Structural analysis of Influenza A virus morphology using convolutional neural networks

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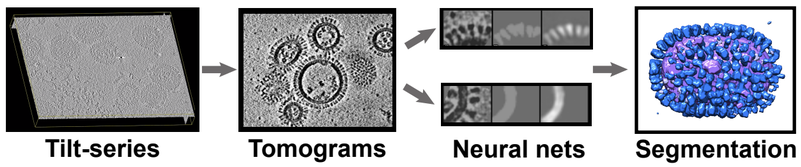
Graphical abstract



Background

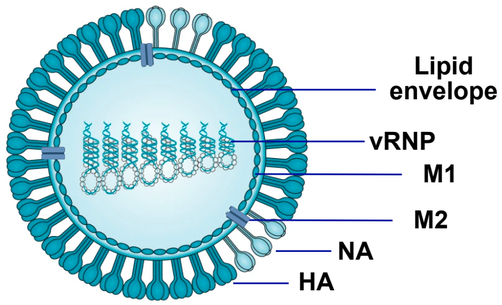


Fig 1. Influenza A virus (Adapted from Ref 1). Influenza A viruses are negative sense RNA viruses that contain two surface glycoproteins, HA and NA; lipid envelope; M1 protein layer; M2 proton channel; eight viral ribonucleoprotein (vRNP) complexes.

Methodology

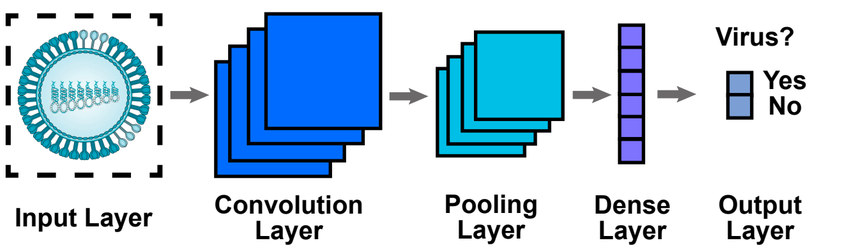


Fig 2. General convolutional neural network workflow. Input cryo-ET data is compiled, pooled, and lastly connected to create an output classifyer.

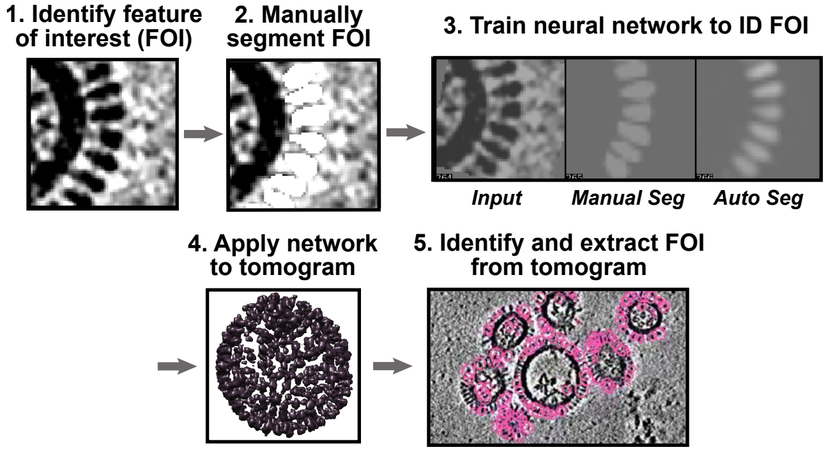


Fig 3. Neural network workflow. Example of the identification, annotation, and application of HA shown. Pink boxes in (5) represent the locations of identified HA from a representative tomogram

Influenza A viruses exhibit diverse morphologies

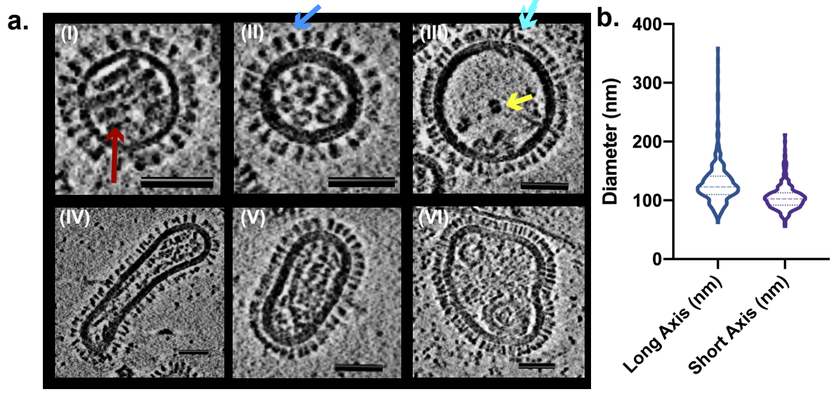


Fig 4. IAV structure and size. a. Examples of pleomorphic IAV particles with **(I)** a solenoid vRNP organization (red arrow); **(II)** bilayers (with M1 protein), surface HA (blue arrow), and an organized (7+1) vRNP core; **(III)** only a monolayer, NA (aqua arrow), and a disorganized vRNP core (yellow arrow); **(IV)** elongated and **(V)** oval virions, and **(VI)** irregular virions. Scale bars = 50 nm. **b.** Diameters of IAV particles measured by long and short axes (n = 311).

M1 is a determinant of vRNP presence and IAV size

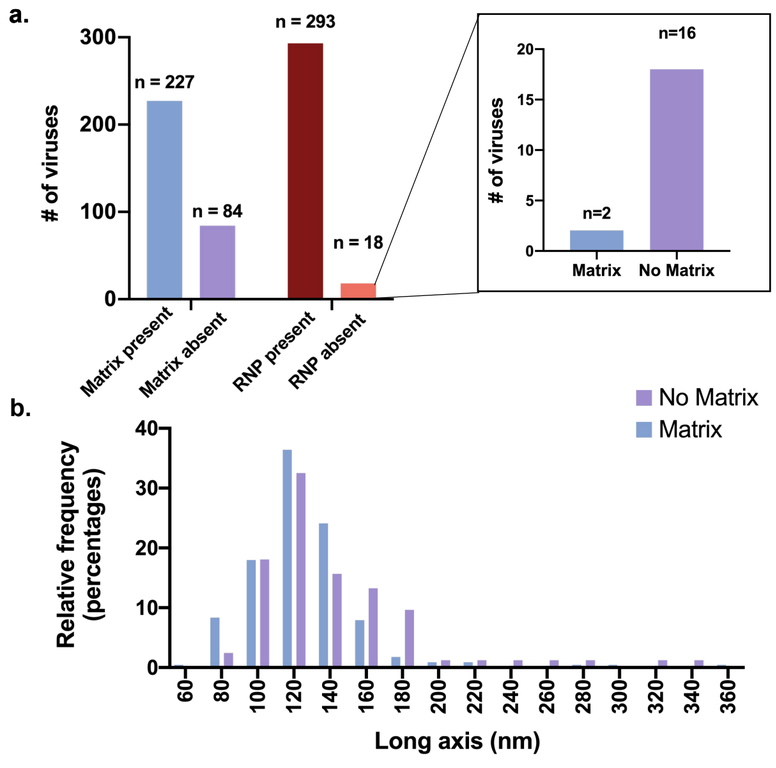


Fig 5. Morphological characterization of IAV virions revealed M1 dependency of RNP presence and virion size. a. Presence of M1 assembly and vRNP. **b.** Histogram of long axis for viruses with and without M1 protein layer

Neural networks accurately recognize viral features

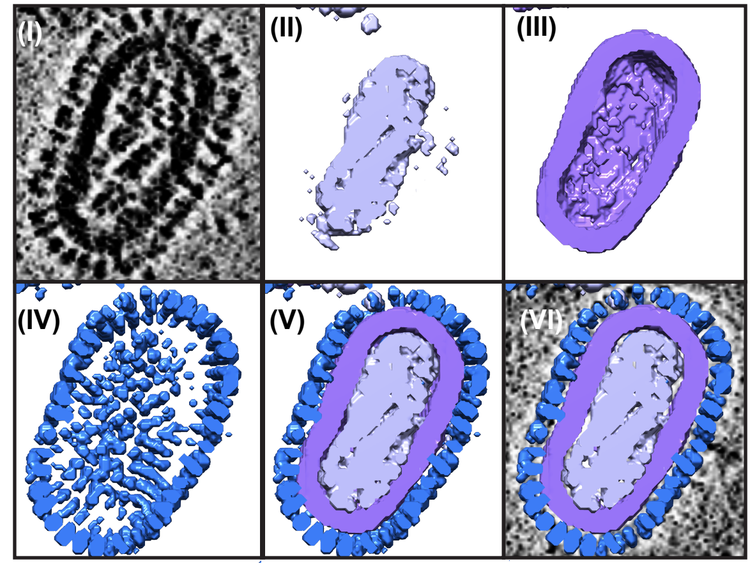


Fig 6. Neural network segmentation of representative IAV virion. (I) Tomographic slice of a virus particle with vRNP complexes, M1 protein assembly, lipid bilayer, and glycoproteins. **(II)** Surface representation of vRNP segmentation in lilac. **(III)** Membrane + M1 layer segmentation in purple. **(IV)** Glycoprotein segmentation in blue. **(V)** Merged segmentation of the virus particle. **(VI)** Merged virus segmentation overlaid on tomogram. Surfaces of virus components were capped to visualize within the glycoprotein and membrane layers.

Conclusions

- Neural networks precisely & accurately segment IAV components
- Cryo-ET data of the IAV strain A/PR/8/34 (H1N1) showed a diverse array of morphologies consisting of mostly spherical or oval virions .
- The size distribution and vRNP presence is associated with the presence of the M1 protein.
- Future work will characterize the surface landscape of IAV particles by quantifying and localizing HA/NA, determine structures of HA and NA using subtomogram averaging, and elucidate the structural basis of antibody resistance.

Acknowledgments

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For further comments, suggestions, and questions, please contact qiuyu.huang@umassmed.edu

References: 1. Krammer *et al.*, ***Nat. Rev. Dis. Primers***, 2018