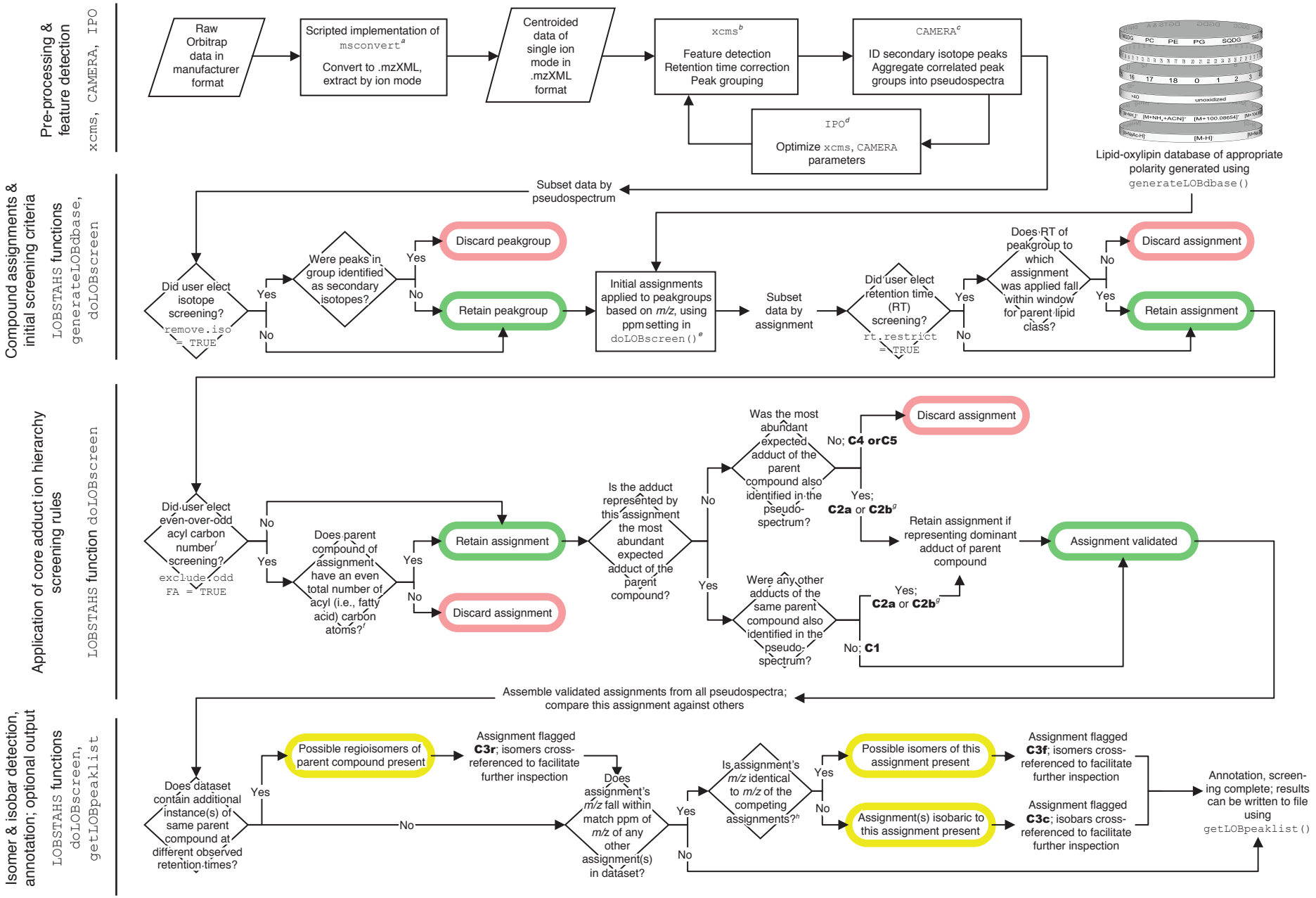
Pre-processing & feature detection
xcms, CAMERA, IPO
Raw Orbitrap data in manufacturer format
Scripted implementation of msconvert[a]
Convert to .mzXML, extract by ion mode
Centroided data of single ion mode in .mzXML format
xcms[b]
Feature detection
Retention time correction
Peak grouping
CAMERA[c]
ID secondary isotope peaks
Aggregate correlated peak groups into pseudospectra
IPO[d]
Optimize xcms, CAMERA parameters
Lipid-oxylipin database of appropriate polarity generated using generateLOBdbase()
Compound assignments & initial screening criteria
LOBSTAHS functions generateLOBdbase, doLOBscreen
Subset data by pseudospectrum
Did user elect isotope screening? remove.iso = TRUE
Were peaks in group identified as secondary isotopes?
Discard peakgroup
Retain peakgroup
Initial assignments applied to peakgroups based on m/z, using ppm setting in doLOBscreen()[e]
Subset data by assignment
Did user elect retention time (RT) screening? rt.restrict = TRUE
Does RT of peakgroup to which assignment was applied fall within window for parent lipid class?
Discard assignment
Retain assignment
Application of core adduct ion hierarchy screening rules
LOBSTAHS function doLOBscreen
Did user elect even-over-odd acyl carbon number[f] screening? exclude.odd FA = TRUE
Does parent compound of assignment have an even total number of acyl (i.e., fatty acid) carbon atoms?[f]
Retain assignment
Discard assignment
Is the adduct represented by this assignment the most abundant expected adduct of the parent compound?
Was the most abundant expected adduct of the parent compound also identified in the pseudo-spectrum?
No; C4 orC5
Discard assignment
Yes; C2a or C2b[g]
Retain assignment if representing dominant adduct of parent compound
Assignment validated
Were any other adducts of the same parent compound also identified in the pseudo-spectrum?
Yes; C2a or C2b[g]
No; C1
Assemble validated assignments from all pseudospectra; compare this assignment against others
Isomer & isobar detection, annotation; optional output
LOBSTAHS functions doLOBscreen, getLOBpeaklist
Does dataset contain additional instance(s) of same parent compound at different observed retention times?
Possible regioisomers of parent compound present
Assignment flagged C3r; isomers cross-referenced to facilitate further inspection
Does assignment's m/z fall within match ppm of m/z of any other assignment(s) in dataset?
Is assignment's m/z identical to m/z of the competing assignments?[h]
Possible isomers of this assignment present
Assignment(s) isobaric to this assignment present
Assignment flagged C3f; isomers cross-referenced to facilitate further inspection
Assignment flagged C3c; isobars cross-referenced to facilitate further inspection
Annotation, screening complete; results can be written to file using getLOBpeaklist()
Yes
No