# Histochemistry as an irreplaceable approach for investigating functional cytology and histology

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## Abstract

In agreement with the evolution of histochemistry over the last fifty years and thanks to the impressive advancements in microscopy sciences, the application of cytochemical techniques to light and electron microscopy is more and more addressed to elucidate the functional characteristics of cells and tissue under different physiological, pathological or experimental conditions. Simultaneously, the mere description of composition and morphological features has become increasingly sporadic in the histochemical literature. Since basic research on cell functional organization is essential for understanding the mechanisms responsible for major biological processes such as differentiation or growth control in normal and tumor tissues, histochemical Journals will continue to play a pivotal role in the field of cell and tissue biology in all its structural and functional aspects.

## Histochemistry and Cytochemistry in recent literature

According to the web definitions, Histochemistry is *the branch of science that deals with the chemical composition of the cells and tissues of the body* or *a science that combines the techniques of biochemistry and histology in the study of the chemical constitution of cells and tissues* (Free online Dictionary by Farlex: http://www.thefreedictionary.com). On the other hand, Cytochemistry is *the study of intracellular distribution of chemicals, reaction sites, and enzymes, often by means of staining reactions, radioactive isotope uptake, selective metal distribution in electron microscopy, or other methods* (Free Merriam-Webster Dictionary: http://www.merriam-webster. com/).

All these definitions seem to suggest that Histochemistry and Cytochemistry are to be considered as ancillary disciplines which may purely describe the chemical composition of cells and tissues through the application of a reasonably wide collection of methods and techniques. An even cursory survey of the articles published in the histochemical Journals during the last few years provides a much more exhaustive representation of the actual scope and potential of Histochemistry and Cytochemistry in the field of cell and tissue biology (as review articles, see for instance[1-3]).

Indeed, the application of cytochemical techniques to light and electron microscopy has increasingly been addressed to elucidate the functional characteristics of cells and tissue under different physiological, pathological or experimental conditions; in fact, the mere description of composition and morphological features has become increasingly sporadic.[4,5]

This is consistent with the evolution of histochemistry over the last fifty years: thanks to the impressive advancements in microscopy sciences,[6-9] the current histochemical approach essentially aims to locate molecules in the very place where they exert their biological roles, and to dynamically describe specific chemical processes in living cells. This is apparent from a review of the articles recently issued on the European Journal of Histochemistry, an example of a publication which is explicitly devoted to functional cytology and histology.

Consistent with the large number of published articles in the literature on histochemical applications (more than 32,000 in 2011-2013, according to http://www.ncbi.nlm.nih.gov/pubmed), most of the papers concerned investigations on different pathologies, with special attention to tumor biology. The large majority of these papers focused on the molecular bases of diseases[10-12] and on carcinogenesis.[13-21] In particular, immunohistochemistry was used as a suitable tool for labeling diagnostic tumor markers[22-27] (often in a multiple way) or prognosis-predicting indicators,[28-29] and for detecting the expression of specific molecules in premalignant lesions.[30]

The distribution and activity of specific proteins was investigated in different animal or plant cells and tissues,[31-37] and was often compared with the ectopic relocation of the same molecules under pathological conditions[25,38-45] or after the application of experimental stimuli or therapeutic agents.[46-51] The immunohistochemical detection of a given protein or the recognition of a specific enzyme activity was never aimed to purely describe cell features in a micro- (or ultramicro-) anatomical perspective. In fact, it clearly emerged that not only the presence of a given molecular species but also its proper subcellular location are essential for assuring cell and tissue normality.

The expression of specific protein marker was assessed during pre- and post-natal development in mammalian species,[52-65] starting from the process of oocytes maturation and elimination.[55,56,61,62] The development of heart and skeletal muscle was the subject of several papers, where special attention was paid to stem cell populations and their molecular characteristics.[66-72] Here too, the unusual localization or translocation of specific proteins was considered as an important evidence,[72] all the more so as it occurs under pathological conditions (*e.g.*, in myotonic dystrophy) or in sarcopenia, *i.e.*, the loss of muscle mass and function physiologically occurring in the aging process.[67,76,77] As demonstrated by ultrastructural immunocytochemistry, the dystrophic phenotype would depend on a general alteration in the post-transcriptional maturation of pre-mRNAs. Interestingly, the accumulation of pre-mRNA processing factors in the myonuclei of dystrophic patients is reminiscent of the nuclear alterations found in age-related sarcopenia. Consistently, it was observed that *in vitro* myoblasts derived from satellite cells of dystrophic patients show cell senescence features and alteration of the pre-mRNA processing pathways earlier than the myoblasts from healthy subjects.[67] These results suggested possible common cellular mechanisms accounting for skeletal muscle wasting in myotonic dystrophy and sarcopenia.[67,72]

The effect of physical exercise on the structural features of skeletal muscle cells (both myocytes and satellite cells) has repeatedly been investigated,[75,76] but so far much less attention has been paid to the consequence of exercise on the myotendineous junction:[77,78] Molecular and ultrastructural analyses demonstrated that the myotendineous junction is able to adapt to increased tensile forces by enlarging the muscle-tendon contact area, thus increasing mechanical resistance.

Exercise training was found to have anti-inflammatory effects and promote atherosclerotic plaque stabilization in a mouse model of diabetic atherosclerosis,[79] while continuous

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cyclic mechanical tension increases the expression of the *ank* gene (codifying for a transporter of inorganic pyrophosphate from cells) in endplate chondrocytes through specific molecular pathways.[80] This confirms that changes in *ank* expression may influence calcification in the intervertebral disc.

The importance of histochemistry for investigating the structural features and function of hard tissues is confirmed by several papers, where immunocytochemistry and RT-PCR or electron microscopy were often performed in an integrated approach.[81-85] The dentin extracellular matrix proteins, DMP1 and DSP (which are produced by odontoblasts involved in dentin mineralization), have been studied in human sound *versus* sclerotic dentin: these proteins were more abundant in carious teeth, suggesting that odontoblasts are actively engaged in the biomineralization of dentin.[84] Human dental pulp cells were isolated and cultured *in vitro*, where reparative dentinogenesis was simulated by different odontogenic inductors:[85] odontoblast differentiation was monitored by histochemical staining procedures and electron microscopy in parallel with biochemical and biomolecular techniques. It was demonstrated that odontoblast markers (such as DMP1, DSP and type I collagen) were expressed in differentiated cells, and that DSP and DMP1 were differently located during odontoblast differentiation. The combined qualitative and quantitative assays used confirmed that this *in vitro* model can usefully elucidate the dynamic processes occurring *in vivo* during tooth repair.

It is worth noting that there was increasing interest for the application of histochemical techniques in regenerative and reparative medicine. An unexpected heterogeneity of adipocyte populations with different potential as sources for stem cells were described in the human trochanteric fat pad,[86] and an interesting article was published on the quantitative assessment of lipid droplet accumulation in cultured adipogenic cells, as a parameter of adipocyte maturation.[87] Either 3D scaffold-free fusion cultures[88] *in vitro* or scaffoldings (either made on natural biomaterials or on synthetic polymers) were used *in vitro* and *in vivo*[89-91] to enhance chondrogenesis. There, immunohistochemistry was instrumental in monitoring proliferation and differentiation of chondrogenic cells through the changes in the expression of specific proteins.

The potential of immuno-histochemistry for elucidating nerve cell differentiation during development was apparent in the review article by Oboti *et al.* on the rodent olfactory system[92] as well as in other papers concerning the nervous system in mammalian species[53,93-100] and in invertebrates.[101,102]

Due to the heterogeneity of the animal models, tissues and experimental conditions, analytical methods need to be continuously adjusted and refined, as demonstrated by the number of technical notes which have recently been published in the *European Journal of Histochemistry*.

An appropriate fixation is the essential precondition for preserving an adequate morphology and performing a correct histochemical analysis: comparing the effects of different procedures on particular cells is therefore important,[103] and fixatives alternative to the traditional ones may be proposed to make the application of diagnostic techniques easier.[104]

The optimization of enzyme-histochemical techniques[105] or a triple immunoenzymatic method employing primary antibodies from same species and same immunoglobulin subclass[106] have been suggested, while new methods have been proposed for the quantitative evaluation of fluorescence signals[107,108] and for imaging dynamic processes *in vitro* or *in vivo*.[109,110]

Diaminobenzidine photo-oxydation has successfully been applied for tracking the intracellular location of fluorescently labeled nanoparticles at transmission electron microscopy;[111] photo-oxidation procedures proved to be suitable also for the simultaneous ultrastructural analysis in the same sample, and for gold immunolabeling in cultured cells,[112] thus allowing to co-localise the photoconversion product and a variety of antigens at the high resolution of electron microscopy.

## Concluding remarks

With the aim of understanding the mechanisms responsible for major biological processes such as differentiation or growth control in normal and tumor tissues, basic research on cell functional organization is fundamental.

Progress in microscopy techniques and the existing wide variety of refined histochemical methods provide unique opportunities for approaching biological processes in living cells by a true molecular biology *in situ*.

This confirms that histochemical Journals maintain their pivotal role in the field of cell and tissue biology in all its structural and functional aspects, and that they should become the means to present original findings or proposing methodological and technological improvements, as well as to openly discuss founding ideas and theories even more than in the past.

## References

1. Lavis LD. Histochemistry: live and in color. J Histochem Cytochem 2011;59:139-45.
2. Pellicciari C, Malatesta M. Identifying pathological biomarkers: histochemistry still ranks high in the omics era. Eur J Histochem 2011;55:e42.
3. Taatjes DJ, Roth J. The Histochemistry and Cell Biology compendium: a review of 2012. Histochem Cell Biol 2013;139:815-46.
4. Pellicciari C. On the future contents of a small journal of histochemistry. Eur J Histochem 2012;56:e51.
5. Wick MR. Histochemistry as a tool in morphological analysis: a historical review. Ann Diagn Pathol 2012;16:71-8.
6. Jahn KA, Barton DA, Kobayashi K, Ratinac KR, Overall RL, Braet F. Correlative microscopy: providing new understanding in the biomedical and plant sciences. Micron 2012;43:565-82.
7. Ishikawa-Ankerhold HC, Ankerhold R, Drummen GP. Advanced fluorescence microscopy techniques--FRAP, FLIP, FLAP, FRET and FLIM. Molecules 2012;17:4047-132.
8. Lidke DS, Lidke KA. Advances in high-resolution imaging--techniques for three-dimensional imaging of cellular structures. J Cell Sci 2012;125(Pt 11):2571-80.
9. Heintzmann R, Ficz G. Breaking the Resolution Limit in Light Microscopy. Methods in Cell Biology 2013;114:525-44.
10. Cenni V, Capanni C, Columbaro M, Ortolani M, D'Apice MR, Novelli G, et al. Autophagic degradation of farnesylated prelamin A as a therapeutic approach to lamin-linked progeria. Eur J Histochem 2011;55:e36.
11. Markelic M, Velickovic K, Golic I, Otasevic V, Stancic A, Jankovic A, et al. Endothelial cell apoptosis in brown adipose tissue of rats induced by hyperinsulinaemia: the possible role of TNF-α. Eur J Histochem 2011;55:e34.
12. Lattanzi G, Marmiroli S, Facchini A, Maraldi NM. Nuclear damages and oxidative stress: new perspectives for laminopathies. Eur J Histochem 2012;56:e45.
13. Tsirakis G, Pappa CA, Kaparou M, Katsomitrou V, Hatzivasili A, Alegakis T, et al. Assessment of proliferating cell nuclear antigen and its relationship with proinflammatory cytokines and parameters of disease activity in multiple myeloma patients. Eur J Histochem 2011;55:e21.
14. Shen Z, Ren Y, Ye D, Guo J, Kang C, Ding H. Significance and relationship between DJ-1 gene and surviving gene expression in laryngeal carcinoma. Eur J Histochem 2011;55:e9.

15. Santoro A, Pannone G, Errico ME, Bifano D, Lastilla G, Bufo P, et al. Role of β-catenin expression in paediatric mesenchymal lesions: a tissue microarray-based immunohistochemical study. Eur J Histochem 2012;56:e25.
16. Asara Y, Marchal JA, Bandiera P, Mazzarello V, Delogu LG, Sotgiu MA, et al. Cadmium influences the 5-Fluorouracil cytotoxic effects on breast cancer cells. Eur J Histochem 2012;56:e1.
17. Rappa F, Unti E, Baiamonte P, Cappello F, Scibetta N.Different immunohistochemical levels of Hsp60 and Hsp70 in a subset of brain tumors and putative role of Hsp60 in neuroepithelial tumorigenesis. Eur J Histochem 2013;57:e20.
18. Huang S, Guo S, Guo F, Yang Q, Xiao X, Murata M, et al. CD44v6 expression in human skin keratinocytes as a possible mechanism for carcinogenesis associated with chronic arsenic exposure. Eur J Histochem 2013;57:e1.
19. Xu XC, Abuduhadeer X, Zhang WB, Li T, Gao H, Wang YH. Knockdown of RAGE inhibits growth and invasion of gastric cancer cells. Eur J Histochem 2013;57: e36.
20. Zhang Y, Tang YJ, Li ZH, Pan F, Huang K, Xu GH. KiSS1 inhibits growth and invasion of osteosarcoma cells through inhibition of the MAPK pathway. Eur J Histochem 2013;57:e30.
21. Donizy P, Halon A, Surowiak P, Pietrzyk G, Kozyra C, Matkowski R. Correlation between PARP-1 immunoreactivity and cytomorphological features of parthanatos, a specific cellular death in breast cancer cells. Eur J Histochem 2013;57:e35.
22. Bujas T, Marusic Z, Peric Balja M, Mijic A, Kruslin B, Tomas D. MAGE-A3/4 and NY-ESO-1 antigens expression in metastatic esophageal squamous cell carcinoma. Eur J Histochem 2011;55:e7.
23. Pilloni L, Bianco P, Difelice E, Cabras S, Castellanos ME, Atzori L, et al. The usefulness of c-Kit in the immunohistochemical assessment of melanocytic lesions. Eur J Histochem 2011;55:e20.
24. Gurzu S, Krause M, Ember I, Azamfirei L, Gobel G, Feher K, et al. Mena, a new available marker in tumors of salivary glands? Eur J Histochem 2012;56:e8.
25. Galia A, Calogero AE, Condorelli R, Fraggetta F, La Corte A, Ridolfo F, et al. PARP-1 protein expression in glioblastoma multiforme. Eur J Histochem 2012;56:e9.
26. Zhang Y, Ye WY, Wang JQ, Wang SJ, Ji P, Zhou GY, et al. dCTP pyrophosphohydrase exhibits nucleic accumulation in multiple carcinomas. Eur J Histochem 2013;57: e29.
27. Soddu S, Di Felice E, Cabras S, Castellanos ME, Atzori L, Faa G, et al. IMP-3 expression in keratoacanthomas and squamous cell carcinomas of the skin: an immunohistochemical study. Eur J Histochem. 2013;57(1):e6.
28. Ning J, Zhang J, Liu W, Lang Y, Xue Y, Xu S. Overexpression of ubiquitin-specific protease 22 predicts poor survival in patients with early-stage non-small cell lung cancer. Eur J Histochem. 2012;56 (4):e46.
29. Piras F, Ionta MT, Lai S, Perra MT, Atzori F, Minerba L, et al. Nestin expression associates with poor prognosis and triple negative phenotype in locally advanced (T4) breast cancer. Eur J Histochem 2011;55:e39.
30. Protrka Z, Arsenijevic S, Dimitrijevic A, Mitrovic S, Stankovic V, Milosavljevic M, et al. Co-overexpression of bcl-2 and c-myc in uterine cervix carcinomas and premalignant lesions. Eur J Histochem 2011;55:e8.
31. Costanzo M, Cisterna B, Zharskaya OO, Zatsepina OV, Biggiogera M. Discrete foci containing RNase A are found in nucleoli of HeLa cells after aging in culture. Eur J Histochem 2011;55:e15.
32. Nemolato S, Van Eyken P, Cabras T, Cau F, Fanari MU, Locci A, et al. Expression pattern of thymosin beta 4 in the adult human liver. Eur J Histochem 2011;55: e25.
33. Bottone MG, Santin G, Soldani C, Veneroni P, Scovassi AI, Alpini C. Intracellular distribution of Tankyrases as detected by multicolor immunofluorescence techniques. Eur J Histochem 2012; 56:e4.
34. Stepinski D. Immunofluorescent localization of ubiquitin and proteasomes in nucleolar vacuoles of soybean root meristematic cells. Eur J Histochem 2012; 56:e13.
35. Kontro H, Hulmi JJ, Rahkila P, Kainulainen H. Cellular and tissue expression of DAPIT, a phylogenetically conserved peptide. Eur J Histochem 2012; 56:e18.
36. Wang L, Wang J, Zhou X, Li J, Shi Y, Han Z, et al. CM2 antigen, a potential novel molecule participating in glucuronide transport on rat hepatocyte canalicular membrane. Eur J Histochem 2012;56:e26.
37. Nemolato S, Ekstrom J, Cabras T, Gerosa C, Fanni D, Di Felice E, Locci A, et al. Immunoreactivity for thymosin beta 4 and thymosin beta 10 in the adult rat oro-gastro-intestinal tract. Eur J Histochem 2013;57:e17.
38. Bertone V, Tarantola E, Ferrigno A, Gringeri E, Barni S, Vairetti M, et al. Altered alkaline phosphatase activity in obese Zucker rats liver respect to lean Zucker and Wistar rats discussed in terms of all putative roles ascribed to the enzyme. Eur J Histochem 2011;55:e5.
39. Song R, Xu W, Chen Y, Li Z, Zeng Y, Fu Y. The expression of Sirtuins 1 and 4 in peripheral blood leukocytes from patients with type 2 diabetes. Eur J Histochem 2011;55:e10.
40. Cui C, Lal P, Master S, Ma Y, Baradet T, Bing Z. Expression of parafibromin in major renal cell tumors. Eur J Histochem 2012;56:e39.
41. Tarantola E, Bertone V, Milanesi G, Capelli E, Ferrigno A, Neri D, et al. Dipeptidylpeptidase-IV, a key enzyme for the degradation of incretins and neuropeptides: activity and expression in the liver of lean and obese rats. Eur J Histochem 2012;56:e41.
42. Natale G, Pompili E, Biagioni F, Paparelli S, Lenzi P, Fornai F. Histochemical approaches to assess cell-to-cell transmission of misfolded proteins in neurodegenerative diseases. Eur J Histochem 2013; 57:e5.
43. Ferreira SA, Vasconcelos JL, Silva RC, Cavalcanti CL, Bezerra CL, Rego MJ, et al. Expression patterns of α2,3-sialyltransferase I and α2,6-sialyltransferase I in human cutaneous epithelial lesions. Eur J Histochem 2013;57:e7.
44. Loreto C, Leonardi R, Musumeci G, Pannone G, Castorina S. An ex vivo study on immunohistochemical localization of MMP-7 and MMP-9 in temporomandibular joint discs with internal derangement. Eur J Histochem 2013;57:e12.
45. Salemi M, Galia A, Fraggetta F, La Corte C, Pepe P, La Vignera S, et al. Poly (ADP-ribose) polymerase 1 protein expression in normal and neoplastic prostatic tissue. Eur J Histochem 2013;57:e13.
46. Rus A, Del Moral ML, Molina F, Peinado MA. Upregulation of cardiac NO/NOS system during short-term hypoxia and the subsequent reoxygenation period. Eur J Histochem 2011;55:e17.
47. Bolekova A, Spakovska T, Kluchova D, Toth S, Vesela J. NADPH-diaphorase expression in the rat jejunum after intestinal ischemia/reperfusion. Eur J Histochem 2011;55:e23.
48. Tomasello G, Sciumé C, Rappa F, Rodolico V, Zerilli M, Martorana A, et al. Hsp10, Hsp70, and Hsp90 immunohistochemical levels change in ulcerative colitis after therapy. Eur J Histochem 2011;55:e38.
49. Dorko F, Spakovská T, Lovasová K, Patlevič P, Kluchová D. NADPH-d activity in rat thymus after the application of retinoid acid. Eur J Histochem 2012;56:e7.

50. Chiusa M, Timolati F, Perriard JC, Suter TM, Zuppinger C. Sodium nitroprusside induces cell death and cytoskeleton degradation in adult rat cardiomyocytes in vitro: implications for anthracycline-induced cardiotoxicity. Eur J Histochem 2012;56:e15.
51. Latella G., Vetuschi A., Sferra R., Speca S, Gaudio E. αvβ6 integrin-TGF-β1/Smad3, mTOR and PPARγ in experimental colorectal fibrosis. Eur J Histochem 2013;57:e40.
52. He S, Yang J. Maturation of neurotransmission in the developing rat cochlea: immunohistochemical evidence from differential expression of synaptophysin and synaptobrevin 2. Eur J Histochem 2011;55:e2.
53. Carrillo LM, Arciniegas E, Rojas H, Ramírez R. Immunolocalization of endocan during the endothelial-mesenchymal transition process. Eur J Histochem 2011;55:e13.
54. de Moraes LO, Lodi FR, Gomes TS, Marques SR, Oshima CT, Lancellotti CL, et al. Immunohistochemical expression of types I and III collagen antibodies in the temporomandibular joint disc of human foetuses. Eur J Histochem 2011;55:e24.
55. Luciano AM, Corbani D, Lodde V, Tessaro I, Franciosi F, Peluso JJ, et al. Expression of progesterone receptor membrane component-1 in bovine reproductive system during estrous cycle. Eur J Histochem 2011;55:e27.
56. Escobar Sanchez ML, Echeverria Martínez OM, Vazquez-Nin GH. Immunohistochemical and ultrastructural visualization of different routes of oocyte elimination in adult rats. Eur J Histochem 2012;56:e17.
57. Shibata S, Fukuoka H, Sato R, Abe T, Suzuki Y. An in situ hybridization study of the insulin-like growth factor system in developing condylar cartilage of the fetal mouse mandible. Eur J Histochem 2012; 56:e23.
58. Bradaschia-Correa V, Barrence FA, Ferreira LB, Massa LF, Arana-Chavez VE. Effect of alendronate on endochondral ossification in mandibular condyles of growing rats. Eur J Histochem 2012;56: e24.
59. Godoy-Guzman C, San Martin S, Pereda J. Proteoglycan and collagen expression during human air conducting system development. Eur J Histochem 2012; 56:e29.
60. Kovsca Janjatovic A, Valpotic H, Kezic D, Lacković G, Gregorovic G, Sladoljev S, et al. Secretion of immunomodulating neuropeptides (VIP, SP) and nitric oxide synthase in porcine small intestine during postnatal development. Eur J Histochem 2012;56:e30.
61. Luciano AM, Lodde V, Franciosi F, Tessaro I, Corbani D, Modina S. Large-scale chromatin morpho-functional changes during mammalian oocyte growth and differentiation. Eur J Histochem 2012;56:e37.
62. Khalili MA, Maione M, Palmerini MG, Bianchi S, Macchiarelli G, Nottola SA. Ultrastructure of human mature oocytes after vitrification. Eur J Histochem 2012;56:e38.
63. Fanni D, Iacovidou N, Locci A, Gerosa C, Nemolato S, Van Eyken P, et al. MUC1 marks collecting tubules, renal vesicles, comma- and S-shaped bodies in human developing kidney tubules, renal vesicles, comma- and s-shaped bodies in human kidney. Eur J Histochem 2012;56:e40.
64. San Martin S, Fitzgerald JS, Weber M, Párraga M, Sáez T, Zorn TM, et al. Stat3 and Socs3 expression patterns during murine placenta development. Eur J Histochem 2013;57:e19.
65. Shibata S, Sakamoto Y, Baba O, Qin C, Murakami G, Cho BH. An immunohistochemical study of matrix proteins in the craniofacial cartilage in midterm human fetuses. Eur J Histochem 2013;57:e39.
66. Sabbatini AR, Mattii L, Battolla B, Polizzi E, Martini D, Ranieri-Raggi M, et al. Evidence that muscle cells do not express the histidine-rich glycoprotein associated with AMP deaminase but can internalise the plasma protein. Eur J Histochem 2011;55:e6.
67. Malatesta M, Giagnacovo M, Renna LV, Cardani R, Meola G, Pellicciari C. Cultured myoblasts from patients affected by myotonic dystrophy type 2 exhibit senescence-related features: ultrastructural evidence. Eur J Histochem 2011;55:e26.
68. Serradifalco C, Catanese P, Rizzuto L, Cappello F, Puleio R, Barresi V, et al. Embryonic and foetal Islet-1 positive cells in human hearts are also positive to c-Kit. Eur J Histochem 2011;55:e41.
69. Zara S, Macchi V, De Caro R, Rapino M, Cataldi A, Porzionato A. pPKCα mediated-HIF-1α activation related to the morphological modifications occurring in neonatal myocardial tissue in response to severe and mild hyperoxia. Eur J Histochem 2012;56(1):e2
70. Zhao Y, Huang H, Jiang Y, Wei H, Liu P, Wang W, et al. Unusual localization and translocation of TRPV4 protein in cultured ventricular myocytes of the neonatal rat. Eur J Histochem 2012;56:e32.
71. Malatesta M. Skeletal muscle features in myotonic dystrophy and sarcopenia: do similar nuclear mechanisms lead to skeletal muscle wasting? Eur J Histochem 2012;56:e36.
72. Malatesta M, Giagnacovo M, Costanzo M, Cisterna B, Cardani R, Meola G. Muscleblind-like1 undergoes ectopic relocation in the nuclei of skeletal muscles in myotonic dystrophy and sarcopenia. Eur J Histochem 2013;57:e15.
73. Malatesta M, Giagnacovo M, Cardani R, Meola G, Pellicciari C. RNA processing is altered in skeletal muscle nuclei of patients affected by myotonic dystrophy. Histochem Cell Biol 2011;135:419-25.
74. Malatesta M, Perdoni F, Muller S, Pellicciari C, Zancanaro C. Pre-mRNA processing is partially impaired in satellite cell nuclei from aged muscles. J Biomed Biotechnol 2010;2010:410405.
75. Hagen KB, Dagfinrud H, Moe RH, Osteras N, Kjeken I, Grotle M, et al. Exercise therapy for bone and muscle health: an overview of systematic reviews. BMC Med 2012;10:167.
76. Montero-Fernández N, Serra-Rexach JA. Role of exercise on sarcopenia in the elderly. Eur J Phys Rehabil Med 2013;49:131-43.
77. Curzi D, Salucci S, Marini M, Esposito F, Agnello L, Veicsteinas A, et al. How physical exercise changes rat myotendinous junctions: an ultrastructural study. Eur J Histochem 2012;56:e19.
78. Curzi D, Lattanzi D, Ciuffoli S, Burattini S, Grindeland RE, Edgerton VR, et al. Growth hormone plus resistance exercise attenuate structural changes in rat myotendinous junctions resulting from chronic unloading. Eur J Histochem 2013;57:e37.
79. Kadoglou NP, Moustardas P, Kapelouzou A, Katsimpoulas M, Giagini A, Dede E, et al. The anti-inflammatory effects of exercise training promote atherosclerotic plaque stabilization in apolipoprotein E knockout mice with diabetic atherosclerosis. Eur J Histochem 2013;57:e3.
80. Xu H, Zhang X, Wang H, Zhang Y, Shi Y, Zhang X. Continuous cyclic mechanical tension increases ank expression in endplate chondrocytes through the TGF-β1 and p38 pathway. Eur J Histochem 2013;57:e28.
81. Kiga N. Histochemistry for studying structure and function of the articular disc of the human temporomandibular joint. Eur J Histochem 2012;56:e11.
82. Kiga N, Tojyo I, Matsumoto T, Hiraishi Y, Shinohara Y, Makino S, et al. Expression of lumican and fibromodulin following interleukin-1 beta stimulation of disc cells of the human temporomandibular joint. Eur J Histochem 2011;55:e11.
83. Carvalho-Filho EP, Stabile AC, Ervolino E, Stuani MB, Iyomasa MM, Rocha MJ. Celecoxib treatment does not alter

recruitment and activation of osteoclasts in the initial phase of experimental tooth movement. Eur J Histochem 2012;56:e43.
84. Martini D, Trirè A, Breschi L, Mazzoni A, Teti G, Falconi M, et al. Dentin matrix protein 1 and dentin sialophosphoprotein in human sound and carious teeth: an immunohistochemical and colorimetric assay. Eur J Histochem 2013;57:e32.
85. Teti G, Salvatore V, Ruggeri A, Manzoli L, Gesi M, Orsini G, et al. In vitro reparative dentin: a biochemical and morphological study. Eur J Histochem 2013;57:e23.
86. Panettiere P, Accorsi D, Marchetti L, Minicozzi AM, Orsini G, Bernardi P, et al. The trochanteric fat pad. Eur J Histochem 2011;55:e16.
87. Rizzatti V, Boschi F, Pedrotti M, Zoico E, Sbarbati A, Zamboni M. Lipid droplets characterization in adipocyte differentiated 3T3-L1 cells: size and optical density distribution. Eur J Histochem 2013;57:e24.
88. Lehmann M, Martin F, Mannigel K, Kaltschmidt K, Sack U, Anderer U. Three-dimensional scaffold-free fusion culture: the way to enhance chondrogenesis of in vitro propagated human articular chondrocytes. Eur J Histochem 2013;57:e31.
89. Musumeci G, Loreto C, Carnazza ML, Coppolino F, Cardile V, Leonardi R. Lubricin is expressed in chondrocytes derived from osteoarthritic cartilage encapsulated in poly (ethylene glycol) diacrylate scaffold. Eur J Histochem 2011;55:e31.
90. Tetè S, Vinci R, Zizzari VL, Zara S, La Scala V, Cataldi A, et al. Maxillary sinus augmentation procedures through equine-derived biomaterial or calvaria autologous bone: immunohistochemical evaluation of OPG/RANKL in humans. Eur J Histochem 2013;57:e10.
91. Porzionato A, Sfriso MM, Macchi V, Rambaldo A, Lago G, Lancerotto L, et al. Decellularized omentum as novel biologic scaffold for reconstructive surgery and regenerative medicine. Eur J Histochem 2013;57:e4.
92. Oboti L, Peretto P, Marchis SD, Fasolo A. From chemical neuroanatomy to an understanding of the olfactory system. Eur J Histochem 2011;55:e35.
93. Porzionato A, Fenu G, Rucinski M, Macchi V, Montella A, Malendowicz LK, et al. KISS1 and KISS1R expression in the human and rat carotid body and superior cervical ganglion. Eur J Histochem 2011;55:e14.
94. Zhu Y, Ning D, Wang F, Liu C, Xu Y, Jia X, et al. Effect of thyroxine on munc-18 and syntaxin-1 expression in dorsal hippocampus of adult-onset hypothyroid rats. Eur J Histochem 2012;56:e22.
95. Lievajova K, Blasko J, Martoncikova M, Cigankova V, Racekova E. Delayed maturation and altered proliferation within the rat rostral migratory stream following maternal deprivation. Eur J Histochem 2011;55:e33.
96. Kawashima M, Imura K, Sato I. Topographical organization of TRPV1-immunoreactive epithelium and CGRP-immunoreactive nerve terminals in rodent tongue. Eur J Histochem 2012;56:e21.
97. Lomoio S, Necchi D, Scherini E. Uneven distribution of NG2 cells in the rat cerebellar vermis and changes in aging. Eur J Histochem 2012;56:e27.
98. Kovsca Janjatovic A, Valpotic H, Kezic D, Lacković G, Gregorovic G, Sladoljev S, et al. Secretion of immunomodulating neuropeptides (VIP, SP) and nitric oxide synthase in porcine small intestine during postnatal development. Eur J Histochem 2012;56:e30.
99. Damico JP, Ervolino E, Torres KR, Sabino Batagello D, Cruz-Rizzolo RJ, Aparecido Casatti C, et al. Phenotypic alterations of neuropeptide Y and calcitonin gene-related peptide-containing neurons innervating the rat temporomandibular joint during carrageenan-induced arthritis. Eur J Histochem 2012;56:e31.
100. Bolekova A, Kluchova D, Tomasova L, Hvizdosova N. Effect of retinoic acid on the nitrergic innervation of meibomian glands in rats. Eur J Histochem 2012;56:e50.
101. Pennati R, Dell'Anna A, Zega G, De Bernardi F. Immunohistochemical study of the nervous system of the tunicate Thalia democratica (Forsskal, 1775). Eur J Histochem 2012;56:e16.
102. Casini A, Vaccaro R, D'Este L, Sakaue Y, Bellier JP, Kimura H, Renda TG. Immunolocalization of choline acetyltransferase of common type in the central brain mass of Octopus vulgaris. Eur J Histochem 2012;56:e34.
103. Rieger J, Twardziok S, Huenigen H, Hirschberg RM, Plendl J. Porcine intestinal mast cells. Evaluation of different fixatives for histochemical staining techniques considering tissue shrinkage. Eur J Histochem 2013;57:e21.
104. Benerini Gatta L, Cadei M, Balzarini P, Castriciano S, Paroni R, Verzeletti A, et al. Application of alternative fixatives to formalin in diagnostic pathology. Eur J Histochem 2012;56:e12.
105. Galvao MJ, Santos A, Ribeiro MD, Ferreira A, Nolasco F. Optimization of the tartrate-resistant acid phosphatase detection by histochemical method. Eur J Histochem 2011;55:e1.
106. Osman TA, Oijordsbakken G, Costea DE, Johannessen AC. Successful triple immunoenzymatic method employing primary antibodies from same species and same immunoglobulin subclass. Eur J Histochem 2013;57:e22.
107. Gomes F.M., Ramos I.B., Wendt C., Girard-Dias W., De Souza W., Machado E.A., et al. New insights into the in situ microscopic visualization and quantification of inorganic polyphosphate stores by 4',6-diamidino-2-phenylindole (DAPI)-staining. Eur J Histochem 2013;57:e34.
108. Giansanti V, Santamaria G, Torriglia A, Aredia F, Scovassi AI, Bottiroli G, et al. Fluorescence properties of the $Na^+$/$H^+$exchanger inhibitor HMA (5-(N,N-hexamethylene) amiloride) are modulated by intracellular pH. Eur J Histochem 2012;56:e3.
109. Fassina L, Magenes G, Inzaghi A, Palumbo S, Allavena G, Miracco C, et al. AUTOCOUNTER, an ImageJ JavaScript to analyze LC3B-GFP expression dynamics in autophagy-induced astrocytoma cells. Eur J Histochem 2012;56:e44.
110. Boschi F, Fontanella M, Calderan L, Sbarbati A. Luminescence and fluorescence of essential oils. Fluorescence imaging in vivo of wild chamomile oil. Eur J Histochem 2011;55:e18.
111. Malatesta M, Giagnacovo M, Costanzo M, Conti B, Genta I, Dorati R, et al. Diaminobenzidine photoconversion is a suitable tool for tracking the intracellular location of fluorescently labelled nanoparticles at transmission electron microscopy. Eur J Histochem 2012;56:e20.
112. Malatesta M, Zancanaro C, Costanzo M, Cisterna B, Pellicciari C. Simultaneous ultrastructural analysis of fluorochrome-photoconverted diaminobenzidine and gold immunolabeling in cultured cells. Eur J Histochem 2013;57:e26.