Ilastik Image Segmentation and Labeling

**Procedure:**

1. Set up new pixel project
2. ‘Feature Selection’ – select all of the circles that are equal or larger than the particles to be labeled in the image
3. ‘Training’ – identify particles and the background using labels
4. Reduce uncertainty by labeling several different locations on the particles and the background.
5. ‘Object Feature Selection’- select the criteria used to predict the particle type (I used either ‘Count and RegionRadii’ or ‘Skewness, Count, and Region Radii’)
6. ‘Object Classification’ – make four labels for singles, doubles, flats, and clusters, then manually label 10 of each so that Ilastik can predict the rest.

**Data:**

1. For each category of nanoparticle (np), 10 were manually labeled so that Ilastik could more accurately predict the unlabeled np’s.

|  |  |
| --- | --- |
| **Type** | **Labeled** |
| Single | 10 |
| Double | 10 |
| Flats | 10 |
| Super | 10 |

**II.** True percentages of each type of particle in the image found when using Count and Region Radii as the qualifiers for ‘Object Feature Selection’ in Ilastik.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ilastik Count & Region Radii** | | | | |
|  | 8/13/12 | 9/5/12 | 10/19/13 | 10/30/13 |
| Single | 70.0% | 52.0% | 66.8% | 91.6% |
| Double | 20.4% | 20.3% | 23.4% | 7.3% |
| Flats | 8.7% | 13.3% | 8.9% | 0.0% |
| Cluster | 0.8% | 14.4% | 1.0% | 2.0% |

**III.** True percentages of each type of particle in the image determined by a previous method.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Old Method** | | | | |
|  | 8/13/12 | 9/5/12 | 10/19/13 | 10/30/13 |
| Single | 75.8% | 57.6% | 71.15% | 92.41% |
| Double | 19.0% | 12.3% | 19.87% | 6.95% |
| Flats | 5.2% | 23.3% | 8.92% | 0.64% |
| Cluster | 0.1% | 6.8% | 0.07% | 0.00% |

**IV.** An extra analysis of the August 13 image was done to observe the differences due to manually labeling different nanoparticle, but still using the same ‘Object Feature Selection’ criteria.

|  |  |
| --- | --- |
| **Aug 13 CRR (extra)** | |
| Single | 69.8% |
| Double | 14.6% |
| Flats | 14.2% |
| Cluster | 1.4% |

**V.** An analysis was also ran of the August 13 image using skewness, count, and region radii for the ‘Object Feature Selection’ criteria. This analysis used the **exact same particle labels** that gave the results for August 13 in table **III**.

|  |  |
| --- | --- |
| **Aug 13 SCRR** | |
| Single | 69.9% |
| Double | 20.7% |
| Flats | 8.6% |
| Cluster | 0.9% |

**VI.** Summary of the difference between methods.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Method Comparison Aug 13** | | | | |
| Type | Different Feature, Same Label | Same Feature, Differenet Label | Ilastik vs. Old Method Aug 13 | Ilastik vs. Old Method Sep 5 |
| Single | 0.10% | 0.20% | 5.75% | 5.60% |
| Double | 0.30% | 5.80% | 1.42% | 8.00% |
| Flat | 0.10% | 5.50% | 3.50% | 10.00% |
| Cluster | 0.10% | 0.60% | 0.72% | 7.63% |

**Notes:** It is apparent from table **II** and **IV** that there is a lot of variance in the results produced by Ilastik. The two labeled images from August 13 were created in the exact same manner except that the manual labeling of np’s was different. Further, this only produced significant effects on larger np’s, which is expected because the ‘Object feature Selection’ criteria (count and region radii) focus on the size and number of pixels in each np. Thus, such criteria can easily identify and predict singles, but may not be the best for our purposes of also labeling doubles, flats, and clusters.

Also the quality of the image plays a major roll in the ability of Ilastik to predict labels for the np’s. The image from **August 13th** was the best image, due to the high resolution, the larger spacing between np’s, and the consistent shade of the background. The image from **September 5th,** had poor resolution, little spacing, and was dominated by large aggregates. The poor spacing resulted in many smaller particles being identified as parts of the large clusters. The image from **October 19th** was also difficult to work with as it was predominantly a very dense layer of small singles. Because the particles were so close, there were several cases in which two separate singles were identified by Ilastik as a double. Also some of the singles were so small that Ilastik could not identify them. The image from **October 30th** was also predominately singles, but had greater spacing between np’s. This allowed for easy labeling and more accurate predictions.

*We noted that both Object Feature Selections used gave very similar results (seen above in* ***II*** *and* ***V****). The deviation from old method is more significant.*

**Extra:**

* Ilastik runs faster with higher resolution images.
* Ilastik runs slower when images contain large numbers of np’s.
* Ilastik runs much slower when skewness is run as one of the criteria for ‘Object feature Selection.
* Shadows of large clusters are often considered background and therefore affect label prediction when using criteria that focus on size (such as count and region radii).