

# DIFERENT BARCODES CODIFICATION FOR EMBRYO MICRO-LABELING

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## ABSTRACT

The development of in vitro reproduction techniques has presented a great concern about the identification of the embryos. In this work, we report the use of a novel barcode branding based on polysilicon and silicon structures for embryo labeling. Four different barcodes types were fabricated based on standard semiconductor techniques and the labeling potential was tested. The codification method was based on a binary code of 8 bits. The results show that these barcodes, microinjected between the pellucid zone and the cell membrane, represents a usefulness technique for embryo labeling.

**KEYWORDS:** Polysilicon, silicon, barcode, microfabrication, embryo labeling

## INTRODUCTION

Nowadays, alternative barcoding structures at microworld level represent a great interest [1]. In assisted reproductive technologies the embryo labeling is a critical factor, each embryo from each donor have to be correctly identified trough all steps of culture and manipulation. Recently, technological solutions have been developed as alternative to manual processes but misidentification errors still occurs [2][3].

Since a functional micrometer size barcode must accomplish technological, biological and optical requirements a sets of constraints must be considered: technology will lead to micrometer-size structures with well-controlled geometries, optical requirements will fix the minimum critical dimensions, and the devices have to be made of a biocompatible material and small enough to no alter the embryo development. Considering all these requirements, we propose a technology based on silicon micromachining techniques to obtain mass-produced, low cost, robust and micrometer-size devices.

Different types of barcodes have been designed to evaluate physical characteristics, robustness, optical readout and labeling capabilities. Therefore, the contribution of this work is to proof in a real application, the use of these small biocompatible 2D and 3D barcodes for embryo labeling, which could be easily defined by photolithography and micromachining processes.

## EXPERIMENTAL

The fabrication of 2D and 3D barcodes is based on standard semiconductor technologies. The 2D and 3D designs differ in the dimensions, shapes and bits representation. One of these barcodes was already tested in macrophage cells for cell tracking applications and reported at  $\mu$ TAS'09 [4] [5]. The 2D devices consist of a start marker and 8 digits that represent 8 bits, giving 256 different encoding values. The marker is an asymmetric structure that allows the correct readout. These 2D barcodes have dimensions fixed to 10  $\mu$ m x 6  $\mu$ m x 1  $\mu$ m, identical or different codes can be fabricated on the wafer by drawing the appropriate pattern on the mask. The smallest features were limited to  $\sim$ 1  $\mu$ m close to the resolution limit of light microscopes. A 1  $\mu$ m thick silicon oxide layer is used as sacrificial layer and a 1  $\mu$ m thick polysilicon layer as a device layer. After device patterning, the barcodes are released by a sacrificial etching in HF vapors and finally collect in a biocompatible medium, Figure 1A.

The 3D silicon-based barcodes were fabricated using consecutive deep reactive ion etching (DRIE) steps [6]. First, a silicon wafer is used as substrate, then a 1.5  $\mu$ m thick silicon oxide layer is thermally grown and a photolithographic step is done to define spots of 3  $\mu$ m x 3  $\mu$ m. The silicon oxide layer is patterned by a reactive ion etching (RIE) process. Finally, every single bit is done by a silicon DRIE process, Figure 1B. Thus, the researchers can freely program the codes along the axis by simply choosing the DRIE conditions to obtain vertical or non-vertical etch profiles. For instance, the bit = 1 is obtained by a vertical profile etching. The bit = 0 requires a non-vertical profile etching in combination with a preceding 300Å thick silicon thermal oxidation in order to protect from underetching the bits already done. If this oxide layer is not used the previous bits would be destroyed. Following this, an additional silicon oxide RIE step is required to remove the 300Å oxide at the bottom before etching the next bit.

Eight to twelve-weeks-old female mice of the hybrid strain B6CBAF1 were used as embryo donors. One-cell embryos were collected from the oviducts 25 h after human chorionic gonadotropin (hCG; Farma-Lepori, Spain) administration, and incubated for 5-10 min at 37°C in Hepes-buffered potassium simplex optimized medium (H-KSOM); [7], selected embryos with two pronuclei and a good morphology were incubated in KSOM culture medium until their use. Embryos were placed into a drop of H-KSOM medium in the micromanipulation dish, and four different barcodes were transferred into a separate drop of 3% (w/v) polyvinilpirrolidone (PVP; Sigma, Spain) in H-KSOM.

Several barcodes were first introduced into a blunt-ended microinjection pipette with an outer diameter of 10  $\mu$ m. The pipette was then moved to the drop containing the embryos and used to drill a hole in the zona pellucida of an embryo with the help of a few piezo pulses, without affecting the embryo development in culture. Next, the barcodes were expelled into the perivitelline space(PVS) of the embryo, as far away from the hole as possible, and the pipette was gently withdrawn, Figure 2.

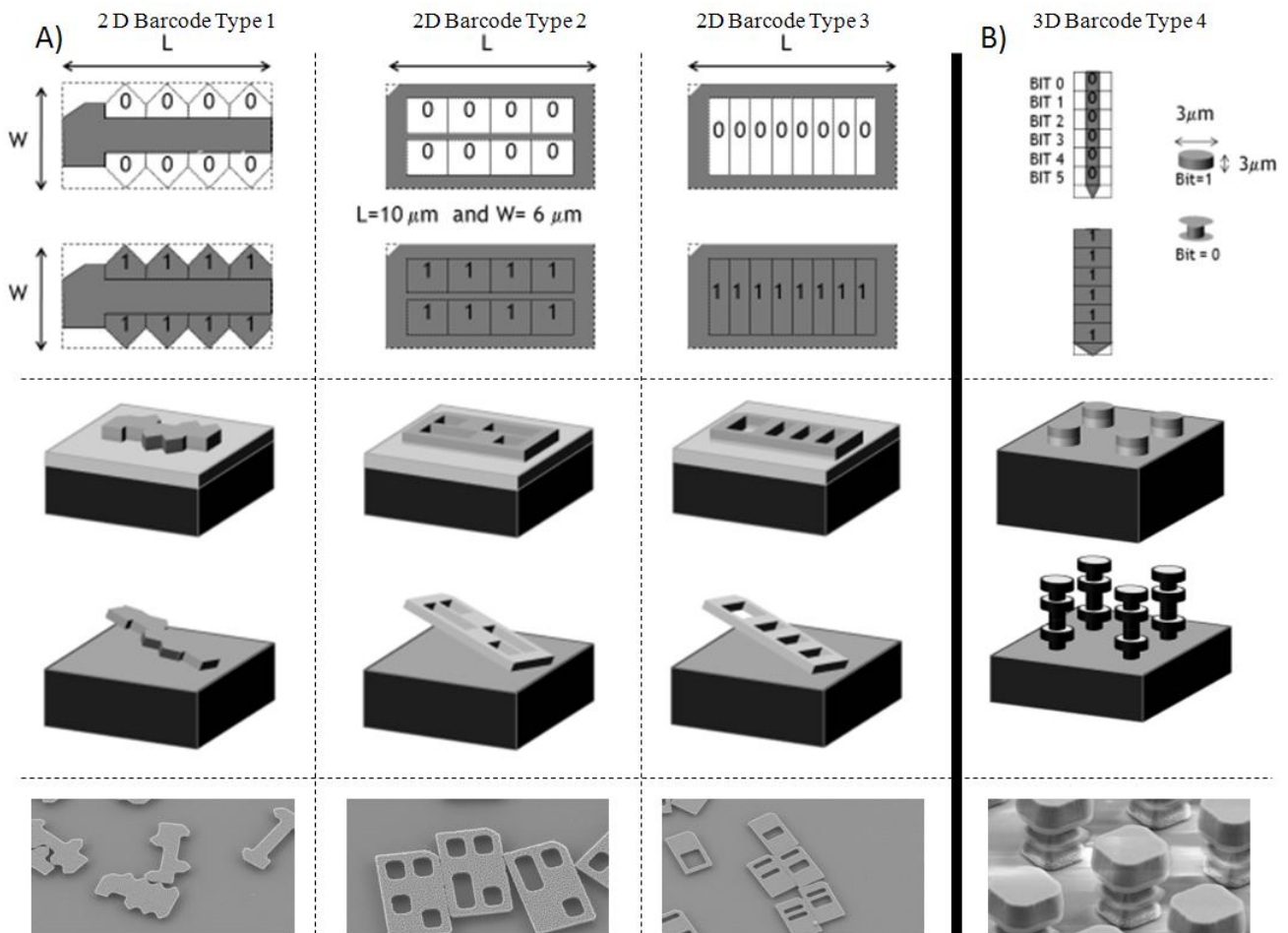


Figure 1: A) Schematic view of 2D silicon barcodes, the asymmetric corners indicates the starting point to the barcode reader. Below, schematic view of barcode fabrication process: first silicon wafers are used as starting material, second silicon oxide layer is deposited as sacrificial layer. LPCVD polysilicon oxide layer is deposited as structural layer. Finally, barcode patterning is achieved by polysilicon dry etching and the barcodes are released in HF vapors. B) Schematic view of 3D silicon barcodes design. Bit=1 and bit=0 representation. Below, schematic view of barcode fabrication process: First, Silicon wafer are used as substrate, then silicon oxide patterning is used as mask for silicon DRIE with vertical profile for bit=1, and non-vertical etching for bit=0. Finally the barcodes are release by long isotropic overetching. The last part shows SEM images of different types of barcodes.

## RESULTS AND DISCUSSION

Four different barcodes have been design, fabricated and tested for embryo microlabeling. The barcodes fabrication technology offers well-controlled shape and dimensions, reproducibility and robustness. The barcodes are large enough to allow their readout by simply optical microscopes and small enough, to be easily manipulate and injected inside embryo cells. In all cases was possible to read the binary code by optical microscopy. However, each design presents their advantages and disadvantages, i.e: whereas 3D barcodes are easily read independent of embryo position, due to the spatial orientation of the code with respect to the reader, the 2D barcodes could presents difficulties to read, if the spatial orientation of the code is not flat with respect to the reader. Also, we found that barcodes type 1 and type 3 are more fragile that barcode type 2 and type 4. The fragility is related to the robustness of barcode design.

## CONCLUSION

Preliminary results show in this work evidence the usefulness of these barcodes for future applications as embryo labeling. At the same way we demonstrate the possibility to employ semiconductor fabrication techniques to produce different types of biocompatible silicon based barcodes as cell microlabeling. Patent pending.

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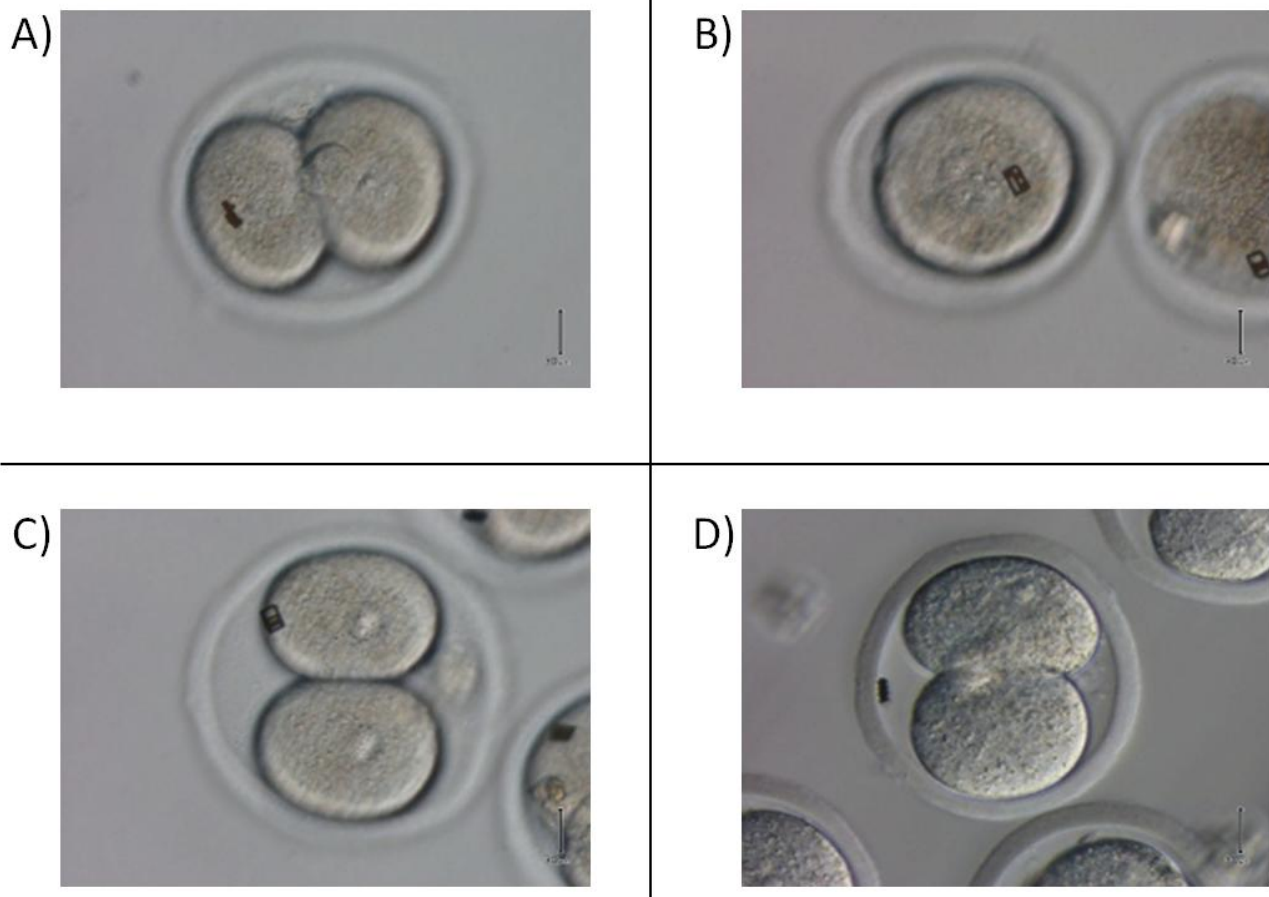


Figure 2: Optical microscope images of mice embryos with: A) 2D barcode type 1; B) 2D barcode type 2; C) 2D barcode type 3; D): 3D barcode, with one or two cell embryo with barcode into PVS.

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