

Chemical Mapping of Ancient Artifacts and Fossils with X-Ray Spectroscopy

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Abstract

The use of synchrotron radiation for the study of ancient objects has seen a significant increase over the last decade. Many of the major synchrotrons now have expertise and instrumentation that are specialized for the study of ancient objects. After giving an overview of these capabilities in the introduction, we focus in this chapter on synchrotron-rapid-scanning X-ray fluorescence (SRS-XRF) imaging of large objects to uncover ancient writings and chemical preservation in fossils. We will also describe the applications of X-ray absorption spectroscopy and new developments in X-ray Raman scattering, which are used to complement SRS-XRF mapping.

Keywords

Ancient materials · Ancient writings · Cultural heritage · Paleontology · Fossils · Archimedes Palimpsest · Archaeopteryx · Synchrotron radiation · X-ray fluorescence imaging · X-ray absorption spectroscopy · X-ray Raman scattering

Introduction

Ancient objects that comprise our planet's natural and cultural heritage provide evidence upon which we reconstruct our past and help us understand the present, but critically such hindsight yields also insight to our future. Objects studied at synchrotron light sources range widely in size (only constrained by experimental parameters), composition, and complexity. While in some cases there might be a very clear line of enquiry, often the questions are less well-defined or are a function of resulting data. It is rare that one tool alone can interrogate multiple lines of enquiry. However, it has become clear over the last decade that synchrotron radiation, with its wide variety of unique techniques, is a powerful tool in the study of ancient materials. Synchrotron-based imaging has critically contributed to answering some of the core questions that had remained unanswerable for many ancient objects. Taking advantage of synchrotron radiation's high brightness, small beam size, energy tunability, monochromaticity, polarization, and coherence, scientists have developed an array of techniques that provide structural and chemical information from sub-Ångströms to decimeter scales.

As synchrotron facilities have dramatically improved over the first half century of operations, the research performed at these facilities has evolved and adapted to uncountable disciplinary niches. Synchrotron facilities are now among the most interdisciplinary research facilities in existence, and the study of ancient objects alone crosses multiple disciplines in both the natural sciences and humanities. Whether the question is in archaeology, paleontology, paleoanthropology, or the many disciplines that comprise the history community, synchrotron radiation might provide an answer. In addition to the dramatic increase in beam performance at synchrotrons, work on ancient materials has also benefited from advances in instrumentation and, importantly, more user-/sample-friendly interface, intuitive operation, and coupling with laboratory instrumentation. These improvements and the expertise of large interdisciplinary teams have made it less daunting to carry out a synchrotron experiment on precious heritage objects. The growing number of publications and reciprocal growth of synchrotron beam lines and instruments adapted or specialized in the study of ancient materials well illustrates this trend. A comprehensive review of the development and trends in synchrotron studies of ancient and historical materials was produced by Bertrand et al. (2012), where a detailed and illustrated description of synchrotron techniques is provided. This chapter also includes a discussion of new approaches, the combination of characterization techniques, and the prevention/mitigation of radiation damage (see also Bertrand et al. 2015). They conclude with perspectives on specific support at synchrotron facilities for conducting studies of ancient materials.

In this chapter, we focus on a much narrower topic, namely, the application of synchrotron-rapid-scanning X-ray fluorescence (SRS-XRF) imaging as well as X-ray absorption spectroscopy (XAS) and X-ray Raman scattering (XRS) that can obtain elemental maps and chemical speciation of relatively large (decimeter scale) objects. We will describe basic concepts and explore the practical considerations of the instrumentation while reviewing some of our work undertaken over the last decade. It is our aim that this information will inspire the creation of interdisciplinary teams that can tackle some of the most exciting and unsolved questions centered on ancient materials. In addition to the X-ray imaging and data scientists, these teams might include conservation scientists, scholars, curators, historians, archaeologists, and paleontologists, who might be neither familiar with nor interested in synchrotron radiation per se. In addition, the long-term behavior of ancient materials raises a range of questions that may inspire modern research in material science (Bertrand et al. 2018). To provide a more general overview that shows how far this field has advanced in recent years, we present here a list of techniques, parameters, and beamlines that are currently configured to study ancient materials and objects. Most generally, the techniques and information they provide can be categorized as follows:

- X-ray fluorescence (XRF) and micro-XRF (μXRF) elemental composition and spatial mapping in the μm to cm range depending on the object size
- X-ray fluorescence micro-computed tomography (XRF-µCT) 3D imaging of material elemental composition
- X-ray absorption spectroscopy (XAS) element-specific chemical speciation

- X-ray Raman scattering (XRS) bulk-sensitive chemical speciation of low-Z objects
- X-ray diffraction (XRD) atomic scale structure information and texture of objects with crystalline phases
- Wide-angle X-ray scattering (WAXS) atomic scale spatial information of noncrystalline systems
- Small angle X-ray scattering (SAXS) mesoscale spatial information of noncrystalline systems
- X-ray micro- and nano-computed tomography (μCT, nCT) 3D morphological/spatial information with resolutions ranging from submicrometer to ~20 nm
- X-ray phase-contrast computed tomography (PCCT) 3D morphological/spatial information of low contrast objects with varying resolution, typically in the few μm range depending on the size of the object
- X-ray-excited optical luminescence (XEOL) chemical speciation
- Differential phase contrast imaging (DPC) 3D morphological/spatial information that enhances small changes in density
- Ptychography (Bragg, reflection, near-field, focused probe, single aperture, and imaging ptychography) nanometric scale 2D and 3D full-field imaging
- Photoluminescence spectroscopy and imaging identification and imaging of luminescent compounds (organics, semiconductors, etc.)
- Fourier-transform infrared spectroscopy (FT-IR) identification of molecules through specific vibrational modes of chemical bonds, with recent development at nanoscale using near-field probing

In Table 1, we provide a list of beamlines, techniques, experimental parameters, and references, which specialize in the study of ancient objects. While we tried to be as inclusive as possible, this is an active and growing field, and there certainly may be other facilities where such work can be performed. In addition, the launch of new sources such as Sirius in Brazil and the Mexican Light Source is expected to provide new capacities to the field.

Synchrotron-Rapid-Scanning X-ray Fluorescence (SRS-XRF) Imaging: Elemental Mapping

Providing information with high spatial resolution on the chemical composition of an ancient object has great value for many reasons. Paintings and manuscripts, for example, can often reveal new and hitherto unseen facets of the object that can be elementally uncovered via compositional differences in inks, pigments, and other materials of which the work consists. This can help resolving obscured drawings and writings or provide new information about the origin, authenticity, degradation, and relative age of the object. It is possibly the first time that such detective work is without doubt "elemental." Paleontological objects (fossilized remains of animals and plants) have the potential to preserve remnant chemistry of the original organism (some constrained by specific biomarkers). Such fossils include mineralized soft

Table 1 List o	f beamlines, techniques, objects,	, and experimental para	umeters at synchrotro	n facilities around the	world
Beamline	Objects / techniques	Energy range	Maximal imaging range	Spatial resolution (pixel / voxel length)	Refs
Advanced Lig	ht Source (https://als.lbl.gov/)				
8.3.2	Engraved mirror, pieces of paintings, Babylonian clay tablets, and Egyptian papyrus / μCT	7-45 keV	Field of view: 25 mm	1 μm diameter	MacDowell et al. (2012)
12.3.2	Miscellaneous artifacts / μXRD, μXRF	6-22 keV	Up to mm	1 μm diameter	Sciau et al. (2006), Liu et al. (2007), Lynch et al. (2007), Mirguet (2009), Leon et al. (2010), Jackson et al. (2013, 2014, 2017), Dejoie et al. (2014a, b, 2015), Leon et al. (2015), and Bertrand et al. (2017)
Advanced Ph	oton Source (https://www.aps.an	d.gov/)			
Ū-I	Metal and ceramic objects incl. figurines, mummies / XRD, XRF, imaging	42-120 keV	$1 \times 2 \text{ mm}^2$	2 × 20 μm ²	Friedman et al. (2008), Young et al. (2010), and Notis et al. (2013)
2-BM	Fossils / µCT	\sim 30 keV pink beam	$4 \times 25 \text{ mm}^2$	1 μm diameter	Schönenberger et al. (2012), Li et al. (2013), and Takahashi et al. (2017)
2-ID-D	Misc artifacts / μXRF, μXAS, ptychography	5-30 keV	$1 \times 1 \text{ mm}^2$	0.1 µm diameter	Nietzold et al. (2018) and Chen et al. (2018)
26-ID	Paint, daguerreotypes, ink, ceramics / XRF	5-30 keV	$10 \times 10 \ \mu m^2$	$0.03 \ \mu m$ diameter	Casadio and Rose (2013)
					(continued)

Beamline	Objects / techniques	Energy range	Maximal imaging range	Spatial resolution (pixel / voxel length)	Refs
Australian Synchrotre	on (https://www.ansto.gov	au/research/facilities	australian-synchrotr/	on/overview)	
XFM	Paintings, pigments, miscellaneous artifacts / XRF, XRF-µCT, XANES	4.1–20 keV	Milliprobe: 600×1200 mm ² Microprobe: 100×100 mm ²	Milliprobe: 50-200 µm ² Microprobe: 1-5 µm ²	Dredge et al. (2015, 2017), MacLeod (2015), Popelka-Filcoff et al. (2015, 2016), Jones et al. (2016), Thurrowgood et al. (2016), Alfeld and de Viguerie (2017), de Jonge et al. (2017), Sharp-Paul
XAS	Misc artifacts /	4-65 keV	Not specified	$0.2 \times 1 \text{ mm}^2$	(2011), Kurkwood et al. (2018), Kyan et al. (2010, 2014, 2018), Paterson et al. (2011), and Howard et al. (2012) Mills (2010)
	XANES, XAS		c		
IMBL	Misc artifacts / µCT	20-120 keV	$10 \times 10 \times 10 \ \mu m^{3}$ voxels	Up to 600 mm width, height not specified	
IR	Paintings, pigments, drawings / FT-IR	0.01–3.1 eV	$3 \times 3 \ \mu m^2$	Not specified	
BESSY II (https://wwv	w.helmholtz-berlin.de/que	llen/bessy/index_en.h	itml)		
BAMline	Gold, drawings, ivory, bone, antler / XRF, nCT	4-60 keV	$30 \times 30 \text{ cm}^2$	XRF: 1 μm nCT: 400 nm diameter	Riesemeier et al. (2005), Reiche et al. (2006, 2011), and Radtke et al. (2013, 2016)
CHESS (https://www.c	hess.cornell.edu/)				
A2, F3, G3, D1	Paintings, manuscripts,	5-80 keV	$600 \times 700 \text{ mm}^2$	30–80 keV (A2): 0.15 mm	Swanston et al. (2012, 2015), Michelin et al. (2013), Rouchon and Bernard (2015),
	daguerreotypes, inscriptions / XRF,			5-30 keV: 5-200 μm	and Alleon et al. (2016a, 2017)
	XRD, XANES, confocal XRF				

Table 1 (continued)

Canadian Light Sour	ce (https://www.lightsourd	ce.ca/)			
07B2-1 (VESPERS)	Bone / XRF, XRD, Laue XRD, XAS, XEOL	6-30 keV	Some millimeters	$\sim 2 \times 2 \mu m^2$	Swanston et al. (2012, 2015)
10ID-1 (SM)	Fossils, metals, paper / STXM	130–2700 eV	Tens of µm	$30 \times 30 \text{ nm}^2$	Michelin et al. (2013), Rouchon and Bernard (2015), Alleon et al. (2016a, 2017), and Gueriau et al. (2017)
11ID-1 SGM	Ancient soils, fossils, stone / XAS	250–2000 eV	Some millimeters	$10 \times 10 \ \mu m^2$ to $500 \times 500 \ \mu m^2$	Robertson and Blyth (2009), Alleon et al. (2016b), and Acksel et al. (2019)
06B1-1 (SXRMB)	Historic mortars, photographs / XAS	1.7–10 keV	Some millimeters	$10 \times 10 \ \mu m^2$	Hormes et al. (2015) and Kozachuk et al. (2018)
Diamond Light Sourc	e (https://www.diamond.a	ic.uk/)			
111, 111-1	Archaeological iron (cannonballs)/in situ XRD	5–25 keV	N/A	Bulk – $0.4 \times 0.4 \text{ mm}^2$	Thompson et al. (2009), Murray et al. (2017), and Simon et al. (2018)
112	Fossils, books, misc. artifacts / radiography, tomography, XRD, WAXS, SAXS, EDXD	53–150 keV	$200 \times 20 \text{ mm}^2$ for tomography, larger objects can be handled for other techniques	1.3-41 μm per pixel for imaging, depending on sample size. Typical probe size for diffraction and scattering: 100-500 μm diameter	http://www.diamond.ac.uk/ Instruments/Imaging-and-Microscopy/ 112.html and Drakopoulos et al. (2015)
113-1, 113-2	Paleoclimatology, fossils, eventually paper / μ CT, full-field microscopy (TXM), grating interferometry ptychography, Bragg-CDI	6-30 keV	Micro-CT 8 × 12 µm Grating 8 × 12 µm TXM 80 × 80 µm Ptycho 300 × 300 µm	25 nm – 5 μm diameter	Rau et al. 2011 and Rau (2017)

Table 1 (continued)					
Beamline	Objects / techniques	Energy range	Maximal imaging range	Spatial resolution (pixel / voxel length)	Refs
114	Misc artifacts / nXRF, nXANES, nXRD mapping, DPC, ptychography	4.5–23 keV	50 × 50 μm	<100 nm diameter	Quinn et al. (2018)
122	Books, parchment, bones / SAXS, μSAXS, grazing incidence SAXS, WAXS	6.0–20 keV	10 × 10 mm	10–200 µ.m diameter	Gonzalez et al. (2012), Možir et al. (2012), and Kennedy et al. (2013)
118	Historical artifacts / μXRF, μXRD, μCT, μXAS	2.05–20.5 keV	$70 \times 30 \text{ mm}^2$ $120 \times 30 \text{ mm}^2$	2 μm diameter 5 μm diameter	Mosselmans et al. (2009) and Diaz-Moreno et al. (2018)
B18	Historical artifacts / XRF, XAS, XRD	2.05–35 keV	$100 \times 40 \text{ cm}^2$	100 µm diameter	Diaz-Moreno et al. (2018) and Dent et al. (2009)
I20-scanning	Historical artifacts / XRF, XAS, XES	4–20 keV	$40 \times 40 \text{ mm}^2$	400 µm diameter	Diaz-Moreno et al. (2018, 2009)
MIRIAM beamline B22	Misc artifacts / IR and THz spectroscopy and microscopy Reflection / transmission 36 to 74	l eV-l meV	$30 \times 30 \ \mu m^2$ at 74× magnifications 128 × 128 μm^2 at 20×	3 μm–15 μm diameter	http://www.diamond.ac.uk/Instruments/ Soft-Condensed-Matter/B22/ publications.html
	mag. MicroATR 20		magnification		

		•			
European Synchrotro	on Radiation Facility (htt	ps://www.esrf.eu/)			
ID19, ID17, BM05	Fossils, various archaeological remains / μCT, PC-μCT	10–250 keV	$18 \times 18 \times 100 \mathrm{cm}^3$	~0.4 μm diameter (ID19) ~100 μm diameter (BM05, ID17)	Tafforeau et al. (2006) and Cau et al. (2017)
ID20	Miscellaneous artifacts including carbonaceous systems / XAS, XES, XRS, DT	4–20 keV	20 × 20 mm	25–500 μm diameter	Huotari et al. (2011, 2017) and Sahle et al. (2017)
ID21	Paintings, glasses, ceramics, papyri, metals, plastics, photographs / XRF, XAS, XRD, FT-IR	2.1–9.1 keV (ongoing development to 11 keV)	$20 \times 20 \text{ mm}^2$	0.3 × 0.7 μm² diameter	Cotte et al. (2017, 2018)
National Synchrotron	I Light Source II (https://	/www.bnl.gov/ps/)			
SRX (5-ID)	Artwork, paint fragments / cross sections, synthetic paints, microfossil / fossils, metal objects / μXRF, μXANES	4.65–22 keV	300 × 300 µm ²	$\sim 1 \times 1 \mu m^2$	Chen-Wiegart et al. (2016) and Chen-Wiegart et al. (2017)
SRX (5-ID)	Paintings / XRF	~10–22 keV	\sim 50 × \sim 50 cm	10 × 10 μm² to 100 × 100 μm²	
FXI (18-ID)	Artwork, paint fragments / cross sections, synthetic paints, microfossil / fossils, metal objects / nCT, nXANES	6-14 keV	$40 \times 40 \mu m^2$ field of view for tomography, image stitching is possible	\sim 30 nm diameter	Manuscript in preparation
					(continued)

Table 1 (continued)					
Beamline	Objects / techniques	Energy range	Maximal imaging range	Spatial resolution (pixel / voxel length)	Refs
PETRA III (http://pho	ton-science.desy.de/facil	ities/petra_iii/index_e	ng.html)		
P02.1	Sheet metals / XRD, XRF	60 keV (fix)	$10 \times 10 \text{ cm}^2$	$100 \times 100 \ \mu m^2 \text{ to}$ $600 \times 900 \ \mu m^2$	Dippel et al. (2015) and Glaser (2018)
P03	Misc objects / GISAXS, XRF	9–22 keV	$\sim 1 \times 1 \text{ cm}^2$	$40 \times 20 \ \mu m^2$ to 0.25 × 0.35 μm^2	Roth et al. (2011) and Buffet et al. (2012)
P05	Fossils / µCT	5-50 keV	FOV $7.2 \times 7.2 \text{ mm}^2$	1 µm diameter	Haibel et al. (2010a, b), Oliveira et al. (2016), Stebner et al. (2016), and Wilde et al. (2016)
P06	Paintings, books / XRF, XANES, XRD	5-50 keV	$80 \times 100 \text{ cm}^2$	300 nm to 200 μm diameter	Schroer et al. (2010), Da Pieve et al. (2013), Monico et al. (2013, 2015), Gay et al. (2015), and Boesenberg et al. (2018)
P07	Bulk metals / XRD, XRF, PDF, CT	50-200 keV	$40 \times 20 \times 10 \text{ cm}^3$	$2 \times 30 \ \mu m^2 \ up \ to$ $1 \times 1 \ mm^2$	Schell et al. 2014, Freudenberg and Glaser (2017), and Glaser (2016)
P09	Iron gall ink / XRF, resonant XRD and scattering	2.7–50 keV	$5 \times 5 \times 10 \text{ mm}^3$	$50 \times 4 \ \mu m^2 \ up \ to$ $20 \times 15 \ mm^2$	Strempfer et al. (2013)
SESAME (http://sesan	ne.org.jo/sesame_2018/)				
XAFS / XRF	Misc artifacts / EXFAS, XANES, XRF	4.5–30 keV	\sim tens of cm	$500 \times 500 \ \mu m^2$	http://www.sesame.org.jo/sesame_2018/ beamlines/xafs-xrf
IR	Misc artifacts / IR spectromicroscopy	0.001–3 eV	~1 cm	Diffraction limited ~3 to 10 μm diameter	http://www.sesame.org.jo/sesame_2018/ beamlines/infrared-beamline-ir

Stanford Synchrotron	Radiation Lightsource	(https://www-ssrl.slad	c.stanford.edu/conten	(
2-3	Cultural / natural heritage, biological, geological, archaeological materials / μXRF, μXAS	4.9–18 keV	$25 \times 25 \text{ mm}^2$	$\sim 2 \times 2 \mu m^2$	Leon et al. (2010), Walton et al. (2010), Sciau et al. (2011), Johnson et al. (2013), and Slotznick et al. (2015)
6-2	Books, parchment, papyrus, fossils, paintings / XRF, XAS, XRS	2.3-17 keV	$60 \times 100 \text{ cm}^2$	diameter diameter	Bergmann (2005, 2007, 2011), Bergmann and Knox (2009), Bergmann et al. (2010, 2012), Larson et al. (2010), Sadeghi and Bergmann (2010), Edwards et al. (2011a, b, 2013, 2014, 2016, 2018), Wogelius et al. (2011), Manning et al. (2013), Anne et al. (2014, 2017, 2018), Barden et al. (2015a, b), Egerton et al. (2015), Harazim et al. (2015), Gueriau et al. (2017), and Gibson et al. (2018)
10-2	Cultural / natural heritage, biological, geological, archaeological materials / XRF, XAS	4.4–22 ke V	$30 \times 60 \text{ cm}^2$	25–100 μm diameter	Johnson et al. (2013, 2016), Slotznick et al. (2015), and Field et al. (2013)
14–3	Misc artifacts / µXRF, µXAS	2.1–5 keV	$25 \times 25 \text{ mm}^2$	5 µm diameter	Paris et al. (2014), Gambardella et al. (2016), Ganio et al. (2018), and Raven et al. (2018)
Swiss Light Source (h TOMCAT	ttps://www.psi.ch/sis/) Fossils, artwork samples / μCT, nCT, PCCT	10-45 keV	5 × 10 mm ² (local tomography samples can be larger)	100 nm – 10 µm	Stampanoni et al. (2006)
					(continued)

Table 1 (continued])				
Beamline	Objects / techniques	Energy range	Maximal imaging range	Spatial resolution (pixel / voxel length)	Refs
Synchrotron SOLI	EIL (https://www.synchrotron-	soleil.fr/en)			
ANATOMIX	Misc artifacts / μCT, nanoCT	5-50 keV	Several cm ²	$40 * 40 \ \mu m^2$ to $40 \times 15 \ mm^2$	Weitkamp et al. (2018)
CRISTAL	Misc artifacts / XRD	4-30 keV	Not specified	$30 \times 400 \ \mu m^2$ to $450 \times 1000 \ \mu m^2$	Gervais et al. (2013) and Moini et al. (2014)
DIFFABS	Misc artifacts / μXRF, μXAS, μXRD	3–23 keV	Contact beamline staff	5–250 µm diameter	Bertrand et al. (2014), Gueriau et al. (2014), Cook et al. (2016), Davesne et al. (2018), and Kergourlay et al. (2018)
DISCO	Semiconductor pigments, corrosion phases, organic materials / µPL	180–1000 nm	Contact beamline staff	100 nm diameter	Bertrand et al. (2013), Echard et al. (2015), Gervais et al. (2015), and Thoury et al. (2016)
GALAXIES	Carbonaceous materials / XRS	2.3–12 keV	$10s \text{ of } \text{cm}^2$	5×10 to $20 \times 80 \ \mu m^2$	Gueriau et al. (2017)
HERMES	Carbonaceous artifacts / nXANES	70–2500 eV	Contact beamline staff	25 nm diameter	Belkhou et al. (2015)
LUCIA	Misc artifacts / µXRF, µXAS	0.6–8 keV	Contact beamline staff	2.5 μm diameter	Robinet et al. (2011), Gueriau et al. (2015), and Réguer et al. (2015)
NANOSCOPIUM	Fossil specimens / nXRF, nXAS	5-20 keV	Contact beamline staff	30–1000 nm diameter	Somogyi et al. (2017)
ODE	Pigments / dispersive µXAS	3.5–25 keV	Contact beamline staff	25*35 μm ²	Gervais et al. (2015)
PSICHE	Misc artifacts / µCT, ED-XRD	15-100 keV	Contact beamline staff	$10 \times 10 \ \mu m^2$ to $17 \times 6 \ mm^2$	Robin et al. (2016)
PUMA	Misc artifacts / μXRF, μXAS, μXRD	4-60 keV	Contact beamline staff	$3 \times 1.5 \ \mu m^2$	
SMIS	Organic materials / FT-IR	1.5–100 μm	Contact beamline staff	$>5 \times 5 \mu m^2$	Echard et al. (2010), Bertrand et al. (2011), and Hoerlé et al. (2016)

tissue that is not seen in the visible light spectrum and is often overlooked in such fossils. This additional information can lead to a better understanding of the pigmentation, anatomy, development and growth stage, diet, and even health of the organism prior to death. However, such information has also helped to constrain the taphonomy (burial history) as well as fossilization processes. These results not only inform us about the ancient organism in life but also provide a more complete understanding of the chemical pathways that lead to the preservation of such remarkable fossils.

The Principle of X-Ray Fluorescence

X-Ray fluorescence (XRF) is one of the most sensitive nondestructive techniques that permits the analysis and quantification of chemical elements and has been used as such for almost a century (Coster and Nishina 1925). In addition to the large laboratory and synchrotron systems, very practical battery-powered light and robust handheld devices are commercially available and are currently used by many disciplines. In XRF, X-rays of sufficiently high energy impinge on the sample and knock out inner shell electrons from the element of interest. An electron from a more outer shell then backfills this inner-shell hole by emitting either Auger electrons (E_{Auger}) or secondary X-rays, the XRF signal. The energy (wavelength) of the XRF photon corresponds to the difference in the binding energies of the two electrons involved. As each chemical element has electrons in shells each with unique binding energies, there is a corresponding distinctive XRF spectrum at characteristically known energies. K emission corresponds to a hole in the innermost K shell, L emission to that of the L shell, and so on. Generally, the strongest lines are labeled as α emission, the second strongest as β , and so on. Hence, without going into the details of separating transitions resulting from different sublevels within a shell, the characteristic transitions are labeled Ka, K\beta, La, Lβ, etc. (Siegbahn notation (Siegbahn 1916)). Figure 1 shows the schematics of the process for K α XRF.

To what extent a chemical element or fluorescence line is suitable for XRF analysis depends on three criteria:

- 1. The XRF line must be sufficiently strong to be detectable at the required elemental concentration.
- 2. The XRF energy has to be sufficiently high to penetrate enough of the sample matrix and environment to be detectable.
- The energy of the XRF line must be discernable from that of other chemical elements in the sample at the resolution of the detector or spectrometer employed.

In practice, for most elements up to the third row transition metals the K α lines resulting from a 2p electron in the L shell filling a 1s hole in the K shell are best suited for XRF analysis, since they have the strongest signal and highest X-ray energy and penetration. For heavier elements, such as lead, for example, the L lines fall into the practical energy range. XRF analyses often focus on trace metals, as they



Fig. 1 Principle of XRF. Schematic presentation of an atom with a nucleus surrounded by a series of electron shells labeled K, L, and M. In the first step (a) the incident X-ray knocks out a 1s electron from the K shell, creating a core hole. A more outer shell electron rapidly fills the K shell hole, and in this process a characteristic XRF photon is emitted. The most likely radiative decay for filling a 1s core hole in the K shell is from a 2p electron in the L shell, resulting in the emission of a K α XRF photon (b). For clarity, not all electrons of each shell are shown in the figure. The right panel (c) shows this process with respect to an electron binding energy level scheme

are chemically important and have distinct XRF lines with sufficient penetration power. Light elements, such as carbon, oxygen, and nitrogen, are typically not detectable in ancient objects, unless a small sample is removed from the object and analyzed using special equipment designed for low-energy X-ray emission and detection. The analysis of these elements also suffers from the additional problem of surface contamination. However, elements in the next periodic row, especially potassium, sulfur, chlorine, and calcium, can play an important role in both cultural heritage objects and fossils (intimately associated with endogenous compounds and even specific biosynthetic pathways), if the matrix of the object allows for enough XRF penetration.

Figure 2 shows the XRF spectrum taken from a parchment leaf of the *Archimedes Palimpsest*, where the measured XRF intensity is plotted as a function of XRF energy. The K α energies increase with the nuclear charge of the elements in the periodic table starting with phosphorous (P) and ending with copper (Cu) in the spectral range shown here. Note that the signal at about 4500 eV can be either from the titanium (Ti) K α line or from the barium (Ba) L α line or both. The argon (Ar) XRF peak originates from the excitation of the natural argon, which is present in the air around the parchment (the atmosphere contains 0.93% Ar). It is important to note, that the XRF lines shown in Fig. 2 are broadened by the Ge detector resolution of ~230 eV FWHM (at 6 keV photon energy). This makes it impossible to discern small energy separations, for example, between typical K α_1 and K α_2 fluorescence lines (5–23 eV difference for the 3D transition metals). While it is possible to use crystal analyzers with much higher spectral resolution that can finely resolve the XRF lines, most XRF analyses today use solid-state detectors and in particular Si drift detectors with a resolution limit of ~180 eV.



Fig. 2 The XRF spectrum as recorded on a parchment leaf of the *Archimedes Palimpsest*. All XRF lines correspond to $K\alpha$ transitions unless indicated otherwise

Elemental Mapping Using XRF

To create spatially resolved two-dimensional elemental maps of an object, a small X-ray beam is rastered across the object, and XRF signals are detected at each spot. (Currently in most synchrotron setups the beam is fixed and the object is rastered.) Generally XRF images are quasi-two-dimensional, and the spatial resolution of the elemental map (the XRF image) along the surface is determined by the footprint of the X-ray beam that strikes the object, while the depth resolution of the image is defined by the penetration depth of the incident beam or the emitted XRF signal. Depending on the matrix of the object, this can vary from less than 1 µm for lowerenergy (~ 3.15 keV) XRF when penetrating rock materials or metal-containing pigments to several millimeters for higher-energy XRF in a light matrix such as papyrus, parchment, or a canvas. To obtain depth information close to the surface of larger objects confocal XRF microscopy can be combined with the 2D XRF scans (Janssens et al. 2004; Woll et al. 2006, 2008; Mantouvalou et al. 2008). Here the incident beam is highly focused, and the fluorescence signal is passed through capillary optics to only see a small spot corresponding to the focused solid angle. The method can provide selected depth information with micron resolution. The experimental challenges in applying these methods are (1) the much slower imaging speed (due to the weaker XRF signal) and (2) the fact that the penetration depth is limited by the matrix and XRF energy.

The critical parameters for creating two-dimensional elemental map of an object of interest are:

- Size and composition of the object
- · Required spatial resolution
- · Concentrations and type of elements of interest
- Surface topography of the object

These parameters determine the X-ray source, optics, detection system, X-ray energy, and beam size and ultimately impact scanning speeds. Commercial systems based on X-ray tubes are now widely available and very practical for many applications, as, for example, the new scanners (e.g., Bruker M6 Jetstream - Bruker Corporation) specialized for the imaging of large paintings with ~100 to 500 μ m spatial resolution. While these commercial systems have practical advantages over synchrotrons, including easy access, lower cost, and higher mobility, they have several limitations that makes their use impossible or impractical for some applications. Specifically, compared to X-rays generated in a tube, synchrotron radiation has:

- (a) Higher intensity
- (b) Smaller beam size
- (c) Smaller beam divergence
- (d) Energy tunability
- (e) Smaller scattering background due to incident beam polarization
- (f) No bremsstrahlung background

All these parameters contribute to an enhanced XRF image quality and further provide some critical advantages. Parameters (a), (b), (e), and (f) mainly benefit the scanning speed, spatial resolution, and sensitivity to low signals and elemental concentrations. Parameter (d) allows for optimizing the XRF imaging to specific sets of elements. When imaging fossils, for example, we use one set of scans specializing on light elements such as phosphorus (P) and sulfur (S) at an incident energy below the Ca K-edge and a second set at higher incident energy to image the trace elements of 3d transition metals and other elements. Another very important advantage of synchrotron radiation is parameter (c), as it allows for producing a highly collimated X-ray beam (also known as pencil beam). In contrast, a short focal depth resulting from the strong focusing used in commercial tube-based systems is required in order to achieve a small beam size. The pencil beam approach is critical for the imaging of objects that lack a good surface flatness, which includes many fossils and wavy parchment or papyrus.

Synchrotron-Rapid-Scanning XRF Imaging of Large Objects

Given the continuing advances in brightness, many synchrotron beamlines have focused on enhancing the spatial resolution and sensitivity of XRF and other imaging. X-ray beam sizes as small as ~ 10 nm are now possible (Mimura et al. 2009; Yan et al. 2011). While such microscopes and nanoscopes specialize on

the imaging of extremely small objects or areas, we will focus here on obtaining elemental maps of an entire area of large (decimeter) objects such as fossils, manuscripts, or paintings. The following example illustrates why synchrotron-rapid-scanning XRF (SRS-XRF) imaging is critical for such work.

A 100 µm resolution image of the size of the Archaeopteryx fossil $(400 \times 500 \text{ mm}^2)$ consists of 2×10^7 pixels. If one wanted to obtain an image of the whole object in less than 12 h, the XRF signal for each pixel needs to be acquired in about 2 milliseconds (ms). The only realistic way to accomplish this is with fast "on-the-fly" readout systems with very little dead time and a powerful synchrotron beam that provides enough XRF signal during these short dwell time times. The first SRS-XRF imaging instrument at SSRL beamline 6-2 was originally designed for imaging of the Archimedes Palimpsest (Bergmann 2005, 2007, 2011; Bergmann and Knox 2009). This instrument, which was operational until 2017, has since been extensively applied in the field of archaeology, medical imaging, geology, biology, and paleontology (Bergmann et al. 2010, 2012; Larson et al. 2010; Sadeghi and Bergmann 2010; Edwards et al. 2011a, b, 2013, 2014, 2016, 2018; Wogelius et al. 2011; Manning et al. 2013; Anne et al. 2014, 2017, 2018; Barden et al. 2015a, b; Egerton et al. 2015; Harazim et al. 2015; Gibson et al. 2018; Popescu et al. 2009a, b, c; Popescu 2009). Being optimized for high speed, one limitation of this instrument was the fact that only 16 channels were recorded for each pixel due to limits in software and hardware.

Other facilities have started to perform SRS-XRF on fossils (see, e.g., Gueriau et al. 2014; Davesne et al. 2018), and advances in detectors and signal processors have enhanced the data acquisition rates, allowing for rapid and continuous scanning of samples with pixel dwell times in the millisecond and lower range (Diaz-Moreno et al. 2018; Vogt and Lanzirotti 2013; Kempson et al. 2014; Barkan et al. 2015; Lanzirotti et al. 2016). Various approaches to these systems are available around the globe such as the large area detector (Maia) employed at the Australian Synchrotron (Ryan et al. 2010; Paterson et al. 2011; Kirkham et al. 2010). The main advantage of these large area detectors is the solid angle, the main disadvantage being the poorer spectral resolution for the XRF analysis and larger scattering background, as these detectors are placed in a backscattering geometry.

In addition to acquiring the whole XRF spectrum at each pixel, ideally one would like to be able to analyze both large and heavy objects with spatial resolutions ranging from $\sim 100 \,\mu$ m for maps of the full object to a few μ m for select areas. We have recently built and commissioned a new SRS-XRF imaging station at beamline 6–2 that combines these two capabilities (Edwards et al. 2018). We focused on making the instrument user-friendly and flexible using state-of-the-art alignment, scanning, detection, and data acquisition/processing instrumentation. Beamline 6–2 spans the energy range from 2.1 to 17 keV, allowing for tender to hard X-ray experiments. The instrument is capable of SRS-XRF imaging large and heavy samples with a maximum scanning range of 1000 × 600 mm² and top load capacity of ~25 kg. The scanning stage has submicron precision and repeatability, which permits targeting of small areas at higher spatial resolution, in addition to rapidly imaging large areas. The change in beam spot size is achieved with a computer-controlled rotatable pinhole cartridge without the need for remounting the object. Currently we use beam sizes ranging from 25 to 100 μ m diameter. With future optics upgrades at the beamline, we will achieve spatial resolutions of a few μ m. Being able to rapidly change the beam size during a single scan is especially beneficial for the study of rare and precious objects that are only available once and/or for a short time that often cannot be sampled or remounted. Figure 3 shows a plan view schematic (left) and photograph of the new SRS-XRF instrument at beamline 6–2 (Edwards et al. 2018).

The beam enters the hutch (from the left in Fig. 3b) and passes through a mirror pitch feedback systems to maintain beam stability. After passing through a set of collimator slits, an ion chamber (I0) and a filter box integrated into the mapping software to provide automatic shutter control for delicate samples, the computer-controlled pinhole assembly at the exit (see segment with yellow kapton window) determines beam spot size variable up to nine different sizes. The sample is placed inside the box, which has a removable frame and can be operated in air (windowless) or under helium with a thin (~17 μ m) commercial polypropylene film stretched across the frame. The in-air operation is used for hard X-ray analysis, and the helium operations are used for tender X-ray analysis of important low-Z elements such as sulfur and phosphorus. Note that thin samples such as parchment leafs are mounted in a separate frame allowing for XRF detection from the front and the back of the leaf (Bergmann and Knox 2009). For the He operations, the detector is placed directly in contact with the polypropylene window to preclude absorption and scattering in air.

To obtain elemental maps, the sample is rastered relative to the incident X-ray beam using high-precision encoded motorized linear motion stages. In addition to the horizontal and vertical scanning stages, the instrument has motorized depth alignment of samples of different thicknesses. In standard scanning operation, the continuous movement is horizontal with a vertical step at the end of each scan line. The scanning speed and readout time can be varied depending on the scan range and sample concentration. In hard X-ray operation, the sample and horizontal motion is set at 45° angle to the incident beam. The XRF signal is measured using a four-element Vortex ME4 silicon drift diode detector positioned at 90° to the incident beam and 45° to the sample surface in order to minimize scattering. For more technical details and examples demonstrating the performance of this operational end-station, see Edwards et al. (2018). As of August 2018, this instrument is also available for general users at SSRL.

In summary, here are some considerations for SRS-XRF imaging of ancient objects:

1. Ancient objects rarely have a surface with flat topography. Therefore, using the pencil beam approach (strong collimation and large focal depth) is best suited for obtaining elemental maps by SRS-XRF imaging. In addition, data correction strategies such as in Alfeld et al. (2017) show promise to correct reabsorption effects and account for the sample surface geometry.





- 2. For a given map size, the scanning times scale between the square and the fourth power of the beam diameter. This depends on the emittance of the synchrotron source, beamline optics, collimation, and pinhole size. Therefore, objects need to be scanned at the lowest acceptable resolution (typically 20–100 μ m), while high-resolution imaging (<1–10 μ m) is reserved for selected small areas of interest. The ideal instrument is suited for SRS-XRF imaging with range of beam sizes available without the need to remount the sample.
- 3. To obtain high-quality elemental maps for light elements (aluminum to potassium) requires a helium-purge sample environment, lower X-ray energy (below the calcium K-edge), and optimized sample-detector geometry. Mapping of second row elements including carbon and oxygen requires a vacuum environment and is limited by surface contamination and low fluorescence yield. Other methods, including X-ray Raman scattering (see section below) might be preferable for these elements.
- 4. Collecting the full XRF spectrum at each pixel (and not just some selected regions of interest) allows for a more detailed analysis resulting in better sensitivity and image quality. Depending on the software and computation power, creating these large datasets can limit the scanning speed.
- 5. For SRS-XRF imaging of thin objects, such as parchment, canvas, and paper, it can be beneficial to place a second detector behind the object. Comparing both signals can be used to separate contributions from the front and the back of the object.
- 6. New scanning protocols including Lissajous curve scans and smart scans using machine learning are currently explored. In the future, these methods could reduce the scanning times for some objects.
- 7. Quantification of trace elements may require single point XRF analysis with better counting statistics at selected locations, after these have been identified with the elemental maps.

In addition to obtaining elemental maps, for some ancient materials and fossils, it is of great value to characterize the chemical speciation for a given element. Such information may include the oxidation state, spin state, coordination, or whether the element is in an organic or inorganic compound. X-ray absorption spectroscopy (XAS) is a very powerful technique to obtain such information in an element-sensitive way, even at very low concentration. The energy tunability of synchrotron source allows for XAS analysis in conjunction with SRS-XRF imaging or separately, and we discuss this technique in the following section.

X-Ray Absorption Spectroscopy: Chemical Speciation Analysis

In X-ray absorption spectroscopy (XAS), the incident photon promotes a core electron (the photoelectron) into an unoccupied state close or above its binding energy (the absorption edge). Generally, XAS is divided into X-ray absorption near-edge structure (XANES) (or equivalently near-edge X-ray absorption fine



Fig. 4 Concept of X-ray absorption spectroscopy. An incident photon is absorbed by an atom promoting a core electron either into the LUMO levels (XANES region) or the continuum (EXAFS region) where it is backscattered from neighboring atoms

structure spectroscopy (NEXFAS)) spectroscopy and extended X-ray absorption fine structure (EXAFS) spectroscopy (Koningsberger and Prins 1988; van Bokhoven and Lamberti 2016); see schematics in Fig. 4. XANES probes the lowest unoccupied molecular orbital (LUMO) energy levels and therefore contains local electronic structure information, such as oxidation state, symmetry, covalency, and the coordination of the absorbing atom. For EXAFS, the energy of the photoelectron is varied over several hundred eV above the absorption edge. This causes small intensity variations due to the interference created from the backscattering of the photoelectron by the nearest neighboring atoms of the absorber. EXAFS therefore provides information on nearest neighbor distances, numbers, and types. While the distance information is generally very precise, the number and type of nearest neighbors is less precise and typically requires some additional information/assumptions about the sample.

In synchrotron-based research, both XANES and EXAFS are widely used and well-established experimental techniques with a strong theoretical basis and a large library of spectra from known compounds. Having an XAS spectrum from an ancient object provides very powerful information. In the worst case, it rules out many types of speciation, electronic and atomic structures of the materials on the object, and in the best case, it can quantitatively identify the chemical speciation of an element within the ancient material. Theoretical tools for interpreting XAS spectra are becoming more and more effective (Newville 2001; Ravel and Newville 2005; Gilmore et al. 2015), and in many cases quantitative analysis can be achieved by using reference spectra from known compounds and using their linear combination to fit the measured spectrum.

XAS is performed in various detection modes, including transmission, fluorescence yield, electron yield, ion yield, high-energy resolution fluorescence detection (HERFD), and X-ray Raman scattering (XRS) (see next section). The K α fluorescence (typically used for elements up to molybdenum) or L α fluorescence detection mode (typically used for rare earths, lead, and other sixth row elements) is most common for dilute systems. The HERFD mode is used to overcome the spectral broadening in the XANES region caused by the short core-hole lifetime in heavier elements (Hämäläinen et al. 1991; Glatzel and Bergmann 2005; De Groot et al. 2009; Friebel et al. 2011; Bauer 2014). Conventional XAS analyses use a standard beamline monochromator (Si (111) and (220) or diamond (111)) to scan the incident photon energy. For the HERFD detection, often a higherresolution incident beam monochromator (Si (311)) and high-resolution crystal analyzer are used to overcome the broadening and provide more detailed speciation information. Experimentally it is more challenging, as the signals are smaller and more sophisticated X-ray optics is needed. XRS is the photon equivalent of electron energy loss spectroscopy (EELS) and is used to obtain XAS information of light element, where the sample or sample environment is not suited for soft X-ray analysis and requires a more penetrating hard X-ray probe. XRS analysis requires similar instrumentation as HERFD XANES, and we will discuss XRS in a later section in this chapter. For XANES characterization in the soft X-ray range, electron and ion yield detection is often used. Here the fluorescence vield is very small, and these detection modes further enhance the surface sensitivity.

In summary, here are some considerations for XAS analyses of ancient objects:

- 1. XAS is a powerful technique for chemical speciation as it provides elementspecific information about the local electronic and atomic structure of the absorbing atom.
- 2. XAS is the most common and widely available X-ray spectroscopy tool at synchrotron sources and is generally easy to perform on ancient materials. Care has to be taken to assess and avoid potential radiation damage in some objects, especially those containing organic materials.
- 3. EXAFS analysis requires about three orders of magnitude more photons than XANES. It is therefore impossible or impractical to perform EXAFS on ancient objects where the concentration of the element of interest is too low or the risk of radiation damage is too high. Microbeam XAS analysis requires a very high X-ray dose which might also prevent the more demanding EXAFS analysis.
- 4. XAS detection on large ancient objects is typically performed in fluorescence mode, as these samples are too thick for transmission and the concentration of the element of interest might be low. For ultrathin samples, a hight spatial resolution variant of XAS called TXM and STXM – (scanning) transmission X-ray microscopy – can be used to assess the speciation of low-Z elements in (Meirer et al. 2013)

Combining XAS and SRS-XRF Imaging: Speciation Analysis and Mapping

For most ancient materials, XAS analysis is performed in fluorescence mode, because the elements of interest typically occur in low concentrations and the

objects are often too thick for transmission XAS. This fluorescence mode XAS uses the same experimental setup as XRF, with the addition of scanning the incident photon energy across the absorption edge of the element of interest. Therefore, XAS is a very practical and complementary tool to SRS-XRF imaging. Ideally, one would obtain a three-dimensional dataset, where the XAS spectrum is recorded for each pixel of the 2D elemental map. However, this is not practical in most cases, as it would take too long to obtain the data cube. Therefore, when combining SRS-XRF mapping with XAS, one can pursue the following strategy consisting of two orthogonal sets of measurements:

- Create a 2D elemental map using SRS-XRF imaging and identify locations of interest on the object map. Perform XAS on these selected areas of interest to obtain additional chemical speciation information. In a fossil, these locations might include the discrete bones to specific parts of a bone (i.e., growth plate), soft tissue, and other structural remains. In a parchment, papyrus, paper document, or painting, this location might include the various inks, pigments, or materials used to originally make/scribe/alter the artefact.
- 2. After obtaining the chemical speciation at selected locations by XANES, identify photon energies in the near-edge spectral region, where the spectral differences for chemical species are most strongly pronounced on the object. Perform SRS-XRF scans at these specific incident photon energies to obtain speciation-sensitive 2D elemental maps. Note that this analysis focuses on XANES, as the spectral changes between different species are too small in EXAFS spectroscopy.

In sulfur XANES, for example, different chemical species have very pronounced and well-separated resonances. We have used this speciation-sensitive 2D elemental mapping method to differentiate between organic and inorganic sulfur maps in the fossilized *Archaeopteryx* feather (the Berlin feather) (Manning et al. 2013) and many other specimens [see also section on Fossils].

In summary, here are some considerations for combining XAS and SRS-XRF imaging for speciation analysis and speciation-sensitive 2D elemental mapping of ancient objects:

- 1. In ancient objects, the changes of XANES features at different locations in the 2D elemental map are typically small. Therefore, additional analyses, including correlation mapping, principle components analysis, and optimization algorithms, are applied to the data to enhance the speciation or imaging contrast.
- 2. Strong resonances and edge shifts are best suited for speciation-sensitive elemental mapping as differences are larger and the XRF signals are stronger.
- 3. Sulfur and some of the 3D transition metal K-edge XANES have the largest speciation sensitivity for mapping of diagnostic XANES features using tender and hard X-rays.

X-Ray Raman Scattering: Carbon Speciation Analysis and Imaging

Carbon is a critical light element in ancient objects, both in archaeological and fossil remains. It is ubiquitous and occurs in a diversity of chemical forms. Characterizing the carbon speciation in an ancient object can provide valuable information including its origin, the materials it is composed of, and the degradation processes that have affected a sample. Ideally, one would like to know the carbon speciation within the object with high spatial resolution. Synchrotron-based XANES (NEXAFS) spectroscopy yields powerful information about carbon speciation, including its structure, bond distances, and configurations (Stohr 1992). Carbon K-edge XANES (280–350 eV) are conventionally performed with soft X-rays, which have a very short penetration depth due to their stronger interaction with matter. This imposes stringent constraints on sample type, preparation, and analytical environment, and the need to keep an ancient object under ambient pressure and temperature precludes the use of soft X-ray techniques for nondestructive studies. X-ray Raman scattering (XRS) is a hard X-ray technique closely related to XAS that overcomes these difficulties, thereby complementing conventional XANES spectroscopy as well as Fourier-transform infrared (FT-IR) spectroscopy, Raman microspectroscopy, and time-of-flight secondary ion mass spectrometry (ToF-SIMS), which are often used to identify organic compounds and document their chemical nature and the degree of carbon organization. In XRS, the incident photon is inelastically scattered (rather than absorbed), and the energy loss promotes a core electron into an unoccupied state close to or above its absorption edge. Therefore the energy transfer in XRS corresponds to the incident X-ray energy in XAS. The concept and schematics of XRS are described in Figs. 5 and 6.

The correspondence between XRS and XANES was predicted and observed more than 50 years ago (Mizuno et al. 1967; Suzuki 1967). Following the pioneering synchrotron-based work in the 1980s and 1990s (Krisch et al. 1997; Tohji and Udagawa 1987, 1989; Watanabe et al. 1996), XRS has now recently developed into a valuable probe for bulk-scale characterization of the speciation of carbon, oxygen, and other light elements in conditions not suited for soft X-ray probes (Bergmann et al. 2002, 2003; Krisch and Sette 2002; Mao et al. 2003; Rueff and Shukla 2010; Wernet et al. 2004). The main experimental challenges for XRS are (1) its low cross section and (2) the need for determining the energy of the scattered as well as the incident X-rays with high resolution. To make the technique practical, XRS requires very intense monochromatic X-rays from a third-generation synchrotron source and a large-acceptance high-resolution analyzer system. Today, many synchrotron facilities have overcome these technical challenges, and routine XRS capabilities are available for the science community, for example, the very large instruments at SSRL and ESRF (Huotari et al. 2017; Sokaras et al. 2012).

Because in XRS the incident photon is scattering and not absorbed, there are differences in the symmetry selection rules for the XANES features. Specifically, the momentum transfer (q) between the incident and scattered photon takes the role



Fig. 5 Left: concept of XRS. The incident photon is inelastically scattered from the sample, and in the process the energy loss promotes a core electron into a LUMO level. Right: X-ray energy loss spectrum from graphite. The analyzer energy is at E' = 6460 eV, and the incident energy E_0 is scanned from 6450 to 7020 eV showing first the quasi-elastic scattering (6460 eV), Compton scattering, and starting at $\Delta E \sim 285 \text{ eV}$ the carbon K-edge XRS

of the polarization vector of the absorbed photon in conventional XANES (Krisch et al. 1997; Schülke et al. 1988). Unlike the polarization vector that has only a directional value, q also has a magnitude. Non-dipole contributions can occur if the product of q and the 1s radius r of the absorbing atom do not fulfill the dipole limit $q^*r \ll 1$. When XRS spectra are used in combination with conventional XANES, it is preferable to perform XRS in the dipole limit at small q. There can also be advantages of performing XRS at large q, because maximizing non-dipole spectral features can enhance the speciation sensitivity. These and other competing parameters determine the best X-ray energy and scattering geometry for a specific XRS experiment (see also discussions in Bergmann et al. (2002), Krisch and Sette (2002), and Sahle et al. (2015)):

- 1. X-ray energies need to be high enough to penetrate through any sample enclosures and into the sample, but low enough to avoid loss in spectral resolution and Bragg analyzer efficiency.
- 2. For horizontal XRS scattering, angles close to 90° need to be avoided as the XRS signal is diminished due to the linear horizontal polarization of the incident synchrotron beam.
- 3. Depending on whether or not non-dipole contributions are desired, the photon energy and momentum transfer *q* should be chosen to maximize the XRS signal, while minimizing Compton scattering background (which also depends on *q*).

Given these and other practical considerations, most XRS experiments are performed in the 5-10 keV at X-ray Raman scattering angles varying from 45°

to 135° using multi-crystal spherically curved spectrometers in Rowland geometry (Huotari et al. 2017; Sokaras et al. 2012). Recently we have performed a study on a series of samples relevant to ancient objects and materials to elucidate the potential of XRS for carbon speciation (Gueriau et al. 2017), which we review in the following section.

These XRS measurements were performed at the GALAXIES beamline at SOLEIL equipped with a double crystal Si(1,1,1) monochromator at a beam size of $\sim 30 \times 80 \ \mu\text{m}^2$ and incident flux of $\sim 1.5 \times 10^{13}$ photons/s on the sample (Rueff et al. 2015). For the best compromise of signal to noise, momentum transfer (q), and the space constraints at the beamline, we selected an XRS scattering angle of \sim 137° and XRS spectrometer energy of 5.95 keV. For the XRS spectrometer, we used a Rowland geometry system based on four 100-mm-diameter spherically bent Si (3.3.3) Bragg crystals with a 1 m radius of curvature at a Bragg angle of 85.6° . With these parameters, the momentum transfer at the carbon K-edge is $q \approx 5.8 \text{ Å}^{-1}$. Estimating the carbon 1s core radius r with a_0/Z , where $a_0 = 0.53$ Å is the Bohr radius and Z = 6 is the charge for the carbon nucleus, results in $q^*r \approx 0.5$. This value is beyond the dipole limit $(q^*r \ll 1)$ resulting in spectral contributions not present in low-q XRS and STXM-based XANES (see discussion below). In Table 2 we describe the samples, and Fig. 6 shows the corresponding XRS spectra. Details of the data collection, analysis, and discussion of the results are provided in Gueriau et al. (2017).



Fig. 6 Left: XRS spectra of three carbon-based artists' black pigments (**a**) and pure graphite at high-*q* compared to a low-*q* spectrum (Bergmann et al. 2002) (**b**). Arrows indicate enhancements of resonances due to our high-*q* geometry. Right: XRS spectra of paleontological samples. From top to bottom: (**a**) fragment of dry skin from a 49,000-year-old mammoth from Siberia; (**b**) 56-million-year-old fossil cocoon from the Rivecourt site, XRS, and STXM spectra. (Figure adapted from Gueriau et al. (2017))

Sample	Description	Origin	Inv. no
Pigment 1	Carbon black – "Carbon black," from no. 6-type oil	Rockwood Inc.	LB-1011
Pigment 2	Charcoal – "Fine charcoal," from beechwood 500 °C	Kremer Inc.	47800
Pigment 3	Lampblack – "Furnace black, lamp black slow drier, lightweight powder, from acetylene"	Kremer Inc.	47250
Pure graphite	Ultra F' purity graphite T.M.I. 5 ppm ultra carbon. Powder 200 mesh. Batch: 169-717	Alfa Johnson Matthey Gmbh	291-955- 3
Rivecourt cocoon	56-million-year-old fossil cocoon	Rivecourt (France) Material taken to the MNHN for study; will later be housed at musée Vivenel (Compiègne, France)	RIV.PPA 1 (field number)
Mammoth	Fragment from the dry skin of a 49,000-year-old fossil mammoth	Lyakhov island (Siberia) MNHN palaeontological collections	MNHN.F. MAQ 287

Table 2 Description of the set of ancient and historic samples used to develop XRS as an analytical tool to study carbon speciation in ancient objects

The top left panel in Fig. 6 shows the comparison between XRS spectra from three artists' black pigments described in Table 2. While Pigments 1 and 3 appear to contain very similar carbon compounds, Pigment 2 exhibits a less intense $1s-\pi^*$ resonance at 285.4 eV and $1s-\sigma^*$ resonance at 292.7 eV. As the area of the 285.4 eV peak relates to the aromaticity, the XRS spectra indicate that the carbonaceous compounds in Pigment 2 are less graphitic than those in Pigments 1 and 3. In the XRS of Pigment 2, one can furthermore see an additional weak spectral feature at 288.7 eV consistent with the presence of stacking defects between the aromatic planes. These findings are consistent with the pigments' manufacturing information, which identifies Pigment 2 as "Fine charcoal," while Pigment 1 and 3 are carbon blacks obtained industrially by the burning or pyrolysis of gas or oil. This illustrates that XRS-based XANES spectroscopy can differentiate between artists' pigment manufacturing processes. Comparison of the two graphite XRS spectra at low q and high q (Fig. 6 left, bottom) shows the contribution of non-dipole features in the highq geometry. Specifically, we observe an enhancement of the C=C 1s- π^* resonance as well as other resonances indicated by arrows. These effects, first reported for oriented graphite (Schülke et al. 1988), show how XRS performed in the non-dipole geometry can enhance the speciation sensitivity of XANES. In fact, it is likely that these non-dipole effects have enhanced the small spectral differences in the $1s-\pi^*$ resonance of the three pigments.

In paleontology, the speciation of light elements, such as carbon and nitrogen, may allow the identification of the signature from relict organic molecules and enable the tracing back of their biological origin and/or their alteration through time. To demonstrate the potential of XRS analysis for such studies, we measured two unprepared paleontological samples (Fig. 6 right). The top spectrum is from a 49,000-year-old mammoth dry skin and the bottom spectrum from a 56-million-year-old fossil cocoon, whose morphology suggests an affinity with leeches.

The spectrum of the mammoth skin (A) shows a small peak at 285.4 eV indicating a low aromaticity of the carbon compounds composing this fossil and a broad and intense feature at 288.7 eV. We assign this second broader feature to the overlapping contributions of $1s-\pi^*$ transitions in amide (288.3 eV) and carboxyl groups (288.75 eV). Absence of features at 290.3 eV and 300 eV indicates the absence of carbonate minerals, which was confirmed independently by powder X-ray diffraction (XRD). A very similar spectrum has been previously reported on a modern collagenous rat-tail tendon (Lam et al. 2012), which corroborates our evidence of the very good chemical preservation of the organic compounds in this ancient mammoth dry skin.

The XRS spectrum of the cocoon (B) shows a small $1s-\pi^*$ resonance at 285.4 eV, attributed to the presence of some aromatic and/or olefin carbons, which is broader and may be related to the presence of graphitic domains disseminated within a globally turbostratic matrix (Bernard et al. 2010). In addition, the XRS spectrum of the paleontological cocoon exhibits a weak shoulder at 287.2 eV. Figure 6 right bottom shows a scanning transmission X-ray microscopy (STXM)-based XANES spectrum collected on an ultrathin section of the same cocoon sample (Kaznatcheev et al. 2007). Compared to the XRS spectrum, it shows the same absorption features, but the peak at 285.4 eV is more intense, and the feature at 287.2 eV is more resolved. These differences are partly due to the lower energy resolution and partly due to additional non-dipole contributions in XRS. The lack of XRS peaks attributable to carbonate minerals is consistent with powder XRD analyses of this material which showed quartz and only trace quantities of calcite. The organic carbon dominates the spectrum.

XRS-Based 2D Speciation Mapping

Our data presented in Fig. 6 and discussed in the previous section show that XRSbased XANES is capable of determining carbon speciation in untreated ancient objects under ambient conditions. Huotari et al. have shown that it is possible to obtain tomographic information with XRS by using a position-sensitive detector in conjunction with the XRS spectrometer (Huotari et al. 2011), and we are currently studying how to use the XRS technique for 2D speciation imaging of ancient objects. The approach to combine XRS with 2D mapping is similar to that discussed above, where XAS and SRS-XRF imaging are combined for speciation-sensitive 2D elemental mapping. Specifically the following strategy can be employed:

1. Perform XRS-based XANES at various locations of interest on the object to obtain details of carbon speciation.

- Identify XRS energy transfers in the XANES region, where the spectral differences for chemical species are most strongly pronounced.
- 3. Because XRS has a large Compton scattering background, identify additional energy transfers below and above the edge to perform background corrections.
- 4. Perform 2D scans at all selected energies to obtain maps. Because of the small signal and large background, additional data analysis such as background correction is required to enhance the quality of the speciation maps.

It is important to note that due to the small signal and large background, XRSbased speciation mapping is experimentally much more challenging than SRS-XRF 2D elemental mapping and XRF-detected XAS-based 2D speciation mapping. Therefore, XRS-based carbon speciation maps are smaller and have lower spatial resolution. Another important limitation for the XRS technique is the sample matrix and carbon concentration. As the XRS signal scales with the number of carbon scatterers, a strongly absorbing matrix will diminish the XRS signal even at relatively high carbon concentration. For example, the 1/e attenuation length of organic compounds at 6 keV is ~ 0.5 mm, whereas the attenuation length of clay is about ten times less. The most carbon-rich shale, for example, contains a few percent carbon, which will diminish the carbon XRS signal by two to three orders of magnitude. The situation is even worse in the presence of some heavier elements. In addition, compared to the XRS signal, the Compton scattering background is less diminished, further worsening the signal-to-noise ratio. This shows that one can only use the XRS technique for ancient objects, which are mainly composed of light elements. A very intense X-ray beam is required to overcome the low X-ray Raman cross section, even when using a highly effective XRS spectrometer. Therefore, the required dose to obtain an XRS spectrum or an XRS-based 2D speciation map is much larger as compared to XRF imaging and conventional XANES. This requires special attention to mitigate and monitor the possible formation of radiation-induced sample damage.

In summary, here are some considerations for XRS analysis and XRS-based 2D speciation mapping of ancient objects:

- XRS is a powerful technique for obtaining bulk-sensitive carbon speciation information in mainly organic ancient objects and materials under ambient conditions with minimal sample preparation. Other elements, in particular oxygen and nitrogen, are also suitable for this technique in some cases.
- 2. Performing XRS at large momentum transfer q results in non-dipole contributions to the XANES, which can enhance the speciation sensitivity.
- 3. The XRS signal is weak and requires a very intense X-ray beam, a largeacceptance XRS spectrometer, and sample-dependent optimized experimental parameters, such as photon energy, energy resolution, and XRS scattering angle.
- 4. XRS-based 2D carbon speciation mapping is possible. Because of the much lower signal and larger background compared to XRF, these maps have fewer pixels and a lower spatial resolution.

5. Given the high X-ray intensity required for XRS, the mitigation of X-ray-induced radiation damage to the object is more challenging as compared to conventional XAS and XRF analyses.

Uncovering Ancient Writings by SRS-XRF

The Archimedes Palimpsest

On July 16, 1907, a big literary find in Constantinople made headlines around the world (Big literary find in constantinople 1907). The Danish philologist Johan Ludvig Heiberg had found a thirteenth-century Byzantine parchment prayer book that underneath the biblical texts revealed unknown writings of the Greek genius Archimedes of Syracuse (c.287–c.212 BCE), considered one of the greatest mathematicians of all time. This famous book known as the *Archimedes Palimpsest* (see Fig. 7) contains Greek versions of seven texts of Archimedes copied by an unknown writer in the tenth century using iron gall ink.

What made Heiberg's finding so significant was the fact he had not only discovered the oldest copy of work by Archimedes, but in this copy were two previously unknown treatises, namely, *The Method* and the *Stomachion* and the original Greek version of *On Floating Bodies*, which had only been known through its Latin translation. In *The Method*, Archimedes describes the use of mechanical methods in a type of physics thought experiment, to determine the volume of various objects. It is an ingenious form of ancient calculus, which some people have argued could have changed the history of science, had it not been hidden for more than two millennia. After World War I, the book had disappeared and was thought to be lost until it resurfaced in Paris in the 1990s. In 1998, the *Archimedes Palimpsest* was



Fig. 7 Photos of the *Archimedes Palimpsest*. Right: view of the palimpsest with one of the Archimedes drawings and writings in the vertical and biblical writings in the horizontal. A palimpsest is a manuscript or piece of writing material on which the original writing has been scraped off to make room for later writings, but of which traces remain. (Christie's Images Ltd. 1998)

auctioned off to a private owner, who has since supported an integrated campaign of conservation, imaging, and scholarship. Over the years, these projects have received extensive press coverage, and it was through an article in the German magazine GEO in 2003, which mentioned the use of iron gall ink in the Archimedes writings, that the Archimedes Palimpsest synchrotron project came to life. By that time, large fractions of the manuscript had been successfully imaged using various optical techniques, most importantly multispectral imaging (see Christens-Barry et al. 2011 and references therein). Yet there were some important gaps that the optical techniques were not able to recover. Furthermore, Heiberg's original photos also had limited quality, and since the time he had taken them, many leafs had degraded even more. Four of them had been overpainted with forgeries of the four Evangelists, copied from miniatures from a 1929 book at the Bibliothèque Nationale in Paris (see Fig. 8). These forgeries cover parts from Archimedes' treatises On Floating Bodies, Equilibrium of Planes, and The Method. In addition to the writings covered by these forgeries, some writings were covered by mold stains, glue, biblical writings, or they were simply too faint to see.

The SRS-XRF imaging at SSRL was specifically developed for the *Archimedes Palimpsest* (Bergmann 2005), and we needed to ensure that no damage would occur to the invaluable parchment leafs. Several concerns had to be addressed before the green light was given by the owner to go forward with the synchrotron-based imaging project (Bergmann 2005, 2007, 2011; Bergmann and Knox 2009). We summarize these precautions here, as they are of general interest for synchrotron studies of ancient objects, especially those made from parchment, papyrus, paper, or other organic materials:

1. Radiation damage tests were performed on a test parchment sample to establish the safe dose for the SRS-XRF imaging. This was done by inspecting the microscopic structure of the exposed fibers and potential changes in coloration.



Fig. 8 Photographs of the four forgery leafs of the Archimedes Palimpsest. From left to right: St. Luke, St. Matthew, St. John, and St. Mark. It is more than likely that the forgers did not know that they were in possession of the *Archimedes Palimpsest*. (Images courtesy to the owner of the *Archimedes Palimpsest*)

- 2. To ensure minimal X-ray exposure, a fast pneumatic shutter was employed, which automatically closed when the parchment was not moving.
- 3. To prevent the parchment from drying or curling up, the storage area and experimental hutch were climate controlled to \sim 22 °C and a relative humidity of 50%.

Besides the safety challenges of handling and protecting the parchment leaves from damage, there were several fundamental challenges for the SRS-XRF imaging of the Archimedes Palimpsest. Except for a few parchment leaves that contain writings from other authors, including the great Attic orator Hypereides (c. 390-322 BCE), in most parts, two iron gall inks were used: the top ink used for the biblical texts and the partly scraped off bottom ink used for the Archimedes text. Unfortunately, both inks are of similar elemental compositions, and it was not possible to discern them by their XRF signal. Furthermore, XANES analysis taken at various locations showed that, not surprisingly, the speciation of iron was the same in both inks, namely, one of its most stable forms, Fe_2O_3 . Because iron K α XRF at 6.4 keV penetrates well through parchment (dried animal skin mainly made of carbon, nitrogen, hydrogen, and oxygen), all four layers of inks, the two layers from the front of the page and two from the back of the page, show up in the iron maps. Fortunately, the biblical writings were perpendicular to the Archimedes text (see Fig. 10), which helped their separation. To mitigate the separation of XRF signals from the inks of the front and the back of each leaf, two XRF detectors were employed and the data further processed (Fig. 9, left and center), see discussion below.

Another serious challenge was the fact that the forgery overpaintings contain heavy elements, including gold. Although these layers are thin and patchy, they absorb the incident beam and even more strongly the XRF signal from the ink. The schematics in Fig. 9 right depicts a small cross section of parchment viewed from the top, with a strongly absorbing patch of forgery paint in red and the iron gall ink in black. When the incident X-ray beam enters from the front (A), the heavy



Fig. 9 Left: top view schematics of the SRS-XRF imaging geometry used for the *Archimedes Palimpsest*. Two XRF detectors are placed perpendicular to the incident beam along the polarization direction of the incident X-rays. A third detector (ion chamber) monitors the transmitted X-ray beam intensity. Center: Photo of the original setup for imaging the St. John forgery leaf in geometry A. Right: top view schematics of two geometries for imaging the forgery pages. In (**a**), the beam enters from the side of the forgery painting, in (**b**) it enters from the backside



Fig. 10 Left: iron XRF image taken from the reverse of the St John leaf. The Archimedes writings are running horizontally and the biblical writings vertically. Writings from both sides of the leaf are visible as well as iron-rich areas of the forgery. Right: selected enlarged areas (see rectangles). (Adapted from Bergmann (2011))

elements in the forgery paint (red) absorb both incident X-rays and the iron XRF signal from the ink (black). Turning the leaf to enter from backside of parchment (B) allows for a stronger XRF signal because the parchment absorbs much less than the forgery ink. Figure 10 shows the iron XRF map from the St. John leaf taken in geometry (B) (mirror image of photo in Fig. 9). The map shows all four layers of writings. The two layers of Archimedes writings in the horizontal direction and the two layers of biblical writings in the vertical direction. Also visible are the parts of the forgery paintings that are rich in iron. The enlarged areas on the right illustrate some of the challenges.

The top right image shows a small area, where the iron-rich part of the forgery painting overlaps with Archimedes text. While some letters are easily discernible, others overlap with the strong signal from the iron in the forgery paint, which makes them difficult or impossible to read. Another example is shown at the bottom right, depicting an area where the Archimedes writings from both sides of the leaf overlap. In principle, scholars can distinguish the two layers by looking in which direction the letters lean, whether they are mirrored, or by making use of other digital images and their textual knowledge. However, in areas like this, it can be of great value to separate the writings from both sides of the parchment leaf.

To do that, one of our colleagues in the *Archimedes Palimpsest* team (Keith Knox) proposed combining the signals from the front and back XRF detectors (Fig. 9 left, middle) with a post-processing algorithm (Bergmann and Knox 2009). The basic idea behind this approach is as follows: When comparing the XRF signals from the inks on the two sides, it is always stronger in the detector to which the ink is closer. By plotting the XRF signal for every pixel in the front detector on a horizontal axis versus that in the back detector on a vertical axis, one obtains a scatter plot, where lower border represents XRF signals close to the front detector, i.e., from the front side of the parchment, and the upper border represents XRF



Fig. 11 Left: visible light image of a boarder section on the St. Mark forgery leaf. Middle and right: deconvoluted iron XRF signal corresponding to the front side (middle) and backside (right) of the leaf. The deconvolution algorithm is described in Bergmann and Knox (2009)

signals closer to the back detector, i.e., from the backside of the parchment. The pixels that have signals in between have contributions from the inks on both sides. Using a linear fit of the two slopes, one can deconvolute the signals into front and backside contributions and thus obtain an approximate separation of signals from the front and backsides. Given the linear approximation and limitations due to the statistical fluctuations of the detector signals, the separation is not perfect. Figure 11 shows the results of applying this algorithm to the iron XRF data in a border section on the St. Mark forgery leaf. To the left is a visible light image, in the middle the deconvoluted iron XRF signal corresponding to the front side, and to the right that corresponding to the backside of the leaf. While the iron in the forgery paint is not perfectly separated and shows up on both XRF images (instead of just the one in the center), one can see a very good separation of the texts from both sides of the leaf. Note that on each side there are both biblical writings (vertical) and the Archimedes writings (horizontal).

In the early morning hours of March 13, 2006, our colleague Reviel Netz from Stanford, one of the historians on the *Archimedes Palimpsest* project looked at the SRS-XRF iron image of the heavily damaged first leaf of the *Archimedes Palimpsest* (see Fig. 12). The image was so striking, that he was able to instantly read and transcribe the final passages of Archimedes' treatise "On Floating Bodies." This was the first time that anyone in modern times had seen these writings in the original Greek. The iron image also revealed the name and colophon (the publisher's emblem) of the Byzantine monk, who had written biblical texts on top. It says:



Fig. 12 Iron XRF image of the first page of the *Archimedes Palimpsest*. The Archimedes writings containing the final passages of "On Floating Bodies" are horizontal and the biblical writings vertical. The colophon by the author Johannes Myronas is shown in the right box. Also discovered was the drawing of a hand pointing upward (left box). (Adapted from Bergmann (2011))

"[This] was written by the hand of presbyter Johannes Myronas on the 14th day of the month of April, a Saturday, of the year 6737." The year is from the Byzantine calendar and corresponds to the year 1229 in our Gregorian calendar. It took almost 777 years to uncover what Johannes Myronas had overwritten just in time to get his book ready for Easter Sunday, on April 15, 1229.

Other significant findings based on SRS-XRF imaging include a diagram by Archimedes and text segments from *The Method* on the St. Matthew and St. John forgery leafs (Bergmann 2011; Netz et al. 2011; Service 2006). The story of the *Archimedes Palimpsest* project was published in a popular book (Netz and Noel 2007), and the SRS-XRF imaging work drew significant attention and media interest around the world (see, e.g., Service 2006; Fildes 2006). As the results became widely known, many ideas of imaging ancient and modern objects were generated, and the SRS-XRF imaging program on ancient objects at SLAC was born. While most subsequent work has focused on fossils (see next section), there were several successful imaging projects of ancient documents. These contacts were made as a consequence of dissemination in the popular media, showing how important this technique was for work on ancient objects, where the scientific communities are widely dispersed.

The Qur³ān Palimpsest

Shortly after the work on the Archimedes Palimpsest, we were contacted through Stanford by the owner of a folio containing writings from the Qur'an. This folio is part of a palimpsest containing two layers of Qur³ān writings. The upper layer of writing is a standard Qur³ān from the seventh or early eighth century AD. Radiocarbon dating assigns the parchment, and therefore the earliest possible time for the lower writing, to the first half of the seventh century. Early Muslim reports assert that different companions of the prophet Muhamad had different versions of the Qur'an, and some reports give the purported variants of their codices. The differences among these codices appear to have motivated an attempt at standardization. According to the collective memory of early Muslims, the companion 'Utman, after becoming caliph, disseminated a version of the holy book, declaring it the standard. This event appears to have taken place sometime during AD 644-650, and almost all extant Qur³ānic manuscripts belong to the textual tradition identified with this 'Utmanic version. The main significance of this Qur'an palimpsest folio is the fact that its lower text does not belong to this 'Utmanic textual tradition (Sadeghi and Bergmann 2010).

In contrast to the *Archimedes Palimpsest*, both upper and lower writings in the Qur³ān palimpsest were written in the same orientation. As the lower writings are still faintly visible, large fractions could be read directly from the visual light images. However, there are some areas where the overlap obscures the lower non-⁴Utmānic writings, for example, the section shown in Fig. 13 top left. XRF analysis of the two inks showed that their chemical composition has significant differences (The XRF imaging is described in more detail in (Bergmann et al. 2012). Both inks are rich in iron, but luckily the lower ink contains zinc and about twice as much copper than the upper ink (see Fig. 13 top right). The iron and zinc XRF maps are shown in the bottom part of Fig. 13. The iron map (left) shows writings from both the upper and lower layer of inks from both sides of the folio. It has therefore limited value for reading the lower text. However, in the zinc map (right) the upper writings are strongly suppressed (the residue is from copper K β XRF), enabling the enhanced reading of the lower text. The circled letter from the upper writings illustrates this.

These XRF images have brought to light features of the writings that were previously not visible and thereby helped the study of this folio of the Qur'ān palimpsest. Based on a detailed textual analysis, Sadeghi argued that the lower non-'Utmānic text, the standard 'Utmānic Qur'ān, and the other known versions of the Qur'ān (belonging to associates of the Prophet) form parallel textual traditions that point to one original source. This comparison can thus illuminate the state of the Qur'ānic text prior to the branching off of these various traditions, shedding light on the progenitor of all of them, the Qur'ānic prototype (Sadeghi and Bergmann 2010).



Fig. 13 Top left: visible light image of a section of the Qur'ān palimpsest. Top left: XRF spectra of the upper and lower inks, showing the absence of Zn and reduced concentration of Cu in the top ink. Note that spectra have been normalized to yield the same Fe K α signals. Bottom XRF images of iron (left) and zinc (right). (Adapted from Bergmann et al. (2012))

Luigi Cherubini's Opera Médée

In 2009 we were contacted by Heiko Cullmann from Berlin, who was working on a critical edition of the original version of the 1797 opera Médée by the French resident Italian Composer Luigi Cherubini (1760-1842). The original score is at the Stanford Green Library, and one of the arias, Médée's aria no. 14 "Du trouble affreux qui me dévore" from the third act, had been blackened out and obscured, presumably with shoe polish by Cherubini himself. An XRF analysis revealed that several different trace metals were present within the ink Cherubini used to write his score, including iron (in the notes) and zinc (in the score lines). Based on this information, we performed full SRS-XRF scans at SSRL beamline 10-2 in collaboration with Sam Webb from SSRL (for more details, see Bergmann et al. (2012)). Figure 14 top shows the visual light image of both sides of the leaf that contains the aria no. 14 with the parts presumed to be obscured by the composer. This document also suffered from the fact that not only had it been overpainted, but there was writing on both sides of the paper which made resolving the musical notes and texts difficult. Fortunately, the iron gall ink used by Cherubini was high in potassium. By collecting XRF images using detectors both in front and behind the manuscript as it was scanned, we obtained K and Fe images of both sides. Because K is a much lower energy XRF emission, correlating K with Fe allowed us to resolve



Fig. 14 Top: visual light image of both sides of the leaf that contains the aria no. 14 "Du trouble affreux qui me dévore" from the final act of Luigi Cherubini's 1797 opera *Médée* showing the parts presumed to be blackened out by the composer. Bottom: the iron XRF images of the same pages, revealing the original score. (Adapted from Bergmann et al. (2012))

ink on the front relative to ink on the back of the page. This allowed us to separate the front of page score from the reverse side. At the bottom of Fig. 14, we show a resolved front and back of the score as a reverse gray-scale image based on the Fe/K correlation analysis, clearly revealing the obscured score. Note that traces of iron are also visible in the shoe polish used to overpainted the score.

The iron XRF maps of the overpainted notes were used by Cullmann to edit the score, producing the most complete version of all the musical numbers in his 2012 critical edition of the original version (Cullmann 2012). In July 2017 one of us (UB) had the pleasure to watch *Médée* and listened to this beautiful aria no. 14 on Air France flight AF0083 from San Francisco to Paris on the in-flight entertainment program. Stanford has been contacted by the Salzburg Festival about obtaining a digital copy of the manuscript including the XRF images to inform a performance planned for summer 2019.

The Syriac Galen Palimpsest

As of the writing of this chapter, we are in the middle of a large project within an international collaboration to image several previously unidentified and unreadable pages of the Syriac Galen Palimpsest (Afif et al. 2018a, b). It contains the Syriac translation of a work on pharmacology, *On Simple Drugs*, by the famous Greek physician and philosopher Galen of Pergamon (c. AD 129–200/216). Arguably the most accomplished of all medical researchers of antiquity, Galen influenced the development of various scientific disciplines, including anatomy, physiology, pathology, pharmacology, and neurology, as well as philosophy and logic. Syriac is an Aramaic dialect that first appeared in the first-century AD in Edessa and later became a major literary language throughout the Middle East from the fourth to the eighth centuries, preserved in a large body of Syriac literature. The significance of this Syriac translation of Galen's work lies in the fact that it shows how Galen's work spread toward East, where he was very well known through the widely used Arabic translations; these Arabic translations were often produced with the help of Syriac, as in the case of *On Simple Drugs*.

Using the SRS-XRF imaging combined with advanced data algorithms, we were able to identify and read several previously unidentified and unreadable pages in the palimpsest. An example is bifolio 108v-101r shown in Fig. 15 with the visual image to the left and a false color XRF image to the right. The palimpsested Syriac Galen writings running horizontal are revealed in the XRF image (green pseudocolor). Publication of this work is anticipated for 2019.

SRS-XRF Imaging of Paleontological Material

Uncovering obscured writings is an obvious choice for SRS-XRF imaging, but it was quickly realized that chemical mapping of other large objects can also be of great value. While some SRS-XRF imaging work at SSRL includes science fields outside of ancient materials, for example, the study of trace metals in brain sections (Popescu et al. 2009a, b, c), most of the work so far has focused on ancient objects and in particular fossils. This work achieved prominence when one of our colleagues from the *Archimedes Palimpsest* project was contacted by Peter Lars Larson, who is known for excavating the most complete *Tyrannosaurus rex* fossil known as *Sue* from the Hell Creek Formation in South Dakota (USA), which is displayed at the Field Museum in Chicago [see CNN documentary *Dinosaur 13*



Fig. 15 Left: visual light image of bifolio 108r-101v of the Syriac Galen Palimpsest. Right: XRF image using the spectral information from several elements and advanced data algorithms to enhance the image. The false color XRF image shows the top writings in pink and the horizontal Syriac Galen writings in green. (Images courtesy of Michelle C. Smith (visible light) and William I. Sellers (X-ray))

https://www.dinosaur13movie.com/). The intimate relationship between the original biochemistry of an organism and its fossilized chemistry had not been explored using the techniques previously deployed on manuscripts. It was hypothesized that fossil chemistry might reveal new insight to the organism, including the burial environment and the processes acting upon the organism in its journey through life, death, fossilization, discovery, curation, and impacts of handling when the object was studied. The "chemical ghosts" of past life had the potential to shed new light on specimens that had been known to science for over a hundred years, but whose secrets were lost to the X-ray portion of the electromagnetic spectrum. One such fossil, well-studied for over a century, was *Archaeopteryx*.

Darwin's Bird: Archaeopteryx

On August 15, 1861, Hermann von Meyer, one of the most important vertebrate paleontologists of the nineteenth century, reported the discovery of a remarkable fossil in a letter to the editor Heinrich Georg Bronn of "Neues Jahrbuch fuer Mineralogie." He wrote: "From the lithographic slates of the faults of Solnhofen, Bavaria I was informed about the slab and counter slab of a fossil, which with

great clarity can be evidenced as a feather that is not distinguishable from bird feathers. In the very well-known organization of pterodactyls, there is nothing known that could be concluded as a plumage; this would therefore be the first remains of a bird of the pre-tertiary" (Translated from von Meyer 1861a). Six weeks later, on September 30, 1861, von Meyer sends a second letter to Bronn, where he writes "Following my letter from August 15, I can now report that I have studied the feather from Solnhofen in detail and I came to the conclusion that it is a true fossilization of the lithographic shale and in complete agreement with a bird feather. At the same time I have received news from Justice Councilor Witte. that an almost complete skeleton of an animal covered with feathers was found. Compared to living birds it shows some differences. I will publish the feather that I studied with an exact drawing. For the description of the animal I suggest the name Archaeopteryx lithographica" (translated from von Meyer (1861b) and in the following called Archaeopteryx). He had derived the name Archaeopteryx from the Greek $d\rho_X \alpha \tilde{\iota} o \zeta$ (archaios) = ancient and $\pi \tau \epsilon \rho \upsilon \xi$ (ptéryx) = feather or wing. These finds mark the discovery of one of the world's most iconic fossil animals also known as Urvogel (German for "original or first bird"). (Note that much of the information about Archaeopteryx presented in this section comes from the very beautiful and comprehensive book Archaeoteryx - Der Urvogel von Solnhofen (its English translation is called "Archaeopteryx – The Icon of Evolution") by Peter Wellnhofer (Wellnhofer 2008; Wellnhofer and Haase 2009)) At the time, the significance of Archaeopteryx was not just its age (the Solnhofen lagersätte is from the Late Jurassic corresponding to ~ 150 million years old, and modern birds were thought to have evolved much later). The anatomy of Archaeopteryx also showed stark differences to modern birds, with some features resembling more a small predatory (theropod) dinosaur. Furthermore, Archaeopteryx was discovered only 2 years after Charles Darwin had published his famous book On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life (Darwin 1859) and was quickly hailed as one of the first socalled missing links in support of Darwinian evolution. While Archaeopteryx is not a direct ancestor of modern birds, it gives insights to the evolution of flight in birds and what is considered by many as an insight into what the first bird might have looked like (Wellnhofer 2008; Wellnhofer and Haase 2009). In the last two decades, numerous other feathered dinosaurs have been discovered in China, notably fossils from Liaoning (Qiang et al. 1998; Hou et al. 1999; Swisher III et al. 1999; Norell and Xu 2005), and our understanding of early bird evolution is steadily improving. The new dinosaur finds are confirming what has long been hypothesized: The evolution of feathers had many other benefits before it was exploited for flight. Archaeopteryx has kept its mythos as an icon of evolution and with only 12 specimens and the single Von Meyer feather (1861) as of October 2018. However, Archaeopteryx is still the top attraction at many Natural History museums around the world, but usually in the form of a specimen cast. In 2001 the paleontologist Lawrence Witmer (https:// people.ohio.edu/witmerl/lab.htm) summarized the iconic standing of this species: "Nothing can challenge the Archaeopteryx as historically, scientifically, politically, and esthetically the most important fossil bird ever discovered" (Wellnhofer 2008;

Wellnhofer and Haase 2009). Adding any new knowledge about *Archaeopteryx* is of course very challenging as these fossils have been meticulously studied for well over a century. Most of what we learn about fossils comes from the fossilized bones and remaining imprints from soft tissue as there is really no hope of preserving complex organic molecules or even DNA. Yet, some questions might be answered by deploying advanced analytical chemistry tools such as SRS-XRF:

- Do fossils contain more than can be seen in the visible portion of the electromagnetic spectrum?
- Is there endogenous chemistry preserved that might be identified as being a "chemical fossil" or "chemical ghost"?
- Can organismal chemistry survive and be preserved through deep geological time?
- What can we learn about the chemistry of the fossil in comparison with related extant (modern) species?
- What can we learn about taphonomy, the process of death, and fossilization?
- Can we image soft tissues that are otherwise not visible to the naked eye?

The owner of the *Thermopolis Archaeopteryx* located in Thermopolis (Wyoming, USA) gave access to this remarkable fossil through Peter Lars Larson (Black Hills Institute). Our team had the unique opportunity to use SRS-XRF imaging to study what was one of the best preserved *Archaeopteryx* specimens. Peter Larson transported the fossil from Wyoming to Stanford and was a key team member in this historic experiment. Figure 16 left shows Peter Larson (right) and Samuel Farrar (left) (Black Hills Institute, South Dakota), taking on the challenging task of mounting a heavy and irreplaceable *Archaeopteryx* specimen in the front hutch of SSRL beam line 6–2. SRS-XRF imaging was performed in two setups, to learn more about the trace elements in both high-Z materials including the 3D transition metals and low-Z materials including phosphorus and sulfur (Bergmann et al. 2010).

In the high-Z setup, we chose an excitation energy of 13.5 keV, and for the low-Z setup we selected an excitation energy of 3.15 keV, below the absorption edge of calcium, an element that compositionally dominates limestone. Figure 16 right shows the two setups. Note that the fossil is housed in a He environment behind a thin cellophane film, which can be seen in Fig. 16. For the high-Z setup, we placed the detector at 90° with respect to the incident beam to minimize the elastic scattering, and the specimen was scanned at a 45° angle. For the low-Z setup, the detector was placed directly against the cellophane layer with the detector window parallel to the sample so to avoid air absorption and minimize argon XRF, and the specimen was scanned at a 3.15 keV is much smaller and does not result in a large background even when the detector is not at the 90° scattering angle). Figure 17 shows a visual light photograph of the *Thermopolis Archaeopteryx* fossil and an artist's depiction (from http://itsnature.org). Characteristic dinosaur-like features such as teeth, clawed functional digits (fingers), and long tail (comprised of



Fig. 16 Left: Samuel Farrar (back left) and Peter Larson (front right) carrying the *Thermopolis Archaeopteryx* specimen into the experimental hutch at beamline 6–2 at SSRL. Top right: top view of SRS-XRF imaging geometry for the analysis of high-Z trace elements including Mn, Cu, Zn, and Br. The detector is positioned at 90° to the incident beam to minimize the scattering background. Bottom right: SRS-XRF imaging geometry for analysis of low-Z elements including sulfur and phosphorus



Fig. 17 Visual light photograph of the exceptionally well-preserved *Thermopolis Archaeopteryx*, showing the skeleton and imprints of the feathers. The inset shows an artist's depiction of the original animal. (Courtesy of Peter Minister)

vertebrae) are clearly visible in the fossil. Before our SRS-XRF imaging results, there had been no chemical evidence supporting the presence of fossilized soft tissue, as the feathers were considered "impressions."

The two marked rectangular sections, where imprints of the wing feathers are visible, are shown enlarged in Fig. 18 left. The phosphorus map of these areas shown in Fig. 18 right reveals a clear signal at the location of the original feather shafts (rachi). Given the concentration level and the fact that feather shafts tend to be more resistant to degradation by bacterial enzymes than the smaller barbules, the feather shaft we observe with remnants of all smaller fibers absent is consistent with what one would predict on the basis of relative stability of keratinous proteins that comprise feathers.

We therefore concluded that the chemical evidence strongly implies that the feather shafts represented the residue of the original organism's biological tissue (Bergmann et al. 2010). We also found similar features with somewhat weaker signals in the sulfur maps. None of the other elemental maps showed unambiguous signatures of preserved soft tissue. However, there were several notable observations from some of the SRS-XRF maps of the high-Z elements shown in Fig. 19.

The manganese map shows diagenetic inorganic Mn-oxide precipitates typical for limestone (Fig. 19). They typically occur along fractures in the stone and can also be seen in the visual light images appearing in a dendritic form with graybluish color. Manganese precipitates appear also to be controlled by the fossil; however, the bones themselves do not show an elevated manganese signal. Copper and zinc is mapped in elevated levels in the bone areas as compared to the limestone



Fig. 18 Left: visual light photograph of two areas where the fossil imprints of wing feathers are apparent. Right: phosphorus map reveals preserved chemistry of the original feather shafts. (Adapted from Bergmann et al. (2010))



Fig. 19 Left to right: SRS-XRF maps of manganese (Mn), copper (Cu), zinc (Zn), and bromine (Br). The Mn map shows inorganic precipitates typical for limestone, while the copper and zinc maps show slightly elevated levels associated with the bone. The Br signal marks a curation artefact where the bone has been carefully repaired with an epoxy resin that also appear lower in Cu and Zn. (Adapted from Bergmann et al. (2010, 2012))

matrix. The concentrations of Zn and Cu in the limestone are low, and there is little evidence for transfer by aqueous fluids in this system. Zinc and copper are key trace elements known in extant bone, including birds, mammals, and fish, and zinc is relatively well-conserved within bone. We concluded that the elevated zinc

levels in the *Thermopolis Archaeopteryx* bone were most likely endogenous to the original organism. Also visible in the zinc map are horizontal lines, which we found to be small traces of zinc contamination in the cellophane, probably from the manufacturing process. Another interesting observation was the enhanced bromine signal in certain bone areas, which was a function of epoxy fill used during the preparation and conservation of the fossil bones. Not surprisingly, these same areas show decreased levels of the key elements associated with bone, including copper, zinc, phosphorus, and sulfur.

The Thermopolis Archaeopteryx shows evidence of organic phosphorus and sulfur in the feather shafts, but no such traces were observed in the feather barbs and barbules. While we see an enhanced iron signal in some of the barbules, it is not obvious whether these are original to the animal or from postmortem diagenesis (the conversion of sediment into rock) (Bergmann et al. 2010). We concluded that the feather shafts were the only soft tissue preserved in the Thermopolis Archaeopteryx. A subsequent SRS-XRF and XAS study focused on the exceptionally preserved \sim 120-million-year-old bird *Confuciusornis sanctus* (western Liaoning, China) (Wogelius et al., 2011). Unlike Archaeopteryx, C. sanctus had no teeth and no long tailbone but shared the sophisticated anatomy adapted to aid tree trunk climbing. Confuciusornis is considered the most primitive known beaked bird. What was remarkable about the C. sanctus results was that we were able to find remnants of several trace elements reflecting original organismal chemistry throughout the C. sanctus feathers, including copper. These results allowed us to identify and map the remnant chemistry of eumelanin pigment that was a key control on feather coloration for this species (Wogelius et al. 2011; Bergmann et al. 2012).

Our team was offered the opportunity to study a second Archaeopteryx fossil, which is perhaps the first to be discovered. This single feather, originally described by Hermann von Meyer in his 1861 publications (von Meyer 1861a), had the potential to provide new insight into pigment chemistry present in this beautifully preserved fossil. The fossil of the single feather is in two parts that are known, respectively, as the Berlin (counterpart) and Munich (part) feathers (see Fig. 20). It was believed that the feather had been shed by the animal before it fossilized. This could have led to a better preservation as no bacteria from the decaying carcass would have been present. The Berlin feather is preserved as a dark carbonaceous film suggesting that organic material might persist, within the darkest areas that are apparent to the left of the distal feather tip. The Munich specimen shows a fainter distribution of organic material to the right of the distal feather tip (a mirror image of the Berlin feather). This distribution similarity suggests that the zonation seen in the Berlin feather is genuine and not an artefact of an uneven split of the fossil between adjacent bedding surfaces. Daniella Schwarz-Wings accompanied the fossil on its visit to SLAC from the Museum für Naturkunde, Leibniz Institute for Research on Evolution and Biodiversity (Berlin, Germany). We performed SRS-XRF imaging on beamline 6-2 using the high-Z and low-Z setups, respectively, and XAS for elemental speciation (Manning et al. 2013). Furthermore, we were able to perform speciation mapping by tuning the incident photon energy to various sulfur XANES features and carry out SRS-XRF imaging (see also the earlier section



Fig. 20 Top: view of the high-Z experimental setup of the Berlin feather at SSRL beam line 6–2. (Note the other fossil bird mounted next to the Berlin feather.) Bottom left: visual light images of Berlin feather counterpart (left) and Munich feather part (right), with the Munich feather image mirrored to permit direct comparison with the Berlin specimen. Bottom right: comparison of sulfur XANES taken from Berlin specimen. From top: feather, sedimentary matrix, and a small bone fragment present in the matrix left of the feather (see Fig. 21e). (Adapted from Manning et al. (2013))

"Combining XAS and SRS-XRF Imaging: Speciation Analysis and Mapping"). Figure 20 right shows sulfur XANES taken at various locations in the sample indicating the sulfur species associated with the various spectral features.

While the XANES of the bone fragment shows mainly inorganic sulfate, both the matrix and the feather show more complex spectra with additional organic sulfur species. The peak energies coincide with theoretical values of various types of organic sulfur in addition to sulfonate as indicated in the figure. The inorganic sulfate and other organic sulfur species in the matrix are either foreign material or keratin-derived oxidation products from unpigmented parts of the feather, diffused away from the feather itself. Higher sulfur levels in the feather relative to the matrix imply mass transfer of sulfur was from the less pigmented parts of the feather outward. To obtain a map of organic sulfur, we set the incident beam energy to



Fig. 21 Berlin feather images using visible light (**a**), SRS-XRF false color images of copper (**b**), nickel (**c**), organic sulfur (all species) (**d**), sulfate only map (**e**), and artist's restoration (**f**). The white arrow on (**e**) indicates the presence of sulfate in a fossil bone fragment just below the sediment surface (scale bar: 10 mm). Brighter signal equates to increased concentration. (Adapted from Manning et al. (2013))

2479.9 eV below the inorganic sulfate resonance, thus minimizing contributions from sulfate. The map is shown in Fig. 21d.

Similar to the SRS-XRF maps of copper (Fig. 21b) and nickel (Fig. 21c), it shows zonation with increased concentration along the outer vanes (especially on the left) of the feather and in the distal tip area. In contrast, the inorganic sulfate map (Fig. 21e), obtained by image subtraction of the 2479.9 eV excitation map from the 2481.5 eV excitation map, shows a very different distribution. Besides the bone fragments that are high in inorganic sulfur, there is not much difference between the matrix and the feather, except the areas in the feather are rich in organic sulfur (distal tip and outer vanes) and lower in inorganic sulfur compared to the matrix. A detailed discussion of the possible origins of the various elemental distributions can be found in (Manning et al. 2013). The main conclusions of this work was that given that copper concentrations in the Berlin feather were comparable to concentrations measured within melanosomes of modern organisms and that the copper bonding environment (via XAS) was consistent with organic chelation, we concluded that the observed trace-metal zoning in the feathers of Archaeopteryx was due to breakdown products from within the original eumelanin, therefore permitting the first distribution of feather pigmentation in Archaeopteryx. Concentrations of Cu and Ni at the feather's distal tip and outer vane indicated that those portions were more heavily pigmented than the inner vane. This suggested a distinct plumage pattern with dark pigment in the outer vain and tips, while the inner vane protected by overlapping feathers remains relatively unpigmented as seen in the artist's restoration (Fig. 21f). This supports a possible visual role for pigments in the feathers of Archaeopteryx, potentially acting as a fitness criterion in the natural selection of this species. However, a potentially more significant finding is the impact of melanin on feather keratin. Pigmented keratin is more resistant to degradation and may be structurally more robust than unpigmented tissue, so the eumelanin distribution in *Archaeopteryx* might reflect a crucial adaptation to protecting and strengthening exposed feather parts during flight. This subtle variation in pigment pattern chemistry observed in this stunning fossil might well offer new insight to this key adaptation of flight in avians.

The chemistry of fossils creates many challenges, not least how to use that information to make interpretations about the physiology of extinct organisms. It is extremely difficult to distinguish between what is original biologically derived chemistry (endogenous) and what has been introduced externally (exogenous) and often compounded by various taphonomic (necrologic, biostratinomic, and diagenetic) processes that occur from immediately after death to over millions of years. In order to say with confidence that a certain element in a fossil is derived from an organism's biochemistry, we first must establish whether that element would likely have been in that living organism and also in that specific tissue type. We can investigate this by studying extant (living)-related species. In the case of *Archaeopteryx* and *C. sanctus*, the closest living relatives are of course modern species of bird. The final section in this chapter outlines a study that our team undertook to characterize melanin pigments in modern bird feathers via SRS-XRF and XAS. Such a systematic study gives valuable complementary data that can be used to validate and interpret results gleaned from fossils.

Modern (Extant) Feathers

Melanin is one of the most widespread pigments in biological tissues, most frequent as the black/brown (eumelanin) and red/yellow (pheomelanin) hues in skin, hair, and feathers (d'Ischia et al. 2013; McGraw 2003; Riley 1997). The concentration and distribution of melanin is consistently shown to correlate with certain metals. In the C. sanctus study (Wogelius et al. 2011), the evidence strongly pointed toward eumelanin as the source of the trace metal (especially zinc and copper) inventory within the preserved feathers. However, there was insufficient data to confidently identify and differentiate the equally significant pheomelanin pigment using synchrotron X-ray analyses, with no comparable data in the literature. Such information would allow more accurate pigment reconstruction within fossils (brown/black versus red/yellow). Therefore, we performed a study of modern feathers known to be pigmented with both eumelanin and pheomelanin (Edwards et al. 2016). The primary difference between eumelanin and pheomelanin was intimate coordination of zinc with sulfur in the pheomelanin structure (Ito et al. 2011). We selected feathers from four genera of birds of prey – Harris hawk (Parabuteo unicinctus), red-tailed hawk (Buteo jamaicensis), kestrel (Falco sparverius, Falco *tinnunculus*), and barn owl (*Tyto alba*); see Fig. 22.

They all displayed strong visual pigmentation of both eumelanin and pheomelanin as determined by high-performance liquid chromatography (HPLC) (Ito et al.



Fig. 22 Photos of the four genera of birds of prey – (a) Harris hawk (*Parabuteo unicinctus*), (b) kestrel (*Falco sparverius/Falco tinnunculus*), (c) barn owl (*Tyto alba*), and (d) red-tailed hawk (*Buteo jamaicensis*) – and the individual feathers analyzed by SRS-XRF and XAS (insets). (Adapted from Edwards et al. (2016) supplementary material)

2011). SRS-XRF elemental maps of feathers from these four birds unequivocally showed that the distribution of calcium, zinc, and copper correlated with visible pigment patterns (Fig. 23). Zn showed a slight anti-correlation to calcium, which came from the fact that Zn affiliates with both eumelanin and pheomelanin but slightly stronger with pheomelanin (Edwards et al. 2016). Furthermore, EXAFS spectroscopy distinguished between the different bonding configuration for zinc in eumelanized and pheomelanized feathers (Edwards et al. 2016). The key difference for pheomelanin is Zn-S bonding occurs in the first shell, compared to the exclusively light element coordination of Zn-N/O in eumelanin.

To see if the inclusion of sulfur in the pheomelanin molecule (as benzothiazine/zole units) could be detected we performed sulfur XANES. The spectra of



Fig. 23 Visual light image (left) of four modern bird feathers compared to SRS-XRF elemental maps of the element calcium (Ca), zinc (Zn), and copper (Cu), where the larger elemental concentration is shown as lighter color. The correlation of these elements with visual pigments is unequivocal. (Scale bars = 1 cm, adapted from Edwards et al. (2016))

all the feathers, both melanized and unpigmented, are dominated by the signal of disulfide and sulfur-carbon bonds within the keratin protein matrix (see Fig. 24). The spectra taken in the strongly pheomelanized regions of the kestrel show a subtle difference compared to those taken in eumelanized and unpigmented feathers. In strongly pheomelanized feathers (kestrel shown as example) the distinctive double peak intensity profile of keratin seen in black and white feathers is reversed (Fig. 24 right). This difference comes from the presence of benzosulfur type moieties known to be present in the molecular structure of pheomelanin. To further constrain the XAS results, SRS-XRF imaging was used to map the distribution of different species of sulfur within the feathers by tuning the energy of the incident X-ray beam to different XANES resonances. Here we saw that the kestrel stripe patterning showed stronger contrast when imaging on the benzosulfur resonances as compared



Fig. 24 (a) HPLC quantification within differently melanized regions of the kestrel feather (see Lynch et al. (2007) for complete study) compared to sulfur XANES from the corresponding locations on the feather. The top spectrum was taken in the dark red area, which is rich in pheomelanin; the middle spectrum in the dark area, rich in eumelanin; and the bottom spectrum in the white area which has no melanin pigments

to the total sulfur and disulfide. This confirmed the presence of sulfur bonding in the first Zn shell only in pheomelanin and not eumelanin that was light element coordinated (N/O) (Edwards et al. 2016). These results might provide insights to aid in the interpretation of pheomelanin pigment distributions in fossil samples in the future.

Conclusion

After giving a more general overview of the burgeoning activities in synchrotronbased research on ancient materials as summarized in Table 1, we have focused in this chapter on the chemical mapping of fossil and ancient materials with synchrotron-based X-ray fluorescence and spectroscopy. We provided the basic background of the techniques and instrumentation and discussed a series of examples, each representing different challenges and the technical and scientific approaches to tackle them. We hope that we also shared the thrill we felt when working in large interdisciplinary teams on some of the most iconic ancient and fossil materials, including the *Archimedes Palimpsest* and *Archaeopteryx*. These and other projects have inspired an increasing worldwide program that uses synchrotron radiation to shine light on questions in archaeology, paleontology, conservation science, and art history. While modern synchrotrons provide now the required beam brightness and future improvements in X-ray imaging and spectroscopy instrumentation and, in particular, in detection, data acquisition and data science will further advance this field. As there are endless important ancient and fossil objects yet to be discovered, this prospect is very exciting.

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Glossary

DPC	Differential phase contrast imaging
eV	Electron volt
EXAFS	Extended X-ray absorption fine structure
FT-IR	Fourier-transform infrared spectroscopy
HERFD	High-energy resolution fluorescence detection
HLCP	High performance liquid chromatography
keV	Kiloelectron volt
LUMO	Lowest unoccupied molecular orbital
nCT	X-ray nano computed tomography
NEXAFS	Near-edge X-ray absorption fine structure spectroscopy
PCCT	X-ray phase-contrast computed tomography
q	Momentum transfer
SAXS	Small angle X-ray scattering
STXM	Scanning transmission X-ray microscopy
ToF-SIMS	Time-of-flight secondary ion mass spectrometry
WAXS	Wide-angle X-ray scattering
XANES	X-ray absorption near-edge structure
XAS	X-ray absorption spectroscopy
XEOL	X-ray-excited optical luminescence
XRD	X-ray diffraction
XRF	X-ray fluorescence
XRS	X-ray raman scattering

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μXRF Micro X-ray fluorescence

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