WELCOME

RIGAKU WEBINAR SERIES

X-RAY COMPUTED TOMOGRAPHY
FOR MATERIALS & LIFE SCIENCE
PREPARING LIFE SCIENCE SAMPLES

IS STARTING NOW.

Presenter: Angela Criswell

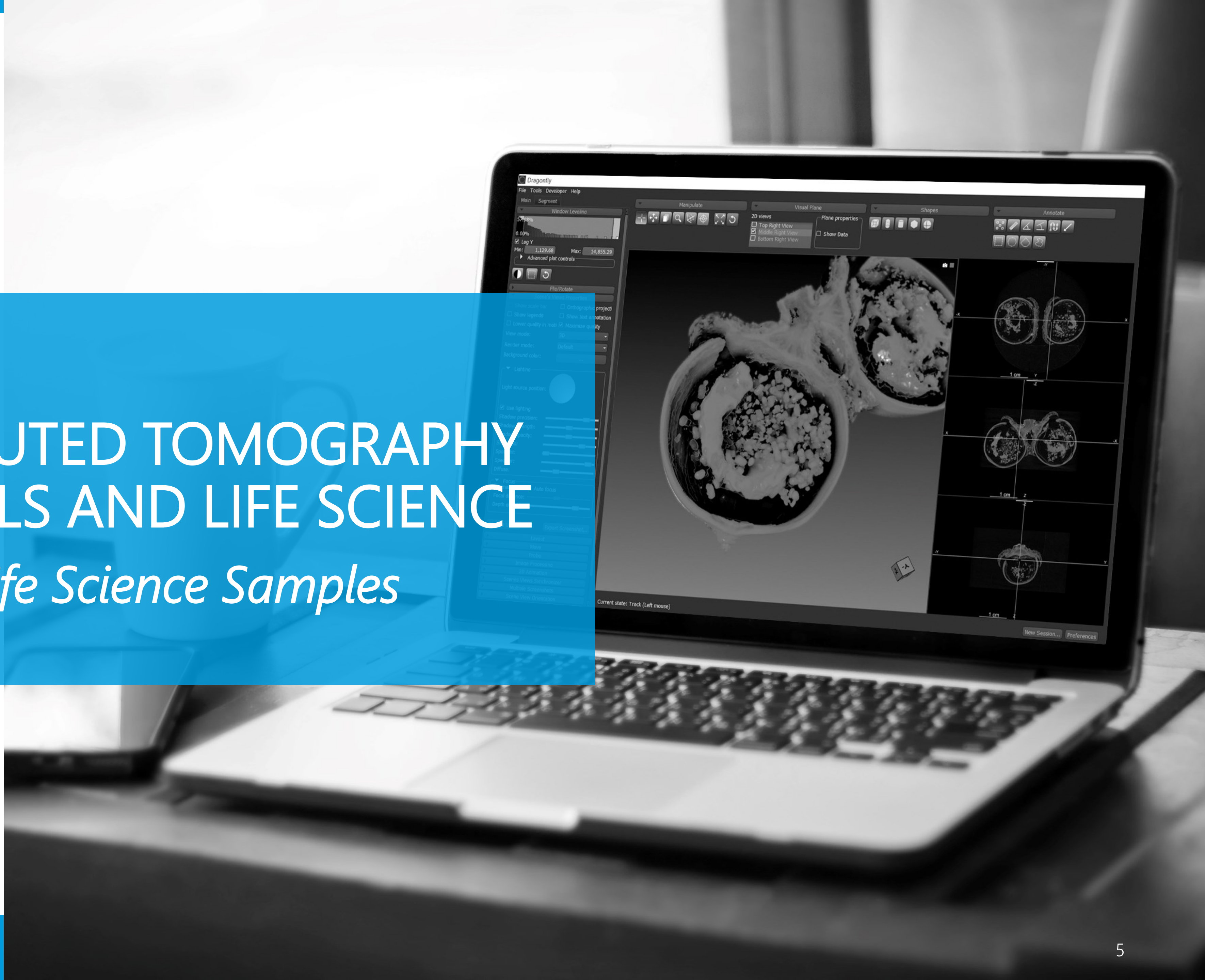
Senior Scientist
Rigaku Americas Corporation

Host: Tom Concolino

Southeast Regional Account Manager
Rigaku Americas Corporation

You can send us questions during the presentation. They will be addressed at the end of the presentation.

A recording of this webinar will be available.
You will receive an email with a link to it tomorrow.



X-RAY COMPUTED TOMOGRAPHY FOR MATERIALS AND LIFE SCIENCE

Preparing Life Science Samples

NOT ALL SAMPLES ARE THE SAME

Some samples require little to no sample preparation

While others require more sample preparation

You will learn:

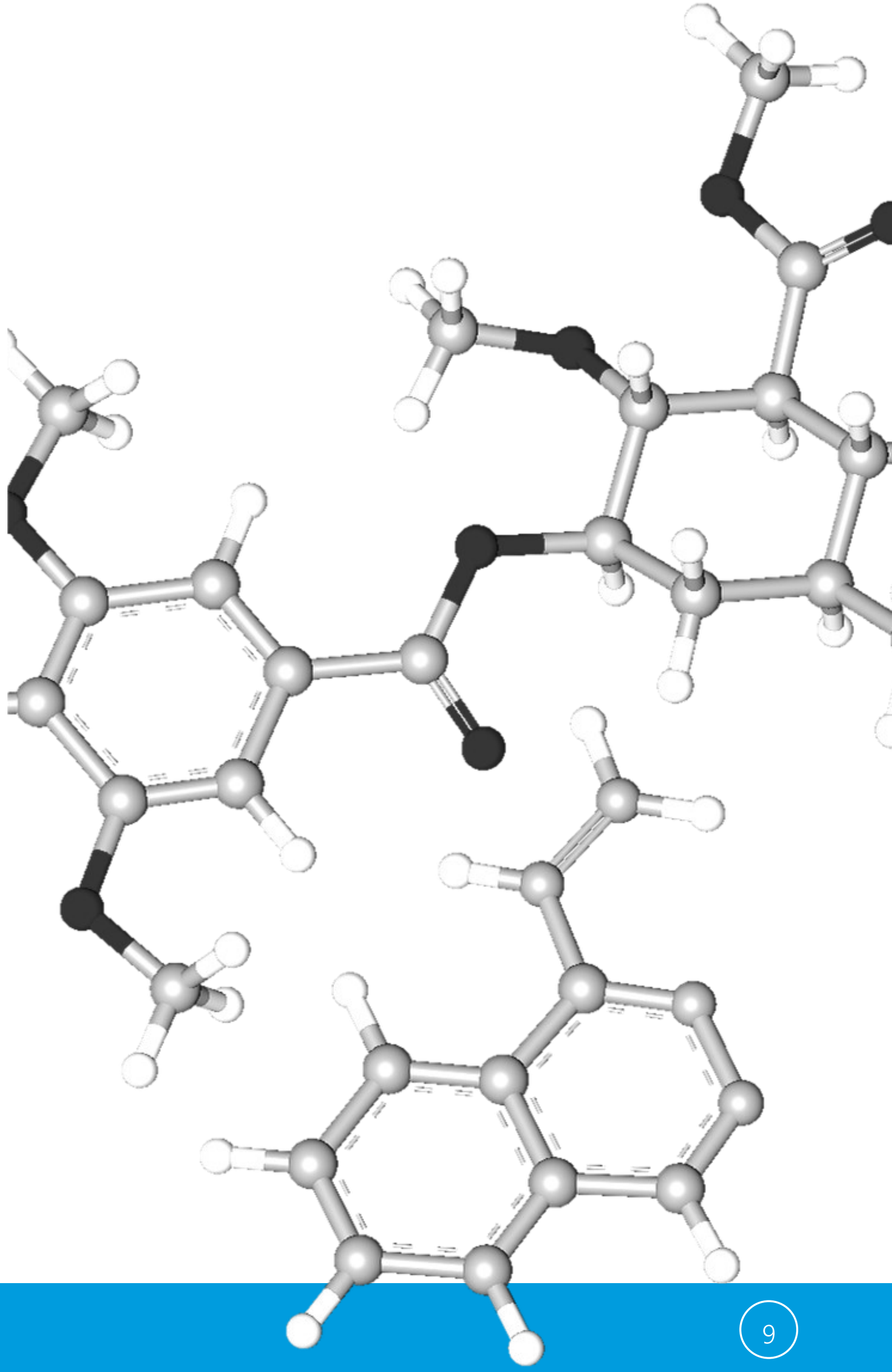
- Sample fixation
- Sample staining
- Life science applications

SAMPLE PRESERVATION

- Use fixatives 'to fix' sample at a point in time and prevent subsequent breakdown of tissue.
  - Stop autolysis
  - Stop microorganism activity
- Types of fixatives
  - Physical fixatives
  - Chemical fixatives

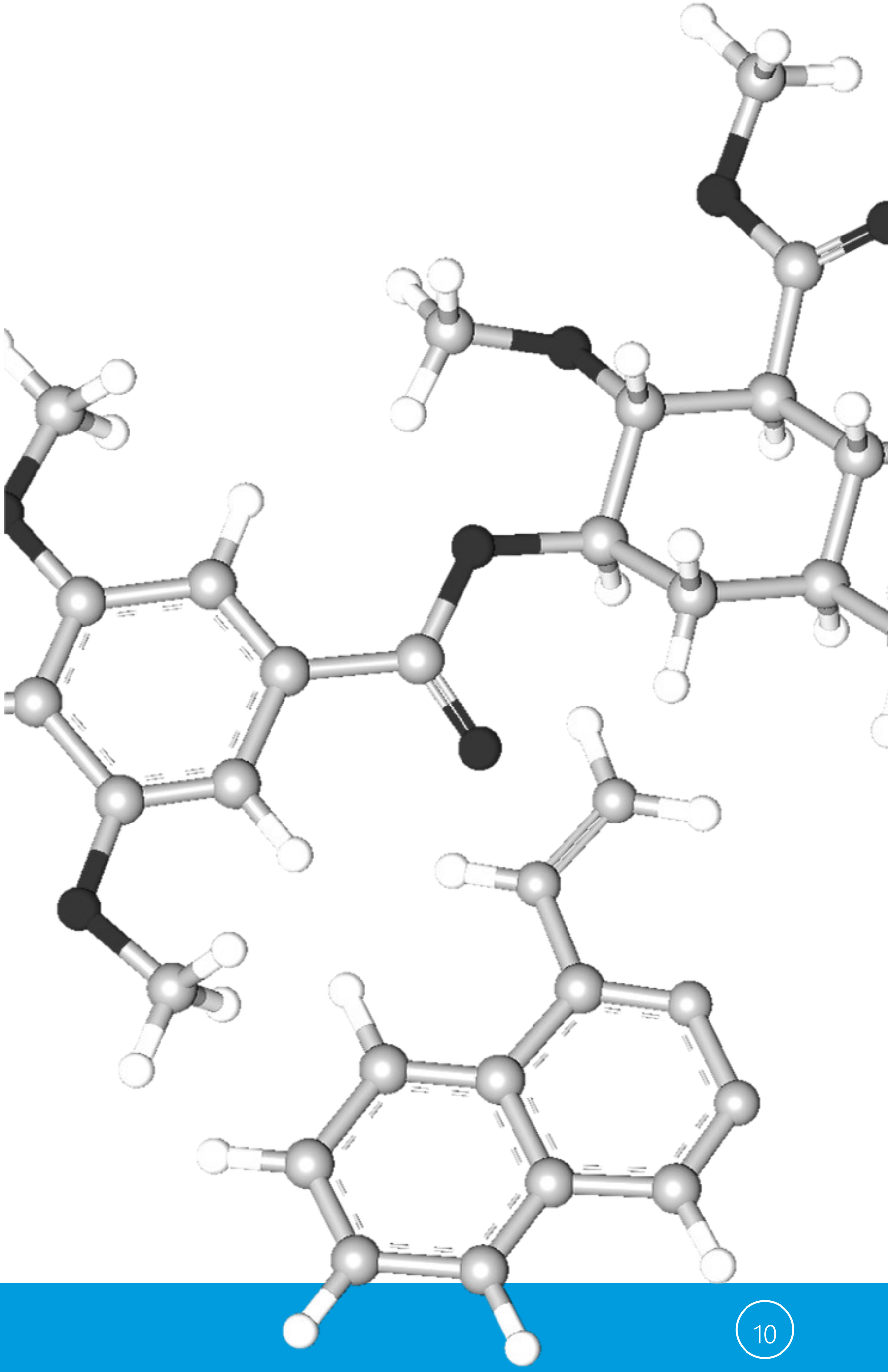
CHEMICAL FIXATION

- Achieved by immersing the sample in a fixative solution
- Fixatives act to minimize the loss of cellular components
- Penetration time ≠ fixation time
- Fixation may cause tissue discoloration
- Fixation may cause tissue swelling or shrinkage
- Fixation may affect downstream processes, like sample staining



TYPES OF FIXATIVES

- Coagulants
  - Dehydrating solutions
- Non-coagulants
  - Cross-linking solutions



COAGULANT FIXATIVES

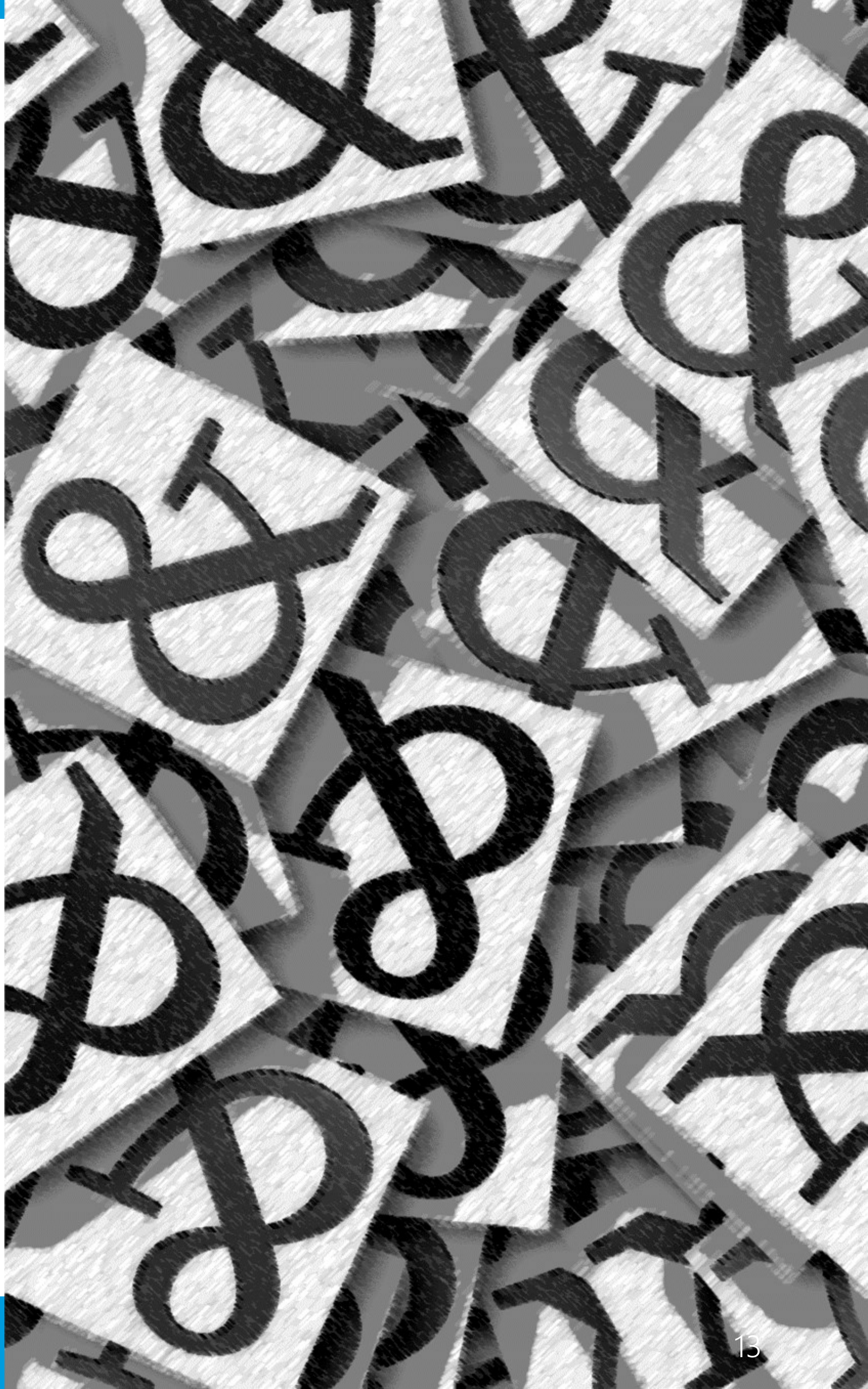
- Remove water from tissues leading to coagulation and denaturalization of proteins, mostly in the extracellular matrix
- Examples:
  - Ethanol solutions
  - Picric acid solutions
  - Carnoy-Lebrun Solution

NON-COAGULANT FIXATIVES

- Form chemical bonds, or cross-links, within and between cellular molecules
- Examples:
  - Formaldehyde
  - Glutaraldehyde
  - Osmium Tetroxide (OsO_4)

COMBINATION FIXATIVES

- Solutions that contain both coagulant and non-coagulant reagents
    - Bouin's Solution
    - Alcoholic Formalin
    - Alcohol-Formalin-Acetic Acid



IDEAL FIXATIVE

- Can be used with a wide variety of tissue
- Should penetrate both small and large specimens rapidly and preserve fixed tissue
- Should promote excellent staining
- Should have a long shelf life (> 1 yr) and be readily disposable or recyclable

Eltoum, I.; Fredenburgh, J.; Myers, R. B.; Grizzle, W. E. *J. of Histotechnology* 2001, *24* (3), 173–190.

FIXATION DURATION

$$d = k\sqrt{t}$$

d - Depth of penetration
k - Coefficient of penetration (diffusion)
t - Time

$$t = \frac{d^2}{k^2} \Rightarrow t = \frac{1\,mm}{k^2}$$

Medawar, P. B. *J. Royal Microscopical Society* 1941, *61* (1–2), 46–57.
Dempster, W. T. *Am. J. Anat.* 1960, *107* (1), 59–72.
Eltoum, I.; Fredenburgh, J.; Myers, R. B.; Grizzle, W. E. *J. of Histotechnology* 2001, *24* (3), 173–190.

FIXATION DURATION

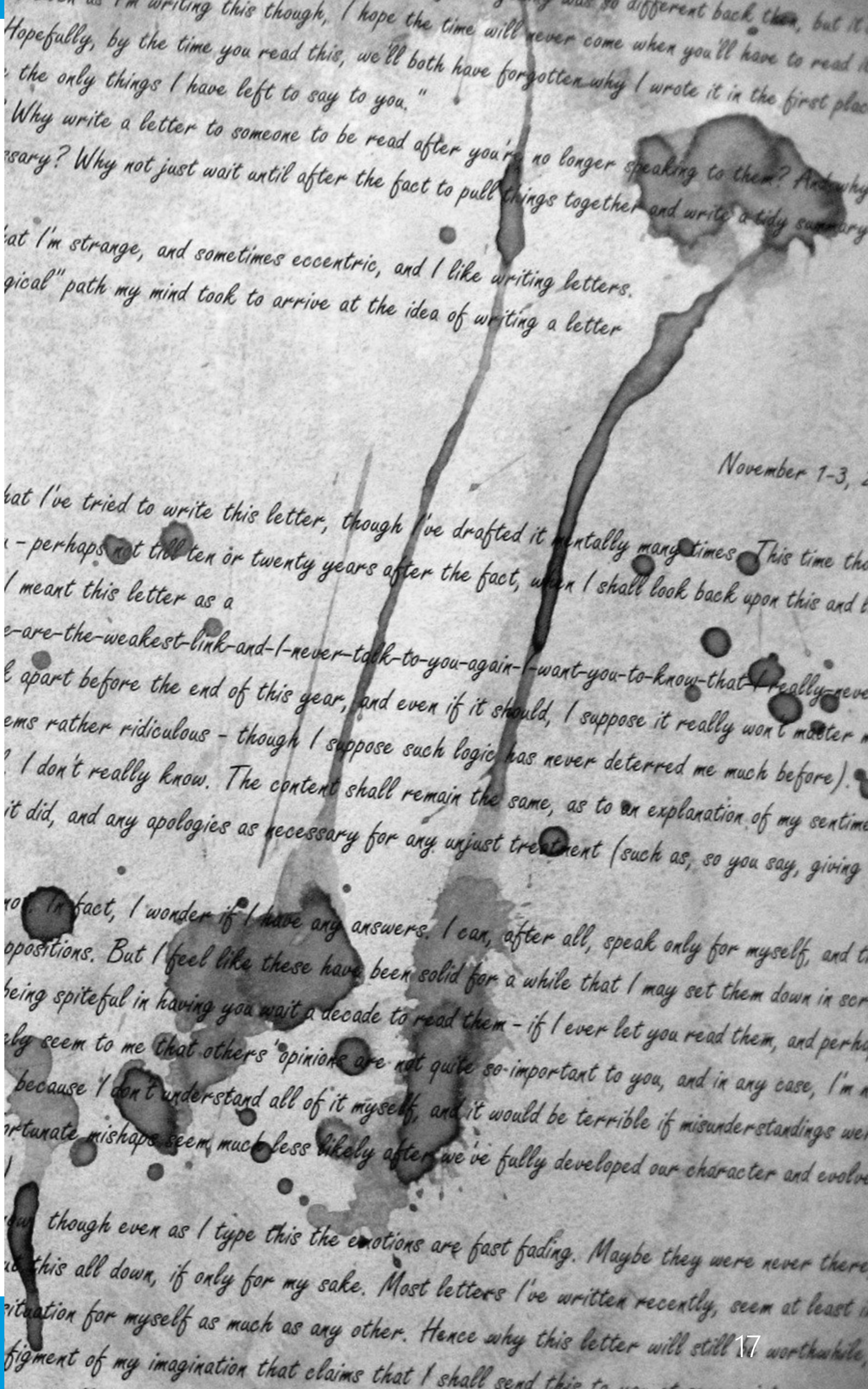
$$t = \frac{1\,mm}{k^2}$$

Fixative	*k*
10% acetic acid	0.25
10% formaldehyde	0.79
100% ethanol	1.0
10% formalin	1.14
1.2% picric acid	3.9
0.4% OsO_4	23.9

Medawar, P. B. *J. Royal Microscopical Society* 1941, *61* (1–2), 46–57.
Dempster, W. T. *Am. J. Anat.* 1960, *107* (1), 59–72.
Eltoum, I.; Fredenburgh, J.; Myers, R. B.; Grizzle, W. E. *J. of Histotechnology* 2001, *24* (3), 173–190.

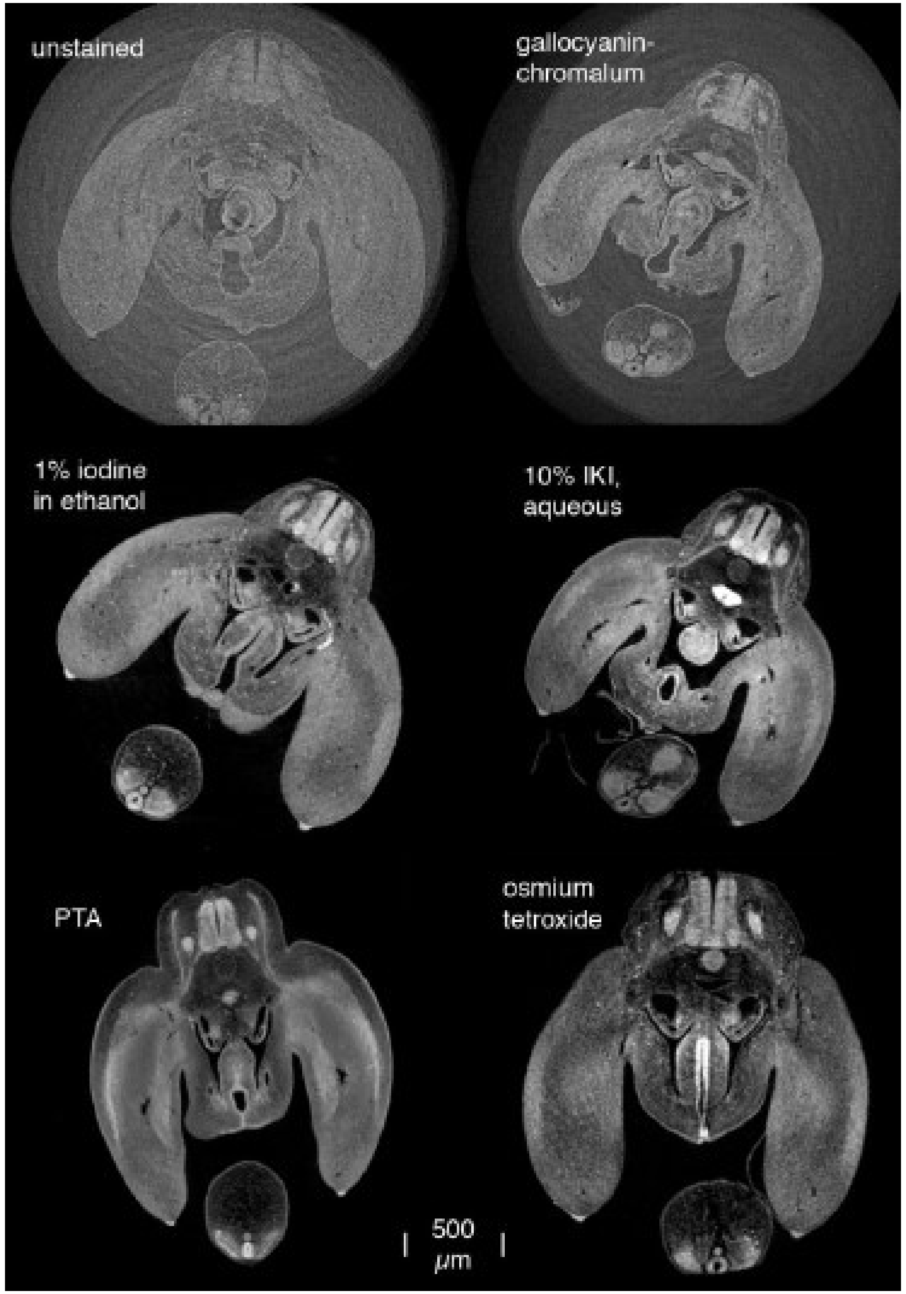
SAMPLE STAINING

- Staining improves contrast by increasing X-ray absorption within the sample
- Types of contrast agents
  - Perfusion – used for *in vivo* imaging
  - Diffusion – used for *ex vivo* imaging



Metscher, B. D. Developmental Dynamics 2009, 238 (3), 632–640

Histogram of pixel values (black) 0 – 255 (white)		Ethanol mean	Tissue mean	Tissue/ ethanol	Tissue st. dev.	Overlap percentile
	Unstained	41	53	1.3	7	17
	Gallocyanin-chromalum	41	66	1.6	10	6
	1% iodine in ethanol	41	100	2.4	18	1
	10% IKI	41	111	2.7	28	2.3
	PTA	41	125	3.1	28	0.4
	Osmium tetroxide	41	107	2.6	30	4



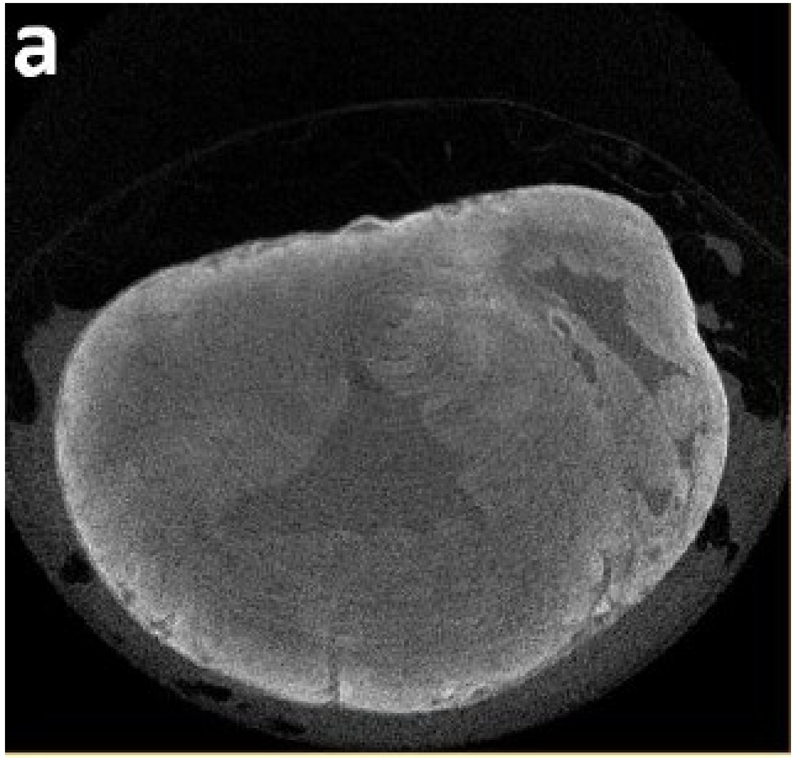
IKI – Lugol's solution (1% I2 + 2% KI)
PTA – phosphotungstic acid

TYPICAL STAINS

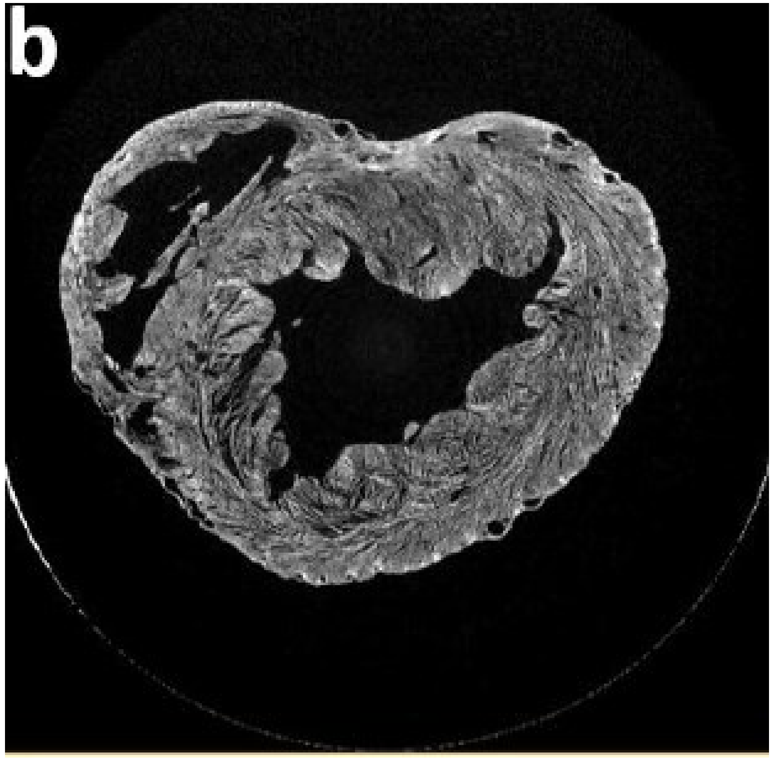
Abbreviation	Staining agent components
PTA	1% (w/v) phosphotungstic acid in water
ePTA	1% PTA (w/v) in 90% ethanol in water (v/v)
IKI (Lugol's solution)	1% iodine metal (I_2) + 2% potassium iodide (KI) in water
I2E, I2M	1% iodine metal (I_2) dissolved in 100% ethanol (I2E) or methanol (I2M)
OsO_4	1-2% osmium tetroxide

NEXT STEPS

- To dry or not to dry?



Wet pig heart



Dried pig heart

Pallares-Lupon, N. et al. 2021. https://doi.org/10.1101/2021.07.29.454121.

NEXT STEPS

- Sample mounts should prevent sample movement during CT data collection
  - Add material to wet mixtures to limit movement

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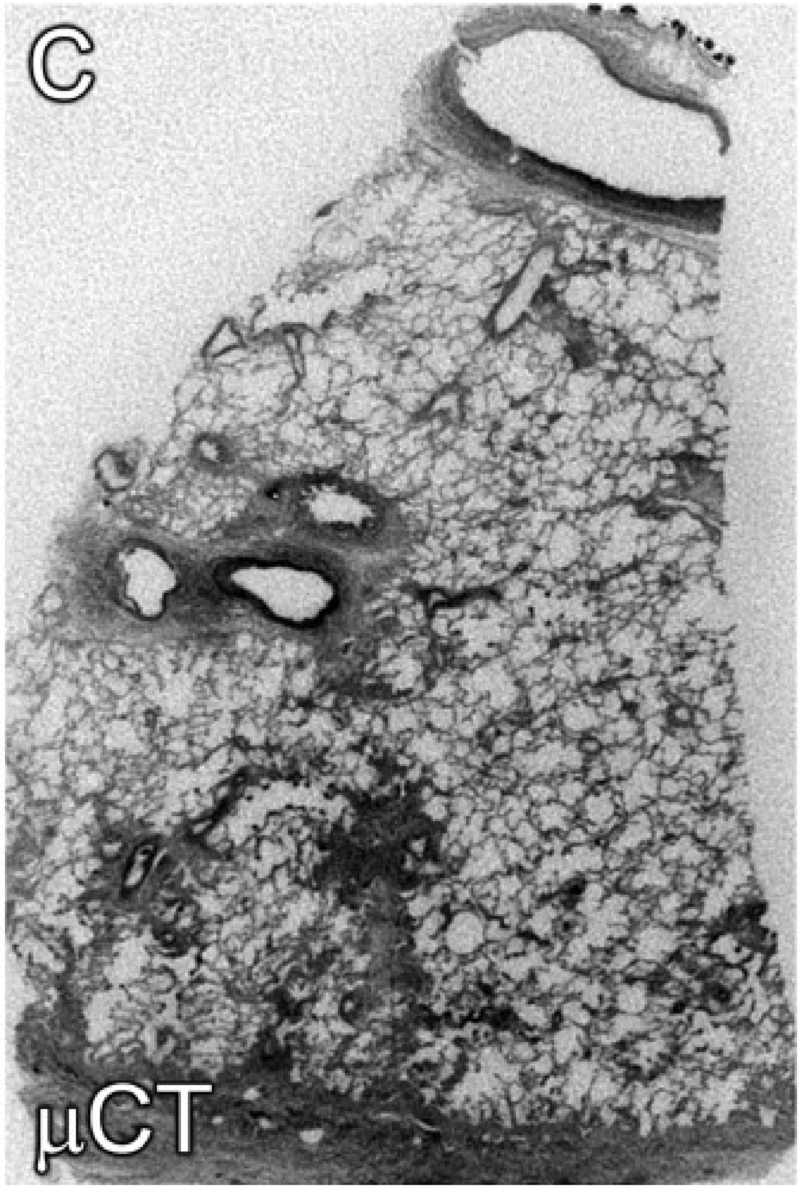
- Sample mounts should prevent sample movement during CT data collection
    - Add material to wet mixtures to limit movement
    - Embed in paraffin, agarose, resin



Scott, A. E. *et al. PLoS One* **2015**, *10* (6), e0126230.

NEXT STEPS

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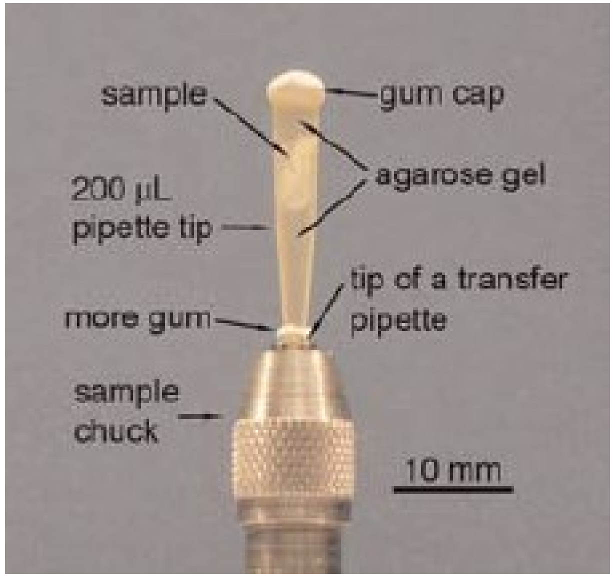

Metscher, B. D. *Cold Spring Harb Protoc* **2011**, *2011* (12), pdb.prot067033.

LET’S LOOK AT SOME EXAMPLES

INSECT - MIDGE

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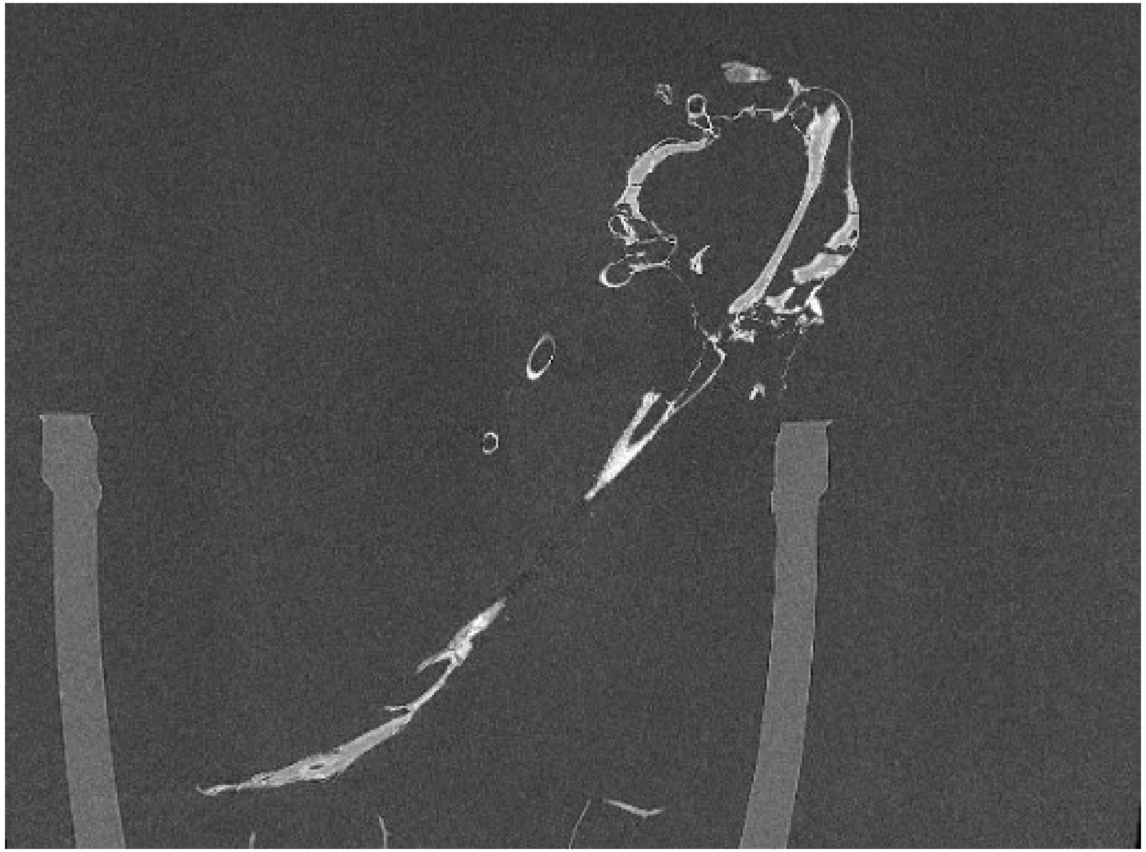
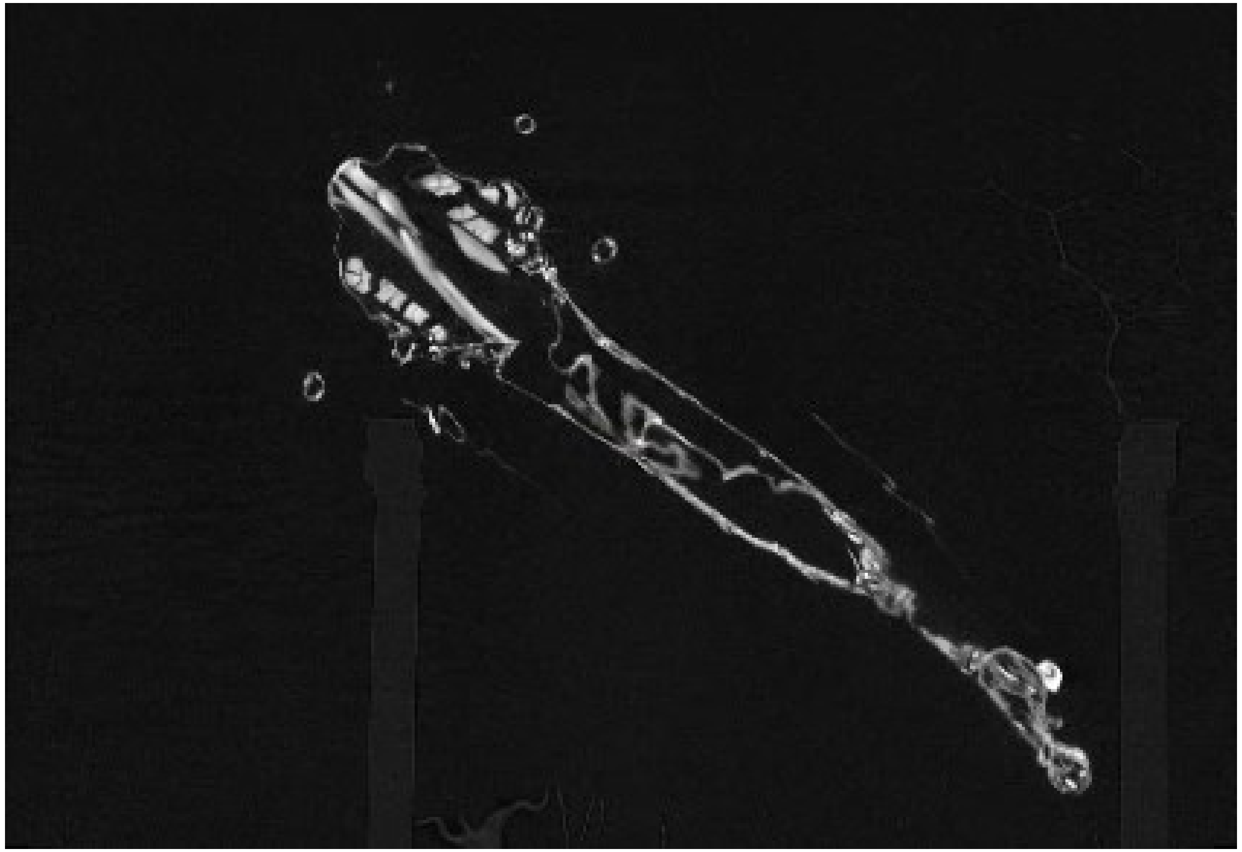


unstained



iodine stained

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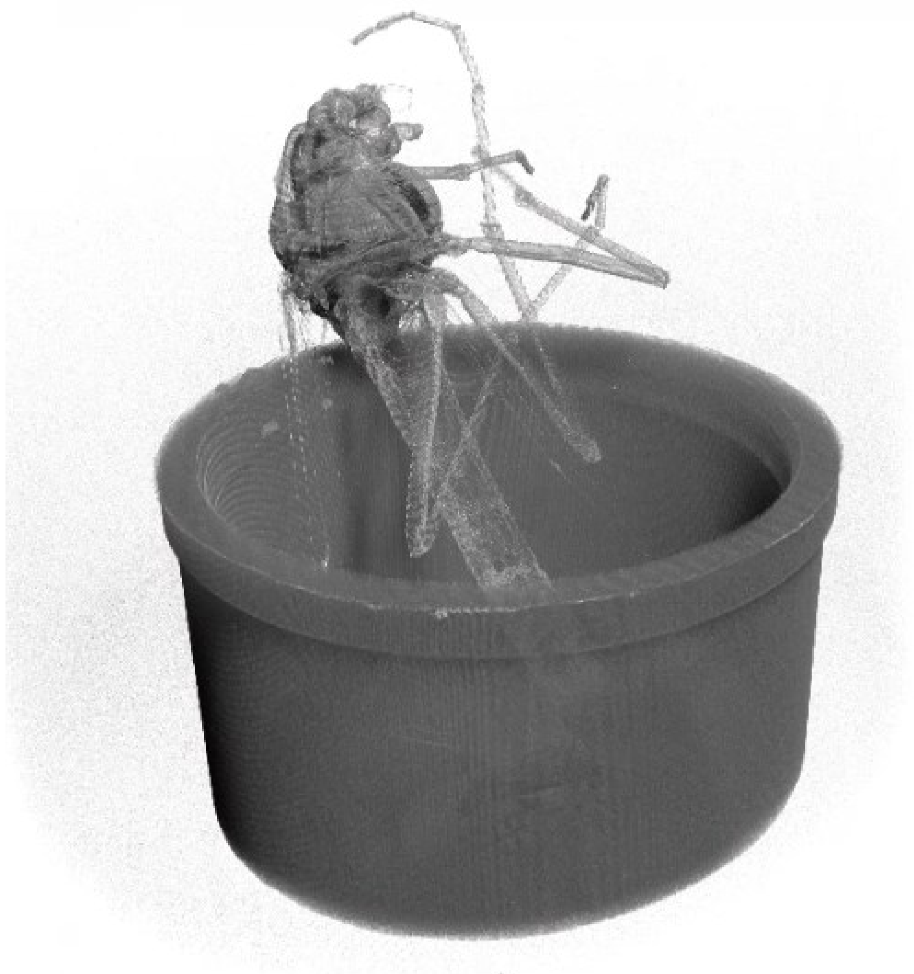
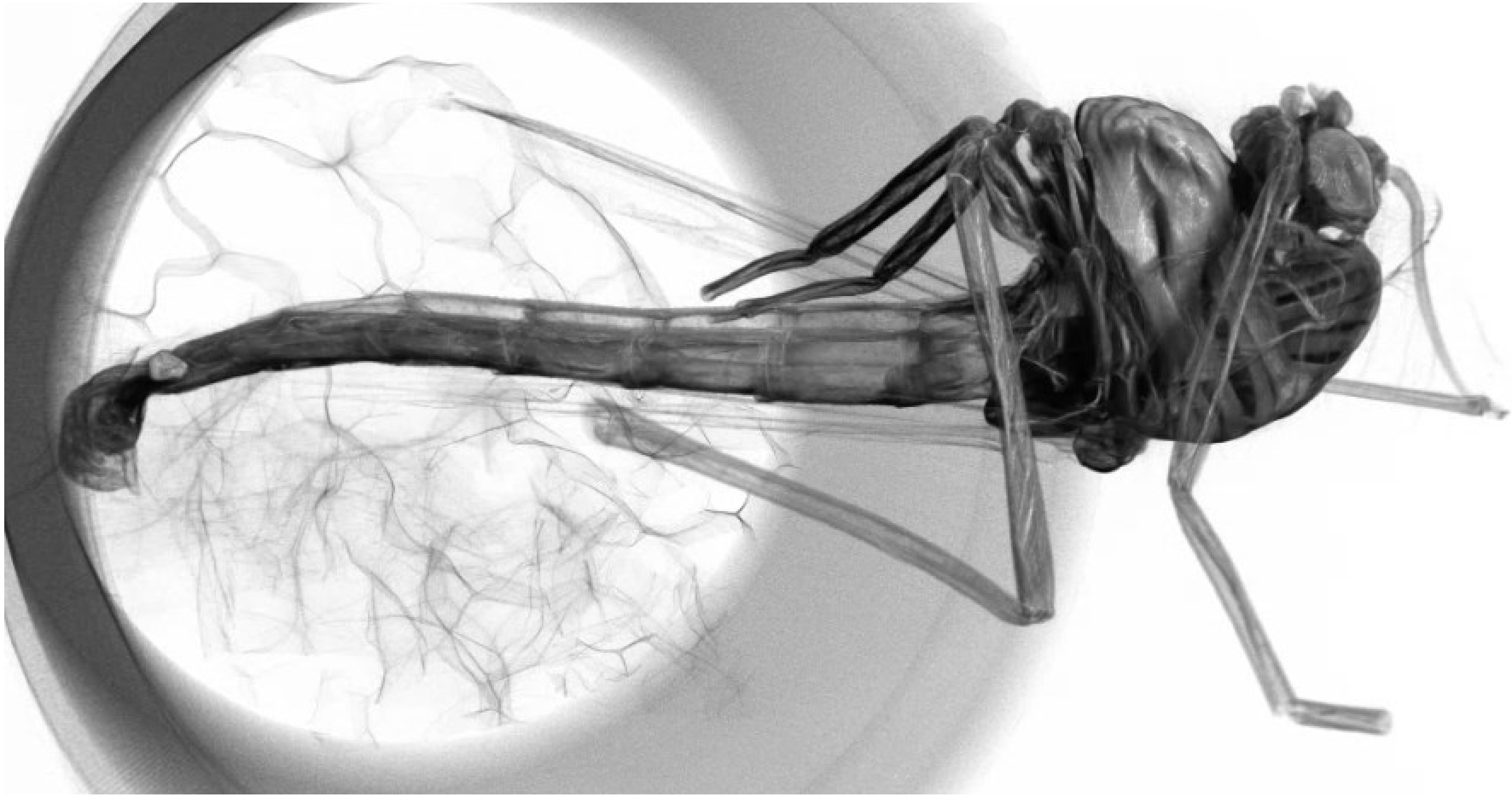


unstained

iodine stained

INSECT - MIDGE



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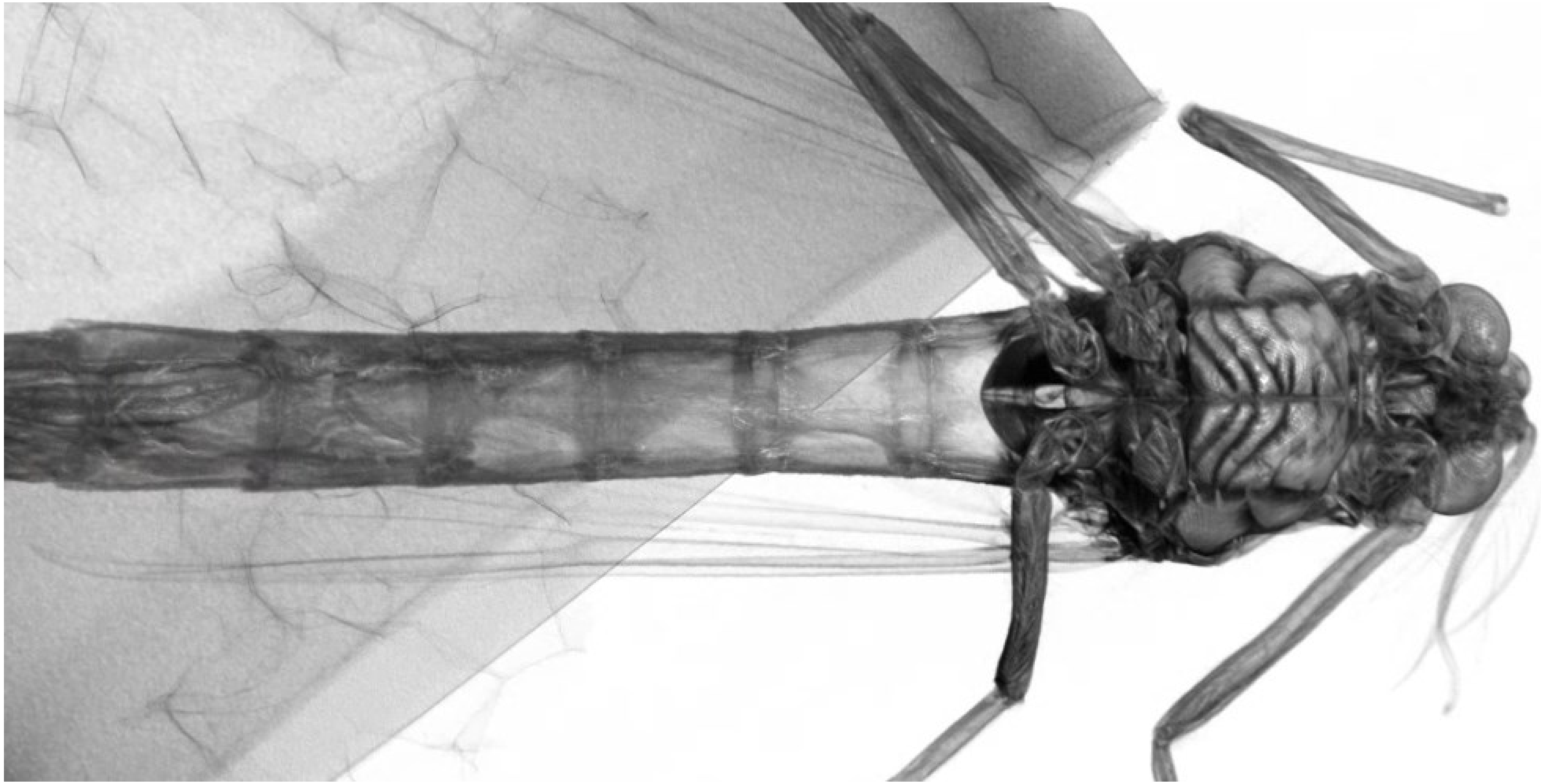
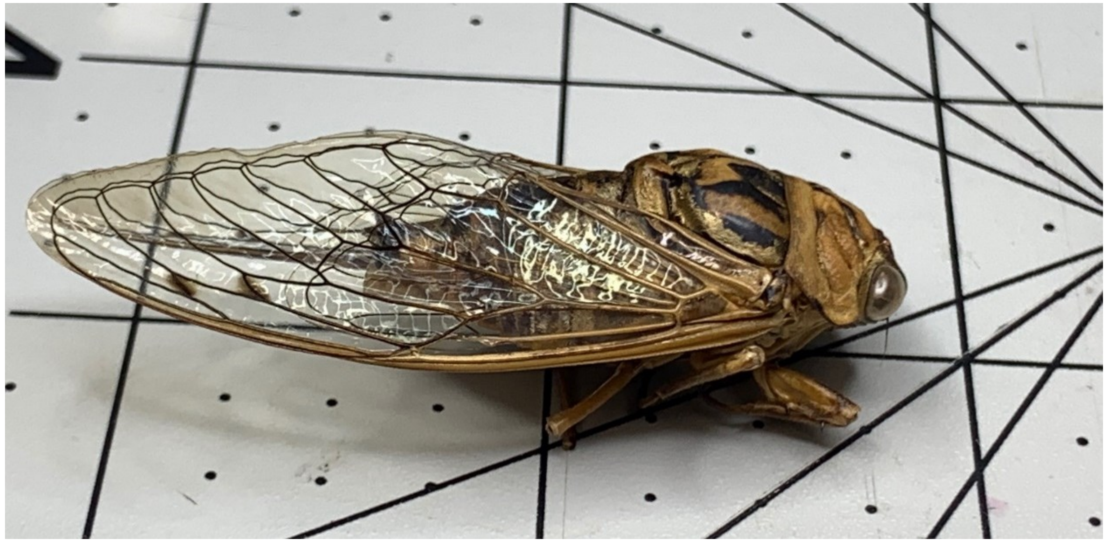
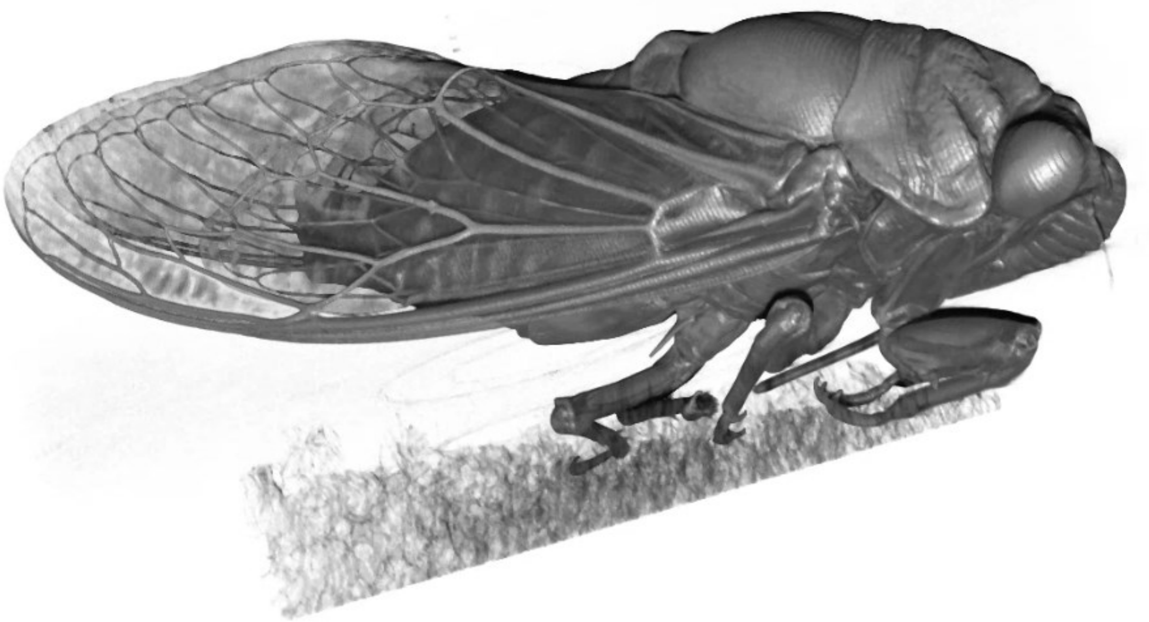


INSECT - MIDGE

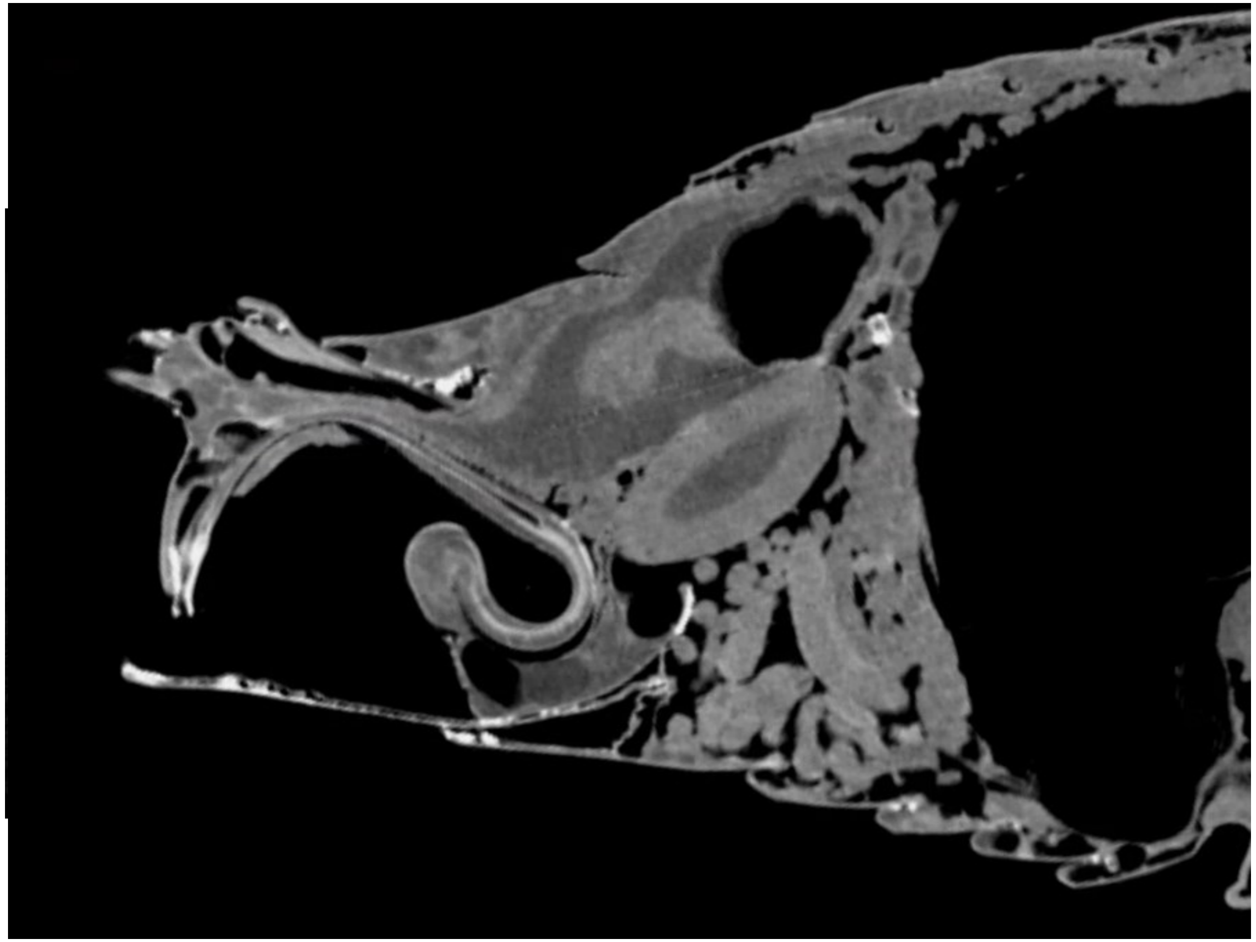
INSECT - CICADA



INSECT - CICADA

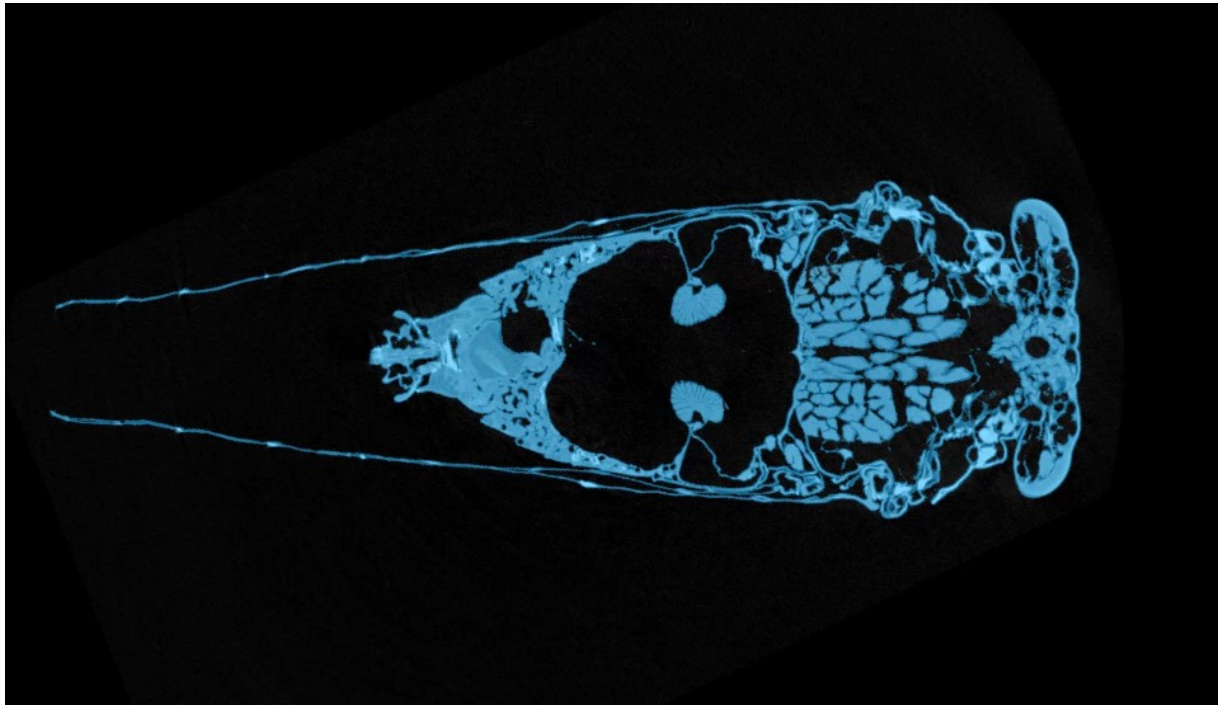


3D volume



2D cross-section

INSECT - CICADA



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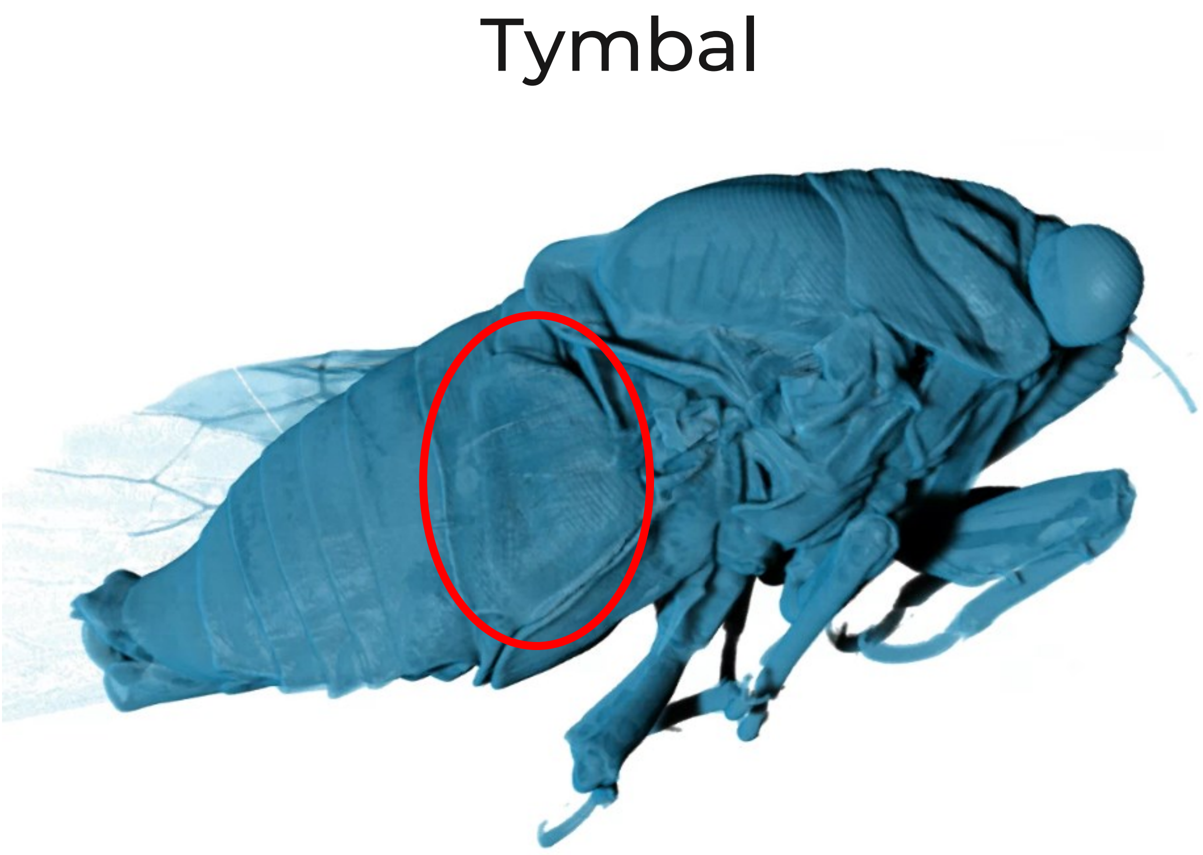
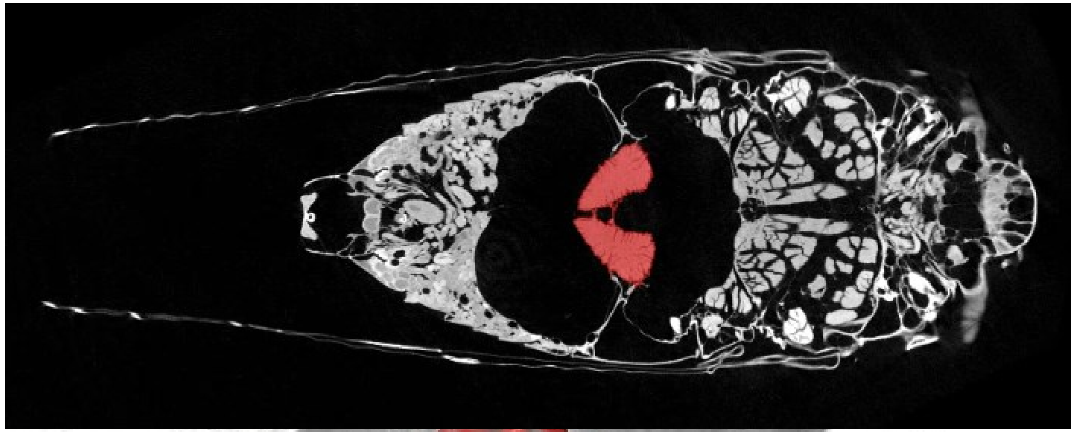
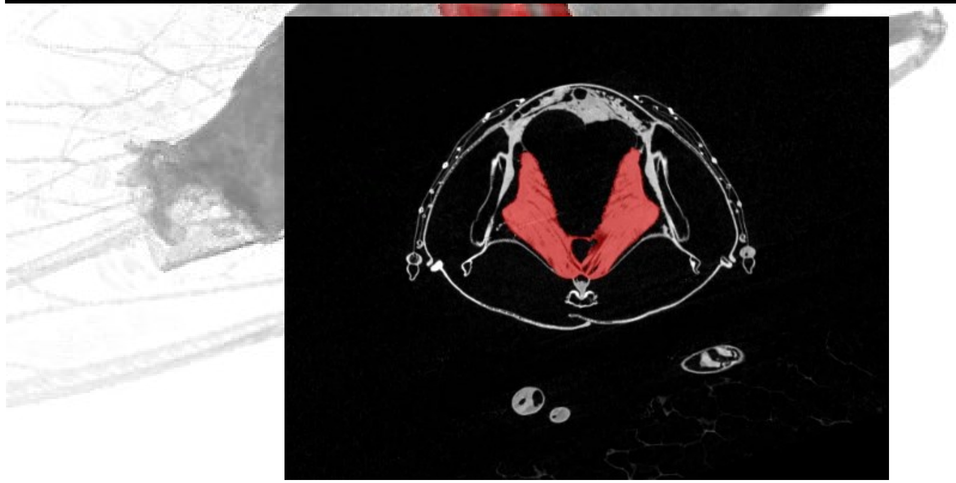
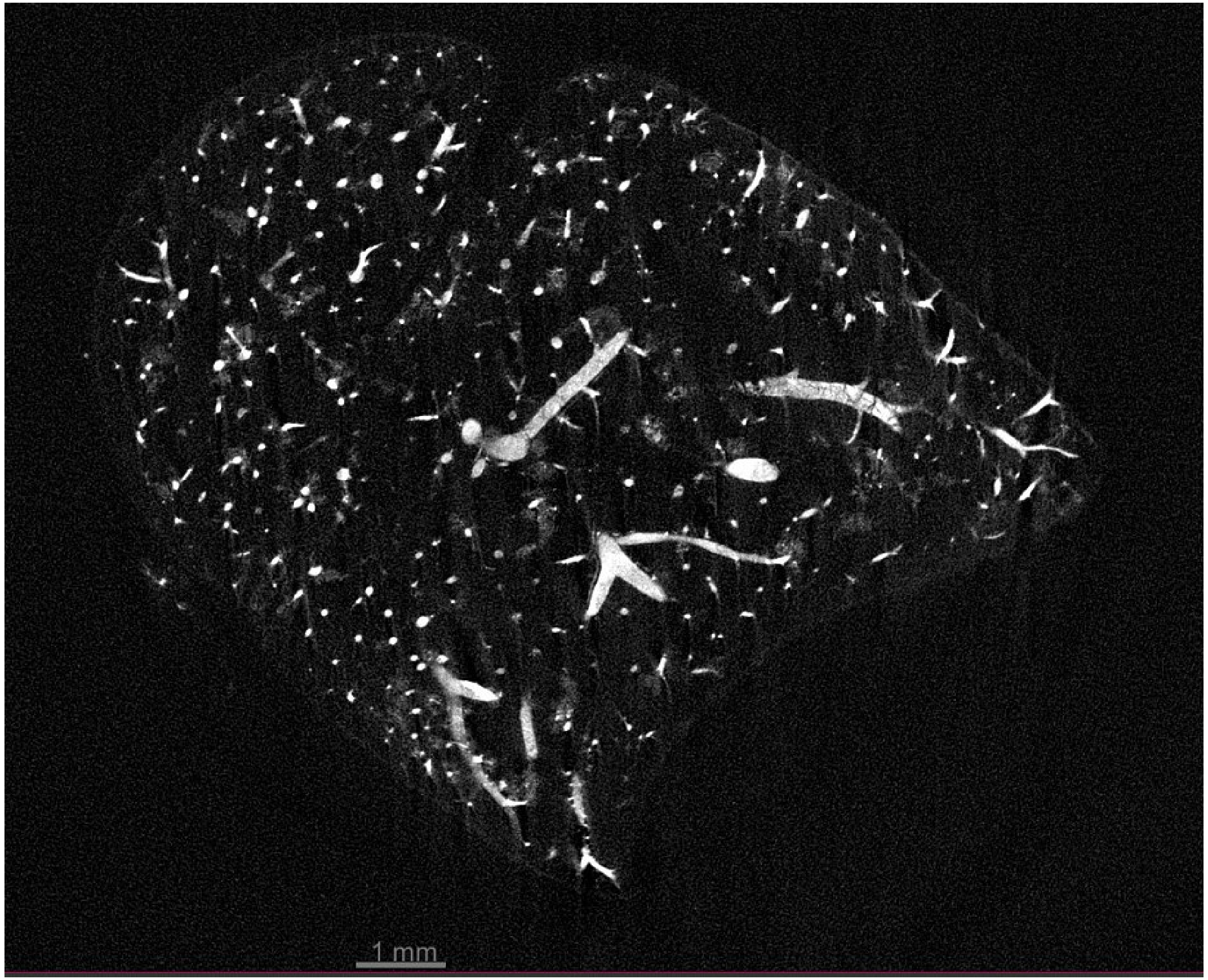






MOUSE LIVER



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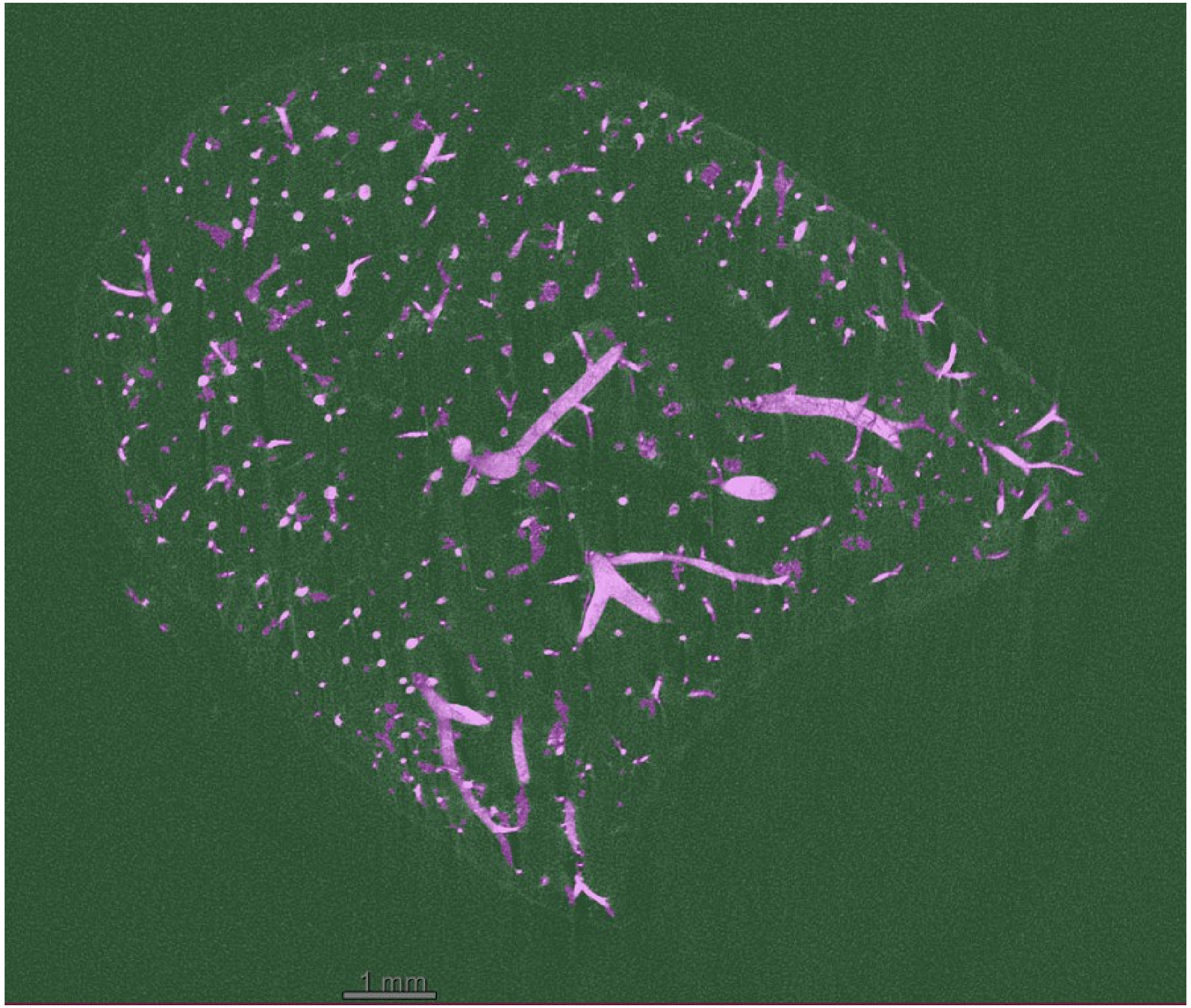

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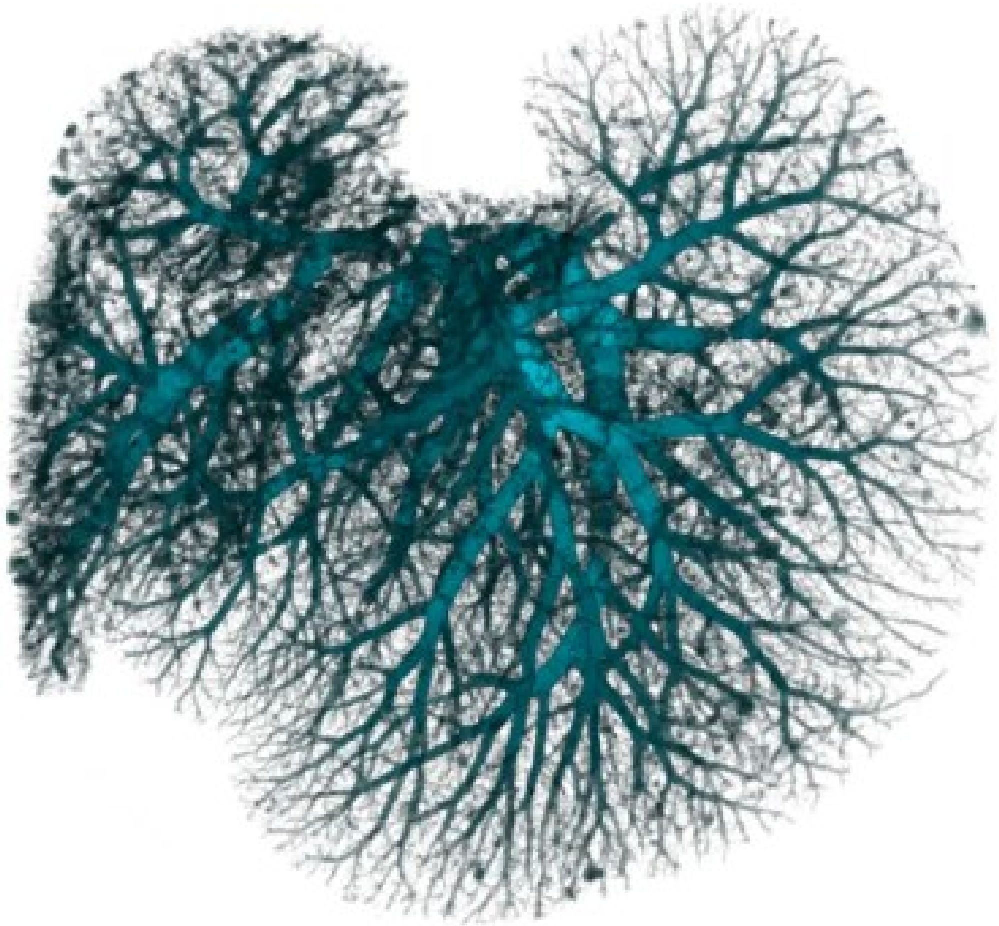
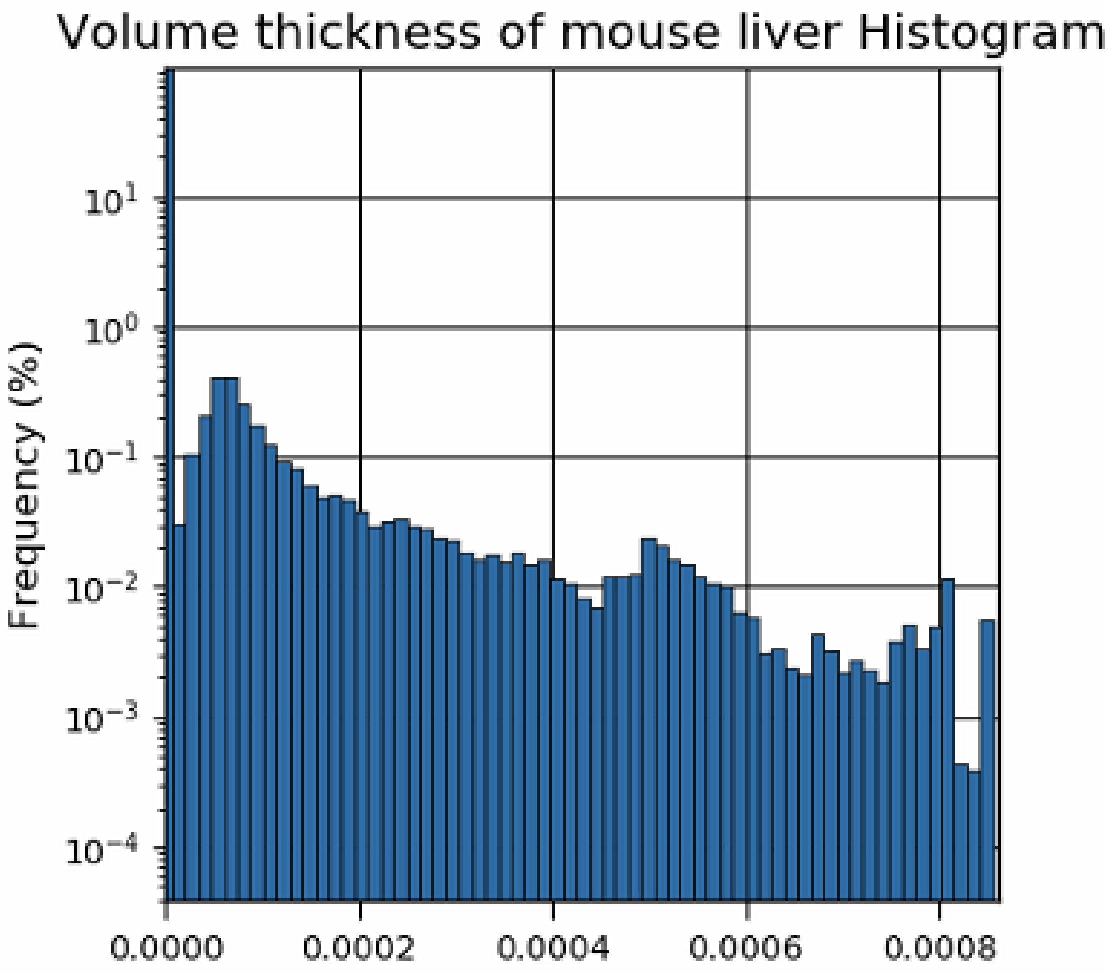
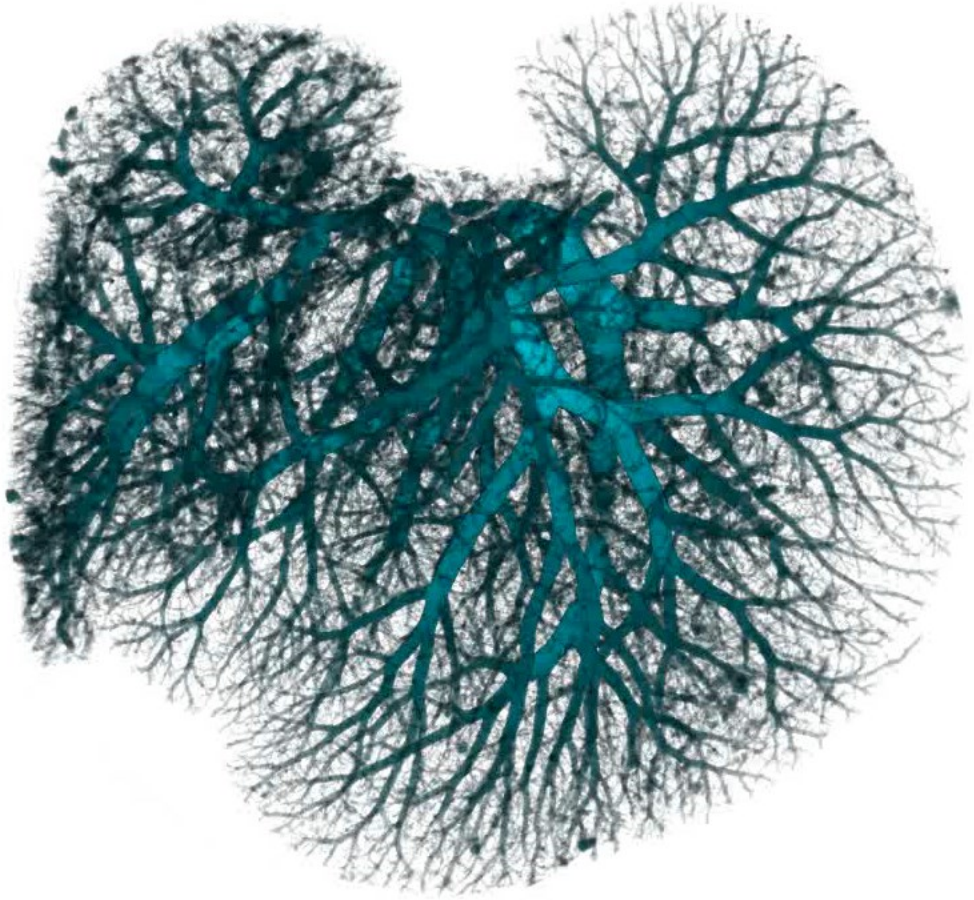



Volume thickness of mouse liver Histogram
Frequency (%)
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
0.0000
0.0002
0.0004
0.0006
0.0008

MOUSE LIVER



INSECT - TERMITE

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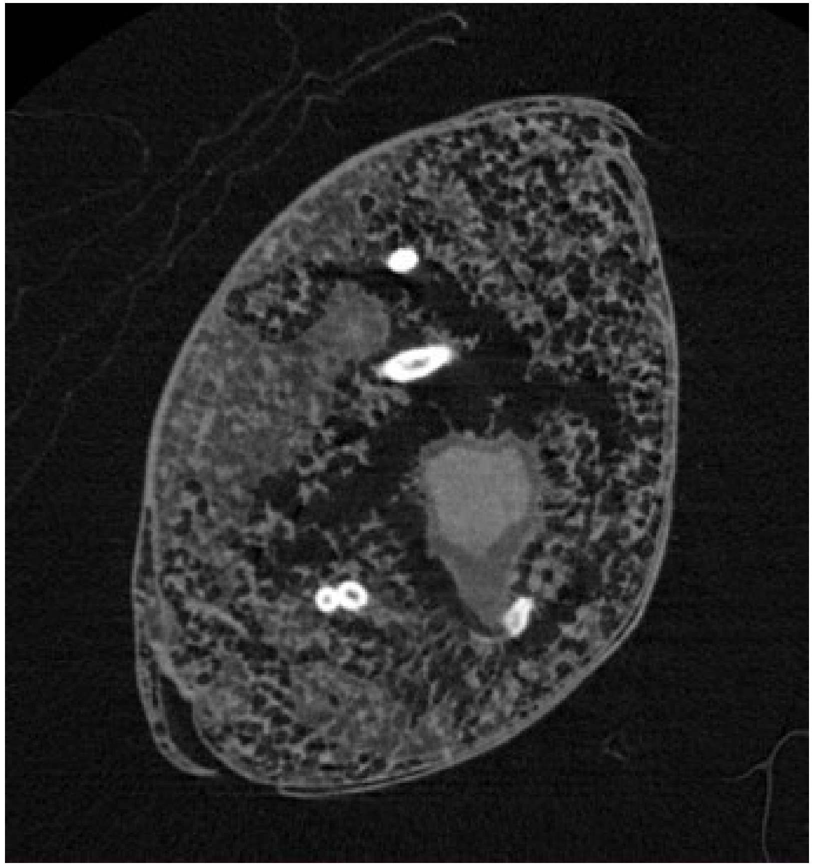

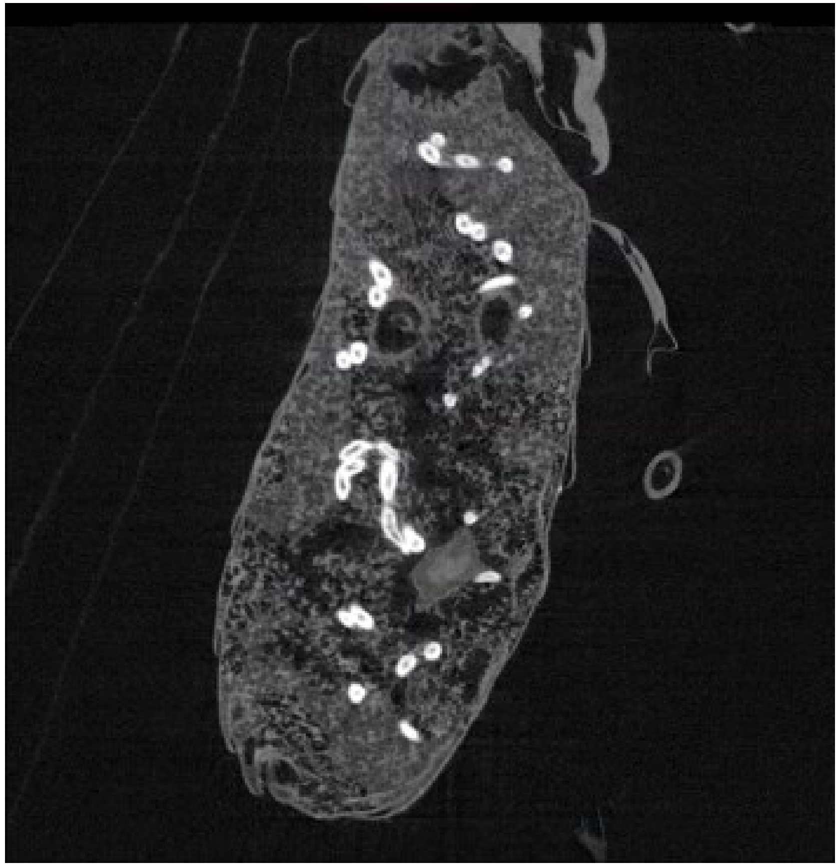

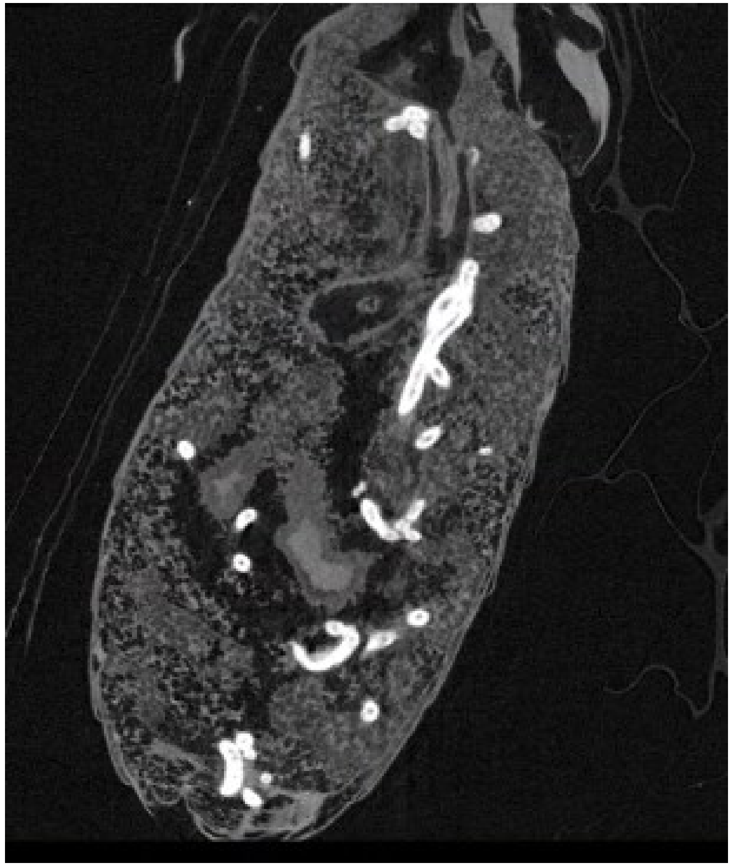

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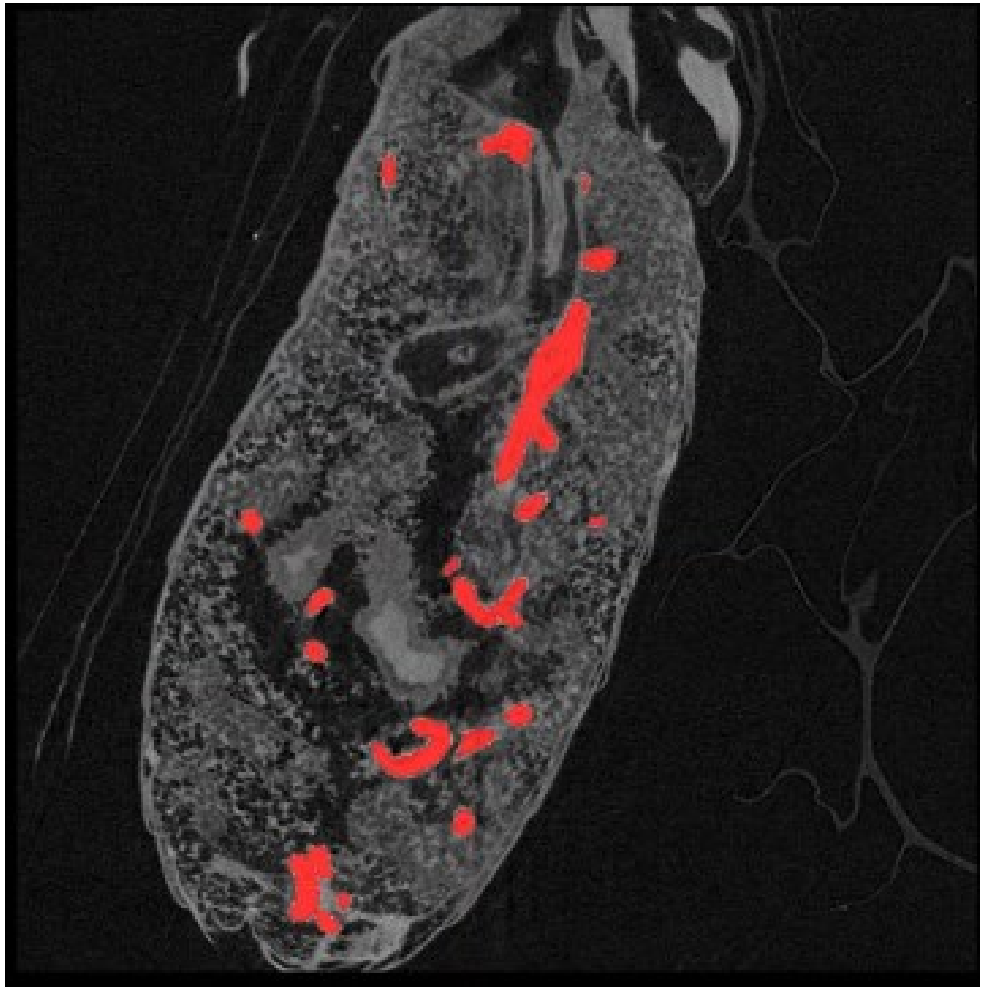
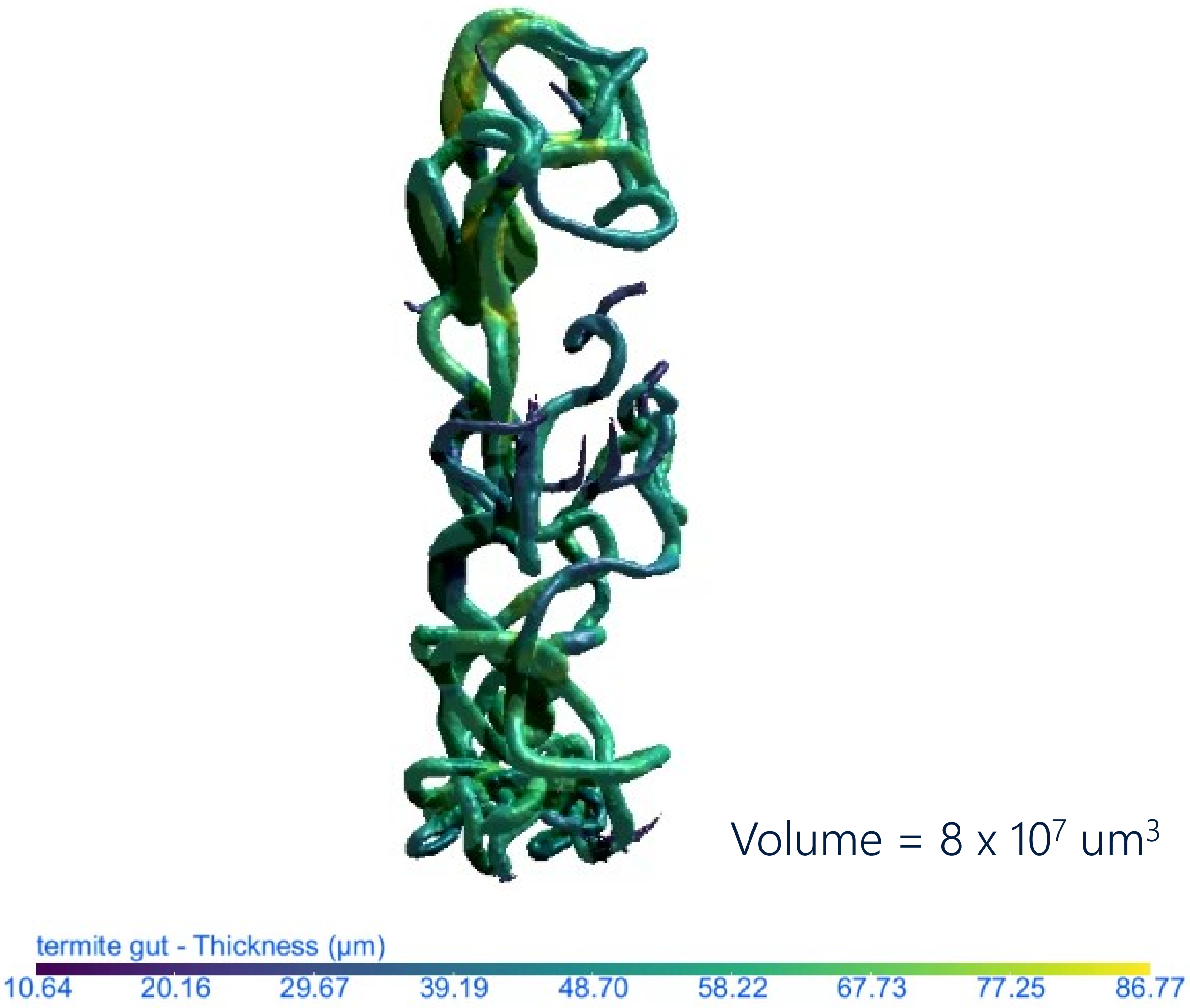
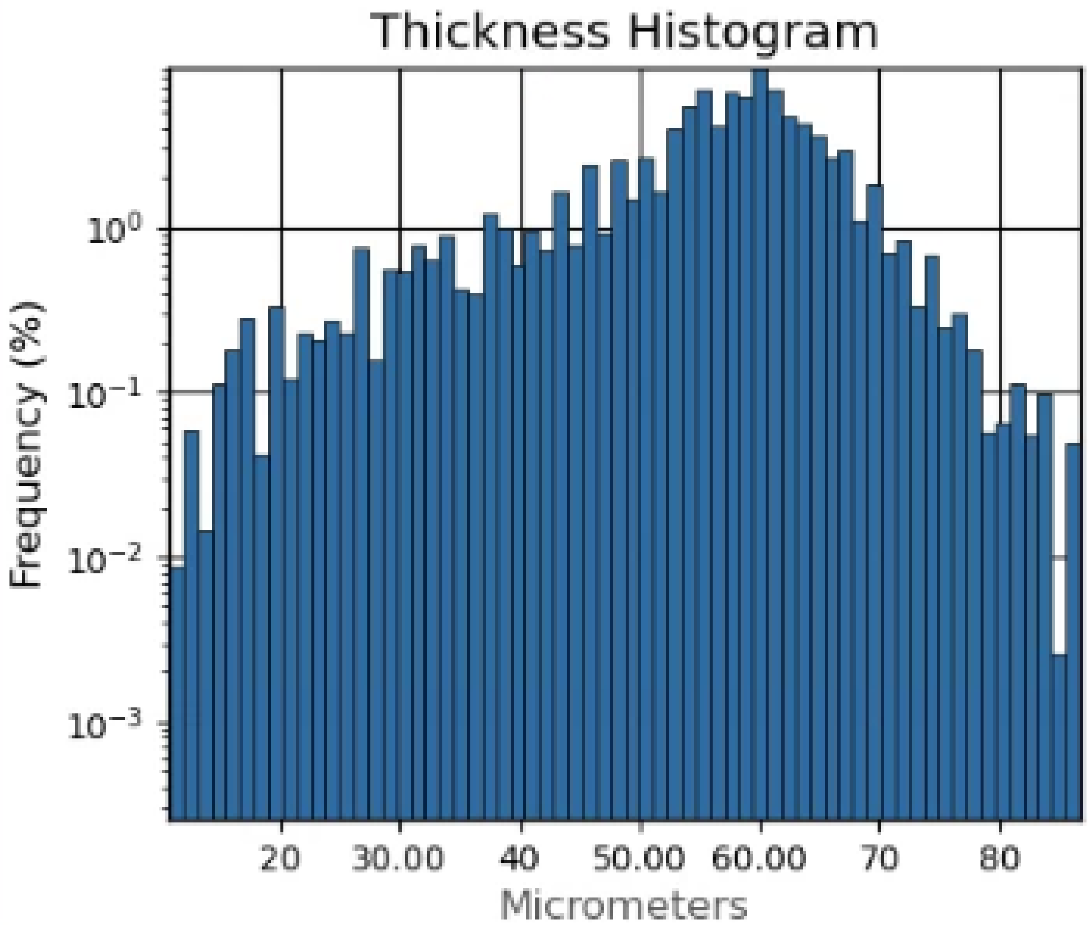


INSECT - TERMITE



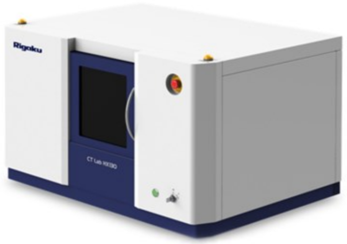
Volume = 8 x 10^7 um^3



Minimum:	10.64 µm
Maximum:	86.77 µm
Mean:	55.41 µm
Standard Deviation:	10.81 µm

IMAGES WERE COLLECTED ON…

nano3DX CT Lab HX CT Lab GX



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https://imaging.rigaku.com/learning/x-ray-ct-webinars-and-workshops

webinar

Q & A SESSION

Angela Criswell

Tom Concolino

We'll follow up with
your questions.

Recording will be
available tomorrow.

Register for
seminar.

X-RAY MICROSCOPY SEMINAR & WORKSHOP

UNIVERSITY OF DELAWARE

WEDNESDAY, MARCH 30, 2022

Year End Survey

will be sent to you tomorrow

THANK YOU FOR JOINING US
SEE YOU NEXT YEAR!