Study Overview:
This study examines vessel diameter and vascular permeability in mouse brain vasculature using intravital two-photon imaging (imaging over ~S1, down to ~0.3mm; T-Z stacks). The researchers compared two groups (control/sham and an experimental group/2XmTBI – imaged 3-6h post-injury/Sx) using various measurements of blood vessels.

Methodology:
1. Imaging:
  - Used galvo-galvo Z-stacks with FITC dextran dye
  - 512x512 pixels (0.3 µm/pixel), Nikon 16X objective (NA 0.8) with 6X zoom
  - 800nm illumination, 50-60mW power output
  - Z-stacks: 5 µm steps, 200-300 µm depth
  - Time series: 10 seconds duration, 10 frames (1Hz) per Z-interval

2. Image Processing:
  - 3D median filter (2 pixels) to reduce PMT shot noise
  - Manual selection of line scan areas (14-50x6 µm), targeting smaller vessels (3-10 µm)

3. Data Analysis:
  - Custom Matlab and Python scripts
  - Calculated full-width half maximum (FWHM) for vessel diameter
  - Measured Fv (interior vessel fluorescence), Fe (exterior fluorescence), and Fi (vessel wall fluorescence)
  - Calculated vessel wall permeability (Pv) as Fe/Fv

4. Statistical Analysis:
  - Mann-Whitney U test (grpA v grpB):
  - FWHM: U statistic = 7.0, p-value = 0.35238
  - Fe/Fv: U statistic = 13.0, p-value = 0.91429
  Both tests fail to reject the null hypothesis, indicating no significant differences between distributions.

  - Kolmogorov-Smirnov test (grpA v grpB):
  - FWHM: statistic = 0.0922, p-value = 0.6163
  - Fe/Fv: statistic = 0.1918, p-value = 0.0167
  The second test shows a significant difference ($p < 0.05$).

Sample sizes are provided: n=157 for one group (6 mice) and n=104 for another group (4 mice).

  - Principal Component Analysis (PCA) on three variables: FeFv, Zmicrons, and mean_FWHM_ums
  - Mann-Whitney U tests and t-tests on PC scores between groups

Key Findings:
1. PCA Results:
   - PC1 explains 52.94% of variance, PC2 explains 30.20%
   - PC1 strongly correlates with all three variables, especially FeFv and Zmicrons

2. Statistical Comparisons:
   - No significant differences between groups for PC1 (p=0.422), PC2 (p=0.062), or PC3 (p=0.462)
   - PC2 shows a trend towards significance (p=0.062)

3. Variable Relationships:
   - Positive correlations between Zmicrons, mean_FWHM_ums, and FeFv
   - FeFv and Zmicrons contribute most to data variation

<u>Page with "From 7-19-24" data:</u>
This page presents statistical test results for two analyses:

Sample sizes are provided: n=157 for A group (6 mice) and n=104 for B group (4 mice).

1. Mann-Whitney U test:
   - FWHM: U statistic = 7.0, p-value = 0.35238
   - Fe/Fv: U statistic = 13.0, p-value = 0.91429
   Both tests fail to reject the null hypothesis, indicating no significant differences between distributions.

2. Kolmogorov-Smirnov test:
   - FWHM: statistic = 0.0922, p-value = 0.6163
   - Fe/Fv: statistic = 0.1918, p-value = 0.0167
   The second test shows a significant difference ($p < 0.05$).
   - K-S test result:
     - Test 2 shows a statistic of 0.1918 with a p-value of 0.0167, which is significant at the $\alpha = 0.05$ level.
   - Interpretation: The K-S test compares the cumulative distribution functions of two samples. A significant result suggests that the two groups (likely control and experimental) have different distributions for the variable being tested.
   - Importance: This significant difference is particularly interesting because it contrasts with the non-significant results from the Mann-Whitney U tests and the PCA analysis. It suggests that there might be subtle differences between the groups that aren't captured by measures of central tendency or linear combinations of variables.
   - Potential implications: a) Distribution shape: The K-S test is sensitive to differences in the shape of distributions, not just central tendency. This could indicate that while the groups may have similar means or medians, they differ in terms of spread, skewness, or other distributional characteristics. b) Subset of data: Given that only one K-S test was significant, this might relate to a specific aspect of the vascular measurements (e.g.,

vessel diameter, permeability, or depth) that shows group differences. c) Biological significance: In the context of brain trauma models, this could suggest subtle alterations in vascular properties that aren't uniform across all vessels or depths, but manifest as changes in the overall distribution of a particular measure.

- Considerations: a) Multiple comparisons: It's important to consider whether this p-value (0.0167) would remain significant after correcting for multiple comparisons, especially if many tests were performed. b) Effect size: While statistically significant, we should also consider the practical significance. The test statistic of 0.1918 suggests a moderate effect size.

Page with "From 12-27-23 - 2D scatter plots":
1. Plot of Vessel diameter (FWHM, microns) vs. Normalized perivessel fluorescence intensity (mean Fe/Fv):
   - X-axis ranges from 10 to 60 (Fe/Fv)
   - Y-axis ranges from 2 to 10 microns (vessel diameter)
   - Two groups are shown: Group A (red) and Group B (blue)
   - Points are scattered without clear separation between groups

2. Plot of Z-depth (microns from surface) vs. Normalized perivessel fluorescence intensity (mean Fe/Fv):
   - X-axis ranges from 10 to 60 (Fe/Fv)
   - Y-axis ranges from 0 to 300 microns (Z-depth)
   - Same two groups are shown (A in red, B in blue)
   - Points are scattered across the plot, with some apparent clustering at certain depths

Both plots suggest overlapping distributions between the two groups, consistent with the statistical tests showing no significant differences in most comparisons.

Page with PCA results:
1. A scatter plot of PC1 vs PC2:
   - Points are colored red and black, likely representing two groups
   - PC1 explains 52.94% of variance
   - PC2 explains 30.20% of variance
   - No clear separation between groups is visible

2. A bar plot showing correlations between PC1 and original variables:
   - Strong positive correlations for all three variables
   - FeFv and Zmicrons have the highest correlations (> 0.8)
   - mean_FWHM_ums has a moderate correlation (~ 0.5)

3. Statistical results comparing PC scores between groups:
   - All three PCs show non-significant differences (p > 0.05)
   - PC2 shows a trend towards significance (p = 0.062)

This analysis suggests that while there are strong relationships between the measured variables, there are no statistically significant differences between the two groups in terms of these principal components.

Interpretation:
1. The lack of significant differences between groups suggests that the experimental condition (possibly a brain trauma model) did not produce large-scale changes in the measured vascular properties.

2. The strong correlations between variables might reflect underlying biological relationships in vascular structure and function. For example, vessel depth (Zmicrons) might be related to vessel size (FWHM) and blood flow characteristics (FeFv).

3. The trend towards significance in PC2 hints at possible subtle differences between groups, which might become significant with a larger sample size or more sensitive measurements.

Limitations and Future Directions:
1. The current analysis may not be sensitive enough to detect subtle changes in vascular properties following the experimental condition.

2. Including additional data such as X, Y coordinates and more detailed time series statistics could potentially increase the sensitivity of the analysis.

3. Using clustering algorithms (e.g., K-means or UMAP) on PC scores could reveal more complex patterns in the data.

4. Standardizing variables before PCA and carefully selecting the number of PCs to retain for clustering could improve the analysis.

5. Validating clustering results using domain knowledge and additional statistical tests would strengthen the findings.

In conclusion, while this study provides valuable insights into the relationships between vascular properties in the mouse brain, it does not demonstrate significant differences between the experimental groups.

Future work could focus on refining the analysis methods and increasing sample sizes to detect potentially subtle effects of the experimental condition on brain vasculature.

→ Classify by vessel type
→ Classify by ROI location
→ Use X,Y data
→ Future expts – collect RBC velocity, neuro-vascular: combine w/ behavior (e.g., whisker-stim)