Single-particle measurements reveal damage to filamentous influenza virions during laboratory handling

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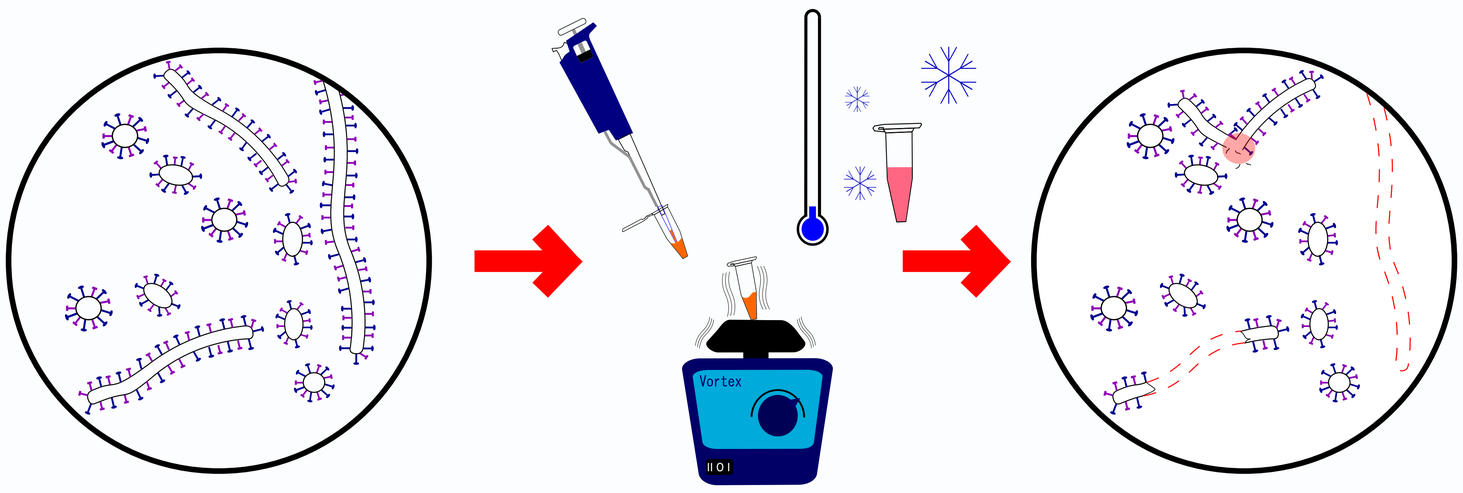
Clinical isolates of influenza virus form both filamentous and spherical virions. Filaments are positively selected in respiratory infections, but it is unclear why.

Studies of filament properties are contradictory. This could be caused by damage from laboratory handling, which has been anecdotally reported[1] but never tested.

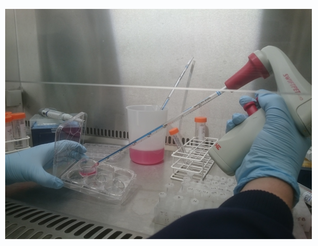
To determine which methods are suitable to analyse filament properties, we assessed how common laboratory techniques affect the concentration and average length of filaments in a population.



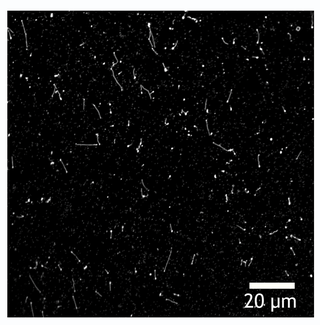
1 - Reviewed in Dadonaite *et al.* (2016) *J Gen Virol* [PMID: 27365089]

Methods

Characterising filament populations by conventional negative stain particle counting is laborious and technically challenging. Filaments are large enough to be resolved by light so we instead chose confocal microscopy.



Infect MDCK cells with A/Udorn/307/72. Harvest virions after 24 hours.



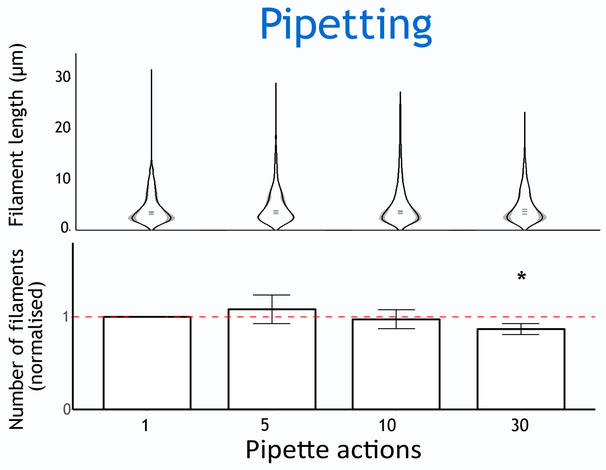
Dilute, centrifuge on to coverslips and immunolabel viral haemagglutinin (HA).

Extract particle lengths in Image J using the ridge detection algorithm[2].

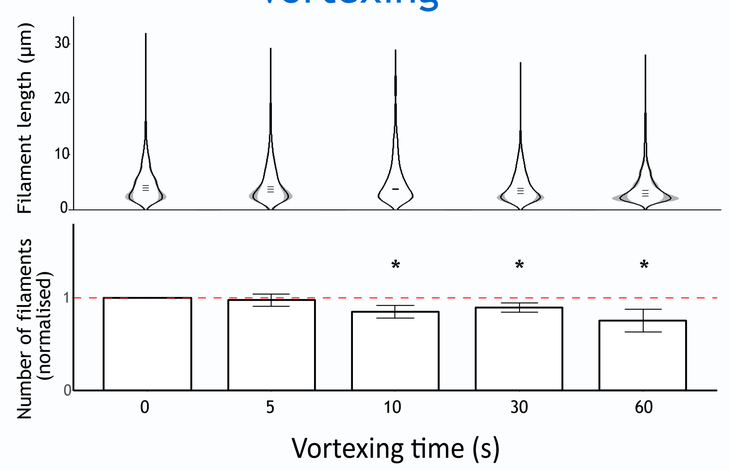
2 - Steger, C., 1998, IEEE Trans. Pattern Anal. Mach. Intell).

Extensive pipetting or vortexing damages filaments

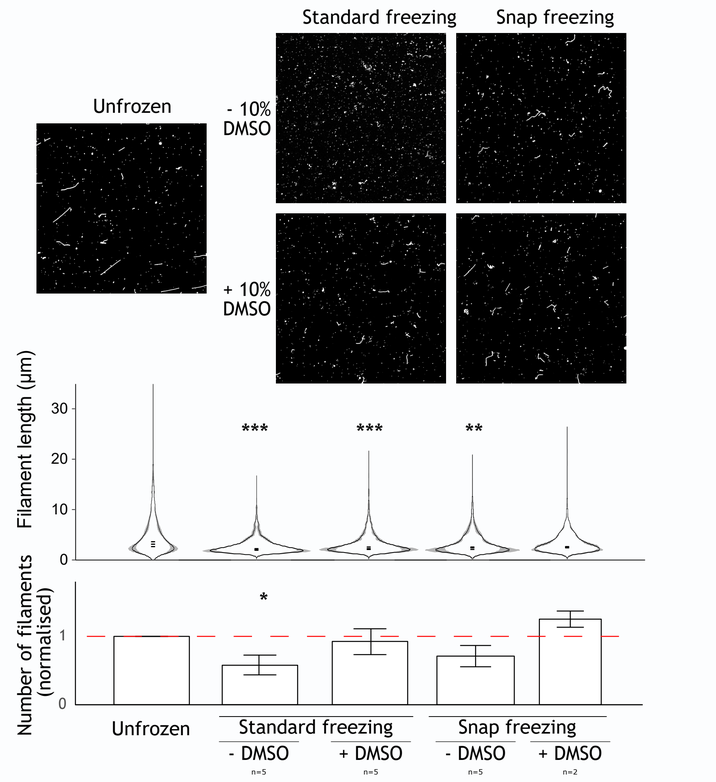
Pipetting



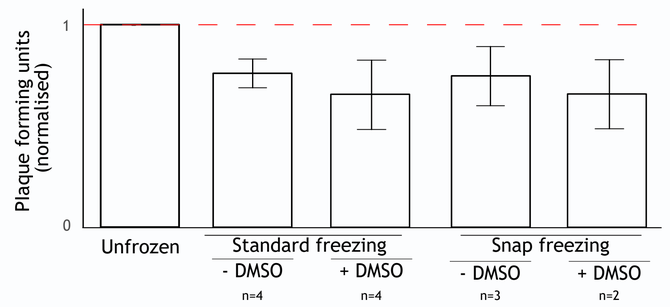
Vortexing



Freezing damage can be reduced by snap freezing or freezing with 10% DMSO

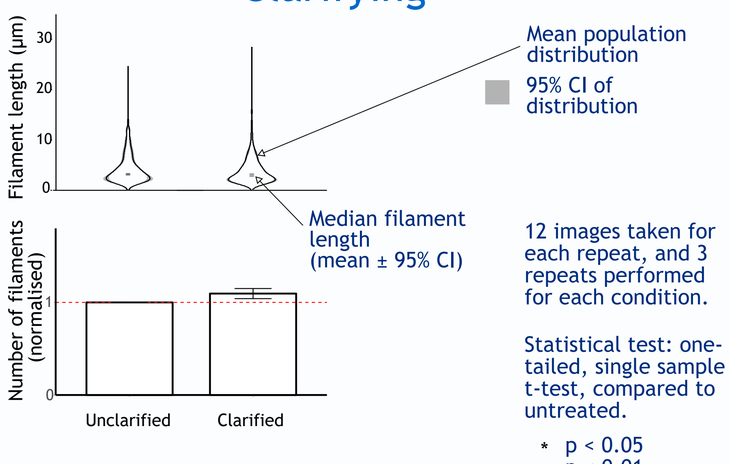


Reducing damage does not rescue infectious titre



Clarification and sonication do not damage filaments

Clarifying

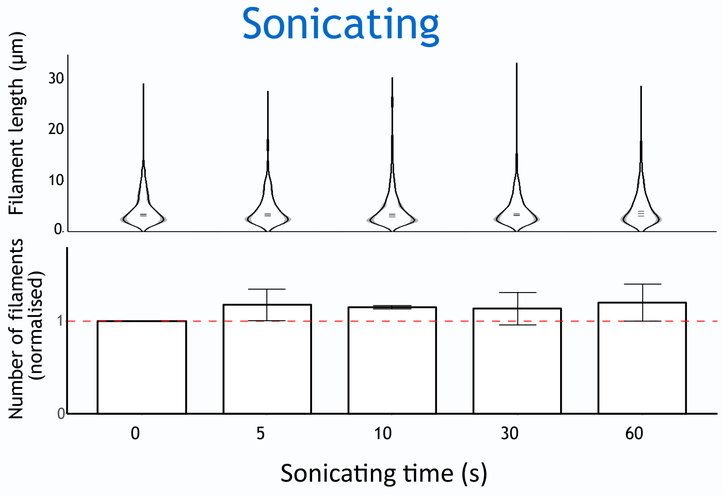


12 images taken for each repeat, and 3 repeats performed for each condition.

Statistical test: one-tailed, single sample t-test, compared to untreated.

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

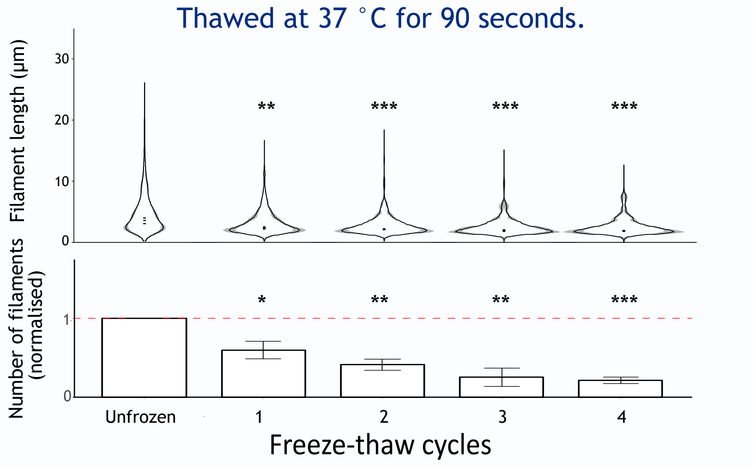
Sonicating

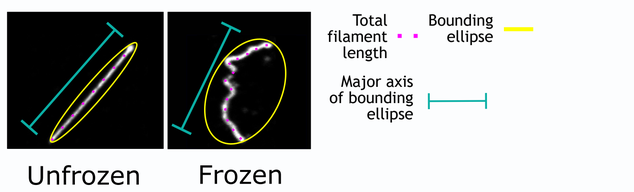


Freezing damages filaments and reduces their median length

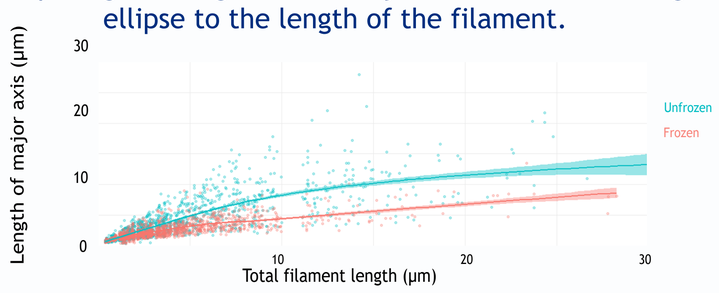
Standard Freezing

1 ml aliquot stored at -80 °C for one hour per cycle.
Thawed at 37 °C for 90 seconds.





Freezing induces "kinks" in the virions which could indicate capsid damage. We quantified this by comparing the length of the major axis of the bounding ellipse to the length of the filament.



Discussion

Filaments can be damaged by routine laboratory handling. This could skew functional analyses into their properties.

Avoiding damaging handling practises such as freezing will improve robustness of future studies.

If freezing can not be avoided, damage can be mitigated by snap freezing or including DMSO.

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