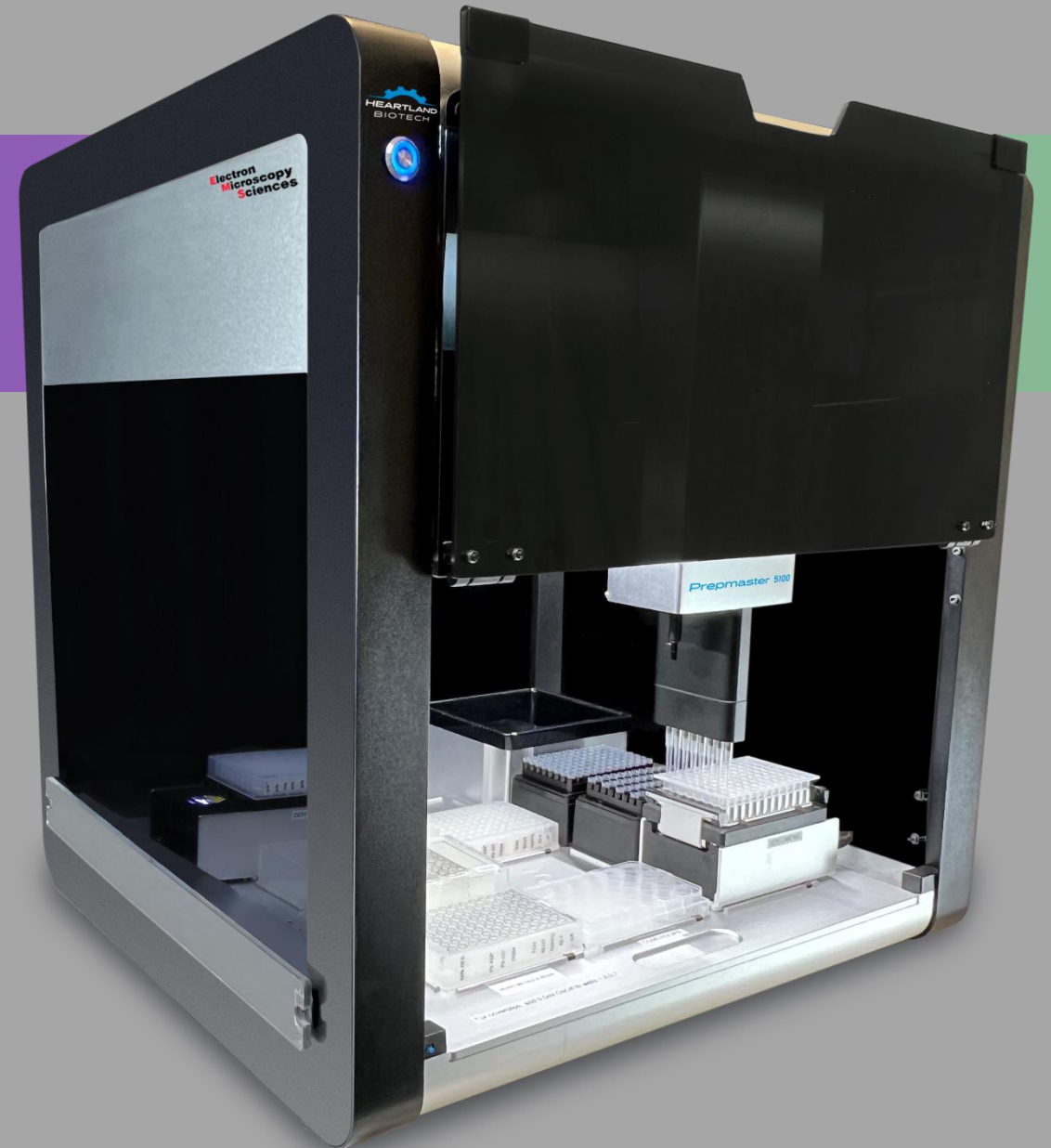
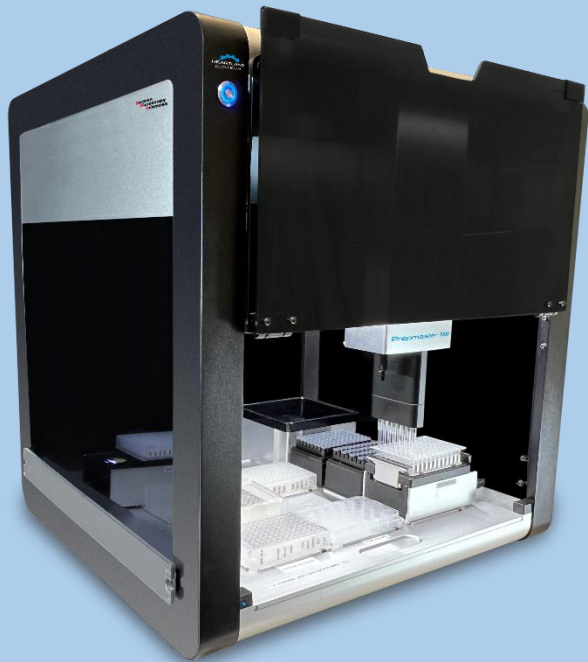


Robotic Preparation of Tissue Specimens for TEM and Volume EM

Thomas E. Strader. M.S. 1,2* , Benjamin K. August2 , Ru-ching Hsia, Ph.D
1. Heartland Biotech, Madison, WI USA 2. University of Wisconsin-Madison, Madison, WI USA 3. Carnegie Institute of Science, Baltimore, MD, USA
*Corresponding author: tom.strader@heartlandbiotech.com



Robotic Preparation of Tissue Specimens for TEM and Volume EM



- Specimen preparation procedures for high-contrast transmission electron microscopy (TEM) and Volume EM (vEM) include multiple contrast enhancing steps making them some of the longest and most complex tissue processing protocols performed for EM.
- Additionally, 4 of the steps for vEM need heating or cooling. These specimen preparation workflows call for use of noxious, hazardous, and often carcinogenic chemicals including uranium salts, arsenic, heavy metals, cyanide, lead and osmium tetroxide.
- These long and complex workflows provide ample opportunity for human error and are currently performed manually in most cases.

Brief Review of Typical Manual Workflow

(from primer)

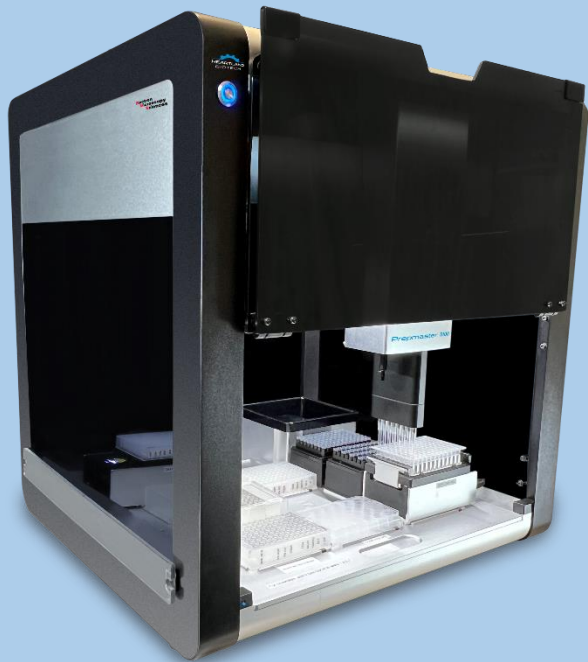
1. Primary fixation (2.5% glutaraldehyde): 1–2 h
 2. Wash (buffer): 30 min to 1 h
 3. Secondary fixation (0.5% osmium tetroxide): 1 h
 4. Dehydration: 70% ethanol, 10 min; 95% ethanol, 10 min; 100% ethanol, 2 × 10 min
 5. Transitional solvent (propylene oxide): 2 × 10 min
 6. Infiltration of resin (50% propylene oxide/50% resin): 1 h minimum
 7. Embedding (100% resin): 1 h minimum
 8. Curing (60–70 °C): 12 h minimum for Spurr's
 9. Thick section (for eight blocks): 20–25 min
 10. Stain thick sections: 5–7 min
 11. Block selection (pathologist, per block): 2 min
 12. Trim blocks (per block): 1 min
 13. Thin section (per two blocks): 10–20 min
 14. Stain grids (eight grids): 30 min
 15. Scope (per grid): 30 min to 1 h
- Total time: 19–21.5 h

Pain Points

- Electron Microscopy lab managers with tight budgets face unprecedented labor shortages and ballooning labor cost.
- The often advanced-degreed scientists they employ are unhappy with having to perform dull and repetitious specimen processing requiring handling of toxic chemicals including arsenic, cyanide, osmium tetroxide and uranium salts.
- Tremendous amounts of severely toxic chemical waste are needlessly being introduced into the environment.



Introducing the Prepmaster™ 5100



All-in-one automated processing system prepares biological specimens for EM applications:

- TEM
- vEM
- Immuno-EM
- SEM

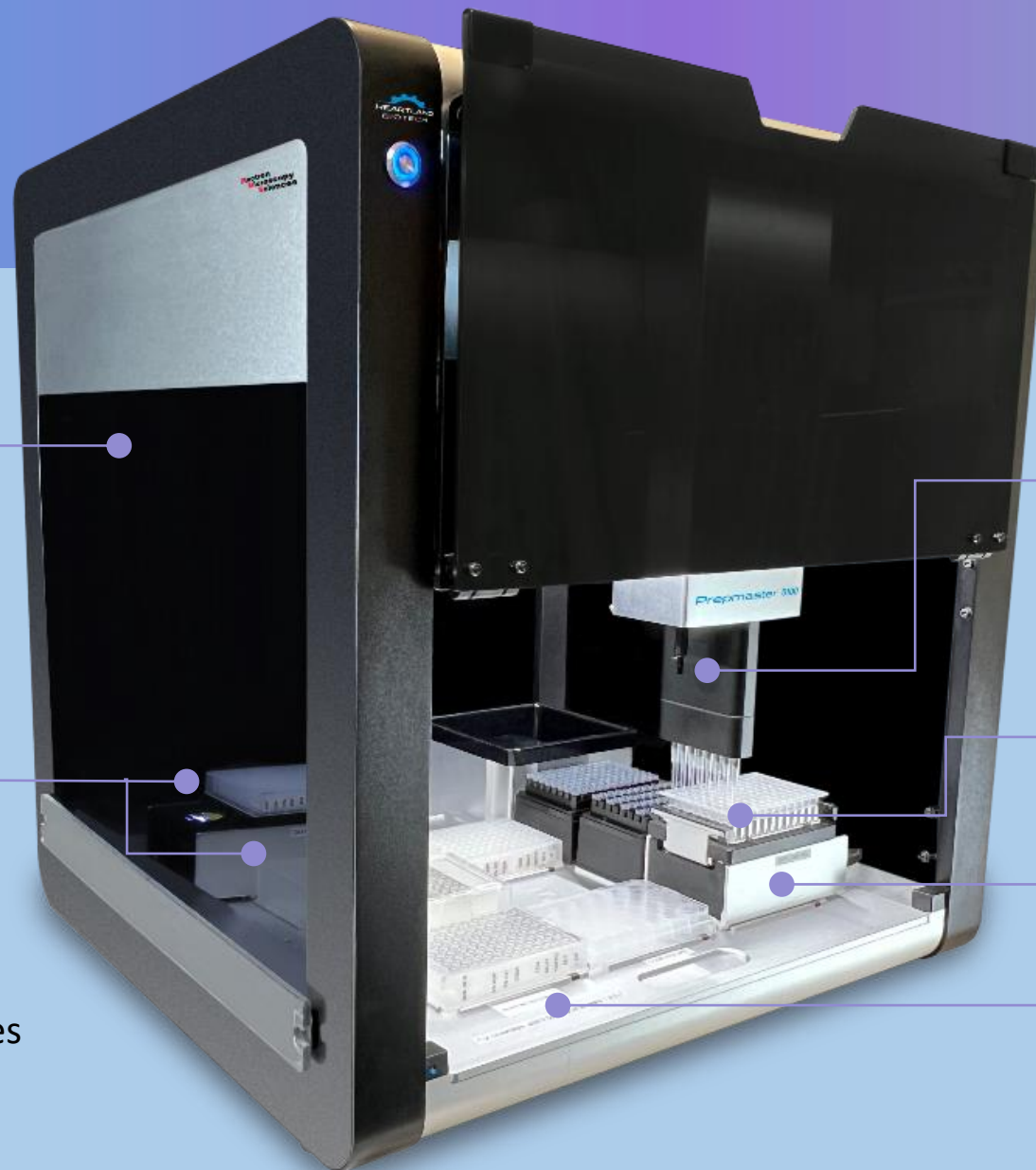
Biological
Tissue
≤1 x 3mm

Cells
seeded
on
coverslips

Organoids

Grids

Features:



99.5% UV-blocked
ventilated, removable
enclosure

Separate heating
& cooling reagent
stations (5° - 60°C)

Laptop control (not shown)
with remote monitoring capabilities

Hydraulic dispensing &
aspiration

Processes up to 8
specimens in parallel

Heated Agitation Station™

Configurable deck

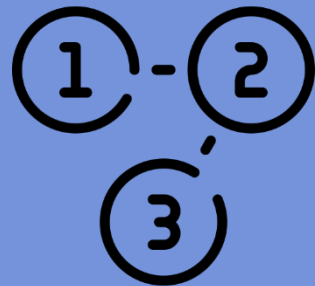
2' x 2' x 2' footprint

Prepmaster Benefits You Can Rely On



Automation

enables
reproducibility,
standardization



Easy

enables quick
workflow
integration



Efficiency

enables
higher lab
throughput



Safety

enables minimized
exposure



Economical

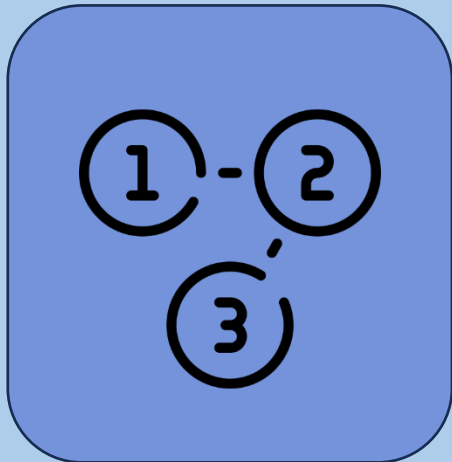
enables
lab budget savings

Prepmaster Ensures Reproducibility



- Consistent preparation = reproducible results
- No sample-to-sample, batch-to-batch, or technician-to-technician variability
- Eliminates human error
- Improved quality & accuracy of results
- Separate developer vs. user login locks protocols for consistency.

Convenient Workflow

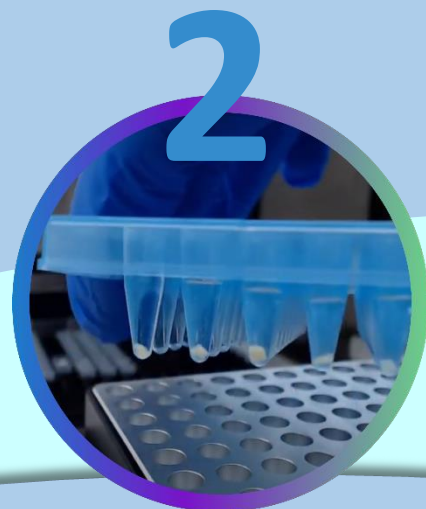


- Easy to set up (<5 mins.)
- Easy to clean up (<1min.)
- Customizable across applications and protocols
- Intuitive interface for protocol development
- Minimal training required
- Easy as 1, 2, 3...

Workflow Efficiency for Greater Lab Productivity



Load
Reagents



Load
Specimens

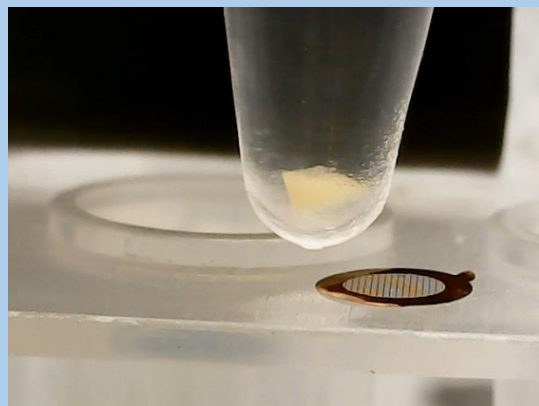


Select Protocol
& Press Start

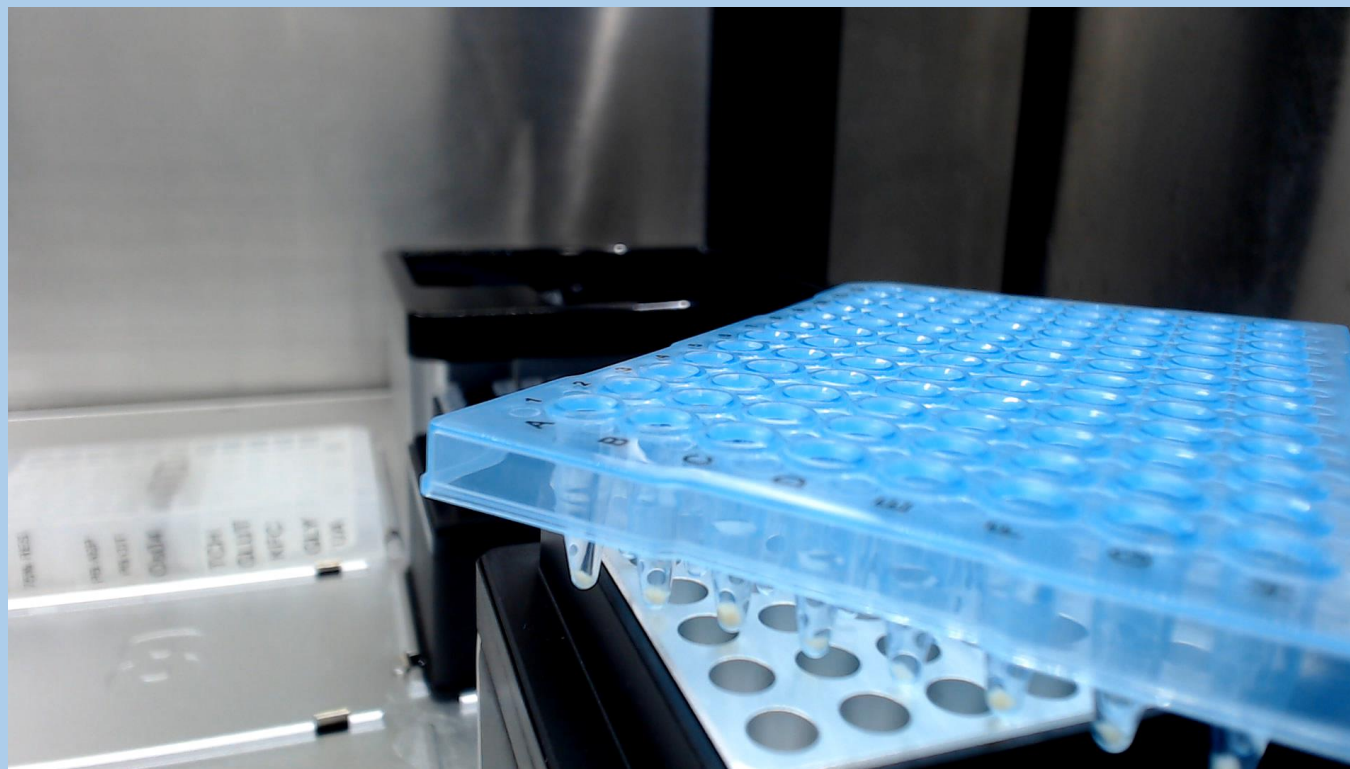
Prepmaster's
walkaway ease
enables greater
lab productivity

Easy Operation

▶ Video

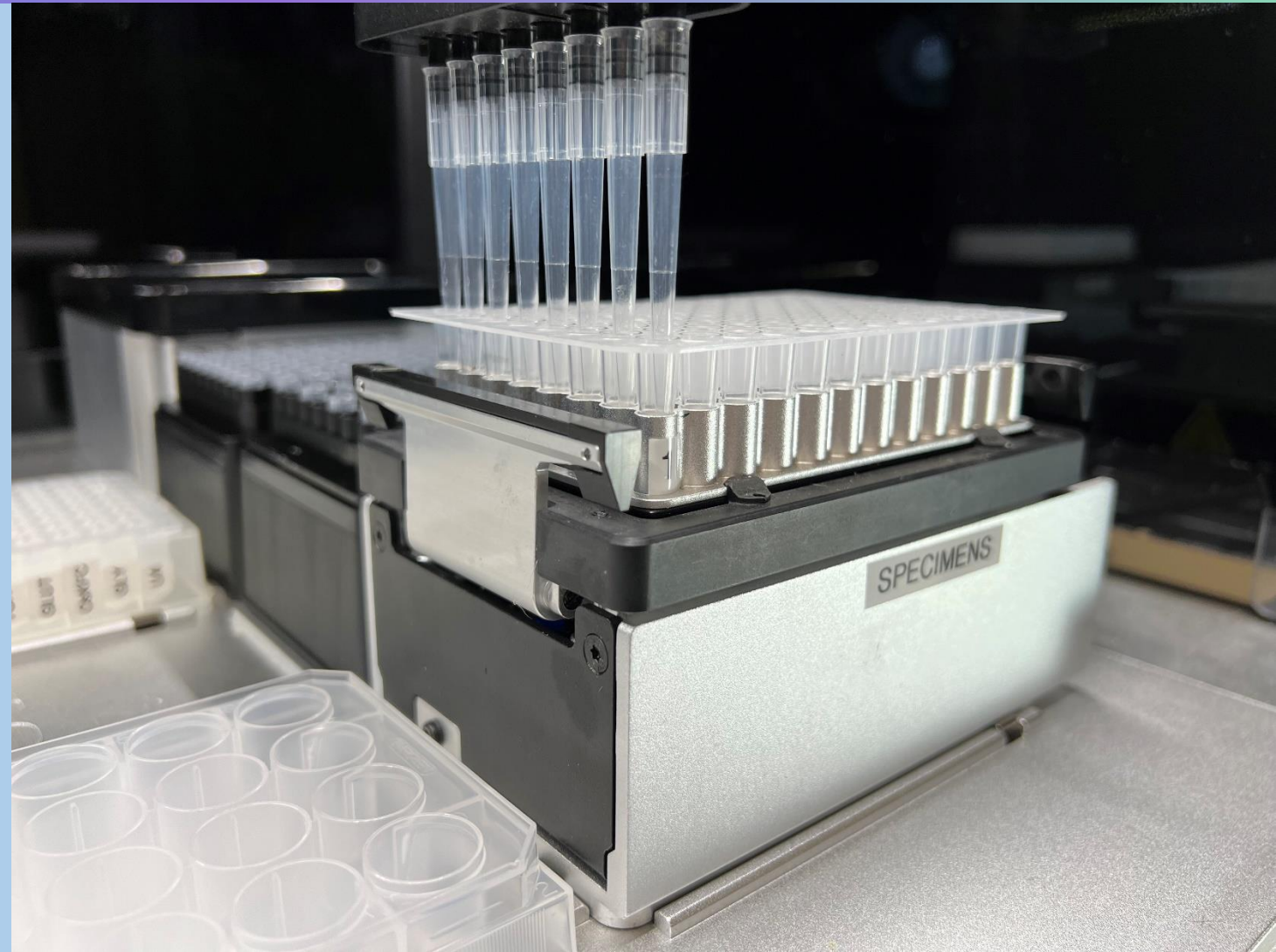


1x3mm specimens placed in
bottom of well in Column 1



Agitation Station™

- Thermal control from RT to 60°C
- Gentle and effective “shaker” agitation @ 300-3000 rpm



Efficiency for Rapid Results



- Hydraulic dispensing and aspiration + Agitation Station accelerate processing without compromising micrograph results
- Press Start and walk away to work on other lab projects
- Run overnight for even greater efficiency; remote monitoring & control
- Prepares 1 or up to 8 samples in parallel

Safety with Minimized Exposure



- Load hazardous and/or toxic reagents onto Prepmaster at beginning and walk away
- Ventilate fumes into your organization's hood or ventilation system
- Press Start and walk away, or run overnight for even less exposure

Cost Effective for Lab Budgets

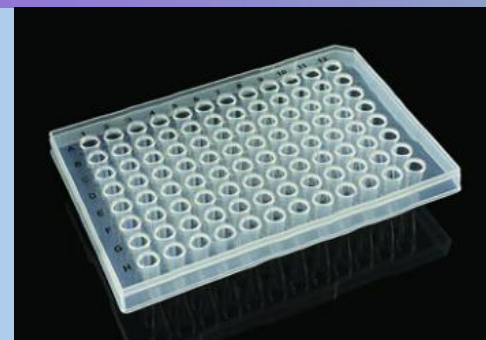


- Uses standard labware; No proprietary, expensive consumables required.
- Up to 90% less reagent use saves purchase, shipping and disposal cost.
- Unattended operation frees time to work on other projects for greater lab productivity

Consumables Cost/Run of 8 Specimens

Item	Approx. Cost
Specimen plate (1)	\$4
Pipette tips (8)	\$1
Water, EtOH	\$3
Osmium (1 ampule)	\$35
Total cost per 8 specimen run*	\$43
Total cost per specimen*	\$5.37

*Excluding shipping and waste disposal



Consumables
available for order
via EMS Diasum

REUSABLE:
Reagent reservoirs, ~\$5 ea.

Results: Consistent, Pristine Ultrastructure

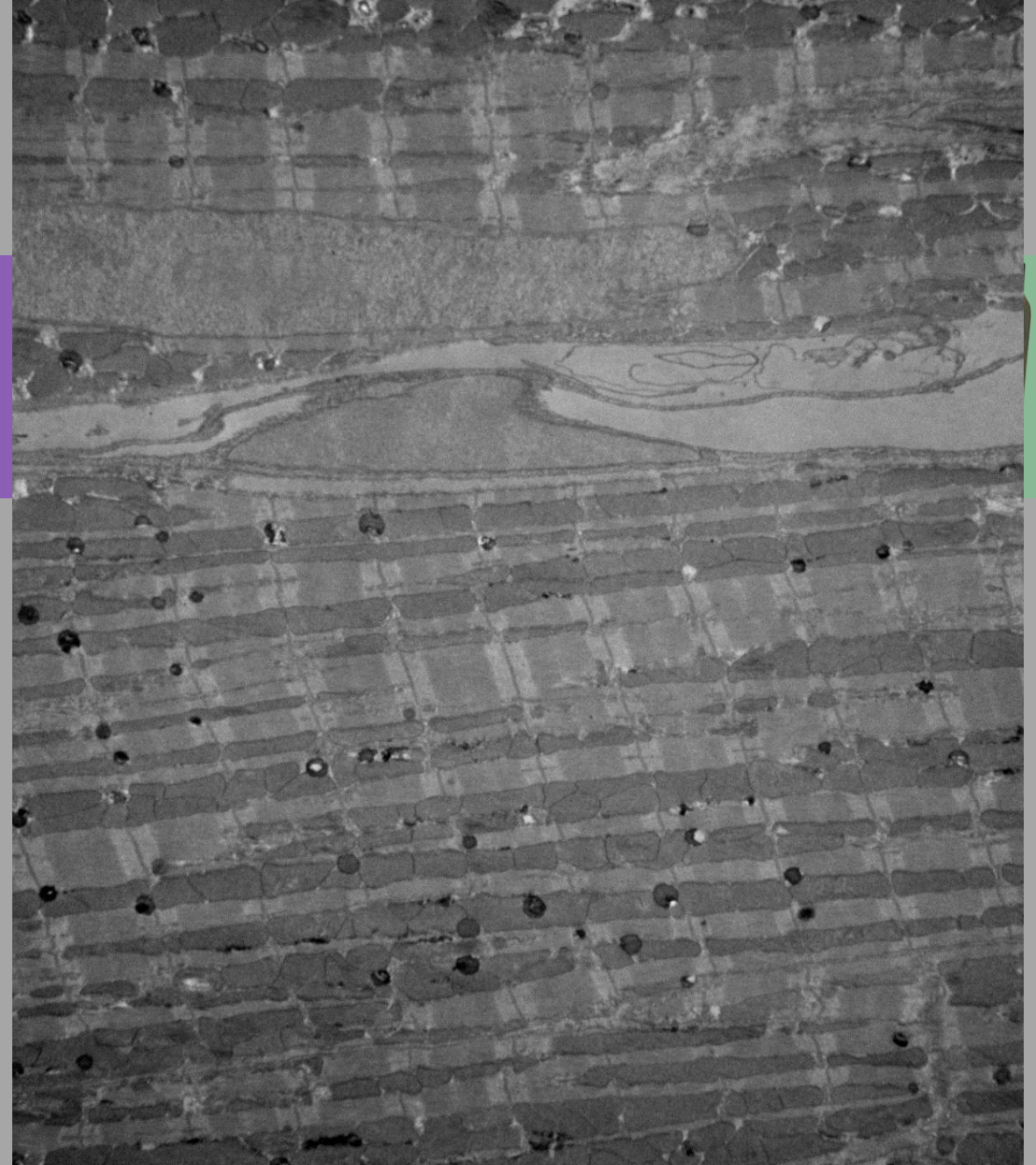
Prepmaster 5100

Results



Ben August, University of Wisconsin-Madison EM Core Facility

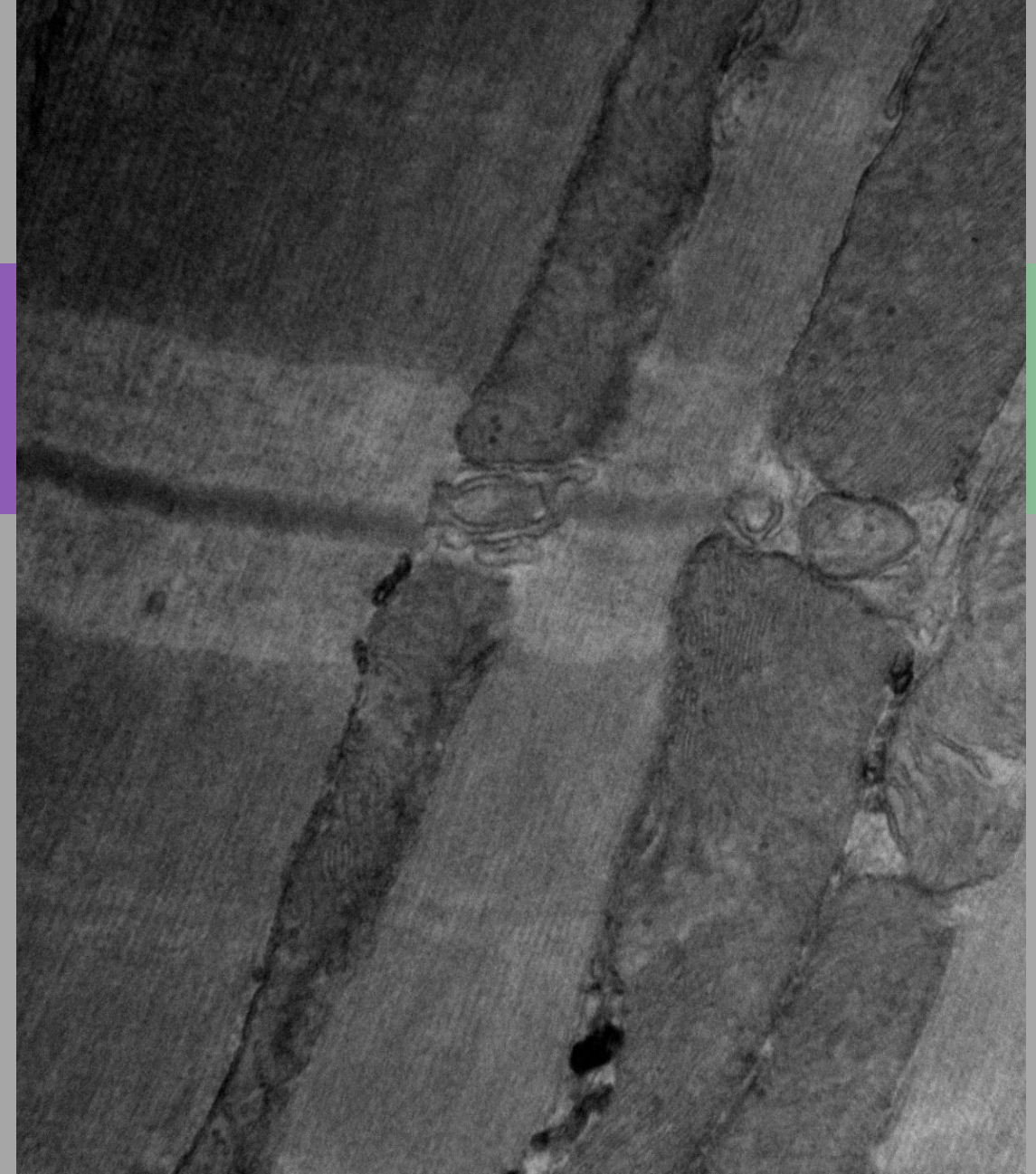
- ◆ Cardiac muscle from UW-Madison
- ◆ Prepared on Prepmaster 5100
- ◆ Ellisman rOTO Protocol
- ◆ Microtomy and imaging by Benjamin K. August
- ◆ FEI CM120 STEM



Cardiac_006
Print Mag: 7870x @ 7.0 in
16:13:17 10/13/2022
TEM Mode: Imaging

2 μ m
HV=80.0kV
Direct Mag: 4400x

- ◆ Cardiac muscle from UW-Madison
- ◆ Prepared on Prepmaster 5100
- ◆ Ellisman rOTO Protocol
- ◆ Microtomy and imaging by Benjamin K. August
- ◆ FEI CM120 STEM



Cardiac_004

Print Mag: 62700x @ 7.0 in

16:10:10 10/13/2022

TEM Mode: Imaging

Microscopist: AMT

01025510_5_00000000_10000000_1.5_Diag_1

200 nm

HV=80.0kV

Direct Mag: 40000x

SMPH TEM LAB

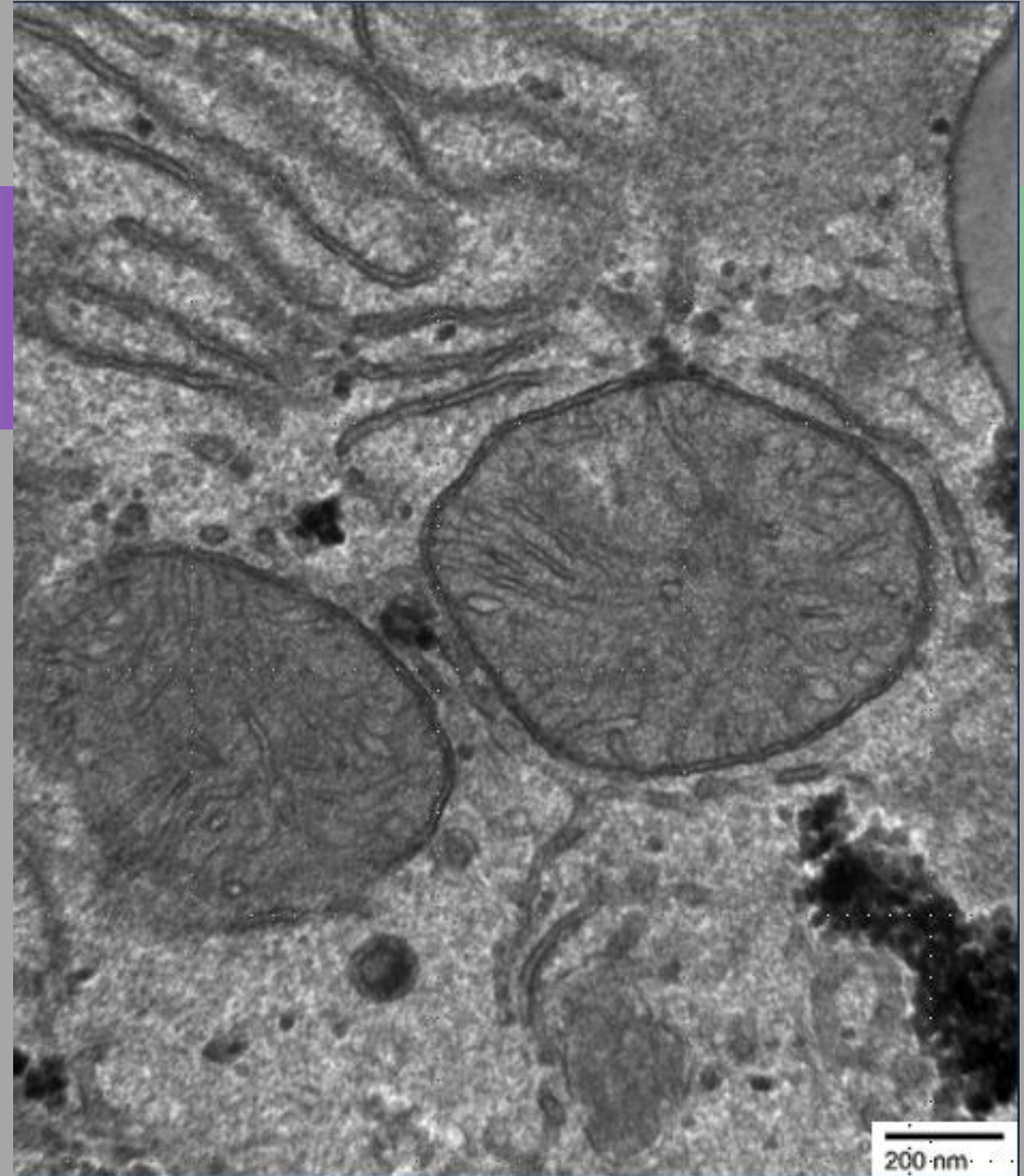
- ◆ Cardiac muscle from UW-Madison
- ◆ Prepared on Prepmaster 5100
- ◆ Ellisman rOTO Protocol
- ◆ Microtomy and imaging by Benjamin K. August
- ◆ FEI CM120 STEM



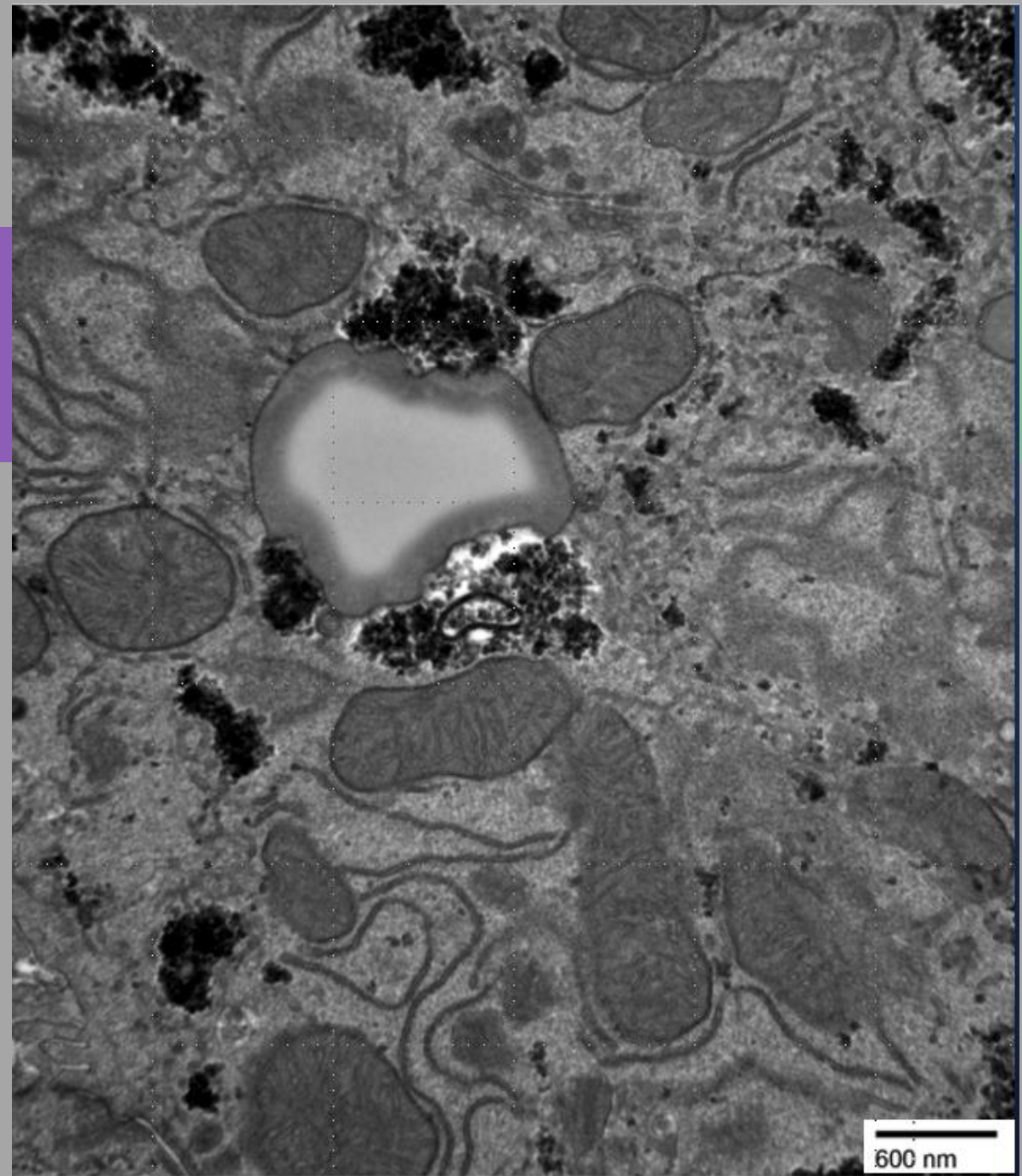
Cardiac_010
Print Mag: 62700x @ 7.0 in
16:18:30 10/13/2022
TEM Mode: Imaging
Microscopist: AMT

200 nm
HV=80.0kV
Direct Mag: 40000x
SMP/TEM LAB

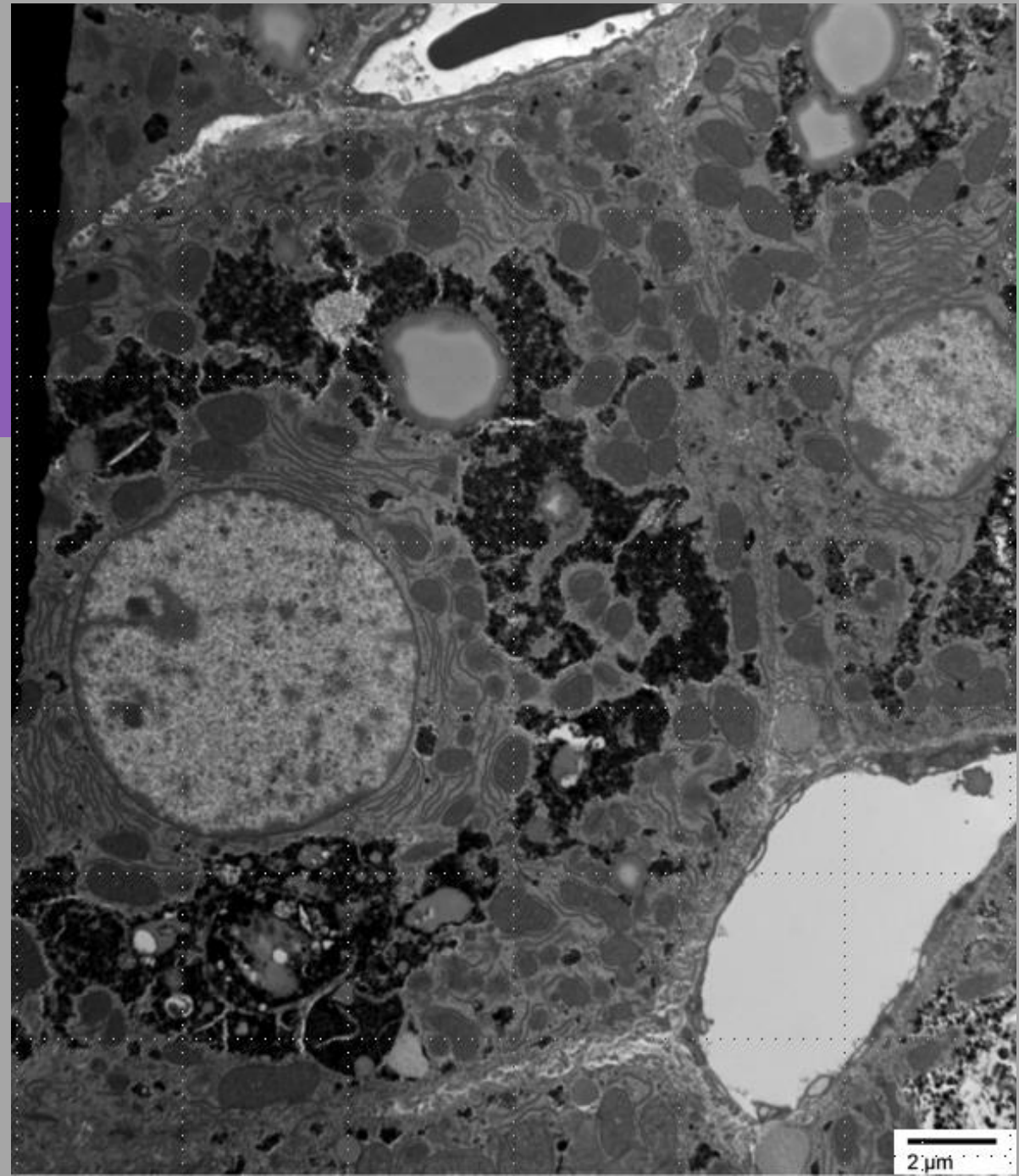
- Liver biopsies
- Ellisman OTO protocol
- UW-Madison

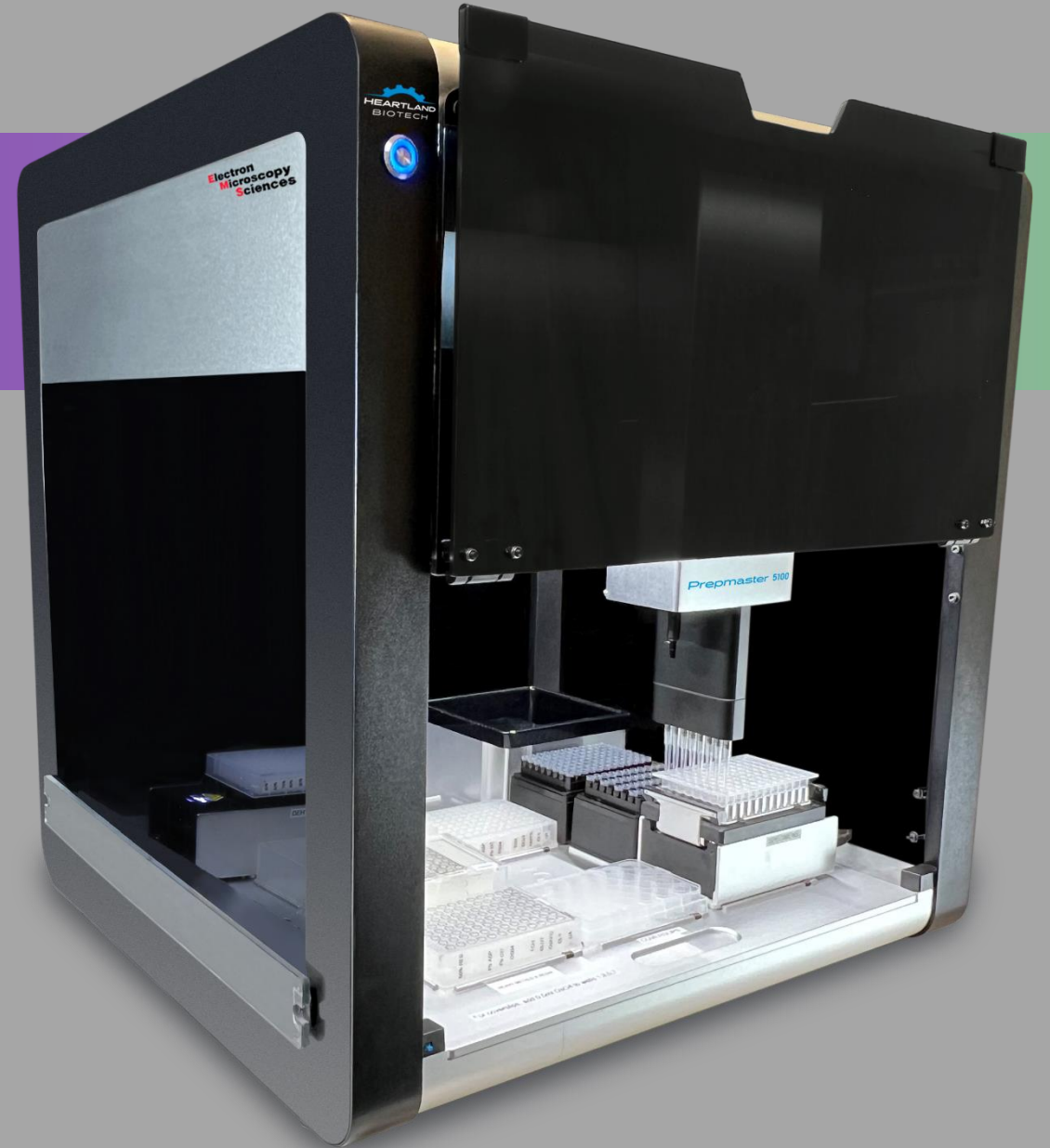
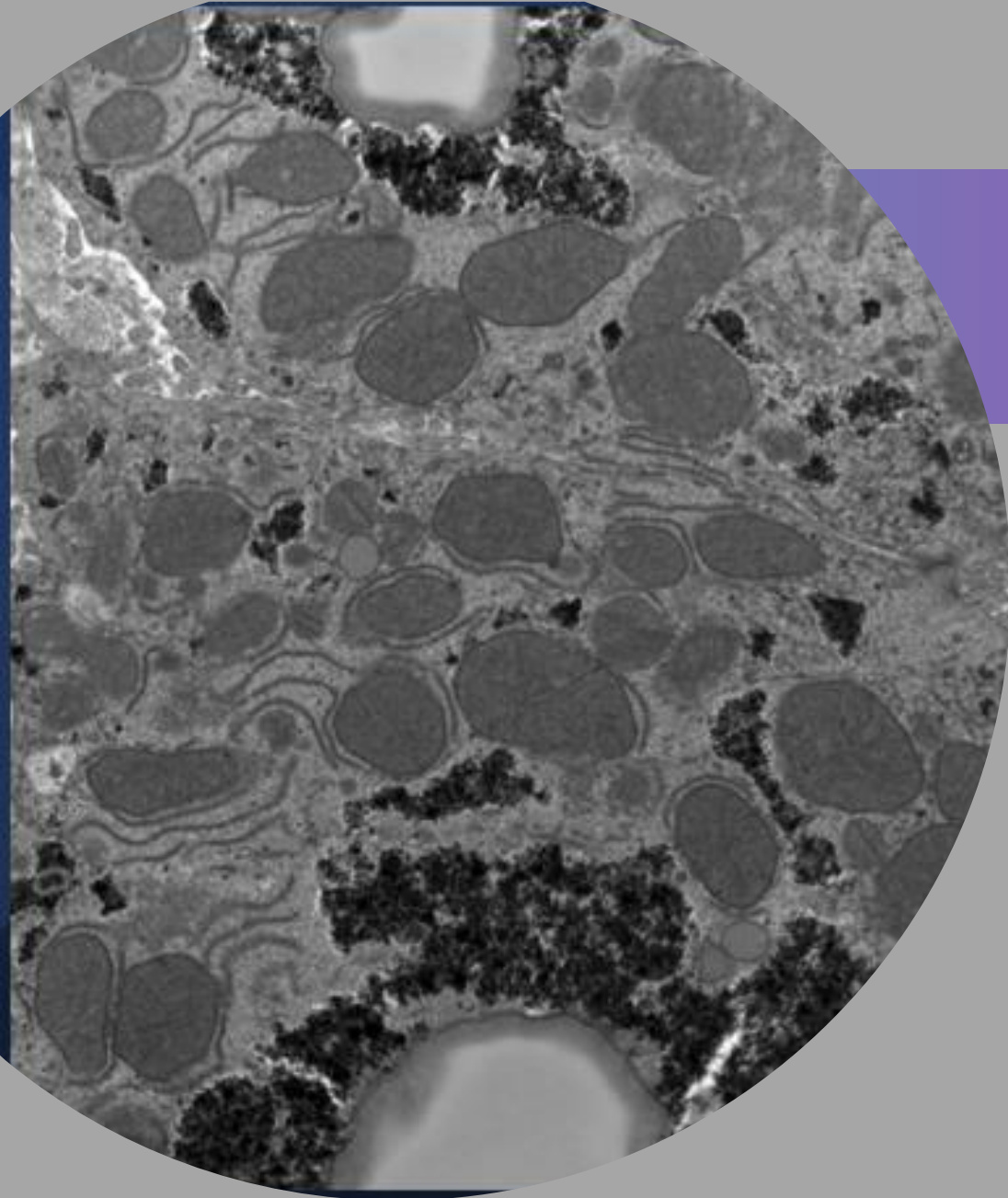


- Liver biopsies
- Ellisman OTO protocol
- UW-Madison



- Liver biopsies
- Ellisman OTO protocol
- UW-Madison



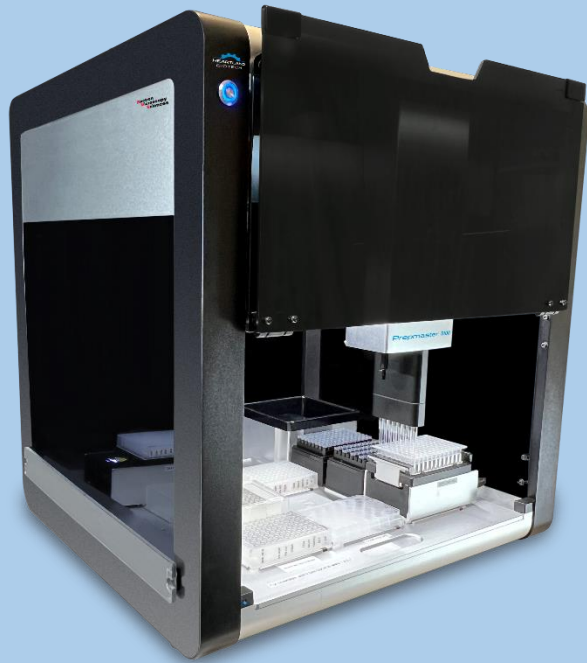


Prepmaster 5100

Prepmaster 5100 provides effortless, error-free, economical and safe execution of your least favorite and most hazardous laboratory operations, specimen prep.



Thank you! Questions?



info@heartlandbiotech.com
www.heartlandbiotech.com
608.770.7649

EMSDiasum.com
info@EMSDiasum.com
215.412.8400