



NextFeed

A new supply chain for sustainable textiles



Our Team



**Shivesh
Sood**

**Product Manager
Engineer**



**Julius
Stein**

**Programmer
Engineer**



**Lurein
Perera**

**Startup Operator
Engineer**

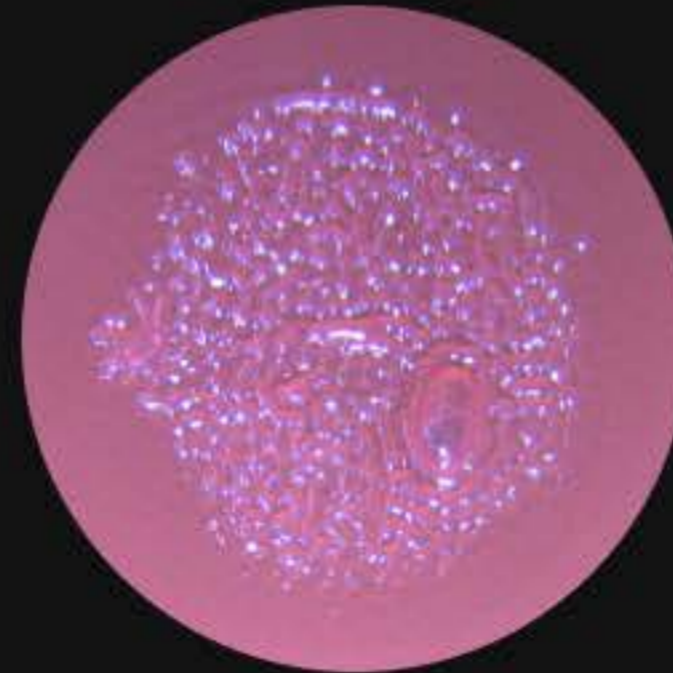
Cellulose = Sustainable Textiles

The next generation of sustainable textiles are made with vats of bacteria and a food source, their feedstock.



FEEDSTOCK AND BACTERIA MIXED

Feedstock is typically comprised of sugarcane and soybean



CELLULOSE COLLECTED

A biofilm of cellulose forms as an output, which is then collected.



PROCESSED INTO TEXTILES

Companies then use cellulose fibers as the base material of a textile

We have to choose between food and fibers.



65%

of global biofeedstock supply
depleted by 2027.

**We set out to separate the
foodstock from the feedstock.**

Nissl Kombucha

E. coli Nissle is added to Kombucha to produce cellulose internally for collection during waste treatment.



↑ WEEKLY DIETARY SUPPLEMENT

In order to sustain a significant colony of E. coli Nissle in the gut, people drink at least one bottle of Kompoocha weekly

↑ CELLULOSE PRODUCED INTERNALLY

Cellulose is produced by the colony, nourished by your digestion, and passes harmlessly through your digestive tract

↑ FIBERS COLLECTED AT WASTE TREATMENT

Waste treatment centers can easily separate the cellulose through mechanical filtration for refinement and use in the production of Rayon, Lyocell, and artificial cotton

**But what can we do to fix the
supply chain today?**





Glycerol Feedstock

Glycerol is a waste product of biodiesel production

**10.28 Billion
Kilograms**

of Biodiesel produced, growing at 10% annually

**913 Million
Kilograms**

of Glycerol produced as a waste product

\$560

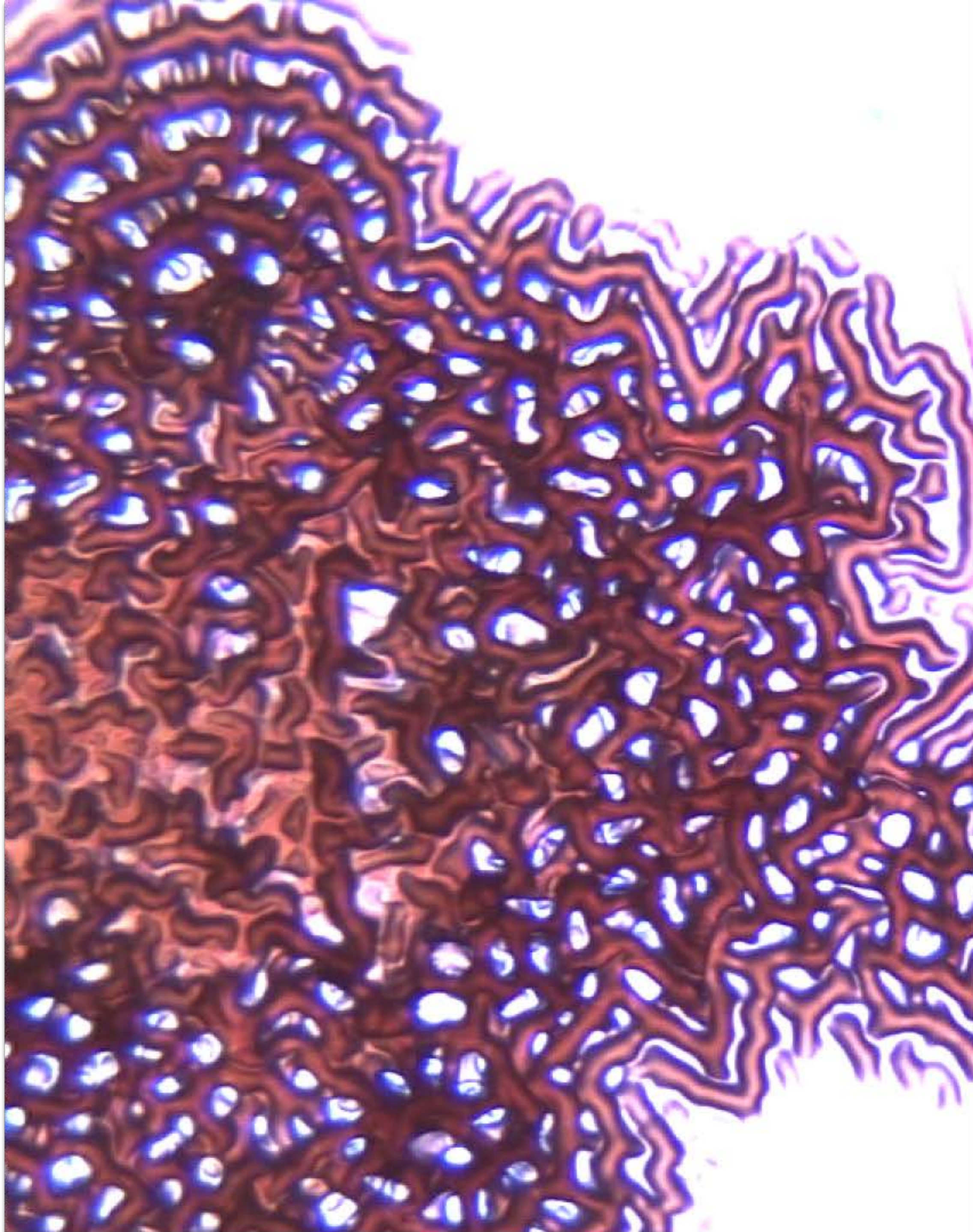
Cost of Glucose
per tonne

\$88

Cost of Glycerol
per tonne

NisslZ





Laboratory Assisted Evolution

Using an LAE approach, we pushed E. Coli Nissle 1917 to evolve an ability to consume Glycerol for colony growth

Left: E. coli Nissle 1917, Single Colony, Congo Red

Process



Daily Steps Checklist

Do OD600 tests on each of the latest incubated generations, using each of their relevant controls as a baseline

Pick the Generation/version that grew in the highest concentration (>1) as the baseline for your next generation (lets call that the selected variant)

First, make a glycerol stock of the selected variant:

Take 500 microliters of glycerol, put in a minitube, cap, then add 500 microliters of the selected variant shake twice and put in the freezer (-80) in the addgene plasmids box, label as ECN GK

Figure out the 3 glycerol concentrations you are going to use for making your next generation from the selected variant, calculate their volumes against 4000 (e.g. 2% concentration is 80 microliters):

Start with 4000 microliters of M9, put into a falcon tube (using the suckergun)

Then subtract the volume of glycerol from your M9 tube (e.g. if using 2%, subtract 80 microliters and dispose) Use the yellow pipette.

Add your glycerol volume to the falcon tube - leave the pipette in the glycerol solution a bit longer than usual for absorption

Mix a bit with pipette pushing

Split this falcon tube into two falcon tubes, each with 2000 microliters of solution. One of these you will incubate with, the other one you will use as a baseline for your OD600 tests, label well.

From your incubation falcon tube, take out 100 microliters of solution

Add 100 microliters of your selected nissle variant

Pipette mix

Add 2 microliters of Streptomycin (StR), which is in the 4C fridge, vial with an orange cap

Incubate

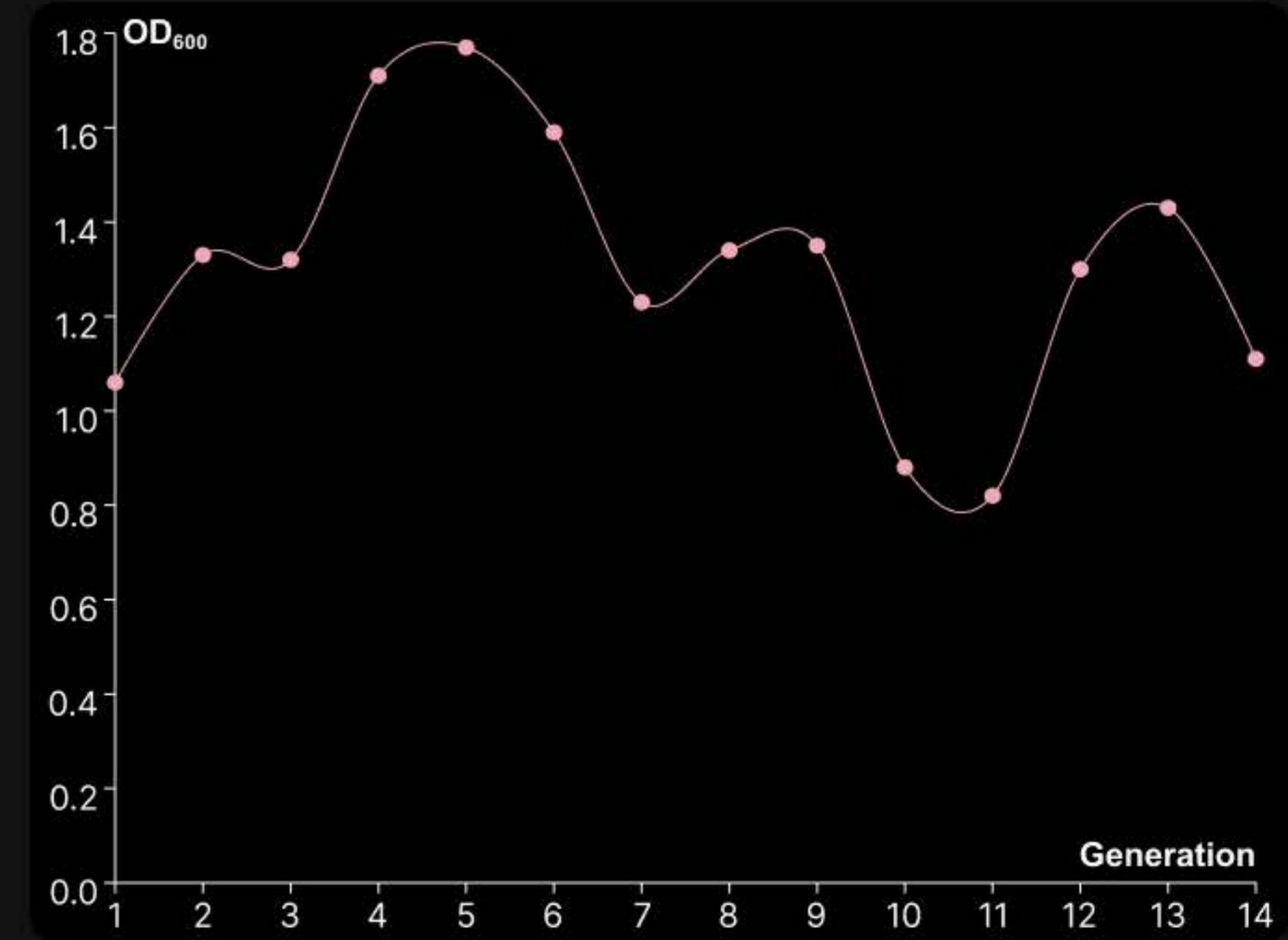
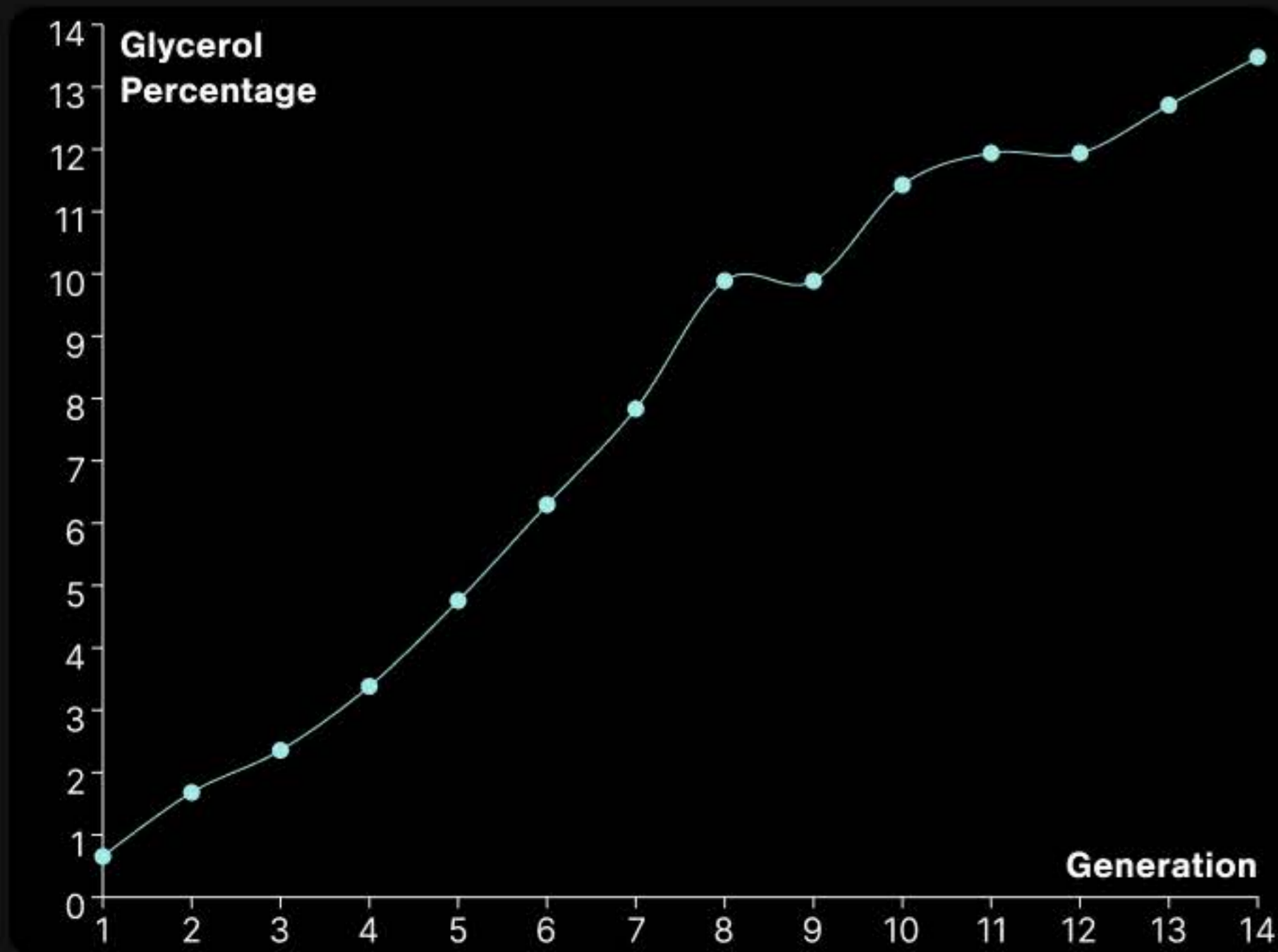
Put the Streptomycin back in the fridge

Make sure to keep your baselines out on our working desk for the next person

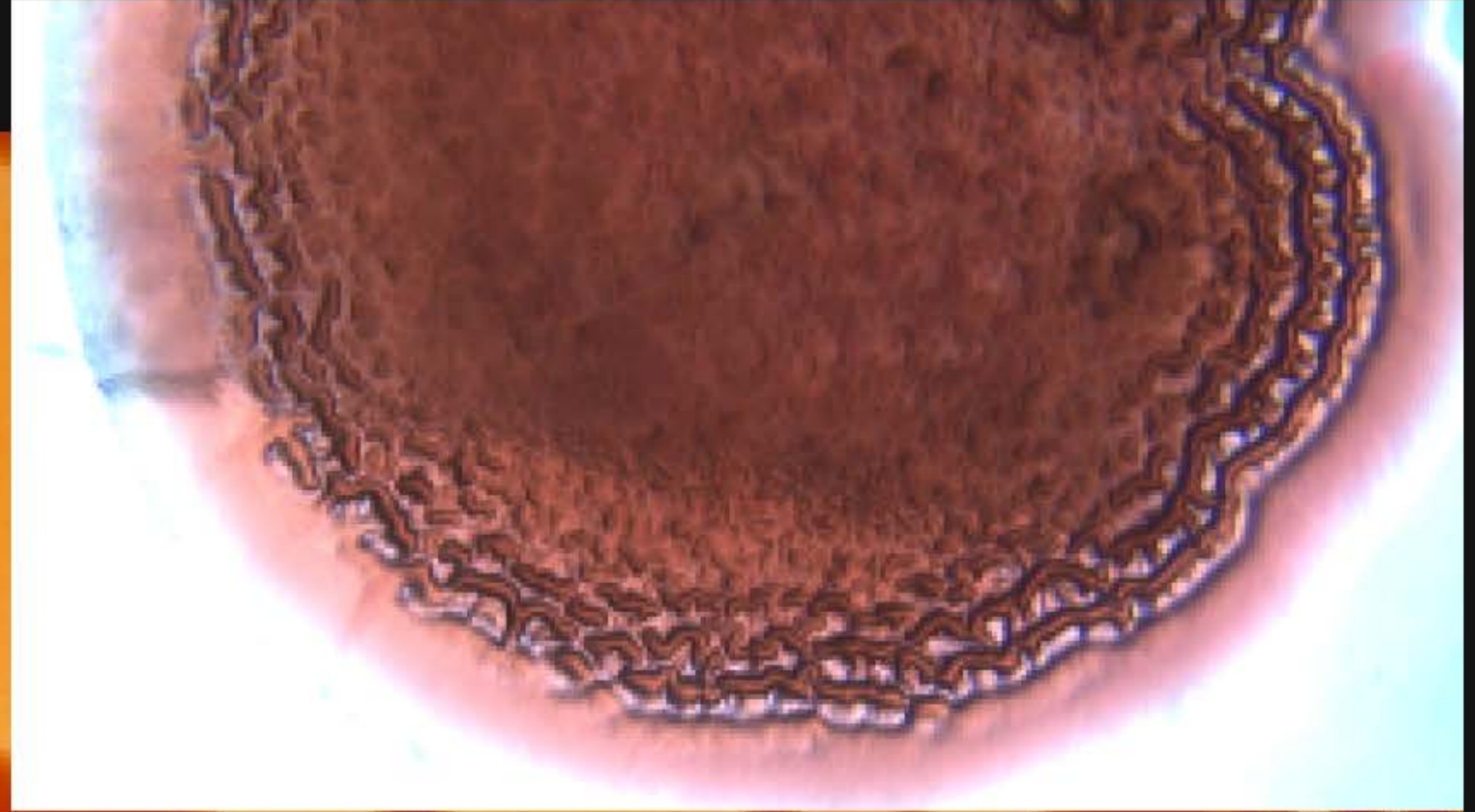


Intermediate Results

With initial cultures failing at Glycerol concentrations 10% and above, we are now growing Nissle at a 15% Glycerol concentration



Cellulose Assay



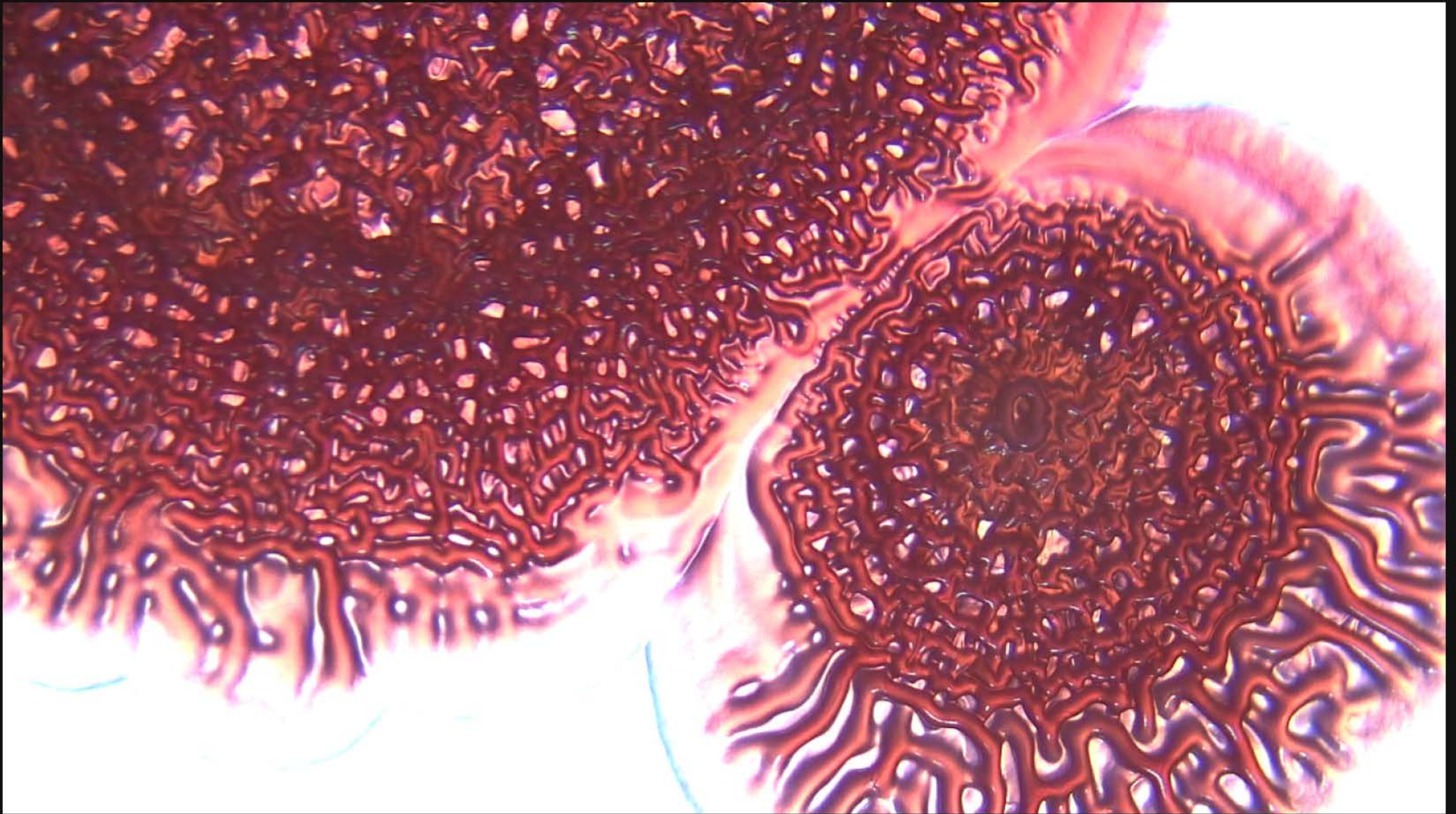
Left: Nissle
1917,
Congo Red

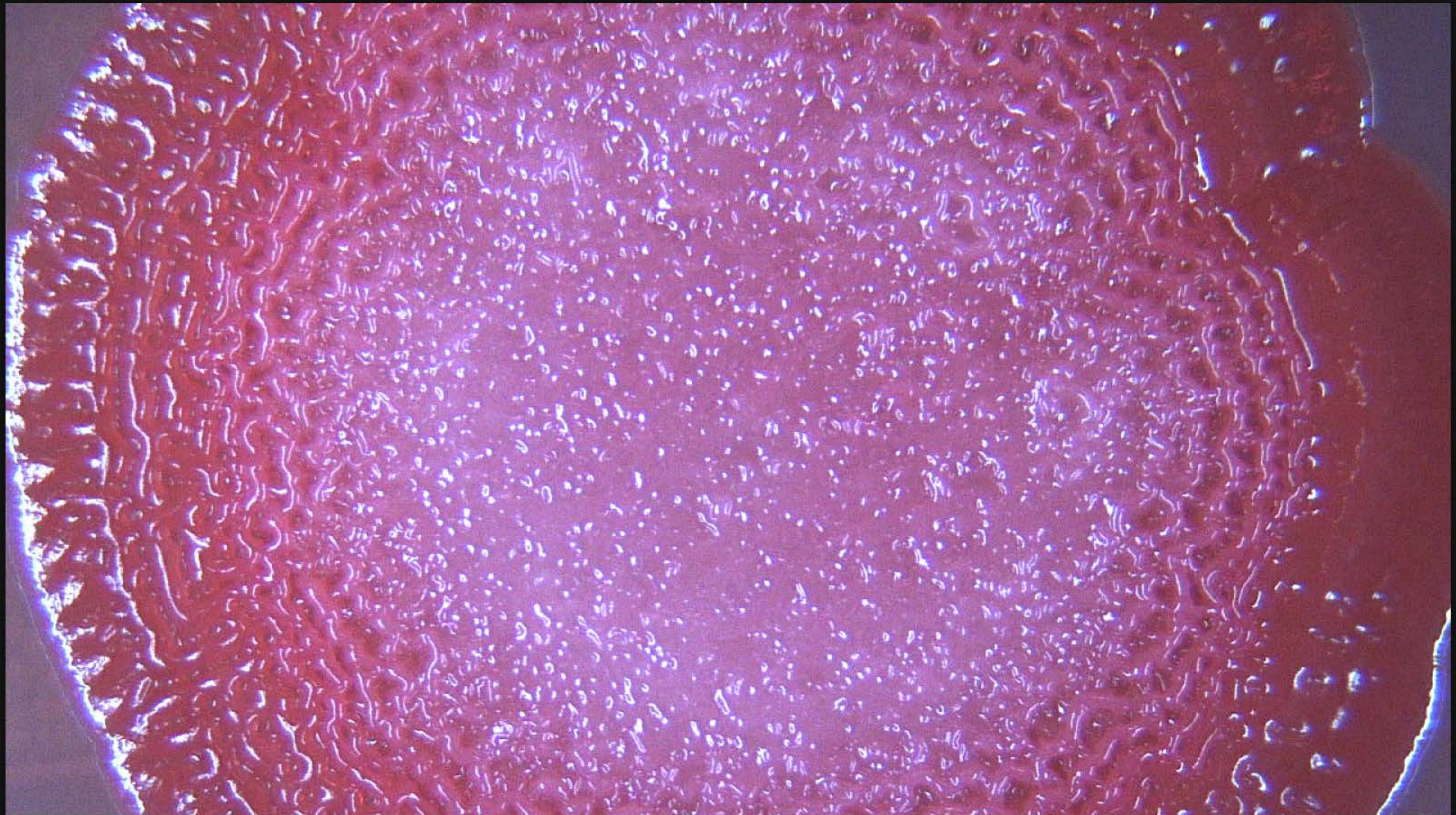
Inset:
Single
Colony,
100x mag

Biofabrication is the future of textiles

but there's a problem.









Thank you.

Special Thanks to Avery Normandin, ALL Team
and Pam Silver's Lab at Harvard Medical School

