Golddigger (Fiji) quantifies EM labeling

Author: Klaas A. Sjollema with Ben N. G. Giepmans (k.a.sjollema@umcg.nl)
Dept. Cell Biology & UMCG Microscopy and Imaging Centre (UMIC), University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

History: 2013/11/01 First version
2015/07/01 Counting module integrated, revised 2016/05/01

Source: Golddigger.ijm,
Golddigger Tools.ijm

Installation: Copy Golddigger.ijm to the ImageJ macros folder and Golddigger Tools .ijm to the ImageJ macros/toolsets folder. Start ImageJ and activate the Golddigger toolset via the "More Tools" menu double arrow icon (button 8 in Fig.1).

Description: Golddigger is an ImageJ macro to detect, mark and count electron dense markers like gold-grains or quantum dots in electron micrographs.

Manual:

1. Open an EM image. For a new image all parameters have to be set, this works best on a small image. For setting up the parameters on large (>25K pixels) image, first use a cropped selection, which should be stored in the same folder as the original. If the parameters are set, the complete image can be analyzed. If the image is part of a series comparable images in one folder and Golddigger was set already for one of these images, just run Golddigger (continue at step 9). It will read a text file which is added by Golddigger in this folder.

2. *Open the Golddigger Toolset* (button 8, Fig.1). Run Golddigger once without any adjustments, it will use default settings (button 1, and OK). The image produced by Golddigger consist of two layers, the first contains all rejected candidate grains (blue circles) the second contains all accepted candidates (red). Check if at least all grains are either blue or red (If so, continue at step4). If not, or if there are far too many grains selected. Run the setup help, question mark button (button 7, Fig.1)

3. Click the question mark button (7 in Fig.1) and subsequently click on a small (gold)grain on a dark background in the image. Golddigger will calculate roughly a set of parameters for the first detection. These values are written in the log window and stored in "parameter.txt" if the user accepts the values.

Fig 1. Golddigger toolset
(1) start Golddigger; (2) show marks and numbers; (3) show marks only; (4) no marks; (5)Return to the original image, removes layers and overlays; (6) change gamma; (7) parameter estimation tool; (8) ImageJ Toolsets.

4. Return to the original image (button 5) and open Golddigger (GD button Fig.1, button1). The main interface will pop-up. The parameters shown can be used for the first, raw detection calculated in step 3. Run Golddigger and evaluate. Positive candidate grains (red, second layer) and negative grains (blue, first layer) are marked. Check if all grains are selected, either blue or red. If not all, or way too many are selected, return to the original image (button 5) and open Golddigger (button 1). Now change one of the first five parameters in the Golddigger interface, typically related to particle size selection (M or P). Run Golddigger with the adapted parameters. Repeat until satisfactory. ***Always use the original image (button 5) before a Golddigger run.*** If satisfactory, the candidate grains have to be sorted properly. For this, the user has to set-up the last 9 parameters.

5. Open Golddigger again and *check "set up mode"* in the interface. Press OK. Golddigger will do a first detection (1000 particles max.) and the "Golddigger Setup" window appears, ***don't change anything and click OK***. A window appears with the message "Evaluate Image then click OK". You now can use the ImageJ functionality like scroll and zoom to see which grains are wrongly selected. Golddigger marked all selected grains with a red circle, all rejected grains with blue. The user can also use the "show label" tools in de Golddigger toolset (Fig.1, buttons 2,3,4) to show labels, numbers or nothing on the image. Check the image first for false negatives (blue encircled).
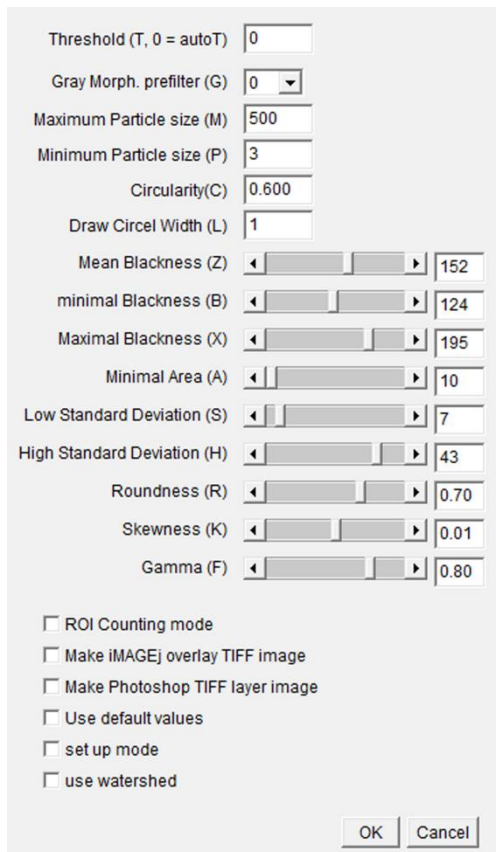
Fig. 2. Golddigger main interface
T: Threshold, if 0 (default) the RenyiEntropy dark auto threshold is used, any other number is a manual threshold setting. **G**: Use of ImageJ Gray morphology filter. 1 (default), 1.5, 2 enhance round-shaped structures. Use if grains are only a few pixels and not very obvious. If 0 no filter is applied.
M, P, C: sets size (square pixels) and circularity used for the analyze particles function of ImageJ (first detection round).
L: Thickness of the marking circles, used in Photoshop.
Z, B, X, A, S, H, R, K, F: used in second round, can be optimized using the set up mode. Blackness is the opposite of brightness.
ROI counting mode: Grain detection, resulting in points. User-defined ROIs in which particles are quantified have to be drawn if asked for. Output is a list of values and an overlay depicting the counts per ROI.
ImageJ overlay Tiff Image: Original image with the red marks only, automatically scaled when zoomed in- or out.
Photoshop TIFF layer: Original image including an overlay containing blue and red marks, and an empty image with the red marks only, which can be used as a layer in Photoshop.
Default values: Sets the default values for G to F.
Set up: Allows change of parameters Z to F and check results per grain.
Watershed: To separate very close particles. Do not use this at large files (> 5k/5k) it will slow down the process.

6. Click OK. A new window appears and all labels on the image are numbered. *Fill in the number of the grain spotted to be wrong* in step 5. *Click OK.* The Golddigger set-up interface appears.

7. Include rejected grains. The interface shows a list of parameters with some numbers. Left of the slider the number represents the actual value of the selected grain and a "<" or ">" sign. Right of the slider the cut-off value is depicted. A candidate grain is selected negative if one or more of the actual values does not meet the cut-off setting. In this case a "#" sign is depicted in front of the parameter. *Change the number of a rejected grain until it meets the criterion and click OK*. The complete image is updated. The more you increased cut-off value, the more grains will be selected. Repeat until all false negatives are selected (red).

8. In case of False positives - Standard deviation, Roundness and Skewness typically have to be changed. Changing the Blackness and Area settings will most likely affect many true positive grains. If the parameters are satisfactory, *skip this step by clicking "cancel"*.

9. Return to the original image (button 5), and *reopen Golddigger* (button 1).
Choose the desired output, if nothing is selected, the output will be a two layer image with the positive and negative labels saved as an ImageJ overlay. This overlay is saved if the image is saved as a Tiff including the overlays.
- ROI Counting mode. In counting mode the user is asked halfway the process to draw ROIs (freehand selection tool), store these in the ROI manager of ImageJ (press T, or cntr. T) and press "*OK*". Golddigger saves the results in the log window and on an overlay of the image.
- ImageJ overlay Tiff image. This shows all positive selections. Marks are automatically scaled and thus visible in very high, and very low magnifications.
- Photoshop Tiff layer image, with the position of all positive grains as red marks on a white background, to be used as an overlay in e.g. Photoshop.

The "Use watershed" option can be selected for images with many close, almost overlapping grains. Water shedding will, however, drastically increase the calculation time and will take long for large images (>25Mb).

Next click OK, Golddigger will start with the final detection. The first 100 grains will be sorted visual for the user, then Golddigger will go into "batchmode", the image will not be visible until the process is finished. This speeds up the process drastically.