

Color Deconvolution: Optimizing Handling of 3D Unitary Optical Density Vectors With Polar Coordinates

Abstract

Objective

Color deconvolution relies on determination of 3D unitary optical density vectors (OD3D) derived from pure constituent stains initially defined as intensity vectors in RGB space. OD3D can be defined in polar coordinates (phi, theta, radius); being always equal to one, radius can be ignored. Easier handling of unitary optical density 2D vectors (OD2D) is shown.

Study Design

OD2D pure stains used in anatomical pathology (AP) were assessed as centroid values (phi, theta) with a measure of variance: inertia based on arc lengths between centroid value and sampled points. These variables were plotted on a stereographic projection plane. In order to assess pure stains OD2D, different methods of sampling RGB pixels were tested and compared: 1) direct sampling of nuclei from preparations using a) composite H&E and b) hematoxylin only; 2) for any pure stain RGB image, different associated 8-bits masks (saturation, brightness and RGB average) were used for sampling and compared. Behaviours of phi, theta and inertia were obtained by moving threshold in 8-bits mask histograms. Phi and theta stability were tested against variable light intensity during image acquisition and by using two different image acquisition systems.

Results

The more saturated RGB pixels are, the stabler phi, theta and inertia values are obtained. Different commercial Hematoxylin have distinct OD2D characteristics. UltraView™ DAB stain shows high inertia and is angularly closer to usual counterstains than ultraView™ Red stain which has also a lower inertia. Superior accuracy is expected from the latter stain. Phi and theta OD2D values are sensitive to light intensity variation, to the used imaging system and to the used objectives. An imageJ plugin was designed to plot and interactively modify OD2D values with instant update of color deconvolution allowing heuristic segmentation.

Conclusion

Utilization of polar OD2D eases statistical characterization of OD3D vectors: conditions of optimal sampling were demonstrated and various factors influencing OD2D stability were explored. Stereographic projection plane allows intuitive visualization of OD3D vectors as well as heuristic vectorial modification. All findings are not restricted to AP but can be applied to bright field microscopy and subtractive color applications in general.

Keywords

color deconvolution, ImageJ, stereographic projection map

Short CV

Gilbert Bigras M.D. FRCP(Path) Ph.D. Medical lead for Edmonton immunohistochemistry department; Clinical Associate Professor University of Alberta Canada; Staff pathologist at Laboratory Medicine Department, Cross Cancer Institute 11560 University Avenue, Edmonton, Alberta Canada. Main clinical activities involved in breast cancer pathology; main research interest: assessment of breast biomarkers with image analysis and ImageJ.

Administrative data

Presenting author: Gilbert Bigras

Organisation: Laboratory Medicine, Cross Cancer Institute, University of Alberta, AB, Canada

co-authors:

From:

<http://www.imagejconf.org/> - **ImageJ User and Developer Conference**

Permanent link:

http://www.imagejconf.org/program/presentations/gilbert_bigras1859921189



Last update: **2012/07/23 06:57**