Is there still room for novelty, in histochemical papers?

C. Pellicciari
Department of Biology and Biotechnology, University of Pavia, Italy

Abstract

Histochemistry continues to be widely applied in biomedical research, being nowadays mostly addressed to detect and locate single molecules or molecular complexes inside cells and tissues, and to relate structural organization and function at the high resolution of the more advanced microscopical techniques. In the attempt to see whether histochemical novelties may be found in the recent literature, the articles published in the European Journal of Histochemistry in the period 2014-2016 have been reviewed. In the majority of the published papers, standardized methods have been preferred by scientists to make their results reliably comparable with the data in the literature, but several papers (approximately one fourth of the published articles) described novel histochemical methods and procedures. It is worth noting that there is a growing interest for minimally-invasive in vivo techniques (magnetic resonance imaging, autofluorescence spectroscopy), which may parallel conventional histochemical analyses to acquire evidence not only on the morphological features of living organs and tissues, but also on their functional, biophysical and molecular characteristics. Thanks to this unceasing methodological refinement, histochemistry will continue to provide innovative applications in the biomedical field.

Introduction

The impact of Histochemistry on biological and medical research is always high,1,2 with widespread application in a large variety of topics. During the last three years, more than 65,000 articles have been published in qualified journals (according to the Web of Science and Scopus databases), and it would be interesting to know how histochemistry has been used, especially which techniques have been employed in these investigations: this would also help to answer the question “was there histochemical originality or novelty, or just instrumental application of accepted protocols?”. A survey of the whole histochemical production would have been a too difficult task; therefore, a review has been made of only the 126 articles published in the European Journal of Histochemistry from January 2014 to present. This journal traditionally accepts manuscript on functional cell and tissue biology in animals and plants, with attention to the processes of cell differentiation, development and senescence, and to the cellular basis of diseases: this should make the article sample sufficiently representative of the possible histochemical applications, though obviously limited by the small number of items considered.

For sake of simplicity, the papers have been divided into six categories, based on their main subject: Tumor biology & markers (14% of the published articles), Non-Tumors diseases (17%), Experimental medicine & Animal models (10%), Stem cells & Development (14%), Tissue Biology in Human and Animals (20%), Methods & Techniques (25%).

Tumor biology & markers, Non-Tumors diseases, Experimental Medicine & Animal Models

In most of the published papers on Tumor biology & markers,11 the expression of specific protein has been investigated (in hepatocellular carcinoma,4,11 uterine lesions,12 colorectal carcinoma,5,10 and other neoplasms11,12). The aim was either to originally define new diagnostic/prognostic markers,11,13,14,15 to identify proteins involved in tumor onset16 or progression,17 or to evaluate the effect of therapy.9 The mechanisms of carcinogenesis have also been studied,1,17-20 and it is worth noting that some new proteins have been identified as potential therapeutic targets for cancer treatment11,14,15 and tailored therapy.20,21 In most of these investigations, well-established immunohistochemical methods have been used (often in parallel with in situ hybridization, RT-PCR, Western blot assays or microarray analyses) to detect and locate single molecular species inside tissues and cells or in the extracellular matrix.

A similar situation may be found in the articles on Non-Tumors diseases.20-42 Here again, multiple immunohistochemical assays have mostly been used to identify pathogenetic factors or to relate the changes in protein expression with tissue remodeling,22-29 or the progression of the disease.23-29,34,35 Interestingly, cartilage, bone and dentin in different pathologies were especially investigated,26,32 which demonstrates the unique role of histochemistry in studying these tissues under normal or pathological conditions.16

The effect of experimental treatments or disease on the fibrous joint tissue31,34 and cartilage45,46 were also investigated in papers falling into the category Experimental Medicine & Animal Models.43-54 In this article group, conventional histochemical techniques, histological examination and morphometry at light and electron microscopy were often used together with immunohistochemistry, to describe the structural organization of brain6 or the skeletal muscle47 in mice strains, and to investigate in rats the effects of a maternal dietary load of alpha-tocopherol on the synapse density and glial synaptic coverage in the hippocampus of adult offspring.46 Enzyme histochemistry and immunolabeling were simultaneously applied to describe the structural and functional organization of fatty livers submitted to a preservation procedure especially suitable for organ transplantation.49

Cultured cell systems have also been used to analyze the effect of ozone at low concentration on cytoskeletal organization, mitochondrial activity and nuclear transcription,52 or to elucidate the molecular pathway responsible for the increased collagen synthesis by fibroblasts in vitro after exposure to the natural flavonoid, apigenin.53 In these articles, the use of multiple molecular and histochemical techniques at light and electron microscopy was effective to detect changes in gene expression and in the structural organization and function of subcellular organelles.

Stem cells & Development, Tissue Biology in Human and Animals

In the papers on Stem cells & Development55-72 the expression of specific proteins was investi-
Methods & Techniques

In the great majority of the papers of all the above mentioned categories, techniques have been used to locate single proteins or nucleic acid sequences in situ (single or multiple immunohistochemistry, in situ hybridization). In these investigations, the scientists obviously preferred to use standardized methods to make their results reliably comparable with the data in the literature. As a consequence, originality resides in the investigated subjects, whereas histochemical novelty may hardly be found and, when seldom present, it is limited to the appropriate protocol adjustment to cope with special sample characteristics.

On the contrary, the papers in the section Methods & Techniques111-120 are histochemically novel either in the proposed procedure and in its application to unusual model systems. The key role of the fixation/embedding procedures for appropriate specimen preparation was demonstrated in some studies,96-104 and particular attention was paid to technical improvements in antigen preservation and retrieval for immunohistochemical applications,105-110 and in multiple histochemical staining.111-113 Imaging techniques were the subject of some articles.114-116 Raman microspectroscopy was used to investigate stenotic aortic valve leaflets to get information on the composition and distribution of accumulated lipids, in the attempt to correlate their presence with mineralization as a precocious diagnostic marker.114 Magnetic resonance imaging (MRI) was used to perform a longitudinal study in vivo on the spine changes occurring in an experimental rat model of ankylosing spondylitis, and the obtained results were validated at the end of the experiments by micro-computerized tomography and histological examination: this non-invasive approach may potentially be applied to follow the progress of this disease and plan therapeutic interventions in humans.115 Advanced MRI techniques, such as MRI microscopy, Magnetic Resonance Spectroscopy, functional MRI, and Diffusion Tensor Imaging may be envisaged as suitable minimally-invasive techniques to investigate in vivo not only the morphological features of living organs and tissues, but also their functional, biophysical and molecular characteristics.116 Among the non-invasive techniques, autofluorescence imaging promise to be particularly suitable and informative for diagnostic applications.117-120 Applications of electron microscopy analyses were used for tridimensional reconstruction of apoptotic nuclei121 and for detecting metal contaminants.122-124 Diaminobenzidine photo-oxidation by fluorescent probes was used for detecting at electron microscopy calcium ions,125 for visualizing endocytotic pathways126 and for tracking nanoparticles inside the cells.127,128 These last articles demonstrate that new varied applications in nanomedicine may be envisaged for ultrastructural histochemistry.129

Concluding remarks

This short survey plainly confirms that histochemical research is presently addressed to identify single molecules,129 and to dynamically describe their intracellular location and movements; in fact, nowadays papers are rarely found where conventional histochemical techniques are used to label or quantify large macromolecular categories, such as whole proteins or nucleic acids in single cells or tissues. Even in the small sample considered, approximately one fourth of the articles describe new or improved histochemical methods or the adaptation to electron microscopy of techniques originally designed for light microscopy; this is consistent with the recent reports on the papers published in another classical Journal of histochemistry.130 Thanks to this unceasing methodological refinement, histochemistry will continue to provide novel applications in the biomedical field.

In the last decade, new technologies have been developed which made it possible to break or bypass the classical Abbe’s diffraction limit.131,132 They are collectively known as super-resolution microscopy, and pushed the optical resolution down to the molecular level.133 This has opened unexpected opportunities for the application of histochemistry at the nanoscale: using highly specific fluorescent labeling by immunocytochemistry, or in situ hybridization, or fluorescent protein probes, the spatial distribution and dynamics of molecules (genome sequences, RNAs or proteins) can be investigated in every subcellular organelle or structure, in living or fixed samples134 with a resolution comparable to electron microscopy and the unique flexibility of multicolor fluorescence microscopy.

So we may surely answer the title question: “yes: there still room for novelty in histochemical papers!”.

References

1. Taatjes DJ, Roth J. The Histochemistry and Cell Biology omnium-gatherum: the year 2015 in review. Histochem Cell Biol 2016;145:239-74.
2. Pelliccari C. Impact of Histochemistry on biomedical research: looking through the articles published in a long-established histochemical journal. Eur J Histochem 2014;58:2474.
3. Pelliccari C. Histochemistry in biology and medicine: a message from the citing journals. Eur J Histochem 2015;59:2510.
4. Guerriero E, Accardo M, Capone F, Colonna G, Castello G, Costantini S. Assessment of the Selenoprotein M (SELM) over-expression on human hepatocellular carcinoma tissues by immunohistochemistry. Eur J Histochem 2014;58:2433.
5. Theunissen W, Fanni D, Nemolato S, Di Felice E, Cabras T, Gerosa C, et al. Thymosin beta 4 and thymosin beta 10 expression in hepatocellular carcinoma. Eur J Histochem 2014;58:2242.
6. Guerriero E, Capone F, Accardo M, Sorice A, Costantini M, Colonna G, et al. GPX4 and GPX7 over-expression in human hepatocellular carcinoma tissues. Eur J Histochem 2015;59:2540.
7. Lindstrom AK, Hellberg D. Immunohistochemical LRIG3 expression in cervical intraepithelial neoplasia and invasive squamous cell cervical cancer: association with expression of tumor markers, hormones, high-risk HPV-infection, smoking and patient outcome. Eur J Histochem 2014;58:2227.
8. Clark ATR, Guimaraes da Costa VML, Bandeira Costa L, Bezerra Cavalcanti CL, de Melo Rêgo MJB, Beltrão EIC. Differential expression patterns of N-acetylglucosaminyl transferases and polylactosamines in uterine lesions. Eur J Histochem 2014;58:2334.
9. Buldak R J, Skonieczna M, Buldak L, Matsiak N, Mielańczyk Ł, Wyróbiec G, et al. Changes in subcellular localiza-

[page 286] [European Journal of Histochemistry 2016; 60:2758]
tion of visfatin in human colorectal HCT-116 carcinoma cell line after cytochalasin B treatment. Eur J Histochem 2014;58:2408.

10. Demirovic A, Cesarec S, Marusic Z, Tomas D, Miloševic M, Hudolin T, et al. TGF-bet al expression in chromophobe renal cell carcinoma and renal oncocytoma. Eur J Histochem 2014;58:2265.

11. Araujo DGB, Nakao L, Gozzo P, Souza CDA, Balderrama V, Gugelmin ES, et al. Expression level of quiescin sulfhydryl oxidase 1 (QSOX1) in neuroblastomas. Eur J Histochem 2014;58:2228.

12. Chene G, Radosovev-Robin N, Tardieu AS, Cayre A, Raeciflis I, Dechelotte P, et al. Morphological and immunohistochemical study of ovarian and tubal dysplasia associated with tamoxifen. Eur J Histochem 2014;58:2251.

13. Zhang J, Luo J, Ni J, Tang L, Zhang HP, Zhang L, et al. MMP-7 is upregulated by COX-2 and promotes proliferation and invasion of lung adenocarcinoma cells. Eur J Histochem 2014;58:2362.

14. Ou J M, Yu ZY, Qiu MK, Dai YX, Dong Q, Shen J, et al. Knockdown of VEGFR2 inhibits proliferation and induces apoptosis in hemangio-derived endothelial cells. Eur J Histochem 2014;58:2463.

15. Fantinato E, Milani L, Sironi G. Sox9 expression in canine epithelial skin tumors. Eur J Histochem 2015;59:2514.

16. Costa VF, Tijoe KC, Nonogaki S, Soares FA, Laurus JR, Oliveira DT. Are podoplanin and ezrin involved in the invasion process of the ameloblastoma? Eur J Histochem 2015;59:2451.

17. Arcucci A, Ruocco MR, Albano F, Granato G, Romano V, Corso G, et al. Analysis of extracellular superoxide dismutase and Akt in ascending aortic aneurysm with tricuspid or bicuspid aortic valve. Eur J Histochem 2014;58:2383.

18. Tonini K, Kasacka I. Quantitative evaluation of CART-containing cells in urinary bladder of rats with renovascular hypertension. Eur J Histochem 2015;59:2446.

19. Sun Y, Zhu L, Huang X, Zhou C, Zhang X. Immunohistochemical localization of nerve fibers in the pseudocapsule of fibroids. Eur J Histochem 2014;58:2249.

20. Hu SS, Mei L, Chen JY, Huang WZ, Wu H. Expression of immediate-early genes in the inferior colliculus and auditory cortex in salicylate-induced tinnitus in rat. Eur J Histochem 2014;58:2249.

21. Caravana VN, Gakipoulou H, Lianos EA. Expression of Ser729 phosphorylated PKC ε in experimental crescentic glomerulonephritis: an immunohistochemical study. Eur J Histochem 2014;58:2426.

22. Demurtas P, Corrias M, Zucca I, Maxia C, Piras F, Sirigu P, et al. Angiotensin II: immunohistochemical study in Sardinian pterygium. Eur J Histochem 2014;58:2426.

23. Janiuk I, Kasacka I. Quantitative evaluation of CART-containing cells in urinary bladder of rats with renovascular hypertension. Eur J Histochem 2015;59:2446.

24. Di Vito A, Scali E, Ferraro G, Mignogna C, Presta I, Camastra C, et al. Elastofibroma dorsi: a histological and immunohistochemical study. Eur J Histochem 2015;59:2459.

25. Goteri G, Altobelli E, Tossetta G, Zizzi A, Avellini C, Licini C, et al. High temperature requirement A1, transforming growth factor beta1, phosphoSmad2 and Ki67 in eutopic and ectopic endometrium and Ki67 in utopic and ectopic endometrium of women with endometriosis. Eur J Histochem 2015;59:2570.

26. Tossetta G, Avellini C, Licini C, Giannubilo SR, Castellucci M, Marzioni D. High temperature requirement AI and fibronectin: two possible players in placental tissue remodelling. Eur J Histochem 2016;60:2724.

27. Snibson D, Mashrah M, Yao Z, Huang J. Aberrant DRK3 expression in the oral leukoplaikia and oral submucous fibrosis: a comparative immunohistochemical study. Eur J Histochem 2016;60:2606.

28. Al-dhohorah T, Mashrah M, Yao Z, Huang J. Aberrant DRK3 expression in the oral leukoplaikia and oral submucous fibrosis: a comparative immunohistochemical study. Eur J Histochem 2016;60:2606.

29. Al-dhohorah T, Mashrah M, Yao Z, Huang J. Aberrant DRK3 expression in the oral leukoplaikia and oral submucous fibrosis: a comparative immunohistochemical study. Eur J Histochem 2016;60:2606.

30. Al-dhohorah T, Mashrah M, Yao Z, Huang J. Aberrant DRK3 expression in the oral leukoplaikia and oral submucous fibrosis: a comparative immunohistochemical study. Eur J Histochem 2016;60:2606.

31. Al-dhohorah T, Mashrah M, Yao Z, Huang J. Aberrant DRK3 expression in the oral leukoplaikia and oral submucous fibrosis: a comparative immunohistochemical study. Eur J Histochem 2016;60:2606.

32. Al-dhohorah T, Mashrah M, Yao Z, Huang J. Aberrant DRK3 expression in the oral leukoplaikia and oral submucous fibrosis: a comparative immunohistochemical study. Eur J Histochem 2016;60:2606.
53. Costanzo M, Szychlinska M A, Tibullo D, Malaguarnara I, Musumeci G. Expression of CHISL1 and CHIT1 in osteoarthritic rat cartilage model. A morphological study. Eur J Histochem 2014;58:2423.

54. Xu HG, Zhang W, Zheng Q, Yu YE, Deng LF, Wang H, et al. Investigating conversion of endplate chondrocytes induced by intermittent cyclic mechanical unconfined compression in three-dimensional cultures. Eur J Histochem 2014;58:2415.

55. Insolia V, Piccolini VM. Brain morphological defects in prolidase deficient mice: first report. Eur J Histochem 2014;58:2417.

56. Ferretti V, Segal-Eiras Á, Barbeito CG, Croce MV. MucSac mucin expression during rat skin development. Eur J Histochem 2015;59:2499.

57. Kato T, Oka K, Nakamura T, Ito A. Decreased expression of Met during differentiation in rat lung. Eur J Histochem 2016;60:2575.

58. Cau F, Pisur E, Gerosa C, Senes G, Ronchi F, Botta C, et al. Interindividual variability in the expression of surfactant protein A and B in the human lung during development. Eur J Histochem 2016;60:2678.

59. Song JH, Lee MY, Kim YJ, Park SR, Kim J, Ryu SY, et al. Developmental immunolocalization of the Klotho protein in mouse kidney epithelial cells. Eur J Histochem 2014;58:2256.

60. Sandhu MA, Saeed AA, Khilji MS, Pasha RH, Muktah N, Anjum MS, et al. Ontogenic development of corticotrophs in fetal buffalo (Bubalus bubalis) pituitary gland. Eur J Histochem 2014;58:2292.

61. Karaca T, Huya Uz Y, Karabacak R, Karakoç I, Demirtas S, Cagatay Cicci A. Effects of hyperthyroidism on expression of vascular endothelial growth factor (VEGF) and apoptosis in fetal adrenal gland. Eur J Histochem 2015;59:2560.

62. Tsukamoto I, Akagi M, Inoue S, Yamagishi K, Mori S, Asada S. Expressions of local renin-angiotensin system components in chondrocytes. Eur J Histochem 2014;58:2387.

63. Fujikawa K, Yokohama-Tamaki T, Morita T, Kato T, Ishii K, Yamada M, et al. Hybridization study of perlecan, DMP1, and COL2A1 mRNAs in the developing rat meniscus: a morphological study. Eur J Histochem 2015;59:2553.

64. Carvalho de Moraes LO, Tedesco RC, Arraes-Aybar IA, Klein O, Merida-Velasco JR, Alonso LG. Development of synovial membrane in the temporomandibular joint of the human fetus. Eur J Histochem 2015;59:2569.

65. Zhao H, Liu P, Wang S, Liu C, Jani P, Lu Y, et al. Trangenic expression of dentin phosphophostatin inhibits skeletal development. Eur J Histochem 2016;60:2578.

66. Liu WJ, Yang J. Preferentially regulated expression of connexin 43 in the developing spiral ganglion neurons and afferent terminals in post-natal rat cochlea. Eur J Histochem 2015;59:2464.

67. Liu WJ, Yang J. Developmental expression of insulin 1, 4, 5-triphosphate receptor in the post-natal rat cochlea. Eur J Histochem 2015;59:2486.

68. Hansson J, Ericsson AE, Axelson H, Johansson ME. Species diversity regarding the presence of proximal tubular progenitor cells of the kidney. Eur J Histochem 2016;60:2587.

69. Vinci L, Ravarino A, Fanos V, Naccarato AG, Senes G, Gerosa C, et al. Immunohistochemical markers of neural progenitor cells in the early embryonic human cerebral cortex. Eur J Histochem 2016;60:2583.

70. Pibiri V, Ravarino A, Gerosa C, Pintus MC, Fanos V, Faa G. Stem/progenitor cells in the developing human cerebellum: an immunohistochemical study. Eur J Histochem 2016;60:2686.

71. Melrose J. The knee joint loose body as a source of viable autologous human chondrocytes. Eur J Histochem 2016;60:2645.

72. Miko M, Danisovic V, Majdi A, Varga I. Ultrastructural analysis of different human mesenchymal stem cells after in vitro expansion: a technical review. Eur J Histochem 2015;59:2528.

73. Bao L, Li Q, Liu Y, Li B, Sheng X, Han Y, et al. Immunolocalization of NGF and its receptors in ovarian surface epithelium of the wild ground squirrel during the breeding and nonbreeding seasons. Eur J Histochem 2014;58:2363.

74. Zhang H, Wang Y, Zhang J, Wang L, Li Q, Sheng X, et al. Testicular expression of NGF, TrkA and p75 during seasonal spermatogenesis of the wild ground squirrel (Citellus dauricus Brandt). Eur J Histochem 2015;59:2522.

75. Li Q, Zhang F, Zhang S, Sheng X, Han X, Weng Q, et al. Seasonal expression of androgen receptor, aromatase, and estrogen receptor alpha and beta in the testis of the wild ground squirrel (Citellus dauricus Brandt). Eur J Histochem 2015;59:2456.

76. Zhang F, Wang J, Jiao Y, Zhang L, Zhang H, Sheng X, et al. Seasonal changes of androgen receptor, estrogen receptors and aromatase expression in the mediopelvic area of the male ground squirrels (Citellus dauricus Brandt). Eur J Histochem 2015;59:2456.

77. Golic I, Velickovic K, Markelic M, Stancic DO, Vargas-Barroso V, Narvaez M, Di Palma M, Agnati LF, et al. Dopamine D1 and D2 receptor immunoreactivities in the arcuate-median eminence complex and their link to the tubero-infundibular dopamine neurons. Eur J Histochem 2014;58:2400.
brown frog during breeding period and pre-hibernation. Eur J Histochem 2014;58:2422.

91. Akat E, Arikant H, Gocmen B. Histochromic and biometric study of the gastrointestinal system of Hyla orientalis (Bediaga 1890) (Anura Hylidae). Eur J Histochem 2014;58:2452.

92. Seliverstova EV, Prutskova NP. Receptor-mediated endocytosis of lysozyme in renal proximal tubules of the frog Rana temporaria. Eur J Histochem 2015;59:2482.

93. Zhang H, Xu O, Zhong S, Ge T, Peng S, Yu P, et al. Heterogeneous vesicles in mucous epithelial cells of posterior esophagus of Chinese giant salamander (Andrias davidianus) . Eur J Histochem 2015;59:2521.

94. Ubeda-Manzano M, Ortiz-Delgado JB, Sarasquete C. The Bromodomain testis-specific gene (Brdt) characterization and expression in gillhead seabream, Sparus aurata, and European seabass, Dicentrarchus labrax. Eur J Histochem 2016;60:2638.

95. Zhang H, Yu P, Zhong S, Ge T, Peng S, Zhou Z, et al. Glycote and synapse analyses in cerebral ganglia of the Chinese mitten crab, Eriocheir sinensis: ultrastructural study. Eur J Histochem 2016;60:2655.

96. Marzo S, Galimberti V, Biggiogera M. Unexpected distribution of KRIT1 inside the nucleus: new insight in a complex molecular pathway. Eur J Histochem 2014;58:2358.

97. Aredia F, Malatesta M, Veneroni P, Bottone MG. Analysis of ERK3 intracellular localization: dynamic distribution during mitosis and apoptosis. Eur J Histochem 2015;59:2571.

98. Ayarza E, Gonzalez M, Lopez F, Fernandez-Donoso R, Page J, Berrios S. Alterations in chromosomal synapses and DNA repair in apoptotic spermatocytes of Mus m. domesticus. Eur J Histochem 2014;58:2557.

99. Ami D, Di Segni  M, Forcella  M, Bernardi P, Favaloro A, Santoro G, et al. Specific association of growth-associated protein 43 with calcium release units in skeletal muscles of lower vertebrates. Eur J Histochem 2014;58:2453.

100. Liu Y, Weng J, Huang S, Shen Y, Sheng X, Han X, Yao Y, et al. Immunoreactivities of PPARgamma2 leptin and leptin receptor in oviduct of Chinese brown frog during breeding period and pre-hibernation. Eur J Histochem 2014;58:2422.

101. Akat E, Arikant H, Gocmen B. Histochromic and biometric study of the gastrointestinal system of Hyla orientalis (Bediaga 1890) (Anura Hylidae). Eur J Histochem 2014;58:2452.

102. Seliverstova EV, Prutskova NP. Receptor-mediated endocytosis of lysozyme in renal proximal tubules of the frog Rana temporaria. Eur J Histochem 2015;59:2482.

103. Zhang H, Xu O, Zhong S, Ge T, Peng S, Yu P, et al. Heterogeneous vesicles in mucous epithelial cells of posterior esophagus of Chinese giant salamander (Andrias davidianus) . Eur J Histochem 2015;59:2521.

104. Ubeda-Manzano M, Ortiz-Delgado JB, Sarasquete C. The Bromodomain testis-specific gene (Brdt) characterization and expression in gillhead seabream, Sparus aurata, and European seabass, Dicentrarchus labrax. Eur J Histochem 2016;60:2638.

105. Zhang H, Yu P, Zhong S, Ge T, Peng S, Zhou Z, et al. Glycote and synapse analyses in cerebral ganglia of the Chinese mitten crab, Eriocheir sinensis: ultrastructural study. Eur J Histochem 2016;60:2655.

106. Marzo S, Galimberti V, Biggiogera M. Unexpected distribution of KRIT1 inside the nucleus: new insight in a complex molecular pathway. Eur J Histochem 2014;58:2358.

107. Aredia F, Malatesta M, Veneroni P, Bottone MG. Analysis of ERK3 intracellular localization: dynamic distribution during mitosis and apoptosis. Eur J Histochem 2015;59:2571.

108. Ayarza E, Gonzalez M, Lopez F, Fernandez-Donoso R, Page J, Berrios S. Alterations in chromosomal synapses and DNA repair in apoptotic spermatocytes of Mus m. domesticus. Eur J Histochem 2014;58:2557.

109. Ami D, Di Segni  M, Forcella  M, Bernardi P, Favaloro A, Santoro G, et al. Specific association of growth-associated protein 43 with calcium release units in skeletal muscles of lower vertebrates. Eur J Histochem 2014;58:2453.
Bottermann K. Wheat germ agglutinin staining as a suitable method for detection and quantification of fibrosis in cardiac tissue after myocardial infarction. Eur J Histochem 2014;58:2448.

114. Bonetti A, Bonifacio A, Della Mora A, Livi U, Marchini M, Ortolani F. Carotenoids co-localize with hydroxyapatite, cholesterol, and other lipids in calcified stenotic aortic valves. Ex vivo Raman maps compared to histological patterns. Eur J Histochem 2015;59:2505.

115. Accart N, Dawson J, Kolbinger F, Kramer I, Beckmann N. Histological validation of non-invasive imaging in an ankylosing spondylitis rat adjuvant model. Eur J Histochem 2016;60:2667.

116. Busato A, Fumene Feruglio P, Parnigotto PP, Marzola P, Sarbati A. In vivo imaging techniques: a new era for histochemical analysis. Eur J Histochem 2016;60:2725.

117. Croce AC, Bottiroli G. Autofluorescence spectroscopy and imaging: a tool for biomedical research and diagnosis. Eur J Histochem 2014;58:2461.

118. Croce AC, Bottiroli G. New light in flavin autofluorescence. Eur J Histochem 2015;59:2576.

119. Di Guardo G. Lipofuscin, lipofuscin-like pigments and autofluorescence. Eur J Histochem 2015;59:2485.

120. Sainz B Jr, Miranda-Lorenzo I, Heeschen C. The fuss over lipo“fuss”cin: not all autofluorescence is the same. Eur J Histochem 2015;59:2512.

121. Salucci S, Burattini S, Falciere E, Gobbi P. Three-dimensional apoptotic nuclear behavior analyzed by means of Field Emission in Lens Scanning Electron Microscope. Eur J Histochem 2015;59:2539.

122. Scimeca M, Orlandi A, Terrenato I, S Bischetti, Bonanno E. Assessment of metal contaminants in non-small cell lung cancer by EDX microanalysis. Eur J Histochem 2014;58:2403.

123. Frontalini F, Curzi D, Giordano FM, Bernhard JM, Falciere E, Coccioni R. Effects of lead pollution on Ammonia parkinsoniana (foraminifera): ultrastructural and microanalytical approaches. Eur J Histochem 2015;59:2460.

124. Scimeca M, Pietrofusci A, Milano F, Anemona L, Orlandi A, Marsella LT, et al. Elemental analysis of histological specimens: a method to unmask nano asbestos fibers. Eur J Histochem 2016;60:2573.

125. Poletto V, Galimberti V, Guerra G, Rosti V, Mocci F, Biggiogera M. Fine structural detection of calcium ions by photoconversion. Eur J Histochem 2016;60:2695.

126. Grecchi S, Malatesta M. Visualizing endocytotic pathways at transmission electron microscopy via diaminobenzidine photo-oxidation by a fluorescent cell-membrane dye. Eur J Histochem 2014;58:2449.

127. Costanzo M, Carton F, Marengo A, Berlier G, Stella B, Arpico S, et al. Fluorescence and electron microscopy to visualize the intracellular fate of nanoparticles for drug delivery. Eur J Histochem 2016;60:2640.

128. Malatesta M, Grecchi S, Chiesa E, Cisterna B, Costanzo M, Zancanaro C. Internalized chitosan nanoparticles persist for long time in cultured cells. Eur J Histochem 2015;59:2492.

129. Malatesta M. Transmission electron microscopy for nanomedicine: novel applications for long-established techniques. Eur J Histochem 2016;60:2751.

130. Pellicciari C, Biggiogera M. (Eds) Histochemistry of single molecules - Methods and Protocols. Humana Press; 2017.

131. Taatjes DJ, Roth J. The histochemistry and cell biology pandect: the year 2014 in review. Histochem Cell Biol 2015;143:339-68.

132. Klein T, Proppert S, Sauer M. Eight years of single-molecule localization microscopy. Histochem Cell Biol 2014;141:561-75.

133. Schermelleh L, Heintzmann R, Leonhardt H. A guide to super-resolution fluorescence microscopy. J Cell Biol 2010;190:165-75.

134. Ilgen P, Stoldt S, Conradi LC, Wurm CA, Rüschoff J, Ghadimi BM, et al. STED super-resolution microscopy of clinical paraffin-embedded human rectal cancer tissue. PLoS One 2014;9:e101563.