## Olfactometer

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version 1.1 07.13.2010

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The odor delivery system consists of one or multiple individual cassettes.

The schematic of a single cassette is shown on Fig. 1. Each cassette is an air dilution odor delivery system. The odorant line is controlled by MFC (range 0-100 cccm/min). To reduce the oxidation of odorants, this line is filled with Nitrogen. In a default state, the gas is flowing through an empty vial. Most of the time these valves are open, thus, they are chosen to be Normally Open (NO) valves. (Note: keeping valves continuously activated leads to heating of the manifold, which may cause slow deformation of Teflon and a potential leak.) During an odor delivery, these valves are closed and a pair of valves connected to an odorant vial are opened; total 4 valves are activated simultaneously. The odorant flow merges the dilution air line, which is controlled by MFC (range 0-1000 sccm/min). The final odorant concentration is equal to the head space vial concentration divided by the ratio of Nitrogen flow and total flow (Nitrogen + air flow). All lines, which are exposed to multiple odorants, are made of Teflon.

The cassette is assembled from 5 valve manifolds (Fig.2). The choice of NResearch valve manifolds is defined by two criteria: first, valve seats are located in the main stream flow, that ensures continuous washing of the odorant residues and negligible dead space; second, it is easy to assemble 5 valves together. Fig. 3 shows the assembly of the olfactometer cassette. The part list is given in Table 1 and 2.

Mass flow controllers (MFCs) in the current configurations are from Bronkhorst. Other MFCs can be

installed. The current electronic board is configured for these MFC, but can be modified for others MFCs, both digital and analog.

The electronics and software are developed by RPMetrix. For details see RPMetrix LASOM2 manual. (Tom Coradetti <tc@rpmetrix.com>)

It takes approximately 0.5-1 sec to stabilize the odor flow concentration. This timing depends on the vial position. To make delivery of all odorants similar and fast, we implemented the final valve (see air flow schematics at Fig. 4 and 5). The final valve is a dual synchronous 3-way valve with 4 ports from NResearch with minimal dead space. In the normal position, the clean air line is connected to the odor port and cassette flow is connected to exhaust. Approximately 1 sec after activation of odorant valves, the final valve can be switched and odorant flow is diverted to the odor port.

All tubing is 1/16 OD, 1/32 ID Teflon tubing. Thin tubing provides good mixing and fast odor delivery; however it requires relatively high inlet pressure (to be checked ...)

The impedances of the exhaust line and odor port line should be matched to prevent pressure jumps during final valve switching. The electronic pressure gauge connected to the exhaust line is used to monitor pressure jumps. Pressure jumps can be also monitor by rotameter readings.

## Major changes in Version 1.1

The recent experiments with multiple odors revealed a potential source of cross contamination. The scenario is the following.

- 1. Open a vial, which is close to MFC. The pressure in  $N_2$  line is increasing.
- 2. Close this vial. The vial will stay with high pressure.
- 3. Open vial far away from MFC. The pressure in  $N_2$  line drops.
- 4. Again close the distant vial and open the first one. The first one had a higher pressure inside, so the gas will expand in the N2 line.

In the previous version (v.1.0), the  $N_2$  line has a dead space, and never been completely washed. It may cause to cross contamination between vials.

Solution: We moved the empty vial and two Normally open valves to the end of N2 line.

We are also working on developing the different timing strategy. We can first close the distant valves and let the pressure in the  $N_2$  line build up, then, some time later, open the vial close to MFC. The timing will be implemented soon in electronics.

I am actively using the current version with an empty vial far away from MFC. Have not found any cross contamination, so far.

## Minor changes:

fitting part # from NResearch is corrected.

<u>Table 1.</u> Purchased parts (for one cassette)

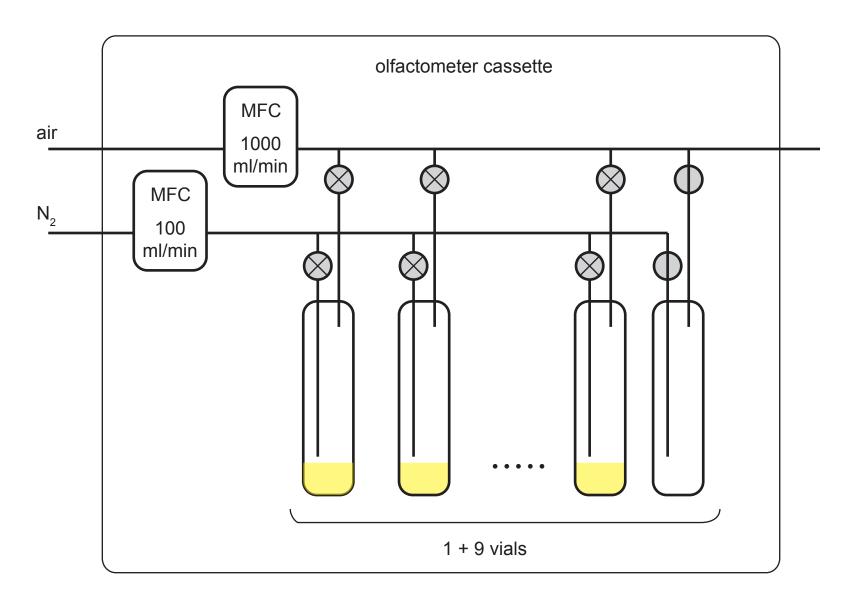
| Fig. | #  | Description                                   | Vendor       | Part #              | Quant.      | Price per unit |
|------|----|---|--------------|---------------------|-------------|----------------|
| 3    | 1  | MFC 1000 sccm/min Air <sup>1</sup>            | Bronkhorst   | IQF-200C-AAD-00-V-A | 1           | \$1,254        |
|      | 2  | MFC 100 sccm/min N <sub>2</sub> <sup>1</sup>  | Bronkhorst   | IQF-200C-AAD-00-V-A | 1           | \$1,254        |
|      | 3  | Valve manifold (5NC valves) <sup>2</sup>      | NResearch    | 161T102HH2          | 2 (or 4)    | \$323.31       |
|      | 4  | Valve manifold (1NO-4NC valves) <sup>2</sup>  | NResearch    | 161T102HH           | 2           | \$323.31       |
|      | 5  | teflon tubing: OD 1/16, ID 1/32               | NResearch    | TBGM109             | 10 ft       | \$1.49/ft      |
|      | 6  | Two piece fitting (1/4-28)                    | NResearch    | 252P109-50          | 1 (50 pack) | \$155.47       |
|      | 7  | Plug (1/4)                                    | NResearch    | FITM128             | 1           | \$1.56         |
|      | 8  | Barb fitting (zero dead volume) (1/4-28-1/16) | NResearch    | FITM331             | 2           | \$2.42         |
|      | 9  | Vial with septa                               | Fischer Sci. |                     | 10 (15)     |                |
|      | 10 | Electronic controlled board                   | RPMetrix     | LASOM2              | 1           | \$1350         |
| 4&5  |    | Dual synchronous 3-way valve(4 ports)         | NResearch    | SH360T042           | 1           | \$162.69       |
|      |    | Pressure regulator                            | Fairchild    | 10202               | 1           | \$90.37        |
|      |    | Pressure regulator (low bleed) <sup>3</sup>   | Fairchild    | 10202B              | 1           | \$100.25       |
|      |    | Shut off manual valve                         |              |                     |             |                |
|      |    | Pressure gauge                                | McMaster     | 4089K734            | 2           | \$16.67        |
|      |    | Rotameter (0-1200 cccm/min)                   | Aalborg      |                     |             |                |
|      |    | Needle valve                                  |              |                     |             |                |

Fittings and mounting of MFC are not specified. Must fit to a specific MFC
These manifolds are special order from NResearch. They came with 6" wire leads and Molex connectors (pins: 43030-0010, housing: 43645-0200). The connectors fit the valve board connectors.
Low bleed pressure regulator is used for N<sub>2</sub> line, to reduce N<sub>2</sub> lost.

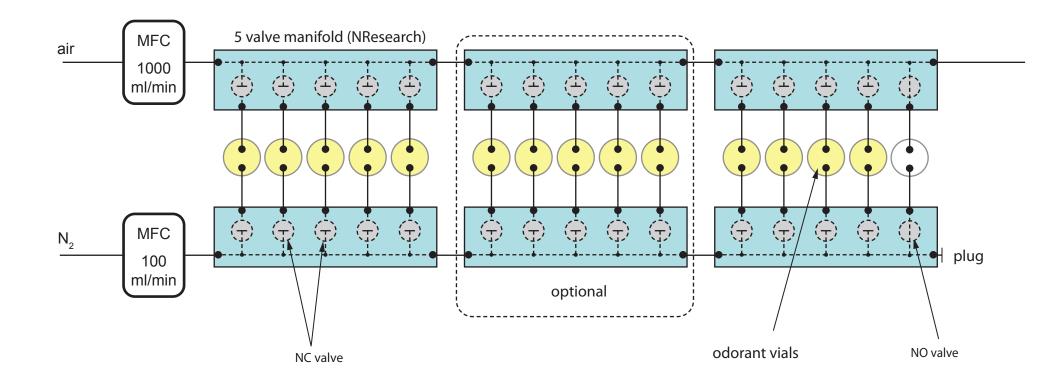
<u>Table 2.</u> Manufactured parts<sup>4</sup>

| Fig | #  | Description          | Quant. | Comment                        |  |
|-----|----|----------------------|--------|--------------------------------|--|
| 3   | 11 | Olfactometer panel   | 1      |                                |  |
|     | 12 | Based support member | 1      | cut from Item or 20x40 profile |  |
|     | 13 | Side member          | 2      |                                |  |
|     | 14 | Support bracket      | 2      |                                |  |
|     | 15 | Vial support         | 2      | printed part                   |  |

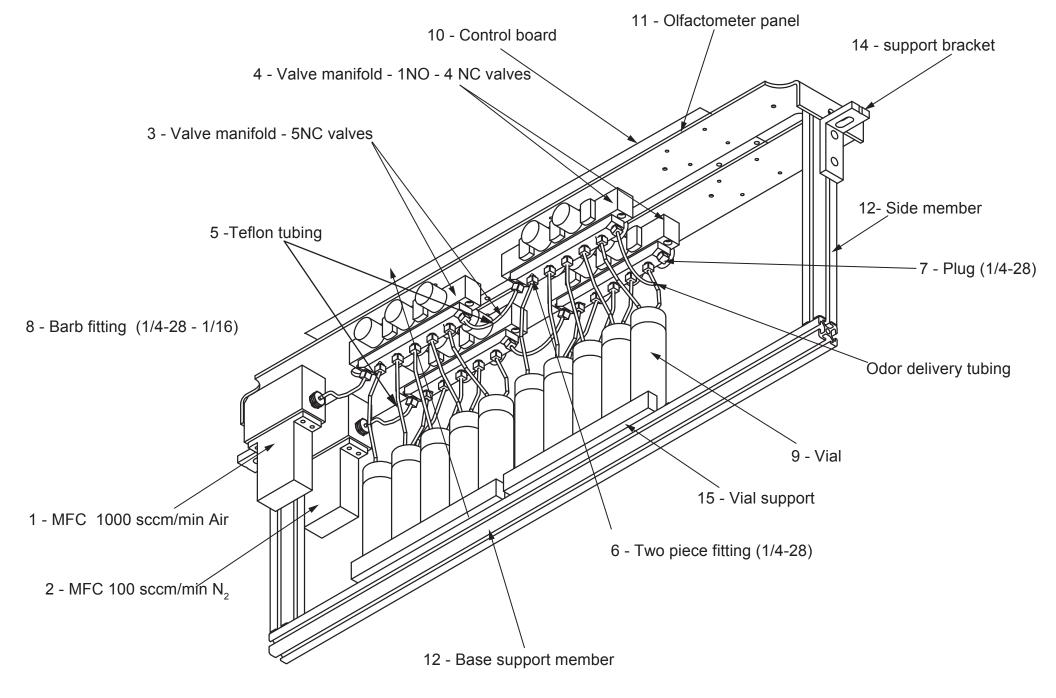
<sup>&</sup>lt;sup>4</sup> Part drawings are available upon request: Tanya Tabachnik <tabachnikt@janelia.hhmi.org>



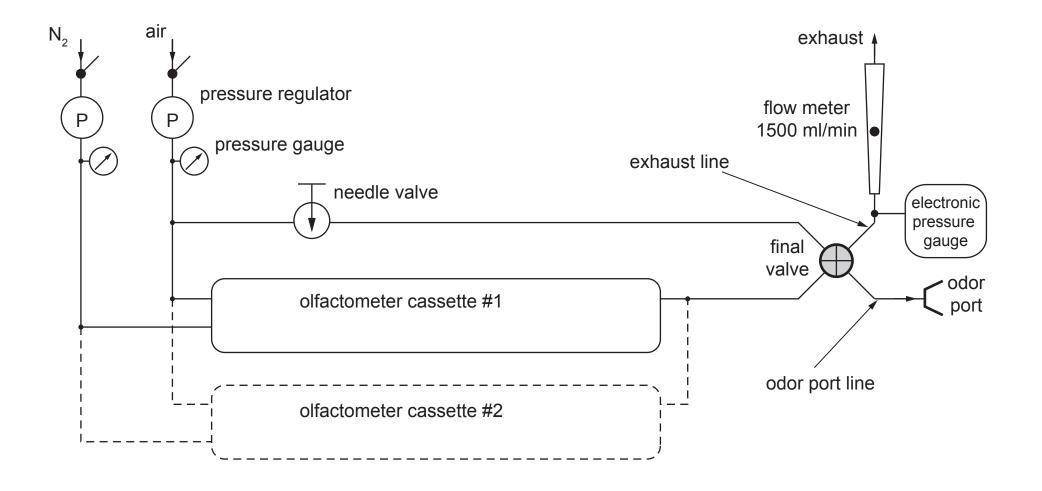
**Figure 1**. Gas flow schematics for the olfactometer cassette. The cassette consists of two Mass Flow Controllers (MFCs), main line (air): 0-1000 cccm/min and the odorant line  $(N_2)$ : 0-100 cccm/min; 10 vials, one empty vial and 9 odorant vials, and 10 pairs of on-off valves. In the default state the valves connected to an empty vial are open. During odor delivery these valves are closed and a pair of valve connected to an odorant vial are open. The concentration of the delivered odor is defined by the concentration of vapors in the vial headspace and the ration between main and odorant lines.



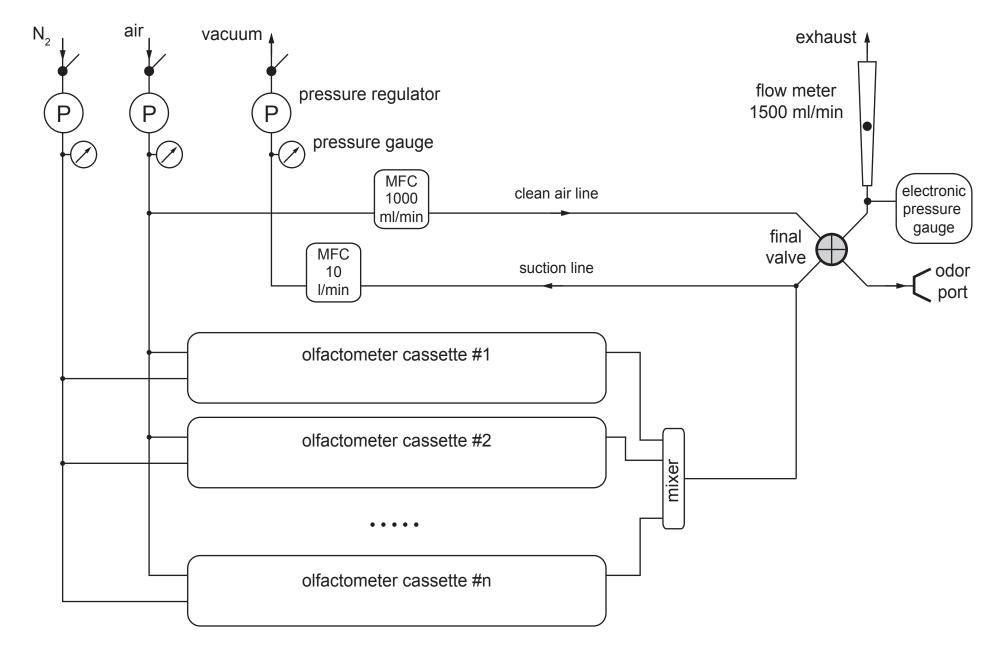
**Figure 2**. Single cassette schematics with NResearch 5 valve teflon manifolds. Implementation of the sceme shown on Fig.1 with NResearch 5 valve manifolds. The main advantage of these maniflds is that the distance between the valve and the main air line is minimal. The air flow continiously wash out the odorant resedues, after a valve is switched off, thus minimizing the chance of cross-contamination. The cassette may have 10 or 15 vials.



**Figure 3**. Single olfactometer cassette assembly schematics. The purchased and manufactured part lists are in Table 1 & 2 .



**Figure 4.** Gas flow connection for a single cassette olfactometer. The cassette is described on Fig.1-3. Extra parts include: pressure regulators for Air and  $N_2$ , pressure gauges, a needle valve, a rotameter - flow meter, a final valve, an electronic pressure gauge. Note: the flow impedances of exhaust and odor port lines need to be equilibrated, to prevent pressure shocks during final valve operation. An electronic fast pressure gauge (not specified) is added to exhaust line to tune the lines and minimize the pressure jumps. The second olfactometer cassette can be connected in parallel with the first one. All gas lines, which are exposed to odorants, are made of Teflon.



**Figure 5.** Gas flow connection for a multi cassette olfactometer (n>2). In addition to the scheme on Fig. 4, the following parts are added to the system: vacuum line, which has its own vacuum regulator and mass flow controller (0-10 l/min), and the clean air line has MFC to regulate the flow. This design allows control of the concentration in a much larger range. Adding additional flows via all cassettes dilute the odorant flow. The main stream is diverted to suction line and only a small portion is delivered to final valve and odor port.