doi:10.1017/S1431927622005025
Microsc. Microanal. 28 (Suppl 1), 2022

Evaluating the chameleon Sample Preparation Device: Case Studies

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The chameleon is a cryo-EM sample preparation device (Figure 1) based on Spotiton [1]. Chameleon's automated workflow reduces user involvement, aims to produce consistent results [2], and potentially addresses particle issues that may arise using current conventional plunge freezing methods [3]. The chameleon uses a picoliter droplet dispenser to apply sample to a self-wicking nanowire grid [4]. A user interface guides the user through the process of setting up the machine. The grids are handled by the robotic tweezers once the user loads four grids onto the grid platform. The chameleon starts by glow discharging a grid, using an internal glow discharge unit, followed by sample application as the grid moves past the droplet dispenser en route to being plunged into liquid ethane. The resulting ice thickness for the sample is determined by the wicking properties of the grids, the glow discharge settings, and the time between sample application and arrival into the ethane (spot-to-plunge-time); these last two parameters can be specified by the user. The user receives feedback from the system in the form of a frame-by-frame video of the grid as it is undergoing wicking (Figure 2).

We will report on using the chameleon in practice for a number of case studies of proteins which have encountered issues using conventional sample preparation systems. These issues include aggregation, ice thickness, preferred orientation, and protein degradation or denaturation. Ideally, the chameleon spot-to-plunge time should be set as short as possible in order to limit the time that particles spend at the air-water interface; currently, the fastest spot-to-plunge time available is fifty-four milliseconds. It is however challenging to achieve uniform and suitably thin ice over large areas of the grid when using very short spot-to-plunge times. In practice we have found that a spot-to-plunge time of around 180 to 260 milliseconds and glow discharge settings of 20 to 40 seconds are usually the optimal parameters. Chameleon also presents a challenge in that reducing the spot-to-plunge time also requires increasing the concentration of the particles in order to visualize sufficient numbers of particles in the thin vitrified film. We will report on both successes and failures of the system and the lessons we have learned.

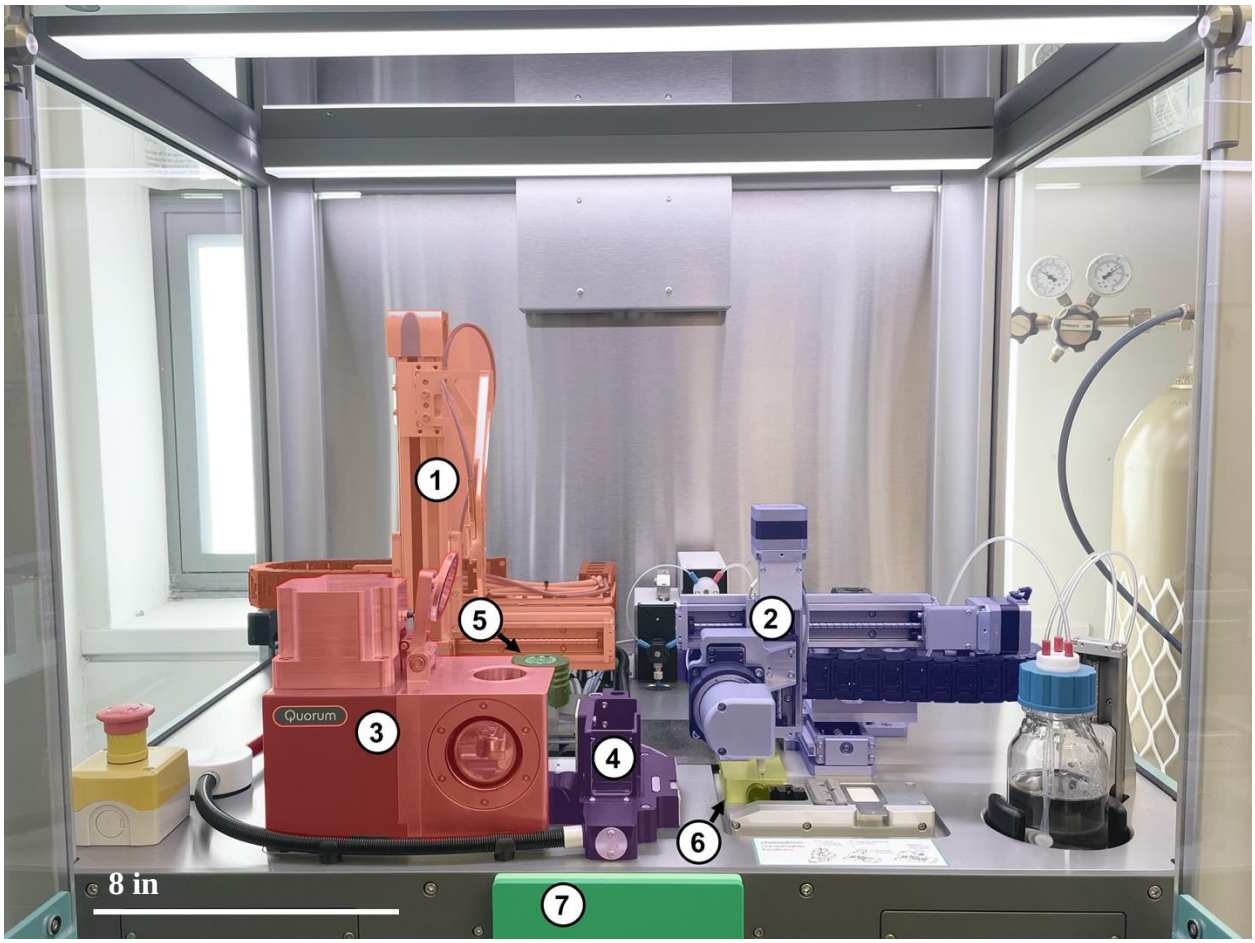

Figure 1. Inside the chameleon sample preparation area with some of the essential components identified. (**1**) The robotic tweezer arms which controls the tweezer's movement. (**2**) The robotic arm which controls the movement of the dispenser. (**3**) The internal glow discharger. (**4**) The humidity shroud where the sample is dispensed and plunged. (**5**) The grid sample holder where the grids are initially placed by the user. (**6**) The camera which shows the sample being dispensed during setup. (**7**) The cryogen compartment housing the nitrogen and ethane.

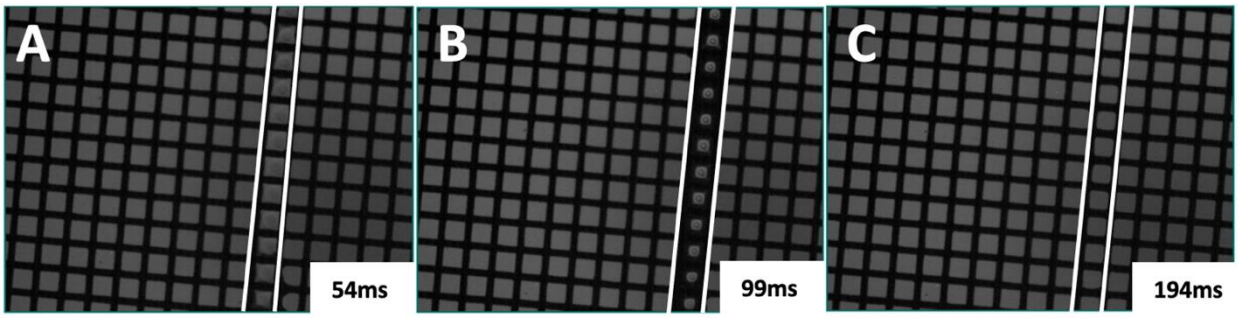

Figure 2. Video frames of the grid at various time points after sample has been applied. The white lines enclose the strip of squares onto which the sample was dispensed. (**A**) After 54 milliseconds squares in

https://doi.org/10.1017/S1431927622005025 Published online by Cambridge University Press

the strip are completely submerged by the dispensed sample. (**B**) At 99 ms, the squares appear smaller as the nanowires wick away the sample. (**C**) At 194 ms, most squares have been wicked just before the gid is submerged into the liquid ethane.

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