This is an Accepted Manuscript of an article published by MRS Advances as follows: Zhanyun, Z, Tse, N, Nel, P & Tobin, M 2017 'Degradation profiles of silk textiles in diverse environments: Synchrotron based infrared micro-spectroscopy analysis', MRS Advances, vol. 2, no. 63, pp. 3939-3949. doi:10.1557/adv.2017.596.

Investigation on deterioration profiling of historic silk (*Bombyx Mori*) using synchrotron based infrared micro-spectroscopy

Abstract: In this paper, synchrotron based infrared micro-spectroscopy was utilized to describe the degradation profile of fibroin contained in silk textiles (*Bombyx mori*). The spatial distributions of deterioration effects in silk samples artificially aged at an assortment of conditions (thermal, hydrolytic and ultraviolet) were distinctly visualised and in accordance with the findings from conventional infrared spectroscopy in references. Further this method was applied on a historic sample from a private museum in Melbourne, and presented consistent results. This established synchrotron IR chemical mapping method could enable museum professionals to better understand the preservation state of historic silk and make informed decisions for conservation.

Key Words: historic silk, degradation, synchrotron infrared micro-spectroscopy, chemical imaging

1 Introduction

The deterioration of historic silk presents significant issues in museum collections, and for scholars using these collections. Used for garments, upholstery, banners or rugs and providing extensive social and historic information, silk is an appreciated material due to its valuable properties e.g. excellent smoothness, lustre, strength and lightness. However, silk textiles are ephemeral organic objects, sensitive to environmental degradation factors that cause deterioration of its intrinsic properties, leading to brittle fabrics which crack and eventually become powdery when touched (Zhu et al., 2014a, Zhu et al., 2013). Conservators concerned with the preservation of historic silk must assess the degradation states in the material when determining suitable approaches for conservation, exhibition, and preservation (Gong et al., 2015b). Thus it is necessary to

understand the degradation mechanisms and to find reliable analytical markers for monitoring the degree of degradation of historic silk textiles (Vilaplana et al., 2014).

Conventional Fourier Transform Infrared Spectroscopy (FT-IR) has been widely used for elucidation of the microstructural change caused by degradation processes in historic silk (Gong et al., 2015a, Aguayo et al., 2014, Akyuz et al., 2014, Garside et al., 2005, Garside et al., 2014, Garside and Wyeth, 2007). With continuous endeavours a number of useful degradation markers derived from IR spectra by juxtaposing the spectra of newly produced, historic samples, and artificially aged model silk samples have been proposed in the literatures. For example, the Amid I (at 1620 cm^{-1}) to Amid II (at 1514 cm^{-1}) intensity ratio ($I_{Amide\ I}$ / $I_{Amide\ II}$) was proposed to monitor the primary structure of historic silks (Zhang and Yuan, 2004). Recently, this marker, alongside with the proportion of band integral located at 1318 cm^{-1} to band integral of CH^3 bending vibration at 1442 cm^{-1} (A_{1318} / A_{1442}) were explored to monitor thermally aged and historic silk samples (Koperska et al., 2014, Koperska et al., 2015).

However, the microstructure of historic silk is extraordinarily heterogeneous due to multifarious ageing effects (Zhu et al., 2014b). Although previous investigations have provided valid estimators for historic silk deterioration, they cannot directly afford a distribution of chemical structure within silk fibres (Ling et al., 2014, Ling et al., 2013). Thus, with the possibility of combining spectral and spatial information, synchrotron based FT-IR will generate more productive data, thereby enabling heterogeneous structural characterization by IR spectra with a conspicuously higher spatial resolution, as well as a spatial chemical visualization of different deterioration indicators.

The present study focuses on chemical imaging of the previously proposed degradation estimators by synchrotron based FT-IR. The deterioration profiles of artificially aged silk samples exposed to different accelerating media were achieved and compared with a genuine historic silk from the Eckfeld Collection in Melbourne, Australia.

2 Experimental section

2.1 Materials

In this work, degummed, undyed and plain weave Bombyx Mori silk fabrics were used, since it is a common practice to use plain weave textiles during the conservation procedures of silk artefacts in leading museums. All the chemical reagents were purchased from Sigma-Aldrich and were of analytical grade. A historic sample from the Eckfeld Collection in Melbourne were used for reference. It is taken from an embroidered panel from a Chinese opera costume as shown in Figure 1 (dimensions: 68 cm H x 11.5 cm W each).

2.2 Sample preparation

Three accelerated ageing regimes were employed as follows. Hydrolytic aged samples were prepared by treating the fresh natural silk fabrics with 5 % NaOH aqueous solution at 35 °C for 5 h (Zhu, 2015). Thermal ageing was operated by exposing the reference silk sample to a temperature of 230 °C in a forced convection oven for 24 h (Zhu and Gong, 2014). Ultraviolet (UV) ageing was performed with a QUV Accelerated Weathering Tester (Q-Lab Corporation) at irradiance 1.2 W/m^2 for 14 d (Zhang and Yuan, 2005). Historic silk sample was scrupulously cleaned with distilled deionized water, acetic ester and petroleum ether sequentially to remove possible contaminations. Before analysis, samples were conditioned in 23 °C, 50 % RH for at least 1 week.

2.3 Synchrotron FT-IR micro-spectroscopy

Absorbance spectra were recorded in transmission mode using a Bruker Hyperion 2000 microscope (Bruker Optic GmbH, Ettlingen, Germany) attached to a Bruker Vertex V80 v FTIR spectrometer at the Australian Synchrotron Infrared Micro-spectroscopy beamline. The microscope was equipped with a set of 36× (N.A. 0.5) reflecting objective and condenser optics, a narrowband mercury cadmium telluride (MCT) detector spanning the wavenumber range of 4000 - 750 cm^{-1}, and motorised microscope apertures and sample stage. Each sample was flattened between the two diamond windows of the Micro Compression Cell (MCC) to prepare for transmission measurements. Potassium bromide powder was used for collection of reference spectra, in order to eliminate spectral fringing that could otherwise result from using the air gap between the two diamonds for the reference. Data were collected from individual

sample areas (5 × 5 microns apertures were used) and custom-defined grid maps (using 5 micron step size) on a number of areas of each sample. All measurements were taken with 6 cm^{-1} spectral resolution and 32 co-added scans for each sample and background measurement. Data collection was performed using the Bruker OPUS 6.5 software package whereas spatially resolved chemical maps were generated by integration of absorbance peak intensities or band integrals of specific functional groups of interest using Bruker OPUS 7.2. The integration peak areas were defined by selecting a baseline between the start and end wavenumbers of the individual absorbance peak.

3 Results and discussion

3.1 Synchrotron based IR spectra

As depicted in Figure 1, the synchrotron IR spectra of samples undergone disparate degradation processes presented consistent characteristics with data from conventional FT-IR (Koperska et al., 2014). With the advantage of rapidly recording an array of spectra from data points with high spatial resolution, these spectra were averages of ten randomly selected points in the mapping area, instead of the arithmetical mean of the data from three separately aged silk samples. In this research, the two aforementioned estimators concerning effects of the oxidation and hydrolysis on the primary structure of fibroin were investigated.

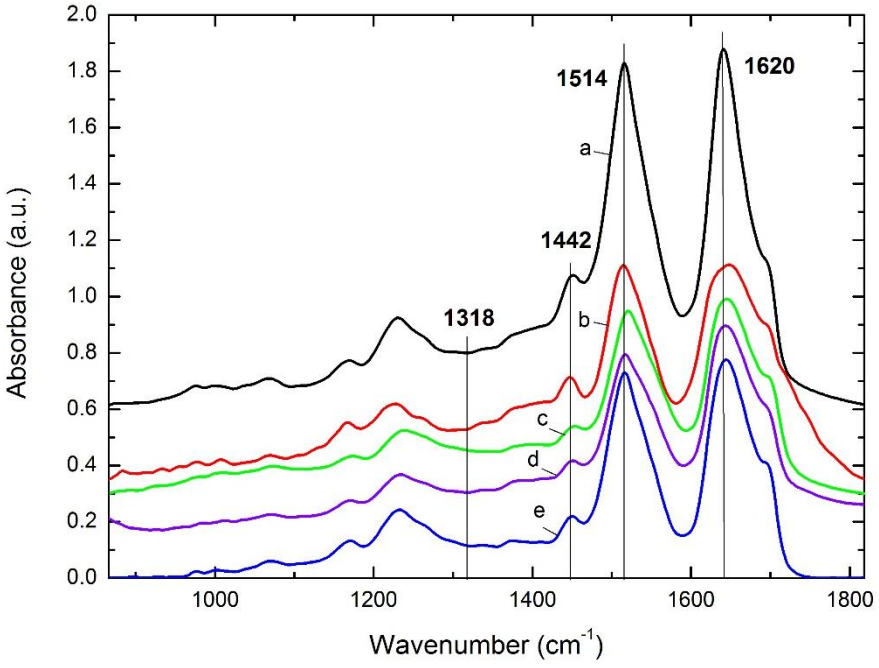

Figure 1 Synchrotron based IR spectra of (a) fresh silk (b) thermal aged silk (c) hydrolytic

aged silk (d) UV aged silk (e) historic silk

3.2 Chemical mapping of the deterioration estimators

The Amid I (at 1620 cm^{-1}) to Amid II (at 1514 cm^{-1}) intensity ratio ($I_{Amide\ I}$ / $I_{Amide\ II}$) estimator has been proven to reflect oxidation of polypeptide to carboxylic, aldehydic or ketones groups in silk fibroin rather than hydrolysis. It can be seen lucidly in Figure 3 that generally this parameter in silk sample mounted after thermal ageing compared with Figure 2. This observation can be explained by radical oxidation mechanism resulting in the formation of new carbonyl group, which was in conformity with previous findings by EPR and Raman Spectroscopy (Gong and Yang, 2013). Figure 4 display that the parameter $I_{Amide\ I}$ / $I_{Amide\ II}$ also increased in silk sample after UV irradiation. The interpretation of this phenomenon could be the formation of carbonyl groups as a result of the photo-oxidation of fibroin to α-keto-acids at the glycine and alanine (Sionkowska and Planecka, 2011). Besides, it is noticeable that the spatial distribution of oxidation is highly anisotropic, which could be visualised explicitly by synchrotron IR imaging, instead of the normally available FT-IR. It is shown in Figure 5 that no significant changes were spotted by this estimator during aging at alkaline hydrolytic conditions as expected.

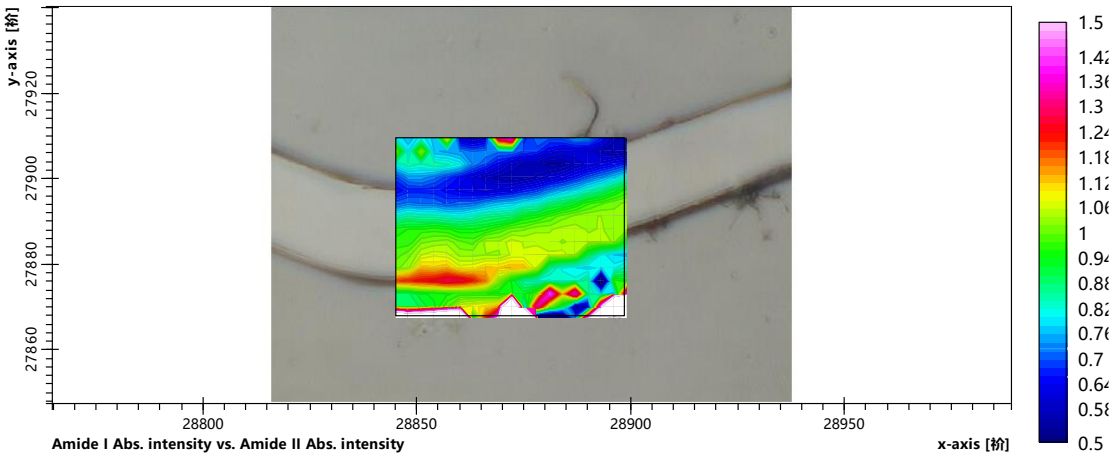

Figure 2 Chemical imaging of fresh silk generated from $I_{Amide\ I}$ / $I_{Amide\ II}$

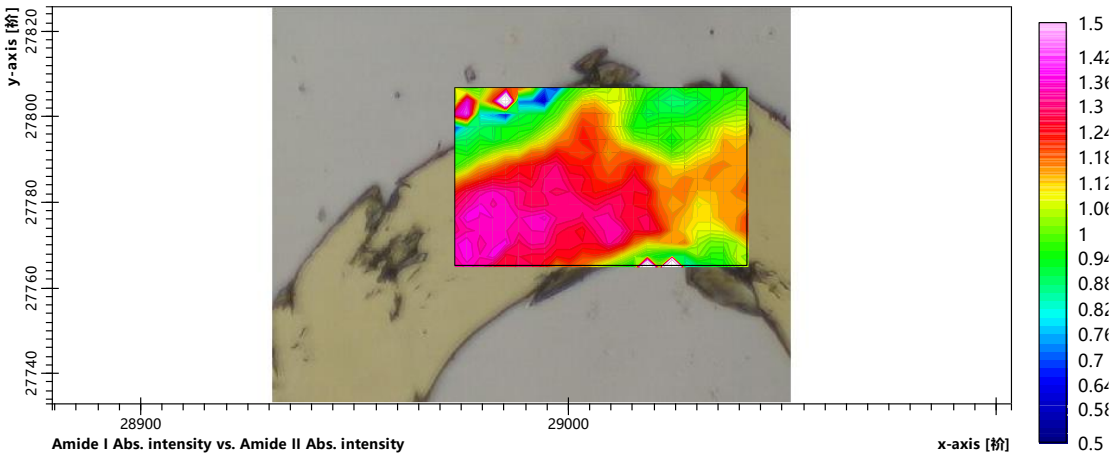

Figure 3 Chemical imaging of thermal aged silk generated from $I_{Amide\ I}$ / $I_{Amide\ II}$

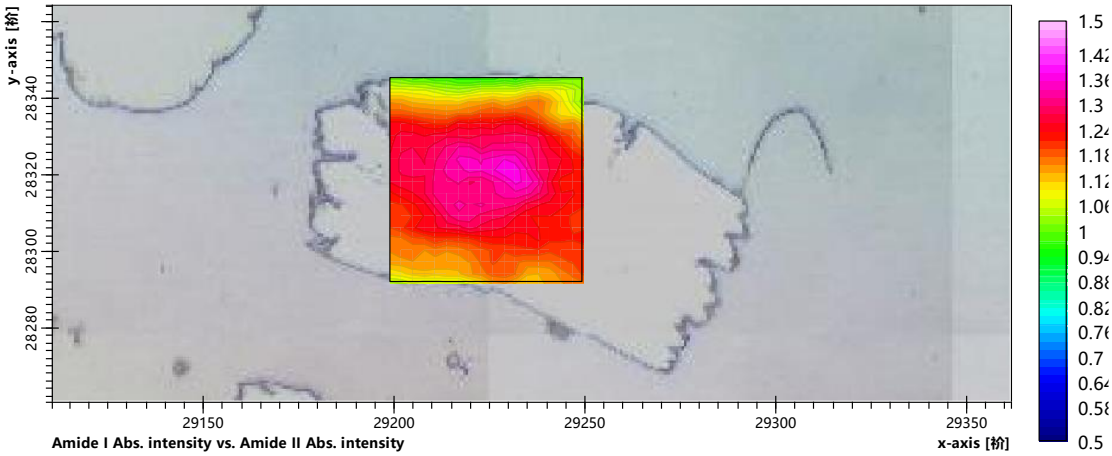

Figure 4 Chemical imaging of UV aged silk generated from $I_{Amide\ I}$ / $I_{Amide\ II}$

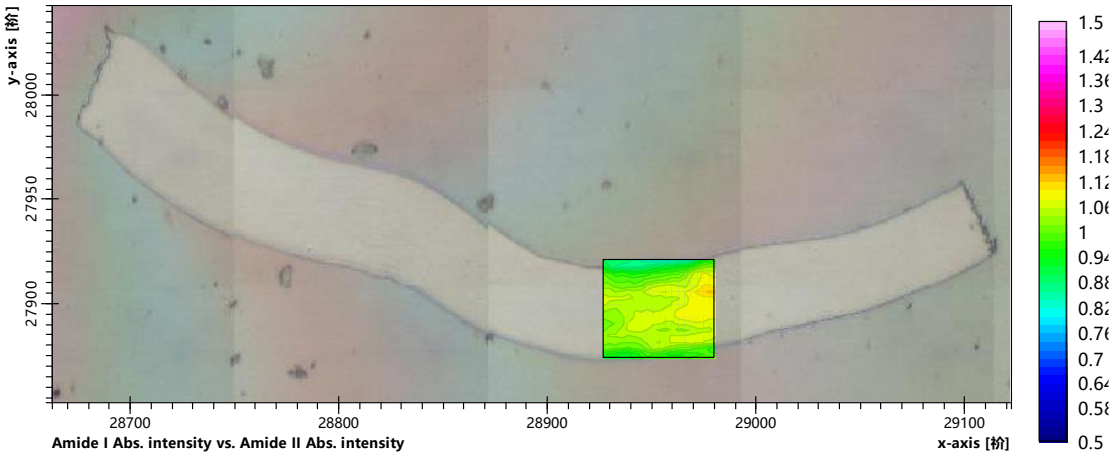

Figure 5 Chemical imaging of hydrolytic aged silk generated from $I_{Amide\ I}$ / $I_{Amide\ II}$

The band of C-H shielded by free dicarboxylic amino acids (at 1318 cm^{-1}) to band of CH^3 bending vibration (at 1442 cm^{-1}) integral proportion (A_{1318} / A_{1442}) degradation estimator was chosen to monitor the development of the vibration bands of new amino and carboxyl groups formed in hydrolysis. Synchrotron IR imaging revealed that this

estimator escalated in hydrolytic aged silk (Figure 7) at nonuniform levels in comparison with fresh silk (Figure 6) due to the formation vast free amino acids after hydrolysis. In contrast, for thermal (Figure 8) and UV (Figure 9) aged silk, this estimator rose only slightly as the main degradation pathway for these samples was oxidation other than depolymerisation.

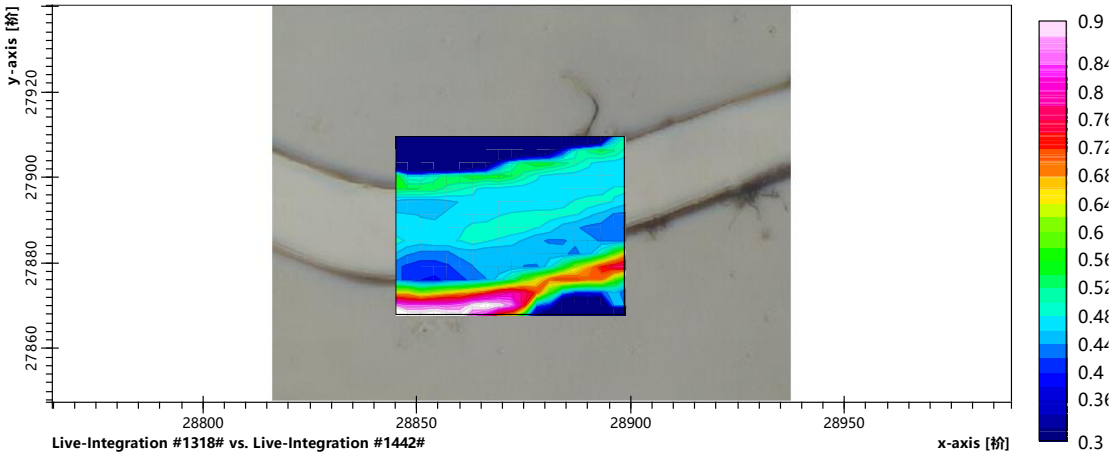

Figure 6 Chemical imaging of fresh silk generated from A_{1318} / A_{1442}

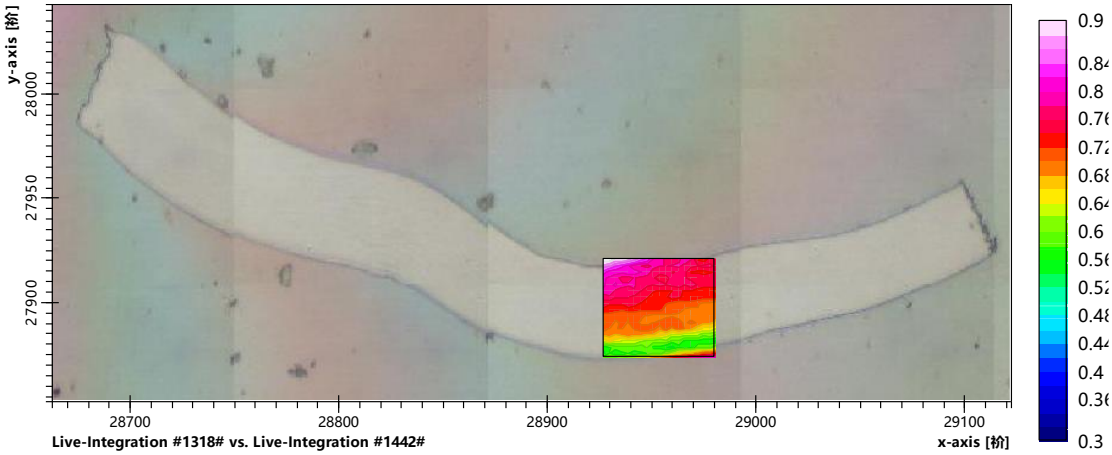

Figure 7 Chemical imaging of hydrolytic aged silk generated from A_{1318} / A_{1442}

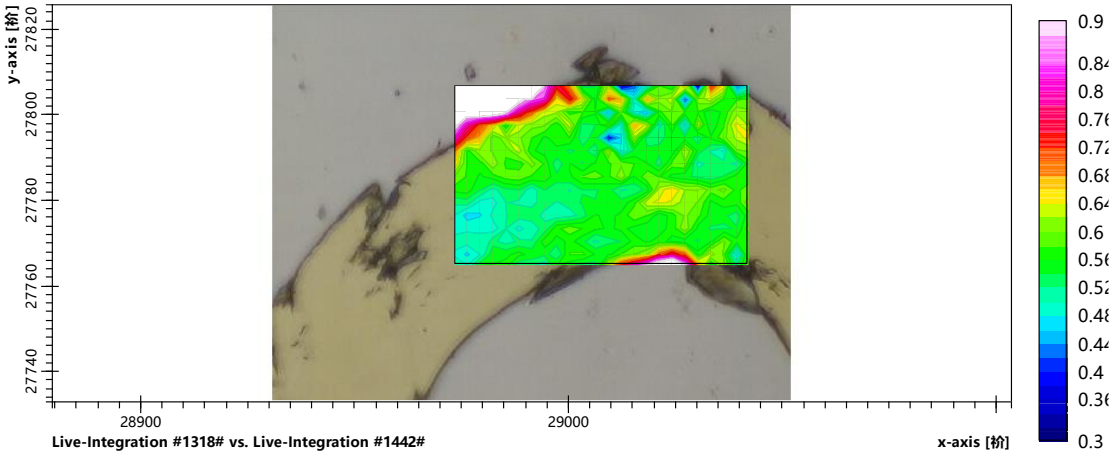

Figure 8 Chemical imaging of thermal aged silk generated from A_{1318} / A_{1442}

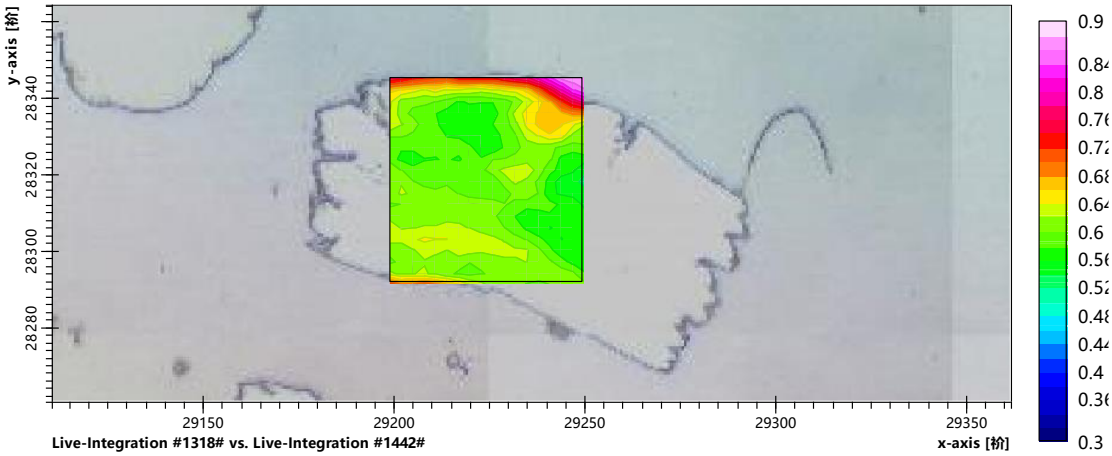

Figure 9 Chemical imaging of UV aged silk generated from A_{1318} / A_{1442}

3.3 Application on historic silk

It is indicated in Figure 10-11 that the primary structure of the historic silk fibroin peptides was disrupted in storage from both oxidation of peptide bond, aliphatic and aromatic side, and peptide bond cleavage through proteolysis. Although quite heterogeneous, the variation trend of the selected two estimators for the historic sample aligned well with the artificially aged samples, vindicating the virtue of synchrotron IR imaging to monitor the distribution of deterioration effects within historic silk fibres.

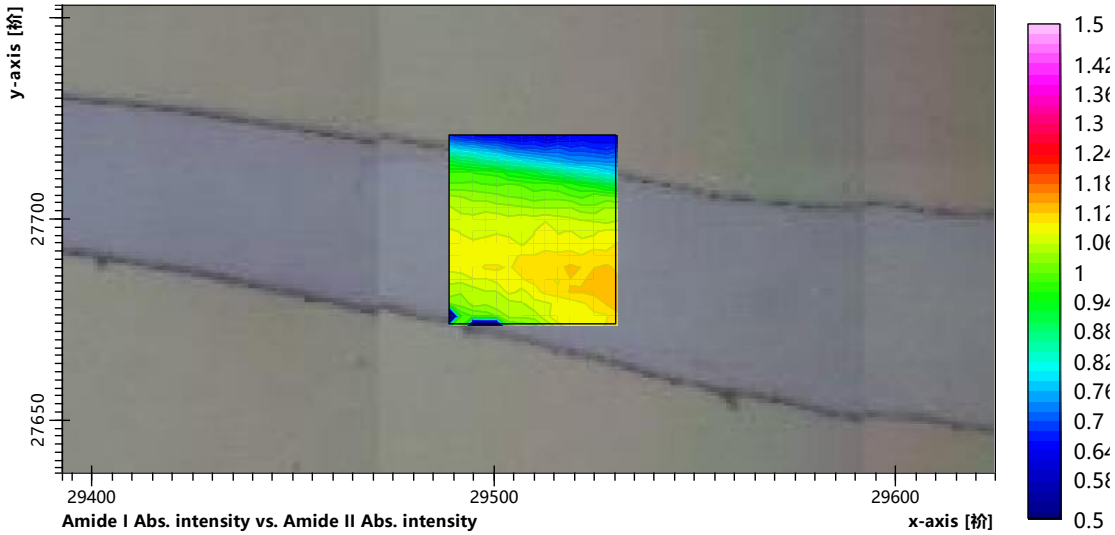

Figure 10 Chemical imaging of historic silk generated from $I_{Amide\ I}$ / $I_{Amide\ II}$

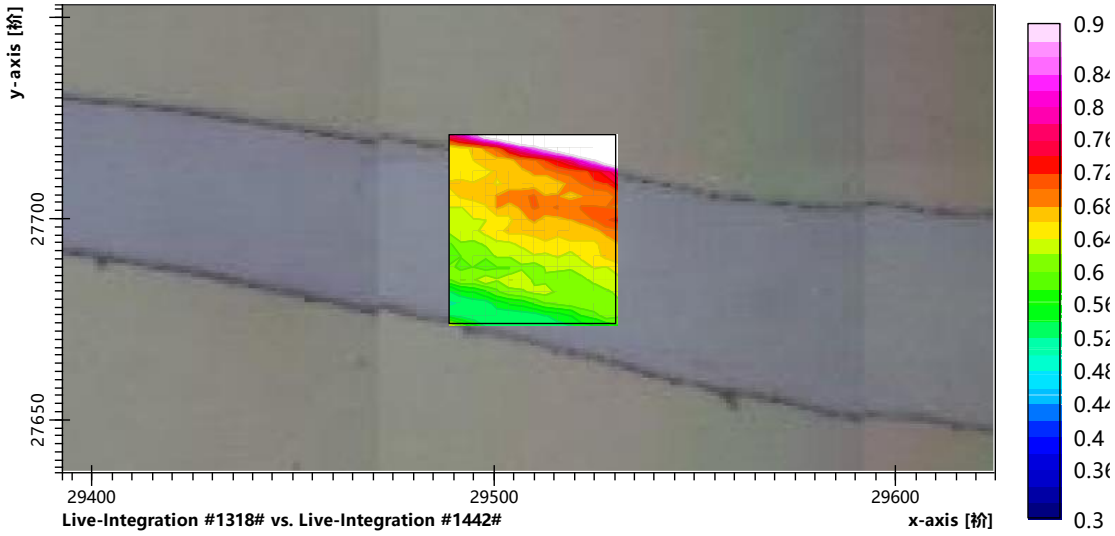

Figure 11 Chemical imaging of historic silk generated from A_{1318} / A_{1442}

4 Conclusions

Synchrotron based infrared micro-spectroscopy was successfully adopted to non-destructively analyse and quantify the spatial distributions of oxidation and hydrolysis stages within artificially aged and historic silk fibres with pre-defined estimators. Such diagnostic method is promising in providing important evidence for conservators and stakeholders to determine manageable storage and exhibition frameworks and risk mitigation strategies for the irreplaceable and vulnerable silk collections.

Acknowledgement

An Australian Synchrotron Beamtime Award (Reference Number: AS152/IRM/9302) is gratefully acknowledged.

References

AGUAYO, T., A., C., I., M. & CAMPOS-VALLETTE, M. 2014. A vibrational approach for the study of historical weighted and dyed silks. *J. Mol. Struct.,* 1075**,** 471-478.
AKYUZ, S., AKYUZ, T., CAKAN, B. & BASARAN, S. 2014. Investigations of the historic textiles excavated from Ancient Ainos (Enez–Turkey) by multiple analytical techniques. *J. Mol. Struct.,* 1073**,** 37-43.
GARSIDE, P., LAHLIL, S. & WYETH, P. 2005. Characterization of historic silk by polarized attenuated total reflectance Fourier transform infrared spectroscopy for informed conservation. *Appl. Spectrosc.,* 59**,** 1242-1247.
GARSIDE, P., MILLS, G., SMITH, J. & WYETH, P. 2014. An investigation of weighted and degraded silk by complementary microscopy techniques. *e-Preserv. Sci.,* 11**,** 15-21.
GARSIDE, P. & WYETH, P. 2007. Crystallinity and degradation of silk: correlations between analytical signatures and physical condition on ageing. *Appl. Phys. A - Mater.,* 89**,** 871-876.
GONG, D., LIU, L. & ZHU, Z. 2015a. Application of Infrared Spectroscopy in Characterizing Fiber Aggregation Structure of Ancient Silk Fabrics. *Science of Sericulture,* 41**,** 0694-0700.
GONG, D. & YANG, H. 2013. The discovery of free radicals in ancient silk textiles. *Polym. Degrad. Stabil.,* 98**,** 1780-1783.
GONG, D., ZHU, Z. & LIU, L. 2015b. Micro-mechanism elucidation of the effects of dehydration on waterlogged historic silk (Bombyx mori) by near-infrared spectroscopy. *Stud. Conserv.,* 60**,** 284-290.
KOPERSKA, M., ŁOJEWSKI, T. & ŁOJEWSKA, J. 2015. Evaluating degradation of silk's fibroin by attenuated total reflectance infrared spectroscopy: Case study of ancient banners from Polish collections. *Spectrochim. Acta A,* 135**,** 576-582.
KOPERSKA, M., PAWCENIS, D., BAGNIUK, J., ZAITZ, M., MISSORI, M., LOJEWSKI, T. & LOJEWSKA, J. 2014. Degradation markers of fibroin in silk through infrared spectroscopy. *Polym. Degrad. Stabil.,* 105**,** 185-196.
LING, S., QI, Z., KNIGHT, D., SHAO, Z. & CHEN, X. 2013. FTIR imaging, a useful method for studying the compatibility of silk fibroin-based polymer blends. *Polym. Chem.,* 4**,** 5401.
LING, S., QI, Z., WATTS, B., SHAO, Z. & CHEN, X. 2014. Structural determination of protein-based polymer blends with a promising tool: combination of FTIR and STXM spectroscopic imaging. *Phys. Chem. Chem. Phys.,* 16**,** 7741-8.
SIONKOWSKA, A. & PLANECKA, A. 2011. The influence of UV radiation on silk fibroin. *Polym. Degrad. Stabil.,* 96**,** 523-528.
VILAPLANA, F., NILSSON, J., SOMMER, D. & KARLSSON, S. 2014. Analytical markers for silk degradation: comparing historic silk and silk artificially aged in different environments. *Anal. Bioanal. Chem.*
ZHANG, X. & YUAN, S. 2004. Research on the infrared spectrometry of aging silk fabrics. *Spectroscopy and Spectral Analysis,* 24**,** 1528-1532.
ZHANG, X. & YUAN, S. 2005. Analytical research on aged silk by SEM. *J. Chin. Electr. Microsc. Soc.,* 22**,** 443-448.
ZHU, Z. 2015. *Elucidation of Micro Pore Structure in Historic Silk and Development of Targeted Conservation Methodology.* University of Science and Technology of China.
ZHU, Z., CHEN, H., LI, L., GONG, D., GAO, X., YANG, J., ZHAO, X. & JI, K. 2014a. Biomass Spectrometry Identification of the Fibre Material in the Pall Imprint Excavated from Grave M1, Peng-state Cemetery, Shanxi, China. *Archaeometry,* 56**,** 681-688.

ZHU, Z. & GONG, D. 2014. Determination of the experimental conditions of the transglutaminase-mediated restoration of thermal aged silk by orthogonal experiment. *J. Cult. Herit.,* 15**,** 18-25.
ZHU, Z., GONG, D., LIU, L. & WANG, Y. 2014b. Microstructure elucidation of historic silk (Bombyx mori) by nuclear magnetic resonance. *Anal. Bioanal. Chem.,* 406**,** 2709-2718.
ZHU, Z., LIU, L. & GONG, D. 2013. Transglutaminase-mediated restoration of historic silk and its ageing resistance. *Herit. Sci.*