Noise-Differential Based Detection of Cells on a Microelectronic Sensor/Actuator Array

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ABSTRACT

We present a new method to count cells on a microelectronic lab-on-a-chip integrating an array of Dielectrophoretic (DEP) cages and embedded optical sensors. Cells are trapped by DEP cages above photodiodes measuring the optical near field. The presence of cells is measured indirectly by the increase in noise power in sensors underlying cells. Compared to previously reported methods, this approach, achieves a 0% error on experimental test runs with 100 cages. The method is inherently insensitive on spatial gradients of illumination and on Fixed Pattern Noise from the embedded photodiodes array. This approach opens up the possibility to count cells in a flow-less portable system without complex external optical add-ons or microscopes.

Keywords: optical sensors, microelectronics, cells, dielectrophoresis, noise

1 INTRODUCTION

Integrated detection of cells and particles in lab-on-a-chip devices is attractive to avoid bulky and expensive add-ons such as microscopes and digital cameras in the perspective of system miniaturization and cost reduction.

We have previously reported [1] a prototype system (DEPArray™) for massively parallel manipulation of cells on a microelectronic lab-on-a-chip based on moving dielectrophoresis (DEP) cages. Organizing the cells with up to 12,000 DEP-cages, it is possible to place them in correspondence of embedded optical (or impedance) sensors. Although we demonstrated detection of individual beads and certain cells [2], in general, just placing cells over the sensors, even compensating for fixed-pattern noise (FPN), reliability is not sufficient to operate the chip without microscope optical inspection.

We present here a new method for reliably detecting the cells with the on-chip optical-sensor array. The specifications of the problem are analyzed in section 2, section 3 presents the working principle proposed approach, while section 4 presents experimental results before drawing conclusions.

2 PROBLEM STATEMENT

2.1 Noise sources and non-idealities due to electronics and system considerations

Several noise sources and other non-idealities impair the possibility of getting a reliable detection of full cages with respect to empty cages.

Thermal noise (random noise), is generally not a big concern. Integrated detectors with fully-differential read-out cater for low-noise levels. Illumination noise (fluctuations of optical power) is generally a more relevant problem. In any case, further averaging of multiple images may help reduce thermal noise value.

Fixed Pattern Noise (FPN) is one important aspect. Although the chip is under uniform illumination, dispersion of parameters from pixel to pixel across the chip cause a different read-out of grey-level. It can be shown that this effect may be interpreted mainly as a pixel-gain variation and reduced by compensating for differences across pixel if they are measure before-hand. However, this is inconvenient because of the additional characterization required and is still not enough to detect cells reliably.

Another problem is non-uniform illumination, which causes spatial gradients of illumination along the chip.

2.2 Optical characteristics of cells

In general, cells are mostly transparent and difficult to see. They do not significantly absorb, but rather refract light acting approximately like lenses, so that light is somehow concentrated at the center of the cell while the borders correspond to darker areas (see Fig. 1).

Accordingly, when the cell is placed over the photodiode embedded in the chip, the overall light it collects depends on the precise position of the cell itself with respect to it, and can be slightly larger or smaller than background illumination. This effect is clearly visible in Fig. 2, where –despite compensation of fixed-pattern noise, cells are sometimes brighter, darker or invisible.

The above effects make it difficult to define a segmentation threshold according to which pixel with higher or lower grey-levels are classified as belonging to “empty” or “full” cages (i.e. cages without or with cells).

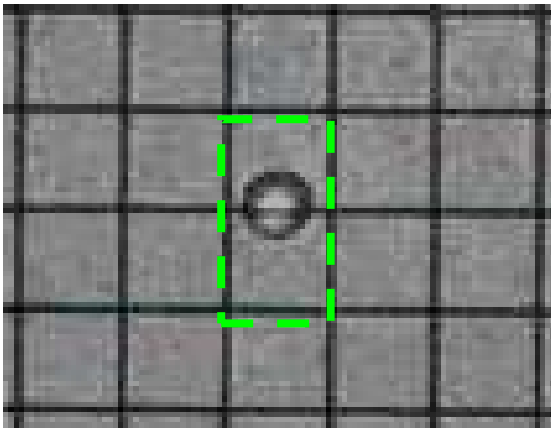

Fig. 1: bright-field microscope image of a K562 cell trapped in the DEPArray™. Cell center appears brighter, while cell borders appear darker, due to refraction of light through the cell and reflection on chip electrodes. Dashed lines corresponds to cage electrodes. Electrode pitch is 20μm.

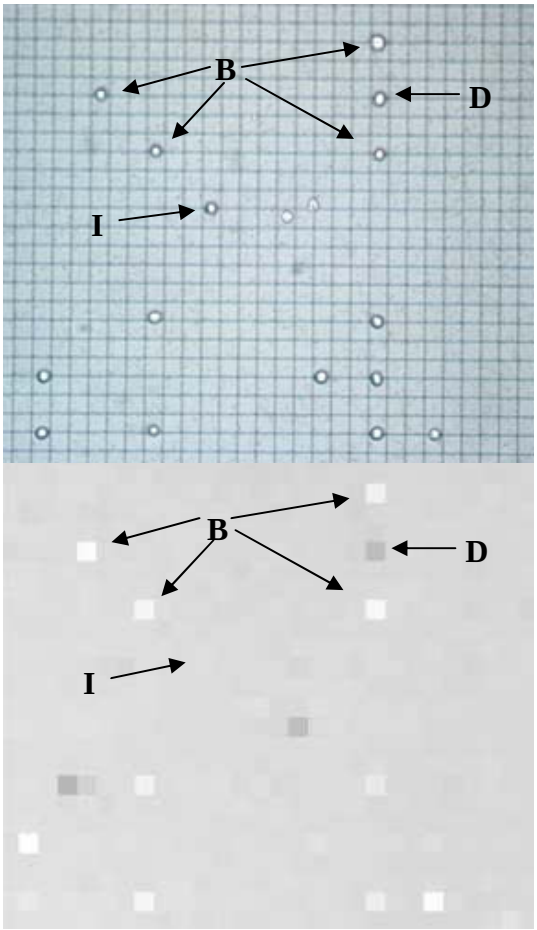

Fig. 2 -top- bright field microscope image of K562 cells trapped in the DEPArray™; -bottom- grey level values detected with the embedded optical sensors: some cells determine brighter pixel (B), some other darker pixels (D), some other are invisible (I).

3 NOISE-DIFFERENCE BASED DETECTION METHOD

As a way to overcome the limitations due to the above mentioned problems we propose to exploit the low-noise characteristic of the embedded optical sensor array to detect differences in noise power when cells are present in the cage above a photodiode.

According to this approach, actuation and sensing are alternated (Fig. 3). During actuation cells are trapped and organized above the photodiodes. Then, actuation is stopped and the output of pixels corresponding to cages, and pixels corresponding to gaps (where cells cannot be present) are recorded, multiple times approximately every 1 or 2 second. Actuation is restored after few images to restore the position of the cells and levitate them in order to prevent sticking to the substrate.

Due to thermal noise the value of each pixel changes from frame to frame, but pixels corresponding to cages trapping a cell experience significantly larger variations.

This may be explained by the fact that due to a combination of Brownian motion, sedimentation and weak local fluid motion (such as electro-thermal flow), cells change slightly their position with respect to the sensors. These slight changes are amplified by the non uniform transmission pattern through the cell, and appear as random variations of larger intensity.

As a result, one can use variations of grey level as a parameter to classify empty versus full cages.

4 EXPERIMENTAL RESULTS

K562 cells were injected into the DEPArray™ chip. The DEP cages were programmed to implement a pattern of cages 1x2 with a gap of 1 or 2 electrodes between neighboring cages. When cells settled after injection, they organized themselves falling into the nearest point of equilibrium for DEP force, represented by the center of the cage. The 1x2 cage allows one to have the cells placed over the photodiodes embedded in the chip corresponding to electrode gaps, as shown in Fig. 3. Actuation and sensing were alternated and grey-level values recorded for cage pixels and reference pixels.

The time series $g_j^{(x,y)}$ representing the grey level at time T_j for pixel (x,y) was recorded. An example of recording of grey-level value over 32 frames for empty and full cages is shown in Fig. 4. The increased noise-power level of full-cages is apparent.

Grey-level values were elaborated and results are shown in *Fig. 5*. For each pixel corresponding to the center of a cage, either full (red lines) or empty (blue lines), the plot shows the value of:

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$$\sigma_{nn}^{k} = \sqrt{\sum_{j=1}^{k} (g_j - M_k)^2} \qquad (1)$$

which we call *non-normalized standard deviation as it* corresponds to the standard deviation on k frames if multiplied by the normalization coefficient $1/\sqrt{k}$ (M_k is the mean value for the pixel over a window of k frames).

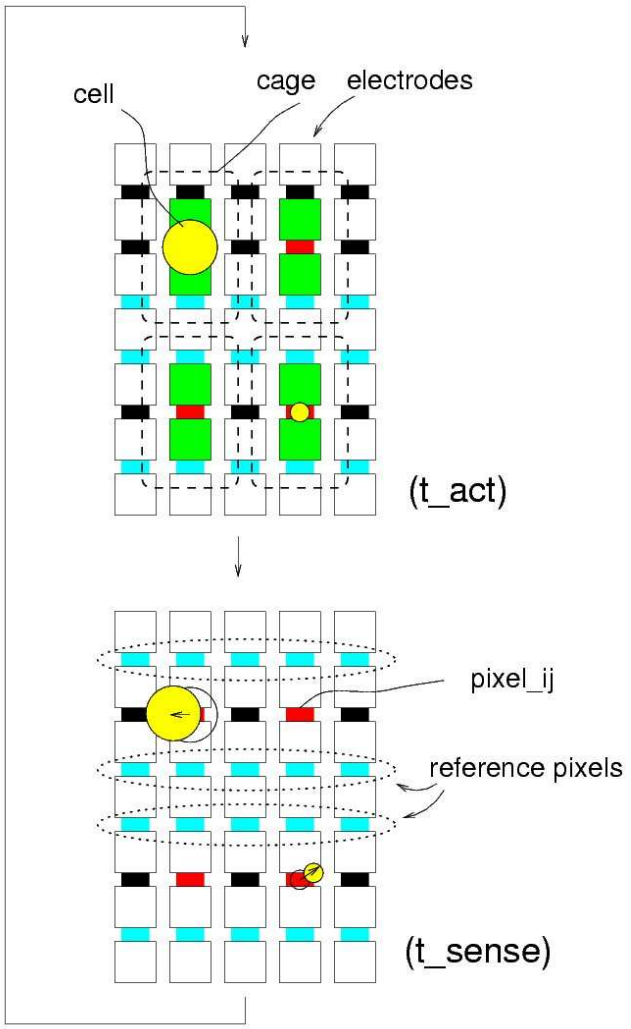

Fig. 3: During actuation cells are trapped in the cages 1x2 electrodes wide, and placed over the photodiodes. For each pixel i,j (red) corresponding to point of equilibrium for cells in cages, the grey value is recorded, along with grey value for reference pixels which are free from cells (light blue).

The classification threshold (yellow line) is obtained from the average of reference pixels' non-normalized standard deviations (magenta line) plus the standard deviation of non-normalized standard deviations of reference pixels themselves over k frames.

Empty/Full cages where verified by optical microscope inspection on an area 30x30 comprising 100 cages.

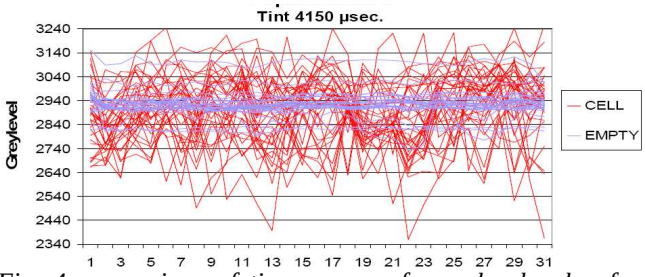

Fig. 4 comparison of time-course of grey-level value for different pixels corresponding to cages with cells (darker red lines) or empty cages (lighter blue lines) X-axis represents frame number.

Fig. 6 shows results of the classification error on a set of cages (same experiment of Fig. 5), going to 0 after about 115 frames (corresponding to about 2-4 minutes).

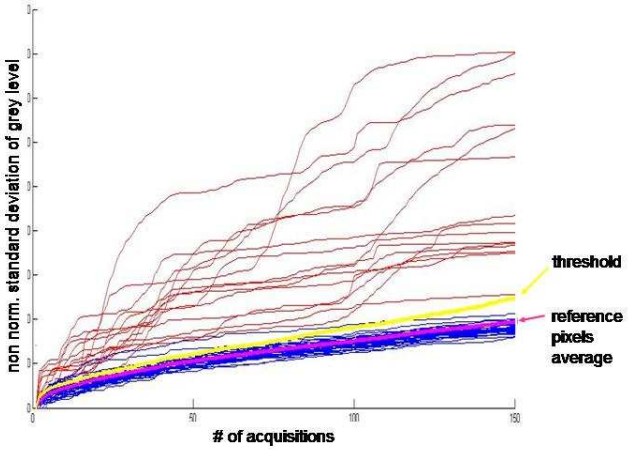

Fig. 5 evolution of the non-normalized standard deviation for each pixel on an increasing number of acquisitions k (reported as x-axis). Pixel corresponding to the center of a cage, either with a cell (red lines) or empty (blue lines), are classified against a threshold value computed from reference pixels average non-normalized standard deviation (magenta line) plus a given margin corresponding to the standard deviation of non-normalized standard deviations of reference pixels themselves over k frames.

5 DISCUSSION

It is noteworthy that this classification method does not depend on the baseline grey level of each photodiode, and is thus intrinsically insensitive to FPN.

Furthermore, it is independent on the actual optical effect caused by the cell. Regardless of whether cells or other particles –such as microbeads- to be detected, cause a darker pixel, a brighter pixel, or an unpredictable darker/brighter pixel, as is more often the case, this method is apt to detect their presence as it is not based on a segmentation based on grey level, but rather on grey-level noise-power.

The method is also robust with respect to optical-power spatial gradient since this has no impact on the noise power of pixels.

Although we did not experience a significant impact on classification performance from illumination noise, the method can be made intrinsically robust also to that kind of noise. In fact, illumination is applied to the whole electrode array. An average of reference pixels may thus be used to compute a frame-by-frame optical-power normalization coefficient. Normalizing the grey-level value of each cage pixel with this normalization coefficient, before computing the pixel noise-power, compensate for optical power variations.

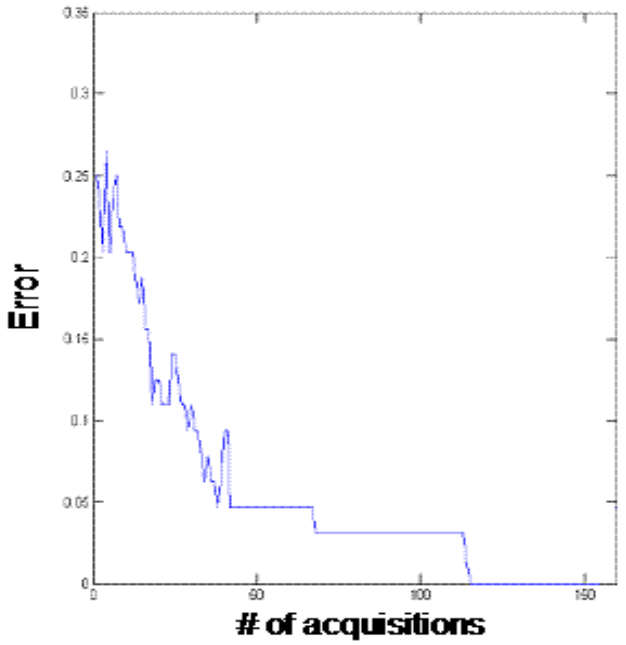

Fig. 6: evolution of the classification error as a function of the number of frames acquired. After about 115 frames classification error (full/empty cage) goes to zero.

6 CONCLUSIONS

We have shown that exploiting the differences in optical power noise of embedded optical sensor in a sensor/actuator array it is possible to obtain highly reliable classification of empty/full cages. On test runs with cells in positions verified by optical inspection, the system is able to classify correctly 100% of empty/full cages.

Information on empty/full cages is, in turn, at the base of several possible developments, including cell-counting, sorting etc, without the use of external microscopes or other optical add-ons.

Further than with optical sensors this approach may be adopted even with other sensing principles such as capacitive or impedentiometric sensing.

REFERENCES

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