Optically Encoded Parallume Suspension Arrays

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Introduction Parallel Synthesis Technologies introduces Parallume™ optical encoding technology - a new type of optical encoding technology, which is not based on organic dyes or quantum dots. The Parallume technology can be used to prepare optically multiplexed bead sets (**Fig. 1**) containing hundreds or thousands of unique optical signatures with a variety of surface linker chemistries suitable for DNA or protein attachment. Sets of Parallume encoded beads with attached DNA, where you specify what type of DNA is used and we attach it to the beads, are also available. Using two-color competitive DNA hybridization as an example, a unique capture probe is attached to a bead with a unique optical signature and the pooled beads hybridized against a two color target mixture (Cy3/Cy5). Sequential imaging of the beads under UV, 532nm and 633nm excitation allows association of the target's Cy3/Cy5 ratio to a given bead's optical code, i.e. the identity of that bead's DNA probe.

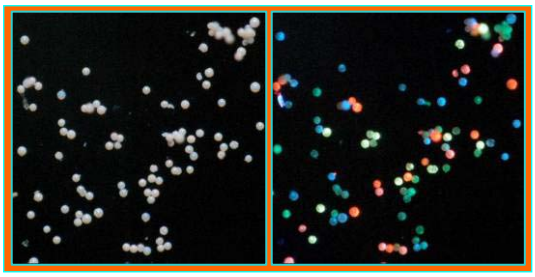

Fig. 1 Photomicrographs of Parallume encoded beads under visible (*left*) and 320nm light (*right*). These beads are ~25 μm in diameter.

How Parallume Works The Parallume optical code serves exactly the same function as an *x*, *y* address on a chip or a physical barcode on sample vials. The Parallume optical encoding technology is based on two to six different rare earth elements, which are present at low levels within a single host compound, which emit six discrete and resolvable colors (**Fig. 2**) when excited at a *single* wavelength. Since the multiple narrow band rare earth emitters display far less spectral overlap than quantum dots or organic dyes (**Fig. 3**), it possible to measure their *relative* fluorescent intensity very accurately. This allows the resolution of a very large number of ratiometric optical codes or optical signatures. In bulk materials, we have statistically demonstrated that we can resolve the relative fluorescent intensity ratios at ~1% which corresponds to the resolution of *ten billion* unique optical signatures. It is extremely important to note that there is no optical cross talk between the Parallume optical code and any reporter dyes. The midrange UV (~320nm) used to excite the Parallume materials does not excite dyes which absorb in the visible region and, conversely, the Parallume materials are not excited by the visible lasers used to excite the organic dyes. The inorganic Parallume material cannot be photobleached even under prolonged laser excitation.

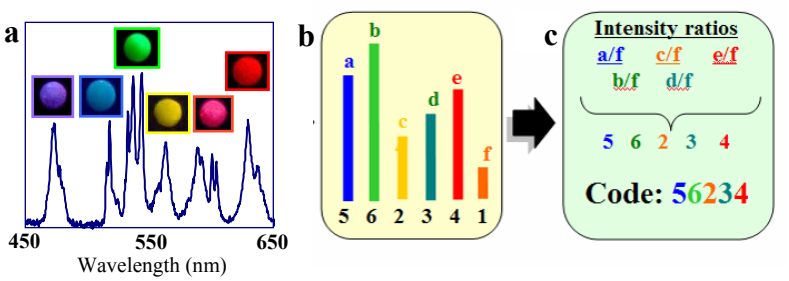

Fig. 2 Beads encoded with Parallume optical encoding technology produce narrow band emission (**a**) throughout the **Fig. 2** Beads encoded with Parallume optical encoding technology entire visible region when excited at a *single* wavelength. The fluorescent intensity of the various emitters within a bead (**b**) relative to one emitter acting as an internal standard gives *n* – 1 optical codes for *n* emitters (**c**).

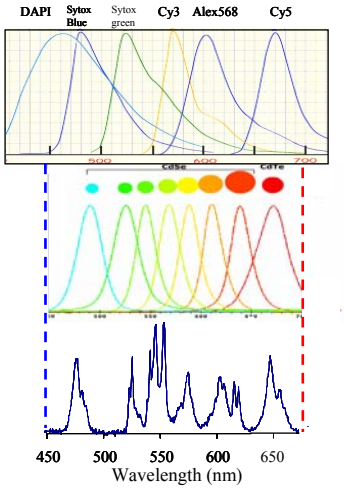

Fig. 3 Emission peaks widths of organic dyes (top), quantum dots (center) and Parallume (bottom).

Using Parallume-impregnated beads, a very large number of diverse and important biochemical reactions, such as hybridization, on-bead PCR, RNA*i* delivery, ELISA, DNA-protein and small molecule-protein interactions can all be studied in a deeply multiplexed suspension array format. In nearly all cases studied, the kinetics and rates of the reactions on the porous Parallume-encoded beads are nearly as fast as the corresponding reactions in solution. The beads may be read with a conventional fluorescent microscope or with one of several different reader designs available from Parallel (see

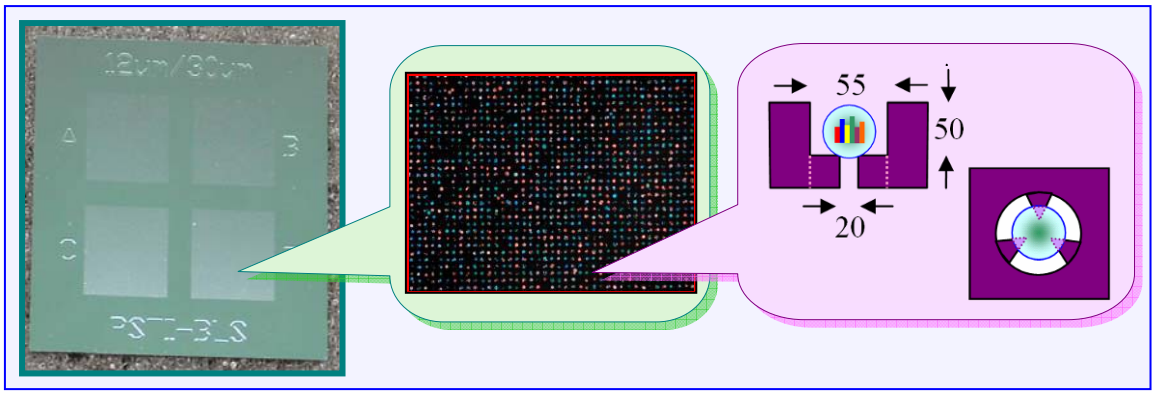

Fig. 4 A 25mm x 25mm silicon Bead Localization Slide (BLS) (*left*), which is fabricated using silicon micromachining techniques at Parallel, optically isolate the encoded beads from one another while maintaining a large fraction of the surface covered with beads (image under UV illumination *center*). By judicious matching of the bead size (e.g. ~50µm) to the dimensions of the hole, the bottom vent and the three prongs, the bead is forced to center itself into the hole in a configuration designed to facilitate washing and drying (*right*). The silicon BLS are reusable and can be sterilized or heated to high temperatures, inexpensive and disposable BLS are also available (**Fig. 5**).

below).

Forms of Parallume: Beads, Nanoparticles and Bulk Materials The Parallume encoded materials may be prepared in many forms including impregnated beads, nanoparticles and pure bulk samples which are all currently available in commercial quantities. For biological reactions, the Parallume optical encoding is conveniently employed in the form of highly porous, thermally stable, spherical glass beads (**Fig. 1**) with DNA or proteins attached to their surfaces. The very large pore size (100-300nm), surface area (~50m^2/g) and pore volume (~1 mL/g) allows diffusion at rates approaching those found in solution. For example, hybridization reactions appear to reach a steady state in <5 minutes.

Bead Content and Surface Chemistry The encoded beads are available with different surface chemistries to attach DNA and proteins (**Appendix 1**). The Parallume encoded beads are synthesized in parallel using an automated procedure. The Parallume material, which is coated onto walls of the large pores within the beads, is subsequently covered with SiO_2 to provide a glass-like surface which can be

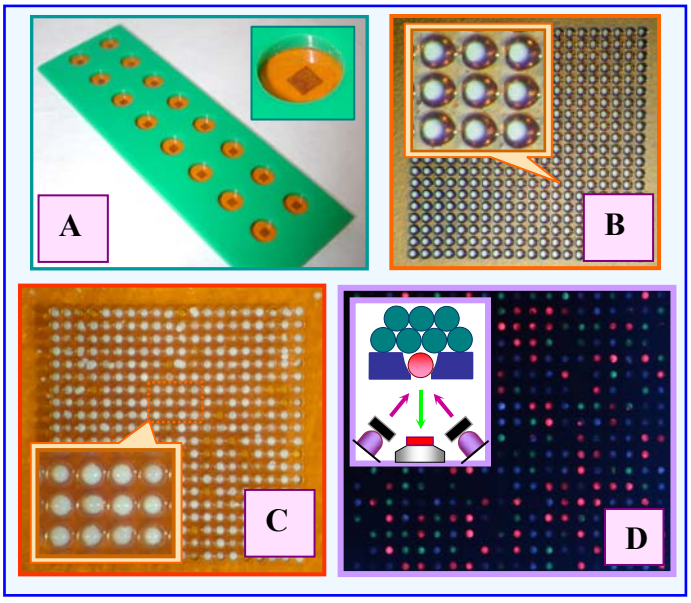

Fig. 5 The BLS holds the beads for incubation, washing, imaging and archiving. The slides (**A**) with wells on 96 or 384 well formats, have (**B**) cup-shaped holes (60µ tops and 40µ bottoms) holding 45-50µ beads on 80µ centers (**C**). After loading an excess of beads into the BLS to ensure filling of all holes in the array, the slide is imaged from the bottom side (**D**) thereby preventing any bead-to-bead optical cross talk.

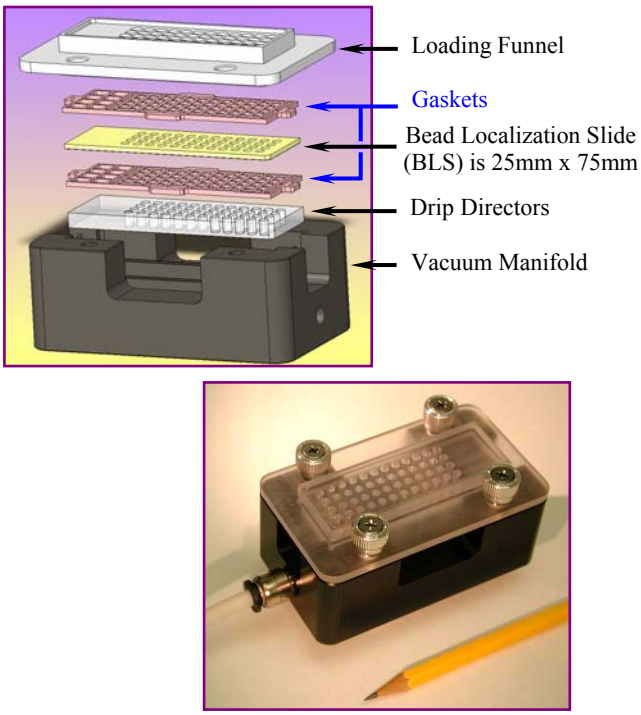

Fig. 6 Schematic exploded view (top) and photograph (bottom) of the BLS within its fixture.

facilitate washing and drying of the beads by virtue of the geometric design of the BLS. The types of BLS slides available range from 8 wells on 9mm centers (96 well format) to 48 wells on 4.5 mm centers (384 well format) are given in **Appendix 2**.

The multiplexed bead samples in the BLS wells may be handled conveniently and incubated, washed, dried, imaged and archived using the fixturing shown in **Fig. 6** to load and filter the beads without any leakage or well-to-well chemical cross talk (**Appendix 3**). A suspension of the beads, in an amount sufficient to cover the bottom of the well, may be added manually or with an automated liquid handler. After the assay and drying the BLS is removed from the fixture and placed directly into the MARS (**Fig. 8**) for optical analysis. The amount of beads transferred need not be carefully measured as long as the bottom of the well is covered with beads (**Fig.**

functionalized and derivatized using standard protocols. The beads are available with large variety of robust surface functionalization such as amines, carboxylates and epoxides. To allow the flexibility of developing your own bead-based chemistries, encoded bead sets with a plain SiO_2 coating are available. Parallel can also provide the Parallume encoded beads with attached materials such as DNA, proteins, antibodies and small molecules or provide protocols for each laboratory to develop their own proprietary content.

Bead Holders In order to conveniently incubate, wash, filter, optically characterize and archive the encoded beads, Parallel offers Bead Localization Slides (BLS) in either a reusable micromachined silicon format (**Fig. 4**) or as a laser-cut, disposable polymer slide (**Fig. 5**) which isolate the beads and prevents the emission from a given bead from shining onto its proximate neighbors. As shown in **Fig. 4**, by matching the diameter of the bead to the hole size in the BLS, loading is readily achieved and the bead is forced to self-center into the well to

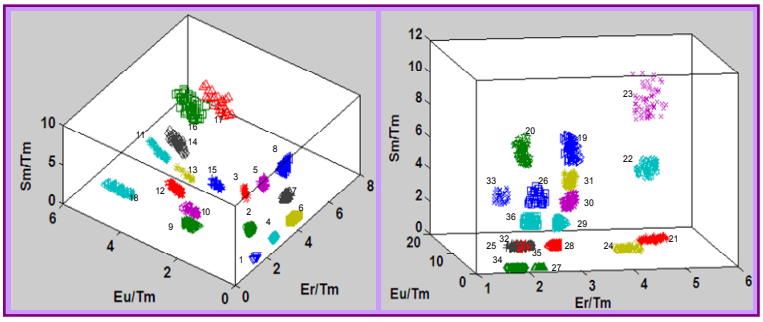

Fig. 7 Examples of optical bins created within the quaternary Sm-Er-Tm-Eu system. Each optical bin is a resolvable optical signature.

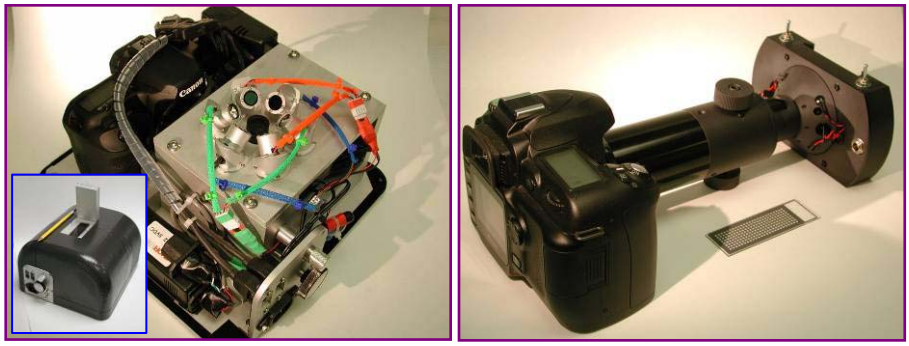

Fig. 8 The least expensive type of Multiplex Assay Reader System (MARS) can resolve ~50 optical codes using a commercial DSLR CMOS detector and is available as a bench top instrument (*left*) or as a portable version (*right*).

5D). The throughput of the BLS system is very high. With a 50-plex assay in each of the 48 wells there are nearly 2500 in individual assays performed simultaneously on the 25mm x 75mm slide.

Optical Characterization of Parallume-Encoded Beads – The Multiplex Assay Reader System (MARS) Many options are available to optically decode the multiplexed bead mixtures and determine the reporter dye levels. A complete range of readers and optical scanners solutions ranging from a Hyperspectral Imaging System capable of reading six or more colors simultaneously to a simple, inexpensive and portable Multiplex Assay Reader System (MARS) capable of reading three colors (**Fig. 8**) are built by Parallel and are available. Importantly, the cost of the reader system scales with multiplexing depth such that the readers for fewer number of codes are less expensive than readers which can resolve larger numbers of codes.

When a multiplexing level of less than 50 is sufficient our least expensive readers employ a commercial DSLR CMOS detector are are available as both laboratory and portable instruments (**Fig. 8**). Hundreds to thousands of optical bins can be created, used and measured by replacing the CMOS detector with a monchrome CCD and multicolor filter wheel. All models are available with motorized stages.

Parallume decoding software is available which identifies the beads based on their color ratios and creates a number of optical bins (**Fig. 7**) into which the beads are sorted, classified and analyzed.

Please call for pricing.

Applications Using Parallume Encoded Beads Parallel has developed a wide variety of chemistries and protocols for the Parallume beads particularly in the areas of nucleic acid and protein detection. The very large pore size in the beads (100-300nm) allows diffusion at rates similar to that found in solution. Only a few possible applications are illustrated in **Fig. 9**. The bead types available are shown in **Appendix 1**, the Bead Localization Slides for incubating, filtering and imaging the bead are given **Appendix 2** while a typical process flow

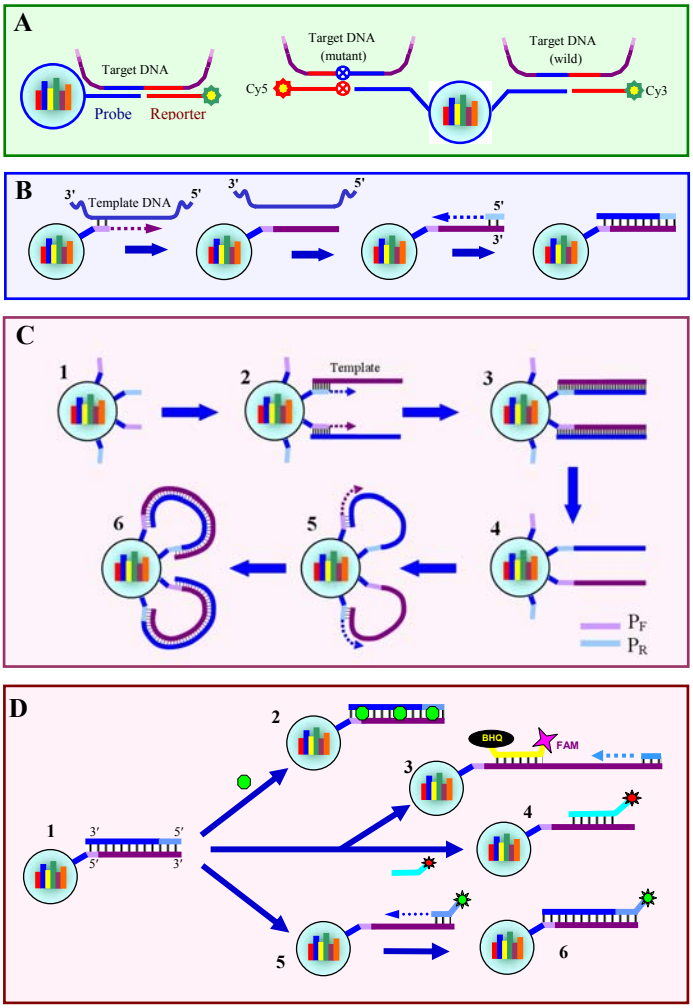

Fig. 9 In addition to such diverse applications as hybridization, functioning as a capture probe to separate and quantitate products in multiplex PCR, the delivery of RNA*i* to cell cultures and the study of protein-ligand or protein–protein interactions, Parallume encoded beads can be used in many others ways. One Parallume-based assay allows the detection of *unlabeled* target DNA (A). For example, it is possible to perform PCR directly on the surface of the encoded beads. If the forward primer is covalently bound to the surface of the bead and the reverse primer in solution (B), then the double-stranded amplicon is tethered to the bead by one strand. If both primers are bound the bead (C), then both strands of the amplicon are covalently bound to the bead. Once the amplicon is bound to the bead it can be detected by a variety of methods (D). Parallume encoded beads are available with SiO_2 surfaces, with chemical linker groups suitable for conjugating nucleic acids or proteins to the beads or with user-specified nucleic acid contents attached to the beads by Parallel.

for performing a multiplex analysis on Parallume-encoded beads is shown in **Appendix 3**.

One particularly important type of reaction, which can be used in conjunction with the mobile MARS (**Fig. 8** *right*) to perform field testing of protein and nucleic acids, is an assay which *can detect unlabeled target DNA* and is shown in **Fig. 9A**.

♦ Please call for pricing for Parallume-encoded beads, with surface functionalization suitable for protein or DNA attachment, or ask for the discounted "Starter Sampler" which contains a milligram each of a red, green and blue emitter beads with $-CO_2H$ surface functionalization.

♦ Please call or see the www.parallume.com for Bead Localization Slides (BLS) and other consumables.

♦ Please call for pricing on the various types of MARS bead reader available

Appendix 1: Bead Types Available

The Parallume encoded beads are available as thermally stable (up to ~800ºC) quartz spheres with a diameter around 50µm. The beads have large pores (125-300nm) which are coated with the Parallume encoding material that is subsequently covered with a layer of SiO_2. Users can derivitize the SiO_2 surface according to their own needs or Parallel provides SiO_2 surfaces with monoamine, polyamine, carboxylate and epoxide functionality. Parallel can prepare beads with attached oligos, dyed oligos or other probes according to your specifications. Please call for pricing.

The below optical encoding schemes are available with the chemically derivitized surfaces discussed above. Since Parallel manufactures all materials, custom formulations are easily prepared. All Parallume-containing beads have SiO_2 surfaces. Please call for pricing.

1. Beads with no Parallume for negative control
2. Beads with each of the single emitters Dy, Er, Eu, Ho, Sm and Tm.
3. Beads with binary combinations of any two emitters
4. Beads with RGB (Sm, Er, Tm) emitters. Used with "color" CCD cameras.
5. Beads with any combination of emitters present.
6. The beads are available in individual vials, mini-centrifuge tubes or microtiter plates.
7. Beads are available in samples as small as 1 mg (~5,000 +/– beads).
8. Beads are available with PEG linkers.

The most popular beads have 750-4000 MW PEG linkers between the surface of the bead and the PEG-pendant, terminal carboxylate groups. When beads are purchased, protocols are provided for the various linker chemistry and oligo and protein attachment.

Appendix 2: Bead Localization Slide Formats Available

Bead Localization Slides (BLS) (**Fig. 4**) hold the beads for washing, filtration, drying and archiving. As shown in **Fig. 4**, the BLS prevent optical cross talk between neighboring beads on the array while maintaining a relatively high percentage of the surface of the holder as sample. The current BLS are available in 25 x 25 mm^2, 25 x 75 mm^2 formats or custom sizes. As shown in **Fig. 4**, a portion of each BLS has holes designed to hold ~40-50µ spherical beads. Other formats are available as custom configurations. Please call for pricing.

Appendix 3: Assay Process Flow for Parallume Suspension Arrays

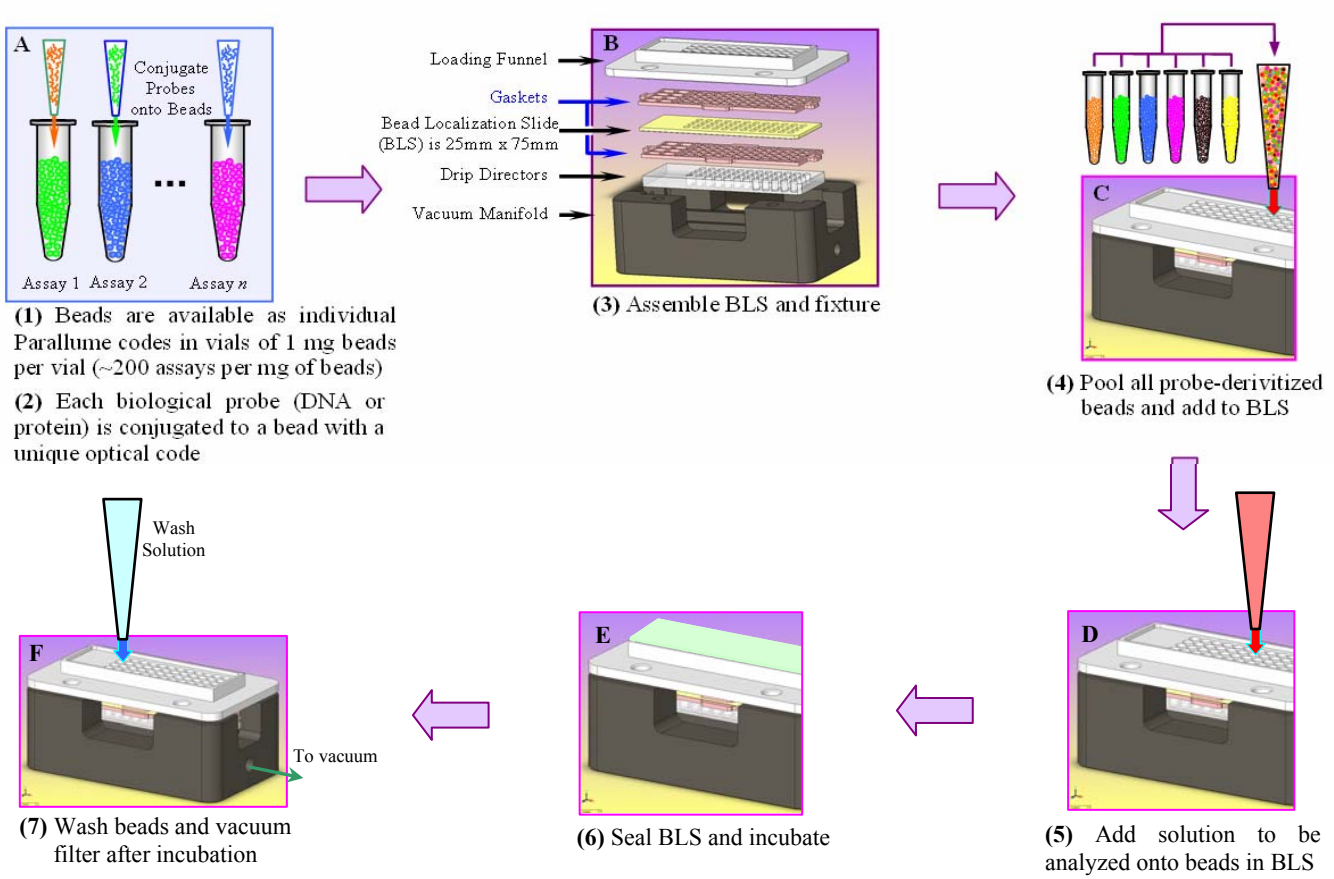

Fig. 10 The process flow for a multiplexed assay on Parallume encoded beads using a BLS and fixture is shown. First, the required number of optical codes for the multiplex depth (i.e. the number of simultaneously assays to be performed) is selected and a different probe biomolecule is attached to each different optical code (A). The BLS (25mm x 75mm) and its fixturing (vacuum holder, gaskets, funnel, drip directors) are assembled (B) where a multiplex assay will be loaded into each of the 48 wells (12 x 4 = 48 wells placed on 4.5mm centers shown). The beads prepared in (A) are pooled (C) and mixed with the sample on which the multiplex assay will be performed and the beads/solution added (D) to one of the 48 wells on the fixture while an additional 47 multiplexed assays are loaded into the remaining 47 wells on the BLS. The fixture containing the BLS is sealed (E) and the solution and beads are incubated before washing the beads with one or more solutions upon completion of the reaction (F). After drying, and optionally affixing the beads into the BLS with permanent or temporary adhesives, the BLS is removed from the fixture and imaged in the Multiplex Assay Reader System (G), or other instrument capable of reading the optical signatures and Reporter dyes emissions on the beads, and optically analyzed to determine the outcome of the diagnostic assay or other reaction.