

Here we offer suggestions to help you prepare your next manuscript. We also include terminology tips to support standardization. If you have questions about your FlowCam, send your instrument serial number to Customer Service at support@fluidimaging.com. Adapted from Owen et al. 2022.

Introducing FlowCam

FlowCam is a flow imaging microscope¹.

💡 Terminology Tip:

¹**Flow imaging microscopy vs. imaging flow cytometry:** Existing papers refer to FlowCam in both ways. While FlowCam borrows principles of flow cytometry, it can create confusion for non-users expecting fluorescent images and multichannel fluorescence data. For this reason, we suggest standardizing on “flow imaging microscopy” to introduce the technique.

Instrument & Sample Details

Samples were analyzed using FlowCam [model]² (Yokogawa Fluid Imaging Technologies, Scarborough, ME, USA³) with VisualSpreadsheet [version number]. Samples consisted of [description of sample collection] and were [description of how samples were treated or stored]. Prior to analysis, samples were [filtered, concentrated/diluted] using [method] to target [type and/or size fraction of particles you're studying].

💡 Terminology Tips:

²**FlowCam models:** Examples of how to refer to current FlowCam models include FlowCam 8100, FlowCam 8400 (488 nm), FlowCam 8400 (532 nm), FlowCam Cyano, FlowCam Macro, FlowCam LO, and FlowCam Nano. If you have questions about which model you have and how to reference it, email your instrument serial number to support@fluidimaging.com.

³**Company name & location:** Our company has changed names and locations over the years, so while you may see it referred to in different ways in older papers, our current name is “Yokogawa Fluid Imaging Technologies, Inc.” and we are located in Scarborough, ME (USA).

Data Acquisition

Samples were processed using [mode(s) of acquisition]⁴ with a [#X]⁵ objective, a [flow cell type]⁶ with a depth of [#] µm, and a [pump type and, if using a syringe pump, specify the syringe barrel volume]. Flow rate was [#] mL min⁻¹, frame rate was [#] frames per second, and raw images were [enabled or disabled]. Typical background intensity mean was [#], and core capture settings included segmentation thresholds of [describe segmentation thresholds], distance to nearest neighbor of [#] µm, and close holes of [#] iterations. A basic capture filter with a minimum diameter [ESD or ABD] of [#] µm was used to [rationale for filter selection].

If applicable: For Trigger mode runs, the Channel 1 acquisition thresholds were [#] to [#], and Channel 2 was [#] to [#]. Particles per Used Image (PPUI) was typically <[#]⁷.

Terminology Tips:

⁴Modes of acquisition: Standard modes on current instruments include AutoImage mode (available on all instruments), Trigger mode (available on instruments with lasers, such as the FlowCam VS-IV and FlowCam 8400), and LO mode (FlowCam LO only). In some papers, authors mention “autotriggering,” which can cause confusion. For this reason, we suggest using “AutoImage mode” (AIM), “Trigger mode” (TM), or Light Obscuration mode (LO).

⁵Objectives: When citing the magnification used, simply refer to the magnification listed on the objective itself with a capital “X” (e.g., 10X, 4X) so that readers know the hardware used. If you want to report how that magnification translates to particle sizes, use the Size Calibration Factor from your Run Summary: this is the pixel size in microns (µm per pixel), and each objective has its own Size Calibration Factor.

⁶Flow cell type: The current standard offering is field-of-view flow cells, indicating that you can see the entire width of the flow cell in Camera View. However, older instruments like FlowCam VS also had non-field-of-view options.

⁷Particles per Used Image (PPUI): PPUI is the average number of particles in an image frame when the laser triggers image capture. Having a PPUI of <1.2 is critical for appropriate fluorescence values to be attributed to each particle.

Classification

Prior to classification, [describe any pre- or post-processing steps used and why, including post-processing steps to remove artifacts]. Images were then classified using [describe classification workflow]⁸. Final classifications were reviewed by [if classifications were quality checked, describe the approach].

⁸Classification workflow: Whether you used tools in VisualSpreadsheet (such as libraries, filters, and classification templates), annotation tools like EcoTaxa or LabelChecker, or your own model, briefly describe your approach to help users visualize how you got from images to insights.

Outputs

Reported outputs included [describe the output values you used, like concentration and particle properties like area-based diameter, etc.]. These outputs were selected because [brief justification]. FlowCam outputs were validated by [if additional validation was performed, describe the process].