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D3.4: Evaluation of evMBDs dynamic parameters using mathematical models

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GLOSSARY OF TERMS

BOW	Biogenic Organotropic Wetsuits
EVs	Extracellular Vesicles
MBDs	Magnetic Bead Devices
evMBDs	EV-membrane coated MBDs
MNPs	magnetic nanoparticles
SPIONs	superparamagnetic iron oxide nanoparticles
MF	magnetic field
S	parameters of logarithmically normal distribution
T	absolute temperature of investigated liquid
K	time from the start of the diffusion of nanoparticles in the measuring channel
η	apparent dynamic viscosity of investigated liquid
C_b	Concentration of MNPs in the mixing buffer
D	Diffusion coefficient
Fo	Fourier number
R/D	external radius/diameter of nanoparticles
B	Magnetic flux density
f	Force influenced magnetic particles
Vmag	Velocity of magnetic particles

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INDEX

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1. Introduction

1.1 Overall and application

BOW aims to produce nanodevices with a biomimetic surface by dressing Magnetic Bead Devices (MBDs) with one or more layers of Extracellular Vesicle (EV) lipid membrane, with designed abilities to produce theragnostic effects. This technology benefits from the complex biological function of the EV coating and the designed functions of the synthetic MBDs and aims to advance towards sustainable production and clinical translation, proving the possibility of recapitulating biomimetic functions on any synthetic nanodevice.
The present document represents **Deliverable 3.4 – Evaluation of evMBDs dynamic parameters using mathematical models.**
It has been developed as part of WP3 "Design and fabrication of a microfluidic system for dressing MBDs with EV membranes".

1.2 RATIONALE

The optimization and advancement of microfluidic devices for the production and characterization of evMBDs are backed by mathematical approaches. These approaches are directed at the mathematical evaluation and prediction of parameters influencing the motility of functionalized evMBDs in microfluidic devices. Based on preliminary experiments involving various magnetic nanoparticles (MNPs) and magnetised liposomes, the critical parameters shaping kinetic profiles of nanoparticle motility in microfluidic systems under the influence of a magnetic field are identified as magnetization and hydrodynamic radius. The parameters of magnetization and hydrodynamic radius play a pivotal role in assessing the motility of MNPs in a liquid environment. Magnetization dictates how nanoparticles respond to an external magnetic field, influencing their behaviour in a magnetic environment. On the other hand, the hydrodynamic radius is instrumental in shaping the diffusion and transport properties of MNPs within the liquid. These parameters are, therefore, indispensable for the design and optimization of applications that depend on the precise control, movement, and manipulation of MNPs in diverse fluid environments. Understanding and fine-tuning these parameters are key steps in enhancing the efficacy and versatility of such applications. This is also especially important because of the heterogeneity of recently manufactured magnetic nano-devices (K.M. Kosuda et.al., 2016) and the necessity to evaluate predominant parameters characterizing suspension of evMBDs. It is necessary for the development of special recommendations to reduce the amount of magnetized material and hence the potential cytotoxicity of the magnetic core, whereas biophysical parameters of EVs wetsuit can be tailored in order to provide appropriate motility.
This targeted approach not only minimizes systemic distribution of cytotoxic compounds but also facilitates effective medical treatments with lower dosages. In this context, the assessment of magnetization parameters and/or hydrodynamic radius of MNPs (and, by extension, hybrid MNPs such as evMBDs) holds special importance. Understanding and

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optimizing these parameters are crucial steps for the successful implementation of therapies relying on magnetic targeting, contributing to enhanced precision, reduced side effects, and improved overall treatment outcomes. biomedical application involving such particles. However, the precise measurement of these characteristics through standard techniques (magnetometry, Raman spectroscopy etc.) is often not sufficiently informative. In order to solve this problem, During RP1, a novel device to test magnetic nano-sized entities such as MNPs, MBD, or evMBDs in water solution was developed (see deliverable D5.3 "First Periodic Progress Report", task T3.3). Starting from video tracking of the diffusion dynamics of the tested magnetic particles, we developed a mathematical method based on machine learning to predict the magnetization of MNPs in solution. We analysed the macroscopic behaviour of a uniform solution of MNP or MNPs/liposomes mixtures - contained in a quartz micro-fluidic capillary under the influence of an external magnetic field. The observed separation dynamics depend intricately on the magnetization and hydrodynamic diameter of the particles. This investigation provides valuable insights into how magnetic nanoparticles, and their mixtures with liposomes, respond and separate within the microfluidic environment when subjected to an external magnetic field. The findings contribute to our understanding of the complex interplay between magnetic forces and fluid dynamics in such systems.

PSPNet-based neural network architectures were trained to predict the relative magnetization of the MBDs and MBD-liposome mixtures, starting from diffusion dynamics, using semantic segmentation of extracted video frames. We achieved the appropriate level of MNP magnetization prediction with percentage error (11,4-24,1%) for the range of nano-object magnetizations (12-63emu/g). However, this approach may lack sufficient accuracy for objects larger than 100 nm. This is because considering the phase transition of the MNP solution, from a fluid of non-interacting Brownian particles to a ferrofluid, becomes challenging when an external magnetic field is applied. In such instances, both the interaction among the MNPs themselves and their interaction with an external magnetic field come into play (Hinderliter P.M. et.al., 2020).

2. Mathematical simulation

2.1 Preliminary mathematical approach for evaluation of evmbds shell thickness

We used an early elaborated model (Makhaniok, A. et.al., 2019) with minor adaptations outlined below. We examin ed the front motility (fig.1) in the channel of two types of superparamagnetic iron oxide nanoparticles (SPIONs) (14,4±3,5nm and 45,3±11,7nm) coated with chitosan and immobilized antimicrobial peptide (Plasmachem GmbH, #3762, #3891) respectively. The advantage of these nanostructures lies in the ability to control the thickness of the immobilized peptide on the surface of the chitosan substrate.

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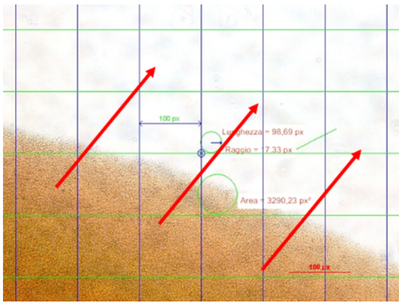

Figure 1. *Image of nanoparticle front motility in the measuring channel.*

The following equations describe the problem of diffusion mass transfer of nanoparticles:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}, \qquad (1)$$

$$C(x,t=0)=0, \quad C(x=0,t>0)=C_b, \quad \partial C/\partial x\big|_{x=L}=0. \qquad (2)$$

Here Cb is the concentration of nanoparticles at the entrance to the channel, D is the diffusion coefficient. We assume that the dependence of the diffusion coefficient from outer radius of MNPs (R) is described by the Einstein-Stokes law:

D = kT/(6€/R). (3)

In the case of monodisperse nanoparticles, D = const, and equations (1) - (3) have the following solution:

$$C(x,t)/C_b = 1 - S^*(x,t), \qquad (4)$$

where $S^*(x,t) = 2\sum_{n=0}^{\infty}\frac{\sin(p_n z)}{p_n}\exp\left(-p_n^2\,\mathrm{Fo}\right)$, $p_n = (2n+1)\frac{\pi}{2}$, $\mathrm{Fo} = \frac{Dt}{L^2}$, $z = \frac{x}{L}$. (5)

The average value for a certain time interval Δt to time t is the value of the concentration of the MNPs in front of width W :

$$\overline{C}_j(t_i) = C_b\left[1 - S_j(t_i)\right], \qquad (6)$$

$$S_j(t_i) = \frac{4}{\omega}\sum_{n=0}^{\infty}\frac{\sin(p_n Z_j)\sin(p_n\omega/2)}{p_n^4}\left\{\exp\left(-p_n^2\left[\mathrm{Fo}_i - \frac{\Delta\mathrm{Fo}}{2}\right]\right) - \exp\left(-p_n^2\left[\mathrm{Fo}_i + \frac{\Delta\mathrm{Fo}}{2}\right]\right)\right\}. \qquad (7)$$

Here, the average value of the measurement time interval, along the channel, Xj and Zj - the absolute and relative coordinates of nanoparticle front,

$$Z_j = X_j / L, \quad \Delta\mathrm{Fo} = D\Delta t / L^2 \text{ и } \mathrm{Fo}_i = Dt_i / L^2. \qquad (8)$$

The direct diffusion problem includes the calculation according to formulas (7) - (8), and estimates nanoparticle concentration distribution along the measuring channel.

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The inverse diffusion problem consists of finding the nanoparticle shell's thickness from the measured nanoparticle concentration profile along the channel (see the results in table 1). We can evaluate the diffusion coefficient D from the condition:

$$\Phi_1(D)=\sum_{j=1}^{N}\left\{\overline{C}_j^{\exp}(t_i)/C_b+S_j(t_i)-1\right\}^2=\min \qquad (9)$$

Where $\overline{C}_j^{\exp}(t_i)$ is the average concentration of the MNPs measured by the first time $t_i \pm \Delta t/2$.

To simulate the experimental data, it is possible to calculate the "reference" values of concentrations according to expressions (7) and (8) and to introduce a normally distributed random error $\varepsilon(\delta)$, described by a Gaussian curve with a standard deviation $\sigma=\delta$, where δ characterizes the error in the input data:

$$\overline{C}_j(t_i,\delta)=\overline{C}_j(t_i,\delta=0)+\varepsilon(\delta)C_b \qquad (10)$$

Each solution is preceded by generating random errors in the initial concentrations obtained by the formulas (7), (8). As a result, the average value and the standard error of the diffusion coefficient are calculated. The transformation of the error uniformly distributed over the interval (-1, 1) is carried out by the Box-Muller method (Box, 1958)), and the search for a global minimum of the function $\Phi_1(D)$ by the Hook-Jeeves method (Bunday, 1984).

Thickness of NP shell (#3762), measured	Thickness of NP shell, simulated	CIH (p=0,05)	Thickness of NP shell (#3981), measured	Thickness of NP shell, simulated	CIH (p=0,05)
97,8	49,7	26,8	106,12	128,9	23,1
26,4	31,1	5,7	33,2	54,3	5,2
16,1	16,9	4,4	19,7	15,5	3,1

Table. 1 *Results of the simulation.*

Thus, this experiment, while only a rough approximation, highlighted limited reliability in determining the thickness of nanoparticle coatings exceeding 100 nm. This observation aligns with literature data [Hinderliter P.M. et.al., 2020]. The utilization of micro-visualization experimental techniques, especially for diffusion transfer, and the corresponding

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extrapolation faces significant limitations in this context. It indirectly suggests that simulating the motility of nanoparticles with a large hydrodynamic radius can be more accurate for those with greater magnetization (size) of the magnetic core. This aspect was further explored and confirmed in our study, specifically in the context of diffusion-convective conditions and the application of an external magnetic field.

2.2 Mathematical approach for evaluation of evmbds diffusion in the microchanel

The important part of our work on evMBDs mathematical characterization is determining the unknown magnetization parameters and average hydrodynamic radius of evMBDs. As planned in Task 3.2. the novel method for testing magnetised nano-object using optical technique in the micro-capillary [Cardellini J. et.al., 2023] paved the way to define crucial parameters of evMBDs, including convective-diffusion transport and behaviour under external MF influence.

We established a mathematical framework to describe the processes of stochastic Brownian motion, associated diffusion, and regular motion influenced by a magnetic field (MF). This includes defining the particle mass flux density under the influence of the MF and specifying the corresponding boundary conditions. Taking the forces acting on the outer boundary of the drop at equilibrium, we calculated particle parameters fig. 2.

Convection-diffusion equation for particle density c

$$\partial c/\partial t + \vec{\nabla}\cdot\vec{i} = 0$$

$$\partial c/\partial t \underbrace{\boxed{+\vec{v}\cdot\vec{\nabla}c}}_{\text{Convection}} \underbrace{\boxed{-D\Delta c}}_{\text{Diffusion}} \underbrace{+b\vec{\nabla}\cdot(c\vec{F}_e)}_{\text{External forces}} = 0$$

Particle flux density under MF – by magnetisation

$$\vec{F}_e = \mu_0 V\chi_e(\vec{H}\cdot\vec{\nabla})\vec{H}$$

$$\begin{cases}\chi_e = 3, & H < \frac{1}{3}M_{sat}\\ \chi_e = M_{sat}/H & H \geq \frac{1}{3}M_{sat}\end{cases}$$

Boundary conditions

$$\vec{i}\cdot\vec{n} = 0$$

Diffusion parameters – by size of nanoparticle

$$D = kT/(6\pi\eta R) \qquad b = D/(kT)$$

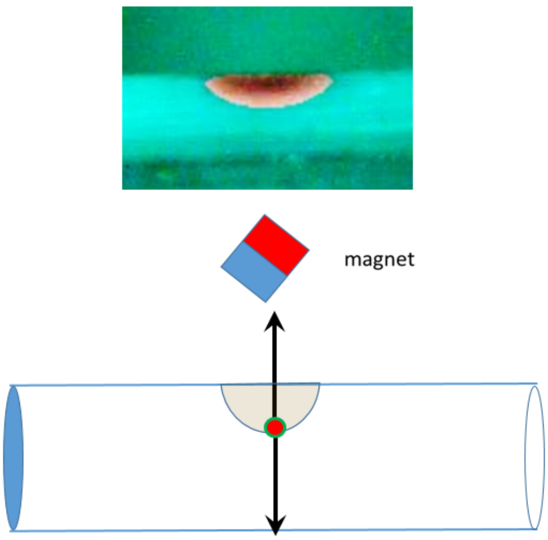

Figure 2. *Scheme of base mathematical workflow and experimental setup.*

As the concentration of the particles in the liquid phase is low and size of particles is small, we will use diffusion approximation according to (L.D. Landau, et.al., 1987). Then, there are three main forces acting on a MNP in the microfluidic channel: (1) the regular fluidic force $\vec{F}_f$, that is exerted by the medium on a moving MNP; (2) the irregular fluidic force $\vec{F}_b$,

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that is produced by fluctuations and is the moving force of Brownian motion and therefore diffusion of the particles; (3) the magnetic force $\vec{F}_m$, that is generated by the applied magnetic field gradient. Other forces as buoyancy, the gravitational force and the interaction between particles can be negligible for MNPs with a low particle concentration in a fluid.

Nanoparticles immersed in liquid have relaxation times in the range of nanoseconds and corresponding distances are also small, so it is accurate to use the stationary approximation for the motion under the influence of regular forces $\vec{F}_f, \vec{F}_m$, n eglecting acceleration of particles and gradients of the forces. Influence of the irregular force $\vec{F}_b$ can be treated then in the form of diffusion force.

Magnetic force influence magnetic particle is equal to

$$\vec{F}_m = \mu_0 V \chi_e (\vec{H} \cdot \vec{\nabla}) \vec{H} \tag{1}$$

where μ_0 is the magnetic permeability of free space, V is the volume of the particle, χ_e is particle effective magnetic susceptibility, and $\vec{H}$ is the applied external magnetic field. χ_e depends on the form of particle, magnetic field, and magnetization saturation. For spherical particles with high magnetic susceptibility χ_e can be approximated as

$$\{\chi_e = 3, H < \frac{1}{3} M_{sat}, \chi_e = M_{sat}/H \; H \geq \frac{1}{3} M_{sat}, \tag{2}$$

where M_{sat} is the saturation magnetization per unit volume of the nanoparticle material.

The regular fluidic force $\vec{F}_f$ for slow particle motion in the liquid is determined by Stokes' law

$$\vec{F}_f = -6\pi\eta R(\vec{v}_p - \vec{v}), \tag{3}$$

where η is the dynamic viscosity of the liquid matrix, R is the effective size of the particle, $\vec{v}(\vec{r})$ is the liquid velocity at a given position $\vec{r} = (x, y, z)$, and $\vec{v}_p$ is the particle velocity.

Stationary motion approximation gives then

$$0 = \vec{F}_f + \vec{F}_m + \vec{F}_b, \tag{4}$$

that can be solved for the $\vec{v}_p$ neglecting $\vec{F}_b$ first—we will take diffusion into account now.

Let $c(\vec{r})$ be density of particles, then full flux $\vec{\imath}(\vec{r})$ of particles will be determined by the following causes:

liquid flow $\vec{\imath}_c = \vec{v}c$—convection;

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Brownian motion that mixes the particles leading to diffusion $\vec{\imath}_b = -D\vec{\nabla}c$, where $D = kT/(6\pi\eta R)$ is diffusion coefficient, k is Boltzmann constant, and T is temperature;
external forces compensated by drag force so that flux is $\vec{\imath}_f = cb\vec{F}_e$, where $\vec{F}_e$ is a force excerted on one particle, $b = D/(kT)$ is particle mobility.
General equation governing particle concentration is

$$\partial c/\partial t + \vec{\nabla} \cdot \vec{\imath} = 0. \tag{5}$$

Using the continuity for the particles number one get convection-diffusion equation in the form

$$\partial c/\partial t + \vec{v} \cdot \vec{\nabla} c - D\Delta c + b\vec{\nabla} \cdot (c\vec{F}_e) = 0. \tag{6}$$

In our case $\vec{F}_e = \vec{F}_f$.

Magnetic field distribution can be obtained from magnetic scalar potential that can be calculated by a triple integral over volume of magnet treating its magnetization M (the magnetic moment per unit volume, characteristic of the magnet material) as constant over volume

$$\Phi(x,y,z) = \frac{\mu_0 M}{4\pi} \iiint \frac{(z-Z)dXdYdZ}{[(x-X)^2+(y-Y)^2+(z-Z)^2]^{3/2}}, \tag{7}$$

and magnetic field outside the magnet is just $\vec{H} = \vec{\nabla}\Phi/\mu_0$.

If the concentration of particles is not so small (for example, in the vicinity of the magnet) we need to include also back reaction of the particles on liquid flow by solving modified Navier–Stokes equation together with continuity equation

$$\vec{\nabla} \cdot \vec{v} = 0,$$

$$\rho(\partial\vec{v}/\partial t + (\vec{v} \cdot \vec{\nabla})\vec{v}) = -\vec{\nabla}p + \eta\Delta\vec{v} + \vec{F}_{vol}, \tag{8}$$

where $\vec{F}_{vol}$ is the volume force, which is equal to the magnetic force on a single particle multiplied by the number of particles per unit volume c

$$\vec{F}_{vol} = c\mu_0 V\chi_e(\vec{H} \cdot \vec{\nabla})\vec{H}, \tag{9}$$

ρ is liquid density, p is pressure.

In particular, we define, additionally, the magnetic parameter U that characterises relative contribution of magnetic forces in process of particle motility in comparison with diffusion influence:

$$U = \mu_0 mH/kT \tag{10}$$

The model underwent testing using established data (Quanliang Cao et. al. 2012), successfully replicating profiles for NP accumulation in a rectangular channel (Figure 3). In

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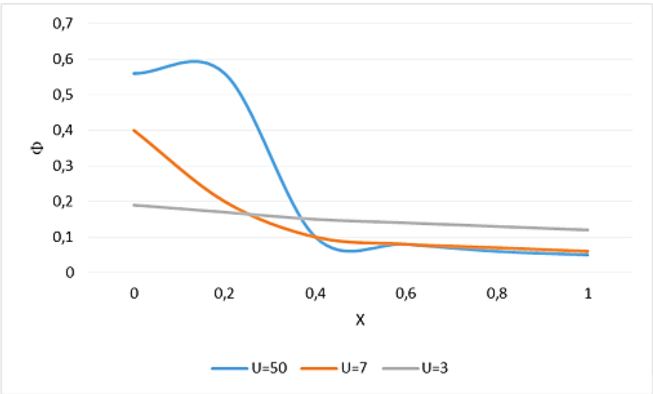
the following section, we utilized experimental data to align the predicted profiles of nanoparticle density distribution with the actual ones. Despite significant disparities between simulated and experimentally measured diameters, potentially stemming from the heterogeneity of magnetic materials distribution within the tested liposomes (no error estimates were provided for diameter determination due to this reason), subsequent calculations using an artificial intelligence approach, as detailed in the next section, yielded much more reliable and accurate results in the diffusion-dominated domain. For conditions dominated by convection, especially applicable to nano-objects exceeding 150 nm in size, the further application to experimental data proved to be more beneficial, as described in Section 2.4.



Experimentaly defined diameter	Calculated diameter
133	217
57	74

Figure. 3 *Evaluation of magnetic susceptibility simulation.*

Table. 2 *Results of the*

2.3 Machine learning/artificial intelligence complementary model for evaluation of magnetized nanoobject in microchannel.

Our third approach, centred around machine learning (artificial intelligence), yielded significantly improved results. The outcomes show a much higher level of consistency with the measured values. This improvement is attributed to the critical nature of experimental data related to magnetization parameters and/or hydrodynamic radius for recently manufactured nano-objects. The heterogeneity of these magnetized products often renders routine examination through standard magnetometry and microscopy insufficiently informative.

In this context, we propose a method to predict such parameters by the behaviour of a uniform solution of magnetic nanoparticles (MNP) or MNP/liposomes mixtures within a micro-fluidic capillary under the influence of an external magnetic field. Videos from these experiments were utilized to generate the necessary time dynamics video-frame dataset

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for subsequent post-processing using machine learning (ML). For semantic segmentation of analysed frames in a specific experiment, neural network architectures based on U-Net were trained. As demonstrated by the multitude of mathematical models, and further confirmed using the model mentioned below, the commonly employed approaches to predict the dynamic behaviour of magnetic particles in fluid under the influence of an external magnetic field can result in significant deviations from parameters directly validated using instrumental tools. The main drawback of this approach lies in the challenge of incorporating the phase transition experienced by a magnetic nano-object solution when subjected to an external magnetic field. Specifically, the transition from a fluid of non-interacting Brownian particles to a ferrofluid poses difficulties. Typically, these processes are examined with the assumption of an unlimited increase in the concentration of magnetic particles in regions with a highly inhomogeneous magnetic field, leading to their dense packing. This, in turn, results in anomalously high viscosity values (characteristic of magnetic fluid) and corresponding viscous stresses during their flow. In such a scenario, magnetic nanoparticles (MNPs) can interact both with each other and with an external magnetic field.

This challenge prompted us to explore the application of artificial intelligence (AI) in analysing video frames extracted from the magnetic separation of MNPs from uniform solutions in microchannels. We demonstrate that this AI approach could serve as a promising complementary methodology for evaluating/predicting some physical parameters in sophisticated systems through image analysis. The response of a uniform solution of MNPs (MNPs or MNP-liposome mixture) under the influence of an external MF have been investigated by an experimental set-up as described in Cardellini J. et.al., 2023. Briefly, a uniform solution consisting of MNPs (MNPs or MNP-liposome aggregates), is contained in a 500 µm-wide capillary and a couple of permanent magnets placed on the capillary's sides generate a high-gradient magnetic field (as it demonstrated on fig.3). The capillary is displaced ca.200 µm from the central axis of the system to allow the concentration of the MNPs on one side of the capillary. A dense plug of MNPs forms on the capillary's wall closest to the magnet and grows over time. A microscope-connected CCD camera tracks the process and the related video-frames are used to feed the neural network model.

Semantic segmentation was chosen to analyse the frames depicting the dynamics of the formation of S and H in a particular experiment. To find the optimal model for the semantic segmentation problem, we screened the following pre-trained models: Unet, Enet, PSPNet, and Unet+Resnet. The networks were fine-tuned for 700 epochs with a learning

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rate of 0.001 and CosineAnnealinglR for improvement of training quality (fig 4 a). To calculate the difference between ground truth and model output we use Binary Cross Entropy loss function (fig 4 b). After thorough analysis, it was found that PSPNet achieves the best validation metrics (fig 4 c-d) – best mean intersection of unit (IOU) and pixel accuracy.

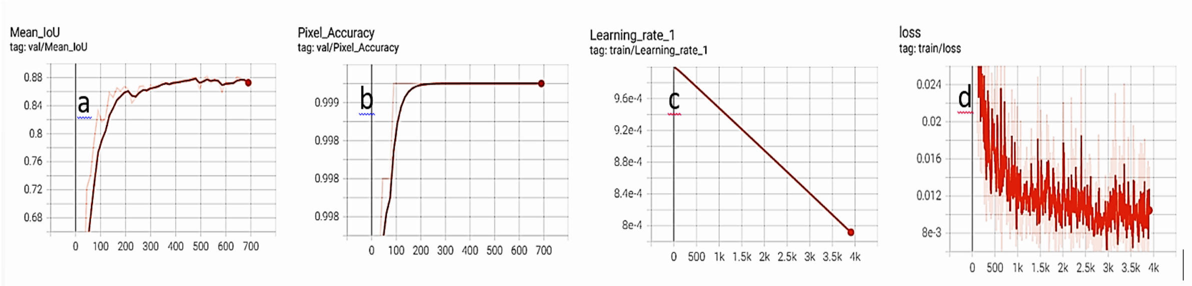

Figure 4. *Mean IOU (a) and pixel accuracy (b) of model validation. Learning rate (c) and loss function value (d) of model training using PSPNet.*

To characterize the MNPs accumulating into the visualized area of the capillary, trained PSPNet model was employed to analyse 2700 consequent images (in 7 experimental sets). The aim was to solve the inverse problem of finding the corresponding RM of particles from the data obtained through the segmentation model (Fig 5.).

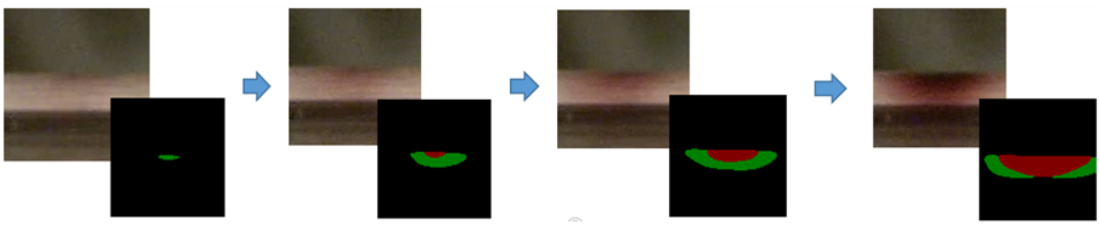

Figure 5. *Upper video-frames are initial images and below is the neural network output. Readapted from Cardellini, J., et al, 2023 (DOI: 10.26434/chemrxiv-2023-q6tf3).*

We analised the accumulation of nanoparticles with varying degrees of magnetization by examining changes in the areas of the spot and halo in pixels. The results showed that even small differences in magnetization values could have a significant impact on the dynamics of changes in geometric parameters (fig.6).

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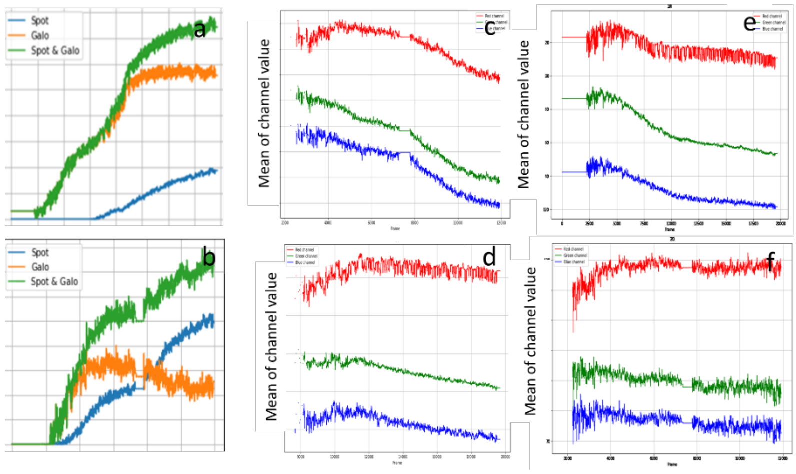

Figure 7. *Dynamic mutual change of areas spot and halo over time for MNPss with magnetization RM48 (a) and RM54 (b). Change of color spectra (RGB channels) for RM48 (c) and RM54 (d) for a spot, for halo: RM48 € and RM54 (f).*

RM (MNPs/ MNPs)	Evaluated magnetizations using AI approach	Approximation error (%)	RM (emu/g /MNP)	Evaluated magnetizations using AI approach	Approximation error (%)
133	104	21,8	68	73	11,4
100	83	17,0	43	78	-
54	61	12,9	24	29	24,1
48	52	11,1			

Table 3. *Results of the simulation of relative magnetization using described approach.*

Thus, the PSPNet artificial neural network was chosen to evaluate the MNPs magnetic accumulation using semantic segmentation of video-frames. Based on the trained neural network, we achieve high-value intersection IOU and pixel accuracy. Overall, the use of neural networks and semantic segmentation with the following analysis using linear regression provides a reasonable level of prediction of MNPs relative magnetization with a percentage error not exceeding 24.1% for the range of magnetization of nano-objects of 17-63 emu/g. This research highlights the potential of neural network application for an evaluation of MNPs parameters.

2.4 Integrated model of nano-object kinetic evaluation in microfluidic chamber using finite element analysis.

While the abovementioned modelling is applicable basically for nano-objects with dimensions up to 100-150nm, evaluating certain parameters for larger objects and cells loaded with evMBDs becomes practically relevant. In such cases, nano-object diffusion

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becomes negligible for particles of several hundred nanometers. Additionally, it is crucial to consider the potential heterogenous joining of liposomes with magnetic MNPs, especially when using more than one nanoparticle covered by the liposomal shell, which can aggregate into active conglomerates. Even in the scenario of separate nanoparticle coverings, fusion or aggregation of several evMBDs into large conglomerates may occur. The parameters of such nano-objects are challenging to evaluate using the methodology of spot accumulation in the capillary. Hence, we proposed another experimental tool (BIOSYS) and a mathematical model that provides the ability to assess how large nano-objects (up to 500nm) and cells will be saturated with magnetized particles. The integrated mathematical model for evaluating the dynamic characteristics of nano-objects under the influence of a magnetic field in a microfluidic chamber is outlined in Figure 8. The model encompasses the distribution of magnetic nanoparticle (MNP) concentration, magnetization, or, conversely, hydrodynamic radius—considered as a parameter for predicting MNP dynamic behaviour. This calculation takes into account the interplay of magnetic fields, fluid flow, and convection-diffusion transfer. Impact parameters, such as fluid velocity, viscosity, magnetic field properties, and more, are considered in the model. This framework provides the capability to determine the parameters of magnetized nano-objects with larger sizes (evMBDs with a size exceeding 100nm) and cells saturated with such nano-objects. Therefore, through the microfluidic chip, we assess the deviation of magnetized nano-particle flux under the influence of an external magnetic field. While we disregard the impact of diffusion effects on this process as they are negligible, we do consider the kinetic parameters of particle motion within the microfluidic device, along with the physical parameters that characterize magnetic permeability and the distribution of the magnetic field gradient. For this second model, we utilize the microfluidic chamber (ChipShop, #881) with a customized microfluidic system (Figure 8a, b, c). In this setup, we observed the displacement of the flow of nano-objects, deviating from the central line and forming an area with a specific distribution of colour density under the influence of an external magnetic field (MF). The model for nanoparticle motion was developed to describe this experiment, employing the methods of finite element analysis. The magnetic particle deviates from the straight flow, drifting due to the influence of two forces: magnetic attraction and fluid drag force. They counterbalance each other for some drift velocity, and if we integrate this over passing time, we get total shift. Shift of 1.5 mm is predicted for one neodymium magnet 1T for 100 nm particles, that is preliminary in correspondence with observations. Overalls these geometrical parameters are proportional to the hydrodynamic radius and fall quickly for smaller-sized particles.

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Firstly, applying multiple magnets to the flow can be explored. Secondly, lowering the flow velocity allows for a longer duration of magnetic attraction. Thirdly, adjusting the geometry by placing two magnets closer to the nanoparticle trajectory can increase the magnetic field gradient. Another variant involves a substantial increase in nanoparticle concentration in the flow; in this scenario, particles will drag the liquid with them, augmenting the shift. Based on our observations, these improvements can potentially enable the evaluation of evMBDs with sizes up to 30-50nm. Beyond this threshold, the results may become blurred by diffusion, which is why the approaches described above are preferred. We also anticipate good results in shift estimation using an artificial intelligence approach to assess the patterning of magnetized nano-objects. This method can help label the fields (areas) occupied in the microfluidic space by nano-objects with relatively uniform magnetization and sizes, providing a more precise definition of the shift of magnetic nanoparticle flow deviation under the influence of a magnetic field (MF). The initial experimental data confirmed the applicability of the model, and the experimental parameters evaluated using finite element analysis (Comsol) were predicted with an appropriate level of reliability (see Table 5).

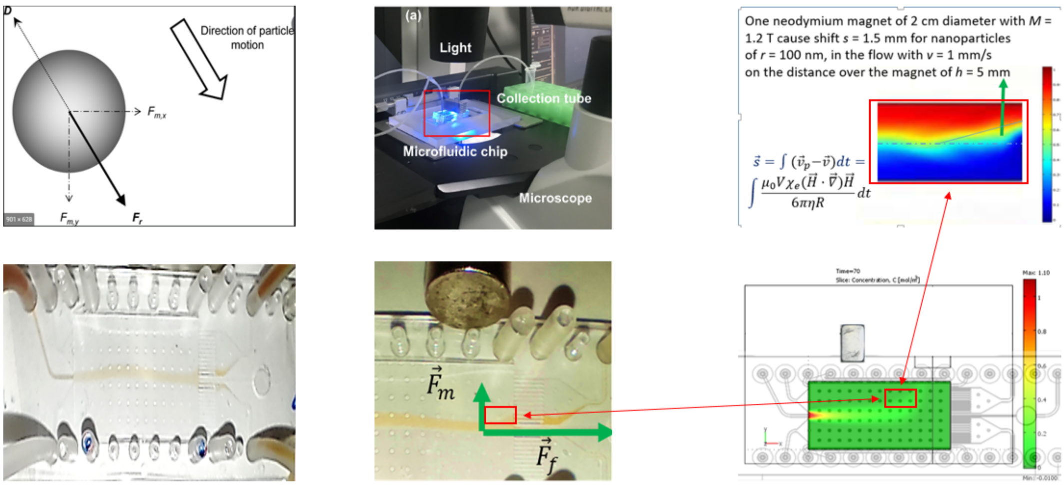

Figure 8. *Schematically outlined workflow of experimental and mathematical evaluation of nanoparticle kinetic in the microfluidic chamber.*

Main equations and parameters for finite element analysis using COMSOL.

Main parameters defined as follows: Magnetic flux density B, Force on the magnetic particles f, Velocity of magnetic particles Vmag, magnetic particle (or cells) concentration field C.

The following equations are used for calculation using Comsol multiphysics:

- Magnetostatic equations:

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Magnet: $-\nabla\left(\mu_0\mu_r\nabla\varphi_m - \vec{B}_r\right) = 0$

Volume: $-\nabla\left(\mu_0\nabla\varphi_m - \mu_0\vec{M}\right) = 0$

- Kelvin magnetic force density (magnetic stress tensor): $f_i = \sum_{j=1}^{3} M_j \frac{\partial B_j}{\partial x_i}$

Where B is magnetic flux density $\vec{B} = -\mu_0\nabla\varphi_m$.,

- χ is volume magnetic susceptibility: $\chi = \left(1-\varepsilon_f\right)\chi_s + \varepsilon_f\chi_f$

In this case the magnetic moment of nano-particles calculated by the equation:

$$\vec{M} = \frac{\chi}{1+\chi}\vec{B}$$

With following approximations of Helmholtz force – (Landau, L. D., Lifshitz, E. M., 1987):

$$\vec{F} = \frac{\mu_0}{2}\chi\nabla\left(H^2\right) - \frac{\mu_0}{2}H^2\nabla\chi$$

$$f_x = \frac{\mu_0\chi}{(1+\chi)^2}\left[B_x\frac{\partial B_x}{\partial x} + B_y\frac{\partial B_y}{\partial x} + B_z\frac{\partial B_z}{\partial x}\right] - \frac{\mu_0\chi}{(1+\chi)^3}B^2\frac{\partial\chi}{\partial x};$$

$$f_y = \frac{\mu_0\chi}{(1+\chi)^2}\left[B_x\frac{\partial B_x}{\partial y} + B_y\frac{\partial B_y}{\partial y} + B_z\frac{\partial B_z}{\partial y}\right] - \frac{\mu_0\chi}{(1+\chi)^3}B^2\frac{\partial\chi}{\partial y};$$

$$f_z = \frac{\mu_0\chi}{(1+\chi)^2}\left[B_x\frac{\partial B_x}{\partial z} + B_y\frac{\partial B_y}{\partial z} + B_z\frac{\partial B_z}{\partial z}\right] - \frac{\mu_0\chi}{(1+\chi)^3}B^2\frac{\partial\chi}{\partial z};$$

Model of adsorption rate of the magnetic particle at the chamber walls and on the bottom (are not used in the example below):

$$R = \left(\alpha + \frac{\beta}{\mu_0}\frac{\chi_s\chi_f}{(1+\chi)^2}B^2\right)C;.$$

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- Velocity of magnetic particles: $\vec{v}_{mag} = \frac{\vec{f}}{6\pi\mu a}$

Where μ is fluid viscosity, a is particle hydrodynamic radius.

- Equation of magnetic particles diffusion: $\frac{\partial C}{\partial t} = \nabla(D\nabla C) - \nabla(\vec{v}_{mag} C) - R$

Where R defines Magnetic particle adsorption equation: $\frac{\partial S}{\partial t} = R$ and C is magnetic particle concentration into fluid, S is magnetic particle concentration/distribution in free space, D is particle diffusivity, R is adsorption rate of the magnetic particle at volume space (not used in these calculations) .

- The summary Integrated Magnetodynamic equations is:

$$\rho\left(\frac{\partial \vec{\upsilon}}{\partial t} + (\vec{\upsilon}\nabla)\vec{\upsilon}\right) = -\nabla p + \mu\Delta\overline{\upsilon} + \mu_0 \sum_{j=1}^{3} M_j \nabla H_j$$

Utilizing this equation in COMSOL with the parameters from Table 4, we conducted a series of simulations for various nanoparticle sizes, some of which are illustrated in Figure 5. The relationship between particle shift at the end of the chamber and the size of particles was derived from these simulations to create a calibration curve. This calibration curve, in turn, was used to determine particle size from the measured shifts presented in Table 5. As evident, the results are quite satisfactory for nanoparticles with sizes exceeding 50nm, considering that NP sizes are typically distributed over a range, and the simulations assume conditions for mostly uniform particles.

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Br	1.3
χ	0.3
µ0	4*pi*10^-7[H/m]
C0_	1
chi__	1[m^3/mol]
Visc	1.e-3[N*s/m^2]
D	1e-10[m^2/s]
d	40e-7[m]
Vp	(pi/6)*d^3
kB	1.38e-23[J/K]
T	310[K]
Mmax	3500[A/m]

Table 4. *Base parameters list.*

Nanoparticle hydrodynamic radius, nm (average)	Mathematically evaluated hydrodynamic radius, nm	Approximation error (%)
19	27,6	7,1
50	63,8	5,7
100	96,4	4,1

Table 5. *Example of calculations.*

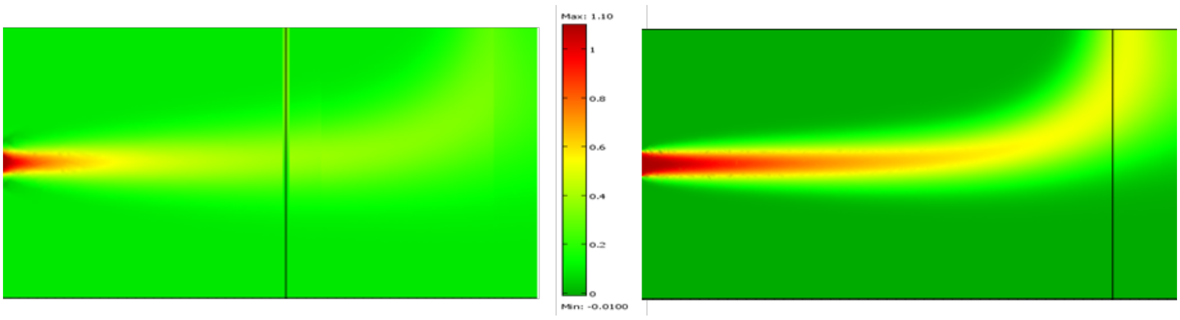

Figure 9. *Example of MNPs kinetic visual 3D simulation using Comsol (a – MNPs 50nm, b – MNPs – 200nm).*

Magnetic flux density B, Force on the magnetic particles f, Velocity of magnetic particles vmag, magnetic particle (or cells) concentration field C, magnetic particles distribution at the scaffold pore's walls S. Further development of this mathematical model can include application of ML/AI for careful processing of visually obtained experimental results using the approach we proposed for the section 2.3.

Thus, using the micro-fluidic chip, we estimate the deviation of the flux of magnetized nano-particles under the influence of an external magnetic field. As we expected, diffusion effects on this process were negligible, but we take into consideration the kinetic parameters of particle motion in the microfluidic device as well as the physical parameters characterizing the magnetic permeability and the distribution of the magnetic field

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gradient. The first experimental data confirmed the model applicability and experimental values/parameters evaluated using finite element analysis (Comsol) were predicted correctly.

3. Conclusion

Magnetic targeting provides precise control over the transport of magnetic nanoparticles (MNPs) in microfluidic chambers. Moreover, assessing associated parameters offers the potential to fine-tune the delivery of therapeutic agents to specific sites, reduce systemic distribution of cytotoxic compounds, and ultimately enable more effective lower-dose medical treatments in vivo. In such applications, the evaluation of magnetization parameters and hydrodynamic properties of nano-(micro-) objects, such as evMNBs, is crucial, but standard techniques often fall short in providing sufficient information. To address this challenge, we developed a number of complementary mathematical approaches based ehaviouring the macroscopehaviourior of a uniform solution of magnetic nano-objects under the influence of an external magnetic field. These approaches predominantly revolve around assessing the equilibrium of magnetic field forces and convection-diffusion effects. For the first time, a machine learning/artificial intelligence approach was employed to predict parameters associated with the magnetization of MNPs.

These mathematical models can be utilized for the subsequent optimization and development of microfluidic devices for the fabrication and/or characterization of evMBDs. They also find application in other studies focused on the biological investigation of nano-sized magnetized objects.

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