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Parchment preservation state of the Prayer book of Mary of Guelders

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Abstract
Illuminated medieval manuscripts are of outstanding value and their preservation is of great importance, not only because of their beauty but also because of the information they contain about medieval society. This work focuses on the evaluation of the parchment's state of preservation of the Prayer book of Mary of Guelders, which comprises about 600 folios. The knowledge gained should support the decision-making process regarding suitable conservation measures. An assessment of the preservation state of the parchment was performed from the macro- down to the microscale. Optical observations of cracks in the parchment and colour measurements preceded chemical analyses. The hydrothermal stability of the fibres was evaluated by means of observations using a micro hot table (MHT). The chemical state of preservation of parchment was evaluated using Laboratory-based Fourier transform (FT) Infrared (IR) analysis in reflection mode as well as synchrotron FTIR imaging in transmission mode at the IRIS beamline at BESSY II/ HZB in Berlin. The study allowed the conclusion that the parchment of the Prayer book of Mary of Guelders was in good state of preservation and indicated that the parchment changes were mainly caused by mechanical stress on the folios due to tight binding of the book and not by chemical processes.

Keywords Parchment · Medieval illuminated manuscript · Conservation state · Optical and electron microscopy · Micro-FTIR analysis and imaging

1 Introduction

Illuminated medieval manuscripts are of outstanding value for our cultural heritage because of their beauty and the multitude of information on medieval societies they bear. Made of parchment, a fragile and complex support material, manuscripts need to be stored and exhibited under special conditions. Parchment is prepared from different animal hides, most commonly from sheep-, goat- or calfskin. Unlike leather, it is not tanned. After drying in air or salt (also named as curing), the hides are washed with cold running water, soaked in a bath of lime $Ca(OH)_2$ and then scraped with a blunt knife to remove hair, tissue and flesh residues. Finally, the hides are dried under tension, mechanically thinned and polished [13, 31]. From the chemical point of view parchment is a protein, basically constituted of collagen molecules. Collagen type I, which is the main component of the skin, has a highly hierarchical structure and consists mainly of three amino acids: glycine, proline and hydroxyproline in a specific 3D arrangement. Three polypeptide chains (amino acids sequence) are twisted and form

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triple-helix molecules, which then arrange together to form fibrils, fibres and finally the tissue [4]. Parchment degrades over time, evidenced by change of physical properties resulting in enhanced fragility. Temperature, humidity, mechanical stress, light radiation or the presence of microorganisms influence the degree of parchment degradation [14]. Three types of collagen degradation are frequently described in the literature and can be observed using vibrational spectroscopy [11, 2, 3, 12, 13, 21, 30]:

(a) Denaturation (structural change): Unfolding of the triple helix is due to a loss of internal hydrogen bonds in the presence of water resulting in the formation of a gel-like substance (gelatine). This process is also named as gelatinization.
(b) Hydrolysis (chemical change): Hydrolysis can be caused by acids or atmospheric pollutants, such as for example SO_2 reacting with water resulting in the chemical breakdown of collagen molecules into smaller polypeptide molecules. Moreover, both, collagen and gelatine can undergo hydrolysis.
(c) Oxidation (chemical change): Oxidation reactions can either take place on the side chains of amino acids or in the main chain, which then lead to its cleavage. Free radicals are needed for this type of reaction, which can be formed e.g. by the action of ultraviolet light on a water molecule.

The illuminated manuscript, the Prayer book of Mary of Guelders, is one of the most famous medieval Netherlandish manuscripts. It is kept today, at least partly, at the *Staatsbibliothek zu Berlin–Stiftung Preußischer Kulturbesitz* (SBB-SPK, Berlin State Library). The Prayer book made of parchment has become very fragile over time and since at least 1975 it has not been bound (as a book) anymore (Fig. 1). Damages that can be observed are abrasion and flaking of paint layers, paint cracks, abrasion of the gilding layers, etc. The major damage was the presence of numerous cracks in the parchment, on painted as well non-painted areas, which is a very unusual damage pattern for this type of manuscript. Because of its high fragility, the manuscript has only occasionally been accessible to the public for decades. An interdisciplinary program was established to both, characterize the paint materials (pigments, colourants) as well as answer questions concerning the state of preservation of the Prayer book. The results of the characterisation of the paint materials were published elsewhere [18].

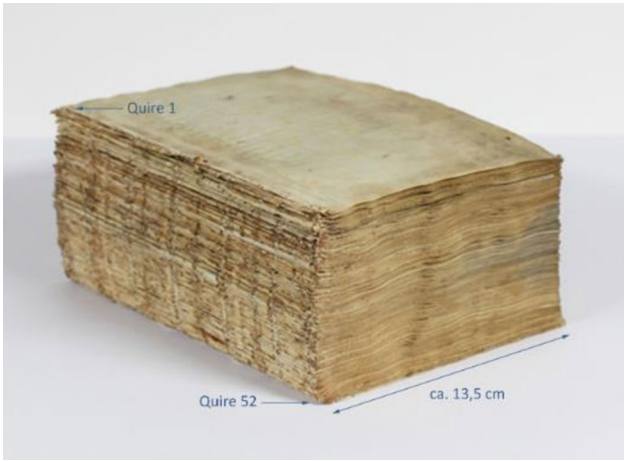

Fig. 1 The unbound part of the manuscript Mary of Guelders as it is kept in the SBB before the interdisciplinary research program (© SBB, M. Hundertmark)

2 Research aims and approach

This study aims at a better understanding of the state of preservation of the parchment of the medieval manuscript, the Prayer book of Mary of Guelders. Different questions are addressed such as: Which types of visible and invisible damages occur on the macroscopic to molecular level? What are potential origins of damage? Is the crack formation rather a consequence of high mechanical stress because of a previous tight bookbinding combined with a frequent use or might it be a chemical alteration of the parchment? Motivated by the unusual damage pattern, a comprehensive inventory of all microscopically visible cracks is carried out for the entire manuscript. In addition to this special measure, the parchment is analysed using a selection of non-invasive methods that are widely used for the examination of parchment degradation. Micro-Hot-Table (MHT) enables determination of shrinkage temperature, which is a widely used parameter for the degree of degradation of collagen in parchment [16, 23, 22, 6, 10, 8]. The colour measurements used by Mühlen-Axelsson et al. [22], for example, to measure heat-induced changes in the parchment are carried out using visible spectrocolourimetry. In addition, the parchment is analysed by infrared spectroscopy (FTIR). With the help of FTIR measurements, even the smallest changes in the secondary structure of the collagen can be recognised and evaluated, which occur during the gradual transition from the strongly hierarchical helical structure to the disordered structure of gelatinised parchment [1, 2, 3, 4, 6, 10, 8, 29]. These investigations are supplemented by artificial ageing tests on parchment mock-up samples. The results of this work informed the decision-making process for the best possible conservation strategy of the Prayer book of Mary of Guelders.

3 Material and methods

3.1 The Prayer book of Mary of Guelders

The Prayer book of Mary of Guelders was manufactured in 1415 in the Augustinian monastery of Arnhem (the Netherlands) for the duchess Mary of Guelders. Owing to the outstanding artistic quality of the miniatures, this Prayer book represents a major work of the Netherlandish book illumination of the SBB, where its major part is kept today. The other part of the manuscript is kept at the Austrian National Library in Vienna. The Berlin part of the manuscript consists of 52 quires made of parchment and contains in total 241 bifolios; each quire being composed of 3 to 6 bifolios.

For the non-invasive investigations, individual bifolios were selected that were representative of the entire manuscript and also stable enough to be analysed without prior consolidation. The bifolios no. 5r, 23v, 114v, 241v were analysed using spectrocolorimetry and the bifolio no. 70v by means of Fourier Transform Infrared Spectroscopy (FTIR) measurements in reflectance mode (refl, Fig. 5, Table 1).

The conservators also chose an inconspicuous spot at the inner rim of the folio 173 of the manuscript (Fig. 2), where the parchment was already slightly torn, and took a tiny sample (smaller than 1 mm^2) from it for invasive analyses. A portion of this sample, with triangular dimensions of about 0.75 x 0.5 x 0.75 mm^3 (see inset Fig.2, 71_15_p1), was prepared for characterisation using FTIR in transmission mode (tr). First, the parchment sample was embedded in epoxy resin (Epofix, Stuers®) and then the resin block was cut such to reveal a cross-section of the parchment. By means of a microtome, thin sections were cut out from the cross-section of the parchment sample (5–10 µm thin) ready for the transmission measurements.

3.2 Parchment references and mock-ups

As the parchment of the Prayer book of Mary of Guelders was most likely prepared from calfskin, the chosen parchment references originate from a calf hide too. Modern parchment (MP) provided by the SBB as well as a naturally aged historical parchment (HP) from a 15th-century book of hours (Flanders – French border region) were studied for comparison. They correspond to the best references that can be provided for such historical objects. For the FTIR refl measurement performed using the lab instrument no prior preparation was needed as the spectra was taken from the parchment surface.

In addition, mock-up samples (MP_DXX) were produced to simulate possible ageing of the historical parchment. As described in Müller et al. [24] they correspond to modern calfskin that was pre-treated following historical procedures and aged afterwards. The modern parchment samples were artificially aged under two different conditions: firstly, in a dry atmosphere (condition I: 70 °C, 30% RH, MP_DXX-I) and secondly in a humid atmosphere (condition II: 80 °C, 80% RH, MP_DXX-II), which can be found in the literature [6]. Different climate chambers had to be used for both ageing methods, one of which can only be heated up to 70 °C. Table 1. summarize the analysed materials and analytical techniques applied in this study. The aging procedures are described in detail in the supplementary information (SI). In addition, the SI contains supplementary information on the characterization of the artificially aged samples using colorimetry, optical and electron microscopy and refl FTIR. All samples were kept at approximately 50–55% relative humidity (RH) after the aging.

3.3 Crack observation in the Prayer book

Number, length, location of cracks as well as their distribution within folios and throughout the entire Berlin part of the Prayer book were recorded using a workbench specially designed for restoration and conservation (MANTIS, 120/90, DI Manfred Meyer) equipped with a stereomicroscope with fourfold magnification. The location of the cracks was classified as illustrated in Fig. 3: outside, within or between the coloured frames and in the yellow (gilded), red or blue areas of the decoration frame. If a crack started in the gilded area and continued into a blue or red area, it counted twice, once for each area. Figure 4 shows a crack in the golden decoration, as they occurred particularly frequently there, as discussed later, and could also have relatively large dimensions (approx. 6 mm long and 0.5 mm wide in this case). The thickness of the individual parchment sheets was measured with an optical microscope and averages 90 ± 20 µm (see Fig. S8 in SI). It was checked whether there was a correlation between the parchment thickness and the number of cracks.

3.4 Macroscopic study of the parchment colour using visible spectrocolorimetry

The parchment colour was evaluated by visible (Vis) spectrocolorimetry using the CIELAB colour space. CIELAB is a device-independent 3D colour space for the precise measurement of colour properties using three colour values (L*a*b*), which enables objective comparison and exact reproduction of colours as well as the numerical determination of colour changes [19]. Vis spectroscopy was performed using the spectrophotometer CM-2006d (Konica Minolta), equipped with pulsed Xenon lamps and a Silicon photodiode

Table 1 Summary of the analysed materials (object and samples) and the methods applied to study them

Sample/ object	Sample name	Analytical techniques						
		Crack doc. (bino)	*Spectro-colorimetry*	*MHT*	*FTIR Refl or Tr*	*OM*	*ESEM*	*Raman**
Prayer book	No. of folio	Yes entire manuscript	Yes folios 5r, 23v, 114v, 241v	No	Yes / refl folio 70v	No	No	Yes* folio 155r
Prayer book Sample from folio 173r	71_15_ p1	No	No	Yes	Yes / tr	No	No	No
Historical parchment (naturally aged)	HP	No	No	No	Yes / refl + tr	No	Yes*	Yes*
Modern parchment - unaged	MP	No	Yes	No	Yes / refl + tr*	Yes*	Yes*	Yes*
Modern parchment - aged	MP_D3-D34 (-I,-II)	No	Yes all	No	Yes / refl D3-D30 (-I,-II)	Yes* D16-II, D34-II	Yes* D3-I,II D16-I,II	No

Lab-FTIR analyses were conducted in reflection mode (refl) and SR-FTIR measurements in transmission mode (tr)

doc documentation, *bino* binocular observation

*Results presented in the supplementary information (SI).

array detector. Measurements were performed using the aperture of 8 mm in diameter and spectra were recorded in the Vis range between 360 and 740 nm.

The colour of the parchment is expressed in the CIELAB colour space by the three coordinates L*(luminosity), a* (green-red-axis in the colour coordinate) and b* (blue-yellow-axis). The colour change of these samples in relation to the modern reference (MP) is quantified by:

$$\begin{aligned}\triangle E &= [(L_{sample} - L_{MP})^2 + (a_{sample} - a_{MP})^2 \\ &\quad + (b_{sample} - b_{MP})^2]^{0.5} \\ &= [\triangle L^2 + \triangle a^2 + \triangle b^2]^{0.5}.\end{aligned}$$

3.5 Microscopic study of the parchment fibres of the Prayer book using Micro Hot Table

Determination of the shrinkage temperature of parchment fibres using the micro-hot table (MHT) technique is a well-established method to assess parchment degradation [15, 23, 6, 10, 8]. MHT analyses of fibres from the Prayer book were performed using a MHT instrument (*LAT GmbH, Garbsen*) with a heating plate for microscopy including PID controller (heating rate 2°/min). The following procedure was applied: a bunch of protein fibres was soaked in demineralised water for a few minutes, sealed between a microscope slide with a recess and a cover glass with rubber cement and heated with 2 °C min^{-1} until the different phases of shrinkage A, B, or C of the shrinkage temperature T_s was reached [17].

Fibres for four sets of measurement were removed from the small remaining sample (71_15_p1, ~3 mm^2) of the Payer Book for MHT analyses. It was not possible to remove long fibres, but only short fibre bundles, making the T_s measurement more difficult. The results were evaluated according to the procedure described by (Larsen [16]).

3.6 Study of chemical and micro-structural changes of collagen in parchment by FTIR spectroscopy

FTIR measurements were performed using two different instruments at the synchrotron facility BESSY II of *Helmholtz-Zentrum Berlin für Materialien und Energie* (HZB). A laboratory FTIR microscope (Lab-FTIR) Nicolet iN10 MX (Thermo Fisher Scientific) was used for non-invasive measurements using a conventional Globar source in reflectance mode (refl). The spectra were collected with Omnic Picta® software using an IR microscope objective with 15x magnification and 0.7 N.A., microscope apertures, even unusual, of(150 x 75), (150 x 150) or (300 x 300) μm^2 in the spectral region between 4000 and 700 cm^{-1} by accumulating 256 or 128 scans per spectrum with a spectral resolution of 4 or 8 cm^{-1}. Background spectra were collected from a gold sample (Au deposited on sandpaper) prior to each measurement and Kubelka-Munk correction was applied to treat the refl spectra. Lab-FTIR measurements were performed on MP and artificially aged parchment (MP_DXX-I,-II) samples

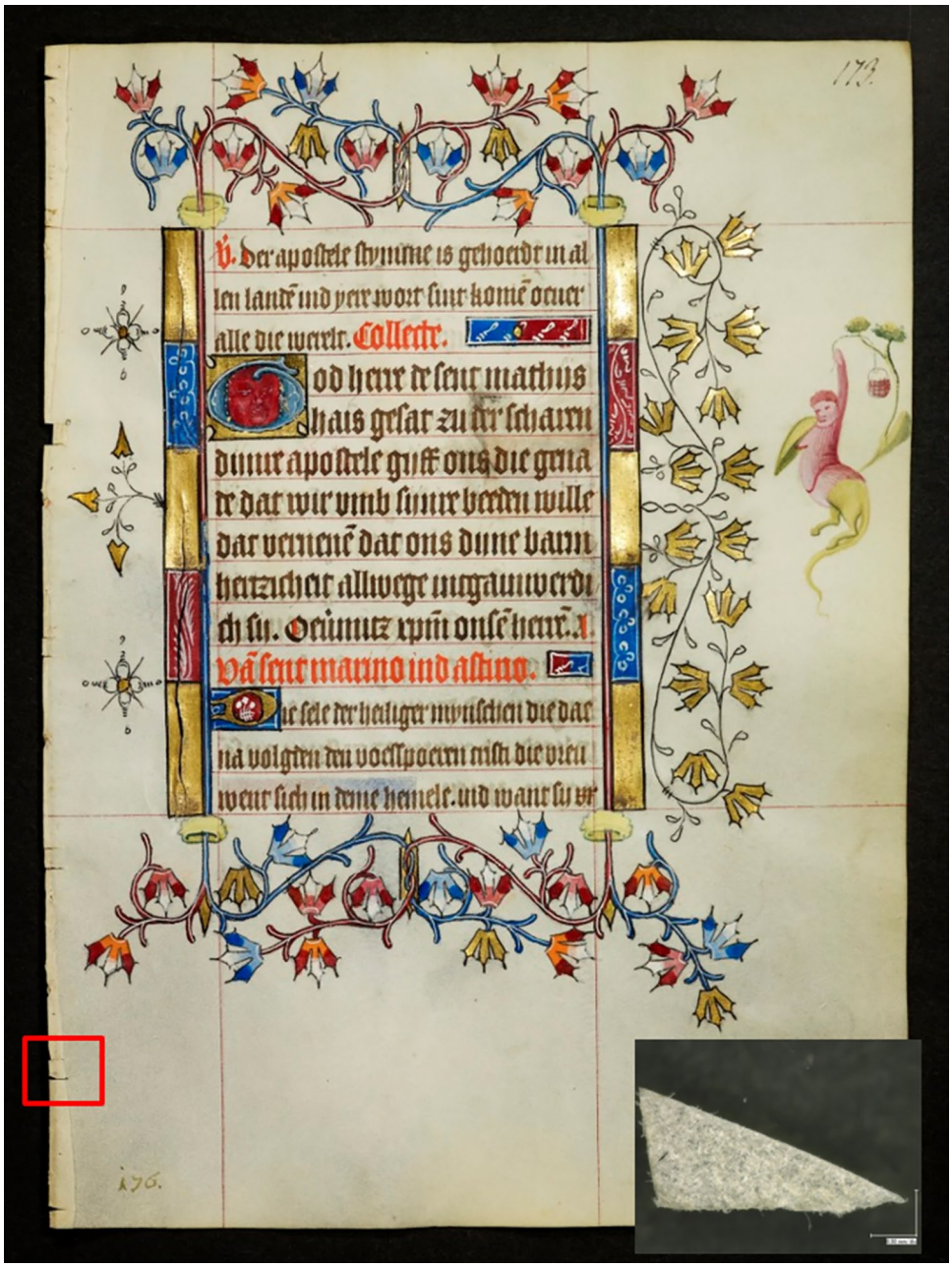

Fig. 2 Folio 173r of the manuscript (18.5 x 13.5 cm^2) with sample location (red frame) and inset showing sample 71_15_p1. Only a part of the sample (the triangular tip) was embedded and prepared as a cross- and thin-section. The scale bar of the whole sample corresponds to a length of 0.5 mm.

as well as on folio (70v) of the medieval manuscript. Point measurements and small 2D mappings were carried out.

Changes of the parchment may occur heterogeneously [28] and therefore different locations on the folios (Fig. 5) of the original manuscript were analysed. The folios were held in an adapted box with inlet and outlet capability to keep the sample at constant relative humidity of 55% RH. Holes were drilled in the box to allow the IR beam to reach and reflect off the sample (Fig. 6). The vast majority of the FTIR refl measurements were carried out on the folio 70v, which are discussed in this article.

In addition, changes can be superficial or can penetrate deeper into the material. Therefore, additional FTIR measurements with synchrotron radiation (SR) as a source were carried out on parchment cross-sections in order to obtain high spatial resolution 2D images of the interior of the samples. The high spatial resolution is required to detect possible variations between the surface and the 'bulk' material. These analyses were performed at the IRIS beamline [27] with a Nicolet Continuμm FTIR microscope coupled to a Nicolet Nexus 870 spectrometer (Thermo Fisher Scientific) in transmission mode (tr) and in the spectral range between

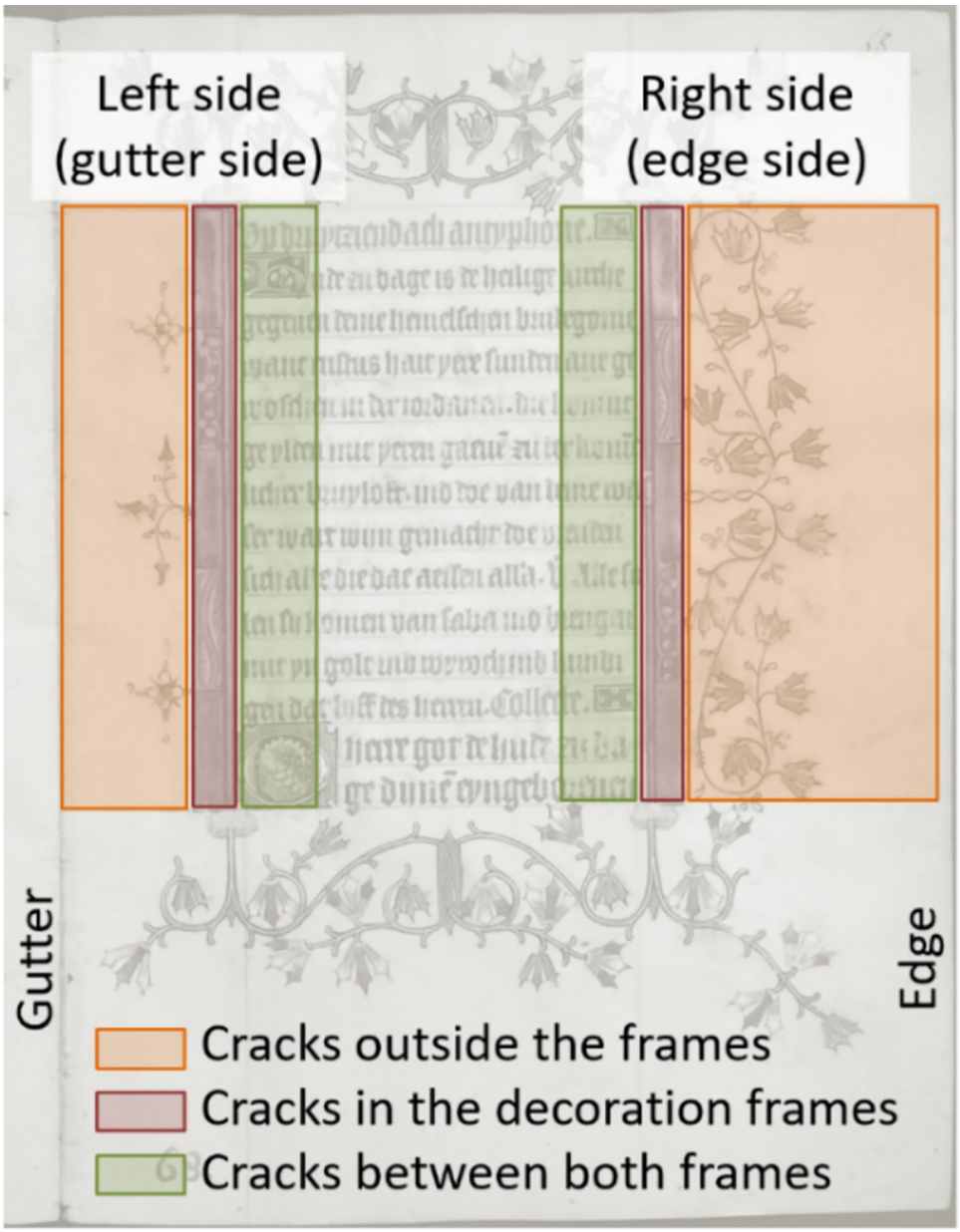

Fig. 3 Different areas of the recto side of the folio where cracks were observed. The cracks were always documented on the recto side of the page, and therefore the left frame is always on the side closer to the gutter and the right frame is closer to the edge of the book

4000 and 650 cm^{-1}. The microtomed thin cross-sections were pressed between two diamonds of a micro-compression cell (Spectra-Tech). In this way, the thickness of the samples could be further reduced and homogenized. Background spectra were collected through KBr grains placed next to the sample in the diamond cell. The following conditions were used: the microscope objectives with 32x magnification and N.A. 0.65, apertures between (10 x 10) and (15 x 15) μm^2, 64 or 128 accumulated scans per spectrum, spectral resolutions of 4 or 6 cm^{-1} with step sizes for 2D mappings as given in Tab.S1. Omnic® software was used for spectral acquisition.

The measurement conditions for both, refl and tr analyses, are summarised in tab. S1 in the SI.

Data treatment was as follows:

Lab-FTIR measurements in reflectance mode. The refl spectra were transformed using Kubelka-Munk. The corrected spectra were pre-treated using Quasar software as follows: baseline correction (rubber band) and Gaussian smoothing (SD 2). The band intensities and positions were determined as peak heights H and wave numbers at the respective maxima of the curve (for amide I at about 1690 ± 10 cm^{-1} and for amide II at about 1570 ± 10 cm^{-1}) using Excel software. The intensity ratios of the amide I to the amide II bands and the difference in the positions of these two bands were calculated. The positions of the vibration band obtained by FTIR refl measurements are shifted

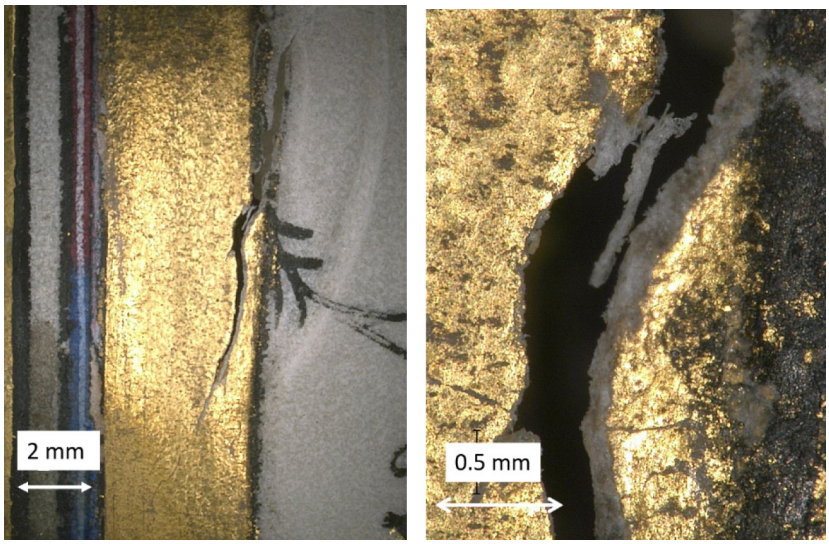

Fig. 4 Cracks found in the parchment of the Prayer book of Mary of Guelders: **a** Folio 150 r – frame of the right side in the gilded area situated at the bottom and **b** detail of crack in gilded layer (© Rathgen research laboratory, Cristina Aibéo)

compared to the expected values known from the literature (see explanation below).

SR-FTIR measurements in transmission mode. Transmission SR-FTIR 2D maps were treated using Quasar software as follows: spectra were pre-treated as described above and then hyperspectral mapping was performed by integrating the absorbance bands in the following selected spectral regions: for amide I: 1680–1615 cm^{-1}, for amide II: 1570–1520 cm^{-1} and for the epoxy resin: 1522–1500 cm^{-1}). Origin® software was used to visualise the spectra.

4 Results and discussion

4.1 Number and locations of cracks

On a single folio, the cracks are mostly concentrated in the region next to the decoration frames. The number of cracks per quire normalized to the number of folios is reported in the Fig. 7. There are only cracks until quire 33 and then they stop abruptly. Furthermore, most of the cracks appear in the gutter side of the folio. The number of single folios per quire is also indicated in the graphic because in some cases, as for example quire 18, the low number of cracks is related to the low number of folios. Nevertheless, from quire 34 on, the

Fig. 5 Photograph of folio 70v of the Prayer book of Mary of Guelders with indications of the analysis locations for Lab-FTIR measurements in reflectance mode (black ovals: m1-7)

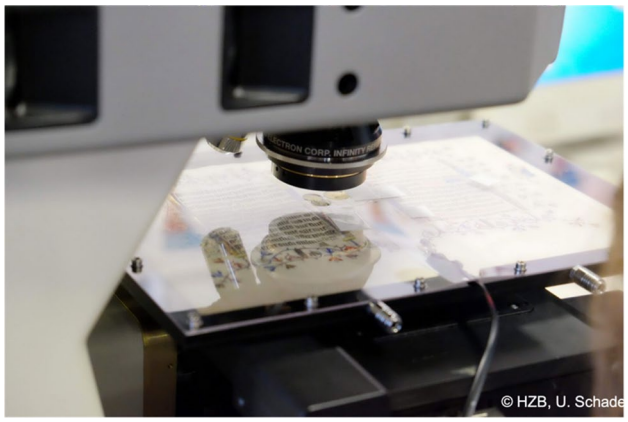

Fig. 6 The folio 70v placed in an adapted box to keep it in constant relative humidity during measurement

number of folios per quire does not change significantly but the cracks disappear completely, a phenomenon that could be linked to the fact that there are less miniatures in this part.

The number of cracks is not related to the thickness of each folio because from quire 10, which begins with folio 67, the thickness of the parchment does not change significantly but the number of cracks increases suddenly.

4.2 Colour changes in the parchment of the Prayer book and the mock-ups

Table 2 summarises the spectrocolometric results obtained for measurements on four folios distributed throughout the manuscript and on the modern reference sample (MP). The colour change of the parchment of the Prayer Book is expressed as ΔE based on the ΔL, Δa and Δb-values with

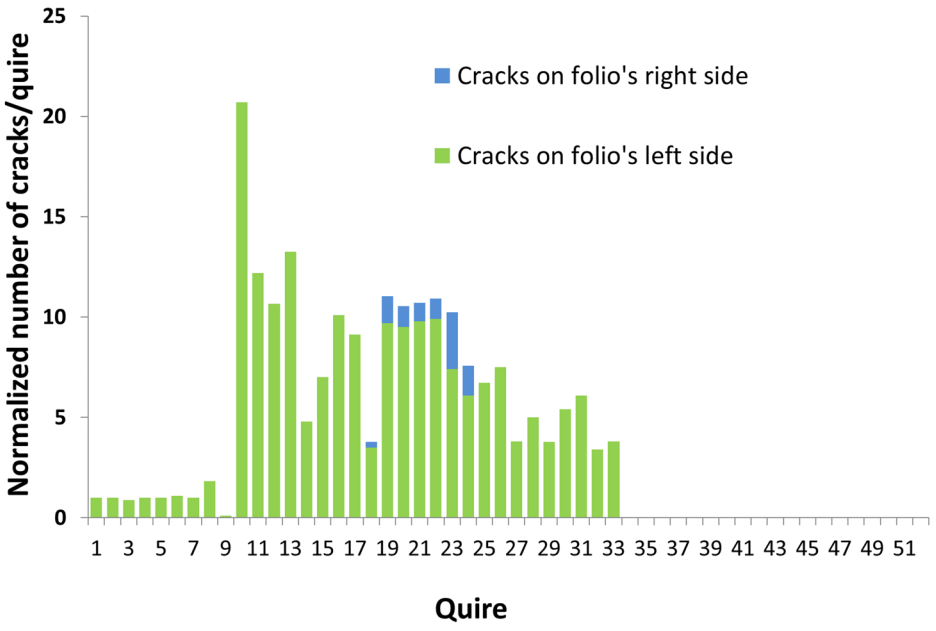

Fig. 7 Number of cracks per quire normalized to the number of folios in a quire: cracks in the left frame and around it (green bars); cracks in the right frame and around it (blue bars)

Table 2 Results of spectrocolorimetric measurements on four folios of the manuscript Mary of Guelders (5r, 23v, 114v, 241v) and on the modern parchment reference (MP)

Samples	L*(D65)	a*(D65)	b*(D65)	ΔE
Manuscript - Prayer book				
114v_c6	80.91	0.62	14.22	11.14
5r_c4	79.97	0.94	8.72	9.41
23v_c8	85.71	0.25	7.96	3.71
241v_c6	85.12	−0.01	9.8	5.04
Mean values ± stdv	**82.93 ± 2.91**	**0.45 ± 0.42**	**10.18 ± 2.80**	**7.33 ± 3.52**
Reference – modern parchment				
MP	88.94 ± 0.34	−1.03 ± 0.13	6.67 ± 0.68	

[Bold] means [mean values]

L*, a* and b* are given in absolute values.

stdv standard deviation

respect to the MP (not aged) parchment reference sample. In Fig. 8, the colour values obtained for the manuscript are compared with those for the artificially aged parchment samples.

The parchment of the Prayer book showed a colour defined by a high L*-value (mean luminosity = 82.93 ± 2.91), an a*-value close to zero (mean green-red-axis value = 0.45 ± 0.42) and a positive b*-value (mean blue-yellow-axis value = 10.18 ± 2.80). This resulted in the following mean delta values, using the values for MP as a reference: $\Delta L = 6.01$, $\Delta a = 1.48$, $\Delta b = 3.51$ and $\Delta E = 7.33$. These values agree quite well with those of the mock-up sample aged for three days under humid conditions (MP_D3-II, Fig. 8). The main colour components contributing to the colour changes were an increase in the b-value ($\Delta b = \Delta b_{sample} - \Delta b_{MP} > 0$), which corresponds to a low degree of yellowing of the parchment, and a decrease in the L-value ($\Delta L = \Delta L_{sample} - \Delta L_{MP} < 0$), which in turn means that the parchment of the manuscript appears darker compared to MP.

4.3 Changes in the parchment fibre structure

The state of preservation of the parchment fibres in the Prayer book was determined by measuring the shrinkage temperature T_s of the fibres (Table 3). The T_s values of samples 1 and 4 are high, indicating a good chemical state of preservation of the fibre protein [15]. Those of the samples 2 and 3 are difficult to interpret, which is probably due to the fact that only fibre bundles and not individual fibres could be taken from the sample. The intervals of T_s could not be distinguished accurately because the movements of the fibres were very slow and difficult to detect. Nevertheless, it was found that generally no gelatine was formed (samples 1–4). This overall stable condition could already be observed by immersing the parchment fibres in demineralised water.

Table 3 Results of the MHT measurement of four fibres' bundles taken from the Prayer book. (–) means that no shrinkage was observed, T_s A: some fibres start to move, T_s B: several fibres move simultaneously, T_s C: the movement stopped.

Measurement	Initial temperature/°C	T_s A/°C	T_s B/°C	T_s C/°C
Sample 1	30.7	51.4		61.8
Sample 2	30.9	52.7		52.7
Sample 3	30.9	–	–	–
Sample 4	32.1	52.9	56	62

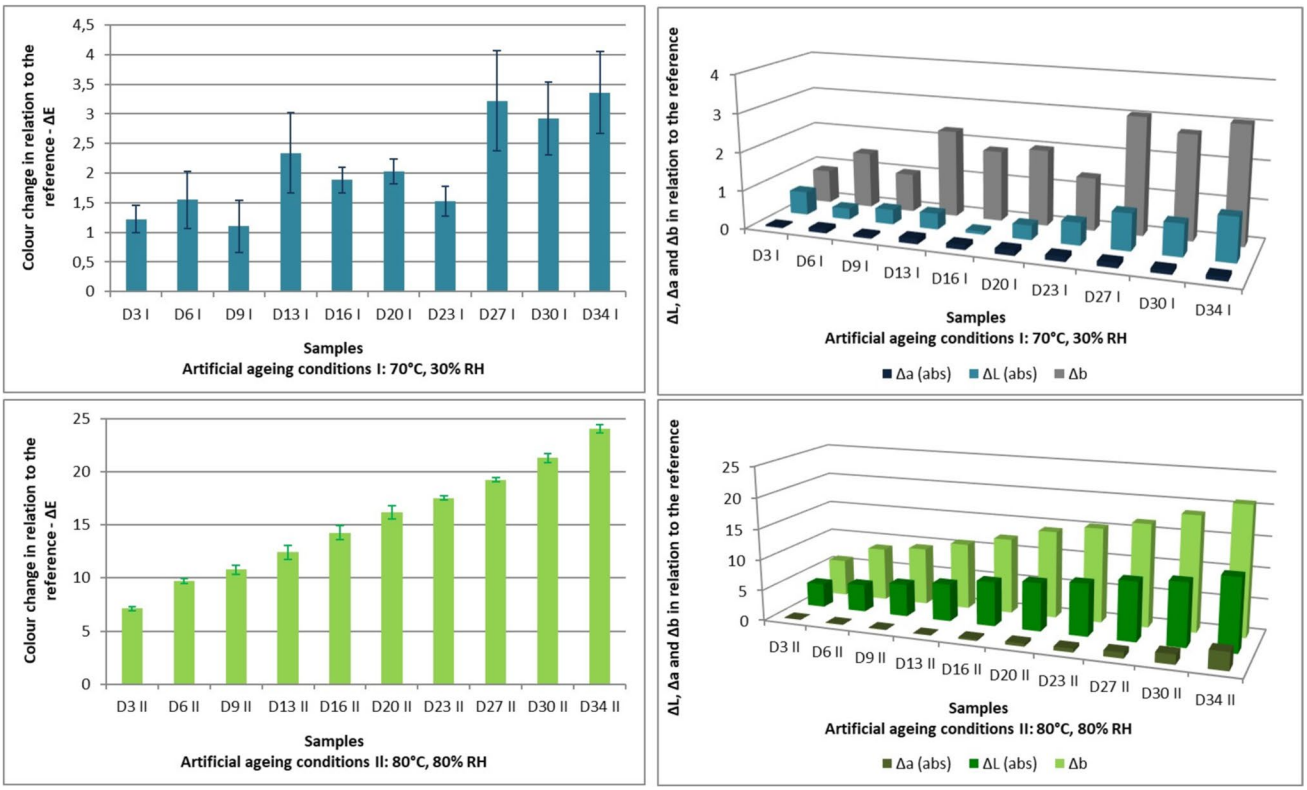

Fig. 8 Colour change, expressed as ΔE (left) and in absolute values of ΔL, Δa and Δb (right) for modern parchment (MP_DXX-I,-II) samples aged under ageing procedures I/II within different time periods (day 3 D3 to day 34 D34). Unaged modern parchment (MP) served as reference

4.4 Chemical state of parchment observed by FTIR spectroscopy

Figure 9 shows the FTIR refl spectra obtained from measurements on the unpainted areas of folio 70v of the Prayer book of Mary of Guelders, on the unaged modern (MP) and naturally aged historical reference (HP) samples, and on the parchment samples artificially aged for three days. The latter were added for comparison purposes, as they showed similar changes to the manuscript in the colorimetric examinations. Vibration bands are indicated in this graph, which are specific for parchment. FTIR measurements were carried out in various locations distributed over the entire page (m1 to m7, see Fig. 5) in order to investigate the heterogeneity of the parchment preservation at the macro level. However, the measuring points are predominantly located near the gilded areas on the gutter side of the leaf, where most of the cracks were found. The FTIR measurements in areas m5 and m6 are not discussed here as they correspond to painted surfaces, and therefore are not suitable for better assessing the state of preservation of the parchment.

FTIR spectra provide a range of valuable information that can be used to infer the state of preservation of the collagen in the parchment. In most cases, the relative intensity, shape and position of the amide I (1630–1660 cm^{-1}) and amide II (1530–1550 cm^{-1}) bands are used for collagen degradation studies [6, 10, 8, 7, 5, 1]. Amide I band results from C=O stretching vibration of the amide group and amide II band is the sum of C-N stretching and N-H in plane bending vibrations [10, 8]. Both are very sensitive to changes in the collagen secondary structure.

Table 4. summarizes the results of the semi-quantitative evaluation of the spectra obtained for manuscript page and MP samples by FTIR refl measurements. The spectral positions of the bands of amide I and amide II are shifted compared to the classical values observed in tr mode (see below). By nature, the refl spectra differ in shape and band position from tr spectra. While the tr spectra display the absorption bands at their correct vibrational energies, the refl spectra are influenced by the structure of the parchment surface. The obtained refl spectrum results from a wavelength-dependent interplay between surface and volume scattering. For the shorter wavelength range volume scattering (diffuse reflectance) is dominant and the spectra looks like an absorption spectrum. With increasing wavelength Fresnel reflection (surface reflection) gains influence, which at the end produces dispersion like features in the vicinity of the absorption sites causing a deviation from the known absorption features.

An increase in the intensity ratio of amide I to amide II bands with respect to MP would indicate hydrolytic changes in the parchment, as Badea et al. [1], for example, found when examining historical parchment. The amide band ratios determined for the various areas of folio 70v fluctuate (on average between 1.5 and 2.3) around the values for MP (2.1 ± 0.1). These deviations are distributed heterogeneously across the sheet without a recognizable pattern. It

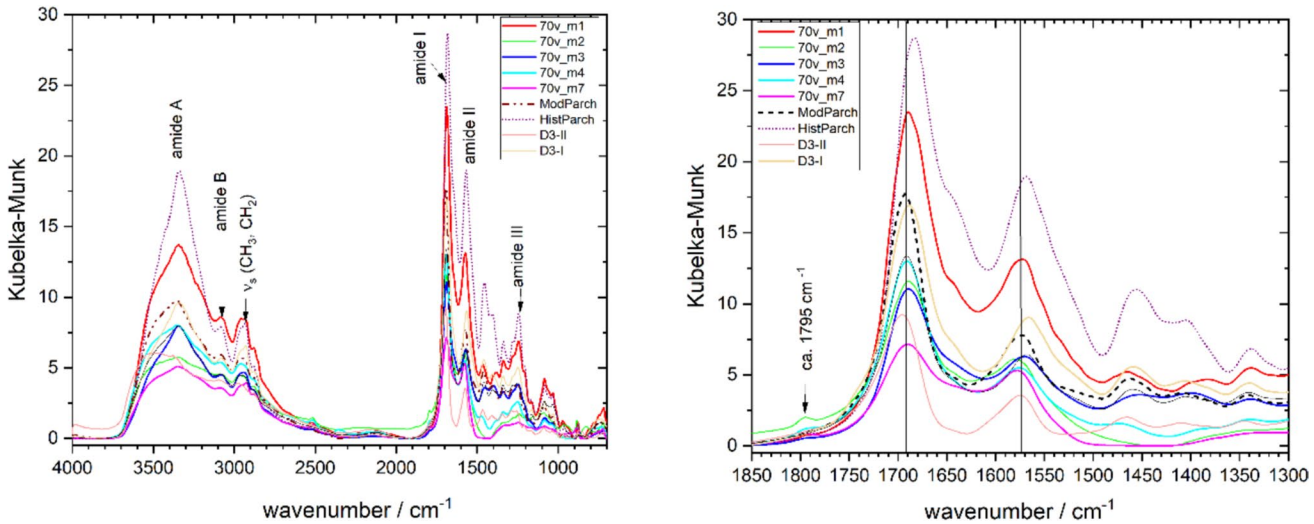

Fig. 9 Selection of FTIR spectra obtained in reflectance mode (Kubelka-Munk correction) using conventional Globar source for folio 70v of the Prayer book at different analysis locations (m1 to m4, m7, see Fig. 5) and for the modern (MP, ModParch_m1) and historical (HP, HistParch_m1) parchment reference samples as well as artificially aged parchment samples (MP_D3-I, D3-II)). Left: full spectral range. Right: detail. The vertical lines represent the amide I and II band positions of MP. A possible explanation for the shift in the vibration bands is given in the main text

is therefore difficult to draw robust conclusions from these parameters.

The positions of the amide I and amide II vibration bands are closely linked to the secondary structure of collagen. An increase in the distance between these two bands, mainly caused by a shift of the amide II band to lower wave numbers, is reported in the literature and is an indicator of collagen denaturation, i.e., the loss of helical structure and the gradual transition to a more disordered structure [1, 10, 8, 7]. In contrast to the phenomenon described in the literature, the band distances determined for the manuscript page are predominantly somewhat smaller than those for MP. This is due to a slight shift of the amide II band to higher wavenumbers (max. + 8 cm^{-1}), which may also be due to small changes in the secondary collagen structure, such as the loss of cross-links [9]. The position of the amide I band varies only within the spectral resolution (max. 5 cm^{-1}). These results indicate a relatively good preservation of collagen secondary structure with only minor local changes.

In addition, the samples artificially aged for 3 days (MP_D3-II) show similar band intensity ratios and distances between amide I to amide II as the manuscript. However, a tendency towards slightly increased band intensity ratios and a slightly enlarged band gap can already be recognised after three days of aging in humid atmosphere, changes that increase with aging time (see tab. S2 in the SI). At analysis location m2 on page 70v, two series of measurements (two lines (A and E) with 6 and 7 individual measurements respectively) were taken in the vicinity of a crack, each starting from the crack. The obtained intensity ratios and positions of the amide I and amide II bands fluctuate a bit more between the two lines than within the lines (see tab. S3 and figs. S6a and S6b in the SI). A very slight trend could be recognised in the observed parameters, which indicates a slightly increased change in the molecular structure in the immediate area of the crack compared to the visually intact material. However, these are only minor differences from which it cannot be concluded that a local microstructural or chemical change in the material could have led to the crack formation.

The refl FTIR spectra of the Prayer book and of the HP reference show a very weak peak at about 1795 cm^{-1}. This could result from CO stretching vibrations of carbonyl compounds such as carboxylic acids or ketones, which can arise, for example, during oxidative cleavage of the main chain of collagen [1, 13]. The appearance of a new band, assigned to C=O groups, was also observed by Manfredi et al. [20] in the study of light ageing of parchment. This peak is absent in the spectra of MP and artificially aged mock-up (MP_DXX-I,-II) samples.

By refl FTIR only the surface of the parchment is measured. However, changes may also extend deeper into the material, including the possibility that the parchment may be better preserved beneath the surface or vice versa. To investigate possible changes inside the parchment, high spatial resolution SR-FTIR spectroscopy was performed in transmission mode on a cross section of the parchment sample from the Prayer book.

The semi-quantitative evaluation of the 2D SR-FTIR mappings was difficult for several reasons: a) possible saturation of the main amide bands, particularly amide I, b) inhomogeneities in the sample were observed, which were

Table 4 Summary of specific parchment parameters obtained by laboratory-FTIR in reflectance mode for folio 70v of the manuscript as well as modern (MP), historical (HP) reference and artificially aged samples (MP_D3-I, _D3-II)

Sample n°	Band positions υ / cm^{-1}		Δ υ	Intensity ratios	
	Amide I	Amide II	(υ Amide I – υ Amide I)	H (Amide I / Amide II)	No. of points
Prayer book					
70v_m1	1690 ± 1	1578 ± 3	112 ± 3	2.3 ± 0.3	24
70v_m2_lineA	1687 ± 3	1580 ± 3	106 ± 3	2.3 ± 0.4	6
70v_m2_lineE	1687 ± 2	1573 ± 3	114 ± 2	1.5 ± 0.1	7
70v_m3	1691 ± 2	1572 ± 2	118 ± 2	1.8 ± 0.1	3
70v_m4	1688 ± 2	1576 ± 3	113 ± 3	2.2 ± 0.2	10
70v_m7	1688 ± 2	1578 ± 3	111 ± 4	1.7 ± 0.2	38
References					
MP	1692 ± 1	1572 ± 1	120 ± 0	2.1 ± 0.1	3
HP	1687 ± 4	1571 ± 4	116 ± 3	1.8 ± 0.3	3
MP_D3-I*	1692 ± 4	1569 ± 4	123 ± 0	2.0 ± 0.2	2
MP_D3-II#	1697 ± 3	1572 ± 2	125 ± 3	2.7 ± 0.5	6

Spectra were evaluated using Quasar and Excel software. The values given are mean values for a certain number of individual measurements (No. of points). The parchment of the manuscript folio 70v was analysed at various locations (m1, m2, m3, m4 and m7; see Fig. 5)

*,#Artificially aged parchment at 70°C, 30% RH (*) and 80°C, 80% RH (#), for three days each (D3).

due to the penetration of resin into sample cavities and/or due to possible mechanical damage to the sample during cutting of thin sections and c) overlapping of amide II and resin bands (see figs. S5a and S5b in the SI). Therefore, semi-quantitative data are not presented for the SR-FTIR mappings in transmission mode.

Figure 10 illustrates the results of qualitative SR-FTIR measurements obtained for the cross section of the parchment sample 71_15_p1 of the Prayer book. Figure 10a shows a microscopic visible image of this parchment sample with the scanned area indicated. The 2D colour maps in Fig. 10b and c show the distributions of amide I in the spectral range from 1680 cm^{-1} to 1615 cm^{-1} and of a main band of epoxy resin at about 1500 cm^{-1} (see figs. S5a and S5b in the SI), which illustrates the penetration of the embedding resin into the parchment. Disregarding the areas of resin penetration, the band intensities of amide I appear to be relatively homogeneously distributed. The amide II band shows the same distribution. Spectra were extracted along a line across the sample (marked as red rectangles in Fig. 10 b and c) to reveal subtle differences in molecular structure between the bulk material and the surface. Care was taken to avoid areas where the resin had penetrated the parchment. The Fig. 10d and e show extracted FTIR spectra in the entire spectral range and in detail for the amide region.

The second derivative was calculated in order to try to enhance interpretation of the extracted spectra as it provides information on the band components [10, 8, 29]. The band components or sub-bands are indicated for analysis point 14/2 in the graph and are at 1660, 1639 and 1690 cm^{-1} and for amide I at 1556 and 1526 cm^{-1}. Alteration of the sub-bands within amide I have been related to cross-linking of collagen in bone during denaturation [27, 9] and the change of the ratio of 1660/1630 components of amide I were used to rank collagen damage (0–5% not damaged, 5–12 % slightly damaged) by Odlyha et al. [25].

For the bulk region (points 14/2 to 14/10) the amide I and amide II band only shift about 5 cm^{-1} to lower wavenumbers which is in the order of spectral resolution (Fig. 10e). Near the surface (points 14/11 to 14/13) slight changes can be recognized such as a more pronounced shift to lower wavenumbers and a decrease of relative intensity of the amide I band. The band shift can be due to a decrease of the participation of the component at about 1660 cm^{-1}assigned to α-helix structure and an increase of the participation of the component in range of 1639 cm^{-1} to 1630 cm^{-1} assigned to disordered structure of collagen (Fig. 10f, [10, 8]). These observations can have several reasons: natural increase in structural disorder at the surface of the material, slight superficial changes or deviations due to the fact that the measurement was partly outside the sample (point 14/13). The 2nd derivative in the area of the amide II band shows a very complex picture and its interpretation probably requires further investigation.

4.5 Assessment of the state of preservation of the parchment of the Prayer book of Mary of Guelders

4.5.1 Crack location and its possible origin

Crack observation of the Prayer book showed that the number of cracks in a bifolio was not related to the thickness of each folio but the cracks were mostly concentrated on the gutter side of the frame and in the decoration frames (Fig. 7 and Fig. S8 in the SI), which could be related to the presence of paint or gilding in these areas. Nevertheless, this does not yet explain the preponderance of the cracks on the gutter side. Otherwise, the same number of cracks would be expected on the outside of the page. Leafing through the book causes a higher mechanical stress on the inner side of the page than on the outer side. This fact, together with the presence of a paint layer, is probably the trigger of the appearance of cracks. The number of cracks in the gilded, red and blue areas of the frame is almost even (Fig. S9 in the SI). There are slightly more cracks (about 10 %) in the gilded areas than in the painted areas, which might be due to the fact that the gilded layer is slightly thicker than the paint layers. However, the thickness of the decorated areas could not be measured, so that this relationship can not be proven. Among the 52 quires, many cracks occur from quire 10 until quire 33, which abruptly stop after the quire 34 (Fig. 7), an observation that could not be explained so far. Possibly this could be related to the fact that the present order of the quires in the Berlin pat of the Prayer book is not corresponding to the original order. The part of the Prayer book now kept in Vienna was placed between the quire 45 and 46 in the orignal version of the Prayer book [26].

4.5.2 State of preservation of the Prayer book, possible origin of degradation and recommendation for preventive conservation

While comparing the characteristics of the parchment and the fibres of the Prayer book to the features of the historical parchment reference and to the aged mock-up samples, the state of preservation of the Prayer book can be estimated and possible origins of the degradation inferred. Colour measurements on the Prayer book showed only slightly different values in comparison to the reference samples. Although a clear yellowing was observed during artificial ageing of the mock up samples, especially under humid conditions, the colour ageing of the parchment of the Prayer book seems low but is difficult to establish because of the low changes and the intrinsic heterogeneity of its colour.

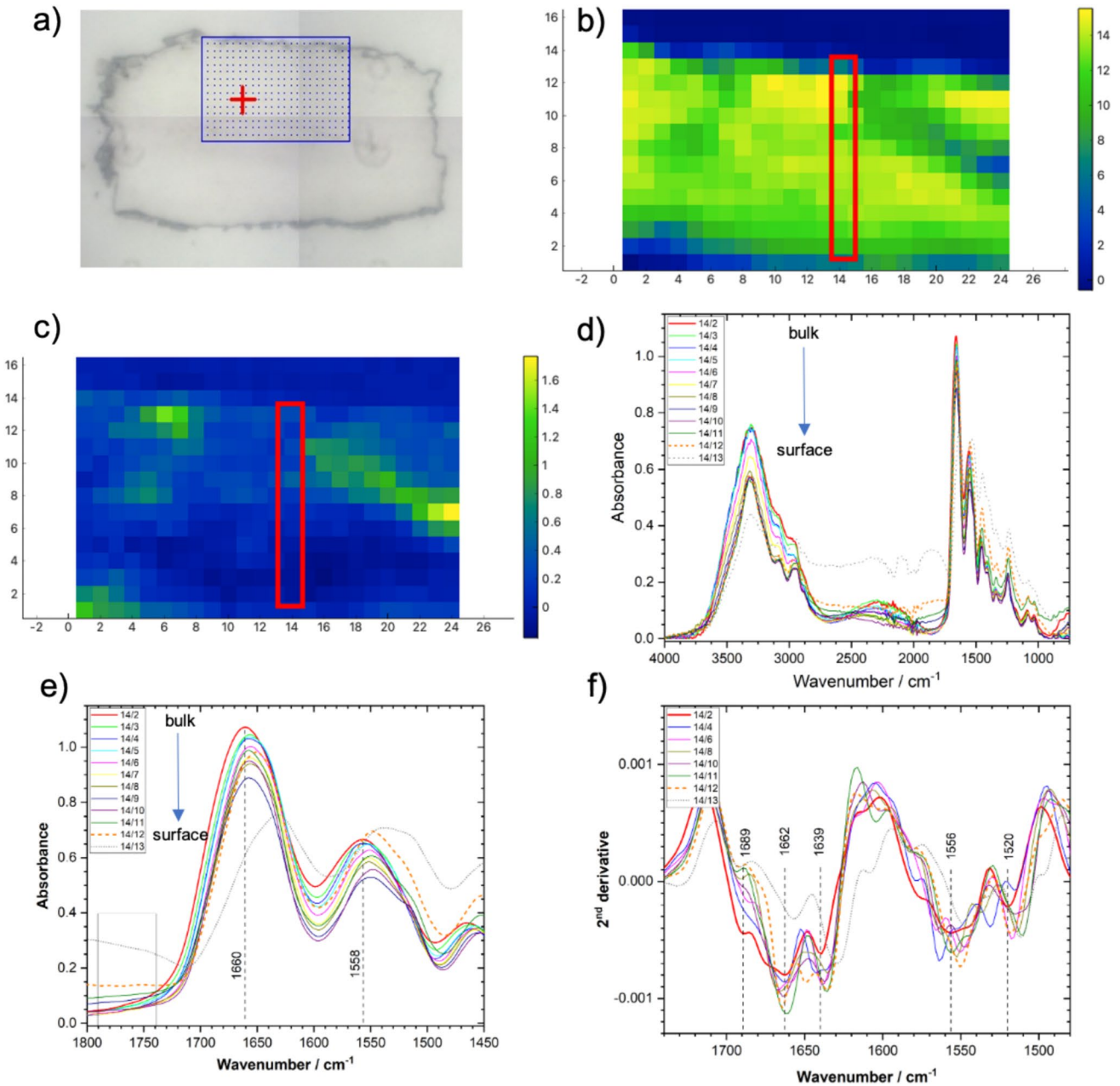

Fig. 10 Results of 2D FT-IR mapping in transmission mode of the manuscript sample 71_080515 (71_15_p1_map1): **a** microscope image of the sample with the scanned area indicated, **b** distribution of the amide I band (1680-1615 cm^{-1}), **c** distribution of a resin band (1522-1500 cm^{-1}) obtained using Quasar software. **d**–**f** selected FTIR-spectra corresponding to a line from the surface in to the bulk material (red rectangle in b and c): **d** entire spectra, **e** detail with positions of amide I and amide II bands of the starting point (14/2) of the line leading from the bulk to the surface marked with dashed lines and **f** second derivative of **e**) for reasons of clarity, only for some of the analyses points. Scan parameters: aperture (12 x 12) μm^2, step size (6 x 6) μm^2, 128 scans, resolution 4 cm^{-1}.

The fibre shrinking temperatures T_s of the Prayer book parchment fibres indicate a good chemical preservation of the fibre structure and no signs of gelatine formation. By contrast, fibres showed important changes during artificial ageing of mock-ups under humid conditions in the form of more or less "melted" fibres (gel-like substance, see figs. S2, S3 in the SI). As no structural changes were observed for the parchment fibres of the Prayer book, the parchment can be classified as well preserved.

FTIR measurements confirmed the good chemical preservation state of the parchment of the Prayer book. Indeed, whereas the mock-up samples aged under humid atmosphere (procedure II) showed slight signs of hydrolysis and denaturation of the parchment with increasing ageing time,

the parchment of the Prayer book does not show this kind of changes. It could be shown that the secondary structure of the collagen is relatively well preserved and shows only minor local changes, which are heterogeneously distributed on the surface of the parchment.

However, cracks were observed in different parts of the Prayer book. They are more frequent on the gutter side of the frame and in the decoration frames of the folios. There seems to be no direct link between the presence of paint layers present in the illuminations of the Prayer book and the state of preservation of the parchment as confirmed with the performed crack statistics. The origin of the cracks in the parchment pages is therefore more likely to be due to mechanical stress because of the tight bookbinding. When leafing through the Prayer book the inner side is more demanded than the outer side of the folios. Mechanical stress is more likely the aging factor rather than chemical alteration. It can be deduced that the parchment of the Prayer book should stay well preserved under the stable climatic conditions combined with a restricted handling. For further conservation of the Prayer book, relative humidity and temperature should be kept as constant as possible to avoid yellowing or darkening of the parchment as well as chemical alteration of the fibres. Furthermore, the Prayer book was bound again according to these recommendations, but not so tight. It is now possible to turn the pages withough bending them and therefore, without causing stress near the gutter.

5 Conclusions

The state of preservation of the medieval illuminated manuscript, the Prayer book of Mary of Guelders, was assessed based on observations by means of various analytical techniques ranging from the macro- down to the microscale. Colour changes in the parchment, modifications of parchment fibres and chemical changes of the parchment were investigated in comparison to modern and historical parchment references. Furthermore, the origin of cracks occurred in the parchment was considered.

The parchment of the Prayer book showed a slight yellowing and darkening in accordance with naturally aged parchment. Some cracks were observed in a part of the Prayer book. The cracks were preferentially observed on the gutter edge (inner side) of the folios. This observation, together with the chemically and structurally good state of preservation of the parchment of the Prayer book, leads to the conclusion that the appearance of the cracks was probably caused by the mechanical stress of leafing through the previously tightly bound Prayer book. Concerning the further conservation of the Prayer book, relative humidity and temperature should be kept as constant as possible to avoid further yellowing or darkening of the parchment as well as chemical alteration of the fibres. Tight book binding should also be avoided and handling extremly restricted.

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Author contributions Ina Reiche and Julia Bispinck-Roßbacher conceived and designed the study. Ellen Egel, Cristina Lopes Aibéo, Ljiljana Puskar, Ulrich Schade, Christine Theuerkauf-Rietz, Katarzyna Schirmacher and Britta Schütrumpf performed the FTIR analyses. Katharina Müller performed the FT-IR data evaluation. Margit Hundertmark performed the crack observation. Ellen Egel and Cristina Lopes Aibéo prepared the mock-up samples and their artificial aging, performed the crack statistics and the colour measurements as well as the evaluation of the colour changes. Andrea Pataki-Hundt performed the MHT observations. Julia Bispinck-Roßbacher decided on the conservation strategy of the Prayer book. Ina Reiche and Katharina Müller wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Data availability All data presented in this manuscript are either provided in the manuscript or in the supplementary material. They are provided by the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest. The authors have no relevant non-financial interest to disclose.

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