Minimum Information about a Flow Cytometry Experiment

MIFlowCyt 1.0

A standard for outlining the minimum information required to report the experimental details of flow cytometry experiments

Document Status

This is an ISAC Recommendation. This document has been reviewed by members of the International Society for Analytical Cytology (ISAC) and other interested parties and has been endorsed by the ISAC President and ISAC Council as an ISAC Recommendation. It is a stable document and may be used as reference material. This document has been produced by the ISAC Data Standards Task Force (ISAC DSTF) and other members of scientific community as acknowledged below. Different editors are responsible for different parts of this specification.

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Abstract

The fundamental tenet of scientific research is that the published results of any study have to be open to independent validation or refutation. The Minimum Information about a Flow Cytometry Experiment (MIFlowCyt) establishes criteria for recording and reporting information about the flow cytometry experiment overview, samples, instrumentation and data analysis. It promotes consistent annotation of clinical, biological and technical issues surrounding a flow cytometry experiment by specifying the requirements for data content and by providing a structured framework for capturing information.

Keywords: flow cytometry, experimental description, checklist, standard

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Introduction

Purpose of this Document

The purpose of this document is to establish criteria to record flow cytometry experiments in a way that provides enough detail to allow for correct interpretation of experimental details including samples, analysis and results.

Scope of this Document

This part of an analytical cytometry standard outlines the minimum information required to record and report flow cytometry experiments (the Minimum Information about a Flow Cytometry Experiment; MIFlowCyt) in order to promote the standardized documentation of experimental details. This document is not intended to address implementation details concerning the format of the provided information. However, these formats are being developed within coordinated efforts [1]. This document is not meant to replace any additional information already collected within any particular group [2]. We expect additional parts of the analytical cytometry standard (e.g., image cytometry) to include much of this material and to be developed in a similar way.

Terminology within this Document

The key words “shall”, “should”, and “may” in this document are to be interpreted as described in RFC 2119 [3] and are also compatible with the IEEE Standards Style Manual [4].

The word *shall* is used to indicate mandatory requirements to be followed in order to conform to the standard and from which no deviation is permitted *(shall* equals *is required to)*.

The word *should* is used to indicate that among several possibilities one is recommended as particularly suitable, without mentioning or excluding others; or that a certain course of action is preferred but not necessarily required; or that (in the negative form) a certain course of action is deprecated but not prohibited *(should* equals *is recommended that)*.

The word *may* is used to indicate a course of action permissible within the limits of the standard *(may* equals *is permitted to)*.

The use of the word “relevant” in this document describes the condition by which information should be provided: “relevant” information is information that is necessary for correct understanding of the context of an experiment component.

Manufacturers, Models, and Products Mentioned within this Document

Manufacturer names, product names, etc. used within this document are fictional and are intended for illustrative purposes only.

Abbreviations used within this Document

ATCC .... American Type Culture Collection
CD .... Cluster of Differentiation

CFSE.. Carboxyfluorescein diacetate succinimidyl ester
DICOM .... Digital Imaging and Communications in Medicine
FCS .... Flow Cytometry Data File
FITC .... Fluorescein isothiocyanate
FMO .... Fluorescence Minus One
FSC .... Forward Scatter
FuGO .... Functional Genomics Ontology (renamed to OBI)
HL7.. Health Level 7
IEEE .... Electrical and Electronics Engineers, Inc.
IL .... Interleukin
LOINC .... Logical Observation Identifiers Names and Codes
MeSH .... Medical Subject Headings
MIFlowCyt .... Minimum Information about a Flow Cytometry Experiment
NCBI... National Center for Biotechnology Information
OBI .... Ontology for Biomedical Investigations (formerly FuGO)
PBMC... Peripheral blood mononuclear cell
PE .... Phycoerythrin
PerCP... Peridinin chlorophyll protein
PMID .... PubMed Identifier
PMT.. Photomultiplier Tube
SA .... Streptavidin
SI................................ Le Système International d'unités (International System of Units)
SNOMED .... Systematized Nomenclature of Medicine
SSC.. Side Scatter
URL .... Uniform Resource Locator
URI .... Uniform Resource Identifier

Glossary

Parameter	A parameter is understood as a type of measurement based on a signal or signals produced by a detector or several detectors of an analytical instrument. A parameter value is a digital representation of an instance of a parameter measurement. The area of the signal detected by the first photomultiplier tube (abbreviated as FL1-A) is an example of a parameter. Parameters may also encompass mathematically transformed measurements such as log fluorescence or calculated measurements such as electronic opacity (radio-frequency impedance divided by DC impedance) or other normalized measurements, such as fluorescence divided by DC impedance or low angle scatter. Note that a parameter description constructed as a concatenation of the detector type, a sequential number and how the signal was processed (e.g., “FL1-W”) is appropriate for engineering studies only and it is not suitable for publishing experimental results, labelling graph axes, etc.
Electronic event	An event is a unit of data representing one particle (e.g., a cell) as detected by flow cytometer. Events are typically stored in list-mode data files. An event is a vector that has a value for each parameter.

List mode data file	A list mode data file is a file containing an ordered list of events. FCS files contain either list mode data or histograms. List mode data may also be stored as simple spreadsheet files, e.g., CSV files.
Fluorescence reagent	A fluorescence reagent is a chemical substance used in the detection of an analyte (e.g., anti-CD25-FITC). Many flow cytometry reagents have separate components playing the role of analyte detector and analyte reporter respectively (antibodies conjugated to fluorochromes) while others such as fluorescent dyes have combined analyte detector/reporter properties.
Analyte detector	A component of a fluorescence reagent plays the role of an analyte detector if it makes the analyte detectable. Typically, the analyte detector chemically binds to an analyte and thus can be used to detect the analyte.
Analyte reporter	A component of a fluorescence reagent plays the role of an analyte reporter if it reports the presents of the analyte. Typically, an analyte detector has fluorescence characteristics that are detectable by a flow cytometry instrument and thus useful for reporting analytes.
Analyte	Material (e.g., a substance or chemical constituent) plays the role of analyte in an experiment if it is the subject of interest of an analytical procedure within the experiment (i.e., it is being quantified in the experiment).
Optical detector	An optical detector is a part of a flow cytometry instrument that serves as a sensor of light or other electromagnetic energy that is being converted to electrical signal. Typically, photodiodes and photomultiplier tubes are used as optical detectors in flow cytometry.

Overview of MIFlowCyt Standard

Standards facilitate both the accurate communication of data by assuring that information is consistently reported, and the creation of tools to communicate and share experimental data [5-7]. MIFlowCyt promotes consistent annotation of biological and technical issues surrounding a flow cytometry experiment by specifying the requirements for data content and by providing a structured framework for capturing information. In addition to the methods and results of the experiments themselves, the standard also covers much of the information that would normally only be kept in the laboratory in which the data was produced, such as who performed the work and the hypothesis that prompted the work.

The elements of the MIFlowCyt standard are:

1. Experiment Overview
2. Flow Sample/Specimen Details
3. Instrument Details
4. Data Analysis Details

A MIFlowCyt-compliant flow cytometry experiment description shall include all relevant information specified in this standard. It is expected that information may be provided by several sources, including by explicit reference to documented protocols (e.g., through a URI) that contain the required information.

Vocabulary for Experimental Description

Where possible, experimental details should be described using terms from an ontology or controlled vocabulary or appropriate standard, such as the Ontology for Biomedical Investigations (OBI) [8]; formerly FuGO [9, 10]), MeSH thesaurus [11], NCBI taxonomy [12], HL7 [13], SNOMED [14], LOINC [15], DICOM [16], etc. and the source of the terms should be noted.

Units for Experimental Description

MIFlowCyt-compliant experiment descriptions shall include SI units. Use of SI metric units facilitates scientific communication, especially in international contexts. Descriptions may utilize alternative units of measure (e.g., Imperial units).

MIFlowCyt Components

A MIFlowCyt-compliant experimental description shall include information specified below. The list states the content of the provided information only; it does not imply the format of the information or whether an item should be directly provided or referenced.

1. Experiment Overview

The experiment overview shall contain the following information:

1.1. Purpose

A brief description of the goal of the experiment. This should include the rationale and hypothesis.

1.2. Keywords

The keywords should include terms from an appropriate vocabulary (e.g., MeSH) to describe the experiment.

1.3. Experiment Variables

Variables are attribute(s) that differ between samples within an experiment due to pre-existing differences in sample states or due to experimental manipulation of the samples. If applicable, a brief description of the conditional and/or manipulated variables in the experiment shall be provided (e.g., smoker vs. nonsmoker, IL-2 treatment vs. no treatment, knockout versus wild-type, varying number of transplanted cells, varying treatment dosage, etc.). The number of instances per experimental group should also be stated.

1.4. Organization

The following shall be specified for the organization performing the experiment:

1.4.1. Name

1.4.2. Address

1.5. Primary Contact

The following shall be specified for the experimental primary contact:

1.5.1. Name

1.5.2. Email Address

The description may include information for additional individuals involved in the experiment, including their contact details and their role.

1.6. Date

The date or time period during which the investigation was performed (i.e., from collecting and treating samples to performing data analysis) shall be stated.

1.7. Conclusions

A brief summary of the interpretation of the results or outcome of the experiment shall be provided if available.

1.8. Quality Control Measures

A description of the quality control measures used such as replicates, calibrations, control assays, etc. shall be provided. If another experiment was performed for the purposes of establishing quality control standards, that experiment may be referenced.

1.9. Other Relevant Experiment Information

Additional information about the experiment should be provided if relevant. This may include funding announcements, related publications (which should be referenced by PMID), URIs, databases, etc.

2. Flow Sample/Specimen Details

The flow sample details shall include a description of each sample material used in the experiment (2.1), (2.2), how they were treated (2.3) and what reagents were used (2.4) to fluorescently label the material. Relations between samples, aliquots, different treatments, and replicates shall be unambiguously described.

2.1. Sample/Specimen Material Description

Sample materials shall be described according to 2.1.1, 2.1.2, or 2.1.3, based on the type of the sample material. Each sample shall be distinguished from other samples within the same experiment.

2.1.1. Biological Samples

The following information about Biological Samples shall be provided:

2.1.1.1. Biological Sample Description

A description of the biological sample shall be provided, including the sample type, if relevant (e.g., C57BL/6 spleen, purified CD4+ lymphocytes, BALB/c thymocyte DNA, control patient PBMCs, protein lysate from lung cancer biopsy, peripheral blood from patient with Type I diabetes, liver biopsy, etc.).

2.1.1.2. Biological Sample Source Description

The source of the biological sample shall be described (e.g., wild-type mouse, C57BL/6 spleen, C57BL/6 splenocytes). If the source is a cell line the description shall include its name, ATCC [17] (or equivalent) number, and cell type.

2.1.1.3. Biological Sample Source Organism Description

2.1.1.3.1. Taxonomy

The source organism shall be specified by genus and species (e.g., *Mus musculus*). The terms should come from an appropriate standard such as the NCBI

taxonomy database [12]. Taxonomy information should also contain the type of subspecies and organism strain, if applicable. A standard taxonomy may be extended or a proprietary taxonomy may be used, especially if detailed identification is relevant and beyond the scope of standard taxonomies.

2.1.1.3.2. Age

The age shall be provided if applicable for the particular organism (e.g., 6 weeks). It may also include the developmental state (e.g., Theiler stage 23).

2.1.1.3.3. Gender

The gender shall be provided if applicable for the particular organism.

2.1.1.3.4. Phenotype

Appropriate phenotype characteristics such as disease state shall be described (e.g., increased tumorigenesis).

2.1.1.3.5. Genotype

Appropriate genotype characteristics shall be described (e.g., p53-/-).

2.1.1.3.6. Treatment

All source organism treatments shall be described (e.g., treatment with cyclooxygenase-2 (COX-2) inhibitor).

2.1.1.3.7. Other Relevant Biological Sample Source Organism Information

Additional information about the source organism should be provided if relevant. This may include anatomic location of the source, visit time/date, or additional details and comments.

2.1.1.4. Other Relevant Biological Sample Information

Additional information about the biological sample should be provided if relevant. This may include relevant information such as *in vivo* or *in vitro* treatment, stimulation, preparation / enrichment protocol, culture/growth protocol, collection time/date, whether the sample was frozen/banked/fixed, etc.

2.1.2. Environmental Samples

The following information about environmental samples (e.g., soil, sea water) shall be provided:

2.1.2.1. Environmental Sample Description

A description about the environmental sample shall be provided. The description should include relevant details such as collection time/date, collection protocol, type of sample (e.g., seawater), etc.

2.1.2.2. Environmental Sample Location

The location of the sample origin shall be specified. This should include longitude and latitude if relevant.

2.1.3. Other Samples

The following information about other samples (i.e., samples not qualifying as Biological Samples or Environmental Samples, such as calibrator samples) shall be provided:

2.1.3.1. Other Sample Description

A description of the sample shall be provided. The description shall include information about the nature of the material in the sample (e.g., dyed plastic beads).

2.2. Sample Characteristics

Key information about the possible and expected sample characteristics should be noted as they provide the rationale for the experimental design, including the choice of appropriate reagents. Sample characteristics description should include the expected and possible types of cells or other particles in the sample material.

2.3. Sample Treatment(s) Description

The description shall include details about treatment agents, which play the role of experiment variables, or shall specify that samples were untreated. For example, a sample treated with an agent might be compared to an untreated sample; when reproducing and interpreting such an experiment, having access to details on treatment agents and conditions is essential in order to query and retrieve samples for further analysis. The treatment description should also contain other relevant treatment details such as, but not limited to, time, temperature, and concentration.

2.4. Fluorescence Reagent(s) Description

The expected and possible types of cells or other particles in the sample material, and their expected and possible measurable characteristics should be noted as these represent the key information for choosing appropriate reagents with respect to the experimental goal. The following information shall be provided about each fluorescence reagent used. Information about the characteristic(s) being measured, as well as details about what plays the role of analyte, analyte detector, and analyte reporter should be descriptive enough to allow for accurate interpretation of the experiment data. Table 1 lists some examples of reagent descriptions.

2.4.1. Characteristic(s) Being Measured

The relative amount of molecules, properties, or processes being evaluated (e.g., CD25, apoptosis, membrane permeability, cell viability, oxidative burst). The characteristic(s) being measured shall be provided whenever there is ambiguity about the analyte being measured, such as when sample processing affects detection of the analyte (e.g., propidium iodide example in Table 1). The optical detector (e.g., FL1) or parameter (e.g., FL1-H, see 3.3.6) used primarily for this measurement shall be indicated.

2.4.2. Analyte

What plays the role of the analyte? Which substance or chemical constituent is the subject of interest of the analytical procedure, what target is specifically bound by the analyte detector (e.g., CD25)?

2.4.3. Analyte Detector

What plays the role of analyte detector? Which component of the fluorescence reagent specifically binds to the analyte to make it detectable (e.g., anti-CD25 antibody)?

2.4.4. Analyte Reporter (Fluorochrome)

What plays the role of analyte reporter? Which component of the fluorescence reagent reports the presents of the analyte to the flow cytometer? What substance (label) is used to generate the measured signal (e.g., FITC)?

2.4.5. Clone Name or Number

If the probe is a monoclonal antibody, the clone name or number shall be provided.

2.4.6. Reagent Manufacturer Name

The reagent manufacturer shall be specified (e.g., MIFlowCyt Reagent Inc.).

2.4.7. Reagent Catalogue Number

The reagent catalogue number shall be specified.

2.4.8. Other Relevant Reagent Information

When secondary antibodies are used, the primary analyte (the analyte bound by the primary antibody) and the reporter component of the secondary antibody (the reporter that generates the measured signal) shall be clearly specified as described in 2.4.2, 2.4.3, and 2.4.4. If relevant, the primary and secondary antibodies shall be described individually (e.g., in the case that the use of a secondary antibody significantly impacts the interpretation of results because of nonspecific fluorescence, etc.). The description should also contain other relevant details which may include lot number, concentration, label incorporation

method, separation technique, antibody staining procedures, and wash steps.

3. Instrument Details

The following details shall be specified about instruments used to analyze samples. It is expected that this information will be automatically provided by flow cytometry instruments in instrumentation description files along with list-mode data files, such as FCS files [18]. For instrument components that are not user configurable and that are part of a standard model, the instrument manufacturer and model number shall be sufficient if this uniquely specifies the required information. For all other cases where the make and model number is not sufficient to specify the required information, the individual component description shall be specified as described below.

3.1. Instrument Manufacturer

The instrument manufacturer shall be specified by stating its name (e.g., MIFlowCyt Cytometry Systems, Inc.). A URL pointing to manufacturer web pages may also be provided.

3.2. Instrument Model

The instrument model shall be specified (e.g., CytMeter Elite-2000). It may include the instrument serial number.

3.3. Instrument Configuration and Settings

The system and its configurations have significant impact on experimental results and as such the following shall be provided:

3.3.1. Flow Cell and Fluidics

The flow cell of the instrument shall be described as follows:

3.3.1.1. Flow Cell Type

The flow cell type (e.g., stream-in-air, cuvette) including the material shall be provided.

3.3.1.2. Other Relevant Flow Cell and Fluidics Information

Other relevant flow cell details should be provided and may include flow cell manufacturer name (e.g., MIFlowCyt Optics, Inc.) and model number (e.g., PRO-CFC-75), flow cell outside shape (e.g., rectangular with an attached sphere, spherical, square) and dimensions, flow channel shape (e.g., rectangular, circular, triangular) and dimensions, sheath flow velocity (e.g., 10 l/s), sheath flow pump type (e.g., gas pressure, vacuum, gear), sample flow velocity (e.g., 7.5 l/s) and sample flow pump (e.g., gas pressure, syringe).

3.3.2. Light Source(s)

Each light source (e.g., laser) shall be described stating the following:

3.3.2.1. Light Source Type

The type of the light source shall be provided (e.g., laser, diode laser, xenon lamp).

3.3.2.2. Light Source Excitatory Wavelength

The excitatory wavelength shall be provided (e.g., 488 nm, 633 nm).

3.3.2.3. Light Source Power at the Excitatory Wavelength

The power of the light source shall be provided (e.g., 180 mW, 50 mW). If possible, this should be measured at the intersection of the light source beam with particles; see also 3.3.3.

3.3.2.4. Light Source Polarization

Polarization shall be specified (e.g., linear, >100:1, in the direction of the sample flow). If possible, this should be specified at the intersection of the light source beam and particles; see also 3.3.3.

3.3.2.5. Light Source Beam

Light source beam dimensions and geometry shall be specified at the flow cell or at the intersection of the beam with particles (e.g., elliptical 22 x 66 µm).

3.3.2.6. Other Relevant Light Source Information

Additional relevant light source details should be provided and may include light source manufacturer name (e.g., MIFlowCyt Lasers, Inc.), light source model name (e.g., 302C Krypton-Ion Laser), light source part number (e.g., I-326), noise (e.g., <1%), pointing and power stability (e.g., <1% over 1 hour), etc.

3.3.3. Excitation Optics Configuration

If possible, the light source power (3.3.2.3), polarization (3.3.2.4), and beam profile (3.3.2.5) should be specified at the intersection of light source beam with particles. If this is not feasible for a particular researcher then details about the excitation optics shall be included to allow for an approximation of the light characteristics at the beam/sample intersection point. This description shall detail all components along the excitation optical path (i.e., from light sources to the flow cell). Each component shall be described stating its type (e.g., beam expander), manufacturer (e.g., MIFlowCyt Optics, Inc.), and model number (e.g., BE03M).

3.3.4. Optical Filters

Each optical filter of the instrument shall be described stating the following:

3.3.4.1. Optical Filter Type

The optical filter type shall be specified (e.g., band pass filter, long pass filter, dichroic long pass filters, short pass filter, dichroic short pass filter, polarizer excitation filter, grating, prism).

3.3.4.2. Transmitted Wavelengths

Light wavelengths transmitted by the filter shall be specified (e.g., 488/25 nm, >670 nm, <620 nm).

3.3.4.3. Optical Filter Installation Date

As optical filters of all types are subject to degradation, filter performance needs to be monitored at intervals to verify continued performance at an acceptable level [19]. The optical filter installation date shall be specified (e.g., January 15, 2007).

3.3.4.4. Optical Filter Manufacturer

The optical filter manufacturer shall be specified (e.g., MIFlowCyt Filter Inc.).

3.3.4.5. Optical Filter Model Number

The optical filter model number shall be specified (e.g., model #1234).

3.3.4.6. Other Relevant Optical Filter Information

Other relevant optical filter details should be provided.

3.3.5. Optical Detectors

Each optical detector (e.g., photomultiplier tube) shall be described stating the following:

3.3.5.1. Optical Detector Name

The optical detector name (e.g., FSC, SSC, FL1, FL2, FL3, FL4, etc.) shall be specified.

3.3.5.2. Optical Detector Type

The type of the optical detector shall be provided (e.g., photodiode, photomultiplier tube).

3.3.5.3. Optical Detector Voltage

The optical detector voltage shall be specified (e.g., 300V, 700V).

3.3.5.4. Optical Detector Amplification Type

The optical detector amplification type shall be specified (e.g., linear, log). For log amplification, the number of decades shall be provided (e.g., 4 decades). For a linear amplification, the linear gain shall be provided (e.g., linear gain of 2.0). Additional details including the actual amplifier profile may be provided.

3.3.5.5. Other Relevant Optical Detector Information

Additional relevant optical detector details should be provided and may include the optical detector manufacturer name and model number (e.g., MIFlowCyt Photonics Inc., PMT #R9220), minimal, maximal, and peak wavelength sensitivity (e.g., 185 nm, 900 nm, and 650 nm), the internal gain (e.g., 10^7), or the rise time (e.g., 2.2ns).

3.3.6. Optical Paths

The full optical path shall be given for each measured parameter where applicable (i.e., excluding time). The optical path shall start with specification of the light source(s), which shall be followed by enumeration of all optical components (e.g., optical filters, beam splitters, mirrors, spectrometer, etc.) that contribute to the particular parameter. If non-imaging components are used, this shall be explicitly noted. The components shall be enumerated corresponding to their order along the light path in the instrument. The description shall include how components are used if relevant (e.g., passed vs. reflected light for a dichroic filter). The optical path description shall specify the optical detector used to measure the particular parameter. It shall also state whether the height, width, or area of the detected signal is used, and specify the threshold value if set. In addition, the collection angle shall be specified for the forward scatter detector. See Table 2 for an example of optical path components and Table 3 for an example of optical path details. As for other sections, we are not specifying the format for providing this information.

3.4. Other Relevant Instrument Details

Additional relevant instrument details should be provided and may include machine-specific information such as information about automated agitation, temperature control, controlled volume dispensing, sampling from microtiter plates, auto-boost, auto-flush, etc. Other custom settings shall be provided if relevant and may include setting name, description, and value.

4. Data Analysis Details

If data analysis has been performed the following details shall be specified:

4.1. List-mode Data File

The list-mode data files (e.g., FCS files [18]) shall be provided directly or details on how they may be requested shall be stated.

4.2. Compensation Details

4.2.1. Compensation Description

A description of the type of compensation used shall be included (e.g., no compensation, hardware compensation, computed compensation) and the spillover or compensation matrix shall be provided when possible (i.e., it may not be available for old data but shall be provided when available). While the spillover matrix is preferred, the compensation matrix is also acceptable. The type of the matrix (i.e., spillover vs. compensation) shall be explicitly stated.

4.2.2. Other Relevant Compensation Information

Additional relevant compensation details shall be provided and may include information such as the FMO control.

4.3. Data Transformation Details

The following shall be described for each data/parameter transformation performed during analysis when the transformation does not qualify as compensation (4.2):

4.3.1. Purpose of Data Transformation

The purpose of each performed transformation shall be specified (e.g., data visualization, background correction, statistical analysis, quantitative flow cytometry, etc.).

4.3.2. Data Transformation Description

Either the exact mathematical formulas/algorithms of each data transformation shall be supplied using an open and freely available specification, or a description of each transformation shall be provided.

4.3.3. Other Relevant Data Transformation Details

Other relevant information about data transformation should be provided and may include specification of software (e.g., name, version, operating system), analysis date, and graphical visualization of the transformation process, which is especially essential for stepwise transformations such as used in quantitative cytometry, i.e., transformation from measured voltage to count of photons, to count of reporter molecules, to count of detector molecules, to count of analytes.

4.4. Gating (Data Filtering) Details

Gating, or data filtering, is a process in flow cytometry in which a subset (subpopulation) of a larger set (population) is defined phenotypically. Gating significantly impacts all statistical and analytical results and thus it is crucial that all the gates be exactly mathematically described (e.g., using Gating-ML [9]). In case the exact gating/filtering description cannot be produced (e.g., software is incapable of exporting an exact description, unknown gate boundaries, probabilistic filtering algorithms, clustering analysis, etc.) detailed membership information should be provided for each gate/subpopulation. This should consist of a complete list of events within each particular subpopulation.

The following information about gating shall be provided, or it shall be specified that no gate was applied:

4.4.1. Gate Description

The subpopulation identified by the gate shall be briefly described (e.g., “IL-4 producing helper T cells of the CD3+CD4+ phenotype”). The gating strategy or a reference to where it is described in detail (e.g., a manuscript) should be provided.

4.4.2. Gate Statistics

Percentage of events within the gate shall be provided specifically stating the denominator. The denominator shall be either the total population of

events (e.g., percentage of lymphocytes based on the total number of events) or another gate (e.g., percentage of CD4+ lymphocytes based on all lymphocytes). When the denominator is another gate, this shall also be exactly specified in recursive fashion so that the gating strategy can be followed up to the original data set. The denominator may not necessarily be a containing (superset) population for some statistics (e.g., the donor / host blood cell ratio).

4.4.3. Gate Boundaries

Either the exact mathematical descriptions of each gate boundary shall be provided using an open and freely available specification, or this information shall be provided in the form of images.

4.4.4. Other relevant gate information

Other descriptive statistics may be provided including the count of events, arithmetic mean, mode(s), median, coefficient of variation (CV), minimum value, maximum value, standard deviation, etc. A description of the relative intensity of staining of the markers defining the subpopulation identified by the gate may also be provided (e.g., CD3 negative, dim, moderate, or bright) [20]. A qualitative description of the subpopulation (e.g., between first and second log decade) and a reference (e.g., publication, individual, or other) for the definition of the qualitative descriptor may also provide useful information.

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Table 1. Fluorescence Reagent Descriptions (Example)

Characteristic(s) being measured	***Analyte***	***Analyte Detector***	***Reporter***	***Manufacturer***
Apoptosis	Phosphatidylserine on the cell membrane outer leaflet OR cells undergoing apoptosis	Annexin V	PE	MIFlowCyt Reagent Inc.
Intracellular protein	Intracellular IL-2	Anti-IL2	FITC	MIFlowCyt Reagent Inc.
Oxidative burst	Reactive oxygen species	Dichloro-dihydroxy-fluorescein diacetate (H_2DCFDA)	2,7-dichloro-fluorescein (DCF)	MIFlowCyt Reagent Inc.
Number of cell divisions	Amino groups of intracellular proteins	Carboxy-fluorescein diacetate succinimidyl ester (CFSE)	fluorescein	MIFlowCyt Reagent Inc.
DNA content or cell cycle	DNA in all cells	Propidium iodide	Propidium iodide	MIFlowCyt Reagent Inc.
Cell viability	DNA in membrane-compromised cells	Propidium iodide	Propidium iodide	MIFlowCyt Reagent Inc.
Membrane potential	Ions or membrane potential	Dihexyloxa-carbocyanine ($DiOC_6$)	Dihexyloxa-carbocyanine ($DiOC_6$)	MIFlowCyt Reagent Inc.
Cell surface protein	CD25	Anti-CD25 antibody conjugated to biotin (anti-CD25-biotin)	PerCP conjugated to streptavidin (SA-PerCP)	MIFlowCyt Reagent Inc.

Table 2. Optical Paths Components (Example)

The optical path details including the usage of the components is described in Table 3.

Item	*Description*	*Manufacturer*	*Model*	*Characteristics*	*Installation Date*
Blue laser	488 nm solid state argon air-cooled	MIFlowCyt Lasers Inc.	ML488-30-I	output power of 30 mW	
Red laser	635 nm diode laser	MIFlowCyt Lasers Inc.	DL635-50-C	output power of 50 mW	
BP 488/10-a	optical filter	MIFlowCyt Filters Inc.	F488052-000	band pass 483-493 nm	Jan. 15, 2006
BP 488/10-b	optical filter	MIFlowCyt Filters Inc.	F488052-000	band pass 483-493 nm	Feb. 2005
DM 560SP	dichroic mirror	MIFlowCyt Filters Inc.	D560052-001	short pass 560 nm	Feb. 28, 2007
DM 640LP	dichroic mirror	MIFlowCyt Filters Inc.	D640052-002	long pass 640 nm	Feb. 28, 2007
670LP	optical filter	MIFlowCyt Filters Inc.	F670052-003	long pass 670 nm	Feb. 28, 2007
BP 530/30	optical filter	MIFlowCyt Filters Inc.	F530052-014	band pass 515-545 nm	Feb. 28, 2007
BP 585/42	optical filter	MIFlowCyt Filters Inc.	F585052-007	band pass 564-606 nm	Feb. 28, 2007
BP 661/16	optical filter	MIFlowCyt Filters Inc.	F661052-009	band pass 653-669 nm	Feb. 28, 2007
Half-mirror	beam splitter	MIFlowCyt Optics Inc.	BSCU-50/50	transmit 50%, reflect 50%, uniform	Feb. 28, 2007
90/10 splitter	beam splitter	MIFlowCyt Optics Inc.	BSCU-90/10	transmit 90%, reflect 10%, uniform	Feb. 28, 2007
FCS diode	photodiode	MIFlowCyt Detection Inc.	PDC8865	4.5V; linear amplification, gain 4.2	
SSC PMT	photomultiplier tube	MIFlowCyt Photonics Inc.	R5916V-58	200 V; linear amplification, gain $2*10^5$	
FL1 PMT	photomultiplier tube	MIFlowCyt Photonics Inc.	R508	500V, 4 decade analog log amplifier; internal gain of 10^6	
FL2 PMT	photomultiplier tube	MIFlowCyt Photonics Inc.	R508	550V, 4 decade analog log amplifier; internal gain 10^6	
FL3 PMT	photomultiplier tube	MIFlowCyt Photonics Inc.	R508	550V, 4 decade analog log amplifier; internal gain 10^6	
FL4 PMT	photomultiplier tube	MIFlowCyt Photonics Inc.	R508	600V, 4 decade analog log amplifier; internal gain 10^6	

Table 3. Optical Path Details for Measured Parameters (Example)

Each optical path starts with blue and red laser light sources combined by a beam combiner and focused on the flow sample using a set of focusing lenses. The light is collected by collection lenses and the optical paths divide based on parameters as shown in the table below. The parameters are the types of measurement as stored in the list mode data file. The optical paths components (defined in Table 2) are ordered as component 1, component 2, component 3, and component 4. The opt. path column indicates whether the passed or the reflected light contributes to the appropriate parameter value.

Parameter	***Comp. 1***	***Opt. path***	***Comp. 2***	***Opt. path***	***Comp. 3***	***Opt. path***	***Comp. 4***	***Opt. path***	***Detector***	***Signal***
FSC-H	BP 488/10-a	passed	N/A	N/A	N/A	N/A	N/A	N/A	FCS diode, collected at 3 degrees	height
SSC-A	DM 560SP	passed	90/10 splitter	reflected	BP 488/10-b	passed	N/A	N/A	SSC PMT	area
FL1-W	DM 560SP	passed	90/10 splitter	passed	BP 530/30	passed	N/A	N/A	FL1 PMT	width
FL2-A	DM 560SP	reflected	DM 640LP	reflected	BP 585/42	passed	N/A	N/A	FL2 PMT	area
FL3-H	DM 560SP	reflected	DM 640LP	passed	half mirror	passed	670 LP	passed	FL3 PMT	height
FL4-H	DM 560SP	reflected	DM 640LP	passed	half mirror	reflected	BP 661/16	passed	FL4 PMT	height