JHC—Journal of Histochemistry & Cytochemistry
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Authors: McCall and Baker


**Supplemental Table 1. Focus scores of samples used.**

| Assay | Status | Focus Score | Age |
|---|---|---|---|
| WB | Non-SS | 0 | 53 |
| | | 0 | 68 |
| | | 0 | 35 |
| | SS | 1.2 | 59 |
| | | 9.5 | 48 |
| | | 1.6 | 63 |
| IF | Non-SS | 0 | 36 |
| | | 0 | 61 |
| | | 0 | 58 |
| | | 0 | 71 |
| | | 0 | 76 |
| | | 0 | 57 |
| | SS | 4.7 | 55 |
| | | 5.9 | 55 |
| | | 1.6 | 47 |
| | | 1 | 48 |
| | | 1 | 57 |
| | | 3.7 | 46 |

This table lists the focus scores (number of lymphocyte foci with >50 lymphocytes per 4 $mm^2$ of salivary tissue) and patient age for each sample used in this study. No individual sample was used for both Western blotting and immunofluorescence. WB: Western Blot; IF: Immunofluorescence; SS: Sjögren's Syndrome.

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**Supplemental Table 2. Antibodies used for Immunofluorescent Studies.**

| **Antibody** | **Company** | **Dilution** | **Application** |
|---|---|---|---|
| Rabbit anti- human vWF | Dako, Carpinteria, CA | 1:500 | IF Primary Ab (Blood) |
| Mouse anti-human PECAM-1 | Millipore, Billerica, MA | 1:250 | IF Primary Ab (Blood, Lymph) |
| Goat anti-LYVE-1 | R&D Systems, Minneapolis, MN | 1:150 | IF Primary Ab (Lymph) |
| Alexa Fluor 488 goat anti-rabbit | Invitrogen, Carlsbad, CA | 1:500 | IF Secondary Ab (Blood) |
| Alexa Fluor 568 goat anti-mouse | Invitrogen | 1:500 | IF Secondary Ab (Blood) |
| Alexa Fluor 488 donkey anti-goat | Invitrogen | 1:500 | IF Secondary Ab (Lymph) |
| Alexa Fluor 568 donkey anti-mouse | Invitrogen | 1:500 | IF Secondary Ab (Lymph) |

This table lists the antibodies that were used for immunofluorescent staining of the human minor salivary gland tissue sections. IF**:** Immunofluorescence; Ab: Antibody.

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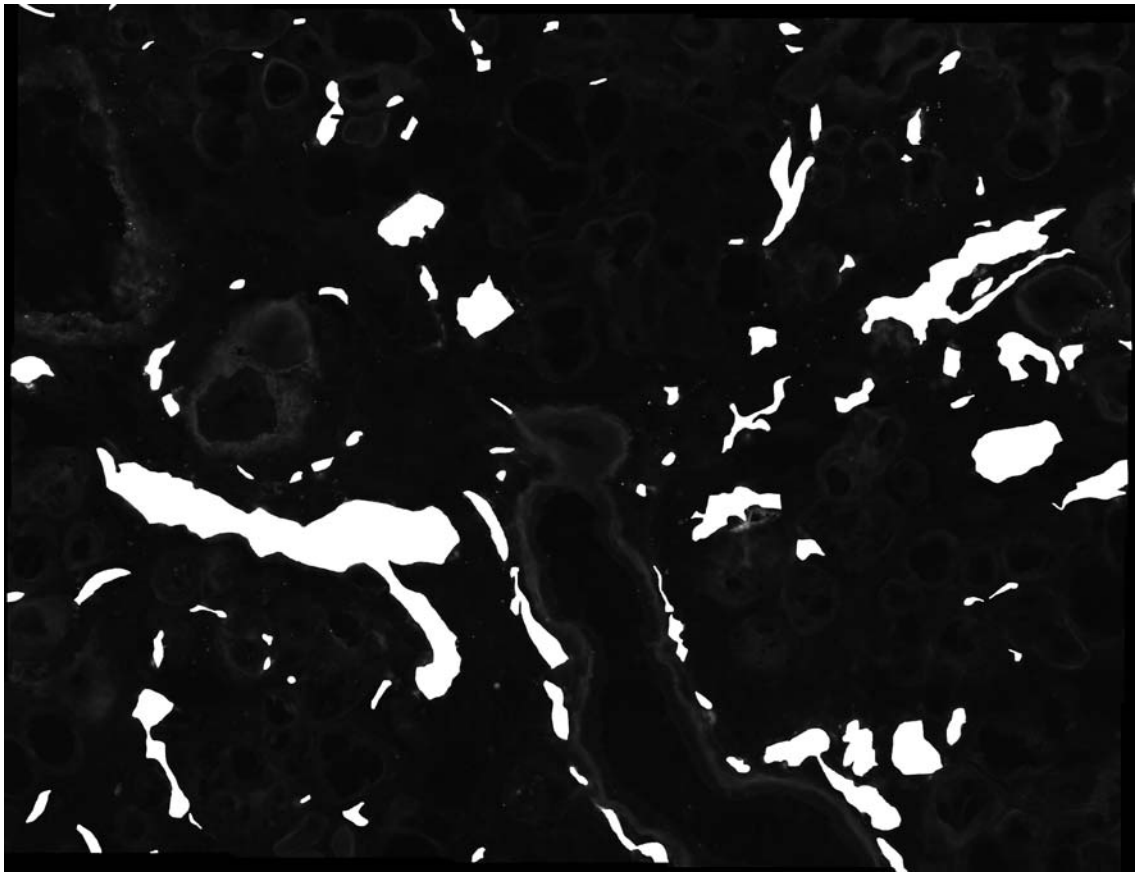
**Supplemental Figure 1. Processed blood vessel image.**



An example of a blood vessel image, processed as described in Materials and Methods. Expression of platelet endothelial cell adhesion molecule-1 (PECAM-1) and von Willebrand factor (vWF) was detected using immunofluorescent microscopy. Blood vessels (PECAM-$1^+$ and $vWF^+$) were highlighted, and the image was analyzed using the script described in Supplemental Text 1.

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**Supplemental Text 1. ImageJ script used for volume fraction analyses.**

```
x = getWidth();                ¥¥ Stores the width of the image in variable ‘x’.

y = getHeight();               ¥¥ Stores the height of the image in variable ‘y’.

highlighted_pixel_count = 0;   ¥¥ Initializes the counter for highlighted pixels.

black_pixel_count= 0;          ¥¥ Initializes the counter for black pixels.

for (i=0; i<= x; ++i){         ¥¥ These ‘for’ statements cycle through every pixel of the image.

    for (j=0; j<=y; ++j){

        if (getPixel(i,j)==4095){          ¥¥ If the pixel is highlighted, a counter is incremented.

            highlighted_pixel_count += 1;

        }

        if (getPixel(i,j)==0){             ¥¥ If the pixel is black , a counter is incremented.

            black_pixel_count += 1;

        }

    }
```

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```
}

print (highlighted_pixel_count /((x*y)- black_pixel_count));          ¥¥ Returns the

volume fraction ratio.
```

This ImageJ script returns the ratio of highlighted pixels (those with maximum intensity) to total imaged area. It accounts for any regions that were erased from the image (regions of no tissue or non-salivary tissue) by removing any black pixels (zero intensity) from the calculated image area. Any statements between '¥¥' and the end of the line are explanatory comments and not part of the executed program.

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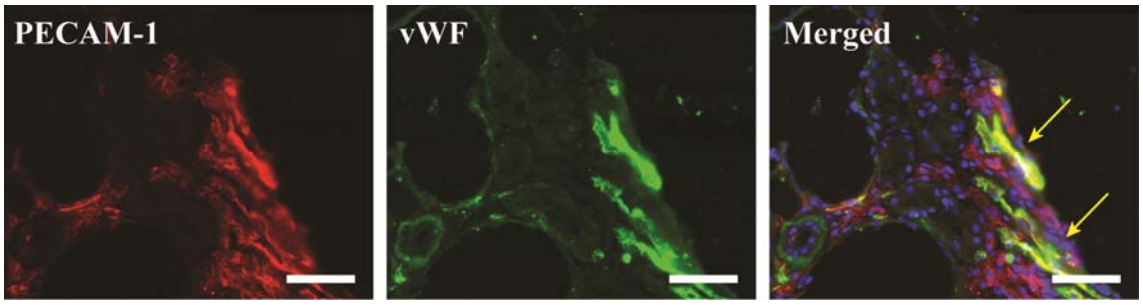
**Supplemental Figure 2. Blood vessels in the minor salivary gland capsule.**




Blood vessels were observed in the capsules encasing the minor salivary glands from both Sjögren's syndrome and non-Sjögren's syndrome patients (the images provided are from a Sjögren's syndrome patient). Five µm frozen human minor salivary gland tissue sections were fixed as described in Materials and Methods. Expression of PECAM-1 and von Willebrand Factor was detected using immunofluorescent microscopy as follows: mouse-anti-PECAM-1 (red); rabbit-anti-von Willebrand Factor (green) and **Hoechst** nuclear stain (blue). Stained structures (red and green) correspond to salivary gland vasculature. Yellow arrows indicate blood vessels in the capsule of the salivary gland. The *xy* cross section images were obtained using a Leica DMI6000B inverted fluorescent microscope. Images are representative of $n = 6$ experiments. All scale bars represent 50 µm.

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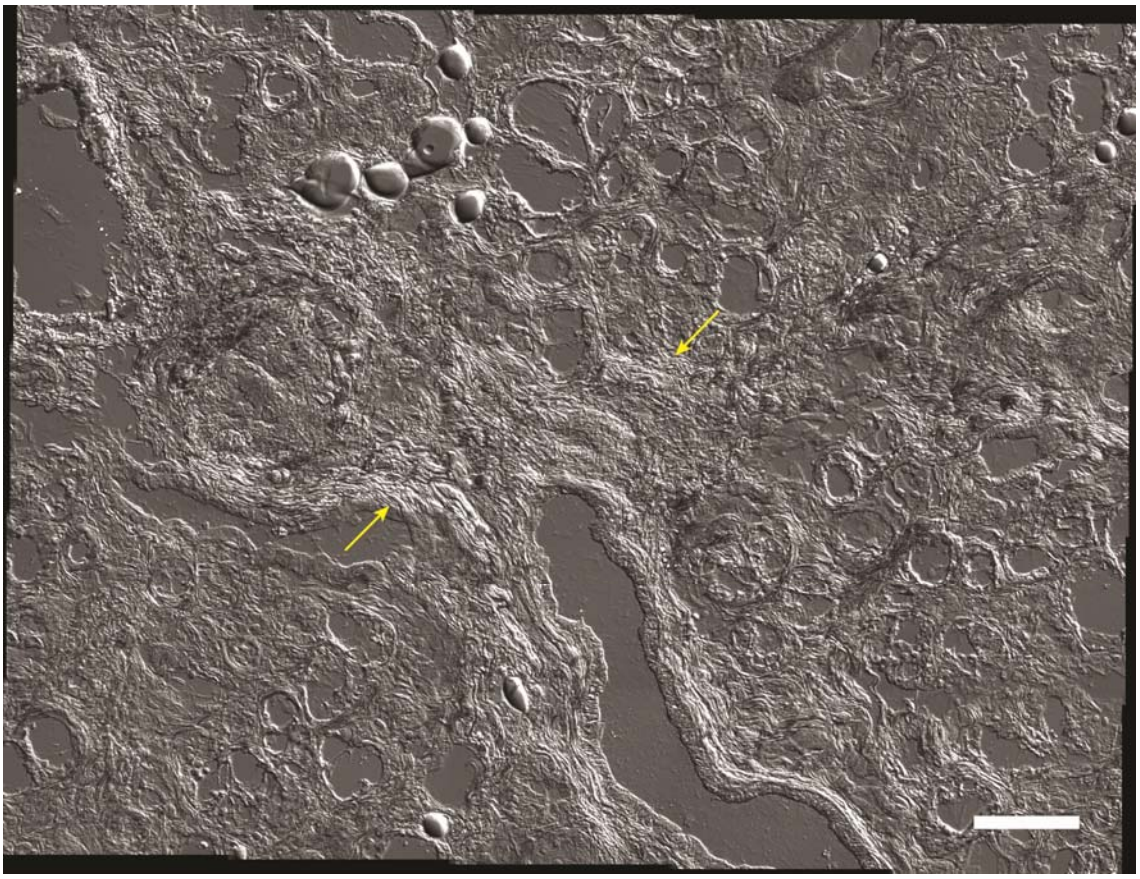
**Supplemental Figure 3. Identification of Connective Tissue.**



Connective Tissue (yellow arrows) was identified in minor salivary glands using DIC imaging (the image provided is from a non-Sjögren's syndrome patient). Five µm frozen human minor salivary gland tissue sections were fixed as described in Materials and Methods. The *xy* cross section images were obtained using a Leica DMI6000B inverted fluorescent microscope. Images are representative of $n = 6$ experiments. Scale bar represents 100 µm.

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**Supplemental Figure 4. Absence of Lymph vessels in Regions of Infiltration.**


No lymph vessels were observed in regions of infiltration in minor salivary glands with Sjögren's syndrome. Five µm frozen human minor salivary gland tissue sections were fixed as described in Materials and Methods. Expression of PECAM-1 and LYVE-1 was detected using immunofluorescent microscopy as follows: mouse-anti-PECAM-1 (red); goat-anti-LYVE-1 (green) and **Hoechst** nuclear stain (blue). Stained structures (red) correspond to salivary gland vasculature. The *xy* cross section images were obtained using a Leica DMI6000B inverted fluorescent microscope. Images are representative of $n = 6$ experiments. All scale bars represent 100 µm.