High-Level Sprout Geometry Extraction for Unstained Assays of In Vitro Angiogenesis

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ABSTRACT

We have developed an automated image analysis system for the quantitative analysis of unstained assays of *in vitro* angiogenesis. The system is designed for fibrin gel bead sprouting assays. The quantification system provides the number of primary sprouts, average branching factor and average length for each bead in an imaged assay.

1. INTRODUCTION

Angiogenesis is a mechanism for the formation of new blood vessels from pre-existing vessels. Additionally, angiogenesis is part of a critical phase in of solid tumor growth; tumor growth is stunted to approximately 1-2mm in diameter without new blood vessel supply. Consequently, to assess the impact of angiogenic and antiangiogenic agents in assays, a reliable and automated system is necessary for quantitative analysis.

The system is designed to detect features, restore features and analyze the features of imaged assays. The high-level geometry of these assays comprise of many beads with several associated sprouts.

In addition to the High-Level Sprout Geometry (HLSG) Extractor, a driver and report generator are implemented to drive functionality on sample images and generate reports on the analyses respectively.

2. DATA STRUCTURES

The following data structures are used to implement the HLSG Extractor.

2.1 Bead Feature

A bead feature is an abstraction of the physical bead coated with endothelial cells in the assay. The geometry of the bead is intuitively circular; subsequently the geometry can is described by the descriptor in Figure 1.

Bead
center: (int, int)
radius: int
sprouts: [Sprout]

Figure 1: Bead Descriptors

Sprout
bead: Bead
centroid: (int, int)
length: int
width: int
segments: [RadialSegment]

Figure 2: Sprout Descriptors

2.2 Sprout Feature

A sprout feature is an abstraction of the blood vessels that develop through angiogenesis from the designed bead. Subsequently, sprout feature extraction is dependent upon bead descriptors. The sprout is actually comprised of a set of pixel segments because of the possibility that a sprout is disconnected.

2.3 Radial Line Segment

Due to the disconnectivity of sprouts, individual sprout segments are represented by a radially defined line segment. That is, we distinguish the end points from its radial distance from the origin of its corresponding bead. Given a line segment, we say that and end point is the *inner point* if it is radially closer than its complementary end point; otherwise, we call the end point the *outer point*.

In addition to the distinguishable end points, a radial line segment is a line fit onto a corresponding blob of pixels which can be considered a sprout segment.

2.4 Driver

The Driver is responsible for parsing input from the client and emulating the encoded actions as functions of the HLSG Extractor. That is, the Driver acts similar to a REPL (Read-Eval-Print-Loop) that reads input from the client, evaluates the input and prints the corresponding output in a loop. The set of commands available to the client is out-

lined Table 1.

3. SYSTEM ARCHITECTURE

The system requires Python version 2.7x with the SimpleCV package. The architecture of the system is based on our methodology for quantitatively analyzing in vitro angiogenesis. The system, however, incorporates modules for driving batch processes as well as a Read-Eval-Print-Loop (REPL) for console interaction. Finally, a module incorporated for generating CSV reports of the analysis. The sequence diagram for the system components are shown in Figure 3.

The REPL module controls the interaction between the user and the system. Commands available in the REPL are shown in Table 1.

Command	Output	Description
extract [file]	HLSG of file	Extracts the HLSG of the given file.
extract [files]	HLSG of files	Extacts the HLSGs of the given files.
exit	Goodbye	Exits the system.

Table 1: Commands

4. METHODOLOGY

Our system enables feature set detection, minor feature restoration and quantitative analysis which can be decomposed into four stages. The first two stages detect feature sets: beads as features and then sprouts as features. In the third stage, the system attempts to restore a few of the sprouts by approximating connections between broken sprout segments. Finally, the system quantitatively analyzes the imaged assays through Sholl Analysis.

4.1 Bead Extraction

Bead extraction is a two-step process. To reduce noise, the system first smooths the image using a Gaussian blur. Second, circles in the image are detected using the circular Hough Transform. The circles detected correspond to the beads in the assay. Subsequently, the origin and radius of the bead is obtained.

4.2 Sprout Extraction

Sprout extraction depends on bead extraction because the beads must be masked before sprout extraction occurs to separate beads from sprouts. We mask the beads given the geometry of the circles. Due to the disconnectivity of sprouts in the assay, we begin by obtaining all sprout segments and represent them as line segments. We distinguish the end points of each line segment for connectivity. The start point, S, is the end point on the line segment such that it is closer to the origin of the line segment's closest bead. The end point, E, is the end point on the line segment such that it is farther from the origin of the line segment's closest bead.

To determine which line segments belong to the same sprout, we use euclidean distance between their start and end points enforced by the constraint that the end point must by closer to the origin of its closest's bead than the target start point. For example, two line segments are part of the same sprout if the distance between the start and end points are within a specified distance parameter, d.

4.3 Sprout Restoration

The process of restoration is dependent on the successful extraction of HLSGs features from the imaged angiogenesis. Restoration is necessary due to the disjoint sprout segments that appear. Disjoint sprout segments can be caused by the an out-of-focus microscope.

4.4 Sholl Analysis

Sholl Analysis is a quantitative method for quantitatively analyzing morphological characteristics of neurons. [1]

5. RESULTS

Display a comparison table with human counts.

6. REFERENCES

[1] D. A. Sholl. Dendritic organization in the neurons of the visual and motor cortices of the cat. *J Anat.*, 87(4):387–406, 1953.

APPENDIX

A. PSEUDO CODE

This section outlines the pseudo-code for the Driver and HLSGExtractor operations.

A.1 Driver

The following pseudo-code outlines the Driver which reads input from the client, evaluates the input as a command, prints the output as a consequence of executing the command and then repeats this sequence of operations.

Algorithm 1 Driver

```
procedure DRIVER
    running ← True
    while running do
        input ← read_input()
        command ← parse(input)
        output ← HLSGExtractor.execute(command)
        print(output)
    end while
end procedure
```

Note that the driver executes while the running flag is true. Consequently, the REPL is responsible for setting this flag false.

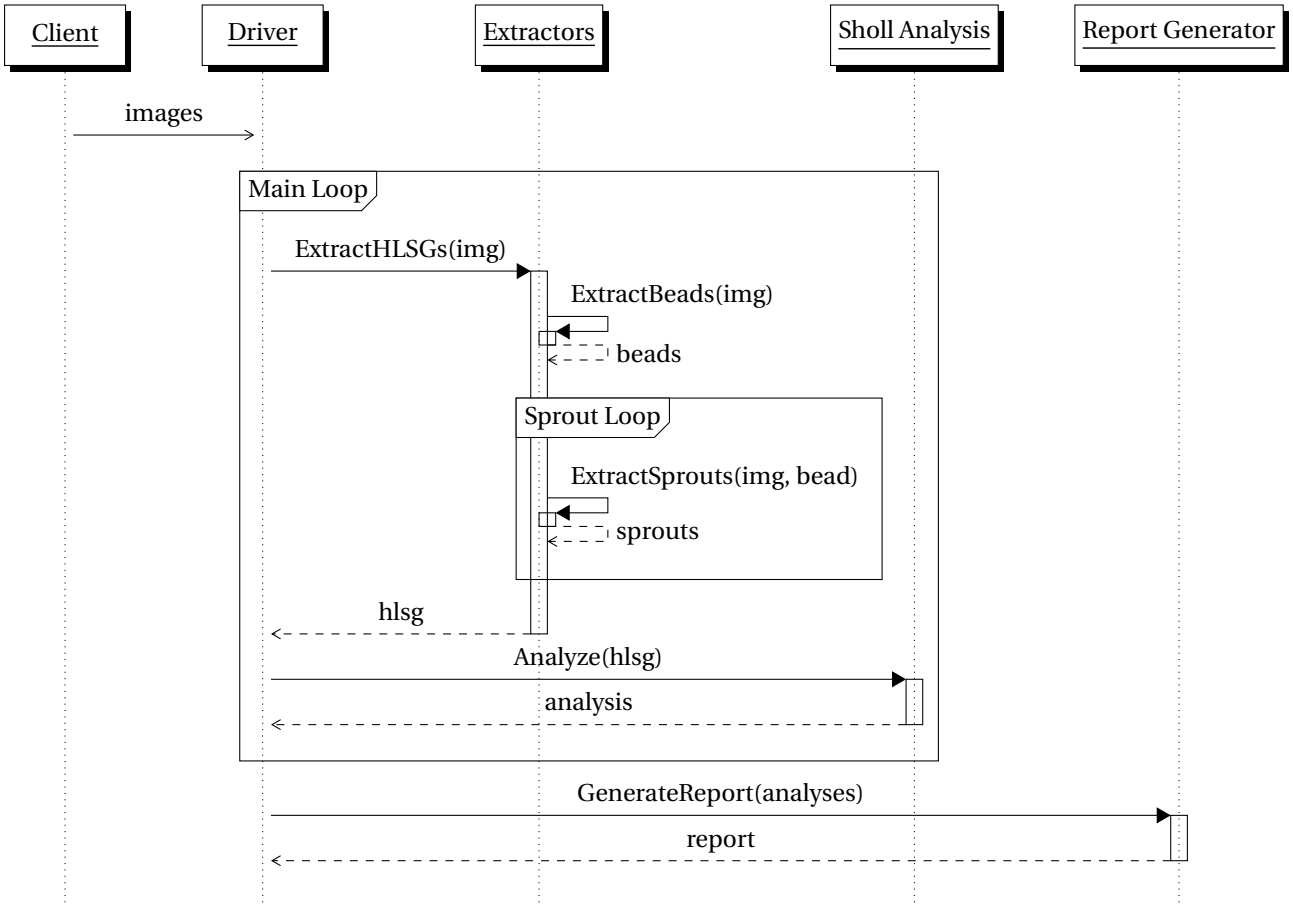

Figure 3: High-Level Architecture

A.1.1 Sprout Extractor

Given an imaged assay and a set of bead features, the algorithm proceeds by masking the beads from the image. Next, a segmentation strategy is used to separate individual sprouts from the collection of globally detected sprouts. Finally, the segmentation strategy yields the detected feature set of sprouts.

Algorithm 2 Sprout Extraction

```
procedure EXTRACTSPROUTS(img, beads)
    maskedImg ← maskBeads(img, beads)
    strategy ← SegmentStrategy(maskedImg,beads)
    sprouts ← strategy.segment()
    return sprouts
end procedure
```

Algorithm 3 HLSG Extraction

```
procedure EXTRACTHLSGS(img)
    beads ← ExtractBeads(img)
    sprouts ← ExtractSprouts(img, beads)
    hlsgs ← MapSproutsToBeads(sprouts, beads)
    return hlsgs
end procedure
```

A.1.2 HLSG Extractor