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| **Document Number:** | **SASoM/METHOD/028.v5** |
| **Title:** | **'MicroVigene' Software: Templates** |
| **Version:** | **v5** |
| **Author:** | **Peter Mullen** |

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| **SOP History** | | |
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| Number | Date | Reason for Change |
| V1 | 01/02/2013 | Original |
| V2 | 01/02/2015 | Update |
| V3 | 01/02/2017 | Update |
| V4 | 01/02/2019 | Update |
| V5 | 01/02/2021 | Update |
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**1.0 Purpose –**
This SOP describes the current procedure for performing creating a 'Spot Template' using the MicroVigene software in Laboratory 248 at the St Andrews School of Medicine (SASoM).

**2.0 Scope –**
This SOP applies to all staff in the SASoM creating a 'Spot Template' using the MicroVigene software.

**3.0 Responsibilities –**
All staff involved with in the creating a 'Spot Template' using the MicroVigene software are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.

**4.0 Procedure –**
Launch the MicroVigene software.

The following SOP assumes that no suitable template is available, in which case a previously created template will be modified. Select any template from the drop-down menu at the top of the Window. If a suitable pre-determined Template (this determines spot size, grid format, etc) is already available for the image in question, simply select from the drop-down menu.

Go to File →Open→Image→filename to select and load the required TIF image. If you are going to 'normalise' a phospho-antibody against its respective 'total' antibody, make sure you process the 'total' antibody first so that it can be 'appended' as a defined 'total protein' beforehand (this is necessary as part of the normalization process when using Platemaps).

The Template can now be modified to suit the spot pattern of the Pad being analysed. From the icons across the top of the menu bar, select the second icon 'Options' to reveal six tabs (Basic, General, Grid, Spot, File, Output).

To enlarge the Image, first click on the Magnifying glass icon and then 'left click' the mouse to enlarge or 'right click to reduce the image size. When finished, click on the White Arrow icon to switch resizing off.

To adjust the Brightness / Contrast of the image, select Image →Contrast from the Menu Bar. Adjust image to render it easiest to display (without affecting data output in any way).

Starting with the **Basic tab**, define the spot pattern for each (i) Slide (Pad), (ii) ROI (Region of Interest), (iii) Grid and (iv) Sub Grid. It is advisable not to use the sub-grid array but rather leave this set as 1 row x 1 column.

**Grid**: Any group of spots of a uniform pattern (corresponding to each pin from the robot) can be seen as a 'Grid' and can be defined as such. This may take the form of, for example, **5 vertical columns** of spots (each column being a single biological sample / lysate / cell culture condition) each comprising of **18 horizontal rows** (1 sample x 6 dilutions x triplicate spots = 18). Samples do NOT need to be spotted in a single Grid in order to be analysed together.

**ROI**: A group of spots in the form of a defined grid is called a Region of Interest (ROI). This ROI can be replicated across the slide wherever samples (the same or otherwise) have been spotted in a similar manner. This ROI will generally correspond to each of the pins used on the robot. If each Slide (Pad) has four similar areas corresponding to each of four Pins, the ROI would be defined as 2 rows x 2 columns (2x2).

**Slide (Pad)**: If the analysis is to be performed on two pads simultaneously then this can be set at 2 rows x 1 column (2 Pads one below the other in a single column).

However, since each pad is generally incubated with different antibodies this should be set at 1x1 and each pad analysed separately.

Spot Detection: the ease at which the software finds spots is determined by the Sensitivity – more spots will be found the higher the number is set (between 0 and 1000). Initially set the spot Sensitivity to 940.

Spot Diameter: Set the spot diameter at 8 and the Max Replicate CV at -1. Tick the 'advanced settings' box to reveal further options.

In the **General tab**, ensure that (i) Setting shows MicroVigene.ini; (ii) Platform shows nylon film; (iii) View shows circles for empty spots; (iv) ROI Mode shows no locate; (v) Palette shows grey; (vi) Barcode shows Code 128; (vii) Direction shows left to right; (viii) Low range shows 0.01; (ix) High range shows 94 and (x) 3D line width shows -1.00. Ensure 'Black on White Barcode' and 'Invert Image' are both ticked.

In the **Grid tab**, the Space settings need to be determined using the Ruler Tool. The spacing between ROIs, both horizontally and vertically, is calculated by measuring the distance between the same spot in adjacent ROIs. These values can then be inserted into Options→Grid tab→Roi gap (in this case, 427). Similarly, the average distance between spots across rows and down columns can be determined and entered into the table Options→Grid tab→Grid (in this case, 23.0). This can be refined at a later stage by physically dragging ROI's to the desired position and saving their locations.

Enter the value 1 for Top, Left, Bottom and Right Margins. Ensure that (i) Min Signal (std) shows -1; (ii) Noise Factor shows -0.5; (iii) Max Spot Shift (pixels) shows 10; (iv) Resolution (micron/pixel) shows 20; (v) Flag Mode shows none; (vi) Signal Mode shows Same Size Circle; (vii) Max Circle Shift (pixel) shows 5; and (viii) Max Saturation (5) shows 10.

In the **Spot tab**, set up general default settings by ensuring that (i) Background shows variable2; (ii) Edge Factor shows -1.00; (iii) Buffer Zone shows 2; (iv) Bkg percentile shows 25; (v) Smooth shows 0; (vi) Bandwidth shows 0; and (vii) Refine via shows none. Set the Dust Threshold at 3, Max Diameter at 5, and Min Mean at 5. Ensure Spots are ticked as 'Positive'. Set the 'Poor' and 'Good' criteria for Min Diameter as 6 & 8; for Max Diameter as 15 & 13; Min Solidity as 45 & 75; Max Circularity as 2.0 and 1.8; and Aspect Ratio as 1.9 & 1.8 respectively. All of these values may require adjusting for individual data sets.

In the **File tab**, ensure that the Template, Platemap, ColourMap and Data all target to their default filepaths of C:\Program Files\VigeneTech\MicroVigene\PlateMap etc. Ensure the 'after loading the pair' shows nothing; autorotate shows nothing; Backup whole file is displayed; Reference has 5 in last 1 chars; and Stitch Mode shows none.

In the **Output tab**, leave all settings as default values.

We can now create a new template by first clicking on 'Apply All' at the bottom of the Window. Click the cursor in the Template box at the top of the Window to remove highlighting and create a new file by giving it a new name. Click 'Save Template' to confirm. This 'Apply All' followed by 'Save Template' should be done after each revision of the template.

Click on the ROI icon and draw a rectangular box containing a grid of spots as defined above (in this case, 18 rows of spots arranged in 5 columns). Click in the red box, hold down the left mouse key and drag the box to the group of spots in the uppermost left hand corner of the Pad. Ensure that the top-left circle within the box is then located directly over the top-left spot of the image below. Release left mouse key. Resize the red box by dragging the bottom right hand corner so that all of the circles lie above their respective spots. Once selected, right click inside it the box again to Show Anchor. Click on Apply All and then Save Template to save changes.

Having defined a ROI and fixed where it should be on the image, first remove it by selecting Edit →Delete All Objects from the Menu Bar. Now select the ROI's icon to reveal multiple ROIs (as defined as above). Select All and then drag and drop the entire group of ROIs so that the upper left ROI lies over the respective spots in the uppermost left hand corner of the Pad. Click outside the ROIs to deselect them and then manually drag each ROI in turn to confirm its positioning. Select the first ROI and then right click to Show Anchor→Apply All→Save Template.

Go to Edit→Roi order from the Menu Bar and sequentially click on each ROI to determine their ordering – this must remain constant for each analysis in order that (i) the Platemap always agrees with the spots, and (ii) spots for the 'phospho' antibody Pad are normalized to identical sample spots from their respective 'total' antibody Pad.

Spot finding can now be attempted by going to Edit→Select All ROIs and then click on the Find All (spots) icon. The software will then attempt to find each spot within the ROIs.

In order to carry out analysis, load a pre-defined Platemap (which contains all the information associating the spots to their respective sample names, characteristics etc) by going to Options→ Basic→ Platemap→select Platemap. After clicking on the View Dilution Curve icon (last icon in the row), the spots will be processed according to the pre-defined Platemap and the data shown in a separate window along with a dilution curve. The formatting of Platemaps is covered in a separate SOP.

*N.B. All of the parameters described above are deemed starting guidelines and are subject to alteration according to the quality of the spots being analysed.*

**5.0 Personal protection –**

A Howie coat must be worn at all times.

**6.0 Spillages –**
This procedure involves analysis of results, therefore no solutions are involved so no spillages should occur.

**7.0 Training –**
All staff should under go training in this technique before performing the procedure.

**8.0 Related documents –**

8.1 MicroVigene User's manual

8.2 SOP SASoM/METHOD/026
MicroVigene' Software: Analysis

8.3 SOP SASoM/METHOD/027
MicroVigene' Software: Platemaps

8.4 SOP SASoM/METHOD/028
MicroVigene' Software: Templates



**9.0 Approval and sign off –**

**Author:**

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 11/02/2021

**Management Approval:**

Name: Peter Mullen

Position: Research Fellow

Signature:  Date: 11/02/2021

**QA release by:**

Name: Alex MacLellan

Position: QA Manager

Signature:  Date: 11/02/2021

# STANDARD OPERATING PROCEDURE

Please sign below to indicate you have read this S.O.P and understand the procedures involved.

| NAME | POSITION HELD | SIGNATURE | DATE |
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