

## Supporting information

# Quantifying degradation of collagen in ancient manuscripts: the case of the Dead Sea Temple scroll

R. Schütz<sup>a,b</sup>, L. Bertinetti<sup>a</sup>, I. Rabin<sup>b</sup>, P. Fratzl<sup>a</sup>, A. Masic<sup>\*a</sup>

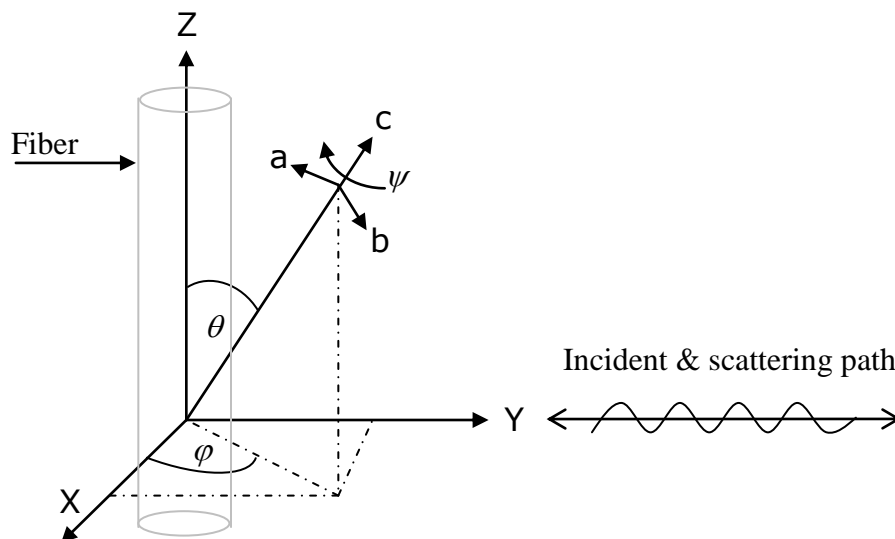
## Theoretical background

Polarized Raman spectroscopy approach has been developed and applied more often in the last decade and in particular on systems of uniaxial symmetry. Since the theoretical description of the technique has been presented in detail by several authors Bower et al.<sup>1,2</sup>, Turrell et al.<sup>3-7</sup>, Sourisseau et al.<sup>8,9</sup>, Ikeda et al.<sup>10</sup>, Rousseau et al.<sup>11,12</sup> and others, here only a compact guide through the basic concepts will be drafted. Scattered Raman intensity of an anisotropic specimen is dependent on the polarization direction of the incident laser and on the polarizability of the particular vibration centers. The latter is described by the polarizability tensor of that particular bond.

$$I_s \sim I_e [e_e \alpha e_s]^2 \quad (1)$$

Where  $\alpha$  is the polarizability tensor in laboratory coordinates,  $e$  = directions of the excitation(<sub>e</sub>) and scattered(<sub>s</sub>) light. We consider the coordinate system as described in figure S1, where the linearly polarized laser light can be oriented in the z-x plane (laboratorial coordinates) by a “polarizer” ( $\lambda/2$ -plate) before it hits the sample. By means of polarization filter- “analyzer”, placed in the path of the scattered light, intensity can be also analyzed by respect to the orientation of scattered polarization. Y is the direction of both the incoming and scattered laser path in a confocal arrangement (Fig. S1).

The sample, which is in this case a cylindrical fiber, is oriented along the z-direction.



**Fig. S1: Definition of the molecular (abc) and the laboratorial (XYZ) coordinates used in the text and Euler Angles used for the transformation.**

Here is an example for the notation, if the incident light would be polarized along the fiber and we would analyze only the scattered intensity with polarization perpendicular to the fiber the measured intensity would be described by the square of the  $\alpha_{ZX}$  component of the polarizability tensor in laboratorial coordinates.

$$\Rightarrow I_{(ZX)} \sim [e_e \alpha e_s]^2 = \left[ \begin{pmatrix} 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} \alpha_{XX} & \alpha_{XY} & \alpha_{XZ} \\ \alpha_{YX} & \alpha_{YY} & \alpha_{YZ} \\ \alpha_{ZX} & \alpha_{ZY} & \alpha_{ZZ} \end{pmatrix} \begin{pmatrix} 1 \\ 0 \\ 0 \end{pmatrix} \right]^2 = (\alpha_{ZX})^2 \quad (2)$$

Now the spectral frequency (1665 1/cm) that we are looking at in this study comes from the C=O stretching known as amide I. The C=O is oriented along the c axis in the molecular coordinates as indicated in Fig. S1. The polarizability response in molecular coordinates of this bond is described by a diagonal polarizability tensor of the form <sup>11</sup>:

$$[\alpha_{abc}] = \alpha_3 \begin{pmatrix} a & 0 & 0 \\ 0 & a & 0 \\ 0 & 0 & 1 \end{pmatrix}, \text{ with } a = \alpha_1/\alpha_3 = \alpha_2/\alpha_3 \quad (3)$$

Due to the stretching symmetry of the Amide I bond the polarizability properties of the tensor do not differ in a or b direction, which means that it is independent on the angle  $\psi$ . However, the polarizability tensor  $[\alpha_{XYZ}]$  in laboratory coordinate frame of the fiber, which corresponds to the hierarchically higher molecular and fibrillar assembling symmetry on the macroscopic level, results from the transformation of the molecular polarizability tensor  $[\alpha_{abc}]$  by means of the so called Euler transformation <sup>1, 10</sup>.

$[\alpha_{XYZ}] = [T^t \alpha_{abc} T]$  : Transformation of coordinates with Euler angles  $(\psi, \varphi, \theta)$

Since the measured Raman scattering is a response of a multitude of vibrational centers it is expressed by an average  $\langle (\alpha_{i,j})^2 \rangle$  which is the integration over the orientation distribution  $N(\psi, \varphi, \theta)$  of the vibrational centers inside the fiber.

$$I_{\text{Raman}(i,j)} \sim \langle (\alpha_{i,j})^2 \rangle = \frac{1}{4\pi^2} \int_0^{2\pi} d\psi \int_0^{2\pi} d\varphi \int_{-1}^1 d(\cos \theta) ([T^t \alpha_{abc} T]_{i,j})^2 N(\psi, \varphi, \theta) \quad (4)$$

In the cylindrical uniaxial fiber symmetry the variation of the distribution function depends only on  $\theta$ , i.e.  $N(\psi, \varphi, \theta) = N(\theta)$ . The distribution regarding the angles  $\varphi$  and  $\psi$  stays constant and the integrals can be reduced to

$$\Rightarrow \langle (\alpha_{i,j})^2 \rangle = \int_{-1}^1 d(\cos \theta) (\alpha_{i,j})^2 N(\theta) \quad (5)$$

An appropriate approach for the representation of the distribution function is given by a series of even Legendre Polynomials.

$$N(\cos \theta) = \sum_l^{\text{even}} \left(1 + \frac{1}{2}\right) \langle P_l \rangle P_l(\cos \theta) \quad (6)$$

$$= \frac{1}{2} [1 + P_2 \frac{5}{2} (3 \cos^2 \theta - 1) + P_4 \frac{9}{8} (35 \cos^4 \theta - 30 \cos^2 \theta + 3) + \dots]$$

Where,  $P_l(\cos \theta)$  are Legendre polynomials and P2, P4 are the first two even Legendre coefficients, which can be determined by Raman scattering. These coefficients are the

average values over the distribution of orientation of the Legendre polynomials and are given as:

$$\langle P_l \rangle = \int_0^\pi \sin \theta P_l(\cos \theta) N(\theta) d\theta \quad (7)$$

The coefficients can be determined experimentally. The relation between the scattered quantities and the Legendre coefficients are given by following equations just by solving the integral in equation (5).

$$\langle \alpha_{xz}^2 \rangle = \alpha_3^2 \left\{ \left[ \frac{a^2 - 2a + 1}{15} \right] - P_2 \left[ \frac{3a^2 + 2a - 1}{21} \right] - 4P_4 \left[ \frac{8a^2 - 16a + 8}{280} \right] \right\} \quad (8)$$

$$\langle \alpha_{xx}^2 \rangle = \alpha_3^2 \left\{ \left[ \frac{8a^2 + 4a + 3}{15} \right] + P_2 \left[ \frac{8a^2 - 2a - 6}{21} \right] + 3P_4 \left[ \frac{8a^2 - 16a + 8}{280} \right] \right\} \quad (9)$$

$$\langle \alpha_{zx}^2 \rangle = \alpha_3^2 \left\{ \left[ \frac{a^2 - 2a + 1}{15} \right] - P_2 \left[ \frac{3a^2 + 2a - 1}{21} \right] - 4P_4 \left[ \frac{8a^2 - 16a + 8}{280} \right] \right\} = \langle \alpha_{xz}^2 \rangle \quad (10)$$

$$\langle \alpha_{zz}^2 \rangle = \alpha_3^2 \left\{ \left[ \frac{8a^2 + 4a + 3}{15} \right] - 2P_2 \left[ \frac{8a^2 - 2a - 6}{21} \right] + 8P_4 \left[ \frac{8a^2 - 16a + 8}{280} \right] \right\} \quad (11)$$

Where  $a$  and  $\alpha_3$  are the principle components of the polarizability tensor on molecular frame as defined above in equation 3.

In case of a high magnification lens (100x) with a wide numerical aperture NA of 0.9 a corrections for resulting scattered intensities has to be applied<sup>3, 13</sup>. A wide NA causes a wide cone of a solid angle  $\Omega = 2\theta_c$  over which the scattered light is collected. The cone angle depends on the reflective index of the material as follows:

$$n \sin \theta_c = NA. \quad (12)$$

Because of the deviation to the 180° backscattering geometry the radiation propagating along the outer parts of the wide cone has got additional polarization components. The corrections for the four significant measured intensities take place in a following way<sup>9</sup>:

$$\mathbf{I}_{(ZX)} \sim \left( \langle \alpha_{ZX}^2 \rangle A + \langle \alpha_{ZY}^2 \rangle B \right) (2C_0 + C_2) + \left( \langle \alpha_{YX}^2 \rangle A + \langle \alpha_{YY}^2 \rangle B \right) (4C_1) + \left( \langle \alpha_{XX}^2 \rangle A + \langle \alpha_{XY}^2 \rangle B \right) (C_2) \quad (13)$$

$$\mathbf{I}_{(ZZ)} \sim \left( \langle \alpha_{ZZ}^2 \rangle A + \langle \alpha_{ZY}^2 \rangle B \right) (2C_0 + C_2) + \left( \langle \alpha_{YZ}^2 \rangle A + \langle \alpha_{YY}^2 \rangle B \right) (4C_1) + \left( \langle \alpha_{XZ}^2 \rangle A + \langle \alpha_{XY}^2 \rangle B \right) (C_2) \quad (14)$$

$$\mathbf{I}_{(XZ)} \sim \left( \langle \alpha_{XZ}^2 \rangle A + \langle \alpha_{XY}^2 \rangle B \right) (2C_0 + C_2) + \left( \langle \alpha_{YZ}^2 \rangle A + \langle \alpha_{YY}^2 \rangle B \right) (4C_1) + \left( \langle \alpha_{ZZ}^2 \rangle A + \langle \alpha_{ZY}^2 \rangle B \right) (C_2) \quad (15)$$

$$\mathbf{I}_{(XX)} \sim \left( \langle \alpha_{XX}^2 \rangle A + \langle \alpha_{XY}^2 \rangle B \right) (2C_0 + C_2) + \left( \langle \alpha_{YX}^2 \rangle A + \langle \alpha_{YY}^2 \rangle B \right) (4C_1) + \left( \langle \alpha_{ZX}^2 \rangle A + \langle \alpha_{ZY}^2 \rangle B \right) (C_2) \quad (16)$$

Where 
$$A = \pi^2 \int_0^{\theta_c} (\cos^2 \vartheta + 1) \sin \vartheta d\vartheta = \pi^2 \left( \frac{4}{3} - \cos \theta_c - \frac{1}{3} \cos^3 \theta_c \right) \quad (17)$$

and 
$$B = 2\pi^2 \int_0^{\theta_c} \sin^3 \vartheta d\vartheta = 2\pi^2 \left( \frac{2}{3} - \cos \theta_c + \frac{1}{3} \cos^3 \theta_c \right) \quad (18)$$

Since  $C_1$  and  $C_2$  are several orders of magnitude lower than  $C_0$  they can be neglected, and, in case of intensity ratios,  $C_0$  is also vanishing from the equations<sup>12</sup>.

Two independent intensity ratios can be then calculated by measuring four linearly polarized spectra:

$$R_1 = \frac{\mathbf{I}_{(ZX)}}{\mathbf{I}_{(ZZ)}} = \frac{\langle \alpha_{ZX}^2 \rangle A + \langle \alpha_{ZY}^2 \rangle B}{\langle \alpha_{ZZ}^2 \rangle A + \langle \alpha_{ZY}^2 \rangle B}; \quad R_2 = \frac{\mathbf{I}_{(XZ)}}{\mathbf{I}_{(XX)}} = \frac{\langle \alpha_{XZ}^2 \rangle A + \langle \alpha_{XY}^2 \rangle B}{\langle \alpha_{XX}^2 \rangle A + \langle \alpha_{XY}^2 \rangle B} \quad (19), (20)$$

The approach of calculating ratios has several advantages. Beside the elimination of all issues related to the polarization dependent instrument throughputs and other constant components such as laser incident intensities, it also reduces the problem to only three unknowns ( $a$ ,  $P_2$ ,  $P_4$ ). However, to solve this problem, a further experimental ratio is needed. It can be provided by a measurement of an anisotropic sample, where it is known that due to a constant distribution function  $R_{\text{iso}}=R_1=R_2$  and  $P_2=P_4=0$ . In our case, gelatin sample can be used as an isotropic sample of collagen based material and “ $a$ ” can be determined.

At this stage with the knowledge of “a” only two unknowns remain, the P2 and P4 of an anisotropic sample can be determined by measurements of the two ratios R1 and R2 and the corresponding distribution of the particular bond can be established. In a case, when the distribution function  $N(\theta)$  is estimated to be following a Gaussian shape with a particular central angle  $\theta$  and a width  $\omega$  (FWHM), the first two even Legendre coefficients P2 and P4 can be calculated for the relevant Gaussians using equation 7. Doing so, each of the P2 and P4 pairs determined through measurement can be linked to a  $\theta_{\max}$  and  $\omega$  pair, which gives a particular distribution function assuming it is a Gaussian.

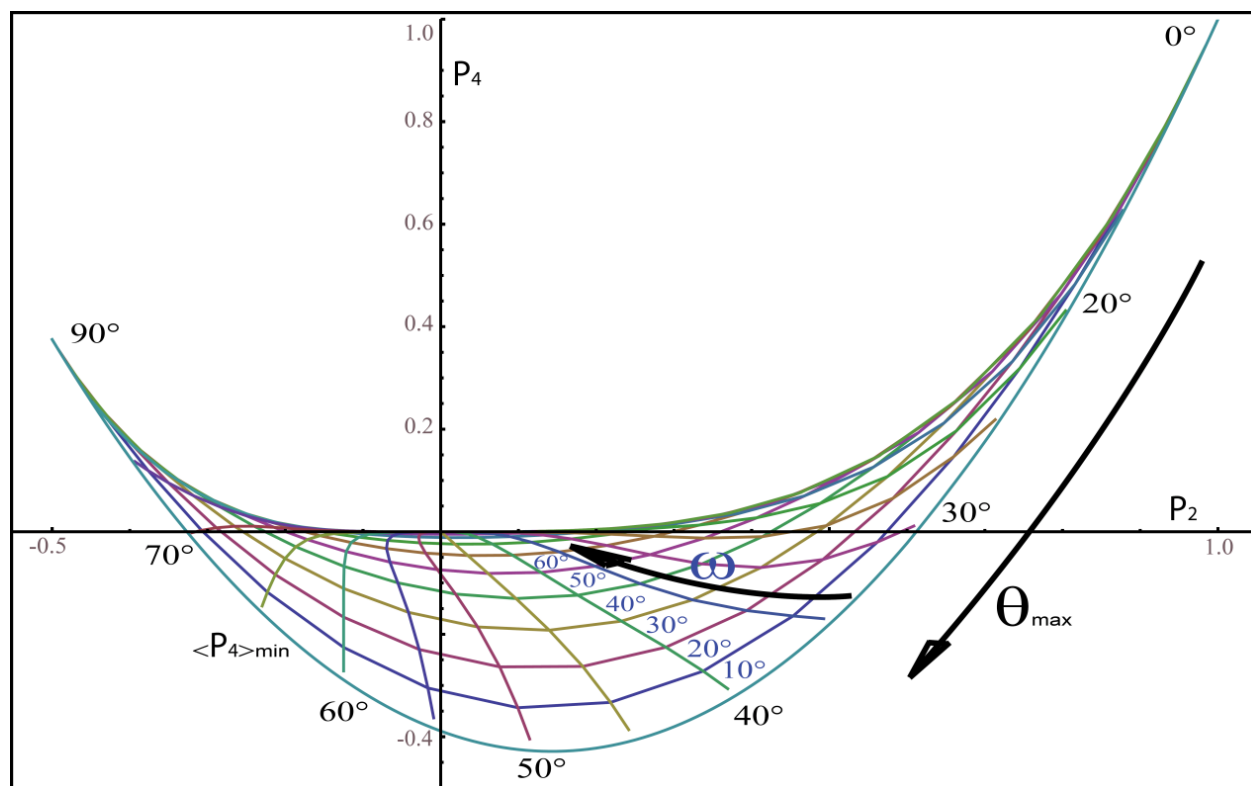


Fig. S2: Iso- $\theta_{\max}$  and Iso- $\omega$  curves of a Gaussian in P2-P4 space.

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