

trates an embodiment of a pressure delivery system. One or more microtiter plates **210** are enclosed in a chamber **270**. A probe **222** to be assayed is contained within each reservoir or well **220** of the microtiter plate **210**. A free end of a capillary tube **100** connects to the well **220** such that it is in contact with the probe **222** which can be dispersed in a fluid form. Multiple such capillaries are bundled at an end **110** distal from the probes **222** to form delivery head **250**.

[**0087**] In one embodiment, compressed air or an inert gas such as nitrogen **280** is pumped into a sealed chamber **270** carrying the microtiter plates. The probes **222** from microtiter plate **220** are forced by hydraulic pressure through the capillary tube to the print head **250**.

[**0088**] In an alternative configuration, the output ends of the capillaries **100** may be placed under a vacuum or a lower pressure than the reservoirs **220**. The print head **250** and substrate holder may be placed within a vacuum chamber, and the capillaries may extend through a wall of the vacuum chamber and to the reservoirs **220**. By lowering the air pressure at the bundled delivery head **250** relative to the pressure at the input end, fluid can be drawn from the reservoirs **220** to the print head **250**.

[**0089**] Once the capillaries are filled with the probe fluids, a constant flow can be maintained and controlled by adjusting the vertical positions of the fluid reservoirs, e.g. the microtiter plates, with respect to the position of the reaction chamber. In the gravity delivery system illustrated in **FIG. 5**, the chemical compounds **222** are dispersed in the wells of a microtiter plate **320**. Capillaries **310** connect at the input end to the microtiter plate **320** and form a delivery head **300** at the output end. By positioning the microtiter plate at a height **340** above the head **300**, differential gravitational force is used to siphon the chemical compound from the wells of the microtiter plate **320** to the end of the delivery head **300**. The height differential may be transiently operated such that once the compound reaches the end of the reaction/delivery head **300**, further flow is ceased by eliminating the height differential. Thus the flow of the chemical compound may be controlled merely by altering the height of the microtiter plate **320** relative to the reaction/delivery head **300**.

[**0090**] Because fluids are negatively or positively charged, a voltage applied between the reservoir and the reaction chamber can be used to control the flow of the fluid through electrostatic and electro-osmotic force (EOF). A voltage source may be connected to an electrically-conductive material on a facet of the input end **102** and to an electrically conductive material contacting the probe-containing liquid near the output ends of the capillary tubes **100**. A voltage regulator may be used to regulate the voltage and thus the rate of deposition of probe molecules.

[**0091**] Another aspect of the invention may have a bundled end, a plurality of reservoirs, and a magnetic field generator that is positioned sufficiently close to the bundled end to move a magnetic probe-containing fluid (such as a fluid containing magnetic beads or paramagnetic beads having probes optionally attached to their surfaces) through the capillaries of the bundle.

[**0092**] II. Probe Immobilization

[**0093**] Immobilization of probe molecules on the substrate is used in preparing a variety of array embodiments of this

invention. Various methods for surface attachment chemistries can be used, as described below.

[**0094**] A. Protected-Aldehyde Silanization Agents

[**0095**] In conventional methods, a surface functionalized aldehyde slide having surface immobilized functional groups with terminal aldehyde groups for attachment of polynucleotides or other biomolecules is prepared in a two-step method consisting of immobilization of an aminoalkyl silane on a substrate to provide terminal amino groups, followed by conversion of the terminal amino groups with glutaraldehyde to terminal aldehyde groups. However, such conventional methods may result in numerous undesired defects and side products, including residual amino groups and unreactive condensation products.

[**0096**] In one embodiment of this invention shown in **FIG. 6A**, a protected aldehyde silane is prepared and used to functionalize a substrate in a one step silanization reaction. Substrates functionalized in this reaction have no residual amino groups, and substantially lack non-aldehyde by-products. An acetal compound comprising a protected aldehyde is prepared by hydrosilylation reaction of triethoxysilane with an alkenyl acetal. A variety of carbon numbers for the alkenyl group may be utilized, providing a variety of alkyl chains for use as a spacer between the silane group and the acetal group, including isomeric mixtures of alkyl chains. The spacer group may also be a polymer or chemical group. The protected aldehyde product may be immobilized on substrates such as glass slides in a one step silanization reaction. The resulting substrate is functionalized with protected aldehyde groups that may be deprotected to provide a surface functionalized by aldehyde groups. Alternatively, a non-protected aldehyde silane may be prepared by hydrosilylation reaction of triethoxysilane with an alkenyl aldehyde. The silane aldehyde may be utilized in combination with the protected aldehyde product to functionalize a substrate.

[**0097**] A substrate may be functionalized with the protected aldehyde silane by a variety of techniques. For example, solution phase reaction of the protected aldehyde silane with the substrate surface may be used. Alternatively, vapor phase deposition of the protected aldehyde silane on the substrate surface may be used. In another embodiment, the substrate is cured after reaction of the protected aldehyde silane with the substrate. Curing may be performed over a wide range of temperatures for a period as long as one day, or longer. These conditions and techniques are well-known to those in the field.

[**0098**] The protected aldehyde silane of the functionalized substrate may be deprotected by a variety of reactions to produce active aldehyde groups. Deprotection may be performed with, for example, trifluoroacetic acid or hydrochloric acid, among others, resulting in a reactive surface aldehyde slide. Such slides are useful for attachment of polynucleotides and other biomolecules, for example, having amino linking groups.

[**0099**] B. Maleimide Silanization Agents

[**0100**] Another composition and method for immobilization of reagents and molecules on the substrate are functional linker groups. In conventional methods, a surface functionalized slide is first prepared having attached functional linker groups with known ability to link, for example,