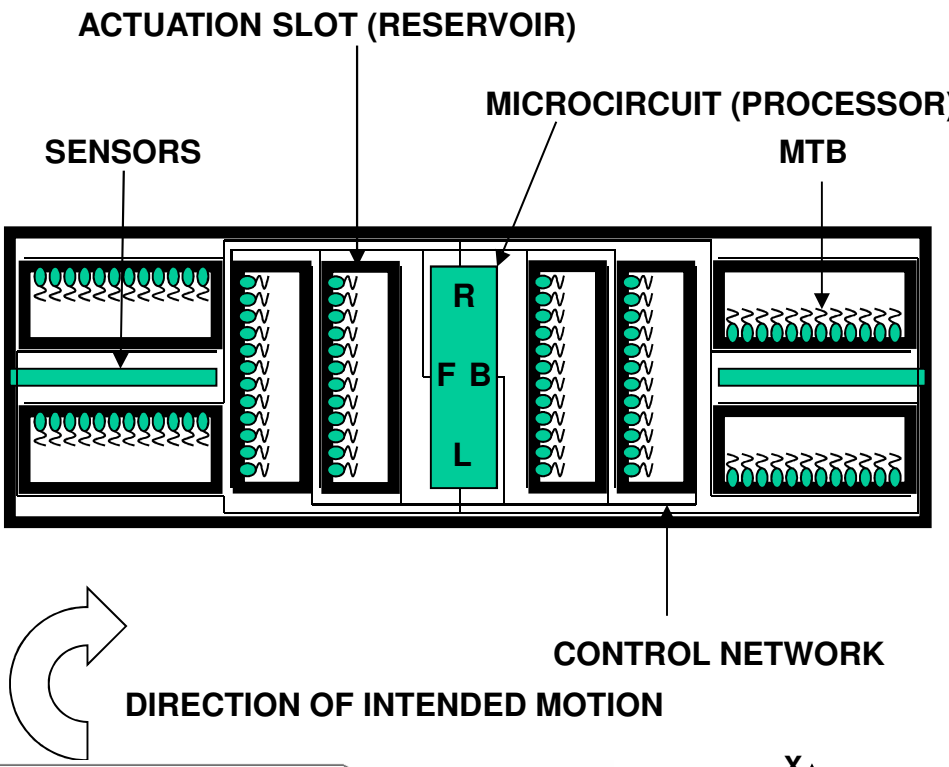
Fig. 9

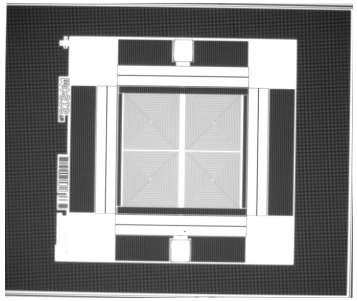




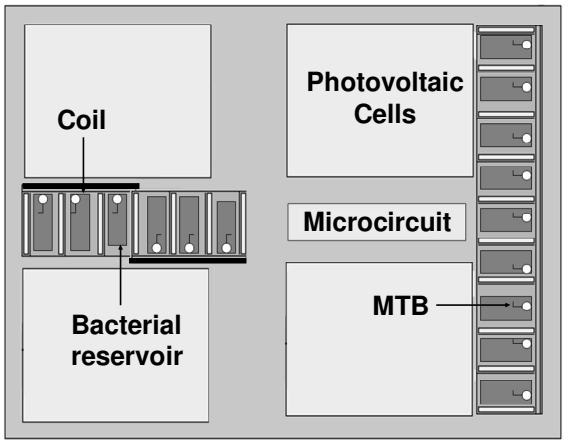
And Beyond Cancer Therapy…

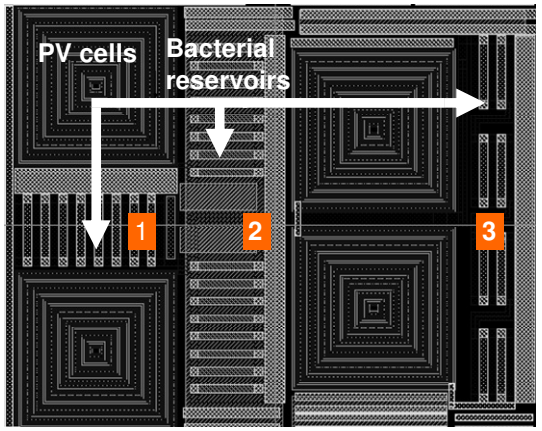
Used as Components in Microsystems

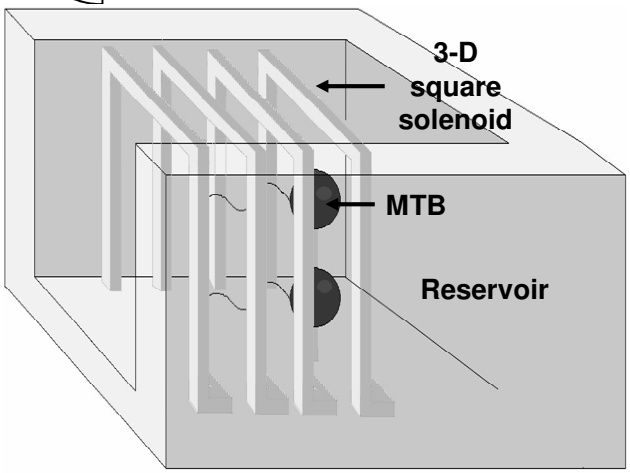


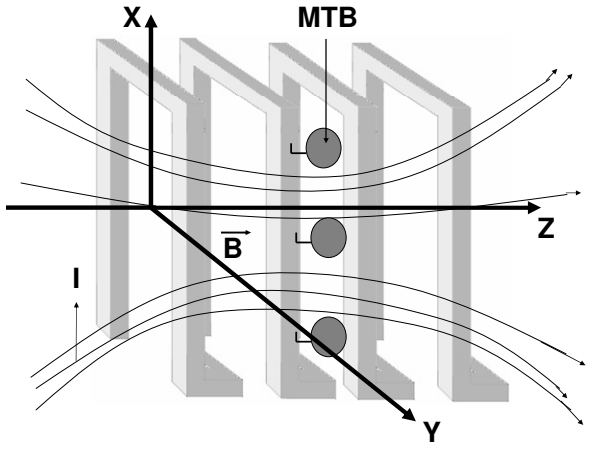


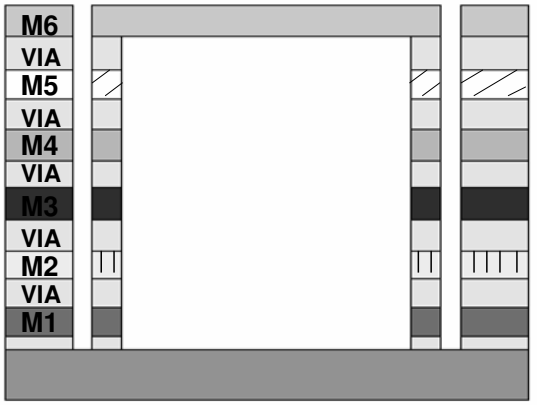
Photovoltaic Cells



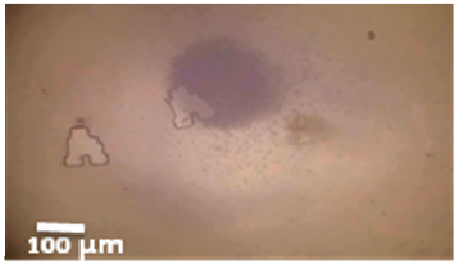




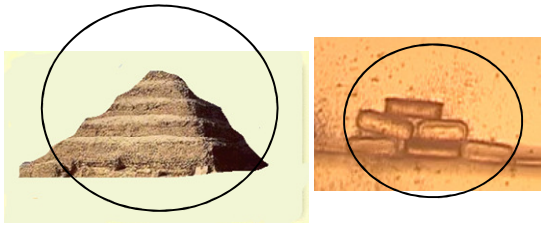


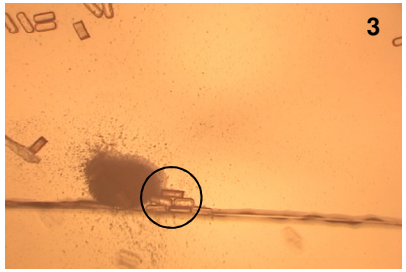


Applications of MTB-based Microsystems

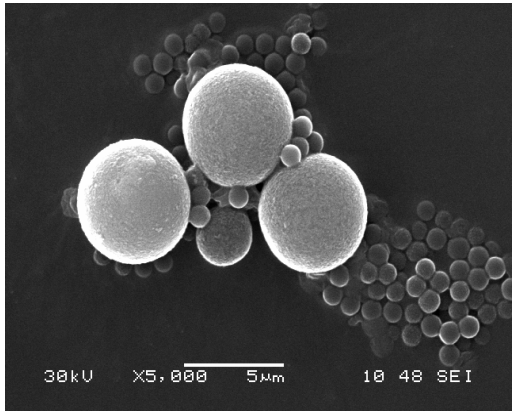


Transport and micromanipulation

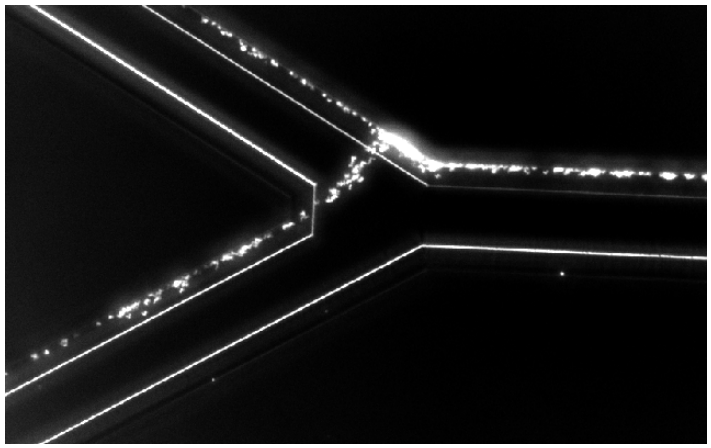




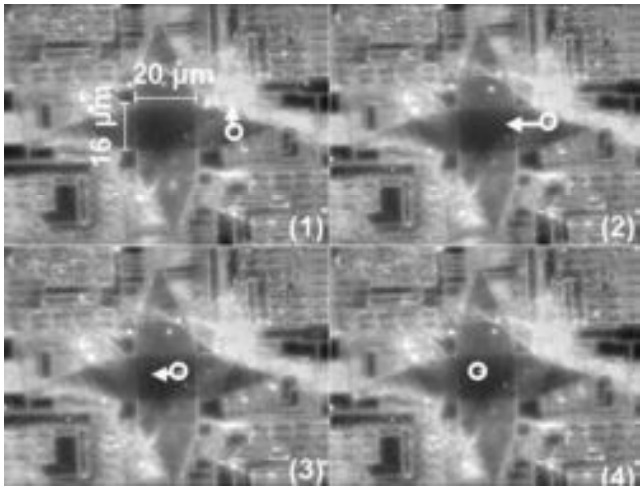
Micro-assembly

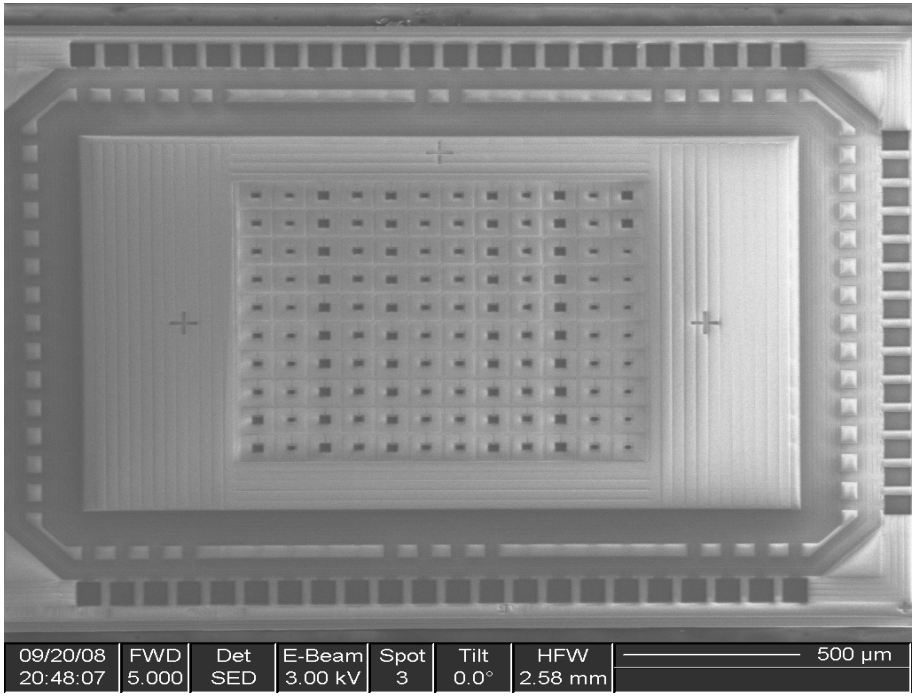


Mixing, Detection, Lab-on-Chip, µTAS, etc…

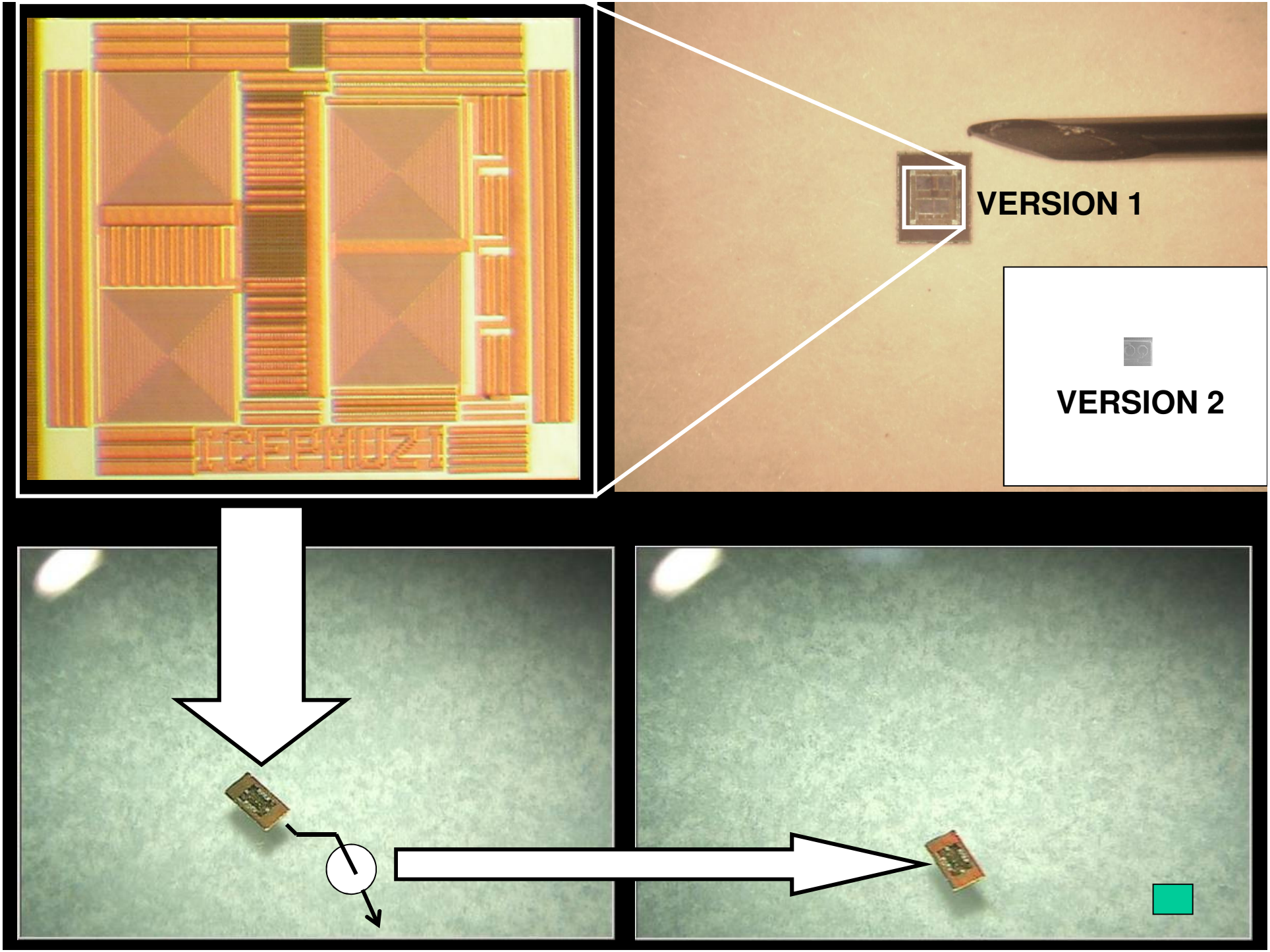


Transport, mixing, etc.





Left - CMOS integrated circuit interacting with MC-1 cells as actuators for controlled manipulation tasks;
Right – CMOS integrated circuit for the fast detection of pathogens using magnetotactic bacteria as controlled displacement sensors (with Prof. Y. Savaria, Polytechnique Montreal)


VERSION 1
VERSION 2

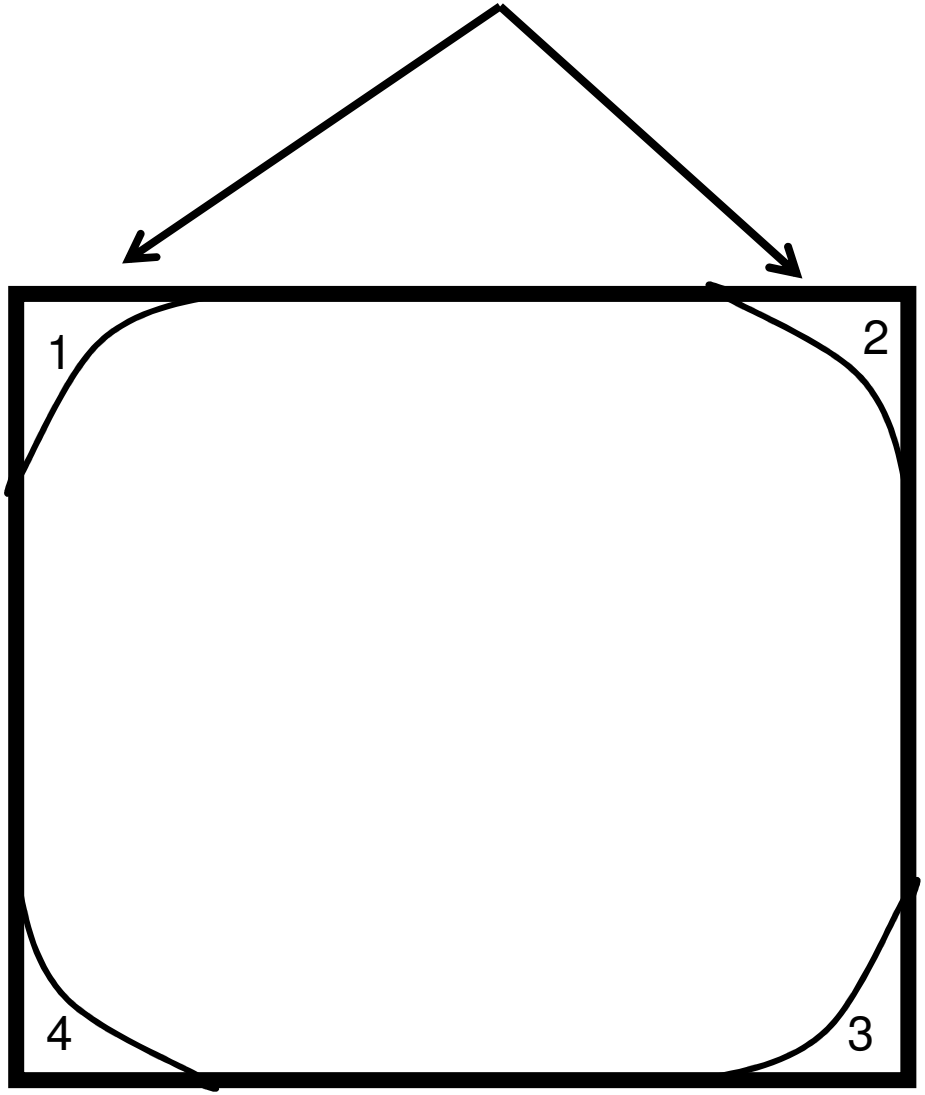
Advantages of Using MTB in Microsystems:

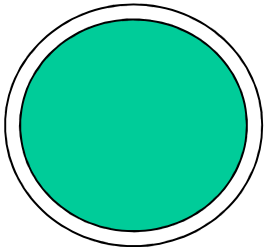
- Shorter distance means lower power for magnetotaxis control.

- Unlike the vascular system (imposed fluidic network), the designer has the freedom to modify the environment for control purpose, reducing power and computation requirement further.

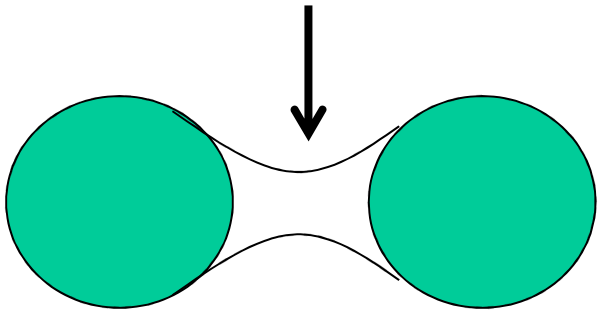
Networked Bacterial Factory

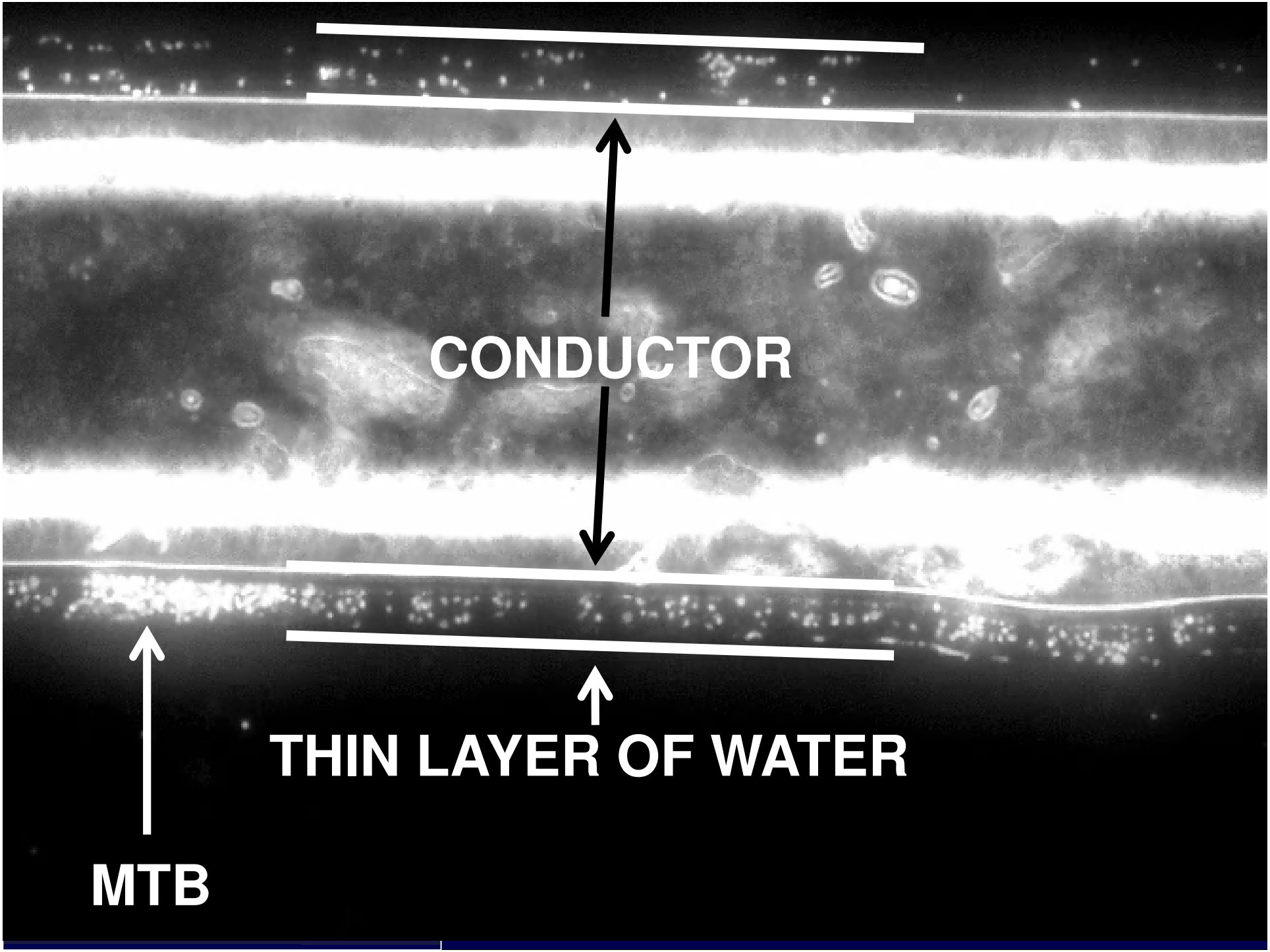
4 Fluidic Channels for Bacterial Carriers

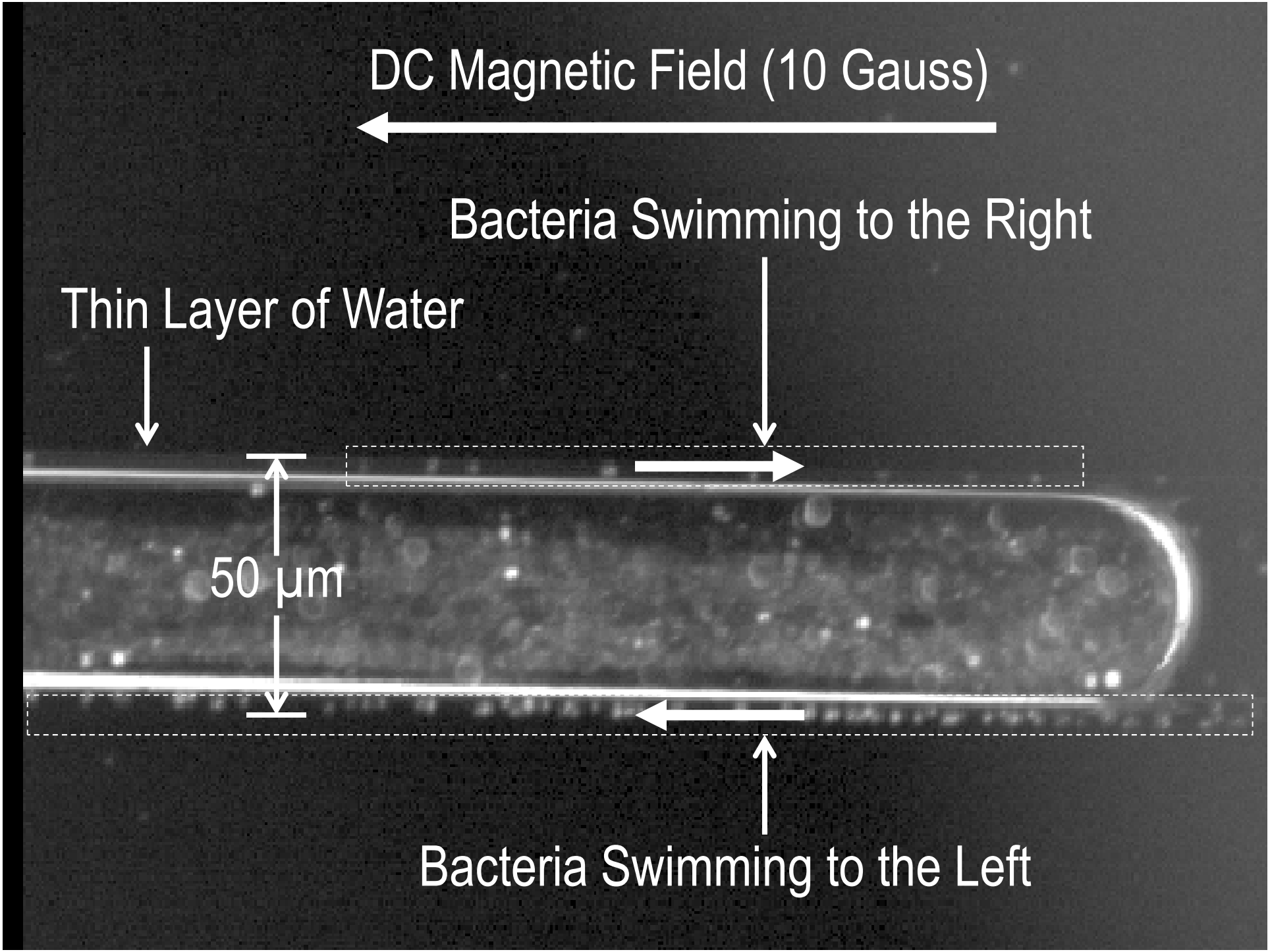


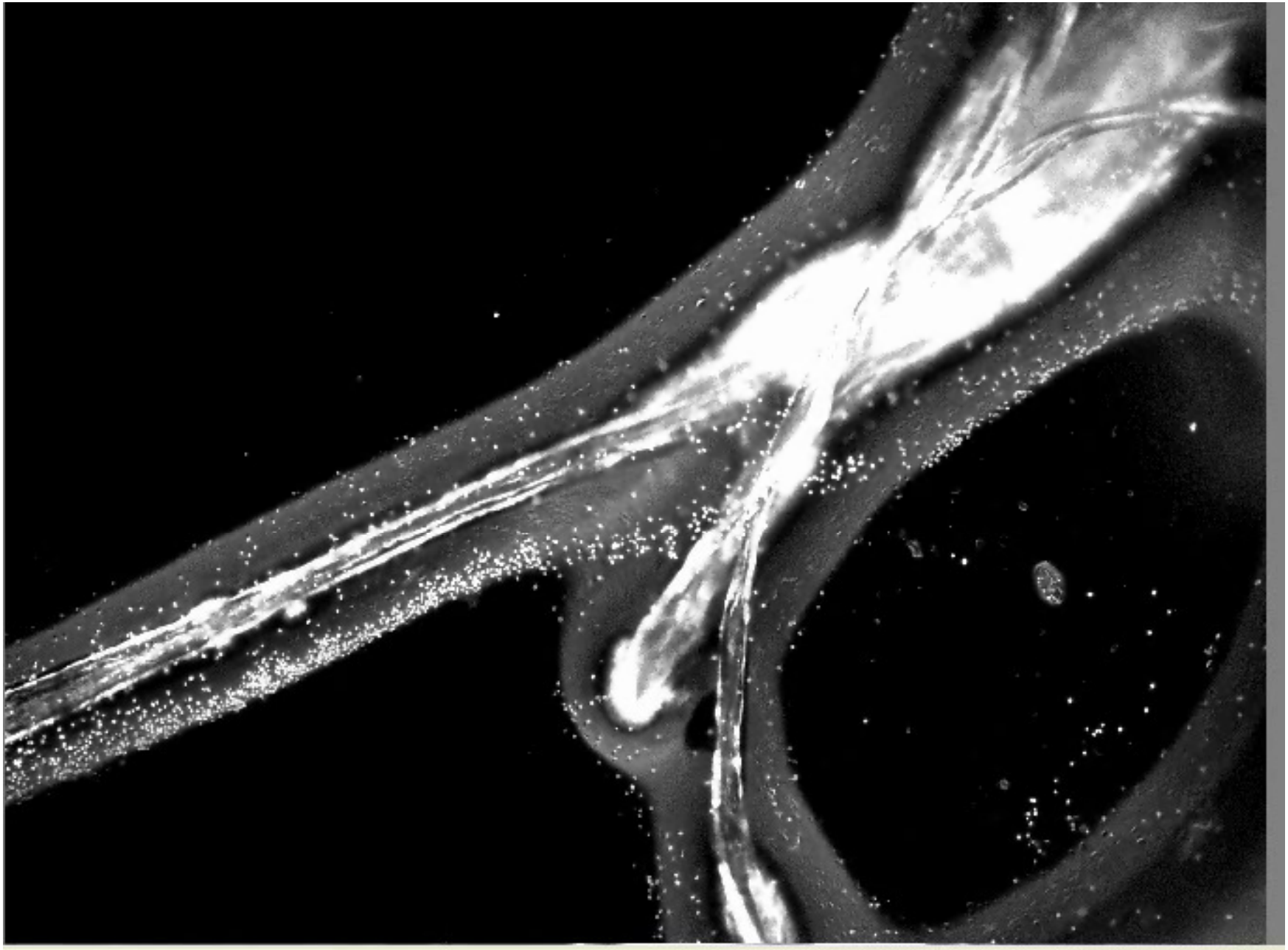


Fluidic Channel
For the Bacteria




CONDUCTOR
THIN LAYER OF WATER
MTB


DC Magnetic Field (10 Gauss)
Bacteria Swimming to the Right
Thin Layer of Water
50 µm
Bacteria Swimming to the Left



Bacterial Highways

Directional Control Methods

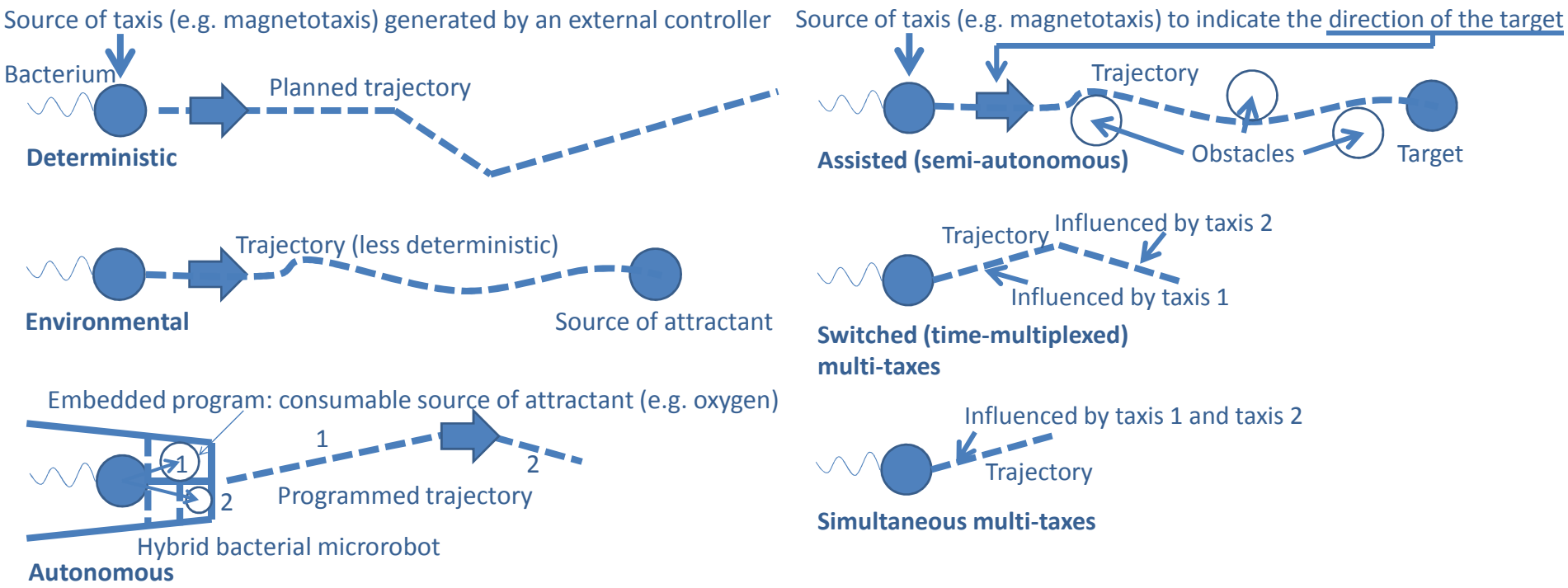
- Taxis-based

  (Positive (toward) nor negative (away)

  - Magnetotaxis
  - Aerotaxis
  - Chemotaxis
  - Phototaxis
  - Etc.

- Structural-based

Taxis-based



Reference: S. Martel, "Bacterial Microsystems and Microrobots," *Biomed. Microdevices*, 2013

Structural-based

- An asymmetric structure can result in a spontaneous and unidirectional motion or rotation of fabricated objects immersed in an active bacterial bath;
- By integrating magnetic fields of various intensities within fluidic microstructures and constraining the swimming paths of the bacteria with thin fluidic layers (incl. around wires), directional controlled micro-transport systems requiring no electrical power can be implemented;
- Magnetic field and structural geometries have been exploited to change the directional preferences of MTB and hence, increasing the level of directional control;
- Restricted geometries and solid planar surfaces can also be exploited to influence the motion behavior of flagellated bacteria;
- The implementation of a solid surface can be used to force the bacteria to swim in circles, etc.…

Swimming Velocity and Stop/Resume Control Methods

- pH level can regulate genes for flagellar motility and as such, control can be done by modifying the property of the liquid medium;
- Phototactic stop/resume is also possible;
- Temperature influence motility;
- Increase in the pitch angle of the MTB helical motion at higher magnetic fields;
- Other environmental approaches are also possible such as the addition of oxygen and arginine that can also play a role on bacterial motility;
- Structural approaches such as the proximity of a solid-liquid interface;
- Modification or an exploitation of the viscosity of the environmental liquid medium;
- Porosity of the surface, etc.

- The knowledge about the causes of the variations in swimming velocity of bacteria to use them to implement novel motile biosensors or measurement/characterization devices.

Embedding Living Technologies

Computational and self-adapting properties of living organisms remain superior to today's technology;

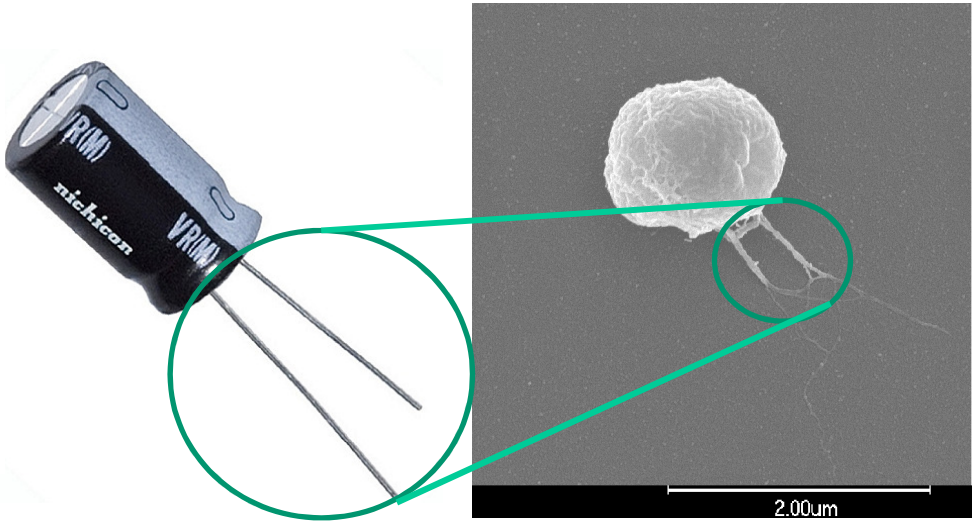
Living systems are composed of physically and chemically embodied agents that are relatively autonomous, self-constructing and self-organizing, adaptable, and robust;

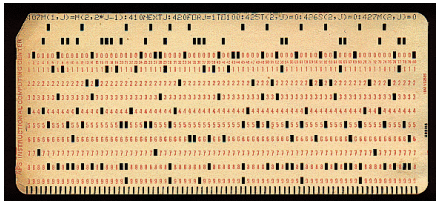
Learning to build future microsystems along these lines would address issues such as design complexity, difficulty of manufacturing, energy management, etc.

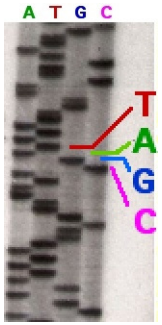
Summary

- Bacteria are self-powered micro-actuated micro-sensors that do not require electrical energy for moving and operating;
- Taxis and structural based methods can be used to control their swimming directions;
- They can be used in micromanipulation, transport, and sensing applications;
- They can transport nano- to few 100's microns objects

Conclusion







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