

INTRODUCTION

- Collagen is a major structural protein and is widely studied in medical research and more recently in forensics, archaeology, and paleontology.
- Mass Spectrometry (MS) has been the benchmark for collagen detection in (ancient) bone, however it is time consuming and not possible 'on site' e.g. at an archeological dig
- Fourier Transform Infra Red (FTIR) is sensitive to collagen organic group vibrational modes and is easily deployed at low-cost.¹
- Our research question is whether FTIR is sufficiently sensitive and precise for collagen decay studies
- We present in this poster a study of collagen decay on artificially aged bone, using FTIR and supplemented by other techniques including Second Harmonic Generation (SHG) imaging, Scanning Electron Microscopy (SEM) and MS.

MATERIALS & METHODS

Bone Preparation & Experiments

Porcine and bovine bones were cleaned and powdered to 250-500 μm in granule size, artificially decayed at three distinct temperatures (between 353–363 K), and examined using FTIR, SHG, SEM and MS.

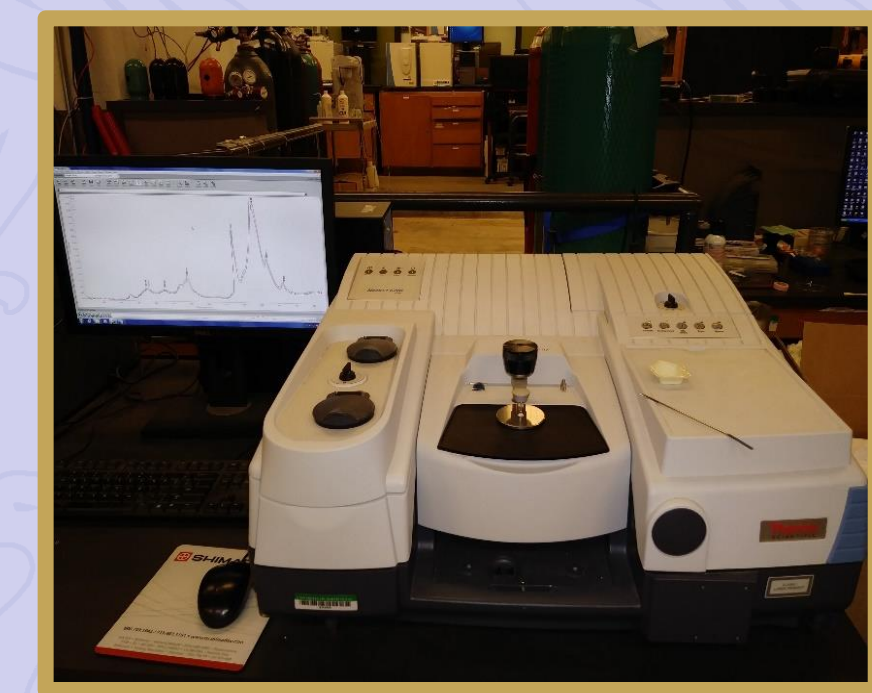


Fig. 1: ATR-IR
Thermo Scientific
Nicolet 6700

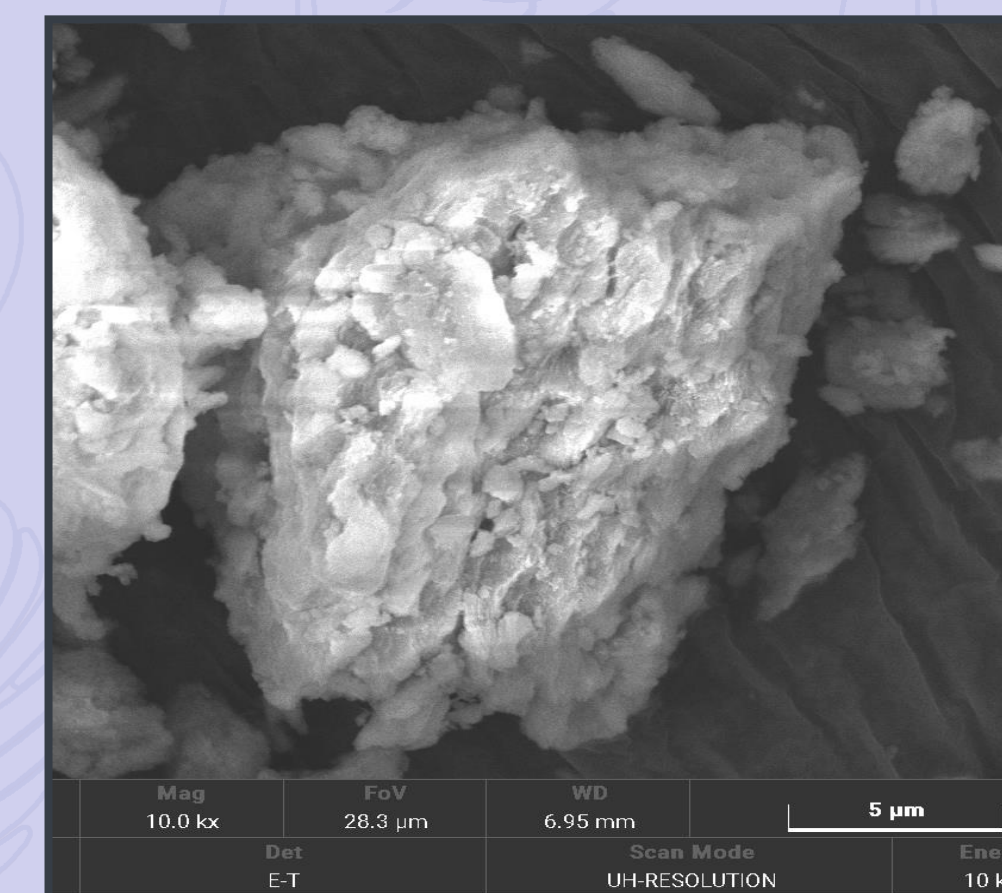


Fig.2 Powderized
bone micrograph

FTIR using non-invasive ATR-IR (Attenuated Total Reflectance-IR) using Thermo Scientific Nicolet 6700 135 spectra of 16 scans each (Fig.1)

Second Harmonic Generation SHG Zeiss Examiner Z1 two-photon excitation laser (signal at $\lambda/2$ of 920 nm) and con-focal microscopy. Parallel channels merged with Fiji s/w

SEM using Tescan focused ion beam microscopy

MS using Waters MALDI Synapt G2-Si (High Definition)

RESULTS & DISCUSSION

Fig. 3: SEM micrographs of bone powder show relative abundances of organic and inorganic elements

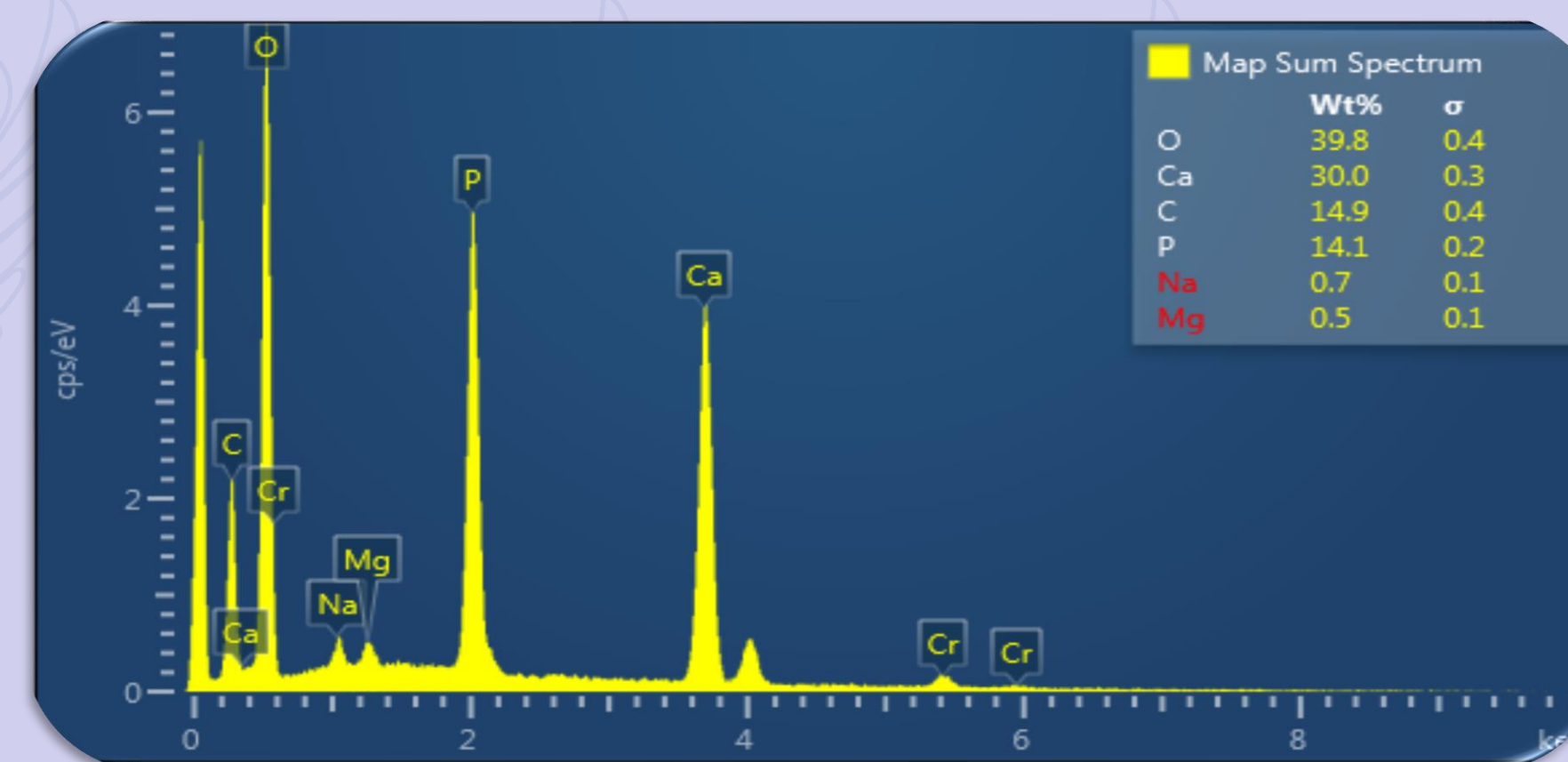


Fig. 4: MS data (below) show relative abundances of principal components

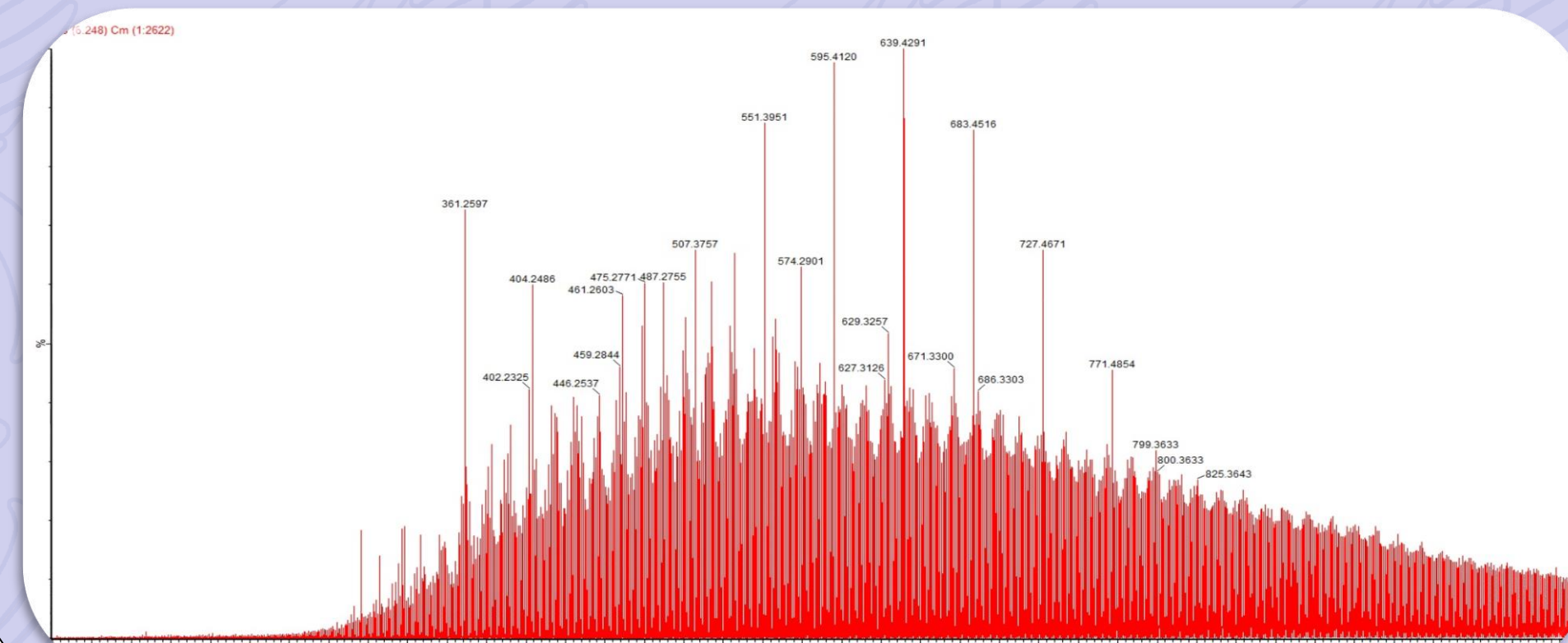
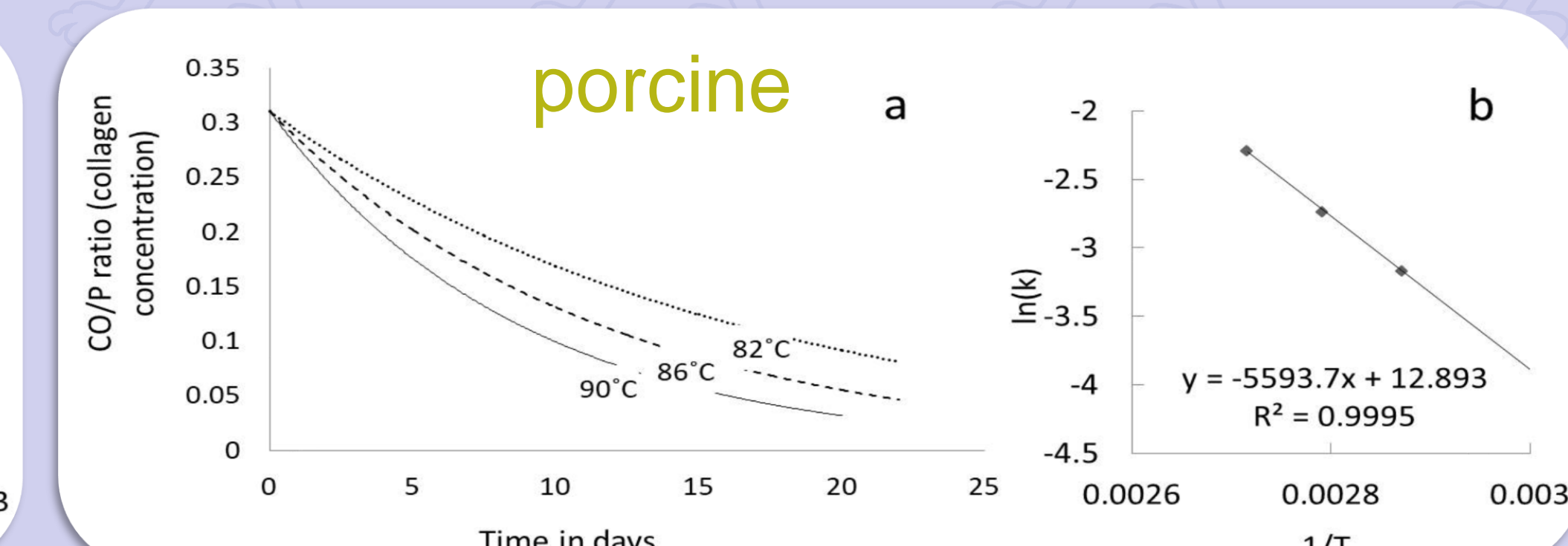
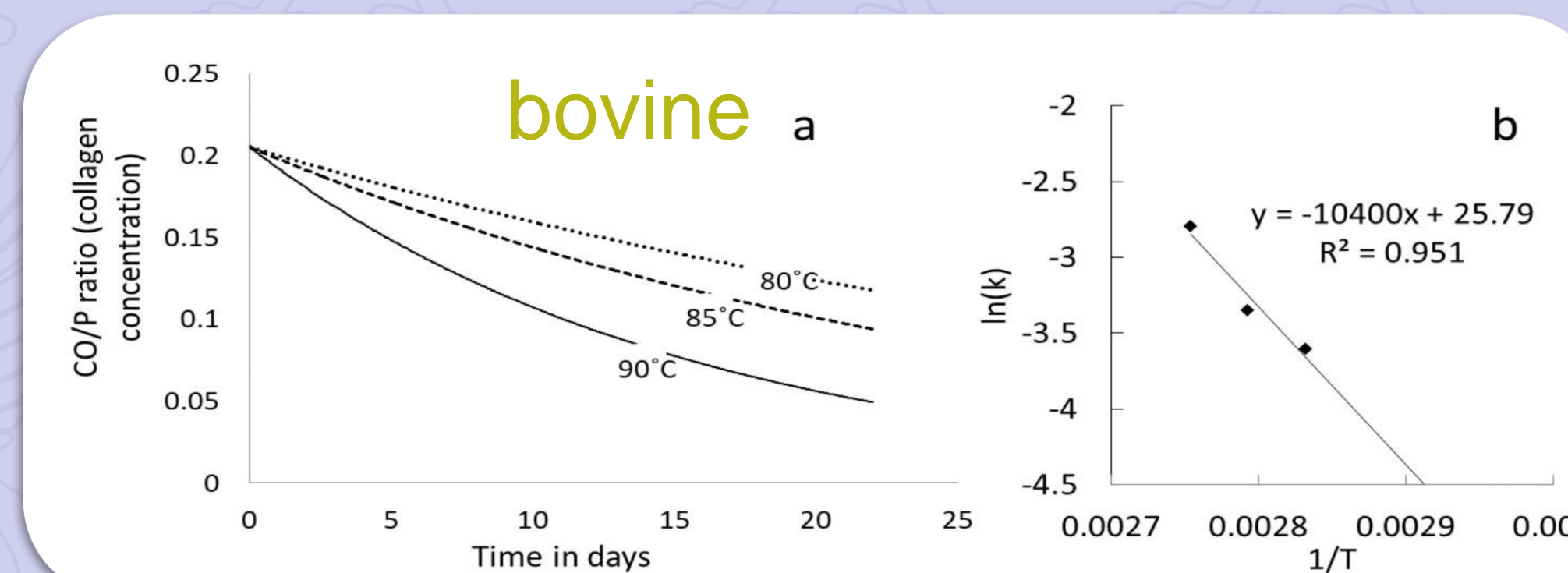
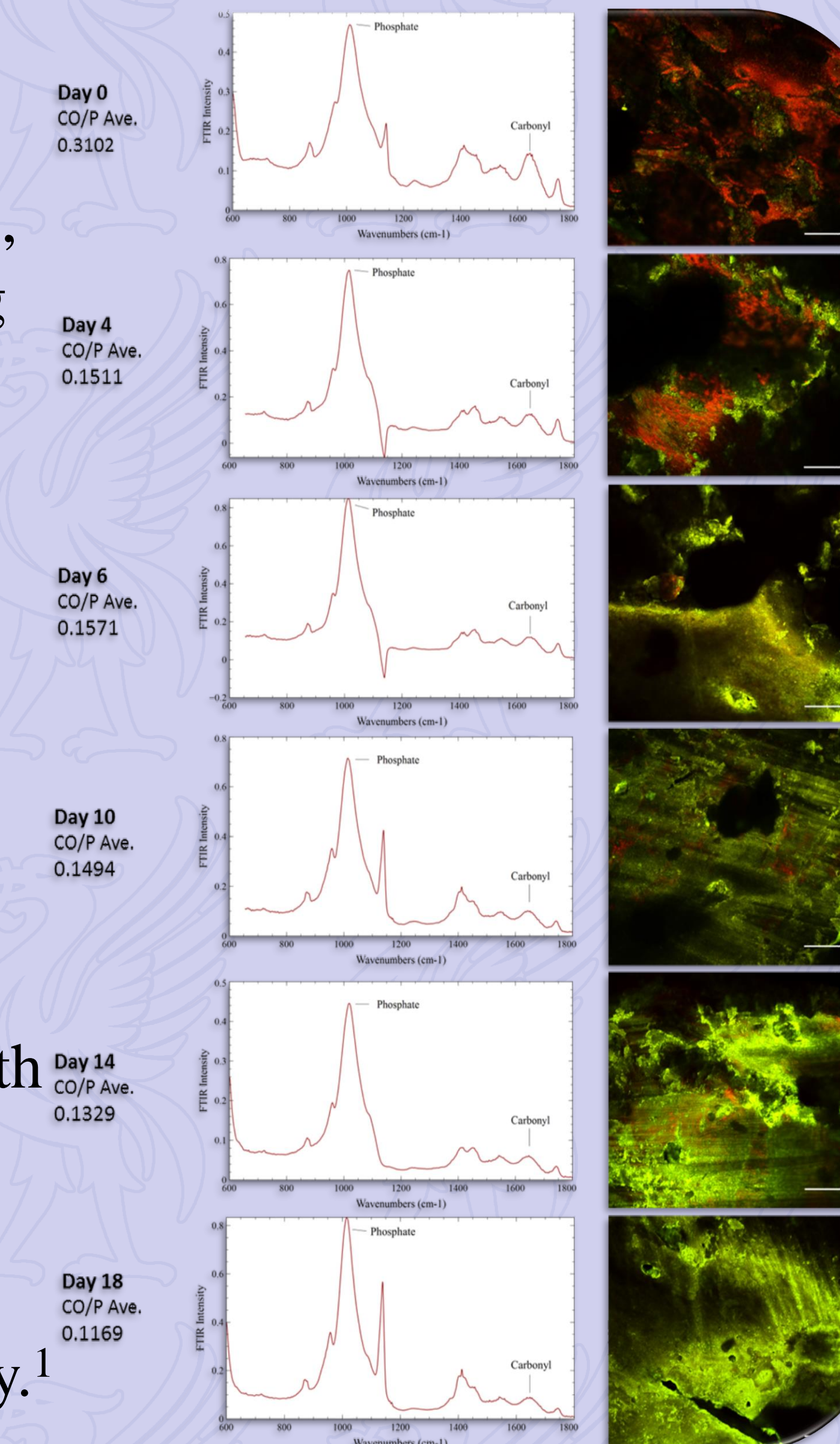


Fig. 6: FTIR tolerance slider adjusted to show CO peak at $\sim 1650 \text{ cm}^{-1}$. $\ln(k)$ versus $1/T$ for the Arrhenius plots. CO/P ratio averages resulted in $R^2 > 0.95$ for both bone datasets.



RESULTS & DISCUSSION

Fig. 5: SHG porcine bone images (right), corresponding **FTIR** spectra (middle), and average CO/P ratios (left). Incubation temperature 359 K. Results show decrease in CO/P ratio with time and temperature attributed to collagen decay.¹



CONCLUSIONS

FTIR using ATR-IR is a convenient tool to characterize **collagen decay** kinetics in artificially decayed bone

Second Harmonic Generation imaging confirmed decay rates and visualized this decay spatially

Relative abundances of collagen types (I, II and III) remain to be confirmed by MS protein sequencing going forward

FTIR features less preparation time, is low cost, and allows on-site results on bone collagen, all of which are beneficial to forensic scientists, archaeologists, and paleontologists.

Reference

- Thomas, B. et al., Second-harmonic generation imaging of collagen in ancient bone, *Bone Reports* (7):137–144, 2017.

Abbreviations key:

FTIR = Fourier Transform InfraRed
ATR = Attenuated Total Reflection
SHG = Second Harmonic Generation
SEM = Scanning Electron Microscope

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