Protocol for plotting calibration curves and beam profiles from film with MATLAB & ImageJ

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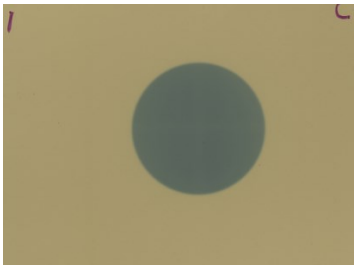
Preliminary checks

- Make sure all 4 .m files are in the working directory
  - CalibrationODtoDose_CCC.m
  - CalibrationGreen_CCC.m,
  - CalibrationBlue_CCC.m,
  - PlotProfileofScannedFilmWithOD_CCC.m
- Make sure all .txt files are in this same directory
  - red.txt
  - green.txt
  - blue.txt

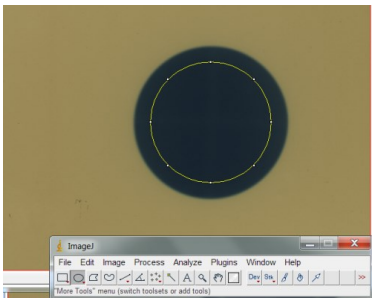
Calibration Curves



1. Scan film with the following:
   - .tif format
   - 16 bits per pixel
   - 150 pixels per inch

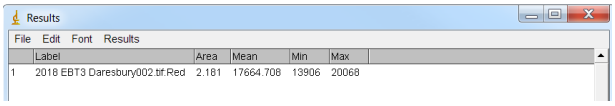
2. Rename all .tif files to their respective irradiated doses (ie 4Gy -> 4.tif)

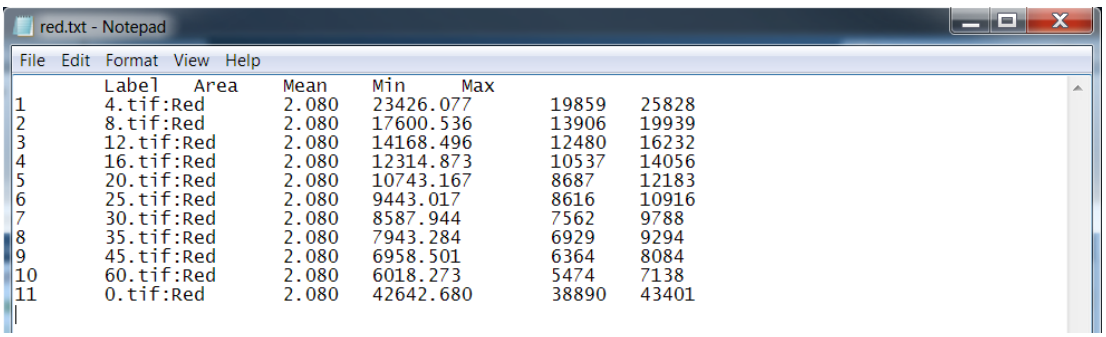
Creating ROIs for each film spot



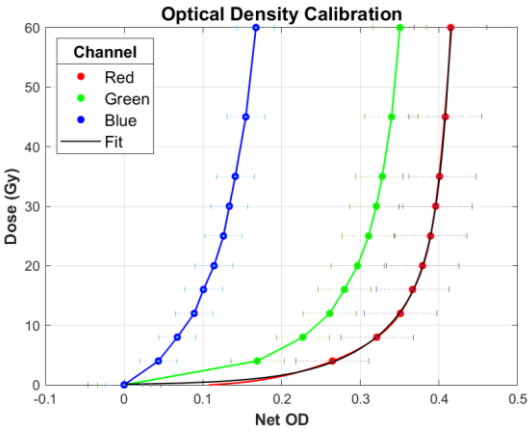
3. Open **ImageJ**, drag and drop all .tif files onto toolbar
   a) Select region of interest (click on the oval tool and draw a circle – hold shift while dragging to maintain/scale diameter for an even circle)
   b) Choose appropriate ROI size for beam spot coverage, this selects area of interest for the Red channel (scroll bar is at left third)
      i.e. Click Edit-> Selection-> Specify, Width = 250, Height = 250, tick for Oval
   c) Press 'ctrl' + 'm' or Analyze -> Measure
      Creates a table of results (Label, Area, Mean, Min, Max)

   Save pixel data in each ROI for each film
   d) Click on next .tif image and press 'ctrl' + 'shift' + 'E' to paste ROI
      It should be the same circle, same dimensions and position
      If needed, drag ROI to necessary position
   e) Again, press 'ctrl' + 'm' or Analyze -> Measure
      Generates a table of results for each film
   f) Save file as red.txt
      Move scroll bar on image to middle (green channel) and repeat d) & e), save as green.txt
      Do the same for the blue channel (scroll bar to right) and save as blue.txt





Analysis

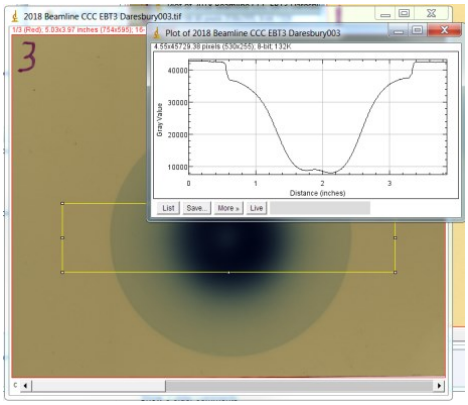


4. Open MATLAB and open all .m files in editor
   a) To generate a plot of the calibration curve, run **CalibrationODtoDose_CCC.m**
      This script reads data from all colour.txt files and generates a plot with:
         NetOD against Dose calibration curves for each channel with error bars
         '**Fit'** line fitted to the red channel

- To see the fit equation, enter 'f' into command window
- The type of fit can be changed in line 57
- To hide the fit, comment out lines 133-135
- To hide from legend table, 156 and uncomment line 157
- For high resolution image uncomment line 164
- To change the plot title, edit line 145
- To view individual channel curves, uncomment lines 70-71 in CalibrationBlue/Green_CCC.m
- The plot is saved automatically in the directory as **date_CalibrationCurve.png**
- Calculation of OD, conversion to dose and errors are calculated using procedures found in literature [1–3]

Beam profile plots

Creating ROIs for relevant film spot



5. Open **ImageJ**, drag and drop the .tif file onto toolbar
   a) Select region of interest (click on the rectangle tool and draw a rectangle on the image
   b) Choose appropriate ROI size for beam spot coverage,
      i.e. Click Edit-> Selection-> Specify, Width = 580, Height = 120

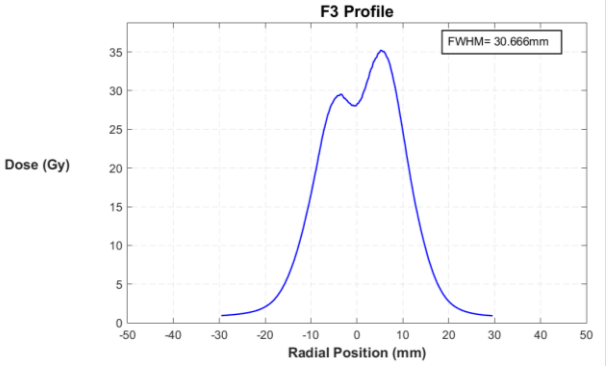
- Ideally the ROI should be larger (better statistics) but also be able to maintain consistency when analysing different beam spot shapes
- Only needs to be done for the Red channel

   c) Press 'ctrl' + 'k' or Analyze -> Plot Profile
      Creates a plot of the grey values by position
   d) Press 'Save...' and rename file, saving data as a .txt file

- You can use 'ctrl' + 'shift' + 'E' to paste ROI from before

Generating the profile plot

- If you have not run the calibration script this session, uncomment line 12
- After running it once, you can comment the line again to speed up the analysis
- This takes the fitted equation from the calibration fits and uses the fit to calculate the doses by pixel

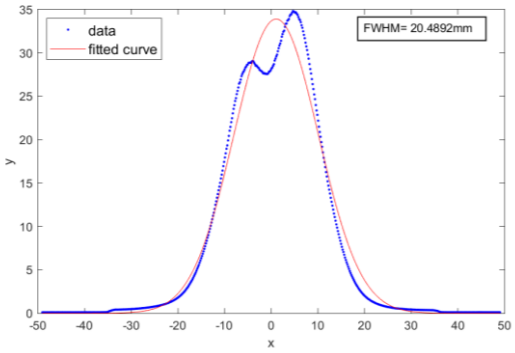


6. Make sure line 7 corresponds to the relevant .txt file
   a) Run **PlotProfileofScannedFilmWithOD_CCC.m**
      Generates a plot with:
         Pixel position in mm
         Dose at each position
         FWHM of the plotted profile

- The FWHM is calculated by 2 methods:
   1. Finds x position of the maximum point and doubles it (default option, works better for symmetrical plots)

2. Fits a Gaussian to the plot (works better for double peaks)
- To use option **2**, you need to uncomment line 57
- You can preview how the Gaussian fit compares to the plot by displaying it by uncommenting line 86

- To change the graph title, edit line 69
- For high resolution image uncomment line 98
- Automatically saves the graph as **filename_date.png**



References

1. Vadrucci, M.; Esposito, G.; Ronsivalle, C.; Cherubini, R.; Marracino, F.; Montereali, R. M.; Picardi, L.; Piccinini, M.; Pimpinella, M.; Vincenti, M. A.; De Angelis, C. Calibration of GafChromic EBT3 for absorbed dose measurements in 5 MeV proton beam and 60Co γ-rays. *Med. Phys.* **2015**, *42*, 4678–4684, doi:10.1118/1.4926558.
2. Sorriaux, J.; Kacperek, A.; Rossomme, S.; Lee, J. A.; Bertrand, D.; Vynckier, S.; Sterpin, E. Evaluation of Gafchromic EBT3 films characteristics in therapy photon, electron and proton beams. *Phys. Medica* **2013**, *29*, 599–606, doi:10.1016/j.ejmp.2012.10.001.
3. Battaglia, M. C. Dosimetry studies for radiation therapy with photons and radiobiology using low-energy protons, University of Seville, 2017.

Disclaimer:

This protocol and all scripts were written by Jacinta Yap to demonstrate methods used for analysis of film irradiations performed at the Clattterbridge Cancer Centre, UK. Any work carried out was for the purposes of my PhD thesis. Please email me if you have any questions.