and, as a result, the structures under study, bones for instance, appeared black. Today, physicians have been trained to work with negatives, in which the image of bones and other structures appears white).

When radioactive elements are detected in histological sections the object under investigation is itself the source of the radiation which influences the emulsion. A black image is then produced, which is a photographic *positive*. Such procedure may be referred to as "autography", that is, according to the Oxford English dictionary (1975), "the reproduction of form or outline of anything by an impression from the thing itself". In the initial work of our group with radioiodine and radiophosphorus 34 years ago, the procedure was called "radioactive autography", two words which were later condensed into "radioautography". Even though the term "autoradiography" is the more popular, the term "radioautography" is the more correct of the two" (Leblond CP *Am J Anat* 160:113-158, 1981).

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b. Applications. Radioautography has played important roles in contributing to our understanding of many fundamental and dynamic processes of cells and tissues including DNA synthesis and cell division, protein synthesis and secretion, function of the Golgi apparatus, mechanisms of exocytosis and endocytosis, and hormone binding to receptors and target cells.

3. Bromodeoxyuridine detection. Cell proliferation has been studied in many tissues by a variety of techniques such as counting mitotic figures and radioautography following [^{3}H]thymidine incorporation into DNA as mentioned above. In 1982, Gratzner et al. described a sensitive, nonisotopic, monoclonal antibody method for detecting DNA replication in single cells (Gratzner HG *Science* 218:474-475, 1982).

a. Methods. In this immunohistochemical procedure, the thymidine analog 5-bromodeoxyuridine (BrdU) - which is incorporated into nuclear DNA during S-phase before mitosis - is localized in tissue sections and reveals cells that have recently undergone cell division. Although first described using an immunofluorescent chromogen, BrdU IHC like other IHC procedures can use a variety of chromogens.

b. Applications. This technique is a sensitive method that is widely used for detecting DNA replication in vitro and in situ.

4. In situ hybridization. It has long been recognized that double-stranded DNA in solution can be denatured ("melted") into single strands by heat or elevation of pH. Reversal of these manipulations allows the single strands to be recombined ("annealed"). This process of molecular **hybridization** is highly specific since only complementary strands will combine. Thus, hybrids between DNA-DNA, DNA-RNA, and RNA-RNA are possible.

a. Methods. These hybridization techniques were then applied *in situ* to cytological preparations on microscope slides to permit detection and localization of RNA-DNA hybrids (Gall JG & Pardue ML. *Proc Natl Acad Sci USA* 63:378-383, 1969) and DNA-DNA hybrids (Pardue ML & Gall JG. *Proc Natl Acad Sci USA* 64:600-604, 1969) at the cell and tissue levels. These studies used radioactive (tritium-labeled) probes, which were detected in the complementary hybrids by radioautography. Subsequently, non-isotopic techniques were employed using enzyme-labeled (e.g., peroxidase-antiperoxidase) or fluorescent-labeled probes, the latter of which is referred to as **FISH** (=**F**luorescence **I**n **S**itu **H**ybridization). EM techniques have also been developed.

b. Applications. These techniques have had great utility in detecting nucleic acids in their cellular environment. Extremely useful in both research and clinical diagnosis, important applications include time course and location (differential gene expression) of mRNA transcripts, location of genes to specific chromosomes, recognizing chromosome abnormalities and pathologies, and diagnosis of genetic diseases.

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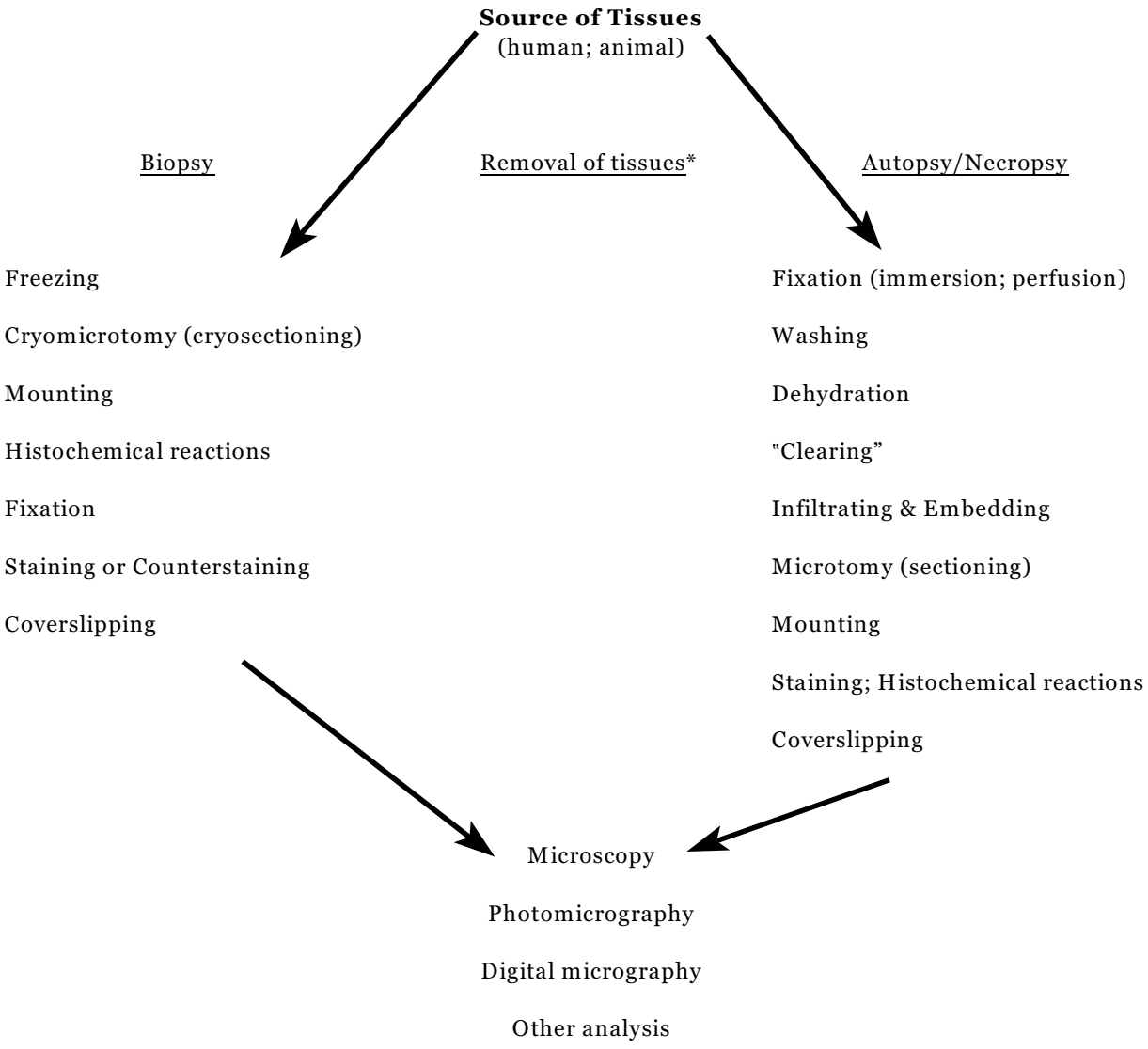
Figure 1-1. Flowchart for the Preparation of Tissues for Microscopic Analysis



*Although the path at the left for biopsy material may be more typical and vice versa, either paths can be used for biopsy and autopsy/necropsy specimens.

Table 1-1. Important Stains and Histochemical Reactions (HR*).

Reagent	Type	Color	Tissue components visualized
Hematoxylin	"basic" dye	blue to bl-black	nuclei, cytoplasmic and extracellular anionic substances (e.g., RER, proteoglycans)
Iron hematoxylin	"basic" dye	bl-black to black	nuclei, mitochondria, muscle striations, RBCs, meiotic chromosomes
PTAH	>"basic" dye	bl-black	terminal bars, intercalated disks
Toluidine blue Methylene blue Azures	basic dyes	blue	cellular & EC anions; metachromatic (purple) with high density polyanions (e.g., mast cell granules, basophil granules, cartilage ECM)
Eosin Orange G Picric acid Fast green Aniline blue	acid dyes	pink-red orange yellow green blue	cellular and EC cationic substances (e.g., Hb, collagen, muscle proteins, keratin tonofibrils)
Alcian blue	basic dye	bl-green	high density polyanions (e.g., GAG's)
PAS	HR*	magenta	*vic*-glycols (e.g., glycogen, mucins, GAG's)
AB-PAS	dye/HR*	bl-green magenta royal blue	anions (e.g., acidic mucins w/out *vic*-glycols) *vic*-glycols (e.g., mucins w/out acidic groups) anions and *vic*-glycols (e.g., mucins w/ both)
Feulgen reaction	HR*	magenta	nuclear DNA (mitochondrial DNA in too low concentration to be detected)
Silver	metal	black	Golgi apparatus, reticular fibers, neurofibrils (argyrophilia: affinity for silver)
Romanowsky dyes	mixture of	dk purple	leukocyte nuclei (P), basophil granules (M),
Wright	acid and		platelet granulomere (P)
Giemsa	basic dyes	blue	cytoplasm of agranulocytes, esp. lymphs (O)
		lt purple	azurophilic granules (P)
		pink-red	RBC cytoplasm, eosinophil granules (O)
Vital stains	nontoxic		phagocytic vacuoles in cells of mononuclear
Trypan blue	colloidal	blue	phagocyte system, e.g., CT macrophages
India ink	suspensions	black	
Lipid stains			
Sudan black	lipid-soluble	black	in situ lipid droplets containing triglycerides,
Oil red O	particles	red	sterols, etc
Osmium tetroxide	metal oxide	black	unsaturated lipids, phospholipids
Elastin stains			elastin, fibers and sheets
Orcein		red	
Resorcin fuchsin		purple	
Verhoeff		black	
Masson trichrome	polychrome	bl-black	nuclei
	(CT stain)	red	cytoplasm, muscle
		green;blue	collagen, mucus

*HR =histochemical reaction