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Chapter 1 : Histological & histochemical methods: theory and practice - John Alan Kiernan - Google Books

Histological and Histochemical Methods by Professor John A. Kiernan is a classic in the histochemical literature since its first edition, in

Histochemistry and cytochemistry are important fields for studying the inner workings of cells and tissues of the body. While visualization techniques have been in use for many years, new methods of detection developed recently help researchers and practitioners better understand cell activity. Histochemical and Cytochemical Methods of Visualization describes the essential techniques that can be used for histochemical investigations in both light and transmission electron microscopy. The book begins by discussing techniques in light microscopy. It reviews classical methods of visualization, histochemical and histoenzymatic methods, and methods used to visualize cell proliferation and apoptosis. Next, the book examines the cytochemical methods used in electron microscopy with traditional techniques, as well as more specialized methods. The final section provides an overview of image analysis and describes how image processing methods can be used to extract vital information. A page insert supplies color illustrations to enhance the text. Techniques will continue to adapt to the latest technological innovations, allowing more and more precise quantification of images. These developments are essential to the biological as well as the medical sciences. This manual is a critical resource for novice and experienced researchers, technicians, and students who need to visualize what happens in the cell, the molecules expressed, the main enzymatic activities, and the repercussions of the molecular activities upon the structure of the cells in the body. Elsevier Health Sciences Format Available: This is a brand new edition of the leading reference work on histological techniques. It is an resource suited to all those involved with histological preparations and applications, from the student to the highly experienced laboratory professional. New to this edition: Self assessment questions and answers. Will help reinforce all of the basics in order to pass course exams, professional certification exams. New material on immunohistochemical and molecular diagnostic techniques. Enables user to keep abreast of latest advances in the field. The authors have aimed to produce a textbook for courses in cellular pathology, both in the United Kingdom and elsewhere. Also, they aimed to produced a book as a practical companion of Theory and Practice of Histological Techniques Bancroft and Stevens, and to produce a laboratory manual containing a full repertoire of standard and non-standard, well-known and not-so-well-known histological techniques. Lavishly illustrated in full color throughout it covers all aspects of liver pathology from the normal, the hepatidities, childhood disease and neoplasms. Throughout the text histopathologic features are correlated with clinical features to provide a practical account of how pathology impacts the diagnosis and management of liver disease. Well written, complete and concise quick reference. Helps the practicing and trainee pathologist solve diagnostic problems at the mircroscope. High quality color images complement the text throughout. Provides the user with a complete visual guide to each entity and assists in the recognition and diagnosis of any tissue sample under the microscope. Provides the trainee and general surgical pathologist with time saving diagnostic clues when dealing with difficult specimens. Glossary of frequently used terms and descriptions used in the pathology report contained at the end of the book. Useful quick reference for trainee pathologists and clinical hepatologists who may be unfamiliar with many of the terms and descriptions. Will assist in the writing and reading of a pathology report. A selection of a few general reading references at the end of each chapter. Directs the trainee or practitioner to the most relevant and authoritative sources of further information and study. A brand new chapter: Focus is on interpretation rather than a mere listing of pathological changes. The initial overview of the biopsy specimen provides critical clues to diagnosis and a highly illustrated guide to ddx will be of enormous help in avoiding diagnostic errors. Expanded coverage throughout, especially of liver tumours, pediatric fatty liver disease, neonatal cholestasis. Keeps the user apprised of the key areas of growth and development in diagnosis and interpretation. Histopathology correlated with molecular genetics and immunohistochemistry whenever clinically relevant. Keeps the user up-to-date with all of the latest tools for performing a complete and accurate diagnosis. Many new high-quality colour illustrations. Helps the user identify a wide variety of pathologic lesions. Text and images

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Chapter 2 : Histological & Histochemical Methods: Theory and Practice by John A. Kiernan

This fifth edition of Histological and Histochemical Methods continues to provide a clear and consistent introduction to the techniques, description and analysis of the chemical and physical principles of fixation, tissue processing, staining, enzyme location, immunohistochemistry and other key procedures.

Carmine[edit] Carmine staining of a parasitic flatworm. Carmine is an intensely red dye used to stain glycogen , while Carmine alum is a nuclear stain. Carmine stains require the use of a mordant, usually aluminum. Coomassie blue[edit] Coomassie blue also brilliant blue nonspecifically stains proteins a strong blue colour. It is often used in gel electrophoresis. Cresyl violet[edit] Cresyl violet stains the acidic components of the neuronal cytoplasm a violet colour, specifically nissl bodies. Often used in brain research. Crystal violet[edit] Crystal violet , when combined with a suitable mordant, stains cell walls purple. Crystal violet is the stain used in Gram staining. DAPI is also not visible with regular transmission microscopy. It may be used in living or fixed cells. DAPI-stained cells are especially appropriate for cell counting. It also imparts a strong red colour to red blood cells. Eosin may also be used as a counterstain in some variants of Gram staining, and in many other protocols. There are actually two very closely related compounds commonly referred to as eosin. Most often used is eosin Y also known as eosin Y ws or eosin yellowish ; it has a very slightly yellowish cast. The other eosin compound is eosin B eosin bluish or imperial red ; it has a very faint bluish cast. The two dyes are interchangeable, and the use of one or the other is more a matter of preference and tradition. Ethidium bromide[edit] Ethidium bromide intercalates and stains DNA, providing a fluorescent red-orange stain. Although it will not stain healthy cells, it can be used to identify cells that are in the final stages of apoptosis â€” such cells have much more permeable membranes. Consequently, ethidium bromide is often used as a marker for apoptosis in cells populations and to locate bands of DNA in gel electrophoresis. The stain may also be used in conjunction with acridine orange AO in viable cell counting. Acid fuchsine[edit] Acid fuchsine may be used to stain collagen, smooth muscle, or mitochondria. Haematoxylin[edit] Haematoxylin hematoxylin in North America is a nuclear stain. Used with a mordant, haematoxylin stains nuclei blue-violet or brown. Hoechst stains[edit] Hoechst is a bis-benzimidazole derivative compound that binds to the minor groove of DNA. Often used in fluorescence microscopy for DNA staining, Hoechst stains appear yellow when dissolved in aqueous solutions and emit blue light under UV excitation. There are two major types of Hoechst: Hoechst and Hoechst The two compounds are functionally similar, but with a little difference in structure. Hoechst contains a terminal hydroxyl group and is thus more soluble in aqueous solution, however this characteristics reduces its ability to penetrate the plasma membrane. Hoechst contains an ethyl substitution on the terminal hydroxyl group i. Starch is a substance common to most plant cells and so a weak iodine solution will stain starch present in the cells. Iodine is one component in the staining technique known as Gram staining , used in microbiology. Malachite green[edit] Malachite green also known as diamond green B or victoria green B can be used as a blue-green counterstain to safranin in the Gimenez staining technique for bacteria. It can also be used to directly stain spores. Methyl green[edit] Methyl green is used commonly with bright-field, as well as fluorescence microscopes [7] to dye the chromatin of cells so that they are more easily viewed. Methylene blue[edit] Methylene blue is used to stain animal cells, such as human cheek cells, to make their nuclei more observable. Also used to stain the blood film and used in cytology. Neutral red[edit] Neutral red or toluylene red stains Nissl substance red. It is usually used as a counterstain in combination with other dyes.

Chapter 3 : Staining - Wikipedia

"Histological and Histochemical Methods is a tour de force wholly suited to the modern age of histology and Professor Kiernan has triumphed again. To cover so much ground clearly and concisely while including the justification of the underlying chemistry makes this book unique.

Published online Feb Kiernan is a classic in the histochemical literature since its first edition, in It is hard to decide what actually is the main reason for the success of this valuable book: The purpose of this book is to teach the chemical, physical and biological principles of fixation, staining and histochemistry. I urge the reader always to determine the reason for every step in a method before doing it [â€¦. This is to encourage an intelligent approach to microtechniques, in which the user reviews the rationale of each method instead of following a list of poorly understood instructions. This is the reason: This fifth edition has been updated with the latest technical developments in the fixation and processing of samples, as well with new staining procedures, although the original organization of the volume has been maintained. The first six chapters are aimed at introducing the microtechniques and at describing the principles and procedures for fixation, processing of soft or hard tissues, embedding and mounting of sections on slides, and single and multiple staining. Professor Kiernan also wrote: Nobody reads this kind of book from beginning to end. This is certainly true; however, Chapters 1 to 6, and chapters 14 Enzyme histochemistry: Chapters 7 to 12 deal with the methods for staining cells in suspension, and with the histochemical techniques for identifying nucleic acids, different functional groups of proteins, carbohydrates and lipids. Chapter 13 concerns the methods for detecting inorganic ions or pigments, such as melanin, lipofoscins or bile pigments. Enzyme histochemistry is the subject of chapters 14 to 16, where special emphasis on hydrolases and oxidoreductases is given. In Chapter 17, the methods for localizing soluble organic compounds of low molecular weight especially amines in bright-filed or fluorescence microscopy are described, while Chapter 18 illustrates staining methods which produce deposits of metals or dark final reaction products: The final Chapter 20 collects a miscellanea of useful recipes for buffers and physiological solutions, and a list of mammalian tissues to be used as positive controls for histochemical and enzyme histochemical reactions. A table of contents precedes the main text of each chapter, and the volume ends with a very rich bibliography about fifty pages , which allows the reader to go deeper into the theory and application of specific methods, referring to the articles where these have originally been proposed. A set of questions and answers which may be interesting and useful especially for students is available at the website www. This book should be present on the bookshelves of every research or analysis Laboratory where histology and histochemistry are routinely used, as an essential reference source of basic and practical information for scientists and technicians. It may also be envisaged as a textbook of histochemical techniques for students in advanced courses of biomedical technology, histopathology and laboratory medicine. Articles from European Journal of Histochemistry:

Chapter 4 : Histological and Histochemical Methods - Theory and Practice

It is common practice to use a recipe book of histological and histochemical methods and follow the procedures. Unfortunately, there is an overall lack of interest in.

Histology Table of contents 1. Introduction to microtechnique 2. Decalcification and other treatments for hard tissues 4. Processing and mounting 5. Histological staining in one or two colours 7. Staining blood and other cell suspensions 8. Methods for connective tissue 9. Methods for nucleic acids Organic functional groups and protein histochemistry Methods for inorganic ions Methods for soluble organic compounds of low molecular weight Metal reduction and precipitation methods Miscellaneous data Bibliography Glossary Index show more Review quote John Kiernan has published extensively on histochemical methods since his first scientific paper in His textbook encompasses his wide personal experience and interest in dyes, staining and histochemical methodologies. It is exceptional that he is the sole author of such a compendium in light of the wide range and amount of material included To understand the mechanisms or pitfalls involved in a specific histochemical technique, one usually can find the answer in only a very select group of books that deal with the theory and practice of histological and histochemical techniques. It was a pleasure to see that John Kiernan continues to update his book. Most practicing histochemists and research students involved in biomedical research and diagnostic histopathology tend to use histochemical methods without understanding the chemistry involved in the staining reactions or the limitations of the procedures. It is common practice to use a recipe book of histological and histochemical methods and follow the procedures. Unfortunately, there is an overall lack of interest in the chemistry of the dyes or how they stain specific products in cells and tissues and the last time that many histochemists considered chemical formulae was often during their undergraduate studies. The current book by Kiernan takes into account the chemistry involved with the premise that such knowledge makes us better histochemists and researchers. Or in the words of the author: A similar situation occurs in recording images with a microscope. If you understand the construction of the instrument and its optical components and set it up accordingly, you will be a much better microscopist and obtain optimal images. The first edition of this book appeared in , with subsequent editions in and This latest edition includes in a single volume an extensive survey of the chemical and physical principles of fixation, staining, and histochemistry. In addition to the classical techniques still in use, the new edition has been expanded to include more recent developments and includes newer procedures that are anticipated to become standard methods in research and diagnostic histopathology. The book encompasses the relations of chemical structures and reactions to fixation, tissue processing, staining, enzyme localization and immunohistochemistry. The first four chapters contain descriptions of the newer reagents and techniques, with subsequent chapters dealing with historical "classical" staining with dyes. The chapter on nucleic acids now includes discussion of in situ hybridization and methods for detecting apoptotic cells. Although the histological and histochemical techniques for animal tissues predominate, there is now some discussion and methodology also applicable to fixation, processing and staining of plant tissues and microorganisms. The bibliography is extensive and encompasses over references. The text is written in classical English spelling and not US spelling e. I do not know how this affects the readership in the major markets in the US. The author tries to correct the inconsistencies of biologists in their spelling of names of dyes. The ending "-ine" should be used when the dye is an amine or a derivative of an amine e. I think that the widespread use of incorrect spelling e. It may be a surprise for the general reader to learn that hematoxylin is not a dye, but only after its partial oxidation to its active ingredient, hematin. This explains why we leave freshly made hematoxylin solution to mature in order for it to become effective if you do not purchase a commercial solution of hematoxylin, which now seems to be common practice. I was also pleased that the correct explanation for the von Kossa technique was given. It is commonly designated as a histochemical method for calcium but it is really a method for phosphate and carbonate, the anions with which the metal is associated in normal and pathological calcified tissues. I personally believe that this text should be found on the bookshelves of every histology and histochemistry laboratory as a basic reference source. It is not a book to be read from start to finish, but should be consulted

by researchers and in particular students before undertaking histochemical staining procedures. The book is very reasonably priced and to be objective, the purchase price is less than the cost of one or two antibodies or most laboratory chemicals, which, unlike a book, have a very limited life.

Chapter 5 : Histological and Histochemical Methods, fifth edition

This book is a compendium of histological and histochemical methods. This is a third edition; the second edition was published in The book is written to provide a rational, theoretical, and practical guide to the most widely used histological and histochemical techniques.

Fixation histology Chemical fixatives are used to preserve tissue from degradation, and to maintain the structure of the cell and of sub-cellular components such as cell organelles e. For electron microscopy, the most commonly used fixative is glutaraldehyde , usually as a 2. These fixatives preserve tissues or cells mainly by irreversibly cross-linking proteins. The main action of these aldehyde fixatives is to cross-link amino groups in proteins through the formation of methylene bridges -CH2- , in the case of formaldehyde, or by C5H10 cross-links in the case of glutaraldehyde. This process, while preserving the structural integrity of the cells and tissue can damage the biological functionality of proteins, particularly enzymes , and can also denature them to a certain extent. This can be detrimental to certain histological techniques. Further fixatives are often used for electron microscopy such as osmium tetroxide or uranyl acetate. However, extraction and analysis of nucleic acids and proteins from formalin-fixed, paraffin-embedded tissues is possible using appropriate protocols. It is often used after surgical removal of tumors to allow rapid determination of margin that the tumor has been completely removed. Processing - dehydration, clearing, and infiltration[edit] The aim of tissue processing is to remove water from tissues and replace with a medium that solidifies to allow thin sections to be cut. For light microscopy, paraffin wax is most frequently used. Since it is immiscible with water, the main constituent of biological tissue, water must first be removed in the process of dehydration. Samples are transferred through baths of progressively more concentrated ethanol to remove the water. This is followed by a hydrophobic clearing agent such as xylene to remove the alcohol, and finally molten paraffin wax , the infiltration agent, which replaces the xylene. Paraffin wax does not provide a sufficiently hard matrix for cutting very thin sections for electron microscopy. Instead, resins are used. Epoxy resins are the most commonly employed embedding media, but acrylic resins are also used, particularly where immunohistochemistry is required. Again, the immiscibility of most epoxy and acrylic resins with water necessitates the use of dehydration, usually with ethanol. Embedding[edit] OCT embedding [13] optimal cutting temperature compound After the tissues have been dehydrated, cleared, and infiltrated with the embedding material, they are ready for external embedding. During this process the tissue samples are placed into molds along with liquid embedding material such as agar, gelatine, or wax which is then hardened. This is achieved by cooling in the case of paraffin wax and heating curing in the case of the epoxy resins. The acrylic resins are polymerised by heat, ultraviolet light, or chemical catalysts. The hardened blocks containing the tissue samples are then ready to be sectioned. Because formalin-fixed, paraffin-embedded FFPE tissues may be stored indefinitely at room temperature, and nucleic acids both DNA and RNA may be recovered from them decades after fixation, FFPE tissues are an important resource for historical studies in medicine. Embedding can also be accomplished using frozen, non-fixed tissue in a water-based medium. Pre-frozen tissues are placed into molds with the liquid embedding material, usually a water-based glycol, OCT, TBS, Cryogel, or resin, which is then frozen to form hardened blocks. Microtome For light microscopy, a steel knife mounted in a microtome is used to cut 4- micrometer -thick tissue sections which are mounted on a glass microscope slide. For transmission electron microscopy, a diamond knife mounted in an ultramicrotome is used to cut nanometer -thick tissue sections which are mounted on a 3-millimeter-diameter copper grid. Then the mounted sections are treated with the appropriate stain. Sections can be cut through the tissue in a number of directions. For pathological evaluation of tissues, vertical sectioning, cut perpendicular to the surface of the tissue to produce a cross section is the usual method. Horizontal also known as transverse or longitudinal sectioning, cut along the long axis of the tissue, is often used in the evaluation of the hair follicles and pilosebaceous units. Frozen section procedure Fixed or unfixed tissue may be frozen and sliced using a microtome mounted in a refrigeration device known as a cryostat. The frozen sections are mounted on a glass slide and may be stained to enhance the contrast between different tissues. Unfixed frozen sections can also be

used for studies requiring enzyme localization in tissues and cells. It is necessary to fix tissue for certain procedures such as antibody linked immunofluorescence staining. Frozen sectioning can also be used to determine if a tumour is malignant when it is found incidentally during surgery on a patient. Sample of a trachea coloured with hematoxylin and eosin Main article: Staining Example of staining [14] in light microscopy: Staining is employed to give both contrast to the tissue as well as highlighting particular features of interest. Where the underlying mechanistic chemistry of staining is understood, the term histochemistry is used. Hematoxylin, a basic dye, stains nuclei blue due to an affinity to nucleic acids in the cell nucleus; eosin, an acidic dye, stains the cytoplasm pink. Uranyl acetate and lead citrate are commonly used to impart contrast to tissue in the electron microscope. There are many other staining techniques that have been used to selectively stain cells and cellular components. One of these techniques involves marking peripheral tumors or surgical margins, in which a certain color of dye is applied to the posterior border of a sample, another to the anterior, etc. Other compounds used to color tissue sections include safranin , Oil Red O , Congo red , Fast green FCF , silver salts, and numerous natural and artificial dyes that usually originated from the development of dyes for the textile industry. Histochemistry refers to the science of using chemical reactions between laboratory chemicals and components within tissue. A commonly performed histochemical technique is the Perls Prussian blue reaction, used to demonstrate iron deposits in diseases like hemochromatosis. Histology samples have often been examined by radioactive techniques. In historadiography , a slide sometimes stained histochemically is X-rayed. More commonly, autoradiography is used to visualize the locations to which a radioactive substance has been transported within the body, such as cells in S phase undergoing DNA replication which incorporate tritiated thymidine , or sites to which radiolabeled nucleic acid probes bind in in situ hybridization. For autoradiography on a microscopic level, the slide is typically dipped into liquid nuclear tract emulsion, which dries to form the exposure film. Individual silver grains in the film are visualized with dark field microscopy. Recently, antibodies have been used to specifically visualize proteins, carbohydrates, and lipids. This process is called immunohistochemistry , or when the stain is a fluorescent molecule, immunofluorescence. This technique has greatly increased the ability to identify categories of cells under a microscope. Other advanced techniques, such as nonradioactive in situ hybridization, can be combined with immunochemistry to identify specific DNA or RNA molecules with fluorescent probes or tags that can be used for immunofluorescence and enzyme-linked fluorescence amplification especially alkaline phosphatase and tyramide signal amplification. Fluorescence microscopy and confocal microscopy are used to detect fluorescent signals with good intracellular detail. Digital cameras are increasingly used to capture histological and histopathological image Common laboratory stains[edit].

Chapter 6 : Histological And Histochemical Methods | Download eBook PDF/EPUB

The gross histological features of the spleen confirmed the presence of a capsule containing numerous collagen fibers surrounded the spleen of the African catfish.

Chapter 7 : Histological and Histochemical Methods : John A. Kiernan :

Histochemistry Staining Methods We offer histochemistry staining that may be performed on paraffin embedded tissue section or frozen tissue sections. And o ur scientists are highly experienced in histochemical staining.

Chapter 8 : Histology - Wikipedia

The relations of chemical structures and reactions to fixation, tissue processing, staining, enzyme location, immunohistochemistry and other procedures are explained in simple, descriptive terms.