Tape-stabilized Cryohistology in Orthopaedic Research: Techniques, Application, and Innovation

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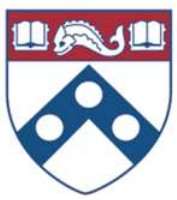

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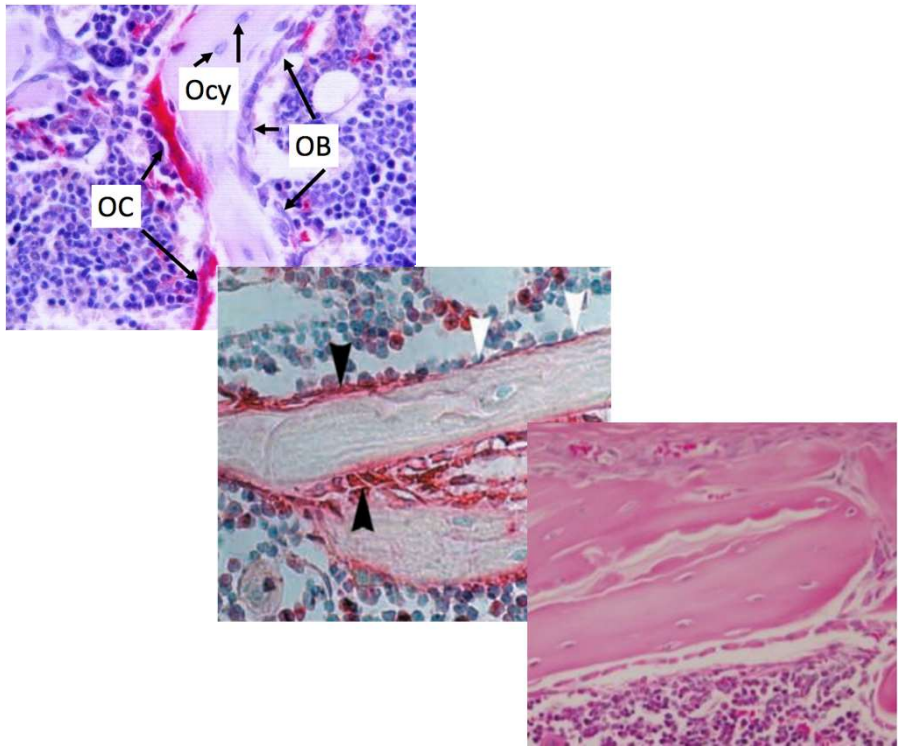
Traditional Bone Histomorphometry

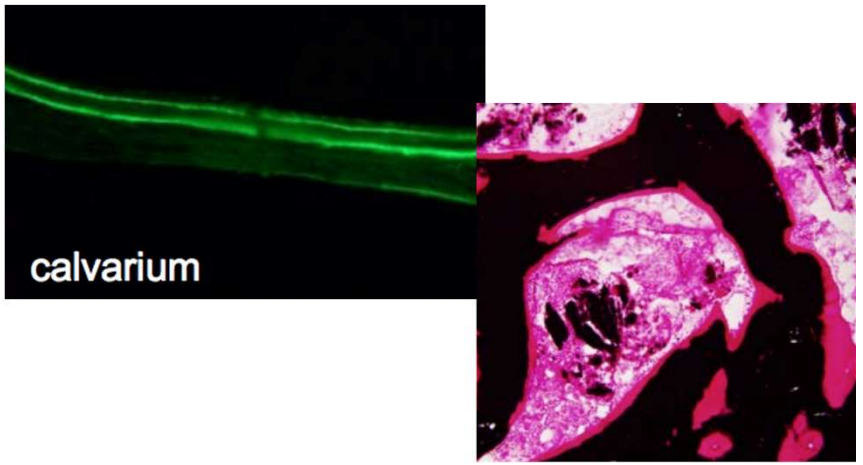
Static

- TRAP stain (osteoclasts) on paraffin section
- Alkaline phosphatase (osteoblasts) on paraffin section
- Chromogenic (e.g., H&E) on paraffin section
- **paraffin sectioning requires decalcification**
- **paraffin processing reduces GFP signal**



Dynamic

- Multiple mineralization labels on plastic section
- Chromogenic (e.g., Von Kossa, trichrome) on plastic section
- **plastic processing reduces GFP signal**



Images taken from: http://www.musculoskeletalcore.wustl.edu/mm/files/Whats_new_in_histo_2014_compressed.pdf

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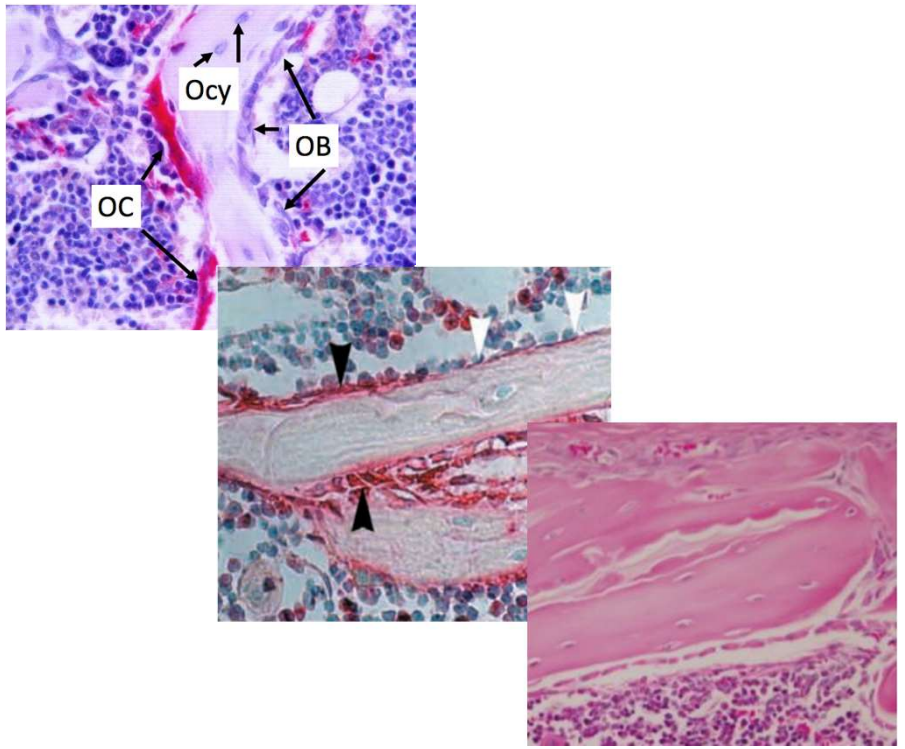

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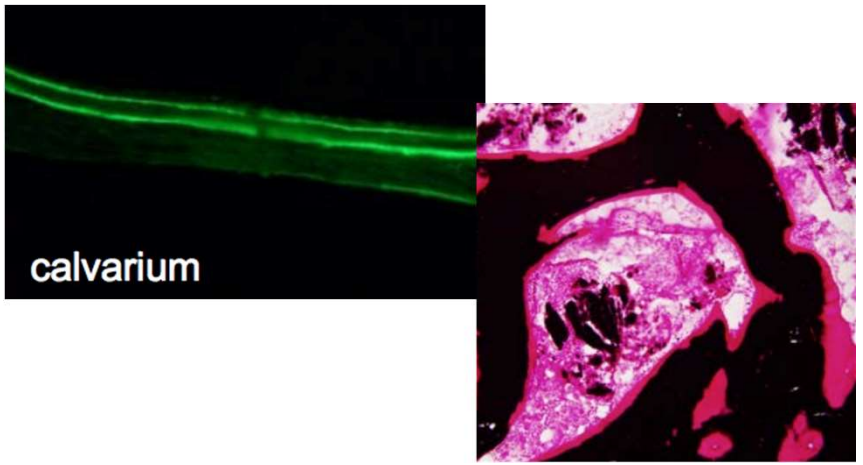

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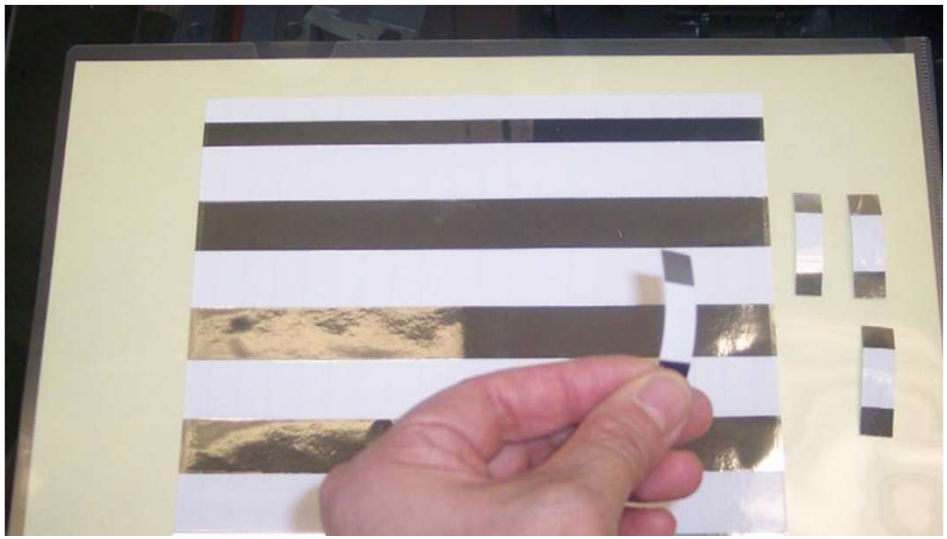
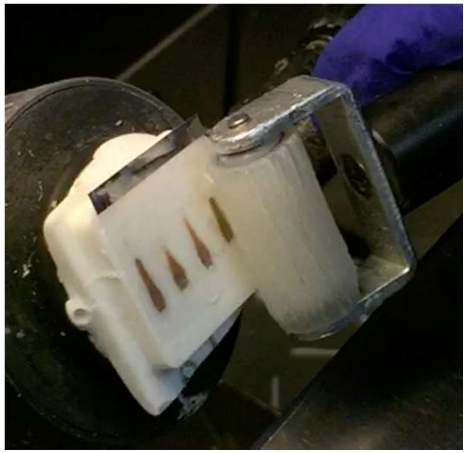
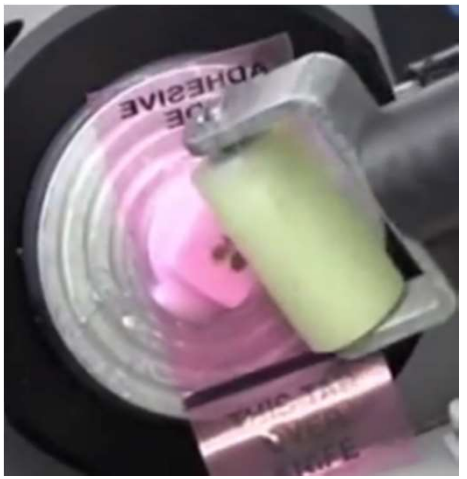
Tape-stabilized Cryosectioning

Cryofilm
Section-lab.jp





CryoJane
Leica Biosystems
“tape-transfer”



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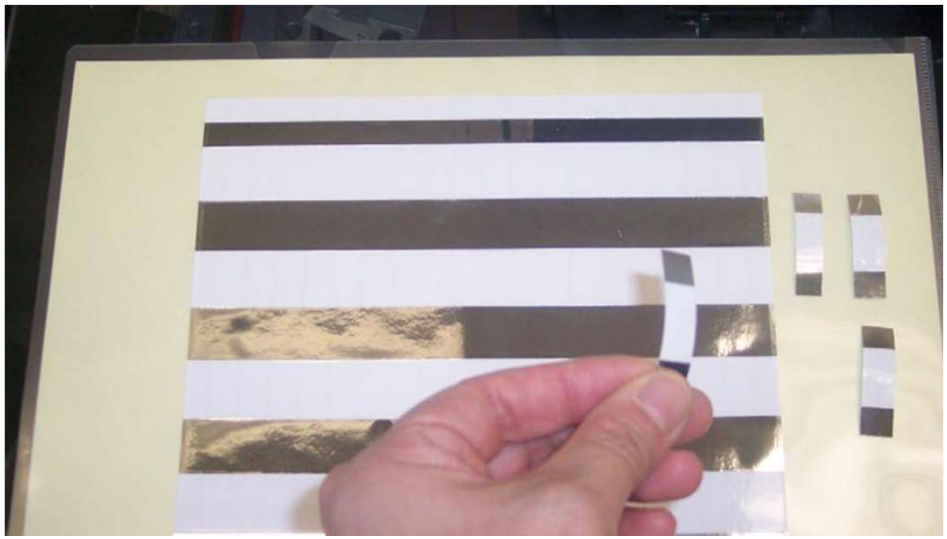
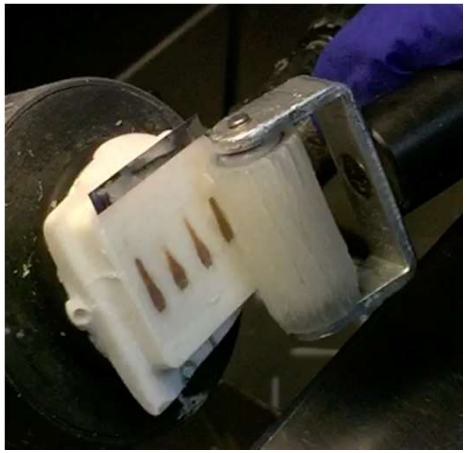
Section-lab.jp





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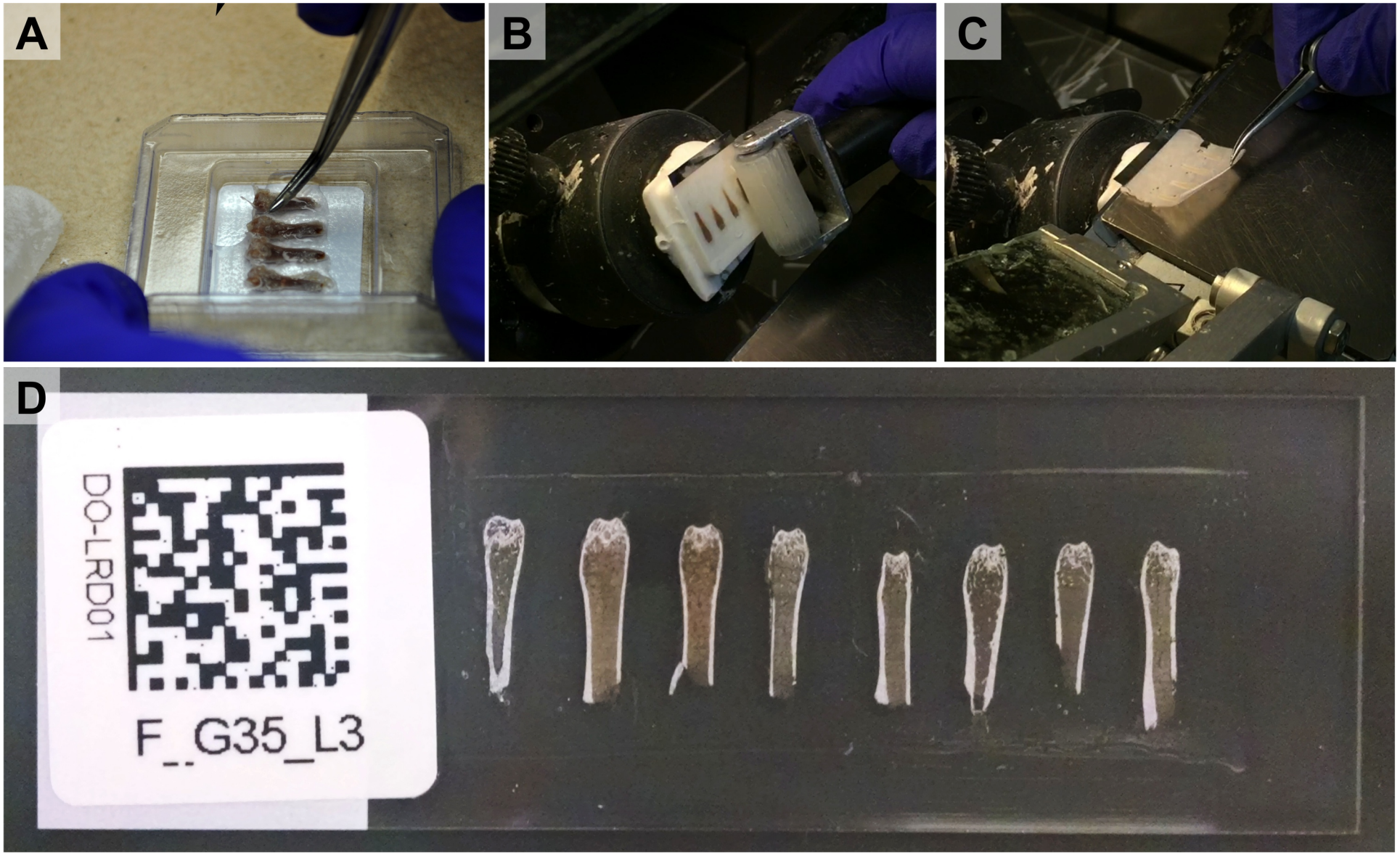
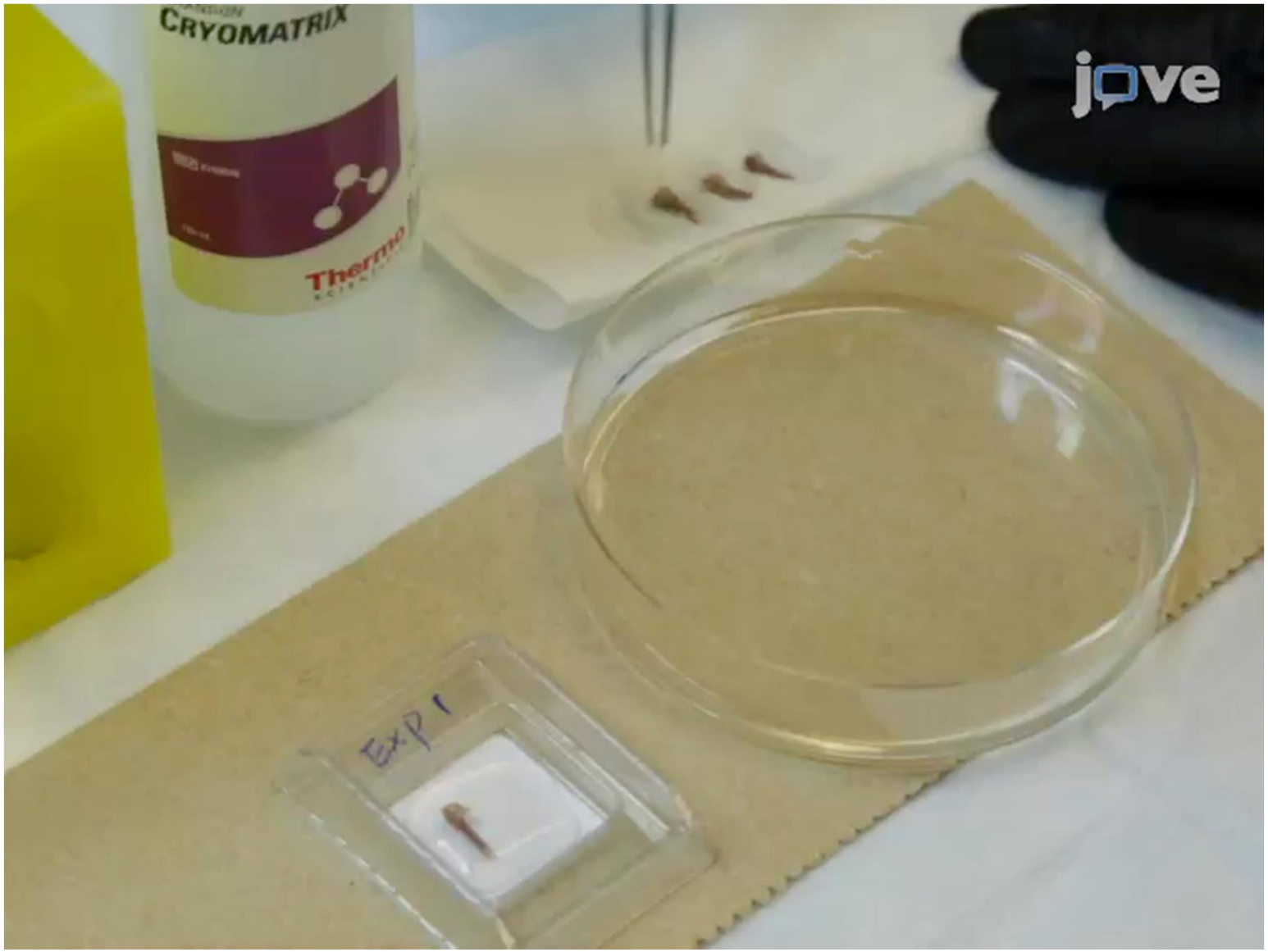
Cryofilm Tape-Stabilized Cryosectioning



Protocol Available: Dyment, N. A. *et al.* High-Throughput, Multi-Image Cryohistology of Mineralized Tissues. *J Vis Exp* e54468–e54468 (2016). doi:10.3791/54468

Multiple Samples in One Block



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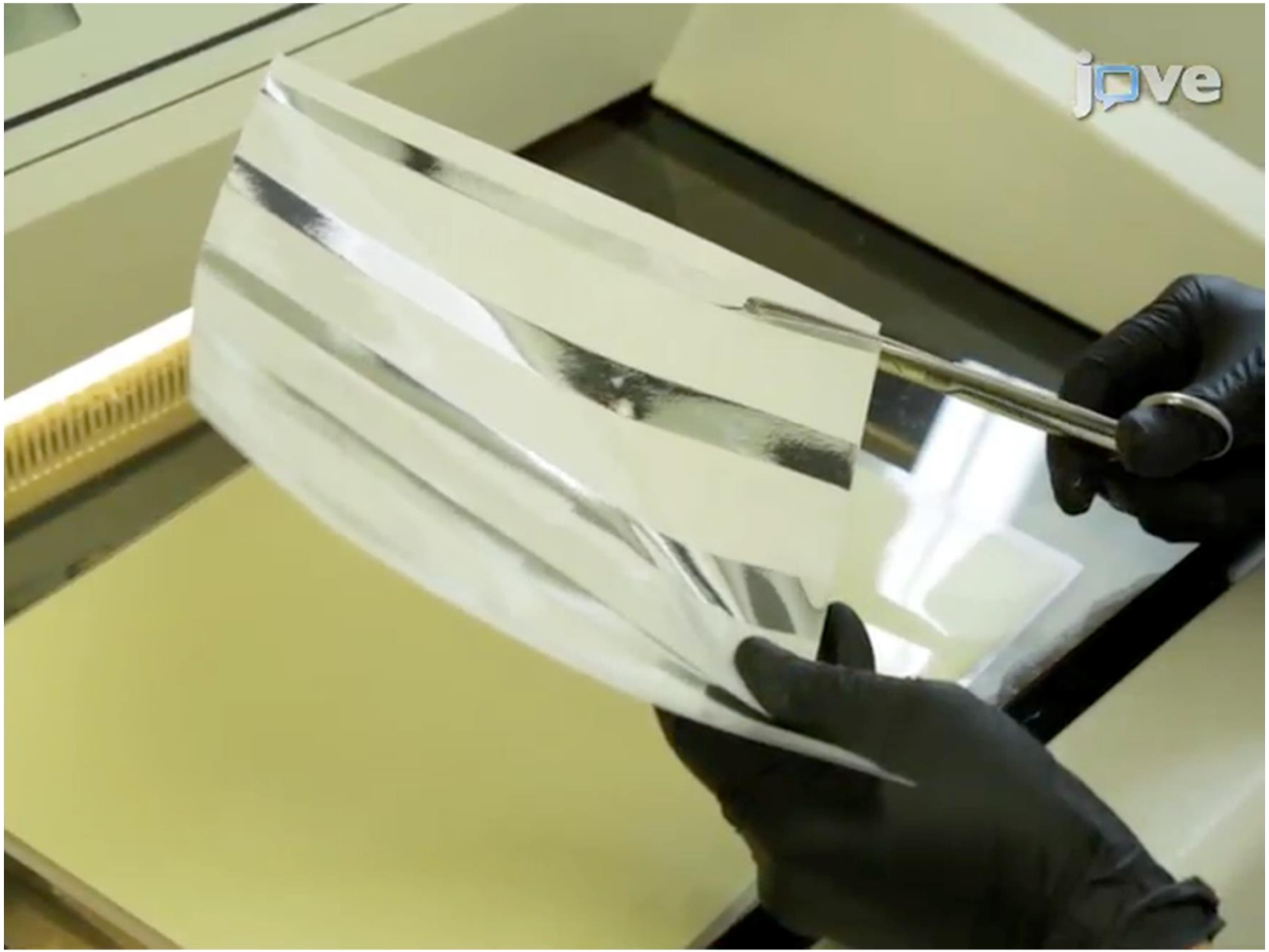
Cryofilm Sectioning: http://section-lab.jp/



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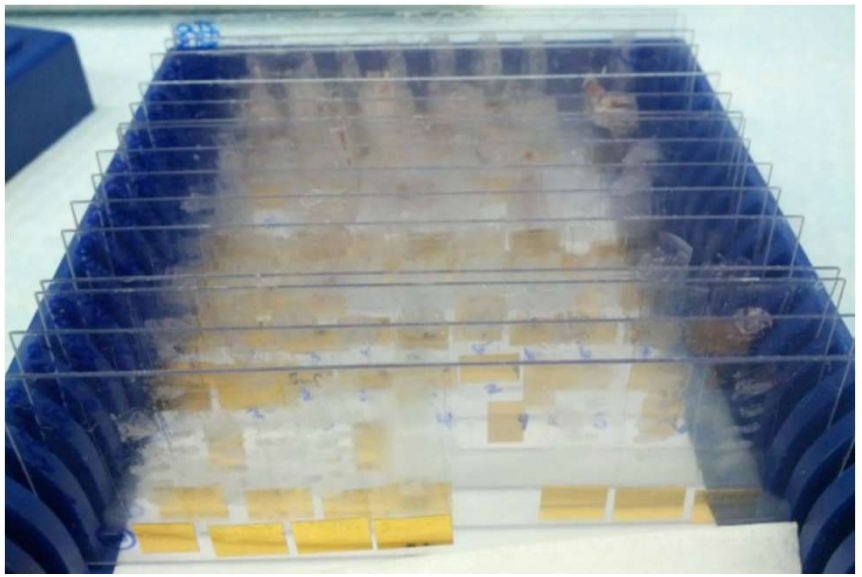
Adhering Cryofilm Sections to Glass Slide

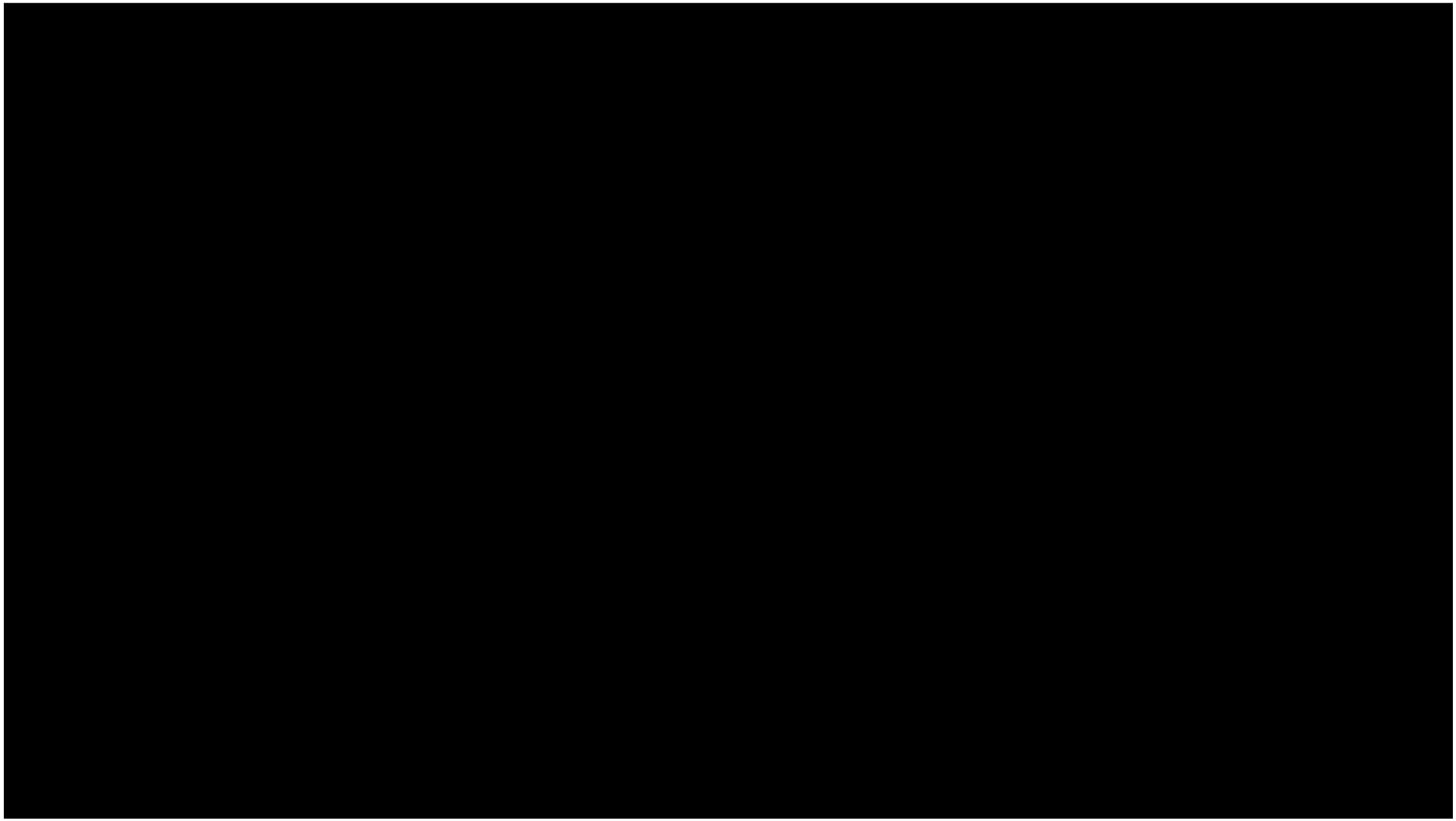
Two methods:

1. Chitosan
2. UV-activated adhesive



Adhering Cryofilm Sections to Glass Slide

Method 1: Chitosan



Adhering Cryofilm Sections to Glass Slide

Method 2: UV-activated adhesive

Comparison of Adhesives for Cryofilm

	Chitosan adhesive	**UV-curing adhesive**
Adhesive mechanism	Evaporation	UV Polymerization
Curing time	> 24 hr	< 20 min
Can sections be removed after adhesive cures?	Yes	No
Is cured adhesive dissolvable?	Yes, in acidic solutions	No
Does adhesive withstand heat antigen retrieval?	No	Yes
Is adhesive auto-fluorescent?	No	Minimal in UV range

Tape-stabilized Cryosectioning

Cryofilm
Section-lab.jp

CryoJane
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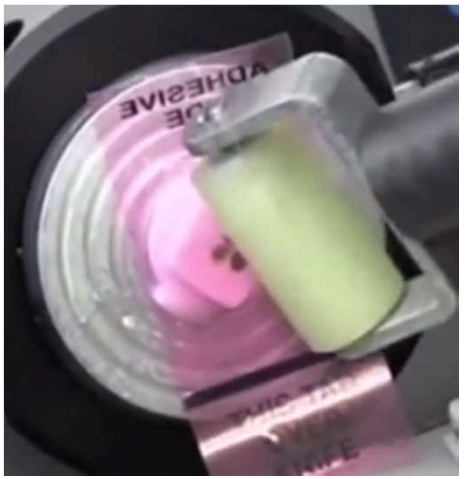
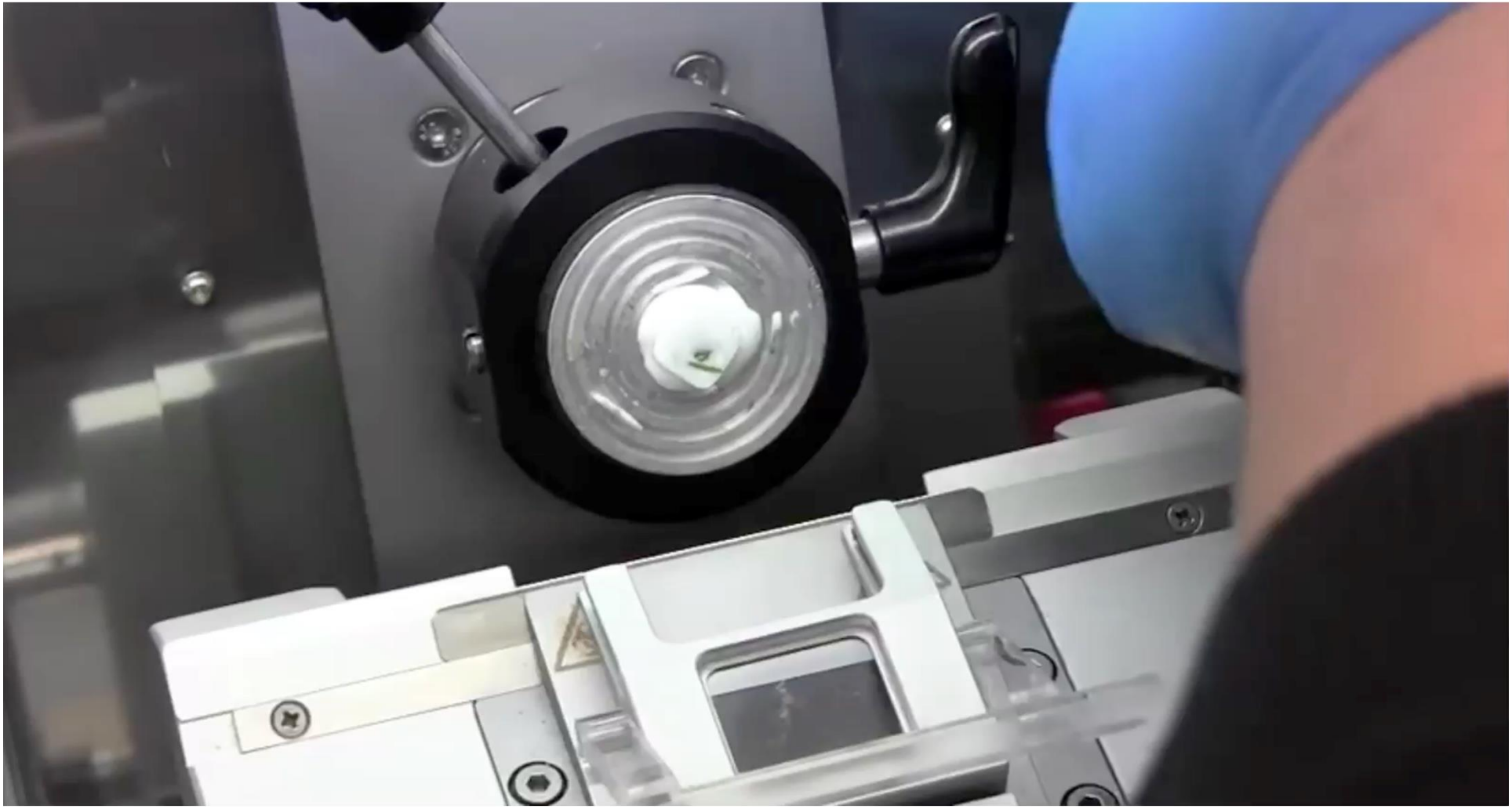
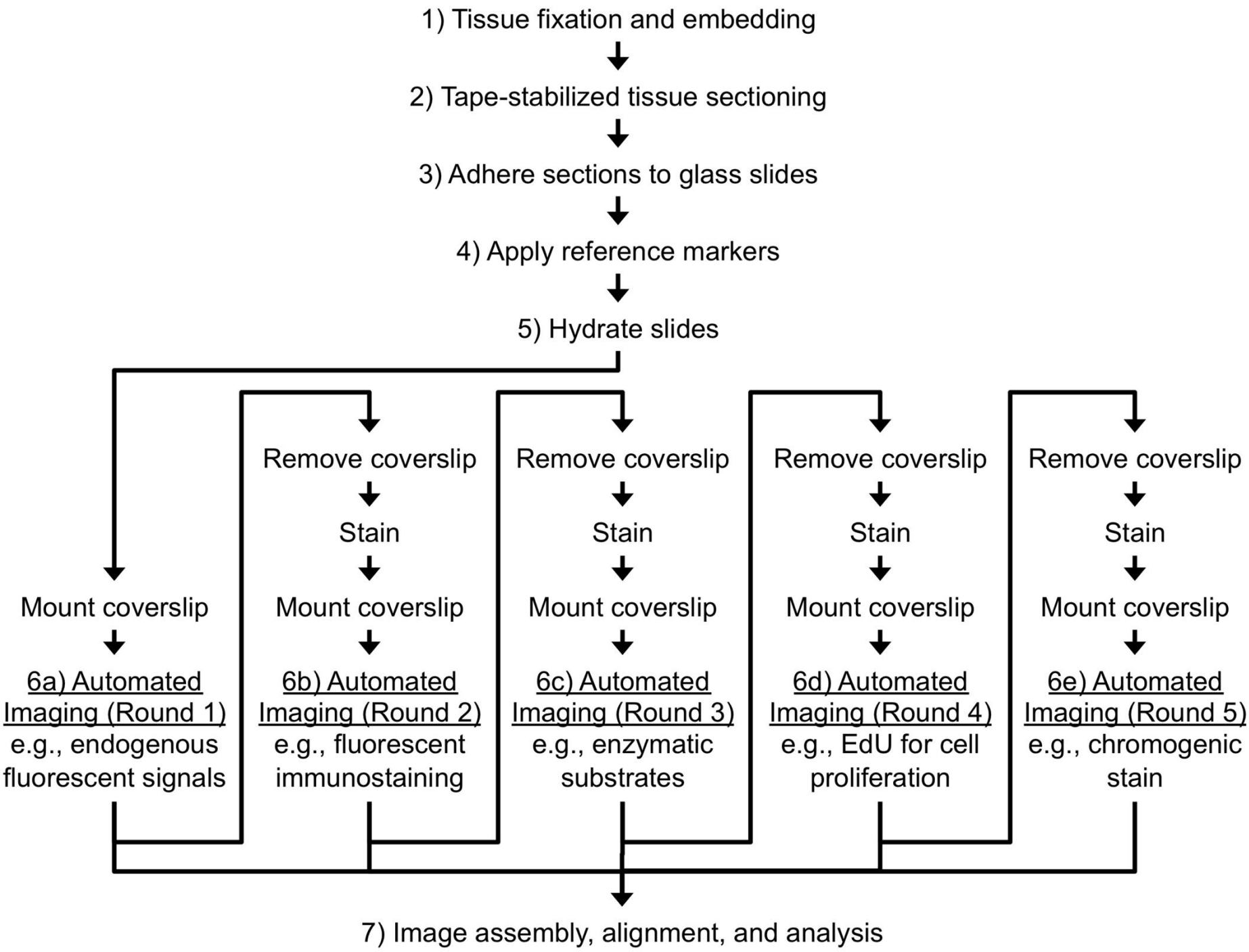
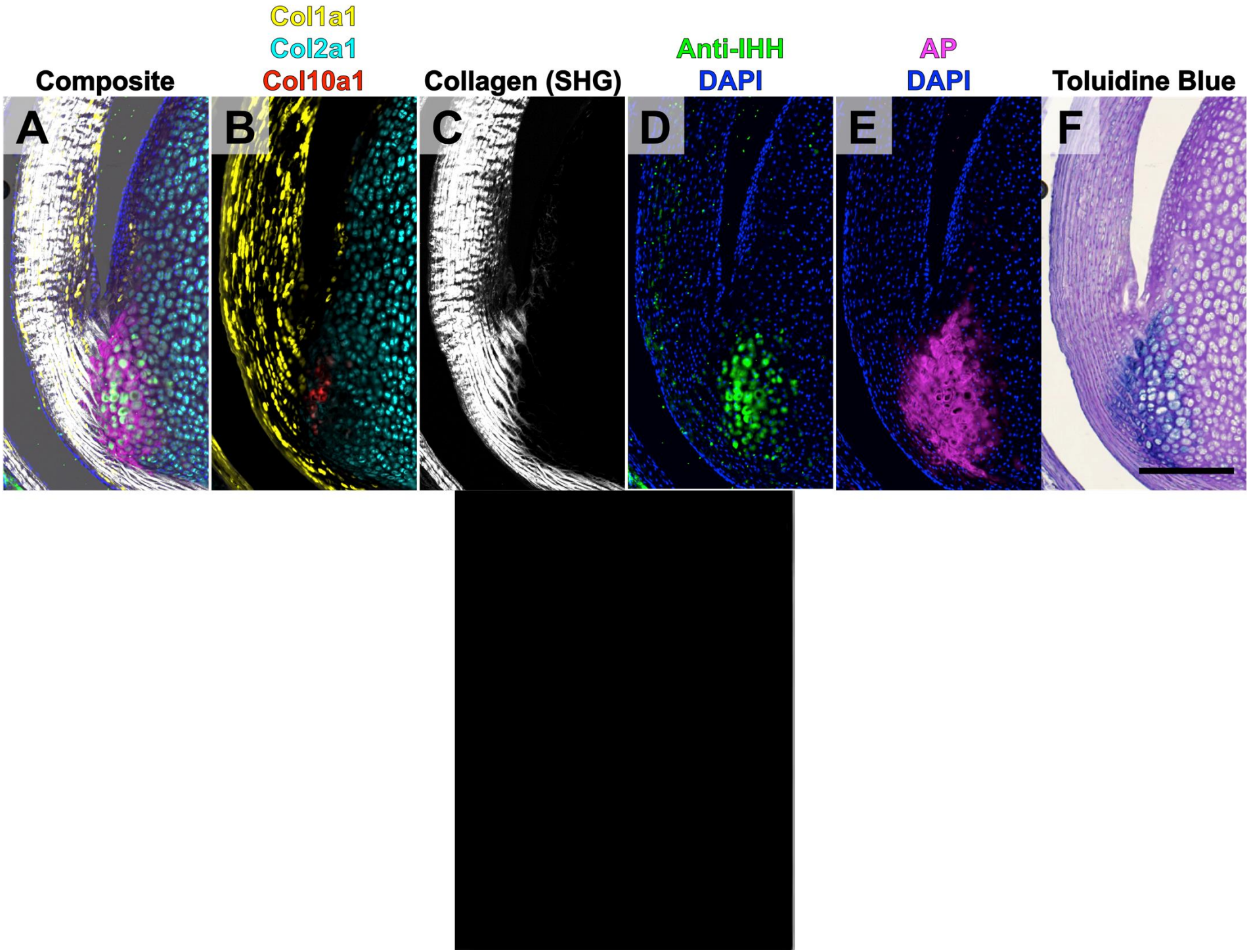


CryoJane Tape-Transfer System

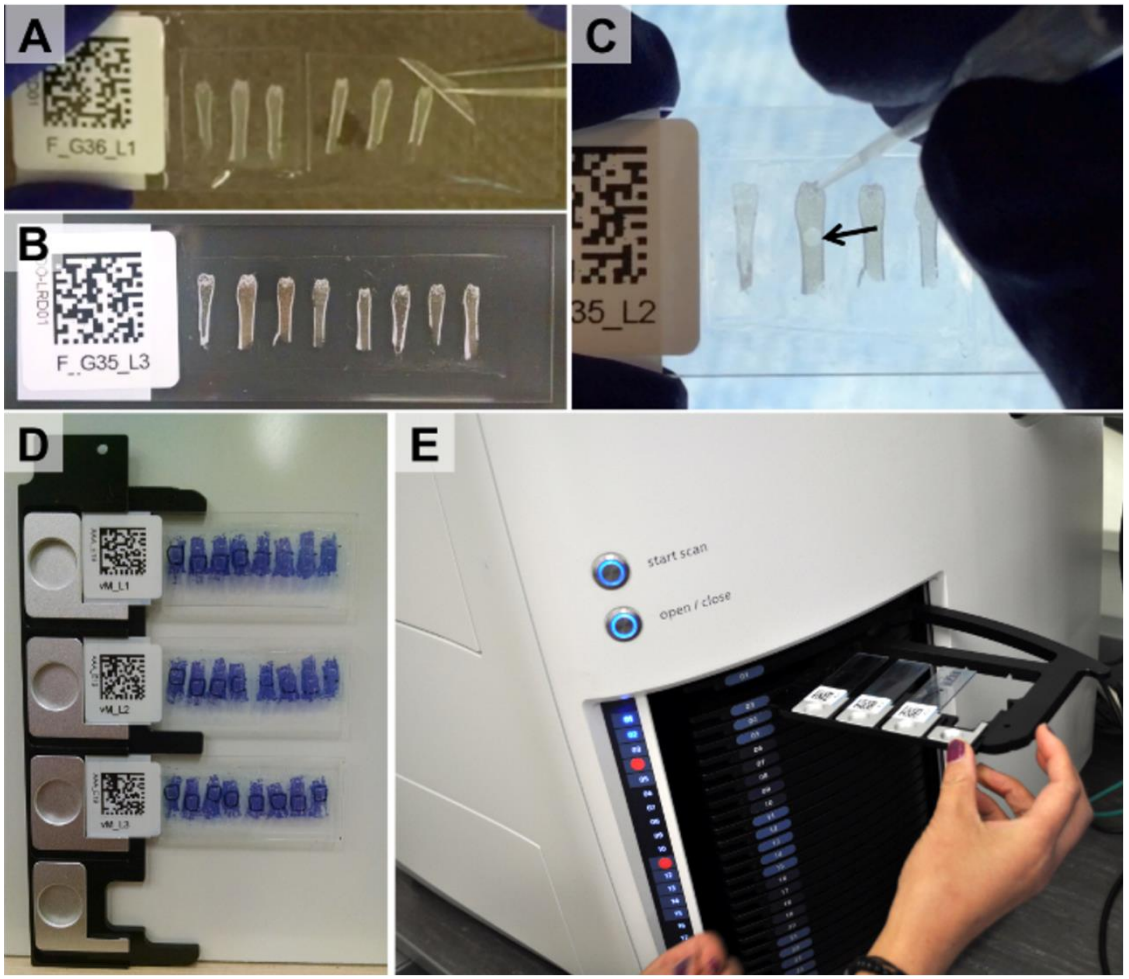


Viriginia Tech, https://www.youtube.com/watch?v=2rVRuG2_AKA&t=166s

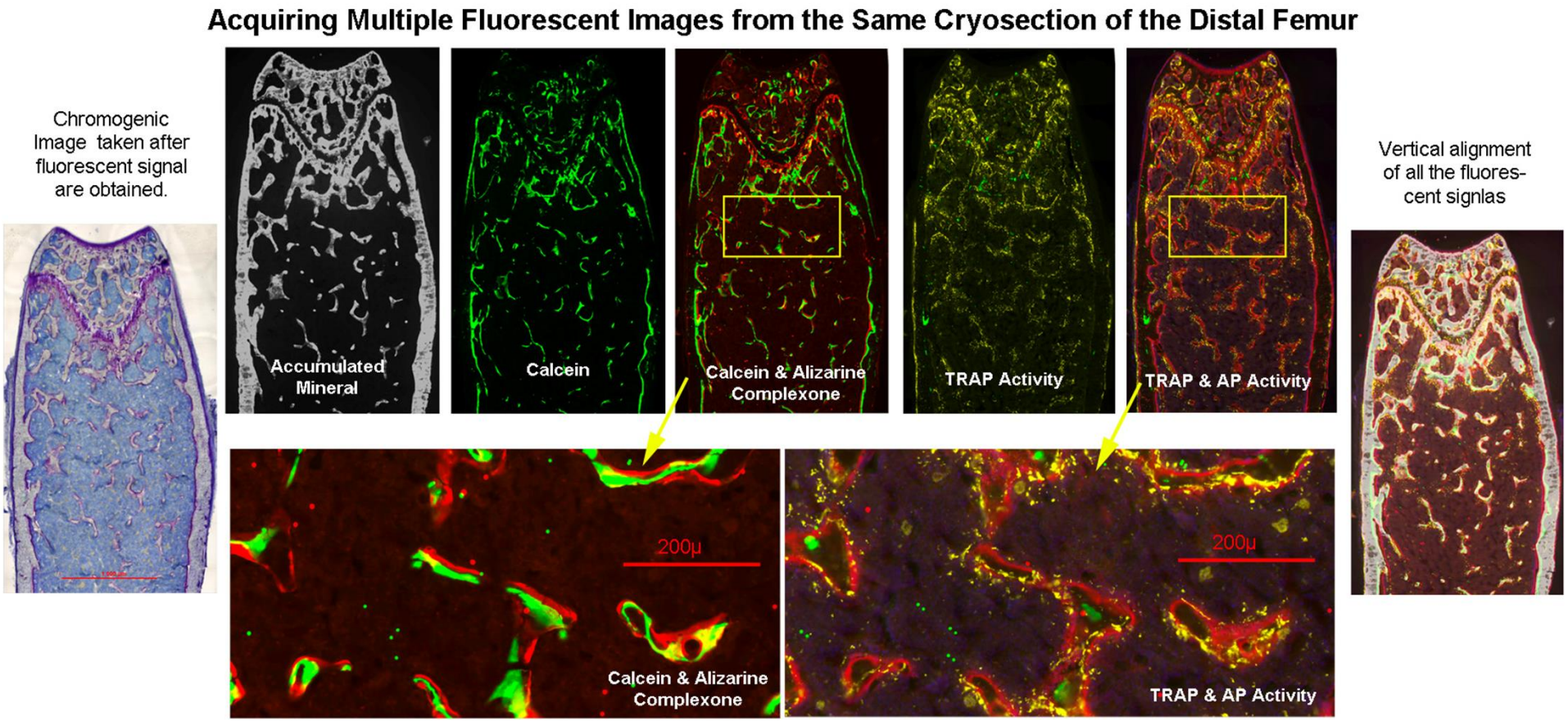

1) Tissue fixation and embedding
2) Tape-stabilized tissue sectioning
3) Adhere sections to glass slides
4) Apply reference markers
5) Hydrate slides
Mount coverslip
6a) Automated Imaging (Round 1) e.g., endogenous fluorescent signals
Remove coverslip
Stain
Mount coverslip
6b) Automated Imaging (Round 2) e.g., fluorescent immunostaining
Remove coverslip
Stain
Mount coverslip
6c) Automated Imaging (Round 3) e.g., enzymatic substrates
Remove coverslip
Stain
Mount coverslip
6d) Automated Imaging (Round 4) e.g., EdU for cell proliferation
Remove coverslip
Stain
Mount coverslip
6e) Automated Imaging (Round 5) e.g., chromogenic stain
7) Image assembly, alignment, and analysis


Composite
Col1a1
Col2a1
Col10a1
Collagen (SHG)
Anti-IHH
DAPI
AP
DAPI
Toluidine Blue
A
B
C
D
E
F

Zeiss Axio Scan.Z1

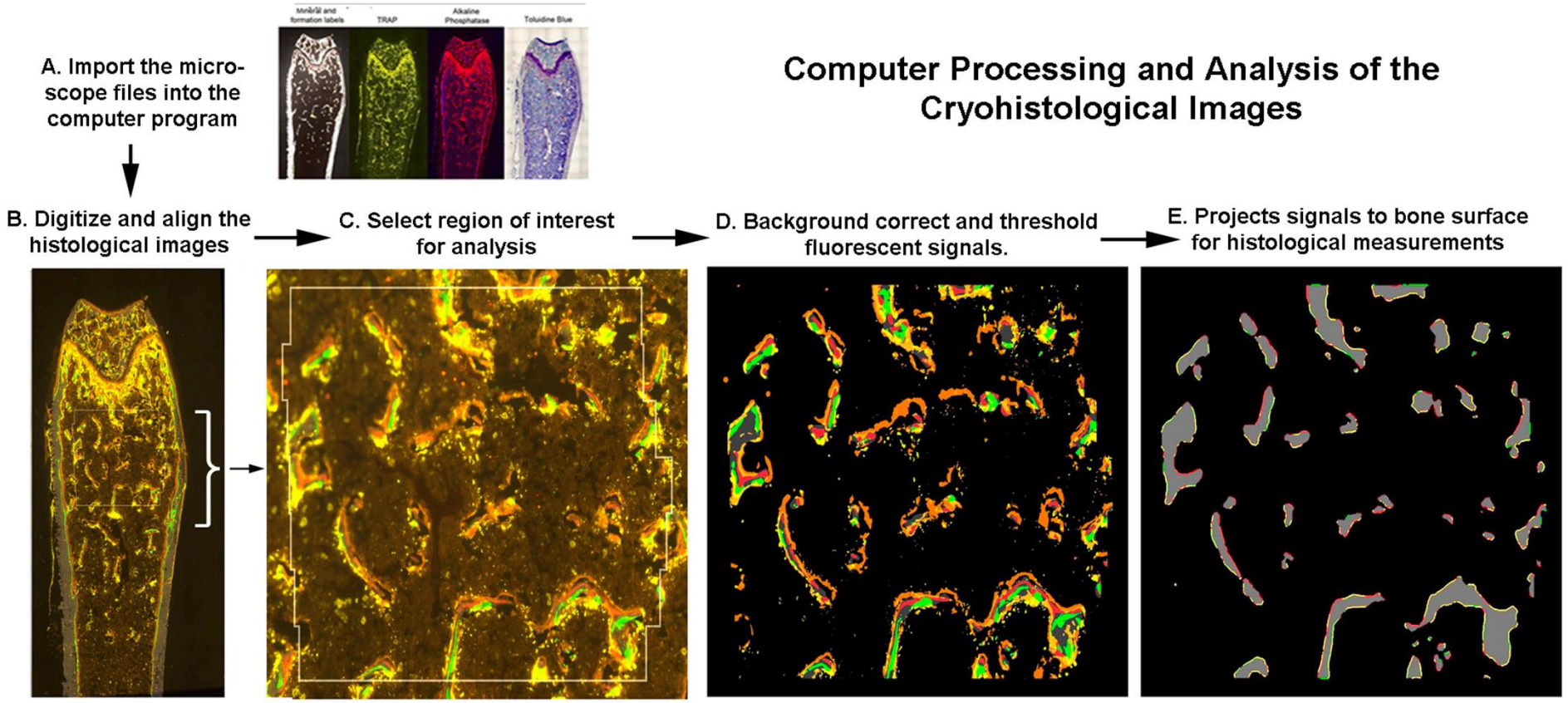


Bonebase.org – KOMP Phenotyping



Bonebase.org – KOMP Phenotyping

Automated Histomorphometry



Cryofilm vs CryoJane Comparison

	Cryofilm	**CryoJane**
Cost	Inexpensive (~$75 per sheet)	Expensive (~$12k capital expense, $2.75/slide)
Learning curve	Easier to master	Takes more time but not difficult
Possible to section mineralized tissues using this system?	Yes	Yes, but pieces of mineralized bone may not transfer completely to the slide
Possible to cut multiple samples embedded in the same block?	Yes	Yes
Possible to conduct multiple rounds of imaging on same section?	Yes	Yes
Possible to use heat antigen retrieval?	Maybe, lower temperature with UV adhesive	Yes