Gel analysis

❖ Now we have the area of each peak. To calculate the concentration in Nano grams relatively depending on the known concentration of standard (sample 1) by Pro Rata calculation method as below:-

The results of our example were:

No.	area	Conc.(ng/µl)
1	9354.610	25
2	4920.610	?
3	1040.983	?
4	2767.569	?
5	2253.447	?

The conc. of sample 2 = $\frac{4920.610 \times 25}{9354.610}$ **= 13.150 ng/µl**

The conc. of sample 3 = $\frac{1040.983 \times 25}{9354.610}$ **= 0.566 ng/µl**

The conc. of sample 4 = $\frac{2767.569 \times 25}{9354.610}$ **= 7.396 ng/µl**

The conc. of sample 5 = $\frac{2253.447 \times 25}{9354.610}$ **= 6.022 ng/µl**

Gel analysis

- If we have 2 or 3 known standard in the plate we can make a standard curve by MS Excel and use the slop formula to calculate the un-known concentrations accurately.

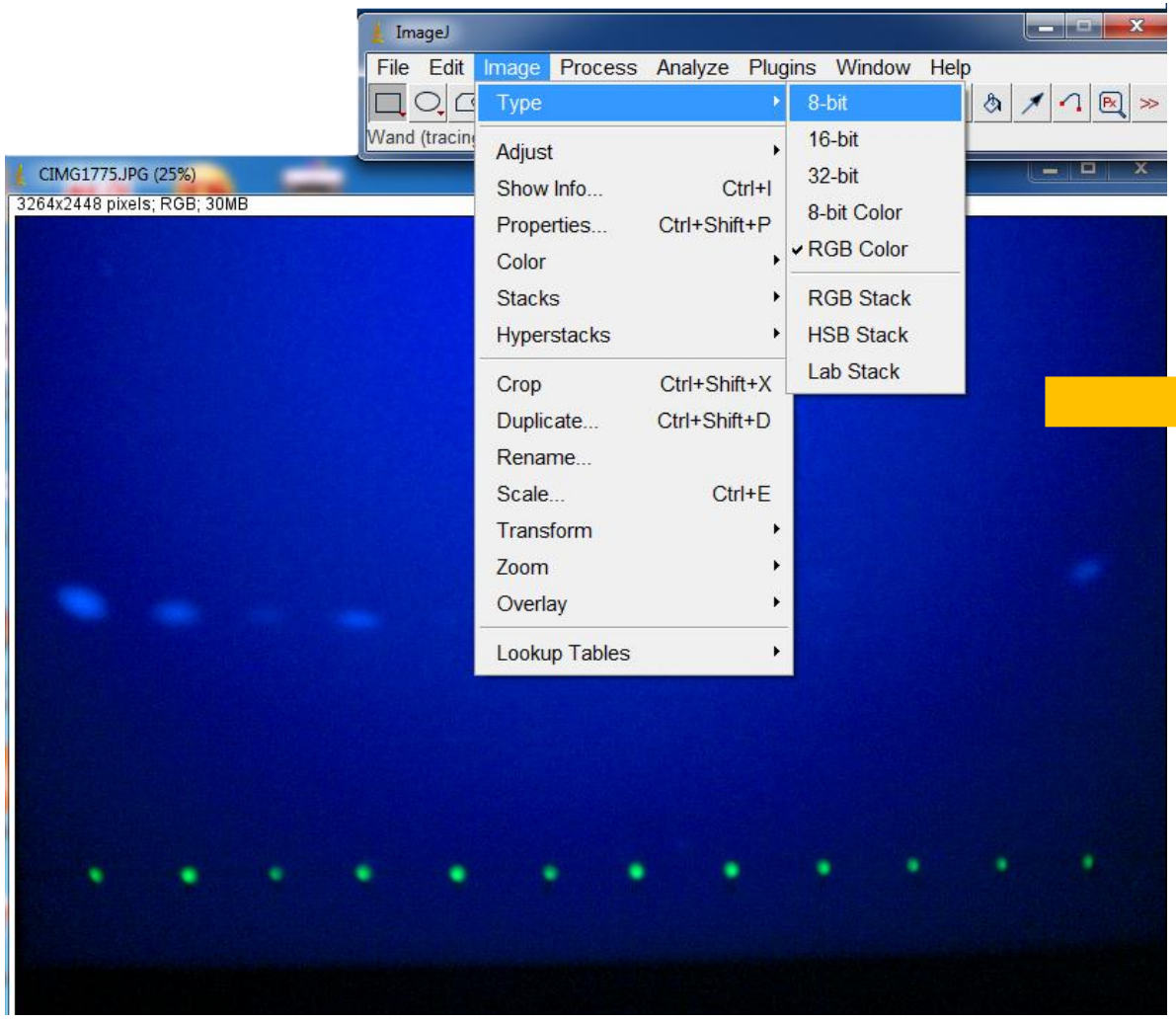
Gel analysis

Other method to quantify TLC spots. This method is better and more accurate when spots are not in straight order.

- Open Image >> convert to gray scale.
- Go to Process >> Subtract Background to reduce noise as could as possible by check Preview >> change the Rolling ball radius value until adjusting background >> OK.
- Draw rectangle around the first spot >> go to Analyze >> Gels >> Select First Lan (or Ctrl+1).
- Transfer rectangle to the second spot by mouse >> go to Analyze >> Gels>> Select next Lan (or Ctrl+2). Repeat this to all spots.

Gel analysis

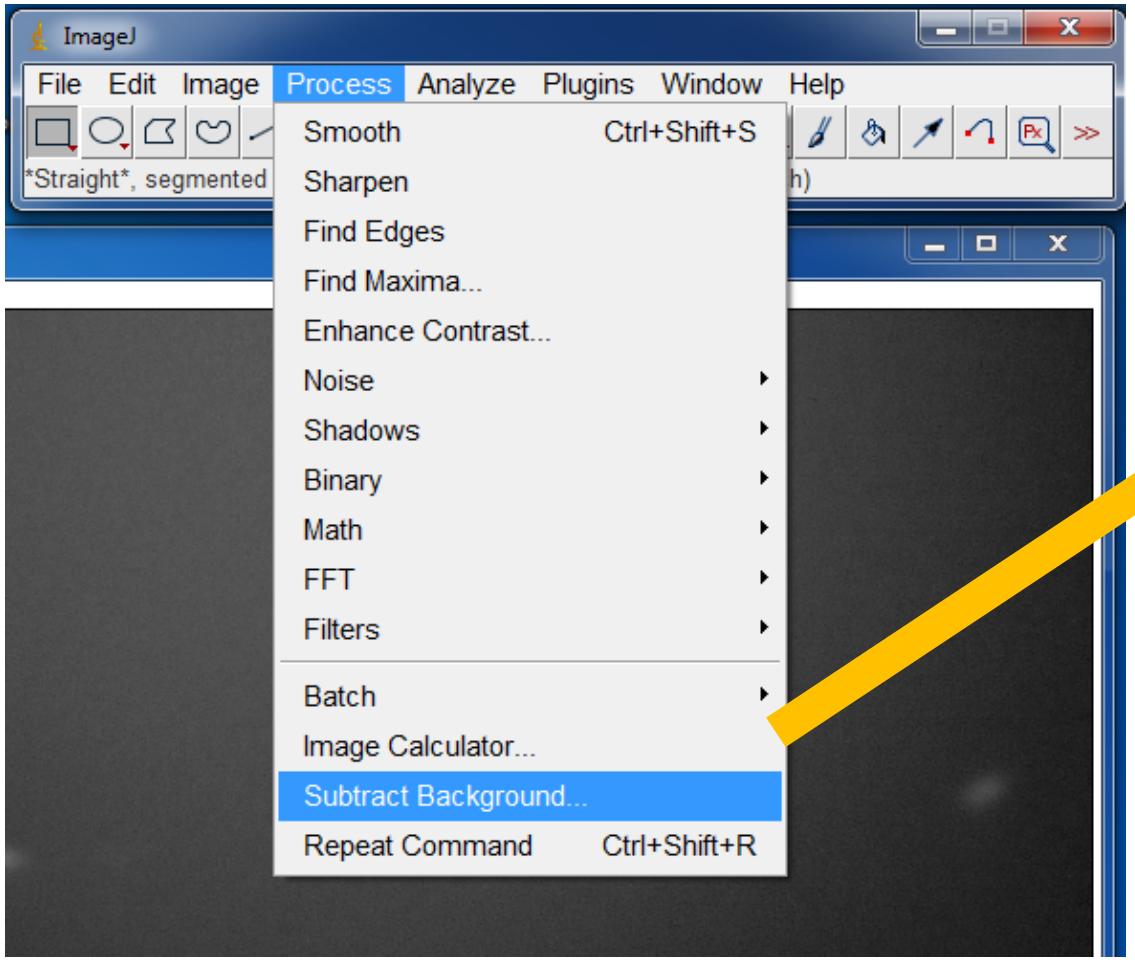
❖open Image >> convert to 8-bit gray scale

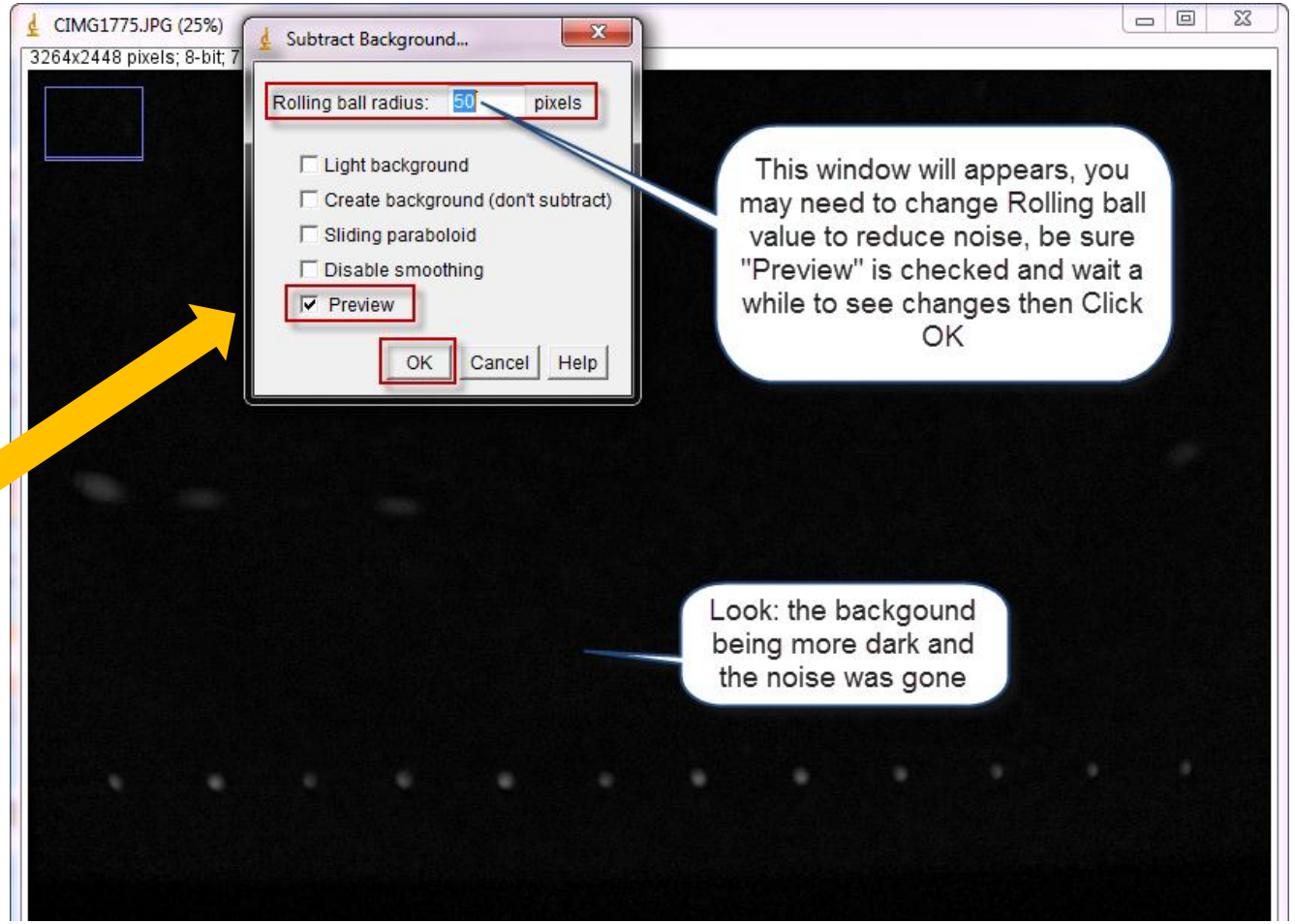


Gel analysis

❖Go to Process >> Subtract Background to reduce noise as could as possible by check

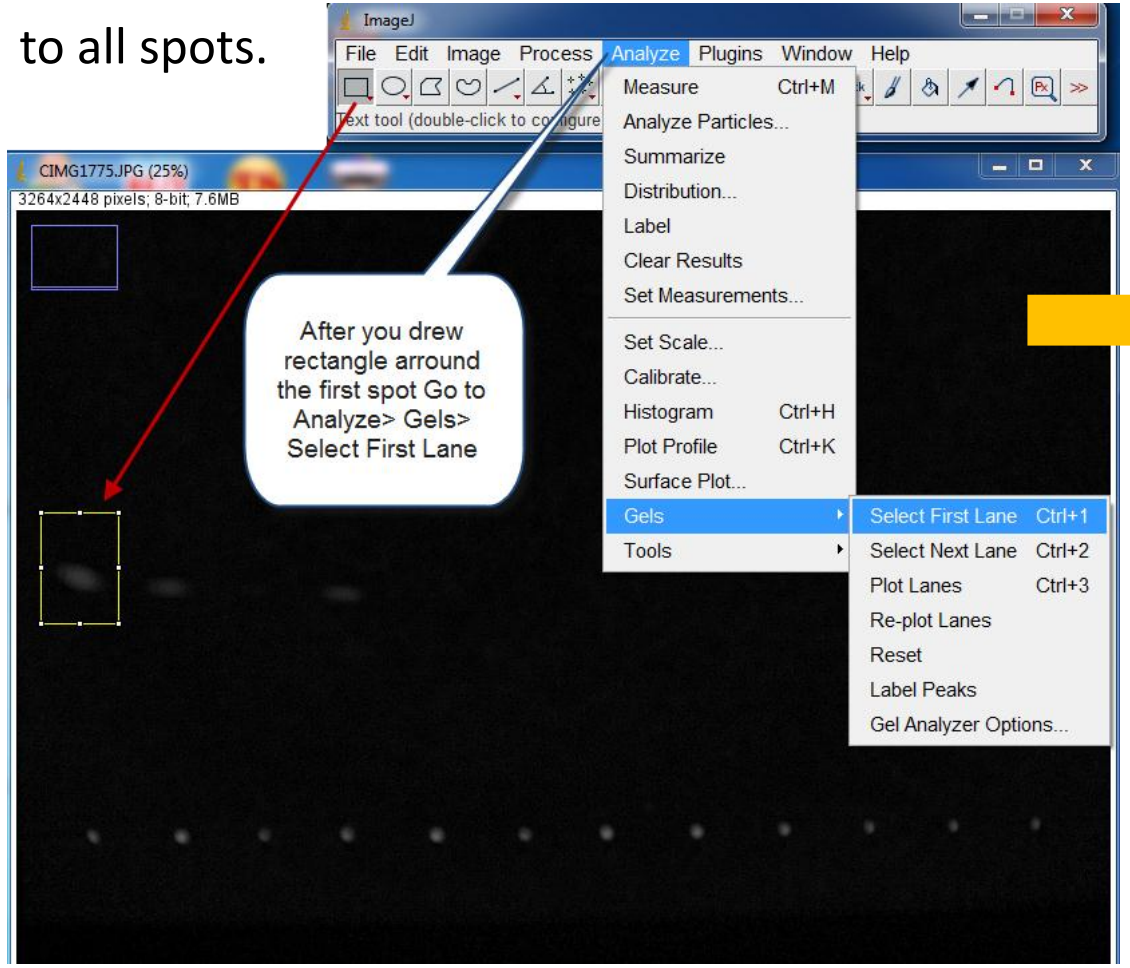
Preview >> change the Rolling ball radius value until adjusting background >> OK.

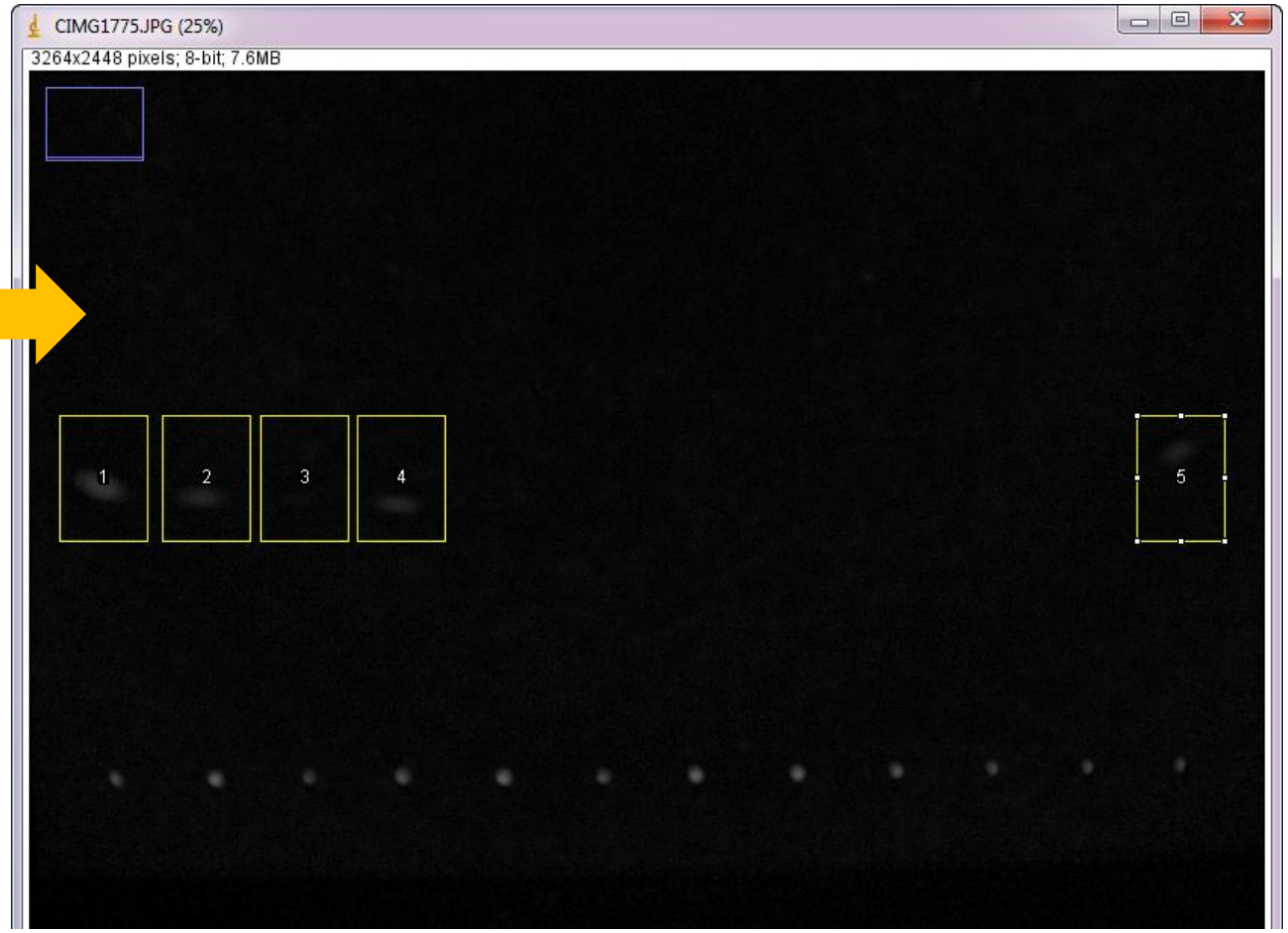




Gel analysis

- Draw rectangle around the first spot >> go to Analyze >> Gels >> Select First Lan (or ctrl+1).
- Transfer rectangle to the second spot by mouse >> go to Analyze >> Gels>> Select next Lan (or ctrl+2). Repeat this to all spots.



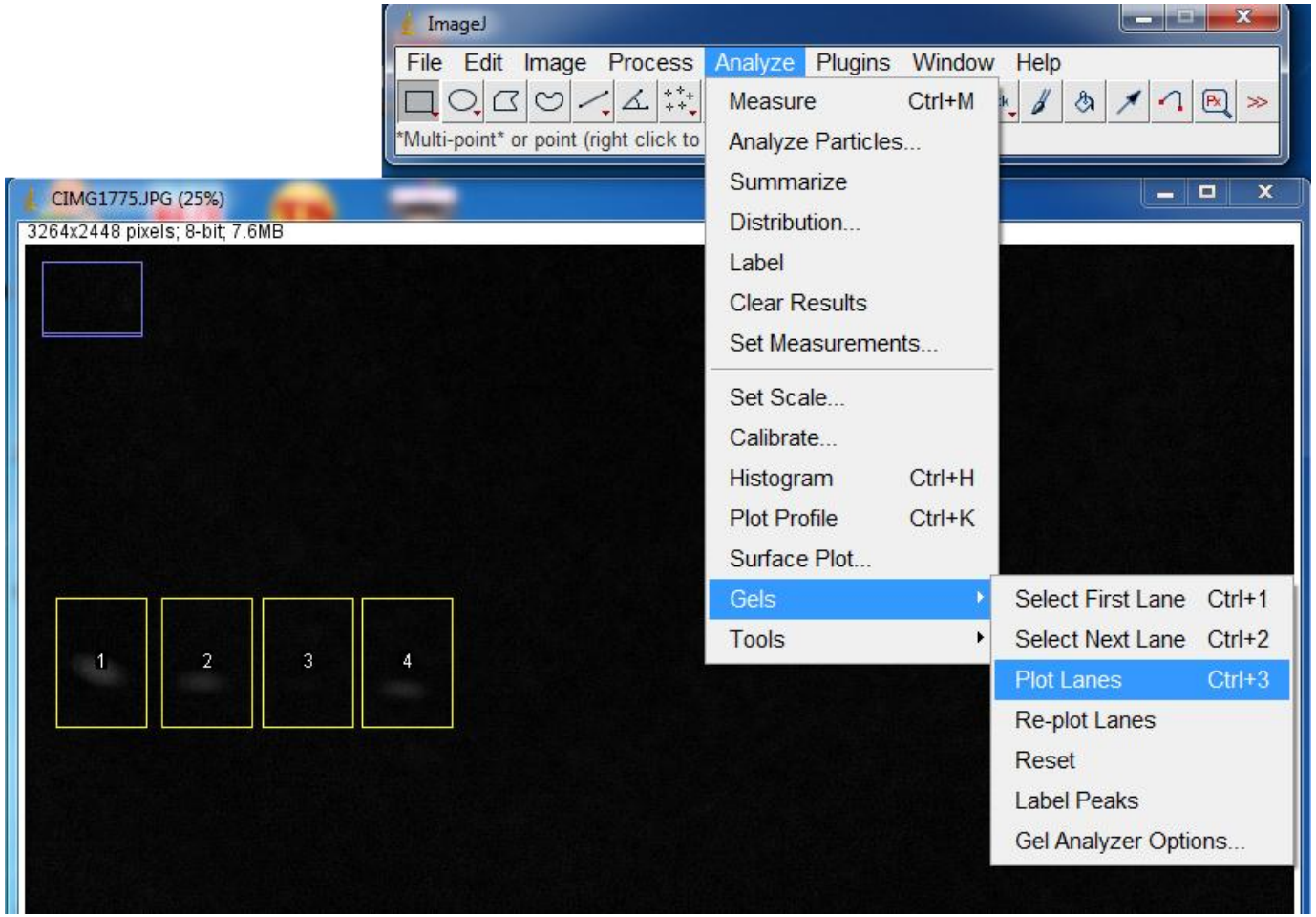


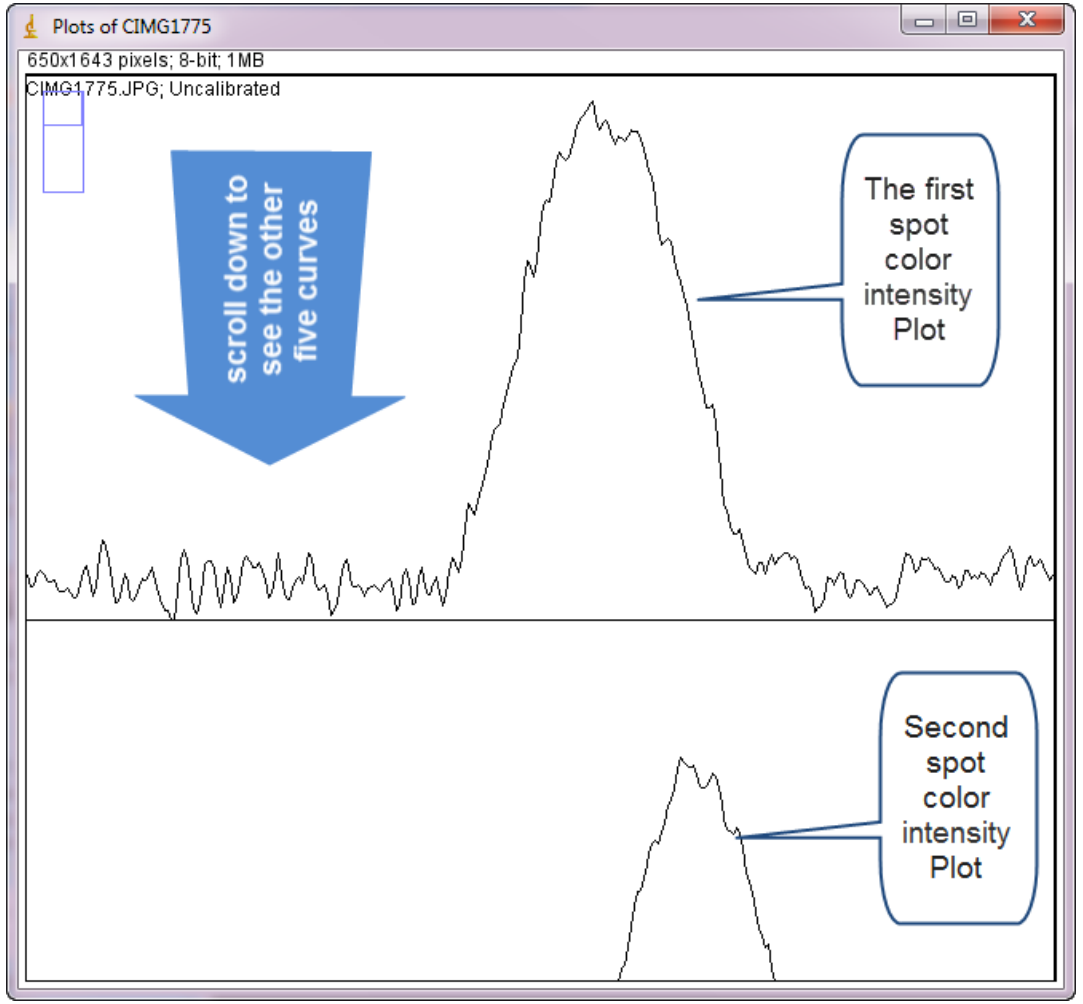
Gel analysis

- Go to analyze >> Gels >> Plot Lanes >> The Plot window will appears and the plots of spots will be ordered vertically.
- Use Line Tool to close the curve area >> Wand Tool to select that area >> and the results will appears in the results window automatically.
- When finish, Go to Analyze >> Gels >> Label Peaks >> each peak will be labeled with area percentage.
- Utilize area or area percentage values and known standard peak concentration to quantify concentration by Pro rata method.

Gel analysis

❖Go to analyze >> Gels >> Plot Lanes (or Ctrl+3) >> The Plot window will appears and the plots of spots will be ordered vertically.





Gel analysis

❖Use Line Tool to close the curve area >> Wand Tool to select that area >> and the results will appears in the results window automatically.