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Microplates based on liquid bridges between glass rods

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ABSTRACT

Microplating that (i) does not necessitate complex or precise machinery to dispense small liquid volumes, (ii) enables fluorescent optical diagnosis, and (iii) permits simple analyte mixing mechanically is desirable. We advance here a novel approach that employs the formation of a liquid bridge held in place by capillary forces between glass rod tubes located parallel to each other. Experimental investigations made on liquid filling characteristics show conformance to theoretical notions. Analytical development showed the presence of regions of minimal uncertainty in the cross-sectional area of the liquid body arising from variations in the contact angle which permit consistent fluorescence measurements. Cyclical translation of the rods relative to each other, which cause rupture and reattachment of the liquid bridge, was found to engender good mixing. Strong linear trends were found in fluorescence signals relative to EGFP fluorophore concentration using standard and optical fiber (which offer targeted) excitation illumination. The open nature of liquid handling in the approach reported here and the positive results obtained portend the ability for development as integrated lab-on-a-chip devices.

1. Introduction

Microplates, essentially test tubes organized in an array on molded plastic plates, are a standard tool in analytical research and clinical diagnostic screening. The quest for more effective ways to dispense and manage the testing of increasingly smaller liquid volumes is an important challenge in microplate instrumentation [1] in which the motivation is clear. Smaller test volumes (i) increase the number of assays that can be conducted per plate thereby increasing throughput and (ii) reduce sample quantity needed per assay which is crucial when the test samples/reagents are scarce or expensive. Miniaturized assays, nevertheless, place greater demands on handling accuracy; and attempts to address this with more complex and precise machinery naturally translate to higher instrumentation costs. There is thus an impetus to develop alternative approaches that do not necessitate complex or precise machinery.

In the quest to achieve this, there is a need not to compromise effective diagnosis in microplate instrumentation. Optical approaches, in particular with the use of fluorescence [2], provide arguably the highest sensitivity measurements with greatest versatility. The availability of an array of detection modalities such as fluorescence resonance energy transfer, fluorescence polarization and time-resolved fluorescence make fluorescent labels an attractive substitute for radioactive isotopes with high potential for establishing miniaturized homogenous assays [3]. For this reason, fluorescence-based assays are used extensively in protein formulation and characterization studies [4]. They are also the method of choice in high throughput screening due to high sensitivity and speed [5]. Fluorescence-based assays that are meant to be used in conjunction with microplates are in continual active development [6–9].

Sample/reagent mixing is another important aspect in microplate instrumentation. The predominant approach currently adopted is for it to be done prior to liquid dispensation into the microplate wells. When assays involve a mixture of different proportions of reagents, this approach can be a bottleneck in the process. A current major thrust is to accomplish mixing within the well itself. A variety of methods, ranging from the use of physical and magnetic agitation [10,11], capillary concentration convection [12], and ultrasonic mixing [13,14], have been reported for this on standard microplates in which the volume is relatively large. A major impediment to small volume mixing lies in the difficulty in generating a turbulent or circulatory flow owing to the low degree of inertia over viscous forces. This is especially true when assays are scaled down from 96-well to 384-well formats [15] or even 1536-well formats. Recently, there have been attempts to use the coalescence of droplets as an avenue for mixing [16,17]. The attractiveness of this approach lies in the complete use of mechanical actuation which facilitates instrumentation in terms of simplicity and robustness.

In short, the availability of a means for microplating that (i) does not necessitate complex or precise machinery to dispense

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small liquid volumes, (ii) enables fluorescent optical diagnosis, and (iii) permits simple analyte mixing mechanically will be desirable. We advance here an approach of open analyte handling on capillary glass rods which apart from convenient liquid handling, possesses also the capabilities of facilitating mixing and fluorescence sensing.

2. Microplate design

The proposed microplate system comprises two capillary tubes resting on small supports at the ends to prevent contact (Fig. 1). Analyte droplets of selected volumes can be deposited between the rods using a pipette and will be retained against the effects of gravity by capillary forces when the appropriate spacing between the rods is used. One capillary tube is able to move relative to the other by attaching it to a glass piece that is, in turn, located on a translator. The glass piece enables optical access from below.

With analyte residing between the capillary tubes, their relative separation as the capillary rods are moved apart will cause the liquid body to be stretched and eventually rupture. This is similar to the formation of a liquid bridge and its rupture under tension [18]. Despite the rupture, the separated liquid bodies remain adhered to the tube surfaces. When the gap between the tubes is restored, the separated liquid bodies will re-attach and re-form as one. A cyclical rupture and reattachment process should create the necessary perturbation in the liquid body to facilitate mixing. Fluorescent analytes can be easily placed in an imager for viewing and analysis. To minimize the risk of transmitted or reflected incident light from reaching the detector, it is often desirable to have the excitation light placed at 90° to the detector [19,20]. In order to possess this feature, an optical fiber is inserted into the capillary tube and moved to the point where its distal end corresponds to the location of the analyte. The attractiveness of this approach is that the optical fiber never touches the analyte and can thus be reused without cleaning. In addition, the fiber tip can be restricted to a specific region to reduce the light flux needed from the source.

3. The behavior of a liquid bridge on two cylindrical rods

The retention of a liquid between two cylindrical rods located close to each other was arguably first advanced by Princen [21] initially from the standpoint of capillary rise based on a two dimensional assumption. A similar analysis conducted on horizontal rods offers relevance to the development here [22]. The evaluation of the complete shape of the liquid bridge is best done computationally [23] and does not offer much insight in the current context. What is more important is the cross-sectional shape at some distance from the ends (see Fig. 2), which is dictated by the pressure inside the liquid and is given by

$$P = P_o - \gamma/R \tag{1}$$

where γ is the surface tension, P_o the external pressure, and R the radius of curvature of the liquid surface. R is positive when the surface is concave outwards ($\alpha + \theta < 90°$) and negative when convex outward ($\alpha + \theta > 90°$); where θ is the contact angle and α is the angle between the line connecting the centers of the cylinders and the radius to the three phase boundary. For a rod radius of r and a distance between the cylinders of $2d$, we have

$$\frac{R}{r} = \frac{1 + d/r - \cos\alpha}{\cos(\theta + \alpha)} \tag{2}$$

From this relation, we can establish the liquid filling behavior. Interestingly, if A_L and A_S are the cross-sectional areas of the liquid bridge and rod respectively, one is able to obtain a neat non-parametric ratio in which

$$\frac{A_L}{A_S} = \frac{2}{\pi}\left\{-\left(\frac{R}{r}\right)^2\left[\left(\frac{\pi}{2} - \theta - \alpha\right) - \sin(\theta+\alpha)\cos(\theta+\alpha)\right] + 2\left(\frac{R}{r}\right)\sin\alpha\cos(\theta+\alpha) + \sin\alpha\cos\alpha - \alpha\right\} \tag{3}$$

If we neglect the end effects, the volume V and length of the liquid bridge L are related to A_L via $V = A_L L$. Next, we consider the effect of pulling the rods apart. Suppose that the thickness of the central section of the liquid is $2h$. A more detailed description of the liquid filling geometry depicted in Fig. 2 is shown in Fig. 3. We can now derive the following relationship:

$$h = [r(1-\cos\alpha) + d]\tan(\theta+\alpha) + r\sin\alpha - \left[\frac{r(1-\cos\alpha)+d}{\cos(\theta+\alpha)}\right] \tag{4}$$

Here, the third term on the right hand side is simply R. Simplifying Eq. (4) more we have

$$\begin{aligned} h &= [r(1-\cos\alpha)+d]\left[\tan(\theta+\alpha) - \frac{1}{\cos(\theta+\alpha)}\right] + r\sin\alpha \\ &= [r(1-\cos\alpha)+d]\left[\frac{\sin(\theta+\alpha)-1}{\cos(\theta+\alpha)}\right] + r\sin\alpha \end{aligned} \tag{5}$$

When extending the bridge of non-Newtonian liquids, it has been shown that thin connecting threads may develop and lead to large separations before total rupture [24]. Hence, we consider

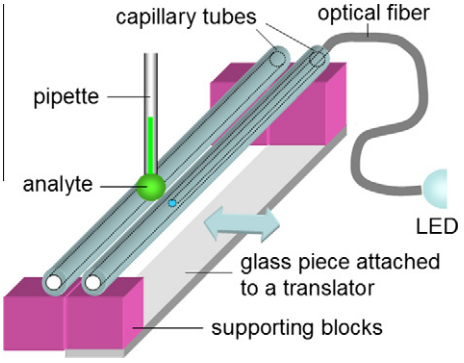

Fig. 1. The dispensation of liquid between two rods, each of radius r and separated by distance $2d$, creates a liquid bridge of length L as viewed from above. The liquid body has a radius of curvature R in the cross-sectional view that contacts the rods at point X and is related to the contact angle θ and angle α.

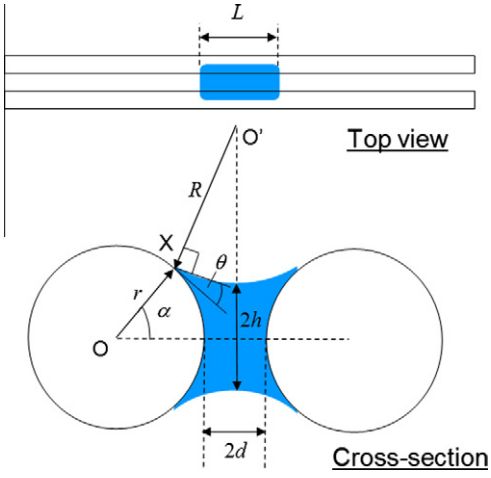

Fig. 2. A geometric description of the liquid bridge forming features which permit estimation of the maximum rod separation before rupture.