

pTop 1.2 User Guide

Version 1.2.0



pFind Group

2017.12.26

❖ Before software installation

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❖ 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).**

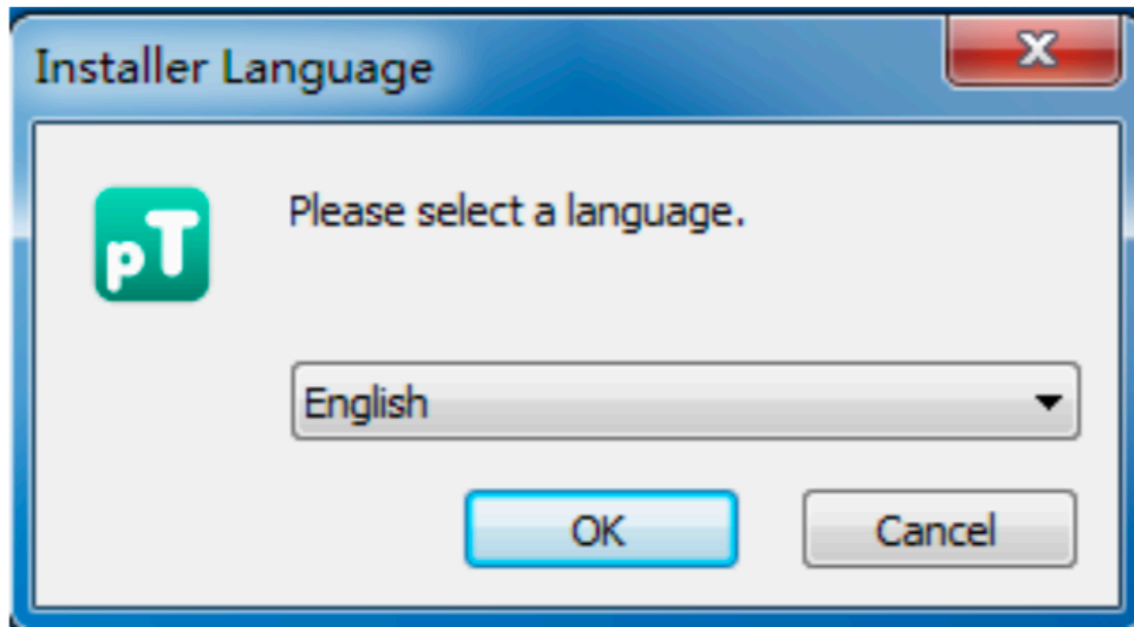


Figure 1. Installer language

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.

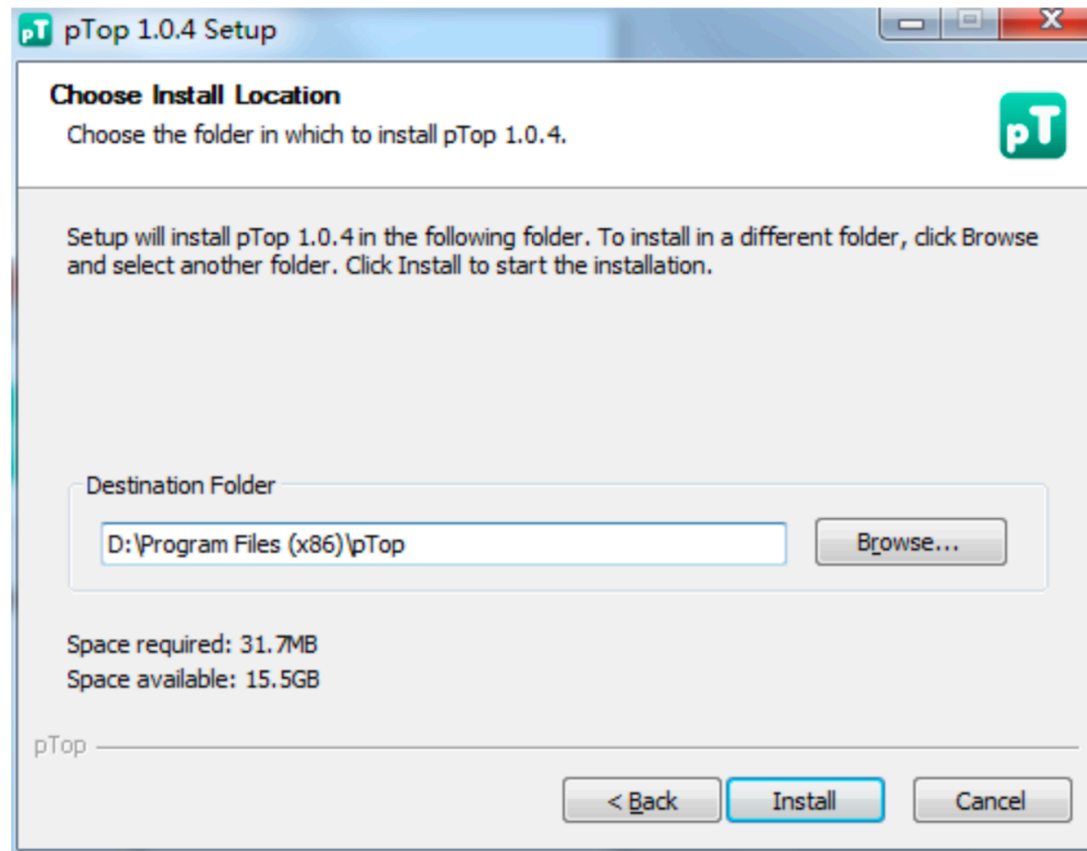


Figure 3. Choose install location

Installation steps

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

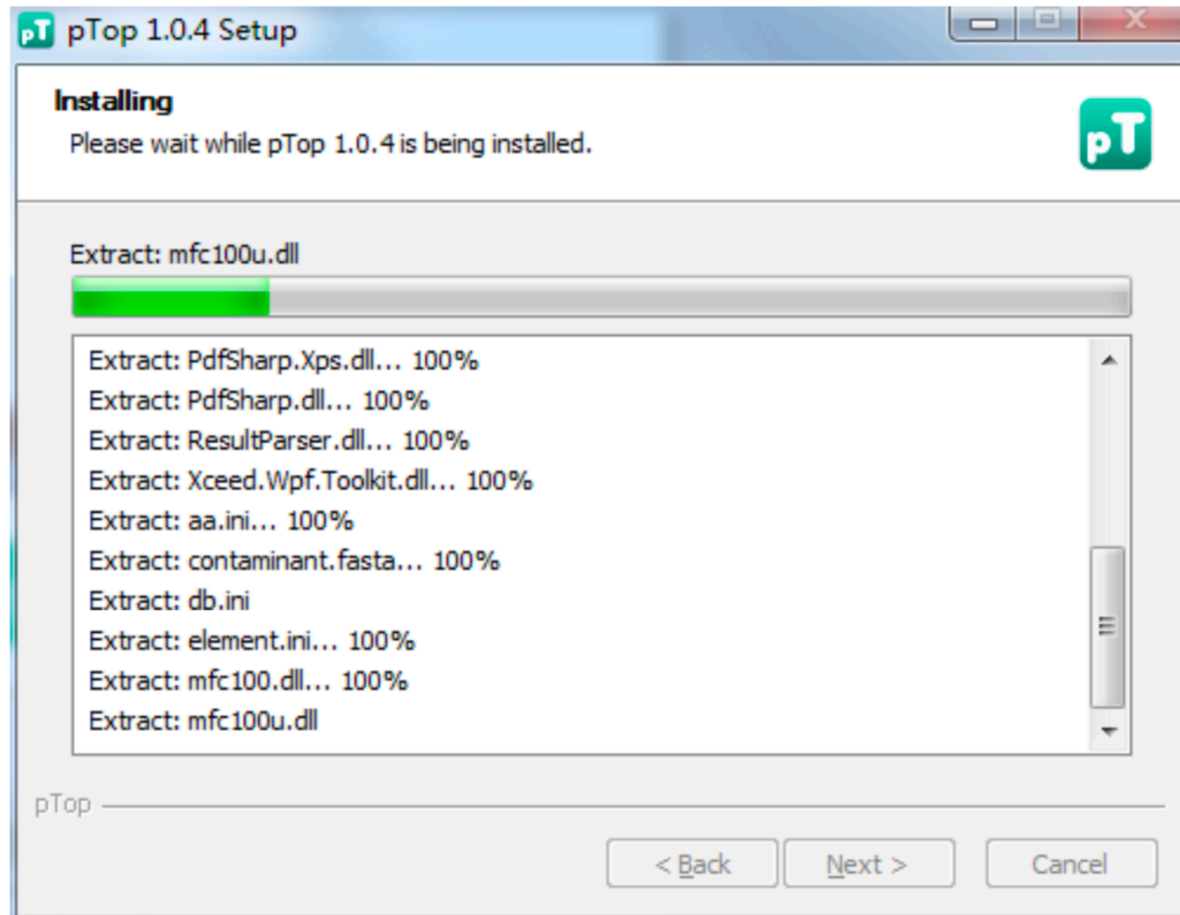
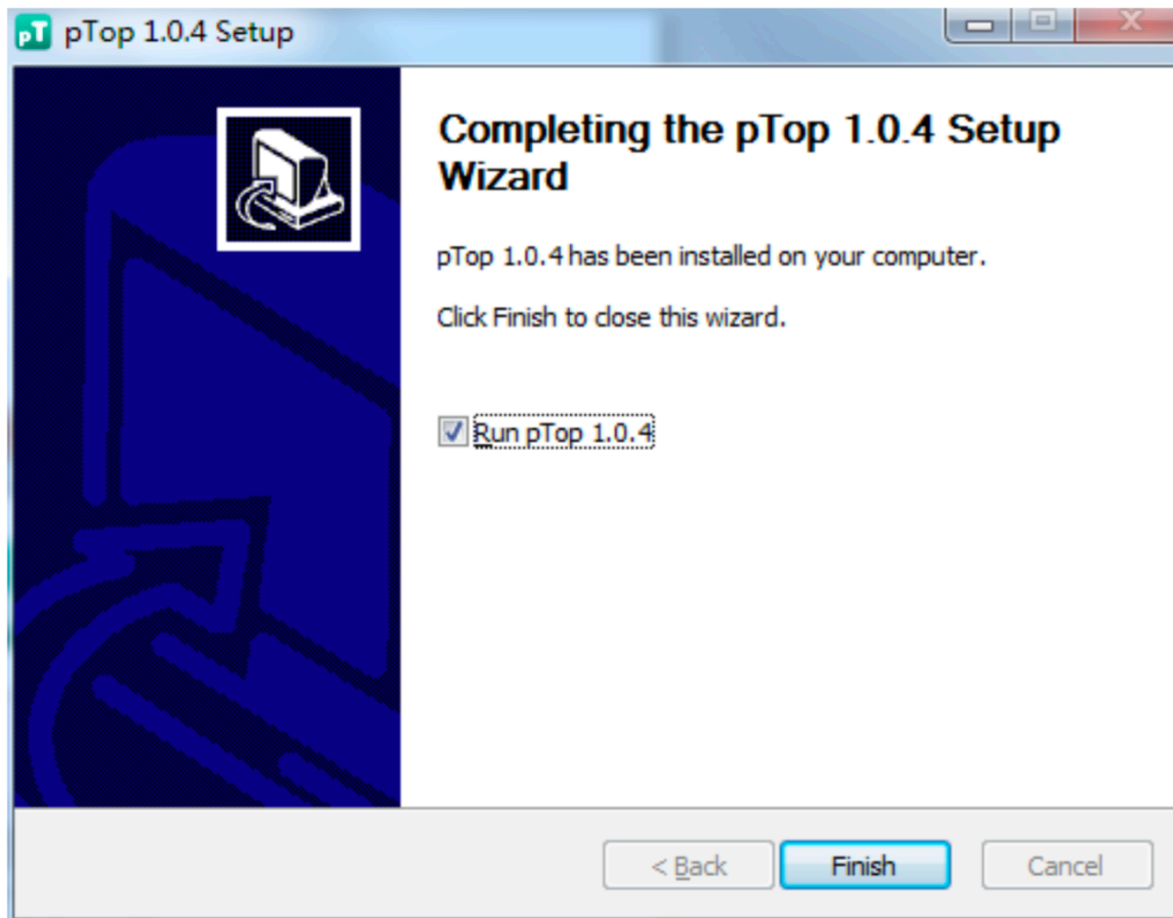


Figure 4. Installing

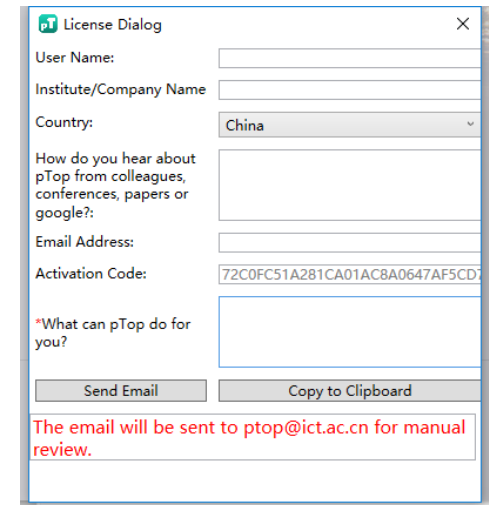
Installation steps

- ❖ 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.

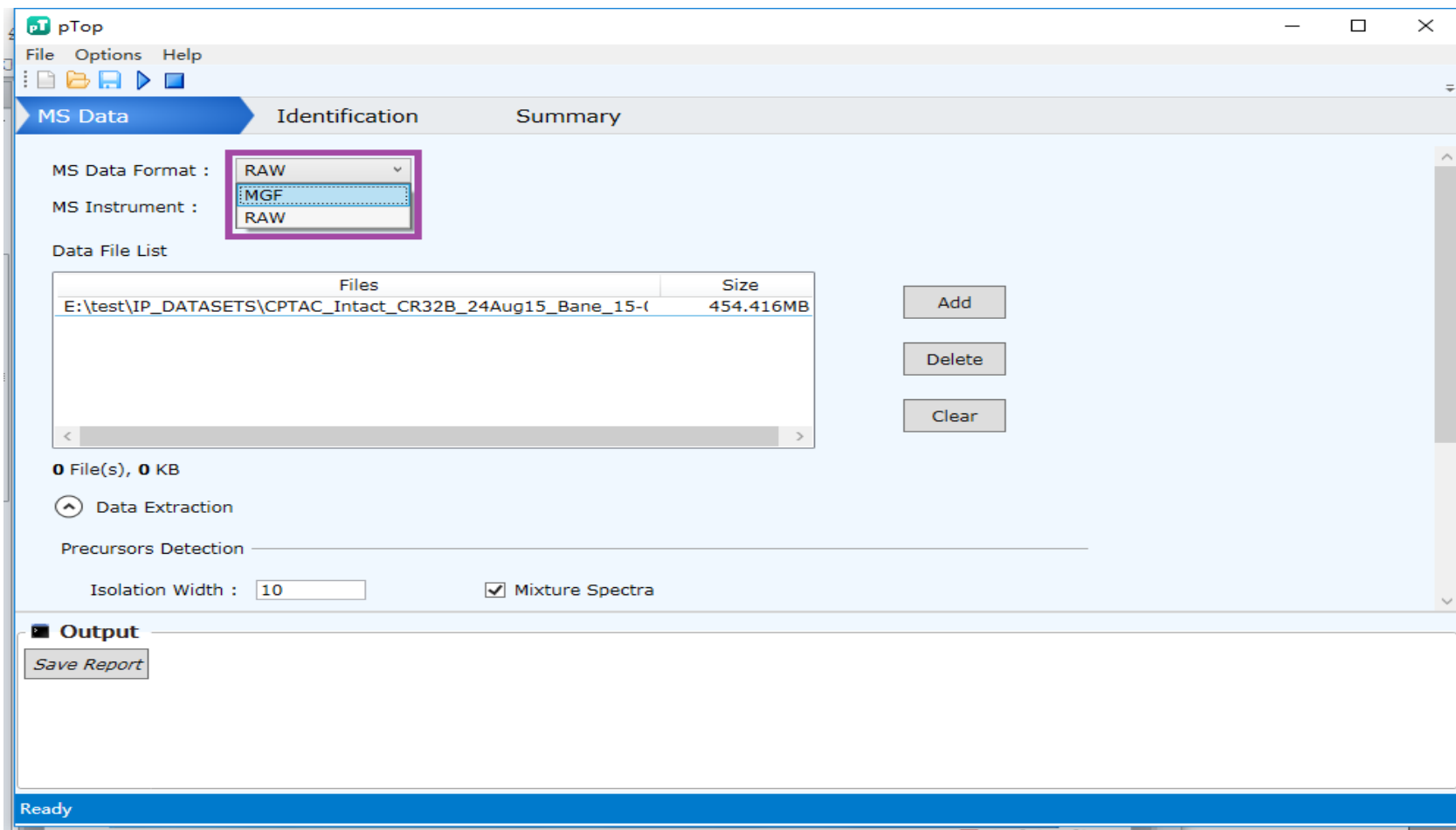


The screenshot shows a 'License Dialog' window with the following fields and controls:

- User Name:
- Institute/Company Name:
- Country:
- How do you hear about pTop from colleagues, conferences, papers or google?:
- Email Address:
- Activation Code:
- *What can pTop do for you?:
- Buttons: 'Send Email' and 'Copy to Clipboard'
- Message: 'The email will be sent to ptop@ict.ac.cn for manual review.'

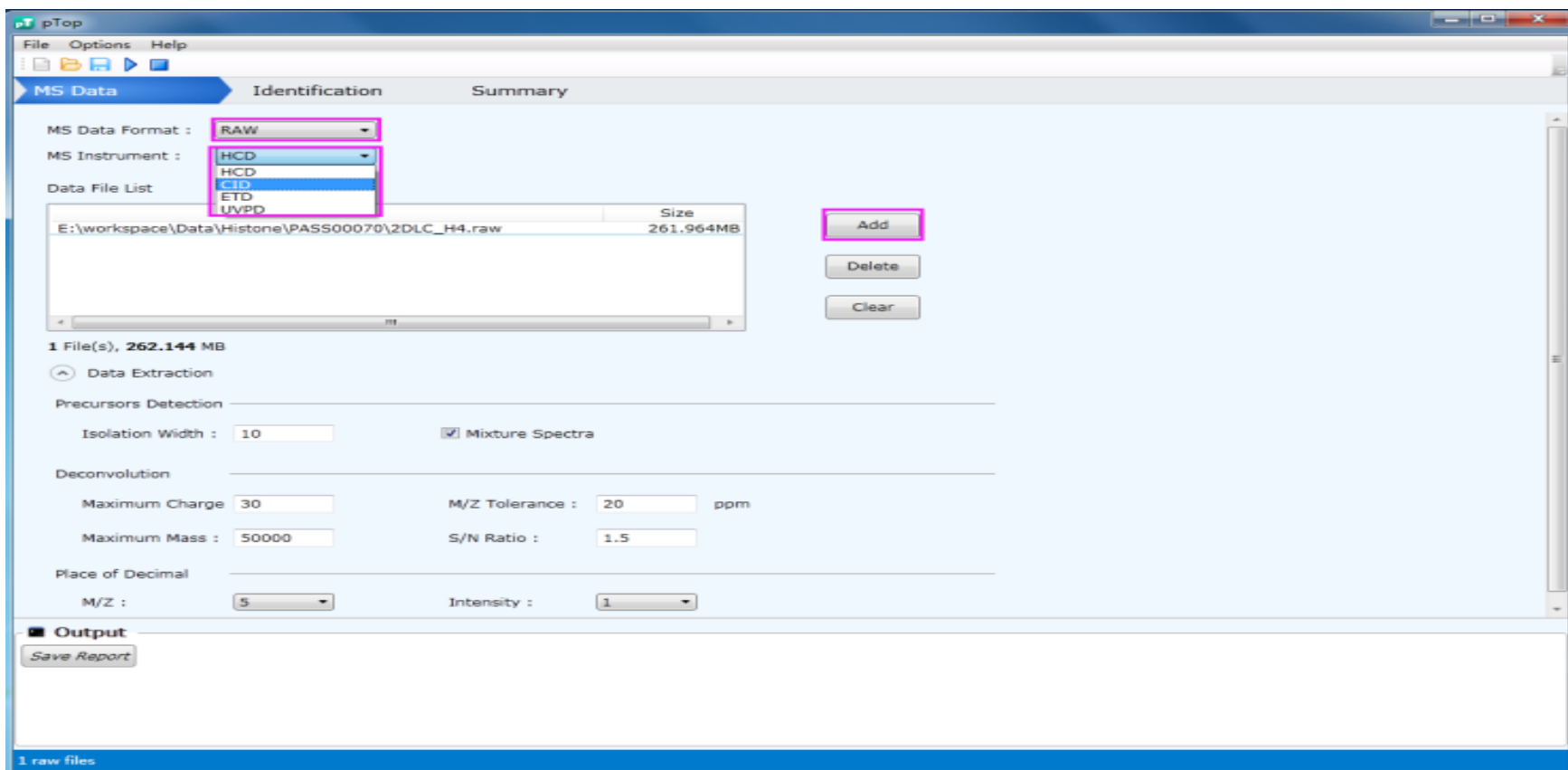
1. Data import and preprocess.

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



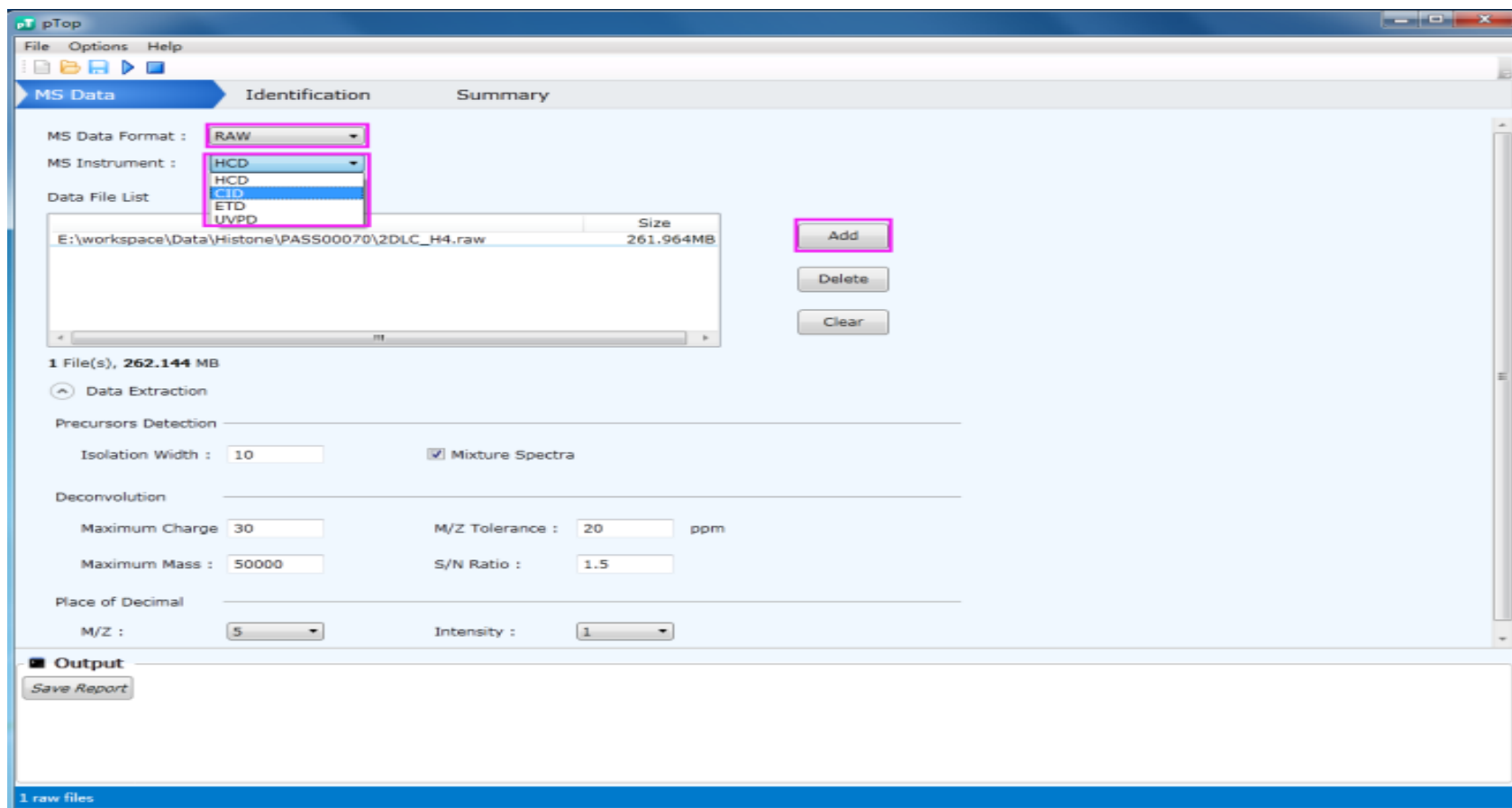
1. Data import and preprocess.

- ❖ 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.

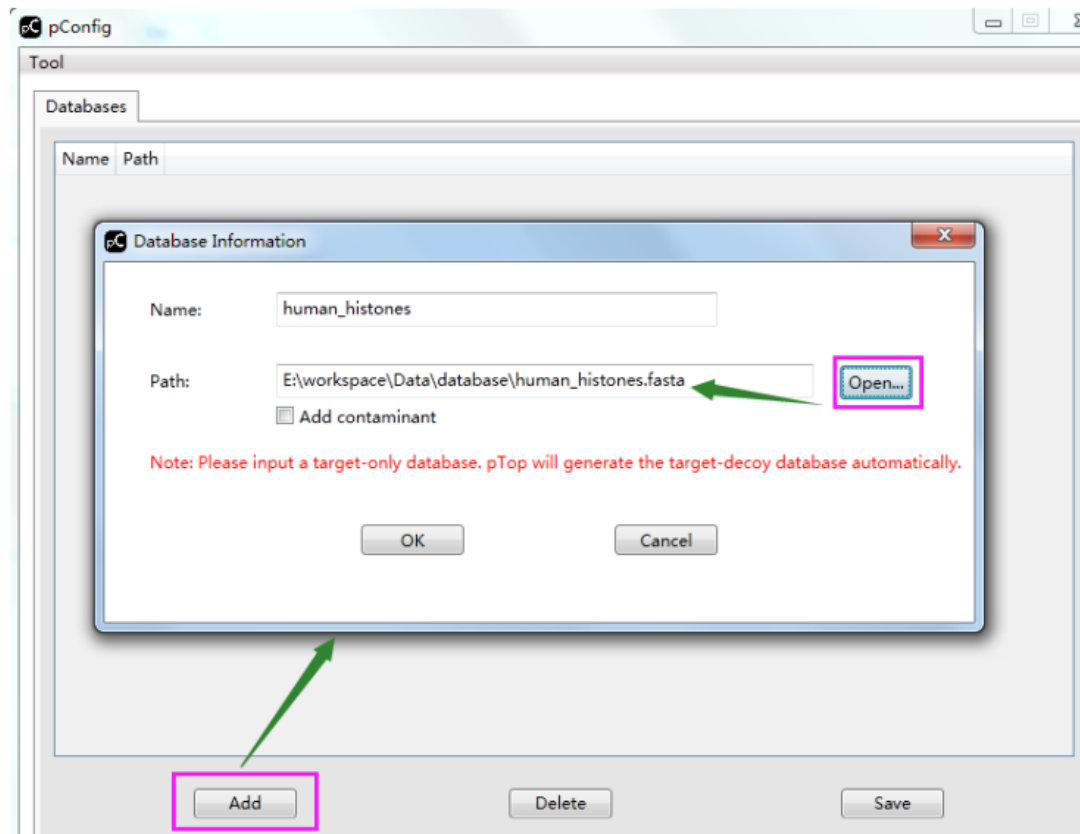
The screenshot displays the pTop software interface, which is used for mass spectrometry data analysis. The window title is "pTop" and it has a menu bar with "File", "Options", and "Help". The main interface is divided into several sections:

- MS Data**: A tabbed interface with "Identification" (active) and "Summary".
- Database Search**: A section where search parameters are configured. The "Database" dropdown menu is highlighted with a pink box and shows "Customize Database...". Other parameters include:
 - Precursor Tolerance: ± 2.2 Da
 - Fragment Tolerance: ± 20 ppm
 - Max Modify Position: 3
- Add Modification**: A section for selecting modifications. It has two columns: "Fixed" and "Variable". A list of modifications is shown in the center, including:
 - Acetyl[AnyN-term]
 - Acetyl[K]
 - Acetyl[ProteinN-term]
 - Amidated[AnyC-term]
 - Amidated[ProteinC-term]
 - Ammonia-loss[AnyN-termC]
 - Biotin[AnyN-term]
 - Biotin[K]
 - C+12[AnyN-term]
 - Carbamidomethyl[C]
 - Carbamyl[AnyN-term]
 - Carbamyl[K]
 - Carboxymethyl[C]
 - Cation_Na[AnyC-term]
 - Cation_Na[D]
 - Cation_Na[F]
- Result Filter**: A section where the False Discovery Rate (FDR) is set to ≤ 1 %.
- Output**: A section with a "Save Report" button.

At the bottom left of the window, it 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 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].
 - Display All:** Checked checkbox.
 - Edit...** button.
- Result Filter:** FDR ≤ 1 %
- Output:** Save Report button

1 raw files

2. Set search parameters

❖ B) Select the modifications.

The screenshot shows the pTop software interface. The 'Identification' tab is active, displaying search parameters and a list of modifications. The 'Database Search' section includes a dropdown for 'Database' set to 'human_histones', 'Precursor Tolerance' set to ± 2.2 Da, and 'Fragment Tolerance' set to ± 20 ppm. The 'Max Modify Position' is set to 3. The 'Add Modification' section has two boxes: 'Fixed' (empty) and 'Variable' (containing Acetyl[AnyN-term], Acetyl[K], Phospho[S], Phospho[T], and Phospho[Y]). A list of modifications is shown in the center, with arrows indicating selection. The 'Display All' checkbox is checked. 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'.

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

Add Modification

Fixed

Variable

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

- 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]

Display All Edit...

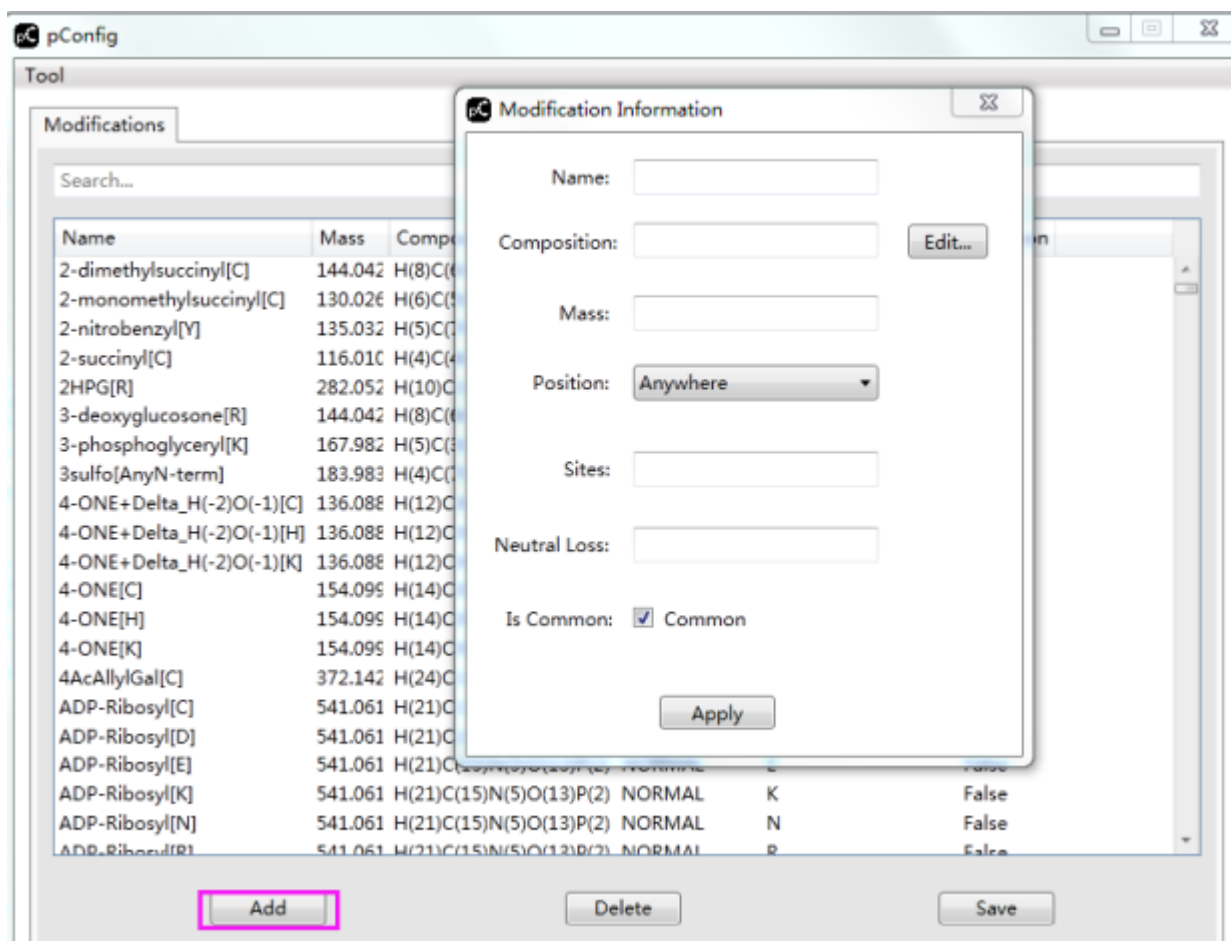
Result Filter
FDR ≤ 1 %

Output
Save Report

1 raw files

2. Set search parameters

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



Searching...

The screenshot displays the pTop software interface, which is used for mass spectrometry data analysis. The window is titled 'pTop' and has a menu bar with 'File', 'Options', and 'Help'. Below the menu bar, there are three tabs: 'MS Data', 'Identification', and 'Summary', with 'Summary' currently selected. The 'MS Data' section contains a table with the following properties and values:

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 another table with the following properties and values:

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 of the window 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 MS Data tab is active, showing search parameters. The Identification tab is also visible, showing a table of search results. A dialog box titled "Work done!" is overlaid on the Identification table, with a "确定" (OK) button. The Output window at the bottom shows the following log:

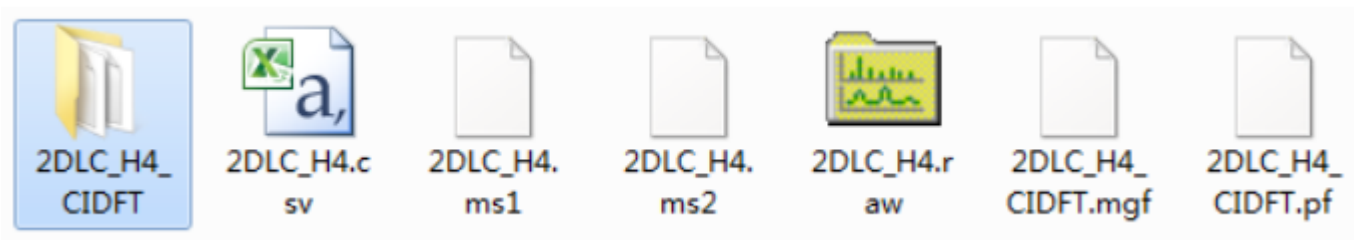
```
[pTop] Database: E:\pTop安装测试\Data\human_histones.fasta  
[pTop] Getting the PTM forms...  
[pTop] Time elapsed: 174.138s  
[pTop] Indexing...  
[Error] Cannot open the database file: E:\pTop安装测试\Data\human_histones.fasta
```

The status bar at the bottom of the window displays "Running".

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

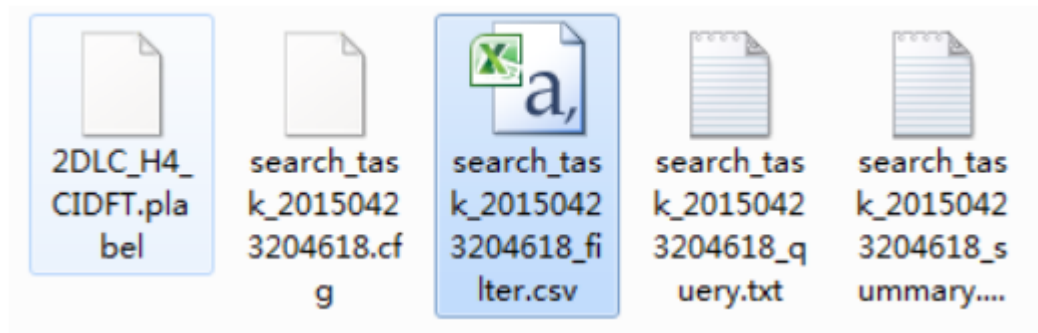
Contents of search results files

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



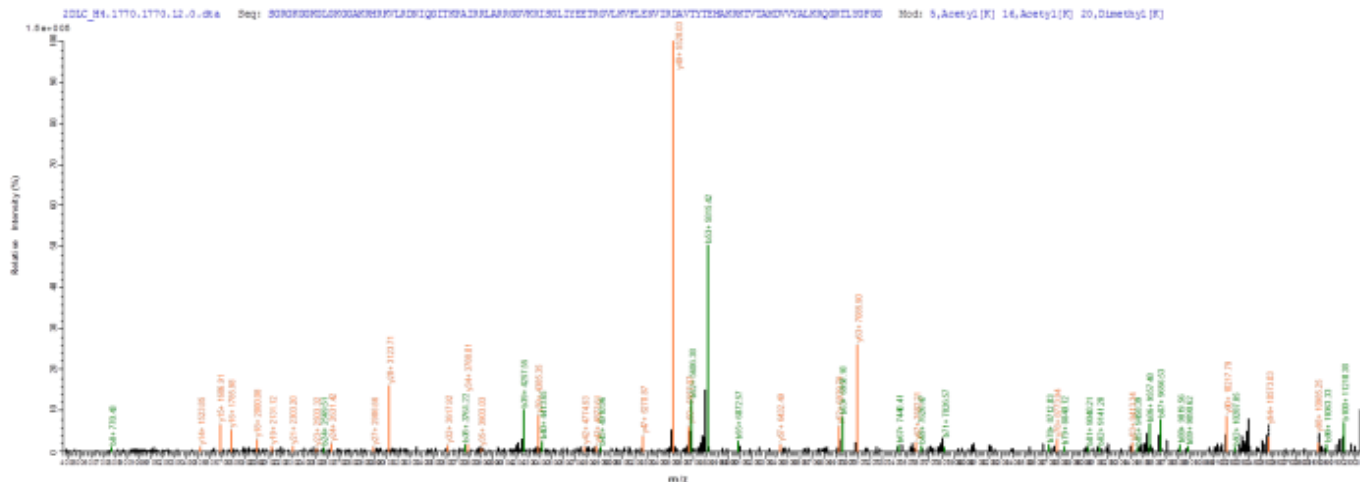
Contents of search results files

- ❖ 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 .**