

July 18, 2024 11:00 AM CST

Dr. Reiner Bleher BioCryo Facility Manager & Research Associate Professor

Automated Electron Microscopy Sample Preparation with the ASP-1000"

Northwestern Exploring Inner Space



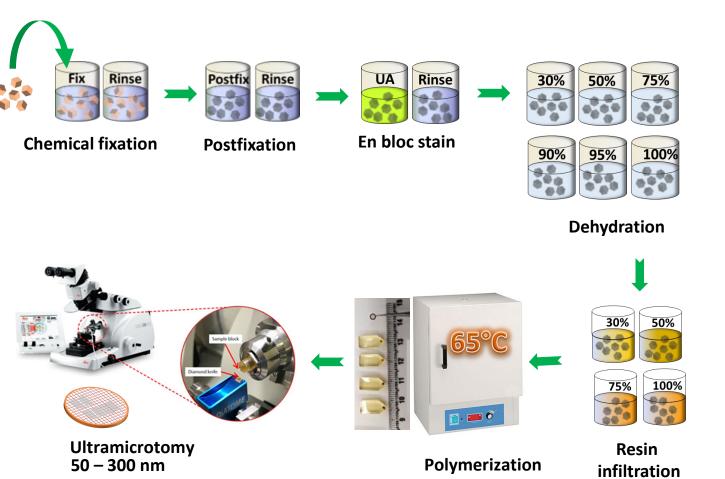
23 years of Excellence...







## **Resin embedment protocol steps**



#### Fixation:

- crosslinking of proteins with glutaraldehyde and/or formaldehyde in buffer
- preservation of ultrastructure/antigenicity for immunolabeling

#### **Postfixation/staining**

- OsO<sub>4</sub> to stabilize and stain lipids (membranes)
- staining of charged sites (DNA, proteins) with UA

#### Dehydration

• replacement of water with an ascending series of ethanol, acetone, or acetonitrile

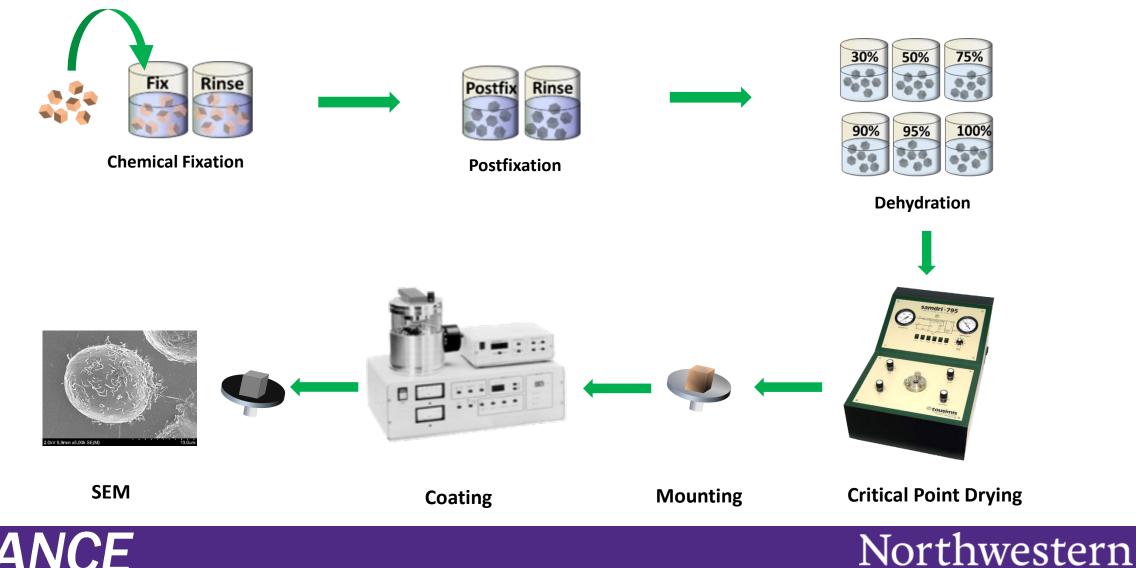


- stainability (LM and EM)
- minimal shrinkage
- hardness/softness for ultramicrotomy
- preservation of antigenicity for immunolabeling
- stability in the vacuum
  - stable when exposed to the electron beam





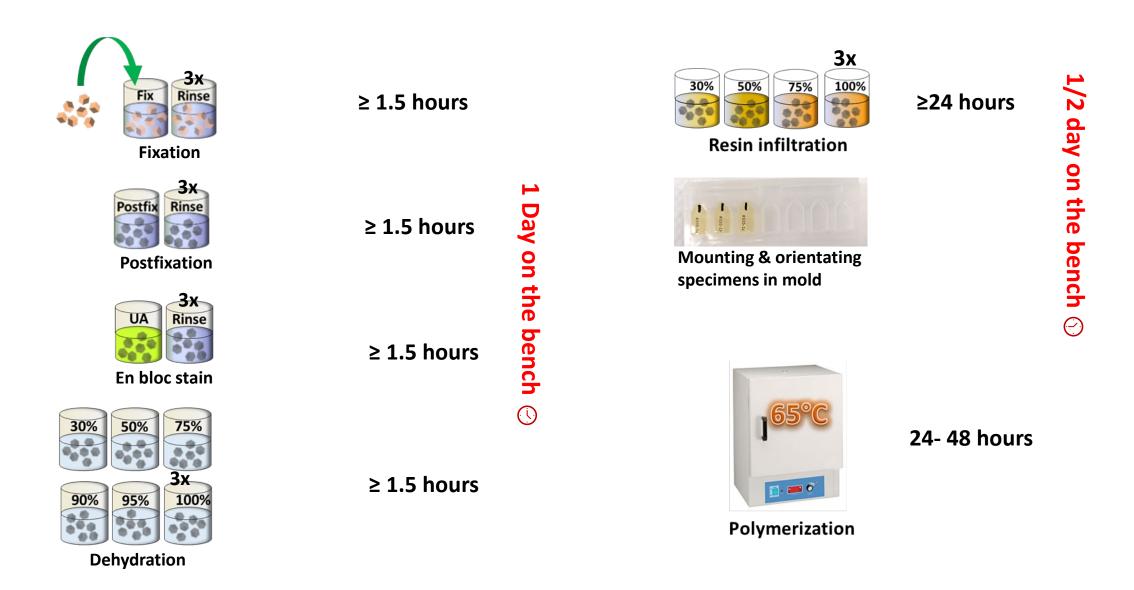
## Sample preparation for SEM



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# Processing protocol steps and time requirements for a standard protocol













(Automated Sample Processor)

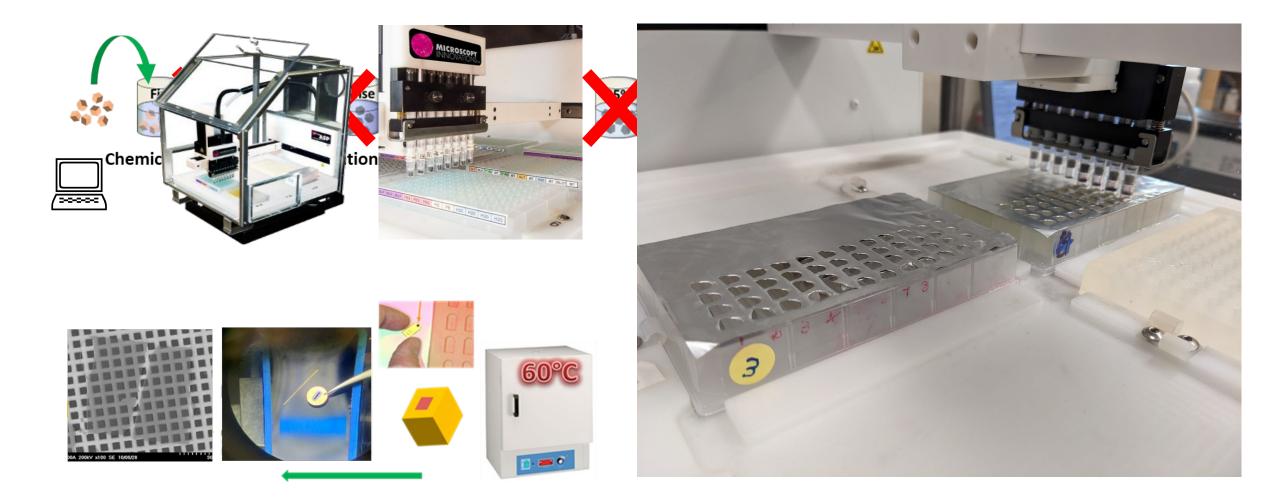
Speed, adaptability, and versatility for automated preparation of biological and soft matter samples







# ASP-1000: automated chemical fixation – dehydration – infiltration







## ASP-1000: automated sample preparation for TEM

The mPrep ASP-1000 processes specimens or grids mounted on the computer-controlled pipette "head" (circled) that moves them between reagents in microtiter plates on the processor tray.





Processor pipette head with 8 specimens entrapped in 8 labeled mPrep/s capsules (arrow points to one specimen).

Head with 8 mPrep/g capsules containing 16 grids (arrow points to one grid).







# Automated sample prep workflow









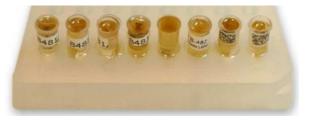
**1. Set up the protocol** 

2. Prepare the plates

3. Mount the samples

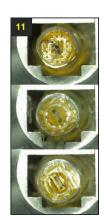


5. Run the protocol



6. Polymerization





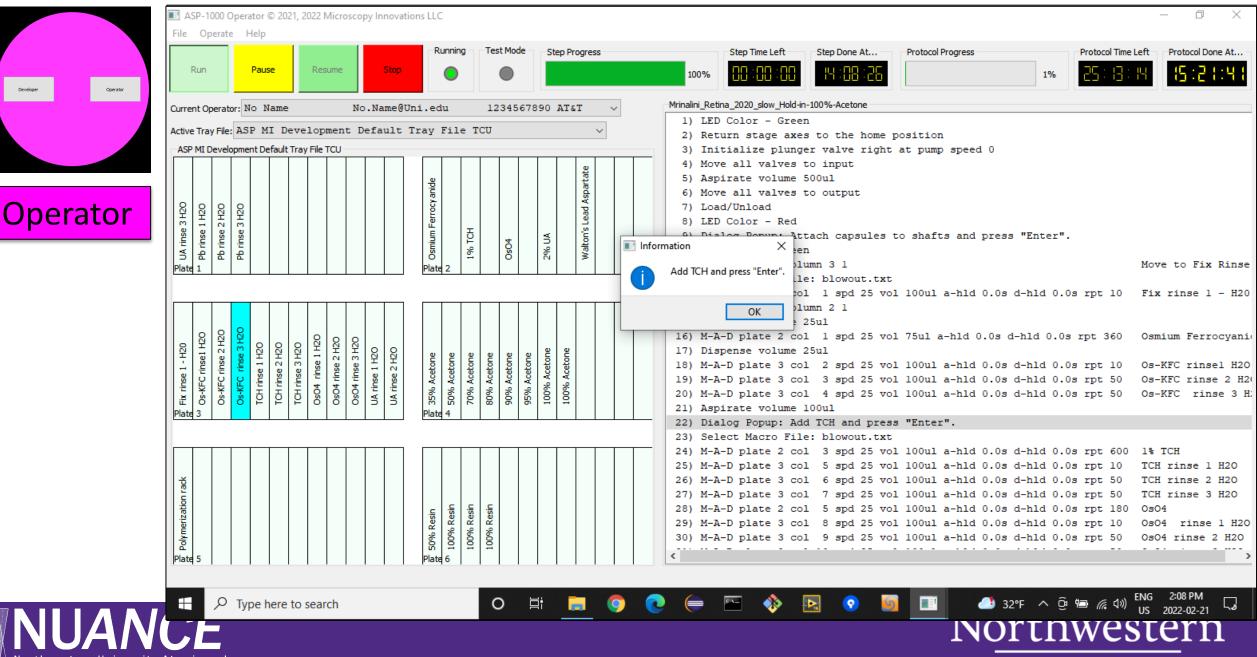
7. Retrieve the cured blocks



ymerization



## **ASP-1000 Dashboard Control**



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## **ASP-1000 Dashboard Control**

	ASP-1000 Developer © 2021, 2022 Micro	scopy Innovations LLC						– a ×
	File Edit Operate Help							
Developer Operator	Run Pause Resume	Stop Running Test Mode	Step Progress	Step Time Left	Step Done At	Protocol Progress	Protocol Time Le	eft Protocol Done At
	Current Developer: No Name	No.Name@Uni.edu 123456789	V TATA O	Dye_Nerve TEM Tissue All 12-well - SL	Gv2*			
	Protocol Step Designer			1) Dialog Popup: TEM	protocol nerve	tissue with Os, UA, EtOH	, Acetone, c	confirm protocol
	Command	Comment		2) LED Color - Green				
		Fix rinse 2		<ol> <li>Return stage axes</li> </ol>	to the home pos	sition		
				<ol> <li>4) Initialize plunger</li> </ol>	-	t pump speed 0		
	Plate Column	Pause (s) LED		5) Move all valves to	-			
Devialence	Plate 3 $\lor$ Column 2 $\lor$	0		6) Aspirate volume 50				
Developer	X (mm) Y (mm)	Z (mm) Setpoint (C)		7) Move all valves to	output			
•	12.7 268	100 20.0		<ol> <li>8) Load/Unload</li> <li>9) LED Color - Red</li> </ol>				
	Speed Vol. (u		Repeat		ch cangulag to	shafts and press "Enter"	1	
	18 100		50			I&T, Begin Nerve Prep pro		send message
	Operator SM			12) LED Color - Green	,			
	operator	o/s capsules:		13) Select Macro File:	blowout.txt			
	Manage Tile Manage	volume = 100 to 150 ul.		14) M-A-D plate 3 col	l spd 18 vol 3	100ul a-hld 0.0s d-hld 0.	0s rpt 50 F	Fix rinse 1
	IVIAXIN	num volume = 150 ul.		15) M-A-D plate 3 col	2 spd 18 vol 3	100ul a-hld 0.0s d-hld 0.	0s rpt 50 F	Fix rinse 2
	Dialog/SMS Text: Active Tray File: ASP MI Develog mPrep	o/g capsules: Le TCU		16) M-A-D plate 3 col	3 spd 18 vol 3	100ul a-hld 0.0s d-hld 0.	0s rpt 50 F	Fix rinse 3
	Usual	volume: 35 ul.	~	17) M-A-D plate 1 col	l spd 18 vol 9	50ul a-hld 0.0s d-hld 0.0	s rpt 300 C	0s04
	Poso4 Cos rinse 1 H2 Os rinse 2 H2 Os rinse 2 H2 Os rinse 1 H2 UA rinse 1 H2 UA rinse 1 H2 UA rinse 2 H2	num volume = 40 ul.		18) Select Macro File:			-	Osmium
	A Second	num volume = 40 ul. HOJ II % 000 000 000 000 000 000 000 000 000	Acetone 2 Acetone 2		-	100ul a-hld 0.0s d-hld 0.	-	Os rinse 1 H2O
	1 Os rinse 0 S rinse 0 S rinse 0 S rinse U A rinse U A rinse U A rinse	90% EF P100% EF P100\%	Acetone		-	100ul a-hld 0.0s d-hld 0.	-	Os rinse 2 H2O
		Plate 2 6 6 7 7			-	100ul a-hld 0.0s d-hld 0.	-	
					-	100ul a-hld 0.0s d-hld 0.	-	
					-	0627, AT&T, Nearing time		-
	rinse 1	Resin Resin Resin Resin			-	100ul a-hld 0.0s d-hld 0.	-	UA rinse 1 H2O
		100% Resin 75% Resin 75% Resin 100% Resin 100% Resin 100% Resin		· · ·	-	100ul a-hld 0.0s d-hld 0.	-	UA rinse 2 H2O
		Plate 4		27) LED Color - Red	9 spa 18 voi .	100ul a-hld 0.0s d-hld 0.	US FPC 100 0	JA FINSE 5 H20
				1 '	to add Aceton	e, Resin (and EtOH), then	nress Ente 7	Time to add reagen
		euch la		29) LED Color - Green	to add Acctoin	c, kesin (and Econ), enen	press Enteri	rime oo add reagen
	Plate 5	Plate 6			l spd 18 vol	100ul a-hld 0.0s d-hld 0.	0s rpt 100 5	50% EtOH
	mPren/s cansules: usually 100 to 150 ul. mP	ren/a cansules: usually 35 ul						





## **ASP-1000 protocol**

1) LED Color - Red 2) LED Color - Green 3) Initialize plunger valve right at pump speed 12 4) Return stage axes to the home position 5) Aspirate volume 500ul 6) Move all valves to output Load/Unload 8) LED Color - Red 9) Dialog Popup: Attach mPrep capsules to pipettor shaft and press "Ent 10) LED Color - Green 11) M-A-D plate 3 col 1 spd 15 vol 100ul a-hld 0.0s d-hld 0.0s rpt 60 Fix rinse 1\_Buffer 12) M-A-D plate 3 col 2 spd 15 vol 100ul a-hld 0.0s d-hld 0.0s rpt 60 Fix rinse 2 Buffer 13) M-A-D plate 3 col 3 spd 15 vol 100ul a-hld 0.0s d-hld 0.0s rpt 60 Fix rinse 3 Buffer 14) M-A-D plate 3 col 4 spd 20 vol 100ul a-hld 0.0s d-hld 0.0s rpt 120 0s04 15) M-A-D plate 3 col 5 spd 15 vol 100ul a-hld 0.0s d-hld 0.0s rpt 60 Osmium rinse 1 H2O 16) M-A-D plate 3 col 6 spd 15 vol 100ul a-hld 0.0s d-hld 0.0s rpt 60 Osmium rinse 2 H2O 17) M-A-D plate 3 col 7 spd 15 vol 100ul a-hld 0.0s d-hld 0.0s rpt 60 Osmium rinse 3\_ H2O 18) M-A-D plate 4 col 1 spd 20 vol 100ul a-hld 0.0s d-hld 0.0s rpt 120 UA 19) M-A-D plate 4 col 2 spd 15 vol 100ul a-hld 0.0s d-hld 0.0s rpt 60 UA rinsel H2O 20) M-A-D plate 4 col 3 spd 15 vol 100ul a-hld 0.0s d-hld 0.0s rpt 60 UA rinse 2\_ H2O 21) M-A-D plate 4 col 4 spd 15 vol 100ul a-hld 0.0s d-hld 0.0s rpt 60 UA rinse 3 H2O 22) M-A-D plate 3 col 8 spd 15 vol 100ul a-hld 0.0s d-hld 0.0s rpt 60 50% ethanol 23) M-A-D plate 3 col 9 spd 15 vol 100ul a-hld 0.0s d-hld 0.0s rpt 60 75% ethanol 24) M-A-D plate 3 col 10 spd 15 vol 100ul a-hld 0.0s d-hld 0.0s rpt 60 90% ethanol 25) M-A-D plate 3 col 11 spd 15 vol 100ul a-hld 0.0s d-hld 0.0s rpt 60 95% ethanol 26) M-A-D plate 3 col 12 spd 15 vol 100ul a-hld 0.0s d-hld 0.0s rpt 120 100% ethanol 27) M-A-D plate 4 col 5 spd 20 vol 100ul a-hld 0.0s d-hld 0.0s rpt 120 100% ethanol 28) M-A-D plate 4 col 6 spd 20 vol 100ul a-hld 0.0s d-hld 0.0s rpt 120 100% ethanol 29) M-A-D plate 4 col 7 spd 25 vol 100ul a-hld 0.5s d-hld 0.0s rpt 120 25% resin 30) M-A-D plate 4 col 8 spd 25 vol 100ul a-hld 1.0s d-hld 1.0s rpt 140 50% resin 31) M-A-D plate 4 col 9 spd 30 vol 100ul a-hld 1.0s d-hld 1.0s rpt 140 75% resin 32) M-A-D plate 4 col 10 spd 35 vol 100ul a-hld 2.0s d-hld 2.0s rpt 160 100% resin w/o accel 33) M-A-D plate 4 col 11 spd 35 vol 100ul a-hld 2.0s d-hld 2.0s rpt 180 100% resin w/o accel 34) M-A-D plate 4 col 12 spd 35 vol 100ul a-hld 2.0s d-hld 2.0s rpt 180 100% resin w acceler 35) Aspirate volume 120ul 36) No-Operation: stage-pause 30 s Comment goes here 37) LED Color - Red 38) Select Plate/Column 6 1 Silicon plate 39) Dialog Popup: Remove capsules and press "Enter". Remove capsules

40) LED Color – Green

																	<b>P</b> 3	63_	202	24/0	)5/1	3	
Plate	1											Plate	2										
et Fix rinse 1_Buffer	<sup>60</sup> Fix rinse 2_Buffer	Fix rinse 3_Buffer	OsO4	Osmium rinse 1_H2O	Osmium rinse 2_H2O	Osmium rinse 3_ H2O	50% ethanol	75% ethanol	90% ethanol	95% ethanol	100% ethanol	ろ Plate	<sup>4</sup> UA rinse1_H20	UA rinse 2_H2O	UA rinse 3_H2O	100% ethanol	100% ethanol	25% resin	50% resin	75% resin	100% resin w/o accelerator	100% resin w/o accelerator	100% resin w accelerator
Plate	5											Silicon plate	Resin	Embec	ment	w Os	wo UA		bugh F	age 2			





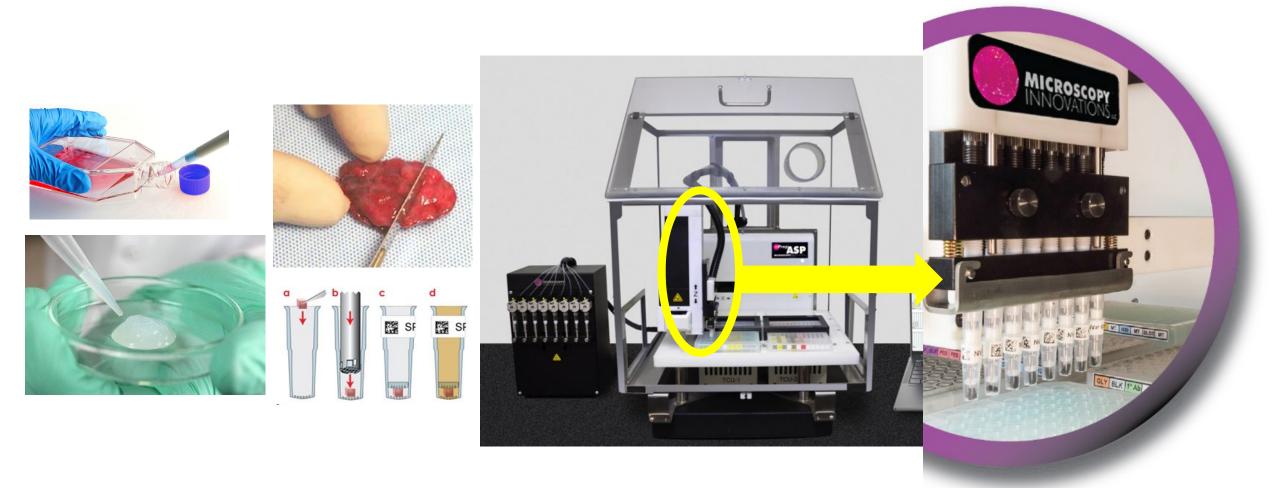
# **Multi-well plate formats**







# ASP-1000: automated sample preparation for TEM









# Sample mounting for ASP-1000



Accurate orientation. Specimens may be oriented using several methods Efficient workflow. Streamlines specimen processing from dissection to reagent processing Reduce handling. Once loaded in capsules, specimens are not touched again for TEM embedding or SEM mounting Easy dissection. Single focal plane keep specimens in focus during dissection and when loading

- specimens in capsules **Wet samples.** Dissect and load specimens wetted by buffers or fixatives
- **Efficient reagent processing.** Directly load capsules onto pipettor from Workstation

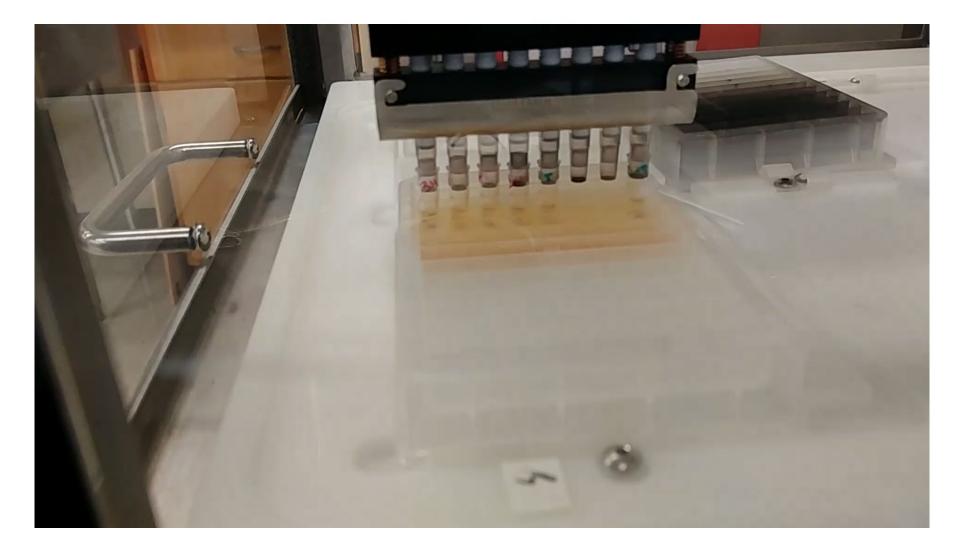
https://www.youtube.com/watch?v=ZG1d1Me70y0





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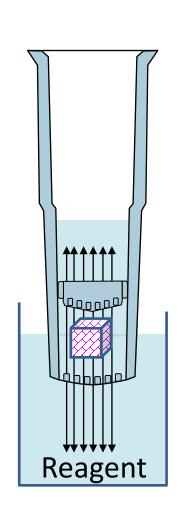
# Automated sample preparation for TEM

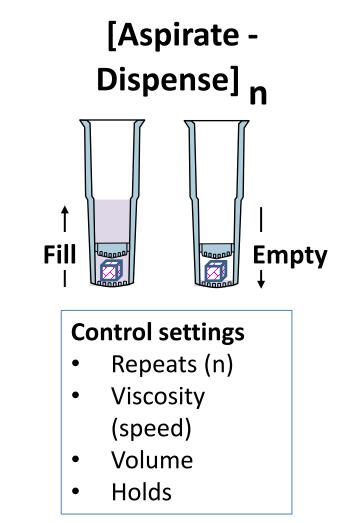


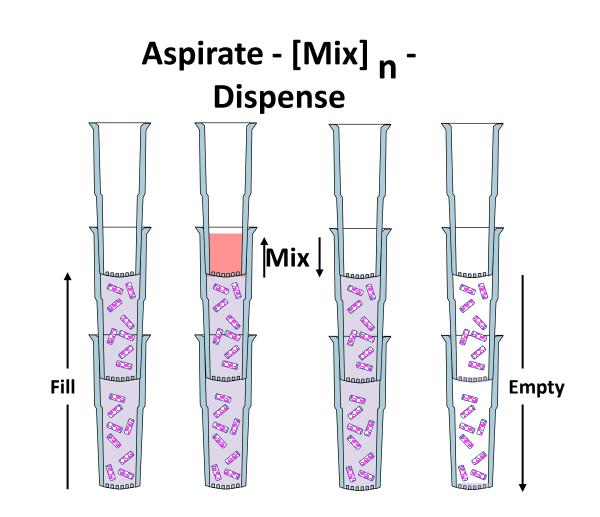




**ASP reagent agitation & mixing** 











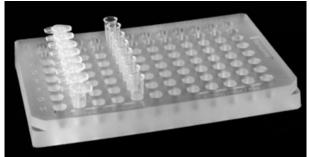


# mPrep system components

mPrep/s capsules



mPrep/bench – seals capsule bottom

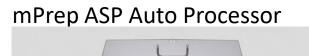


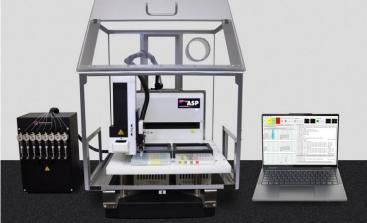
## mPrep/g capsules



Orientation Workstation







Device Compatibility









Specimen & Grid Holder for CPD and

Cryo









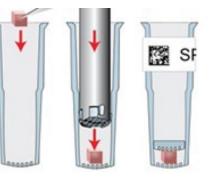


# **ASP-1000 workflow**



1. Set up the protocol







4. Attach the capsules



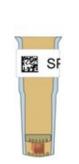
5. Run the protocol

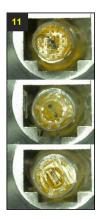


3. Mount the samples



6. Polymerization





7. Retrieve the cured blocks

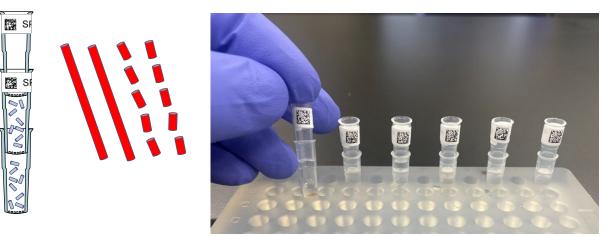




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# Automated TEM specimen preparation for clinical pathology

#### 8 biopsy segments in each stacked capsule



ARUP Labs: CAP, ISO-15189 & CLIA-certified National Reference Lab

- Up to 128 renal/muscle specimens 8 pieces/capsule
- Renal, Skeletal and Cardiac Muscle, Nerve, Cilia

Nanoscale Characterization Experimental Center

🗱 SI

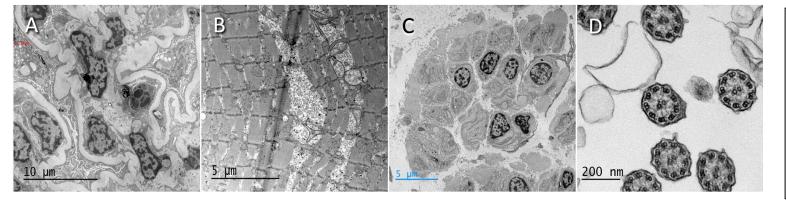




### 2 hours: glut rinse->Os->Ur->100% resin

An Efficient Clinical TEM Workflow Using Automated Specimen Processing Flint et al., M&M 2024 in press.

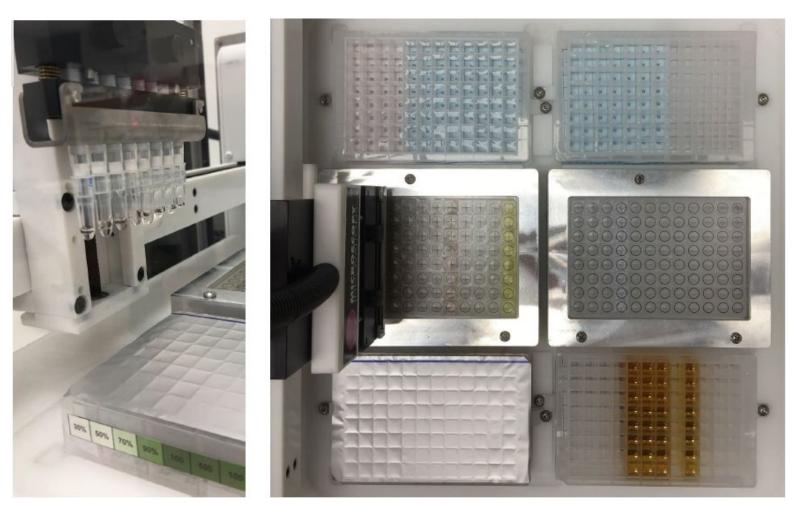
Creating Efficient Workflows for EM Labs with Automated Specimen Preparation Goodman et al., Microscopy Today, Jan 2024





## Automated heavy metal tissue staining for serial block face imaging with the ASP-1000

	ASP-1000 Robot Protocol	
S	Steps	Time each step
our	4X Buffer	15 minutes
Ч О	2% Buffered reduced Osmium	4 hours
<u>es 4</u>	4X Water	15 minutes
tak	1% aqueous TCH	45 minutes
on	4X Water	15 minutes
zati	2% Aqueous OsO4	2 hours
eriz	4X Water	15 minutes
lym	1% Aqueous UA	4 hours
bo	1% Aqueous UA	2 hours
sin	4X Water	15 minutes
protocol without resin polymerization takes 40 hours	Walton's Lead Aspartate	2 hours
hou	4X Water	15 minutes
wit	25%, 50%, 75%, 90% Acetone	15 minutes
0	3X 100% Acetone	15 minutes
oto	25%, 50%, 75% Hard Plus in Acetone	30 minutes
pr j	3X 100% Hard Plus	2 hours
The	1X 100% Hard Plus	Overnight
	Resin Embedding in Hard Plus	48 hours



McClain, Melainia, Morgan Harwood, and Steph Nowotarski. "Automated heavy metal tissue staining for serial block face imaging with the ASP-1000." In Microscopy and Microanalysis Conference. 2019.





# Automated heavy metal tissue staining for serial block face imaging with the ASP-1000

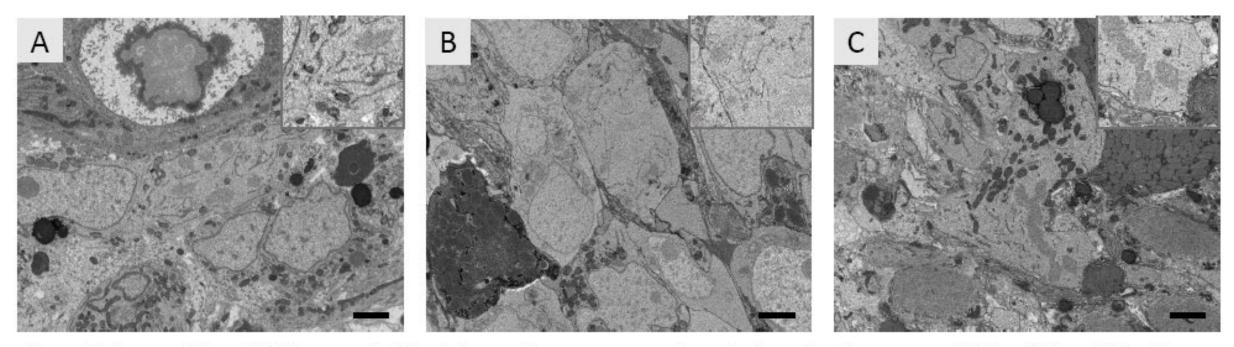


Figure 3. Images (A) and (B) from serial block face volumes processed on the bench using protocol [2] for (A) and [3] with extended staining times for (B). Image (C) from ASP-1000 robot protocol shows equivalent staining and ultrastructure preservation. Inserts show close-ups of the staining in each image. Scale bar is 2 um.

McClain, Melainia, Morgan Harwood, and Steph Nowotarski. "Automated heavy metal tissue staining for serial block face imaging with the ASP-1000." In Microscopy and Microanalysis Conference. 2019.







## vEM – Rat brain cortex

"Artificial Intelligence (AI) segmentation enabled with consistent ASP preparation" 2A В لقوة معما ASP-1000 prep: D • 7.5 hrs to resin curing • 1 hr hands-on Mitochondria Myelin Synapses effort

Creating Efficient Workflows for EM Labs with Automated Specimen Preparation. Goodman et al., Microscopy Today, Jan 2024





## Automated vs. manual preparation of brain tissue for vSEM

	М	anual	Auton	nated
Reagent	Exchanges	Time (min)	Exchanges	Time (min)
Karnovsky fix	perfuse	Store 5C	perfuse	Store 5C
Buffer	6	30	30/30/30*	3
Tannic acid (some)	1	15	450	15
OsO4 - KFeCN	1	180	1800	60
Water	5	25	90/90/90/90/90	15
1% TCH	1	60	1800	60
Water	5	25	90	3
2% OsO4	1	180	900	30
Water	5	25	90/90/90/90/90	15
2% Uranyl Acetate	1	1,200	1800	60
Water	5	25	90/90/90	9
Lead Aspartate	1	40	900	30
Water	5	25	45/45/45	9
Graded ethanols	15	105	630	53
Acetone	2	30	270/270	9
Epoxy-acetones	2	600	900	30
100% epoxy	1	90	120/120/120	30
ansfer tissue to molds	1	45	NA	0
Resin cure 60C	Into oven	2 days	Into oven	Overnight
Time : Effort	4 elapsed d	ays : 2 days work	1 elapsed day	: 1 hour work

Figure 1. Table 1: Reagent protocol, times, and handson labor effort for manual and ASP preparation. Figure 1: Perspective projections of cortex prepared manually (top) and with ASP (bottom). ~60 x 60 x 20 µm deep, from 350 70 nm thick slices. Images acquired in 25 hr each.

**Table 1:** Protocol, reagent exchanges and incubation times for reagentexchanges. \*30/30/30 indicates 30 exchanges in 3 different reagent wells.

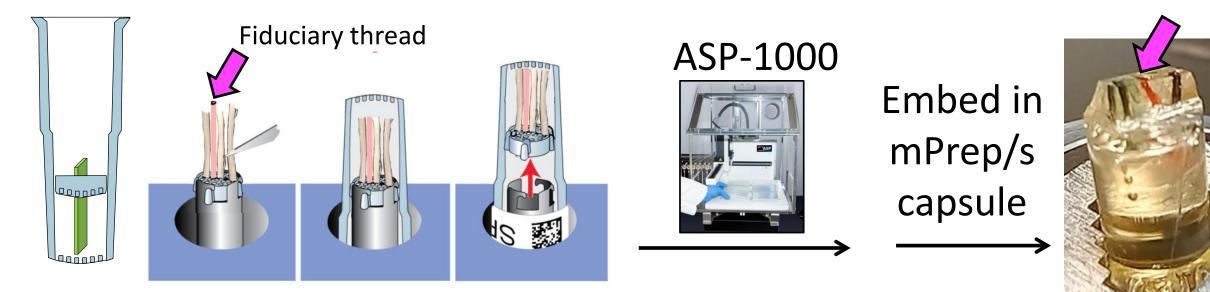
Benson, Emily, Grahame Kidd, Jay Campbell, and Steven Goodman. "Serial block-face SEM of brain tissue using rapid automated preparation." Microscopy and Microanalysis 26, no. S2 (2020): 1372-1373.



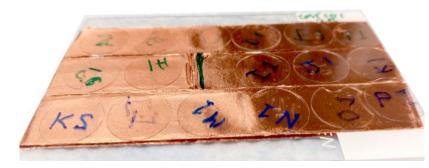


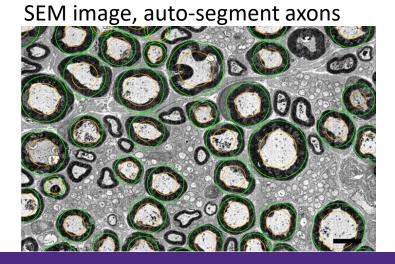


## **vEM–Peripheral Nerve Fibers**



1 μm sections on coverslips, UA-Pb stained, on copper tape





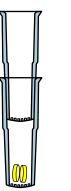
Kidd JK & Benson EK Volume and Large Field of View EM... Preclinical Therapeutic Testing Microscopy Microanalysis 29(1):1083 (2023)

Creating Efficient Workflows for EM Labs with Automated Specimen Preparation Microscopy Today, Jan 2024

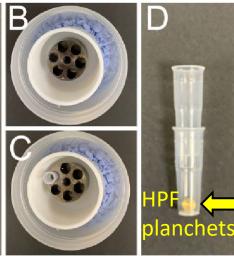


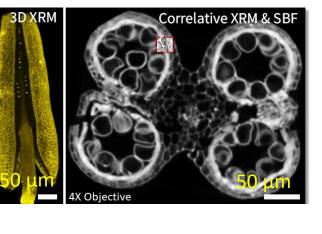


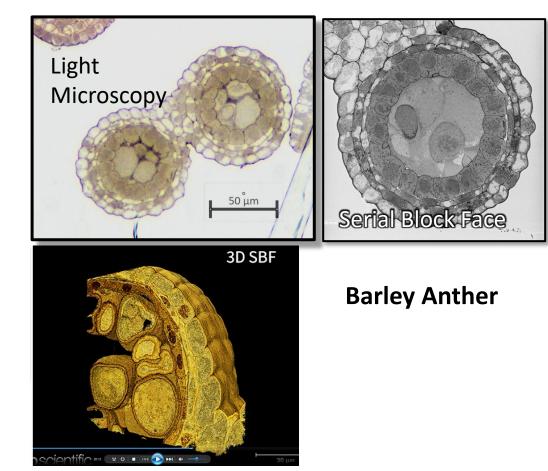
## HPF-Freeze Sub: LM-XRM-SBF



mPrep/s capsules.... "...loss-less handling of small plant samples ... buoyant plant specimens remained immersed throughout preparation"







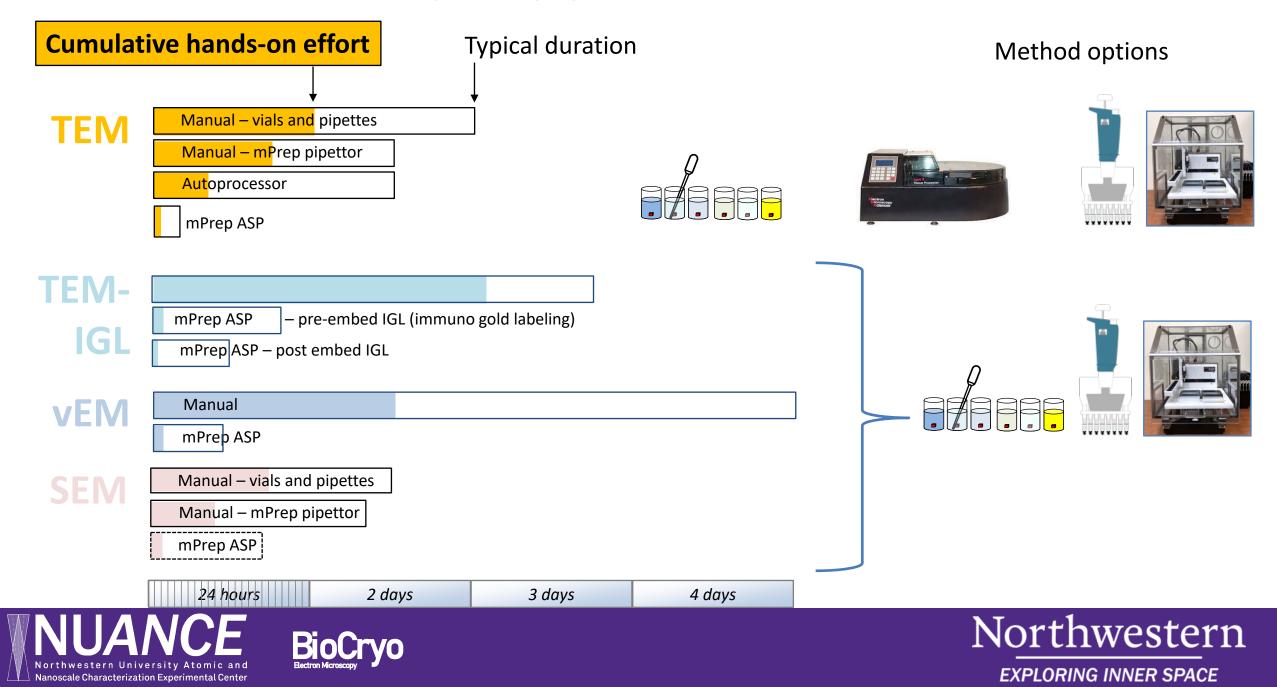
A versatile enhanced freeze-substitution protocol for volume electron microscopy. Belanger S, Berensmann H, Baena V, Duncan K, Meyers BC, Narayan K, Czymmek KJ, Frontiers Cell & Develop Biology, 10 (2022)







Specimen prep time, effort & methods



## **Benefits of automated sample preparation**

#### **Enhanced productivity**

 high time efficiency and scalability → researchers have more time for other tasks and have more samples ready in less time

#### Improved consistency

repeatable, standardized sample prep → minimized variability between samples

#### **Reduced errors**

manual handling errors are eliminated → maximum reliability of results

#### **Increased safety**

 APS is connected to exhaust and manual handling of chemicals is reduced → minimized risk of exposure of researchers to toxic chemicals and vapors



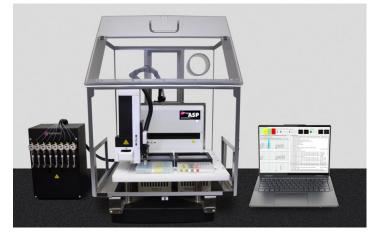


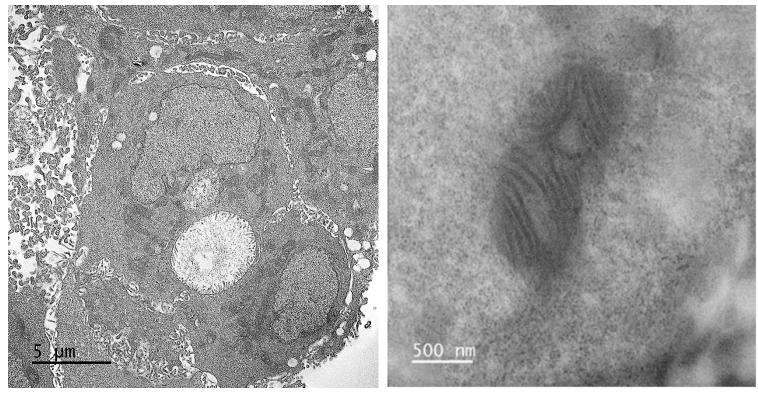


nPrep System

## New BioCryo development: ASP protocol for cell monolayers on coverslips







TEM image of resin embedded HT29 cells and of a mitochondrion in a 300 nm thick section. The sample was processed with the ASP-1000. The image was recorded with the JEOL 3200FS.

The sample was provided by Yi Li, Shana Kelley lab, CZI.







# Thank you for your attention!

# **Questions?**

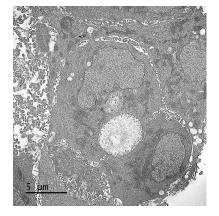


#### **Contact:**

#### **Reiner Bleher, PhD**

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https://nuance.northwestern.edu/facilities/biocryo/index.html





