Histochemistry as a versatile research toolkit in biological research, not only an applied discipline in pathology

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Abstract

The impressive progress of histochemistry over the last 50 years has led to setting up specific and sensitive techniques to describe dynamic events, through the detection of specific molecules in the very place where they exist in live cells. The scientific field where histochemistry has most largely been applied is histopathology, with the aim to identify disease-specific molecular markers or to elucidate the etiopathological mechanisms. Numerous authors did however apply histochemistry to a variety of other research fields; their interests range from the microanatomy of animal and plant organisms to the cellular mechanisms of life. This is especially apparent browsing the contents of the histochemical journals where the articles on subjects other than pathology are the majority; these journals still keep a pivotal role in the field of cell and tissue biology, while being a forum for a diverse range of biologists whose scientific interests expand the research horizon of histochemistry to ever novel subjects. Thus, histochemistry can always receive inspiring stimuli toward a continuous methodological refinement.

Introduction

In the Merriam-Webster dictionary (https://www.merriam-webster.com/dictionary/histochemistry), Histochemistry is defined as “a science that combines the techniques of biochemistry and histology in the study of the chemical constitution of cells and tissues”, while in the English Oxford Dictionary (https://en.oxforddictionaries.com/definition/histochemistry) it is “the branch of science concerned with the identification and distribution of the chemical constituents of tissues by means of stains, indicators, and microscopy”. These definitions seem to assert that histochemistry has the main function to statically describe the chemical composition of tissues and cells, in the framework of their structural organization.

Actually, since the end of the 19th Century and for some decades, histochemistry was a discipline where new dyes and staining methods were seen as a means to discriminate morphology through the light microscope, irrespective of their capability to detect specific chemicals at the tissue or cellular level. However, already in the first half of the 20th century the classical textbooks on histochemistry by Lucien Lison and David Glick focused on the chemistry of cells and tissues, in the attempt to improve the knowledge on cell biochemistry through the application of chemically specific reactions on sample sections. Based on this tenet, in 1958 A.G. Everson Pearse wrote that “Histochemistry is concerned particularly in the correlation of structure with function”, and cleverly foresaw that “if advances are to be made in our knowledge of the mechanisms of pathological processes then new methods must be invented which will describe the organization of cellular events and which will be sensitive enough to detect early functional changes”.

Actually, the impressive, still ongoing progress of histochemistry over the last 50 years has led to setting up specific and sensitive techniques suitable for describing dynamic events, with attention to the detection of specific molecules in the very place where they exist in live cells.

In the attempt to ever more precisely describe the “chemistry” of a diseased tissue, refined techniques to detect single molecular species are routinely used on sections, generally from fixed and embedded samples. In situ hybridization and especially immunohistochemistry are widely applied to identify and localize specific nucleic acid sequences or proteins, and multiple techniques have been developed to simultaneously detect several different molecules on the same section. The more traditional multicolor immunofluorescence approach has in recent years been paralleled by mass cytometry where antibodies tagged with unique rare-earth-metal isotopes of defined atomic mass allow to localize up to more than 30 different proteins in a single tissue section.

As a complementary technique to the conventional histological examination and immunohistochemistry, enzyme histochemistry on cryosections effectively links biochemistry with morphology through the detection of an ongoing enzyme activity in its topographical localization: this makes it possible to obtain a metabolic mapping of tissues so that cell metabolic changes may be noticed as a consequence of pathological events or experimental treatments, even in the absence of apparent changes in the histological or immunohistochemical features.

Applications of histochemistry in the recent scientific literature

It was an obvious consequence that the scientific field where histochemistry has most largely been applied was histopathology, with the scope of identifying disease-specific structural indicators, or of elucidating the etiopathological mechanisms. This also occurs nowadays. In fact, browsing the scientific literature during the last ten years (source: the Scopus database, https://www.scopus.com/), it is easy to realize that the great majority (about 80%) of the published histochemical articles dealt with applications in human pathology (Figure 1). Describing the molecular organization of pathological tissues proved to be crucial to precisely diagnose a disease, and often to select the most appropriate therapeutic approach; furthermore, the microscopical observation of the spatial cellular organization and possible heterogeneity of tissue is especially important in cancer where different cell subpopulations may coexist, and their location in the tumor microenvironment may provide indication on the actual condition and the progression of the disease.

On the contrary, during the same time span, about 20% only were the published papers where histochemistry was explicitly reported as the main approach for investigating biological subjects other than human pathology (such as e.g., cell biology, zoology, animal and human anatomy, botany, developmental biology, etc.), despite the wide range of investigated topics. There is no reason to conclude that histochemistry has limited application outside pathology; more likely, histochemical techniques are used, sometimes unconsciously, by scientists who see histochemistry as an ancillary discipline even worthless to be
mentioned. Perhaps, as Raymond Coleman wrote, “histochemistry is often mistakenly perceived as an archaic discipline, and its contributions to cell and molecular biology are not always given the credit it deserves”.

Interestingly, if we focus on strictly histochemical journals, the percentage of articles on non-pathology topics is much higher, from 50 to 70% of the published papers in the journals considered in Figure 2a.

Taking as an example the European Journal of Histochemistry, we may observe that the articles on human tumor or non-tumor diseases or on experimental medicine were, as a mean value, less than 40%, whereas especially in the last couple of years, the papers published on other biological areas did approach 70% (Figure 2b).

This evidence suggests that indeed the attention is still high for the histochemical detection of markers for tumor16-27 or non-tumor28-32 diseases, or for the use of histochemical methods to investigate the biological bases of a disease through approaches of experimental medicine.33-36 Numerous are, however, the authors who applied histochemistry to many other research fields.

Basic biological processes such as DNA damage and repair, cell migration and cytoskeletal organization, or epithelial-mesenchymal transition were effectively described through specific histochemical reactions using experimental models in vitro or in vivo.37-42 The biology of stem cells was extensively investigated through the labelling by specific molecular markers, during development and in the adult.43-46 The molecular organization of cells and tissues was carefully analyzed47-63 especially in poorly described species of mammals55-57 and non-mammalian vertebrates58-61 or in invertebrates,62,63 while immunohistochemistry was crucial to elucidate the topographic distribution of cell lineages in different organs,64-66 especially in the nervous system,67-71 and during embryogenesis and the pre- or postnatal development,72-79 or to evaluate the effects of environmental or pharmacological stress conditions.80-85

To expand the application potential of the histochemical approach and to increase the informational capacity of the histochemical evidence, novel techniques were set up86-95 or original applications of established histochemical methods were described, using tissue and cell models from a variety of organisms (invertebrates, vertebrates or plants).96-104

Concluding remarks

Similar indications can be obtained examining the subjects of the articles pub-

Figure 1. Number of scientific articles where histochemistry was used during the last ten years (source: https://www.scopus.com/). Most of the published papers (70 to 80%) were on pathology subjects.

Figure 2. a) Mean percentage values of the histochemical articles published on pathology or non-pathology subjects in all the scientific journals indexed in the Scopus database or in some histochemical journals, during the last ten years. b) Percentage of scientific articles on pathology or non-pathology subjects, or on methods published in the European Journal of Histochemistry from 2009 to present.
lished in other histochemical journals (see e.g.105-111), and this attitude should especially be taken into account by scientists in basic biology whose interests range from the microanatomy of organisms to the cellular mechanisms of life, and who often use histochemistry and microscopy in their researches: sometimes, the results of their investigations can hardly be considered for publication in strictly discipline-oriented journals, whereas the histochemical journals may be the ones where their articles may fittingly be published to be read by a naturally open-minded and scientifically diversified readership.

Thus, histochemical Journals would keep their pivotal role in the field of cell and tissue biology, while becoming -even more than in the past- a forum for a diverse range of researches: sometimes, the results of their articles may be the ones where their articles would be vital to give histochemistry driving stimuli toward an unceasing methodological refinement.112

References
1. Wick MR. Histochemistry as a tool in morphological analysis: a historical review. Am J Diagn Pathol 2012;16:71-8.
2. Lison L. Histochimie animale. Paris: Gautier-Villars Publishers; 1936.
3. Glick D. Techniques of histo- & cytchemistry. New York: Interscience Publishers; 1949.
4. Pearse AGE. Extension of the limits of cellular pathology: the role of enzyme histochemistry. J Clin Path 1958;11:520-34.
5. Musumeci G. The old and the new concepts of histochemistry. J Histol Histopathol 2015;2:10.
6. Pelliccari C, Biggiogera M. Histochemistry of single molecule: Methods and protocols. Methods in molecular biology. vol. 1560. Humana Press, New York; 2017.
7. Pelliccari C. Histochemistry today: detection and location of single molecules. Eur J Histoch 2017;61:2855. doi: 10.4081/ejh.2017.2855
8. Giesen C, Wang Hao, Schapiro D, Zivanovic N, Jacobs A, Hattendorf B, et al. Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. Nat Methods 2014;11:417-22
9. Meier-Ruge WA, Bruder E. Current concepts of enzyme histochemistry in modern pathology. Pathobiology 2008;75:233-43.
10. Van Noorden CJ. Imaging enzymes at work: metabolic mapping by enzyme histochemistry. J Histochem Cytochem 2010;58:481-497.
11. Molenaar RJ, Khurshed M, Hira VVV, Van Noorden CJF. Metabolic mapping: Quantitative enzyme cytochemistry and histochemistry to determine the activity of dehydrogenases in cells and tissues. J Vis Exp 2018;(135). doi: 10.3791/56843.
12. Riva MA, Manzoni M, Isimbaldi G, Cesana G, Pagni F. Histochemistry: historical development and current use in pathology. Biotech Histochem 2014;89:81-90.
13. Blom S, Paavolainen L, Bychov D, Turkki R, Mäki-Teeri P, Hennamo A. Systems pathology by multiplexed immunohistochemistry and whole-slide digital image analysis. Sci Rep 2017;7:15580.
14. Coleman R. The impact of histochemistry - a historical perspective. Acta Histochem 2000;102:5-14.
15. Fanni D, Manchia M, Lai F, Gerosa C, Ambu R, Faa G. Immunohistochemical markers of CYP3A4 and CYP3A7: a new tool towards personalized pharma-cotherapy of hepatocellular carcinoma. Eur J Histochem 2016;60:2614.
16. Caocci G, Greco M, Fanni D, Senes G, Littera R, Lai S, et al. Immunohistochemical expression of heparanase isoforms and syndecan-1 proteins in colorectal adenomas. Eur J Histochem 2016;60:2590.
17. Waisberg J, Theodoro TR, Matos LL, Orlandi FB, Serrano RL, Saba GH, et al. Immunohistochemical expression of heparanase isoforms and syndecan-1 proteins in colorectal adenomas. Eur J Histochem 2016;60:2590.
18. Chen W, Liang J, Huang C, Lai Y, Lai J, et al. Characterizing the activation of the Wnt signaling pathway in hilar cholangiocarcinoma using a tissue microarray approach. Eur J Histoch 2016;60:2536.
19. Tabola R, Zaremba-Czogalla M, Baczynska D, Cirocchi R, Stach K, Grabowski K, et al. Fibroblast activating protein-a expression in squamous cell carcinoma of the esophagus in primary and irradiated tumors: the use of archival FFPE material for molecular techniques. Eur J Histoch 2017;61:2793.
20. Sferra R, Pompili S, Festuccia C, Brindisi M, Pompili D, Patacchiola F, et al. The possible prognostic role of histone deacetylase and transforming growth factor β/Smad signaling in high grade gliomas treated by radiochemotherapy: a preliminary immunohistochemical study. Eur J Histoch 2017;61:2732.
21. Pirrone C, ChiaraValli AM, Marando A, Conti A, Rainero A, Pistochini A, et al. OTX1 and OTX2 as possible molecular markers of sinonasal carcinomas and olfactory neuroblastomas. Eur J Histoch 2017;61:2730.
22. Cioca A, Ceausu AR, Marin I, Raica M, Cinpean AM. The multifaceted role of podoplanin expression in hepatocellular carcinoma. Eur J Histoch 2017;61:2707.
23. Dávila-Rodríguez MI, Cortés-Gutiérrez EI, Hernández-Valdés R, Guzmán-Cortés K, De León-Cantu RE, Cerda-Flores RM, et al. DNA damage in acute myeloid leukemia patients of Northern Mexico. Eur J Histoch 2017;61:2831.
24. De Souza Albuquerque MS, Da Silva-Filho AF, Ferraz Cordeiro M, Deodato de Souza MF, Quirino MLW, Amorim Lima LR, et al. GalNAc-T15 in gastric adenocarcinoma: Characterization according to tissue architecture and cellular location. Eur J Histoch 2018;62:2931.
25. Moudi B, Heidari Z, Mahmoudzadeh-Sagheb H, Alavian SM, Lankarani KB, Farrókh P, et al. Concomitant use of heat-shock protein 70, glutamine synthetase and glypican-3 is useful in diagnosis of HBV-related hepatocellular carcinoma with higher specificity and sensitivity. Eur J Histoch. 2018;62:2859
26. Rabinovich I, Martins Sebastião AP, Silveira Lima R, Cicero de Andrade U, Schunemann E Jr, Furlan Anselmi K, et al. Cancer stem cell markers ALDH1 and CD44+/CD24- phenotype and their prognosis impact in invasive ductal carcinoma. Eur J Histoch 2018;62:2943.
27. 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 Histoch 2016;60:2604.
28. Mangas A, Yajeya J, González N, Ruiz I, Pernia M, Geffard M, et al. Gemst: a taylor-made combination that reverts neuroanatomical changes in stroke. Eur J Histoch 2017;61:2790.
29. Carotti S, Perrone G, Amato M, Vespasiani Gentilucci U, Rigli D, Francesconi M, et al. Reelin expression in human liver of patients with chronic hepatitis C infection. Eur J Histoch 2017;61:2745.
30. Ferroni L, Gardin C, De Pieri A, Sambataro M, Seganfreddo E, Goret C, et al. Treatment of diabetic foot ulcers with Therapeutic Magnetic Resonance (TMR®) improves the quality of granulation tissue. Eur J Histoch 2017;61:2800.
31. Maxia C, Murtas D, Corrias M, Zucca I, Minerba L, Piras F, et al. Vitamin D and vitamin D receptor in patients with ophthalmal pterygium. Eur J Histochem 2017;61:2837.

32. Al-Hazza T, Masharrah M, Yao Z, Huang J. Aberrant DKK3 expression in the oral leukoplaikia and oral submucous fibrosis: a comparative immunohistochemical study. Eur J Histochem 2016;60:2629.

33. Hashimoto K, Oda Y, Nakamura F, Kakinoki R, Akagi M. Lectin-like, oxidized low-density lipoprotein receptor-1-deficient mice show resistance to age-related knee osteoarthritis. Eur J Histochem 2017;61:2762.

34. Diomede F, Thangavelu SR, Merciario I, D’Orazio M, Bramanti P, Mazzon E, Trubiani O, et al. Porphyromonas gingivalis lipopolysaccharide stimulation in human periodontal ligament stem cells: role of epigenetic modifications to the inflammation. Eur J Histochem 2017;61:2826.

35. Costanzo M, Boschi F, Carton F, Conti G, Covi V, Tabaracci G, et al. Low oxygen concentrations promote adipogenesis in human adipose-derived adult stem cells. Eur J Histochem 2018;62:2969.

36. Di Giancamillo A, Andreis ME, Taini P, Veronesi MC, Fanos V, Faa G. Stem/progenitor cells in the developing human cerebellum: an immunohistochemical study. Eur J Histochem 2016;60:2686.

37. Costanzo M, Boschi F, Carton F, Conti G, Covi V, Tabaracci G, et al. Low oxygen concentrations promote adipogenesis in human adipose-derived adult stem cells. Eur J Histochem 2018;62:2969.

38. 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;60:2609.

39. Cortés Gutiérrez EJ, García-Villena C, Aguilar-Lemarroy A, Vallejo-Ruiz V, Piña-Sánchez P, Zapata-Benívides P, et al. Expression of the HPV18/E6 oncoprotein induces DNA damage. Eur J Histochem 2016;60:2773.

40. Zuppingter C, Gibbons G, Dutta-Passecker P, Segisier A, Most H, Suter TM. Characterization of cytoskeleton features and maturation status of cultured human iPSC-derived cardiomyocytes. Eur J Histochem 2017;61:2763.

41. Oswald J, Büttner M, Jasinsky-Bergner S, Jacobs R, Rosenstock P, Kielstein H. Leptin affects filopodia and cofillin in NK-92 cells in a dose- and time-dependent manner. Eur J Histochem 2018;62:2848.

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

43. Diomede F, Thangavelu SR, Merciario I, D’Orazio M, Bramanti P, Mazzon E, et al. Porphyromonas gingivalis lipopolysaccharide stimulation in human periodontal ligament stem cells: role of epigenetic modifications to the inflammation. Eur J Histochem 2017;61:2826.

44. 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.

45. Costanzo M, Boschi F, Carton F, Conti G, Covi V, Tabaracci G, et al. Low oxygen concentrations promote adipogenesis in human adipose-derived adult stem cells. Eur J Histochem 2018;62:2969.

46. 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.

47. Soštarić-Zuckermann IC, Severin K, Huzak M, Holsťter M, Gudan Kuriš A, Artukovic B, et al. Quantification of morphology of canine circumanal gland tumors: a fractal based study. Eur J Histochem 2016;60:2699.

48. 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;60:2609.

49. Hannon J, Ericsson AE, Axelson H, Johansson ME. Species diversity regarding the presence of proximal tubular progenitor cells of the kidney. Eur J Histochem 2016;60:2557.

50. Ma B, Yin C, Hu D, Newman M, Nicholls PK, Wu Z, et al. Distribution of non-myelinating Schwann cells and their associations with leukocytes in mouse spleen revealed by immunofluorescence staining. Eur J Histochem 2018;62:2890.

51. Shi Z, Greene KW, Nicholls KP, Hu D, Tirnitz-Parker JEE, Yuan Q, et al. Immunofluorescent characterization of non-myelinating Schwann cells and their interactions with immune cells in mouse mesenteric lymph node. Eur J Histochem 2016;61:2827.

52. Maruyama-Koide Y, Mikawa S, Rüegg MA, Wada K, Yagi Y. Distribution of choline acetyltransferase (ChAT) immunoreactivity in the brain of the teleost Cyprinus carpio. Eur J Histochem 2018;62:2932.

53. Xi L, Wang C, Chen P, Yang Q, Hu R, Zhang H, et al. Expressions of IL-6, TNF-α and NF-κB in the skin of Chinese brown frog (Rana dybowskii). Eur J Histochem 2017;61:2834.

54. Batista-Manzanaro N, 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.

55. Verderame M, Limatola E, Scudiero R. Metallothionein expression and synthesis in the testis of the lizard Podarcis sicula under natural conditions and following estrogenic exposure. Eur J Histochem 2017;61:2777.

56. Casha A, Vaccaro R, Toni M, Cioni C. Distribution of choline acetyltransferase (ChAT) immunoreactivity in the brain of the teleost Cyprinus carpio. Eur J Histochem 2018;62:2932.

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

58. Accogli G, Schillitani G, Mentino D, Desantis S, Lazzarini R. Characterization of the skin mucus in the common octopus Octopus vulgaris (Cuvier) reared paralarvae. Eur J Histochem 2017;61:2815.

59. Fede C, Albertini 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.

60. Frick C, Martin HL, Brueder J, Lang K, et al. Characterization of proteins concerned with the secretory machinery in goat ceruminous glands. Eur J Histochem 2017;61:2828.
Breer H. Topographic distribution pattern of morphologically different G cells in the murine antral mucosa. Eur J Histochem 2017;61:2810.

65. Polakovičová S, Csóborényová M, Fillová B, Borovský M, Maršík L, Kvasilová A, et al. Merkel-like cell distribution in the epithelium of the human vagina. An immunohistochemical and TEM study. Eur J Histochem 2018;62:2836.

66. 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.

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

68. Mangas A, Vajey 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.

69. Kálmán M, Oszwald E, Adorján I. Appearance of β-dystroglycan precedes the formation of glio-vascular end-feet in developing rat brain. Eur J Histochem 2018;62:2908.

70. Nishida K, Nomura Y, Kawamori K, Kato T, Oka K, Nakamura T, Ito A. Elemental analysis of histological specimens: a method to unmask nanostructures. Eur J Histochem 2016;60:2573.

71. Zhang H, Liu P, Wang S, Liu C, Jani P, Shibutani M. Identification of 5-fluorouracil photoproducts in DNA from colorectal cancer tissues. Eur J Histochem 2016;60:2678.

72. Dall'Aglio C, Polisca A, Cappai MG, Mercati F, Troisi A, Pirino C, et al. Immunohistochemistry detected and localized cannabinoid receptor type 2 in bovine fetal pancreas at late gestation. Eur J Histochem 2017;61:2761.

73. Cau F, Pisu E, Gerosa C, Senes G, Ronchi F, Bottà C, et al. Interindividual variability in the expression of surfactant protein A and B in the human lung during development. Eur J Histochem 2016;60:2588.

74. Monti M, Calligaro A, Behr B, Rejo Perà R, Redi CA, Wossidlo M. Functional topography of the fully grown human oocyte. Eur J Histochem 2017;61:2769.

75. Maeda Y, Miwa Y, Sato I. Expression of CGRP, vasculogenesis and osteogenesis associated mRNAs in the developing mouse mandible and tibia. Eur J Histochem 2017;61:2750.

76. Liu J, Cai L, He Y, Yang J. Apoptosis pattern and alterations of expression of apoptosis-related factors of supporting cells in Köllicker’s organ in vivo in early stage after birth in rats. Eur J Histochem 2017;61:2706.

77. Liu W, Wang C, Yu H, Liu S, Yang J. Expression of acetylated tubulin in the postnatal developing mouse cochlea. Eur J Histochem 2018;62:2942.

78. Favorito R, Monaco A, Girimalli MC, Ferrandino I. Effects of cadmium on the glial architecture in lizard brain. Eur J Histochem 2017;61:2734.

79. Monaco A, Capiello T, Girimalli MC, Schiano V, Ferrandino I. Neutropodization in zebrafish embryos and adults after cadmium exposure. Eur J Histochem 2017;61:2833.

80. Aidos L, Pinheiro Valente LM, Sousa V, Lanfranchi M, Domenechhi C, Di Giancamillo A. Effects of different rearing temperatures on muscle development and stress response in the early larval stages of Acipenser baerii. Eur J Histochem 2017;61:2850.

81. Giacoppo S, Gugliandolo A, Trubiani O, Pollastro F, Grassi G, Bramanti P, et al. Cannabinoid CB2 receptors are involved in the protection of RAW264.7 macrophages against the oxidative stress: an in vitro study. Eur J Histochem 2017;61:2749.

82. Varricchio B, O, Pollastro F, Grassi G, Bramanti P, et al. Localization of cannabinoid CB2 receptors in the developing mouse brain. Eur J Histochem 2018;62:2870.

83. Khotimchenko V, Antimonova O, Fedorova E, Shavlovsky M, Krutikov A, Mikhailova E, et al. Fluorescent characterization of amyloid deposits in the kidneys of mdx mice. Eur J Histochem 2018;62:2870.

84. Röhle I, Hüttner FJ, Plendl J, Drexes B, Zentek J. Comparison of different histological protocols for the preservation and quantification of the intestinal mucus layer in pigs. Eur J Histochem 2018;62:2874.

85. Costanzo M, Carton F, Marengo A, Bertler 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.

86. Boschi F, De Sanctis F. Overview of the optical properties of fluorescent nanoparticles for optical imaging. Eur J Histochem 2017;61:2830.

87. Carotti B, Carton F, Marengo A, Berlier G, Stella B, Arpicco S, et al. The biocompatibility of bone cements: progress in methodological developments. Eur J Histochem 2016;60:2689.

88. Poletto V, Galimberti V, Guerra G, Rosti V, Moccia F, Bigioggeri M. Fine structural detection of calcium ions by photoconversion. Eur J Histochem 2016;60:2685.

89. Bobek G, Stait-Gardner T, Price W, Makris A, Hennessy A. Quantification of placental change in mouse models of preeclampsia using magnetic resonance microscopy. Eur J Histochem 2018;62:2688.

90. Scimeca M, Bissetti S, Lamsira HK, Bonfiglio R, Bonanno E. Energy Dispersive X-ray (EDX) microanalysis: A powerful tool in biomedical research and diagnosis. Eur J Histochem 2018;62:2841.

91. Croce AC, Bottiroli G. Lipids: Evergreen autofluorescent biomarkers for the liver functional profiling. Eur J Histochem 2017;61:2808.

92. Casiraghi E, Cossa M, Huber V, Rivoltini L, Tozzi M, Villa A, et al. MIAQuant, a novel system for automatic segmentation, measurement, and localization comparison of different biomarkers from serialized histological slices. Eur J Histochem 2017;61:2838.

93. Gusel’nikova V, Antimonova O, Fedorova E, Shavlovsky M, Krutikov A, Mikhailova E, et al. Fluorescent characterization of amyloid deposits in the kidneys of mdx mice. Eur J Histochem 2018;62:2870.

94. Röhe I, Hüttner FJ, Plendl J, Drexes B, Zentek J. Comparison of different histological protocols for the preservation and quantification of the intestinal mucus layer in pigs. Eur J Histochem 2018;62:2874.

95. Costanzo M, Carton F, Marengo A, Bertler 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.

96. Boschi F, De Sanctis F. Overview of the optical properties of fluorescent nanoparticles for optical imaging. Eur J Histochem 2017;61:2830.

97. Carton F, Calderan L, Malatesta M. Incubation under fluid dynamic conditions markedly improves the structural preservation in vitro of explanted skeletal muscles. Eur J Histochem 2017;61:2862.

98. Dall’Oca C, Maluta T, Micheloni GM, Cengarle M, Morbioni G, Bernardi P, et al. The biocompatibility of bone cements: progress in methodological approach. Eur J Histochem 2017;61:2673.

99. Amaroli A, Ferrando S, Pozzolini M, Gallus L, Parker S, Benedicenti S. The
earthworm Dendrobaena veneta (Annelida): A new experimental organism for photobiomodulation and wound healing. Eur J Histochem 2018;62:2867.

100. Kawai M, Kataoka Y, Sonobe J, Yamamoto H, Maruyama H, Yamamoto T, et al. Analysis of mineral apposition rates during alveolar bone regeneration over three weeks following transfer of BMP-2/7 gene via in vivo electroporation. Eur J Histochem 2018;62:2947.

101. Brechú-Franco AE, Laguna-Hernández G, De la Cruz-Chacón I, González-Esquinca AR. In situ histochemical localisation of alkaloids and aceto genins in the endosperm and embryonic axis of Annona macroprophyllata Donn. Sm. seeds during germination. Eur J Histochem 2016;60:2568.

102. Laguna-Hernández G, Rio-Zamorano CA, Meneses-Ochoa IG, Brechú-Franco AE. Histochemistry and immunolocalisation of glucokinin in antidiabetic plants used in traditional Mexican medicine. Eur J Histochem 2017;61:2782.

103. Antonini E, Zara C, Valentini L, Gobbi P, Ninfali P, Menotta M. Novel insights into pericarp, protein body globoids of aleurone layer, starchy granules of three cereals gained using atomic force microscopy and environmental scanning electronic microscopy. Eur J Histochem 2018;62:2869.

104. Coleman R. Acta Histochemica celebrates 60 years of publication (1954-2014). Acta Histochem 2014;116:1-4.

105. Taatjes DJ, Roth J. The Histochemistry and Cell Biology pandect: the year 2014 in review. Histochem Cell Biol 2015;143:339-68.

106. Piasecka M. Editorial. Folia Histochem Cytobiol 2015;53:175-6.

107. Taatjes DJ, Roth J. The Histochemistry and Cell Biology omnium-gatherum: the year 2015 in review. Histochem Cell Biol 2016;145:239-74.

108. Hewitt SM. Time Advances, people and language change, but the fundamentals remain the same. J Histochem Cytochem 2016;64:5-6.

109. Taatjes DJ, Roth J. In focus in HCB. Histochem Cell Biol 2017;148:217-8.

110. Taatjes, DJ, Roth J. In focus in HCB. Histochem Cell Biol 2018;150:575-8.

111. Pellicciari C. Is there still room for novelty, in histochemical papers? Eur J Histochem 2016;60:2758.