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Easier and Safer Biological Staining: High Contrast UranyLess Staining of TEM Grids using mPrep/g Capsules

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Uranyl acetate (UA) has been used for decades in life science electron microscopy as a positive and negative stain [1, 2]. But due to recent regulations (especially in Europe and Japan) there has been considerable effort to find less toxic and non-radioactive replacements, including stains based on Oolong tea extracts [3], platinum blue [4], and gadolinium [5]. This report demonstrates a safe and non-radioactive stain that provide the contrast, broad utility, rapid staining, and ease of use of UA.

UA is commonly followed by lead citrate [1,6] in a multistep protocol that also includes multiple rinses. This protocol requires extensive forceps handling to transfer grids into and out of grid boxes, onto and between stains and rinses, and back into storage. Due to their fragility, it is difficult to not damage some grids during all this handling. Moreover when several grids are prepared they are easily mixed-up and obtaining identical stain timing is difficult, thereby reducing experimental reproducibility.

We demonstrate a new low-toxicity "Uranyless" stain developed by Delta Microscopy with Chromalys (France) that is composed of lanthanides (La, Dy, Gd) and other compounds that provide a high affinity to stain biological structures at a near neutral 6.4-6.8 pH to not disrupt sensitive structures. We combine UranyLess with mPrep/g capsule processing to make staining easier, more efficient and reproducible. Each mPrep/g capsule holds 1 or 2 grids and connects to lab pipettors to precisely deliver reagent to the grids held within. Capsules connect individually or can be stacked together onto single or multi-channel pipettors to enable staining any desired number of grids (Fig. 1). Since grids are stored and stained in the same capsule, this eliminates the potential for loss, damage or mix-up. Reproducibility is enabled since any number of grids can be prepared simultaneously with the same protocol, or even with multiple protocols by simultaneously drawing reagents from different microtiter wells in one 96-well plate.

Tissues were prepared with a standard protocol: 2% glutaraldehyde in 0.2M pH 7.4 PO_4 buffer for 2 hrs, buffer rinses, 1 hr buffered 1% OsO_4 postfix, serial ethanols, and embedding in Epon/Araldite. 80 nm sections on 200 mesh Cu grids were inserted into mPrep/g capsules. The grids were stained by pipetting into mPrep/g capsules: UranyLess 2 minutes, 3 water rinses, lead citrate 2 minutes, 3 water rinses, and air drying in the capsule. Negative stain specimens were prepared by inserting carbon-coated Formvar-filmed 300 mesh grids into mPrep/g capsules, pipetting in bacteriophage T6 suspension and holding for 1 minute, contrasting with UranyLess for 1 minute, and then removing the mPrep/g capsules and blotting grids in the capsule to remove excess water prior to imaging with an Hitachi HT7700 at 80 keV.

UranyLess and mPrep/g capsules provide rapid high contrast positive staining of animal and plant tissues (Figs 2-3), and high contrast negative staining (Fig 4) comparable to UA without radioactivity and with less toxicity. The mPrep/g capsules also provide a sealed staining environment that eliminates the need for hydroxide pellets to reduce lead precipitation [1]. In summary, mPrep/g capsule grid staining with Uranyless and lead citrate staining provides ease, quality and reproducible results.

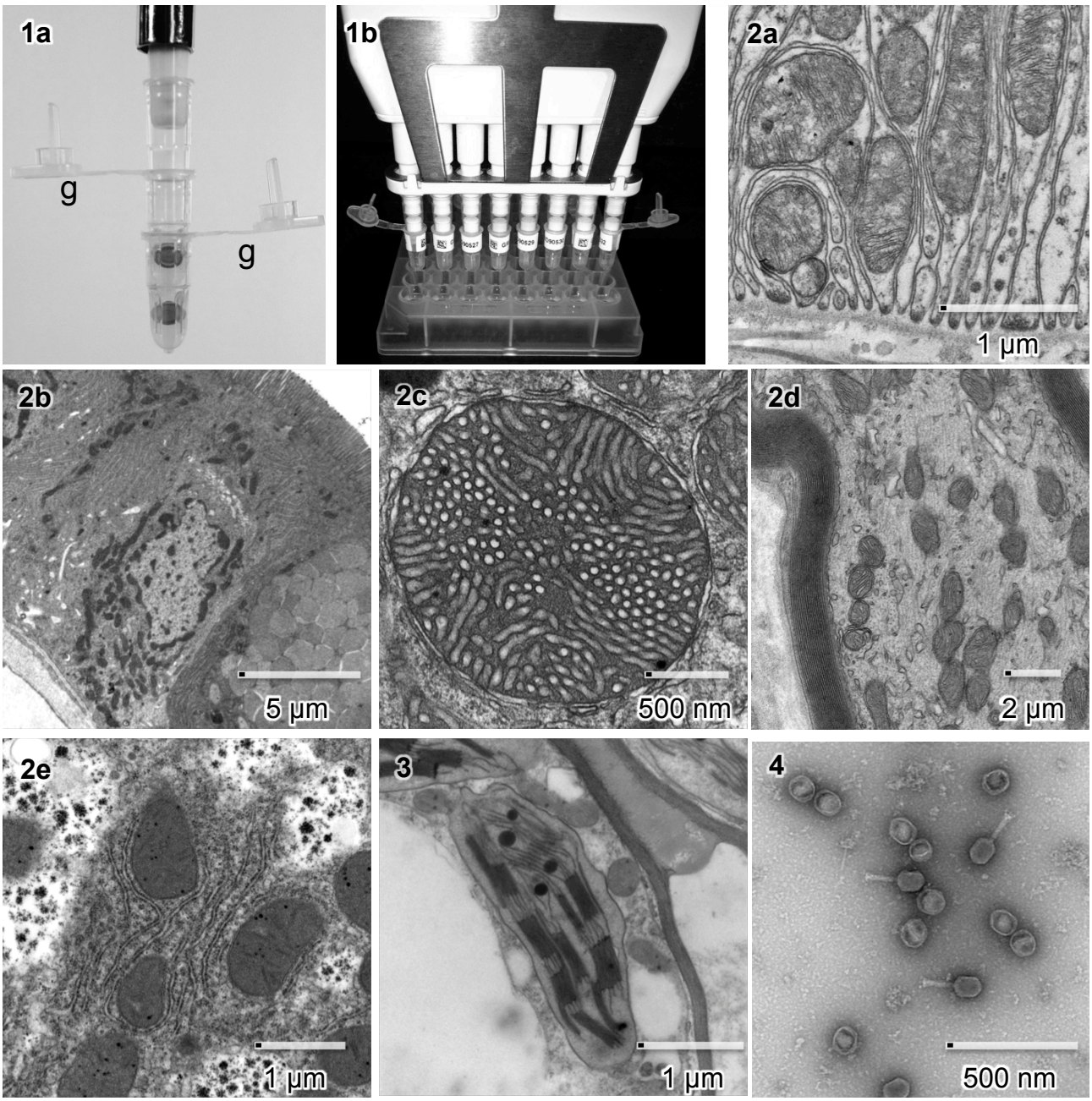

Fig 1: a) Two mPrep/g capsules (g) stacked onto a single channel pipettor for staining 2-4 grids. b) Eight labeled mPrep/g capsules on 8-channel pipettor for staining 8-16 grids, from reagents in microtiter plate.
Fig 2: Animal tissues: a) kidney, b) intestine, c) ovarian follicle, d) myelinated neuron, e) hepatocyte.
Fig 3: Spinach leaf thylakoid plant tissue. Fig 4: Negative stained T6 bacteriophage.

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