Model-Based Estimation of Specimen Thickness on Pathology Slides from the Stain Intensity Histogram

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A model of randomly-distributed uniform-density spheres in a thin layer is used to generate a relationship between thickness and intensity histogram, in which thickness (measured in sphere diameter) is determinable from the histogram. It is proposed that this same relationship may be approximately true for pathology slides of specimens containing irregularly stained nuclei. A brief empirical investigation provides some evidence for that being true. If this relationship is even approximately true, it might provide a post-hoc estimator of the actual thickness of a pathology slide specimen from the image alone.

keywords: quantitative histology, automated image analysis, computer assisted techniques

I. The Problem

Variability in thickness of pathology slides can cause significant variation in measured values of various stains, which can make collection and interpretation of quantitative measures difficult. It is a well known problem that obtaining specimens that are a consistent thickness over a long period of time and different equipment operators is a difficult task. Sometimes it is important to know, or even estimate, the actual specimen thickness obtained when one request a “four-micron” nominal thickness.

Actual specimen thickness could be obtained by use of a confocal microscope, but that is expensive, time-consuming, often-unavailable, and requires the original specimen itself. This paper proposes a technique which is fast, can be done entirely computationally, and requires just the image, not the original specimen.

II. Equipment

The model images were generated using the NIH-Image program on a PowerComputing Powerbase 240 (Macintosh Clone).

III. The Model

Uniformly shaded spheres were placed randomly (but not overlapping) on an image and a “slice” taken of various “depths”. All distances are measured below in pixels, and the spheres had a diameter of 30 pixels. Figure 1 below illustrates a slice 45
pixels thick through this image, and various simulated “light-paths” through the “slice”.

Figure 1: simulation of a 45 pixel thick slice through a material containing a distribution of 30 pixel diameter circles.

For this paper, a virtual section 400 pixels in height, 400 pixels in width, and varying (10-100) pixels “thick” was analyzed. 250 spherical “nuclei”, 30 pixels in diameter, were randomly distributed across a volume 430 x 430 x 130 pixels in size, centered on the 400x400x100 pixel region being measured. The overlap of 15 pixels on all sides was to allow spheres have centers located outside the region of interest but with their extreme portion still intersecting the region of interest.

Six such distributions were created, and each distribution analyzed for the total content inside a layer ranging from pixel level 1 to 10 (9 pixels “thick”) to pixel range 1-100 (99 pixels “thick”).

A view of the simulated sample is shown below, as is the histogram average of the 6 runs.
Figure 2: Representative region, view from “top” of a 100 pixel deep “slice”, with spheres 30-pixels in diameter distributed throughout the volume.

Figure 2 shows a “top view” of this simulated sample. Note that what is being shown here is the absolute count of pixels, darker being a higher count, not an exponentially decreasing brightness as would result if this were an actual sample being viewed optically.

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Figure 3, shows stacked histograms for sample thicknesses from 10 to 90 pixels (1/3 diameter to 3.33 diameters of the spheres.) Note the uniformly sharp cut-off running horizontally at a density of 30 pixels, corresponding to exactly one uncut sphere in the light path.

A sample histogram, corresponding to a vertical slice through the stacked histogram image at thickness = 60, with the same color coding, is shown below.
IV. Interpretation

In this perfect model of uniformly dense spheres, viewed in absolute integrated density along the light path through the “specimen”, thicknesses under one sphere diameter do not have a clear “cut-off” value. The sphere diameter is 30 pixels. An example histogram of slice 25-pixels thick through these spheres is below:

Figure 4: A cut through the stacked histogram shows the histogram of darkness values (in pixels) measured for a 400x400 x 60 pixels “thick” slice through the spheres.
Figure 5 -- 20 pixel thickness histogram

Once the thickness exceeds a sphere diameter, a cut-off will be visible, as shown in the next histogram, for a section 45 pixels thick.

Figure 5, 45 pixel (1.5 diameter) thickness histogram

As the thickness continues to increase, the cut-off remains fixed, but the fraction of the histogram area in region B increases, both absolutely and relative to region A. (i.e., there is an increasing chance that two spheres are in the light path, and a decreasing chance that only one sphere is in the light path.) The next figure shows this.

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V. Discussion

In this model of uniformly dense spheres and measurements that are linear with depth (not logarithmic), it is apparent that there are multiple measures of the histogram of gray-scale values that can be used to deduce the sample thickness, in terms of sphere-diameter.

The nuclear diameter on an actual pathology slide can, of course, be measured directly from the image, if one assumes that the image slice is arbitrary and the diameters visible on the slide (in the x-y plane) are the same as the diameter distribution along the Z-plane (depth).

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In the perfect model described, however, with uniformly increasing centroids of the histogram distributions, clean cut-off values, and uniformly increasing ratios of region B to region A, as well as uniformly increasing ratios of region A2 to region A1, it is possible to work backwards from a histogram distribution to deduce the sample “thickness”.

VI. Extensions

Obvious next inquiries would include analyzing the impact of spheres of various sizes, say normally distributed, or ellipsoids, not spheres, or thin hollow shells, not filled shells.

Also, an empirical investigation of existing images, especially a series of known thickness sequence, would be a good follow up.

It would be convenient to redo this work in the visible light scale, where brightness falls exponentially with integrated stain along the light path.

VI. Empirical Examples

(Note: one must remove the background for this to work)

{This section to be completed later}

VIII. Operational Code and Web Site

Code for this work is (or will soon be) posted on the author’s web site, http://www-personal.umich.edu/~schuette/imaging