# Twenty years of histochemistry in the third millennium, browsing the scientific literature

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ABSTRACT

Over the last twenty years, about 240,000 articles where histochemical techniques were used have been published in indexed journals, and their yearly number has progressively increased. The histochemical approach was selected by researchers with very different scientific interests, as the journals in which these articles were published fall within 140 subject categories. The relative proportion of articles in some of these journal categories did change over the years, and browsing the table of contents of the *European Journal of Histochemistry,* as an example of a strictly histochemical journal, it appeared that in recent years histochemical techniques were preferentially used to mechanistically investigate natural or experimentally induced dynamic processes, with reduced attention to purely descriptive works. It may be foreseen that, in the future, histochemistry will be increasingly focused on studying the molecular pathways responsible for cell differentiation, the maintenance or loss of the differentiated state, and tissue regeneration.

**Key words:** Histochemistry; scientific literature.

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## Introduction

At the beginning of the year 2000, Raymond Coleman published in *Acta Histochemica* two interesting commentaries on the role and fate of histochemistry in the new millennium.[1,2] He complained that non-histochemists often perceive histochemistry "as an archaic term primarily associated with stains and staining techniques" so that its right nature and importance are made unclear. Then, he proposed to "adopt a new and creative terminology to describe" the discipline in order "to popularize the viewpoint that histochemistry and cytochemistry remain at the forefront of modern cell biology". To do this, he even hypothesized that the names of histochemical Societies and histochemical Journals had to be changed, to make them more modern and attractive. Twenty years later, we realize that such a "creative semantic change" (in Coleman's words) did not extensively occurred, and perhaps the scientists' attitude toward histochemistry is not so different from the one in 2000.

## Articles published in 2000-2020, where histochemistry was used

Browsing the Web of Science database for the articles of the last twenty years where the words "histochemistry", "immunohistochemistry", "*in situ* hybridization", "lectin histochemistry" or "enzyme histochemistry" appear in the title, abstract or author keywords, we observe that about 240,000 papers have been published in indexed journals: their number has progressively increased from about 9300 in 2000 to 15,000 in 2019 (14,000 to date, in 2020) (Figure 1A). This confirms that during these years, histochemistry has even increased its impact on the scientific literature, especially in the biological and medical fields.[3]

The journals in which these articles were published cover more that 140 subject categories, thus strengthening that the histochemical approach is (sometimes unconsciously) chosen by researchers with largely different scientific interests. Most of the "histochemically classified" articles were published on journals belonging to

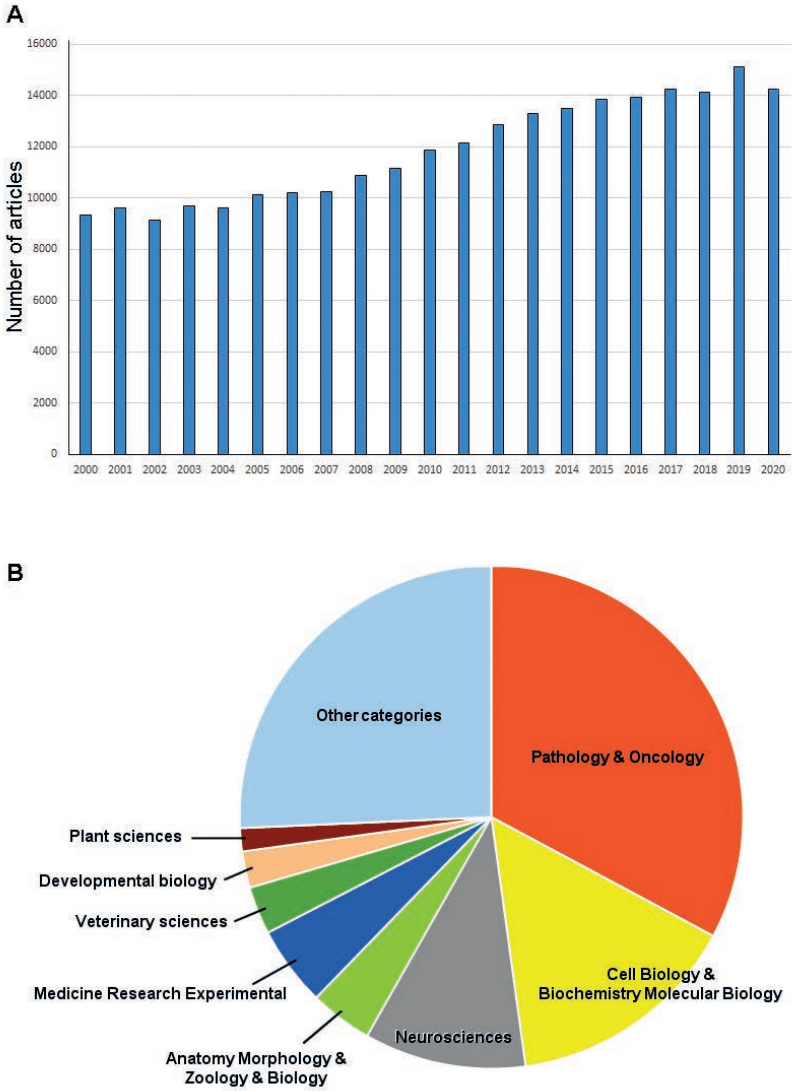


**Figure 1. A) Number of articles where histochemical techniques were used, and which have been published in indexed journals in the time interval 2000-2020. B) Percentage of the articles that appeared in indexed journals of different categories during 2000-2020 (source: the Web of Science database).**

life sciences: in fact, about 75% of the papers appeared in the journals' categories Pathology & Oncology (34%), Cell Biology & Biochemistry Molecular Biology (15%), Neurosciences (10%), Medicine Research Experimental (5%), Anatomy Morphology & Zoology & Biology (4%), Veterinary Sciences (3%), Developmental Biology (2.5%) and Plant Sciences (1.5%) (Figure 1B). It is interesting to observe that the relative proportion of articles in some of these journal categories did change over the years (Figure 2). The percentage of articles in Pathology & Oncology was around 25% in 2000 but increased to 40% in 2016 (about 36% in 2020): this was especially due to the progressively larger number of articles published in journals of Oncology (whose percentage doubled in 2020, from the 12% in 2000). The use of histochemical techniques also increased in the journals of the category Medicine Research Experimental (from 3% to 9% of the published articles). On the contrary, the percentage of articles in the category Neurosciences decreased from 17% to 6%; this negative trend also occurred for the articles in Cell Biology & Biochemistry Molecular Biology (from 18% to 14%) and those in Developmental Biology (from about 4% in 2000 to 1% in 2020). Minor fluctuations were only observed for the categories Morphology & Zoology & Biology, Veterinary Sciences, and Plant Sciences.

Considering the subject categories, it is obvious that the great majority of the papers in which histochemistry was used have not been published in strictly histochemical journals; in these latter ones, the authors' interests and articles' subjects may have progressively been changing as well, during these last twenty years.

## How the articles' subjects changed in a histochemical journal

To test this hypothesis, I browsed the tables of contents of the *European Journal of Histochemistry*, as an example of a long-established histochemical journal that has traditionally been open to a wide assortment of subjects, from cytology and histology in animals and plants, to human and veterinary medicine, to developmental biology.

A total of 880 scientific articles have been published from January 2000 to date. Due to the relatively small number of papers, they have been divided into three groups (the first one from 2000 to 2008, the second one from 2009 to 2017, and the third one from 2018 up to now), and the articles have been assigned to the following topics: Methods, Tumor and non-tumor diseases, Cell biology, Neurosciences, Animal biology (including Zoology, Microanatomy and Normal Histology), Experimental research and medicine, Veterinary sciences, Developmental biology and Plant Sciences (Figure 3).

The articles dealing with new methods or technical refinements were numerous and their percentage increased mainly in the last three years[4-19] when improvements in all the different steps of

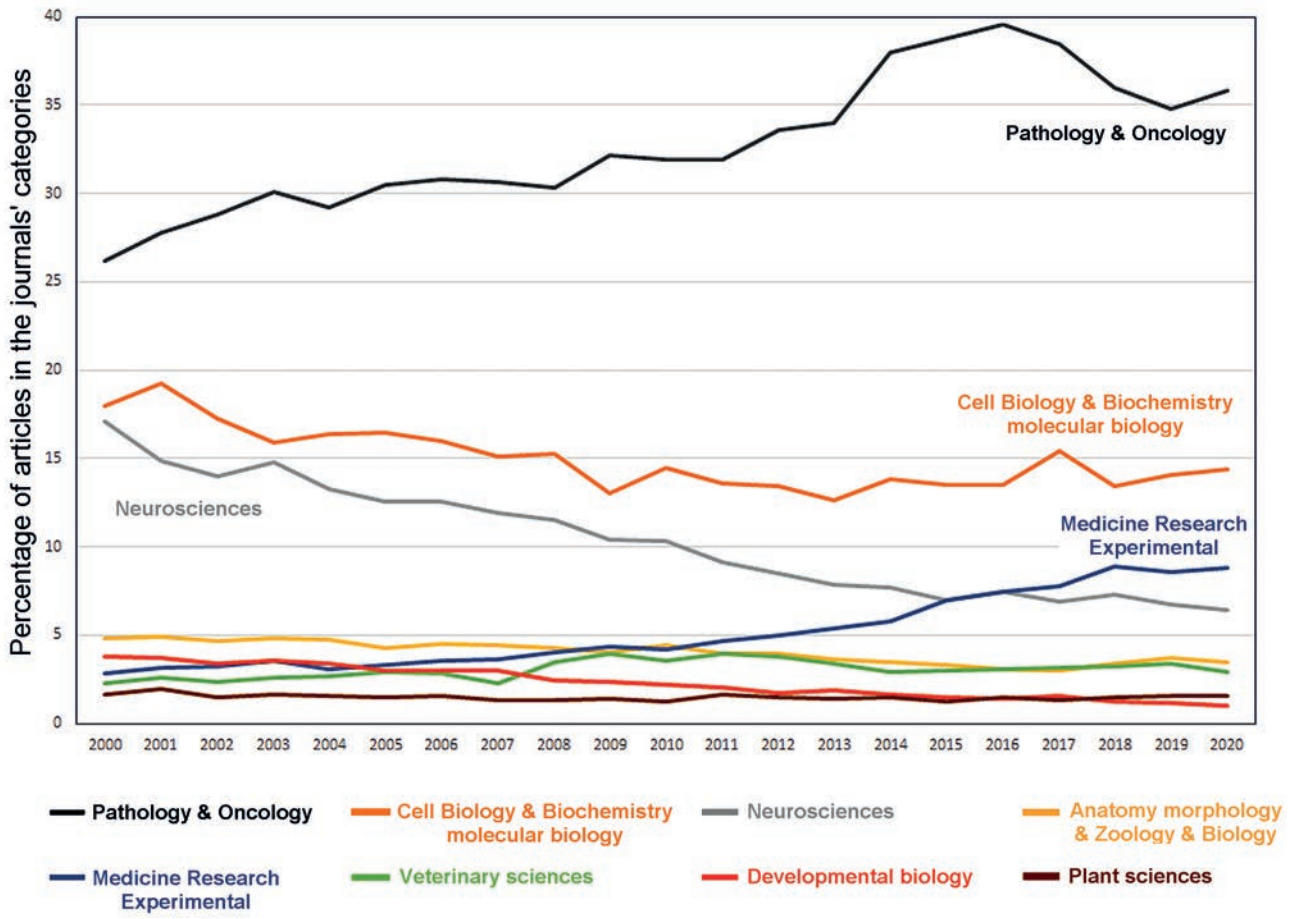


**Figure 2. Percentage of articles published yearly in journals of the different categories considered in Figure 1B.**

tissue preparation were proposed, from fixation[4-8] and embedding,[9] to antigen retrieval[10-11] and staining.[12-15] Several papers dealt with ultrastructural cytochemistry,[9,15,16] imaging techniques[17-19] and microanalysis.[20] This is not surprising for a histochemical journal, as setting up refined techniques is crucial for their appropriate and targeted application to visualize specific chemical species under different detecting procedures.

In recent years, there was no significant change in the percentage of articles published in Neurosciences[21-29] and Veterinary sciences,[30-31] whereas the one of those on Animal biology[33-43] decreased.

No doubt, the scientific field where histochemistry has been (and still is) most largely used is histopathology; this was observed in the present survey of the whole scientific production of the last twenty years (Figure 1B), and it is confirmed by the fraction of articles on tumors[44-58] and non-tumor diseases[59-69] recently published in our journal. It is worth noting that in 2000-2009 more than 90% of the articles on tumors were aimed at describing disease-specific markers suitable for diagnosis or prognosis, whereas in a few papers only attempts were made to study the molecular mechanisms responsible for tumor onset and progress; these latter processes were, on the contrary, investigated in more than 35% of the articles published in the last three years. Histochemistry was used in parallel with molecular techniques as the proper approach to mechanistically explain the molecular basis of different diseases; this special attention to the cellular mechanisms responsible for pathological processes may also explain the progressive decrease of the published articles on basic Cell biology. Consistently with the trend observed in Figure 2, the papers on Experimental research and medicine have become more numerous: investigations were performed on animals *in vivo*[70-81] or on cultured cells,[82-89] as experimental systems for human pathologies, to elucidate the effects of the administration of physical or pharmacological agents. The papers on Developmental biology also increased: besides those on embryological development,[90-96] several ones were focussed on stem cells[97-101] and tissue regeneration.[102-106] The application of histochemistry in the field of Plant sciences seems to have revived especially in the last year.[107-110]

As a general observation, we realize that histochemical techniques were preferentially used to investigate natural or experimentally induced dynamic processes at the molecular level, with reduced application in purely descriptive works.

## Concluding remark

The long history of histochemistry started in 1829 with the seminal work *Essai de Chimie Microscopique Appliquée à la Physiologie (ou l'art de transporter le laboratoire sur le porte-objet dans l'étude des corps organisés)* by Francois-Vincent Raspail;[111] since then, histochemistry developed and evolved in parallel with the growth and progress of life sciences. The reason for the extensive use of histochemistry by researchers in a variety of scientific fields is certainly due to the unique opportunity this discipline offers to specifically locate molecules in the tissues, cells and subcellular sites where they are present and exert their structural and functional roles. Thanks to ultrastructural cytochemistry[16] and the tremendous improvement in super-resolution microscopy,[112,113] it has become possible to track histochemically labelled molecules at the nanoscale by microscopy imaging, and this has often been crucial to reach a mechanistic explanation of the cell functions in organs and tissues, under physiological or pathological conditions.

It is easy to foresee that, in the years to come, histochemistry will be even more oriented toward the understanding of the biocomplexity by elucidating the molecular pathways responsible for cell differentiation, the maintenance or loss of the differentiate state, and tissue regeneration. These topics will likely be the subjects of the large part of the manuscripts that, in the future, will be submitted to the histochemical journals, which will continue to be an open forum for scientists active in biomedical research, and to exert a promoting action on the development of histochemistry in its technical improvements and novel applications.

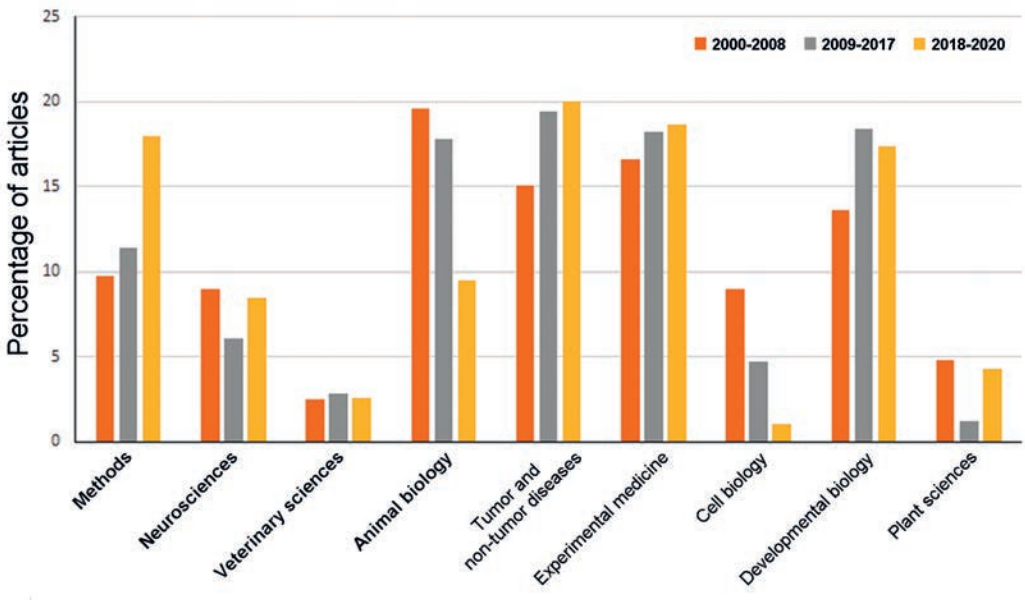


**Figure 3. Number of articles published in the European Journal of Histochemistry from 2000 to 2008, 2009 to 2017, and 2018 to present.**

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Received for publication: 28 December 2020. Accepted for publication: 28 December 2020

European Journal of Histochemistry 2020; 64:3213
doi:10.4081/ejh.2020.3213