

CRYOEM 001 : NEGATIVE STAIN SUPPLEMENT

NCCAT Embedded Training — Master Class series

October 14, 2020

<https://dx.doi.org/10.1251/bpo70>

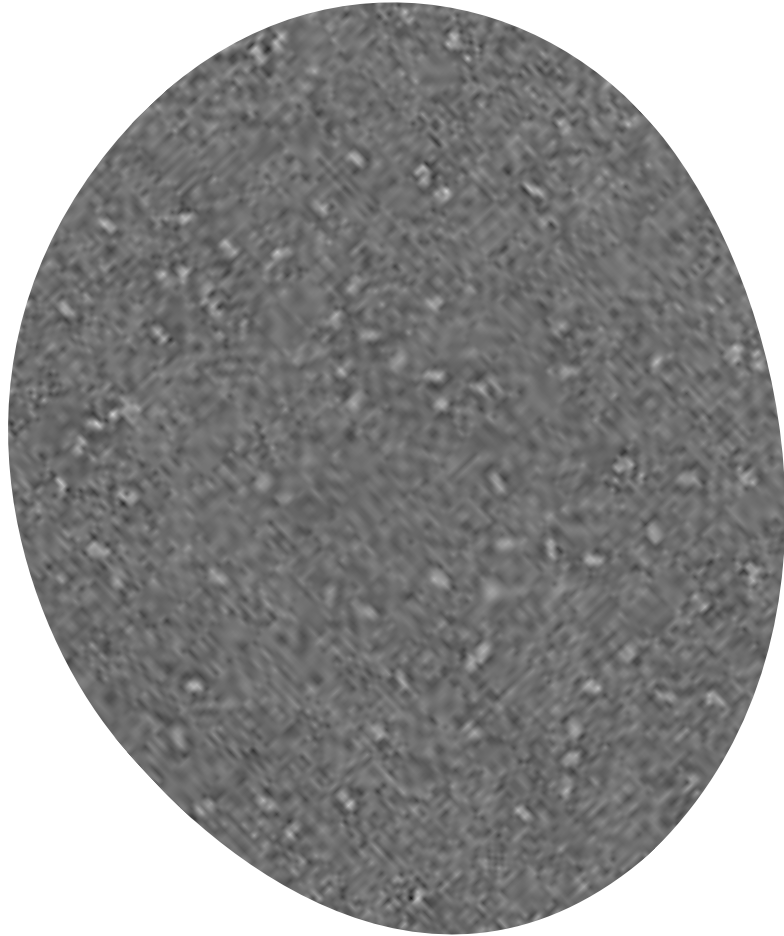
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Negative stain -extra module

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Chapters

- 0:05 Title
- 0:30 Adsorbing Samples to the Carbon Substrate
- 1:07 Staining Using the Side Blot Method
- 2:25 Staining Using the Flicking Method
- 3:24 Staining Using the Rapid Flushing Method
- 4:12 Results: The Ideal Negative Stain for EM is Sample Dependent
- 5:36 Conclusion

<https://dx.doi.org/10.3791/57199>

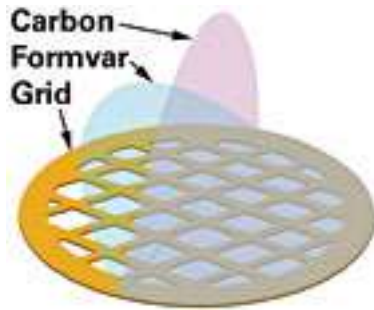
Grids

Ultrathin Carbon Type-A:
Carbon support film of
approximately 3nm
thickness

Carbon Type-A:
Carbon support film
of 15 to 25nm
thickness

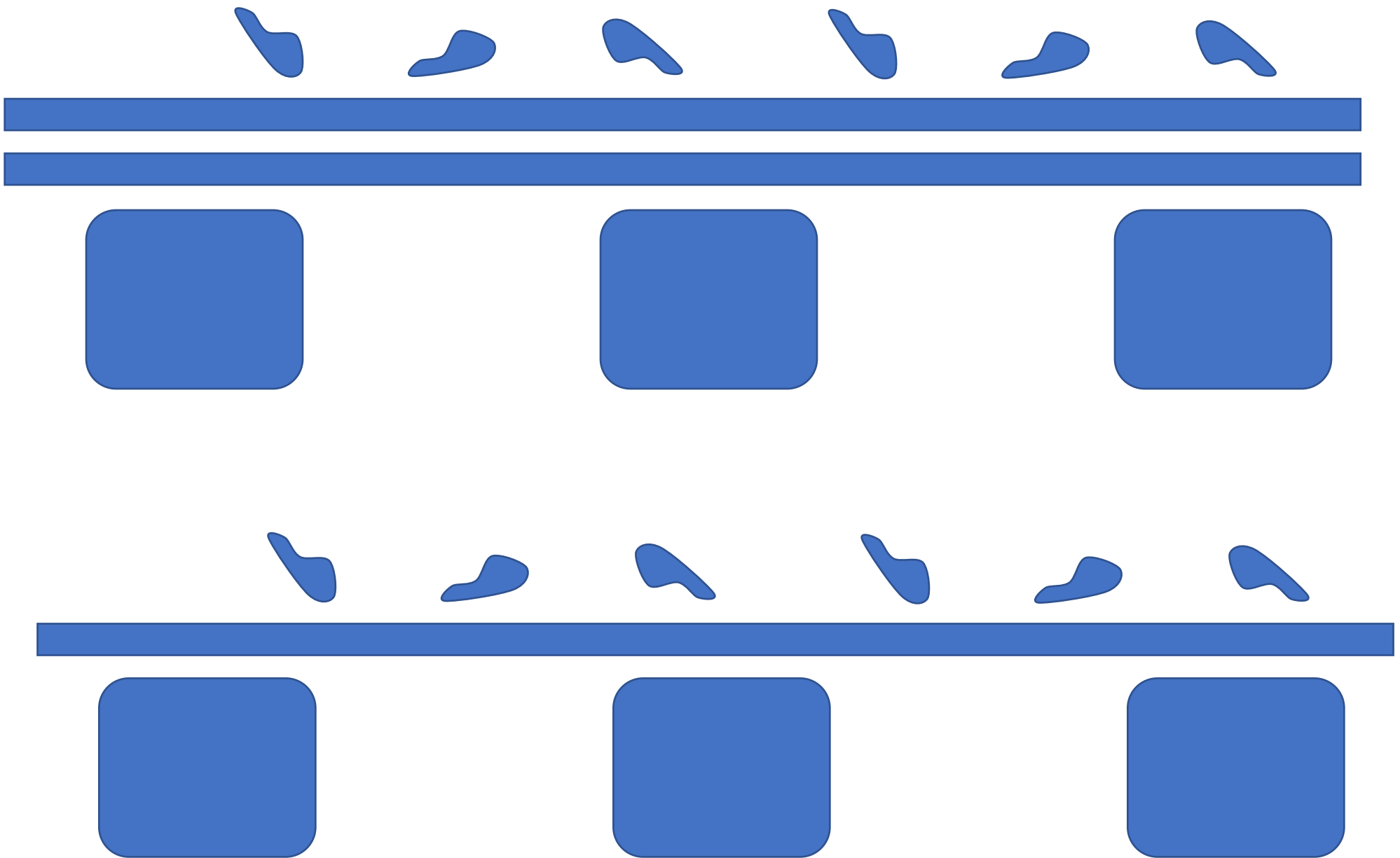
Carbon Type-B: A Formvar film coated with a “heavier” layer of carbon. This is the strongest and most versatile support film we produce. It is stable in the EM under all operating conditions including high magnification with high beam intensity. The films can withstand vigorous specimen preparation techniques. If the carbon surface is hydrophobic, specimen suspensions can be applied to the Formvar surface.

- Continuous carbon
- Thickness is variable but “thick”



01814-F

Carbon Type-B, 400 mesh, Copper approx.
grid hole size: 42µm



Hexane
Chloroform
Amyl acetate

Ethanol/water

Glow discharging

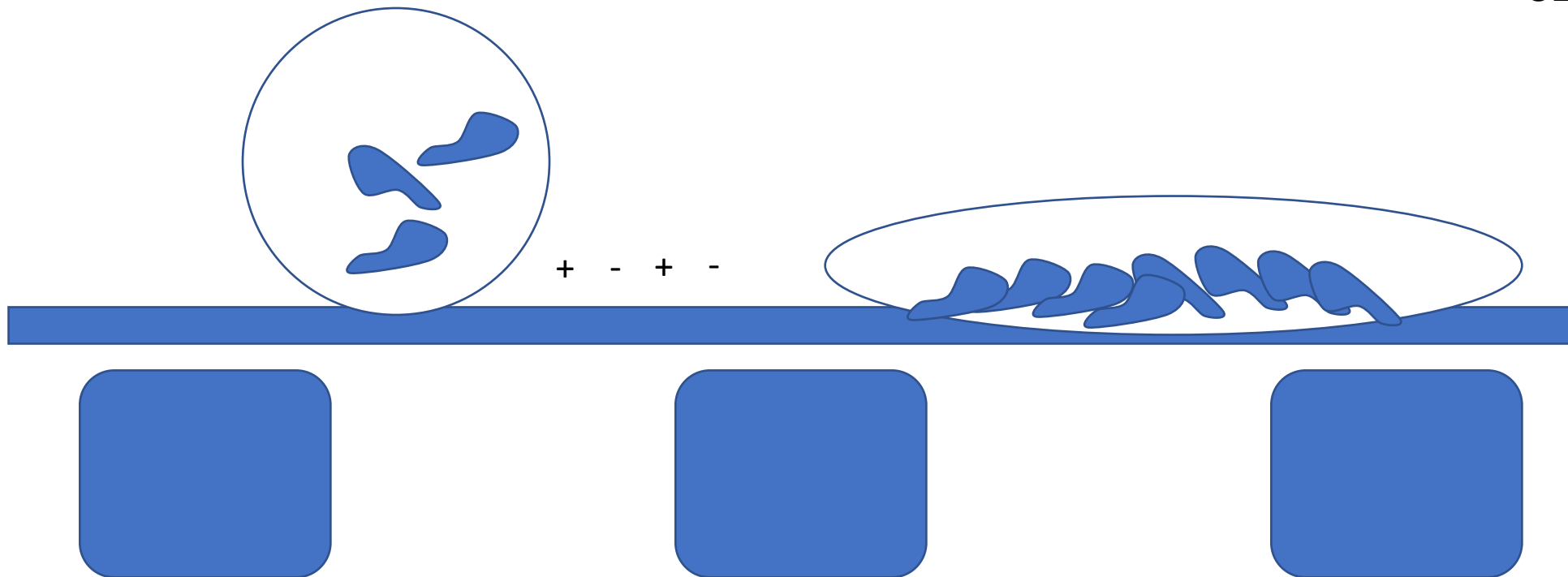
Air - N₂/O₂

O₂

H₂

H₂/O₂

O₂/Ar

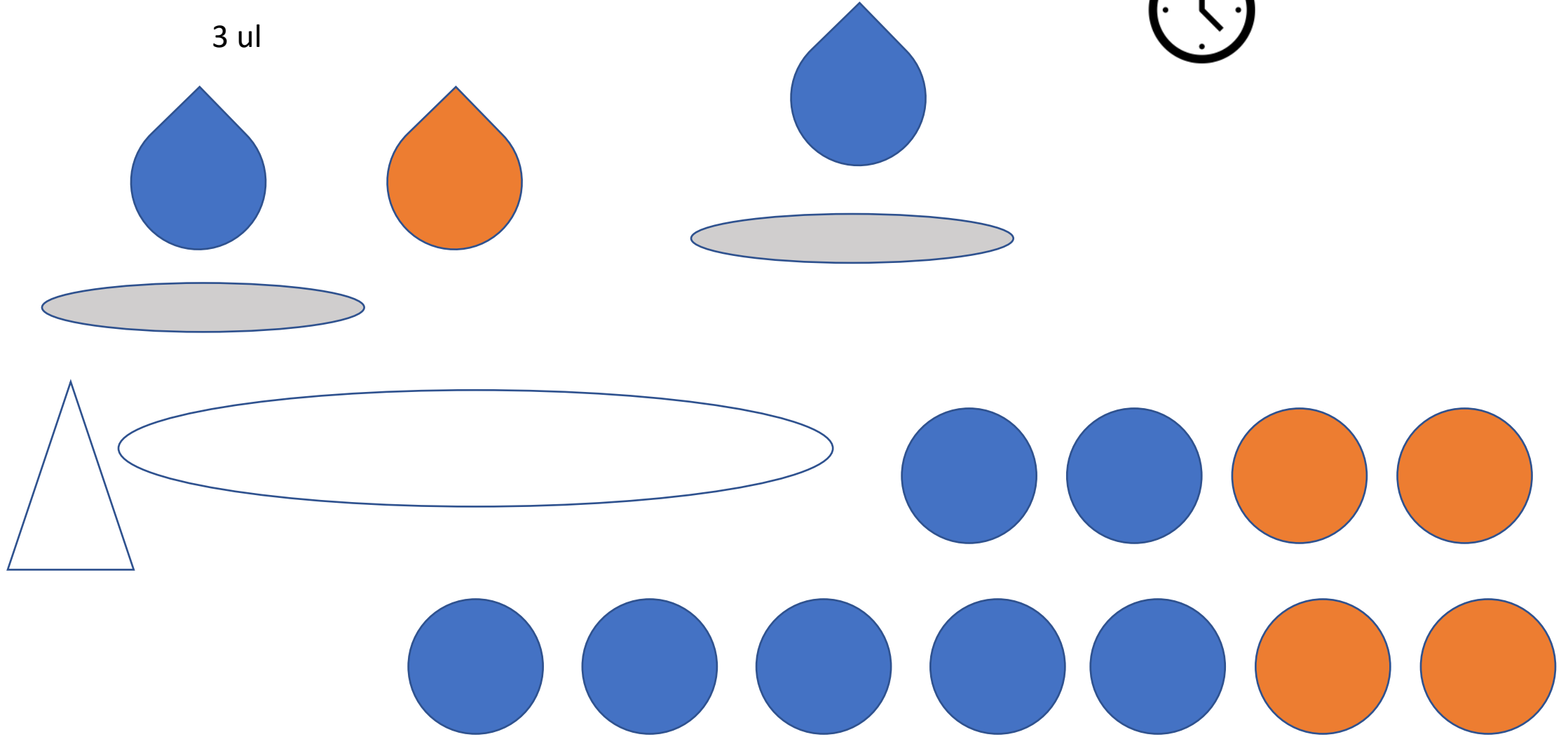


Which side?



How to stain?

3 ul



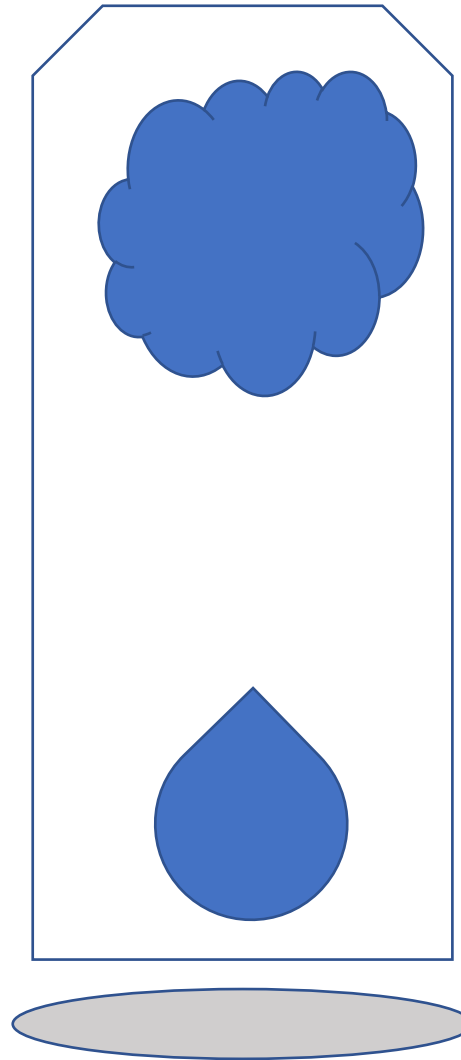
How to stain?

1:10 serial dilution sample

Side blot or direct blot is equivalent
-try to be as reproducible as possible

Always easier to dilute

- protein
- spare buffer
- water
- stain



But what if I'm too dilute?

What is my stain?

Ur UA
 UF pH 3-4

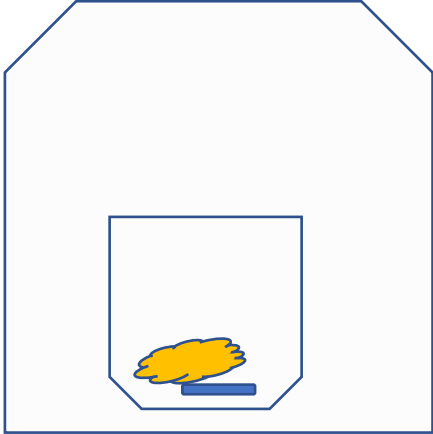
NH₄Mo pH 6

PTA pH 7

UF protocol



5 mL H₂O
Boil it



Measure
UF

0.1M NaOH
pH 4

pH strips

Filter

Aliquot

LN₂

-80C
Away from light

Which elements?

Ur

UA
UF

pH 3-4

NH₄Mo

pH 6

PTA

pH 7

Periodic Table of the Elements

Atomic Number → 1
Name → Hydrogen
Electrons per shell → 1
Symbol → H
Atomic Weight → 1.008

State of matter (color of name): GAS, LIQUID, SOLID, UNKNOWN

Subcategory in the metal-metalloid-nonmetal trend (color of background):
 Alkali metals, Alkaline earth metals, Transition metals, Lanthanides, Actinides, Transition metals, Metalloids, Reactive nonmetals, Noble gases, Unknown chemical properties

1 IA 1 H Hydrogen 1.008	2 IIA 4 He Helium 4.003											13 IIIA 5 B Boron 10.81	14 IVA 6 C Carbon 12.01	15 VA 7 N Nitrogen 14.01	16 VIA 8 O Oxygen 15.99	17 VIIA 9 F Fluorine 18.99	18 VIIIA 10 Ne Neon 20.18
3 Li Lithium 6.94	4 Be Beryllium 9.01	5 V Vanadium 50.94	6 Cr Chromium 51.99	7 Mn Manganese 54.94	8 Fe Iron 55.85	9 Co Cobalt 58.93	10 Ni Nickel 58.69	11 Cu Copper 63.55	12 Zn Zinc 65.38	13 Al Aluminum 26.98	14 Si Silicon 28.09	15 P Phosphorus 30.97	16 S Sulfur 32.06	17 Cl Chlorine 35.45	18 Ar Argon 39.95		
19 K Potassium 39.10	20 Ca Calcium 40.08	21 Sc Scandium 44.96	22 Ti Titanium 47.88	23 V Vanadium 50.94	24 Cr Chromium 51.99	25 Mn Manganese 54.94	26 Fe Iron 55.85	27 Co Cobalt 58.93	28 Ni Nickel 58.69	29 Cu Copper 63.55	30 Zn Zinc 65.38	31 Ga Gallium 69.72	32 Ge Germanium 72.64	33 As Arsenic 74.92	34 Se Selenium 78.96	35 Br Bromine 79.90	36 Kr Krypton 83.80
37 Rb Rubidium 85.47	38 Sr Strontium 87.62	39 Y Yttrium 88.91	40 Zr Zirconium 91.22	41 Nb Niobium 92.91	42 Mo Molybdenum 95.94	43 Tc Technetium 98.91	44 Ru Ruthenium 101.07	45 Rh Rhodium 102.91	46 Pd Palladium 106.38	47 Ag Silver 107.87	48 Cd Cadmium 112.41	49 In Indium 114.82	50 Sn Tin 118.71	51 Sb Antimony 121.76	52 Te Tellurium 127.60	53 I Iodine 126.91	54 Xe Xenon 131.29
55 Cs Cesium 132.91	56 Ba Barium 137.33	57-71 Lanthanides	72 Hf Hafnium 178.49	73 Ta Tantalum 180.95	74 W Tungsten 183.84	75 Re Rhenium 186.21	76 Os Osmium 190.23	77 Ir Iridium 192.22	78 Pt Platinum 195.08	79 Au Gold 196.97	80 Hg Mercury 200.59	81 Tl Thallium 204.38	82 Pb Lead 207.2	83 Bi Bismuth 208.98	84 Po Polonium 209	85 At Astatine 210	86 Rn Radon 222
87 Fr Francium 223	88 Ra Radium 226	89-103 Actinides	104 Rf Rutherfordium 261	105 Db Dubnium 262	106 Sg Seaborgium 263	107 Bh Bohrium 264	108 Hs Hassium 265	109 Mt Meitnerium 266	110 Ds Darmstadtium 267	111 Rg Roentgenium 268	112 Cn Copernicium 269	113 Nh Nihonium 270	114 Fl Flerovium 271	115 Mc Moscovium 272	116 Lv Livermorium 273	117 Ts Tennessine 274	118 Og Oganesson 276
57 La Lanthanum 138.91	58 Ce Cerium 140.12	59 Pr Praseodymium 140.91	60 Nd Neodymium 144.24	61 Pm Promethium 144.91	62 Sm Samarium 150.36	63 Eu Europium 151.96	64 Gd Gadolinium 157.25	65 Tb Terbium 158.93	66 Dy Dysprosium 162.50	67 Ho Holmium 164.93	68 Er Erbium 167.26	69 Tm Thulium 168.93	70 Yb Ytterbium 173.05	71 Lu Lutetium 174.97			
89 Ac Actinium 227	90 Th Thorium 232.04	91 Pa Protactinium 231.04	92 U Uranium 238.03	93 Np Neptunium 237.05	94 Pu Plutonium 244.06	95 Am Americium 243.06	96 Cm Curium 247.07	97 Bk Berkelium 247.07	98 Cf Californium 251.08	99 Es Einsteinium 252.08	100 Fm Fermium 257.10	101 Md Mendelevium 258.10	102 No Nobelium 259.10	103 Lr Lawrencium 260.10			

CRYOEM 001 : SINGLE PARTICLE MASTERCLASS

Introduction to cryoEM: SPA

Building a cryoEM toolkit

EM compatible samples

EM support films and grids

Sample preparation

Tools of the trade:

microscopes and detectors

Microscope operations

Data collection strategies

Data assessment & QC

Data processing:

cryoEM IT infrastructure

On-the-fly feedback

3D Reconstruction

Visualization and validation