Bone & Soft Tissue Grossing Practices & Techniques

Nicole A. Cipriani, MD

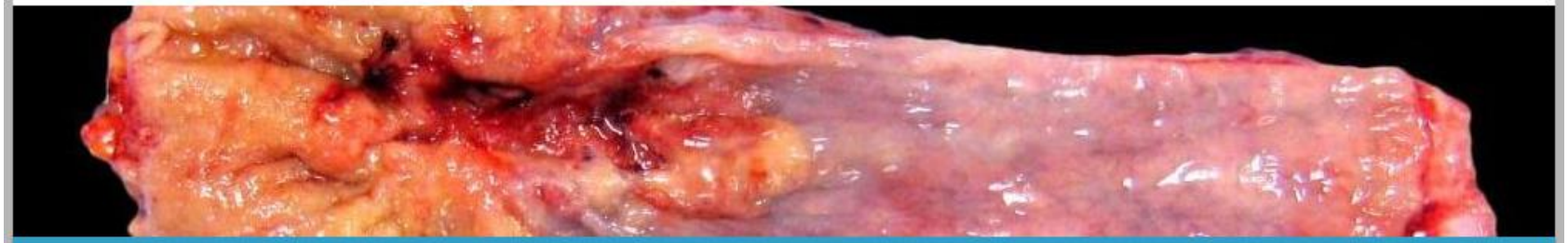
01-13-20

AT THE FOREFRONT
UChicago Medicine

Gross Pathology Manual

By The University of Chicago Department of Pathology

https://voices.uchicago.edu/grosspathology



Bone & Soft Tissue

UChicago Medicine Gross Pathology

Bone & Soft Tissue Pathologists are:

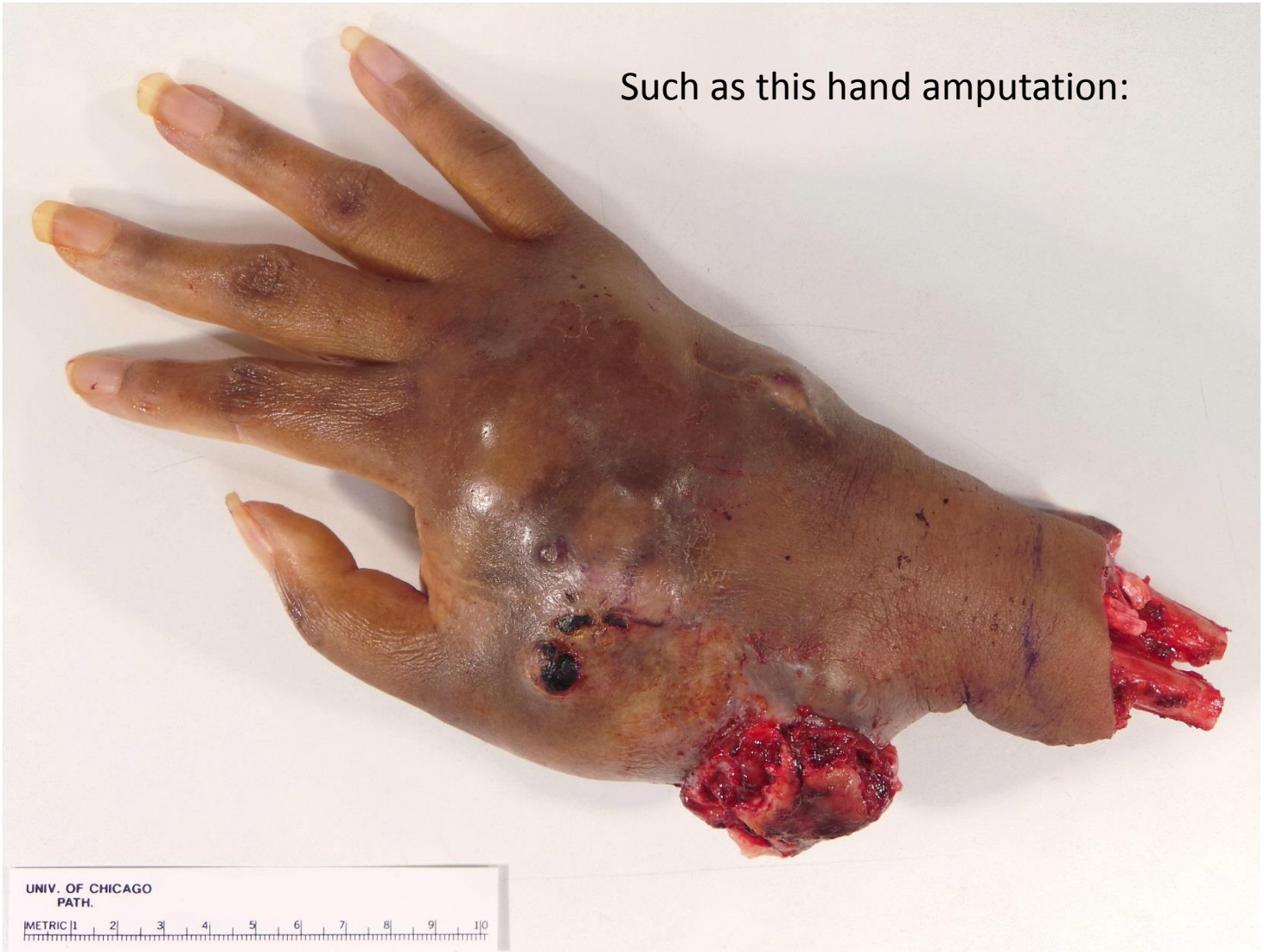
- Peter Pytel, MD
- Nicole Cipriani, MD
- Thomas Krausz, MD

Remember: For any tumor adjacent to / involving bone, if any part of the tumor is SOFT and does not NEED decalcification, please isolate 1-2 sections and submit as **non-decalcified** tumor (important for possible molecular testing). Then proceed with decal as necessary for the remainder of the case. Alternatively, if molecular testing is anticipated, fix and decalcify a portion of tumor in **EDTA**.

Recent Blog Posts

- **Protected: 11-1-2019 Surg Path Meeting** November 1, 2019
- **Protected: 10-1-19 Surg Path Meeting** October 2, 2019
- **Protected: 9-3-19 Surg Path Meeting** September 3, 2019
- **Protected: 8-1-19 (7-31-19) Surg Path Meeting** August 28,

Challenge: Closely associated bone, soft tissue, and tumor



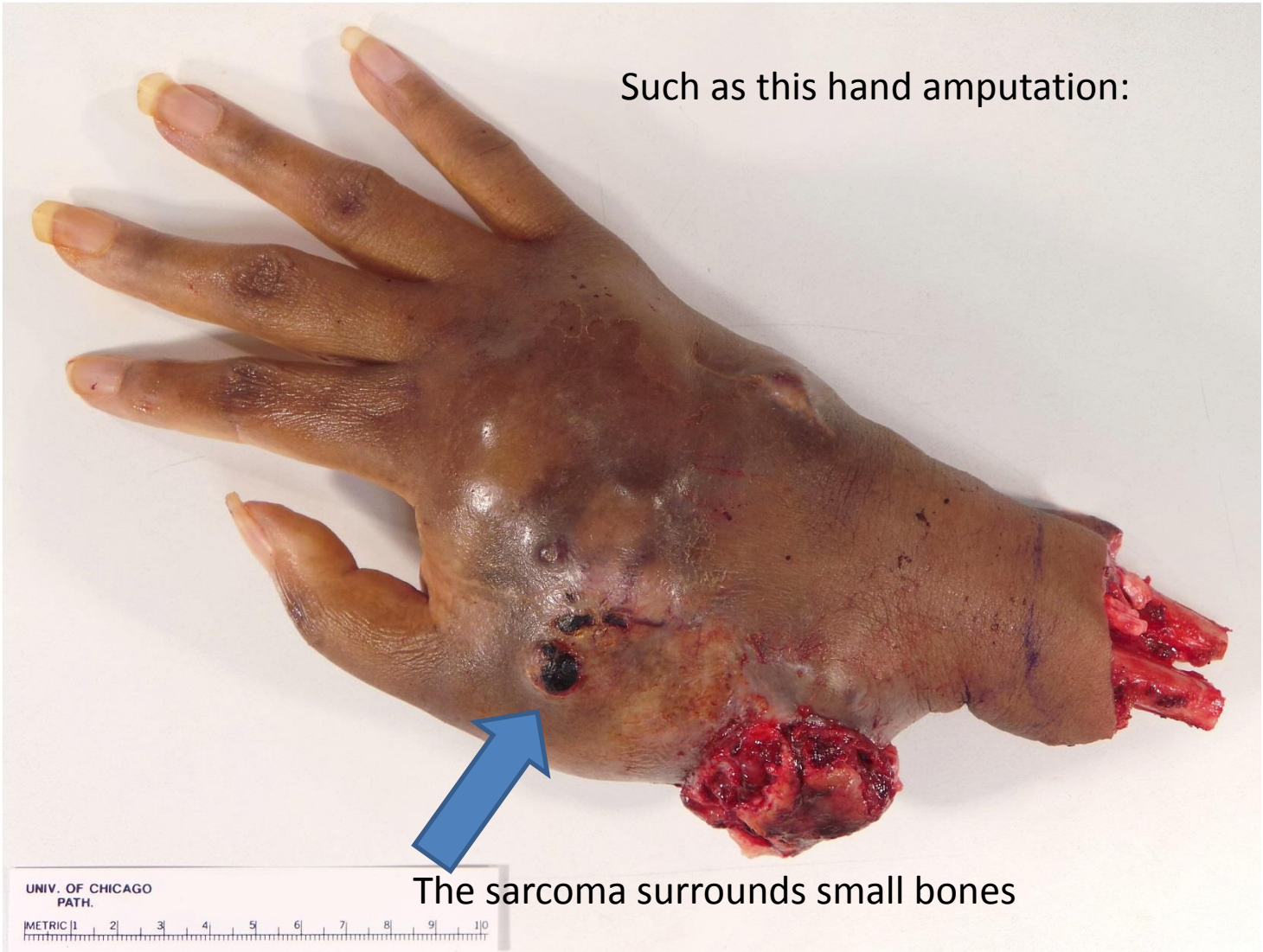
Challenge: Closely associated bone, soft tissue, and tumor

- With large specimens such as this, ideally they should be triaged **same-day** as receipt
  - Prefer to “open up” the tumor to start fixation
  - Do not immerse entire specimen in formalin and expect tissues to fix properly
- If very late in the day, can place in fridge over night and address first thing in morning

OPTIONS for grossing:

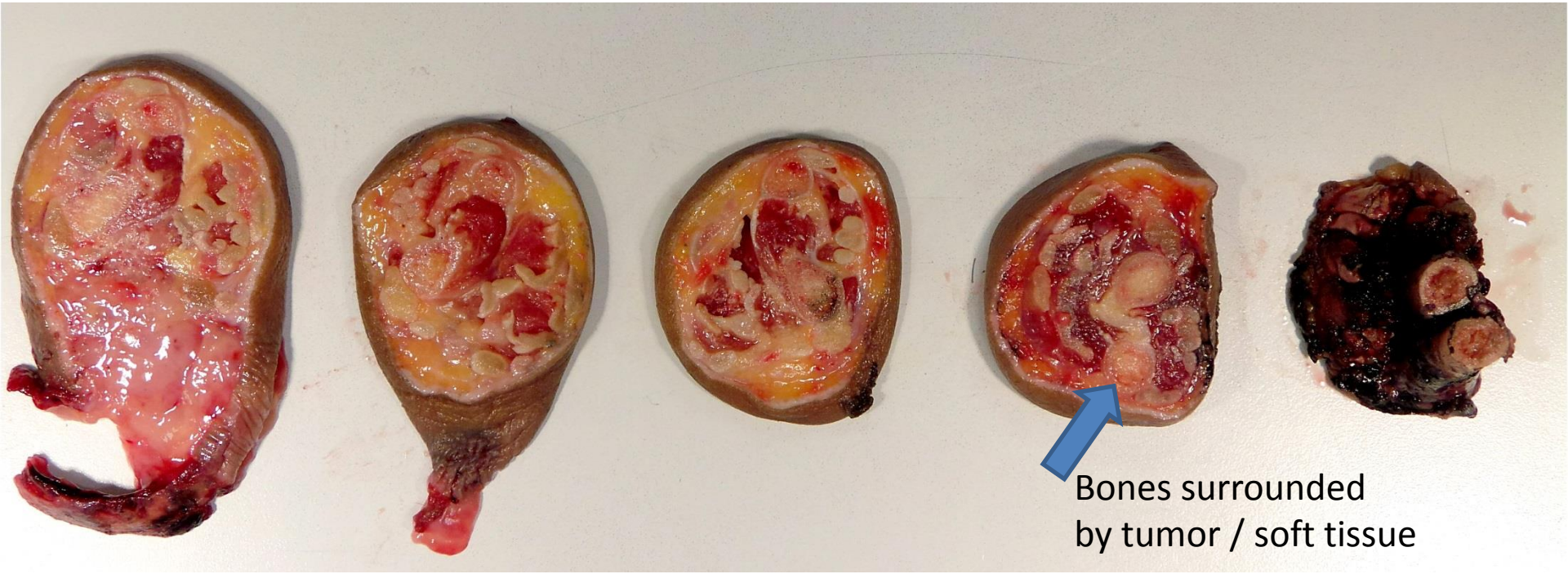
What	How	Pros	Cons	When to consider
Score	Score the soft tissue and cut through bone with saw	• Can be done fresh or fixed • Preserves relationships of bony / soft structures	• May result in uneven sections depending on skill of grosser / complexity of specimen	• When most pathology is in the soft tissue and there is a single / simple core of bone (mandible)
Shave	Shave off as much soft tissue as you can and gross soft tissue and bone separately	• Can be done fresh or fixed	• Does NOT preserve relationships of bony / soft structures	• When most pathology is in the bone (osteosarcoma)
Freeze	Freeze the FRESH specimen in liquid nitrogen (until hard) or deep freezer (overnight) then cut entire specimen with saw	• Preserves relationships of bony / soft structures • Usually results in even sections	• Can only be done FRESH – time sensitive • May distort morphology	• When soft and hard parts and tumor are intimately admixed (soft tissue tumor surrounding and invading bone OR bone tumor invading soft tissue)
Fix & Decal	Fix the specimen fully, decalcify it entirely, and cut with regular long blade	• Preserves relationships of bony / soft structures • Usually results in even sections	• Requires decalcification of entire specimen and eliminates the possibility of molecular testing	• When bone is sparse, thin, delicate, and would suffer fragmentation from the above (maxilla, alveolar ridge)

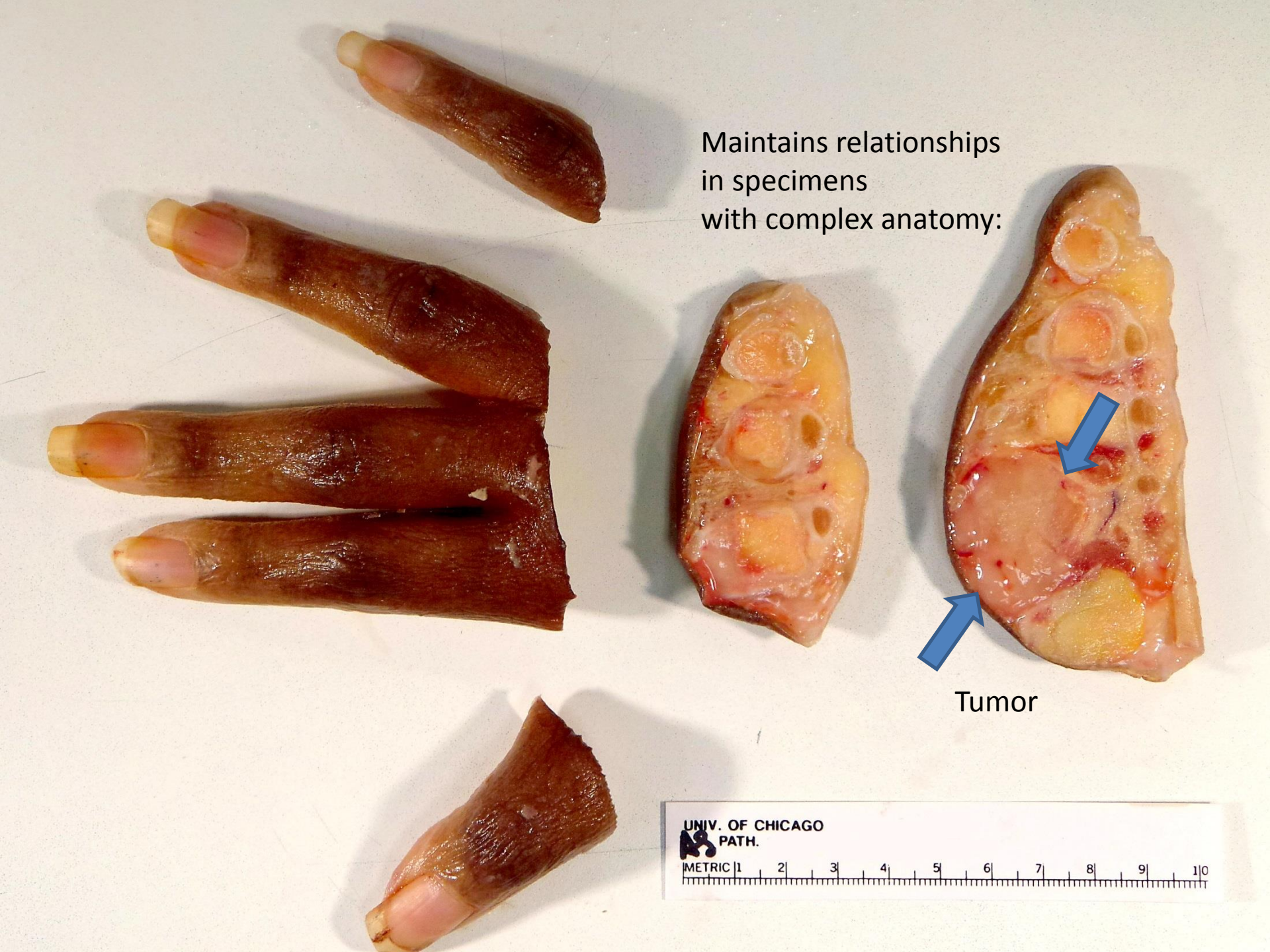
Challenge: Closely associated bone, soft tissue, and tumor



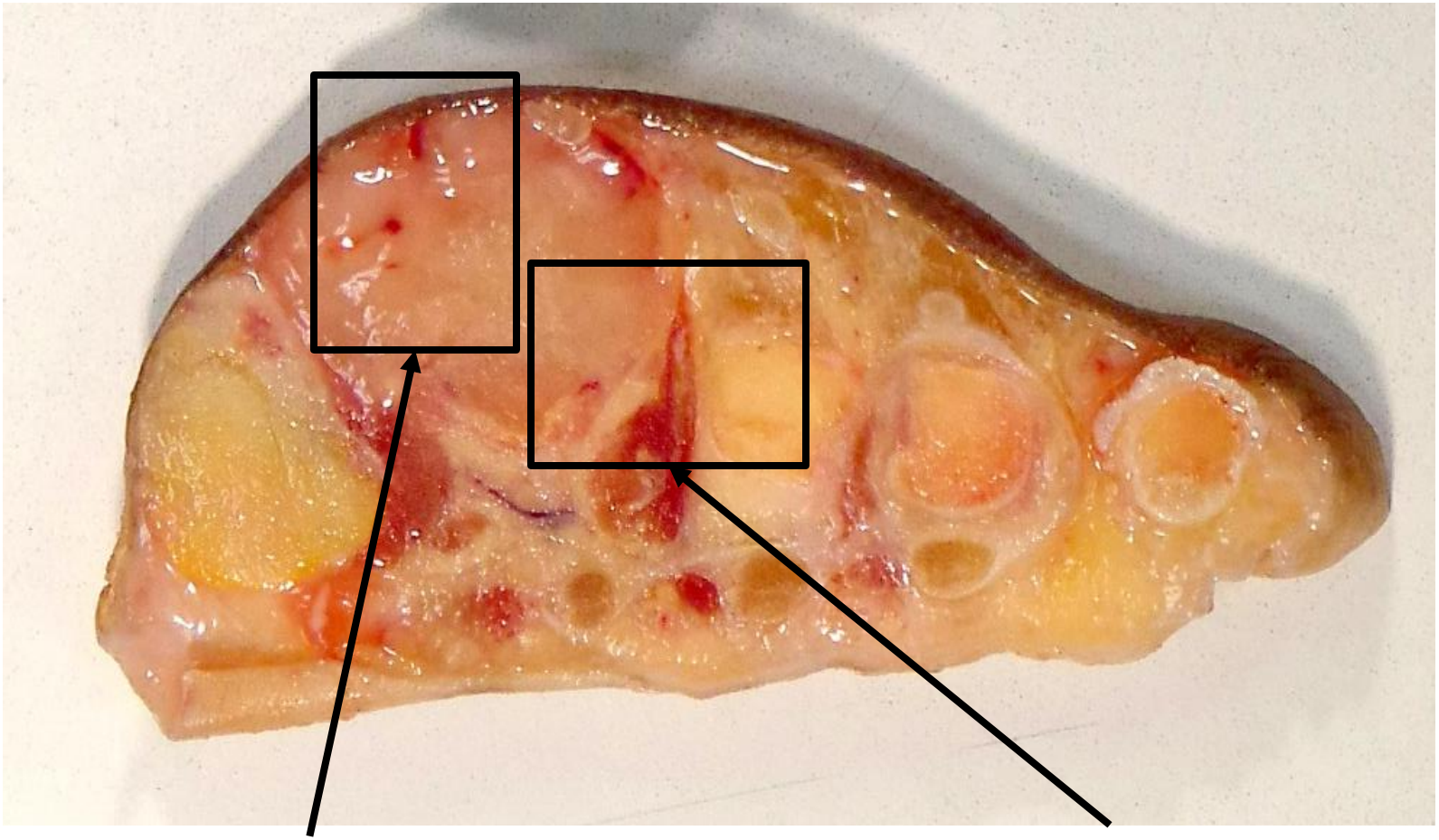
Example of *Freeze*:

Good when soft and hard parts and tumor are *intimately associated*




Maintains relationships
in specimens
with complex anatomy:
Tumor
UNIV. OF CHICAGO
PATH.
METRIC 1 2 3 4 5 6 7 8 9 10

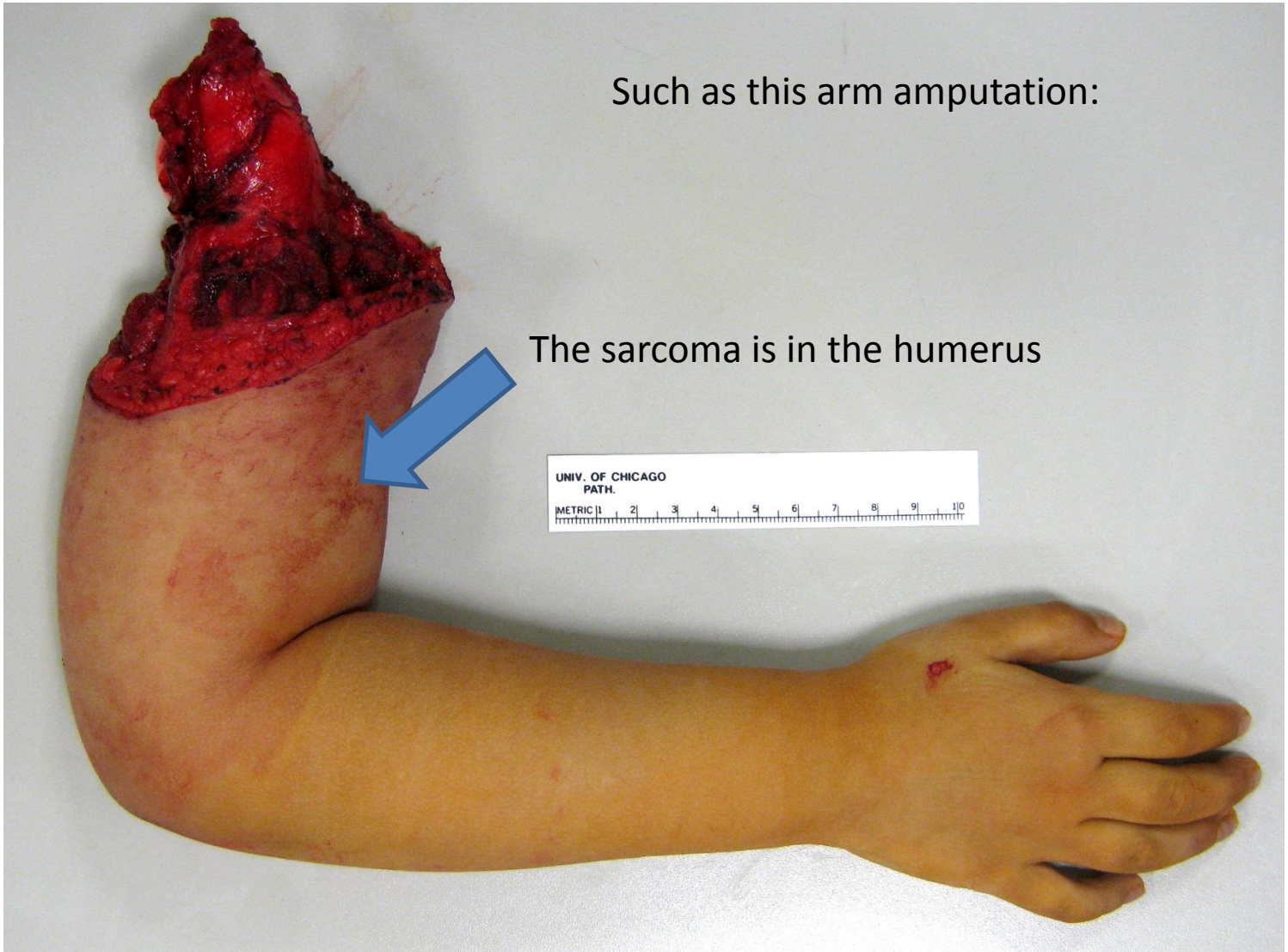
These slices were ~1 cm in thickness.
Too thick for submitting in cassettes.
What are our options? (after fixation)



Isolate areas of soft tumor without bone.
Do NOT decalcify.
Thin down with knife.

Isolate select areas of bone OR tumor interface with bone.
Decalcify ONLY those sections.
Thin down with knife after decal.

Challenge: Closely associated bone, soft tissue, and tumor



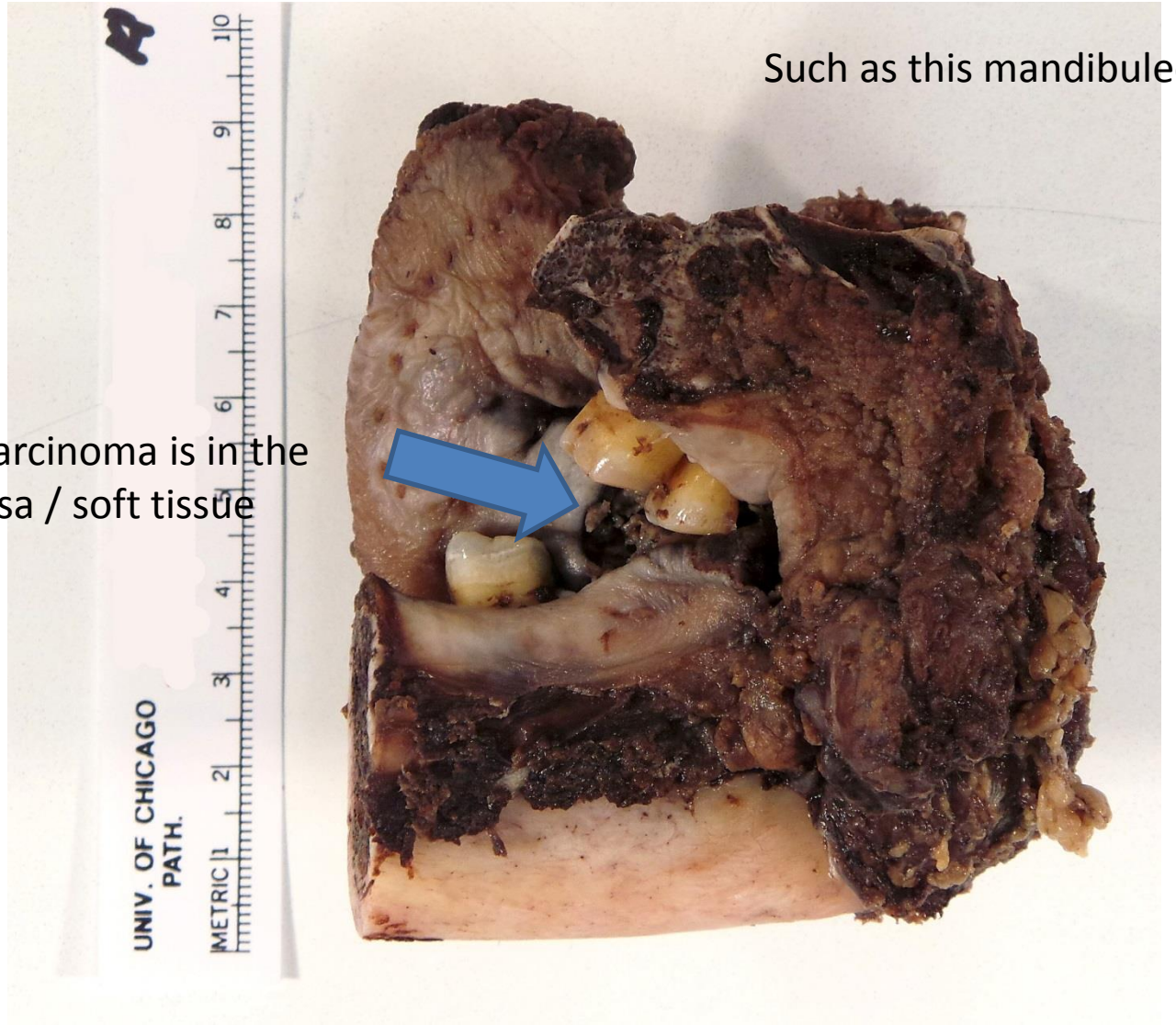
Example of *Shave*:

Good when most pathology is in the *bone*

Bone was isolated and then cut with saw:

Challenge: Closely associated bone, soft tissue, and tumor

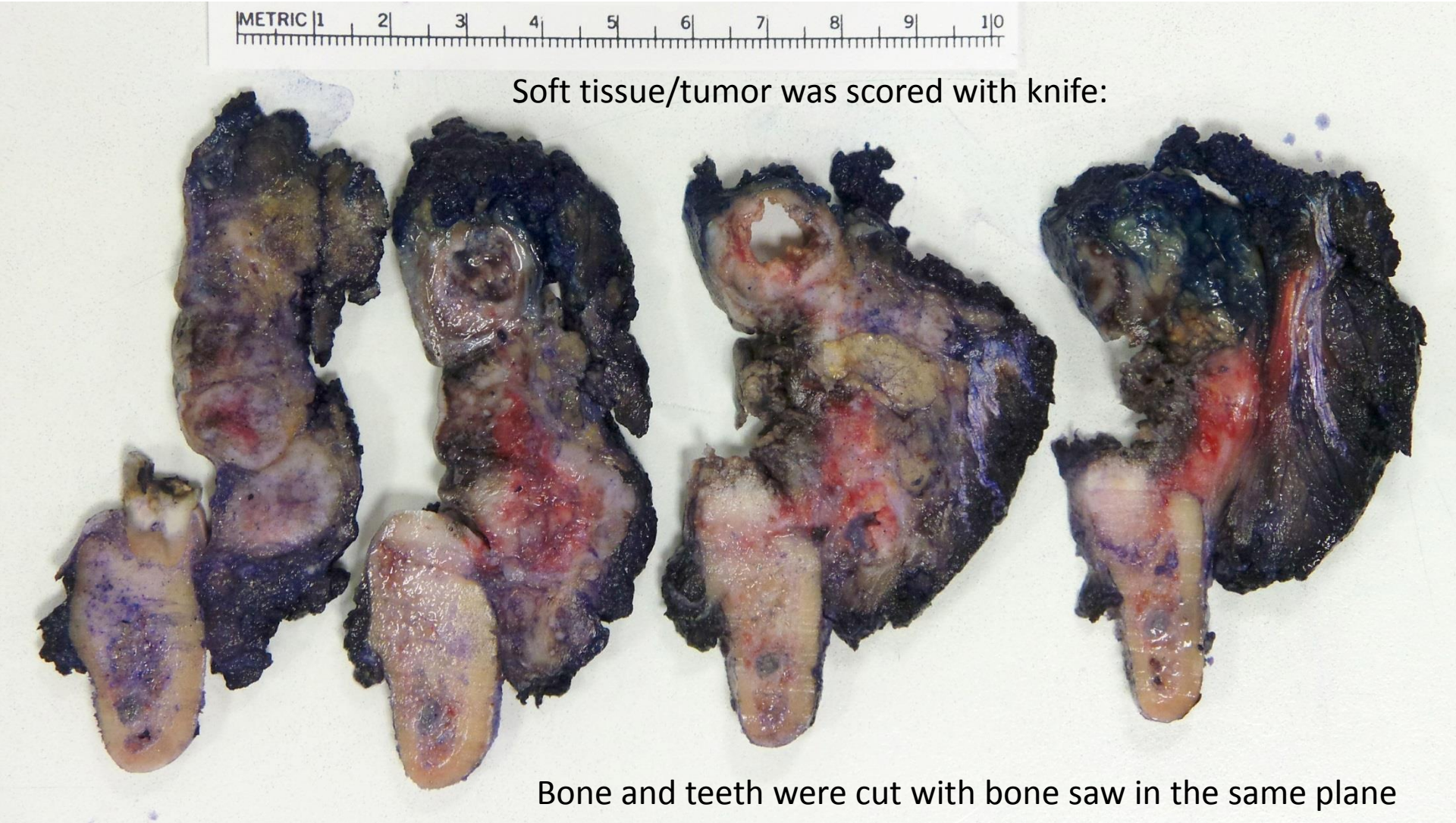
Such as this mandibulectomy:



The carcinoma is in the mucosa / soft tissue

Example of *Score*:

Good when most pathology is in the *soft tissue*
and there is a single / simple core of bone



If these slices were ~1 cm in thickness...
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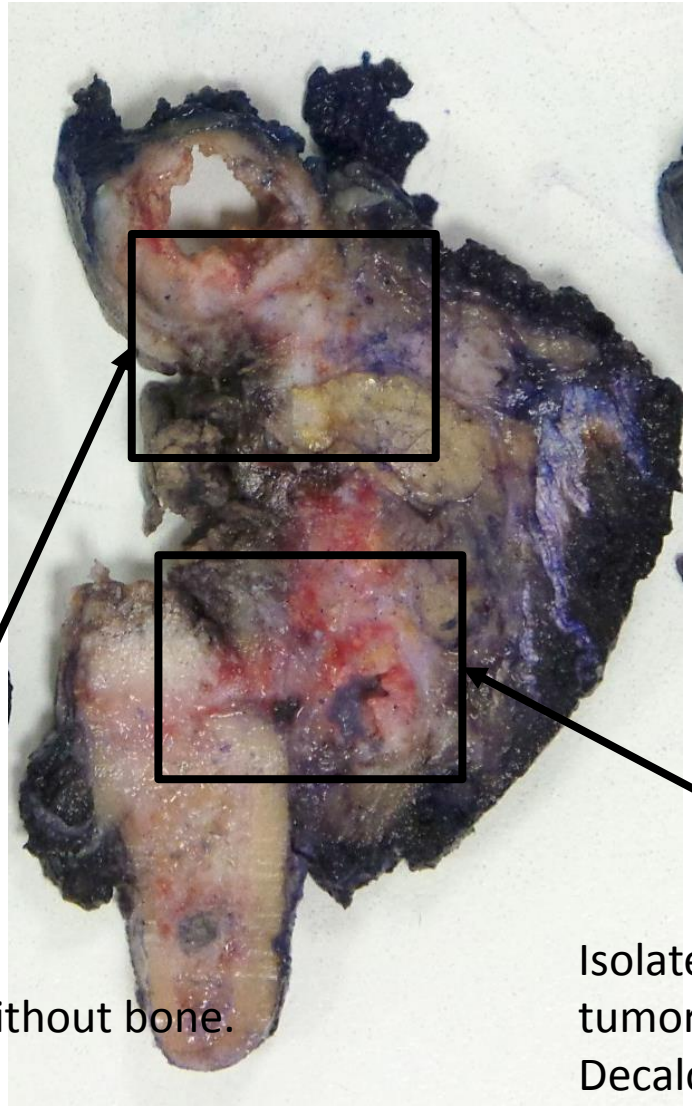


Isolate areas of soft tumor without bone.
Do NOT decalcify.
Thin down with knife.

Isolate select areas of bone OR
tumor interface with bone.
Decalcify ONLY those sections.
Thin down with knife after decal.

Example of *Fix & Decal*:

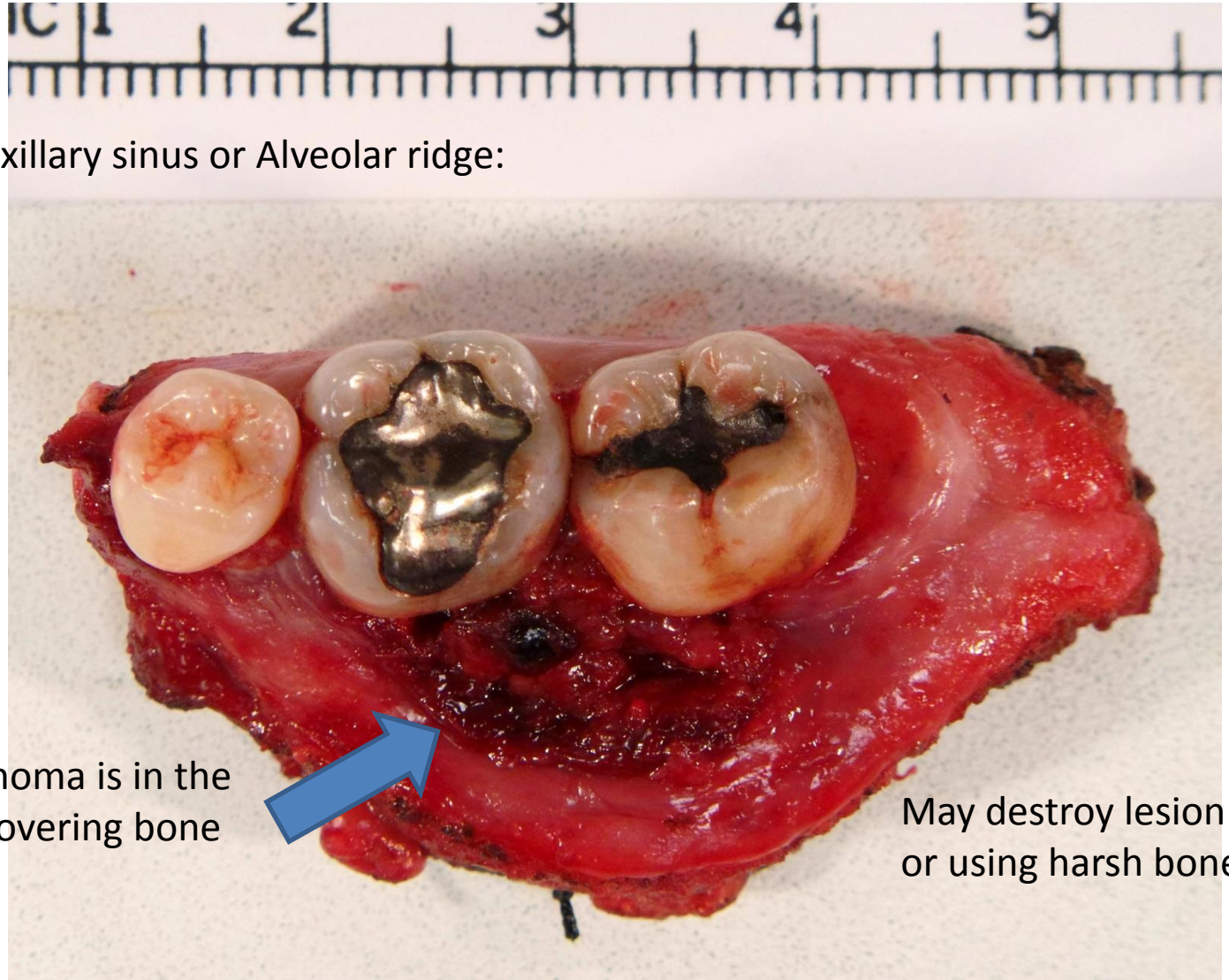
Good when bone is *sparse, thin, delicate,*
and would otherwise suffer fragmentation

- If you want to decalcify a **whole** specimen
  - (after formalin fixation of course)
- DISCUSS WITH AN **ATTENDING** FIRST
- DETERMINE IF THERE IS A **PRIOR** NON-DECALCIFIED BIOPSY
- Consider all options before deciding on this "last ditch" approach

Example of *Fix & Decal*:

Good when bone is *sparse, thin, delicate,*
and would otherwise suffer fragmentation

Palate/maxillary sinus or Alveolar ridge:



The carcinoma is in the mucosa covering bone

May destroy lesion by shaving or using harsh bone saw

Example of *Fix & Decal*:

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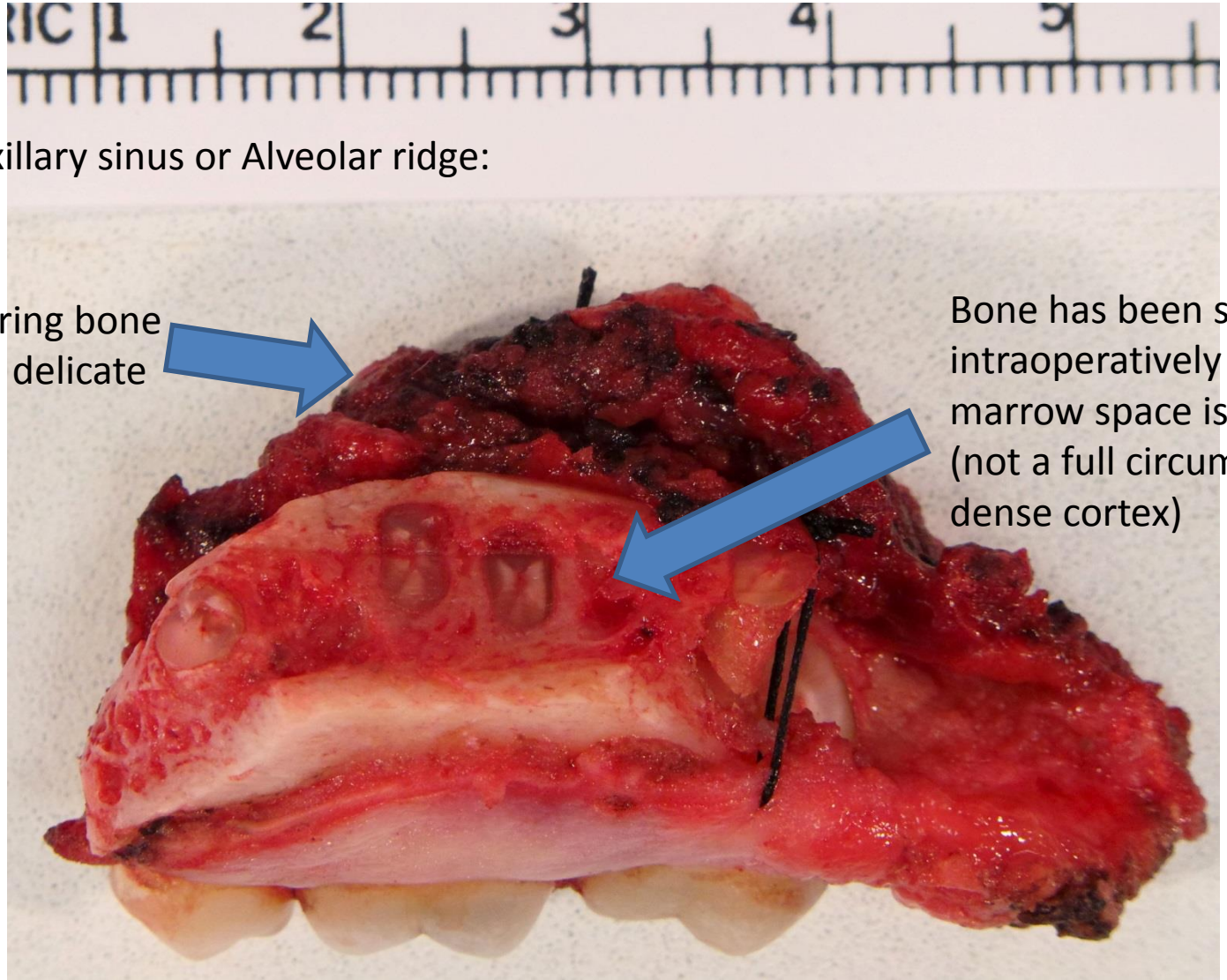
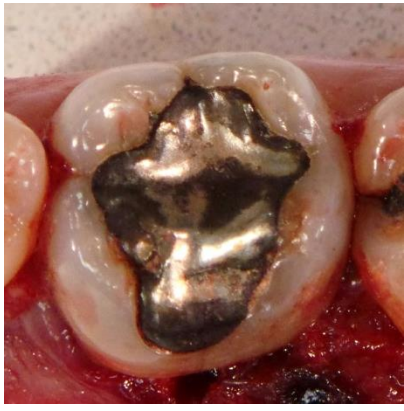
Palate/maxillary sinus or Alveolar ridge:



Example of *Fix & Decal*:

Good when bone is *sparse, thin, delicate,*
and would otherwise suffer fragmentation

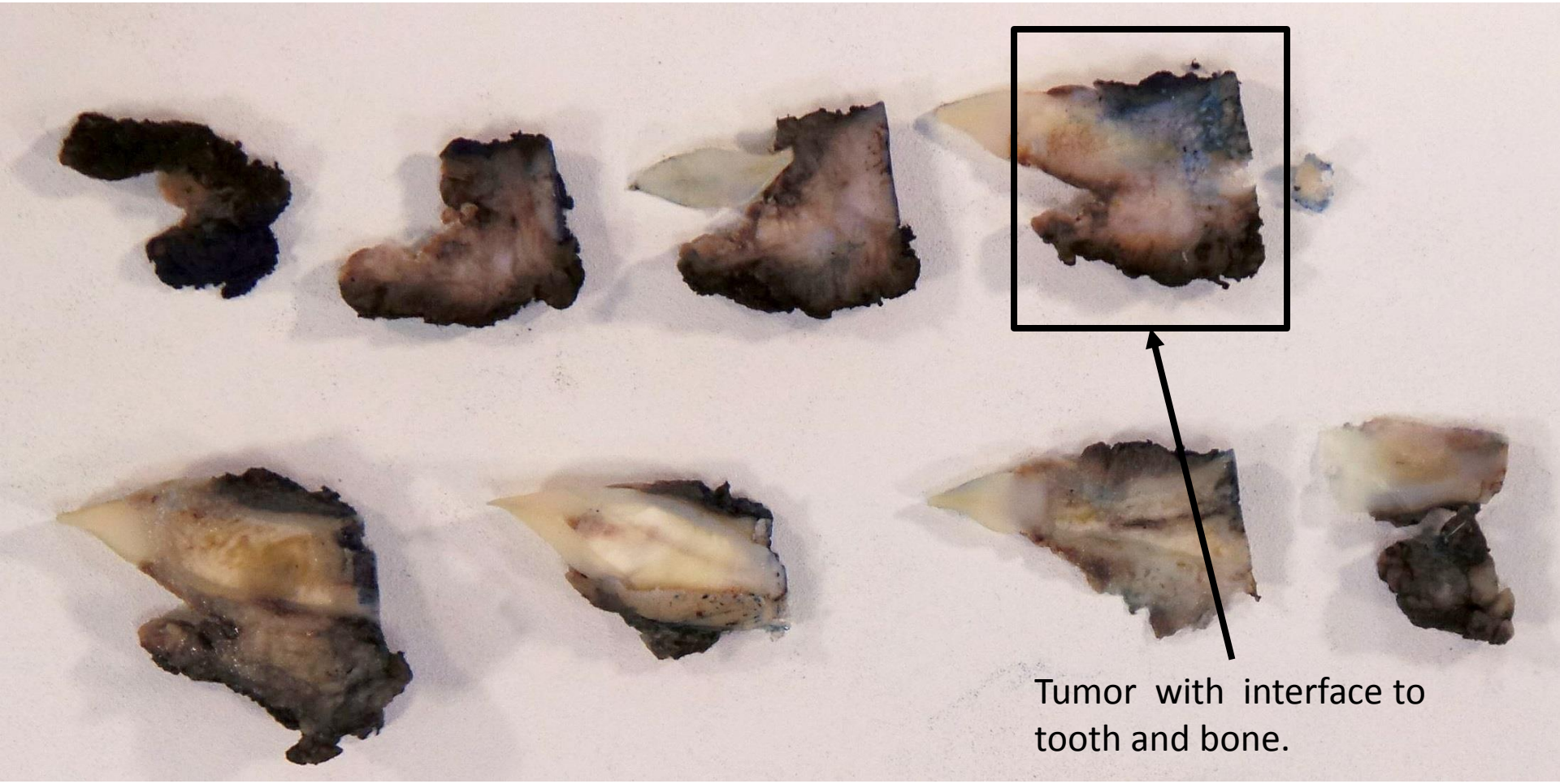
- In these circumstances, you may want to consider full fixation and full decalcification
- Teeth and bone fix/decalcify/cut similarly
- Fillings and cement do NOT fix/decalcify/cut
  - These need to be removed
- Sometimes decal “thins out” the tooth / bone so that fillings can be popped out



Example of *Fix & Decal*:

Good when bone is *sparse, thin, delicate,*
and would otherwise suffer fragmentation

Now you have full sections of all important components:



Score, Shave, Freeze, Fix & Decal

- Questions, comments?

Challenge: Need for molecular testing

- Not a problem with soft tissues
- Becomes a potential problem with bony / calcified tissues in which molecular testing is needed for
  - **Neoplasms** (to detect mutations)
  - **Infections** (to detect organisms)
    - Valves, synovium, bone
    - Molecular testing is less utilized here so we won't focus on it
  - You name it
- **Need decalcifier that preserves nucleic acids**
  - NGS, PCR, FISH

Decal

- Decalcification can be performed for
  - Bone, teeth, otherwise calcified tissues
  - That cannot cut with your knife (and therefore also cannot cut with a microtome in histology)
  - Don’t forget about Decal in:
    - Atherosclerotic arteries in limbs/hearts
    - Calcified heart valves
    - Partially calcified tumors
- **FULL FIXATION** IS ALWAYS PERFORMED **BEFORE** DECALCIFICATION

How to Decalcify

- ***Never*** decalcify an ***entire*** specimen before consulting with an attending first
  - Check to see if there is a pre-existing recent non-decalcified biopsy
  - Determine extent of HCL vs EDTA (will discuss)
- ***Always*** try to ***isolate*** soft lesional tissue to submit WITHOUT decalcification:
  - Tumor next to bone
  - Soft tumor within bone
  - Noncalcified tumor

How to Decalcify

- If there is **no way** to submit tumor without decalcification:
  - Bone core for metastasis
  - Predominantly ossified tumor (osteosarc)
  - Tumor diffusely infiltrating bone with no soft component
- If it is a biopsy:
  - Decalcify entirely in **EDTA after fixation**
- If it is a medium-large specimen:
  - Decalcify at least 1 (if not more) sections in **EDTA after fixation**
  - **WHY?**

Decal Solutions & Limitations

- **Strong acids**
  - **Hydrochloric acid***, nitric acid
  - Fastest (hours to days) but destroys nucleic acids
  - **ALL BONY/CALCIFIED TISSUES HAVE ROUTINELY BEEN DECALCIFIED IN HCL at UChicago**
- **Weaker organic acids**
  - Formic acid
  - Slower and somewhat more gentle but not great at nucleic acid preservation
- **Chelating agents**
  - Ethylenediaminetetraacetic acid (**EDTA**)
  - Slowest (hours to days to weeks) but the most gentle and best for preserving nucleic acids

How to evaluate nucleic acids?

- DNA *quantity*:
  - Qubit2.0 Fluorometer (ThermoFisher)
  - **100 ng quant** is needed for in-house assays
- DNA *quality*:
  - fragment analysis (TapeStation 2200, Roche)
- DNA *quantity* and *quality*:
  - qPCR (Kapa hgDNA Quantification and QC kit)

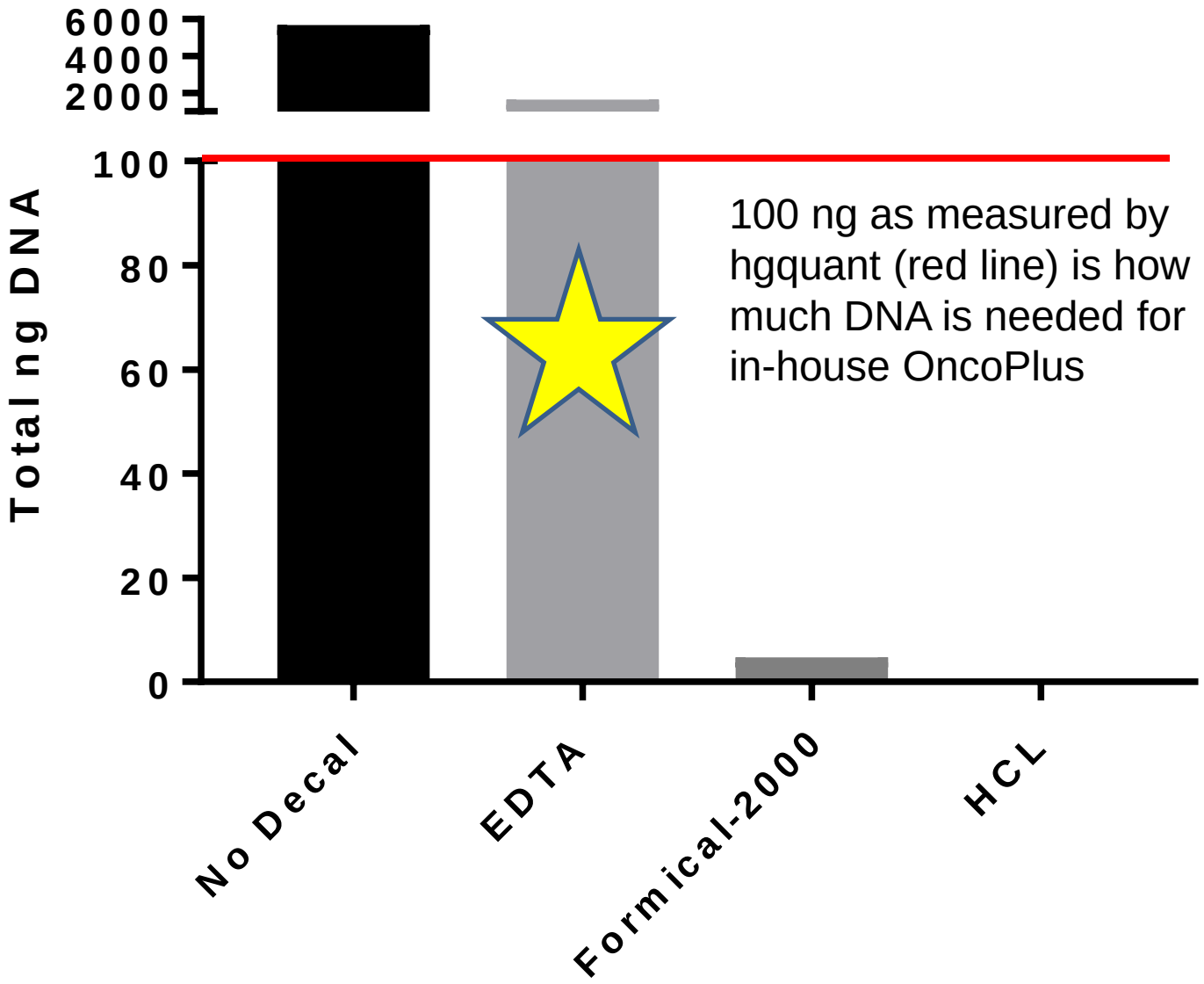
Prior Internal Study

HCL vs Formic Acid vs 5% EDTA vs None

Qubit ng/ul	total Qubit ng	hgquant ng/ul	hgquant total ng	Decal
LOW	0	0.0055281	**0.41460761**	**HCL**
55.4	4155	0.06351738	**4.76380316**	Formical-2000 (**Formic Acid**)
89.8	6735	22.004029	**1650.30217**	**EDTA (5%)**
106	7950	75.7562345	**5681.71759**	**No Decal**

Courtesy Lauren Ritterhouse, MD

Prior Internal Study



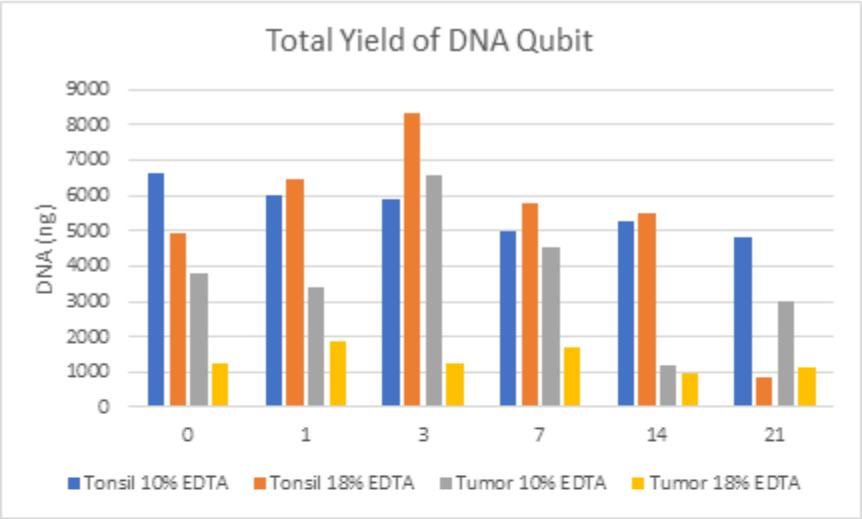
Courtesy Lauren Ritterhouse, MD

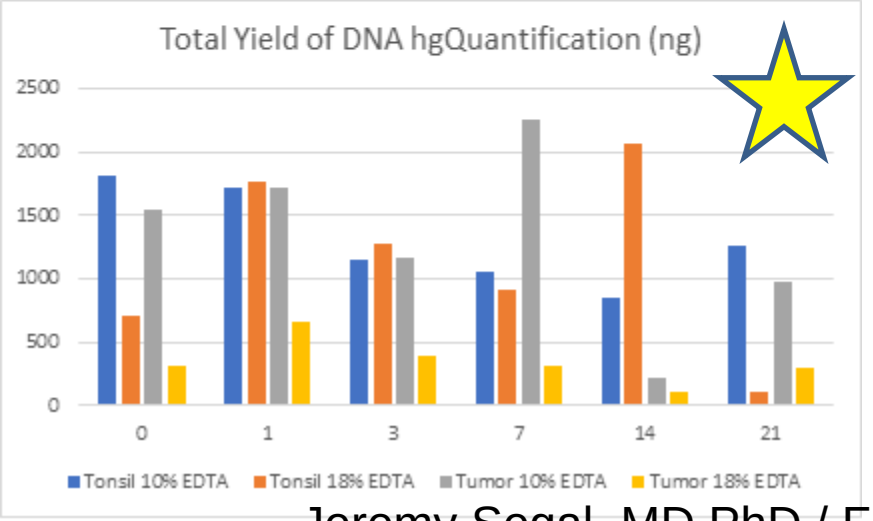
Recent Study

10% EDTA vs 18% EDTA

- Tonsil and Solid tumor tissue
- 0 days through 21 days
- In 10% and 18% EDTA

- All were >100 ng quant
- 18% had less DNA **quantity** than 10% but still adequate after **3 STRAIGHT WEEKS**

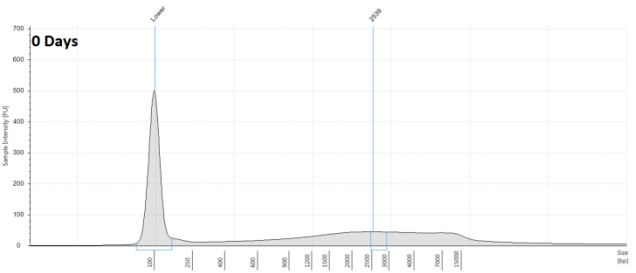


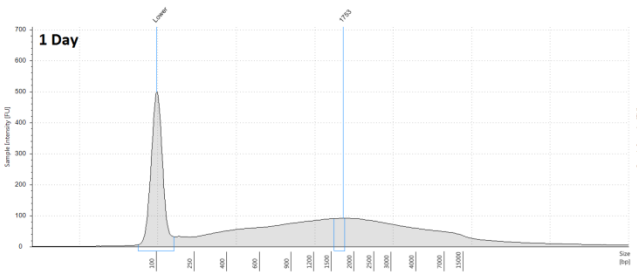


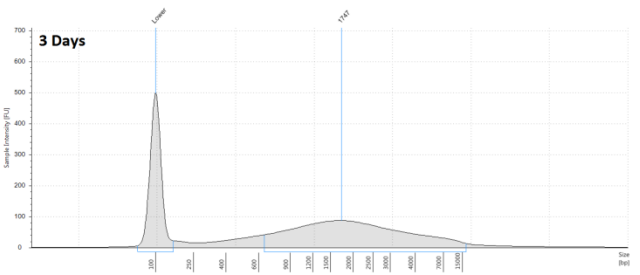
Jeremy Segal, MD PhD / Filippo Galbo

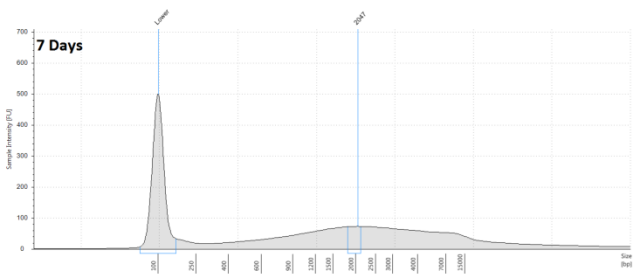
Recent Study

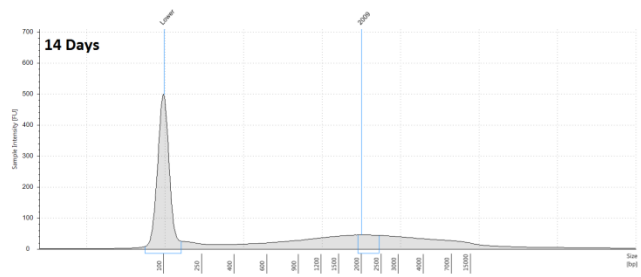
DNA **quality** of the six **18% EDTA** tumor specimens remained adequate for sequencing across time points (**through 21 days**)

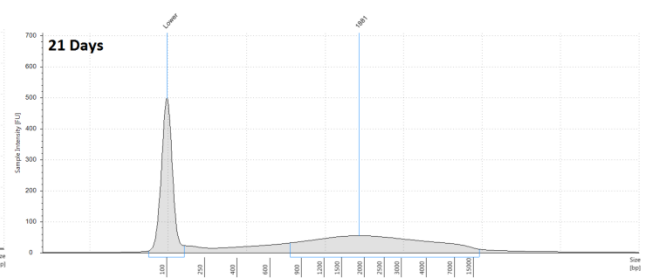


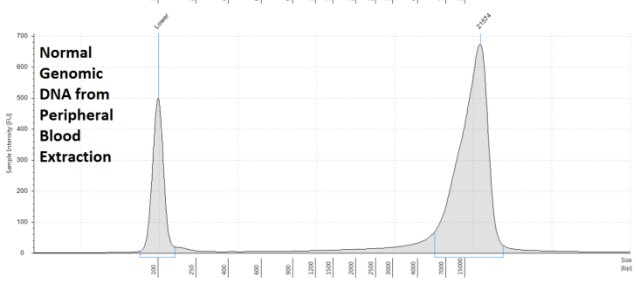












Jeremy Segal, MD PhD / Filippo Galbo

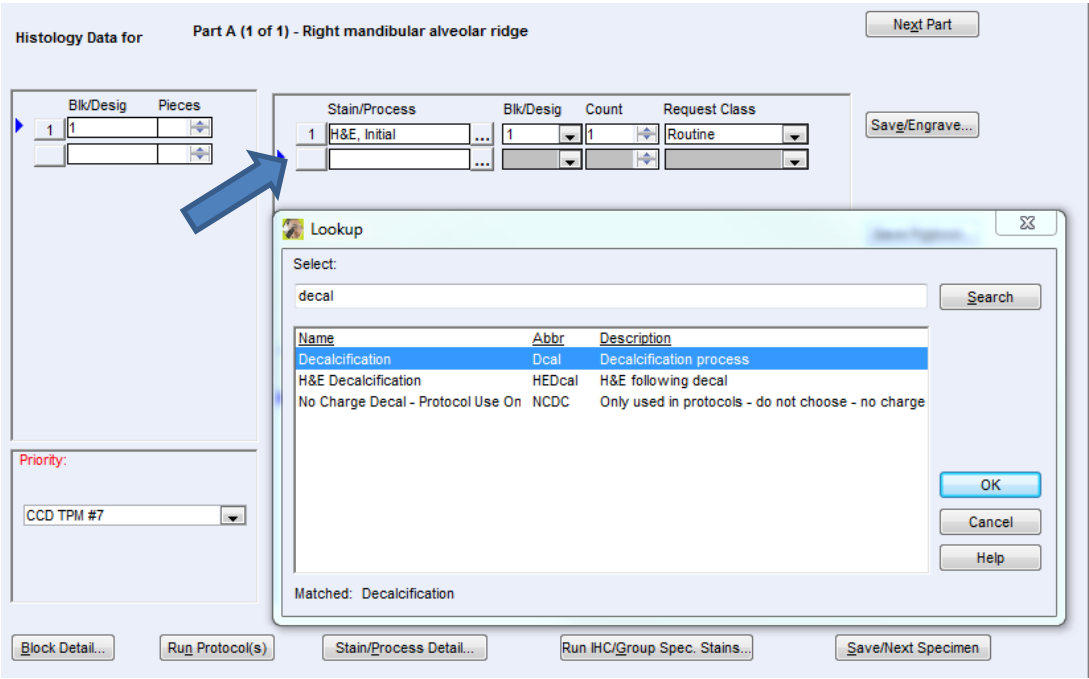
The point is…

- Always critically evaluate specimens that need decalcification
- If it is a biopsy for *possible* tumor and **all** needs to be decalcified -> use **EDTA** 18%
- If it is a medium-large specimen and you can grossly see tumor:
  - First choice: If there is soft tumor:
    - Submit as much soft tumor as you need that does NOT need decal
    - Submit some following HCl
    - If you have DEFINITE soft tumor, EDTA may not be needed (since you already have non-decalcified tumor)
    - Ask an attending if questions
  - Second choice: If there is NO soft tumor:
    - Submit some cassettes following EDTA 18%
    - Submit some following HCl

How to document Decal in CoPath?

- MUST enter Decalcification ***charge*** for each **container** (not necessarily each block) in which you use decal (either HCl or EDTA)
  - Generates a billing charge

- In **Histology Data Entry/Edit**
- In Stain/Process
- Search for decal
- Select “Decalcification” (Dcal)
- The block designation does not matter, as you only need one entry per PART, regardless of how many cassettes you Decal



How to document Decal in CoPath?

- MUST dictate in your **cassette summary** *which* cassettes were decalcified and in *which* solution
  - Must do this for all cassettes
  - Confirms that the charge is legitimate

If you don't specify EDTA vs HCl, we assume HCl:

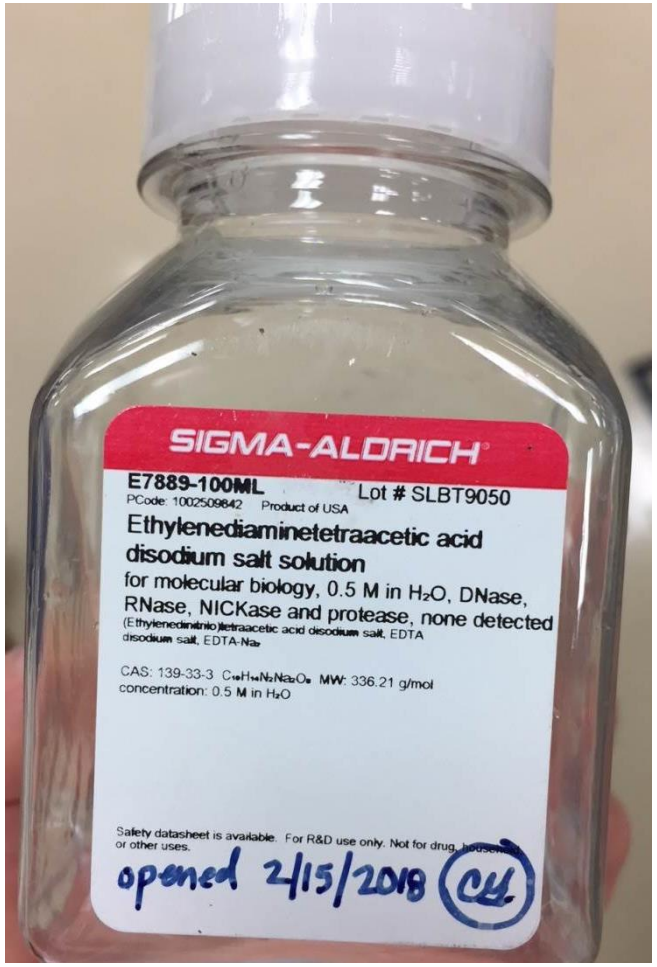
Cassette Summary
B1-6: Representative tumor from slice #1, EDTA-decalcified (see diagram)
B7: Marrow margin, following decalcification (from slice #4)
B8-9: Nearest anterior margin, perpendicular, following decalcification
B10-12: Nearest posterior margin, perpendicular, following decalcification
B13-14: Nearest medial margin, perpendicular, following decalcification
B15-16: Nearest lateral margin, perpendicular, following decalcification
B17-19: Skin, entirely, with lesion, following decalcification
B20-40: Full face of bone with tumor, following decalcification (see diagram, slice #2)
B41-59: Full face of bone with tumor, following decalcification (see diagram, slice #4)

- Entering Decal in **Vantage** alerts Histology to a possible delay but does not generate a charge

Options

10% EDTA

18% EDTA



HCl