

lymphocytes, bacterial cells, recombinant cells and cells infected with a pathogen. Further, although this section describes analysis of cells, the reader will appreciate that the same methods can be used for analysis of other biological entities, such as viruses and organelles.

**[0167]** In one aspect, the method includes partitioning a sample comprising a plurality of separable cells into at least  $10^3$  separate reaction chambers in an MPD, where after partitioning at least two chambers comprise exactly one cell each. Often the sample is partitioned into at least  $10^4$  separate reaction chambers, at least  $2 \times 10^4$  separate reaction chambers or at least  $3 \times 10^4$  separate reaction chambers. The number of cells introduced into the MPD and/or the number of chambers in the MPD are selected so that most or virtually all of the chambers contain either no cells or a single cell. This can be determined from the Poisson distribution (based on the number of chambers in the device and number of cells injected into the device) or empirically (e.g., by detecting the number of chambers that contain cells). Usually at least 90% of the chambers contain zero or one cell, often at least 99% of the chambers contain zero or one cell, and in some cases virtually all of the chambers contain zero or one cell.

**[0168]** Each of the plurality of chambers contains the same reagents for conducting the analysis. All or some of these reagents can be added to the sample or cells prior to injection into the device and/or can be prepositioned in the chambers and/or provided in inactive form, as described above. Because the reagents are constant, any chamber-to-chamber differences in analytical results are due to the presence of different cells (or no cell) and reflect differences in the properties of the cells. By detecting different signals from different chambers, a property or properties of cells in chambers can be determined and compared. This method finds a variety of applications in which it is informative to determine that a sample contains a cell having two or more properties detectable in separate assays. This method also finds a variety of applications in which the cell of interest is a rare cell in a background of many other cells.

**[0169]** The nature or type of reagents used will depend on the type of assay contemplated and specific properties to be detected. Generally the properties that can be assayed can be divided into two groups: properties determined based on the presence or absence of a nucleic acid target sequence and properties determined based on something other than the presence or absence of a nucleic acid target sequence. In many applications both a nucleic acid analysis and detection of a different type of property are carried out.

**[0170]** In embodiments in which the analysis of cell properties includes detecting a nucleic acid sequence (i.e., one, two or more target sequences are detected for an isolated cell) reagents suitable for nucleic acid analysis include those used for nucleic acid amplification (including but not limited to the PCR, SPIA, Invader, and other amplification methods described in this disclosure or known in the art) and those used for detection (including but not limited to, FRET based methods and other detection methods described in this disclosure or known in the art). In one embodiment the UA amplification/detection methods described in Section C (v) are used.

**[0171]** Methods are known in the art for assay of a multitude of cell properties other than or in addition to the characteristics of nucleic acids. For example, proximity ligation and FRET-based assays can be used to detect the presence of proteins or epitopes in a cell; presence, activation or change in

enzymatic activities; intracellular organelle function; pathogen (e.g., viral) infection; intracellular signaling; protein-protein interactions; protein-DNA interactions; colocalization of proteins cell cycle; metabolic reactions such as generation of reactive oxygen species; mitochondrial membrane potential; apoptosis; intracellular organelle function; changes in representations of cell types in cell populations; and subcellular localization of macromolecules.

**[0172]** In some embodiments of the invention the cell(s) is destroyed in the course of the process of detecting the cell property. Alternatively, numerous fluorescence based assays that can be carried out on living cells can readily be adapted for use in the present invention and/or reagents used in such assays may be used in the methods of the present invention. See, e.g., Dirks et al., 2003, *Visualizing RNA molecules inside the nucleus of living cells*, *Methods*, 29:51-7; Santangelo et al., 2004, *Dual FRET molecular beacons for mRNA detection in living cells*, *Nucleic Acids Res.*, 32:e57; Awais et al., 2004, *A genetically encoded fluorescent indicator capable of discriminating estrogen agonists from antagonists in living cells*, *Anal Chem.*, 76:2181-6; Nohe et al., 2004, *Analyzing for co-localization of proteins at a cell membrane*, *Curr Pharm Biotechnol.*, 5:213-20; Thoren et al., 2004, *Membrane binding and translocation of cell-penetrating peptides*, *Biochemistry*, 43:3471-89; Balaji et al., 2004, *Live cell ultraviolet microscopy: a comparison between two- and three-photon excitation*, *Microsc Res Tech*, 63:67-71; Gardiner, 2002, *Spatial and temporal analysis of Rac activation during live neutrophil chemotaxis*, *Curr Biol.*, 12:2029-34; Moshinsky et al., 2003, *A Widely Applicable, High-Throughput TR-FRET Assay for the Measurement of Kinase Autophosphorylation: VEGFR-2 as a Prototype Journal of Biomolecular Screening*, 8:447-452; U.S. Pat. Nos. 4,822,733, 5,622,821, 5,639,615, and 5,656,433 [describing the Invitrogen LanthaScreen™ TR-FRET Kinase Assays for Tyrosine and Serine/Threonine Kinases]; Zhang et al., (2004), *Detection of mitochondrial caspase activity in real time in situ in live cells*, *Microsc Microanal.*, 10:442-8; Martin-Fernandez et al., 2004, *Adenovirus type-5 entry and disassembly followed in living cells by FRET, fluorescence anisotropy, and FLIM*, *Biophys J.*, 87:1316-27; Zorov et al., 2004, *Examining intracellular organelle function using fluorescent probes: from animalcules to quantum dots*, *Circ Res.*, 95:239-52; Mongillo et al., 2004, *Fluorescence resonance energy transfer-based analysis of cAMP dynamics in live neonatal rat cardiac myocytes reveals distinct functions of compartmentalized phosphodiesterases*, *Circ Res.*, 95:67-75, Chigaev et al., 2004, *Conformational regulation of alpha 4 beta 1-integrin affinity by reducing agents. "Inside-out" signaling is independent of and additive to reduction-regulated integrin activation*, *J Biol Chem.*, 279:32435-43; Zaccolo et al., 2004, *Use of chimeric fluorescent proteins and fluorescence resonance energy transfer to monitor cellular responses*, *Circ Res.*, 94:866-73; Nohe et al., 2004, *Analyzing for co-localization of proteins at a cell membrane*, *Curr Pharm Biotechnol.*, 5:213-20; Thoren et al., 2004, *Membrane binding and translocation of cell-penetrating peptides*, *Biochemistry*, 43:3471-89; Balaji et al., 2004, *Live cell ultraviolet microscopy: a comparison between two- and three photon excitation*, *Microsc Res Tech.*, 63:67-71; Gardiner, 2002, *Spatial and temporal analysis of Rac activation during live neutrophil chemotaxis*, *Curr Biol.*, 12:2029-34.

**[0173]** In addition, many cell-based assays developed for use in Laser Scanning Cytometry technology (e.g., Com-