

## 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,<sup>1-3</sup> 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*,<sup>4,21</sup> the expression of specific protein has been investigated (in hepatocellular carcinoma,<sup>4,6</sup> uterine lesions,<sup>7,8</sup> colorectal carcinoma,<sup>9,10</sup> and other neoplasms<sup>11,15,21</sup>). The aim was either to originally define new diagnostic/prognostic markers,<sup>4,6,8,10-12</sup> or to identify proteins involved in tumor onset<sup>7</sup> or progression,<sup>5,21</sup> or to evaluate the effect of therapy.<sup>9</sup> The mechanisms of carcinogenesis have also been studied,<sup>13-20</sup> and it is worth noting that some new proteins have been identified as potential therapeutic targets for cancer treatment<sup>14,16</sup> and tailored therapy.<sup>20,21</sup> 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*.<sup>22-42</sup> Here again, multiple immunohistochemical assays have mostly been used to identify pathogenetic factors or to relate the changes in protein expression with tissue remodeling,<sup>22,29-33</sup> or the progression of the disease.<sup>23-28,34,35</sup> Interestingly, cartilage, bone and dentin in different pathologies were especially investigated,<sup>36-41</sup> which demonstrates the unique role of histochemistry in studying these tissues under normal or pathological conditions.<sup>42</sup>

The effect of experimental treatments or disease on the fibrous joint tissue<sup>43,44</sup> and car-

Correspondence: Carlo Pellicciari,  
Dipartimento di Biologia e Biotecnologie  
"Lazzaro Spallanzani",  
Università degli Studi di Pavia, Via A. Ferrata 9,  
27100 Pavia, Italy.  
E-mail: carlo.pellicciari@unipv.it

Key words: Basic and applied histochemistry; biomedical research; histochemical methods

Received for publication: 12 December 2016.

Accepted for publication: 15 December 2016.

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0).

©Copyright C. Pellicciari, 2016  
Licensee PAGEPress, Italy  
*European Journal of Histochemistry* 2016; 60:2758  
doi:10.4081/ejh.2016.2758

tilage<sup>45,46</sup> were also investigated in papers falling into the category *Experimental Medicine & Animal Models*.<sup>43-54</sup> 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 brain<sup>46</sup> or the skeletal muscle<sup>47</sup> 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.<sup>48</sup> 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.<sup>49</sup>

Cultured cell systems have also been used to analyze the effect of ozone at low concentration on cytoskeletal organization, mitochondrial activity and nuclear transcription,<sup>52</sup> or to elucidate the molecular pathway responsible for the increased collagen synthesis by fibroblasts *in vitro* after exposure to the natural flavonoid, apigenin.<sup>53</sup> 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 & Development*<sup>55-72</sup> the expression of specific proteins was investi-

gated during fetal development,<sup>55</sup> with particular reference to skin,<sup>56</sup> lung,<sup>57,58</sup> kidney,<sup>59</sup> pituitary and adrenal gland,<sup>60,61</sup> developing cartilage and bone,<sup>62-65</sup> and cochlea.<sup>66,67</sup> The presence of progenitor stem cells has been detected in the kidney,<sup>68</sup> cerebral cortex<sup>69</sup> and cerebellum<sup>70</sup> during human embryogenesis. Viable chondrogenic stem cells have been detected in the knee joint loose body,<sup>71</sup> and the ultrastructural features of mesenchymal stem cells after *in vitro* expansion have been reviewed.<sup>72</sup> Histochemistry proved to be especially suitable also for the essentially descriptive articles on *Tissue Biology in Human & Animals*.<sup>73-98</sup> Here, several proteins and protein receptors have been studied in different organs and tissues from mammals (rodents,<sup>73-82</sup> bat,<sup>83</sup> dog,<sup>84</sup> primates and human<sup>85-88</sup>), as well as from lower vertebrates<sup>89-94</sup> and invertebrates.<sup>95</sup> Few papers described the peculiar behavior of nuclear proteins involved in RNA transcription and maturation<sup>96</sup> or in apoptotic cell death.<sup>97-98</sup>

## Methods & Techniques

In the great majority of the papers of all the above mentioned categories, techniques have been used to locate single proteins or nuclei 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 & Techniques*<sup>99-129</sup> 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,<sup>99-104</sup> and particular attention was paid to technical improvements in antigen preservation and retrieval for immunohistochemical applications,<sup>105-110</sup> and in multiple histochemical staining.<sup>111-113</sup> Imaging techniques were the subject of some articles.<sup>114-116</sup> 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.<sup>114</sup> 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.<sup>115</sup> 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.<sup>116</sup> Among the non-invasive techniques, autofluorescence imaging promise to be particularly suitable and informative for diagnostic applications.<sup>117-120</sup> Applications of electron microscopy analyses were used for tridimensional reconstruction of apoptotic nuclei<sup>121</sup> and for detecting metal contaminants.<sup>122-124</sup> Diaminobenzidine photo-oxidation by fluorescent probes was used for detecting at electron microscopy calcium ions,<sup>125</sup> for visualizing endocytotic pathways<sup>126</sup> and for tracking nanoparticles inside the cells.<sup>127,128</sup> These last articles demonstrate that new varied applications in nanomedicine may be envisaged for ultrastructural histochemistry.<sup>129</sup>

## Concluding remarks

This short survey plainly confirms that histochemical research is presently addressed to identify single molecules,<sup>130</sup> 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.<sup>1,131</sup> 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.<sup>131,132</sup> They are collectively known as super-resolution microscopy, and pushed the optical resolution down to the molecular level.<sup>133</sup> 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 samples<sup>134</sup> 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. Pellicciari 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. Pellicciari C. Histochemistry in biology and medicine: a message from the citing journals. *Eur J Histochem* 2015;59:2610.
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, Matysiak N, Mielańczyk Ł, Wyrobietec G, et al. Changes in subcellular localiza-

- 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, Milosevic M, Hudolin T, et al. TGF-beta1 expression in chromophobe renal cell carcinoma and renal oncocytoma. *Eur J Histochem* 2014;58:2265.
  11. Araujo DGB, Nakao L, Gozto 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, Radosevic-Robin N, Tardieu AS, Cayre A, Raoufils 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;58:2262.
  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 hemangioma-derived endothelial cells. *Eur J Histochem* 2014;58:2263.
  15. Fantinato E, Milani L, Sironi G. Sox9 expression in canine epithelial skin tumors. *Eur J Histochem* 2015;59:2514.
  16. Costa YF, Tjioe KC, Nonogaki S, Soares FA, Lauris JR, Oliveira DT. Are podoplanin and ezrin involved in the invasion process of the ameloblastomas? *Eur J Histochem* 2015;59:2451.
  17. Porzionato A, Rucinski M, Macchi V, Sarasin G, Malendowicz LK, De Caro R. ECRG4 expression in normal rat tissues: expression study and literature review. *Eur J Histochem* 2015;59:2458.
  18. Waisberg J, Theodoro TR, Matos LL, Orlandi FB, Serrano RL, Saba GT, et al. Immunohistochemical expression of heparanase isoforms and syndecan-1 proteins in colorectal adenomas. *Eur J Histochem* 2016;60:2590.
  19. Caocci G, Greco M, Fanni D, Senes G, Littera R, Lai S, et al. HLA-G expression and role in advanced-stage classical Hodgkin lymphoma. *Eur J Histochem* 2016;60:2606.
  20. Al-dhohorah T, Mashrah M, Yao Z, Huang J. Aberrant DKK3 expression in the oral leukoplakia and oral submucous fibrosis: a comparative immunohistochemical study. *Eur J Histochem* 2016;60:2629.
  21. Fanni D, Manchia M, Lai F, Gerosa C, Ambu R, Faa G. Immunohistochemical markers of CYP3A4 and CYP3A7: a new tool towards personalized pharmacotherapy of hepatocellular carcinoma. *Eur J Histochem* 2016;60:2614.
  22. 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.
  23. Hu SS, Mei L, Chen JY, Huang ZW, Wu H. Expression of immediate-early genes in the inferior colliculus and auditory cortex in salicylate-induced tinnitus in rat. *Eur J Histochem* 2014;58:2294.
  24. Karavana VN, Gakiopoulou H, Lianos EA. Expression of Ser729 phosphorylated PKC epsilon in experimental crescentic glomerulonephritis: an immunohistochemical study. *Eur J Histochem* 2014;58:2308.
  25. 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.
  26. 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.
  27. Janiuk I, Kasacka I. Quantitative evaluation of CART-containing cells in urinary bladder of rats with renovascular hypertension. *Eur J Histochem* 2015;59:2446.
  28. Di Vito A, Scali E, Ferraro G, Mignogna C, Presta I, Camastra C, et al. Elastofibroma dorsi: a histochemical and immunohistochemical study. *Eur J Histochem* 2015;59:2459.
  29. 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 of women with endometriosis. *Eur J Histochem* 2015;59:2570.
  30. Tossetta G, Avellini C, Licini C, Giannubilo SR, Castellucci M, Marzioni D. High temperature requirement A1 and fibronectin: two possible players in placental tissue remodelling. *Eur J Histochem* 2016;60:2724.
  31. Knabl J, Vattai A, Hüttenbrenner R, Hutter S, Karsten M, Jeschke U. RXR $\alpha$  is upregulated in first trimester endometrial glands of spontaneous abortions unlike LXR and PPAR $\gamma$ . *Eur J Histochem* 2016;60:2665.
  32. Vetuschi A, D'Alfonso A, Sferra R, Zanelli D, Pompili S, Patacchiola F, et al. Changes in muscularis propria of anterior vaginal wall in women with pelvic organ prolapse. *Eur J Histochem* 2016;60:2604.
  33. Cutroneo G, Vermiglio G, Centofanti A, Rizzo G, Runci M, Favaloro A, et al. Morphofunctional compensation of masseter muscles in unilateral posterior crossbite patients. *Eur J Histochem* 2016;60:2605.
  34. Renna LV, Cardani R, Botta A, Rossi G, Fossati B, Costa E, et al. Premature senescence in primary muscle cultures of myotonic dystrophy type 2 is not associated with p16 induction. *Eur J Histochem* 2014;58:2444.
  35. Severi C, Sferra R, Scirocco A, Vetuschi A, Pallotta N, Pronio A, et al. Contribution of intestinal smooth muscle to Crohn's disease fibrogenesis. *Eur J Histochem* 2014;58:2457.
  36. Orsini G, Majorana A, Mazzoni A, Putignano A, Falconi M, Polimeni A, et al. Immunocytochemical detection of dentin matrix proteins in primary teeth from patients with dentinogenesis imperfecta associated with osteogenesis imperfecta. *Eur J Histochem* 2014;58:2405.
  37. Loreto C, Galanti C, Musumeci G, Rusu MC, Leonardi R. Immunohistochemical analysis of matrix metalloproteinase-13 in human caries dentin. *Eur J Histochem* 2014;58:2318.
  38. Loreto C, Psaila A, Musumeci G, Castorina S, Leonardi R. Apoptosis activation in human carious dentin. An immunohistochemical study. *Eur J Histochem* 2015;59:2513.
  39. Leonardi R, Perrotta RE, Loreto C, Musumeci G, Crimi S, Dos Santos JN, et al. Toll-like receptor 4 expression in the epithelium of inflammatory periapical lesions. An immunohistochemical study. *Eur J Histochem* 2015;59:2547.
  40. Cobo T, Obaya A, Cal S, Solares L, Cabo R, Vega JA, et al. Immunohistochemical localization of periostin in human gingiva. *Eur J Histochem* 2015;59:2548.
  41. Di Nisio C, Zizzari VL, S Zara, Falconi M, Teti G, Tetè G, et al. RANK/RANKL/OPG signaling pathways in necrotic jaw bone from bisphosphonate-treated subjects. *Eur J Histochem* 2015;59:2455.
  42. Musumeci G, Castrogiovanni P, Mazzone V, Szychlinska MA, Castorina S, Loreto C. Histochemistry as a unique approach for investigating normal and osteoarthritic cartilage. *Eur J Histochem* 2014;58:2371.
  43. Shinohara Y, Okamoto K, Goh Y, Kiga N, Tojyo I, Fujita S. Inhibition of fibrous adhesion formation in the temporomandibular joint of tenascin-C knockout mice. *Eur J Histochem* 2014;58:2337.
  44. Okamoto K, Kiga N, Shinohara Y, Tojyo I, Fujita S. Effect of interleukin-1beta and dehydroepiandrosterone on the expression of lumican and fibromodulin in fibroblast-like synovial cells of the human temporomandibular joint. *Eur J*

- Histochem 2015;59:2440.
45. Di Rosa M, Szychlinska MA, Tibullo D, Malaguarnera L, Musumeci G. Expression of CHI3L1 and CHIT1 in osteoarthritic rat cartilage model. A morphological study. *Eur J Histochem* 2014;58:2423
  46. Xu HG, Zhang W, Zheng Q, Yu YF, 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.
  47. Insolia V, Piccolini VM. Brain morphological defects in prolidase deficient mice: first report. *Eur J Histochem* 2014;58:2417.
  48. Kocsis T, Baan J, Muller G, L Mendler, Dux L, Keller-Pintér A. Skeletal muscle cellularity and glycogen distribution in the hypermuscular Compact mice. *Eur J Histochem* 2014;58:2353.
  49. Salucci S, Ambrogini P, Lattanzi D, Betti M, Gobbi P, Galati C, et al. Maternal dietary loads of alpha-tocopherol increase synapse density and glial synaptic coverage in the hippocampus of adult offspring. *Eur J Histochem* 2014;58:2355.
  50. Tarantola E, Bertone V, Milanesi G, Gruppi C, Ferrigno A, Vairetti M, et al. Dipeptidylpeptidase-IV activity and expression reveal decreased damage to the intrahepatic biliary tree in fatty livers submitted to subnormothermic machine-perfusion respect to conventional cold storage. *Eur J Histochem* 2014;58:2414.
  51. Suchankova J, Legartova S, Sehnalova P, S Kozubek, Valente S, Labella D, et al. PRMT1 arginine methyltransferase accumulates in cytoplasmic bodies that respond to selective inhibition and DNA damage. *Eur J Histochem* 2014;58:2389.
  52. Ferreira MJr, Batista SA, Vidigal PVT, Cordeiro AAC, Oliveira FMS, Prata LO, et al. Infection with CagA-positive *Helicobacter pylori* strain containing three EPIYA C phosphorylation sites is associated with more severe gastric lesions in experimentally infected Mongolian gerbils (*Meriones unguiculatus*). *Eur J Histochem* 2015;59:2489.
  53. Costanzo M, Cisterna B, Vella A, Cestari T, Covi V, Tabaracci G, et al. Low ozone concentrations stimulate cytoskeletal organization, mitochondrial activity and nuclear transcription. *Eur J Histochem* 2015;59:2515.
  54. Zhang Y, Wang J, Cheng X, Yi B, Zhang X, Li Q. Apigenin induces dermal collagen synthesis via smad2/3 signaling pathway. *Eur J Histochem* 2015;59:2467.
  55. Ambu R, Vinci L, Gerosa C, Fanni D, Obinu E, Faa A, et al. WT1 expression in the human fetus during development. *Eur J Histochem* 2015;59:2499.
  56. Ferretti V, Segal-Eiras Á, Barbeito CG, Croce MV. Muc5ac mucin expression during rat skin development. *Eur J Histochem* 2015;59:2462.
  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, Pisu 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, Mukhtar 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, Hulya Uz Y, Karabacak R, Karaboga I, Demirtas S, Cagatay Cicek A. Effects of hyperthyroidism on expression of vascular endothelial growth factor (VEGF) and apoptosis in fetal adrenal glands. *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, Baba O, Qin C, Shibata S. An in situ hybridization study of perlecan, DMP1, and MEPE in developing condylar cartilage of the fetal mouse mandible and limb bud cartilage. *Eur J Histochem* 2015;59:2553.
  64. Carvalho de Moraes LO, Tedesco RC, Arraez-Aybar LA, Klein O, Mérida-Velasco JR, Alonso LG. Development of synovial membrane in the temporomandibular joint of the human fetus. *Eur J Histochem* 2015;59:2569.
  65. Zhang H, Liu P, Wang S, Liu C, Jani P, Lu Y, et al. Transgenic expression of dentin phosphoprotein inhibits skeletal development. *Eur J Histochem* 2016;60:2587.
  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 inositol 1, 4, 5-trisphosphate 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;60:2567.
  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;60:2563.
  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, Danišovič L, Majidi 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 medial preoptic area of the wild male ground squirrels (*Citellus dauricus* Brandt). *Eur J Histochem* 2016;60:2621.
  77. Golic I, Velickovic K, Markelic M, Stancic A, Jankovic A, Vucetic M, et al. Calcium-induced alteration of mitochondrial morphology and mitochondrial-endoplasmic reticulum contacts in rat brown adipocytes. *Eur J Histochem* 2014;58:2377.
  78. Romero-Fernandez W, Borroto-Escuela DO, Vargas-Barroso V, Narváez 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.

79. Escobar ML, Echeverría OM, García G, Ortíz R, Vázquez-Nin GH. Immunohistochemical and ultrastructural study of the lamellae of oocytes in atretic follicles in relation to different processes of cell death. *Eur J Histochem* 2015;59:2535.
80. Mangas A, Yajeya J, González N, Duleu S, Geffard M, Coveñas R. NO-tryptophan: a new small molecule located in the rat brain. *Eur J Histochem* 2016;60:2692.
81. Merigo F, Boschi F, Lasconi C, Benati D, Sbarbati A. Molecules implicated in glucose homeostasis are differentially expressed in the trachea of lean and obese Zucker rats. *Eur J Histochem* 2016;60:2557.
82. Ayarza E, González M, López F, Fernández-Donoso R, Page J, Berrios S. Alterations in chromosomal synapses and DNA repair in apoptotic spermatocytes of *Mus m. domesticus*. *Eur J Histochem* 2016;60:2677.
83. Strobel S, Encarnação JA, Becker NI, Trenczek TE. Histological and histochemical analysis of the gastrointestinal tract of the common pipistrelle bat (*Pipistrellus pipistrellus*). *Eur J Histochem* 2015;59:2477.
84. Di Giancamillo A, Andreis ME, Taini P, Veronesi MC, Di Giancamillo M, Modina SC. Cartilage canals in newborn dogs: histochemical and immunohistochemical findings. *Eur J Histochem* 2016;60:2701.
85. Cutroneo G, Centofanti A, Speciale F, Rizzo G, Favalaro A, Santoro G, et al. Sarcoglycan complex in masseter and sternocleidomastoid muscles of baboons: an immunohistochemical study. *Eur J Histochem* 2015;59:2509.
86. Torres-da-Silva KR, Da Silva AV, Barioni NO, Tessarin GW, De Oliveira JA, Ervolino E, et al. Neurochemistry study of spinal cord in non-human primate (*Sapajus* spp.). *Eur J Histochem* 2016;60:2623.
87. Fede C, Albertin G, Petrelli L, Sfriso MM, Biz C, De Caro R, et al. Expression of the endocannabinoid receptors in human fascial tissue. *Eur J Histochem* 2016;60:2643.
88. Fede C, Albertin G, Petrelli L, Sfriso MM, Biz C, De Caro R, et al. Hormone receptor expression in human fascial tissue. *Eur J Histochem* 2016;60:2710.
89. Caprara G A, Perni S, Morabito C, Mariggiò MA, Guarnieri S. Specific association of growth-associated protein 43 with calcium release units in skeletal muscles of lower vertebrates. *Eur J Histochem* 2014;58:2453.
90. Liu Y, Weng J, Huang S, Shen Y, Sheng X, Han Y, et al. Immunoreactivities of PPAR $\gamma$ 2 leptin and leptin receptor in oviduct of Chinese brown frog during breeding period and pre-hibernation. *Eur J Histochem* 2014;58:2422.
91. Akat E, Arikan H, Gocmen B. Histochemical and biometric study of the gastrointestinal system of *Hyla orientalis* (Bedriaga 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, Guo X, 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. Úbeda-Manzanaro M, Ortiz-Delgado JB, Sarasquete C. The Bromodomain testis-specific gene (*Brdt*) characterization and expression in gilthead 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. Gliocyte 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, González M, López F, Fernández-Donoso R, Page J, Berrios S. Alterations in chromosomal synapses and DNA repair in apoptotic spermatocytes of *Mus m. domesticus*. *Eur J Histochem* 2016;60:2677.
99. Ami D, Di Segni M, Forcella M, Meraviglia V, Baccarin M, Doglia SM, et al. Role of water in chromosome spreading and swelling induced by acetic acid treatment: a FTIR spectroscopy study. *Eur J Histochem* 2014;58:2330.
100. Korzhhevskii DE, Sukhorukova EG, Kirik OV, Grigorev IP. Immunohistochemical demonstration of specific antigens in the human brain fixed in zinc-ethanol-formaldehyde. *Eur J Histochem* 2015;59:2530.
101. Rieger J, Janczyk P, Hünigen H, Plendl J. Enhancement of immunohistochemical detection of *Salmonella* in tissues of experimentally infected pigs. *Eur J Histochem* 2015;59:2516.
102. Pinto AJ, de Amorim IF, Pinheiro LJ, Madeira IM, Souza CC, Chiarini-Garcia H, et al. Glycol methacrylate embedding for the histochemical study of the gastrointestinal tract of dogs naturally infected with *Leishmania infantum*. *Eur J Histochem* 2015;59:2546.
103. Percival KR, Radi ZA. A modified Verhoeff's elastin histochemical stain to enable pulmonary arterial hypertension model characterization. *Eur J Histochem* 2016;60:2588.
104. Dall'Oca C, Maluta T, Cavani F, Morbioli GP, Bernardi P, Sbarbati A, et al. The biocompatibility of porous vs non-porous bone cements: a new methodological approach. *Eur J Histochem* 2014;58:2255.
105. Perdoni F, Falleni M, Tosi D, Cirasola D, Romagnoli S, Braidotti P, et al. A histological procedure to study fungal infection in the wax moth *Galleria mellonella*. *Eur J Histochem* 2014;58:2428.
106. Fede C, Fortunati I, Petrelli L, Guidolin D, De Caro R, Ferrante C, et al. An easy-to-handle microfluidic device suitable for immunohistochemical procedures in mammalian cells grown under flow conditions. *Eur J Histochem* 2014;58:2360.
107. Kammoun M, Cassar-Malek I, Meunier B, Picard B. A simplified immunohistochemical classification of skeletal muscle fibres in mouse. *Eur J Histochem* 2014;58:2254.
108. Schläfli AM, Berezowska S, Adams O, Langer R, Tschan MP. Reliable LC3 and p62 autophagy marker detection in formalin fixed paraffin embedded human tissue by immunohistochemistry. *Eur J Histochem* 2015;59:2481.
109. Paulsen IM, Dimke H, Frische S. A single simple procedure for dewaxing, hydration and heat-induced epitope retrieval (HIER) for immunohistochemistry in formalin fixed paraffin-embedded tissue. *Eur J Histochem* 2015;59:2532.
110. Chen W, Liang J, Huang L, Cai J, Lei Y, Lai J, et al. Characterizing the activation of the Wnt signaling pathway in hilar cholangiocarcinoma using a tissue microarray approach. *Eur J Histochem* 2016;60:2536.
111. Šoštarić-Zuckermann IC, Severin K, Huzak M, Hohšteter M, Gudan Kurilj A, Artuković B, et al. Quantification of morphology of canine circumanal gland tumors: a fractal based study. *Eur J Histochem* 2016;60:2609.
112. Cabibi D, Giannone A G, Mascarella C, Guarnotta C, Castiglia M, Pantuso G, et al. Immunohistochemical/histochemical double staining method in the study of the columnar metaplasia of the oesophagus. *Eur J Histochem* 2014;58:2326.
113. Emde B, Heinen A, Godecke A,

- 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, Sbarbati 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, Falcieri 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, Falcieri E, Coccioni R. Effects of lead pollution on Ammonia parkinsoniana (foraminifera): ultrastructural and microanalytical approaches. *Eur J Histochem* 2015;59:2460.
124. Scimeca M, Pietrojusti 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, Moccia 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, Arpicco 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.