

## Introduction

Dynamic Field Gradient Focusing (DFGF) is a process used to separate molecules based on their electrophoretic mobilities. A linear electric field is applied to a channel that exerts a force on charged molecules in the opposite direction of flow. Because the slope of the field causes the force to increase in magnitude as a molecule approaches the outlet, eventually the force exerted by the field will oppose in equal magnitude the force exerted by the flow, causing the molecule to focus at a specific point in the channel. A common issue with designs similar to mine is that they depend on a membrane to supply the field. However, membranes allow small molecules to pass through them rather than focus. Our design seeks to eliminate this problem by placing electrodes directly in the channel, eliminating the need for a membrane as can be seen in Figure 1.

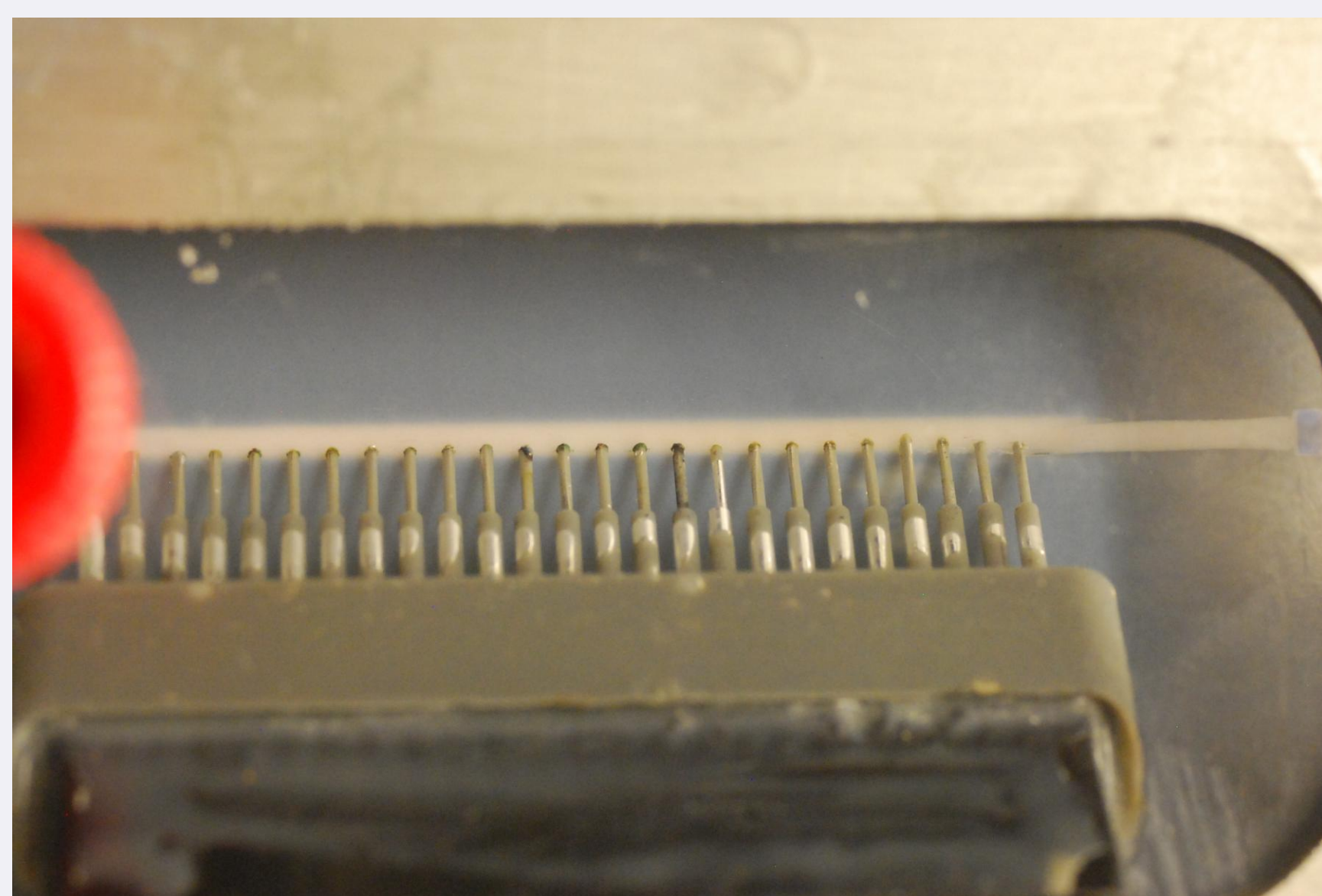


Figure 1: A picture of the unloaded apparatus.

The channel is 200 microns deep and one millimeter wide. In total there are 25 equally spaced electrodes. The volume is roughly four microliters.

## Run Conditions

For our experiments, we chose to run with 300 mM Bis Tris titrated with EACA to pH 8.7 for its high buffering capacity and low conductivity. We ran with Amaranth, Bromophenol Blue, and Methyl red both for their distinct color relationship and their small sizes to show that this apparatus was capable of separating molecules that others could not. For these conditions, we found a field with a top end potential of 15.5 V/cm to work the best. The field can be seen in Figure 3. The voltage is controlled by using a device that allows us to set the potential at the first, last, and middle electrodes, forming a linear field from three specified points.

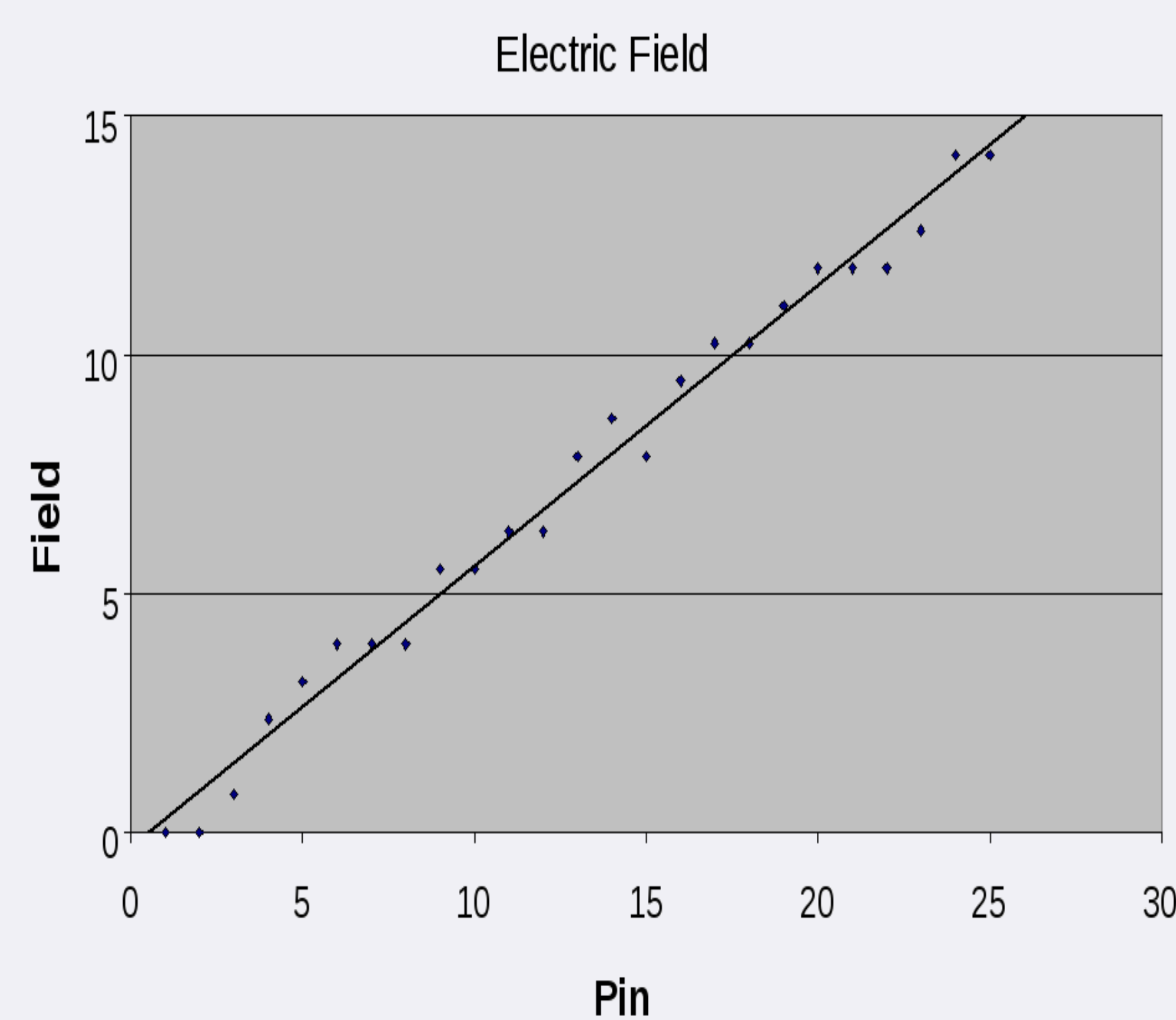


Figure 2: A graph of the electric field.

## Solution For Electrolysis

An early emerging flaw with our in channel design was that of water undergoing hydrolysis at the electrodes due to our applied field, water would produce hydronium and oxygen gas on the anodes, and hydroxide anion and hydrogen gas on the cathode. These would be visible as in-channel bubbles that could potentially disrupt our separations. To solve this issue, we designed the apparatus such that a house vacuum could be pulled under the channel. Porous ceramic was placed in between the channel and the vacuum, and a 200 micron thick layer of Teflon was used in between the channel and the ceramic. This allowed the gases to dissolve in the Teflon, and the vacuum to remove the gases in the Teflon via the vacuum. This can be done continuously while the apparatus is running, permanently preventing bubble formation. However, due to twice as much hydrogen gas being produced on the single cathode in our system, gas bubbles can often be seen near the outlet. This presently does not adversely affect experiments due to the focusing usually taking place towards the center of our channel.

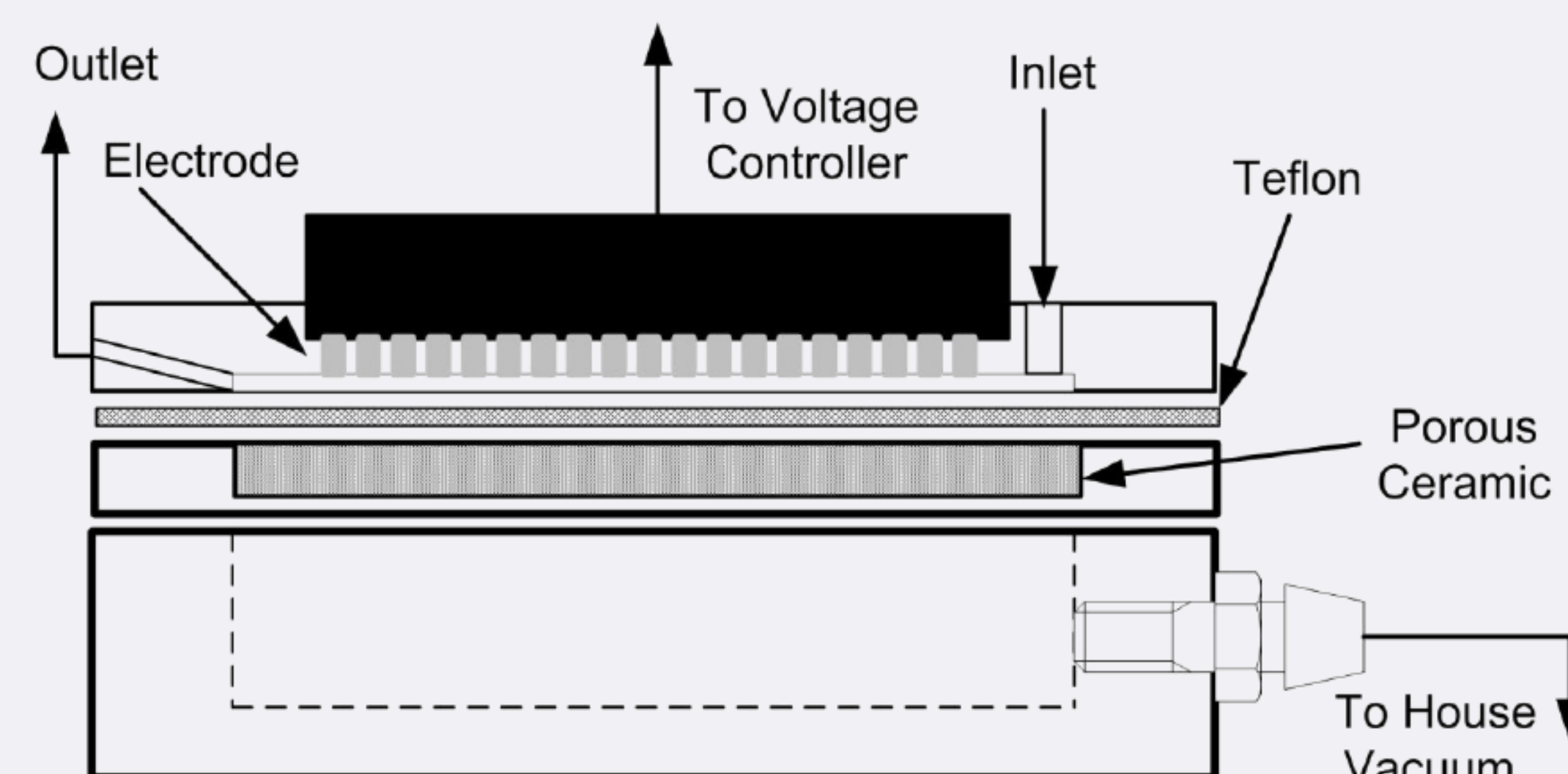


Figure 3: A side view of the apparatus

## Results

We successfully achieved a three part separation of the dyes. We began our runs by filling the entire channel (roughly 4 microliters) with set concentrations of the dyes (0.075 mg/ml for BpB and MR, 0.0375 for Am) and then allowed it to focus for several hours in the field shown in Figure 2.

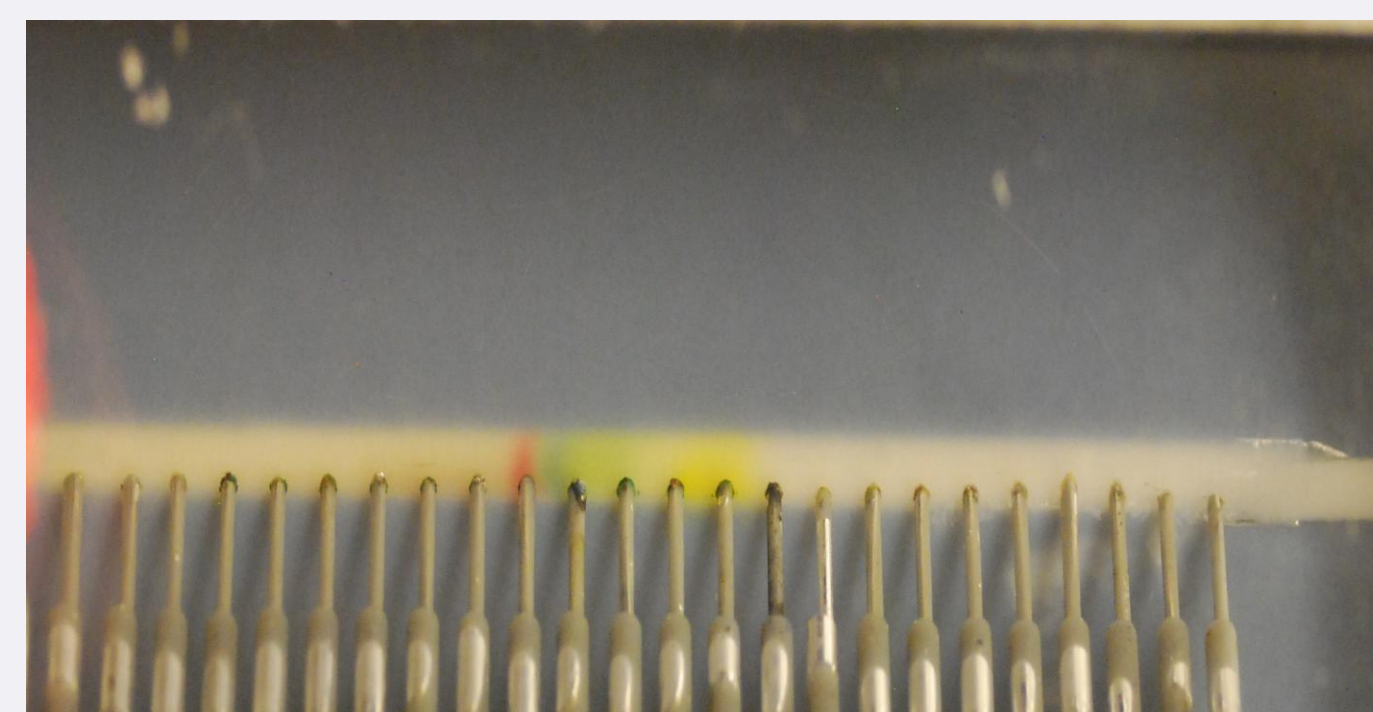


Figure 4: Amaranth, BpB, and Methyl Red after one hour in the chamber.

Typically, it takes two to three hours to fully focus and separate. Larger concentrations of dye can take more time while smaller concentrations tend to come apart more readily. Larger concentrations of dye can also disrupt the field by drawing too much current, so best results were usually achieved with small load sizes.

This is a picture taken after one hour in the chamber. The dyes are already beginning to stack out. Methyl Red (the yellow) is the broadest due to its small size.

Once in the chamber, the dye is stable for long periods of time, this is helpful in that it allows our variable field to be tweaked over long periods of time during runs to either further separate the samples, or even potentially elute.

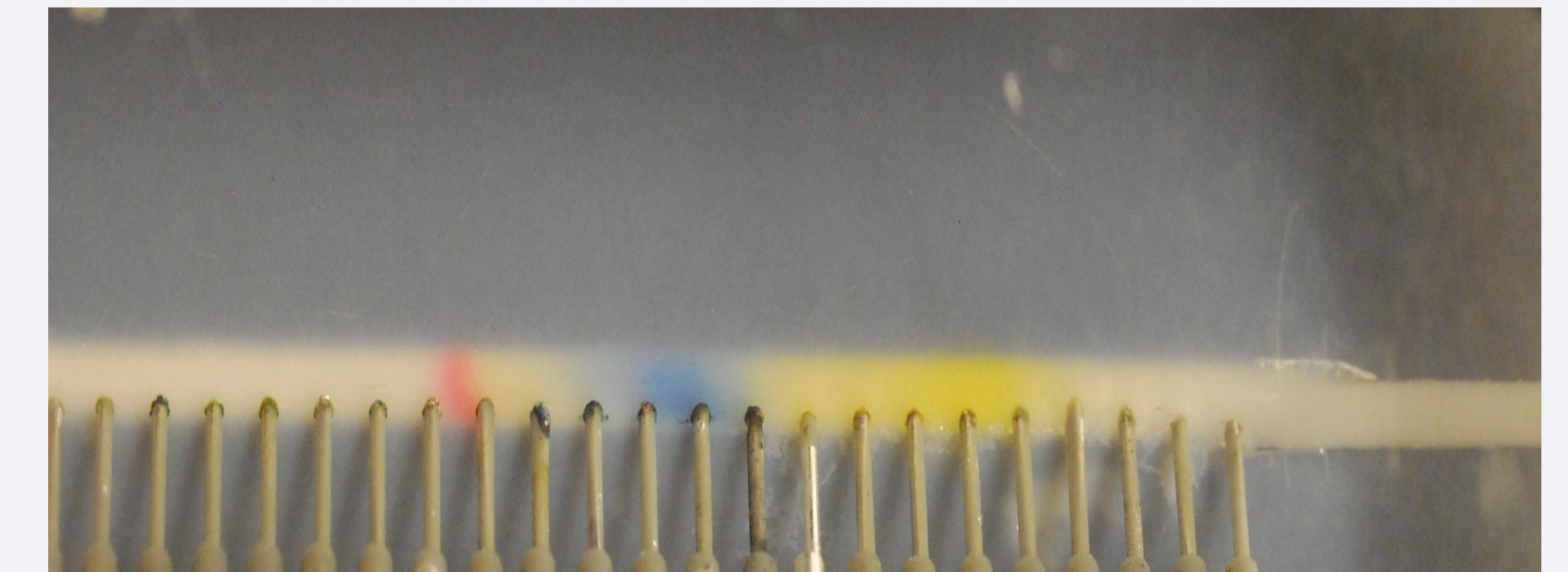


Figure 5: A focused Sample

The focused and separated samples here are still stable after being left with the field on for over ten hours. The stability also allows us to constantly feed in lower concentrations while allowing the sample to build up over time, which could have useful applications in focusing a low abundance species among a high abundance species.

## Conclusion

Our experiments showed that in channel electrodes could be used to bypass the need for a membrane. This allowed us to focus and separate three small dyes, the smallest of which (Methyl Red) had a molecular weight of 269.3 g/mol. We were also able to solve some problems associated with in channel electrodes such as gas production due to electrolysis. Our apparatus also kept stable conditions for long periods of time without notable loss of sample or degradation.

## Future Work

Presently we are moving onto similar designs with smaller channels. Our current 200 micron deep channel requires packing to achieve decent resolution while focusing. However, packing can slow down the focusing process to a large degree due to the samples having to navigate through it, this can be seen in the broadness of the Methyl Red. We have already developed a 25 micron deep channel that does not need to be packed and we have begun running tests on it. It has been made by imprinting a channel on a 200 micron thick piece of Teflon, removing the need for the acrylic to have the channel embedded into it, this will also allow us to change channel size and depth as needed. We are also looking into the possibility of using an external electrode for the cathode. This would eliminate any possible interferences caused by gas bubble formation near the outlet and make eluting the separated samples more simple.

## Acknowledgements



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