

Twitter Thread by Kevin McKernan



Kevin McKernan

@Kevin_McKernan



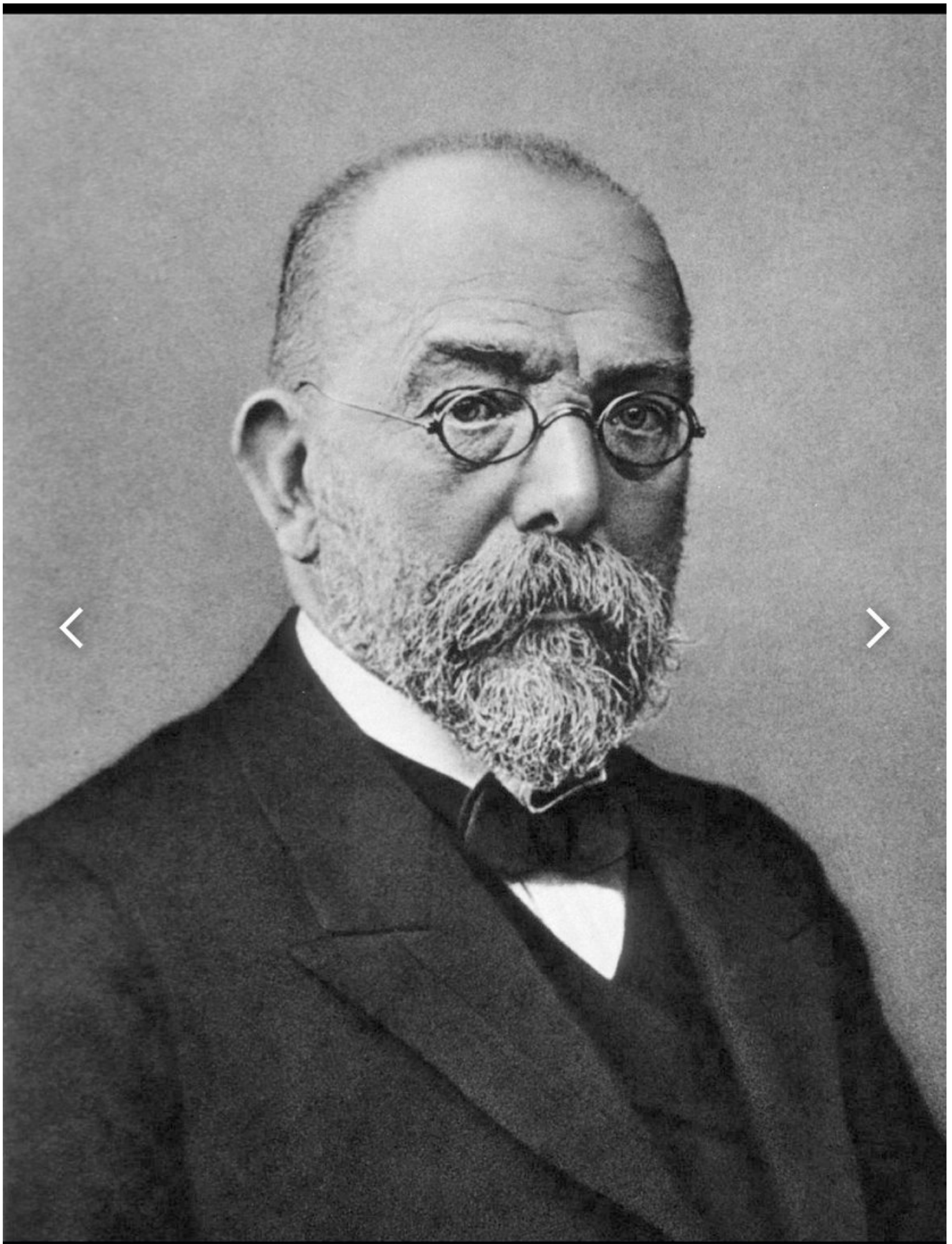
OK Koch heads... bring your hurt.

I'm glad you folks exist as we need people questioning everything.

However, I have found the "virus doesn't exist" arguments unconvincing.

The virus isn't the only cause of disease may have some ground.

I remain open to new papers on this.



Robert Hermann Koch (11 December 184...

[Details](#)

Unknown author • [Public domain](#)

Set some rules.

This was established in 1884. Watson & Crick discovered DNA structure in 1953.

He did not contemplate unculturable organisms... the vast majority of organisms are not culture-able.

Viruses by definition cannot be cultured. They require a host cell to culture.

Koch's Postulates:

① The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.

Susp
path

② The microorganism must be isolated from a diseased organism and grown in pure culture.

③ The cultured microorganism should cause disease when introduced into a healthy organism.

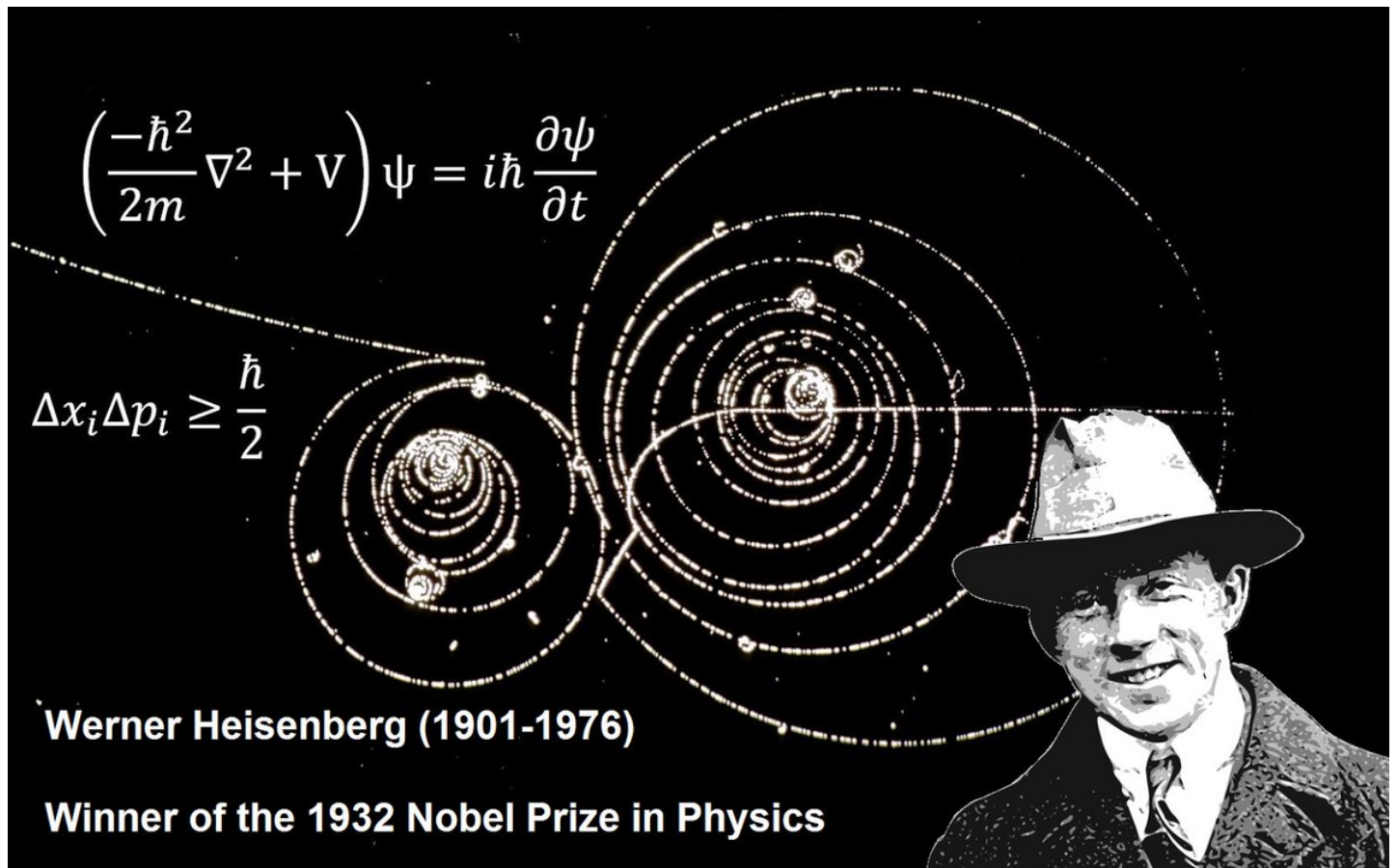
④ The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Koch's postulates of

This is where the debate gets dirty.

In order to culture the virus you need to find cells/organisms it can infect but not be a pathogen to those cells.

But Koch wants to demonstrate the pathogen creates symptoms of the disease which be counterproductive to culture.



This is like the uncertainty principle.

The cell line/model organism you choose to culture the virus, can't be extremely affected by the virus or the culture won't succeed.

Therefore the vector for the disease is unlikely to be the best place to look for the disease symptoms.

As a result we have folks discounting Vero cell (monkey kidney cells) studies for viral isolation.

By this same logic, all animal models are thus insufficient as well.

Any argument that Vero cells are not identical to patients must also reject all animal models.

Medical ethics prevents us from infecting real patients with a virus suspected of pandemic potential.

This leaves us with Ex-vivo culture of sick and healthy patients samples.

Fairly invasive research. Needs IRB approval.

<https://t.co/kvyPWepzFT>

This extracts epithelial cells from patients respiratory tract and cultures those cells as a model for viral infection and replication.

It has been done in C19 and I'm open to Koch Folks scrutinizing it's short comings.

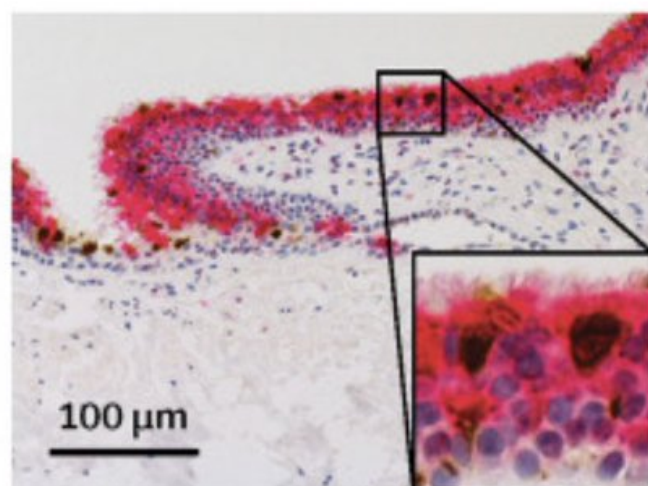
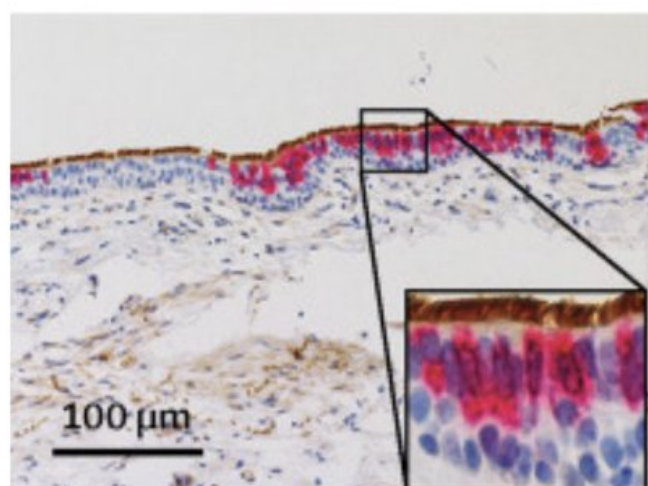
A

Ciliated cell

NP/Ac-Tub

Club cell

NP/CC10

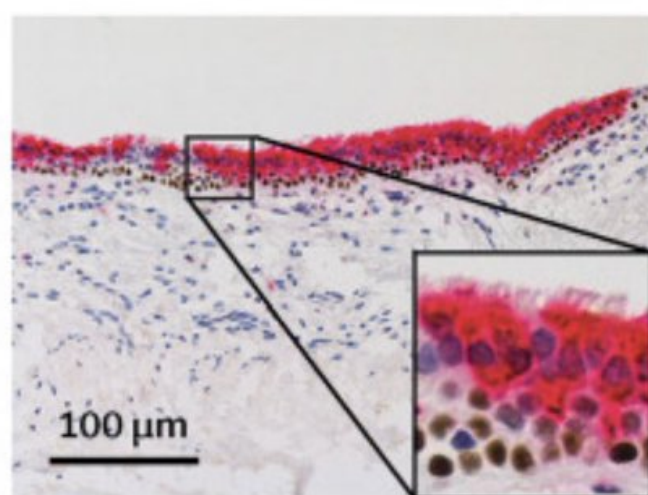
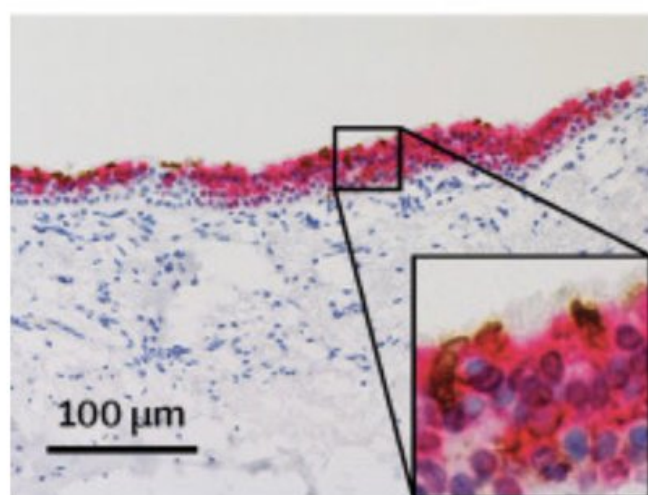


Goblet cell

NP/MUC5AC

Basal cell

NP/p63a

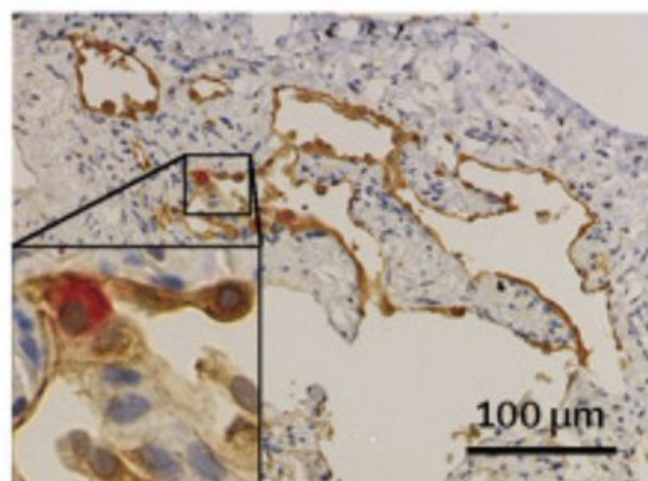
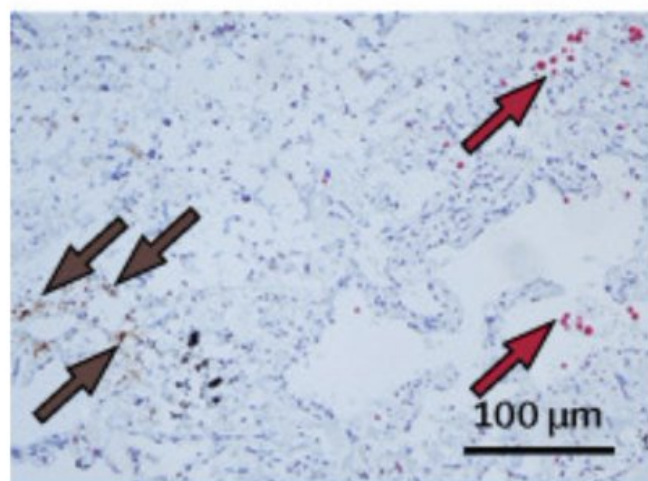
**B**

Macrophage

NP/CD68

Epithelial cell

NP/AE1/3



Ideally, we would have highly purified virus from the diseased and be able to put it into a human cell line and demonstrate replication and illness and re-isolate and confirm its the same.

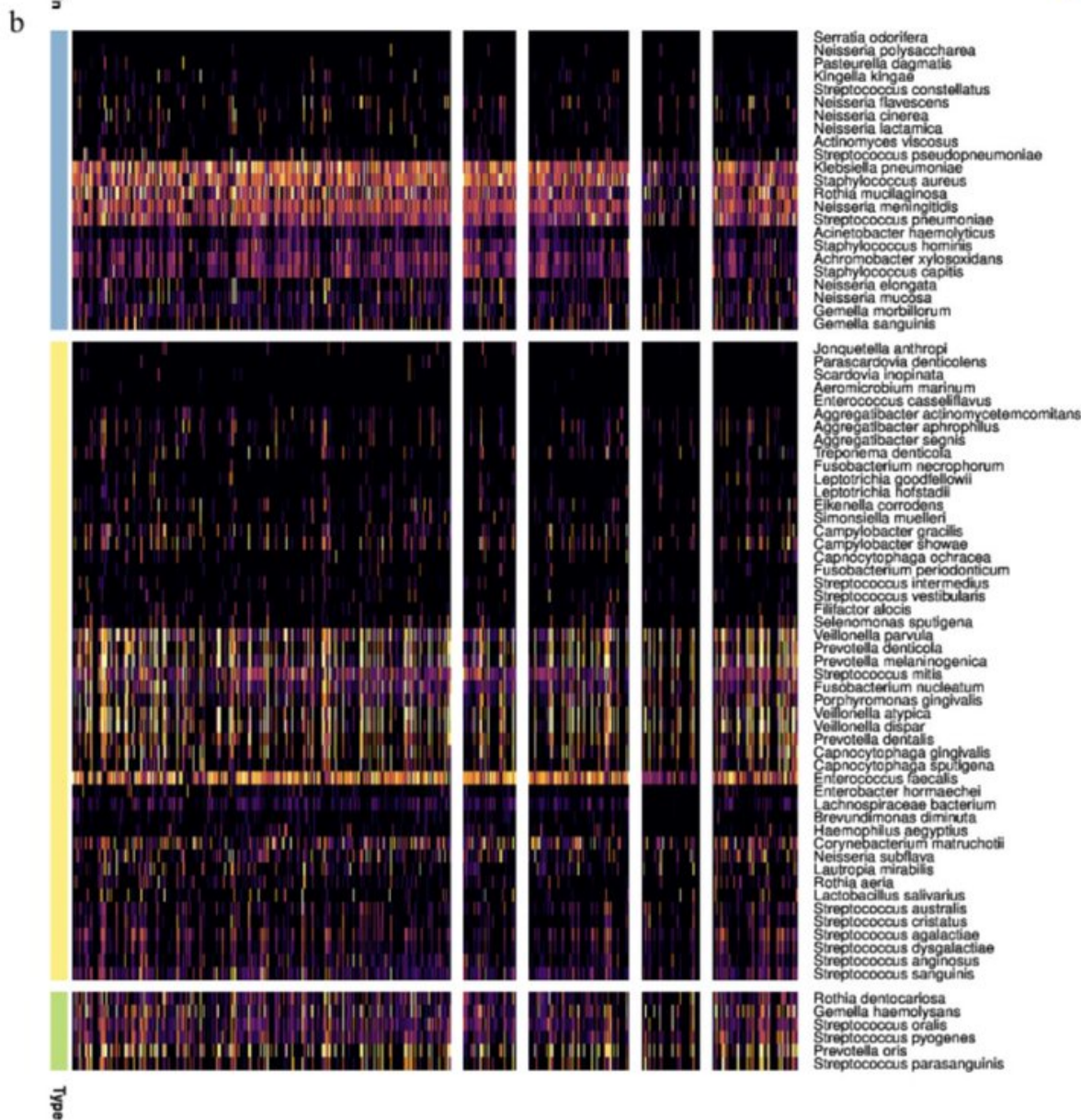
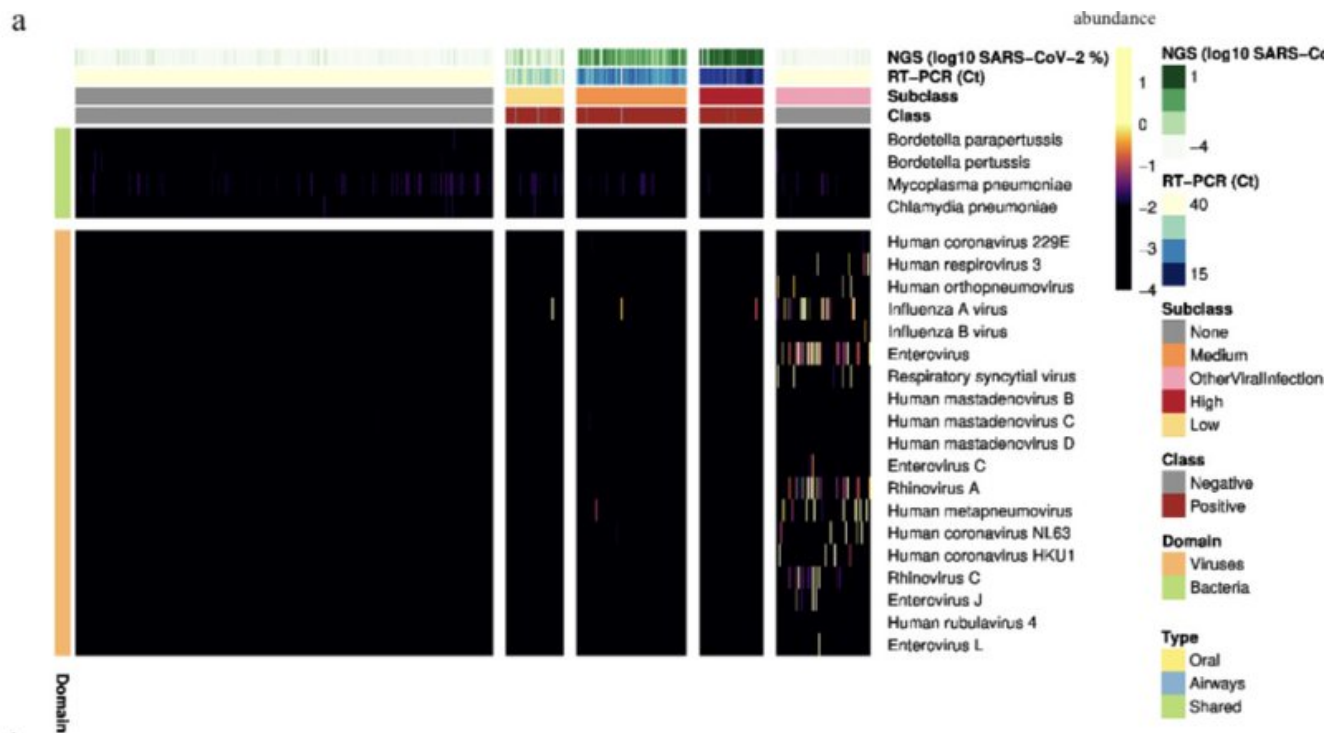
Since we can't infect humans, we resort to human cell lines.

We have new tools for understanding purity today.

We can sequence all RNA in a patient and see the complete virome present.

Based on the sequence we can predict proteins we should see with Mass spec after successful viral replication in a host.

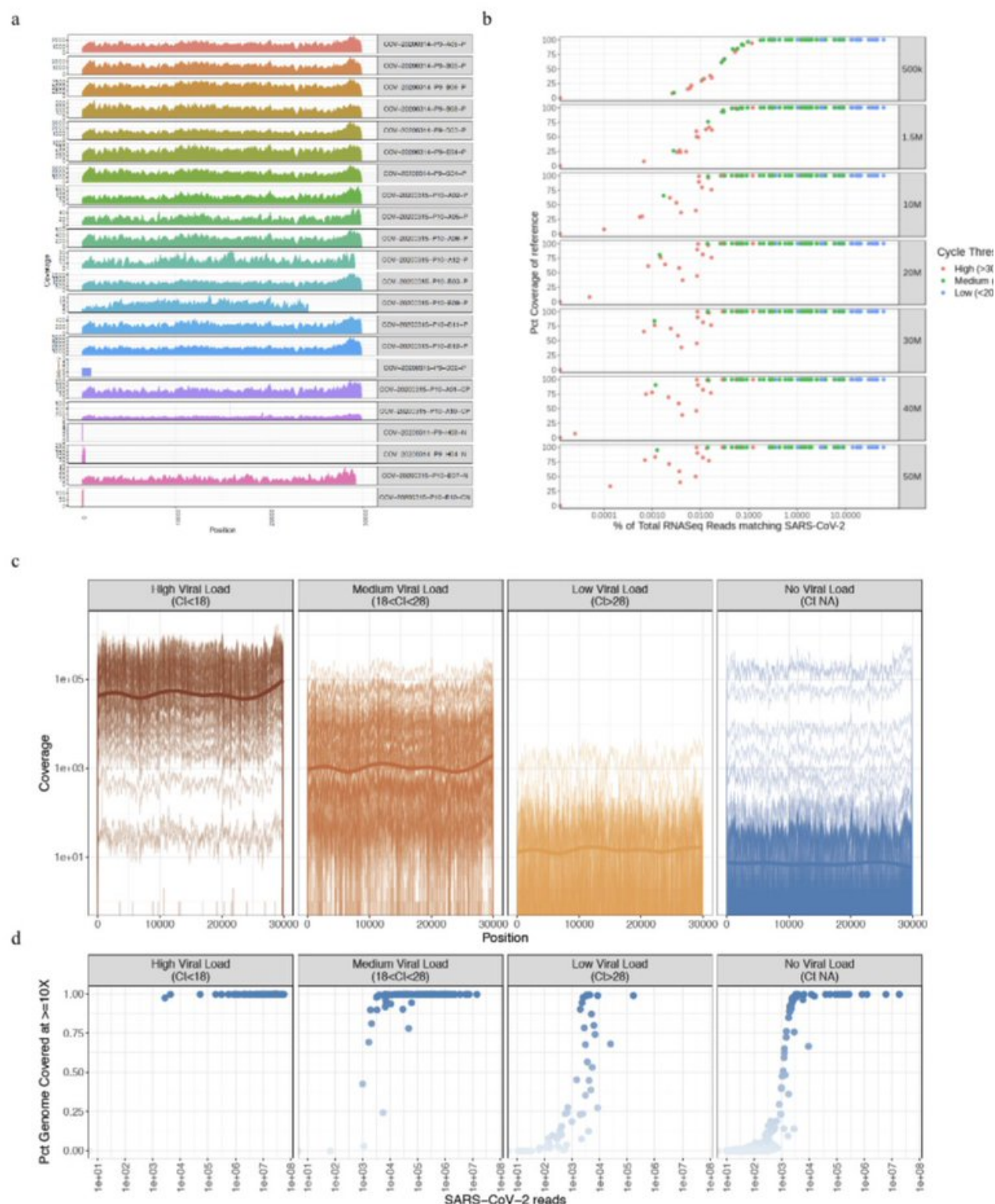
<https://t.co/xmbh4BGBv5>



Masons lab goes on to build beautiful whole transcriptome pictures of each SARs patient.

Over 96% of the patients had C19 sequence in their Bronchial Lavage (BAFL). This is not Vero cell culture. Patient cells.

Whole SARs genomes from patients and it's the dominant RNA present.



Supplementary Figure 6. Viral genomes from RNA-seq data and titration of coverage (a) The coverage plot of the SARS-CoV-2 genomes (viral coordinate on bottom, colored by sample) from a representative set of clinical positive samples. Sample names with the suffixes CN and N are clinical negative (buffer), P are qRT-PCR positive, and C are Vero E6 cells with virus. (b) Downsampling (right annotation) of the samples and mapping to the SARS-CoV-2 genome to gauge the percent coverage (y-axis) as a function of the viral quantification by qRT-PCR (Ct thresholds, low < 20, medium 20-30, and high > 30). (c) Average coverage statistics for the low, medium, and high Ct samples, as well as mean coverage for each of these samples. (d) The cycle threshold (x-axis) vs. the coverage of the genome (y-axis) for the total RNA-seq.

Some will say... you don't know it's the causative virus from this. Highest prevalence does not = causative agent.

But since we have whole transcriptomes we can see there is no other sensible hypothesis. No other viruses present.

The RNA has been isolated but not the virus.

On the flip side, images of fuzzy crowns on EM, I find unconvincing.

You can't identify a virus with a photograph. All taxonomy is ultimately DNA/RNA based.

So how do we catch the virus in the act of infection?

Spatial transcriptomics-

This is the art of sequencing in situ.

You infect cells with the purified virus and you perform spatial transcriptomics on those cells.

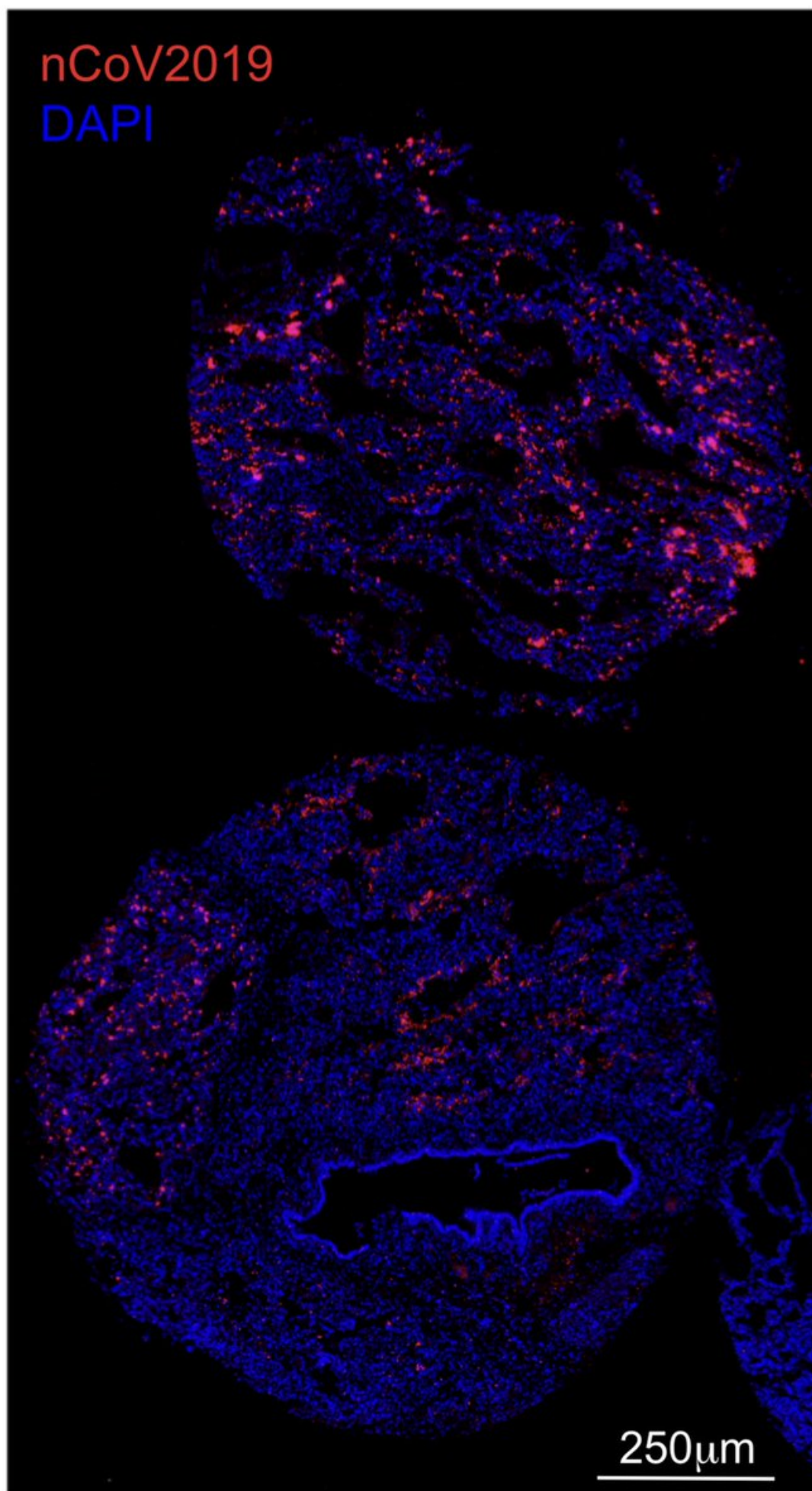
This is a new, explosive and exciting field that SOLiD sequencing is still being used for.

<https://t.co/joyNHqCJB2>

A

nCoV2019

DAPI



It's worth understanding the technique.

It enables you to harvest RNA from cells and maintain their spatial coordinates in the cell with 10um and eventually 1um resolution.

It's unlikely to get below the diffraction limit of light (250nm) in terms of resolution.

Here it deployed on C19 patient biopsies.

We have spike protein histochemistry co-localized with C19 RNA sequence in infected patients.

Not all biopsies succeeded. Lots of tissue heterogeneity but a good piece of the puzzle.

<https://t.co/NCrmqAHRDe>

