Nail Unit Specimen Processing and the Diagnosis of Common and Important Nail Lesions

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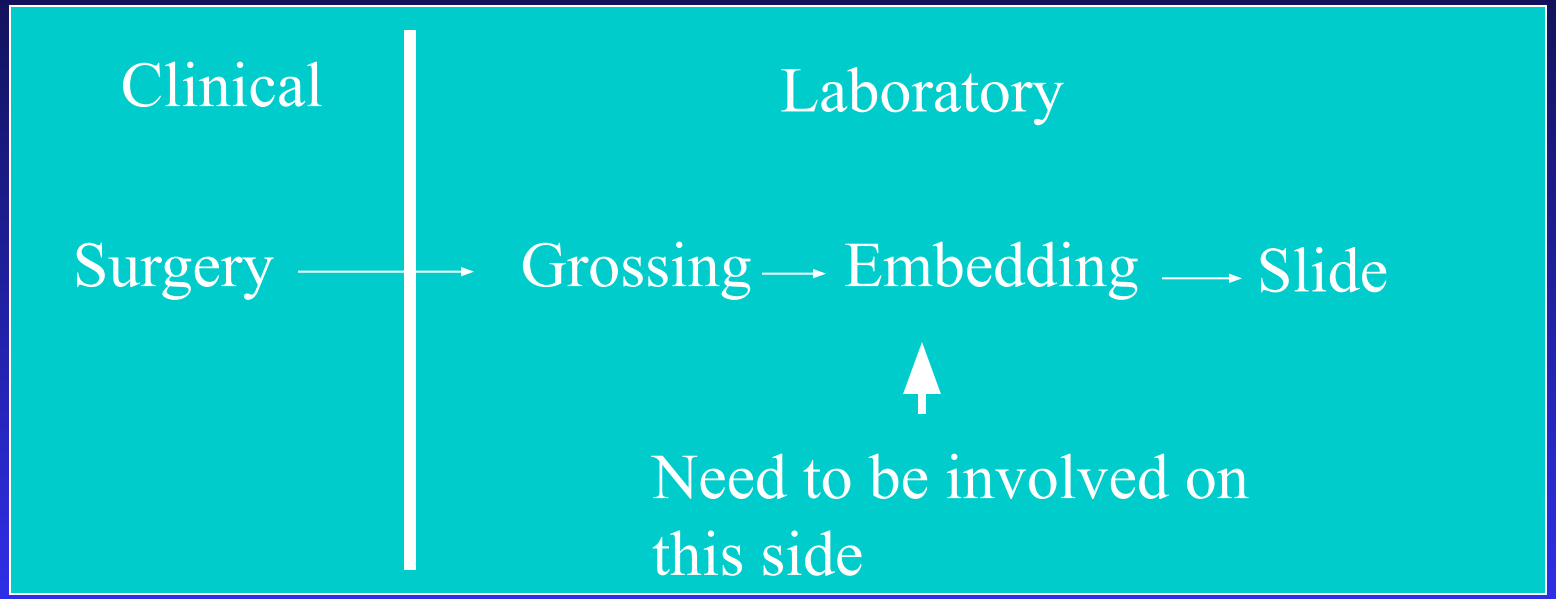
Oregon Health and Sciences University
and
Medical Director
CTA Lab

Objectives

- Nail
  - Tissue submission/processing
  - Fungal identification including mold

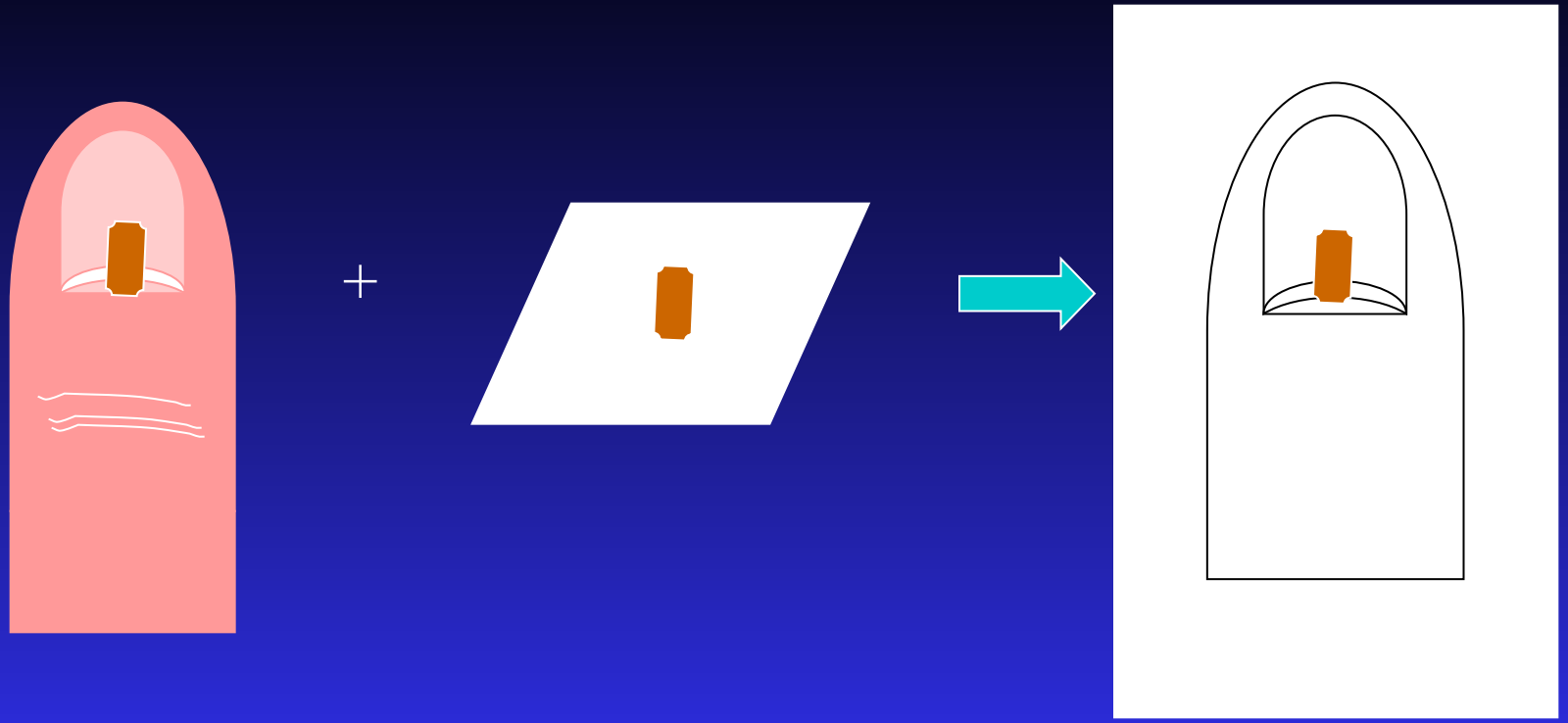
What can the nail surgeon do to submit a bed/matrix specimen for appropriate interpretation?

Need to be involved in lab prep



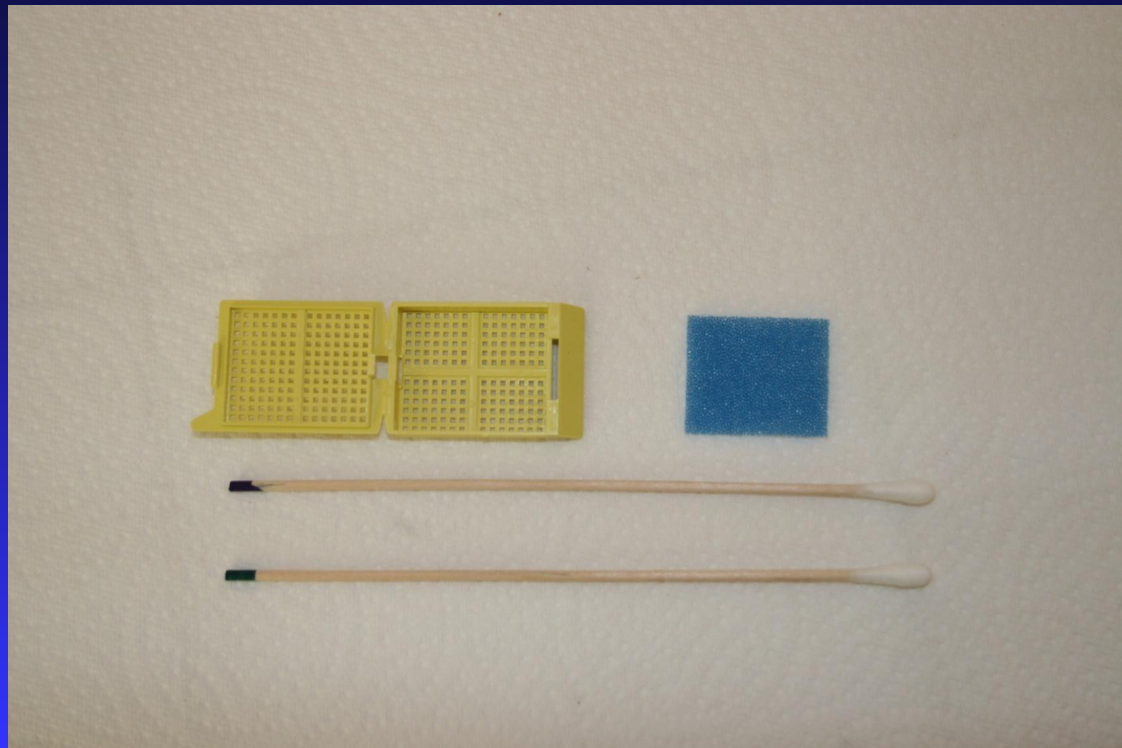
Need concise and clear guidelines for specimen submission:

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



Print template at www.cta-lab.com

Histology Materials



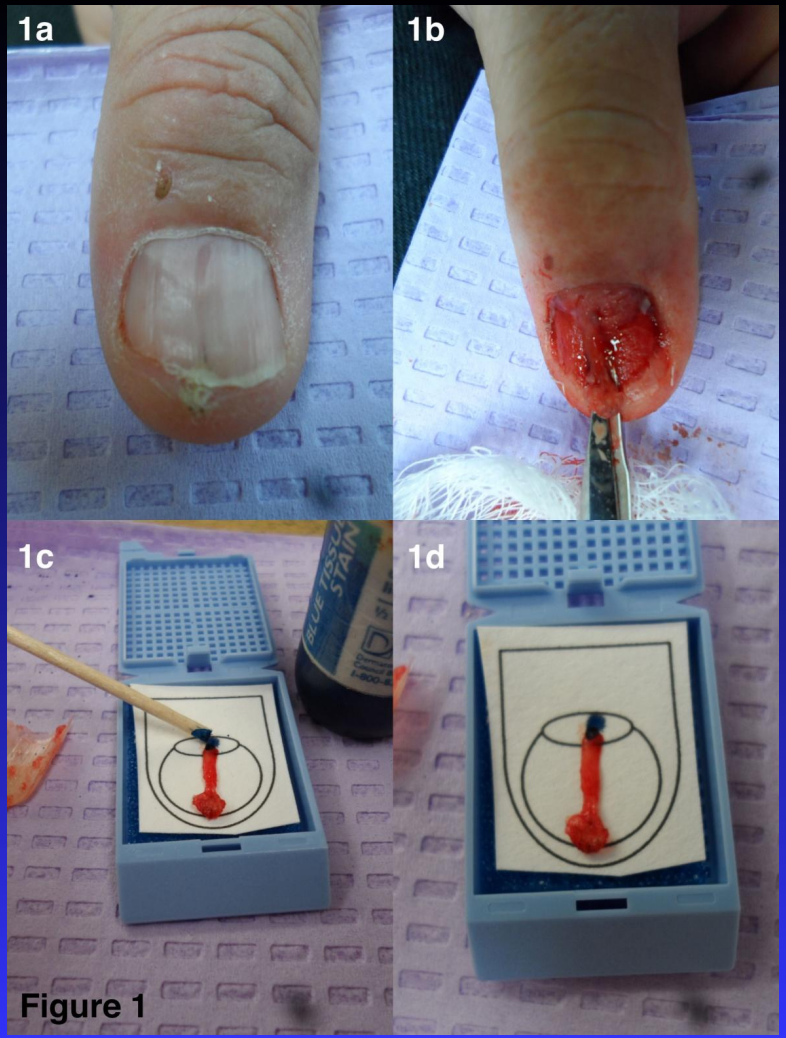


Figure 1

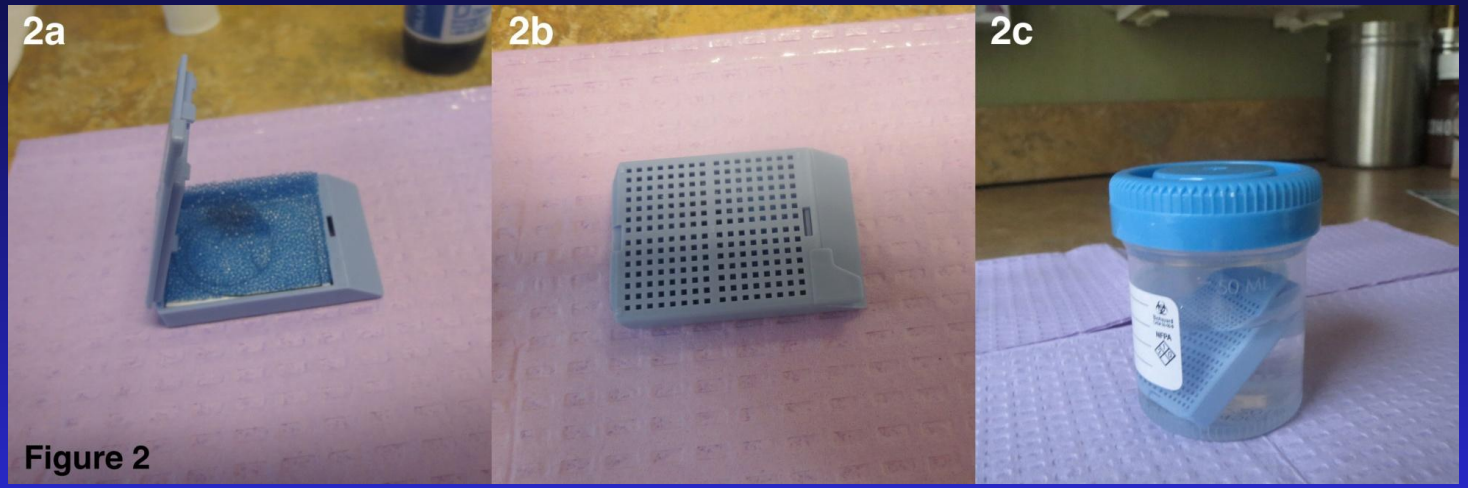
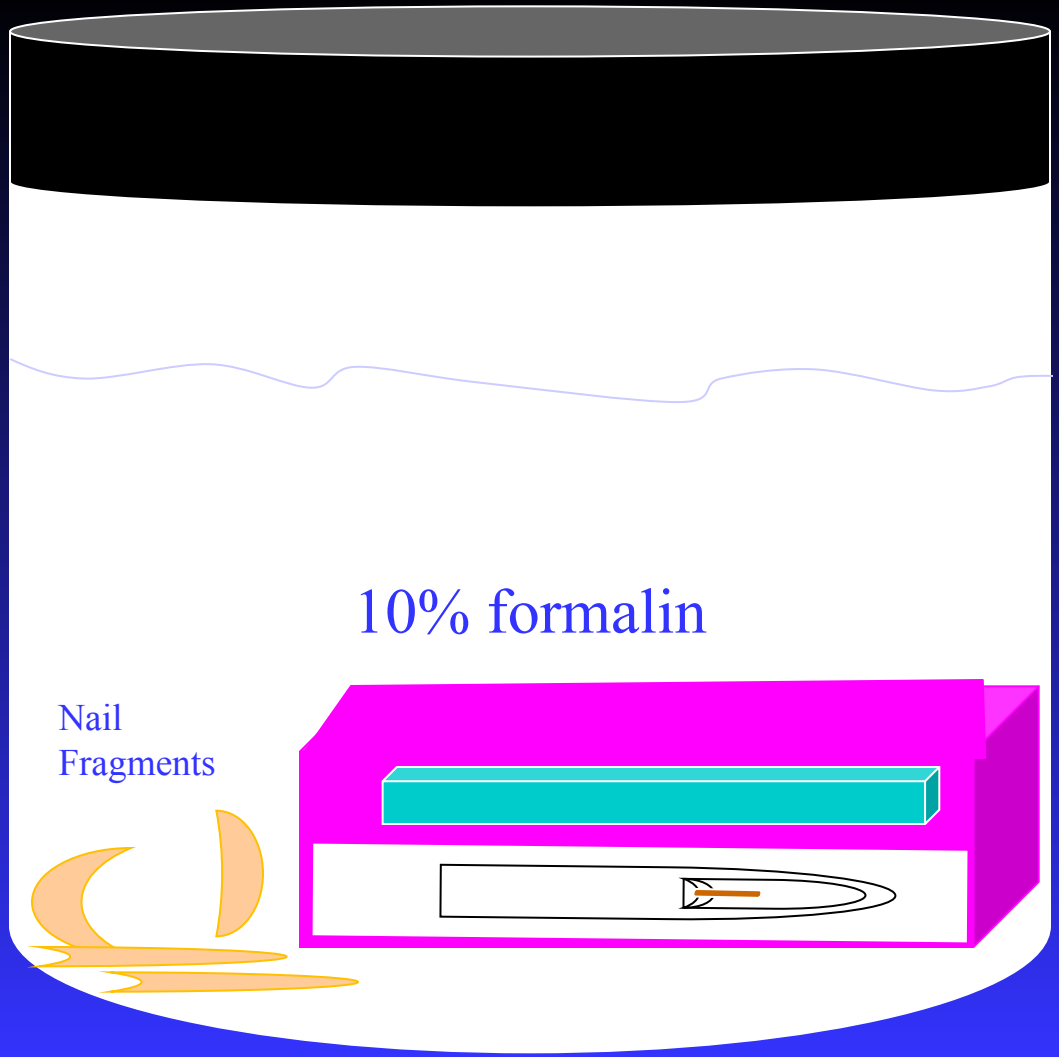


Figure 2


10% formalin
Nail
Fragments

Each specimen is different

Pathologist review before grossing

- Number tissue blocks
- Unstained slides or levels at the start
- Special stains
- Importance of nail
- Reserve nail for culture

Think about the differential diagnosis when grossing

Think about the differential diagnosis when grossing

- Onychopapilloma
- Onychomatricoma
- Digital myxoid/mucous cyst
- Squamous cell carcinoma
- Longintudinal melanonychia

Onychopapilloma

Onychopapilloma

- Clinical
  - Logintudinal erythronychia (redness)
  - Distal nail split

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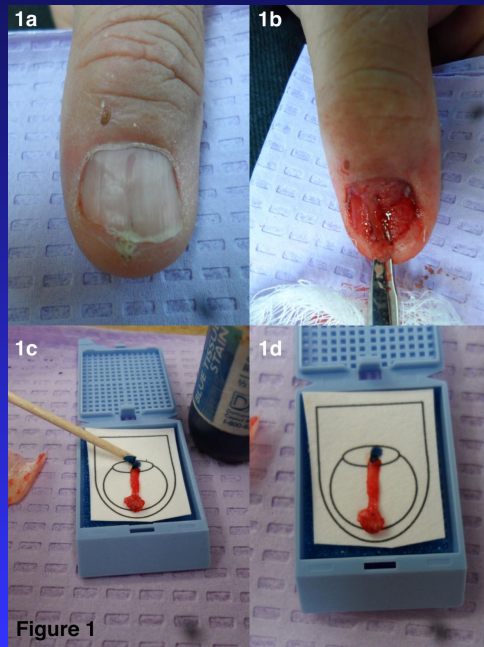
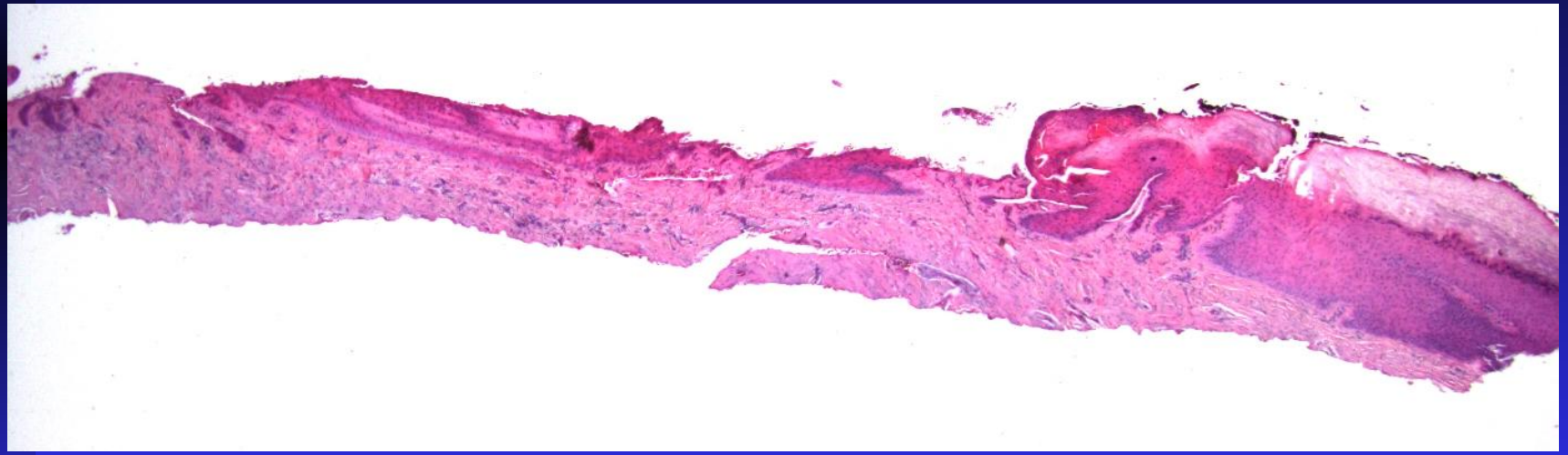
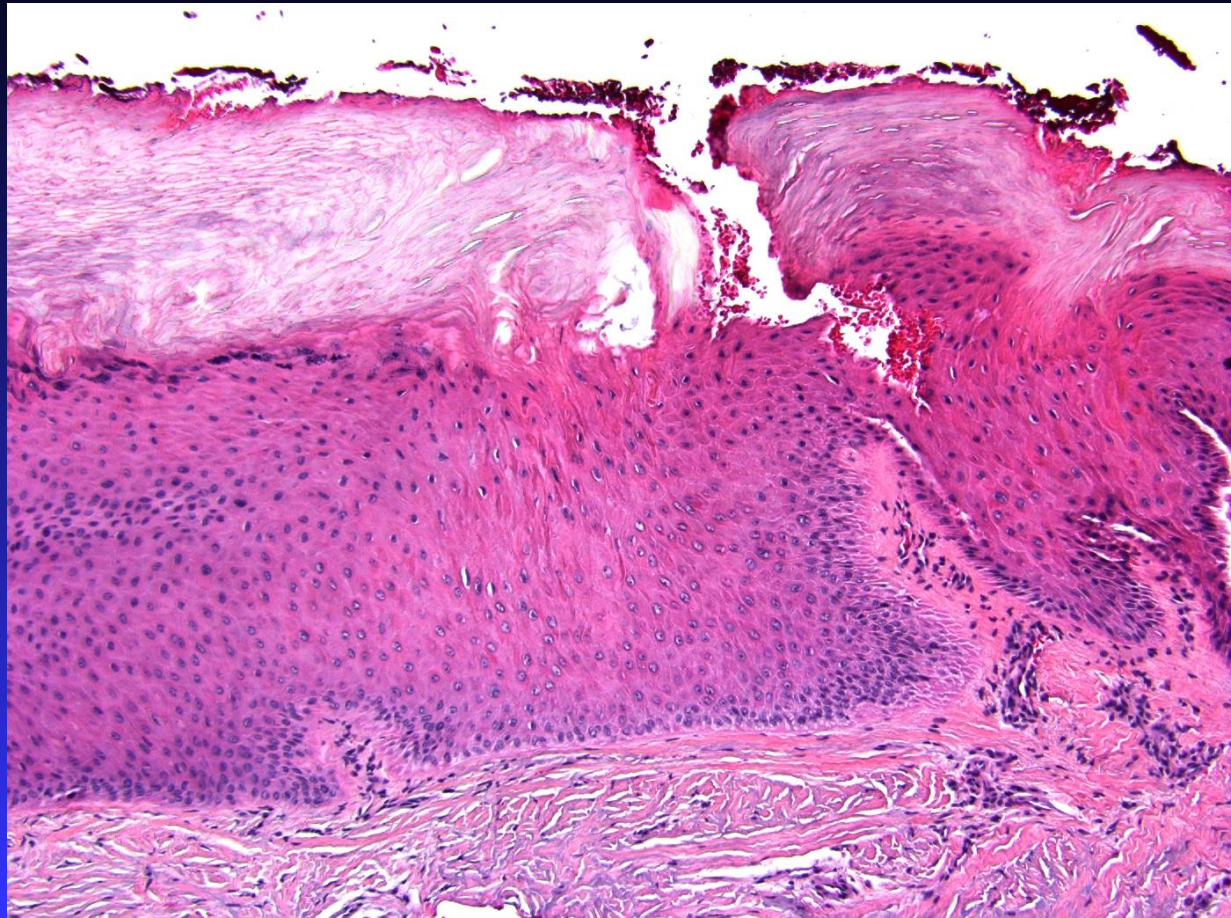
Embed proximal to distal



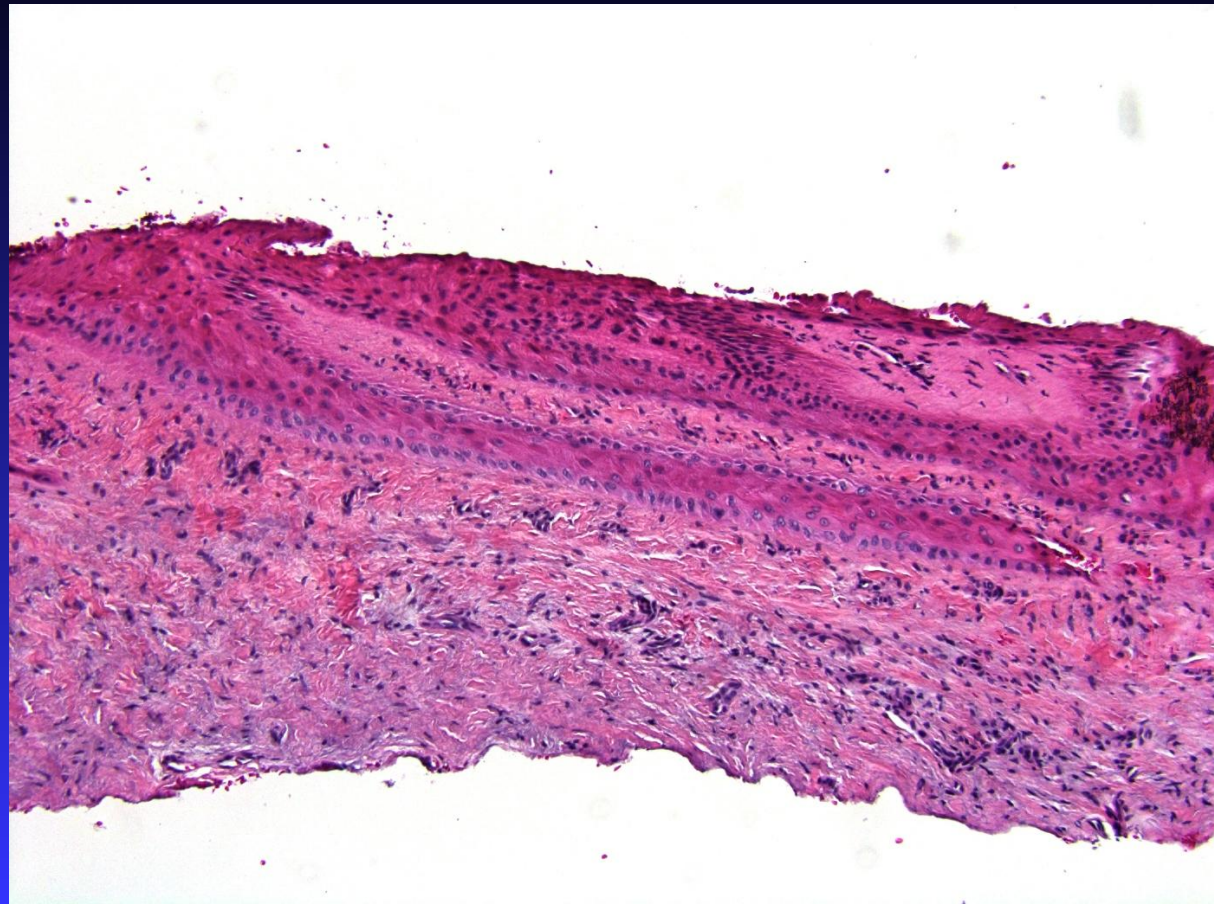
Onychopapilloma—Keratin Producing



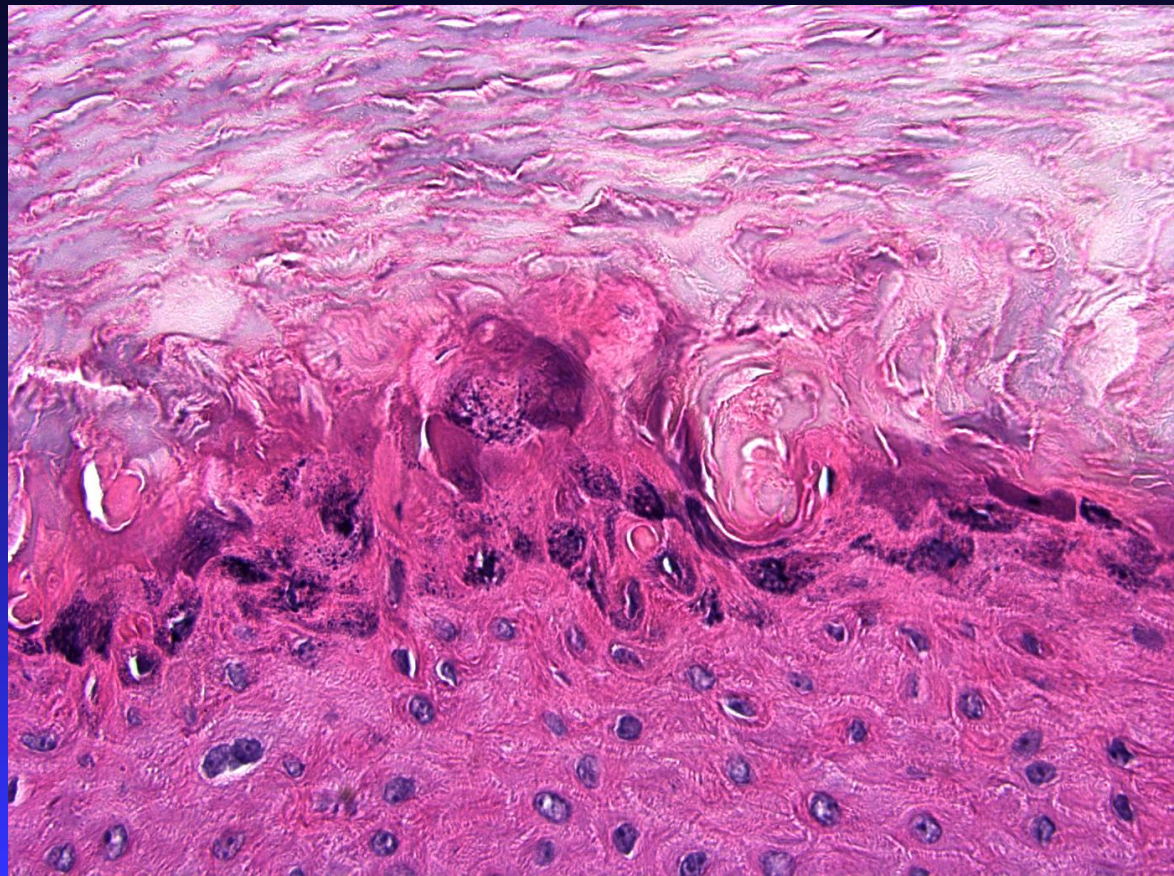
Onychopapilloma—Keratin Producing



Onychopapilloma



Onychopapilloma—Not a wart



Onycomatricoma

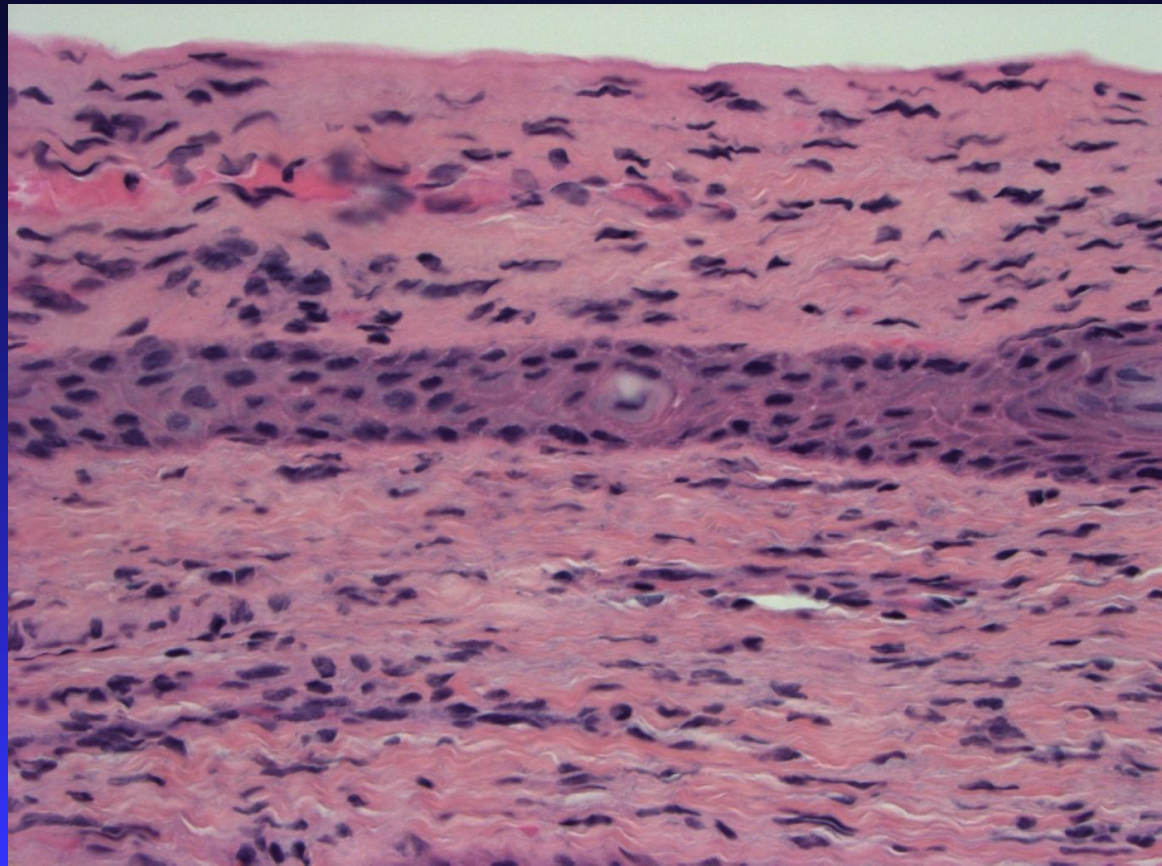
Onycomatricoma

- Examine nail for holes—Transverse sections of dystrophic nail

Onychomatricoma

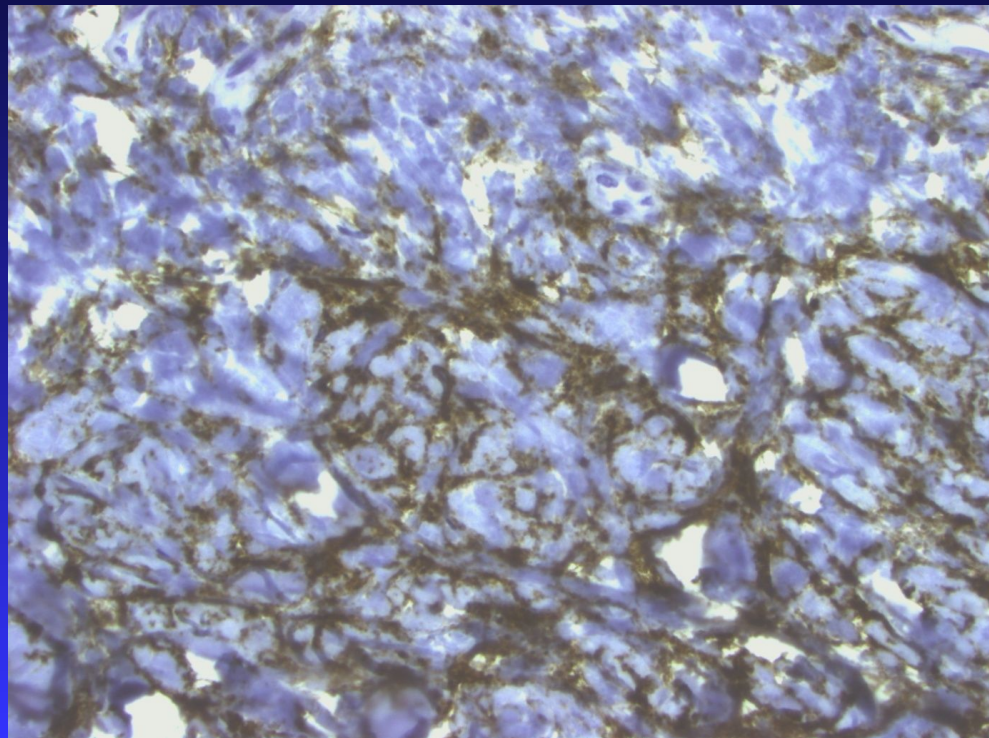
Epithelial and dermal components

Onychomatricoma



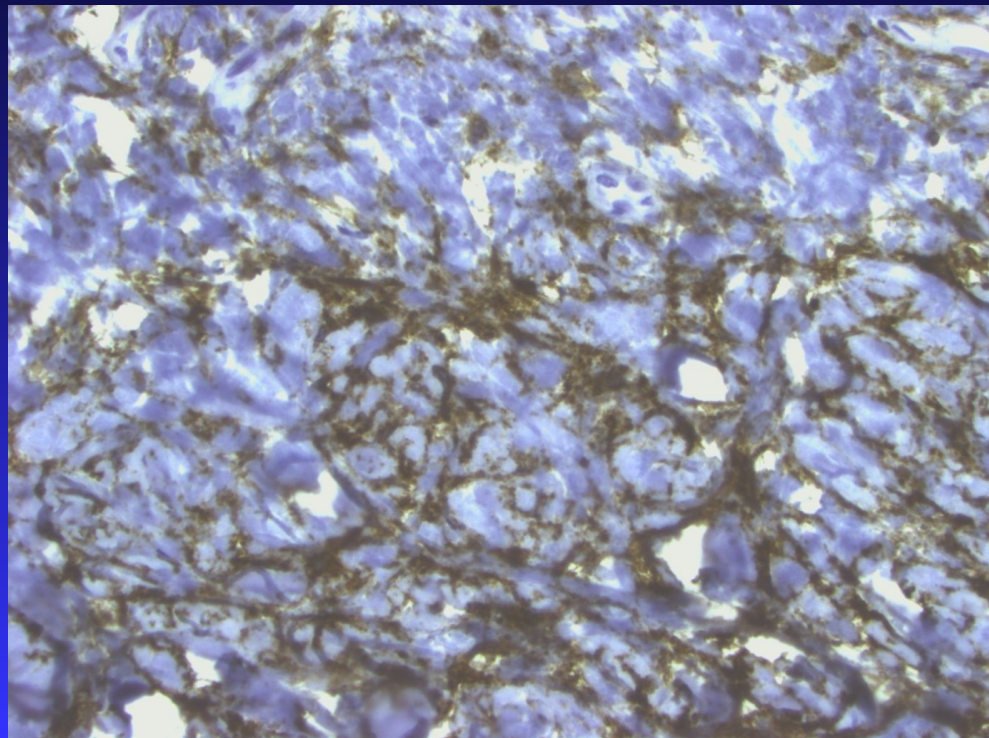
Onychomatricoma

- Onychodermis/onychofibroblasts
- CD10+



Onychomatricoma

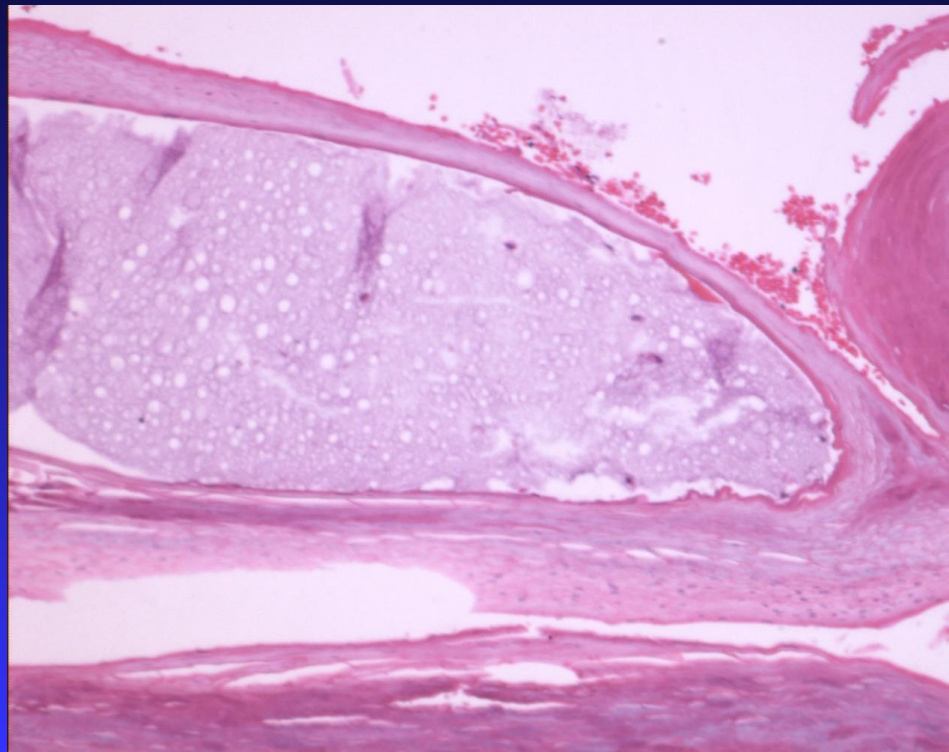
- Onychodermis/onychofibroblasts
- CD10+
- CD13+



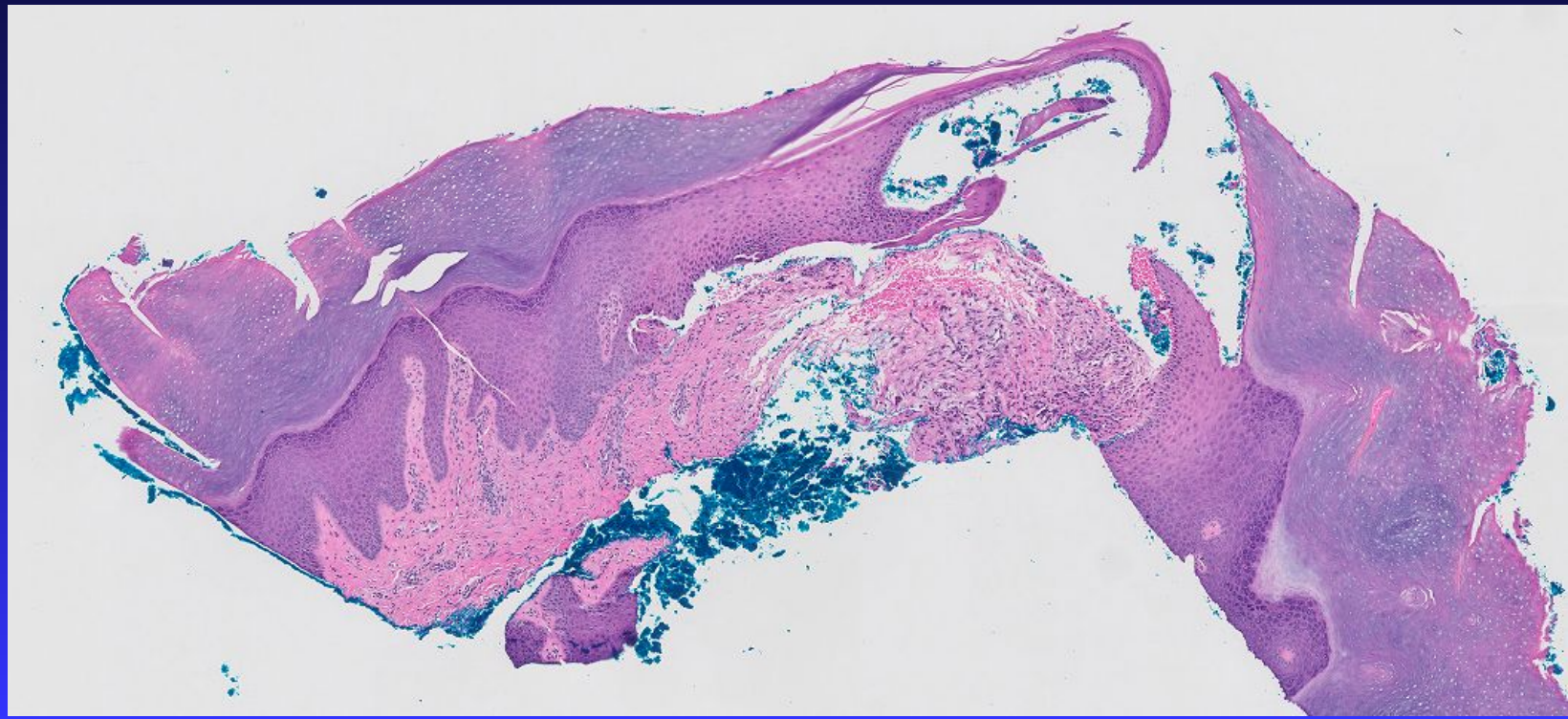
Digital Myxoid/Mucous Cyst

Digital Myxoid/Mucous Cyst

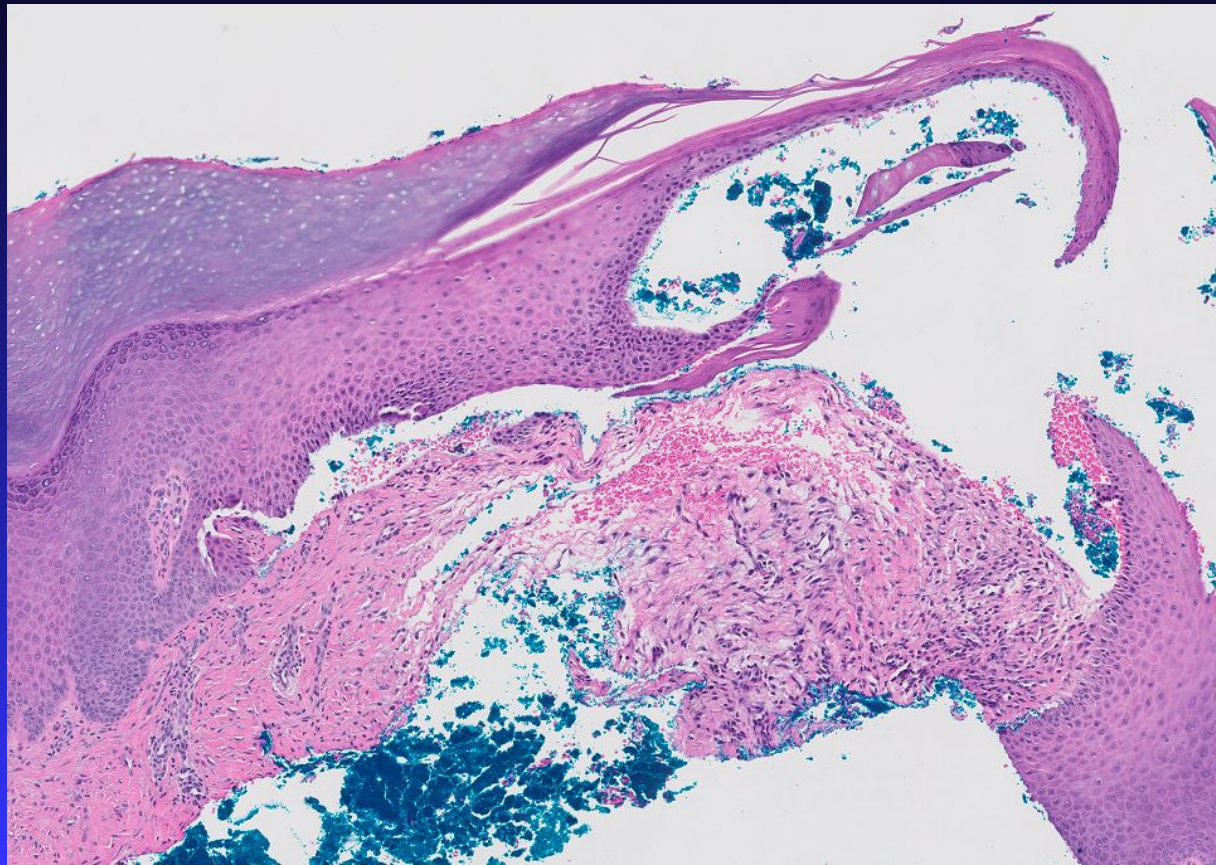
- Mucin may be anywhere



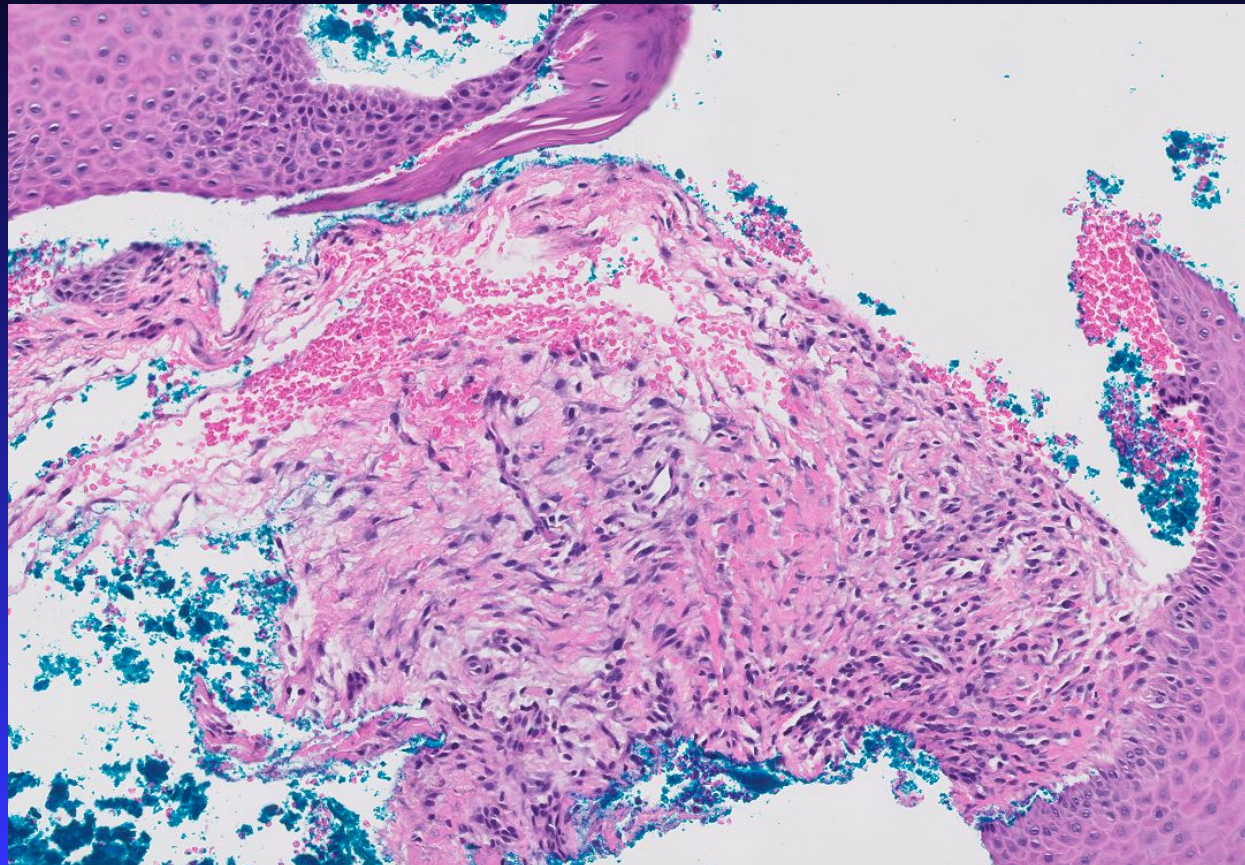
H&E may show only scar and reactive change



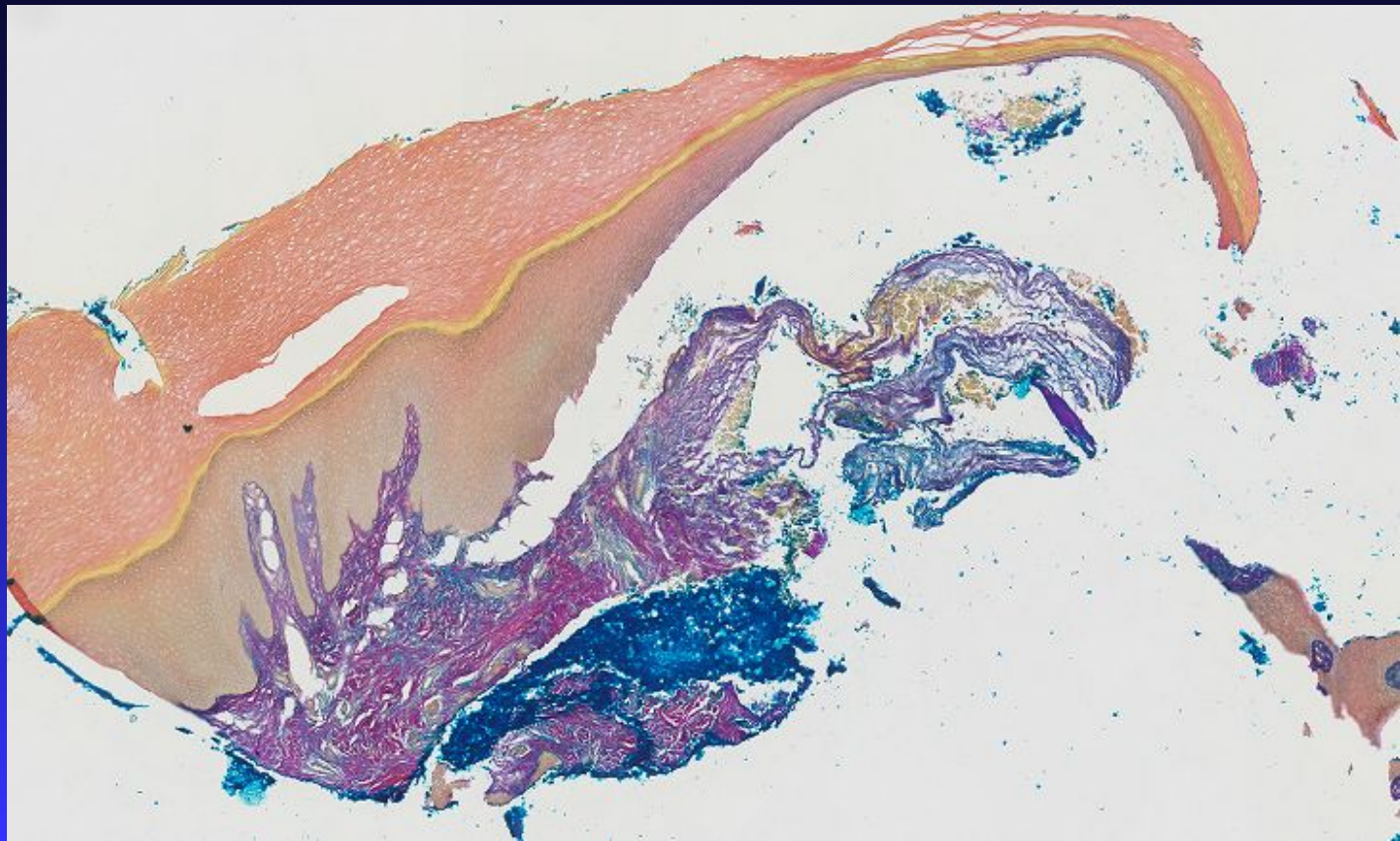
H&E may not show mucin



H&E may not show mucin



Mucin stain often required

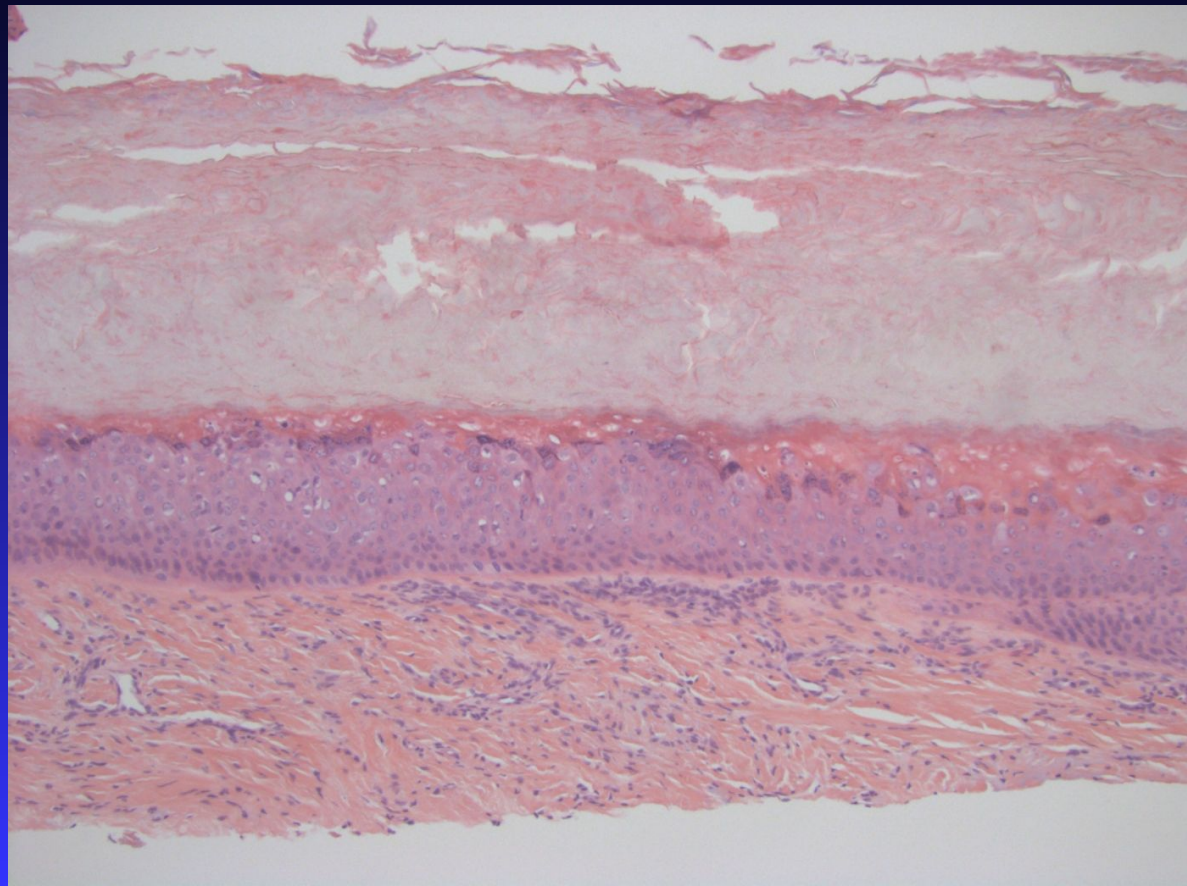


Think about the diagnosis when grossing

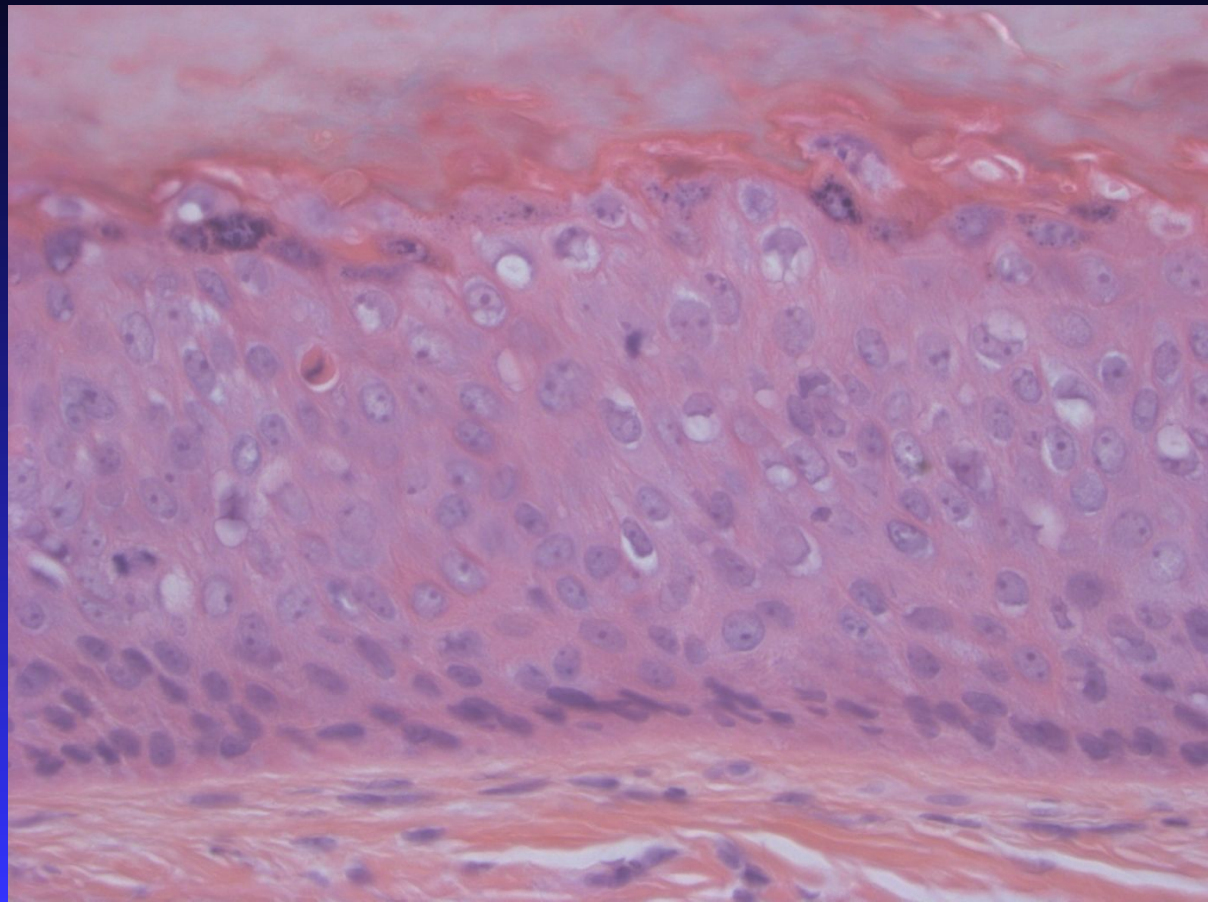
- Squamous cell carcinoma
  - Sampling
  - HPV-Verruca etiology

Squamous cell carcinoma

Squamous cell carcinoma

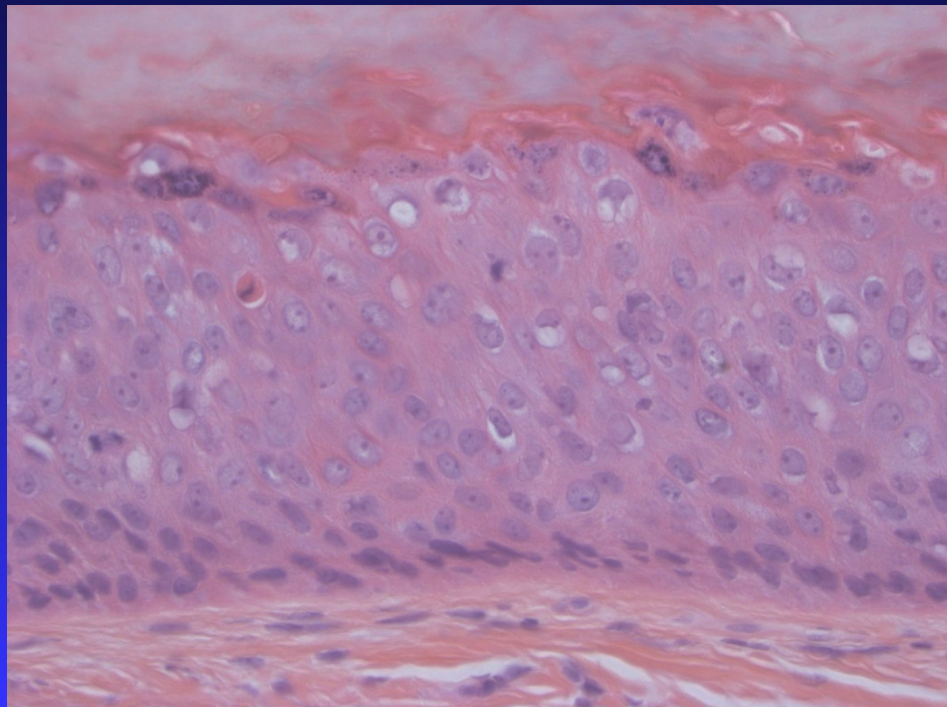


Squamous cell carcinoma in-situ

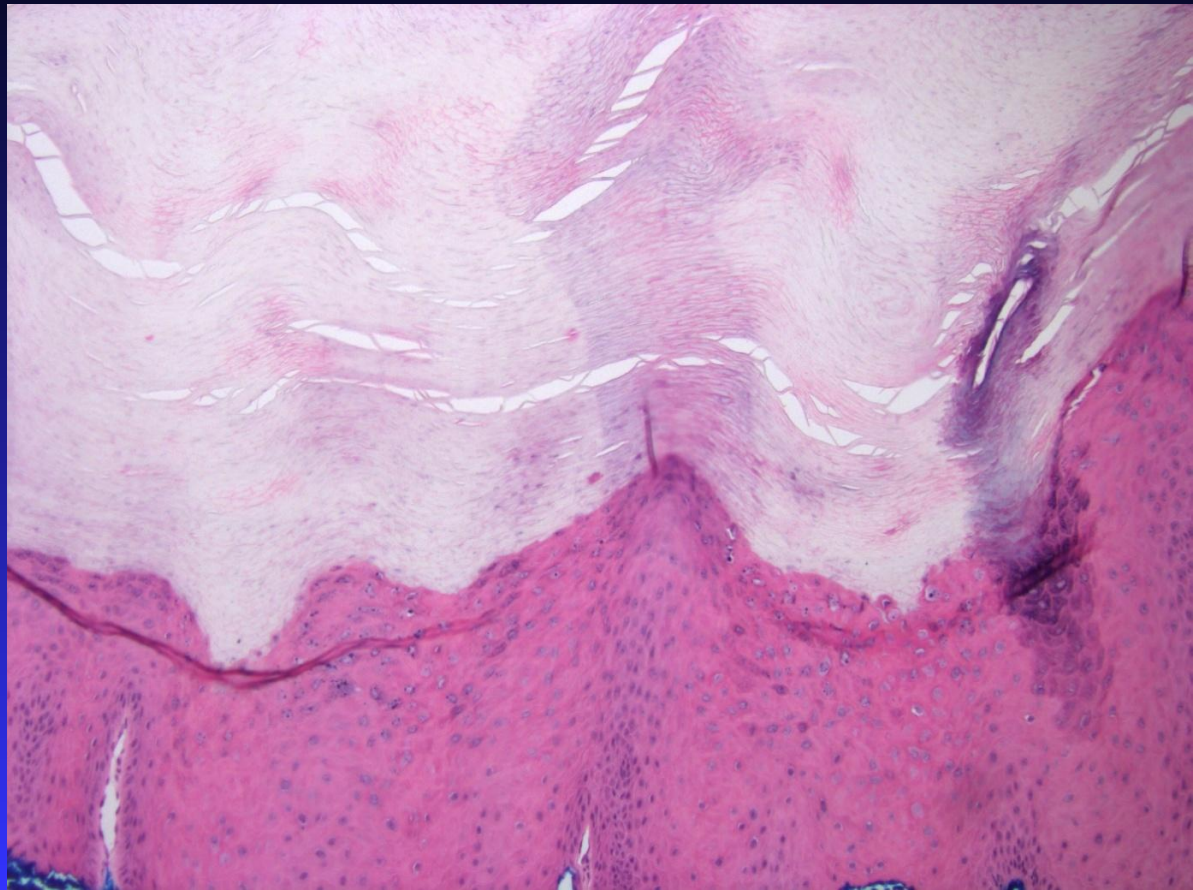


Squamous cell carcinoma in-situ

- Human Papillomavirus (HPV) features



SCC versus Wart/Verruca



SCC versus Wart/Verruca

- Clinical correlation often necessary
  - Immunosuppression (esp HIV)
  - If it is destroying bone, it is not benign!
  - Sample more if suspicious

HPV In-situ Hybridization (ISH)

- HPV Subtypes
  - Low risk--Verruca
  - High risk—Squamous cell carcinoma
  - Pan HPV test—Benign and malignant

HPV In-situ Hybridization (ISH)

- HPV Subtypes
  - Low risk--Verruca
  - High risk—Carcinoma
  - Pan HPV test—Does not contain all subtypes

HPV In-situ Hybridization (ISH)

Comment

Although in an ideal world we would always expect a positive pan-HPV in situ hybridization stain when we see a positive Low-risk HPV stain, we have observed this situation (i.e., a negative pan HPV stain in the face of a positive Low-risk HPV stain) on several occasions in our laboratory. The label "Pan-HPV" is a bit misleading, because although it detects multiple Low-risk and High-risk HPV subtypes, it clearly does not detect every possible type of HPV. Additionally, our Low-risk HPV probe detects HPV type 44, which is an HPV type that is not detected in the Pan-HPV probe that we have available to us. As such, infection with Low-risk HPV type 44 would readily explain the findings in this case.

The HPV in situ hybridization stains were performed using the RNAscope method, which targets HPV-associated RNA in the nucleus and cytoplasm of the target cells. This method has vastly superior sensitivity to the previous methods used at ProPath (which were based on detection of HPV DNA). Unlike DNA-based methods, RNAscope signals can be observed in both the nucleus and cytoplasm. The HPV RNAscope probes used at ProPath are directed at the following HPV types:

Pan-HPV RNAscope probe: Detects HPV types 6, 11, 16, [illegible], 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
Low-risk HPV RNAscope probe: Detects HPV types 6, 11, [illegible], 42, 43, 44
High / Intermediate risk HPV RNAscope probe: Detects HPV types [illegible], 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68

Thank you for allowing us to study this case.

REFERENCES:

Schache AG, Liloglou T, Jones TM et al: Validation of a novel diagnostic standard in HPV-positive oropharyngeal

HPV In-situ Hybridization (ISH)

- HPV Subtypes
  - Low risk--Verruca
  - High risk—Carcinoma
  - Pan HPV test—Do not use since does not contain all of the subtypes

Think about the diagnosis when grossing

- Longintudinal melanonychia
  - Identify source of clinical pigmentation

Histopathology of benign activation

- Epithelial pigmentation
- Melanophages
- No or only a slight increase in junctional melanocyte density

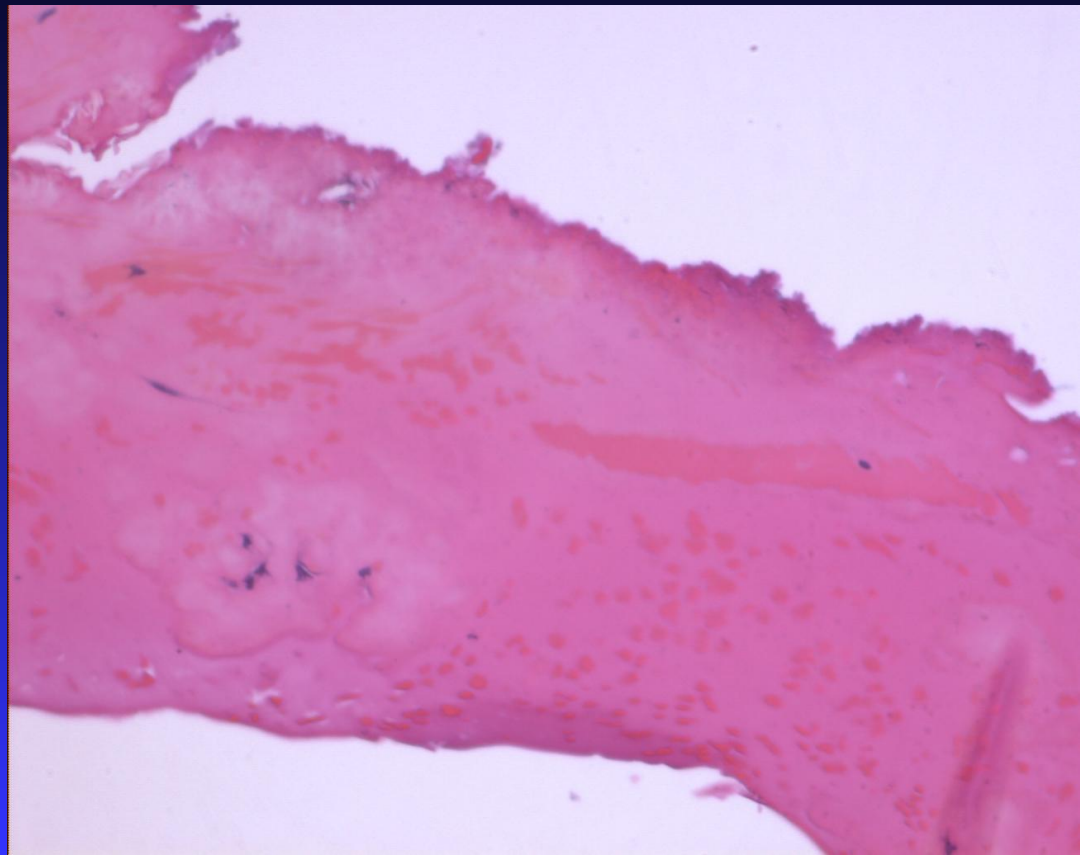
Features of melanotic macule can be subtle.

- H&E with initial levels
- MelanA IHC
- Fontana-Masson
- PAS fungus
- Unstained slides

H&E for melanonychia

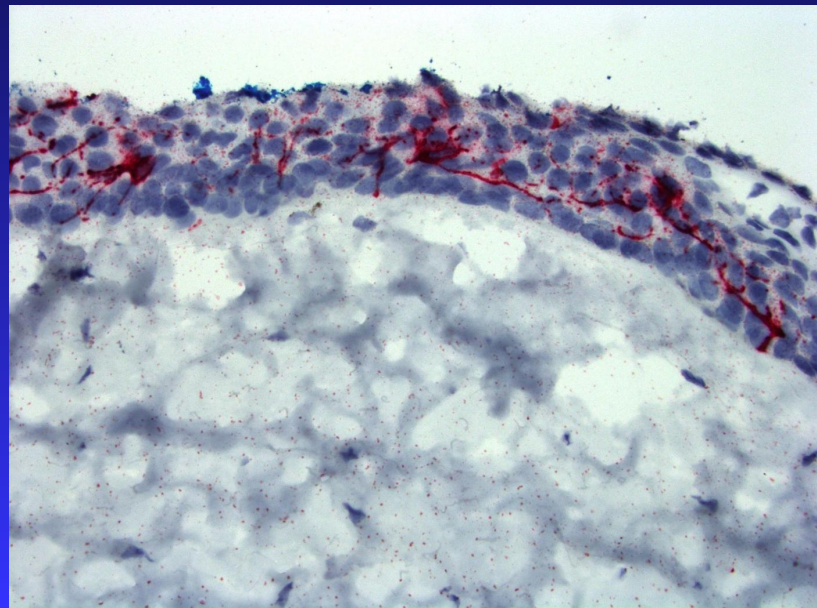
- H&E level sections
  - Blood
  - Exogenous material
  - Medication deposition

Blood in nail plate

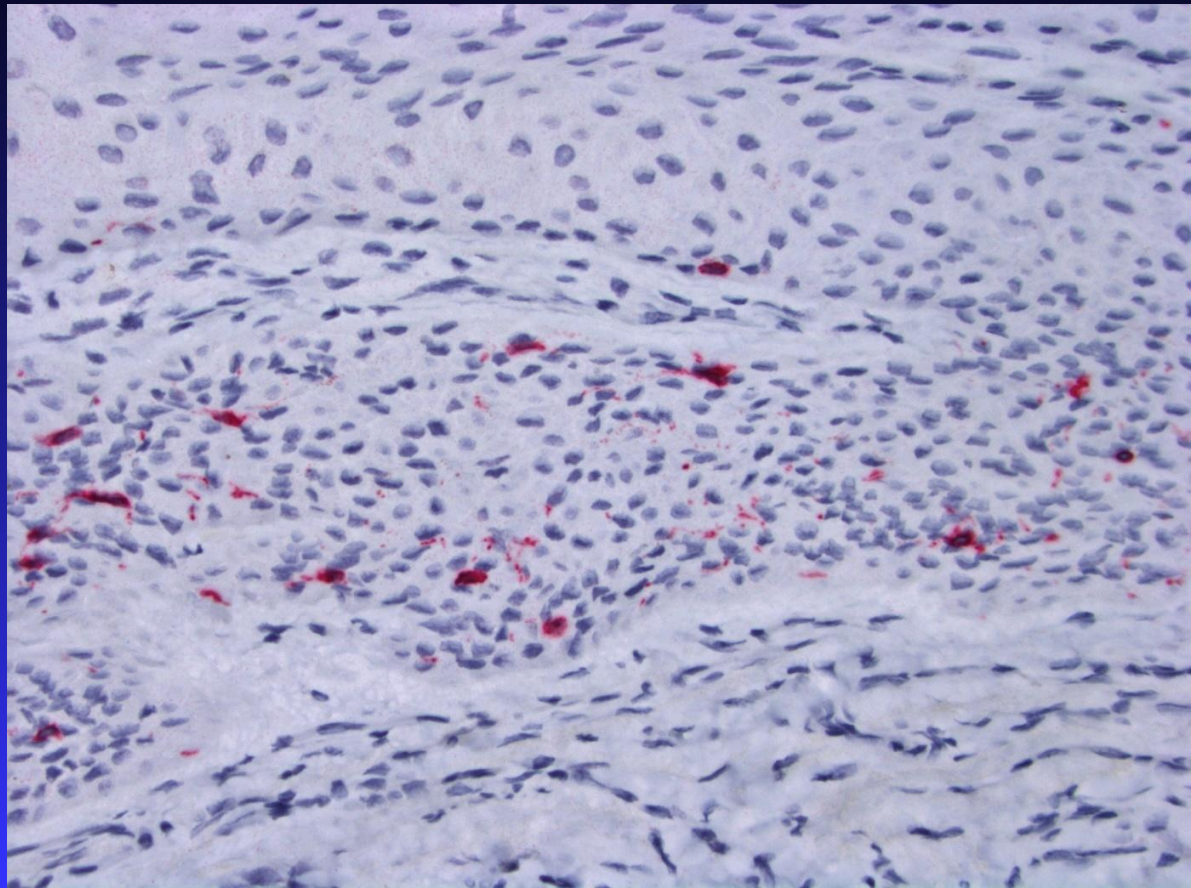


MelanA/Mart1 for melanonychia

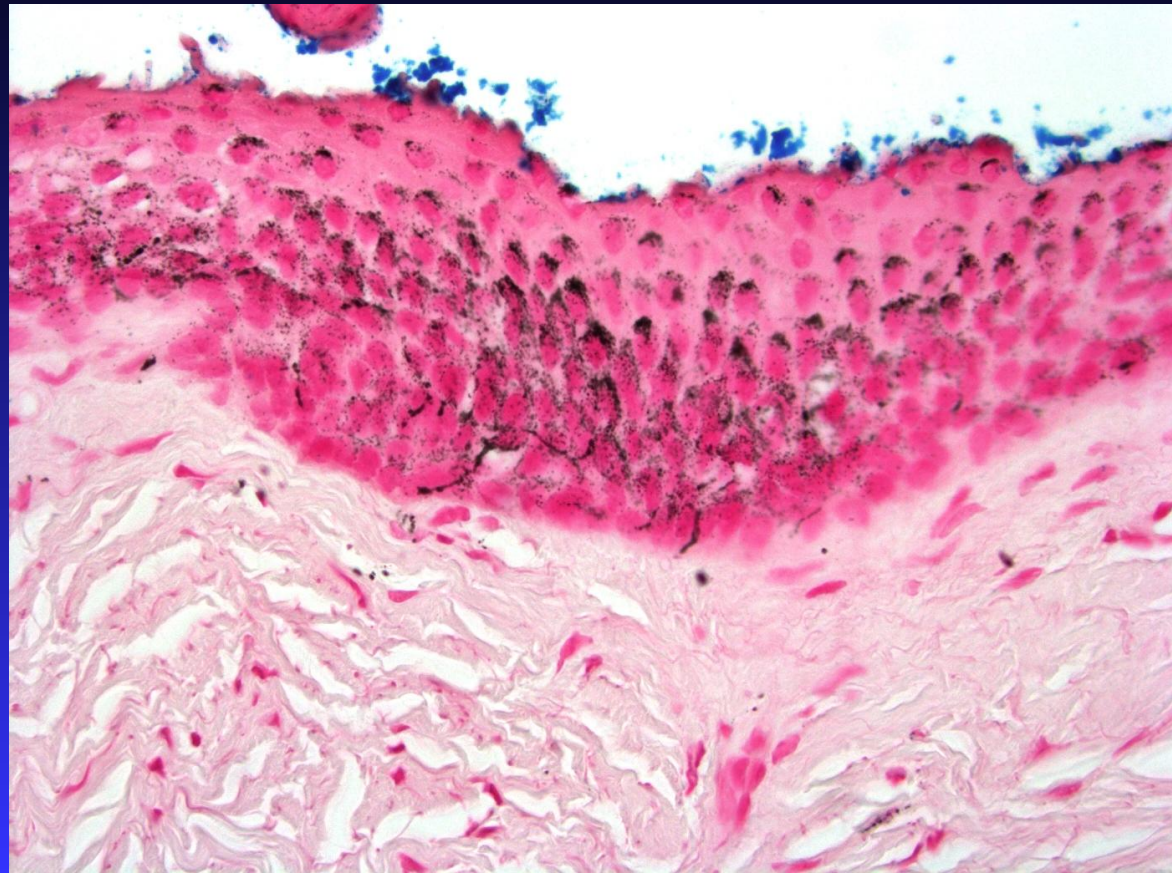
- Melanocytes density may vary highly, especially in melanoma in-situ
- Use a red chromogen



Variable density of melanocytes

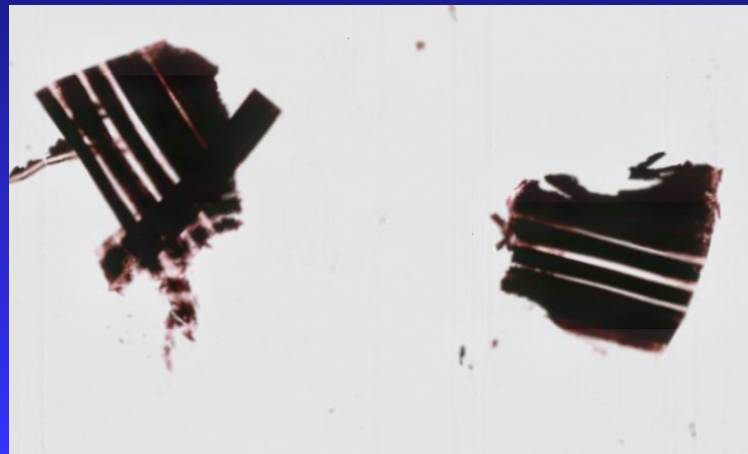


Fontana-Masson for melanonychia

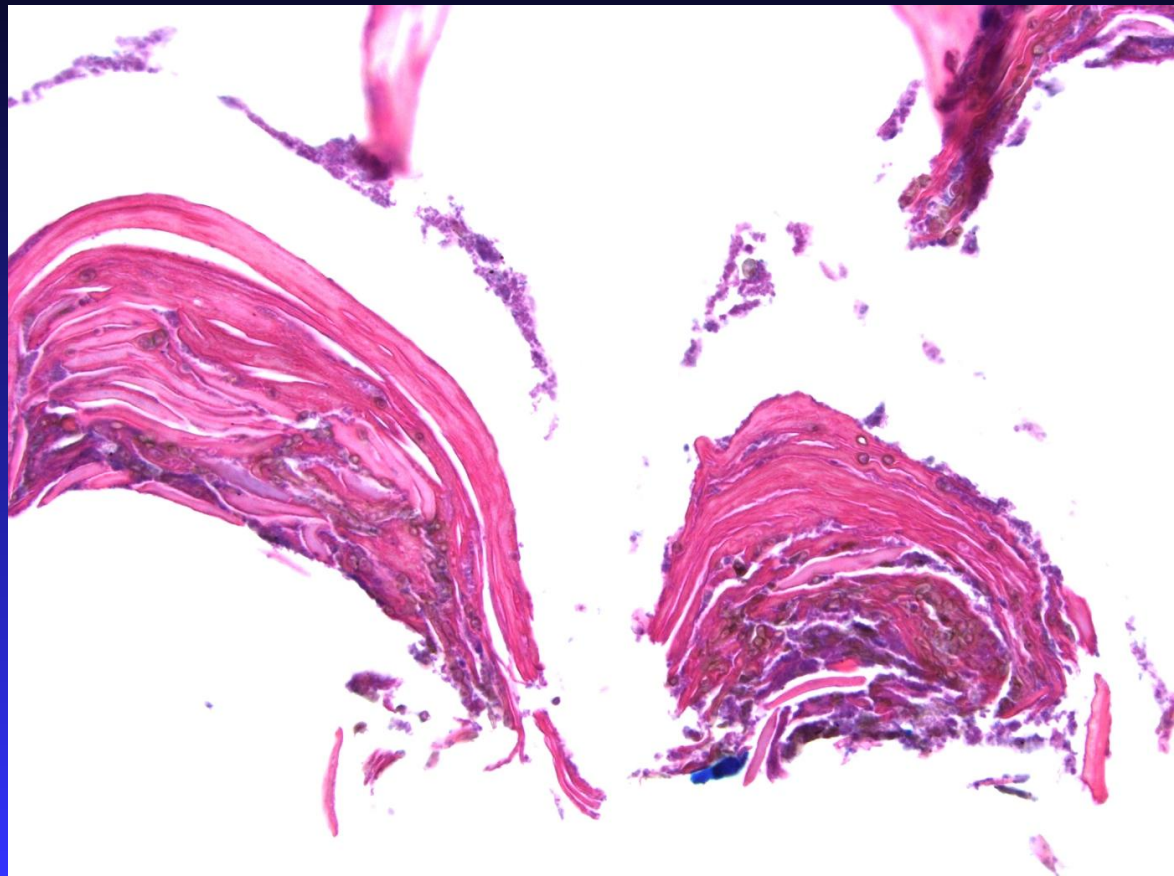


Special stains for pigment do not work in nail plate

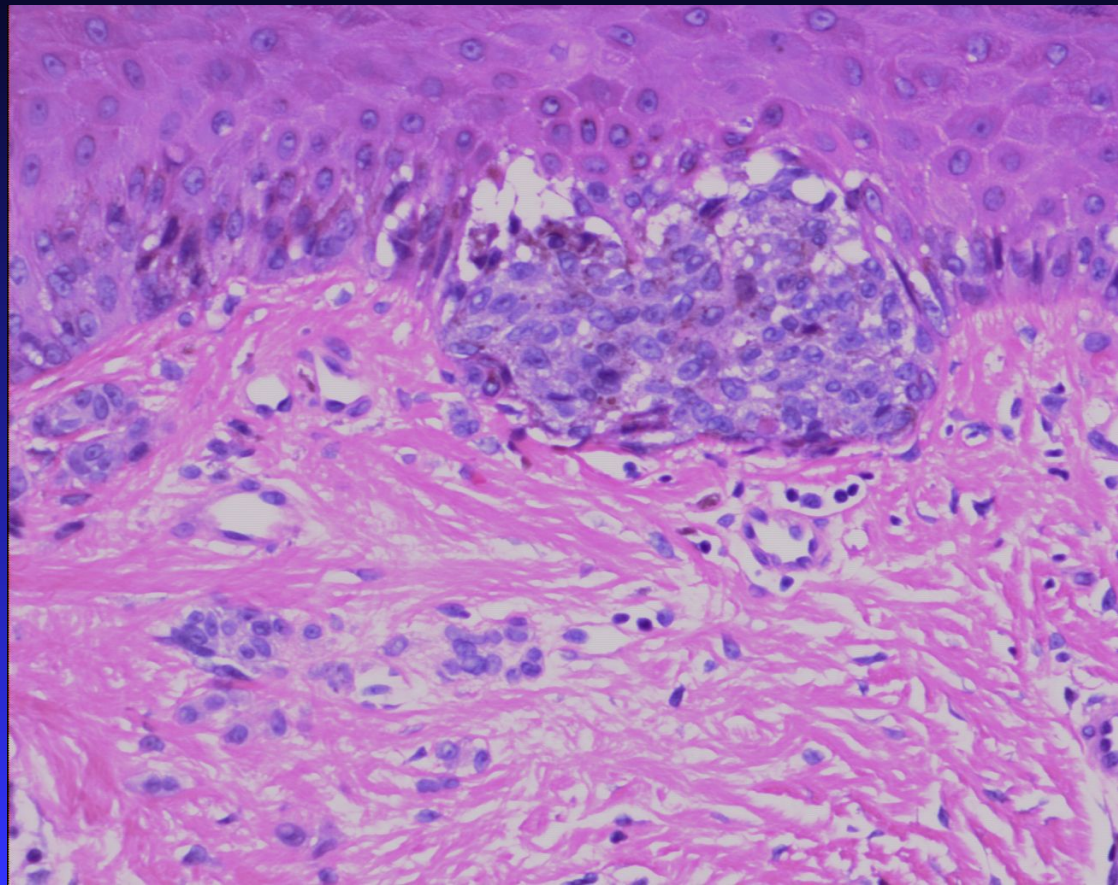
- Perl's iron—Fe^{2+} still in heme
- Fontana-Masson—overstains plate—must be diluted



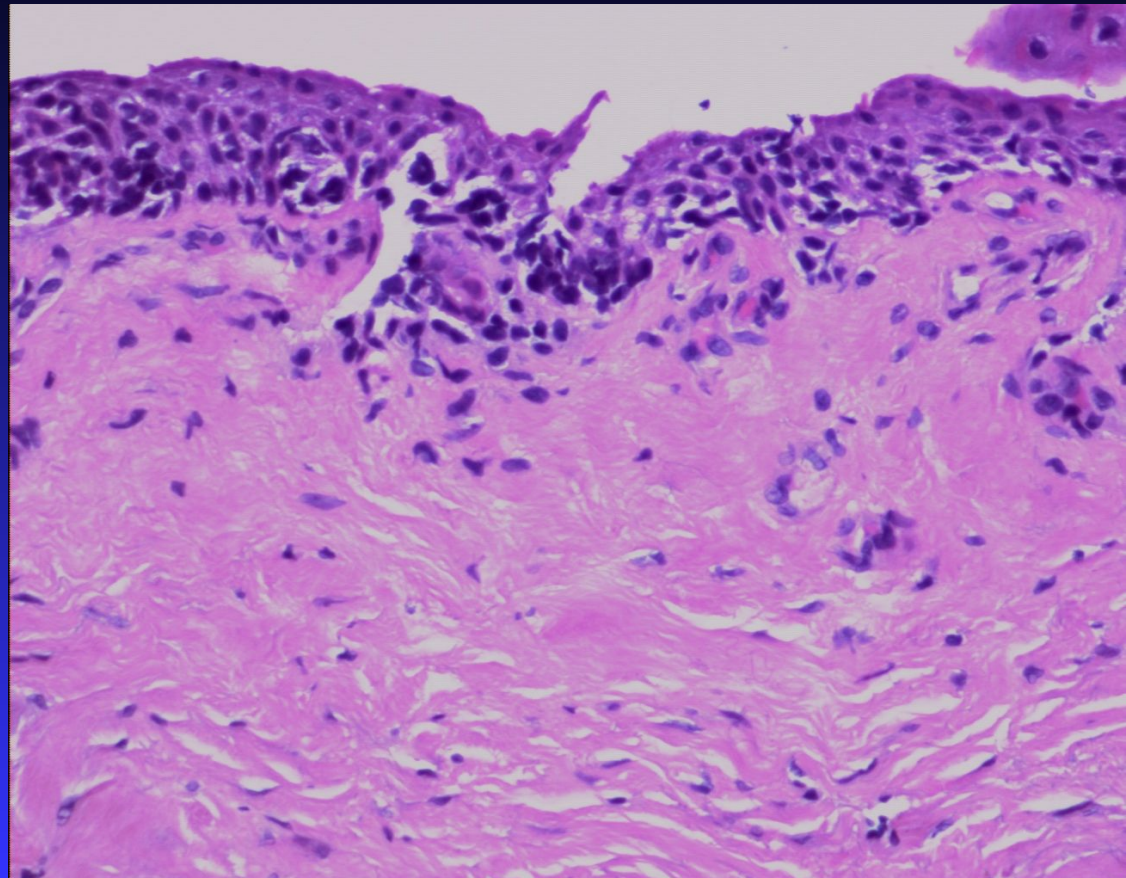
Pigmented fungus



Benign melanocytic nevus

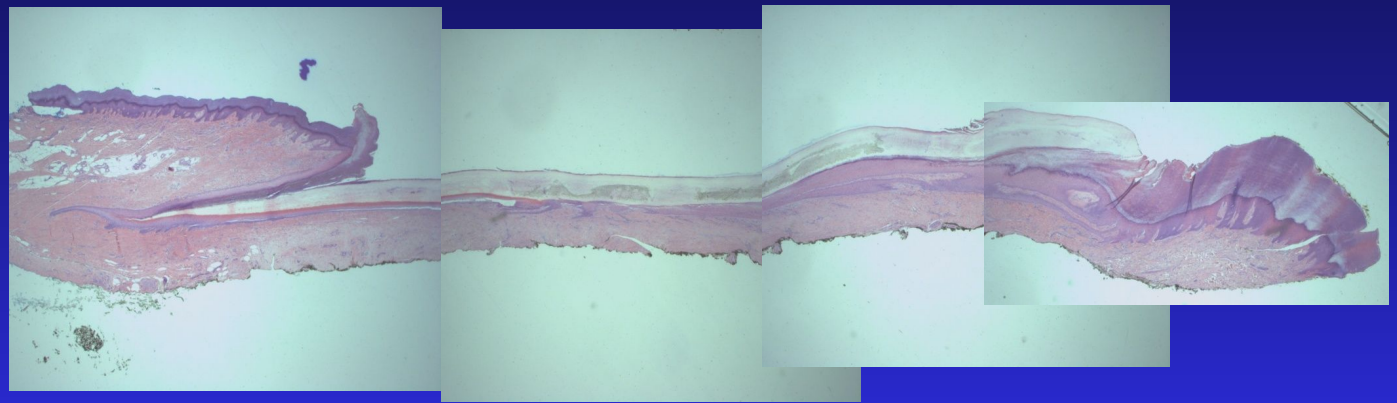


Melanoma in-situ



Hutchinson's Sign

Hutchinson's Sign



Hutchinson's Sign

- J Am Acad Dermatol. 2001 Feb;44(2):305-7.
- **Two kinds of Hutchinson's sign, benign and malignant.**
- Kawabata Y, Ohara K, Hino H, Tamaki K.
- Department of Dermatology, Faculty of Medicine, University of Tokyo, Japan. KAWABATA-der@h.u-tokyo.ac.jp
- We examined 6 subungual melanomas in situ and 18 melanocytic nevi and compared pigmentation of the nail plates and hyponychium with the use of a dermatoscope. Hutchinson's sign on the hyponychium was not always evidence of subungual melanoma because it can be seen in both diseases. However, there was a wide difference in their dermatoscopic features. We believe that observation of pigmentation on the hyponychium with the use of a dermatoscope contributes to the precise diagnosis of subungual melanoma.

Dr. Rich's Differential Diagnosis

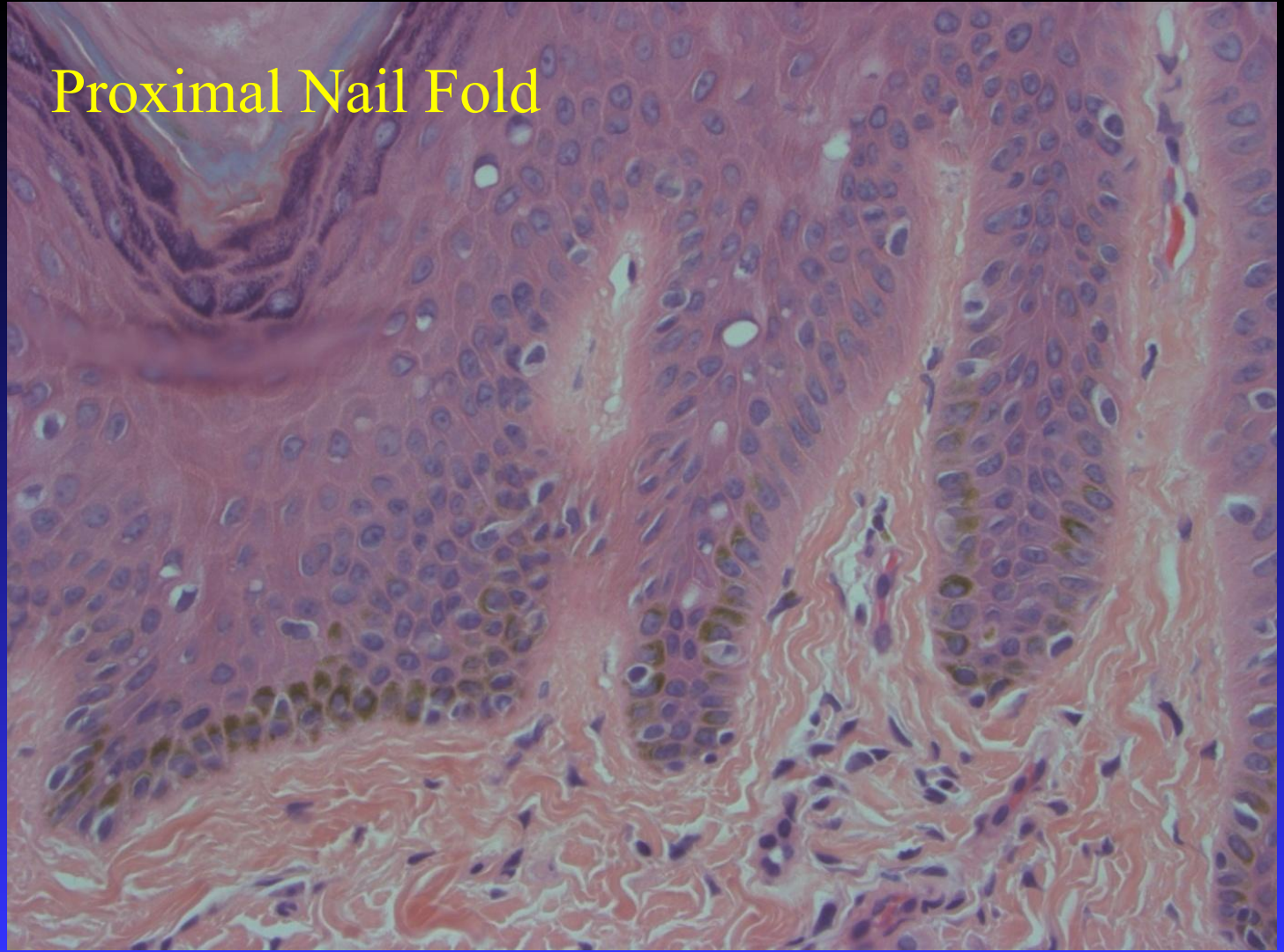
- Trauma pigment
- Nevus
- Lentigo
- R/O Melanoma

Biopsy

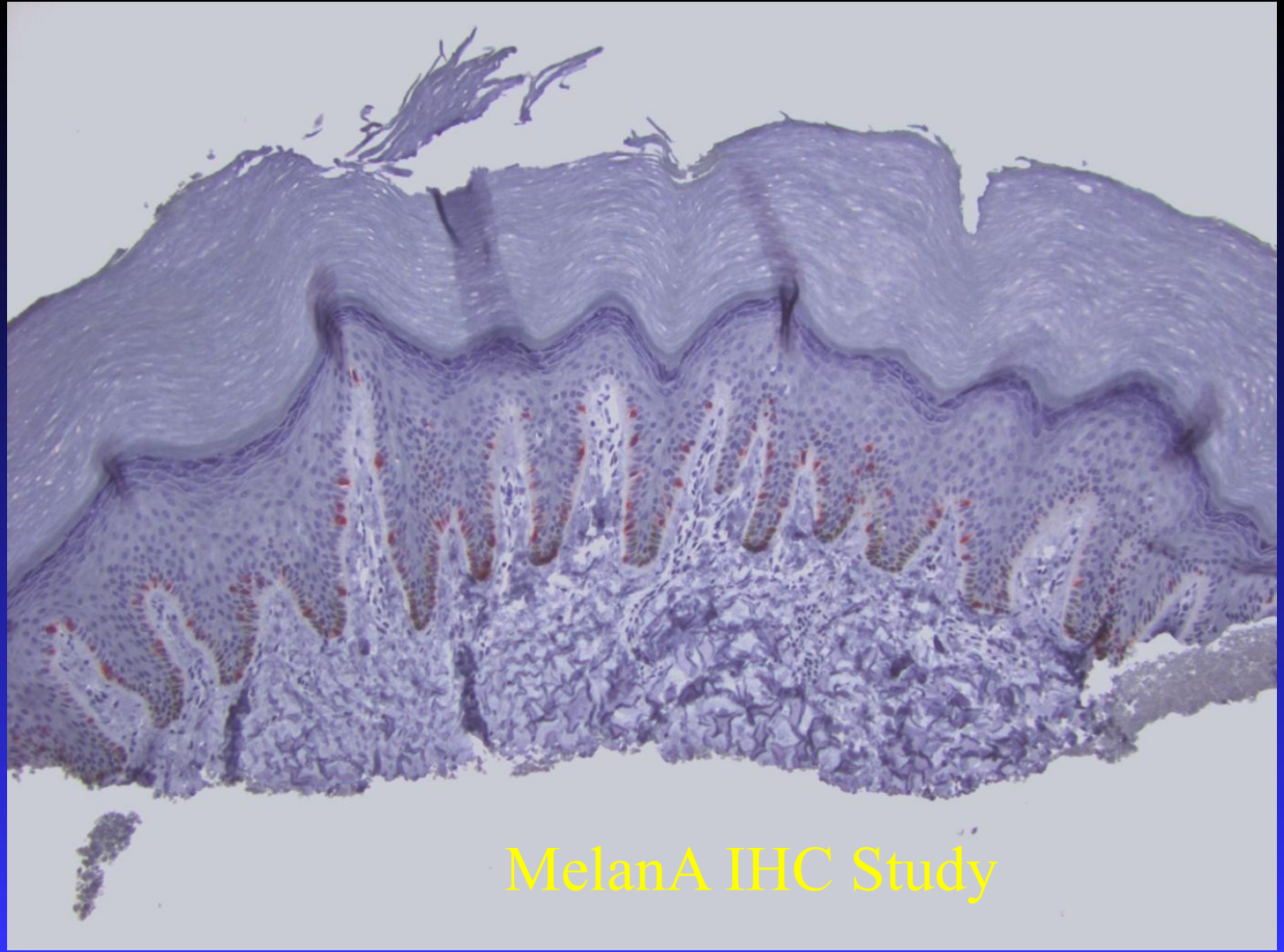
- Nail plate reflected and matrix sampled
- Proximal nail fold sampled

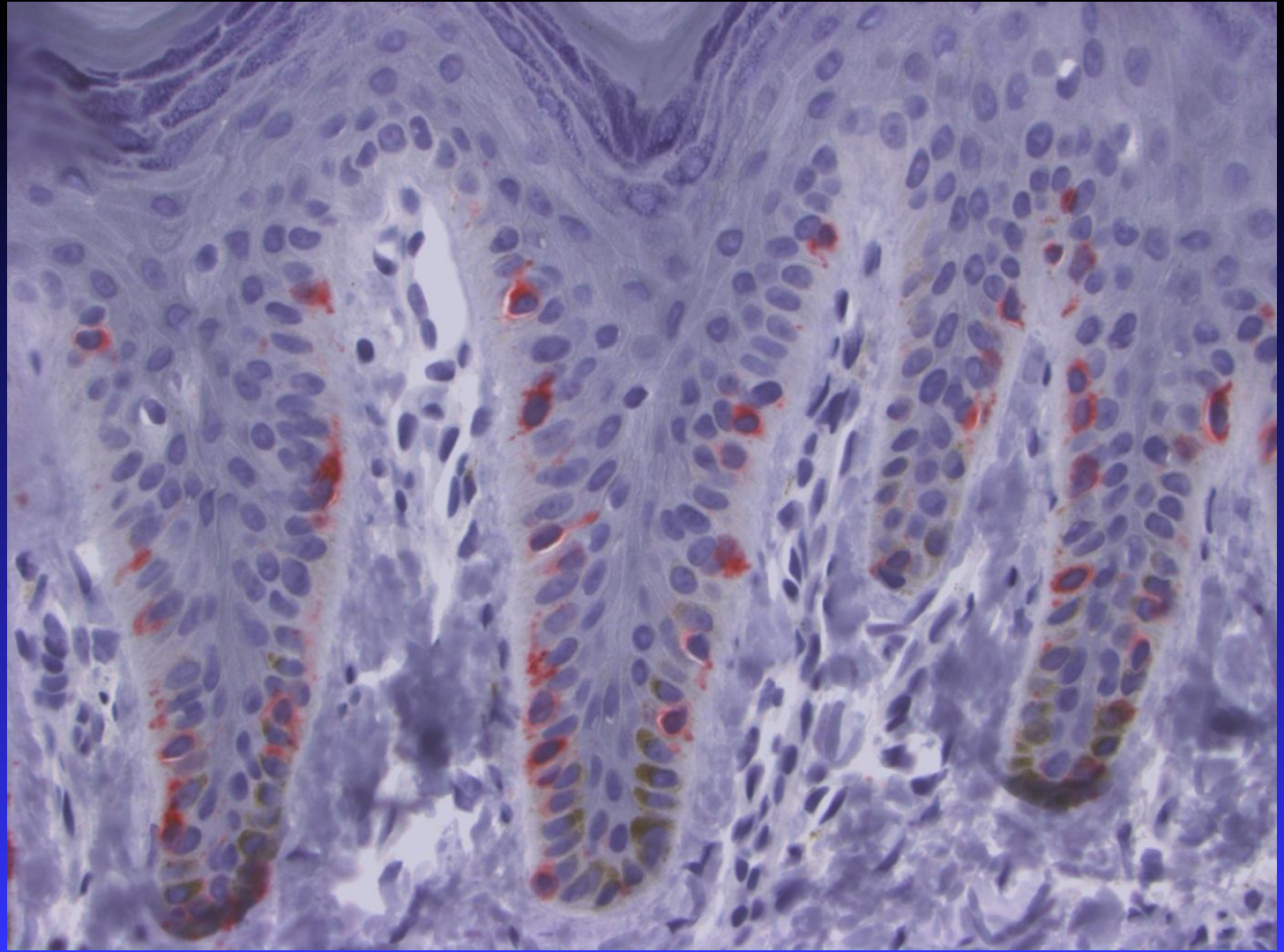
Proximal Nail Fold

Proximal Nail Fold

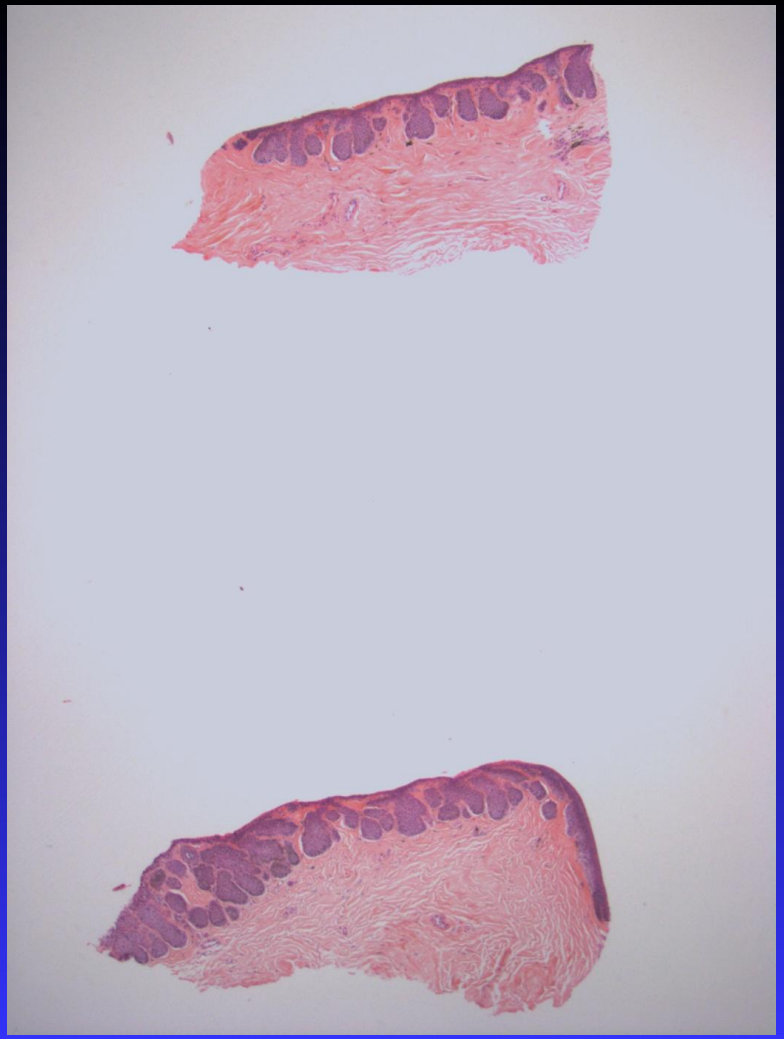



Fontana-Masson Stain


MelanA IHC Study

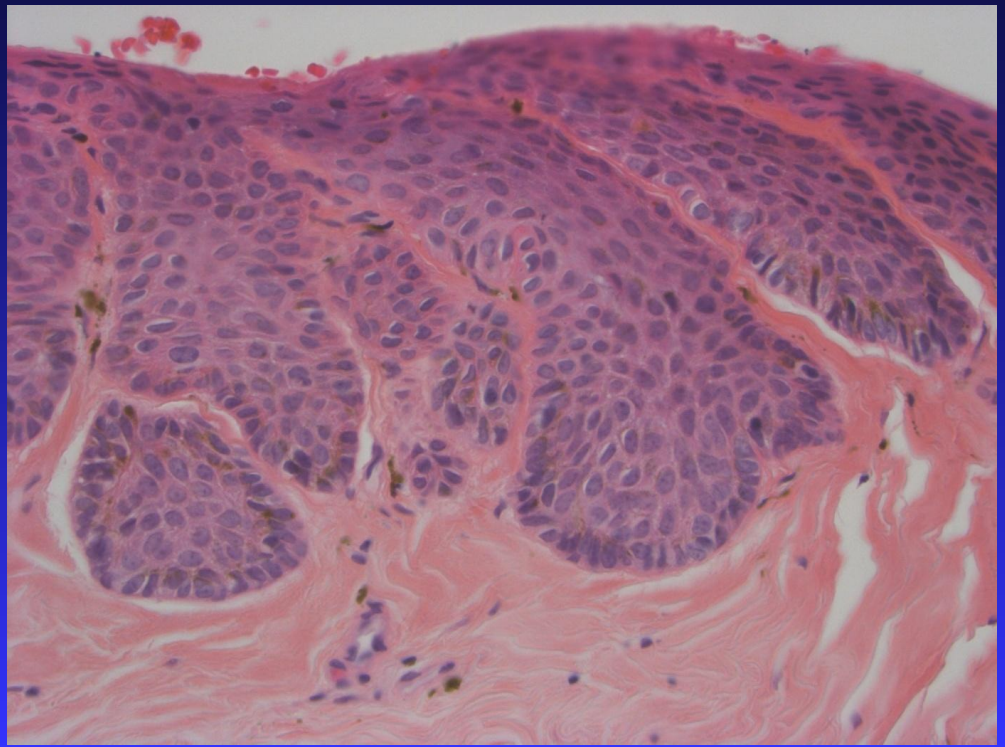


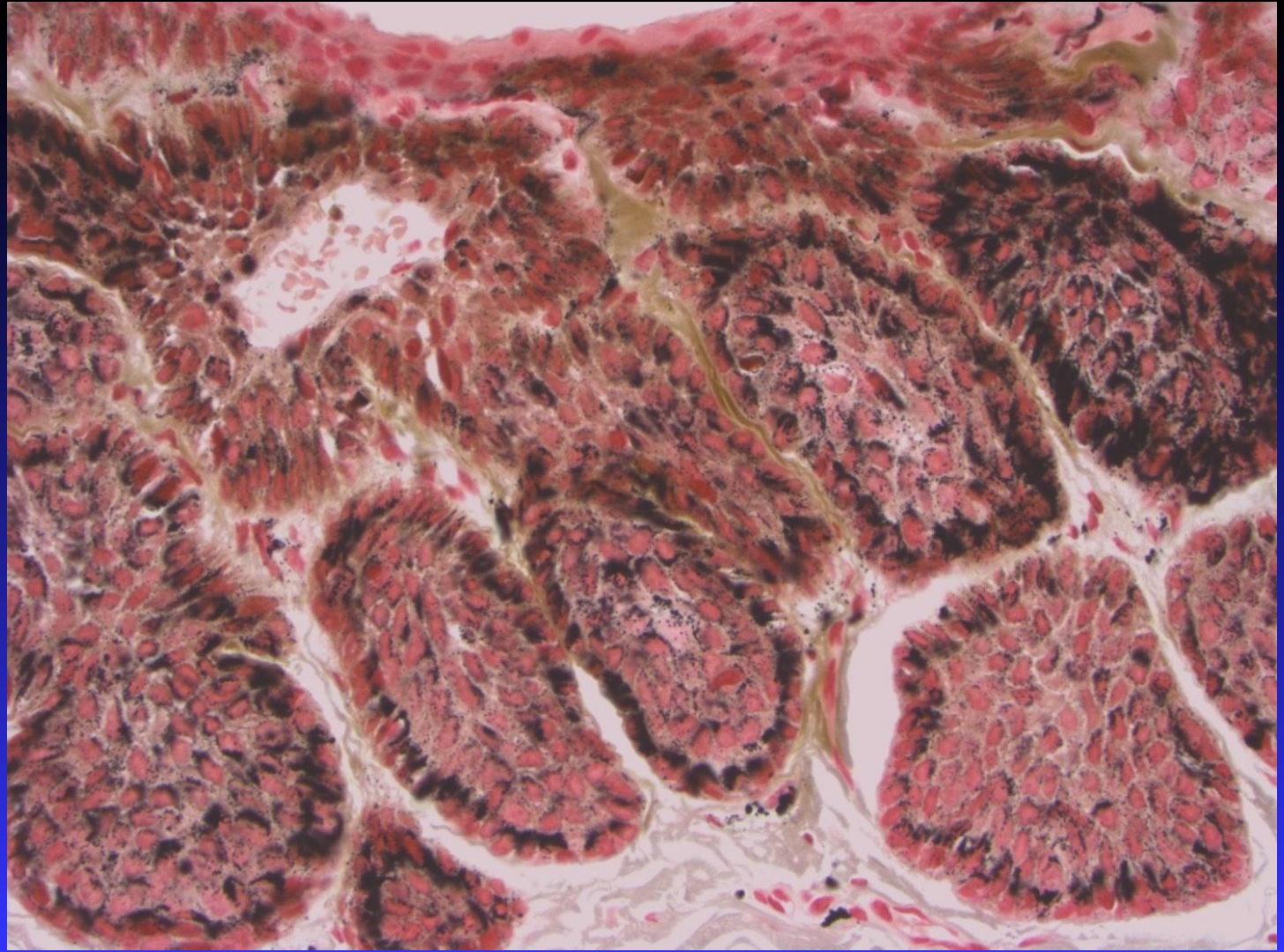
Nail Matrix

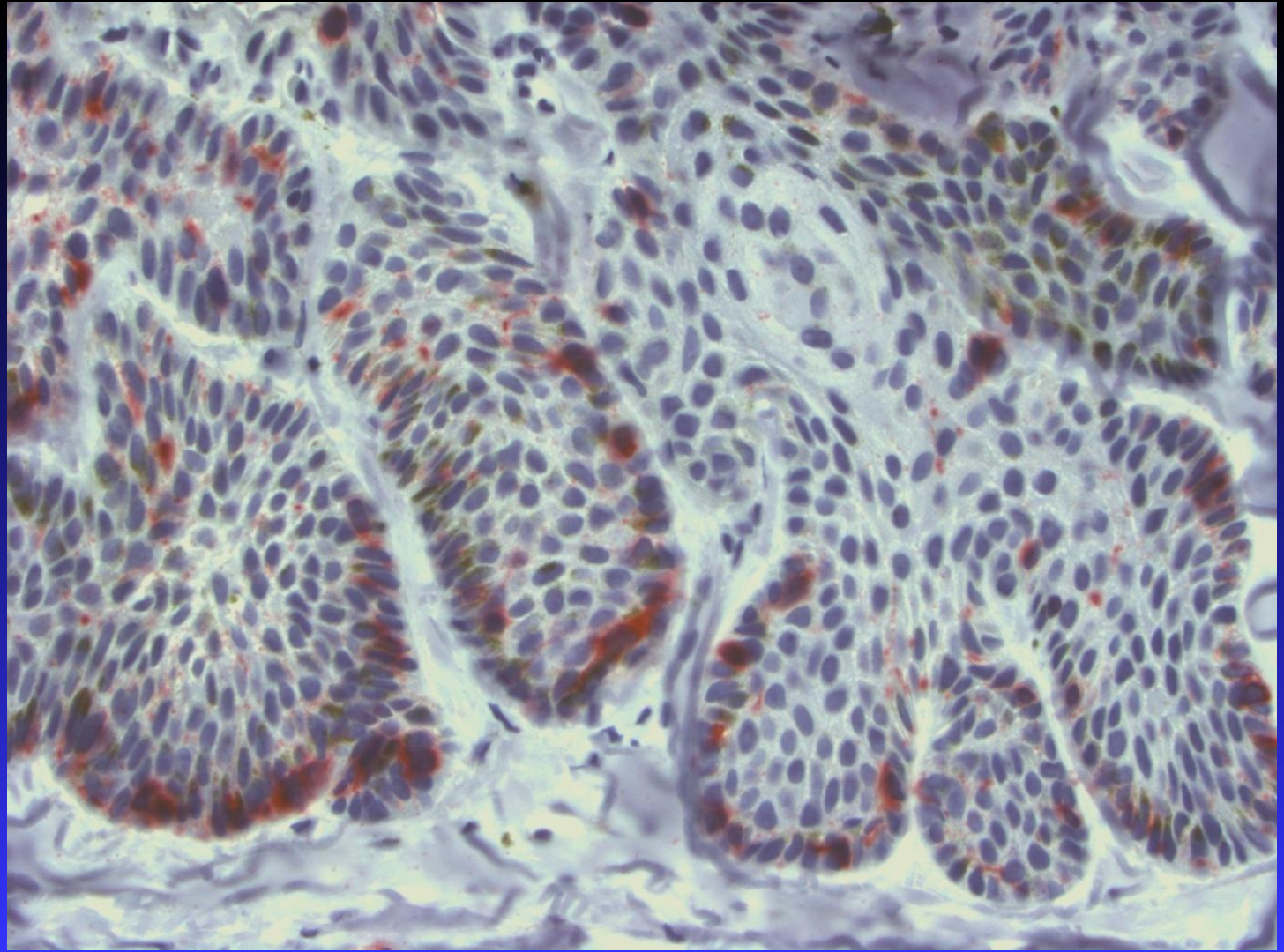


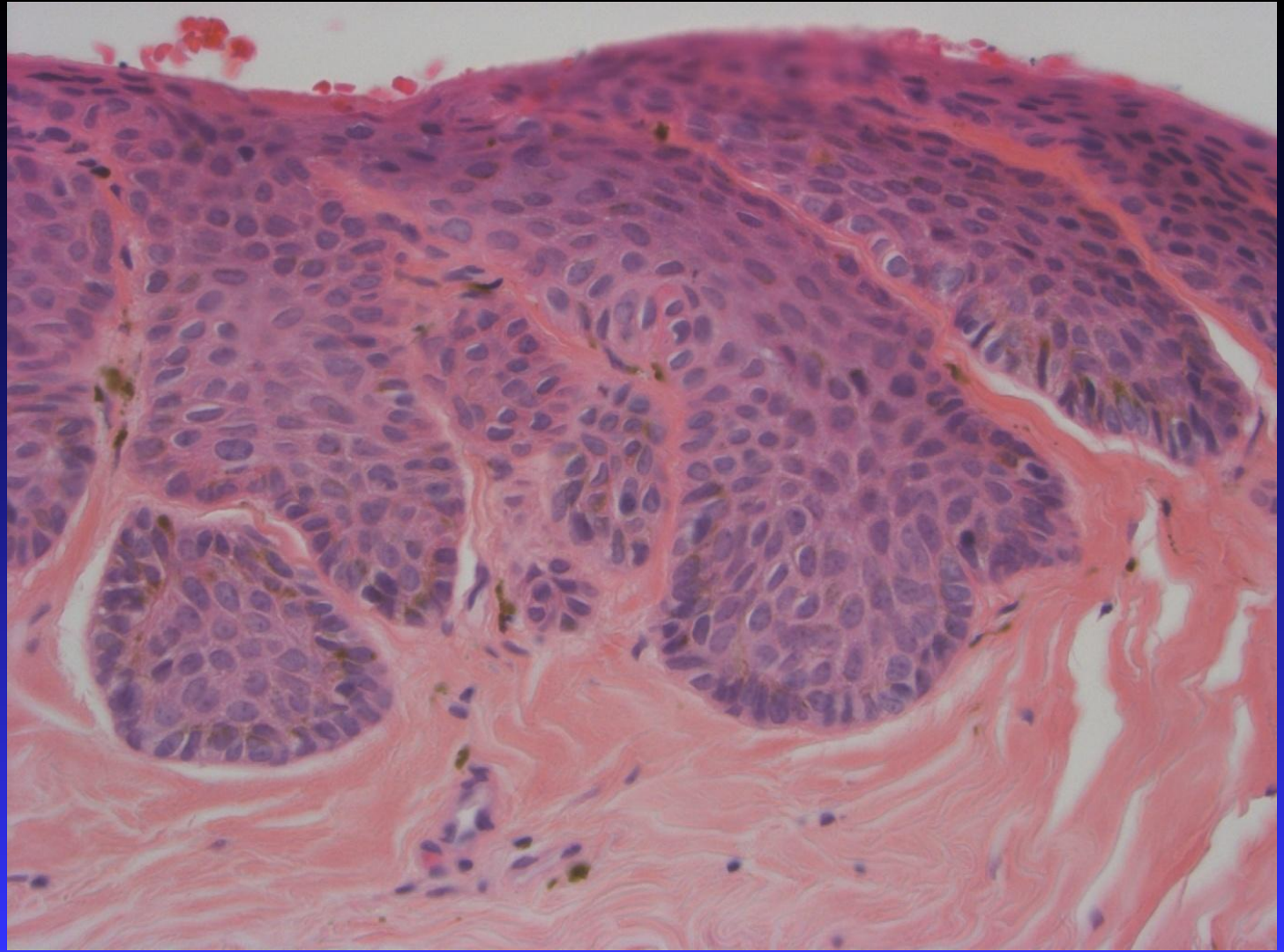

Nail Matrix

Onychocytic matricoma with a Hutchinson's sign









Onychocytic Matricoma

Am J Dermatopathol. 2012 Feb;34(1):54-9. doi: 10.1097/DAD.0b013e31822c3d8b.

Onychocytic matricoma presenting as pachymelanonychia longitudinal. A new entity (report of five cases).

Perrin C[1], Cannata GE, Bossard C, Grill JM, Ambrossetti D, Michiels JF.

Author information

Abstract

Among the tumors of the epidermal appendages, only rare tumors have been proved as differentiating in the direction of the nail. Beside onychomatricoma, we report a new matrical tumor of the nail: onychocytic matricoma (acanthoma of the nail matrix producing onychocytes). The main differential diagnosis of onychocytic matricoma is seborrheic keratosis. However, if attention is paid to the nature of the different layers of the tumor and the peculiar microanatomy of the nail matrix, the differentiation is not difficult. Onychocytic matricoma is a localized (monodactylous) longitudinal melanonychia which is slightly raised. The term pachymelanonychia is used to define the 2 clinical features of the tumor. Pachyonychia indicate a localized thickening of the nail plate, and melanonychia indicate its longitudinal pigmented band. Onychocytic matricoma is composed of a basal compartment with a varying admixture of prekeratogenous cells and keratogenous cells. Endokeratinization originating in the deep portion of the tumor and nests of prekeratogenous and keratogenous cells in concentric arrangement are a characteristic feature. Three major patterns can be identified as follows: acanthotic, papillomatous, keratogenous type with retarded maturation. Given the peculiar thickening of the nail plate observed both in pigmented onychomatricoma and onychocytic matricoma, the term pachymelanonychia longitudinal could be proposed to specify clinically these 2 lesions, which the clinician sometimes mistakes for melanoma.

Onychocytic Matricoma

Observation | March 2014

Onychocytic Matricoma: A New, Important Nail-Unit Tumor Mistaken for a Foreign Body FREE

Karolyn A. Wanat, MD[1]; Erika Reid, MD[1]; Adam I. Rubin, MD[1]

[1]Department of Dermatology at the Hospital of the University of Pennsylvania, Philadelphia

JAMA Dermatol. 2014;150(3):335-337. doi:10.1001/jamadermatol.2013.6358.

Onychocytic matricoma (OCM) is a benign acanthoma of the nail unit that presents with localized thickening of the nail plate and melanonychia.[1] This newly described entity has suggestive clinical features and distinctive histopathologic changes.

REPORT OF A CASE

A man in his 40s presented with a history of traumatic injury to the nail unit, after which he noted a dark line under the nail, which he assumed to be a splinter. It persisted for 3 years without any notable change. The patient reported removing portions of it when he would clip the nail back.

Physical examination demonstrated a 2-mm-wide black longitudinal streak extending to the distal lunula with localized nail plate thickening on the right second digit (Figure 1A and B). Dermatoscopic findings were consistent with a foreign body under the nail (Figure 1C and D). Nail clippings of the nail plate were performed to sample the distal portion of the lesion and demonstrated parakeratosis associated with pigmentation.

Onychocytic matricoma versus Seborrheic keratosis of the nail unit

Onychocytic matricoma vs Nail unit seborrheic keratosis

- Semantic difference
- Seborrheic keratosis is very common
- More important is to make sure this is not subtle, pigmented squamous cell carcinoma

Nail Fungus Diagnostics

- Sampling is an issue
  - Subungal debris is better than nail plate for sampling.

JAAD
Journal of the American Academy of Dermatology

Subungual debris cytopathology increases sensitivity of fungus detection in onychomycosis

Christian S. Jordan, MD, PhD, Brandon Stokes, CHT, Curtis T. Thompson, MD

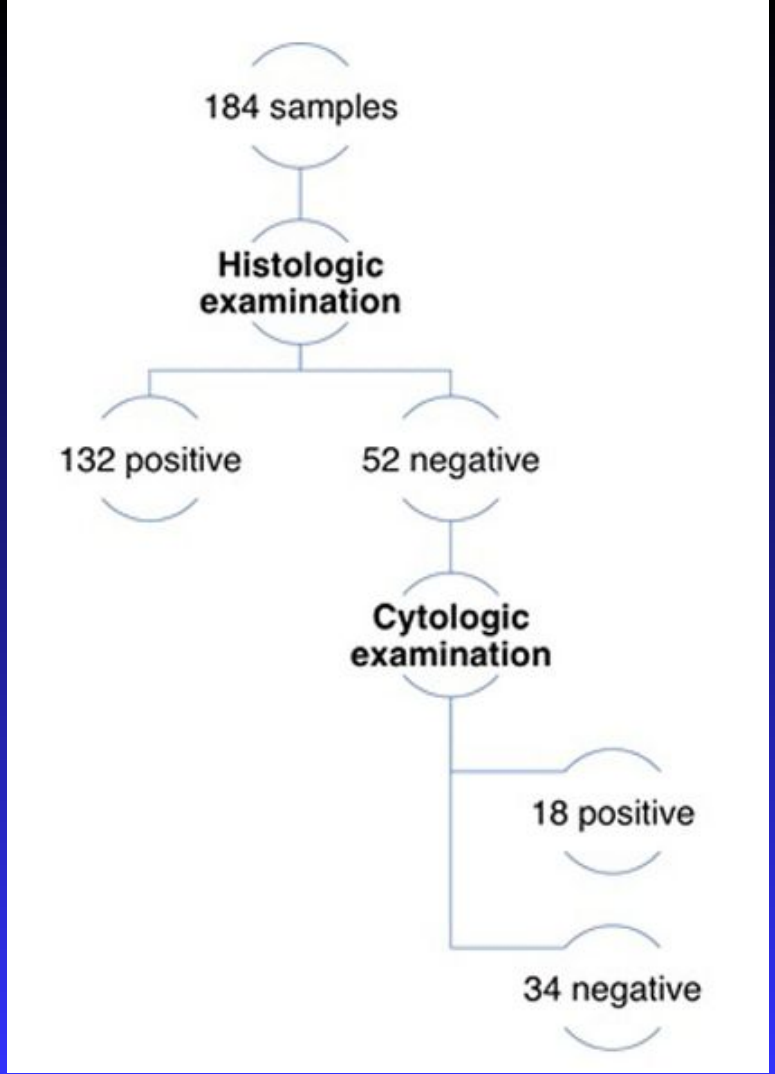
Centrifuge
(Cytospin, Fisher HealthCare)

Centrifuge with slide

Centrifuge with slide

J Am Acad Dermatol
Volume 75, Number 1

Fig 1. Onychomycosis. Microscopic examination of PAS-stained subungual debris. (Original magnification: ×400.) Subungual debris was collected by centrifugation of the formalin in which nail clipping specimens were submitted. Microscopic examination of a thin-layer preparation of PAS-stained subungual debris reveals multiple darkly staining fungal forms associated with a single keratin aggregate.


184 samples
Histologic examination
132 positive
52 negative
Cytologic examination
18 positive
34 negative

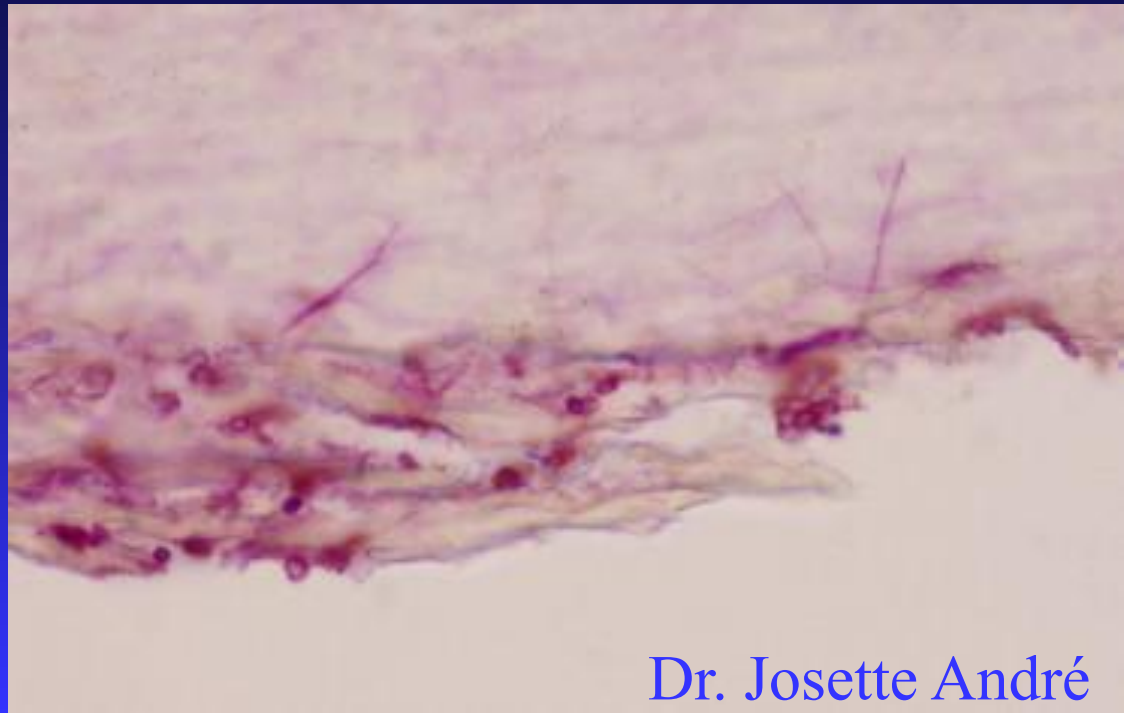
Submit specimen dry in a small envelope

- Test nail plate first
- If plate negative, then centrifugre and PAS

Mold

Mold vs Dermatophyte

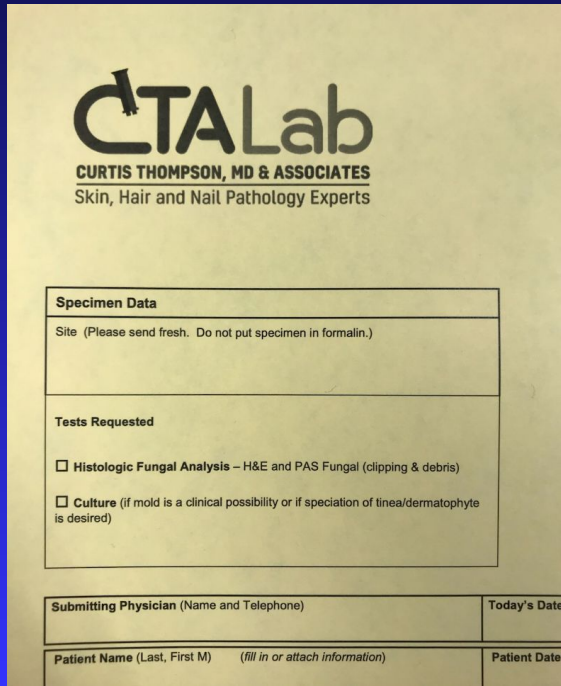
- Invades vertical to nail plate.



Dr. Josette André

Mold

- Clinical suspicion
- Culture with cycloheximide-free media
  - Must notify lab of possibility



CTALab
CURTIS THOMPSON, MD & ASSOCIATES
Skin, Hair and Nail Pathology Experts

Specimen Data
Site (Please send fresh. Do not put specimen in formalin.)
Tests Requested ☐ **Histologic Fungal Analysis** – H&E and PAS Fungal (clipping & debris) ☐ **Culture** (if mold is a clinical possibility or if speciation of tinea/dermatophyte is desired)

Submitting Physician (Name and Telephone)	**Today's Date**
Patient Name (Last, First M) *(fill in or attach information)*	**Patient Date**

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