Automatic Processing and Analysis of the Quality Healing of Derma Injury

Elena Semenova, Oleg Gerasimov, Elizaveta Koroleva, Nafis Ahmetov, Tatyana Baltina, and Oskar Sachenkov(✉)

Kazan Federal University,
Kremlevskaya str. 18, 420000 Kazan, Russia
4works@bk.ru

Abstract. Automation of analyzing the biological data can increase the quality of analyses and decrease spending time. Analyze of the microscope's bitmaps is usual task in biology. To illustrate the proposed method we used analyzing collagen in dermis snapshots. Methodic to automatic analyses of microscope snapshots is presented. Object of analysis can be determine by color vector. Then the snapshot can be binarized and meshed. For every element we can restore distribution of the mean intercept length. Orientation of the objects can be calculated using approximation of the mean intercept length. Equation to estimate the quality of collagen recovery was presented. We used the method on samples of three types: no ficin group (N), ficin group (F), immobilized ficin (Fi). We tested 10 bitmaps for every group and we got results for all bitmaps according described technique. Quality of collagen recovery values was: for N group – 48% ± 8%, for F group 78 ± 7%, for Fi group 68 ± 9%. It can be concluded that ficin positively influence on dermas recovery. Received results are consistent with published results.

Keywords: Snapshot analysis · Fabric tensor · Collagen

1 Introduction

Automation of analyzing the biological data can increase the quality of analyses and decrease spending time. Analyze of the microscope's bitmaps is usual task in biology. In many cases there are typical problems to solve: count number of some biological object, analyze orientation of some biological object in snapshot. In paper we introduce method to analyze density and orientation of some biological object. To illustrate the proposed method we used analyzing collagen in dermis snapshots.

Let's introduce biological problem. Bacterial cells in biofilms are extremely resistant to drug treatment and to attacks from the immune system, which leads to chronic recurrent infections [1–3]. Many opportunistic bacteria (i.e. Staphylococcus, Micrococcus, Klebsiella, Pseudomonas, etc.) form biofilms on chronic and acute dermal wounds, preventing their cure and causing reinfection and sepsis [1, 3, 4]. Degradation of the biofilm matrix backbone, for example via enzymatic lysis, is an advantageous approach for controlling the growth and development of biofilms [5]. It was shown [6, 7] that protolytic enzymes are one of the most effective enzymes in the

K. Arkusz et al. (Eds.): BIOMECHANICS 2018, AISC 831, pp. 107–113, 2019.
https://doi.org/10.1007/978-3-319-97286-2_10

destruction of biofilm matrix by hydrolyzing both matrix proteins and adhesins (proteins that ensure the attachment of cells to hard surfaces and to other bacteria), and for cleavage of signal peptides of intercellular communication of Gram-positive bacteria [6], especially good for this purpose plant proteolytic enzyme ficin [8].

To estimate the quality of derma's recovery we can analyze the collagens orientation. It was shown, that in normal case collagens orientation is chaotic. While in case of injured derma the collagen fibers aligns in direction of maximum stress [9]. In this case we can estimate the quality of healing using relative density and orientation of the collagen fibers [10–12]. To calculate the orientation of the collagen fabric tensor was used [13–15].

2 Materials and Methods

2.1 Mean Intercept Length

Collagen distribution in area can be analyzed as an orthotropic media. To estimate main direction of collagen orientation we decided to use mean intercept length (MIL) [13, 16].

The MIL in a material can be calculated as the average distance, measured along a straight line $\vec{n}$, between two components of the material. In our case two components of the material are collagen and not collagen. MIL can be approximated by quadratic form [16], which can be described by the relationship:

$$L^{-2}(\vec{n}) = \vec{n} \cdot \tilde{M} \cdot \vec{n}, \tag{1}$$

where n is the unit vector in the direction of the mean intercept length measurement and length $L(\vec{n})$ is the mean intercept length.

In this case the problem of defining the orientation of collagen equivalent to eigenvalue and eigenvectors problem for tensor M. We introduce degree of anisotropy, it can be calculated as aspect ratio of the eigenvalues:

$$r = \frac{\lambda_2}{\lambda_1}, \tag{2}$$

where λ_1 - 1st eigenvalue (the largest one), λ_2 - 1st eigenvalue (the lowest one).

If the aspect ratio equal to one it means that there are no certain orientation for collagen, it's distribution is chaotic. Eigenvector related to the 1st eigenvalue (in case of aspect ratio lower than one) describes direction of the collagen's elongation.

2.2 Approximation

The MIL in a material can be stored as vector:

$$\vec{x}_i = (x_i, y_i);\ i = \overline{1, n} \tag{3}$$

In this case we can present quadratic form in general case as:

$$f(\vec{x}) = \vec{x} \cdot A \cdot \vec{x}^T + \vec{x} \cdot B + C = 0, \tag{4}$$

where $A = \begin{pmatrix} A_{11} & 2A_{12} \\ 2A_{12} & A_{22} \end{pmatrix}, B = \begin{pmatrix} B_1 \\ B_2 \end{pmatrix}$

The simplest non-geometric fit in this case is the one minimizing:

$$J(\vec{x}) = \sum_{i=1}^{n} (f(\vec{x}))^2 \rightarrow \min \tag{5}$$

To justify this method it can be noted that $f(\vec{x})$ equivalent to zero if and only if the points $\vec{x}$ lies on the curve, and $J(\vec{x})$ is small when the point lies near the curve. We introduce the vector of unknown parameters of the quadratic form (4):

$$\vec{\eta} = (A_{11}, A_{22}, A_{12}, B_1, B_2, C)^T \tag{6}$$

In this case the problem of the minimization (5) of the $J(\vec{x})$ can be presented:

$$J(\vec{x}, \vec{\eta}) = \sum_{i=1}^{n} (f(\vec{x}, \vec{\eta}))^2 \rightarrow \min \tag{7}$$

That's lead to:

$$\frac{\partial J(\vec{x}, \vec{\eta})}{\partial \eta_i} = 0 \ (i = 1, 6) \tag{8}$$

This problem is equal to system of linear equations [17]:

$$S \cdot \vec{\eta} = \vec{b} \tag{9}$$

But the matrix S is singular. Usually to solve this problem some additional equations added to system of linear equations (i.e. absolute value of vector $\vec{\eta}$ should be equal to one). But in case of analyze of the eigenvectors it's no use and it is allowed to decrease dimension of the vector $\vec{\eta}$.

$$S^* \cdot \vec{\eta}^* = \vec{b}^*, \ \vec{\eta}^* = (A_{11}, A_{22}, A_{12}, B_1, B_2)^T \tag{10}$$

Here constant C is just scale factor of our approximation. Quadratic form A is second rank symmetric tensor. We analyze the collagen orientation using by eigenvalues of the tensor A.

2.3 Algorithm of the Analyze

We used specific algorithm to analyze the micro photos. Generally the algorithm of the photo analyze can be described as:

(1) Determination segment of color vector of the analyzing object
(2) Binarization of the photo
(3) Meshing the photo
(4) For every element we calculate relative content of the object, construct the MIL and it's approximation by ellipse, then find eigenvalues and eigenvectors.
(5) We receive vector field of object distribution.

To identify collagen (paragraph 1 in algorithm) we used equation:

$$||C(i,j) - C_{col}|| < \varepsilon, \tag{11}$$

where C(x, y) – color of bitmap in point (i, j), C_{col} – color of the collagen, ε – color tolerance.

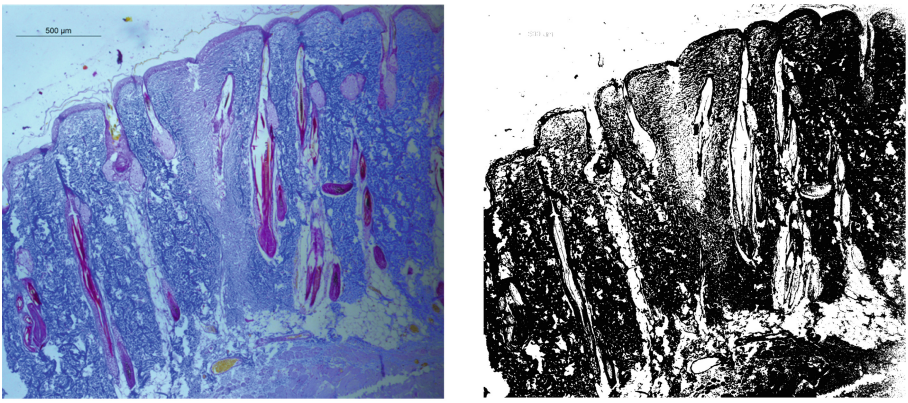
According to color distance photos was binarizied (paragraph 2 in algorithm). It was not classic binarization, because we used three colors: 0 – collagen, 1 – connective tissue, 2 – other not analyzing tissue. Then bitmap was meshed by structured grid and for every element MIL was constructed and approximated by quadratic form (paragraph 4 in algorithm). After that eigenvalues and eigenvectors was calculated and analyzed (paragraph 5 in algorithm). To analyze the quality of the derma we normalized eigenvectors by degree of anisotropy (2). To estimate quality of collagen recovery Q in all area we used equation:

$$Q = \frac{\int\limits_{|\vec{F}(x,y)| \le \delta} dS}{\int\limits_{S} dS}, \tag{12}$$

where $|\vec{F}(x,y)| \le \delta$ mean part of area where magnitude of the vector field is lower than set threshold δ.

3 Results and Discussion

Presented algorithm was used on analyze the quality of derma's recovery. For this purpose we analyzed the micro photos of derma. For analyze in Eq. (11) we used color tolerance equal to 0.05 and in Eq. (12) we used threshold equal to 0.8. On Fig. 1 presented example of original photo (Fig. 1a) and binarized (Fig. 1b). Then the binarized picture was meshed and for every mesh element MIL was build and approximated. On Fig. 2 an example of oriented mesh element presented (white dots - collagen). Red arrow on Fig. 2 shows the first eigenvector (relative to the largest eigenvalue), green arrow shows the second eigenvector (relative to the lowest



a b

Fig. 1. Example of origin and binarized picture.

eigenvalue). On Fig. 2a presented mesh element with collagen density 2% and degree of the anisotropy 0.27, it means that in the first eigenvector direction elongation is greater by 73% than in the second eigenvector. It shows that orientation of collagen is not chaotic. On Fig. 2b presented mesh element with collagen density 42% and degree of the anisotropy 0.80, it means that in the first eigenvector direction elongation is greater by 20% than in the second eigenvector. This difference is not significant and it means that orientation of collagen is chaotic. Analogic calculations was done for every element of the mesh.

As a result of the calculations vector field can be restored. To simplify analyze, on Fig. 2c vector field was restored for the first eigenvectors normalized by degree of anisotropy. Quality of collagen recovery can be estimated by Eq. (12), for presented results Q was equal to 0.54. It mean that more than half (54%) of collagen objects are oriented (not chaotic) in the presented sample. Of course this parameter has integral nature and it is important to understand areas of chaotic and not chaotic collagens orientation. But for primary quick analyze this parameter is significant. We can specify the area of resulting vector field for detailed analyze. More than, methods for analyze of the result can be easily expanded, because as a result of calculation we got vector field. It means that all mathematical methods to analyze vector fields can be used.

We used our method on samples of three types: no ficin group (N), ficin group (F), immobilized ficin (Fi). We tested 10 bitmaps for every group and we got results for all bitmaps according described technique. Quality of collagen recovery values was: for N group – 48% $\pm$ 8%, for F group 78 $\pm$ 7%, for Fi group 68 $\pm$ 9%. it can be concluded that ficin positively influence on dermas recovery. This effect is known [7, 8] and this is just confirmation of the methodic.

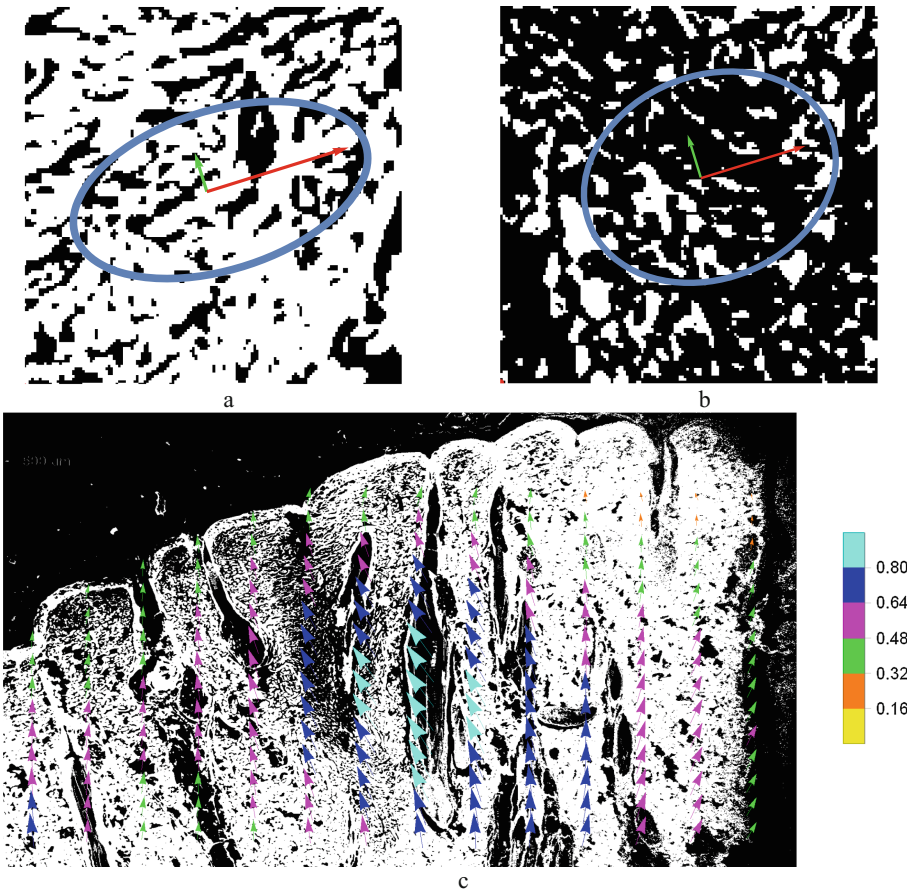


Fig. 2. Illustration of fabric tensor and it's eigenvectors: a – fabric tensor in mesh element with aspect ratio 0.27 and density 90%, b – fabric tensor in mesh element with aspect ratio 0.80, density 68%, c – vector field normalized by aspect ratio with the origin bitmap.

4 Conclusion

Methodic to automatic analyses of microscope snapshots is presented. Object of analysis can be determine by color vector. Then the snapshot can be binarized and meshed. For every element we can restore distribution of the mean intercept length. Orientation of the objects can be calculated using approximation of the mean intercept length. Equation to estimate the quality of collagen recovery was presented. Described technique was used to analyze snapshots of derma. Received results are consistent with published results. This fact shows effectiveness of the method. Traditionally analyze of the collagen distribution is manual and quality of the analyze depends on specialist's experience. Algorithm can be easily transferred in some software for the automatic processing of the picture, for example ImageJ.

Acknowledgements. This work was supported be the grant of the President of the Russian Federation №МК-1717.2018.1

References

1. Yang, L., Liu, Y., Wu, H., Song, Z., Hoiby, N., et al.: Combating biofilms. FEMS Immunol. Med. Microbiol. **65**, 146–147 (2012)
2. Richards, J.J., Melander, C.: Controlling bacterial biofilms. ChemBioChem **10**, 2287–2294 (2009)
3. Donlan, R.M.: Biofilms - microbial life on surfaces. Emerg. Infect. Dis. **8**, 881–890 (2002)
4. Worthington, R.J., Blackledge, M.S., Melander, C.: Small-molecule inhibition of bacterial two-component systems to combat antibiotic resistance and virulence. Future Med. Chem. **5**, 1265–1284 (2013)
5. Blackledge, M.S., Worthington, R.J., Melander, C.: Biologically inspired strategies for combating bacterial biofilms. Curr. Opin. Pharmacol. **13**, 699–706 (2013)
6. Sharafutdinov, I., Shigapova, Z., Baltin, M., Akhmetov, N., Bogachev, M.: HtrA protease from bacillus subtilis suppresses the bacterial fouling of the rat skin injuries. Bionanoscience **6**, 564–567 (2016)
7. Leroy, C., Delbarre-Ladrat, C., Ghillebaert, F., Compere, C., Combes, D.: Effects of commercial enzymes on the adhesion of a marine biofilm-forming bacterium. Biofouling **11**, 11–22 (2008)
8. Baidamshina, D.R., Trizna, E.Y., Holyavka, M.G., Boqachev, M.I., Artyukhov, V.G., et al.: Targeting microbial biofilms using Ficin, a nonspecific plant protease. Sci. Rep. **7**, 46068 (2015)
9. Corr, D.T., Hart, D.A.: Biomechanics of scar tissue and uninjured skin. Adv. Wound Care **2**, 37–43 (2013)
10. van Zuijlen, P.P., Ruurda, J.J., van Veen, H.A., van Marle, J., van Trier, A.J., Groenevelt, F., Kreis, R.W., Middelkoop, E.: Collagen morphology in human skin and scar tissue: no adaptations in response to mechanical loading at joints. Burns **29**(5), 423–431 (2003)
11. Rawlins, J.M., Lam, W.L., Karoo, R.O., Naylor, I.L., Sharpe, D.T.: Quantifying collagen type in mature burn scars: a novel approach using histology and digital image analysis. J. Burn Care Res. **27**(1), 60–65 (2006)
12. Hooman, K., Zhong, Z., Calvin, N., Janette, Z., Xinli, Z., Joyce, W., Kang, T., Chia, S.A.: Quantitative approach to scar analysis. Am. J. Pathol. **178**(2), 621–628 (2011). https://doi.org/10.1016/j.ajpath.2010.10.019
13. Shigapova, F.A., Baltina, T.V., Konoplev, Y.G., Sachenkov, O.A.: Methods for automatic processing and analysis of orthotropic biological structures by microscopy and computed. Int. J. Pharm. Technol. **8**(3), 14953–14964 (2016)
14. Gerasimov, O., Shigapova, F., Konoplev, Y., Sachenkov, O.: Evaluation of the stress-strain state of a one-dimensional heterogeneous porous structure. IOP Conference Series: Materials Science and Engineering, vol. 158, no. 1, p. 012036 (2016)
15. Gerasimov, O., Shigapova, F., Konoplev, Y., Sachenkov, O.: The evolution of the bone in the half-plane under the influence of external pressure. In: IOP Conference Series: Materials Science and Engineering, vol. 158, no. 1, p. 012037 (2016)
16. Harrigan, T.P., Mann, R.W.: Characterization of microstructural anisotropy in orthotropic materials using a second rank tensor. J. Mater. Sci. **19**, 761–767 (1984)
17. Chernov, N., Ma, H.: Computer vision, least squares fitting of quadratic curves and surfaces, pp. 287–302 (2011)