Calculating Optical Density Using MCID

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Abstract

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Guidelines

Overall Notes:

- Measure all regions twice (on separate days)
    - Use Pearson coefficient to determine how similar they are
    - If Pearson coefficient is 0.9, then average them together
    - If not, measure regions again and re-calculate the Pearson's coefficient to each old sample
    - Average the samples that have Pearson's coefficient of 0.9
- Measure all samples for given region on the same day
    - Light will change readings, will never be the same day-to-day
- Close the door to the scope room to help control for lighting differences
- Lightbox is fluorescent, so as it warms up it will change lumens
    - let it warm up fully before starting anything
- If a region is done during the morning, and you are going to complete another region during the afternoon, leave everything on while you eat luch

Cover the lightbox with the blue lab bench sheet

Protocol

Step 1.

If this is the first time, create a folder labeled "Your Name" on the desktop

Step 2.

1. Open MCiD using the icon on the desktop

- Stretch the program to cover both screens: the picture should be 1 whole screen

Step 3.

1. Settings --> Display Format --> Mono (12 bit) --> ok

Step 4.

1. Settings --> Input Selection --> QICAM – 12 Lightbox Camera --> ok

- Check that lightbox camera is on (blue and green lights) (switch is on top of camera)
- Check that color camera (on top of other scope) is also on, because they are daisy-chained together (switch is on top of camera)

Adjust magnification and focus

Step 5.

1. film is on lightbox for this

Adjust magnification and focus

Step 6.

1. Digitize button – looks like a video camera

- Focus using the camera
- use ink on film to determine if it is in focus
- If need to adjust magnification, use the crank that the camera arm is attached to
- has to be on digitize (dialog box open) in order to see it move
- click ok to take image

Establish flat field correction

Step 7.

1. remove film for this step

Establish flat field correction

Step 8.

1. Change pseudocolor (grayscale bar on top menu) to SPECT2VIS

Establish flat field correction

Step 9.

1. Adjust light intensity using intensity turner on the light box until the blank field of view looks bluish-pink

- lumens 1000

Establish flat field correction

Step 10.

1. Ctrl+click on the Digitize icon --> Flat Field Correction

Establish flat field correction

Step 11.

1. If there is already a flat field correction established, click “Clear”

Establish flat field correction

Step 12.

1. Click “Acquire” to establish a new flat field correction
2. Do not change settings!!! (but just in case, they should match the below):

- Correction source: Digitize
- Correction method: Pixel by pixel
- Frame average: 4
- Smoothing and Median filters should be clicked

1. Click “ok”

Create a Density Calibration

Step 13.

film is back on the lightbox for this part, looking at the standards slide

Create a Density Calibration

Step 14.

1. Study type (on left-side menu) --> Standards

Create a Density Calibration

Step 15.

1. Operations (on left-side menu) --> Calibration

- ROD – relative optical density

Create a Density Calibration

Step 16.

Create a Density Calibration

Step 17.

1. Calibrations (on top menu bar) --> Density

- Change to Cal Stds
- input lowest to highest values (zero first)
- Select a density standards file --> ok
- If you need to create a new calibration table, click “None”
- Enter numbers from data sheet into “Standards unit” column
- Data sheet nCi/mg x Decay rate = # entered
- Click cursor in “Value Read” column next to first standard value
- Digitize the standards
- Get a zero reading (from plain film), then procedure through the standards from lightest to darkest

Create a Density Calibration

Step 18.

1. Select curve type (on dialog box)

- cubic spline (smoothed) is usually used
- pick the one that fits the data best (by eye)

Create a Density Calibration

Step 19.

1. Extrapolate (under curve graph)

- will tell computer how to interpret data that falls outside the range of the standards
- “current curve type” for both min and max

Sampling

Step 20.

1. Operations --> Sample

Sampling

Step 21.

1. Have laptop nearby with Excel spreadsheet open (don’t use MCiD spreadsheet)

- example:

Animals	Sect 1 VP Bkgd	Sect 2 VP Bkgd
1		
2		

Sampling

Step 22.

1. change pseudocolor to grayscale

Sampling

Step 23.

1. Choose whether unilateral or bilateral measuring

- Usually use unilateral

Sampling

Step 24.

1. Choose whether free-hand or use shape (rectangle or oval)

- For VP, use oval
- useful:
- ctrl + drag = changes size
- alt + drag = rotates
- ctrl + click shape on menu = get measurements (to keep consistent across days/accidental changes)
- F4 clears image, not data

Sampling

Step 25.

1. Click on region of interest and background (2 clicks) to get data

- if you want, hit F4 to clear the image, then re-click to make sure you are getting close numbers
- Numbers in red are **extrapolated** from the standards

Sampling

Step 26.

1. Input data into excel spreadsheet

Sampling

Step 27.

1. Move to next slice and repeat process

Sampling

Step 28.

1. When completely done with that animal, clear all the data using “Clear” on sheet

Shut down

Step 29.

1. turn off camera on the microscope and cover the scope

Shut down

Step 30.

1. turn off camera on the lightbox

Shut down

Step 31.

1. put lens cover on the camera

Shut down

Step 32.

1. wipe down the light box with sparkle and kimwipes

Shut down

Step 33.

1. place blue lab bench sheet on the light box, soft side down

Shut down

Step 34.

1. Shut down computer

10. At end of experiment

Step 35.

get images

10. At end of experiment

Step 36.

1. use micrometer to determine sizes