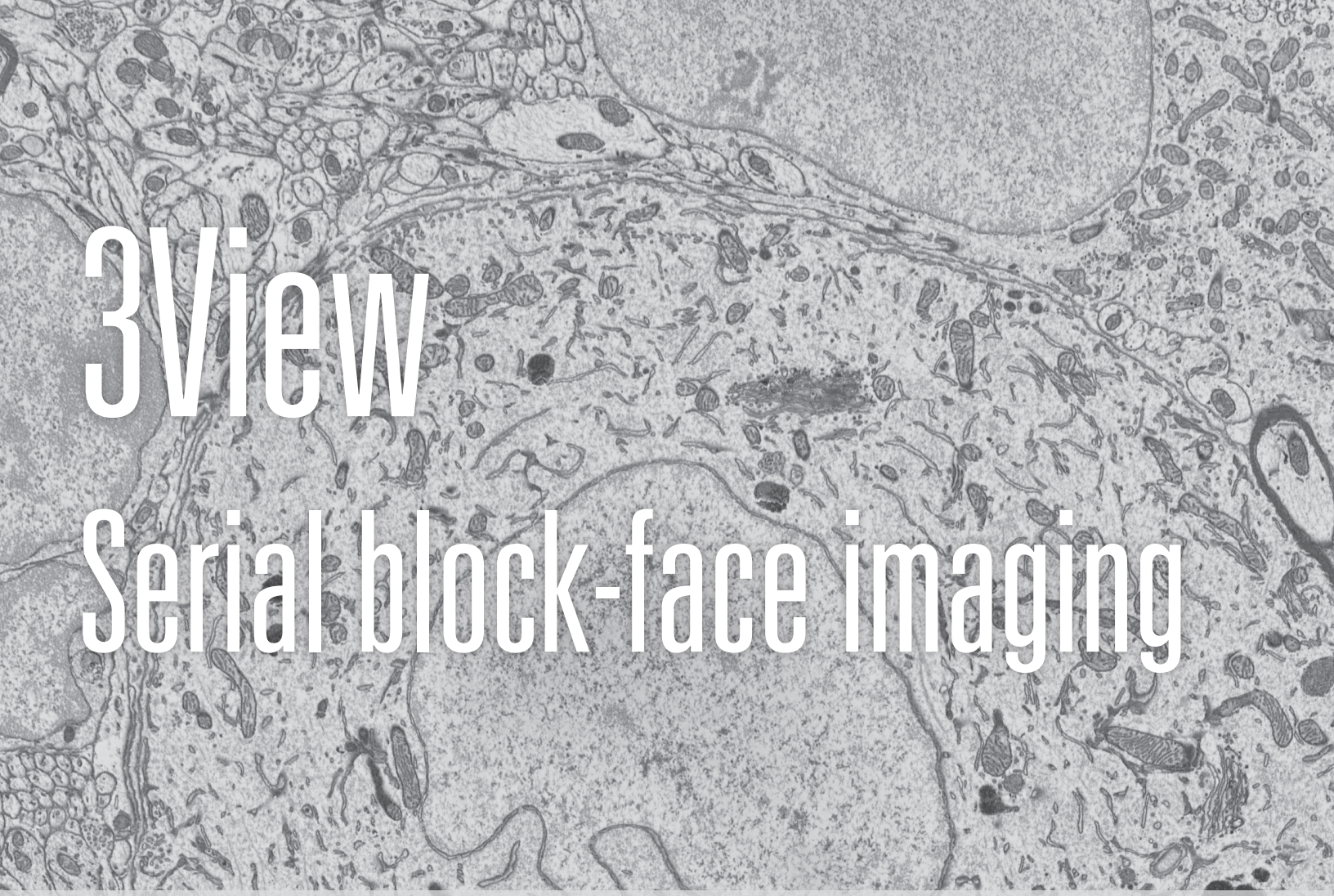
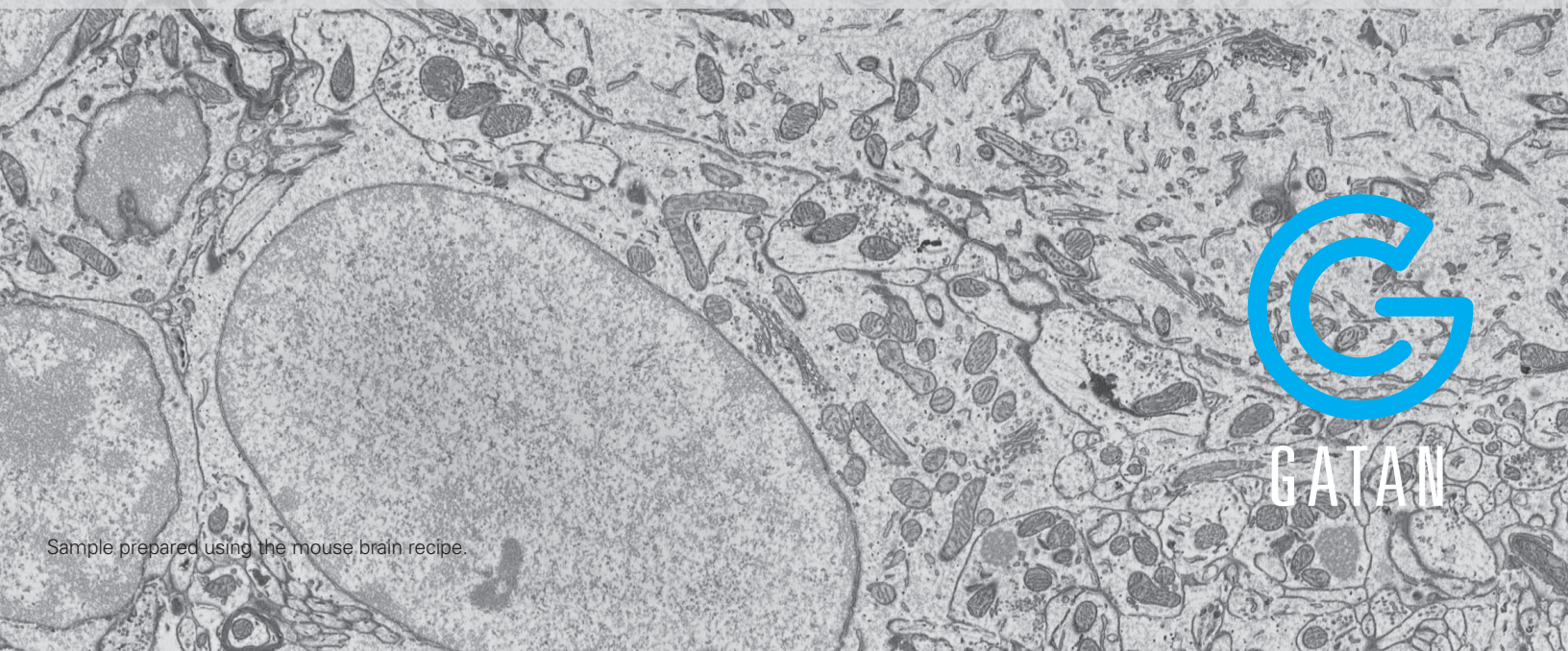


3View

Serial block-face imaging

3View® 2.XP system sample recipes

Basic preparation recipes for your samples, to aid automated sectioning and image capture of your 3D ultrastructure.



GATAN

Sample prepared using the mouse brain recipe.

Serial Block-Face Imaging Recipes

Arabidopsis root tip

Publication: *Developing 3D SEM in a broad biological context, VIB Ghent*

Primary fixation:
- 2 h; 0.1 M phosphate buffer (pH 6.8) , with 3% glutaraldehyde and 2% paraformaldehyde
- Unspecified timing; encase individual samples in agarose blocks
- Overnight, fresh fixative; same formula

Washing: 5 x 3 min, cold; 0.15 M cacodylate buffer

Post-fixation staining:
- 1 h, on ice; 0.15 M cacodylate buffer with 0.2% ruthenium red and 2% osmium tetroxide

Washing: 5 x 3 min; ultrapure water

Mordant: 20 min, RT; freshly prepared thiocarbohydrazide solution (1% w/v in ultrapure water)

Washing: 5 x 3 min; ultrapure water

Second staining: 30 min, RT; 2% osmium

Washing: 5 x 3 min, RT; ddH_2O

En bloc stain:
- Overnight, 4 °C; 2% uranyl acetate
- 5 x 3 min; ultrapure water
- 30 min, 60 °C; Waltron's lead aspartate (by dissolving 20 mM lead nitrate in a 30 mM L-aspartic acid solution)
- 5 x 3 min; ultrapure water

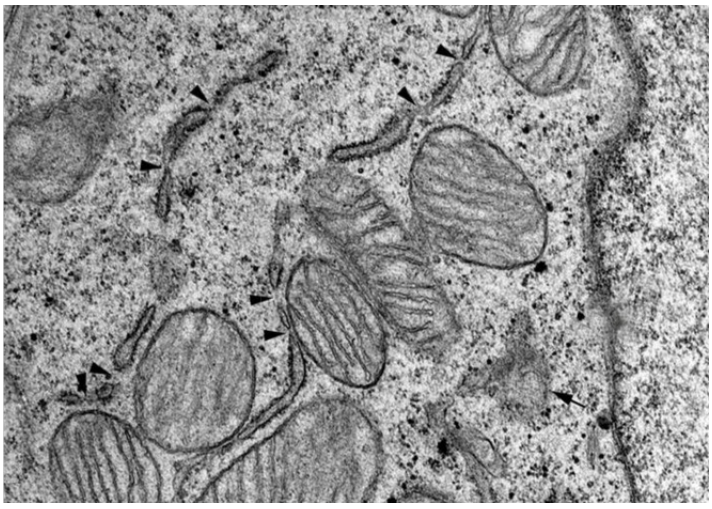
Dehydration:
- 30 min each, ice cold; 30%, 50%, 70%, 90%, 100%, 100% ethanol in water
- 30 min each, ice cold; 100%, 100%, acetone

Resin infiltration:
- 2 h each; 30%, 50%, Spurr's resin in propylene oxide
- 100% each; overnight, 8 h, overnight

Embedding: 24 h, 60 °C; fresh Spurr's resin

ER in mammalian cells

Publication: *Puhka M, Joensuu M, Vihinen H, Belevich I, Jokitalo E. Progressive sheet-to-tubule transformation is a general mechanism for endoplasmic reticulum partitioning in dividing mammalian cells. Klumperman J, ed. Molecular Biology of the Cell. 2012;23(13):2424-2432. doi:10.1091/mbc.E10-12-0950., IOB, Helsinki*



Primary fixation: 20 min, RT; 0.1 M sodium cacodylate buffer (pH 7.4) with 1.5% glutaraldehyde and 2% formaldehyde

Washing: 1 min; 0.1 M sodium cacodylate buffer

Post-fixation staining: 1 h, on ice; freshly prepared 0.3 M cacodylate buffer with 4% aqueous osmium tetroxide + 3% potassium ferrocyanide

Washing: 1 min; ddH_2O

Mordant: 10 min; thiocarbohydrazide

Washing: 1 min; ddH_2O

Second staining: 1% osmium tetroxide

Washing: 1 min; ddH_2O

Dehydration:
- 5 min each, ice cold; 20%, 50%, 70%, 90%, 100%, 100%, acetone in ddH_2O
- 10 min, RT; 100% acetone

Resin infiltration:
- At least 2 h; 100% Durcupan

Embedding: 60 °C for 48 h; fresh Durcupan

Serial Block-Face Imaging Recipes

Collagen fibrils

Publication: *Nature protocols - Using transmission electron microscopy and 3View to determine collagen fibril size and three-dimensional organisation, Manchester University, OTOTO*

Primary fixation:
- 15 min whole tissue, RT; 0.1 M cacodylate buffer (pH 7.2) with 2.5% glutaraldehyde
- 2 h for dissected region, 4 °C; 0.1 M cacodylate buffer (pH 7.2) with 2.5% glutaraldehyde

Washing: 5 x 3 min, RT; 0.1 M cacodylate buffer

Post-fixation staining: 1 h, RT; 0.1 M cacodylate buffer with 2% osmium tetroxide + 1.5% potassium ferrocyanide

Washing: 5 x 3 min, RT; ddH_2O

Mordant: 2 x 2 h, 4 °C (replace solution after 2 h and repeat); 1% tannic acid in 0.1 mM cacodylate buffer

Washing: 5 x 3 min, RT; ddH_2O

Second staining: 40 min, RT

En bloc stain:
- 16 h/overnight; 1% aqueous uranyl acetate
- 3 x 5 min, RT; ddH_2O
- Centrifuge 5000 g, 5 min; 1% aqueous uranyl acetate

Dehydration:
- 10 min each, RT; 30%, 50%, 70%, 90%, 100%, 100%, 100%, 100% ethanol in ddH_2O
- 10 min, RT; 100% propylene oxide

Resin infiltration:
- 30% for 4 h, 50% overnight, 67% for 1 h, 75% for 1 h, 80% for 1 h, 100% for 1 h, 100% for 1 h, 100% for 1 h resin in propylene oxide

C. elegans

Primary fixation: Unspecified timing; 0.1 HEPES buffer (pH 7.4) with 2.5% glutaraldehyde and 2% paraformaldehyde

Washing: 5 x 3 min; cold HEPES buffer

Post-fixation staining: Unspecified timing; freshly prepared 0.2 M ice cold HEPES buffer with 4% aqueous osmium tetroxide + 3% potassium ferrocyanide

Washing: 5 x 3 min, RT; ddH_2O

Mordant: Unspecified timing; thiocarbohydrazide

Washing: 5 x 3 min, RT; ddH_2O

Second staining: Unspecified timing; 2% osmium tetroxide in ddH_2O

Washing: 5 x 3 min, RT; ddH_2O

En bloc stain:
- Overnight; 1% aqueous uranyl acetate
- 5 x 3 min, RT; ddH_2O
- 1 x 30 min, 60 °C; lead aspartate
- 5 x 3 min, RT; ddH_2O

Dehydration: 5 min each, on ice; 20%, 33%, 47%, 60%, 73%, 87%, 100%, ethanol in ddH_2O

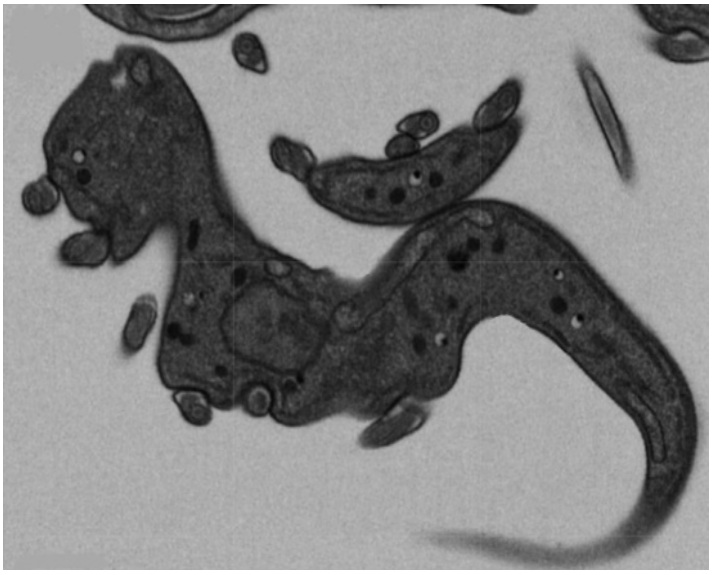
Resin infiltration: 50% for 30 min, 100% for 4 h, 100% overnight, resin in ethanol

Embedding: 60 °C for 24 h; freshly prepared Embed812

Serial Block-Face Imaging Recipes

Trypanosomes

Publication: *Journal of cell science – A cell-body groove housing the new flagellum tip suggests an adaptation of cellular morphogenesis for parasitism in the bloodstream form of Trypanosoma brucei, Oxford Brookes, OTO for TEM*



Primary fixation:
- In suspension, 3 – 5 min; glutaraldehyde, 2.5%
- Resuspend and centrifuge 3 min; 0.1 M phosphate buffer with 2.5% glutaraldehyde, 2% paraformaldehyde, 0.1% tannic acid
- 2 h, RT; same formula

Washing: 0.1 M phosphate buffer

Post-fixation staining:
- 1 h, RT; 0.1 M phosphate buffer with 1% osmium tetroxide

Washing: Rinse (unspecified)

En bloc stain: 40 min, 2% uranyl acetate

Dehydration: Ascending acetone series

Embedding: Agar 100 resin

Primary acinar cells

Customer data: *University of Liverpool, OTOTO*

Primary fixation:
- 1 h, RT; 0.1 M cacodylate buffer (pH 7.4) with 2% paraformaldehyde, 2% glutaraldehyde, 2 nm calcium chloride
- 2 h, RT; 0.1 M phosphate buffer with 2.5% glutaraldehyde, 2% paraformaldehyde, 0.1% tannic acid

Washing: 5 x 3 min, RT; 0.1 M cacodylate buffer with 2 mM calcium chloride

Post-fixation staining: 1 h, RT; 0.1 M sodium cacodylate buffer with 2% osmium tetroxide + 1.5% potassium ferrocyanide

Washing: 5 x 3 min, RT; ddH_2O

Mordant: 1 x 10 min; thiocarbohydrazide in ddH_2O

Washing: Extensive, RT; ddH_2O

Second staining: 40 min, RT; 2% osmium tetroxide in ddH_2O

Washing: 5 x 3 min, RT; ddH_2O

En bloc stain:
- Overnight, 4 °C; 1% uranyl acetate in ddH_2O
- 5 x 3 min, RT; ddH_2O
- 1 x 30 min, 60 °C; lead aspartate
- 5 x 3 min, RT; ddH_2O

Dehydration: 5 min each, RT; 30%, 50%, 70%, 90%, 100%, 100% ethanol in ddH_2O

Resin infiltration:
- Overnight; 50% resin in ethanol
- 1 h, 1 h 30 min; 67%, 75%, 100%, 100% resin in ethanol

Embedding: At least 48 h, 60 °C; graded hard TAAB premix 812

Serial Block-Face Imaging Recipes

Tsetse fly mid-gut (trypanosomes)

Customer data: *University of Liverpool, OTOTO*

Primary fixation:
- 30 min whole tissue; 0.1 M phosphate buffer (pH 7.4) with 0.1% tannic acid + 3% sucrose
- 90 min for dissected region; same formula

Washing: 3 x 3 min, RT; 0.1 M phosphate buffer

Post fixation staining: 1 h, RT; 0.1 M phosphate buffer with 2% osmium tetroxide + 1.5% potassium ferrocyanide

Washing: 5 x 3 min, RT; ddH_2O

Mordant: 1 x 10 min; thiocarbohydrazide in ddH_2O

Washing: 5 x 3 min, RT; ddH_2O

Second staining: 30 min, RT; 2% osmium tetroxide in ddH_2O

Washing: 5 x 3 min, RT; ddH_2O

En bloc stain:
- Overnight at 4 °C, 1% uranyl acetate
- 5 x 3 min, RT; ddH_2O
- 1 x 30 min, 60 °C; lead aspartate

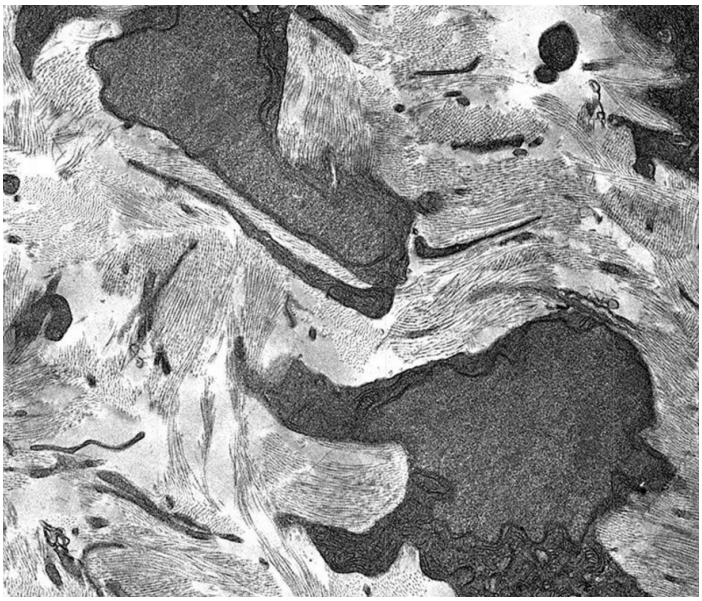
Dehydration:
- 10 min each, RT; 30%, 50%, 70%, 90%, 100%, 100%, 100%, 100% ethanol in ddH_2O
- 10 min each, RT; 100%, 100% propylene oxide

Resin infiltration:
- 30% for 4 h, 50% overnight, 67% for 1 h, 75% for 1 h, 80% for 1 h, 100% for 1 h, 100% for 1 h, 100% for 1 h, resin in propylene oxide

Embedding:
- At least 48 h, 60 °C, graded hard TAAB premix 812

Cornea

Publication: *PNAS100 – Three-dimensional aspects of matrix assembly by cells in the developing cornea, Manchester University, OTOTO*



Primary fixation: 3 h; 0.1 M sodium cacodylate buffer (pH 7.2) with 2.5% paraformaldehyde, 2% glutaraldehyde

Post-fixation staining: 1 h; 0.1 M sodium cacodylate buffer with 1% osmium tetroxide + 1.5% potassium ferrocyanide

Mordant: 2 h; 1% tannic acid

Second staining: 1 h; 1% osmium tetroxide

Washing: "appropriate washes"

En bloc stain: 1 h; 1% uranyl acetate

Washing: "appropriate washes"

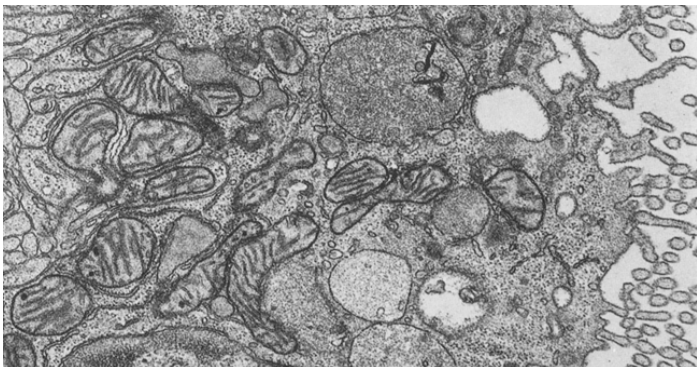
Dehydration: Ethanol dehydration with unspecified method

Embedding: Araldite CY212 resin with unspecified cure time

Serial Block-Face Imaging Recipes

Liver and renal cortex of rat and mouse

Publication: *Electron staining of the cell surface coat by osmium-low ferrocyanide. W.F. Neiss 1983, Institute of anatomy, University of Wurzburg, OTO*



Primary fixation:

- 5 min perfusion; 0.1 M sodium cacodylate-HCl buffer (pH 7.4), with 2.5% glutaraldehyde, 1% paraformaldehyde, and 17 mM $CaCl_2$
- Immerse for 3 h, 20 °C; 0.1 M sodium cacodylate -HCl buffer (pH 7.4), with 3% glutaraldehyde

Washing: Overnight, with several changes of solution; 0.1 M sodium cacodylate-HCl buffer (pH 7.4), with 3% glutaraldehyde

Post-fixation staining: 30 min, 20 °C, vibrating platform, dark; prepared 90 min in advance; 0.2 M sodium cacodylate buffer with 40 mM osmium tetroxide and 20 mM potassium ferrocyanide

En bloc stain: 10 min; saturated uranyl acetate in 50% ethanol and lead nitrate (pH 11.8)

Dehydration: 2 min each; 50%, 70%, 95%, 100%, 100%, acetone

Resin infiltration:

- 33% for 5 min, 66% for 25 min, Durcupan "Medium 1" in acetone, agitated at 20 °C
- 2 x 30 min, at 60 °C; 100% Durcupan "Medium 1"

Embedding: 60 min at 60 °C; 100% Durcupan "Medium 2"

Kidney

Publication: *Resolution of the three dimensional structure of components of the glomerular filtration barrier. Starborg, Kargill, et al, Manchester*

Primary fixation:

- RT, timing unspecified; HEPES buffered mammalian ringer solution containing 0.5% $LaNO_3.6H_2O$ and 0.5% $DyCl_3.6H_2O$
- 0.1 M cacodylate buffer (pH 7.3) with 2.5% glutaraldehyde and 2% sucrose

Washing: Timing unspecified; HEPES buffer

Post-fixation staining: 1 h, RT; 0.1 M cacodylate buffer with 1% osmium tetroxide

Washing:

- Timing unspecified; 0.1 M sodium cacodylate buffer
- Timing unspecified; distilled water

En bloc stain: 12 h, 4 °C; 2 – 3% uranyl acetate

Dehydration: Unspecified timing; graded series of ethanol

Resin infiltration:

- Unspecified timing; araldite resin mixtures in propylene oxide
- Unspecified timing; 100% araldite

Chromosomes

Publication: *Staining and embedding of human chromosomes for 3-D serial block-face scanning electron microscopy. Mohammed Yusuf, Bo Chen, Teruo Hashimoto, Ana Katrina Estandarte, George Thompson, and Ian Robinson, UCL, Manchester*

Primary fixation: Timing unspecified; 0.1 M cacodylate buffer (pH 7.2), with 2.5% glutaraldehyde (by volume)

Washing: Wash twice, timing unspecified; 0.1 M cacodylate buffer (pH 7.2)

Post-fixation staining: 30 min; platinum blue

Washing: 2 x 5 min; Milli-Q water

Dehydration: 15 min each; 30%, 50%, 75%, 100%

Resin infiltration: Unspecified timing; agar 100 resin (hard)

Embedding:

- Immerse in 150 µL resin for 10 h at 60 °C; agar 100 resin (hard)
- Layer with 500 µL resin for 16 h, unspecified temperature; agar 100 resin (hard)

Serial Block-Face Imaging Recipes

Whole mouse brain

Publication: *High-resolution whole-brain staining for electron microscopic circuit reconstruction, Max Planck Institute for Medical Research, Heidelberg, BROPA*

Primary fixation:

- Perfusion, 30 mL at approximately 0.5 mL/s, freshly prepared 30 min prior; 0.1 M cacodylate buffer (pH 7.2) with 0.25 M (2.5%, w/v) glutaraldehyde and 0.12 M sucrose
- Keep wet during brain removal; same formula
- Immersed for 48 – 72 h, 2 °C, no agitation

Washing: 5 x 8 – 12 h; 0.1 M cacodylate buffer (pH 7.2) with 0.12 M sucrose

Post-fixation staining: 96 h, RT, dark, gyratory rocker 10 rpm; 0.1 M cacodylate buffer (pH 7.4) with 40 mM osmium tetroxide, 35 mM potassium ferrocyanide and 2.5 M formamide

Mordant: 72 h, RT, dark, gyratory rocker 10 rpm; 0.1 M cacodylate buffer (pH 7.4) with 40 mM osmium tetroxide

Washing: 4 h, RT, dark, gyratory rocker 10 rpm; 0.1 M cacodylate buffer

Second staining: 72 h, RT, dark, gyratory rocker 10 rpm; unbuffered solution of 0.32 M pyrogallol (pH 4.1)

Washing: 4 h, RT, dark, gyratory rocker 10 rpm; 0.1 M cacodylate buffer

En bloc stain: 96 h, RT, dark, gyratory rocker 10 rpm; unbuffered solution of 0.04 M osmium tetroxide

Dehydration: 18 – 24 h each; 10%, 25%, 50%, 75%, 100%, ethanol in water

Resin infiltration:

- 18 – 24 h; 100% propylene oxide
- 18 – 24 h each; 25%, 50%, 75%, 100%, modified Spurr's epoxy in propylene oxide

Embedding: In custom silicon mold, 48 h, 60 °C; modified Spurr's resin formulation

Mouse brain

Publication: *NCMIR methods for 3D EM: a new protocol for preparation of biological specimens for serial block face scanning electron microscopy, University of San Diego (NCMIR)*

Primary fixation:

- 5 min, 35 °C, whole tissue; 0.15 M cacodylate buffer (pH 7.4) with 2.5% glutaraldehyde, 2% formaldehyde (fresh from paraformaldehyde), and 2 mM $CaCl_2$
- Immerse for 2 – 3 h on ice; using same solution
- If required, cut into 100 µm thick sections in ice cold 0.15 M cacodylate buffer with 2 mM $CaCl_2$

Washing: 5 x 3 min; cold cacodylate buffer with 2 mM $CaCl_2$

Post-fixation staining: 1 h, on ice; freshly prepared 0.3 M cacodylate buffer with 4% aqueous osmium tetroxide + 3% potassium ferrocyanide

Washing: 5 x 3 min; ddH_2O

Mordant: 20 min, RT; thiocarbohydrazide

- Preparation: Add 0.1 gm to 10 mL ddH_2O, agitate in 60 °C oven for 1 h; filter through 0.22 µm filter

Washing: 5 x 3 min; ddH_2O

Second staining: 30 min, RT; 2% osmium tetroxide in ddH_2O

Washing: 5 x 3 min; ddH_2O

En bloc stain:

- Overnight, 4 °C; 1% aqueous uranyl acetate
- 5 x 3 min, RT; ddH_2O
- 1 x 30 min, 60 °C; lead aspartate; prepared by dissolving 0.66 gm lead nitrate in 10 mL 0.03 M aspartic acid; adjust pH to 5.5, and then oven 30 min 60 °C

Dehydration:

- 5 min each, ice cold; 20%, 50%, 70%, 90%, 100%, 100%, acetone in ddH_2O
- 10 min, RT; 100% acetone

Resin infiltration:

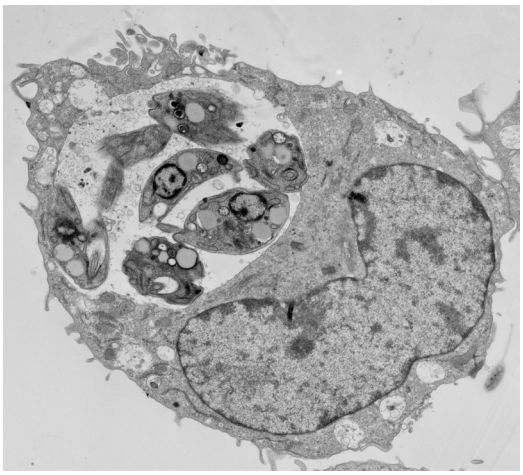
- Overnight; 100% Durcupan
- 2 h; fresh 100% Durcupan

Embedding: 60 °C for 48 h; fresh Durcupan

Serial Block-Face Imaging Recipes

Leishmania mexicana

Publication: *Methods Cell biology – Scanning and three dimensional electron microscopy methods for the study of Trypanosoma brucei and Leishmania mexicana flagella, Oxford Brookes, OTO*



Primary fixation:

- In suspension, 5 min, RT; glutaraldehyde, 2.5%
- Resuspend and centrifuge at 800 g for 10 min; 2.5% glutaraldehyde
- 2 h, RT; 0.1 M phosphate buffer with 2.5% glutaraldehyde, 2% paraformaldehyde

Post-fixation staining: 1 h, RT; 0.1 M phosphate buffer with 1% osmium tetroxide

Washing: Wash at least 3x with distilled water

En bloc stain: Overnight at 4 °C, in dark; 2% magnesium uranyl acetate

Dehydration:

- <100% – 15 min each, RT; 100% – 30 min each, RT
- 30%, 50%, 70%, 90%, 100%, 100%, 100% acetone in water by volume

Resin infiltration:

- Resin in acetone; 33% for 3 h, 50% for 3 h, 67% for 3 h, 100% overnight, 100% for 3 h, 100% for 3 h
- 3 h each, RT; two additional infiltrations of 100% resin

Embedding: 24 h, 70 °C; agar 100 resin

AP-3View 2XP Recipes-FL1-UK-Feb16