

1. Multiple compartments permit particles, e.g. biological particles (i.e. cells and chromosomes), to be separated and/or combined for microexperiments;
2. The compartments are connected by channels having dimensions which are large enough to manipulate particles, but small enough to substantially preclude particle drifting;
3. The compartments are connected to external ports to allow the introduction and circulation of experimental fluids, e.g. cell and chromosome suspensions, culture media, and reagents;
4. The chamber is enclosed by optical windows of a quality effective to permit particle trapping and imaging at the selected wavelengths;
5. The chamber compartments and channels are shallow to allow concentrated samples to be processed and analyzed;
6. The chamber design provides efficient heat transfer between the compartments and external temperature control devices.

Referring now to FIG. 1, there is shown a laser and optics system 10 in accordance with one embodiment of the present invention. Manipulation chamber 12, hereinafter discussed for FIGS. 2-7, has trapping laser beams 14a, 14b from laser beam 14, split by beam splitter 16 and directed by dichroic mirrors 18, 20 through chamber 12. Alignment camera 22 observes a portion of the beams in the optical loop to maintain the two beams in coaxial alignment and for adjusting the distance between the two beam waists formed by microscope objectives 24, 26 for trapping particles in chamber 12. An imaging system formed of an illumination source 28, mirrors 28, 30, zoom lens 32, and imaging camera 34 enable a video display to be formed for the operator to direct particle movement within chamber 12 and to observe the progress of the microexperiments.

It will be appreciated that laser beam 14 is stationary in the preferred embodiment and manipulation chamber 12 is positioned to effect the relative movement of particles trapped by beams 14a, 14b within chamber 12. Thus, the laser optics are simplified and large relative movements of the particles within chamber 12 can be obtained.

Manipulation chamber 12 is comprised of a central channel section 42 (FIG. 2), upper window 92 (FIG. 4), a lower window (not shown), inner holder 110 (FIGS. 5 and 6), and an outer shell 140 (FIG. 7). Central channel section 42 is sandwiched between upper window 92, which may provide structural support and external ports for particle and fluid access, and a lower window for sealing. The resulting assembly is mounted in holder 110 for clamping the assembly, including central section 42, providing temperature sensor access 116, and providing a piezoelectric crystal 128 for dislodging particles within central section 42. An outer shell 140 connects to the positioning stage and pumps heat between the chamber and the surrounding environment.

Central channel section 42, depicted in FIG. 2, is a sheet 44 of material that defines manipulation compartments 74-78, interconnecting channels 82-86, process flow channels 58-62, and external ports 46-55. The number and configuration of compartments and channels is exemplary only, and is not intended to limit the scope of this invention. Sheet 44 may be formed from stainless steel shim stock and machined with a suitable laser beam, or may be formed from ceramic sheet which is photoetched to produce the desired flow configurations. Photoetching permits more precise machining to

be done but the ceramic stock can only be lapped to a thickness of about 250 micrometers, while the stainless steel shim stock can be obtained with a thickness of about 100 micrometers. Sheet 44 further defines connection holes 72 (ten places) for glue injection close to manipulation area 68 and fiducial marks 88 for calibrating the optical manipulator video and position control systems.

Process flow channels 58-62 enable various particles and fluids to be injected and removed through selected external ports 46-55. As shown in FIG. 3, particles are maneuvered in manipulation area 68 through flow channels 58, 60, 62 into manipulation compartments 74, 76, 78, respectively. Compartments 74, 76, 78 define volumes suitable for microexperiments containing a selected number of particles or for introducing test fluids, e.g. culture media or reagents, adjacent the particles to be tested. Connections between experimental compartments is controlled through interconnection channels 82-85 by the use of valve compartments 75, 77 which may be bubble valves operated by the introduction of a suitable fluid through channels 59, 61, respectively, using positive displacement pumps or syringes. Exemplary dimensions are shown in Table A.

TABLE A

Process Flow Channels 58-62	0.114 mm wide
Manipulation Compartment 74, 76, 78	0.305 mm × 0.305 mm
Valve Compartment 75, 77	0.200 mm × 0.200 mm
External Ports 46-55	1.02 mm dia.

In one embodiment, the window cover sheets for central section 42 may include electrode surfaces for applying an electric field within one or more of the manipulation compartments 74, 76, and 78 for use in experimental techniques, such as cell electrofusion and electroporation. One possible electrode configuration is schematically shown in partial cross section in FIG. 3A. Electrode area 65 is formed on window 90 within an area defined by manipulation chamber 73 formed in flow sheet 44. Connecting electrode pad 67 is also formed along an edge of window 90 and is connected through conducting strip 66 with electrode area 65. An edge notch 64 is formed in flow sheet 44 for connecting pad 67 with external circuitry.

It will be appreciated that flow sheet 44 is formed of an insulating material, such as ceramic, for use with electroded surfaces on window 90. Electroded surfaces 65, 66, and 67 are conventionally formed, e.g. by vacuum deposition and photolithography techniques, and are preferably of a transparent material, such as gold or platinum, in the required thickness. Electrode surfaces may be formed on either one or both of the window surfaces covering any given manipulation chamber 73 and connecting pads 67 formed at any convenient location about the perimeter of manipulation chamber 12 (FIG. 1).

An isometric view of an upper window for covering central flow sheet 44 is shown in isometric view in FIG. 4. Upper window 92 serves as a structural support for central channel section 42 while providing optical access to sheet 42 for imaging and optical trapping. The embodiment shown in FIG. 4 includes holes 96 for introducing glue for attaching window 92 to sheet 42. Window 92 includes external port assemblies 98 having connectors 106 (ten places as shown) in registry with external ports 46-55 in channel section 42. Connectors