

[0170] The poly-avidin layers were further characterized by using avidin that was labeled with an electrochemiluminescent label (on average 0.3 Sulfo-TAG NHS labels per protein). The electrodes were treated with one of three solutions: (i) 75 ug/mL avidin, (ii) 75 ug/mL avidin and 25 ug/mL BSA or (iii) 75 ug/mL avidin and 25 ug/mL biotin-BSA. All the solutions contained 0.0035% Triton X-100. The electrodes were washed with water, immersed in MSD Assay Buffer and ECL was measured. The electrode treated with all the components of poly-avidin (avidin and biotin-BSA) gave an ECL signal (**150981**) that was roughly twice that observed for avidin alone (**85235**) or avidin with unlabeled BSA (**65570**), demonstrating that cross-linking was required for the improved performance of poly-avidin. It was also observed that the intensity of ECL was much more evenly distributed across the electrode for the poly-avidin electrodes than for the other electrodes.

[0171] In a different experiment the labeled and immobilized avidin or poly-avidin layers were i) not washed or ii) exposed to a solution containing BSA for 2 hours and then extensively washed with phosphate buffered saline. In this experiment, the avidin concentration was 0.5 mg/mL, the ratio of avidin to biotin-BSA was 16:1 and the labeled avidin was mixed with unlabeled avidin (at a 1:100 ratio) to reduce the overall signals. The experiment was carried out on both non-treated electrodes and electrodes that were treated with an oxygen plasma. The table below shows that the use of poly-avidin substantially reduced the loss of avidin from the surface after extensive washes and exposure to protein-containing solutions.

	Unmodified Electrodes				Plasma-Treated Electrodes			
	Avidin		Poly-Avidin		Avidin		Poly-Avidin	
	Signal	% Left	Signal	% Left	Signal	% Left	Signal	% Left
No Wash	21,107		26,618		10,871		18,512	
Wash	9,545	45	18,845	71	3,332	31	14,024	76

[0172] After immobilizing assay reagents on surfaces for use in solid phase assays (e.g., by applying solutions comprising the assay reagents to the surfaces, most preferably, by patterned depositions of these solutions to form an array of assay domains comprising the assay reagents), assay performance is often improved by washing the assay electrodes to remove unbound assay reagents. This washing step is particularly important when unbound assay reagent may interfere with an assay (e.g., unbound antibodies may interfere by competing with the capture of analytes to antibodies on the surface). Preferably, this washing step is carried out using a procedure that minimizes the ability of unbound reagents to adsorb in other undesirable locations. For example, after immobilization of an antibody on an assay domain on an electrode in an assay module, the washing step will preferably minimize the adsorption of unbound antibody to non-electrode surface (antibody adsorbed on non-electrode surfaces interfering with binding assays by competing for the binding of analyte with antibody immobilized on the electrode). Even more importantly, in array type

measurements involving a plurality of assay domains specific for different analytes of interest, the washing step should

[0173] minimize the diffusion of an unbound assay reagent from one assay domain and its adsorption on a different assay domain (this process leading to assay cross-talk).

[0174] We have found that we can prevent the undesired adsorption of assay reagents outside pre-defined locations by localized washing of assay domains using a concentric tube dispense/aspirate fixture. **FIGS. 7a** and **7b** depict one embodiment wherein a washing fixture was constructed that consists of a single concentric tube structure which may be used to wash a single assay domain in an assay module or to sequentially wash multiple assays domains in an assay module by positioned the concentric tube structure over each assay electrode. It should be understood, however that the invention is not limited to a single concentric tube device but can, preferably, employ an array of concentric tubes, preferably, arranged in the same pattern and spacing as the assay domains. Preferably, wash fluid is dispensed through inner tube **705** and aspirated through outer tube **710**. In operation, as the fluid transitions from the inner tube to the outer, it preferably passes over the assay domain surface, washing the assay domain in an area confined by the diameter of the outer tube. The figure shows the concentric tube being used to wash a carbon ink electrode **720** patterned on substrate **730**, the exposed surface of electrode **720** being defined by patterned dielectric layer **725** which acts as a boundary to form a fluid containment region on electrode **720**. By analogy, the concentric tubes may be used to wash assay

domains on a variety of other surfaces, the assay domains being preferably but not necessarily defined by a fluid boundary. The tubes are preferably configured so that the outer tube removes fluid with a high enough efficiency so as to prevent the spread of fluid to regions outside the domain being washed. In alternate embodiments, the functions of the inner and outer tubes may also be reversed such that the wash fluid is dispensed through the outer tube, and aspirated up the center via the inner tube. These arrangements of tubes prevent unbound assay reagents on the assay domains from contacting other surfaces of the assay module.

[0175] In another alternate embodiment, a tube structure having three concentric tubes is used to pattern and wash assay reagents on assay domains. A first tube (preferably the inner tube) is used to microdispense assay reagents on an assay domain. This tube is preferably linked to a low volume fluid dispensing controller such as a microsyringe (optionally, having a solenoid valve flow controller) or piezoelectric dispenser. The second tube (preferably the middle tube) is used to dispense bulk washing reagents on the assay domain. The third tube (preferably the outer tube) is used to aspirate