New Forceful Magnetic Bioseparation using GIAMAG Magnet Systems

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ABSTRACT

Magnetic bioseparation is an important area of biotechnology. Various techniques are used with a wide range of possible applications in bioscience research. Magnetic micro- or nanospheres can be functionalized with appropriate ligands, such as antibodies, with a high affinity to the target, like cells, bacteria or DNA/RNA. In order to realize magnets with efficient separation capabilities, it is important to have a strong force $\boldsymbol{F_M}$ acting on the magnetic bodies. $\boldsymbol{F_M}$ is proportional to the product of the magnetic field and the field gradient. Many permanent magnets on the market have large magnetic fields, but relatively weak field gradients. GIAMAG magnets have unique and patented designs that produce both very large magnetic fields and high field gradients, resulting in the most forceful magnetic separators available on the market.

INTRODUCTION

This paper will describe some historical and recent advances in magnetic micro-nanoparticle separation and manipulation using very forceful magnet systems. In the 1970s, prof. John Ugelstad at NTH in Trondheim, Norway, became the first to succeed in making uniformly micrometer-sized polystyrene microspheres [1]. Later the microspheres were made magnetizable by depositing nanometer-sized iron oxide in pores inside the spheres [2]. These "Ugelstad spheres" exhibited superparamagnetic behavior, in that they could be easily magnetized by an applied magnetic field, but reverted back to an unmagnetized state once the field was removed. Thus, they experienced a magnetic force when subjected to a local field gradient, and they could be used to catch and separate magnetically labeled biomaterials from a carrier fluid using an external magnetic field. In 1983, the microspheres were used to treat bone marrow cancer, see figure1. The method, briefly, is to remove some of the bone marrow from cancer patients, adding the functionalized magnetic beads, removing cancer cells in vitro and re-infusing the treated bone marrow to the patient. These magnetic beads, later denoted Dynabeads, revolutionized separation methodologies in the 1980s [3,4]. At present, such magnetic beads are still used in countless scientific applications and cited in numerous published articles, especially in the life science areas.

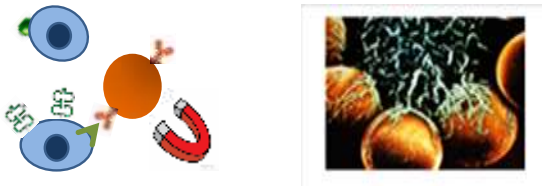

Figure 1. Magnetic bioseparation of bone marrow cancer cells as explained in the text. Left: Schematic illustration of magnetic beads covered with antibodies specifically attach to the cancer cells. The magnetic beads with the cancer cells can then be removed by an external magnetic field. Right: A historical picture showing a SEM micrograph of a blood cancer cell bound to Dynabeads M-450, diameter 4.5 μm. (Picture taken by H. Danielsen at the Norwegian Radium Hospital, 1985).

Magnetic beads typically consist of a polymer with embedded superparamagnetic nanoparticles of magnetic iron oxides. The beads have a significant magnetization in an applied field and zero, or nearly zero, magnetization in the absence of an applied field. The beads are commercially available with diameters ranging from 50 nm to 10 microns and with a large range of surface functionalizations. Recent development in bioseparation is the use of smaller magnetic beads down to about 50 nm. Nanoparticles are used extensively for different bioseparation purposes [5], see figure 2 below. Magnetic beads with multi - biofunctional coatings, like antibodies, are thus used in a wide range of fields these days, such as biotechnology, biomedicine, microbiology and microfluidics where they are used to label, transport and separate various biomaterials, and for specific drug delivery.
This makes them very suitable for attaching to various biosystems such as proteins (5−50 nm), viruses (20−450 nm), genes (2 nm wide and 10−100 nm long), or whole cells (10–100 μm).

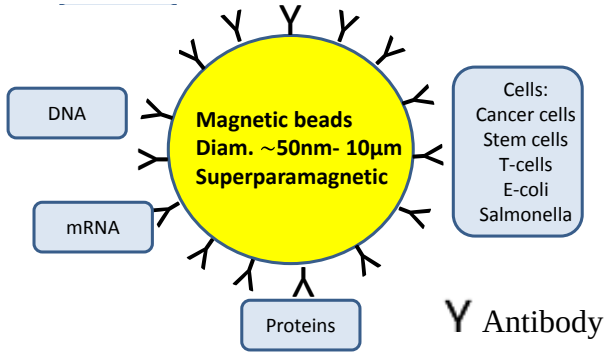

Figure 2. Schematic the wide use of magnetic bioseparation. The magnetic micro- and nanoparticles need to be functionalized, i.e. treated with appropriate surface coatings, like antibodies, to be able to target various biosystems as discussed in the text.

The two main steps in the process are thus targeting and separation. Firstly, the magnetic beads bind to a target and are secondly separated from the solution using high-gradient magnets. Many of the current methods rely on separation of micron-sized magnetic beads. Reducing the size of the beads to nm range (50-150nm) can lead to vastly increased sensitivity and selectivity of targeting. However, the magnetic separation process itself becomes considerably more challenging due to reduced magnetic force on the beads. There exist on the market today solutions for magnetic nanoparticle separation that are very efficient, such as MACS [6]. These techniques, however use magnetic column filtration which can be resource demanding.

Giamag Technologies has newly developed a column-free magnetic nanoparticle separation with a high yield using the patented Giamag magnet design by one of the authors (ATS) [7]. At present, there are no other magnets in the market enabling magnetic separation and capture of magnetic nanoparticles of this size so directly. The challenges in magnetic nanoparticle separation and the need for this novel magnet design, is discussed below.

THEORY, SIMULATIONS AND EXPERIMENTS

The calculation of the force F_M on a magnetic particle is straightforward and performed below using the notation outlined in figure 3.

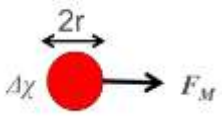

Figure 3. Schematic the parameters used to calculate the force on a magnetic particle as discussed in the text.

$$\boldsymbol{F_M} = \frac{4\pi r^3}{3} \Delta\chi \, \boldsymbol{B} \cdot \nabla \boldsymbol{B} / \mu 0 \, . \qquad 1$$

Here, r is the radius of the magnetic particle, $\Delta\chi$ is the difference in the susceptibility of the magnetic particle and the surroundings, $\boldsymbol{B} \cdot \nabla \boldsymbol{B}$ is the product of the magnetic field $\boldsymbol{B}$ and field gradient $\nabla \boldsymbol{B}$ and μ_0 is the vacuum permeability. As we shall see, magnets with high field gradients are needed to catch and separate out nanoparticles as the magnetic field $\boldsymbol{B}$ strength is more or less fixed to a maximum of about 2 Tesla.

One can make a rough estimate of why it is difficult to separate out magnetic particles in the nanometer range: colloidal particles experience thermal forces leading to Brownian motion, the thermal force is of the order $\boldsymbol{F_{Therm}} \sim k_B T/r$, where r is the radius of the particle. If the magnetic force is much smaller than thermal forces, there is no separation. The minimum magnetic force needed to separate out particles is roughly given by, $\boldsymbol{F_M} \sim \boldsymbol{F_{Therm}}$, which gives an estimate of the minimum magnetic field gradient necessary for particle separation to occur:

$$|\nabla \boldsymbol{B}| \sim \frac{3 k_B T \mu_0}{4\pi \Delta\chi B r^4} \propto \frac{1}{r^4} \, . \qquad 2$$

In reducing the particle size from 1 μm to 100 nm it means that the magnitude of $\nabla \boldsymbol{B}$ must increase by a factor 10^4 in order to achieve separation. For example, for a magnetite nanoparticle the saturation magnetization $M_s \approx \Delta\chi \frac{B}{\mu_0} \approx 4\,10^5 \mathrm{A/m}$ is achieved for field strength around 1T [8]. For a particle of size $r = 50\mathrm{nm}$ this implies $|\nabla \boldsymbol{B}| \approx 400\mathrm{T/m}$. Many permanent magnets on the market have high magnetic fields, but far lower magnetic field gradients.

A new design of a magnet system denoted GIAMAG (GIAnt MAgnet field Gradient) has thus been patented [7] and realized [9] with an unprecedented value of the product of the magnetic field strength $\boldsymbol{B}$ and the field gradient $\nabla \boldsymbol{B}$. Existing magnet systems can just pull efficiently magnetic microparticles from solutions, whereas GIAMAG can extract single nanosized magnetic particles. The principle design of GIAMAG is outlined in figure 4.

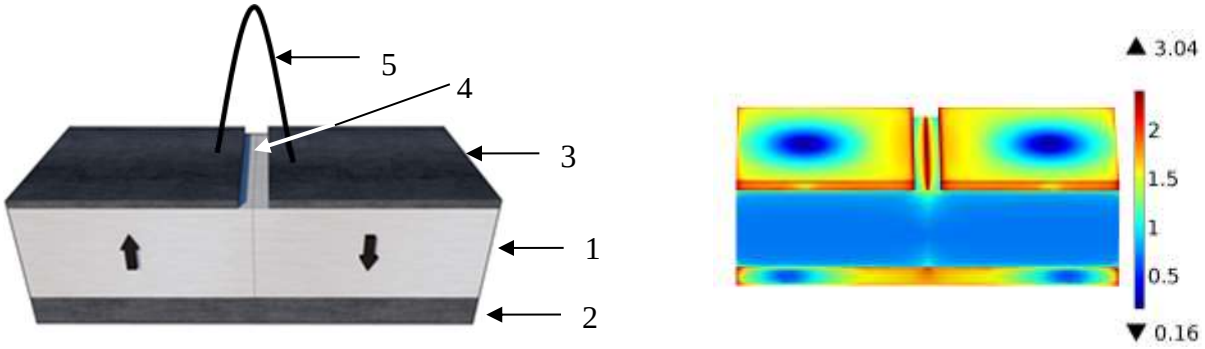

Figure 4. Left: Schematic outline of the original design of the GIAMAG magnet. The system consists of the following key components: Two adjacent permanent magnets (1) with opposite direction of magnetization, a yoke (2) and masks (3). The design involves a small gap (4) producing a very strong magnetic field gradient (5). Right: Computer simulation of the magnetic field strength distribution on the surface with N52 grade Neodymium–iron–boron magnets. The color scale is given in Tesla.

The magnet system consists of two permanent magnets, like Neodymium–iron–boron, with opposite magnetization in the so-called Kittel open domain structure [9] shunted with a yoke at the lower side made of a special low carbon steel. There is a strong magnetic stray field in the vicinity of the line where the surfaces of the magnets come together on the top. The essential feature with the present work was to improve the design and trap more of the magnetic stray field using two masks of thin sheets of a soft magnetic material, like permendur [12], with a high induction of saturation placed on the surface of the neighboring magnetic poles. The main feature of the masks is permeability. Permendur has relatively high permeability when the material is at saturation magnetization. The masks will thus guide the magnetic field toward the gap and focus it in the gap. With this construction both the maximum magnetic field $\boldsymbol{B}$ and especially the gradient,$\nabla \boldsymbol{B}$**,** was significantly increased compared to the open Kittel structure [9].

Magnets with other geometries have also been designed, tested and patented, see figure 5.

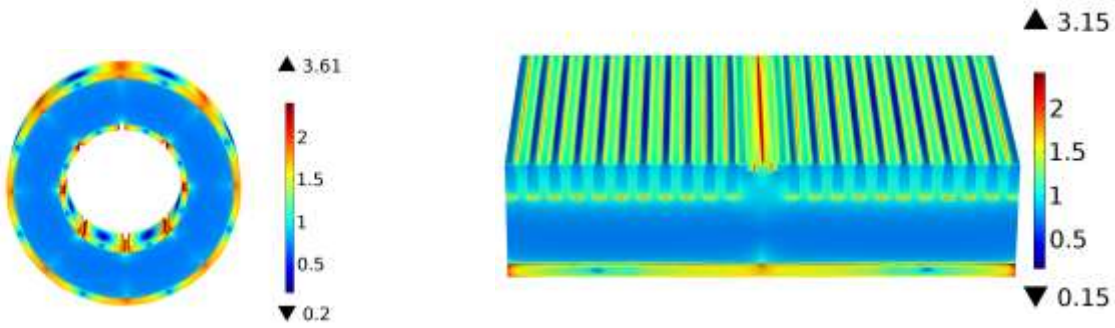

Figure 5. Left: A cylindrical GIAMAG magnet design where 8 gaps run axially. The yoke is placed on the outside and the masks on the inside. The color scale is given in Tesla. Right: A GIAMAG magnet design with many separate masks. The color scale is given in Tesla.

Figure 6 shows schematically an experimental setup to measure how fast 50 nm bead magnetic nanoparticles can be separated with GIAMAG. The magnetic iron oxide core size of the nanoparticles is about 40 nm. The magnet setup had a magnitude of $\boldsymbol{B} \cdot \nabla \boldsymbol{B}$ ~ 900 T^2/m. To our knowledge, this value is at least a factor of ten up from existing magnet design for separation.

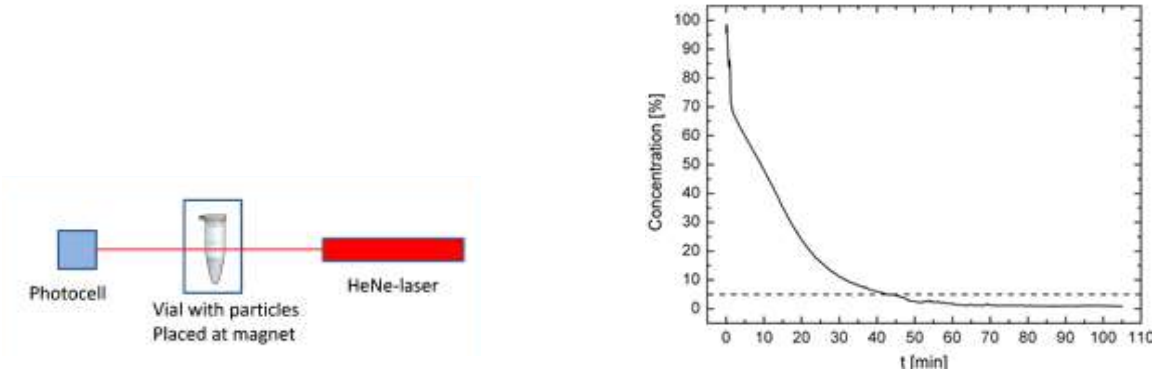

Figure 6. Left: Experimental setup to measure how fast 50 nm beads with a 40 nm iron oxide nanoparticle core can be separated with GIAMAG. The vial was a 2 ml microcentrifuge tube strapped to the surface of the magnet. The amount of light from the laser beam detected by the photocell is a measure of the remaining dispersed particle concentration. Right: The measured concentration of the nanoparticles still in the solution versus time.

DISCUSSION

The GIAMAG magnets developed in the present work differ from the Kittel open domain structure as follows:

- the surfaces of the neighboring magnet poles are covered with a mask made of sheets of a soft magnetic material
- the soft magnetic material of the mask is selected on the basis of the magnitudes of the induction of saturation and magnetic permeability for optimizing $\boldsymbol{B} \cdot \nabla \boldsymbol{B}$
- between the sheets of the mask there is an adjustable air gap located symmetrically to the line where the magnets meet
- the size and the form of the air gap between the sheets of the mask are selected in order to achieve the desired form and a gradient of the magnetic field

Simulations have been performed on the various geometries discussed above. Typically, one obtains values of $\boldsymbol{B} \cdot \nabla \boldsymbol{B} \sim 500 - 1000$ Tesla^2/m. This is roughly a factor of at least 10 up from conventional magnet separation set-ups.

CONCLUSIONS

In conclusion, the GIAMAG magnet design described here allows generating forceful magnets enabling separation of magnetic particles down to nanosizes. The design also allows different geometries, rectangular, radial and axial shapes. The choice of mask material and its thickness is important for optimizing the GIAMAG magnet systems. GIAMAG has also found uses in other areas than bioseparation, like in tracer technology and cleaning of polluted water [12].

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