



# Using 3D segmentation to characterize the ultrastructural determinants of biophotonic reflectivity in cephalopod skin

Stephen Senft<sup>1</sup>, Teodora Szasz<sup>2</sup>, Hakizumwami B. Runesha<sup>2</sup>, Roger T. Hanlon<sup>1</sup>

<sup>1</sup>Marine Biology Laboratory, <sup>2</sup>Research Computing Center, The University of Chicago

## Background

The optical properties of cephalopod skin provide the basis for the sophisticated camouflage capabilities of these remarkable animals. Embedded in their skin are several classes of cells that contain biological nanoparticles whose material properties and shapes enable them to diffuse and reflect light in unusual ways. Together two of these cell types — **leucocytes and iridocytes** — jointly produce one of the most extreme white reflectivities known in the animal world. However the nanoparticles within each cell differ dramatically in shape: leucocytes contain spheroids (that act as broadband reflectors), whereas iridocytes contain stacked platelets (that act to reflect narrow sets of wavelengths). Earlier work [Mäthger et al., 2013] focused on the optical properties of the leucocytes. However, we would like to understand the relative roles of both optical components in producing the bright white reflectance observed at the macroscopic level. Hence it is necessary to attempt to quantify the ways in which these mixtures of platelets and spheres contribute to tissue reflectivity. Below we report our preliminary results towards this aim.

Serial Block Face (SEM) Imaging (sectioning courtesy Gatan, Inc.) was used to examine the three-dimensional ultrastructure of the fin spot of a European cuttlefish (*Sepia officinalis*). This **3D electron microscopy** technique (with an isotropic voxel resolution of 50 nm) captured several leucocyte and iridocyte cells in their entirety, and in addition clearly revealed the high refractive index particles contained within them.

With the aim of being able to **computationally model the optical behavior of these sets of particles** (i.e. how they scatter and reflect incoming photons of various energies), we have been attempting to segment each particle in 3D to obtain its location, thickness, orientation and spacing. We have isolated from the full [1024x1024x750] data set several sub-volumes [192<sup>3</sup> voxels] (Fig. 1). Each contains an enriched population of particles: mainly plates or spheroids (although other regions contain more complex objects). Higher resolution electron microscopy (not shown) suggests that most of the particles are disjoint from each other, although some adjacent platelets or spheres may in fact be fused (as opposed to artificially blended together due to limited spatial sampling).

## The dataset that we segmented

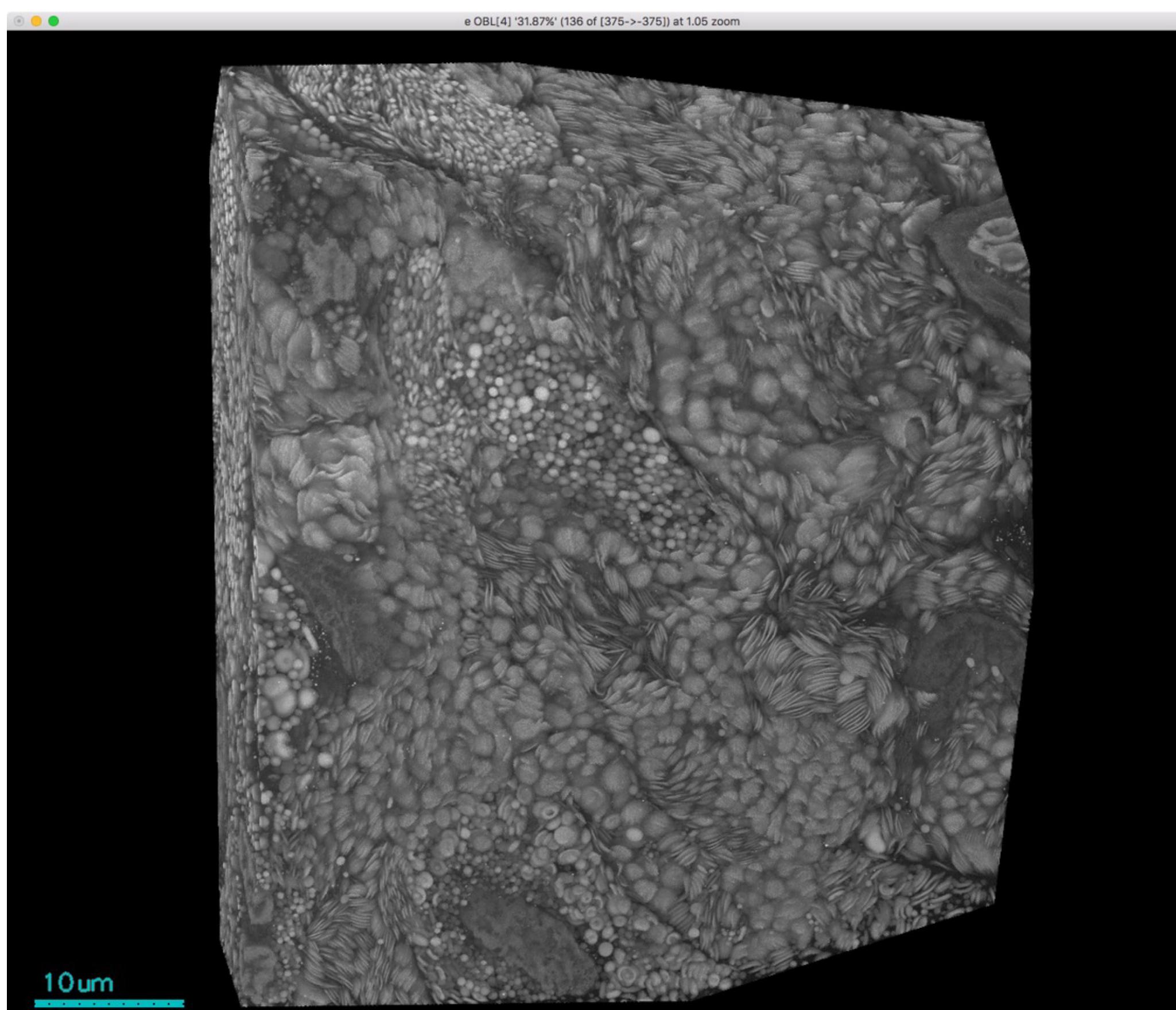
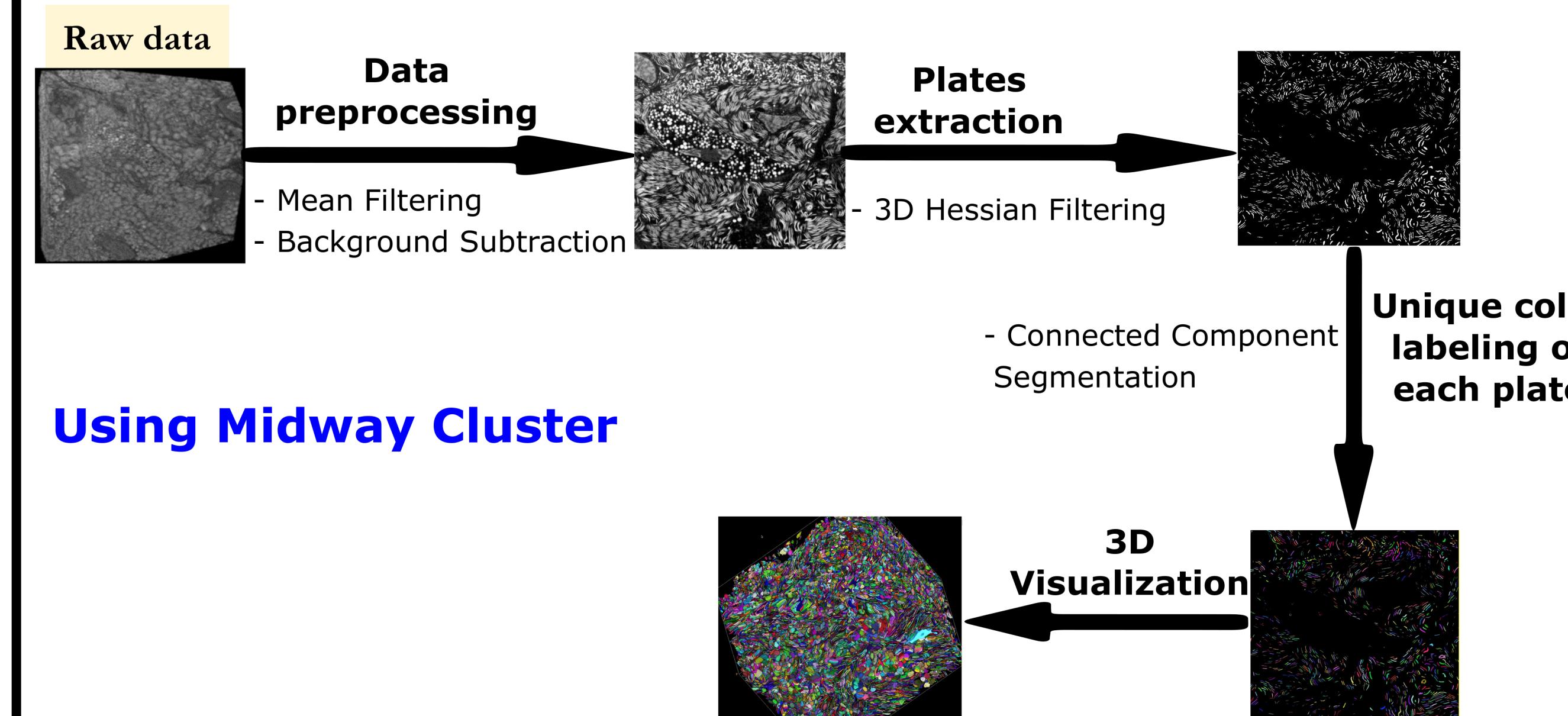


Fig. 1: The original dataset, before segmentation. The dataset is 1024 X 1024 X 738.

## Method – developed on Midway cluster



### Using Midway Cluster

Fig. 2: Image processing pipeline applied on Midway cluster

3D Hessian methods were used to segment the plates – the volume was filtered with a Gaussian kernel, followed by calculation of second order gradients, which approximates the second order derivatives of the image. Then, we used the `ConnectedComponentImageFilter` class of Insight Toolkit (ITK) module on Midway. This class labels the objects in a binary image, and each distinct object is assigned a distinct color label.

## Results

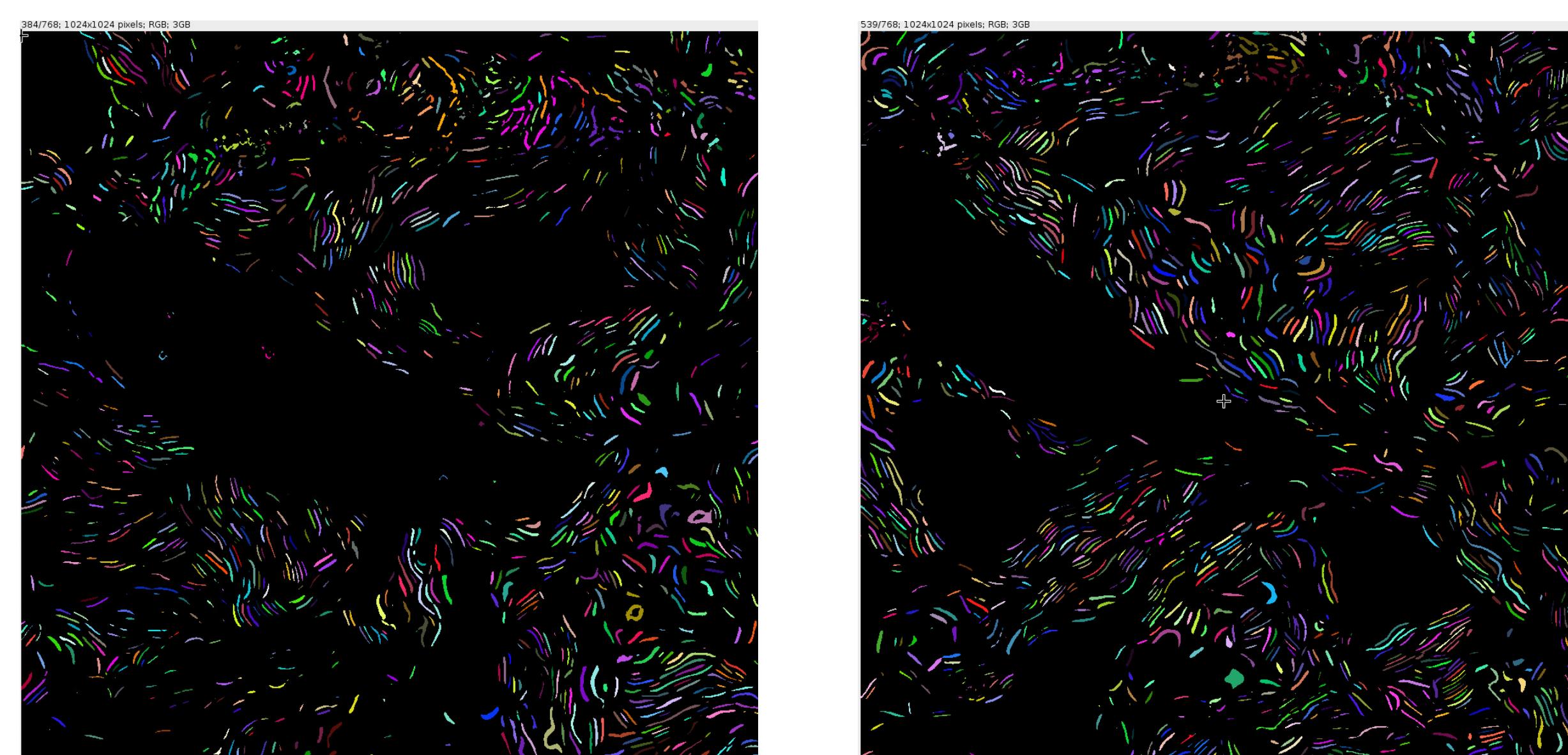


Fig. 3: Results after color labeling the detected plates. The two figures present two slices extracted from the larger 3D volume

As observed in Fig. 3, we were able to segment iridocyte plates separately from leucocyte spheres, using a 3D Hessian filter. (The black regions on the presented images are where the excluded leucocyte spheres were located.)

To segment leucocyte spheres separately, we used a Watershed method [Li et al., 2007]. The results of the segmentation of leucocytes is presented in Fig. 4.

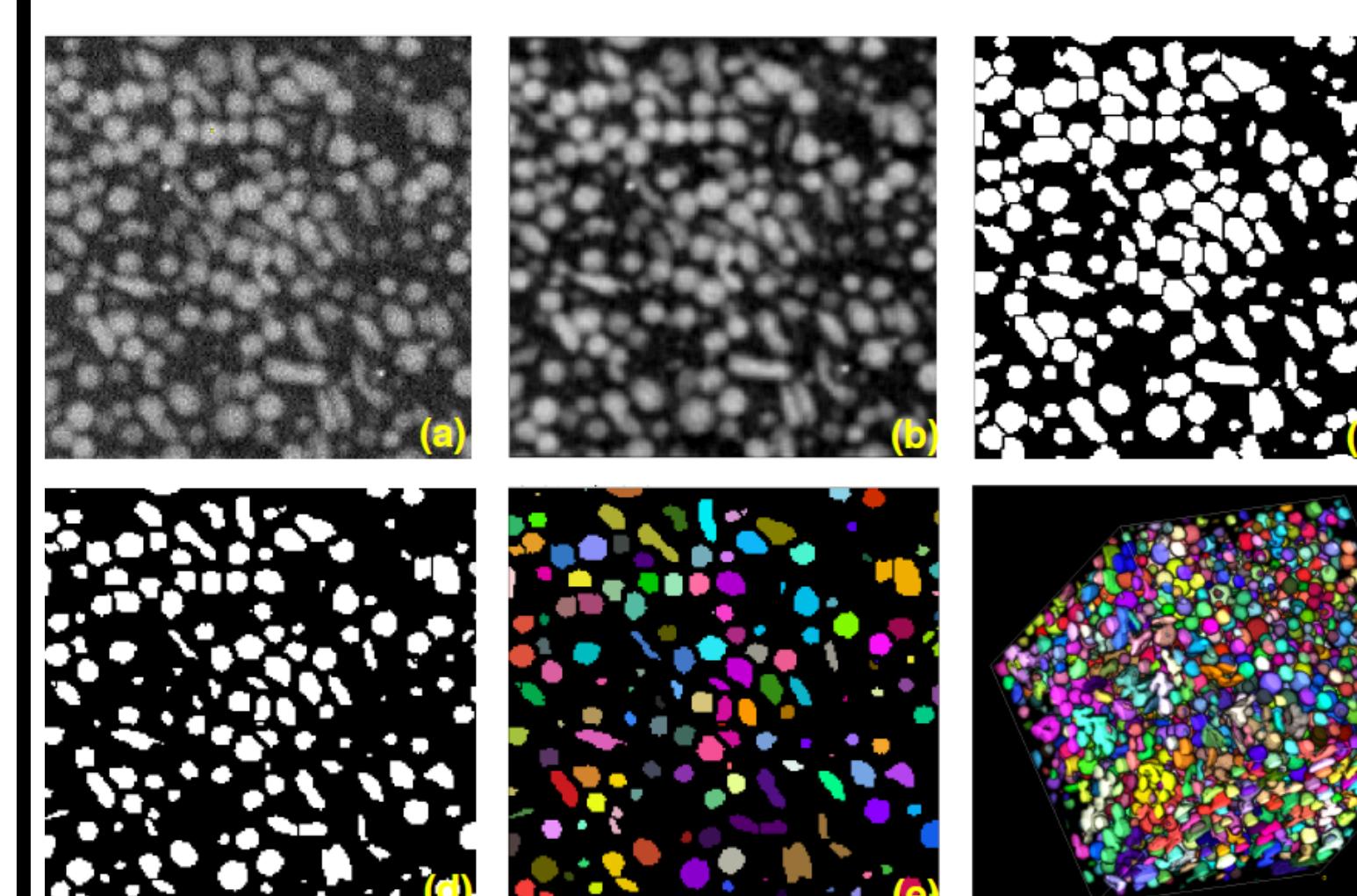
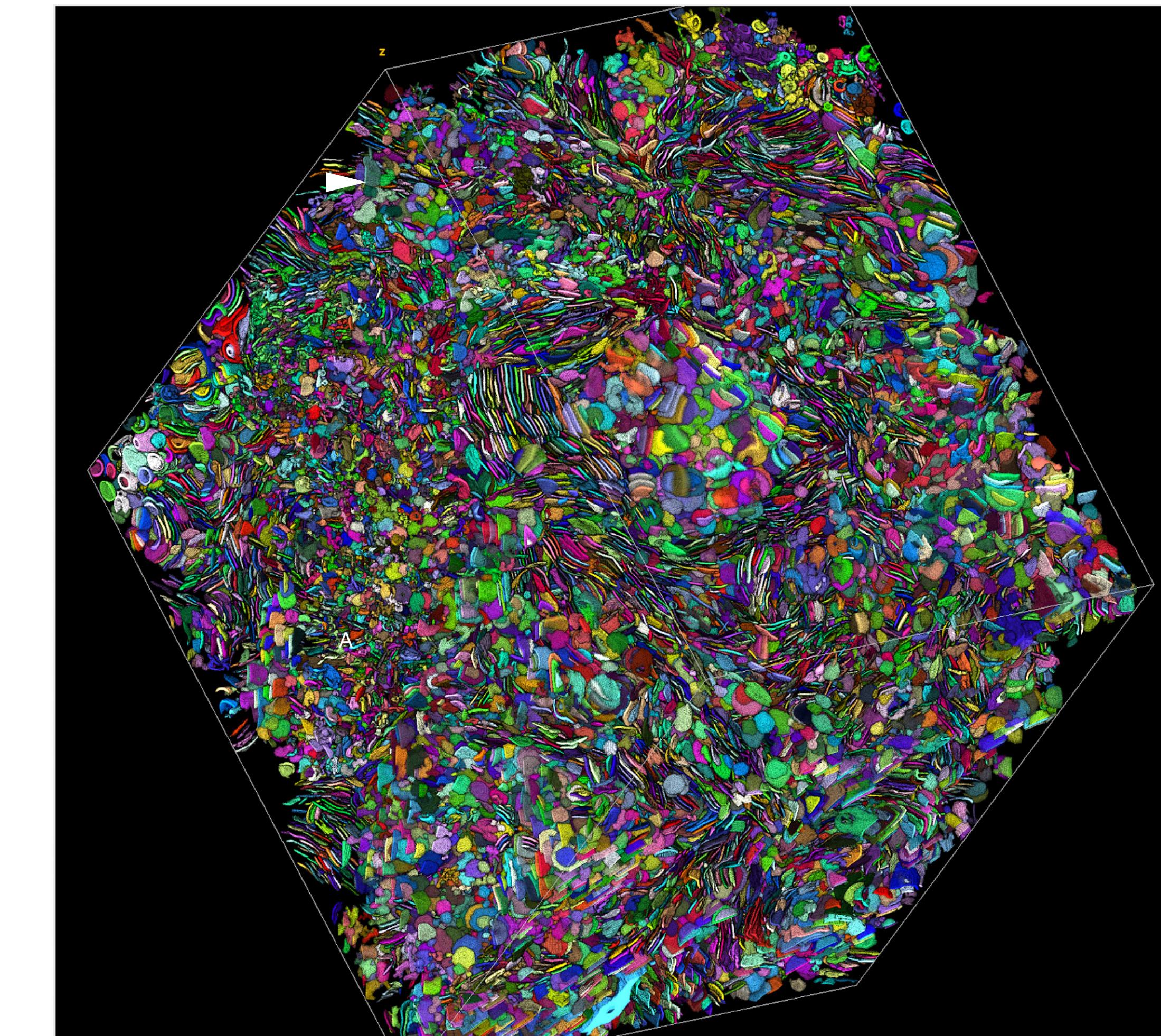


Fig. 4: Segmentation pipeline of leucocyte cells: (a) original data; (b), (c) preprocessed data using median filter and contrast enhancement, (d) segmented data after applying 3D Watershed method, (e) color labeled data, (f) 3D reconstruction of the segmented data

## 3D Reconstruction of the segmented sub-cellular plates



Number of detected plates in this region of tissue: 191349

## Future Work

### Higher Level Organization of the Plates in 3D and Possible Optical Consequences

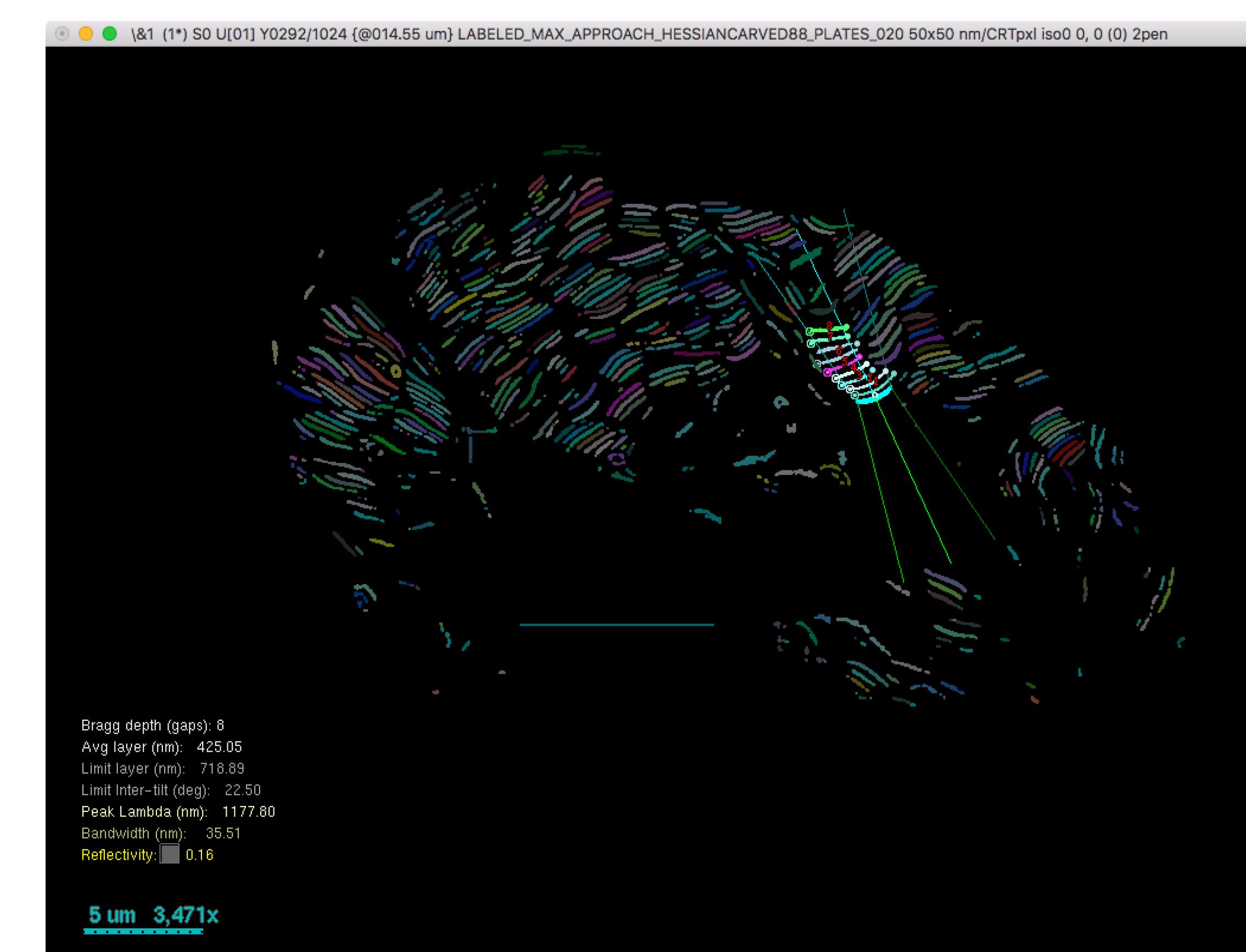


Fig. 5: Preliminary work on characterizing the reflective properties of the plates. Subsets of stacked plates differing in spacing and orientation will reflect light differently.

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