Journal of Colloid and Interface Science 426 (2014) 56–63

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Transparency microplates under impact

Chun Yat Lau [a], Zulhanif Roslan [a], Brandon Huey-Ping Cheong [a], Wei Seong Chua [a], Oi Wah Liew [b], Tuck Wah Ng [a,*]

[a] *Laboratory for Optics and Applied Mechanics, Monash University, Clayton VIC3800, Australia*
[b] *Cardiovascular Research Institute, Yong Loo Lin School of Medicine, National University of Singapore, National University Health System, Centre for Translational Medicine, 14 Medical Drive, Singapore 117599, Singapore*

ARTICLE INFO

Article history:
Received 28 February 2014
Accepted 17 March 2014
Available online 2 April 2014

Keywords:
Microplate
Transparency
Impact
Contact angle

ABSTRACT

Transparency microplates enable biochemical analysis in resource-limited laboratories. During the process of transfer, the analytes tittered into the wells may undergo spillage from one well to another due to lateral impact. Sidelong impact tests conducted found the absence of non-linear effects (e.g., viscoelastic behavior) but high energy loss. Finite element simulations conducted showed that the rectangular plate holding the transparencies could undergo *z*-axis deflections when a normal component of the force was present despite constraints being used. High speed camera sequences confirmed this and also showed the asymmetrical *z*-axis deflection to cause the contact line closer to impact to displace first when the advancing condition was exceeded. Capillary waves were found to travel toward the contact line at the opposite end, where if the advancing contact angle condition was exceeded, also resulted in spreading. The presence of surface scribing was found to limit contact line movement better. With water drops dispensed on scribed transparencies, immunity from momentum change of up to 9.07 kgm/s on impact was possible for volumes of 40 μL. In the case of glycerol drops immunity from momentum change of up to 9.07 kgm/s on impact extended to volumes of 90 μL. The improved immunity of glycerol was attributed to its heightened dampening characteristics and its higher attenuation of capillary waves. Overall, scribed transparency microplates were able to better withstand spillage from accidental impact. Accidental impact was also found not to cause any detrimental effects on the fluorescence properties of enhanced green fluorescent protein samples tested.

1. Introduction

Standard microplates (microtiters), which are essentially test tubes arranged in an array on molded plastic plates, are the tools of choice for liquid handling in analytical research and clinical diagnostic screening [1]. One aspect of microplate instrumentation involves finding more effective ways to dispense and manage the testing of increasingly smaller liquid volumes [2]. Smaller test volumes (i) increase the number of assays that can be conducted per plate thereby increasing throughput, and (ii) reduce the sample quantity needed per assay which is crucial when the test samples/reagents are scarce or expensive. Nanoliter spotting can achieve this [3,4] but is subject to evaporation and thus requires handling in humidified cassettes [4]. When microliter volumes are affordable, the use of capillary-based microplates has been shown to offer distinct advantages [5–7]. Another aspect of microplate instrumentation that is actively developed is in the creation of microplates that are cost effective enough to be available for the use in resource-limited laboratories so that diagnostic outcomes can be achieved in a more timely fashion. Paper, by virtue of its strong wicking capabilities and low cost, has been advanced to address this [8].

The ability to derive effective signals from the reagents tested and its detection are also crucial in microplate instrumentation apart from good small liquid volumes retention features. Optical approaches, in particular with the use of fluorescence, are widely accepted to offer the highest sensitivity measurements with greatest versatility. For this reason, they are used extensively in protein formulation and characterization studies, and are the method of choice in high throughput screening due to high sensitivity and speed [9–11]. The use of paper as liquid handling media does not offer the ability to conduct optical sensing well.

Transparency sheets, which are normally associated with use on overhead projectors, have recently been reported for application as microplates [12,13]. In the non-scribed version of the method, an array of holes is created on a hydrophobic sheet which was then

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* Corresponding author. Fax: +61 3 9905 1825.
E-mail address: engngtw@gmail.com (T.W. Ng).

http://dx.doi.org/10.1016/j.jcis.2014.03.048
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affixed to the transparency (see Fig. 1(a)). This disposable unit can then be attached temporarily onto a reusable plexiglass base [12]. In the scribed version, circles are scribed directly on the surface of the transparency directly to create the boundaries to hold the drop in place (see Fig. 1(b)). The advantage offered by the latter approach is the reduction in cost and time needed to fabricate the transparency microplates [13].

After the desired analytes are dispensed into microplate wells, it is typical to transfer the microplate to various stations where operations such as incubation and sensing are carried out. During this process, it is possible for spillage to occur from one well to another, leading to cross-contamination of the analytes. Impact or sudden acceleration is arguably the most significant cause for this. Thus, we investigated the effect of lateral impact on the liquid retention capability of transparency microplates. The tests were conducted on water and glycerol, the former being a universal diluent and the latter for its wide use in pharmaceutical formulations. From this, we sought to define the limits with which impact energy can be applied in order for these microplates to operate properly. Using enhanced green fluorescent protein as a model, we also examined potential loss-of-function effects that impact may have on droplet samples by assessing fluorescence properties before and after impact.

2. Perturbations of liquid body on solid surfaces

The ability of a liquid drop to remain in a stationary position, particularly when its volume is small, is dependent on the pinning of its contact line. A contact line is governed by the ability of the contact angles associated with it to vary over a finite range. If we assume quasi-static states, the drop will not move on a flat surface until the contact angles reach their critical values.

When an obstacle is present at the contact line, its tilted edge will cause an apparent critical advancing angle θ'_a that is larger than the angle without the obstacle θ_a (see Fig. 2(a) and (b)). The tilted edge of the obstacle will cause an apparent critical receding angle θ'_r that is smaller than the angle without the obstacle θ_r (see Fig. 2(c) and (d)). This will then require that

$$\theta'_r \leqslant \theta \leqslant \theta'_a \tag{1}$$

This inequality was first highlighted by Gibbs [14]. The apparent advancing angle behavior was first demonstrated with pedestals interacting with the liquid body [15], and later with surface topologies [16–20] and bubbles [21,22]. It is based on this, that the scribed shape on the surface of the transparency (Fig. 1(b)) is able to sustain a larger drop volume or retain its position on a slowly tilted incline better without breaching the scribed boundary [13].

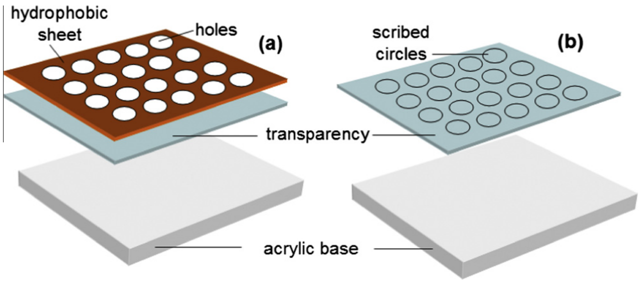

Fig. 1. Two schemes of conducting transparency microplating, wherein either (a) a hydrophobic sheet with holes cut out is attached to a transparency, or (b) the transparency is scribed with circles. Both designs (which are disposable) are attached to a plexiglass base (that is reusable).

The study of the dynamic behavior of drops, alternatively, has been done mainly from monitoring the spreading (and sometimes jetting) character of drops falling at different velocities on to solid surfaces [23–25]. These studies have revealed that the spreading behavior is strongly dependent on whether the dynamically changing contact angles have breached their limiting conditions. With a drop dispensed on a surface and the latter vibrated, a similar capacity of the dynamically changing contact angles to cause spreading has been observed [26,27]. For a drop on an incline, in which hysteresis causes the contact angles to attain advancing and receding states when static, vibration has been shown to cause the drop to climb up the incline through the unequal dynamic alteration of the contact angles [28].

In the studies conducted to observe the spreading and movement of drops, through impact on to surfaces or through vibration of the surfaces, capillary waves are present. A capillary wave is one that travels along the phase boundary of a fluid and whose dynamics are dominated by the effects of surface tension. Capillary waves have significant effect on surface waves [29], the latter which can be observed under forced vibration and through laser diffraction [30,31]. Stokes [32] had established very early on that the initial amplitude a_o of capillary waves would attenuate with distance x via

$$a = a_o e^{-\alpha x} \tag{2}$$

where α is the attenuation coefficient. The attenuation coefficient can be related to viscosity η, gravitational acceleration g, density ρ, surface tension σ, and $k = 2\pi/\lambda$, for λ being the wavelength of the wave, using [33]

$$\alpha = \frac{4k^2\eta\left(kg + \frac{\sigma k^3}{\rho}\right)^{1/2}}{(\rho g + 3\sigma k^2)} \tag{3}$$

3. Methods

3.1. Preparation of scribed well microplates

A desktop cutter plotter (Hefei, model JK361) was used to prepare the scribed transparencies. The layout and dimensions of the wells were prepared via a sign making software (Artcut 2009). Each well was set at 6.9 mm diameter to be consistent with that of conventional 96-well microplates. The wells were then scribed on to standard polyester transparencies of 0.11 mm thickness (Avery model 2505) using plotting speed and force of 150 m/s and 50 g respectively.

3.2. Application of impact and visualization

The setup is depicted schematically in Fig. 3. The impact was supplied through a pendulum which has a striking mass of 3.1 kg. A rectangular acrylic piece functioned as the base of the microplate on which the transparency was attached using double-sided tape. The former was held in place using clamps. Liquid drops (ranging in volume from 40 μL to 60 μL) were then dispensed using a manual pipette (Biohit mLine Mechanical Pipette, 10–100 μL) onto the transparency at a location nearest to the edge where the striker would impact. This indicates the worst case scenario for the drop to be displaced on impact. The tests were conducted using water (MilliQ) and glycerol $C_3H_8O_3$ (USB Corporation, 16374) on scribed and non-scribed transparencies as shown in Fig. 1. The behavior of the drop upon impact was monitored using a high speed camera (Fastcam) at 1000 frames per second. In order to obtain sufficient illumination, we used ultrabright LED lamps for illumination. The low heat generated from these