either invasive medical testing on the apparently healthy subjects, or else wait a sufficient amount of time to be sure they do not manifest some type of carcinoma over subsequent years.

[0008] Various formats have been used in the prior art to present patient samples for assays. Those formats have included fluidic systems, such as flow cytometry based systems, and static systems. FIG. 1 shows a plan view of a three well plate as used in prior art static imaging systems. A slide includes three equally sized well regions. By way of example, each well region 10 is a 1.45 cm square. The resulting total well area for the slide is 6.3 cm^2. The periphery of the wells is 17.4 cm. The percentage of the overall slide occupied by the three wells is 23.6%. An estimated number of cells per slide is in the range from 1.25 to 1.5 million cells.

[0009] FIG. 2 shows a plan view of a twelve well plate as used in the prior art. A slide includes twelve equally sized well regions 12. By way of example, each well region is a circle with a 0.5 cm diameter. The resulting total well area for the slide is 2.4 cm^2. The periphery of the wells is 19 cm. Finally, the percentage of the overall slide occupied by the three wells is 12.8%. An estimated number of cells per slide is in the range from 500 to 600 thousand cells.

[0010] Despite intensive effort, the detection of CTCs has heretofore remained challenging. What is required is a sensitive, specific system which can efficiently, expeditiously and inexpensively detect CTCs.

SUMMARY OF THE INVENTION

[0011] Apparatus, system and method are provided for the identification of various objects, particularly CTCs.

[0012] As such, in one aspect, the invention provides a system for assaying cells. The system includes a well of the present invention, an illumination system, an imaging system, an analysis module having functionality for analyzing cell selection criteria, and a user output. In ~~embodiments~~some examples the system may include, but is not limited to, a scanning system, an image storage system, and an analysis system The analysis system preferably identifies desired objects, such as complete cells, based on various criteria, which may include cell nuclear area or volume, CD-45 negative status, and cytokeratine positive status. Preferably included is a slide bearing a well for containing the cells during the imaging step, the well including a planar bottom surface, a border at the periphery of the well defining sides for the

well, the border being adjacent the bottom surface of the well and providing a fluidic seal there between.

[0013] In another ~~aspect~~example, ~~the invention provides~~disclosed herein is a well for assaying cells which is disposed on a surface of a substrate. The well includes a planar bottom surface, and a border forming a periphery of the well, the border being adjacent the bottom surface and providing a fluidic seal therebetween. In one aspect, the present invention refers to a well for assaying cells which is disposed on a surface of a substrate, the well comprising:

a) planar bottom surface; and

b) a border forming a periphery of the well, the border being adjacent the bottom surface and providing a fluidic seal therebetween, wherein the well is configured to received a monolayer of at least 1.5 million cells within the border, and wherein the planar bottom surface of the well has an area of at least 7.0 cm^2. ~~Embodiments of the invention~~Examples disclosed herein provide for a single imaging well, providing for substantially a monolayer of objects, e.g., cells. The well has an area preferably greater than 7.5 cm^2, more preferably greater than 10 cm^2, and most preferably substantially 11.7 cm . The perimeter of the well is preferably substantially 12.5 cm, more preferably substantially 14.5 cm and most preferably substantially 15.7cm, correspondingly. The percentage of the top surface of the slide covered by the well is preferably substantially 40%, more preferably 53% and most preferably substantially 62%. The wells are sized so as to permit the imaging of a monolayer of preferably 1.6 to 1.9 million cells, more preferably 2.1 to 2.6 million cells, and most preferably, 2.5 to 3 million cells, respectively. The preferred imaging wells have a total of four sides. By reducing the number of sides (as compared to the prior art, for example, the 3 well slide has 12 sides) and their perimeter, the edge effects associated with the side wall boundary are minimized.

[0014] In one implementation, the approach used herein to identify CTCs in high definition (HD-CTCs) is distinct in that it does not rely on any single protein enrichment strategies. Instead, all nucleated cells are retained and immunofluorescently stained with monoclonal antibodies targeting cytokeratin (CK), an intermediate filament found exclusively in epithelial cells, a pan leukocyte specific antibody targeting CD45, and a nuclear stain, DAPI. The nucleated blood cells are imaged in multiple fluorescent channels to produce high quality and high resolution digital images that retain fine cytologic details of nuclear contour and cytoplasmic distribution. This enrichment-free strategy results in high sensitivity and high specificity, while adding high definition cytomorphology to enable detailed morphologic

characterization of a CTC population known to be heterogeneous. An advantage of this approach is that multiple analysis parameters can be pursued to identify and characterize specific populations of interest.

[0015] ~~Embodiments of the instant inventions~~Examples described herein have been used to assay samples using "HD-CTCs" in metastatic cancer patients. The key innovative aspects of this assay are its simplicity, with minimal processing to the blood specimen, and its ability to enable

professional morphologic interpretation with diagnostic pathology/cytopathology quality imagery.

[0016] In yet another aspect, the invention provides a method for performing a cellular assay. The method includes contacting a sample having a population of cells with the well of the present invention, and analyzing the population of cells via the system of the present invention, thereby preforming the cellular assay. In ~~embodiments~~examples, the analysis includes characterization of cell types within the population of cells, such as CTCs.

[0017] In yet another aspect, the invention provides a method of detecting a CTC in a sample. The method includes contacting the well of the present invention with the sample, analyzing the population of cells via the system of the present invention; and detecting a CTC based on the analysis, thereby detecting a CTC in the sample. In ~~embodiments~~examples, more than 2, 5,7, 10, 15, 20 or 50 circulating tumor cells are detected per ml of sample.

[0018] In yet another aspect, the invention provides a method for diagnosing cancer or providing a prognosis for cancer in a subject. The method includes contacting a well of the present invention with a sample including a population of cells from the subject, analyzing the population of cells via the system of the present invention, detecting a CTC in the cell population, characterizing the CTC, and determining a diagnosis or prognosis based on the characterization, thereby diagnosing or providing a prognosis for cancer in the subject.

[0019] In yet another aspect, the invention provides a method for determining responsiveness of a subject to a chemotherapeutic regime. The method includes contacting the well of the present invention with a sample including a population of cells from a subject, analyzing the population of cells via the system of the present invention, detecting a CTC via the analysis, and characterizing the CTC to determine efficacy of administration of a chemotherapeutic agent, thereby determining responsiveness of the subject to the therapeutic regime.

[0020] In yet another embodiment the invention provides a kit. The kit includes at least one well of the present invention, reagents for immunologically determining the presence of cytokeratin or CD45 in a cell, and instructions for utilizing the kit the detect a CTC in a sample.

[0021] The apparatus, systems and methods described herein demonstrate the first use of the HD-CTC assay in a controlled prospective protocol which addressed the reliability and robustness of the assay, compares sensitivity in a split sample comparison with the Cellsearch® assay, and establishes the incidence of HD-CTCs and HD-CTC clusters in

patients with metastatic breast, prostate, and pancreatic cancers as well as normal controls. Importantly, the definition of "HD-CTC" preferably requires one or more of the requirements that the cell(s) have an intact nucleus, express cytokeratin and not CD45, be morphologically distinct from surrounding benign white blood cells (WBCs), and have cytologic features consistent with intact morphologically abnormal epithelial cells suitable for downstream analysis.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] FIG. 1 is a plan view of a prior art 3 well plate.

[0023] FIG. 2 is a plan view of a prior art 12 well plate.

[0024] FIG. 3 is a functional block diagram of the overall HD CTC system.

[0025] FIG. 4 is a component level diagram of the scanning and imaging components of the system.

[0026] FIG. 5 shows possible image slices through the measured parameter space, representing filtering of cell based on these parameters.

[0027] FIG. 6 is a plan view of a representative slide and well.

[0028] FIG. 7 is a perspective view of a representative slide and well.

[0029] FIG. 8 shows the mean observed SKBR3s plotted against expected SKBR3s.

[0030] FIG. 9 shows a comparison of CTC counts between two separate processors on 9 different cancer patient samples. CTC/mL counts ranged from 0 to 203.

[0031] FIG. 10 shows comparative test data of the systems, apparatus and methods described here, versus the CellSearch® product.

[0032] FIG. 11 shows test results graphing the quantity of CTCs for various samples, for prostate, pancreatic, breast tumors, and a comparison to healthy population.

[0033] FIG. 12 shows the quantity of CTCs for various patient samples relative to breast cancer.

[0034] FIG. 13 shows the normalized nuclear area versus nuclear area for white blood cells (WBCs) and CTCs, including a blow-up of the base-line region.

DETAILED DESCRIPTION

[0035] Before the present compositions and methods are described, it is to be understood that this invention is not limited to particular compositions, methods, and experimental conditions described, as such compositions, methods, and conditions may vary. It is also to