A Raman Algorithm to Estimate Human Age From Protein Structural Variations in Autopsy Skin Samples: the Proteins' Biological Clock

Daisuke Miyamori

Kyoto Prefectural University of Medicine

Takeshi Uemura

Kyoto Prefectural University of Medicine

Wenliang Zhu

Kyoto Institute of Technology

Kei Fujikawa

Kyoto Prefectural University of Medicine

Satoshi Teramukai

Kyoto Prefectural University of Medicine

Giuseppe Pezzotti

Kyoto Institute of Technology

Hiroshi Ikegaya (✉ ikegaya-ky@umin.ac.jp)

Kyoto Prefectural University of Medicine

Research Article

Keywords: RPF, spectroscopy, Pearson

Posted Date: December 31st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-132925/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Scientific Reports on March 15th, 2021. See the published version at <https://doi.org/10.1038/s41598-021-85371-7>.

Abstract

The recent increase of the number of unidentified cadavers has become a serious problem in the world. As a simple and objective method for age estimation, we attempted to utilize Raman spectrometry for forensic identification. Raman spectroscopy is an optical-based vibrational spectroscopic technique that provides detailed information about molecular composition and structure. Building upon our previous proof-of-concept study, Raman spectra of abdominal skin sample were measured in 132 autopsy cases and protein-folding intensity ratio (R_{PF}) was calculated. There was a strong negative correlation between age and R_{PF} with a Pearson correlation coefficient $r = 0.878$. The results of cross validation suggested that Model 2 (the square of R_{PF}) is the best model with lowest mean squared error (127.9) and highest coefficient of determination (0.743) among the four models. Our results indicate that there is a high correlation between the age and R_{PF} . The Raman biological clock of protein folding can be used as a simple and objective forensic age estimation method of unidentified cadavers.

1. Introduction

Recently, the number of the unidentified cadavers is increasing due to disasters and terrorisms episodes worldwide, as well as a consequence of social internationalization ^{1,2}.

Among the various items needed for cadaver identification, age is among the most important ones. Most of the conventional age estimation methods, including anthropological methods and dental method ³ are mainly used in highly corrupted or skeletonized corpses. Closing of cranial suture ⁴, change of the pubic bone ⁵, morphology of the ribs ⁶, the developmental stages of the seven left permanent mandibular teeth ⁷ and so on represent some examples of those methods. Some of the methods provide high estimation accuracy ⁸⁻¹⁰. However, they are mainly subjective and require highly technical skills and experience ^{2,11-13}. Recently, Computed Tomography and Magnetic Resonance Imaging are also used in this area ¹⁴⁻¹⁷. However, these methods require very expensive equipment, so their use is not yet widely adopted.

Age estimation is somewhat thought to be necessary only for highly corrupted corpses, but actually many fresh corpses which requires age estimation are found on a daily basis. For age estimation in those fresh cadavers or living individuals, in addition to above mentioned methods, bone X-ray examination, physical examination, dental eruptions and so on ¹⁸, molecular biological methods are also used. For instance, among the alterations that occur to our body as we age, many relate to proteins, and attach to intrinsic variations of their molecular structure. Stadtman ¹⁹ proved earlier how the age-related increase in oxidized proteins might reflect the age-dependent accumulation of unrepaired DNA damage. The most advanced and objective concept of "age predictor" in modern forensic science matches the so-called epigenetic (or DNA, or Horvath's) clock, ²⁰⁻²² which is based on DNA methylation of CpG dinucleotides and can be used to estimate the age of a suspect based on blood remains. Usable on a wide spectrum of different tissues, the median error in age prediction of this method is 3.6 y, with a Pearson correlation coefficient, $R = 0.96$, to chronological age ²¹. The stronger the association of the two variables, the closer

the Pearson correlation coefficient, r ; this coefficient being either +1 or -1 depending on whether the relationship is positive or negative, respectively. The Horvath's clock indeed leads to a remarkably precise age estimation as compared to other proposed methods (e.g., telomere length, p16INK4a, or microsatellite mutations)^{23–25}, which show distinctly lower correlation coefficients and only relate to specific types of cell rather than directly to chronological age. The epigenetic clock was also found to relate to Body Mass Index (BMI) with a good correlation, $R = 0.42$, for analyses conducted on liver tissue²¹.

However, though the Horvath's method proved highly objective and has remarkable accuracy among the various age estimation methods, special technical skills, expensive reagents and equipment are still required as well as time and efforts.

Looking for a different approach, we examined the possibility of using Raman spectroscopy of human skin in the attempt to establish a reliable and easily accessible method of age estimation in the forensic medicine. Our spectroscopic method relies on the collection of incisional skin samples during forensic autopsy, and spectroscopic examination of structural characteristics of both proteins and lipids contained in the dermis. Aging of structures of skin has been deeply studied by employing a number of different analytical techniques^{26,27}. Our previously published proof-of-concept study attempted to label all the skin-emitted Raman bands and to locate any biophysical link between vibrational fingerprints and specific structural variations associated with human age²⁸. The existence of such links was indeed phenomenologically proved for protein structures, but it is yet hindered by insufficient statistics. The final proof for our spectroscopic findings thus relies on their statistical validations.

The main purpose here is a statistical validation of the presence of Raman spectral markers for precise age identification from human skin samples. In doing so, we used the same spectral deconvolution algorithm previously proposed²⁸. In that previous study, we have studied the Raman spectra of various skin samples to find a candidate of ideal structural change for age estimation with small number of cases. Accordingly, we found that protein folding may be a good potential "biological clock". In this study, upon increasing the number of autopsy samples (i.e., $n = 132$) and concurrently taking care that wide age range are included, we attempted to assess the statistical exactness of age estimation by Raman spectrometry. The statistical validation presented here could be attractive for the forensic community because the Raman method is label-free and directly links vibrational features at the molecular scale to practical forensic purposes.

Figure 1 gives schematic drafts of different secondary structures of proteins.

These self-explanatory figures also include drafts of the specific vibrational modes that we monitored in this study, as well as the Raman spectroscopic links in terms of vibrational frequencies of different structural assemblies. The structure of skin upon aging has been thoroughly studied by means of numbers of different analytical techniques^{26,27}. Yet, explicit biophysical links are missing between the observed vibrational fingerprints and the specific structural variations associated with human age. With reference to the relative intensities of specific Raman emissions from different protein mesostructures (α -

helix structure and disordered random coil protein structures in Fig. 1), we defined the protein-folding intensity ratio (R_{PF}). R_{PF} represents the ratio of Raman intensities related to the C = O stretching mode (i.e., in the overall Amide I vibrational spectrum depicted in Table S2) in disordered protein structures (Raman signal seen at 1681 cm^{-1}) to α -helix structure (Raman signal at 1652 cm^{-1})^{29–37}. Some literatures^{29,30} reported that proteins irreversibly fold upon aging. Accordingly in our previous study²⁸, the Raman features representing those folding processes were located as parameters sensing biological age, although their age-sensing precision was yet unknown to us.

The aim of this study was to statistically validate the Raman spectroscopic parameters of protein folding in the human skin as age-predicting parameters.

2. Results

The data from total of 132 subjects (92 males and 40 females) were analyzed.

Table 1 shows the summary statistics of age and R_{PF} . R_{PF} of our samples ranged 0.16–0.90. The mean years of age and R_{PF} were 53.4 and 0.41, respectively. On the other hand, median years of age and R_{PF} were 53.5 and 0.39, respectively. As shown in Table 2, both estimates for R_{PF} and R_{PF}^2 were statistically significant at the 5% significance level. The results of cross validation suggested that Model 2 is the best model with lowest mean squared error (127.9) and highest coefficient of determination (0.743). There was a strong negative correlation between age and R_{PF} with a Pearson correlation coefficient $r=-0.878$. Complete plots for all age intervals are given in Fig. 3 for the R_{PF} . Figure 3 also showed the predicted values of age and the 95% prediction intervals. Table 3 showed the 95%, 80%, and 50% prediction intervals. For instance, if R_{PF} would be 0.3, the 95%, 80%, and 50% prediction intervals are 46.7–91.3 years of age, 54.5–83.5 years of age and 61.4–76.6 years of age, respectively. And if R_{PF} would be 0.5, the 95%, 80%, and 50% prediction intervals are 15.9–60.6 years of age, 23.7–52.8 years of age and 30.6–45.9 years of age, respectively. There was no statistically significant difference between male and female samples. Moreover, the specific cause of death was not seen in the outliers.

Table 1
Descriptive statistics of Age and R_{PF}

	Age	R_{PF}
Mean	53.4	0.41
Standard deviation	24.2	0.16
Median	53.5	0.39
Range	0–93	0.16–0.90
n = 132		
RPF: protein-folding intensity ratio		

Table 2
Result of regression analysis for Model 2 (R_{PF} , R_{PF}^2)

	Estimate	SE	t value	P
Intercept	130.0	7.0	18.45	< 0.0001
R_{PF}	-233.3	30.6	-7.63	< 0.0001
R_{PF}^2	99.4	29.9	3.32	0.001
R_{PF} : protein-folding intensity ratio, R_{PF}^2 : the square of R_{PF}				
SE: standard error, P: p-value				

Table 3
 R_{PF} and prediction interval by confidence levels

R_{PF}	Prediction interval (years old)		
	50%	80%	95%
0.3	61.4–76.6	54.5–83.5	46.7–91.3
0.4	45.0–60.2	38.1–67.1	30.4–74.9
0.5	30.6–45.9	23.7–52.8	15.9–60.6
0.6	18.2–33.5	11.3–40.4	3.5–48.2
0.7	7.7–23.1	0.7–30.1	0–37.9
R_{PF} protein-folding intensity ratio			

3. Discussion

In order to quickly identify unidentified cadavers, age estimation methods that can be used by non-experts at the places where bodies found need to be developed. Recently, biomarkers, such as Prostate specific antigen and Thyroid hormones, skin imaging and ultrasonography are reported as alternative age estimation methods to the conventional subjective methods^{38–42}. Especially skin imaging and ultrasonography are very elementary and can be used by non-experts at scenes^{38,41}. However, unfortunately, their estimation accuracy is relatively low.

In this study, a large number of analytical data indeed showed that R_{PF} , related to folding in peptides and proteins, as a reliable marker for the estimation of human age.

The reason for the observed precision of age-predictor, R_{PF} , might reside in the fact that protein oxidation and misfolding mainly affect the frequency shift of the sub-bands used for age assessment, while they alter little the Raman intensity.

Although the outputs of this study were validated by a statistically analyzable number of patients and the shown equations are presently used at Kyoto Prefectural University of Medicine for estimating the age of unidentifiable bodies, the our present method yet requires a substantial amount of time and skill.

Recently, hand-held Raman spectrometric devices have been developed⁴³. This device is now available in each prefectural police in Japan for drug detection at crime scenes. Considering from our results, this equipment appears a promising choice also for age estimation of unidentified cadavers for instantaneous assessments at the crime scene by simply detecting R_{PF} related bands.

There are some limitations in our study. First, although there was no statistically significant difference between male and female samples, this may yet be related to our sample size. Therefore, we need to continue increasing the sample number. Second, although the specific causes of death were not related to outliers, we could not perform a statistical analysis because of the diverse causes of death. In this context too, we also have to increase the number of analyses for this purpose. Third, the confident intervals of age prediction were relatively wide especially in young people. Many methods that use growing and development characters are reported as reliable for individuals under 20 years of age^{44,45}. Therefore, we consider that age estimation should be done by the combination of many ways to obtain the better accuracy avoiding false judgment. Finally, our sampling was restricted to Japanese donors living in the same area and climate. Nevertheless, it is evocative and could be possible to confirm with future experiments whether or not (or to which extent) the Raman biological clock of protein folding could be common to all human beings.

4. Materials And Methods

4 – 1 Materials

One hundred thirty-two cadaveric skin samples were obtained from forensic autopsies at different ages spanning from few months to 93 y old (cf. Table S1 in the supplementary information). Relatives of all the subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board of Kyoto Prefectural University of Medicine, Japan (ERB C-191). Samples were taken in the autopsies conducted within 48 hours.

Several samples for each patient were taken from sub-umbilical region of abdominal skin in the body, which usually remain unexposed to solar irradiation. Sampling included only Japanese subjects. A list of donors with information of age, height and weight at death, sex is given in Table S1. In the statistical characterization, no distinction was made about sampling, thus including in the Raman analysis any available sample irrespective of the cause of death. Skin samples were $10 \times 10 \text{ mm}^2$, and encompassed the full thickness of the skin from stratum corneum to hypodermis.

4 – 2 Spectroscopy

The samples were stored in a humid and evacuated environment at around 0°C until use. Raman spectroscopy was conducted within less than 5 days after the autopsy. The Raman excitation source in this experiments used a 532 nm Nd:YVO₄ diode-pumped solid state laser operating with a power of 200 mW (SOC JUNO, Showa Optronics Co. Ltd., Tokyo, Japan). An objective lens with a numerical aperture of 0.5 was used both to focus the laser beam on the sample surface and to collect the scattered Raman light. A pinhole aperture of 100 μm was adopted with employing an objective lens with a magnification of 100x. For each studied sample, several tens of spectra were collected from the epidermis to dermis (100–600 μm depth from the skin surface) and obtained an average spectrum whose sub-bands were best-fitted to Gaussian-Lorentzian bells⁴⁶. Table S2 shows a complete description of the sub-bands labeled in Fig. 2 for the protein spectral zone, which includes the frequency range at which these specific sub-bands were located and their vibrational origins. In order to have a reliable sampling of the bands per each donor, a number of Raman spectra in the order of > 100 needed to be averaged. Although the spectral acquisition procedure could be automatized, scanning of wide spectral range through dermis to epidermis resulted quite time consuming (about 20 h of measurement per donor).

4 – 3 Statistics

We evaluated correlation between age and R_{PF} using the Pearson correlation coefficient. To build a prediction model, we performed regression analysis letting age as a dependent variable for four candidate models. Independent variables of those models were follows: R_{PF} in Model 1, R_{PF} and R_{PF}^2 (the square of R_{PF}) in Model 2, R_{PF} , R_{PF}^2 and sex in Model 3, and R_{PF} , sex and R_{PF} by sex interaction in Model 4. The goodness-of-fit of each model was assessed using 10-fold cross validation method (repeated 100 times), estimating mean squared error and coefficient of determination. After selecting a best model, we estimated 95% prediction intervals for predicted ages.

Declarations

Acknowledgments: We thank Mr. Akasaka for the support of sample collection.

Author Contributions: Conceptualization, H.I. and G.P.; methodology, W.Z.; validation and formal analysis, D.M., T.U., W.Z.; statistical analysis; K.F., S.T.; investigation, D.M., T.U., W.Z.; resources, D.M., T.U., W.Z.; data curation, K.F., S.T.; writing—original draft preparation, G.P., H.I., S.T.; writing—review and editing, H.I.; visualization, G.P.; supervision, G.P., H.I., S.T.; project administration, H.I.; funding acquisition, H.I. All authors have read and agreed to the published version of the manuscript.

Additional Information

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Funding: This work was supported by Grant-in-Aid for Scientific Research(C), Japan Society for the Promotion of Science (JP17K09270).

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Cattaneo, C. *et al.* Unidentified cadavers and human remains in the EU: an unknown issue. *International journal of legal medicine*.**113**, N2–3 (2000).
2. Cattaneo, C. *et al.* Unidentified bodies and human remains: an Italian glimpse through a European problem. *Forensic science international***195**, 167 e161–166, doi:10.1016/j.forsciint.2009.11.008 (2010).
3. Verma, M., Verma, N., Sharma, R. & Sharma, A. Dental age estimation methods in adult dentitions: An overview. *J Forensic Dent Sci*.**11**, 57–63 https://doi.org/10.4103/jfo.jfds_64_19 (2019).
4. Ruengdit, S., Troy Case, D. & Mahakkanukrauh, P. Cranial suture closure as an age indicator: A review. *Forensic science international*.**307**, 110111 <https://doi.org/10.1016/j.forsciint.2019.110111> (2020).
5. Brooks, S. T. Skeletal age at death: the reliability of cranial and pubic age indicators. *American journal of physical anthropology*.**13**, 567–597 <https://doi.org/10.1002/ajpa.1330130403> (1955).
6. Yoder, C., Ubelaker, D. H. & Powell, J. F. Examination of variation in sternal rib end morphology relevant to age assessment. *J Forensic Sci*.**46**, 223–227 (2001).
7. Bedek, I., Dumancic, J., Lauc, T., Marusic, M. & Cukovic-Bagic, I. New model for dental age estimation: Willems method applied on fewer than seven mandibular teeth. *International journal of legal*

- medicine*.**134**, 735–743 <https://doi.org/10.1007/s00414-019-02066-5> (2020).
8. Braga, J. & Treil, J. Estimation of pediatric skeletal age using geometric morphometrics and three-dimensional cranial size changes. *International journal of legal medicine*.**121**, 439–443 <https://doi.org/10.1007/s00414-007-0170-x> (2007).
 9. Franklin, D., Cardini, A., O'Higgins, P., Oxnard, C. E. & Dadour, I. Mandibular morphology as an indicator of human subadult age: geometric morphometric approaches. *Forensic Sci Med Pathol*.**4**, 91–99 <https://doi.org/10.1007/s12024-007-9015-7> (2008).
 10. Melo, M. & Ata-Ali, J. Accuracy of the estimation of dental age in comparison with chronological age in a Spanish sample of 2641 living subjects using the Demirjian and Nolla methods. *Forensic science international*.**270**, 276 e271-276 e277, doi:10.1016/j.forsciint.2016.10.001 (2017).
 11. Wolff, K., Vas, Z., Sotonyi, P. & Magyar, L. G. Skeletal age estimation in Hungarian population of known age and sex. *Forensic science international*.**223**, 374 e371-378, doi:10.1016/j.forsciint.2012.08.033 (2012).
 12. Dorandeu, A. *et al.* Age-at-death estimation based on the study of frontosphenoidal sutures. *Forensic science international*.**177**, 47–51 <https://doi.org/10.1016/j.forsciint.2007.10.012> (2008).
 13. Cunha, E. *et al.* The problem of aging human remains and living individuals: a review. *Forensic science international*.**193**, 1–13 <https://doi.org/10.1016/j.forsciint.2009.09.008> (2009).
 14. Dubourg, O., Faruch-Bilfeld, M., Telmon, N., Savall, F. & Saint-Martin, P. Technical note: age estimation by using pubic bone densitometry according to a twofold mode of CT measurement. *International journal of legal medicine*.**134**, 2275–2281 <https://doi.org/10.1007/s00414-020-02349-2> (2020).
 15. Ekizoglu, O. *et al.* Forensic age estimation via magnetic resonance imaging of knee in the Turkish population: use of T1-TSE sequence. *International journal of legal medicine*.<https://doi.org/10.1007/s00414-020-02402-0> (2020).
 16. Bjork, M. B. & Kvaal, S. I. CT and MR imaging used in age estimation: a systematic review. *J Forensic Odontostomatol*.**36**, 14–25 (2018).
 17. Monum, T. *et al.* Age estimation from ossification of sternum and true ribs using 3D post-mortem CT images in a Japanese population. *Leg Med (Tokyo)*.**43**, 101663 <https://doi.org/10.1016/j.legalmed.2019.101663> (2020).
 18. Andreas Schmeling, P. M. G. Jose Luis Prieto and María Irene Landa. Forensic Age Estimation in Unaccompanied Minors and Young Living Adults, Forensic Medicine - From Old Problems to New Challenges (IntechOpen, 2011).
 19. Stadtman, E. R. Protein oxidation and aging. *Science*.**257**, 1220–1224 <https://doi.org/10.1126/science.1355616> (1992).
 20. Horvath, S. DNA methylation age of human tissues and cell types. *Genome Biol*.**14**, R115 <https://doi.org/10.1186/gb-2013-14-10-r115> (2013).
 21. Horvath, S. *et al.* Obesity accelerates epigenetic aging of human liver. *Proc Natl Acad Sci U S A*.**111**, 15538–15543 <https://doi.org/10.1073/pnas.1412759111> (2014).

22. Horvath, S. *et al.* Accelerated epigenetic aging in Down syndrome. *Aging Cell*.**14**, 491–495 <https://doi.org/10.1111/accel.12325> (2015).
23. Collado, M., Blasco, M. A. & Serrano, M. Cellular senescence in cancer and aging. *Cell*.**130**, 223–233 <https://doi.org/10.1016/j.cell.2007.07.003> (2007).
24. Forster, P. *et al.* Elevated germline mutation rate in teenage fathers. *Proc Biol Sci*.**282**, 20142898 <https://doi.org/10.1098/rspb.2014.2898> (2015).
25. Nordfjall, K., Svenson, U., Norrback, K. F., Adolfsson, R. & Roos, G. Large-scale parent-child comparison confirms a strong paternal influence on telomere length. *Eur J Hum Genet*.**18**, 385–389 <https://doi.org/10.1038/ejhg.2009.178> (2010).
26. Lipman, E. A., Schuler, B., Bakajin, O. & Eaton, W. A. Single-molecule measurement of protein folding kinetics. *Science*.**301**, 1233–1235 <https://doi.org/10.1126/science.1085399> (2003).
27. Wright, A. J., Narine, S. S. & Marangoni, A. G. Comparison of experimental techniques used in lipid crystallization studies. *Journal of the American Oil Chemists' Society*.**77**, 1239–1242 <https://doi.org/10.1007/s11746-000-0194-2> (2000).
28. Pezzotti, G. *et al.* Raman spectroscopy of human skin: looking for a quantitative algorithm to reliably estimate human age. *J Biomed Opt*.**20**, 065008 <https://doi.org/10.1117/1.JBO.20.6.065008> (2015).
29. Alix, A. J. P., Pedanou, G. & Berjot, M. Fast determination of the quantitative secondary structure of proteins by using some parameters of the Raman Amide I band. *Journal of Molecular Structure*.**174**, 159 [https://doi.org/10.1016/0022-2860\(88\)80151-0](https://doi.org/10.1016/0022-2860(88)80151-0) (1988).
30. McKersie, B. D. & Thompson, J. E. Lipid crystallization in senescent membranes from cotyledons. *Plant Physiol*.**59**, 803–807 <https://doi.org/10.1104/pp.59.5.803> (1977).
31. Gaber, B. P. & Peticolas, W. L. On the quantitative interpretation of biomembrane structure by Raman spectroscopy. *Biochim Biophys Acta*.**465**, 260–274 [https://doi.org/10.1016/0005-2736\(77\)90078-5](https://doi.org/10.1016/0005-2736(77)90078-5) (1977).
32. Bouwstra, J. A., Gooris, G. S., Dubbelaar, F. E. & Ponc, M. Phase behavior of lipid mixtures based on human ceramides: coexistence of crystalline and liquid phases. *J Lipid Res*.**42**, 1759–1770 (2001).
33. Damien, F. & Boncheva, M. The extent of orthorhombic lipid phases in the stratum corneum determines the barrier efficiency of human skin in vivo. *J Invest Dermatol*.**130**, 611–614 <https://doi.org/10.1038/jid.2009.272> (2010).
34. Jyothi Lakshmi, R. *et al.* Tissue Raman spectroscopy for the study of radiation damage: brain irradiation of mice. *Radiat Res*.**157**, 175–182 (2002). doi:10.1667/0033-7587(2002)157[0175:trsfts]2.0.co;2
35. Parker, F. S. Application of Infrared Raman and Resonance Raman Spectroscopy in Biochemistry. (Plenum Press 1983).
36. Huleihel, M. *et al.* Novel spectral method for the study of viral carcinogenesis in vitro. *J Biochem Biophys Methods*.**50**, 111–121 [https://doi.org/10.1016/s0165-022x\(01\)00177-4](https://doi.org/10.1016/s0165-022x(01)00177-4) (2002).
37. ur Rehman, I. M. & Rehman, Z. S. Vibrational Spectroscopy for Tissue Analysis (CRC Press, 2012).

38. Ichioka, H. *et al.* Estimation of Cadaveric Age by Ultrasonography. *Diagnostics (Basel)*.**10**, <https://doi.org/10.3390/diagnostics10070499> (2020).
39. Miyamori, D. T. *et al.* H. Relationship between thyroid hormone levels and age in post-mortem cases. *Romanian Journal of Legal Medicine*.**26**, 12–15 <https://doi.org/10.4323/rjlm.2018.12> (2018).
40. Tsuboi, H., Miyamori, D., Ishikawa, N., Ichioka, H. & Ikegaya, H. Relationship between serum prostate-specific antigen and age in cadavers. *SAGE Open Medicine*.**8**, 2050312120958212 <https://doi.org/10.1177/2050312120958212> (2020).
41. Tsuboi, H. *et al.* Age estimation based on visual parameters of the skin of cadavers. *Skin Res Technol*.**25**, 532–537 <https://doi.org/10.1111/srt.12683> (2019).
42. Uemura, T., Akasaka, Y. & Ikegaya, H. Correlation of polyamines, acrolein-conjugated lysine and polyamine metabolic enzyme levels with age in human liver. *Heliyon*.**6**, e05031 <https://doi.org/10.1016/j.heliyon.2020.e05031> (2020).
43. RIGAKU, R. P. F. (
44. Olze, A., Hertel, J., Schulz, R., Wierer, T. & Schmeling, A. Radiographic evaluation of Gustafson's criteria for the purpose of forensic age diagnostics. *International journal of legal medicine*.**126**, 615–621 <https://doi.org/10.1007/s00414-012-0701-y> (2012).
45. Wade, A., Nelson, A., Garvin, G. & Holdsworth, D. W. Preliminary radiological assessment of age-related change in the trabecular structure of the human os pubis. *J Forensic Sci*.**56**, 312–319 <https://doi.org/10.1111/j.1556-4029.2010.01643.x> (2011).
46. Yuan, X. & Mayanovic, R. A. An Empirical Study on Raman Peak Fitting and Its Application to Raman Quantitative Research. *Appl Spectrosc*.**71**, 2325–2338 <https://doi.org/10.1177/0003702817721527> (2017).

Figures

Figure 1

Explanatory schema of the different secondary structures involved with the spectroscopic measurement of human age based on the protein folding mechanism. Three kinds of protein mesostructures (shown in Table S2) are detected by Raman spectroscopy. Our previous study 28 suggested that the rate of random coiled disordered protein structures (Raman signal seen at 1681 cm^{-1}) to α -helix structure (Raman signal at 1652 cm^{-1}) was age dependent.

Figure 2

Average Raman spectra from skin samples from patients listed in Table S1 for selected age intervals (shown in inset). Spectroscopic intervals related to proteins are shown. In the protein spectral zone, the labels α and d refer to α -helix and disordered structures, respectively. The vibrational origins of all labeled bands are listed in Table S2 of the Supplementary information and visualized in Figure 1.

Figure 3

Prediction curve using the Model 2 (the square of RPF). Complete plots for all age intervals are given for protein-folding intensity ratio (RPF) and also the 95% prediction intervals are shown. There was a strong negative correlation between age and RPF with a Pearson correlation coefficient $r = 0.878$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SRsuppl.docx](#)