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

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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 pr

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

List mode data file	A list mode data file is a file contai

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

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

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)

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

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

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 Relegmber del Numa.7

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

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16.

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	

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	