



# Characterizing the ultrastructural determinants of biophotonic reflectivity in cephalopod skin: a challenge for 3D segmentation

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## 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 in 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 each optical component 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*). The 3D electron microscopy (with an isotropic voxel resolution of 50 nm) captured several leucocytes and iridocytes in their entirety, and in addition clearly revealed the particles contained within them (Fig. 1).

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 [192^3 voxels] (Fig. 2 and Fig. 3). Each contain 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, however some adjacent platelets or spheres may in fact be fused (as opposed to blended together due to limited spatial sampling).

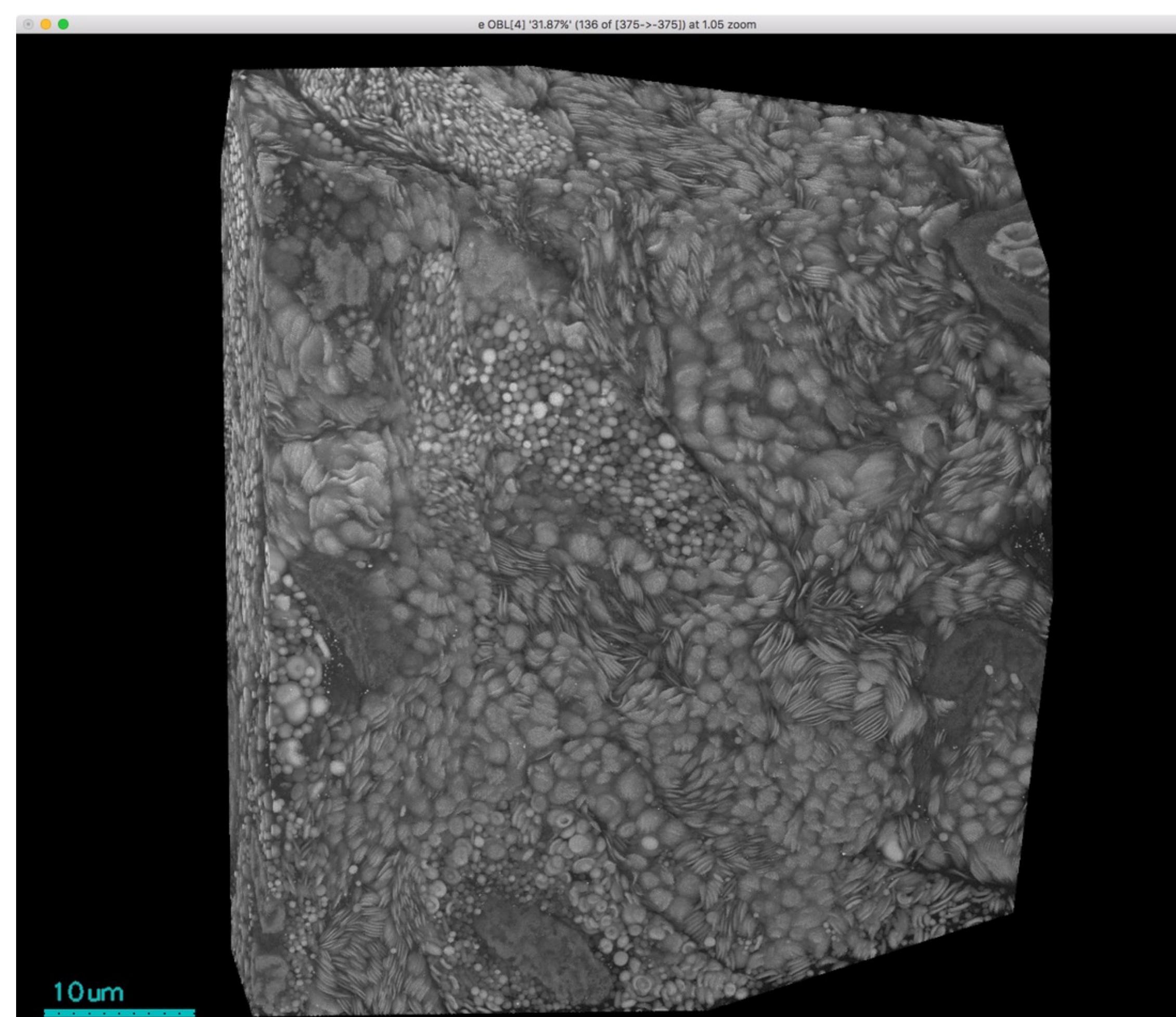


Fig. 1: The dataset to be segmented: [1024 X 1024 X 750]. Due to the complexity of the full dataset, we selected volumes of interest containing mainly plates or mainly spheres.

## Segmentation of the iridocytes using Frangi method

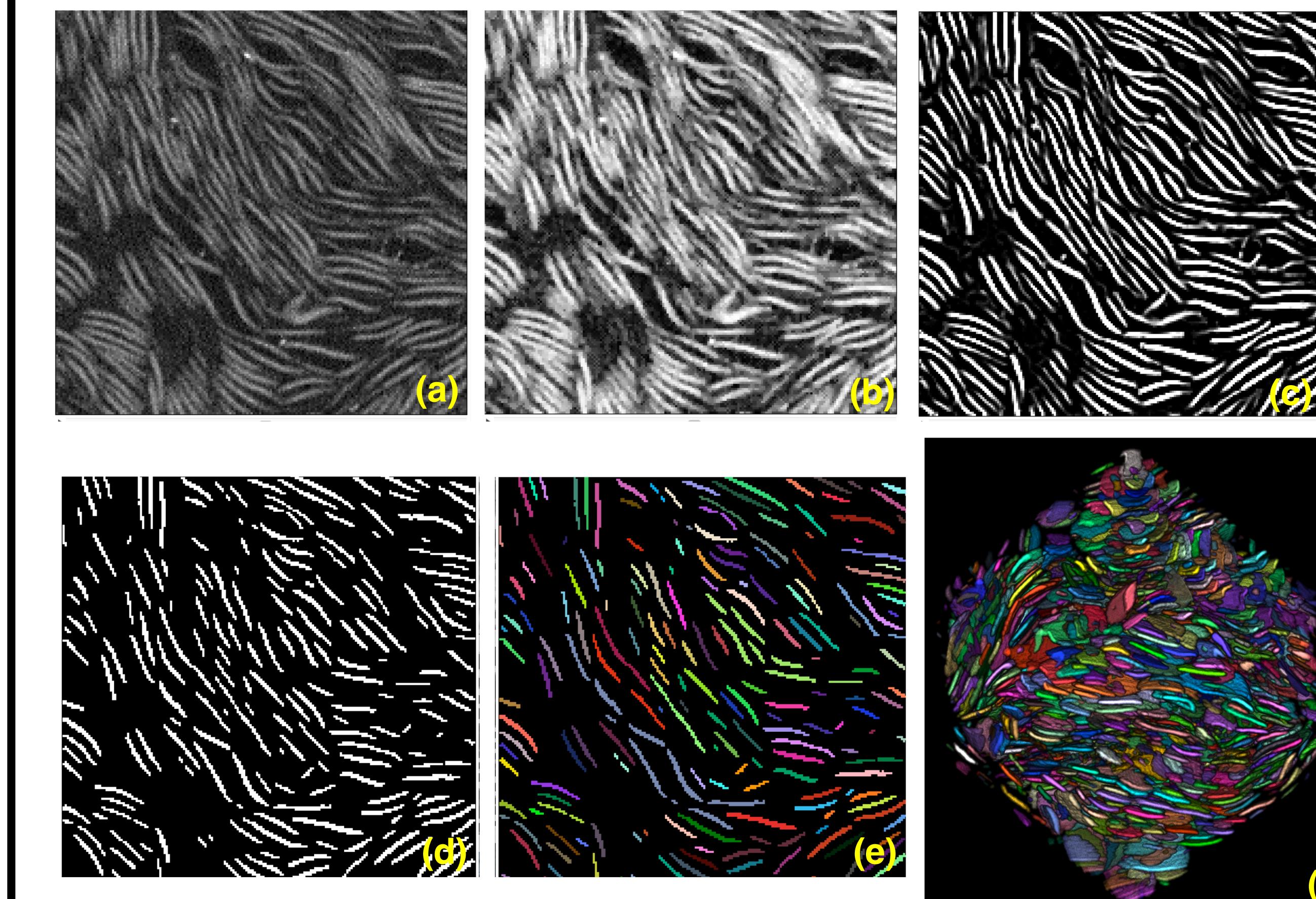


Fig. 2: Segmentation pipeline of the iridocytes cells: (a) original data; (b), (c) preprocessed data using median filter and contrast enhancement; (d) segmented data after applying Frangi method; (e) color labeled data; (f) 3D reconstruction of the segmented data.

Frangi method [Frangi et al., 1998] was used for segmenting the plates, that uses the eigenvalues of the Hessian to determine locally the likelihood that a vessel-like structure is present.

Detected spheres: 3081

## Segmentation of the leucocytes using 3D Watershed method

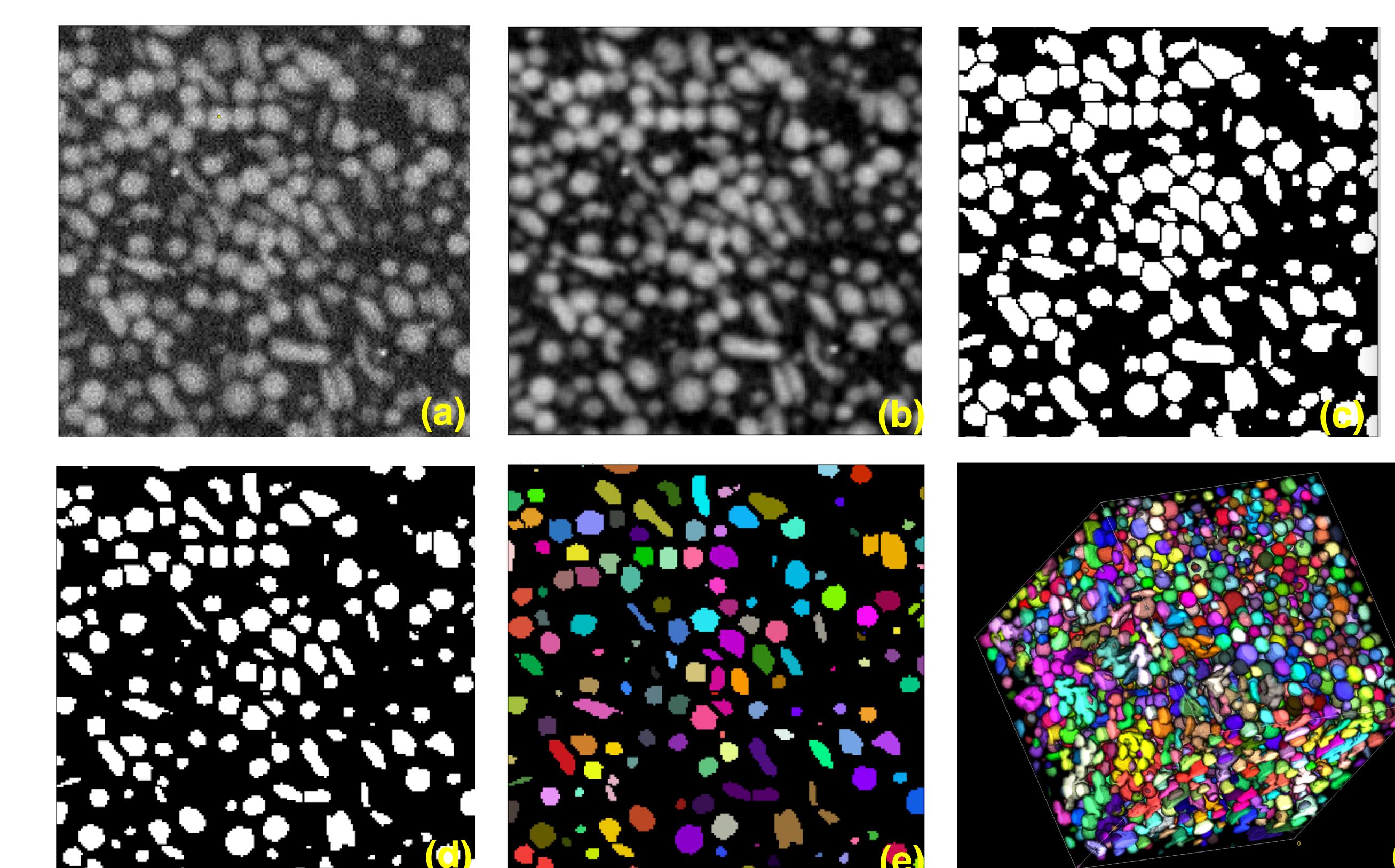


Fig. 3: Segmentation pipeline of the leucocytes cells: (a) original data; (b), (c) preprocessed data using median filter and contrast enhancement; (d) segmented data after applying 3D Watershed method; (e) color labeled data; (f) 3D reconstruction of the segmented data.

Watershed method [Li et al., 2007] was used for segmenting the spheres. The method is a morphological gradient-based technique, where the gradient map of the image is considered as a relief map in which different gradient values correspond to different heights. If we punch a whole in each local minimum and immerse the whole map in water, the water level will rise over the basins. When two different body of water meet, a dam is built between them. The whole image is segmented by creating such dams that are called watersheds.

Detected spheres: 3086

## Future work

### Segmentation of the entire dataset

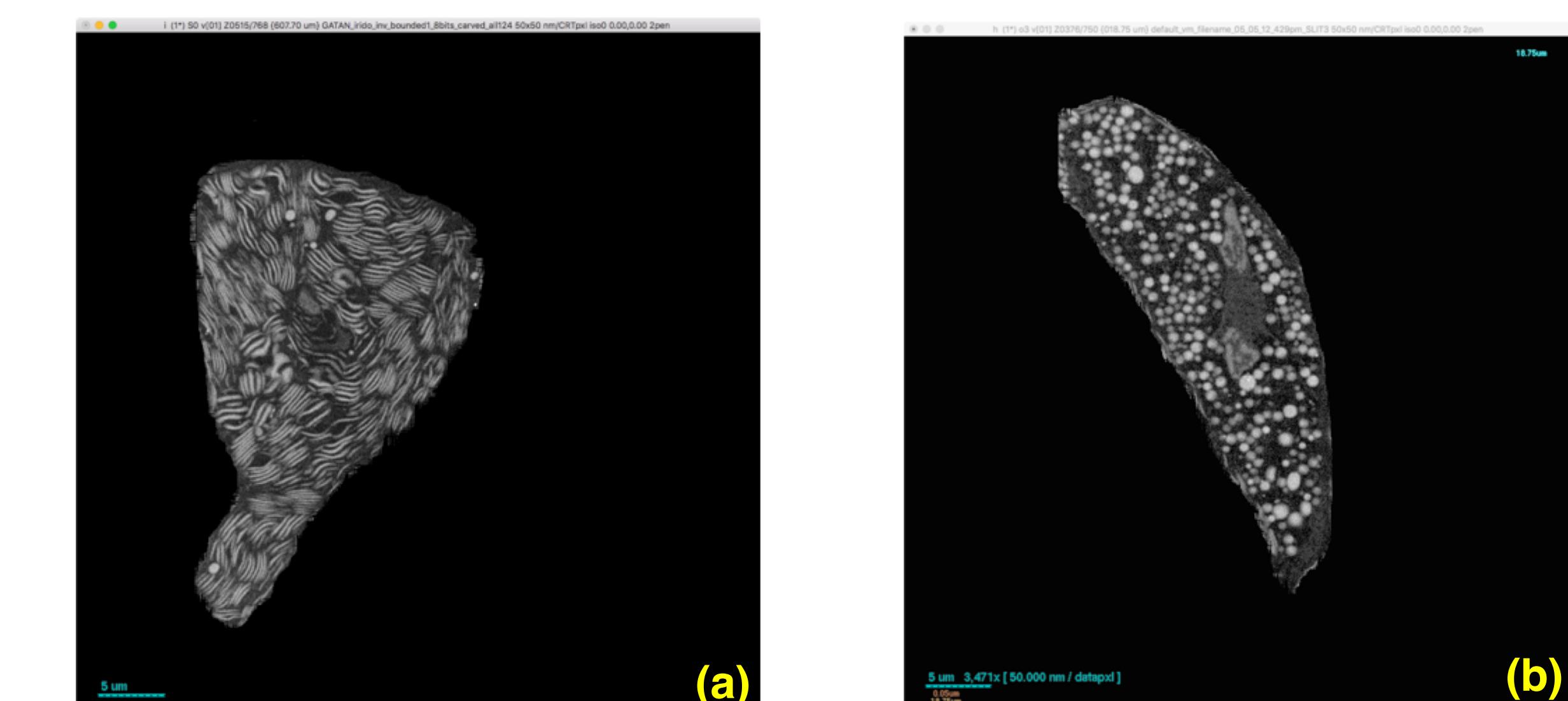


Fig. 4: Individual cells manually segmented from the full dataset; (a) one containing platelets and the other (b) containing spheres

Ideally, we would like to have a method that can obtain this information in 3D for any of the shapes in our data. Thus far we have found that we have to use a combination of approaches: Frangi methods (designed to detect tubular structures) and Watershed methods (designed to separate touching blobs). For the Frangi method, we find that by using the ImageJ Frangi package and the Insight Toolkit (ITK), for the 3D implementation there are limitations (the plates are not segmented correctly) in each of several available software packages, such that we have had to resort to using 2D Frangi on a series of image planes. We nonetheless have been able to segment many (we estimate about 2/3) of the plates in the sub-volumes. With a “true” 3D Frangi method we would expect to have more precision.

## Segmenting other unusual shapes using Frangi method

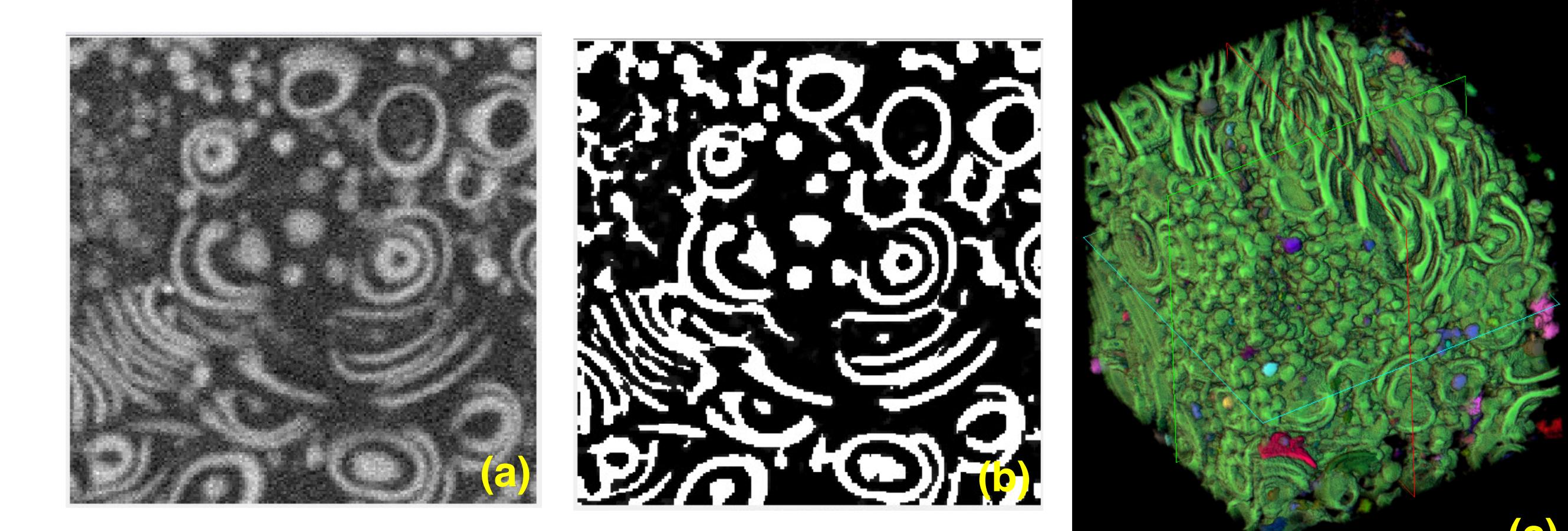


Fig. 5: Segmentation pipeline of the unusual shaped cells: (a) original data; (b) segmented data after applying Frangi method; (c) 3D reconstruction of the segmented data.

The above sub-volume contains a mix of spherical cells and plates that emerge into a spherical structure. The segmentation technique is challenging since Frangi method (designed to detect vessel-like structures) will deform the sphere structures. On the other hand, watershed will detect too many catching basins in the image, dividing the long plates.

Our future work will focus on improving the segmentation of this kind of volumes and divide the full dataset in volumes that will be segmented adaptively using one of the aforementioned segmentation methods.

## Acknowledgements

Funding AFOSR Grant #: FA9550-14-1-0134  
Research Computing Center