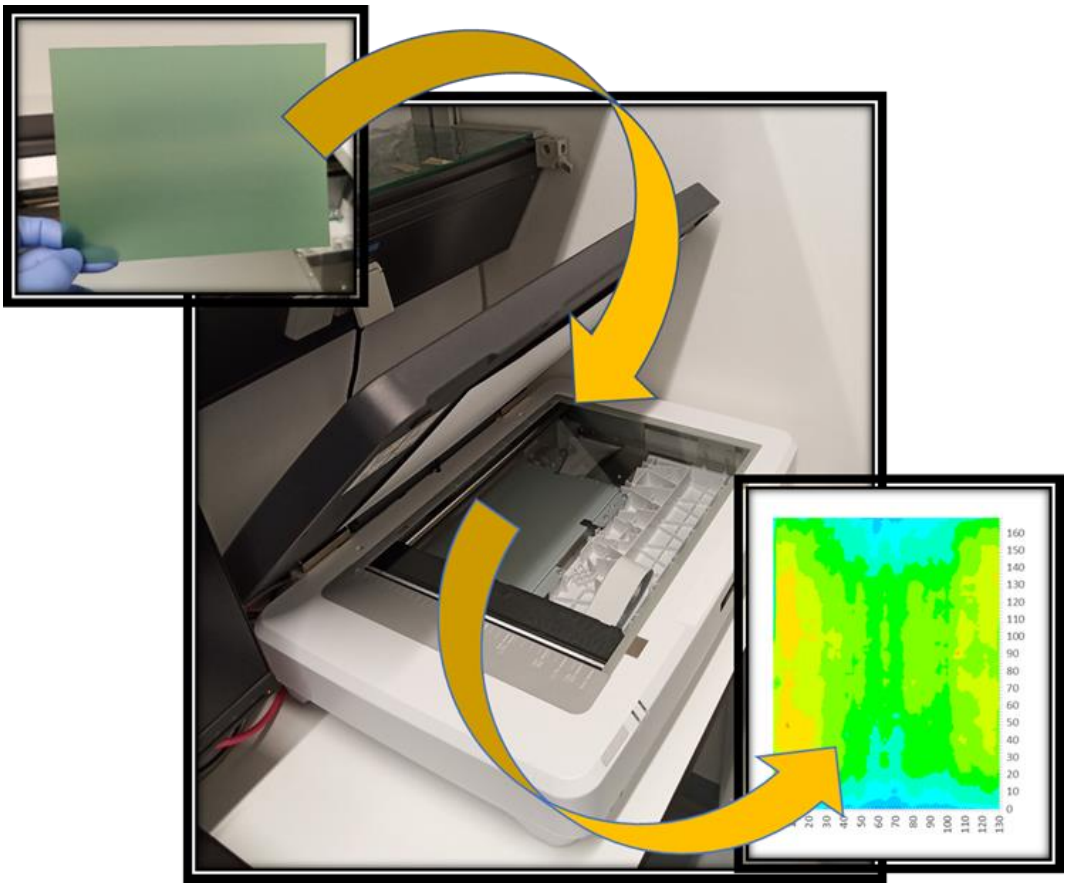
Joint FAO/IAEA Programme
Nuclear Techniques in Food and Agriculture

Dose mapping by scanning Gafchromic film to measure the absorbed dose of insects during their sterilization



Food and Agriculture Organization of the United Nations
International Atomic Energy Agency
Vienna, 2020

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Dose mapping by scanning Gafchromic film to measure the absorbed dose of insects during their sterilization

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Introduction

For the success of a programme using the Sterile Insect Technique (SIT), the dose delivered to the insects for their sterilization need to be accurately quantified and controlled. Therefore, programmes need to have an established dosimetry system to accurately measure absorbed dose and estimate if the associated confidence interval is within the permissible. Ideally, it would be desirable that all the insects that have been irradiated in a container have the same dose. In practice, because of the characteristic of the interaction of radiation with matter, there is a systematic pattern of dose variation within the container and, therefore, not all insects receive the same dose. Dose distribution within the container is determined by "dose mapping", which typically is conducted by placing several dosimeters at known locations throughout the container. Dose mapping provides operators of SIT irradiators with the information of the dose within the irradiation container, including areas of maximum and minimum dose, the dose uniformity ratio (maximum dose/ minimum dose), and areas were the dose rate is relatively uniform so that a suitable volume for the canister can be selected to provide the DUR required.

The absorbed dose that is used to induce sterility is of prime importance to programmes that release sterile insects. Absorbed dose can be measured using any dosimetry system, but many dosimeters are relatively large limiting the resolution that can be achieved. Radiochromic films can be used to measure dose over the area of the sheet used and have good resolution, in the order of tens of micrometres. Whilst individual 10 × 10 mm dosimeters can be read with a densitometer, much finer resolution can be achieved by scanning the film on a flatbed colour scanner and using the colour channel information.

The development of better system for dose distribution within an irradiation container and the development of an accurate dose-response curve for the target insect using precise dosimetry is a prerequisite of any programmes releasing sterile insects. This manual describes the operational procedures to develop dose maps by scanning Gafchromic film and the calibration of the system, to be used in the insect irradiation process for SIT programmes.

Materials

1. Gafchromic® EBT-XD, MD-V3 or HD-V2 film
2. Fine marker pen, scissors, knife or paper cutter, gloves etc. to handle, mark and cut film to size
3. Paper envelopes or sheets of copy paper to protect the cut films
4. Polymethyl methacrylate (PMMA, acrylic, Plexiglas®, Lucite®) sheet, 2-4 mm thick, cut to size as support and build-up material for the film
5. Flatbed colour scanner with software (best is Epson 12000 XL with transparency head)
6. Analysis software (FilmQA™ Pro / tsplit + Excel)

Gafchromic® film

Gafchromic® EBT-XD (0.1-60 Gy), MD-V3 (1-100 Gy) and HD-V2 (10-1000 Gy) films have a dose response range appropriate for insect sterilization for SIT purposes. Are usable in both low energy X ray and gamma irradiators and have a resolution of about 10 µm. The films are self-developing and have low sensitivity to visible and near UV light. Whilst the HD-V2 film has the active layer exposed, and is therefore sensitive to water, in the EBT-XD and MD-V3 films the active layer is sandwiched between two acetate backing sheets giving it a reasonable protection from water and allowing it to be immersed temporarily in water for dose mapping. EBT-XD and HD-V2 films are available in 20 x 25 cm (8 x 10 inch) sheets while MD-V3 is available in 12.5 x 12.5 cm (5 x 5 inch) sheets.

Flatbed colour scanner

In principle the films can be scanned on any flatbed colour scanner but better quality with lower uncertainty can be achieved by using a good quality scanner. The most suitable scanner currently available is the Epson Expression 12000XL (either PH or GA version), which is a professional grade A3 scanner. Good quality consumer grade scanners (such as the A4 size Canoscan 9000F Mk II) are considerably cheaper and still give good results. The most important feature to have is the possibility in the scanning software to turn off all colour correction settings to prevent distortion of the colour in the scan.

Resolution

Scanners can work at very high resolutions, typically 2400 × 2400 dots per inch (dpi), equivalent to 94.5 dots per millimetre or just over 10 µm square. This produces very large data files, too big to handle comfortably on a standard PC, and much finer resolution than can practically be used. A resolution of 1 × 1 mm, equivalent to 25 dpi, is usually sufficient for dose mapping (2 × 2 mm if the film is large or 12.5 dpi),. Scanners do not usually operate at such low resolution, so the lowest available resolution that is an exact multiple of 25 dpi should be used (usually 50, 75 or 100 dpi, avoid the common 72 dpi as it results in a strange scaling). The tsplit program includes a facility to combine adjacent pixels to reduce the resolution to the required level, so keep a record of the scanning resolution actually used.

Colour depth

Scanners usually operate at either 24 or 48 bits per pixel colour depth. Use the highest colour depth available on the scanner.

Image file format

The scanned image must be saved as an uncompressed tiff file (`.tif` or `.tiff`). Never save the file as jpg as this is a lossy compression format that distorts the image colours and will affect the dose map adversely. It must be saved directly as tiff and not be converted to another format at any point.

Software

Commercial software (FilmQA™ Pro) is available from Ashland. This works specifically with the Epson 12000XL scanner and automates most of the mapping process. If you are using this software, follow the supplied instructions.

Good results can also be achieved with a little more effort using custom made free software (tsplit) combined with Excel or another spreadsheet program. tsplit is composed of a script file to automate

the running and two executable files. tsplit must be run from the terminal command prompt. It is most convenient to place the scan files in a folder with a short name in the root of C:\ (e.g. C:\temp\scan) to make them easy to access from the command prompt. Avoid folder or file names containing spaces, use _ (underscore) instead if you need to separate words. The following instructions assume the use of Windows 10.

There are two options for using the tsplit programs.

1. To use the tsplit files in any folder in Windows the files need to be saved in the Program Files (x86) folder. This required administrator rights. Create a sub-folder in Program Files (x86) called tsplit. Copy the three files (tsplit.bat, csv_reduce.exe and tiff8_to_csv.exe) into this folder. The PATH environment variable has to be set so that Windows can find the files. Press the Windows key or click Start (Fig. 1) and type **`Edit the system environment variables`**. Select this option and in the **`System Properties`** window, **`Advanced`** tab, select **`Environment variables...`** at the bottom. In **`Environment Variables`**, double click the Path variable name in the lower Window (**`System variables`**) to open **`Edit environment variable`**. Click **`New`** and type the path of the new folder you made (C:\Program Files (x86) \tsplit\) and select OK.

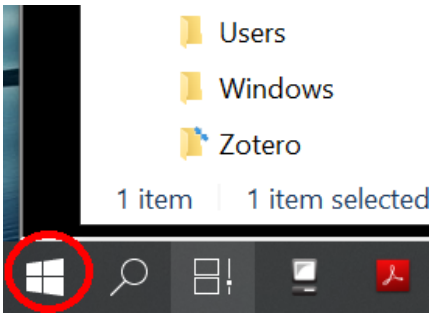


Fig. 1. Start windows option

2. If you do not have administrator rights on the computer, copy the three files (tsplit.bat, csv_reduce.exe and tiff8_to_csv.exe) into the folder containing the tiff file. The programs will run in this folder without further installation, but they must be copied into the folder containing the tiff files each time a new folder is used.

All the software, including the templates mentioned below, are available in a zip file tsplit.zip. Extract the files to a suitable folder (e.g. C:\temp\) for use. The template files can be copied to the template folder of Excel by double clicking the copy_templates.bat file.

Dose for mapping

The central dose for the mapping exercise does not need to match the dose that will later be used to irradiate the insects. As the purpose of mapping is to get the ***relative*** dose (rate) at different points, any dose can be used. Select a dose appropriate to the film to be used, 20-50 Gy for MD-V3 or EBT-XD, 50-150 Gy for HD-V2.

Calibration

The film system needs to be calibrated. This is done at the same time as the dose mapping, using pieces of the film cut to about 20 × 20 mm. A series of films are exposed to a series of doses spanning the central dose expected for the dose mapping, from about - 50% to +50%. The selected calibration doses should be in a geometric sequence with at least one or two doses above the highest expected dose; include also a zero-dose control film piece. Doses outside this calibration range will be estimated by extrapolation, leading to larger errors, but doses this far from the central dose are of little interest

to SIT anyway. These films are most conveniently irradiated in the same geometry as the map but can alternatively be irradiated at the calibration position of the irradiator.

Analysis

The step-by-step procedure for scanning dose maps and converting the data to relative and absolute dose are given below.

The reciprocal of the colour channel data (from the tif scan) is related to the dose. This relationship can be used to build a calibration and then convert the dose map to absolute dose. For moderate doses the green channel usually gives the best fit, but for high or low doses it may be necessary to use the red or blue channel. The channel to use can be determined by inspection of the R^2 value of the regression. Blocks of adjacent pixels can be combined to adjust the final resolution; this is determined by the original scan resolution and the final resolution required. For large maps a resolution of 2 mm is suitable, for smaller maps a resolution of 1 mm can be used. 50 dpi represents approximately 2 pixels per millimetre, so to get a final resolution of 1 mm the number would be 2 (Table 1). Starting from 100 dpi to get a final resolution of 2 mm the number would be 8.

Table 1. Number of blocks of adjacent pixels that can be combined to get resolutions of 1 or 2 mm, according the original scan resolution (dpi)

dpi	1 mm resolution	2 mm resolution
50	2	4
75	3	6
100	4	8

The value of this number can lie between 2-25. 2 means a block of 2 x 2 pixels, so a total of four values are combined to give each final value. Omit the number completely if you do not want adjacent pixels combined. The maximum value of the number in each cell of the spreadsheet depends on the colour depth (bits per pixel, 24 or 48 bpp) and the number of adjacent pixels combined. For 48 bpp and 2 x 2 pixels combined, the maximum is approximately 260 000.

To make it easier to determine where the film data is, in the spreadsheet use Conditional Formatting on the Home ribbon. Select one cell just to the right of the data and enter the value 220 000 (for the case of 2 x 2 pixels combined, 48 bits per pixel; a value must be determined in other cases). Select all the data and then click Conditional Formatting > Highlight Cells Rules > Greater than and then select the cell containing the number 220 000 and press enter. All cells corresponding to the white background (where there is no film) will be coloured pink. The value of 220 000 can be adjusted if the highlighting does not show the data correctly. Columns and rows that are all pink can be deleted but leave at least one pink row and column all around the data so that you can still see the edge of the film.

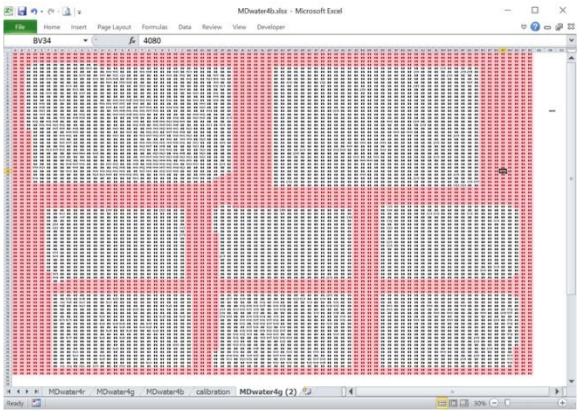


Fig. 2. Spreadsheet showing pink cells (where there is no film) and white cell with data of film reading.

To create the calibration, starting on the green channel spreadsheet make a small table below the scan data with the columns Film Reading (FR), 2*10^6/FR and Dose (in this case 6 x 6 blocks were combined). Enter the dose values used for the calibration in the Dose column (Gray), starting with the 0 control. In the Film Reading column, using the AVERAGE formula, take the average of the 6 x 6 block of cells in the middle of the film for the dose corresponding to the Dose value. When you have the averages taken from all the calibration films, calculate 2*10^6/FR for each value and plot a scatter graph of Dose against 2*10^6/FR and add the linear trend line with the equation and R^2 value (If a different number of pixels were combined the value of 2*10^6 can be adjusted to give numbers between 1 and 10 for convenience). The R^2 value should be 0.99 or greater. If you do not get a good fit, omit the control film (0 Gy). If the fit is still not good, repeat the calibration procedure on the red and blue channel data to select the largest R^2 value. As a last resort try using the log of dose (omitting the zero dose) and/or try a quadratic fit. If one point is atypical, that is, is out of line in relation of the other points, check that the AVERAGE points to the correct film and check the actual data in case there is a flaw in the film. It may be necessary to repeat one or more films if damage is apparent.

Table 2 Average film reading data for each calibration dose, Intercept value and Gradient value of the equation

Film Reading	2*10^6/FR	Dose
1636609	1.222	0
1146829	1.744	50
1000685	1.999	75
904945	2.210	100
828073	2.415	125
	Intercept	**-129.303**
	Gradient	**103.9125**

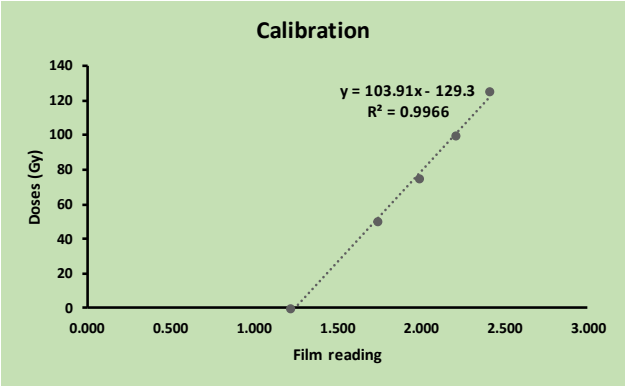
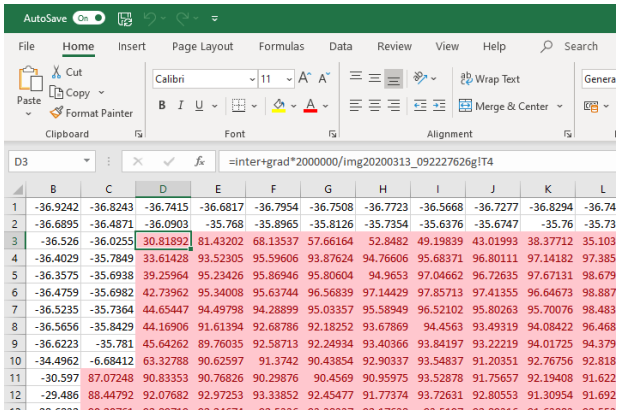


Fig. 3. Regression equation and coefficient of determination (R^2) of film readings vs irradiation dose

Once you have selected the best fit, use the trend line equation to calculate the actual dose values in the map. Type the gradient and intercept values from the trend line into empty cells and label them. Then on a new spreadsheet, starting in cell B2, calculate the dose value from the top left data point from inside the film scan. Copy the formula across and down the spreadsheet to include every cell from the dose map. In the top row enter sequential numbers (1, 2, 3 …) to label the width of the mapping film and likewise in column A for the height of the film. When the pixel combining is performed, the pixel just outside the film may be combined with the first pixel inside the film, giving a much lower dose value, so inspect the resulting dose value and trim off any rows or columns affected (Figure 4).



	B	C	D	E	F	G	H	I	J	K	L
1	-36.9242	-36.8243	-36.7415	-36.6817	-36.7954	-36.7508	-36.7723	-36.5668	-36.7277	-36.8294	-36.74
2	-36.6895	-36.4871	-36.0903	-35.768	-35.8965	-35.8126	-35.7354	-35.6376	-35.6747	-35.76	-35.73
3	-36.526	-36.0255	30.81892	81.43202	68.13537	57.66164	52.8482	49.19839	43.01993	38.37712	35.103
4	-36.4029	-35.7849	33.61428	93.52305	95.59606	93.87624	94.76606	95.68371	96.80111	97.14182	97.385
5	-36.3575	-35.6938	39.25964	95.23426	95.86946	95.80604	94.9653	97.04662	96.72635	97.67131	98.679
6	-36.4759	-35.6982	42.73962	95.34008	95.63744	96.56839	97.14429	97.85713	97.41355	96.64673	98.887
7	-36.5235	-35.7364	44.65447	94.49798	94.28899	95.03357	95.58949	96.52102	95.80263	95.70076	98.483
8	-36.5656	-35.8429	44.16906	91.61394	92.68786	92.18252	93.67869	94.4563	93.49319	94.08422	96.468
9	-36.6223	-35.781	45.64262	89.76035	92.58713	92.24934	93.40366	93.84197	93.22219	94.01725	94.379
10	-34.4962	-6.68412	63.32788	90.62597	91.3742	90.43854	92.90337	93.54837	91.20351	92.76756	92.818
11	-30.597	87.07248	90.83353	90.76826	90.29876	90.4569	90.95975	93.52878	91.75657	92.19408	91.622
12	-29.486	88.44792	92.07682	92.97253	93.33852	92.45477	91.77374	93.72631	92.80553	91.30954	91.692

Fig. 4. Spreadsheet showing areas with negative values and lower doses values for edge effect of the film

The resulting doses in grey can be plotted using a surface plot in Excel, or further processed to give dose relative to any desired point. A convenient way is to select the minimum acceptable dose in the map and express all doses as a proportion of this dose. Each dose point then represents the effective DUR at that point.

Procedure

1. Determine the geometry of the container or canister that you wish to map. Depending on the shape of the irradiation container, the film should be placed parallel to the irradiation source, covering as possible, the whole container (Figure 5).
2. Decide on the film to be used for mapping: if the map will be in water you must use EBT-XD or MD-V3 film or seal the HD-V2 film inside a waterproof covering. Size is also important; MD-V3 sheets are only 125 × 125 mm, whereas the EBT-XD and HD-V2 sheets are approximately 200 × 250 mm.
3. Cut film to fit the volume to be mapped. Place the film in a close fitting envelope or cover in paper to protect it from dust and dirt. If HD-V2 film is to be used in water, seal in close fitting polythene.

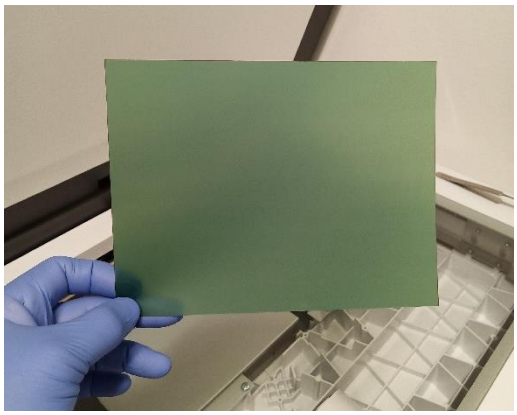


Fig. 5. GAFchromic film for dose mapping in a Gammacell 2020

4. Prepare the support/build-up material. For gamma rays, 4 mm build-up material is required, for X ray the film acts as its own build-up material (particularly if using EBT-XD or MD-V3 film) so the PMMA is only required as a support. The same supports can be used to hold the calibration pieces

by marking the middle of the PMMA and holding each calibration piece in place with a small piece of sticky tape.

5. Cut the calibration pieces, 20 × 20 mm, at least 6 pieces to give 6 calibration points; more can be used to get a better calibration. As only the central 10 × 10 mm will be used for the calibration, each film can be marked near the edge with the dose that will be applied (Figure 6). Place each piece in its own envelope, labelled with the dose and date, or seal in polythene if needed. It is important that the calibration pieces are prepared in the same manner as the map sheet.

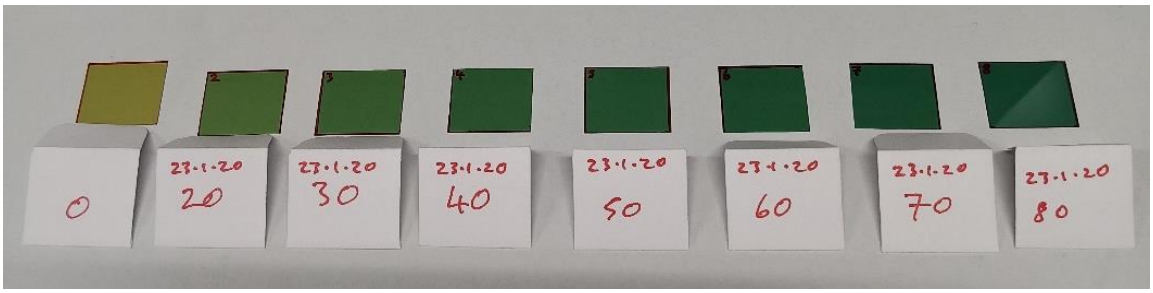


Fig. 6. 20 x 20 cm GAFchromic films irradiated at different doses used for dosimetry calibration and dose mapping

6. Prepare support/build-up material of the same thickness for the calibration if it is necessary to calibrate at a different position.
7. Determine the exposure time for the mapping dose. Calculate times for the calibration spanning the mapping time (approximately from -50% to +50%). The exposure time will be calculated according to the dose rate given by reference dosimetry (e.g. alanine, ionization chamber or Fricke).
8. Place each calibration piece in turn, in polythene if it is HD-V2 film to be in water, between the PMMA support and position it at the reference location in the irradiator, where the dose rate is known from the primary calibration; if the primary calibration was without rotation, these exposures should also be without rotation to ensure a known dose and if the primary calibration was done with material in the canister the same material (insects or dummy) should be included for exposing these calibration pieces to ensure the same dose rate. Expose it for the appropriate time to give the correct dose for the calibration series. Continue until all calibration pieces have been exposed.
9. Place the mapping sheet between the support PMMA and position it in the location where the dose map is required. Fill the rest of the irradiation canister with insects or a suitable dummy material. Expose for the time calculated for the central dose, using rotation if this is supported by the irradiator.

10. If mapping or calibration was done with bare EBT-XD or MD-V3 film in water, carefully dry the film immediately after exposure and return to the storage envelope or paper.
11. Allow the films to develop for about 24 hours.
12. Remove the map and calibration pieces from their storage envelopes and place in sequence on the bed of the scanner. They should be aligned carefully parallel with the edges of the scanner, in the centre line of the scanner bed. Leave a gap of at least several millimetres between each piece of film.

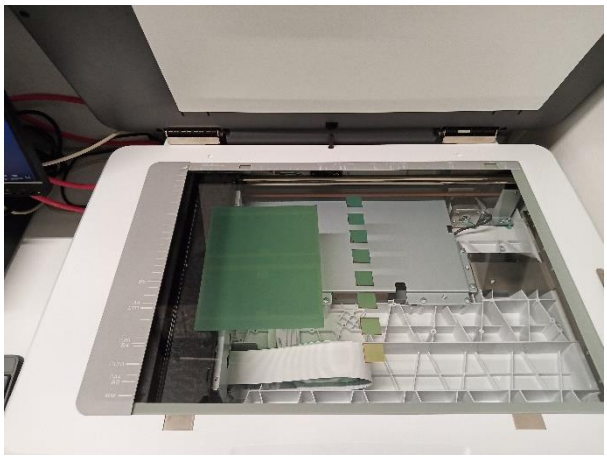


Fig. 7. Scanning of GAFchromic films irradiated for calibration and the complete film irradiated a central dose for dose mapping

13. Using the scanner software, scan the film. Where possible use 48-bit per pixel, 50, 75 or 100 dpi, with all colour correction features switched off. Don't use the common default dpi of 72, this gives an awkward scale. Check that the scan includes all the films with some white border around. Save the file as an uncompressed tif file to the selected folder (e.g. C:\temp\scan\), using a file name without spaces.
14. Open a terminal window (click the search button bottom left, type cmd and press enter) and change to the folder containing the scan tif file (to change directory use the command cd followed by the path, e.g. **`cd c:\temp\scan`**). The prompt changes to show the current location (c:\temp\scan).
15. View the files in this directory to check that the scan file is present (**`dir`** to list files).
16. Extract the colour channel data using tsplit. tsplit takes as parameters (optional) the number of adjacent pixels to be combined and the tif file name WITHOUT THE .tif EXTENSION (Figure 8), e.g.

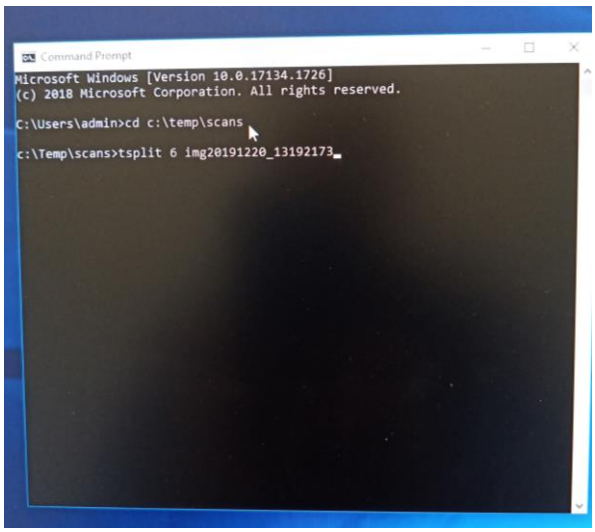


Fig. 8. Extraction of the .tif file from the scanner and conversion to CSV file for analysis

c: \temp\scans>tsplit 6 img20191220_13192173

17. The number of pixels to be combined is determined by the original scan resolution and the final resolution required (see above). The value of this number can lie between 2-25. Omit the number completely if you do not want adjacent pixels combined.
18. When the program runs it first checks the tif file then extracts one colour at a time. The files have the same name as the original tif but with the reduction number and colour added at the end, with the extension .csv, e.g. map_scan2r.csv, map_scan2g.csv and map_scan2b.csv for the three colour channels red, green, and blue. Make sure you have the three files and they are not empty!

19. Open the three csv files in Excel. Drag the green and blue tabs to the workbook containing the red data and save the file in xlsx format.
20. Create the calibration as above under Analysis and the dose map.
21. Select all the data of the dose map, including the row and column labels, and create a surface plot. With the data selected, go to the Insert ribbon and select the dropdown icon in the bottom right corner of the Charts menu. Select All Charts, Surface and select the third or fourth option (Figure 9).
22. The default format for this surface plot does not look very good. An Excel chart template is available to format it with a "heat" plot (blue is cold for low doses to red is hot for high doses) or in grey scale for black and white printed publications (black is low dose; white is high dose). The templates, Heat_map_1.0-2.0(DUR).crtx and Grey_map_60-110%.crtx, must be saved to the folder C:\Users\%username%\AppData\Roaming\Microsoft\Templates\Charts, where %username% is your username on the computer. To do this automatically, double click the batch file copy_templates.bat. Close and reopen Excel for the templates to be available.

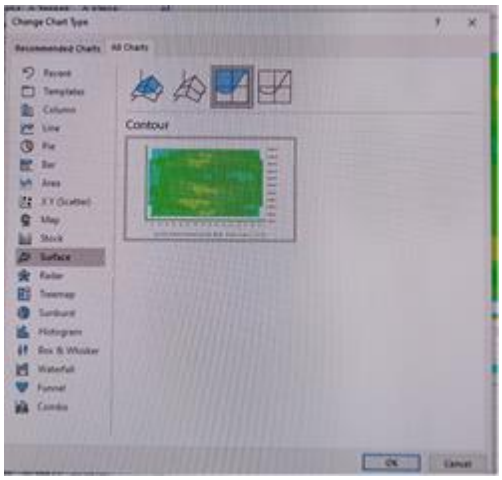


Fig. 9. Surface chart, selecting chart template option for heat map

23. To format the chart using the template, click to select the complete chart. In the Chart Tools, Design tab, select Change Chart Type. In the Chart Type window, select Templates and click on the Heat_map_1.0-2.0(DUR) or Grey_map_60-110% template, click OK. The chart will be reformatted (Figure 10).

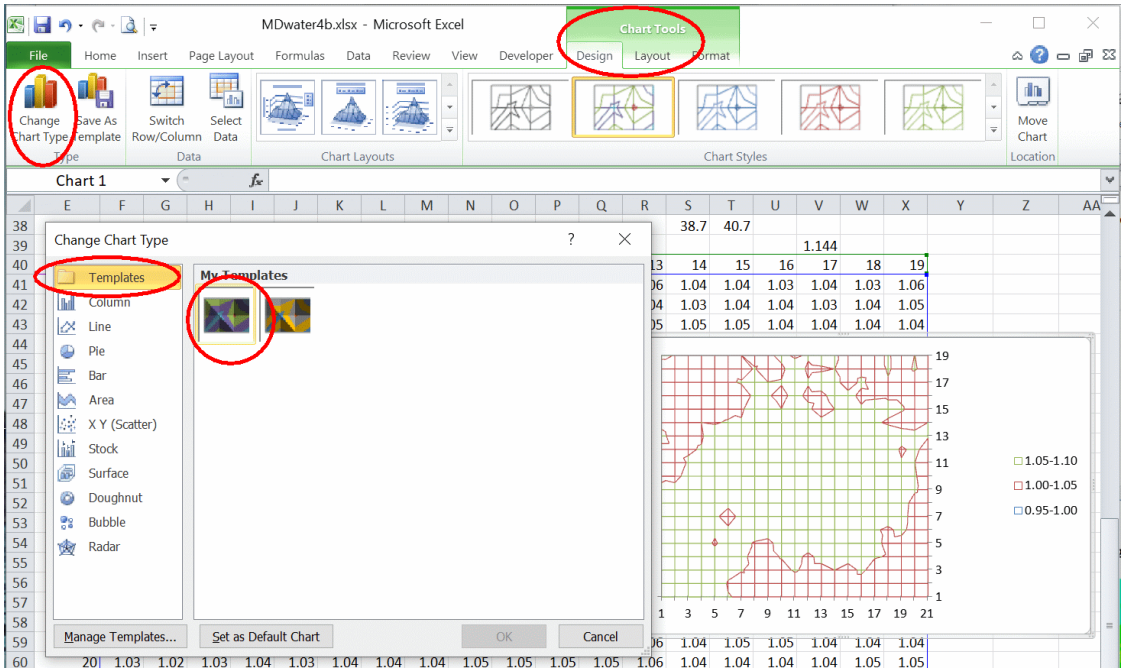


Fig. 10. Chart tool option for heat map (Heat_map_1.0-2.0.**) template in excel**

24. There are ten colour steps available. Initially they are set to 1.0-2.0 in 0.1 steps. To change this, in the Chart Tools, Layout tab select Vertical (Value) Axis in the left-hand most dropdown box and select Format Selection. Adjust the Axis Options to give the range you need. In the screenshot below the values are from 1.00 to 1.1 in ten steps of 0.01

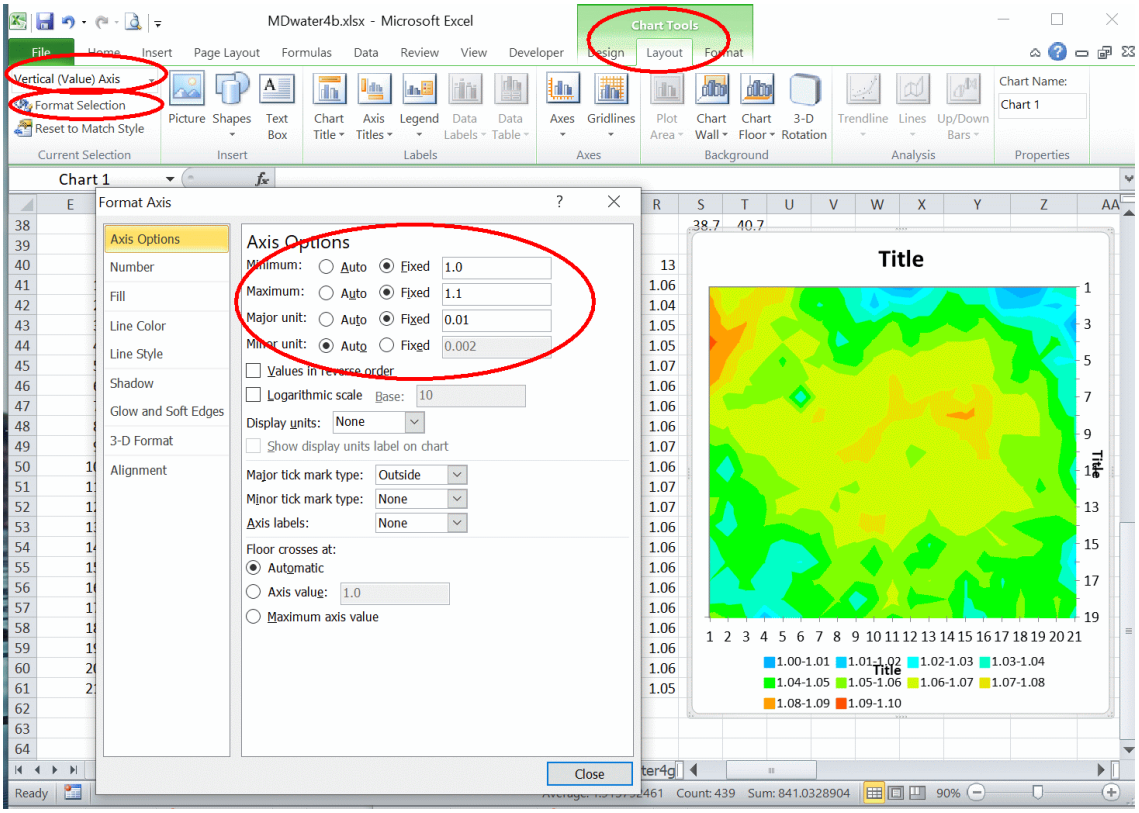


Fig. 11. Heat_map_1.0-2.0 Chart, showing values from 1.0 a 1.1 with intervals of 0.01 for dose mapping

The dose map graph is a large file. To use the dose map in a Word document without transferring all the data and making the Word file very large, complete the formatting in Excel, including background and title, copy the chart and Paste Special, Other and select Enhanced Meta File (.emf).

Recommendations

Dose mapping by scanning Gafchromic® film can be used to select the volume of the canister or irradiation container where the dose uniformity is such that the dose variation within the volume will not affect the target objective of the irradiation application. Also, it can be used to select the reference point for routine dosimetry where the minimum dose is observed.

This mapping system allows measuring the absorbed radiation dose by the insects during sterilization in a reliable, practical and economical manner.

Relevant references

GAFchromic film data sheets from the Ashland web site ETB XD, MD-V3, HD-V2

FilmQA™ Pro user guide PDF from Ashland. (http://www.gafchromic.com/filmqa-software/filmqapro/index.asp)

Lewis D., Micke A., Yu X, Chan M. 2012. An Efficient Protocol for Radiochromic Film Dosimetry combining Calibration and Measurement in a Single Scan, Medical Physics, 39 (2012) 10, pp. 6339

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