

Article

Assessment of Skin Deep Layer Biochemical Profile Using Spatially Offset Raman Spectroscopy

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Featured Application: This study employs a spatially offset spectroscopic approach with a potential applicability to in vivo skin cancer screening.

Supplementary Materials:

Gelatin-gel based phantom matrix protocol:

1. Add porcine gelatin to deionized water (5 gr gelatin in 200 ml H₂O) and place the mixture in a glass beaker on a hot/stirring plate;
2. Heat the mixture of gelatin and water to a temperature of 50°C, while stirring with a magnetic stirring bar until the gelatin dissolves completely and the solution turns transparent;
3. Water-bath gelatin-water solution in a larger beaker and add 10 ml of IL (20% emulsion, Sigma-Aldrich);
4. Cover beakers with foil and stir for 30 minutes;
5. Let cool down to room temperature;
6. Repeat steps 1-4 for top layer using: 1 gr of gelatin in 40 ml H₂O, 5 ml IL;
7. Let cool down to room temperature;
8. Pour mixture at the top of bottom layer.

A parafilm layer was placed at the top of gelatin matrix either between steps 7 and 8 (tissue phantoms type B) or at the end of the procedure (tissue phantoms type A).

Citation: Vardaki, M.Z.; Seretis, K.; Gaitanis, G.; Bassukas, I.D.; Kourkouvelis, N. Assessment of Skin Deep Layer Biochemical Profile Using Spatially Offset Raman Spectroscopy. *Appl. Sci.* **2021**, *11*, 9498. <https://doi.org/10.3390/app11209498>

Academic Editor: Jongsung Lee

Received: 9 September 2021

Accepted: 10 October 2021

Published: date

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Table S1. Raman band assignments of healthy eyelid and scalp human skin layers.

| Raman band (cm ⁻¹) | Assignment | Skin layer | Reference |
|--------------------------------|--|------------------|-----------|
| 492 | nucleic acids (guanine, thymine); amino acids (cystine, glycine) from proteins | all | [1] |
| 620 | Ring breathing (Phenylalanine) | all | [2] |
| 643 | Ring breathing (tyrosine), C-S stretching (cysteine) | all | [2] |
| 722 | C-S stretching (protein); C-N stretching of choline (membrane phospholipid head—phospholipids); nucleic acids (adenine); CH ₂ rocking | all | [1,2] |
| 757 | symmetrical stretching O-P-O, phospholipids | all | [2] |
| 852 | v(C-C) of proline, v(C-C) vibration of the collagen backbone | all | [3-7] |
| 872 | v(C-C) of hydroxyproline | all | [3-6] |
| 939 | Proline, Hydroxyproline, v(C-C) vibration of the collagen backbone | all | [3-6] |
| 972 | =C-H bending mode in unsaturated fatty chains | Subcutaneous fat | [8] |
| 1004 | C-C stretching of aromatic ring breathing mode of phenylalanine | all | [1] |
| 1031 | CH in-plane bending (phenylalanine) | all | [9] |
| 1063 | C-C bending modes of the lipid skeleton | | [8] |
| 1083 | v(C-C) or v(C-O), phospholipids (lipid assignment), phosphate vibrations (phosphodiester groups in nucleic acids), nucleic acids | Subcutaneous fat | [10] |
| 1123 | C-C skeletal stretching of acyl backbone of lipids | Subcutaneous fat | [1,8] |
| 1170 | CH, Tyrosine, Phenylalanine | all | [2] |
| 1245 | Amide III (β-sheet and random coil conformations)—C-N stretching and CH ₂ wagging; PO ₂ asymmetric stretching in nucleic acids | all | [1] |
| 1258 | Lipids (doublet with the 1297 peak), amide III, adenine, cytosine | Subcutaneous fat | [10] |
| 1268 | Amide III (α-helix conformation)—C-N stretching and N-H in-plane bending (proteins); ¼ C-H ethylene deformations—cis | all | [1] |

| | | | |
|-------------|--|------------------|--------|
| | conformation from unsaturated fatty acids (triolein and phospholipids); | | |
| 1303 | CH modes (CH ₂ twisting and wagging) of lipids and collagen; C – H bending (cis conformation) of lipids; amide III mode (α -helix conformation) – C-N stretching and N-H in-plane bending (proteins) | Subcutaneous fat | [1] |
| 1318 | CH ₂ twisting and wagging of proteins and lipids; amide III (C-N asymmetric stretching and C-H deformation) of proteins; nucleic acids (ring breathing mode of guanine) | all | [1] |
| 1339 | ν CC, CH bend (Phe, Trp), purine stretching (DNA, RNA) | all | [2,11] |
| 1439 | Cholesterol, fatty acids, δ CH ₂ , δ CH ₃ | Subcutaneous fat | [10] |
| 1450 | CH modes (CH ₂ and CH ₃ deformations – bending and scissoring) – proteins and lipids (including cholesterol) | all | [1,10] |
| 1554 | Amide II (ν (C-N) and δ (N-H)) | all | [12] |
| 1656 - 1662 | C = O stretching of amide I (α -helix, β -sheet and random coil conformations) of structural proteins; C = C alkyl stretching of lipids – cis conformation; C = C stretching of squalene (strong peak at 1670 cm ⁻¹) | all | [1] |
| 1746 | C=O stretching mode (ester group in glycerol heads of TAG) | Subcutaneous fat | [8] |

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