SAND2013-10316P

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Analysis of Autophagy Induction in Cells

U.S. DEPARTMENT OF ENERGY

NNSA National Nuclear Security Administration

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Qualitative Comparisons to Quantitative Analysis

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- Qualitatively, cells with induced autophagy (30 µM PP242) appear dramatically different than negative controls (DMSO)
  - In the absence of autophagy, GFP signal is widely distributed throughout the cell, while only a small fraction of the GFP is segregated into puncta. Significant RFP signal from numerous puncta is observed.
  - When autophagy is induced, the GFP signal becomes concentrated into numerous puncta. The relative number of RFP puncta appears to decrease.
- Quantitative results are obtained by processing each image with a wavelet-based spot-detection (a.k.a. segmentation) algorithm
  - The algorithm determines which pixels have significant “spot-like” signal, that is signal which is punctate.

Sort 1 DMSO

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Raw Image Overlay

Pixels with Punctate Signal

Sort 1 PP242

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Raw Image Overlay

Pixels with Punctate Signal

Classification Schemes

- Numerous metrics were examined to see which were best able to classify between the presence and absence of autophagy.
  - Number of pixels found to have punctate signal
  - Number of distinct puncta
  - Average size of the puncta
  - Sum of the signal intensity of all pixels found to have punctate signal
  - Average signal intensity of pixels found to have punctate signal
  - Average signal intensity of the peak pixels (brightest signal pixel in each puncta)
- Metrics were examined for each channel (GFP, RFP) independently, as well as comparing the ratio of that metric for the two channels.

Best Classifier

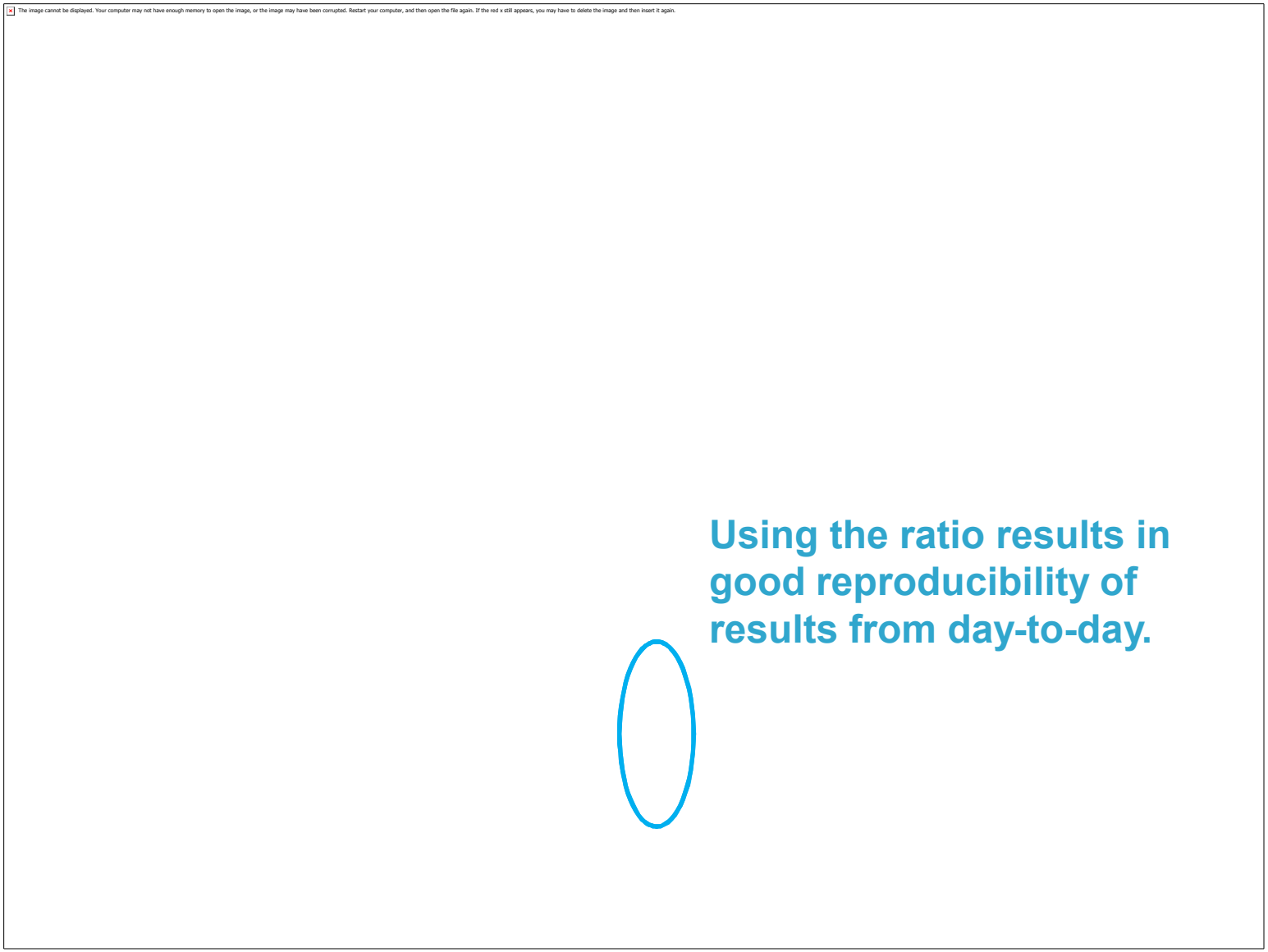
- The best classifier was probably the number of pixels found to have punctate signal.
  - Sum of the signal intensity of all pixels found to have punctate signal was about as good, as it is largely dependent upon the number of signal pixels found.
  - The GFP channel was a stronger classifier than the RFP channel. However, as the trends are opposite, the ratio of GFP channel/RFP channel is even better.
    - Taking the ratio also has the advantage of automatically compensating for variations in transfection/expression or in the fraction of the image area occupied by cells.
- Sort 1 cells seem to have better reproducibility to their results.

Area with Signal

Dose Dependence

- The metrics were also evaluated as the dosages of the potentially active compounds were varied.
- 30 µM PP242 was tested on three separate occasions, and the results were found to be highly reproducible for the ratio of signal area.
- Examining the dose dependence ensures the validity of the response, that the observed changes are due to the compound being evaluated

Area with Signal

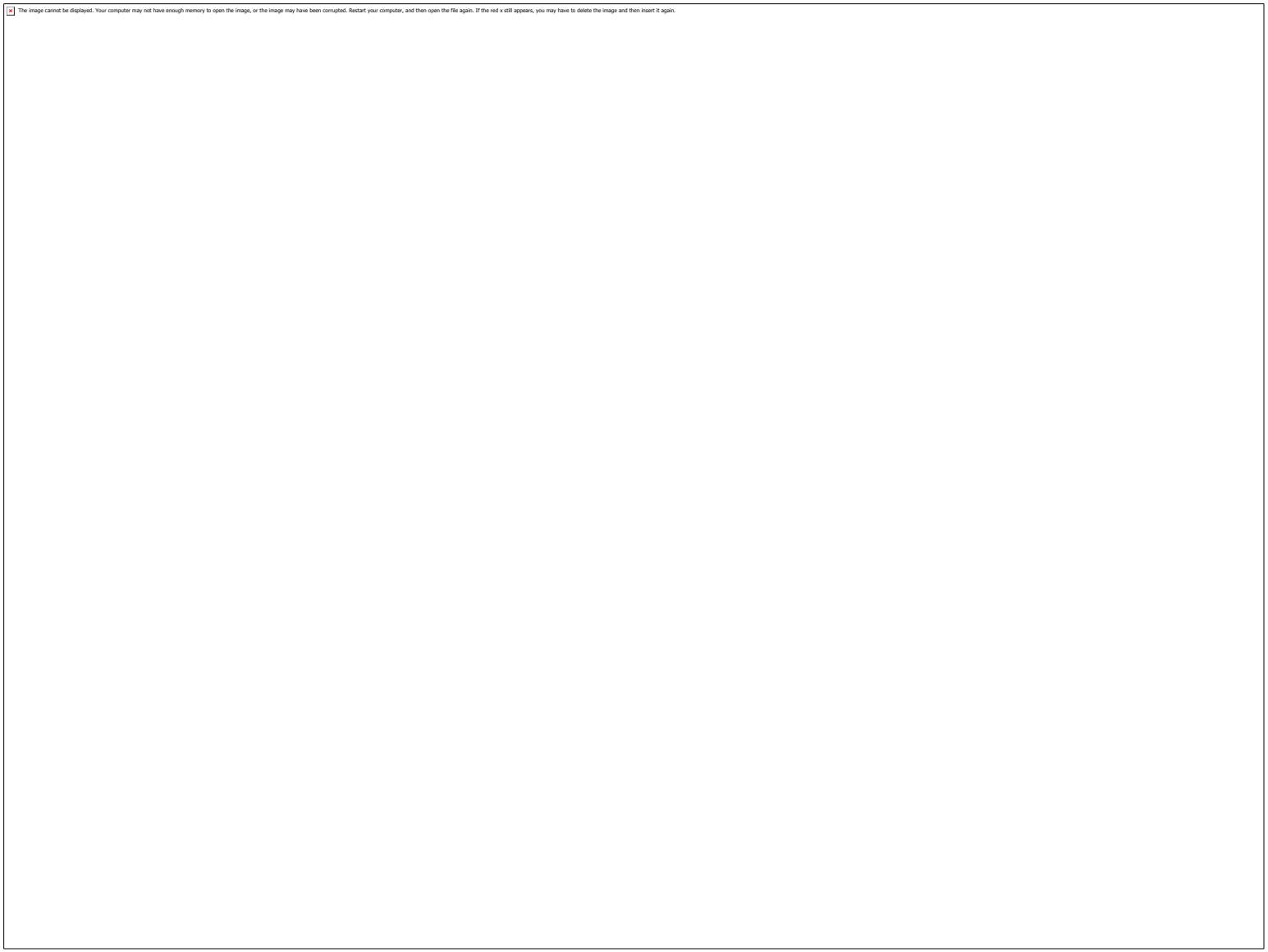


Using the ratio results in good reproducibility of results from day-to-day.

Additional Compounds

- Resveratrol and Curcumin were also evaluated as potential inducers of autophagy
- Responses may be time-dependent, and the delay after exposure may have been insufficient to observe autophagy.
- Note that in the following plot, multiple concentrations are present (same color). Therefore, apparent lack of autophagic response from some examples of a compound may only indicate insufficient dosage.
- At higher concentrations, PP242 is clearly distinct from DMSO.
- While not as clear, Resveratrol appears to be distinct from DMSO as well, but somewhat different behavior than PP242.

Area with Signal



Sort 1 Resveratrol 100 µm 6 hrs

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Raw Image Overlay

Pixels with Punctate Signal

Sort 1 Curcumin 10 µm

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Raw Image Overlay

Pixels with Punctate Signal

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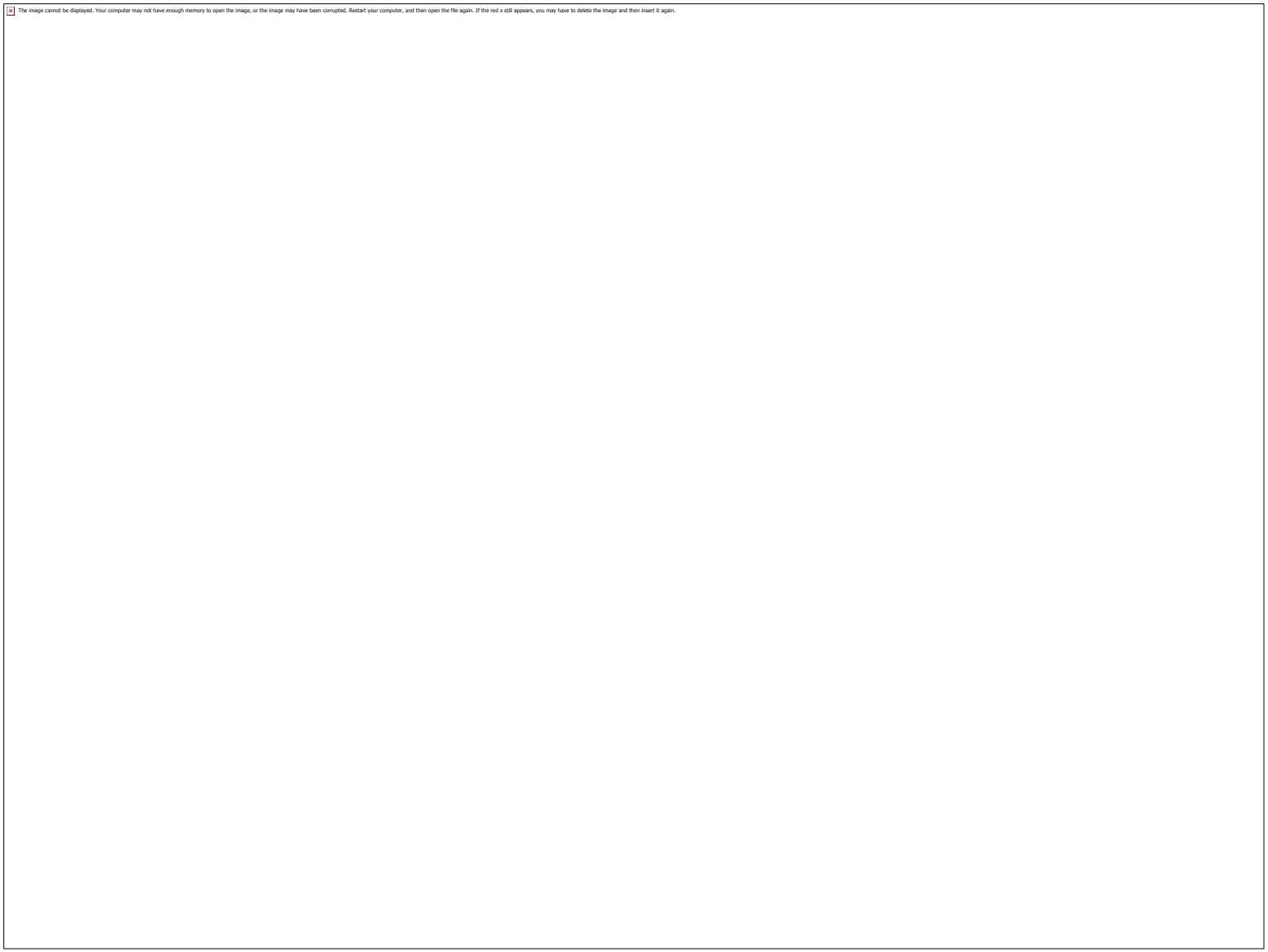
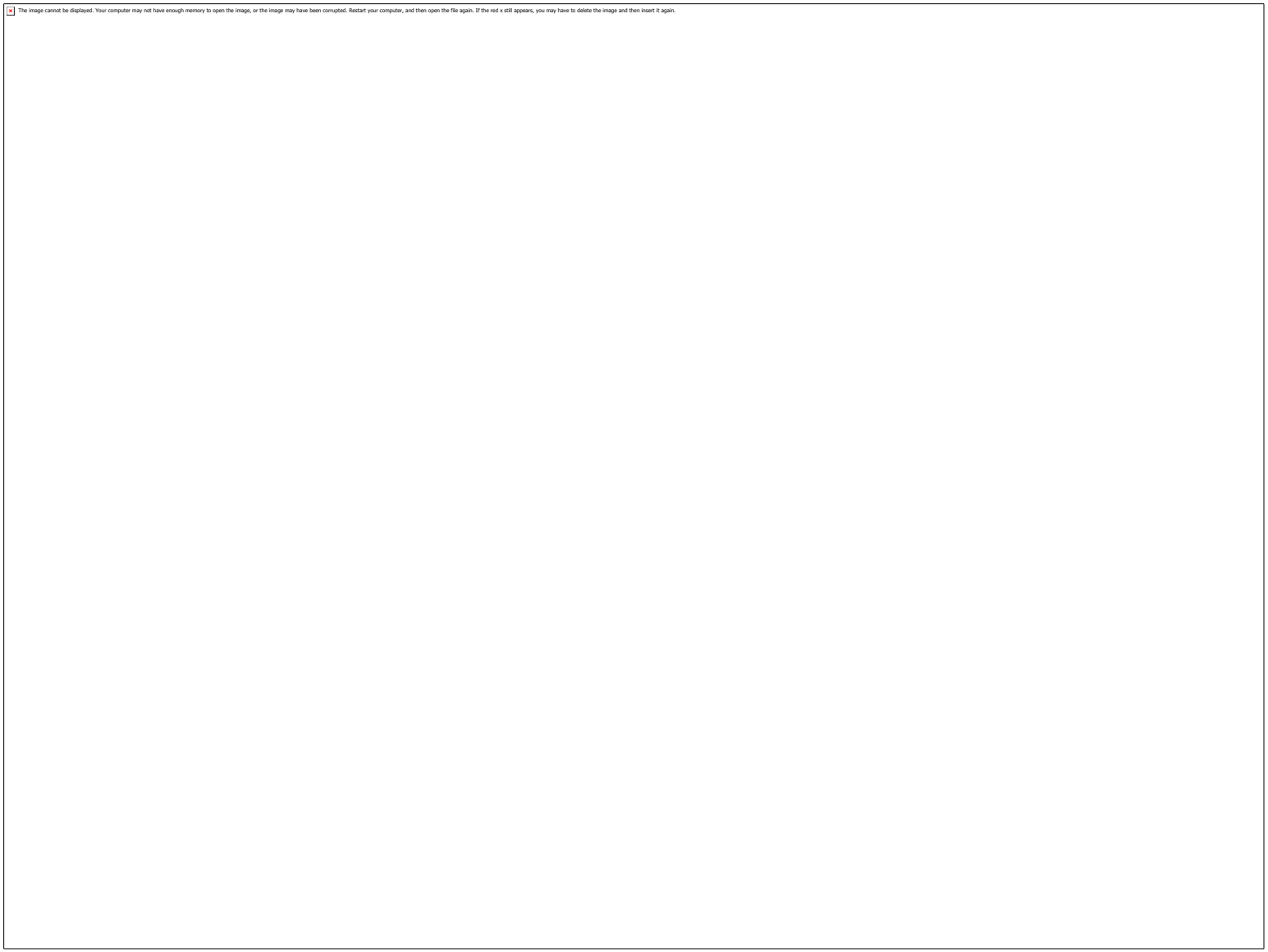
ADDITIONAL DATA

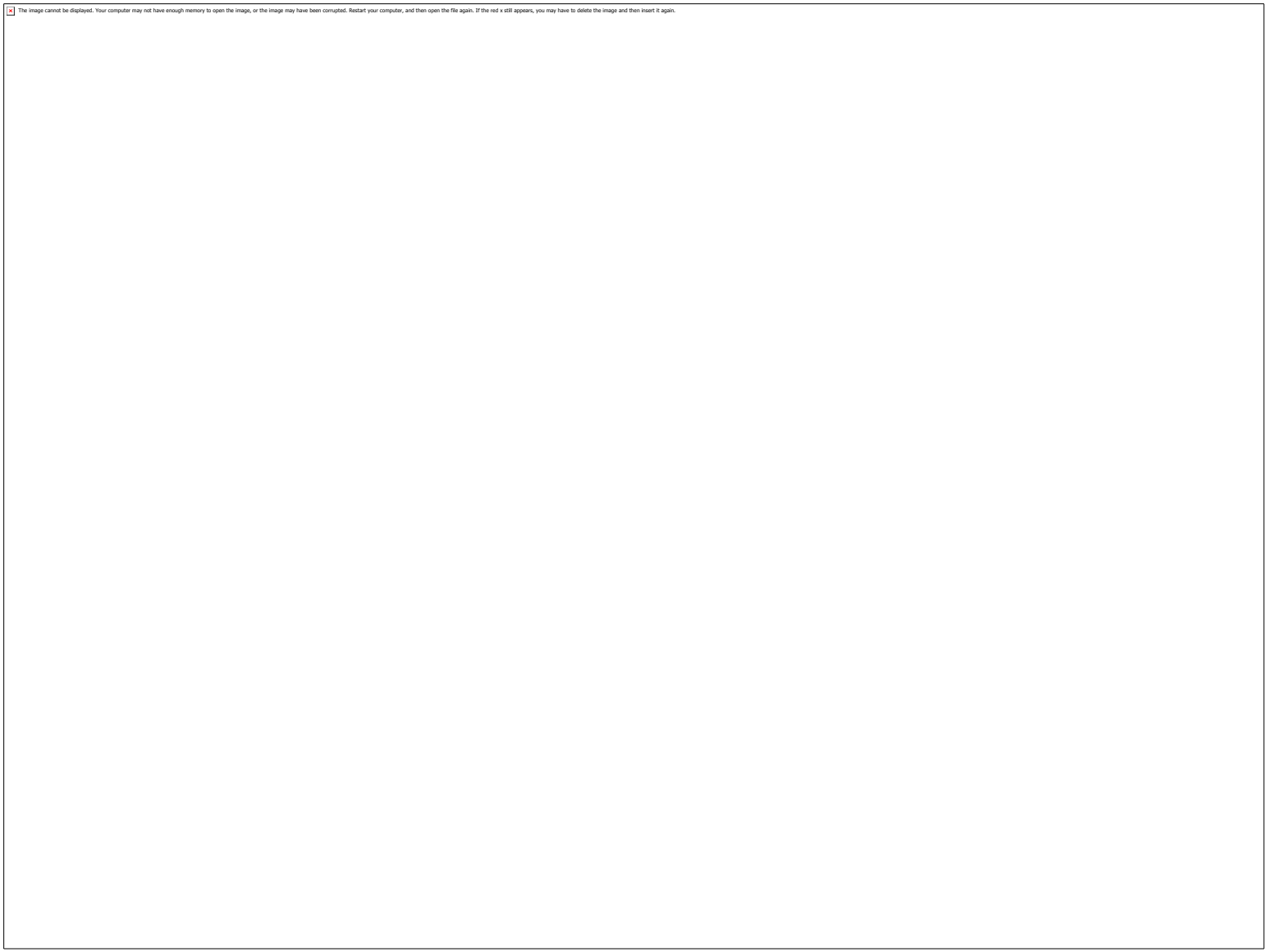
Number of Puncta



Number of Puncta



Puncta Size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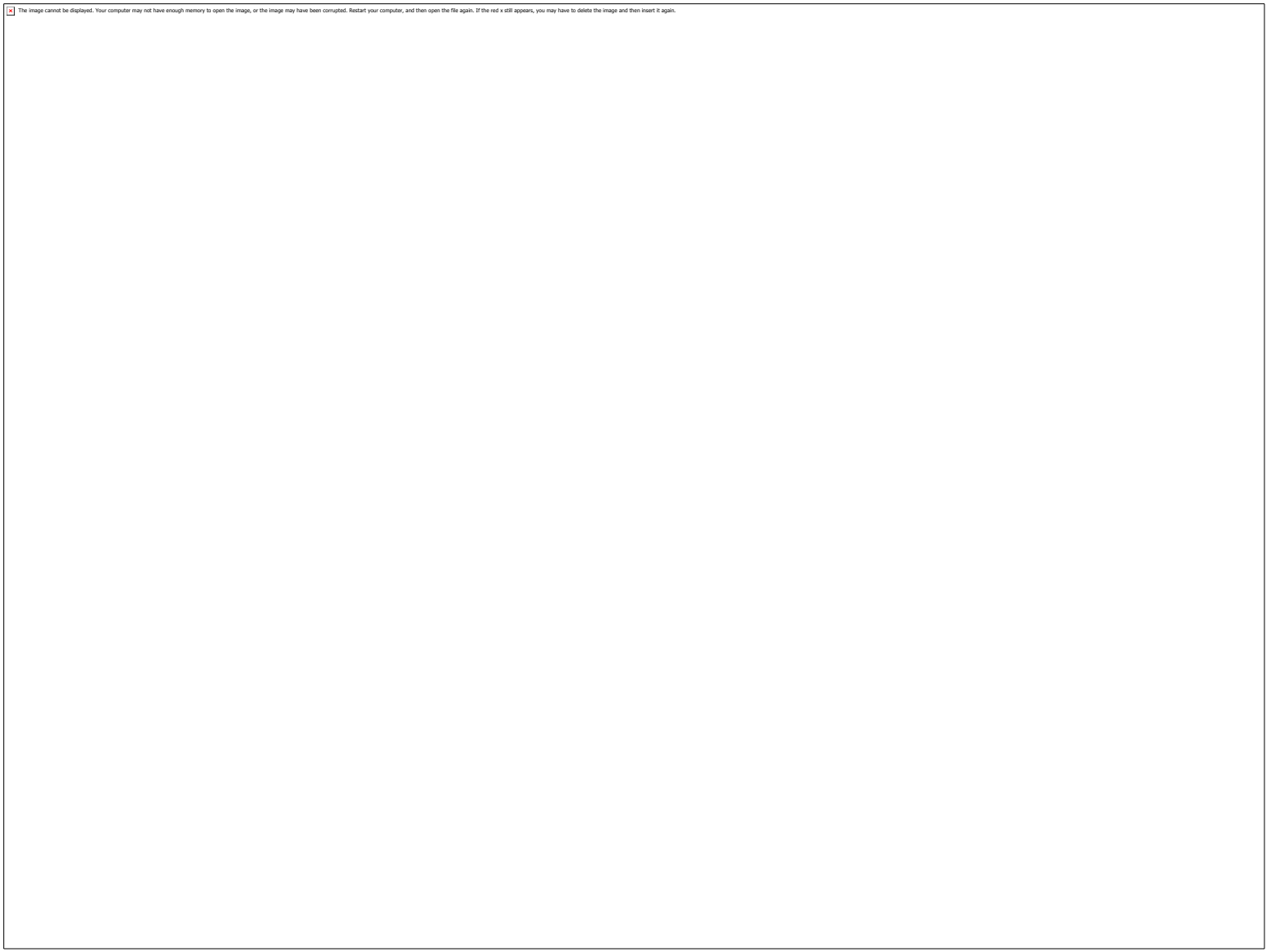
Puncta Size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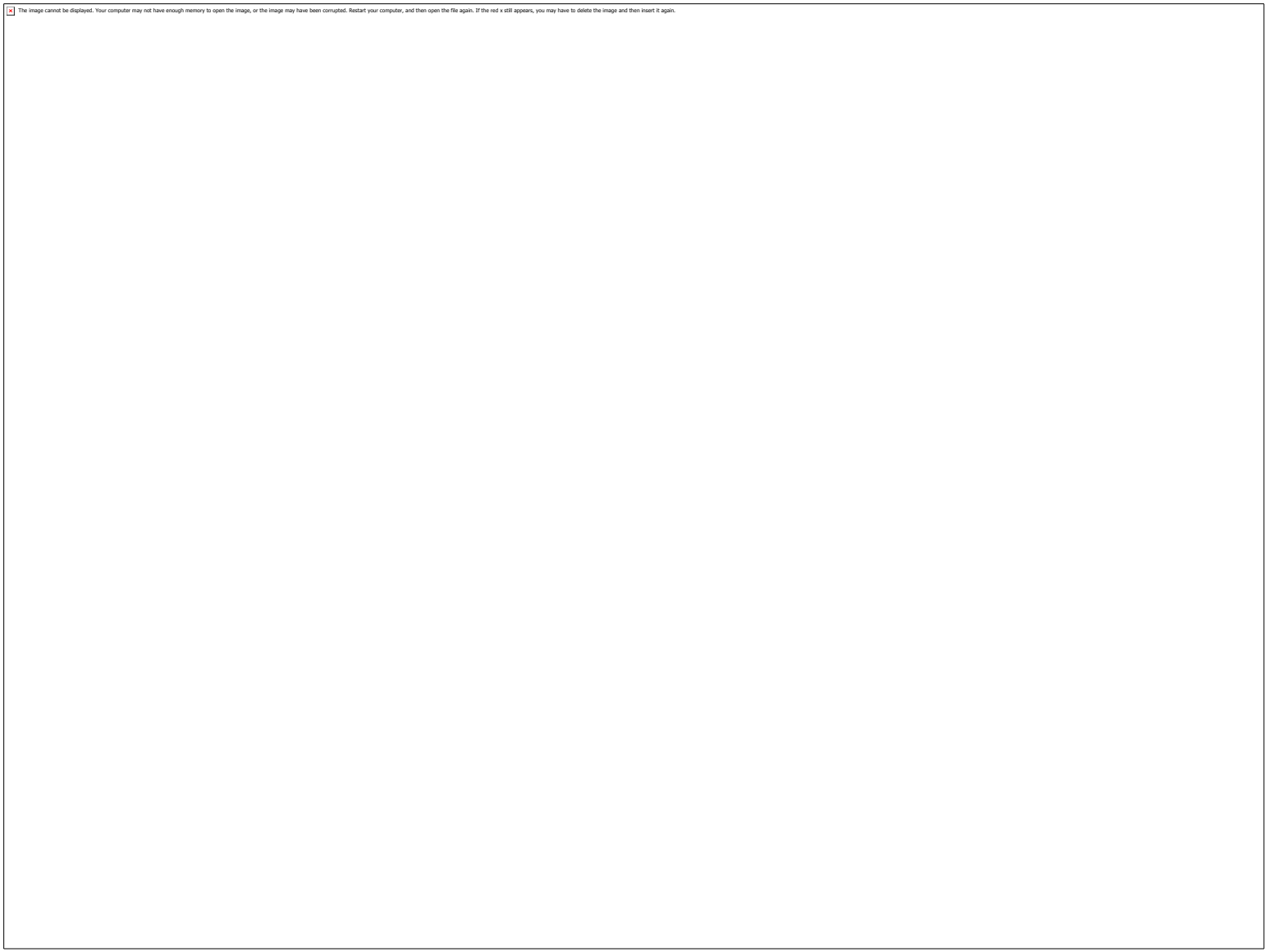
Total Signal Intensity



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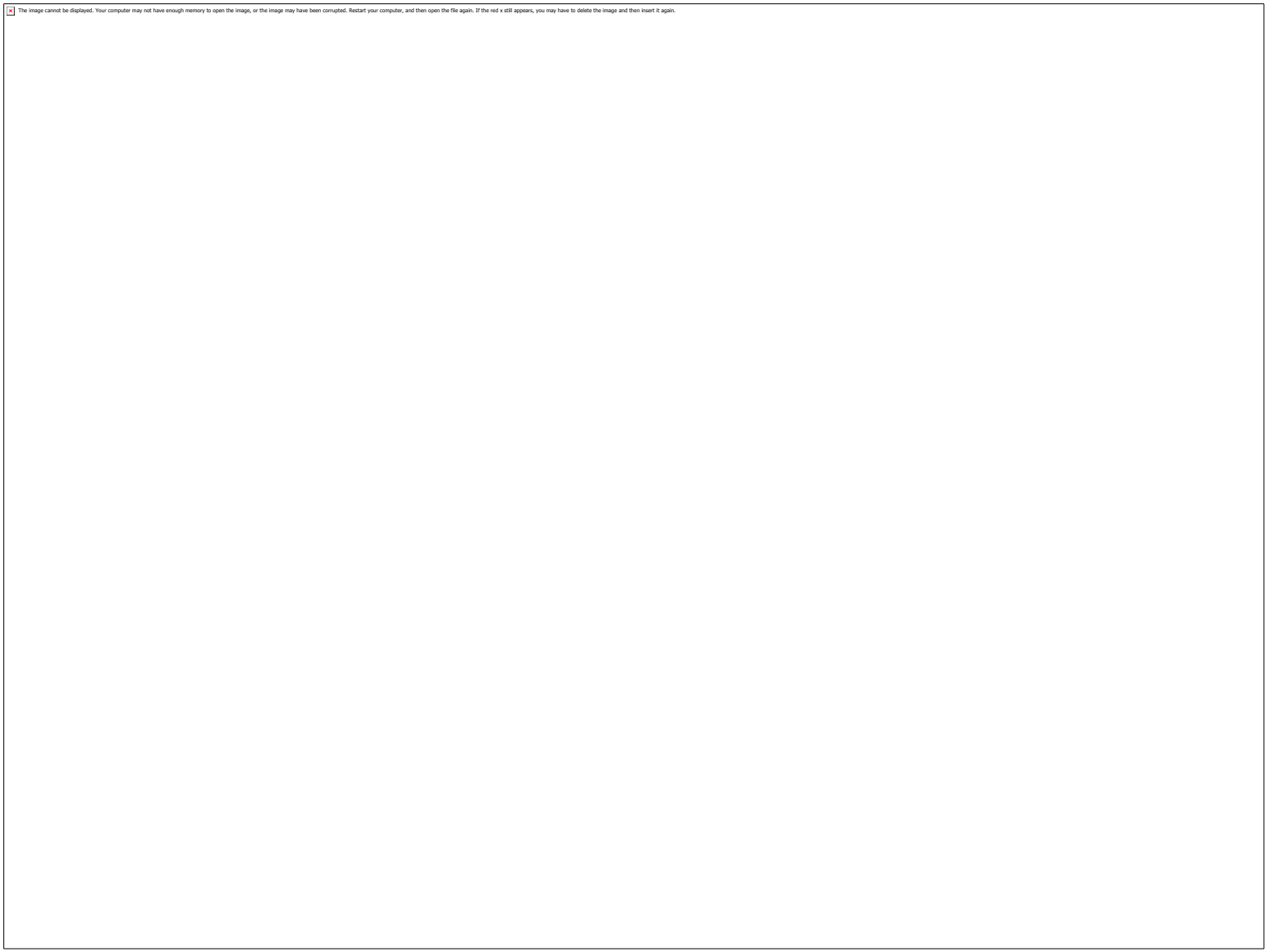
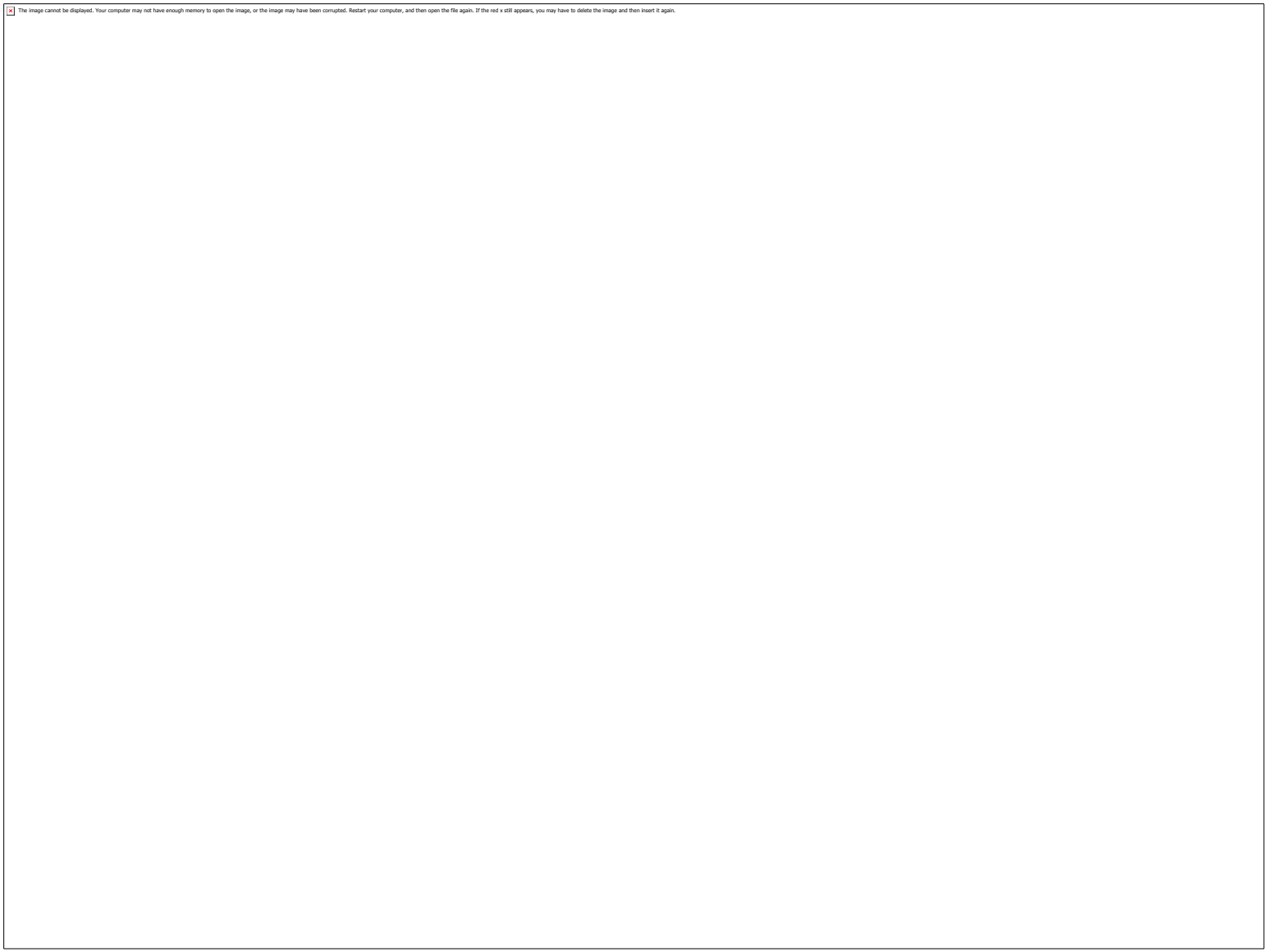


Mean Signal Pixel Intensity



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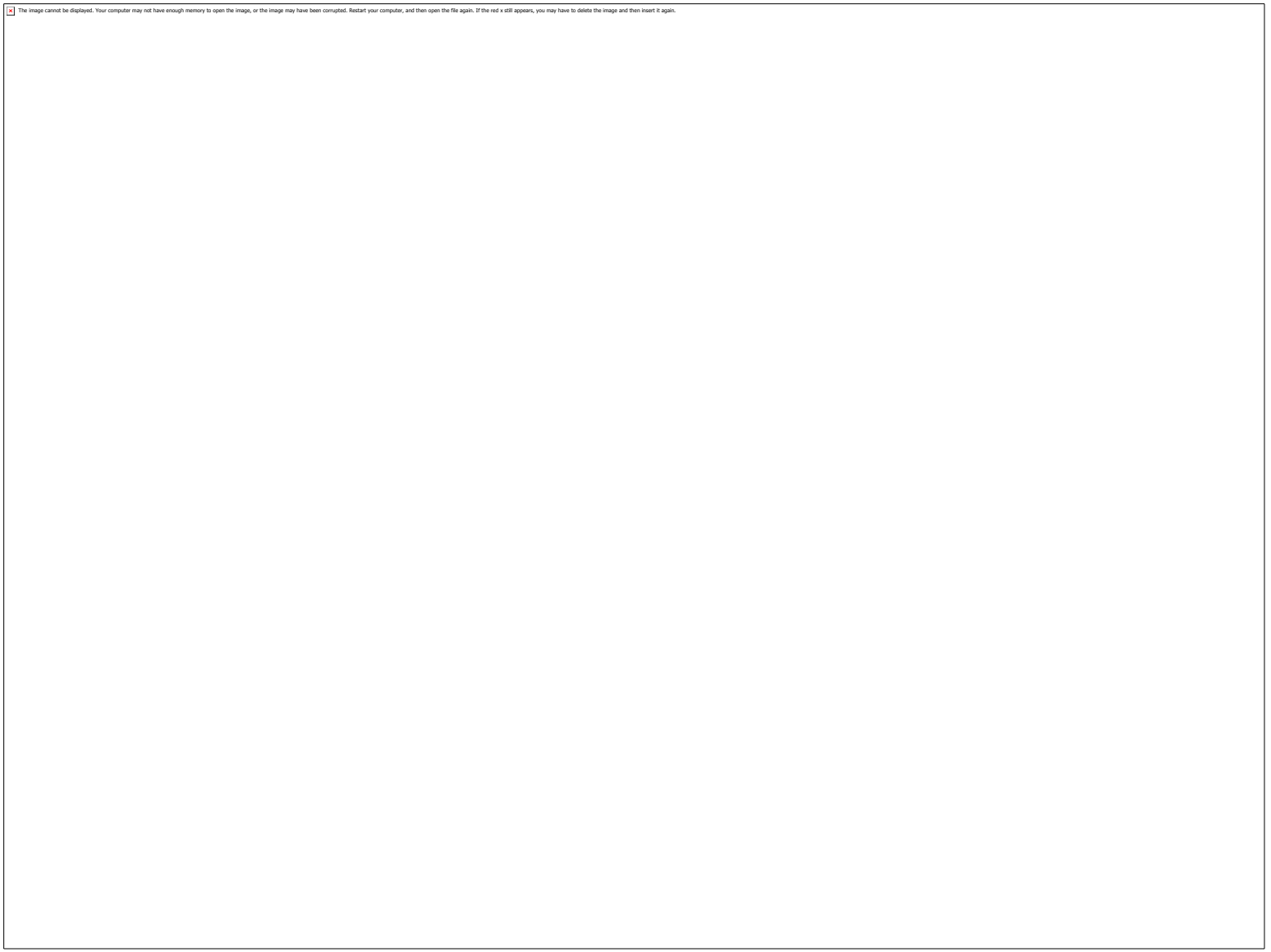
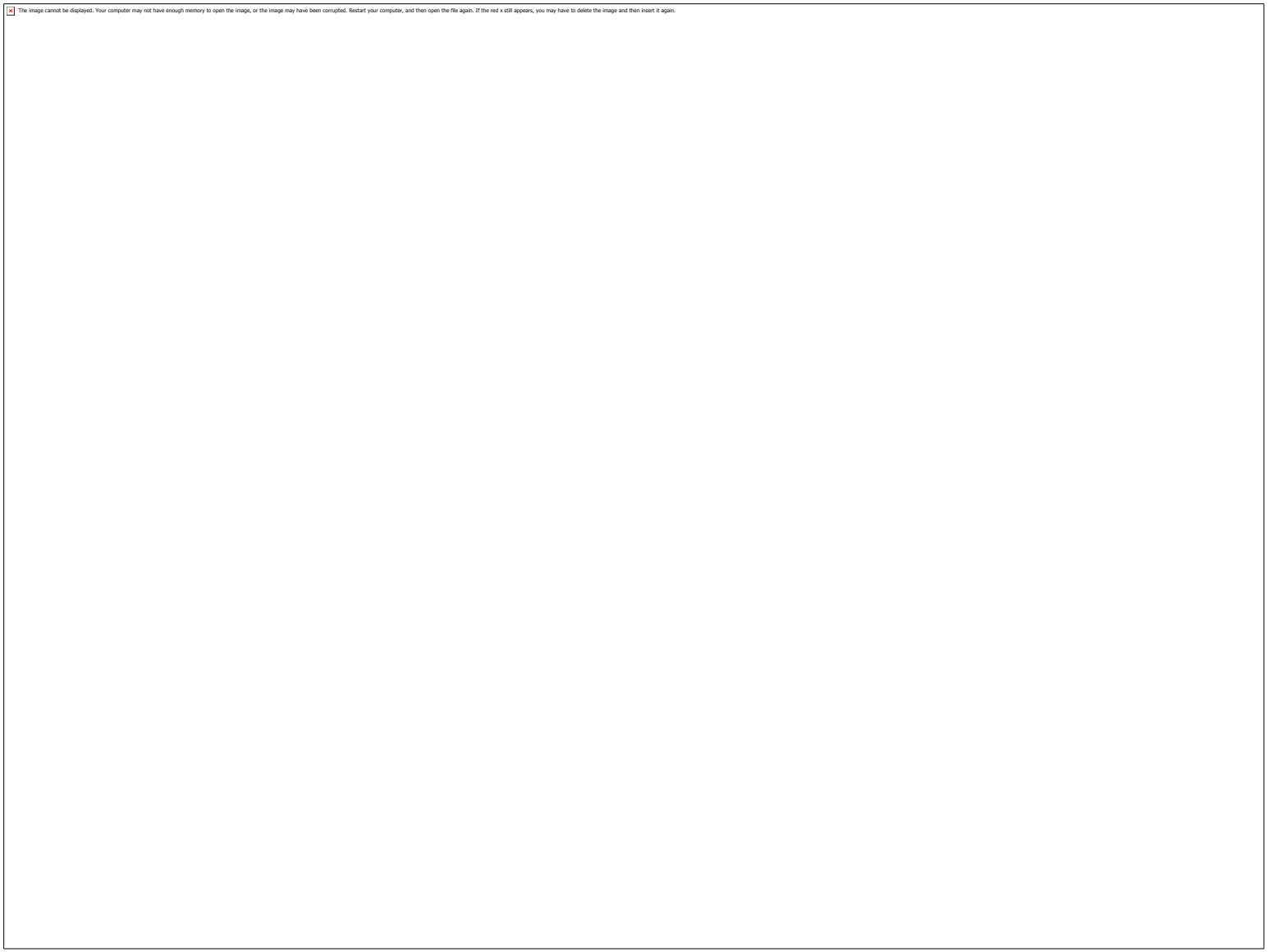
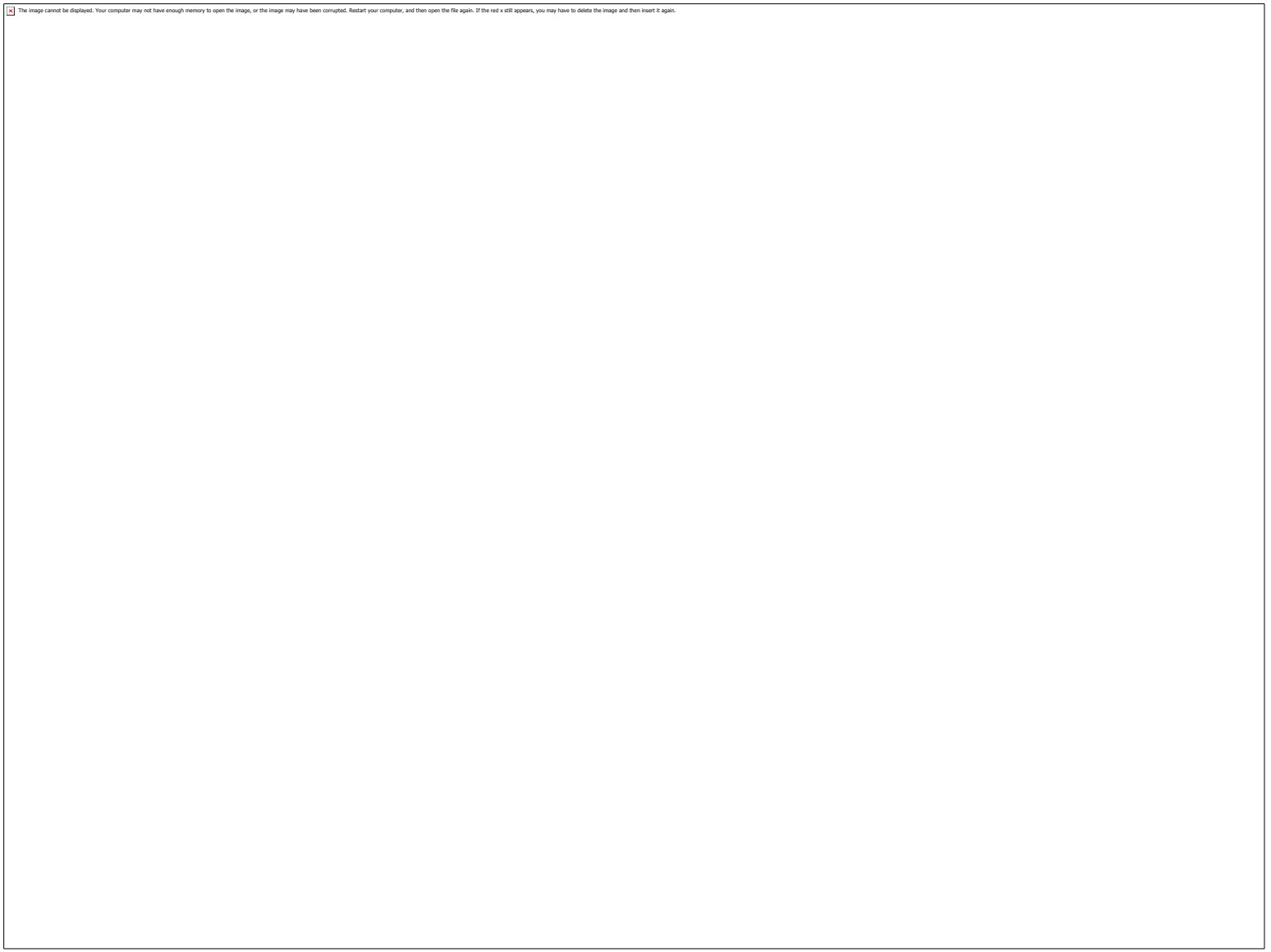


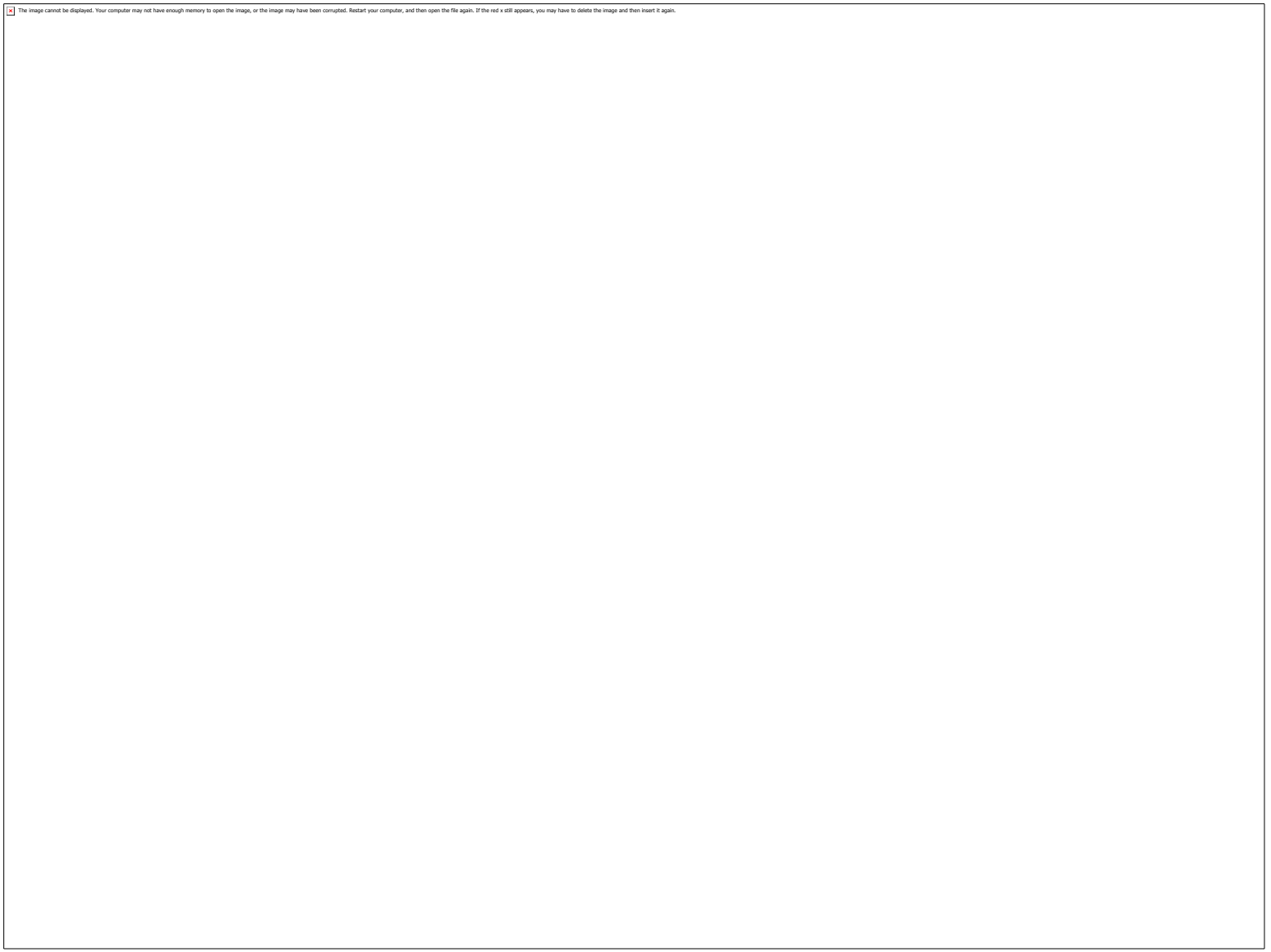
Puncta Total Peak Intensity



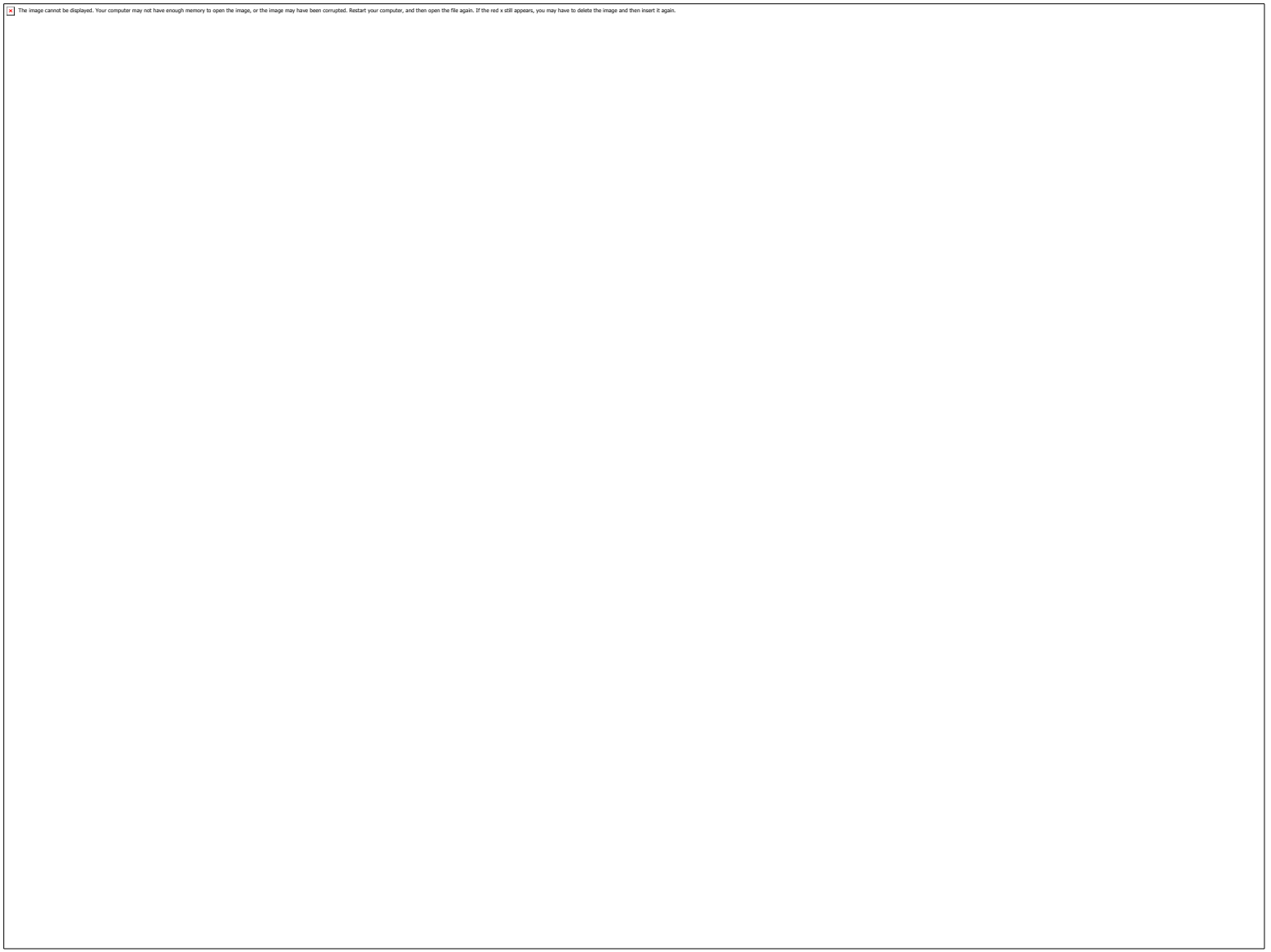
Puncta Total Peak Intensity



Puncta Mean Peak Intensity



Puncta Mean Peak Intensity



Results provided by Biophagy

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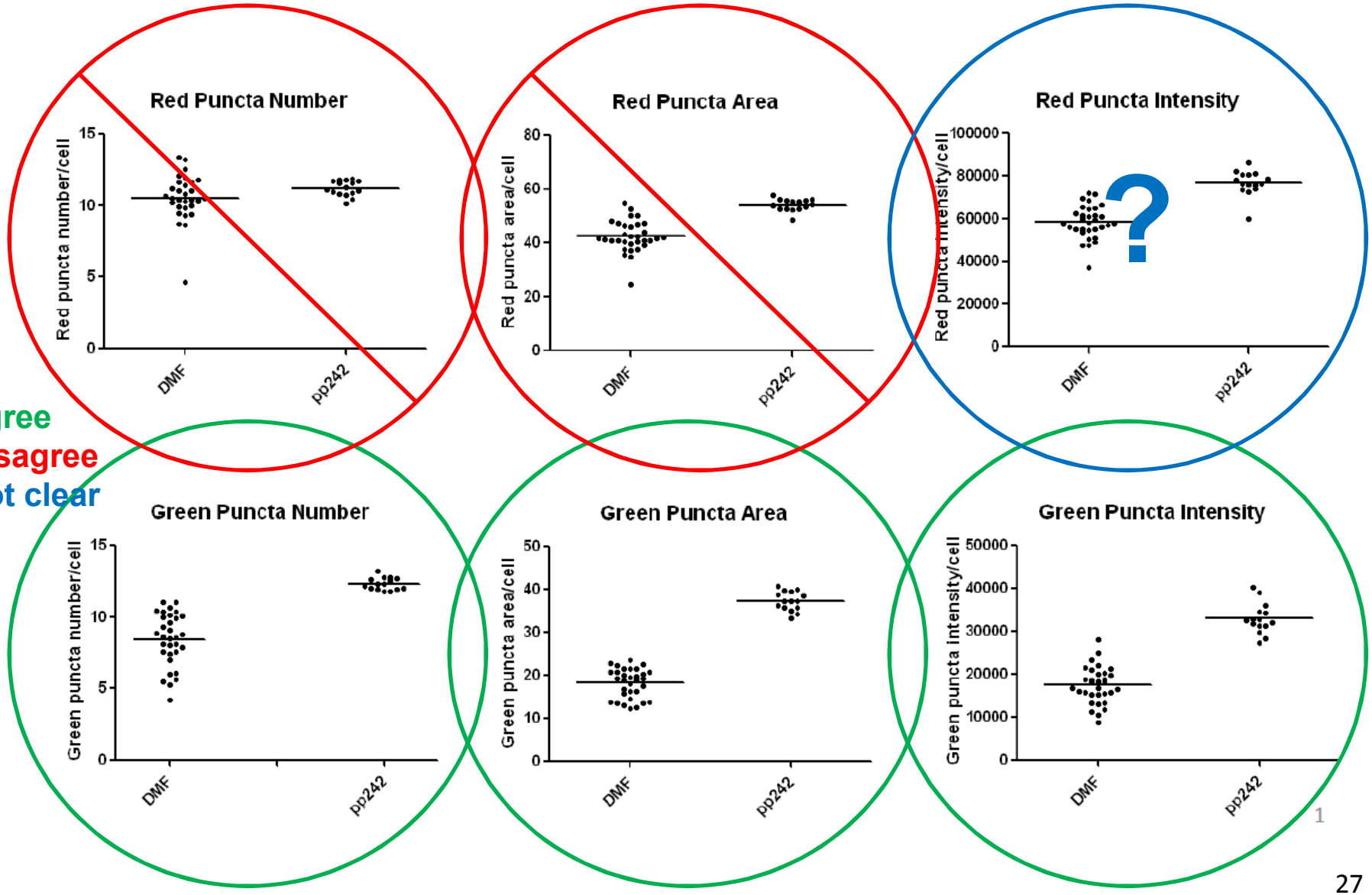


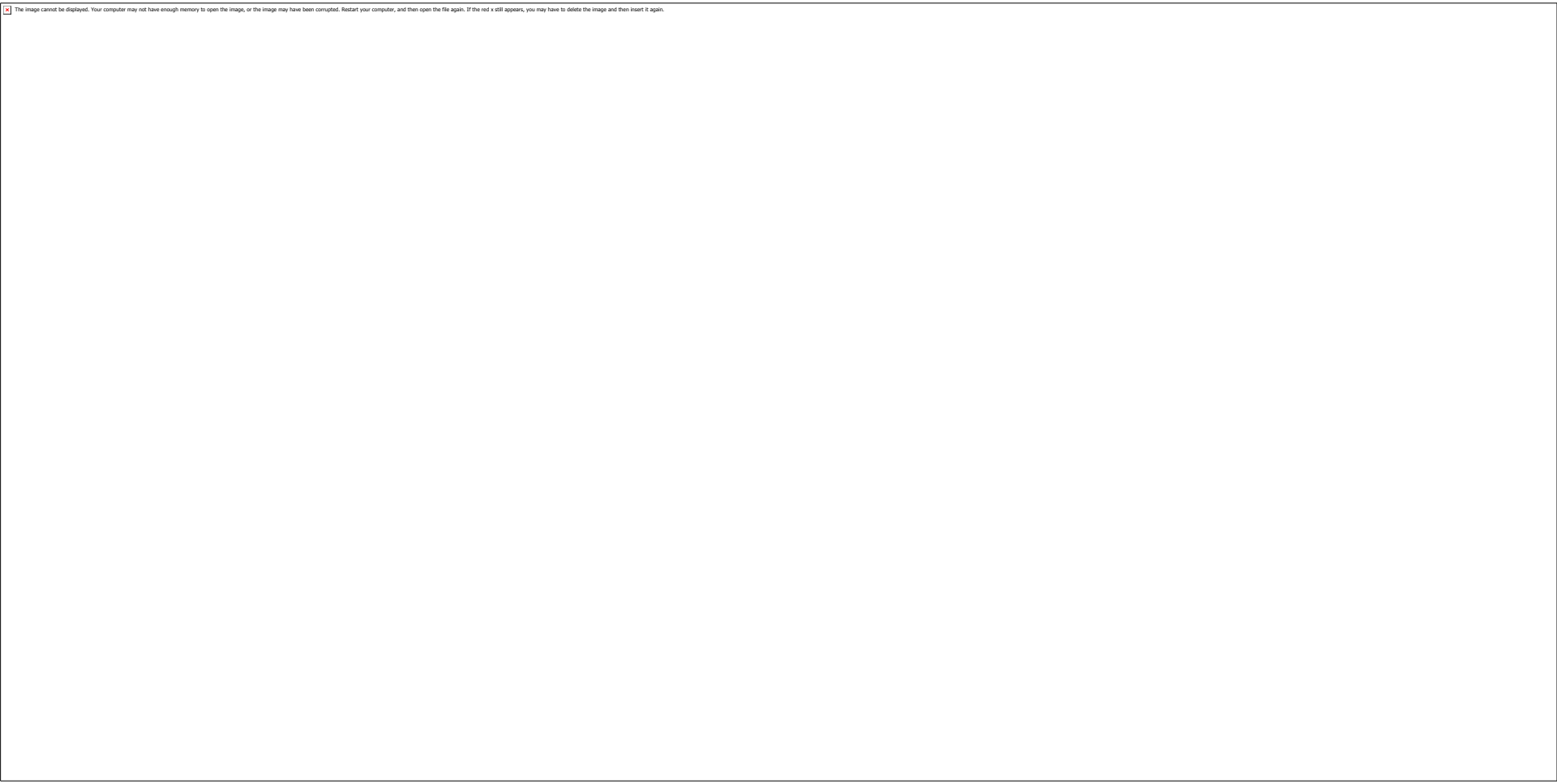
Agree
Disagree
Not clear

1

Representative Overlays

- Overlays shown (left) are always from the frame of the z-stack with the most total puncta (GFP puncta + RFP puncta)
- Corresponding images on the right indicate which pixels were detected as signal in each channel.

SORT 1 DMSO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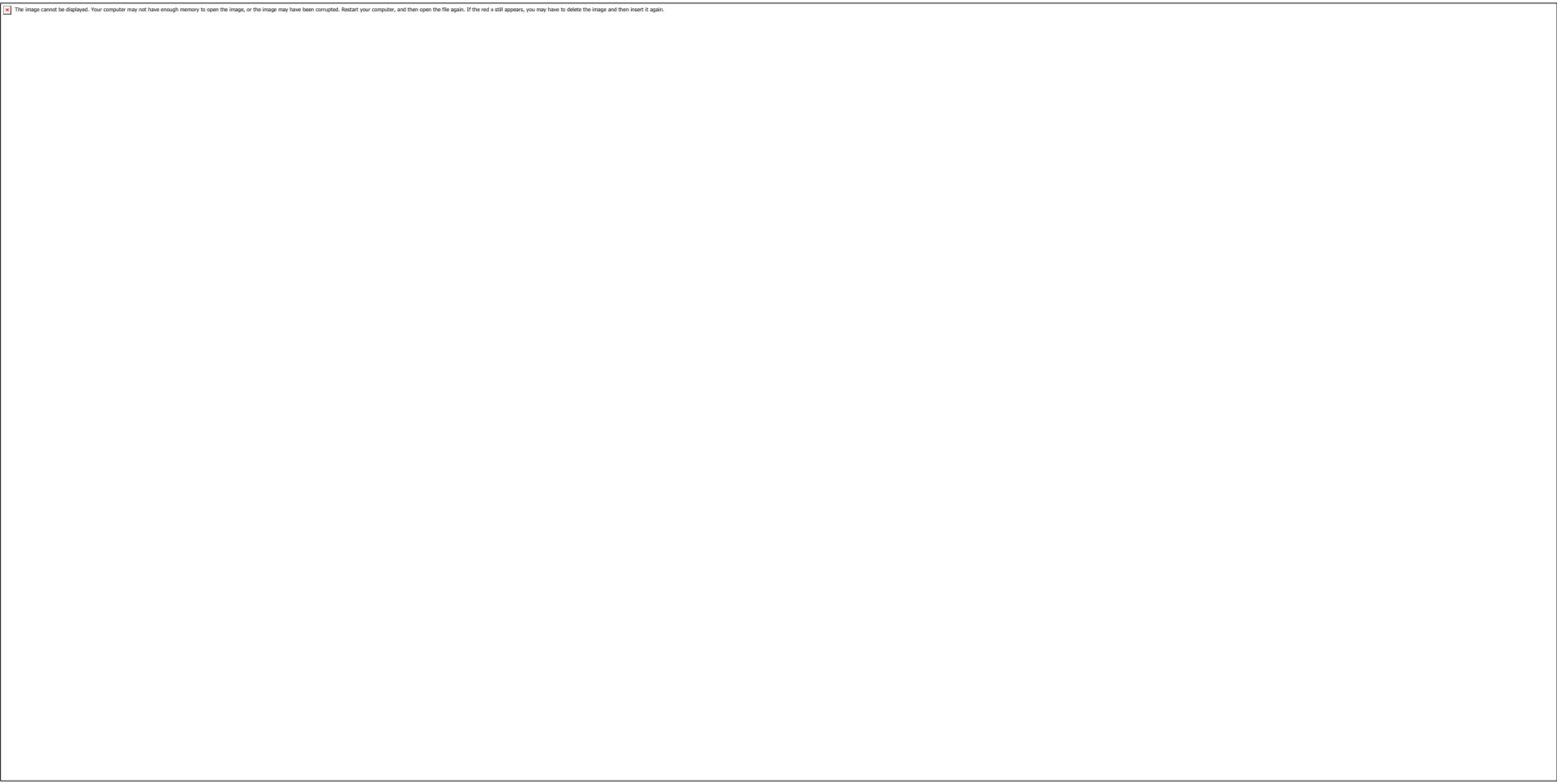
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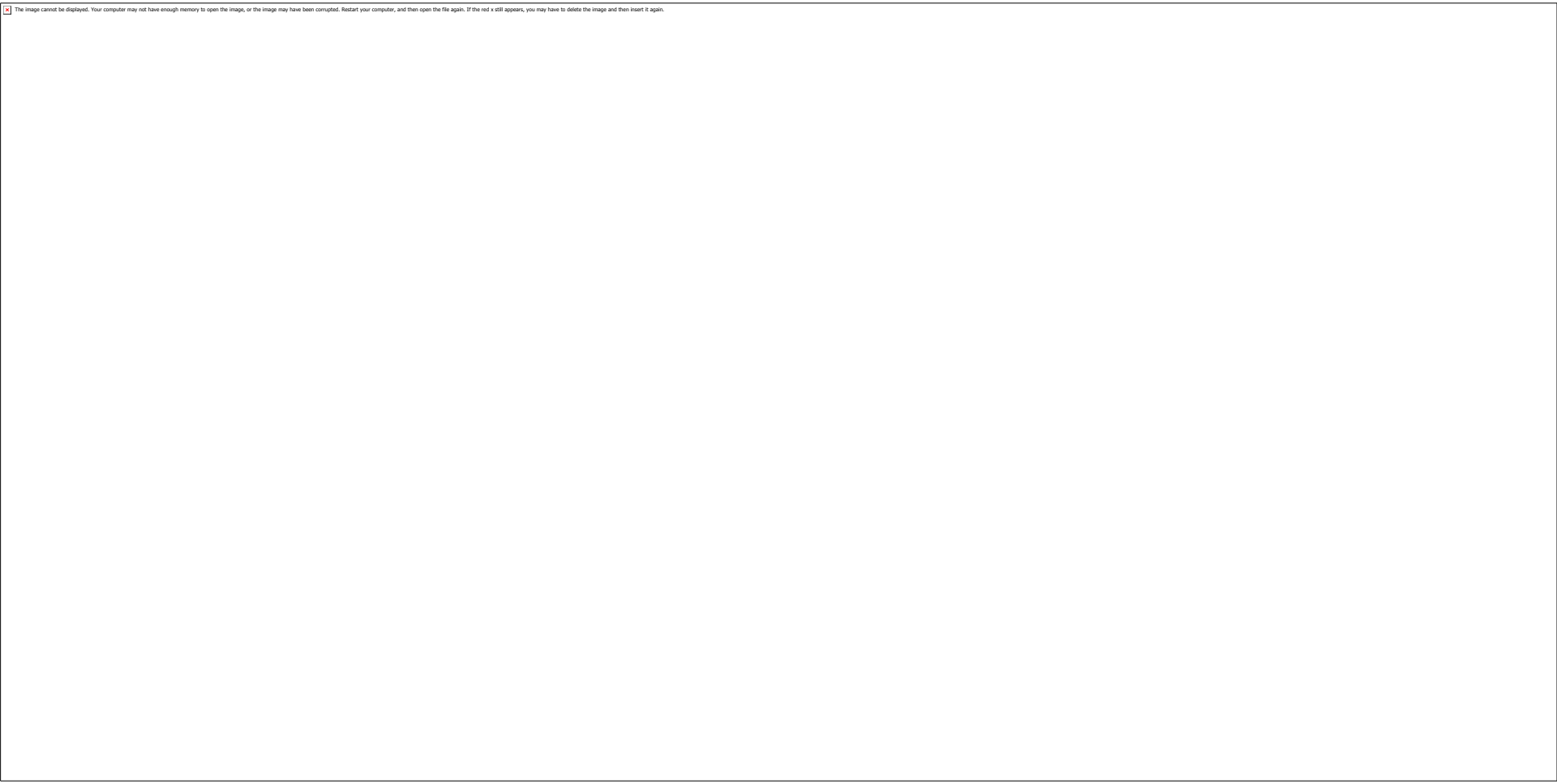
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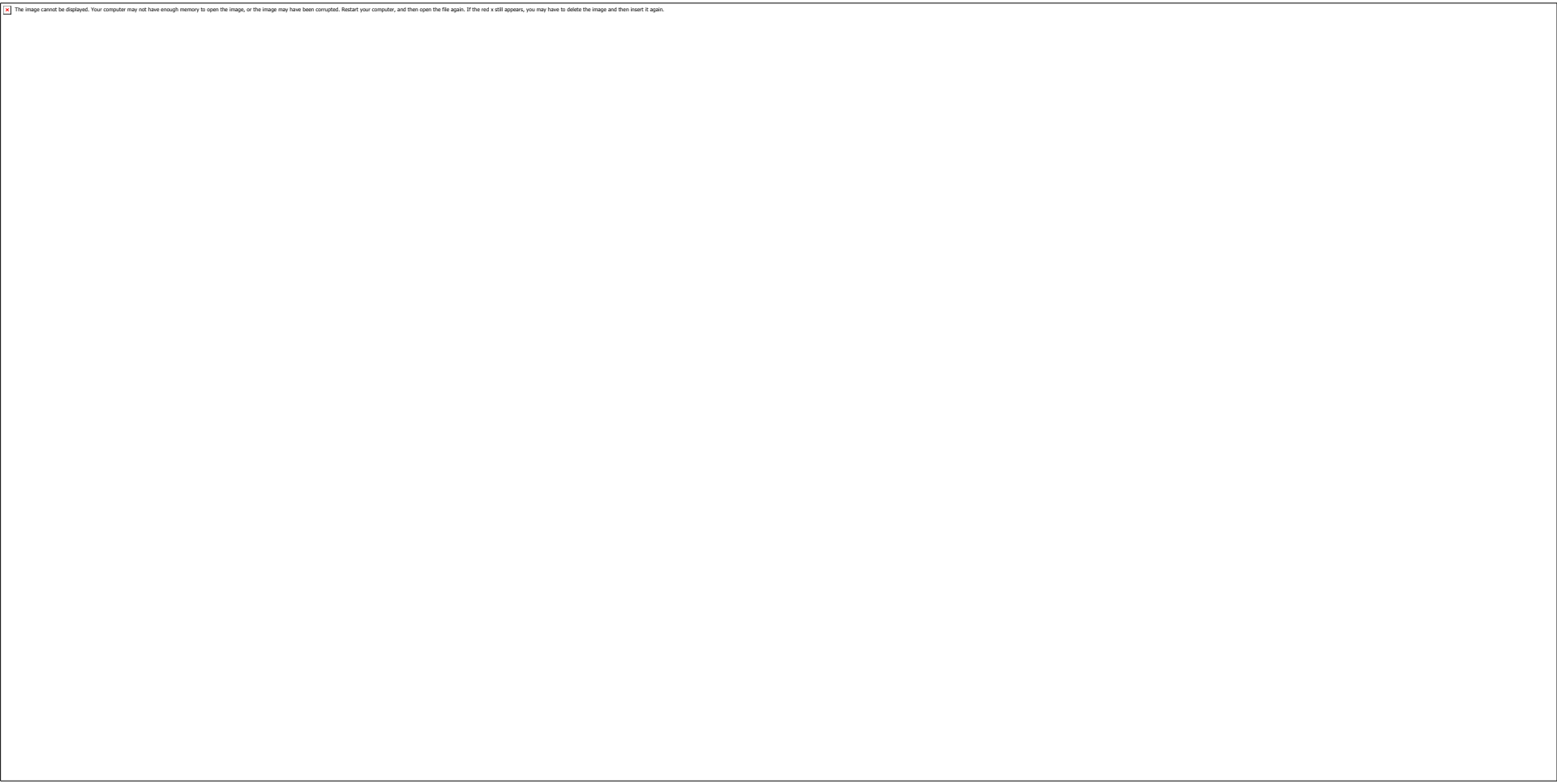
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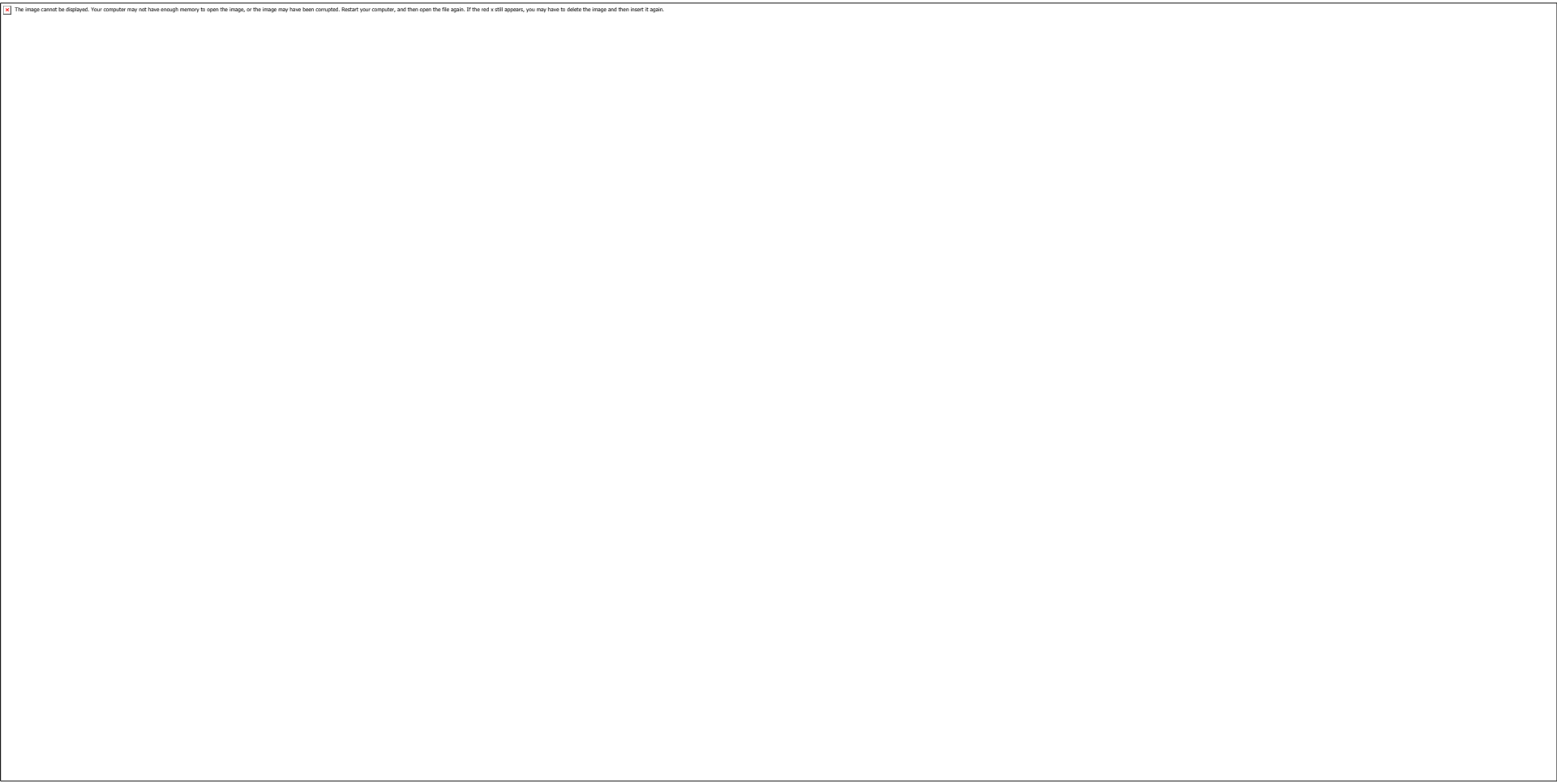
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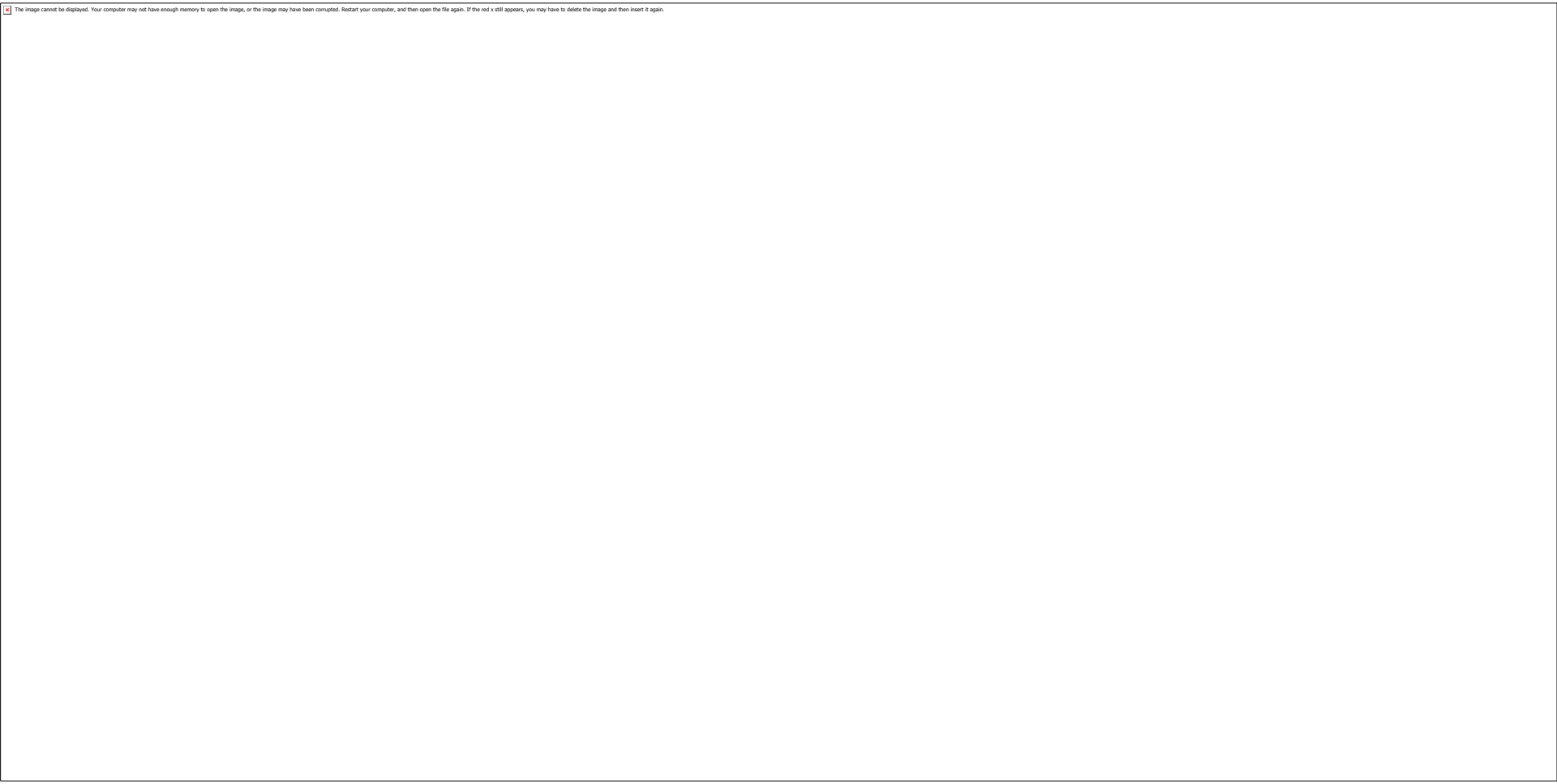
SORT 1 PP242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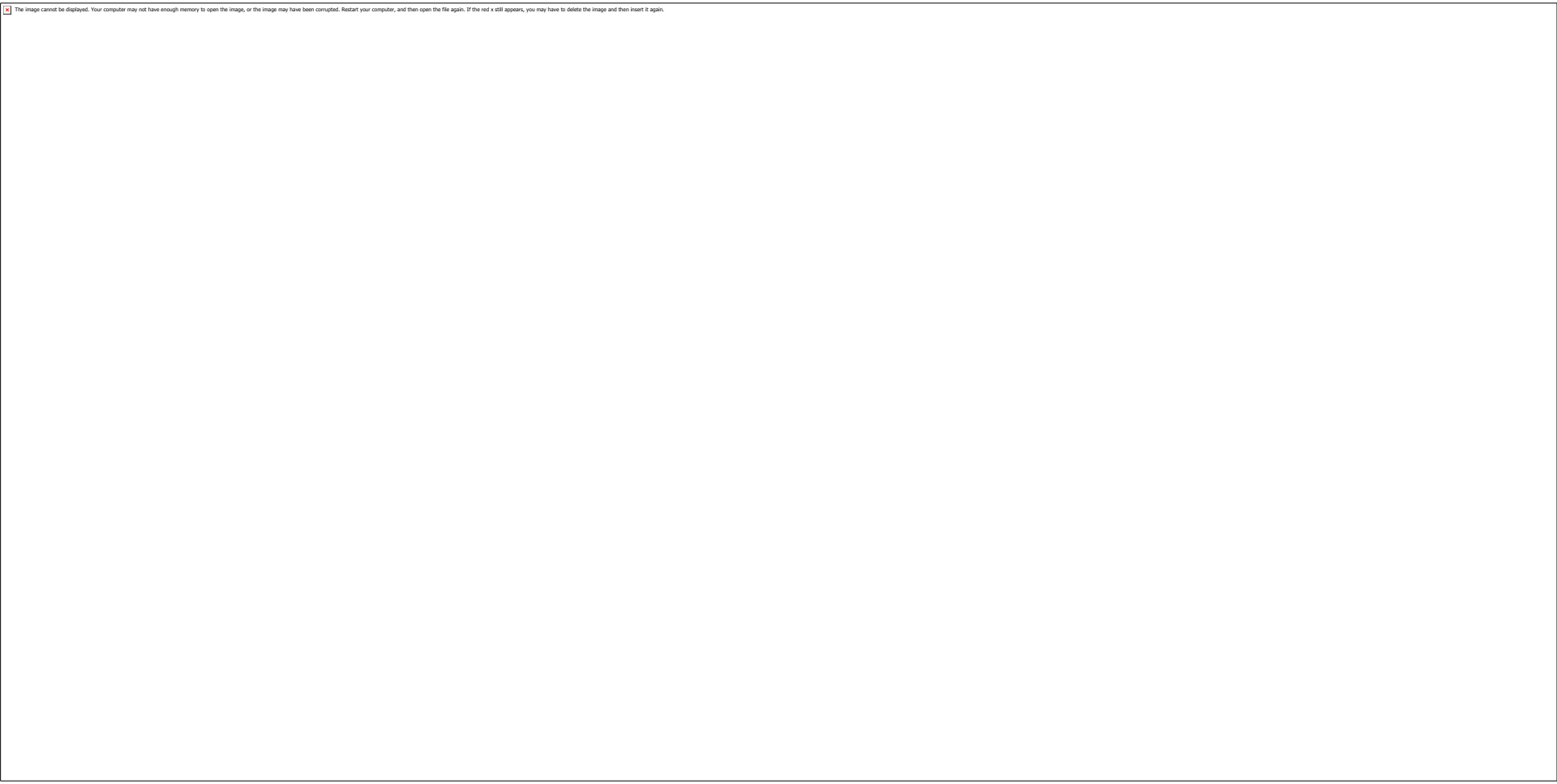
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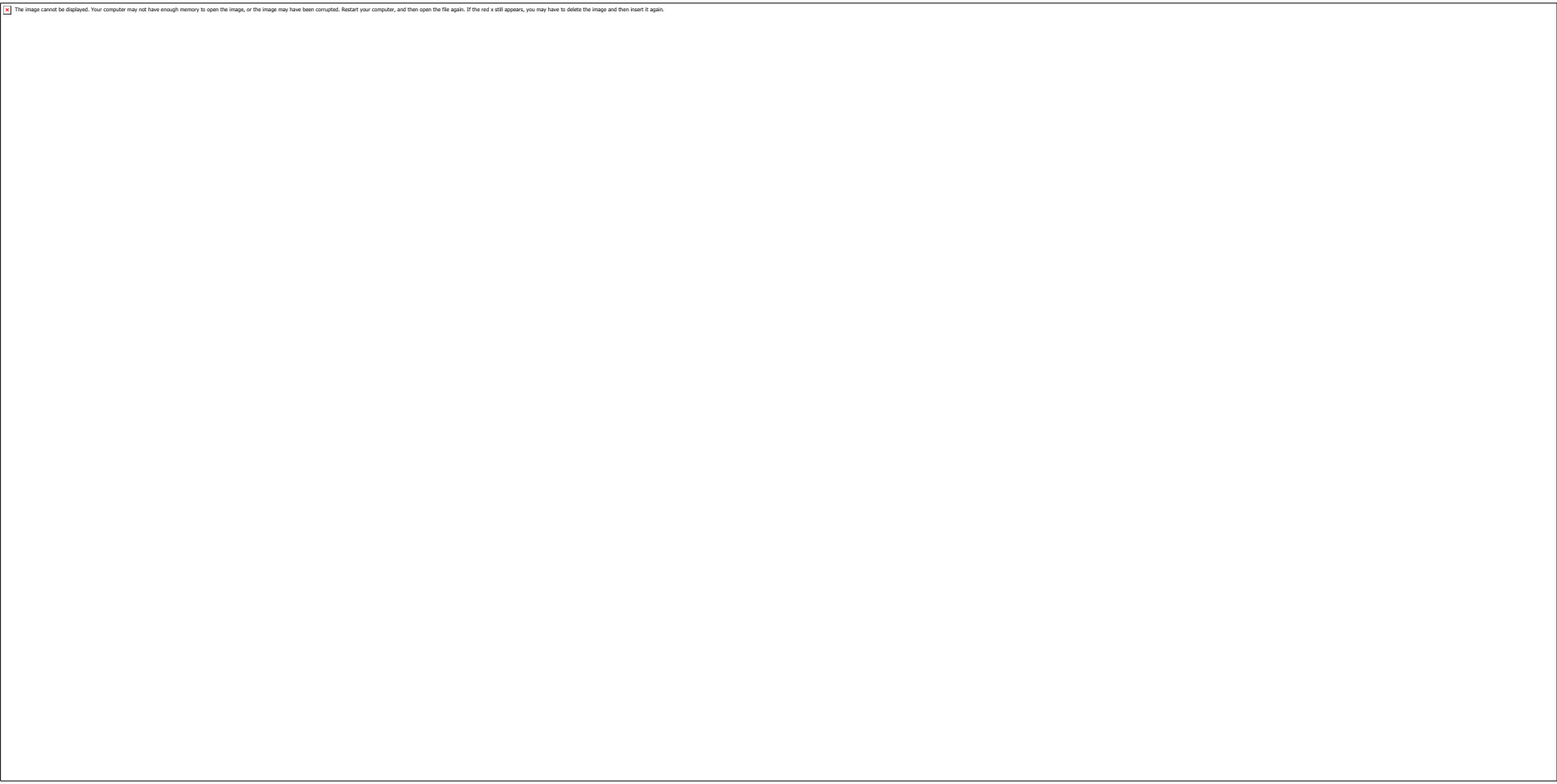
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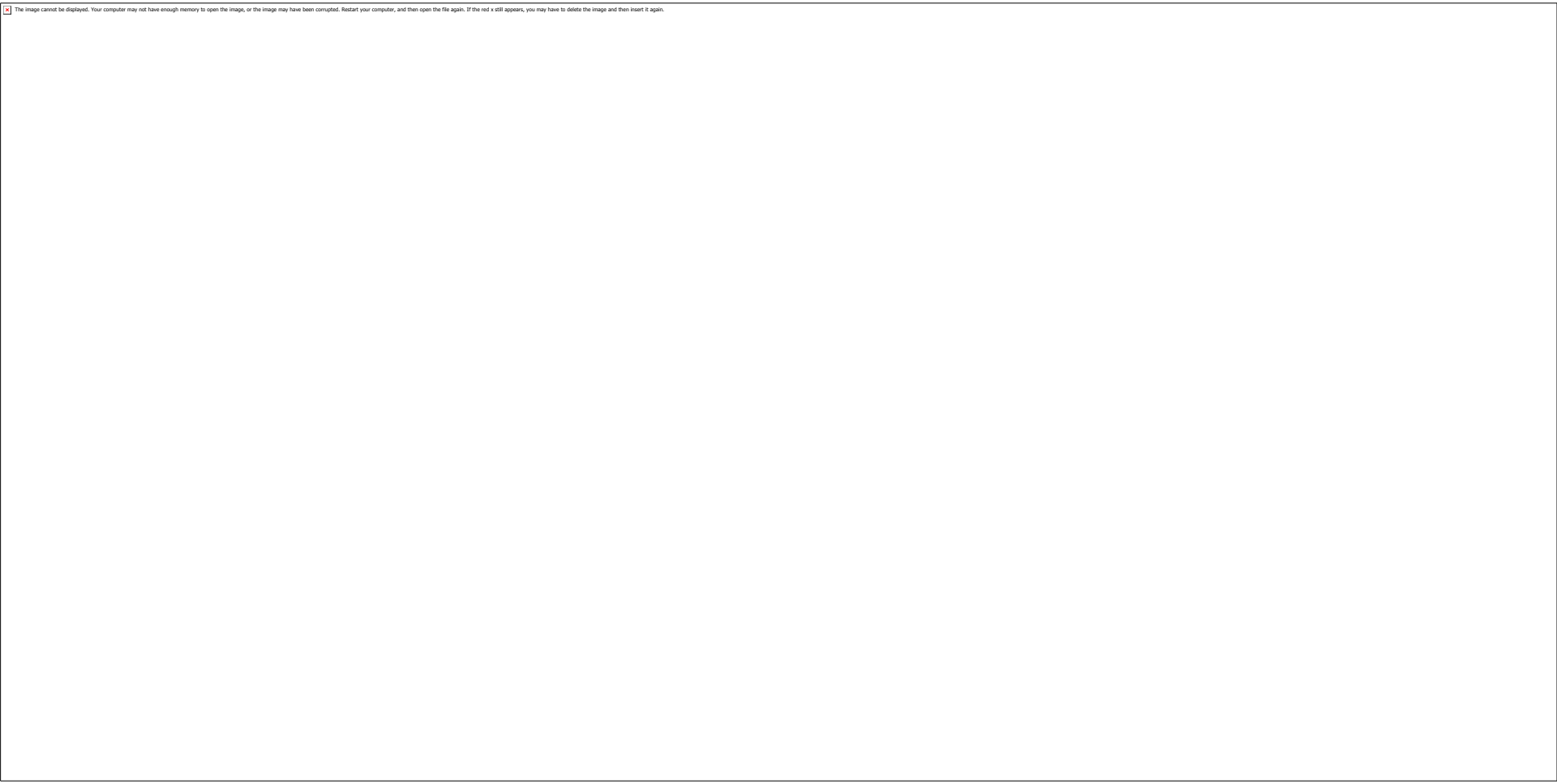
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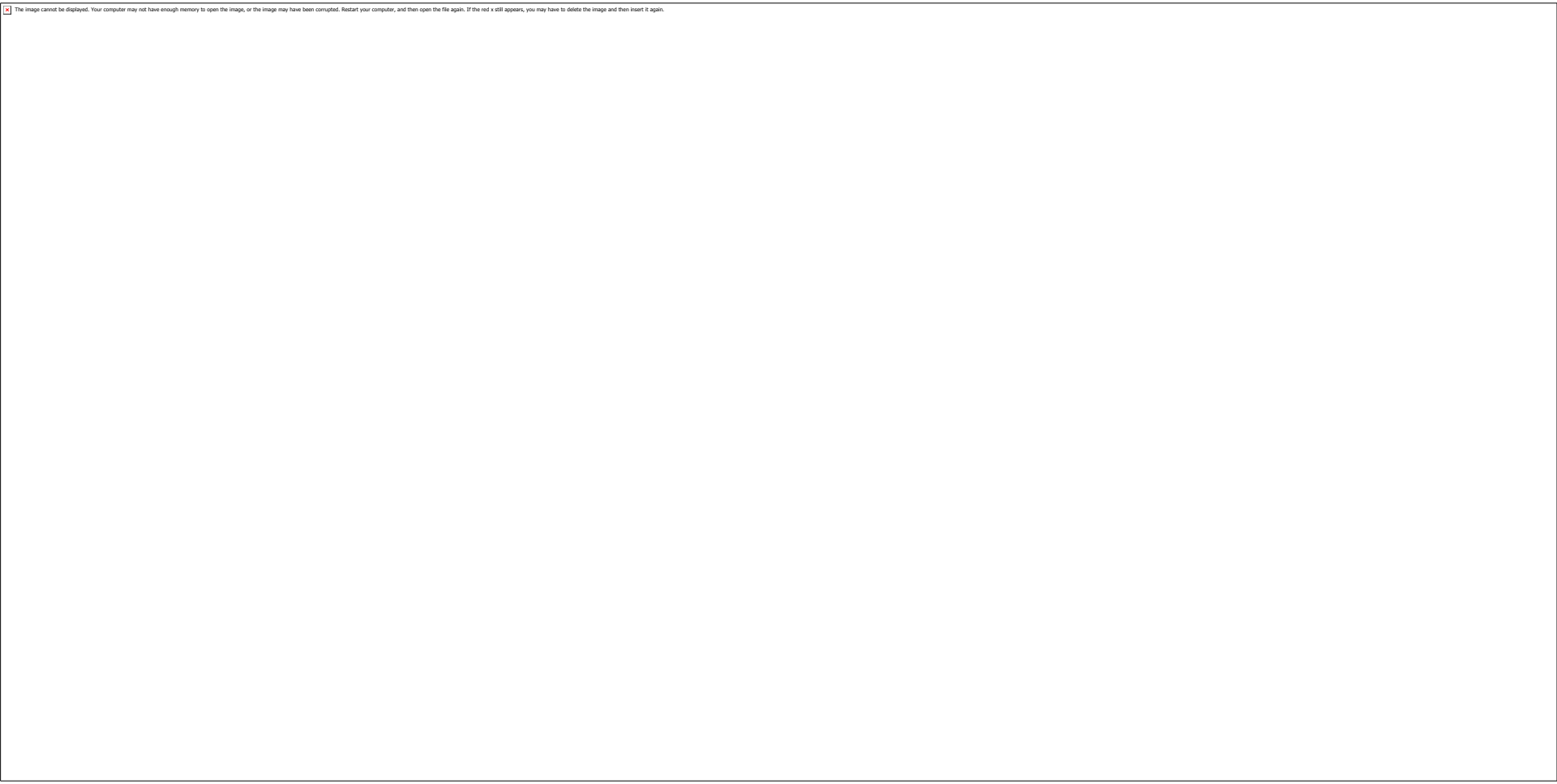
SORT 2 DMSO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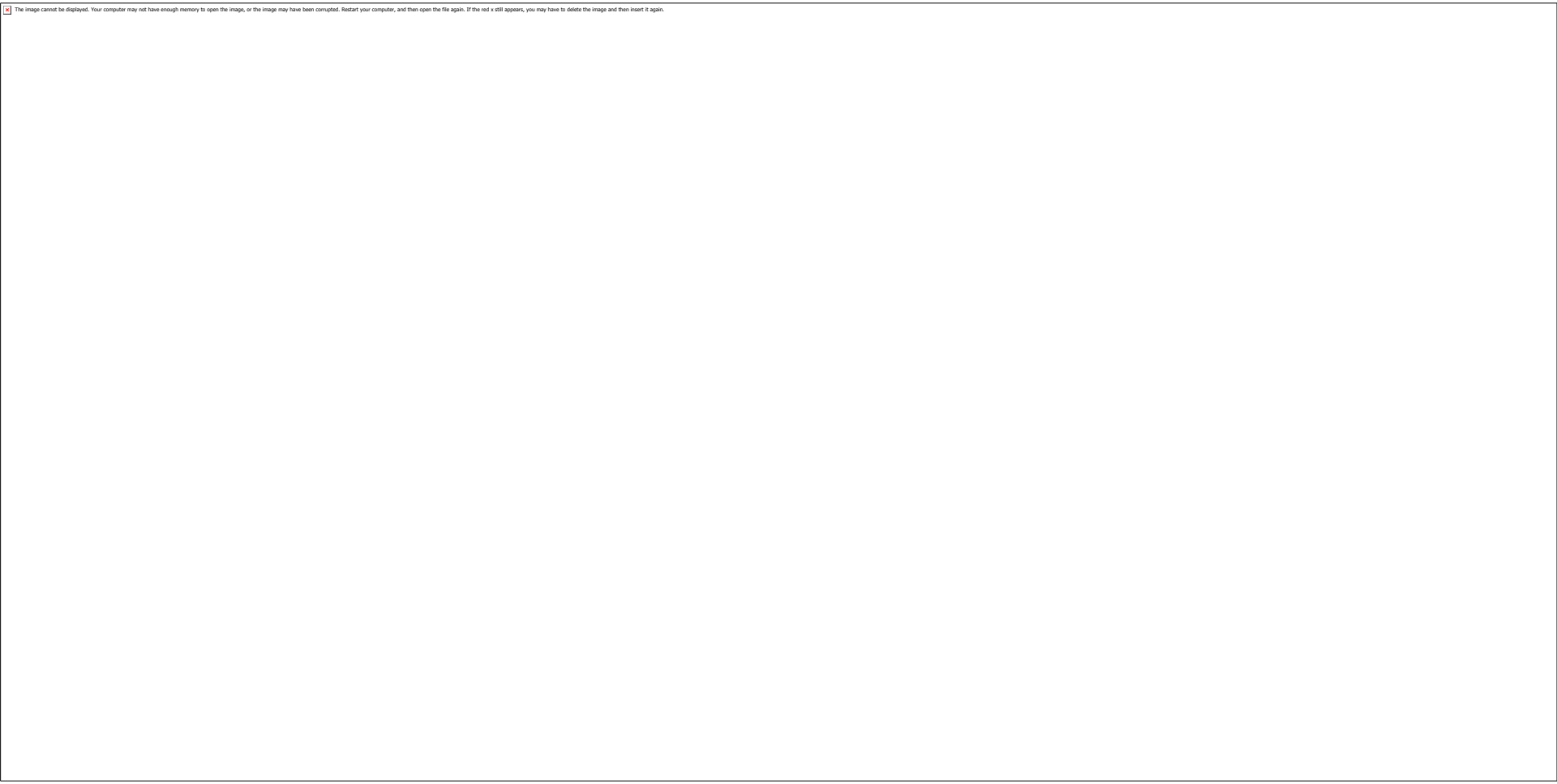
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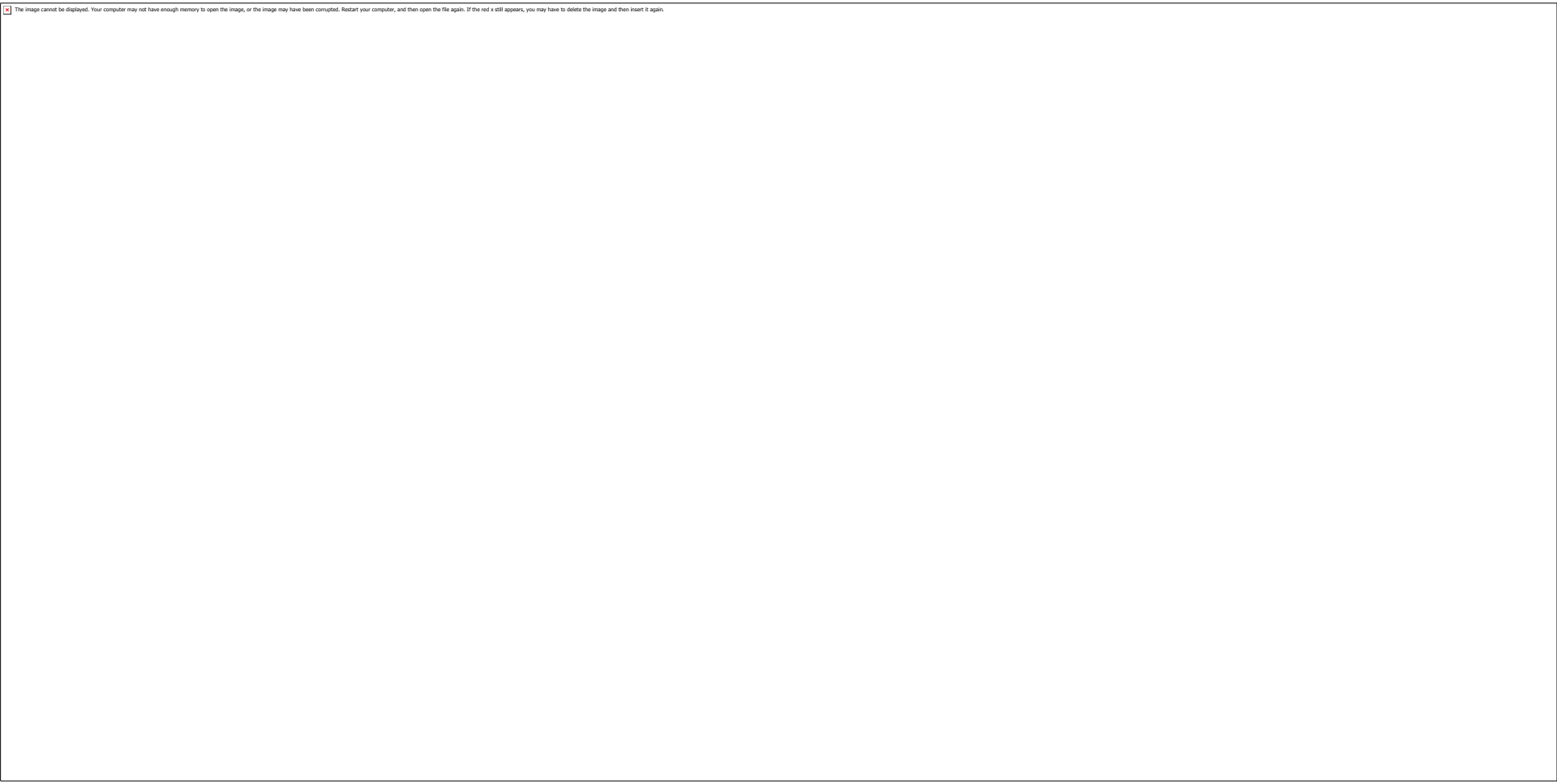
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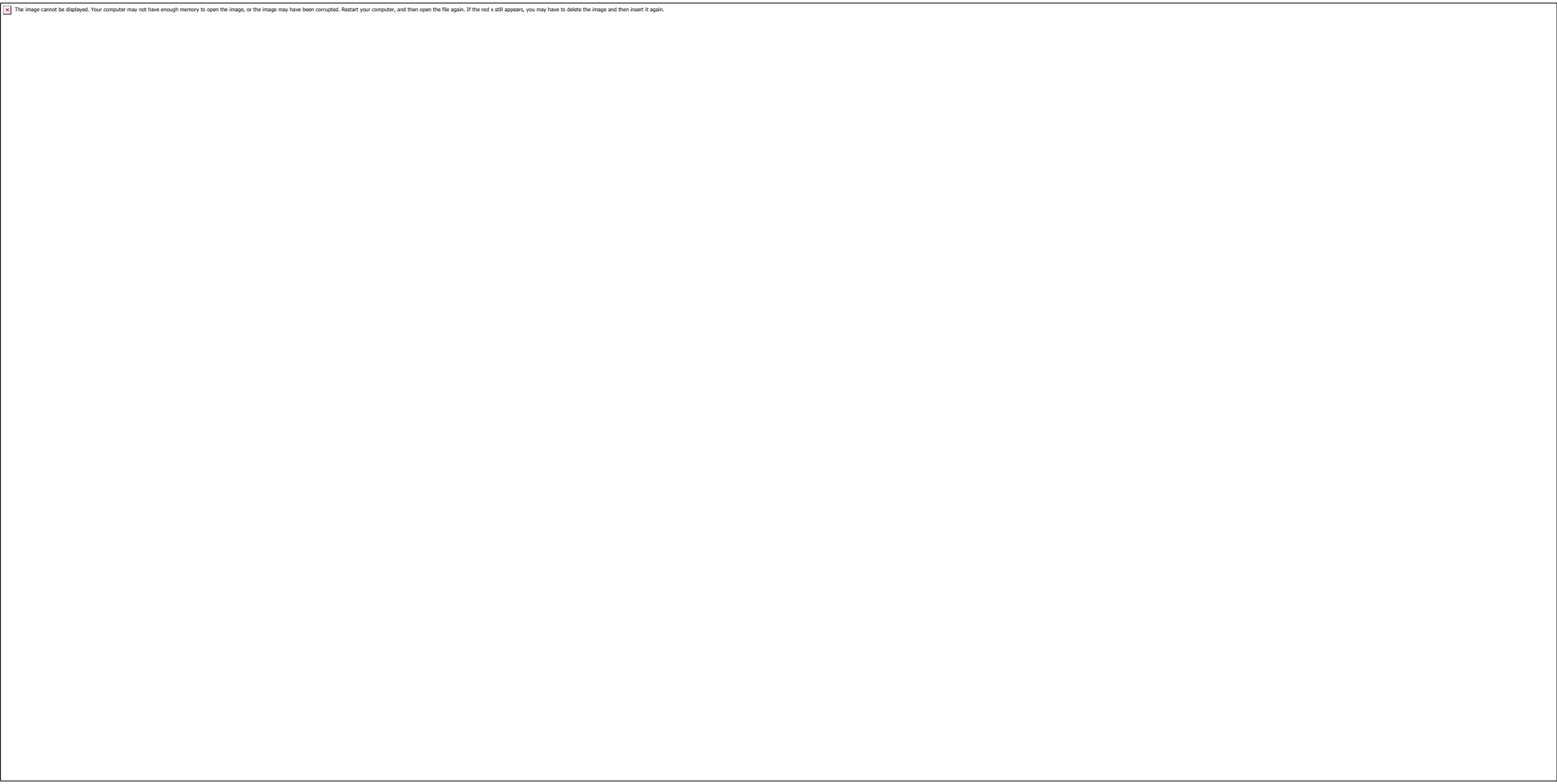
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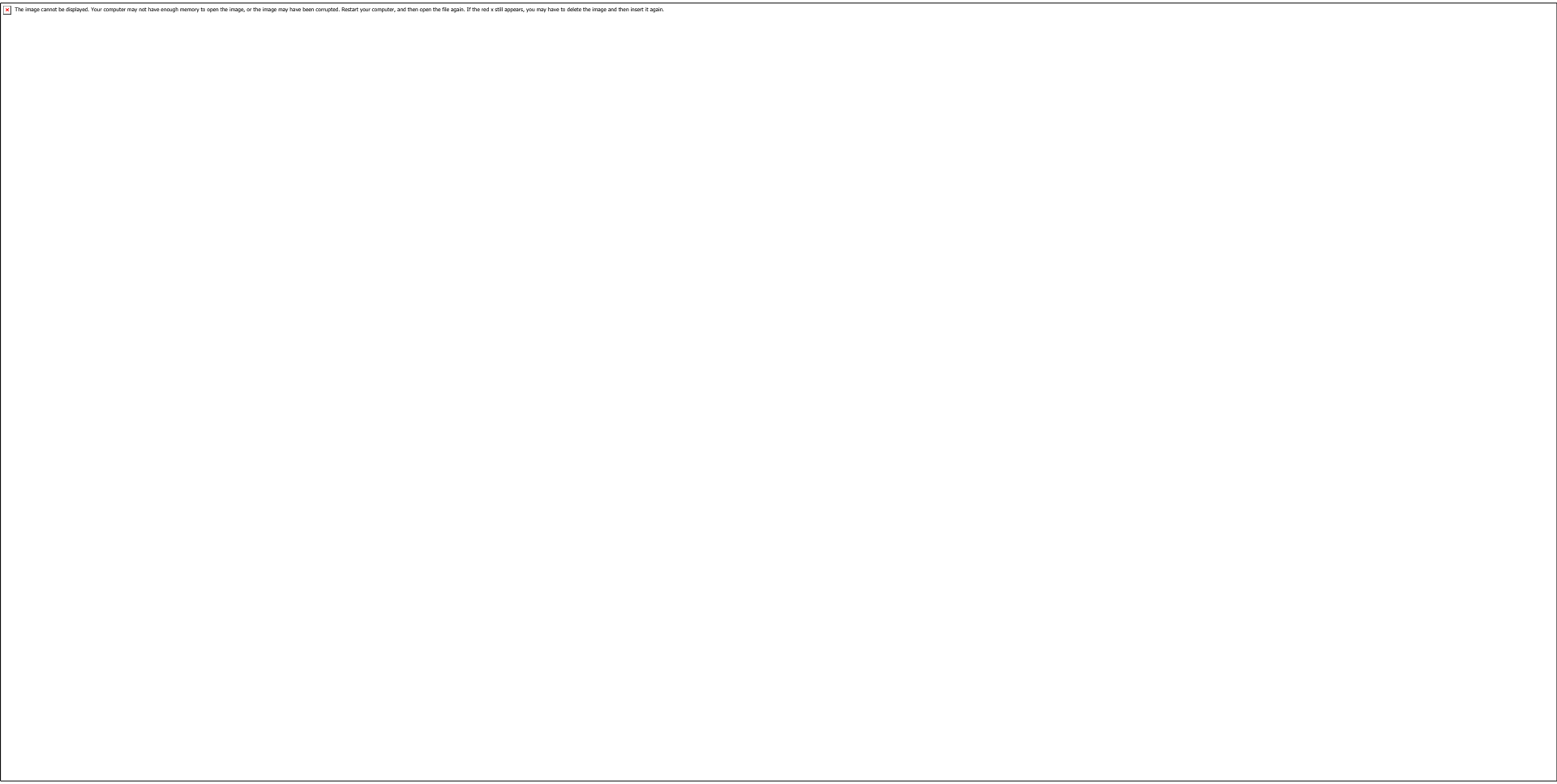
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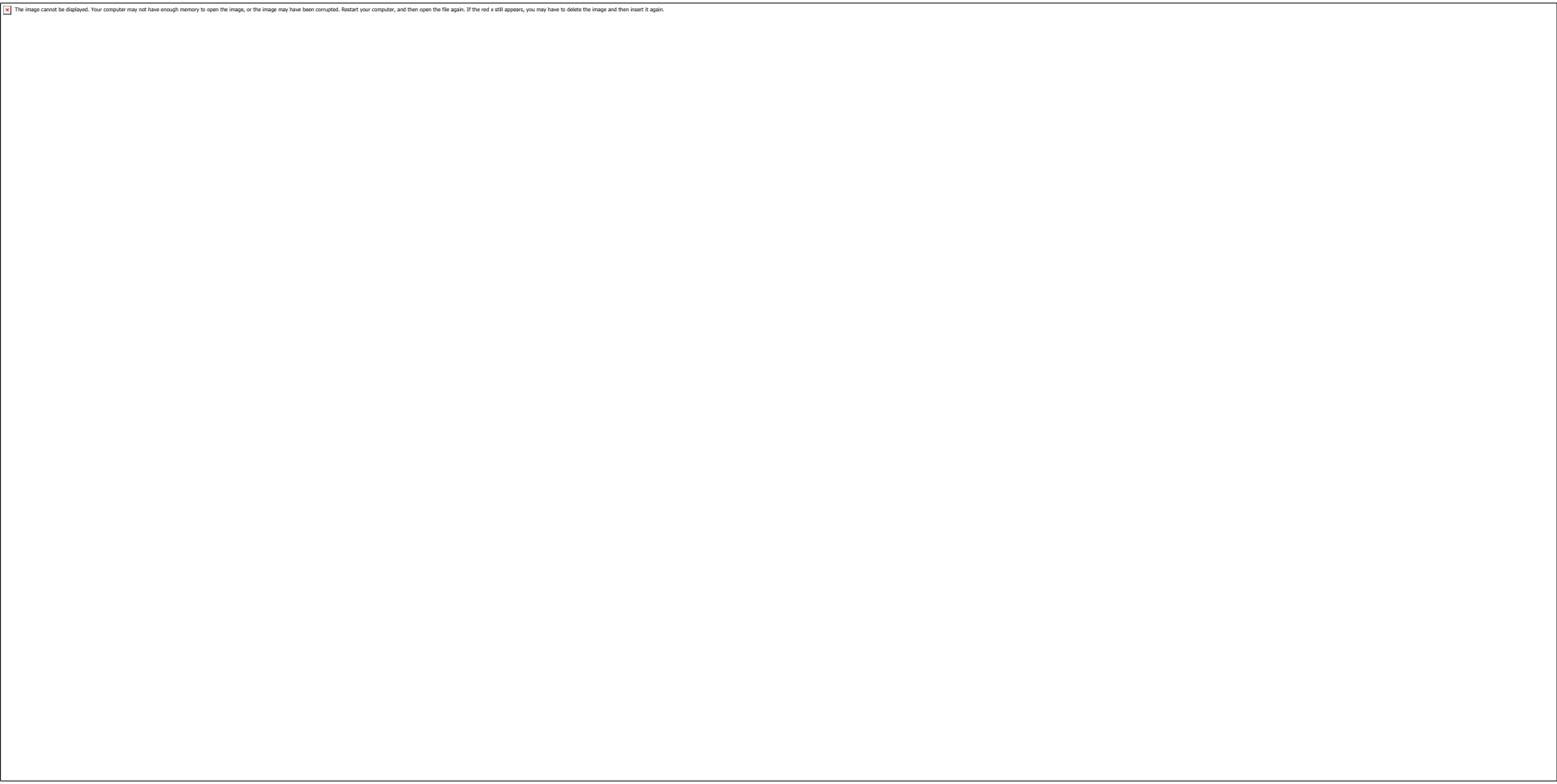
SORT 2 PP242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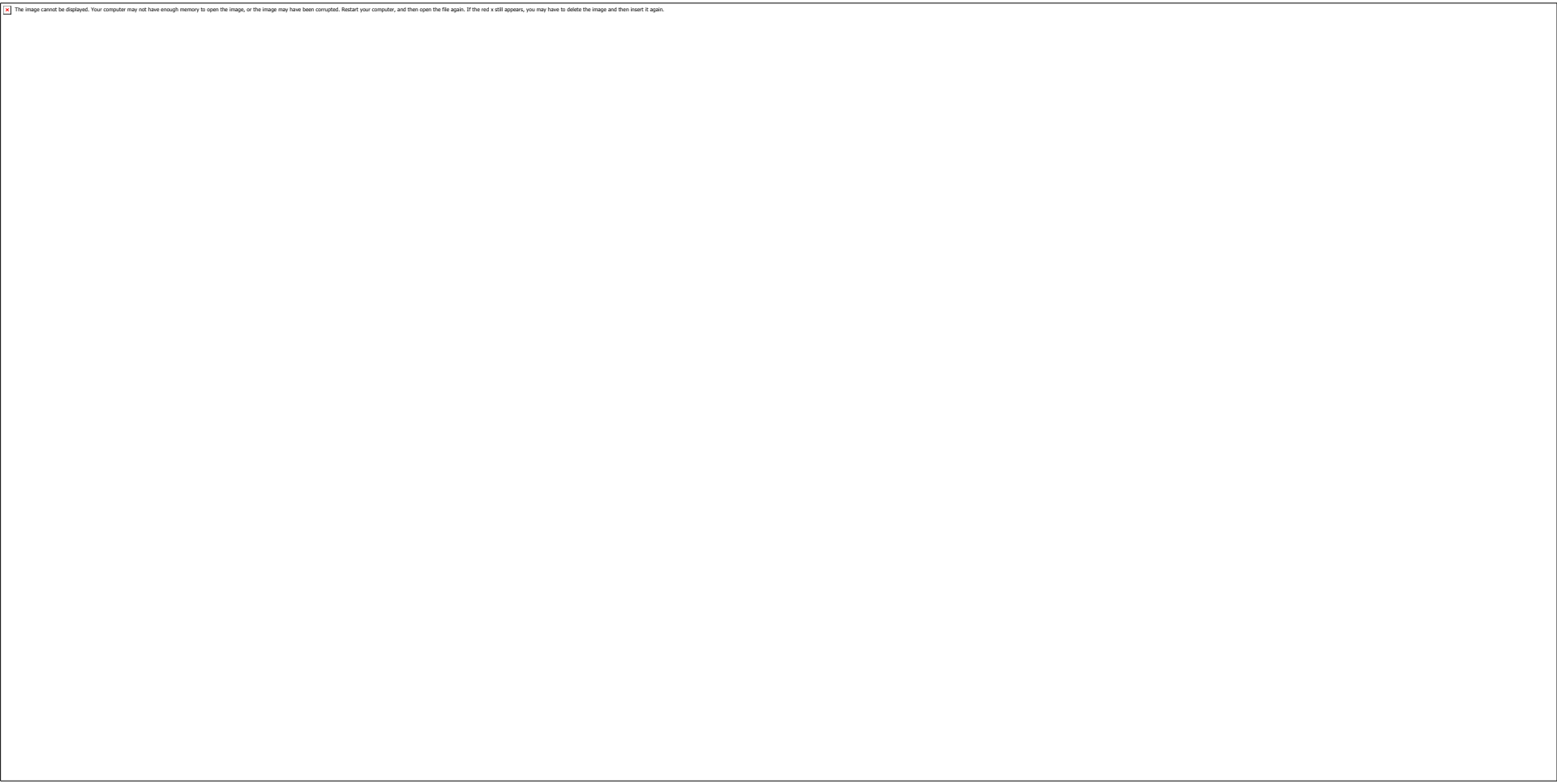
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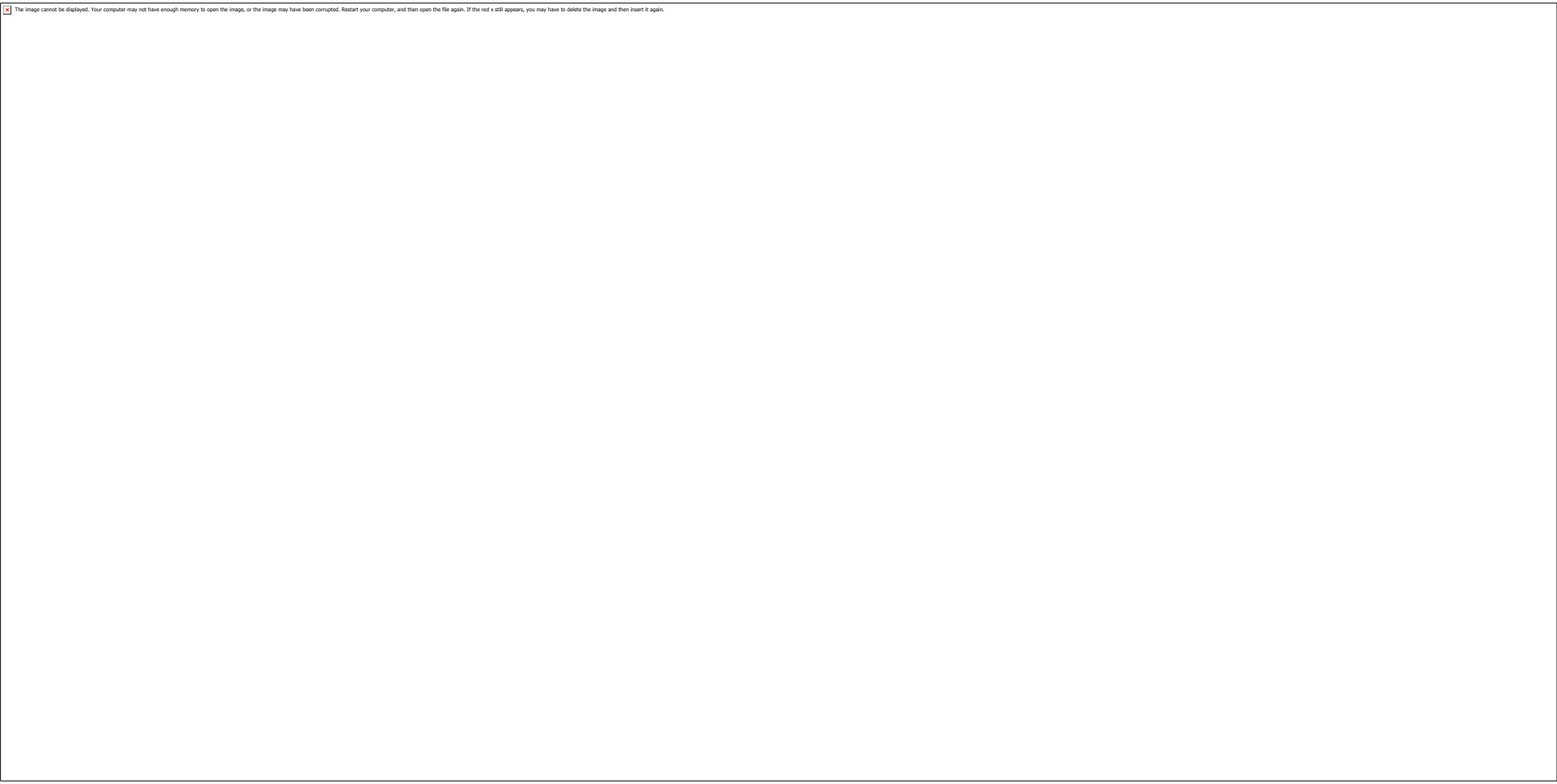
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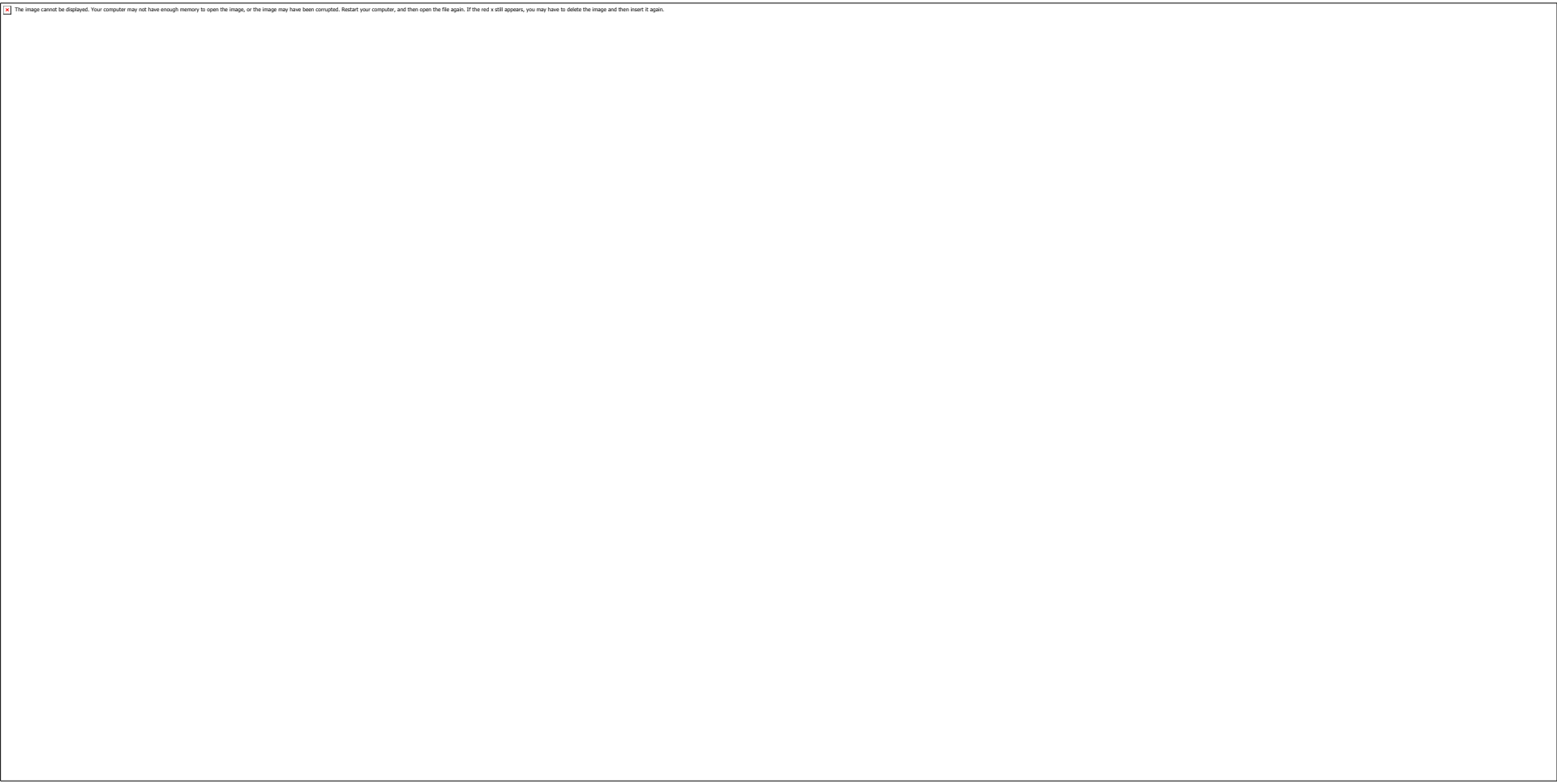
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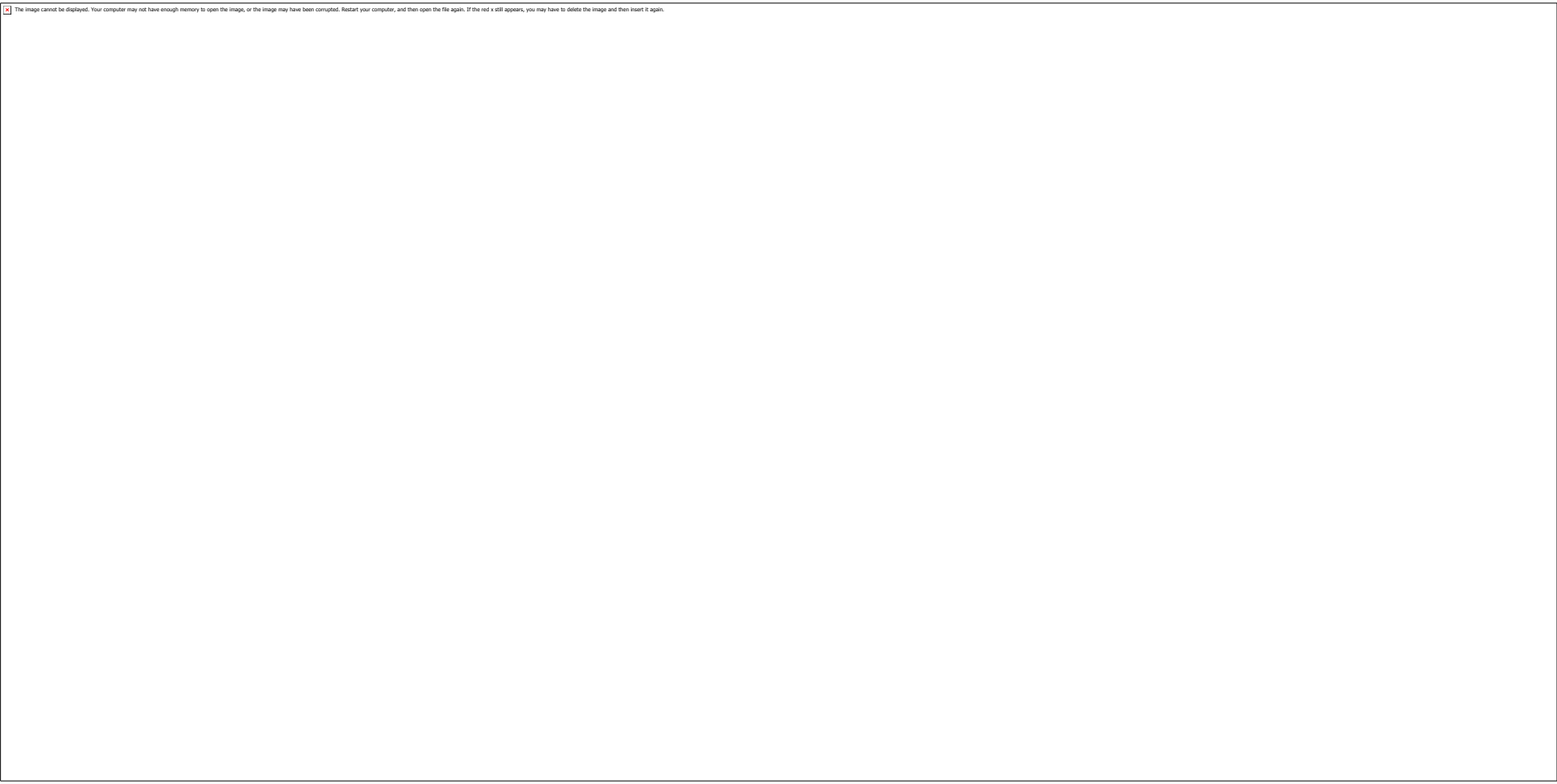
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For Review and Approval

- Presentation may include alternate representations of the above data replotted for clarity
- Overlays shown are representative, alternate images from any of the movies analyzed here may be presented