

How to count mites using image analysis software

Aim

Count **number of red mite** caught in cardboard traps in case of high population

Material required



- **Camera** + camera stand (if possible)
- Computer **software** Image J (<https://imagej.net/Welcome>)
- **Petri dish(es)**

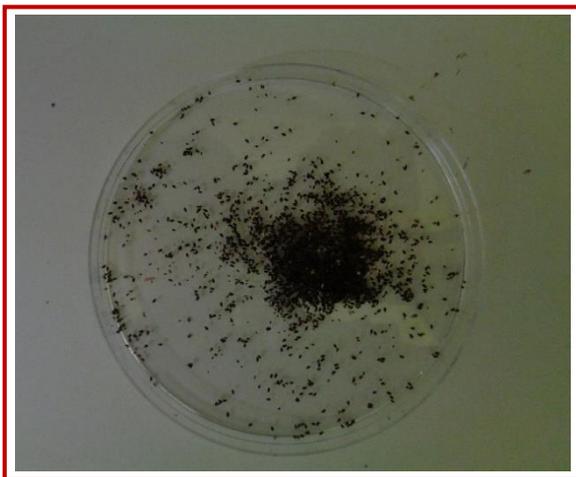
Collecting mites

Remove the cardboard inserts from each trap and place them into separate small bag for each trap. Then freeze the bags to kill the mites. The mites can then be poured from the bags into petri dishes. Make sure all the mites are removed from the cardboard but tapping the cardboard to dislodge them.

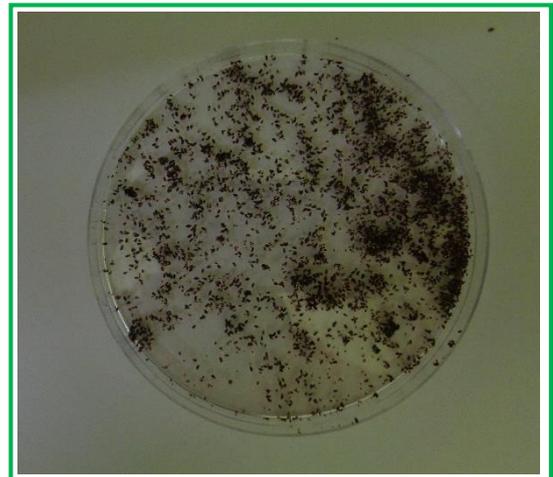
Distributing mites

In case there are too many mites to count manually, the mites can be distributed into one or more Petri dishes. The **mites need to be distributed over the petri dish** as evenly as possible to avoid clusters of mites. A computer software will then analyse the pictures taken from the Petri Dishes and estimate the number of mites. You do not need to use petri dishes if you do not have any, just pouring onto white paper will suffice, but try to keep them within a small area so they can fit into a photo.

NOT OK



OK

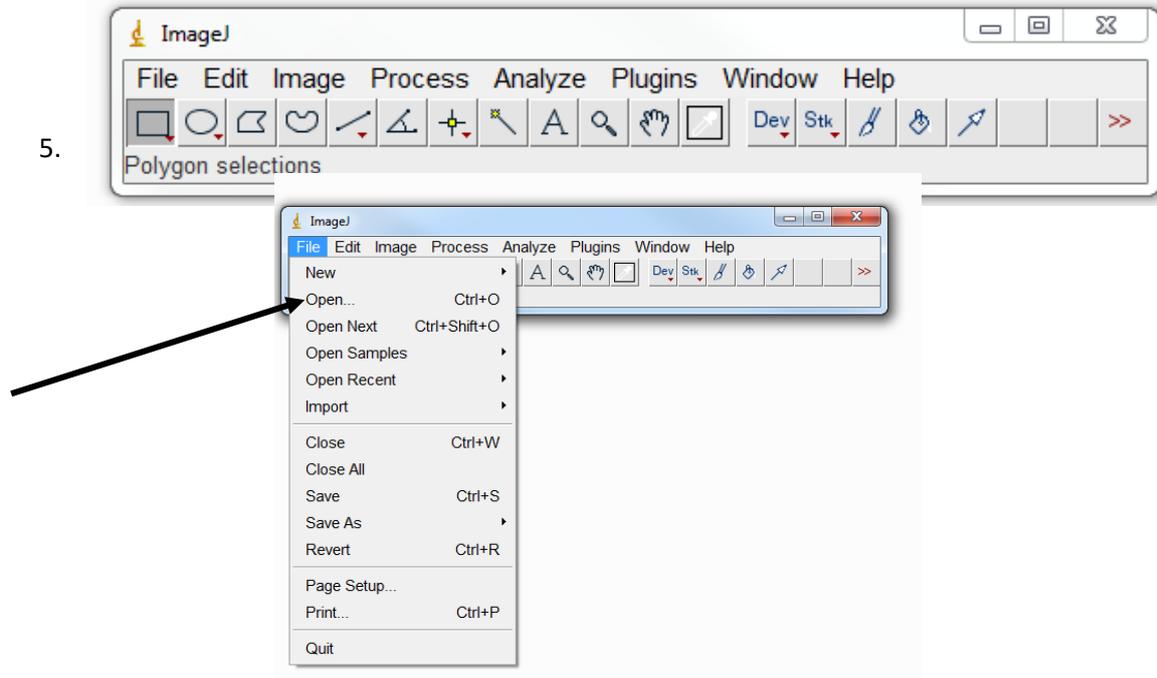


Taking pictures

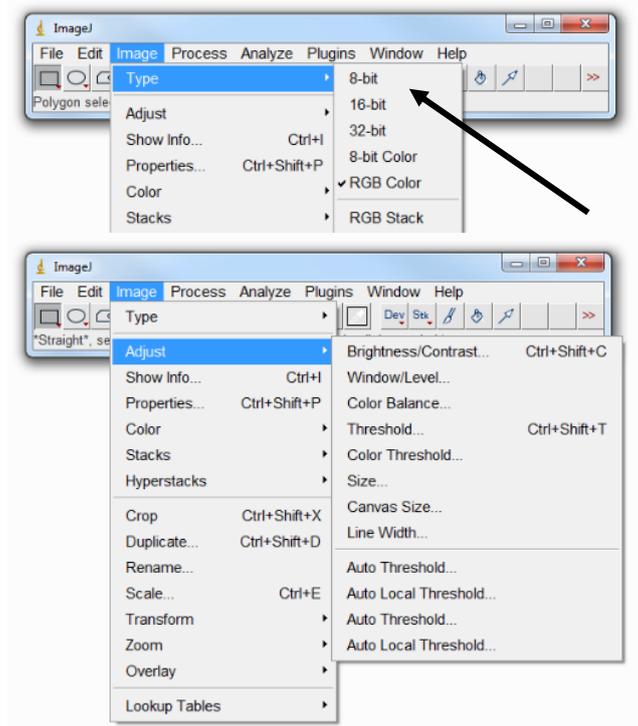
A camera stand is helpful but not necessary. The Petri dish (8.5 cm diameter) needs to be just **within the picture frame** on a **light-coloured background** (e.g. white paper). Shadows and dirty backgrounds should be avoided as this can give false results when counting with the software.

Manual for the use of Image J (software)

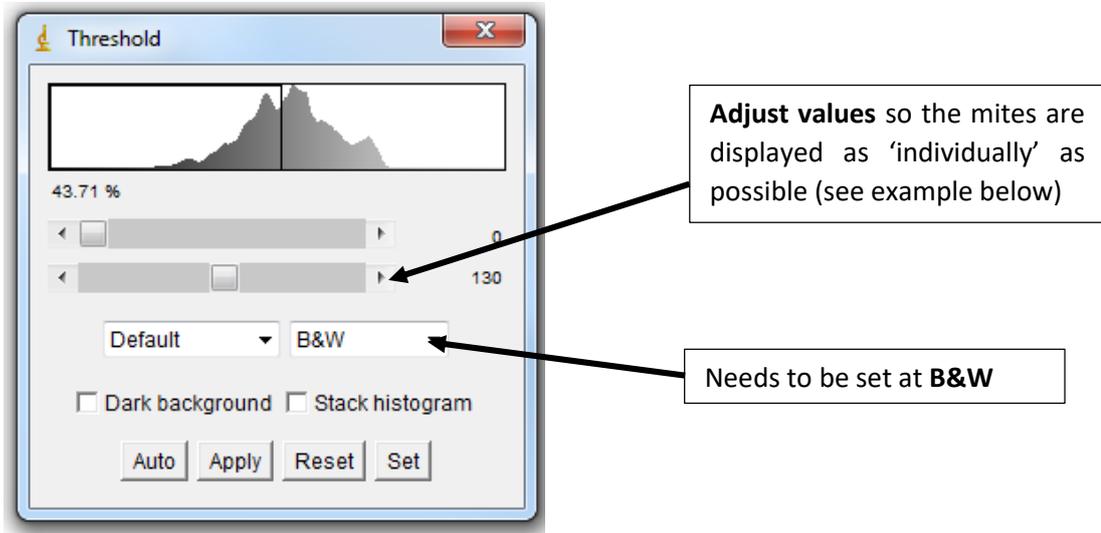
1. Download the Image J software for free using this link: <https://imagej.net/downloads>
2. Open the program
3. Click "OK" when you receive following error: "Plugin configuration error:..."
4. You get the following display:



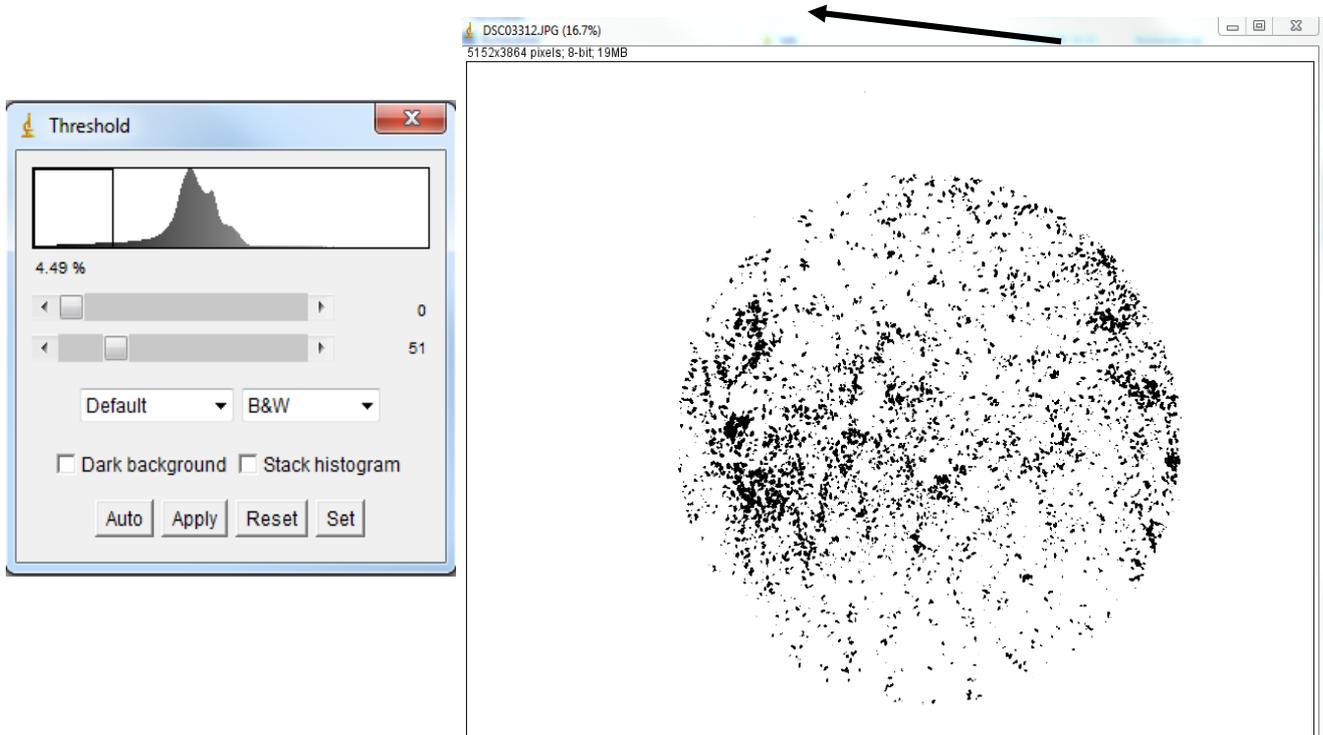
6. Indicate the picture you want to analyse.
7. Click "Image" → "Type" → "8-bit"



8. Click “Image” → “Adjust” → “Threshold
9. Following display appears:



Example :

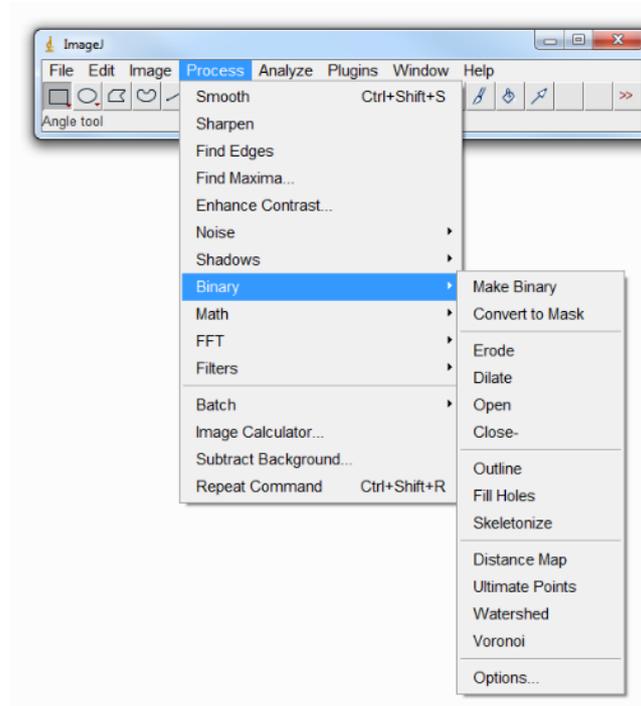


(OPTIONAL – IF MITES LOCATED TOO CLOSELY AND UNABLE TO BE SPREAD)

Use the “Watershed” option:

Click “Process” → “Binary” → “Watershed”

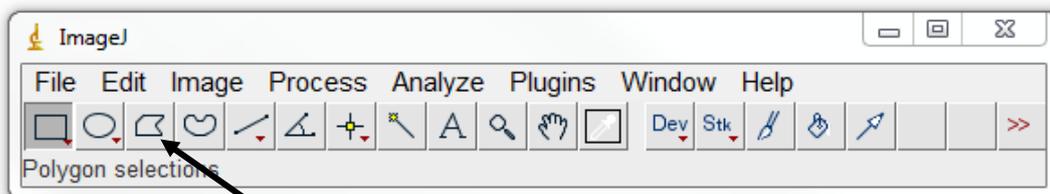
However, it is preferred to rather use an additional Petri-Dish to spread the mites (and add up the numbers) instead of using the “watershed” option

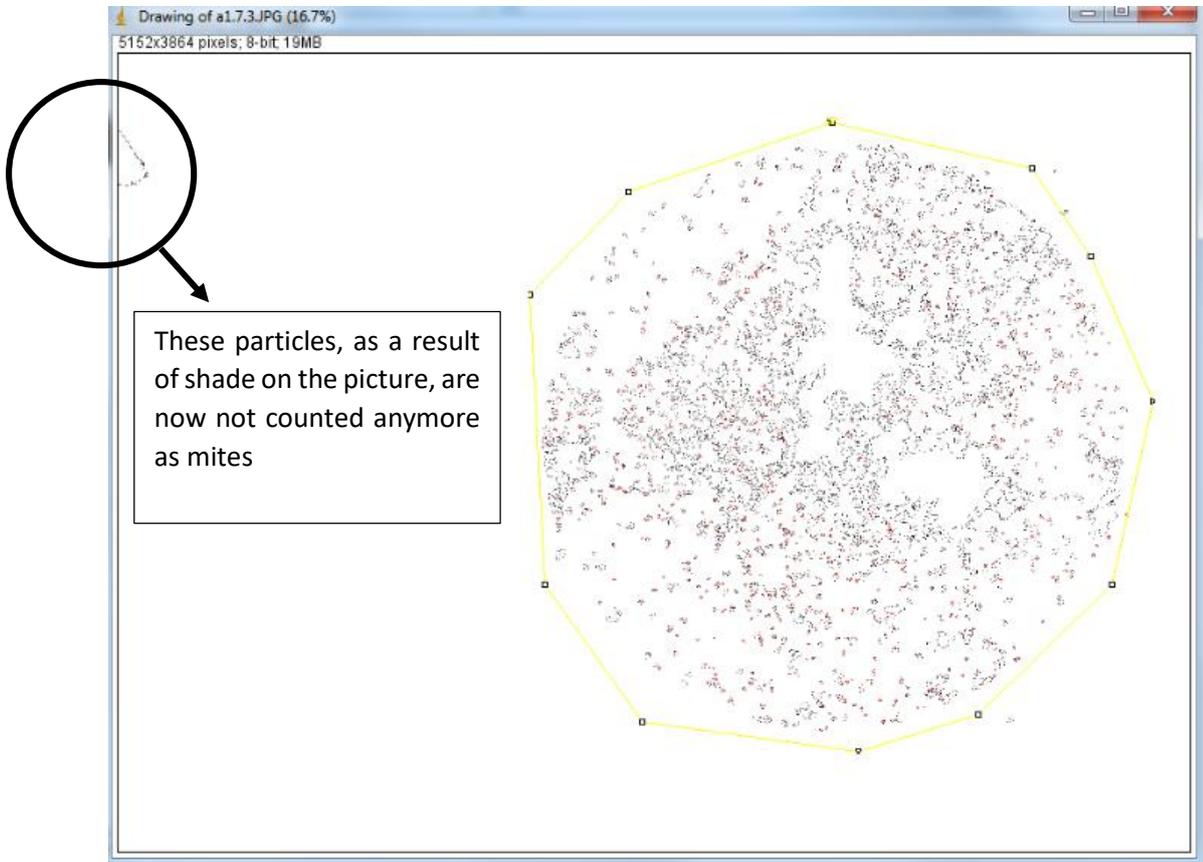


(OPTIONAL – IF SHADOW/DIRT ON PICTURE/PETRI DISH)

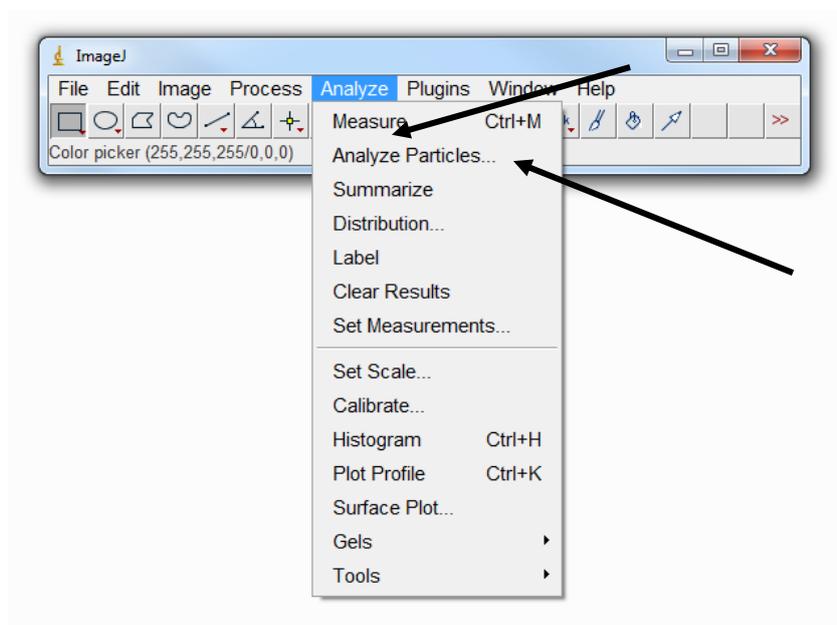
In cases where you have shadow/dirt on the picture/Petri Dish, this needs to be removed, otherwise this is counted as ‘particles’.

In this case you need to select the part of the picture that needs to be analysed, i.e. the petri dish itself. You can do this by selecting the area of the Petri Dish (yellow line) by using the polygon selections tool.

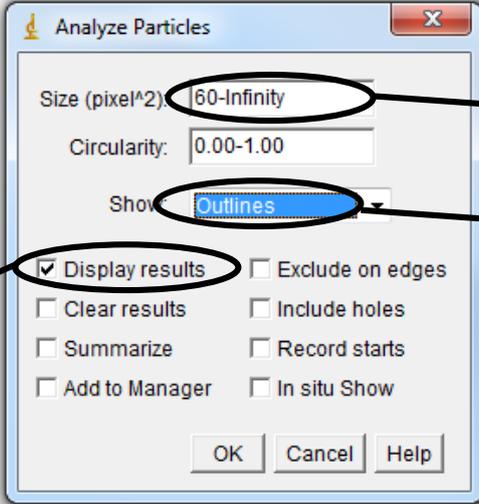




10. To estimate the number of mites click “Analyze” → “Analyze Particles”



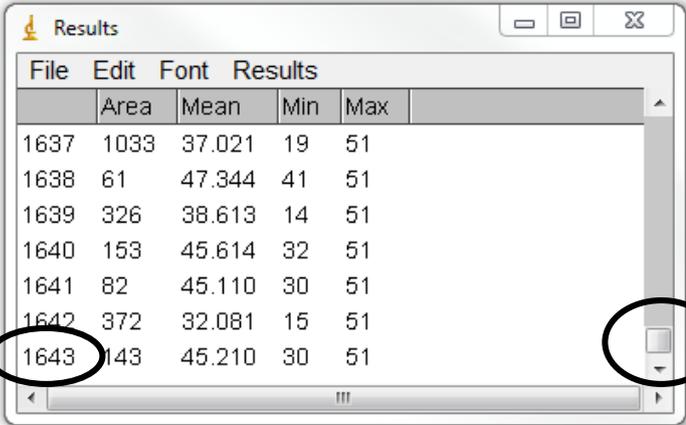
11. Make following adjustments on the display as illustrated below and click “OK”.



The 'Analyze Particles' dialog box is shown with the following settings and annotations:

- Size (pixel²):** Set to '60-Infinity'. Annotation: 'Change from "00" to "60"'. The '00' is circled in the original image.
- Circularity:** Set to '0.00-1.00'.
- Show:** Set to 'Outlines'. Annotation: 'Change from "Nothing" to "Outlines"'. 'Nothing' is circled in the original image.
- Display results:** Checked. Annotation: 'Needs to be checked!'. The checkbox is circled in the original image.
- Exclude on edges:** Unchecked.
- Clear results:** Unchecked.
- Include holes:** Unchecked.
- Summarize:** Unchecked.
- Record starts:** Unchecked.
- Add to Manager:** Unchecked.
- In situ Show:** Unchecked.

12. The results appear in a separate window



The 'Results' window displays a table with the following data:

File	Area	Mean	Min	Max
1637	1033	37.021	19	51
1638	61	47.344	41	51
1639	326	38.613	14	51
1640	153	45.614	32	51
1641	82	45.110	30	51
1642	372	32.081	15	51
1643	143	45.210	30	51

Annotations for the Results window:

- An arrow points to the last row (1643) with the text: 'Make sure you note the **last number** completely at the bottom'.
- An arrow points to the scrollbar with the text: 'Scroll to the bottom'.

REMEMBER

Close the “Results” window after analyzing each picture. Otherwise, the program will keep adding up the numbers!