Introduction to EBImage,
an image processing and analysis toolkit for R

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Contents

1 Reading/displaying/writing images

The package EBImage is loaded by the following command.

```
> library("EBImage")
```

The function `readImage` is able to read images from files or URLs. Current supported image formats are JPEG, PNG and TIFF.

```
> f = system.file("images", "lena.png", package="EBImage")
> lena = readImage(f)
```

Images can be displayed using the function `display`. Pixel intensities should range from 0 (black) to 1 (white).

```
> display(lena)
```

Color images or images with multiple frames can also be read with `readImage`.

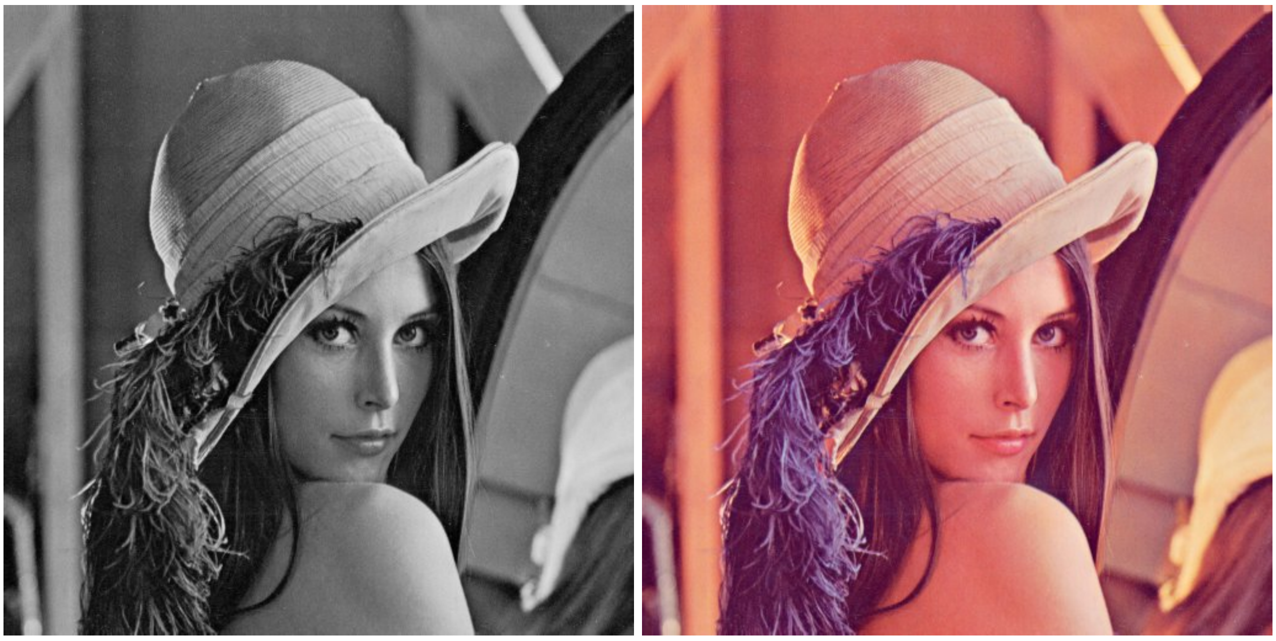
Figure 1: `lena`, `lenac`

```
> lenac = readImage(system.file("images", "lena-color.png", package="EBImage"))
> display(lenac)
> nuc = readImage(system.file('images', 'nuclei.tif', package='EBImage'))
```

```
image 510 x 510 x 0, tiles 0 x 0, bps = 8, spp = 1 (output 1), config = 1, colormap = no
image 510 x 510 x 0, tiles 0 x 0, bps = 8, spp = 1 (output 1), config = 1, colormap = no
image 510 x 510 x 0, tiles 0 x 0, bps = 8, spp = 1 (output 1), config = 1, colormap = no
image 510 x 510 x 0, tiles 0 x 0, bps = 8, spp = 1 (output 1), config = 1, colormap = no
```

```
> display(nuc)
```

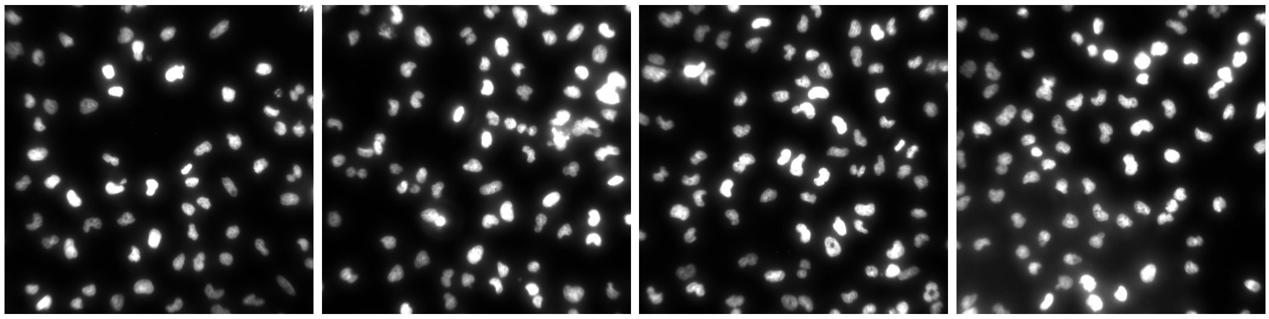
Figure 2: `nuc`

Images can be written with `writeImage`. The file format is deduced from the file name extension. This is useful to convert image formats, here from PNG format to JPEG format.

```
> writeImage(lena,  'lena.jpeg', quality=85)
> writeImage(lenac, 'lenac.jpeg', quality=85)
```

2 Image objects and matrices

The package EBImage uses the class Image to store and process images. Images are stored as multi-dimensional arrays containing the pixel intensities. All EBImage functions are also able to work with matrices and arrays.

```
> print(lena)

Image
  colormode: Grayscale
  storage.mode: double
  dim: 512 512
  nb.total.frames: 1
  nb.render.frames: 1

imageData(object)[1:5,1:6]:
          [,1]      [,2]      [,3]      [,4]      [,5]      [,6]
[1,] 0.5372549 0.5372549 0.5372549 0.5372549 0.5372549 0.5490196
[2,] 0.5372549 0.5372549 0.5372549 0.5372549 0.5372549 0.5490196
[3,] 0.5372549 0.5372549 0.5372549 0.5372549 0.5372549 0.5137255
[4,] 0.5333333 0.5333333 0.5333333 0.5333333 0.5333333 0.5098039
[5,] 0.5411765 0.5411765 0.5411765 0.5411765 0.5411765 0.5333333
```

As matrices, images can be manipulated with all R mathematical operators. This includes `+` to control the brightness of an image, `*` to control the contrast of an image or `^` to control the gamma correction parameter.

```
> lena1 = lena+0.5
> lena2 = 3*lena
> lena3 = (0.2+lena)^3
```

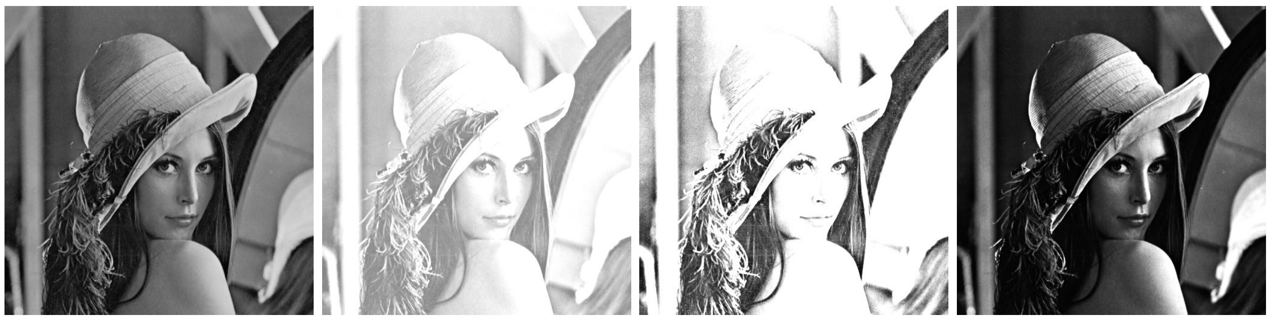

Figure 3: `lena`, `lena1`, `lena2`, `lena3`

Others operators include `[` to crop images, `<` to threshold images or `t` to transpose images.

```
> lena4 = lena[299:376, 224:301]
> lena5 = lena>0.5
> lena6 = t(lena)
> print(median(lena))

[1] 0.3803922
```

Images with multiple frames are created using `combine` which merges images.

```
> lenacomb = combine(lena, lena*2, lena*3, lena*4)
> display(lenacomb)
```

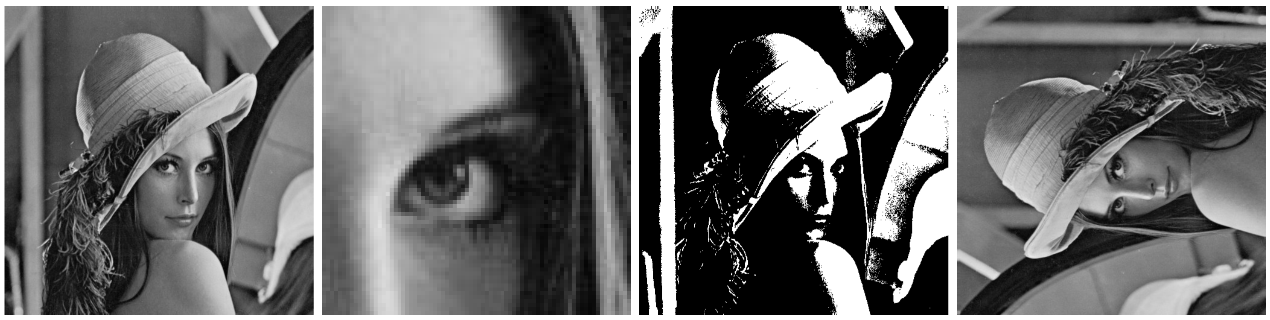
Figure 4: `lena`, `lena4`, `lena5`, `lena6`

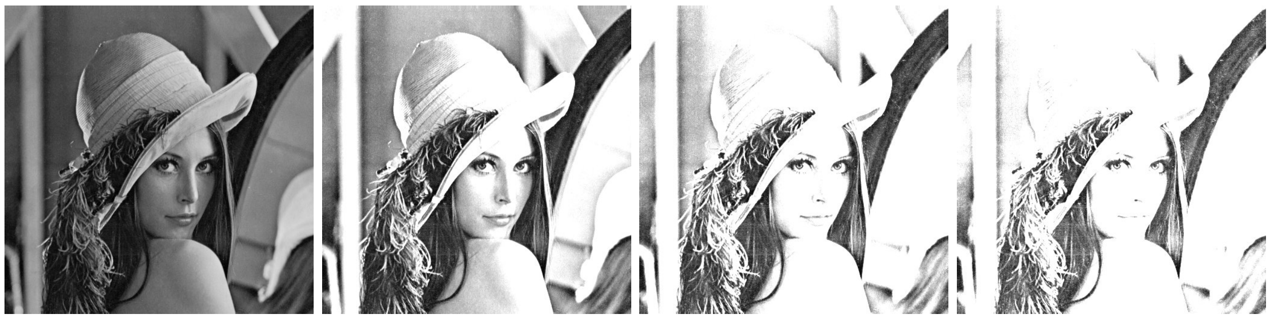
Figure 5: `lenacomb`

3 Spatial transformations

Specific spatial image transformations are done with the functions `resize`, `rotate`, `translate` and the functions `flip` and `flop` to reflect images.

```
> lena7 = rotate(lena, 30)
> lena8 = translate(lena, c(40, 70))
> lena9 = flip(lena)
```

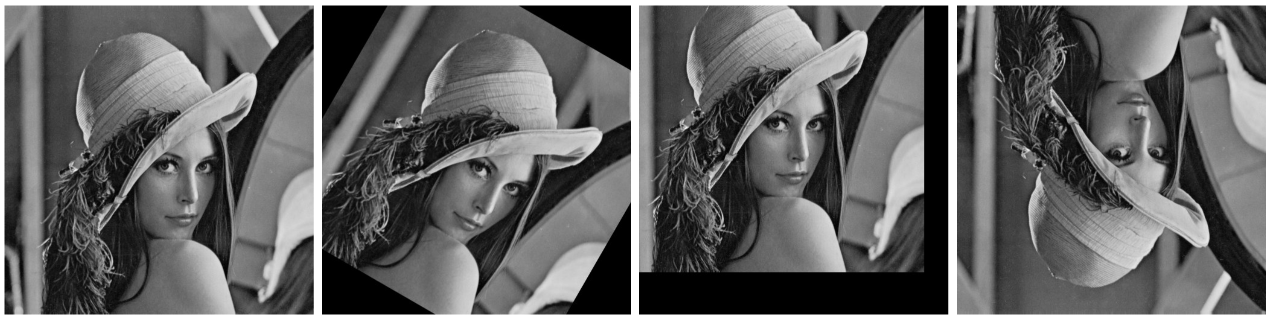
Figure 6: `lena`, `lena7`, `lena8`, `lena9`

4 Color management

The class `Image` extends the base class `array` and uses the `colormode` slot to store how the color information of the multi-dimensional data should be handled.

As an example, the color image `lenac` is a 512x512x3 array, with a `colormode` slot equals to `Color`. The object is understood as a color image by `EBImage` functions.

```
> print(lenac)
```

```
Image
  colormode: Color
  storage.mode: double
  dim: 512 512 3
  nb.total.frames: 3
  nb.render.frames: 1

imageData(object)[1:5,1:6,1]:
          [,1]      [,2]      [,3]      [,4]      [,5]      [,6]
[1,] 0.8862745 0.8862745 0.8862745 0.8862745 0.8862745 0.8901961
[2,] 0.8862745 0.8862745 0.8862745 0.8862745 0.8862745 0.8901961
[3,] 0.8745098 0.8745098 0.8745098 0.8745098 0.8745098 0.8901961
[4,] 0.8745098 0.8745098 0.8745098 0.8745098 0.8745098 0.8705882
[5,] 0.8862745 0.8862745 0.8862745 0.8862745 0.8862745 0.8862745
```

The function `colorMode` can access and change the value of the slot `colormode`, modifying the rendering mode of an image. In the next example, the `Color` image `lenac` with one frame is changed into a `Grayscale` image with 3 frames, corresponding to the red, green and blue channels. The function `colorMode` does not change the content of the image but changes only the way the image is rendered by `EBImage`.

```
> colorMode(lenac) = Grayscale
> display(lenac)
```

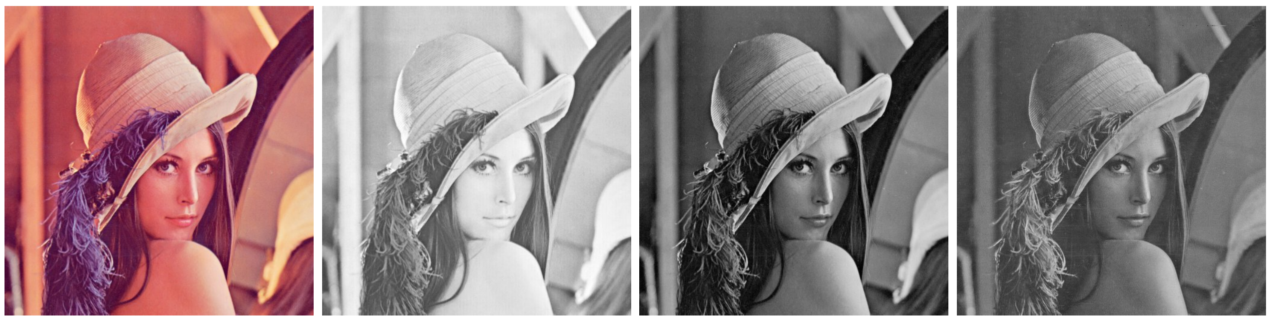

Figure 7: `lenac`, rendered as a `Color` image and as a `Grayscale` image with 3 frames (red channel, green channel, blue channel)

The color mode of image `lenac` is reverted back to `Color`.

```
> colorMode(lenac) = Color
```

The function `channel` performs colorspace conversion and can convert `Grayscale` images into `Color` ones both ways and can extract color channels from `Color` images. Unlike `colorMode`, `channel` changes the pixel intensity values of the image. The function `rgbImage` is able to combine 3 `Grayscale` images into a `Color` one.

```
> lenak = channel(lena, 'rgb')
> lenak[236:276, 106:146, 1] = 1
> lenak[236:276, 156:196, 2] = 1
> lenak[236:276, 206:246, 3] = 1
> lenab = rgbImage(red=lena, green=flip(lena), blue=flop(lena))
```

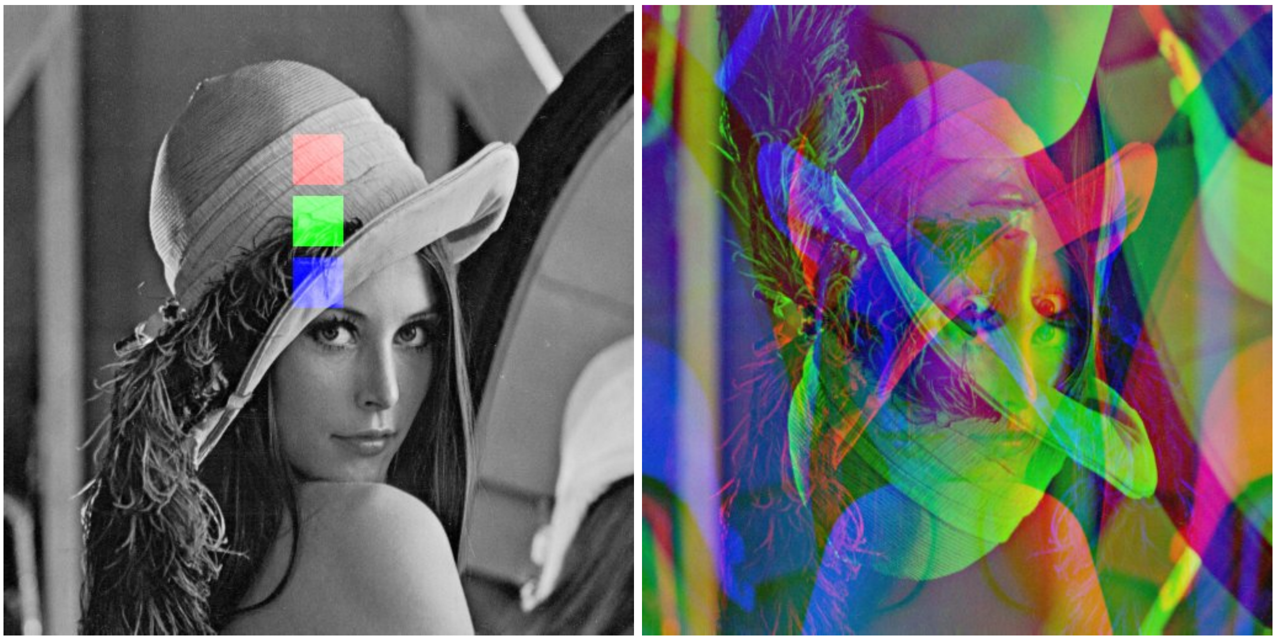

Figure 8: `lenak`, `lenab`

5 Image filtering

Images can be linearly filtered using `filter2`. `filter2` convolves the image with a matrix filter. Linear filtering is useful to perform low-pass filtering (to blur images, remove noise, ...) and high-pass filtering (to detect edges, sharpen images, ...). Various filter shapes can be generated using `makeBrush`.

```
> flo = makeBrush(21, shape='disc', step=FALSE)^2
> flo = flo/sum(flo)
> lenaflo = filter2(lenac, flo)
> fhi =  matrix(1, nc=3, nr=3)
> fhi[2,2] = -8
> lenafhi = filter2(lenac, fhi)
```

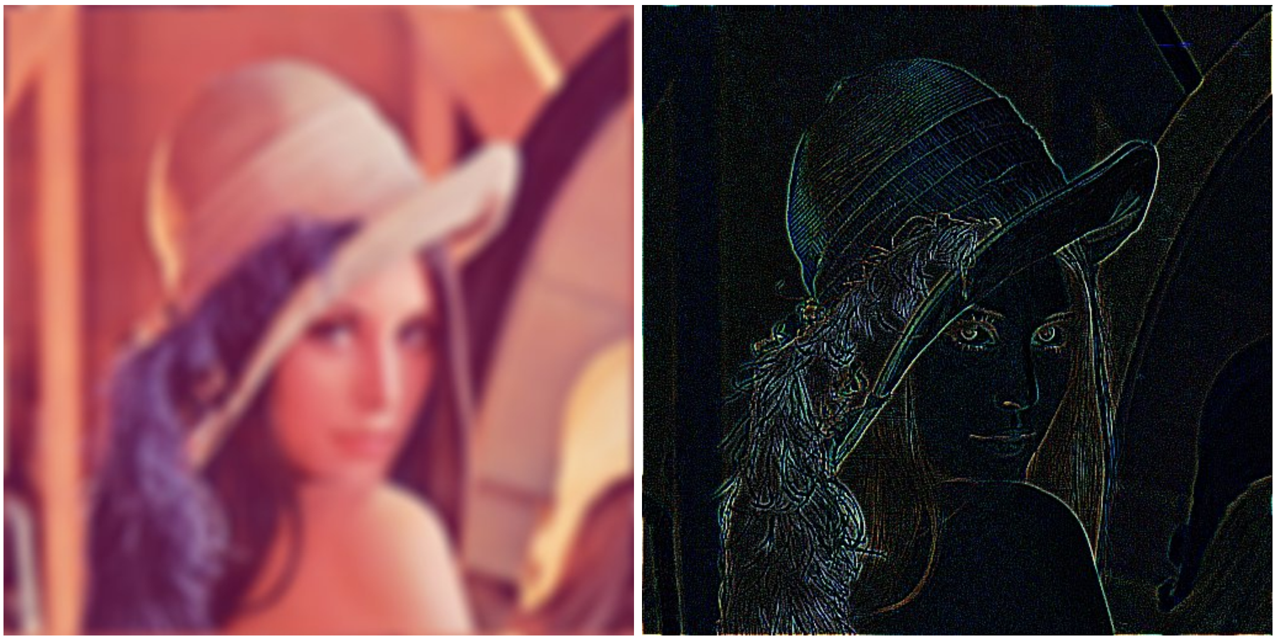

Figure 9: Low-pass filtered `lenaflo` and high-pass filtered `lenafhi`

6 Morphological operations

Binary images are images where the pixels of value 0 constitute the background and the other ones constitute the foreground. These images are subject to several non-linear mathematical operators called morphological operators, able to `erode` and `dilate` an image.

```
> ei = readImage(system.file('images', 'shapes.png', package='EBImage'))
> ei = ei[110:512,1:130]
> display(ei)
> kern = makeBrush(5, shape='diamond')
> eierode = erode(ei, kern)
> eidilat = dilate(ei, kern)
```

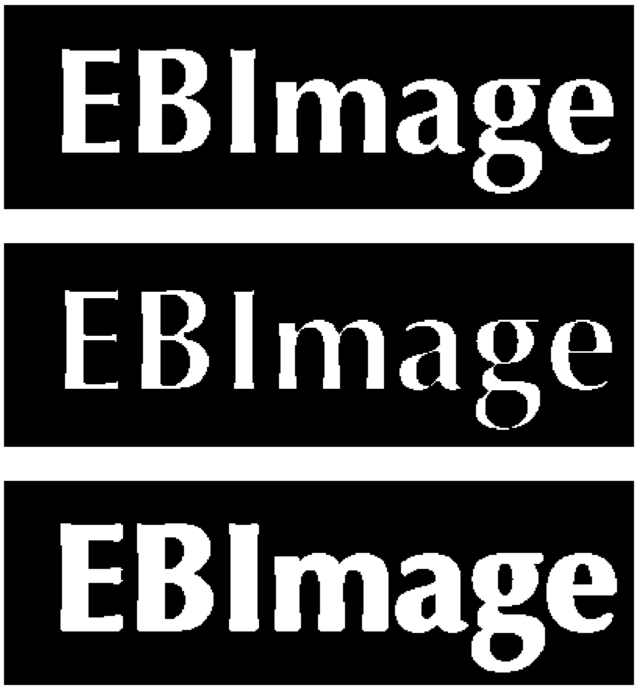

Figure 10: `ei` ; `eierode` ; `eidilat`

7 Segmentation

Segmentation consists in extracting objects from an image. The function `bwlabel` is a simple function able to extract every connected sets of pixels from an image and relabel these sets with a unique increasing integer. `bwlabel` can be used on binary images and is useful after thresholding.

```
> eilabel = bwlabel(ei)
> cat('Number of objects=', max(eilabel),'\n')

Number of objects= 7

> nuct = nuc[,,1]>0.2
> nuclabel = bwlabel(nuct)
> cat('Number of nuclei=', max(nuclabel),'\n')

Number of nuclei= 74
```

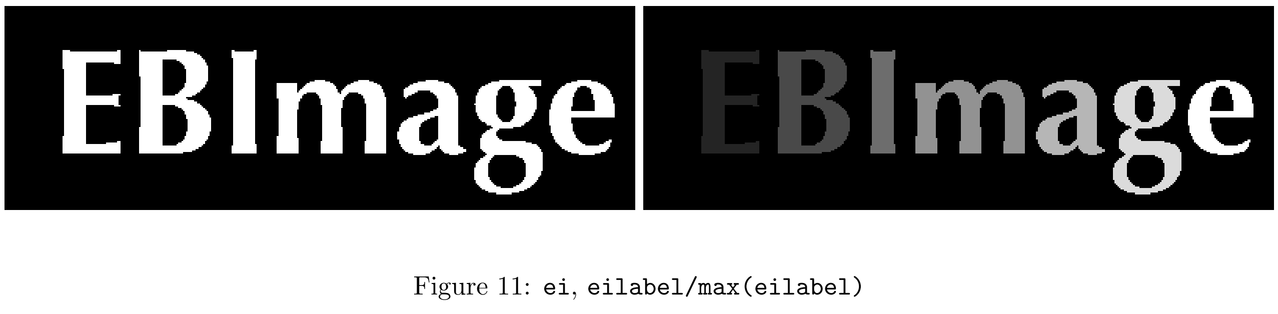

Figure 11: `ei`, `eilabel/max(eilabel)`

Since the images `eilabel` and `nuclabel` range from 0 to the number of object they contain (given by `max(eilabel)` and `max(nucabel)`), they have to be divided by these number before displaying, in order to fit the [0,1] range needed by `display`.

The grayscale top-bottom gradient observable in `eilabel` and `nuclabel` is due to the way `bwlabel` labels the connected sets, from top-left to bottom-right.

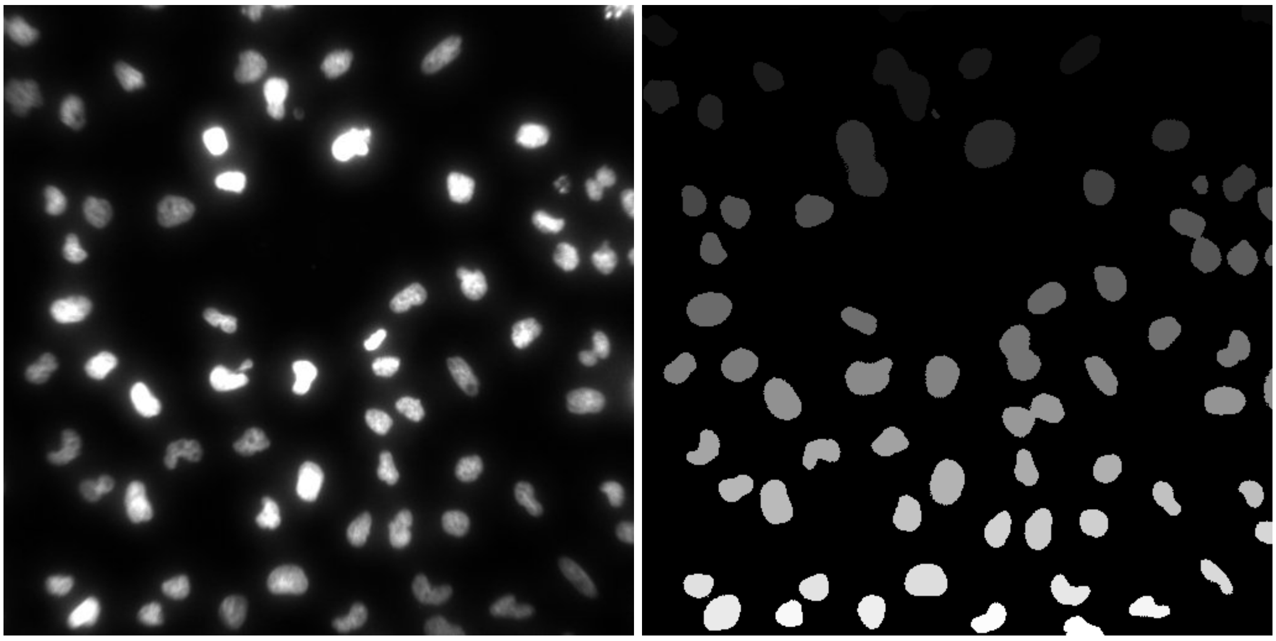

Figure 12: `nuc[ , ,1]`, `nuclabel/max(nuclabel)`

Adaptive thresholding consists in comparing the intensity of pixels with their neighbors, where the neighborhood is specified by a filter matrix. The function `thresh` performs a fast adaptive thresholding of an image with a rectangular window while the combination of `filter2` and `<` allows a finer control. Adaptive thresholding allows a better segmentation when objects are close together.

```
> nuct2 =  thresh(nuc[,,1], w=10, h=10, offset=0.05)
> kern = makeBrush(5, shape='disc')
> nuct2 = dilate(erode(nuct2, kern), kern)
> nuclabel2 = bwlabel(nuct2)
> cat('Number of nuclei=', max(nuclabel2),'\n')

Number of nuclei= 77
```

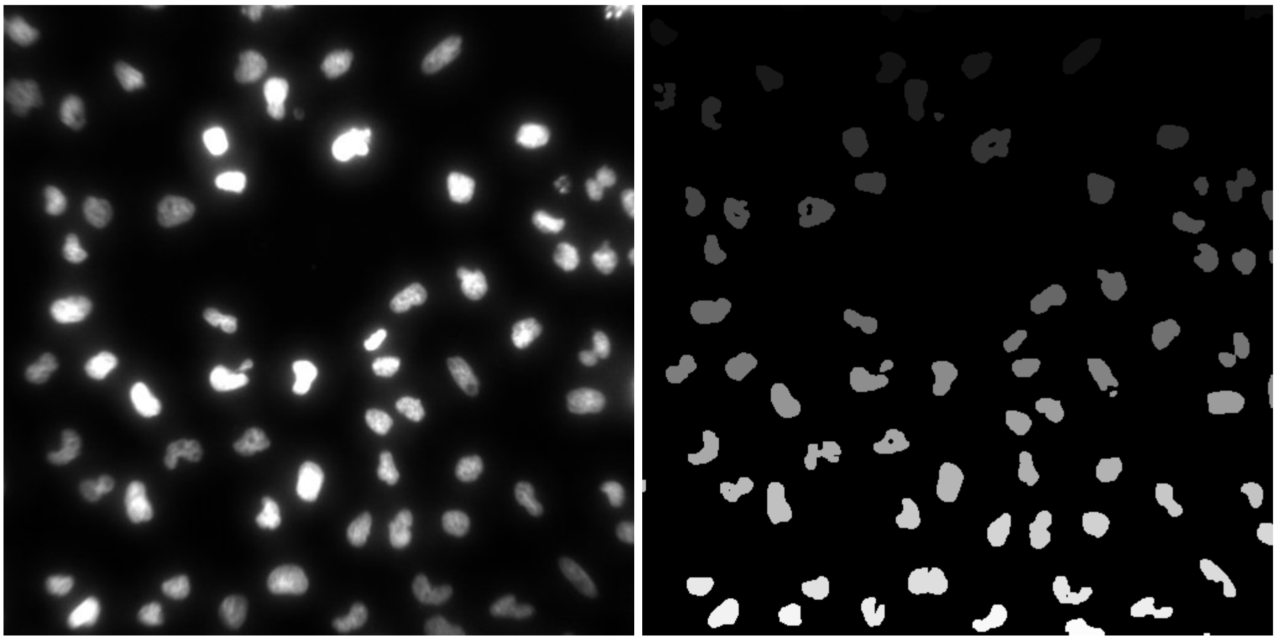

Figure 13: `nuc[ , ,1]`, `nuclabel2/max(nuclabel)`

8 Object manipulation

Objects, defined as sets of pixels with the same unique integer value can be outlined and painted using `paintObjects`. Some holes are present in objects of `nuclabel2` which can be filled using `fillHull`.

```
> nucgray = channel(nuc[,,1], 'rgb')
> nuch1 = paintObjects(nuclabel2, nucgray, col='#ff00ff')
> nuclabel3 = fillHull(nuclabel2)
> nuch2 = paintObjects(nuclabel3, nucgray, col='#ff00ff')
```

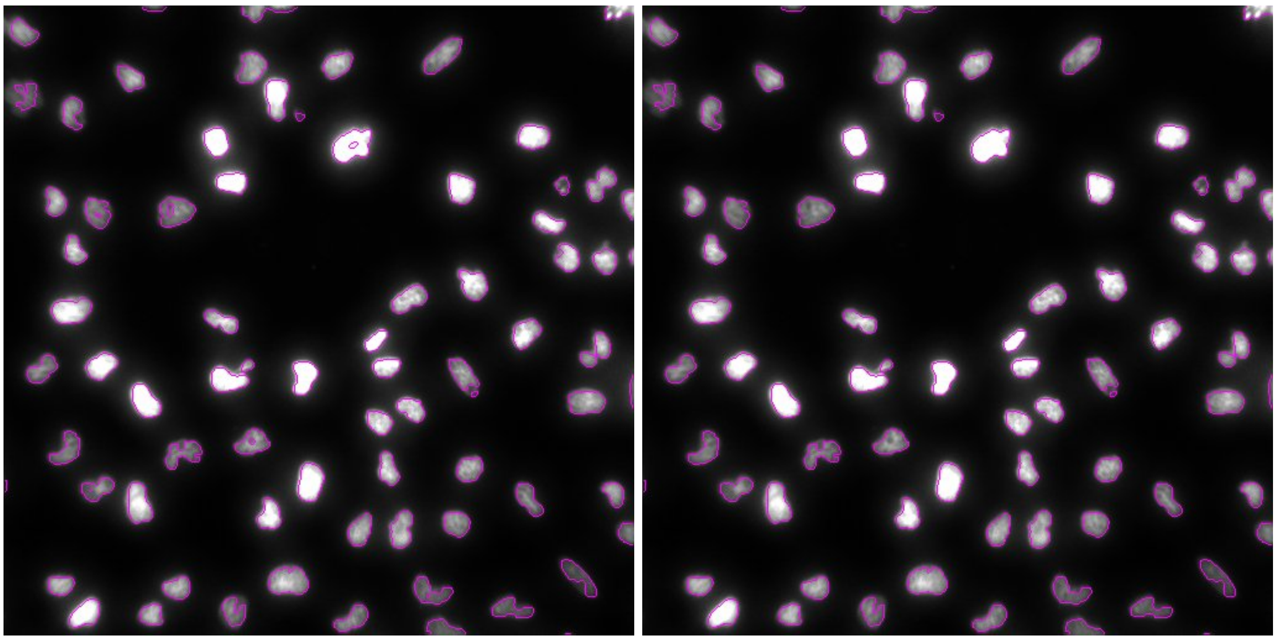

Figure 14: `nuch1`, `nuch2`

A broad variety of objects features (basic, image moments, shape, Haralick features) can be computed using `computeFeatures`. In particular, object coordinates are computed with the function `computeFeatures.moment`.

```
> xy = computeFeatures.moment(nuclabel3)[, c("m.cx", "m.cy")]
> xy[1:4,]

       m.cx      m.cy
1 122.37079  2.808989
2 211.70062  4.910494
3 497.94009  5.474654
4  16.75851 22.907121
```

9 Cell segmentation example

This is a complete example of segmentation of cells (nucleus + cell bodies) using the functions described before and the function propagate, able to perform Voronoi-based region segmentation.

Images of nuclei and cell bodies are first loaded:

```
> nuc = readImage(system.file('images', 'nuclei.tif', package='EBImage'))

image 510 x 510 x 0, tiles 0 x 0, bps = 8, spp = 1 (output 1), config = 1, colormap = no
image 510 x 510 x 0, tiles 0 x 0, bps = 8, spp = 1 (output 1), config = 1, colormap = no
image 510 x 510 x 0, tiles 0 x 0, bps = 8, spp = 1 (output 1), config = 1, colormap = no
image 510 x 510 x 0, tiles 0 x 0, bps = 8, spp = 1 (output 1), config = 1, colormap = no

> cel = readImage(system.file('images', 'cells.tif', package='EBImage'))

image 510 x 510 x 0, tiles 0 x 0, bps = 8, spp = 1 (output 1), config = 1, colormap = no
image 510 x 510 x 0, tiles 0 x 0, bps = 8, spp = 1 (output 1), config = 1, colormap = no
image 510 x 510 x 0, tiles 0 x 0, bps = 8, spp = 1 (output 1), config = 1, colormap = no
image 510 x 510 x 0, tiles 0 x 0, bps = 8, spp = 1 (output 1), config = 1, colormap = no

> img = rgbImage(green=1.5*cel, blue=nuc)
```

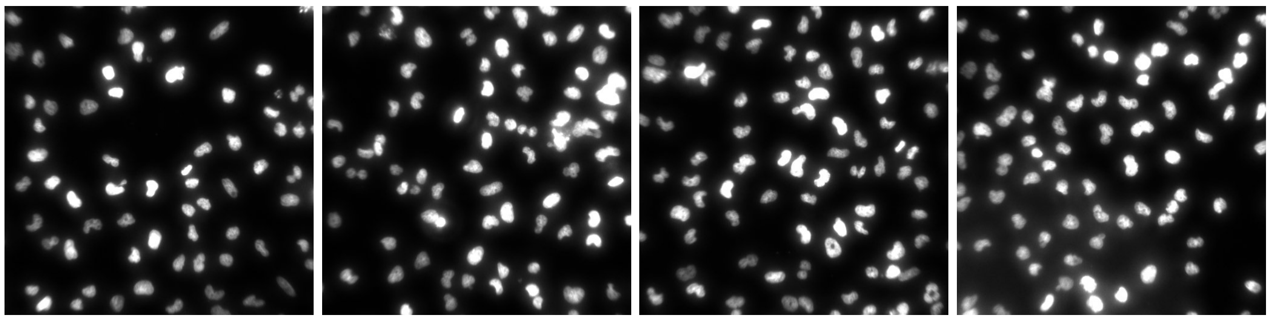

Figure 15: nuc

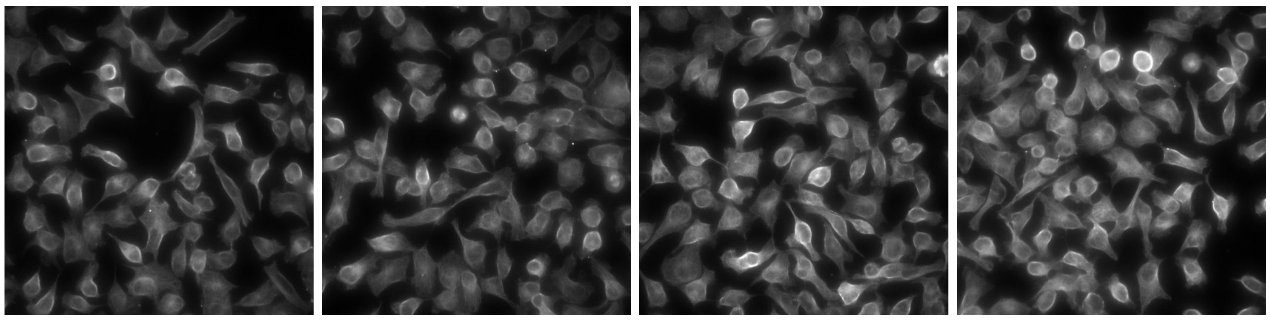

Figure 16: cel

Nuclei are first segmented using thresh, fillHull, bwlabel and opening, which is an erosion followed by a dilatation.

```
> nmask = thresh(nuc, w=10, h=10, offset=0.05)
> nmask = opening(nmask, makeBrush(5, shape='disc'))
> nmask = fillHull(nmask)
> nmask = bwlabel(nmask)
```

Cell bodies are segmented using propagate.

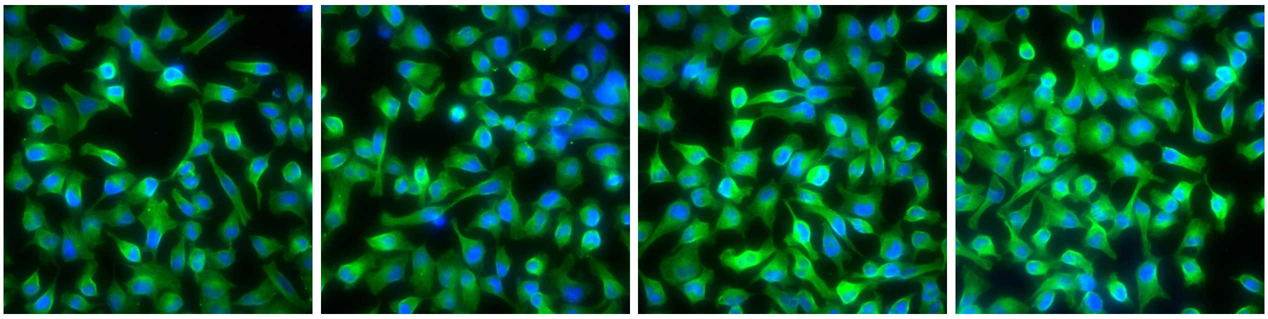

Figure 17: img

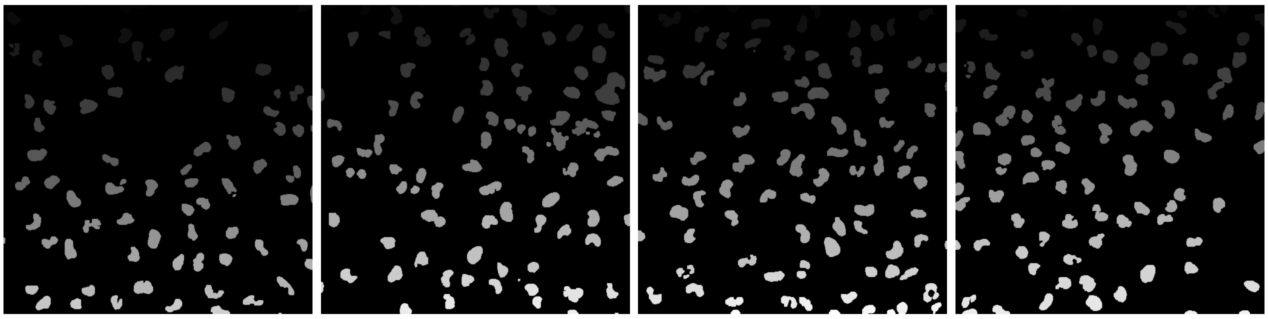

Figure 18: nmask/max(nmask)

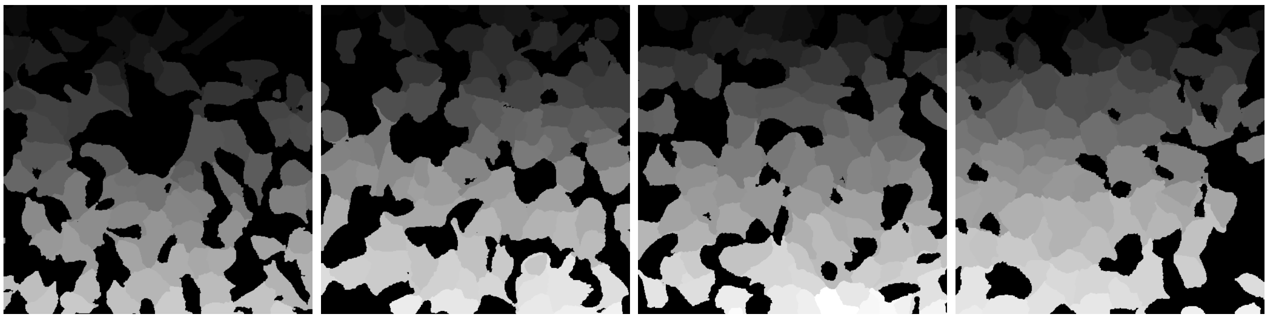

Figure 19: cmask/max(cmask)

```
> ctmask = opening(cel>0.1, makeBrush(5, shape='disc'))
> cmask = propagate(cel, seeds=nmask, mask=ctmask)
```

Cells are outlined using paintObjects.

```
> res = paintObjects(cmask, img, col='#ff00ff')
> res = paintObjects(nmask, res, col='#ffff00')
```

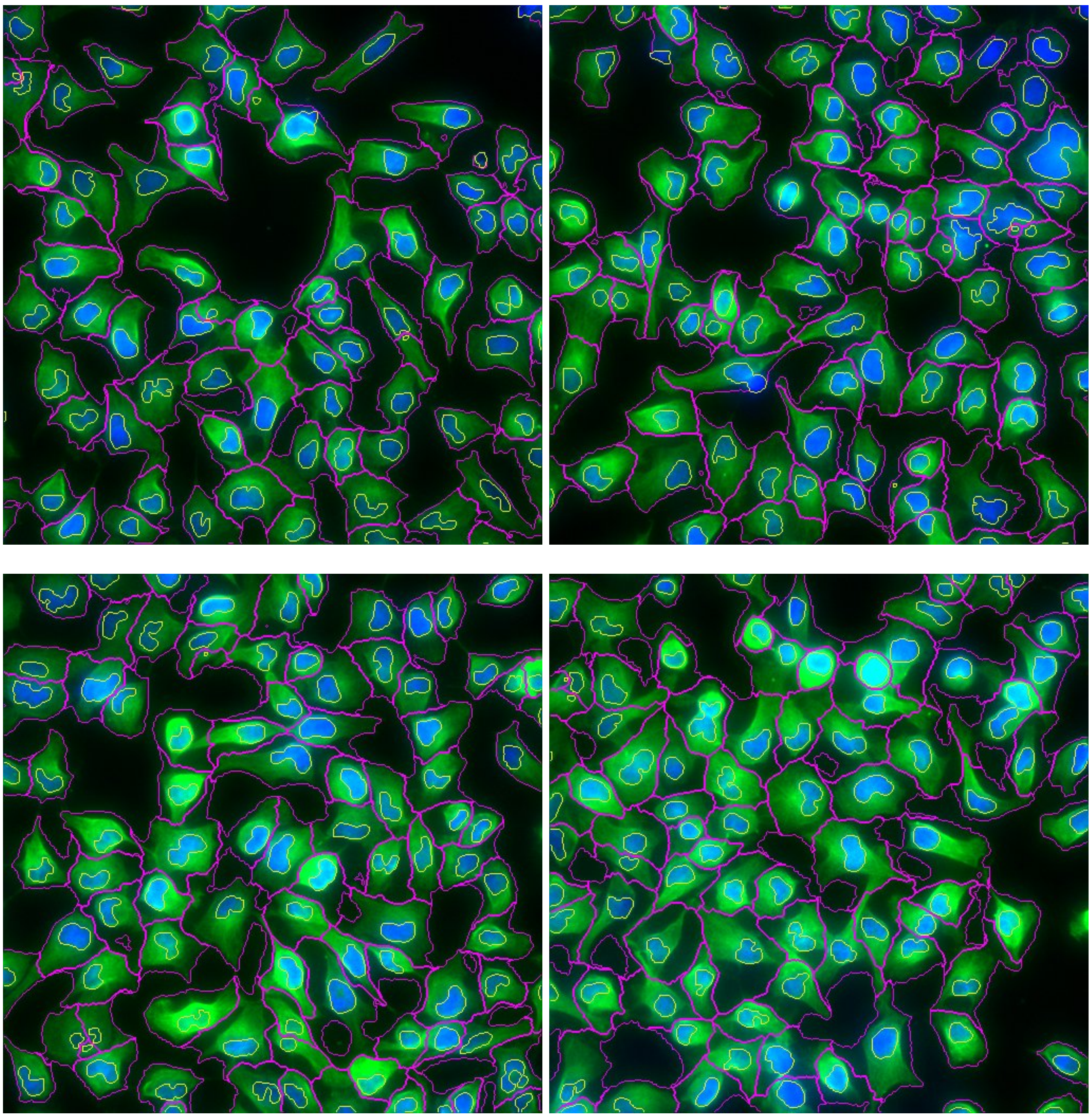
Figure 20: Final segmentation `res`