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Time-resolved CryoEM Using Spotiton

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Almost every aspect of cryo electron microscopy (cryoEM) has been automated over the last few decades. One of the challenges that remains to be addressed is the robust and reliable preparation of vitrified specimens of suitable ice thickness. To this end we have been developing an approach that uses piezo dispensing of picoliter volumes coupled to ‘‘self-blotting” nanowire EM grids [1][2][3][4] to vitrify very small amounts of sample without the use of externally applied blotting paper. The prototype instrument, Spotiton, has recently been developed into a commercial system (chameleon, SPT Labtech Ltd.) This approach to vitrification has the additional advantage of significantly reducing the time the sample spends as a thin liquid film prior to vitrification when compared to manual or automatic plunging machines, which appears to ameliorate the deleterious effects on the sample that arise from interaction with the air-water interface [5].

Here, we present a further development of the Spotiton system that allows the preparation of cryoEM sample grids for the study of short-lived molecular states. Using independent piezo electric dispensers, two samples are deposited as streams of ~50 pL droplets within 10 ms of each other onto overlapping regions of a nanowire grid (Fig. 1). Mixing occurs as the sample volumes are thinned by capillary action of the nanowires on the grid surface and is stopped upon vitrification in liquid ethane, between ~100-500 ms later. In addition, by adjusting the firing direction of one of the dispensers, we are able to prepare control grids with the two samples unmixed (Fig. 2). We demonstrate the utility of this approach for four different cases where short lived states are of high interest: (i) binding of ribosomal subunits; (ii) binding of promoter DNA to RNA polymerase; (iii) binding of Ca++ to a potassium channel; (iv) conformational rearrangements of dynamin lipid tubes driven by GTP hydrolysis. Our results show that this method of time-resolved mixing is efficient and effective and has widespread potential [6].

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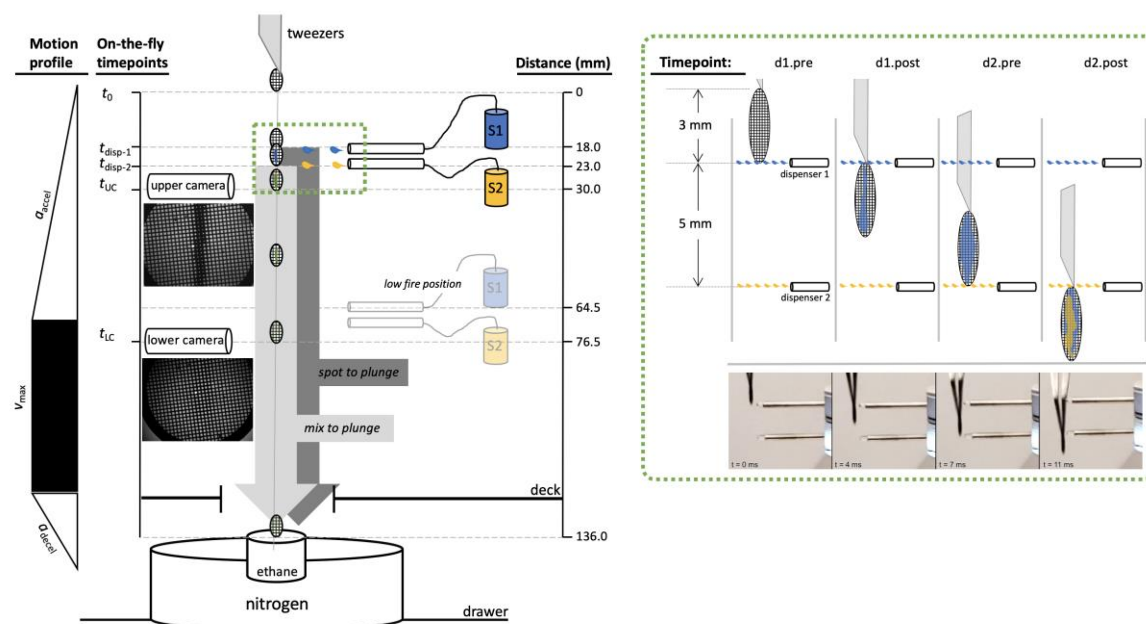

Figure 1. Schematic of time-resolved Spotiton operation. (a) Diagrammatic overview of the distances (fixed) and elapsed times (variable) relevant to spraying and mixing two samples on a moving grid. Simultaneous dispensing of both samples is triggered after the grid plunge begins. Inset images are representative video captures from the two system cameras. (b) Magnified view of (green-dashed) boxed area in (a) showing the locations of the grid and dispensing tips at a series of time-points with corresponding images of the tips and grid below. Estimated relative elapsed times are indicated on each image. Objects in (a) and (b) are not to scale.

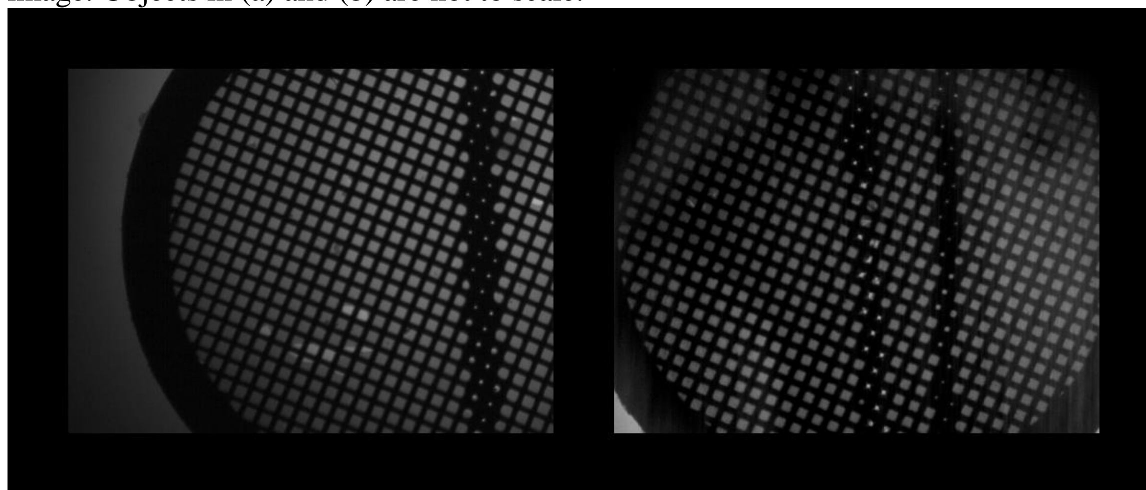

Figure 2. Samples can be deposited onto the same path (left) so as to mix on the grid or onto separate paths (right) to provide for controls, or more simply two samples on a single grid.

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