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FILING RECEIPT

Date Mailed: 01/08/2007

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The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US60/874,561**

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Early Publication Request: No

** SMALL ENTITY **

Title

Methods of generating library emulsions

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR § 1.53(c)

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☐	Yes, the name of the U.S. Government agency and the Government contract number are:		
METHOD OF PAYMENT			
☒	A check in the amount of \$100.00 (#23483) is enclosed to cover the filing fees of the Provisional application.		
☒	The Commissioner is hereby authorized to charge additional fees or credit any overpayment to Deposit Account No. 50-0311, Reference No: 28855-528 PRO B.		

Respectfully submitted,

Date: December 12, 2006

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TRA 2232511v.1

METHODS OF GENERATING LIBRARY EMULSIONS

5

FIELD OF THE INVENTION

The invention described herein pertains to methods of directly generating emulsions from libraries.

10

BACKGROUND

Microfluidic devices are essentially miniaturized chemistry laboratories, featuring a series of micrometer-sized channels etched into a chip in which nanoliter-sized samples of fluids can be analyzed. Such analyses can provide a wealth of information for
15 biomedical and analytical chemistry studies. Because of their incredibly small sample sizes — thousands of times smaller in volume than a typical droplet — microfluidic “labs on a chip” are highly prized for providing rapid analysis at relatively low costs.

High throughput molecular screening (HTS) is the automated, rapid testing of thousands of distinct small molecules or probes in cellular models of biological
20 mechanisms or disease, or in biochemical or pharmacological assays. Active compounds identified through HTS can provide powerful research tools to elucidate biological processes through chemical genetic approaches, or can form the basis of therapeutics or imaging agent development programs. HTS has experienced revolutionary changes in technology since the advent of molecular biology and combinatorial chemistry, and the
25 incorporation of modern information management systems. Current HTS instrumentation allows screening of hundreds of thousands of compounds in a single day at a rate orders of magnitude greater than was possible a decade ago. However, there are still bottlenecks which currently limit HTS capacity, such as (a) compound collection maintenance, tracking, and disbursement, and (b) rapidity, accuracy, and content of assay
30 instrumentation.

The manipulation of fluids to form fluid streams of desired configuration, discontinuous fluid streams, droplets, particles, dispersions, etc., for purposes of fluid delivery, product manufacture, analysis, and the like, is a relatively well-studied art. For example, highly monodisperse gas bubbles, less than 100 microns in diameter, have been

produced using a technique referred to as capillary flow focusing. In this technique, gas is forced out of a capillary tube into a bath of liquid, where the tube is positioned above a small orifice, and the contraction flow of the external liquid through this orifice focuses the gas into a thin jet which subsequently breaks into equal-sized bubbles via a capillary instability. A similar arrangement can be used to produce liquid droplets in air.

Microfluidic systems have been described in a variety of contexts, typically in the context of miniaturized laboratory (e.g., clinical) analysis. Other uses have been described as well. For example, International Patent Application Publication No. WO 01/89788 describes multi-level microfluidic systems that can be used to provide patterns of materials, such as biological materials and cells, on surfaces. Other publications describe microfluidic systems including valves, switches, and other components.

Precision manipulation of streams of fluids with microfluidic devices is revolutionizing many fluid-based technologies. Networks of small channels are a flexible platform for the precision manipulation of small amounts of fluids. The utility of such microfluidic devices depends critically on enabling technologies such as the microfluidic peristaltic pump, electrokinetic pumping, dielectrophoretic pump or electrowetting driven flow. The assembly of such modules into complete systems provides a convenient and robust way to construct microfluidic devices. However, virtually all microfluidic devices are based on flows of streams of fluids; this sets a limit on the smallest volume of reagent that can effectively be used because of the contaminating effects of diffusion and surface adsorption. As the dimensions of small volumes shrink, diffusion becomes the dominant mechanism for mixing leading to dispersion of reactants; moreover, surface adsorption of reactants, while small, can be highly detrimental when the concentrations are low and volumes are small. As a result current microfluidic technologies cannot be reliably used for applications involving minute quantities of reagent; for example, bioassays on single cells or library searches involving single beads are not easily performed. An alternate approach that overcomes these limitations is the use of aqueous droplets in an immiscible carrier fluid; these provide a well defined, encapsulated microenvironment that eliminates cross contamination or changes in concentration due to diffusion or surface interactions. Droplets provide the ideal microcapsule that can isolate reactive materials, cells, or small particles for further manipulation and study. However, essentially all enabling technology for microfluidic systems developed thus far has focused on single phase fluid flow and there are few equivalent active means to manipulate droplets requiring the development of droplet handling technology. While significant advances have been made

in dynamics at the macro-or microfluidic scale, improved techniques and the results of these techniques are still needed. For example, as the scale of these reactors shrinks, contamination effects due to surface adsorption and diffusion limit the smallest quantities that can be used. Confinement of reagents in droplets in an immiscible carrier fluid
5 overcomes these limitations, but demands new fluid-handling technology.

The described microfluidic technologies use of aqueous droplets in an immiscible carrier fluid. The aqueous droplets in an immiscible carrier fluid can also be deemed emulsions. An emulsion is a mixture of two immiscible (unblendable) substances. One substance (the dispersed phase) is dispersed in the other (the continuous phase). Examples
10 of emulsions include butter and margarine, espresso, mayonnaise, the photo-sensitive side of Photographic film, and cutting fluid for metalworking. In butter and margarine, a continuous lipid phase surrounds droplets of water (water-in-oil emulsion). Emulsification is the process by which emulsions are prepared.

Emulsions tend to have a cloudy appearance, because the many phase interfaces
15 (the boundary between the phases is called the interface) scatter light that passes through the emulsion. Emulsions are unstable and thus do not form spontaneously. Energy input through shaking, stirring, homogenizers, or spray processes are needed to form an emulsion. Over time, emulsions tend to revert to the stable state of oil separated from water. Surface active substances (surfactants) can increase the kinetic stability of
20 emulsions greatly so that, once formed, the emulsion does not change significantly over years of storage. Homemade oil and vinegar salad dressing is an example of an unstable emulsion that will quickly separate unless shaken continuously. This phenomenon is called coalescence, and happens when small droplets recombine to form bigger ones. Fluid emulsions can also suffer from creaming, the migration of one of the substances to
25 the top of the emulsion under the influence of buoyancy or centripetal force when a centrifuge is used.

Emulsions are part of a more general class of two-phase systems of matter called colloids. Although the terms colloid and emulsion are sometimes used interchangeably, emulsion tends to imply that both the dispersed and the continuous phase are liquid.

30 There are three types of emulsion instability: flocculation, where the particles form clumps; creaming, where the particles concentrate towards the surface of the mixture while staying separated; and breaking, where the particles coalesce and form a layer of liquid.

Currently, methods of generating an emulsion library comprised of many different elements is time and cost prohibitive as the number of elements required increases. Thus, there is a need for a need for an improved methods to directly emulsify library elements from standard library storage geometries (e.g. 96 well plates, etc).

5

DETAILED DESCRIPTION

The present invention provides methods for directly generating an emulsion library. Preferably, the emulsions exist as droplets on a microfluidic device. Such microfluidic devices are generally known in the art. An example of a preferred microfluidic device is that provided by RainDance Technologies, Inc. ("RDT"), as described in PCT Application No. US2006/021286, filed June 1, 2006, entitled "Microfluidic Devices and Methods of Use," PCT Application No. US2006/021280, filed June 1, 2006, entitled "Microfluidic Devices and Methods of Use in the Formation and Control of Nanoreactors," and PCT Application No. US2006/021380, filed June 1, 2006, entitled "Microfluidic Devices, Methods of Use, and Kits for Performing Diagnostics" each of which are incorporated herein by reference in their entirety.

In an embodiment, in order to create a monodisperse emulsion directly from a library well, a nozzle is formed directly into the fitting used to connect the collection syringe to a syringe tip (e.g. capillary tubing), as shown in Figure 1. The nozzle can be made from one or two oil lines providing constant flow of oil into the nozzle, a connection to the capillary tubing, and a connection to the collection syringe. Aspiration of the sample can be accomplished by running the collection syringe in withdrawal mode at a flow rate (Q3) above the flow rate of the two oil syringes (Step 1 in Figure 1). The difference in flow would correspond to the flow rate aspirated from the sample well. When the appropriate volume of sample has been loaded into the capillary tubing, the capillary tubing would be removed from the sample well, an air bubble, and possibly a cleaning solution would be aspirated (Step 2 in Figure 1). When almost all of the sample has been emulsified, the collection syringe withdrawal rate would either be reduced below the oil flow rates, stopped, or set to infuse at some nominal rate (Step 3 in Figure 1). The remaining sample, air, cleaning solution, etc, left in the capillary would be flushed back out into a cleaning well and the outside of the capillary would be cleaned at the "wash station." When the capillary is completely clean, the process would repeat for the next library element.

In an embodiment, the nozzle can be formed through using small bore tubing (glass, Teflon, peek capillaries) or micro-fabrication or molding processes such as PDMS soft lithography, glass etching, hot embossing, or similar high resolution fabrication technology.

5 The present apparatus can be readily adapted for clinical applications or work where cross contamination must be eliminated, since the region from the nozzle to the syringe are isolated from the sample stream (e.g., the oil wets these surfaces and keeps the sample from directly contacting aqueous sample). In an embodiment, the sipper tip can be designed as a disposable item (like a robotic sampler sipper tips) and automatically
10 replaced between samples.

 In an embodiment, multiple nozzle/syringe pairs can be operated in parallel, thus increasing throughput. This allows simultaneous sampling of multiple wells/samples during a single process step. Each sample can be collected into a separate syringe.

15

METHODS OF GENERATING LIBRARY EMULSIONS

ABSTRACT

The present invention provides methods of directly generating emulsions from libraries. The invention provides that these emulsions can be droplets for use on microfluidic devices.

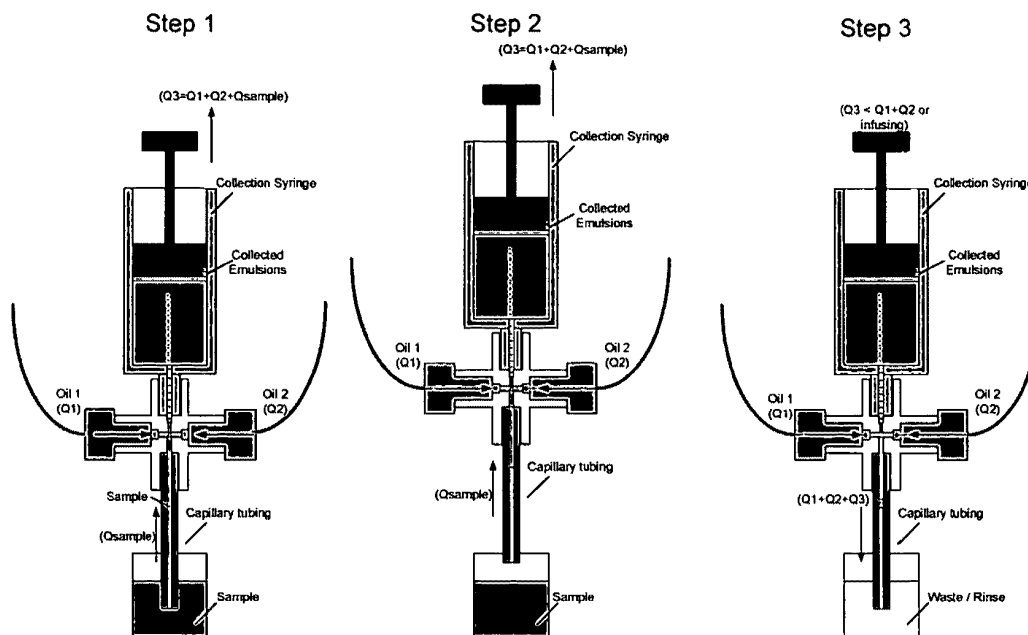


Figure 1

TRA 2232502v.1

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