

Protocol for Ribosome Identification

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Polyribosomes can be identified based on size, shape, pattern, and opacity. The slides should be viewed at very high contrast, such that actin is barely visible. The screen width should be about 2 μm when scanning for clusters and counting, but magnification is often necessary for identification.

Size: A structure can be counted as a polyribosome if it consists of at least three distinct opaque spots. A spot in a cluster that is counted as a single ribosome should be in the range of 0.01-0.03 μm in diameter, not including the edges. Because of their small size, individual ribosomes can not be seen on more than one section, although a particularly large cluster may spread across two or even three sections.

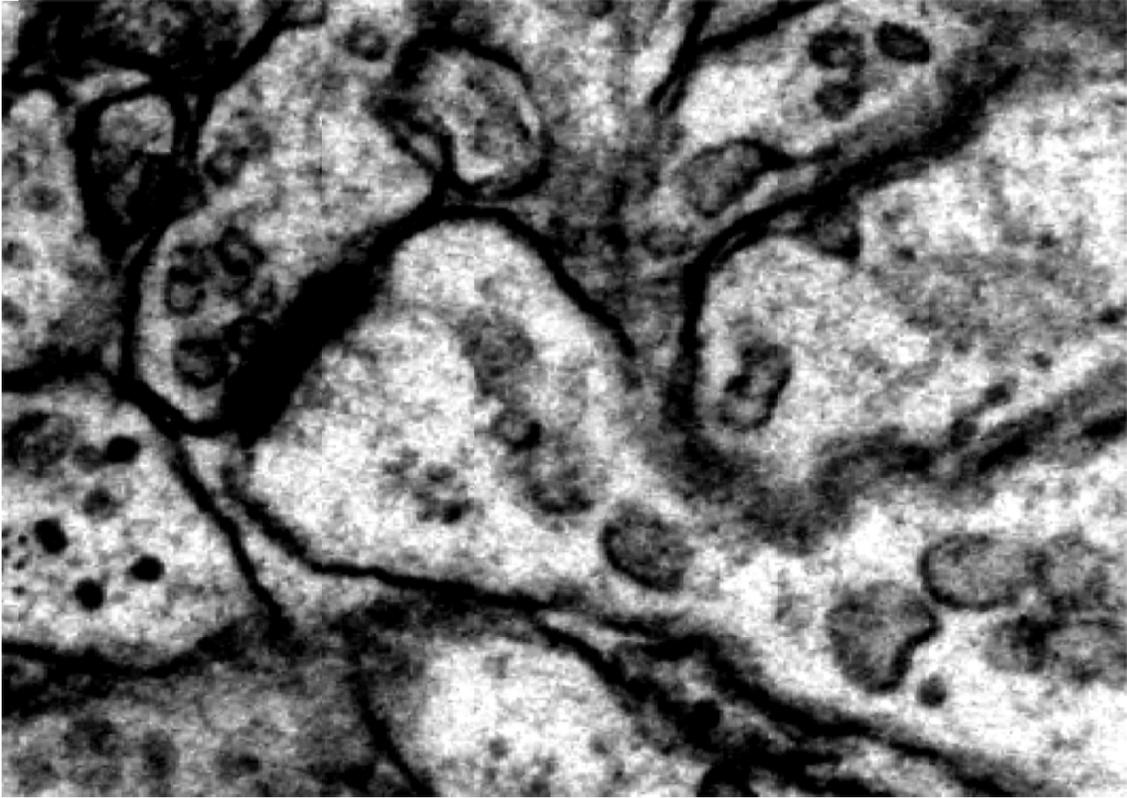
Shape: Individual ribosomes do not appear round; most are heart-shaped or oval. The edges are fuzzy, and there is generally grey matter between the dark-staining spots.

Pattern: Clusters of ribosomes come in many shapes, but a few are seen often. The most common are: 1) 5 or 6 in a star shape, 2) a spiral or circle, and 3) a staggered double line. Larger clusters (>15) tend to have more chaotic patterns.

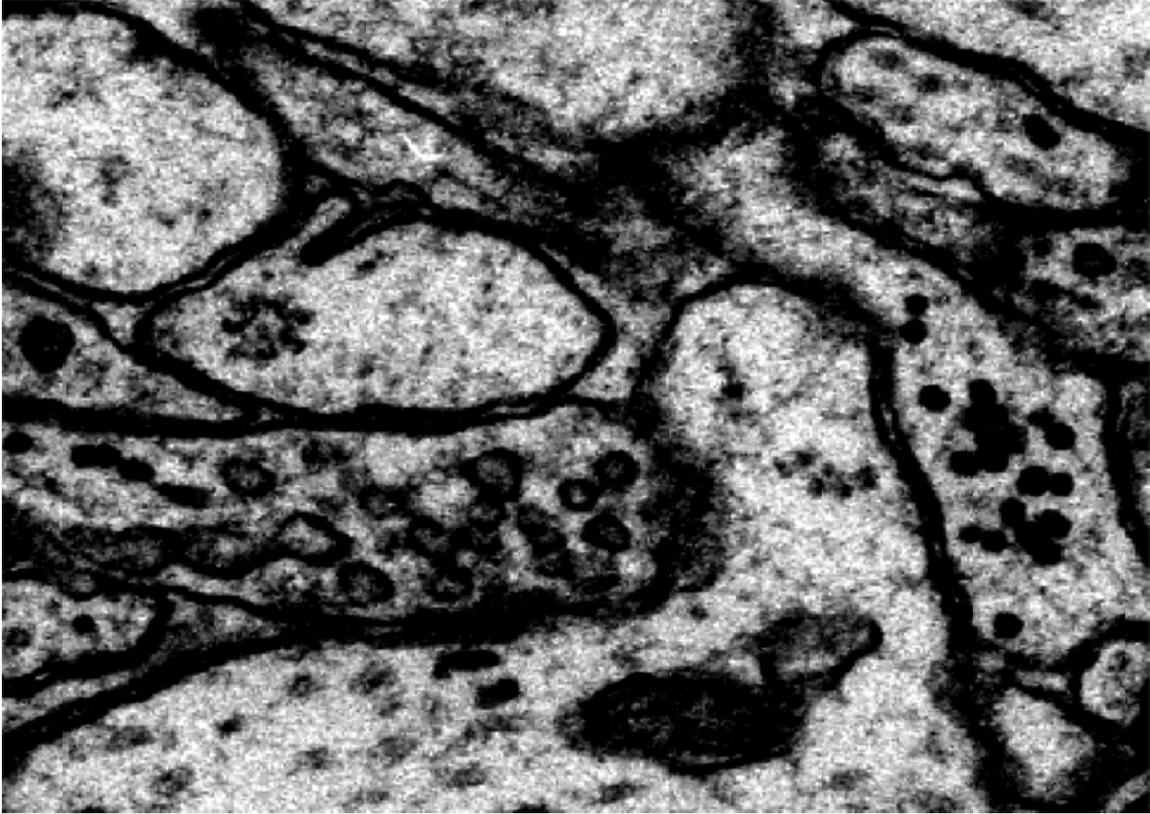
Opacity: A cluster must have several spots that stain as dark as the plasma membrane, although the surrounding lighter area may be counted as part of the structure.



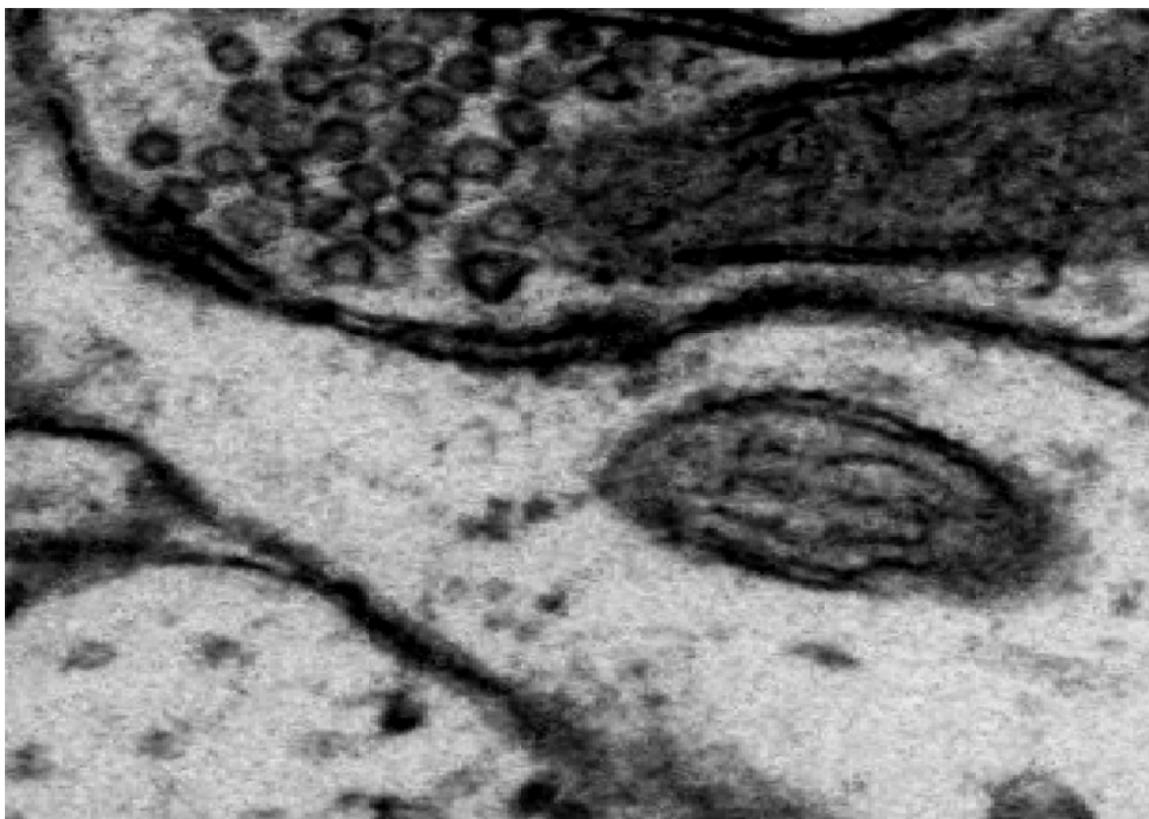
This cluster is a circle of six, perhaps the beginning of a spiral. Notice the irregularly shaped dark spots surrounded by grey matter. The three individual spots to the lower right of the cluster are too light and discrete to be ribosomes.



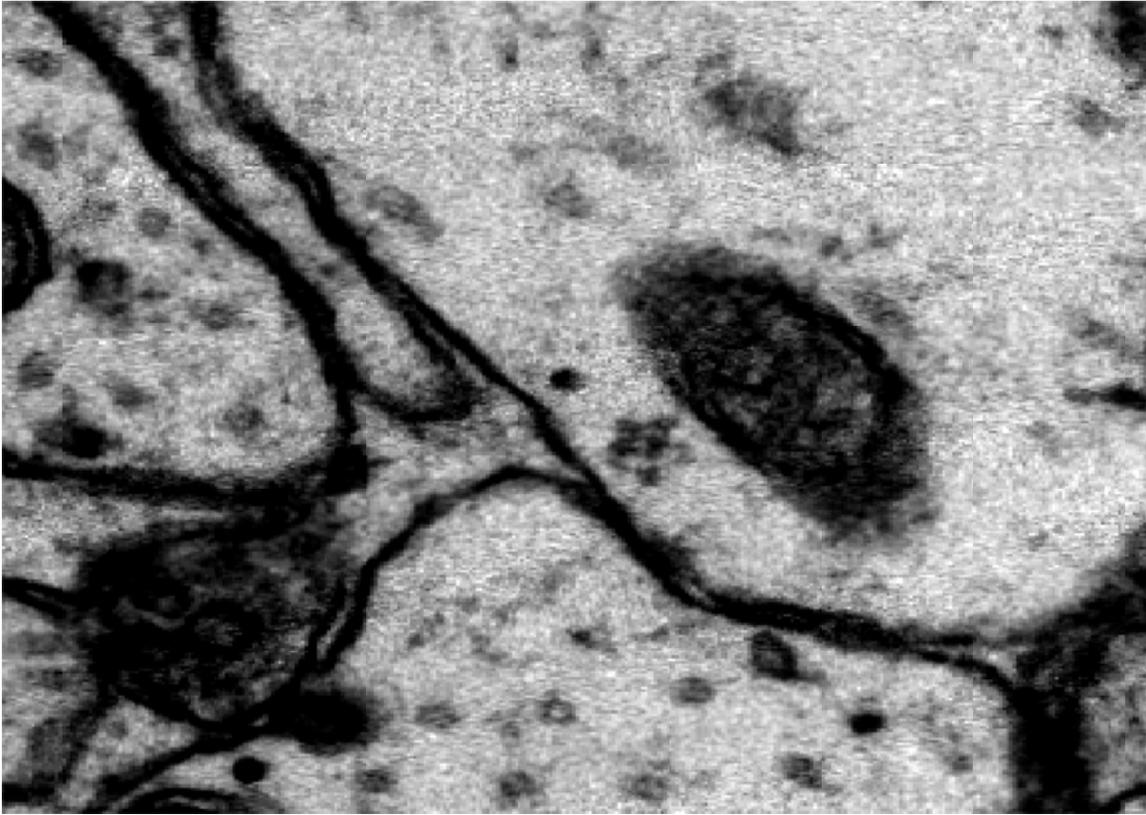
The structure on the left is a polyribosome, which would be counted as six. The structure on the right looks similar, but is continuous with ER in the next section and is therefore not counted.



A spiral and a staggered line. It doesn't get any better than this.



The four ribosomes to the left of the mitochondria are clear. Six more are counted beneath these, although only one is opaque. Below this four or five more may be counted, but only because their proximity to the other clusters makes them candidates.



A star of five next to a mitochondrion. Note the dark centers and fuzzy edges, and the grey matter. The large black spot above the cluster is too large and round to be a ribosome, and too far away from the polyribosome.