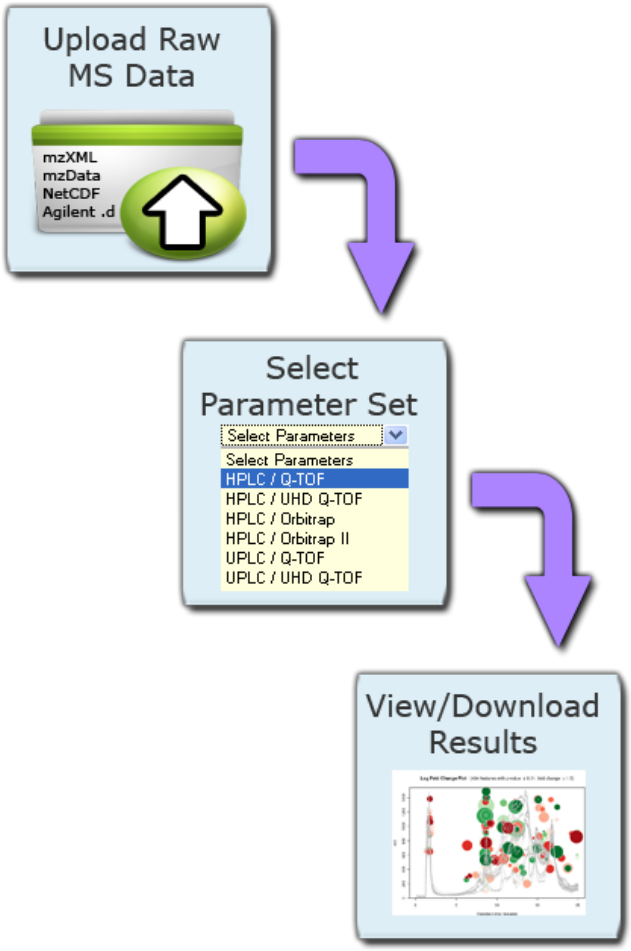
XCMS Online Usage Instructions

Overview

XCMS Online is an innovative platform with an intuitive graphical interface which allows users to easily upload and process LC/MS data for untargeted metabolomics profiling. XCMS Online provides a complete metabolomics workflow including feature detection, retention time correction, alignment, annotation and statistical analysis. To facilitate the usage, predefined parameter sets for different instrument setups (e.g. HPLC/QTOF, UPLC/QTOF, HPLC/Orbitrap) can be selected. Customized settings can be created from these templates. Results can be browsed online in an interactive, customizable table that shows feature statistics, EICs, box plots, and putative METLIN ID's for each metabolite. All results including the images may also be downloaded as a zip file for offline analysis.



You will need a user account to use the system however user accounts are free and only require a valid e-mail address to register. To process datasets using XCMS Online you will need to create a job which involves uploading the datasets, defining parameters and submitting the job. You will be notified of your job status, including errors/warnings that you can correct prior to resubmitting a job. After processing is complete, you will be notified via e-mail that your results are ready for review.

All screen shots taken from v. 1.22.01.

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All screen shots taken from v. 1.22.01.

1. **Create User Account** – You will initially need to register your e-mail account to be able to use the system. After you register you will receive an e-mail at the account you entered with validation instructions. Upon validation you will have access to the system. You may then login using the username and password combination you used to register.

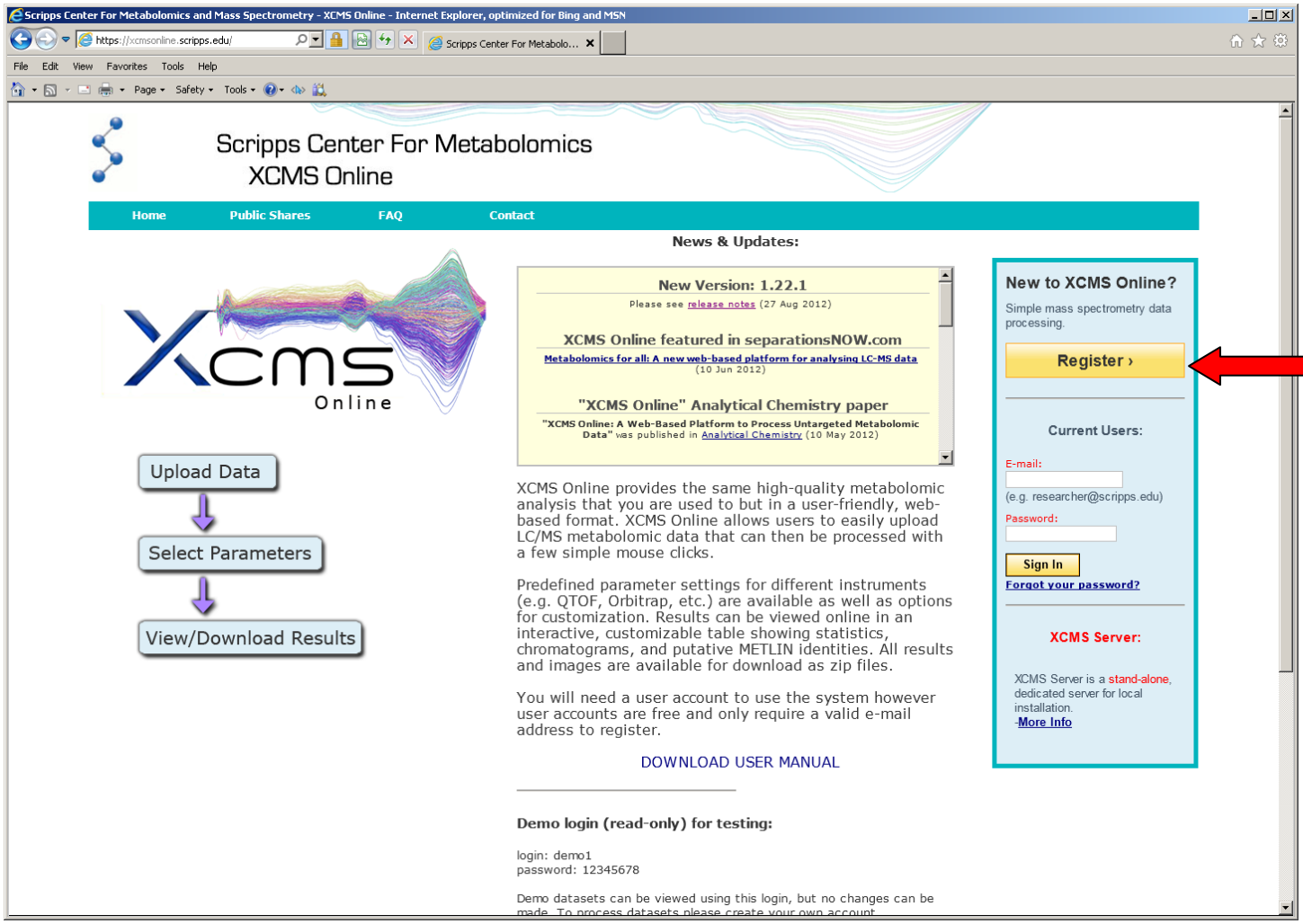

Figure 1 – Create user account from home page by clicking on the "Register" button

All screen shots taken from v. 1.22.01.

2. **Create Job** – As a first-time user, you will want to begin by creating a new job. Click on "Create Job" in the top navigation menu (Figure 2)

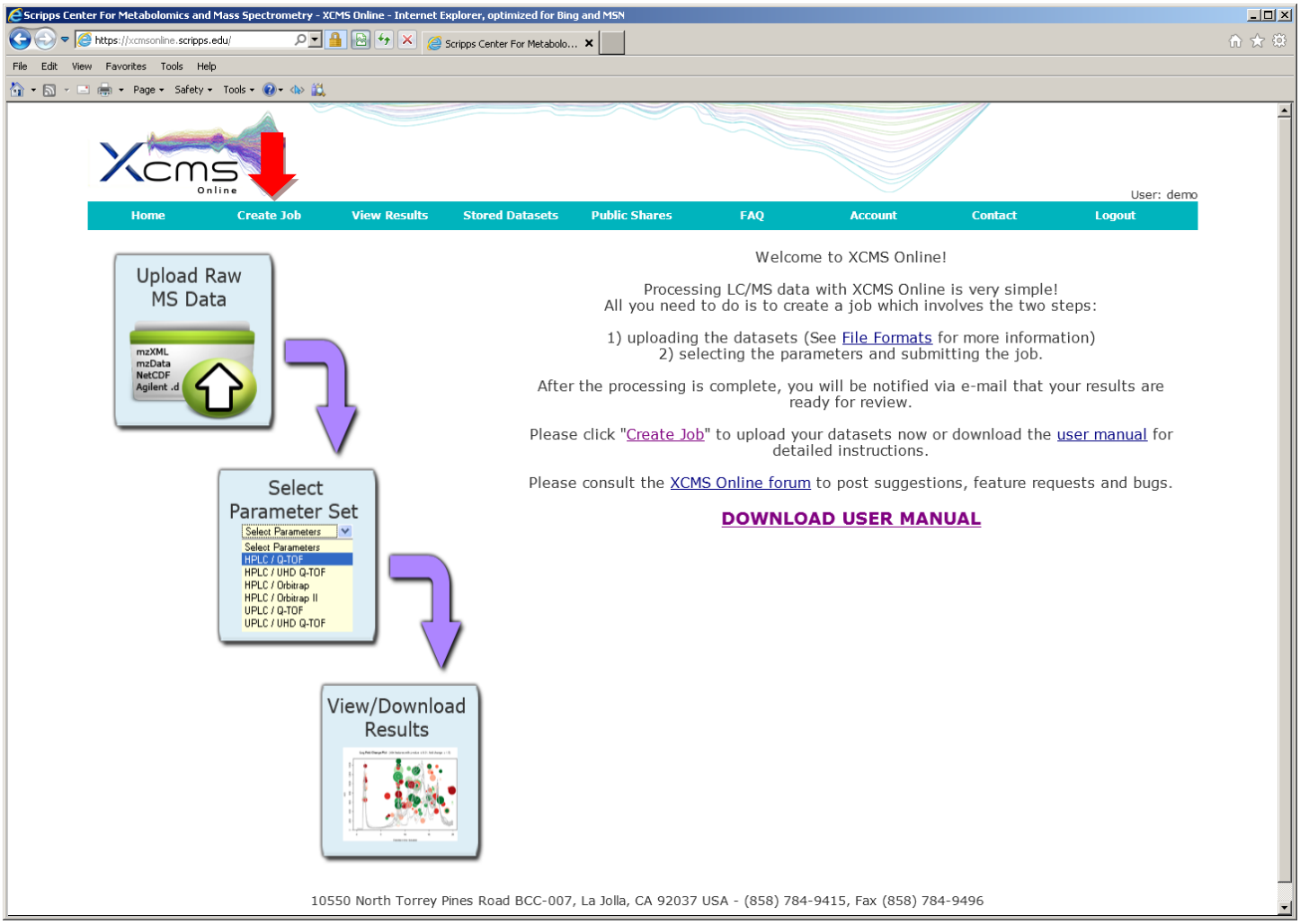

Figure 2 – Create New Job by clicking on "Create Job" in the top navigation menu

All screen shots taken from v. 1.22.01.

3. **Uploading the Datasets** - The create job page will open and you will be able to start defining the job (see Figure 3). There is a step navigation wizard directly below the top navigation bar that will serve as a guide. Upon initial load, a default job name will be created based on your user name and the current date. *You may click the job name to edit.*

Upload Dataset 1

Initially you will need to upload a dataset. **Dataset 1** (left-hand side) is often defined as the control data set.

a) Click the "Upload Dataset" button to initiate an upload. A separate window will open. This will load the Java module which is required for large uploads. Even if you receive a warning (see Appendix – Figure A1), click "Run". After the applet runs, you will be presented with an upload dialog box (see Figure 4).

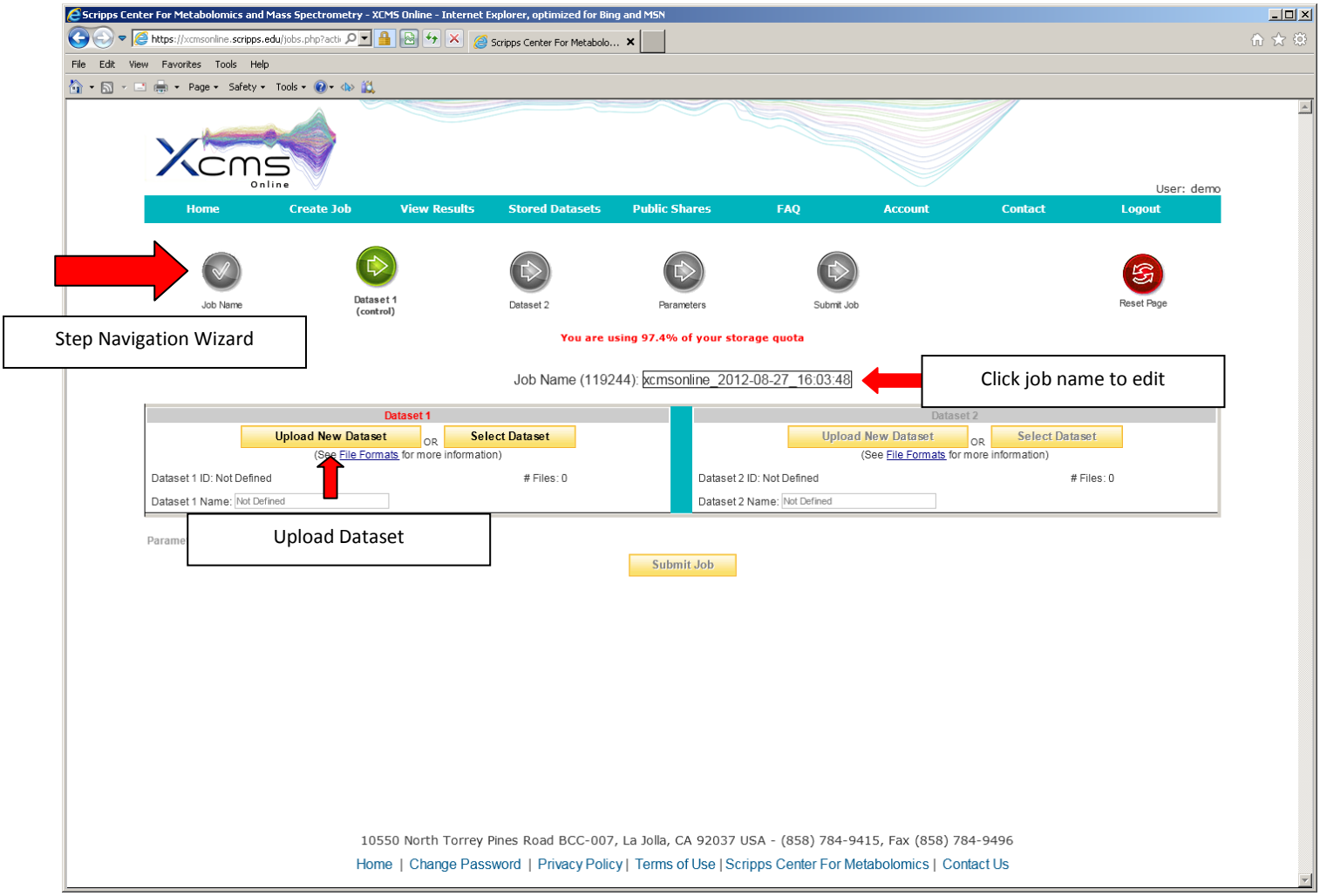

Figure 3 – Initial job creation page

All screen shots taken from v. 1.22.01.

b) **Select files/folders to be included in the dataset** from the file tree (Figure 4). Individual files will appear in the file list as you navigate the file tree. After you locate the files for this dataset, drag them to the "Drop Files Here" area (lower-right) and they will immediately start compressing. Alternatively you can click the "+" symbol (see "Add Files" in Figure 4) to select the files you want to include. When you are satisfied all files are included in this dataset, you may press the "Upload" button to start the upload. You may need to maximize window size or use scroll bars to display all text. The animated line under a file or folder appears during file zipping. You may need to wait until zipping finishes prior to clicking the upload button.

Note: Valid files the system can accept include mzXML, mzData, mzData.XML, netCDF, wiff (AB SCIEX), CDF and folders with ".d" extension (Agilent) at present. Additional formats may be included in future versions.

c) Depending on the speed of your Internet connection, you may choose to continue with the job setup. At this point you can minimize the upload in progress and continue. Alternatively you can drag the upload dialog box to another part of the screen so you can access the main create job page. Simply use the minimize button (single bar) in the upper right-hand corner of the window (**See Figure 4**). Note: minimize button may be different depending on operating system.

Note: If you close the dialog box, you will need to start again.

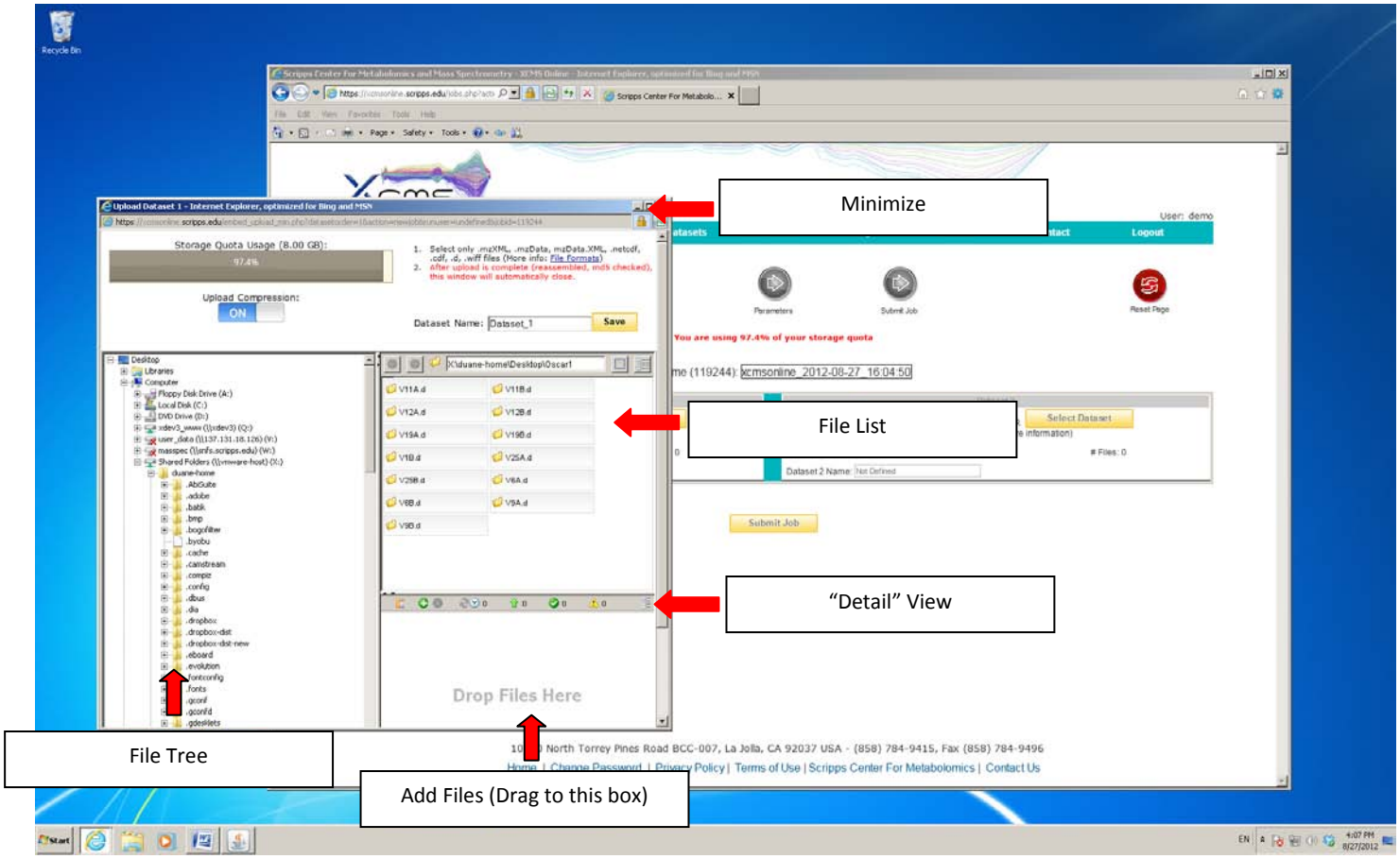

Figure 4 – Upload Dataset Dialog

All screen shots taken from v. 1.22.01.

Upload Dataset 2

At this point, you can repeat step number 3 to begin the **second dataset upload.** The Dataset 2 section can be found to the right of the Dataset 1 section.

Note: You must have unique names for each dataset (even if the id is different). You can modify the dataset names after an upload has started by clicking the "Edit" button to the right of the respective dataset name (Figure 5).

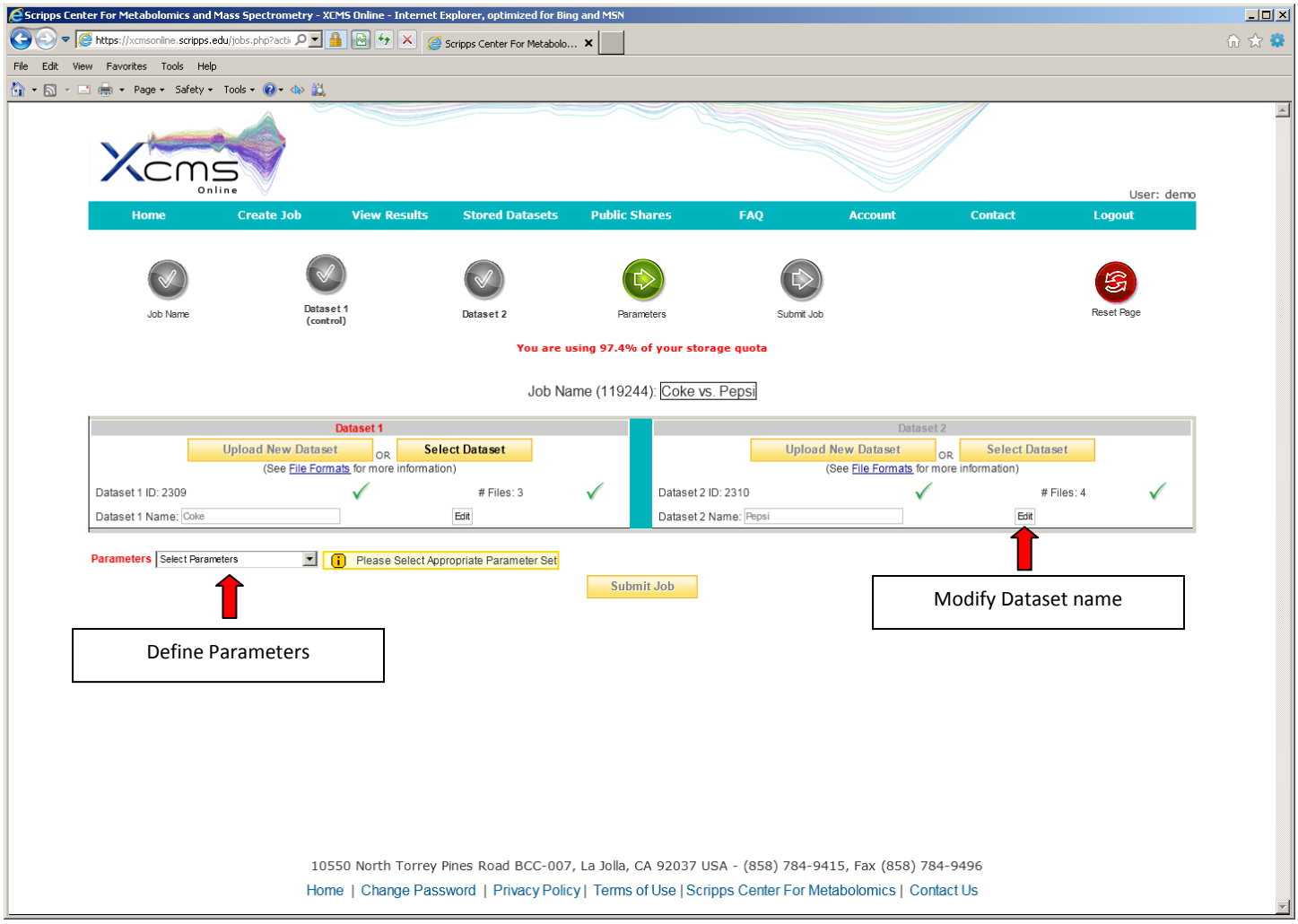

Figure 5 – Screen after uploads are complete

4. **Select Parameter Set** - After uploads are defined (still in progress or complete), you have to define the parameters that correspond to this job. Some **template parameter sets (e.g. HPLC/TOF, UPLC/TOF, HPLC/OrbiTrap) have been predefined**. You can **simply select one of those predefined parameter sets** or customize them to your particular needs. To begin defining parameters, click on the Parameter drop down box (Figure 5). After you select a template, the dropdown will change and you will be able to view or edit the selected parameter set (Figure 6).

4a. Define Custom Parameters ***-skip this step if you do not wish to define custom parameters-*** If you select the "View/Edit" button to view the parameter set you will be presented with a modal dialog box where you can define how the datasets are processed. Methods and properties are available for all aspects of dataset processing. **(See Appendix B for more details)**

Note: If you choose to change any of the settings in a template parameter set, you will need to create a new parameter name for processing (Figure 6). Otherwise your changes will not be saved as you can not overwrite a template parameter set. The name for a custom parameter set can be set in the "General" tab.

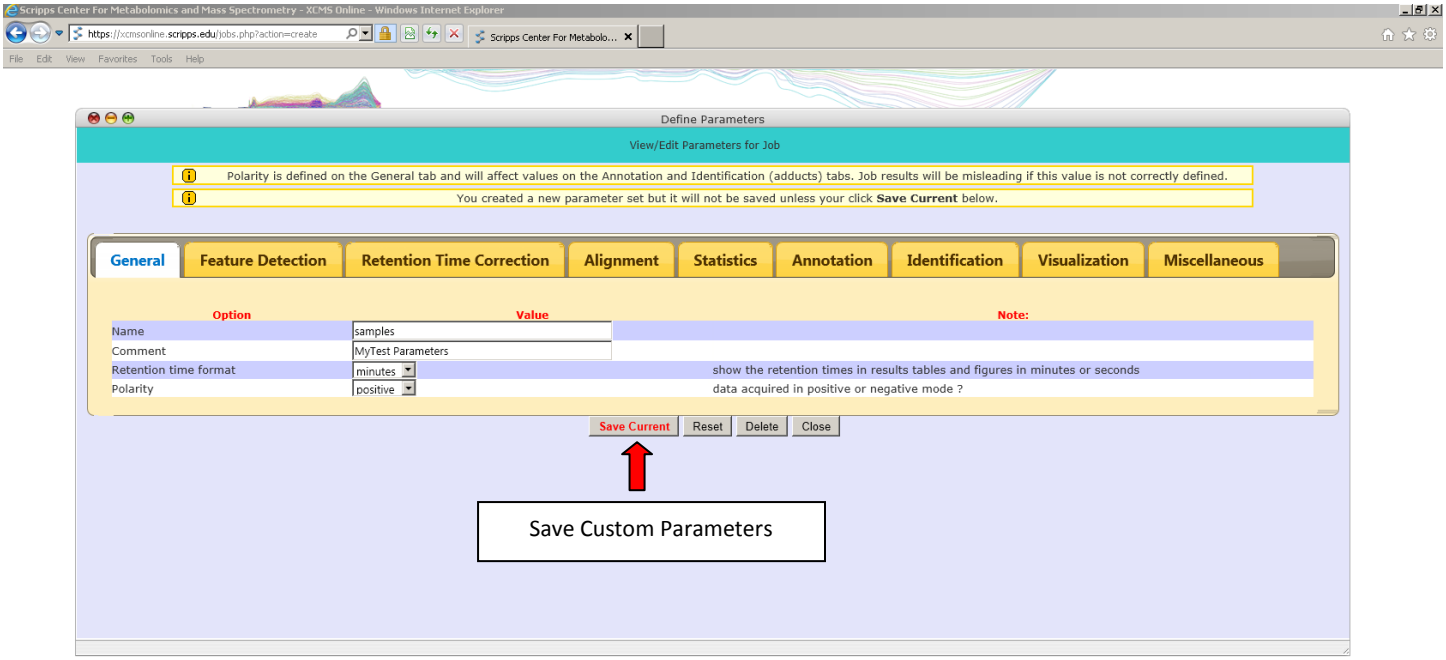

Figure 6 – View/Edit Parameters for Job

5. **Submit the job! -** At this point you can review all relevant details about the job prior to submission (e.g. job name, dataset names, correct file count, correct parameters). Once you are satisfied, click the "Submit Job"

button to queue the job for processing. The "Submit Job" button can be found at the bottom of the screen (Figure 6).

Note: Even if you still have datasets uploading, you can pre-submit the job so it will automatically queue once the datasets complete the upload.

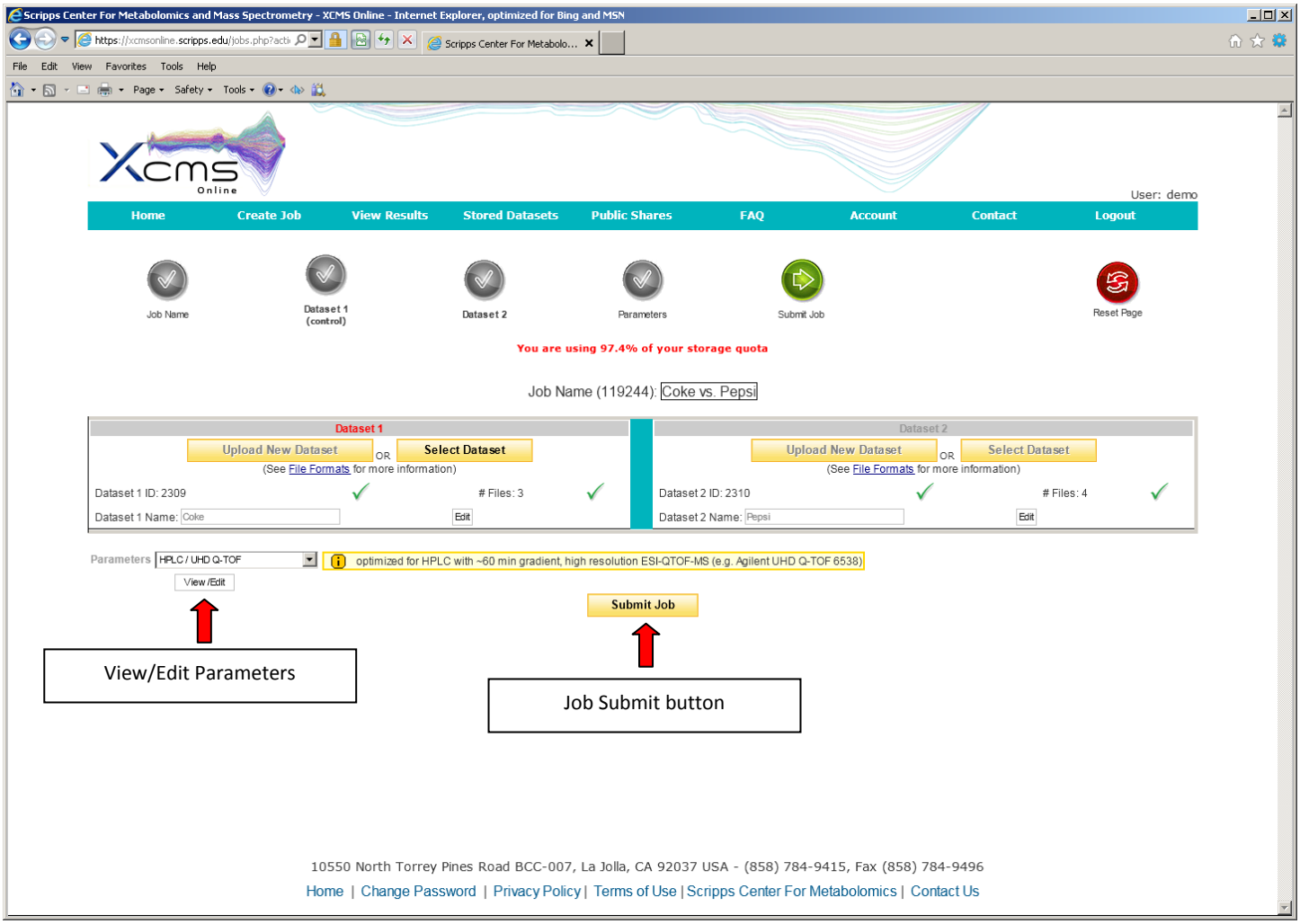

Figure 6 – Screen after parameter selection

After you click "Submit Job", you will have a final opportunity to view all settings for this job prior to submission. A modal dialog box will appear on your screen (Figure 7) with job settings. Carefully review the information as this cannot be changed after submission (You would need to create a new job – although your data sets will already be stored). If you are satisfied with the displayed information, click the "Submit Job" button in the lower-right corner of the dialog box, otherwise click cancel and make the necessary changes.

Note: It is important to not close the browser if you have uploads in progress as these will cancel. There is a timer on the page that will automatically redirect the entire page to the "View Results" section when all uploads are complete. In addition, ***you will receive an e-mail when your job has successfully queued****.*

All screen shots taken from v. 1.22.01.

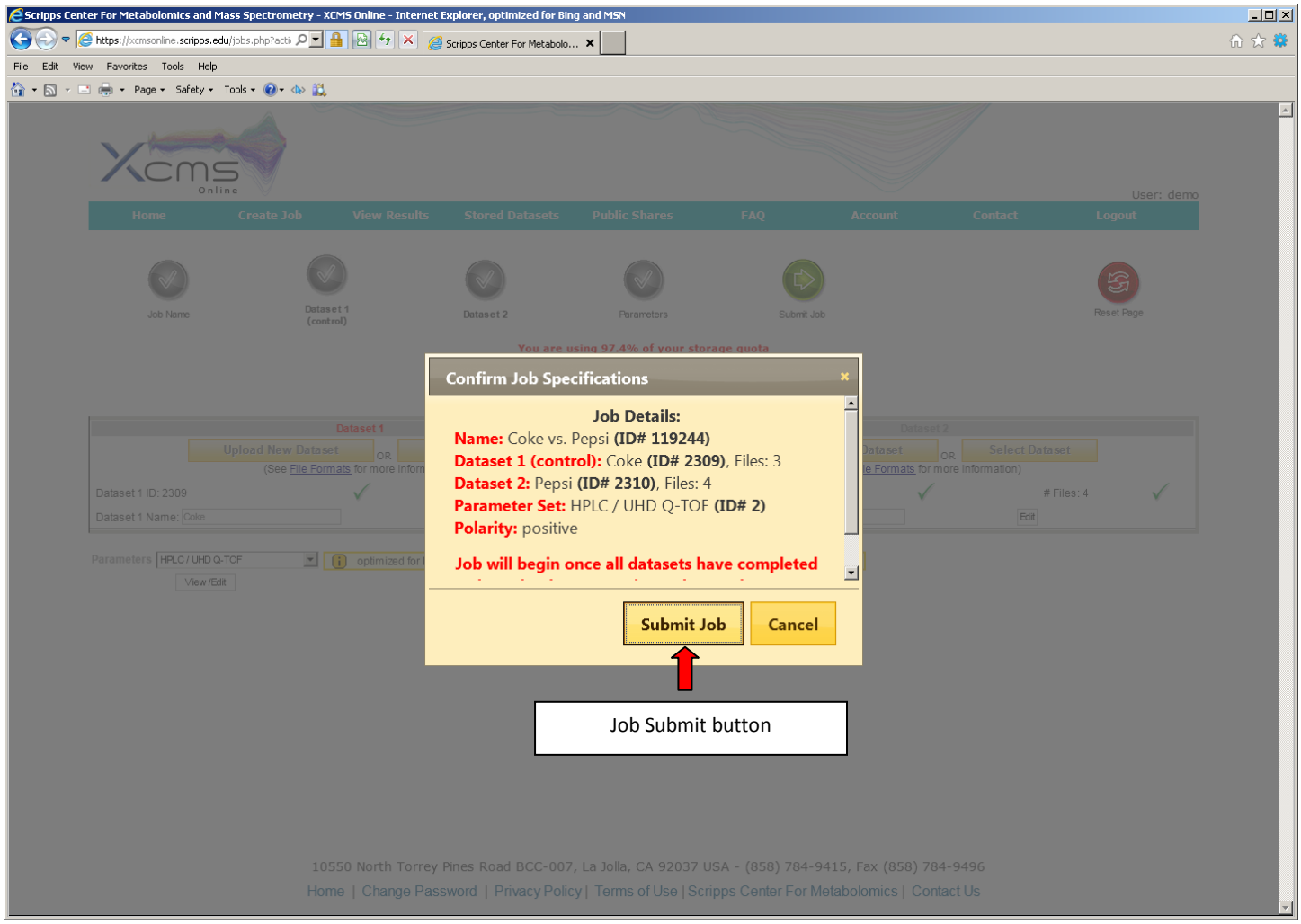

Figure 7 – Job Confirmation Dialog

All screen shots taken from v. 1.22.01.

View Results

After you submit your job, you will be forwarded to the "View Results" page where you can see details of your submitted jobs, including progress percentage, datasets used and parameter set used. The page will automatically refresh if you have pending jobs however you will receive an e-mail when each submitted job is complete, if alerts are enabled (default is to send all alerts). Once complete, you may view the results by clicking the green "VIEW" button (Figure 8). You may also include publically-available jobs in "View Results" by selecting "View Public Shares" from the header (See Figure 8).

1. **Click green "VIEW" button on the View Results page**

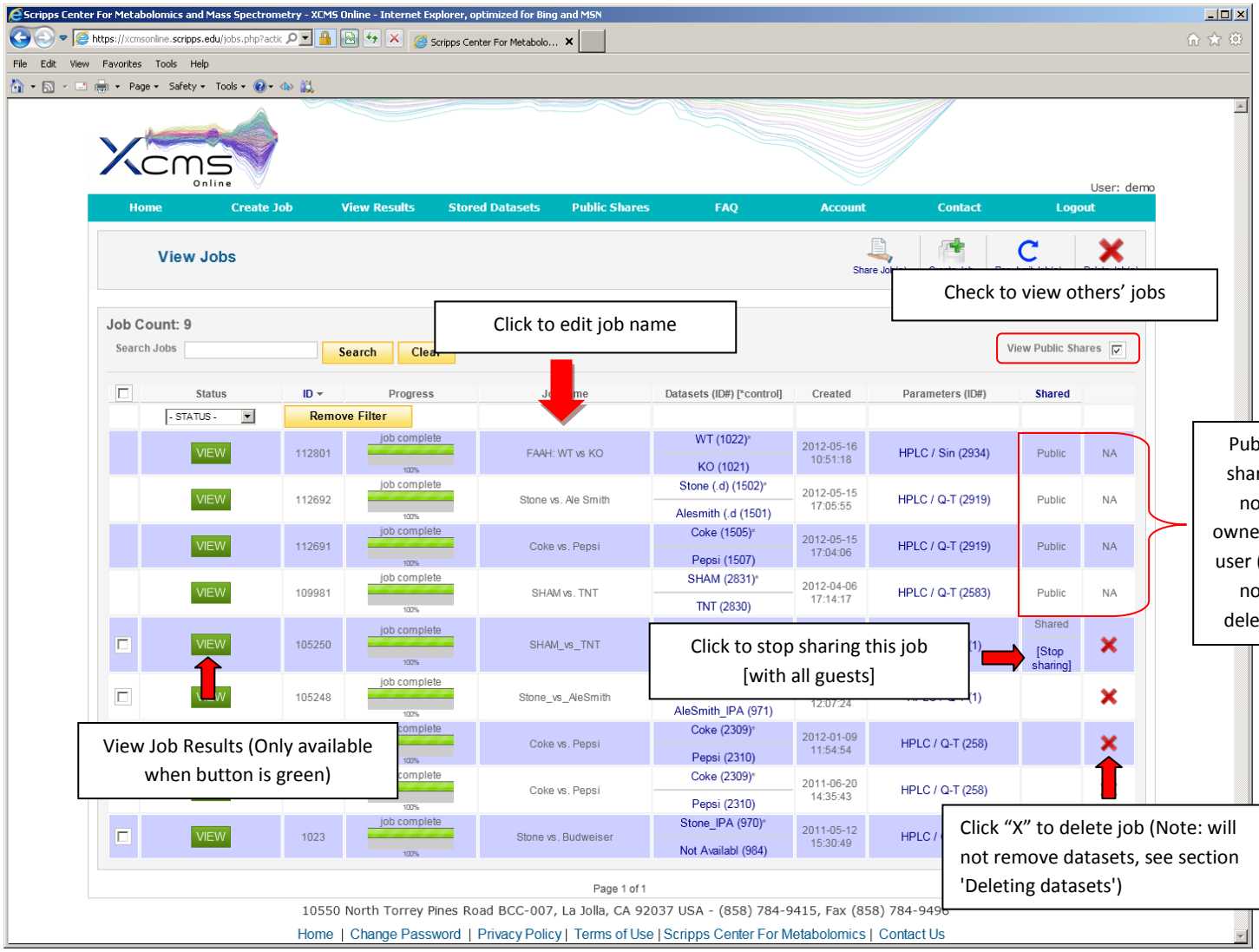

Figure 8 – View Results Page

All screen shots taken from v. 1.22.01.

The initial results screen provides a summary of the job details replete with graphs (total ion chromatograms (TIC) before and after retention time correction), retention time deviation vs retention time, log fold change bubble plot and multidimensional scaling (MDS). You can also download the full zipped results using the link at the bottom of the page. Note: This will file will not include any online annotations you input in the feature result table (see next step).

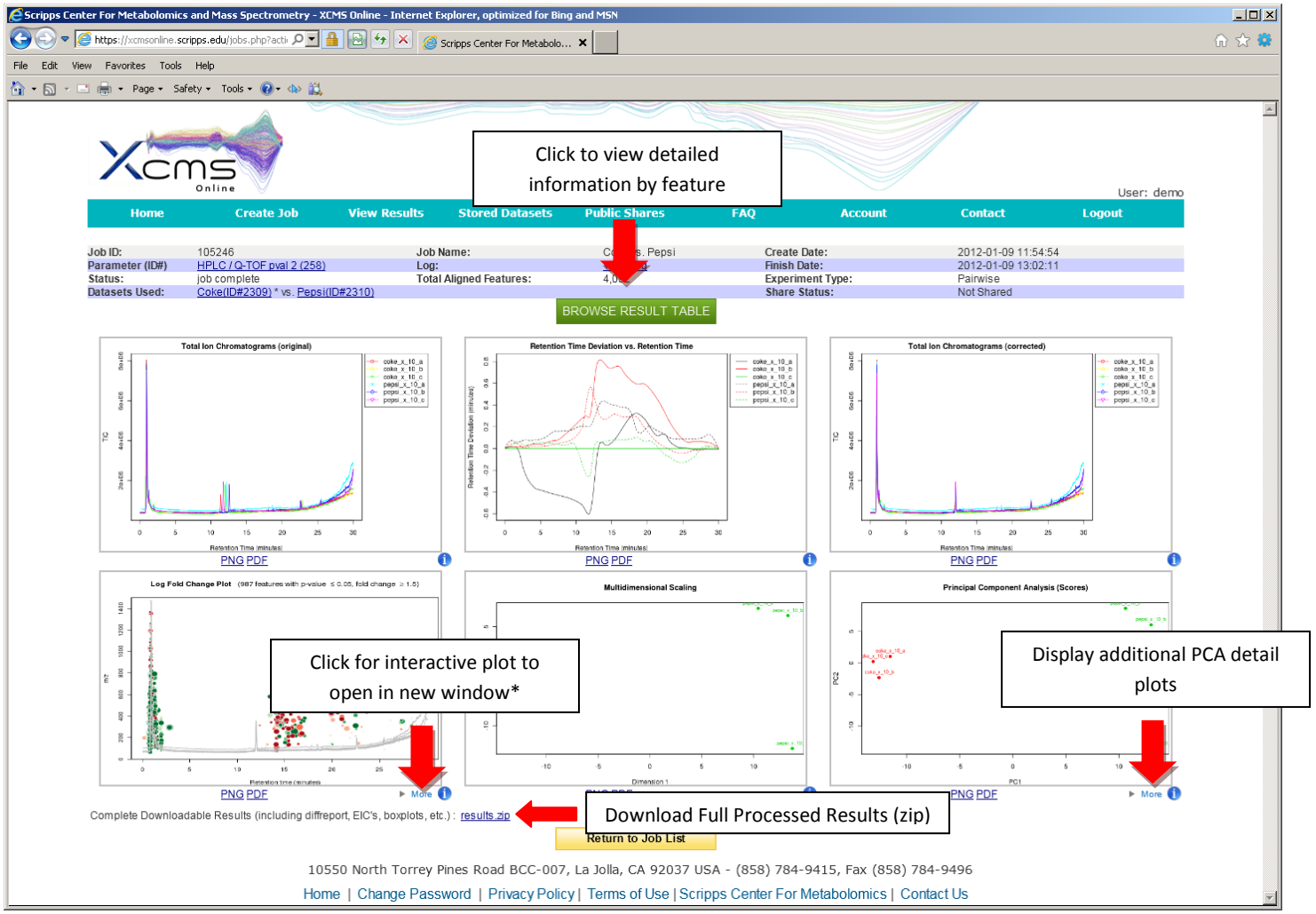

Figure 9 – Job Summary Information

(* Interactive plot only available in certain browsers – see FAQ for more information)

2. Click "BROWSE RESULT TABLE" button (Fig. 9) to View Feature Details

If this is the first time viewing the feature detail results of the current job, the data will be loaded into the database for viewing and annotating (which may cause a brief delay); subsequent viewing will be faster. As you navigate through each feature (you can use arrow keys), details about each feature (*m/z*, retention time, EIC, box-and-whisker plot and putative METLIN ID's) is displayed to the right of the table.

All screen shots taken from v. 1.22.01.

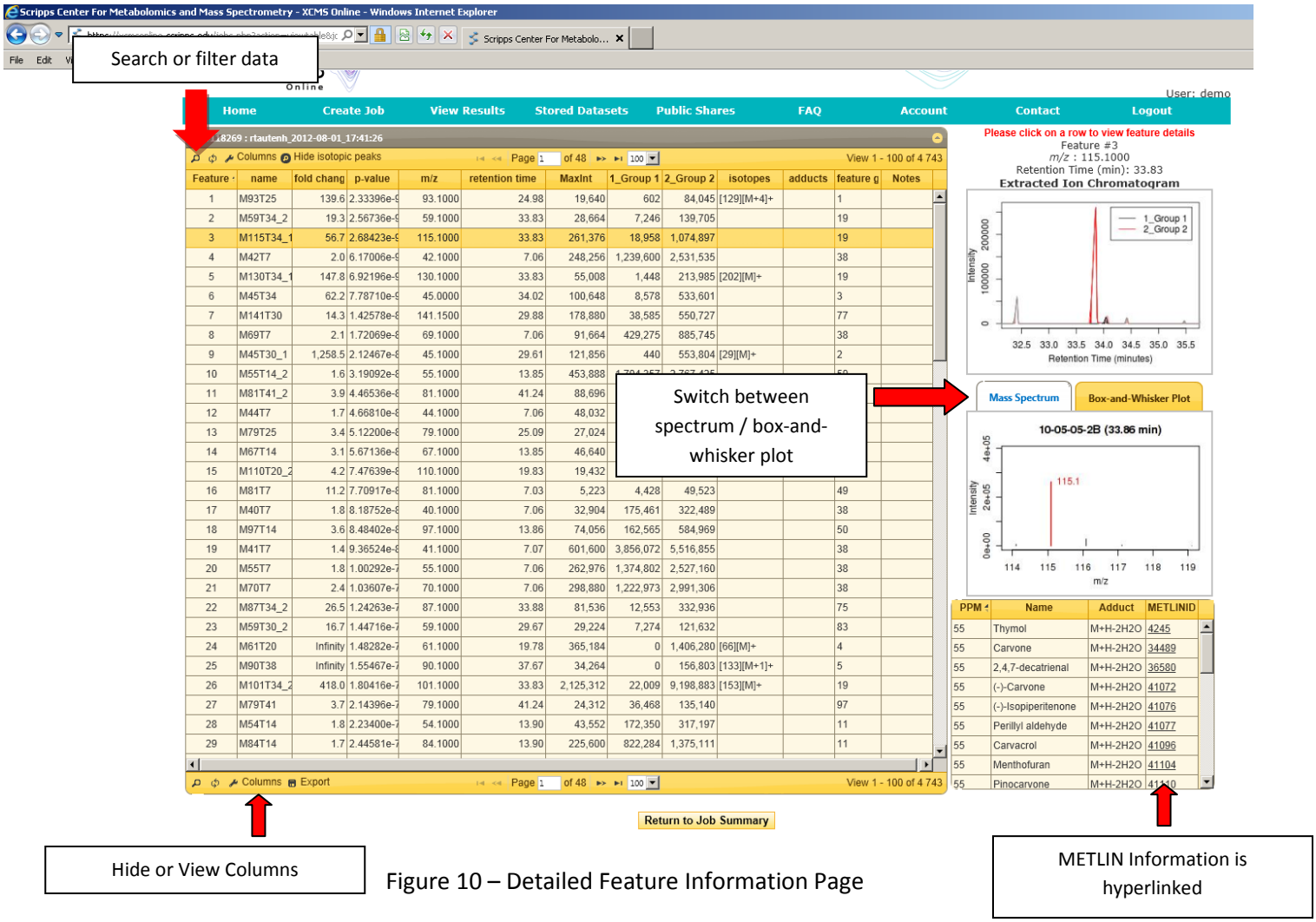

Figure 10 – Detailed Feature Information Page

Frequently Used Features:

- Searching / filtering is available by clicking the magnifying glass icon (upper-right of table)
- You can hide or display specific columns by clicking the icon in the lower-left (See Figure 10)
- Clicking any column header will sort the table in ascending or descending order by that field
- Column order and width can be configured by dragging and dropping column headers
- Double clicking row or pressing “Enter” will open notes dialog box (information saved in “Notes” column)

All screen shots taken from v. 1.22.01.

Currently storage of user data (e.g. uploaded datasets, custom parameters and jobs results, including annotations) is limited (See Account menu for usage). To free space for additional uploads, you will likely need to delete jobs and/or datasets. The check your account storage, navigate to the Account menu (See Figure 11).

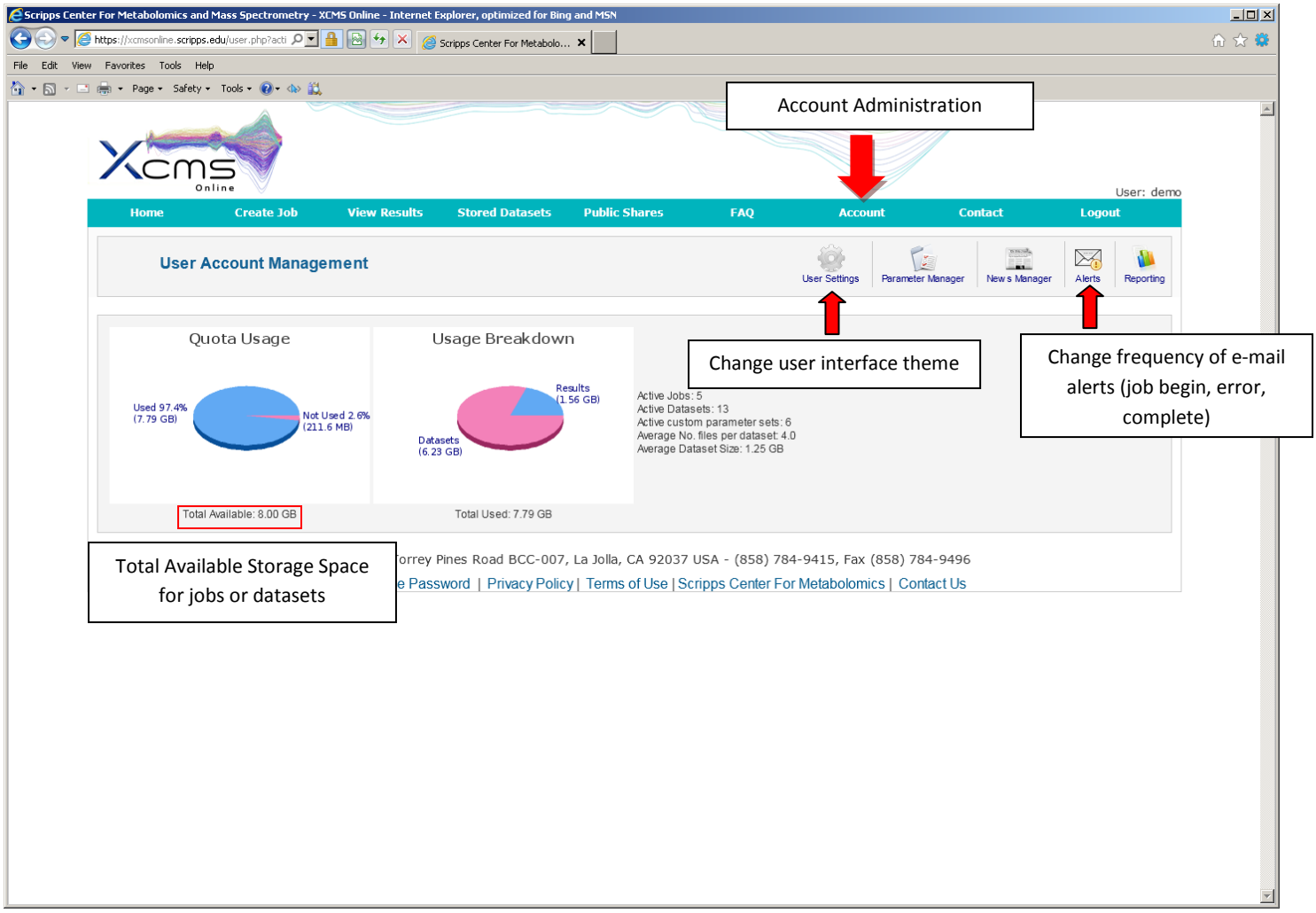

Figure 11 – Account View

All screen shots taken from v. 1.22.01.

Deleting Jobs:

On the view results page (See Figure 8), you have the option to delete a job (far right column) but the underlying dataset will not be deleted with a single click (as you may wish to create another job with different parameters and compare results). Deleting the actual datasets can be accomplished by following the steps in the section "Deleting Datasets".

Deleting Datasets:

1. Click on "Stored Datasets" in the top navigation bar to enter the stored datasets area. This page will list all datasets previously uploaded. From here you may delete the entire dataset by clicking the "X" in the far right column (See Figure 12). A confirmation dialog box will inform you of the impending action and allow you to cancel. Note: To rerun jobs based on deleted data, you will need to re-upload the data; we cannot retrieve deleted datasets.

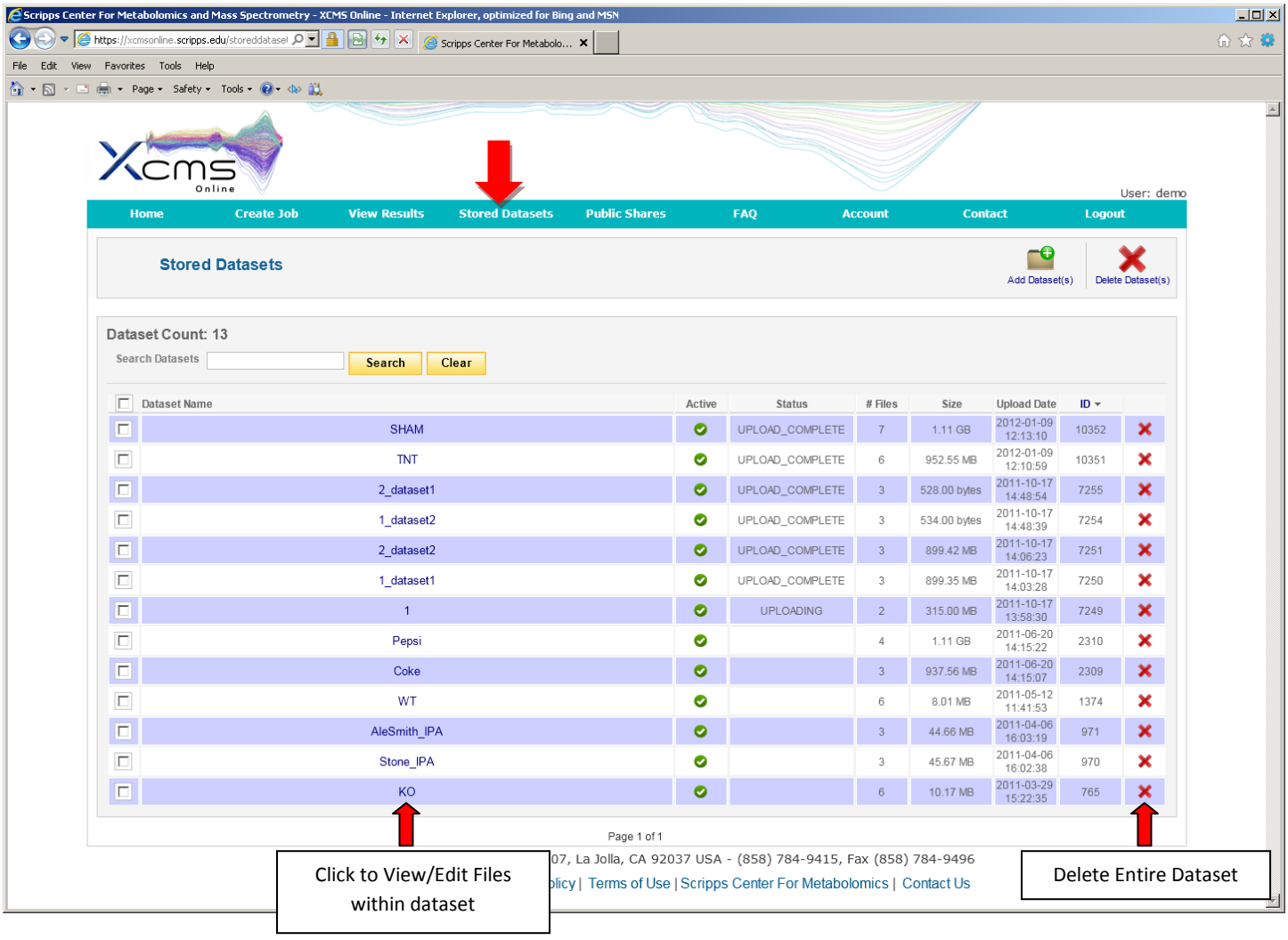

Figure 12 – Create New Job by clicking on "Create Job" in the top navigation menu

All screen shots taken from v. 1.22.01.

Administering Datasets:

Clicking on the "Edit/View Dataset" button (See Figure 12) will present the dataset edit view (See Figure 13). From here you can remove individual files that were uploaded as part of a dataset. Simply click on the "X" in the far right column. You may also change the dataset name or add comments to be stored with the dataset. In the bottom center of screen, you may add files to the current dataset by clicking the "Add Files" button (Figure 13).

Note: The following actions are planned but not included in XCMS Online as of version 1.20.7-Beta:

- Adding the same files to multiple datasets (upload once but include in separate datasets – possibly as part of iterative analysis)
- Multiple delete files (selection boxes where a user may want to delete several, but not all, files within a dataset)
- Connection to cloud storage (theoretically unlimited datasets could be stored for a user or lab for indefinite period of time)

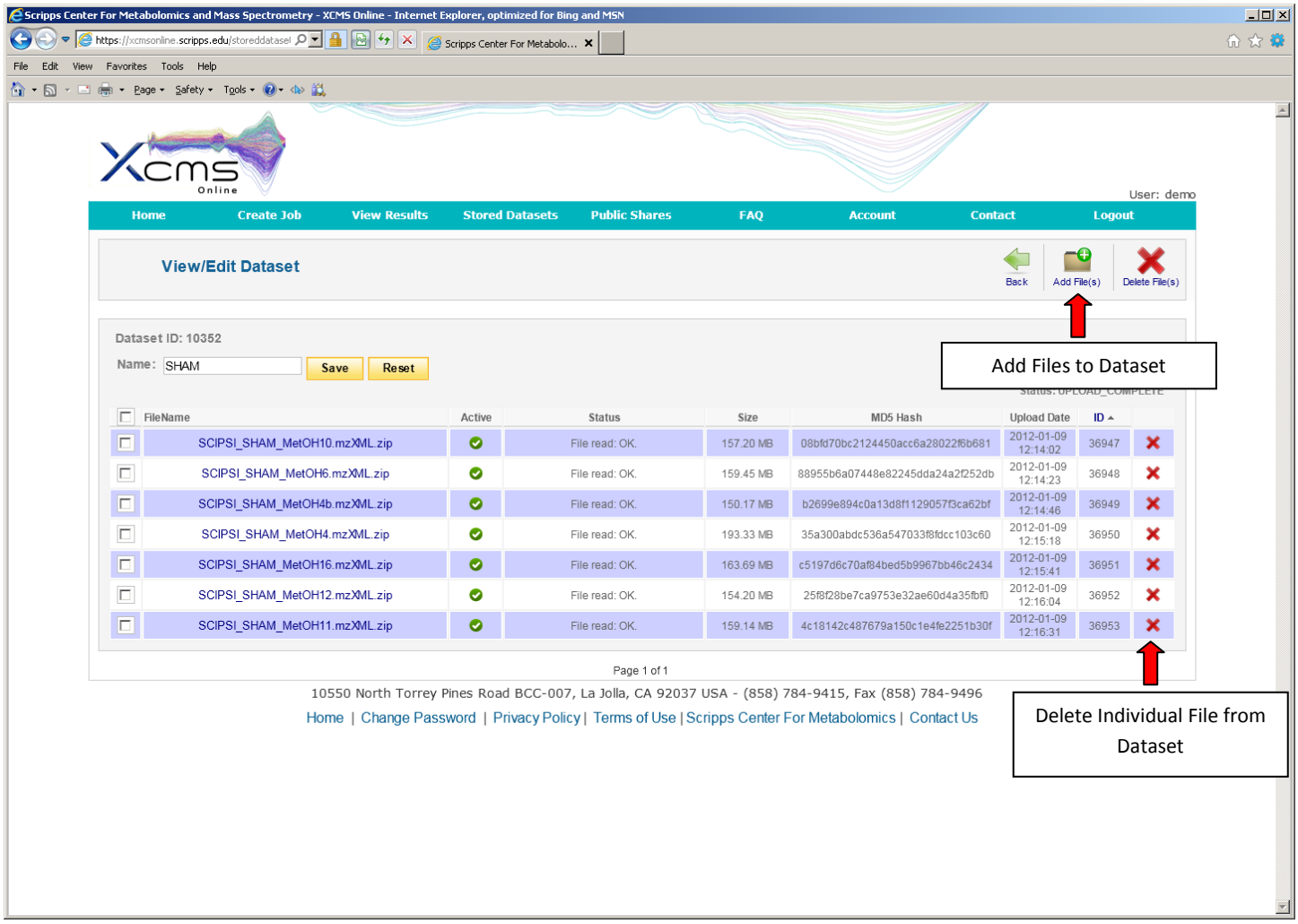

Figure 13 – Edit Dataset Files

All screen shots taken from v. 1.22.01.

Job Sharing:

After you have completed an experiment, you may wish to share it with another user or the public. This can be accomplished from the "View Results" menu. You first need to select the box on the left hand side that corresponds to the job(s) you wish to share and then click the "Share Job(s)" icon in the header (See Figure 14).

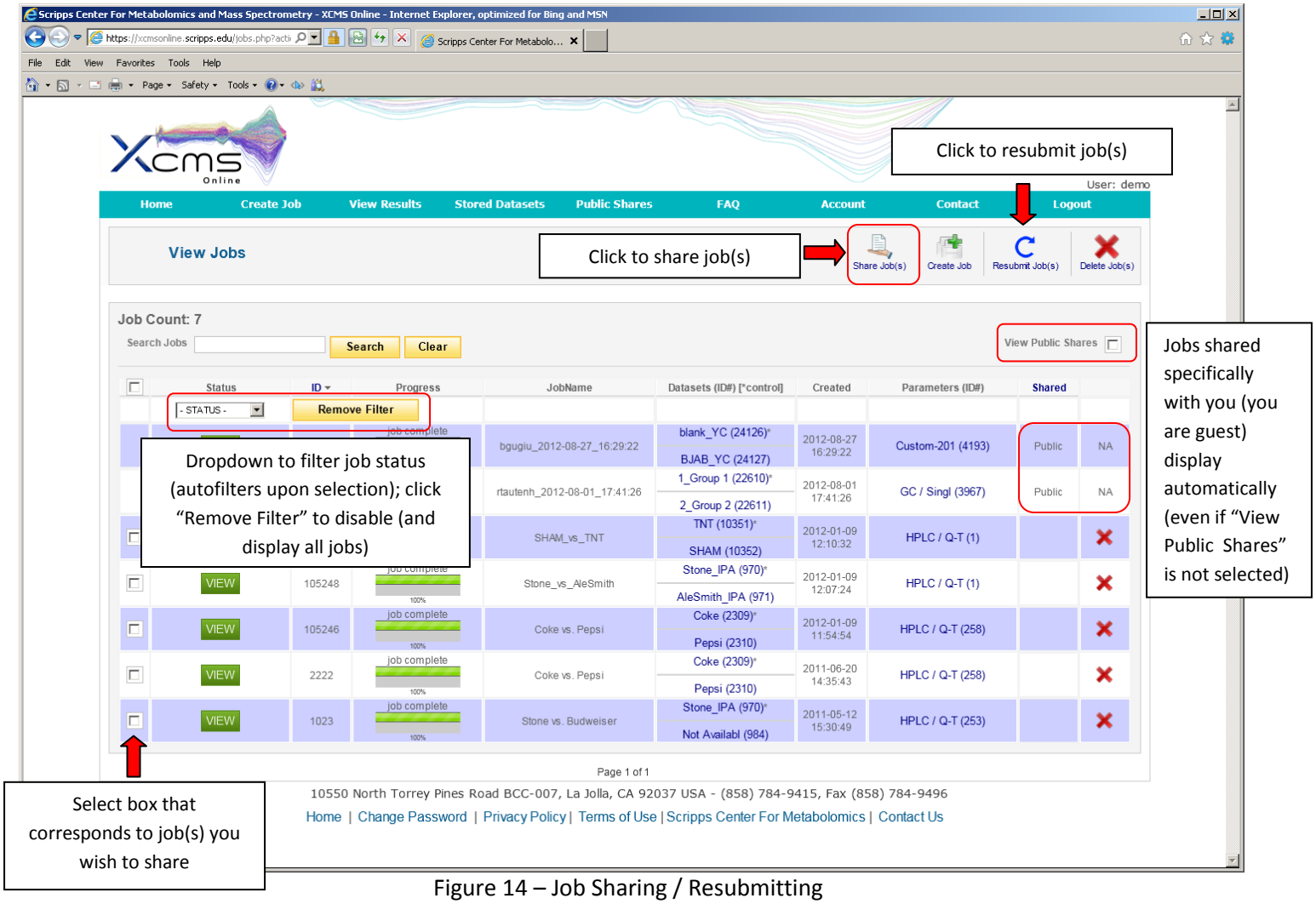

Figure 14 – Job Sharing / Resubmitting

When you click the "Share Job(s)" icon and new dialog box will appear on the screen where you will need to define the job sharing parameters (See Figure 15). Each job may be shared multiple times (with multiple users and with distinct expiration dates). Figure 15 demonstrates the detail view where you define the expiration date (by typing in YYYY-MM-DD format or selecting calendar icon) and select if the job is shared with the public or a specific user. You must also define if the job to be shared may be edited by your guest. Upon submitting the job share dialog, you will return to the "View Results" page and the system will automatically send an e-mail to your guest inviting him/her to login to the system to view the shared job. You will receive a carbon copy of the invitation e-mail. After job is shared the shared column on "View Results" page will show the job is shared (See Figure 8) along with a link to stop sharing the job.

Notes:

- We recommend view-only if job shared with public.
- Only job owner can delete job or share with guests

All screen shots taken from v. 1.22.01.

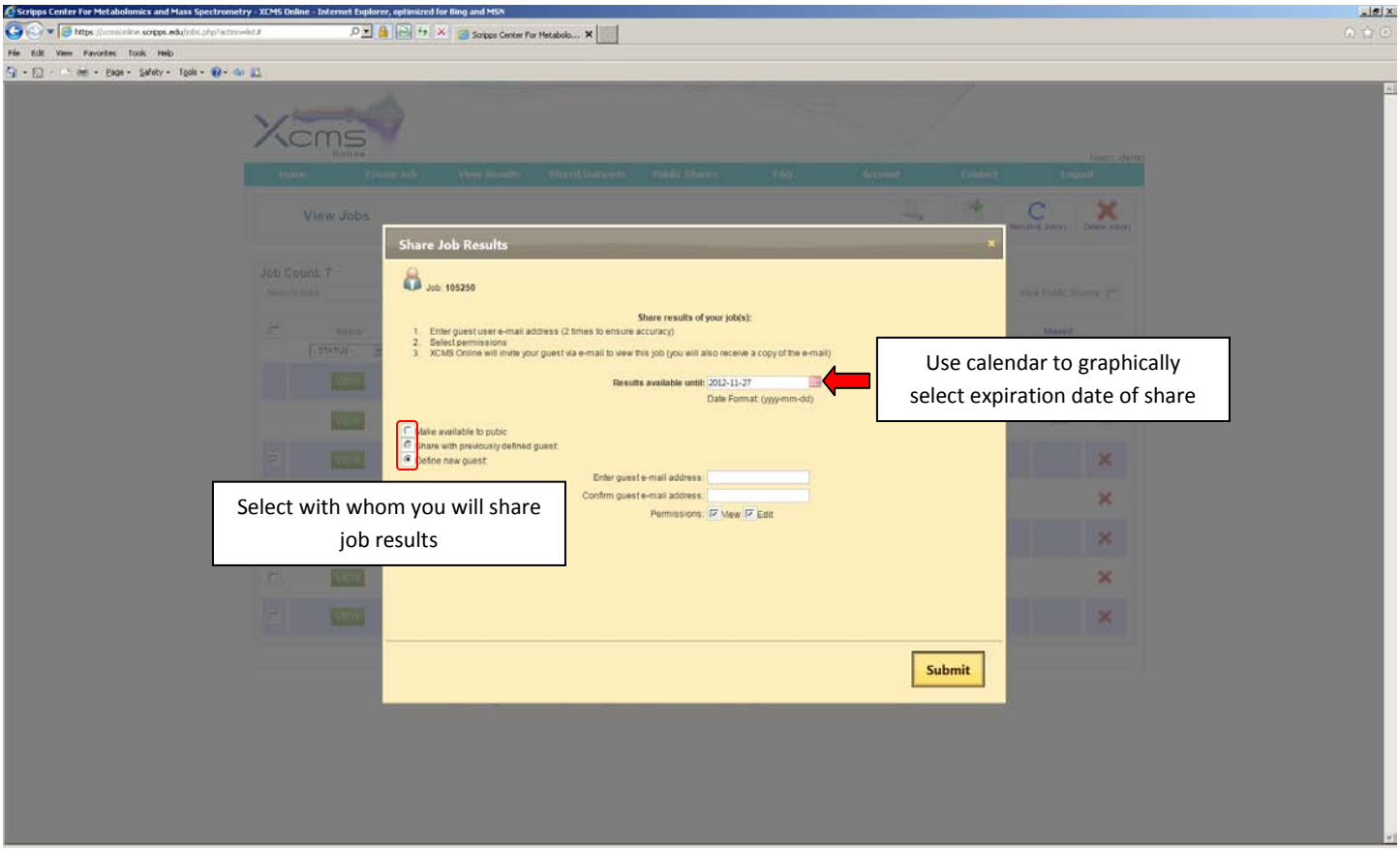

Figure 15 – Job Sharing Detail

Public Shares:

The "Public Shares" page is available on the top navigation menu (See Figure 16). This page displays all public jobs that have publication information and have been released by administrator. This section will enable academic users to publish results and reference the source experiment (including parameters, datasets, etc.). Users can view, filter, download and analyze the raw data just like the author. A user must create an account to view the results however summary information is available to general public. We ask that you reciprocally cite XCMS Online when you point researchers to the public shares:

> R. Tautenhahn, G.J. Patti, D. Rinehart, and G. Siuzdak. **XCMS Online: A Web-Based Platform to Process Untargeted Metabolomic Data,** *Analytical Chemistry*, 2012

If you wish to have your results added to this list, you must first share the job with the public and then contact administrator (use contact link) with additional information:

- Author(s)
- Job number
- Publication citation (journal/link
- Any notes you wish to include

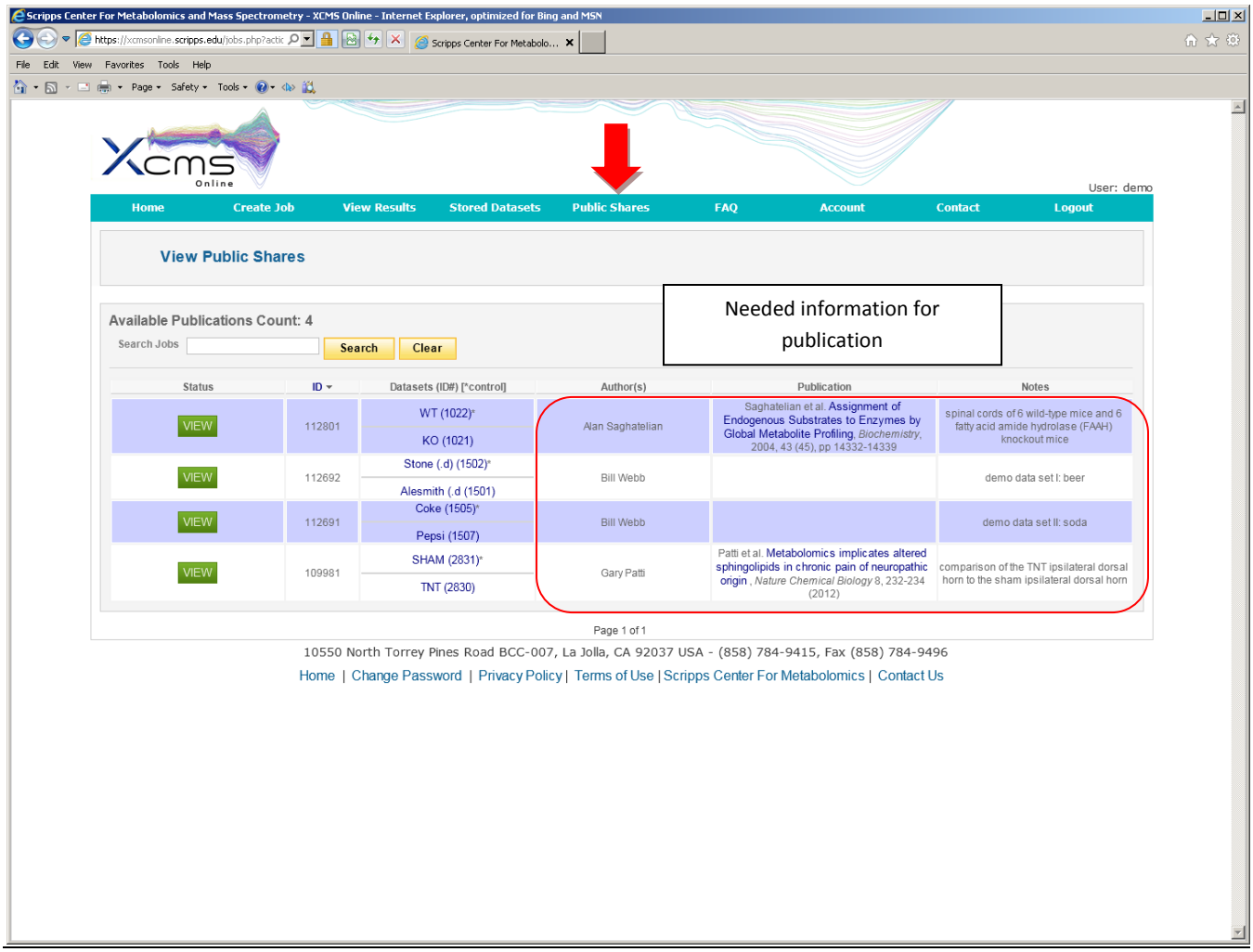

Figure 16 – Public Shares

Resubmitting Job:

If you previously ran a job, you may wish to resubmit the job. You can resubmit a job from the "View Results" page by selecting the job(s) in the left hand and then the "Resubmit Job(s)" icon in the header (See Figure 14). Number of jobs user may simultaneously resubmit may be limited by administrator. Typical scenarios for resubmitting are:

- Job ran but one of the datasets was missing a file (User would need to add files to existing dataset prior to resubmitting – see Figure 13)
- Job had error and did not complete the first time due to backend error (Administrator would need to resolve backend issue prior to resubmitting)

Note: If job is resubmitted (and was previously successfully completed), any user notes that were added to the diff report tables will be deleted.

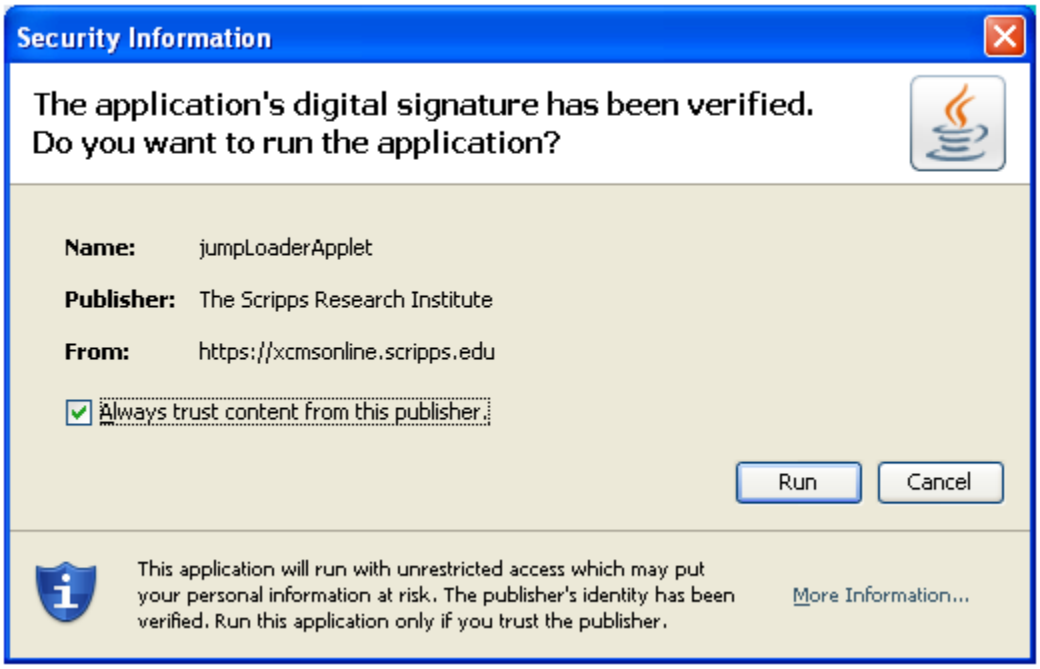

Figure A1 – Java Warning Dialog

All screen shots taken from v. 1.22.01.

Appendix B – Custom Parameter Creation

If you wish to create custom parameters for your experiment you will need to create a job following the steps described in the quick start guide. Once you elect to create your own parameter set, you will want to modify the default properties of a pre-defined parameter set (Step 4a). Each tab has categorized information for how XCMS Online will analyze your datasets.

Tab	Options Available
General	• Job Name, Comment • Retention time format (minutes or seconds) • Polarity (positive or negative)
Feature Detection	Choose method (CentWave or matchedFilter): CentWave: • PPM • Minimum / Maximum peak width matchedFilter: • FWHM (full width at half maximum of matched filtration Gaussian model peak) • Step size to use for profile generation Note: Additional options are available by clicking "Advanced Options" (Contextual help available on site)
Retention Time Correction	Choose method (obiwarp, peakgroups or none): Obiwarp: Step size PeakGroups: • Linear or non-linear alignment • Extra peaks to allow in retention time correction groups • Number of missing samples to allow in retention time correction groups Note: Additional options are available by clicking "Advanced Options" (Contextual help available on site)
Alignment	• Minimum fraction of samples necessary in at least one of the sample groups for it to be a valid group • Allowable retention time deviations (in seconds) • Width of overlapping *m/z* slices to use for creating peak density chromatograms Note: Additional options are available by clicking "Advanced Options" (Contextual help available on site)
Statistics	• p-value threshold (highly significant features) [included] • fold change threshold (highly significant features) • p-value threshold (significant features) [excluded] Note: Additional options are available by clicking "Advanced Options" (Contextual help available on site)
Annotation	• Search for isotopes only or isotopes + adducts • *m/z* absolute error • ppm error
Identification	• ppm tolerance for database search • adducts (user may select multiple adducts by holding down "Ctrl" key)
Visualization	• EIC width (default width for ion chromatograms, in seconds)
Miscellaneous	• Other options