Oct 18, 2019

Mesangial Index Quantification V.2

Frank Brosius[1]

[1]University of Arizona

1 Works for me dx.doi.org/10.17504/protocols.io.8e7hthn

Diabetic Complications Consortium
Tech. support email: rmcindoe@augusta.edu

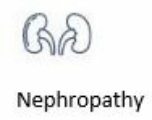
Lili Liang

ABSTRACT

Summary:

This protocol describes the procedures for quantifying the percent area of the glomerulus that is PAS stained and is expressed as the mesangial index.

Diabetic Complication:



EXTERNAL LINK

https://www.diacomp.org/shared/document.aspx?id=64&docType=Protocol

MATERIALS TEXT

Equipment:

Universal Imaging MetaMorph® Imaging System, Molecular Devices
Scientific grade digital color CCD camera
Microscope and lens

BEFORE STARTING

Pre-Operating Instructions:

Camera and Microscope should be calibrated and values loaded into MetaMorph® program

1 Using the camera or MetaMorph® software digitize 15 cortical glomeruli per case with a 40 X lens. Glomeruli should be chosen for a similar diameter of maximal size. Save images as uncompressed tiff files.

2 Open the glomerulus tiff file in MetaMorph®. Scale the image in such a way that the entire glomerulus can be seen on the screen (50-75%).

3 Using the polygon tool carefully outline the glomerular tuft. Double click to close the polygon tool.

4 From the tool bar choose **Measure, Calibrate Distances** and in the **Apply** window choose the calibration file for the camera from which the image was taken. Then choose **Apply**.

5 The area of the glomerular tuft can then be calculated by choosing **Measure** from the tool bar and then **Region Measurements**. Record the glomerulus area displayed.

6 To calculate the area that is PAS stained the tuft will have to be removed from the background. Select the outlined tuft and then from the tool bar choose **Edit, Duplicate, Image.** Close the full size image.

7 Size the edited image to150–200%.

8 From the tool bar select **Measure** and **Set Color Threshold**.

9 In the **Set Color Threshold** window select **Set By Example**.

10 Using **Display** on the tool bar and **Adjust Digital Contrast** the color brightness and contrast can be adjusted to best suit thresholding the area of PAS stain.

11 Once the image is adjusted use the cursor to choose the area of staining.
Continue clicking the cursor over the area until the entire PAS stained area is highlighted. As each pixel is selected every pixel that color is also selected. Care must be taken to ensure that ***only*** PAS stained tissue is highlighted.

♦ **NOTE:** If an area is mistakenly selected selecting **Undo Last Click** in the **Set Color Threshold** box will remove the last selection.
♦ To clear all selections check the box next to **Reset color threshold range on next click** in the **Set Color Threshold** box. The next pixel chosen in the image will clear the screen. Continue selecting pixels.
♦ To toggle between the selected and unselected screen, select **OFF** and **Inclusive** in the **Set Color Threshold** box

12 Once all the PAS stained area is selected choose **Measure** and **Integrated Morphometry Analysis**.

13 In the **Integrated Morphometry Analysis** box under **Set Up Parameters For**: choose **Measuring** and then **Total Area** and then **Classifying** and **Total Area**.

14 Under **Display** choose **Summary**.

15 Under **Show/Log Data** choose **Current**.

16 Now choose **Measure** and the “area of PAS staining” will be the last cell in the **Summary** window under **Total**.

17 To close the **Integrated Morphometry Analysis** box choose **Reset Current** and then **Close**.

18 The percent of the glomerulus that is PAS stained is calculated as:

$$\left(\frac{\text{Area of PAS staining}}{\text{Total area of glomerulus}}\right) \text{X } 100$$