Twitter Thread by Kevin McKernan

Kevin McKernan
@Kevin_McKernan

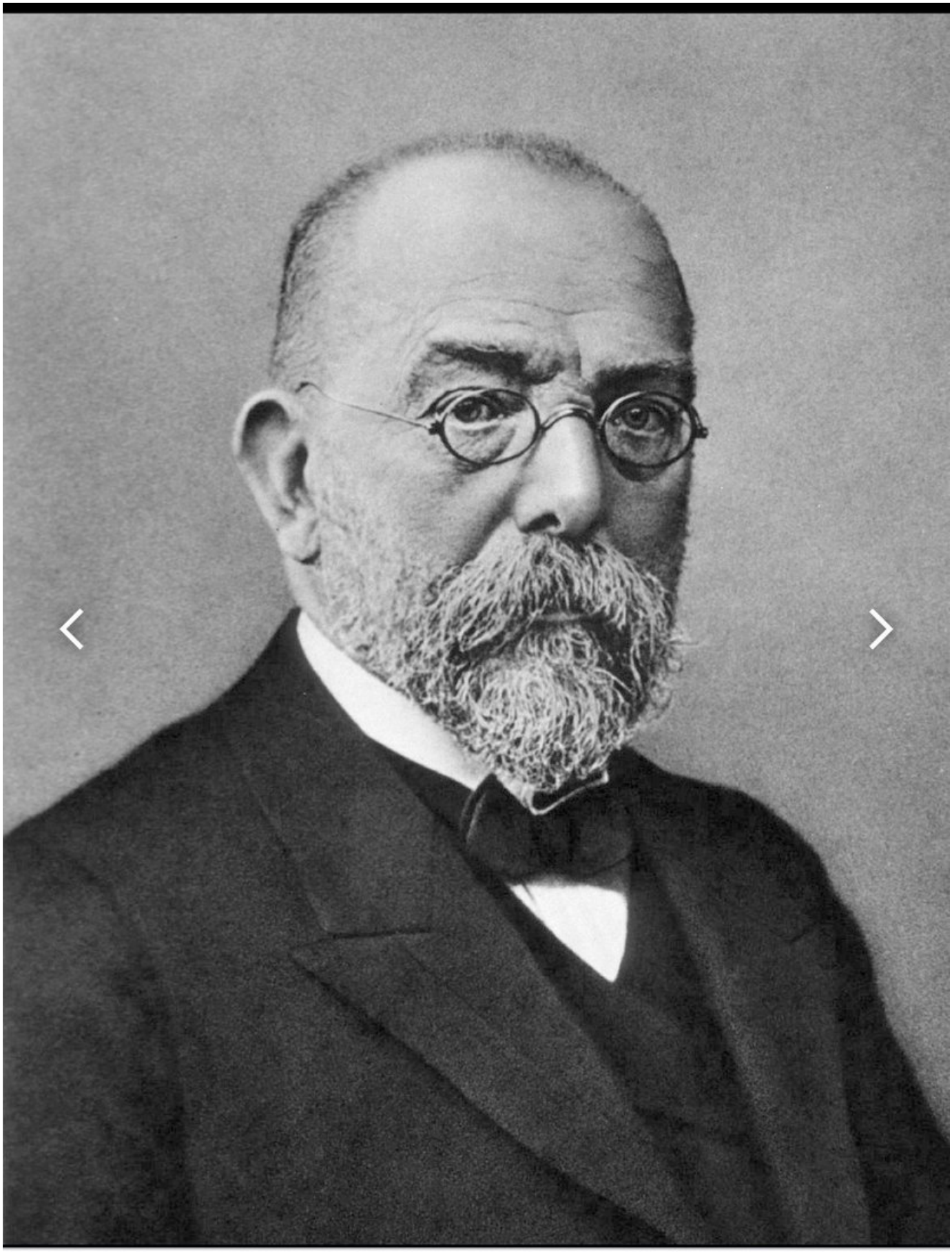
OK Koch heads... bring your hurt.

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.

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.

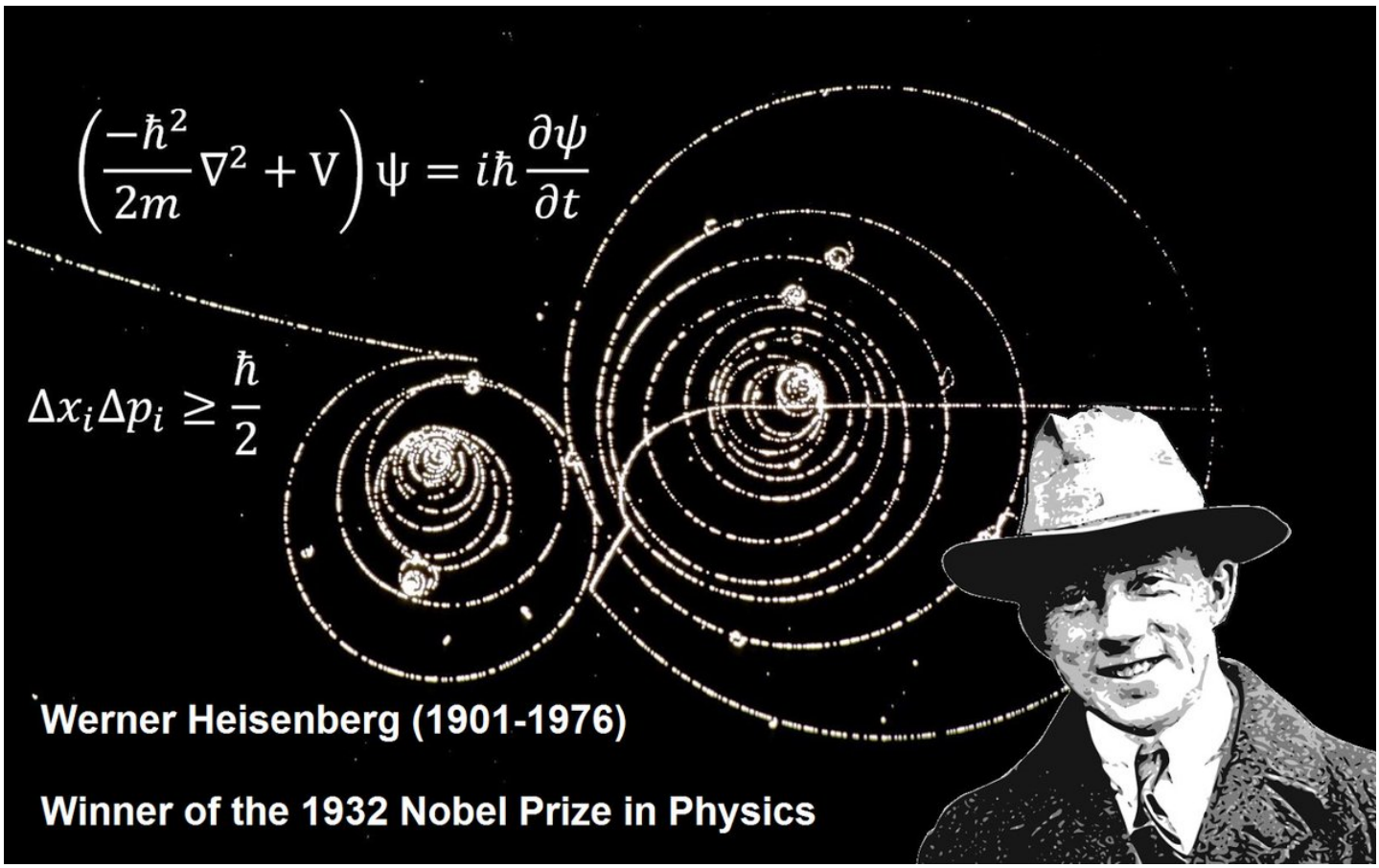
④ The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

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.

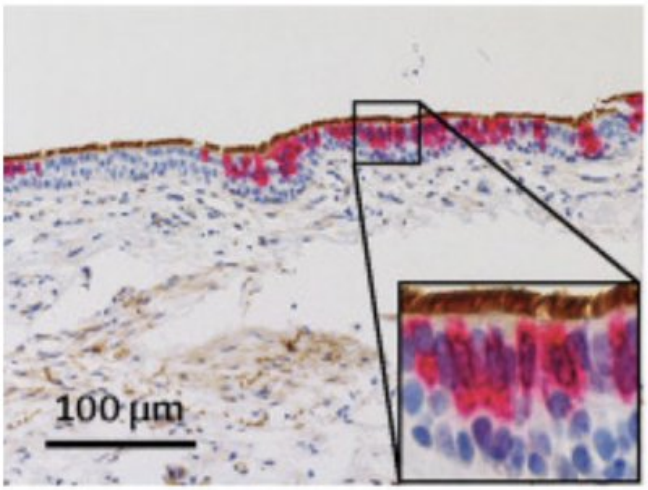
https://t.co/kvyPWepzFT

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

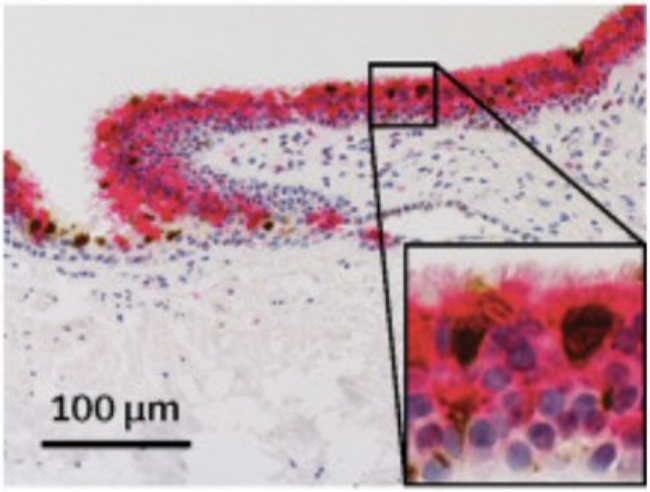
It has been done in C19 and I’m open to Koch Folks scrutinizing it’s short comings.

A

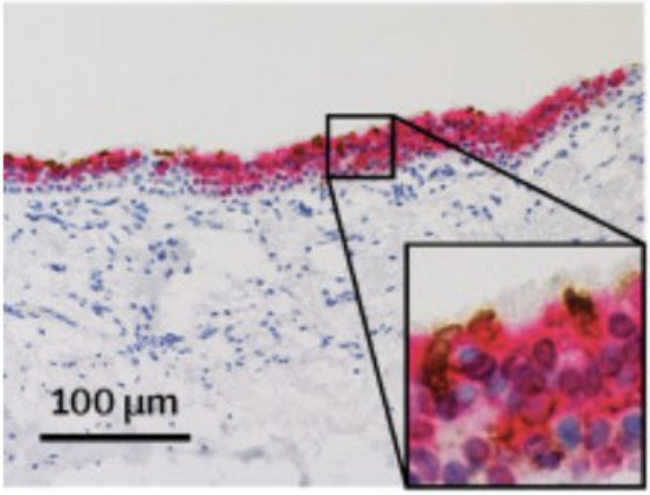
Ciliated cell NP/Ac-Tub



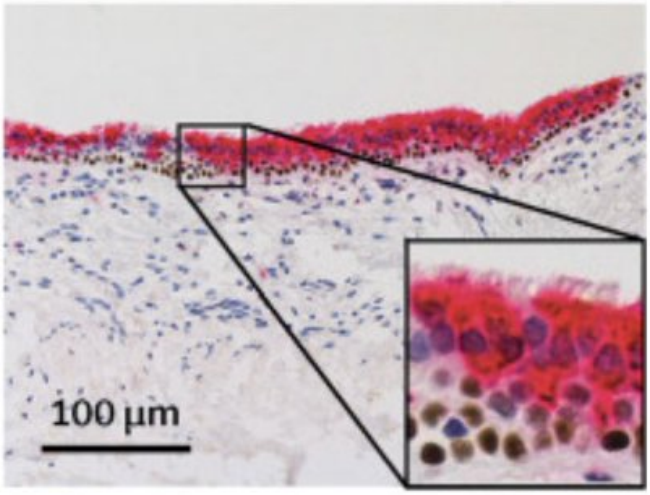
Club cell NP/CC10



Goblet cell NP/MUC5AC

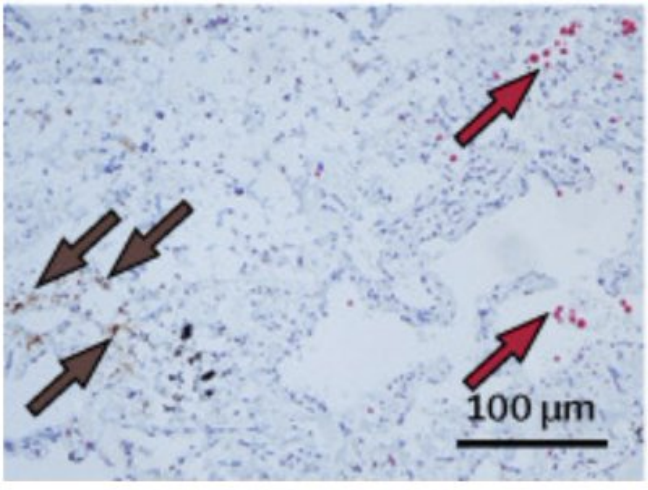


Basal cell NP/p63a

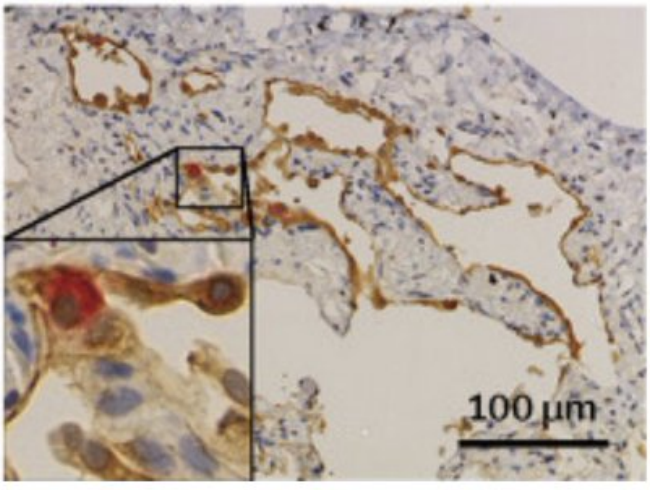


B

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.

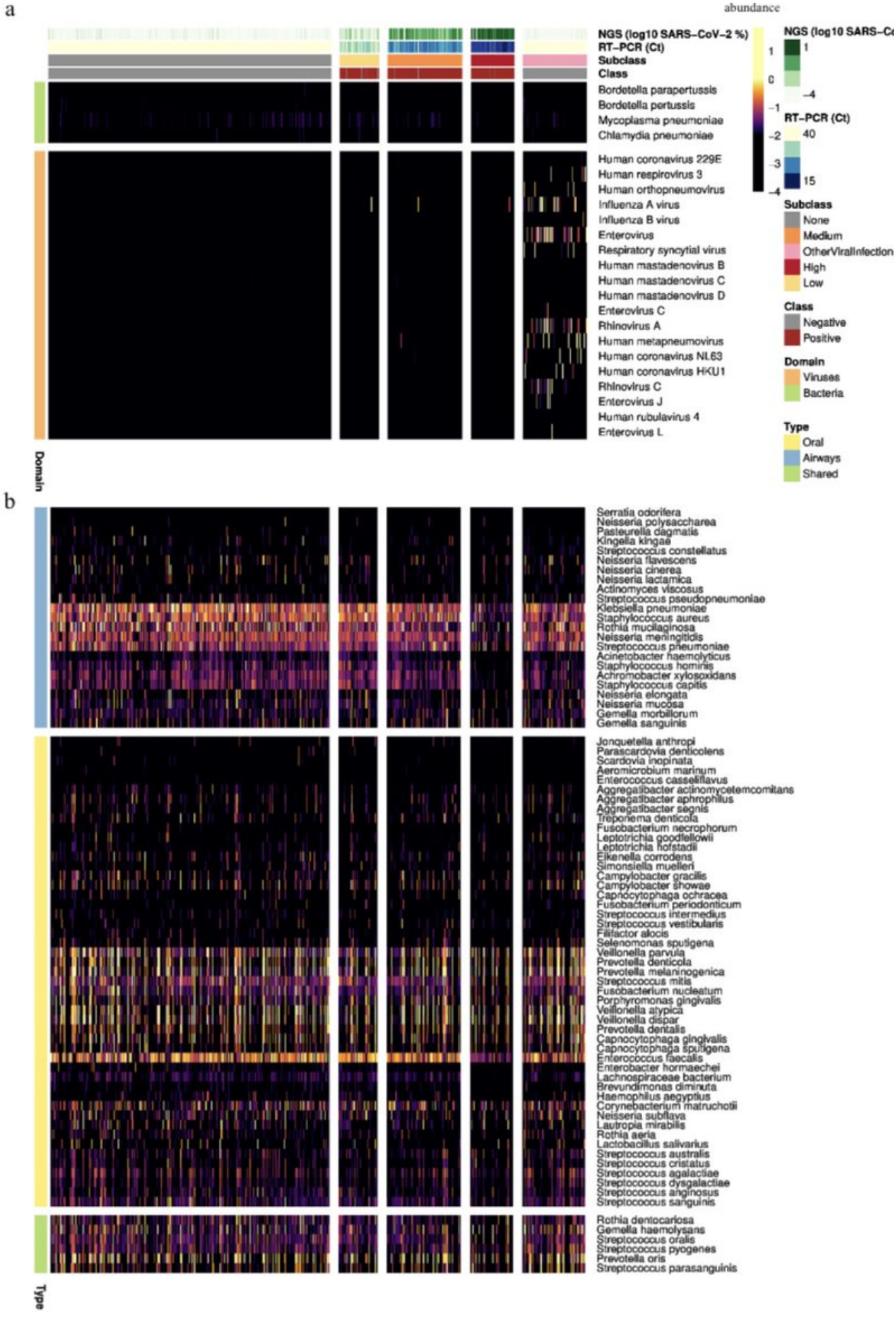
Since we can’t infect humans, we resort to human cell lines.

We have new tools for understanding purity today.

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

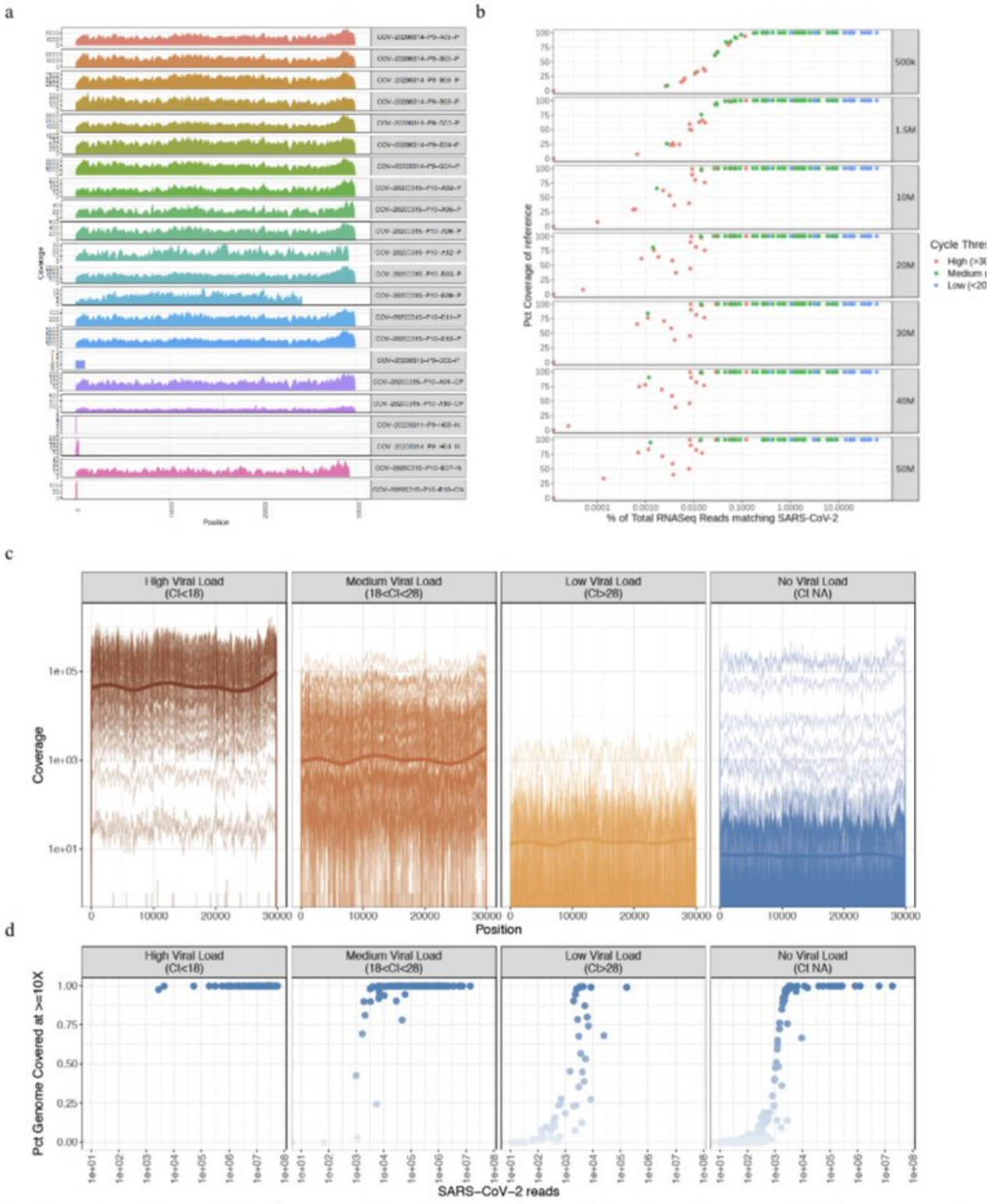
Based on the sequence we can predict proteins we should see with Mass spec after successful viral replication in a host.

https://t.co/xmbh4BGBv5


a
abundance
NGS (log10 SARS−CoV−2 %)
RT−PCR (Ct)
Subclass
Class
Bordetella parapertussis
Bordetella pertussis
Mycoplasma pneumoniae
Chlamydia pneumoniae
Human coronavirus 229E
Human respirovirus 3
Human orthopneumovirus
Influenza A virus
Influenza B virus
Enterovirus
Respiratory syncytial virus
Human mastadenovirus B
Human mastadenovirus C
Human mastadenovirus D
Enterovirus C
Rhinovirus A
Human metapneumovirus
Human coronavirus NL63
Human coronavirus HKU1
Rhinovirus C
Enterovirus J
Human rubulavirus 4
Enterovirus L
Domain
NGS (log10 SARS−C
1
0
−1
−2
−3
−4
RT−PCR (Ct)
40
15
Subclass
None
Medium
OtherViralInfection
High
Low
Class
Negative
Positive
Domain
Viruses
Bacteria
Type
Oral
Airways
Shared
b
Serratia odorifera
Neisseria polysaccharea
Pasteurella dagmatis
Kingella kingae
Streptococcus constellatus
Neisseria flavescens
Neisseria cinerea
Neisseria lactamica
Actinomyces viscosus
Streptococcus pseudopneumoniae
Klebsiella pneumoniae
Staphylococcus aureus
Rothia mucilaginosa
Neisseria meningitidis
Streptococcus pneumoniae
Acinetobacter haemolyticus
Staphylococcus hominis
Achromobacter xylosoxidans
Staphylococcus capitis
Neisseria elongata
Neisseria mucosa
Gemella morbillorum
Gemella sanguinis
Jonquetella anthropi
Parascardovia denticolens
Scardovia inopinata
Aeromicrobium marinum
Enterococcus casseliflavus
Aggregatibacter actinomycetemcomitans
Aggregatibacter aphrophilus
Aggregatibacter segnis
Treponema denticola
Fusobacterium necrophorum
Leptotrichia goodfellowii
Leptotrichia hofstadii
Eikenella corrodens
Simonsiella muelleri
Campylobacter gracilis
Campylobacter showae
Capnocytophaga ochracea
Fusobacterium periodonticum
Streptococcus intermedius
Streptococcus vestibularis
Filifactor alocis
Selenomonas sputigena
Veillonella parvula
Prevotella denticola
Prevotella melaninogenica
Streptococcus mitis
Fusobacterium nucleatum
Porphyromonas gingivalis
Veillonella atypica
Veillonella dispar
Prevotella dentalis
Capnocytophaga gingivalis
Capnocytophaga sputigena
Enterococcus faecalis
Enterobacter hormaechei
Lachnospiraceae bacterium
Brevundimonas diminuta
Haemophilus aegyptius
Corynebacterium matruchotii
Neisseria subflava
Lautropia mirabilis
Rothia aeria
Lactobacillus salivarius
Streptococcus australis
Streptococcus cristatus
Streptococcus agalactiae
Streptococcus dysgalactiae
Streptococcus anginosus
Streptococcus sanguinis
Rothia dentocariosa
Gemella haemolysans
Streptococcus oralis
Streptococcus pyogenes
Prevotella oris
Streptococcus parasanguinis
Type

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

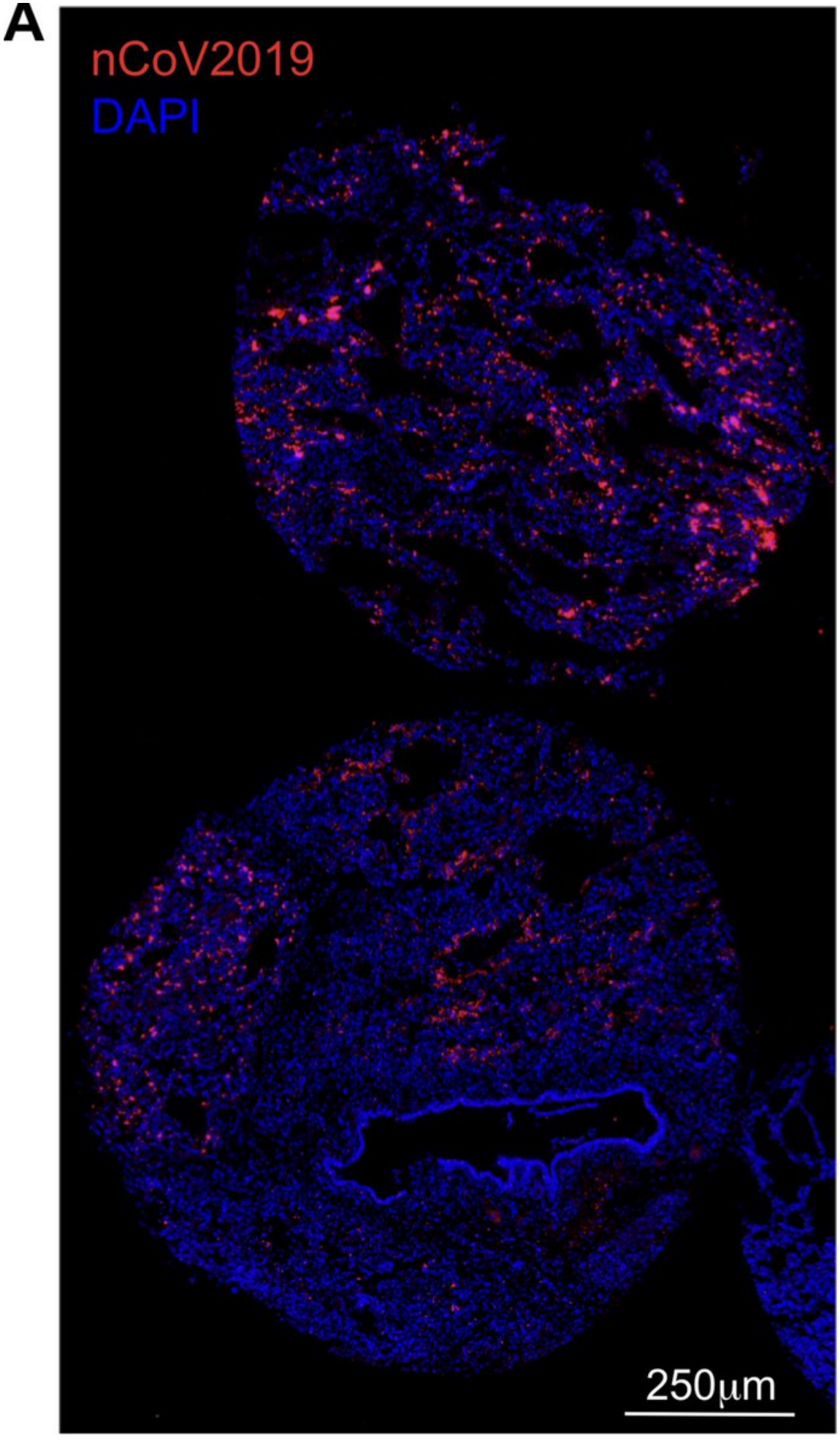
Spatial transcriptomics-

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/ioyNHqCBJ2


A
nCoV2019
DAPI
250μm

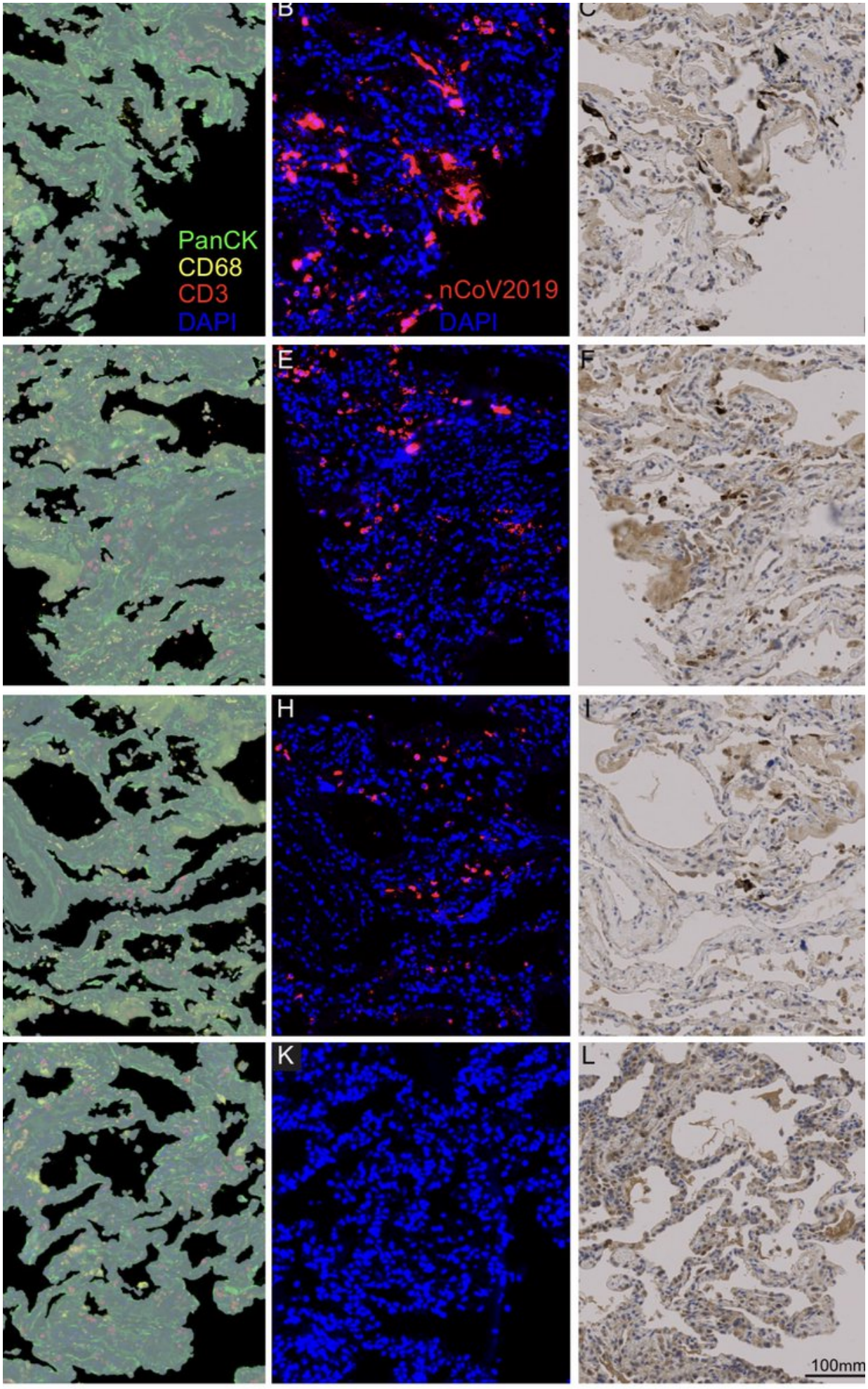
It’s worth understanding the technique.
It enables you to harvest RNA from cells and maintain their spatial coordinates in the cell with 10um and eventually 1um resolution.
It’s unlikely to get below the diffraction limit of light (250nm) in terms of resolution.

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


PanCK
CD68
CD3
DAPI
B
nCoV2019
DAPI
C
E
F
H
I
K
L
100mm