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Cryo-EM Optimal Box Size

The Ando Lab
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General Notes

1. Literature references:

   - Rosenthal, Peter B., and Richard Henderson. 'Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy.' Journal of molecular biology 333.4 (2003): 721-745. [1]
   - A discussion board:
     https://www.jiscmail.ac.uk/cgi-bin/webadmin?A2=ccpem;e433702b.1412

2. Common microscopes and their parameters:

   - Talos Arctica/Glacios
   - Krios

Derivation

Box Size

The derivation is expanded from Rosenthal et al., 2003 [1] but before we dive into the derivation we will start at the final result and discuss it's implications.

The result from Rosenthal et al., [1] states that the optimal box size for cryo electron microscopy three-dimensional particle reconstruction should obey the following relationship:

$$BoxSize \geq ParticleDiameter + 2R \tag{1}$$

$$R = \lambda \Delta f u + C_s \lambda^3 u^3 \tag{2}$$

where R represents YYY, λ is the energy of the electron beam, and u = $[\frac{1}{m}]$ where m is the highest resolution expected in meters . . . . WHERE DO THE HIGHER ORDER TERMS COME FROM?! SEE THIS REF [2] - ALTHOUGH HOW TO PURCHASE?!

The calculation (**WHAT CALCULATION**) speed will increase with with decreasing box size with the caveat that fast fourier transforms (FFT's) have the unique behavior of operating faster with **YYY** as a power of 2, 3, or 4.

EXPLAIN ABOVE BETTER

Boxes per grid and particle density

Reference: https://math.stackexchange.com/questions/3007527/how-many-squares-fit-in-a-circle

Once an ideal box size has been calculated an estimation of ideal particle density, given a known grid size hole, can be estimated to further improve grid preparation optimization. The problem boils down to the simple question: How many squares can you fit in a circle?

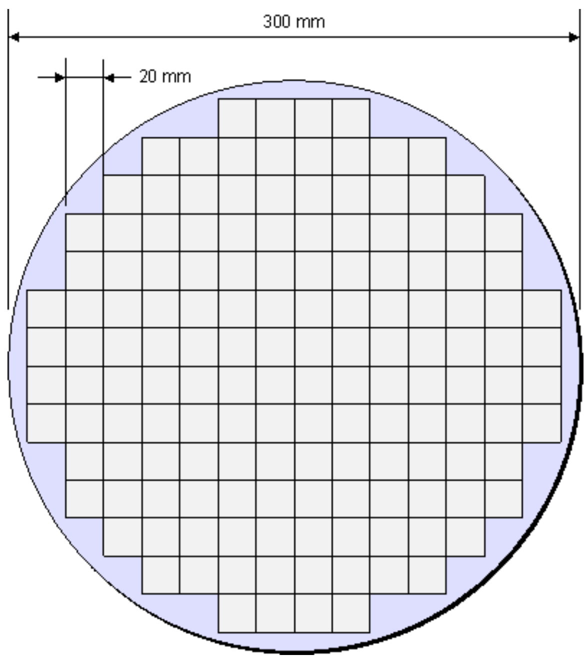

Figure 1: An illustration, including arbitrary units, detailing the general principle of the geometric solution desired for calculating the number of boxes that could fit within a given grid size hole. These calculations will allow for an estimation of ideal particle density(i.e. sample concentration).

The following derivation was obtained form the reference above.

gd = Grid diameter
gr = Grid radius
gc = Grid circumference
ga = Grid area
SEL = Square Box Edge Length
BA = Box area

$$Boxes\ Per\ Grid = \frac{\pi(\frac{gd}{2})^2}{BA} - \frac{\pi gd}{\sqrt{2BA}} \tag{3}$$

The above can be rewritten considering the following:

$$\frac{gd}{2} = gr \tag{4}$$

Therefore ...

$$\pi gd = gc \tag{5}$$

&

$$\sqrt{BA} = SEL \tag{6}$$

Finally..

$$Boxes\ per\ Grid = \frac{ga}{BA} - \sqrt{\frac{1}{2}}(\frac{gc}{SEL}) \tag{7}$$

where the first term describes the maximum number of boxes you could fit per grid if the grid was a perfect square and the second term accounts for the loss of boxes per grid due to the circular shape of the grid hole (i.e. squares in a circle do not fit perfectly, of course).

To derive this relationship, recall gr is the grid radius and SEL is the square box edge length. Furthermore, the 'unusable' area of the grid corresponds to the area within $\frac{SEL}{\sqrt{2}}$ of the perimeter (half the diagonal of each box). We can then estimate the number of boxes (N) we can fit on the grid:

$$N(r, L) \approx \frac{\pi(r - \sqrt{1/2}(SEL))^2}{(SEL)^2} = \frac{\pi(gr)^2}{(SEL)^2} - \frac{2\pi(\sqrt{1/2}SEL)}{(SEL)^2} + \frac{\pi(SEL)^2}{2(SEL)^2} \tag{8}$$

which can be written more simply as:

$$N(r, L) = \pi(\frac{gr}{SEL})^2 - \frac{2\pi(gr)}{\sqrt{2}(SEL)} + \frac{\pi}{2} \tag{9}$$

and because $\pi/2 \approx 1.57$ it can be dropped from the estimate. The estimate then becomes:

$$N(r, L) = \pi(\frac{gr}{SEL})^2 - \frac{2\pi(gr)}{\sqrt{2}(SEL)} \tag{10}$$

and finally..

$$N(r, L) = \pi\frac{(gr)^2}{BA} - \frac{2\pi(gr)}{\sqrt{2}(BA)} \tag{11}$$

Substitute $\lambda = \frac{gr}{SEL}$ and the above becomes:

$$N(\lambda) = \pi\lambda^2 = \sqrt{\frac{1}{2}}2\pi\lambda = \pi\lambda(\lambda - \sqrt{2}) \tag{12}$$

which is actually quite a conservative estimate.

Python Code

Located at https://github.com/Mill6159/EM_BoxSize_Prediction.

References

[1] Peter B Rosenthal and Richard Henderson. "Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy". In: *Journal of molecular biology* 333.4 (2003), pp. 721–745.

[2] John CH Spence. *High-resolution electron microscopy*. OUP Oxford, 2013.