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# Electrostatic readout of DNA microarrays with charged microspheres

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**DNA microarrays are widely used for gene-expression profiling, single-nucleotide polymorphism (SNP) detection, disease diagnosis, and pathogen detection<sup>1-3</sup>. A persistent challenge in this area is the lack of microarray screening technology positioned for integration into routine clinical care<sup>4,5</sup>. Herein, we describe a sensitive and label-free electrostatic readout of DNA or RNA hybridization in a microarray format. The electrostatic properties of the microarray are measured from the positions and motions of charged microspheres randomly dispersed over the surface. We demonstrate non-destructive electrostatic imaging with 10- $\mu$ m lateral resolution over centimeter length-scales, which is four orders of magnitude larger than that practically achievable with conventional scanning electric force microscopy. Changes in surface charge density as a result of specific DNA hybridization can be detected and quantified with 50 pM sensitivity, single base-pair mismatch selectivity, and in the presence of a complex background. Comparison with fluorescence readout under identical conditions indicates that electrostatic detection provides comparable sensitivity. Moreover, no more than the naked eye is needed to readout the microarray, potentially enabling the broad application of multiplexed genome-scale assays.**

Effective medical care is often limited by the failure to diagnose diseases in resource-limited settings. For example, tuberculosis kills 2 million people each year, and according to the 2006 Global Health Diagnostics Forum, 400,000 lives could be saved if a rapid and accurate diagnostic requiring minimal instrumentation were available<sup>6</sup>. DNA microarray-based assays promise rapid on-site identification of multiple pathogens, which is particularly important for proper treatment of patients afflicted by multiple diseases or drug-resistant strains of diseases<sup>3,7</sup>. However, microarray assays typically rely on fluorescence detection, which requires time-consuming chemical labeling, reverse transcription, high-power excitation sources, and sophisticated instrumentation for scanning. Consequently, microarray assays tend to be performed by a few dedicated centers rather than individual labs, and especially not by clinics in developing countries<sup>2,8</sup>. Many label-free DNA detection techniques such as surface plasmon resonance<sup>9,10</sup>, electrochemical sensing<sup>11,12</sup>, fluorescent polymers<sup>13</sup>, atomic force microscopy<sup>14,15</sup>, microcantilevers<sup>16</sup>, and electronic depletion of a field effect transistor (FET)<sup>17,18</sup> have been introduced in efforts to circumvent some of the problems inherent to chemical labeling<sup>19-21</sup>. However, none of these have gained widespread use because each requires either complex device fabrication or sophisticated instrumentation for readout. Additionally, none are compatible with conventional DNA microarrays where up to one million sequences can be interrogated in a single experiment<sup>21</sup>.

We report a fundamentally different, electrostatic-based DNA or RNA detection method that is label-free, can be massively multiplexed, and may render microarrays sufficiently cost effective for broad world health applications. In its first implementation, we demonstrate 50 pM sensitivity, single-base mismatch selectivity, and native RNA expression profiling in a conventional microarray format. Complementary oligonucleotide binding strongly affects the electrostatic charge of the surface due to the negatively charged DNA phosphate backbone<sup>15,18</sup>. In this approach,

hybridization is measured electrostatically using charged microspheres that are highly responsive to changes in charge density on the microarrayed surface (Fig. 1a).

Interactions between the microspheres and substrate can be imaged by a variety of optical methods that provide a rapid indicator of DNA hybridization. The role of each silica microsphere is analogous to that of an electrostatic force microscope (EFM) tip where the vertical deflection of the tip is used to report local electrostatic surface properties. EFM, however, is a serial technique that is practically limited to a field of view of  $100 \mu\text{m}^2$ <sup>15,23</sup>. Even state-of-the-art parallel scanning probe microscopy systems, which have as many as 4096 integrated cantilevers, cannot address the length scales required for microarray analysis, not to mention the technological obstacles involved in operating such systems in EFM mode<sup>24</sup>. The particle-based technique described here is capable of parallel sampling of a microarray surface over centimeter length scales, which is the largest area to be electrostatically imaged with micron-scale resolution to date.

Due to non-linear screening interactions in aqueous environments, charged microparticles are responsive to a limited range of surface charge densities (at experimental conditions, the range is  $\sim 10$  to  $10^6 e/\mu\text{m}^2$ )<sup>25</sup>. Thus, the charge density of hybridized spots must be tuned within this optimal range. This is achieved by positively biasing the glass support using an aminosilane modification, which balances the negative charge contributed by both the glass surface and the printed ssDNA molecules (Fig. 1a). Substrates are rendered thiol-reactive using a heterofunctional crosslinking reagent, and an oligoethylene glycol surface functionalization is used to minimize non-specific adsorption. The optimal print concentration of ssDNA probes with the greatest sensitivity was determined by measuring the charge of each spot across a dilution series of spots.

In a typical experiment, a prepared substrate is mounted in a well chamber and hybridized. A fluorescence image of a representative spot after hybridization with Cy3-*A'* (see Supplementary Table 1 for sequences) is shown in Figure 1b. Note that fluorescence imaging and labeling of target DNA are not required, but are only performed for comparison. Unlabeled, and negatively charged 5.6  $\mu\text{m}$  diameter silica microparticles are then added and allowed to sediment above (or otherwise interact with) the array over a period of 20 minutes. Microspheres uniformly distribute across the entire surface (Fig. 1c), and adsorb to the positively charged background. However, over sufficiently negatively charged areas, they adopt an equilibrium height that is dictated by a balance between gravitational and local electrostatic forces. To determine the precise heights and positions of the population of levitated microspheres, a collection of dual wavelength reflection interference contrast microscopy (RICM) images (Fig. 1d) were then acquired covering the entire array area<sup>26</sup>. Image acquisition was automated using a motorized translation stage, and a software-driven autofocus routine. At each stage position, 20 images were acquired (0.4 fps) yielding 20,000 images/ $\text{mm}^2$ . On average, there were 20 microspheres per field of view ( $30 \times 30 \mu\text{m}$ ) resulting in 400,000 microparticle observations/ $\text{mm}^2$ . The interference images corresponding to individual microspheres were used to determine their position with 1 nm vertical resolution and 16 nm lateral resolution<sup>27</sup>. Although this is an optical technique, the resolution is not diffraction limited; it is determined by the particle position resolution and the density of particle observations.

A quantitative spatial map of the surface charge density is generated by compiling the set of 3D particle position measurements. The imaged area is divided into a grid of squares (10  $\mu\text{m}$ ;  $40 \pm 7$  position observations per square), and the median height (5 nm s.e.m.) of particles was used to calculate the surface charge density within each square through an electrostatic force model (Supplementary Information) (Fig. 1e). The dashed line in the electrostatic charge density image (Fig. 1e) indicates the spot

perimeter as determined by fluorescence (Fig. 1b). Importantly, the highest charge density in Figure 1e is predominantly located within the confines of the dashed line, confirming agreement of both readouts.

To quantify the electrostatic response across a range of DNA surface densities, a series of spots was printed using binary mixtures of oligonucleotide sequences,  $A$  and  $B$ , with linearly varying molar ratios. In each series, the total ssDNA concentration was maintained while linearly adjusting the mole fraction of  $A$  from 0 to 1. Since both  $A$  and  $B$  strands are electrostatically and sterically identical, the hybridization efficiency at each spot remains constant<sup>28</sup>. Therefore, after hybridization, the density of  $AA'$  duplexes is linearly related to the density of  $A$  strands at each spot, and the total DNA density varies linearly. The charge density map of two of these series, printed with total ssDNA concentrations of 5 and 6  $\mu\text{M}$ , show a gradual increase in charge density as a function of the mole fraction of  $A$  (Fig. 2a). The charge density corresponds with fluorescence intensities and doubles as the mole fraction of  $A$  changes from 0 to 1 (Fig. 2b). The measured surface charge densities agree with results quoted in the literature for comparable systems<sup>16,18</sup>.

To investigate the specificity of electrostatic-based imaging, we examined two cases where non-specific binding can affect electrostatic response. First, a  $2 \times 2$  array of  $A$  and  $B$  spots was hybridized with 50 nM  $A'$ , 50 nM  $B'$ , or both. This resulted in a specific increase in charge density ( $3 \times 10^3 e/\mu\text{m}^2$ ) of complementary spots relative to non-complementary spots, which were only mildly charged ( $150 e/\mu\text{m}^2$ ) (Fig. 2c). Second, probe sequences of  $A_{12}$  ( $5' - \text{TACCA}\underline{\text{C}}\text{ATCATC} - 3'$ ) and  $A_{12M}$  ( $5' - \text{TACCA}\underline{\text{A}}\text{ATCATC} - 3'$ ) differing by a single-base were imaged before and after hybridization with 50 nM  $A_{12}'$  (Fig. 2d). Each of these spots can be distinguished electrostatically, which is important for diagnostic and genotyping applications involving single nucleotide polymorphisms (SNP). Additionally, by hybridizing

overnight, 100 pM AA' hybridization could also be differentiated electrostatically (Fig. 2e). These results indicate that surface charge imaging is a powerful approach to selectively detect DNA hybridization in a conventional microarray format.

However, practical diagnostics require rapid and simple quantitative readouts that do not utilize dedicated instrumentation or intensive image processing. To develop such a readout strategy we take advantage of the fact that silica microspheres respond to the sign of the surface charge in an easily observable manner. If the surface is negatively charged, microparticles remain laterally mobile and can be easily visualized by monitoring the intensity variance in a time series of brightfield images (Fig. 1f). Alternatively, positively charged areas can be identified by the presence of electrostatically adhered microspheres. This can be rapidly imaged using darkfield (Fig. 1g) or brightfield microscopy. Therefore, adhesion of charged particles provides an exceedingly simple "litmus" test to map the sign of the surface charge.

To develop this binary readout into a quantitative assay, we generated a gradient of surface charge densities by printing a series of DNA spots with a linear range of concentrations (1-25  $\mu$ M). This effectively tunes the net charge of DNA spots from positive to negative (Fig. 3a). When the negative charge contribution from ssDNA is equivalent to the positive charge contribution of the aminosilane background, the spot will be neutral. This is analogous to an equivalence point and, for the present purposes, is defined as such. Upon hybridization with DNA, complementary spots become more negatively charged causing a shift in the location of the equivalence point along a series of spots relative to a non-complementary control series (Fig. 3b). This shift can be easily imaged by examining the mobility of microspheres using low-magnification optics, without the need for complex instrumentation. In these experiments, DNA can be detected at a concentration as low as 50 pM using 30 mer capture sequences under standard hybridization conditions, with a dynamic range extending over 3 orders of

magnitude (Fig. 3c). Direct comparison between fluorescence and electrostatic detection on the same substrates, under identical conditions reveals comparable figures of merit, indicating that sensitivity is primarily limited by hybridization, not the readout. State-of-the-art fluorescence imaging for microarrays using confocal scanners and specialized hybridization conditions has been reported to achieve sensitivities of 1-5 pM [www.affymetrix.com]. As was demonstrated by the classic Millikan oil drop experiment, particle-based electrostatic readout can resolve the fundamental unit of charge (one electron) (see Supplementary Note). Thus similar detection limits as fluorescence may also be anticipated using electrostatic readout under appropriately optimized substrate and hybridization conditions. Notably, the electrostatic approach enables array readout over large areas such as a 1"×3" microscope slide (3000 spots/cm<sup>2</sup>) using darkfield imaging of adsorbed microspheres (Fig. 3d). Since microparticles are unlabeled, detection can be rapidly performed with low-power, low-magnification systems or even the naked eye (Fig. 3e).

Since gene expression profiling is the most widely implemented application of microarray technology, it is important to demonstrate that electrostatic readout can be applied to physiological samples to identify a specific transcript in a complex background of cellular mRNA. We focused on detection of human aldolase A (ALD), and methionine-tRNA synthetase mRNA in purified but unamplified poly(A)-RNA extracted from human breast adenocarcinoma MCF-7 cells. Prior to measurements, the unamplified poly(A)-RNA was randomly fragmented to 60-200 bp in length to match the length of probe strands, and hybridization was performed for 20 min with 100 ng of RNA in 30  $\mu$ l of 1  $\times$  SSC heated to 60°C (Fig. 4a). Brightfield variance (Fig. 1f) of a 4×4 array interrogated with 2.34  $\mu$ m diameter silica spheres indicates the electrostatic response of the MARS probe spot is larger than that of the ALD probe spot after hybridization (Fig. 4b). To date, the only other demonstration of primary mRNA detection in surface array format without amplification or labeling has been performed



using microcantilever arrays<sup>16</sup>. However, microcantilevers cannot be multiplexed to the level of standard DNA microarrays due to the technological difficulties and prohibitive cost associated with microfabrication, chemical modification, and integration of large numbers of cantilevers<sup>16</sup>.

This particle-based electrostatic detection method offers multiple advantages over existing microarray detection methods, which will greatly broaden the applicability of microarrays. First, expression profiling and SNP detection using primary mRNA can now be performed rapidly, circumventing reverse transcription and fluorescent tagging protocols. Second, electrostatic detection is compatible with conventional microarrays as well as unconventional arrays fabricated on injection-molded plastic or embedded within microfluidic architectures<sup>29,30</sup>. Third, imaging by pixel-by-pixel statistical averaging of colloidal particle positions is not diffraction limited, which suggests a strategy to take advantage of DNA nanoarrays that can hold  $10^4$  more features per unit area than conventional microarrays<sup>15,30,31</sup>. Finally, microarrays consisting of proteins<sup>32</sup>, small molecules<sup>33</sup>, polymers<sup>34</sup>, and heterogeneous catalysts<sup>35</sup> are rapidly coming online, and would benefit from such a general transduction platform compatible with nearly every analyte of interest.

## METHODS

**Microarrays.** Oligonucleotide microarrays were generated using a conventional robotic spotter to deposit ssDNA on activated glass coverslips. Substrates were prepared by etching for 15 min in piranha (1:3 30% H<sub>2</sub>O<sub>2</sub>:H<sub>2</sub>SO<sub>4</sub>), washing 6 times in ultrapure (18.2 MΩ/cm) water, and three times in ethanol. The silica surface was then functionalized with aminopropyltrimethoxysilane (Fluka >97%) by incubation in a 2% (vol/vol) ethanol solution for 1 hr. Subsequently, substrates were rinsed with ethanol, dried under a stream of N<sub>2</sub>, and annealed at 80°C for 1 hr. Finally, substrates were activated with succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (Pierce, USA) by incubating in a 2 mg/ml DMSO solution overnight.

Microarrays were generated by using an in house robotic arrayer (Functional Genomics Facility, UC Berkeley) with silicon microcontact printing pins (Parallel Synthesis, Santa Clara, CA). The DNA print concentrations ranged from 1 to 25  $\mu\text{M}$  in  $3\times\text{SSC}$ . After printing, substrates were passivated against non-specific DNA adsorption by incubating in a 2 mg/ml 11-mercaptoundecyl-hexa(ethylene glycol) DMSO solution overnight. Finally, substrates were rinsed in ethanol, dried with a  $\text{N}_2$  stream and stored under dry  $\text{N}_2$  until final use. Similar results were obtained by immobilizing ssDNA on aminosilane treated coverslips with UV crosslinking ( $300 \text{ mJ/cm}^2$ ), and followed by blocking with an *N*-hydroxysuccinimide methyl-capped ethylene oxide reagent (Methyl-PEO<sub>12</sub>-NHS ester, Pierce, USA).

**Oligonucleotides.** All HPLC-purified DNA sequences (Supplementary Table 1) were purchased from Integrated DNA Technologies ([www.IDTDNA.com](http://www.IDTDNA.com)). Disulfide modified DNA was treated with a tris(2-carboxyethyl)phosphine hydrochloride reducing gel (Pierce, IL, USA) before use. Hybridizations with target DNA in the nM range of concentrations were performed in  $1\times\text{SSC}$  solution where the analyte DNA was heated to  $\sim 70^\circ\text{C}$  and then allowed to cool for 20 min. Alternatively, hybridization was allowed to proceed overnight when pM target DNA concentrations were present in order to maximize signal. After hybridization, microarrays were rinsed with 50 ml  $1\times\text{SSC}$  followed by a rinse with 50 ml deionized (18.1  $\text{M}\Omega/\text{cm}$ ) water. Importantly, we considered the possibility that dehybridization may occur in the deionized water during rinsing and particle-based readout. This is not the case, and hybridized DNA is stable under low ionic strength conditions for weeks, which is also confirmed by literature precedent.<sup>18</sup> Nonetheless, DNA crosslinking reagents (such as psoralen) covalently couple duplexes and eliminate the possibility of dehybridization.

**Microarray Imaging.** Silica particles (Bangs Labs, USA) with diameters ranging from 5.68  $\mu\text{m}$  to 0.97  $\mu\text{m}$  were used for imaging. To collect darkfield images, silica particles were allowed to sediment onto the surface of the microarray. The substrate was subsequently inverted for a period of 10 minutes and then a 4X objective was used to collect darkfield images of the substrate. The scattering from 2.34  $\mu\text{m}$  diameter silica particles was sufficiently intense that a cell phone camera could be used to image a side-illuminated microarray through a magnifying glass. To collect brightfield variance data, 2.34  $\mu\text{m}$  silica particles were allowed to sediment for  $\sim 20$  min, and then particle positions were measured by collecting time-lapse images for a total of 30 seconds. The variance in these images was used to distinguish between laterally mobile and immobile silica microparticles.

**RICM.** Dual wavelength RICM was performed using a Nikon TE-2000 inverted fluorescence microscope fitted with a 100X plan apo oil immersion objective (NA 1.3). Sample illumination was provided by a mercury arc lamp and filtered using a dual-band pass (460-480 nm and 510-

550 nm) excitation filter (Chroma, Rockingham, VT). The aperture diaphragm was set to provide a small illumination numerical aperture (INA 0.496) and, hence, minimal angular variation in the incident light. This is important for maximizing the contrast between interference fringes. The field diaphragm was partially closed to facilitate reproducible focusing on the coverslip-water interface. Reflected light was imaged onto separate halves of a single CCD camera (Quantix 57, Roper Scientific, USA) using a DualView (Optical Insights LLC, USA) image splitter interposed between the body of the microscope and the camera. Microspheres imaged by dual wavelength RICM produce interferograms that depend on the particle height and the illumination wavelength regardless of the presence of neighbouring microspheres. The height of an individual microsphere is estimated by correlating interferograms from respective color channels with interferograms calculated according to an image formation theory. The absolute height is determined by finding the pair of correlation maxima that coincide across each color channel. Image pairs lacking unique agreement correspond to malformed or partially imaged particles and are excluded from further analysis. Lateral positions within an image are measured independently in each color channel by image correlation. By accounting for the lateral location where the image was acquired, microspheres across the entire array could be localized. A distributed image processing framework was developed that allowed processing of datasets to generate 3-D particle positions using a flexible number of workstations as each application demanded.

**Large Area Scanning.** The acquisition protocol for RICM imaging of a microarray involved several steps that were automated using Matlab together with  $\mu$ Manager ([www.micro-manager.com](http://www.micro-manager.com)). An ASI-MS2000 translation stage equipped with a linear encoder was used for translating the sample with 200 nm precision over the imaged area. At each location, an autofocus routine was used to ensure reproducible focusing, and 40 images were acquired at a rate of 0.4 Hz. The frame rate was chosen to ensure the vertical fluctuations of beads were uncorrelated from frame to frame.

**Expression Profiling.** Both breast adenocarcinoma (MCF-7) poly(A) RNA and RNA fragmentation reagents were acquired from Ambion (Austin, TX). RNA fragmentation was performed using a zinc acetate reagent according to the manufacturer's instructions. All hybridizations were performed with 100 ng of RNA in 30  $\mu$ l of 1 X SSC buffer heated to 70 C°. Probe sequences used for expression profiling were obtained from [www.Affymetrix.com](http://www.Affymetrix.com).

**Three-dimensional Particle Tracking.** Surface charge density can be quantitatively determined using an electrostatic model (see Supplementary Methods) to interpret the heights of microspheres above the array. In order for this approach to succeed, the three-dimensional position of tens of the thousands of microparticles must be determined over centimeter length

scales. RICM was used here because it facilitates determination of the absolute separation distance between the microarray surface and individual particles with 5 nm resolution without complications due to closely neighboring particles. A caveat of RICM is that image contrast is generated using a single wavelength according to the phase of interfering rays; absolute phase information has been lost introducing ambiguity into height measurements<sup>26</sup>. To overcome this problem, dual wavelength RICM was developed<sup>26</sup>. Recently, we introduced a RICM-based method capable of localizing the three dimensional positions of a population of microspheres using an image correlation technique<sup>27</sup>. By adapting this methodology to the analysis of dual wavelength RICM images, the absolute three-dimensional particle positions can be determined unambiguously.

*Note: Supplementary information is available on the Nature Biotechnology website.*

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**Figure 1. Electrostatic microarray readout using microparticle probes. a,**

A suspension of negatively charged silica microspheres is gravitationally sedimented over a microarray surface. The positions and motions of a population of microspheres are used to image the surface charge of the microarray and detect hybridization. This is because areas displaying double stranded DNA are highly negatively charged compared to areas displaying single stranded DNA, and both contrast with the positively charged background.

**b,** Typical epifluorescence image of a microspot displaying DNA *A* after hybridizing with 50 nM Cy3-labelled *A'* (20 min, 1X SSC). **c,** Brightfield image after 5.6  $\mu\text{m}$  diameter silica microspheres are allowed to gravitationally sediment for 20 min. The dashed line indicates the spot's perimeter as determined by fluorescence. **d,** Representative reflection interference contrast microscopy (RICM) image of 5.6  $\mu\text{m}$  diameter silica spheres. Such interferograms are used to measure the height of microspheres, and, consequently, the magnitude of electrostatic repulsion over the surface. **e,** The charge density map, as compiled from RICM observations of microspheres (black dots). **f,** Over negatively charged areas, microspheres are laterally mobile as indicated by the variance of brightfield intensity over time (pixel size is  $3 \times 3 \mu\text{m}$ ). **g,** Microspheres that remain adhered after agitating the surface can be observed by darkfield microscopy to identify positively charged regions. Images **b-g** correspond to the same spot and were collected in 100  $\mu\text{M}$  NaCl.

**Figure 2. Electrostatic response to DNA surface density. a,**

A graded DNA density was generated by printing spots with a mixture of specific, *A*, and control, *B*, DNA while maintaining a constant total DNA concentration (5 or 6  $\mu\text{M}$  as indicated). Charge density and fluorescence images of the same array are shown after hybridization with 50 nM *A'*. **b,** Plot of the average charge density and fluorescence intensity in spots along the 5  $\mu\text{M}$  lane in **a**. The dashed lines

are linear fits. The charge density roughly doubles as the molar fraction of *A* increases from 0 to 1. This is consistent with the expectation that complementary DNA binding should double the ssDNA charge density. **c**, Electrostatic response of a 2×2 array of *A* and *B* DNA to specific hybridization with target DNA *A'*, *B'*, or both *A'* and *B'* strands. **d**, Image of  $A_{12}$  (5' - TACCACATCATC-3') and  $A_{12M}$  (5' - TACCAAATCATC-3') spots before and after hybridization with 50 nM *A'* for 20 min which indicates that electrostatic imaging can resolve a single base-pair mismatch. **e**, Epifluorescence and electrostatic images of *A* and *B* spots after overnight hybridization with 100 pM *A'* DNA. All hybridizations performed in 1X SSC and images were collected in 100 μM NaCl.

**Figure 3. Simplified readout using charged microparticles.** **a**, A series of microarray spots are printed with a gradient of ssDNA densities to titrate the surface charge from net positive to net negative. After hybridization (in 1X SSC), complementary spots become more negatively charged. In each series, the change in DNA density can be identifying by a shift in the number of negatively biased spots relative to a control series. **b**, Schematic and experimental data demonstrates this concept. Images of the variance in brightfield intensity over 30 s indicate where sedimented 2.34 μm diameter silica spheres remain mobile. Negatively charged areas appear bright due to the lateral motion of microspheres repelled by the surface. Relative to the control DNA series, *B*, two additional spots in the *AA'* row appear negatively biased, indicating a specific change in charge density due to hybridization. **c**, The observed shift is dependent on the concentration of target *A'*. Plot compares this label-free readout with fluorescence data obtained on the same substrate under identical conditions. Inset points were hybridized overnight, and all others were performed for 20 min. **d**, Darkfield and epifluorescence (inverse contrast) micrographs of a representative area from a 7000 spot microarray

hybridized (20 min, 1X SSC, 50 nM A'). This suggests that this assay is compatible with conventional microarrays that cover  $\text{cm}^2$  areas. **e**, Photograph of a side-illuminated microarray after hybridization and development with 2.34  $\mu\text{m}$  diameter silica spheres. On average there are 200-300 microparticles/spot. Inset, right, shows a digitally magnified region of the array (inverse contrast with subtracted background). Bright areas indicate regions of high DNA density. All images collected in 100  $\mu\text{M}$  NaCl.

**Figure 4. Label-free expression profiling with primary mRNA.** **a**, Scheme of procedure used to measure mRNA expression in breast adenocarcinoma MCF-7 cells. **b**, A brightfield intensity variance image of 2.34  $\mu\text{m}$  silica microspheres shows the differential expression of human aldolase A gene (ALD) and human methionine-tRNA synthetase (MARS) gene in a 4x4 array of spots (in 100  $\mu\text{M}$  NaCl). This indicates that MARS is more highly expressed compared to ALD in this sample of cells.

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