

## **Invited Review**

# **Tissue transfers: Substrates for cytology and cytochemistry of animal tissues**

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**Summary.** The Tissue Transfer Technique (TTT) is a novel method of sampling animal tissue that can be used to study tissue morphology, chemistry and physiology. This review provides an overview of the technique and demonstrates its use to detect the tissue distribution of specific epitopes, lectin binding sites and nucleic acids as well as its application as an organ monolayer in culture. These applications are compared and contrasted with standard histological techniques including the "Tissue Printing Technique" developed to sample plant tissue.

**Key words:** Tissue transfer, Immunocytochemistry, Cytochemistry

### **The evolution of tissue sampling techniques**

The ability to sample biological material while maintaining *in vivo* architecture and chemical composition is fundamental to both basic science and clinical studies. The need to preserve tissue architecture and cytological detail typically requires the fixation of dissected tissue followed by dehydrating, embedding and sectioning with a microtome. This procedure is exacting, laborious and time consuming. When rapid sampling and analysis are required, frozen sections can be made, though cryosections suffer a loss of both cytological and histological detail.

In cases where architectural detail is not required, "touch preps" using glass slides applied to the freshly cut surface of an organ or tissue allows for the rapid collection of cellular constituents for both cytological and cytochemical analysis. An interesting outgrowth of this approach is the technique of "tissue printing" in which a membrane substrate is substituted for the glass slide. Tissue printing allows the collection of both cellular fluids and cellular constituents while preserving the spatial distribution of these components (Reid et al., 1992).

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The tissue printing technique has found its primary application in the botanical sciences where rigid cells walls actually make a physical impression on the membrane. The tissue printing of plants involves the transfer of cellular fluids onto the membrane substrate while retaining their spatial distribution in the original specimen. Once imbibed and immobilized on a membrane substrate, it was possible to detect and characterize the macromolecular constituents of the intracellular fluid using a variety of techniques such as Northern, Southern and Western blotting. By comparing a histological section (or a physical impression) from the same region as that used to prepare the tissue print, it is possible to correlate tissue structure with the localization of macromolecules (Varner and Ye, 1994). While the tissue printing procedure has found widespread acceptance as a sampling method for botanical specimens, its application with animal tissue has been limited (reviewed by Reid, 1992). Several studies with animal tissues have demonstrated that it is possible to transfer macromolecules and cells from frozen sections to membrane substrates but such preparations show limited histological resolution (Blount et al., 1986; Okabe et al., 1993). Tissue printing has also been used to isolate cells from brain tissue (Hernandez Bronchund et al., 1988) where individual nerve cells can be blotted onto membranes with their processes intact (Barres et al., 1990) and are accessible for electrophysiology. It is also possible to use membranes as a substrate for the collection of isolated cells using either plating or suction (Seshi et al., 1986). While tissue printing with membranes is interesting and useful for some applications, its utility for routine sampling for histological and cytological work is limited. The availability of a method for the rapid sampling of both large and small portions of animal tissue that both preserved histological architecture and allowed cytological and biochemical analysis awaited the next development in the evolution of histological sampling techniques, the tissue transfer technique.

### **The tissue transfer technique**

The observation that membrane substrates could be

used to directly sample biological components led to studies that explored the use of membrane substrates for sampling animal material and the development of a method known as the "Tissue transfer Technique". Unlike tissue prints, tissue transfers sample intact layers of cells from freshly cut animal tissue and transfers them to a membrane while preserving organ architecture and cytomorphology (Matyas et al., 1995a). A comparison of the tissue transfer technique with other methods routinely used to sample tissues for histomorphology is presented in Table 1.

### The basic method

The material to be sampled is cut with a scalpel or single edge razor blade to expose a freshly cut surface. A piece of membrane is placed onto a sheet of aluminium foil. Tissue transfers can be carried out using a variety of membrane substrates, the most common being nylon and nitrocellulose. Nitrocellulose is used if the transfers are to be viewed by transmitted light microscopy, they are dehydrated, cleared in xylene. However, dehydration must be brief as nitrocellulose dissolves in high grades of ethanol and acetone. The freshly cut surface of the material is placed cut-face-down on the membrane. Slight pressure is applied with a gloved hand allowing the entire cut surface of the material to come in contact with the membrane. After 30 seconds, the material is carefully removed from the membrane either by carefully lifting the tissue off with forceps or by inverting the material-membrane complex and peeling off the membrane (Fig. 1A). If destined for histomorphology, the tissue transfer can then be immersed in the desired fixative for 5-15 minutes depending on tissue type. Subsequently the transfer can be prepared for either light or electron microscopy. For electron microscopy the transfer can be treated as one would treat a tissue specimen and standard protocols can be applied (Matyas et al., 1995a). For light microscopy, specimens can be used for incident light examination, transmitted light microscopy, or immunofluorescence. Multiple transfers can be made from the same cut surface though with diminishing returns (Matyas et al., 1995a). This allows the collection of serial specimens that can be used as controls as well as treated, stained or probed using different procedures for comparative purposes.

### Tissue transfers in cytochemistry

The following section illustrates that tissue transfers can be used for conventional microscopy, enzyme cytochemistry, lectin cytochemistry, immunocytochemistry, and *in situ* hybridization cytochemistry demonstrating the utility of tissue transfers for various applications in cytophysiology and clinical pathology. To compare the quality of cytochemistry on tissue transfers, cryosections have been processed in parallel with tissue transfers.

Kidney is used here as a representative organ since it is composed of several cell and tissue types that have a characteristic arrangement within the organ and its composition can be demonstrated using four different cytochemical systems: enzyme/substrate-, immuno-, lectin-, and *in situ* hybridization cytochemistry (Bancroft, 1982; Helwig et al., 1984; Hanei et al., 1994; Statlin et al., 1992). An example of the ability of tissue transfers to capture and illustrate both the macro and micro-architecture of the rabbit kidney is illustrated in Fig. 1B. Major architectural features such as the renal cortex, medulla and pelvis are clearly visible. Careful inspection reveals tissue morphology throughout the transfer. Particularly prominent are whole glomeruli, renal tubules and accumulations of fat.

### Enzyme cytochemistry

Acid phosphatase is an enzyme present in rabbit kidney glomeruli, blood vessels, and renal tubules (Helwig et al., 1984). Acid phosphatase staining can be observed on tissue transfers and cryosections using the Naphthol AS-BI phosphate/basic fuchsin substrate/detection system for frozen sections (Bancroft, 1982). Acid phosphatase activity is observed within convoluted tubules and collecting ducts on both cryosections and tissue transfers; staining is also seen in the glomeruli of tissue transfers (Fig. 2b,d) but not in 6  $\mu\text{m}$  thick cryosections. Although not generally recognized, glomerular acid phosphatase activity has been described (Helwig et al., 1984). Interestingly, when acid phosphatase activity is compared between kidney cryosections of varying thickness, it is only detected in sections 40  $\mu\text{m}$  to 60  $\mu\text{m}$  thick (Fig. 2c), which suggests

**Table 1.** Comparison of the tissue transfer technique with other methods.

METHOD OF TISSUE PREPARATION	CYTOLOGICAL DETAIL	HISTOLOGICAL DETAIL	RETENTION OF ENZYME ACTIVITY	RETENTION OF EPITOPE ACTIVITY	RETENTION OF mRNA FOR ISH
Frozen section	2	2	5	5	5
Touch prep	5	1	N/A	N/A	3
Paraffin section	4	4	3	3	3
Plastic section	5	5	2	2	2
Tissue transfer technique	4	3	5	5	5
Scanning EM	1	3	N/A	N/A	N/A
Transmission EM	5	5	1	1	1

\* Quality is rated from poor: 1, to excellent: 5.

## Tissue transfers

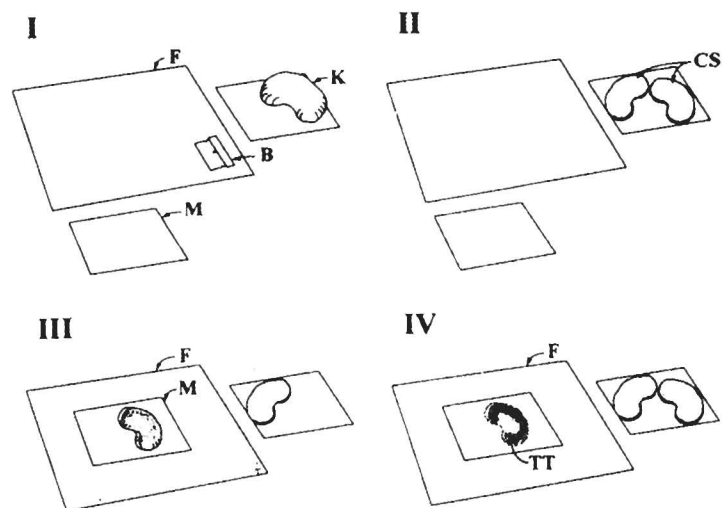
that both tissue transfers and thick cryosections sample sufficient tissue to detect this enzyme. Virtually no background staining is observed on either cryosections or tissue transfers provided that fresh sodium nitrite (used to prepare the coupling dye) is added to the enzyme/substrate reaction mixture.

*Immunocytochemistry*

The application of tissue transfers in immunocytochemistry is illustrated using a panel of primary antibodies to both nuclear (DNA, histone) and cytoplasmic (cytokeratin, vimentin, tubulin, centrosome) antigens coupled with one of several detection methods including fluorescence, enzyme-coupled color reagents, and chemiluminescence. Negative controls are prepared by omitting primary antibodies. Fluorescein isothiocyanate (FITC)-conjugated secondary antibodies are detected by indirect immunofluorescence while secondary antibodies conjugated to biotin are reacted with avidin-biotin-horseradish peroxidase (ABC-HRP) complex and detected by the peroxidase-diaminobenzidine (or chloronaphthol) color reaction (Sambrook et al., 1989). Both indirect immunofluorescence and ABC-HRP detection give clear localization of nuclear and cytoplasmic antigens on tissue transfers and the localization of antigens are comparable between cryosections and tissue transfers. Figs. 2e,g, 3a,b are a gallery of images showing immunostaining of both cytoplasmic and nuclear antigens using FITC and ABC-HRP detection systems. Again, virtually no background is observed on cryosections or tissue transfers. In general, chemiluminescence is not a sensitive detection system for either tissue transfers or cryosections.

*Lectin cytochemistry*

Lectin cytochemistry is illustrated using peanut agglutinin (PNA), a lectin that binds specifically to a galactose dimer present in cortical collecting duct cells (Statlin et al., 1992; Hanai et al., 1994). In the illustrated specimen, fluorescein-conjugated PNA was incubated for 30 min with the tissue transfers and the tissue transfers were examined by epifluorescent microscopy. PNA reactivity is localized to the apical side of the



**Fig. 1. A.** Diagram showing the basic method of animal tissue transfer (I-IV). F: Aluminum foil; K: kidney; R: razor blade; CS: cut surface; M: membrane substrate; TT: tissue transfer. **B.** Tissue Transfer. An unmounted, air-dried, kidney transfer made on a nylon membrane from a whole rabbit kidney cut in the coronal plane is illustrated. This transfer was stained with toluidine blue O. The kidney cortex (C), medulla (M), and pelvis (P) are clearly visible. Reflected light microscopy. Original magnification  $\times 1,8$

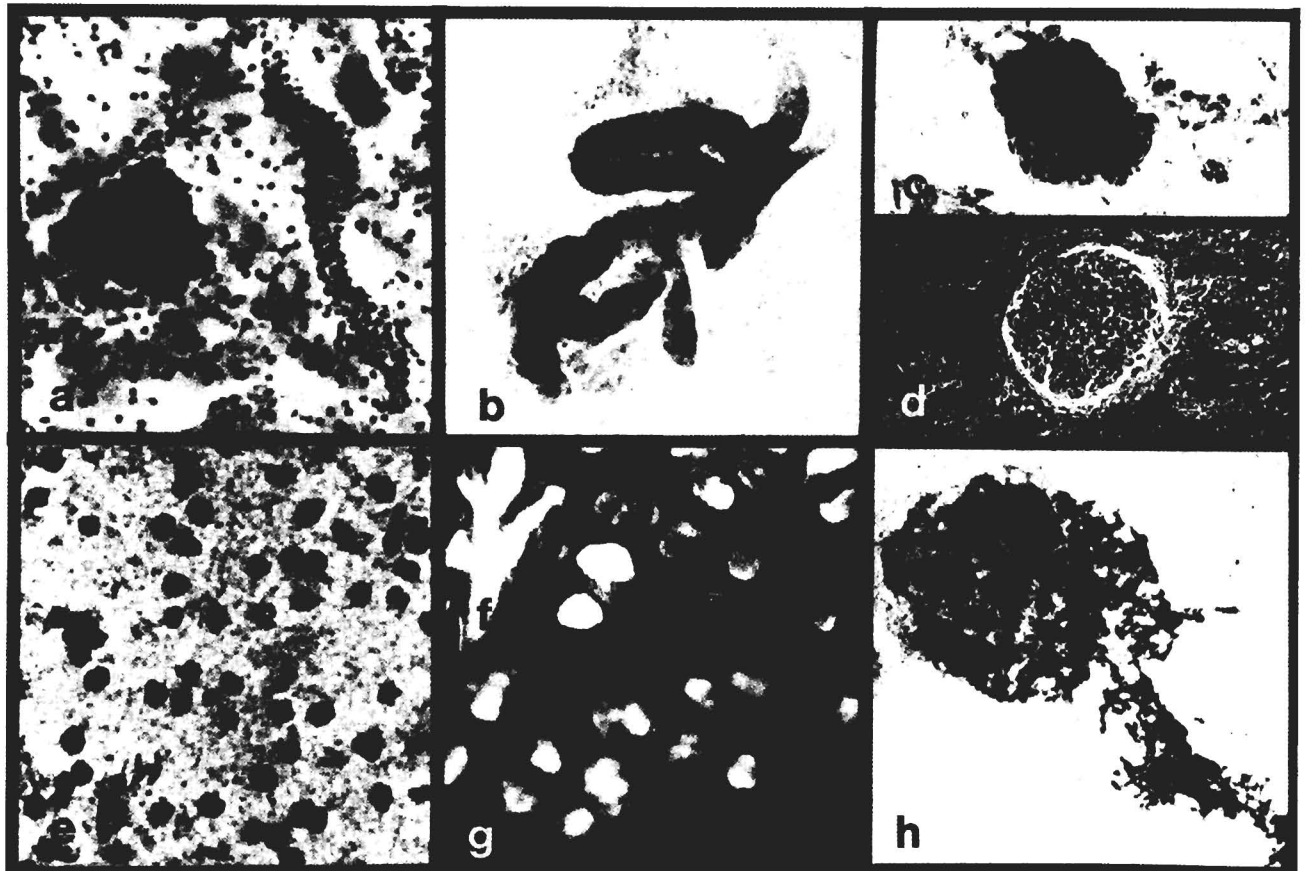
collecting ducts and signal intensity is comparable on tissue transfers (Fig. 3c,d) and cryosections with no observable background. In contrast to the fluorescent label, PNA is undetectable on either tissue transfers or cryosections with HRP-conjugated PNA, this is likely due to the relative insensitivity of the peroxidase chemistry.

#### *In situ* hybridization

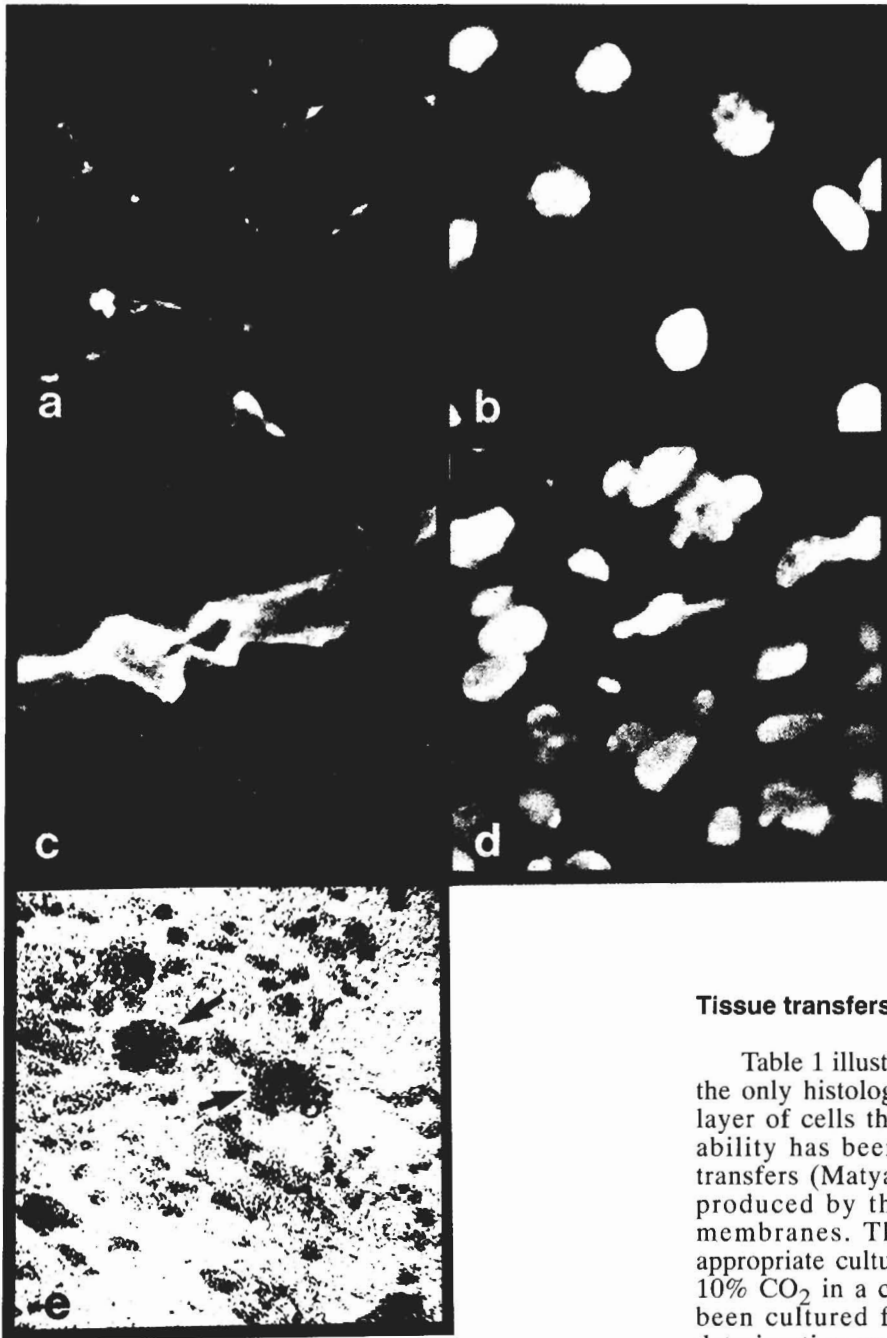
*In situ* hybridization on tissue transfers is illustrated here using a 300 nt. digoxigenin-labelled RNA probe complementary to 188 nt. for U2@ mRNA, which encodes for a protein involved in splicing heteronuclear RNA in mammalian cells (Darnell et al., 1990). The expression of U2 is ubiquitous and significantly large to be useful as a positive control for *in situ* hybridizations. The U2 probe has been labelled by *in vitro* transcription including digoxigenin-conjugated uridine. U2 RNA was hybridized and washed at 50°C and was detected with

anti-digoxigenin antibody conjugated with alkaline phosphatase. Negative controls have been prepared by predigesting the tissue transfers and cryosections with RNase. The hybridization signal for U2 is comparable on tissue transfers and cryosections (Fig. 3e) and virtually no background was observed. Thus, like tissue prints (Varner and Ye, 1994), tissue transfers are good substrates for a variety of cytochemical techniques.

While comparable to cryosections in many ways, there are several notable limitations in using tissue transfers as substrates for cytochemistry. First, unlike tissue sections, tissue transfers are not entirely uniform in thickness. In some circumstances, the thicker parts of these preparations may limit cytological resolution (however, this can also be an advantage, see below). A second limitation is that some fixatives, dehydrating agents, and clearing agents (notably acetone and absolute ethanol) are incompatible with nitrocellulose membranes, causing them to dissolve. It is possible to dehydrate tissue transfers with isopropanol rather than



**Fig. 2.** Gallery of histochemical stains on tissue transfers. **a.** Tissue transfer of rabbit kidney, hematoxylin and eosin stained. Glomerulus on the left, renal tubule on the right. x 20. **b.** Tissue transfer, acid phosphatase/substrate reaction, no counterstain: renal tubules. x 20. **c.** Tissue transfer, acid phosphatase/substrate reaction, no counterstain: glomerulus and tubules. x 20. **d.** Sixty  $\mu$ m-thick cryosection, acid phosphatase substrate reaction, no counterstain: glomerulus and tubules. x 20. **e.** Tissue transfer, anti-histone primary antibody, biotin conjugated secondary antibody, avidin-biotin-HRP, DAB detection, no counterstain: tubular cells. x 20. **f.** Tissue transfer, anti-DNA primary antibody, FITC-conjugated secondary antibody: tubular cells. x 40. **g.** DAPI counterstain showing nuclei in the same field as (f). **h.** Tissue transfer, anti-vimentin primary antibody, biotin conjugated secondary antibody, avidin-biotin-HRP, DAB detection, no counterstain: glomerulus. x 20



**Fig. 3.** Gallery of histochemical stains on tissue transfers. **a.** Tissue transfer, anti-vimentin primary antibody, FITC-conjugated secondary antibody: part of glomerulus. x 40. **b.** DAPI counterstain showing nuclei in the same field as (a). **c.** Tissue transfer, FITC-conjugated PNA: collecting ducts. x 40. **d.** DAPI counterstain showing nuclei in the same field (c). **e.** In situ localization of U2 mRNA. Arrows show glomeruli. x 10

#### Tissue transfers as organ monolayers in culture

Table 1 illustrates that the tissue transfer technique is the only histological technique that produces an intact layer of cells that can be maintained in culture. This ability has been demonstrated using kidney tissue transfers (Matyas et al., 1995b). Tissue transfers are produced by the standard technique using sterile membranes. The transfers are then placed in the appropriate culture media and incubated at 37 °C with 10% CO<sub>2</sub> in a closed chamber. Kidney transfers have been cultured for up to 96 hrs with no significant deterioration as assayed by electron microscopy. The demonstration that tissue transfers can be cultured for extended periods illustrates that these preparations have utility in both anatomical and physiological studies. In particular, cultures of tissue transfers may be useful for investigating mechanisms of native cell-to-cell interactions. In addition, tissue transfers from malignant tissues can be used to study drug sensitivity of component cell populations. Such application could prove useful in determining specific drug treatments for individual patients as well as in the development of new drugs.

ethanol however some antigens are best preserved when fixed in acetone. Hence, some routine methods are not fully compatible with tissue transfers. It should be noted that nylon membranes resist dissolution in these solvents, but they cannot be completely cleared; hence, nitrocellulose remains the membrane of choice for transmitted-light microscopy. For epifluorescent microscopy nylon membranes are excellent since membrane opacity does not affect the sensitivity of the detection system.

### Unique advantages and applications of the tissue transfer technique

One unique feature and a major advantage of tissue transfers is that it has the flexibility to be able to sample very large areas of tissue as well as very small areas. The ability to sample areas larger than included in a conventional section can facilitate detecting regional differences within an entire organ eg. prostate, kidney or tissue and improve the chance of detecting components that are in low concentrations or are confined to small portions of a particular structure. The ability to produce large tissue transfers is particularly useful for studying regional differences in a whole organ and can be used to map tumour sites in pathological specimens such as those of the prostate. In this context, one yet unexplored application of the tissue transfer is in the sampling of tissue at a surgical site within a patient during and following surgery. For example, following tumour removal, it is possible to sample all the exposed tissue remaining in the patient. Thus it is possible to examine for residual tumour material over a region far greater than that encompassed by conventional quick sections.

The tissue transfer contains information that places it in between that which can be obtained from a gross specimen and that found in a conventional thin section. As such, the tissue transfer have both research and clinical applications. Further, tissue transfers can also be utilized in an educational context as a teaching tool for demonstrating tissue and organ architecture as well as aiding the student in appreciating how successive levels of histological organization are integrated together. Another important feature and advantage to the tissue transfer process is that tissue transfers can be prepared and processed in a fast and cost efficient manner. Since they are thin, porous, flexible, and dimensionally stable, tissue transfers can also be collected (or archived) and processed in bulk in, for example, plastic bags like Northern and Southern blots. For large numbers of samples this can be economical.

The ability to preserve both macro- and micro-architecture coupled with the ability to visualize specific macromolecules while retaining tissue and organ context, combined with the potential benefits of both a large working section and multiple sample processing, makes the tissue transfer technique useful for augmenting conventional histological methods. Furthermore, since tissue transfers can be maintained in culture (Matyas et al., 1995b), the ability to perform cytochemistry on tissue transfers facilitates studies of cell, tissue, and organ metabolism.

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