High-Level Sprout Geometry Extraction and Analysis of In Vitro Angiogenesis

Gio Borje and Craig Steinke

UC Irvine

July 23, 2013

Overview

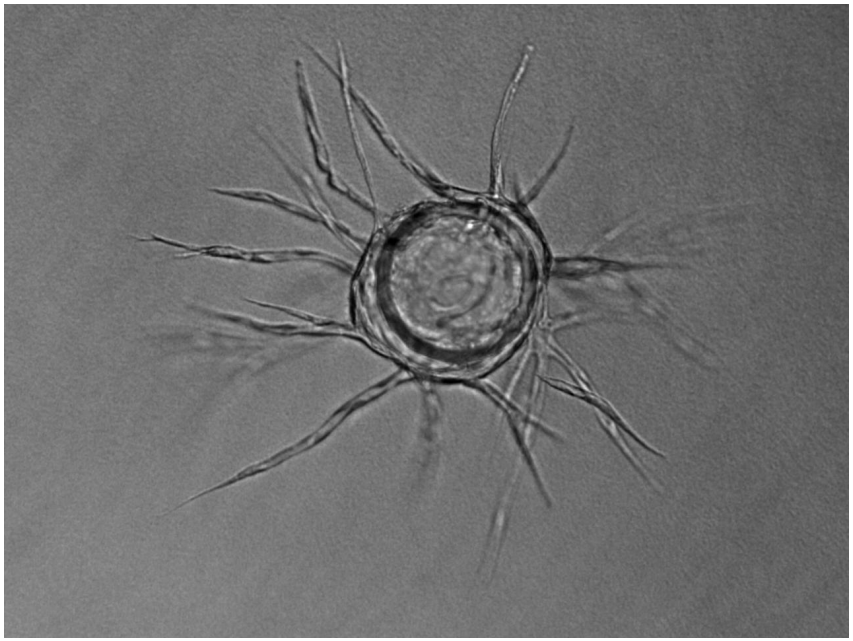


Figure: Before

Spreadsheet Report
Sprout Counts
⋮
Branching Factor

Figure: After

Outline

1. Motivation
2. Fibrin Gel Bead Sprouting Assay (FGBSA)
3. Methodology
4. Results

Motivation

Solid tumors have an avascular (no nearby blood vessels) growth phase that allows for an approximate maximum size of 1-2mm in diameter[1].

[1] Robert S. Kerbel. Tumor angiogenesis: past, present and the near future. *Carcinogenesis*, 21(3):505515, 1999.

Motivation

Solid tumors have an avascular (no nearby blood vessels) growth phase that allows for an approximate maximum size of 1-2mm in diameter[1].

Think of the size of *very coarse sand*.

[1] Robert S. Kerbel. Tumor angiogenesis: past, present and the near future. *Carcinogenesis*, 21(3):505515, 1999.

Motivation

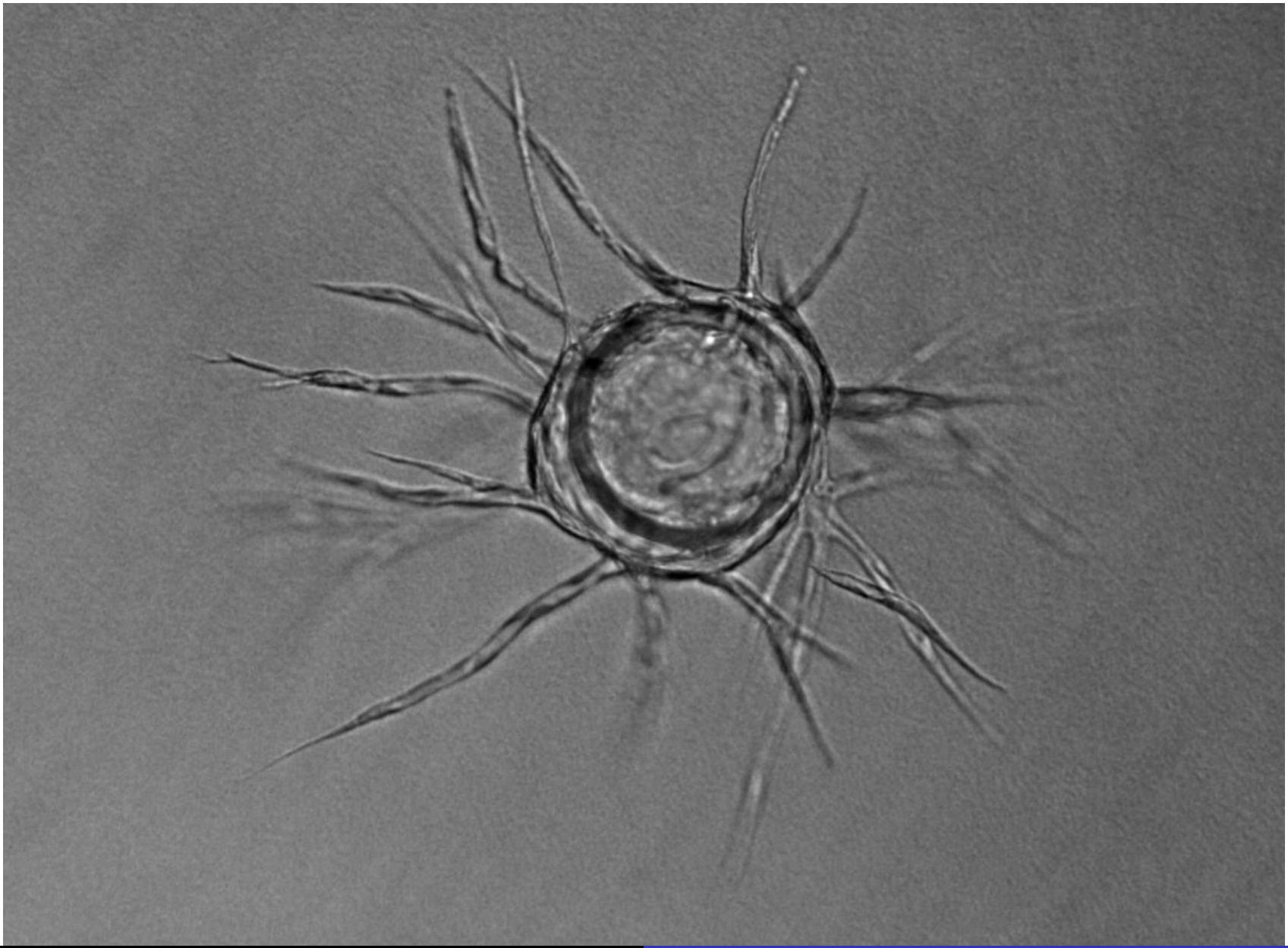
Solid tumors have an avascular (no nearby blood vessels) growth phase that allows for an approximate maximum size of 1-2mm in diameter[1].

Think of the size of *very coarse sand*.

Tumor angiogesesis enables relentless tumor growth and metastasis.

[1] Robert S. Kerbel. Tumor angiogenesis: past, present and the near future. *Carcinogenesis*, 21(3):505515, 1999.

FGBSA Image



Current Methodology

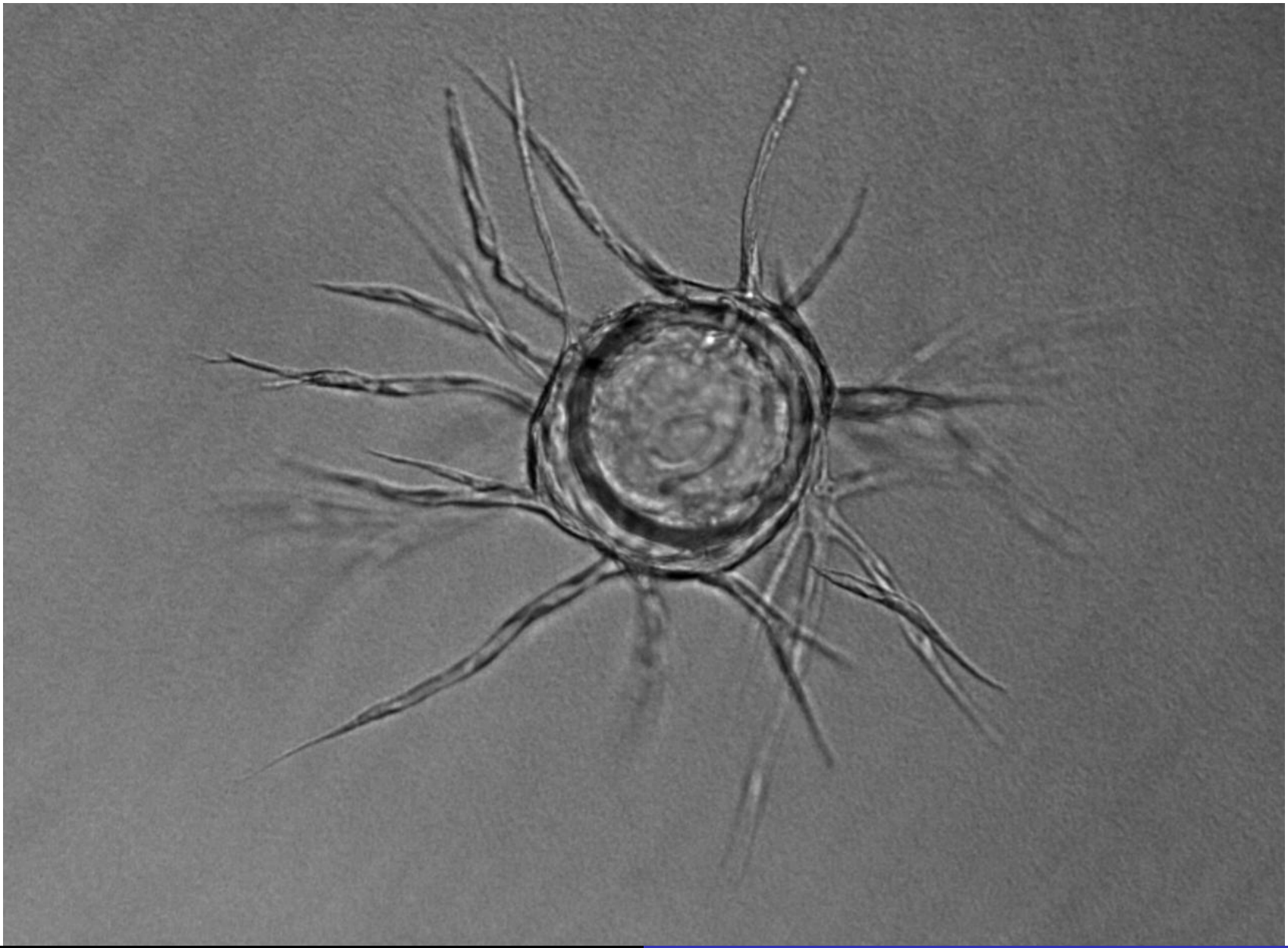
1. Sprout Restoration
2. Sholl Analysis

Current Methodology

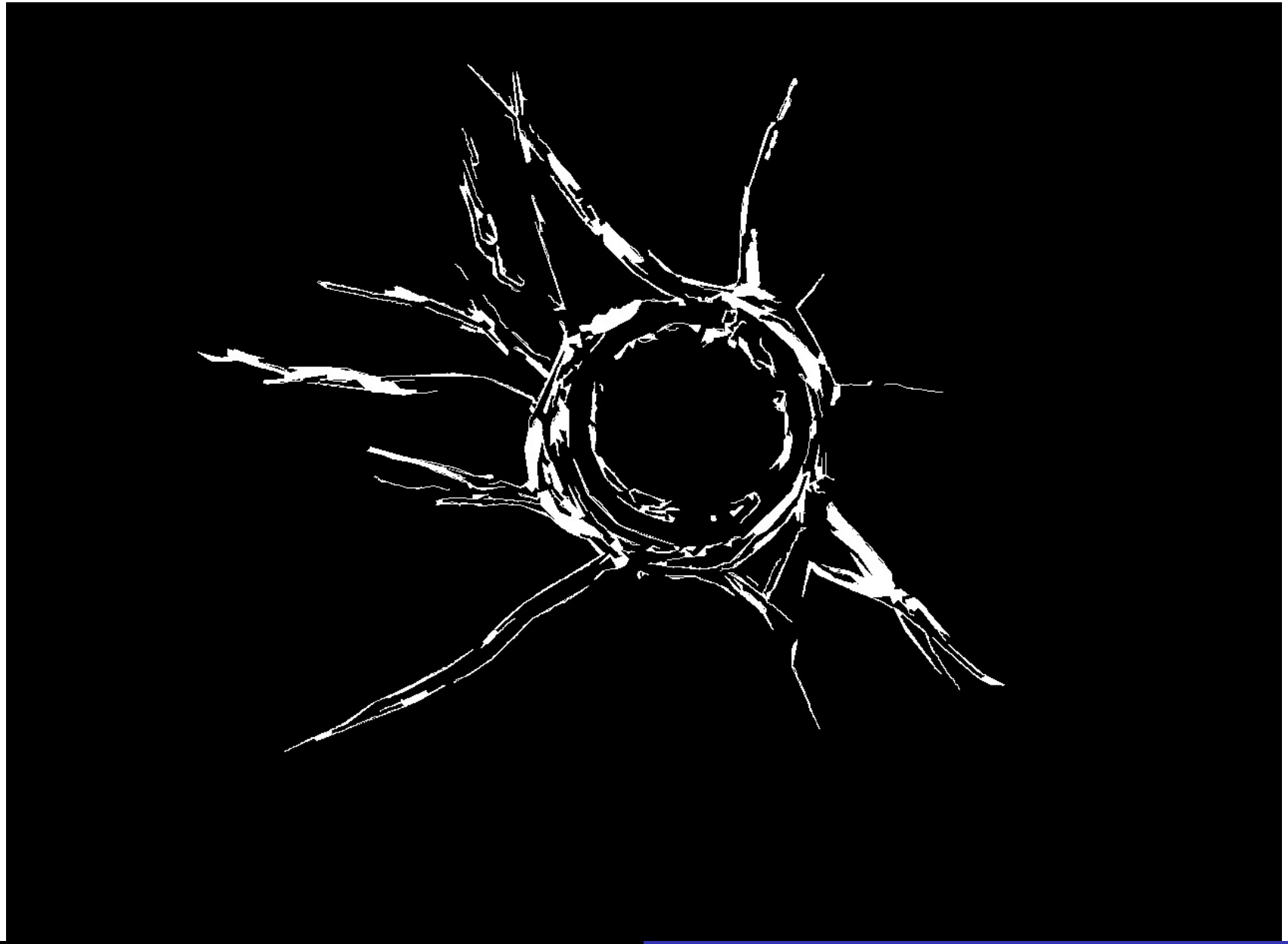
1. Edge Detection and Polygon Approximation
2. Bead Detection (Hough Transform)
3. Non-Sprout Detection
4. Dilation for Approximate Centerline
5. Thinning and Pruning
6. **Sholl Analysis**

Expanded sprout restoration methods

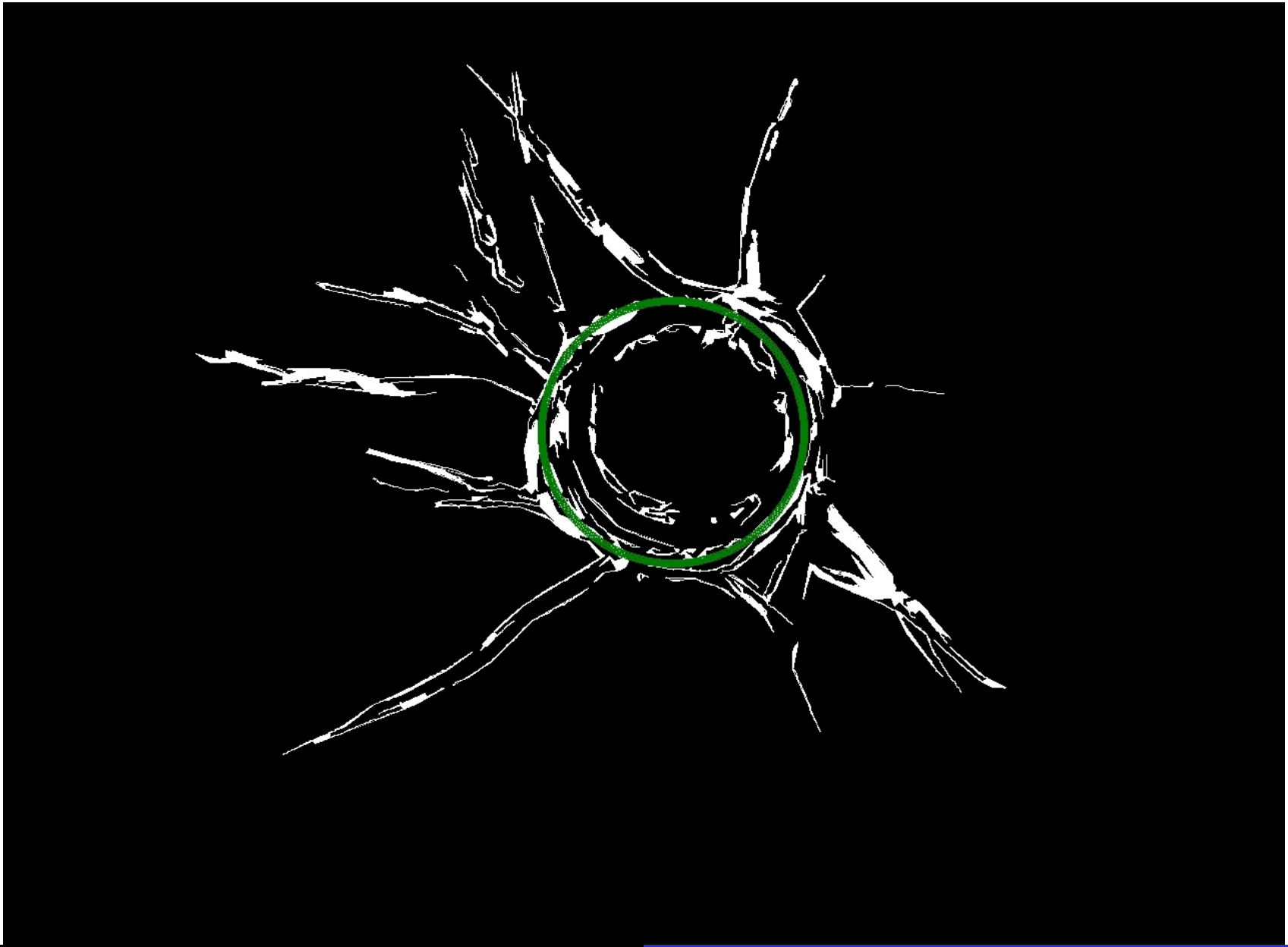
Original Image



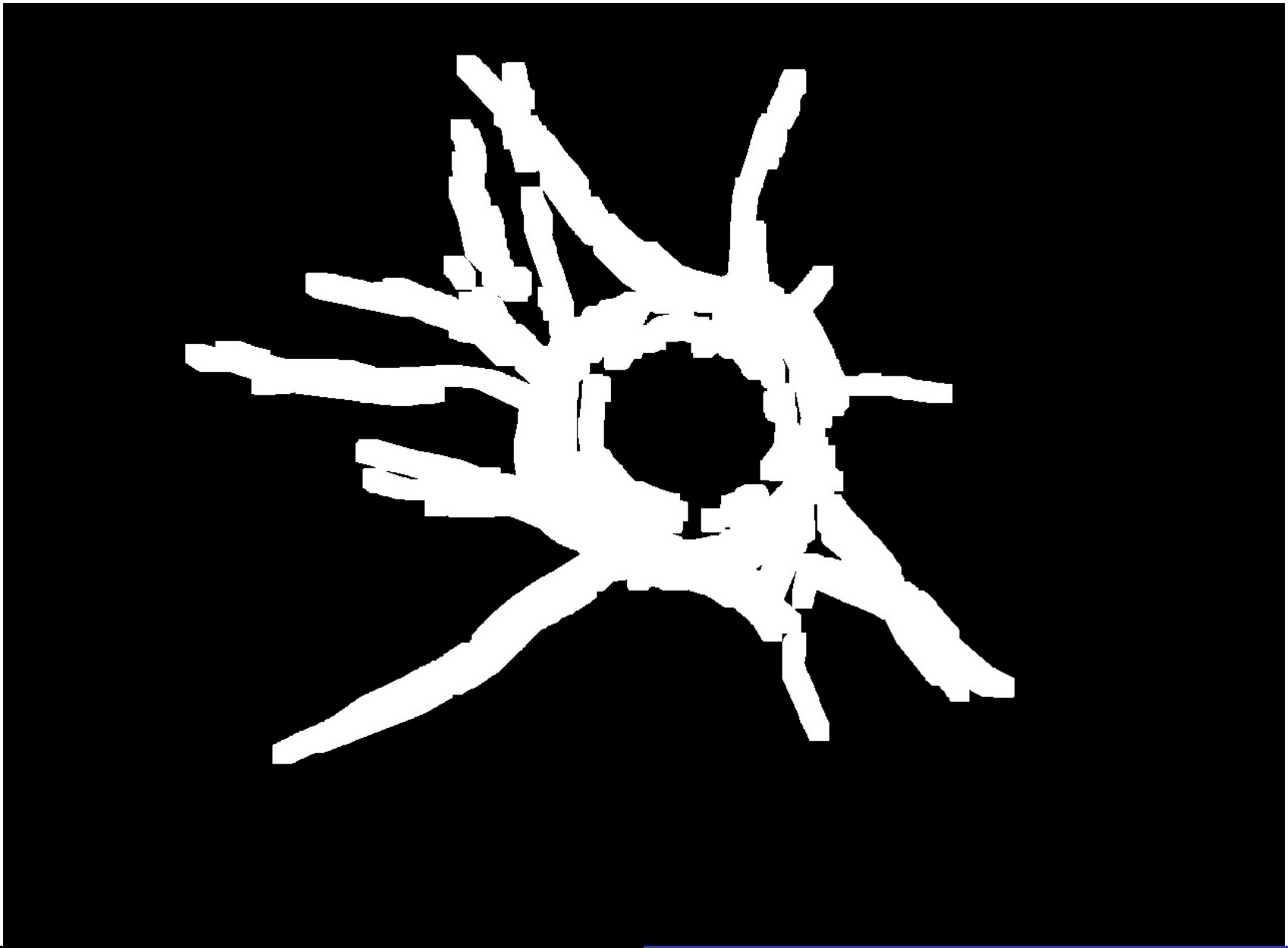
Edge Detection and Polygon Approximation



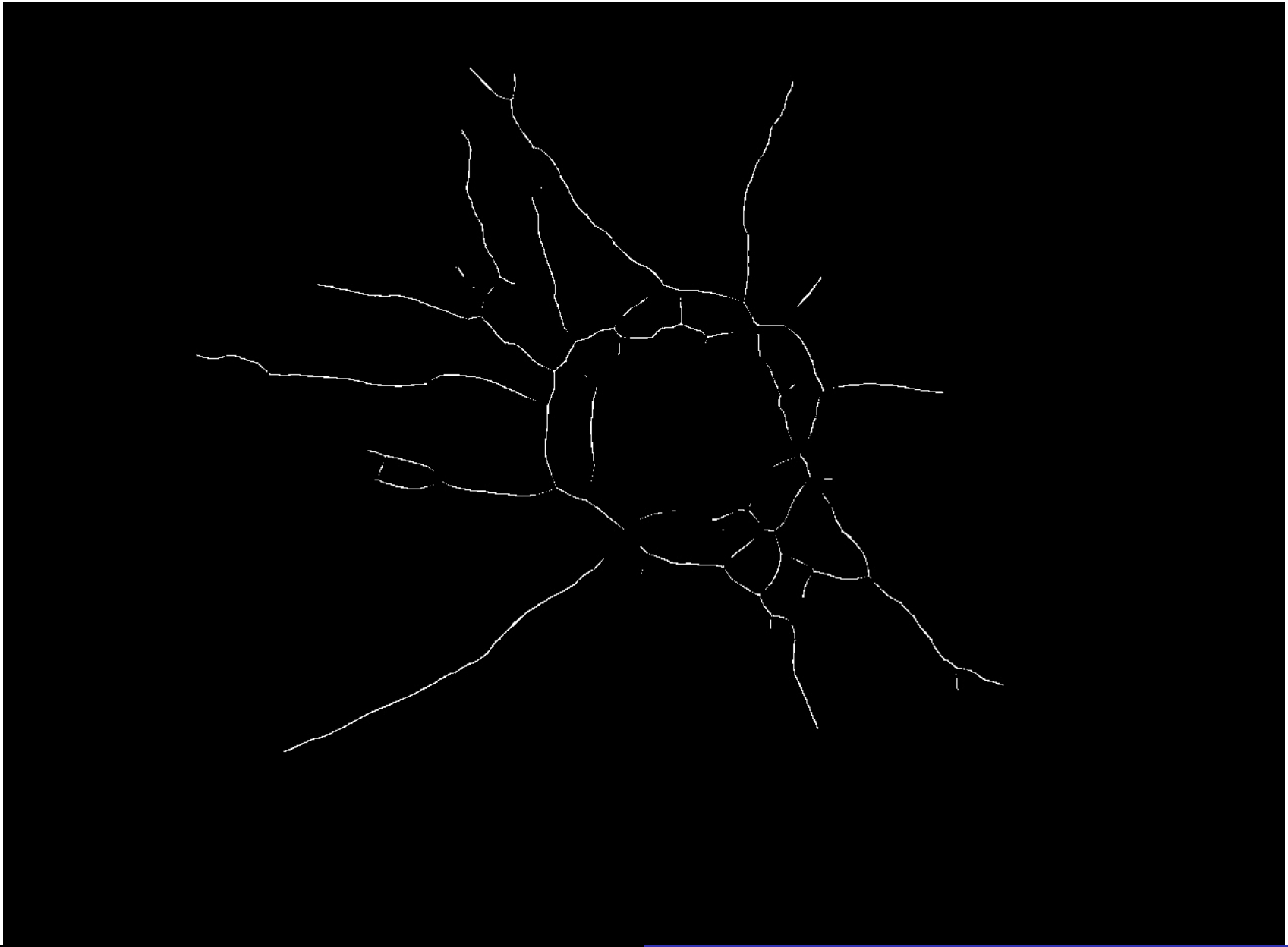
Bead Detection (Hough Transform)



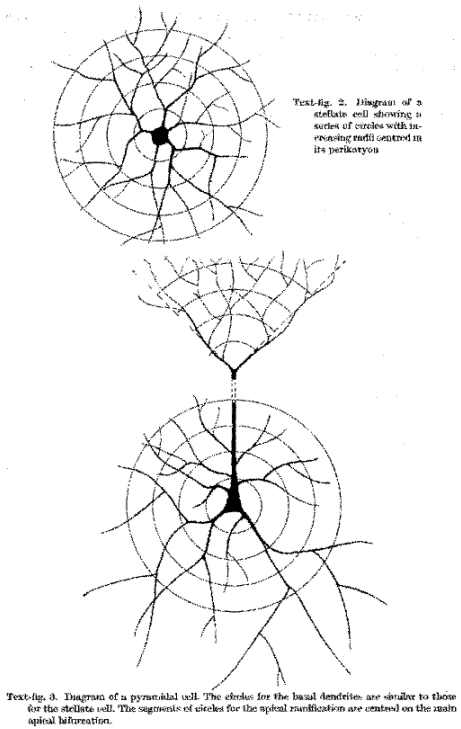
Dilation for Approximate Centerline



Thinning and Pruning



Sholl Analysis: Why

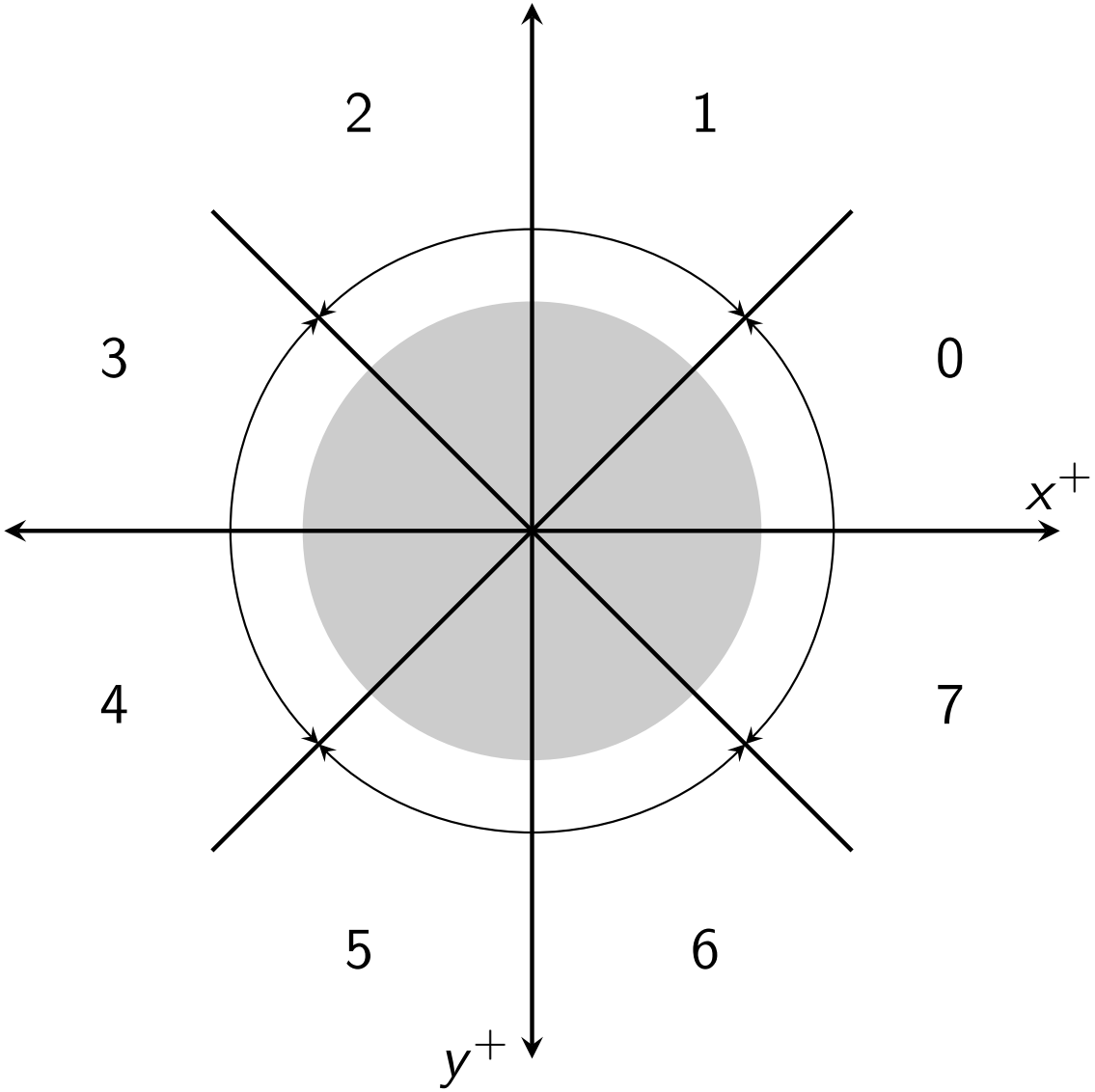


Text-fig. 3. Diagram of a pyramidal cell. The circles for the basal dendrites are smaller to those for the stellate cell. The segments of circles for the apical ramification are centred on the main apical bifurcation.

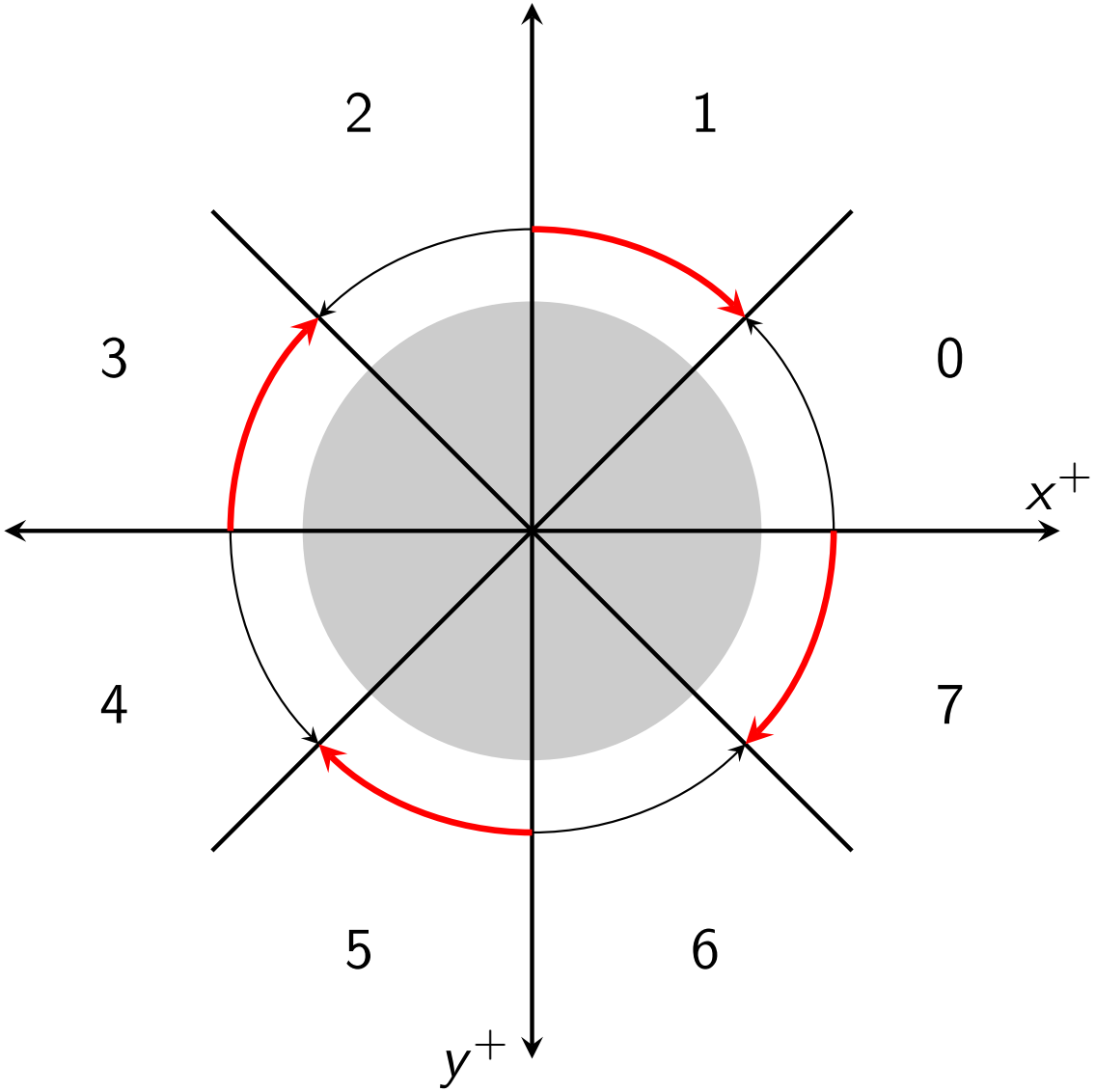
- No tracing required
- Morphometric descriptors can be obtained

Figure: D. A. Sholl. Dendritic organization in the neurons of the visual and motor cortices of the cat. *J Anat.*, 87(4):387406, 1953.

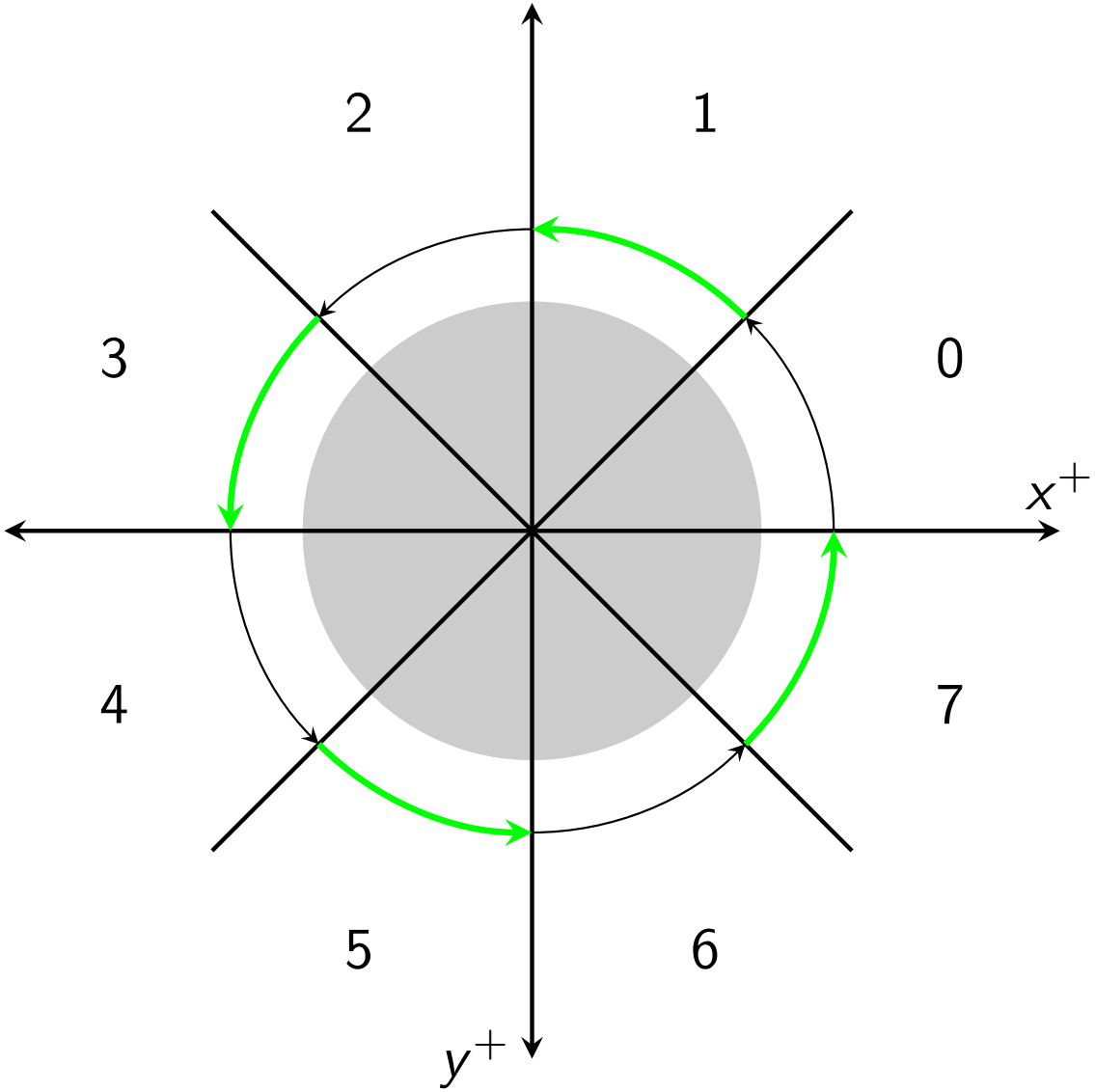
Sholl Analysis: Implementation



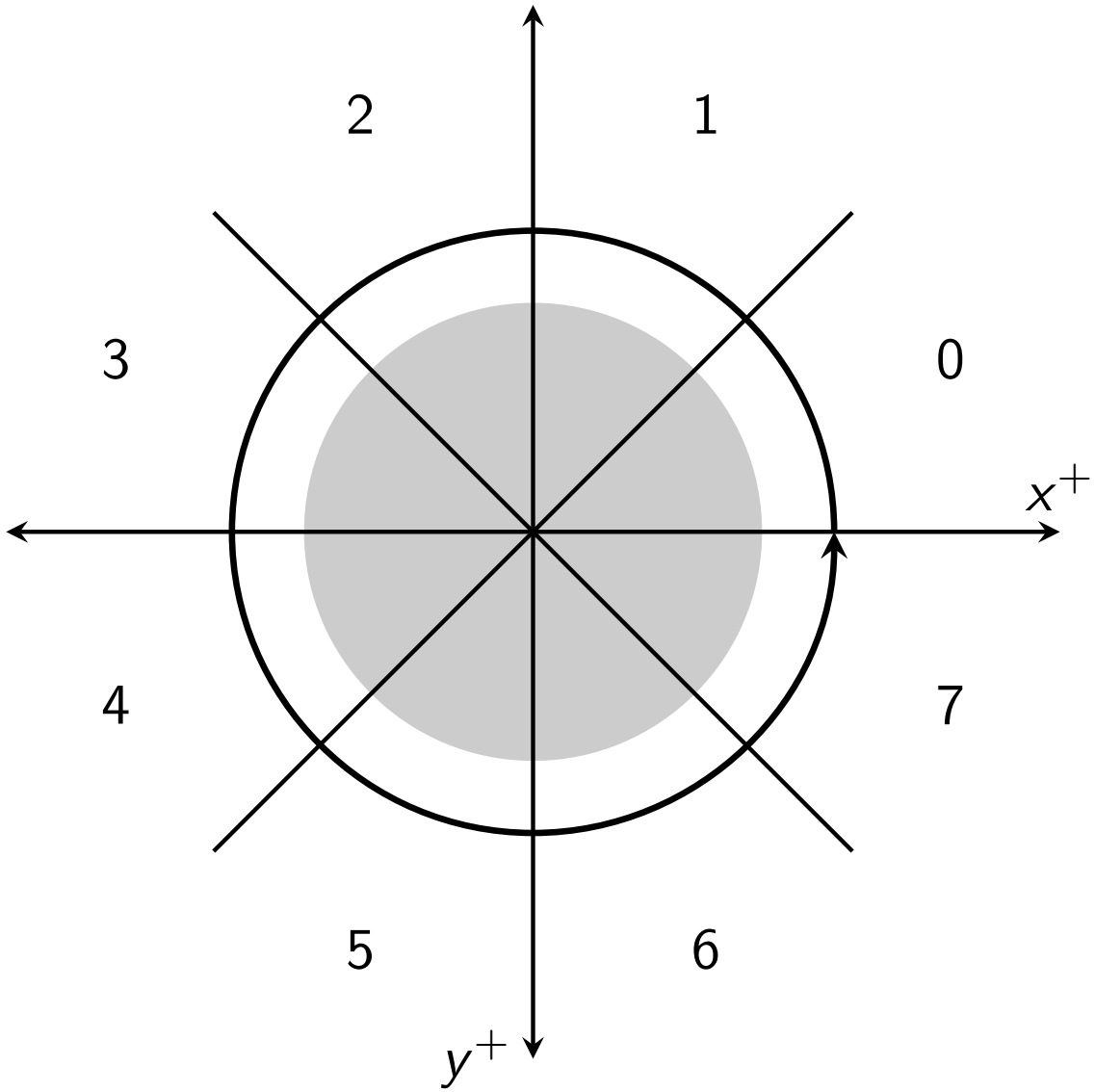
Sholl Analysis: Bresenham Circle Algorithm



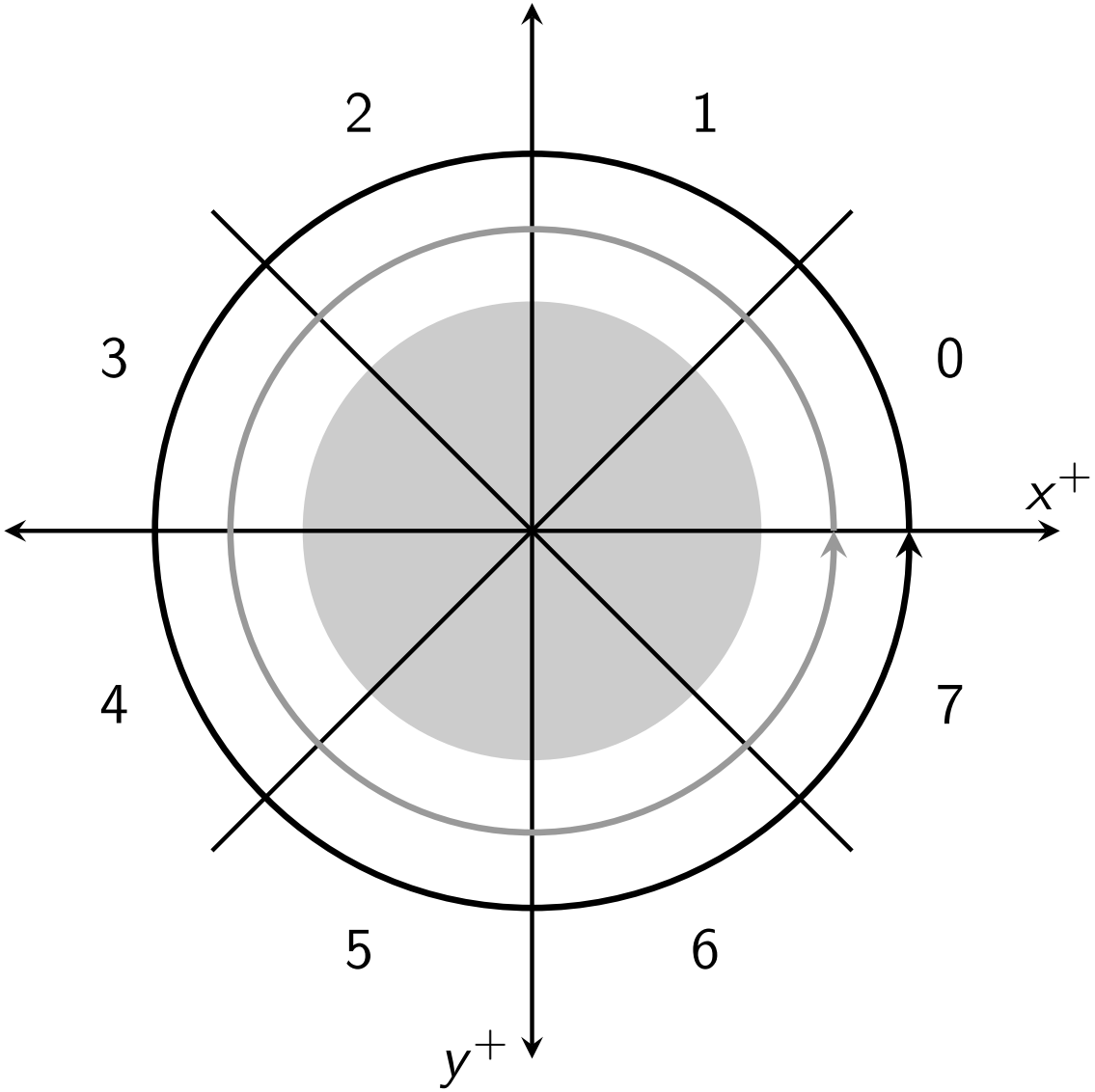
Sholl Analysis: Unordered Property of Circle Algorithm



Sholl Analysis: Ordered Bresenham Circle Algorithm



Sholl Analysis: Concentric Circles Analysis

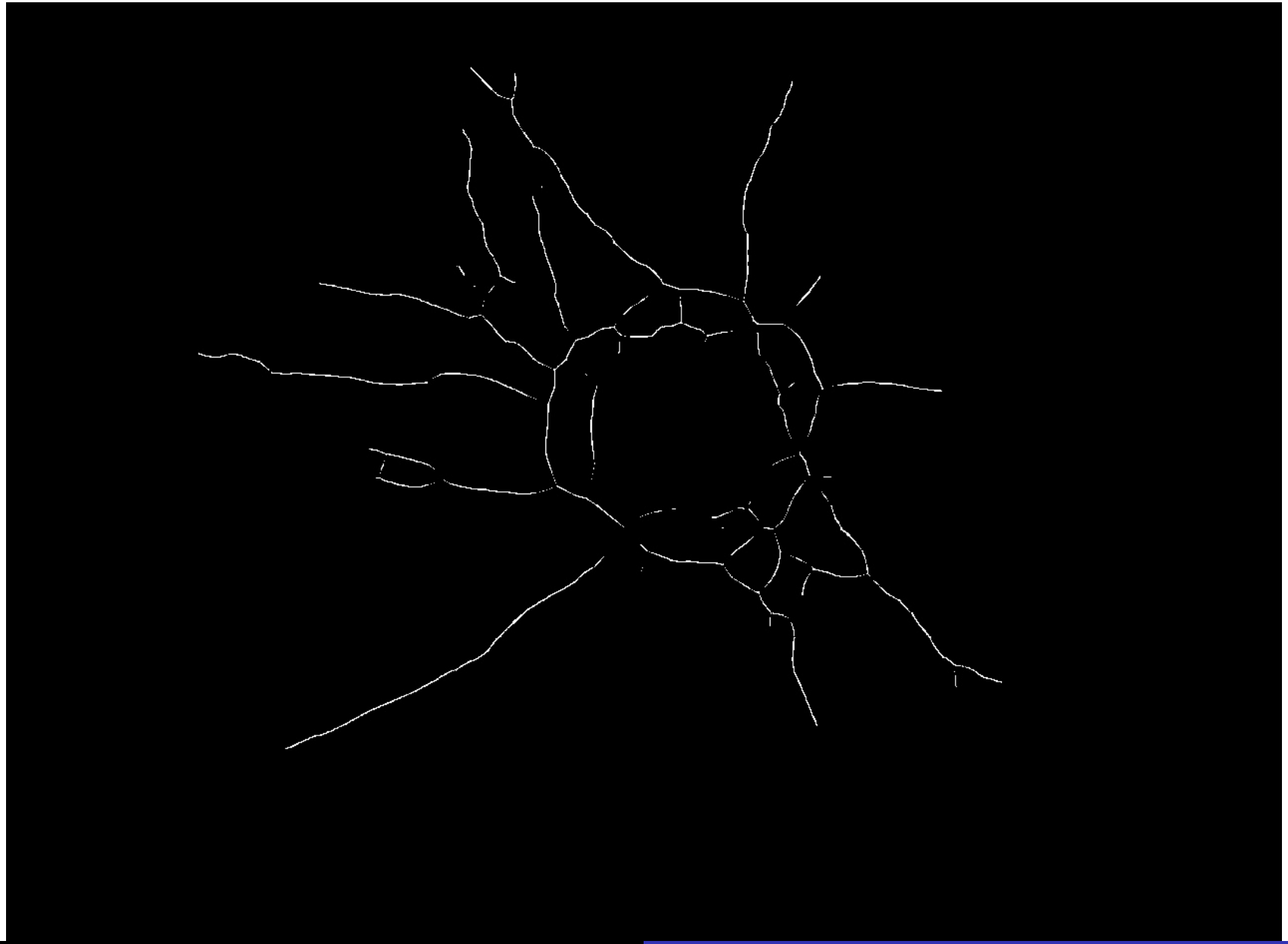


Sholl Analysis: Descriptors

Important descriptors

1. Sprout enumeration
2. Average sprout length
3. Branching factor (Shoenen Ramification Index)
4. Critical Value

Skeleton Reference



Sholl Analysis: Sprout Enumeration

Problem 1 Non-continuous sprouts

Problem 2 Bad initial radius

Solution Bounded crossings integration

- Mean of n crossings
- Median of n crossings

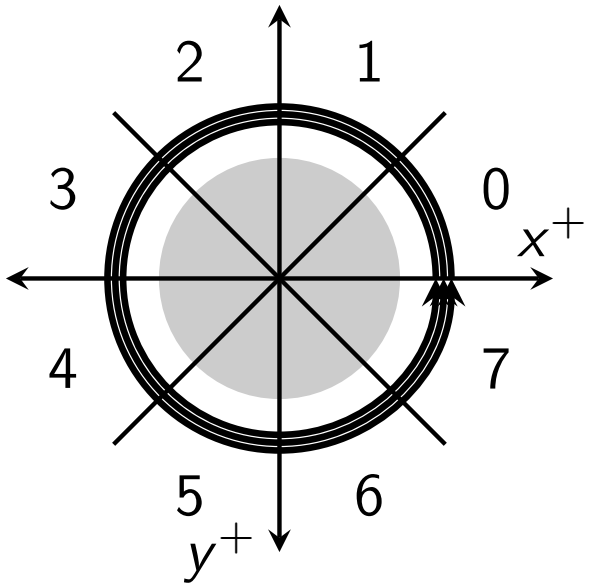


Figure: Crossing integration over intervals of size 3

Results

Resulting sprout counts are compared with an expert observer using 15 images.

$$\text{RMSE} = \sqrt{\frac{1}{n}\sum_{i \leftarrow 1}^{n}(\hat{\mathbf{Y}}_i - \mathbf{Y}_i)^2} \tag{1}$$

Method Variant	RMSE
Median Integration Method	1.736
Ignoring isolated points	1.79
Mean Integration Method Benchmark	1.93
LIS on Large Image	3.45

Table: Results of Method Variants

Further Work

1. Gather results for median integration method
2. Distinguish individual sprouts
3. Sprout tracing similar to neuron tracing; see Meijering E. Neuron tracing in perspective. *Cytometry A* 2010;77A: 693704.
4. Three-dimensional reconstruction using multiple image depths

Thank you. Questions?