A quartz nanopillar hemocytometer for high-yield separation and counting of CD4+ T lymphocytes

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We report the development of a novel quartz nanopillar (QNP) array cell separation system capable of selectively capturing and isolating a single cell population including primary CD4+ T lymphocytes from the whole pool of splenocytes. Integrated with a photolithographically patterned hemocytometer structure, the streptavidin (STR)-functionalized-QNP (STR-QNP) arrays allow for direct quantitation of captured cells using high content imaging. This technology exhibits an excellent separation yield (efficiency) of ~95.3 ± 1.1% for the CD4+ T lymphocytes from the mouse splenocyte suspensions and good linear response for quantitating captured CD4+ T-lymphoblasts, which is comparable to flow cytometry and outperforms any non-nanostructured surface capture techniques, i.e. cell panning. This nanopillar hemocytometer represents a simple, yet efficient cell capture and counting technology and may find immediate applications for diagnosis and immune monitoring in the point-of-care setting.

1. Introduction

Capture, separation and counting of rare cells is one of the most essential tasks required for studying biological characteristics of these cells and applying these cells to transplantation, anti-tumor cell therapy, etc.1–2 The nanometre-sized magnetic bead method (Dynabeads®, Invitrogen, USA) is widely used for capturing and isolating specific cells, i.e., T-lymphocytes and circulating tumor cells (CTCs). It utilizes capture-agent-coated magnetic beads to immunologically recognize the specific cells in the blood followed by passing the cell–bead complex through a magnetic field for cell isolation. However, this approach still needs further improvement in capture efficiency and is limited by the viability and functionality of the cells isolated.3–5 Other variants such as cell separation using magnetic nanowires (NWs) have been demonstrated.6–8 To address the need to overcome the exposure of separated cells to magnetic field and to develop cost-effective material-based system, we recently demonstrated a novel platform to separate CD4+ T lymphocytes using streptavidin (STR)-functionalized silicon nanowire (SiNW) arrays with a separation efficiency of greater than 93%.9 These great properties suggest that the silicon NW array is a promising platform for sensing biomaterials and for molecular bioelectronics, due to good biocompatibility of the silicon surface and ease of control over the density, diameter, and length of SiNWs. Wang et al. demonstrated that three-dimensional (3D)-nanostructured SiNWs on a substrate coated with antibody against epithelial cell adhesion molecule (anti-EpCAM) exhibited high capture efficiency when employed to isolate EpCAM positive rare tumor cells.9,10 Despite a significant improvement in the separation efficiency of SiNW-based capturing/isolating devices, a reliable and semi-automated platform for simultaneous capture and counting of primary cells is yet to be realized.

Herein, we propose to develop nanostructured quartz for quantification of captured cells via cost-effective methods that will provide valuable information about managing and early stage detection of cancer and human immunodeficiency virus (HIV) infected patients.11–16 The quartz nanopillar (QNP) array-based cell separation system we developed is capable of selectively capturing/isolating a single cell population, and counting isolated cells with a photolithographically patterned grid structure on the STR-functionalized-QNP (STR-QNP) arrays on one chip. In addition, we first demonstrate the ability to visualize and quantify the captured T-cells on a large-scale STR-QNP array. Our technology possesses a number of key merits as compared to those in previous reports, including our previous report on SiNW-based cell capturing/isolating devices.13–16 First, we succeeded in separating CD4+ T lymphocytes extracted from mouse spleen using transparent QNP arrays, and present a separation efficiency greater than ~95%. Second, we established a system capable of separating lymphocytes and simultaneously...
performing specific cell counting in a chip. Third, we further extend this platform to large-area cell capture and counting, making it a practical tool for studying rare cells. Fourth, this study demonstrates informative analysis of captured cells including cell size, shape, and distribution through high-content fluorescence imaging. Fifth, this research is not limited to separating CD4+ T lymphocytes; our proposed approach is also applicable to other lymphocytes such as B, NK, and NKT cells, as well as circulating tumor cells (CTCs). It may also enable in situ measurement of cytokine or chemokines secreted from these cells via spotting the cognate antibodies on the same QNP substrate.

2. Experimental details

2.1 QNP array preparation

Fig. 1a–h show a schematic diagram of the QNP fabrication process and mechanism of capturing CD4+ T lymphocytes on the STR-functionalized QNP arrays. A monolayer of polystyrene (PS) nanoparticles (NPs) was prepared using spin coating on a quartz substrate. In this method, the NP monolayer acts as an etching mask. The colloidal PS beads (about 100 nm in diameter) were purchased from Polysciences Inc. in the USA. The typical etching process consists of three steps: (1) size reduction of the PS NPs with CF4 plasma treatment, (2) nanostructuring with CF4 + H2 plasma etching, and (3) removal of the residual PS NPs using the piranha cleaning process (96% H2SO4 : 30% H2O2 ¼ 1 : 1). The length of the QNP arrays is controlled by the etching time. The diameter and length of synthesized QNP on a quartz substrate were found to be in the range of 30 to 80 nm and 200 to 1800 nm, respectively, which strongly depended on the plasma etching times.

2.2 CD4+ T-lymphocyte separation from mouse splenocytes using STR-QNP arrays

The QNP surface was treated with O2 plasma for 60 s to present the hydroxyl groups on the surface of the QNP arrays. The 3-aminopropyltriethoxysilane (APTES, Sigma-Aldrich, USA) was treated in ethanol for 30 min at room temperature to functionalize the QNP surface with amine groups. The amine groups of the QNP arrays were reacted with glutaraldehyde (GA, Sigma-Aldrich, USA) to confer an aldehyde group that sequentially reacted with streptavidin (STR, Sigma-Aldrich, USA). For the preparation of mouse splenocytes, C57BL/6 mice (6–8 weeks) were purchased from Nara Biotech Co. in Korea. The extracted spleens were passed through a nylon wool column to deplete B-lymphocytes, remaining CD4+ T, CD8+ T, NK, and NKT cells in mouse splenocytes. A certain quantity of cells (~10^6 cells per mL) was counted using a conventional hemocytometer (Hausser Scientific Co., USA). Prior to loading the cell suspension in the culture medium, the cell population (10^6 cells per plate) was first reacted with biotinylated anti-CD4 mAb (clone GK 1.5, eBioscience Inc. USA) and incubated at 4°C for 20 min. Non-specifically unbound cells with biotin-conjugated mAbs were washed out, while separated CD4+ T cells could bind to QNP surfaces due to adhesion enabled by the antigen–antibody interaction. Remnants of unbound CD4+ T-lymphocytes were then enumerated using a flow cytometer (Becton Dickinson, USA) after being washed several times in phosphate buffered saline (PBS) solution on a two-dimensional (2-D) rocker and analyzed using CellQuest™ Pro software (BD Bioscience, USA).

2.3 Large-scale STR-QNP arrays for cell counting devices

Cell-counting reservoirs with nine circular wells (5 mm in diameter) were made by molding a polydimethylsiloxane (PDMS) elastomer. A mixture of GE RTV 615 PDMS (GE Silicones, USA) prepolymer part A and part B (10 : 1) was prepared, homogenized, and applied to a blank Petri dish (55 mm in diameter). After degassing for 1 h, the PDMS mold was cured at about 80°C for 60 min. The solidified PDMS mold was cut off in the size of 25 × 25 mm² and the cell-counting reservoir holes (5 mm in diameter) were drilled with a stainless steel hole punch. For large-scale application of the STR-functionalized QNP arrays, CD4+ T-lymphoblast (human T-lymphoblast cell line, CCRF-CEM) was purchased from American Type Culture Collection (ATCC) in the USA. Prior to introducing the CD4+ T-lymphoblast cells into the PDMS wells, the CD4+ T-cells were premixed with biotinylated anti-human CD4-Ab (clone OKT4, eBioscience Inc., USA) and then stored at 4°C for 20 min. A solution of CD4+ T cells conjugated with biotinylated anti-human CD4-Ab (~40 mL in PBS) was pipetted into each eight wells. Cell counts were manually performed using a conventional hemocytometer (Hausser Scientific Co. USA) within 10% error. A series of counted and differently diluted cells in RPMI-1640 medium with a final volume of about 50 mL for each well was introduced into eight wells (1 × 1, 1 × 2, 1 × 3, 2 × 1, 2 × 2, 2 × 3, 3 × 1, and 3 × 2) with cell populations of approximately 500, 1000, 1500, 2000, 2500, 3000, 3500, and 4000 cells per mL, respectively. After incubation in a 37°C incubator for 40 min, the PDMS wells were washed out 1 × PBS with Tween-20 (PBST, KPL Inc., USA) for

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at least three times to remove unbound CD4+ T-lymphoblast cells. Subsequently, all of the immobilized T-lymphoblast cells were stained with a mixture of fluorescein isothiocyanate (FITC)-labeled CD3-Ab and allophycocyanin (APC)-labeled CD4-Ab (eBioscience Inc., USA) to identify the CD4+ T-cells with CD4+ and CD3+ expression. To fix the captured cells, 4% paraformaldehyde (PFA, Santa Cruz Biotechnology Inc., USA) in PBS was loaded into the wells for 15 min, followed by washing out in PBST. The stained STR-QNP arrays were finally given a threestep cleaning process: PBS, PBS in DI water (1 : 1), and DI water. Both CD3-FITC and CD4-APC stained cells on the STR-QNP arrays were then scanned using an Axon Genepix Scanner 4000B (Molecular Devices, USA) with 635 nm and 532 nm excitation wavelengths. The finest resolution of this microarray scanner is as low as about 5 μm. The scanned images of the captured CD4+ T cells were visualized using Genepix 6.0 software (Molecular Devices, USA). The visualized CD4+ T-cells images exported from the Genepix 6.0 were analyzed in order to quantify the population of the captured CD4+ T cells using CellProfiler/C212 (http://www.cellprofiler.org), cell image analysis software.26

3. Results and discussion

3.1 CD4+ T-lymphocyte separation from mouse whole splenocytes

We fabricated the QNP arrays using the combination of colloidal lithography and plasma etching.27 After preparing the QNP arrays, we employed 3-aminopropyltriethoxysilane (APTES) and glutaraldehyde (GA) sequentially to immobilize streptavidin (STR) onto the QNP surfaces. Then, a cell suspension containing CD4+ T-lymphocytes premixed with biotinylated anti-CD4 mouse antibodies (mAbs) was introduced onto the STR-functionalized QNP arrays prior to the cell separation process (see details in the Experimental section). As shown in Fig. 2a–c, captured CD4+ T lymphocytes, highlighted in red, firmly adhere onto the STR-functionalized QNP arrays via a biotin-STR binding since this conjugation between STR and biotinylated CD4 mAb has a high binding affinity (Kd = 10−15 M).3 We noticed that the bound CD4+ T lymphocytes on the surface were in the range of 3 to 5 μm in diameter as shown in Fig. 2 (upper panels). To further investigate the binding between separated cells and the STR-QNP array and to examine the interface between the cell and the substrate surface, gallium (Ga+) ion milling of the separated T-lymphocytes was performed using a focused ion beam (FIB, FEI Co., USA). As shown in the electron micrographs for the cross-sectional views in Fig. 2 (lower panels), we observed that the captured CD4+ T lymphocytes were tightly bound on the STR-QNP arrays with no air pockets or cracks at the interface.

Fig. 3a presents the fluorescence-activated cell sorting (FACS) results of CD4+ T lymphocyte separation using an STR-QNP array and compared to the results with a bare glass wafer (top and bottom left panels). According to the flow cytometric analysis shown in Fig. 3a (left panel), the percentages of the CD4+ T lymphocytes (CD4+/CD3+ double positive) and the non-CD4+ T lymphocytes (CD4/CD3 and CD3+) in the whole mouse splenocyte mixture used for the experiment were determined to be 37.2% and 62.8%, respectively. After introducing this cell suspension onto the STR-functionalized QNP arrays, we observed a significant decrease in the percentage of CD4+ T lymphocytes in the cell suspension to 1.5% (left bottom panel in Fig. 3a), indicating that the STR-QNP arrays completely separated the CD4+ T lymphocyte from the mixed cell population. The separation yield (efficiency), which is defined by the percentage of cells captured to cells initially loaded, for different cell separation platforms—STR-QNP arrays and the STR-functionalized glass wafer—were calculated using flow cytometric analysis. The average separation efficiency of CD4+ T lymphocytes captured on STR-QNP arrays from whole mouse

![Fig. 2](image-url) SEM images of CD4+ T lymphocytes bound on the STR-functionalized QNP arrays. (a) Tilted SEM image of CD4+ T lymphocytes with low magnification on STR-QNP arrays. (b and c) Side and top-view SEM images of region A in (a) with high magnification, respectively. (d–f) Cross-sectional SEM images of surface-bound CD4+ T lymphocytes on QNP arrays. (d) Low, and (e and f) high magnification image of separated cells of regions B and C in (d), respectively. The samples were prepared using a focused ion beam (Ga+ ion milling). All of the CD4+ T lymphocytes bound on the surface are highlighted in red for easy differentiation.
lymphocytes was determined to be \( \sim 95.3 \pm 1.1\% (n = 3) \), which is the highest value compared to other reports in the literature.\(^3\)\(^9\)\(^10\) The performance is even slightly better than that obtained with STR-SiNWs.\(^3\) For comparison, the bare glass wafer with no nanostructures gave rise to a cell separation efficiency of \( \sim 65.8 \pm 2.3\% \). The elevated cell separation efficiency of STR-QNP arrays compared to previous reports in the literature can be explained as follows. First, it was evident that nanostructures on the quartz substrate increased the contact area between cells and the substrate surface compared to flat glass wafer.\(^3\)\(^9\) As a result, QNP arrays can separate a larger number of loaded cells, thereby increasing cell separation efficiency. Second, the higher separation efficiency of the STR-QNP arrays compared to our prior results on STR-SiNW arrays is likely due to the relatively higher surface area per cell on the QNP arrays than on the STR-SiNW arrays. The QNP arrays possessed approximately 500 to 800 QNPs per cell as seen from Fig. 2c, while the STR-SiNW structure only had 3 to 4 SiNWs per cell. Consequently, we observed that the QNP array did capture more cells, yielding higher cell separation efficiency. Additionally, we verified that the O\(_2\) plasma treatment unquestionably assisted STR immobilization on the QNP arrays (see Fig. S1 in the ESI†).

To investigate the effect of the diameter-to-length ARs in STR-functionalized QNP arrays, four STR-QNP arrays with different ARs (1 : 1, 1 : 4, 1 : 5, and 1 : 10 diameter-to-length) were prepared using the same fabrication processes. Fig. 3b shows the capture efficiency of four different QNP arrays before and after capturing processes together with a summary of the results in the bar graph and table. As shown in Fig. 3b (bar graph and table in the right panel), the capture efficiency of STR-QNP arrays increased from \( \sim 84.3 \pm 4.8\% \) to \( \sim 95.3 \pm 1.1\% \) as increasing ARs from 1 : 1 to 1 : 4. By further increasing the AR to 1 : 10, the capture efficiency of the STR-QNP arrays then decreased to \( \sim 79.5 \pm 2.2\% \) for the STR-QNP with an AR of 1 : 10. We speculate that the enhancement of capture efficiency with increasing AR from 1 : 1 to 1 : 4 may be attributed to

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Fig. 3  Flow cytometric analysis of CD4\(^+\) T lymphocytes after cell separation. (a) Flow cytometric analysis of CD4\(^+\) T lymphocytes using cell suspensions after binding to STR-QNP arrays (bottom left panel) and glass wafer (top left panel). Average cell separation efficiency is shown using bar graphs (right panel). (b) Flow cytometric analysis of CD4\(^+\) T lymphocytes before and after cell separation for different diameter-to-length aspect ratios (ARs) from 1 : 1 to 1 : 10 (top and bottom left panels) with averaged separation efficiency shown in bar graph (top right) and table (bottom right).
increased interaction between nanopillars and the microvillus of CD4+ T cells.9,11 However, this should not help any more when the AR is sufficiently large and provides maximum contact area. As a result, we expect to see a plateau of capture efficiency with further increasing AR. But we observed a decrease of capture efficiency with increasing AR to 1 : 10. This could be due to the reduced nanopillar size caused by plasma thinning of nanosphere masks and consequently reduced contact area between pillars and cellular microvillus. Our observations are consistent with previous studies performed using various types of cells including platelets and fibrinogen.58–60

Furthermore, to identify that the cells separated using STR-QNP arrays were CD4+ T lymphocytes and to ensure dead cells, the captured cells were stained by phycoerythrin (PE)-conjugated anti-CD4 mAb, 4′-6-diamidino-2-phenylindole (DAPI), and propidium iodide (PI). The cells' images were then detected using fluorescence microscopy (Eclipse Ti-U, Japan). DAPI generally binds to all nuclei of the cells, while PI binds to only dead cells. Fig. 4 shows optical and fluorescence images of stained CD4+ T-lymphocytes with PE-labeled CD4 mAb (shown in red of Fig. 4c and d), DAPI (shown in blue of Fig. 4f and h), and PI (shown in red of Fig. 4g and h). As shown in Fig. 4c and d, we confirmed that most cells were CD4+ T lymphocytes from indication of PE-labeled CD4 mAb positive.

3.2 STR-QNP arrays as a miniaturized cell counting device (hemocytometer)

For potential use as a miniaturized cell counting device, we prepared STR-QNP arrays with integrated photolithography-patterned grids. Fig. 5 shows the images of STR-QNP arrays with a patterned grid structure (left panels) and the comparison of cell counts between FACS analysis and the QNP-based hemocytometer analysis (right panels). We measured the population of CD4+ T lymphocytes captured on the patterned hemocytometer on STR-QNP arrays using optical microscopy and compared it to the results from the flow cytometry analysis.7,18 To create the grid on the hemocytometer shown in Fig. 5a–d, we first used photolithography to define the microscale grids on the QNP array that define square patterns with a single square of 200 × 200 μm² over a total area of 3 × 3 mm², followed by a pattern transfer process using wet-chemical etching with buffered hydrofluoric acid (BHF). Then, we separated the CD4+ T lymphocytes and averaged the numbers of captured/isolated CD4+ T lymphocytes in all four-unit areas to achieve statistic consistency in a manner similar to a conventional hemocytometer (Hausser Scientific Co., USA). The formula for the calculation of the captured cells was (4 unit areas) × (1/4) × (1/unit volume) with a unit volume of ~1.87 × 10⁻⁴ cm³. The total population of captured/isolated CD4+ T lymphocytes using STR-QNP arrays was thus determined to be about 8.2 × 10⁵ ± 5.6 × 10⁴ cells per mL (n = 3). As shown in Fig. 5e, we confirm that the cell counts obtained from the STR-QNP-based hemocytometer are in close agreement with that determined by the flow cytometer analysis (CD4+ T lymphocytes ~9.4 × 10⁵ ± 6.9 × 10⁴ cells per mL, n = 3). From the hemocytometer experiments using the STR-functionalized QNP arrays, we found that our cost-effective and simply structured QNP array system has promising potential in that it eliminates the use of bulky and expensive equipments (such as flow cytometers) to quantify the cell population, and obviates the need for additional sample processing procedures (such as fluorescence probe staining).

3.3 Large-scale STR-QNP arrays as a cell counting device

To further assess the possibility of extending this platform to large-scale cell capturing and counting, STR-QNP arrays (25 × 25 mm², see Fig. S3a in the ESI†) were prepared. We selected human T-lymphoblast cancer cells (CCRF-CEM) as the target cells for detection and counting. FACS analysis indicated that

![Fig. 4](image-url) Optical and fluorescence image of CD4+ T lymphocytes bound on the surface of STR-QNP arrays wafer. (a and b) Low and high magnification optical images of CD4+ T lymphocytes on STR-QNP arrays. (c and d) Low and high magnification of fluorescence images of separated cells on the surface, where the separated cells were stained by PE-labeled CD4-mAb (red color) for identification of CD4+ T lymphocytes, indicating that all of the captured cells are undoubtedly CD4+ T lymphocytes. (e) Optical image of CD4+ T cells. (f and g) Fluorescence images of CD4+ T lymphocytes stained DAPI (blue color) and PI (red color). (h) Overlapped fluorescence image of DAPI and PI on optical image. No dead cells (red-colored cells in (g)) were observed from the fluorescence images of DAPI and PI.
these cells comprise both CD4+ CD3+ and CD4+ CD3- phenotypes while almost all cells express CD4, making it most suitable for studying CD4 T cell counting. For visualizing and counting the captured cells on the large-scale area of the STR-QNP arrays, we used an Axon Genepix Scanner 4000B. T cells were introduced to each polydimethylsiloxane (PDMS) well in the range of approximately 500 to 4000 cells per well. Fig. 6a and b (see also Fig. S3a–c in the ESI†) show scanned fluorescence images of immobilized CD4+ T-lymphoblast cells on large-scale STR-QNP arrays. Fig. 6c shows the quantitative analysis of these images using high content cell biology analysis software, CellProfilerTM.26 The size distribution in pixels of immobilized CD4+ T-lymphoblasts provided from the analysis using CellProfilerTM is shown in Fig. 6d. Using immunofluorescence staining with fluorescein isothiocyanate (FITC)-conjugated anti-CD3 Ab (green) and allophycocyanin (APC)-labeled CD4-Ab (red), we verified that captured CD4+ T-lymphoblasts are positive for CD4 and many express CD3 as shown in Fig. 6a. As shown in Fig. 6a and S4a–c (ESI†), the population and size of surface-bound CD4+ T-lymphoblasts on the STR-QNP arrays increase with increasing the quantity of cells loaded into the well. It was noted that with increasing cell number two or more CD4+ T-lymphoblast cells agglomerate to one another as shown in the 2 × 3, 3 × 1, and 3 × 2 wells in Fig. 6c. This leads to underestimation of cell counts and affects cell separation efficiency when the cells of interests are not so rare (>2000 per well). Fig. 6e shows the numbers of the T-lymphoblast cells loaded in the wells as compared to the cell counts measured using the STR-QNP-based hemocytometer. As shown in Fig. 6e, cell separation efficiency was found to be in the range of 55 to 75%. These lower nominal capture efficiencies observed in large-scale STR-functionalized QNP arrays could be due to several factors. The values computed using FACS analysis correspond to the percentage of CD4+ T cells removed by nanopillar arrays, but the imaging cytometry analyses count the CD4+ T cells that still remained on the substrate after several washing steps. With respect to human T cell capture experiments, although the cell line was identified as a CD4+ phenotype, but a small fraction of these cells express no or low levels of CD4 antigen. In addition, cell size, shape and adhesion force all vary among different cell samples and alter the interaction between cells and nanostructures to some extent, and thus affect the actual counts of cells remaining on the nanopillar substrate. Previous reports have also been pointed out that various types of cells represented different cellular responses when the topographic features of the surface were varied.9,28–31 With increasing cell loading ranging from ~500 to ~2500 cells, the cell count increased linearly until reaching 4000 cells and then remain nearly unchanged with further increasing cell loading. To automatically quantitate the captured cell population, the cells were analyzed according to size distribution shown in the data generated by the CellProfilerTM software. Our observation shows that captured CD4+ T-lymphoblasts were in the size range of 225–1806 mm2 and those greater than 2500 mm2 were believed to be cell agglomerates. Fig. 6f shows the distribution of captured cells greater than 10 pixels in each well. This indicates that loading of more than ~2500 cells to a well causes cells to agglomerate and decreases the cell counting accuracy. On the basis of these findings, algorithms can be used in the image analysis program to reduce this effect and extend the linear range as in Fig. 6e. Moreover, we demonstrated that the use of a high-content imaging approach allowed for more rapid32–34 and quantitative analysis of cell markers or signaling proteins using fluorescence signals from captured cells on the STR-QNP arrays. In addition, the captured specific cells can be easily visualized and quantitated in a semi-automated manner over a large-area providing a practical means for direct counting of rare cells.34 Immobilizing cells on the surface has a number of advantages including ease of monitoring over time and the possibility to
analyze cell morphology, migration and function. In this report, we describe the experiments to employ CD4+ T lymphocytes as target cells. However, its generic platform is not limited to the study of CD4+ T lymphocytes. First, the conjugation of biotin to antibodies against a variety of cell surface markers allows for the application to capturing and isolating many other types of cells. Second, this platform can be extended to the study of cellular functions such as the production of cytokines/chemokines, which are essential for the body's immune responses and homeostatic control in both health and disease. Many cytokines and chemokines have been identified as dysregulated in various diseases, and thus such a cell capture and functional analysis technology would have great value for clinical diagnosis and immune monitoring. Another promising application is the detection of CTCs. CTCs are the disseminating tumor cells circulating in the peripheral blood of patients and have been found to be an early biomarker to detect cancer metastasis. Also the quantification of CTCs in patient blood offers valuable information for monitoring the progression or therapeutic responses of cancer patients.

4. Conclusion

In summary, we have succeeded in developing a new technology platform for capturing and counting specific cells via a miniaturized quartz nanopillar array hemocytometer. We also presented the first demonstration of direct visualization and automated quantitation of captured T-cells over a large-scale chip, representing a practical tool for low-cost and high-throughput rare cell numeration. The STR-QNP arrays offer an

**Fig. 6** Microarray-scanned images of immobilized T-lymphoblast (CCRF-CEM) on large-scale STR-QNP arrays. (a) Low magnification and (b) enlarged images (white box region shown in the first row) of fluorescence-stained T-lymphoblast (CCRF-CEM), which was pre-mixed with biotin-conjugated anti-human CD4-Ab prior to the cell separation process, for eight wells (1 × 1, 1 × 2, 1 × 3, 2 × 1, 2 × 2, 2 × 3, 3 × 1, and 3 × 2) with FITC-labeled anti-human CD3-Ab (green color), thereby indentifying these cells as CD4+ T cells (double positive CD3+/CD4+). The original images taken from a microarray scanner were converted into a gray color, as shown in (b), for further analysis. The visualized T-lymphoblast images (shown in (a) and (b)) exported from Genepix software were analyzed to quantify the population of the captured CD4+ T cells using cell image analysis software, CellProfiler™ (ref. 26). (c) CellProfiler™ generated outlined images of separated T-lymphoblast cells on STR-QNP arrays for cell counting. The red-marked regions provided from CellProfiler™ in the 2 × 3, 3 × 1, and 3 × 2 wells were excluded from cell counting due to poor image quality. (d) Cell size distribution in pixels of immobilized T-lymphoblast cells on STR-QNP arrays for each well (5 μm in a pixel), showing that the cell size increased with increase in loaded cell population. (e) Comparison of cell population of immobilized T-lymphoblast (CCRF-CEM) cells on STR-QNP arrays at each well. The cell population was modified on the basis of the cell size distribution shown in (a). (f) Cell size and population distribution where the cell size is >10 pixels (1 pixel is as low as ~5 μm) at eight different wells. The cell size of immobilized T-lymphoblasts was analyzed using CellProfiler™ software.
excellent platform for the separation and isolation of a wide variety of cells such as regulatory T lymphocytes, circulating tumor cells or tumor stem cells. With further development, our system can monitor the function of captured single cells and detect the secretion of immune effector molecules such as cytokines and chemokines. Thus, the quartz nanopillar array hemocytometer may serve as a foundational platform to develop tools for studying the biology of rare cells from mixed populations or tissue samples.

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