Doctoral School on Engineering Sciences
Università Politecnica delle Marche

Extended summary

Measurement techniques based on image processing for the assessment of biomedical parameters

Curriculum: Ingegneria meccanica e gestionale

Author

Natascia Bernacchia

Tutor(s)

Enrico Primo Tomasini

Lorenzo Scalise

Date: 14-02-2014

Abstract. Biomedical imaging represents an important topic in the field of diagnosis and clinical research. Image analysis and processing software also helps to automatically identify what might not be apparent to the human eye. The technological development and the use of different imaging modalities create more challenges, as the need to analyse a significant volume of images so that high quality information can be produced for disease diagnosis, treatment and monitoring, in clinical structures as well as at home.
All the measurement systems routinely used in clinical environment require to be put in direct contact with the subject, which in some cases can be uncomfortable or even non-suited for long monitoring. On the other hand, in some cases contact could alter shape or composition of the samples under study, and state-of-the-art techniques could require a lot of time and provide very low resolution.
This doctoral thesis presents a series of new experimental applications of the image analysis and processing in the biomedical field. The aim was to develop and validate new methodologies, based on image analysis, for non contact measurement of quantities of different nature. The study is focused on the extraction of morphological characteristics of cell aggregates to assess of the regeneration processes in infarcted hearts, the design of a non contact methodology to measure mechanical properties of rabbit patellar tendons subjected to tensile tests, the development of new methods for the monitoring of physiological parameters (heart and respiration rate, chest volume variations) through the use of image acquisition systems, as Kinect™ device and a digital camera.
The experimental setups, designed in this work, were validated, showing high correlation respect to the reference methods. Imaging systems, although so different in many aspects, have demonstrated to be suitable for the respective tasks, confirming the feasibility of the imaging approach in the biomedical field.
Keywords. Up to five keywords or phrases in alphabetical order, separated by commas.

1 Introduction and aims

Biomedical imaging is becoming an essential aid for physician in diagnostics and monitoring, also remotely in order to reduce follow-up incidence [1]. The new technological development and the use of different imaging modalities, create more challenges, as the need to process and analyze a significant volume of images so that high quality information can be produced for disease diagnoses and treatment [2].
The aim of this thesis is to study and present some different image systems and processing techniques, in order to develop experimental methodologies aiming to the measurement of biological and physiological parameters.
Heart imaging is one of the most challenging research topics in biomedical field. Automation of histology is still in its infancy; much work still needs to be done in this area [3]. X-ray micro-tomography will be assessed as a high-resolution technique able to provide detailed slices of the heart [4]. Monitoring and tracking injected cells require the use of non-destructive strategies able to identify location, time of cell survival and fate. The aim will be to assess regenerative processes in infarcted myocardium through a measurement of level of tissue regeneration and cell engraftment achieved with different treatment protocols.
Patellar tendon failure is a very interesting theme, especially in the sports world, because this tissue is used in autologous reconstruction of the cruciate ligament. Specimen length and cross-sectional area appear to influence mechanical characteristics of tendons [5]. Tendons are constituted by soft tissue so contact could alter shape and real cross-section. In this thesis an experimental method is proposed for the non contact characterization of the mechanical proprieties of rabbit patellar tendons based on the use of a fixed lens camera and a customized gripping system.
It is commonly known that heart and respiratory rate are routinely monitored in patients recovered in clinics and their variations need to be reported to the subject or to the caregivers. Today these parameters are measured respectively with electrocardiographs and spirometers, that need to be put in contact. This thesis proposes a novel measurement method based on the use of the Kinect™ device for non contact measurement of the heart and respiratory rates. This technology is based on infrared emitter and receiver and it is mainly used for motion detection. Another task will be the assessment of air flow.
Imaging techniques are able to automatically identify what might not be visible to the human eye [6], thus reducing the need for more invasive procedures. In this context the last activity find application. It is based on the propagation of the cardiac pulse wave in all blood vessels of the human body, causing apparently invisible variations in the light reflection. The observations will be realized acquiring a video of the patient face with a digital camera [7] and exploiting the potentiality of face detection algorithms [8].
Moreover the first two research topics involve grayscale images with very high resolution, performed on ex-vivo anatomical segments. Kinect based method makes use of color intensity images representing a depth map, while camera provides RGB video sequences, both acquiring human beings.
This work can provide both spatial and temporal analysis to detect patterns and characteristics. In particular X-ray micro-CT was used to conduct a static analysis on infarcted rat hearts. The activity on patellar tendons is based on tensile tests, that are performed in a steady state. Despite that a Kinect device and a digital camera were employed for the study of time-varying phenomenon (heart and respiratory rate).

2 Research focus and methodologies

The study of regenerative processes in infarcted hearts and stem cells migration from the injection site to the infarcted area were conducted employing many protocols, thus dividing rat hearts in several groups through different criteria: stem cell nature (r-CPCs or h-MSCs), cell treatment (ITS, Feridex, both, none), time to sacrifice (24-48 h, 12-13 d, 21 d, 30 d). Rat hearts were placed in the sample holder. X-ray microtomography acquisitions were carried out at European Synchrotron Radiation Facility (ESRF) in Grenoble [9]. Monochromatic radiation with an energy of 15keV, working in 3D parallel tomography modality, was used. The exposure time was equal to 0.3 s per projection, 2000 projections were taken over an angular range of 360°. Resolution was of 10.112 μm.

Image processing was realized with a purpose-written algorithm, based on the identification of an optimal segmentation threshold (minimizing RMSE of detected cells volumes), image segmentation [10] of the heart sections, calculation of cells area distribution along the three anatomical planes. The aspects of main interest that were explored are:

– Measurement of heart wall (old and new) thickness in the ventricular area.
– Detection of the heart zone in which stem cells are more concentrated (axial direction).
– Quantitative measurements of dimensional and morphological parameters on cells belonging to this slice (after labelling).

Structures were classified in three groups: single cells, medium and big aggregates. In particular roundness and form factor of the detected structures were computed following the equations (1) and (2):

$$FormFactor = \frac{4 \cdot \tau \cdot Area}{Perimeter^2} \quad (1) \qquad Roundness = \frac{4 \cdot Area}{\tau \cdot Lenght^2} \quad (2)$$

The experimental set-up developed to conduct tensile tests on rabbit patellar tendons was validated with nylon wires [11] and it is reported in Figure 1.

In particular a Marlin F-131 camera (Allied Vision Technologies) was used as imaging system (resolution from calibration of 0.05 mm). It can be interfaced with a computer desktop through IEEE 1394 firewire connection. A new system was designed for sample clamping to allow a preconditioning phase [12], revealing an hysteretic behavior, and the tensile testing (elongation rate of 0.3 mm/s) with a Servo-pneumatic test machine 966-804-23 made by Si-Plan Electronics Research Ltd©.

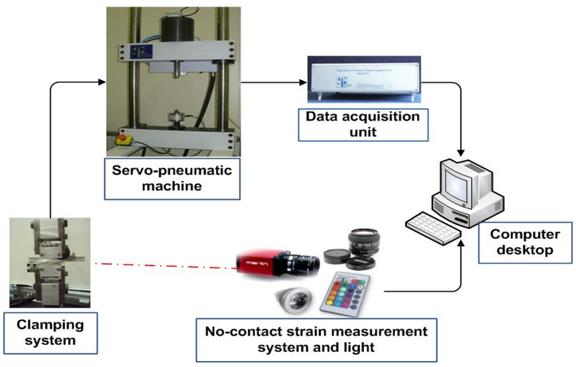

Figure 1. Experimental setup scheme for tendons tensile tests

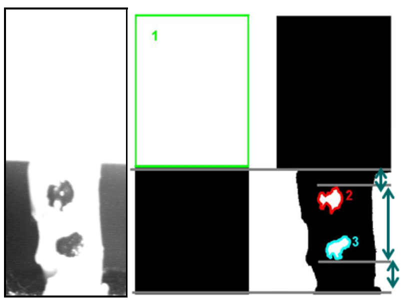

Figure 2. Image segmentation

Image analysis on camera frames was conducted selecting a Region Of Interest (ROI), enhancing contrast [10] and performing image segmentation, in order to separately identify the sample and the targets over it (Figure 2) [15].
Mechanical properties of interest for a tendon [13], that is viscoelastic [14], are: Young modulus, ultimate strength, ultimate strain. Applied load and sample elongation were acquired by the test machine, but the camera provided no contact measurement of local longitudinal strain (as distance between the targets), making possible to check any slippage, and cross-sectional area (through image segmentation), useful to calculate the real withstanded strength and to evaluate the transverse necking.

In the previous paragraph the importance to measure heart and respiratory rate contactless was already discussed. Image-based systems were proposed, in particular using Kinect™ [16] and a Microsoft® digital camera [17] (both with a sampling frequency of 30 fps and at a distance of 1,20 m, chosen after calibration, and 50 cm respectively) were used and compared respect to the gold standard methods (ECG and spirometer, or respiratory belt). The developed experimental set-ups are showed in Figure 3 and 4.

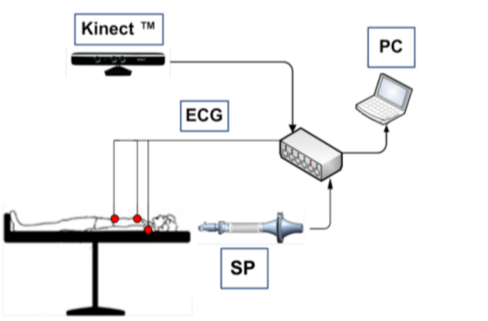

Figure 3. Set-up for tests with Kinect™

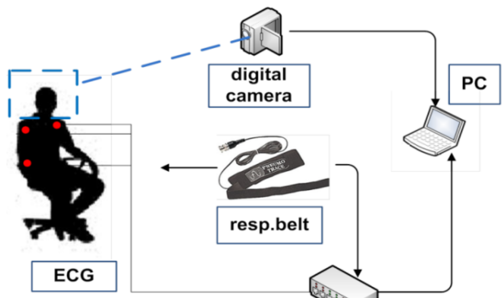

Figure 4. Set-up for tests with camera

The data acquisition unit PowerLab 4/25T (ADInstruments) allowed to record simultaneously more channels.
Kinect is provided by infrared emitter and sensor that capture the depth of the different objects in the environment, so, after a calibration procedure (plane resolution of 2 x 2 mm) it was also possible to evaluate respiratory volume exchanges measuring chest wall displacement [18]. The technique based on the camera exploited the propagation of the cardiac pulse wave in all blood vessels of the human body, causing apparently invisible variations in the light reflection [7].
Signals for the subsequent analysis were extracted respectively for the two methods:
- Selecting three ROIs over Kinect frames and averaging pixels depth values.
- Selecting a ROI over camera frames with an optimized version of "Face Parts Detection" algorithm [19], separating the three color planes, averaging color pixel values, normalizing the traces.

The subsequent steps are the following:
- Kinect: application of Independent Component Analysis (ICA) [20]. One component was subjected to a wavelet decomposition (DWT) [26] and respiratory rate was extracted. Another component was treated performing power spectral density (PSD) [10] and evaluating the frequency of its peak in a range of 0.75-4 Hz [7]. Volume changes, instead, were found performing the integral of chest area displacement.
- Camera: application of ICA. Components were treated performing PSD [10] and evaluating which of the peaks are higher, in order to choose the signal for further analysis.

The trace was decomposed with DWT, and peaks are detected to extract heart rate. A sort of tachogram was obtained, to conduct heart rate variability analysis, and its spectrum was computed [21] in order to find the peak frequency in the range of the High Frequencies (0.15-0.4 Hz) [22], that corresponds to respiratory rate.

3 Results and discussion

The first study showed the assessment of the cardiac tissue regeneration after stem cell injection, using X-ray micro-CT. For almost all the rats (8 out of 10), the maximum cell density zone results to be in the ventricular area, showing that cells migrate from the injection site to infarcted tissue. Figure 5 shows a good correlation [23] between volumes obtained with reference and innovative methods, reporting a root mean square error of 1112478,49 μm^3, were the mean value of cells volume is 978313172,21 μm^3. Studying the effect of time from cells injection, it was found that in the early phase there is a clear increase in the integral, indicating a proliferation of cardiac progenitor cells. In the following period a sensible reduction is shown, in agreement with reference unpublished data. Treatment protocols were studied. Feridex despite the fact that helps the in-vivo visualization of labeled cells, on the other hand interactions of these labelled cells with macrophages are not completely clear [24]. ITS grow factor seems to enhance tissue regeneration. Heart wall thickness was measured both in the pre-existing tissue and in the regenerated one, showing a clearly visible presence of new cells in infarcted area for almost all the samples.

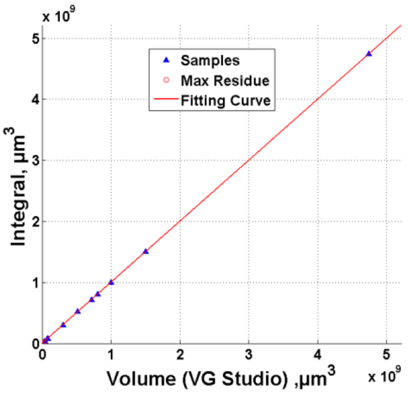

Figure 5. Scatter plot analysis

Table 1. Roundness and Form factor (m ± SD).

Sample	Roundness	Form factor
S1	0.51 ± 0.05	0.61 ± 0.11
S3	0.57 ± 0.27	3.48 ± 4.35
S4	0.72 ± 0.17	2.70 ± 3.61
S5	0.65 ± 0.22	0.95 ± 0.24
S6	0.80 ± 0.08	1.02 ± 0.06
S7	0.54 ± 0.14	0.86 ± 0.44
S8	0.65 ± 0.29	2.31 ± 3.43
S9	0.71 ± 0.19	1.53 ± 1.98
S10	0.43 ± 0.20	0.59 ± 0.24
S11	0.68 ± 0.12	1.03 ± 0.46

Roundness and form factor are very interesting in order to evaluate if the cell structures in the slice are mainly uniform (circular) or rugged (elongated). In Table 1 the mean and standard deviation of roundness and form factor of all the cell structures found in the samples were reported. For what concerns roundness, values closer to 1 suggest that the prevalent morphology corresponds to single cells or small aggregates.

The experimental set-up designed for tensile tests on tendons was validated, comparing strain with that provided by the machine, and a Pearson squared correlation coefficient R^2 of 0.99 was obtained, showing a very good correlation.

In Figure 6 stress/strain curve of quasi-static tensile tests are visible. The upper one was assessed using only data provided by the machine with a fixed CSA, instead of the lower one where CSA and strain measurements are those measured with digital camera. Both the curves show a mechanical behavior comparable with that showed by previous studies in this research field [25].

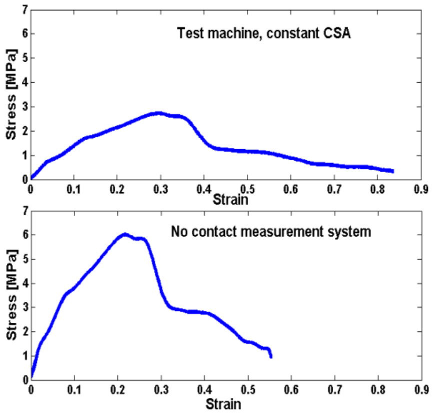

Figure 6. Stress/strain curves for non-contact tendons analysis

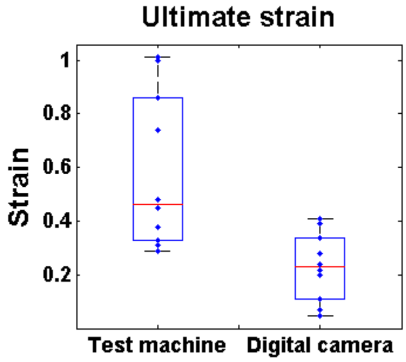

Figure 7. Boxplot of ultimate strain for the tendons samples

Figure 6 suggests that data sets belonging to test machine and new method are comparable to each other, and the variability of the results are often caused by phenomenon, like slippage, observable only with the aid of a useful no contact measurement system, as a digital camera. Preliminary tests on equine Achilles tendons indicate the feasibility of the new approach and give some important keypoints for future clamping system design: these samples can withstand larger loads, until 103 N [27].

Kinect-based approach, in the second phase, (N=8 subjects, 32 tests) was applied for a test duration of 120 s (frame size: 320 x 240). Mean heart and respiratory rate (HR and RR) were compared for each concerns new and gold standard methodologies, using Bland-Altman test [28] [29], as it can be seen in Figure 8 and 9.

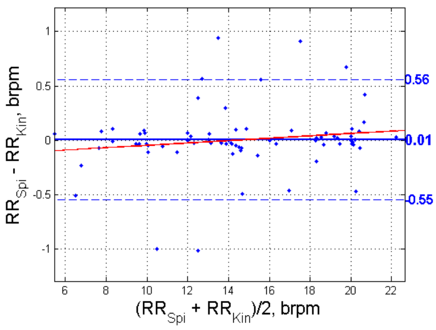

Figure 8. Bland-Altman analysis for RR

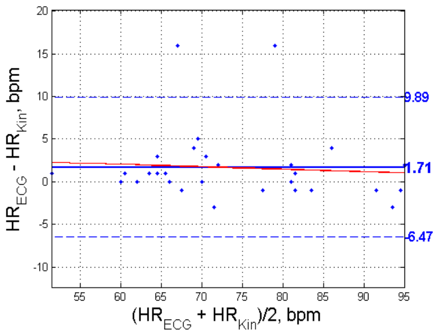

Figure 9. Bland-Altman analysis for HR

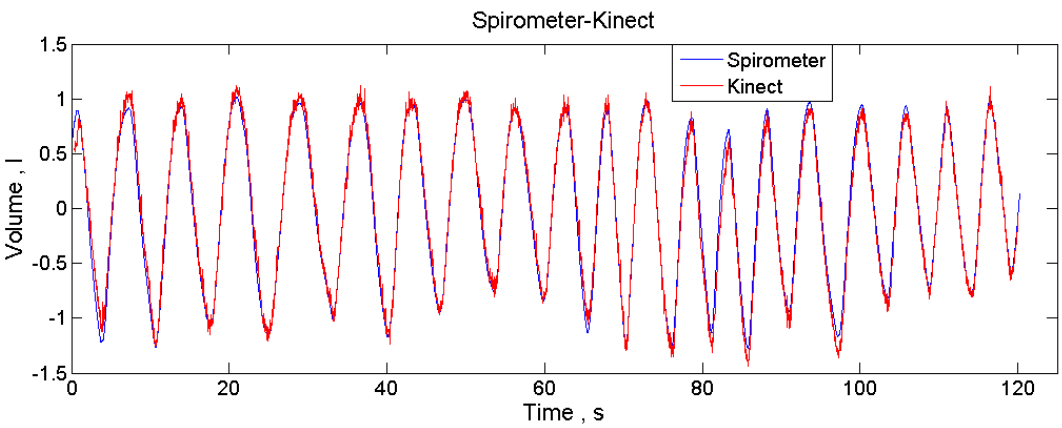

Figure 10. Volume measured by spirometer and Kinect

A bias was found of 0.01 brpm for RR, and 1.71 bpm for HR, in both the cases very close to zero. Pearson correlation coefficient resulted 0.99 and 0.94 respectively for respiratory and heart rate. The uncertainty analysis has provided a value of 1 brpm for the first parameter and 10 bpm for the second one (k=2) [23]. The third realized task was to measure volume changes of the chest through Kinect device (N=8 subjects), it showed in Figure 10.

Validation of camera-based measurement method was performed testing 18 subjects in sitting position (43 tests) [30]. In Figure 11 HR measurements are considered. In particular Pearson's coefficient [23] was 0.96, obtaining high correlation with reference data. A comparison between RR extracted with the customized algorithm, and values obtained by the RB signal was made (Figure 12). Pearson's coefficient in this case was 0.81.

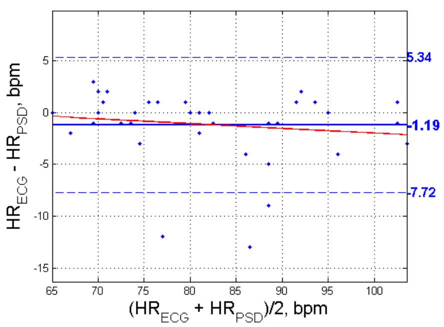

Figure 11. Bland-Altman analysis for HR

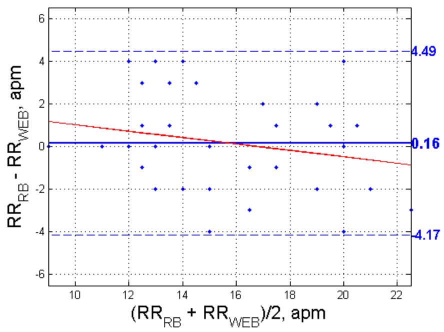

Figure 12. Bland-Altman analysis for RR

A bias was found of -1.19 bpm, for HR calculated from power spectral density. RR assessment reported a bias of 0.16 brpm. In all the cases it is very close to 0. The uncertainty analysis has provided a value of 8 bpm for the same two procedure to measure heart rate (k=2) [23]. An uncertainty value of 4 brpm was calculated for what concerns respiration assessment (k=2) [23].

A preliminary step of this activity was conducted on hospitalized preterm neonates (7 subjects) [31] [32], with the aim to provide a non contact heart rate measurement. A good correlation was obtained, as proved by a Pearson's coefficient [23] of 0.94.

Also long term monitoring was performed acquiring a video of 6 min in order to achieve better conditions for HRV analysis. The HR trend is better described if a time-window of 60 s is considered, after many attempts with different length (30, 40, 50, 60 s). Poincarè is reported for camera based method in Figure 14, and showed a comparable data dispersion respect to ECG data.

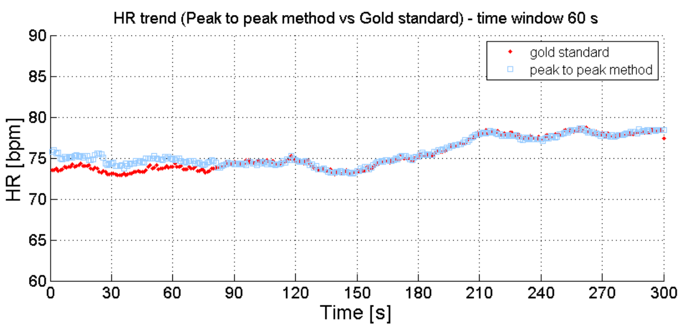

Figure 13. HR trend (time window of 60 s

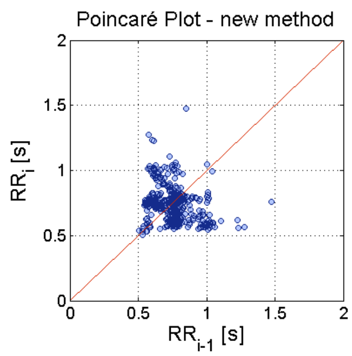

Figure 14. Poincarè plot of the RR intervals

4 Conclusions

The thesis explored the possibility to realize experimental contactless measurement methods making use of image analysis techniques and processing in the biomedical field.
The study on infarcted hearts, after stem cells injection, aimed to assess myocardium regeneration, requiring to look at each cell inside the samples. X-ray micro-CT represents the most expensive but also precise imaging method (resolution of 10 μm). Obviously there is an established working time and tests are almost expensive.
Tendons have viscoelastic behavior, so their mechanical properties depend on cross sectional area. The measurement of this variable together with local sample strain was performed using a fixed lens camera, allowing a good resolution (0.05 mm), a continuous measurement during the test and a check of any sample slippage. This device is more expensive (about 400 euros) than a simple digital camera.
Kinect™ was used, exploiting depth map provided by the infrared sensors, to evaluate chest volume variations and retrieve heart and respiratory rate. The pixel size, from calibration procedure, was of 2 mm, and the decrease in resolution also corresponds to a cost reduction (100-150 euros).
Vital signs were also measured with a new measurement method based on the acquisition of a video of the subject face, using a digital camera. The cost is less than 100 euros, providing good results.
In the future experiments on infarcted hearts an important observation will be to place markers on the samples, in order to completely recognize heart orientation. A target would be to count cellular aggregates inside the whole sample, but many efforts are needed to correlate slice by slice, in terms of computational requirements.
Diseases tendons could be studied, in order to test the efficacy of grafts or therapies, and to design a new and custom clamping system for samples of larger size, such as equine Achilles tendons. The use of a second camera could allow the estimate of cross-section as an ellipse, thus reducing uncertainty.
The novel approach based on Kinect™ will be definitely improved studying its sensitivity to the subject posture (sitting, standing) and to the presence of clothes.
The camera-based non contact measurement method will be optimized improving the algorithm potentiality, making it more efficient, also in critical ambient condition (illumination, movement), and less time consuming.
All new experimental set-ups showed high correlation respect to the gold standard and demonstrated to be suitable for the respective tasks, confirming the feasibility of the imaging approach in the biomedical field.
In particular the studies conducted on infarcted hearts and tendons find application in research environments or laboratory. The other two techniques for the measurement of the heart and respiration rate, with Kinect™ and a digital camera, are instead very actual and interesting for the possibility to extend their use for clinical, but also home monitoring.
All the measurement systems routinely used for diagnosis and monitoring, require to be put in direct contact with the subject, which in some cases can be uncomfortable or even non-suited for long monitoring [33], especially in case of skin diseases, burns or not collaborative people. On the other hand, in some cases contact could alter shape or composition of the samples under study, and state-of-the-art techniques could require a lot of time and provide very low resolution. Contactless measurements are really challenging and interesting in the biomedical field for these reasons.

References

[1] J.D. Bronzino, "The Biomedical Engineer Handbook", s.l.: Springer, 2000.
[2] F.P. Branca, "Fondamenti di ingegneria Clinica", s.l.: Springer, Vol. 1, 2000.
[3] H.K. Huang, "Biomedical image processing.", Critical Reviews in Bioengineering, Vol. 5 (3), pp. 185-271, 1981.
[4] A. Giuliani, "3D visualization of transplanted stem cells in infarcted rat hearts by high-resolution X-ray microtomography.", Il Nuovo Cimento C, Vol. 2, pp. 157-167, 2012.
[5] K. Legerlotz, G.P. Riley, H.R. Screen, "Specimen dimensions influence the measurement of material properties in tendon fascicles", Journal of Biomechanics, Vol. 43(12), pp. 2274–2280, 2010.
[6] http://www.embs.org/about-biomedical-engineering/our-areas-of-research/biomedical-imaging-a-image-processing (15/01/2014).
[7] M.Z. Poh, D.J. McDuff, R.W. Picard, "Advancements in noncontact, multiparameter physiological measurements using a webcam", IEEE Trans. Biomed. Eng., Vol. 58 (1), pp. 7–11, 2010.
[8] P. Viola, M. Jones, "Rapid object detection using a boosted cascade of simple features", Proc of IEEE Comp Vis and Pattern Recogn (CVPR), Vol. 1, pp. I-511 - I-518, 2001.
[9] A. Giuliani, C. Frati, A. Rossini, V. S. Komlev, C. Lagrasta, M. Savi, S. Cavalli, C. Gaetano, F. Quaini, A. Manescu e F. Rustichelli, "High-resolution X-ray microtomography for three-dimensional imaging of cardiac progenitor cell homing in infarcted rat hearts.", J Tissue Eng Regen Med, Vol. 5 (8), pp. e168-e178, 2011.
[10] J.L. Semmlow, "Biosignal and Biomedical Image Processing - MATLAB-Based Applications", s.l.: Signal Processing and Communication Series, 2004.
[11] UNI EN ISO 527-1/1997, Plastics – Determination of tensile properties, 1997.
[12] S.L. Woo, R.E. Debski, J. Zeminski, S.D. Abramowitch, S.S.C. Saw, J.A. Fenwick, "Injury and repair of ligaments and tendons", Annual Review of Biomedical Engineering, Vol. 2, pp. 83-118, 2000.
[13] R.F. Ker, "Mechanics of tendon, from an engineering perspective", International Journal of Fatigue, Vol. 29 (6), pp.1001-1009, 2007.
[14] J. H.-C. Wang, "Mechanobiology of tendon.", Journal of Biomechanics, Vol. 39, pp. 1563-1582, 2006.
[15] L.Scalise, B. Lonzi, N. Bernacchia, "A camera-based experimental method for mechanical test on patellar tendons", This paper is submitted for SEM, 2014, Location : Greenville (USA).
[16] http://www.microsoft.com/en-us/kinectforwindows/ (20/12/2013).
[17] http://www.microsoft.com/hardware/it-it/p/lifecam-studio/Q2F-00016 10/01/2014.
[18] M.-C. Yu, J.-L. Liou, S.-W. Kuo, M.-S. Lee, Y.-P. Hung, "Noncontact Respiratory Measurement of Volume Change Using Depth Camera", IEEE Eng Med Biol Soc Proc., pp. 2371-2374, 2012.
[19] http://www.mathworks.it/matlabcentral/fileexchange/36855-face-parts-detection. 10/01/2014.
[20] P. Comon, "Independent Component Analysis: a new concept?", Signal Processing, Vol. 36 (3), pp. 287-314, 1994.
[21] S.M. Kay, S.L. Marple, "Spectrum analysis - A modern perspective", IEEE Proc, Vol. 69 (11), pp. 1380 - 1419, 1981.
[22] J. Vila, F. Palacios, J. Presedo, M. Fernandez-Delgado, P. Felix, S. Barro, "Time-frequency analysis of heart-rate variability," IEEE Eng. Med. Biol. Mag., Vol. 16 (5), pp. 119–126, 1997.
[23] International Organization for Standardization, "Guide to the Expression of Uncertainty in Measurement", Switzerland, First ed., 1993, corrected and reprinted 1995.
[24] F. Arfelli, "Synchrotron light and imaging systems for medical radiology.", Nuclear Instruments and methods in Physics Reasearch, Vol.454, pp. 11-25, 2000.
[25] A.A. De Moura Meneses, C.J.G. Pinheiro, P. Rancoita, T. Schaul, L.M. Gambardella, R.

Schirru, R.C. Barroso, L.F. De Oliveira, "Assessment of neural networks training strategies for histomorphometric analysis of synchrotron radiation medical images.", s.l. : Nuclear instrument and methods in physics research, Section A, Vol. 621(1-3), p 662-669, 2010.

[26] L. G. Gamero, J. Vila, F. Palacios, "Wavelet transform analysis of heart rate variability during myocardial ischaemia," Med. Biol. Eng. Comput., Vol. 40 (1), pp. 72-78, 2002.

[27] N. Crevier, P. Pourcelot, J.M. Denoix, D. Geiger, C. Bortolussi, X. Ribot, M. Sanaa, "Segmental variations of in vitro mechanical properties in equine superficial digital flexor tendons.", Am. J. of Vet. Res., Vol. 57 (8), pp. 1111-1117, 1996.

[28] J.M. Bland, D.G. Altman, "Statistical methods for assessing agreement between two methods of clinical measurement", Lancet, Vol. 327 (8476), pp. 307–310, 1986.

[29] N. Bernacchia, L.Scalise, P. Marchionni, I. Ercoli, L. Casacanditella, E.P. Tomasini, "No-contact measurement of heart and respiration rates based on Kinect ™", This paper is submitted for IEEE Int Symposium on Medical Measurements and Applications (MeMeA), 2014, Location : Lisbona (Portugal).

[30] L. Scalise, N. Bernacchia, P. Marchionni, I. Ercoli, E.P. Tomasini, "Non contact assessment of vital signs: LDV vs image-based". This paper appears in Laser Florence, Firenze, 9-10 November 2013, Lasers Med Sci, Vol. 28, p. 1418, 2013.

[31] L. Scalise, N. Bernacchia, I. Ercoli, P. Marchionni, "Heart rate measurement in neonatal patient using a webcamera.", Proc. of IEEE Int Symposium on Medical Measurements and Applications (MeMeA 2012), pp. 1-4, 2012.

[32] N. Bernacchia, I. Ercoli, P. Marchionni, L. Scalise, "Video processing for heart rate measurement in infants". This poster appears in : International Computer Vision Summer School 2012, Poster Session.

[33] E. Zimlichman, J. Terrence, D. Argaman, Z. Shinar, H. Brown, "Effect of contactless continuous patient monitoring in a medical - surgical unit on intensive care unit transfers: a controlled clinical trial", Am J Respir Crit Care Med, Vol. 185, 2012.