Background

The goal of microdissection is to capture pure populations of cells for downstream molecular analysis. Manual microdissection is time consuming, labor-intensive, and limited by manual dexterity, making it impractical for accumulation of sufficient tissue for meaningful analysis. Laser Capture Microdissection (LCM) eliminates the manual component, but remains too time-consuming and costly for routine use.

Target Activated Microdissection (TAM) offers fast, precise, operator-independent capture of desired cell types. The procedure begins with immunohistochemistry to stain target cells.

A primary antibody binds a specific target biomarker molecule (antigen).

A secondary antibody with peroxidase activity is added and binds the primary antibody.

Diaminobenzidine (DAB) is added and oxidized by the peroxidase, forming a brown precipitate stain within the desired targets.

Once the tissue slide has been IHC stained, it is ready for microdissection with TAM. This involves three main steps:

- Laser capture microdissection (LCM) eliminates the manual component, but remains too time-consuming and costly for routine use.
- The specific objectives are:
  1. Develop a quantitative method to accurately assess efficiency and specificity of transfer.
  2. Demonstrate specific capture of nuclei from IHC stained cells when IHC biomarkers are not in the cell nucleus.

Objectives

A method to quantitatively assess the efficiency and specificity of transfer is needed to evaluate the advantages TAM offers in speed, ease, and specificity. Such a method would also aid in selection of appropriate transfer films and dosimetry, preferably based on image analysis of the stain distribution and prior knowledge of the stained target localization, in order to achieve optimal dissection of specific tissue targets.

The specific objectives are:

1. Develop a quantitative method to accurately assess efficiency and specificity of transfer.
2. Demonstrate specific capture of nuclei from IHC stained cells when IHC biomarkers are not in the cell nucleus.

Evaluating Transfer Efficiency

The absorption spectrum of oxidized DAB is shown to the right. The blue box indicates the region excited by the flashlamp. DAB's spectral properties are such that it absorbs more strongly in the blue wavelength range than in the red. We can take advantage of this to differentiate stained tissue from unstained tissue by splitting the image into its RGB channels and computing the blue over red ratio as shown below.

Unoxidized DAB (substrate)
Oxidized DAB

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1. A primary antibody binds a specific target biomarker molecule (antigen).
2. A secondary antibody with peroxidase activity is added and binds the primary antibody.
3. Diaminobenzidine (DAB) is added and oxidized by the peroxidase, forming a brown precipitate stain within the desired targets.

When uniformly illuminated by a brief pulse from a Xenon flashlamp, the immunostain heats and focally melts the overlying EVA.

After cooling (ms), the EVA forms a bond with the tissue targets, which are then removed when the transfer tape is peeled from the slide.

Evaluating Transfer Specificity

Nuclear fluorescent staining allows visualization of nuclei to determine specificity. DAPI is a common fluorescent stain that binds DNA, excites in the UV wavelength range with a maximum at 358 nm, and emits in the blue wavelength range with a maximum of 461 nm.

As a replacement for DAPI, we tested and chose DRAQ5, a fluorescent stain that allows visualization of nuclei to determine specificity. As a replacement for DAPI, we tested and chose DRAQ5, a fluorescent stain that binds DNA, excites in the red with a 647 nm maximum and emits in the far-red >665 nm. By selecting captured targets from the brightfield image using the same method described above and masking the fluorescence image as shown below for a mouse pancreas islet, we can determine the specificity of capture.

Conclusions and Future Work

We report here the use of a fluorescent DRAQ5 counterstain of cell nuclei and transmission and fluorescent microscope image analysis to evaluate specificity (only stained cells and their nuclei) and efficiency (fraction of stained captured) of transfer as TAM parameters are varied. From these microscope images, we can determine those pixels with DAB stain above some threshold and those unthreshold pixels with DAB stain above some threshold and all cell nuclei in the tissue sections before and after microdissection, as well as on the transfer tape with the captured targets, using a 20x objective.

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References