

pTop 1.2 User Guide

Version 1.2.0

pFind Group

2017.12.26

❖ Before software installation

❖ Installation steps

❖ Software registration

❖ How to run pTop 1.2

- Create a new search task
- View search results

Before software installation

❖ Hardware requirements

- 2 GB or higher recommended memory

❖ Software requirements

- Windows 7 or above
- Xcalibur (2.1 or above) or MSFileReader
- .NET Framework 4.5 environment

Installation steps

- ❖ **Step 1: Select the installer language(Figure 1). Now it only supports English and Chinese(Simplified).**

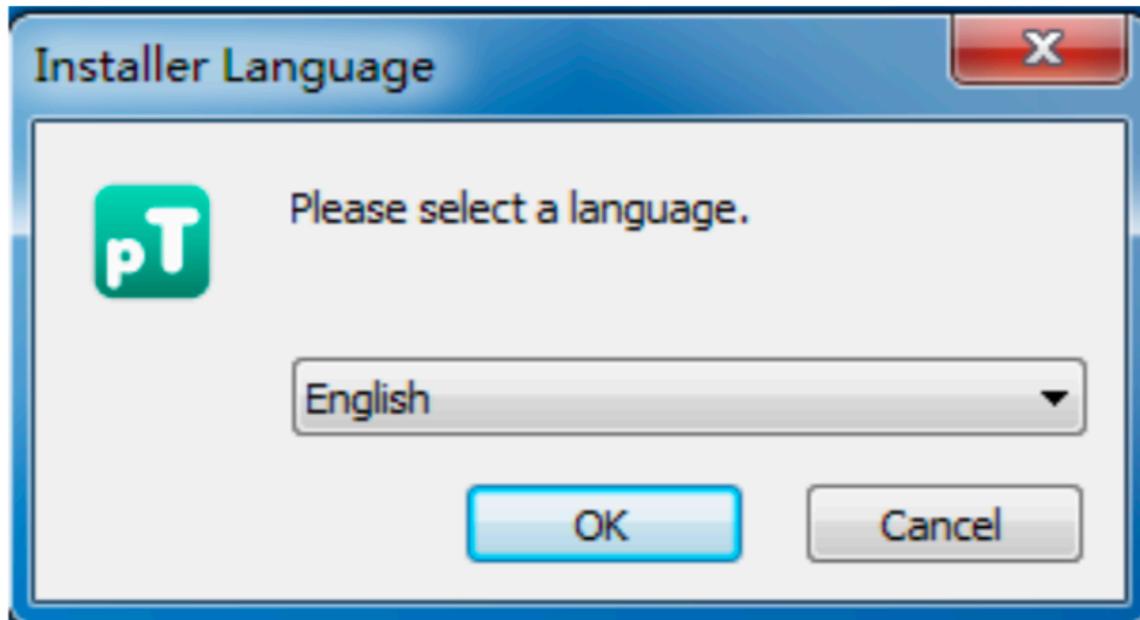


Figure 1. Installer language

Installation steps

- ❖ Step 2: Click Next to start the setup.

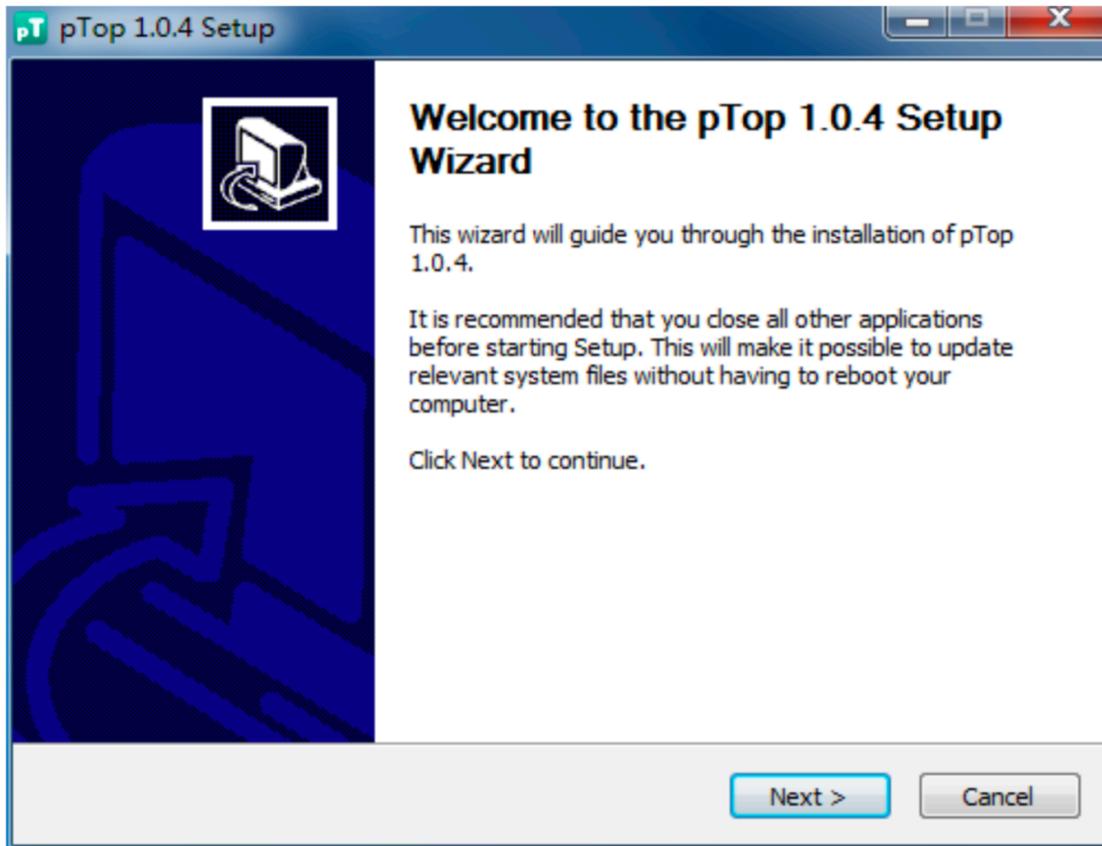


Figure 2. Welcome to the setup wizard

Installation steps

- ❖ Step 3: Choose the install Location. And D drive disk is recommended.

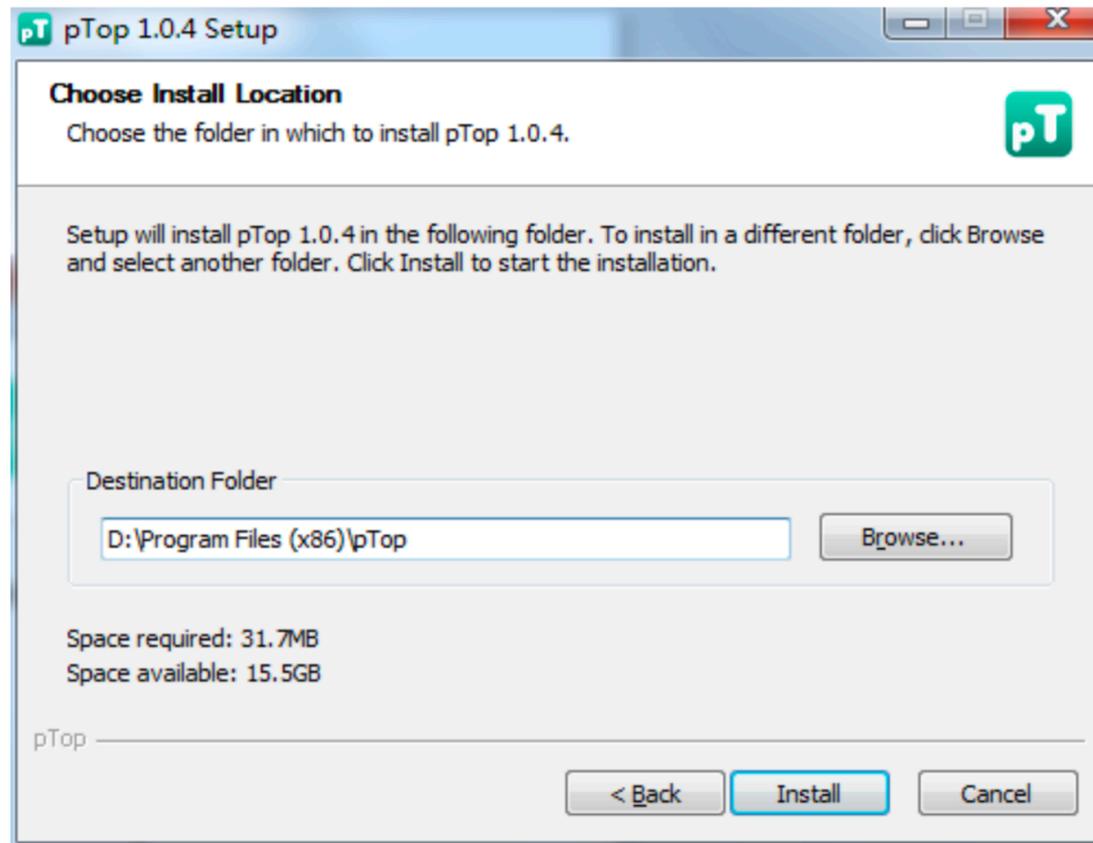


Figure 3. Choose install location

Installation steps

- ❖ Step 4: Just wait a few seconds, the Installation will be finished.

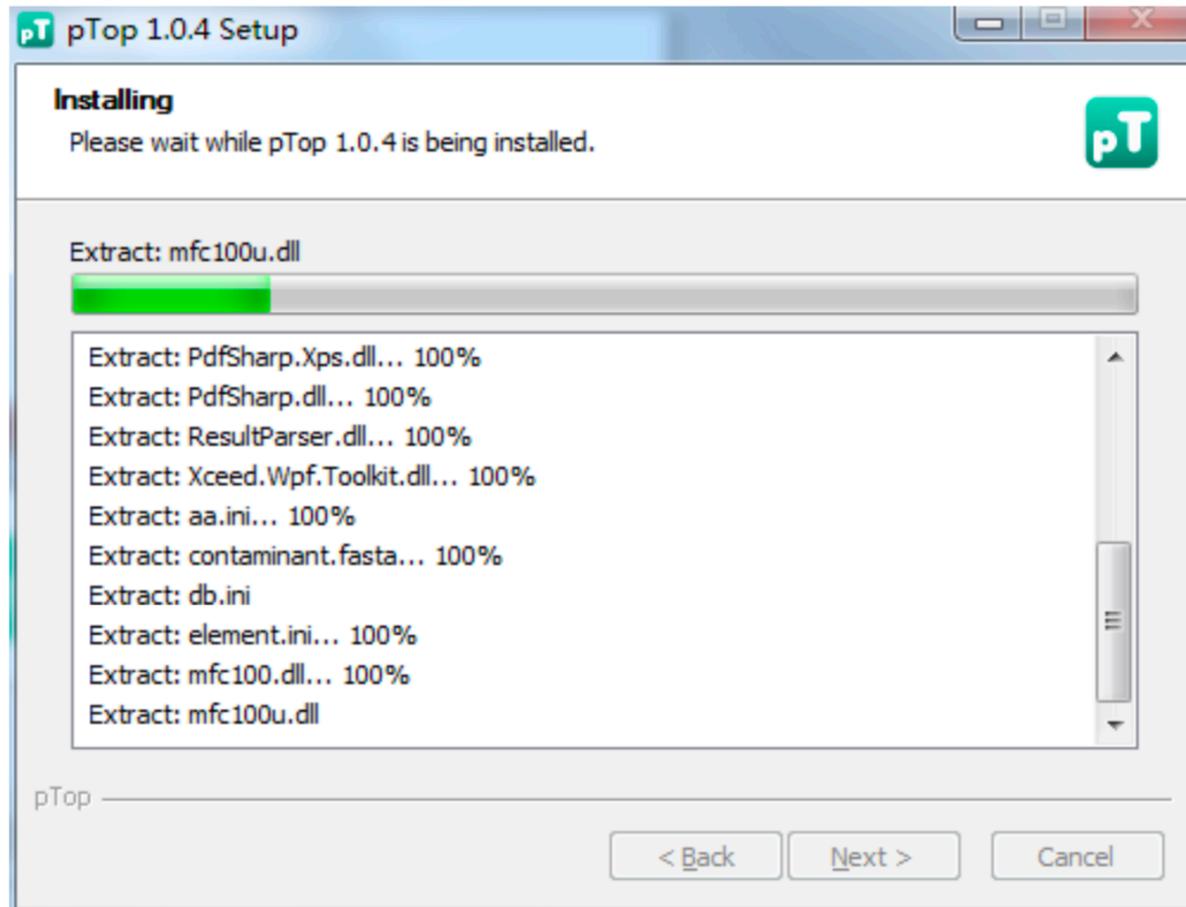
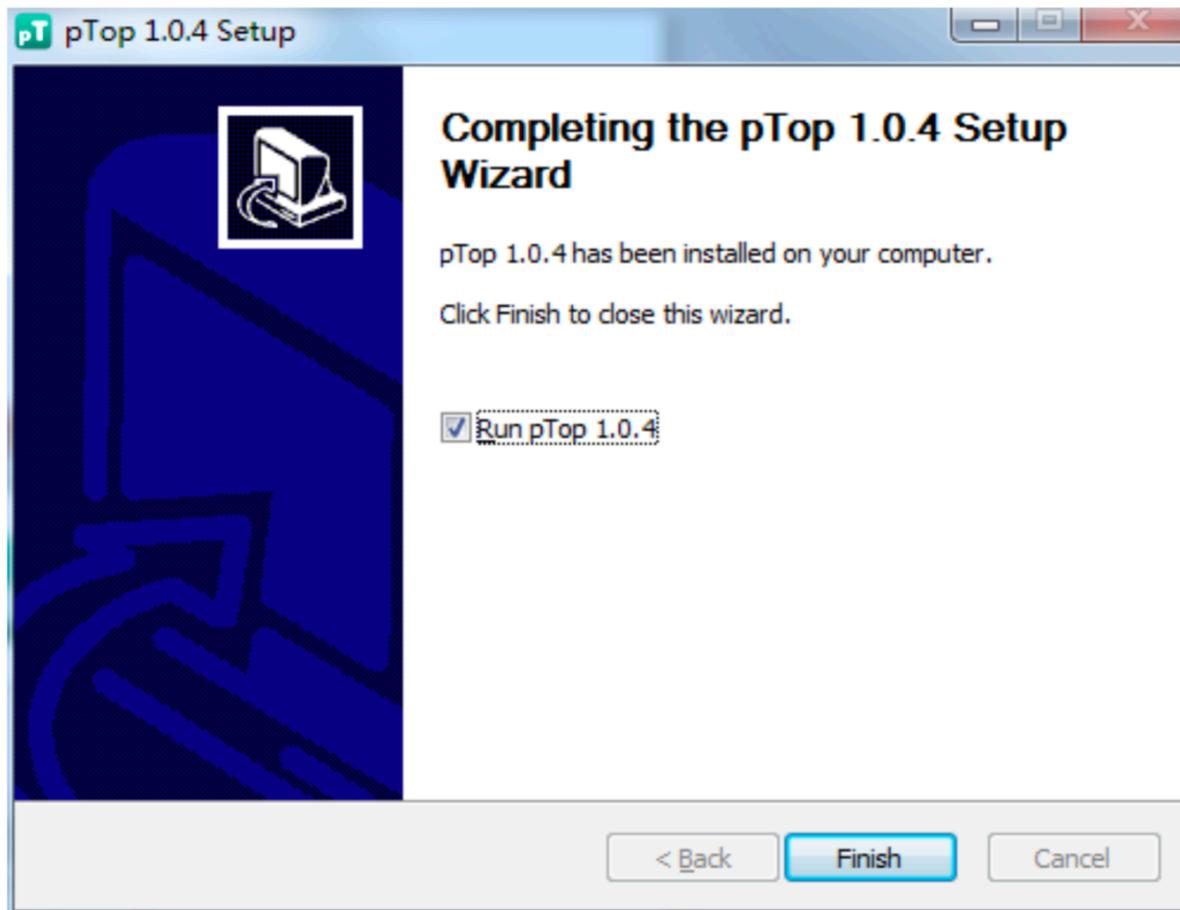


Figure 4. Installing

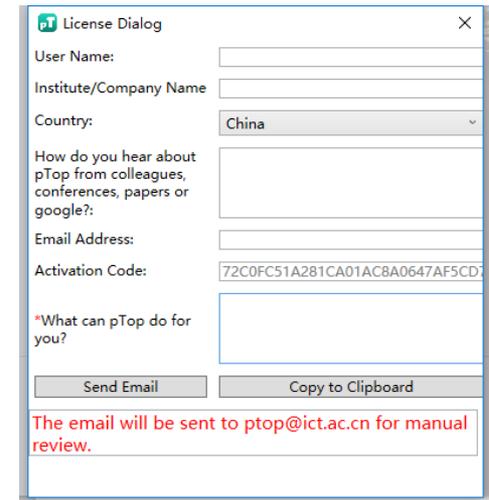
Installation steps

- ❖ Step 5: Finally, you can check the box of run pTop and then click Finish to start pTop.



Software registration

- ❖ Click pTop.exe and fill in personal information in the following information panel.
- ❖ Send the information to ptop@ict.ac.cn
- ❖ Apply for pTop.license.
- ❖ Put the pTop.license into pTop installation directory under the **\pTop\bin** folder.

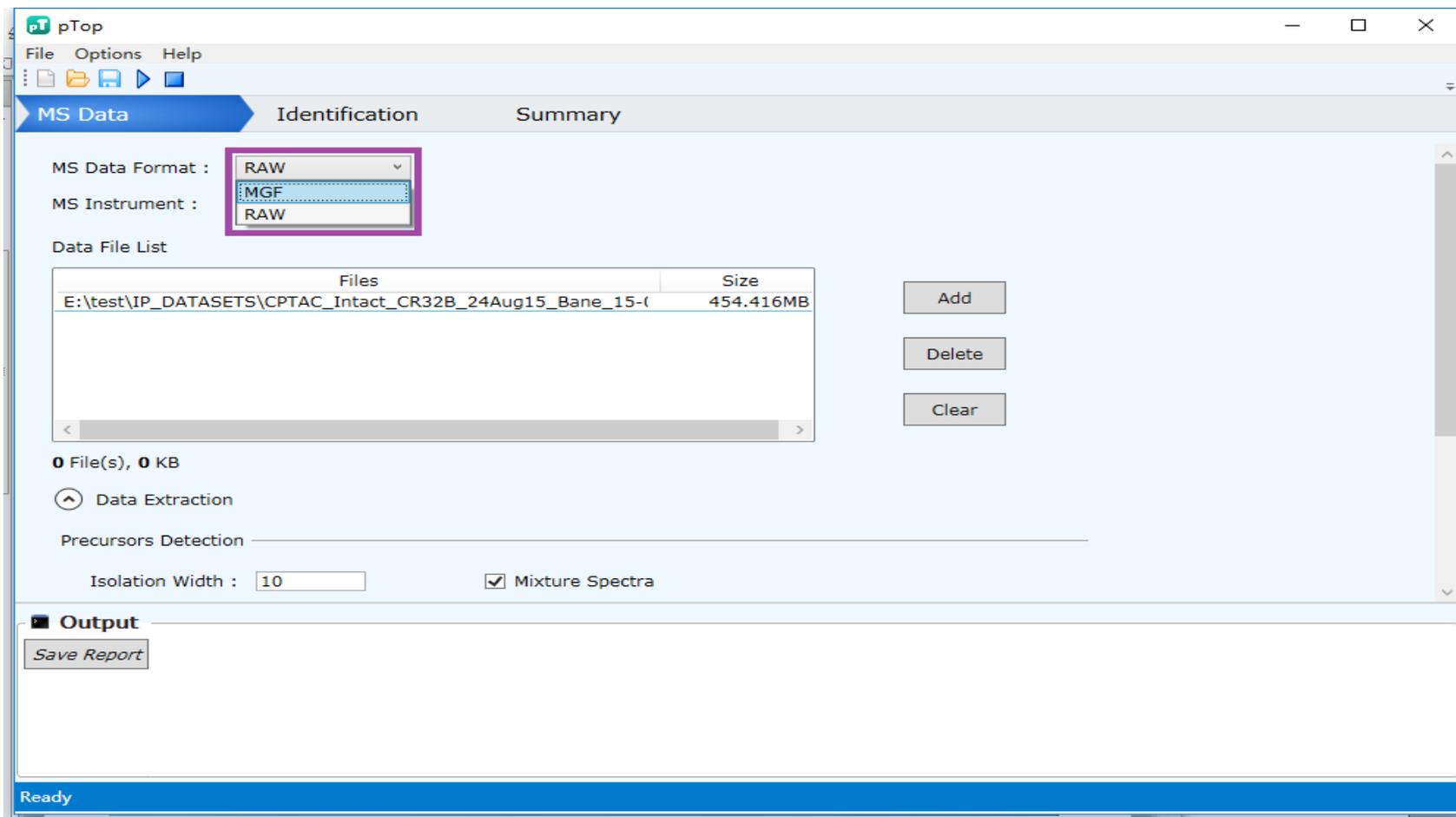


The screenshot shows a 'License Dialog' window with the following fields and content:

- User Name: [Empty text box]
- Institute/Company Name: [Empty text box]
- Country: [Dropdown menu showing 'China']
- How do you hear about pTop from colleagues, conferences, papers or google?: [Empty text box]
- Email Address: [Empty text box]
- Activation Code: [Text box containing '72C0FC51A281CA01AC8A0647AF5CD']
- *What can pTop do for you?: [Empty text box]
- Buttons: 'Send Email' and 'Copy to Clipboard'
- Footer text: 'The email will be sent to ptop@ict.ac.cn for manual review.'

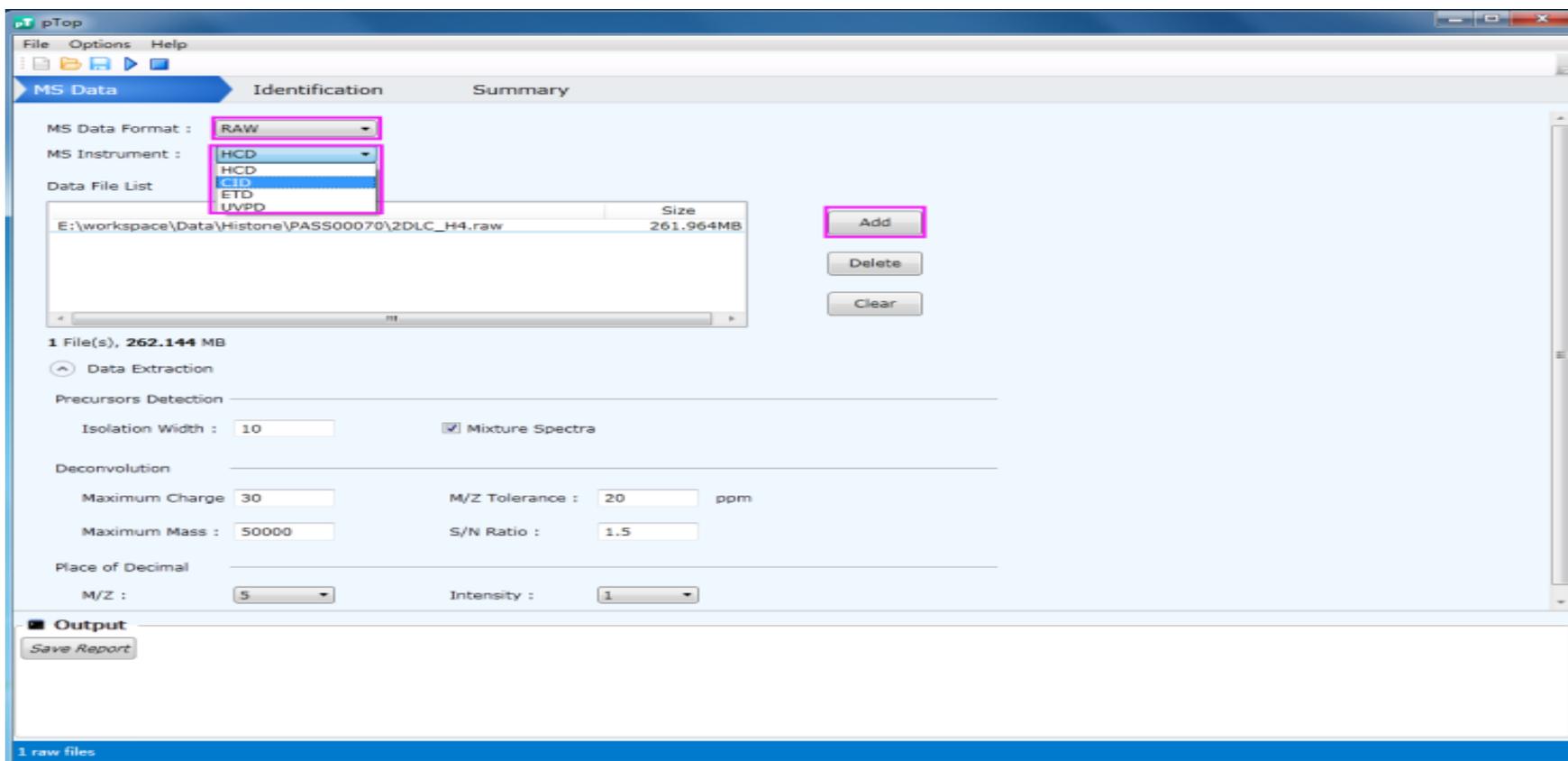
1. Data import and preprocess.

- ❖ **MS Data format: following formats are supported by pTop: RAW, MGF and PF.**



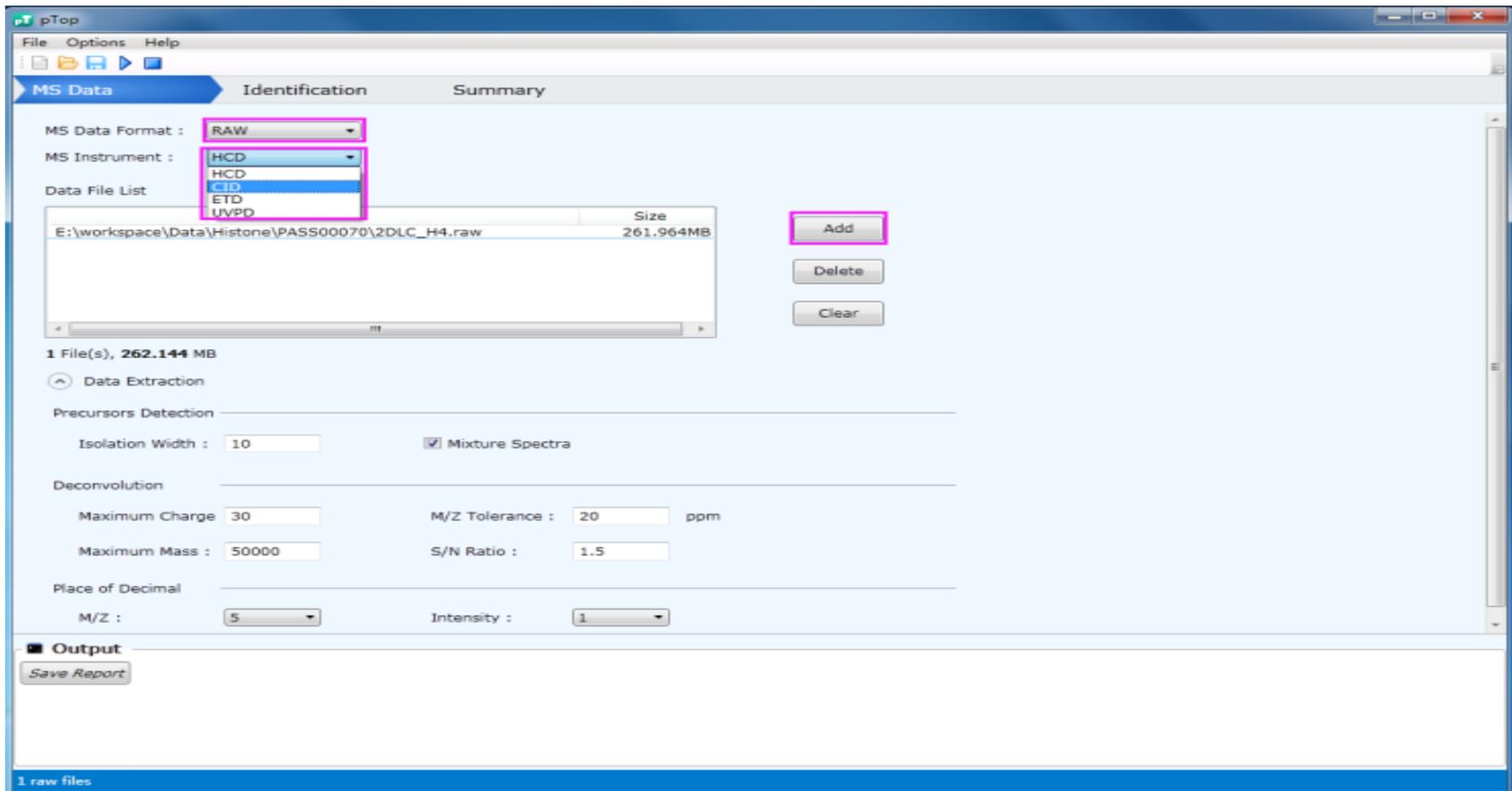
1. Data import and preprocess.

- ❖ MS Instrument determines which fragment ion series will be used for scoring. Now HCD, CID, ETD and UVPD are supported.



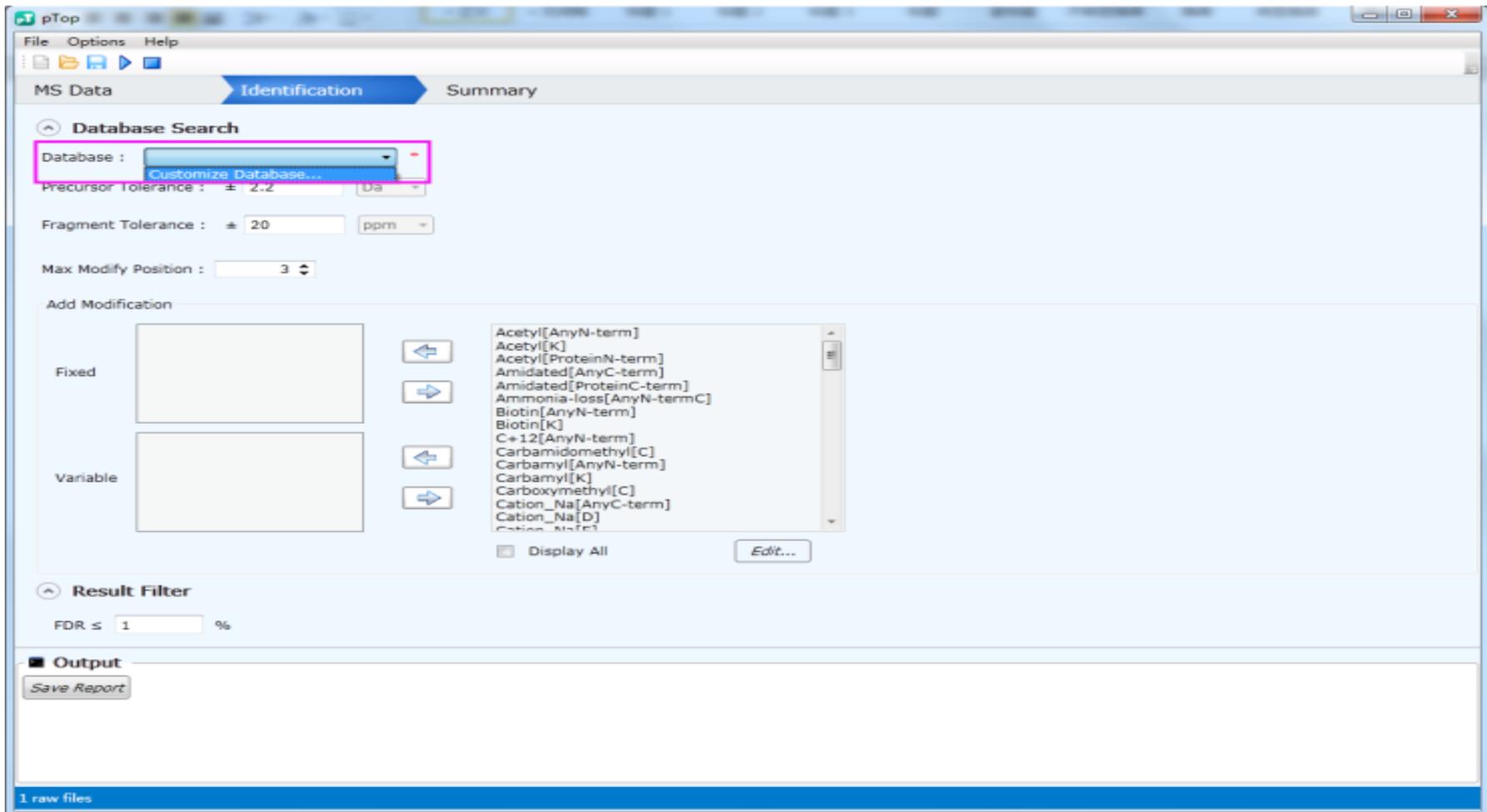
1. Data import and preprocess.

- ❖ Click Add to put the paths of input files in the list, the path or folder containing the tandem mass spectra.



2. Set search parameters

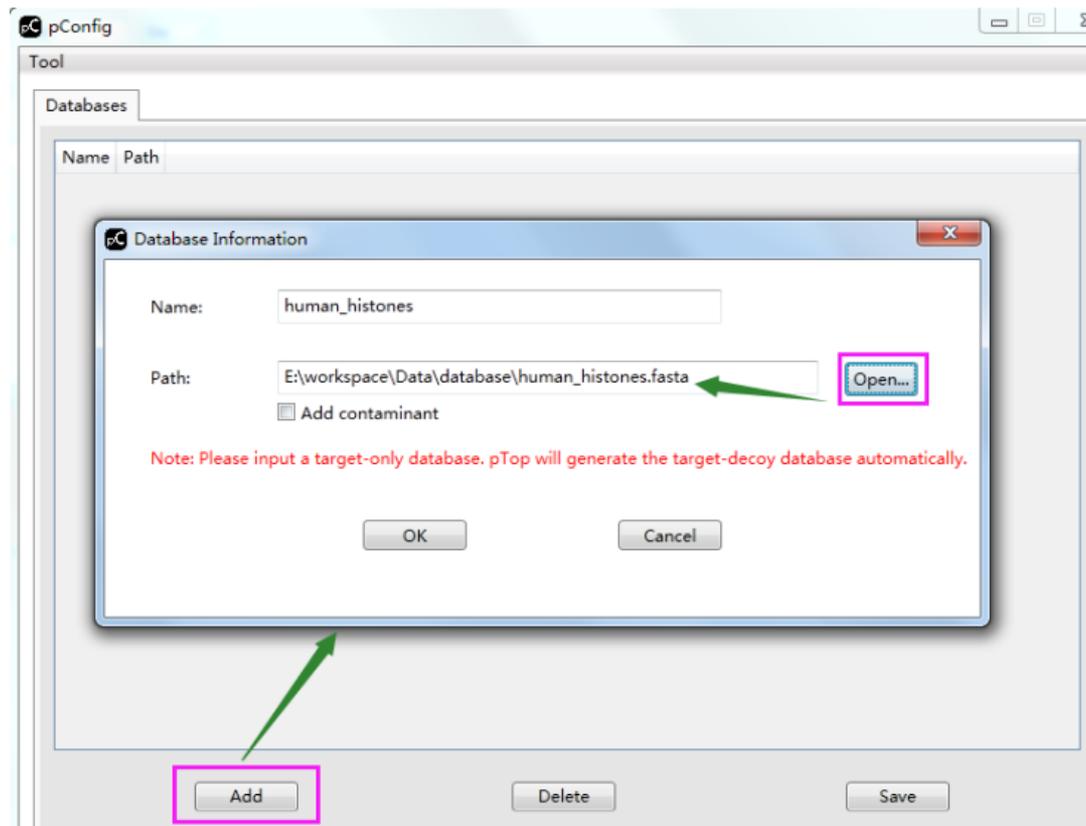
❖ A) Select and import database.



The screenshot displays the pTop software interface, specifically the 'Identification' tab. The 'Database Search' section is highlighted with a pink box, showing a dropdown menu for 'Database' with the option 'Customize Database...' selected. Below this, the 'Precursor Tolerance' is set to 2.2 Da, and the 'Fragment Tolerance' is set to 20 ppm. The 'Max Modify Position' is set to 3. The 'Add Modification' section shows a list of modifications, including Acetyl, Amidated, Biotin, C+12, Carbamidomethyl, Carbamyl, Carboxymethyl, Cation_Na, and Cation_Na. The 'Result Filter' section shows 'FDR ≤ 1 %'. The 'Output' section has a 'Save Report' button. The status bar at the bottom indicates '1 raw files'.

2. Set search parameters

- ❖ A) Select and import database.
 - Add contaminated proteins to the database if it doesn't contain them.



2. Set search parameters

❖ B) Select the modifications.

The screenshot shows the pTop software interface in the Identification tab. The Database Search section is configured with the following parameters:

- Database: human_histones
- Precursor Tolerance: ± 2.2 Da
- Fragment Tolerance: ± 20 ppm
- Max Modify Position: 3

The Add Modification section shows a list of modifications. The 'Fixed' section is empty. The 'Variable' section contains:

- Acetyl[AnyN-term]
- Acetyl[K]
- Phospho[S]
- Phospho[T]
- Phospho[Y]

The list of modifications includes:

- Ala->Trp[A]
- Ala->Tyr[A]
- Ala->Val[A]
- Ala->Xle[A]
- Amidated[AnyC-term]
- Amidated[ProteinC-term]
- Amidine[AnyN-term]
- Amidine[K]
- Amidino[C]
- Amino[Y]
- Ammonia-loss[AnyN-termC]
- Ammonia-loss[N]
- Ammonia-loss[ProteinN-termS]
- Ammonia-loss[ProteinN-termT]
- Ammonium[AnyC-term]
- Ammonium[N]

The 'Display All' checkbox is checked, and the 'Edit...' button is visible. The Result Filter section shows FDR ≤ 1 %.

The Output section has a 'Save Report' button.

At the bottom, it indicates '1 raw files'.

2. Set search parameters

❖ B) Select the modifications.

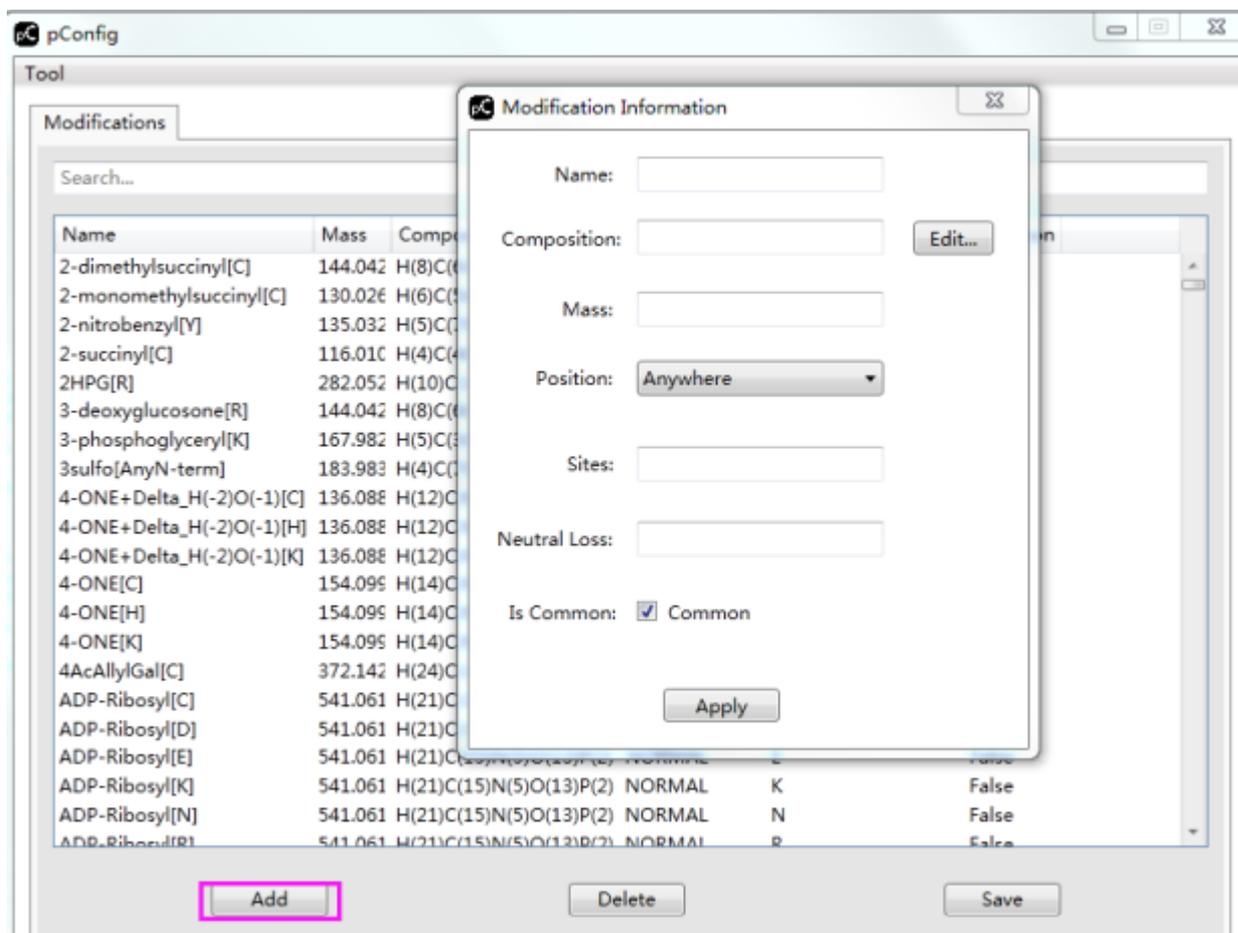
The screenshot shows the pTop software interface with the following settings and actions:

- Database Search:**
 - Database: human_histones
 - Precursor Tolerance: ± 2.2 Da
 - Fragment Tolerance: ± 20 ppm
 - Max Modify Position: 3
- Add Modification:**
 - Fixed:** (Empty box)
 - Variable:** Acetyl[AnyN-term], Acetyl[K], Phospho[S], Phospho[T], Phospho[Y]
 - Modification List:** Ala->Trp[A], Ala->Tyr[A], Ala->Val[A], Ala->Xle[A], Amidated[AnyC-term], Amidated[ProteinC-term], Amidine[AnyN-term], Amidine[K], Amidino[C], Amino[Y], Ammonia-loss[AnyN-termC], Ammonia-loss[N], Ammonia-loss[ProteinN-termS], Ammonia-loss[ProteinN-termT], Ammonium[AnyC-term], Ammonium[N]
 - Selection:** Left arrows point to Ala->Trp[A] and Amidine[K]. Right arrows point to Acetyl[K] and Phospho[Y].
 - Buttons:** Display All, Edit...
- Result Filter:** FDR ≤ 1 %
- Output:** Save Report button

1 raw files

2. Set search parameters

- ❖ B) Select the modifications.
 - You can add a custom modification.



Searching...

The screenshot shows the pTop software interface. The window title is 'pTop'. The menu bar includes 'File', 'Options', and 'Help'. Below the menu bar, there are icons for file operations. The main area is divided into two tabs: 'MS Data' and 'Identification', with 'Summary' selected. The 'MS Data' section contains a table with the following data:

Property	Value
Format	RAW
Instrument	HCD
Data File List	E:\workspace\Data\Histone\PASS00070\2DLC_H4.raw
Isolation Width	10
Mixture Spectra	True
Model	svm
Max Charge	30
M/Z Tolerance	20
Max Mass	50000
S/N Ratio	1.5
Decimal Places of M/Z	5
Decimal Places of Intensity	1

The 'Identification' section contains a table with the following data:

Property	Value
Database	human_histones
Precursor Tolerance	±2.2 Da
Fragment Tolerance	±20 ppm
Max Modify Position	3
Fixed Modifications	Acetyl[A nyN-term] Acetyl[K]
Variable Modifications	Phospho[S] Phospho[T] Phospho[Y]
FDR	1

At the bottom of the 'Identification' section, there are three buttons: 'Save', 'Start', and 'Stop'. Below this is an 'Output' section with a 'Save Report' button. The status bar at the bottom indicates '1 raw files'.

Searching completed

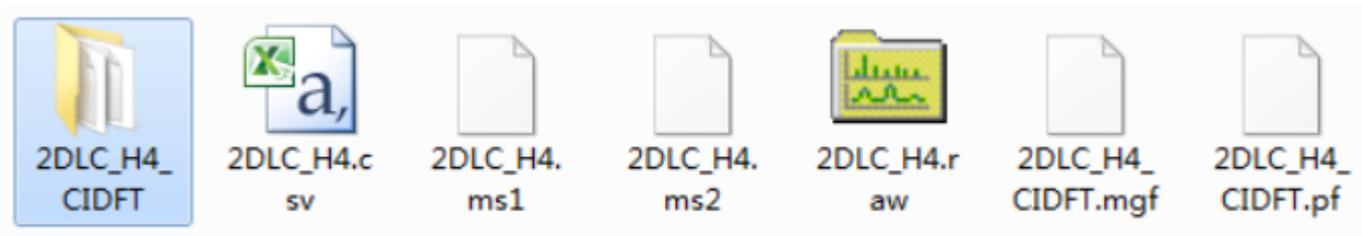
The screenshot shows the pTop software interface. The main window has three tabs: MS Data, Identification, and Summary. The Identification tab is active, displaying a table of search parameters. A dialog box titled "Work done!" with a close button and a "确定" (OK) button is overlaid on the table. The Output window at the bottom shows a log of operations and an error message.

Property	Value
Database	human
Precursor Tolerance	±3.2 D
Fragment Tolerance	±20 p
Max Modify Position	2
Fixed Modifications	Acetyl
Variable Modifications	
FDR	1%

```
[pTop] Database: E:\pTop安装测试\Data\human_histones.fasta
[pTop] Getting the PTM forms...
[pTop] Time elapsed: 174.138s
[pTop] Indexing...
[Error] Cannot open the database file: E:\pTop安装测试\Data\human_histones.fasta
```

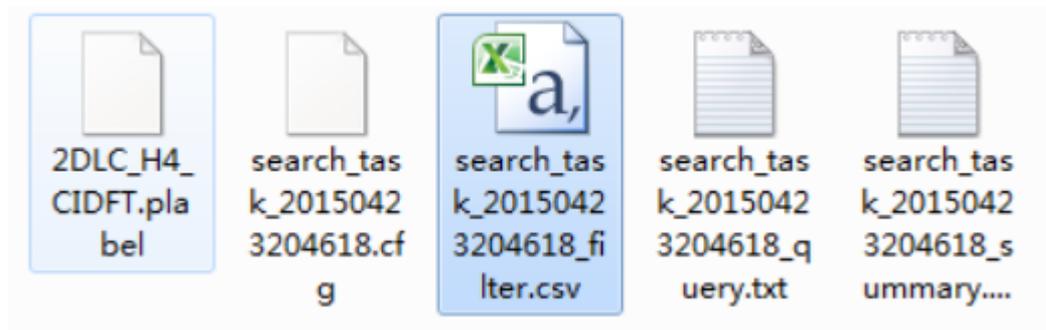
Contents of search results files

❖ In the same path of the input data, you can see a folder with the same name of MGF file.



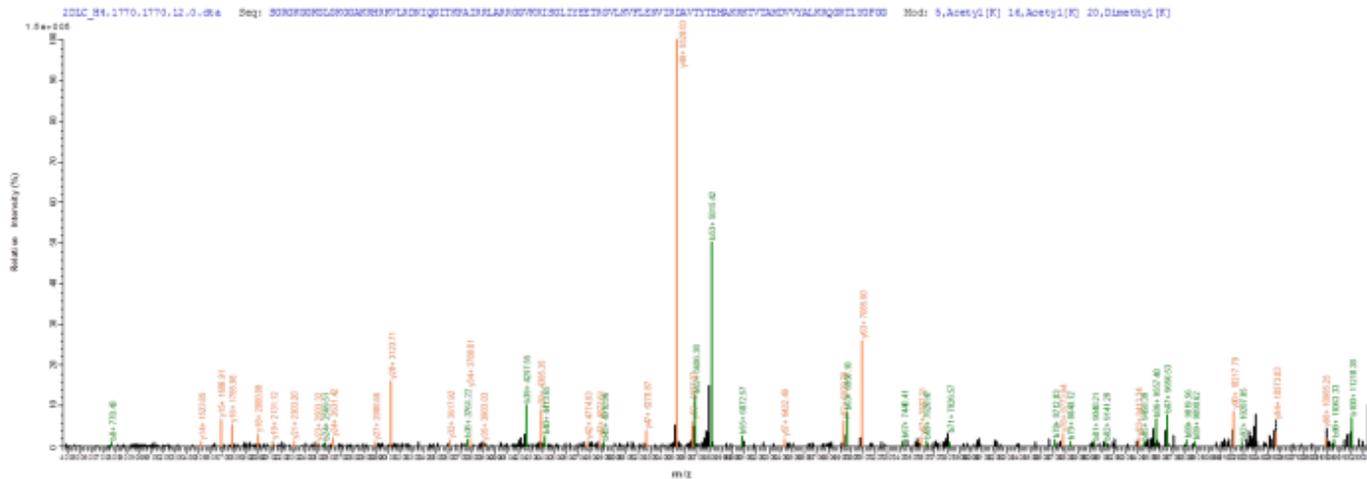
Contents of search results files

- ❖ In the folder, there are 5 files for each search. They are .plabel, .cfg, filter.csv, query.txt and summary.txt. And the finally identification reports are list in the filter.csv file.



View search results

❖ pLabel: Result statistics





❖ **Thank you for using pTop 1.2!**

❖ **If you have any questions, please contact ptop@ict.ac.cn or rxsun@ict.ac.cn .**