Chinmayee Govinda Raj

Real-time Autonomous Instrumentation for Lab-based Microbe Experimental Evolution

1
00:00:00,240 --> 00:00:10,810
[Music]

2
00:00:17,150 --> 00:00:14,180
I'm June my Raj I'm from I just started

3
00:00:19,160 --> 00:00:17,160
my ph.d program at Georgia Tech this

4
00:00:21,350 --> 00:00:19,170
work is my master's thesis work that I

5
00:00:24,740 --> 00:00:21,360
carried out at NASA Ames before I

6
00:00:27,920 --> 00:00:24,750
started at Georgia Tech this is a

7
00:00:31,060 --> 00:00:27,930
real-time autonomous instrumentation for

8
00:00:33,919 --> 00:00:31,070
lab based microbe experimental evolution

9
00:00:37,340 --> 00:00:33,929
so basically extremophiles have somehow

10
00:00:39,619 --> 00:00:37,350
adapted to learn in to learn to live in

11
00:00:41,479 --> 00:00:39,629
uncomfortable environments it might be

12
00:00:43,639 --> 00:00:41,489
extreme temperatures it might be extreme

13
00:00:46,520 --> 00:00:43,649

amounts of salts it might be extreme

14
00:00:49,399 --> 00:00:46,530
acids acidic environments extremely

15
00:00:50,989 --> 00:00:49,409
basic environments and lack of oxygen no

16
00:00:52,639 --> 00:00:50,999
matter what you think they they have

17
00:00:54,829 --> 00:00:52,649
learned to live in those environments

18
00:00:57,200 --> 00:00:54,839
through natural selection natural

19
00:00:58,849 --> 00:00:57,210
selection is a process of adaptation of

20
00:01:01,219 --> 00:00:58,859
an organism to its environment by

21
00:01:04,160 --> 00:01:01,229
selectively passing on the changes in

22
00:01:05,990 --> 00:01:04,170
its genetic Constitution experimental

23
00:01:08,000 --> 00:01:06,000
evolution that we are interested in is

24
00:01:10,220 --> 00:01:08,010
the process of mimicking natural

25
00:01:12,620 --> 00:01:10,230
selection artificially by the process of

26
00:01:14,690 --> 00:01:12,630
exposing microbial community to

27
00:01:17,780 --> 00:01:14,700
intentional stressors to improve the

28
00:01:20,090 --> 00:01:17,790
resistance through artificial mutation

29
00:01:22,790 --> 00:01:20,100
basically experimental evolution is

30
00:01:26,360 --> 00:01:22,800
forced natural selection using lab based

31
00:01:28,310 --> 00:01:26,370
procedures how do you carry out

32
00:01:30,230 --> 00:01:28,320
experimental procedures basically it

33
00:01:32,180 --> 00:01:30,240
just grew up microbes you subject them

34
00:01:34,550 --> 00:01:32,190
to a stressor that you're interested in

35
00:01:36,890 --> 00:01:34,560
remove unfit microbes and you retrain

36
00:01:39,230 --> 00:01:36,900
the process and at some nth iteration

37
00:01:42,410 --> 00:01:39,240
you have a microbial colony that has

38
00:01:44,600 --> 00:01:42,420

adapted to your stress despite having no

39
00:01:49,700 --> 00:01:44,610
information as to how to do it initially

40
00:01:51,530 --> 00:01:49,710
in the initial stages so the proof of

41
00:01:54,890 --> 00:01:51,540
experimental evolution is this data

42
00:01:58,190 --> 00:01:54,900
basically they use we use

43
00:02:00,710 --> 00:01:58,200
ecoli microbes for by exposing them to

44
00:02:02,660 --> 00:02:00,720
40 seconds and 60 seconds of UVC

45
00:02:04,820 --> 00:02:02,670
exposure which is supposed to kill them

46
00:02:06,890 --> 00:02:04,830
but they actually learned to grow

47
00:02:09,680 --> 00:02:06,900
despite the UV stresses and in the

48
00:02:10,669 --> 00:02:09,690
seventh iteration they had almost ran to

49
00:02:12,840 --> 00:02:10,679
the power 6

50
00:02:15,000 --> 00:02:12,850
you know factors of increase

51
00:02:16,860 --> 00:02:15,010
the growth rate so this has proof that

52
00:02:19,380 --> 00:02:16,870
experimental evolution actually works

53
00:02:22,320 --> 00:02:19,390
and this is proof that lab in the lab a

54
00:02:26,970 --> 00:02:22,330
natural environment can be created and

55
00:02:29,040 --> 00:02:26,980
this can be made to work this is this

56
00:02:31,170 --> 00:02:29,050
work is a black box approach I am an

57
00:02:33,450 --> 00:02:31,180
instrumentation engineer and I in learnt

58
00:02:35,790 --> 00:02:33,460
biology for the further part of this

59
00:02:38,070 --> 00:02:35,800
project so basically it's a black box

60
00:02:40,200 --> 00:02:38,080
approach we have a microbe that's

61
00:02:42,630 --> 00:02:40,210
radiation by the way we're exposing

62
00:02:45,300 --> 00:02:42,640
we're exposing them to radiation and

63
00:02:48,090 --> 00:02:45,310

it's learning to grow stronger and

64
00:02:50,640 --> 00:02:48,100
robust despite the radiation we are not

65
00:02:53,400 --> 00:02:50,650
focusing on what is happening to the DNA

66
00:02:54,990 --> 00:02:53,410
when this process is happening and this

67
00:02:56,520 --> 00:02:55,000
is a black box approach we are not

68
00:02:58,740 --> 00:02:56,530
interested in what's happening here we

69
00:03:00,090 --> 00:02:58,750
just care that this is happening and we

70
00:03:02,460 --> 00:03:00,100
just care about producing an

71
00:03:06,410 --> 00:03:02,470
instrumentation technique that can allow

72
00:03:12,000 --> 00:03:09,510
the problem with manual experimental

73
00:03:14,850 --> 00:03:12,010
evolution processes is that it's tedious

74
00:03:17,790 --> 00:03:14,860
it's extremely time-consuming it's

75
00:03:19,920 --> 00:03:17,800
highly prone to human errors it's it has

76
00:03:23,910 --> 00:03:19,930
high reproducibility and repeatability

77
00:03:26,580 --> 00:03:23,920
issues and that affects your moods and

78
00:03:29,300 --> 00:03:26,590
also it kind of hurts that a one-celled

79
00:03:31,380 --> 00:03:29,310
organism dictates a multicellular

80
00:03:34,110 --> 00:03:31,390
magnificent organism such as ourselves

81
00:03:37,170 --> 00:03:34,120
in the lab it's not supposed to dictate

82
00:03:40,800 --> 00:03:37,180
our schedule so what do we do we ought

83
00:03:43,800 --> 00:03:40,810
to be the process so this was the first

84
00:03:47,130 --> 00:03:43,810
generation device that was designed at

85
00:03:49,400 --> 00:03:47,140
NASA Ames it had four sub components it

86
00:03:52,470 --> 00:03:49,410
had sensors environmental controls

87
00:03:54,960 --> 00:03:52,480
fluidic systems and data storage using

88
00:03:56,730 --> 00:03:54,970

Arduino so that's how it looked and yes

89
00:04:00,090 --> 00:03:56,740
I repaired it when I started at NASA

90
00:04:01,380 --> 00:04:00,100
Ames it had temperature sensing in

91
00:04:04,890 --> 00:04:01,390
optical density which was done

92
00:04:06,930 --> 00:04:04,900
automatically eco light likes to grow in

93
00:04:09,930 --> 00:04:06,940
37 degree searches so it had that kind

94
00:04:12,300 --> 00:04:09,940
of sensing UVC system was the stressor

95
00:04:13,860 --> 00:04:12,310
that was used and temperature also could

96
00:04:17,580 --> 00:04:13,870
be used because it was also a sensing

97
00:04:19,560 --> 00:04:17,590
parameter the fluidic system included a

98
00:04:22,770 --> 00:04:19,570
one chamber growth this is the one

99
00:04:25,980 --> 00:04:22,780
growth one chamber growth chamber it had

100
00:04:27,990 --> 00:04:25,990
parasitic pumps for in letting fluids

101
00:04:30,689 --> 00:04:28,000
letting the nutrients continuously and

102
00:04:32,490 --> 00:04:30,699
it had education because e coli tends to

103
00:04:33,839 --> 00:04:32,500
stick to the walls and that messes with

104
00:04:36,749 --> 00:04:33,849
the optical density and we don't like

105
00:04:40,140 --> 00:04:36,759
that so agitated magnetically so the

106
00:04:42,809 --> 00:04:40,150
cells are in in the limbo state and in

107
00:04:44,850 --> 00:04:42,819
between media and also Arduino control

108
00:04:48,270 --> 00:04:44,860
so everything was automated and nothing

109
00:04:52,920 --> 00:04:48,280
I included minimal human interaction

110
00:04:55,260 --> 00:04:52,930
actually that had its own problems it

111
00:04:59,040 --> 00:04:55,270
had only sent to two types of sensors

112
00:05:01,980 --> 00:04:59,050
and it only allowed one stressor to be

113
00:05:04,890 --> 00:05:01,990

applied and also one grow chamber was

114
00:05:07,140 --> 00:05:04,900
not really that fancy because if you do

115
00:05:09,749 --> 00:05:07,150
multiple chambers you can do multi

116
00:05:11,700 --> 00:05:09,759
parameter growth and you can compare

117
00:05:15,059 --> 00:05:11,710
much populations and that that was not

118
00:05:17,909 --> 00:05:15,069
allowed in this so that's why my work

119
00:05:21,270 --> 00:05:17,919
was to enable parallel growth cultures

120
00:05:22,950 --> 00:05:21,280
so have multiple chambers and there was

121
00:05:24,960 --> 00:05:22,960
absolutely new biochemical information

122
00:05:27,059 --> 00:05:24,970
as to what is happening inside the

123
00:05:29,550 --> 00:05:27,069
chamber during the evolution process and

124
00:05:32,520 --> 00:05:29,560
that's why I added a set of wet

125
00:05:34,469 --> 00:05:32,530
chemistry sensors and also enable

126
00:05:36,749 --> 00:05:34,479
dynamic application of stressors in

127
00:05:39,209 --> 00:05:36,759
response to sensor parameters and all

128
00:05:40,740 --> 00:05:39,219
these sensors were added along with cell

129
00:05:43,589 --> 00:05:40,750
density which was already previously

130
00:05:46,680 --> 00:05:43,599
done I added dissolved oxygen electrical

131
00:05:50,490 --> 00:05:46,690
conductivities pH and ORP sensors to the

132
00:05:53,129 --> 00:05:50,500
system adding sensors is not that easy

133
00:05:57,059 --> 00:05:53,139
because you need to select the self

134
00:05:58,559 --> 00:05:57,069
sensors and also have a set of my the

135
00:06:00,480 --> 00:05:58,569
fluidics card needs to be completely

136
00:06:01,110 --> 00:06:00,490
changed so that it can accommodate the

137
00:06:04,020 --> 00:06:01,120
sensors

138
00:06:06,300 --> 00:06:04,030

so this fluidics card like you see for

139
00:06:08,760 --> 00:06:06,310
chambers for growth chambers the

140
00:06:10,379 --> 00:06:08,770
circular one is a growth chamber and the

141
00:06:12,029 --> 00:06:10,389
square ones and there are the sensor

142
00:06:14,640 --> 00:06:12,039
chamber slots where a sensors can be

143
00:06:16,920 --> 00:06:14,650
sensor probes could be inserted and it's

144
00:06:19,260 --> 00:06:16,930
a six layer design that was integrated

145
00:06:23,430 --> 00:06:19,270
and it looked like this once it was put

146
00:06:25,339 --> 00:06:23,440
together and this these are the sensor

147
00:06:27,659 --> 00:06:25,349
circuits we bought off the shelf

148
00:06:30,180 --> 00:06:27,669
these are basically called a class

149
00:06:32,430 --> 00:06:30,190
sensor stamps they're really stamp size

150
00:06:35,459 --> 00:06:32,440
they're really that small and its really

151
00:06:37,290 --> 00:06:35,469
cute that's why they have a slide in my

152
00:06:38,600 --> 00:06:37,300
presentation and this is how it looks on

153
00:06:40,459 --> 00:06:38,610
the breadboard design

154
00:06:41,809 --> 00:06:40,469
it's very compact it's very efficient

155
00:06:45,469 --> 00:06:41,819
and I'll show you the data you'll be

156
00:06:48,140 --> 00:06:45,479
impressed in the process of adding

157
00:06:49,850 --> 00:06:48,150
sensors we found pretty good set micro

158
00:06:52,520 --> 00:06:49,860
sensors but the electrical conductivity

159
00:06:55,490 --> 00:06:52,530
sensor that we looked at was not really

160
00:06:57,379 --> 00:06:55,500
compact enough so I designed a sensor

161
00:06:59,719 --> 00:06:57,389
electrocuted electrical conductivity

162
00:07:02,029 --> 00:06:59,729
sensor called the meek on microbio

163
00:07:05,839 --> 00:07:02,039

electrical conductivity probe it's

164
00:07:08,510 --> 00:07:05,849
basically this is a 1 ml syringe to be

165
00:07:11,029 --> 00:07:08,520
honest and this is a point eight

166
00:07:13,249 --> 00:07:11,039
millimeter nichrome wire so these

167
00:07:15,679 --> 00:07:13,259
together make the electrical

168
00:07:18,080 --> 00:07:15,689
conductivity probes and these were much

169
00:07:20,360 --> 00:07:18,090
less invasive as to what we could buy

170
00:07:23,929 --> 00:07:20,370
off-the-shelf and this is a circuit that

171
00:07:26,540 --> 00:07:23,939
controls it and this is the data that

172
00:07:29,629 --> 00:07:26,550
shows that the commercial probe EC probe

173
00:07:31,820 --> 00:07:29,639
was the me con probe was functioning

174
00:07:34,159 --> 00:07:31,830
exactly as well as the commercial probe

175
00:07:39,550 --> 00:07:34,169
in the in the range that we were

176
00:07:42,230 --> 00:07:39,560
interested in that's impressive data FYI

177
00:07:45,379 --> 00:07:42,240
so in the second generation device all

178
00:07:48,469 --> 00:07:45,389
these parts in yellow added added more

179
00:07:51,079 --> 00:07:48,479
color to it so for the sensing instead

180
00:07:53,300 --> 00:07:51,089
of two parameters it added four more so

181
00:07:55,999 --> 00:07:53,310
the total of six sensing parameters was

182
00:07:57,740 --> 00:07:56,009
enabled and if you have a we'd a sense

183
00:08:00,140 --> 00:07:57,750
it you have a way to environmentally

184
00:08:03,740 --> 00:08:00,150
control it so environmental control

185
00:08:04,760 --> 00:08:03,750
system increased from two to seven two

186
00:08:08,029 --> 00:08:04,770
to six sorry

187
00:08:10,249 --> 00:08:08,039
two to six now and fluid mechanics was

188
00:08:11,480 --> 00:08:10,259

like inner chamber flow was allowed pump

189
00:08:13,100 --> 00:08:11,490
was still there

190
00:08:14,959 --> 00:08:13,110
there was an exposure chamber and a

191
00:08:18,379 --> 00:08:14,969
sensor chamber added to accommodate the

192
00:08:21,070 --> 00:08:18,389
sensors I like Raspberry Pi along with

193
00:08:23,839 --> 00:08:21,080
Arduino Arduino space a microprocessor

194
00:08:25,100 --> 00:08:23,849
raspberry PI's an entire computer it can

195
00:08:27,829 --> 00:08:25,110
control the entire system without

196
00:08:29,540 --> 00:08:27,839
failing really badly and that

197
00:08:32,060 --> 00:08:29,550
constituted the second generation device

198
00:08:35,180 --> 00:08:32,070
that I'd have loved and this is

199
00:08:39,550 --> 00:08:35,190
important data because two reasons one

200
00:08:43,519 --> 00:08:39,560
it showed that I was awake for 24 hours

201
00:08:45,319 --> 00:08:43,529
and also this was the data that proved

202
00:08:47,340 --> 00:08:45,329
to my adviser that I'm capable of

203
00:08:49,410 --> 00:08:47,350
graduating

204
00:08:52,020 --> 00:08:49,420
so the optical density data was

205
00:08:53,760 --> 00:08:52,030
collected over 24 hours every R I had to

206
00:08:56,280 --> 00:08:53,770
collect samples and put it through an

207
00:08:59,430 --> 00:08:56,290
optical density sensor and record what

208
00:09:02,040 --> 00:08:59,440
was the optical density dissolved oxygen

209
00:09:03,750 --> 00:09:02,050
is the most interesting part here so the

210
00:09:05,970 --> 00:09:03,760
dissolved oxygen started at some point

211
00:09:09,060 --> 00:09:05,980
and then it went to zero at

212
00:09:10,560 --> 00:09:09,070
approximately six hours so after six

213
00:09:13,740 --> 00:09:10,570

hours everything that happened was

214
00:09:16,470 --> 00:09:13,750
anaerobic niccola is not supposed to

215
00:09:19,080 --> 00:09:16,480
grow in an anaerobic environment but it

216
00:09:21,870 --> 00:09:19,090
did grant it it did not have a really

217
00:09:25,080 --> 00:09:21,880
good growth rate as it had previously

218
00:09:26,880 --> 00:09:25,090
when it was Arabic but it did have an

219
00:09:28,950 --> 00:09:26,890
increase in the growth rate even after

220
00:09:31,770 --> 00:09:28,960
six hours when oxygen was completely

221
00:09:33,990 --> 00:09:31,780
depleted of it well also what's

222
00:09:36,690 --> 00:09:34,000
interesting is that add that switch

223
00:09:40,140 --> 00:09:36,700
between aerobic and anaerobic the pH

224
00:09:42,750 --> 00:09:40,150
slope changed drastically and there is

225
00:09:44,370 --> 00:09:42,760
one more slope change that I do not know

226
00:09:45,020 --> 00:09:44,380
there is a biochemist chair please help

227
00:09:47,760 --> 00:09:45,030
me

228
00:09:50,430 --> 00:09:47,770
there is also electrical contacts would

229
00:09:52,110 --> 00:09:50,440
be change at six RS there was a dip in

230
00:09:54,720 --> 00:09:52,120
the small dip in the electrical

231
00:09:58,380 --> 00:09:54,730
conductivity that that gray line that

232
00:10:00,450 --> 00:09:58,390
shows that there was a short-term

233
00:10:02,910 --> 00:10:00,460
stagnation in the exchange of ions

234
00:10:05,220 --> 00:10:02,920
between the media and the cells but

235
00:10:07,320 --> 00:10:05,230
again it regained that it's somehow

236
00:10:09,600 --> 00:10:07,330
learned to do that and despite having no

237
00:10:11,520 --> 00:10:09,610
oxygen and then there was again an

238
00:10:13,410 --> 00:10:11,530

increase in the electrical conductivity

239
00:10:18,870 --> 00:10:13,420
that means the ions were still produced

240
00:10:21,510 --> 00:10:18,880
and excreted out of the system so that

241
00:10:23,700 --> 00:10:21,520
means this system can be used to

242
00:10:26,820 --> 00:10:23,710
eliminate the black box that I had spoke

243
00:10:29,670 --> 00:10:26,830
about initially so basically you have a

244
00:10:32,570 --> 00:10:29,680
system that can enable application of

245
00:10:34,890 --> 00:10:32,580
radiation stressor temperature stressor

246
00:10:35,730 --> 00:10:34,900
increase amount of salts or decrease

247
00:10:38,190 --> 00:10:35,740
amount of sauce

248
00:10:40,290 --> 00:10:38,200
I said you can control the pH and you

249
00:10:42,300 --> 00:10:40,300
can control the amount of oxygen and all

250
00:10:45,270 --> 00:10:42,310
these stressors can be applied at the

251
00:10:48,360 --> 00:10:45,280
same time in combination or individually

252
00:10:50,640 --> 00:10:48,370
and you will have a microbial population

253
00:10:53,010 --> 00:10:50,650
that has learned to grow despite these

254
00:10:54,630 --> 00:10:53,020
stressors that means the intermediate

255
00:10:56,790 --> 00:10:54,640
black box the changes in the protein

256
00:10:58,319 --> 00:10:56,800
structure the metabolic pathways it took

257
00:10:59,939 --> 00:10:58,329
to and after that change

258
00:11:03,210 --> 00:10:59,949
and the genetic sequences that were

259
00:11:05,280 --> 00:11:03,220
adapted can be studied with devices like

260
00:11:08,699 --> 00:11:05,290
this because it allows intermediate

261
00:11:10,739 --> 00:11:08,709
changes between population that has no

262
00:11:12,989 --> 00:11:10,749
resistance to a population that has

263
00:11:16,519 --> 00:11:12,999

excellent resistance to all the stresses

264
00:11:20,039 --> 00:11:16,529
that you apply in stay in small stages

265
00:11:23,369 --> 00:11:20,049
also this kind of study can be used to

266
00:11:25,530 --> 00:11:23,379
help and enhance the ecosystem for

267
00:11:28,590 --> 00:11:25,540
cycling food water air and waste

268
00:11:31,319 --> 00:11:28,600
so basically no organism can survive on

269
00:11:33,900 --> 00:11:31,329
its own period it needs other organisms

270
00:11:36,269 --> 00:11:33,910
to support it so that that includes

271
00:11:38,460 --> 00:11:36,279
humans so if we want to have extended

272
00:11:40,379 --> 00:11:38,470
presence in space if we want to travel

273
00:11:43,590 --> 00:11:40,389
really long distances and through space

274
00:11:46,079 --> 00:11:43,600
and time then we need to have a set of

275
00:11:49,379 --> 00:11:46,089
microbes that will support us by

276
00:11:53,400 --> 00:11:49,389
providing a cycling procedure or water

277
00:11:59,309 --> 00:11:53,410
air food water and waste so that kind of

278
00:12:01,739 --> 00:11:59,319
study will envel envelopes apply

279
00:12:03,900 --> 00:12:01,749
microgravity and high radiation levels

280
00:12:06,809 --> 00:12:03,910
and then those microbes can be actually

281
00:12:10,549 --> 00:12:06,819
sent with the astronauts to survive in

282
00:12:14,009 --> 00:12:10,559
space flight mission control environment

283
00:12:16,350 --> 00:12:14,019
for future work this device is intended

284
00:12:18,809 --> 00:12:16,360
to carry out the Iggy regimes that I

285
00:12:22,340 --> 00:12:18,819
just mentioned with individual or

286
00:12:25,019 --> 00:12:22,350
combinational stressors six stressors or

287
00:12:27,090 --> 00:12:25,029
employable stressors like with those

288
00:12:30,559 --> 00:12:27,100

sensors can be like free ion

289
00:12:33,359 --> 00:12:30,569
concentration osmotic ion stress acidity

290
00:12:35,579 --> 00:12:33,369
metal ion presence very nutrient

291
00:12:39,179 --> 00:12:35,589
availability like oxygen thermal

292
00:12:41,579 --> 00:12:39,189
stressors dissolved oxygen again as more

293
00:12:43,919 --> 00:12:41,589
stressors that can be added that means

294
00:12:46,109 --> 00:12:43,929
more sensors there is no such thing as

295
00:12:48,329 --> 00:12:46,119
too many sensors in the system so

296
00:12:50,579 --> 00:12:48,339
reactive oxygen species can be included

297
00:12:53,460 --> 00:12:50,589
and very usually availability can be

298
00:12:55,530 --> 00:12:53,470
added to the system also this was a

299
00:12:58,319 --> 00:12:55,540
proof-of-concept so we did it for one

300
00:13:00,509 --> 00:12:58,329
chamber system so a multi chamber system

301
00:13:02,879 --> 00:13:00,519
for intercultural comparisons and much

302
00:13:04,859 --> 00:13:02,889
population studies can be expanded so

303
00:13:07,739 --> 00:13:04,869
the single chamber can be expanded to

304
00:13:10,560 --> 00:13:07,749
four or six as per you like it and have

305
00:13:12,210 --> 00:13:10,570
those studies carried out

306
00:13:14,040 --> 00:13:12,220
extended sister for growth and testing

307
00:13:17,520 --> 00:13:14,050
of other bacterial species so this was

308
00:13:19,920 --> 00:13:17,530
done for e.coli so any any system any

309
00:13:23,010 --> 00:13:19,930
ill organism that doesn't require like

310
00:13:25,320 --> 00:13:23,020
beyond 200 to 300 degrees temperatures

311
00:13:27,630 --> 00:13:25,330
can be used that will not melt the

312
00:13:29,340 --> 00:13:27,640
fluidic system and you can still use

313
00:13:42,090 --> 00:13:29,350

other bacterial species to carry out

314
00:13:44,400 --> 00:13:42,100
this processes questions okay I really

315
00:13:48,390 --> 00:13:44,410
love the potential for this hardware but

316
00:13:51,630 --> 00:13:48,400
I have a ton of questions which I'll

317
00:13:55,020 --> 00:13:51,640
probably so how do you guys deal with

318
00:13:59,460 --> 00:13:55,030
sterility it's like how does the

319
00:14:03,150 --> 00:13:59,470
hardware and all sterilization so this

320
00:14:05,250 --> 00:14:03,160
was this was a microfluidic system so

321
00:14:08,310 --> 00:14:05,260
basically what we did is we assembled it

322
00:14:10,080 --> 00:14:08,320
in the presence of a flame in a Bunsen

323
00:14:12,270 --> 00:14:10,090
burner flame and before that it was

324
00:14:14,850 --> 00:14:12,280
autoclaved this system can be autoclaved

325
00:14:17,130 --> 00:14:14,860
in my presence itself i autoclave this

326
00:14:22,050 --> 00:14:17,140
at least seven to eight times and it

327
00:14:24,270 --> 00:14:22,060
still remain intact actually and so with

328
00:14:26,730 --> 00:14:24,280
the with the experimental evolution

329
00:14:31,650 --> 00:14:26,740
portion of it if you wanted to do like

330
00:14:35,030 --> 00:14:31,660
very long extended you know thousand

331
00:14:37,190 --> 00:14:35,040
generation plus experiments

332
00:14:40,040 --> 00:14:37,200
the fluidics pumps I'm assuming or you

333
00:14:42,920 --> 00:14:40,050
know moving media out and then putting

334
00:14:45,310 --> 00:14:42,930
it back okay so it's not like a chemo

335
00:14:48,310 --> 00:14:45,320
static system No

336
00:14:51,620 --> 00:14:48,320
well we avoided that kind of a system

337
00:14:54,020 --> 00:14:51,630
because of the limitations in the

338
00:14:56,750 --> 00:14:54,030

fluidic paths that it allowed so this

339
00:14:59,630 --> 00:14:56,760
system was basically you can have a

340
00:15:01,430 --> 00:14:59,640
secondary chamber which will store the

341
00:15:03,890 --> 00:15:01,440
first generation of a first generation

342
00:15:06,950 --> 00:15:03,900
culture and then it can be made to

343
00:15:08,690 --> 00:15:06,960
reflow into the fluid it's into the

344
00:15:10,820 --> 00:15:08,700
floating system and it has the ports for

345
00:15:31,250 --> 00:15:10,830
it okay very cool I'll have to talk to

346
00:15:33,230 --> 00:15:31,260
you if you have a larger system the way

347
00:15:38,870 --> 00:15:33,240
I would see it as like the sterilization

348
00:15:41,450 --> 00:15:38,880
will be harder and also if the system is

349
00:15:44,180 --> 00:15:41,460
smaller you can see the growth in a very

350
00:15:46,490 --> 00:15:44,190
short amount of time and if the system

351
00:15:48,770 --> 00:15:46,500
is really large it would actually

352
00:15:50,840 --> 00:15:48,780
simplify the application of sensors but

353
00:15:53,780 --> 00:15:50,850
that also means that you need to have a

354
00:15:57,910 --> 00:15:53,790
really robust agitation system to keep

355
00:16:00,560 --> 00:15:57,920
the media homogeneous throughout the

356
00:16:02,810 --> 00:16:00,570
chamber which is why we switch to

357
00:16:04,820 --> 00:16:02,820
smaller and smaller chambers so that

358
00:16:15,470 --> 00:16:04,830
there's not much of magnetic magnetic

359
00:16:23,220 --> 00:16:21,210
hey um I wonder if you think you can use

360
00:16:25,770 --> 00:16:23,230
this system to study Khan soldier of

361
00:16:28,550 --> 00:16:25,780
different species living together either

362
00:16:31,500 --> 00:16:28,560
by differentiating them with different

363
00:16:33,630 --> 00:16:31,510

chromophoric probes for example while

364
00:16:36,510 --> 00:16:33,640
they're growing or any way on any other

365
00:16:40,020 --> 00:16:36,520
means which necessitates some another

366
00:16:43,770 --> 00:16:40,030
form of detection like microscopy or

367
00:16:45,900 --> 00:16:43,780
different wavelengths so you want to add

368
00:16:48,750 --> 00:16:45,910
some kind of an imaging system into this

369
00:16:50,070 --> 00:16:48,760
is that what you asked yeah because you

370
00:16:51,960 --> 00:16:50,080
need if you want to grow different

371
00:16:55,550 --> 00:16:51,970
species together you need to track all

372
00:16:58,530 --> 00:16:55,560
these parameters for every other species

373
00:17:01,890 --> 00:16:58,540
so I was wondering if you think this

374
00:17:03,720 --> 00:17:01,900
could be done in a very efficient way so

375
00:17:06,570 --> 00:17:03,730
the center of parameters data that I

376
00:17:08,520 --> 00:17:06,580
showed was actually real-time so during

377
00:17:11,330 --> 00:17:08,530
the growth of e.coli those parameters

378
00:17:14,730 --> 00:17:11,340
were recorded so if you want individual

379
00:17:17,070 --> 00:17:14,740
parameters over a duration of time for

380
00:17:20,430 --> 00:17:17,080
multiple species there has to be some

381
00:17:23,280 --> 00:17:20,440
kind of chemical barrier between those

382
00:17:28,560 --> 00:17:23,290
systems so you can actually isolate the

383
00:17:30,420 --> 00:17:28,570
sensor parameters but it's not living

384
00:17:33,270 --> 00:17:30,430
together in a solution it's like you

385
00:17:34,830 --> 00:17:33,280
need to separate them and if you put

386
00:17:37,470 --> 00:17:34,840
them together in a solution you will get

387
00:17:39,480 --> 00:17:37,480
the resultant yeah media okay count

388
00:17:50,520 --> 00:17:39,490

chemistry and the other individual