USING SYNCHROTRON RADIATION TECHNIQUES AS A TOOL IN INVERTEBRATE PALEONTOLOGY

Jerit Mitchell, MSc student

University of Regina

June 8

CAP Congress 2022

University of Regina

Canadian Association of Physicists

Association canadienne des physiciens et physiciennes

MOTIVATION

Jurassic Park: Fact or Fiction?

- Paleontology research has become increasingly analytical in the last decade, employing techniques and analysis usually governed by chemists and physicists
- Insects make up 80% of known species and give information on diversity of life on Earth millions of years ago.

What is Amber?

- Amber is fossilized tree resin
- Small creatures can get trapped before the resin hardens.
- Similar properties to glass, which acts as a shield for inclusions.
- Best preservation of organic, soft tissue material in life-like 3-D

Turtle Beetle from Baltic Amber (44 million years old)

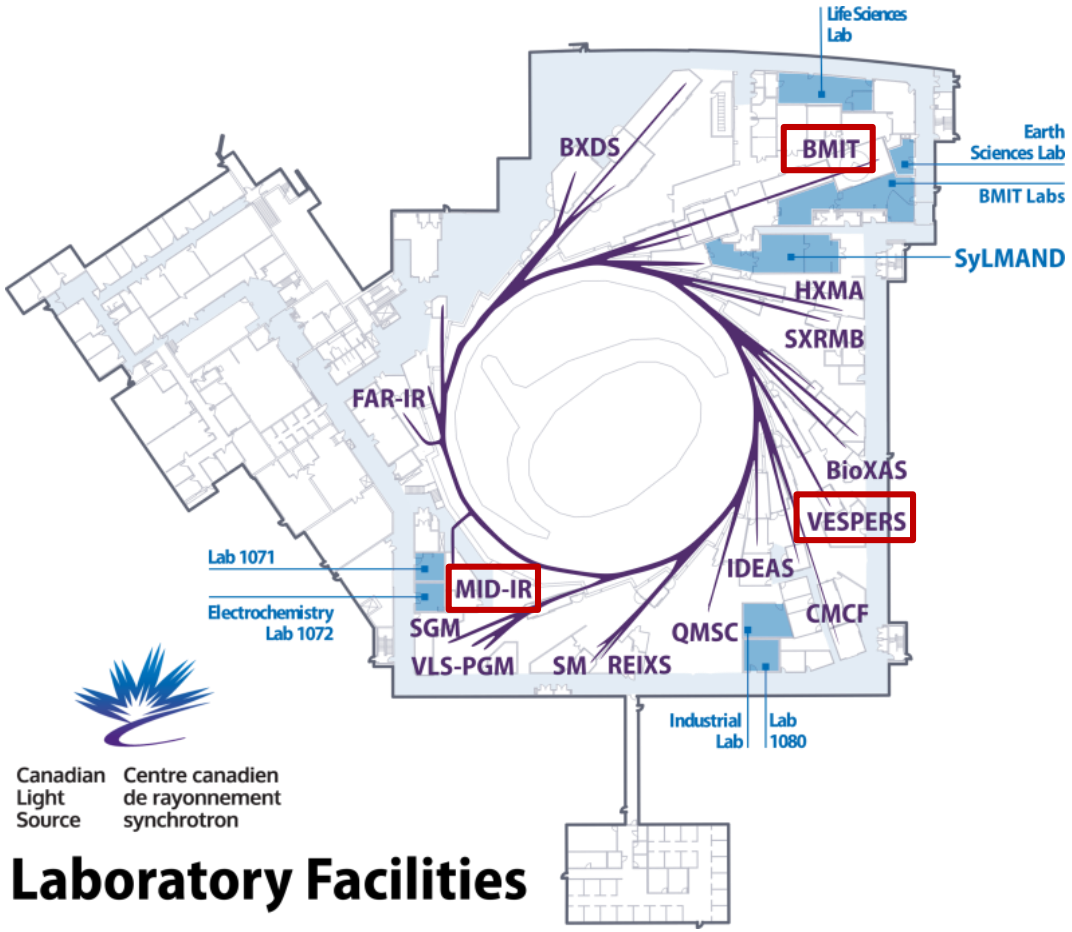
Scale Bar = 1mm
Leaf Beetle from Baltic Amber.

THE SYNCHROTRON ADVANTAGE

Synchrotron radiation is produced by acceleration of charged particles in specialized light source facilities

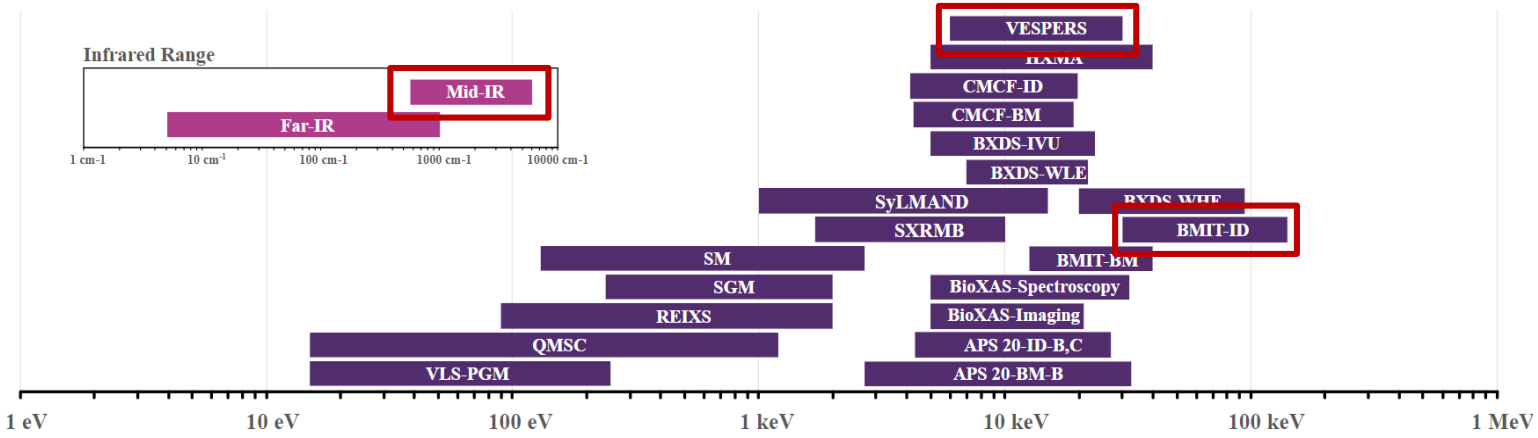
Synchrotron radiation is useful since its:

- Suited for wide range of analysis using specialized beamlines
- Non-destructive
- High Brightness => better resolution + faster acquisition times
- Useful for heterogeneous geological samples



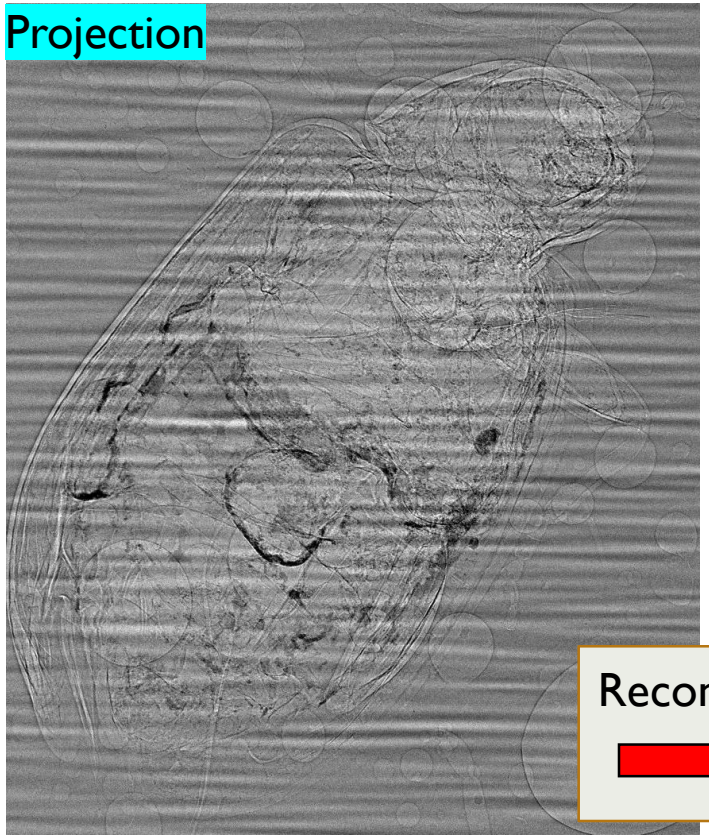
Selected synchrotron techniques:

- **Computed Tomography (CT)**
- **Fourier Transform Infrared Spectroscopy (FTIR)**
- **X-Ray Fluorescence (XRF)**



COMPUTED TOMOGRAPHY (CT)
PHYSICS PRINCIPLES

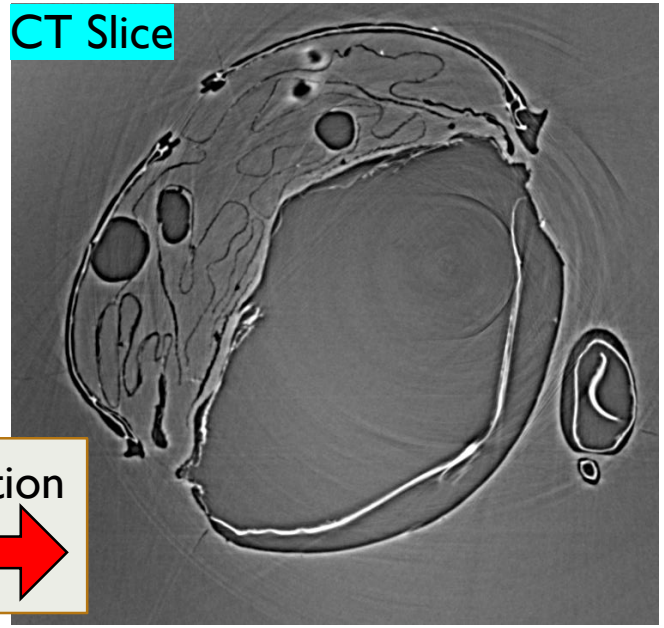
Biomedical Imaging and Therapy Facility



Reconstruction

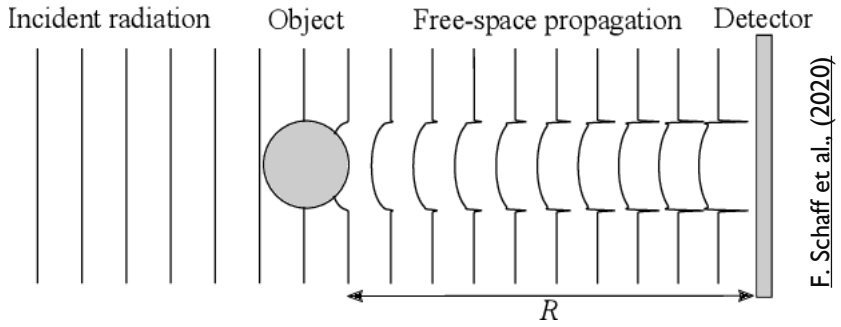
CT Basics

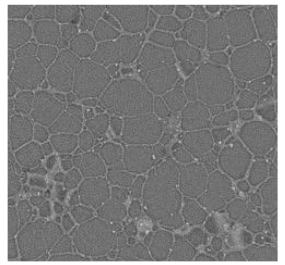
- Bulk X-Ray absorption through a sample, reconstructed over 180° in slices
- Creates a lifelike 3D model containing density information



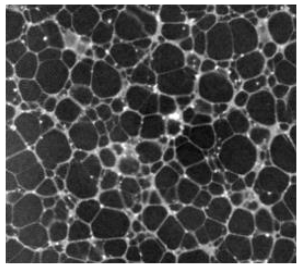
Propagation Phase Contrast (PPC)

- Records interference of waves refracted from the sample in order to highlight structures with small changes in indices of refraction
- Synchrotron light provides highly coherent X-Rays





PPC

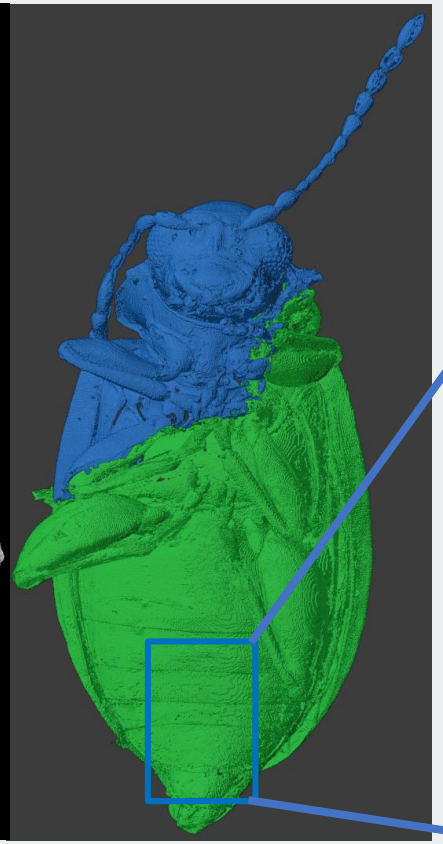


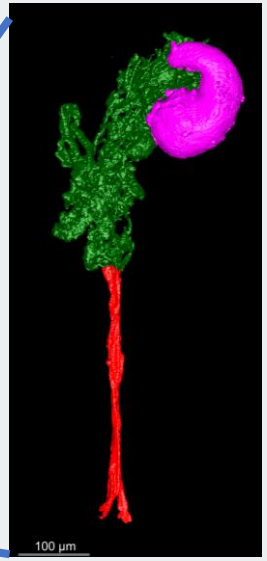
CT RESULTS

Leaf beetle from Baltic Amber (44 Ma)
- Genitalia is preserved, useful for taxonomy
- Used for organic preservation studies

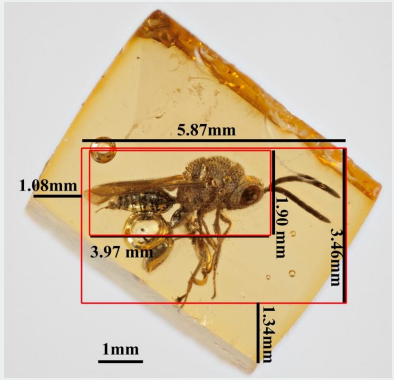
Wasp from Dominican Amber (16 Ma)
- Better preservation than Baltic amber
- Individual muscle fibers are preserved
- Used for chemical studies

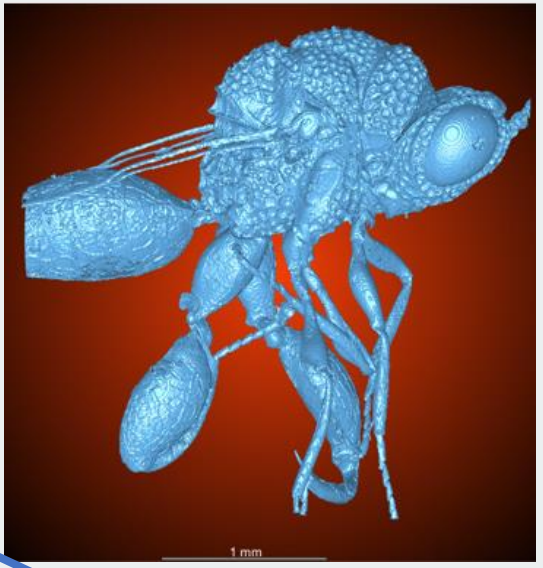
Leaf beetle

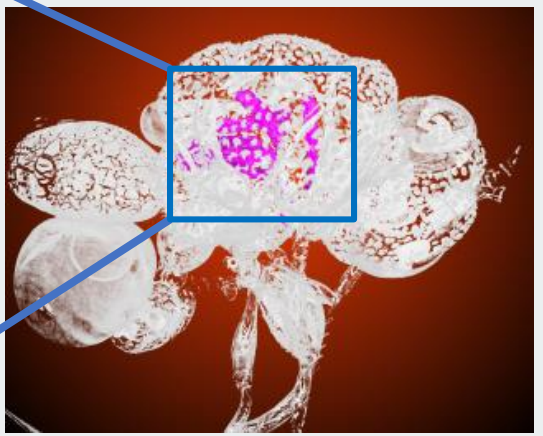




Chalcidoid wasp







INFRARED SPECTROSCOPY (FTIR)
PHYSICAL PRINCIPLES

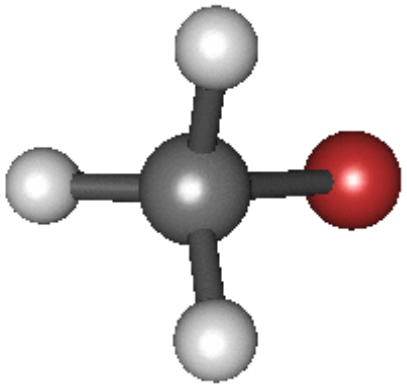
IR Basics

- Atomic bonds ≈ Harmonic oscillator with characteristic vibrational frequency
- The main vibrational excitations for molecules occur in the infrared region (wavenumber range 4000 cm^{-1} – 400 cm^{-1})
- Provides a finger prints for organic functional groups in a sample

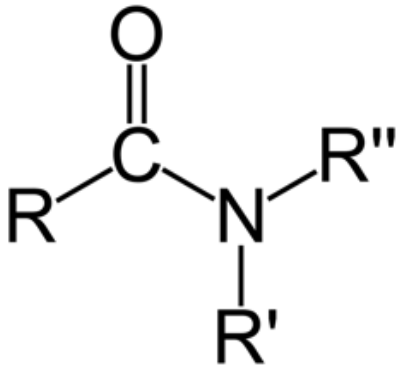
Synchrotron Fourier Transform Spectroscopy

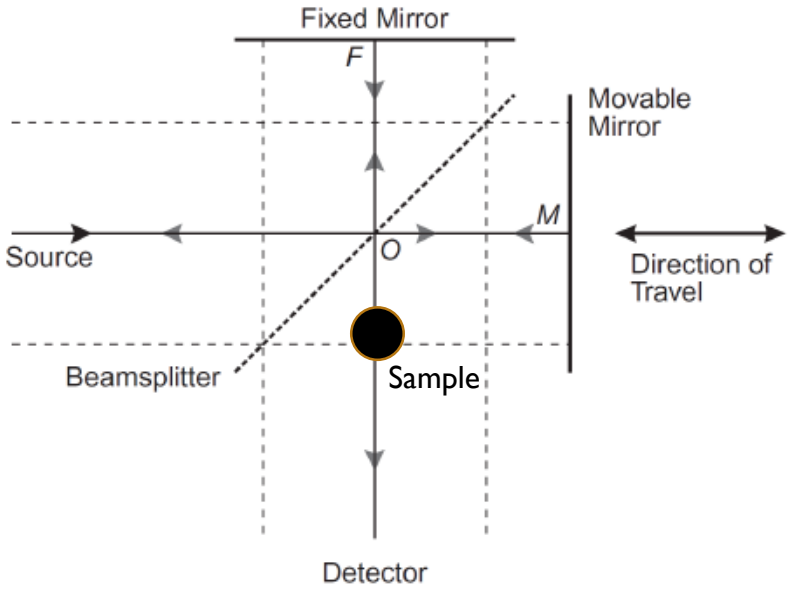
- Using a polychromatic synchrotron IR beam can speed up spectral acquisition and improve signal-to-noise ratio
- A Michelson interferometer is used with a movable mirror to record the spectrum in position space, which can be converted back to frequency space

"Stretching Mode ν_s"



Amide Functional Group

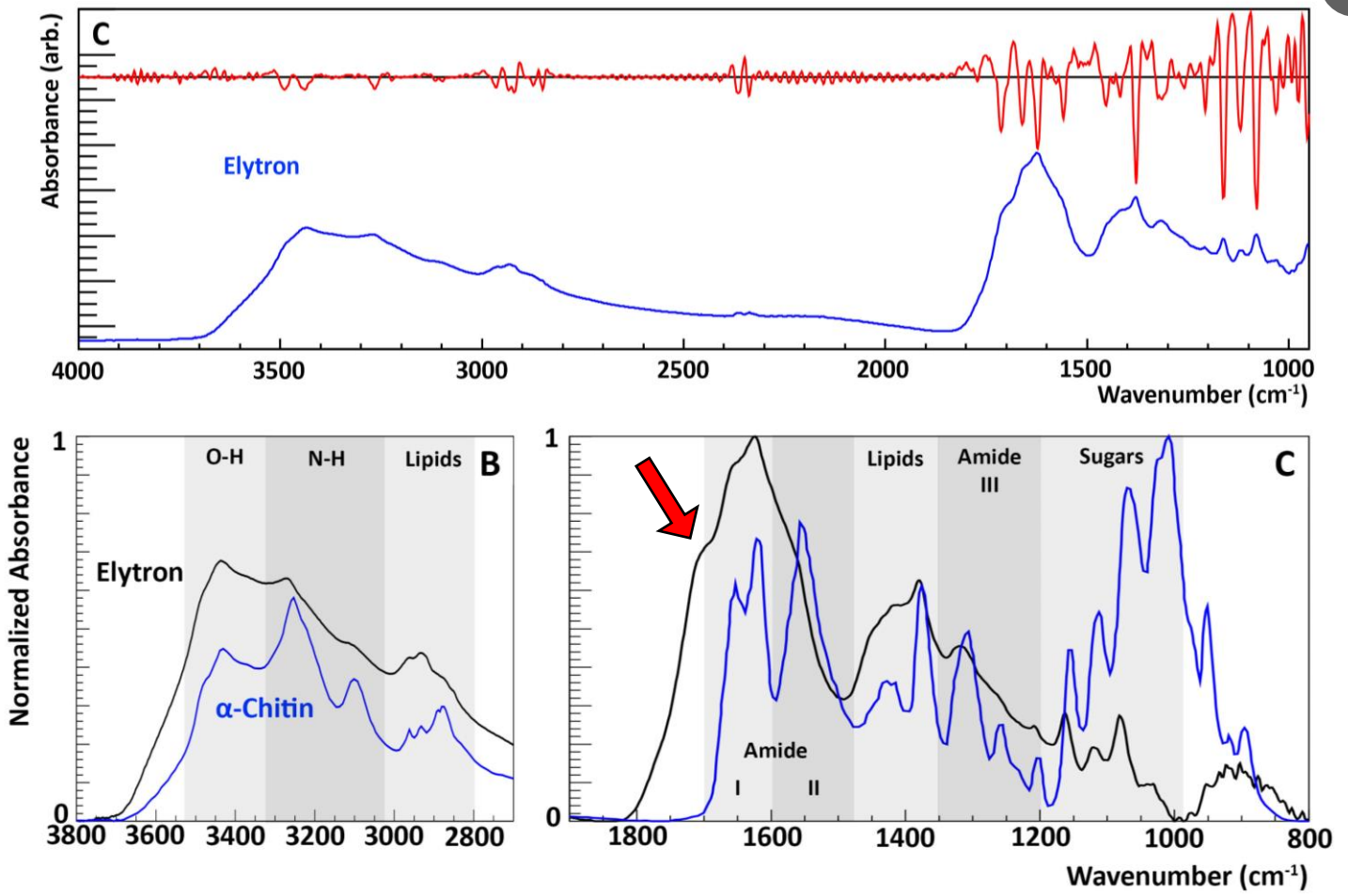




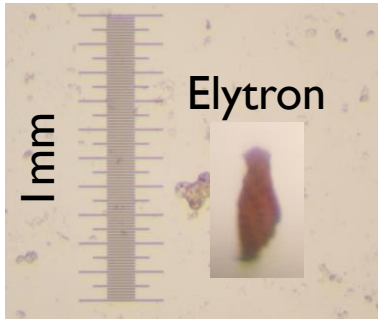
Mid Infrared Spectromicroscopy

FTIR RESULTS

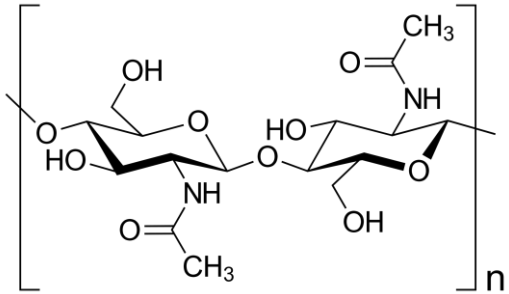
Elytron Peak Frequency (cm^{-1})	α-chitin Reference (Hassainia[19]) Peak Frequency (cm^{-1})	α-chitin Reference (Cárdenas[18]) Peak Frequency (cm^{-1})	Band Assignment	Organic Component
3490	3483*	3479	ν(O-H)	
3439	3428	3448	ν(O-H)	
3266	3254	3268	ν_{as}(N-H)	Amide A
3099	3100	3102	ν_{s}(N-H)	Amide B
2967	2960	2965	$\nu_{as}(CH_3)$	Lipid
2927	2932	2927	$\nu_{s}(CH_2)$	Lipid
2873	2876	2883	$\nu_{as}(CH_3)$	Lipid
2850	-	-	$\nu_{s}(CH_2)$	Lipid
1772	-	-	?	
1713	-	-	ν(C=O)	Oxidation
1661	1652	1660	ν(C=O)	Amide I
1623	1621	1627	ν(C=O)	Amide I
1558	1556	1558	ν(C-N) + δ(N-H)	Amide II
1453	-	-	$\delta(CH_2)$	Lipid
1418	1428	1422	$\delta(CH_2)$	Lipid
1379	1376	1376	δ(CH) + $\delta(C\text{-}CH_3)$	Lipid
1320	1308	1312	ν(C-N) + δ(N-H)	Amide III
1259	1260	1255	δ(N-H)	Amide III
1207	1207	-	δ(N-H)	Amide III
1163	1156	1157	ν_{as}(C-O-C, ring)	
1120	1114	1113	ν(C-O)	Sugar
1080	1069	1072	ν(C-O)	Sugar
1032	1029*	-	ν(C-O)	Sugar
1015	1008	1021	ν(C-O)	Sugar



- Chitin is a sugar-based molecule that strengthens insect exoskeletons
- Organics will be altered through decay; broadening of peaks and introduction of new peaks in the IR spectrum
- Oldest reported chitin from an insect (44 Ma), and is not highly degraded



Chitin



X-RAY FLUORESCENCE (XRF)
PHYSICAL PRINCIPLES

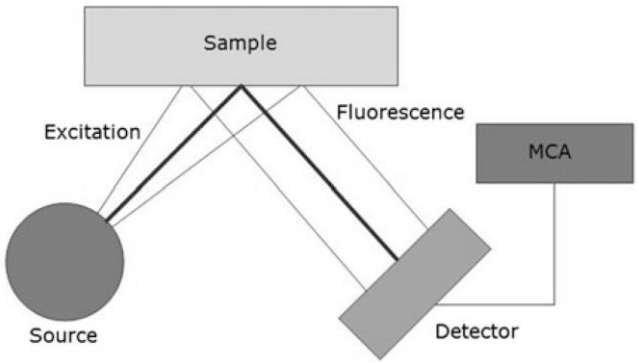
XRF Basics

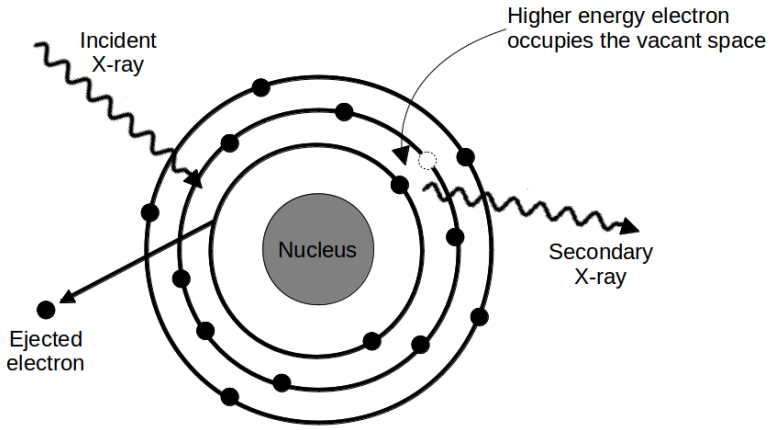
- Ionized atoms will de-excite, emitting characteristic characteristic fluorescent X-Rays
- All of the emitted X-Rays from a sample enter a detector where their energy is recorded in a histogram

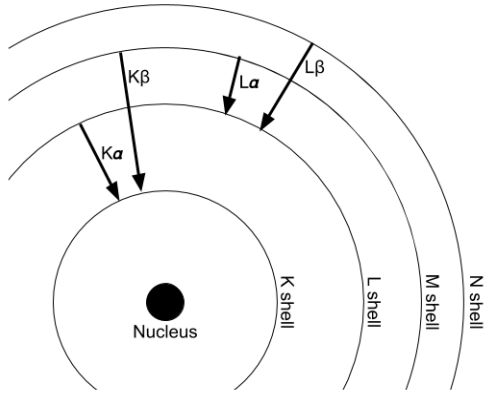
Chemical Mapping/Imaging

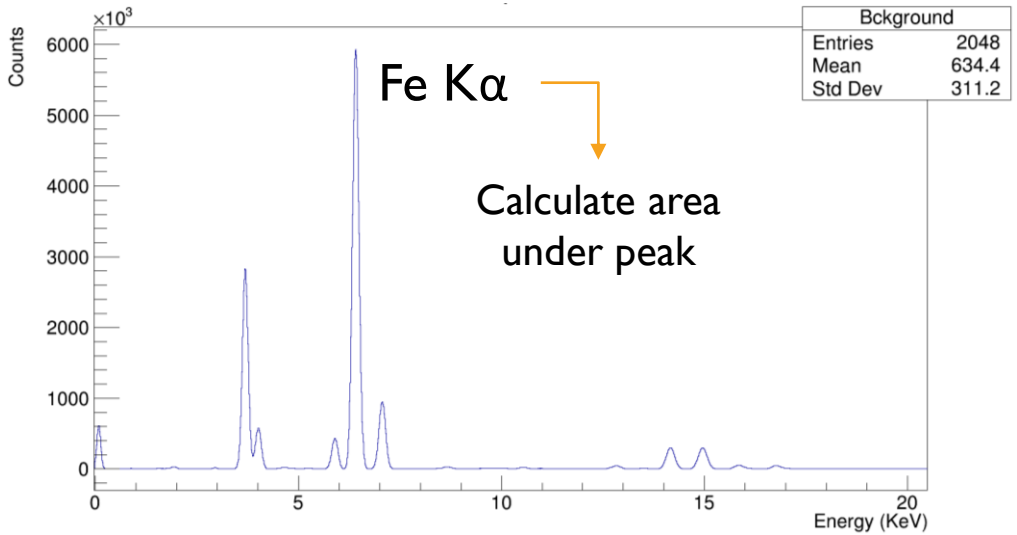
- If the spectrum is measured at each location on a grid of points, we can create a chemical intensity map for a given element
- Fossils are heterogeneous and important for how minerals track through fossilization

Soft X-ray Microcharacterization Beamline



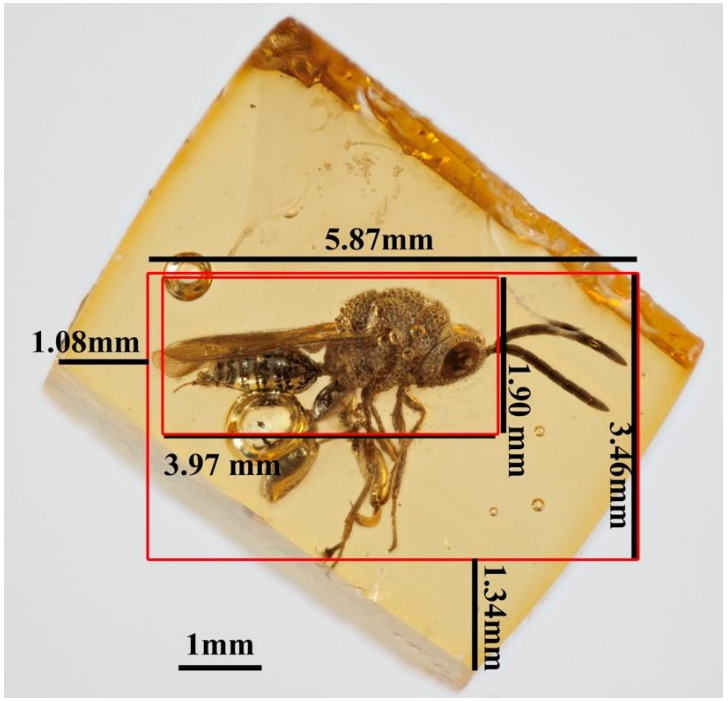


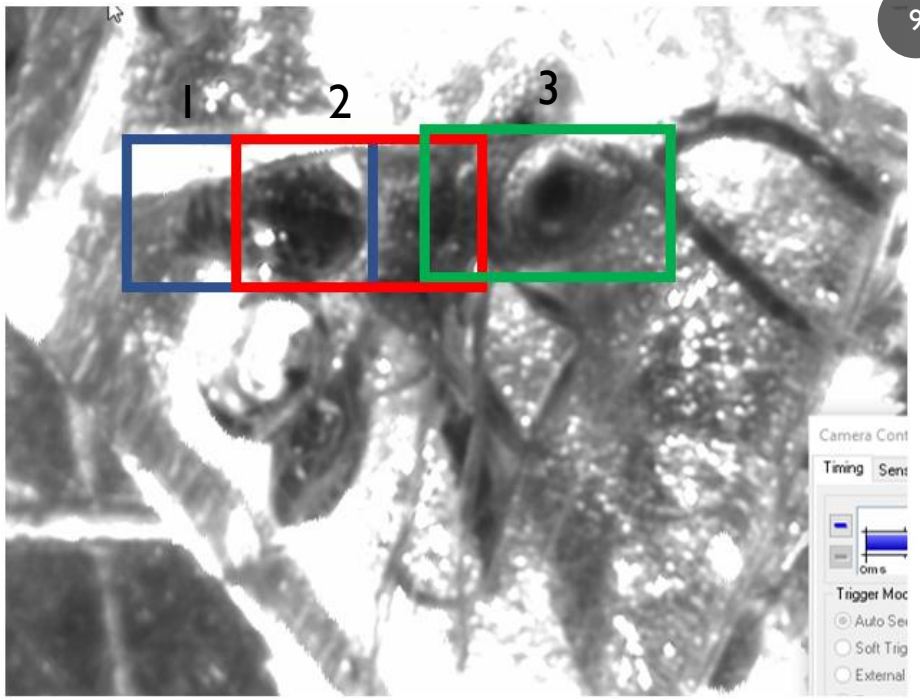


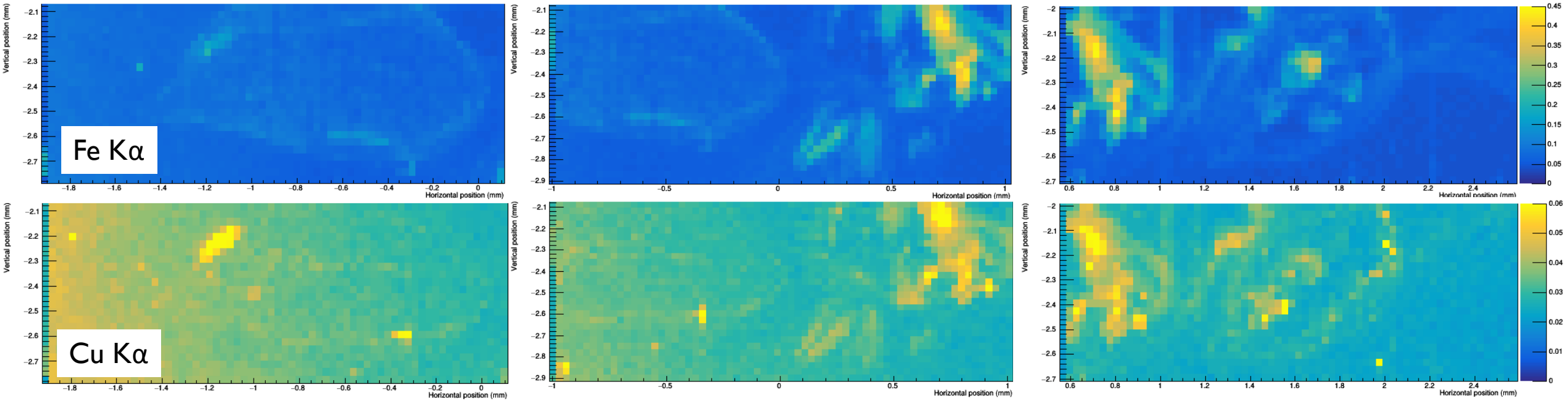


XRF RESULTS

- High concentrations of iron and copper, other elements negligible
- Iron is related to preservation of soft tissues. The flight muscles show up as extremely rich in iron.
- Copper can show original colour patterns of insect exoskeletons. It is associated with the iron here.







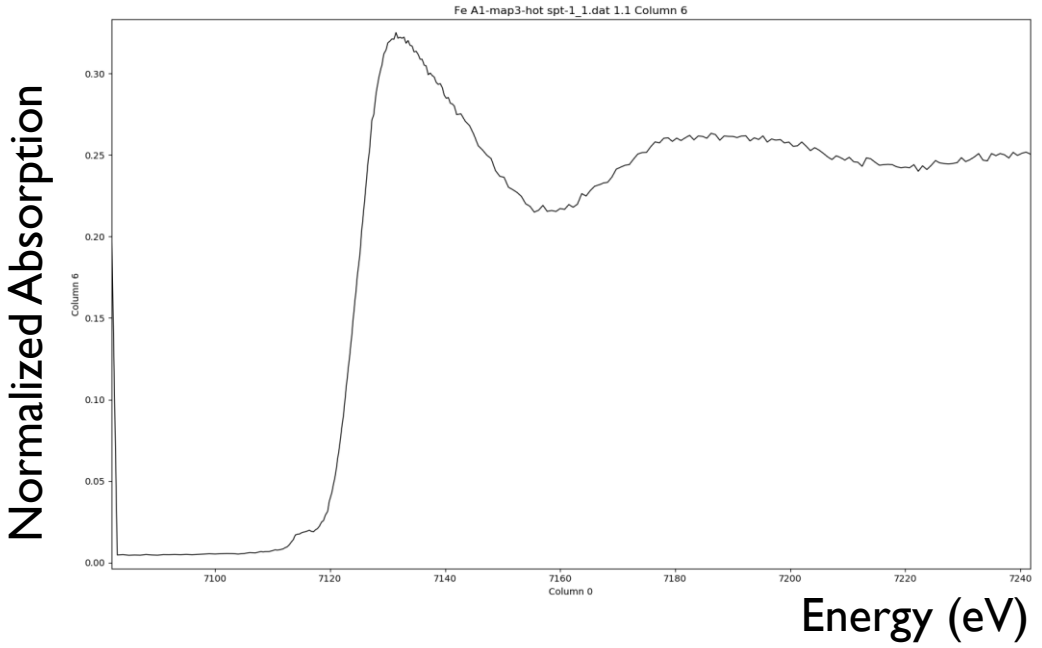
CONCLUSIONS

- **Synchrotron radiation** can be used to improve resolution and acquisition times of a wide collection of imaging and chemical analysis techniques that are non-destructive for precious fossils
- Using **CT,** we can model objects and their interior in 3D with micron resolution:
  - We were able to digitally extract ancient insects that are trapped amber allowing us to understand their evolution and structure
- Using **FTIR**, we can characterize organic signatures in a sample:
  - We were able to find organic chitin preserved in a 44 Ma beetle, extending the temporal range for this level of preservation
- Using **XRF**, we can determine qualitatively and quantitatively the elemental composition of a sample
  - We were able to determine possible mechanisms of preservation of muscle fibers in a 19 Ma wasp related to high concentrations of iron and copper

Organic material and soft tissues may be more commonly preserved in fossils than conventional paleontology states

Other techniques include:

- Synchrotron X-Ray Absorption Near Edge Structure (**XANES**)



ACKNOWLEDGEMENTS

Thank you to:

My supervisors:
Dr. Ryan McKellar
Dr. Mauricio Barbi
Canadian Light Source and beamline scientists
Dr. Andris Bukejs for taxonomic analysis
MITACS Accelerate for funding

University of Regina | Faculty of Science

RSM ROYAL SASKATCHEWAN MUSEUM

Canadian Light Source | Centre canadien de rayonnement synchrotron

Mitacs

REFERENCES

- P. Griths, J. Haseth. (2007)."Fourier Transform Infrared Spectrometry", Second Edition. Wiley.
- B. C. Smith. (2011)."Fundamentals of Fourier Transform Infrared Spectroscopy". CRC Press.
- M. Haschke. (2014)."Laboratory Micro-X-Ray Fluorescence Spectroscopy: Instrumentation and Applications". Springer.
- B. Beckho, B. Kanngieer, N. Langho, R. Wedell, H. Wol. (2008)."Handbook of Practical X-Ray Fluorescence Analysis". Springer.

CT RESULTS

Insects from Baltic Amber (44 Ma)

- Amber and decay artifacts can be removed using 3D rendering software
- Two new fossil species!

DRAGONFLY
OBJECT + RESEARCH + SYSTEMS

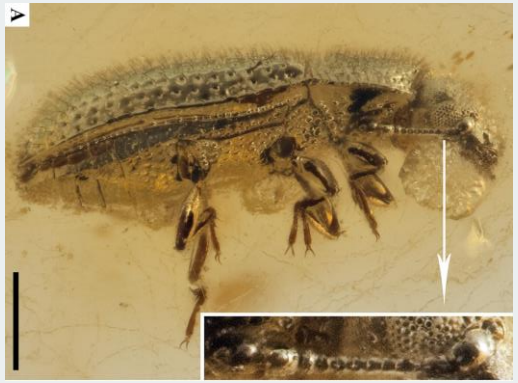
The first described turtle beetles from Eocene Baltic amber, with notes on fossil Chelonariidae (Coleoptera: Byrrhoidea)

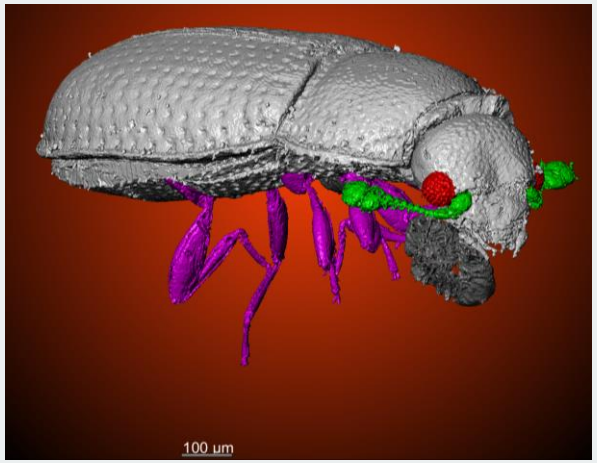
Vitalii I. Alekseev[1,2], Jerit Mitchell[3], Ryan C. McKellar[4,5,6], Mauricio Barbi[3], Hans C. E. Larsson[7], and Andris Bukejs[8]

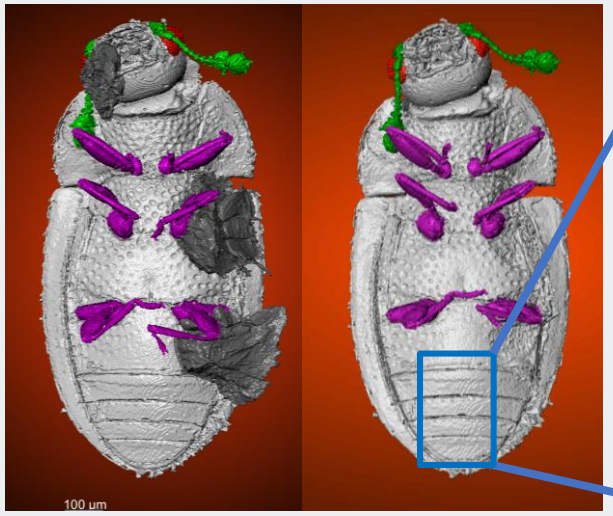
First fossil representative of Cerylonidae (Coleoptera: Coccinelloidea) described using X-ray micro-computed tomography, from Eocene Baltic amber

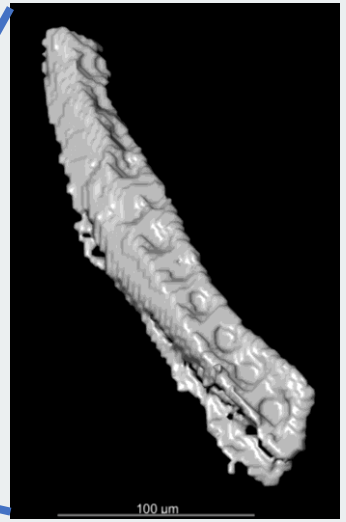
ANDRIS BUKEJS[10], ADAM ŚLIPIŃSKI[2], JERIT L. MITCHELL[3], RYAN C. MCKELLAR[4,5,6], MAURICIO BARBI[3], HANS C.E. LARSSON[7] & VITALII I. ALEKSEEV[8,9,10]

Minute Bark Beetle

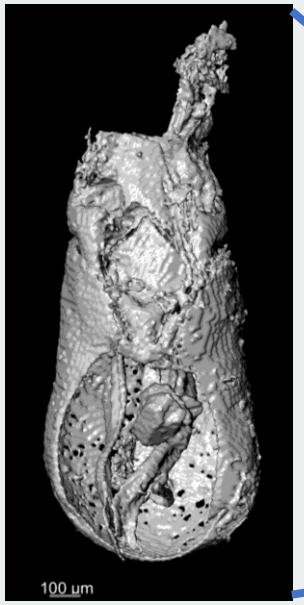




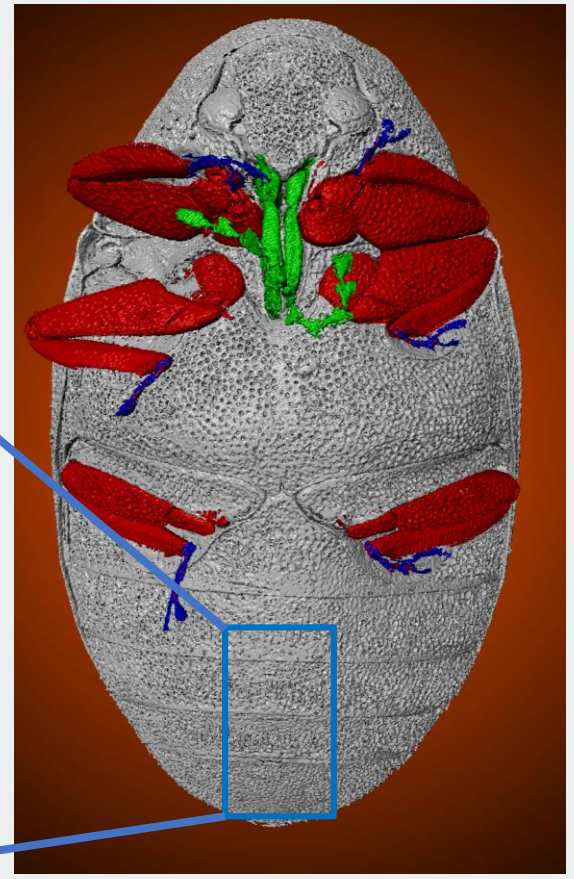




Genitalia is critical to taxonomic analysis



Turtle Beetle



FTIR RESULTS

- The spectral second derivative can be used to deconvolute peaks
- Amides contain sub bands due to transition dipole coupling => can give info on protein secondary structure
- This spectrum contains predominantly α–helix structure and therefore is likely due to a human contaminant