

High Capacity Nano-optical Diffraction Barcode Tagging for Biological and Chemical Applications

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ABSTRACT

We describe a new non-contact high capacity optical tagging technique for encoding small beads, based on the use of nanostructured barcodes. The tags are generated from a number of superimposed diffraction gratings. With one-dimensional diffraction, capacity for up to 68,000 distinguishable tags has been demonstrated on a glass substrate, with a theoretical capacity of up to 10^9 tags. Extension into two dimensions increases this limit to 10^{18} tags. Manufacture of such tags on a polymer material compatible with biochemistry has also been demonstrated, which allows reading in a high throughput microfluidic system.

Keywords: optical tagging, diffraction, nanostructured elements, combinatorial chemistry

1 INTRODUCTION

The rapid advances in high throughput screening, combinatorial chemistry, genomic and proteomic sciences have stimulated dramatic development of new encoding strategies for bead-based assays. Several optical encoding methods are currently used in these applications [1], including fluorescence, IR [2], Raman [3] and optical [4], [5] tagging on microbeads. Methods using magnetic tagging are also being investigated [6]. In particular, multicolor optical coding has been achieved by embedding quantum dots of zinc sulfide-capped cadmium selenide nanocrystals into beads [7-10] while patterns can also be written in fluorescently dyed beads by spatially selective photo-bleaching to create spatially selective fluorescent tags [11]. Here we describe a new method for encoding small beads which allows for non-contact reading. The tagging technique is based on fabricating a nano-structured pattern on the surface of the particle, which is only a few microns in size, read by detecting the spatial distribution of laser light diffracted by the tag, as detailed elsewhere [12].

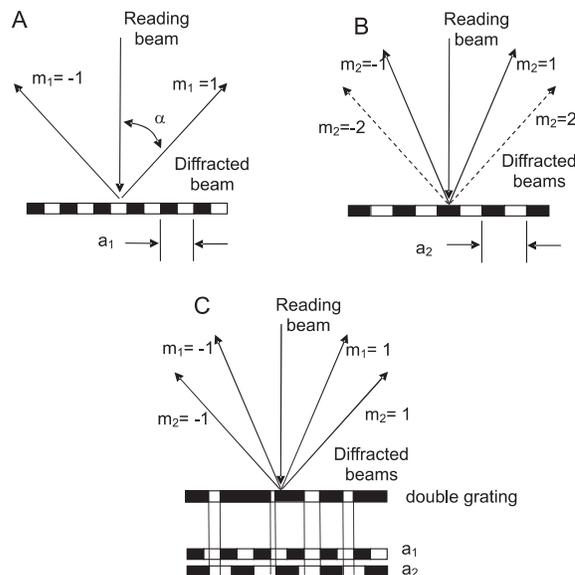


Figure 1: A) first order diffraction from grating with pitch a_1 ; B) first and second order diffraction from grating with pitch a_2 . Higher order diffraction is normally less intense and for encoding applications higher orders may be eliminated by threshold detection; C) first order diffraction from combinatorial grating made by superposition of gratings with pitches a_1 and a_2

2 CONCEPT AND THEORY

2.1 Basic concept

In a classical single diffraction grating, information may be encoded in the pitch or spacing of the grating, a . When such a grating is illuminated with light at wavelength λ , for example at normal incidence, a series of diffracted beams of different order m is created, according to the equation $a \sin \alpha = m\lambda$. Here α is the angle of diffraction and m is the diffraction order. Therefore, a measurement of the first order diffraction angle, at $m = 1$, gives direct information about the pitch of the grating a . This principle is shown in Fig.

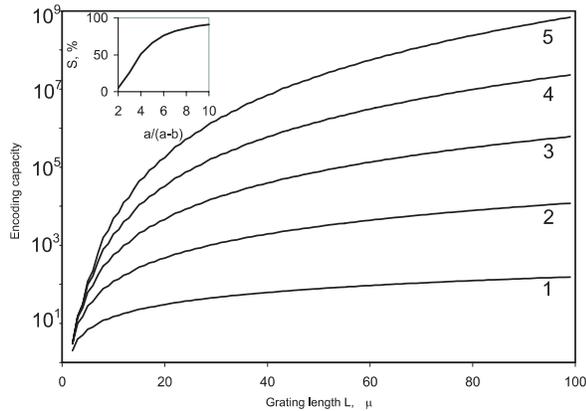


Figure 2: Encoding capacity of a diffractive barcode tag as a function of the length of the tag for different numbers of superimposed gratings. The number on each curve corresponds to the number of superimposed gratings. The inset shows the level of intensity discrimination S necessary to ensure error-free identification of tags

1. The number of distinguishable tags that can be manufactured depends on the ability to resolve a difference between two diffraction patterns. Two diffracted beams at diffraction angles θ_1 and θ_2 can be resolved if they satisfy the Rayleigh criterion that the maximum of one appears at the minimum of the other. Using this criterion it is possible to calculate the capacity of diffractive bar-codes with a single grating as a function of the grating length (neglecting all diffraction orders above $m = 1$). The results are presented in Fig. 2, curve (1).

A much larger capacity of tag can be obtained if several gratings are superimposed on the bead, as shown on Fig. 2. Each of the superimposed gratings produces its own set of diffracted beams independently of the other gratings on the tag. If the tag has k superimposed gratings, the number of possible distinguishable codes c is then given by the number of possible combinations of k beams in n possible positions, using the combinatorial formula $c = \frac{n!}{(n-k)!k!}$, shown for several superimposed gratings in curves (2)-(5), Fig. 2. In practice the maximum number k_{max} of superimposed gratings in the tag which can be distinguished by diffraction is limited by the resolution δ of the tag's fabrication process and may be estimated as $k_{max} \simeq \lambda/(2\delta)$. It is possible to increase the tag capacity even further by moving to grating periods in two dimensions, creating capacities equal to the square of those in one dimension.

2.2 Reading requirements

This analysis assumes that the data is encoded only in the first order diffraction spots. In practice, however, higher diffraction orders will be present, which could be confused with the first order beams, leading to misreading of the tag. In general intensities of high-order diffraction beams depend

on the grating aspect ratio and in practice are much smaller than intensities of the first order beams. These can be distinguished from first order beams by setting a discrimination threshold that depends on the physical characteristics of the diffraction grating, in particular on the ratio $a/(a-b)$, where b is width of the transparent grating elements (see Fig. 1). The discrimination threshold level S was calculated for an ideal grating made of perfectly transparent and perfectly opaque strips for different values of $a/(a-b)$, by calculating the intensity of the brightest higher order diffracted beam as a fraction of the first order beam intensity (see inset of Fig. 2).

Some further reading problems can appear when gratings are superimposed. There is generally some interplay between gratings and appearance of extra 'ghost' beams not present in individual gratings. Our modeling shows that intensities of the ghost beams are generally much smaller than that of the main beams. They depend on the aspect ratio of the gratings and could become a serious factor at high levels of superposition. Tag capacity limitations arising from the ghost beams for high order tags will have to be investigated further, but does not appear to be a major factor in the experiments reported below.

3 EXPERIMENTAL

3.1 Metal on glass barcodes

In order to demonstrate this tagging concept a chip library of chromium gratings was manufactured on a glass substrate using direct write electron beam lithography. The library of gratings contains almost 7,400 unique barcode tags, $50 \times 50 \mu\text{m}$, separated by gaps of $200 \mu\text{m}$. SEM images of these tags showing the range of different superimposed gratings are presented in Fig. 3. With an available nanofabrication resolution δ of about 100 nm we have been able to demonstrate tags up to order three (containing three superimposed gratings) that are fully distinguishable by diffraction. This provides a capacity of about 68,000 distinguishable tags. Higher order tags have also been fabricated, but they sometimes show fails in pattern reproduction which spoils the quality of diffraction (Fig. 3(iv))

An example of the diffraction patterns created by these tags is presented in Fig. 4. The gratings were read using light from a HeNe laser (633 nm) incident at normal angle to the sample. The diffraction pattern was observed on a screen parallel to the grating and captured using a CCD array detector. Fig. 4(a) shows how the diffraction pattern changes with grating pitch; increasing in complexity from the simplest single grating tag. The series of diffraction patterns (A to J) demonstrates how it is possible to uniquely distinguish between ten different tags containing only a single grating. In the photographs, the first order diffraction spots are highlighted by the solid circles, while the positions of the much weaker second order diffraction spots is indicated by dashed circles.

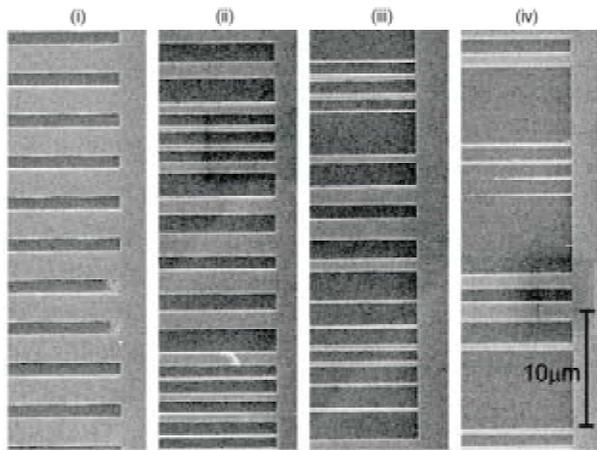


Figure 3: SEM images of barcode tags of different order: (i) single grating tag; (ii) two superimposed gratings; (iii) three superimposed gratings; (iv) four superimposed gratings (note nano-lithography resolution limiting quality of this grating).

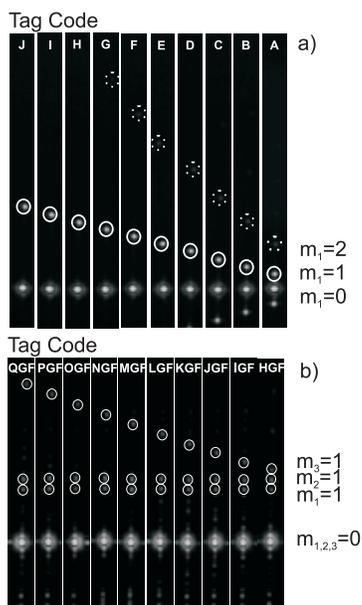


Figure 4: Diffraction patterns created by a single grating tag (a), and tags containing three different gratings (b). Moving from left to right shows how a progressive decrease in the pitch of one of the gratings changes the diffraction pattern

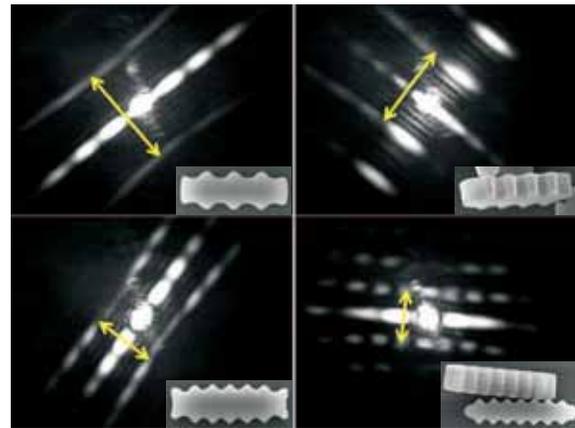


Figure 5: Four different diffraction patterns from barcode tags made from SU8 containing 100 ppm nickel nanoparticles, together with SEM images of the actual particles.

Fig. 4(b) shows diffraction patterns from different tags containing three superimposed gratings (see patterns HFG to QFG). Here, the first grating diffracts exactly as grating G in Fig. 4(a), while the second grating diffracts as grating F. The third grating differs from pattern to pattern. The first diffraction pattern is from a grating which diffracts as grating H in Fig. 4(a), but then changes step by step to give a higher and higher diffraction angle. In this way we have resolved diffraction patterns with up to three superimposed gratings. Further increase in the number of superimposed gratings on the tags led to increasing read errors due to the limited resolution of the grating fabrication process.

3.2 Polymer barcodes

We also produced biologically compatible barcodes using photopolymer SU8. This polymer is a suitable media for the covalent attachment antibodies, proteins or oligonucleotides to the particle surface and therefore can be used for a large variety of biochemical applications. The barcode patterning on SU8 was achieved by photolithography. In order to improve the diffraction efficiency, the polymer was mixed with metal nanoparticles of gold, silver or nickel. This has led to a substantial improvement in the quality of the diffraction patterns. For instance Fig. 5 shows four different nickel-doped polymer tags and their corresponding diffraction patterns which can be fully resolved even if the tag is immersed in water. Since photolithography is a relatively low resolution manufacturing technique, it is not suitable for the manufacture of high capacity tags. In order to create high capacity tags in SU8, we will use a nano-embossing technique, which imprints the pattern into the polymer with a high resolution master prepared by e-beam lithography. We also designed a high-throughput microfluidic on-a-chip transport system for the tags (see Fig. 6).

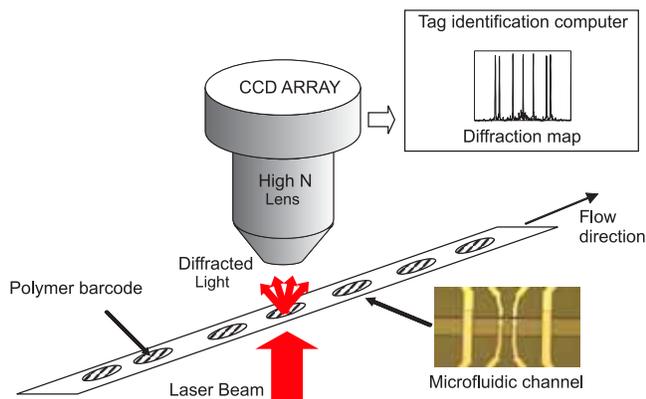


Figure 6: Schematic of a microfluidic transport system with barcode reader.

4 CONCLUSION

In conclusion we have demonstrated a new optical, non-contact tagging technique based on superimposing large numbers of miniature diffraction gratings on a tag. With a 50 nm nanofabrication resolution now routinely available, the technique is capable of creating distinguishable tags containing at least 5 superimposed grating and encoding up to 10^9 tags, each of which is only $100\ \mu\text{m}$ long and a few μm wide. To demonstrate this technique we manufactured a library of $50\ \mu\text{m} \times 50\ \mu\text{m}$ tags on a glass wafer. With nanofabrication resolution of about 100nm it has been possible to resolve tags containing at least three superimposed gratings providing capacity for more than 68,000 tags. An enormous increase in capacity will be possible if two sets of mutually perpendicular gratings are used. Combinatorial analysis shows that up to 10^{18} different barcode tags can possibly be fabricated with such two-dimensional superimposed gratings up to order five. We have also demonstrated bio-compatible tags, suitable for chemical attachment and use in a high throughput microfluidic system.

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