

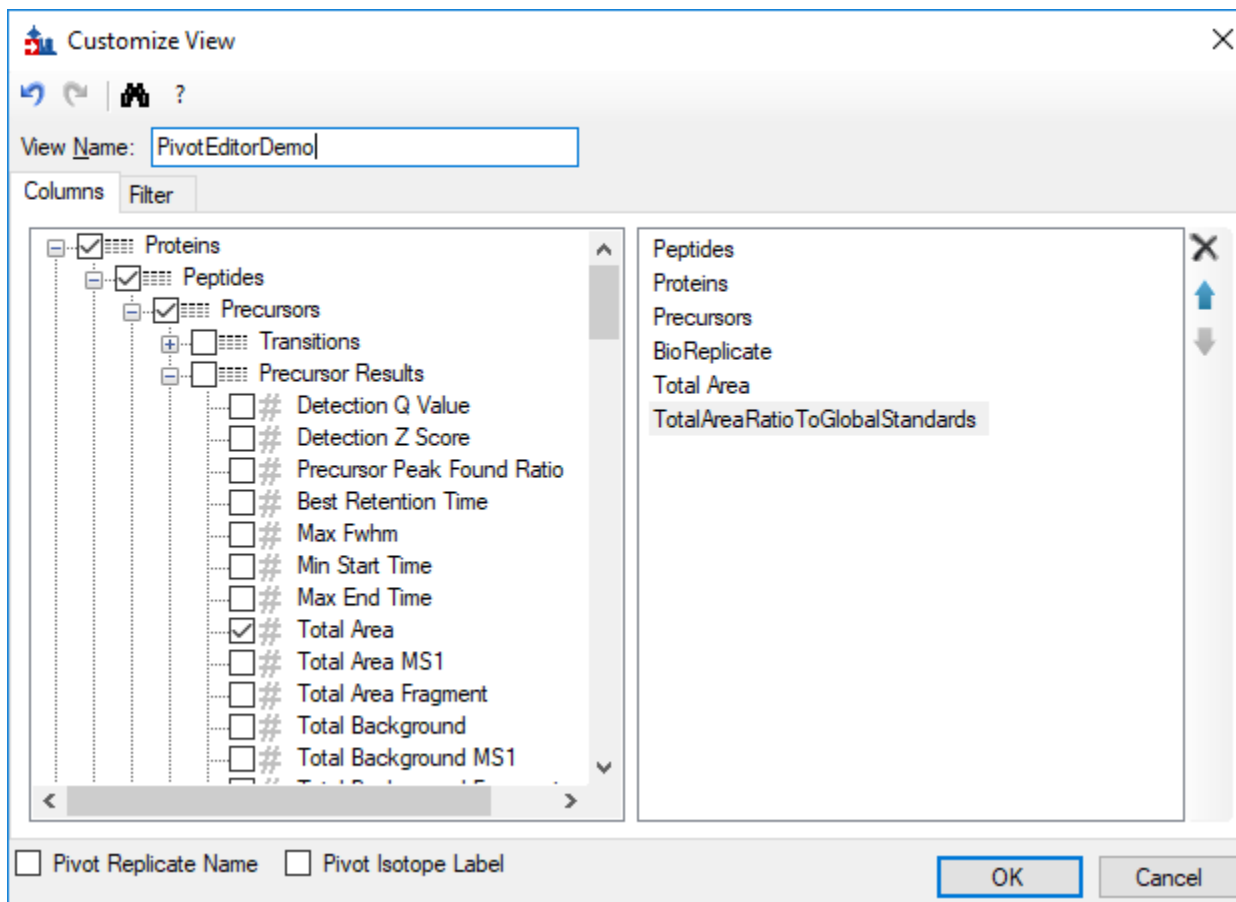
Skyline Pivot Editor

Available in Skyline-Daily November, 2017

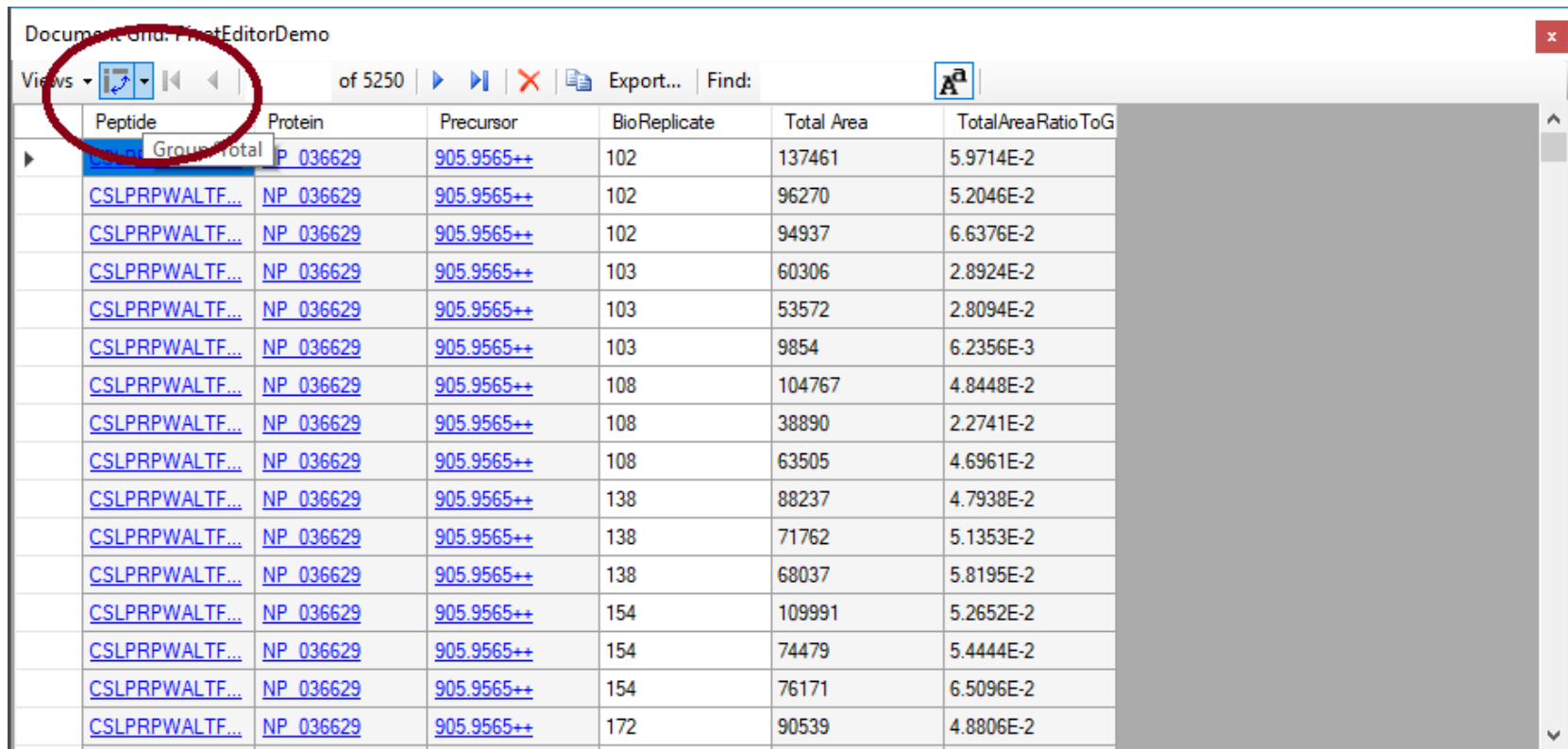
Scenario: Want to compare average CV of Total Area, and Total Area normalized to global standards.

- Using “Rat_plasma.sky.zip” from the Grouped Studies tutorial:
<https://skyline.gs.washington.edu/tutorials/GroupedStudies1.zip>
- The data file has a replicate annotation called “BioReplicate” which represents the individual rats that the samples were taken from.
- If we group by precursor and BioReplicate, whatever normalization method produces the lower CV is probably more precise and accurate.
- In the next slides, we will look at how the CV of the precursor result “Total Area” compares to “TotalAreaRatioToGlobalStandards”.

Create a view that has all of the columns that you are going to want to group and summarize



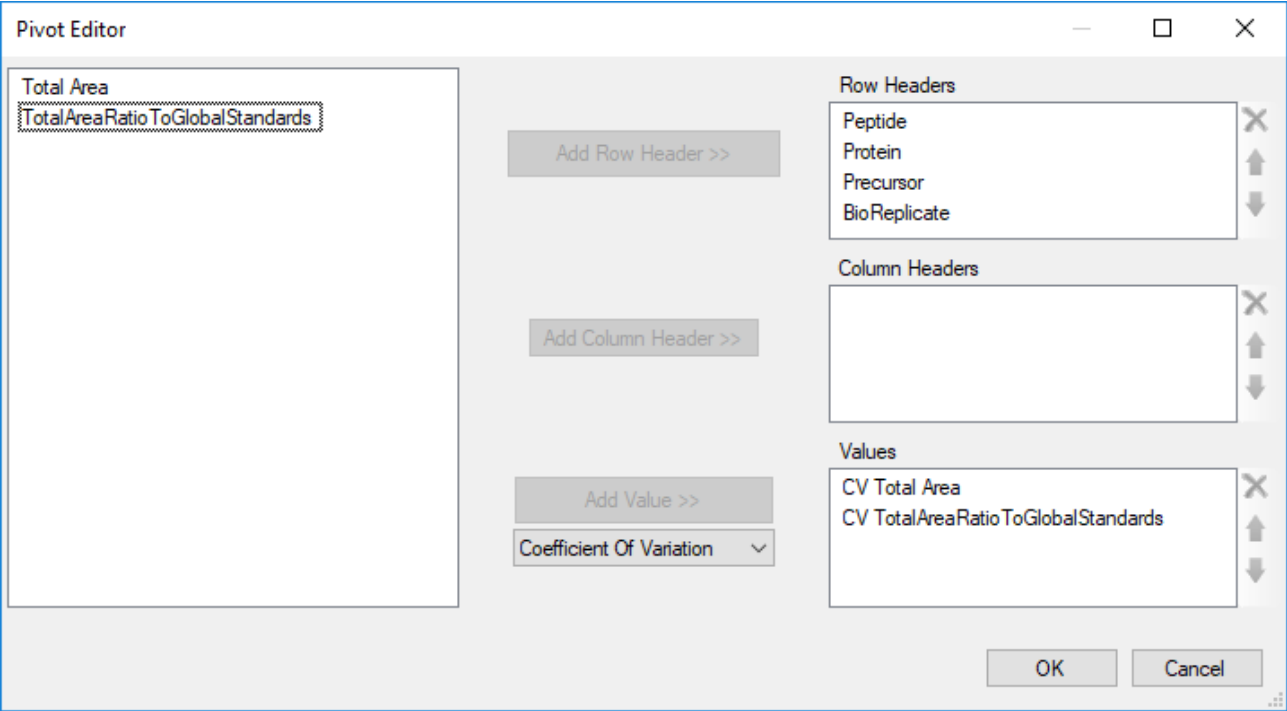
Push the Group/Total button to launch the pivot editor



The screenshot shows a software window titled "Document Grid: PivotEditorDemo". The interface includes a toolbar with a "Views" dropdown, a "Group/Total" button (circled in red), and navigation icons. Below the toolbar is a table with the following columns: Peptide, Protein, Precursor, BioReplicate, Total Area, and TotalAreaRatioToG. The table contains 15 rows of data, each representing a peptide entry with its corresponding protein, precursor, bio-replicate, total area, and ratio to the group total.

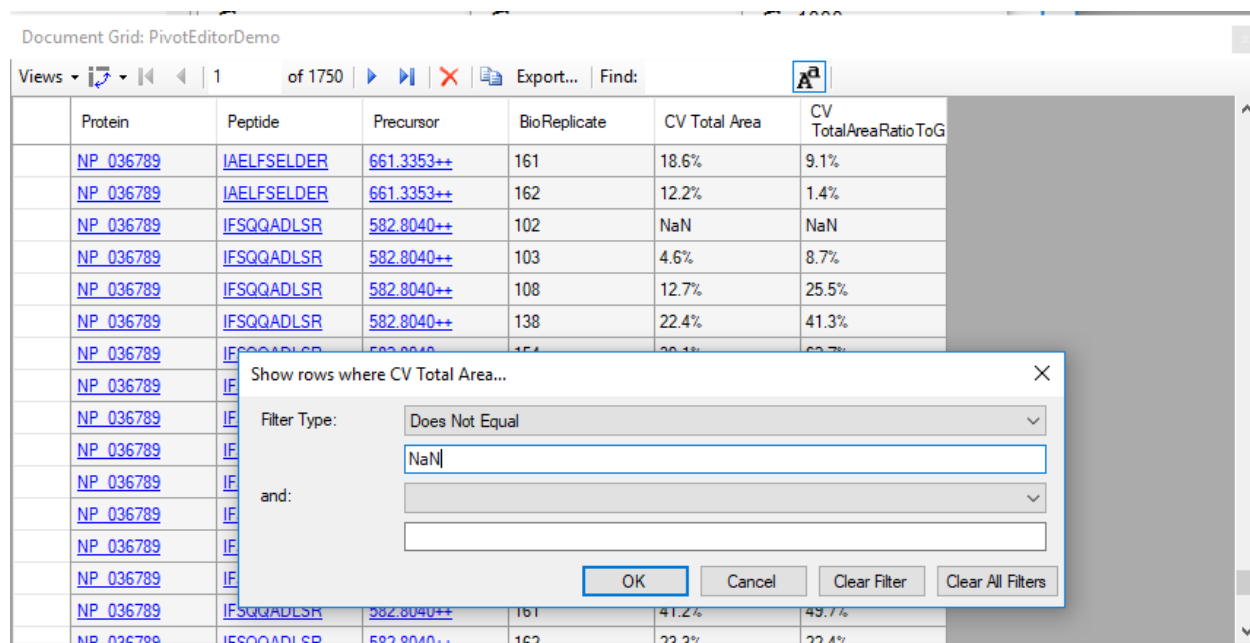
| Peptide | Protein | Precursor | BioReplicate | Total Area | TotalAreaRatioToG |
|----------------|-----------|------------|--------------|------------|-------------------|
| Group Total | P_036629 | 905.9565++ | 102 | 137461 | 5.9714E-2 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 102 | 96270 | 5.2046E-2 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 102 | 94937 | 6.6376E-2 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 103 | 60306 | 2.8924E-2 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 103 | 53572 | 2.8094E-2 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 103 | 9854 | 6.2356E-3 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 108 | 104767 | 4.8448E-2 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 108 | 38890 | 2.2741E-2 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 108 | 63505 | 4.6961E-2 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 138 | 88237 | 4.7938E-2 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 138 | 71762 | 5.1353E-2 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 138 | 68037 | 5.8195E-2 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 154 | 109991 | 5.2652E-2 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 154 | 74479 | 5.4444E-2 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 154 | 76171 | 6.5096E-2 |
| CSLPRPWALTF... | NP_036629 | 905.9565++ | 172 | 90539 | 4.8806E-2 |

Choose which columns you want to group on, and what aggregate operations you want to apply to the other columns.



For each precursor, we want to calculate the CV within each BioReplicate

Filter out NaN (not a number)



The screenshot shows a software interface titled "Document Grid: PivotEditorDemo". It displays a table with columns: Protein, Peptide, Precursor, BioReplicate, CV Total Area, and CV TotalAreaRatioToG. The table contains 17 rows of data. A dialog box titled "Show rows where CV Total Area..." is open over the table. The dialog box has a "Filter Type:" dropdown set to "Does Not Equal" and a text input field containing "NaN". There are also "and:" and "or:" dropdowns, and buttons for "OK", "Cancel", "Clear Filter", and "Clear All Filters".

| Protein | Peptide | Precursor | BioReplicate | CV Total Area | CV TotalAreaRatioToG |
|-----------|-------------|------------|--------------|---------------|----------------------|
| NP_036789 | IAELFSELDER | 661.3353++ | 161 | 18.6% | 9.1% |
| NP_036789 | IAELFSELDER | 661.3353++ | 162 | 12.2% | 1.4% |
| NP_036789 | IFSQQADLSR | 582.8040++ | 102 | NaN | NaN |
| NP_036789 | IFSQQADLSR | 582.8040++ | 103 | 4.6% | 8.7% |
| NP_036789 | IFSQQADLSR | 582.8040++ | 108 | 12.7% | 25.5% |
| NP_036789 | IFSQQADLSR | 582.8040++ | 138 | 22.4% | 41.3% |
| NP_036789 | IFSQQADLSR | 582.8040++ | 151 | 20.1% | 32.7% |
| NP_036789 | IFSQQADLSR | 582.8040++ | 152 | 22.2% | 32.4% |
| NP_036789 | IFSQQADLSR | 582.8040++ | 161 | 41.2% | 49.7% |
| NP_036789 | IFSQQADLSR | 582.8040++ | 162 | 22.2% | 32.4% |

Some precursors were only detected in one of the replicates for a given subject. The CV of one value is NaN. We need to filter these values out now so that they do not cause problems when we try to take the average later.

Use the New Pivot button to add another level of grouping/totalling

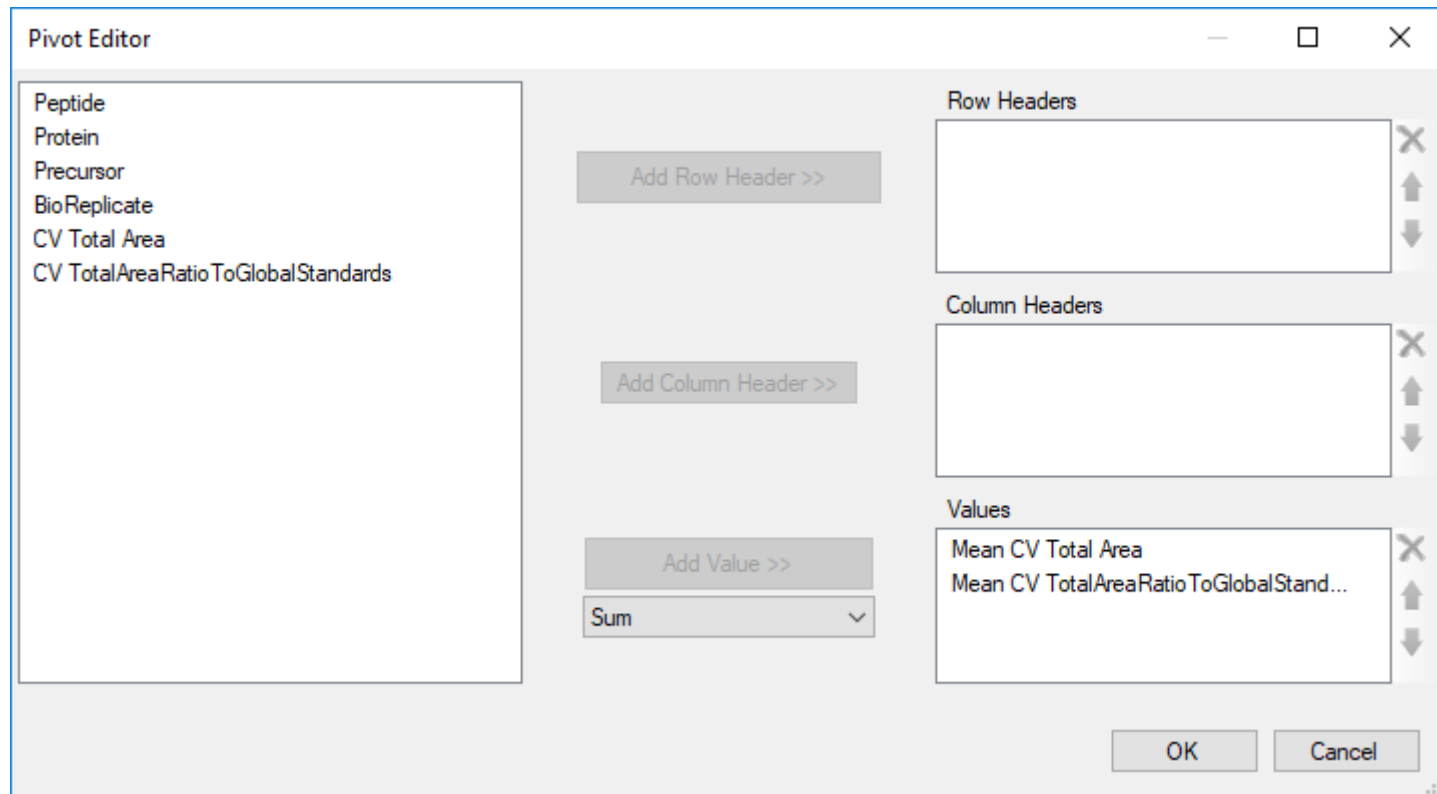
Document Grid: PivotEditorDemo

Views | 1 of 1744 | Export... | Find: (Filtered from 1750)

Transforms
New Pivot...
Remember current layout...

| | cursor | BioReplicate | CV Total Area | CV TotalAreaRatioToG |
|---------------------------|----------------------------|--------------|---------------|----------------------|
| NP_036629 | 905.9565++ | 102 | 22.1% | 12.1% |
| NP_036629 | 905.9565++ | 103 | 66.4% | 61% |
| NP_036629 | 905.9565++ | 108 | 48.2% | 36.6% |
| NP_036629 | 905.9565++ | 138 | 14.1% | 9.9% |
| NP_036629 | 905.9565++ | 154 | 23.1% | 11.7% |
| NP_036629 | 905.9565++ | 172 | 21% | 3.8% |
| NP_036629 | 905.9565++ | 196 | 15.5% | 11.2% |
| NP_036629 | 905.9565++ | 146 | 37.7% | 19.3% |
| NP_036629 | 905.9565++ | 147 | 18.3% | 8.9% |
| NP_036629 | 905.9565++ | 148 | 14.5% | 4.3% |
| NP_036629 | 905.9565++ | 159 | 37.8% | 28.9% |
| NP_036629 | 905.9565++ | 160 | 15% | 1.6% |
| NP_036629 | 905.9565++ | 161 | 21% | 2.8% |
| NP_036629 | 905.9565++ | 162 | 68.6% | 63.5% |
| NP_037030 | 574.7844++ | 102 | 35% | 12.4% |
| NP_037030 | 574.7844++ | 103 | 25.9% | 14.2% |

For the next level of pivoting, just get one with the mean of each of the CV columns.



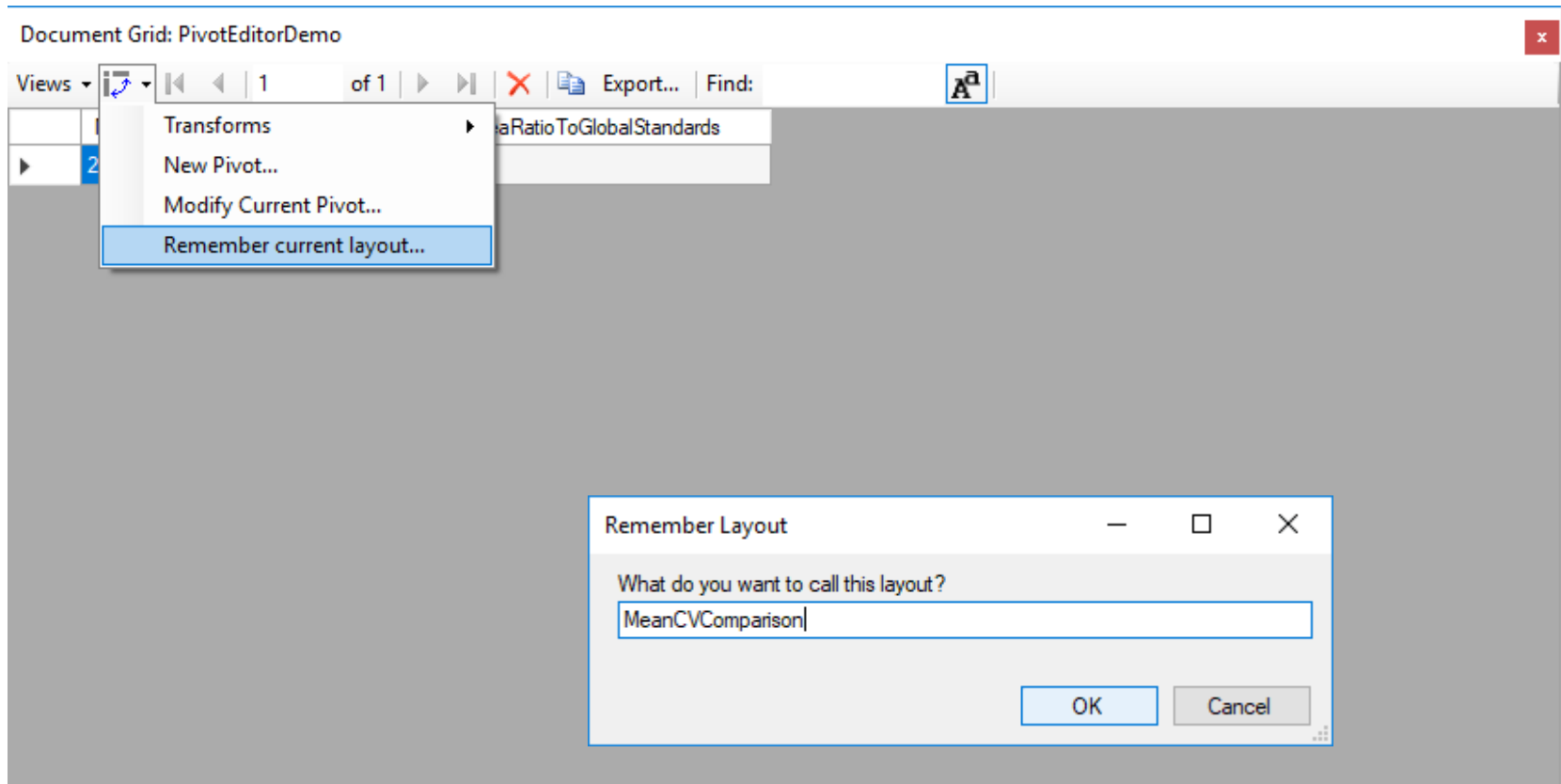
The result is a single row with the mean CVs

Document Grid: PivotEditorDemo

Views 1 of 1 Export... Find: |

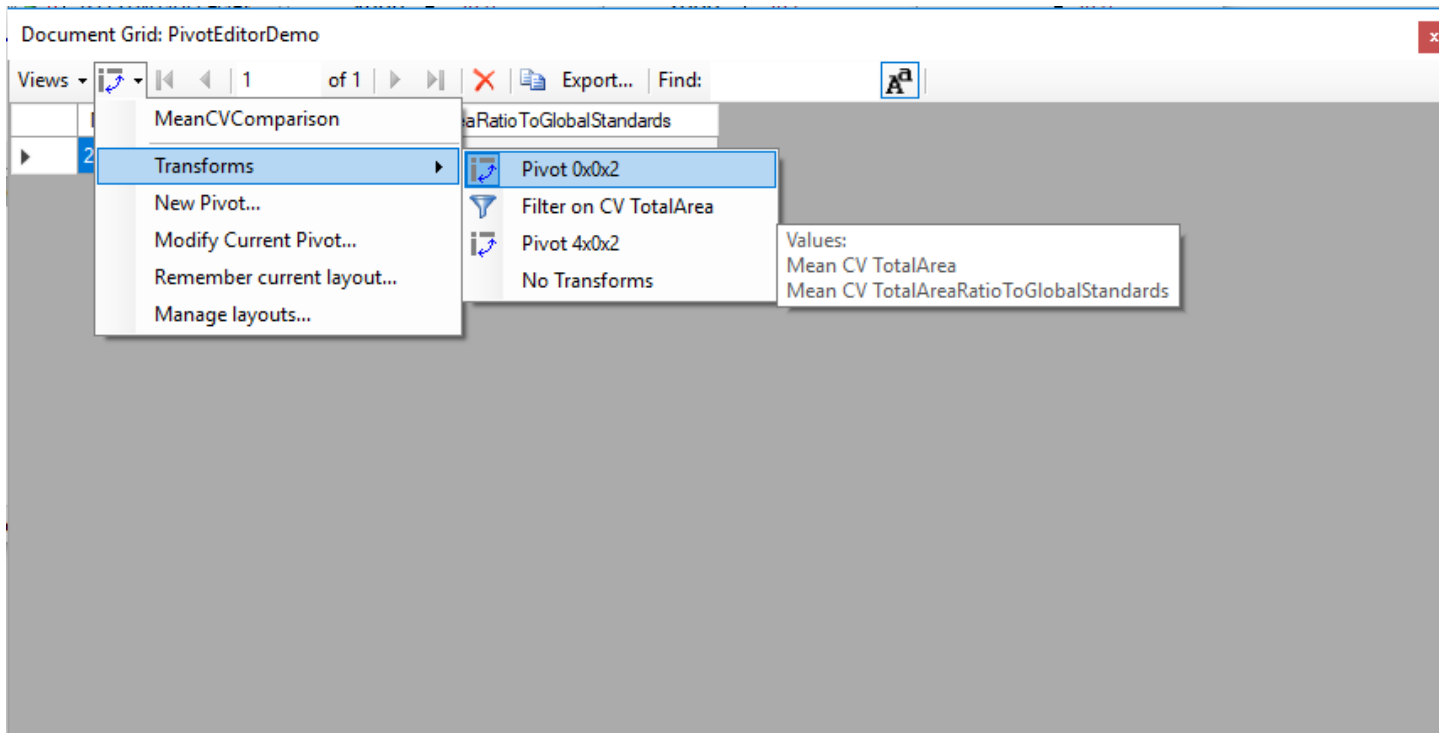
| | Mean CV Total Area | Mean CV TotalAreaRatioToGlobalStandards |
|---|--------------------|---|
| ▶ | 21.5% | 16.5% |

The “Remember Layout” menu item allows us to save the set of transforms that we have applied.



The Layout consists of the set of pivots, filters, sorts, and column width and formats that have been applied.

The Transforms sub-menu shows you the set of pivots and filters that have been applied.



Reading from bottom to top, we started with the plain view "PivotEditorDemo", and then did a pivot with 4 row headers and 2 values, and then applied a filter, and then applied a pivot with 0 row headers and 2 values. The tooltip shows you a more detailed explanation of what the pivot was.

You can use the Transforms sub-menu to go back before some of the transforms were applied.

Document Grid: PivotEditorDemo

Views | 1 of 1750 | Export... | Find: | A

MeanCVComparison

Transforms

New Pivot...

Modify Current Pivot...

Remember current layout...

Manage layouts...

Pivot 0x0x2

Filter on CV TotalArea

Pivot 4x0x2

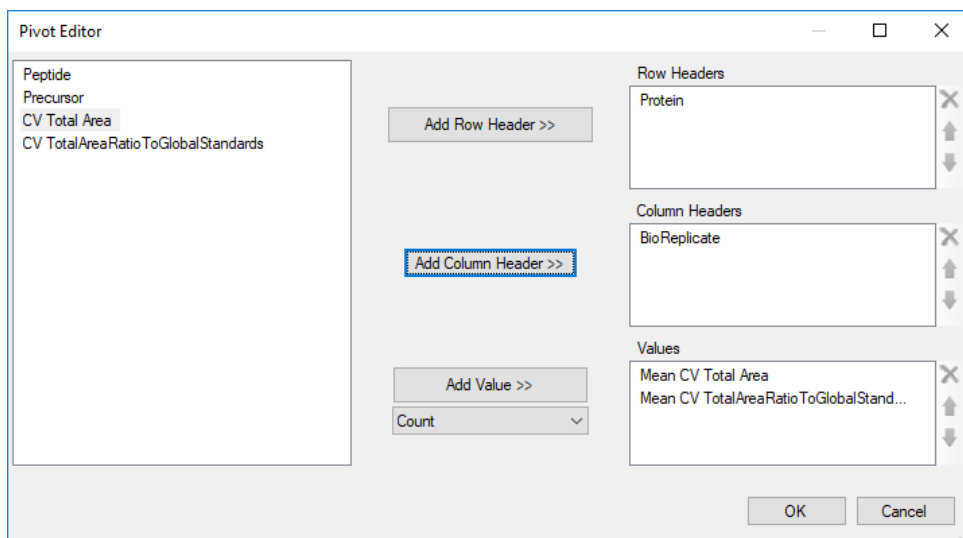
No Transforms

| | cursor | BioReplicate | CV Total Area | CV TotalAreaRatioToG |
|---------------------------|--------------------------------|----------------------------|---------------|----------------------|
| | | | 1% | 12.1% |
| | | | 4% | 61% |
| | | | 2% | 36.6% |
| | | | 1% | 9.9% |
| | 9565++ | 154 | | |
| NP_036629 | CSLPRPWALTF... | 905.9565++ | 172 | |
| NP_036629 | CSLPRPWALTF... | 905.9565++ | 196 | |
| NP_036629 | CSLPRPWALTF... | 905.9565++ | 146 | |
| NP_036629 | CSLPRPWALTF... | 905.9565++ | 147 | |
| NP_036629 | CSLPRPWALTF... | 905.9565++ | 148 | |
| NP_036629 | CSLPRPWALTF... | 905.9565++ | 159 | 37.8% 28.9% |
| NP_036629 | CSLPRPWALTF... | 905.9565++ | 160 | 15% 1.6% |
| NP_036629 | CSLPRPWALTF... | 905.9565++ | 161 | 21% 2.8% |
| NP_036629 | CSLPRPWALTF... | 905.9565++ | 162 | 68.6% 63.5% |
| NP_037030 | LGGEEVSVACK | 574.7844++ | 102 | 35% 12.4% |
| NP_037030 | LGGEEVSVACK | 574.7844++ | 102 | 25.9% 14.2% |

Row Headers:
Protein
Peptide
Precursor
BioReplicate

Values:
CV Total Area
CV TotalAreaRatioToGlobalStandards

We can then add a new pivot to this earlier step, and demonstrate column headers.



In this case, we want one row per protein, and we want separate columns for each BioReplicate.

One row per protein, and separate columns for the BioReplicates.

Document Grid: PivotEditorDemo

Views 1 of 48 Export... Find:

| Protein | 102 Mean CV Total Area | 102 Mean CV TotalAreaRatioToG | 103 Mean CV Total Area | 103 Mean CV TotalAreaRatioToG | 108 Mean CV Total Area | 108 Mean CV TotalAreaRatioToG | 138 Mean CV Total Area |
|------------------------------|---------------------------|----------------------------------|---------------------------|----------------------------------|---------------------------|----------------------------------|---------------------------|
| NP_036629 | 22.1% | 12.1% | 66.4% | 61% | 48.2% | 36.6% | 14.1% |
| NP_037030 | 29.6% | 11.5% | 22.8% | 11.9% | 39.7% | 18.8% | 26.8% |
| NP_602308 | 7.4% | 17.7% | 0.5% | 6.8% | 18.3% | 14.7% | 6.6% |
| NP_001012027 | 12.5% | 19.4% | 12.8% | 18.5% | 19.8% | 15.4% | 11.5% |
| NP_036870 | 14.2% | 12.1% | 42.7% | 39% | 33.5% | 25.6% | 18.2% |
| NP_037244 | 34.4% | 25.3% | 55.4% | 49.2% | 45.7% | 19.3% | 28.1% |
| NP_001010968 | 13.4% | 13% | 8% | 8.3% | 48.3% | 44.9% | 17.5% |
| NP_444180 | 13.1% | 12.3% | 43.8% | 38% | 29.9% | 27.9% | 19.9% |
| NP_872279 | 17.9% | 18.5% | 15.3% | 6.4% | 13.6% | 12.6% | 17.5% |
| NP_001121161 | 22.5% | 13.4% | 19.7% | 12.2% | 32.4% | 11.9% | 20.9% |
| NP_058716 | 44.2% | 48.4% | 66.9% | 56.8% | 33.9% | 46.6% | 40.4% |
| NP_036745 | 14.7% | 16.4% | 16% | 3.7% | 21.3% | 11.7% | 18.3% |
| NP_446290 | 21.6% | 18.2% | 18.8% | 12.9% | 20.9% | 12.7% | 20.2% |
| NP_037001 | 26.1% | 30.6% | 14.1% | 10.9% | 26.2% | 16.8% | 35.3% |
| NP_150641 | 39.3% | 45.4% | 20.4% | 9.4% | 20.6% | 2.7% | 27.7% |