Quotient Bio Analytical Sciences
an LGC business

Challenges for flow cytometry in regulated bioanalysis: Quality assurance and regulatory considerations

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Scope

- The science of instrument and assay validation is increasingly and rightly being discussed, agreed, published and reviewed.

- This presentation is not about the science, but how flow fits in with regulatory expectations
  - An awesome flow project is nothing if it doesn't stand up to regulatory scrutiny

- Pivotal to regulatory acceptance is the definition, protection and storage of raw data
  - Working toward a consensus with EBF Topic Team 32

- How should QA teams approach flow data?
  - As compared to other immunochemistry assays and mass spectrometry

Defining raw data

- "…any laboratory worksheets, records, memoranda, notes, or exact copies thereof, that are the result of original observations and activities of a study and are necessary for the reconstruction and evaluation of the report of that study."

- Why is defining raw data important?
  - Increasing complexity of electronic data acquisition systems
  - Flow cytometry becoming more widely used in the regulated environment
  - Must be able to prove that data aren't fraudulent

  - The Steven Eaton case; fraud exposed by reconstruction of studies through electronic raw data (mass spectrometry):
    - Acquisition times and dates
    - File names
    - Multiple runs

MHRA
Regulating Medicines and Medical Devices

Immunoassay data flow

Run Assay

- e.g. ELISA
- e.g. ECLIA

Read Assay

- e.g. BMG Labtech PHERAstar
- e.g. MSD Sector Imager 6000

Process Raw Data

- Import copy of raw data
- Process, report and store

Watson™ Bioanalytical LIMS

Immunoassay raw data

- ✓ Electronic – original data as written to the instrument database during acquisition
- ✓ Copy made to electronic project folder
- ✓ Copy made to paper

- ✓ Human readable
- ✓ Raw data values can be manually typed into alternative regression/data processing software at later date

User: [redacted] Path: C:\Program Files\BMG\PHERAstar\User\Data\ Test ID: 7342

Test Name: [redacted] Date: 06/11/2013 Time: 14:50:34

ID1: [redacted]

ID2: Exp1

Absorbance Absorbance values are displayed as OD

1. Raw Data 450 [A/1] - Raw Data 630 [A/2]

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.8753	2.8959	0.0691	0.0672	2.4523	2.4278	0.0439	0.0422	1.5309	1.5212	0.0265	0.0251
B	2.7561	2.719	0.0204	0.0204	1.9236	1.9192	0.0154	0.0144	1.1042	1.1237	0.0136	0.0129
C	2.1338	2.0916	2.4179	2.4344	1.2715	1.2845	1.5949	1.5668	0.7439	0.7172	0.8996	0.9209
D	1.3289	1.3115	1.0016	0.9974	0.7387	0.7414	0.5741	0.6171	0.4101	0.4217	0.3259	0.3315
E	0.7515	0.7143	0.3485	0.3514	0.4036	0.3988	0.2038	0.2036	0.2392	0.2424	0.1193	0.118
F	0.3962	0.3783	2.4296	2.434	0.2224	0.2179	1.6425	1.56	0.1274	0.1292	0.9238	0.9277
G	0.2091	0.2111	1.0181	1.0421	0.1227	0.1222	0.6622	0.6049	0.0726	0.0735	0.3338	0.3417
H	0.1179	0.1201	0.3708	0.378	0.071	0.0729	0.2179	0.2158	0.0465	0.0475	0.1223	0.1194

COPY

Test Name: [redacted] Date: 06/11/2013 Time: 14:50:34

ID1: [redacted] Exp1

Absorbance **Microplate View**

PHERAstar FS, 471-0075, 06/11/2013, 14:50:34

	1	2	3	4	5	6	7	8	9	10	11	12
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Legend:

1. Raw Data 450 [A/1] - Raw Data 630 [A/2]

COPY

MS data flow

Run Assay

- e.g. LCMS
- e.g. BioMS™

} Raw data generated

Process Raw Data

- Integration

Process Integrated Data

- Import processed data
- Further process, report and store

Watson™ Bioanalytical LIMS

MS raw data

✓ Electronic – original data as written to the instrument database during acquisition

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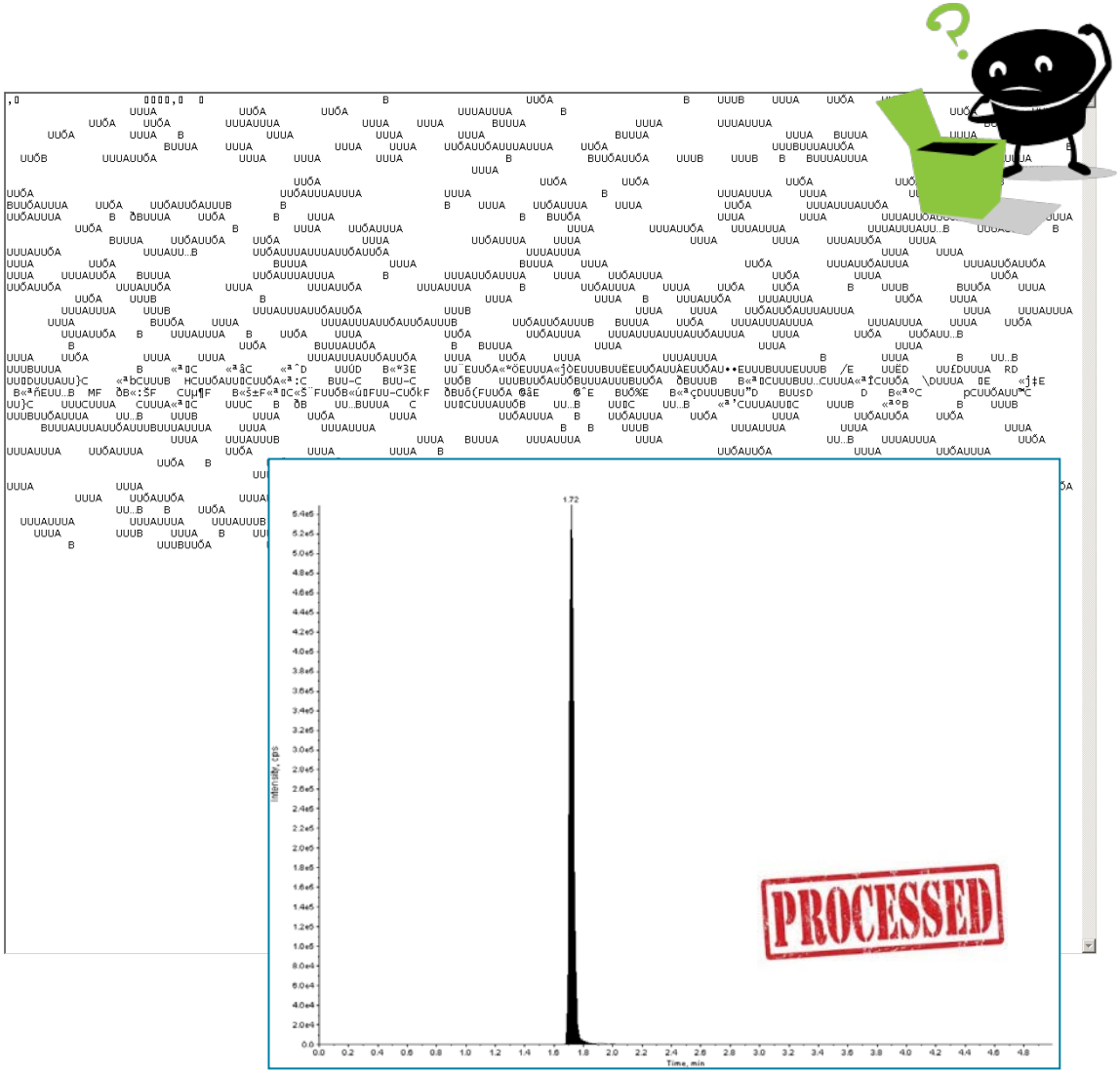
✗ Copy made to paper

✗ Human readable

✗ Raw data values can be manually typed into alternative regression/data processing software at later date



Flow data flow

Run Assay

- Acquire data using instrument software – raw data generated

Process Raw Data

- Gating using instrument or stand-alone software
- Generate final results or data for further processing (cytometric bead arrays)

Further Processing

- Import final results for storage
- Further process data, report and store (cytometric bead arrays)

Flow raw data

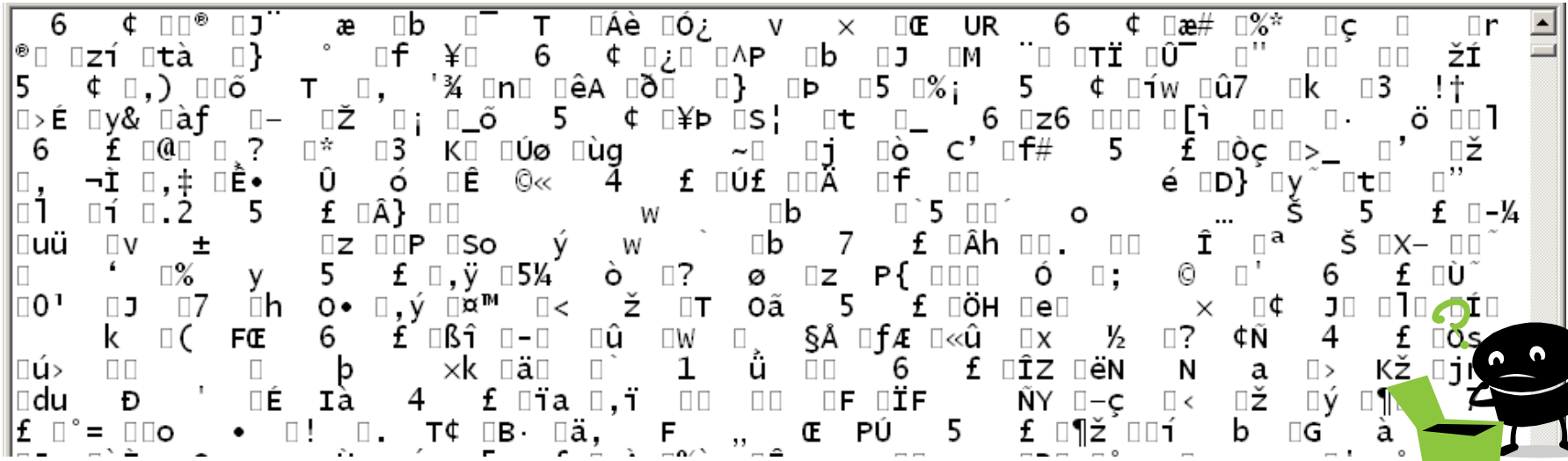
- Flow raw data is defined at QBAS according to the following principles
  - raw data are as written (electronically) during data acquisition
  - data are described as 'processed' following any form of interpretation which is not an automated (and validated) part of the acquisition process

- For example
  - Flow: Data file written on acquisition (normally .fcs but could be proprietary file type) is raw data.
    - Gated data = processed
  - ELISA: Calculation of 450nm minus 630nm OD data can be considered as part of raw data if calculation is performed automatically on acquisition by validated software
    - Any other calculations on the raw data which are not defined as part of the acquisition process constitute processed data
  - Mass Spec: Automatic peak integration by validated software
    - Subsequent re-integration by an analyst = processed data (the original integration should not be overwritten)

The .fcs file

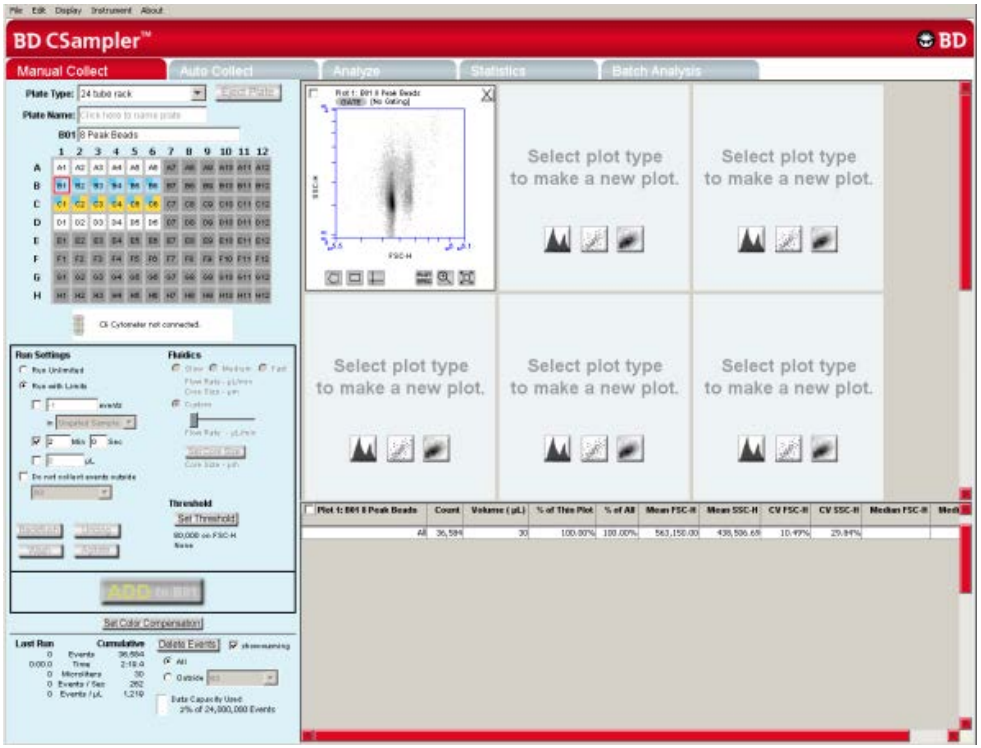
- The .fcs file lends itself well to hosting raw data on most systems where data are written directly to .fcs on acquisition
  - Universal format so not dependant on legacy software
  - Processing software operates and saves data independently from .fcs files (the workspace)

  - But – not human readable so can't store as paper raw data
  - Need validated and secure electronic systems for control and storage



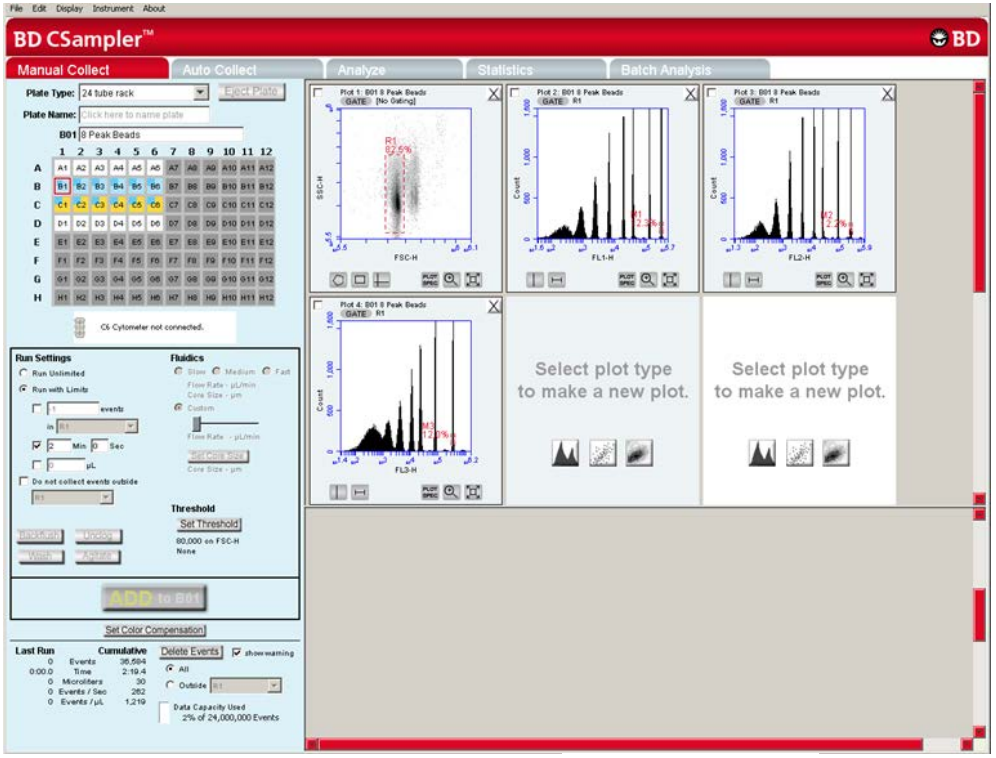
What if…

- …your software doesn't automatically write to .fcs on acquisition?
  - E.g. BD Accuri CFlow Plus
  - Writes to proprietary .c6 files
  - These contain data embedded in .fcs format, which can be exported to .fcs files if desired
  - These .fcs files will be *copies* of the raw data. The .c6 file remains the raw data.
  - Need to maintain the software as a legacy system to allow recovery of raw data



And what If…

- …your software over-writes the original file after processing?
  - ‘Workspace’ is saved in the same file as the raw data
  - Embedded raw data is not changed
  - But traceability of the original file created at the time of acquisition is lost when processing is saved
  - Therefore .c6 file should be saved as ‘RAW’ on completion of acquisition, then saved separately as ‘PROCESSED’ before processing



PROCESSED

QA approach to flow data

- QA teams are dealing with more and more diverse technologies, particularly in the immunoassay field
  - Flow is just one, also molecular biology, Singulex
- Drawing parallels with other familiar data flows is helpful to understand how to manage flow data
- Scientifically challenging in some cases, particularly where extensive gating is required
  - There needs to be recognition and acceptance that scientific judgment is an important part of the flow process (even where all-important gating rules are in operation)
  - May not be possible to re-create processed data with 100% accuracy (even when same analyst re-gates same raw data).
- Printed (or controlled .pdf) copies of the processed data are therefore important

Consensus

- Your feedback and opinions are very valuable – I and EBF TT32 would love to hear from you!
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- Booth C1

Thank you!

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