

SpindleUtil

Manual

By T47, Dec 2014

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Introduction

- SpindleUtil is a set of MATLAB scripts to facilitate the process of spindle line-scan quantitation. It is aimed to improve the productivity by replacing the “traditional” way of manually draw lines in MetaMorph and process data by hand using Excel.
- SpindleUtil makes it much easier in rotating the image to position poles vertically. It also introduces box-scan, as well as the line-scan as a comparison.
- SpindleUtil automates the quantitation, data visualization, comparison, and data management, making it all done in MATLAB.

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Introduction

- In this manual, we will demonstrate the basic setup and process. Using a test dataset, the interface and output files will be illustrated.
- For future expansions, the steps after the FITC/TexRd ratio plot needs to be developed and tested.
- All scripts are named with “spindle_” prefix, making it easier to identify in MATLAB
- You should “Set Path” first to include those scripts (Add with subdirectories).

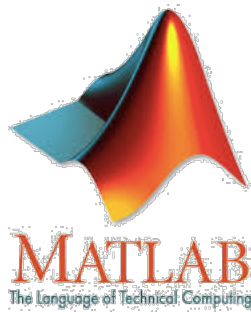
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Without SpindleUtil

- Before SpindleUtil became available, the image quantitation is done with MetaMorph and Excel. Lines are drawn in MetaMorph, suffering problems with inconsistent spindle boundaries. Data was exported to a huge Excel sheet, making plots and comparisons are thus hard and cumbersome.



Line-scan
Quantitation



Plots
Comparison

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With SpindleUtil

- SpindleUtil aims to take over the whole process using MATLAB, eliminating the complicated and time-consuming data transfer.
- SpindleUtil takes advantage of an interactive interface for spindle rotation and boundary setup, which is faster than in MetaMorph or ImageJ.
- SpindleUtil quantitates the intensity from the original TIFF, and overlays them to each other using normalized inter-pole distance. This is much faster than Excel.
- SpindleUtil automatically saves all figures, log, and workspace data to an "analysis" folder in an organized way.

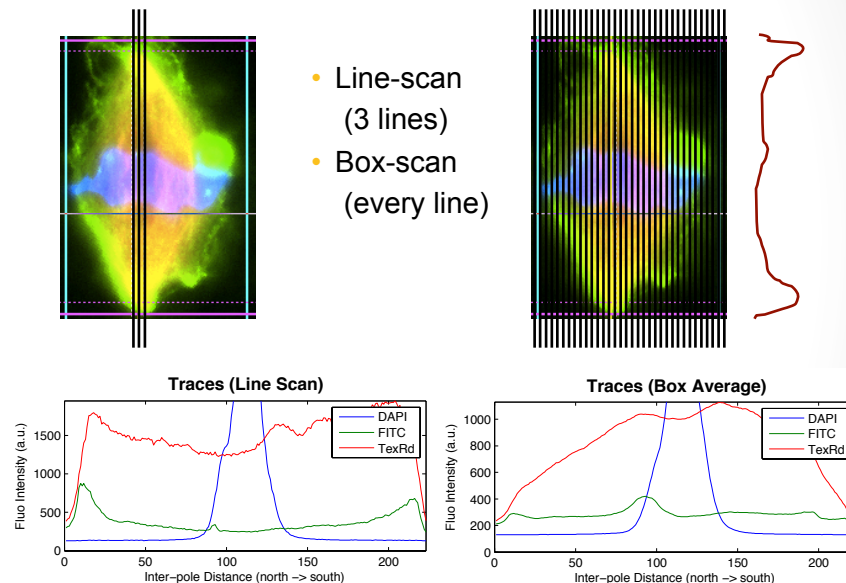
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Box-scan Quantitation

- One advantage of using SpindleUtil is its box-scan quantitation, especially when comes to deformed spindle geometry, e.g. splayed poles.
- Line-scan quantitation only takes account for the center line (pole-pole line) and 2 side-lines. This is representative in regular spindles, but may be inaccurate and losing information.
- Box-scan draws as many lines as possible in the spindle (including the 3 lines in line-scan), and thus averaging the whole spindle. This is useful in e.g. splayed pole case where the pole is not focused.

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Box-scan Quantitation



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Section 1: Image Processing

- In this section, we will go over the pipeline of image processing, which is from raw TIFF files to quantitated traces saved in MATLAB session.
- Related scripts are in folder:
Scripts/SpindleQuantify/ImageProcess
- However, you will be using a master function `spindle_mat_analysis`, instead of any individual functions in the above folder.

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`spindle_mat_analysis`

- This is an all-in-one function to perform the analysis pipeline.
- Usage:

```
spindle_mat_analysis (dir_name,  
CEN_LINE_OFFSET, POLE_PORTION, y_lim,  
NUM_BIN)
```
- `dir_name` is required, all others are optional.
- Example:

```
spindle_mat_analysis ("3. M+F2MCAK");
```

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spindle_mat_analysis

- Arguments:
 - **dir_name:** (type string), the name of the folder containing all the TIFF images.
 - **CEN_LINE_OFFSET:** (type int), the distance between side-lines and the center line used in line-scan. If not specified, default of 5 will be used.
 - **POLE_PORTION:** (type double), the portion along the pole-pole axis considered as pole region. If not specified, default of 1/24 will be used.
 - **y_lim:** (type double), the y axis upper limit in the ratio plots. If not specified, default of 2.0 will be used.
 - **NUM_BIN:** (type int), the number of bins used to group ratio data points. If not specified, default of 100 will be used, meaning one ratio data point for every 1%.

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More about the arguments

- **dir_name:**
 - First, make sure your “current working directory” is correct (see left panel in MATLAB window). Go to the parent directory of your image-file folder (so that the target folder is a child of the current working directory).
 - Second, use the target directory name as `dir_name`. For example, “3. M+F2MCAK” (exactly as is, including whitespaces).

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More about the arguments

- `dir_name`:
 - All TIFF files should meet the format of `"Prefix_XXX_fluroChannel.TIF"`:
 - Prefix should be exactly same as the parent folder name (i.e. `dir_name`).
 - ID number is 3-digit, (filled with 0s). It needs to be neither continuous (of files in the same folder), nor starting with `001`.
 - Fluorophore Channel should be one of the following: `"w1DAPI"`, `"w2FITC"`, `"w3Texas Red"`. This is same as default by microscope image output.
 - Extension is `"TIF"`, not `"TIFF"`.
 - Valid image name example: `"3. M+F2MCAK_001_w2FITC.TIF"`.
- `"nd"` and `"_thumb"` files in the folder will be ignored.

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More about the arguments

- `dir_name`:
 - The script is expecting that for each spindle (set), 3 images of each channel are present. Thus, the number of total legit TIF images should be multiples of 3.
- Possible error messages:
 - `ERROR: directory not found.`
 - `ERROR: TIFF images set (of 3) incomplete.`
`14 non-thumb TIF files present.`
 - `ERROR: TIF and directory name mismatch.`
`3. M+F2MCAK/5. M+715AA_001_w1DAPI.TIF`

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More about the arguments

- `CEN_LINE_OFFSET`:
 - This defines the distance between the 3 lines used in line-scan. In line-scan, one center line is drawn, and two side-lines (one on each side) `CEN_LINE_OFFSET` pixels away are automatically drawn.
 - Default is 5 (pixels), as inherited from older MetaMorph approaches.

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More about the arguments

- `POLE_PORTION`:
 - This defines the portion of spindle considered as “pole region”. Default is 1/24, which means the inter-pole distance will be divided to 24 sections, where the 1st and 24th section are considered “north” and “south” pole, while the 2nd-to-23rd sections are “body”.
 - This number is useful when comparing a distribution of “pole” (where MTs are enriched) to rest of spindle. Currently, no calculation in the output files is dependent on this argument. But it can be used in marking the boundary on plots.
- *Use 1/24, not 24.*

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More about the arguments

- `y_lim`:
 - This defines the upper limit of y-axis in all ratio plots. Default is 2.0, which means ratio of 0-to-2.0 will be shown. Ratios higher than 2.0 will appear “cut-off” on the plot. You may specify a lower number, e.g. 1.5, if max ratio of the trace is low.
- `NUM_BIN`:
 - This defines the number of bins used in grouping/normalizing the ratio trace across different spindle length. See “calculate the ratio” page.

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spindle_mat_analysis

- A successful launch will show the following message in MATLAB console:

```
Load files from directory: 3. M+FMCAK
Load 15 non-thumb TIF images from directory.
Group into 5 spindles {DAPI, FITC, TexRd}.
```

Instructions:

```
First rotate image to where poles are aligned vertically (north-south),
then draw box to encompass full spindle
(two horizontal, two vertical, one vertical center line).
```

Controls:

```
Keys: up/left: counterclockwise 1°/5°; down/right: clockwise 1°/5°;
r: reset; p: pass; q: save & next;
x: abort (premature terminate, data lost).
Lines: 1/2: horizontal (top / bottom boundary);
3/4: vertical (left / right boundary);
5: vertical (linescan center).
```

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Define spindle from image

- For each spindle set (of 3 images representing each channel), we will select a region that defines the spindle. This is done using an interactive interface.
- A window will pop out (see next page), with a cross-cursor and key descriptions.
- We will first rotate the image, then draw 5 lines to define the spindle.

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First rotate image to where poles are aligned vertically (north-south), then draw box to encompass full spindle (two horizontal, two vertical, one vertical center line).

Rotation: 0°

Adjust Channel
Display: +/-
1/2, 3/4, 5/6;
t: reset color.

Keys: up/left : counterclockwise 1°/5°; down/right : clockwise 1°/5°; r: reset; p: pass; q: save & next; x: abort.
Lines: 1/2: horizontal (top / bottom boundary); 3/4: vertical (left / right boundary); 5: vertical (linescan center).

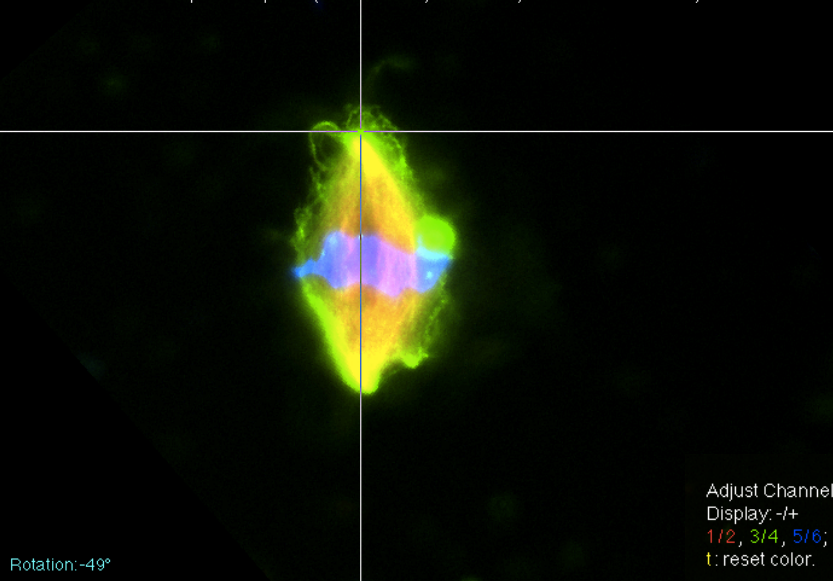
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Rotate the spindle

- This is to make the line drawing easier. The goal is to rotate the image so that the spindle is oriented/aligned north-to-south (vertical).
- To do so, use the following control keys:
 - **Up**: rotate **counterclockwise** by 1 degree;
 - **Down**: rotate **clockwise** by 1 degree;
 - **Left**: rotate **counterclockwise** by 5 degree;
 - **Right**: rotate **clockwise** by 5 degree.
- Use left/right first to rotate the image, then refine by up/down. You can check the orientation by overlaying the cursor, which has vertical cross-line.
- The current rotation angle is shown in bottom-left corner (cyan).

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First rotate image to where poles are aligned vertically (north-south).
then draw box to encompass full spindle (two horizontal, two vertical, one vertical center line).



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Keys: **up/left** : counterclockwise 1°/5°; **down/right** : clockwise 1°/5°; **r**: reset; **p**: pass; **q**: save & next; **x**: abort.
Lines: **1/2**: horizontal (top / bottom boundary); **3/4**: vertical (left / right boundary); **5**: vertical (linescan center).

Draw 2 horizontal lines

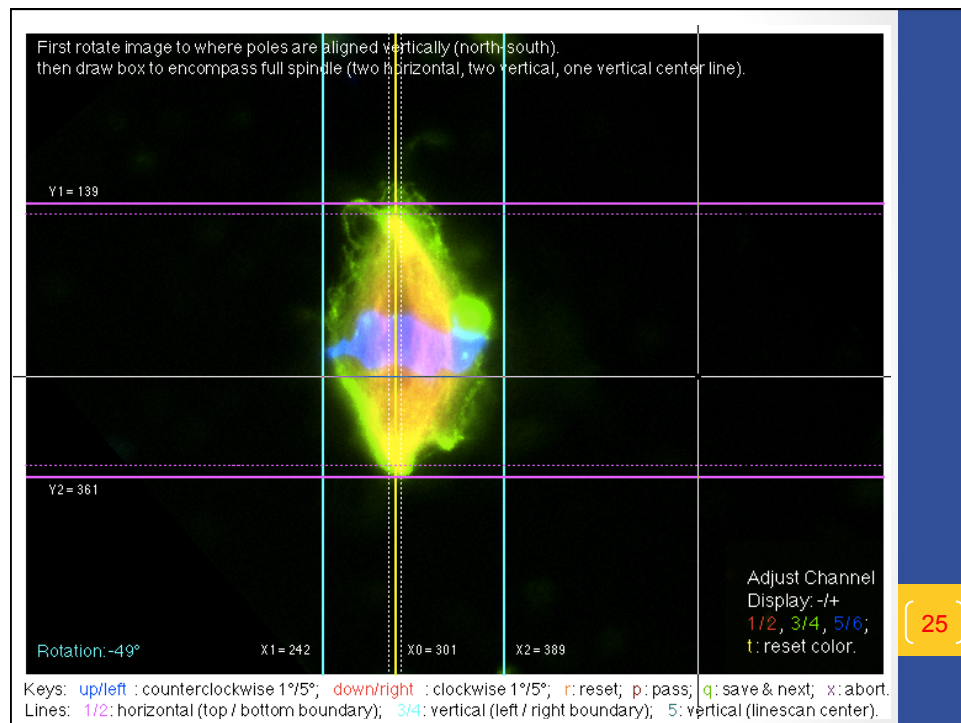
- These first 2 lines are horizontal and defines the north and south boundary. They are shown in magenta. It should be drawn exactly at the outer tip of the pole (names `y1` and `y2`).
- The `POLE_PORTION` defined pole region will be automatically shown by dashed lines inside the 2 horizontal lines you drawn.

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Draw 3 vertical lines

- These next 2 lines are vertical and defines the left and right boundary. They are shown in cyan. It should be drawn exactly at the widest point of the spindle (named `x1` and `x2`).
- The last vertical line mimics the line-scan, where it should be through both poles. So it's not necessarily at the center. It is shown in yellow (named `x0`), and the `CEN_LINE_OFFSET` defined two side-lines are shown in dashed lines.

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Interactive interface

- At this point, the line drawing is complete. Hit “**q**” to save and quit, which leads you to the next spindle.
- Other keys:
 - **r**: reset, restores the original image and erases all lines;
 - **p**: pass, the current spindle is considered bad (quality), thus it will be excluded from the following analysis (does not count in ratio calculation); Any rotation or lines drawn will be ignored.
 - **x**: abort: quit the `spindle_mat_analysis` function without saving any data (all will be lost).

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Interactive interface

- Note:
 - The “close window” button is disabled to avoid mistaken clicks. Neither does “[command+w](#)” works.
 - The MATLAB console window may gain focus when you click keys too fast. Use “[command+`](#)” to switch back to it.
 - **You can't go back to a spindle once “q” or “p”.** So think twice before passing/finishing a spindle.
 - You can use [1/2](#), [3/4](#), and [5/6](#) key pairs to adjust the display of R, G, B channels. This only alters the display (contrast), not affecting the data in the image to be quantified. Use [t](#) to reset color display (not reset rotation or lines).

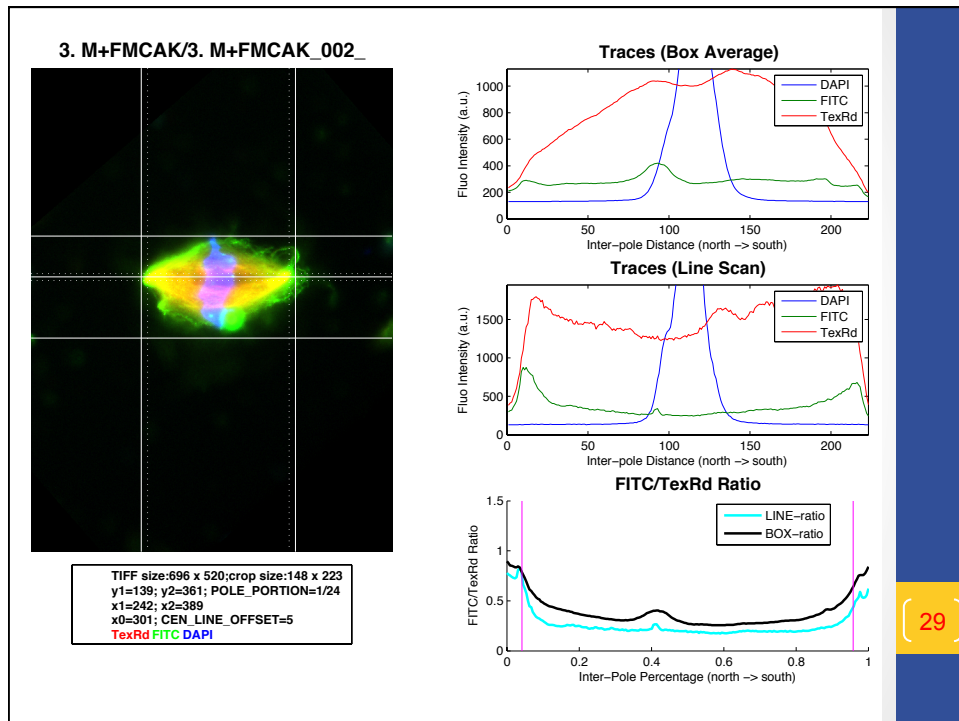
Adjust Channel
Display: +/-
[1/2](#), [3/4](#), [5/6](#);
[t](#): reset color.

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Spindle output

- Once you've done the definition for all spindles in the folder, an output file (.eps) will be saved for each spindle. See example next page.
- The output file includes the image of the spindle (rotated), the 5 lines drawn on top of the image, parameters used, and the quantitated traces. Although printing out the output is costly (color printing, black background), it is a good record.
- Outputs are saved to “Prefix_analysis/summary” with same name as spindle, e.g. “3. M +F2MCAK_analysis/summary/3. M +F2MCAK_002.eps” (not same folder as TIF data).

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Spindle output

- First 2 plots are the fluorescence intensity from pole-to-pole, averaged by box-scan and line-scan, respectively.
- X-axis is the pole length in pixels; Y-axis is the averaged fluorescence. Again, box-scan averaged all columns while line-scan only samples 3 columns.
- The 3rd plot is the FITC/TexRd ratio (assuming that FITC channel is protein of interest and Texas Red is labeled MTs). Ratio at each pixel position is calculated by dividing the FITC intensity by TexRd. X-axis is normalized to 0%-to-100% and the POLE_PORTION line is drawn for reference.

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Section 2: Data Processing

- In this section, we will go over the pipeline of data processing, which is from quantitated traces to normalized FITC/TeXRd ratio.
- Related scripts are in folder:
Scripts/SpindleQuantify/DataProcess
- However, you will still be using a master function `spindle_mat_analysis`, instead of any individual functions in the above folder (continuously from section 1).

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Calculating the ratio

- As described in the previous section, FITC/TeXRd ratio of each spindle is calculated by simple division.
- However, the ratio of each spindle cannot be averaged directly since the length of each spindle (pole-to-pole distance in pixels) are different, thus the number of points in the (0%-to-100%) range is different.
- To solve the problem, we group the data points in the ratio trace to 100 bins first, then average the binned ratio trace across spindles.
- This is done by `spindle_mat_average_ratio` function, with a default `NUM_BIN = 100`.

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Calculating the ratio

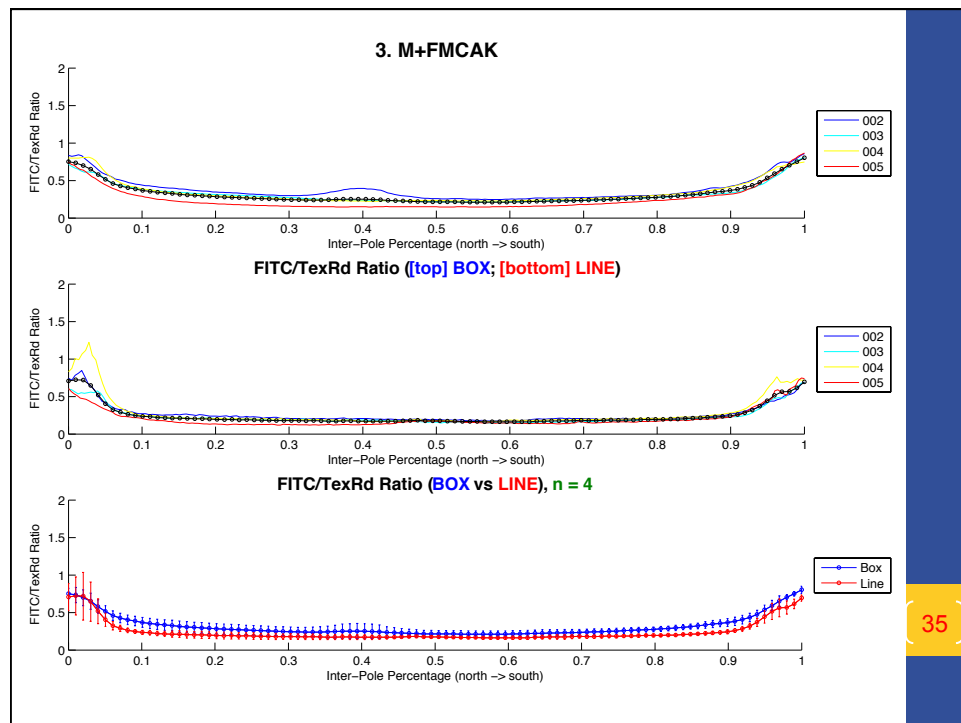
- Briefly, the grouping into bins is simple:
 - Divide 0%-100% into 100 bins (1% each);
 - Find the data points in the original ratio in each bin;
 - Assign ratio of each (1%) bin by averaging the ratio data points fall in the bin.
- Usually, the pole-to-pole distance in pixels is larger than 100. So each 1% bin will have at least one ratio data points.
- In the end, an average of ratio of all spindles is calculated and plotted (as well as standard deviation).

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Calculating the ratio

- An example output is in next page.
- For box-scan and line-scan, the averaged ratio (across spindles) is colored in black and overlaid to all individual ratio traces.
- The averaged ratio is plotted against each other (box-scan vs. line-scan) with error bars.
- Overall, the box-scan and line-scan agrees in trends, while box-scan has smaller errors.

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Calculating the ratio

- Notes:
 - The averaged ratio is across all “good” spindles in the folder, not discriminating “regular vs. splayed”. For such selection, see “pick spindles to plot” page.
 - Ratio plot is saved as “Prefix_analysis/ratio.eps”.
 - Screen log is saved as “Prefix_analysis/log.txt”.
 - MATLAB data is saved as “Prefix_analysis/save.mat”. (see “MATLAB data structure” page)

Pick spindles to plot

- You may need to pick a subset of spindles in a folder and calculate statistics of the subset, instead of the mixture of all “good” spindles.
- To do so, you will use an all-in-one function:

- Usage:

```
obj_select = spindle_mat_pick (spd_data,  
                                str_title, file_name, y_lim,  
                                NUM_BIN)
```

- **spd_data** is required, all others are optional.

- Example:

```
obj_splayed = spindle_mat_pick (spd_data,  
                                “splayed”);
```

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spindle_mat_pick

- Arguments:

- **spd_data**: (*type spd_data struct*), the **spd_data** variable from **save.mat**.
- **str_title**: (*type string*), the title of the plot, e.g. “MCAK-715AA splayed”. If not specified, default of folder name will be used.
- **file_name**: (*type string*), the saved .eps file name. If not specified, default of “ratio_pick” will be used.
- **y_lim**: (*type double*), the y axis upper limit in the ratio plots. If not specified, default of 2.0 will be used.
- **NUM_BIN**: (*type int*), the number of bins used to group ratio data points. If not specified, default of 100 will be used, meaning one ratio data point for every 1%.

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More about the arguments

- `spd_data`:
 - First, load the `save.mat` from any “prefix_analysis” folder. This loads the previously saved data into MATLAB session. The `spd_data` variable in `save.mat` is the data structure holding all spindle information and quantitated data.
- `str_title` and `file_name`:
 - These allows you to specify the figure title and saved figure name for easier comparison when printed out.
- `y_lim` and `NUM_BIN`:
 - Same as before.

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More about the arguments

- `obj_select`
 - This is the return variable that holds the data. It is a struct with following fields:
 - `list_chosen`: all spindle IDs used in the average ratio calculation;
 - `ratio_box_mean`: averaged ratio of box-scan;
 - `ratio_box_std`: standard deviation of box-scan ratio;
 - `ratio_box_mean`: averaged ratio of box-scan;
 - `ratio_box_std`: standard deviation of box-scan ratio;
 - Use a variable name that is identifiable, e.g. “obj_splayed”.
 - See “MATLAB data structure” page.

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spindle_mat_pick

- First, you will be asked to type in the selection:

Type in spindle IDs (MATLAB expression, e.g. [1:10,12]).
Only spindles not marked as `is_bad` will be selected.
Input:

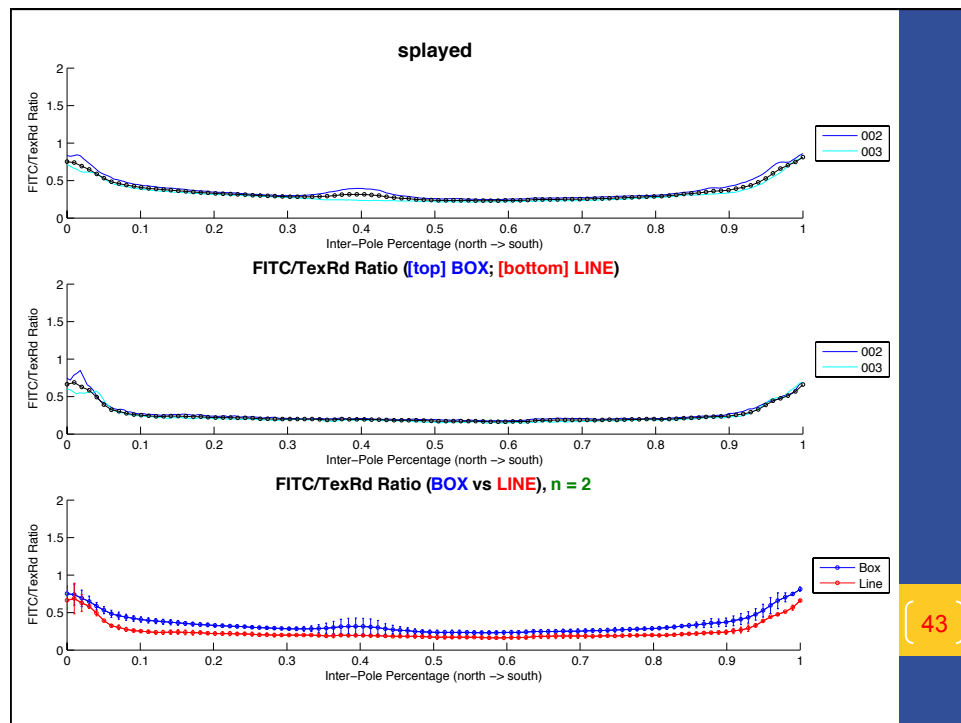
- Use MATLAB expression for ranges, e.g. [1:10], or [1:10, 12], or [1:10, 12:15], etc. Do not forget the square brackets.
- The function will automatically go through `spd_data`, and get all good (not "`is_bad`") spindles in your range, and put together a new `list_select` for averaging and plotting.

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spindle_mat_pick

- The result figure is similar to the calculated ratio figure, but only with the spindles you just picked.
- It is saved as the name you input (or "`ratio_pick.eps`" if left blank), at the same folder as current working directory.
- The number of picked spindles is shown in the title of 3rd panel ("`n =` ");

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MATLAB data structure

- There are several variables in save.mat:

Workspace	
Name	Value
CEN_LINE_OFFSET	5
dir_name	'3. M+FMCAK'
list_bad	1x1 cell
list_good	1x4 cell
NUM_BIN	100
obj_good	1x1 struct
POLE_PORTION	0.0417
spd_data	1x5 cell
y_lim	2

- Input arguments of CEN_LINE_OFFSET, POLE_PORTION, y_lim, NUM_BIN and dir_name are saved.

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MATLAB data structure

- `spd_data` is the main variable holding all data. It is a 1xn cell, where n is the total number of spindles (good and bad together) originally in the folder.
- For each entry of `spd_data` (e.g. `spd_data{2}`), it is a struct as below:

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MATLAB data structure

Variables - spd_data{1, 2}

spd_data spd_data{1, 2}

1x1 struct with 16 fields

Field	Value	Min	Max
raw_file	'3. M+FMCAK/3. M+FMCAK_002_'		
rotation_angle	-48	-48	-48
box_coord	[144.0000, 363, 244, 381, 298]	144.0...	381
img_raw	520x696x3 uint16	<Too ...	<Too ...
is_bad	0		
data_box	220x3 double	129.3...	2.0009...
data_line	220x3 double	130	3.6003...
data_label	1x3 cell		
img_box	220x138x3 uint16	121	4077
CEN_LINE_OFFSET	5	5	5
POLE_PORTION	0.0417	0.0417	0.0417
ratio_box	220x1 double	0.2470	0.8577
ratio_line	220x1 double	0.1740	0.8477
plot_x	220x1 double	0	1
ratio_box_100	100x1 double	0.2474	0.8374
ratio_line_100	100x1 double	0.1754	0.8293

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spd_data entry

- **raw_file:** (*type string*) the prefix of the spindle image file names.
- **rotation_angle:** (*type int*) the final rotation angle (in degrees).
- **box_coord:** (*type array double*) the coordinates of 5 drawn lines after image rotated, i.e. [y1, y2, x1, x2, x0].
- **img_raw:** (*type matrix uint16*) the raw TIFF image, height-by-width-by-3, 3 as in (R,G,B) channels. This serves as a backup of the TIFF files.
- **img_box** (*type matrix uint16*) the cropped TIFF image, only showing the spindle, i.e. [y1:y2, x1:x2, :] of img_raw.

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spd_data entry

- **CEN_LINE_OFFSET:** (*type int*) the input argument.
- **POLE_PORTION:** (*type double*) the input argument.
- **data_label:** (*type cell string*) the label of color channels, i.e. {'TexRd', 'FITC', 'DAPI'} as in (R,G,B).
- **is_bad:** (*type boolean*) the flag for whether this spindle is considered bad quality.
- If it were "passed" in interactive interface, **is_bad = True (1)**, and no quantitation data (img_box, rotation_angle, box_coord, data_box, data_line, ratio_box, ratio_line, ratio_box_100, ratio_line_100, plot_x) is available (all empty).

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spd_data entry

- **data_box** and **data_line**: (type *matrix double*) the averaged/projected fluorescence intensity from pole to pole in 3 channels, using box-scan and line-scan respectively; same column order as **data_label**.
- **ratio_box** and **ratio_line**: (type *array double*) the FITC/TexRd ratio from corresponding projected data, i.e. `data_box(:,2)./data_box(:,1)`.
- **plot_x**: (type *array double*) the 0-to-100% normalized x-axis positions, used for plotting **ratio_box** and **ratio_line** on 0-100% scale.
- **ratio_box_100** and **ratio_line_100**: (type *array double*) ratios of **ratio_box_100** and **ratio_line_100** grouped into **NUM_BIN** bins.

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obj_good

Variables - obj_good

obj_good

1x1 struct with 5 fields

Field	Value	Min	Max
list_chosen	1x4 cell		
ratio_box_mean	1x100 double	0.2085	0.8017
ratio_box_std	1x100 double	0.0300	0.1094
ratio_line_mean	1x100 double	0.1604	0.7205
ratio_line_std	1x100 double	0.0105	0.3181

- **obj_** variables are objects that hold data for the result of ratio averaging of a selected set of spidles.
- **obj_good** is saved after image analysis automatically. As the name suggests, it is the result of all “good” spindles.
- **obj_select** is returned by **spidnle_mat_pick** to reflect the subset the user picked.

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obj_good

- **list_chosen:** (type cell string) the list of all chosen spindle IDs of this object. For each individual entry, the 3-digit ID is saved as string, i.e. '001', not (int) 001.
- **ratio_box_mean** and **ratio_line_mean:** (type array double) the averaged ratio trace across all spindles in list_chosen, using box-scan or line-scan, respectively.
- **ratio_box_std** and **ratio_line_std:** (type array double) the standard deviation of the averaged ratio trace across all spindles in list_chosen, using box-scan or line-scan, respectively.
- Note: ratio_box_mean, ratio_line_mean, ratio_box_std and ratio_line_std are all of length NUM_BIN.

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Function Description

- Image processing:
 - `spd_data = spindle_read_folder(dir_name, CEN_LINE_OFFSET, POLE_PORTION);`
Scan through and checks all TIF files in a input folder, generate and match a list of TIF files, call `spindle_box_select` for each spindle.
 - `im_input = spindle_read_TIFF(file_id, file_id2, file_id3);`
Load 3 TIF files into MATLAB and save as matrix.

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Function Description

- Image processing:
 - `spd_data = spindle_box_select(file_id, CEN_LINE_OFFSET, POLE_PORTION);`
Calls `spidnle_read_TIFF` to load image, calls `spindle_draw_box` to interactively define spindle, calls `spindle_quantitate` to project data traces, and builds each `spd_data` entry.
 - `[y1, y2, x1, x2, x0, rot_angle, is_pass] = spindle_draw_box(im_input, rot_angle, file_id, CEN_LINE_OFFSET, POLE_PORTION);`
Interactive interface for rotation and line drawing.

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Function Description

- Image processing:
 - `[data_box, data_line] = spindle_quantitate(im_input, rot_angle, coord_xy, CEN_LINE_OFFSET);`
Quantitates and projects box-scan and line-scan to get 1D fluorescence intensity.

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Function Description

- Data processing:

- `spindle_mat_analysis(dir_name, CEN_LINE_OFFSET, POLE_PORTION, y_lim, NUM_BIN);`

Calls `spindle_read_folder` to process all images in folder, calls `spindle_mat_summary` to print out summary for each spindle, calls `spindle_mat_calc_ratio` to group ratio traces into bins, calls `spindle_mat_plot_ratio` to plot average ratio, and saves log and workspace file.

- `idx = spindle_mat_find_ID(spd_data, spindle_ID);`

Finds index of target spindle ID in `spd_data`.

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Function Description

- Data processing:

- `spindle_mat_summary(spd_data_sub);`

Print out one-page summary for a specific `spd_data` entry, including raw image, lines, intensity plots, and ratio.

- `spd_data = spindle_mat_calc_ratio(spd_data, list_good);`

Calculates FITC/TeXRd ratio for all “good” entries in `spd_data`.

- `[spd_data, ratio_box_mean, ratio_box_std, ratio_line_mean, ratio_line_std] = spindle_mat_average_ratio(spd_data, list_good, NUM_BIN)`

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Function Description

- Data processing:

Groups ratio trace data points into NUM_BIN of bins.

- `[spd_data, ratio_box_mean, ratio_box_std, ratio_line_mean, ratio_line_std] = spindle_mat_plot_ratio(spd_data, list_plot, y_lim, NUM_BIN, str_title, file_name, dir_name);`

Plots a selected list of spindles for averaged ratio.

- `obj_chosen = spindle_mat_pick(spd_data, str_title, file_name, y_lim, NUM_BIN)`

Let user choose a subset of spindles and calls `spindle_mat_plot_ratio`.

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Function Description

- For historical reasons, data processing scripts has prefix "`_mat_`". This is due to a previous version processing data output from MetaMorph, with a set of scripts with prefix "`_excel_`". Those are no longer in use.

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