Quantifying the Fitness of Influenza Drug-Resistant Mutants from Plaque Assay Data

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Background

- An increasing fraction of circulating and emerging influenza strains are resistant to the anti-flu adamantanes and neuraminidase inhibitors (NAIs).
- To assist in developing public health measures to contain a potential flu pandemic, it is crucial to obtain quantitative measures of the parameters responsible for the fitness impairments/enhancements of these drug-resistant strains.
- Differences in fitness between strains is typically assessed by comparing the diameter of the plaques produced by different strains in infection plaque assay experiments.

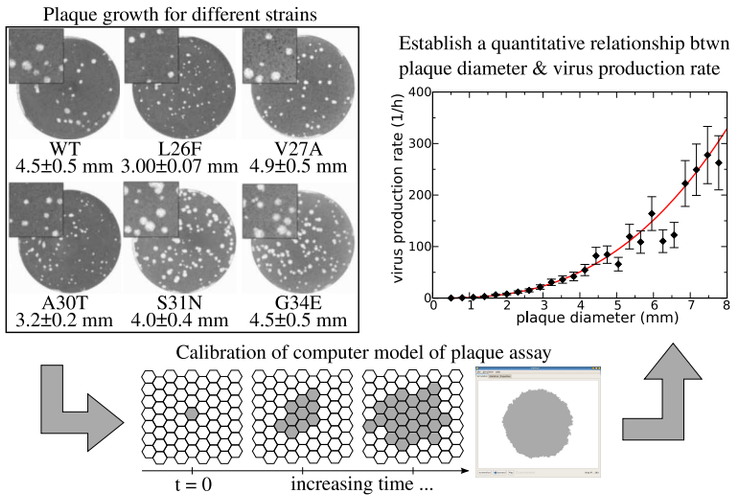
Long-term goal

- To quantify the fitness impairments (e.g. virion-cell binding rate, virion production and release rate) of NAI-resistant mutants.
- To use this information to predict the speed and severity of emergence of drug-resistance for various drug regimens.
  - ▷ Aid in developing accurate epidemiological models to predict the spread of drug-resistant strains within a population as a function of treatment measures adopted.

Approach

- Develop a computer model of an in vitro plaque assay.
- Obtain **reliable and consistent** experimental data (e.g., plaque diameter over time) which gives us a stable target: it is the dynamics our model needs to reproduce.
- Combine these 2 modalities as a tool to rapidly convert plaque diameter measurements to e.g. virus-cell binding rate or virus production rate for a given strain.

Mapping plaque diameter to an infection parameter



Plaque assay models

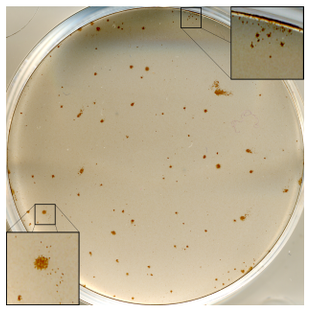


Figure 1: In vitro

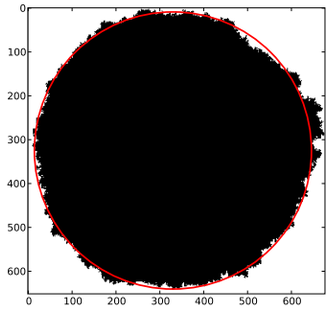


Figure 2: In silico

The average diameter of the plaques in the in vitro assay is compared with the diameter of plaques produced by the in silico model averaged over many runs.

Plaque diameter

- The diameter (d) of the plaque was determined using

$$\pi\left(\frac{d}{2}\right)^2 = N_{\text{infected}} \cdot \text{Area}_{\text{cell}}$$

$$d = \sqrt{\frac{4 \cdot N_{\text{infected}} \cdot \text{Area}_{\text{cell}}}{\pi}}$$

where N_{infected} is the number of infected cells, and $\text{Area}_{\text{cell}}$ is the surface area of a single cell.

- The centre of the plaque was determined using the centre of mass of all infected cells, namely

$$(x_{\text{centre}}, y_{\text{centre}}) = \left(\sum_{i=1}^{N_{\text{infected}}} \frac{x_i}{N_{\text{infected}}}, \sum_{i=1}^{N_{\text{infected}}} \frac{y_i}{N_{\text{infected}}}\right)$$

Infection rate effect on plaque diameter

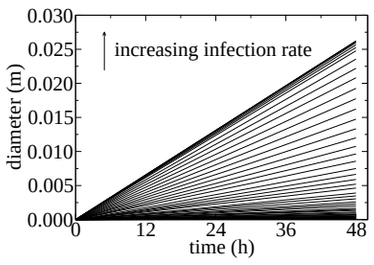


Figure 3

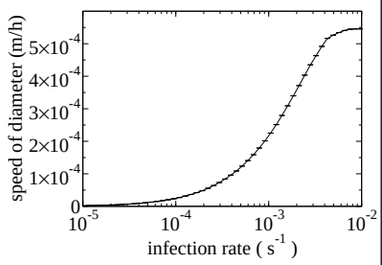


Figure 4

Figure 3: Plaque diameter over time for different values of the infection rate $\in [10^{-5}, 10^{-2}]$ s^{-1} (evenly spaced in $\log_{10}$ space).

Figure 4: Rate of change of the diameter (slope of diameter vs time graph) as a function of the infection rate.

Observed diameter vs infection rate

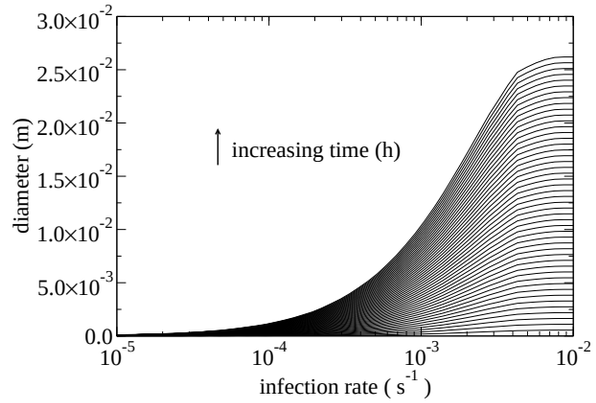
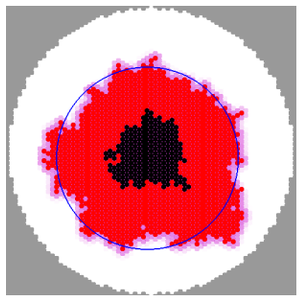


Figure 5: The effect of the infection rate on the plaque diameter observed at different times ranging from 1 to 48 hours. The arrow shows the direction of increasing times of observation.

Conclusion and future work

- From the in silico model, we determined that the rate of change of the plaque diameter is proportional to the infection rate.
- Here, we assumed neighbour-to-neighbour (n2n) infection. This is likely a reasonable assumption because the agar overlay placed on top of the cell culture in vitro prevents infectious virus from diffusing much further than the immediate neighbours.
  - However, the n2n description does not allow us to extract true biological parameters such as virion-cell binding rates, virion production and release rate, etc.
  - In future work, we will use a more sophisticated in silico plaque assay model based on MASyV's `ma_virions` client [**1**] where infection proceeds as infected cells release virus which in turn infect other cells.



Acknowledgements

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References

[**1**] Beauchemin, CAA, MASyV: A Multi-Agent System Visualization package. Open-source GNU GPL software available on `http://masyv.sourceforge.net`.