

Centrifuging Nail Specimen Debris Increases Sensitivity of Fungus Detection

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Objectives—Nail Unit Processing

- Nail bed/matrix
- Nail plate
- New method for fungal detection

Need to be involved in lab prep

Clinical

Laboratory

Surgery → Grossing → Embedding → Slide

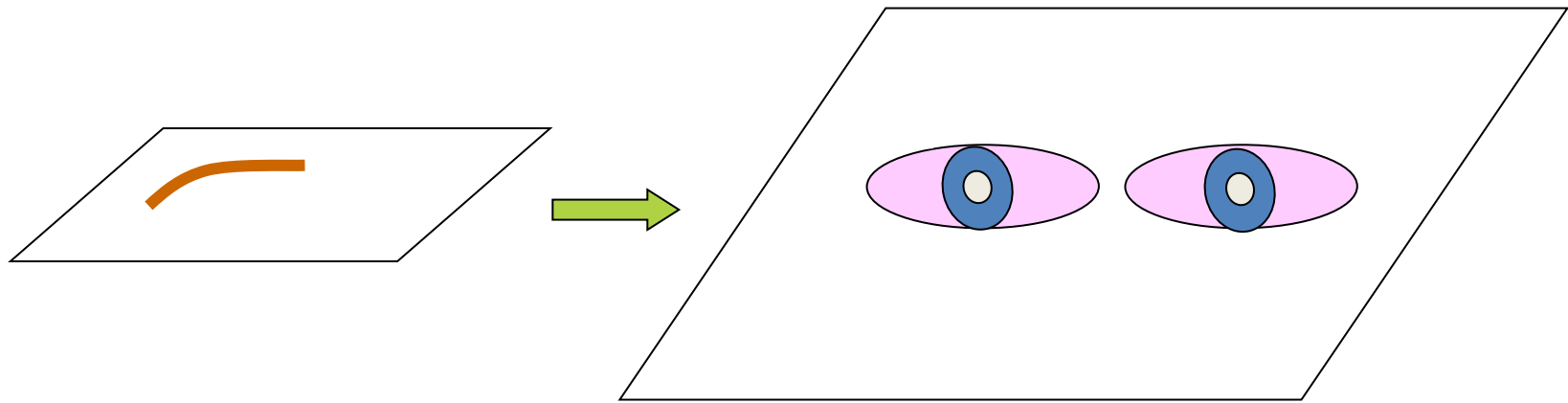


Need to be involved on
this side

Goal in Submitting Nail Bed and Matrix Biopsy

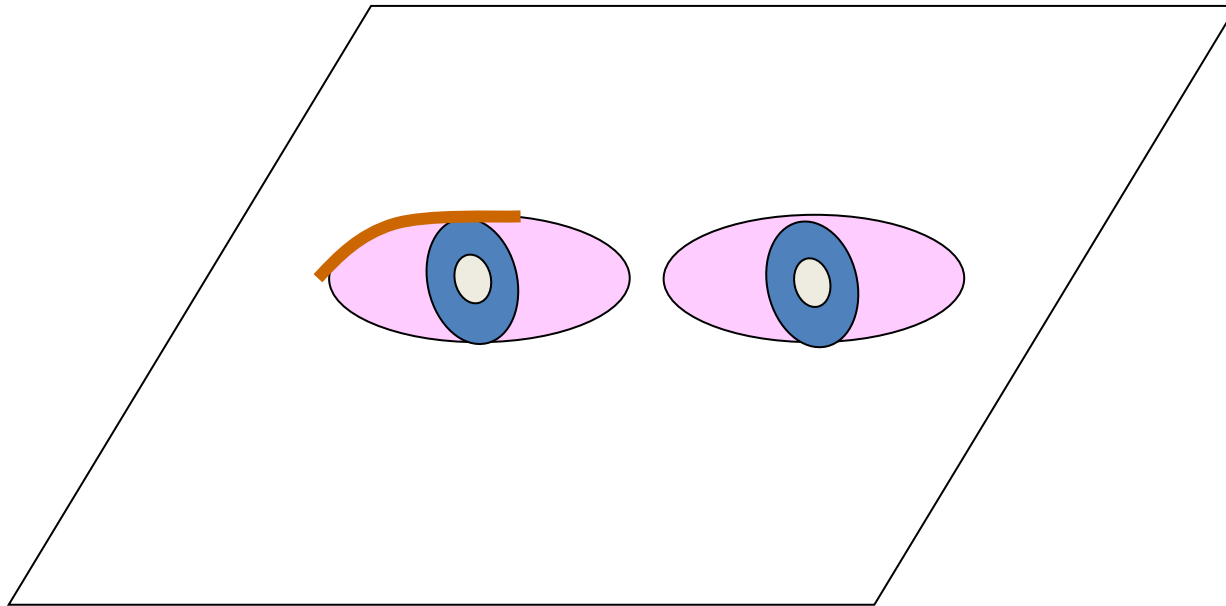
- Orientation of tissue
- Clear information to laboratory
- Reproducible among different laboratories

Idea—Orientation of Tissue

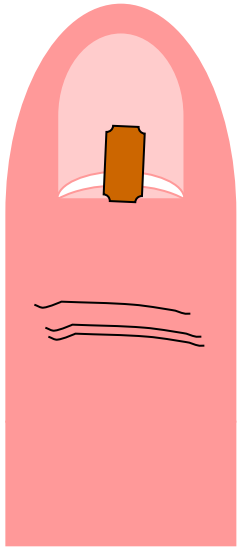


Cartoon of Eyes

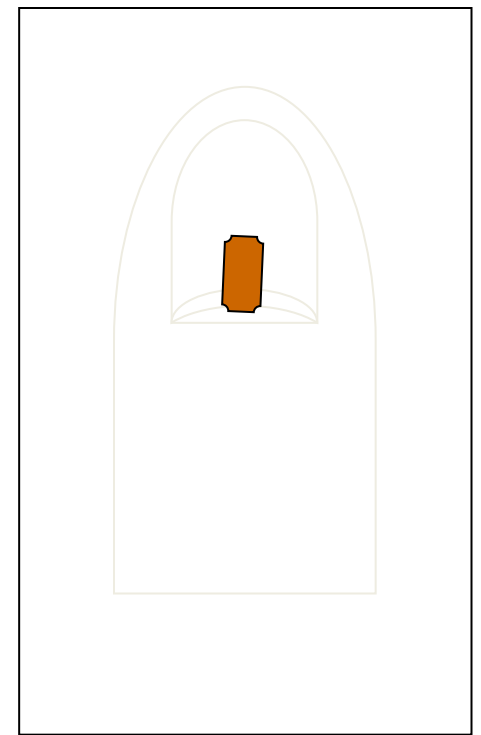
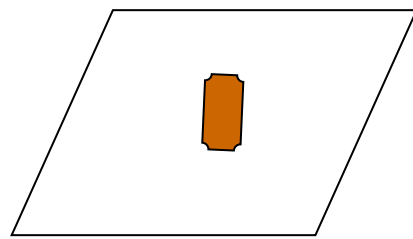
Idea—Orientation of Tissue



Cartoon of Eyes

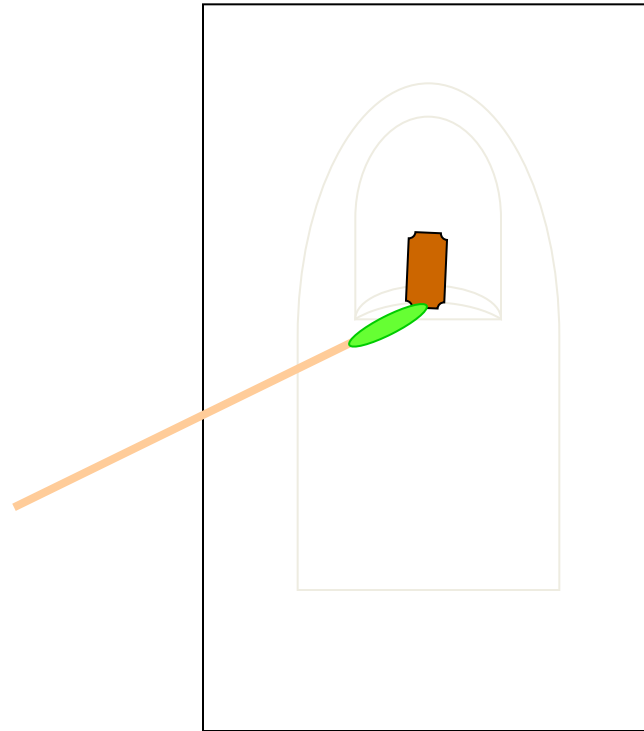


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Cartoon of Nail

Inking to maintain orientation



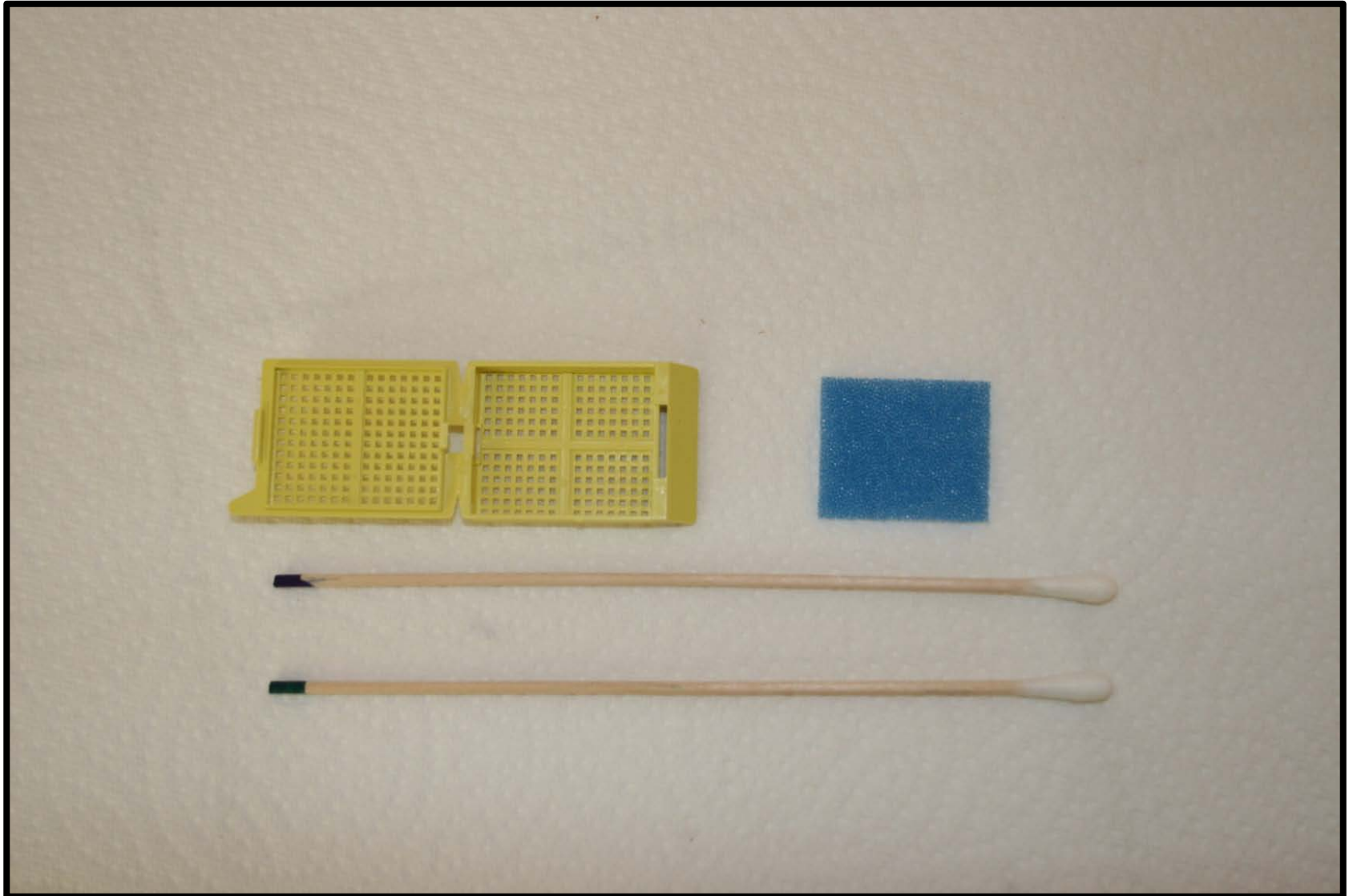
Orientation of soft tissue



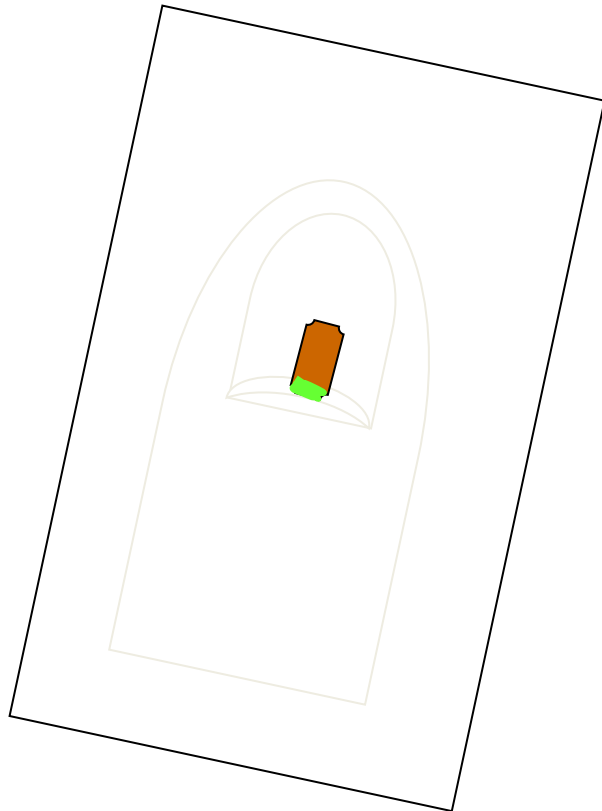
Orientation is helpful whether the lesion is inflammatory or neoplastic



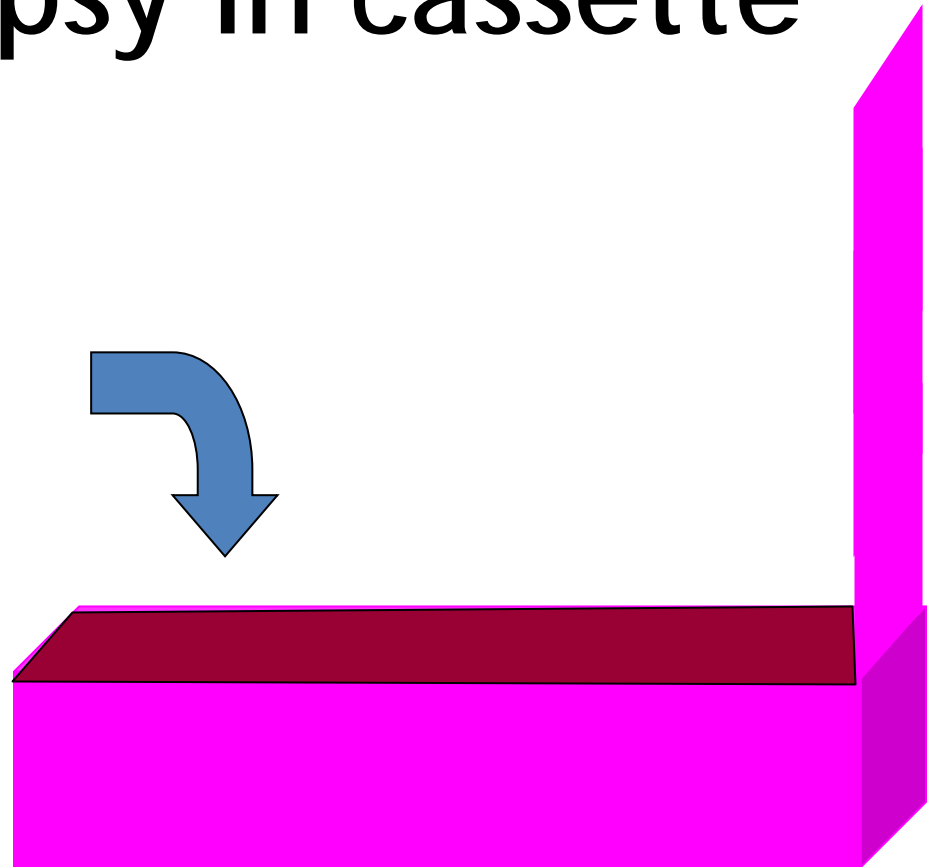
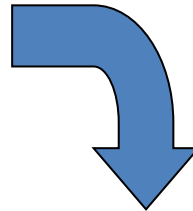




Place biopsy in cassette

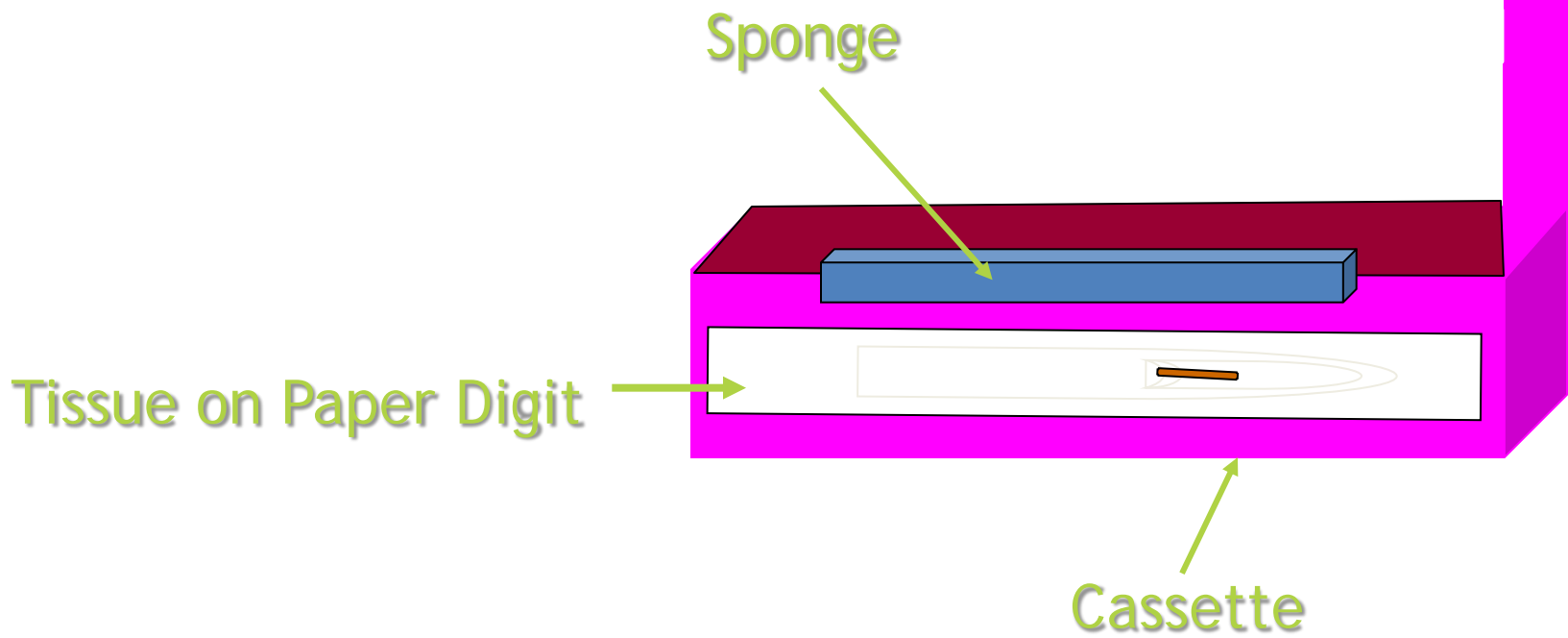


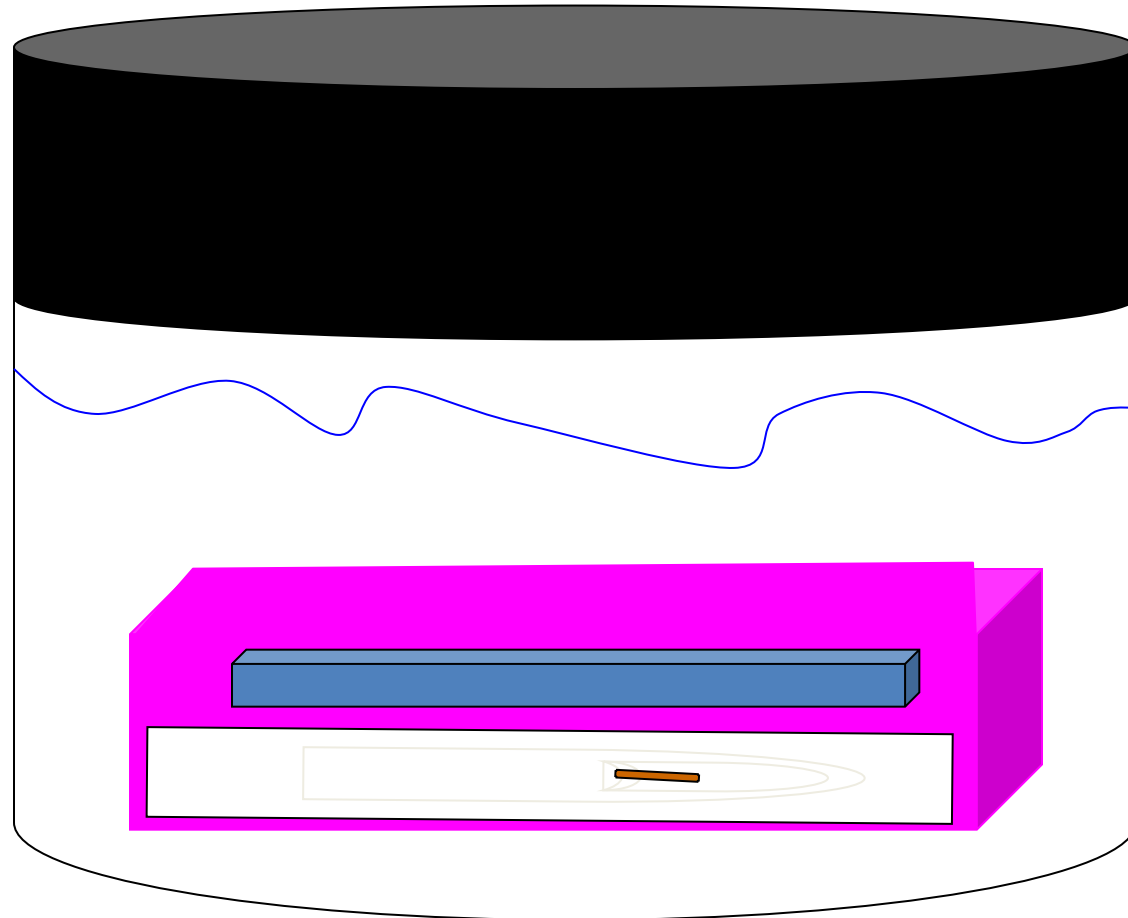
Tissue on Paper Digit



Cassette

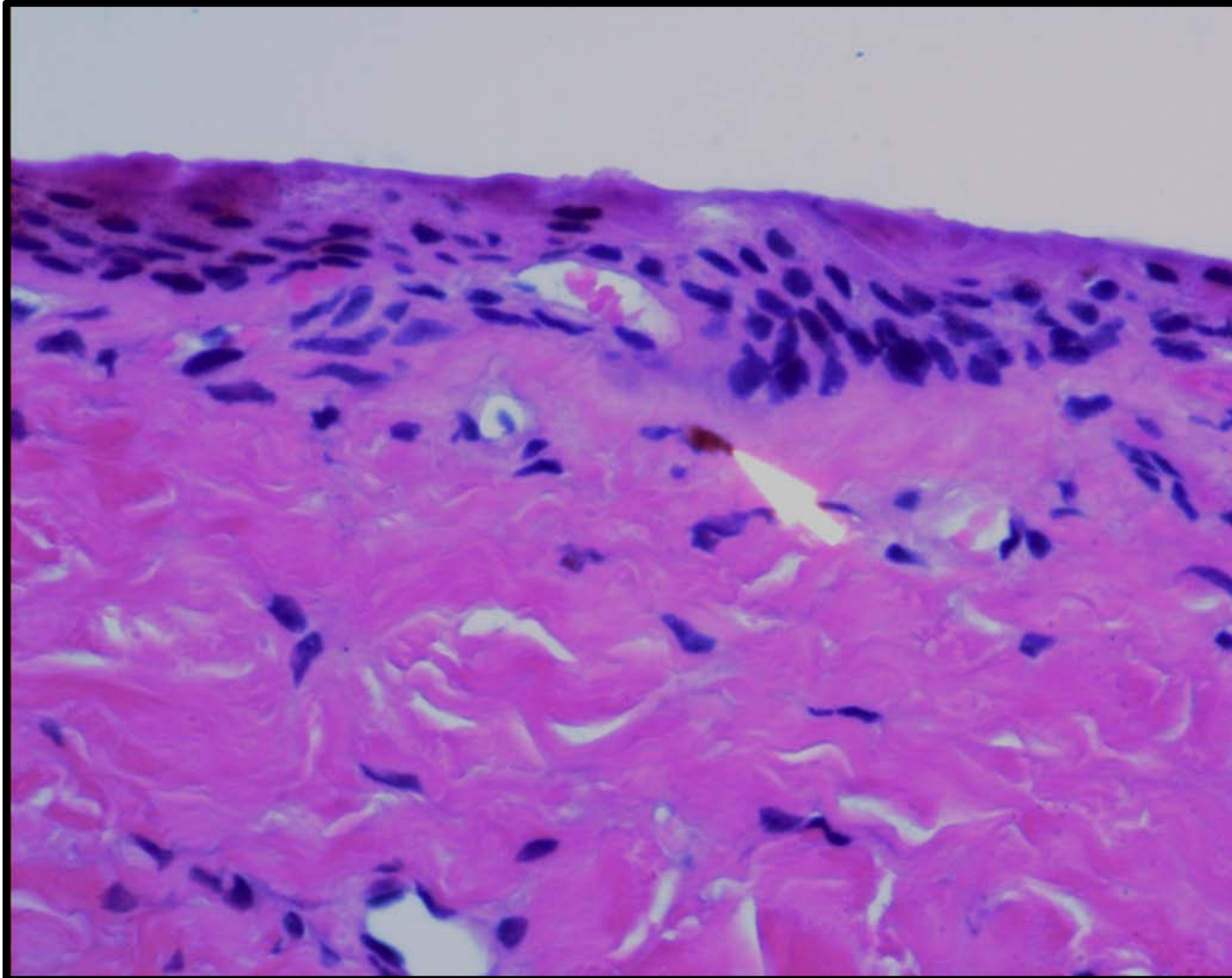
Cover with sponge to hold in place

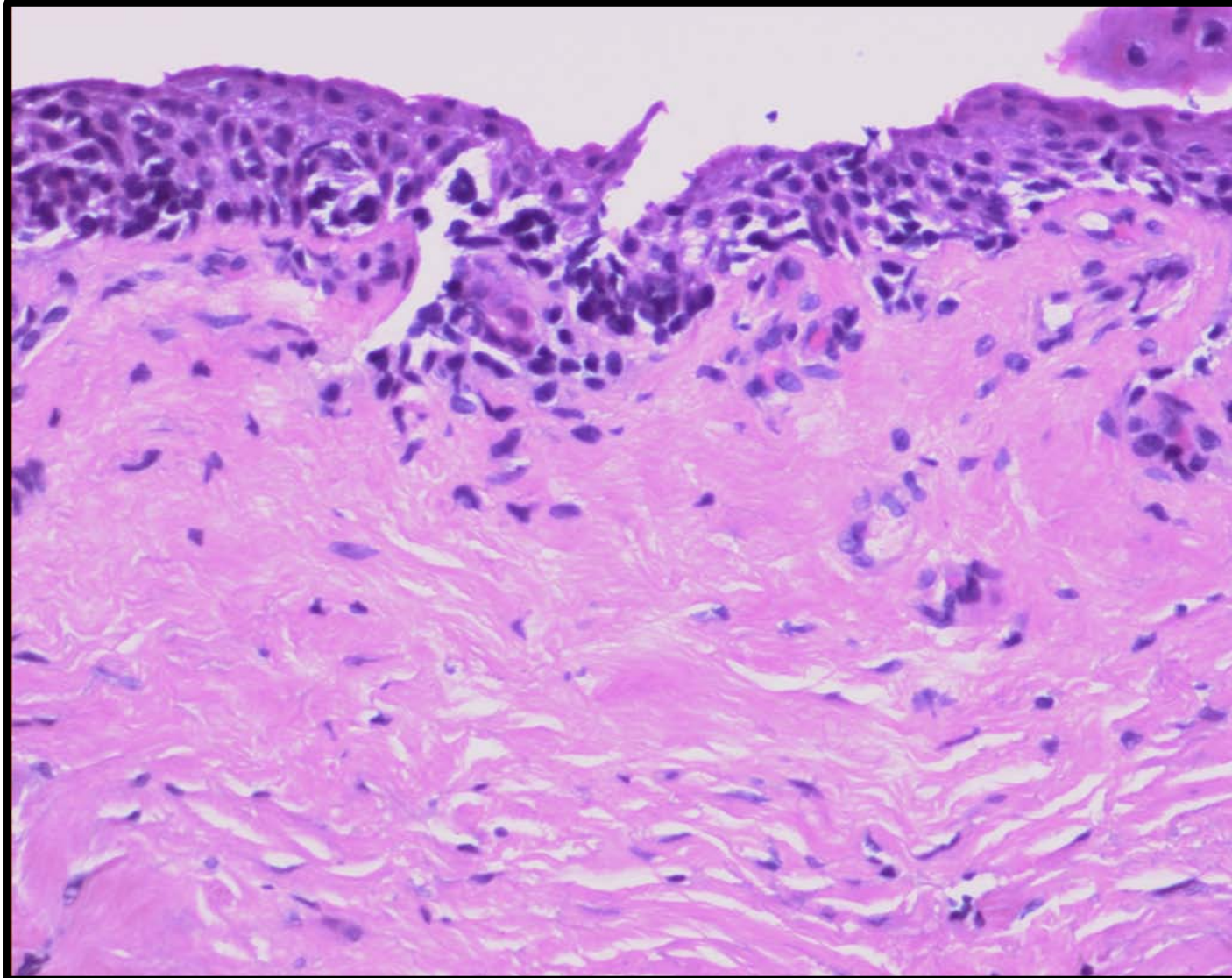


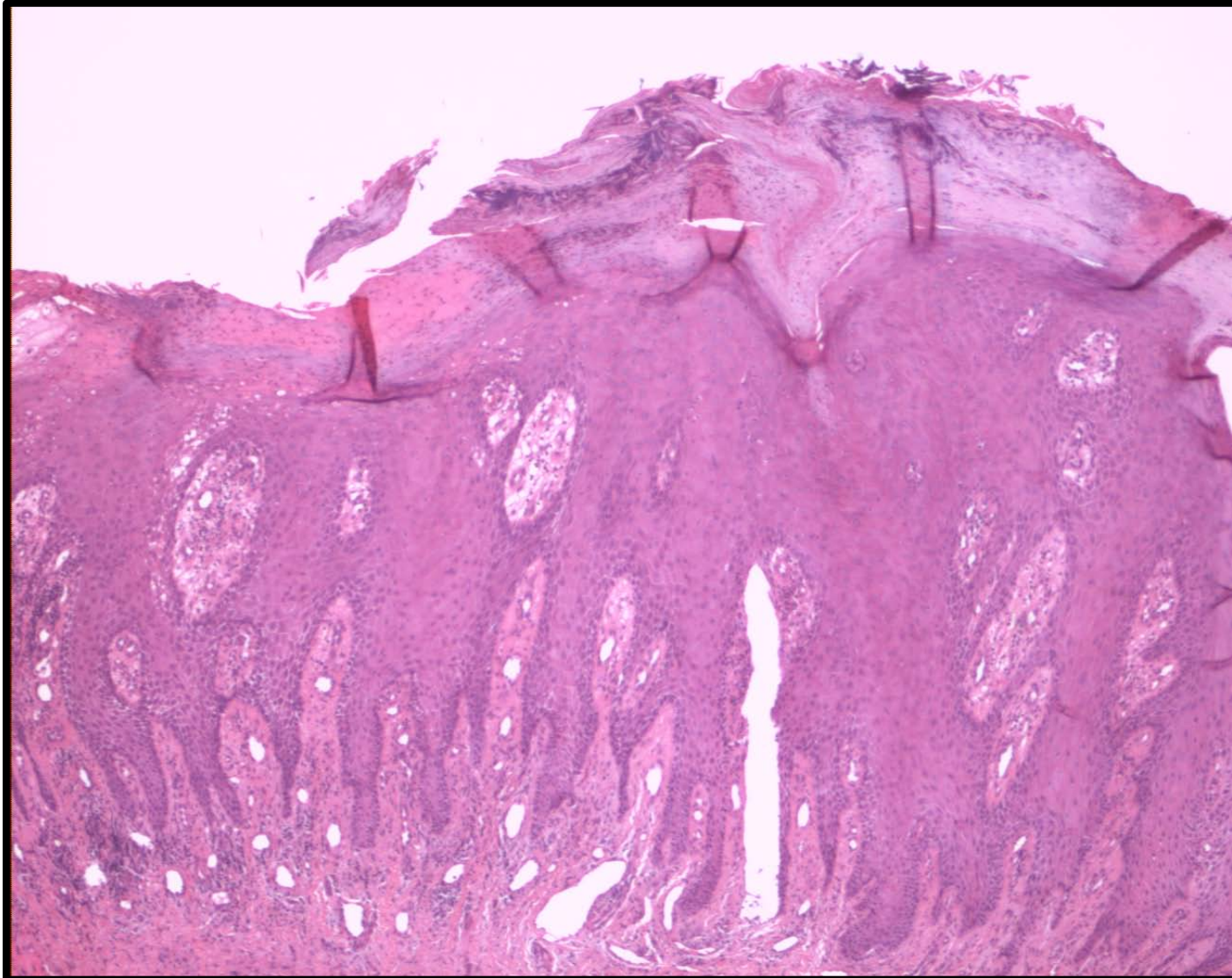


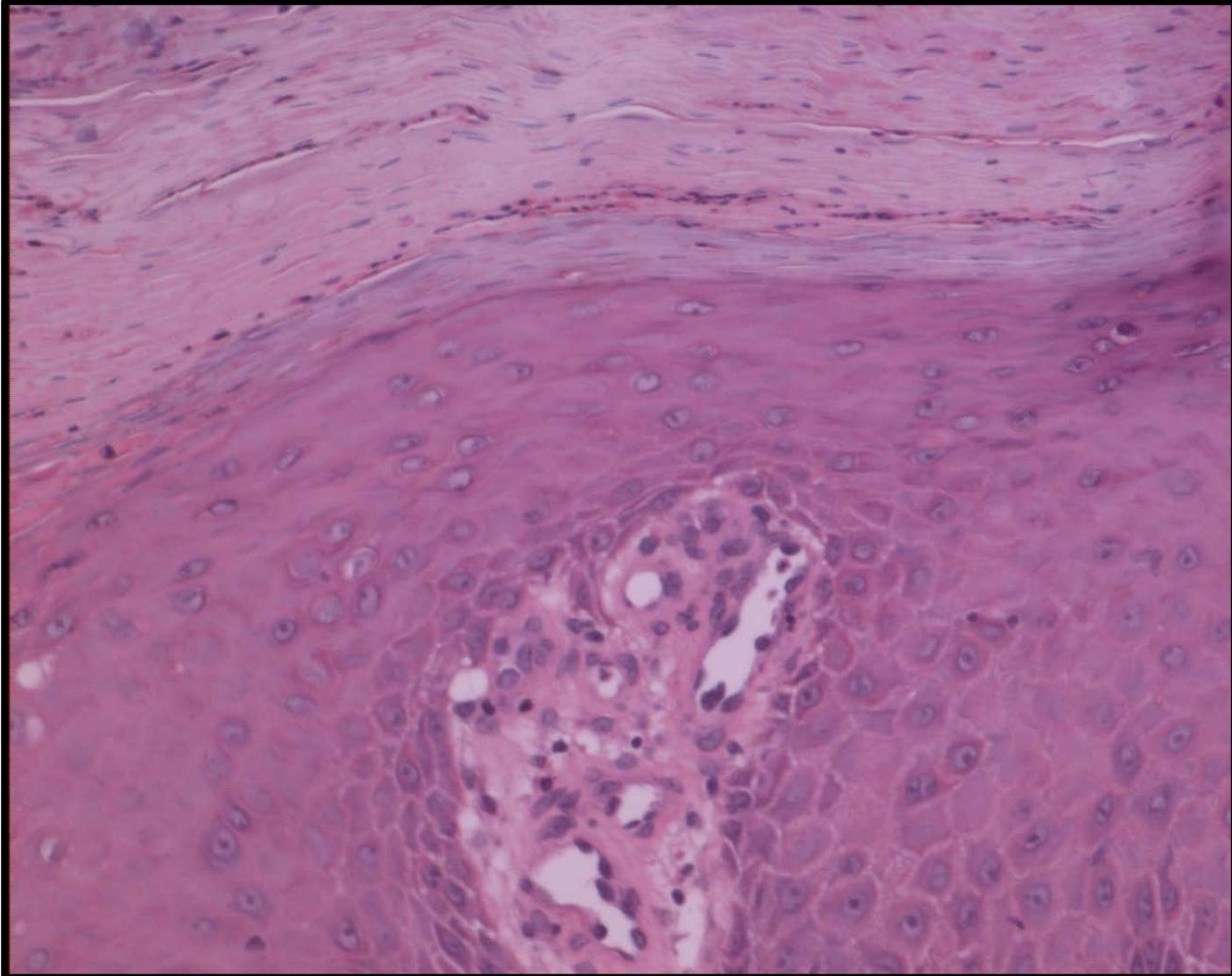
Summary of Matrix/Bed Biopsy

- Use cartoon diagram
- Ink one end for orientation

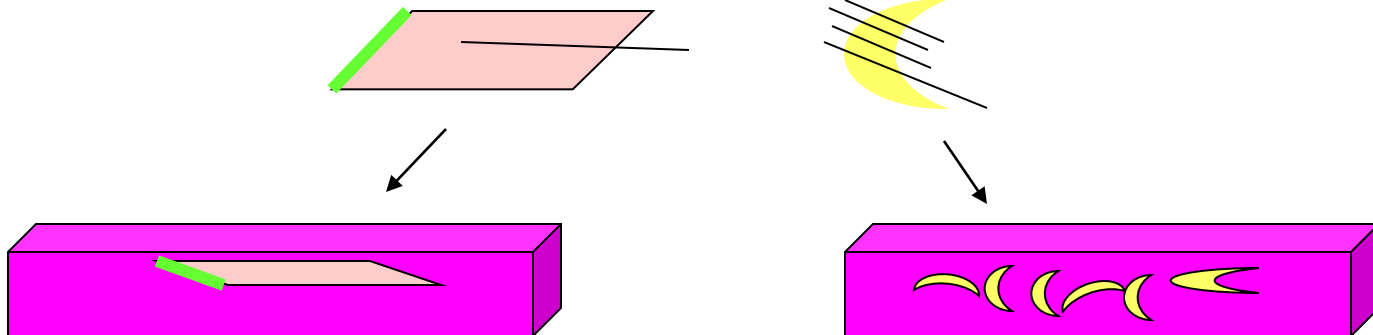








Submit nail plate separately from soft tissue

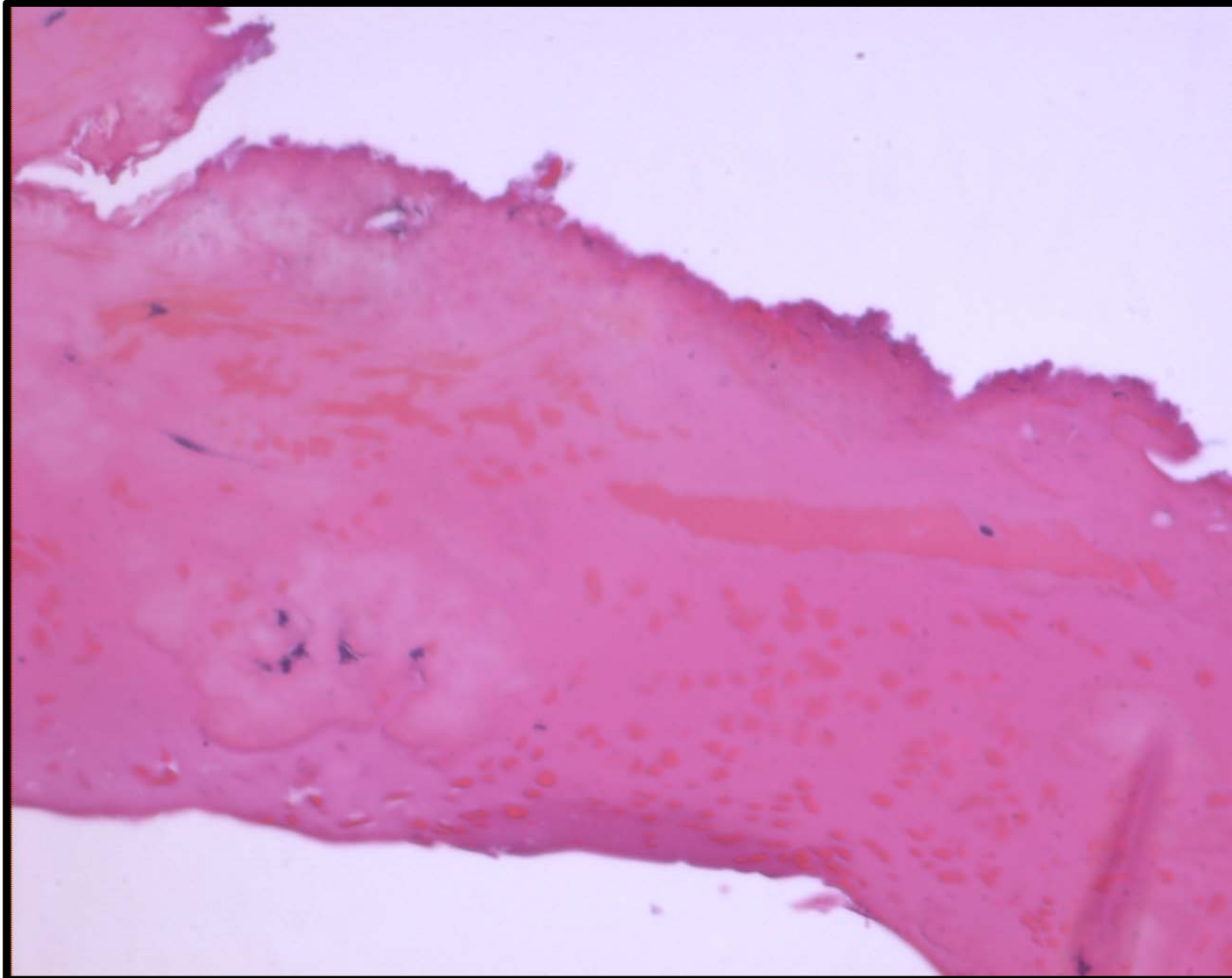


Pigment in the nail plate

- Blood
- Melanin
- Fungus
- Exogenous pigment
- Medications

Blood

- Evident on standard H&E.
 - Benzidine stain works but not necessary
 - Carcinogenic
 - Perl's iron stain does not work in the nail.
 - Iron not released from heme.
- May still be a subjacent neoplasm or dermatitis (fungus).



Nail fungus diagnostics

- Calcofluor White Stain
- PAS
- KOH
- Culture

Comparison of diagnostic methods in the evaluation of onychomycosis. Weinbert JM and Koestenblatt MS, Dermatology Online Journal 7:23G, 2001.

Nail sampling is an issue

- Subungual debris is better than nail plate for sampling.
 - Issue—Not all patients have subungual debris.

Chang A *et al.* A modified approach to the histologic diagnosis of onychomycosis. JAAD 57:849-53, 2007.

New Method: Centrifuging Debris

- Formalin sample is reduced in volume in a large volume centrifuge.
- Pellet moved to Cytospin (Fisher HealthCare) centrifuge.
- Debris is centrifuged directly onto a glass slide.

CytoSpin Centrifuge



Centrifuge with slide



Centrifuge with slide



Study—204 Cases

- 132 cases positive on initial nail PAS
- 72 cases negative
 - 52 cases centrifuged after negative nail PAS
 - 20 cases (10%) not centrifuged, usually because nail was submitted dry.

Study—204 Cases

- 18 (of 52) cases positive on centrifuge
- Total 150 positive cases—nail and centrifuge

Study—204 Cases

- Sensitivity increase of 12%.
- 74% nails positive for fungus

Study—52 Centrifuged Cases

- 18 cases positive on centrifuge
- 34 negative on centrifuge
 - Bacteria—20 cases
 - No bacteria—14 cases

Study—Bacteria

- 59% of negative cases were associated with bacteria, usually coccal forms.
- Suggests that bacteria, probably *Staphylococcus* species, are pathogenic in nail disease.

Study—Conclusion

- Positive cases—sensitivity increase of 12%
- Negative centrifuge cases often associated with bacteria (59%)

Conclusion

- Cartoon orientation of bed/matrix biopsies
- Separate nail plate—blood issues
- New fungal diagnostics via centrifuging

Gratitude:

Phoebe Rich, Oregon

Monica Lawry, California

Hendrik Uyttendaele, New York

Brandon Stokes—Centrifuge Technique

Rodd Takiguchi—Centrifuge Study