[0152] The appearance of chambers fluorescening blue indicates that there is at least one bacterial pathogen present in the sample. The appearance of chambers fluorescening green indicates that there is at least one fungal pathogen present in the sample. The appearance of chambers fluorescesing red indicates that there is at least one viral pathogen present in the sample.

[0153] If none of the chambers is emits blue, green or red fluorescence when illuminated at the proper wavelengths this is an indication that none of the pathogens tested for are present (typically assays would also include a positive control). If, however, red fluorescence was detected, this would be an indication that a virus is present. Further testing could then be carried out to identify the viral pathogen. For further testing, the values of the MPD are opened and sub-samples allowed to pool by diffusion or active mixing, producing the amplicon pool. A portion of the resulting amplicon pool is withdrawn from the MPD and divided into fifty (50) aliquots each containing reagents for identification of one of the 50 viruses assayed for in the initial part of the screen. An exemplary detection method uses 50 molecular beacons (one in each aliquot) that each recognize a different virus specific sequence in the amplicon. By determining which of the molecular beacons bind a target sequence present in the amplicon pool (or the portion contained in an aliquot) the identity of the pathogen is determined.

[0154] 4. Additional Applications

[0155] It will be appreciated to the reader that the methods of the invention can be used in many applications not specifically described, including, for example and not limitation, detection of gene mutations (substitutions, deletions, translocations, amplifications, etc.) in samples from cancer patients and others. Many of these assays can be carried out without using the optional pooling step and subsequent analysis steps.

D. Partitioning, Detection and Analysis of Proteins and Other Biomolecules

[0156] Proteins and other biomolecules can be partitioned in a manner analogous to that described above for nucleic acid molecules. Any suitable method can be used to produce an amplification product indicative of the presence of a protein. In one method, a proximity ligation procedure is used. The proximity ligation procedure is analogous in certain respects to the use of padlock probes, described above in Section C, but is used for detecting proteins and other analytes. The proximity ligation procedure uses specific protein binding agents linked to oligonucleotides. Examples of specific protein binding agents include, but are not limited to, antibodies (defined as any specific binding agent comprising a CDR, including phage display antibodies, single chain antibodies, monoclonal antibodies, and the like) and nucleic acid aptamers. The proximity ligation procedure is described in Landegren et al., 2003, supra; Landegren et al., 2004, Molecular tools for a molecular medicine: analyzing genes, transcripts and proteins using padlock and proximity probes, *J Mol Recognit.* 7:194-7; Gullberg et al., 2004, Cytokine detection by antibody-based proximity ligation, *Proc Natl Acad Sci USA* 101(22):8420-4; Fredriksson et al., 2002, Protein detection using proximity-dependent DNA ligation assays, *Nat Biotechnol.* 20:448-9; and Landegren, 2002, Methods and kits for proximity probing *United States Patent Application* 20020064779. Briefly, a pair of protein binding agents that recognize different epitopes of a target protein are used. Each of the binding agents is attached (e.g., via streptavidin-biotin linkage) to a synthetic DNA strand that includes a PCR primer binding site. The synthetic DNA strands are brought into proximity when both binding agents bind the same target molecule. A connector oligonucleotide that hybridizes to sequences at the ends of both of the synthetic DNAs is added in excess, bringing termini of the DNA strands together so that they can be joined by ligase. In the presence of PCR reagents and primers that recognize the primer binding sites on the two DNA strands, a region of the ligated sequence may be amplified and detected by real time PCR. In contrast, unligated strands are not amplified and therefore not detected in the assay.

[0157] It will be appreciated that the assay also may be used to assay for non-protein molecules that are specifically bound by a nucleic acid aptamer, antibody or other binding agent. Proximity ligation methods can also be used to detect nucleic acid targets. In this approach, nucleotide sequences complementary to the target are used rather than protein binding agents.

E. Partitioning of Cells

[0158] In another aspect of the invention, individual cells are isolated by partitioning using a MPD, and one or more properties of one or more of the individual cells are determined. Using this method, analysis of individual cells can be carried out without background from other cells in a sample.

[0159] Virtually any property of an individual cell can be assayed. For illustration, cell properties include

[0160] the presence or absence of a target nucleic acid sequence in the cell (where the nucleic acid is RNA or DNA; recombinant or naturally occurring; cellular or viral; nuclear, cytoplasmic or from an organelle);

[0161] the presence or absence of a protein or epitope in the cell or on the cell surface;

[0162] secretion by the cell of a protein or non-protein molecule, for example in response to a stimulus;

[0163] metabolic reactions or changes in cell metabolism, for example in response to a stimulus;

[0164] other properties of cells (which will be recognized by those of skill in the art);

[0165] combinations of two, three, or more than three different properties (e.g., the presence in a cell of two different target nucleic acid sequences; the presence in a cell of a target nucleic acid sequence and a protein epitope; a change in a metabolic property of a cell and expression of a nucleic acid sequence in the cell; a cell surface epitope and secretion of a cytokine by the cell in response to a stimulus, etc.).

[0166] For this analysis, a liquid sample containing a plurality of separable cells is introduced into the MPD and the cells partitioned. A "separable cell" is a cell that is physically separated from other cells and can be partitioned into a chamber without other cells. In some cases (e.g., blood cells, lymphocytes, spermatocytes, oocytes, yeast, certain bacteria or other microorganisms) seperable cells can be obtained from a patient or other source with little processing. In other cases (e.g., liver biopsy, cultured cells, blastocyte) it will be necessary to disrupt a tissue or aggregate mechanically, enzymatically, or using other methods well known in the art. See, e.g., Ausubel et al., 2004, Current Protocols In Molecular Biology, Greene Publishing and Wiley-Interscience, New York; Chapter 25. Examples of cells that can be assayed in this method include eukaryotic cells, human cells, animal cells, plant cells, fetal cells, embryonic cells, stem cells, blood cells,