Cellular Biophysics
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Review: Paperfuge for hematology

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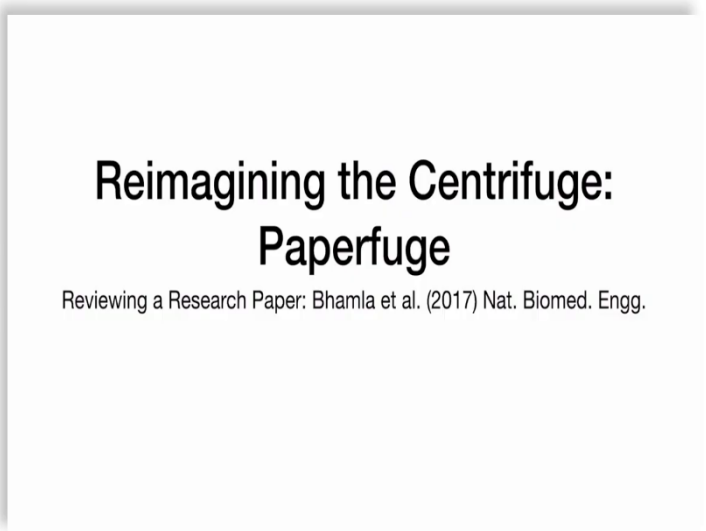

So, we have spoken about centrifugation so far. In terms of centrifuges that we can find in a research lab, but what if we know the principles of centrifugation? And we want to think, how can we make one from scratch? And can we make one that is then capable of being cheap and yet doing our job. Now, sometimes this is a contradiction. If you make it very cheap, then it does not have the features that the complex one has. And this is the theme that Assad Bhamla and company attempted to address in the paper in nature biomedical engineering.

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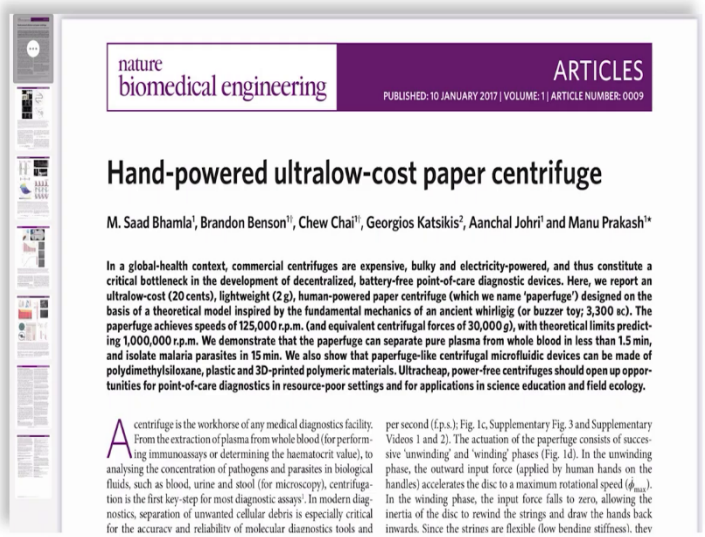

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Hand-powered ultralow-cost paper centrifuge

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In a global-health context, commercial centrifuges are expensive, bulky and electricity-powered, and thus constitute a critical bottleneck in the development of decentralized, battery-free point-of-care diagnostic devices. Here, we report an ultralow-cost (20 cents), lightweight (2 g), human-powered paper centrifuge (which we name 'paperfuge') designed on the basis of a theoretical model inspired by the fundamental mechanics of an ancient whirligig (or buzzer toy; 3,300 BC). The paperfuge achieves speeds of 125,000 r.p.m. (and equivalent centrifugal forces of 30,000 g), with theoretical limits predicting 1,000,000 r.p.m. We demonstrate that the paperfuge can separate pure plasma from whole blood in less than 1.5 min, and isolate malaria parasites in 15 min. We also show that paperfuge-like centrifugal microfluidic devices can be made of polydimethylsiloxane, plastic and 3D-printed polymeric materials. Ultracheap, power-free centrifuges should open up opportunities for point-of-care diagnostics in resource-poor settings and for applications in science education and field ecology.

A centrifuge is the workhorse of any medical diagnostics facility. From the extraction of plasma from whole blood (for performing immunoassays or determining the haematocrit value), to analysing the concentration of pathogens and parasites in biological fluids, such as blood, urine and stool (for microscopy), centrifugation is the first key-step for most diagnostic assays[1]. In modern diagnostics, separation of unwanted cellular debris is especially critical for the accuracy and reliability of molecular diagnostics tools and

per second (f.p.s.); Fig. 1c, Supplementary Fig. 3 and Supplementary Videos 1 and 2). The actuation of the paperfuge consists of successive 'unwinding' and 'winding' phases (Fig. 1d). In the unwinding phase, the outward input force (applied by human hands on the handles) accelerates the disc to a maximum rotational speed (ϕ_{max}). In the winding phase, the input force falls to zero, allowing the inertia of the disc to rewind the strings and draw the hands back inwards. Since the strings are flexible (low bending stiffness), they

This paper is titled Hand-powered ultralow-cost paper centrifuge and we are going to review it and want to discuss it in the context of the limitations and advantages of such an approach. They claim that their paperfuge or paper powered, hand powered paper centrifuge to the key part of the rotating rotor is made of paper can achieve 1.25 lakh rpm, approximately 30,000 g and upper limit of 1 million or 10 lakh rpm is maximally achievable according to the, in fact managed to separate plasma in 1.5 minutes, let us see the evidence.

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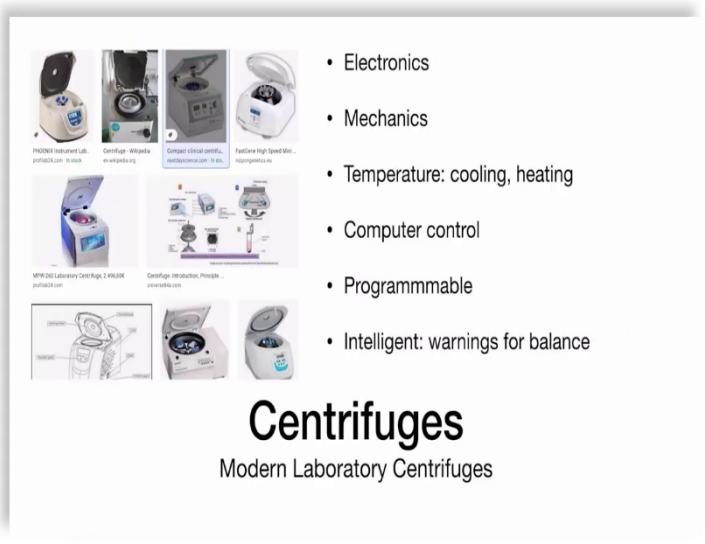

So, conventional laboratory centrifuges have complex electronics, they have an electric motor, usually that drives the mechanics rotors, sprinter, ball bearings, lots of engineering goes into it. They are also at times capable of having thermal components with either cooling

or heating or both, because sometimes your sample is thermo labile or thermo stable only at a certain specific temperature. And being able to control this is important, which is why a good research lab has a good centrifuge at least. Modern centrifuges also computer controlled with programmable interfaces, they may even be intelligent, they will give you a warning, alert you when things go wrong. Like for example, tubes are balanced and so on so forth.

But all these things are not always available. Many of you may be in colleges that are watching this and maybe that these kinds of centrifuges are not available in your college. And it is a bit of a pity, but that is how it is.

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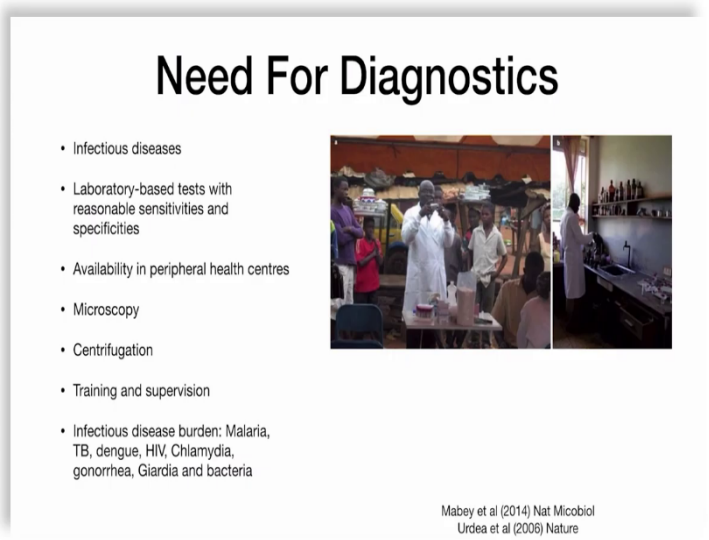

But when we go to medical diagnostics, it turns out that for infectious diseases, to run laboratory-based tests of reasonable sensitivity and specificity, we need microscopy and centrifugation. Now, not all these devices are available in peripheral health centers in remote areas.

Even in India, you can see that there is a big difference between urban and rural and even within urban areas, there will be parts of the city which do not have very good facilities for health and parts that too. So, to tackle this difference, we need both equipment and training, as well as supervision.

Because the disease burden of malaria, TB, dengue, HIV, chlamydia, gonorrhea, giardia and bacterial infections is so high in countries like ours, that something needs to be done. And this is reviewed by Mabey et al Nature Microbiology and Urdea et al at Nature. The paradox is that these devices are commercial, you can buy them, but that means the primary health

care center needs money to buy them, which means they need a budget, which means they need somebody to pay for it. Now, not everyone has the money. Not every country has the budget for it. Not every country has the plan to put diagnostic centers everywhere. This is a tragedy for humanity.

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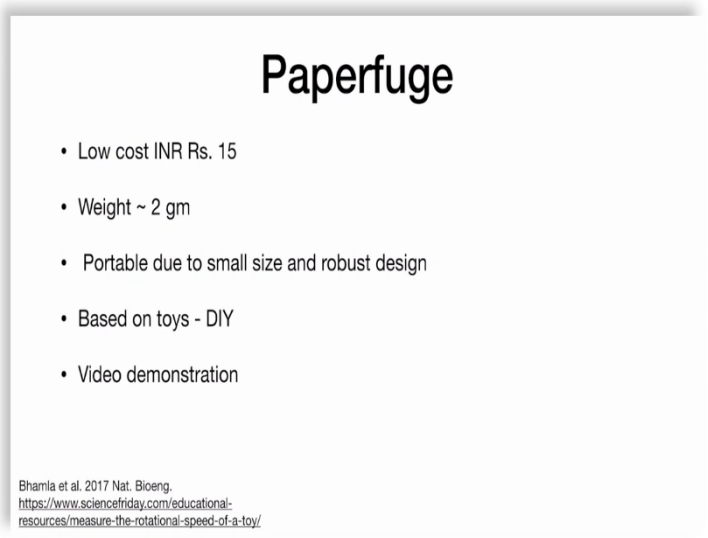

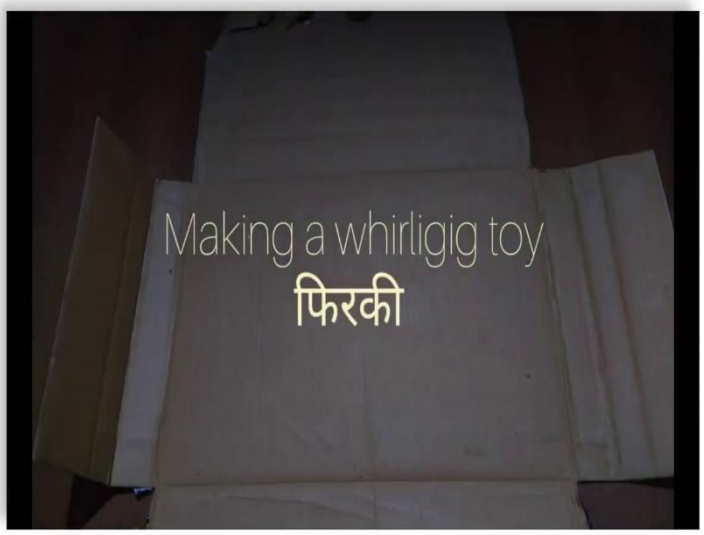


Materials
1. Cardboard
2. Scissors
3. Twine/thread 1-2ft
4. Pen and scale
5. Circular template

And scientists like Bhamla et al, they decided to make a low cost centrifuge costing only rupees 15 in terms of parts, weighing only 2 grams and made in such a way that it is portable and robust. They in fact used it as a platform based on a toy, but do it yourself. So, the video demonstration I am going to show you is about exactly this toy. I took a cardboard piece. So, I tried to mimic what we did earlier, took scissors, thread, pen and something circular, and marked a circular pattern on the cardboard piece cut it.

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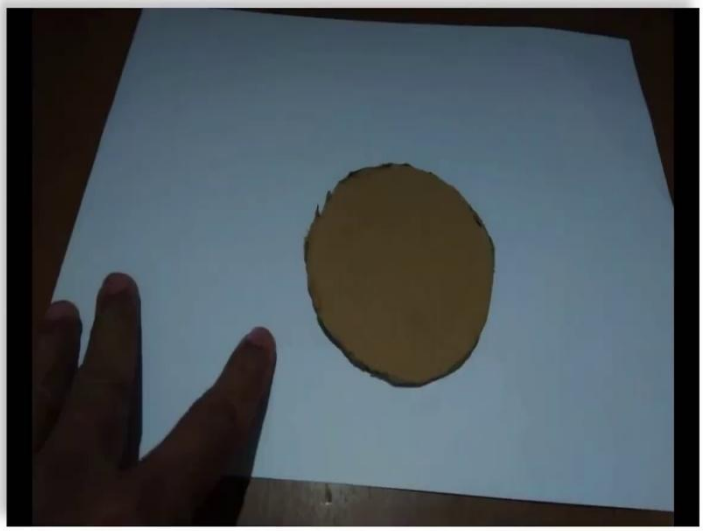

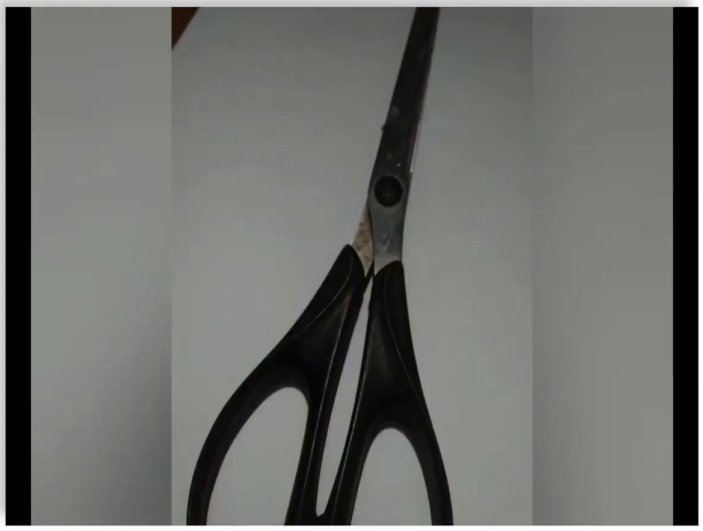

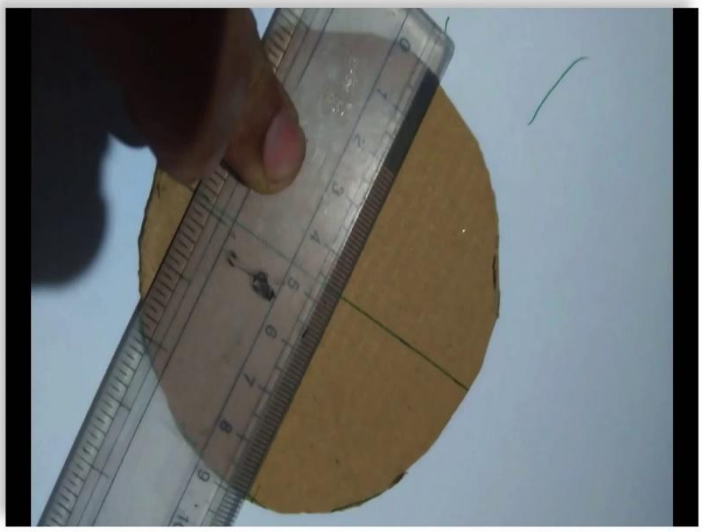

Smoothly to the edges of the cut with a scissor and then, with a scale or a ruler, I made lines along the diameter, so as to find the approximate center.

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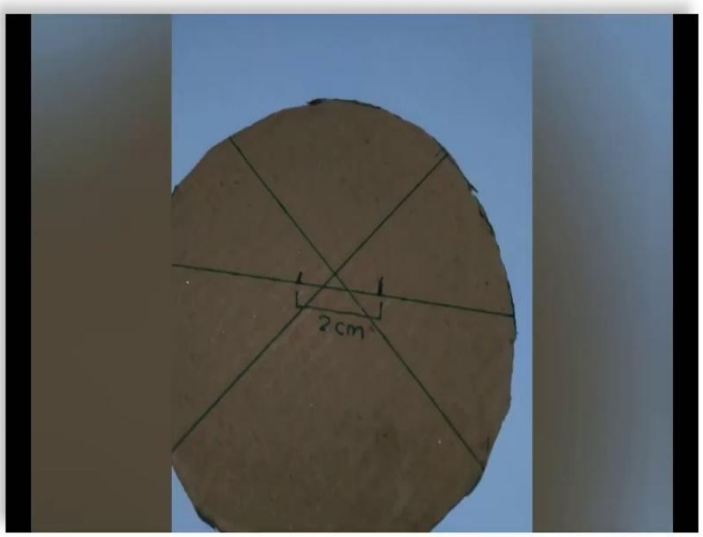

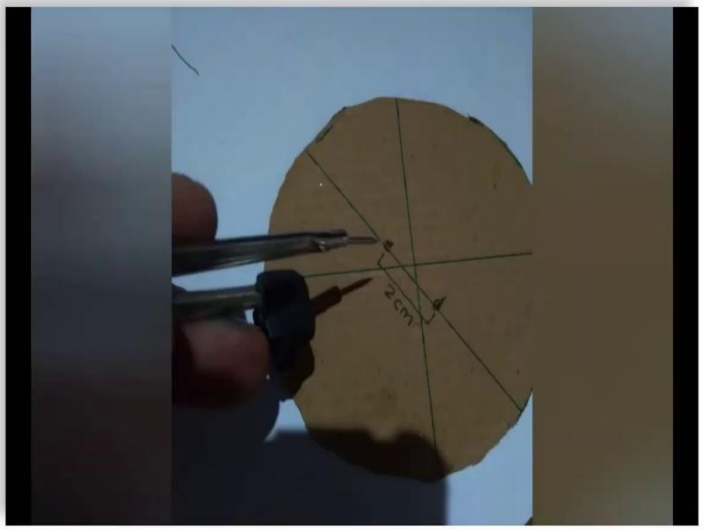

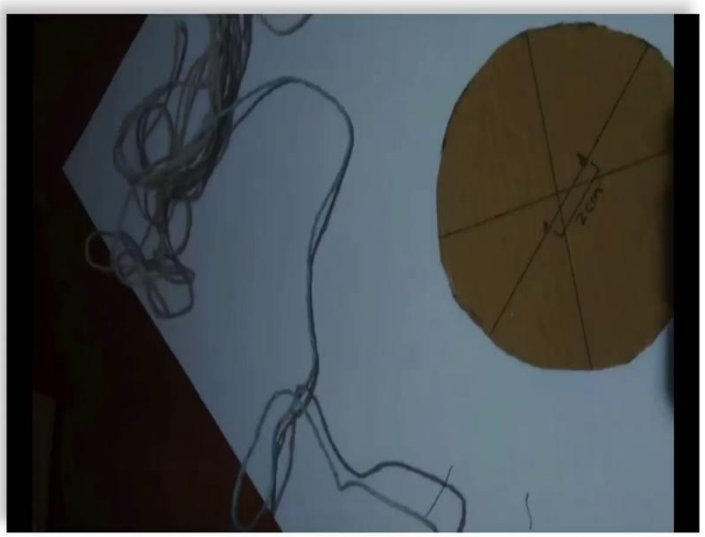

Once I had the center, initially, I got some wrong centers, I marked two holes, setup separated by two centimeters using a compass and inserted thread through it. That was my basic toy.

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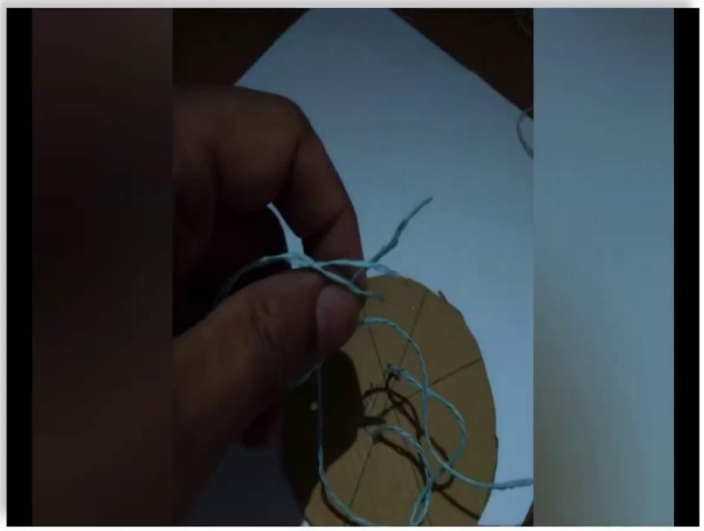

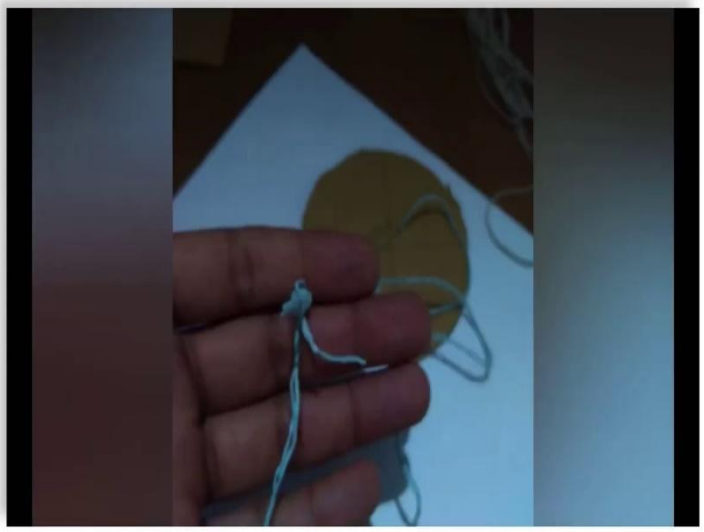


Making a whirligig toy
फिरकी

And I tied it up. Now I have my basic toy. But this basic toy can be made a bit more sophisticated as the people in the paper have described.

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Paperfuge: Materials

- Card paper- cut into 2 circles diameter = 10 cm
- Two holes (diam 3 mm) spaced by 2.5
- Adapters for holes to prevent thread cutting
- Braided nylon fishing line (high tensile strength)
- Wooden or PVC pipe handles
- Drinking straws as holders for centrifuge capillaries sealed at outer end
- Straw holders glued to disc along radius
- Velcro to hold 2 discs together and as safety and reduce drag

And I have made a version of it, which you can see now. So, this is a small mimic of the paper future that we have made with the handles made out of PVC pipe, nylon thread, fishing thread, if you see it in focus now.

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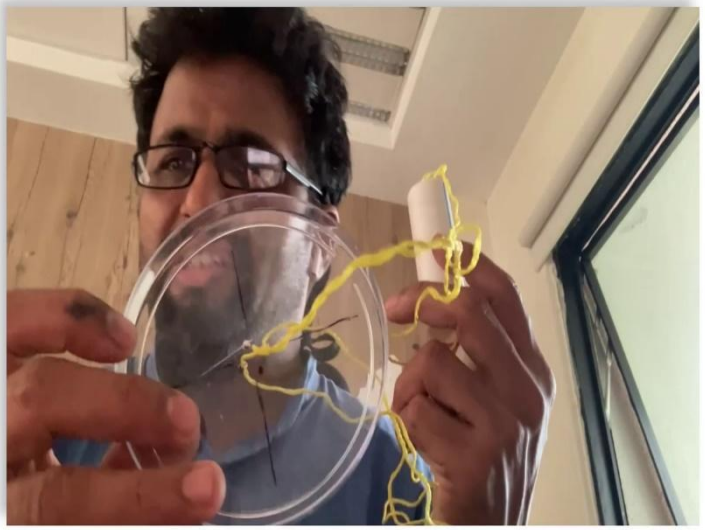

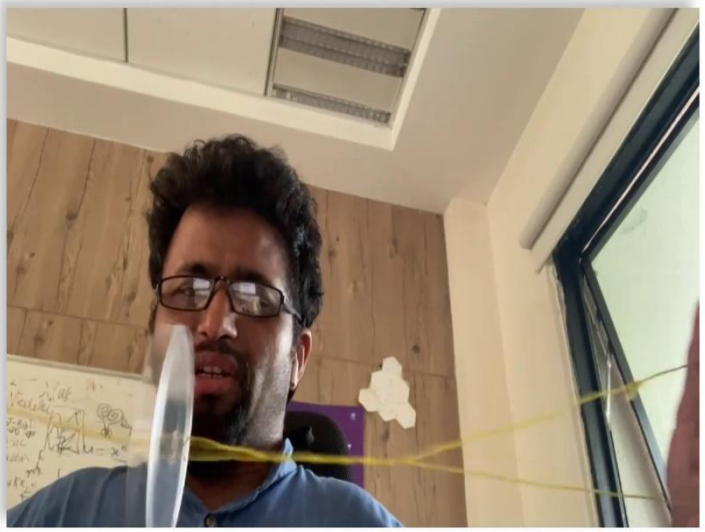

And disk that is made out of a cap of a petri dish, 80 mm petri dish and we have marked the two centers as we had described in the description earlier. And you will notice that this is surprisingly capable of working quite well. So, we need a little bit of practice then you torque it and you will find that this expansion and contraction motion gives you quite high rpm then I do not know if you can hear it. But this is really fast.

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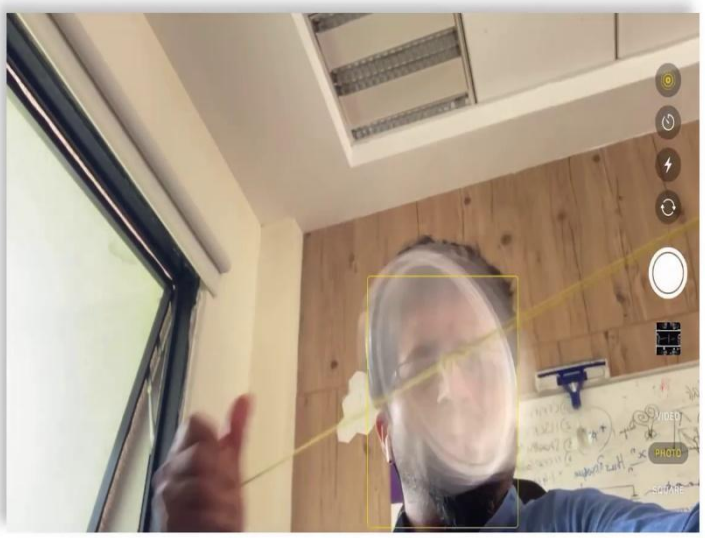

According to the calculations in the paper, they can at times even reach 10,000 rpm and that is sounding. Now on this motion, what is it called? Let us see.

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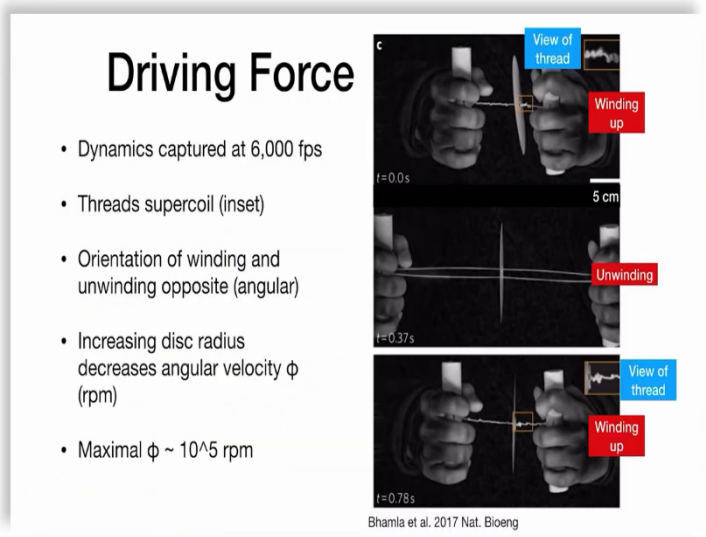

So, as it turns out, when they used a high speed camera, what I have here is a normal 40 frames per second camera, they use 6000 frames per second camera. And they could see what you saw already, maybe by that the thread can super coil. And the orientation of winding and unwinding is opposite meaning to say that when it is winding up, the rotation goes one direction, clockwise you say and when you unwind it, it goes in the opposite direction. The force that is exert is usually to unwind, because as you noticed, that my device compresses when I am trying to let the super coiling takeover.

This is the part which we refer in the paper to as winding up and the pulling apart is called unwinding which leads to rotation in the other direction that momentum or may I say inertia, results in it over torquing and going in the other direction leading to again super coiling and the cycle goes on. We also found that the discrete is of its very small, the angular velocity is very high and as the disc radius increases, you get lower angular velocity.

Now, think about this for a second that if you are trying to centrifuge a tube, let us say Eppendorf tubes, these micro centrifuge tubes, then you want at least a minimum discreteness because if you do not have it, then you are unlikely to be able to make this any use of making any use of it.

But on the other hand, you also want it to go fast for the applications we are looking for. So, the maximal rpm that they could observe was one lakh rpm. That is a lot. That is a lot by the way and you can go back if you have access to a laboratory or when you go back to your laboratories in school or college you can take a look at this.

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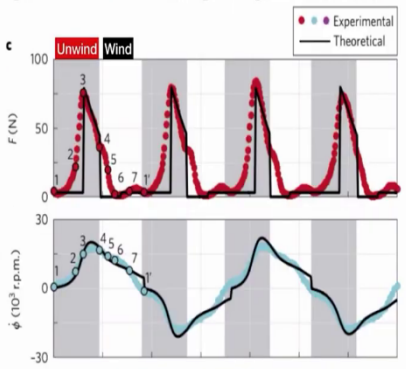
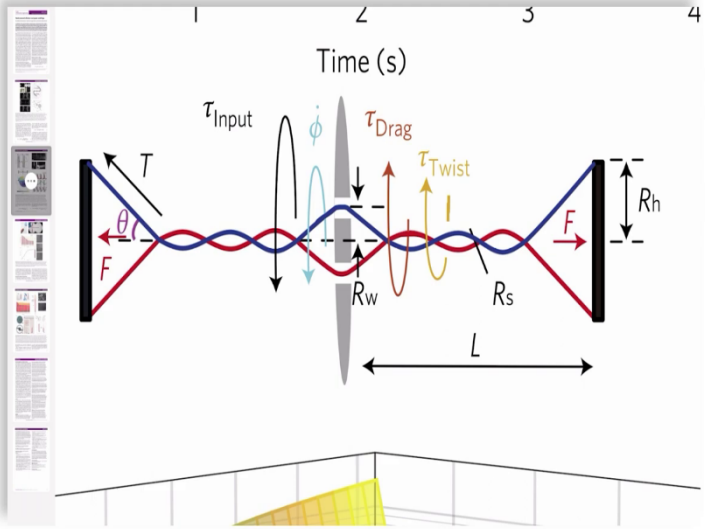
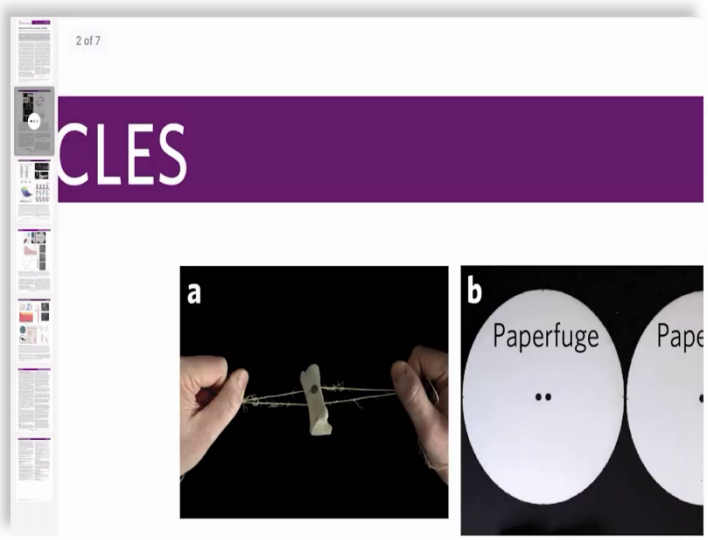
Force and Angular Velocity Dynamics

- Force transducer used to measure unwinding force
- Maximal rotations
- Inertia drives re-winding
- Theoretical model



Bhamla et al. 2017 Nat. Bioeng





In their paper, they demonstrated using a force transduction device that the pulling force, the unwinding force is in the range of about 75 Newton's. The part where there is a drop in the force is also a part corresponding to the winding phase that winding phase leads to slightly slower rotations and a switch to the other direction this is what I was referring to. So, what you are looking at in the two graphs upper and lower are force and the corresponding rpm for the same time series.

The interesting part of this is that they also developed a theoretical model of the physics of torque of the thread of the viscous drag that the centrifuge experiences as it moves through this fluid of air and finally, the motion itself, which is driven by the behavior of the user. So, if we look at the paper, they are describing T input, that is the behavior of the user, the twist due to the thread, and T drag, tau drag, these are the three torques that they are interested.

The radius of winding, radius of the hands and the theta angle have formed by the hand, thread and hands are considered to be important. So, twist drag and input.

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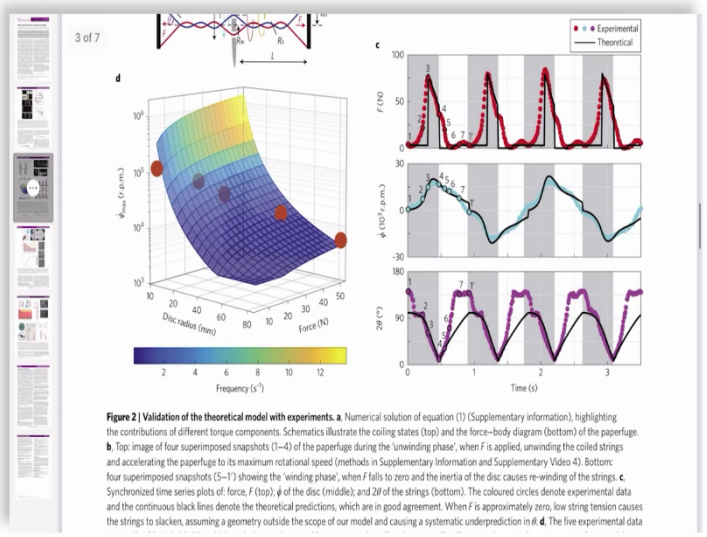
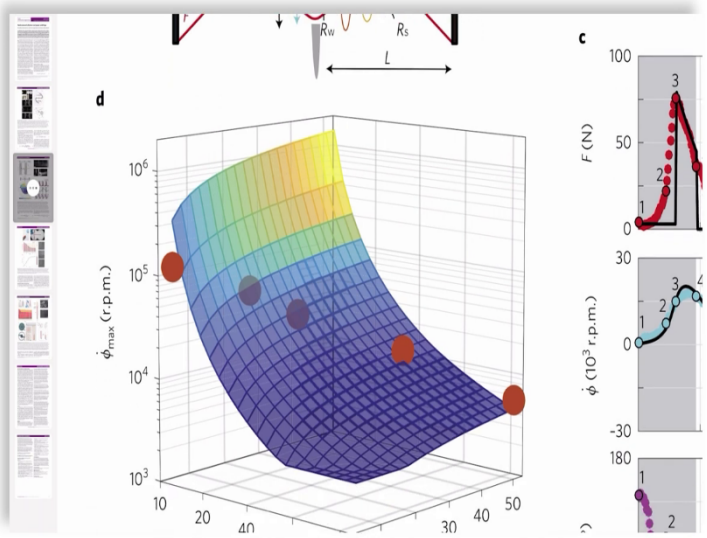
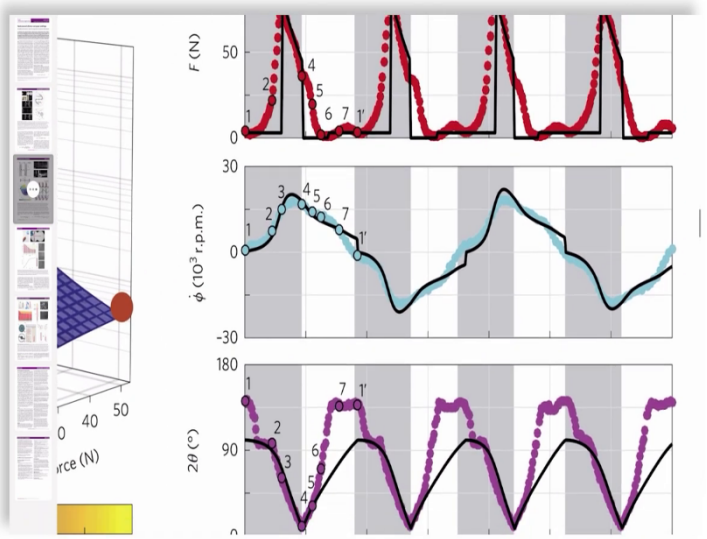
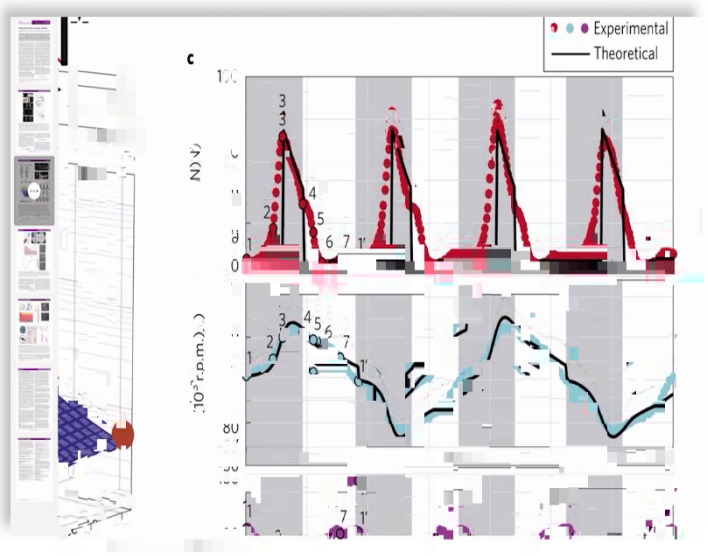


Figure 2 | Validation of the theoretical model with experiments. a, Numerical solution of equation (1) (Supplementary information), highlighting the contributions of different torque components. Schematics illustrate the coiling states (top) and the force–body diagram (bottom) of the paperfuge. **b**, Top: image of four superimposed snapshots (1–4) of the paperfuge during the 'unwinding phase', when F is applied, unwinding the coiled strings and accelerating the paperfuge to its maximum rotational speed (methods in Supplementary Information and Supplementary Video 4). Bottom: four superimposed snapshots (5–1′) showing the 'winding phase', when F falls to zero and the inertia of the disc causes re-winding of the strings. **c**, Synchronized time series plots of: force, F (top); $\dot{\phi}$ of the disc (middle); and 2θ of the strings (bottom). The coloured circles denote experimental data and the continuous black lines denote the theoretical predictions, which are in good agreement. When F is approximately zero, low string tension causes the strings to slacken, assuming a geometry outside the scope of our model and causing a systematic underprediction in θ. **d**, The five experimental data







Now, what they also do is they test whether the experimental data of rotation, disk radius and force match with each other. And as you can see, in this surface graph, they appear to indeed match. As with a lot of theory, you can calculate a lot theoretically, once you have the equations, but experiments are harder to do.

But in this sort of empirical approach, it is very important to know if theory and experiment match that is also the case in the force, angular rotations and two theta degree measures, where they all qualitatively follow the same behavior as the experiment. the circles are the experiments, the black lines are the theory.

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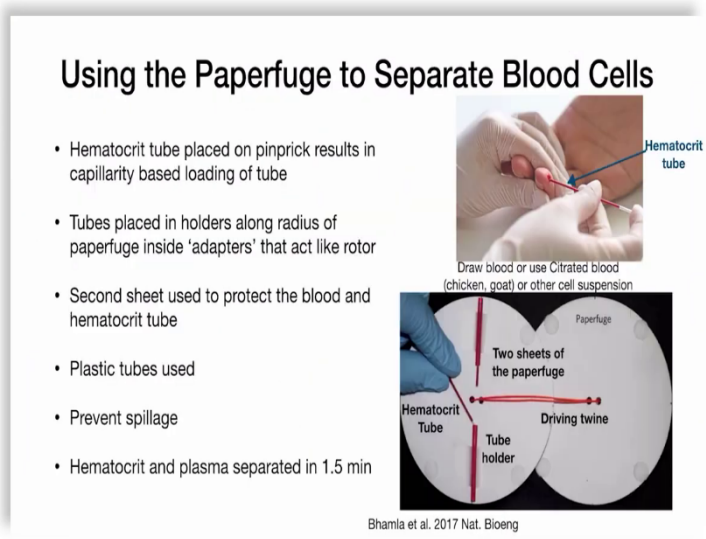

So, what did they use it for? I said this earlier that it depends on the application. In that case, they followed the hematocrit. Now you remember, I have spoken quite a bit about hematology and very basic, simple elementary medical tests that are conducted when you go to do a blood test.

Hematocrit is something that is a very old fashioned measure, because you take a hematocrit tube, pinprick person, take a capillary bleed, place the tube either in a centrifuge in specialized hematocrit centrifuge, and spin it and measure the percentage of the red blood cell there to the total amount the height of the blood column, naturally, this process must involve heparinized or citrated blood to prevent clotting.

So, when you take the blood like this, it has to be already containing citrate, the amount of citrate, added is constant so that you do not make a it does not make a difference to your calculations of hematocrit. Anyway, these are relative measures. So, relative to the total volume is all you are interested in. But if you can imagine that if you put too much citrate, you will change hematocrits. The tubes are placed in the holders along the radius of the paperfuge and the adapters are straws like sucking straws.

A second sheet remember we said that the published report talks about two paper sheets is used to protect the blood and the hematocrit tube, these plastic tubes to prevent breakage and damage and injury to the user and they use the straws to prevent spillage. All of them are sealed at one end using acrylic adhesive. What they found is that hematocrit in plasma

separation 1.5 minutes that is amazing because typically in experimental work, this takes about two minutes. So, it is very comparable.

Now of course they optimize the conditions. And this is important in any kind of experimental approach to find the best situation that works.

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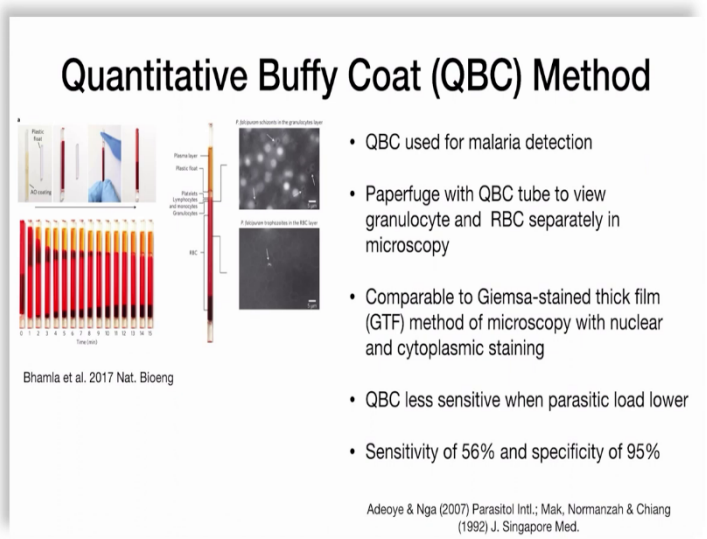

And they also went ahead to use for demonstration to check whether the Buffy coat can be obtained from this paper centrifuge for diagnosing malaria. And they did this using QBC quantitative Buffy coat tube, which is already pre-coated on the inside with Acridine or Acridine orange is a DNA staining dye, AO as it is referred to here, the citrated blood is introduced into the tube, a little plastic floater is added into it that spreads the Buffy layer therefore giving you a bigger resolution because otherwise the Buffy is very small, Buffy layer usually include white blood cells, Buffy coat and those cells were centrifuged using the paperfuge.

Initially, as you see over here in the left corner, the float drops down because of centrifugation but then because of density, it reaches a equilibrium position and spreads the Buffy coat. The bright red color is RBC as you expect. The platelets lymphocytes and monocytes and granulocytes are spread in the upper thin layer and plasma layer is on top of it.

The float is the one that separates the plasma and Buffy coat. In comparative studies between the quantitative Buffy coat method and the Giemsa-stained thick film that a GTF method, it was found that QBC is much less sensitive, leading to say the lowest number of parasites that

can be detected using QBC is only 56 percent compared to the gold standard, which is Giemsa-stained, stained thicker.

The specificity of the element meaning if there is something you will find it and you can distinguish it from other parasites let us say malaria from dengue is 95 percent. This is based on studies by Adeoye and Nga in Parasitology International and Mark, Normanzah and Chiang in 1992 from Journal of Singapore Medicine.

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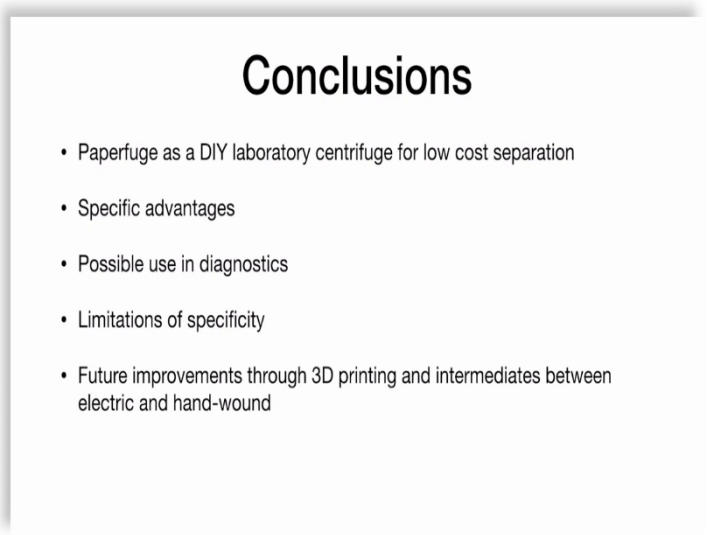

So, in summary, we can say that the paperfuge is a wonderful, do it yourself laboratoriy interface for low cost separation. It has very specific advantages that relate to the applications that it was used for making it a possible additional tool for diagnostics, especially in low resource settings meaning when there is less funding available for basic clinical diagnostics.

But it is also limited in terms of the application because it has not been demonstrated or other, so if you would like to try this, this is something that you believe we do in a small school or college laboratory. And future improvements are definitely possible through 3D printing and intermediates between electrical hand wound machines. So we stop here, and we continue on some problems.