9/28/11 From lab notebook R-619

Trip to San Francisco

Visit with Richard Sweet of Stanford Electronics Lab. Stanford U., California.

Saw Sweet the afternoon of 12 August, the following information was gained:

a. He thinks the idea is completely feasible and that a 10 set separator could be easily obtainable.

b. He suggest operating the interstream deflection by charging electrode at ground in order to avoid interference with the Coulter detector.

c. Was operated at a drop freq. from 250 kc/sec to 10 kc/sec and pressure of 60 psi to 11 psi

d. Agree that the "pots" could be moved farther away from nozzle to increase deflection this will be limited by droplet slowing down in air resistance.

e. Forgot that giving droplet ranging charge & variable deflection voltage would work.

f. Sweet graciously loaned me a nozzle vibrator assembly, and will send diagrams of drive system, and charging voltage amplifier.

g. Used a Krohn-Hite 0C4-10 amplifier to drive vibrator (nozzle).

Original notes filed under "Cell Separator"

Visit with Dr. Donald Glaser, Rm. Lab., Un. of Cal. at Berkeley

1) Stanford U. - California.

Saw Sweet the afternoon of 12 August, the following information was gained:

a. He thinks the idea is completely feasible and that a 10 pot separator would be easily obtainable.

b. He suggest operating the interstream deflection ~~or~~ charging electrode at ground in order to avoid interference with the Coulter detector.

c. Has operated at a drop freq. from 250 kc/sec to 10 kc/sec and pressure of 60 psi to 11 psi.

d. Agrees that the "pots" could be moved farther away from nozzle to increase deflection this will be limited by droplets slowing down in air resistance.

e. I got that giving droplets varying charge & varying deflection voltage wouldn't work.

f. Sweet graciously loaned me a nozzle vibrator assembly, and will send diagrams of drive system, and charging voltage amplifier.

j. Used a Krohn-Hite OCA-10 amplifier to drive vibrator (nozzle).

Original notes filed under "Cell Separator"

2) Visit with Dr. Donald Glaser, Virus Lab., Un. of Cal. at Berkeley

Met with him the 13 of August, was briefly shown his counter setup which he uses to [illegible] (measure vol.) of small biological particles i.e. bacteria ("bugs" he calls them)

30. 18 Aug '64 (cont.)

2) cont. the following information was gained:

a) Thinks the cell separator idea is feasible and may be extremely valuable. He had one particular reservation. "Can sufficient numbers of cells be separated to perform certain kinds of experiment ie ~10^8?" For other types of experiments he says 10^4 would be plenty.

Fluorescent Sorting

b) He gave a idea as to how we might separate specific virus and bacteria from others

Tag antibodies with a flourescent dye. Let these antibodies attach themselves to their specific virus or bacterium. Then flow the suspension past a U.V. light to activate and a P.M. tube to detect the now flourescent virus or bacterium; use the P.M. output to place a charge on the proper drop deflecting it into a receptacle.

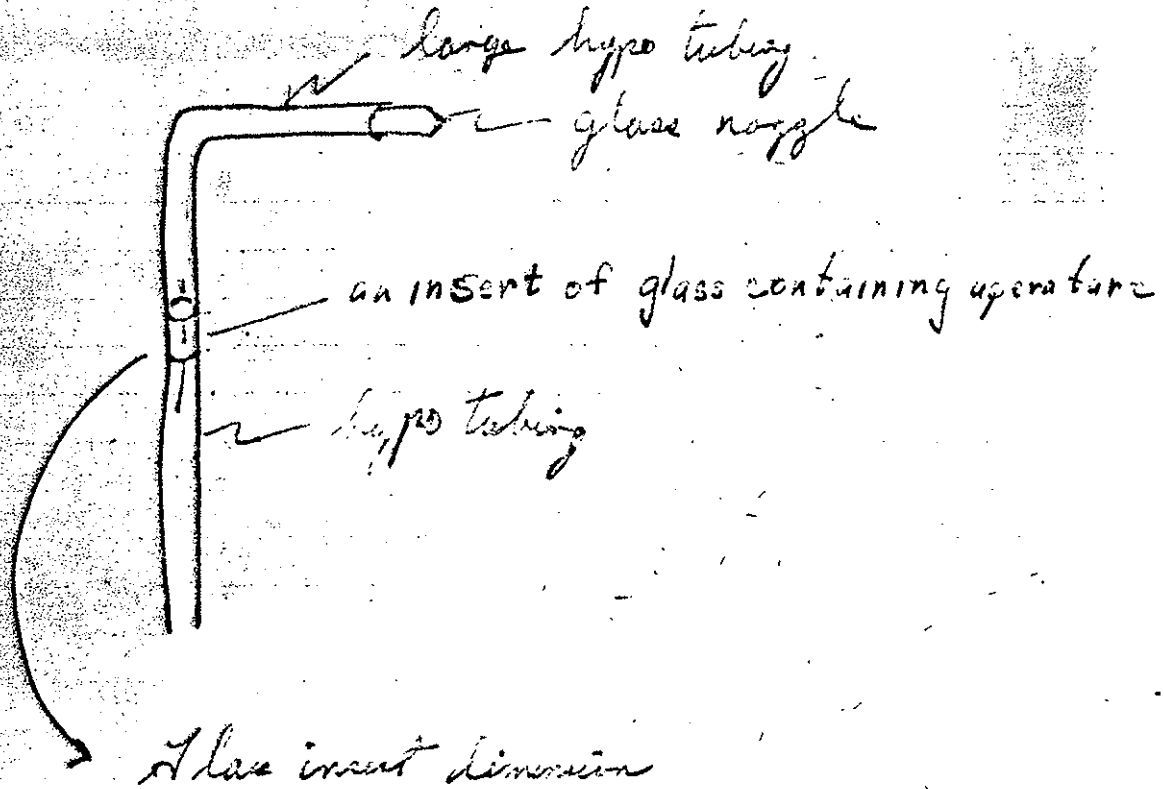
c.) He suggests that a polystyrene sphere may be rendered partially conducting by picking up charges on its surface with a high Zeta potential. I don't understand Zeta potential.

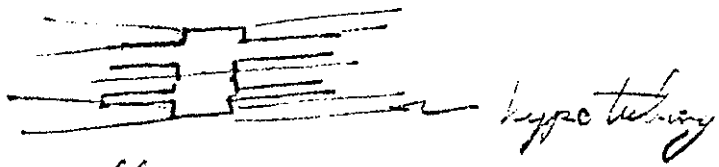
d) He asked to be sent our annual reports and to be kept informed on progress of cell separator.

Mack J. Fulwyler 18 Aug '64

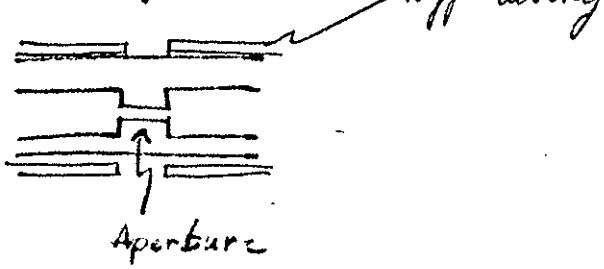
4 Ideas for incorporation of aperature into drop-gun.

1)





or better yet



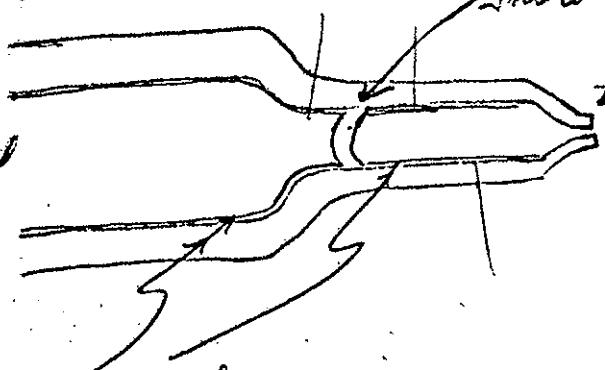
Mack J. Fulwyler 18 Aug '64

32 19 Aug '64

Combined Aperture Nozzle System

For the cell separator it would be advantageous for several reasons to combine the aperture and nozzle into the same glass tube as shown below:

1) Take a piece of glass capillary tubing of appropriate ID & OD and draw it out as shown below



The tubing could be bent down to form and attached to the magneto-strictive vibrator element as in Sweets operation.

To be plated out nickel or other conductor

2) Some advantages of this scheme:

a) Laminar flow between aperture & nozzle allowing accurate timing of drop-cell sensing and drop formation

b) Aperture is structurally strong

c) Aperture can be located near nozzle

d) Can use Sweets drop formation system as it exists now.

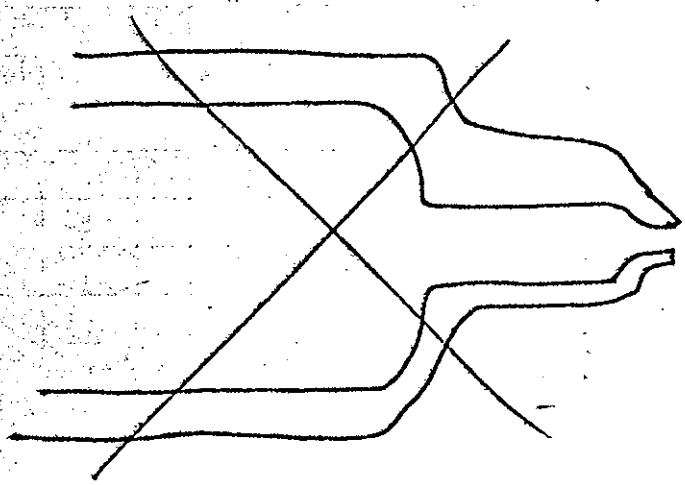
Mack Fulwyler 19 Aug '64

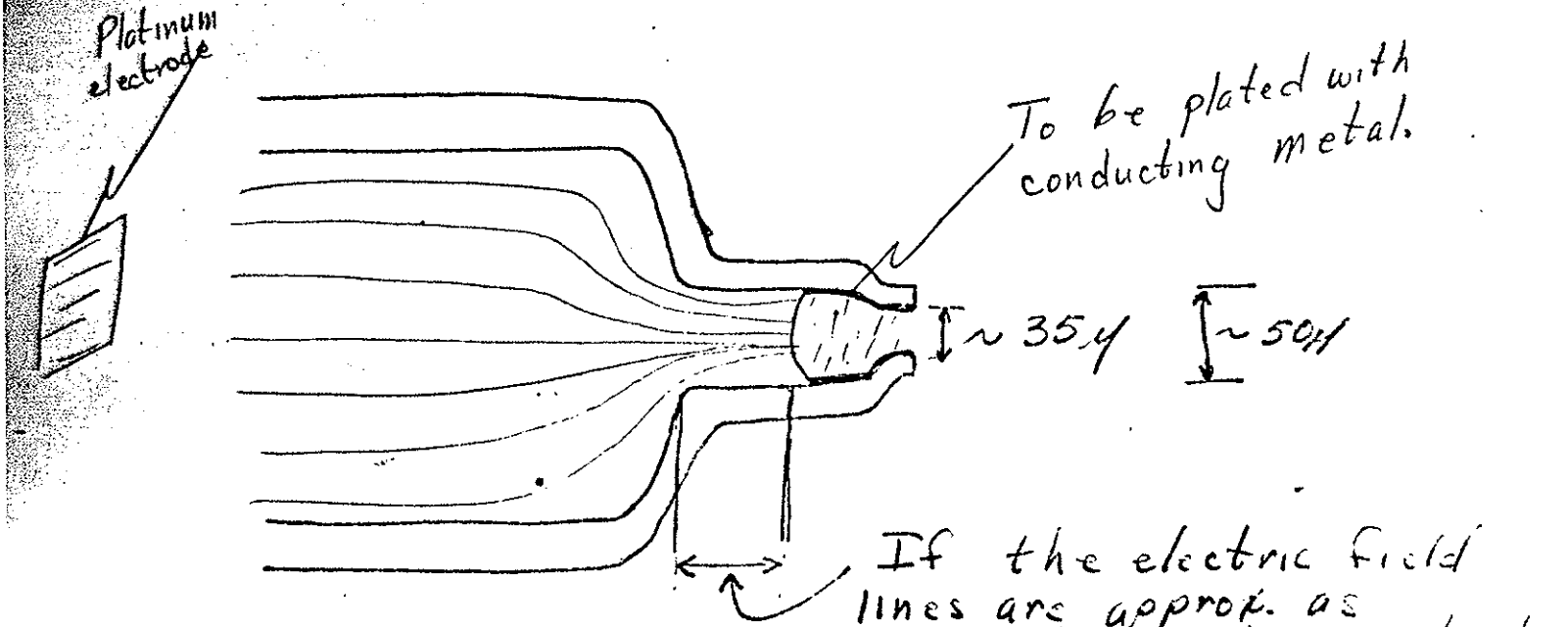
MF

Aug '64 Scheme #2 Combined Aperture Nozzle System. 38

Refering to the preceding page the following idea is suggested.

1) As before form a nozzle-aperture as ~~this~~ below:



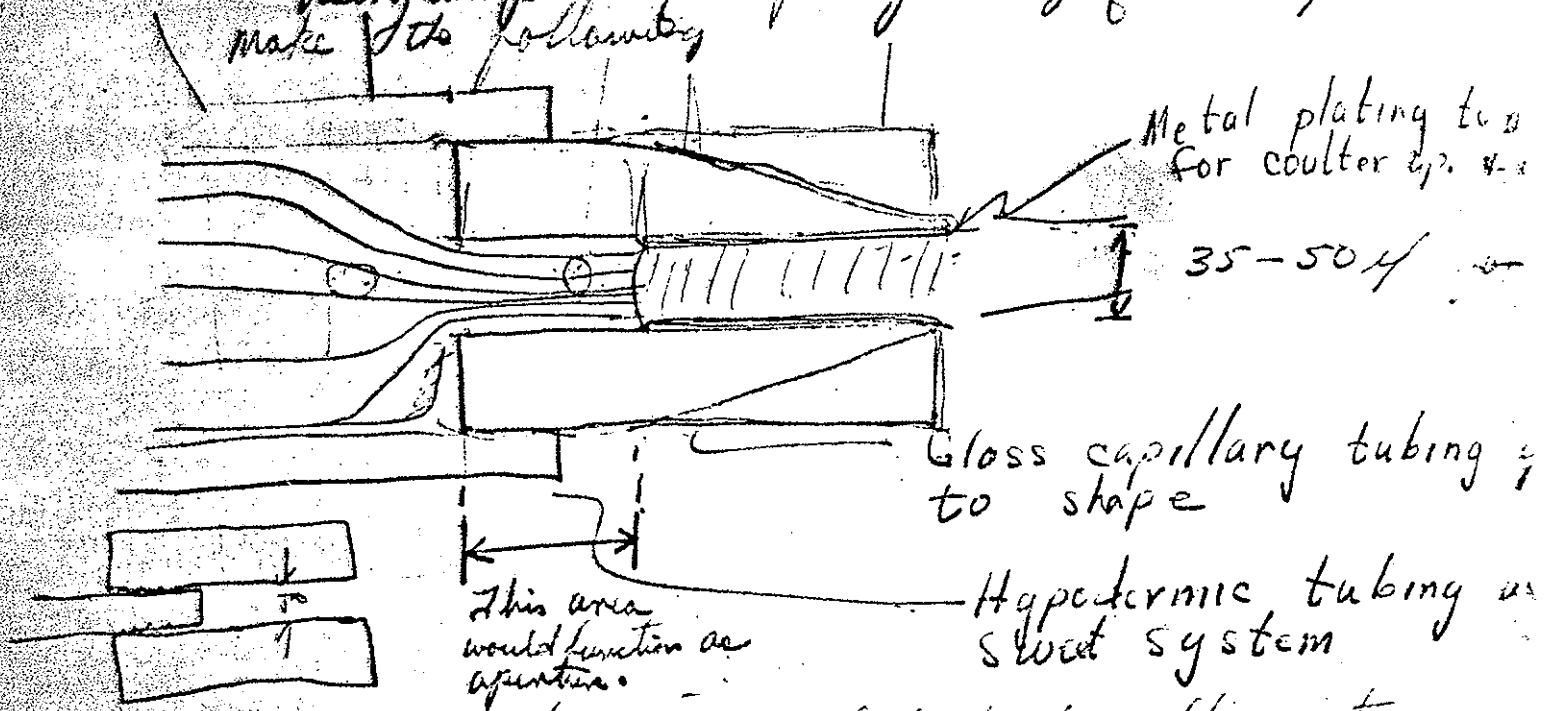


If the electric field lines are approx. as shown, this ~~portion~~ constriction would become an a Coulter aperture and could be used to detect particles and measure volumes.

2) With this scheme it would be possible to see plugging by looking through aperture — Also this would require one plating operation.

3½ 20 Aug '64 Scheme #3 Nozzle-aperture system

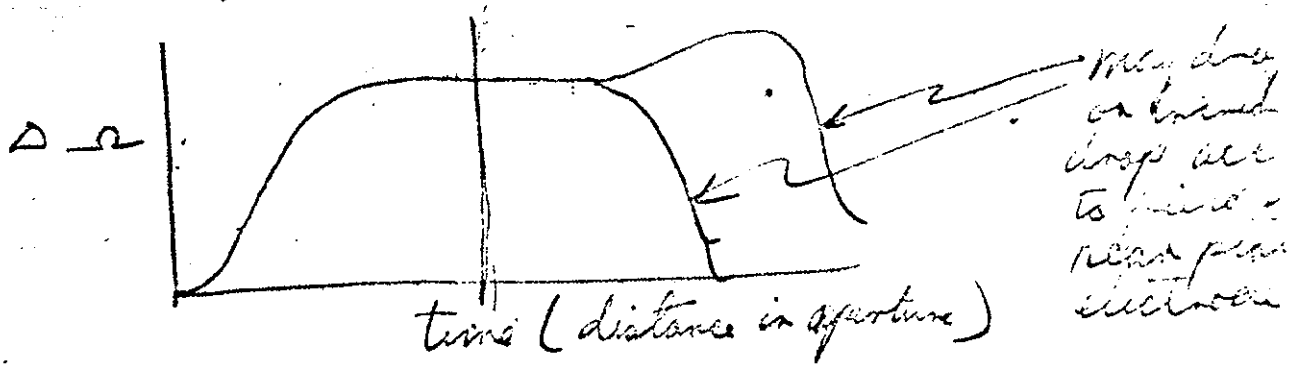
Using a piece of capillary tubing of ~ 50μ I.D. make the following



1) If hypo. tubing is one electrode of coulter system and plating is the second the electric field lines might be as shown

2) This would be simply made requiring no drawing of cap. tubing.

3) Should give a pulse shape of

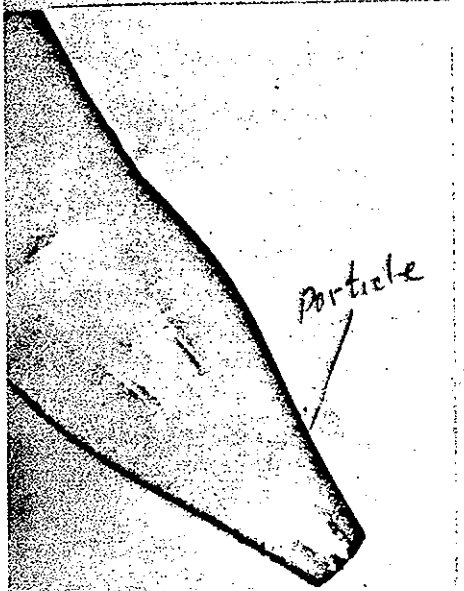


4) Could use Sweet's droplet formation system of vibrating hypo needle.

Mack F. Fulwyler 20 Aug '64

Photographs of Sweet's Nozzle

d) What is might be interesting to photograph sweets nozzle to determine shape of the tubing inside, and then to photograph the emerging stream. The following pictures resulted:



a) This photo was taken before I tried to pass water thru it.

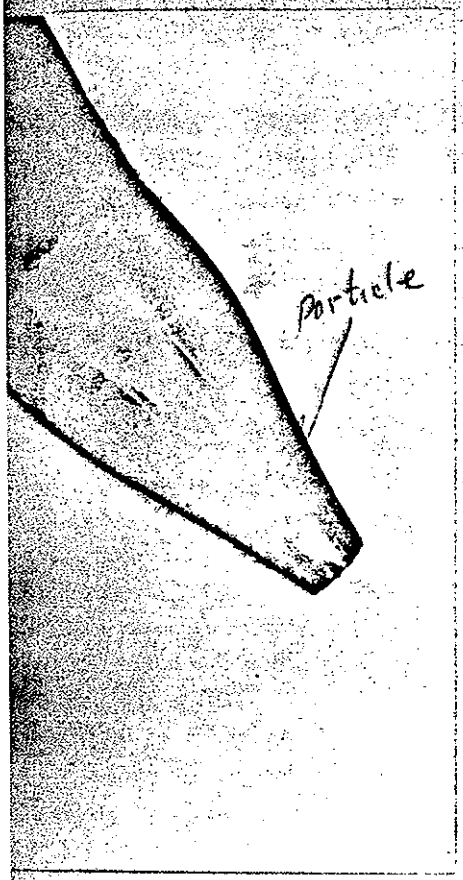
b) Note the dust particle.

a) I then forced water through with a syringe

b) Note that the particle has be forced toward the nozzle and that the emerging stream is perturbed. Compare this with photos on following page

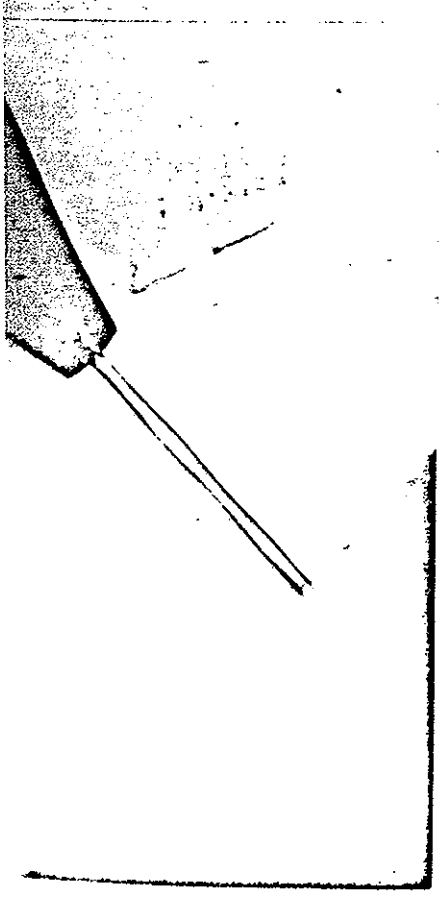
c) [illegible] the emerging stream it could be said that it [illegible] to the eye [illegible]

tried to photograph the emerging streak. The following picture resulted.



a) This photo was taken before I tried to pass water through.

b) Note the dust particle.



a) I then forced water through with a syringe

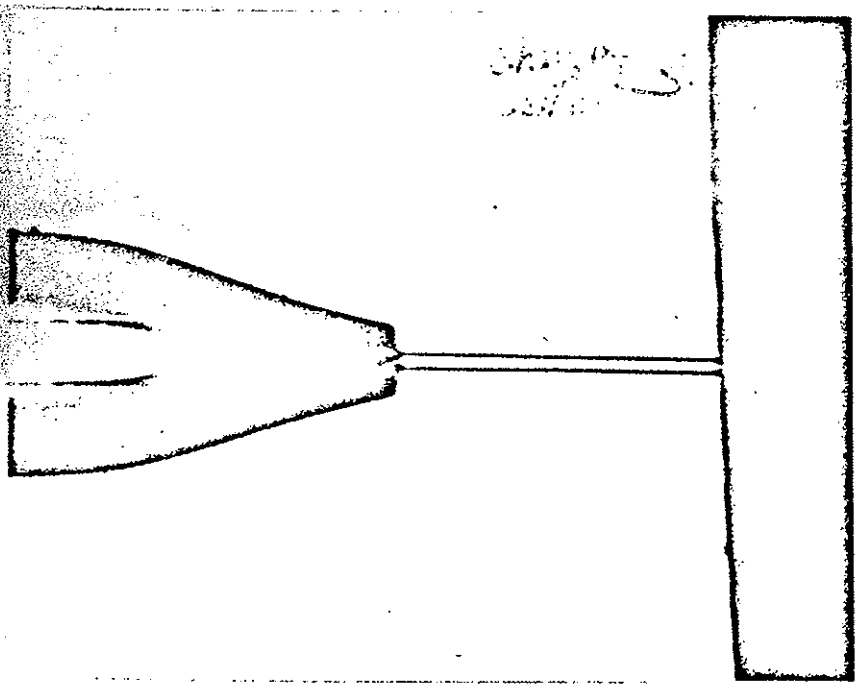
b) Note that the particle has been forced toward the nozzle and that the emerging stream is perturbed. Compare this with photos on following page

c) On observing the emerging stream it could be seen that it was not [illegible] the axis [illegible] was [illegible]

36 20 Aug '64 (continued.)

2) After cleaning the plug & device to replace the nozzle & stream.

a) In this photo you'll notice that the [illegible] has been removed.



a) Notice the uniformity of the emerging stream.

Mack J. Fulwyler 21 Aug '64

August 64

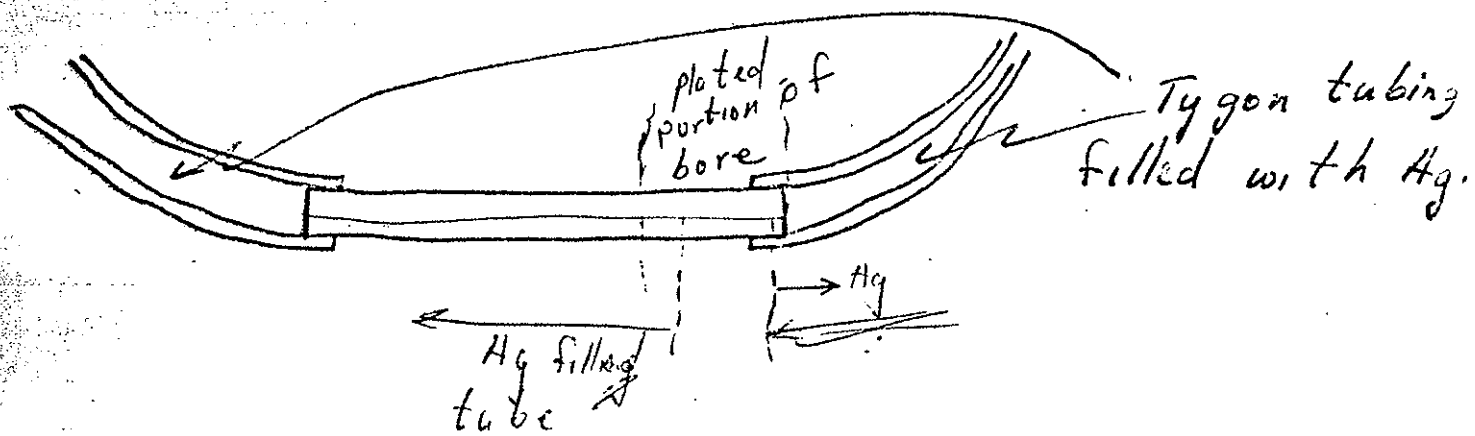
Attempt to plate capillary bore with Platinum.

1) Using after talking to Roesch about building this nozzle-aperture scheme (No. 3 pg. 34) I acquired 25 grams of Liquid Bright Platinum and 25 gms of Liquid Bright Gold.

2) I then partially filled a 50γ capillary tube with Liquid Platinum and plated it to ~200°C for ~2 hrs.

3) After "plating" it appears that the bore was clear as it passed air and mercury.

4) I readied to measure conductivity of the bore plating & set up the following.



Using this scheme I attempted to measure the conductivity of the plating. I was unable to measure a resistance different from ∞

.4 Scheme #4 for aperture-nozzle

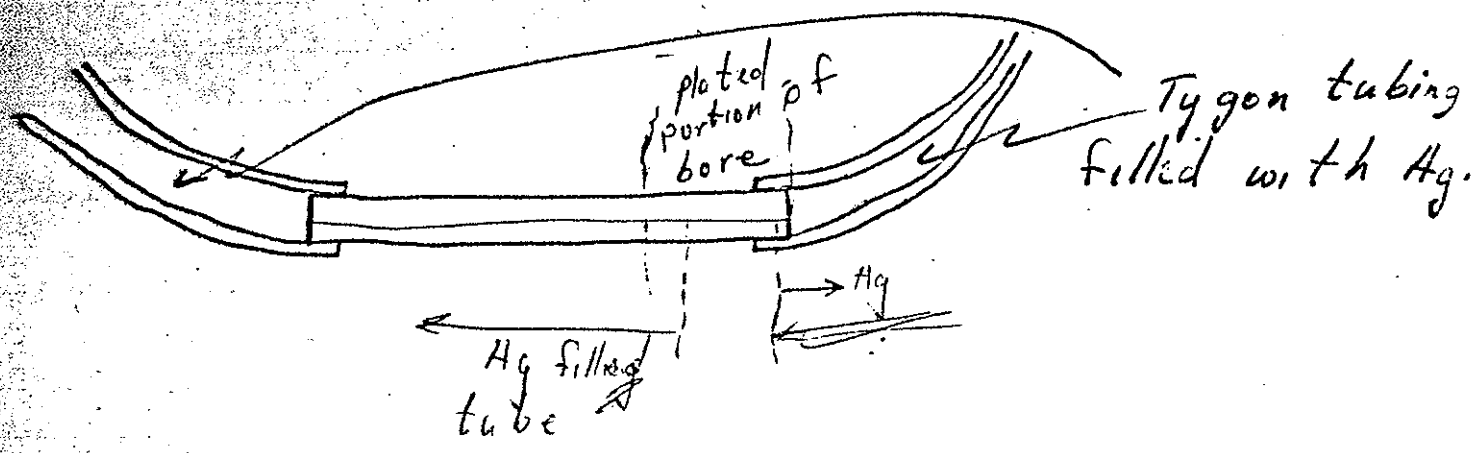
1) Because of the possibility of not being able to plate the inside of the capillary bore [illegible] I have decided to [illegible]

1) Using, after talking to Roesch about building the nozzle-aperture scheme (No. 3 pg. 34) I acquired 25 grams of Liquid Bright Platinum and 25 gms of Liquid Bright Gold.

2) I then partially filled a 50γ capillary tube with Liquid Platinum and plated it to ~200°C for ~2 hrs.

3) After "plating" it appears that the bore was clear as it passed air and mercury.

4) In order to measure conductivity of the bore plating I set up the following.



Using this scheme I attempted to measure the conductivity of the plating. I was unable to measure a resistance different from ∞.

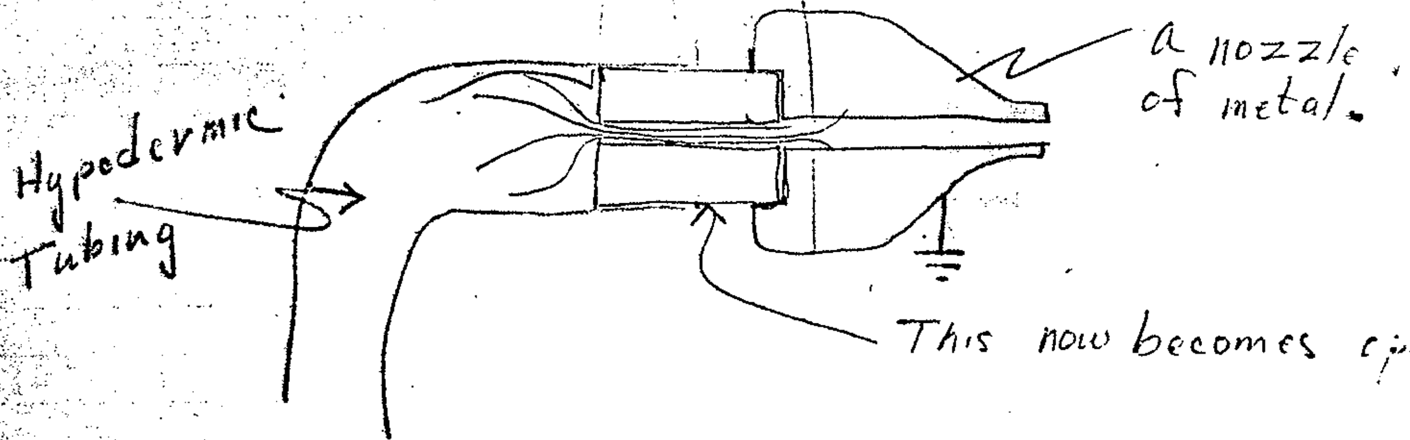
4 Scheme #4 for aperture-nozzle

1) Because of the possibility of not being able to plate the inside of the capillary bore with a conducting layer of platinum I have developed the following idea

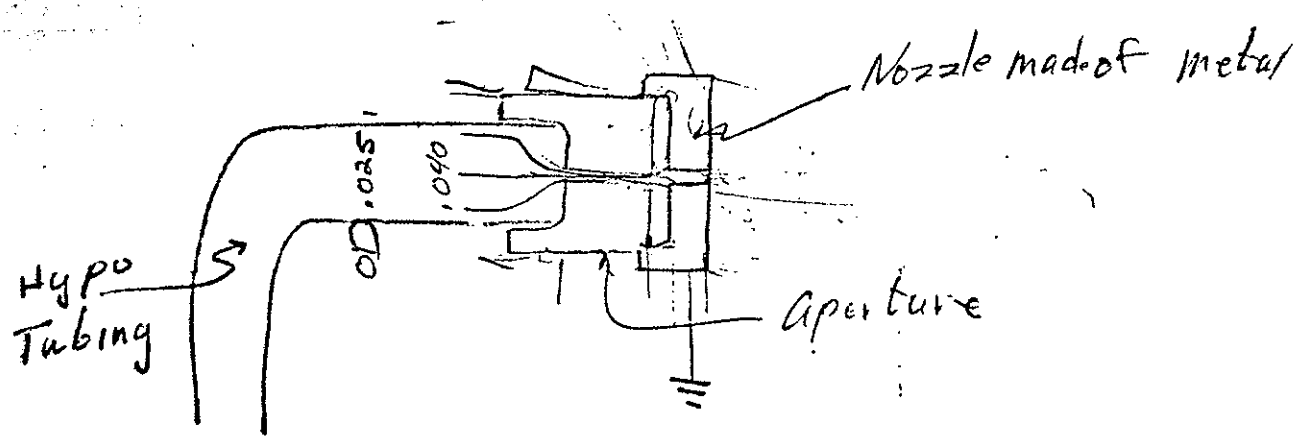
see next page

38 - 31 Aug '64 (continued) Scheme #4 - Aperture, Nozzle

2)



3) A variation on the scheme



31 Aug '64 Because I couldn't measure the conductivity of the liquid Platinum - as I thought - it might be that the firing temp. I used was too low (< 200 20°) I painted a strip of the stuff on a microscope slide and fired it ~1/2 hrs at 200°C and it was still black + grey looking. So I heated it in a bunsen flame causing it to turn silvery in color.

Mack J. Fulwyler 31 Aug '64

1'65 Successful Separation

After succes completion of the charging system I found it necessary to electrostatically shield the gun from the charging signal.
The Electrostatic shielding apparently removed the charging noise.
I then calculated an estimated delay time under a certain set of operating conditions of ~ 320 μsec. I then tried a separation

The first attempt at separation used Dow polystyrene spheres, diameter 7 — 14.4 μ, using a delay (charging pulse delay) of ~200 μsec I began to catch the separated spheres. This was done on 6 April and was the first sign of success. The operating parameters were

Time delay - 200 μsec
Charging Pulse duration ~ 100 μsec
Fluid pressure — 35 P.S.I.
Oscillator Freq — 72 kc

After successful completion of the charging system I found it necessary to electrostatically shield the gun from the charging signal.

The Electrostatic shielding apparently removed the charging noise.

I then calculated an estimated delay time under a certain set of operating conditions $\tau \sim 325$ μsec. I then tried a separation.

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Time delay – 200 μsec
Charging Pulse duration ~ 100 μsec
Fluid pressure – 53.5 P.S.I.
Oscillator Freq – 72 kc
Voltage – 50 v R.M.S.
Charging pulse amplitude 30 v

84

8 April '65

5. The first separation attempt took place 7 Apr. and involved sep. of Red blood cells (mice) from 3.49μ polystyrene spheres. All operation condition were as before. I obtained a very good separation but was still not satisfied.

6. So I tried to separate pigeon nucleus pollen (washed & filtered) from my R.B.C.

Operating conditions were

Charging Pulse Delay - 200 μsec

" " Duration - 100 μsec

" " Amplitude - 30 volts

Fluid Pressure - 53 PSI

Sec Avg/Volt = $\frac{\text{Take}}{52 \text{ V.R.M.S.}}$

7. I set the threshold to cut on approx. the top of the P.M.T. peak. The separation fluid was applied to [illegible]

and involved sep. of Red blood cells (mine) from 3.49μ polystyrene spheres. All operation condition were as before. I obtained a very good separation but was still not satisfied.

6. So I tried to separate paper mulberry pollen (washed & filtered) from my R.B.C.

Operating conditions were

Charging Pulse Delay - 200 μsec

" " Duration - 100 μsec

" " Amplitude - 30 volts

Fluid Pressure - 53 PSI

Osc. Freq/Volt = $\frac{7.2 \text{ KC}}{52 \text{ V. R.M.S.}}$

7. I set the threshold to cut on approx. the top of the p.m.p. peak. The separation of this was excellent with little or no residual R.B.C. contamination of the separated p.m.p. fraction.

The results are filed.

86. 8 Apr '65

Separation of Human R.B.C. from Mouse

1. Jean Beaman prepared a mixture of mouse and human RBC. for separation.

2. I then attempted separation using the same settings listed on page 84.

3. Although things didn't go smoothly (I had trouble with plugging, noise etc) the separation of was accomplished. The spectra are filed.

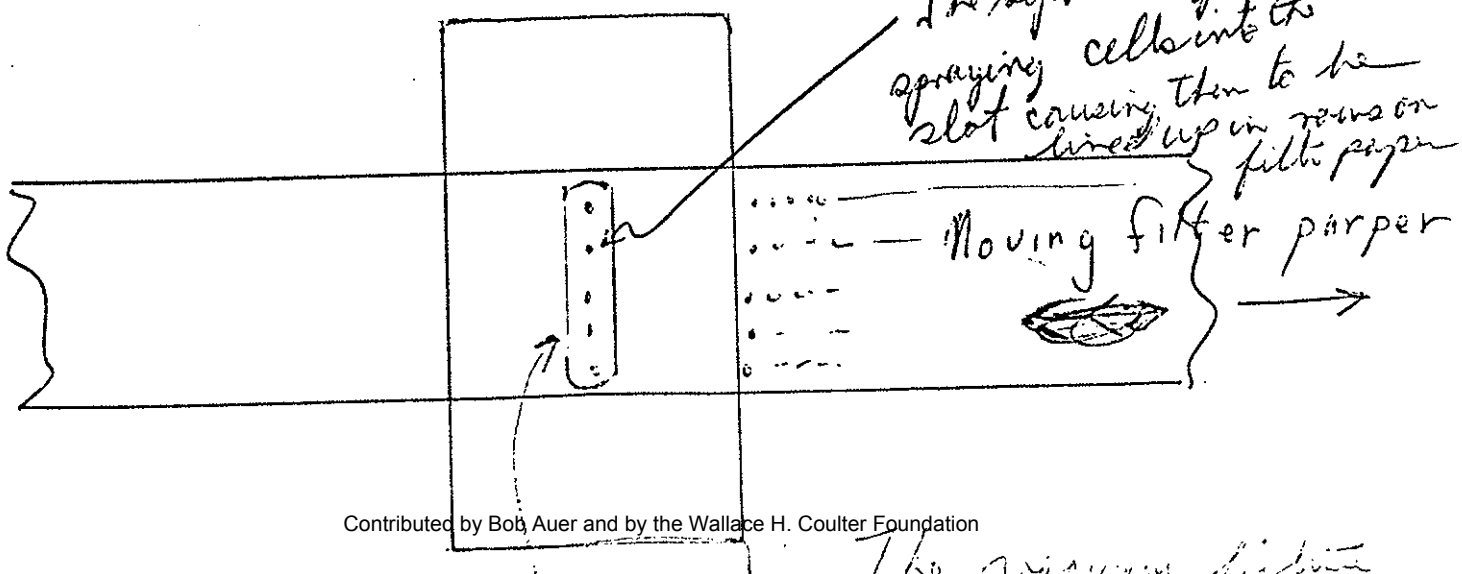
4. Wright mentioned the possibility of using the separator to separate ~~[illegible]~~ or concentrate the abnormal, cancer indicating cells ~~[illegible]~~ making detection much more rapid and reliable.

8 Apr — Mack J. Fulwyler

'65 New Ideas

Last Saturday (1 May '65) Wright + I discussing the separator system and developed the following ideas.

1. For the system of the future which will place the cells on a moving strip of filter paper according to their volume, Wright suggests using a vacuum device to pull the saline into the filter paper leaving the cells high and dry. The cells can then be stained and otherwise treated. The device might look like this:



38 3 May '65 (Continued)

Two parameter sorting

Idea

Coincident detection system. If we are successful in designing an optical detection system it may be possible to operate the two sensing systems in coincidence. For example if we have fluorescently stained cells mixed with non-stained cells this would enable us to select stained cells of any volume from the mixture.

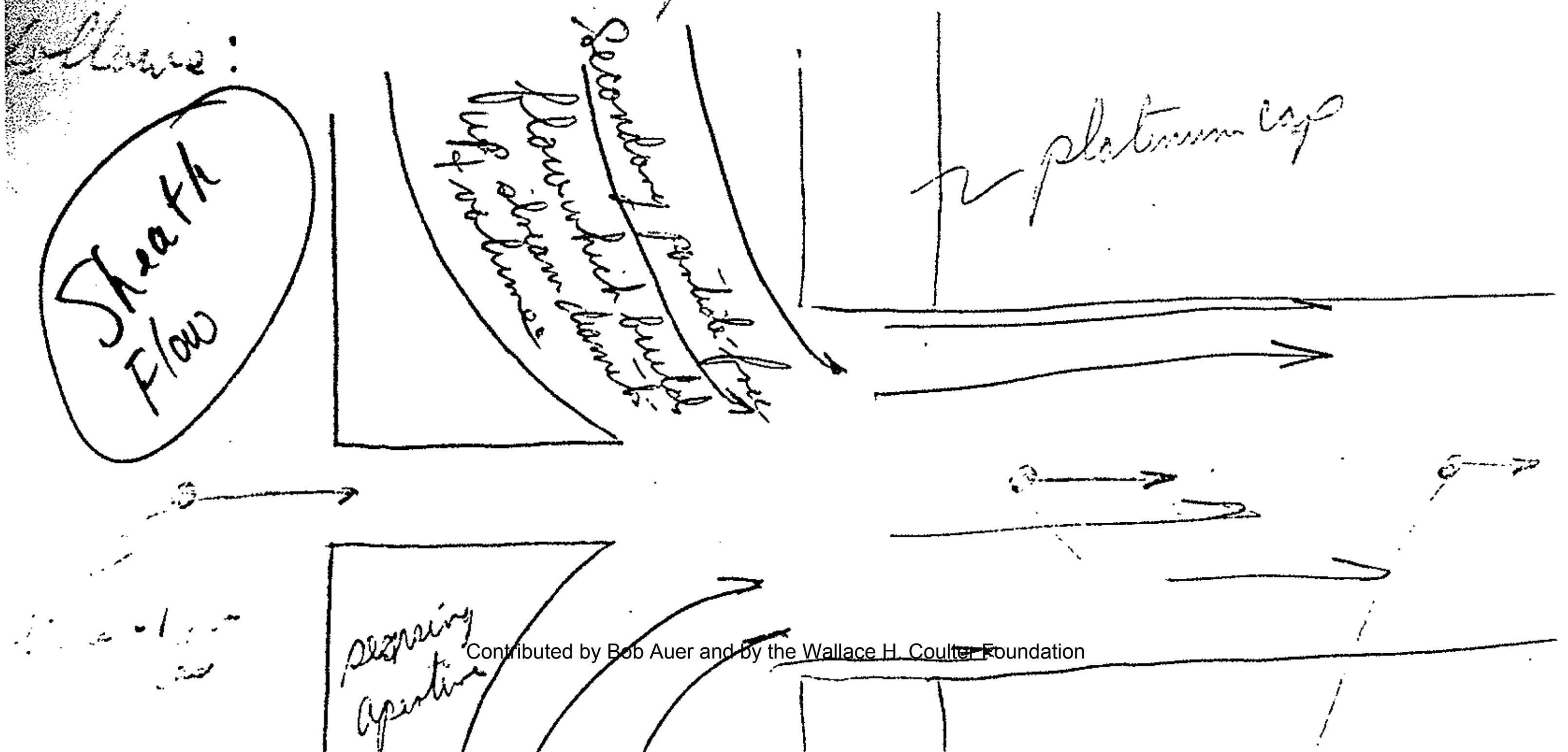
For example the coincidence idea would enable us to si
stained cells of the size from the mixture (or unst
cells)

'65 (continued)

Ideas

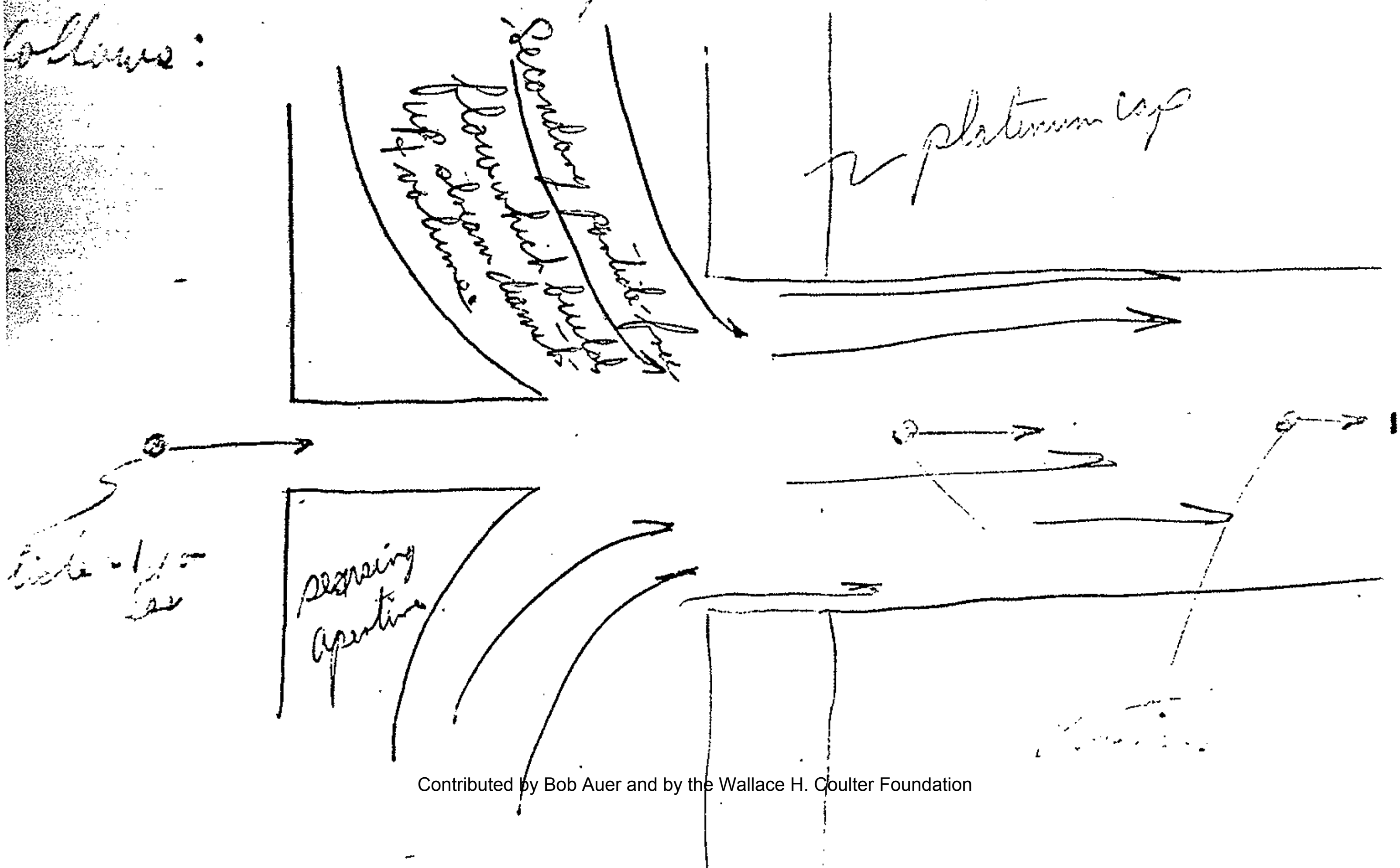
Because of the positive pressure system and tem filtration system of the separator I think should have a better chance of developing very small particle spectrometer and separator n anyone else. The positive pressure ld be 200 psi or more) enables us to force uspension through a very tiny aperture against ge capillary forces. The filtration system y reduce plugging to manageable levels.

may be possible to adapt the separator system work with small apertures (~10 μ diameter) follows:



... of the positive pressure system and
...tive filtration system of the aperture, I think
should have a better chance of developing
very small particle spectrometer and aperture
... anyone else. The positive pressure
...ld be 200psi (or more) enables us to force
...uspension through a very tiny aperture against
... capillary forces. The filtration system
... reduce plugging to manageable limits.

... may be possible to adapt the aperture system
work with small apertures (~10µ diam.)
...follows:



 3 May '65 continued.

The problem with the present unit is that the diameter of the jet is ≤ that of the sensing aperture. At jet diameters of ~10μ I fear the huge surface tension forces would cause the jet to break into random sized drops before I could cause the jet to break up smoothly; the idea on the preceding page may circumvent the problem by allowing the jet diameter to be larger (hence manageable). The secondary stream then would allow us to break up a manageable jet column into manageable sized droplets.

3 May '65 Mack J. Fulwyler
Dwight H. Langham

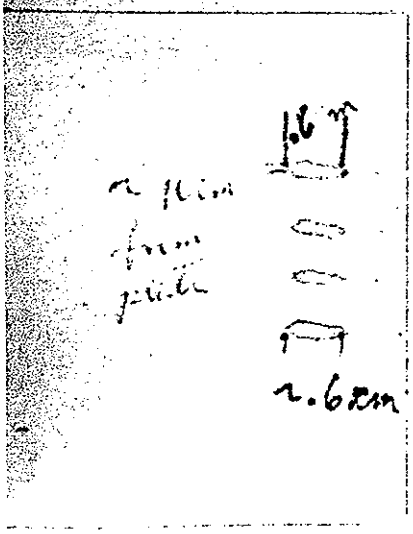
The coincidence system mentioned on page 88 may be used for any two independently measurable characteristics of a cell.

3 May '65 · Mack J. Fulwyler

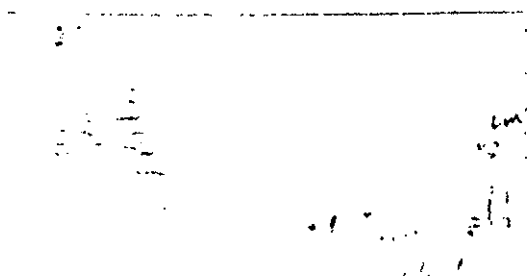
Ink

In order to get some idea how big a spot the impact point deflected droplet might be, I filled the separation reservoir with water soluable blue ink. I then pulsed the charging system and caught the deflected droplet on blotter paper. Operating condition were, 53 psi, 72 KC/sec, 30 volt charging pulse

Results.



The ~~filter~~ blotter paper was ~ 10 cm from lower edge of diflection plate. I estimate the spread of the droplets in the deflection plane to be ~ 6 mm & perpendicular to the diflection plane to be < 1 mm.

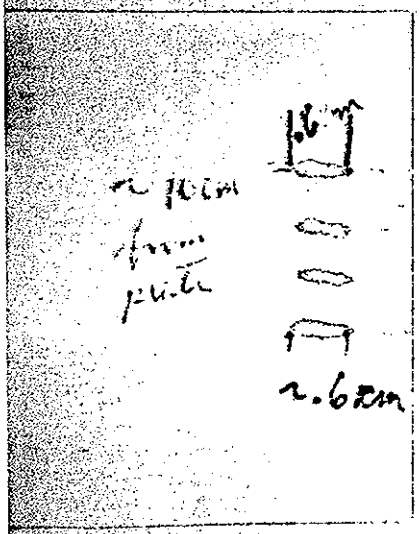


Here the blotter paper was ~ 2 cm from lower edge of diflection plate. I estimate the spread to be ~ 2 mm // to diflection plane & ~0.2 mm ⊥ to diflection plane.

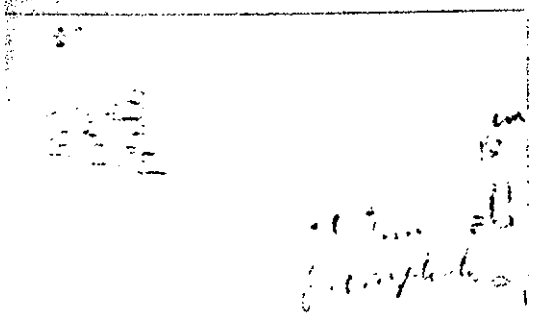
Here the blotter paper was ~ 2 cm from plate.

In order to get some idea how big a mist the impact point deflected droplet might be, I filled the separation reservoir with water soluble blue ink. I then pulsed the charging system and caught the deflected ink droplet on blotter paper. Operating conditions were; 53 psi, 72 KC/sec, 30 volt charging pulse

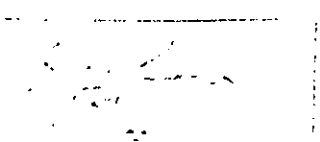
Results.



The blotter paper was ~ 10 cm from lower edge of deflection plate. I estimate the spread of the droplets in the deflection plane to be ~ 6 mm & perpendicular to the deflection plane to be < 1 mm.



Here the blotter paper was ~ 2 cm from lower edge of deflection plate. I estimate the spread to be ~ 2 mm // to deflection plane & x 0.2 mm ⊥ to def. plane.



Here the blotter paper was ~ 2 cm from plate. I attempted to magnify with the paper, but not very much. Here the dispersion made

32 20 May Separation Rate Experiment

1. Fluid flow rate -

Pure saline under 53 psi, 72°C & ~40

50 gauge.

Flow rate — 4.7 cc / 4 min 26 sec - 4.7 cc

— 4.6 cc / 4 min 24 sec — 4.6 cc

— 4.2 cc / 4 min — 42 cc

1st min — 1.06 cc/min

2nd min — 1.045 cc/min

3rd min — ~~1.05~~ 1.05 cc/min

Average — 1.05 cc/min -

June '65

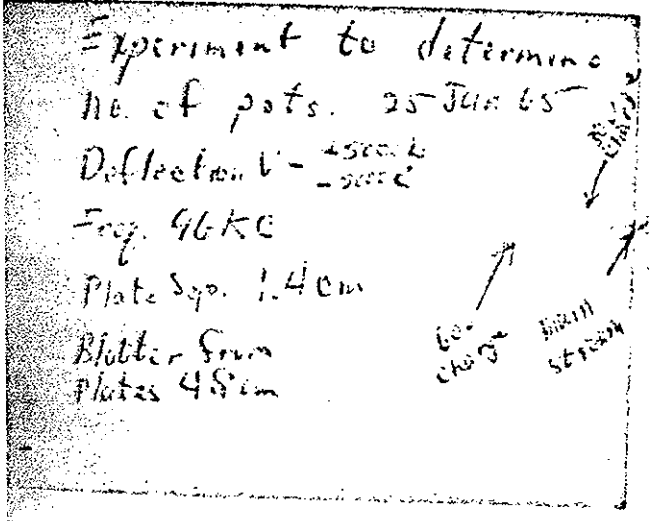
No. of Pots

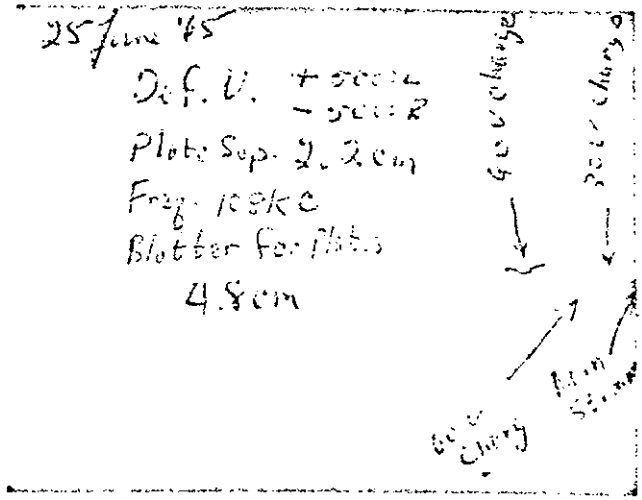
This experiment has several purposes:

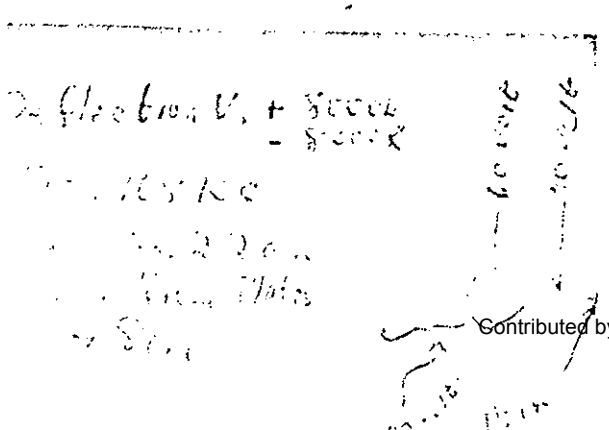
1.) to see just how many pots might be possible for the new charging system (+ & −) still to be received.

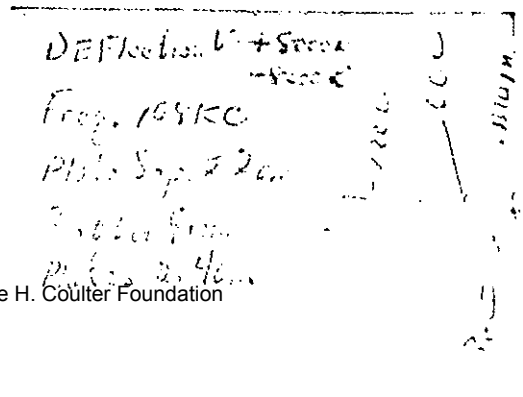
2.) to see just what deflects result at what distances from the gun for various operating conditions.

Result:









This experiment has several purposes:

1.) to see just how many pots might be possible for the new charging system (+ & −) still to be received.

2.) to see just what deflects result at what distances from the gun for various operating condition.

Result:

Experiment to determine no. of pots. 25 Jun 65
Deflection V − +5000 & −5000 R
Freq. 96 KC
Plate Sep. 1.4 cm
Blotter from Plates 4.8 cm
60 charge
Main stream

25 June '65
Def. V. +5000 & −5000 R
Plate Sep. 2.2 cm
Freq. 108 KC
Blotter for Plates 4.8 cm
60 V charge
30 V charge
60 V charg
Main stream

Deflection V. +8000 & −8000 R
Freq. 105 KC
Plate Sep. 2.2 cm
Blotter from Plates 4.8 cm
60 volt
30 volt

Deflection V +5000 & −5000 R
Freq. 105 KC
Plate Sep. 2.2 cm
Blotter from Plates 2.4 cm
120 V
60 V
30

Model 2 Gun

The first gun of the model 2 design has built and operates very well. There appear a few obvious improvements that are feasible; discuss them with Tom Carrol.

The mounting of a glass disc to the platinum while compressing an O-ring proved easier than [illegible]. I am further considering the possibility drilling (or lapping) a hole through a platinum [illegible] laminate. A precision platinum machinist [illegible] main shops thinks he may be able lap a hole through glass; if so a platinum laminate may be the solution.

[illegible] hole through the platinum was a stepped-hole [illegible] after electropolishing had an entry side diameter approx. 78µ and an exit diameter of approx. 70µ. [illegible] glass disc was somewhat square in cross section [illegible] of 87µ and a maximum [illegible] 91µ.

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my Red Blood Cells using [illegible]
which appears to have no effect on [illegible]
formation.

In trying to obtain (settle on) suitable
droplet formation the following results were
obtained

Run #1 Model 2

Droplet Forming Parameters, in buffered saline
aperture current 5 [illegible].

a. At 32 p.s.i. a smooth - stable stream
is obtain which can be made to break
at
40 k.c. - 60 v. R.M.S.

b. At 25 PSI. A smooth stable stream
stream with breaks nicely at
24.3 KC 27 v. R.M.S.

c. 20 PSI A smooth stable stream
which breaks poorly at
35.6 KC 12 v. R.M.S.

d. 35 P.S.I.
Nice good formation