doi:10.1017/S1431927614008174
Microsc. Microanal. 20 (Suppl 3), 2014

High Throughput Multi Parameter TEM Chemical Processing Protocol Development with the mPrep-s Capsule System: *Schmidtea mediterranea*

Melainia McClain[1]

1. Electron Microscopy Core Facility/Stowers Institute for Medical Research, Kansas City, Missouri USA.

It is widely accepted that chemical processing protocols for transmission electron microscopy (TEM) must be optimized for each different organism or tissue type for a particular research need. While processing conditions have been established for many tissues, there are some organisms and tissue types that are not well preserved with standard protocols and aren't represented in current literature for a particular research need. This study demonstrates a high throughput strategy for multi-parameter protocol testing in one processing run using the mPrep capsule and micropipettor-based system from Microscopy Innovations [1], and a quick comparison method using a silicone micro-well insert from Ibidi [2].

Freshwater planarian worm *Schmidtea mediterranea* were processed with 16 parameter variations based on a published protocol [3] which included four molarities of sodium cacodylate buffer (25 mM, 50 mM, 100 mM, 200 mM), secondary fixation in buffered osmium tetroxide (OsO_4) with and without the additive potassium ferricyanide (PFC) for membrane enhancement, and embedding in either Araldite (502) or Spurr's resin. Reagents were loaded into either 96 or 24 well plates for processing using a color coded paper template created in Excel and attached to each plate as a guide (Fig. 1). Planarian specimens were loaded into mPrep-s capsules and attached to a color coded 12 channel pipette (Fig. 1), which was used to pipet reagents from the well plates into the corresponding mPrep capsule for incubations. One piece of tissue from each of the eight conditions was embedded together in one micro-well of an Ibidi silicone insert for Araldite resin, and one micro-well for Spurr's resin. The resulting block faces produced sections that fit within the 2X1 mm opening in a slot grid (Fig. 2). Sections with a silver interference color were cut with a Diatome Histo diamond knife on a Leica UTC and placed on a formvar/carbon coated slot grid for comparison in the TEM (Fig. 2).

The 25 mM and 50 mM buffer flame cells showed defined membranes and clear ultrastructural detail (Fig. 2). However, some of the 25 mM flame cells showed swelling artifacts and some of the 50 mM flame cells showed a more electron dense cytoplasm that obscured ultrastructural details as compared to the 25 mM. Both the 100 mM and 200 mM buffer samples showed significant shrinking and electron dense cytoplasm. Spurr's resin produced more fully infiltrated samples than Araldite. While the use of PFC did not have a discernable effect on the Spurr's samples, the 50 mM and 100 mM PFC Araldite samples showed significantly more electron dense cytoplasm than the same buffer molarities without PFC.

The mPrep system facilitated the controlled and consistent testing of many conditions, which greatly increased the chances of obtaining optimized specimen preservation in one processing run. Embedding eight different samples in one block substantially reduced microtomy and imaging time. Using this approach for whole planarian worms created a set of embedded test samples which can be examined for preservation of any organ system or cell population without having to perform several test runs [4].

References:

[1] mPrep/s capsules (Catalog #S0812), Microscopy Innovations LLC, Marshfield, WI, USA.
[2] 4 well micro-inserts (Catalog # 90406), Ibidi LLC, Munich, Germany and Verona, WI, USA.

[3] James A McKanna, Fine Structure of the Protonephridial System in Planaria II. Ductules, Collecting Ducts, and Osmoregulatory Cells, Zeitschrift für Zellforschung **92** (1968), p. 524-535.

[4] Thanks to Hanh Vu for providing *S. mediterranea* samples and a starting protocol, Steven Goodman and Tom Strader from Microscopy Innovations for product support, and the Stowers Institute for Medical Research.

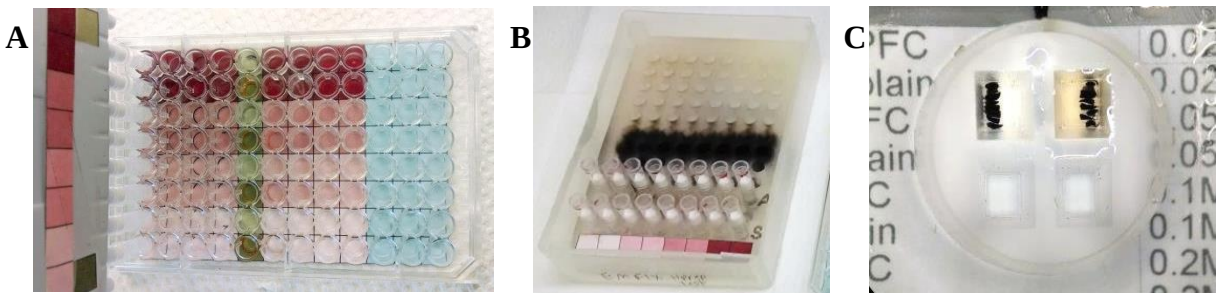

Figure 1. (A) Samples in capsules attached to a color coded micropipettor and lined up with the corresponding colored well on the 96 well plate for reagent changes. **(B)** Capsules in silicone bench for incubation. (C) Ibidi cell culture micro-well insert with samples embedded in Spurr's and Araldite resin.

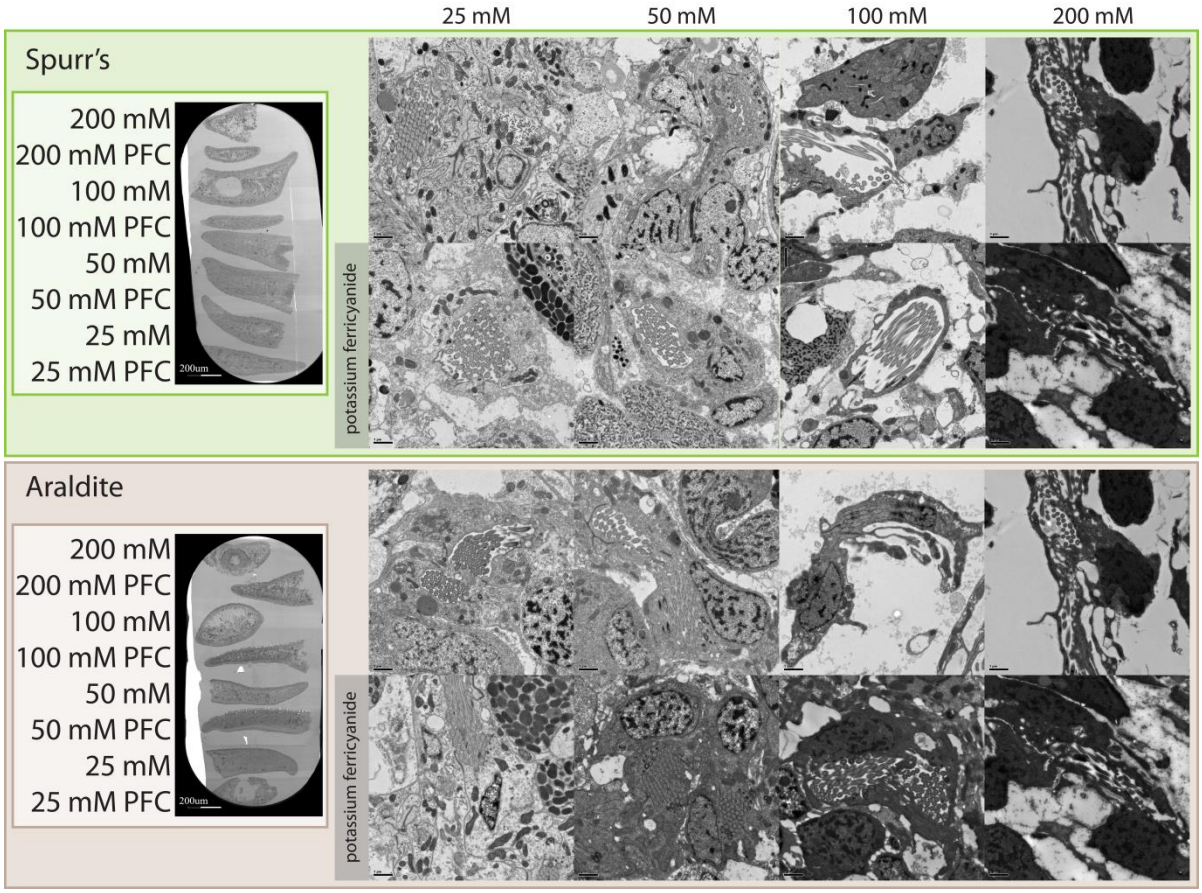

Figure 2. (Left) TEM image of a section from each block face mounted on a slot grid showing eight samples in each section, labeled with buffer molarity and potassium ferricyanide (PFC). Scale 200nm. (Right) TEM images of a representative flame cell from each condition, scale 1um.

https://doi.org/10.1017/S1431927614008174 Published online by Cambridge University Press