**Reviewer #1:**   
*Manuscript Summary:*  
This manuscript describes an effort to construct a low gravity flow cytometer. The article focuses on what is seemingly mundane topics such as sample delivery and containment that are routine at normal gravity. Other than some simple pulse data, no actual flow cytometry data is presented. The authors state the instrument is not capable of performing cellular measurements beyond a WBC detection that simply showed random pulses. No description of controls or other details provided. This paper needs to be rewritten with an attempt to provide enough information for evaluation of the work.

**Responses:**

-With respect to data presentation – the representative data corresponds to the 3 major test objectives of our parabolic flights: (1) Successful blood-saline (and dye mix) in reduced gravity, (2) Ability to detect fluorescent particle samples loaded into the system under reduced gravity, and (3) Demonstration of the cytometer to detect various types of flowing samples (including pre-stained WBCs).

-With respect to controls – controls are identified within the protocol as ground performances of the same demonstrations (see 2.3.1.). There are no comparable ‘gold standards’ for the sample loader or micromixer, although we agree that a quantitative flow cytometer being tested should ideally be compared to a commercial ground cytometer using identical samples to demonstrate fidelity of measurement.

**Changes**

-Changed title to better reflect the purpose of paper as an example guide to parabolic flight hardware assembly/demonstration (i.e., less about the novelty of a reduced-gravity ready cytometer).

-Added supplementary Figures with additional data traces and analyses in support of the presented method.

-Fixed typos in sentence describing controls in 2.3.1

*Major Concerns:*  
1. There is no discussion of specific performance of target metric What would a successful instrument be able perform, what assays, what cell rates, what sensitivities? Etc...

**Response/change(s):** Performance metrics of interest added to caption of Fig. 7. This particular system did not have quantified performance targets

2. There are many control experiments that could be performed to compare prototype performance with existing cytometer performance on the ground.

**Response:** See 1st response above**.**

3. There is no details provided on where components were purchased or made. The methods of how work was accomplished was provided as a randomly highlighted protocol list.

**Response/change(s):** Component sources, when included, are described in the Materials List. We have opted not include custom engineering/machining procedures. Highlights in the text indicate procedures selected for filming in conjunction with JoVE editorial staff.

4. The discussion of past and current efforts of relevant point of care flow cytometers (including those that were designed for low gravity such as the original NPE instrument) was not provided in any detail.

**Response:**  From our understanding, the NPE analyzer was built to meet design specifications that came out of the NASA/American Cancer Society High-Resolution Flow Cytometry Project. However, we are unable to find any evidence that the NPE instrument was ever actually tested in reduced gravity, and therefore we did not consider it initially as an example of a past demonstration that newcomers can look to for parabolic flight guidance. We did list flow cytometers we find ‘relevant’ in the introduction, based on documented reduced-gravity demonstrations (specifically including those from Crucian, Sams, and Y-C Tai, plus the recent publication on the Microflow1). Current NASA scientists focused on in-flight diagnostics (i.e., co-authors on this paper) reviewed the cited list of past examples before manuscript submission and did not determine that relevant cytometers had been excluded.

**Change:** Expanded list of ‘relevant’ cytometers to include those tested in reduced *or designed with reduced gravity in mind.* The NASA/ACS High-Resolution Flow Cytometry Project publications did produce useful guidelines still high relevant today, and thus we agree that the NPE instrument should at least be mentioned.

*Minor Concerns:*  
1. The paper seemed to be very roughly written and just and excerpt from a report.  
  
*Additional Comments to Authors*  
N/A  
  
  
**Reviewer #2:**   
The manuscript presents a modular method for the construction and operation of a prototype blood diagnostic device in microgravity, based on a flow-based optical system. The design, development and testing is straight forward, systematically described and in-flight experimental procedures were mentioned in detail. Such "point of care" biomedical analytic equipment is without any doubt of highest relevance for future long-term space flights.  
  
The manuscript is very well written, however, some improvements could be recommended:  
  
-The authors described that "countless POC devices have been developed to mimic laboratory scale counterparts, but most have narrow applications and few have demonstrable use in an in-flight, reduced-gravity environment." To substantiate this statement, a short table summarizing the previous development, their field of applications, advantages and disadvantages would be useful.

**Response:** It’s not clear to us whether ‘previous development’ in the above comment refers to miniaturization efforts in general (i.e., **all** POC) or POC specifically utilized in the reduced-gravity environment. Note that the quoted sentence is from the Long Abstract and not the main body of the introduction where previous in-flight *in vitro* diagnostics are already described.

**Change(s):** Changed the text describing the previous cytometers for more clarity. Tabulating the information did not seem to produce favorable results (i.e, took up more space than it saved and didn’t contribute significantly to understandability).

- The long list of examples between line 148 and line 167 could be presented also in a short table.  
The authors did not describe the flight profile of the "modified privately operated, narrow-body jet airliner", the type of the airlines and the quality of microgravity.

**Response/change(s):**

-Significantly condensed the information in tabulated form (Table 1).

-Flight profiles for reduced-gravity testing do not vary significantly between flights. Added references for descriptions of typical trajectory and NASA web post regarding the flight week. JoVE discouraged us from including specifics about the plane make/model.  
  
- In the discussion, a short comparison with previous developments (e.g. in fulfilling specific requirements) is missing.

**Response/change(s):** Added paragraph – new 3rd paragraph in the Discussion.

**Reviewer #3:**   
*Manuscript Summary:*   
No comments  
  
*Major Concerns:*  
No major concerns  
  
*Minor Concerns:*  
- Reference 17 is applied to two different articles. The reference for Laval University's work is missing.

**Response:** We cannot find any published references for the Laval University technology.

**Change(s):**

**-**Deleted second ‘17’ citation.

-Added new ref (webpage) for the Laval U work plus a paper describing the technology outside the context of reduced gravity.

- Line 231: the word 'fluid' is repeated twice.

**Change(s):**

**-**Deleted extra ‘fluid’.

*Additional Comments to Authors:*  
Excellent manuscript providing clear experimental steps and very useful direct experience that will be appreciated by any investigator testing hardware in parabolic flights.t

**Other changes:**

-Deleted an unintended ‘3’ in near the end of the Long Abstract

-Several typos fixed throughout

-Thiel et al. (2012) was present twice in the Reference list (eliminated 1 entry.

-Re-evaluated inclusion of some of the References (particularly the previous 1-4, which have been removed)

-Reference list almost entirely reordered, with several other references added.