Geometry in digital molecular arrays†

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The development of digital molecular devices arises through the appropriate geometric positioning of a molecular assay. A detailed evaluation of the digital media reveals the critical aspects of geometric positioning in terms of developing an analytically-robust system for molecular analysis. This study reveals an explicit digital compact disc based assay for molecular affinity events.

The development of digital systems for molecular diagnostics has invoked the conceptualization of molecular-based digital machines. The realization of such machines has been accomplished by adaptation of digital media, such as the compact disc (CD), for use in biomolecular analyses.[1] The remarkable engineering of the CD player offers a multidimensional system for the molecular scientist, as it promises a cost-effective solution for microfluidic devices,[2] molecular diagnostics,[3–7] and cell processing.[8] In terms of diagnostic use, the standard disc player (CD or DVD player) has been shown to provide a viable analytical device for screening a number of protein and DNA based analyses.[1b,c,3] This article evaluates the features critical for the development of a true digital molecular assay system.

To date, three different architectures, as given by types IA, IB and II (Fig. 1), have been developed for CD-based molecular analyses. In principle, a molecular analyte can be used to create (type IA), process (type IB), or modulate (type II) a string of digits. The substantial differences between these approaches, in terms of their engineering, data collection, and data management, necessitate a thorough comparison to guide the future development of digital molecular devices. To demonstrate, we examined application of types I–II to digital molecular analysis. Based upon their suitability for digital architectures, conclusions can be drawn as to the basic structure best fit for contemporary digital machines with their benefits to advance data manipulation and information flow. In this study, we evaluate the performance of type I and II systems on both a theoretical and experimental level.

A digital molecular assay must be able to perform two tasks: detect a molecular change and relay this detection *via* digital code to other digital machines. This digital code may arise by either the generation of new digital information or the alteration of existing digital code. It is the source of this digital information that delineates the major types of digital molecular devices explored to date. All type I devices use the generation of a digital signal for the creation of raw analytical data. This format may be seen in two examples. The first, type IA, uses reflected light from a pattern of analytes to report a positive biomolecular signal through binding of a reporter, resulting in the creation of light intensity.[4] In this system, the affinity event creates the digital signal, as given by its geometric positioning into byte structures. The second, type IB, uses constructive interference to alter the wave-based reflection of light to generate a digital signature.[5] In this model, the processing of light (reflection) is displayed in a geometric arrangement to generate byte-like patterns. The difference between type IA and IB lies in the function by which the optical event is translated into a digital signal. Both type I systems begin with a theoretically blank digital structure (background, Fig. 2), and the generation of signal occurs when positive biomolecular events take place. To make such a system digital, reporter molecules must be deposited in an array whereby the return of positive binding events creates an interpretable digital code. Type II devices, on the other hand, work upon a pre-existing digital template. As shown in Fig. 1, it is the modification of bits within the returned signal during transmission that acts as the analytical event through creation of calculable error.[3]

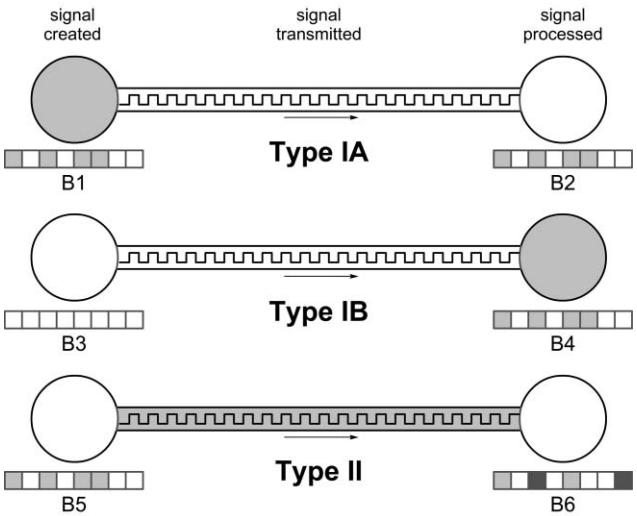

Fig. 1 Schematic representation of the three digital architectures of molecular analysis. Each system, types IA, IB, and II, is represented with two spheres connected by a conduit. The digital signal is created in the left sphere and transmitted through the conduit to a second sphere on the right, where it is processed. A set of six bytes, B1–B6, are presented wherein B1, B3 and B5 are bytes created by the system and bytes B2, B4 and B6 are bytes that have been processed. Grey-shaded regions within the representations denote the regions in molecular analysis interacts with the digital system. Coloring within each byte indicates 0 in white, 1 in light grey, and modified bits in dark grey.

Conventional wisdom may suggest that the generation of a signal (type IA/B) would be optimal for ascertaining a molecular entity, much like traditional spectroscopy. Because type I devices

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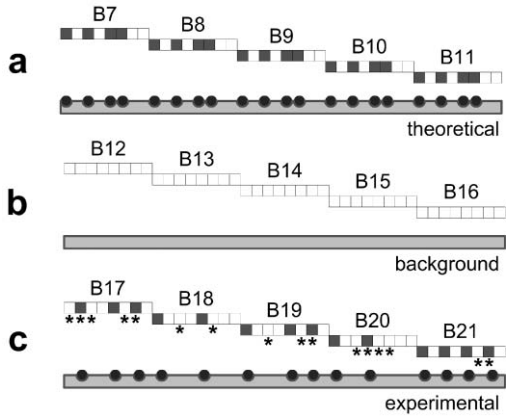

Fig. 2 Operation of a type I device. (a) Theoretically-expected outcome. (b) A background reading. (c) An exemplary experimental outcome. The symbol * denotes null bits, which can be assigned neither 0 nor 1. Circles represent regions of the assay where positive affinity events occur.

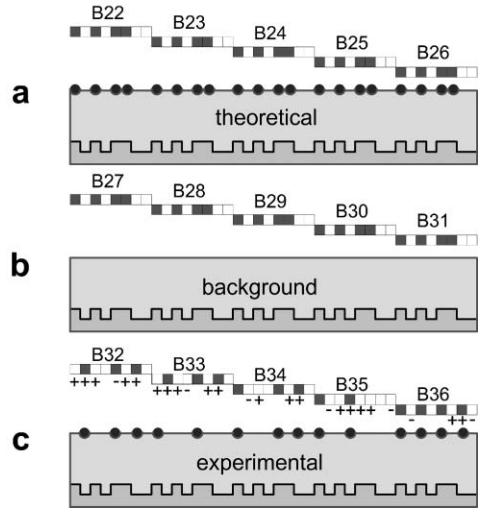

Fig. 3 Operation of a type II device. (a) Theoretically-expected outcome. (b) A background reading. (c) An exemplary experimental outcome. The symbols + and − denote basic and redundant error, respectively. Redundant error depicts the return of the same bit (*i.e.*, 0 to 0 or 1 to 1), while basic error is given by a change of 0 to 1 or 1 to 0. Circles represent regions were positive affinity events occur.

serve to create an information stream, the nature of information coded within a given set of assays is regulated by the geometric assembly of each assay. A major limitation of geometric arrays within digital systems is treatment of error when a given assay fails to execute properly. The creation of error for such a digital device is represented by bits that are erroneous, or null bits, which can be assigned neither 0 nor 1. For instance, byte B17 (Fig. 2) contains five null bits when compared to the theoretical equivalent, byte B7. When the position of such errors is stochastic, they cannot be included in a digital architecture, as stochastic events are not addressed by digital machines.[9] As a digit is determined by either a 0 or 1, there is no room to address intermediate states (*i.e.*, 1/2 or 0.75). Therefore, for type I systems the precision of geometric deposition upon the assay surface is critical to its function as a digital machine.

For molecular analysis, the generation of error in reading or writing events poses the same particular problems of data loss. The engineering of type I molecular digital devices must create repeated arrays in order to compensate for positional error. To achieve such a self-correcting system, expansion in the size of the data set is required, whereby a single assay is represented within a track of redundant analyses. For traditional analog processes, this problem is identical to the need for precision, wherein a dynamic range in error is approached by repetitive analysis. In terms of a digital machine, however, the comparison is not absolute.

The ability to determine precision in a digital device can become exceedingly complex. This issue is addressed by type IB molecular digital devices, where signal creation occurs at the processing side of an assay. Here a molecular event, either reaction or association, causes a signal to be recognized by the creation of fractional wavelengths for interferometric analysis.[5] Like the type IA system, processor-created data requires the installation of complex algorithms to correct for positional error.

Type II assays provide a distinct departure from type I systems, as error generation is intrinsic within the creation of digital data. As illustrated in Fig. 3, data delivered through a type II assay is present in the background, thus providing a direct mimic of the theoretical experiment. A biomolecular event creates error during the analysis, thereby altering the transmitted digital information. For digital sequences, the byte change is developed in terms of two possibilities, as given by redundant or basic error (Fig. 3). Comparison of the background or theoretical to experimental bytes identifies error. This is the key component through which type II systems gain function. The experimentally observed byte B32 can be compared with byte B22 (Fig. 3). Rather than generating null bytes, both basic and redundant error are returned as functioning digital code.

In effect, the utility of type I molecular digital devices relies on the geometric fidelity of particle placement, whereas type II systems can incorporate algorithms to manage geometric inaccuracies within their operating system.[3] The question arises, then, whether geometrical arrangement of particles on a surface may be arrayed with sufficient precision for adoption of type I systems to a digital architecture. To probe this question, we developed the two formats into CD-based prototypes.

Fig. 4 illustrates a type I system, where a biotin moiety was distributed to match a repeated pattern on a gold surface. This surface was addressed through an alkylthiol tether *via* templated deposition, as given by the rows of theoretical bytes in Fig. 4.[10] After binding of strepatvidin-linked gold beads, the device was imaged *via* electron microscopy to reveal a variety of observed patterns. Fig. 4c–d are indicative of positional error that results from such arrays, in which the difference between the theoretical and observed bytes results in bit patterns that may not be quantized digitally. The statistical deviation of this system was analyzed by examining 10^5 repeated bytes from Fig. 4. By compiling the fidelity of experimental results with the theoretically expected readout, less than 25% of all bits were returned correctly (Fig. 5). Fig. 6 depicts the number of correct bits obtained for each byte of data. As shown, less than 1% of the bytes were returned with the correct theoretical sequence (*i.e.* 8 correct bits per byte.)

A type II system was developed using a recently described protocol (Fig. 7).[3] A biotin moiety was evenly distributed across an assay surface of a polycarbonate CD. Biotin was tethered

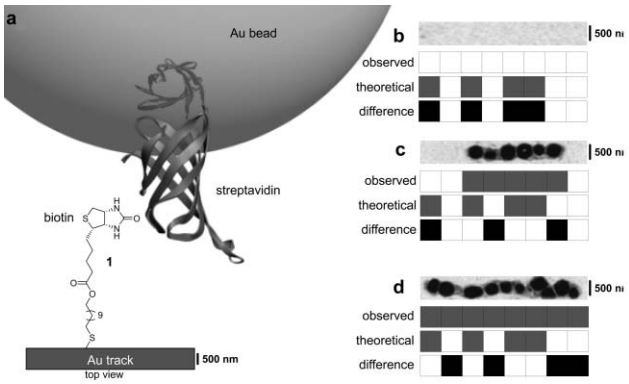

Fig. 4 Design of a model type I device. (a) Biotinylated ligand **1** is applied to a gold-coated glass slide through microcontact printing that depicts the byte pattern repeated in this manuscript (B1). Streptavidin-linked gold beads are exposed to the patterned surface. (b) An exemplary background byte, where no pattern was deposited. Top row shows an electron microscopy (EM) image of the track. Observed and theoretical rows are used to calculate the difference byte below. (c–d) Two examples of experimental bytes imaged by EM. Note that the difference between (c) and (d) can not be modelled algebraically.

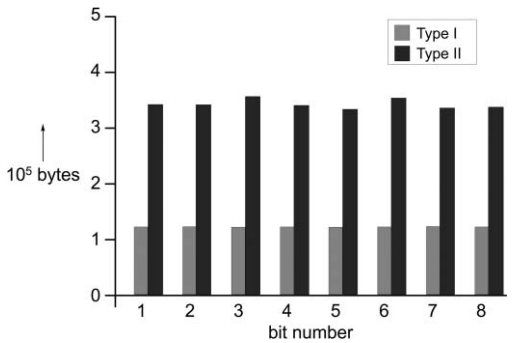

Fig. 5 Number of bytes obtained correctly at a given bit. A total of 500 000 bytes were examined. Data for the type I and II systems were collected using the prototypes provided in Fig. 4 and 7, respectively.

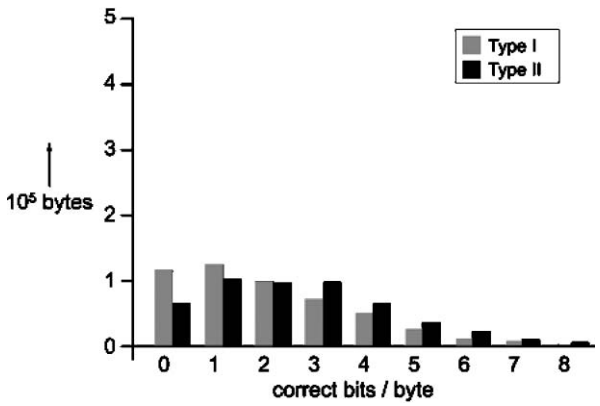

Fig. 6 Number correct bits obtained per byte. A total of 500 000 bytes were examined. Data for the type I and II systems were collected using the prototypes provided in Fig. 4 and 7, respectively.

via **2**, which was doped into to the polycarbonate surface *via* a polycarbonate tail, as depicted in Fig. 7b. A streptavidin solution was exposed to the doped surface in an assay region,

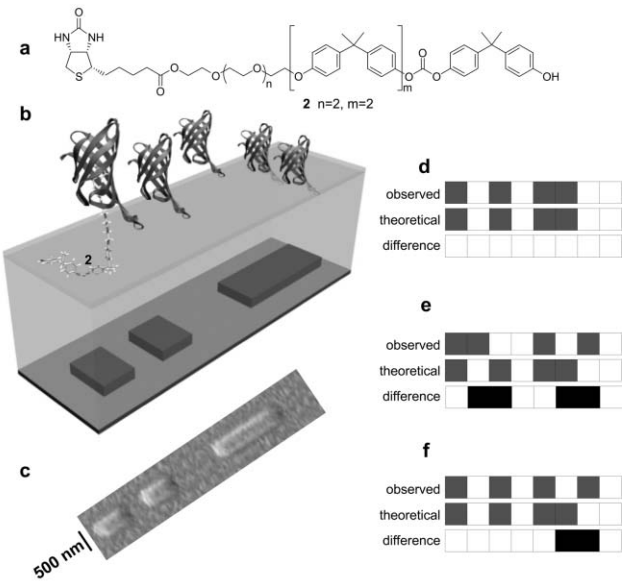

Fig. 7 Prototype of a type II device. This device is similar to that published previously.[3] (a) Affinity-polycarbonate doping label **2**, consisting of biotin tethered to a polyethyleglycol–polycarbonate tail. (b) A projection view of the composite. A data CD is engineered to contain the repeated byte used in this manuscript (B1) within its digital layer. Affinity doping label **2** is applied to a CD surface as a thin film. Streptavidin is exposed to the disc in an assay region, which serves to modify the reading of the internal digital structure. (c) An EM image of the aluminium digital layer, prior to polycarbonate molding. (d) An exemplary background byte, where no streptavidin was deposited. Observed and theoretical rows are used to calculate the difference byte below. (e–f) Two exemplary experimental bytes. Observed and theoretical rows are used to calculate the difference byte. Difference signal indicates molecular interaction.

and the digital information was read off of the CD as previously described.[3] As seen in Fig. 5, greater than 60% of the bits returned correctly. However, the number of correct bits per byte (Fig. 6) was comparable to the type I system, returning a distribution that contained a modest improvement in the number of correct bytes.

The difference between the type I (Fig. 4) and type II (Fig. 7) systems arises not from the distribution of error, but from its interpretation. Because type I systems are generating new digital code, errors in the data are disallowed, whereas in type II systems, where digital code is modified, error in the data is interpreted as a positive binding event. The distribution of error seen in type II systems arises through a data structure that autonomously detects a molecular binding event.[4] Here, the fidelity (as given by correct bits per byte) provides an analytical readout that is directly correlated to the affinity event. In contrast, all incorrect bits in type I systems are returned as null bits, which are inadmissible for digital computation. This phenomenon precludes the use of type I systems as digital molecular devices without the inclusion of massive redundancy. As a result, most type I systems to date have employed secondary devices to convert an analog signal to digital code.[4–5] While not inherently superior for molecular diagnostics, digital readout offers the advantage of data collection and

dissemination and networking structures available to contemporary computation.

The development of digital molecular media provides unique advantages for biomolecular science, several of which arise through the adaptation of intelligent software design to directly process digital readout. Here, we illustrate that one of the key features to the development of digital molecular machines, as suggested herein, arises from geometric concerns. In conclusion, we find that type II systems provide a superior platform to develop digital molecular devices to develop facile interfaces between the machines that conduct molecular analyses and digital communications.

Experimental

Type I system (as depicted in Fig. 4)

A 1 × 3 cm gold-coated glass slide was stamped with biotinylated linker **1** using an established microcontact printing (m-CP) technique with a pattern representing the byte 10101100.[11] A dilute solution of **1** (0.1 mM in ethanol) was applied to the gold surface by dipping, and the excess was removed by flushing with N_2. The thiol-exposed stamp was then transferred onto the gold surface by physical contact for 2 s. This patterned substrate was then exposed to a freshly prepared diazomethane solution (*ca.* 1 M in ether) and washed with ethanol (3 × 5 mL). Diazomethane treatment enhanced the removal of non-covalently linked mercaptans. The surface was treated with streptavidin-labelled 0.5 micron OD gold microspheres for 30 min in 20 mM phosphate buffered saline (PBS, 150 mM NaCl) at pH 7.4. The slide was removed and washed with PBS (3 × 5 mL) and examined using electron microscopy.

Type II system (as depicted in Fig. 7)

An aluminium CD was created from a glass master with a repeating data byte of 10101100 packaged between the appropriate tracker and header information using the standard stamper-injection molding process.[12] The surface of this disc was coated with biotinylated receptor **2** by spraying with a thin film of **2** (10 mM in ethanol) followed by incubation at 35 °C for 2 h. The resulting disc was washed with ethanol (3 × 5 mL) and dried with a cotton cloth. Once dry, the surface of the disc was treated with 100 nM streptavidin in 20 mM PBS with 150 mM NaCl at pH 7.4. The disc was removed and washed with PBS (3 × 5 mL) and examined on a compact disc player using established raw data collection routines.[3] Electron microscopic images were collected on the raw electroplated surface prior to molding with polycarbonate.

Notes and references

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