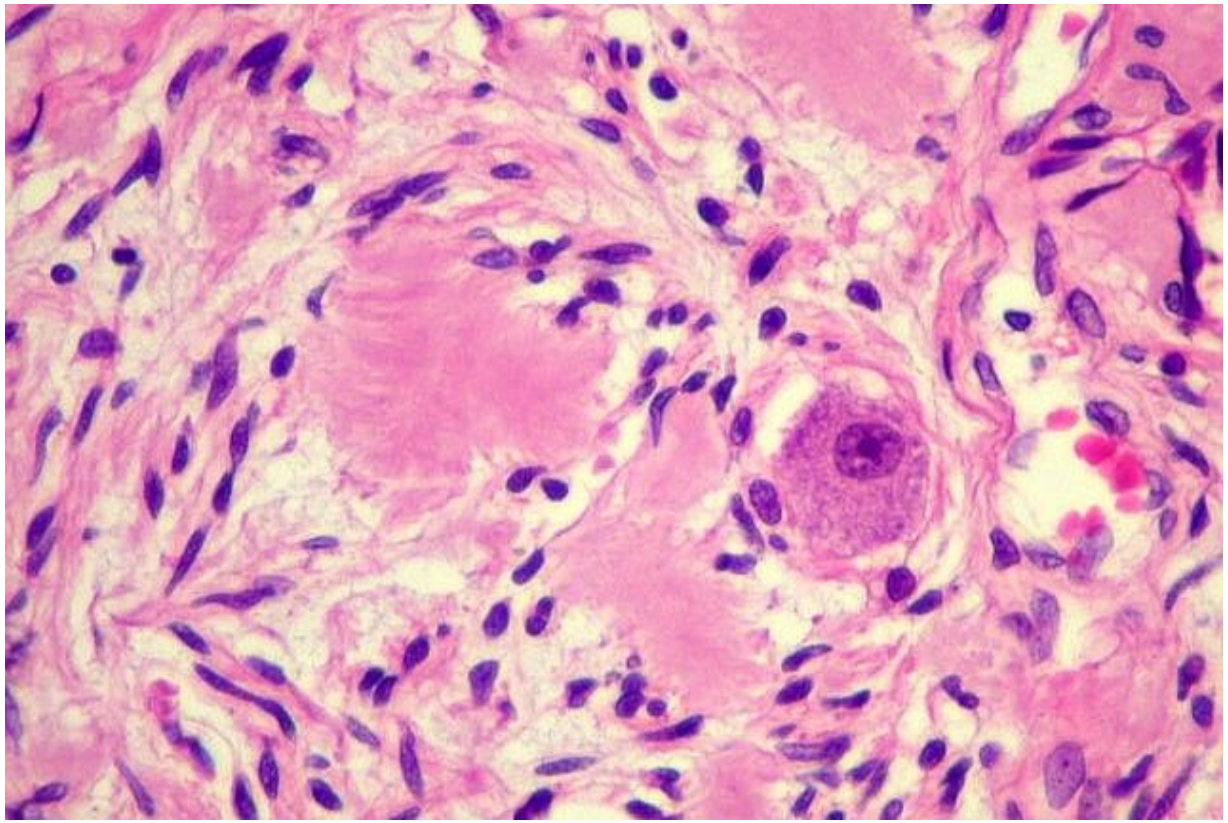
Segmentação e estimação de células em imagens teciduais com coloração por H&E

Bruno Mingoti (20204165)
Ramiro Gurgel (20100428)

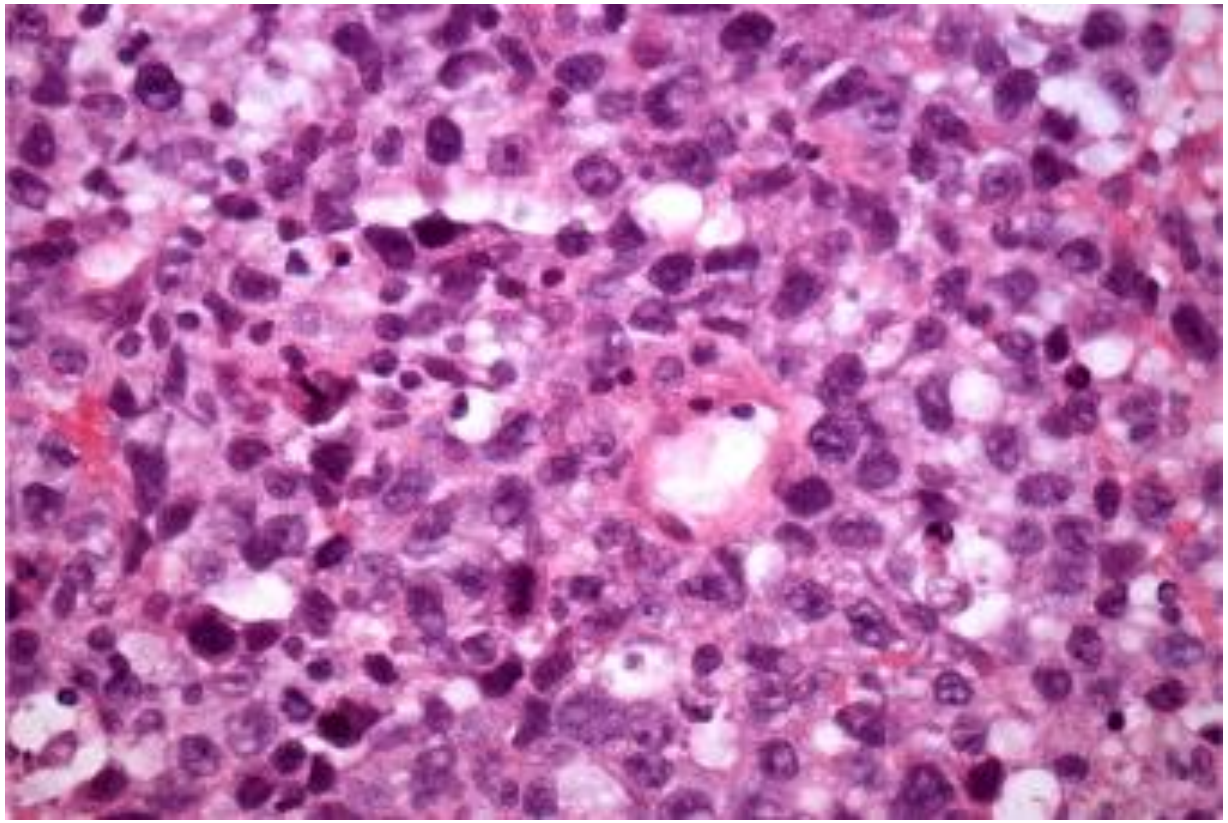
EEL7825 – Projeto Nível I em Controle e Processamento de Sinais I

EEL / CTC / UFSC

Introdução



(coloração por hematoxilina-eosina do nervo simpático)



(coloração por hematoxilina-eosina de linfócitos)

Motivação

"Para a contagem de reticulócitos, a técnica manual é tida, desde a década de 1940, como método padrão ouro"

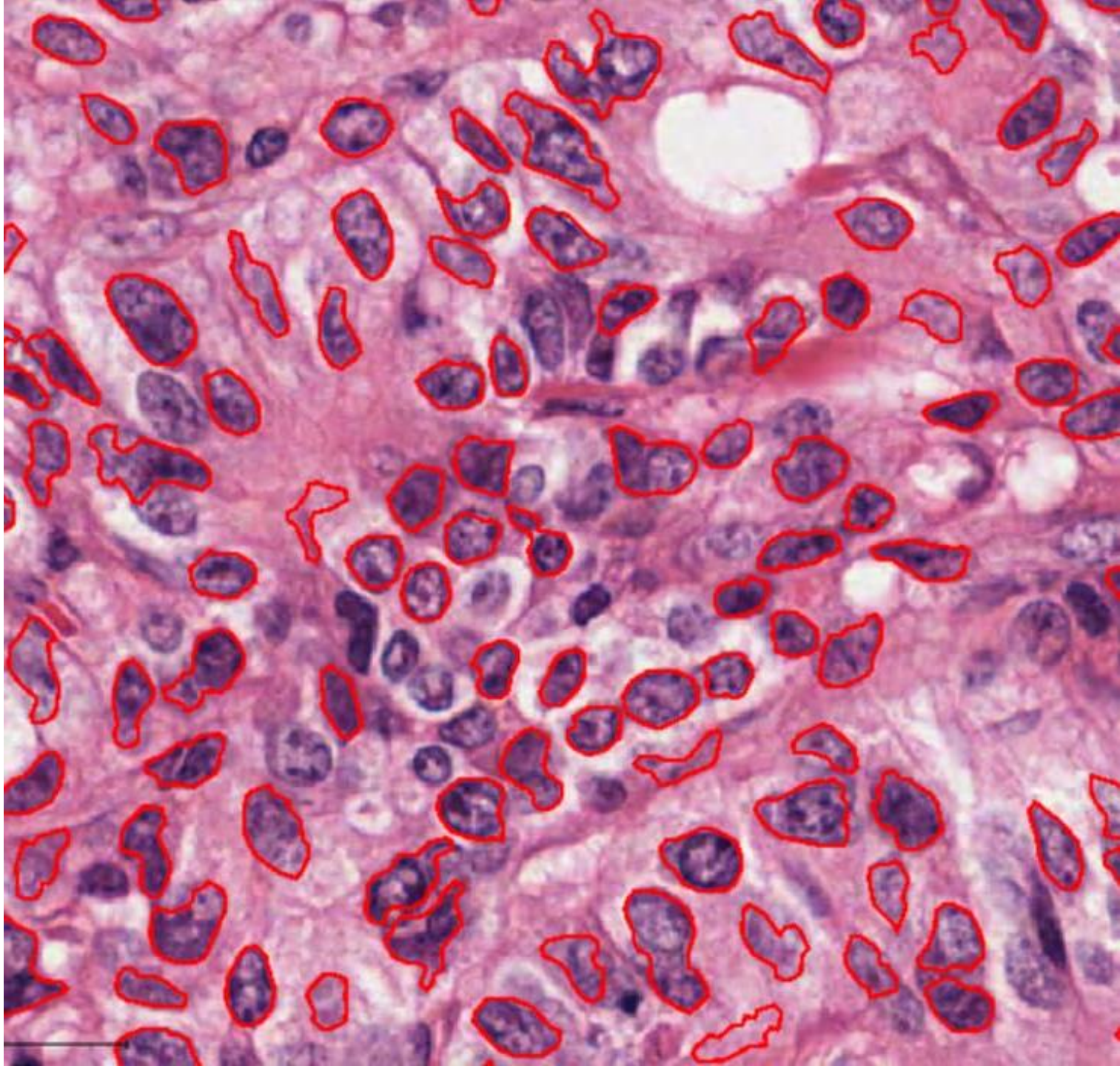
Miyake, Cecilia Emica Tanaka. Reticulócitos: da contagem manual à citometria de fluxo. 2011. Disponível em: https://acervodigital.ufpr.br/handle/1884/32922.

"Em relação ao método utilizado para contagem de reticulócitos, observou-se no presente estudo que todos os laboratórios executam a técnica manual,"

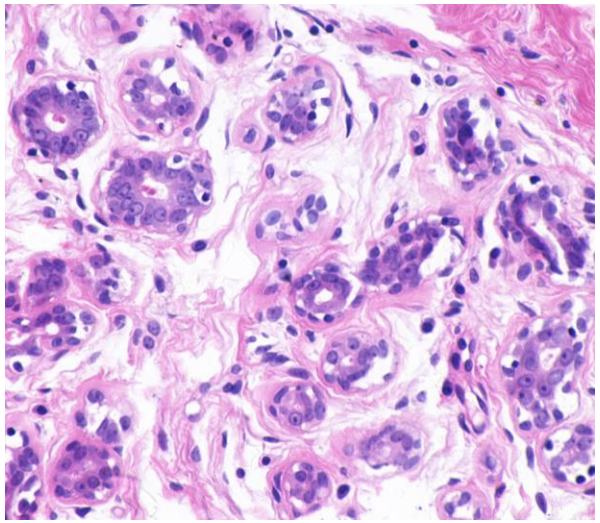
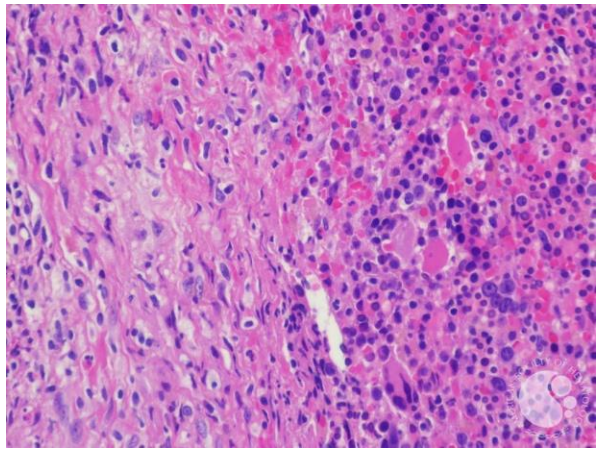
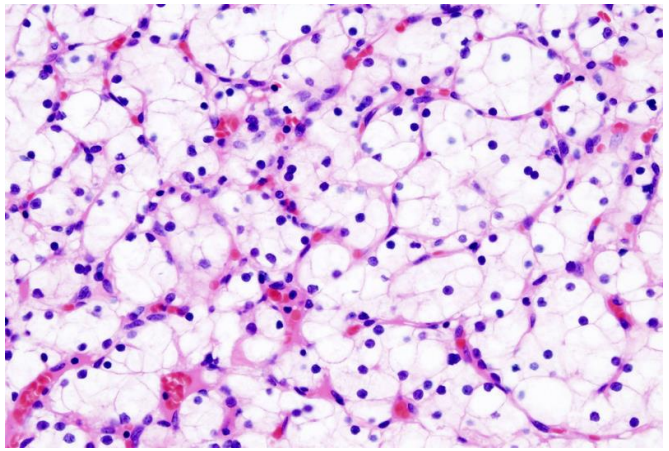
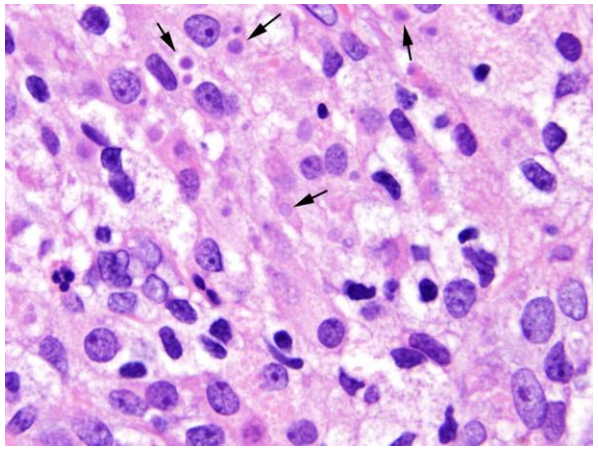
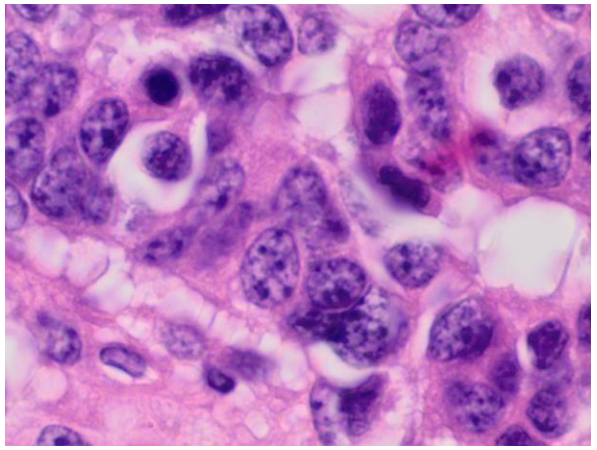
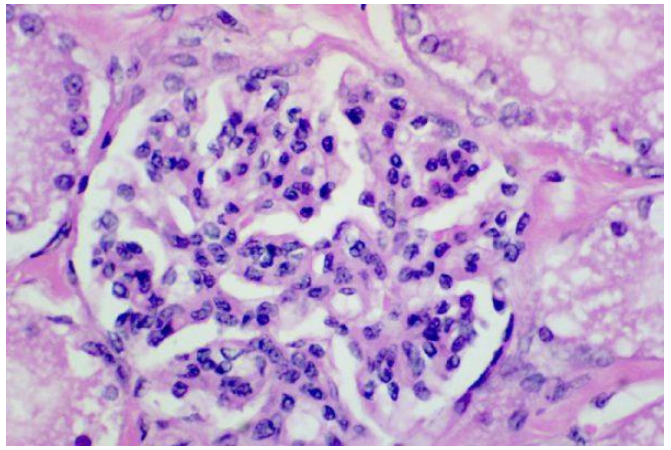
Gois, J. C. de; Sutana, V. L.; Figueiredo, R. C. de; Rios, D. R. A. Contagem de Reticulócitos na Prática Clínica: um Exame Pouco Utilizado. Revista Médica de Minas Gerais, [S.l.], v. 29, 2019. Disponível em: https://rmmg.org/artigo/detalhes/2487

Objetivos e Ferramentas utilizadas

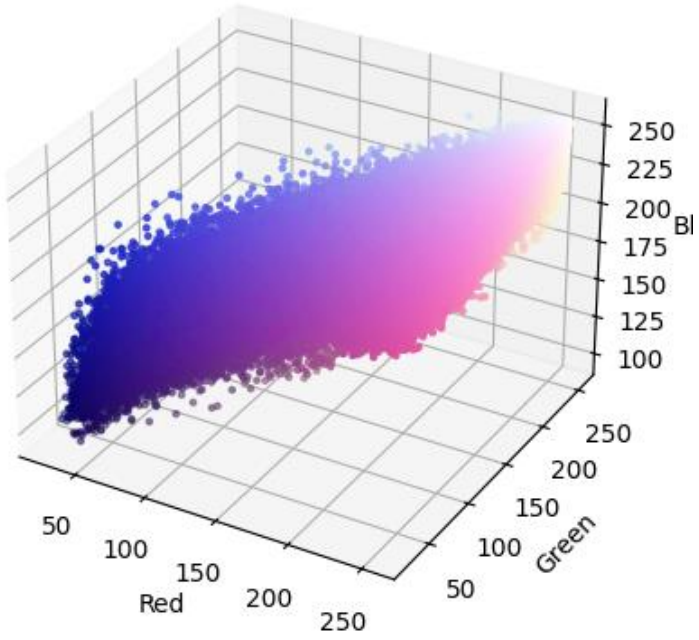
- **Jupyter Notebook**
- **OpenCV**
- **Scikit Image**
- **Flask**



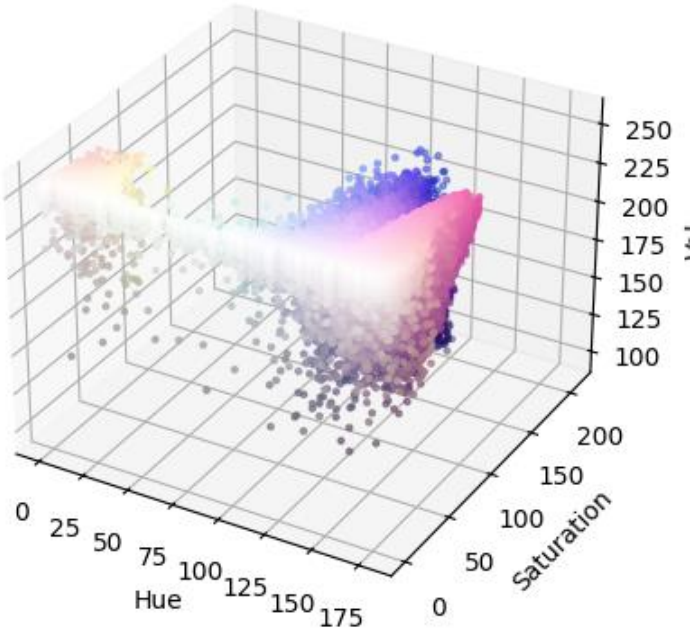
Avaliação das imagens H&E Stain

1º Método: HSV + Watershed

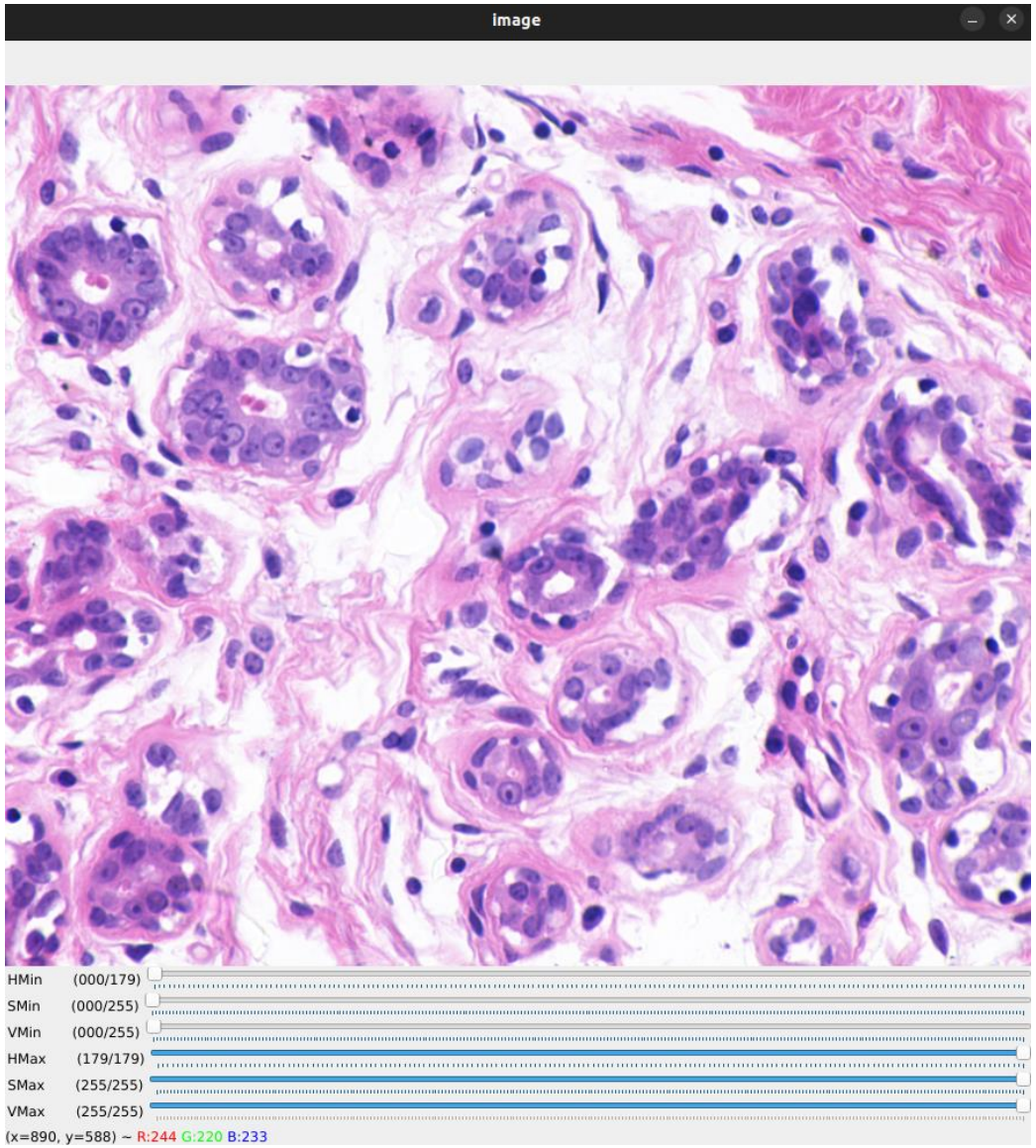


RGB

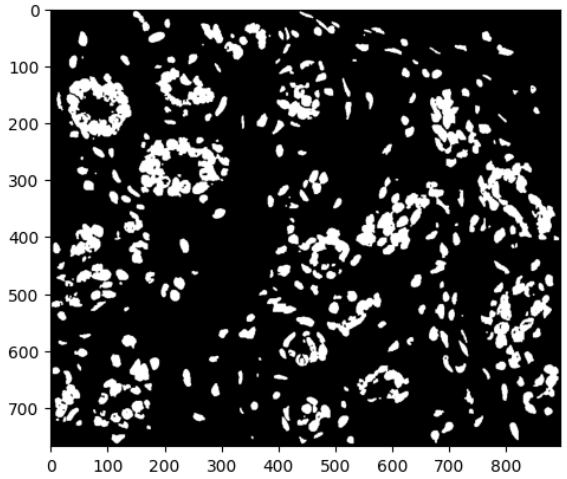


HSV

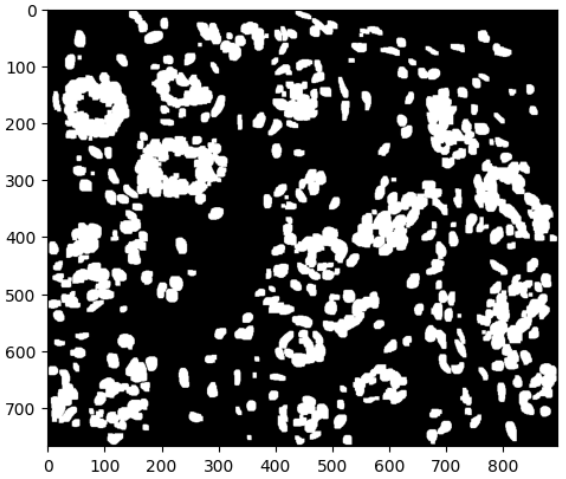
1º Método: HSV + Watershed



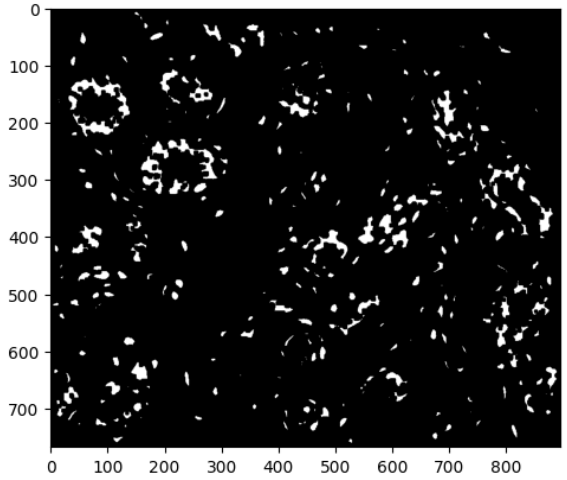
1º Método: HSV + Watershed



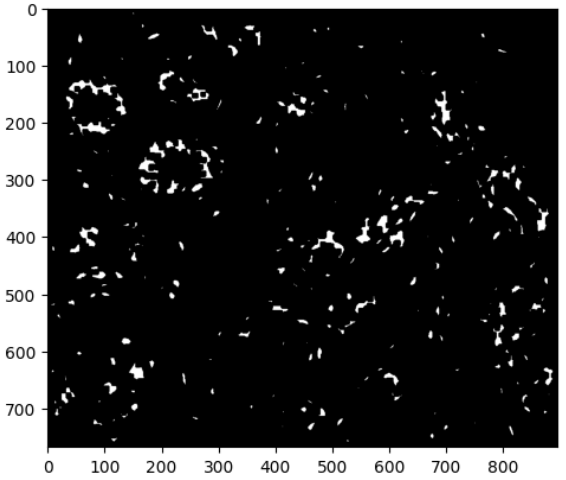
Threshold



Dilate

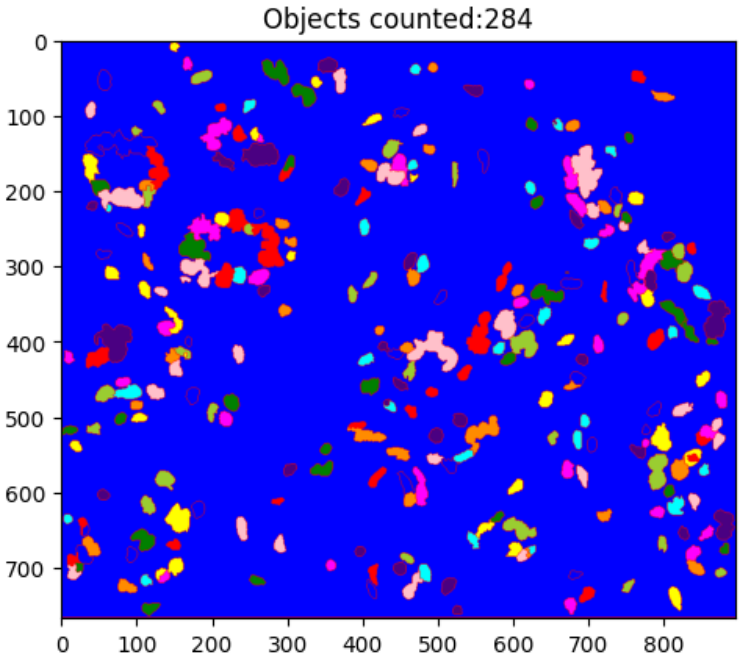


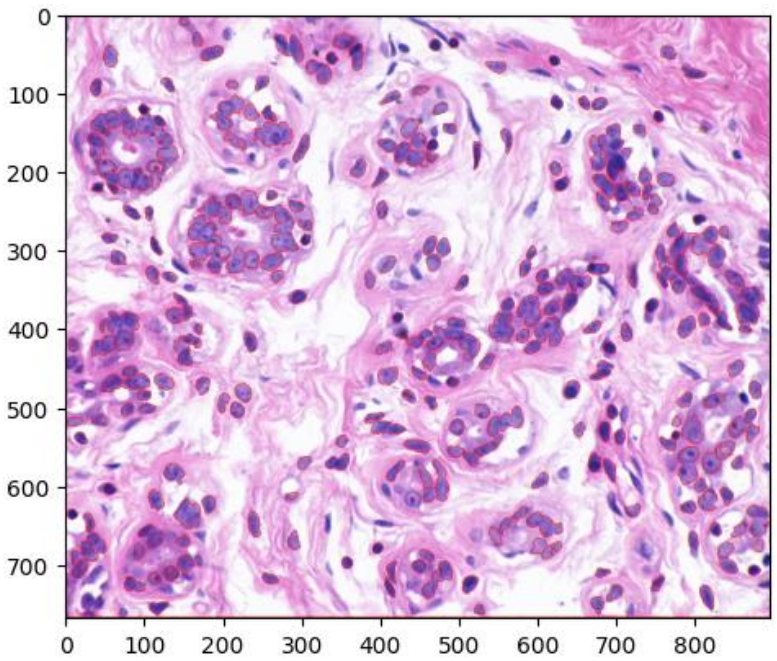
Distance Transformation



Erode

1º Método: HSV + Watershed





Substract, connectedComponents, watershed

2º Método: OD + Watershed

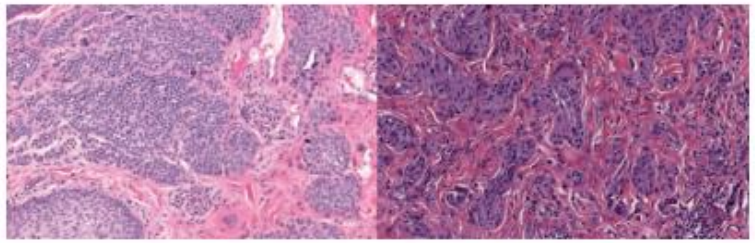
A METHOD FOR NORMALIZING HISTOLOGY SLIDES FOR QUANTITATIVE ANALYSIS

Marc Macenko [1], *Marc Niethammer* [1,2], *J. S. Marron* [3,4], *David Borland* [5], *John T. Woosley* [6], *Xiaojun Guan* [5], *Charles Schmitt* [5], and *Nancy E. Thomas* [4,7]

Departments of [1]Computer Science,[2]Biomedical Research Imaging Center, [3]Statistics and Operations Research, [4]Lineberger Comprehensive Cancer Center, [5]Renaissance Computing Institute, [6]Pathology and Laboratory Medicine, [7]Dermatology University of North Carolina, Chapel Hill, NC

ABSTRACT

Inconsistencies in the preparation of histology slides make it difficult to perform quantitative analysis on their results. In this paper we provide two mechanisms for overcoming many of the known inconsistencies in the staining process, thereby bringing slides that were processed or stored under very different conditions into a common, normalized space to enable improved quantitative analysis.



Disponível em: https://wwwx.cs.unc.edu/~mn/sites/default/files/macenko2009.pdf

2º Método: OD + Watershed

$$OD = -log_{10}(I) \tag{1}$$

where I is the RGB color vector with each component normalized to $[0,1]$. This transformation provides a space where a linear combination of stains will result in a linear combination of OD values [3]. The relationship between intensity and OD is demonstrated in figures 2(a) and 2(b), using data acquired from images of hematoxylin and eosin stained melanoma slides.

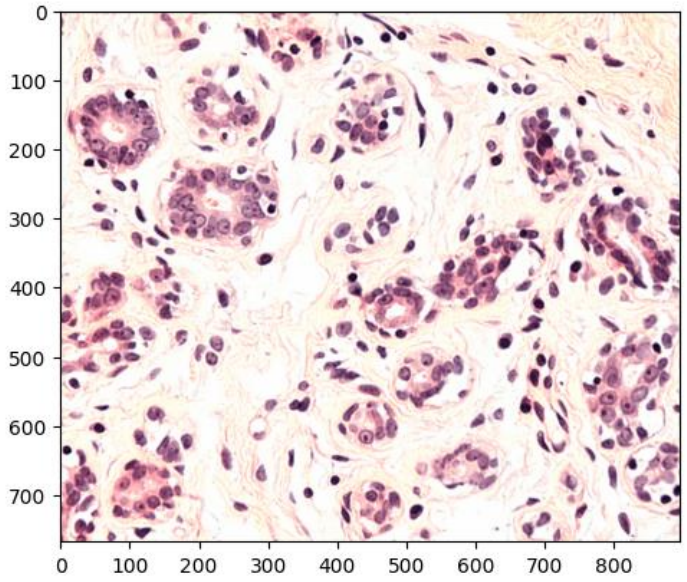
Once the correct vectors are determined by some method, a simple color deconvolution scheme similar to [4] is used to transform the color values into quantitative values of interest:

$$OD = VS \implies S = V^{-1}OD \tag{2}$$

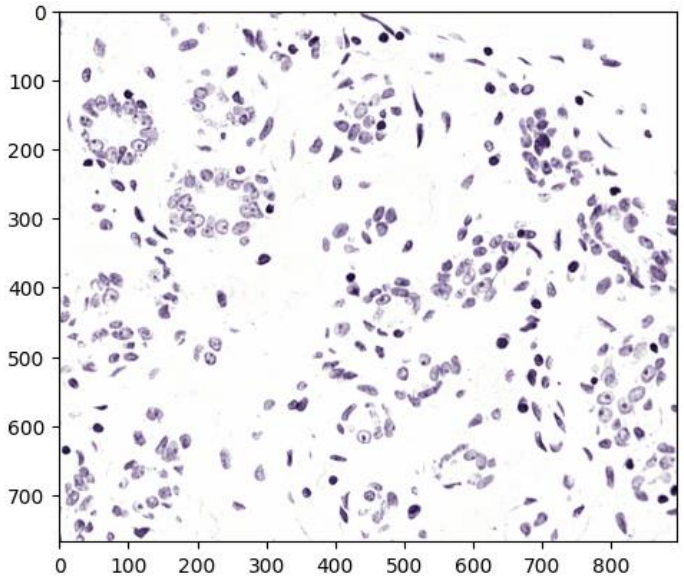
where OD is the optical density value observed and V and S are the matrices of the stain vectors and the saturations of each of the stains, respectively.

Input: RGB Slide
1 Convert RGB to OD
2 Remove data with OD intensity less than β
3 Calculate SVD on the OD tuples
4 Create plane from the SVD directions corresponding to the two largest singular values
5 Project data onto the plane, and normalize to unit length
6 Calculate angle of each point wrt the first SVD direction
7 Find robust extremes (α^{th} and $(100-\alpha)^{th}$ percentiles) of the angle
8 Convert extreme values back to OD space
Output: Optimal Stain Vectors

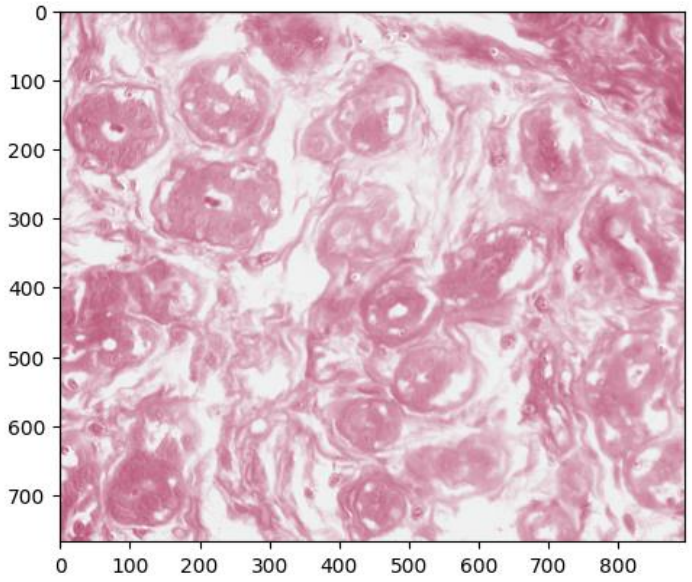
2º Método: OD + Watershed



Normalização

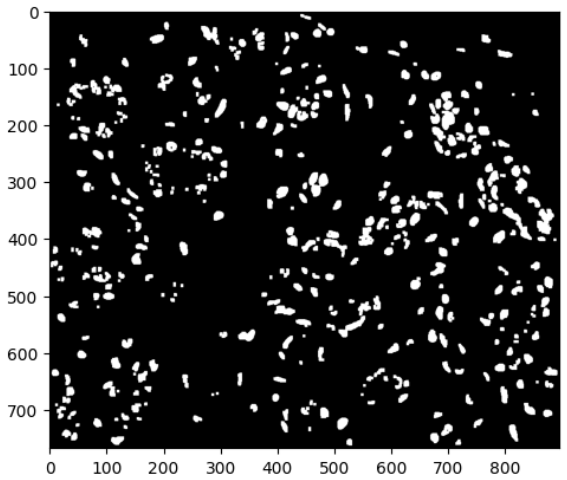


Hematoxilina

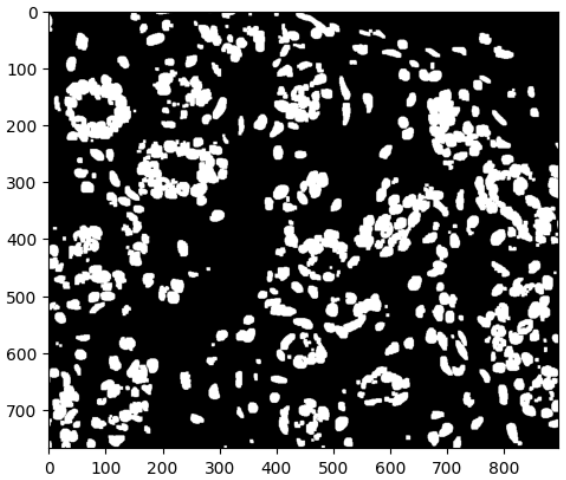


Eosina

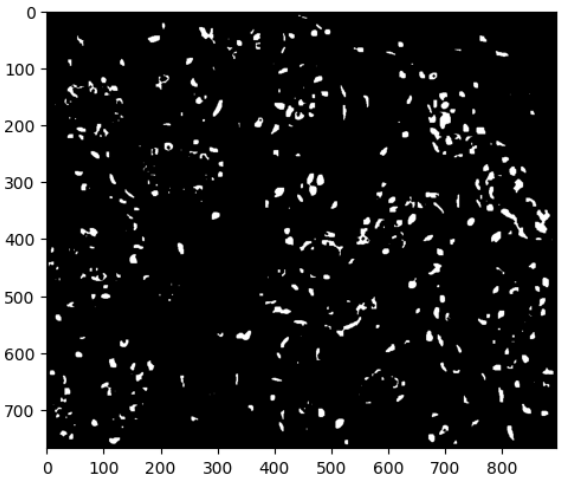
2º Método: OD + Watershed



Threshold

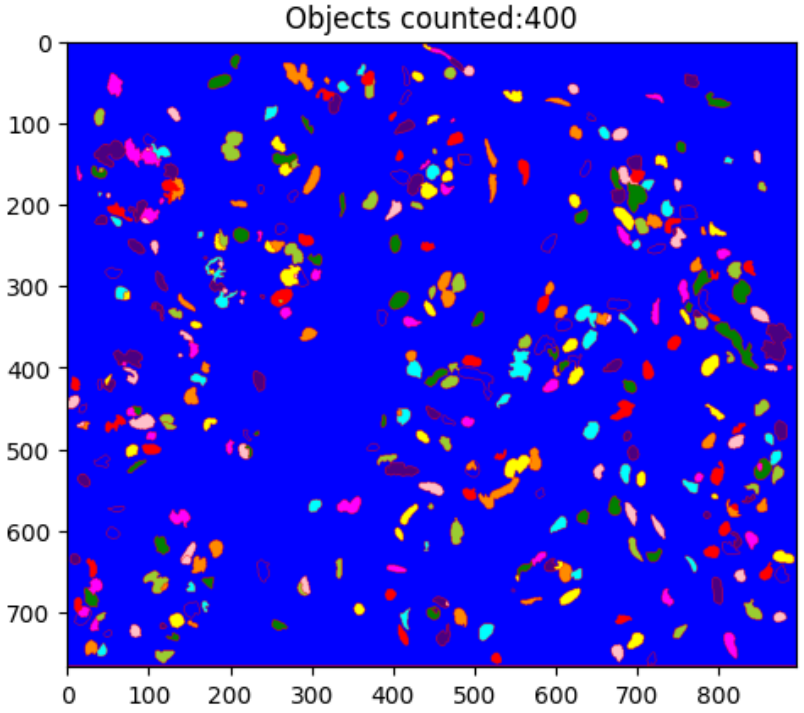


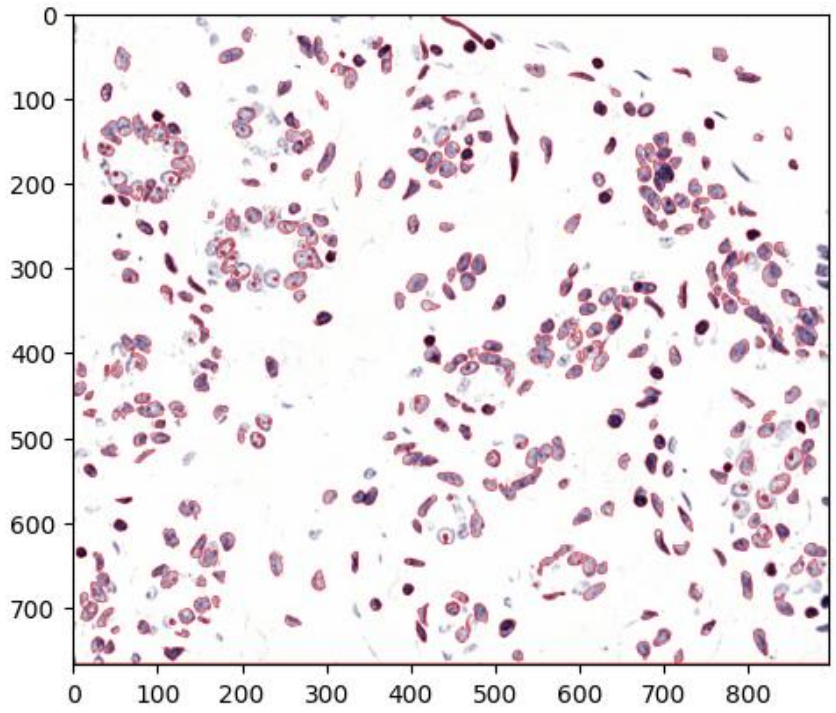
Dilate



Distance Transformation

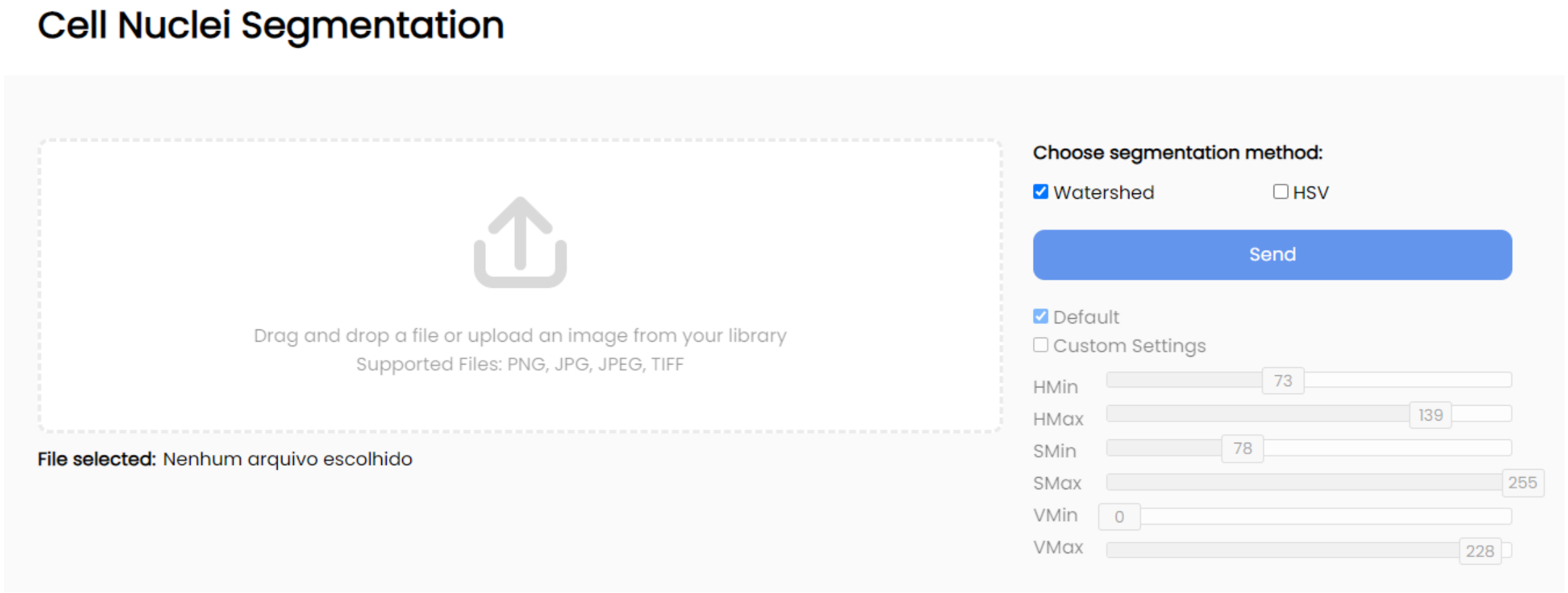
2º Método: OD + Watershed





Substract, connectedComponents, watershed

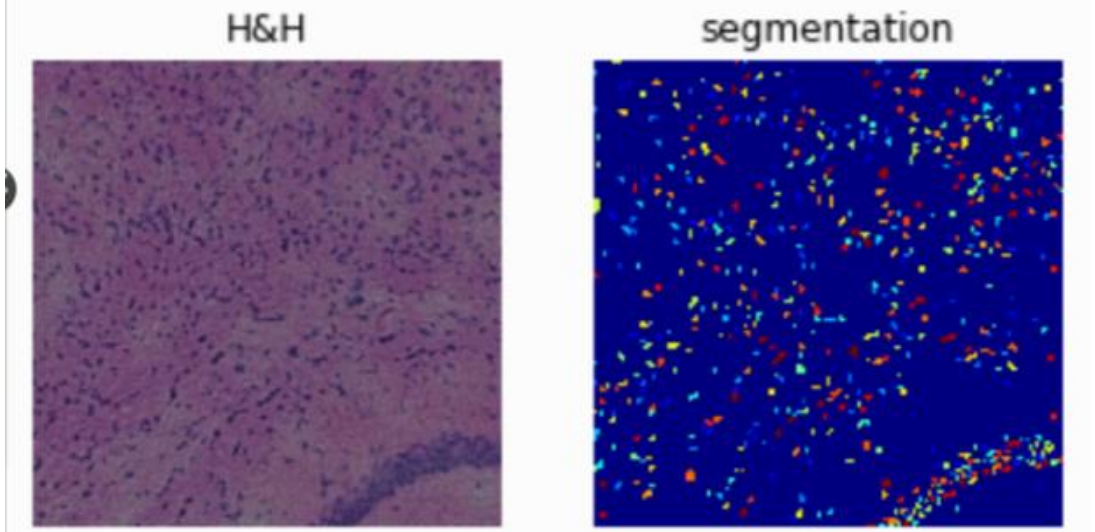
Aplicação Web



Validação por comparação de resultados

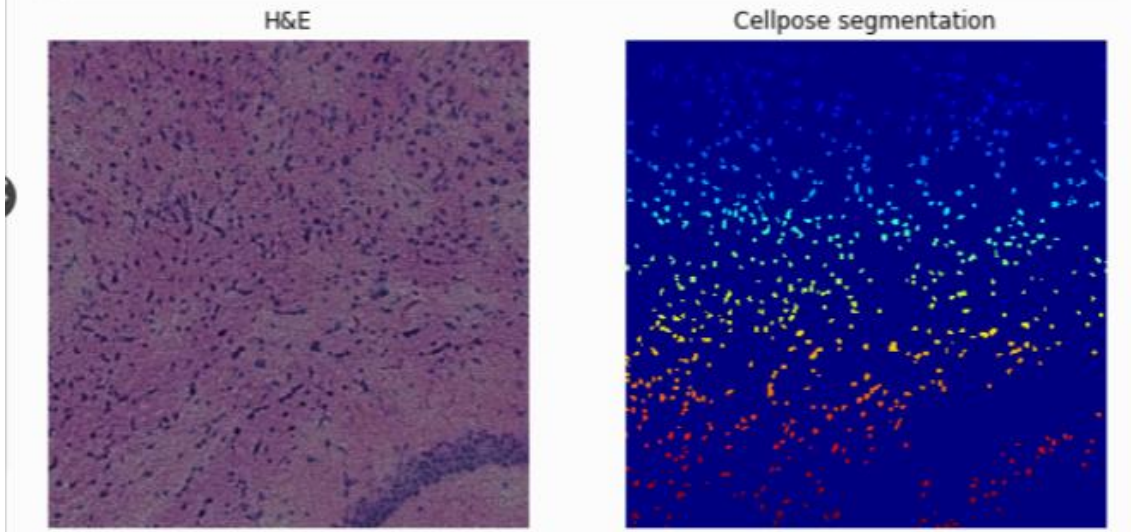
SquidPy + StarDist

```
ImageContainer[shape=(1000, 1000), layers=['image',
Number of segments in crop: 632
```



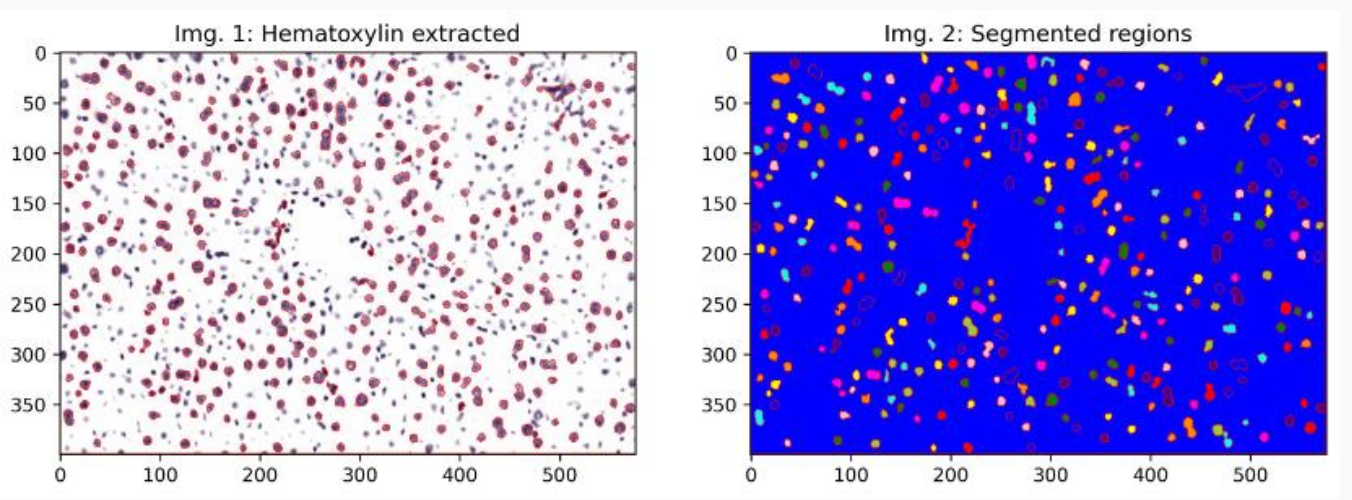
SquidPy + Cellpose

```
ImageContainer[shape=(1000, 1000), layers=['image', '
Number of segments in crop: 647
```

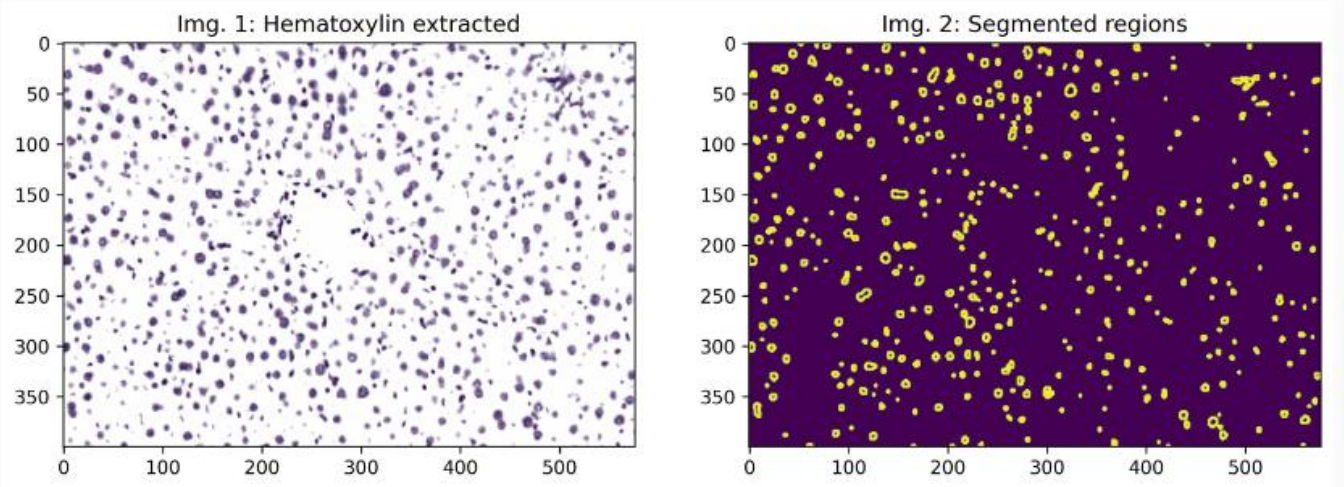


Validação por comparação entre métodos

Total: 321 núcleos



Total: 392 contagens



Conclusão

	Area	equivalent_diameter	orientation	MajorAxisLength	MinorAxisLength	Perimeter	MinIntensity	MaxIntensity	MeanIntensity	Solidity
count	400.000000	400.000000	400.000000	400.000000	400.000000	400.000000	400.000000	400.000000	400.000000	400.000000
mean	34.239227	6.212710	2.530926	8.984010	4.911137	24.066408	79.612500	198.892500	136.695453	0.392077
std	24.516158	2.238189	47.159644	3.967707	1.905496	11.225977	33.163563	32.107213	33.295574	0.044385
min	1.253094	1.263127	-89.874520	1.583094	0.861727	2.931296	9.000000	86.000000	37.067961	0.184592
25%	18.326500	4.830516	-37.283741	6.601743	3.493762	17.679058	59.750000	180.000000	117.750000	0.369603
50%	30.491954	6.230856	2.127771	8.591774	5.004249	23.649173	79.500000	204.000000	142.700690	0.406350
75%	44.745898	7.547992	40.917462	10.914979	6.027273	28.574799	103.000000	220.000000	161.283971	0.425051
max	188.381798	15.487258	90.000032	36.466737	12.823888	91.227421	155.000000	254.000000	193.404762	0.457000

- **Uso da função regionProps e cCálculo da mediana + desvio padrão para eliminar segmentações equivocadas (muito pequenas ou muito grandes);**
- **Ainda está sendo realizado contato com o Laboratório de Biologia para obtenção de mais informações, como:**
  - **Validar metodologias utilizadas;**
  - **Entender qual a margem de erro aceita pela comunidade científica;**
  - **Obtenção de imagens anotadas para validação dos métodos desenvolvidos.**