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Optical Imaging Fiber-Based Single Live Cell Arrays: A High-Density Cell Assay Platform

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A high-density, ordered array containing thousands of microwells is fabricated on an optical imaging fiber. Each individually addressable microwell is used to accommodate a single living cell. A charged coupled device (CCD) detector is employed to monitor and spatially resolve the fluorescence signals obtained from each individual cell, allowing simultaneous monitoring of cellular responses of all the cells in the array using reporter genes (*lac*Z, EGFP, ECFP, DsRed) or fluorescent indicators. Yeast and bacteria cell arrays were fabricated and used to perform multiplexed cell assays with resolution at the single-cell level. Monitoring gene expression in single yeast cells carrying a two-hybrid system was used to detect in vivo protein−protein interactions. The single-cell array technology provides a new platform for monitoring the unique multiple responses of large populations of individual cells from different strains or cell lines. The rich data acquired by the cell array has the potential to be employed as a new tool for cell biology research as well as to improve cell-based high-throughput screening (HTS) applications, such as the validation of new disease-associated cellular targets and the early-stage evaluation of potential drug candidates.

Large-scale, cell-based assays are employed for studying cellular functions, such as metabolism or gene expression patterns in living cells. Cell-based assays are becoming an essential step in high-throughput screening (HTS)-based drug discovery processes.[1−6] Cell-based assays are employed for drug discovery in two ways: first, to identify new potential therapeutic targets; and second, to evaluate the therapeutic potential of new lead compounds (typically discovered by cell-free HTS) by examining their effects on living cells. The cell responses are resolved using several approaches, including monitoring transcription or translation events using reporter genes,[7] monitoring activation of membrane bound receptors by measuring intracellular ions (Ca^{2+}, H^+),[8,9] and monitoring cell viability.[10] Fluorescence techniques are usually used to monitor cell responses. These techniques are highly sensitive and allow measurements of different cellular events using reporter proteins or fluorescent dyes.[11]

The currently used vessels for high-throughput, cell-based assays are 96- and 384-well plates; recently, 1536-well plates assays were adapted for cell-based HTS assays.[12,13] Assay miniaturization may speed drug discovery processes and reduce costs by reducing the reagent volume used for each individual assay (∼1 μL in a 1536-well plate). One fundamental limitation of plate-based assays is that an average response of many cells (i.e., $\sim 10^3$ cells in a 1536-well plate) is measured. Although the average cell response provides valuable information about overall biological effects, such as the effect a drug candidate may have on living cells, it provides only partial information about the real cellular effects, since the response of each cell can vary depending on its physiological and genetic state (e.g., age, mutations). Furthermore, in well-based assays, each well can be used to measure only one type of activity and, therefore, is limited in the information it can provide about multiple cellular events. The need to maximize the information each assay can provide is also economically important in HTS processes in which only small quantities of lead compounds (i.e., drug candidates) are available because of their high production price and the small scale on which they are prepared.

One way to measure multiple cellular responses in single cells is to use multiprobe, fluorescence microscopy.[14] Recently, this assay technology was integrated into an automated microscopy system (ArrayScan), allowing analysis of multiple responses of cells in a well-based format.[11] Although there are other technologies for analysis of single or a few cells,[15−25] none of them allow

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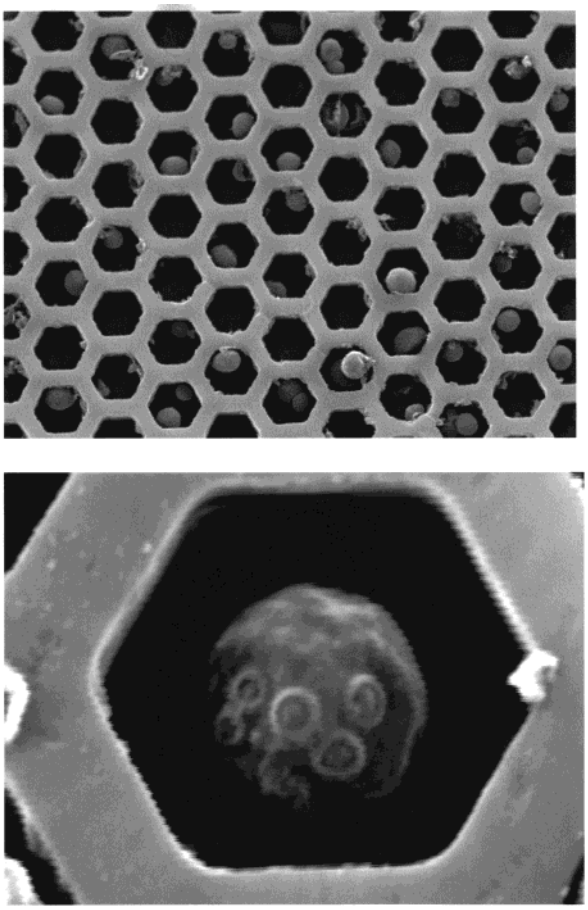

Figure 1. Loading cells into wells. Scanning electron micrograph (SEM) of (A) an array of yeast cells in microwells and (B) a single yeast cell in a microwell. Each microwell has a diameter of 6 μm.

the simultaneous and repetitive monitoring of multiple cellular responses of thousands of individual cells.

In this paper, we present a new technology for large-scale, single-cell multiresponse analysis. An ordered microwell array, wherein each microwell accommodates a single living cell, is used as a platform to simultaneously carry out thousands of single cell assays. A schematic representation of the cell array technology is shown in Figure 2. Previously, we demonstrated the feasibility of the microwell array to monitor physiological responses in single mouse fibroblast cells (NIH 3T3) by monitoring pH changes in each cell microenvironment.[26] Here, we describe the potential use of the microwell array for cell-based HTS applications using several strains of yeast (*Saccharomyces cerevisiae*) or bacteria (*Escherichia coli*). Our approach takes cell-based assays to the single cell level. One of the key advantages of this approach is that no registration of each individual cell position in the array is needed prior to beginning the experiment. The cells are encoded with a unique dye or genetic signature corresponding to their strain and then randomly dispersed into the microwell array. The different strains of cells are located by using a simple and rapid optical decoding scheme. The cells' genetic or physiological responses are measured simultaneously while retaining the ability to individually address each cell. Fluorescence detection is employed to monitor gene expression using the reporter genes *lac*Z, EGFP, ECFP, and DsRED. Alternatively, fluorescent indicators may be employed as previously described.[26] The simple fabrication and operation of the cell array allows for rapid, simultaneous and continuous optical analysis of a large number of individual cell assays.

EXPERIMENTAL SECTION

Strains, Media, and Plasmids. Standard methods were used for the growth and maintenance of yeast and bacteria. The yeast *S. cerevisiae* strains used for this study were AH109[pGBKT7-53 + pGADT7-T] and AH109[pGBKT7 + pGADT7], the positive and negative control strains of the yeast two-hybrid system (Clontech). Strains SS11[27] and YPH499[28] were provided by C. Freudenreich, Department of Biology, Tufts University, and xy222-1A (ATCC 26675) was purchased from ATCC. The *E. coli* strain used in this study was Epicurian Coli XL1-Blue (Stratagene). The plasmids pEGFP, pECFP, and pDsRed (Clontech) were used for the *E. coli* transformation.

Cell Array Fabrication. Etched imaging fibers (Illumina, San Diego, CA), containing 24 000 microwells each with a diameter of 5 μm (for yeast cell array fabrication) or 50 000 microwells each with a diameter of 2.5 μm (for bacteria cell array fabrication) were used. Microwell arrays were also prepared as previously described[26] using imaging fibers (Galileo ElectroOptics, Sturbridge, MA) containing ~8000 individual fibers with core diameter of 6 μm. The fiber's distal end (microwells side) was inserted into a 10-mm-long polyurethane tube (Small Parts, Miami Lakes, FL). Cell suspension (5 μL) was introduced into the tube, and the fiber was centrifuged horizontally at 4000 rpm for 15 s. The tube was removed, and the array was immediately inserted into cell media. During the cell array fabrication and use, care was taken to avoid evaporation of the media from the microwells by keeping the distal end of the fiber covered with medium.

Bacteria Cell Genetic Encoding. *E. coli* XL1-Blue competent cells (Stratagene) were transformed according to the Stratagene instructions manual. The transformed cells were grown in LB medium (GibcoBRL) supplemented with 0.1 mg/mL ampicillin (Fisher). The cells were washed twice in saline before use.

Yeast Cell Encoding. Yeast cell cultures were grown in glucose (2%)-supplemented YPD medium (Difco) for 48 h. Cultures (500 μL) containing ~10^8 cell/mL were washed twice in 2% glucose in 10 mM Na−HEPES (Sigma), pH 7.4 (glucose−HEPES). Then the cells were resuspended in 500-μL aliquots of 250 μg/mL Concanavalin A (ConA)−(Alexa Fluor 350, Alexa Fluor 660, Tetramethylrhodamine, Fluorescein, Texas Red) conjugate (Molecular Probes, Eugene, OR) solutions made in glucose−HEPES and agitated for 1 h at room temperature. The cells were washed three times in glucose−HEPES before use.

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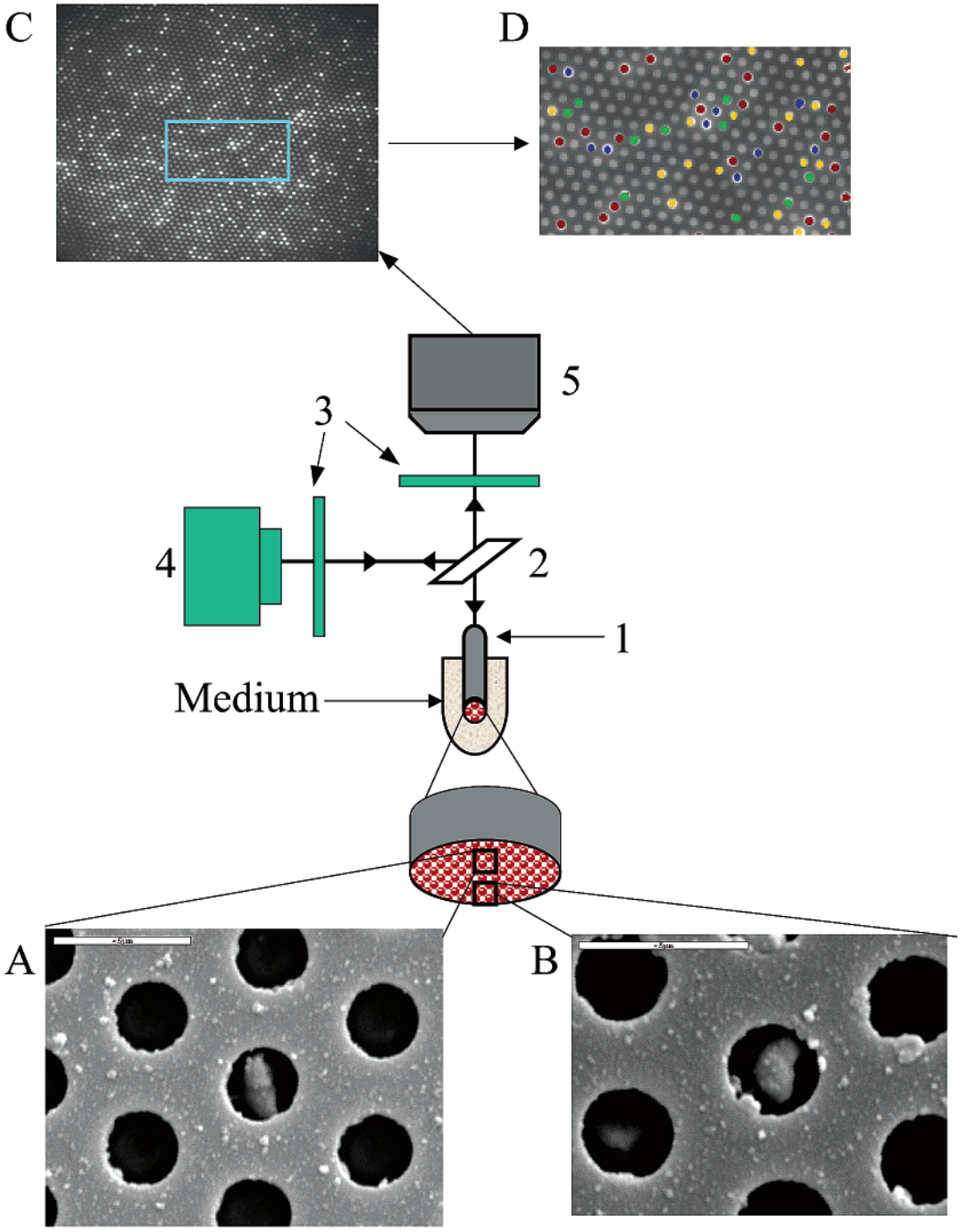

Figure 2. Schematic diagram of imaging fiber-based single cell array. Cells are distributed into the microwell array on the distal end of an etched imaging fiber (1). (A,B) SEM images of single *E. coli* cells inside 2.5-μm wells. The fluorescence signals were obtained using a light source (4), filters (3), dichroic mirror (2), and CCD camera (5); (C) typical fluorescence image of the emission signals obtained when the cell array is excited at the appropriate wavelength; and (D) a portion of the fluorescent image after data analysis (decoding).

Instrumentation Setup. Fluorescence images were acquired using a Zeiss epifluorescence microscope equipped with a mercury lamp connected to a CCD camera, model TEA/CCD-512 (Roper Scientific, Trenton, NJ), both controlled by IPLab software (Scanalytics, Fairfax, VA). IPLab software was also used for the data analysis. Omega band-pass filters and dichroic mirrors were used for fluorescence measurements (Omega Optical, Brattleboro, VT).

Cell Array Decoding. Fluorescence emission signals were acquired for 1 or 2 s using the appropriate excitation wavelength corresponding to the dyes or fluorescent proteins used to encode the cells. For each image, overlay segments (a different color for each image) were drawn. All of the segments were copied to a single image (Figures 3F and 6A) and overlapping segments (usually indicating the presence of more than one cell in a single microwell) were marked in black and were not analyzed.

Yeast Two-Hybrid System Experimental Protocol. The two-hybrid positive and negative strains and the xy222-1A wild-type strain (not containing two-hybrid plasmids) were grown in glucose (2%)-supplemented YPD medium or, for the positive control strain

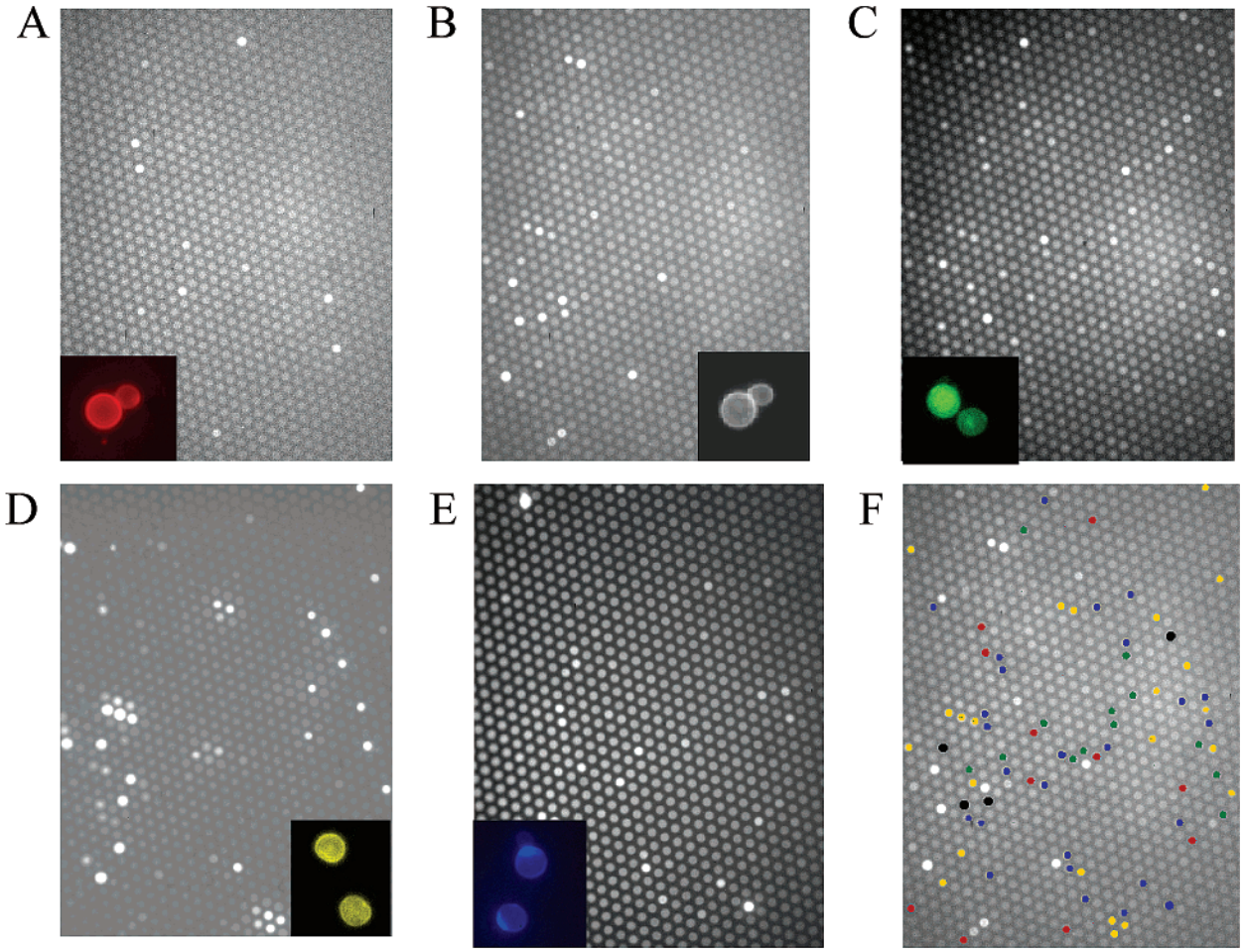

Figure 3. Encoding yeast cells with fluorescent dyes. Decoded yeast cell array containing five different strains, each encoded with a unique ConA−fluorescent dye conjugate. The decoding fluorescence emission images were used to identify the location of (A) ConA−Texas Red-encoded AH109[pGBKT7 + pGADT7] cells with image taken at 630 nm using 590-nm excitation light, (B) ConA−Tetramethylrhodamine-encoded YPH499 cells with image taken at 580 nm using 540-nm excitation light, (C) ConA−Alexa Fluor 660-encoded xy222-1A cells with image taken at 680 nm using 650-nm excitation light, (D) ConA−Fluorescein-encoded SS11 cells with image taken at 530 nm using 490-nm excitation light, and (E) ConA−Alexa Fluor 350-encoded AH109[pGBKT7-53 + pGADT7-T] cells with image taken at 440 nm using 360-nm excitation light. All images were acquired with a 1-s CCD acquisition time. Inserts A−E show fluorescence images and pseudocolored images of encoded cells with colors corresponding to their decoding color. The decoded image (F) is obtained by copying segments from all the images to image (B). Black segments indicate overlapping segments (two cells in one microwell).

experiment (Figure 5), SGd/-Leu/-Trp medium (Clontech) for 12 h. The strains were decoded: the positive strain with ConA-Texas Red, negative strain with ConA-Alexa Fluor 660, and wild-type strain with ConA-Alexa Fluor 350. The yeast array was fabricated and decoded. Then the distal face of the array was placed in 5 mM 5-dodecanoylaminofluorescein di-β-D-galactopyranoside (C_{12}FDG) (Molecular Probes) solution made in glucose−HEPES or SGd/-Leu/-Trp medium. For the measurements, the array was placed in medium without substrate. Images were acquired with 2 s exposure time at 530 nm using 490-nm excitation light. After the first measurement, the array was again placed in the substrate solution for subsequent measurements.

Scanning Electron Micrographs (SEM). The cells in the array were fixed with 2.5% glutaraldehyde (Sigma) followed by 1% osmium tetroxide (Fisher), dehydrated in ethanol using a gradual series of ethanol/water solutions (70/30, 80/20, 90/10, and 100%; 15−20 min each), and critical-point-dried with liquid CO_2 (Tousimis Samadri 790, Rockville, MD). The cell arrays were gold-coated and examined using a scanning electron microscope (JEOL SM 840, Peabody, MA).

RESULTS AND DISCUSSION

Yeast And Bacteria Cell Array Fabrication. Optical imaging fibers are composed of thousands (e.g., 3000−100 000) of identical individual fibers coherently bundled together. The ordered microwell array is fabricated on the distal end of an optical imaging fiber using a wet-chemical etching process developed in our laboratory.[29] The microwells are formed by the different etching rates of the individual fiber cores (pure silica) and clad (germania-doped silica) materials. Since the etching rate is higher for the core material, an array of identical microwells is created with diameters corresponding to the individual fiber cores. By varying the fiber structure (i.e., the individual fiber's core diameter) and etching time, the diameter and depth of the microwells can be controlled and tailored to accommodate different types and sizes of cells. The cells in the microwells are individually addressed on the basis of the ability of each individual fiber to carry its own light signal from one end of the fiber to the other, allowing imaging fiber bundles to analyze individual cells in the array.

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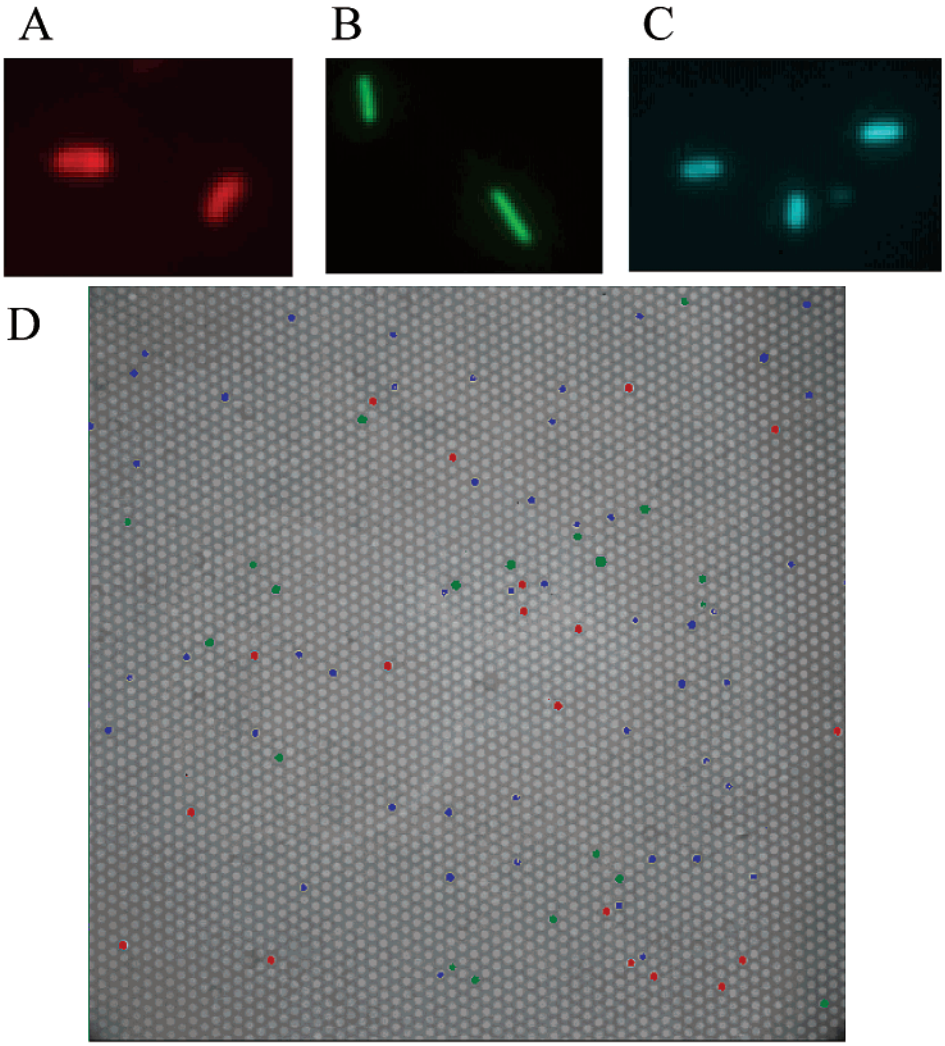

Figure 4. Genetic encoding of bacteria cells with fluorescent proteins. Bacterial array containing three different genetically encoded *E. coli* cells. (A) *E. coli* (pDsRed) expressing the red fluorescent protein, emission images taken at 575 nm using 540-nm excitation light; (B) *E. coli* (pEGFP) expressing the green fluorescent protein, emission images taken at 530 nm using 490-nm excitation light; and (C) *E. coli* (pECFP) expressing the cyan fluorescent protein, emission images taken at 480 nm using 440-nm excitation light. Fluorescence images were pseudocolored using IPLab software. The three transformants were distributed into the microwell array, and the decoded image (D) was obtained by imaging the cell array as described in Figure 3 using the different fluorescent proteins' emission and excitation wavelengths.

Previously, we used a 7000-fiber array containing 7-μm microwells to accommodate NIH 3T3 mouse fibroblast cells.[26] In the present work, fiber bundles containing 24 000 individual 5-μm or 8000 individual 6-μm microwells are used to fabricate yeast cell arrays (Figure 1A), and fiber bundles containing 50 000 individual 2.5-μm microwells are used to fabricate bacterial cell arrays (Figure 2A,B). The number of microwells in each array is limited by the imaging fiber diameter but represents a packing density of 10^7 microwells/cm^2. In principle, this density would allow one to carry out approximately one-half million individual cell assays in an area equivalent to a single well from a 96-well microtiter plate.

The yeast cell array is prepared by randomly dispersing the cells on the tip of the optical fiber containing thousands of microwells with diameters slightly larger than the cells (5−6 μm). No surface modification is needed in order to place and maintain yeast or fibroblast cells[26] in the microwells, which considerably simplifies the preparation of the cell arrays and enables the measurement of the natural cell responses without interference caused by immobilization procedures. To put the yeast cells into the microwells, 5 μL of a cell suspension containing ~10^4 cells was placed into a short plastic tube connected to the distal end of the imaging fiber. The fiber was centrifuged horizontally and the cells were forced into the microwells. The number of the occupied wells can be controlled by varying the number of cells placed in the tube. Using this method, cell arrays were fabricated, in which up to 80% of the wells were occupied by cells. In these arrays, ~95% of the occupied wells contain single cells. In Figure 1, a scanning electron micrograph (SEM) of a small area of a yeast cell array demonstrates that most of the occupied microwells contain single yeast cells (Figure 1A) and, at higher magnification, a single yeast cell in an individual microwell is visualized (Figure 1B). Single cells are located inside the wells, and each cell is isolated in its own microwell; therefore, multiple responses of thousands of individual cells can be monitored with the optical fiber.

For fabricating *E. coli* arrays, the microwell surface was modified with polyethylenimine (PEI) to allow better adhesion of

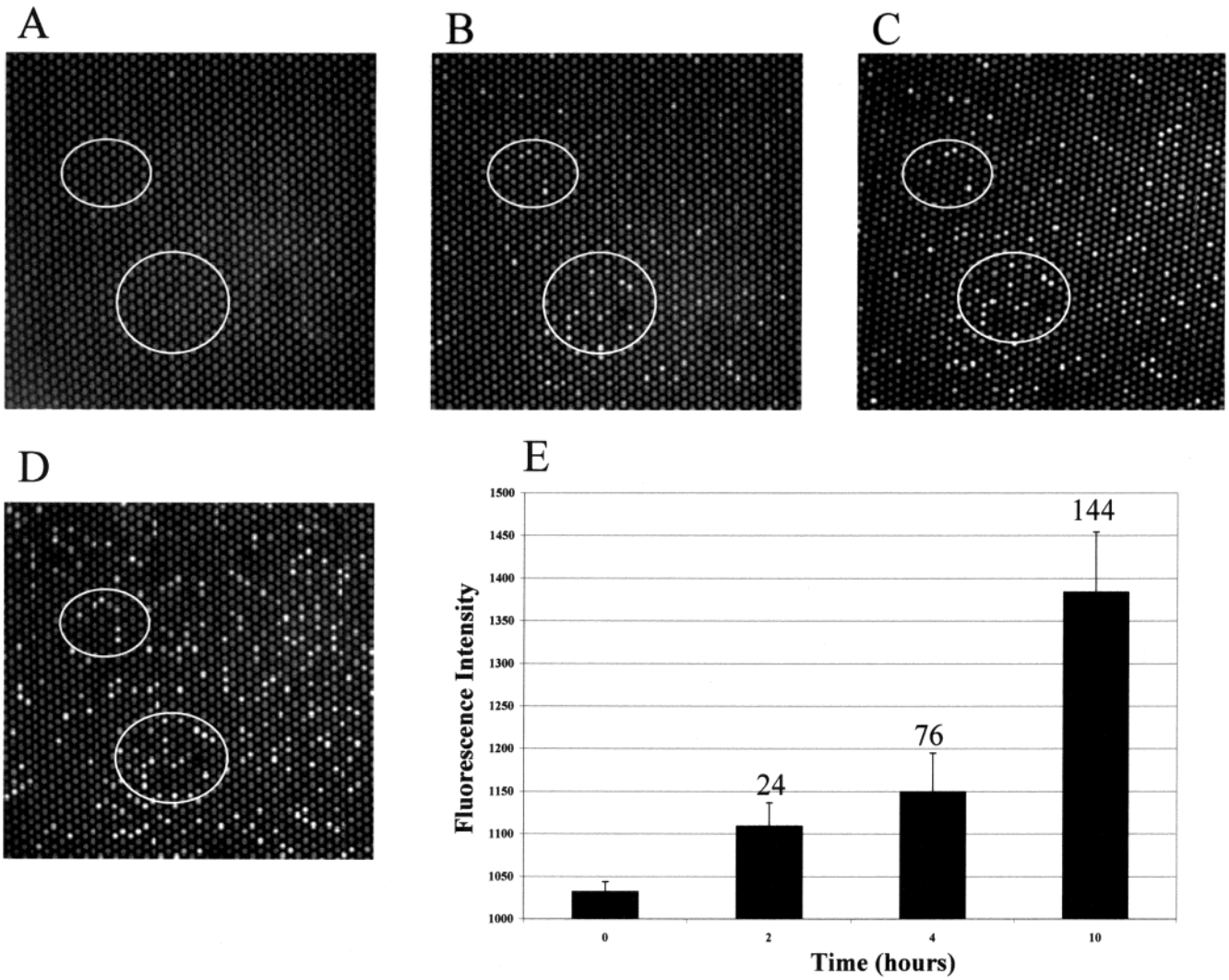

Figure 5. Measuring a single yeast cell's gene expression. Detecting protein−protein interactions by measuring the *lac*Z reporter gene expression in a yeast strain carrying the positive two-hybrid system control plasmids. (A) The cells were distributed into the microwells, and images were taken before the array was incubated with β-galactosidase substrate C_{12}FDG and (B) after 2, (C) 4, and (D) 10 hours. The circular "region of interest" marks unique patterns of cell location and shows the increase in fluorescence signal over time and the increase in the number of cells producing a detectable signal. (E) The average cell array fluorescence signal and the number of cells (shown at top of each bar) in the entire array responding above the background fluorescence signal (obtained before the substrate was added).

the cells to the microwells through electrostatic interactions. This modification is needed, because the *E. coli* rod shape is not perfectly matched to the shape of the microwell and its interaction energy is reduced. The cells are distributed into the 2.5-μm microwell array using the same procedure used to dispense the yeast cells into the array. In these arrays, because of the size disparity between the cells and wells, more wells contained multiple cells. Therefore, to increase the chance of getting single cells in the wells, less dense arrays (~50% occupancy) were fabricated. By using imaging fibers specifically designed to match the bacterial cells size, it should be possible to fabricate much denser single cell arrays. A SEM of *E. coli* cells inside the wells indicates that the bacterial cells are situated inside the wells in different positions, either "horizontally" (Figure 2A) or "vertically" (Figure 2B); in both cases, the cells inside the wells are isolated and can be individually addressed.

Cell Array Registration and Analysis. The overall principle of the cell array operation and analysis is shown in Figure 2. After the cells are dispensed into the microwells on the distal end of the imaging fiber, an epifluorescence microscope coupled to a CCD detector is used to interrogate each cell's fluorescent signals. Light at a specified wavelength is introduced into the proximal end of the fiber array and is conducted down all the fibers and excites fluorescent indicators attached to or localized within the cells. The fluorescent signals emitted from the cells at the imaging fiber's distal end are transmitted back through the individual fibers to the imaging fiber's proximal end where they are filtered and then captured by a CCD camera. This configuration allows the manipulation of the cell's environment (i.e., exposure to lead compounds, substrates, inhibitors, or dyes) at the distal end while continuously monitoring the individual cell responses at the fiber's proximal end. The raw data obtained from these measurements (Figure 2C) are analyzed using commercially available, imaging analysis software (Figure 2D). The different spectral signals are defined and automatically calculated.

Unlike a typical well plate assay, in which each well is indexed and registered prior to the experiment, we randomly distribute the cells into the array and use an optical encoding scheme to

later identify their location within the array. Two new approaches for the optical encoding of cells were developed. In the first approach, each cell is encoded with a unique signature dye. Spectrally resolved concanavalin A (ConA)−fluorescent dye conjugates were used to encode different yeast strains. The strong interaction between the lectin ConA and the mannoproteins on the yeast cell wall[30] enables a very efficient labeling of yeast cells, as shown in Figure 3A−E inserts. After the encoded cells are distributed into the microwells, a decoding procedure is employed. The array is exposed to sequential excitation light at several wavelengths, matched to the encoding dye's optical characteristics, and the emitted light signals from the labeled strains are detected and used to locate the positions of the different strains within the array. The encoding/decoding scheme is demonstrated in Figure 3A−E, which shows a yeast array consisting of five different yeast strains, each encoded with a different ConA−fluorescent dye conjugate. Figure 3F shows the decoded array. This encoding scheme can be expanded by employing other encoding dyes or by using combinations of encoding dyes. This encoding method was previously employed for bead-based arrays in which over 100 bead types were produced, each with a unique optical signature.[31] Other cell types can be encoded using a similar methodology with different commercially available lectin−fluorescent dye conjugates.

In the second encoding approach, genetic signatures were used to encode the cells. This approach is based on genetically engineering cells to express reporter genes coding for optically detectable proteins (e.g., intrinsically fluorescent or luminescent proteins or enzymes that cleave fluorogenic substrates). We demonstrate this approach using three plasmids, each coding for fluorescent proteins with different excitation and emission wavelengths (red, green, and cyan), to transform *E. coli* cells such that each cell constitutively expresses its own unique fluorescent protein. The three transformants were randomly distributed into the microwells, and the imaging process scheme described above was used to identify the location of the different transformants in the array (Figure 4). The genetic encoding scheme can be expanded to provide additional unique optical signatures by using other fluorescent proteins with (a) different spectral properties, such as different excitation or emission wavelengths or different quantum yields and (b) different biochemical properties, such as lower stability.[32] Alternatively, vectors can be designed that enable the coexpression of two or more fluorescent proteins, wherein the resulting combined fluorescence signal can be used as the cell's genetic signature. This genetic encoding scheme can be easily adapted to many types of cells, since compatible vectors for expression of fluorescent proteins in bacteria, fungi, and mammalian cells are commercially available.

The main advantage of both encoding schemes is that the identity of each cell is not determined by its position in the array but by its optical signature. Thus, if a microwell contains more than one cell, the fluorescence signal obtained will not match the decoding scheme and, therefore, will be ignored, as seen in Figure 3F. Moreover, this approach is fault-tolerant because it eliminates registration or pipetting mistakes, which occur in typical well-plate assays.

Once the array has been decoded, it is ready for use. Cell responses are measured by using fluorescent indicators to detect cellular responses, such as changes in intracellular or extracellular ion concentrations. Alternatively, cells can be transformed with a promoter−reporter system in which the promoter activity is monitored using a reporter gene different from the one used for encoding. The advantage of genetic encoding is that it can be used as an internal marker for the transcriptional and translational processes in each cell and, therefore, provides a better way to measure, evaluate, and understand gene expression profiles in living cells.

Yeast Cell Arrays For Monitoring Cellular Responses and Gene Expression. Several yeast-based assays were recently developed for HTS applications,[2,33,34] because yeast cells can provide an inexpensive and easy alternative to mammalian cells. Yeast cells are simple to grow, maintain, and assay and are readily genetically modified. In many cases, the yeast screening assays involve the use of reporter genes, such as the bacterial *lac*Z gene coding for the enzyme β-galactosidase.[35] One of the most valuable yeast systems in which this reporter gene is used is the yeast two-hybrid system. The system was originally designed to explore in vivo protein−protein interactions by genetically engineering the cells to activate transcription in response to such interactions, resulting in the expression of the *lac*Z reporter gene.[36] Since then, several variations of the two-hybrid system have been proposed for detecting DNA−protein or RNA−protein interactions.[37,38−39]

Yeast two-hybrid systems still suffer from several limitations, preventing their extensive use for HTS. Traditionally, screening for cells carrying interacting proteins (positive response) is based on isolating the blue colonies growing on X-gal agar plates. This screening technique makes the assay complex, time-consuming, and long (it typically take a few days to obtain the screening results). Furthermore, the blue color of the colonies allows only semiquantitative analysis and, although several 96-well plate assays have been proposed,[40] they are not suitable for live cell analysis, since lysis of the cells is required.

Here, we demonstrate the suitability of the fiber-optic based, single cell array for yeast two-hybrid system applications. First, we examined if individual yeast cells could express the *lac*Z reporter gene while in the microwells. The two-hybrid system positive control strain cells, which express the p53 and SV40 large T-antigen interacting proteins,[41] were distributed into the microwells, and the single cell *lac*Z gene expression was measured two (Figure 5B), four (Figure 5C) and 10 hours (Figure 5D) after incubation with the β-galactosidase substrate, C_{12}FDG.[42] Fluorescence signals from a few individual cells were detected after 2 h

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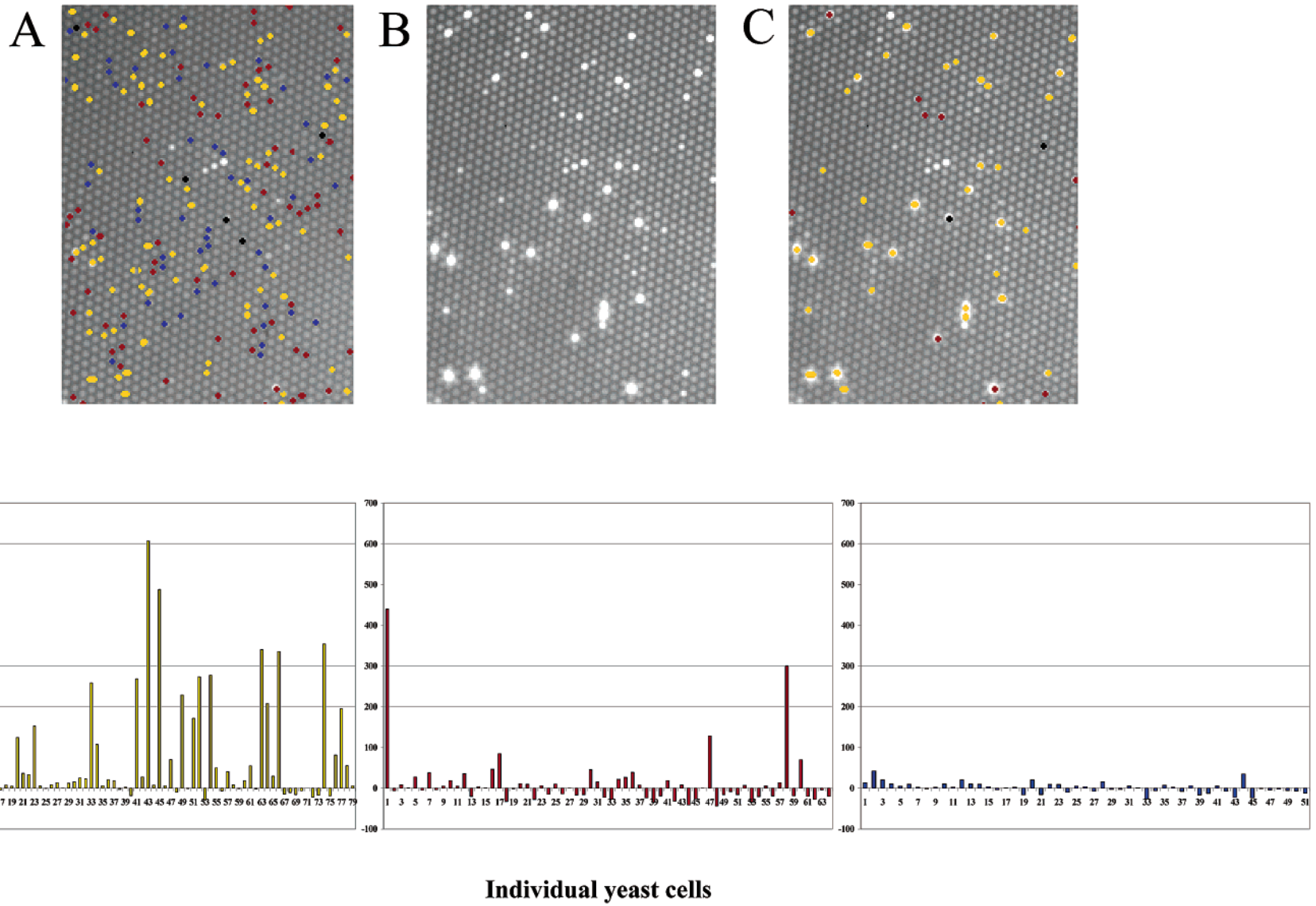

Figure 6. Multiplexed gene expression measurement from different yeast two-hybrid strains cells. Three strains, wild-type strain (xy222-1A), positive two-hybrid control strain, and negative two-hybrid control strain, were encoded with ConA−Alexa Fluor 350, ConA−Texas Red, and ConA−Alexa Fluor 660, respectively. (A) The decoded signals are overlaid on the fluorescence image of the yeast array before it was incubated with the substrate. The distribution of wild-type (blue), positive (yellow), and negative (red) control strains are shown. Black dots mark a microwell with dual cell occupancy. The bright signals in the center were obtained from the fiber-optic intrinsic fluorescence and were later used as a marker for the array area being analyzed. (B) Fluorescence emission image, at 530 nm using 490-nm excitation light, 4 h after the yeast array was incubated with C_{12}FDG. (C) The brightest signals were matched with their decoding signals, indicating that gene expression accrued mostly in the positive strain. (D) Individual cells' fluorescence signals after 4 h incubation with C_{12}FDG: yellow bars, positive strain; red bars, negative strains; blue bars, wild type strain. The fluorescence signal was calculated by subtracting the background signals obtained before incubation with C_{12}FDG (average signals: positive cells, 959 ± 32; negative cells, 966 ± 33; wild-type cells, 946 ± 15) from the fluorescence signal obtained after 4 h of incubation.

(Figure 5B) but enhanced signals from many more cells were detected after 4 and 10 hours (Figure 5C,D). The results show that the cells are alive and can be monitored continuously while expressing genes inside the microwells.

To demonstrate the multiplexing capabilities of the cell array platform, a *lac*Z expression in single yeast cells from three different strains, located in the same array, was measured. The positive strain (interacting proteins), the negative strain (expressing the GAL4 DNA-binding and GAL4 activation domains without any fused interacting proteins), and the wild-type strain (not containing two-hybrid plasmids), were uniquely encoded with three different ConA−dye conjugates and were randomly distributed into the array (Figure 6). After decoding (Figure 6A), the cell array was incubated for 4 h with the β-galactosidase substrate C_{12}FDG, and the fluorescence signals were measured (Figure 6B). In most cases, the location of cells expressing a high level of β-galactosidase matched the decoding signals of the positive control strain (yellow). In a few cases, a high level of β-galactosidase matched the decoding signal of negative control strain cells (red) but no expression was obtained from wild-type strain cells (Figure 6C). Signals from the individual cells were analyzed using imaging software (Figure 6D) showing that 33% of the positive strain cells generate an increase in signals higher than 100 units, compared to less than 5% of the negative control strain cells and with no such signals from wild-type strain cells (Figure 6D). The different expression levels obtained from the individual positive and negative strain cells demonstrate the high level of diversity of individual cells within the cell population. It should also be noted that some of the cells could not be decoded (Figure 6A) because of encoding signals that did not match the expected values. With the large number of cells in this array, these cells can simply be ignored.

The live yeast cell array's fast, sensitive, and accurate results can provide a platform for real-time measurement of responses from cells carrying two-hybrid systems. For example, "reverse two-hybrid" strains[43] carrying known interacting proteins can be used to screen for small molecules that disrupt therapeutically important protein−protein interactions.[34,44,45] The yeast cell array platform can accommodate different reverse two-hybrid strains (each carrying different known interacting proteins) and can be used for HTS of small molecules against multiple targets. In a similar way, a mammalian cell array could be used for applications such as absorption, diffusion, metabolism, excretion (ADME) and toxicity HTS tests by accommodating several different cell lines, each with a promoter−reporter fusion designed to indicate if the tested drug candidate can efficiently affect living cells.[46]

Although in HTS microtiter-based assays the information about the overall cell responses can be obtained in a shorter time because of aggregate signal responses, the cell array platform can provide much more detailed information, since effects at the single cell level are detected. Moreover, the imaging fiber bundles carrying the cell arrays can be ordered to match 96 or 384 separate cell arrays to the wells of a matched microtiter plate. This configuration would facilitate the testing of different potential drugs on different cell strains simultaneously and should enable increased throughput. Significantly more detailed information about different cellular processes would be obtained from each microtiter plate well by incorporating separate indicators to measure multiple parameters within each cell.

As was shown in Figures 3 and 4, by using multiple excitation and emission filters, five different encoding dyes (Figure 3) or three different fluorescence proteins (Figure 4) were measured simultaneously on the same array. In addition, as was shown in Figure 6, both the encoding dye fluorescence and the *lac*Z expression were measured from each individual cell. On the basis of these results, we can estimate that using suitable indicators, four or five different parameters can be measured for each individual cell in the array. Although multiple fluorescence responses from individual cells can be measured using systems such as fluorescence activated cell sorters (FACS), they are limited in the number of different fluorescence signals they can measure as a result of the limited number of laser lines that can be used simultaneously.

Another important advantage of the cell array approach is that unlike FACS, which measures signals from random cells during their very short transit times through the optical interrogation zone, the cell array platform can provide continuous analysis of the same individual cells and can provide valuable information about the kinetics of cellular responses in each individual cell.

CONCLUSIONS

The fiber-optic-based, cell array technology described here is highly versatile and is well-suited for both cell biology research and biotechnology cell-based HTS applications. The key advantage of this system is that multiple responses from a large population of individual cells from different strains or cell lines can be repeatedly analyzed simultaneously. This assay platform provides a means to perform cell assays with thousands of replicates, thereby reducing spurious false positive and false negative results as well as providing reliable and accurate results. This unique technology could give new insight toward understanding cellular mechanisms and could significantly contribute to improving drug discovery processes.

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