KET-VR5 Image Data Wrangling

January 31, 2024

1 Ketamine VR5 - SINGLE LABELED DATA ONLY

Jonathan Ramos 1/26/2024 I'm glad these data came just as I finished the sleep dep set so some of code is still fresh in my brain. For these data, the format of the csvs is quite different (due to the difference in the way PIPSQUEAK vs POLYGON spit out csvs). Col names are different and some label names need to be changed; in particular, some stain type names are simply called "hand drawn" if the user added ROIs that were not detected by the polygon algorithm. This causes probems because all hand drawn ROIs of any stain type are all called "hand drawn." This has been an on going issue with polygon, but we have a work around.

In the filename col, all files follow a consistent naming scheme: - *_2.tif : PV - *_3.tif : cFos - *_4.tif : Npas4 - *_5.tif : WFA

Additionally, since there is no subject ID col, we can construct it from informatively named filenames instead. For this project, filenames follow the following format:

(rat number)_(brain region)_(bregma)_(n).tif

In this notebook I will wrangle all the data into one spot (data is distributed over ~600 small csvs), clean things up, normalize intensity and count mean cell ns.

1.1 Cleaning, Wrangling Data

1.1.1 Loading data, stitching sets together

[1]:
```
import numpy as np
import pandas as pd
import glob

# load cohort key (there's a few empty rows at the end)
df_key = pd.read_csv('KETAMINE_COHORT KEY.csv').dropna()

# load data; getting it all in one spot
df_full = pd.concat([pd.read_csv(f) for f in glob.glob('CSV_FILES/*/*.csv')])

# col names begin with a whitespace char; let's remove all ' ' chars from col␣
↪names
df_full.columns = [col.replace(' ', '') for col in df_full.columns]

# let's take a look
```

```
df_full
```

```
/var/folders/b2/3h2lpxx14kgb12pp_7pltxnc0000gn/T/ipykernel_59275/1229073174.py:2
: DeprecationWarning:
Pyarrow will become a required dependency of pandas in the next major release of
pandas (pandas 3.0),
(to allow more performant data types, such as the Arrow string type, and better
interoperability with other libraries)
but was not found to be installed on your system.
If this would cause problems for you,
please provide us feedback at https://github.com/pandas-dev/pandas/issues/54466

  import pandas as pd
```

[1]:

```
    cell_number     roi_id   roi_source roi_type   CoM_x   CoM_y  \
0             1  000-00000  Parvalbumin     OVAL  120.64  307.06
1             2  000-00001  Parvalbumin     OVAL  403.99  379.52
2             3  000-00002  Parvalbumin     OVAL  363.44  463.29
3             4  000-00003  Parvalbumin     OVAL   68.43  322.91
4             5  000-00004  Parvalbumin     OVAL  386.72  251.18
..          …          …          …      …       …       …
73           74  FFF-00073   hand-drawn     OVAL  491.39   60.52
74           75  FFF-00074   hand-drawn     OVAL  497.35   15.91
75           76  FFF-00075   hand-drawn     OVAL  437.04  376.98
76           77  FFF-00076   hand-drawn     OVAL  472.11   432.2
77           78  FFF-00077   hand-drawn     OVAL  352.86  214.76

    pixel_area  background mean_intensity median_intensity  … feret_angle  \
0        665.0    285.8023       636.1711         677.3578  …         0.0
1        468.0    285.8023       412.1381         395.8835  …        90.0
2        399.0    285.8023       369.5932          366.851  …         0.0
3        550.0    285.8023       518.8725         540.6805  …         0.0
4        524.0    285.8023       832.9809         920.6865  …         0.0
..         …           …            …              …  …         …
73       130.0    541.3023       435.1857         415.7151  …         0.0
74        65.0    541.3023       413.2948         392.1778  …         0.0
75       130.0    541.3023       493.2017          462.339  …         0.0
76       104.0    541.3023        472.384         433.4397  …         0.0
77        96.0    541.3023       422.3084         387.4282  …         0.0

    feret_min circularity aspect_ratio roundness  solidity skewness  kurtosis  \
0        28.0      0.9387       1.1447    0.8205    0.9419  -0.4228   -0.6905
1        24.0      0.8789       1.1555    0.7613    0.8797   0.4731   -0.5155
2        20.0      0.8698       1.4266    0.6299    0.9027   0.3051   -0.1709
3        22.0      0.8089       1.6476     0.535    0.8814  -0.4078   -0.4258
4        26.0      0.9146       1.0654    0.8539    0.9066  -0.6879   -0.6308
..         …          …            …         …         …        …         …
```

```
73        28.0     0.1835      1.1447   0.1604   0.1841   1.3612    1.5127
74        28.0     0.0918      1.0617   0.0933   0.0921   3.3638   15.8851
75        28.0     0.1835      1.1447   0.1604   0.1841   1.4819    2.5275
76        28.0     0.1468      1.1447   0.1283   0.1473   1.6255    3.4102
77        28.0     0.1355      1.1447   0.1184   0.1360   1.8271    3.7074

                   filename                 analysis_date
0     PE-11-7_PFC_3.9_A_2.tif  Thu Jan 25 15:09:00 PST 2024
1     PE-11-7_PFC_3.9_A_2.tif  Thu Jan 25 15:09:00 PST 2024
2     PE-11-7_PFC_3.9_A_2.tif  Thu Jan 25 15:09:00 PST 2024
3     PE-11-7_PFC_3.9_A_2.tif  Thu Jan 25 15:09:00 PST 2024
4     PE-11-7_PFC_3.9_A_2.tif  Thu Jan 25 15:09:00 PST 2024
..                        …                             …
73   KET-10-4_PFC_3.5_C_4.tif  Mon Jan 22 16:14:32 PST 2024
74   KET-10-4_PFC_3.5_C_4.tif  Mon Jan 22 16:14:32 PST 2024
75   KET-10-4_PFC_3.5_C_4.tif  Mon Jan 22 16:14:32 PST 2024
76   KET-10-4_PFC_3.5_C_4.tif  Mon Jan 22 16:14:32 PST 2024
77   KET-10-4_PFC_3.5_C_4.tif  Mon Jan 22 16:14:32 PST 2024

[24613 rows x 30 columns]
```

1.1.2 Relabeling incorrect data

[2]:
```python
# there were some issues with the naming/cohort key

# PE-12-7 was incorrectly labeled as PE-12-3; this was confirmed by JR and AG␣
 ↪by checking slide books/hard copies of behavior data
df_full['filename'] = df_full.filename.replace({'PE-12-3': 'PE-12-7'},␣
 ↪regex=True)

# similarly KET-8-2 was incorrectly labeled as KET-8-5; this was confirmed by␣
 ↪JR and AG  by checking slide books. this wouldn't really
# change anything since they received the same treatment but let's just change␣
 ↪it to the correct label anyway
df_full['filename'] = df_full.filename.replace({'KET-8-5': 'KET-8-2'},␣
 ↪regex=True)

# check result
assert df_full.filename.str.contains('PE-12-3').sum() == 0
assert df_full.filename.str.contains('PE-12-7').sum() != 0
assert df_full.filename.str.contains('KET-8-5').sum() == 0
assert df_full.filename.str.contains('KET-8-2').sum() != 0
```

1.1.3 Building the necessary cols

In particular we will need a rat_n (sid) col, stain_type col, and a treatment col. the filename col functions as the image name (iid) col.

We need the following cols - rat_n (sid) - treatment - filename (fid) - imagename (iid) - stain_type - CoM_x - CoM_y - mean-background

[3]:

```
# creating a new rat_n col
df_full['rat_n'] = df_full.filename.apply(lambda x: x.split('_')[0])\
    .replace({' ': ''}, regex=True) # for some reason, we have more leading whitespace chars

# some checks. we want be sure that the structure of all our rat_n labels is consistent
# in particular, we expect something of the form 'PE-12-7', that is we have exactly
# two dashes '-' separating some letters, followed by two numbers
assert df_full.rat_n.apply(lambda x: len(x.split('-')) == 3).sum() == len(df_full)
assert df_full.rat_n.apply(lambda x: x.split('-')[0].isalpha()).sum() == len(df_full)
assert df_full.rat_n.apply(lambda x: x.split('-')[1].isnumeric()).sum() == len(df_full)
assert df_full.rat_n.apply(lambda x: x.split('-')[2].isnumeric()).sum() == len(df_full)

# building a cohort key dictionary from df_key
treatment = dict(zip(df_key.Subject, df_key.TX.replace({' ': '_'}, regex=True)))

# creating new treatment col by mapping from cohort key dict
df_full['treatment'] = df_full.rat_n.map(treatment)

# creating new stain_type col from filename
stains = {
    '.*_2.tif$' : 'PV',
    '.*_3.tif$' : 'cFos',
    '.*_4.tif$' : 'Npas4',
    '.*_5.tif$' : 'WFA'
}
df_full['stain_type'] = df_full.filename.replace(stains, regex=True)

# check that stain_type col contains the appropriate labels
assert set(df_full.stain_type.unique()) == set(stains.values())

# building image name (iid) from file name (fid) col
df_full['image_name'] = df_full.filename.replace({'_[0-9]\.tif': ''}, regex=True)

df_subset = df_full[['rat_n', 'treatment', 'stain_type', 'filename', 'image_name', 'CoM_x', 'CoM_y', 'mean-background']]
```

```
# let's take a look
df_subset
```

[3]:

```
     rat_n treatment stain_type                     filename  \
0    PE-11-7   VR5_KET         PV    PE-11-7_PFC_3.9_A_2.tif
1    PE-11-7   VR5_KET         PV    PE-11-7_PFC_3.9_A_2.tif
2    PE-11-7   VR5_KET         PV    PE-11-7_PFC_3.9_A_2.tif
3    PE-11-7   VR5_KET         PV    PE-11-7_PFC_3.9_A_2.tif
4    PE-11-7   VR5_KET         PV    PE-11-7_PFC_3.9_A_2.tif
..       …         …          …                          …
73  KET-10-4   VR5_SAL      Npas4   KET-10-4_PFC_3.5_C_4.tif
74  KET-10-4   VR5_SAL      Npas4   KET-10-4_PFC_3.5_C_4.tif
75  KET-10-4   VR5_SAL      Npas4   KET-10-4_PFC_3.5_C_4.tif
76  KET-10-4   VR5_SAL      Npas4   KET-10-4_PFC_3.5_C_4.tif
77  KET-10-4   VR5_SAL      Npas4   KET-10-4_PFC_3.5_C_4.tif

             image_name   CoM_x   CoM_y mean-background
0     PE-11-7_PFC_3.9_A  120.64  307.06         354.873
1     PE-11-7_PFC_3.9_A  403.99  379.52        127.1692
2     PE-11-7_PFC_3.9_A  363.44  463.29         86.6384
3     PE-11-7_PFC_3.9_A   68.43  322.91        233.9562
4     PE-11-7_PFC_3.9_A  386.72  251.18        547.2813
..                  …       …       …               …
73   KET-10-4_PFC_3.5_C  491.39   60.52       -106.9829
74   KET-10-4_PFC_3.5_C  497.35   15.91       -120.9047
75   KET-10-4_PFC_3.5_C  437.04  376.98        -49.6622
76   KET-10-4_PFC_3.5_C  472.11   432.2        -67.7978
77   KET-10-4_PFC_3.5_C  352.86  214.76       -119.9755

[24613 rows x 8 columns]
```

1.1.4 Dropping nans, duplicates

[4]:

```
# which cols have nans, how many?
print('Nan per col:')
print(df_subset.isna().sum())
# it looks like we have no nans! nothing to drop here.

# how many duplicated rows do we have?
print('\nTotal n of duplicated rows:')
print(df_subset.duplicated().sum())

# it looks like we have 15 duplicated rows. let's take a look
df_full[df_subset.duplicated()].head(15)

# I'm not concerned about dropping these duplicates, so let's just toss em
df_cleaned = df_subset[~df_subset.duplicated()]
```

```python
assert df_cleaned.duplicated().sum() == 0
```

```
Nan per col:
rat_n              0
treatment          0
stain_type         0
filename           0
image_name         0
CoM_x              0
CoM_y              0
mean-background    0
dtype: int64

Total n of duplicated rows:
15
```

1.2 Removing cells with negative intensity

[124]:
```python
df_cleaned.loc[:, 'mean-background'] = df_cleaned['mean-background'].astype('f')
df_positive = df_cleaned[df_cleaned['mean-background'] > 0]
mint_gt_bg = df_full.query('mean_intensity > background')
print('total number of cells: \t\t\t\t\t\t', len(df_full))
print('number of cells with positive mean-background:\t\t\t', len(df_positive))
print('number of cells with mean_intensity greater than background: \t',␣
 ↪len(mint_gt_bg))
```

```
total number of cells:                                          24613
number of cells with positive mean-background:                  7352
number of cells with mean_intensity greater than background:    7321
```

[115]:
```python
# why don't the above print statements agree?
npas4 = df_full.query('stain_type == "Npas4"')
wrong_math = npas4[npas4['mean-background'].astype('f') > 0].
 ↪query('mean_intensity < background')[['roi_id', 'stain_type', 'filename',␣
 ↪'mean_intensity', 'background', 'mean-background']]
wrong_math['correct_subtraction (by hand)'] = wrong_math.mean_intensity -␣
 ↪wrong_math.background
# wrong_math.to_csv('wrong_math.csv')

df_copy = df_full.copy(deep=True)
df_copy['correct_subtraction'] = df_full.mean_intensity - df_full.background
df_copy['difference'] = df_copy['mean-background'].astype('f') - df_copy.
 ↪correct_subtraction
# df_copy[['rat_n', 'treatment', 'stain_type', 'image_name', 'filename',␣
 ↪'image_name', 'CoM_x', 'CoM_y',\
#             'mean_intensity', 'background', 'mean-background',␣
 ↪'correct_subtraction', 'difference']]\
```

```
#         .to_csv('Ketamine_single_label_corrected_subtraction.csv')

# descriptive stats of the differences
df_copy['difference'].describe()
```

[115]:
```
count    24571.000000
mean         0.636140
std          6.153627
min        -96.231972
25%         -0.892497
50%          0.113886
75%          1.771140
max        286.666326
Name: difference, dtype: float64
```

For now, let's just continue on with the mean-background col that Polygon provides us with. I'll just look at the mean-background col and take only rows with positive values in this col

[128]:
```
df_cleaned = df_cleaned[df_cleaned['mean-background'].astype('f') > 0].
↪copy(deep=True)
```

[128]:
```
      rat_n treatment stain_type                   filename  \
0    PE-11-7   VR5_KET         PV    PE-11-7_PFC_3.9_A_2.tif
1    PE-11-7   VR5_KET         PV    PE-11-7_PFC_3.9_A_2.tif
2    PE-11-7   VR5_KET         PV    PE-11-7_PFC_3.9_A_2.tif
3    PE-11-7   VR5_KET         PV    PE-11-7_PFC_3.9_A_2.tif
4    PE-11-7   VR5_KET         PV    PE-11-7_PFC_3.9_A_2.tif
..       …       …       …                   …
4   KET-10-4   VR5_SAL      Npas4   KET-10-4_PFC_3.5_C_4.tif
20  KET-10-4   VR5_SAL      Npas4   KET-10-4_PFC_3.5_C_4.tif
36  KET-10-4   VR5_SAL      Npas4   KET-10-4_PFC_3.5_C_4.tif
38  KET-10-4   VR5_SAL      Npas4   KET-10-4_PFC_3.5_C_4.tif
65  KET-10-4   VR5_SAL      Npas4   KET-10-4_PFC_3.5_C_4.tif

            image_name   CoM_x   CoM_y  mean-background
0      PE-11-7_PFC_3.9_A  120.64  307.06       354.872986
1      PE-11-7_PFC_3.9_A  403.99  379.52       127.169197
2      PE-11-7_PFC_3.9_A  363.44  463.29        86.638397
3      PE-11-7_PFC_3.9_A   68.43  322.91       233.956207
4      PE-11-7_PFC_3.9_A  386.72  251.18       547.281311
..                  …       …     …             …
4     KET-10-4_PFC_3.5_C   13.18  218.34        22.122801
20    KET-10-4_PFC_3.5_C  322.85  114.05        81.903999
36    KET-10-4_PFC_3.5_C  289.63  251.79       138.050598
38    KET-10-4_PFC_3.5_C   251.2  324.81        48.534698
65    KET-10-4_PFC_3.5_C  168.35  491.08         2.141600
```

```
[7352 rows x 8 columns]
```

1.3 Normalizing Intensity

all parameterized functions will get set aside into module for future use and standardization.

```
[5]: def normalize_intensity(df, norm_condition):
         '''
         computes the mean of rows of the norm_condition and divides mean-background␣
      ↪by this mean,
         normalizing all data to the mean of the norm_condition. sets normalized␣
      ↪value into new
         column called "norm mean-background" and returns new dataframe containing␣
      ↪normalized intensity.
         '''
         df_norm = df[df.treatment == norm_condition]
         norm_mean = df_norm['mean-background'].astype('f').mean()

         df_norm = df.copy(deep=True)
         df_norm['norm mean-background'] = df['mean-background'].astype('f') /␣
      ↪norm_mean

         # quickly check that the mean of the norm condition is set to about 1.00000
         # this is never exatly 1 due to small rounding errors from floating point␣
      ↪operations
         assert round(df_norm[df_norm.treatment == norm_condition]['norm␣
      ↪mean-background'].mean(), 5) == 1

         return df_norm

     def prism_reorg(df, group):
         '''
         Takes just the norm_mean-background intensity col per rat, groups by␣
      ↪treatment
         and
         '''
         treatments = np.unique(df.treatment)
         reorg = []

         for t in treatments:
             df_treat = df[df.treatment == t]
             norm_int_ratn = []
             treatment_ratns = np.unique(df_treat.rat_n)

             for rat in treatment_ratns:
                 norm_int = df_treat[df_treat.rat_n == rat]['norm mean-background']
                 df_normint = pd.DataFrame({t: norm_int}).reset_index(drop=True)
```

```
            norm_int_ratn.append(df_normint)

        # concat "vertically"
        df_ratn_cols = pd.concat(norm_int_ratn, axis=0).reset_index(drop=True)

        # write csv to disk
        reorg.append(df_ratn_cols)

    # concat "horizontally"
    df_prism_reorg = pd.concat(reorg, axis=1)


    # write csv to disk
    df_prism_reorg.to_csv(f'{group}_{np.unique(df.stain_type).item()}_{t}_PRISM.
 ↪csv')

    return df_prism_reorg

def prism_reorg(df, group):
    '''
    Takes just the norm_mean-background intensity col per rat, groups by␣
 ↪treatment
    and
    '''
    treatments = np.unique(df.treatment)
    reorg = []

    for t in treatments:
        df_treat = df[df.treatment == t]
        norm_int_ratn = []
        treatment_ratns = np.unique(df_treat.rat_n)

        for rat in treatment_ratns:
            norm_int = df_treat[df_treat.rat_n == rat]['norm mean-background']
            df_normint = pd.DataFrame({t: norm_int}).reset_index(drop=True)
            norm_int_ratn.append(df_normint)

        # concat "vertically"
        df_ratn_cols = pd.concat(norm_int_ratn, axis=0).reset_index(drop=True)

        # write csv to disk
        reorg.append(df_ratn_cols)

    # concat "horizontally"
    df_prism_reorg = pd.concat(reorg, axis=1)

    return df_prism_reorg
```

```

```

1.4 Counting Mean Cell Ns

Again, all parameterized functions will get set aside into module for future use and standardization

[6]:
```python
def count_imgs(df, sid, iid):
    '''
    takes a dataframe and counts the number of unique strings that occur in the
    "image_name" col for each rat in "rat_n" col
    args:
        df: pd.core.frame.DataFrame(n, m)
            n: the number of rows,
            m: the number of features
        sid: str, denoting the name of the col containing unique subject ids
        iid: str, denoting the name of the col containing unique image ids
    return:
        df_imgn: pd.core.frame.DataFrame(n=|sid|), m=2)
            n: the number of rows, equal to the cardinality of the sid set
            (the number of unique ID strings in sid)
            this df contains 2 cols: a sid col, and an iid col containing counts
    '''
    assert iid in df.columns

    df_imgn = df.groupby([sid])[[sid, iid]]\
        .apply(lambda x: len(np.unique(x[iid])))\
        .reset_index(name='image_n')

    return df_imgn

def count_cells(df, cols):
    '''
    takes a df and counts the number of instances each distinct row
    (created by unique combinations of labels from columns indicated
    by cols arg); counts are reported in a new col called "cell_counts"
    args:
        df: pd.core.frame.DataFrame(N, M); N: the number of rows, M: the
            number of cols (assumed to have already been split by stain_type)
        cols: list(n), n: the number of cols over which to count distinct rows
    return:
        df_counts: pd.core.frame.DataFrame(N,M+1)
    '''
    df_counts = df.value_counts(cols)\
        .reset_index(name='cell_counts')\
        .sort_values(by=cols)

```

```
    return df_counts

def sum_cells(df, cols, iid):
    '''
    takes cell count df, groups by cols denoted in cols list and computes sum
    of cell_counts col for each group. Adds new column "cell_count_sums"
    containing sums.
    args:
        df: pd.core.frame.DataFrame(N, M), N: the number of rows (N=|id_col|),
            M: the number of cols, must contain col called "cell_counts"
        cols: list(M-2), list containing col name strings that define each␣
 ↪group
            for group by and reduction (in this case summing)
        iid: str, denotes
    return:
        df_sums: pd.core.frame.DataFrame; dataframe containing summed cell
            counts per subject id.
    '''
    # remove image id col (we want to sum counts across all images per rat)
    reduce_cols = list(filter(lambda x: x != iid, cols))

    if 'scaled_counts' in df.columns:
            # group by, reduce
        df_sums = df.groupby(by=reduce_cols)[cols]\
            .apply(lambda x: np.sum(x.scaled_counts))\
            .reset_index(name='cell_count_sums')

    else:
        # group by, reduce
        df_sums = df.groupby(by=reduce_cols)[df.columns]\
            .apply(lambda x: np.sum(x.cell_counts))\
            .reset_index(name='cell_count_sums')

    return df_sums

def average_counts(df_sums, df_ns, cols, sid, iid):
    '''
    takes df of cell count sums and df of image ns, and computes the mean cell
    n (divides cell count sums by the number of images) for each subject.
    args:
        df_sums: pd.core.frame.DataFrame(ni, mi), ni: the number of rows
            (ni=|sid|), mi: the number of cols (mi = |cols|); must
            contain a col "cell_count_sums".
        df_ns: pd.core.frame.DataFrame(nj, mj), nj: the number of rows
            (nj=|sid|), mj: the number of cols (mj=2); must contain a col
            "image_n"
        cols: list(n), n: the number of cols (contains all cols necessary to
```

```
            create every unique group combination)
        sid: str, denoting the name of the col containing unique subject ids
        iid: str, denoting the name of the col containing unique image ids
    return:
        mean_cell_ns: pd.core.frame.DataFrame(N,M), N: the number of rows (N=
        |sid|), M: the number of cols (M=|cols|+2)

    '''
    # list of cols with out image id, since it was removed during the reduction␣
↪step
    reduce_cols = list(filter(lambda x: x != iid, cols))

    # compute mean cell n
    mean_cell_ns = df_sums.join(df_ns.set_index(sid), on=sid, how='inner')\
        .sort_values(by=reduce_cols)
    mean_cell_ns['mean_cell_n'] = mean_cell_ns.cell_count_sums / mean_cell_ns.
↪image_n

    # reorder so that subject id is the first col
    col_reorder = [sid] + list(filter(lambda x: x != sid, list(mean_cell_ns.
↪columns)))
    mean_cell_ns = mean_cell_ns[col_reorder]

    return mean_cell_ns

def mean_cell_n(df_stain, df_full, cols, sid, iid, return_counts=False):
    '''
    wrapper function to compute mean cell ns; magnification/zoom factor
    is assuemd to be equal across all images. NOTE that we count total image
    ns based on full cleaned dataset: it may be the case the not every image
    contains every stain type combination, and we must still count images
    with 0 cells of a particular stain type towards the total number of images.
    args:
        df_stain: pd.core.frame.DataFrame; df containing data for a given stain␣
↪type
        df_full: pd.core.frame.DataFrame; df containing data for full (cleaned)␣
↪set
        cols: list, contains str denoting col names for grouping
        sid: str, col name denoting col containing unique subject ids
        iid: str, col name denoting col containing unique image ids
        return_counts: bool, flag for added utility during debugging
    return:
        mean_cell_ns: pd.core.frame.DataFrame; df containing final mean cell ns
        cell_counts: pd.core.frame. DataFram; df containing cell counts per
            image (for debugging)

    '''
```

```
    # count n of unique image names per subject
    img_ns = count_imgs(df_full, sid, iid)

    # count n of cells per image for each subject
    cell_counts = count_cells(df_stain, cols)

    # sum cell counts across all images for each subject
    cell_sums = sum_cells(cell_counts, cols, iid)

    # compute mean cell count per image for each subject
    mean_cell_ns = average_counts(cell_sums, img_ns, cols, sid, iid)

    if not return_counts:
        return mean_cell_ns

    return (cell_counts, mean_cell_ns)
```

1.5 Time to run it!

Normalize Intensity, write to disk

[7]:
```
grp = 'VR5_KET'
for stain in df_subset.stain_type.unique():

    # split by stain
    df_stain = df_subset[df_subset.stain_type == stain]

    # normalize to FR1_SAL
    df_norm = normalize_intensity(df_stain, norm_condition='FR1_SAL')
    df_norm.to_csv(f'{grp}_{stain}_single_NORM.csv')

    # reorganize into cols for prism
    df_prism = prism_reorg(df_norm, grp)
    df_prism.to_csv(f'{grp}_{stain}_PRISM.csv')

# let's take a look at one of our final output dataframes, organized for entry␣
 ↪into prism
print(stain)
df_prism
```

```
WFA
```

[7]:

	FR1_KET	FR1_SAL	VR5_KET	VR5_SAL
0	3.573138	5.740431	-1.730680	-0.584066
1	-0.228130	0.071669	-1.073475	-0.080524
2	0.373468	0.019555	-0.500109	-0.956723
3	-1.247351	3.060095	1.072946	-0.567922
4	0.126912	3.312895	-1.526467	-0.273813

```
..         …        …        …        …
354        NaN       NaN -1.250855       NaN
355        NaN       NaN  1.005051       NaN
356        NaN       NaN -1.339845       NaN
357        NaN       NaN -1.134914       NaN
358        NaN       NaN -0.498651       NaN

[359 rows x 4 columns]
```

Count mean cell ns, write to disk

[8]:
```python
# count n of unique image names per subject
sid = 'rat_n'
iid = 'image_name'
cols = ['treatment', 'stain_type', sid, iid]

# wrapper fn calls
for stain in df_cleaned.stain_type.unique():

    # split by stain type
    df_stain = df_cleaned[df_cleaned.stain_type == stain]

    # compute mean cell ns
    df_means = mean_cell_n(df_stain, df_cleaned, cols, sid, iid)

    # write to disk
    df_means.to_csv(f'{grp}_{stain}_mean_cell_ns.csv')

# let's take a look at one of our final output dataframes
print(stain)
df_means
```

WFA

[8]:

	rat_n	treatment	stain_type	cell_count_sums	image_n	mean_cell_n
0	KET-10-12	FR1_KET	WFA	32	5	6.40
1	KET-9-1	FR1_KET	WFA	29	4	7.25
2	PE-11-1	FR1_KET	WFA	20	5	4.00
3	PE-11-2	FR1_KET	WFA	25	5	5.00
4	PE-11-3	FR1_KET	WFA	46	5	9.20
5	PE-12-1	FR1_KET	WFA	26	5	5.20
6	PE-12-2	FR1_KET	WFA	39	5	7.80
7	PE-12-7	FR1_KET	WFA	47	5	9.40
8	KET-10-1	FR1_SAL	WFA	35	5	7.00
9	KET-10-5	FR1_SAL	WFA	32	5	6.40
10	KET-8-2	FR1_SAL	WFA	33	5	6.60
11	KET-9-2	FR1_SAL	WFA	48	5	9.60

12	KET-9-4	FR1_SAL	WFA	46	5	9.20
13	KET-9-5	FR1_SAL	WFA	45	5	9.00
14	KET-9-6	FR1_SAL	WFA	40	5	8.00
15	KET-10-14	VR5_KET	WFA	38	5	7.60
16	KET-8-7	VR5_KET	WFA	32	4	8.00
17	PE-11-4	VR5_KET	WFA	35	5	7.00
18	PE-11-5	VR5_KET	WFA	30	5	6.00
19	PE-11-6	VR5_KET	WFA	52	5	10.40
20	PE-11-7	VR5_KET	WFA	38	5	7.60
21	PE-13-2	VR5_KET	WFA	39	5	7.80
22	PE-13-3	VR5_KET	WFA	40	5	8.00
23	PE-13-6	VR5_KET	WFA	55	5	11.00
24	KET-10-2	VR5_SAL	WFA	31	5	6.20
25	KET-10-3	VR5_SAL	WFA	35	5	7.00
26	KET-10-4	VR5_SAL	WFA	26	5	5.20
27	PE-13-1	VR5_SAL	WFA	66	5	13.20
28	PE-13-11	VR5_SAL	WFA	52	5	10.40
29	PE-13-9	VR5_SAL	WFA	48	5	9.60

2 Negative Intensity?

I noticed that in the test output of the normalized intensities reshaped for prism, we were getting some negative values for normalized intensity. Negative values don't really make sense here. This would mean that the observed effect of the treatment results in cells actually being **dimmer** than background.

The only way for negative numbers to arise here is if the mean intensity before background subtraction was **less than** the background at the time the image was captured. If the average signal in a selected region was less than (or not different from) background, that is its signal to noise ratio (SNR) is less than 1, it is unclear to me why we would consider this selection as an ROI.

This means that either, selections were made where cells actually showed less fluorescence than background (did the stain work?), or background regions were poorly selected (did the background selection include ROIs?).

2.0.1 Distribution of dim selections across stain types

[18]:
```
df_full = df_full[~df_full.duplicated()]
df_full['mean_intensity'] = df_full.mean_intensity.astype('f')

df_lt = df_full.query('mean_intensity < background')

print('total number of cells where mean intensity < background, per stain:')
print(df_lt.stain_type.value_counts())

print('\npercent of cells where mean intensity < background, per stain:')
print(df_lt.stain_type.value_counts() / df_full.stain_type.value_counts() * 100)
```

```python
print('total number cells with negative mean-background')
```

```
total number of cells where mean intensity < background, per stain:
stain_type
Npas4    11324
cFos      4828
WFA        644
PV         466
Name: count, dtype: int64

percent of cells where mean intensity < background, per stain:
stain_type
Npas4    91.736876
PV       27.622999
WFA      55.517241
cFos     51.241775
Name: count, dtype: float64
total number cells with negative mean-background
```

[18]:

```
7       -98.858
8      -55.6183
10     -29.2319
11     -56.5307
12     -119.333
         …
73    -106.9829
74    -120.9047
75     -49.6622
76     -67.7978
77    -119.9755
Name: mean-background, Length: 17262, dtype: object
```

2.0.2 Distribution of dim selections across rats

[10]:

```python
print('\npercent cells where mean intensity < background, per rat:')
print(df_lt.rat_n.value_counts() / df_full.rat_n.value_counts() * 100)
```

```
percent cells where mean intensity < background, per rat:
rat_n
KET-10-1     65.707434
KET-10-12    68.518519
KET-10-14    63.389831
KET-10-2     53.207547
KET-10-3     60.782443
KET-10-4     68.148148
```

```
KET-10-5     63.955638
KET-8-2      62.178218
KET-8-7      69.841270
KET-9-1      72.389791
KET-9-2      74.835526
KET-9-4      81.145251
KET-9-5      69.240506
KET-9-6      59.211823
PE-11-1      70.720000
PE-11-2      75.552050
PE-11-3      75.396825
PE-11-4      71.587302
PE-11-5      82.324841
PE-11-6      79.788839
PE-11-7      81.153305
PE-12-1      69.776119
PE-12-2      71.132765
PE-12-7      68.951194
PE-13-1      80.444965
PE-13-11     66.795367
PE-13-2      71.444824
PE-13-3      79.567308
PE-13-6      79.648241
PE-13-9      78.071334
Name: count, dtype: float64
```

2.0.3 A Closer Look at Pixel Area

[11]:
```
df_full = df_full[~df_full.duplicated()]
df_max = df_full.groupby(['stain_type', 'image_name'])[['stain_type',␣
↪'image_name', 'pixel_area']]\
    .apply(lambda x: np.max(x.pixel_area))\
    .reset_index(name='max_pixel_area')\

df_avg_max = df_max.groupby('stain_type')[['stain_type', 'max_pixel_area']]\
    .apply(lambda x: np.average(x.max_pixel_area))\
    .reset_index(name='mean_max_pixel_area')

df_avg_max
```

[11]:

	stain_type	mean_max_pixel_area
0	Npas4	1961.263514
1	PV	673.175676
2	WFA	1163.337838
3	cFos	402.837838

[12]:
```python
# means seem inflated by outliers; let's take a look at medians
df_median_max = df_max.groupby('stain_type')[['stain_type', 'max_pixel_area']]\
    .apply(lambda x: np.median(x.max_pixel_area))\
    .reset_index(name='median_max_pixel_area')

df_median_max
```

[12]:

	stain_type	median_max_pixel_area
0	Npas4	520.5
1	PV	676.0
2	WFA	1147.5
3	cFos	385.5

[13]:
```python
# those means seem a little high. what is the maximum pixel area per stain type?
# means area likely driven up by outliers
df_full.groupby('stain_type')[['stain_type', 'pixel_area']]\
    .apply(lambda x: np.max(x.pixel_area))\
    .reset_index(name='max_pixel_area')
```

[13]:

	stain_type	max_pixel_area
0	Npas4	28596.0
1	PV	1342.0
2	WFA	2264.0
3	cFos	763.0

[14]:
```python
# max pixel area for one cell of 28000 does not even make sense
# let's investigate those unusual, very large Npas4 cells
# something weird happened in these images
df_full.query('stain_type == "Npas4" & pixel_area > 1500')
```

[14]:

	cell_number	roi_id	roi_source	roi_type	CoM_x	\
4	5	200-00004	General Segmentation v1	POLYGON	238.33	
5	6	200-00009	General Segmentation v1	POLYGON	245.39	
4	5	200-00018	General Segmentation v1	POLYGON	270.94	
5	6	200-00021	General Segmentation v1	POLYGON	270.94	
6	7	200-00022	General Segmentation v1	POLYGON	270.94	
7	8	200-00023	General Segmentation v1	POLYGON	270.94	
0	1	200-00000	General Segmentation v1	POLYGON	271.7	
0	1	200-00001	General Segmentation v1	POLYGON	250.45	
0	1	200-00000	General Segmentation v1	POLYGON	239.68	
8	9	200-00008	General Segmentation v1	POLYGON	279.49	
10	11	200-00010	General Segmentation v1	POLYGON	279.49	
8	9	200-00008	General Segmentation v1	POLYGON	253.38	

	CoM_y	pixel_area	background	mean_intensity	median_intensity	…	\
4	249.59	28596.0	832.9028	717.443420	680.6517	…	
5	259.26	27314.0	737.6275	621.745789	571.9613	…	

4	238.52	27429.0	780.4174	675.169312	638.1753	…
5	238.52	27429.0	780.4174	675.169312	638.1753	…
6	238.52	27429.0	780.4174	675.169312	638.1753	…
7	238.52	27429.0	780.4174	675.169312	638.1753	…
0	252.53	23805.0	720.1612	618.770630	582.1547	…
0	273.55	26636.0	665.9866	580.254883	543.9764	…
0	285.52	26845.0	316.7702	267.418701	233.0273	…
8	250.82	27853.0	703.4017	579.480103	526.3982	…
10	250.82	27853.0	703.4017	579.480103	526.3982	…
8	251.91	27063.0	494.2210	430.552399	388.4379	…

	roundness	solidity	skewness	kurtosis	filename	\
4	0.1091	1.0	1.5587	2.9482	PE-11-5_PFC_3.9_B_4.tif	
5	0.1042	1.0	2.0482	5.4282	PE-11-4_PFC_3.6_D_4.tif	
4	0.1046	1.0	1.7441	3.9845	PE-11-6_PFC_3.9_A_4.tif	
5	0.1046	1.0	1.7441	3.9845	PE-11-6_PFC_3.9_A_4.tif	
6	0.1046	1.0	1.7441	3.9845	PE-11-6_PFC_3.9_A_4.tif	
7	0.1046	1.0	1.7441	3.9845	PE-11-6_PFC_3.9_A_4.tif	
0	0.0908	1.0	1.7792	4.2285	PE-11-4_PFC_3.8_B_4.tif	
0	0.1016	1.0	1.6087	3.1387	PE-13-1_PFC_3.6_E_4.tif	
0	0.1024	1.0	3.1471	15.0826	KET-9-5_PFC_3.5_C_4.tif	
8	0.1063	1.0	2.0342	5.1542	KET-10-4_PFC_4.1_A_4.tif	
10	0.1063	1.0	2.0342	5.1542	KET-10-4_PFC_4.1_A_4.tif	
8	0.1032	1.0	2.9071	12.5434	KET-10-3_PFC_3.7_D_4.tif	

	analysis_date	rat_n	treatment	stain_type	\
4	Thu Jan 25 15:00:50 PST 2024	PE-11-5	VR5_KET	Npas4	
5	Thu Jan 25 14:55:25 PST 2024	PE-11-4	VR5_KET	Npas4	
4	Thu Jan 25 15:03:53 PST 2024	PE-11-6	VR5_KET	Npas4	
5	Thu Jan 25 15:03:53 PST 2024	PE-11-6	VR5_KET	Npas4	
6	Thu Jan 25 15:03:53 PST 2024	PE-11-6	VR5_KET	Npas4	
7	Thu Jan 25 15:03:53 PST 2024	PE-11-6	VR5_KET	Npas4	
0	Thu Jan 25 14:56:51 PST 2024	PE-11-4	VR5_KET	Npas4	
0	Thu Jan 25 15:43:05 PST 2024	PE-13-1	VR5_SAL	Npas4	
0	Thu Jan 25 13:03:10 PST 2024	KET-9-5	FR1_SAL	Npas4	
8	Mon Jan 22 16:20:19 PST 2024	KET-10-4	VR5_SAL	Npas4	
10	Mon Jan 22 16:20:19 PST 2024	KET-10-4	VR5_SAL	Npas4	
8	Mon Jan 22 15:02:22 PST 2024	KET-10-3	VR5_SAL	Npas4	

	image_name
4	PE-11-5_PFC_3.9_B
5	PE-11-4_PFC_3.6_D
4	PE-11-6_PFC_3.9_A
5	PE-11-6_PFC_3.9_A
6	PE-11-6_PFC_3.9_A
7	PE-11-6_PFC_3.9_A
0	PE-11-4_PFC_3.8_B

```
0     PE-13-1_PFC_3.6_E
0     KET-9-5_PFC_3.5_C
8    KET-10-4_PFC_4.1_A
10   KET-10-4_PFC_4.1_A
8    KET-10-3_PFC_3.7_D

[12 rows x 34 columns]
```

[52]:
```
df_negative_counts = df_lt.groupby(['filename', 'stain_type'])[['filename',␣
↪'stain_type']]\
    .apply(lambda x: len(x.filename))\
    .reset_index(name='negative intensity counts')\
    .sort_values(by='negative intensity counts')
```

[57]:
```
df_counts = df_full.groupby(['filename', 'stain_type'])[['filename',␣
↪'stain_type']]\
    .apply(lambda x: len(x.filename))\
    .reset_index(name='counts')\
    .sort_values(by='filename')

df_negative_counts.merge(df_counts).to_csv('negative_intensity_counts_by_file.
↪csv')
```

3 Dealing with negative intensities

3.1 Median Split?

[290]:
```
npas4 = df_full[df_full.stain_type == 'Npas4']
print(npas4.median_intensity.astype('f').median())

npas4.query('median_intensity == 437.85144')
print(len(npas4))

med_med_gt0 = npas4['mean-background'].astype('f') + npas4.median_intensity.
↪astype('f').median() > 0
print(med_med_gt0.sum())

med_med_gt0 = npas4['mean-background'].astype('f') + npas4.mean_intensity.
↪astype('f').median() > 0
print(med_med_gt0.sum())
```

```
437.85144
12344
12311
12311
```

[163]:
```
# median of median intensity
test_img = npas4.query('filename == " PE-12-2_PFC_3.6_D_4.tif"')
med_int = test_img.median_intensity.astype('f').median()
med_int
```

[163]: 444.28497

[267]:
```
test_img_neg = test_img[test_img['mean-background'].astype('f') < 0]
test_img_neg_sorted = test_img_neg.sort_values(by='mean-background')
m =␣
↪list(test_img_neg_sorted['mean-background'])[test_img_neg_sorted['mean-background'].
↪count() // 2]

test_img['adjusted_mean-background'] = test_img['mean-background'].astype('f')␣
↪+ (-1*m)
test_img
```

```
/var/folders/b2/3h2lpxx14kgb12pp_7pltxnc0000gn/T/ipykernel_59275/525166263.py:5:
SettingWithCopyWarning:
A value is trying to be set on a copy of a slice from a DataFrame.
Try using .loc[row_indexer,col_indexer] = value instead

See the caveats in the documentation: https://pandas.pydata.org/pandas-
docs/stable/user_guide/indexing.html#returning-a-view-versus-a-copy
  test_img['adjusted_mean-background'] = test_img['mean-background'].astype('f')
+ (-1*m)
```

[267]:

	cell_number	roi_id	roi_source	roi_type	CoM_x	\
0	1	200-00000	General Segmentation v1	POLYGON	417.96	
1	2	200-00001	General Segmentation v1	POLYGON	267.27	
2	3	200-00002	General Segmentation v1	POLYGON	45.88	
3	4	200-00003	General Segmentation v1	POLYGON	37.63	
4	5	200-00004	General Segmentation v1	POLYGON	132.09	
..	…	…	…	…	…	
105	106	FFF-00105	hand-drawn	OVAL	266.4	
106	107	FFF-00106	hand-drawn	OVAL	276.06	
107	108	FFF-00107	hand-drawn	OVAL	10.99	
108	109	FFF-00108	hand-drawn	OVAL	348.03	
109	110	FFF-00109	hand-drawn	OVAL	102.48	

	CoM_y	pixel_area	background	mean_intensity	median_intensity	…	\
0	499.13	350.0	572.0286	582.638611	554.322	…	
1	458.34	757.0	572.0286	886.685120	871.4989	…	
2	450.17	193.0	572.0286	488.809296	461.5495	…	
3	407.37	297.0	572.0286	453.547211	430.6313	…	
4	404.08	284.0	572.0286	499.338806	465.2783	…	
..	…	…	…	…	…	…	

```
105  376.93       132.0   572.0286      518.463501         484.7022  …
106  350.38       123.0   572.0286      474.718506         440.8519  …
107  274.34        88.0   572.0286      457.285492         450.8587  …
108   55.62        59.0   572.0286      456.607208         418.0623  …
109  505.66       260.0   572.0286      525.071594         499.7756  …

    skewness  kurtosis                 filename  \
0     0.7936    0.2897   PE-12-2_PFC_3.6_D_4.tif
1     0.1222   -1.2742   PE-12-2_PFC_3.6_D_4.tif
2     1.4261    2.5188   PE-12-2_PFC_3.6_D_4.tif
3     1.6214    3.1709   PE-12-2_PFC_3.6_D_4.tif
4     0.8968   -0.0045   PE-12-2_PFC_3.6_D_4.tif
..       …         …                        …
105   1.2488    1.4826   PE-12-2_PFC_3.6_D_4.tif
106   2.0996    5.5607   PE-12-2_PFC_3.6_D_4.tif
107   1.1905    1.4763   PE-12-2_PFC_3.6_D_4.tif
108   2.0806    5.3494   PE-12-2_PFC_3.6_D_4.tif
109   1.1052    1.4702   PE-12-2_PFC_3.6_D_4.tif

                    analysis_date    rat_n  treatment stain_type  \
0     Thu Jan 25 15:35:18 PST 2024  PE-12-2    FR1_KET      Npas4
1     Thu Jan 25 15:35:18 PST 2024  PE-12-2    FR1_KET      Npas4
2     Thu Jan 25 15:35:18 PST 2024  PE-12-2    FR1_KET      Npas4
3     Thu Jan 25 15:35:18 PST 2024  PE-12-2    FR1_KET      Npas4
4     Thu Jan 25 15:35:18 PST 2024  PE-12-2    FR1_KET      Npas4
..                              …        …          …        …
105   Thu Jan 25 15:35:18 PST 2024  PE-12-2    FR1_KET      Npas4
106   Thu Jan 25 15:35:18 PST 2024  PE-12-2    FR1_KET      Npas4
107   Thu Jan 25 15:35:18 PST 2024  PE-12-2    FR1_KET      Npas4
108   Thu Jan 25 15:35:18 PST 2024  PE-12-2    FR1_KET      Npas4
109   Thu Jan 25 15:35:18 PST 2024  PE-12-2    FR1_KET      Npas4

             image_name  adjusted_mean-background       snr
0     PE-12-2_PFC_3.6_D                107.973000  1.018548
1     PE-12-2_PFC_3.6_D                405.441010  1.550071
2     PE-12-2_PFC_3.6_D                 14.707001  0.854519
3     PE-12-2_PFC_3.6_D                -20.220901  0.792875
4     PE-12-2_PFC_3.6_D                 25.610504  0.872926
..                    …                        …        …
105   PE-12-2_PFC_3.6_D                 46.297802  0.906359
106   PE-12-2_PFC_3.6_D                  0.525200  0.829886
107   PE-12-2_PFC_3.6_D                -15.935799  0.799410
108   PE-12-2_PFC_3.6_D                -18.632698  0.798224
109   PE-12-2_PFC_3.6_D                 51.222301  0.917911

[110 rows x 36 columns]
```

[270]:
```
# ok so we keep 59 cells by adding back in the median of the mean_intensity to␣
↪all cells
test_img[test_img['adjusted_mean-background'] > 0].shape
```

[270]: (59, 36)

3.2 Let's try a different approach: dividing by background, rather than subtracting it

This way, we end up with a signal to noise ratio (SNR) of intensity for each cell that can never be negative. ROI intensity can then be interpretted as a percent of background (SNR < 1 means that particular cell was dimmer than background). From here we can set/adjust a threshold based on SNR and assess how representative the new ROI selections/exclusions are of the cells we actually see in an image. Then, the data taken to be normalized to some treatment condition will be the intensity SNR, and the mean of the norm group will be set to 1.

After visual inspection by AG, JR, and BS, of the "worst offenders" (images with the greatest number of negative intensities), the following SNR cutoffs were deteremined to be appropriate: - Npas4: 0.8 - PV: 0.8 - WFA: 0.85 - cFos: 0 (we want to keep all the data)

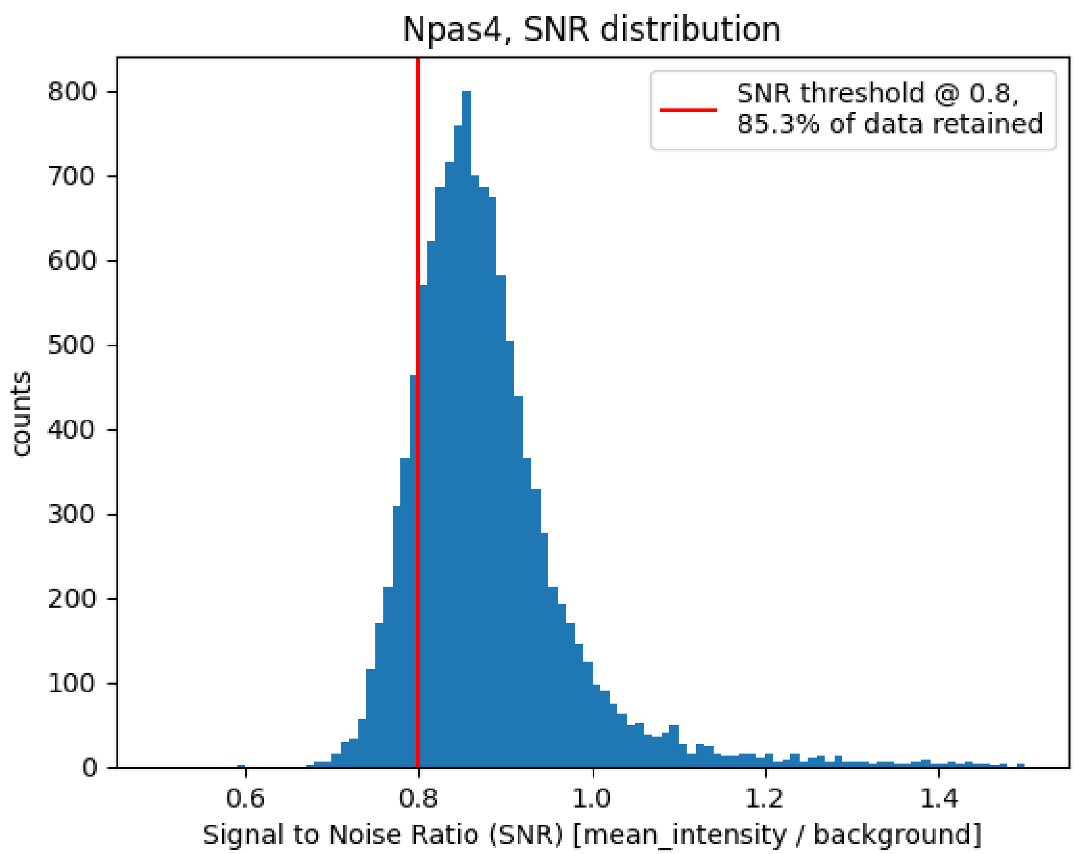
In the following cells, I will plot exploratory histograms of the intensity SNRs for each stain type (single label only) and denote the determined cutoff with a red vertical line.

3.2.1 Npas4

[294]:
```
npas4 = df_full.query('stain_type == "Npas4"').copy(deep=True)

npas4['snr'] = npas4.loc[:, 'mean_intensity'].astype('f') / npas4.loc[:,␣
↪'background'].astype('f')
print(npas4.query('snr > 0.8').__len__() / npas4.__len__())
ret = np.round((npas4.query('snr > 0.8').__len__() / npas4.__len__()) * 100, 1)
plt.title('Npas4, SNR distribution')
plt.xlabel('Signal to Noise Ratio (SNR) [mean_intensity / background]')
plt.ylabel('counts')
plt.axvline(x=0.8, c='r', label =f'SNR threshold @ 0.8,\n{ret}% of data␣
↪retained')
plt.legend()
plt.hist(npas4.snr, 100, range=[0.5, 1.5])
plt.savefig('Intensity_SNR_Distribution_Npas4.png', dpi=600)
plt.show()
```

0.853451069345431



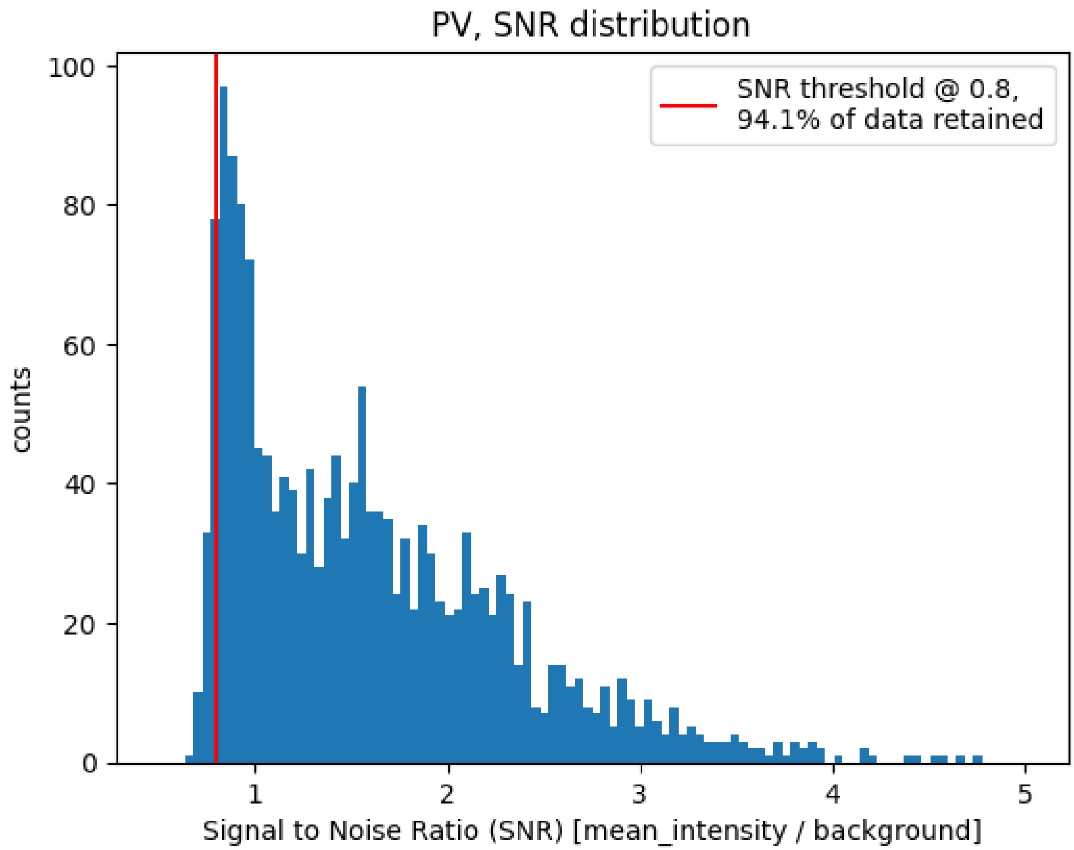
3.2.2 PV

[293]:
```
pv = df_full.query('stain_type == "PV"').copy(deep=True)

pv['snr'] = pv.loc[:, 'mean_intensity'].astype('f') / pv.loc[:, 'background'].
↪astype('f')
print(pv.query('snr > 0.8').__len__() / pv.__len__())
ret = np.round((pv.query('snr > 0.8').__len__() / pv.__len__()) * 100, 1)

plt.title('PV, SNR distribution')
plt.xlabel('Signal to Noise Ratio (SNR) [mean_intensity / background]')
plt.ylabel('counts')
plt.axvline(x=0.8, c='r', label =f'SNR threshold @ 0.8,\n{ret}% of data␣
↪retained')
plt.legend()
plt.hist(pv.snr, 100, range=[0.5, 5])
plt.savefig('Intensity_SNR_Distribution_PV.png', dpi=600)
plt.show()
```

```
0.941315945465323
```



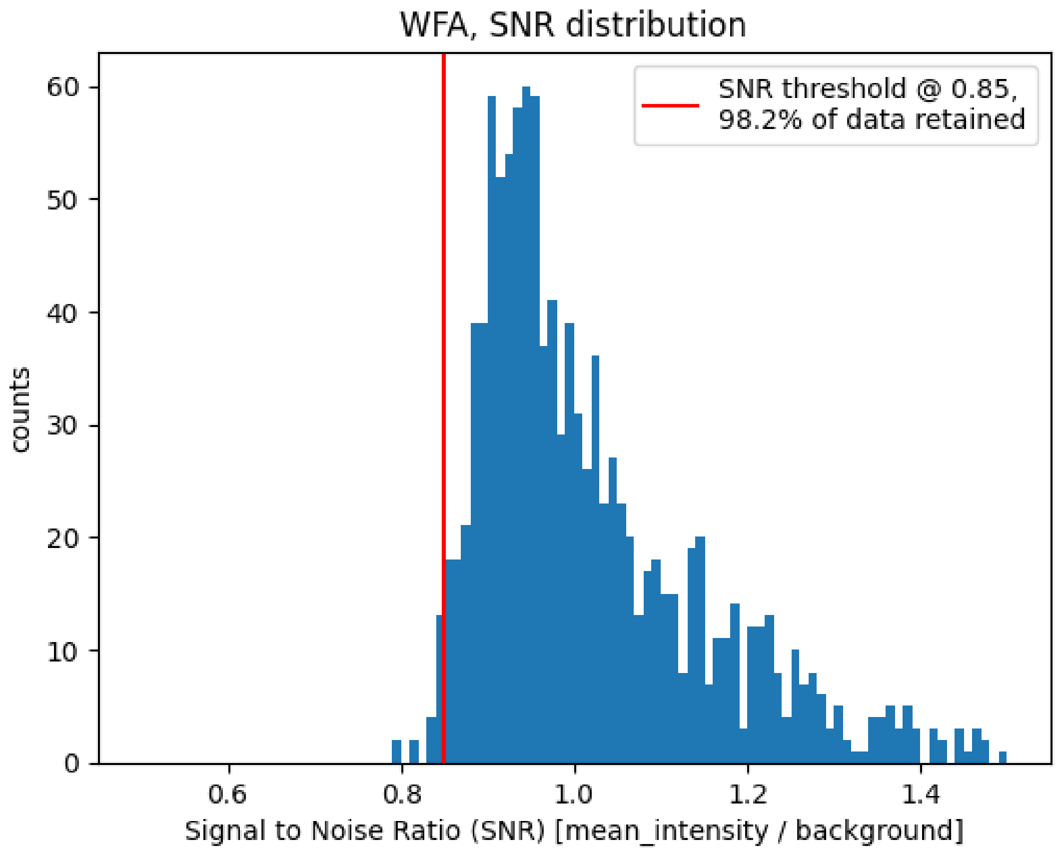
3.2.3 WFA

[291]:
```
wfa = df_full.query('stain_type == "WFA"').copy(deep=True)

wfa['snr'] = wfa.loc[:, 'mean_intensity'].astype('f') / wfa.loc[:,␣
↪'background'].astype('f')
print(wfa.query('snr > 0.85').__len__() / wfa.__len__())
ret = np.round((wfa.query('snr > 0.85').__len__() / wfa.__len__()) * 100, 1)

plt.title('WFA, SNR distribution')
plt.xlabel('Signal to Noise Ratio (SNR) [mean_intensity / background]')
plt.ylabel('counts')
plt.axvline(x=0.85, c='r', label =f'SNR threshold @ 0.85,\n{ret}% of data␣
↪retained')
plt.legend()
plt.hist(wfa.snr, 100, range=[0.5, 1.5])
plt.savefig('Intensity_SNR_Distribution_WFA.png', dpi=600)
plt.show()
```

```
0.9818965517241379
```



3.2.4 cFos

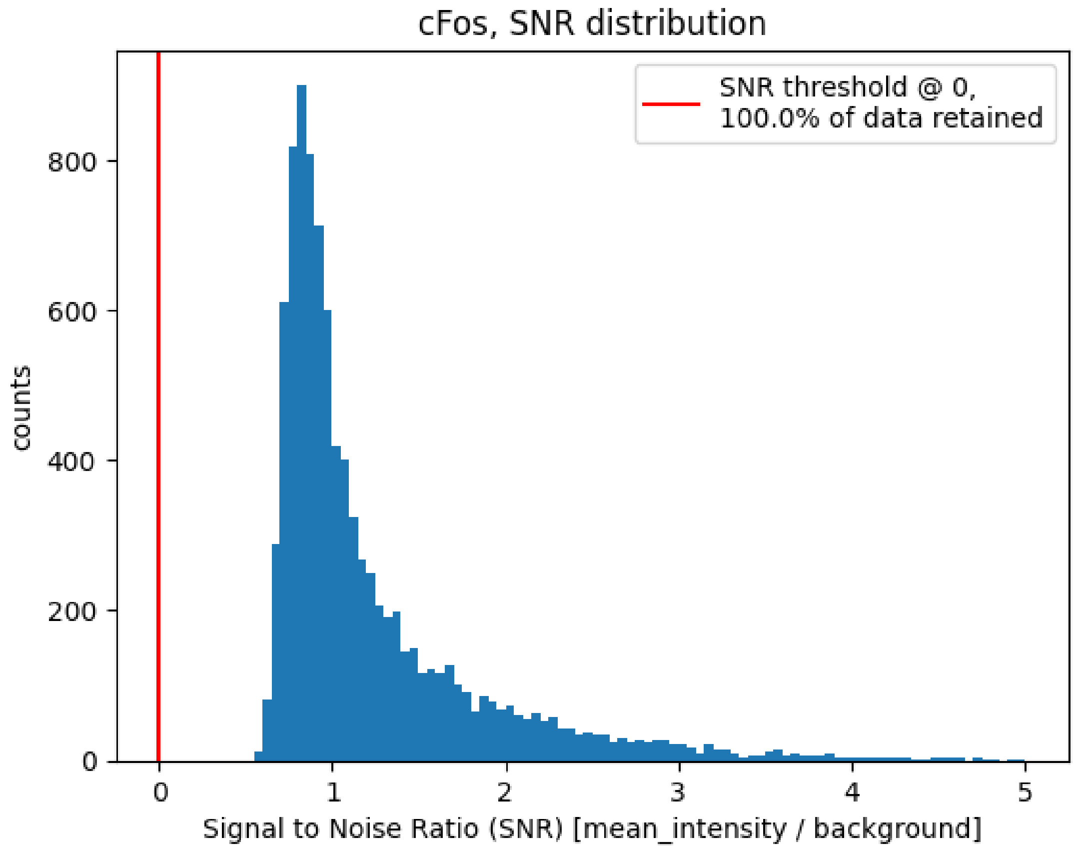
Upon visual inspection of some of the "worst offenders" for cFos, it was determined that all selected ROIs reasonably reflect the presence of true cells and so no SNR threshold will be applied.

[292]:
```
cfos = df_full.query('stain_type == "cFos"').copy(deep=True)

cfos['snr'] = cfos.loc[:, 'mean_intensity'].astype('f') / cfos.loc[:,␣
 ↪'background'].astype('f')
print(cfos.query('snr > 0').__len__() / cfos.__len__())
ret = np.round((cfos.query('snr > 0').__len__() / cfos.__len__()) * 100, 1)

plt.title('cFos, SNR distribution')
plt.xlabel('Signal to Noise Ratio (SNR) [mean_intensity / background]')
plt.ylabel('counts')
plt.axvline(x=0, c='r', label =f'SNR threshold @ 0,\n{ret}% of data retained')
plt.legend()
plt.hist(cfos.snr, 100, range=[0, 5])
plt.savefig('Intensity_SNR_Distribution_cFos.png', dpi=600)
plt.show()
```

```
0.9997877308427086
```



Once I receive some updated data, I will repeat the wrangling/cleaning process for the single labeled ketamine data again, but this time implementing SNR thresholding as described above.