Before using plugin

1. Taking images, it is the best to have only one cell in each images. Like this:

2. The cell images have to be bright enough otherwise the plugin would not get the cell area right later (left: wrong and right: right)

3. Combine all cell and gelatin images in separate stack (cell images on one stack and gelatin on images another stack images have to be 8 bits grey the plugin only works on stacks.)

4. Before doing actual degradation quantification, you need to perform a pilot experiment. Which is taking a series of gelatin images with different exposure.
Like this:

The reason for this is you need to find the best exposure, which the plugin would not miscount the degradation. As you see the gelatin images from left to right with the highest to lowest exposure the plug in will quantify the degradation differently. The exposure too high you will get more degradation then it should be, exposure too low, you will get less degradation or even no degradation. In the case, the middle one is the best one to fits the real degradation. It is necessary to do this every time you start using new batch of gelatin. As there may be difference on the level of FITC labelling.

**Start using the plugin**

After you install the plugin by copying the plugin into plugins folder in imageJ folder. Open imageJ, then Plugins>Gelatin>Gelatin degradation.

The plugin will ask to open the myc image, this is actually ask you to open cell stack, here click OK and find you cell stack. Then the plugin will ask to you to find gelatin images, click ok and find gelatin stack.

Now the plugin should run automatically, and you will get the degradation quantification and images for degradation at end.