# THE 2-D PROCESSING OF IMAGES PROVIDED BY CONFOCAL MICROSCOPE

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***Abstract:***

*This paper discuses possibilities of software 2-D processing of optical cuts provided by confocal microscope. It introduce new mathematical tools for construction a 2-D image. Each optical cut will represent by different colour, namely in case, when data will be of the grayscale type. All characteristics would improve after the cuts composition. It may be presumed that similar mathematical tools might also be employed for spatial reconstructions of these preparations.*

**Keywords:** graphic space, physical and logical voxel, physical and logical space, mapping, multifocal image, optical cut

## 1. Introduction

The display of preparate is realised by a beam of laser rays in the confocal microscope . The laser beam which is parallel to an objective optical axis comes through the semi-permeable mirror and it is refracting to an objective focus. If the focus is situated on the preparation surface, the ray is reflected by preparation and it recurs through object-lens back. Because this reflected ray goes from an objective focus, it goes through image space of object-lens parallel with its optical axes again. It comes on mirror, which reflects it parallel with optical ocular axes. The ray is refracted into a pictorial focus. There is a pin hole in the focal picture plane of ocular, which has a chink in focus. The ray goes through the chink and it impacts on a physical pixel of scanner.

The rays reflected by preparation out of focal plane can come this way into ocular too, but cannot be reflected parallel with optical axis after going through objective. Nor they are reflected by mirror in direction of ocular optical axes. Therefore they can not be refracted into focus by ocular. On pictorial focal plane they fall out of focus, they are intercepted by shutter and they cannot cause defocusing image. This microscope has the sharpness zone very narrow and practically it does not display the preparation points, which are situated in set of unsharpness. Therefore we obtain the optical cut of observe preparation with very small altitudes.

## 2. The graphic space, universal co-ordinate system

We work again with two-dimensional reconstructions in this article, but each optical cut will represent by different colour, namely in case, when data will be of the grayscale type. All characteristics would improve after the cuts composition. Three-dimensional colour system RGB belongs to the most used colour systems. Following definitions are connected with colours and palettes in this system. Following constructs and their properties can be further used for three dimensional reconstruction of all sorts of objects.

**2.1. Definition:** Let $I=\langle i_1;i_2\rangle; J=\langle j_1;j_2\rangle;\ K=\langle k_1;k_2\rangle$ be intervals, $D_x=\{x_i\}_{i=0}^{m}; m>1$ is equidistant division of interval $I$, $D_y=\{y_i\}_{i=0}^{n}; n>1$; is equidistant division of interval $J$, $D_z=\{z_k\}_{k=0}^{s}; s>1$ is equidistant division of interval $K$. Block $F_{i,j,k}=\langle x_i;x_{i+1})\times\langle y_j;y_{j+1})\times\langle z_k;z_{k+1})$; $i=0,1,..,m-1$, $j=0,1,..,n-1$, $k=0,1,..,s-1$ is called a physical voxel. Numbers $v_x=x_{i+1}-x_i$; $v_y=y_{j+1}-y_j$ $v_z=z_{k+1}-z_k$ are called dimensions of physical voxel $F_{i,j,k}$ respectively. Block $I\times J\times K$ together with divisions $D_x, D_y, D_z$ are called a graphic space, in detailed notation $\mathcal{G}_3=(I\times J\times K, D_x, D_y, D_z)$.

Following statements are resulting from this definition:

**2.2. Theorem:** Corresponding proportions of all physical voxels $F_{i,j,k}$ of the same graphic space $\mathcal{G}_3$ are equal.

**2.3. Theorem:** The set
$$\mathcal{F}_3=\{F_{i,j,k}=\langle x_i;x_{i+1})\times\langle y_j;y_{j+1})\times\langle z_k;z_{k+1})\mid i\in\{0,..,m-1\}, j\in\{0,..,n-1\}, k\in\{0,..,s-1\}\}$$
of all physical voxels of graphic space $\mathcal{G}_3$ is a decomposition of graphic space $\mathcal{G}_3$.

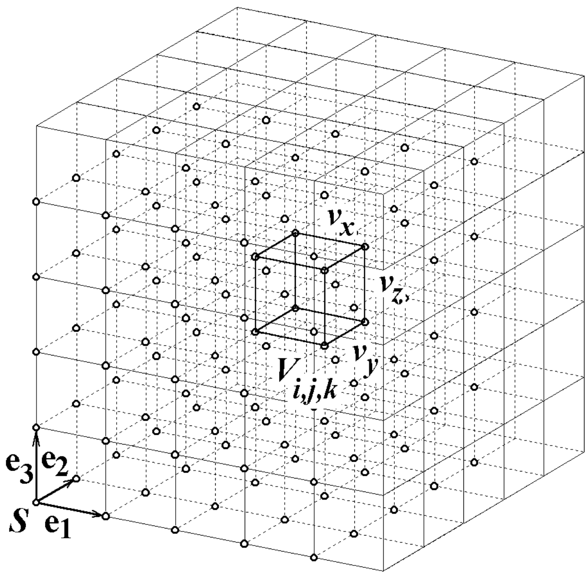


**Fig. 1: The graphic space with a vertex mapping**

**2.4. Theorem:** Let $\mathcal{G}_3$ be the graphic space, $\mathcal{F}_3$ the set from theorem 2.3. The relation ρ defined on $\mathcal{G}_3$ by reference
$$\rho(A,B)\Leftrightarrow(\exists F_{ijk}\in\mathcal{F}_3)[A\in F_{ijk}\wedge B\in F_{ijk}]$$
is an equivalence on $\mathcal{G}_3$.

**2.5. Definition:** Let $\mathcal{G}_3$ be the graphic space. A factor set $\mathcal{F}_3=\mathcal{G}_3/\rho$, where ρ is the equivalence from previous theorem, is called physical space of the space $\mathcal{G}_3$. As resolution of physical space $\mathcal{F}_3$ we understand the resolution relevant to graphics space $\mathcal{G}_3$.

**2.6. Definition:** Let $\mathcal{G}_3$ be the graphic space, $\mathcal{F}_3$ its physical space, $v_x$; $v_y$; $v_z$ the dimensions of its physical voxels $F_{i,j,k}$ respectively. Further lets

$$\text{for } c<v_x \text{ is } {}_cI=\{r_k\in\mathbf{R}\mid\forall k\in\{0,1,...,m-1\}: r_k\in\langle x_k;x_{k+1})\wedge r_k-x_k=c\}$$

for $d < v_y$ is ${}_dJ = \{s_k \in \mathbf{R} | \forall k \in \{0,1,...,n-1\}: s_k \in \langle y_k; y_{k+1}) \wedge s_k - y_k = d\}$
for $e < v_z$ is ${}_eJ = \{t_k \in \mathbf{R} | \forall k \in \{0,1,...,s-1\}: t_k \in \langle y_k; y_{k+1}) \wedge t_k - z_k = e\}$

and $P = [c,d,e]$. Then the set ${}_P\mathbf{L}_3 = {}_cI \times_d J \times_e J$ we call a logical space, its elements ${}_PL_{i,j,k}$ are called logical voxels.

**2.7. Theorem:** Let $\mathcal{F}_3$ be the physical space of the graphic space $\mathcal{G}_3$, let ${}_P\mathbf{L}_3$ be any logical space of the same graphic space and ${}_P\varphi : \mathcal{F}_3 \rightarrow {}_P\mathbf{L}_3$ is a mapping, where for all $i = 0,1,..,m-1$, $j = 0,1,...,n-1$, $k = 0,1,...,s-1$ is ${}_P\varphi(F_{i,j,k}) = {}_PL_{i,j,k} \Leftrightarrow {}_PL_{i,j,k} \in F_{i,j,k}$. Then the mapping ${}_P\varphi$ is a bijection.

**2.8. Definition**: The mapping ${}_P\varphi : \mathcal{F}_3 \rightarrow {}_P\mathbf{L}_3$ from previous theorem is called a mapping of physical space.

**2.9. Definition:** The mapping ${}_V\varphi : \mathcal{F}_3 \rightarrow {}_V\mathbf{L}_3$, where $V = [x_0; y_0; z_0]$, is called a vertex mapping. The mapping ${}_S\varphi : \mathcal{F}_3 \rightarrow {}_S\mathbf{L}_3$, where $S = \left[\frac{1}{2}(x_0 + x_1); \frac{1}{2}(y_0 + y_1); \frac{1}{2}(z_0 + z_1)\right]$, is called a center mapping.

In many graphical application it is not necessary to discriminate between physical and logical voxels again, in colour spaces allways it is not necessary to introduce these conception. If we want to reduce a number of colours in already existing image, we can use the fact that it is possible to represent the colour by orderly triad of natural numbers (components R, G, B). Leat us consider that, it is possible to define the metric on the set of these triads (e.g . Euclidean). The chromatic set is orderly, but the metric space too. The colour, that we can not use for some reason, is possible to replace it by the nearest colour, in sense of used metric. Mapping of colour space enables to carry out all sorts of operation in these spaces, e.g. cuts or selection of pallets in such a way, how it is described further. In cases, we need to use "smooth connection" between single colours (and it is a case of these papers), these constructs are necessary too.

**2.10. Definition**: Let $\mathcal{F}_3$ is the physical space, $F_{i,j,k}$ its physical voxel. The ordered triad $[i,j,k]$ is caled the co-ordinate of physical voxel $F_{i,j,k}$.

**2.11. Definition**: Let $\mathbf{L}_3$ is a logical space of the physical space $\mathcal{F}_3$. Lets us sign $\mathbf{e}_1 = (v_x;0;0); \mathbf{e}_2 = (0;v_y;0); \mathbf{e}_3 = (0;0;v_z); S = L_{000}$. Then ordered pentad $\langle \mathbf{L}_3; S; \mathbf{e}_1; \mathbf{e}_2; \mathbf{e}_3 \rangle$ is called the universal co-ordinate system of the logical space $\mathbf{L}_3$ .

**2.12. Definition**: Let $\mathbf{L}_3$ be the logical space, $\langle \mathbf{L}_3; S; \mathbf{e}_1; \mathbf{e}_2; \mathbf{e}_3 \rangle$ its universal co-ordinate system. Furher let $\mathcal{F}_3$ be the physical space, for which exists the inverse mapping $\varphi^{-1} : \mathcal{F}_3 \rightarrow \mathbf{L}_3$. The system $\langle \mathbf{L}_3; S; \mathbf{e}_1; \mathbf{e}_2; \mathbf{e}_3 \rangle$ is called universal co-ordinate system of logical space $\mathbf{L}_3$ which is induced by mapping $\varphi$. We denote it $\langle \mathbf{L}_3; S; \mathbf{e}_1; \mathbf{e}_2; \mathbf{e}_3 \rangle_\varphi$.

## 3. Palets and colour multifocal image

Image $O$ can bee defined as the mapping $O:\mathcal{F}_2 \to C_r$ of scanner plane $\mathcal{F}_2$ into so-called chromatic set $C_r$:

**3.1. Definition:** Let $\mathcal{F}_2$ be a physical plane and $C_r = \{c \in N; 0 \le c < r; r > 1\}$. A mapping $O:\mathcal{F}_2 \to C_r$ is called a picture matrix or a short picture. The set $C_r$ is called an $r$-chromatic set. If $O:F_{i,j} \to c$, the number $c$ is called the value or colour of $F_{i,j}$. Under the resolution of the picture we understand the resolution of the incident physical plane.

Multifocal image can bee defined as a sequence of images:
**3.2. Definition:** The sequence $\{^{(k)}O\}k = 1,..,n$ of the images is called a multi-focal image (or, more precisely, an $n$- focal image).

The multifocal image $\{^{(k)}O\}k = 1,..,n$ which is acquired of a confocal microscope, can consist as far as of several tens images. If the input data are saved as Gray Scale, the chromatic set consists from 256 gray tone $(r = 256)$. If they are True Color, then $r = 256^3$. In case, that data type is grayscale, it is acceptable to colour data with suitable colours. After the composition all sorts of characteristics excel better. The $RGB$ system belongs to the most used colour systems. Following definitions describe the colours and pallets in this system.

**3.3. Definition:** The chromatic set $C_r$, for which is $r = z^n; z > 1$, is called $n$-chromatic set. Specially for $n = 3$ we have a trichromatic set. The number $z$ we call a basis of the chromatic set.

**3.4. Theorem:** Let $C_r$ be a chromatic set of trichromatic system with the basis $z$, $c_0; c_1; c_2; c_i = 0,1,...,z-1$. Then there exists a bijection $\beta : C_r \to C_z^3$ such that for all $c \in C_r$ it is $\beta(c) = (c_0; c_1; c_2) \Leftrightarrow \quad c = c_0 z^0 + c_1 z^1 + c_2 z^2$. On the set $C_z^3$ there exists an ordering $(c_0; c_1; c_2) < (d_0; d_1; d_2) \Leftrightarrow \; c_0 z^0 + c_1 z^1 + c_2 z^2 < d_0 z^0 + d_1 z^1 + d_2 z^2$.

**3.5. Definition:** The set $C_z^3$ from previous theorem is called trichromatic system.

The theorem 3.2. makes possible to construct physical space $\mathcal{F}_3$ over the trichromatic system, where coordinates of physical voxel are given by orderly triad $\beta(c) = (c_0; c_1; c_2)$. We use this space for the construction of palettes. Assume that the first component of sequenced triad of trichromatic system determines red quantity, the second green quantity and the third the blue quantity of colours for mixing the required hue. Then we get system $RGB$, which every hue is identified by orderly triad $(R; G; B)$.

**3.6. Definion**: Let $C_r$ be $r$-chromatic set, let $P \subseteq C_r$ be its least two elements subset, let $<_P$ be the ordering of the set $P$. Then the set $P$ is called a palette selected from $r$-chromatic set $C_r$.

**3.7. Definition:** Let $\mathcal{F}_3$ be the physical chromatic space, let $F_{i,j,k}$, $F_{l,m,n}$ be physical voxels and let $\overline{F}_{i,j,k}$, $\overline{F}_{l,m,n}$ be its closings. The voxel $F_{l,m,n}$ is called the neighbour of voxel $F_{i,j,k}$ just then, when $\overline{F}_{l,m,n} \cap \overline{F}_{i,j,k} \neq \emptyset$.

**3.8. Definition:** Let $N_r$ be chromatic set of trichromatic system, let $\mathcal{F}_3$ be physical space of this system and $P \subseteq N_r$ is a palette selected from this system. Let there further exist two physical voxels $F_{i,j,k}$, $F_{l,m,n}$ in the palette at most, they have exactly one different neighbour from themself (so called initial resp. ending voxel of the palette). Other voxels have at least two neighbours different from themself. Then the palette $P$ is called a smooth palette.

For construction of the smooth palettes we can use the parametric defined connected curves $\alpha \subset \mathcal{G}_3$ , namely by following way: Let $\mathcal{F}_3$ be the physical chromaticc space, $(S;\mathbf{e}_1;\mathbf{e}_2;\mathbf{e}_3)$ its universal co-ordinate system induced by random mapping. In this co-ordinate system there is definied continuous curve by parametric equations $x = \varphi(t)$; $y = \psi(t)$; $z = \tau(t)$; $t \in \langle t_1;t_2 \rangle$. Lets denote $A_1 = [\varphi(t_1);\psi(t_1);\tau(t_1)] \in \mathcal{G}_3$, $A_2 = [\varphi(t_2);\psi(t_2);\tau(t_2)] \in \mathcal{G}_3$. Let $F^{(1)}$ be the physical voxel, for which it is $A_1 \in F^{(1)}$. $F^{(2)}$ is the physical voxel, for which it is $A_2 \in F^{(2)}$. Then the construction of the smooth palette consists of recursive halving of interval $\langle t_1;t_2 \rangle$.

## 4. The composition of a focused image from transparent optical cuts

For the composition of a focused image, some operations can be used, known from image processing. According to the previous description of the confocal microscope, we could say, that for composition of the sharp picture it is enough to sumarise pixels values of separate components of multifocal picture. According to the previous principle for every physical pixel it should exist at most one image ${}^{(k)}O$, for that is ${}^{(k)}O(F_{ij}) > 0$, in other words the intersection of particular optical cuts should be empty. However, practically in reality, it is complicated, because there are two optical cuts of the same preparation with non empty intersection. This fact can have two causes:

1. Even the height of sharpness zone of confocal microscope is minimal, it is not nought. The same point of preparation can then be focalizated in two cuts.

2. Scanned preparation is transparent, e.g. there is a luminous ray at its separate layers, which is partial repulsed and which partial goes through preparation, so that the preparation can be repulsed by other layers.

If we consider the object as transparent, there is a colour mixture of the light in repulse at diferent layers of preparation. This fact can be considered by weighing of separate component in the definition of generalization relevant image operations
I generalize the operation with images for any number of components and add their weighting. In the following section, $\mathbf{O} = \{{}^{(k)}O\}$ $k = 1;...;n$ is the multifocal image in system $RGB$. I define:

**4.1. Definition:** Compression weighted sum of images as an image, for which chromatic components is

$$_{m}c_{i,j} = {}^{(1)}c_{i,j}; m = 0,1,2 \text{ for } n = 1,$$

$$\Sigma\,{}^{(n+1)}_{\;m}c_{i,j} = Trunc\left[p \cdot \Sigma^{n}_{m}c_{i,j} + (1-p) \cdot {}^{(n+1)}_{\;m}c_{i,j}\right]; p \in (0;1)$$

(this operation makes possible to model transparency preparation),

**4.2. Definition:** Compression weighted product of images as an image, for which chromatic components is

$$_{m}c_{i,j} = {}^{(1)}c_{i,j}; m = 0,1,2 \text{ for } n = 1$$

$$\Pi\,{}^{(n+1)}_{\;m}c_{i,j} = \begin{cases} \Pi^{n}_{m}c_{i,j} & \text{for } {}^{(n+1)}_{\;m}c_{i,j} = 0 \\ Trunc\left[\frac{1}{256}\,\Pi^{n}_{m}c^{p}_{i,j} \cdot {}^{(n+1)}_{\;m}c^{1-p}_{i,j}\right] & \text{for } {}^{(n+1)}_{\;m}c_{i,j} > 0 \end{cases}; \quad p \in (0;1)$$

(this operation displays thin layers of preparation),

**4.3. Definition:** Compression weighted inverse product of images as an image, for which chromatic components is

$$_{m}c_{i,j} = {}^{(1)}c_{i,j}; m = 0,1,2 \text{ for } n = 1$$

$$\Pi\,{}^{(n+1)}_{\;m}c_{i,j} = \begin{cases} \Pi^{n}_{m}c_{i,j} & \text{for } {}^{(n+1)}_{\;m}c_{i,j} = 0 \\ Trunc\left[\frac{256}{\Pi^{n}_{m}c^{p}_{i,j} \cdot {}^{(n+1)}_{\;m}c^{1-p}_{i,j}}\right] & pro \quad {}^{(n+1)}_{\;m}c_{i,j} > 0 \end{cases}; \quad p \in (0;1)$$

(this operation displays thick layers of preparation),

**4.4. Definition:** disjunction of images as an image, for which chromatic components are

$$_{m}c_{i,j} = {}^{(1)}c_{i,j}; m = 0,1,2 \text{ for } n = 1,$$

$$\vee\,{}^{(n+1)}_{\;m}c_{i,j} = Max\left\{\vee\,{}^{(n+1)}_{\;m}c_{i,j};\ {}^{(n+1)}_{\;m}c_{i,j}\right\}$$

(this operation builds up most contrasty image)

## 5. Results

The results reflect, that it is possible to obtain images which are appropriate for all sorts of purposes by using above-cited operation.

Meaning of palettes is demonstred on fig. 2 - protozoon from genus Paramethyum. There are fluent palette determination by Lissajouss curve

$$\begin{aligned} R &= Trunc(145 - 90\cos 6t) \\ G &= Trunc(145 - 90\sin 5t) \quad t \in \langle 0;2\pi \rangle \\ B &= Trunc(145 - 90\sin 13t) \end{aligned}$$

on fig. 2

**Fig.2: Details highlighting by high frequency palette**

Frequences of used goniometrical functions are quite high, which results in very intense change of colours in a palette. Thereby it can excel a small change of image's details. On the other side it is not possible to presume pixels height from colours' pixel, because it is not sure, that colour is identified with explicit altitudes.

The choice of transparency can mainly influence the image of observed object and its approximation to reality when the operation compression sum is used. The preparation is displayed by using the same operations and the same transparency on fig 3., both images differ by used transparency only. The object on the left is non-transparent, the same object is displayed on the right, in the way how it would look, if it had been transparent from 80%

Fig. 4. demonstrates the use of compression product. It is evident from the definition's formula of this operation, that the value of processed pixel declines by every multiplication. If more images ${}^{(k)}O$ have nonzero value in the given physical pixel $F_{i,j}$ (it is in case, that thick layer of the preparation is situated here), the value of product in this pixel falls into the predetermined limit. If we in this case colour the pixel by the background colour, as result we would get the image, that contains only thin preparation layers and would not contain thick layers. The thickness of displayed layers

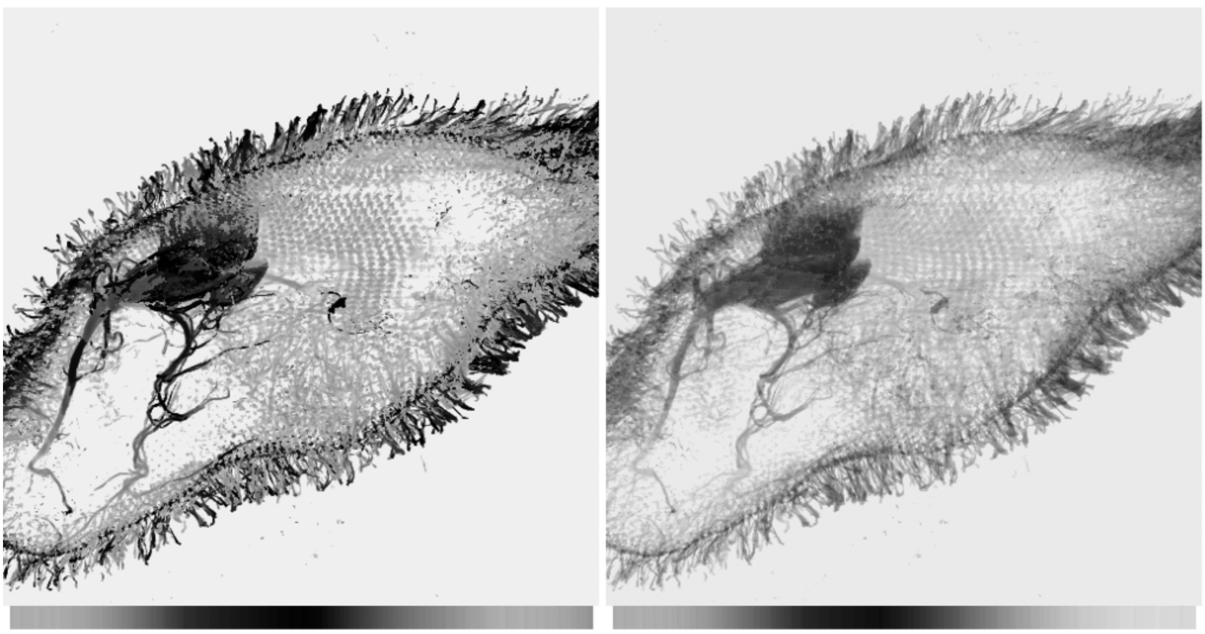

**Fig. 3: Modelling preparation transparency by compression sum**

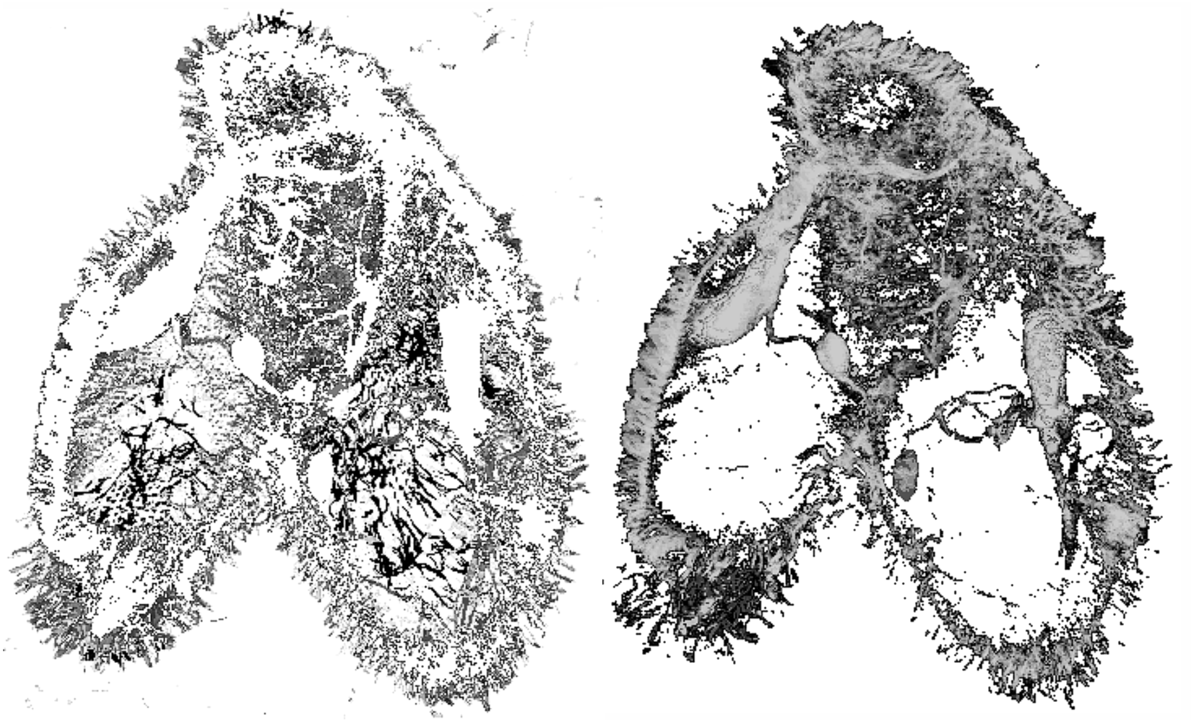

**Fig. 4: Displaying of the thin and thick layers by product and inverse product**

depends on selected parameter $p$. In fig. 4. on the left we see the thin layers, which are displayed by compression product for $p = 0.15$.

Fig. 4. on the right demonstrates the use of inverse product. From the definition expresion of this operation it is again evident, that the value of processed pixel grows by every division. If a small number of images ${}^{(k)}O$ has nonzero value in given physical pixel $F_{i,j}$ (such in the case, that thin layer preparation is situated here), the value of inverse product would stay down over the predetermined limit. If we colour the pixel by background colour in this case, as result we would get the image, which would not contain only thick layers of preparation and does not contain thin layers. Volume of displayed layers depends on selectiton of parameter $p$ again.

It may be presumed that similar mathematical tools might also be employed for spatial reconstructions of these preparations.

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