A METHOD FOR PRINTING HETEROGENEOUS PATTERNS OF CELLS AND BIOLOGICAL MOLECULES BY MAGNETICALLY DIRECTED ASSEMBLY

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INTRODUCTION

Micron-sized colloidal particles with magnetic properties have been used in a wide variety of biomedical applications. Some of the most promising applications for magnetic particles are in cell sorting and cell separation based on high gradient magnetic fields, because they can be conveniently performed with only a large ferromagnet and magnetic beads that recognize and bind to specific cells. An interesting modification of this technique is using a substrate patterned with ferromagnetic film to trap cells from suspension. Ferromagnetic traps are easily patterned on glass or Silicon substrates with micrometer resolution using standard photolithographic methods. The advantage to using ferromagnetic traps to print cells is that the highly non-linear behavior of magnetic materials can be exploited to generate both attractive and repulsive forces on colloidal particles. The ability to generate repulsion, in particular, allows for the possibility of printing heterogeneous patterns of cells.

In this paper, a method for printing an ordered array of superparamagnetic colloidal particles onto a lithographically defined pattern of ferromagnetic film is demonstrated for applications in printing heterogeneous cellular and chemical patterns. Printing of magnetic particles has been widely utilized to visualize areas of changing magnetization since the work of Francis Bitter. On one hand, magnetic forces can be weaker than competing forces in colloidal systems such as electrostatic, van der Waals and surface tension forces. However, magnetically driven assembly of colloidal particles has significant advantages over other methods [see ref 1 for overview]. One advantage is that magnetic forces in liquids can act at much longer range than surface tension, and van der Waals interactions. Another advantage is that magnetic forces can exploit highly non-linear behavior of magnetic materials to allow switching between attractive and repulsive forces, something that has not been widely employed in colloidal printing methods.

EXPERIMENTAL METHODS

Ferromagnetic islands were patterned in 50-nm thick thermally evaporated Cobalt film. An array of Cobalt islands is illustrated in Figure 1 (left). The islands were patterned on Silicon and Pyrex wafers using standard photolithographic lift-off methods. In the lift-off process, an image is defined by exposing photoresist to ultraviolet radiation through a patterned mask. The photoresist pattern is developed, and then metal is evaporated onto the patterned wafer. Finally, the remaining photoresist is stripped to produce a pattern of metallic film. While negative or multi-layer resists are frequently utilized to obtain an undercut resist profile, positive Shipley 1813 yielded satisfactory results.

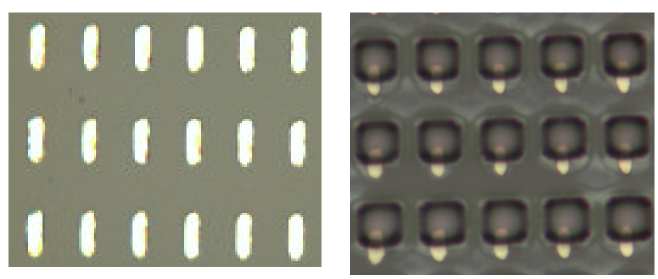

FIG 1: Optical image of rectangular Cobalt islands patterned by lift-off process with 15–μm length and 5-μm width (Left). Optical image of a template of 10-μm square wells aligned above the ends of magnetic islands with dimensions 15-μm long and 5-μm wide (right).

A protective layer of SU-8 2005 film was photolithgraphically patterned and aligned on top of the previously patterned ferromagnetic layer, as illustrated in Figure 1 (right), in order to better control the spatial distribution and number of beads that populate the template. The thickness of the protective layer is approximately equal to the diameter of the superparamagnetic beads.

The patterned Cobalt film was magnetized to saturation with a permanent magnet. Magnetic Force Microscopy of the islands revealed multi-domain structure of the remanent state with one large elongated domain in the center, aligned parallel to the magnetized direction. Experiments on decorating the patterned substrate with superparamagnetic colloidal particles were conducted in a bath of DI water and observed with a Leica DM LFS microscope through a fluid-immersion lens. Commercially available superparamagnetic beads (2.8-µm diameter from Dynal Biotech, and 7.0-µm diameter from Spherotech) were injected into the bath while the process of bead assembly onto the template was observed. During decoration experiments, the bath was agitated using various techniques, including ultrasonic vibration, flow rinsing, and manual shaking of the bath in order to increase the probability that each particle finds its nearest potential well. The decoration experiments usually lasted a few minutes.

External uniform magnetic fields were applied to the bath in order to bias the dipole moments of all the magnetic beads in a particular direction. Magnetic fields were generated with a solenoid coil and iron core in the range of 5 – 200 Oe, which is enough to bias the bead's moments but not enough to reverse the template magnetization.

RESULTS

In the absence of external magnetic field bias, the bead's equilibrium position depends strongly on its size relative to the island's size. Previous theoretical models predicted that small beads are attracted to the magnetic poles near the ends of the islands, whereas larger beads tend towards the center of the island [2]. Moreover, models indicated that beads slightly larger than the islands do not tend to settle on top of one another. Experiments with islands of various shapes and sizes support this conclusion. For example, when 5-µm diameter circular islands illustrated in Figure 2 were decorated with 2.8-µm and 7-µm beads, the smaller beads congregated next to and on top of one another, whereas the larger beads occupied the islands one by one.

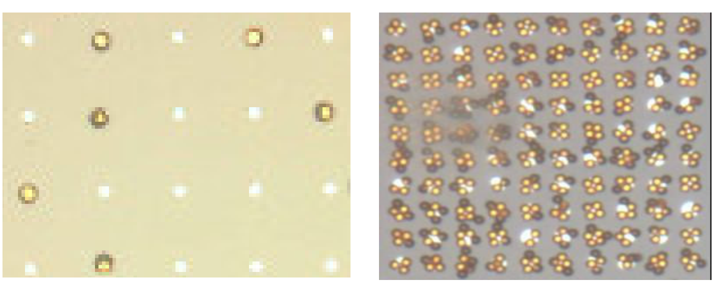

FIG 2: Optical image of solitary 7-µm beads assembling on the center of 5-µm circular Cobalt islands (Left); and 2.8-µm beads assembling on the edges of the islands (Right).

While a pattern of ferromagnetic islands alone is suitable for arranging magnetic particles in a general area, its control over the number of beads that populate an island is relatively poor. Moreover, in the case when the beads are slightly larger than the islands, they are often easily washed away when the bead solution is removed. If a magnetic pattern is used in conjunction with a mechanical template of micro-wells, however, the spatial distribution and number of beads can be more precisely controlled and beads that are trapped inside wells are not easily washed away.

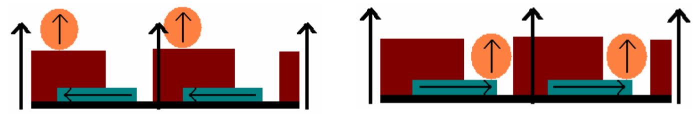

FIG 3: External field bias can induce repulsion (Top) or attraction (Bottom) between particles and the exposed end of the island based on the island's magnetization.

In addition, external magnetic field bias can be used in this case to attract as well as repel the beads. A conceptual illustration is shown in Figure 3. When the magnetic islands are magnetized in one direction, the particles are attracted to the exposed end of the islands. When the islands are magnetized in the opposite direction, the particles are repelled from the exposed end of the islands and attracted to the covered end where they can later be washed away.

Figure 4 shows experimental confirmation of this process. The top image depicts magnetic beads populating the lattice site micro-wells due to the island's favorable magnetized state. Vigorous washing and agitation does not remove the beads. If the island is magnetized in an unfavorable state, then magnetic beads will aggregate on top of the protective layer but not in the micro-wells as shown in Figure 3 (Bottom). This example suggests that magnetism rather than surface forces dominates the assembly process.

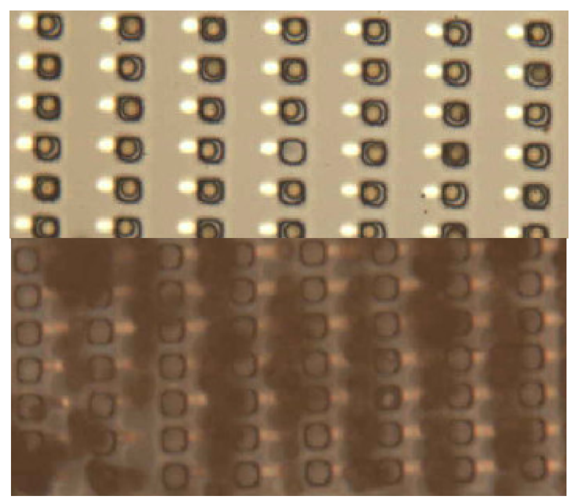

Figure 4: Optical image of 7-µm colloidal beads solitarily confined to a template of 10-µm wells (Top); and beads avoiding the wells (Bottom).

CONCLUSIONS

This paper details a method for printing superparamagnetic colloidal particles onto a magnetic pattern, and initial results suggest that magnetically driven assembly of colloidal particles has significant advantages over other techniques. In particular, the ability to generate repulsive forces shows potential for assembling defects, which may be used to print heterogeneous patterns of magnetic particles carrying cells or biological molecules.

REFERENCES

1. Y. Yin, Y. Lu, B. Gates, Y. Xia, *J. Am. Chem. Soc.* **123**, 8718 (2001).
2. B. B. Yellen, G. Friedman, A. Feinerman, *J. Appl. Phys*. **91**, 855 (2002).